Revealing the Secrets of African Annonaceae

Systematics, Evolution and Biogeography of the Syncarpous Genera *Isolona* and *Monodora*

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Dit onderzoek is uitgevoerd binnen de onderzoekschool Biodiversiteit

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Proefschrift
ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit
Prof.dr. M.J. Kropff
in het openbaar te verdedigen
op maandag 21 april 2008
des namiddags te vier uur in de Aula

Thomas L.P. Couvreur (2008)

Revealing the Secrets of African Annonaceae: Systematics, Evolution and Biogeography of the Syncarpous Genera *Isolona* and *Monodora*

PhD thesis Wageningen University, The Netherlands
With references – with summaries in English and Dutch.

ISBN 978-90-8504-924-1

to my parents

Contents

1 General Introduction

"Biodiversity is our most valuable but least appreciated resource"
Wilson, E.O., 1992

BIODIVERSITY

The Unit: Species

Biological diversity, or simply biodiversity, surrounds (and inhabits) each and every one of us. Mankind has depended and will always depend on biodiversity for his survival. In fact, mankind itself is part of earth's biodiversity. Vital substances such as food, medicine or shelter are all primarily derived from the diversity of species occurring on our planet, and that is why Wilson (1992) refers to it as "our most valuable resource". But what exactly *is* biodiversity? It is fairly simple to understand what biodiversity is *as a whole*: it represents all living organisms, a bit like a dictionary representing all known words for a language. The tricky question lies in the determination of what biodiversity is composed of? The United Nations Convention on Biological Diversity defines biodiversity as "the variability among living organisms from all sources […]. This includes diversity within species, between species and of ecosystems." Thus, from this definition we may conclude that species play a crucial role in defining biodiversity. Indeed, a 'species' is the direct outcome of evolution and represents the *fundamental unit* in the vast field of biology, from molecular biology to ecology as well as in evolution (de Queiroz, 2005). But what is a species? Interestingly, there is yet no satisfactory definition of what constitutes a species (Hey, 2001; Hey, 2006), even though this question is viewed as the "oldest and most fundamental in biology" (Dobzhansky, 1935). Over the last decades, a great deal of time and ink has been spent on trying to solve this problem, not just by biologist but also by philosophers. There exist over 20 species definitions based on different biological properties (for a review see Mayden, 1997). For example, the biological species concept uses the idea of reproductive isolation (Mayr, 1940) while the ecological species concept emphasizes the occupation of a distinct ecological niche (Van Valen, 1976). Any of these definitions has advantages as well as drawbacks, or are simply inapplicable to a certain extent. It is likely that the species problem will never have a clear solution: "The fact is that species are hard to define for a variety of reasons related to the various ways they can be truly indistinct, and no criterion that presumes to delineate natural boundaries can overcome this." (Hey, 2006).

The theory of evolution by natural selection presented by Darwin (1859) was mainly based on the observation that species do not represent fixed entities as previously thought, but evolve through time. This led Dobzhansky (1955, p. 183) to state that if anyone could actually

provide a "universally applicable, static definition of species" it would shed serious doubt over the whole modern theory of evolution. Despite this problem, the term species is widely used among researchers in biology as if a general, but ill-definable, consensus had been reached. It is important to recognize the difficulty in defining the word species, and to accept that the concept of 'species' is more a working hypothesis than a real entity. As in most fields of biology, when the reality is too complicated to capture (be it a definition or a process) one has to clearly define the working hypotheses or assumptions used in a study. In a taxonomic revision this means explicitly stating the species concept used (Luckow, 1995). In this thesis we follow the phylogenetic species concept (Cracraft, 1983; Nixon and Wheeler, 1990) in that the *hypothesis* of a species is formulated by identifying the smallest aggregation of individuals diagnosable by a unique combination of *constant* (or fixed) character states observable by ordinary morphological means (Snow, 1997). "Ordinary morphological means" refers to morphological characters that are immediately visible on the herbarium sheets or require no more that 30x magnification (e.g. hairs). The consistency of character states across individuals of the same species is interpreted as reliable and indicative of the existence of a common history shared among them (Luckow, 1995; Snow, 1997). In addition, we do not consider a species to be polyphyletic, i.e. a group of individuals with several different most recent common ancestors.

The Tool: Systematics

Putting the debate of 'what are species' aside, it is generally accepted that the fundamental unit of biodiversity is the species (Claridge et al., 1997). Estimates of the Earth's total number of species varies greatly, from 4 to 100 million species (Pennisi, 2003), but 14 million is a widely accepted number. The fact that we cannot provide a better estimate underlines how little we know about what builds up biodiversity. Identifying, describing, classifying and naming Earth's biodiversity as well as understanding the evolutionary relationships between species is the major role of the branch of biology called "systematics" or "taxonomy"[1]. Systematics is one of the oldest scientific disciplines and every culture has developed some kind of system to classify the plants and animals they encounter and use. How much have we already described? Well, not much. Estimates vary once again, ranging from 1.4 to 1.7 million described species (Pennisi, 2003). Thus much work is still needed. Unfortunately, our time is limited. In the past decades the use of the term "biodiversity crisis" has been on the increase in the media. In the coming decades we shall see a major loss of species diversity, almost completely caused by human actives. We are still far from

[1] There appears to be some confusion between the words taxonomy and systematics (De Queiroz, 1988). Some consider them as two separate entities, with systematics concerned with the study of relationships between living organisms and taxonomy with the identification and classification of species into a scheme of words (e.g. Wiley, 1981). Others suggest that taxonomy encloses systematics and classification (De Queiroz, 1988). In this thesis I use the term systematics to refer to the action of describing the species as well as studying their evolutionary relationships.

inventorying and understanding the world's biodiversity and yet it is already disappearing at a quick and steady pace. There are three good reasons why the loss of biodiversity and the ecosystems in which they occur in should not be ignored. First, the undescribed but disappearing biodiversity could provide valuable resources yet to be exploited. We have gained so much already from the meager ca. 12% [2] of described biodiversity, who knows what we can find in the rest. Second, some species occupy a more important role in a particular ecosystem than others. Such species are called "keystone" species (Mills et al., 1993). The basic idea is that if a keystone species goes extinct or disappears from the ecosystem this will have repercussions on many other species that depend on it (e.g. an animal species that disperses the seeds of numerous different tree species). Thus the loss of even a fairly low number of species could lead to the collapse of entire ecosystems because of these close interrelationships and existing interdependences of species. Finally, the loss of species and their ecosystems will lead to the loss of the evolutionary potential from which this diversity is generated. Loss of entire ecosystems will affect the evolutionary ability to re-diversify the planet. Tropical ecosystems in particular are often referred to as "evolutionary powerhouses" because they have contributed immensely to the production of present day biodiversity. Some studies demonstrated the importance of the evolutionary potential of tropical regions not only for the tropics but also for temperate ecosystems. The "out of the tropics" pattern shows that diversity often originates in the tropics and then expands into temperate regions (Jablonski, 1993; Jablonski et al., 2006). Thus, this crisis not only eliminates potentially important (and undescribed) species but also threatens to alter the potential for *rediversification* of the planet once the crisis has passed. Michael Soule puts it this way (1980): "Death is one thing, the end of birth is something else". We need to understand in order to conserve, in other words we need to know how many species there are, their relationships as well as their evolutionary history and origins. In that respect, systematics plays a pivotal role. With systematic knowledge we can devise better-informed conservation strategies for protecting not only species richness but also the evolutionary processes they represent in a given ecosystem.

Hotspots

It is well known that biodiversity is not distributed equally on the planet. Some regions contain many more species than others (e.g. deserts vs. tropical rain forests). Many systematists and biogeographers have mapped and described regions with exceptionally high concentrations of endemic species. More recently, the focus has been on those regions that in addition face exceptional levels of habitat loss (Myers, 1988; Mittermeier et al., 1998; Myers et al., 2000; Kuper et al., 2004). Such regions are called "biodiversity hotspots" and are a major focus of biodiversity conservation efforts. Initially, 25 such hotspots were identified

[2] This percentage was obtained by taking a total number of species of 14 million and 1.7 million currently described species.

totaling as little as 1.4% of Earth's surface and containing as much as 45% of all land plant species (Myers et al., 2000). Although the methods used to identify hotspots are sometimes controversial (see Myers and Mittermeier, 2003), the impact of the hotspots concept in terms of investment in conservation has been huge. Many international conservation organizations (such as Conservation International or even the World Bank) use hotspots as a primary goal for their investments. The recognition of hotspots allowed the conservation community to focus their efforts and funding to specific regions and maximizes the "number of species saved per dollar".

However, defining and mapping these regions is merely the first step. Even though some regions have been recognized as hotspots they remain largely unknown in terms of species composition. For example, the Eastern Arc Mountains and the Coastal Forests hotspot of Kenya and Tanzania are known to be one of the most diverse regions of the planet. This biological richness is clearly illustrated by the family Annonaceae (see below). An analysis based on distributional data of Tanzanian Annonaceae indicated that the highest diversity in terms of number of species and genera were found in lowland rain forests along the coast and in the montane rain forests of the Eastern Arc mountains (Couvreur et al., 2006; Fig. 1.1). Yet, during my taxonomic research on the plant genera *Isolona* and *Monodora* of the Annonaceae family I described no less than four new species of Annonaceae from Tanzania alone (Couvreur et al., 2006) and even discovered an entirely new genus of Annonaceae (Couvreur et al., in prep). This, however, is not exceptional as many new species and genera are still awaiting their discovery in this hotspot. Finally, we also need to have a better understanding on how these regions of exceptional diversity arose: when and how did they originate? As indicated above, a better understanding of these regions will facilitate more efficient and effective conservation.

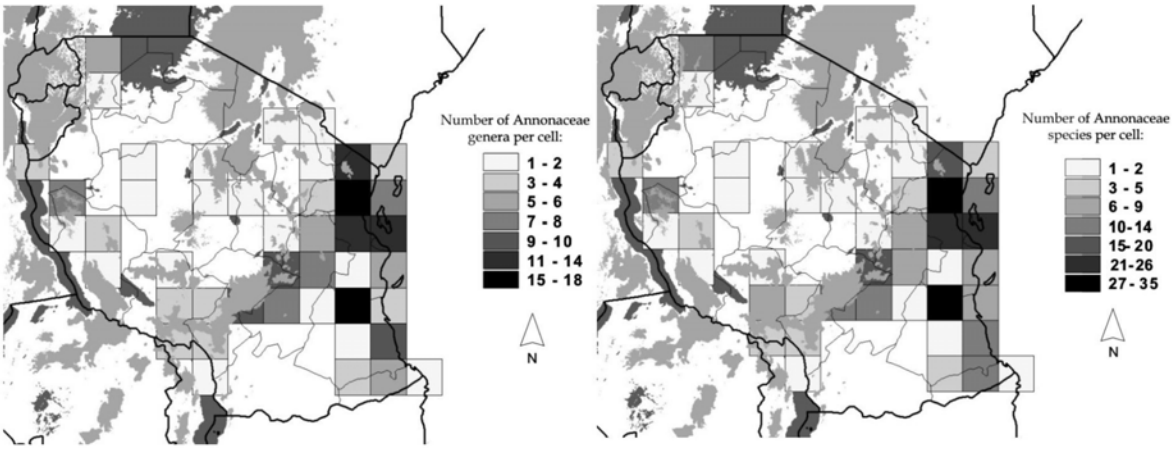


Figure 1.1. Generic and species diversity map of Annonaceae in Tanzania. Regional administrative boundaries are indicated. Symbols: Light grey patches, land above 1400 m; Dark grey patches, water. (Modified from Couvreur et al, 2006).

African Annonaceae

Annonaceae is a tropical family of trees, shrubs, and lianas belonging to the order Magnoliales (APGII, 2003). It is the most diverse family of this comparatively "primitive" order, comprising some 130 genera and ca. 2500 species (Chatrou et al., 2004). They form an easily recognizable and natural group with alternate distichous leaves, a trimerous perianth, numerous stamens and one to numerous carpels (Keßler, 1993). Classification within the family and delimitation of genera, however, has been much debated (Koek-Noorman et al., 1990). Annonaceae are almost entirely tropical, with three main centres of distribution. The combined area of South-East Asia, Australia, and the Pacific islands is the richest in genera (ca. 60) and species (ca. 1100; (Keßler, 1993). In terms of genera, tropical Africa and the Neotropics are equivalent (ca. 40), but the latter holds more species, ca. 900 compared to ca. 500 respectively (Mols, 2004). Only two genera occur in North America: *Asimina* and *Deeringothamus*. Due to their pantropical distribution, great morphological diversity, and key ecological role in tropical forests (Gentry, 1993), systematic and evolutionary studies of Annonaceae taxa provide important insights into the evolution of tropical floras (e.g. Pirie et al., 2006; Erkens et al., 2007).

African Annonaceae have been largely understudied in recent years when compared to the major focus on Neotropical and South-East Asian taxa, coordinated during the past two decades by the Nationaal Herbarium Nederland (NHN) - Utrecht and - Leiden branches, respectively. In 2002, the NHN-Wageningen branch initiated a long-term project focusing on the systematics of African Annonaceae. The African genera were treated as a whole for the last time over 100 years ago by Engler and Diels (1901). Since then, regional accounts have been published in the second half of the last century such as those for Flore du Gabon (Le Thomas, 1969) and Flora of Tropical East Africa (Verdcourt, 1971). Besides that, small proportions of African species have been part of monographic research (e.g. Chatrou, 1998; Maas et al., 2003). Thus, it is apparent that the state of our taxonomic knowledge of African Annonaceae is rather poor.

Distribution

Most African Annonaceae are restricted to lowland or montane rain forests across Africa and in Madagascar (Fig. 1.2). A minority of species, however, have adapted to slightly more arid conditions and can be found in thickets or savanna type vegetations.

Even though rain forests appear homogeneous across tropical Africa, species composition varies greatly between different regions. In Africa, the most striking difference is that between the Eastern Arc Mountains and Coastal rain forests of East Africa and the larger rain forest block in West-Central Africa called the Guineo-Congolian floristic region (White, 1979). Numerous studies have shown that even though both areas present strong botanical affinities, very few species co-occur in both East and West-Central Africa, i.e. the distribution of genera

is often disjunct (Moreau, 1933; Brenan, 1978; Iversen, 1991; Wasser and Lovett, 1993; Burgess et al., 1998; Burgess and Clarke, 2000). This disjunction is also very clear in Annonaceae, with almost no overlap between the two regions in terms of species distribution (Couvreur et al., 2006). However, many of these endemic species belong to genera that do co-occur in both areas, suggesting some kind of common history between the two areas that was investigated in Chapter 4 of this thesis.

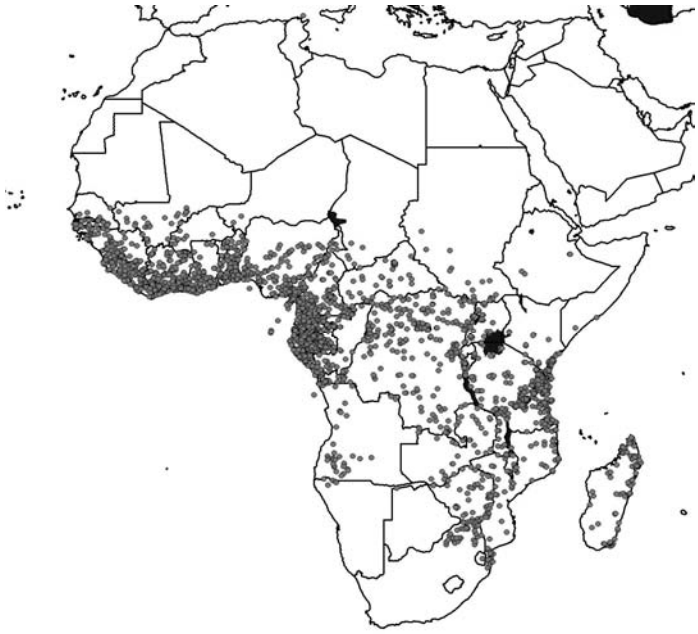


Figure 1.2. Annonaceae distribution in Africa and Madagascar. Map based on 7400 georeferenced herbarium specimens. Strong collection and databasing biases are found for West Africa (Senegal to Nigeria) and western Central Africa (Cameroon, Equatorial Guinea and Gabon), as well as for Tanzania.

Isolona *and* Monodora

Isolona and *Monodora* are large to small trees and sometimes shrubs occurring in lowland and montane rain forests from West to East Africa. *Isolona* is also found in Madagascar. Both genera have a unique character within Annonaceae: the female reproductive parts that enclose the ovules, called carpels, are fused, a state referred to as *syncarpy*. The rest of the Annonaceae as well as most taxa in the Magnoliales are characterized by freely arranged carpels or *apocarpy*. The presence of such a unique character has lead many scientists to suggest a close relationship between the two genera even though they are otherwise morphologically very distinct. In *Isolona*, the six petals are clearly fused into a tube and the lobes are equal in size and shape. In contrast, the six petals in *Monodora* are only fused at the very base and differentiate into an inner whorl of small petals and an outer whorl of larger petals. In *Monodora*, the flowers are conspicuous and brightly coloured yellow and purple, which is why the species are sometimes referred to as orchid trees. *Isolona* on the other hand

has smaller flowers uniformly coloured light yellow to red. An additional difference can be found in the pollen grains. In *Monodora* the pollen unit is composed of four unified pollen grains (called tetrads), while in *Isolona* the pollen unit is composed of one grain only (called monads).

Syncarpy has been regarded as a major innovation (Endress, 2001) in the evolution of flowering plants (or angiosperms) in having numerous evolutionary advantages over apocarpy. The main advantage comes from a the fact that fused carpels allow for a centralized distribution of the pollen tubes to the ovules (Endress, 1982). Thus one pollen tube can reach all the carpels and ovules, significantly enhancing fertilization and hence seed production. This common space has also been thought to enhance competition between pollen grains, providing stronger selection for fitter pollen grains and thus fitter offspring (Mulcahy, 1979; Endress, 1982; Mulcahy and Mulcahy, 1987; Armbruster et al., 2002). Other advantages have been proposed such as protection of the ovules from predators (Stebbins, 1974) and better dispersal abilities (Endress, 1982). In Chapter 3 of this thesis, *Isolona* and *Monodora* were used as models to study the evolutionary origins of this unique character within Annonaceae and Magnoliales in general.

MOLECULAR PHYLOGENIES

The most important tool used to investigate the evolution of *Isolona* and *Monodora* throughout the present project were *molecular phylogenies* based on DNA sequence data. Phylogenetic trees allow us to visualize in a clear fashion the differences as well as the similarities between a group of organisms (e.g. species, genera, etc.) or any defined taxonomic unit (Felsenstein, 2004). We can thus identify clades of taxa that share derived traits, generating hypotheses of relatedness through a suite of nested relationships. Consequently, phylogenies also provide us with hypotheses of the evolutionary history of changes that occurred in a studied group: closely related taxa will share common genetic and morphological character states (e.g. homologous character states, in contrast to similar states that have evolved independently or homoplasious character states) that originated in and have been transmitted via a *common ancestor*. Thus, a phylogenetic hypothesis allows us to identify and test whether a specific character state evolved only once or on multiple occasions (Harvey and Pagel, 1991). Additionally, molecular phylogenies can also be used to pinpoint evolutionary events in time, such as speciation, by rendering the trees ultrametric using molecular dating methods (see below). Such methods open the doors to integrated historical biogeographic studies as shown in Chapter 4 (Donoghue and Moore, 2003).

Molecular Phylogeny Inference

There are numerous methods to infer phylogenetic trees (Felsenstein, 2004). In general, these can be divided into four different types, two non model-based methods:

phenetics and maximum parsimony (MP), and two model-based methods: maximum likelihood (ML) and Bayesian inference. In this thesis, two methods to estimate tree structure were used: MP and Bayesian inference.

The maximum parsimony method (MP) relies on the principle of parsimony (introduced by William of Ockham in the 14th century): given a range of plausible alternative hypotheses, the simplest one (i.e the one which needs the fewest presumptions) should be preferred. In terms of phylogenetic inference this means selecting the tree which invokes the minimum total amount of evolution or character state change (Cavalli-Sforza and Edwards, 1967; Felsenstein, 2004). In other words, apparent homology of character states is more likely to be true homology than homoplasy.

However, DNA is a changing entity, as it mutates. The passing on of mutations to next generations is the driving force behind DNA evolution through time. It is possible to represent this change via *models of DNA sequence evolution*. Such models range from simple to complex (reviewed in Whelan et al., 2001; Felsenstein, 2004), and are the basis for model-based methods of tree inference. By describing such evolutionary changes we provide a probabilistic model of how the data (D) arose, in this case DNA sequence data for a group of organisms. Consequently, a probability (P) of observing the data *given* particular values of the model parameters (θ_j) can be calculated using $P(D/\theta_j)$, in other words how "likely" is it to observe the data given a particular set of parameters. Different values of the parameters will yield different probabilities, which together define the likelihood function. A high likelihood indicates a good fit of the parameters to the observed data. The "maximum likelihood estimates" (MLE) represent a set of *point estimate* parameters that maximize the likelihood. The maximum likelihood method as applied in molecular phylogenetics was introduced by Joe Felsenstein (1981). The favored phylogenetic hypothesis is the tree topology with the branch lengths having the highest cumulative likelihood.

Bayesian inference also depends on the likelihood function but the parameters of the model are not fixed to point non-random estimates but are allowed to vary *randomly* within the limits of a pre-defined distribution, or *prior distribution* $P(\theta_j)$. We shall come back to this shortly. The Bayes theorem (introduced by Reverend Thomas Bayes 1702-1761) dictates that the prior distribution $P(\theta_j)$ is combined with the likelihood $P(D/\theta_j)$ to provide the so-called posterior probability distribution $P(\theta_j / D)$:

$$P(\theta_j / D) = \frac{P(\theta_j \cap D)}{P(D)} = \frac{P(D/\theta_j) \times P(\theta_j)}{\sum_i P(D/\theta_i) \times P(\theta_i)}$$

The posterior probability distribution is central to Bayesian inference. It represents the probability of the parameters given the data and is also known as the *inverse probability*. In contrast to MLE, the answer is not just one point estimate, but is a distribution of probabilities integrating to one. Thus not only a best estimate is obtained but also an idea of the probability

of the alternative hypotheses, which in phylogenetic reconstruction are the trees with different probabilities. The posterior probability can be summarized into a point estimate in numerous ways, such as taking the mean, medium or mode of the distribution. In phylogenetics, there exist numerous methods for summarizing the trees sampled from the posterior, such as the majority rule consensus tree, the maximum clade credibility tree or using a network approach (cf. Holland et al., 2004).

The denominator $P(D)$ represents the marginal probability of the data and is used as a normalizing constant to make the posterior distribution $P(\theta_j / D)$ integrate to one. Basically, it involves summing over all possible hypotheses, which is impossible for most practical cases. For example in phylogeny reconstructions, $P(D)$ equals the sum over all possible tree topologies and integration over all branch lengths in those trees as well as over all parameters in the model of DNA evolution (Yang, 2005). Fortunately, the development of the Markov chain Monte Carlo algorithms (e.g. the Metropolis-Hastings algorithm; Metropolis et al., 1953; Hastings, 1970) provided a powerful way to sidestep the calculation of the normalizing constant which, together with evermore powerful computers, has contributed to the widespread use of Bayesian inference in recent years (Huelsenbeck et al., 2002).

Priors

The long-standing debate surrounding the use of Bayesian statistics is not over the theorem itself, which is mathematically valid, nor is the prior probability *per se* at stake. What creates the debate is the question of *how to choose* the prior value. Prior probabilities are generally based on previous independently derived information *without* looking at the data at hand. This means that different investigators with different experiences or beliefs could choose different prior values for the same analysis, leading to a certain level of *subjectivity*. The problem is to know if and how these subjective choices in priors will lead to different results and thus alternative conclusions when analyzing the same data (Felsenstein, 2004). Bayesian statisticians view prior probabilities as a strength precisely because one can incorporate prior beliefs or results into the analysis under a solid statistical framework (Huelsenbeck et al., 2002; Beaumont and Rannala, 2004). If the researcher has accumulated significant knowledge and experience, why not include that into the analyses instead of discarding it?

An additional advantage, one exploited throughout this thesis, is that *uncertainty* in a parameter can be easily described by allowing the values of this parameter to vary instead of being fixed. Especially in the field of biology, very few parameters are known with undeniable certainty, and at best we can provide an estimate of that value, e.g. the rates of change between nucleotides when using the general time-reversible model of DNA evolution. Thus being able to *describe* this uncertainty provides a much-needed level of realism to any phylogenetic analysis.

From the above it is clear that if one accepts to undertake a Bayesian analysis, prior choice is of crucial importance. There are two main types of priors: informative and uninformative. An informative prior makes use of information from previous research or the investigator's initial beliefs in different hypotheses or parameter values, and is generally referred to as the *subjective Bayesian approach* (Alfaro and Holder, 2006). Such priors will place higher probabilities over specific parameter regions than over others (Fig. 1.3 B and C). By increasing or decreasing the variance of the distribution one can also provide an indication of confidence in the prior values (Schultz and Churchill, 1999), see e.g. the difference between Figure 1.3 B and C.

Uninformative priors, also known as vague priors, will have a probability distribution that *minimizes* the effect of the prior over the posterior distribution, in the hope that the strength of the data will override the prior. This is also known as the *objective Bayesian approach* (Berger, 2004). For example, a flat or uniform prior assigns equal probability to the entire parameter space (Fig. 1.3 A). The term "uninformative prior" is, however, slightly misleading because stating that all values have an equal probability is very different from stating that nothing is known about the prior. Thus no prior is truly non-informative (Yang, 2005).

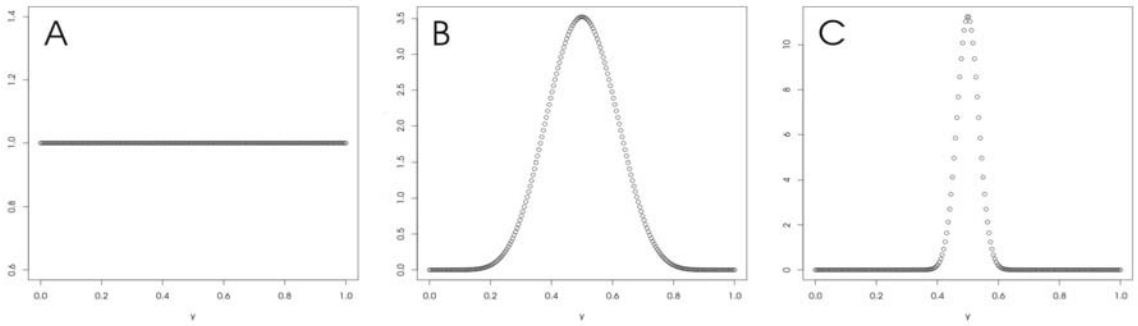


Figure 1.3. Different prior probability distributions used for binomial data modeled as a beta distribution. A. A flat prior (α=1 and β=1 (see paragraph below)); gives equal prior probability to all possible values. B. An informative prior (α=10 and β=10); gives a higher probability to values situated around 0.5, although with some uncertainty as can be noted by the relatively large width of the distribution. C. An informative prior (α=100 and β=100); gives a much higher probability to values situated around 0.5 than in B, as can be noticed by the smaller width of the distribution.

Another important aspect concerning priors is the type of mathematical distribution they follow, which is directly related to the type of data analyzed. For two-sided or binomial data such as a coin flipping experiment or a two-state morphological character, a beta distribution (with variables α and β) is typically used. The two variables define the shape of the distribution (see Fig. 1.3). Beta distributions are a family of distributions which are non-zero and are bound between 0 and 1. Such a property is ideal when one wants to model a probability, which by definition varies between 0 and 1, e.g. probability of getting heads in a coin tossing experiment or probability of evolving from state 0 to state 1 in a two-state morphological character. Other families of distributions include the normal distribution which is unbound from $-\infty$ to $+\infty$, while the exponential, logarithmic or gamma distributions all vary

between 0 and +∞. The gamma distribution is used as a substitute for the normal distribution and is particularly useful when modeling parameters that cannot deal with negative values such as a rate of substitution (see Chapter 2). A problem arises when we try to use a flat prior on an unbound prior distribution such as a gamma or normal distribution. If a prior has to be flat from, for example, 0 to +∞ the probability density will be zero over the whole of parameter space (Felsenstein, 2004). So, in these situations we are forced to use a prior that is informative and therefore will be subjective and thus must be appropriately chosen (see Chapter 2).

Molecular Dating

The timing of evolutionary events using DNA sequence data is referred to as molecular dating. Because DNA mutations take place continuously, past speciation events can be dated by analyzing the differences that have accumulated between the DNA of two species since their divergence. The *molecular clock* hypothesis first formulated by Zuckerkandl and Pauling (1962) postulates that the amount of difference between the DNA molecules of two species is proportional to the time elapsed since the divergence from their common ancestor (i.e. the speciation event). The word "clock" refers to a *constant* "tick" of the rate of the nucleotide substitution for the gene or DNA regions through evolutionary time. Thus, not only can evolutionary events be timed, but the mechanisms and processes of evolution can be 'unraveled' (Bromham and Penny, 2003). However, it was shown early on that a constantly ticking molecular clock was not universal and that the ticking rate, hence the rate in which random mutations occur, could be different in different evolutionary periods of a lineage and in different lineages within the phylogeny (Langley and Fitch, 1974; Welch and Bromham, 2005). The molecular clock is then referred to as "sloppy" or "relaxed". This observation gave rise to alternative dating methods depending on how rates vary within the phylogeny (for review see Rutschmann, 2006). In one case rates of substitution are assumed to be similar between closely related species, but become increasingly different as lineages are more distantly related (Gillespie, 1991). This property is called 'rate autocorrelation' and is the main alternative assumption used for estimating divergence dates in land plants (e.g. Wikström et al., 2001; Sanderson et al., 2004; Ramirez et al., 2007) and also within Annonaceae (Richardson et al., 2004; Pirie et al., 2006; Erkens et al., 2007). The alternative to the autocorrelation property is of course that rates among adjacent branches in a phylogeny are not correlated, i.e. perform as uncorrelated relaxed clocks. This property has been recently implemented into the Bayesian-based program BEAST (Drummond et al., 2006; Drummond and Rambaut, 2007).

For the molecular clock to start ticking (in a strict or relaxed fashion) we need to introduce a temporal scale into the phylogeny from which the timing of the events will be derived. This scale can be *relative*, i.e. the dates have no historical meaning (Loader et al., 2007), or *absolute*, i.e. the dates have a historical meaning and can be correlated to the

geological timescale of events. In the absolute framework, the choice of the calibration point(s) is (are) of crucial importance. Non-molecular information used for calibrating phylogenies can be taken from the fossil record (assign a fossil with its associated date to a specific position in the tree) or geological events (assign the origin of a clade based on the geological age of the region where that clade occurs). Whatever the information used, uncertainty will always be present. The Bayesian framework allows us to incorporate calibration uncertainty directly into the analyses. Thus, instead of assigning a non-variable age (i.e. a point calibration) to a specific node, we can assume a probability distribution that best fits our prior knowledge depending on the type of calibration used (see Fig. 1.4 for a brief overview). Therefore, the calibration date will be drawn randomly from the probability distribution at each iteration of the MCMC chain. This effectively accommodates for age uncertainty in a statistically sound framework (Ho, 2007).

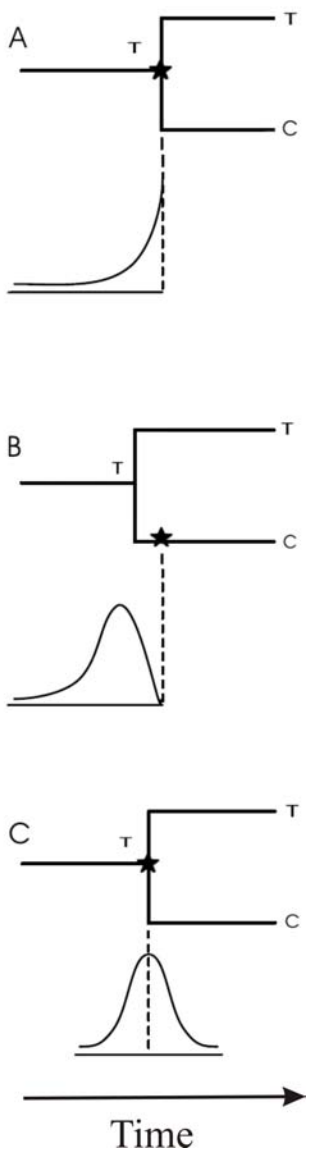


Figure 1.4. Alternative prior distributions used to calibrate a molecular phylogeny. 'T' represents an ancestral state, while 'C' is the derived state found in the non-molecular calibration information represented by a star. The star can either be fossil or geological information.

A. *Exponential distribution.* This type of distribution can be used when we are confident about the assignment of the fossil to a certain node in the tree. The probability increases with a growing similarity between the fossil age and the calibrated node.

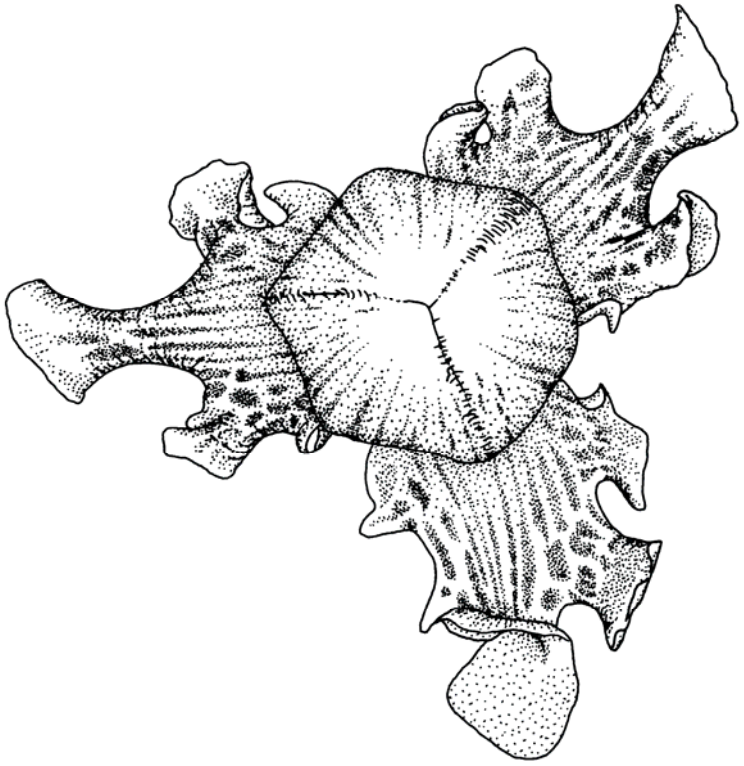
B. *Lognormal distribution.* This distribution is the most appropriate for modeling fossil data. The fossil record documents the earliest appearance of a morphological trait that characterizes a clade, here for example trait 'C'. In the absence of a complete fossil record, we are unsure whether the fossil represents the age of the split or the age when the trait became abundant and consequently the splitting event occurred earlier. In this case the star is placed just after the node. The lognormal distribution assumes that the actual divergence date is most likely to have occurred prior to the age of the fossil evidence.

C. *Normal distribution*. This distribution is ideal when we import a date for a node obtained from a previous molecular dating analysis referred to as a *secondary calibration* approach. Secondary calibrations are used as an alternative when no other type of information is available from direct observations (lack of fossil record or useful geographical events). It can also be used to model certain biogeographic events.
Figures modified from Ho (2007).

THESIS GOALS AND OUTLINE

The goal of this PhD project was to focus on the evolution of the African genera *Isolona* and *Monodora* as well as resolve their phylogenetic relationships with the other African genera. The thesis is divided in two major parts "character evolution and biogeography" and "systematics". The first part deals with the two major evolutionary questions that surround both genera: the evolution of syncarpy and the historical biogeography of African rain forests. The first chapter of the first part is a methodological one, in which we introduce and investigate the influence of selecting different prior values in the Bayesian-based posterior mapping method for the study of character evolution. In Chapter 3, we tackle the evolutionary history of syncarpy in Annonaceae. In this chapter we first clarify the phylogenetic relationships between most of the African genera by means of DNA sequence data, especially those related to *Isolona* and *Monodora*. We then investigate the evolution of syncarpy and other important morphological characters within African Annonaceae by using the posterior mapping method. In Chapter 4 we use African Annonaceae as a model for understanding the evolutionary origins of the East African rain forest endemic flora. A species-level molecular phylogeny was generated for *Isolona* and *Monodora* and, together with the generic one from Chapter 3, the divergence ages of the various observed evolutionary splits are dated by means of a Bayesian molecular dating method.

The taxonomic part of this thesis begins with a detailed study of pollen morphological variation in a monophyletic clade comprising *Isolona* and *Monodora* and three other African genera (Chapter 5). We investigate the taxonomic utility of such characters between and within genera. The last part of this thesis comprises the monographic treatment of the 34 species recognized in *Isolona* and *Monodora*. It starts of with a detailed introduction to the biology of both genera, then goes on to the actual species descriptions.



Chapter 2

2 Substitution Rate Prior Influences Posterior Mapping of Discrete Morphological Characters: an Unconventional Remedy ♣

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Abstract. Posterior mapping is an increasingly popular hierarchical Bayesian based method to infer character histories along phylogenies, notably of morphological characters. It implements the Mk model of discrete character evolution in a continuous time Markov chain. The parameters of the Mk model, namely the rate of substitution and the directional bias, are sampled from a prior distribution defined by two hyperparameters each. The prior distribution of the directional bias is modelled as a beta distribution, which allows for the use of a uniform prior. However, a gamma distribution is placed on the rate of substitution, and therefore cannot be assigned a uniform prior. A lack of understanding of how the prior distribution on the rate of substitution affects the outcome of posterior mapping has lead researchers to ignore their impact on the results in recent studies of morphological character evolution. We specified alternative prior distributions for the rate of substitution to reconstruct the character history of two contrasting morphological characters in the pan-tropical plant family Annonaceae: a slow evolving character, carpel fusion, and a faster evolving palynological character. Our analyses showed that the prior distributions had a marked effect on the results in terms of average number of character state changes. In contrast, however, the alternative priors did not influence the posterior probability distributions, suggesting that the data overruled the priors. We show that the explanation for these contrasting observations, e.g. different average number of character state changes with same posterior probability distribution, are related to the transformation of the continuous gamma distribution into discrete rate categories, or discretization, which is directly dependent on the priors specified. The most probable character history for both characters was affected differently by the prior. For the slower evolving character, the same character history always had the highest posterior probability independent of the priors. In contrast, the faster evolving character showed different most probable character histories depending on the prior. These differences could be related to the level of homoplasy exhibited by each character. An empirical Bayesian approach is demonstrated to be a useful alternative whereby the data is used to estimate the prior value of the gamma distribution placed on the substitution rate in posterior mapping.

♣ Manuscript in preparation

INTRODUCTION

Posterior mapping, or Bayesian inference of character evolution, is a novel way to map character histories along phylogenies (Nielsen, 2002; Huelsenbeck et al., 2003; Bollback, 2005). A character history reveals more information about the evolution of a specific character than just the reconstruction of ancestral states at the nodes of the tree. Additionally, it provides information about the number of changes, the timing and placement, and the type of change that occurred along the tree(s) (Huelsenbeck et al., 2003; Bollback, 2005). Posterior mapping was originally developed for DNA sequence data (Nielsen, 2002) but its use has since been extended to morphological characters (Huelsenbeck et al., 2003). In contrast to the widely used maximum parsimony optimization method, which optimizes characters by minimizing the number of state changes across a fixed topology, posterior mapping simultaneously accommodates for both mapping as well as phylogenetic uncertainty, i.e. alternative reconstructions within and between equally likely trees respectively (Schluter et al., 1997; Huelsenbeck et al., 2003; Ronquist, 2004). In addition, this method also allows for character states to change along a branch, which is especially important for long branches for which the probability of change is much higher (Cunningham et al., 1998; Huelsenbeck et al., 2003; Bollback, 2005).

The following four steps are involved in the process (for a detailed review of each step see Bollback, 2005). First, a substitution model defining the probabilities of change from one state to another is characterized. For discrete morphological characters, which is the main interest of this study, a continuous-time Markov chain implementing the Mk model of Lewis (2001) has been proposed (Huelsenbeck et al., 2003). The continuous-time Markov chain contains a transition matrix defined by two parameters: the rate of substitution of the morphological character (θ) and a bias parameter governing the direction of change between each character state (Л). To accommodate for uncertainty over these parameters, a hierarchical Bayesian approach is adopted whereby the rates are assigned a prior distribution from which the prior values will be sampled. The prior probability distribution of the rate of substitution θ is modelled as a gamma distribution with hyperparameters α_S and β_S, while a beta distribution with hyperparameters α_B and β_B is placed on the directional bias л. In both cases the values of the hyperparameters α and β will define the mean (E) and the standard deviation (SD) of the distributions (Schultz and Churchill, 1999; Huelsenbeck et al., 2003). Drawing values from continuous distributions is computationally too intensive, so the two prior probability distributions are broken into several discrete, equally probable, categories (i.e. discretization, Yang, 1994; Huelsenbeck et al., 2003). Each category is represented by the mean value of its range. The second step in the process is to estimate the likelihood of each state at each node of the tree including the root. In step three, a combination of states at each node of the tree is simulated and fixed given the likelihood assigned in the previous step (Felsenstein, 1981). Finally, in step four, for each branch of the tree a character history is simulated (i.e. realization), using the transition matrix conditional on the starting and ending states of the branch. The elapsed time between each character state change is drawn from an

exponential distribution using the inverse transformation method (Huelsenbeck et al., 2003; Bollback, 2005). Phylogenetic uncertainty is accommodated for by simulating the model on a subset of trees sampled from the posterior distribution using the Markov chain Monte Carlo method (MCMC, Metropolis et al., 1953; Hastings, 1970; Huelsenbeck et al., 2003). As a result we can calculate the average number of transformations (in total, as well as between specific states) that have occurred after 'x' realizations. For each character history we can also calculate the frequency of occurrence over all simulations, i.e. its posterior probability (Huelsenbeck et al., 2003). We can thus identify not only which character history is the most probable, but also the character histories that are less or equally probable given the data.

As for all Bayesian analyses, specifying prior values can be problematic and many researchers feel uneasy in doing so (Huelsenbeck et al., 2002; Alfaro and Holder, 2006; Buschbom and Barker, 2006). This apprehension could come from a lack of understanding of the effect of the priors on the final results. Moreover, in recent studies that apply posterior mapping to study the evolution of morphological and ecological characters, the values of the hyperparameters are not reported (Chaverri et al., 2005; Lewis and Lewis, 2005; Jones et al., 2006; Smedmark et al., 2006; McLeish et al., 2007) or their impact on the final results is ignored (Leschen and Buckley, 2007) under the false impression that the data will always overrule the priors. The nature of the beta distribution placed on the directional bias prior (JI) allows for the use of a so-called flat or uninformative prior ($\alpha_B=\beta_B=1$). Probabilities are uniform over the whole parameter space providing an adequate and widely used alternative to the lack of prior knowledge. For this study the influence of the prior on the directional bias (JI) will not be addressed. In contrast, and most importantly, the gamma distribution placed on the rate of substitution θ cannot accommodate for uniform priors. Any combination of the two hyperparameters, α_S or β_S, will be subjectively defining the mean E(T) and the standard deviation SD(T) of the prior distribution. For morphological character evolution, the impact of this prior distribution on the realizations has received meagre attention and to our knowledge has not been thoroughly assessed using empirical data. Schultz and Churchill (1999), using simulated data, showed that certain combinations of priors on θ and JI can influence the outcome of simulations. In contrast, Huelsenbeck et al. (2003), applied different substitution rate priors, a slow and fast mean rate E(T), with a flat prior on the directional bias (JI), on two different empirical datasets. They noticed that the posterior probabilities of the character histories were independent of the prior used. How the priors affect the outcome of the realizations in terms of average number of transformations and the posterior probability of a character history remains unclear. With the advent of user-friendly software (e.g. SIMMAP; Bollback, 2006) enabling a more widespread application of this method it is important to assess the impact priors have on the outcome of the analyses and to renew awareness of this issue.

To this end we undertook a detailed empirical study of two morphological characters found within the flowering plant family Annonaceae (Magnoliales, APGII, 2003). Recent molecular phylogenetic studies (Mols et al., 2004; Richardson et al., 2004; Pirie et al., 2006) revealed a well supported clade with on average twice the level of sequence divergence (the

so-called long branch clade, LBC) when compared to a second major clade with lower levels of sequence divergence (the so-called short-branch clade) (see Fig. 3.2, Chapter 3). The LBC is generally characterized by long branches subtending species-rich clades (Pirie, 2005). The long branches of the LBC offer an ideal situation for applying posterior mapping to the study of the evolution of morphological characters, given the flaws that might be expected when applying maximum parsimony optimization to character reconstruction. Two contrasting morphological characters found within the LBC were selected. (1) A potentially slow evolving character, carpel fusion, which has two states: apocarpy and syncarpy. Syncarpy is defined as the congenital fusion of the female reproductive units of the flower termed carpels (Carr and Carr, 1961; Endress, 1990), and has only rarely evolved within early-diverging magnoliids (sensu APGII, 2003). In Annonaceae, however, syncarpy has evolved within two strongly supported African sister genera *Isolona* and *Monodora* (Endress, 1982; Endress, 1990; Chapter 3). (2) A potentially faster evolving character: pollen unit, with two states: single (pollen composed of a single grain) and compound (pollen composed of two, four or more grains). The single state is considered ancestral within Annonaceae with reversals being fairly common (Doyle and Le Thomas, 1996; Chapter 3).

The aim of the present study was to explore the impact of the prior distribution on the substitution rate θ on the average number of transformations, the posterior probability distribution, as well as the posterior probability of each character history by subjecting empirical data to the posterior mapping method. Thus, this study was not designed to compare results between maximum parsimony optimization and posterior mapping. For such comparisons the reader is referred to Huelsenbeck et al. (2003). Finally, in the light of such an analysis we provide guidelines on prior specification in posterior mapping.

MATERIAL AND METHODS

Phylogenetic Analysis

The results presented here are derived from a DNA sequence data matrix of the Annonaceae family (Couvreur et al., in press; Chapter 3) totalling 66 taxa sampled across the family. The dataset was composed of six chloroplast markers, three non-coding (*trnLF*, *trnSG* and *psbA-trnH*) and three coding (*ndhF*, *rbcL* and partial *matK*), totalling 7945 characters. Gaps were coded as separate characters. All phylogenetic analyses were run using the Metropolis-coupled Monte Carlo Markov chain (MCMCMC) algorithm implemented in MrBayes (ver. 3.1.2; Ronquist and Huelsenbeck, 2003) under the best partitioning strategy identified using the Bayes factor (Nylander et al., 2004) and following Brandley et al. (2005). For each partition, the best performing evolutionary model was identified using the Akaike information criterion (AIC; Akaike, 1973) using MrModeltest (Nylander, 2004). Three separate runs of five million generations each were undertaken and stationarity as well as convergence between the MCMC runs was checked using both Tracer v. 1.3 (Rambaut and Drummond, 2003) and the online program AWTY (Wilgenbusch et al., 2004).

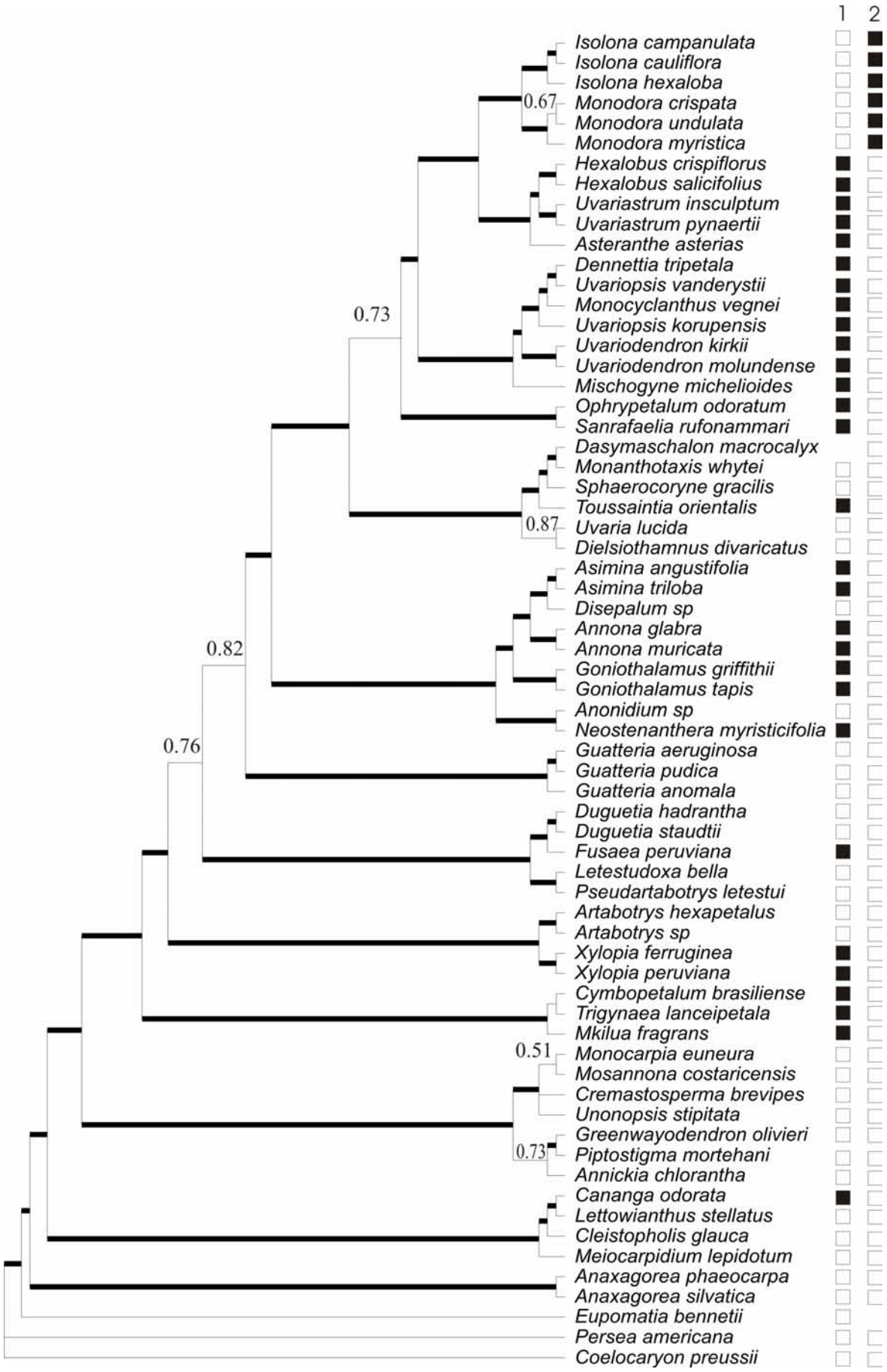
Influence of the Substitution Rate Prior

The impact of alternative prior distributions on the substitution rate (θ) was studied by subjecting the carpel fusion and pollen characters to the posterior mapping method as implemented in the program SIMMAP version 1.0 beta 2.3 (build 12092006, Bollback, 2006). Both characters were scored for each taxon using literature or personal observations (Fig. 2.1). All characters were unordered.

SIMMAP allows the user to specify two hyperparameters (α_S and β_S) that define the prior gamma distribution placed on the substitution rate θ and one hyperparameter (α_B) for the beta distribution placed on the directional bias Л. For the latter, a flat prior was used in all analyses ($\alpha_B=\beta_B=1$). To compare the effect of the prior distributions on θ we must be sure to compare them equally, i.e. make sure they have either the same mean (E(T)) or standard deviation (SD(T)). For the prior gamma distribution we have $E(T) = \frac{\alpha_S}{\beta_S}$ and $SD(T) = \sqrt{\frac{\alpha_S}{\beta_S^{\,2}}}$ (Yang, 1994; Huelsenbeck et al., 2003). Formulating these equations as a function of α_S and β_S leads to $\alpha_S = \frac{E(T)^2}{SD(T)^2}$ and $\beta_S = \frac{E(T)}{SD(T)^2}$. This formula allows us to find the values of the hyperparameters α_S and β_S for any required combination of E(T) and SD(T).

Six different combinations of E(T) and SD(T) for the prior distribution on θ were tested. A slow, medium, and fast mean rate (E(T)= 1, 5 and 10, respectively) were each combined with a high and low confidence (SD(T)= 1 and 5, respectively). These values and associated terminology were chosen relative to the biology of the morphological characters considered for this specific analysis.

The influence of the priors on the posterior distribution was first evaluated by using the "number of realizations sampled from priors" function in SIMMAP with 10,000 realizations for each combination of E(T) and SD(T). These 10,000 draws from the prior distribution represent a valid estimation of the posterior distribution probability of each rate category. The posterior distribution for each combination was visualized in Tracer v. 1.3 (Rambaut and Drummond, 2003) by converting the SIMMAP output file to a Tracer file using the python script "convert2tracer.py" found on the SIMMAP website (Bollback, http://www.simmap.com/simmap/pgs/helperscripts.html). The prior gamma distribution was broken into 60 rate categories, each of which represents an equal probability density (Yang, 1994). As the areas under the probability curve are equalized, the resulting categories have different widths. For each combination of E(T) and SD(T) two different graphs were produced. A posterior density histogram was normalized by dividing the number of counts within each rate category by the width of that category, with the total surface area of the rectangles equalling one. This representation allows for the overlay of the prior gamma probability density as a reference. A second graph represents the number of times each rate category was sampled out of 10,000 draws, i.e. the posterior probability of each category.


1
2
Isolona campanulata
Isolona cauliflora
Isolona hexaloba
0.67
Monodora crispata
Monodora undulata
Monodora myristica
Hexalobus crispiflorus
Hexalobus salicifolius
Uvariastrum insculptum
Uvariastrum pynaertii
Asteranthe asterias
Dennettia tripetala
Uvariopsis vanderystii
0.73
Monocyclanthus vegnei
Uvariopsis korupensis
Uvariodendron kirkii
Uvariodendron molundense
Mischogyne michelioides
Ophrypetalum odoratum
Sanrafaelia rufonammari
Dasymaschalon macrocalyx
Monanthotaxis whytei
Sphaerocoryne gracilis
Toussaintia orientalis
0.87
Uvaria lucida
Dielsiothamnus divaricatus
Asimina angustifolia
Asimina triloba
Disepalum sp
Annona glabra
0.82
Annona muricata
Goniothalamus griffithii
Goniothalamus tapis
Anonidium sp
Neostenanthera myristicifolia
0.76
Guatteria aeruginosa
Guatteria pudica
Guatteria anomala
Duguetia hadrantha
Duguetia staudtii
Fusaea peruviana
Letestudoxa bella
Pseudartabotrys letestui
Artabotrys hexapetalus
Artabotrys sp
Xylopia ferruginea
Xylopia peruviana
Cymbopetalum brasiliense
Trigynaea lanceipetala
Mkilua fragrans
0.51
Monocarpia euneura
Mosannona costaricensis
Cremastosperma brevipes
Unonopsis stipitata
Greenwayodendron olivieri
0.73
Piptostigma mortehani
Annickia chlorantha
Cananga odorata
Lettowianthus stellatus
Cleistopholis glauca
Meiocarpidium lepidotum
Anaxagorea phaeocarpa
Anaxagorea silvatica
Eupomatia bennetii
Persea americana
Coelocaryon preussii

<--Figure 2.1. Majority rule consensus tree of the last 30,000 trees sampled after five million generations of the MCMCMC run. Posterior probabilities under 0.95 are displayed at nodes. Thick branches indicate support > 0.95 PP. The distribution of species with compound pollen (1, black squares) and syncarpy (2, black squares) are represented along the tips of the phylogeny. Missing squares indicate absent observations; the species was scored as uncertain for that character.

The simulation of the continuous-time Markov chain was then realized 1,000 times over the last 201 trees imported from the MrBayes analysis for both characters. The total number of character transformations, and the number of transformations between each state were averaged over all realizations. Finally, the actual number of times a particular character history occurred throughout the 201,000 realizations was calculated (for example, how many times was "one gain and one loss" simulated). A perl script (Vriesendorp and Couvreur, unpublished) was written in order to extract that information from the SIMMAP output files. Dividing the number of occurrences by the total number of realizations gives the posterior probability of each character history (abbreviated as PP_c, not to be confused with the PP of the nodes in the phylogenetic tree). To reduce the large range of values between PP_cs (5^{e-6} to 0.9) the negative logarithm of the PP_c for each of the characters histories was plotted on a 2D graph using Kyplot (Koishi Yoshioka, v.2 beta 15, www.woundedmoon.org/win32/kyplot.html).

Maximum parsimony optimization results were also provided as a reference only. The majority rule consensus tree from the Bayesian analysis was used for subsequent analyses using Mesquite v. 1.11 (Maddison and Maddison, 2006). Both characters were treated as unordered.

RESULTS

Phylogeny

The partition strategy strongly supported under the Bayes factor was run for five million generations with three independent runs. The posterior probabilities of all splits were indistinguishable between independent runs as visualized with AWTY (results not shown), suggesting convergence between them. In addition, all three runs reached stationarity after 250,000 generations with all of the parameters converging to the same values as visualized with Tracer. The majority rule consensus tree (treeBASE number in prep) was generally well resolved and well supported (Fig. 2.1, Couvreur et al., in press or Chapter 3). For a detailed discussion about the phylogenetic relationships in Annonaceae resulting from this analysis see Couvreur et al. (in press) or Chapter 3.

	Rate parameter θ	Bias parameter Л	Total average # of transformations	State-to-state # of transformations	
				0=>1	1=>0
Pollen unit					
Parsimony	---	---	8	6	2
E(T)=1 SD(T)=1	3.12	0.49	10.40	7.06	3.34
E(T)=1 SD(T)=5	8.16	0.49	22.06	12.26	9.81
E(T)=5 SD(T)=1	5.33	0.49	13.59	8.36	5.23
E(T)=5 SD(T)=5	7.79	0.49	20.82	11.66	9.15
E(T)=10 SD(T)=1	**9.96**	**0.49**	**26.55**	**14.24**	**12.31**
E(T)=10 SD(T)=5	**9.57**	**0.49**	**25.66**	**13.87**	**11.79**
*Carpel fusio*n					
Parsimony	---	---	1	1	0
E(T)=1 SD(T)=1	**0.97**	**0.49**	**1.27**	**1.15**	**0.12**
E(T)=1 SD(T)=5	**1.05**	**0.49**	**1.39**	**1.20**	**0.19**
E(T)=5 SD(T)=1	4.65	0.48	3.48	2.18	1.31
E(T)=5 SD(T)=5	2.09	0.49	1.91	1.45	0.46
E(T)=10 SD(T)=1	9.72	0.44	8.36	4.39	3.97
E(T)=10 SD(T)=5	5.42	0.47	4.35	2.57	1.78

Table 2.1: Average number of transformations estimated for each combination of the mean rate value (E(T)) and the level of confidence (SD(T)) estimated after 1000 simulations on the 201 last trees sample from the MCMC run. The maximum parsimony numbers of transformations were taken from a single most parsimonious tree arbitrarily chosen out of the seven found. The bold values represent a centered posterior distribution around the mean rate as visualized with the posterior distribution graphs in Figure 2.2.

Influence of the Rate Prior θ

The average number of total transformations as well as the average number of transformations from one state to another, for each of the two characters under six different combinations of E(T) and SD(T), are summarized in Table 2.1. For both characters the average number of total transformations as well as state-to-state changes is higher with the faster rate prior, i.e. higher E(T). Thus, for carpel fusion the average total number of transformations changed from 1.39 (prior set at a low rate: E(T)=1, SD(T)=5) to 4.35 (prior set at a high rate: E(T)=10, SD(T)=5). If SD(T) is narrowed to one, the differences are even greater (1.27 to 8.36). Finally, averages for similar values of E(T) showed marked differences according to the different values of SD(T), except for two cases indicated in Table 2.1 (bold part), when the averages did not differ greatly.

The density distributions and posterior probabilities of the rate categories for the two characters are illustrated in Figure 2.2. Each graph is split in two, with a common X axis indicating the 60 rate categories and their respective widths. For a few combinations of E(T) and SD(T), however, the mean value of the range of some categories was extremely small ($< 1 \times 10^{-5}$). As a result, these categories were never sampled during the simulation. Those categories were assigned a rate and sampling value of zero, resulting in less than 60 categories being represented (Fig. 2.2 a, d, e and j).

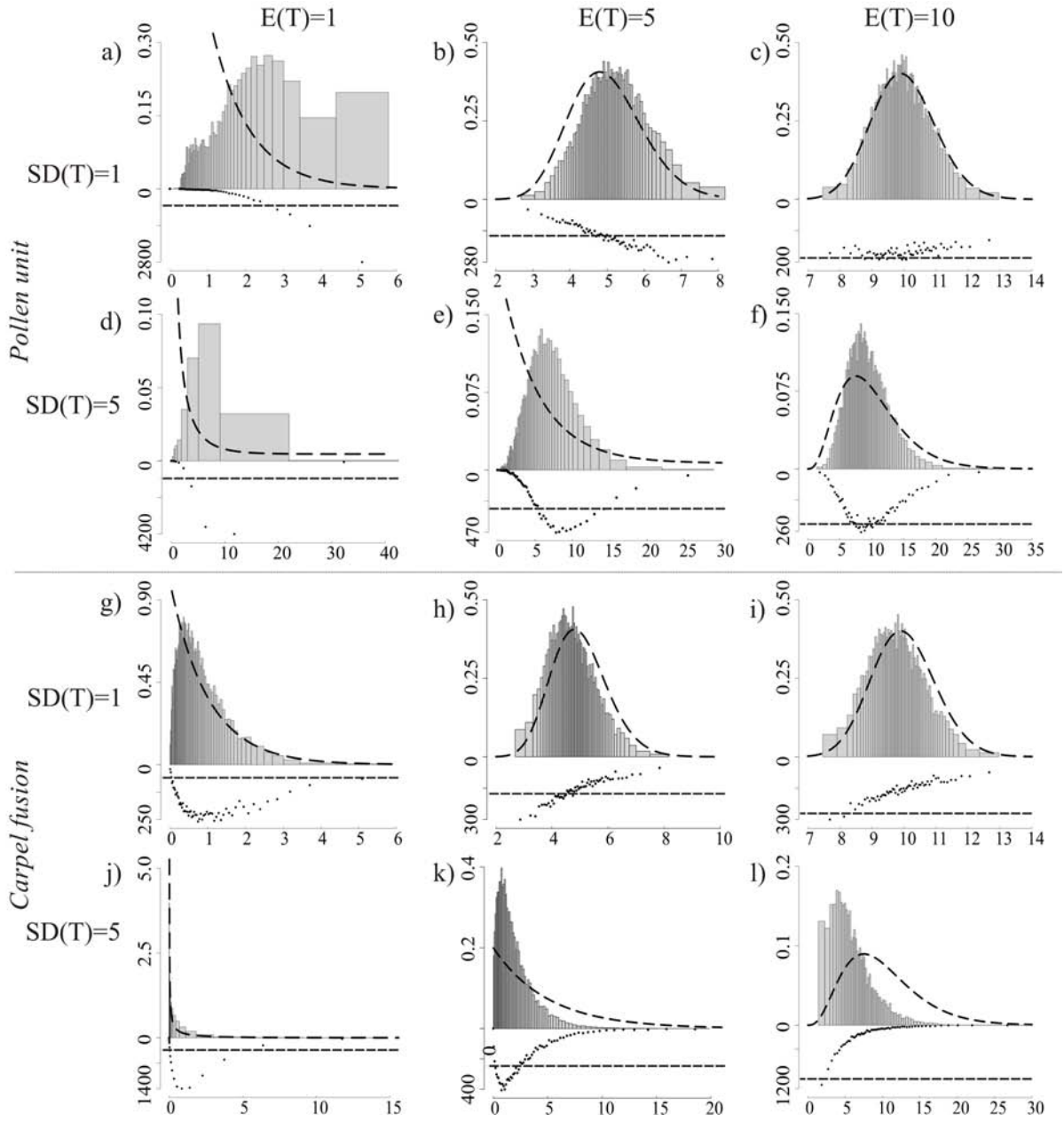


Figure 2.2. Posterior probability density distributions and posterior probabilities of each rate category given each combination of E(T) and SD(T) for both characters. The bars of the histogram represent the posterior distribution densities given the prior and the data for each rate category. The continuous gamma distribution was made discrete by breaking it into 60 equal probable rate categories (Yang, 1994). Each category is represented by the mean of the portion of the gamma distribution included in the rate category. The dashed lines of the upper graph represent the prior gamma probability density. The total area of the histogram as well as the prior distribution equal one. The dashed lines in the lower graph represent the prior distribution and are flat across categories (each rate category has an equal probability). X axis: rate of substitution. Upper Y axis: density scale, lower Y axis: sampling frequency of each discrete rate category.

As expected, the mean rate and confidence values (E(T) and SD(T), respectively) have an effect on the parameter space sampled, which is clearly visible when comparing the range of values between the different X-axes (Fig. 2.2). The mean rate value E(T) determines where

in parameter space the values are sampled while the confidence SD(T) designates the extent of the range. When the confidence was high (SD(T)=1; Fig. 2.2 a-c, g-i) the range of rate values sampled in parameter space was narrow, for example between 0 and 6 for E(T)=1. In contrast, with a low confidence, the values that were sampled encompassed a wider range of rate values (SD(T)=5, Fig. 2.2 d-f; j-l), for example between 0 and 40 for E(T)=1).

The upper part of the graph represents the density histograms for each combination of E(T) and SD(T), and is overlaid with the prior gamma density distribution. For the pollen character, the posterior density histograms did not fit the prior gamma distributions with the low and median mean rate (E(T)=1 and 5, Fig. 2.2 a, b, d, e), for any value of SD(T). In those cases the highest rate categories provided most of the density, i.e. the largest portion of the sampled part of parameter space, giving an unbalanced aspect to the histogram. With a high mean rate, however, there was a better fit between the prior and posterior densities (Fig. 2.2 c and f). In contrast, for the carpel fusion character a better fit to the prior distribution was found for the low mean rate (E(T)=1, Fig. 2.2 g and j). The high and medium mean rates generated unbalanced histograms with most of the probability density placed on the lower rate categories.

The lower graphs in Figure 2.2 indicate the actual sampling frequency (i.e. posterior probability) of each rate category out of the 10,000 draws. In all cases with the confidence set to be low (SD(T)=5), the categories around the same rate values were more thoroughly sampled leading to an almost identical shape in their distributions (Fig. 2.2 d-f and j-l). For the pollen unit the highest sampling frequency was found for the categories around the rate value of 10 (Fig. 2.2 d-f). For the carpel fusion character this rate value was around 1 (Fig. 2.2 j-l). When a high confidence was specified (SD(T)=1), the shape of the frequency distributions changed with different mean rates. For the pollen unit, higher rate categories were the most sampled under low and medium mean rates, giving a skewed shape to the distribution (Fig. 2.2 a, b). For the fast mean rate, each category was more evenly sampled (Fig. 2.2 c). Inversely, for the carpel fusion character, the lower categories were always the most sampled under fast and medium mean rates (Fig. 2.2 h, i).

Character History Space and Transformation Bias

The exploration of character history space by the 201,000 realizations is shown in Figures 2.3 and 2.4, split according to the different combinations of E(T) and SD(T). These figures simultaneously represent all the different character histories, their respective frequencies, and the transformation bias, as explored during the analysis. The character history space explored for the pollen unit (Fig. 2.3) is much larger than for carpel fusion (Fig. 2.4), visible from the difference in number of gain/loss combinations. In both cases the space explored by the simulation is larger under a low confidence (SD(T)=5; Fig. 2.3 d-f and Fig. 2.4 d-f) than under a high confidence (SD(T)=1; Fig. 2.3 a-c and Fig. 2.4 a-c). In other words, faster transformation scenarios are sampled when our confidence is low and this is independent of the mean rate prior used. However, a large majority of the character histories occur only a few

times (low PP_c), which is indicated by the bright yellow and green colours.

	Pollen unit			**Carpel fusion**		
	0=>1	1=>0	PP_c	0=>1	1=>0	PP_c
E(T)=1 SD(T)=1	6	2	0.307	1	0	0.867
	7	3	0.157	2	1	0.063
	7	2	0.103	2	0	0.042
	8	4	0.075	3	2	0.007
	8	3	0.041	1	1	0.007
	6	3	0.036	3	1	0.005
E(T)=1 SD(T)=5	6	2	0.062	1	0	0.848
	7	3	0.056	2	1	0.067
	8	4	0.049	2	0	0.036
	9	5	0.042	3	2	0.015
	10	6	0.032	1	1	0.006
	11	7	0.021	3	1	0.005
E(T)=5 SD(T)=1	7	3	0.118	1	0	0.295
	8	4	0.115	2	1	0.285
	9	5	0.084	3	2	0.145
	6	2	0.066	4	3	0.055
	10	6	0.050	2	0	0.035
	8	5	0.044	3	1	0.033
E(T)=5 SD(T)=5	7	3	0.060	1	0	0.695
	6	2	0.056	2	1	0.138
	8	4	0.052	2	0	0.045
	9	5	0.042	3	2	0.040
	10	6	0.033	3	1	0.013
	11	7	0.027	4	3	0.013
E(T)=10 SD(T)=1	14	10	0.037	4	3	0.134
	13	9	0.035	3	2	0.130
	15	11	0.033	5	4	0.105
	12	8	0.030	2	1	0.088
	14	11	0.029	6	5	0.066
	13	10	0.027	7	6	0.034
E(T)=10 SD(T)=5	9	5	0.030	1	0	0.300
	10	6	0.029	2	1	0.220
	8	4	0.029	3	2	0.125
	11	7	0.027	4	3	0.065
	7	3	0.025	5	4	0.032
	12	8	0.024	2	0	0.031

Table 2.2: The first six character histories with the highest posterior probability (PP_c) for each combination of E(T) and SD(T).

The highest posterior probabilities (dark red squares) are returned for character transformation scenarios that are slightly biased towards gains (0 => 1), as these are positioned above the diagonal in all plots. In contrast, the character history space that is explored is skewed towards a slight excess of losses over gains. This pattern is similar for all plots in Figures 2.3 and 2.4.

The different prior values had a contrasting influence on the identification of the most probable character history. For carpel fusion, the same character history (1 gain and 0 losses; Table 2.2) was assigned the highest PP_c independent of the priors used (except in one extreme case, Table 2.2). This is graphically visible in Figure 2.4 where the darkest red square is mainly situated at 1 gain and 0 losses. However, for the pollen unit, different values of E(T) always returns different most probable character histories (Table 2.2, Fig. 2.3). Moreover, with a SD(T) of 5, many alternative character histories received an almost equal PP_c value (Table 2.2). This is also visible in Figure 2.3 where numerous dark-red squares cover a large number of squares. Finally, in Figure 2.3 d the cloud is broken in two at around 30 transformations from 0 to 1 and 0 to 1. The squares above 30 represent very high rates of substitution (> 60 transformations over the tree).

DISCUSSION

Influence of the Prior Gamma Distribution on θ

It has been shown that priors do influence Bayesian inference in phylogenetic reconstruction (Zwickl and Holder, 2004; Yang and Rannala, 2005). However, the role played by the prior gamma distribution on the substitution rate θ in posterior mapping is still largely unclear (Schultz and Churchill, 1999; Huelsenbeck et al., 2003). Judged by the number of recent articles that did not report on the selection and quantification of prior values (Chaverri et al., 2005; Lewis and Lewis, 2005; Jones et al., 2006; Smedmark et al., 2006; McLeish et al., 2007) it would seem that researchers consider its impact minimal.

Our study showed that the prior distribution on the rate parameter θ had a significant impact on the results for both characters analyzed. The differences were obvious in the two results reported here: the average number of transformations of character states (Table 2.1) and the most probable character history (Table 2.2). In the latter case, the prior had a stronger impact on the pollen character than on the carpel fusion character (Table 2.2) and will be discussed later. In the former the different combinations of E(T) always produced different outcomes (Table 2.1). These differences would result in alternative interpretations for the evolution of this character. In contrast, however, we have also shown that the posterior distributions were not influenced by the different prior values. If the prior on θ would have an effect on the posterior probability distribution we would expect (1) a good match between the prior and posterior density distributions and (2) an even sampling of the rate categories around the mean rate prior, and this for every prior combination used. This would effectively indicate a lack of information in the data about the posterior distribution (i.e. the posterior

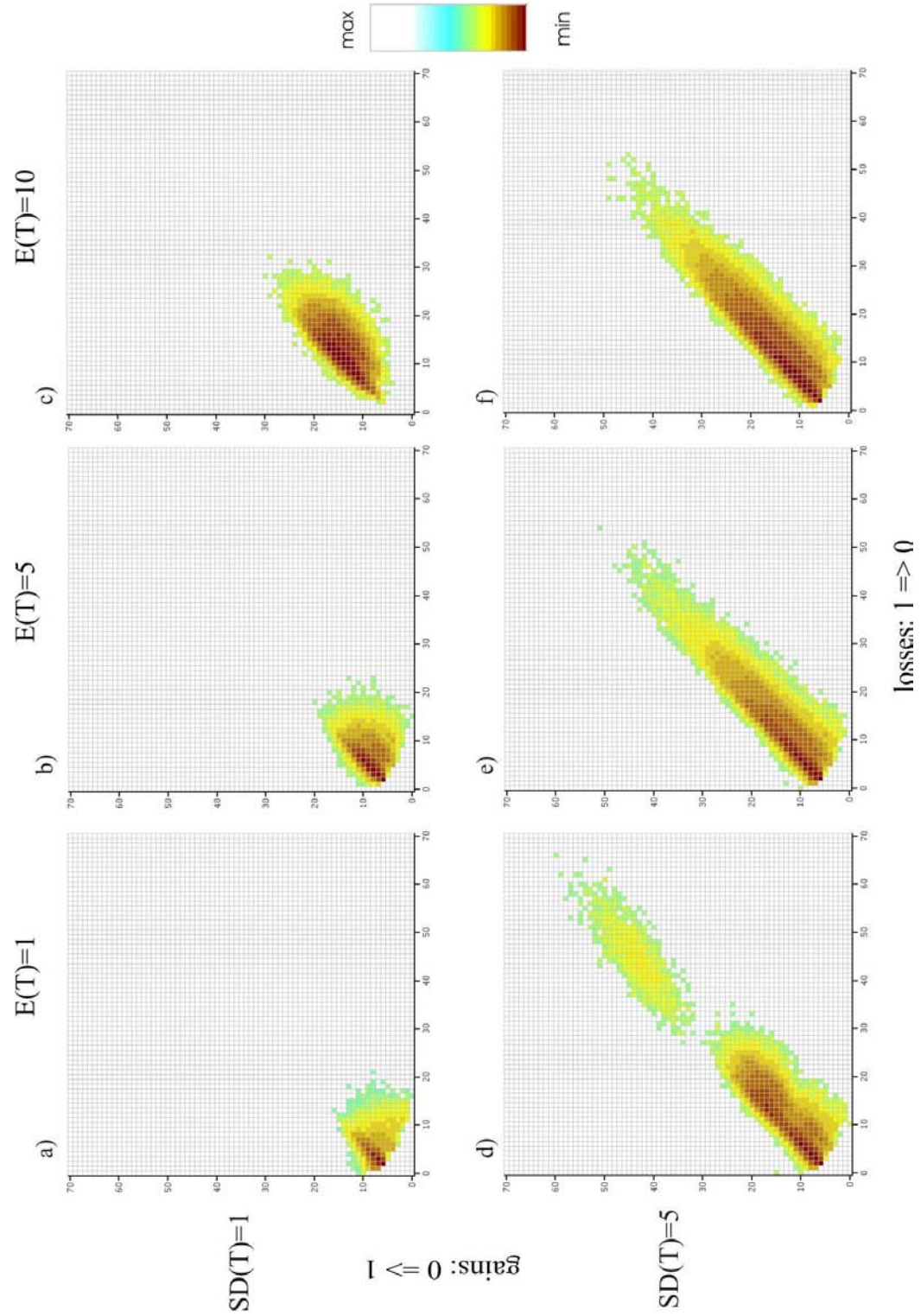


Figure 2.3: Negative logarithm of the posterior probabilities for all character histories that have occurred during the simulation for the *pollen character* for all six combinations of E(T) and SD(T). The X axis represents the total number of transformations from 1 to 0 (i.e. number of gains) and the Y axis from 0 to 1 (i.e. number of losses). It is important to note that as we used the negative logarithm the lowest values (dark red) represent the highest PPcs. The colours for the PPc are not the same across the graphs, as they represent the values for each independent analysis.

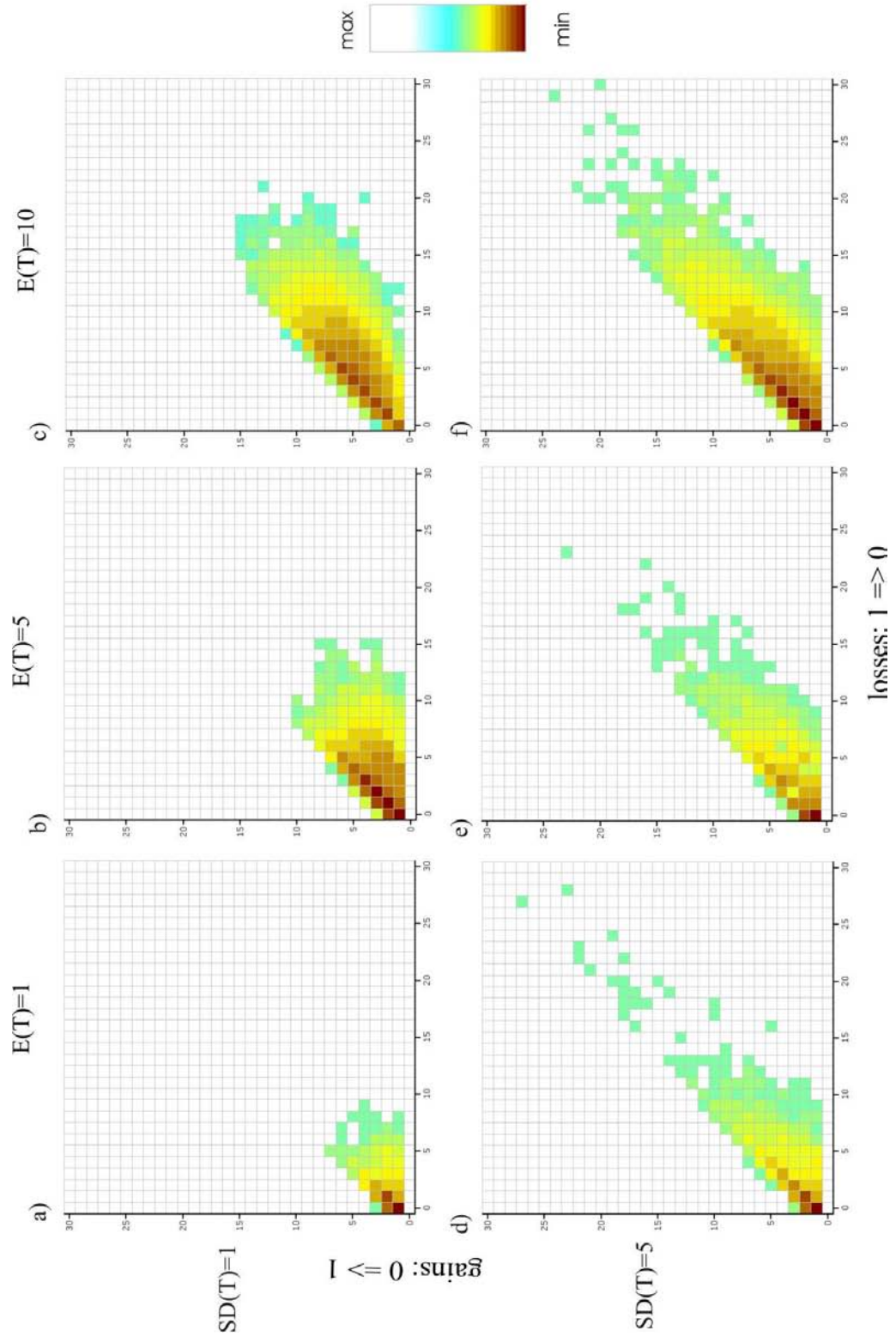


Figure 2.4: Negative logarithm of the posterior probabilities for all character histories that have occurred during the simulation for the *carpel fusion character* and for all six combinations of E(T) and SD(T). The X axis represents the total number of transformations from 1 to 0 (i.e. number of gains) and the Y axis from 0 to 1 (i.e. number of losses). It is important to note that as we used the negative logarithm the lowest values (dark red) represent the highest PP_cs. The colours for the PP_c are not the same across the graphs, as they represent the values for each independent analysis.

distribution returns the prior distribution). This is clearly not observed in our results and is especially apparent under low confidence regimes (SD(T)=5, Fig. 2.2). In those cases, the density histograms (upper graphs, Fig. 2.2) and the frequency distributions (lower graphs, Fig. 2.2) of the posterior probabilities displayed an almost identical shape. This suggests that, for the two characters studied here, the posterior distribution is mainly influenced by the data and not by the prior, a situation where it is generally recognized that priors will not influence the results (Alfaro and Holder, 2006). Thus, how can we explain the observed differences in averages if the prior had no effect on the posterior? A more detailed look at the data, as presented in Figure 2.2, provides us with an answer. The situations of a high and a low confidence however require two different explanations. With a high confidence (SD(T)=1), values are drawn from significantly different regions in parameter space and the posterior distributions are generally skewed (Fig. 2.2, a-c and g-i). If different regions are sampled with a changing E(T), different character histories will be realized thus leading to the different observed averages. With a low confidence however (SD(T)=5) the explanation is less intuitive because parameter space as well as the posterior distributions were equivalent independent of E(T) (lower graphs, Fig. 2.2 d-f and j-l). The critical explanatory factor in this case is the width of the rate categories. The continuous gamma distribution is discretized into 60 equally probable categories, i.e. an equal surface area (Yang, 1994; Huelsenbeck et al., 2003). This effectively means: small widths around the mean and increasingly larger widths away from the mean (the marginal regions). Each category is then assigned a fixed rate value equal to the mean of the range. Thus, these widths are directly dependent on the shape of the prior probability distribution, and therefore on the values assigned to the hyperparameters α_S and β_S. It is the generation of different width ranges within an equal parameter space for different prior values that produces the observed disparities in the average number character transformations. For example, for the pollen character, categories around the rate value 10, which is the most frequently sampled category for any value of E(T), present large widths for E(T)=1 (Fig. 2.2d), medium widths for E(T)=5 (Fig. 2.2e) and smaller widths for E(T)=10 (Fig. 2.2f). Because each width is represented by its mean, the different discretization around the most frequently sampled rate value will have a direct effect on the average number of transformations. Thus, if we would be able to sample from continuous distributions, we could predict that for these characters the priors would not influence the results. However, this is not the case, and discretization is the most commonly used method for sampling from a continuous distribution and is widely used in phylogenetics (Yang, 1994; Yang, 1996; Drummond et al., 2006).

Finally, discretization is also responsible for another counter intuitive result. For the pollen unit, a higher average number of transformations was returned under the slow mean rate prior when compared to the medium one (Table 2.1), although we would expect a lower average for the slow rate. Figure 2.2d shows the ranges of the two last rate categories generated under E(T)=1 and SD(T)=5, which encompassed a large range of values and were represented by their mean of 11.78 and 32.24. The latter rate value is the largest rate category generated. Although it had a low posterior probability (sampled 65 times out of 10,000

draws), this was still enough during the course of a long simulation to produce a few high transformations (>60). These high transformations are clearly visible in Figure 2.3 d, where character history space is split into two at around 30 gains and 30 losses. These high generated transformations are responsible for returning an average superior to the one for E(T)=5. For carpel fusion, this broken cloud effect is also visible but to a lesser extent (Fig. 2.4, broken at 15 gains and 15 losses), and was not marked enough to produce a superior average than for E(T)=5 (Table 2.1). The broken cloud effect seems to be related to mean rate values close to zero coupled with high standard deviations and requires further investigation.

The main conclusion is that although the priors do not influence the posterior distribution, they will exert an indirect and noticeable effect on the results via the discretization of the continuous distribution. These results are significant as the average number of transformations are the main results provided by SIMMAP and are the ones that are generally reported and used for the interpretation of character evolution (e.g. Smedmark et al., 2006).

Levels of Homoplasy

Although we have shown that the prior distribution on the substitution rate will affect the average number of transformations, we have also shown that it had a contrasting influence when identifying the most probable character history (Table 2.2). For carpel fusion, the same character history was always assigned the highest PP_c independent of the prior used, except with an extremely unrealistic prior combination (Table 2.2 and Fig. 2.4 c). On the other hand, for the pollen unit, different character histories were most probable between the different values of E(T) as well as within the same analysis (several sub-equally probable character histories, Table 2.2). These differences are also visible in the character history space when using the appropriate prior values. For the pollen character the red squares (indicating a high PP_c) cover a much wider space (Fig. 2.3 c and f) than for the carpel fusion character which is concentrated around one gain - zero losses (Fig. 2.4 c and f). One explanation for these differences is the level of homoplasy present in the data for each character. A characters consistency index c_i (Farris, 1969) provides a simple measure of the overall homoplasy of the character (Sanderson and Donoghue, 1996). For pollen unit and carpel fusion the c_i was 0.13 and 1.0, respectively. Thus, a character with high levels of homoplasy (a low c_i) had several equally most probable histories, and when the c_i of the character was high, a single most probable history was significantly favoured. Homoplasy is positively correlated with the rate of evolution of a character (Archie, 1996; Donoghue and Ree, 2000): the higher the rate, the lower the c_i. For fast evolving characters, the levels of homoplasy will always be high and thus many equally most probable character histories will be found. This appears to be the case for the pollen character used here. However, this relationship might not always be straightforward. For example, the characters studied in Huelsenbeck et al. (2003), seastars with or without larval feeding (Hart et al., 1997) and, absence or presence of a horned soldier in aphid species (Stern, 1998), were also shown to have a high rate of transformation amongst

states (E(T)=10), as judged from their posterior distributions. In addition, the characters had a relatively low c_i (0.33 for the aphid dataset, and 0.25 for the seastar dataset). However, even under two contrasting mean rate priors (E(T)=1 and 10), the same character history had the highest PP_c: four gains and zero losses for larval feeding, one gain and two losses for horned soldiers. In this case, despite relatively high levels of homoplasy, one character history was significantly favored over the others. Thus, the relationship between homoplasy and rates of evolution is complex as noted by Sanderson and Donoghue (1996). In the examples provided by Huelsenbeck et al. (2003), it would appear that even though the characters were fast evolving and likely to be more homoplasious, the signal provided by the data was strong. In that case the priors seemed to have little influence on identifying the most probable character history as has been previously suggested for Bayesian analyses in general (e.g. Alfaro and Holder, 2006). It is important to stress that this concerns the most probable character history, a result not immediately available when using SIMMAP. The exact influence of the level of homoplasy and the strength of the data on the outcome of the analysis is beyond the scope of this paper. Simulation studies could be undertaken in order to address this question.

Specification of the Gamma Prior Distribution on θ

How should one specify the parameters of the prior gamma distribution (the hyperparameters) in posterior mapping? Choosing appropriate priors for Bayesian analyses is a hard task, inciting ongoing debate (Kass and Wasserman, 1996; Carlin and Louis, 2000; Van Dongen, 2006). However our analyses give us a detailed understanding of the influence of the prior distribution on θ, de facto providing some insights on prior specification in posterior mapping. Several observations can be deduced from our analyses that would help to clarify the choice of priors. First, there is a prior mean rate (E(T)) that better suits the data than others. For example, in all cases with SD(T) = 5, the sampling frequency (lower graphs, Fig. 2.2) was maximal roughly around the same rate value, regardless of the E(T) (just under 10 for the pollen unit and around 1 for carpel fusion). These values could be interpreted as the "appropriate" mean rate for the character given the data. These appropriate values are also found when the confidence level is changed. Ideally, the posterior distribution is sampled evenly around the specified mean (normal distribution) and not centered on the highest or lowest categories (skewed distribution). Under high confidence (e.g. a low SD(T)) we narrow down the possible parameter space. In that case a skewed posterior distribution could result in only part (or none) of the "true" parameter space being sampled. Second, we also observed that when the appropriate substitution rate was selected, little difference was observed between a high and a low confidence (SD(T)=1 or 5) on the average number of transformations (Table 2.1). This implies that the mean of the prior rate value (E(T)) is more important than the associated standard deviation (SD(T)). Thus, in agreement with Huelsenbeck et al. (2003), it would seem that the data contains some information over the rate of substitution and that a prior distribution generating a skewed posterior probability distribution is not appropriate given the data.

The idealized Bayesian approach dictates that one should choose priors by using external knowledge independent of the data at hand. In some cases choosing a prior value on θ could be fairly straightforward. This would be the case for a character such as carpel fusion. Syncarpy was inferred to have evolved once with no losses or c. four times with four losses within Annonaceae (Table 2.1). Our prior knowledge suggests however that an evolutionary scenario of four gains is highly improbable and unrealistic because syncarpy has rarely evolved in magnoliids with reversals being even rarer (Endress, 1982; Endress, 1990). In contrast, for some characters prior knowledge would not be able to clearly indicate which result to expect. We can be confident that the pollen character evolves faster that the carpel character (e.g. an independent dataset suggested high homoplasy, Doyle and Le Thomas, 1996), but we would be unable to favor one prior value over the other in a well-informed way (Table 2.1).

Here we recommend an empirical Bayes or "data-dependent" approach in posterior mapping whereby the data, via the posterior distribution, is used to estimate the hyperparameters of the gamma prior distribution on θ. Strictly speaking, this is at odds with Bayesian philosophy as we use the data to inform our prior. A philosophical discussion on this issue is beyond the scope of this paper, but we do provide some justification of its use. The empirical Bayes approach is a well known method, and is generally referred to in Bayesian statistics textbooks (e.g. Berger, 1985; Carlin and Louis, 2000). Berger (1985, page 112) goes to suggest that "the problem [of data-dependent priors] does not seem so bad if a slightly different perspective is adopted" in that if the evaluation of the prior seems reasonable then little concern should be taken over its specification (using the data or not). For the two characters used here the appropriate prior values provided by the posterior distributions (E(T)=1 for carpel fusion; E(T)=10 for pollen unit) did indeed seem reasonable given our prior knowledge. Moreover, this approach, e.g. using the data to estimate the prior, has been used in previous analyses of character evolution (estimation of rate values via maximum likelihood or the likelihood surface; Pagel et al., 2004) as well as for phylogenetic inference (branch length prior specification; Yang and Rannala, 2005). Finally, informal documents such as the online manuals SIMMAP (Bollback, 2006) and BayesTraits (Pagel and Meade, http://www.evolution.rdg.ac.uk/BayesTraits.html) recommend checking posterior probabilities for skewness and adjusting prior values accordingly. Thus, although this approach might seem unconventional, it does provide a useful remedy to prior specification, especially in the case of posterior mapping.

When undertaking posterior mapping with discrete morphological characters we suggest a two step empirical Bayes prior specification method. First, select an as good as possible estimation of the mean rate (E(T)) in combination with a large SD(T) and sample from the prior distribution. Second, by visualizing the posterior distribution (in Tracer 1.3, for example), the appropriate mean rate prior (E(T)) can be identified corresponding to the rate with the highest posterior probability (Fig. 2.2 d-f; j-i). As we have shown, once the appropriate mean rate value is estimated (e.g. E(T)=10 for the pollen unit; E(T)=1 for carpel fusion) the SD(T) will have a small and negligible influence on the average number of

transformations. However, the purpose of a prior distribution on the rate of substitution is meant to accommodate for uncertainty over the parameter. Thus, even with the empirical Bayes approach suggested here, it is nonetheless important to accommodate for uncertainty over the rate of substitution by allowing for some variance in the prior distribution (SD(T)).

Finally, although we demonstrated that priors have an effect on the outcome, *how* exactly the priors will affect the results in certain situations still needs to be explored. Many questions need further investigation: for example how will the priors affect the results with different degrees of homoplasy, rate heterogeneity of a morphological character, tree shape, sampling of taxa and characters. Answering these questions using simulated data would allow for a better understanding of the precise role priors play in posterior mapping.

ACKNOWLEDGEMENTS: Timo van der Niet is deeply thanked for critically reading and significantly improving earlier versions of the manuscript. Jonathan Bollback is thanked for comments and discussions around the posterior mapping method and suggestions for improving the manuscript. Bastienne Vriesendorp is also thanked for her help in writing the perl scripts. Gort Gerrit is also acknowledged for help with the statistical software R and production of the graphs in Figure 2.2.

3 Evolution of Syncarpy and other Morphological Characters in African Annonaceae: a Posterior Mapping Approach ♣

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Abstract. The congenital fusion of carpels, or syncarpy, is considered a key innovation as it is found in more than 80% of the angiosperms. Within the magnoliids, however, syncarpy has rarely evolved. Two alternative evolutionary origins of syncarpy were suggested in order to explain this rare occurrence in magnoliids: multiplication of a single carpel vs. fusion of a moderate number of carpels. The magnoliid family Annonaceae provides an ideal situation to test these hypotheses as two African genera, *Isolona* and *Monodora*, are syncarpous in an otherwise apocarpous family with multicarpellate and unicarpellate genera. In addition to syncarpy, the evolution of six other morphological characters was studied. Well-supported phylogenetic relationships of African Annonaceae and in particular those of *Isolona* and *Monodora* were reconstructed. Six chloroplast regions were sequenced and analyzed using maximum parsimony and Bayesian inference methods. The Bayesian posterior mapping approach to study character evolution was used as it accounts for both mapping and phylogenetic uncertainty, and also allows multiple state changes along the branches. Our phylogenetic analyses recovered a fully resolved clade comprising twelve endemic African genera, including *Isolona* and *Monodora*, which was nested within the so-called long-branch clade. This is the largest and most species-rich clade of African genera identified to date within Annonaceae. *Isolona* and *Monodora* were inferred with maximum support to be sister to a clade characterized by genera with multicarpellate apocarpous gynoecia, supporting the hypothesis that syncarpy arose by fusion of a moderate number of carpels. This hypothesis was also favoured when studying the floral anatomy of both genera. Annonaceae provide the only case of a clear evolution of syncarpy within an otherwise apocarpous magnoliid family. The results presented here offer a better understanding of the evolution of syncarpy in Annonaceae and within angiosperms in general.

♣ In press at Molecular Phylogenetics and Evolution

INTRODUCTION

Syncarpy is defined as the congenital fusion of carpels (Carr and Carr, 1961; Endress, 1990) and is regarded as a key innovation in the evolution of flowering plants (Endress, 2001). It is thought to present numerous evolutionary advantages over the alternative state, apocarpy (free carpels), such as an increased pollination efficiency (Endress, 1982; Armbruster et al., 2002). Syncarpy is a derived state in angiosperms (Soltis et al., 2005) and is found in over 80% of all angiosperm species (Endress, 1982), mostly confined to the Monocotyledons and the eudicots (Armbruster et al., 2002). In contrast, syncarpy is rare in the early-diverging magnoliids (sensu APGII, 2003), appearing in only a few groups such as Canellaceae and *Takhtajania* (Winteraceae). Interestingly, syncarpy has also evolved in two African genera within the mainly apocarpous pantropical magnoliid family Annonaceae: *Isolona* and *Monodora* (Deroin, 1997; Endress, 1982, 1990; Guédès and Le Thomas, 1981). This rare occurrence of syncarpy within the magnoliids provides an interesting framework to study the evolution of this important character. Two evolutionary scenarios have been suggested (Endress, 1990): (1) syncarpy arose by reduction to a unicarpellate state followed by multiplication of this single carpel, i.e. branching of a single carpel primordium (multiplication hypothesis), or (2) syncarpy arose by congenital fusion of initially numerous free carpels (fusion hypothesis). Endress (1990) concluded that the former hypothesis was more probable, following two lines of evidence. First, the carpels of the syncarpous magnoliids are fused up to the stigma, rather than partially, suggesting multiplication of a single carpel. Second, Endress (1990) suggested that most of the syncarpous magnoliid clades, including *Isolona* and *Monodora*, would be sister to unicarpellate taxa. Assuming an ancestral state of several carpels, he indicated that it is morphogenetically easier to first evolve a unicarpellate state and from there to evolve into a syncarpous gynoecium, in contrast to directly evolving a syncarpous gynoecium from a multicarpellate state. Within the Annonaceae, support for this view was expressed by Verdcourt (1996) and van Heusden (1992), who suggested that two unicarpellate taxa, the monotypic East African genera *Dielsiothamnus* and *Sanrafaelia* are closely related to *Isolona* and *Monodora* based on morphological characters.

In contrast, Deroin (1997) favoured the fusion hypothesis (i.e. a multicarpellate origin) based on the analysis of the gynoecial vasculature which provided evidence for fusion, not multiplication, of carpels within *Isolona* and *Monodora* (Deroin, 1985). He also stated that, given the low percentage of unicarpellate species in Annonaceae (c. 10% of species), the reduction from several to one carpel didn't stand out as a probable evolutionary step within the family. Based on extensive floral anatomical studies within the family, Deroin (1997) suggested two evolutionary series both starting with the plesiomorphic state of a small number of free carpels (possibly three). In the first series this ancestral state underwent a moderate augmentation of carpel number (to 3-20), which would have preceded the evolution of syncarpy. The other trend was characterized by a larger increase in carpel number (>20) leading to the evolution of pseudosyncarpy, where carpels are free in the flower but post-

genitally fuse during fructification to form a syncarpous fruit (Briechle-Mäck, 1994; Chatrou and He, 1999; Chatrou et al., 2000). Pseudosyncarpy has originated multiple times within the family, in lineages that are sister to those with multiple carpels, which are free both in flower and in fruit. The pseudosyncarpous lineages are not related to *Isolona* and *Monodora* (Richardson et al., 2004). Moreover, pseudosyncarpy is likely to be a non-homologous character, as anatomical studies have shown that, e.g. in *Annona* and *Fusaea*, the development of the fruits takes place along different developmental pathways (Briechle- Mäck, 1994; Chatrou et al., 1999). We therefore adopt the view that pseudosyncarpy is a different feature altogether that will not be considered further in this paper.

If Deroin's (1997) hypothesis on the origin of syncarpy is correct, and assuming no extinction, taxa characterized by a moderate amount of carpels (2-20) would be expected to be sister to *Isolona* and *Monodora*. In contrast, adopting the multiplication hypothesis one would expect unicarpellate taxa to be sister to the syncarpous genera as suggested by Endress (1990). In order to test these hypotheses it is important to know the exact phylogenetic relationships of *Isolona* and *Monodora* with their related genera.

Study reference	Fries (1959)	Walker (1971/1972)	van Heusden (1992)		van Setten (1992)		This study
Characters used	*Flowers*	*Pollen*	*Flowers*		*Fruits and seeds*		*DNA*
Informal group name	*Hexalobus* group	*Hexalobus* tribe	*Hexalobus* group	*Uvariastrum* group	Gr.13	Gr. 14	ALBC
Genus							
Monodora	✓	✓	✓			✓	✓
Isolona		✓	✓			✓	✓
Hexalobus		✓	✓		✓		✓
Uvariastrum		✓	✓		✓		✓
Asteranthe		✓	✓				✓
Uvariopsis		✓		✓	✓		✓
Uvariodendron		✓		✓	✓		✓
Dennettia		✓		✓	✓		✓
Monocyclanthus		✓		✓			✓
Mischogyne		✓		✓	✓		✓
Sanrafaelia							✓
Ophrypetalum	✓	✓	✓				✓
Toussaintia			✓				
Lettowianthus	✓						
Polyceratocarpus				✓			
Cleistochlamys	✓	✓					
Meiocarpidium				✓			
Dielsiothamnus				✓			
Diclinanona		✓	✓				
Asimina			✓				
Deeringothamnus			✓				

Table 3.1: Previous classifications of *Isolona* and *Monodora* and other Annonaceae genera based on different morphological/palynological characters and DNA sequence data. African genera are in bold type. ALBC= African long-branch clade (see results).

The infrafamilial classification of Annonaceae has always been problematic mainly due to the absence of unambiguous floral, fruit and seed characters (Doyle and Le Thomas, 1996; Walker, 1971). Recent morphological cladistic analyses (Chatrou et al., 2000; Doyle and Le Thomas, 1994; Doyle and Le Thomas, 1996; Johnson and Murray, 1995) as well as molecular phylogenetic studies using DNA sequence data (Doyle et al., 2000; Mols et al., 2004; Pirie et al., 2006; Richardson et al., 2004) have proved very useful in the elucidation of the generic and higher-level relationships. In numerous analyses *Anaxagorea* was inferred as sister to the rest of the Annonaceae using morphology (Doyle and Le Thomas, 1996) as well as molecular data (Doyle et al., 2000; Doyle et al., 2004; Richardson et al., 2004; Scharaschkin and Doyle, 2005). The next-diverging clade after *Anaxagorea* is referred to as the ambavioids (Doyle and Le Thomas, 1996). This clade is mainly composed of genera presenting unique or unusual morphological characters (Doyle and Le Thomas, 1994; Doyle and Le Thomas, 1996), and is characterized by plesiomorphic palynological characters (heteropolar-sulcate pollen with poorly differentiated granular infratectum, Le Thomas, 1980, 1981). Finally, two major well-supported clades containing most of the genera have been recovered (Richardson et al., 2004): the so-called long-branch clade (LBC) and short-branch clade (SBC). The LBC is characterized by taxa having inaperturate pollen and is equivalent to the 'inaperturate clade' of Doyle and Le Thomas (1996). The long branches subtend species rich clades and have on average twice the level of sequence divergence when compared to the SBC. The latter is equivalent to the *Malmea-Piptostigma-Miliusa* (MPM) clade of Doyle and Le Thomas (1996).

Based on different morphological studies, *Isolona* and *Monodora* have been suggested to be closely related to numerous other African genera (Table 3.1). However, each of these studies provided different groupings of these genera and were not based on any formal analysis of morphological data, but on the intuitive assembly of groups of genera. Walker (1971; 1972) recognized, based on a wide survey of Annonaceae pollen, a close relationship between twelve strictly African genera, including *Isolona* and *Monodora*, and one South American genus (*Diclinanona*). These were placed into the *Hexalobus* tribe, characterized by tetrad pollen grains, except for *Cleistochlamys* and *Isolona* that have monads. Later, it was recognized that these genera all share inaperturate pollen grains (Le Thomas, 1980). The inclusion of *Cleistochlamys* from East Africa and *Diclinanona* in the *Hexalobus* tribe, however, was considered doubtful (Walker, 1971). Classifications based on floral (van Heusden, 1992) and fruit morphology (van Setten and Koek-Noorman, 1992) also recognized the close affinity between the genera of Walker's *Hexalobus* tribe. Both the *Uvariastrum* and *Hexalobus* groups of van Heusden, as well as groups 13 and 14 of van Setten (Table 3.1) bore many similarities.

In addition to the syncarpy character, African Annonaceae have several morphological characters that are mostly uncommon within the family. *Asteranthe, Hexalobus, Isolona*, *Monodora* and *Sanrafaelia* all have conspicuously or at least basally fused petals. *Dennettia* and *Uvariopsis* differ from the usual Annonaceae floral structure of six petals in two whorls, in having a single whorl of three or four petals, respectively, while *Monocyclanthus* has one whorl of six equal and free petals. *Uvariopsis* is also exceptional being monoecious which is

rare within Annonaceae, while *Polyceratocarpus* is androdioecious. *Hexalobus* is unique in having plicate petals (folded in bud and paper like). Finally, *Toussaintia* has a long *Magnolia*-like receptacle and numerous spirally arranged petals, unusual for Annonaceae, whereas one species in *Mischogyne* uncommonly has long-stipitate carpels. Except for *Isolona* and *Monodora* that appeared strongly supported as sisters within the LBC, none of the other African genera presented in Table 3.1 were sampled in the molecular phylogeny of Richardson et al. (2004). The morphological cladistic analysis of Doyle and Le Thomas (1994, 1996) had a wider sampling of African genera, but most of the relationships were unsupported by bootstrap analyses. Thus, the relationships of *Isolona* and *Monodora* with these genera remained unclear and, because of these unusual characters, were hard to define from morphology alone.

Therefore, the first aim of this study is to clarify the phylogenetic position of *Isolona* and *Monodora*, and the evolutionary relationships of African genera of Annonaceae in general, based on the extensive sampling of Richardson et al. (2004) supplemented with all African genera mentioned above. The second aim of the study is to test the different evolutionary hypotheses on the evolution of syncarpy within Annonaceae, based on the newly assessed phylogenetic reconstruction. Given the large morphological diversity in African Annonaceae, we also studied the evolution of a few additional morphological characters of interest within the African genera, or important for Annonaceae classification in general.

MATERIALS AND METHODS

Taxon sampling

Preliminary analyses indicated that most of the Africa genera, in particular those related to *Isolona* and *Monodora,* belonged to the LBC. Thus, we focused on sampling within the LBC (see Appendix). Based on Richardson et al. (2004) 18 out of the c. 30 genera of the LBC were sampled, representing all major lineages. All African genera in the LBC were included. The other major clades of Annonaceae (SBC, Ambavioids, and *Anaxagorea*) were represented by seven genera out of c. 45 for the SBC, four genera from the c. eight genera in the ambavioid clade and two species of *Anaxagorea*. In total, 31 out of 40 Annonaceae genera occurring in Africa-Madagascar were included in our analysis. This sampling included all the African genera indicated in Table 3.1, except for *Cleistochlamys* and *Polyceratocarpus*. When available, two or three species per genus were included to give an indication of their monophyly. Finally, three species from the Magnoliales and Laurales were chosen as outgroups: *Eupomatia* (Eupomatiaceae sister to Annonaceae, Qiu et al., 2000; Sauquet et al., 2003)), *Coelocaryon* (Myristicaceae), and *Persea* (Lauraceae). Vouchered specimens used in this study are listed in the Appendix A (end of thesis).

DNA Extraction, PCR Amplification and Sequencing

DNA extractions were performed using a modified cetyl trimethyl ammonium bromide (CTAB, Doyle and Doyle, 1987) following Bakker et al. (1998). The universal primers C/D and E/F (Taberlet et al., 1991) were used to amplify and sequence the *trnL* intron and *trnL-trnF* spacer. The *psbA-trnH* intergenic spacer was amplified and sequenced using primers *psbA* and *trnH* (GUG) (Hamilton, 1999). The *trnS-trnG* intergenic spacer was amplified and sequenced using primers trnS (GCU) and trnG (UCC) (Hamilton, 1999). Partial *matK* sequences were amplified and sequenced using primers 390F and 1326R (Cuénoud et al., 2002) and int-F2 (Erkens et al, 2007). The *ndhF* gene was amplified and sequenced in three overlapping pieces using primers 1F, 972F, 972R and 2110R (Olmstead and Sweere, 1994) and the Annonaceae specific LBC-intF, LBC-intR (Erkens, 2007). PCR reactions were performed with 30-50 ng of genomic DNA, 0.4% of BSA, 0.2 µM of each primer, 0.2 mM dNTP PCR mix (Promega, Madison, WI), 3 µM $MgCl_2$, 1X PCR buffer (Promega, Madison, WI), and 0.5 U of *Taq* DNA polymerase (Promega, Madison, WI) in a total volume of 50 µl. The PCR program was as follows: 35 thermal cycles at 94 °C for 1 min, 50-55 °C for 50 s, 72 °C for 50s and a final extension at 72 °C for 3 min. Sequences were edited using Staden (http://staden.sourceforge.net/) and aligned manually. Gaps were coded following the simple coding model of Simmons and Ochoterena (2000). Microsatellites were excluded from the analysis, as these structures probably originate through slipped-strand mispairing (Levinson and Gutman, 1987) and are highly homoplastic.

Maximum Parsimony

Maximum Parsimony (MP) analyses were performed on each of the six markers separately and on the combined dataset using PAUP* (version 4.10b; Swofford, 2002). Heuristic searches were performed with 100 random sequence addition iterations, saving 100 trees in each iteration, with tree bisection-reconnection branch-swapping. After completing the iterations, all trees found were then used as starting trees for another round of swapping with a tree limit of 5000. The strict consensus tree was computed on the remaining trees. Relative support for each node was assessed by performing 100 bootstrap replications (Felsenstein, 1985; Salamin et al., 2003) for each marker separately, and 1,000 replications for the combined dataset with TBR branch swapping (10 random addition sequences, saving 10 trees per replicate). To justify the combined analysis, we compared the bootstrap consensus trees between each individual marker to check for any well supported incongruences between them. For the complete dataset, conflict between the most parsimonious trees was also visualized using the consensus network approach (Holland et al., 2004) as implemented in Splitstrees 4 (Huson and Bryant, 2006).

Bayesian Analysis

All analyses were run using the Metropolis-coupled Monte Carlo Markov chain algorithm as implemented in MrBayes (ver. 3.1.2; Ronquist and Huelsenbeck, 2003) with the program's default parameters for the priors. For each model, four separate runs were started from random trees. Each run was composed of one cold and three heated chains with the temperature parameter T set to 0.05 to ensure good mixing. Gap characters were always included in the analysis. When analyzed separately from the rest of the sequence data, they were set to follow the model implemented in MrBayes for binary data, using the "lset coding=variable" command. Six alternative partition strategies varying from simple to complex were considered for this study (Table 3.2). The best performing evolutionary model for each partition was identified under two different model selection criteria, the hierarchical likelihood ratio test (hLRT) and the Akaike information criterion (AIC; Akaike, 1973) as implemented in MrModelTest (Nylander, 2004). The parameters for each partition were allowed to evolve independently using the "unlink" command. An initial analysis for each of the six partition strategies was run for 2 million generations sampling every 100th generation. To decide which partition strategy best agreed with the data, the Bayes factor (Nylander et al., 2004) and following Brandley et al. (2005) was computed using the harmonic means after each MCMC run provided by the sump function in MrBayes. The best partition strategy was then rerun with the same parameters, but with three separate runs and for five million generations. In order to assess that the MCMC reached stationarity we examined the loglikelihood (lnL) plots using Tracer v. 1.3 (Rambaut and Drummond, 2003). In particular, we searched for evidence that model likelihoods and parameter estimates reached stationarity after a burn-in period. Convergence between the runs was checked by looking at the correlation of the posterior probability for each clade (= split) between each of the three runs as suggested by Huelsenbeck et al. (2001) and implemented in the online program AWTY (Wilgenbusch et al., 2004; http://ceb.csit.fsu.edu/awty). When, after comparing each independent run, clade posterior probabilities were not significantly different, we assumed our runs had reached convergence (Huelsenbeck et al., 2001).

Partition strategy	Number of partitions	Partition identity
T0	1	All data combined
T1	2	Sequence characters / gap characters
T2	3	Coding regions / non-coding regions / gap characters
T3	3	Sequences evolving under GTR+ G / GTR+G+Inv / gap characters
T4	7	*rbcL* / *matK* / *ndhF* / *trnLF* / *trnSG* / *psbA* /gap characters
T5	11	Separate codon positions for *rbcL*, *matK*, *ndhF* / non-coding regions / gap characters

Table 3.2. Partitioning strategies explored for this study.

Character Choice

Characters were scored at the generic level. Besides carpel fusion and carpel number, five additional morphological characters were selected, based either on their presumed utility for Annonaceae classification, or on their importance from an evolutionary point of view (Table 3.3). Definition and scoring of the states of each character were mainly taken from Doyle and Le Thomas (1996).

Within Annonaceae genera carpel number can be variable (van Heusden, 1992). As each genus is only represented by one to three species, this variation must be taken into account in the coding scheme. Based on our own observations and on reports in the literature, we have assessed that most genera have an intrageneric variation either ranging from 2 to 20 carpels or being more than 20 carpels, which is in complete agreement with van Heusden's (1992, p. 27) and Deroin's (1997) observations. Thus, carpel number variation within the family can be appropriately represented by three discrete categories (1; 2-20 and >20). For *Isolona* and *Monodora* the number of fused carpels ranged from 6-14 (Deroin, 1997; personal observations, see Chapter 6). For genera not sampled in Doyle and Le Thomas (1996), characters were scored using literature and/or herbarium material (see Appendix A, (end of thesis). In case of the occurrence of polymorphic states within a genus the character was coded as uncertain (?), unless a published molecular or morphological phylogeny allowed the unambiguous identification of the state at the crown node of the genus (see Appendix A for references to such studies).

	State	**0**	**1**	**2**
	Character			
1	Carpel fusion	apocarpous	syncarpous	
2	Carpel number	1	2-20	> 20
3	Habit	trees/shrub	liana	
4	Petal aestivation (in bud)	imbricate	valvate	
5	Petal fusion	free	fused	
6	Pollen unit	single	compound	
7	Exine infratectum	granular	intermediate	columellar

Table 3.3. List of variable characters scored on all specimens included in the Appendix.

Posterior Mapping, Prior Specification and Ancestral State Reconstruction

Analyses of character evolution using maximum parsimony optimization have been shown to deal poorly with long branches, mainly because it only allows for one character state change per branch (Cunningham et al., 1998; Huelsenbeck et al., 2003). Given the preliminary result that most African genera belonged to the long branch clade, the selected morphological characters were subjected to a Bayesian posterior mapping approach (Bollback, 2005; Huelsenbeck et al., 2003) which allows for multiple state changes along

branches. In addition, it can simultaneously accommodate for mapping as well as phylogenetic uncertainty (Huelsenbeck et al., 2003). The software SIMMAP version 1.0 beta 2.3 (build 12092006; Bollback, 2006) was used as it implements the posterior mapping method originally described for DNA sequences (Nielsen, 2002) and extended to morphological characters by Huelsenbeck et al. (2003) by using a continuous time Markov chain applying a Mk model of character evolution (Lewis, 2001). Two parameters define the state to state transition rate matrix (Lewis, 2001): (1) a substitution rate parameter θ (i.e. speed of evolutionary change for the character) and a bias rate parameter Π (i.e. symmetry of forward and backward evolutionary change). To accommodate for uncertainty, both parameter values are drawn from a prior probability distribution modelled as a gamma distribution for the substitution rate θ, and a beta distribution for the bias rate Π. Each prior distribution is governed by two hyperparameters defining the mean (E) and the standard deviation (SD) and have to be specified in SIMMAP. For the bias rate Π a flat prior (both hyperparameters equal one) was used for all analyses. However, for the substitution rate, a gamma distribution does not allow the use of flat priors and any value of the hyperparameters will subjectively provide an indication on the E and SD of the substitution rate (hereon E(T) and SD(T)). A detailed study of the influence of these hyperparameters on the outcome of the results showed that they should not be chosen randomly as their influence is always significant (Couvreur et al. Chapter 2). It was also shown that the posterior probability distribution provides a valid way to check if the priors are correct given the data (Huelsenbeck et al., 2003; Couvreur et al., submitted; Chapter 2). Thus, the values of the hyperparameters for the prior gamma distribution were selected independently for each character and following Couvreur et al. (Chapter 2) by using the "number of realizations sampled from priors" function in SIMMAP with 10,000 draws with a large SD(T) (= 10). By checking the resulting posterior distribution we can objectively identify which values of E(T) represent the highest sampling (e.g. highest posterior probability), and thus adjust (i.e. optimize) the value of E(T). In addition, Couvreur et al. (Chapter 2) showed that once the optimized hyperparameters were chosen, the influence of the SD(T) was negligible. With each optimized mean value (E(T)), and with a standard deviation of SD(T)=2, 100 realizations on the 2000 last trees sampled from the MCMC run under the chosen partition strategy were undertaken as they provided a good representation of the overall phylogenetic uncertainty. The total and state-to-state average number of transformations was recorded for each character.

In addition to the character history, we assessed the ancestral state at different nodes using a hierarchical Bayesian ancestral state reconstruction method (Huelsenbeck and Bollback, 2001) as implemented in the "posterior ancestral states" function of SIMMAP. This method has the advantage of not being conditioned on a single tree or set of fixed parameter values, but approximates the posterior probability of the ancestral states by sampling from their prior distributions (Huelsenbeck and Bollback, 2001). The same values of E(T) and SD(T) as above and on the same 2000 trees with 100 draws from the prior were used. The ancestral states for seven different crown nodes in the phylogeny were estimated: the *Isolona-*

Monodora clade (A), the *Monodora* clade (B); the *Uvariodendron* clade (C), the ALBC (see results) (D), the ALBC-Uvarioid clade (E); the Uvarioid clade (F), and the LBC (G). For each character state the marginal posterior probabilities were calculated (PP_{as}).

RESULTS

Maximum Parsimony Analyses

Maximum parsimony statistics for each individual marker and for the combined dataset are given in Table 3.4. Bootstrap analyses for each marker were compared (results not shown) and no well supported conflicts (> 70 % bootstrap support) were found. The parsimony analysis for the combined dataset returned seven equally most parsimonious trees. The consensus network tree indicated conflicts between the trees within the SBC only, and therefore is of no concern for this study focusing on the LBC (results not shown).

Marker	*rbcL*	*matK*	*trnLF*	*trnSG*	*psbA-trnH*	*ndhF*	*combined*
# of taxa	66	62	66	62	65	60	66
# of characters	1387	844	1282	1064	587	2000	7945
% PI	14.7	29.1	25.9	27.5	41.0	36.9	27.6
CI	0.546	0.688	0.709	0.715	0.566	0.5	0.586
RI	0.751	0.78	0.775	0.8	0.685	0.69	0.708

Table 3.4: Maximum parsimony statistics for each individual marker and the combined data set after a heuristic search: 100 random sequence addition iterations, saving 100 trees for each iteration, and tree bisection-reconnection branch swapping. All trees found were then used as starting trees for another round of swapping with a tree limit of 5000.

Bayesian Analysis

The hLRT and AIC methods always selected the same evolutionary models for each partition or marker except for the *psbA-trnH* region (HKY+G with hLRT and GTR+G with AIC). The AIC approach has proven to have important advantages over the hLRT (Posada and Buckley, 2004) and so the model selected by the former was used. For all the partition strategies stationarity was reached after 150,000 generations (as visualized with Tracer) and the lnL were the same between independent runs. All strategies generated consensus trees with identical topologies if considering the PP's above 0.5. Small fluctuations in posterior probabilities occurred for moderately supported clades, but the strongly supported ones (PP≥0.99) remained constant. The Bayes Factor always indicated strong support (BF>200) in favour of partition strategy T5 over the other five strategies considered (results not shown). This partition strategy was then rerun for five million generations with three independent runs. The posterior probabilities of all splits were indistinguishable between independent runs as visualized with AWTY (results not shown). All three runs reached stationarity after 250,000 generation, all of the parameters converging to the same values as seen with Tracer.

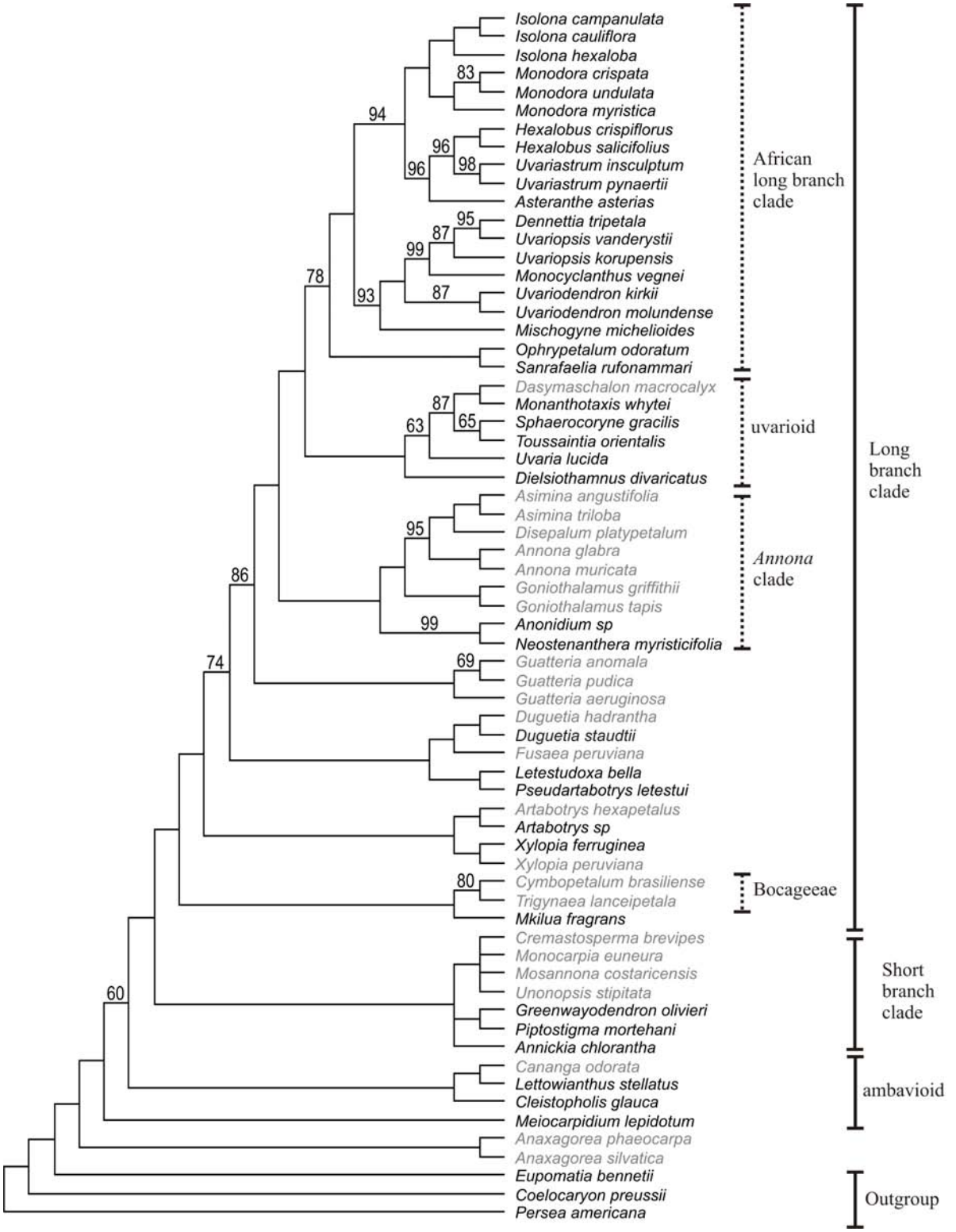

Figure 3.1: Strict consensus tree of the seven most parsimonious trees. Support values under 100% as well as major groups are indicated. African taxa are in bold.

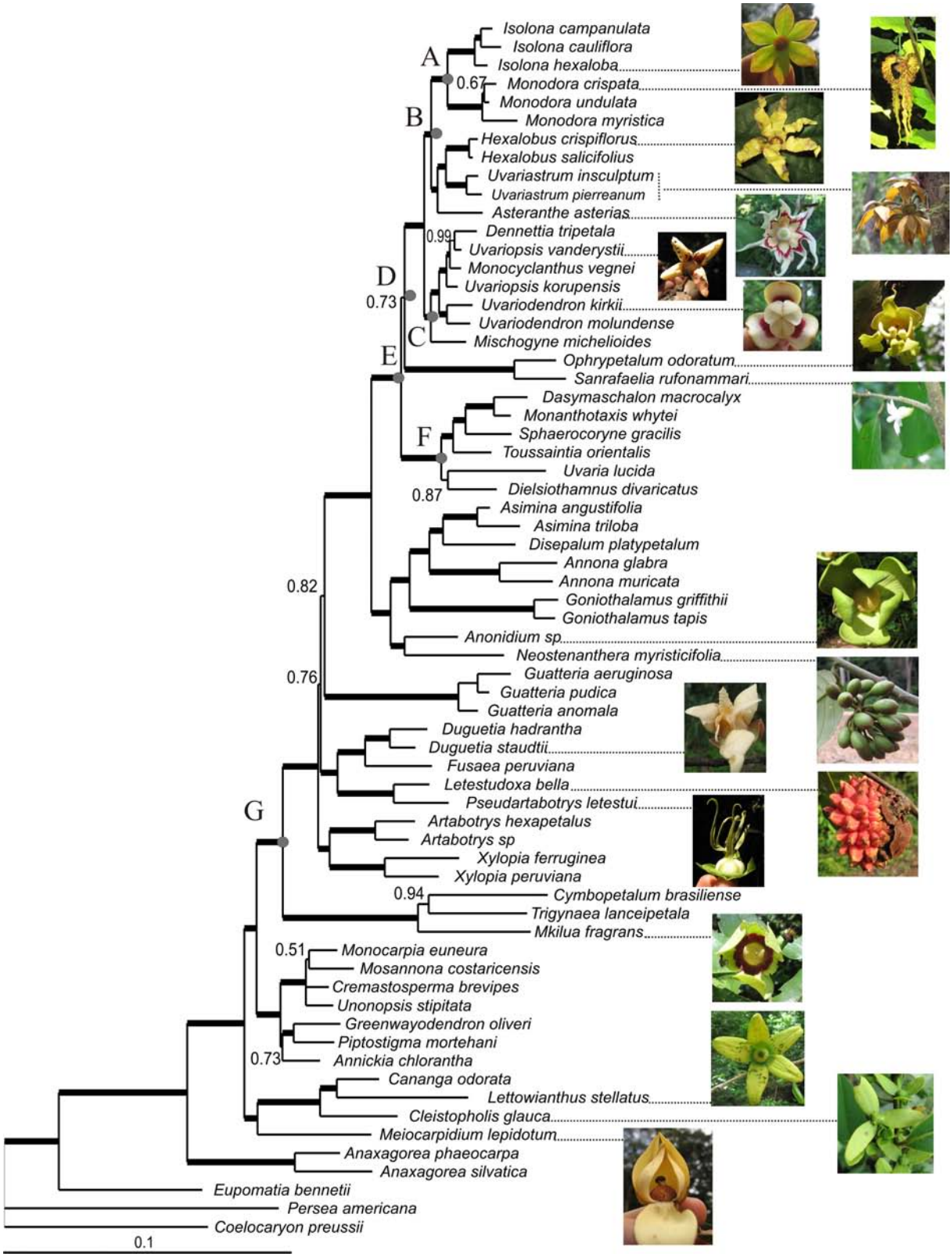

Figure 3.2: Majority rule consensus tree of the last 30,000 trees from the Bayesian analysis using the partition strategy T5 with five million generations. Posterior probabilities lower than 1.00 are displayed at nodes. Thick branches indicate maximum support. Letters indicate the nodes for which ancestral states for the six morphological characters were calculated. A: *Isolona-Monodora* clade; B: *Monodora* clade; C: *Uvariodendron* clade; D: ALBC; E: uvarioid-ALBC clade; F: uvarioid clade; G: LBC. Photographs of flowers of African genera providing an overview of the morphological diversity.

Phylogeny

Three major clades were recovered with maximum support in both MP and Bayesian analyses (Figs. 3.1 and 3.2): the short and long-branch clades and the ambavioids. In all analyses *Anaxagorea* was sister to the rest of the family. *Lettowianthus* clustered in the ambavioids, with maximum support in both analyses. The position of *Meiocarpidium* is ambiguous. In the MP analysis it was recovered as sister to all Annonaceae excluding *Anaxagorea*, though with weak bootstrap support, while the Bayesian analysis recovered it with maximum support as sister to the ambavioids. The rest of the African taxa all fell into the long-branch clade. Twelve of these genera formed a clade referred to as the African long-branch clade (ALBC hereafter). The clade comprising two monotypic East African genera, *Sanrafaelia* and *Ophrypetalum*, was moderately supported (79% BS; 0.73 PP) as sister to the rest of the ALBC genera, which received maximum support (100% BS; 1.00 PP). In addition, two subclades containing five genera each, received strong support (both 92% BS and 1.00 PP). The first, termed the *Monodora* clade, included the monophyletic syncarpous genera *Isolona* and *Monodora,* as well as a sister clade containing *Hexalobus*, *Uvariastrum* and *Asteranthe*. The second subclade, termed the *Uvariodendron* clade, contained *Mischogyne*, sister to a clade with *Monocyclanthus*, *Uvariodendron* and the monotypic genus *Dennettia* nested within *Uvariopsis.* These results are supported both by MP and the Bayesian analyses, except for the position of *Monocyclanthus*. In the MP analysis it is recovered as sister to the *Uvariopsis-Dennettia* clade (87% BS), while in the Bayesian analysis, *Monocyclanthus* is nested within *Uvariopsis* (1.00 PP). The two remaining genera sampled, *Dielsiothamnus* and *Toussaintia* clustered together with the Asian/African genera *Uvaria*, *Sphaerocoryne,* the large African genus *Monanthotaxis*, and the South-East Asian genus *Dasymaschalon* with maximum support in both analyses. The position of *Dielsiothamnus* differs between the MP and Bayesian analyses, being sister to the rest of the uvarioid taxa in the MP reconstruction (63% BS) or grouped with *Uvaria* (0.87 PP), both being sister to the rest of this clade (1.00 PP), in the Bayesian analysis. Finally, *Mkilua* was recovered with maximum support as sister to two Neotropical genera *Cymbopetalum* and *Trigynaea*.

Character Mapping and Ancestral State Reconstruction

For each character the average number of transformations as well as the average number of state-to-state transformations is indicated in Table 3.5. The posterior probabilities for the ancestral states of the six characters and for nodes A-G shown in Figure 3.2 is given in Table 3.6.

DISCUSSION

Marker Utility

Although *psbA-trnF* has the highest number of parsimony informative characters (PIC, Table 3.4) its independent analysis generated a largely unsupported tree (results not shown). Erkens (2007b) showed that within the LBC of Annonaceae the *psbA-trnH* marker is saturated, i.e. the phylogenetic pattern is obscured by too much sequence variability. In contrast, *ndhF* with a slightly smaller amount of PICs (-5%, Table 3.4), generated the best resolved tree of the six markers used, especially within ALCB. Finally, and in agreement with Erkens (2007b), the combined analysis of *ndhF* and *trnLF* regions was sufficient to generate well supported clades within the LBC, but lacked some resolution within the ALBC. The highest support values for clades in the ALBC were returned only when all markers were combined. For detailed analyses of the utility of these markers for phylogenetic reconstruction in the LBC see Erkens (2007b).

Although infra-familial classification of Annonaceae has proved problematic for a long time generic relationships are becoming clearer with the advent of molecular phylogenetics (Doyle et al., 2004; Erkens et al., 2007a; Pirie et al., 2006; Richardson et al., 2004). The results presented here shed much needed light on the phylogeny of the African Annonaceae that had not been or were poorly sampled in previous analyses. In agreement with Richardson et al. (2004) our analyses recovered the four major clades found with the Annonaceae: the long- and short-branch clades, the ambavioid clade, and *Anaxagorea* as sister to the rest of the family.

Phylogenetic Relations within African Annonaceae

Our results indicate that *Isolona* and *Monodora* belong in a larger clade of 12 African endemic genera, which we shall refer to as the African long-branch clade (ALBC, Figs. 3.1 and 3.2, Table 3.1). This result is significant as all the genera thought to be closely related to the syncarpous clade have been included. The few non-sampled African genera do not appear related to this clade based on evidence provided by published (Richardson et al., 2004) or unpublished molecular phylogenies (Bygrave, unpub.). The ALBC appears to be the largest and most species-rich clade of African genera across the whole family (c. 80 species in total).

The other strongly supported clades containing endemic African genera identified in previous analyses (Richardson et al., 2004) contained from one or two (e.g. monotypic *Mkilua* or *Anonidium-Neostenanthera* clade in the LBC) to at least five genera at the base of the SBC (*Greenwayodendron-Annickia* clade). As previously indicated, the ALBC is very diverse morphologically, making it hard to provide a general circumscription characterizing this group. One morphological character, however, that is common to all genera in the ALBC and not present in the uvarioids is a sessile or shortly stipitate monocarp, although this state is found in other genera throughout the Annonaceae (e.g. *Meiocarpidium*; van Setten and Koek-

Noorman, 1992). Pollen morphology seems to provide some help as the ALBC is identical to the *Hexalobus* tribe recognized by Walker on the basis of palynological data (Walker, 1971; Walker, 1972), but excluding the ambiguously placed *Cleistochlamys* and *Diclinanona*.

Interestingly, the monotypic endemic East African genus *Mkilua* was recovered as sister to a clade (Figs. 3.1 and 3.2) equivalent to the Bocageeae tribe as defined by Johnson and Murray (1995). The Bocageeae contains seven Neotropical genera and c. 61 species (Johnson and Murray, 1995). The sister position of *Mkilua* was also recovered based on a morphological cladistic analyzes of the tribe (Johnson and Murray, 1995) as well as with molecular data including a wider sampling than the one presented here (4 out of the 7, unpublished results). Together they represent the earliest diverging clade within the LBC. This sister relationship between an endemic East African genus with a larger Neotropical one is unique within Annonaceae, and should be further investigated in order to understand the underlining biogeographic events leading to such a situation.

Relationships within the ALBC

A small clade composed of two monotypic genera from Tanzania and Kenya, *Ophrypetalum* and *Sanrafaelia*, is sister to the rest of the ALBC. *Sanrafaelia* has very small flowers with united petals, few stamens (c. 10) and one carpel, while *Ophrypetalum* has free, thick and distinctly clawed petals, the inner ones with a brush-like appendage on the inner side, as well as numerous stamens and a moderate number of carpels. *Sanrafaelia* was thought to be closely related to *Xylopia* based on the comparative analysis of floral anatomy (Deroin, 2000), a relationship not supported here. The affinity of these two monotypic genera with the ALBC, although moderately supported in both analyses, is not surprising as they share tetrad pollen grains (Verdcourt, 1996; Walker, 1972) and a sessile monocarp (van Setten and Koek-Noorman, 1992) with the rest of the clade. *Ophrypetalum* was already placed in the *Hexalobus* group by van Heusden (1992) based on floral characteristics such as prominently veined petals, short stamens, and a single to a moderate number of carpels (Table 3.1). The weak support for their inclusion in the ALBC comes from the very short branch leading from the most recent common ancestor (MRCA) of the ALBC to the MRCA of the ALBC excluding *Ophrypetalum* / *Sanrafaelia*. In the Bayesian trees sampled from the MCMC, their positions differed, being either nested in the uvarioids or being sister to the uvarioids and the ALBC. Moreover, the first three tree topologies with the highest posterior probabilities (as seen in the .tprobs output file from MrBayes) supported this relationship (*Ophrypetalum-Sanrafaelia* sister to the rest of the ALBC) receiving a cumulative PP of 0.157.

The second group within the ALBC, the *Uvariodendron* clade (node C, Fig. 3.2), contains taxa with free petals. Our results corroborate the previous hypothesis that *Dennettia* belongs in *Uvariopsis* (Kenfack et al., 2003). The conflicting position of the monotypic West African *Monocyclanthus*, either sister to *Uvariopsis* in the MP analysis or nested within *Uvariopsis* in the Bayesian analysis (PP=1.0), needs further investigation at the

morphological level.

Furthermore, previous cladistic analyses have placed *Uvariopsis* in two drastically different positions within the family. Based on morphological data alone (i.e. excluding all palynological data), *Uvariopsis* was recovered as sister to the rest of Annonaceae due to numerous ancestral characters (Doyle and Le Thomas, 1995). However, when palynological data was included, *Uvariopsis* was nested within the family (Doyle and Le Thomas, 1995; Doyle and Le Thomas, 1996; Doyle and Le Thomas, 1997), a position strongly supported in this analysis. This stresses once again the usefulness of palynological data for understanding Annonaceae evolution and classification (Doyle and Le Thomas, 1997).

The last well supported clade within the ALBC contains two subclades (node B, Fig. 3.2): the two syncarpous genera *Isolona* and *Monodora* (node A, Fig. 3.2); and the *Hexalobus* clade with three genera. Thus even with a complete sampling of closely related genera, *Isolona* and *Monodora* remain strongly supported as sister taxa. Besides the shared syncarpy character, this relationship is not immediately obvious as they both have very divergent morphological characters (e.g. *Monodora* has conspicuous flowers with unequal inner and outer petals as well as pollen in tetrads, while *Isolona* has fused equal length petals and pollen in monads; photos in Fig. 3.2). In the second subclade, *Hexalobus* is sister to *Uvariastrum* with maximum support in both analyses being in turn sister to *Asteranthe* (Figs. 3.1 and 3.2). The former relationship (between *Hexalobus* and *Uvariastrum*) was not found when using morphological characters (Doyle and Le Thomas, 1996) as *Uvariastrum* was sister to *Hexalobus* and the syncarpous group (*Asteranthe* was not sampled) although with no support. In addition, using just *rbcL* sequence data Doyle et al. (2000) recovered *Hexalobus* with strong support (BS=97%) at the base of the uvarioids, which was sister to *Isolona* and *Monodora*. However, our analysis using just *rbcL* still recovered *Hexalobus* and *Uvariastrum* as sister with moderate support (BS=69%) but this clade was unresolved in relation to the rest of the genera of the ALBC and to the strongly supported uvarioid clade (results not shown). This contrasting result could be due to our wider sampling of the ALBC, especially the inclusion of *Asteranthe*.

Relationships of the ALBC with Other Groups

Deeper phylogenetic relationships within the ALBC are strongly supported in both the MP and Bayesian analyses (Figs. 3.1 and 3.2). The ALBC was recovered as sister to the clade comprising the genus *Uvaria* with maximum support (Figs. 3.1 and 3.2). The *Uvaria* clade is equivalent to the uvarioid clade of Doyle and Le Thomas (1996). This strong relationship between the ALBC and the uvarioids was not reconstructed when using morphological data in the cladistic analysis of Doyle and Le Thomas (1996) where the uvarioids were sister to the rest of the 'inaperturates', or ambiguously resolved (because of the position of *Hexalobus*, see above) when using *rbcL* sequence data alone (Doyle et al., 2000). Our data thus confirm the nested position of the uvarioids within the inaperturate clade (i.e. the LBC). Finally, the

uvarioid clade and the ALBC together are sister to a clade containing *Annona* with maximum support (Figs. 3.1 and 3.2). It is worth mentioning a character related to arrangement of branches on the trunk. The distichous phyllotactic pattern (in contrast to spiral phyllotaxis) occurs in all genera of the ALBC, but is also a synapomorphy for a more inclusive clade comprising the ALBC, uvarioids and the *Annona* clade (Johnson, 2003).

The Ambavioids

The ambavioid clade is the second clade branching off after *Anaxagorea*, and contains genera from different continents with very few macromorphological affinities (van Heusden, 1992). Our analysis identified two extra genera belonging to this clade both being African and monotypic: *Meiocarpidium* and *Lettowianthus* (Figs. 3.1 and 3.2). Their position within the ambavioids is in agreement with their palynological features. Both genera have a type of pollen regarded as ancestral being heteropolar-sulcate with a poorly differentiated granular exine (Le Thomas, 1980, 1981). Floral anatomy studies have also underlined the ancestral characteristics of *Meiocarpidium* (Deroin, 1989; Deroin and Le Thomas, 1989). However, the position of *Meiocarpidium* remains uncertain as it adopts two alternative positions: being sister to the rest of the Annonaceae, excluding *Anaxagorea*, in the MP analysis with weak support (BS=60) to being sister to the ambavioids in the Bayesian analysis with maximum support. This latter position would thus be the favoured hypothesis but different molecular markers should be used to further clarify its taxonomic relationships. Moreover, all other ambavioids and *Anaxagorea* have an irregular endosperm rumination, thought to be ancestral (Doyle and Le Thomas, 1996) but *Meiocarpidium*, on the other hand, has a regular endosperm lamellate in four parts (van Setten and Koek-Noorman, 1992). In previous estimates of divergence dates of Annonaceae (Doyle et al., 2004; Erkens et al., 2007c; Richardson et al., 2004) this character was used to assign a fossil seed from the Nigerian Maastrichtian with regular lamelliform ruminations (Chesters, 1955) as a calibration point for the stem of the LBC and SBC (i.e. excluding the ambavioids and *Anaxagorea*). The early diverging placement of *Meiocarpidium* would suggest that regular lamelliform ruminations could have evolved much earlier thus shedding doubt over the accurate placement of this fossil within the Annonaceae phylogeny.

Bayesian Character Evolution

Syncarpy. Endress (1990) used two main lines of evidence when favouring the multiplication hypothesis. The first argument stated that it was easier from a morphogenetic point of view to reach a syncarpous state by first evolving to a single carpel state by reduction, than to directly evolve from a multicarpellate gynoecium. The results presented here provide no support for this argument as the syncarpous *Isolona-Monodora* clade is not inferred to be sister to any of the unicarpellate taxa, i.e. *Dielsiothamnus* and *Sanrafaelia*.

Character	# states	Bias	Rate	Average # of total transformations	Average # of transformations from					
					0=>1	0=>2	1=>0	1=>2	2=>0	2=>1
1: carpel fusion (E(T)=1)	2	0.49	1.06	1.33	1.18	-	0.15	-	-	-
2: carpel number (E(T)=16)	3	0.33	15.77	17.12	0.48	1.15	0.88	3.24	2.70	8.66
3: habit (E(T)=9)	2	0.42	8.93	13.66	6.03	-	7.63	-	-	-
4: petal aestivation; E(T)=27	2	0.49	26.96	90.72	44.54	-	46.18	-	-	-
5: petal fusion; E(T)=6	2	0.45	5.89	8.48	3.59	-	4.89		-	-
6: pollen unit; E(T)=10	2	0.49	9.87	24.47	13.18	-	11.29	-	-	-
7: exine infratectum; E(T)=24	3	0.33	23.88	27.06	3.42	5.90	3.90	4.46	5.57	3.90

Table 3.5: Average total and between-state number of transformations, for each character using the optimized value of E(T) and a confidence of SD(T)=2, after 200'000 realizations of the continuous time Markov chain. For explanation of character states see Table 3.3.

Clade		*Monodora*	*Monodora-Hexalobus*	*Uvariodendron*	ALBC	ALBC and uvarioid	uvarioid	LBC
Character	states							
1: carpel fusion	0	0.0002	0.9851	1	0.9997	0.9999	1	0.9999
	1	0.9998	0.0149	0	0.0003	0.0001	0	0.0001
2: carpel number	0	0	0	0.0005	0.0074	0.0019	0.0055	0.0007
	1	1	1	0.7269	0.5105	0.016	0.0017	0.0015
	2	0	0	0.2726	0.4822	0.9821	0.9928	0.9978
3: habit	0	0.9994	0.9999	0.9999	0.9814	0.9111	0.0491	0.817
	1	0.0006	0.0001	0.0001	0.0186	0.0889	0.9509	0.183
4: petal aestivation	0	0.0033	0.0004	0.0005	0.0113	0.0179	0.0545	0.1865
	1	0.9967	0.9996	0.9995	0.9887	0.9821	0.9455	0.8135
5: petals fusion	0	0.0001	0.001	0.993	0.4831	0.8482	0.9962	0.9974
	1	0.9999	0.999	0.007	0.5169	0.1518	0.0038	0.0026
6: pollen unit	0	0.0811	0.0005	0	0.0028	0.016	0.9402	0.8603
	1	0.9189	0.9995	1	0.9972	0.984	0.0598	0.1397
7: exine infratectum	0	0.0002	0	0.0011	0.0075	0.0473	0.3998	0.6429
	1	0.9995	0.9986	0.6102	0.9349	0.6978	0.5941	0.0152
	2	0.0003	0.0014	0.3887	0.0576	0.2549	0.0061	0.3419

Table 3.6: Hierarchical Bayesian estimation of the posterior probability of ancestral states for six characters, with 100 draws from the prior distribution and over the last 2,000 trees from the MCMC run. The values of SD(T) and E(T) of each character are the same as in Table 3.5. For explanation of character states see Table 3.3.

Rather, the molecular phylogeny shows that the syncarpous clade is strongly supported as sister to a clade characterized by a moderate number of carpels (2-20; Figs. 3.1 and 3.2). Moreover, the ancestral state of the *Monodora* clade was inferred to be apocarpic with 2-20 carpels (node B, Table 3.6), while a unicarpellate state was never inferred as probable for any node within the ALBC (nodes A-D; Table 3.6). Finally, syncarpy arose on average 1.18 times in the simulations and a reversal to apocarpy 0.15 times, when the character was mapped onto 2,000 trees from the MCMC run. Thus, it is most likely that syncarpy arose only once during the evolutionary history of Annonaceae.

The second argument was based on the observation that most Magnoliid syncarpous gynoecia presented carpels completely fused up to the stigma, implying the internal doubling of an initially single carpel (Endress, 1990). This argument was supported by the floral ontogeny of *Monodora crispata* as studied by Leins and Erbar (1982). Both authors showed that the gynoecium of *Monodora crispata* starts its development as a single primordium. However, floral anatomy studies clearly demonstrated that the gynoecia of both *Isolona* and *Monodora* were composed of several (6-14) fused carpels (Deroin, 1997). Deroin (1997) argued that in flower ontogeny studies, being an external observation of the structure, the gynoecia only appeared as one unit precisely because they are congenitally fused.

Our phylogenetic and character evolution analyses coupled with the anatomical observations of Deroin (1997) provide strong support that syncarpy in Annonaceae originated by congenital fusion of a moderate number of carpels, and not by multiplication of a single carpel as suggested by Endress (1990). *Takhtajania* (Winteraceae) was the only other genus cited by Endress (1982; 1990) as having a syncarpous gynoecium within a mainly apocarpous family. It has been shown that syncarpy in *Takhtajania* is bicarpellate with an apical placentation and thus differs morphologically from that of *Isolona* and *Monodora,* being multicarpellate with parietal placentation (Deroin and Leroy, 1993). However, *Takhtajania* was inferred to be sister to the rest of Winteraceae (Endress et al., 2000; Karol et al., 2000), in turn sister to Canellaceae (and not to Myristicaeae as suggested by Endress (1990); Soltis and Soltis, 2004), also having bicarpellate syncarpous gynoecia. These relationships suggest that syncarpy is not derived within Winteraceae, but ancestral. Thus, the genera *Isolona* and *Monodora* would provide the only case of an isolated evolution of syncarpy from apocarpy within an otherwise apocarpous family. The evolutionary history of genes involved in carpel development in plants has been addressed in recent years (Scutt et al., 2006). However, the precise mechanisms of carpel fusion and what genes trigger its onset still have not been studied in an evolutionary context (Scutt et al., 2006). As we have shown here, Annonaceae could provide a model family to address this question of fundamental importance for the understanding of angiosperm evolution.

Finally, the ecological and evolutionary advantages of the evolution of syncarpy within the family are a matter for speculation. Erkens (2007) demonstrated that *Isolona* and *Monodora* each showed an increase in their diversification rate suggesting a more rapid radiation. The reasons for radiations in plant genera are hard to pin point, but syncarpy as a key innovation would be a good candidate (Erkens, 2007).

Evolutionary trends in carpel number. The evolution of carpel number has been used previously to infer hypotheses about the evolution of important features such as syncarpy (see above) and pseudosyncarpy within the Annonaceae (Deroin, 1997), but also in other magnoliids (Endress, 1982, 1990). Based on floral anatomy studies three independent evolutionary trends were hypothesized for carpel number from a supposed ancestral state of three carpels within Annonaceae (Deroin, 1997): one reduction scenario to the unicarpellate state present in a few genera (e.g. *Sanrafaelia*), and two increment scenarios: one moderate increment to 2-20 carpels and one more significant one to more than 20 carpels. Our results suggest that the presence of numerous carpels is the ancestral state for the LBC (node G, Table 3.6). In addition, reduction of carpel number accounted for three times more transitions between states than increments (12.1 against 4.8 times respectively, Table 3.5), which is especially true for the transition from numerous to moderate (8.66 times, Table 3.5). Thus, the general trend in Annonaceae is a decrease in carpel number with a reduction from numerous to few carpels and more rarely to a single carpel (e.g. in the ALBC), which is in agreement with Doyle and Le Thomas (1996) but in contrast to Deroin (1997). Our results are also in agreement with the general trend observed with a wider sampling within the basal angiosperms (De Craene et al., 2003), but contrasts with the trend observed within the core-eudicot order of the Saxifragales, where two or three increments of carpel number from the ancestral bicarpellate state were inferred (Soltis et al., 2005).

Origin of the lianescent habit. Our results suggest three independent origins of lianas within Annonaceae based on the phylogeny of the extant taxa. This is one fewer than inferred by Doyle and Le Thomas (1996) as the liana genus *Toussaintia* was placed in their pseudosyncarp clade based on morphological data. Here, *Toussaintia* is strongly recovered as part of the uvarioid clade which is mainly composed of lianas and confirms the *rbcL* sequence data (Doyle et al., 2000). Our data suggests two reversals from a lianescent to a self-standing state (PP_{as} of liana for the uvarioid clade equal to 0.95, Table 3.6) in *Dielsiothamnus* and *Dasymaschalon*. This type of reversal has been shown to be unusual in other plants (Rowe et al., 2004; Rowe and Speck, 2005). Moreover, reversal from the liana state is also supported by the average number of transformations being 6.03 gains and 7.63 losses (Table 3.5), when this character was mapped on 2000 trees. However, not all genera belonging to this clade have been sampled (Richardson et al., 2004) leaving relationships unclear and preventing any solid conclusions. It is also important to underline that a precise definition of a liana is still vague within Annonaceae as noted by Doyle and Le Thomas (1996).

Petal aestivation transformations. Fries (1959) considered petal (and sepal) aestivation an important character for higher level Annonaceae classification since he used it to divide the family into two main tribes. However, recent analyses led to the conclusion that petal aestivation is very homoplasious (Doyle and Le Thomas, 1996). This is corroborated here as this character had by far the highest average number of transformations (90.72, Table 3.5) with an equal average number of gains and losses when compared to the other characters

studied. This strengthens even more the idea that this character provides very little useful taxonomic information, at least in the LBC. The valvate state was recovered as ancestral for Annonaceae (results not shown) confirming previous results (Doyle and Le Thomas, 1996; Scharaschkin and Doyle, 2006).

Development of sympetaly. The congenital fusion of petals is thought to be a key innovation mainly in higher angiosperms (e.g. asterids: Endress, 2001). In the long branch clade, sympetaly evolved a number of times in isolated species within genera like *Disepalum* and *Fusaea* (Chatrou and He, 1999; Johnson, 1989) or in small clades of large genera such as in the former *Raimondia*, now included in *Annona* (Westra, 1995). In the SBC sympetaly has also evolved, though only a few times, as in *Haplostichantus* (van Heusden, 1994). Our results show, however, that sympetaly is a significant character within the ALBC. The ancestral state of the clade is ambiguous with the fused state having a slightly higher PP_{as}= 0.51 (Table 3.6). Thus two scenarios are suggested: (1) sympetaly is ancestral to the whole ALBC with three independent reversals to the free state, or (2) free petals would be the ancestral state with two independent transitions to fused: once in *Sanrafaelia* and once in the *Monodora* clade with a reversal to the free state in *Uvariastrum*. The average number of transitions (Table 3.5) from the fused to the free state is slightly higher (4.89) than from free to fused (3.58) indicating a slight bias towards loss of the fused state. This could provide some support for the former hypothesis.

Pollen unit evolution. The ALBC genera have tetrads as pollen dispersal units, except for *Isolona* which has monads (Walker, 1971; Walker, 1972). The monad state of *Isolona* was first thought to be an intermediate state leading to the tetrad state found in the rest of the ALBC genera and in *Annona* and *Anonidium* (Le Thomas, 1980, 1981). The ancestral state of the *Isolona-Monodora* clade as well as the ALBC was inferred to have tetrads (node A, PP_{as}=0.92 and 0.99 respectively, Table 3.6) confirming that the monads in *Isolona* are a reversal. This was also suggested by Doyle and Le Thomas (1996). Based on morphological data, two main transformations from monads to compound pollen grains were initially suggested: one in the annonoid and one in the xylopioid groups with *Xylopia* linked to *Neostenanthera* and *Cananga* (Doyle and Le Thomas, 1994). The ancestral state of the ALBC-uvarioid clade was strongly supported as compound (node E, PP_{as}=0.93, Table 3.6) as was the *Annona*-uvarioid-ALBC clade (results not shown). Thus, our results imply that only one major evolutionary step towards compound pollen took place in the *Annona*-uvarioid-ALBC group, with a major reversal to monads in the uvarioid clade. The other trend suggested would no longer be valid as *Cananga* was placed within the ambavioids and *Neostenanthera* has been recovered as sister to *Anonidium* within the *Annona* clade (Doyle et al., 2000; Richardson et al., 2004), thus producing an isolated occurrence of compound pollen in *Xylopia* being now sister to single grained *Artabotrys*. The other origins of compound pollen within Annonaceae are also isolated cases such as in *Pseuduvaria* (Su and Saunders, 2003), *Fusaea* (Le Thomas et al., 1994) or *Cananga*.

Pollen exine. The pollen infratectum structure, being granular, intermediate or columellar, has been a key character in understanding the evolution of African Annonaceae (Doyle and Le Thomas, 1994; Le Thomas, 1980, 1981). It was first thought that the intermediate infratectal state was transitional from granular, being ancestral in Annonaceae, to columellar (Doyle and Le Thomas, 1995; Le Thomas, 1980, 1981). However the nested position of *Isolona*, *Hexalobus, Monodora* and *Uvariastrum*, all with intermediate infratecta, in the morphological analysis of Doyle and Le Thomas (1996) led the authors to suggest that the intermediate state was in fact derived from the columellar state. This result was also supported with *rbcL* sequence data (Doyle et al., 2000). Our results indicate, however, that the intermediate state is ancestral for the ALBC and also for the *Uvariodendron* clade but with moderate support (PP_{as}= 0.93 and 0.61 respectively, Table 3.6) and evolved three times into the columellar state (in *Asteranthe, Ophrypetalum* and in the *Uvariodendron* clade). In addition, the ALBC-uvarioid clade was also inferred to have intermediate infratectum however with a moderate PP_{as} (node E, Table 3.6, PP_{as}=0.7). Finally, the ancestral state of the LBC was inferred to be granular, but also with moderate support (node G, Table 3.6, PP_{as} =0.65). Thus our data provides support for the former hypothesis, in that an intermediate infratectum is transitional between granular and columellar states within the LBC and with a reversal in the uvarioid clade.

ACKNOWLEDGEMENTS: We thank Thierry Deroin and Timo van der Niet for useful comments on a previous version of the manuscript. Two anonymous reviewers have also provided very constructive comments significantly improving an earlier version of the manuscript. The National Geographic Society is also thanked for funding a field trip to Gabon for TLPC and SMSM in 2005. Finally, Ann Robertson, David Johnson and the Missouri Botanical Garden in St Louis are deeply thank for providing good quality DNA material for some of the African species.

4 Unraveling the Evolutionary Origins of the East African Rain Forest Tree Flora ♣

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Abstract. Some regions on earth support outstanding levels of biodiversity. Understanding the evolutionary processes that led to the development of such areas has fascinated evolutionary biologists and is pivotal for determining appropriate conservation strategies. The rain forests of coastal and montane eastern Africa, both representing biodiversity hotspots, are exceptional in having one of the highest concentrations of endemic plant species on the planet. How this unique biodiversity arose is still largely unexplained, but is thought to be the result of climatic and geological events affecting East Africa over the last 30 million years. Annonaceae form a characteristic plant family in the rain forests of eastern Africa, in having numerous endemic genera as well as endemic species. Using plastid DNA, two molecular phylogenies were generated: a generic level phylogeny and a species level phylogeny. Trees were dated using a Bayesian approach accounting for calibration uncertainty. We show that the endemic East African lineages within an Annonaceae clade have ancient and multiple temporally significantly different origins. The different estimated ages for the origin of these endemic lineages (c. 33, 16 and 8 Myr) coincide with periods of renewed African aridity that would have recurrently isolated the East African rain forests from the larger Guineo-Congolian rain forest block in West-Central Africa. We argue that the high levels of plant endemicity observed in the East African rain forests is a result of a slow accumulation of lineages originating from multiple and pre-Pleistocene vicariance events. Regional and recent (Pleistocene) forest fragmentation is generally invoked to explain high levels of biodiversity in African rain forests. Our results suggest, however, that forest fragmentation did play a major role but at a much larger continental scale and over longer periods of time, spanning the Oligocene and Neogene. This diversification scenario is clearly different from the explosive speciation scenario demonstrated to explain high levels of plant endemicity in other African and Neotropical hotspots, and provides an alternative explanation for the exceptional high biodiversity of certain areas.

♣ Manuscript in preparation

INTRODUCTION

It is well established that some regions on earth support significantly more biodiversity than others. Unfortunately, many of these regions also correlate with high population pressure or other high levels of threat to the biodiversity and are referred to as "biodiversity hotspots" (Myers, 1988; Cincotta et al., 2000; Myers et al., 2000; Sechrest et al., 2002). Besides the important role of identifying these hotspots (Myers et al., 2000; Kuper et al., 2004; Mittermeier et al., 2004), understanding the evolutionary history and biogeographic processes behind the origin of such areas plays a central role towards their urgent conservation (Myers et al., 2000; Mace et al., 2003).

The East African rain forests are botanically one of the most diverse regions on the planet. Previously thought to represent a single biodiversity hotspot (Myers et al., 2000), they are now considered to be two distinct hotspots: the Eastern Afromontane Hotspot and the Coastal Forests of Eastern Africa Hotspot (Mittermeier et al., 2004). The Eastern Arc Mountains are a disjunct chain of ancient mountain ranges (c. 100 Ma old) situated 40-450 km from the Indian Ocean. They run from southern Kenya (Taita Hills) to south-central Tanzania (Udzungwa Mountains) and rise to slightly over 2000 m a.s.l. (Lovett, 1993b; Burgess et al., 2007). The Coastal Forests of East Africa consist of small patches of fragmentary lowland forests not bigger than 370 km^2, running down the East coast from southern Somalia to southern Mozambique (Burgess et al., 1998; Burgess and Clarke, 2000). They also include the lowland rain forests at the base of the Eastern Arc Mountain range (e.g. Usambara or Uluguru mountains, Burgess et al., 1998). The coastal and montane rain forests of eastern Africa have an exceptional density of endemic plants, which is one of the highest on the planet (Myers et al., 2000). Concentrated in a total area of only c. 7000 km^2 they support c. 3500 plant species, of which c. 1100 (= 31%) are endemic (Burgess et al., 1998; Burgess and Clarke, 2000; Burgess et al., 2007). How this diversity arose is still largely unexplained (Burgess et al., 1998; Burgess and Clarke, 2000; Burgess et al., 2007).

It is well documented that the East African rain forests present strong affinities with the more extensive Guineo-Congolian rain forest situated in West-Central Africa (Moreau, 1933; Brenan, 1978; Iversen, 1991; Wasser and Lovett, 1993; Burgess et al., 1998; Burgess and Clarke, 2000). Both regions are separated by a north to south arid corridor. Indeed, many endemic species are representatives of widespread genera co-occurring in the Guineo-Congolian region. In addition, the endemic East African genera are thought to be closely related to Guineo-Congolian genera.

These disjunctions are generally explained by vicariance of a once large pan-African rain forest (Axelrod and Raven, 1978; White, 1979; Coetzee, 1993; Wasser and Lovett, 1993; Burgess et al., 1998; Jacobs et al., 1999; Burgess and Clarke, 2000; Morley, 2000; Burgess et al., 2007), the existence of which is corroborated by fossil evidence (Axelrod and Raven, 1978; Coetzee, 1993; Maley, 1996; Jacobs et al., 1999; Morley, 2000). The possible ad-hoc explanation of long-range dispersal is generally ignored in the literature, and is unlikely if only for the multiple, recurring patterns of east-west disjunctions that strongly suggest a

common underlying cause (Iversen, 1991). The precise role the break-up of this pan-African rain forest played in the generation of high levels of endemicity, however, is still unclear. A first hypothesis suggests that the East African forest endemics originated from a single vicariance event induced by the onset of East African aridification, separating West and Central African forest areas from East African ones during the Oligocene – Early Miocene (c. 33-20 Myr) (Axelrod and Raven, 1978; Wasser and Lovett, 1993; Burgess and Clarke, 2000; Morley, 2000). In contrast, a second hypothesis suggests that the endemic taxa originated from multiple recurrent isolations and reconnections of West-Central and East African forest from the mid-Tertiary (c. 33 Myr) onwards (Hamilton and Faden, 1974; Coetzee, 1993; Wasser and Lovett, 1993; Burgess et al., 1998; Jacobs et al., 1999). Finally, it has also been argued that speciation may have been driven by local rain forest expansion and contraction due to Pleistocene climatic fluctuations (1.8 – 0 Myr) (Coetzee, 1993; Fjeldsa and Lovett, 1997; Morley, 2000; Burgess et al., 2007). Distinguishing between these hypotheses enhances our understanding of the evolutionary history that shaped this region, which is of crucial importance if one wants to conserve not only the large number of endemics but also the phylogenetic diversity they represent (Sechrest et al., 2002; Mace et al., 2003).

Dated molecular phylogenies of rain forest-restricted taxa with a disjunct West-Central and East African distribution provide a powerful way to test these hypotheses. If a single vicariance event explains the origin of endemicity in East Africa, either the West-Central and East African taxa, respectively, should be reciprocally monophyletic (Fig. 4.1a), or multiple splits between West-Central and East African taxa should all be dated to the same age (Fig. 4.1b) coinciding with the beginning of aridification around the Oligocene – Early Miocene (c. 33-20 Myr). In contrast, if a chronological sequence of multiple vicariance events is at cause, the splits should be dated to significantly different time periods during the middle and late Tertiary (Fig. 4.1c). Pleistocene speciation should also be evident as splits in the phylogeny that occurred during this period.

Annonaceae (Magnoliales, APGII, 2003) form a large pan-tropical family of trees, shrubs or lianas mainly restricted to rain forests. In East Africa they have many endemic genera as well as endemic species in both the montane and coastal forests (Burgess and Clarke, 2000; Couvreur et al., 2006). Moreover, in the coastal forests Annonaceae is a characteristic family, having the highest number of endemic genera (six in total) and containing one of the highest numbers of endemic species (over 60, c. 5% of the total) (Burgess and Clarke, 2000).

A family level molecular phylogeny revealed that the African genera were not monophyletic (Richardson et al., 2004). However, in a more recent study including a wider sampling of the African taxa, Couvreur et al. (in press, Chapter 3) identified a clade containing 11 strictly African genera nested within the Annonaceae Long Branch Clade (LBC, Richardson et al., 2004). This African clade represents the largest and most species rich group of African genera in the family (Couvreur et al., in press). These genera mainly grow in lowland and montane rain forests and present a variety of distributional patterns across Africa. Out of the 11 genera, *Asteranthe* (two species), *Ophrypetalum* and *Sanrafaelia*

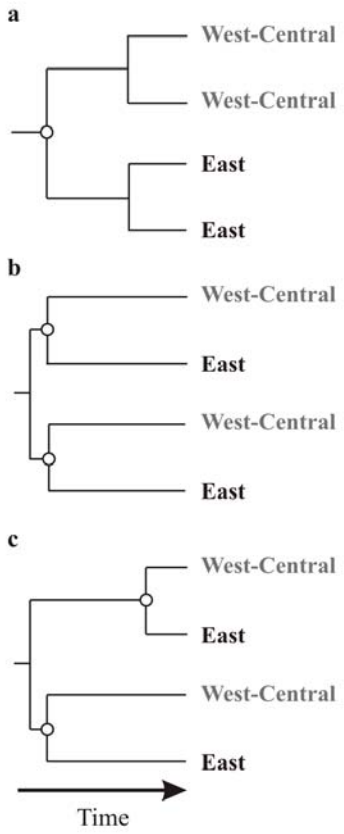


Figure 4.1: Alternative phylogenetic hypotheses on the origin of East African rain forest tree lineages. b) Multiple origins dated to same period, hence to the same event; c) Multiple origins dated to significantly different periods, hence to different events. Open circles indicate moment of West-Central/East split.

(both monotypic) and are endemic to the East African rain forests (Verdcourt, 1971; Verdcourt, 1996; Clarke et al., 2000; Couvreur et al., 2006) and two genera (*Mischogyne* and *Monocyclanthus*) are endemic to the Guineo-Congolian region. Finally, seven genera are mostly Guineo-Congolian but have a few endemic species growing in the East African rain forests. *Monodora* and *Uvariodendron* have an almost equal number of distinct species in both regions (c. seven), while *Hexalobus, Isolona, Uvariastrum* and *Uvariopsis* are represented by one to three endemic species in the East African rain forest (Couvreur et al., 2006). Their propagules being dispersed by rain forest dwelling mammals, (e.g. gorillas Rogers et al., 2004), these taxa are not normally associated with long-distance dispersal (van Setten and Koek-Noorman, 1992) making the crossing of wide arid corridors such as the one existing between the Central en East African rain forest highly improbable. Finally, the two sister genera *Isolona* and *Monodora* are of particular interest in that they have been the subject of a recent morphological revision (Chapter 6). Thus a detailed knowledge of the exact number of total species as well as their precise distribution ranges is now known. Both genera are widespread in Africa, but present a clear disjunction with distinct species in both East and West-Central Africa (Couvreur et al., 2006). In addition, they are both characterized by large syncarpous fruits, a rare feature in Annonaceae and even more strongly suggesting a

low capacity for long-range dispersal (Balcomb and Chapman, 2003). Given the low capacity for dispersal, the abundance and taxon richness of this Annonaceae clade in the East African rain forests as well as its distribution across Africa, this group provides a perfect model to investigate the origin of East African rain forest endemic lineages.

The aims of this study was first to test if the East African endemic lineages arose via one or multiple biogeographic events since c. 30 million years, and second try to correlate the resulting pattern to known geological and climatic events that have affected the African continent during that period.

MATERIAL AND METHODS

Molecular Data

For this study two different DNA sequence datasets were used. First, in order to date the origin of the East African endemic genera, a previously published DNA sequence data matrix (dataset A, see Appendix C, end of thesis) of the African clade was used (Couvreur et al., in press, treeBASE SN3554). This dataset, totalling 64 taxa, included all the genera of the African clade (11 genera) and presented a thorough sampling of the genera within the LBC. The dataset was composed of six plastid markers, three non-coding (*trnLF*; *trnSG* and *psbA-trnH*) and three coding (*ndhF*, *rbcL* and partial *matK*) totalling 7945 characters.

Second, in order to date the splits within genera, a species-level phylogeny of the two sister genera *Isolona* (15 out of c. 21 species) and *Monodora* (13 out of 14 species) was generated (dataset B, see Appendix D, end of thesis, treeBASE number SN3633). For both genera all the known East African species were included.

A modified cetyl trimethyl ammonium bromide (CTAB) protocol of Doyle and Doyle (1987) following Bakker et al. (1998) was used for DNA extraction. The universal primers C/D and E/F (Taberlet et al., 1991) were used to amplify and sequence the *trnL* intron and *trnL-trnF* spacer. The *psbA-trnH* intergenic spacer was amplified and sequenced using primers *psbA* and *trnH* (GUG) (Hamilton, 1999). The *trnS-trnG* intergenic spacer was amplified and sequenced using primers trnS (GCU) and trnG (UCC) (Hamilton, 1999) and the *trnD-trnT* marker was also amplified and sequenced using primers $trnT^{GGU}$ and $trnD^{GUC}$ (Shaw et al., 2005). In addition, only the last part of the *ndhF* gene was sequenced as it has been shown to be more variable than the more conserved 5' region thus more appropriate for species-level analyses (Erkens et al., 2007). The Annonaceae specific primer LBC-intF (Erkens et al., 2007) was used in combination with the usual 2110R primer (Olmstead and Sweere, 1994) amplifying a region of c. 620 bp. PCR reactions were performed with 30-50 ng of genomic DNA, 0.4% of BSA, 0.2 µM of each primer, 0.2 mM dNTP PCR mix (Promega, Madison, WI), 3 µM $MgCl_2$, 1X PCR buffer (Promega, Madison, WI), and 0.5 U of *Taq* DNA polymerase (Promega, Madison, WI) in a total volume of 50 µl. The PCR program was as follows: 35 thermal cycles at 94°C for 1 min, 50-55 °C for 50 s, 72°C for 50s and a final extension at 72°C for 3 min.

For both datasets, sequences were edited using the program Staden (http://staden.sourceforge.net/) and aligned manually. Gaps were coded following the simple coding model of Simmons and Ochoterena (2000). Microsatellites were excluded from the analysis, as these structures probably originate through slipped-strand mispairing (Levinson and Gutman, 1987) and are likely highly homoplasious.

Phylogenetic Reconstruction and Divergence Time Estimates

All analyses were performed in a Bayesian framework using the software BEAST ver. 1.4.5 (Drummond et al., 2006; Drummond and Rambaut, 2007). This method simultaneously estimates divergence times, tree topology and rates, providing a clear advantage over previous relaxed clock methods (Ho et al., 2005) that estimate tree topology and divergence dates separately (e.g. Sanderson, 1997; Thorne et al., 1998; Sanderson, 2002). Both datasets were partitioned into the number of markers used by directly editing of the XLM file and following Couvreur (http://tlpcouvreur.googlepages.com/beastpartitioning). The best performing evolutionary model for each marker was identified under two different model selection criteria, the hierarchical likelihood ratio test (hLRT) and the Akaike information criterion (AIC; Akaike, 1973) as implemented in MrModelTest (Nylander, 2004). For both datasets two independent analyses were undertaken to check for convergence of the MCMC chains. In order to assess that the MCMC chain reached stationarity we examined the lnL plots using Tracer v. 1.3 (Rambaut and Drummond, 2003). In particular, we searched for evidence that the model likelihood and parameter estimates had reached stationarity after a burn-in period.

Since informative Annonaceae fossils are quite scarce past calibrations within the family have largely relied on the fossil taxon *Archaeanthus* (98 Myr, Dilcher and Crane, 1984) that provides a minimum age for the stem node of the Magnoliaceae (Doyle et al., 2004; Richardson et al., 2004; Pirie et al., 2006). Using this age as well as a wider taxon sampling within Annonaceae (80 genera out of c. 110, 205 taxa) and *rbcL* and *trnLF* sequences, Pirie (pers. com.) provided an age estimate of 90.93 Myr for the stem of Annonaceae when using the Penalized Likelihood (PL) method of Sanderson (2002). This estimated date is in perfect accordance with previously published date estimates for the origin of Annonaceae (Richardson et al., 2004). Thus 90.93 Myr was used as a calibration point for dataset A. Secondary calibration (calibrating a node with a date provided by a previous analysis) is a commonly used alternative given the absence of a method for direct calibration (e.g. fossil or geological, Berry et al., 2004; Renner, 2005; Zhou et al., 2006). However, it has been shown that, unless particular care is taken, secondary calibration can generate internal inconsistencies leading to unreliable dates (Shaul and Graur, 2002). BEAST accommodates for calibration uncertainty by applying a prior probability distribution on the age, e.g. a prior distribution defined in terms of it's mean and standard deviation (Drummond et al., 2006). A wide variety of probability distributions are available. For example, it is suggested that for direct fossil calibrations (which provides a minimum age for a split) a lognormal prior distribution should be used with the probability of the nodal age decreasing with time (Ho,

2007). For this study, however, a normal probability distribution was used as it is thought to better reflect uncertainty related to secondary calibration points (Ho, 2007). For dataset A a mean of 90.93 and a standard deviation of 1 were specified. This effectively encloses a range of possible ages from c. 89 to 93 Myr. In dataset B, a normal distribution was also used for the age of the stem node of *Isolona* and *Monodora*, with the mean taken from the analysis of dataset A. The standard deviation was set to contain the lower and higher boundaries of the 95% highest posterior density values (Fig. 4.2) effectively accommodating for age uncertainty. Both sequence datasets deviated from a strict molecular clock and rates between adjacent branches were uncorrelated as shown by the values of the parameters "coefficient of variation" and "covariance" respectively (Peng et al., 2006). Thus divergence times were estimated under a lognormal non-correlated relaxed clock method and using the Yule model of speciation as implemented in BEAST 1.4.5. In both cases user-specified chronogram trees were used as starting trees and were obtained using the r8s program (Sanderson, 2003). Finally, taxon subsets were specified for each clade of interest that permitted the recording of the mean time of the most recent common ancestor (t_{MRCA}), the 95% highest posterior density intervals (HPD) and the effective sampling size (ESS). Analyses were undertaken by sampling every 1000th generation, and were considered complete once the ESSs of each parameter were above 200 as suggested in the BEAST manual (see results for the total number of generations).

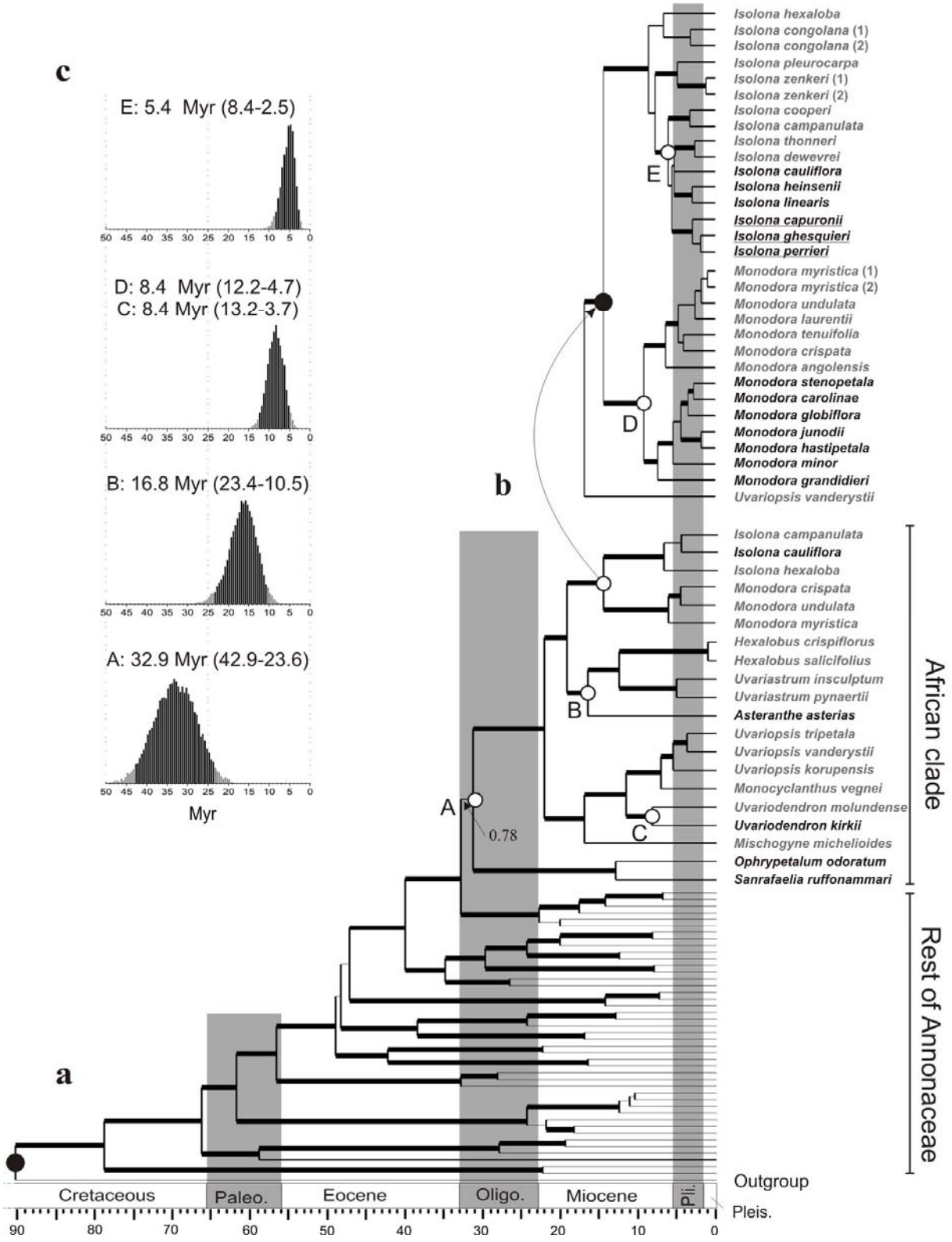
Lineage Through Time Plots

In order to provide an indication of diversification rates in East Africa, we generated a log-lineage through time plot (l-LTT) for the East African *Monodora* species clade. This clade is fully sampled and well supported (see results), which is an ideal situation for the generation of l-LTT plots (Nee et al., 1994; Ricklefs, 2007). The mean age maximum clade credibility tree resulting from the analysis in BEAST of dataset B was imported into the APE package (Paradis et al., 2004) using the R statistical environment. We pruned the tree down to having just the East African *Monodora* clade (7 tips) using the 'drop.tip' function. This pruned ultrametric tree was then used to produce the l-LTT plots with the package LASER (Rabosky, 2006).

RESULTS

Phylogenetic Analyses

For dataset A, a total of 30 million generations were needed in order to reach appropriate ESS levels (<200). For dataset B, 5 million generations were sufficient. The chronograms and phylogenetic relationships resulting from both analyses are presented in Figure 4.2 a-b. At the generic level (dataset A) the resulting phylogenetic relationships as well as corresponding branch support were identical to those found in Couvreur et al. (in press,


c
E: 5.4 Myr (8.4-2.5)
D: 8.4 Myr (12.2-4.7)
C: 8.4 Myr (13.2-3.7)
B: 16.8 Myr (23.4-10.5)
A: 32.9 Myr (42.9-23.6)
50 45 40 35 30 25 20 15 10 5 0
Myr
b
a
A
0.78
B
C
D
E
Isolona hexaloba
Isolona congolana (1)
Isolona congolana (2)
Isolona pleurocarpa
Isolona zenkeri (1)
Isolona zenkeri (2)
Isolona cooperi
Isolona campanulata
Isolona thonneri
Isolona dewevrei
Isolona cauliflora
Isolona heinsenii
Isolona linearis
Isolona capuronii
Isolona ghesquieri
Isolona perrieri
Monodora myristica (1)
Monodora myristica (2)
Monodora undulata
Monodora laurentii
Monodora tenuifolia
Monodora crispata
Monodora angolensis
Monodora stenopetala
Monodora carolinae
Monodora globiflora
Monodora junodii
Monodora hastipetala
Monodora minor
Monodora grandidieri
Uvariopsis vanderystii
Isolona campanulata
Isolona cauliflora
Isolona hexaloba
Monodora crispata
Monodora undulata
Monodora myristica
Hexalobus crispiflorus
Hexalobus salicifolius
Uvariastrum insculptum
Uvariastrum pynaertii
Asteranthe asterias
Uvariopsis tripetala
Uvariopsis vanderystii
Uvariopsis korupensis
Monocyclanthus vegnei
Uvariodendron molundense
Uvariodendron kirkii
Mischogyne michelioides
Ophrypetalum odoratum
Sanrafaelia ruffonammari
African clade
Rest of Annonaceae
Outgroup
Pleis.
Cretaceous
Paleo.
Eocene
Oligo.
Miocene
Plii.
90 80 70 60 50 40 30 20 10 0

<-- Figure 4.2. Maximum clade credibility chronogram, with nodes represented by their mean ages estimated under a relaxed lognormal non-correlated molecular clock assumption. East African endemic taxa are indicated in black, West and Central African taxa in grey, taxa endemic to Madagascar in black and underlined. Solid circles indicate nodes used for calibration of the trees. Open circles indicate nodes for which divergence dates were estimated. Thick branches represent nodes with > 0.95 posterior probability support. Geological time period abbreviations: Paleo.: Paleocene; Oligo.: Oligocene; Pli.: Pliocene; Pleis.: Pleistocene.
a, genus-level chronogram showing phylogenetic relationships within the African clade.
b, species-level chronogram of the two sister genera *Isolona* and *Monodora.*
c, posterior distributions of the estimated ages. The 95% highest posterior density intervals are indicated with black bars and given between brackets after the mean. These distributions were used to significantly accept or reject congruence of split ages. The posterior distribution for node C is not represented but is equivalent to that of node D.

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but see Chapter 3 of this thesis, section "results" for a detailed description of the phylogenetic relationships). The species-level phylogeny (dataset B) of the two sister genera *Isolona* and *Monodora* is represented in Figure 4.2b (see also Chapter 6, Introduction). *Monodora* has two well-supported clades (PP=1.00): a West African clade, and an East African clade. Relationships within the West African clade are weakly supported except for the sister position of *M. angolensis* (PP=1.00). In the East African clade, *M. grandidieri* is strongly supported as sister to the rest of the taxa. Moreover, *M. globiflora* with *M. carolinae* and *M. stenopetala*, as well as *M. hastipetala* with *M. junodii* form well-supported subclades (PP=1.00). Within *Isolona*, the Malagasy species form a highly supported clade nested within the genus. The East African taxa are also strongly supported as nested, however, their relationships with the other clades remains unresolved.

Divergence Times

Both datasets deviated from the strict molecular clock model as indicated by the Coefficient of variation of rates (value not abutting against zero) (Table 4.1). Moreover, rates between adjacent branches were uncorrelated as shown by the rate of covariance which was centered on zero (Table 4.1). These values indicated that a lognormal non-correlated relaxed clock method best fits the data (Peng et al., 2006; Drummond et al., 2007).

Five different origins of endemic East African lineages were identified (Fig. 4.2a, b; nodes A-E). Node A represents the origin of the *Sanrafaelia*/*Ophrypetalum* clade with a mean age of 32.9 Myr (95% highest posterior distributions (HPD): 42.9-23.6 Myr) and node B that of *Asteranthe* with a mean age of 16.8 (HPD: 23.4-10.5). Node C indicates the origin of the East African endemic species *Uvariodendron kirkii*, with a mean of 8.4 Myr (HPD: 13.2-3.7). Two further nodes are indicated in Figure 4.2b with the first one corresponding to the origin of the East African *Monodora*'s (node D), with a mean of 8.4 Myr (HPD: 12.2-4.7). Because of the lack of resolution in distinguishing the origin of the East African species of *Isolona* (Fig. 4.2b), only a minimum age for this potential split can be provided corresponding to node E with a mean of 5.4 Myr (HPD: 8.4-2.5).

	Coefficient of variation	Covariance
mean dataset A		
(30 million generations, sampled every 1000th)	**0.525**	**7.33E-02**
95% HPD lower	0.45	-0.101
95% HPD upper	0.602	0.238
mean dataset B		
(5 million generations, sampled every 1000th)	**0.753**	**-4.52E-02**
95% HPD lower	0.521	-0.254
95% HPD upper	1.012	0.186

Table 4.1: Mean and 95% of the highest posterior distributions (HPD) of the Coefficient of variation and Covariance parameters for dataset A (genus-level phylogeny) and B (species-level phylogeny).

As judged by the 95% HPD's of each age estimate (Fig. 4.2c) these nodes are dated to three significantly different periods of time as the 95% HPD's are not overlapping. For node C, D, and E the 95% HPD's are largely overlapping, indicating no significant differences in their estimations. Finally, the origin of the Malagasy *Isolona*'s was dated to 2.7 Myr (HPD: 0.8-5).

Lineage Through Time Plot

Under a stochastic constant net diversification rate (speciation minus extinction) process an l-LTT plot should be linear. The one for the East African *Monodora* species (Fig. 4.3) is not linear, but instead shows a steady increase in speciation from the crown node (6.8 Myr) until a certain deflection point at around 4 Myr. After that point a clear decrease in diversification rate occurred.

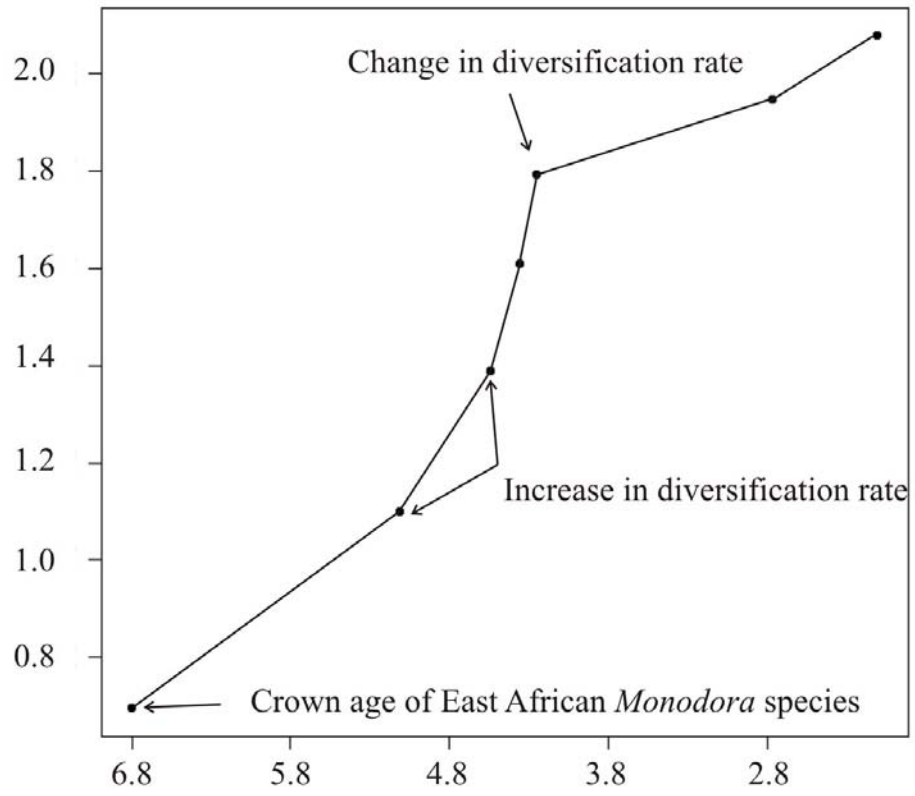

Figure 4.3. Log lineage-through-time plot for the East African *Monodora* clade. The Y-axis is the natural logarithm of the cumulative number of lineages extant at any point in time. The X-axis represents the absolute age in millions of years (Myr) before present.

DISCUSSION

Vicariance Driven Speciation

The resulting chronograms in Figure 4.2a and b are congruent with the model presented in Figure 4.1c, indicating that the endemic East African lineages within the African Annonaceae clade are not monophyletic and thus have multiple origins. These origins are dated to three significantly different time periods spanning the Oligocene and Miocene (Fig. 4.2c). As mentioned above, a large majority of the species found within this clade are restricted to rain forests and present no long-range dispersal potential. Thus the observed recurrent vicariance events are likely the result of a series of connection-isolation events between the East African and Guineo-Congolian forests. This enabled the alternating exchange between and isolation of populations. There is substantial fossil evidence for the existence of pan-African rain forests at different periods in time during the Neogene (Axelrod and Raven, 1978; Williamson, 1985; Jacobs and Kabuye, 1987; Maley, 1996; Morley, 2000). Even if the precise history of vegetation change in East Africa is complex (Jacobs et al., 1999), the estimated dates coincide well with known periods of renewed aridity and/or continental uplift in East Africa generally thought to have played a role in the breaking up of the pan-African rain forest. During the Paleocene-Eocene periods (65-34 Myr) the climate

was warm (Zachos et al., 2001) and rain forest extended across Africa (Fig. 4.3a; Axelrod and Raven, 1978; Coetzee, 1993; Morley, 2000). Moreover, Africa was isolated from other continental landmasses allowing for the existence of a circular oceanic current which brought significant humidity to the continent (Fig. 4.4a).

The first split observed in the African clade is dated to the early Oligocene (mean: 32.9; Fig. 4.2a, Node A), which coincides with a drastic global cooling period (the 'big chill' or terminal Eocene cooling event) involving the development of permanent continental ice-sheets in Antarctica (Coetzee, 1993; Zachos et al., 2001). This cooling induced a drier climate at equatorial latitudes in Africa leading to numerous extinctions, for example in the palm genus *Nypa* (Morley, 2000). These drier and cooler climates were also responsible for the retraction and fragmentation of the Eocene pan-African rain forest (Coetzee, 1993; Morley, 2000) leading to the observed split. After this cooling, global temperatures rose again (Zachos et al., 2001) and by the end of the Oligocene until the Mid Miocene climatic optimum (17-15 Myr) rain forest again extended from coast to coast (Fig. 4.4b; Axelrod and Raven, 1978; Morley, 2000). After that, global temperatures started to drop and ice sheets developed again in Antarctica (Zachos et al., 2001). Additionally, an important geological event for Africa was the closure of the Tethys sea caused by the collision of the African plate into the Eurasian one at around 18-17 Myr. This brought an end to the moist influence of the latitudinal oceanic circulation system (Axelrod and Raven, 1978). The renewal of colder climates coupled with drier conditions in Africa induced a new period of aridity resulting in the spread of savannas at the expense of rain forests (Axelrod and Raven, 1978) and coincides well with the second dated split (Fig. 4.2a, Node B; mean 16.5 Myr). The third significant split (mean: 8.4 Myr) occurred in two lineages, *Uvariodendron* (Node C) and *Monodora* (Node D), and took place shortly after the initiation of geological activity in the western East African Rift System which uplifted the central Tanganyikan plateau (c. 10 Myr) (Lovett, 1993a; Chorowicz, 2005; Sepulchre et al., 2006). Such uplifting has been shown to play a significant role in the aridification of eastern Africa (Sepulchre et al., 2006). In addition, this estimated date also coincides with a period of large savanna extension in East Africa as judged by an increase in biomass of plants using C4 photosynthesis pathways (8-6 Myr) (Cerling et al., 1997). Thus the reported dates provide evidence that the East African rain forests have been marked by cycles of connections with the Guineo-Congolian forest during relatively moist periods followed by isolation linked to aridification.

Forest fragmentation is generally postulated to be a driving force behind plant speciation in Africa and is mainly invoked at more regional and recent scales as being the result of Quaternary climatic fluctuations (c. 2.5 Myr) (Coetzee, 1993; Sosef, 1994; Fjeldsa and Lovett, 1997; Morley, 2000; Leal, 2004). However, it has been documented that for the last c. 2.5 Myr the East African coastal and montane rain forests have been ecologically stable with little variation in forest distribution (Wasser and Lovett, 1993). The main mechanism promoting this stability is attributed to the continued supply of moisture derived from the Indian Ocean even during the climatic fluctuations of the Quaternary (Marchant et al., 2007). Moreover, our results indicate that the East African species of *Isolona* and *Monodora* all have

a pre-Pleistocene origin. Additionally, the l-LTT plot for the East African *Monodora* clade (Fig. 4.3) shows that most of the diversification occurred before c. 4 Myr after which there was an obvious decrease in diversification rates. Such a decline can be explained either by an increase in extinction or a decrease in speciation. Whatever the reasons, the decline in diversification rate indicates that the climatic fluctuations during the Pleistocene have played no role in the generation of the East African *Monodora* diversity.

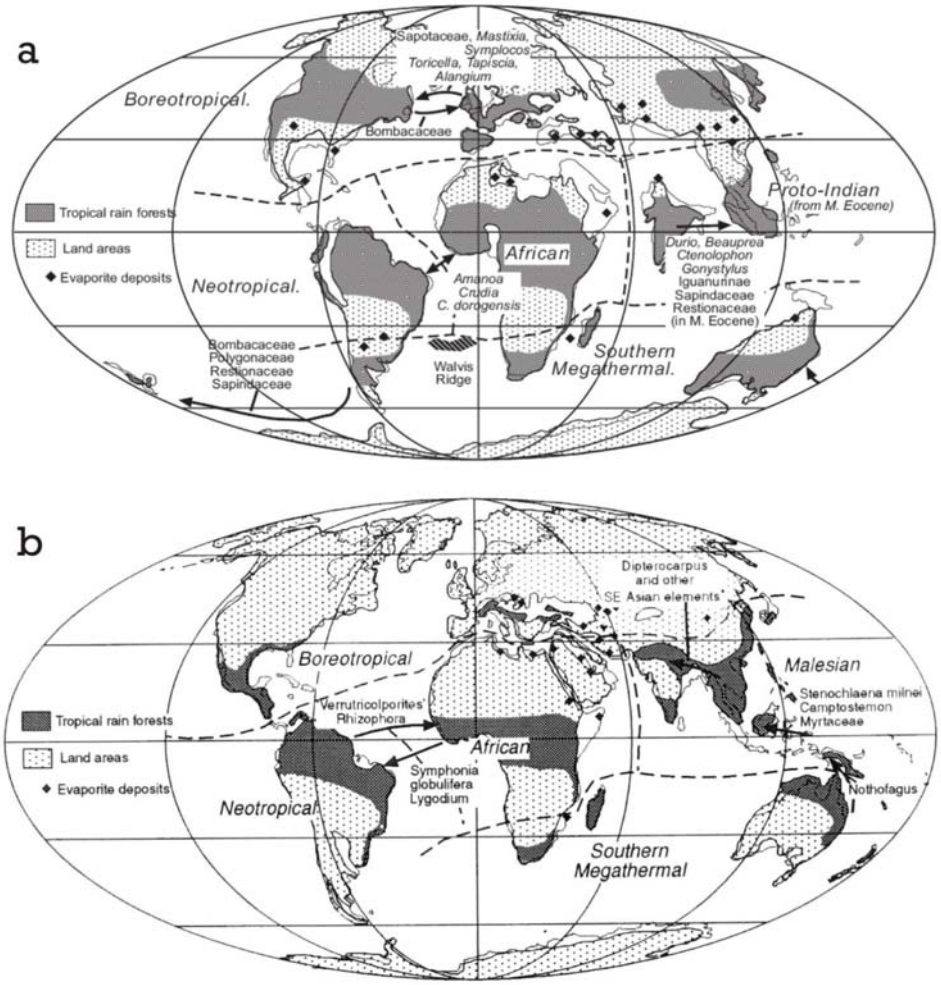

Figure 4.4. Global distribution of closed canopy tropical rain forest and geological positions of land masses. a) during the Late Paleocene/Early Eocene thermal maximum (c. 50-55 Myr). Note the isolated position of Africa from any other major land masses. b) during the Mid Miocene climatic optimum (15-17 Myr). Note the collision of the Africa plate with the Asian one. Maps reproduced with permission from "Origin and Evolution of Tropical Rain Forests", R.J. Morley, 2000. John Wiley & Sons Limited ©.

Given that woody elements in the East African endemic flora share a distribution pattern similar to that of taxa within the African Annonaceae clade (Brenan, 1978; Wasser and Lovett, 1993; Burgess and Clarke, 2000), we argue that the mode of speciation described here explains most of the endemicity found in the East African rain forest tree flora today. Our data, therefore, provide strong evidence that it is a larger continental-scale fragmentation between, rather than within, West/Central and East African forests that has played a major

role in generating plant endemicity in the East African rain forest flora. It may be that other, possibly herbaceous, groups speciated in East Africa as a result of Pleistocene climatic change but this is certainly not the case for Annonaceae and is likely also true for other woody representatives of the rain forest flora. These results provide for the first time an alternative explanation to the rapid and recent diversification scenario documented for endemic plants in other hotspots (Richardson et al., 2001a; Richardson et al., 2001b; Klak et al., 2004; Hughes and Eastwood, 2006).

Conservation of East African Taxa

Most East African forest lineages are species-poor (Burgess and Clarke, 2000). Our results further suggest that these rain forests contain large amounts of pre-Pleistocene derived lineages having evolutionary histories that date back to the Early Oligocene. A large portion of these endemic species have small distributional ranges (Burgess et al., 1998) and have been red-listed with some level of threat to their survival (an estimated 22-25% of the total number of endemic species). Thus, in addition to their ancient origins and species-poor composition, these lineages are highly vulnerable to extinction. For example, the monotypic genus *Sanrafaelia* is restricted to the lowland rain forests of a single mountain, the East Usambaras (Couvreur et al., 2006), and has a conservation status of "critically endangered" (Gereau and Luke, 2003). The extinction of such a lineage would lead to the loss of ancient evolutionary history including unique biological characters (Mace et al., 2003). In terms of phylogenetic diversity, if just the two species *Ophrypetalum odoratum* and *Sanrafaelia ruffomannari* (see Fig. 4.2a) were to go extinct we would have to wait c. 10 000 years[1] with no plant extinctions in the coastal and montane rain forests of East Africa just to rebuild this lost diversity. This situation can be contrasted to the "charismatic" hotspots such as the Cape Floristic Province (Myers et al., 2000), where relatively few lineages account for most of the species richness and endemicity (Linder, 2003) most of them having undergone recent explosive bursts of diversification (Richardson et al., 2001b; reviewed in Linder, 2003).

Isolation of East African Endemic Lineages

Finally, our results also suggest that once the East African lineages were isolated they did not migrate back into the Guineo-Congolian region during the existence of pan-African rain forests. If the isolated East African lineages would have successfully migrated back into the Guineo-Congolian region we would expect, for each significant splitting event, lineages containing reciprocal monophyly of East and West-Central endemic taxa (Figure 4.5a). This is clearly not the case, because each East African lineage, once isolated apparently never produced additional extant West-Central African lineages even though their were multiple opportunities (Fig. 4.5b). For example, the *Ophrypetalum*/*Sanrafaelia* lineage (Fig. 4.2a) has

[1] Total number of plant species in the East African rain forests (c. 3500) / age of the clade (c. 33 Myr).

been isolated for 33 million years. During that time at least two other occasions arose when they could have migrated back into the Guineo-Congolian region. Given that no extant taxa have originated from this lineage, it would seem that it failed to do so. Why these 'back migrations' did not occur is hard to explain without further investigation. It is possible that East African lineages did migrate back, but were driven to extinction under ecological pressures (e.g. competitive exclusion, no available niches). Adaptation to the slightly more xeric environments found in East Africa could also have impeded there migration back to the higher rainfall regimes of West-Central Africa. Whatever the reasons, this would indicate that the continental scale vicariance events shown here drove speciation and endemism in East Africa, but contributed little in generating endemism in the Guineo-Congolian rain forests.

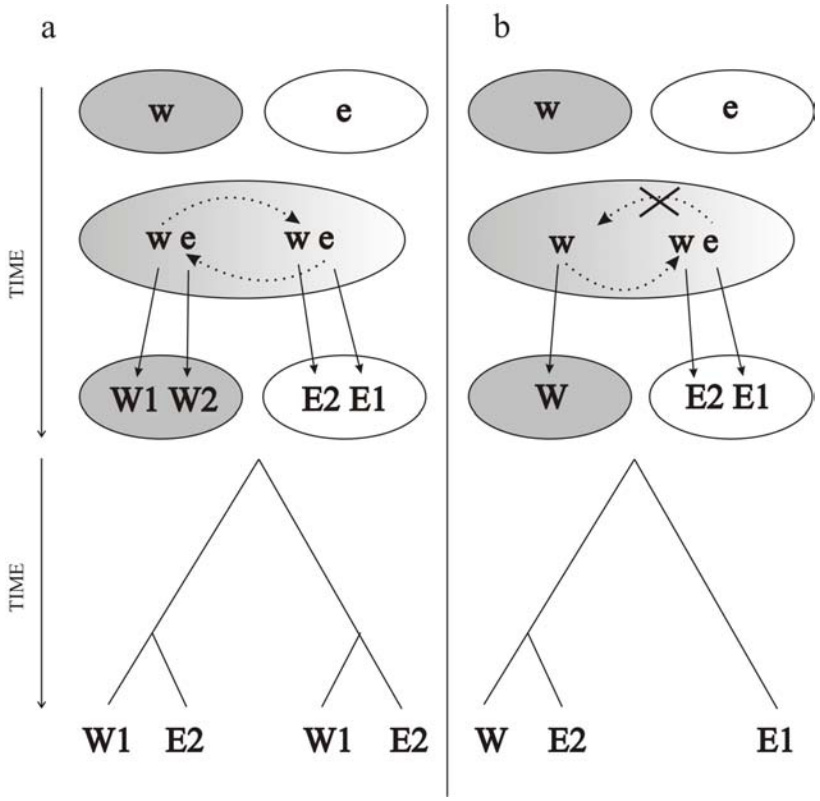

Figure 4.5: Hypothesised phylogenetic patterns resulting from a) bi-directional migration between the West-Central Guineo-Congolian forest and the Eastern rain forests, and b) uni-directional migration from the Guineo-Congolian forest into the Eastern rain forests. In grey: West-Central Guineo-Congolian rain forest block. In white: Eastern African rain forest block. In grey and white: pan-African rain forest connecting both regions. Dashed arrows: migration. Small letters: ancestral areas; w: west lineages; e: east lineages. Capitals: present distribution.

Origin of the Malagasy Isolona*'s*

The presence of *Isolona* in Madagascar can be considered a bit puzzling for several reasons. First, East African *Isolona* species are restricted to Tanzania and southern Kenya c. 1200 km from the nearest Malagasy coast. Other genera within the African clade such as *Monodora* or *Uvariodendron* are also found along the Mozambique shore just 400 km from the Malagasy one but are nevertheless absent from Madagascar. Second, as noted above,

Isolona has a virtually no long-range dispersal capacity given the nature of its syncarpous fruits. The Malagasy species of *Isolona* form a strongly supported clade nested within the rest of the genus, implying a single dispersal event from Africa. Single dispersal events from mainland Africa to Madagascar have been documented in other tropical African lineages such as *Acridocarpus* (Davis et al., 2002) or *Begonia* (Plana et al., 2004). Unfortunately, phylogenetic relationships of this clade with the other clades in *Isolona* are not resolved so we cannot state whether the Malagasy clade is sister to the East African clade or a West Africa clade. The origin of this Malagasy clade was estimated to be about 2.7 Myr (HPH: 0.8-5). This date excludes an origin via vicariance as Madagascar started its separation from mainland Africa during the middle Jurassic attaining its present position at around 120-130 Myr (Rabinowitz and Woods, 2006). In the absence of the existence of land bridges (Rabinowitz and Woods, 2006), especially recent ones, long-range dispersal between East Africa and Madagascar would appear to be the only viable explanation. None of the remaining genera within the African clade are found in Madagascar, thus successful dispersal from Africa to Madagascar happened just once in c. 33 Myr of evolution. In conclusion, dispersal across the Mozambique Channel, although possible, is a very rare event in the biogeographic history of this clade.

CONCLUSION

The evolutionary origin of the East African rain forest tree flora suggested here is in marked contrast to the recent and rapid speciation previously documented for endemic plants in other hotspots (Richardson et al., 2001a; Richardson et al., 2001b; Klak et al., 2004; Hughes and Eastwood, 2006). Threatened hyper-rich biota are often grouped under the same concept (hotspots), but have evidently not originated through similar evolutionary processes. Our results suggest that the rain forests of East Africa may contain numerous Oligocene-Miocene derived lineages. Unfortunately, a large majority of them are species poor (Lind and Morrison, 1974; Burgess and Clarke, 2000) making these evolutionary old lineages much more vulnerable to extinction. These differences should be carefully and urgently taken into consideration during future conservation planning (Purvis and Hector, 2000; Sechrest et al., 2002; Mace et al., 2003).

ACKNOWLEDGEMENTS: We thank J. J. Wieringa, B. F. Jacobs, J.C. Lovett, Q. Luke, D. J. Harris, E. Haston and R. T. Pennington for useful comments and discussions. We also acknowledge A. Robertson, D. Johnson and the Missouri Botanical Garden for providing plant DNA material. We are grateful to F. M. Mbago, R. E. Gereau, L. Ngok Balak, Y. Issembe, R. Niangadouma and M. Botermans for their excellent help and assistance in the field. The governmental authorities of Gabon and Tanzania (COSTECH) as well as national park directors are thanked for granting collection permits. Funding for fieldwork came from the Netherlands Organization for Scientific Research (N.W.O.), National Geographic Society, Alberta Mennega Stichting, Hugo de Vries Fonds and Air France-KLM.

5 Pollen Morphology of a Monophyletic Clade of Five African Annonaceae Genera ♣

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Abstract. Pollen morphology has played a major role in elucidating infrafamiliar-level systematics and evolution within Annonaceae, especially within the African genera. The *Monodora* clade is composed of five genera, *Asteranthe*, *Hexalobus*, *Isolona*, *Monodora* and *Uvariastrum*, which are restricted to Africa and contain together c. 50 species. A molecular phylogeny of the family showed that the monophyly of the *Monodora* clade is strongly supported and that it is part of a larger clade of 11 African genera. In order to support classification a detailed survey was made of the pollen morphological variation within the *Monodora* clade, using scanning and transmission electron microsopy. For the two most species-rich genera, *Isolona* and *Monodora*, a molecular species-level phylogeny was used to asses the taxonomic usefulness of the pollen characters. The survey showed a wide range of pollen morphological diversity. The most conspicuous variation concerned the occurrence of monads without a thicker outer foliation in the basal exine layer in *Isolona* in contrast to tetrads with a thicker outer foliation in *Asteranthe*, *Hexalobus*, *Monodora* and *Uvariastrum*. At the infrageneric level, *Hexalobus*, *Isolona* and *Monodora* showed the largest diversity, with various pollen types based on tectum morphology. *Hexalobus* is exceptional with three types within only five species. The pollen types defined in this study are hardly useful in characterizing major groups identified within both *Isolona* and *Monodora*, but they do illustrate relationships within smaller groups.

♣ Submitted to Grana

INTRODUCTION

Annonaceae is a pantropical family of trees, shrubs, and lianas belonging to the order Magnoliales (APGII, 2003). With c. 130 genera and *c.* 2500 species (Chatrou et al., 2004) it is the most diverse family of the order, not only at the macromorphological level but also at the pollen morphological level (Sampson, 2000). African Annonaceae have been relatively understudied and many genera require updated revisions. However, the pollen morphology of the African genera (Le Thomas and Lugardon, 1976; Le Thomas, 1980; Le Thomas, 1981), as well as that of the rest of the family (Walker, 1971a; Walker, 1971b; Walker, 1972) received significant attention, which has played a considerable role in understanding the evolution of the family (Doyle and Le Thomas, 1994; Doyle and Le Thomas, 1997; Doyle et al., 2000).

The genus *Anaxagorea* is sister to the rest of the family on the basis of morphological, (Doyle and Le Thomas, 1994; Doyle and Le Thomas, 1996), molecular (Richardson et al., 2004) and combined data (Doyle et al., 2000; Doyle et al., 2004). *Anaxagorea* is characterized by monosulcate pollen with a granular infratectum. This ancestral pollen type gave rise to columellate monosulcate pollen (malmeoids, *Malmea*), disulcate pollen (miliusoids, *Miliusa*) and inaperturate pollen in tetrads (e.g. *Annona*, *Monodora*) and polyads (e.g. *Xylopia*), with a reversal to monads with a granular infratectum in the uvarioid clade (Doyle and Le Thomas, 1996; Doyle et al., 2000; Doyle, 2005). The genera with inaperturate pollen (Doyle and Le Thomas, 1996) represent a strongly supported monophyletic clade referred to as the "long branch clade" (LBC) by Richardson et al. (2004). The LBC showed more molecular divergence than the other well supported "short branch clade" (SBC) which is equivalent to the *Malmea-Piptostima-Miliusa* (MPM) clade of Doyle and Le Thomas (1996).

Genus	Number of species	Geographic distribution	Gynoecium type	Petals
Asteranthe	2	East Africa	apocarpic	fused
Hexalobus	5	West-Central/East Africa	apocarpic	fused
Isolona	20	West-Central/East Africa and Madagascar	syncarpic	fused
Monodora	14	West-Central/East Africa	syncarpic	fused
Uvariastrum	8	West-Central/East Africa	apocarpic	free

Table 5.1: Species richness, geographical distribution and morphological diversity of the *Monodora* clade genera.

Asteranthe Engl. & Diels, *Hexalobus* A.DC., *Isolona* Engl., *Monodora* Dunal and *Uvariastrum* Engl. are five tropical African genera of trees and shrubs that form a monophyletic clade within the LBC called the *Monodora* clade (Table 5.1). *Isolona* is also found in Madagascar while *Asteranthe* is restricted to East Africa (Kenya and Tanzania). Most of the species of these genera grow in lowland and montane rain forests although a few species are adapted to slightly more xeric conditions, especially those found in East Africa (e.g. *Asteranthe asterias*, *Hexalobus mossambicensis*, *Uvariastrum hexaloboides*).

Isolona and *Monodora* are unique in Annonaceae in having a syncarpous gynoecium (Deroin, 1997; Couvreur et al., in press), which is also rare within the Magnoliales (Endress, 1982). In the past, numerous morphological studies have indicated that *Asteranthe*, *Hexalobus, Isolona, Monodora* and *Uvariastrum* are closely related (floral morphology: van Heusden, 1992; fruit and seed morphology: van Setten and Koek-Noorman, 1992). Walker (1971b; 1972) was the first to propose an informal classification of the Annonaceae based on a large generic pollen survey using especially light microscopy (LM) characters. He placed the above five genera in the *Hexalobus* tribe characterized by tetragonal tetrads (except for *Isolona*, which has monads). This tribe also included seven other African genera (of which *Cleistochlamys* has monads) and the South American genus *Diclinanona*. Finally, in a cladistic analysis based on pollen and macromorphological characters, Doyle and Le Thomas (1994) recovered *Hexalobus* and *Uvariastrum* as sister to both *Isolona* and *Monodora* (*Asteranthe* was not included in the analysis). A recent molecular phylogeny based on six plastid markers including many previously unavailable African genera (Couvreur et al., in press, Chapter 3, Figs. 3.1 and 3.2) confirmed that *Asteranthe*, *Hexalobus, Isolona, Monodora* and *Uvariastrum* form a strongly supported monophyletic group, referred to as the *Monodora* clade, nested within the LBC. The *Monodora* clade grouped within a larger clade composed of eleven strictly African genera (*Dennettia* was sunken into *Uvariopsis*; Kenfack et al., 2003), referred to as the African long branch clade (ALBC, Couvreur et al., in press; Chapter 3), which includes all genera of Walker's *Hexalobus* tribe (except for *Cleistochlamys* and *Diclinanona*) and *Sanrafaelia* (described in 1996).

The close match between the taxa included in Walker's *Hexalobus* tribe (1971b) and the molecular phylogeny (Couvreur et al., in press) indicate the value of pollen characters at infra-familar-level classification (Doyle and Le Thomas, 1997). Here, we take a more in depth look at the *Monodora* clade to determine the value of these characters within the ALBC with reference to Figures 3.1 and 3.2 Additionally, and using a species level phylogeny of the two most species-rich genera in this study, *Isolona* and *Monodora* (Table 5.1), we also assess the usefulness of these characters for infra-generic classification.

MATERIAL AND METHODS

Sampling and Pollen Preparation

In total 78 samples (see Appendix 5.1, page 103) were analyzed representing 46 out of

the 49 species found within the *Monodora* clade. All species within *Asteranthe* (2), *Hexalobus* (5) and *Monodora* (14) were sampled, whereas 19 out of the 20 *Isolona* species and 6 out of the 8 *Uvariastrum* species were studied. Pollen samples were taken from herbarium or alcohol collections preserved at the following herbaria: BR, C, COI, EA, FHO, G, MO, P and WAG.

Annonaceae pollen is very fragile and the acetolysis method for pollen preparation (Erdtman, 1960) is often too drastic and breaks the pollen grains making observations difficult. Following Couvreur et al. (2006), we used an alternative method based on three consecutive baths of crushed mature stamens in n-hexane, an organic solvent. Material preserved in 70% alcohol was given an extra bath in 100% alcohol prior to the n-hexane baths. The samples were then gold-coated and examined using scanning electron microscope (SEM). When possible, the size of five pollen grains per sample was measured (Table 5.3). In addition, transmission electron microscopy (TEM) was carried out for a limited number of species within *Hexalobus*, *Isolona and Monodora* (Table 5.2). For a few specimens (marked with an asterisk in the Appendix 5.1) results were derived from SEM and TEM images (mostly unpublished) provided by Annick Le Thomas (Muséum National d'Histoire Naturelle, Paris). The delimitation of pollen types is based on ornamentation only, as this is the only informative character available for all specimens examined.

Maximum parsimony optimization of the pollen types was undertaken on the majority rule consensus tree from the Bayesian analysis (see Chapter 6; Fig. 6.14) using Mesquite v. 1.11 (Maddison and Maddison, 2006). The pollen type was treated as unordered.

Terminology

In Annonaceae, the delimitation of the various exine layers is still unclear (Gabarayeva, 1995; Doyle, 2005). The main problem comes from the presence of conspicuous foliations under the infratectum. Different interpretations have been proposed based on different criteria (Fig. 5.1). A first view defines the exine of Annonaceae pollen as lacking an endexinous part, thus consisting only of an ectexinous part composed of three layers: the tectum, the infratectum and a layer composed of conspicuous foliations termed the basal layer (Le Thomas and Lugardon, 1976; Le Thomas, 1980). These conclusions were based on the observation that the tectum, infratectum and basal layer have the same electron density (Le Thomas, 1980). Gabarayeva (1995) had a different view. She took an ontogenetic approach and defined the thick outer foliation of the basal layer as the foot layer (ectexinous) and the thinner inner foliations together as the endexine, the foot layer developing earlier than the endexine. For the sake of consistency we adopt the definition of Le Thomas (1980) in the present paper.

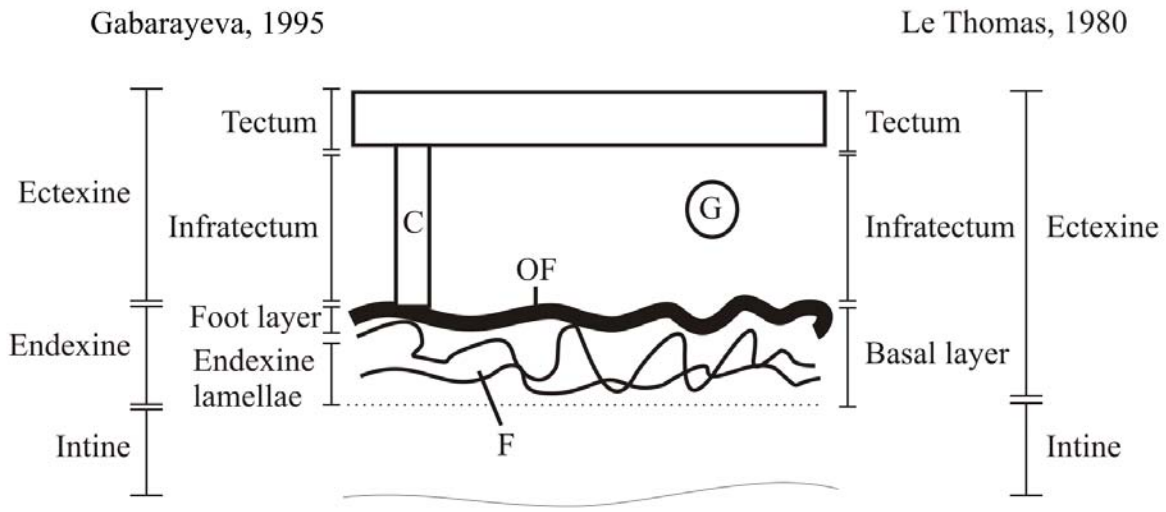

Figure 5.1: Alternative terminologies used for pollen wall structures in Annonaceae. C = columella, G = granule, OF = outer foliation, F = foliations, T = tectum.

Le Thomas (1980; 1981; 1983) used scanning electron microscopy (SEM) as well as transmission electronic microscopy (TEM) to study the variation of tectal and infratectal characters in African Annonaceae genera. She described three major types of infratectum: 1) granular, 2) columellate and 3) intermediate. The intermediate state includes a range of cases, all considered to be intergrading (Doyle, 2005): columellae composed of fused granules, columellae mixed with granules, and infratectum consisting of radially elongated, ellipsoidal elements.

Further terminology follows Punt et al. (2007).

RESULTS

Measurements and descriptions for each species are summarized in Table 5.2 (page 83) and Table 5.3 (page 88).

1. *Asteranthe* (Plate 1)

 # species studied: 2/2
SEM: Pollen in acalymmate tetragonal tetrads, 105–140 µm in diameter. Constituent monads inaperturate, P = 50–63 µm, E = 66–84 µm, P/E = 0.75–0.76. Ornamentation foveolate; foveolae 0.9–1.8 µm.
TEM (A. asterias): Exine 3.4 µm thick. Tectum 0.9 µm. Infratectum 1 µm, columellate. Basal layer consisting of 2–4 loose, undulate foliations; outer foliation thicker, 0.35 µm. Tectum, infratectum and thick outer foliation of basal layer absent between monads, the contact zones consisting of thin foliations only.
Species included: *A. asterias*, *A. lutea*.

Previous observations: LM: Walker (1972), Le Thomas (1974); SEM and TEM: Le Thomas (1974; 1980).

2. *Hexalobus* (Plates 2–4)

 # species studied: 5/5
SEM: Pollen in acalymmate, tetragonal tetrads, 56 (63.8) 79 µm in diameter. Constituent monads inaperturate, P = 28 (33.6) 42 µm, E = 38 (51.8) 61 µm, P/E = 0.54 (0.65) 0.74. Ornamentation granular to gemmate (type A), areolate-verrucate to/or rugulate (type B), or psilate with perforations (type C).
TEM: Exine 2.4–3.9 µm thick. Tectum 0.7–1.4 µm. Infratectum 1.5–1.9 µm, granular or columellate/granular. Basal layer consisting of 2–4 undulate foliations; outer foliation not to clearly thicker, 0.1–0.4 µm. Tectum, infratectum and thick outer foliation of basal layer absent between monads, the contact zones consisting of tightly packed thin foliations only.
Previous observations: LM: Walker (1971b; 1972), Le Thomas (1974); SEM and TEM: Le Thomas & Lugardon (1976), Le Thomas (1980).

Hexalobus type A (Plate 2)
SEM: Ornamentation granular to gemmate; granules/gemmae 0.4–1.5 µm in diameter.
TEM (*A. bussei*): Exine 2.1–4 µm thick. Tectum 0.7 µm, consisting of granules/gemmae, hardly or not distinguisable from the granular infratectum. Basal layer consisting of 2–4 undulate foliations; outer foliation not much thicker, 0.15 µm.
Species included: *H. bussei*, *H. mossambicensis*.

Hexalobus type B (Plate 3)
SEM: Ornamentation areolate-verrucate to/or rugulate; muri 0.6–1.9 µm wide.
TEM (*H. crispiflorus*): Exine 2.4–3.9 µm thick. Tectum 1.2 µm. Infratectum 1.5 µm, columellate/granular. Basal layer consisting of 2–3 undulate foliations; outer foliation not or slightly thicker, 0.1–0.3 µm.
Species included: *H. crispiflorus*, *H. salicifolius*.
Note: Both species included into the *Hexalobus* pollen type B exhibit a continuous ornamentation range from areolate-verrucate (Plate 3D, E, H) to rugulate (Plate 3B, F, I).

Hexalobus type C (Plate 4)
SEM: Ornamentation psilate; perforations 0.1–0.4 µm in diameter.
TEM: Exine 3.6 µm thick. Tectum 1.4 µm. Infratectum 1.9 µm, columellate/granular. Basal layer consisting of 2–3 undulate foliations; outer foliation hardly to clearly thicker, 0.1–0.4 µm.
Species included: *H. monopetalus*.

3. *Isolona* (Plates 5–7, 12)

 # species studied: 19/20
SEM: Pollen grains solitary, inaperturate, apolar, subspheroidal, L (length) = 32 (39.7) 46 µm, B (width) = 25 (33.0) 42 µm, L/B = 1.07 (1.20) 1.33 µm. Ornamentation scabrate to verrucate (type A), finely rugulate (type B) or more coarsely rugulate (type C).
TEM: Exine 1–2.3 µm thick, proximally reduced. Tectum 0.4–1.5 µm, sometimes hardly or not distinguishable as a separate layer. Infratectum granular to columellate/granular, 0.5–1.5 µm. Basal layer consisting of 2–3 undulate foliations; outer foliation not thicker, < 0.1 µm.
Previous observations: LM: Walker (1971b); SEM and TEM: Le Thomas & Lugardon (1976), Le Thomas (1980).

Isolona type A (Plates 5, 12A)
SEM: Ornamentation scabrate to verrucate; scabrae/verrucae 0.1–3 µm in diameter.
TEM (*I. humbertiana*, *I. thonneri*): Exine 1–1.4 µm thick. Tectum 0.4–1.5 µm, consisting of scabrae/verrucae, hardly or not distinguisable from the granular infratectum. Basal layer consisting of 2–3 foliations; outer foliation not thicker, < 0.1 µm. Foliations tightly packed at the distal side, but loose and undulate proximally.
Species included: *I. capuronii*, *I. deightonii*, *I dewevrei*, *I. heinsenii*, *I. humbertiana*, *I. madagascariensis*, *I. perrieri*, *I. pilosa*, *I. thonneri*.

Isolona type B (Plates 6, 12C)
SEM: Ornamentation finely rugulate; muri 0.2–0.8 µm wide.
TEM (*I. campanulata*, *I. hexaloba*): Exine 1–1.9 µm thick. Tectum 0.4 µm. Infratectum columellate/granular, 0.5–0.7 µm. Basal layer consisting of 2–3 moderately undulate foliations; outer foliation not thicker, < 0.1 µm; proximal foliations loose.
Species included: *I. campanulata*, *I. cooperi*, *I. hexaloba*, *I. pleurocarpa*, *I. zenkeri*.

Isolona type C (Plates 7, 12B, D)
SEM: Ornamentation rugulate; muri 0.6–1.5 µm wide.
TEM (*I. congolana*, *I. ghesquierei*): Exine 2–2.3 µm thick. Tectum 0.4–0.8 µm. Infratectum granular to columellate/granular, 0.9–1.5 µm. Basal layer consisting of 2–3 moderately undulate foliations; outer foliation not thicker, < 0.1 µm. In *I. ghesquierei* the proximal exine consists of 2–3 loose foliations, often with the inclusion of granules (data proximal exine *I. congolana* not available).
Species included: *I. cauliflora*, *I. congolona*, *I. ghesquierei*, *I. lebrunii*, *I. linearis*.

4. *Monodora* (Plates 8–10)

 # species studied: 14/14
SEM: Pollen in acalymmate, tetragonal tetrads, 54 (77.8) 110 µm in diameter. Constituent

monads inapertuarate, P = 24 (40.4) 60 µm, E = 30 (41.9) 52 µm, P/E = 0.80 (0.96) 1.18. Ornamentation psilate with small perforations (type A1), rugulate/locally psilate with small perforations (type A2) or rugulate with relatively large perforations (type B).
TEM: Exine 3–3.6 µm thick. Tectum 0.6–1.6 µm. Infratectum 0.6–1.1 µm, columellate/granular. Basal layer consisting of 2–5 loose, undulate foliations; outer foliation thicker, 0.3–0.4 µm. Tectum, infratectum and thick outer foliation of basal layer absent between monads, the contact zones consisting of tightly packed thin foliations only.
Previous observations: LM: Walker (1971b), SEM and TEM: Le Thomas (1980; 1983).

Monodora type A1 (Plate 8)
SEM: Ornamentation psilate; perforations up to 0.4 µm in diameter.
TEM (*M. myristica*): Exine 3 µm thick. Tectum 1.6 µm. Infratectum 0.6 µm, columellate/granular. Basal layer consisting of 2–4 loose, undulate foliations; outer foliation thicker, 0.3 µm.
Species included: *M. laurentii*, *M. minor*, *M. myristica*, *M. stenopetala*, *M. tenuifolia*, *M. undulata.*

Monodora type A2 (Plate 9)
SEM: Ornamentation rugulate/locally psilate; perforations 0.1–0.5 µm in diameter.
TEM (*M. crispata*): Exine 3.2 µm thick. Tectum 0.8 µm. Infratectum 0.7 µm, columellate/granular. Basal layer consisting of 3–4 loose, undulate foliations; outer foliation thicker, 0.4 µm.
Species included: *M. carolinae*, *M. crispata*, *M. grandideri*, *M. zenkeri*.

Monodora type B (Plate 10)
SEM: Ornamentation rugulate; perforations 0.3–1 µm in diameter.
TEM (*M. angolensis*): Exine 3.6 µm thick. Tectum 0.6 µm. Infratectum 1.1 µm, columellate/granular. Basal layer consisting of 4–5 loose, undulate foliations; outer foliation thicker, 0.4 µm.
Species included: *M. angolensis*, *M. globiflora*, *M. hastipetala*, *M. junodii*.

5. *Uvariastrum* (Plate 11)

 # species studied: 6/8
SEM: Pollen in acalymmate, tetragonal tetrads, 52 (74.2) 107 µm in diameter. Constituent monads inaperturate, P = 22 (34.2) 47 µm; E = 33 (46.7) 64 µm, P/E = 0.67 (0.73) 0.79.
Ornamentation rugulate, sometimes locally psilate; perforations up to 0.5 µm.
TEM (*U. pierreanum*, *U. pynaertii*): Exine 3.5–5.6 µm thick. Tectum 1.1–1.8 µm. Infratectum 2–3.2 µm, columellate/granular. Basal layer consisting of 4–6 loose, undulate foliations; outer foliation thicker, 0.6–0.7 µm. Tectum, infratectum and thick outer foliation of basal layer absent between monads, the contact zones consisting of thin foliations only.

Species included: *U. germainii*, *U. hexaloboides*, *U. insculptum*, *U. pierreanum*, *U. pynaertii*, *U. zenkeri*.
Previous observations: LM: Walker (1971b), Le Thomas (1974); TEM and SEM: Le Thomas (1980; 1983).

genus	species	tectum thickness	columella length	columella width	granule size	outer foliation
Asteranthe	*asterias**	0.9	1.0	0.7	---	0.35
Hexalobus	*bussei*	0.7	---	---	0.5-1.3	0.15
	crispiflorus	1.2	1.5	1.1	0.1-0.3	0.1-0.3
	monopetalus	1.4	1.9	1.2	0.1-0.3	0.1-0.4
Isolona	*campanulata*	0.4	0.5	0.4	0.2-0.3	< 0.1
	congolana	0.6	0.9	0.6	0.4-0.8	---
	ghesquierei	0.4-0.8	---	---	1.3-1.5	---
	hexaloba	0.4	0.7	0.5	0.1-0.4	< 0.1
	humbertiana	0.5-1.5	---	---	0.4-0.9	< 0.1
	*thonneri**	0.4	---	---	0.1-0.4	< 0.1
Monodora	*angolensis*	0.6	1.1	0.8	0.5	0.4
	crispata	0.8	0.7	0.7	0.3-0.8	0.4
	*myristica**	1.7	0.6	0.7	0.15-0.35	0.3
Uvariastrum	*pierreanum**	1.1	2.0	1.3	0.2-0.7	0.7
	*pynaertii**	1.8	3.2	1.8	0.3-0.9	0.6

Table 5.2: TEM pollen data for species of *Asteranthe*, *Hexalobus*, *Isolona*, *Monodora* and *Uvariastrum* (*Monodora* clade). All measurements in μm. Asterisks indicate data obtained from the archive of Annick Le Thomas.

DISCUSSION

Intergeneric Variation

The almost complete species-level sampling of the 5 genera within the monophyletic *Monodora* clade showed a wide pollen morphological diversity. The most conspicuous variation concerns the exclusive occurrence of tetrads with a thickened outer foliation in the basal exine layer in *Asteranthe*, *Hexalobus*, *Monodora* and *Uvariastrum* in contrast to the monads without a thickened outer foliation in *Isolona*. Canright (1963) described the monads of *Isolona* as monosulcate, while Walker (1971c) characterized them as inaperturate. Le Thomas (1980) confirmed the latter view, stating that the pollen grains do not possess any distinct (distal) apertural structure, but instead show a clearly reduced proximal exine in cross-section (*I. hexaloba*, *I. thonneri*; TEM). We observed such a proximal thinning also in *I. campanulata*, *I. ghesquierei* and *I. humbertiana* (Plate 12). We did not find it in *I. congolana*, however, possibly because the plane of sectioning was not through the proximal face of the

pollen wall (Plate 12B). Le Thomas (1980, 1983) considered the inaperturate *Isolona* monads with their proximal exine thinning as transitional between (distally) aperturate monads and inaperturate tetrads with fused thin proximal exines. Le Thomas et al. (1986) interpreted the proximally reduced monads of *Isolona* as derived from a former tetrad stage. The phylogenetic analysis by Couvreur et al. (in press) clearly showed *Isolona* to be nested within the 'African long branch clade' (ALBC; Chapter 3, Figs. 3.1 and 3.2), which, except for *Isolona*, is characterized by tetrad pollen. This topology confirms that the *Isolona* monads indeed represent a derived state relative to the tetrads. Then, the reduced proximal exine of *Isolona* pollen is a relic of the thin proximal exine of an ancestral tetrad condition. The observation of Le Thomas (1980, p. 322, 340) that *I. thonneri* pollen has a prolonged developmental tetrad stage fits very well in this view: the longer the tetrad stage lasts, the less space/time there is for proximal exine growth. An explanation for the presence of tetrads and monads within the *Monodora* clade could come from the occurrence of different pollen vectors. Unfortunately, very little is known about the pollination biology within the *Monodora* clade, and more data is needed in order to adequately tackle these questions. A similar case of evolution from tetrads to monads, also unexplained, occurred in the Winteraceae, in the genus *Zygogynum* s.s. (van der Ham and van Heuven, 2002).

The locally reduced exine of *Isolona* pollen seems to be fundamentally different from that found in the miliusoid clade of the 'short branch clade' (SCB, Mols et al., 2004). Most genera in the miliusoid clade have monad pollen, in which an exine thinning, if present, probably has a distal position, and therefore would represent an apertural structure. The scarce tetrads in the miliusoid clade, present in *Mitrephora*, *Petalolophus* and *Pseuduvaria*, appeared to be derived (twice), being nested in monad subclades (Mols et al., 2004).

Further intergeneric variation pertains to the structure of the infratectum. Both basic angiosperm types of infratectal structure, columellate and granular (Le Thomas, 1980; Le Thomas, 1981), are represented within the *Monodora* clade. *Asteranthe* is the only genus characterized by a strictly columellate infratectum, while *Isolona* and *Hexalobus* contain species with a strictly granular infratectum. All other representatives of the clade possess an intermediate infratectum type, showing columellae mixed with granules. So, the latter type is the commonest within the *Monodora* clade. *H. monopetalus* was previously thought to have an exclusively columellate infratectum (Le Thomas and Lugardon, 1976; Le Thomas, 1980; Le Thomas, 1981), but granules are clearly present in the material studied by us (different from that used by Le Thomas). Thus, *H. monopetalus* is better defined as having an intermediate infratectum type, though with a dominance of columellae.

All genera of the *Monodora* clade share a basal layer consisting of foliations which is, however, also common to numerous other African genera with tetrad pollen (Le Thomas, 1980). Except for *Isolona*, all genera in the *Monodora* clade show a relatively thick outer foliation. Contrary to Doyle and Le Thomas (1994), *Hexalobus* also shows a thickened outer foliation, though less obviously so than the other genera.

The deviating pollen of *Isolona* within the *Monodora* clade implies that the monophyly of this clade as indicated by molecular evidence, cannot be demonstrated using a

pollen morphological criterium.

Infrageneric Variation

Isolona, *Hexalobus* and *Monodora* exhibit the largest amount of pollen morphological variation, especially with regard to ornamentation, each of these three genera being subdivided into several pollen types (Plates 2–10). *Hexalobus* is remarkable in that three types occur in five species only. The two species belonging to *Hexalobus* type B (*H. crispiflorus* and *H. salicifolius*; Plate 3) show large infraspecific variation of the ornamentation which ranges from areolate-verrucate to rugulate which is unique within the *Monodora* clade. Interestingly, *Uvariastrum,* the sister genus of *Hexalobus*, exhibits hardly any variation of the ornamentation (Plate 11). Why there is such a contrast between these two small genera is hard to explain. Both genera have similar distributions, mainly in the Guineo-Congolian region in West-Central Africa, with one or two species occurring in East Africa. Both have the same amount of morphological variation, e.g. *Hexalobus* does not present a strikingly larger amount of variation in its flowers than *Uvariastrum*. As with the presence of tetrads and monads within the *Monodora* clade, an explanation for the wide ornamentation range within *Hexalobus* might be the occurrence of different pollination syndromes. For instance, pollen ornamentation has in some cases been shown to be correlated with the type of pollen vectors (e.g. Osborn et al., 1991; Hesse, 2000; Tanaka et al., 2004). However, very little is known about pollinators within the *Monodora* clade.

Taxonomic Significance of Pollen Characters

Given that *Isolona* and *Monodora* are the two most species-rich genera (together 34 out of 49 species) within the *Monodora* clade, species level molecular phylogenies should provide a reasonable guideline in assessing the usefulness of pollen characters for infrageneric classification within this clade (Bayesian majority rule tree of both genera: see Chapter 6, Fig. 6.14). When the different pollen types, which are based on pollen ornamentation, are optimized on the trees using the maximum parsimony method (Fig. 5.2), there appears to be no taxonomic information for the deeper relationships within both genera, i.e. no major clade is characterized by a particular pollen type. The largest clade within *Isolona* contains all three pollen types. Within *Monodora,* both the West-Central and the East African clades contain representatives of each pollen (sub)type.

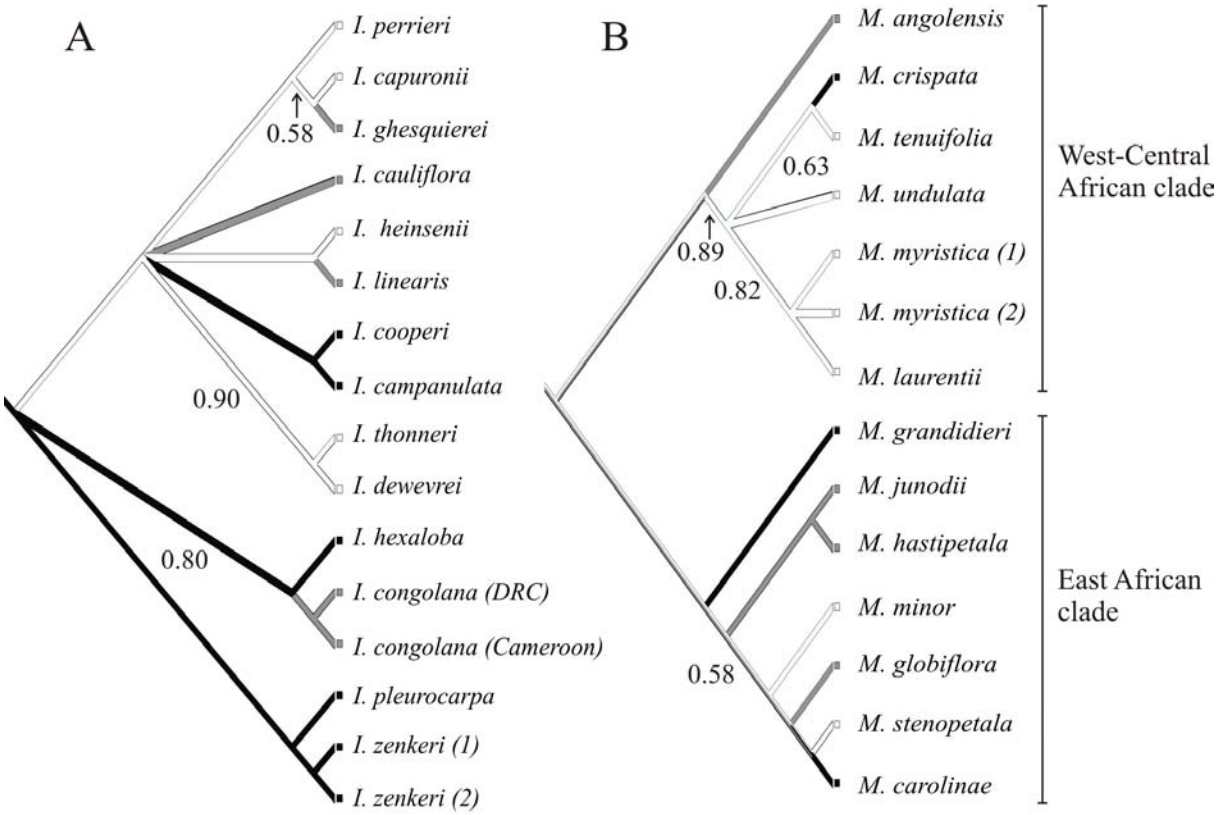

Figure 5.2. Maximum parsimony optimization of the various pollen types on the Bayesian majority rule consensus tree (see Chapter 6, Fig. 6.14). A. *Isolona*: white: type A; black: type B; gray: type C. B. *Monodora*: white: type A1; black: type A2; gray: type B. Posterior probabilities > 0.90 are indicated below the branches.

However, pollen characters appear more informative within smaller groups of species. Several groups of closely related species have similar pollen morphologies. For example, the West-Central clade within *Monodora*, excluding *M. angolensis*, contains species with quite dissimilar macromorphologies (see Chapter 6), except maybe for *M. myristica* and *M. undulata*, which are less disparate. Some species have a unique macromorphology (*M. tenuifolia*, *M. laurentii*) or resemble more distantly related species (*M. crispata* with *M. angolensis* or *M. grandidieri*). On the other hand, pollen morphology shows little variation within this group, all species belonging to pollen type A, and most of them to subtype A1), which is in agreement with the molecular data (Fig. 5.2). Species found in the two early diverging clades within *Isolona* are also united by the same pollen type (B) with the exception of *I. congolona* (type C).

Strongly supported sister species in general possess the same pollen type (Fig. 5.2), except for *I. heinsenii* and *I. linearis*, and *I. congoloana* and *I. hexaloba*. In the latter case, this difference might be explained by a shift in habitat, with *I. congolana* generally growing in montane forests above 900 m, while *I. hexaloba* is restricted to lowland rain forests below 700 m. Indeed, such a difference in habitat could imply a difference in pollinating vectors which could have led to these differences. In the former, the variation is harder to explain because both species are restricted to the montane forests of the Eastern Arc in Tanzania,

although hardly occurring in sympatry (see Chapter 6; Couvreur et al., 2006).

Pollen morphology has also been very useful to distinguish morphologically similar species. Verdcourt (1986) identified a small Eastern Arc Mountain population in Tanzania as being part of the West-Central African species *I. hexaloba*. Pollen morphology, however, provided support for the description of a new species (Couvreur et al., 2006), *I. linearis*, which is strongly supported by the molecular phylogeny, i.e. *I. linearis* does not cluster with *I. hexaloba*.

Thus, pollen characters at the infrageneric level would appear to have a mixed utility. It provides little information for characterizing major clades with genera, but does seem to contain information between closely related species. In addition they can be used to a certain extent to support taxonomic decisions.

The taxonomic significance of pollen characters within the other three genera is hard to assess without a molecular phylogeny. A case worth mentioning is that of *H. bussei* and *H. mossambicensis.* Both species show many morphological as well as ecological differences. The latter species is a shrub or a small tree distributed in the xeric southern part of East Africa, while the former is a large rain forest tree endemic to Cameroon. Furthermore, *H. bussei* has the largest flowers within the genus, while *H. mossambicensis* has the smallest flowers. Despite numerous differences, both species present strong pollen morphological affinities, having a granular to gemmate exine ornamentation (Plate 2). In view of the macromorphological differences as well as the large geographical separation, it is hard to suggest close relationship between both species. Clearly this case deserves further investigation.

ACKNOWLEDGEMENTS: Annick Le Thomas and Thierry Deroin are deeply thanked for allowing access and use of the pollen archives of Annick Le Thomas in P. Marc Sosef and James Richardson are also thanked for critically reading through earlier versions of the manuscript. We are grateful to Wim Star and Ben Kieft (NHN-Leiden) for preparing the TEM views and plates, respectively.

Genus species	pollen type	tetrad size	P monad	E monad	P/E monad	ornamentation	width of muri	size of gemmae, scabrae, verrucae	size of perforations, foveolae
Asteranthe									
asterias		105	50	66	0.76	- foveolate	---	---	1-1.8
lutea		140	63	84	0.75	- foveolate	---	---	0.9-1.7
Hexalobus									
bussei	H-A	79	42	61	0.69	- granular to gemmate	---	0.4-1.5	---
mossambicensis	H-A	56	28	52	0.54	- granular to gemmate	---	0.4-1.5	---
crispiflorus	H-B	64	31	55	0.56	- areolate-verrucate to/or rugulate	0.6-1.9	---	---
salicifolius	H-B	59	29	53	0.55	- areolate-verrucate to/or rugulate	1.0-1.9	---	---
monopetalus	H-C	61	28	38	0.74	- psilate, with perforations	---	---	0.1-0.4
Isolona									
capuronii	I-A	---	---	---	---	- scabrate, scabrae not fused, at same level	---	0.7-1.2	---
deightonii	I-A	---	32	25	1.28	- scabrate, scabrae not fused, superimposed	---	0.1-0.6	---
dewevrei	I-A	---	40	31	1.29	- scabrate, scabrae rarely fused, at same level	---	0.2-0.8	---
heinsenii	I-A	---	41	38	1.08	- scabrate, scabrae not fused, at same level	---	0.8-1.1	---
humbertiana	I-A	---	46	41	1.12	- verrucate, verrucae often fused, at same level	---	1-3	---
*madagascariensis**	I-A	---	33	26	1.27	- verrucate, verrucae often fused, at same level	---	0.6-2.1	---
perrieri	I-A	---	45	35	1.29	- scabrate, scabrae somet. fused, at same level	---	0.5-1	---
pilosa	I-A	---	37	33	1.12	- scabrate, scabrae rarely fused, superimposed	---	0.1-0.5	---
thonneri	I-A	---	35	29	1.21	- scabrate, scabrae often fused, somet. elongate	---	0.5-1	---

campanulata	I-B	---	41	33	1.24	- finely rugulate, perforations small	0.5-0.8	---	0.2.-0.3
cooperi	I-B	---	44	33	1.33	- finely rugulate, perforations small	0.3-0.5	---	0.2
hexaloba	I-B	---	45	39	1.15	- finely rugulate, perforations small	0.3-0.6	---	0.1-0.3
pleurocarpa	I-B	---	36	27	1.33	- finely rugulate, perforations small	0.3-0.5	---	0.2-0.3
zenkeri	I-B	---	45	42	1.07	- finely rugulate, perforations small	0.2-0.5	---	< 0.1
cauliflora	I-C	---	36	30	1.20	- rugulate, rarely with perforations	0.6-1.0	---	< 0.2
congolana	I-C	---	36	32	1.13	- rugulate, with perforations	0.6-1.2	---	0.4-0.7
ghesquierei	I-C	---	40	34	1.18	- rugulate, somet. with perforations	1.0-1.5	---	0.2-0.5
lebrunii	I-C	---	46	35	1.31	- rugulate, with perforations	0.8-1.5	---	0.2-0.4
linearis	I-C	---	37	31	1.19	- rugulate, with perforations	0.8-1.2	---	< 0.1
Monodora									
laurentii	M-A1	92	48	52	0.93	- psilate, perforations regular in size	---	---	0.2-0.4
minor	M-A1	72	41	45	0.92	- psilate, perforations regular in size	---	---	0.2-0.5
myristica	M-A1	82	42	48	0.87	- psilate, perforations regular in size	---	---	0.1-0.2
stenopetala	M-A1	54	27	31	0.87	- psilate, perforations regular in size	---	---	0.1-0.3
tenuifolia	M-A1	110	60	52	1.15	- psilate, perforations regular in size	---	---	0.1-0.3
undulata	M-A1	104	52	44	1.18	- psilate, perforations regular in size	---	---	< 0.1
carolinae	M-A2	71	45	41	1.10	- rugulate, locally psilate	0.5-3.0	---	0.2-0.4
crispata	M-A2	66	39	34	1.15	- rugulate, locally psilate	0.3-2.5	---	0.1-0.4
grandidieri	M-A2	70	33	38	0.87	- rugulate, locally psilate	1.0-3.0	---	0.1-0.2

zenkeri	M-A2	85	40	47	0.85	- rugulate, locally psilate	1.0-2.0	---	0.1-0.5
angolensis	M-B	63	31	33	0.94	- rugulate, perfor. bigger and denser than in type A	0.7-1.0	---	0.3-0.7
globiflora	M-B	80	44	48	0.92	- rugulate, perfor. bigger and denser than in type A	1.0-1.5	---	0.3-1
hastipetala	M-B	54	24	30	0.80	- rugulate, perfor. bigger and denser than in type A	0.5-0.7	---	0.3-0.5
junodii	M-B	86	40	44	0.91	- rugulate, perfor. bigger and denser than in type A	0.8-1.1	---	0.3-0.9
Uvariastrum									
germainii		52	22	33	0.67	- rugulate, rarely with perforations	0.8-1.0	---	0.1-0.2
hexaloboides		60	30	40	0.75	- rugulate, rarely with perforations	2.0-2.5	---	0.2-0.3
insculptum		81	35	47	0.74	- rugulate, with perforations	0.7-1.0	---	0.1-0.2
pierreanum		63	30	44	0.68	- rugulate, with perforations	2.1-3.0	---	0.2-0.5
pynaertii		107	47	64	0.73	- rugulate, without perforations	1.5-2.1	---	---
zenkeri		82	41	52	0.79	- rugulate, locally psilate, with perforations	1.5-2.0	---	0.1

Table 5.3: SEM pollen data for species of *Asteranthe*, *Hexalobus*, *Isolona*, *Monodora* and *Uvariastrum* (*Monodora* clade). All measurements in µm; P = length polar axis, E = equatorial diameter (except for *Isolona*: see description). Asterisks indicate data obtained from the archive of Annick Le Thomas.

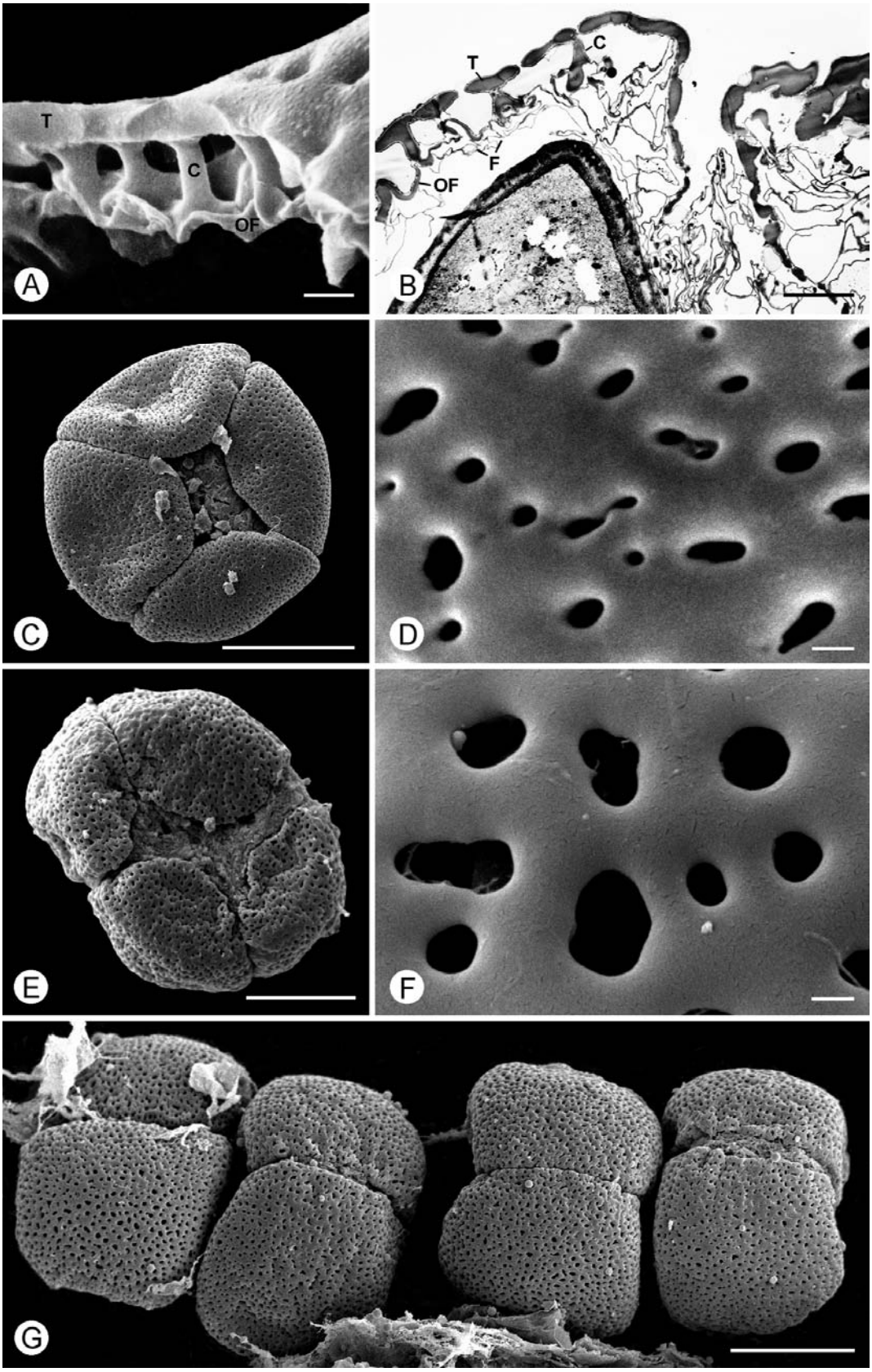

Plate 1. Pollen of *Asteranthe*. **A, B.** *A. asterias* (Sacleux 712): cross-sections of pollen wall showing columellate infratectum (SEM and TEM). **C, D.** *A. asterias* (Robertson 3878): tetrad and detail of foveolate tectum. **E–G.** *A. lutea* (Couvreur 46): tetrad, detail of foveolate tectum, and four tetrads in anther locule. Bar: 10 μm (C, E, G), 5 μm (F), 1 μm (A, B, D). Abbreviations: see Figure 5.1.

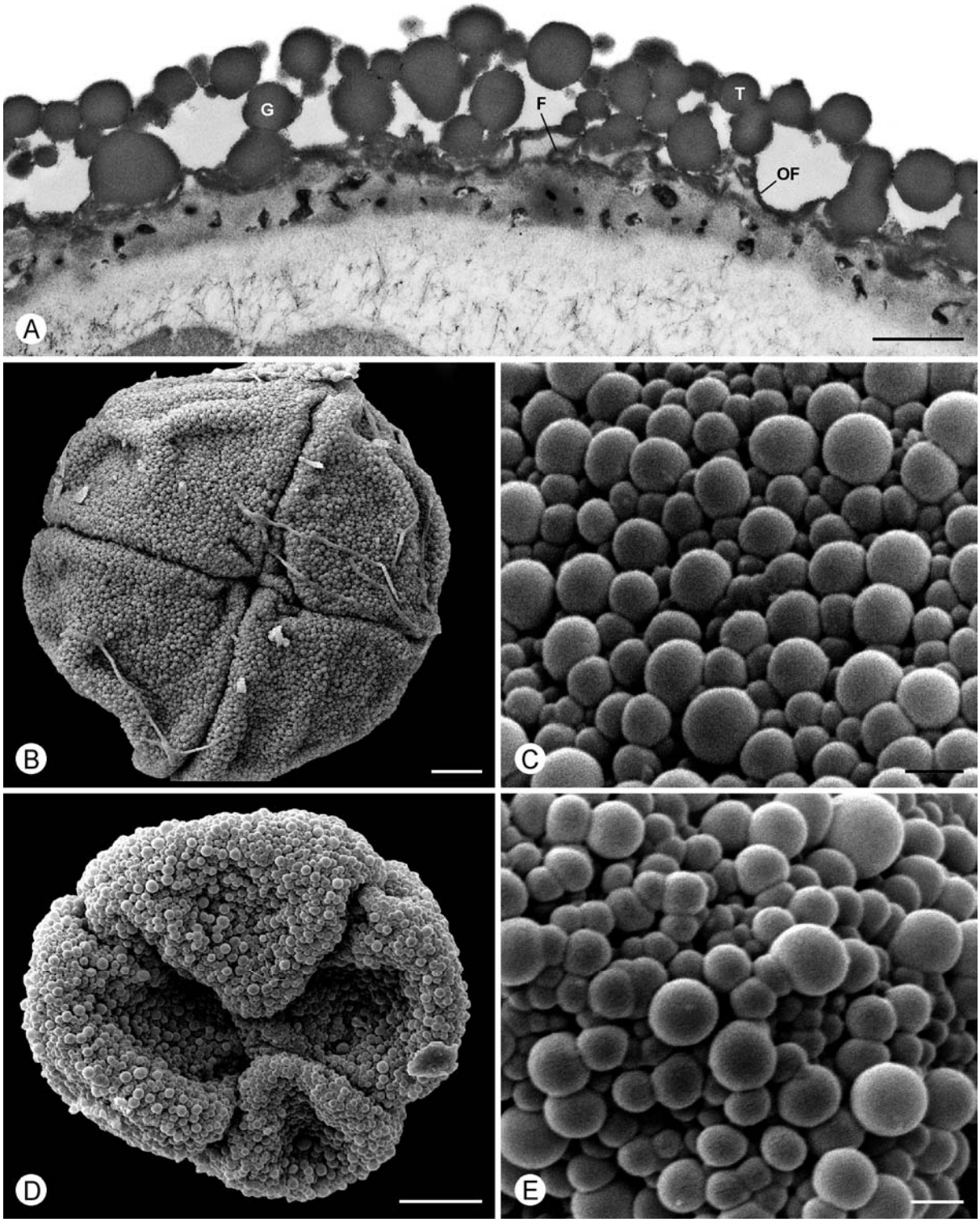

Plate 2. Pollen type A of *Hexalobus*. **A–C.** *H. bussei* (Bos 5370): cross-section of pollen wall (TEM), tetrad and detail of granular to gemmate tectum. **D, E**. *H. mossambicensis* (Gomes e Sousa 4897): tetrad and detail of of granular to gemmate tectum. Bar: 10 μm (B, D), 1 μm (A, C, E). Abbreviations: see Figure 5.1.

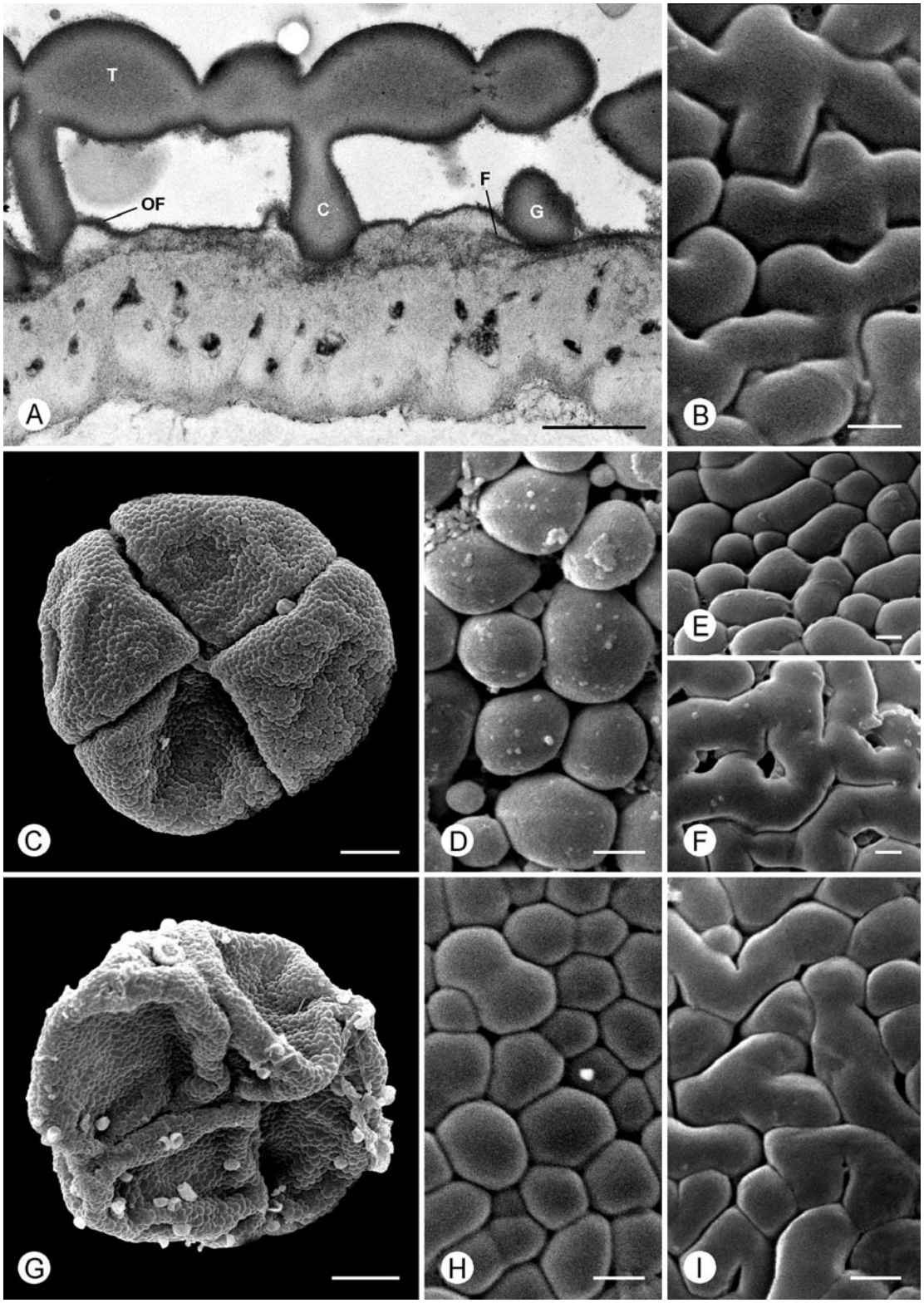

Plate 3. Pollen type B of *Hexalobus*. **A, F.** *H. crispiflorus* (Liben 2390): cross-section of pollen wall (TEM) and detail of rugulate tectum. **B.** *H. crispiflorus* (Hoyle 789): detail of areolate-verrucate to rugulate tectum. **C, D.** *H. crispiflorus* (J.J. de Wilde 7909): tetrad and detail of areolate-verrucate tectum. **E.** *H. crispiflorus* (Chevalier 13385): detail of areolate-verrucate tectum. **G, H.** *H. salicifolius* (Zenker 3330): tetrad and detail of areolate-verrucate tectum. **I.** *H. salicifolius* (Letouzey 8122): detail of areolate-verrucate to rugulate tectum. Bar: 10 μm (C, G), 1 μm (A, B, D–F, H, I). Abbreviations: see Figure 5.1.

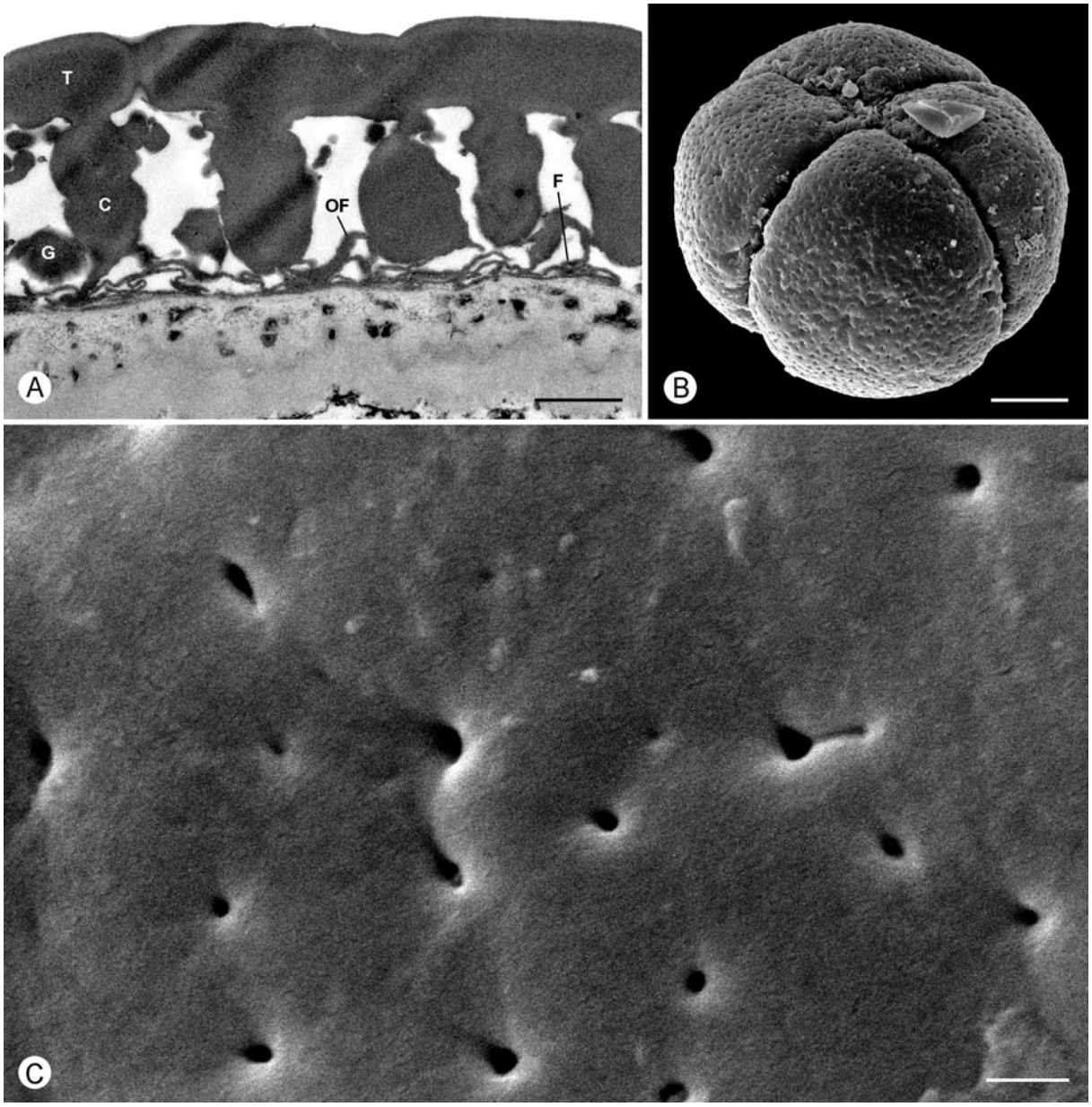

Plate 4. Pollen type C of *Hexalobus*. **A–C.** *H. monopetalus* (Breteler 7288): cross-section of pollen wall (TEM), tetrad and detail of psilate-perforate tectum. Bar: 10 µm (B), 1 µm (A, C). Abbreviations: see Figure 5.1.

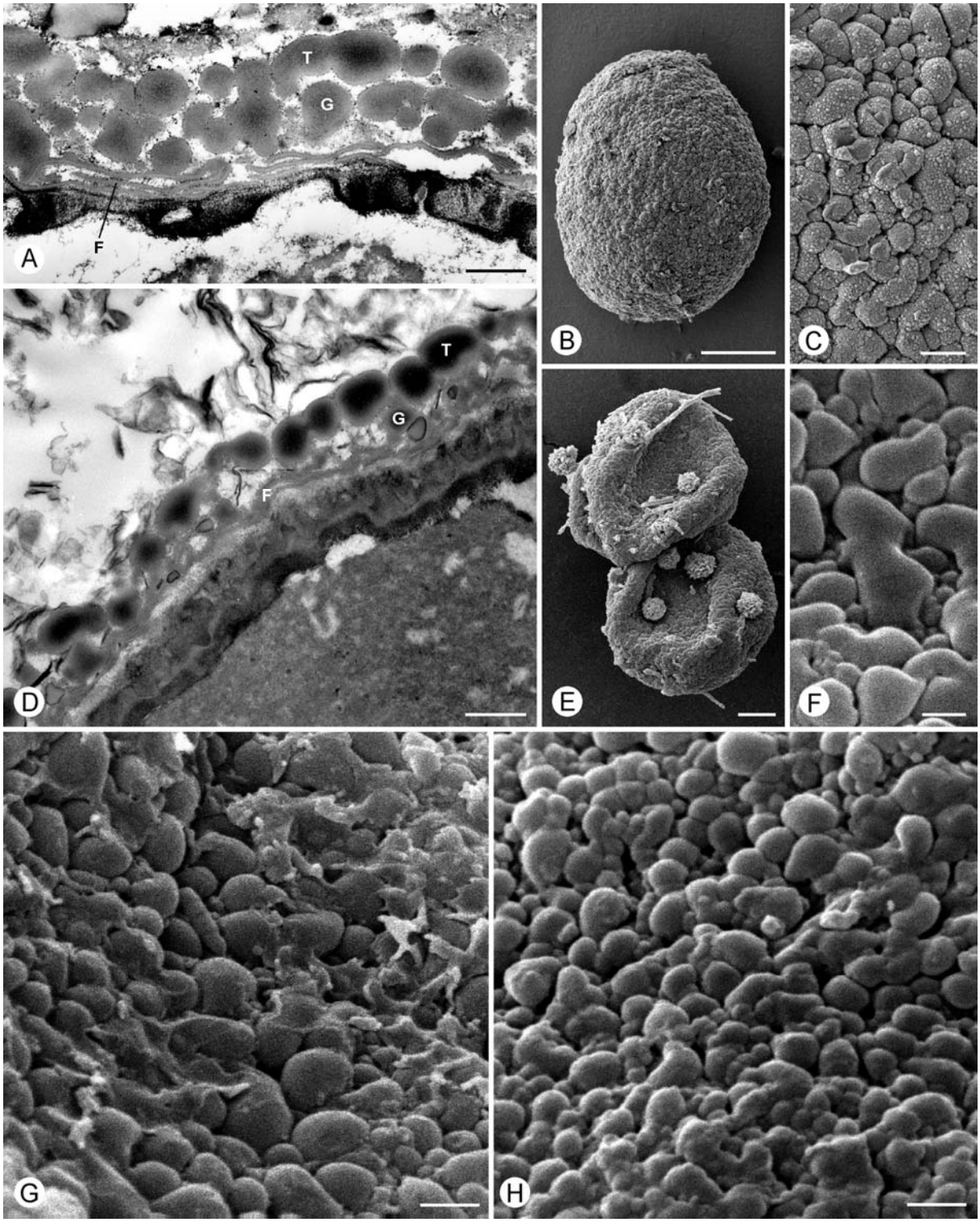

Plate 5. Pollen type A of *Isolona*. **A.** *I. thonneri* (Letouzey 10205): cross-section of pollen wall (TEM). **B, C.** *I. thonneri* (Letouzey 12111): pollen grain (monad) and detail of scabrate tectum. **D–F.** *I. humbertiana* (Perrier 1511): cross-section of pollen wall (TEM), two pollen grains (monads; note Ubisch bodies), detail of verrucate tectum. **G.** *I. capuronii* (Service Forestier de Madagascar 8941): detail of scabrate tectum. **H.** *I. pilosa* (Le Testu 8602): detail of scabrate tectum. Bar: 10 µm (B, E), 1 µm (A, C, D, F, G, H). Abbreviations: see Figure 5.1.

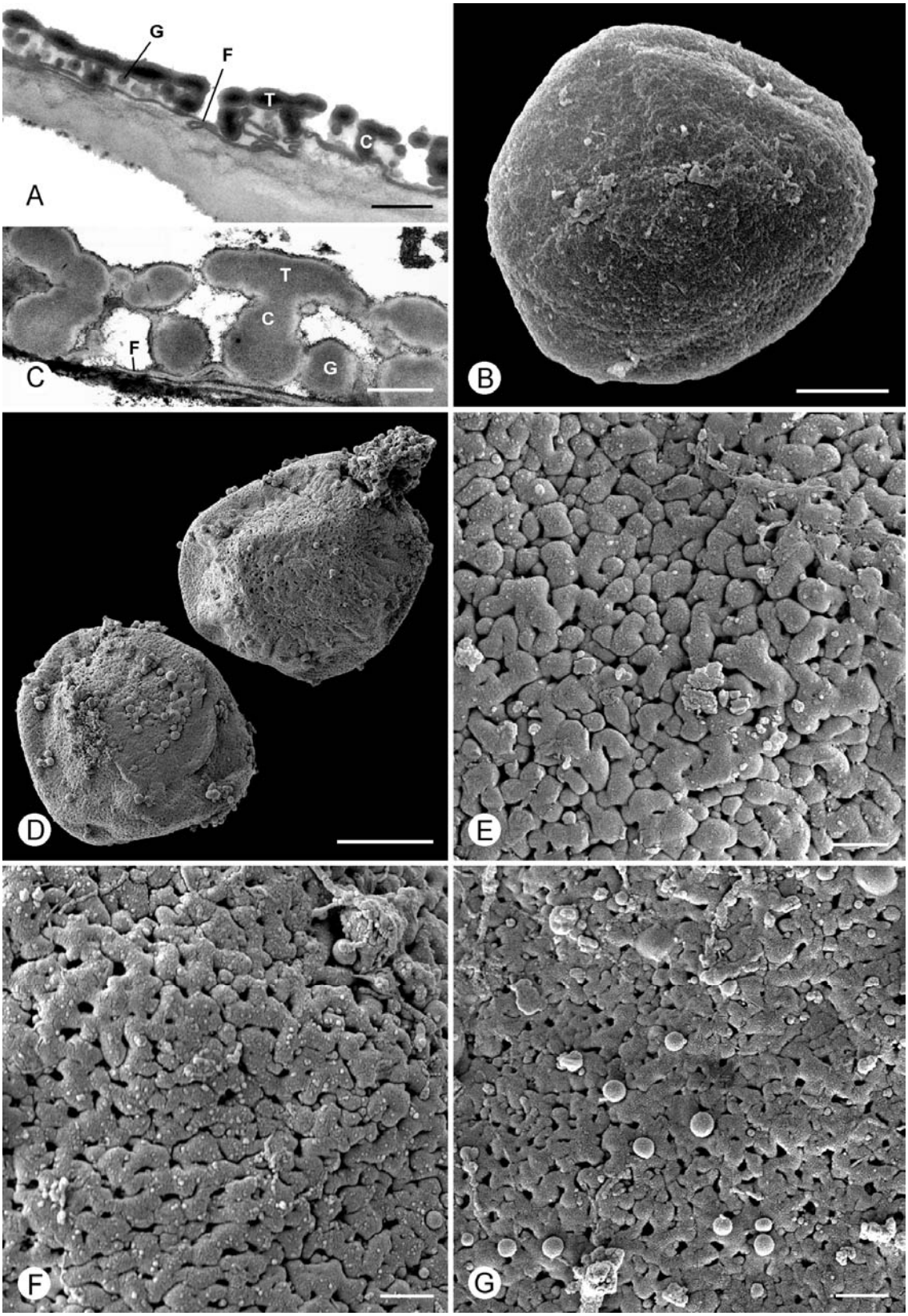

Plate 6. Pollen type B of *Isolona*. **A, D, F.** *I. campanulata* (De Koning 6748): cross-section of pollen wall (TEM), two pollen grains (monads) and detail of finely rugulate tectum. **B, E.** *I. hexaloba* (J.J. de Wilde 839 WALK-B): pollen grain (monad) and detail of finely rugulate tectum. **C.** *I. hexaloba* (Letouzey 10419): cross-section of pollen wall (TEM). **G.** *I. cooperi* (Bos 1609): detail of finely rugulate tectum. Bar: 10 μm (B, D), 1 μm (A, C, E–G). Abbreviations: see Figure 5.1.

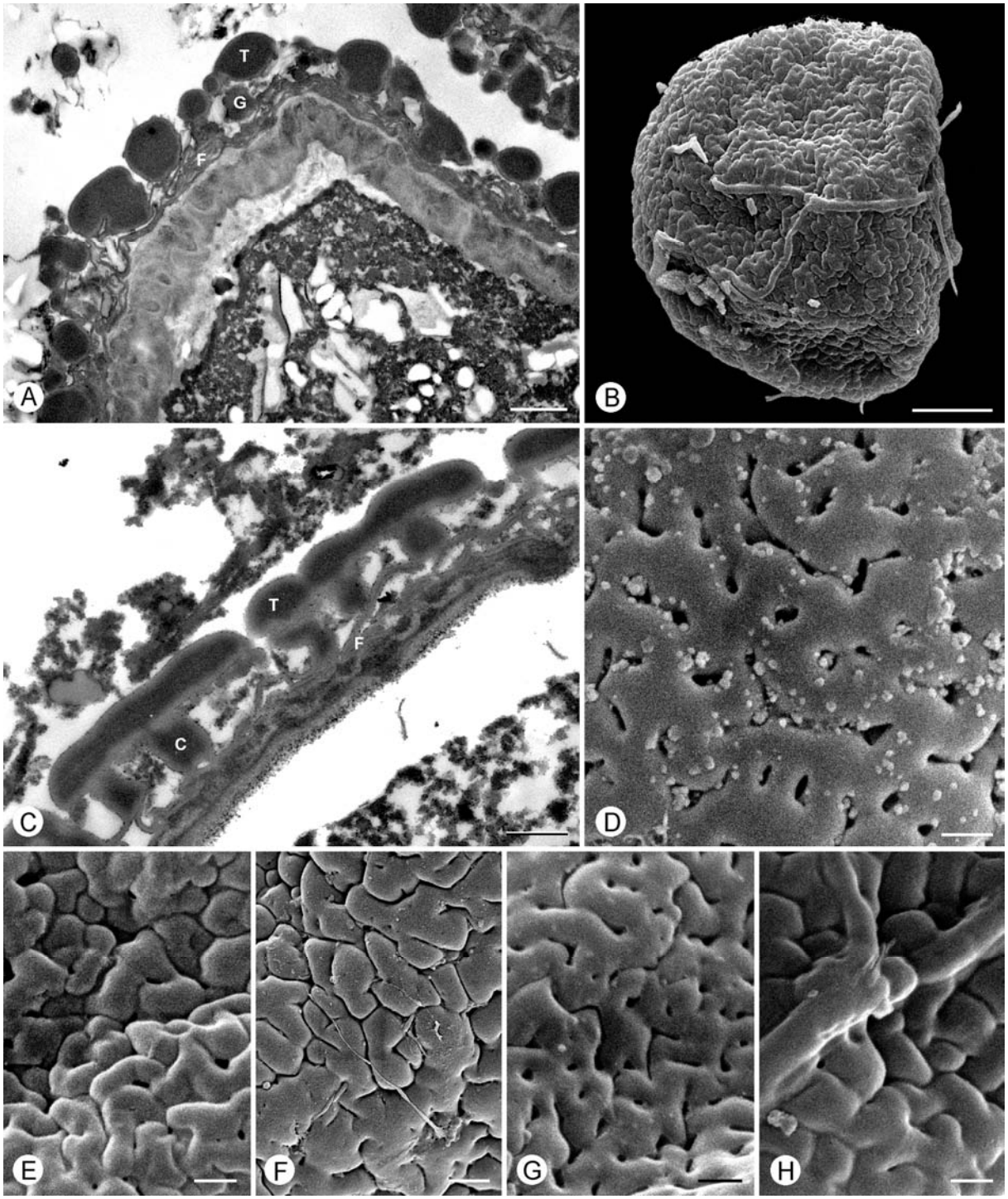

Plate 7. Pollen type C of *Isolona*. **A, B, H.** *I. ghesquierei* (Service Forestier de Madagascar 8587): cross-section of pollen wall (TEM), pollen grain (monad) and detail of rugulate tectum. **C, D.** *I. congolana* (Leeuwenberg 9550): cross-section of pollen wall (TEM) and detail of rugulate tectum. **E.** *I. cauliflora* (Polhill 4782): detail of rugulate tectum. **F.** *I. linearis* (Frimodt-Möller TZ59): detail of rugulate tectum. **G.** *I. lebrunii* (Deville 234): detail of rugulate tectum. Bar: 10 μm (B), 1 μm (A, C–H). Abbreviations: see Figure 5.1.

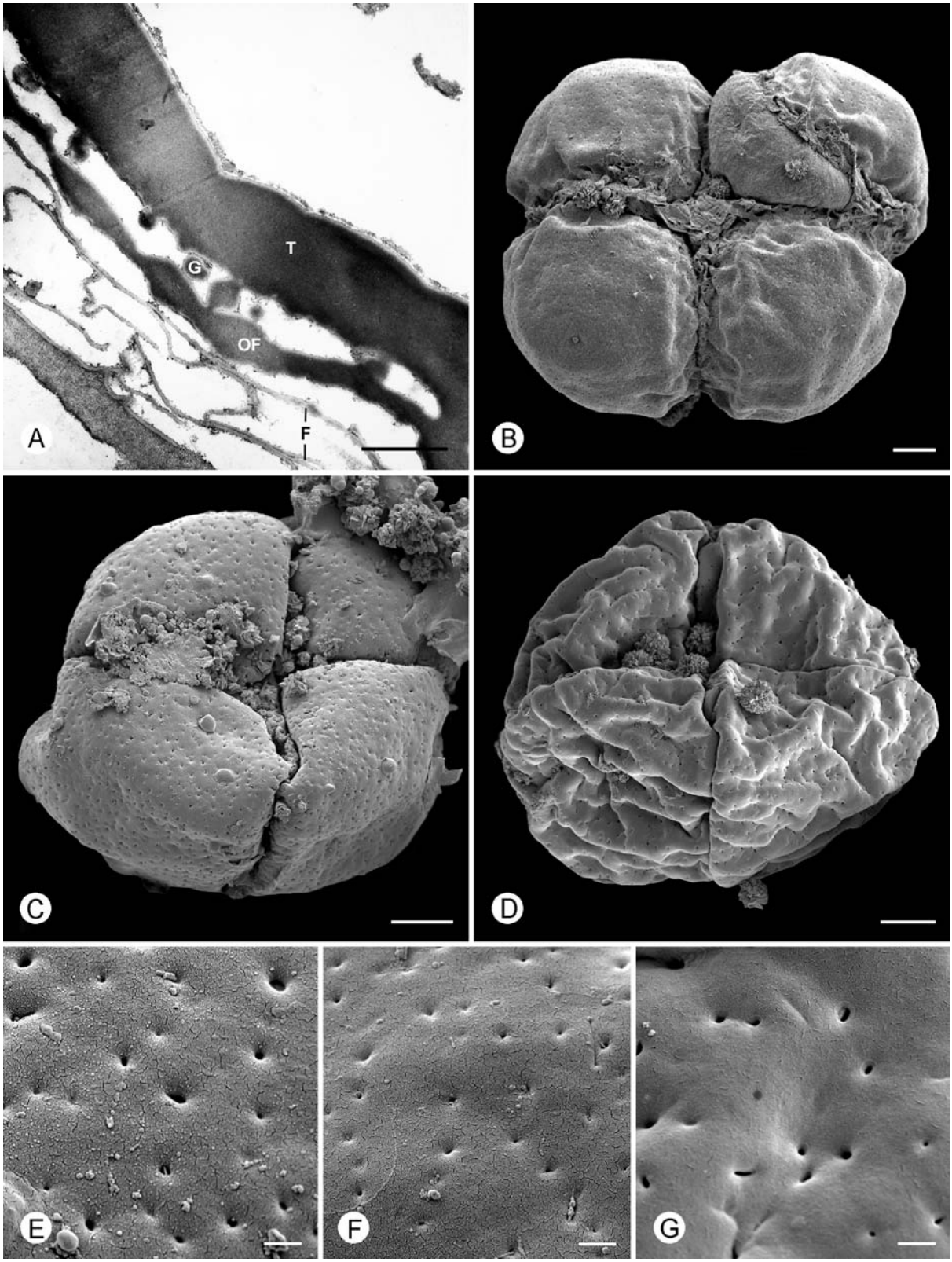

Plate 8. Pollen type A1 of *Monodora*. **A.** *M. myristica* (Letouzey 11474): cross-section of pollen wall (TEM). **B, F.** *M. undulata* (Bos 2306): tetrad (note Ubisch bodies) and detail of psilate-perforate tectum. **C, E.** *M. myristica* (De Koning 1146): tetrad and detail of psilate-perforate tectum. **D, G.** *M. minor* (Mgaza 783): tetrad and detail of psilate-perforate tectum. Bar: 10 μm (B–D), 1 μm (A, E–F). Abbreviations: see Figure 5.1.

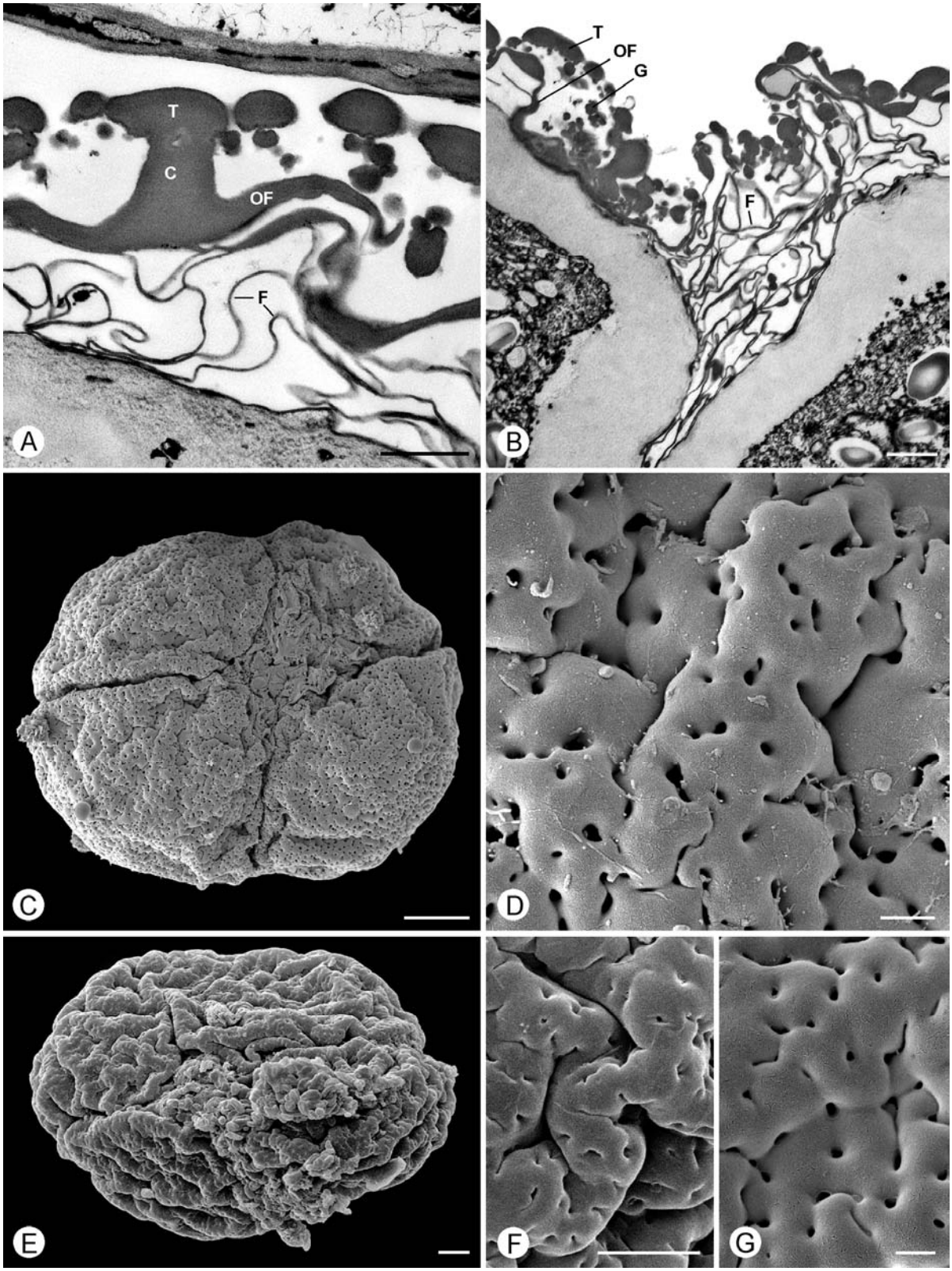

Plate 9. Pollen type A2 of *Monodora*. **A–D.** *M. crispata* (W.J. de Wilde 867): cross-section of pollen wall (TEM), contact zone between two monads showing presence of thin foliations and absence of thicker outer foliation (TEM), tetrad (note Ubisch bodies) and detail of rugulate (locally psilate) tectum. **E, F.** *M. zenkeri* (Breteler 2747): tetrad and detail of folded rugulate (locally psilate) tectum. **G.** *M. carolinae* (Philipson 4940): detail of rugulate (locally psilate) tectum. Bar: 10 µm (C, E), 5 µm (F), 1 µm (A, B, D, G). Abbreviations: see Figure 5.1.

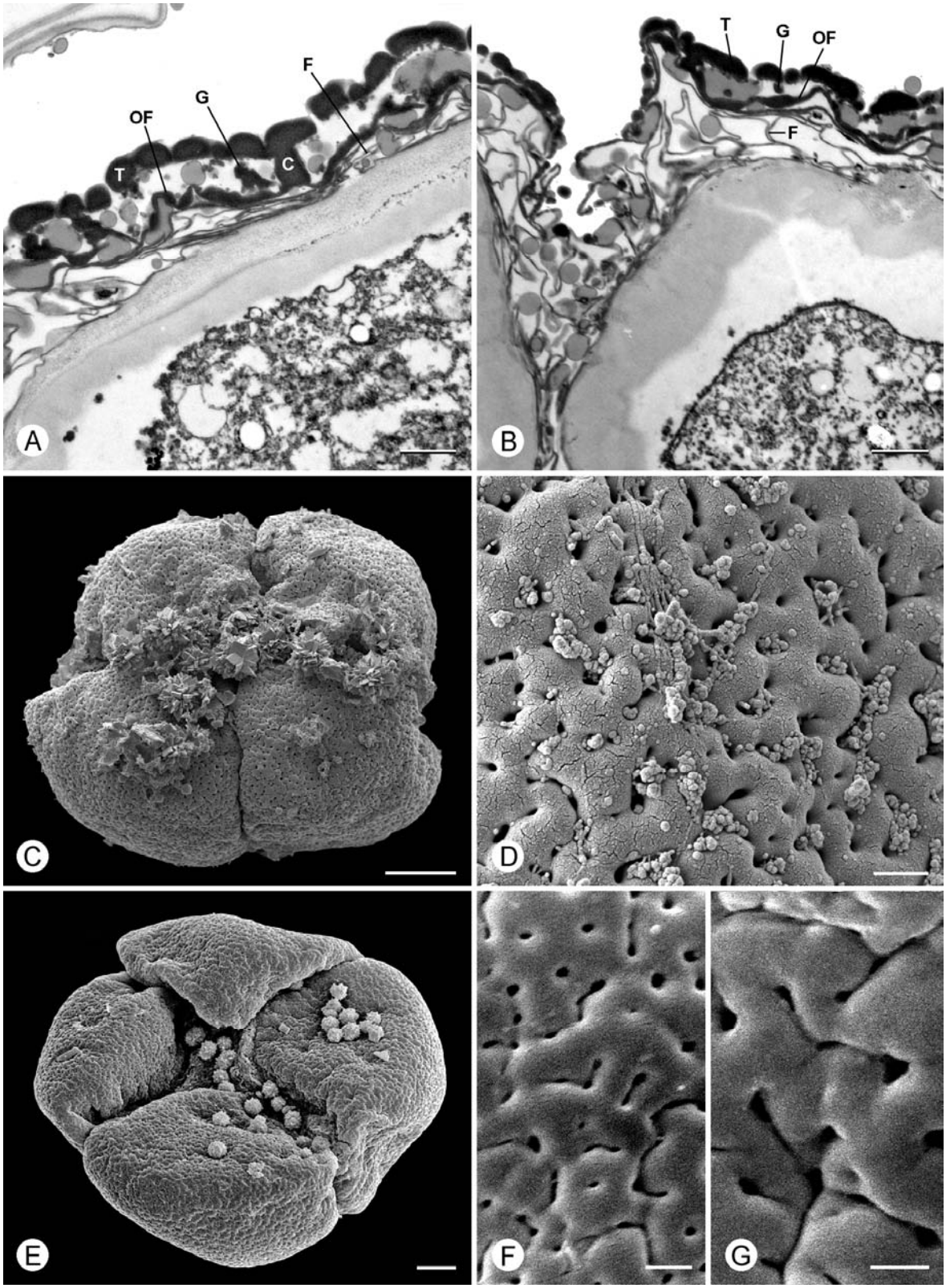

Plate 10. Pollen type B of *Monodora*. **A–D.** *M. angolensis* (Van Valkenburg 2688): A. cross-section of pollen wall (TEM); B. contact zone between two monads showing presence of thin foliations and absence of thicker outer foliation (TEM); C. tetrad (note Ubisch bodies); D. detail of rugulate tectum. **E, F.** *M. junodii* (Torre and Paiva 9035): tetrad (note Ubisch bodies) and detail of rugulate tectum. **G.** *M. globiflora* (Luke 3136): detail of rugulate tectum. Bar: 10 μm (C, E), 2 μm (A, B), 1 μm (D, F, G). Abbreviations: see Figure 5.1.

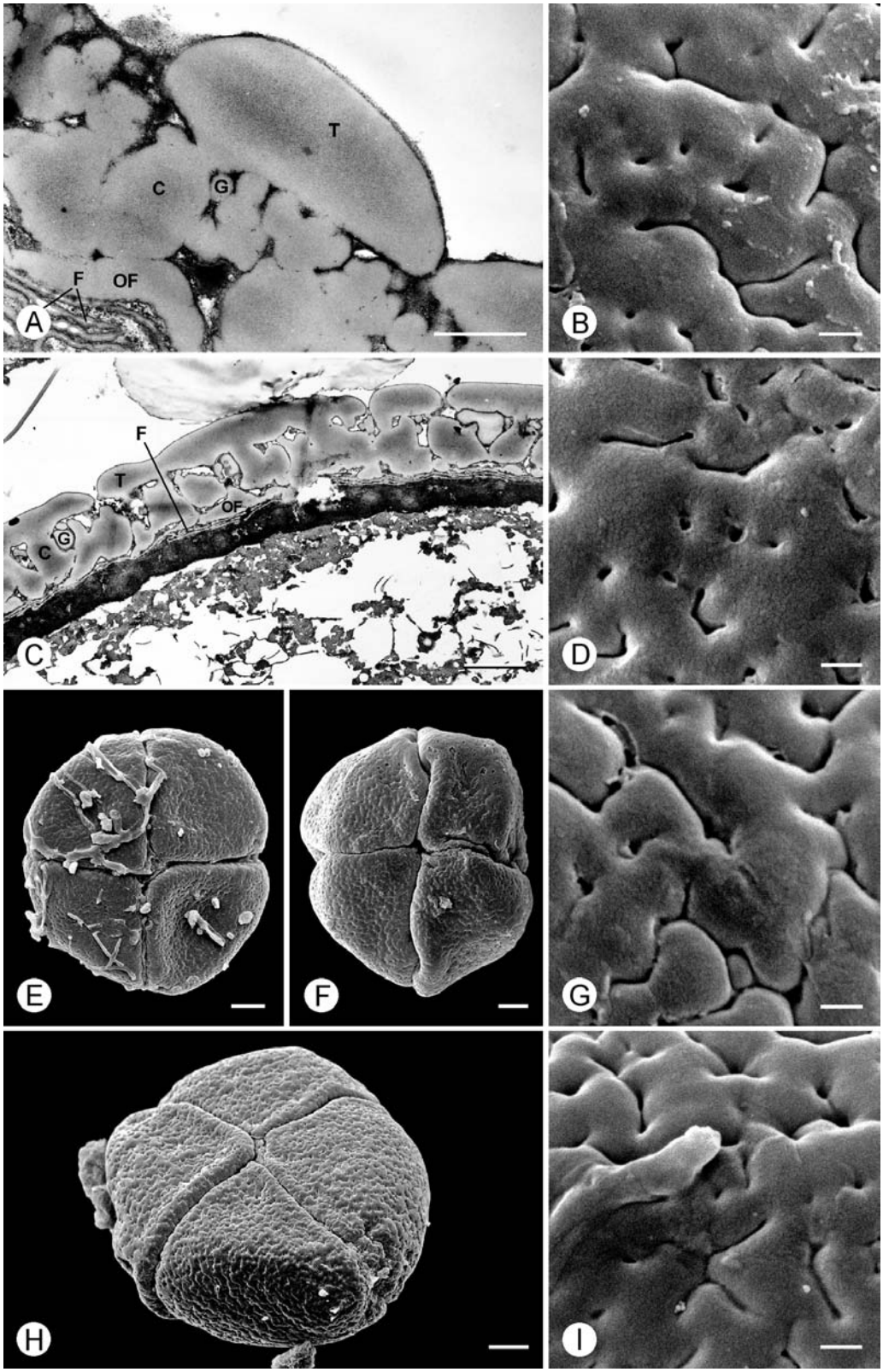

Plate 11. Pollen of *Uvariastrum*. **A.** *U. pyneartii* (Le Testu 8473): cross-section of pollen wall (TEM). **B, F.** *U. insculptum* (Breteler 5811): detail of rugulate tectum and tetrad. **C.** *U. pierreanum* (Letouzey 10225): cross-section of pollen wall (TEM). **D, H.** *U. zenkeri* (Bos 6266): detail of rugulate tectum and tetrad. **E, I.** *U. germainii* (Lebrun 5977): tetrad and detail of rugulate tectum. **G.** *U. hexaloboides* (Breteler 11894): detail of rugulate tectum. Bar: 10 μm (E, F, H), 1 μm (A–D, G, I). Abbreviations: see Figure 5.1.

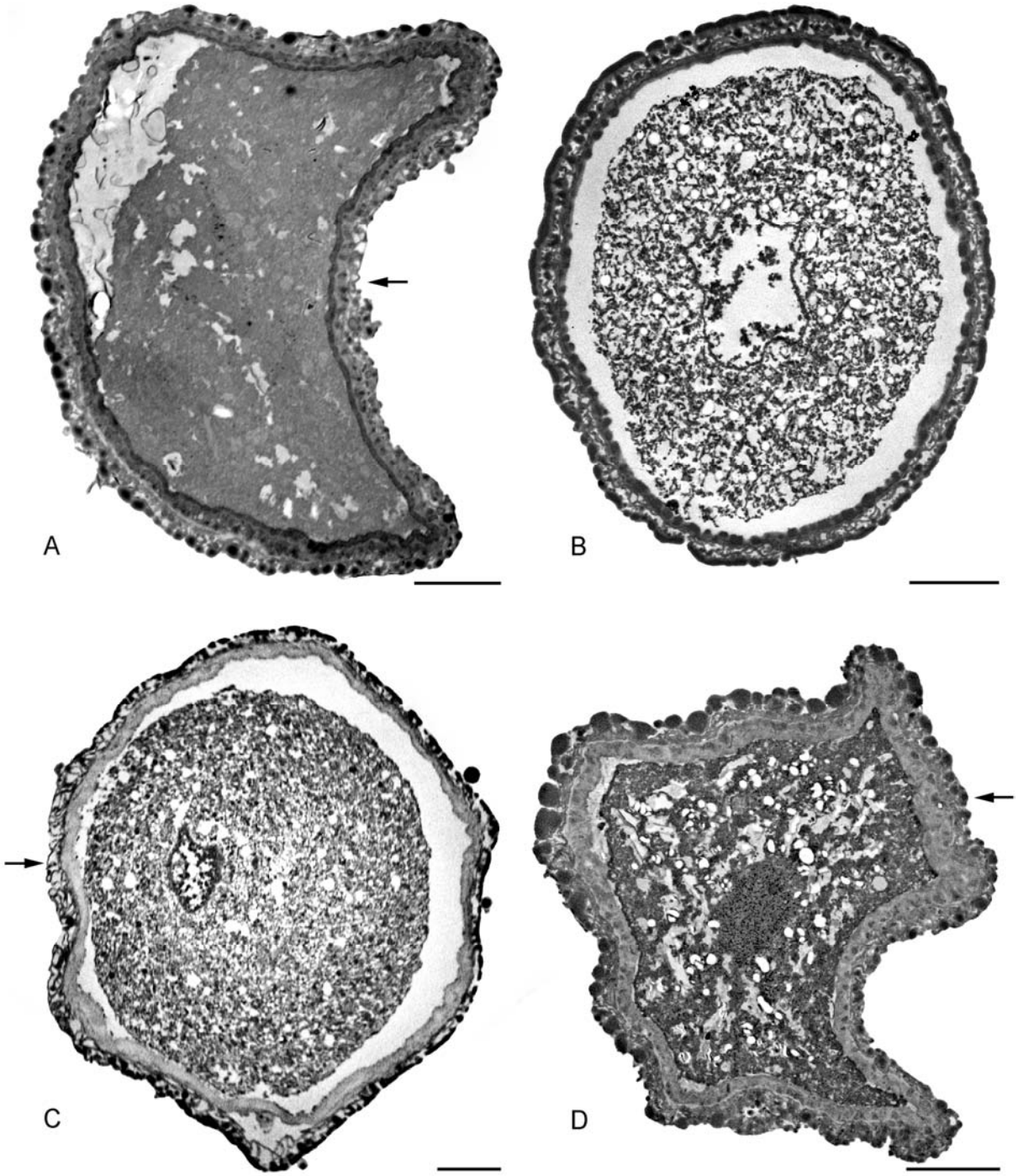

Plate 12. Pollen grains (monads) of *Isolona* (TEM). **A.** *I. humbertiana* (Perrier 1511): pollen wall showing proximal thinning (arrow). **B.** *I. congolana* (Leeuwenburg 9550): note absence of proximal thinning, which might be due to orientation of section. **C.** *I. campanulata* (De Koning 6748): pollen wall showing proximal thinning (arrow). **D.** *I. ghesquieri* (Service Forestier de Madagascar 8587): pollen wall showing proximal thinning (arrow). Bar: 5 μm (A–D).

Appendix 5.1: Voucher information and specimens used for TEM. Pollen photos from specimens marked with an asterisk (*) were provided by A. Le Thomas (Muséum National d'Histoire Naturelle, Paris).

genus	species	herbarium voucher	country	herbarium acronym	TEM
Asteranthe	*asterias*	Robertson 3878	Kenya	WAG	
Asteranthe	*asterias*	Sacleux 712*	Tanzania	P	X
Asteranthe	*lutea*	Couvreur 46	Tanzania	WAG	
Hexalobus	*bussei*	Zenker 3889	Cameroun	P	
Hexalobus	*bussei*	Bos 5370	Cameroun	WAG	X
Hexalobus	*crispiflorus*	Schaijes 3596	Congo	BR	
Hexalobus	*crispiflorus*	Hoyle 789	Sudan	FHO	
Hexalobus	*crispiflorus*	Chevalier 13385	Guinea	P	
Hexalobus	*crispiflorus*	Pobéguin 844	Guinea	P	
Hexalobus	*crispiflorus*	Espirito Santo 3841	Guinea-Bissau	WAG	
Hexalobus	*crispiflorus*	Jongkind 4386	Ivory Coast	WAG	X
Hexalobus	*crispiflorus*	J.J. de Wilde 7909	Cameroun	WAG	
Hexalobus	*crispiflorus*	Liben 2390	Congo	WAG	X
Hexalobus	*monopetalus*	Gillman 1090	Tanzania	EA	
Hexalobus	*monopetalus*	Brenan 7856	Zambia	FHO	
Hexalobus	*monopetalus*	Schlieben 7432	South Africa	G	
Hexalobus	*monopetalus*	Breteler 7288	Togo	WAG	X
Hexalobus	*monopetalus*	Diarra 367*	Mali	P	X
Hexalobus	*mossambicensis*	Gomes e Sousa 4897	Mozambique	COI	
Hexalobus	*mossambicensis*	Pedro 5189	Mozambique	EA	
Hexalobus	*salicifolius*	Letouzey 8122	Cameroun	BR	
Hexalobus	*salicifolius*	Zenker 3330	Cameroun	UPS	
Isolona	*campanulata*	De Koning 6748	Ivory Coast	WAG	X
Isolona	*capuronii*	Service Forestier de Madagascar 8941	Madagascar	P	
Isolona	*cauliflora*	Polhill 4782	Kenya	C	
Isolona	*congolana*	Leeuwenberg 9550	Cameroun	WAG	X
Isolona	*congolana*	Lejoly 4961	Congo	BR	
Isolona	*cooperi*	Bos 1609	West Africa	WAG	
Isolona	*deightonii*	Bernardi 8691	Ivory Coast	US	
Isolona	*dewevrei*	Merello 1346	Ghana	US	
Isolona	*ghesquierei*	Service Forestier de Madagascar 8587	Madagascar	P	X
Isolona	*heinsenii*	Schlieben 1539	Tanzania	G	
Isolona	*hexaloba*	J.J. de Wilde 839 WALK-B	Gabon	WAG	
Isolona	*hexaloba*	Sosef 2244	Gabon	WAG	
Isolona	*hexaloba*	Letouzey 10419*	Cameroun	P	X
Isolona	*humbertiana*	Perrier 1511	Madagascar	P	X
Isolona	*madagascariensis*	Service Forestier de Madagascar 11409*	Madagascar	P	
Isolona	*lebrunii*	Deville 234	Congo	BR	
Isolona	*linearis*	Frimodt-Möller TZ59	Tanzania	C	
Isolona	*perrieri*	Du Puy MB512	Madagascar	P	
Isolona	*perrieri*	Perrier 18714*	Madagascar	P	
Isolona	*pilosa*	Le Testu 8602	Gabon	WAG	
Isolona	*pilosa*	Le Testu 8740	Gabon	WAG	
Isolona	*pleurocarpa*	Leeuwenberg 9784	Cameroun	WAG	
Isolona	*thonneri*	Letouzey 10205*	Cameroun	P	X
Isolona	*thonneri*	Letouzey 12111	Cameroun	WAG	
Isolona	*zenkeri*	Sosef 2232	Gabon	WAG	
Isolona	*zenkeri*	Cabalion 144	Congo	WAG	
Monodora	*angolensis*	Hart 1593	Congo	WAG	
Monodora	*angolensis*	Van Valkenburg 2688	Gabon	WAG	X

Monodora	*angolensis*	Tisserant 1858*	Central African Republic	P	
Monodora	*carolinae*	Philipson 4940	Tanzania	C	
Monodora	*crispata*	W.J. de Wilde 867	Ivory Coast	WAG	X
Monodora	*globiflora*	Luke 6724	Tanzania	EA	
Monodora	*globiflora*	Luke 3136	Tanzania	MO	
Monodora	*grandidieri*	Luke 10104	Mozambique	MO	
Monodora	*grandidieri*	Lesley 163	Kenya	WAG	
Monodora	*grandidieri*	Sacleux 958*	Kenya	P	
Monodora	*hastipetala*	Philipson 4958	Tanzania	MO	
Monodora	*junodii*	Torre & Paiva 9035	Mozambique	WAG	
Monodora	*laurentii*	De Giorgi 1617	Congo	BR	
Monodora	*minor*	Mgaza 783	Tanzania	EA	
Monodora	*myristica*	De Koning 1146	Ivory Coast	WAG	
Monodora	*myristica*	Letouzey 11474*	Cameroun	P	X
Monodora	*stenopetala*	Simaõ 1196	Mozambique	COI	
Monodora	*tenuifolia*	De Koning 982	Ivory Coast	WAG	
Monodora	*tenuifolia*	Letouzey 4978*	Cameroun	P	
Monodora	*undulata*	Bos 2306	Liberia	WAG	
Monodora	*undulata*	Letouzey 10120*	Cameroun	P	
Monodora	*zenkeri*	Breteler 2747	Cameroun	WAG	
Uvariastrum	*hexaloboides*	Breteler 11894	Zambia	WAG	
Uvariastrum	*germainii*	Germain 213	Congo	P	
Uvariastrum	*germainii*	Lebrun 5977	Congo	P	
Uvariastrum	*insculptum*	Aké Assi 16772	Ivory Coast	G	
Uvariastrum	*insculptum*	Breteler 5811	Ivory Coast	WAG	
Uvariastrum	*pierreanum*	Letouzey 10225*	Cameroun	P	X
Uvariastrum	*pynaertii*	Le Testu 8473*	Gabon	P	X
Uvariastrum	*zenkeri*	Thomas 4334	Cameroun	US	
Uvariastrum	*zenkeri*	Bos 6266	Cameroun	WAG	

6 Monograph of the African Genera *Isolona* and *Monodora* (Annonaceae) ♣

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Abstract. The genera *Isolona* Engl. and *Monodora* Dun. (Annonaceae) consist of 20 and 14 species, respectively, occurring across tropical Africa. Both genera are unique within the family due to the presence of a syncarpous gynoecium. *Isolona* and *Monodora* have been recovered as sister genera when using molecular data. In *Isolona*, the flowers are medium sized, generally yellow or red, and the petals are basely fused into a tube dividing into six equal-sized lobes. In *Monodora* the flowers are large and mutli-coloured. The petals are only slightly fused basely with the lobes differentiated into large outer and smaller inner petals. The inner petals are connivent forming a dome covering the androecium and gynoecium and are thought to play a role in pollination. This monograph provides unpublished results on floral anatomy as well as an almost complete species-level molecular phylogeny of both genera. In addition, previous published relevant information for both genera is reviewed and discussed on morphology, pollination biology, dispersal, palynology and ethnobotany. Keys to the flowering specimens of both genera, as well as a key to the fruiting specimens in *Monodora*, are provided. One new combination is proposed. The conservation status of each species is also assessed. For both genera, 16 species were assigned to some level of threat.

♣ In preparation for Systematic Botany Monographs

"*Monodora myristica* […] represents one of the most gigantic and certainly one of the most splendid forest trees of the whole of tropical Africa."
F.M.J. Welwitsch, 1859

INTRODUCTION

Isolona and *Monodora* are two closely related tropical African genera with 20 and 14 species respectively. They consist mainly of trees and shrubs from 2-3 m up to 30-40 m tall. Both genera are exceptional within Annonaceae because of their syncarpous flowers and fruits (Deroin, 1997), i.e. where the carpels are congenitally fused into a unique structure (Carr and Carr, 1961). In a recent molecular phylogeny, the two genera were found to be nested within a larger clade composed of nine other endemic African genera (Couvreur et al., in press).

Unlike most Annonaceae, both genera have one whorl of fused petals. In *Isolona* the fusion is conspicuous, leading to the formation of a tube enclosing the receptacle, with six undifferentiated petal lobes. In *Monodora*, the fusion is inconspicuous and the flower assumes the common Annonaceae pattern having "inner" and "outer" petals.

The last revision of *Isolona* and *Monodora* dates back to that of Engler and Diels published in 1901. Since then, only accounts of these genera have been published in regional floras (Boutique, 1951b; e.g. Cavaco and Keraudren, 1958; Le Thomas, 1960; Robson, 1960; Verdcourt, 1971). Hence, the present monograph represents the first comprehensive taxonomic revision of both genera since 1901.

TAXONOMIC HISTORY

The taxonomic history of both *Isolona* and *Monodora* are linked, mainly because of certain important morphological affinities which led to some confusion about their distinction in the early stages of their discovery. The most striking similarity is the occurrence of a syncarpous flower (Deroin, 1997) in an otherwise apocarpous family. The distinct gynoecia of these two genera led many authors to place them in a separate subfamily, the Monodoroideae (Dunal, 1817; Candolle, 1824; Hutchinson, 1923; Fries, 1959). Only Bentham (1862) disagreed and included *Monodora* (*Isolona* was not officially described yet, see below) in the tribe Mitrephoreae because of the spreading outer petals and inner petals being connivent over the receptacle. However, the isolation of the Monodoroideae in a separate subfamily was not supported by palynological data (Walker, 1971), floral and fruit morphology (Van Heusden, 1992; van Setten and Koek-Noorman, 1992), morphological cladistic analyses (Doyle and Le Thomas, 1994; Doyle and Le Thomas, 1996) or molecular data (Richardson et al., 2004; Couvreur et al., in press; see Chapter 3). The close sister relationship between the two genera has, however, never really been questioned.

Gaertner (1791) was the first to describe a species that was later accommodated in *Monodora*: *Annona myristica*. A few years later another species, now known as *Monodora*

undulata, was described under the name *Xylopia undulata* by Palisot de Beauvois (1804). The genus *Monodora* was created by Dunal (1817) who included two species: *Annona myristica* that was combined to *M. myristica*, from Africa, and *M. microcarpa,* from Australia. The latter, however, proved to be a synonym of *Diospyros cargillia* F.Muell. (Ebenaceae). The main characters used for the distinction of *Monodora* were the unique ovary with multiple ovules and the unique fruit with numerous scattered seeds. Dunal (1817) indicated, however, that only a precise anatomical study would reveal the true nature of the *Monodora* gynoecium and determine whether it is composed of one carpel or of multiple fused carpels. Interestingly, in the same publication Dunal (1817) combined *Xylopia undulata* into *Unona undulata*. In 1867, Bentham described the new species *Monodora grandiflora*, citing however *X. undulata* as a synonym rendering this name invalid. A year later, *Monodora grandiflora* was synonymized into *M. myristica* by Baillon (1868). However, the type of *Xylopia undulata* is not conspecific with *M. myristica* but with *M. brevipes*, a species described by Bentham in 1867. This led to the new combination *M. undulata* (P.Beauv.) Couvreur presented in this monograph.

In 1832, Alphonse de Candolle described the genus *Hexalobus* characterized by the conspicuously and basally fused petals, and included two species: *H. senegalensis* and *H. madagascariensis*. The latter was the first description of a species later to be accommodated in *Isolona* by Diels (1925). Baillon (1868) was the first to describe, although he was unsure of whether this placement was correct, a species later to be transferred to *Isolona* within *Monodora.* He created *M. madagascariensis* indicating that it might be conspecific with *Hexalobus madagascariensis* of De Candolle. Subsequently, new species initially described in *Monodora* (M. *congolana* De Wild. & T. Durand, *M. dewevrei* De Wild. & T. Durand, *M. thonneri* De Wild. & T. Durand) that were later transferred to *Isolona*. In 1896, Pierre validly described a new species named *Monodora hexaloba* placing it in the invalidly published 'section' *Isolona* becoming the first record of the name *Isolona*. In 1897, Engler recognized the need to create a new genus and described the genus *Isolona* Engl. *M. madagascariensis* Baill. was transferred to *Isolona madagascariensis* (Baill.) Engl. However, no mention was made of *H. madagascariensis* A.DC. that was still considered as a distinct species. Hutchinson (1923) chose the type of *Isolona* apparently by selecting the first described species, which is *I. madagascariensis* (Baill.) Engl. with the associated type (*Bernier 131*). In 1925, Diels recognized that both *H. madagascariensis* A.DC. and *I. madagascariensis* (Baill.) Engl. were conspecific. He corrected Engler's combination to *Isolona madagascariensis* (A.DC.) Engl. This recombination is invalid, however, because it is a later homonym of *I. madagascariensis* (Baill.) Engl. Since Baillon explicitly states the species described by him not to be the same as that of De Candolle, Baillon's name is the valid basionym, and the recombination by Engler in 1897 blocks the transfer of *H. madagascariensis* A.DC to *Isolona*. Thus the correct name for the species is *Isolona madagascariensis* (Baill.) Engl. and *H. madagascariensis* A.DC. should be treated as a heterotypic synonym.

The first revision of *Monodora* was published in the Flora of Tropical Africa (Oliver, 1868) and counted five species. In 1901, Engler and Diels provided an updated revision of

both *Isolona* and *Monodora* (excluding the Malagasy species of *Isolona*) which counted seven species in *Isolona* and 11 in *Monodora*. Subsequently new species were added to both genera by Diels (1907; 1908; 1925), De Wildeman (1909; 1911), Boutique (1951a), Keay (1952), Cavaco and Keraudren (1957) and Couvreur et al. (2006). Table 6.1 provides an overview of the most important taxonomic events in the history of both genera.

Year	Taxonomic event
1791	Description of *Annona myristica* by Gaertner
1804	Description of *Xylopia undulata* by Palisot de Beauvois
1817	Creation of *Monodora* by Dunal, containing *M. myristica* based on *A. myristica*
1832	Description of *Hexalobus madagascariensis* by A. De Candolle
1867	Bentham invalidly describes *M. grandiflora* by citing *Xylopia undulata* as a synonym
1868	*Monodora grandiflora* treated as a synonym of *M. myristica* by Baillon
1868	Description of *Monodora madagascariensis* by Baillon
1896	Description of *Monodora hexaloba* in section *Isolona* by Pierre
1897	Creation of *Isolona* by Engler
1901	Monograph of African Annonaceae (excl. Madagascar) by Engler and Diels
1923	Selection of the type species of *Isolona*: *I. madagascariensis* (Baill.) Engl. by Hutchinson
1925	Diels publishes *Isolona madagascariensis* (A.DC.) Engl., based on *Hexalobus madagascariensis* A.DC. with *Isolona madagascariensis* (Baill.) Engl. cited as a synonym, which proved invalid

Table 6.1. Major taxonomic events in the taxonomic history of *Isolona* and *Monodora*.

MORPHOLOGY

To arrive at the monograph of *Isolona* and *Monodora* over 2000 herbarium specimens originating from the following herbaria were examined: A, B, BM, BR, BRLU, C, COI, CSRS, DSM, E, EA, F, FHI, FHO, G, G-DC, H, HBG, K, L, LBV, LISC, LISJC, LISU, LMA, M, MA, MO, NHT, NY, OWU, P, PRE, S, SRGH, U, UPS, US, WAG, YA and Z. For some species, spirit material associated with herbarium collections in WAG was also observed. To assess species boundaries, the taxonomic utility of a large number of morphological characters was evaluated. Throughout this taxonomic study the phylogenetic species concept (Cracraft, 1983; Nixon and Wheeler, 1990) was followed in that the hypothesis on the delimitation of a species is formulated by identifying the smallest aggregation of individuals diagnosable by a unique combination of constant (or fixed) character states observable by ordinary morphological means (Snow, 1997). “Ordinary morphological means” refers to morphological characters that are immediately visible on the

herbarium sheets or require no more that 30x magnification (e.g. hairs). The consistency of character states across individuals of the same species is interpreted as reliable and indicative of the existence of a common history shared among them (Luckow, 1995; Snow, 1997). In addition, we do not consider a species to be polyphyletic, i.e. a group of individuals with several different most recent common ancestors.

Habit. The species of *Isolona* and *Monodora* vary from small to large, sometimes scandent trees. They are never lianas, even though some label descriptions indicate this. What has been observed is that some species start off as a small tree, but if growing near some kind of support (a big rock for example) it will have the tendency to grow against it becoming scandent, sometimes giving the appearance of a liana. The tallest individuals are found in the species *Monodora myristica* and *Isolona hexaloba*, both growing to maximum heights of 40 and 30 meters, respectively.

The architecture of *Monodora* trees follows Troll's Model (Hallé et al., 1978) which is one of the two common types found within Annonaceae, the other one being the closely comparable Roux Model. In the Troll Model the trunk axis is built secondarily from the proximal segment of a horizontal (or successive) axis (Hallé et al., 1978). Johnson (2003) indicated a direct correlation between the architectural model and the phyllotaxis of the primary axis (the trunk): species with the Roux model have spiral phyllotaxis; those with the Troll Model have distichous phyllotaxis. In that respect both *Isolona* and *Monodora* were described as having distichous phyllotaxis on the main axis.

Stem and branches. The trunk of *Isolona* and *Monodora* is usually straight. In some species of *Isolona* such as *I. hexaloba* it is clearly fluted. The bark varies in colour from light grey to dark brown, is striate or smooth, and can peel off easily in some species of *Monodora*. The trunk diameter at breast height (dbh) of mature individuals varies from ca. 3 cm up to one meter (e.g. some specimens of *M. myristica*).

Older branches are generally glabrous, although in some species they can be hairy (*I. deightonii*). The texture is generally horizontally striate or less often lenticellate (e.g. *M. grandidieri*). Young branches can be glabrous or hairy and usually dry black. Some species have a blue-greyish wax layer that is easily wiped off and is also found on very young leaves, petioles, flowering pedicels and fruits. This is mainly observed in fresh material but in *M. minor* it is very obvious in dried material too.

Most species of *Monodora* are deciduous. This is, however, not constant within species apparently depending on the seasonality of the different regions: seasons either weakly marked by rain and dry seasons (no shedding) or very marked with clear rainy and dry seasons (shedding). In *Isolona* leaf shedding has not been observed and is not apparent from herbarium sheets.

Branch growth type has been observed in *M. myristica* (Lamoureux, 1975) and *M. tenuifolia* (Njoku, 1963) and is described as sympodial, but appears as monopodial (pseudomonopodial). After expansion of a young twig at leaf flush, the terminal meristem

aborts. The axillary bud of the uppermost leaf then continues growth at the next leaf flush displacing the old bud to an axillary position, thus giving the appearance of monopodial growth. No growth type observations have been recorded for *Isolona*.

***Leaves*.** As for all Annonaceae, the leaves of both *Isolona* and *Monodora* are alternate, exstipulate and petiolate. The leaf lamina is simple, with entire margins and generally dorsiventral, i.e. both surfaces differ from each other. The size of the lamina can vary considerably between and within species of *Monodora*. The within-species variation is especially apparent between old leaves and younger ones on flower-bearing branches. The largest leaves are found in *M. myristica* where they sometimes attain a length of 50 cm. Within *Isolona* the variation is less extreme with the size being generally smaller than in *Monodora*. The apex of the leaf lamina is acuminate with the acumen being variable in length, or sometimes acute, rarely obtuse. An emarginate or rounded apex is occasionally observed on one or two leaves per specimen, but would appear to be related to a growth problem. The base of the leaf lamina is usually decurrent to obtuse or rounded, and sometimes cordate (e.g. in *M. myristica, M. grandidieri*, *I. pilosa*, Figure 6.1). When young, the lamina is typically pending, papyraceous or membranous and light green, when older they become horizontally spread, coriaceous and darker green. Leaves are either glabrous or covered with short appressed or straight hairs, which is normally invariable within species. In two hairy species, *M. grandidieri* and *I. heinsenii*, a fraction of collected specimens were, however, completely glabrous. Furthermore, a general trend towards a progressive loss of hairs is observed during maturity resulting in older foliage being glabrous.

In a few species the petioles can be very short (< 3 mm, e.g. *I. hexaloba, M. grandidieri*) or long in others (up to 14 mm in *M. myristica*, up to 10 mm in *I. lebrunii*). The insertion of the lamina is either on the top or on the side of the petiole (Fig. 6.1), and proves a useful taxonomic character in *Isolona*, especially for the glabrous species. Within *Monodora* there is hardly any variation, with just one species having an insertion on the top (*M. grandidieri*), all others having the lamina inserted to the side of the petiole.

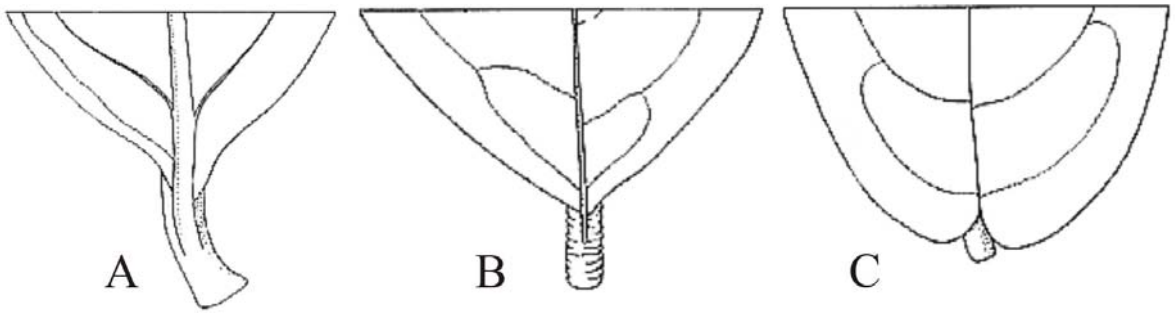

Figure 6.1. Three types of leaf bases and insertions found within *Isolona* and *Monodora*. A. Decurrent leaf inserted on the side of the petiole. B. Cuneate leaf inserted on top of the petiole. C. Cordate leaf inserted on top of the petiole.

Leaf venation. The midrib is always raised adaxially in both *Isolona* and *Monodora*. This provides a very useful character, as most Annonaceae have an impressed or sunken midrib. Only a few other African genera such as *Ophrypetalum* have raised midribs. Abaxially, the mid-rib is always prominent.

The secondary veins are usually arcuate, but can sometimes be straight only curving upwards towards the margins. The angle of divergence of the secondaries from the mid-rib is always acute. Venation is brochidodromous: secondaries joined together at the margin in a series of arches and interarches, i.e. loop forming. The tertiary venation is intermediate between percurrent and reticulate. *Monodora globiflora* is exceptional in being conspicuously percurrent, i.e. tertiaries are parallel between them.

Inflorescences. Both genera have two contrasting patterns in the origin of the inflorescence, representing the two main types found within Annonaceae: axillary and terminal (Fries, 1959).

Isolona is defined by a single-flowered rhipidium developing from the axillary leaf meristems (Fig. 6.2). Sometimes two or three additional single-flowered rhipidia are produced from extra-axillary meristems (Fig. 6.2, arrow). Following the terminology of Maas et al. (2003), both the peduncle and the sympodial rachis (Fig. 6.2 a and b) are indistinguishable from each other and minute, bearing 2-6 lower bracts. The upper bract, i.e. the bract above the flower articulation, is in general absent or abscises early in the development leaving a scar. The fact that the presence of this upper bract is not constant within a genus is rare within Annonaceae (but see Chatrou and Pirie, 2005). Sometimes, the lower bract is clasping to the pedicel. In *I. campanulata* the upper and lower bracts can sometimes be foliaceous and petiolate which is unique within the genus. Cauliflory, e.g. flowers developing on stems or old branches from retarded buds, is found within other Annonaceae genera (Fries, 1959), but is unusual in *Isolona*. It does occur, however, in *I. cauliflora* and has been observed in *I. cooperi* (pers. obs., living specimen in Botanical Garden of Utrecht University, The Netherlands). Flagelliflory only occurs in *I. cauliflora* in which the internodes of the sympodial rachis become elongated producing long inflorescences (up to 2 meters). The flagellae depart from the trunk and continue to grow on the forest floor. Within Annonaceae, flagelliflory originated independently in other unrelated genera such as *Anaxagorea* (Maas and Westra, 1984, 1985), *Duguetia* (Maas et al., 2003) and *Hornschuchia* (Johnson and Murray, 1995). It is unclear, however, what sort of evolutionary advantage such a state provides within Annonaceae.

In contrast, *Monodora* has solitary, terminal flowers with a mid- or sub-apical bract and no articulation (Fig. 6.3 A, B). The growth of the leaf continues from the axillary meristem, displacing the flowering pedicel to a leaf-opposite position. This happens almost simultaneously, thus stage A in Figure 6.3 is never seen as such. Independent of species, the inflorescences can sometimes appear axillary as they develop terminally on a minute, axillary and leafless shoot. Sometimes the flowers can be supra- or sub-axillary (Fig. 6.3 D, C) by coalescence of either the petiole or the pedicel, respectively. Additionally, the pedicels of

Monodora are pendulous. *M. minor* is exceptional as it possesses an inflorescence with two or three flowers in a rhapidium. The successive new flowers are borne from the bracts' axillary meristem. Moreover, the inflorescences are not pendulous but are erect above the foliage. Finally, cauliflory or flagelliflory is never found within *Monodora*.

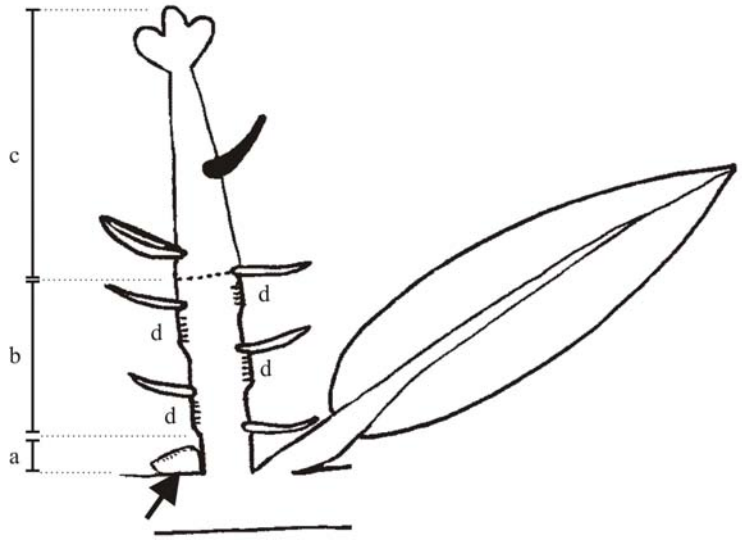

Figure 6.2. Inflorescence structure in *Isolona*. a. peduncle, b. sympodial rachis, c. flowering pedicel, d. scars of dropped flowers. Arrow indicates extra-axillary meristem. White bracts = lower bracts, black bract = upper bract.

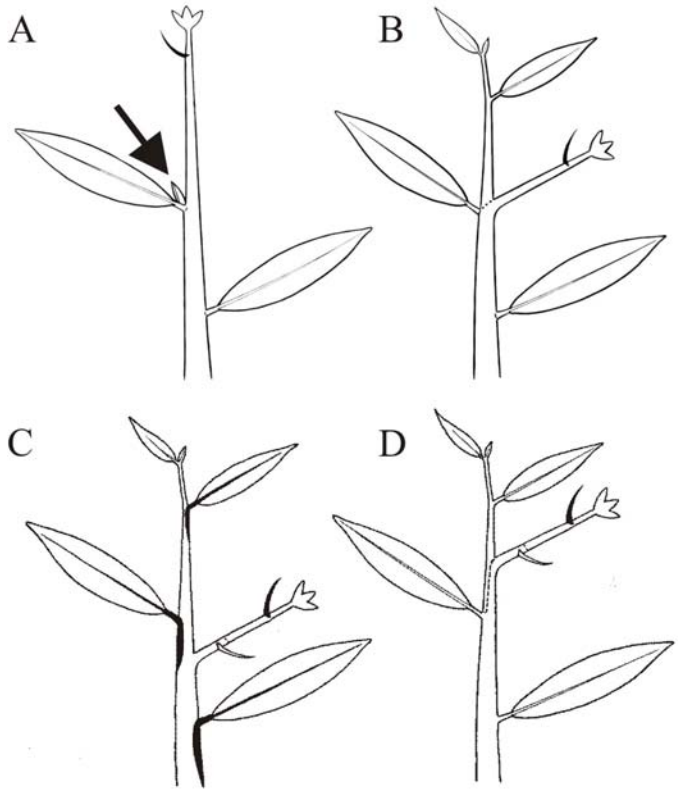

Figure 6.3: Inflorescence position in *Monodora*. A. Terminal flower with a sub-basal bract; arrow indicates axillary bud. B. Seemingly leaf-opposed position of flower due to overtopping by the axillary shoot. C. Supra-axillary position (fused petiolar part indicated in black). D. Sub-axillary position (dotted lines indicates fused part of axillary shoot). Modified from Maas et al. (2003).

***Flowers*.** As in most Annonaceae, *Isolona* and *Monodora* possess radially symmetric, bisexual flowers.

***Perianth*.** Flowers in both genera have one whorl of three valvate sepals, and one whorl of six basally fused petals. In *Isolona*, fusion of the petals is conspicuous, the tube being tightly appressed against the receptacle and the stamens, with six undifferentiated petal lobes (Fig. 6.4 C). In *Monodora,* however, the petals are inconspicuously fused for only a short length, ca. 1-3 mm (Fig. 6.4 B), occasionally up to 6-8 mm in *M. myristica* and *M. undulata* (Fig. 6.4 A). The free lobes are then differentiated into three "inner petals" alternating with three "outer petals", which is in fact the typical floral structure in Annonaceae.

In both genera the sepals are smaller than the petals. In *Monodora* the sepals vary in size and are oblong to ovate, with straight to clearly crisped or undulate margins. In *Isolona* they are usually small and inconspicuous (ca. 2-3 mm long), broadly to narrowly ovate, and generally appressed against the tube (Fig. 6.4 C). However, in a few species the sepals become large and papyraceous like in *I. campanulata* and *I. deightonii*.

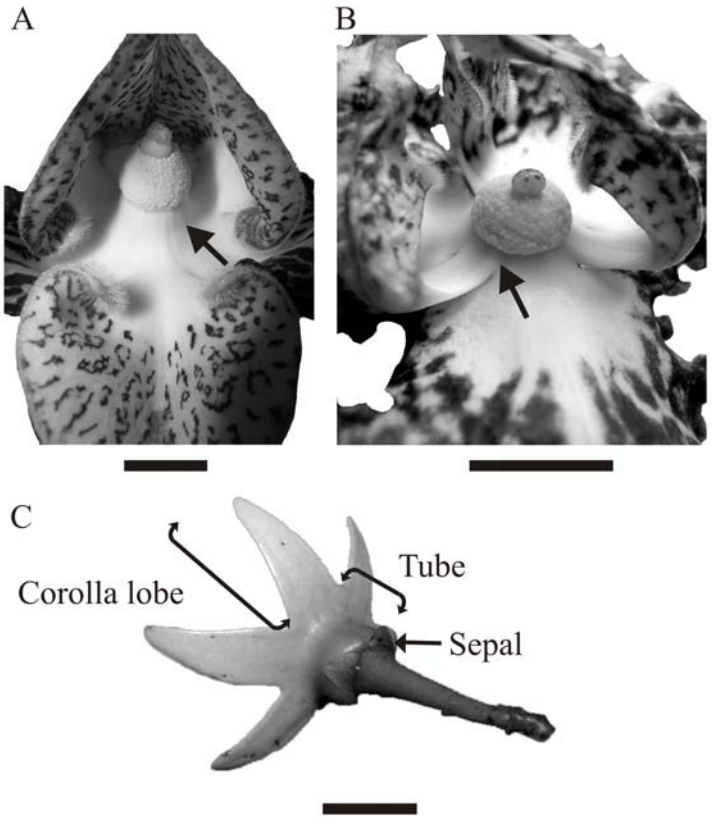

Figure 6.4. Petal fusion in *Isolona* and *Monodora*. A. Distinctly and basally fused petals, reflexed downwards along upper part of pedicel, inner and outer petals clearly differentiated (*M. myristica*). B. Indistinctly and basally fused petals, not reflexed, inner and outer petals clearly differentiated (*M. tenuiflolia*). C. Distinctly fused petals forming a tube around the receptacle, inner and outer corolla lobes equal in size, small sepals (*I. cooperi*). Scale bars: A-C: 10 mm.

The corolla lobes (Fig. 6.4 C) in *Isolona* provides one of the most important characters for species identification. Variation between species can be found in shape (linear to triangular), hairiness of inner and outer surfaces (glabrous all over, hairy inside or outside only, hairy all over), consistency of the lobes (coriaceous (e.g. *I. cooperi*), membranous or papyraceous (e.g. *I. ghesquieri*)), length of the lobes (short (e.g. *I. deightonii*) to very long (up to ca. 50 mm in *I. le-testui*)) and ratio between length of lobes and tube (usually the lobes are slightly to much longer than the tube, but in a few species the tube can be longer than the lobes (*I. deightonii* or in some specimens of *I. campanulata*)). In young flowers of *Isolona,* however, the lobes are usually shorter than the tube.

It is important to underline that lobe length can be very variable within species because of their continuous growth during development, even after anthesis, until abscission. The same phenomenon was also observed in *Cananga odorata*, the petals growing from ca. 10 mm up to 60 mm (Deroin, 1988). Herbarium notes related to field observations have been made on *I. hexaloba* by *C. Tisserant 220* and *R. Letouzey 5072* both indicating a significant variation in lobe size from the same individual. This was confirmed by my own observations in Gabon (*Sosef 2244*). This type of variability has previously led to the description of numerous species, citing a difference in flower size as the main distinguishing character (e.g. *I. seretii*, *I. brunellii*, both now synonymized with *I. hexaloba*). Colour of the corolla varies from bright yellow to dark red depending on the species and the stage of development. Generally young flower buds are light green becoming bright yellow, and then red to dark red at anthesis. Some species do not become red at anthesis, e.g. *I. cooperi* and *I. campanulata*. The reddening of the corolla generally starts from the inner side of the tube upwards, or occasionally from the tip of the lobes downwards (personal observations as well as numerous herbarium labels).

In *Monodora*, the outer petals are longer than the inner ones and vary in shape from narrowly oblong to ovate, or rarely linear (*M. stenopetala*). The margins are usually weakly or strongly undulate, sometimes crisped (*M. crispata*) or straight (e.g. *M. zenkeri*, *M. junodii*). The base of the outer petals is usually truncate but can be cordate. In *M. zenkeri* the base is uniquely characterized by the presence of two small lobes. When immature the outer petals are orientated straight downwards, slowly curving upwards during development, becoming horizontally spread or arching down at anthesis. The colour of the petals is reminiscent of some orchids, being in general white streaked with red or purple and yellow, hence the name Orchid tree for *M. tenuifolia*. Those of *M. hastipetala* are plain white without streaks, whereas in *M. junodii* they are plain red to purple.

The inner petals are always shorter than the outer ones and, in agreement with Bentham (1867), are one of the most taxonomically useful structures for identification. Each inner petal has a basal claw with a variably shaped lamina which can be either ovate (Fig. 6.5 A and C), cordate (Fig. 6.5 B and F), triangular (Fig. 6.5 E) or even cochleariform in *M. tenuifolia* (Fig. 6.5 D). The margins are usually straight (Fig. 6.5 A-D), undulate (Fig. 6.5 F), or crisped only in *M. crispata* (Fig. 6.5 E). Hairy appendages can be found in certain species. Those of *M. tenuifolia* are exceptional as they are positioned halfway up the petal lamina

providing an easily observed character. *M. myristica* and *M. undulata* both have hairy lobes at the base of the lamina of the inner petals (Fig. 6.4 A). The colour of the inner petals is generally the same as that of the outer petals. In some species the base of the inner petal lamina is distinctly yellow or red. The claw is always white and glabrous or sparsely hairy.

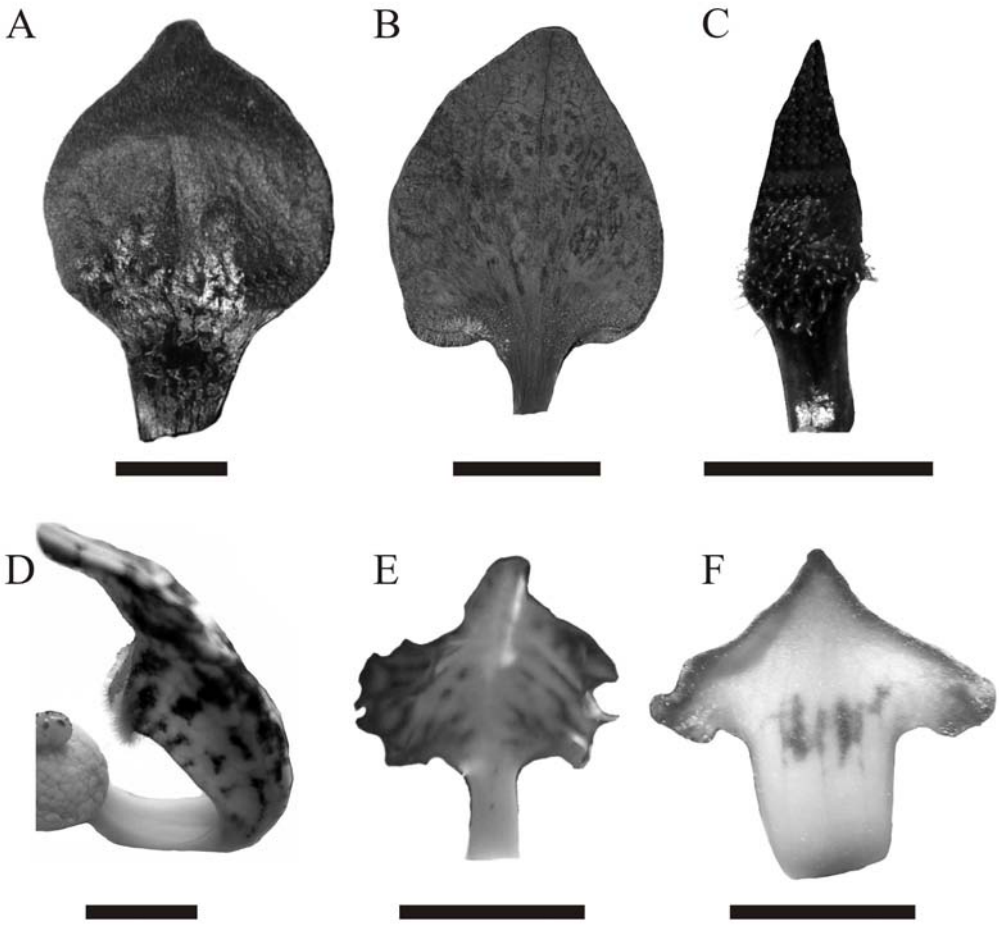

Figure 6.5: Morphology of inner petals in *Monodora*. A. Clawed-ovate inner petal (inner surface, *M. junodii*) B. Clawed-cordate inner petal, claw much shorter than lamina (inner surface, *M. myristica*), C. Clawed-ovate inner petal with attenuate apex (inner surface, *M. hastipetala*), D. clawed-cochleariform, with two hairy appendages halfway up the lamina (side view, *M. tenuifolia*), E. Clawed-circular with claw as long as lamina (outer surface, *M. crispata*), F. Clawed-cordate with claw longer than lamina (outer surface, *M. grandidieri*). Scale bars A-F: 10 mm.

The inner whorl of petals is marginally connivent forming a mitriform dome over the reproductive structures. The connivance can either be along most of the margins (Fig. 6.6 A) forming an almost completely closed dome, or just at the petal apices forming a dome that is basally open (Fig. 6.6 B). In some species, however, the inner petals are not connivent along the margins, but are appressed against each other at the middle of the lamina, forming an apically and basally opened dome (Fig. 6.6 C). Only *M. tenuifolia* does not exhibit connivance between inner petals (Fig. 6.4 B). The inner surface of the inner petals can be glabrous, covered with short (e.g. *M. junodii*) to long straight hairs. The hairs of *M. laurentii* are ribbon-like, which is unique within *Monodora*.

Within *Monodora*, the reported presence of glands on the inner side of the petals is not conspicuous, except in *M. junodii* (Fig. 6.5 A). However, anatomical studies to precisely characterize them have not been undertaken yet. In *Isolona* glands have not been observed nor reported in the literature.

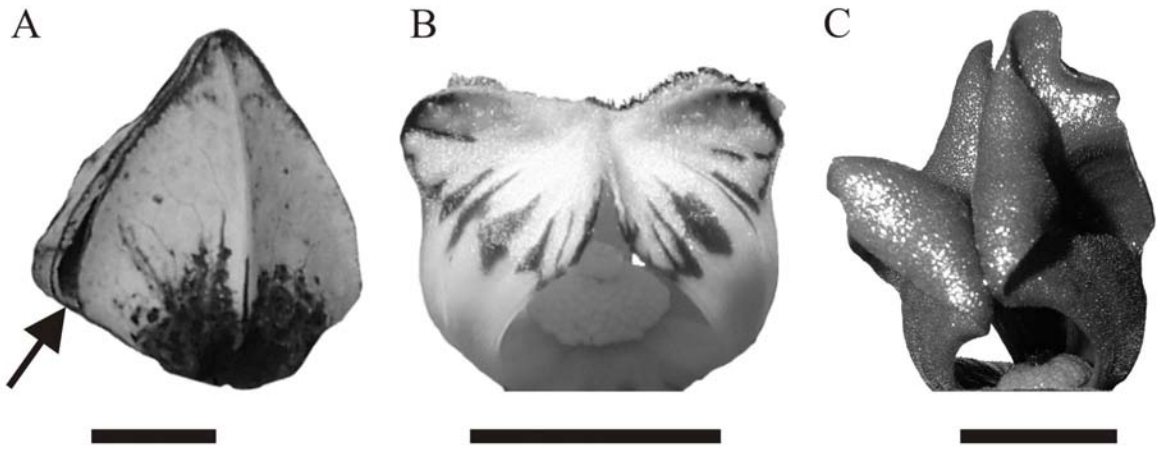

Figure 6.6: Arrangement of inner whorl of petals over the reproductive parts. A. Connivance along most of the margin, completely closed dome with basal access only, arrow indicates the line of connivance (*M. myristica*); B. Connivance along the apical part of the inner petal lamina, dome largely open at base (*M. carolinae*); C. Appressed at the center of the lamina, dome open (*M. junodii*). Scale bars A-C: 10 mm.

***Androecium*.** In *Isolona*, the staminate part of the receptacle is tightly compressed by the corolla tube, and is always slightly convex. In *Monodora*, the shape of the staminate part of the receptacle varies from flat or slightly convex (Fig. 6.6 B-C) to strongly convex in *M. myristica* and *M. undulata* (see Fig. 6.4 A). As for most Annonaceae, stamens are spirally arranged in both genera. In *Isolona* the number of stamen whorls is invariably three or four. In contrast, *Monodora* exhibits more variation, from three (*M. hastipetala* and *M. stenopetala*) to up to 18-20 whorls in *M. myristica*. The stamens of both genera are broad and flattened (sometimes elongated in a few species of *Monodora*) with a very short filament. The two thecae are united by a connective which is prolonged apically into a shield-like structure of 0.1-0.2 mm long, referred to here as the connective shield (Fig. 6.7), which can be glabrous to hairy.

Several species within *Monodora* have been shown to have septate anthers, i.e. the sporogenous cells are partitioned by transverse or longitudinal walls of sterile tissue (Lecompte, 1896; Tsou and Johnson, 2003). Septate anthers are less common than the aseptate anthers within angiosperms. However, in Annonaceae both forms can be found (Tsou and Johnson, 2003). Tsou and Johnson (2003) classified *Monodora minor* as having a T-type septum in which two or three layers of sterile cells are formed between each sporogenous cell (in contrast to the P-type septum characterized by three or more layers). In *Monodora*, the septae can only be observed using anatomical sections, this in contrast to other genera (e.g.

Neostenanthera, van Heusden, 1992). In *Isolona* the anthers appear to be aseptate seen from the outside, but anatomical sections are still needed to confirm this.

The length of the stamens ranges from 1-2 mm in *Isolona*, and from 0.5-1 mm in *Monodora* (exceptionally 1-2 mm in *M. myristica*). The connective shield of the innermost whorl is generally elongated over the ovary wall in *Isolona*, but is not or weakly elongated in *Monodora*.

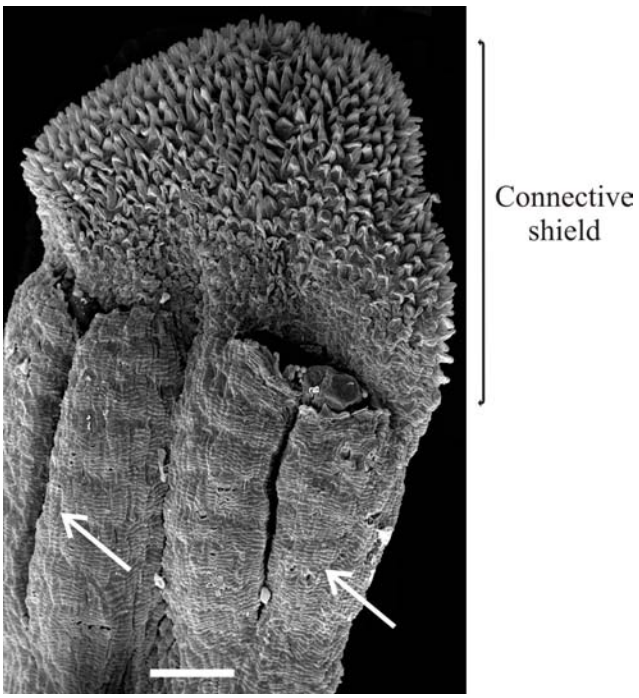

Figure 6.7. Connective shield. Apical part of a *Monodora tenuifolia* stamen. Arrows indicate the two thecae.

***Pollen*:** In Chapter 5 of this thesis, Couvreur et al. present a comprehensive study of pollen morphology and ultrastructure for both *Isolona* and *Monodora* as well as for three sister genera.

***Gynoecium*:** The carpels in both *Isolona* and *Monodora* are fused into a syncarpous gynoecium. This is a unique character in Annonaceae, and is rare in the early diverging magnoliid group (Endress, 1990; see Chapter 3). See the section on floral anatomy for more details.

Fruits. The fruits in both *Isolona* and *Monodora* are syncarpous. The shape is generally ovoid to ellipsoid or globose. In *Monodora* the diameter ranges from ca. 2 cm (*M. hastipetala*) to ca. 15 cm (*M. myristica*). In *Isolona* it varies between 2 and 10 cm in diameter. The surface of the fruits (Fig. 6.8) ranges from smooth to rugose or exceptionally farinose (*M. undulata*), and can be either conspicuously 6-7 ribbed (e.g. *I. heinsenii,* Fig. 6.8 F, *M. crispata,* Fig. 6.8 B), or with multiple inconspicuous ribs (e.g. *I. zenkeri, M. myristica*), or

irregularly ribbed (e.g *I. cauliflora,* Fig. 6.8 D*, M. angolensis,* Fig. 6.8 A). The pericarp of both genera is thick and woody (> 5 mm) or thin (< 4 mm). When it is thin, the pericarp usually constricts around the seeds in dried material only (e.g. *I. cooperi,* Fig. 6.8 E) but occasionally this also occurs in fresh material (e.g. *I. campanulata*). Fruits are generally glabrous, but in some of the most hairy species the fruits can be hairy too (*I. pilosa* or *M. grandidieri*).

Fruits of *Monodora* have a strong taxonomic utility with each species having its unique set of characters (see key). In *Isolona*, however, there is less taxonomic information, with some species being indistinguishable when in fruit.

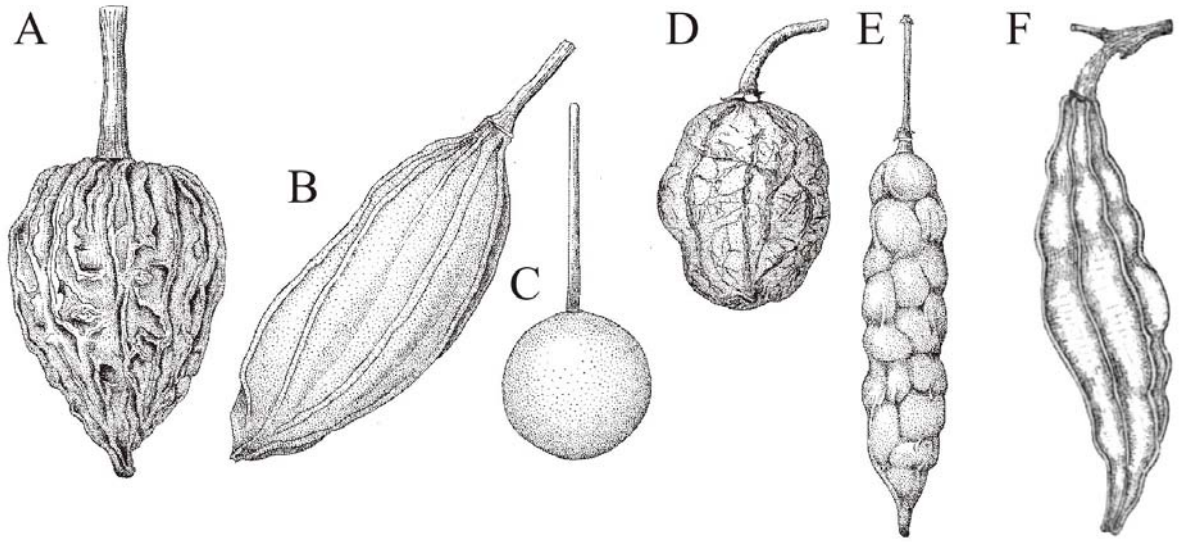

Figure 6.8. Fruit morphology in *Isolona* and *Monodora*. A. Fruit ovoid with irregularly ribbed pericarp (*M. angolensis*). B. Fruit conic with regularly ribbed pericarp *(M. crispata*). C. Fruit globose with smooth pericarp (*M. tenuifolia*). D. Fruit globose with irregularly ribbed pericarp (*I. cauliflora*). E. Fruit conic with pericarp constricting around seeds (*I. cooperi*). F. Fruit conic with regularly ribbed pericarp (*I. heinsenii*). A-E reproduced from van Setten and Koek-Noorman (1992) with permission from E. Schweizerbart'sche Verlagsbuchhandlung (Naegele u. Obermiller); http://www.schweizerbart.de.

***Seeds*.** The seeds are arranged in no apparent order in the syncarpous fruit of both *Isolona* and *Monodora*, which is unique within the family where seed arrangement is always uni or biseriate. Seed number ranges from ca. 10 in the smallest fruits to numerous in the larger ones (up to ca. 600 in *M. myristica*, Chapman and Chapman, 1996). Seed shape is generally ellipsoid (Fig. 6.9: a, d, e, f, h, i) to hemi-spherical (Fig. 6.9: b, c, g). The length of the seed is generally less than 20 mm, but can become to 30 mm in *I. lebrunii* (Fig. 6.9: d). The testa is always smooth in *Monodora* (Fig. 6.9: e-i), but varies from smooth (Fig. 6.9: c-d) to rough (Fig. 6.9: b) to rugose (Fig. 6.9: a) in *Isolona*. The colour of the testa varies from light to dark brown. The raphe can be either thickened or not. In *Monodora*, only two species have a conspicuously thickened raphe (Fig. 6.9: g and h), while in *Isolona* this feature is more common. In the species without a thickened raphe it is of a darker colour than the testa (for example Fig. 6.9: d, e, i). The hilum is located near the micropyle and is generally narrowly elliptical or oblong. Some seeds give off a strong spicy or sweet scent when crushed or

opened (e.g. *I. cooperi or M. myristic*a).

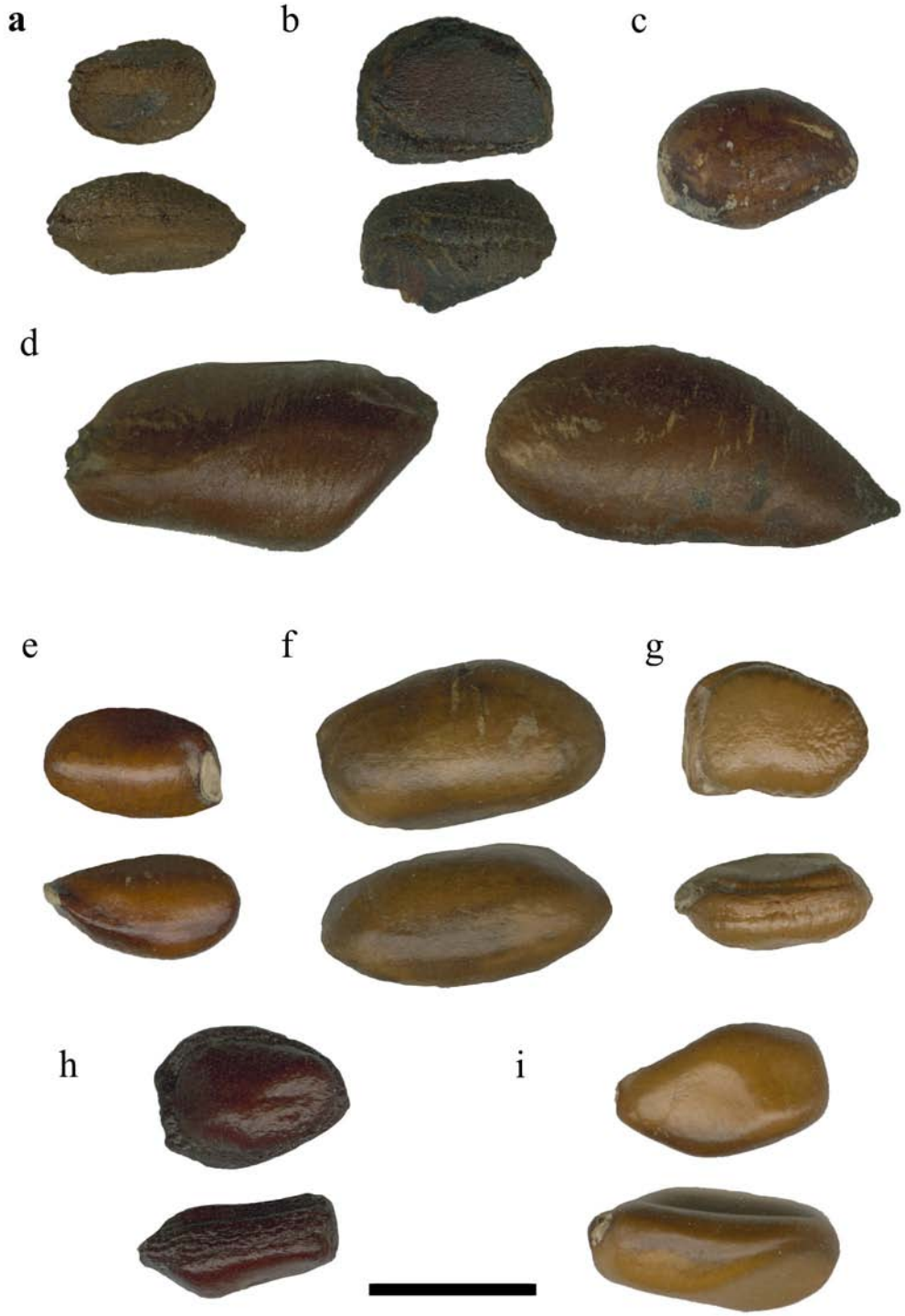

Figure 6.9: Seeds morphology in *Isolona* and *Monodora*. a. Seeds with finely rugulose testa and a thickened raphe (*I. campanulata*); b. Seeds with rough testa, raphe thickened (*I. cooperi*); c. Seed with smooth testa, raphe not thickened (*I. zenkeri*); d. Seeds large with smooth testa, raphe not thickened (*I. lebrunii*); e. Seeds smooth, raphe not thickened (*I. angolensis*); f. Seeds large with smooth testa; raphe not thickened (*M. myristica*); g. Seeds smooth with smooth and light brown testa, raphe thickened (*M. tenuifolia*); h. Seeds with smooth and dark brown testa, raphe thickened (*M. minor*); i. Seeds with smooth testa, raphe not thickened (*M. undulata*). Color scale = scale bar: 10 mm.

FLORAL ANATOMY

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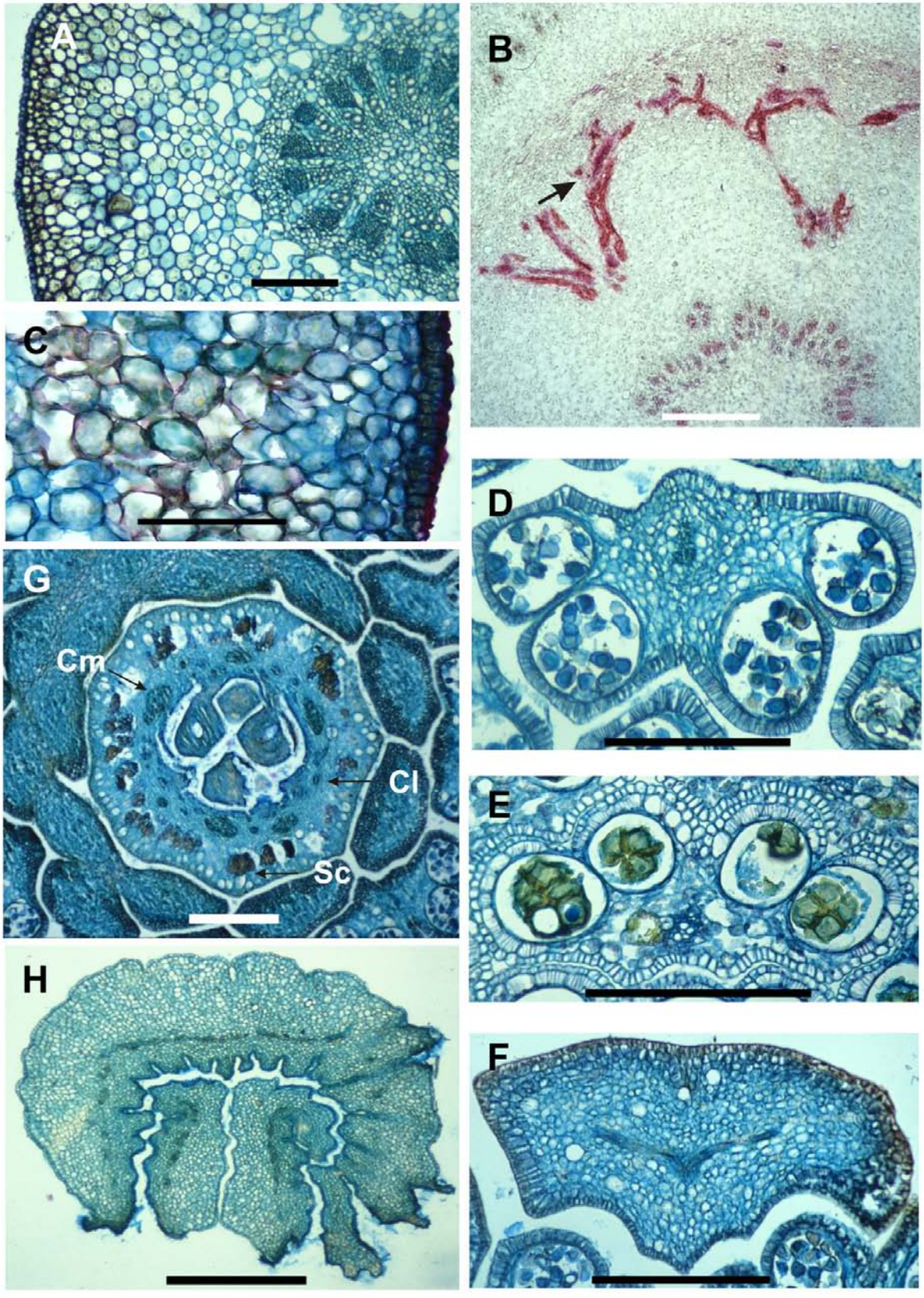
Materials and Methods

Four specimens of *Monodora* (*M. crispata* (De Koning 5131, WAG), *M. minor* (Couvreur 36, WAG), *M. myristica* (De Koning 1146, WAG) and *M. undulata* (Aké Assi s.n., P)) as well as three specimens of *Isolona* (*I. campanulata* (Aké-Assi s.n., P), *I. cooperi* (Bos 1609, WAG) and *I. heinsenii* (Couvreur 10, WAG)) were selected covering a wide geographical range across Africa (from West to East). Specimens were rehydrated by an aqueous 20% NH_4OH solution at 60°C, postfixed in F.A.A.. They were then dehydrated in a butanol series (Gerlach, 1984) and infiltrated in paraffin (Histomed, melting point: 60°C). Microtome sections were made at a thickness of 15-20 µm and were stained by a combination of Astrablue 0.5% / Ziehl's Fuchsine 10% (or sometimes by Toluidine Blue after Sakai or only Safranine, but results were less satisfactory), then dehydrated and mounted in Eukitt. All anatomical slides are kept in the plant anatomical slide library of the Museum National d'Histoire Naturelle in Paris (Dept. Systematics and Evolution). Floral vasculature was reconstituted by drawing the serial sections using a camera lucida, and then by superimposing tracing papers over them.

Comparative Histology

Pedicel. Both genera are characterized by a glabrous and sometimes papillose epidermis with wide intercellular spaces in the parenchyma, large secretory cells and sclereids scattered or into small clusters (Fig. 6.10: A). The pedicel contains 12 to 25 circularly arranged vascular bundles. In transverse section the phloem is rounded and has outer fibers during anthesis. The xylem poles are wedge-shaped with vessels in 1 to 4 lines. Finally, as for most Annonaceae, the pith contains incomplete sclerenchymatic diaphragms which are especially apparent in *Isolona*.

Perianth. Sepal and petal histology is comparable to that of the leaf lamina being composed of undifferentiated parenchyma cells. The inner part of the petal base however, appears to be slightly altered possibly in relation to pollination aspects. In *Isolona cooperi*, parenchyma found on the inner side of the perianth tube appears to be very rich in starch with a thick and cutinized epidermis (Fig. 6.10 C). This combination would suggest a cantharophilous (e.g. beetle pollinated) adaptation.


A
B
C
D
G
Cm
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Sc
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<-- Figure 6.10. Floral anatomy in *Isolona* and *Monodora*. A. Transversal section of the lower pedicel region in *Isolona cooperi*. B. Transversal section of the perianth region in *Monodora crispata*. The arrow indicates a girdling bundle. C. Transversal section showing a detail of the tube in *Isolona cooperi* (adaxial side). Note the thick epiderm and starch cells (in red). D. Transversal section of the anther in *Isolona cooperi* (floral centre above) E. Transversal section of the anther in *Monodora crispata* (floral centre below). F. Transversal section of the connective shield in *Isolona cooperi*. Note the 'v' shape of the vascular bundle. G. Transversal section of the gynoecium in *Isolona cooperi*. Cm: Carpel medium bundle; Cl: carpel lateral bundle; Sc: secretory cells. H. Transversal section of the stigmatic plate in *Monodora crispata*. Note the separation between the three stigmas. Scale bars: C; 0.1 mm; E, A: 0.2 mm; B, D, G, H: 0.5 mm.

***Stamen*.** The two genera exhibit to a certain extent different anther histology. In *Isolona*, the epidermis is discontinuous as it does not cover the locules and stomia and, the endothecium and the hypodermis are separated by intermediate cells being indistinguishable (Fig. 6.10 D). In *Monodora,* the epidermis is continuous throughout the stamen and the endothecium is sharply distinguished from the hypodermis with no intermediate tissues (Fig. 6.10 E). However, *Isolona* and *Monodora* do present common traits such as a spongy parenchyma, large but scattered secretory cells and amphiphloic vascular bundles that dichotomize in the connective shields (Fig. 6.10 F).

***Carpel*.** The gynoecium in both genera is syncarpous (for a review see Deroin, 1997) and almost always trimerous, or slightly derived from a trimerous condition. In both genera only the three tallest carpels bear stigmas. The stigma is characterized as involute (Deroin, 1991), i.e. they are fused into a single thick, radially striate lamina, always trimerous in outline (Fig. 6.10 H).

The histology of the gynoecium is not well differentiated at anthesis. However, it is worth noting the glabrous epidermis as well as the presence of the two differentiated parenchymatous zones in the ovary wall (Fig. 6.10 G). The outer layer is composed of wide parenchyma and contains scattered secretory cells. The inner row has smaller parenchyma and contains the vascular system which is composed of alternating large and small vascular bundles. The large ones represent the median carpel bundles (Cm, Fig. 6.10 G), while the smaller ones represent the lateral carpel bundles (Cl, Fig. 6.10 G) which are often fused in synlateral bundles. Moreover, this venation is merged in a tangential meshwork of mainly phloemian thin traces.

Comparative vascular anatomy of the receptacle (Fig. 6.11)

***Sepals*.** In *Isolona*, the lateral sepal bundles are not (E) or shortly fused (F). In *Monodora*, the lateral sepal bundles are generally fused into synlaterals (A-D), and assume the appearance of horizontal girdling bundles (Fig. 6.10 B) which interconnects the perianth vascular system *(sensu* Sporne, 1977).

Petals. Fusion of the lateral petal bundles is slightly variable in both genera. However, as a rule those of *Isolona* are free (E-F), i.e. petals are fed by a unique trace (except in *I. heinsenii* where they are fused, G). This implies that sympetaly in *Isolona* is parenchymatous in nature. In contrast, the lateral bundles of *Monodora* are fused into synlaterals (B-D), except in *Monodora crispata* where each petal is independently irrigated (A). Thus, it appears that the degree of fusion of the petals observed at the macro-morphological scale is reversed at the anatomical one.

Cortical vascular system. In both genera, the floral vascular system is rather regular and is normally characterized by a petal-staminal cortical vascular system (CVS), where the petal and stamen bundles are fused. This represents the most common type of CVS in Annonaceae. In *I. heinsenii*, the CVS was found to follow the perianth-stamen cortical vascular pattern. The CVS in *Monodora* is wider both in the horizontal and vertical planes than in *Isolona* and, frequently involves large parts of the sepal bundles (especially synlateral ones, but sometimes the median bundles too, as in *M. minor*).

Androecium. There are generally 18 stamen trunks irrigating the androecium in both genera. However, in *M. undulata,* they are 12 dichotomizing bundles (1-24 in D) which are fused to the CVS, with 12 free additional superimposed bundles (25-36) feeding the stamens. This combined androecial vasculature was already recognized in another Annonaceae genus *Asimina* by Smith (1928). In *I. cooperi*, the pentamerous gynoecium leads to a slightly altered number of stamen trunks (20 instead of 18). In *I. heinsenii*, the alternative CVS pattern allows the recognition of only 9 stamen trunks.

Gynoecium. Vascularisation results from a rearrangement of the central stele, whose bundles bend and branch towards the centre so that a plexus is built, in which the gynoecial traces are inserted. The occurrence of this generally phloemian plexus just below the ovary floor might be paralleled with the evolution towards numerous carpels crowded on a flat receptacle, since the same structure can be recognized in *Cananga, Piptostigma* and *Xylopia*.

Carpels. In *Isolona* six carpels form the syncarpous gynoecium, except in *I. cooperi* which was found to be pentamerous (i.e. composed of 5 carpels). In *Monodora* the number of fused carpels is variable ranging from 6 to 14, more or less ordered in 2-3 levels. As for most Annonaceae, the ovules are fed by the median carpel bundles, while the seeds are linked to the lateral -or synlateral- bundles.

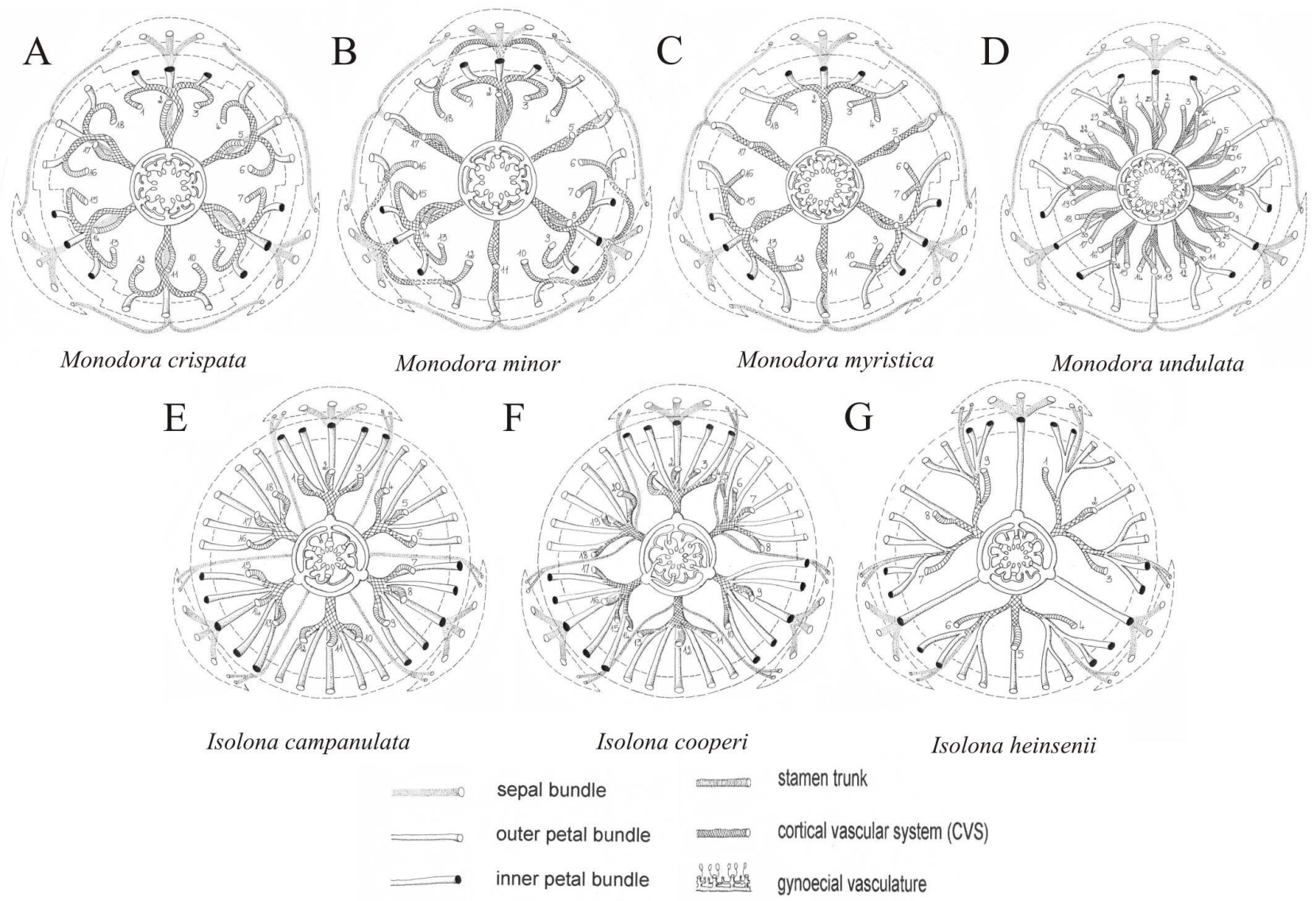

Figure 6.11: Diagrams of the different vascular systems found with *Isolona* and *Monodora*. Drawings by Thierry Deroin.

POLLINATION BIOLOGY

Studies on pollination within both genera are scarce. The flowers are generally thought to be insect pollinated as in most Annonaceae (Silberbauer-Gottsberger et al., 2003). Only one published detailed study has been carried out on pollination in *Monodora*. Lamoureux (1975) studied the floral biology of *M. myristica* on individuals growing in the Botanic Garden of Bogor in Indonesia, thus not in its natural environment. Flowers in *M. myristica* are long-lived, lasting about 25 days with an anthesis period of ca. 12 days. The gynoecium is apparently protogynous, with the production of a viscous transparent liquid covering the stigmas. Such exudate was also observed in many other species of *Monodora* (pers. obs.; Meinke, 2008) and appears before stamen maturity. Lamoureux (1975) observed that the position of the outer petals changes slowly during the initial stages of development. At first they are tightly appressed against the inner petals preventing access to the reproductive organs which are contained in a pollination chamber. They soon start to reflex upwards from the hanging flower occupying an arched position at female anthesis, allowing access sideways in between the inner petals to the chamber. The stigmas eventually dry up and fall, after which male anthesis is initiated with the release of pollen. The colour of the streaks of the outer petals changes during the male anthesis turning from brown-purple to bright red. Finally, the stamens drop off and, at least in the Bogor trees, the flower abscises. Lamoureux (1975) indicates that the individuals in Bogor are all part of the same clone and have never set fruit, even in the presence of visiting insects or when artificially pollinated. This would suggest that a self-incompatibly mechanism is operational in *Monodora*. In its native range it is thought that *M. myristica* is fly pollinated (Keßler, 1993), a pollination vector that has also been reported for *M. tenuifolia* (Meinke, 2008) and *M. crispata* (Gottsberger, 1985). Although no detailed studies have been undertaken, and most conclusions were based on field observations, *Monodora* flowers present several characters common to the fly pollination syndrome: the floral structure, particularly the mitriform inner petals, petal pigmentation being usually streaked with dark red-purple and anthesis being diurnal (van der Pijl, 1961). However, the flowers do not emit an unpleasant odour but a sweet scent and would thus be myophylous rather than sapromyophylous (pollinated by flies attracted by unpleasant odors). Myophyly is a common pollination syndrome in the “basal angiosperms” (see Thien et al. 2000). Within Annonaceae fly pollination is thought to be more common in the Paleotropics than in the Neotropics (Gottsberger 1999; Silberbauer-Gottsberger et al. 2003) and has been documented in several species of the South-East Asian genus *Pseuduvaria* (Silberbauer-Gottsberger et al. 2003; Su and Saunders 2006) and in the African species *Uvariopsis bakeriana* (Meinke 2008). In the Neotropics it has been documented from a species in *Annona* (Gottsberger 1999). However, more studies have to be undertaken to confirm myophyly within *Monodora*.

No pollination studies have been published for *Isolona*. A few field observations have been made, but these do not shed light on possible pollinators. The stamens of *Isolona* are not accessible via the top because they are compressed by the tube and are protected by the

connectives. In *I. hexaloba* holes in the lower parts of the tubes were observed, apparently caused by the feeding of some kind of insect possibly trying to gain access to the stamens and nutritious pollen. The flowers of *Isolona* emit a sweet scent, and change colour during maturation, from green to yellow to red. Anatomical studies revealed a tube rich in starch which could imply pollination by beetles (see above).

More studies should be performed on both genera because given their divergent floral and pollen morphology (see Chapter 3 and 5) they might be expected to exhibit contrasting pollination syndromes. Their divergent morphologies could be the result of adaptation to different pollinators, which could be a major evolutionary development having caused the split between these two sister genera.

FRUIT AND SEED DISPERSAL

Fruit and seed morphology suggests that seeds of both genera are dispersed by medium- to large-bodied vertebrates. In a recent study undertaken in Uganda, Balcomb and Chapman (2003) confirmed the critical role played by large arboreal frugivores, such as chimpanzees or mangabeys, dispersing over 85% of the seeds in *M. myristica*. Such large-bodied frugivores were the only ones capable of breaking open the hard pericarp. In general, seeds deposited away from the parent tree had a higher recruitment success than those left under the parent tree, indicating the important role of dispersal (Balcomb and Chapman, 2003). A small percentage of seeds was also dispersed by medium-bodied primates such as small monkeys (ingested after the fruit was handled by the larger frugivores) via single seed spitting (i.e. not eaten, but kept in mouth patches and spat out one by one). Such dispersal was shown to be less effective when compared to seeds ingested and deposited in dung by the larger frugivores. Post dispersal seed predation by beetles or rodents also had a major influence in the successful establishment of a seedling. In this respect, secondary dispersal via dung beetles, inadvertently including the seeds in their buried dung, has been shown to play a positive role in enhancing seed survival in *M. myristica* (Shepherd and Chapman 1998). If the seed is buried at an optimum depth (dependent on the species) and in a favourable location for germination, it can escape predation as well as density dependent selection from the other seeds.

Other important large-bodied vertebrates shown to eat *Monodora* fruits include the western lowland gorillas (*Gorilla gorilla gorilla* and *G. g. deilhi*) in the Congo basin, Gabon and Cameroon (Rogers et al., 2004) and elephants (*Loxodonta africana*, Balcomb and Chapman, 2003).

No observations have been made on seed dispersal in *Isolona*, but given the similar fruit morphology it can be assumed that large mammals may also represent an important vector.

DISTRIBUTION AND HABITATS

Isolona and *Monodora* grow from tropical West to East Africa, with *Isolona* also occurring in Madagascar. When both genera are taken together, there appear to be three main centres of diversity. The most diverse centre is situated in western Central Africa (Fig. 6.12 A and B, Cameroon to Gabon). In this centre most of the species have relatively wide distributions (Table 6.2). The only exception is *M. zenkeri*, which is endemic to the southern half of Cameroon. The second centre of diversity is found in Tanzania (Fig. 6.12 E) and coincides with the montane forests of the Eastern Arc Mountains and the coastal forests, a pattern common to most Annonaceae genera (Couvreur et al., 2006). In contrast to the first centre, narrow endemics are frequent, most of the species being endemic to smaller geographic regions (Table 6.2). Finally, a smaller centre of diversity is located in northern Democratic Republic of Congo, near the town of Yangambie. This could, however, also be the result of a collecting bias, since that area was intensively sampled.

Both genera are adapted to wet and humid climatic conditions, growing in dry-land vegetation but also near fresh water swamps or rivers. *Isolona* is mainly found in lowland rain forest and occasionally in montane rain forests. *Monodora* also grows in lowland and montane rain forests, but is also found in more xeric ecosystems such as savannas or thickets, especially in East Africa. It is apparent by the much wider distribution of *Monodora* across Africa (Fig. 6.13 A, B) that it has adapted to a wider range of habitats than *Isolona*.

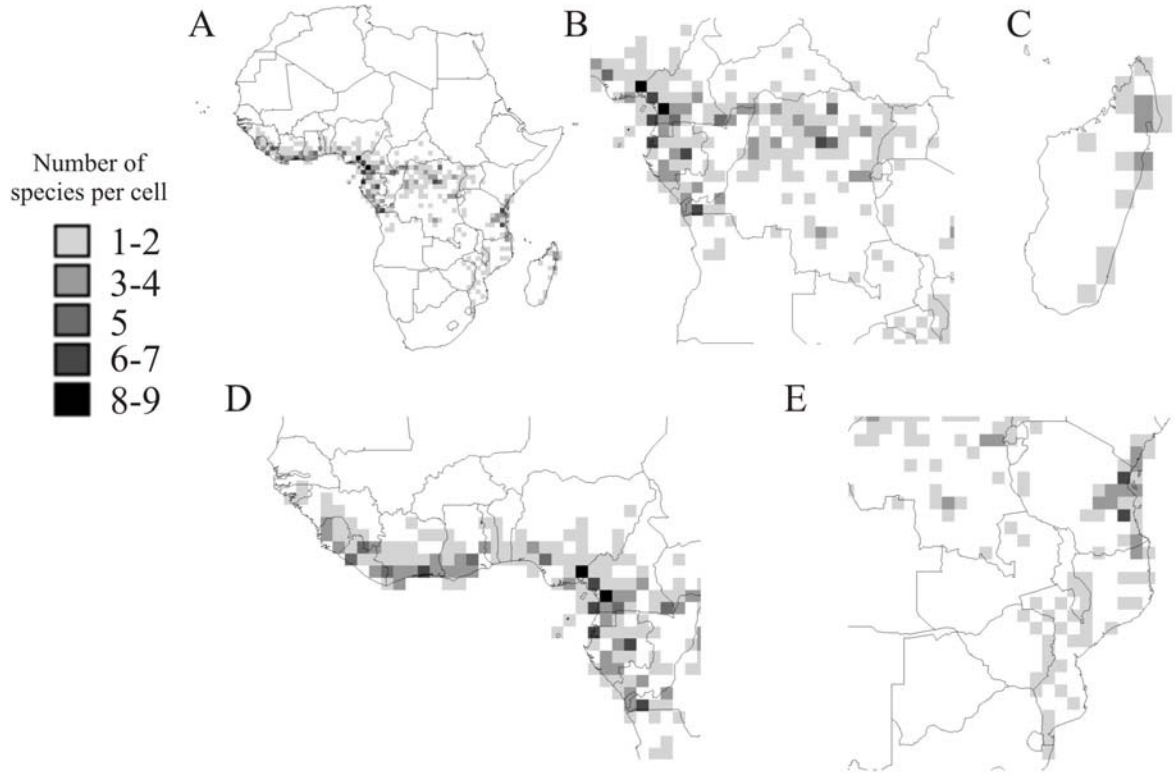

Figure 6.12. Species richness in 100x100 km cells for both *Isolona* and *Monodora*. A. Africa and Madagascar; B. Central Africa; C. Madagascar; D: West and west Central Africa; E. East Africa.

Geographical region/Country	Total number of species (endemics) in *Monodora*	Total number of species (endemics) in *Isolona*	Total
WEST AFRICA	**5(0)**	**5(0)**	**10(0)**
Ghana	4	4	8
Guinea	3	0	3
Ivory Coast	5	4	9
Liberia	4	2	6
Sierra Leone	4	2	6
Togo	2	0	2
WEST CENTRAL AFRICA	**7(1)**	**11(3)**	**18(4)**
Angola	2	2	4
Cameroon	6(1)	9	15(1)
Equatorial Guinea	5	0	5
Gabon	5	7	12
Nigeria	5	6	11
Republic of Congo	4	4	8
Sao Tomé & Principe	2	0	2
CENTRAL AFRICA	**4(0)**	**7(0)**	**12(0)**
Burundi	0	1	1
Central African Republic	3	3	6
Democratic Republic of Congo	4	6	10
Sudan	2	0	2
Uganda	2	1	3
EAST AFRICA	**9(7)**	**3(3)**	**12(10)**
Kenya	3	1	4
Malawi	3	0	3
Mozambique	5	0	5
Tanzania	8(2)	3(1)	11(3)
Zambia	2	0	2
Zimbabwe	1	0	1
MADAGASCAR	**0**	**5(5)**	**5(5)**

Table 6.2. Species richness and endemism of *Isolona* and *Monodora* in each major geographical region and country in mainland Africa and Madagascar.

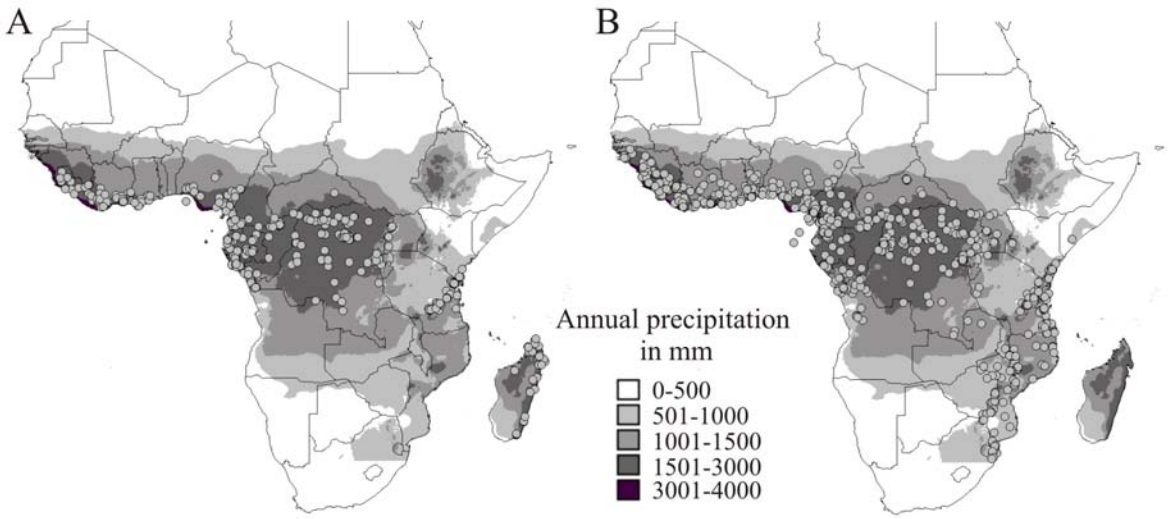

Figure 6.13. Distribution map of *Isolona* and *Monodora* overlaid with annual precipitation. A. *Isolona*; B. *Monodora*.

Except for the tallest species, *Isolona* and *Monodora* grow in forests with a low canopy (ca. 20 m) and are rarely found in primary forests with a canopy higher than 30 m. This can be in association with some degree of disturbance, like flooding or to some extent human impact.

Monodora generally grows on rocky soils, whereas *Isolona* is found on sandier soils. In East Africa both genera are found on coral rags or rocky soils.

ETHNOBOTANY

For species in both genera, a variety of ethnobotanical uses have been reported. The seeds of some *Monodora* species are of widespread and economic importance (e.g. *M. myristica, M. undulata*, Burkill, 1985). The seeds are ground into a powder and used as a condiment in food. The flavour of the seed resembles that of nutmeg (*Myristica fragrans*). In addition, seeds, leaves, bark and roots are also used in medicine for humans but also for dogs (in Sierra Leone). In *M. myristica* the essential oils showed antifungal and antibacterial activity (Tatsadjieu et al., 2003). Finally, *M. crispata*, *M. myristica* and *M. tenuifolia* are also cultivated as ornamental plants in gardens across the tropics and in green houses in temperate regions because of their large and spectacular flowers.

In *Isolona*, seeds have not been reported to be used as a condiment, but the powder of the bark is used in medicine and witchcraft. The hard wood of certain species is used in construction (Burkill, 1985).

MOLECULAR PHYLOGENETICS

Materials and Methods

Taxon sampling. A total of 33 samples representing 27 ingroup and 3 outgroup species were included in the analysis. Of the 14 species recognized in *Monodora* 13 were sampled, with *M. myristica* represented by two specimens. For *M. zenkeri* only herbarium material was available and PCR amplification of the DNA extracted from this was unsuccessful. In *Isolona*, 14 out of the 20 recognized species were included, with *I. zenkeri* and *I. congolana* represented by two specimens each. Amplification of the DNA of the six missing species from herbarium material was unsuccessful. *Uvariopsis vanderystii*, *Guatteria pudica* and *Fusaea peruviana* were selected as outgroups (Couvreur et al., in press).

Character sampling. Five plastid makers were sequenced: *trnL-trnF*, *psbA-trnH*, *trnS-trnG*, partial *ndhF* (last 600 bp of whole marker) and *trnD-trnT*. PCR protocols for these markers are the same as in Chapter 3. For the amplification of *trnD-trnT* the protocol was the same, except for the annealing temperature that was set at 53-58 °C depending on the sample. However, amplification of *trnD-trnT* failed for ten samples. Additionally, amplification of *ndhF* failed for three samples.

Phylogenetic analyses. Maximum Parsimony (MP) analyses were performed on the combined dataset using PAUP* (version 4.10b; Swofford, 2002). Heuristic searches were performed with 100 random sequence addition iterations, saving 100 trees in each, with tree bisection-reconnection branch-swapping. After completing the iterations, all trees found were then used as starting trees for another round of swapping with a limit of 5000 trees. The strict consensus tree was computed using the remaining trees. Relative support for each node was assessed by performing 1000 bootstrap replications (Felsenstein, 1985; Salamin et al., 2003) with TBR branch swapping (ten random addition sequences, saving ten trees per replicate).

For the Bayesian analyses, the same approach as in Chapter 3 was adopted here.

Results

Infra-generic relationships. Couvreur et al. (in press, or see Chapter 3) clarified the phylogenetic relationships of a number of African Annonaceae genera. *Isolona* and *Monodora* were recovered as strongly supported sister genera (Couvreur et al., in press) nested within the so-called long branch clade (Richardson et al., 2004). In addition, both genera belonged to the largest and most species-rich clade of endemic African genera referred to as the African Long Branch clade. This clade can be characterized by tetrad pollen grains (except for *Isolona* which has reverted to a monad state) and a sessile monocarp base (Couvreur et al. in press; or Chapter 3). Sister to *Isolona* and *Monodora* were three small genera namely *Asteranthe* (endemic to East Africa), *Hexalobus* and *Uvariastrum*.

Species-level relationships. Both the MP and Bayesian analyses returned the same tree topology, but the Bayesian one (Fig. 6.14) provided higher overall branch support. Higher posterior probability values when compared to bootstrap values is normal in such analyses (Suzuki et al., 2002). The Bayesian majority rule consensus tree has been used for the interpretation. The partial *ndhF* sequence had the highest number of phylogenetically informative characters (PICs) but generated an unresolved overall tree, the monophyly of *Monodora* being the only strongly supported relationship. The marker *trnS-trnG* provided the best overall support for a single marker with the greatest number of strongly supported nodes. Finally, *trnD-trnT* had the lowest number of PICs and returned a largely unresolved tree. Table 6.3 provides the main statistics for each marker.

marker	*trnL-trnF*	*trnS-trnG*	*psbA-trnH*	*part. ndhF*	*trnD-trnT*	Total
# of included characters	967	778	534	627	1252	4163
PIC	44	62	26	57	19	219
% of PICs	**4.6**	**8.0**	**4.9**	**9.1**	**1.5**	**5.3**

Table 6.3. Main statistics of the five plastid markers used in the phylogenetic analysis of *Isolona* and *Monodora*. PIC = phylogenetically informative character.

With the combined dataset, the species-level phylogenetic relationships within *Isolona* and *Monodora* were generally well supported (Fig. 6.14). The monophyly of both genera was strongly supported in the Bayesian and MP analyses.

Within *Monodora* two major, well-supported clades were identified: a West-Central African clade and an East African clade. Resolution within the West-Central clade was poor. The sister position of *M. angolensis* to the rest of the clade is the only strongly supported relationship (posterior probability = 1.00). The rest of the West-Central taxa form a moderately supported clade (PP=0.89). Finally, *M. myristica* forms a moderately supported clade with *M. laurentii*, while the positions of *M. crispata*, *M. tenuifolia and M. undulata* are not resolved. Within the East African clade, all relationships received maximum support, except for the sister relationship of *M. minor* with a clade containing *M. carolinae, M. globiflora* and *M. stenopetala*.

Within *Isolona*, only sister species relationships received strong support, while deeper relationships often remain unresolved. It is unclear still why there is a difference in resolution between the two genera. Only one major clade was strongly supported and contained the Malagasy, East African and two West-Central taxa. The monophyly of the sampled Malagasy taxa (3/5) was strongly supported, while the relationships between the three East African species remained unresolved. This phylogeny provides support for a few taxonomic decisions taken within this monographic study: (1) the synonymy of *I. maitlandii* (specimen *I.*

congolona from Cameroon, APPENDIX B) with *I. congolana*, (2) the distinctiveness of *I. hexaloba* from *I. pleurocarpa*, previously considered as synonyms.

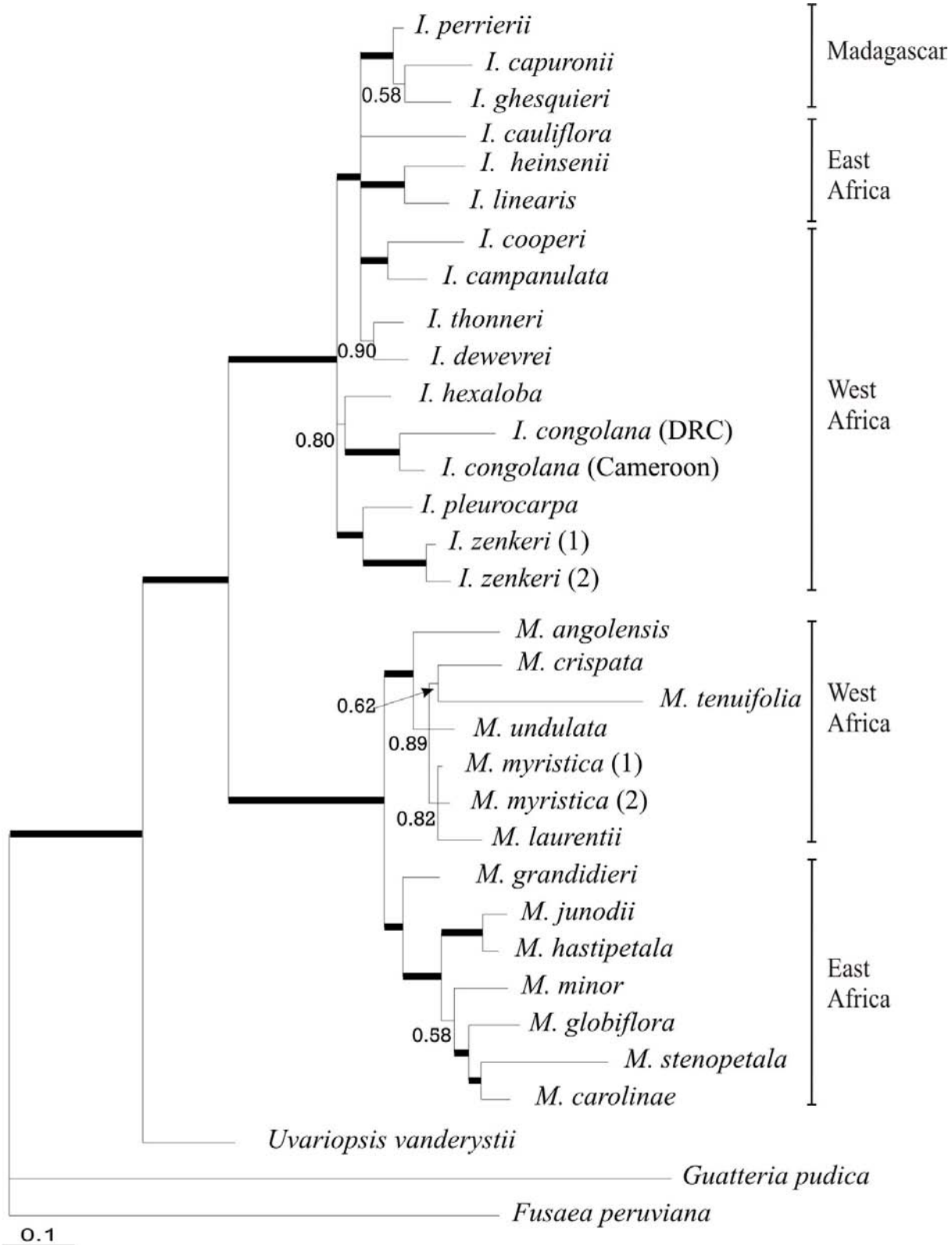

Figure 6.14. Bayesian majority rule consensus tree of *Isolona* (14/20 species) and *Monodora* (13/14 species) using five plastid markers. Thick branches indicate posterior probabilities of > 0.95.

Chapter 6

CONSERVATION STATUS

Materials and Methods

Following the IUCN guidelines (IUCN, 2004) an attempt was made to assign a category of threat to each species: extinct: EX, extinct in the wild: EW, critically endangered: CR, endangered: EN, vulnerable: VU, near threatened: NT, least concern: LC, data deficient: DD. Five criteria can be used to evaluate to which category a species belongs: A. Population reduction, B. Geographic range, C. Small population and decline, D. Very small or restricted population, E. Quantitative analysis. In absence of other information, it has been shown that herbarium collections constitute valuable information and can be used to determine categories of threat using criterion B (Schatz, 2002; Willis et al., 2003). Geographical distribution of each species was calculated either in terms of "Extent of Occurrence" (EOO: shortest continuous imaginary boundary, represented as a convex polygon enclosing a set of distribution points) or of "Area of Occupancy" (AOO: actual area occupied by a taxon within its more general EOO and is usually taken as a measure of species distribution size, see IUCN, 2004) using herbarium specimens. A large amount of the specimens studied were geo-referenced using online gazetteers (e.g. Fuzzy Gazetteer) or information on collecting localities of botanists in different countries or regions (Bamps, 1982; Polhill, 1988; Pope, 1998). The software ArcView ver. 3.3 (ESRI, 2002) was used to produce distribution maps for each individual species and the add-in script provided by IUCN (Justin Moat, Royal Botanic Garden Kew) allowed the calculation of the EOO and AOO. The cell area was always set to the largest permissible value which is just under 10 km^2 (cell width = 3.16 km, cell area = 9.98 km^2). Setting a cell size width of 3.2 km or larger will not allow any taxa to be listed as Critically Endangered where the threshold AOO under criterion B is 10 km^2 (Standards and Petitions Working Group, 2006). Because it is thought that herbarium specimens underestimate the real AOO, the highest cell size width was chosen in order to minimize this underestimation (Roy Gereau, pers. comm.). Finally, this cell size value was also used in other large scale conservation assessments (e.g. Plant Conservation Assessment in the Eastern Arc Mountains and Coastal Forests of Tanzania and Kenya, http://www.mobot.org/MOBOT/Research/tanzania/cepf2.shtml). Finally, projected continuing decline of population was assessed by determining the presence of each species within protected areas (national parks, forest reserves, nature reserves, etc).

Information about distribution of *Isolona* and *Monodora* species largely depended on herbarium collections. They often provided the only source used to arrive at recommendations for the IUCN red data list categories (IUCN, 2004). However, during a field trip to Tanzania in 2006, I was able to make limited observations on local population density for a few species, which was taken into account when assigning the categories of those species. Logging in tropical Africa is generally intense, even in certain protected areas. However, recent efforts by local governments to create national parks (e.g. Gabon, Tanzania) and to conserve large portions of their tropical rain forest provide some hope for the future of species included in

these parks.

Results

Just under half of the total number of species from both genera has been assigned to some level of threat (12 species or 60% in *Isolona* and four species or 28% in *Monodora*). Three of them (*I. capuronii*, *M. hastipetala* and *I. le-testui*) were assigned to the "critically endangered" category because of the small EOO and/or AOO and the exclusive occurrence in unprotected areas. *M. zenkeri* could easily join this category in the near future. For the two *Isolona* species this decision was also based on the early date of the most recent collection, and there is a fair possibility that they are both now extinct, although specific field surveys must be undertaken in order to comply with the IUCN (2004) criteria for that category.

The conservation assessments provided here are uncertain because of the lack of direct field observations. However, they do serve as a first guideline for protection and generate awareness for certain situations. It is important to remember that conservation assessments (and thus categories) are not fixed for any species, and can undergo significant changes with reports of new discoveries or losses of populations or extra field information.

GENUS DESCRIPTION: *ISOLONA*

Isolona Engl. in Engl. & Prantl, Nat. Pflanzenfam. Nachtr. 1: 161. 1897. — TYPE SPECIES: *Isolona madagascariensis* (Baill.) Engl. — TYPE: MADAGASCAR. Antsiranana: Diego-Suarez, *A.C.J. Bernier131* (holotype: P!).

Trees or treelets up to 30 m tall; young branches densely covered with erect or appressed hairs to glabrous. *Petioles* 1-15 mm long, leaf lamina inserted on side or on top, broadly to narrowly grooved adaxially. *Leaf lamina* elliptic to ovate or obovate, base decurrent to rounded, apex acute to acuminate, papyraceous to coriaceous; midrib raised adaxially, prominent abaxially; secondary veins 6-20 pairs, straight or uniformally curving upwards, loop forming towards margin; tertiary veins intermediate between rticulate and percurrent. *Flowers* bisexual, regular. *Inflorescence* a single, axillary, 1-flowered or rarely many flowered rhipidium, occasionally one or two additional rhipidia are present, growing on leafy branches, older leafless branches or on the trunk. *Flowering pedicels* variable in size and slender. *Lower bracts* 2-6, minute. *Upper bract* present, viariable in size, or sometimes absent. *Sepals* 3, valvate, free, ovate to broadly ovate, margins straight. *Petals* 6, clearly fused at base into a tube shorter than or rarely longer than the corolla lobes; tube urceolate or not; *corolla* lobes spreading horizontally or vertically, linear to elliptic, coriaceous to papyraceous, rarely membranous; yellow to red at anthesis. *Receptacle* slithgly convex. *Stamens* short and broad, glabrous, shielded by a truncated and broadened apical prolongation of the connective being glabrous or hairy; anthers with extrorse dehiscence; pollen in monads, inaperturate. *Carpels* ca. 6, congenitally fused into a syncarpous ovary; stigma capitate, bi-lobed; ovules numerous, bi-seriate; placentation parietal. *Fruiting pedicels* thick and woody to slender. *Fruits* syncarpous, globose to conical. *Seeds* numerous, ellipsoid to broadly ellipsoid; testa smooth or rugose; raphe thickened or flat; hilum elliptical to oblong; rumination laminate in four parts. Base chromosome number n=8.

Distribution: Throughout tropical West and Central Africa, in Tanzania, Kenya and Madagascar (see Fig. 6.13 A). *See appendix C for photos (end of thesis)*

KEYS TO THE SPECIES OF *ISOLONA*

I. Key to Flowering Specimens in Africa (excluding Madagascar)

1. Leaves or young branches hairy. 2
1. Leaves and young branches completely glabrous. 6

2. Flowers on shoots up to 2 m long departing from trunk or in many flowered inflorescences (Tanzania and Kenya). 3. *I. cauliflora*
2. Flowers solitary, on young or old branches. 3

3. Sepals > 8 mm long, papyraceous; corolla tube longer than lobes. 6. *I. deightonii*
3. Sepals < 8 mm long, coriaceous; corolla tube shorter than or as long as lobes. 4

4. Corolla glabrous outside. 4. *I. congolana*
4. Corolla hairy outside. 5

5. Flowering pedicels < 4 mm long; secondary veins > 14 pairs (Central Africa). 17. *I. pilosa*
5. Flowering pedicels > 4 mm long, secondary veins < 14 pairs (Tanzania). 9. *I. heinsenii*

6. Leaf lamina inserted apically on the petiole. 7
6. Leaf lamina inserted sideways on the petiole. 8

7. Corolla smooth in dried material; margins of corolla lobe flat; flowers emitting a strong sweet scent even in dried material. 5. *I. cooperi*
7. Corolla clearly verrucose in dried material; margins of corolla lobe curving inwards; flowers not emitting a strong sweet scent. 20. *I. zenkeri*

8. Corolla lobes > 50 mm long and > 10 times as long as wide. 13. *I. le-testui*
8. Corolla lobes < 35 mm long and < 10 times as long as wide. 9

9. Sepals conspicuous, > 4 mm long, papyraceous; upper bract present halfway up the pedicel sometimes leaf-like. 1. *I. campanulata*
9. Sepals not conspicuous, < 4 mm long, coriaceous; upper bract absent or minute. 10

10. Leaf lamina with acute to obtuse base. 11
10. Leaf lamina with decurrent to narrowly cuneate at base. 13

11. Petioles ≥ 6 mm long. 12. *I. lebrunii*
11. Petioles < 6 mm long. 12

12. Petioles < 4 mm long; flowering pedicels > 10 mm long (West-Central Africa). 10. *I. hexaloba*
12. Petioles > 4 mm long; flowering pedicels < 10 mm long (Tanzania). 14. *I. linearis*

13. Corolla lobes > 4 times as long as wide. 19. *I. thonneri*
13. Corolla lobes < 4 times as long as wide. 14

14. Flowering pedicels < 10 mm long; corolla lobes with rounded tip, the margins sparsely covered with hairs. 7. *I. dewevrei*
14. Flowering pedicels > 10 mm long; corolla lobes with acute tip, the margins glabrous. 18. *I. pleurocarpa*

II. Key to Flowering Specimens in Madagascar

1. Sepals > 3 mm long. 2. *I. capuronii*
1. Sepals < 3 mm long. 2

2. Corolla lobes drying conspicuously papery, thin; flowering pedicels sparsely covered with appressed hairs. 8. *I. ghesquierei*
2. Corolla lobes not papery, coriaceous; flowering pedicels glabrous. 3

3. Pedicels < 5 mm long. 15. *I. madagascariensis*
3. Pedicels > 5 mm long. 4

4. Leaf tip clearly rounded; pedicels thin, > 1 mm in diameter; corolla lobes < 4 mm long, narrowly elliptic to narrowly oblong, narrowed at base. 11. *I. humbertiana*
4. Leaf tip pointed; pedicels thick, < 1 mm in diameter; corolla lobes > 4 mm long, linear to very narrowly elliptic, not narrowed at base. 16. *I. perrieri*

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1. Isolona campanulata Engl. & Diels, Monogr. Afrik. Pflanzen-Fam. 6: 83. Fig. 27C, 1901. — TYPE: CAMEROON. North Province: Bangwe, 17 October 1899, *J. Conrau 93* (holotype: B!; isotype: K!). *Figure 6.26 D-G*

Isolona soubreana A.Chev., Explor. Bot. Afrique Occ. Franç. 1: 12. 1920. — TYPE: IVORY COAST. Sassandra: route de Soubré a Grabia, 23 June 1907, *A. Chevalier 19088* (lectotype designated here: P!; isotype: WAG!).

Isolona leonensis Sprague & Hutch., Kew Bull. 1916: 151. 1916. — TYPE: SIERRA LEONE. Northern Province: Yonibana, 30 October 1914, *N.W. Thomas 4230* (lectotype designated here: K!).

Tree or shrub to 10(-15) m tall; trunk with d.b.h. up to 10(-15) cm, narrowly fluted frequently to the base of the crown; bark dark brown; young branches dark green, drying black, glabrous; old branches light brown drying brown to black, glabrous, striate. *Petioles* 3-4(-6) mm long, 1-1.5 mm in diameter, glabrous, leaf lamina inserted on side, grooved adaxially. *Leaf lamina* 10-15(-18) cm long, 3-7 cm wide, length:width ratio 2.2-3.9; narrowly elliptic to elliptic or narrowly obovate to obovate, base decurrent to narrowly cuneate, apex acuminate, acumen ca.

1 cm long, subcoriaceous to papyraceous, glabrous on both sides, dark green above, light green below, sometimes with black lenticels; midrib raised and glabrous adaxially, prominent and glabrous abaxially; secondary veins 7-12 pairs, prominent beneath, glabrous. *Inflorescences* sometimes with 1(-2) additional rhipidia, on leafy branches. Flowering *pedicels* (5-)12-30(-35) mm long, ca. 0.5 mm in diameter. Lower bracts 1-4, 2-3 mm long, 1-2 mm wide. Upper bract sub-basal to halfway up the pedicel, sometimes leaf-like, 2-19 mm long, 1-4 mm wide, sessile to petiolate, petiole 1-2 mm long, base cuneate, apex rounded, papyraceous, glabrous. *Sepals* 4-9 mm long, 3-5 mm wide, length:width ratio 1-3, broadly ovate to elliptic, base truncate, apex acute, glabrous, persistent on young fruits. *Corolla* green when immature turning bright yellow at anthesis; tube 9-20 mm long, 4-5 mm in diameter, lobe:tube ratio 1-2; lobes 6-20 mm long, 2-7 mm wide, length:width ratio 2-5, triangular to narrowly triangular, apex acute, glabrous, papyraceous. *Receptacle* ca. 4 mm in diameter. *Stamens* ca. 1.2 mm long; connective shields ca. 0.1 mm long, glabrous, those of innermost stamens extended over adjacent ovary wall. *Ovary* ca. 2 mm long, ca. 2 mm in diameter; stigma ca. 1 mm in diameter, capitate, glabrous. *Fruiting pedicels* 2-5 cm long, 2-3 mm in diameter, glabrous. *Fruits* 4-7.5 cm long, 2-3.5 cm in diameter, length:width ratio 1.5-3; narrowly ovoid to ovoid, not apiculate, smooth with lumpy aspect on fresh and dried material, green turning deep yellow when mature; pericarp ca. 1 mm thick. *Seeds* 8-15 cm long, 5-10 mm in diameter, ellipsoid to transversely ellipsoid, packed in white pulp; testa rugose, dark brown, raphe thickened; hilum 5-6 mm long, 1-1.5 mm wide, narrowly elliptical.

Distribution: Disjunct, from Sierra Leone to Ghana and in south-eastern Nigeria, western Cameroon and one specimen collected in Gabon (see Map 1); in primary and secondary rain forest, and along rivers; at 0-500 m altitude.

Phenology: Flowers and fruits collected all year round.

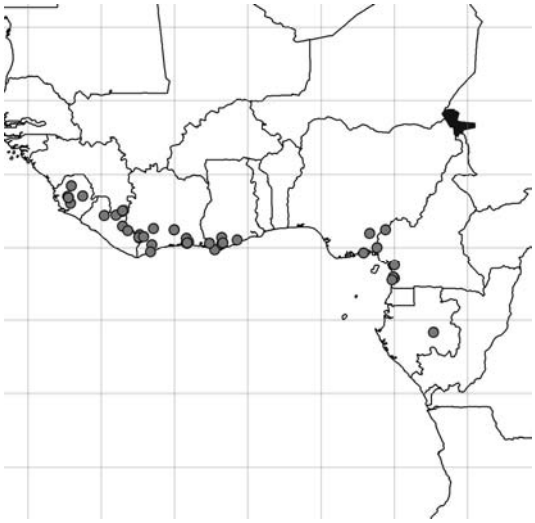
Uses: A root decoction is drunk in Sierra Leone against rheumatism. In Ivory Coast it is used for bronchial troubles, to allay fever, and against worms. A powder made from the bark is used as an aphrodisiac.

IUCN conservation status: LC. *Isolona campanulata* is well represented in herbaria from both West and Central Africa. It occurs in several protected areas such as national parks (Banco and Tai in Ivory Coast, Korup in Cameroon) as well as in forest reserves (Ankasa, Bonsa Ben and Subri River in Ghana; Angédédou in Ivory Coast; Kienke in Cameroon). Furthermore, it has been collected frequently in recent years, which is why the category of “least concern” is applied here.

Notes: *Isolona campanulata* can be distinguished by its fairly large glabrous sepals, as well as the sometimes leaf-like upper bract. The flowers are campanulate with the lobes acute at the apex. The flower shape suggests a close affinity with *I. deigthonii* (not sampled in the

molecular study), but also with *I. cooperi* by the shape of the lobes and the fruit; the latter relationship is supported by molecular data.

Extra references: Pellegrin, Bull. Soc. Bot. France 94: 387. 1947; Keay, Fl. W. Trop. Afr. ed. 2, 1(1): 53.1947; Aubréville, Fl. Forestière de la Côte d'Ivoire ed. 2, 1: 152; Fig. 44: 6-9. 1959; Irvine, Woody plants of Ghana 11. 1961; Le Thomas, Fl. Gabon 16: 353, Fig. 67: 4-7. 1969; Keay, Trees of Nigeria 28. 1989; Aké Assi, Boissiera 57: 98. 2001.



Map 1: Distribution of *Isolona campanulata*.

ADDITIONAL SPECIMENS EXAMINED:

CAMEROON: Entre Ekondo Nente et Loe, 15 km NW de Ekongo Titi, 3 June 1973, *R. Letouzey 15078* (P); **Littoral Province**. Lombe amp, Tissongo Study Area, Doula-Edea Reserve, June 1976, *P.G. Waterman 830* (K); **North Province**. Bangwe, 7 October 1899, *G. Conrau 93* (B, K); **South Province**. 12 km from Kribi, N of Ebolowa road, between Kribi Airfield and the Kienke River, 18 June 1969, *J.J. Bos 4866* (BR, K, LMA, M, MO, P, POZG, WAG, YA); 15 km SE of Kribi, several km N of Ebolowa road, Kienke Forest Reserve, Bidou Plantation at Bidou II, 30 June 1969, *J.J. Bos 4947* (BR, LMA, MO, P, WAG, YA); 10 km from Kribi, S of Lolodorf road, on Northern bank of the Kienke River, 9 July 1970, *J.J. Bos 7069* (WAG); **South-West Province**. Korup National Park, collected between The Ndian River and PAMOL field and 2.5 km on transect "P", 50 m, 21 April 1985, *D.W. Thomas 4763* (BR, K, MO, NY, P, US, WAG); Takamanda Forest Reserve, 500 m, 30 April 1987, *D.W. Thomas 7354* (MO, P, WAG).

CENTRAL AFRICAN REPUBLIC: Unknown. May 1949, *G.M.P.C. Le Testu 2990* (WAG).

GABON: Ogooué-Lolo. région de Lastoursville, Lastoursville, June 1930, *G.M.P.C. Le Testu 8136* (BM, BR, EA, P, WAG).

GHANA: Ashanti Region. Bonsa-Ben forest reserve, December 1940, *C. Vigne 4753* (FHO); Subri River FOREST RESERVE, February 1941, *C. Vigne 4757* (FHO); **Greater Accra Region**. Swedru, 28 February 1927, *J.M. Dalziel 8300* (K); **Western Region**. Reserve Forestière de Ankasa, 4 January 1967, *J.B. Hall 36269* (K); Near Axim, February 1934, *Forest Reserve Irvine 2194* (K); Subri reserve, February 1941, *C. Vigne 337* (US).

IVORY COAST: Abidjan. 6 March 1947, *L. Aké Assi 12498* (G); Foret du Banco, 25 June 1980, *L. Aké Assi 15174* (G); Foret du Banco, 1 June 1981, *L. Aké Assi 15905* (G); Foret du Banco, 1 September 1987, *L. Aké Assi 17705* (G, MO); Adiopodoumé; ORSTOM terrain, 26 March 1990, *P. Albers 63* (WAG);, 14 March 1928, *A. Aubréville (Ivory Coast series) 6* (B, HBG, P, WAG); Foret de Banco, 13 January 1969, *P. Bamps 1837* (BR, P); About 600 m. on route de 3 étages from Banco Centre, 14 May 1975, *W.J. van der Burg 310* (WAG); Banco Forest Reserve, 17 June 1975, *W.J. van der Burg 581* (WAG); Adiopodoumé. Forêt de l'Orstom sur le plateau, 23 February 1990, *C. Chatelain 130* (CSRS); Foret d'Adiopodumé, 18 January 1967, *G.A. Cremers 540* (K, P); Adiopodoumé, 12 December 1967, *G.A. Cremers 644 e* (BR, P); 18 January 1967, *G.A. Cremers 644* (BR, P); Adiopodoumé, about 17 km W of Abidjan, ORSTOM terrain, 2 July 1978, *A.J.F.M. Dekker 82* (WAG); Adiopodoumé, 20 August 1987, *L. Gautier & D. Béguin 566* (CSRS, G, MO); 22 January 1968, *C. Geerling 1939* (C, K, MO, WAG); ORSTOM, Adiopodoumé, 20 m, 25 May 1970, *J. de Koning 560* (WAG); Banco Forest Reserve., 22 December 1972, *J. de Koning 997* (WAG); Banco Forest Reserve. In North-west part, 19 January 1973, *J. de Koning 1022* (WAG); Banco Forest Reserve, 30 January 1973, *J. de Koning 1056* (WAG); 9 February 1973, *J. de Koning 1098* (WAG); Abidjan. Experimental Station ORSTOM, Adiopodomé, 28 April 1973, *J. de Koning 1634* (WAG); Abidjan. Experimental Station ORSTOM, Adiopodoumé, 27 May 1973, *J. de Koning 1711* (WAG); Abidjan. Banco Forest Reserve, in southern part, 13 June 1973, *J. de Koning 1792* (WAG); Abidjan. Experimental station ORSTOM, Adiopodoumé, 24 August

1973, *J. de Koning 2151* (WAG); Abidjan. Experimental station ORSTOM, Adiopodoumé, 22 October 1973, *J. de Koning 2484* (WAG); Abidjan. Experimental Station ORSTOM, Adiopodoume, 2 January 1974, *J. de Koning 3058* (WAG); Abidjan. Experimental Station ORSTOM, Adiopodoume. Seedlings, seed source Banco Forest, 14 March 1974, *J. de Koning 3426* (WAG); Abidjan. Experimental Station ORSTOM, Adiopodoumé, 23 March 1974, *J. de Koning 3471* (U, WAG); Abidjan. Adiopodoume forest, 28 January 1975, *J. de Koning 5250* (WAG); Abidjan. Experimental Station ORSTOM, Adiopodoume, 22 March 1975, *J. de Koning 5579* (WAG); Abidjan. Banco Forest Reserve. Near cottage, 24 June 1975, *J. de Koning 5865* (WAG); Abidjan. Banco Forest Reserve, near cottage, 27 March 1976, *J. de Koning 6748* (WAG); Abidjan. Banco Forest Reserve. Route du Rail, 5 April 1976, *J. de Koning 6764* (WAG); Abidjan. Banco Forest Reserve, 16 April 1976, *J. de Koning 6785* (WAG); Forêt de l'Anguédédou, about 15 km NW of Abidjan, 0-40 m, 24 December 1958, *A.J.M. Leeuwenberg 2278* (K, L, P, WAG, Z); Adiopodoumé, 13 May 2001, *P. Martin LN 23* (G, MO, WAG); 17 March 1961, *J. Miège s.n.* (G); 17 March 1961, *J. Miège s.n.* (G); Adiopodoumé. bord de route à l'interieur du centre, 10 November 1997, *J. Munzinger 6* (MO, P); Adiopodoumé, 12 November 1997, *J. Munzinger 63* (MO, P, UCJ, WAG); Banco forest, 7 March 1965, *J. Raynal 13640* (BR, COI, K, L, P); Adiopodoumé, foret de l'Orstom, 7 April 1987, *A. de Rouw 348* (WAG); Surroundings of Adiopodoumé, ORSTOM, 17 km W of Abidjan, 12 May 1969, *C. Versteegh 22* (MO, U, WAG); Adiopodomé, in the middle of the O.R.S.T.O.M. forest, 7 July 1956, *J.J.F.E. de Wilde 76* (FHO, WAG); Réserve de l'Anguédédou, 10 August 1956, *J.J.F.E. de Wilde 202* (WAG); Ca. 1 km NW of ORSTOM, Adiopodoumé, Forêt d'IDERT, 18 May 1963, *W.J.J.O. de Wilde 16* (EA, K, WAG, Z); Forêt du Banco, 12 January 1961, *H.C.D. de Wit 9017* (WAG); Forest behind ORSTOM buildings, 15 January 1961, *H.C.D. de Wit 9045* (WAG); **Agboville**. Azaguié, 18 March 1951, *G. Roberty 14257* (COI, G, MO, Z); **Daloa**. 40 km SE Issia, 11 August 1975, *J.B. Hall GC 45389* (MO); **Divo**. Lamto, 17 July 1972, *J.-L. Devineau 409* (LAMTO); 1 March 1972, *R. Spichiger 333* (CSRS, LAMTO); 5 May 1980, *R. Vuattoux s.n.* (LAMTO); **Guiglo**. Taï, along the border of the Cavally, 14 June 1981, *M.M. Barink 20* (WAG); About 16 km. WSW of Toulepleu, 5 km. SW of Klobli. Along Nimoi Dain River, 9 September 1975, *W.J. van der Burg 1011* (WAG); **San Pedro**. 80 km S of Soubré, 3 April 1968, *C. Geerling 2481* (MO, WAG); San Pedro, no date, *G. Thoiré 24 1* (K); 26 November 1901, *G. Thoiré 252* (P); **Soubré**. Tai, Parc National de, 27 September 1975, *L. Aké Assi 13075* (G); Route de Soubré à Grabia, 23 June 1907, *A.J.B. Chevalier 19088* (P, WAG); Bassin de la moyenne Sassandra, Soubré, 19 June 1907, *A.J.B. Chevalier 19138* (P); **Tabou**. Pauléoula, 9 November 1979, *M. Knecht 948* (G); **Unknown.** Rasso, 14 December 1931, *A. Aubréville (Ivory Coast series) 570* (P); Molokotosso, 9 February 1957, *A. Aubréville (Ivory Coast series) 4089* (P); 9 February 1957, *A. Aubréville (Ivory Coast series) 4098* (P).

LIBERIA: Bong. Central Province, Salala distr. Piatah, 9 December 1947, *J.T. Baldwin jr. 12509* (K, MO); **Grand Gedeh**. Mim Timber Co (Fijnhout) Tchien. Virgin forest, 250 m, 15 May 1970, *J. de Koning 505* (WAG); **Nimba**. Nimba Monts, 30 November 1966, *J.G. Adam 20812* (P); Nimba Expedition, South Nimba, 21 June 1965, *J.G. Adam 21576* (K, UPS); Nimba, Grassfield, 5 May 1973, *J.G. Adam 27400* (MO); Yéképa, foret frontier de la Guinee, 13 October 1975, *J.G. Adam 29855* (MO); Ganta, 15 June 1936, *G.W. Harley 938* (K).

NIGERIA: Akwa-Ibom State. Eket District, 1912, *P.A. Talbot 3261* (BM); **Cross River State**. Iyamoyong Forest Reserve (Ogoja Province, Obubra District), 25 April 1959, *A. Binuyo FHI 41274* (BR, FHI, FHO, K, WAG).

SIERRA LEONE: Eastern Province. Iaiama-Senehum road, 24 September 1952, *N.H.A. Cole 53* (K, MO); **Northern Province**. 30 October 1914, *N.W. Thomas 4230* (K); Yonibana, 30 October 1914, *N.W. Thomas 4259* (K); Mamaka, 2 November 1914, *N.W. Thomas 4593* (K); Mamaka, 11 October 1914, *N.W. Thomas 4690* (K); Mamaka, 97m, 11 November 1915, *N.W. Thomas 4962* (K); **Southern Province**. Sendugu, 106m, 22 June 1914, *N.W. Thomas 678* (K); Mano, 1915, *N.W. Thomas 9894* (K); **Unkwon.** Near Kongohun, 6 April 1939, *F.C. Deighton 3676* (K); near Jau (Tunkia), 28 October 1949, *F.C. Deighton 5238* (K); Bamaka, 2 November 1914, *N.W. Thomas 4648* (K).

2. Isolona capuronii Cavaco & Keraudren, Bull. Jard. Bot. État, Brux. 27: 80. 1957. — TYPE: MADAGASCAR. Toamasina: Ambodiatafana, 20 January 1954, *Service Forestier de Madagascar 8941* (holotype: P!; isotypes: P!, WAG!). *Figure 6.21 C*

Tree, height unknown, d.b.h. unknown, young branches light green, glabrous; old branches black, striate, glabrous. *Petioles* 4-6 mm long, 1-1.5 mm in diameter, glabrous, leaf lamina inserted on side, broadly grooved adaxially. *Leaf lamina* 9-12 cm long, 3-3.5 cm wide, length:width ratio 3-3.4, narrowly elliptic, base cuneate, apex acuminate, acumen c. 10 mm long; midrib raised and glabrous adaxially, prominent and glabrous abaxially; secondary veins 9-10 pairs, glabrous. *Inflorescences* sometimes with an additional rhipidium, on leafy branches. *Flowering pedicels* ca. 15 mm long, ca. 1 mm in diameter, glabrous. Lower bracts 2-4, 1-2 mm wide. Upper bract absent. *Sepals* 4-5 mm long, 3-4 mm wide, length:width ratio 1-1.3, triangular, base truncate, apex acute, papyraceous, glabrous, drying light brown to light yellow. *Corolla* glabrous; tube 7-9 mm long, 4-5 mm in diameter, tube:lobe ratio 0.3-0.5,

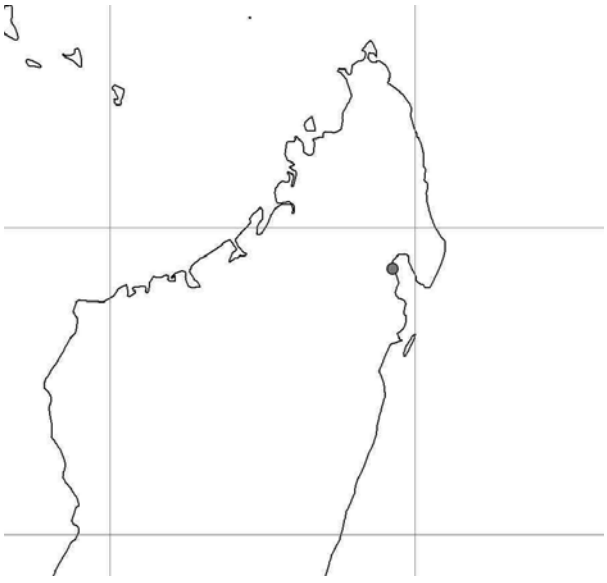
lobes 18-22 long, 5-6 mm wide, length:width ratio 3-3.8, narrowly elliptic, base truncate and slightly narrowed, apex acute, coriaceous. *Receptacle* ca. 4 mm in diameter. *Stamens* ca. 2 mm long; connective shield ca. 0.2 mm long, glabrous, those of inner most row elongated over ovary wall. *Ovary* ca. 2 mm long, ca. 1.5 mm in diameter; stigma ca. 1.5 mm in diameter, glabrous. *Fruits* unknown.

Distribution: Known from only a single collection from north-western Madagascar (see Map 2); in primary rain forest; at ca. 50 m altitude.

Phenology: Mature flowers found in January.

IUCN conservation status: CR B1ab(iii). The single collection of *I. capuronii* dates from 1954. Just over 3% of Madagascar has a formal protected status. The “critically endangered” status is thus applied. It could be possible that this species has gone extinct regarding the lack of recent collections and the only record being positioned near populated areas.

Notes: *Isolona capuronii* is only known by the type which bears flowers but lacks fruits. It is easily distinguished from other Malagasy *Isolona*’s by the large papyraceous sepals that dry light brown-yellow as well as by the coriaceous petal lobes.



Map 2: Distribution of *Isolona capuronii*.

3. Isolona cauliflora Verdc., Kew Bull. 25: 34. 1971. — TYPE: TANZANIA. Tanga: East Usambara Mountains, 6 May 1924, *A. Peter 40024* (holotype: B!). *Figure 6.15*

Tree up to 8 m high; trunk with d.b.h. up to 10 cm; bark striate, smooth, grey to brown; young branches drying black covered with short erect hairs; old branches grey, generally glabrous, striate. *Petioles* 3-5 mm long, 1-1.5 mm in diameter, glabrous sparsely to densely covered

with short erect hairs, leaf lamina inserted on top, narrowly grooved adaxially. *Leaf lamina* 12-23 cm long, 5-9 cm wide, length:width ratio 2-3, narrowly elliptic to elliptic or narrowly obovate to obovate, base rounded, apex acuminate, acumen 1-2 cm long, coriaceous, glabrous on both sides, dark green adaxially, lighter green abaxially; midrib raised and glabrous adaxially, prominent and glabrous abaxially; secondary veins 10-16(-18) pairs, glabrous. *Inflorescences* cauliflorous, multi-flowered; main flowering axis up to 2 m long, departing from the trunck and growing on forest floor; young parts green, fleshy, densely covered with short appressed hairs; old parts black, woody, glabrous. *Flowering pedicels* 12-33 mm long, 1-1.5 mm in diameter, densely covered with short appressed hairs, dark green to red. *Lower bracts* 2-6, ca. 1 mm long, ca. 1 mm wide, densely covered with short appressed hairs outside, glabrous to sparsely covered with short appressed hairs inside, larger lower bracts rarely present, ca. 5 cm long, ca. 4 cm wide, length:width ratio 1.25, broadly elliptic, glabrous, light green. *Upper bract* basal, 1-2 mm long, 1-1.5 mm wide, covered with short appressed hairs outside, glabrous to sparsely covered with short appressed hairs inside. *Sepals* 3-6 mm long, 2-4 mm wide, length:width ratio 1.-1.5, triangular, apex acute to acuminate, densely coverd with short appressed hairs outside, glabrous inside, green, pressed against tube, persistent in fruit. *Corolla* light green when immature to light or dark red at anthesis; tube 4-6 mm long, 6-10 mm in diameter, lobe:tube ratio 2.5-6.5, coverd with short appressed hairs, glabrous inside; lobes 13-32 mm long, 4-10 mm wide, length:width ratio 2.5-4.5, narrowly ovate to ovate, narrowed at base, apex acute, covered with short appressed hairs on both sides, coriaceous, three lobes spreading horizontally, the other three spreading vertically. *Receptacle* 3-5 mm in diameter. *Stamens* ca. 1.2 mm long; connective shields ca. 0.2 mm long, glabrous, those of innermost row stretched and pressing against ovary wall. *Ovary* ca. 2 mm long, ca. 2 mm wide; stigma 2.5-3 mm in diameter, very sparsely covered with erect hairs. *Fruiting pedicels* 23-30 cm long, 5-9 mm in diameter, glabrous, warty, dark red. *Fruits* sometimes buried in forest soil, 20-45 mm long, 20-43 mm in diameter, length:width ratio 1-1.5, broadly ovoid, irregular longitudinally 6(-7) ribbed, coarsely rugulose all over, apicule absent, sparsely covered with short erect hairs when immature, glabrous when mature, light to dark red or brown at maturity; pericarp 1-2 mm thick. *Seeds* 10-15 mm long, 5-7 mm in diameter, ellipsoid, packed in white pulp; testa smooth, light brown; raphe not thickened; hilum 4-5 mm long, 2-3 mm wide, broadly elliptical.

Distribution: South-eastern Kenya and north-eastern Tanzania (see Map 3); in lowland rain forests, along rivers and streams, on rocky soils; at 20-500 m altitude.

Phenology: Mature flowers collected from May to June and from September to December. Mature fruits collected in February, May and June as well as from November to December.

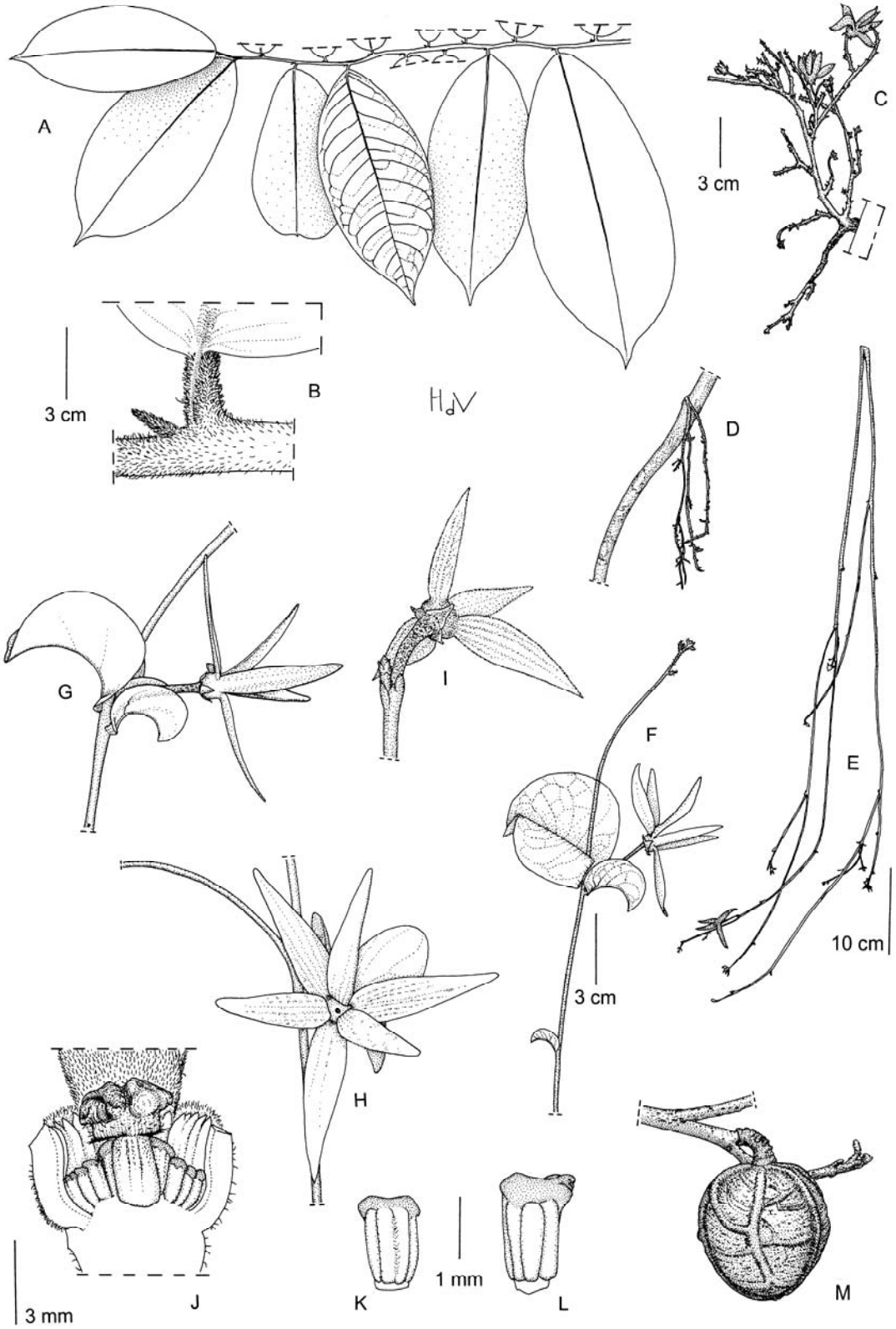


Figure 6.15. *Isolona cauliflora*. A. Leaved branch. B. Detail of petiole. C. Many-flowered inflorescence. D. Illustration of cauliflorous inflorescence. E. Flageliflorous inflorescence. F. Detail of inflorescence with leaf-like bracts. G. Detail of leaf-like bracts. H. Flower (top view). I. Flower and sepals (bottom view). J. Transversal section of receptacle and ovary topped by stigma. K. Stamen, front view. L. Stamen from inner most row (semi side view). M. Fruit. Drawings by Hans de Vries.

IUCN conservation status: EN B2ab(iii). *Isolona cauliflora* is moderately represented in herbaria and has been collected several times recently. It can be found in several protected areas such as forest reserves (Gongoni, Manga, Longuza and Kimboza in Tanzania) and one nature reserve (Shimba Hills in Kenya). However, *Isolona cauliflora* is found in less than 5 locations and has an Area of Occupancy under 500 km^2, and is therefore assigned the "endangered" category.

Notes: *Isolona cauliflora* is easily distinguishable by the long inflorescences reaching 2 m long and lying on the forest floor or by its multi-flowered inflorescences. It does not show any close relationship with the two other East African *Isolona* species, but does show a slight resemblance to *I. congolana* by the shape of the corrolla. The position of *I. congolana* in the molecular phylogeny is unresolved, but clusters in a large group with the other two East African species, the Malagasy clade and a few other West Africa taxa. The phylogeny does not support, however, a close relationship to *I. congolana*. As mentioned by Verdcourt (1971) the presence of climbing hooks on the notes of the holotype in B must be an error as it has not been observed on the type specimen or in any other collection.

Extra references: Verdcourt, Fl. Trop. E. Afr., Annonaceae: 125. 1971.

Map 3: Distribution of *Isolona cauliflora*.

ADDITIONAL SPECIMENS EXAMINED:

KENYA: Coast. Shimba hills National Reserve, Makadara picnic site, 400 m, 26 December 1982, *J.B. Gillett 23997* (EA, K); Summit of Mangea Hill, 500 m, 25 February 1987, *W.R.Q. Luke 278* (EA, K); Shimba hills, Mwele, 16 October 1991, *W.R.Q. Luke 2930* (K, MO); Shimba Hills, Makadara, 400 m, 16 September 1982, *R.M. Polhill 4782* (B, BR, C, K); Gongoni Forest Reserve, 1.2 km NW of NE corner along N boundary, 20 m, 12 November 1989, *S.A. Robertson 5950* (EA); Kaya Muhaka (Kaya Kambe), 30 m, 31 May 1990, *S.A. Robertson 6263* (EA, K); Shimba Hills NR, Makadara forest, 350 m, 4 June 2005, *S.A. Robertson 7555* (EA, K, WAG); Shimba hills Naturel Reserve, Makadara Forest, 350 m, 12 December 1982, *S.A. Robertson 3459* (K).

TANZANIA: Morogoro. Kimboza Forest reserve, 2 km after Kimboza village, 45 km from Morogoro, 250 m, 25 November 2006, *T.L.P. Couvreur 70* (DSM, MO, NHT, WAG); near road to Malanga village, just after Mkuyuni village, near Kimboza Forest Reserve, 350 m, 26 November 2006, *T.L.P. Couvreur 87* (DSM, MO, WAG); Kimboza Forest Reserve. c. 48 km Morogoro to Matombo Road, 300-350 m, October 1983, *W.A. Rodgers 2627* (K); 400 m, October 1983, *W.A. Rodgers 2672*

(K); **Tanga**. Kiwanda, island in Zigi river +- 1 km NE of Kiwanda (edge of Longuza Forest Reserve), 250 m, 5 June 1996, *D.M. Johnson 1946* (OWU); Kiwanda, Sigi river, 200 m, 10 November 1981, *S.P. Kibuwa 5456* (NHT); Marimba Forest Reserve near Kiwanda Village, 250 m, 15 November 1995, *F.M. Mbago 1425* (DSM); East Usambara Mountains, Longuza to Sigi river, 1.5 km below Longuza, 6 May 1926, *A. Peter 40024* (B); East Usambara Mts, November 1917, *A. Peter 22011* (B); Manga Forest Reserve, Frontier plot 29, 165m, 31 July 1997, *Simon, V. 6* (C, L, NHT).

4. Isolona congolana (De Wild & Th.Dur.) Engl. & Diels, Monogr. Afrik. Pflanzen-Fam. 6: 84. 1901. *Monodora congolana* De Wild & Th.Dur., Bull. Soc. Roy. Bot. Belg. 38: 13. 1899. — TYPE: DEMOCRATIC REPUBLIC OF CONGO. Equateur: Lukandu, 19 November 1896, *A. Dewèvre 1103* (holotype: BR!; isotypes: BR-3 sheets!). *Figure 6.16*

Isolona maitlandii Keay, Kew Bull. 7: 155. 1952. — TYPE: CAMEROON. North-West Province: Ngong, June 1931, *T.D. Maitland 1555* (holotype K!; isotypes: BM!, FHO!) ***syn. nov.***

Tree up to 20(-30) m tall; trunk with d.b.h. up to 45 cm; bark smooth, pale grey-brown; young branches dark brown to black, striate, densely cover with short appressed hairs, beconing glabrous in older branches. *Petioles* 4-7 mm long, 1.5-2.5 mm in diameter, desenly covered with short erect hairs when young, becoming sparsely covered with short erect hairs when older except at the base; leaf lamina inserted on top, narrowly grooved adaxially. *Leaf lamina* 13-19 cm long, 4-5 cm wide, length:width ratio 3.5-4.5, narrowly obovate or narrowly oblong, base broadly cuneate to rounded, apex acuminate, acumen 5-10 mm, sub-coriaceous, sparsely covered with short appressed hairs when young becoming glabrous when older; midrib sparsely covered with short erect hairs adaxially, beconing glabrous when older, prominent and sparsely to very sparsely covered with short erect hairs abaxially; secondary veins 11-14 pairs, sparsely covered with short erect hairs to glabrous below. *Inflorescences* sometimes with 1(-2) additional 1-flowered rhipidia, on leafy branches. *Flowering pedicels* 10-23 mm long, 0.7-1 mm in diameter, sparsely covered with short erect hairs to glabrous. *Lower bracts* 2-3, ca. 1 mm long, ca. 1 mm wide, sparsely covered with short erect hairs. *Upper bract* at base of pedicel, 0.5-1 mm long, ca. 0.5 mm wide, sparsely covered with short erect hairs. *Sepals* ca. 3 mm long, ca. 2 mm wide, length:width ratio ca. 1, transversely ovate, base truncate, apex acute, glabrous to sparsely covered with short appressed hairs, green, falling in fruit. *Corolla* green when immature to red at anthesis; tube 4-10 mm long, 4-7 mm in diameter, lobe:tube ratio 1.1-4, glabrous on the outside, densely covered with short erect hairs on the inside; lobes 9-20 mm long, 3-7 cm wide, length:width ratio 3-4.3, narrowly ovate or narrowly elliptic, base truncate, apex narrowly rounded, glabrous or rarely sparsely covered with short erect hairs on the outside, densely covered with short appressed hairs on the inside especially around the tube and towards the margins, coriaceous, spreading horizontally and then curving up or downwards, margins wavy on dried material. *Receptacle* 3-4 mm in diameter. *Stamens* ca. 2 mm long; connective shields ca. 0.2 mm long, densely covered with short erect hairs, those of innermost row extended over ovary wall. *Ovary* ca. 2 mm long, ca. 1.5 mm wide; stigma 2-2.5 mm in diameter, densely covred with short erect hairs. *Fruiting pedicels* 22-30 mm long, 4-8 mm in diameter, glabrous, woody. *Fruits* 6-8 cm

long, 4-5 cm in diameter, length:width ratio 1.5-2, broadly ellipsoid to ellipsoid, apicule absent, irregularly ribbed, surface coarsely verrucose to smooth, glabrous, green; pericarp 3-5 mm thick. *Seeds* 15-25 mm long, 10-15 mm in diameter, irregularly ellipsoid, embedded in a thick white pulp; testa smooth, brown; raphe not thickened; hilum 5-6 mm long, 3-4 mm wide, elliptical.

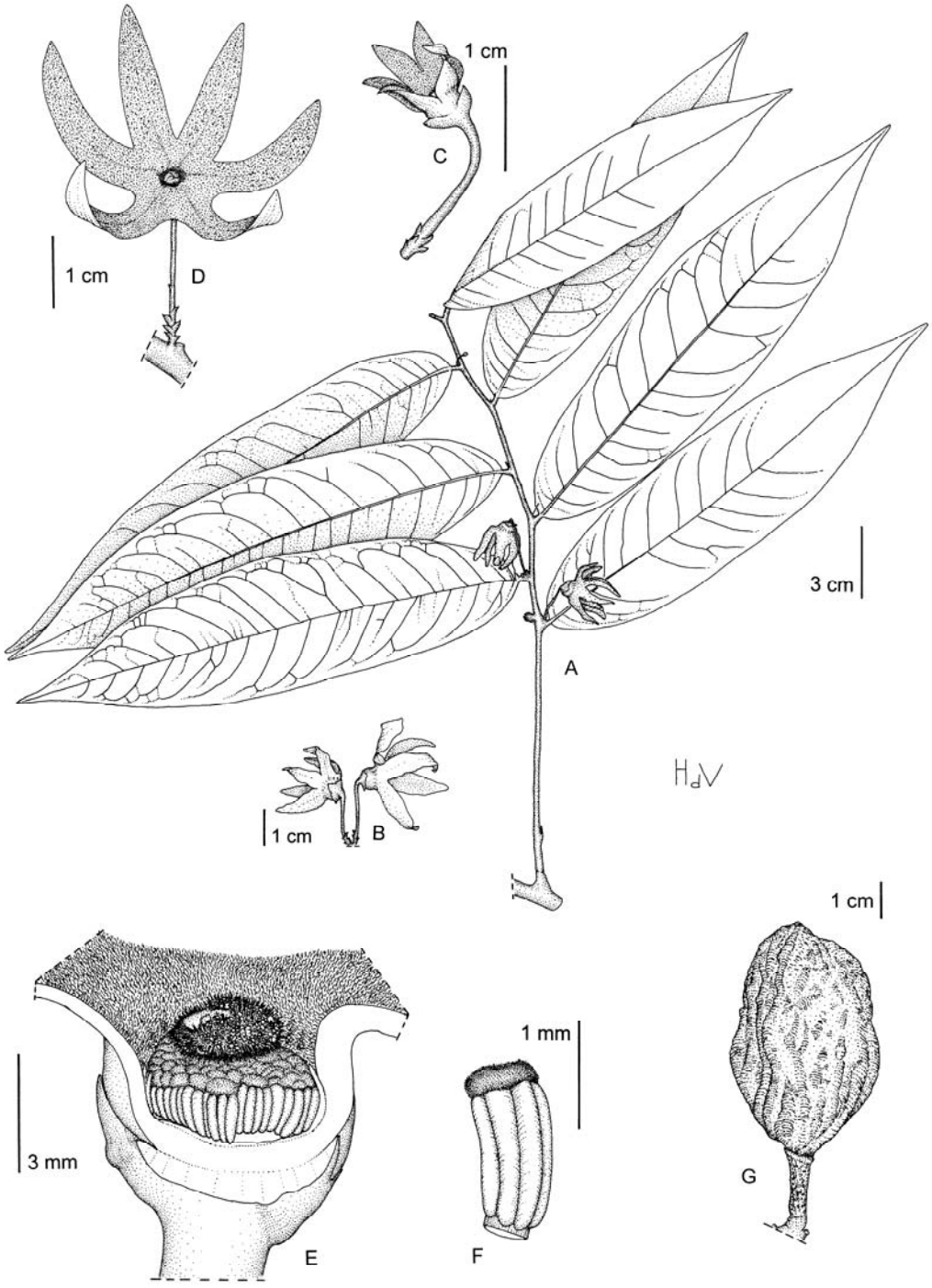


Figure 6.16. *Isolona congolana.* A. Flowering branch. B. Flowers. C. Young flower bud. D. Flower. E. Transversal section of flower showing receptacle, stigma and stamens. F. Stamen. G. Fruit. Drawings by Hans de Vries.

Distribution: Western Cameroon, Central African Republic, and Democratic Republic of Congo (see Map 4); in lowland or montane primary or secondary rain forests, along rivers or farm bush land; at (450-)800-1700 m altitude.

Phenology: Mature flowers collected from February to June and from September to December. Mature fruits collected from September to January, from March to April and in June.

Vernacular names:
Cameroon: Ndin.
Democratic Republic of Congo: Inaolo-a-likamba (dial. turumbu); Mwenaya-luembe (Kaniama); Wingo (dial. lomogo); Bofimingo (Boende); Tsikosokosa (dial. tshiluba).

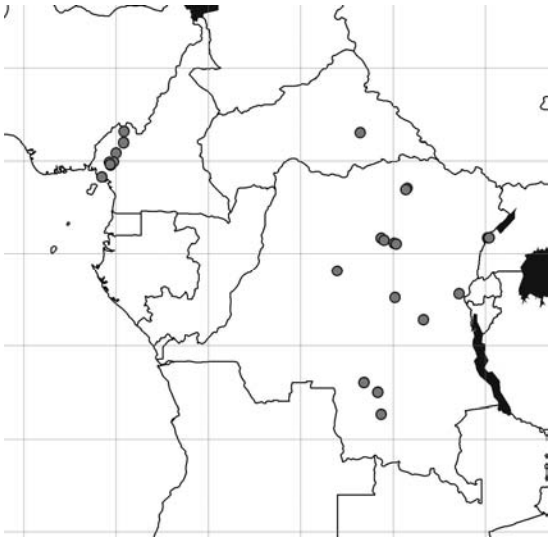
Uses: Crushed seeds used as soap. The wood is soft and used in crafts.

IUCN conservation status: NT. *Isolona congolona* has a wide but disjunct distribution in the Congo basin and is represented by a moderate number of herbaria specimens. It is hard to evaluate the current state of threat in the Democratic Republic of Congo because of the recent war. The Area of Occupancy suggests the endangered category, but there are significantly more than 5 locations, thus we prefer to assign it to "near threatened" category instead.

Notes: *Isolona maitlandii* was described from the mountain ranges north of Mount Cameroon in western Cameroon. Despite the fact that it is geographically isolated from the rest of the species (mainly occurring in the Democratic Republic of Congo) there are no morphological differences to be found that would distinguish it from *I. congolana*. Indeed they both share the characteristic narrowly oblong to narrowly elliptic leaves, as well as a corolla that is densely covered with hairs on the inside and glabrous on the outside. This decision to unite both species is also supported by molecular data as one specimen from both populations were sampled and together were recovered as monophyletic with maximum support. Additionally, the molecular tree indicates that *I. congolana* is sister to *I. hexaloba* but this relationship is poorly supported.

ADDITIONAL SPECIMENS EXAMINED:
CAMEROON: Littoral Province. Manengouba Mountains, 4 km WNW of Nkongsamba, 1200 m, 4 April 1972, *A.J.M. Leeuwenberg 9550* (B, BR, C, EA, FHI, GC, H, HBG, K, LD, LISC, M, MO, P, PRE, UPS, US, WAG, YA); **North-West Province**. Bamenda, Ngong, 900 m, June 1931, *T.D. Maitland 1555* (BM, FHO, K); Bamenda, Wae, 1067m, April 1931, *T.D. Maitland 1596* (K); **South-West Province**. Kupe-Muanenguba Division. Nyasoso. Max's trail upo to Phil Lane's plot, 800 m, 19 March 1996, *M. Etuge 1794* (K, WAG); Kupe-Muanenguba Division. Kupe Village. White trail (above Kupe village) towards Madam Kupe, 700 m, 28 May 1996, *M. Etuge 2000* (BR, K, MO, P, SCA, WAG, YA); Kupe-Muanenguba Division, Edip, 1300 m, 21 November 1998, *M. Etuge 4488* (K, WAG, YA); Buea, 1000 m, 6 June 1898, *H. Lehmbach 224* (B); **West Province**. 6 km. W. of Dschang, on road to Fongo Ndeng, along the road, 15 May 1978, *E. Westphal 10012* (P, WAG).
CENTRAL AFRICAN REPUBLIC: Haute-Kotto. Haut-Kotto, région de Yalinga, Haut Oubangui, 9 February 1923, *G.M.P.C. Le Testu 4656* (BM, P, WAG).
DEMOCRATIC REPUBLIC OF CONGO: Equateur. Esanga, 6 December 1937, *E. Collart 102* (B, BR, K, MO, US, WAG); Bena Kamba, November 1896, *A. Dewèvre 1103* (BR); Angodio, May 1931, *J. Lebrun 2968* (A, BR); **Kasai**. 24 October 1957, *L. Liben 3852* (BR, K, WAG); Mwene-Ditu, 24 October 1957, *L. Liben 3880* (BR, K, WAG); **Katanga**

(Shaba). Kaniama, 17 May 1950, *J. Delvaux 147* (BR); Mondoye, 1100 m, October 1951, *R. Desenfans 2131* (BRLU); Foret de la Witsimaie, Mwene-Ditu. Terr. de Kanda kanda, 24 September 1948, *Y. Hardy 9* (BR); Kamiana, November 1934, *Herman 2063* (BR); Kaniama-Haut Lomami, 900 m, 26 September 1947, *W. Mullenders 1164* (BR, BRLU, COI); **Orientale**. Bambesa, 5 January 1957, *P. Gérard 2624* (BR, WAG); Bambesa (Babeye), 13 September 1962, *P. Gérard 5272* (BR, WAG); Yangambi, ile Tutuku, 470 m, 19 March 1940, *R.G.A. Germain 278* (BR, EA, MO, NY, WAG); Ile Tutuku, 1 October 1940, *R.G.A. Germain 356* (BM); Yangambi, 2 June 1943, *R.G.A. Germain 16839* (BR); Kisangani, Ile Kongolo à la confluence de la Lindi avec le fleuve Zaïre, 6 April 1979, *J. Lejoly 4961* (BR, BRLU, WAG); Yalutcha, en amont d Yangambi, 18 October 1947, *J.J.G. Léonard 1495* (BR); Yangambi, 2 June 1943, *J. Louis 16839* (BR, P); Ile de Kongolo, pres de Kisangui, 12 September 1979, *Mandango 1921* (BR); Ile de Tundulu, en amount de Kisangui, 5 May 1983, *L. Pauwels 6675* (BR, WAG); Bitale Terr. Kalehe, 48 km road Kavumu-Walikale, 1750 m, 9 December 1955, *R. Pierlot 1053* (BR); **Uknown.** no date, *Galoux 97* (BRLU).
UGANDA: North Buganda. Nyabarogo River, Wasa area, Toro, October 1940, *W.J. Eggeling 4055* (K); **Western Province**. Sempaya Escarpment Ioso, April 1943, *W.J. Eggeling 5240* (EA).



Map 4: Distribution of *Isolona congolana.*

5. Isolona cooperi Hutch. & Dalziel ex Cooper & Record, Bull. Yale Univ. School For. 31: 15. 1931. — TYPE: LIBERIA. Montserrado: near Firestone plantations, along Dukwai road, 7 May 1929, *G.P. Cooper 417* (holotype: A!; isotypes: BM!, FHO!, K!). *Figure 6.17*

Tree or shrub to 6-10(-18) m high; trunk with d.b.h up to 20 cm; bark coarsely rugulate, brown; young branches drying black, glabrous, smooth; old branches dark brown, glabrous, striate. *Petioles* 1-5 mm long, 2-3 mm in diameter, glabrous, verruculose, leaf lamina inserted on top, very weakly and narrowly grooved adaxially. *Leaf lamina* (15-)17-27(-29) cm long, 6-12(-15) cm wide, length:width ratio 2.2-3.9; oblong or narrow oblong to obovate or narrow obovate, base rounded to broadly cuneate, apex acuminate, acumen 1-2 cm long, subcoriaceous, glabrous on both sides, pale green below, dark green somewhat shiny above; midrib raised and glabrous adaxially, very prominent and glabrous abaxially; secondary veins 9-18 pairs, prominent below, printed above, straight then abruptly curved or uniformly curved upwards, loop forming towards margin. *Inflorescences* sometimes with an extra 1-flowered rhipidium, on leafy branches and/or trunk. *Flowering pedicels* 14-25 mm long, ca. 0.5 mm in diameter, glabrous. *Lower bracts* 2-4, 0.5-1 mm long, glabrous. *Upper bract* absent. *Sepals* ca. 2 mm long, ca. 2 mm wide, length:width ratio ca. 1, very broadly ovate, base truncate, apex rounded, glabrous, pressed against tube; falling when in fruit. *Corolla* green when

immature to bright yellow and emitting a very strong sweet scent at anthesis; tube 6-11 mm, 3-5 mm in diameter, lobe:tube ratio 1.2-1.9, cupulate, glabrous; lobes 8-15 mm long, 4-6 mm wide, length:width ratio 1.6-3.2, narrowly oblong to oblong, apex acute, rounded at tip, glabrous, coriaceous, spreading horizontally, margins flat. *Receptacle* 4-5 mm in diameter. Stamens ca. 2 mm long; connective shield ca. 0.2 mm long, glabrous, those of innermost row extended over adjacent ovary wall. *Ovary* ca. 2.5 mm long, ca. 1.5 mm wide; stigma ca. 1.5 mm in diameter, glabrous. *Fruiting pedicels* 16-27 mm long, ca. 2 mm in diameter, glabrous. *Fruits* (3-)8-9 cm long, 1.5-3.0 cm in diameter, length:width ratio 2-4.5, ellipsoid to narrowly ellipsoid, apex apiculate, apicule ca. 1 cm long, smooth, glabrous, orange with white spots when mature; pericarp 1-2 mm thick, constricted over seeds in dried material but not in fresh material. *Seeds* 10-15 mm long, 5-10 mm in diameter, ellipsoid, packed in white pulp; testa rough, dark brown; raphe slightly thickened; hilum 4-5 mm long, 1.5-2 mm wide, elliptical.

Distribution: Liberia to Ghana, most common in Ivory Coast, once collected in western Cameroon (see Map 5); in primary or secondary lowland rain forest and along rivers, on sandy soil; at 0-300 m altitude.

Phenology: Mature flowers collected from January to July. Mature fruits collected from November to September.

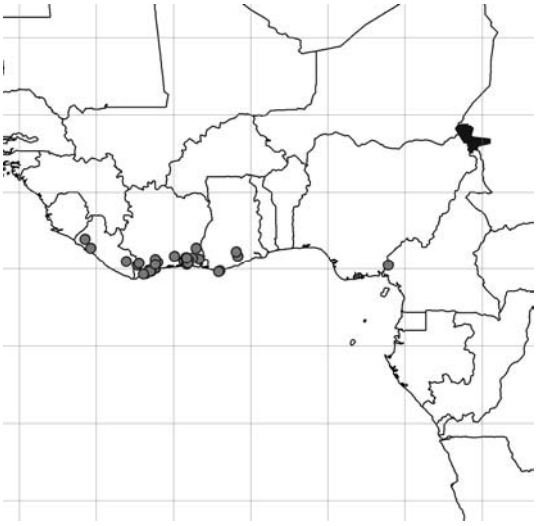
Vernacular names:
Ghana: Nzotala (Nzima).
Liberia: Ku-ghe (Bassa).

Uses: The bark has magical use in Liberia.

IUCN conservation status: LC. *Isolona cooperi* is found in three forest reserves (Monogaga and Esen Epam, Ivory Coast; Cape Three Points, Ghana) and in two national parks (Banco, Ivory Coast; Korup, Cameroon). The species is well represented in herbaria with numerous recent collections. The “Least concern” category is therefore applied.

Notes: *Isolona cooperi* emits an extraordinary strong sweet smell at anthesis which persists in herbarium material and even in spirit collections. It presents close morphological similarities to *I. hexaloba* by the shape of its flowers as well as to *I. campanulata* by the shape of the fruits; the latter relationship is strongly supported by molecular evidence. *Isolona cooperi* is distinguished by the leaves having rounded to broadly cuneate bases, being inserted on top of the short petioles.

Extra references: Aubréville, Fl. Forestière de la Côte d'Ivoire ed. 2, 1: 154, Fig. 44: 6-9. 1959; Irvine, Woody plants of Ghana 12. 1961; Aké Assi, Boissiera 57: 98. 2001.



Map 5: Distribution of *Isolona cooperi*.

ADDITIONAL SPECIMENS EXAMINED:

CAMEROON: South Province. Bipindi, February 1910, *G.A. Zenker s.n.* (F); **South-West Province**. Korup Forest Dynamics Plot, Korup National Park, 10 March 1998, *D. Kenfack 1063* (MO, WAG).
GABON: Estuaire. Environ de Libreville, no date, *T.-J. Klaine s.n.* (Z).
GHANA: Eastern Region. Kade A.R.S, 28 March 1968, *J.B. Hall GC 38267* (K); Esen Epam Forest Reserve, footpath from main road to East along "big tree", 300 m, 27 April 1994, *C.C.H. Jongkind 1443* (MO, UPS, WAG); **Western Region**. Axim-Sekondi Road, 17 February 1934, *G.K. Akpabla 115* (K); Cape 3 points forest reserve, 3 April 1973, *J.B. Hall GC 44349* (MO).
IVORY COAST: Abidjan. Foret du Yapo, 9 December 1969, *L. Aké Assi 11009* (G); Foret du Banco, 16 July 1980, *L. Aké Assi 15200* (G); Forêt du Téké. Abidjan-Adzopé km 31, 8 February 1969, *P. Bamps 2024* (BR); Forêt du Téké, 10 January 1970, *P. Bamps 2306* (BR); Forêt du Téké, 29 January 1970, *P. Bamps 2379* (BR); forêt du Téké, 6 km N of Anyama, 5m, 22 May 1975, *H.J. Beentje 178* (WAG); Near ORSTOM, Adiopodoumé, 24 February 1962, *L. Bernardi 8249* (K, US, WAG); About 30 km SE of YakasséMé, 25 April 1974, *F.J. Breteler 7458* (B, MO, P, US, WAG); Adiopodoumé, 27 December 1987, *L. Gautier & D. Béguin 738* (CSRS, MO); Téké forest, 30 km N of Abidjan, on the right side of the road to Adzopé, 50 m, 19 January 1970, *J. de Koning 9* (WAG); Téké forest, 30 km N of Abidjan, 30 m, 28 January 1970, *J. de Koning 149* (WAG); Km 38 new road Abidjan-Ndouci, 14 June 1979, *A.P.M. de Kruif 145* (UCJ, WAG); Surroundings of Anyama, 28 km N of Abidjan, 22 August 1969, *C. Versteegh 706* (MO, WAG); Adiopodoumé, Botanic gardens, 29 January 1961, *H.C.D. de Wit 9117* (WAG); **Aboisso**. 5 km NNW of Nganda-nganda, 100 m, 24 July 1975, *H.J. Beentje 223* (WAG); **Adzopé**. On border of Comoé river, ca. 15 km NW of Mbasso, ca. 60 km NE of Adzopé, 27 July 1963, *W.J.J.O. de Wilde 572* (WAG); **Agboville**. Foret du Téké, 28 February 1967, *L. Aké Assi 9516* (G); Réserve botanique de Yapo, 12 November 1980, *L. Aké Assi 15728* (BR); Foret de Yapo, 23 September 1987, *L. Aké Assi 17738* (G, MO); 45 km N of Abidjan, Abbé forest, 22 November 1968, *F.J. Breteler 6084* (K, WAG); Forêt du Yapo. relevé nº33, au niveau du layon H7 de la Sodefor, 14 November 1991, *C. Chatelain 784* (G); Abidjan. Yapo Forest, 17 January 1974, *J. de Koning 3108* (WAG); 17 January 1974, *J. de Koning 3111* (WAG); Yapo, Bégué, May 1949, *Service Forestier de la Côte d'Ivoire 2990* (P); Forêt du Yapo, 26 July 1991, *H.G. Téré 1946* (G, WAG); 24 December 1956, *J.J.F.E. de Wilde 1021* (WAG); **Grand-Lahou**. Foret de Mopri, 23 January 1966, *L. Aké Assi 8489* (G); **San Pedro**. Grand Bereby, 10 February 1970, *L. Aké Assi 11046* (G); San Pedro, nouvelle piste vers le nord, 25 March 1970, *P. Bamps 2577* (BR, K); F.C. de Monogaga, Nord. forêt de haut de pente dégradée, 12 March 1997, *C. Chatelain 1404* (CSRS); Forêt Classée de Monogaga, just S of Sassandra-San Pedro road, 25 March 2000, *C.C.H. Jongkind 4736* (WAG); 25 March 2000, *C.C.H. Jongkind 4743* (WAG); between San Pedro and Grand Bérébу, 40 m, 25 March 1970, *J. de Koning 301* (WAG); **Sassandra**. 10 km. N. of Sassandra. Near left border of Sassandra river, 10 May 1975, *W.J. van der Burg 257* (WAG); Between Sassandra and Gagnoa, after the Fuyt plantation, 100 m, 7 April 1973, *J. de Koning 1233* (WAG); 100 m, 21 February 1959, *A.J.M. Leeuwenberg 2774* (K, WAG); 61 km N of Sassandra, W of Niapidou, 100 m, 18 March 1959, *A.J.M. Leeuwenberg 3111* (K, L, WAG); 35 km SW of Guéyo, 100 m, 28 March 1962, *A.J.M. Leeuwenberg 3753* (WAG); On border of river Niegré, ca. 64 km N of Sassandra near village Baléko, 16 June 1963, *W.J.J.O. de Wilde 249* (WAG); **Tabou**. Tai-Grabo. 15 km au S. de la Hana, 21 March 1969, *P. Bamps 2246* (BR, K); Entre Djiroutou et le mont Niénoukoué. Guiroutou, 50 m au Sud du layon 'Mont Niénokoué'; à 4 Km du campement écotouristique, March 1999, *A. Menzies 360* (G); Entre Djiroutou et le mont Niénoukoué. Guiroutou, 50 m au Sud du layon 'Hana'; à 3.5 Km du campement écotouristique, March 1999, *A. Menzies 375* (G); **Unknown.** Brotoko, May 1935, *A. Aubréville 2369* (P); Molokoto, 26 February 1957, *A. Aubréville (Ivory Coast series) 4122* (P); Region Yapo-Nord, 60 km septentrionem de Abidjan, 14 March 1962, *L. Bernardi 8617* (P); Environs de Mbasso, région de Moyen Comoé, 24 December 1909, *A.J.B. Chevalier 22634* (BR); Provence d'Attié, Mautéza, 28 June 1907, *A.J.B. Chevalier 17441* (P); Soumié, 27 April 1931, *Service Forestier de la Côte d'Ivoire 450* (P).

LIBERIA: Grand Gedeh. Near Kanweake, a village about 70 km S. of Chiehn (Zwedru village), 26 March 1962, *J.J.F.E. de Wilde 3644* (K, S, WAG); **Montserrado**. Dukwia Reserve, 7 May 1929, *G.P. Cooper 417* (A, BM, F, FHO, K); 10 miles north of Bomi Hills, 29 January 1971, *W. Goll 73* (WAG).

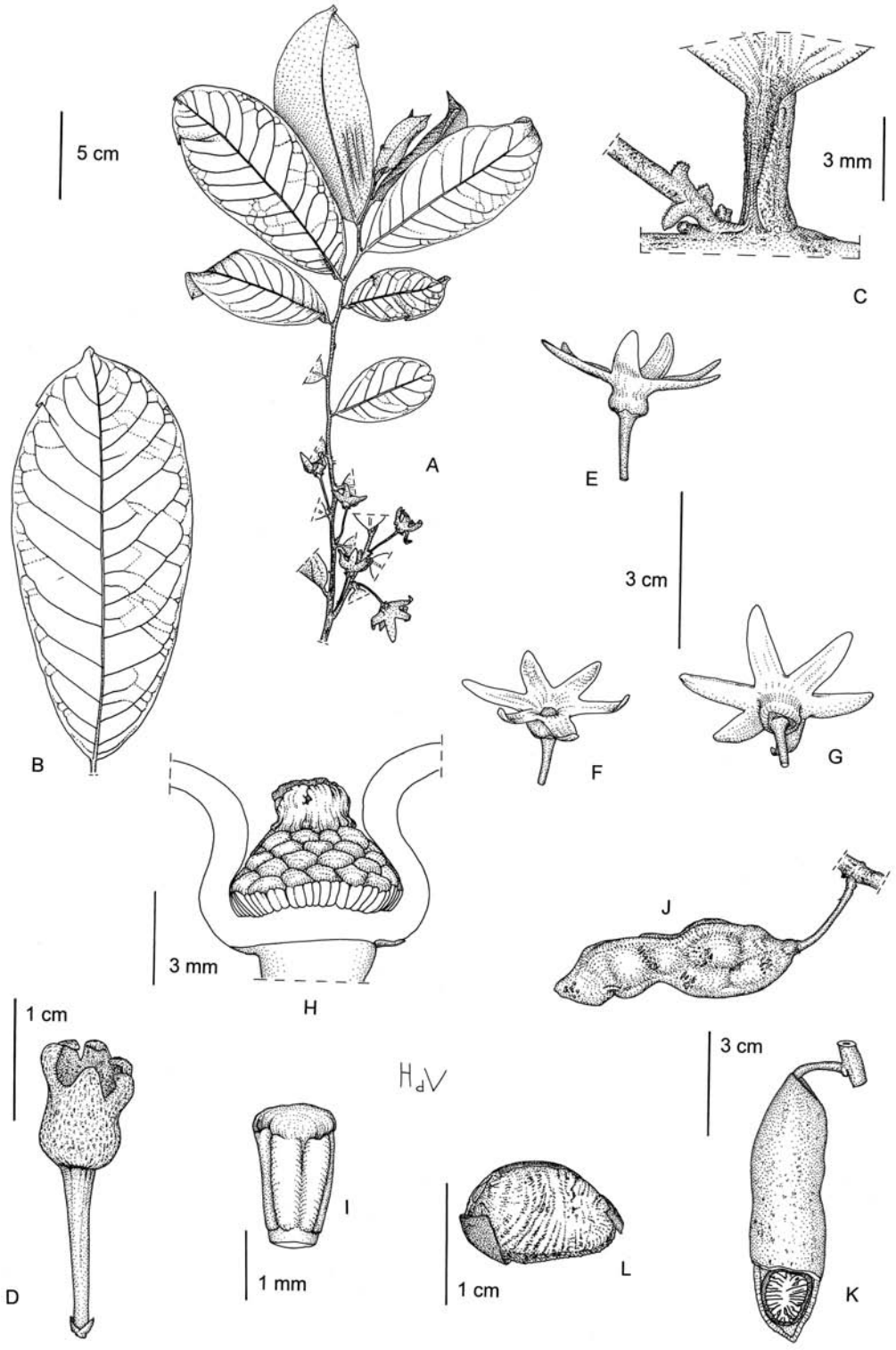


Figure 6.17. *Isolona cooperi*. A. Flowering branch. B. leaf (adaxial view). C. Detail of petiole and axillary rhipidium. D. Flower bud. E. Flower (Side view). F. Flower (semi side view). G. Flower (bottom view). H. Transversal section showing androecium and stigma. I. Stamen (front view). J. Fruit (dried). K. Fruit, part of pericarp removed showing transversal riuminate section of seed (fresh). L. Seed with partial outer tegument removed showing ruminations. Drawings Hans de Vries.

6. Isolona deightonii Keay, Kew Bull. 7: 156. 1952. — TYPE: SIERRA LEONE. Eastern Province: Daru, Tunkia, 8 March 1945, *F.C. Deighton 4112* (holotype: K!; isotype: P!). *Figure 6.18*

Tree up to 5 m high; d.b.h. unknown; bark grey-brown; young branches densely covered with short curly reddish hairs; old branches dark brown, striate, sparsely to densely covered with short curly hairs. *Petioles* 2-4 mm long, 1.5-2 mm in diameter, densely covered with short curly hairs, leaf lamina inserted on top, weakly grooved adaxially. *Leaf lamina* 13-21 cm long, 4-6 cm wide, length:width ratio 3-3.4, narrowly elliptic to narrowly obovate, base obtuse to broadly cuneate, apex acuminate, acumen ca. 1 cm long, subcoriaceous, glabrous adaxially, sparsely covered with erect hairs abaxially; midrib raised and sparsely to densely covered with short curly hairs adaxially, prominent and densely covered with short curly hairs abaxially; secondary veins 11-15 pairs, straight then abruptly curved upwards near margin, densely covered with short curly hairs on both sides. *Inflorescences* on leafy branches. *Flowering pedicels* 4-6 mm long, ca. 1 mm in diameter, densely covered with short appressed hairs. *Lower bracts* 1-2, ca. 1 mm long, ca. 1 mm wide, densely covered with short erect hairs; margins covered with short appressed hairs. *Upper bract* at base of pedicel, ca. 1 mm long, ca. 1 mm wide, covered with short appressed hairs. *Sepals* 8-10 mm long, 7-10 mm wide, length:width ratio 1-1.1, very broadly ovate, base truncate, apex acute, papyraceous, densely covered with short cirly hairs on both sides and along margins, green, falling in fruit. *Corolla* green; tube ca. 14-17 mm long, 4-5 mm in diameter, lobe:tube ratio 0.7-0.9, densely covered with short appressed hairs; lobes 8-12 mm long, 5-6 mm wide, length:width ratio 1.6-2, triangular, apex acute, sparsely covered with short appressed hairs, papyraceous. *Receptacle* ca. 5 mm in diameter. *Stamens* ca. 2 mm long; connective shields ca. 0.5 mm long, covered with short erect hairs, those of innermost stamens extended over adjacent carpel wall. *Ovary* ca. 2 mm long, ca. 1 mm in diameter; stigma ca. 2 mm in diameter, covered with short erect hairs. *Fruiting pedicels* 5-8 mm long, 3-4 mm in diameter, woody, sparsely covered with short appressed hairs. *Fruits* ca. 6 cm long, ca. 4 cm in diameter, length:width ratio ca. 1.5, globose, glabrous, smooth, longitudinally and irregularly ribbed, yellow; pericarp 2-3 mm thick. *Seeds* 10-13 mm long, 5-8 mm in diameter, transversely ellipsoid; testa rugose, light brown; raphe very slightly thickened, slightly rugose; hilum 5-7 mm long, 2-3.5 mm wide, elliptical.

Distribution: Sierra Leone and Ivory Coast (see Map 6); in rain forests; altitude unknown.

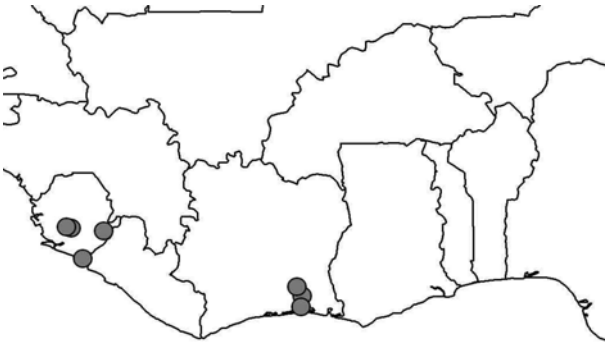
Phenology: Mature flowers present in March-April, September and December. Mature fruits collected in September and December.

Vernacular names:
Sierra Leone: Kpende-golei (Mende).

IUCN conservation status: EN B2ab(iv). *Isolona deightonii* is represented by seven collections mainly from unprotected areas, and has an area of occurrence (AOO) of only 70 km^2. Only one collection of *I. deightonii* originated from a protected area namely the classified forest of Anguédédou in Ivory Coast, north of Abidjan. In 2004, however, ca. 400 of the ca. 7000 ha of this protected forest was logged. Moreover, the species has not been collected since 1967 suggesting that the "endangered" (EN) category is appropriate.

Notes: *Isolona deightonii* can be distinguished from other species of *Isolona* by its large, papyraceous and hairy sepals and by the tube being longer than the lobes. This species bears a resemblance with *I. campanulata*; both having large papyraceous sepals, although those of the latter species are smaller and glabrous. Keay (1954) suggests that *I. deightonii* is related to *I. congolana*, *I. heinsenii* and *I. pilosa* due to the characteristic hairiness. However, molecular data does not support a close relationship between the hairy forms in *Isolona*.

Extra references: Keay, Fl. Trop. W. Afr. ed. 2, 1: 53. 1954; Aké Assi, Cont. Et. Fl. C. d'Iv. 1: 14. 1963 ; Aké Assi, Boissiera 57: 98. Fig. 5. 2001.



Map 6: Distribution of *Isolona deightonii*.

ADDITIONAL SPECIMENS EXAMINED:
IVORY COAST: Abengourou. Foret pres de Rubino, 30 September 1958, *L. Aké Assi 4937* (G); **Abidjan**. Foret d'Anguédédou, 19 March 1967, *L. Aké Assi 9545* (G, MO); **Agboville**. Yapo-Nord forest, 15 March 1962, *L. Bernardi 8691* (G, K, M, MO, S, US, WAG).
NIGERIA: Taraba State. Sardauna (Division Mawbilla); Plateau Ngel. Nyaki Forest Reserve, 1524m, 2 September 1976, *J.D. Chapman 4573* (FHO).
SIERRA LEONE: Eastern Province. Daru (Tukia), 8 March 1945, *F.C. Deighton 4112* (K, P); Jama, 22 September 1935, *F.C. Deighton 3072* (K); **Southern Province**. Kpende-Golei (Mende), 6 April 1939, *F.C. Deighton 3675* (K, P); Njala (Kori), December 1955, *C.T. Pyne 83* (K, P).

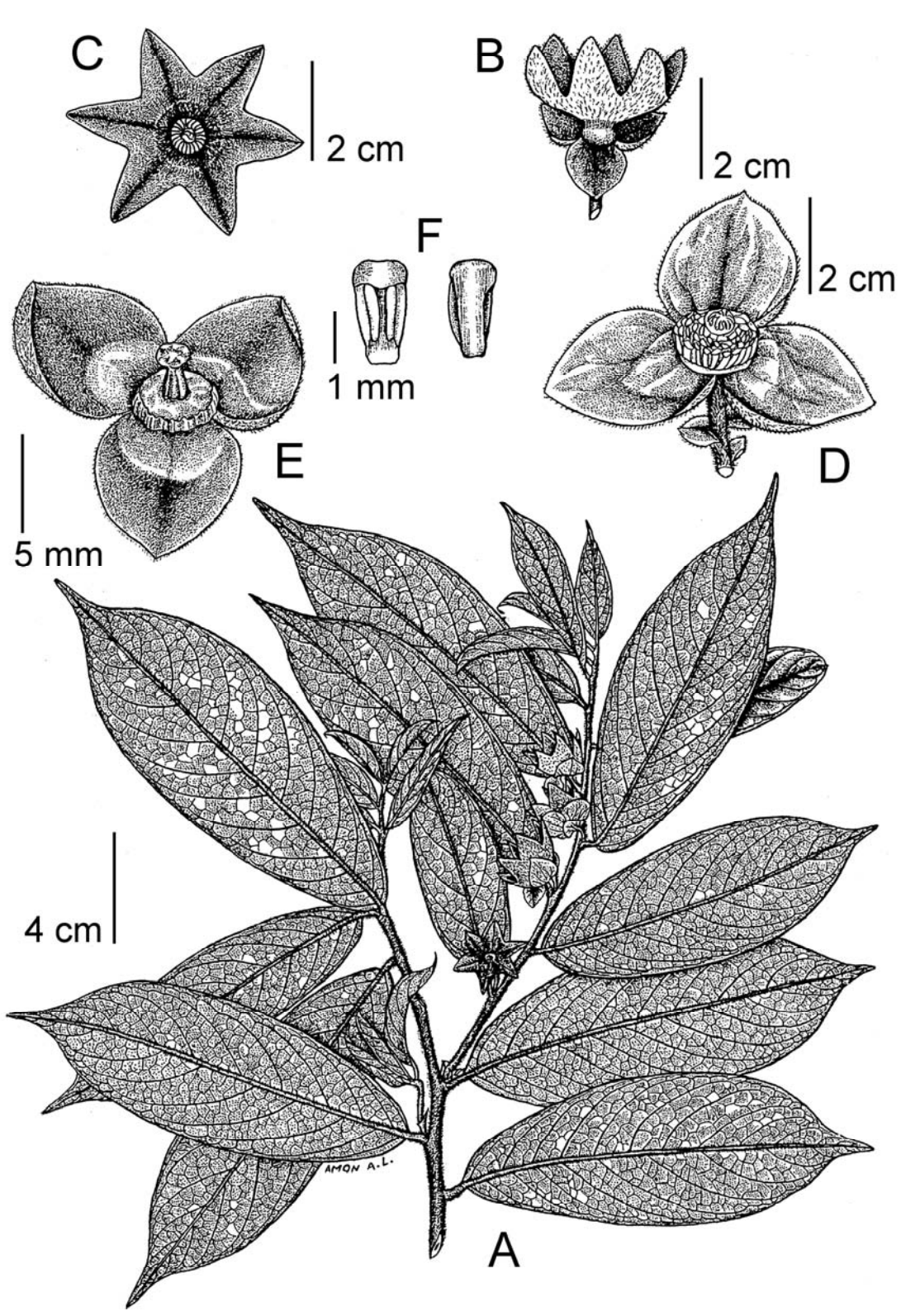


Figure 6.18. *Isolona deightonii*. A. Flowering branch. B. Flower. C. Flower (top view). D. Flower, details of sepals, androecium and stigma. E. Flower with ovary and stigma. E. Stamens front and back view. Reproduced with permission from Éditions des Conservatoire et Jardin botaniques de la Ville de Genève, Drawings by M. Lazare Amon Aya.

7. Isolona dewevrei (De Wild. & Th.Dur.) Engl. & Diels, Monogr. Afrik. Pflanzen-Fam. 6: 83. 1901. *Monodora dewevrei*, De Wild & Th.Dur., Bull. Soc. Roy. Bot. Belg. 38: 11. 1899 — TYPE: DEMOCRATIC REPUBLIC OF CONGO. Lemba-Luki, *A. Dewèvre 365* (holotype: BR!; isotypes: BR-3 sheets!). *Figure 6.19*

Tree or shrub to 15 m tall; trunk with d.b.h. up to 20 cm; old branches light brown, glabrous; young branches drying black, glabrous. *Petioles* 4-10(-15) mm long, 1-1.5 mm in diameter, glabrous, verrucose, leaf lamina inserted on side, broadly grooved adaxially. *Leaf lamina* 10-17 cm long, 4-7 cm wide, length:width ratio 2.5-3, narrowly obovate to obovate or elliptic to narrowly elliptic, base decurrent or rarely narrowly cuneate, apex acuminate, acumen ca. 1 cm, papyraceous, glabrous; midrib raised but proximally depressed and glabrous adaxially, prominent and glabrous abaxially; secondary veins 9-14, uniformally curving upwards, glabrous. *Inflorescences* on leafy branches, sometimes on older branches. *Flowering pedicels* 2-7 mm long, 1-1.2 mm in diameter, sparsely covered with short erect hairs to glabrous. *Lower bracts* 2-5, ca. 0.5 mm long, 0.2-0.5 mm wide, glabrous; margins with short and straight hairs. *Upper bract* near the base of the pedicel, ca. 0.9-1.1 mm long, 0.6-0.9 mm wide, glabrous; margins with short and straight hairs. *Sepals* 2-3 mm long, 3-4 mm long, length:width ratio 0.8-1, broadly ovate, base truncate, apex acuminate, pressed against corolla tube, glabrous, margins with short and straight hairs, falling in fruit. *Corolla* green turning yellow with red center at anthesis; tube 3-4 mm long, 4-6 mm in diameter, lobe:tube ratio 2.3-3.7; lobes 7-14(-17) mm long, 5-7 mm wide, length:width ratio 2-3, narrowly elliptic to elliptic, narrowed at base, apex rounded, glabrous, membranous, margins with sparsely short and curly hairs, curving inwards with margins curved outwards. *Receptacle* ca. 4 mm in diameter. *Stamens* 1.5-1.8 mm long; connective shields ca. 0.1 mm, glabrous, those of innermost whorl elongated and pressing against the ovary wall. *Ovary* 1-1.2 mm long, ca. 1 mm in diameter; stigma ca. 2.5 mm in diameter, capitate, glabrous to very sparsely covered with short erect hairs. *Fruiting pedicels* 9-10 mm long, 2-3 mm in diameter, woody, glabrous. *Fruits* 6-7 cm long, 4-5 cm in diameter, length:width ratio ca. 1.5, ovoid, smooth but very finely ribbed, glabrous, green; pericarp 2-3 mm thick. *Seeds* 10-20 mm long, 10-15 mm in diameter, ellipsoid, packed in a pale yellow pulp; testa smooth, dark brown; raphe slightly thickened, rugose, dark brown; hilum 8-10 mm long 3-4 mm wide, elliptical.

Distribution: Disjunct, Ivory Coast, western Ghana, Cameroon, and Democratic Republic of Congo (see Map 7); an understorey tree in evergreen moist forest; at 0-860 m altitude.

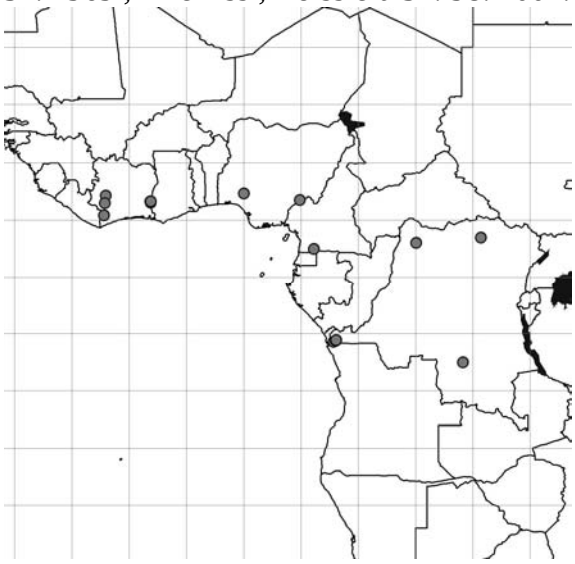
Phenology: Mature flowers collected in January, March, April, September and November. Mature fruits collected in January, February, April, and from June to September.

Vernacular names:
Democratic Republic of Congo: Divignia, Divinia (Kiobo et Luki).

IUCN conservation status: LC. Although disjunct, *Isolona dewevrei* is widely distributed and occurs in one national park (Bia, Ghana), and two forest reserves (Ajure-Ofosu in Nigeria; Haut-Sassandra in Ivory Coast). It was collected recently in both Ghana and Ivory Coast. The “least concern” category is therefore applied.

Notes: *Isolona dewevrei* presents close affinities with *I. thonneri* especially in leaf and fruit morphology. However, *Isolona dewevrei* can be distinguished by its elliptic and shorter corrolla lobes with hairy margins as well as a flowering pedicel sparsely covered with short hairs. It is, however, very hard to tell both species apart based on fruit or vegetative characters alone. Molecular data also indicate a close relationship between these species.

Extra references: De Wild., Ann. Mus. Congo Belge, Bot. ser. 5, tome 3: 393. 1912; Boutique, Fl. Congo Belge 2: 261. 1951; Aké Assi, Cont. Et. Fl. C. d’Iv. 1: 14. 1963; Le Thomas, Fl. Gabon 16: 354. 1969 ; Aké Assi, Boissiera 57: 98. 2001.



Map 7: Distribution of *Isolona dewevrei*.

ADDITIONAL SPECIMENS EXAMINED:
CAMEROON: South Province. Ngongondje hill, near Akonetye, 2 30'S of Ebolowa, 650 m, 28 August 1979, *A. Koufani 123* (P); **South-West Province**. Piste Munker-Gayama, 40 km NNW, 8 July 1975, *R. Letouzey 13984* (K, MO). **DEMOCRATIC REPUBLIC OF CONGO: Bas-Congo**. Luki, parc forestier de la Nkula, 21 June 1978, *H. Breyne 3340* (BR); Kiobo, 350 m, 18 January 1940, *C. Donis 56* (BR); Sandanda, 350 m, 18 January 1940, *C. Donis 58* (BR); Luki, 27 January 1948, *L. Toussaint 186* (BR, COI); Luki, à N'tola, près du poste météo, 28 September 1946, *L. Toussaint 2028* (BR, BRLU, WAG); Vallé de N'kula. Luki, 3 November 1946, *L. Toussaint 2117* (K, P); 8 October 1947, *L. Toussaint 2482* (BR, MO); Inéac-Luki, 11 February 1959, *J. Wagemans 2160* (K); **Equateur**. Mbongo, 1910, *J. Claessens 735* (BR); **Katanga (Shaba)**. Kaniama-Haut Lomani, 860 m, September 1947, *W. Mullenders 2369* (BR); **Orientale**. Bambesa, 19 April 1952, *P. Gérard 198* (BR); **Unknown.** 39 km de Boma, chemin de fer du Mayumbe. Pemba? Sans doute au Mayumbe, no date, *A. Dewèvre 365* (BR); Kiola, 27 September 1945, *C. Donis 369* (K).
GHANA: Western Region. Brong Ahafo Region. Bia National Park, 24 September 1982, *D.K. Abbiw 253* (MO, WAG); Bia National Park, 26 September 1976, *J.B. Hall GC 46522* (K, MO, US); Bia National Park and Production Reserve. Game and Wildlife Adufa Camp. South 11 km along main logging road, 150-190 m, 29 February 1996, *M. Merello 1346* (G, MO, UPS, WAG).
IVORY COAST: Soubré. Forest of Kouléahinou, 13 January 1956, *L. Aké Assi 3438* (G); **Tabou**. Mont Niénokoué, 14 March 1975, *L. Aké Assi 12830* (G); **Vavoua**. F.C. du Haut-Sassandra, Centre. forêt très dégradée, relevé FNK20, 5 April 1995, *F.N. Kouamé 1451* (CSRS); **Unknown.** Moloko[to?]sso, 10 February 1957, *A. Aubréville (Ivory Coast series) 1957/ 89* (P).
NIGERIA: Ondo State. Akure Ofosu Forest Reserve, 21 November 1961, *J.K. Adebusuyi 43592* (K).

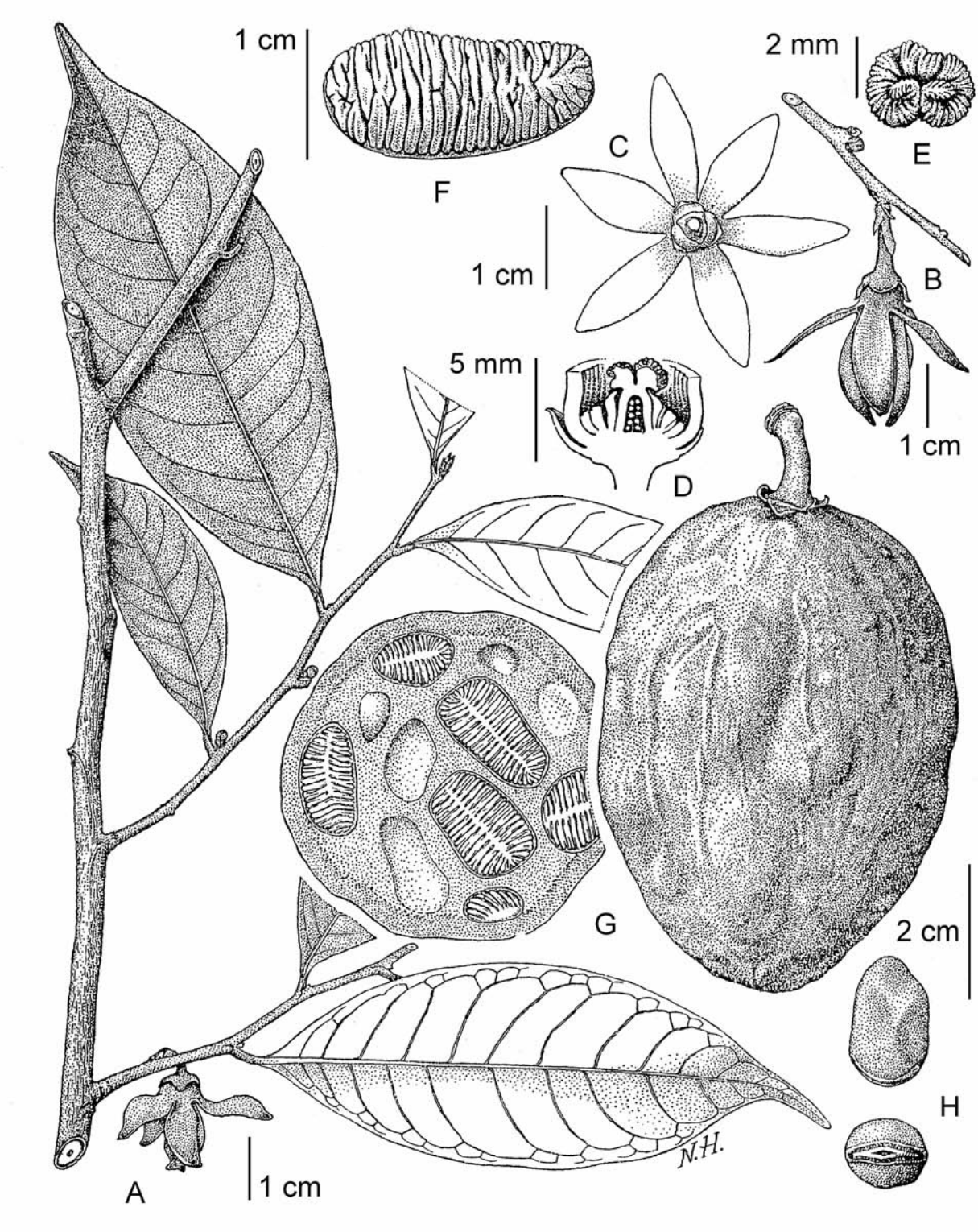


Figure 6.19. *Isolona dewevrei*. A. Flowering branch. B. Flower. C. Flower (top view). D. Transversal section of flower. E. Detail of stigma (top view). F. Transversal section of seed. G. Fruit (left) and longitudinal section of fruit (right). H. Seed, side view (top), detail of hilum (bottom). Modified from Contribution à l'étude de la flore de la Côte d'Ivoire, Aké Assi, Fig. 1. 14. 1963.

8. Isolona ghesquierei Cavaco & Keraudren, Bull. Jard. Bot. État, Brux. 27: 78. 1957. — TYPE: MADAGASCAR. Diego-Suarez: Maromandia, 11 November 1956, *Service Forestier de Madagascar 12451* (holotype: P!; isotypes: P-2 sheets!). *Figure 6.20 A-C*

Isolona ghesquierei var. *longipedicellata* Cavaco & Keraudren, Bull. Jard. Bot. État, Brux. 27: 78. 1957. — TYPE: MADAGASCAR. Betampona, *R.P.R. Capuron 8587* (holotype: P!; isotypes: P!, WAG!). ***syn. nov.***

Tree up to 15 m high; trunk with d.b.h. up to 15 cm; young branches drying black, smooth, glabrous; old branches dark grey, striate, glabrous. *Petioles* 6-9 mm long, 1 mm in diameter, glabrous, leaf lamina inserted on side, weakly grooved adaxially. *Leaf lamina* (6-)9.5-16 cm long, (2-)3-6.5 cm wide, length:width ratio 2-3, elliptic to obovate, base acute to broadly cuneate, apex acuminate, acumen 5-15 mm long, rounded, glabrous; midrib glabrous on both sides, secondary veins 7-11 pairs, uniformly curving upwards, glabrous. *Inflorescences* on leafy branches. *Flowering pedicels* 4-10(-16) mm long, 1-1.5 mm in diameter, very sparsely covered with short appressed hairs. *Lower bracts* 2-3, ca. 0.5 mm long, ca. 0.5 mm wide, glabrous, margins sparsely covered with short appressed hairs. *Upper bract* absent. *Sepals* ca. 2 mm long, ca. 3 mm wide, length:width ratio ca. 0.6, very broadly ovate, base truncate, apex cuspidate, pressed against tube, glabrous, margins sparsely covered with short appressed hairs, falling in fruit. *Corolla* dark red at anthesis; tube 6-11 mm long, 4-6 mm in diameter, lobe:tube ratio 4-8, glabrous; lobes 15-45 mm long, 5-11 mm wide, length:width ratio 2.5-4, narrowly elliptic to elliptic, narrowed at base, apex acute with rounded tip, glabrous, membraneous when dried, wavy and pointing downwards, margins sparsely covered with short erect hairs. *Receptacle* ca. 4 mm in diameter. *Stamens* ca. 1.5 mm long; connective shield ca. 0.1 mm long, glabrous, those of innermost stamens extended over adjacent ovary wall. *Ovary* ca. 2 mm long ca. 1.5 mm wide; stigma ca. 2 mm in diameter, glabrous to very sparsely covered with short appressed hairs. *Fruiting pedicels* ca. 10 mm long, 4-5 mm in diameter, woody, glabrous. *Fruits* 4-7 cm long, 4-5 cm in diameter, length:width ratio 1-1.5, globose to ovoid, smooth, glabrous, green; pericarp ca. 4 mm thick. *Seeds* 15-19 mm long, 8-10 mm in diameter, ellipsoid; testa smooth, light brown; raphe not thickened, darker brown; hilum 4-6 mm long, 3-4 mm wide, elliptical.

Distribution: Eastern Madagascar (see Map 8); in primary and secondary rain forests; at 0-1100 m altitude.

Phenology: Mature flowers collected in February and from October to December. Mature fruits collected in March and from May to July.

IUCN conservation status: EN B2ab(iii). *Isolona ghesquierei* is not well represented in herbaria, but has been collected recently. Moreover, it occurs in one national park (Marojejy). However, its AOO is less than 200 km^2 thus the “endangered” category seems applicable.

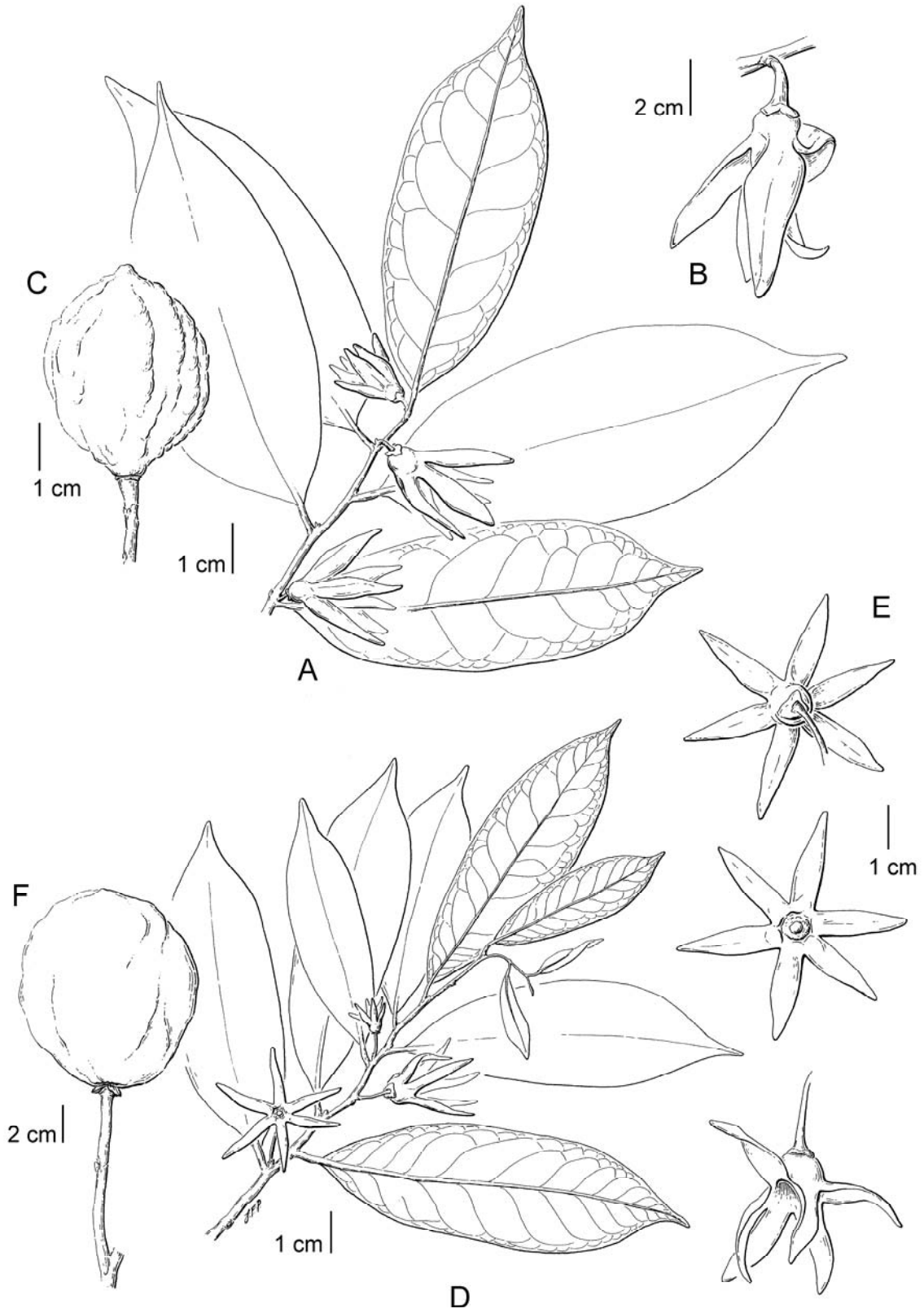
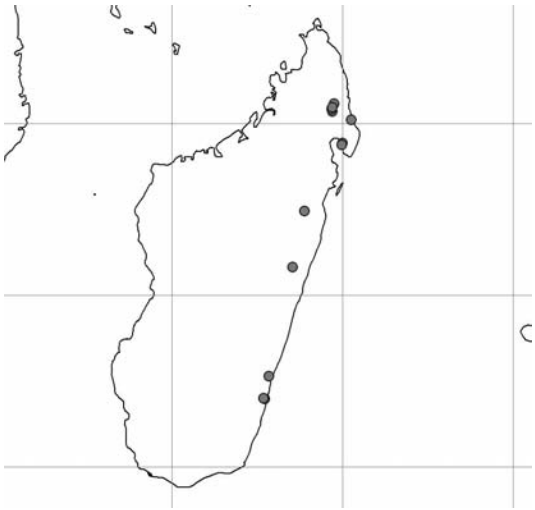


Figure 6.20. *Isolona ghesquierei*. A. Flowering branch. C. Flower (side view). C. Fruit. *Isolona perrieri*. D. Flower branch. E. Flower, bottom view (top); top view (middle), side view (bottom). F. Fruit. Drawings by Joanne Porck.

Notes: *Isolona ghesquierei* can be recognized by its often large and conspicuous papery corrolla lobes, and generally short and flowering pedicels sparsely covered with hairs. All other *Isolona*'s in Madagascar have glabrous flowering pedicels. The variety *longipedicellata*, recognized because of a longer pedicel, is no longer maintained as pedicel length seems to be quite variable and a more or less continuous variation was observed.



Map 8: Distribution of *Isolona ghesquierei*.

ADDITIONAL SPECIMENS EXAMINED:
MADAGASCAR: Antsiranana (Diego Suarez). Environ de Lehenen-Tsahebe (Haute Antsahabe, affluent r.g. de le Lokeho) entre Sambave et Andape, 19 October 1966, *R.P.R. Capuron SF(MDG) 24934* (WAG); Path from Mandena to the summit of Marojejy. Ridge top forest btw camp 2 and 3 Vicinity: Marojejy RNI, 700-1000 m, 2 October 1994, *B. Lewis 1256* (MO); Marojejy RNI, Analamboahangy Andrakata, Andapa. Aux environs de Manenobasy, 1171m, 24 January 1995, *F. Rasoavimbahoaka 504* (MO); Réserve naturelle Intégrale de Marojejy, Antampo Ambaliade, Andrakala, Andapa, 1150 m, 4 May 1994, *F. Rasoavimbahoaka 237* (P, WAG); Sous prefecture d'Andapa, Commune Rurale d'Andapa, Foret domaniale de Masiaposa, 890 m, 10 November 1995, *D. Ravelonarivo 883* (MO, WAG); Réserve Naturelle Intégrale de Marojejy, Massif d'Ambatosoratra, 1000-1400 m, 16 July 1996, *S.G. Razafimandimbison 270* (MO, WAG); Foret Andougozabe Antalaha, 18 November 1956, *Service Forestier de Madagascar SF(MDG) 12451* (P); **Fianarantsoa**. Manombo Reserve Spécialle, parcelle #1, à l'ouest de la rivière, point d'eau du campment., 30 m, 24 August 1995, *P.-J. Rakotomalaza 443* (MO); Analampaniby-Ilakata-Vohipeno, 25 October 1952, *Service Forestier de Madagascar SF(MDG) 6373* (P); J.B. num 16; Village le plus proche Manombo, 16 November 1955, *Service Forestier de Madagascar SF(MDG) 16157* (P); **Mahajanga (Majunga)**. Mont Vatovavy (entre Antsenavolo et Kianjavato), bassin de la Mananjary, 300-572m, 23 October 1964, *R.P.R. Capuron SF(MDG) 23704* (WAG); **Toamasina (Tamatave)**. Sous prefecture Vavatenina; commune: Ambodimangavalo, Moango. Dans l'Aire protegée de Zahamena, 800 m, 6 May 2003, *R. Rakotondrajaona 286* (MO); Vatomandry, Ambalabe, Tobin'i Foara, a l ouest de Toby Foara, 500 m, 17 March 2005, *A.A. Razanatsima 15* (MO); Masoala Peninsula ca 6 km NNE of Ambanizana along trail leading from Ambanizana River to the summit of Ambohitsitondroina, 550 m, 24 December 1989, *G.E. Schatz 2889* (C, K, MO, WAG); Masoala Peninsula, Ambanizana, "S Trail" (S of Androka River) climbing into hills SE of Ambanizana, 350 m, 29 October 1992, *G.E. Schatz 3364* (MO, WAG); **Unknown.** Amlooumangavalo, 11 February 1958, *Ramarokoto SF(MDG) 9483* (P); Doauy-Auala, 24 June 1951, *Service Forestier de Madagascar SF(MDG) 3655* (P); Foret orientale: Reserve Naturelle Num I (Betempora, Dct de Tametave), 450 m, 6 November 1953, *Service Forestier de Madagascar SF(MDG) 8587* (P, WAG).

9. Isolona heinsenii Engl. & Diels, Notizbl. Bot. Gart. Berlin-Dahlem 2: 301. 1899. — TYPE: TANZANIA. Tanga: Usambara Mountains, Derema, *E. Heinsen 19* (holotype B!; isotypes: K!, WU). *Figure 6.21*

Tree or shrub to 6(-12) m tall; trunk with d.b.h up to 10-15 cm; bark smooth, green-grey; branches spreading horizontally; young branches black, densely covered with short appressed hairs or rarely glabrous; old branches grey, sparsely covered with short appressed hairs to

glabrous, striate. *Petioles* 3-5 mm long, 1-2 mm in diameter, densely covered with short appressed hairs or rarely glabrous, leaf lamina inserted on side, weakly grooved adaxially. *Leaf lamina* (8.5-)15-22 cm long, 3-8 cm wide, length:width ratio 2-3.5, narrowly obovate to obovate or narrowly elliptic to elliptic, base broadly cuneate to rounded, apex acuminate, acumen ca. 1 cm long, papyraceous, glabrous to very sparsely covered with short appressed hairs abaxially, glabrous adaxially, dark green adaxially, light green abaxially; midrib sparsely covered with short appressed hairs to glabrous adaxially, densely to sparsely covered with short appressed hairs or rarely glabrous abaxially; secondary veins 8-13 pairs, sparsely covered with short appressed hairs to glabrous. *Inflorescences* on leafy branches. *Flowering pedicels* 5-12 mm long, ca. 2 mm in diameter, densely or rarely sparsely covered with short appressed hairs, light green. *Lower bracts* 1-3, ca. 1 mm long, ca. 0.5 mm wide, covered with short appressed hairs. *Upper bract* near the base of the pedicel or absent, ca. 0.5 mm long, ca. 0.5 mm wide, covered with short appressed hairs or rarely glabrous. *Sepals* 3-5 mm long, 2-3 mm wide, length:width ratio 1-2, narrowly to broadly triangular, base truncate, apex acute, densely covered with short appressed hairs or rarely sparsely covered with short appressed hairs, green, in mature flowers reflexed outwards, falling when in fruit. *Corolla* green when immature to light yellow with light red in center of tube at anthesis; tube 3-5 mm long, 4-6 mm in diameter, lobe:tube ratio 2.7-4.8, cupulate, covered with short appressed hairsv or rarely glabrous outside, glabrous inside; lobes 15-19 mm long, 2-4 mm wide, length:width ratio 4.5-6.3, lorate to narrowly oblong, base truncate, apex round to acute, densely covered with short appressed hairs on the outside, less so on inside or rarely glabrous, coriaceous, spreading horizontally and then curving inwards, the margins folded outwards. *Receptacle* 4-5 mm in diameter. *Stamens* ca. 1 mm long; connective shield ca. 0.1 mm long, glabrous, light yellow, those of innermost whorl elongated and stretched against the ovary wall. *Ovary* 1-2 mm long, ca. 1 mm wide; stigma 3-4 mm, glabrous to sparsely covered with short erect hairs, light green. *Fruiting pedicels* 2-3 cm long, 3-4 mm in diameter, densely to sparsely covered with short erect hairs. *Fruits* 4-10 cm long, 1-3 cm in diameter, length:width ratio 3.6-4.5, narrowly ellipsoid, apiculate, apicule ca. 1 cm, 6-ribbed, glabrous, heavily speckled with white and yellow; pericarp ca. 3 mm thick. *Seeds* 15-20 mm long, ca. 10 mm in diameter, irregularly ellipsoid; testa smooth, light brown; hilum not seen.

Distribution: Eastern Tanzania, Eastern Arc Mountains (see Map 9); in montane rain forests, occasionally in lowland rain forests; at (500-)1000-1440 m altitude.

Phenology: Mature flowers collected from October to May. Mature fruits collected from April to May and from September to October.

IUCN conservation status: VU B2ab(iii). *Isolona heinsenii* is represented by a fair amount of specimens in herbaria, and has been collected recently. It can also be found in two protected areas: the Udzungwa national park and the Amani nature reserve, both of which are in good condition. However, it never grows in dense populations and can consequently

become threatened because of its small Area of Occurrence (ca. 220 km^2), which is why we apply the “vulnerable” category accordingly.

Notes: *Isolona heinsenii* is generally densely hairy, especially on young branches, petioles, pedicels and corollas. Their are, however, a few specimens that are very sparsely hairy to completely glabrous on the leaves and young branches, and these seem to occur at lower altitudes (500-900 m). The floral parts are also glabrescent which on first view might give the impression of a different species. However, the flowers are otherwise identical to those of the hairy specimens.
Isolona heinsenii can be distinguished by the generally densely covered hairy leaves and flowers, petal lobes curving inwards over the receptacle and conspicuously 6-ribbed fruits. Molecular data strongly supports a sister relationship with *I. linearis*. The latter species has many morphological differences with *I. heinsenii* (glabrous, fruits not ribbed). However, they do share the same habitat, both occurring in the montane rain forest of the Eastern Arc Mountains, with *I. linearis* mainly found in the South (Udzungwas) and *I. heinsenii* in the North (Usamabras), although some overlap does exist.

Extra references: Engl. & Diels, Monogr. Afrik. Pflanzen-Fam. 6: 84. 1901; Brenan & Greenway, Tanganyika Territory Ch. Lis. 2: 42. 1949; Verdcourt, Fl. Trop. E. Afr., Annonaceae 125. 1971.

Map 9: Distribution of *Isolona heinsenii*.

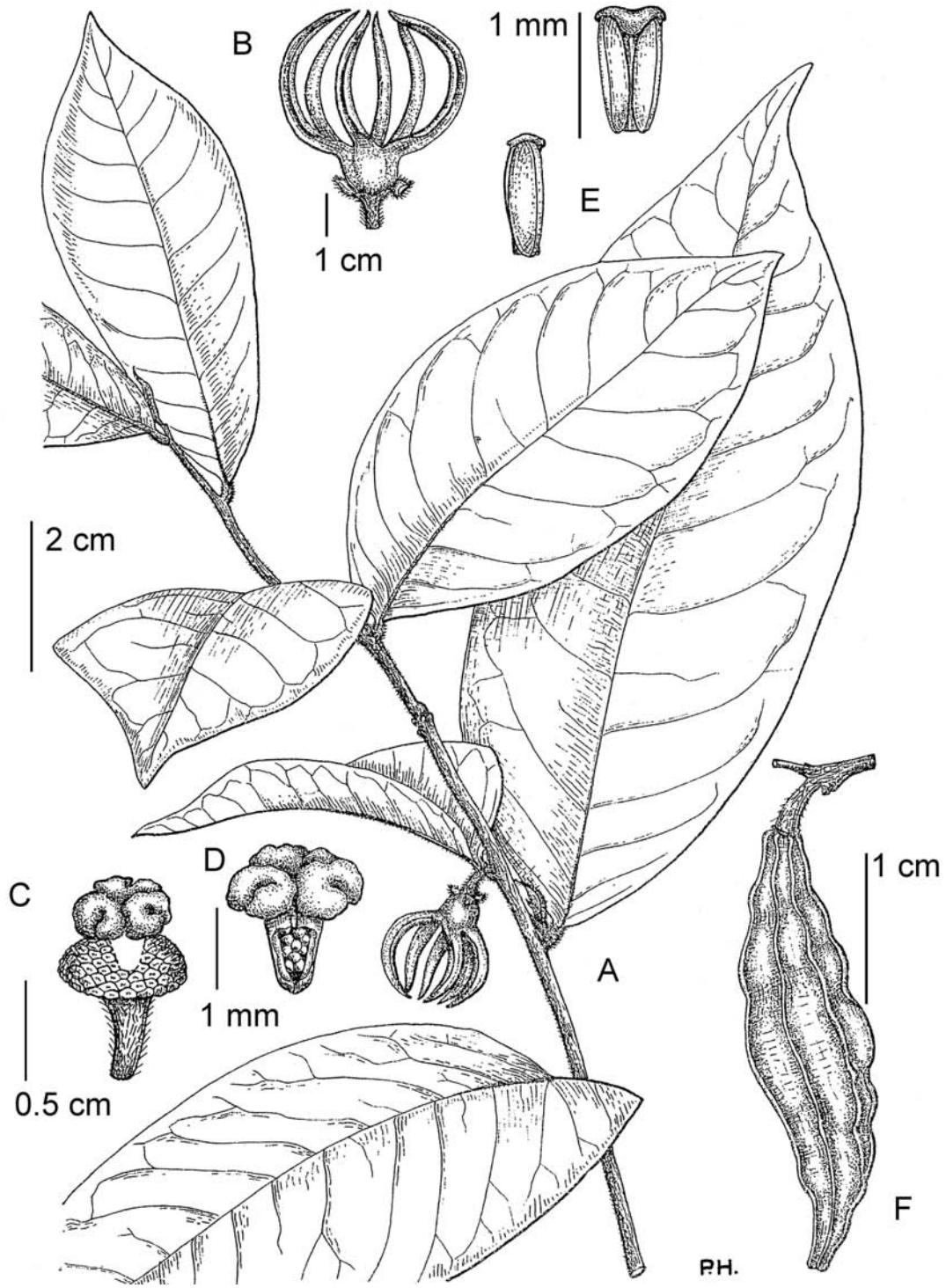


Figure 6.21. *Isolona heinsenii*. A. Flowering branch. B. Flower. C. Androecium and stigma. D. Gynoecium. E. Stamens (front and side view). F. Fruit. Modified from Flora of Tropical East Africa, Verdcourt, Fig. 29. 1971.

ADDITIONAL SPECIMENS EXAMINED:

TANZANIA: Iringa. Sanje, Uzungwa, 510 m, 20 October 1983, *J.C. Lovett 188* (K); Lulanda Forest Reserve, 29 March 1988, *W.R. Mziray 176* (NHT); Udekwa Village EW Kilombero FOREST RESERVE, October 1982, *W.A. Rodgers 2278* (DSM, K); Luhomero massif, Lofia River catchement, 12 August 1985, *W.A. Rodgers 4355* (K); Luhomero massif, Lofia River catchement, 17 August 1985, *W.A. Rodgers 4524* (K); **Lindi**. Kiwengoma Forest,, Northern edge of the Matumbi Highlands, 17 February 1990, *Frontier-Tanzania Coastal Forest Research Programe 881* (C, MO); **Morogoro**. Udzungwa

mts NP Mt Luhomero, 1440 m, 27 September 2000, *W.R.Q. Luke 6721* (EA, K); Lower Kihansi Hydropower Project, 30 August 1997, *F.M. Mbago 1630* (DSM); Kanga Mts, 90 km N of Morogoro Town, 2 December 1987, *L.B. Mwasumbi 13840* (DSM, K, MO); Ubangala (Mbangala) North of Uahenge Station, 20 February 1932, *H.J.E. Schlieben 1787* (BM, BR, G, HBG, K, M, P, S); Mahenge, Umgebung der station Mahenge, 900-1000 m, 10 December 1931, *H.J.E. Schlieben 1539* (BM, BR, G, HBG, K, M, P, S, Z); Mtibwa Forest Reserve, 420 m, November 1954, *S.R. Semsei 1912* (FHO); Magombera Forest Reserve, 3 November 1961, *S.R. Semsei 3383* (K); **Tanga**. Mongo, 304m, December 1912, *B.L. Institut Amani 3853* (FHO, K); Amani Nature Reserve, Sangarawe division tea estate, 1100 m, 11 November 2006, *T.L.P. Couvreur 10* (DSM, MO, WAG); Amani Nature Reserve. 1 km along the Mbomole trail, 1100 m, 13 November 2006, *T.L.P. Couvreur 31* (DSM, MO, WAG); forest above Amani-Monga about 1 mile NNE of Amani, East Usambaras, 950 m, 23 April 1953, *R.B. Drummond 3415* (B, BR, EA, K, P, S); East Usambara Mountains, 8 October 1905, *A. Engler 3402* (B); Amani Lab Plantation 11, 23 January 1933, *P.J. Greenway 3353* (FHO, K); East Usambara Mountains, Amani, 914m, 18 December 1928, *P.J. Greenway 1055* (K); East Usambara Mountains, Amani Laboratory Plantation 11, 23 January 1933, *P.J. Greenway 3335* (K); East Usambara Mountains, Amani to Monga, 975m, 2 November 1936, *P.J. Greenway 4702* (BR, EA, K); East Usambara Mountains, Derema, no date, *E. Heinsen 19* (B, K); Muheza District, Amani, East Usambaras, 900 m, 20 January 1983, *S.P. Kibuwa 6001* (NHT, UPS); E Usambara, Derema forest, remnant above Dodwe falls to the NE, 8 November 1998, *L.B. Mwasumbi 112425* (DSM); East Usambara Mountains, Amani 25 January 1906, *A. Peter 359* (K); East Usambara Mountains, January 1914, *A. Peter 309* (B, K, WAG); March 1919, *A. Peter 7968* (B, K); 24 November 1916, *A. Peter 18322* (B, K, WAG); January 1917, *A. Peter 52291* (B); Kwamkoro Forest Reserve, 850 m, 7 January 1987, *C.K. Ruffo 2017* (K); Kwamkoro Forest Reserve near Sawmill, 25 May 1987, *C.K. Ruffo 2269* (K); East Usambara Mountains, Derema, no date, *G. Scheffler 145* (B, BM, K); Amani, October 1929, *K.E. Toms 2* (K); East Usambara Mountains, between Derema and Amani, Kwamkuyu / Dodwe Falls over hanging St Paulia locality, 1000 m, 27 December 1956, *B. Verdcourt 1742* (BR, K); East Usambara Mountains, Amani, 900 m, January 1903, *O. Warnecke 231* (A, BM).

10. Isolona hexaloba (Pierre) Engl. in Engl. & Prantl, Nat. Pflanzenfam. Nachtr. 1: 161. 1897. — *Monodora hexaloba* Pierre, Fig. Herb. L. Pierre, del. E. Delpy 5/1896. — TYPE : GABON : Estuaire, Environ de Libreville, 17 February 1896, *T.J. Klaine 360* (holotype: P!; isotypes: B!, K!, P!, WAG!). *Figure 6.22*

Isolona bruneelii De Wild., Ann. Mus. Congo Belge, Bot. ser. 5, 3: 82, tab. 10. 1909. — TYPE: DEMOCRATIC REPUBLIC OF CONGO. Orientale: Dikila, December 1906, *A. Bruneel s.n.* (holotype: BR!; isotypes: BR!, S!).

Isolona seretii De Wild., Ann. Mus. Congo Belge, Bot., ser. 5, 3: 82, tab. 9. 1909. — TYPE: DEMOCRATIC REPUBLIC OF CONGO. Equateur: near Nala, March 1907, *F. Seret 792* (holotype: BR!; isotype: BR!).

Isolona solheidii De Wild., Ann. Mus. Congo Belge, Bot. ser. 5, 3: 83, tab. 8. 1909. — TYPE: DEMOCRATIC REPUBLIC OF CONGO. Orientale: surroundings of Yambuya, 1906, *A.F. Solheid s.n.* (holotype: BR!; isotype: BR!).

Isolona pleurocarpa var. *nigerica* Keay, Kew Bull. 7: 157. 1952. — TYPE: NIGERIA. Ijebu District: Shasha Forest Reserve, 8 April 1935, *P.W. Richards 3343* (holotype: BM!; isotypes: BR!, G!, MO!, S!).

Tree up to 25-30 m high; trunk with d.b.h. up to 50 cm, fluted; bark smooth, brown or grey greenish; young branches drying black, glabrous, old branches light grey, glabrous. *Petioles* 2-4 mm long, 2-3 mm in diameter, glabrous, leaf lamina inserted on side, grooved adaxially. *Leaf lamina* 10-28 cm long, 3-11 cm long, length:width ratio 2.3-4, narrowly ovate to ovate or narrowly elliptic to elliptic, base acute or rarely obtuse, apex acuminate, acumen 1-2 cm long,

coriaceous, shiny dark green above, light green below, glabrous; midrib proximally depressed adaxially, glabrous on both sides; secondary veins 8-16 pairs, uniformally curving upwards, loop forming towards margins, glabrous. *Inflorescences* often with an additional 1-flowered rhipidium, on leafy branches. Flowering pedicels (7-)13-25(-30) mm long, ca. 0.5 mm in diameter, glabrous, green. *Lower bracts* 2-4, ca, 0.5 mm long, ca. 0.4 mm wide, glabrous, margins covered with short appressed hairs. *Upper bract* at the base of the pedicel, 2-5 mm long, 1 mm wide, glabrous, margins covered with short erect hairs. *Sepals* 1-4 mm long, 2-4 mm wide, length:width ratio 0.5-1, transversely elliptic to elliptic, base truncate, apex shortly acuminate, glabrous, green, margins with short erect hairs, pressed against tube, falling in fruit. *Corolla* white to yellow or green when immature to dark red at anthesis; tube 4-10 mm long, 4-7 mm in diameter, lobe:tube ratio 1-5, glabrous; lobes 6-25 mm long, 4-12 mm wide, length:width ratio 1.3-2, elliptic or ovate, narrowed at base, apex acute to rounded, glabrous, coriaceous, spreading horizontally. *Receptacle* 4-6 mm in diameter. *Stamens* 1.8-2 mm long; connective shield ca. 0.1 mm long, glabrous, those of innermost row elongated over ovary wall. *Ovary* ca. 1 mm long, ca. 0.5 mm wide; stigma ca. 2 mm in diameter, glabrous, light red at anthesis. *Fruiting pedicels* 20-25(-29) mm long, 3-5 mm in diameter, woody. *Fruits* 3-7 cm long, 2.5-4 cm in diameter, broadly ovoid, lumpy, irregularly and transversely ribbed, glabrous, light green to dark purple-red when mature; pericarp 3-4 mm thick. *Seeds* 8-15 mm long, 4-6 mm in diameter, transversely ellipsoid; testa rugose, light brown; raphe thickened, darker brown; hilum 3-5 mm long, 2-3 mm wide, elliptical.

Distribution: Ghana, Nigeria, Cameroon, southern Central African Republic, Gabon, Congo (Brazzaville), Democratic Republic of Congo and the extreme north-east of Angola (see Map 10); in primary and secondary forests, near rivers, but also found in semi deciduous forests; at 0-700 m altitude.

Phenology: Flowers and fruits collected all year round.

Vernacular names:
Angola: Muamba (Kikongo).
Cameroon: Ndin (West Province).
Central African Republic: Monyingo (Bambindjere), Nzingodengwe (lissango).
Democratic Republic of Congo: Bompafwa, Ehai (Dundusana); Mossombe (dial. kundu); Bundjingu (Kawa); Edale, Efondi (Yambata); Embebu (dial. mangettu); Pembedjingo (Likimi); Loopa (dial. turumbu; Mongambo (Mobwasa); Pombodjingo (Musa).
Nigeria: Aghako-élé (Benin), Aghako-eze (Edo).
Republic of the Congo: M'Belzok (Bakimele).

IUCN conservation status: LC. *Isolona hexaloba* is very well represented in herbaria and is widely distributed in Central Africa, less so in West Africa, and can be found in numerous protected areas. Therefore the “least concern” category is appropriate.

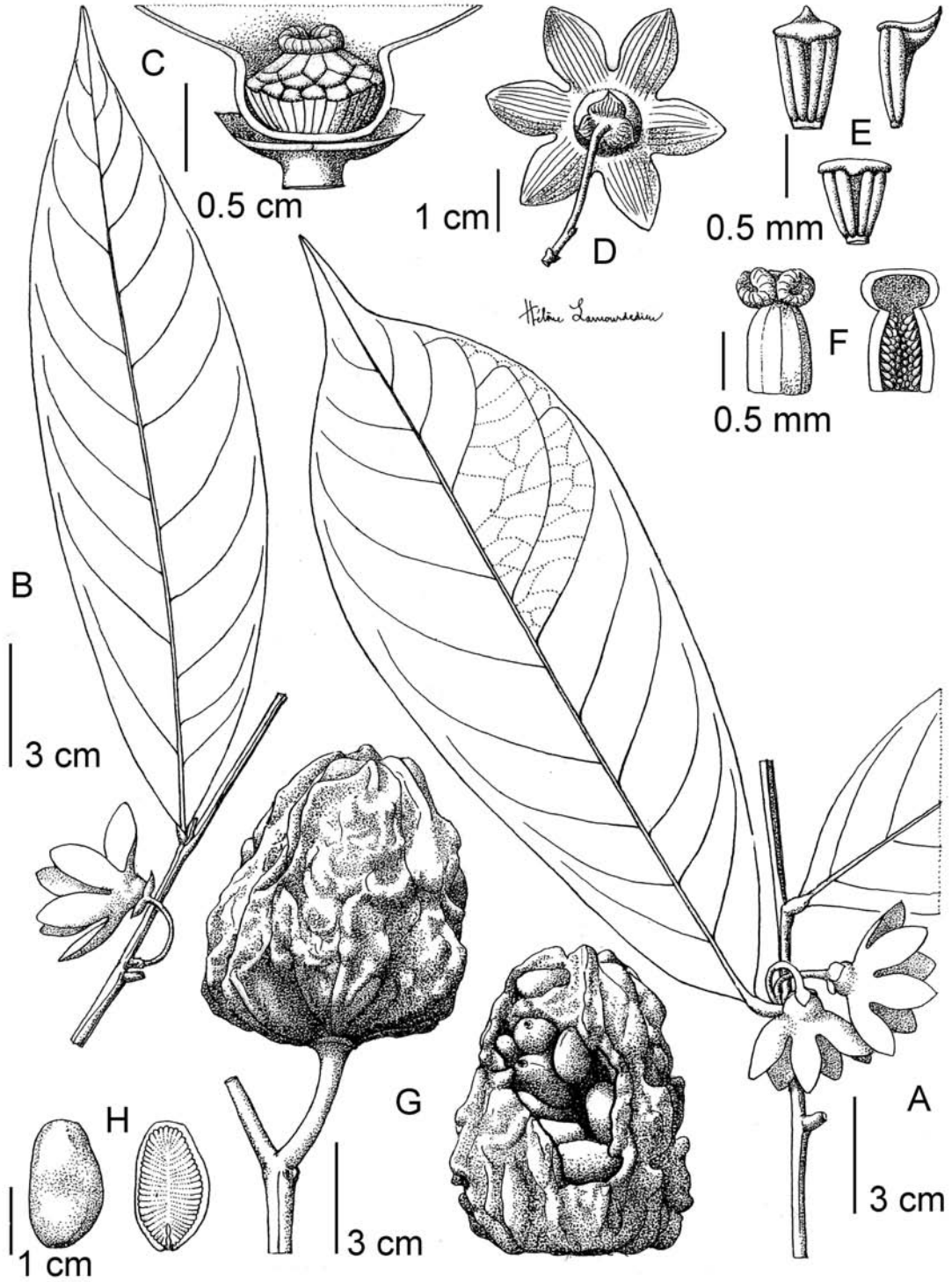
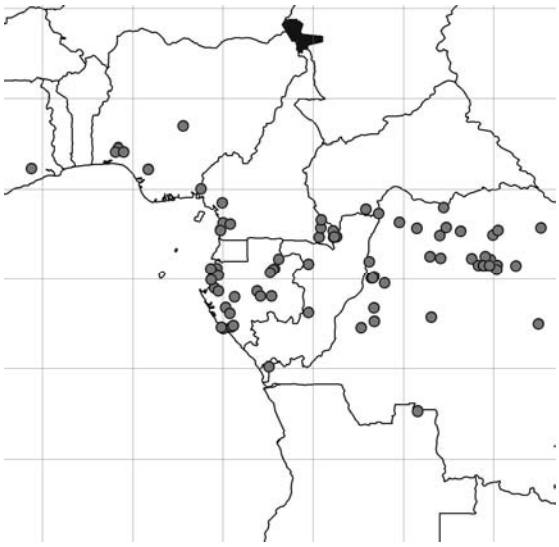


Figure 6.22. *Isolona hexaloba*. A-B. Flowering branch. C. Transversal section of flower showing androecium and stigma. D. Flower (bottom view). E. Stamens inner row (2 top); stamens of inner most row (top) and outermost row (bottom). F. Gynoecium and transversal section of gynoecium. G. Fruits. H. Seed (left) and transversal section of seed showing ruminate endosperm (right). Modified from La Flore du Gabon, Le Thomas, Fig. 66. 1969.

Notes: *Isolona hexaloba* can be distinguished by the short and grooved petiole with the lamina inserted on the side. The flower lobes are elliptic to obovate, with a narrowed base. However, it is a very polymorphic species and has been described under several other names, now all synonyms. Most of these new species were mainly based on the different lengths of the leaves and flowers. Detailed field notes describe that even after anthesis the flower lobes continue to grow until flower abscission. The color also changes from light green to yellow and becoming red at anthesis, and dark red just before abscission. *I. hexaloba* resembles *I. cooperi* by the shape of the flower lobes, but is distinguishable by the insertion of the leaf lamina on a shorter petiole and lacks the strong sweat smell. The molecular phylogeny, however, indicates a moderately supported sister relationship with *I. congolana*.
In 1896, Pierre validly described, by means of a detailed drawing by Delpy and without descriptive text (Art. 44, St Louis code; and for a detailed discussion on the validity in Pierre's case see Breteler, 2005), a new species named *Monodora hexaloba* placing it in the invalidly published 'section' *Isolona* (there is no provision in the botanical code for validating names of infrageneric groups by an illustration without a descriptive text), becoming the first record of the name *Isolona*.

Extra references: Pellegrin, Bull. Soc. Bot. France 94: 387. 1947; Tisserant & Sillans, Not. Syst. 15 : 325. 1958; Le Thomas, Fl. Gabon 16 : 354; Fig. 66. 1969; Keay, Trees of Nigeria 30. 1989.



Map 10: Distribution of *Isolona hexaloba*.

ADDITIONAL SPECIMENS EXAMINED:
ANGOLA: Cabinda. Maiobe, Reserva Indigena de Chiaca, 70 m, 14 May 1952, *F. Càmeira 21* (COI, LISC, LISJC, LUA); **Lunda Norte**. Lunda région, Locality: Dundo, near Luachimo river, 28 October 1946, *J. Gossweiler 13738* (B, BM, COI, US).
CAMEROON: East Province. About 14 km W of Yenga Port Gentil, a village about 35 km NNE of Moloundou, 21 April 1971, *R. Letouzey 10703* (BR, COI, HBG, K, WAG); A 25 km environ á l'Ene de Mikel village situé á 85 km au N de Moloundou sur road de Yokadouma, 24 February 1971, *R. Letouzey 10419* (P, WAG); a 20 km Sud de Mboy I, 45 km l'Est de Yokadouma, 16 May 1963, *R. Letouzey 5072* (K, P, WAG); **Littoral Province**. Route forestière SNCB, about 25 km S of Yabassi, 11 May 1976, *R. Letouzey 14910* (C, K, MO, WAG); **South Province**. Near Bipaga II, km 40 road Kribi-Edéa, 30 m, 30 December 1982, *A.P.M. de Kruif 998* (WAG); 22 km on road Kribi to Campo, 12 km past Gross Batanga, 30 m, 24

February 1994, *J.J. Wieringa 2327* (MPU, U, WAG); 17 km S. of the Lobé River, along the road to Campo, 18 March 1975, *J.J.F.E. de Wilde 8088* (BR, EA, HBG, K, LG, MA, MO, P, PRE, SRGH, U, WAG, YA); Place de Bipinde, November 1901, *G.A. Zenker s.n.* (P); **South-West Province**. Korup National Park, P plot, subplot 28F, 100 m, 31 October 2005, *X.M. van der Burgt 791* (BR, G, K, MO, P, SCA, WAG, YA); **Unknown.** 22 March 1910, *G.A. Zenker s.n.* (K).

CENTRAL AFRICAN REPUBLIC: Grima, 28 December 1961, *G. Guigonis 2339* (P); **Lobaye**. Region Mbaiki et Boukoko, Boukoko, 12 September 1947, *C. Tisserant 220* (BM, P, WAG); Boukoko, 14 April 1953, *C. Tisserant 82* (P); **Sangha**. Lindjombo, within 3 km radius of Lindjombo, 385m, 1 November 1988, *J.M. Fay 8683* (MO); Ndakan: Upland forest 3 km of Sango River, Tansect 2, 400 m, 10 May 1988, *A.H. Gentry 62692* (MO); Ndakan: upland forest 3 km E of Sango River, Transect 1, 400 m, 10 May 1988, *A.H. Gentry 62678* (MO); 45 km S of Lidjombo, E of Sangha river, Ndakan study area, 350 m, 1 November 1990, *D.J. Harris 2647* (K, MO, P); Sanha Economique. Dzanga-Sangha Reserve. Ndakan gorilla study area M4000 to M400, 4 October 1988, *D.J. Harris 1322* (K).

DEMOCRATIC REPUBLIC OF CONGO: Bandundu. Ingende, 12 April 1959, *C.M. Evrard 6130* (BR); road de Bolia-Iboko, limite des Terr. Inongo et Bikoro, 16 April 1959, *C.M. Evrard 6186* (BR); Nioki, 1948, *A. Flamigni 7079* (K); Bankaie (terr. Inongo), 11 September 1953, *G. Gilbert 14794* (BR); 29 September 1953, *G. Gilbert 14878* (BR); **Equateur**. Dikila, 1 December 1906, *A. Bruneel s.n.* (BR, S); Mobwasa, April 1910, *J. Claessens 615* (BR); Rubi, May 1921, *J. Claessens 652* (BR); Coquilhatville (Mbadaka), 7 November 1934, *G. Coûteaux 311* (BM, K); Yambata, March 1949, *S. De Giorgi 1749* (BR); Bas Uele, 9 November 1934, *A. De Wulf 312* (BR); Equateur, Monkako, Iwana, 13 October 1957, *C.M. Evrard 2820* (FHO); Monkoto, Iwama, 13 October 1957, *C.M. Evrard 2821* (BR, K, UPS); Ngondo sur Ngiri, 11 March 1959, *C.M. Evrard 5891* (K); Station Ineac, Boketa, 19 March 1955, *C.M. Evrard 5241* (BR); Eala, October 1936, *J.H.P.A. Ghesquière 3378* (BR, FHO, K, MO, US); 1930, *J. Lebrun 1379* (B, BR, G, GH, P, WAG); *J. Leemans 466* (K, P); Ter. Gemma (Bozene), 28 April 1941, *C. Leontovitch 202* (BR, K); Bantoie, pres d'Eala, 7 June 1936, *J. Louis 2197* (B, K); Eala, October 1936, *J. Louis 3378* (BM); Environ de Likimi, 20 April 1910, *L. Malchair 279* (BR); 19 October 1910, *L. Malchair 439* (BR); Djombo, 21 November 1912, *Mengé, A. 34* (BR); Dundusana, October 1913, *M.G. Mortehan 583* (BR); Mobwasa, October 1913, *F.J. Reygaert 1134* (BR); Sur Nala, March 1907, *F. Seret 792* (BR); Eala, 1930, *P.J. Staner 1295* (B, BR, G, GH, K); **Maniema**. Urega (Maniema), July 1932, *J. Lebrun 5786* (K, P); **Orientale**. 23 km along road from Kisangani to Bengamisa, 2 March 1973, *J. Bokdam 3980* (WAG); 21 June 1973, *J. Bokdam 4188* (KIS, WAG); Yangambi, 5 May 1960, *D. Bolema 67* (BR, WAG); Kawa, March 1921, *J. Claessens 284* (B, K, WAG); Yangambi, 12 February 1960, *R. Devred 4114* (BR, WAG); Yangambi, 15 March 1960, *R. Devred 4167* (BR); Yangambi,14 September 1950, *C. Donis 2721* (K); Yangambi, 19 September 1950, *C. Donis 2771* (BR, UPS); Yangambi, plateau, au bord de la falaise, 25 September 1950, *C. Donis 2801* (BR, MO); Yangambi, 28 September 1950, *C. Donis 2824* (BR, EA); Yangambi, 4 October 1950, *C. Donis 2863* (BR); Yangambi, 20 October 1950, *C. Donis 2951* (BR); Yangambi, 11 October 1951, *C. Donis 3143* (BR, S); Yangambi, 20 December 1951, *C. Donis 3214* (BR); Yangambi, 21 December 2005, *C. Donis 3245* (BR); Yangambi, 2 January 1951, *C. Donis 3279* (BR); Yangambi, 11 January 1952, *C. Donis 3383* (BR); Yangambi, 23 January 1952, *C. Donis 3476* (FHO, K); Station Ineac, Boketa, 31 August 1955, *C.M. Evrard 1732* (BR); Yaosuka, July 1938, *G. Gilbert 1310* (BR, K, MO, P, US); Yangambi, 1944, *G. Gilbert 1102* (BM, K, P); Yangambi, 1944, *G. Gilbert 1286* (K); Yangambi, 1944, *G. Gilbert 9196* (K, P); Yangambi, 1944, *G. Gilbert 99* (K, P); Yangambi, 1944, *G. Gilbert 7993* (K, P); Yangambi, no date, *G. Gilbert 8213* (BR, US); Yangambi, March 1938, *G. Gilbert 979* (BR); Yangambi, no date, *G. Gilbert 7809* (BR); Yangambi, Bloc du 7 km, 4 August 1952, *R. Gutzwiller 198* (Z); Yangambi. Parc Yasuka, no date, *J. Homes 147* (BRLU, WAG); Yangambi, 13 November 1957, *A. Léonard 146* (BR, K, UPS); Yangambi, 16 December 1957, *A. Léonard 198* (BR); Yangambi, 24 December 1957, *A. Léonard 208* (BR, WAG); Stanleyville, arboretum, September 1944, *P. Liégeois 122* (K); Yalembe, 7 January 1927, *D.H. Linder 1885* (A, B, K); Yangambi, 7 km road de Ngazi, 4 December 1935, *J. Louis 766* (B, K); à 5 km au N de Yanbambi, 25 February 1937, *J. Louis 1382* (BM); Route Yangambi Bengamissa, 52 km, 6 April 1936, *J. Louis 1602* (BR, C, EA, MO); Yangambi, 8 May 1939, *J. Louis 1839* (BR, MO, US); Yangambi km 8, 400 m de la route de Ngazi a l est, 470 m, 9 August 1936, *J. Louis 2401* (BM, BR, BRLU, C); Yangambi, km 8 de la road Ngazi, 17 August 1936, *J. Louis 2454* (BR); Yangambi, résever flore Isalowe, 11 February 1937, *J. Louis 3265* (BR, NY, S, WAG); Yangambi, a la hauteur de 8 km de la road de Ngazi, 470 m, 26 July 1937, *J. Louis 5650* (BR); Yangambi, au km 5 de la road de Ngazi, 19 August 1937, *J. Louis 5814* (BR); Yangambi, reserve floristique Isalowe, 9 September 1937, *J. Louis 5954* (BR, FHO); Yangambi, reserve floristique Isalowe, 470 m, 16 September 1937, *J. Louis 6076* (BR, FHO, S, WAG); Yangambi, plateau van Luweo (riv), 19 February 1938, *J. Louis 7974* (BR, NY); Yangambi, au bord affluent Lusambila, 470 m, 16 March 1938, *J. Louis 8420* (BR); Yangambi, aux pieds des Falaises de Isalowe, 8 April 1938, *J. Louis 8793* (BR, C, COI, MO, P); Yangambi, entre Lilanda et Yamboa, 21 April 1938, *J. Louis 8985* (BM); Yangambi, cirque source de la Mbutu, 17 July 1938, *J. Louis 10388* (BR, S); Yangambi, le long de l' Isalowe, 18 August 1938, *J. Louis 10872* (EA, K, NY, U); Yangambi, 8 November 1938, *J. Louis 12491* (FHO, MO, P); Yangambi, plateau de la Lusumbila, 470 m, 20 January 1939, *J. Louis 13735* (B, BR, COI); Yangambi, 10 December 1939, *J. Louis 16342* (BR); Yangambi, 4 September 1952, *E. Madoux 347* (BR); 13 September 1952, *E. Madoux 397* (BR); 20 September 1952, *E. Madoux 439* (BR); 25 May 1981, *N.B. Ndjele 356* (BR); 25 May 1981, *N.B. Ndjele 388* (BR); Chemin de Bula a Banalia, environ de Bula, 9 January 1926, *F.H.E.A.W. Robyns 1318 a* (BR); environ de Yambuya, 1906, *A.F. Solheid s.n.* (BR); **Unknown.** April 1921, *J. Claessens 573* (BR, WAG); km 65 road de Weko-Bengamisa, 6 December 1949, *R.G.A. Germain 5439* (BR); Entre Businga et Banzyville (Ubangui), January 1031, *J. Lebrun 2011* (BR, P); Musa ausrey (?), 8 February 1913, *Sparano 23* (BR).

GABON: Estuaire. Sibang, 31 October 1951, *F. Bernard SRFG 289* (LBV); Environs de Libreville, 17 February 1896, *T.-J. Klaine 360* (B, K, P, WAG); Donghila, 1902, *T.-J. Klaine 2689* (K, P); North of Libreville, Foret de la Mondah. Parcelle des conservateurs, 26 October 2005, *M.S.M. Sosef 2032* (WAG); 32 km along the road Ntoum-Cocobeach, near village No Ayong. Along track to Debarcadere, 10 m, 30 January 1993, *J.J.F.E. de Wilde 11009* (LBV, U, WAG); Nyonyie survey, sondage F2, 5 July 1990, *C.M. Wilks 2124* (MO, WAG); Nyonyie. Transect F1, 14 July 1990, *C.M. Wilks 2241* (LBV, WAG); **Moyen-Ogooué**. Lac Gomé, July 1952, *D.N. Bois SRFG 826* (LBV); Eastern part of the Presidential Reserve

Wonga-Wongué, c. 100 km S of Libreville. Forest patch leading what locally is called "Little Bambam", 1 March 1983, *J.J.F.E. de Wilde (WALKB-series) 839* (BR, K, LBV, MO, WAG); **Ngounié**. Agouma, December 1925, *G.M.P.C. Le Testu 5836* (BM, BR, EA, P, WAG); La Bendolo, March 1926, *G.M.P.C. Le Testu 5862* (BM, BR, EA, P); Doudou Mountains National Parc, c. 30 km S of Mandji, E of Mont Igoumbi, 18 November 2005, *M.S.M. Sosef 2372* (WAG); **Nyanga**. 32 road-km N of Igotchi-Mouenda, Bakker timber concession, 13 May 1997, *G.D. McPherson 16967* (G, LBV, MO, UPS, WAG); Inventory; chantier CEB, ca 50 km SW of Doussala, 23 August 1985, *J.M. Reitsma 1373* (LBV, NY, WAG); c. 50 km SE of Forestry Camp Doussala. Inventory, 20 February 1986, *J.M. Reitsma 1921* (LBV, MO, NY, U, WAG); c. 50 km SW of Forestry Camp Doussala. Inventory, 480 m, 21 October 1985, *J.M. Reitsma 1703* (LBV, NY, WAG); **Ogooué-Ivindo**. M'passa, 21 April 1978, *J. Florence 1028* (P); Makokou. Transect 13, 480 m, 18 July 1981, *A.H. Gentry 33383* (MO, WAG); Makokou. Transect 11, no date, *A.H. Gentry 33342* (MO); Bélinga, mines de fer, bord de l'Ivindo, 20 July 1966, *N. Hallé & A. Le Thomas 115* (BR, K, P); Ipassa, 10 km S of Makokou, 29 April 1972, *A. Hladik 2092* (US); Ipassa Reserve, IRET Research Station, SW of Makokou, 4 November 2005, *M.S.M. Sosef 2211* (WAG); c. 30 km down the Ivindo River from the IRET Research Station, SW of Makokou, 7 November 2005, *M.S.M. Sosef 2244* (WAG); **Ogooué-Lolo**. Région de la "forêt des abeilles"; campement rivière Makandé (2 km en amont de son embouchure dans l'Offoué), 8 March 1999, *F. Hallé 4611* (MPU, WAG); région de Lastoursville, Liyança, November 1929, *G.M.P.C. Le Testu 7697* (BM, BR, P); région de Lastoursville, Lissacho, December 1930, *G.M.P.C. Le Testu 8563* (BM, BR, P); Ngoungui, no date, *G.M.P.C. Le Testu 9862* (WAG); **Ogooué-Maritime**. Gamba, near airport, 6 November 1998, *F.J. Breteler 14580* (WAG); Yenzi, near Gamba, 25 November 1994, *J.J.F.E. de Wilde 11200* (LBV, WAG).
GHANA: Eastern Region. Atewa Range Forest Reserve, 26 October 1978, *J.B. Hall 46996* (K).
NIGERIA: Edo State. Sapoba, 1930, *J.D. Kennedy 1568* (A, BEDF, F, FHO, L, M, MO, S, US); no date, *J.D. Kennedy 1508* (US); **Kogi State**. Near Onda, 22 February 1946, *A.P.D. Jones FHI 16981* (FHO); **Ogun State**. About 2 miles south of Osho, 5 April 1946, *A.P.D. Jones FHI 17243* (FHO); **Osun State**. Shasha Forest Reserve, 8 April 1935, *P.W. Richards 3343* (BM, BR, G, MO, S); **Oyo State**. About 1 1/2 mile south of Abeku on steep side ridge with Lovoa-Kheya, 10 March 1946, *A.P.D. Jones FHI 16813* (FHO).
REPUBLIC OF THE CONGO: Cuvette. National Park Odzala, Foret d'Andzoyi, 14 March 1994, *F. Dowsett-Lemaire 1701* (BR); **Unknown.** Quesso (Province), 26 April 1971, *F. Grison 38* (P); Zanaga, région Ingolo II, monts N'Doumou, 17 May 1972, *P. Sita 3331 bis* (P); Sembé, forêt environs du Poste, 2,500 km du garage RNTP, 18 September 1972, *P. Sita 3460* (WAG); Mayombe, forest environ deNgoungui 2, 15 March 1975, *P. Sita 3900* (P); Chaillu; région de Komono, env. de Mbaya, road de Mossendjo, 15 November 1976, *P. Sita 4044* (P, WAG).

11. Isolona humbertiana Ghesq. ex Cavaco & Keraudren, Bull. Jard. Bot. État, Brux. 27: 78. 1957. — TYPE: MADAGASCAR. Mahajanga: Ankaladina, *J.M.H.A. Perrier de la Bâthie 1511* (holotype: P!; isotypes: B!, P!). *Figure 6.23 A-C*

Tree up to 18 m high; trunk with d.b.h. up to 20 cm; young branches drying black, glabrous; old branches grey, striate, glabrous. *Petioles* 4-7 mm long, ca. 1 mm in diameter, glabrous, leaf lamina inserted on side, faintly grooved adaxially. *Leaf lamina* 5-10(-15) long, 2.5-6 cm wide, length:width ratio 2.3-3.1, narrowly elliptic to elliptic or narrowly obovate to obovate, base narrowly cuneate to decurrent, apex acuminate, acumen 10-15 mm long, rounded at tip, coriaceous, glabrous, sometimes shinny adaxially; midrib glabrous on both sides; secondary veins 6-12 pairs, uniformally curving upwards, glabrous. *Inflorescences* sometimes with an additional rhipidium, on leafy branches. *Flowering pedicels* 5-10 mm long, 0.5-1 mm in diameter, glabrous. *Lower bracts* 2-3, 0.4-0.5 mm long, ca. 0.4 mm wide, sometimes last bract fused with basal half of pedicel, glabrous; margins glabrous to very sparsely covered with short erect hairs. *Upper bract* absent. *Sepals* 2-3 mm long, 2-3 mm wide, length:width ratio ca. 1, very broadly ovate, base truncate, apex obtuse, glabrous, persistent in fruit. *Corolla* yellow to light yellow when young becoming red at anthesis; tube 4-6 mm long, ca. 4 mm in diameter, lobe:tube ratio 2.5-4.5, glabrous; lobes 12-23 mm long, 4-6 mm wide, length:width ratio 3-5.5, narrowly elliptic or narrowly oblong, narrowed at base, apex acute, glabrous, coriaceous. *Receptacle* ca. 4 mm in diameter. *Stamens* ca. 2 mm long; connective shield ca. 0.2 mm long, yellow, glabrous, those of innermost whorl slightly elongated over ovary wall. *Ovary* ca. 2 mm long, ca. 1.5 mm wide; stigma ca. 1.5 mm in diameter, glabrous,

whitish. *Fruiting pedicels* 12-15 cm long, 3-4 mm in diameter, woody. *Fruits* ca. 4-8 cm long, 3-4 cm wide, oblong to very broadly ovoid, smooth, apex apiculate, apicule ca. 3 mm long; pericarp 2-3 mm thick. *Seeds* 15-20 mm long, 9-13 mm in diameter, ellipsoid, packed in white pulp; testa smooth, light brown; raphe not thickened, slightly darker brown; hilum 7-8 mm long, 4-5 mm wide, elliptical.

Distribution: Northern Madagascar (see Map 11); in dense primary rain forests, on rocky soils; at 500-900 m altitude.

Phenology: Mature flowers collected from November to January. Mature fruits collected in May and from September to October.

Vernacular names:
Madagascar: Roimbary, Ombary, Ambery, Klilo (Malgash).

IUCN conservation status: EN B2ab(ii,iv). *Isolona humbertiana* is only represented by 7 collections, the last one dating from 2001. It is found in one protected area namely the Marojejy national park. The Area of Occupancy being less than 50 km^2 and the fact it is found in only four locations, justifies its placement in the “endangered” category.

Notes: *Isolona humbertiana* presents a slight similarity to *I. ghesquierei* because of the shape of the corolla lobes, but differs by the glabrous flowering pedicels and papyraceous corolla.
Perrier de la Bâthie 1511 and *1511bis* were collected at the same locality, but he identified *1511* as a new species, and *1511bis* as *I. madagascariensis*, indicating that the latter differs by “division plus longues d’un centimètre, la fleur plus grande, entièrement purpurine à l’intérieur” [lobes longer than 1 centimeter, the flower bigger and completely red inside]. Moreover, the leaf base of *1511* is rounded compared to cuneate in *1511bis*, which lead to conserve these two specimens as distinct over the years. However, the color and size differences mentioned are also commonly encountered variations within other species of *Isolona*, where the young corollas are yellow gradually becoming red and enlarge while reaching anthesis (f.e. as in *I. hexaloba*). After careful examination it was decided that the two specimens belong to the same species.

ADDITIONAL SPECIMENS EXAMINED:
MADAGASCAR: Antsiranana (Diego Suarez). Plantes du Prac national du Marojejy, sous prefecture d Andapa, commune rurale de Doany, fokontany de Betsomanga, versant nord ouest de Marojejy Camp, 0.2Km a l'est du Camp I, au point 003, 860 m, 14 October 2001, *L. Gautier 3830* (G, MO); Marojejy RNI, Andapa. Environ 3.5 km vol d oiseau de Marovato et 2 km a vol d oiseau de Sarahandrano soit 8 km a pied., 692-759m, 2 May 1995, *F. Rasoavimbahoaka 629* (MO, P); **Mahajanga (Majunga)**. Antralardina (or Antralachina) sur le Betsiboka (Boiny), January 1903, *J.M.H.A. Perrier de la Bâthie 1511* (B, P); Antralachina sur le Betsiboka (Boiny), January 1903, *J.M.H.A. Perrier de la Bâthie 1511 bis* (P); **Toamasina (Tamatave)**. Near Mbu village, 10 km W of Wone which is on the Kumba-Mamfe road, 500-750 m, 20 September 1993, *S.T. Malcomber 2521* (MO, P); Anipasimazava-Besakay-Brickaville (Ambila-Lemaito), 1 November 1951, *Service Forestier de Madagascar SF(MDG) 4898* (P); **Unknown.** Tsilaiza, Mitsinjo, 16 December 1951, *Service Forestier de Madagascar SF(MDG) 5382* (P, WAG).

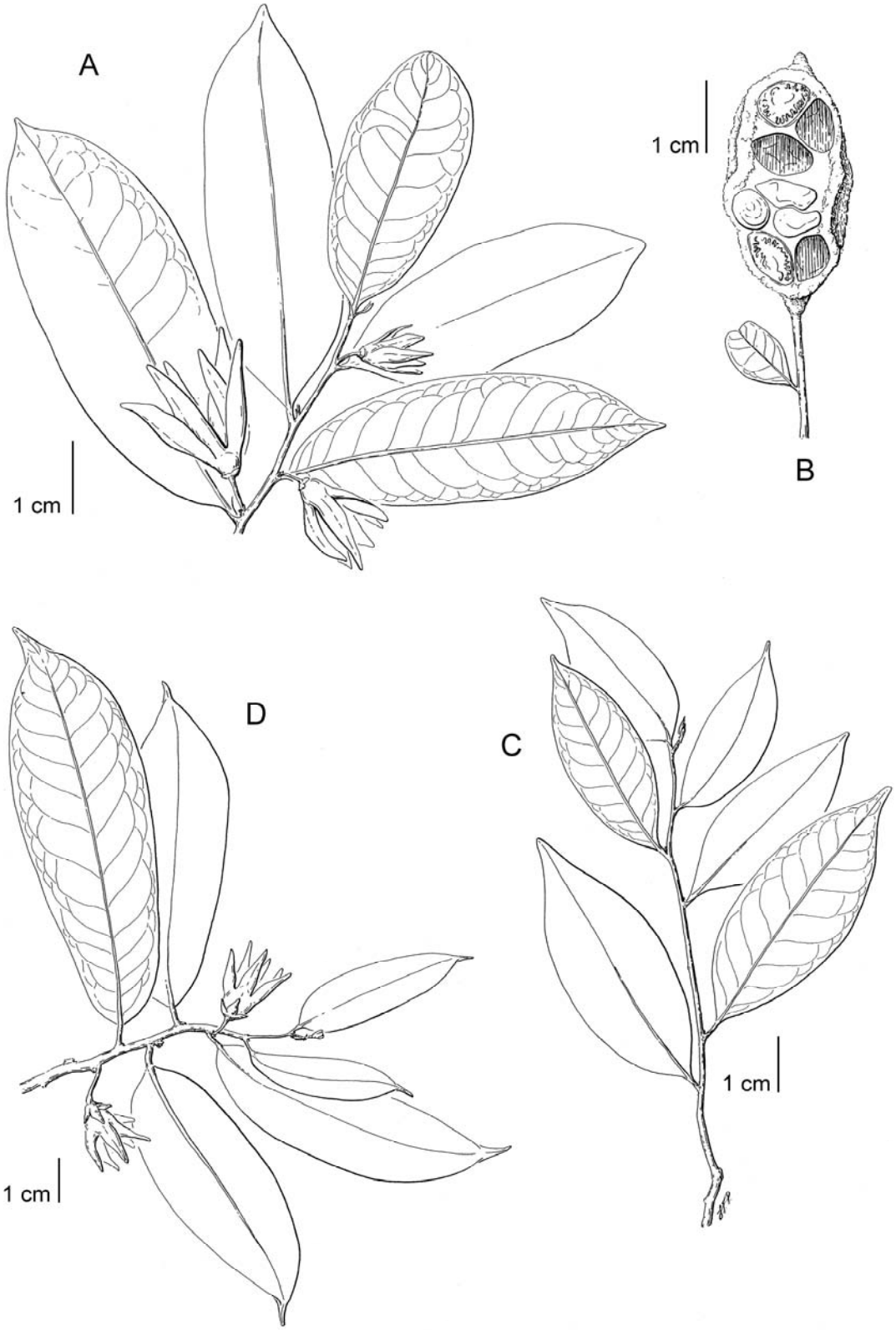
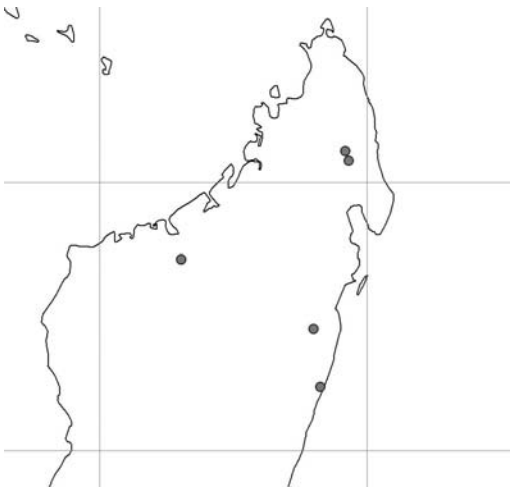


Figure 6.23. *Isolona humbertiana*. A. Flowering branch. B. Fruit. C. Flowerless branch. *Isolona capuronii*. D. Flowering branch. Drawings by Joanne Porck.



Map 11: Distribution of *Isolona humbertiana*.

12. Isolona lebrunii Boutique, Bull. Jard. Bot. Brux. 21: 96. 1951. — TYPE: DEMOCRATIC REPUBLIC OF CONGO. Kinshasa: Djugu, 7 February 1931, *J. Lebrun 3886* (holotype: BR!; isotype: K!).

Tree up to 15 m high; trunk with d.b.h. up to 40 m; young branches finely rugulose, blackish grey, glabrous, old branches grey, striate, glabrous. *Petioles* 6-10 mm long, ca. 2 mm in diameter, glabrous, leaf lamina inserted on the side, faintly grooved adaxially. *Leaf lamina* 10-25 cm long, 3.5-8 cm long, length:width ratio 3-4, narrowly oblong to narrowly obovate, base acute, apex acute, papyraceous, glabrous; midrib proximally depressed adaxially, glabrous on both sides; secondary veins 6-12 pairs, uniformally curving upwards, glabrous. *Inflorescences* on leafy branches. *Flowering pedicels* 7-13 mm long, ca. 0.5 mm in diameter, glabrous. *Lower bracts* 2-4, ca. 0.5 mm long, ca, 0.5 mm wide, glabrous; margins with short erect hairs. *Upper bract* positioned at base to halfway up the pedicel, ca. 1 mm long, ca. 0.8 mm wide, glabrous; margins with short erect hairs. *Sepals* 2-3 mm long, 2-3 mm wide, very broadly ovate, base truncate, apex acute, glabrous; margins with short erect hairs, pressed against tube, falling in fruit. *Corolla* green when immature, yellow with a red center at anthesis; tube 5-8 mm long, 4-6 mm in diameter, lobe:tube ratio 1.5-3, glabrous on both sides; lobes (7-)10-20 mm long, 3-9 mm wide, length:width ratio 2.3-3, narrowly obovate to obovate, abruptly narrowed at base, apex acute to rounded, glabrous, papyraceous; margins wavy when dried, glabrous. *Receptacle* 3-4 mm in diameter. *Stamens* 1.2-1.6 mm long; connective shield ca. 0.2 mm long, glabrous, those of innermost whorl elongated against ovary wall. *Ovary* ca. 2 mm long, ca. 1.5 mm wide; stigma ca. 2 mm in diameter, sparsely covered with short erect hairs. *Fruiting pedicels* 15-20 mm long, 5-6 mm in diameter, rugulose, glabrous. *Fruits* 7.5-9.5(-15) cm long, 5.5-6.5(-10) cm in diameter, length:width ratio 1.5-1.9, broadly ovoid to ovoid, smooth, finely ribbed, glabrous, green; pericarp 3-5 mm thick. *Seeds* 20-30 mm long, 10-15 mm in diameter, ellipsoid; testa smooth, light brown; raphe not thickened; hilum 8-10 mm long, 3-5 mm wide, narrowly elliptical to narrowly oblong.

Distribution: Eastern Democratic Republic of Congo and Burundi (see Map 12); in moist montane rain forests; at 1800-2000 m altitude.

Phenology: Mature flowers collected from January to April, July and September to October. Mature fruits collected from January to February and September to October.

IUCN conservation status: EN B2ab(iv). *Isolona lebrunii* is poorly represented in herbaria with less than 20 collections, and has not been collected since 1971 maybe partially due to the civil war in the region. It occurs in two reserves (Mt Bei and Djugu both in the Decmocratic Republic of Congo). The area of occupancy is about 100 km^2, thus the category of "endangered" seems appropriate.

Notes: *Isolona lebrunii* can be characterized by its long glabrous petiole, the acute leaf lamina base and flower lobes that are abruptly narrowed at the base. It would appear close to *I. hexaloba* by the shape of the corolla lobes, but the latter is easily distinguished by the much shorter petiole and non-wavy margins in dried material.

Map 12: Distribution of *Isolona lebrunii*.

ADDITIONAL SPECIMENS EXAMINED:
BURUNDI: Bubanza. Budanza, Mabaye, Lua, Frontiere du Rwanda, 1650 m, 22 June 1969, *J. Lewalle 3781* (K).
DEMOCRATIC REPUBLIC OF CONGO: Katanga (Shaba). Bendera, barrage de la Kiymbi, 1675m, 17 September 1959, *A. Schmitz 6504* (BR); **Kinshasa**. Djugu (kibali), 1750 m, 7 February 1931, *J. Lebrun 3886* (BR, K); Kalehe, 5 March 1959, *A. Léonard 3306* (K); **Nord-Kivu**. Mahanga Terr. Masisi, 12 January 1959, *A. Léonard 2487* (BR, WAG); **Orientale**. Lakwa-Foret de Djugu, 13 March 1958, *P. Bamps 131* (K); 27 km N of Kisangani, 9 April 1971, *J. Bokdam 3142* (KIS, WAG); Lakwa Djugu, 5 March 1959, *A. Devillé 228* (K); Lekwa (Djugu), 1 April 1959, *A. Devillé 234* (BR); Djugu (Kilali-Ituri), 1780 m, 7 February 1931, *J. Lebrun 3884* (BR); Djugu Reserve, 25 October 1951, *F. Smeyers 41* (BR, K, MO, WAG); Djugu Lekwa, 1700 m, 10 July 1946, *A. Taton 153* (BR); Nioka, Réserve Ineac Mt Bei, 1800 m, 5 September 1946, *A. Taton 282* (BR); road Nioka-Djugu, 9 December 1947, *A. Taton 715* (K, P); **Sud-Kivu**. Lac Edouard et Kivu, Ile Idjwi, no date, *A. Michelson 302* (K); Mikonzi, 1950 m, 12 February 1957, *R. Pierlot 1482* (K); Bukavu-Stanleyville, Terr. de Kalehe, 2150 m, 1959, *R. Pierlot 3208* (BR, WAG).

13. Isolona le-testui Pellegrin, Bull. Mus. Nat. Hist. Paris 26: 657. 1920. — TYPE: GABON. Nyanga: Tchibanga, 29 Novembre 1907, *G.M.P.C. Le Testu 1252* (holotype: P!; isotypes: BM!, P!). *Figure 6.25 A-D*

Tree or shrub up to 10 m high; young branches drying black, glabrous; old branches light brown, glabrous, striate. *Petioles* 2-3 mm long, ca. 2 mm in diameter, glabrous, leaf lamina inserted on side, narrowlly grooved adaxially. *Leaf lamina* 10-14.5 cm long, 4-5 cm wide, length:width ratio 2.5-3.3, narrowly elliptic to elliptic, base acute to obtuse, apex acuminate, acumen 1-1.5 cm, papyraceous, glabrous, dark green; midrib glabrous on both sides; secondary veins ca. 12 pairs, uniformally curving upwards, glabrous. *Inflorescences* on leafy branches. *Flowering pedicels* 10-13 cm long, 0.5-0.8 mm in diameter, glabrous to sparsely covered with short erect hairs. *Lower bracts* 2-4, ca. 0.5 mm long, ca. 0.5 mm wide, glabrous; margins covered with short erect hairs. *Upper bract* inserted basally to the pedicel, ca. 0.5 mm long, ca. 0.5 mm wide, glabrous; margins covered with short hairs. *Sepals* 2-4 mm long, ca. 2-3 mm wide, length:width ratio 1-1.3, broadly ovate, base truncate, apex acuminate, glabrous; margins covered with short erect hairs. *Corolla* green at base with red lobes; tube ca. 5 mm long, lobe:tube ratio 10-50; lobes 50-100 mm long, 3-6 mm wide, linear, base truncate, apex rounded, glabrous, papyraceous. *Receptacle* ca. 4 mm in diameter. *Stamens* 1-1.5 mm long; connective shield ca. 0.1 mm long, glabrous, those of innermost row caudate and pressing against ovary wall. *Ovary* ca. 2 mm long, ca. 1.5 mm wide; stigma ca. 2 mm in diameter, sparsely covered with short erect hairs. *Fruits* unknown.

Distribution: Gabon and the Republic of Congo (see Map 13), altitude unknown.

Phenology: Mature flowers collected in November.

IUCN conservation status: CR B1ab(iv) + 2ab(iv). *Isolona le-testui* is only known from two collections. The last one was made in 1975. None of the collections are found in protected areas thus the “critically endangered” category seems justified.

Notes: *Isolona le-testui* is characterized by its very long and linear corolla lobes and an acute to obtuse leaf lamina base. It has many affinities with *I. thonneri* which has, however, much shorter corolla lobes and a decurrent leaf lamina base.

ADDITIONAL SPECIMENS EXAMINED:
GABON: Nyanga. Tchibanga, Forêt du Mayombe, 29 November 1907, *G.M.P.C. Le Testu 1252* (BM, P).
REPUBLIC OF THE CONGO: Niari. Niari-ouest, région Banda, Vallé de la Ngouanga, 5 November 1975, *P. Sita 3996* (P, WAG).

Map 13 : Distribution of *Isolona le-testui*.

14. Isolona linearis Couvreur, Adansonia 28: 253. 2006. — TYPE: TANZANIA. Iringa: Luhega Forest Reserve, 20 January 1997, *C. Frimodt-Møller TZ 59* (holotype: C!; isotype: K!). *Figure 6.24*

Tree to 15 m tall; trunk with d.b.h. up to 60 cm; bark dark brown to black, smooth; young branches drying black, glabrous, smooth; old branches dark brown to grey, glabrous, striate. *Petioles* 4-5 mm long, 1.0-1.5 mm in diameter, glabrous, leaf lamina inserted on side, narrowly grooved adaxially. *Leaf lamina* 14-18(-25) cm long, 5-7(-9) cm wide, length:width ratio 2.3-3.3, narrowly oblong to oblong or narrowly elliptic to elliptic, base rounded to narrowly cuneate, apex acuminate, acumen 5-17 mm long, coriaceous, glabrous; midrib glabrous on both sides; secondary veins 11-13 pairs, uniformly curved upwards, glabrous. *Inflorescences* sometimes with one additional rhipidium, on leafy branches. *Flowering pedicels* (3-)5-10 mm long, 1-1.5 mm in diameter, glabrous. *Lower bracts* 2-6, ca. 0.5 mm long, ca. 0.5 mm wide, glabrous, margins sparsely covered with short erect hairs. *Upper bract* inserted basally or subbasally on the pedicel, 1-2 mm long, ca. 1 mm wide, glabrous; margins sparsely covered with short erect hairs. *Sepals* 2-3 mm long, ca. 3 mm wide, length:width ratio 0.7-1, transversely elliptic to elliptic, base truncate, apex acuminate; margins sparsely covered with short erect hairs, green, persistent in fruit. *Corolla* green when immature, red at anthesis; tube 4-5 mm long, lobe:tube ratio 2.5-7.5, glabrous, verrucose when dried; lobes 10-30 mm long, 3-4(-8) mm wide, length:width ratio 2.5-7, linear to narrowly elliptic or rarely elliptic, narrowed at base, apex rounded, sparsely covered with short appressed hairs when very young, glabrous at anthesis, papyraceous. *Receptacle* 3-5 mm in diameter. *Stamens* ca. 1.1 mm long; connective shield ca. 0.1 mm long, glabrous, those of innermost whorl slightly extended over ovary wall. *Ovary* ca. 2 mm, ca. 1 mm wide; stigma ca. 2.5 mm in diameter, glabrous. *Fruiting pedicels* 5-15 mm long, ca. 3 mm in diameter, glabrous. *Fruits* ca. 5 cm long, 2-3 cm in diameter, length:width ratio 1.5-2.5, irregularly elliptic, apex apiculate,

apicule ca. 1 cm long, furrowed, green tinged with white; pericarp 2-3 mm thick. *Seeds* 10-15 mm long, ca. 5 mm in diameter, transversely ellipsoid; testa smooth, light brown; raphe thickened, dark brown; hilum not seen.

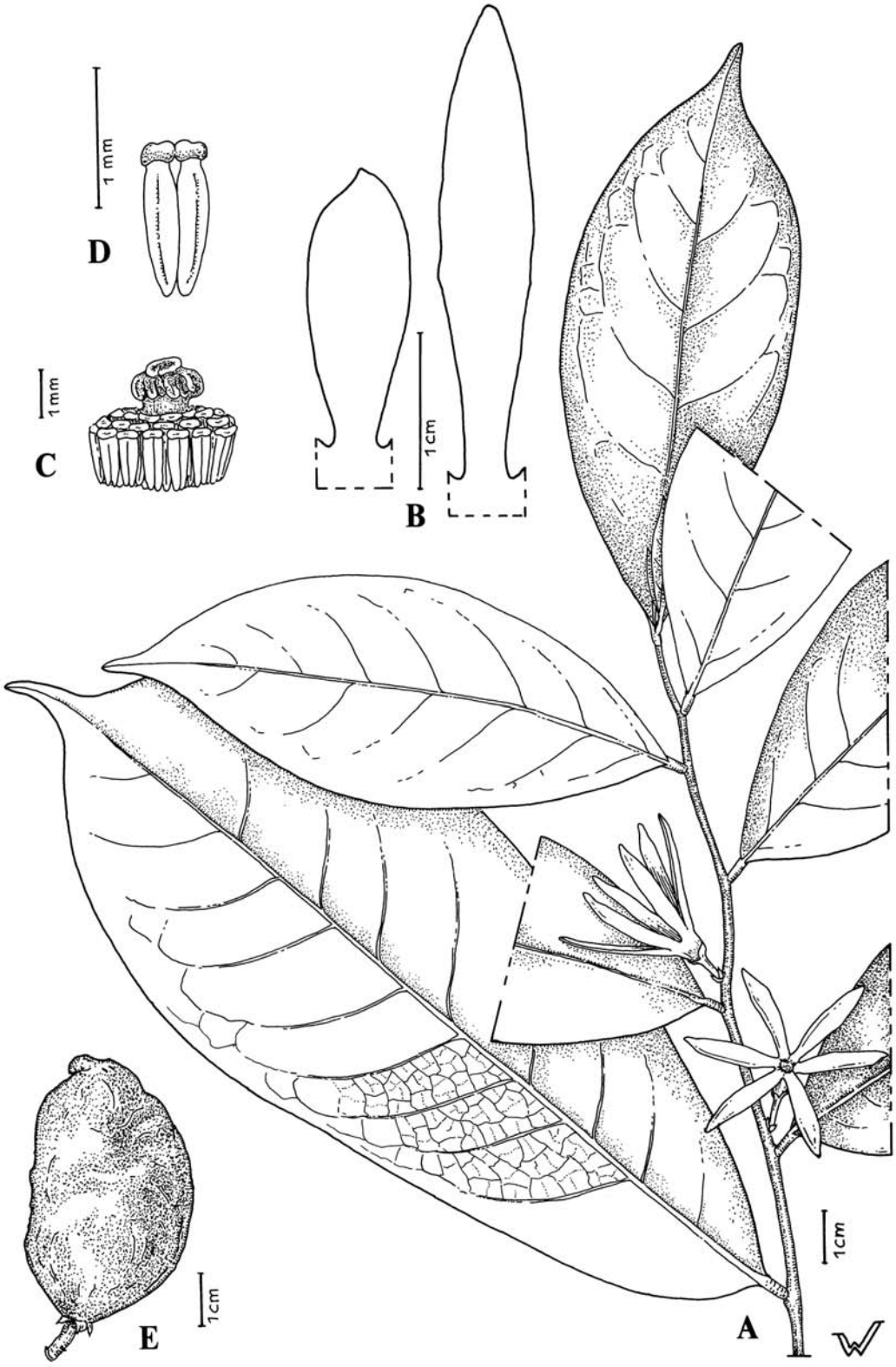


Figure 6.24. *Isolona linearis*. A. Flowering branch. B. Lobe variation. C. Androecium and stigma. D. Stamen. E Fruit. Drawings by Wil Wessel-Brand.

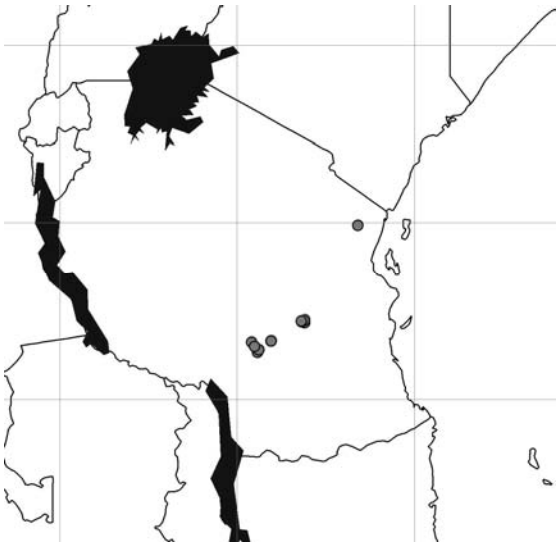
Distribution: Tanzania (Eastern Arc Mountains; see Map 14); in moist montane forest; at 1100-1700 m altitude.

Phenology: Mature flowers collected from November to February, from April to July and in September. Mature fruits collected from November to December.

Vernacular names:
Tanzania: Mulinditi (Kihehe).

IUCN conservation status: EN B2ab(iv). *Isolona linearis* is moderately represented in herbaria, but has numerous recent collections. Most of these originate from protected areas: one natural reserve (Amani), one national park (Udzungwas) and two forest reserves (Lulanda and Luhega). However, the area of occupancy is quite small (ca. 100 km^2) and there are only three main locations. The "endangered" category is therefore recommended.

Notes: The shape and size of the corolla lobes in *I. linearis* vary, with the predominant shape being linear. One other striking feature is that some flowers of various specimens of *I. linearis* have five corolla lobes instead of six, which could indicate a tendency towards pentamery (photo Luke 7732 (EA), Frimodt-Møller TZ59 (C), and Frimodt-Møller TZ5 (C) all indicates "5-merous flowers"). This tendency is also observed in *I. campanulata* Engl. & Diels (Adam, 1971). *Isolona linearis* would appear closely related to *I. heinsenii* by the shape of the corolla lobes and by their disposition, curving uniformly over the receptacle. However, *I. linearis* is always completely glabrous, and the sepals are generally smaller than those of *I. heinsenii*. Besides, they are both montane forest species. This resemblance is also reflected in the molecular phylogeny (Fig. 9), where they appear as sister species.



Map 14: Distribution of *Isolona linearis*.

ADDITIONAL SPECIMENS EXAMINED:
TANZANIA: Iringa. Mufindi, Lulanda Forest, 1450 m, 6 December 1987, *T.C.E. Congdon s.n* (P); Mufindi area, Lulanda Forest Reserve, 1650 m, 16 February 1979, *P.J. Cribb 11470* (K); Luhega Forest Reserve, 1650 m, 21 February 1996, *Frimodt-Möller, C. NG 64* (C); 1650 m, 18 February 1996, *Frimodt-Möller, C. NG 5* (C, K); 1650 m, 18 February 1996, *Frimodt-Möller, C. NG 12* (C); 1650 m, 21 February 1996, *Frimodt-Möller, C. NG 66* (C); 1650 m, 20 January 1997, *Frimodt-Möller, C. TZ 59* (C, K); Udzungwa Scarp Forest Reserve, 1550-1700 m, 18 December 1997, *Frimodt-Möller, C. TZ 679* (C); Mufindi, Lulanda, 1500 m, 24 November 1998, *R.E. Gereau 2552* (F, MO, NY); Lulanda, 1500 m, 25 January 1989, *R.E. Gereau 2882* (BR, MO, P, WAG); Luhega Forest Reserve, 1650 m, 20 February 1997, *V. Horlyck TZ 359* (C); 1650 m, 18 February 1997, *V. Horlyck TZ 325* (C, K); Lulando Forest. Isolated patch of closed high forest on Uzungwa escarpment, 1430 m, 6 April 1986, *J.C. Lovett 592* (MO); Mufindi Lulanda Forest, 1450 m, 23 November 1987, *J.C. Lovett 2469* (MO); Udzungwa Montain National Park, Camp 242-pt 243, 1150 m, 5 October 2001, *W.R.Q. Luke 8157* (EA, K); **Morogoro**. East Udzungwa National Park, in forest south of Mwanihana hill. c. 2 km south of last camping site on Mwanihana trail, 1400 m, 30 November 2006, *T.L.P. Couvreur 102* (DSM, WAG); Udzungwa Mountains, Udzungwa Scarp Forest reserve. Mbawi. Ruaha route, 22 February 2000, *Ndangalasi, H.J. 377* (OWU); Mwanihana Forest Reserve above Sanje Village, moist forest on edge of Gologolo mountains overlooking the Kilombero flood plain, 1250 m, 17 June 1986, *J.C. Lovett 861* (MO); Udzungwa mts NP Mt Luhomero, 1200 m, 23 September 2001, *W.R.Q. Luke 7732* (BR, EA); Ulanga distr. Ridge above Sanje Falls, 1150 m, 24 July 1983, *R.M. Polhill 5143* (EA, K, MO); Mwanihana Forest Reserve Above Sanje village, 1400-1700 m, 10 October 1984, *D.W. Thomas 3899* (MO); **Tanga**. Ambangulu Forest Reserve, N of Tamota Village near the estate of Kunga and Kieti village, 1225m, 18 May 1999, *M.A. Mwangoka 533* (MO, WAG); **Unknown**. no location, 1650 m, 23 February 1996, *K.S. Mikkelsen 384* (K).

15. Isolona madagascariensis (Baill.) Engl., in Engl. & Prantl, Nat. Pflanzenfam. Nachtr. 1: 161. 1897. *Monodora madagascariensis* Baill., Adansonia 8: 299. 1867. — TYPE: MADAGASCAR. Antsiranana (Diego Suarez): Diego-Suarez, *A.C.J. Bernier 131* (holotype: P!).

Hexalobus madagascariensis A.DC., Mem. Soc. Phys. Genève 5: 213. 1832. — TYPE: MADAGASCAR. no location indicated, no collector indicated, ex herbarium L'Héritier (holotype: G!).

Tree up to 8 m tall; trunk with d.b.h. up to 10 cm; young branches drying black, glabrous; old branches dark brown, striate, glabrous. *Petiole* 5-6(-8), 1.5-2 mm in diameter, glabrous, leaf lamina inserted on side, very faintly grooved adaxially. *Leaf lamina* 13-22 cm long, 5-8 cm wide, length:width ratio: 2.5-2.7, elliptic (obovate), base acute to narrowly cuneate, apex acuminate, acumen 1-2.5 cm, coriaceous, glabrous; midrib glabrous on both sides; secondary veins 9-13, uniformally curved upwards, glabrous. *Inflorescences* on leafy branches. *Flowering pedicels* 4-5 mm long, 1-1.5 mm in diameter, glabrous. *Lower bracts* 2-4, 0.5 mm long, ca. 0.5 mm wide, glabrous. *Upper bract* absent. *Sepals* ca. 2 mm long, ca. 2 mm wide, length:width ratio ca. 1, depressed ovate, base truncate, apex rounded, glabrous; margins with short straight hairs, falling in fruit. *Corolla* white-yellow when immature to light red at anthesis; tube 4-5 mm long, 4-5 mm wide, lobe:tube ratio: 1.7-2.7, clearly urceolate around receptacle, glabrous; lobes 7-12 mm long, 3-4 mm wide, length:width ratio: 2.3-3.6, narrowly ovate to narrowly oblong, narrowed at base, apex attenuate, glabrous, coriaceous; margins glabrous. *Receptacle* ca. 4 mm in diameter. *Stamens* 1-1.2 mm long, connective shield ca. 0.2 mm long, glabrous, those of innermost whorl elongated and pressing against ovary wall. *Ovary* ca. 2 mm long, 1.7 mm wide; stigma ca. 3 mm in diameter, glabrous. *Fruiting pedicels* ca. 5 mm long, 3-4 mm in diameter, glabrous. *Fruits* ca. 5 cm long, 4.5-5 cm wide, length:width ratio ca. 1, globose, apex rounded, finely ribbed, glabrous, green; pericarp 7-9

mm thick. *Seeds* 12-16 mm long, 7-9 mm in diameter, transversely ellipsoid; testa smooth, light brown; raphe not thickened; hilum 6-7 mm long, 2-4 mm wide, elliptical to oblong.

Distribution: Northern Madagascar (see Map 15); in lowland rain forests, along rivers, on sandy soil; at 0-200 m altitude.

Phenology: Mature flowers collected from November to January. Mature fruits collected in March and May.

Vernacular names:
Madagascar: Ambavilahy, Ambavilavaravina, Hombavy (Malgash).

ICUN conservation status: NT. *Isolona madasgascariensis* is only represented by 8 specimens found in 5 localities. Five of these collections were made on the Nosy Be Island in the strict nature reserve of Lokobe. This reserve appears to be in good condition (it will even become a national park soon), with apparent little threat to its destruction. We thus assign it to the "near threatened" category, mainly because no collections have been made this past decade, thus it could be at threat. Future field work will help provide a better assessment.

Notes: See taxonomic history in the Introduction for a discussion about the correct name and authors for this species. *Isolona madagascariensis* can be characterized by its large, elliptic leaves with acuminate apex, its relatively short, thick and glabrous pedicel, short elliptic lobes as well as a clearly urceolate tube in herbarium material. In this respect it would seem close to *I. perrieri*.

Map 15: Distribution of *Isolona madagascariensis*.

ADDITIONAL SPECIMENS EXAMINED:
MADAGASCAR: Antsiranana (Diego Suarez). Nosy Be. Réserve Intégrale de Lokobe, 0-50 m, 17 March 1994, *P. Antilahimena 50* (MO, P, WAG); Nosy-Be, Reserve Naturelle Intégrale de Lokobe, 0-60 m, 16 September 1994, *P. Antilahimena 161* (BR, MO, WAG); Diego-Suarez, no date, *A.C.J. Bernier131* (P); Analamazava, part of Binara Range, SW

of Daraina (Vohemar), Faritany, Antsiranana, 200-1180 m, 23 January 1991, *D.M. Meyers 255* (K, MO, P); Forêt de Lokobe au S.E. d'Hell-Ville, Ambanoro, Nossy-Bé, 200 m, 22 May 1998, *J. Rabenantoandro 7* (MO, WAG); Nossi-Be, 28 September 1960, *Ramamonjisoa, N. 11635* (P); Sambirano: Nossibe: Foret de Lokobe, 1 November 1954, *Service Forestier de Madagascar SF(MDG) 11409* (P); **Toamasina (Tamatave)**. Réserve forestière de Tampolo (Fénérive), 9 December 1989, *C.M. Evrard 11253* (BR, P).

16. Isolona perrieri Diels, Notizbl. Bot. Gart. Berlin-Dahlem 9: 357. 1925. — TYPE: MADAGASCAR. Antsiranana: Sambirano, May 1905, *J.M.H.A. Perrier de la Bâthie 4951* (holotype:P!; isotype: B!). *Figure 6.20 D-F*

Tree up to 10-15 m high; trunk with d.b.h. up to 20 cm; bark mottled light and dark grey with thin black lines; young branches drying black, glabrous; old branches grey, glabrous. *Petioles* 5-8 mm long, 1-1.2 mm in diameter, glabrous, leaf lamina inserted on side, faintly grooved adaxially. *Leaf lamina* 10-18 cm long, 3-4 cm wide, length:width ratio 3-4.2, narrowly elliptic, base narrowly cuneate to decurrent, apex acuminate, acumen 5-15 mm long, glabrous, green glossy; midrib glabrous on both sides; secondary veins 8-12 pairs, uniformally curving upwards, glabrous. *Inflorescences* rarely with an additional rhipidium, on leafy branches, sometimes on older branches. *Flowering pedicels* (4-)10-15 mm long, ca. 0.5 mm in diameter, glabrous. *Lower bracts* 2-4, ca. 0.5 mm long, ca. 0.5 mm wide, glabrous. *Upper bract* absent. *Sepals* usually persistent in fruit, ca. 2 mm long, ca. 2 mm wide, length:width ratio ca. 1, depressed ovate, glabrous, yellow; margins glabrous. *Corolla* green or light yellow when immature, bright yellow to purple red at anthesis; tube 2-6 mm long, 3-5 mm in diameter, lobe:tube ratio 2.5-5, glabrous; lobes (8-)11-20 mm long, 2-4 mm wide, length:width ratio 4-7, linear to very narrowly elliptic, coriaceous, spreading horizontally, sometimes curved and then spreading, glabrous. *Receptacle* ca. 4 mm in diameter. *Stamens* ca. 1.2 mm long; connective shield ca. 0.1 mm long, glabrous, those of innermost stamens extended or rarely slightly so over adjacent ovary wall. *Ovary* ca. 2 mm long, ca. 1.5 mm wide; stigma ca. 1.8 mm in diameter, glabrous, red at anthesis. *Fruiting pedicels* ca. 3.5 cm long, woody. *Fruits* 9-13 cm long, 5.5-7 cm in diameter, length:width ratio 1-1.5, globose to obconic, apex rounded to apiculate, apicule ca. 5-8 mm, striate, green, glabrous; pericarp ca. 5 mm thick. *Seeds* 22-25 mm long, 10-12 mm in diameter, ellipsoid, packed in white pulp; testa smooth, light brown; raphe not thickened, brown; hilum 7-8 mm long, 3-3.5 mm wide, elliptical.

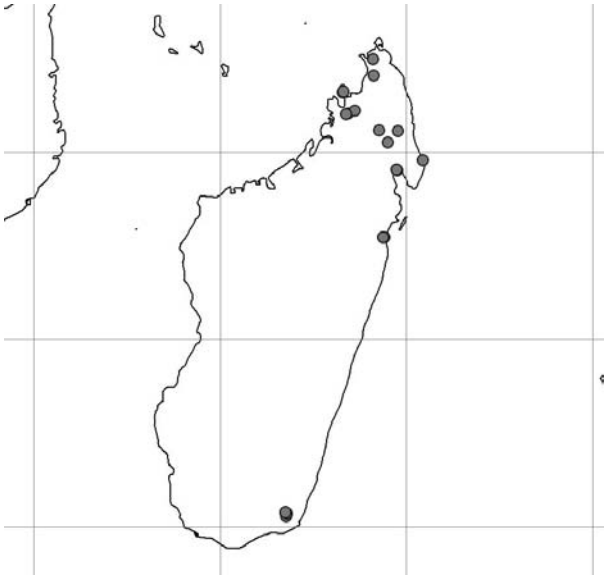
Distribution: Northern and southeastern Madagascar (see Map 16); in primary and secondary rain forest; at 200-1100 m altitude.

Phenology: Mature flowers collected from January to May and in November. Mature fruits collected from April to July and in October.

Vernacular names:
Madagascar: Mbavy, Hombavy (Malgash).

IUCN conservation status: VU B2ab(iii). *Isolona perrieri* has a fair number of specimens in herbaria, and has been collected a few times these past two decades. It grows in numerous protected areas such as national parks (Andohahela), special reserves (Ankarana, Anjanaharibe Sud, Montagne d'Ambre, Nosy Mangabe) and strict nature reserves (Lokobe on the Nosy Be Island). However, the Area of Occupancy is smaller than 200 km^2 and the species is found in less than 10 locations, thus the "vulnerable" category appears to be the best option.

Notes: *Isolona perrieri* is distinguishable by the narrowly elliptic leaves, very thin, long and glabrous flowering pedicels, as well as linear to narrowly elliptic corolla lobes, that spread horizontally. On dried material the corolla of some specimens is characteristically spread out into a star shape. It appears close to *I. madagascariensis* by the shape of the corolla lobes and seems to be considered conspecific by the indigenous people because of the overlap in some vernacular names. However, it is distinct by the length and thickness of the flowering pedicels. The small population growing in the exterm south of the isalnd presents no clear morphological differences with the northern populations so it is conserved within *I. perrieri*.



Map 16: Distribution of *Isolona perrieri*.

ADDITIONAL SPECIMENS EXAMINED:

MADAGASCAR: Antsiranana (Diego Suarez). Montagne d'Ambre national park, 800-1100 m, 19 April 1993, *O. Andrianantoanina 70* (P, WAG); Foret du Lokobé, versant ouest, rive droite de l'Andranobe, 120 m, 6 December 1989, *T. Deroin 200* (MO); Ambahatra, cours superieur. Crete entre les deux bras de l'Ambahatra, 1.1 Km au N du point coté 1528, 1220 m, 14 March 1999, *L. Gautier 3563* (G, WAG); Reserve Naturelle Marojejy, along the trail to the summit of Marojejy Est, NW of Mandena between the first and the second camps, 600-660 m, 6 October 1988, *J.S. Miller 3414* (MO, U, WAG); Reserve Naturelle Marojejy, W portion of the base of Mt. Beondroka, 200-550 m, 22 October 1989, *J.S. Miller 4353* (K, MO, U); Sambirano, Nossi-Be, Lokobe, 1932, *J.M.H.A. Perrier de la Bâthie 18714* (P); Tambirano, Mont Antsatrtro, base du Massif de Manongarivo; Antsatrat, 0-500 m, May 1909, *J.M.H.A. Perrier de la Bâthie 4951* (B, P); Ambohitralanana, no date, *Ranjokiny SF(MDG) 9995* (B, P); Prefecture d'Antalaha, Sous prefecture d'Andapa, commune rurale de Bealampona, village de Mandritsarahely. Sud ouest d'Andapa, Reserve Spéciale d'Anjanaharibe-Sud, suivant la piste vers Ranomafana, 5.5 km Sud-Ouest de Befingotra. Campement #1, 875m, 18 October 1994, *D. Ravelonarivo 400* (MO, WAG); District d'Ambilobe, Canton de Mahamasina. N. of Mahamasina, Réserve Spéciale d'Ankarana, 50 m, 14 June 1995, *S.G. Razafimandimbison 82* (MO, WAG); Sambirano: Vallé de la Beandrona a l'Est d'Ambanja, 9 November 1958, *Service Forestier de Madagascar SF(MDG) 18925* (P); **Toamasina (Tamatave)**. Island of Nosy Mangabe, 5 km S of Marosantsetra

in the Bay of Antongil, 0-200 m, 24 February 1990, *E. Carlson 48* (G, K, MO, WAG); Island of Nosy Mangabe, 5 km S of Marosantsetra in the Bay of Antongil, 19 July 1990, *Carlson, E. 339* (K, MO); 25m, 9 December 1989, *Carlson, E. 7* (MO); 10.3 Km of Fenoarivo, Station Forestiere de Tampolo. Vicinity of Station and along 1 km of forestry raod leading E, 10 m, 29 February 1992, *R.D. Noyes 963* (P); Nosy Mangabe, a 520 ha island in the Bay of Angongil, 5 km. from Maroantsetra, 0-330 m, 9 January 1989, *G.E. Schatz 2550* (MO, S, WAG); Tampolo, near Tanambas, 3m, 22 March 1955, *Service Forestier de Madagascar SF(MDG) 13073* (P); Village le plus proche: Tampolo; Canton: Ampasina, 22 March 1957, *Service Forestier de Madagascar SF(MDG) 16896* (BR, COI, P); **Toliara (Tulear)**. SE Madagascar, of national road XI. Andohahela parcel 1, path over Col Antanatana to Iminminy, 800 m, 7 December 1989, *D.J. Du Puy MB 512* (MO, P); Toliara, NW of Tolanaro, Reserve naturelle Intergrale num 11 (Andohahela) parecelle I, NW of Eminiminy, beside river Itrotroky, 500-1000 m, 13 February 1993, *S.T. Malcomber 2165* (P); Reserve naturelle Intergrale d'Andohahela, parcelle 1, au bord de la riviere Andranohela, a 8 km d'Eminiminy, 435m, 12 January 1995, *N. Messmer 41* (G); Reserve Intégrale num 11. Andohahela, parcelle 1, vicinity of Eminimy, 200-700 m, 24 May 1993, *B. Randriamampionona 341* (MO, P, WAG); Reserve Intégrale num 11. Andohahela, parcelle 1, vicinity of Eminimy, 200-700 m, 24 May 1993, *B. Randriamampionona 365* (P, WAG); Reserve Intégrale num 11. Andohahela, parcelle 1, vicinity of Eminimy, 200-700 m, 24 May 1993, *B. Randriamampionona 366* (P, WAG).

17. Isolona pilosa Diels, Bot. Jahrb. Syst. 41: 328. 1908. — TYPE: DEMOCRATIC REPUBLIC OF CONGO. Kasai Oriental: Lualaba, [ca. 4°58' S, 23°16' E], *L.C. Ledermann 11* (holotype: BR!; isotype: K!). *Figure 6.25 E-G*

Isolona theobromina Exell, Journ. Bot. 64, Suppl. Polypet: 10. 1926. — TYPE: ANGOLA. Cabinda: Pango Munga, 7 January 1916, *J. Gossweiler 6112* (holotype: BM!; isotypes: COI!, LISJC!, LISCU!).

Tree up to 13 m tall; trunk with d.b.h. up to 50 cm; young branches light brown, densely covred with curly hairs, becoming glabrous in older branches. *Petioles* 3-9(-12) mm long, 3-4 mm in diameter, densely covered with curly hairs, leaf lamina inserted on side, very narrowly grooved adaxially. *Leaf lamina* 19-24(-27) cm long, 6-10 cm wide, length:width ratio 2.5-3, obovate to narrowly obovate, base cordate to rounded, apex acuminate, acumen 1-2 cm long, papyraceous, densely covered with appressed hairs abaxially, sparsely covered with appressed hairs to glabrous adaxially; midrib densely covered with curly hairs abaxially, sparsely covered with curly hairs adaxially; secondary veins 15-20 pairs, covered with short appressed hairs abaxially, glabrous adaxillay, uniformally curving upwards. *Inflorescences* sometimes with an additional rhipidium, on leafy branches. *Flowering pedicels* 2-4 mm long, 1-2 mm in diameter, densely covered with short erect hairs. *Lower bracts* 2-4, 2-4 mm long, 1-2 mm wide, length:width ratio ca. 2, ovate, densely covered with short appressed hairs outside and along the margins, glabrous inside. *Upper bract* absent. *Sepals* 2-5 mm long, 2-4 mm wide, length:width ratio 1-1.5, ovate, base truncate, apex acuminate, densely covered with short appressed hairs outside and along margins glabrous inside, not appressed against tube, persistent in fruit. *Corolla* yellow; tube 5-10 mm long, 3-4 mm in diameter, lobe:tube ratio 1-2.6, densely to sparsely covered with short appressed hairs outside, very sparsely to densely covered with short straight hairs inside; lobes 8-13 mm long, 3-5 mm wide, length:width ratio 2.4-2.7, oblong or elliptic, apex acute, curving inwards over the receptacle, densely covered with short appressed hairs outside, towards the apex and along the margins inside, coriaceous. *Receptacle* ca. 4 mm in diameter. *Stamens* ca. 2 mm long; connective shield ca. 0.1 mm long, sparsely covered with short appressed hairs to glabrous, those of innermost whorl slightly

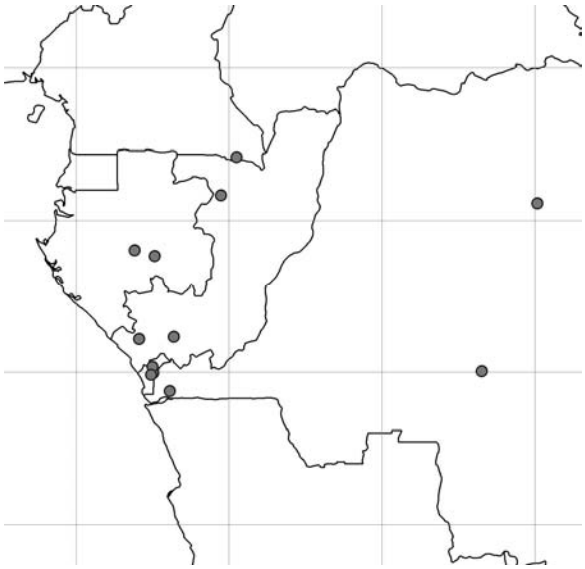
elongated and pressed against ovary wall. *Ovary* ca. 2.2 mm long, ca. 1 mm wide; stigma ca. 2 mm in diameter, glabrous. *Fruiting pedicels* 2-8 cm long, 2-3 mm in diameter, densely covered with short appressed hairs. *Fruits* 3-6 cm long, 2-4 cm in diameter, length:width ratio 2.5-4, ellipsoid, apex cuspidate, longitudinally ribbed, sparsely covered with short appressed hairs; pericarp 2-3 mm thick. *Seeds* 13-15 mm long, 6-8 mm in diameter, transversely ellipsoid; testa smooth, dark brown raphe slightly thickened, very dark brown, hilum not seen.

Distribution: Disjunct, Gabon, south-eastern Cameroon, Cabinda (Angola) and Republic of the Congo, as well as eastern Democratic Republic of Congo (see Map 17); in primary rain forest, also found in swampy forests; only recorded once at 420 m.

Phenology: Mature flowers collected from December to March and in June. Mature fruits collected from December to February.

IUCN conservation status: VU B2ab(iii, iv). *Isolona pilosa* is poorly represented in herbaria but has been collected in various countries in West-Central Africa generating a high value for the Area of occupancy. Only one collection was made in the last 30 years (in 1998) in the Odzala national park in the Republic of Congo. Thus the “vulnerable” category seems appropriate.

Notes: *Isolona pilosa* is the most hairy species within *Isolona*. It is easily distinguishable by its densely hairy adaxial midrib, even in older leaves, as well as its short and densely hairy flowering pedicels with the corolla lobes being completely hairy on the outside and near the margins on the inside. The inner part of the tube is glabrous, which distinguishes it from other hairy species such as *I. congolona*.



Map 17: Distribution of *Isolona pilosa*.

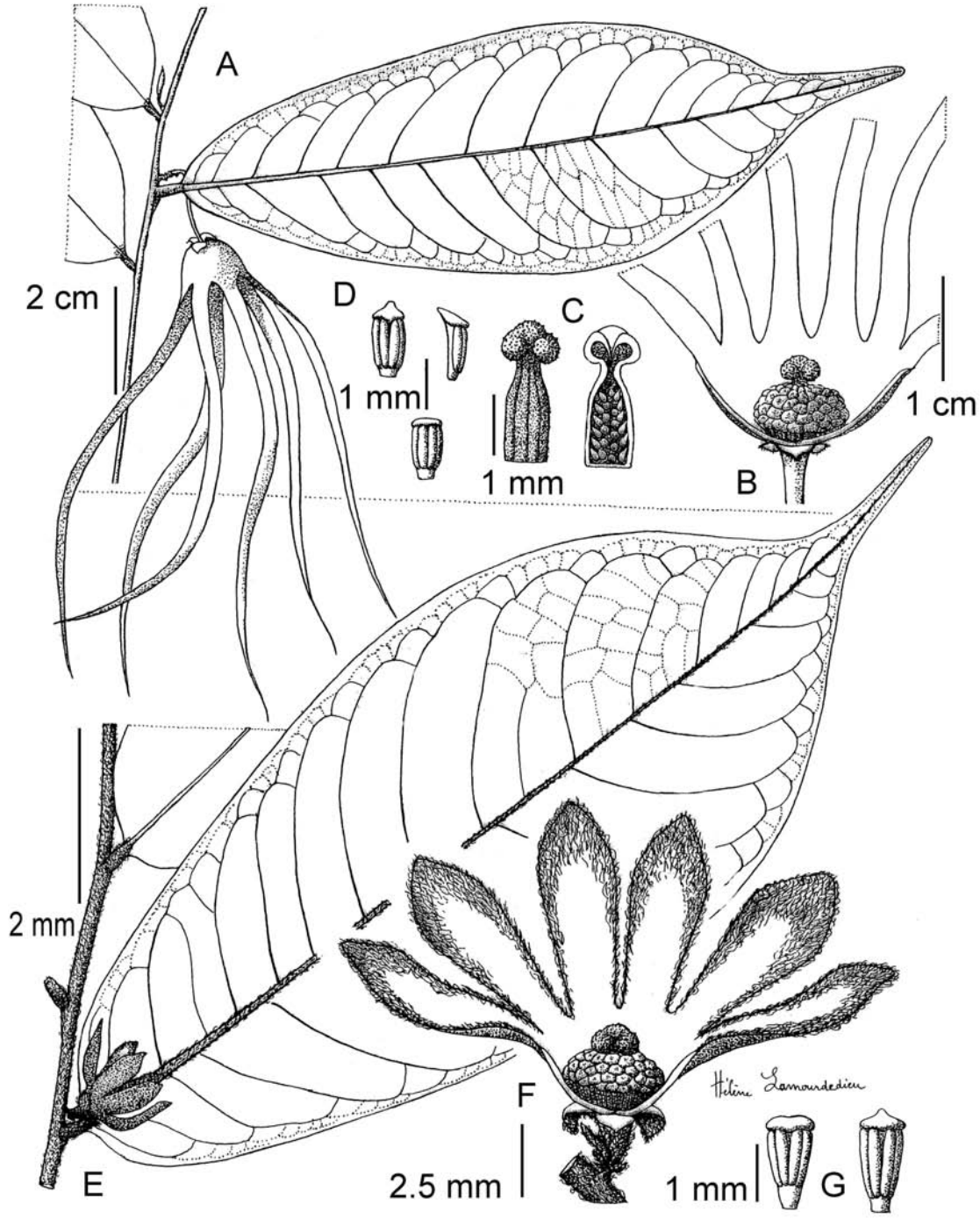


Figure 6.25. *Isolona le-testui*. A. Flowering branch. B. Opened flower showing androecium and stigma. C. Carpel (left) and transversal section of carpel (right). D. Stamens of innermost row (2 top) and stamen of outermost row (bottom). *Isolona pilosa*. E. Flowering branch. F. Opened flower showing androecium and stigma. G. Stamen of outermost row (left) and innermost row (right). Modified from Flore du Gabon, Le Thomas, Fig. 65, 1969.

Extra references: Exell & Mendonça, Consp. Fl. Angola. 1, 1: 31. 1937; Pellegrin, Bull. Soc. Bot. France. 94: 387. 1947; Paiva, Mem. Soc. Brot. 19: 117. 1966.

ADDITIONAL SPECIMENS EXAMINED:
ANGOLA: Cabinda. Maiombe, Panga Mungo, 7 January 1916, *J. Gossweiler 6112* (BM, COI, LISJC, LISU); Maiombe, Buco Zau, 20 January 1917, *J. Gossweiler 6940* (BM, COI, LISJC, LISU); Nkanda Mbaku, Luali-Chilcanga (riv.), 20 June 1924, *J. Gossweiler 9063* (B, BM, K, LISJC, US).
CAMEROON: South Province. Region near Station Molundu Dscha (Ngoko). Nginda 21 km north Molundu, 7 January 1911, *G.W.J. Mildbraed 4193* (HBG).
DEMOCRATIC REPUBLIC OF CONGO: Bas-Congo. Luki, entre Kisavu et Kiyengo, 3 December 1948, *C. Donis 2180* (BR, WAG); **Kasai Oriental**. Distr. Lualaba-Kasai, am Sankuru, Kondué, 420 m, March 1906, *C.L. Ledermann 11* (B, K); **Orientale**. Kisangani, Ile de Kongolo, a la confluence e la Lindi avec le fleuve Zaire, 31 October 1978, *J. Lejoly 4234* (BR, BRLU); 16 December 1978, *J. Lejoly 4441* (BR, BRLU).
REPUBLIC OF THE CONGO: Entre Loudima et Sibiti, February 1957, *J. Koechlin 4176* (BR); Mayombe, environ de Ngoungui 2, 15 March 1975, *P. Sita 3899* (P); **Cuvette**. Odazala National Park, 30 December 1993, *F. Dowsett-Lemaire 1603* (BR); **Kouilou**. Chantier forestier Gouttex Ngoungui II. Piste Vounda-Kakamoeka, 27 January 1976, *P. Cabalion 133* (P, WAG).
GABON: Ogooué-Lolo. Region de Lastoursville, Iméno, December 1930, *G.M.P.C. Le Testu 8602* (BM, BR, P, WAG); Region de Lastoursville, Matongo, April 1931, *G.M.P.C. Le Testu 8740* (BM, BR, EA, P, WAG).

18. Isolona pleurocarpa Diels, Bot. Jahrb. Syst. 39: 485. 1907. — TYPE: CAMEROON. South Province: Bipinde, July 1904, *G.A. Zenker 3217* (holotype B!; isotypes BR!, G!, K!, M!, S!, WAG!).

Isolona leucantha Diels, Bot. Jahrb. Syst. 39: 485. 1907. — TYPE: CAMEROON. South Province: Bipinde, April 1904, *G.A. Zenker 3038* (holotype: B!; isotypes: COI!, G!, HBG!, K!, M!, MO!, P!, S!, WAG!). ***syn. nov.***

Tree up to 15-30 m high; trunk with d.b.h up to 40(-60) cm, fluted; bark thick, black with grey markings;; young branches drying black, glabrous; old branches dark grey, rugulose longitudinally, glabrous. *Petioles* 4-8(-12) mm long, ca. 1 mm in diameter, glabrous, leaf lamina inserted on side, broadly grooved adaxially. *Leaf lamina* 8.5-15.5 cm long, 3-6 cm wide, length:width ratio 2.5-3.5, narrowly elliptic to elliptic or narrowly obovate to obovate, base decurrent to narrowly cuneate, apex acuminate, acumen 1-1.5 cm long, subcoriaceous to papyraceous, glabrous, light green abaxially, dull dark green adaxially; midrib proximally depressed and glabrous on both sides; secondary veins 9-12 pairs, uniformally curving upwards, glabrous. *Inflorescences* on leafy branches. *Flowering pedicels* 10-20(-23) mm long, ca. 1 mm in diameter, glabrous. *Lower bracts* 2-6, ca. 1 mm long, ca. 1 mm wide, glabrous; margins with short appressed hairs. *Upper bract* inserted in the basal half of the pedicel, ca. 1 mm long, ca. 1 mm wide, glabrous; margins with short appressed hairs. *Sepals* 2-3 mm long, 2-3 mm wide, length:width ratio 0.6-1, depressed ovate to broadly ovate, base truncate, apex acuminate, glabrous, green; margins with short appressed hairs, appressed against tube, falling in fruit. *Corolla* bright green-white when immature, yellow with red tube at anthesis; tube (6)9-15 mm long, lobe:tube ratio 1.6-3, verruculose when dried, glabrous; lobes 10-23 mm long, 5-10 mm wide, length:width ratio 1.6-3.5, narrowly ovate to ovate, narrowed at base, apex acute, glabrous, papyraceous, the margins undulate-wavy. *Receptacle* ca. 4 mm in diameter. *Stamens* 1.7-1.9 mm long; connective shield ca. 0.2 mm long, glabrous, those of innermost row stretched and pressing against ovary wall. *Ovary* ca. 2.2 mm long, ca. 2 mm wide; stigma ca. 2.5 mm in diameter, sparsely covered with short erect hairs. *Fruiting pedicels* ca. 3 cm long, 3-4 mm in diameter, woody, rugulose in longitudinal lines, glabrous.

Fruits ca. 5 cm long, ca. 4 cm in diameter, length:width ratio ca. 1.25, globose, apicule absent, conspicuously longitudinally 6-8 ribbed, rugulose all over, glabrous, green when immature. *Seeds* not seen.

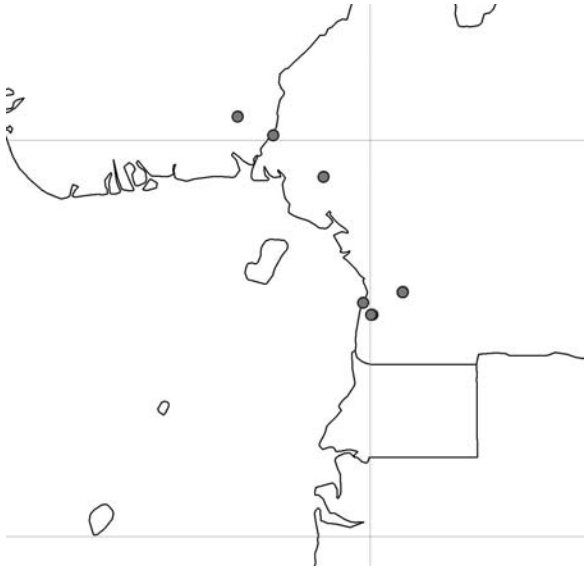
Distribution: Western Cameroon and south-eastern Nigeria (see Map 18); in lowland rain forests; at 0-100 m altitude.

Phenology: Mature flowers collected from January to May and in October. Mature fruits found in July, immature fruits collected in February and March.

IUCN conservation status: EN B2ab(iii). *Isolona pleurocarpa* is moderately represented in herbaria. Most of the collections come from a single locality (Bipindi) with a clear decrease in collections in recent years. It has only been collected in one forest reserve (Southern Bakundu in Cameroon) and one national park (Korup in Cameroon). The “endangered” category is thus deemed appropriate.

Notes: *Isolona pleurocarpa* was synonymized with *I. hexaloba* by Le Thomas in the Flore du Gabon (1969). However, there are clear differences especially in the petioles, which are thick and short in *I. hexaloba* but elongated and thin in *I. pleurocarpa*. Corolla lobes in *I. pleurocarpa* are thin and wavy with a cuneate apex, whereas *I. hexaloba* has thin non-undulate and apically rounded corolla lobes. Fruits of *I. pleurocarpa* were described based on a scan kindly provided by the Berlin Herbarium (B) and may not be completely accurate, but one can clearly see that the fruit of *I. pleurocarpa* is rounded and ribbed, whereas the fruit of *I. hexaloba* is conical and lumpy. Even though the protologue clearly describes the flowers, there are no signs of flowers either on the holotype or on numerous isotypes (only specimen cited by Diels). Moreover, the label on *Zenker 3217* refers only to fruits and does not describe flowers.
Although *I. pleurocarpa* and *I. leucantha* where published by Diels in the same article, we see no clear differences between them and reduce *I. leucantha* to a synonym of *I. pleurocarpa*. However, flowering material of *I. leucantha* (only represented by the type) was very scarce or in poor condition. Extra material might be able to justify the new status in the future.

Extra references: Keay, Fl. W. Trop. Afr., ed. 2, 1, 1: 53. 1947.



Map 18 : Distribution of *Isolona pleurocarpa*.

ADDITIONAL SPECIMENS EXAMINED:
CAMEROON: South Province. Elephant Mont, halfway the hill, 100 m, 22 October 2001, *T.R. van Andel 4177* (KRIBI, SCA, WAG, YA); 20 km SE. of Kribi, NE. of Mt. Elephant, 10 February 1970, *J.J. Bos 6298* (WAG); Colline Nkolo Manga (20 km SE Kribi) Feuille IGN 1/200.000 Kribi, 16 April 1968, *R. Letouzey 9341* (P, WAG); Place de Bipindi, January 1918, *G.A. Zenker 22* (P); Bipindi, Mimfia, November 1919, *G.A. Zenker 95* (BM); Bipindi, 1895, *G.A. Zenker 1716* (B, FHO, G, HBG, M, P, WAG); Bipindi, 1904, *G.A. Zenker 2732* (US); Bipindi, April 1904, *G.A. Zenker 3038* (B, COI, G, HBG, K, L, M, MO, P, S, WAG); Bipindi, July 1904, *G.A. Zenker 3217* (B, BR, G, K, M, S, WAG); Bipindi, 1907, *G.A. Zenker 3375* (B, BM, BR, COI, G, HBG, L, M, MO, S, US, WAG, Z); Bipindi, 1908, *G.A. Zenker 3433* (BR, COI, G, M, MO, P, S, US, Z); Bipindi, 1908, *G.A. Zenker 3540* (G, K, L, M, MO, US); 1909, *G.A. Zenker 3921* (B, BR, COI, G, L, M, MO, P, S, US, Z); Bipinde, 1913, *G.A. Zenker 4704* (BM, BR, G, K, L, M, P, S, Z); Bipindi, November 1896, *G.A. Zenker s.n.* (F); July 1907, *G.A. Zenker s.n.* (F); May 1907, *G.A. Zenker s.n.* (F); May 1906, *G.A. Zenker s.n.* (F); Bipindi, Mimfia forest, November 1919, *G.A. Zenker s.n.* (US); **South-West Province**. S. Bakundu Forest, 3 km from Kindongi Camp (8 km from road), 50 m, 2 May 1972, *A.J.M. Leeuwenberg 9784* (B, BR, C, FHI, H, HBG, K, LD, LISC, M, MO, P, PRE, SCA, UPS, WAG, YA); Korup Reserve, tansect Q, 13 April 1978, *D.W. Thomas 349* (K); **Unknown**. Mbiave, January 1913, *G.A. Zenker 267* (A, B, BR, C, G, M, MO, U, US, WAG).
NIGERIA: Cross River State. Akamkpa Rubber Estate, calabae River Division, 16 March 1959, *M.G. Latilo FHI 41347* (K).

19. Isolona thonneri (De Wild & Th.Dur.) Engl. & Diels, Monogr. Afr. Pflanzenfam. 6: 83. 1901. — *Monodora thonneri* De Wild & Th.Dur., Bull. Soc. Roy. Bot. Belg. 38: 12. 1899. — TYPE: DEMOCRATIC REPUBLIC OF CONGO. Equateur: Massanga-Mondeva, 20 October 1896, *F. Thonner 104* (holotype BR!; isotypes: B!, BR!). *Figure 6.26 H-J*

Isolona dewevrei De Wild., Ann. Mus. Congo Belge, Bot., Sér. 5, 3: 393. 1912, not (De Wild. & Th. Dur.) Engl. & Diels

Tree up to 10 m tall; trunk with d.b.h. up to 25 cm; bark smooth; young branches drying black, glabrous; old branches brown, glabrous, striate. *Petioles* 3-8 mm long, 1-2 mm in diameter, glabrous, leaf lamina inserted on side, grooved adaxially. *Leaf lamina* 11-20 cm long, 4-7.5 cm long, length:width ratio 2.3-3.6, narrowly obovate to obovate or narrowly elliptic to elliptic, base decurrent or rarely cuneate, apex acuminate, acumen 10-20 mm long, sub-coriaceous to coriaceous, glabrous, dark green; midrib glabrous on both sides; secondary

veins 9-12 pairs, clearly impressed adaxially, straight then abruptly curved upwards or uniformly curved upwards, glabrous. *Inflorescences* sometimes with an additional rhipidium, on leafy branches. *Flowering pedicels* 5-18 mm long, ca. 1 mm in diameter, glabrous. *Lower bracts* 2-6, ca. 1 mm long, ca. 1 mm wide, glabrous; margins with short erect hairs. *Upper bract* inserted near the base of the pedicel, ca. 1.5 mm long, ca. 1 mm wide; margins with short erect hairs. *Sepals* 2-3 mm long, 1-2 mm wide, length:width ratio 1-2, ovate, base truncate, apex acute, glabrous, dark green, curved outwards on fresh material, sometimes persistent on fruit. *Corolla* green when immature, yellow with reddish center at anthesis; tube 3-6 mm long, 3-4 mm in diameter, lobe:tube ratio 3.3-7.8, glabrous; lobes hanging vertically, 14-31 mm long, 3-5 mm wide, length:width ratio 4-10, narrowly elliptic or lorate to linear, base truncate to slightly narrowed, apex acute, glabrous, membranous. *Receptacle* ca. 4-5 mm in diameter. *Stamens* ca. 1.5 mm long; connective shield ca. 0.1 mm long, glabrous, those of innermost row elongated and pressing against the ovary wall. *Ovary* ca. 2 mm long, ca. 1.5 mm wide; stigma ca. 3 mm in diameter, very sparsely covered with short erect hairs. *Fruiting pedicels* 8-10 mm long, 3-4 mm in diameter, glabrous. *Fruits* 3.5-6 cm long, 2-3.5 cm in diameter, length:width ratio 1.4-1.8, ellipsoid, apicule absent, smooth, not ribbed, glabrous; pericarp 2-3 mm thick. *Seeds* 15-18 mm long, 8-9 mm in diameter, ellipsoid; testa smooth, dark brown; raphe weakly thickened, very dark brown; hilum 5-7 mm long, 2-3 mm wide, elliptical.

Distribution: Southern Nigeria, southern Cameroon, Gabon, and Democratic Republic of Congo (see Map 19); in lowland rain forests, especially near rivers and swamps; at 450-750 m altitude.

Phenology: Mature flowers collected from January to April and from August to December. Mature fruits found in January, March to April and in October.

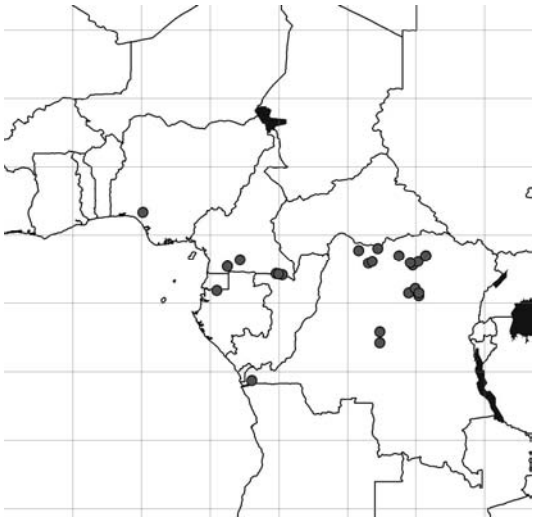
Vernacular names:
Democratic Republic of Congo: Bundjingi (Banalia); Eka (Dundusana); Konadala (dial. azande); Loopa (dial. turumbu); Mundzingu (dial. babua).

Uses: The hard wood is used in construction as well as for making xylophones in the Democratic Republic of Congo (Boutique, 1951).

IUCN conservation status: VU B2ab(iv). *Isolona thonneri* is moderately represented in herbaria. Most of the collections originate from the Democratic Republic of Congo were collected before the mid 1970's. The more recent collections (3 in 2001) were made in Cameroon and Gabon. It was collected in one national park (Crystal Mountains in Gabon) and one forest reserve (Ohosu in Nigeria). Even though the Area of occupancy (AOO) is less than 500 km^2 the number of locations is higher than 5 but less than 10, suggesting that the "vulnerable" category is more appropriate.

Notes: *Isolona thonneri* is characterized by its long, narrowly elliptic to linear and glabrous corolla lobes. It has a strong affinity with *I. le-testui*, the latter having however significantly longer lobes. It also bears a similarity to *I. linearis* from Tanzania. When in fruit, it is indistinguishable from *I. dewevrei*. The latter relationship is confirmed by the molecular phylogeny.

Extra references: Th. & H. Dur., Syll. 23. 1909; De Wild., Bull. Jard. Bot. État Brux. 5: 273. 1916; Boutique Fl. Congo Belge 2: 262. 1951; Keay, Fl. W. Trop. Afr., ed. 2, 1, 1: 53. 1954; Keay, Trees of Nigeria: 30. 1989.



Map 19: Distribution of *Isolona thonneri*.

ADDITIONAL SPECIMENS EXAMINED:
CAMEROON: East Province. near Ndongo, about 40 km WNW of Moloundou, 16 March 1973, *R. Letouzey 12111* (BR, HBG, K, P, WAG); 16 March 1973, *R. Letouzey 12115* (BR, K, P, WAG); **South Province**. Colline Ongongondje near Akonekye, 15 km NW of Ambam, 23 March 1970, *R. Letouzey 10205* (BR, COI, HBG, K, P, WAG); near Ndongo, about 45 km WNW of Moloundou, 15 March 1973, *R. Letouzey 12085* (K, P); Inselberg d'Akookas, près du village d'Akookas à 38 km au sud-est d'Ebolowa, 750 m, 15 March 2001, *I. Parmentier 1961* (BRLU, WAG); Inselberg d'Akookas, pres du village d'Akookas a 38 km au sud est d'Ebolowa, 750 m, 15 March 2001, *I. Parmentier 1943* (BRLU, WAG); **Unknown**. Prés Banana, 10 km ENE de Moloundou, 17 April 1972, *R. Letouzey 10682* (P, WAG).
DEMOCRATIC REPUBLIC OF CONGO: Bas-Congo. Luki, Parc de la Nkila, 25 September 1979, *Nsimundele 534* (BR); **Equateur**. Bas Uele, 11 August 1935, *A. De Wulf 996* (BR); entre Businga et Banzyville, January 1931, *J. Lebrun 2032* (K); Dundusana, November 1913, *M.G. Mortehan 738* (BR); March 1913, *F.J. Reygaert 185* (BR); Mssanga (près de Monveda), 450 m, 24 September 1896, *F. Thonner 104* (BR); Bogolo (près de Businga), 450 m, 20 October 1896, *F. Thonner 106* (B, BR); **Kinshasa**. La Kulu, 1928, *J.F. Van den Brande 233* (K); **Orientale**. 23 km along road from Kisangani to Bengamisa, 2 March 1973, *J. Bokdam 3976* (KIS, WAG); Environs de Bambesa, 1933, *H.J.A.E.R. Brédo 713* (BR); 1933, *H.J.A.E.R. Brédo 773* (BR); 1933, *H.J.A.E.R. Brédo 544* (BR); Bambesa, 1 October 1956, *P. Gérard 2444* (BR); 25 March 1953, *P. Gérard 600* (BR); 1943, *G. Gilbert 891* (BR); Madengedenge, April 1937, *G. Gilbert 253* (BR); Entre Buta et Titule (Kumu entered), April 1931, *J. Lebrun 2656* (K, P); Yangambi, 500 m, 27 February 1938, *J. Louis 8231* (BM, BR, C, EA, FHO, K, MO, P, US); Cirque source de la N'gula, 470 m, 5 July 1939, *J. Louis 15475* (BR, MO, NY, US); Environs de Bambesa, 1936, *R. Pittery 157* (BR); Environs de Bambesa, Uele, 1936, *R. Pittery 162* (BR); 1936, *R. Pittery 163* (BR); Chemin de Bula a Banalia, environ de Bula, 1926, *F.H.E.A.W. Robyns 1318* (BR, FHO, WAG); Environ de Stanleyville, entre Tshopo et Lindi, 28 January 1926, *F.H.E.A.W. Robyns 1433* (A, BR, P); Bambesa, 23 March 1939, *J.-M. Vrydagh 34* (BR, K); 18 April 1940, *J.-M. Vrydagh 363* (BR).
REPUBLIC OF THE CONGO: Unknown. Pemba, rive droite du Niari Madingou, March 1973, *J. Koechlin 4306* (BR).
GABON: Woleu-Ntem. Crystal Mountains, Inselberg Milobo, 700 m, 22 October 2001, *L. Ngok Banak 235* (BRLU, LBV, MO, WAG).

NIGERIA: Edo State. Ohosu Reserve, December 1938, *R.H. Hide 38/ 1* (FHO).

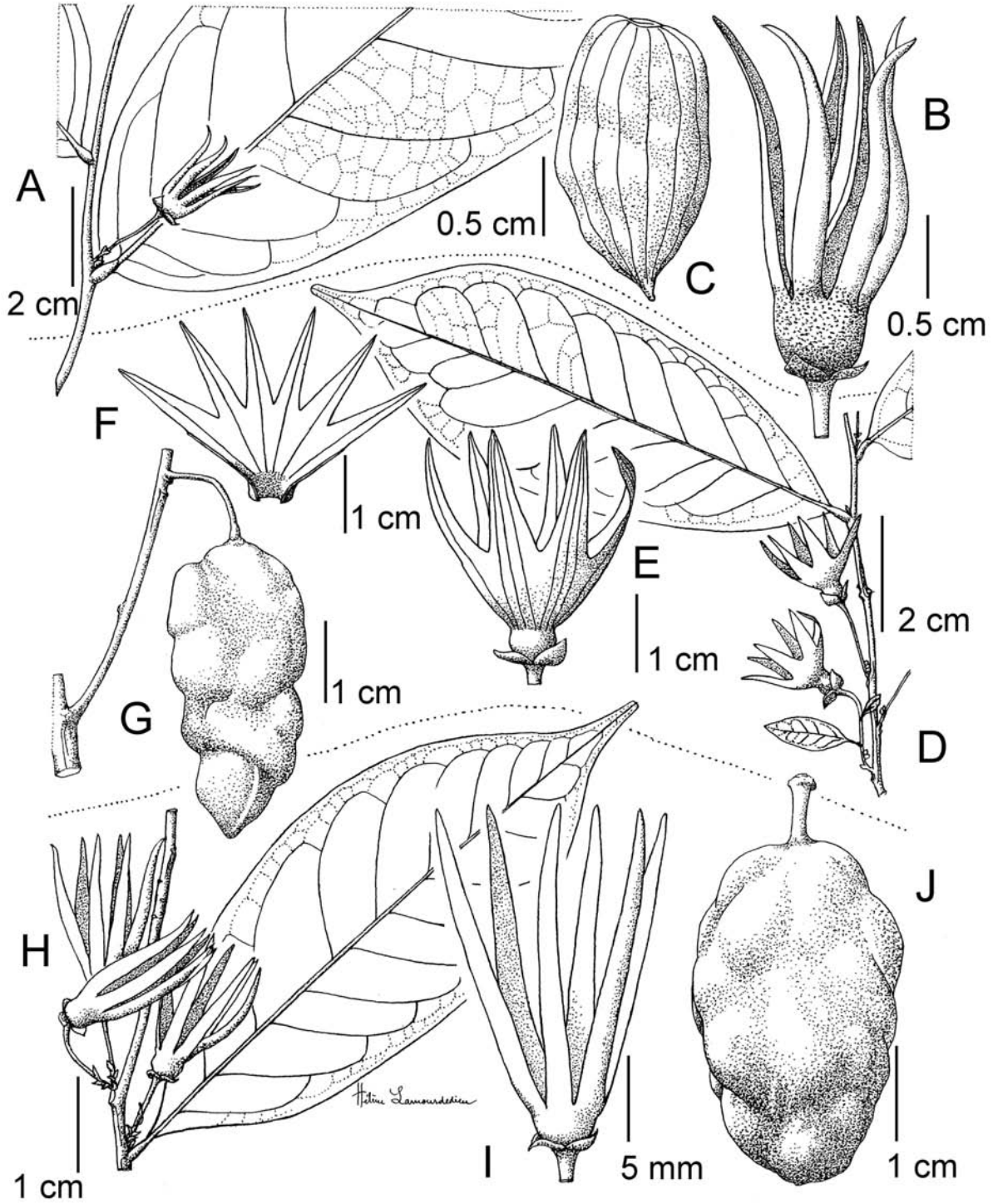


Figure 6.26. *Isolona zenkeri*. A. Flowering branch. B. Flower. C. Fruit. *Isolona campanulata*. D. Flowering branch. E. Flower. E. Corolla lobe opened. G. Fruit. *Isolona thonneri*. H. Flowering branch. I. Flower. J. Fruit. Drawings by Hélene Lamourdedieux.

20. Isolona zenkeri Engl., Notizbl. Bot. Gart. Berlin-Dahlem 2: 301. 1899. — TYPE: CAMEROON. South Province: Bipindi, 1896, *G.A. Zenker, 1186* (holotype: B!; isotypes: BM!, G!, K!, WU!). *Figure 6.26 A-C*

Diospyros oblongicarpa Gürke, Bot. Jahrb. Syst. 43: 200. 1909, p.p. quoad *Zenker 3471*.

Tree or shrub to 7(-15) m high; trunk with d.b.h. up to 15 cm; outer bark brown to dark brown; younger branches drying black, glabrous; old branches brown, finely rugulose, glabrous. *Petioles* 2-5(-6) mm long, ca. 2 mm in diameter, glabrous, verrucose, leaf lamina inserted on top, very faintly grooved adaxially. *Leaf lamina* 16-23 cm long, 6.5-8.5 cm wide, length:width ratio 2.5-2.7, narrow obovate to oblanceolate or rarely oblong, base rounded to acute, apex abruptly acuminate, acumen 1-2 cm long, coriaceous, glabrous; midrib glabrous on both sides; secondary veins 11-13 pairs, uniformally curving upwards, glabrous. *Inflorescences* rarely with an additional rhipidium, usually on leafy branches but occasionally on old leafless branches. *Flowering pedicels* 3-7 mm, 0.5-1 mm in diameter, glabrous, brown-red. *Lower bracts* inserted at the base of the pedicel, 2-4, minute, glabrous; margins densely or sparsely covered with short appressed hairs. *Upper bract* absent. *Sepals* 2-5 mm long, 2-4 mm wide; length:width ratio 1-1.4, very broadly ovate to ovate, base truncate, apex acute, glabrous, brown-red or green. *Corolla* light green to light yellow when immature, light red at anthesis, with small oil cells present on fresh and boiled material, giving a verrucose appearance to the dried material; tube 4-7 mm long, 3-4 mm in diameter, lobe:tube ratio 2.5-3.6, glabrous; lobes 15-25 mm long, 3-4 mm wide, length:width ratio 3.5-6.3, narrowly oblong to lorate, apex acute, glabrous, coriaceous, hanging over receptacle, margins folded inwards. *Receptacle* 4-5 mm in diameter. *Stamens* ca. 2 mm long; connective shield ca. 0.2 mm long, glabrous, those of innermost stamens extended over adjacent ovary wall. *Ovary* ca. 3 mm long, ca. 1.5 mm wide; stigma ca. 2 mm in diameter, glabrous. *Fruiting pedicels* 5-15 cm long, 2-3 mm in diameter, glabrous, woody. *Fruits* 3-6.5 cm long, 1.5-3 cm in diameter, length:width ratio 1.5-2.5, globose to ellipsoid, smooth, faintly ribbed longitudinally, glabrous, green turning yellow; pericarp 2-3 mm thick. *Seeds* 15-20 mm long, 8-10 mm in diameter, broadly ellipsoid; testa smooth, light brown; raphe very slightly thickened, brown; hilum 4-5 mm long, 2-2.5 mm wide, oblong to elliptical.

Distribution: Cameroon and Gabon (see Map 20); in understory of rain forests near streams or in secondary forests; at 0-800 m altitude.

Phenology: Mature flowers present from March to May, July to August and November to January. Mature fruits collected in November to March, May and August.

IUCN conservation status: LC. *Isolona zenkeri* is fairly well represented in herbaria, and many of the collections are recent (the most recent one from 2006). This species does not seem to grow in dense populations, but is fairly common in Gabon and Cameroon. *Isolona*

zenkeri grows in two protected areas: The Doudou Mountains in Gabon and in the Campo-Ma'an national park in southern Cameroon. The category of "Least Concern" is assigned here.

Notes: *Isolona zenkeri* can be distinguished by the coriaceous corolla lobes with incurved margins as well as by the presence of oil cells giving a verrucose appearance to the dried corolla. The molecular data strongly supports *I. pleurocarpa* as sister to *I. zenkeri*. Both species are, however, morphologically very distinct and present no apparent similarities, except maybe in their pollen morphology (see Chapter 5).

Extra references: Engl. & Diels, Monogr. Afrik. Pflanzen-Fam. 6: 83. 1901; Pellegrin, Bull. Soc. Bot. France. 94: 387. 1947; Le Thomas, Fl. Gabon 16: 358. 1969.

Map 20: Distribution of *Isolona zenkeri*.

ADDITIONAL SPECIMENS EXAMINED:
CAMEROON: South Province. no date, *E. Annet 359* (WAG); 6 km N. of km 46 Kribi-Lolodorf, 12 March 1970, *J.J. Bos 6522* (BR, K, LMA, MO, P, WAG, YA); Campo Ma'an area, Ebianemeyong, 460 m, 24 May 2002, *M. Elad 1545* (KRIBI, SCA, WAG); Campo-Ma'an area, Bibabimvoto, along transect T4, 40 m, 1 February 2000, *M. Elad 1269* (WAG); Approx 2 km S of Kwambo and 6 km WSW of Bipindi, 160 m, 19 January 1987, *S.D. Manning 1453* (MO); Campo-Ma'an area, Bibabimvoto, 60 m, 13 May 2000, *G.P. Tchouto Mbatchou 2855* (KRIBI, WAG, YA); Campo-Ma'an area, Bibabimvoto, along transect T8, 40 m, 24 August 2000, *G.P. Tchouto Mbatchou 3009* (KRIBI, SCA, WAG, YA); Bipinde, 1896, *G.A. Zenker 1186* (B, BM, G, K); Bipindi, June 1906, *G.A. Zenker s.n.* (S, WAG); Bipindi, 1912, *G.A. Zenker 4405* (G, HBG, K, MO); December 1907, *G.A. Zenker s.n.* (F); May 1906, *G.A. Zenker s.n.* (F); 1908, *G.A. Zenker 3471* (US).
GABON: Estuaire. Sibang, 4 December 1901, *T.-J. Klaine 2583* (BM, BR, K, P); 8 January 1902, *T.-J. Klaine 2678* (A, G, L, P); Environs de Libreville, 1902, *T.-J. Klaine 2875* (BR, K, P); Libreville, Sibang Arboretum, 50 m, 9 November 2005, *M.S.M. Sosef 2250* (WAG); Libreville, Sibange Arboretum, 30 m, 8 July 1986, *D.W. Thomas 6342* (LBV, MO, P); La Nkoulounga, 26 January 1959, *G. Touzet 122* (P); Cristal Mountains, about 3 km along the track Alen Nkomo-Andok Foula, 30 m, 21 November 1986, *J.J.F.E. de Wilde 8884* (BR, LBV, MO, WAG); **Ngounié**. slope at the waterfall in the Waka River, 330 m, 25 November 1984, *J.C. Arends 451* (LBV, WAG); Forêt des Echiras, Mouteti, November 1924, *G.M.P.C. Le Testu 5117* (BM, BR, EA, K, P); région de Mouila, Tamba Naghi, October 1926, *G.M.P.C. Le Testu 6332* (BM, BR, EA, P); SW of Fougamou, Koumounabwali Massive, 250 m, 11 December 1995, *J.J.F.E. de Wilde 11554* (LBV, WAG); **Ogooué-Lolo**. Lastoursville, January 1930, *G.M.P.C. Le Testu 7871* (BM, BR, P); Koulamoutou, March 1930, *G.M.P.C. Le Testu 8001* (BM, BR, EA, P); Bounounou, January 1931, *G.M.P.C. Le Testu 8685* (BM, BR, P); 1.5 km E of Lastoursville Railway bridge, 260 m, 25 November 1988, *L.J.G. van der Maesen 5829* (LBV, WAG); c. 60 km N of Lastoursville, "Milolé" logging concession of CEB (UFA2-UFG2-lot 2), foothills of Ngota Mountain., 330 m, 28 January 2008, *J.J. Wieringa 6225* (LBV,

WAG); c. 60 km N of Lastoursville, "Milolé" logging concession of CEB (UFA2-UFG2-lot 2), top of Ngota Mountain., 605 m, 28 January 2008, *J.J. Wieringa 6248* (LBV, WAG); **Ogooué-Maritime**. 20 road- km SE of Igotchi-Mouenda, Bakker timber concession, 25m, 18 May 1997, *G.D. McPherson 17043* (MO); Doudou Mountains National Parc, c. 5 km S of Camp Peny (CBG), 100 m, 14 November 2005, *M.S.M. Sosef 2291* (LBV, WAG); Doudou Mountains National Parc, c. 40 km S of Mandji, 200 m, 16 November 2005, *M.S.M. Sosef 2322* (LBV, WAG); 34 km along an exploitation track in NW direction from Doussala, 140 m, 30 November 1986, *J.J.F.E. de Wilde 9066* (LBV, MO, WAG); **Unknown.** 2 April 1910, *G.A. Zenker 8001* (BM).

REPUBLIC OF THE CONGO: Unknown. 3 km O de Coto Vuidou (Prémayombo), 1 February 1976, *P. Cabalion 144* (WAG); Region de Kotto-Vindou, 16 March 1975, *P. Sita 3901* (P); Chaillu, nord ouest d'Irogo, région de Minanga, piste de la Ngounié. 30 km de Irogo, 9 February 1976, *P. Sita 4013* (P).

GENUS DESCRIPTION: *MONODORA*

Monodora Dunal, Monogr. Anonac. 79. 1817. — TYPE SPECIES: *Monodora myristica* (Gaertn.) Dunal. — TYPE: JAMAICA. Specimen cultivated in Jamaica obtained from Banks (holotype: BM!).

Trees or shrubs to 40 m tall; young branches glabrous to densely hairy; old branches mostly glabrous, sometimes sparsely hairy. *Petioles* 2-14 mm long, leaf lamina inserted on side rarely on top, broadly to narrowly grooved adaxially. *Leaf lamina* ovate to obovate, base rounded to cuneate or sometimes cordate, apex acuminate to acute, papyraceous to coriaceous; midrib raised, glabrous adaxially, prominent abaxially; secondary veins 8-17 pairs, uniformly curving upwards or straight and curving upwards only near the margins, loop forming towards margin; tertiary veins intermediate between reticulate and percurrent, rarely percurrent. *Flowers* bisexual, regular. *Inflorescence* terminal, leaf opposed to supra- or sub-axillary, solitary or rarely 2-3 together, appearing from young leaved or leafless shoots, pendulous or rarely curving upwards and overtopping the foliage, white to yellow streaked with red to purple. *Flowering pedicels* variable in length, slender. *Lower bract* absent. *Upper bract* variable in size, margins undulate to straight. *Sepals* 3, valvate, free or sometimes slightly fused at base, oblong to ovate, margins undulate to straight. *Petals* 6, partially connate at base. *Outer petals* 3, longer than inner petals, oblong to ovate, without claw, margins undulate to straight, rarely crisped. *Inner petals* 3, apically connivent or appressed at center, rarely free, over reproductive receptacle, rhomboid to cordate, clawed; claw shorter or longer than inner petal, glabrous. *Receptacle* strongly convex to flat. *Stamens* short and broad, sometimes elongated, glabrous, shielded by a truncated and broadened apical prolongation of the connective being glabrous to hairy; dehiscence extrorse; pollen in tetrads, inaperturate. *Carpels* 6-12, congenitally fused into a syncarpous ovary, glabrous; stigmas sessile, bi-lobed, slightly capitate; ovules numerous, bi-seriate; placentation parietal. *Fruiting pedicels* thick and woody to slender. *Fruits* syncarpous, globose to conical, sometimes conspicuously ribbed, smooth to rugose. *Seeds* numerous, ellipsoid to broadly ellipsoid; testa smooth; raphe thickened or not; hilum elliptical; rumination laminate in four parts. Base chromosome number n=8.

Distribution: West and Central Tropical Africa, East and South-East Tropical Africa (see Figure 6.13 B). *See Appendix D for photos (end of thesis)*

Chapter 6

KEYS TO THE SPECIES OF *MONODORA*

I. Key to Flowering Specimens

1. Inner petals with two conspicuously hairy appendices around the centre of the lamina. 12. *M. tenuifolia*
1. Inner petals without two conspicuously hairy appendices around the centre of the lamina. 2

2. Flowers 2-3 together, in a rhipidium. 9. *M. minor*
2. Flowers single, not in a rhipidium. 3

3. Margin of outer petals straight. 4
3. Margin of outer petals clearly undulate or crisped. 7

4. Outer petals up to 2 times as long as wide, obovate to elliptic. 5
4. Outer petals at least 3.5 times as long as wide, linear to narrowly elliptic. 6

5. Outer petals with two small lobes at the base (Cameroon). 14. *M. zenkeri*
5. Outer petals without lobes at base (East Africa). 7. *M. junodii*

6. Outer petal 3.5-4 as long as wide; inner side of inner petals set with ribbon-like hairs of c. 1 mm long; stamens in 6-7 rows (West Africa). 8. *M. laurentii*
6. Outer petals 10-20 times as long as wide; inner side of inner petals densely set with non ribbon-like hairs; stamens in 3-4 rows (Malawi and Mozambique). 11. *M. stenopetala*

7. Inner petal laminas > 17 mm long; claw inconspicuous, its length less than 1/3 of that of the lamina; receptacle strongly convex. 8
7. Inner petal laminas < 17 mm long; claw conspicuous, its length more than 1/3 of that of the lamina; receptacle slightly convex to flat. 9

8. Upper bract margins clearly undulate, elliptic to obovate, apex acute to acuminate; flowering pedicels 5-25 cm long. 10. *M. myristica*
8. Upper bract margins straight, very broadly ovate, apex rounded; flowering pedicels 3-5 cm long. 13. *M. undulata*

9. Leaves cordate at base. 5. *M. grandidieri*
9. Leaves acute to rounded at base. 10

10. Flowering pedicels hairy; leaves with conspicuous tertiary venation, generally hairy on both sides. 4. *M. globiflora*
10. Flowering pedicels glabrous; leaves with intermediate tertiary venation, glabrous. 11

11. Inner petals with conspicuously attenuate apex, leaves spatulate; stamens in 3-4 rows (eastern Tanzania). 6. *M. hastipetala*
11. Inner petals with acute to rounded or emarginated apex; leaves not spatulate; stamens in more than 5 rows. 12

12. Inner petals with hairs 2-3 mm long on inner surface; outer petals crisped. 3. *M. crispata*
12. Inner petals glabrous on inner surface; outer petals undulate. 13

13. Sepal margins glabrous, undulate (West-Central Africa; western Tanzania). 1. *M. angolensis*
13. Sepal margins hairy, straight (eastern Tanzania and Mozambique). 2. *M. carolinae*

II. Key to Fruiting Specimens

1. Fruits smooth, not ribbed or inconspicuously ribbed. 2
1. Fruits rugose or conspicuously ribbed. 9

2. Leaves cordate at base. 5. *M. grandidieri*
2. Leaves acute to rounded at base. 3

3. Leaves with conspicuous parallel tertiary venation, leaves generally hairy on both sides. 4. *M. globiflora*
3. Leaves with intermediate tertiary venation, leaves glabrous. 4

4. Pericarp > 4 mm thick. 5
4. Pericarp < 3 mm thick. 7

5. Fruits ovoid, surface farinose, drying light brown. 13. *M. undulata*
5. Fruits globose, surface not farinose, drying black. 6

6. Fruiting pedicels 5-25 cm long, > 1 cm in diameter, inconspicuously finely ribbed. 10. *M. myristica*
6. Fruiting pedicels < 5 cm long, < 0.6 mm in diameter, smooth. 12. *M. tenuifolia*

7. Young leaves and branches covered with a blue-greyish wax layer; fruiting pedicels > 5 cm long, < 3 mm in diameter. 9. *M. minor*
7. Young leaves and branches without a blue-greyish wax layer; fruiting pedicels < 5 cm long, > 3 mm in diameter. 8

8. Fruits > 3 cm long when ripe. 7. *M. junodii*
8. Fruits < 3 cm long when ripe. 6. *M. hastipetala*

9. Fruits rugose or irregularly ribbed. 10
9. Fruits conspicuously 6- to 7-ribbed. 11

10. Fruits ellipsoid, rugose. 11. *M. stenopetala*
10. Fruits conic, irregularly ribbed. 1. *M. angolensis*

11. Pericarp < 2 mm thick, with thin and not clearly raised ribs; seeds with thickened raphe. 8. *M. laurentii*
11. Pericarp > 2 mm thick; with broad and clearly raised ribs; seeds with undifferentiated raphe. 3. *M. crispata*

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1. Monodora angolensis Welw., Ann. Conselho. Ultramario 587. 1859. — TYPE: ANGOLA. Malanje: Pungo Andongo, May 1855, *F.M.J. Welwitsch 774* (holotype: LISC, isotypes: BM!, COI!, G!, K!). *Figure 6.27 A-E*

Monodora durieuxii Wild., Études Fl. Bas-et Moyen-Congo 1: 122. 1903. — TYPE: DEMOCRATIC REPUBLIC OF CONGO. Equateur: Wangata, 14 January 1896, *A. Dewèvre 613* (holotype: BR!, isotypes: BR! 2 sheets).

Monodora le-testui Pellegrin, Bull. Soc. Bot. France 94: 386. 1947. — TYPE: GABON. Ogooué-Lolo: Lastoursville, April 1929, *G.M.P.C. Le Testu 7222* (holotype: P! isotypes: BM!, BR!, LBV!, LISC!, P!).

Monodora louisii Boutique, Bull. Jard. Bot. État Brux. 21: 97. 1951. — TYPE: DEMOCRATIC REPUBLIC OF CONGO. Orientale: Yangambi, 15 November 1937, *J. Louis 6612* (holotype: BR!; isotypes: B!, C!, MO!, NY!, P!, WAG!). ***syn. nov.***

Monodora brevipes auct. non Bentham, Tisserrant & Sillans, Not. Syst. 15 : 325. 1958.

Monodora gibsonii Bullock ex Eggeling, Burtt Davy and Bolton, Check-Lists For Trees & Shrubs Brit. Empire No. 1, Uganda 20. 1935. *nomen nudum*.

Tree to 20 m high; trunk with d.b.h. up to 80 cm; outer bark ash-grey to black, vertically shallowly furrowed; young branches drying black, glabrous; old branches dark grayish or black with white lenticels, glabrous. *Petioles* 2-8(-10) mm long, ca. 1 mm in diameter, glabrous, leaf lamina inserted on side, grooved adaxially. *Leaf lamina* (4-)6-15(-20) cm long, 2-6(-7.5) cm wide, length:width ratio 1.8-3.5, narrowly obovate to obovate or narrowly elliptic to elliptic, base cuneate to obtuse, apex acuminate, acumen 0.5-1 cm long, coriaceous to papyraceous, glabrous; midrib glabrous on both sides; secondary veins 8-12(-16) pairs, uniformly curving upwards, glabrous. *Flowers* single, leaf opposed, pendulous. *Flowering pedicels* (8-)20-40(-80) mm long; 0.5-0.8 mm in diameter, glabrous, light green. *Upper bract* subapical or apical, 4-12(-17) mm long, 3-12 mm wide, length:width ratio 1-2.5, very broadly ovate to narrowly ovate, base decurrent, apex attenuate, glabrous, tinged red-brown, greenish towards the base, margins undulate. *Sepals* 4-10(-15) mm long, 2-6 mm wide, length:width ratio 1.5-3, narrowly ovate to ovate, base truncate, apex acute, glabrous, green specked with red and purple, curved upwards at anthesis, falling in fruit, margins undulate. *Outer petals* (17-)20-40(-50) mm long, 10-30 mm wide, 1.5-2.5, oblong to ovate, base truncate, apex acute, glabrous, arching over receptacle, white at base, medium red brown with pale yellow spots towards apex, margins undulate. *Inner petals* 4-11 mm long, 5-16 mm wide, length:width ratio 0.4-1.4, clawed cordate to triangular or rhomboid, base truncate to cordate, apex acuminate to rounded or emarginated, glabrous except for the margins, white tinged with yellow minutely purple mottled along the margins, connivent by the tips arching over the receptacle, margins straight, upper part densely hairs with short curly intermingled hairs; claw 3-9 mm long, 2-6 mm wide, claw:inner petal ratio 0.7-2, glabrous, white. *Receptacle* 3-5 mm in diameter, flat or slightly convex. *Stamens* in 9-11 rows, 0.7-1 mm long; connective shield ca. 0.2 mm long, glabrous, those of innermost whorl extending slightly over ovary wall. *Ovary* 1-1.5 mm long, ca. 0.8 mm wide; stigma 0.9-1.2 mm in diameter, glabrous. *Fruiting pedicels* 25-65(-85) cm long, 5-7 mm in diameter, woody, glabrous. *Fruits* 3.5-5.5 cm long, 3.5-4 cm wide, ovoid or rarely globose, apex acute to apiculate, apicule ca. 5 mm long, irregularly and grossly ribbed, glabrous, green with white spots; pericarp 3-4 mm thick. *Seeds* 9-13 mm long, 5-8 mm wide, transversely ellipsoid, packed in a white pulp; testa smooth, brown; raphe not thickened, dark brown; hilum 4-5 mm long, 1-1.5 mm wide, elliptical.

Distribution: Widespread throughout tropical Central Africa east to Uganda, western Tanzania and northern Zambia, one collection from Ivory Coast (see Map 21); in primary, secondary and montane rain forests, gallery forest and sometimes in dry forests, at 0-1800 m altitude.

Phenology: Mature flowers and fruits collected throughout the year.

Vernacular names:
Central African Republic: Nzingodengwe (lissango).

Democratic Republic of Congo: Bamba, Ingambule (Nouvelle-Anvers); Bobusu (Banzyville); Bofafwa (dial. kundu); Bfumba (ikelemba; Boma-nzanga (dial. banza); Bompimpimbo (dial. m'bole); Boniningo, Onininго (dial. turumbu); Bonjungola (Bokuma); Divinia (Luki); Djala, Lidjale, Mondjali (Likimi); Ifafua, Mongangila (Eala); Kabaya (dial. swahili); Ngapet (dial. azande); Sefufoi (dial. fiote); Wingola (Bolima)

Uses: Wood used to make tools and for indigenous lighters. Leaves used to prepare infusions for diarrhea and constipation. Squashed fruits used as shampoo or soap.

IUCN conservation status: LC. *Monodora angolensis* is probably a fairly common species considering the fact it is very well represented in herbaria. Moreover, it is widely distributed in Central/East Africa. It has been collected from three national parks (Lopé-Okanda and Doudou Mountains in Gabon; Manovo-Gounda St Floris in Central African Republic) and in one forest reserve (Budong in Uganda). The category “least concern” is recommended.

Notes: *M. louisii* is lumped into *M. angolensis* because the only morphological difference is the fruit which is ovoid in *M. angolensis* and globose in *M. louisii* but this character can be variable as it would seem to be directly linked to the number and the position of the ovules that were fertilized.
Monodora angolensis is a widely distributed species, and thus is quite polymorphic in its dimensions which explains the fairly large number of synonymized species names. The shape of the inner petals is also variable, the apex varying from acuminate to emarginate or even cordate. However, it is distinct from the other species of *Monodora* by the following unique combination of characters: glabrous leaves and branches, flat or weakly convex receptacle, clawed, non-undulated and glabrous inner petals. It appears closely related to *M. crispata*, the latter having crisped inner and outer petals, as well as long hairs on the inside of the inner petals. Molecular data indicate that this species is sister to the rest of the monophyletic West African species group.
The notes on the BR specimens all indicate that the flowers have a very strong scent, similar to that of a violet. Some specimens indicate that *M. angolensis* grows on ant nests.

Extra references: Welwitsch, Trans. Linn. Soc. London 27: 10, Fig. 1. 1869; Hiern, Catalogue of African Plants collected by F. Welwitsch 13. 1853-1861; Oliver, Fl. Trop. Afr. 1: 38. 1868; Engler & Diels, Monogr. Afrik. Pflanzen-Fam. 6: 88, Fig. 29C. 1901; Boutique, Fl. Congo Belge 2: 265. 1951; Tisserant & Sillans, Not. Syst. 15: 324. 1958; Robson, Fl. Zamb. 1: 148, Fig. 16D. 1960; Le Thomas, Fl. Gabon 16 : 346. 1969; Verdcourt, Fl. Trop. E. Afr., Annonaceae 119. 1971.

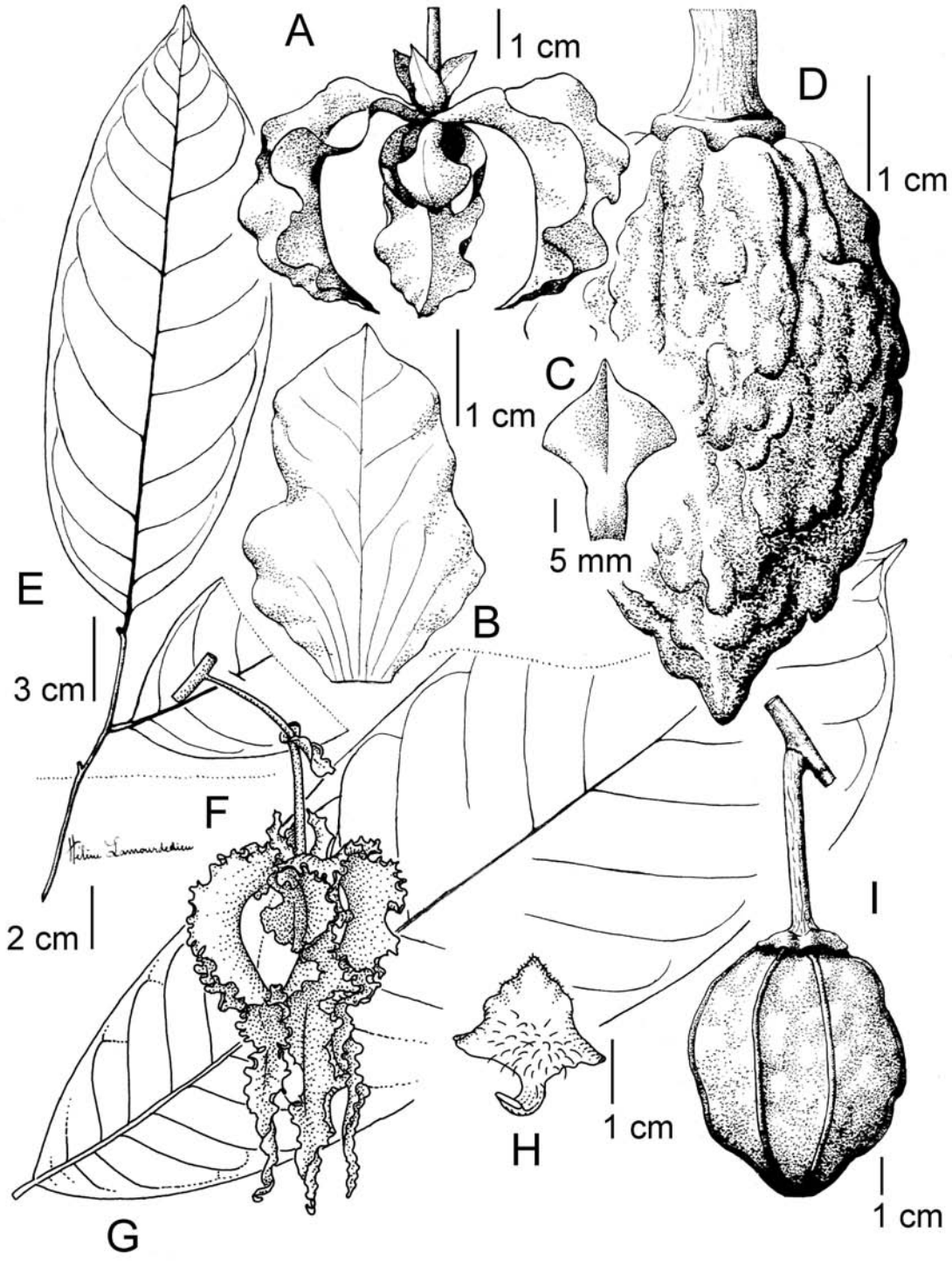
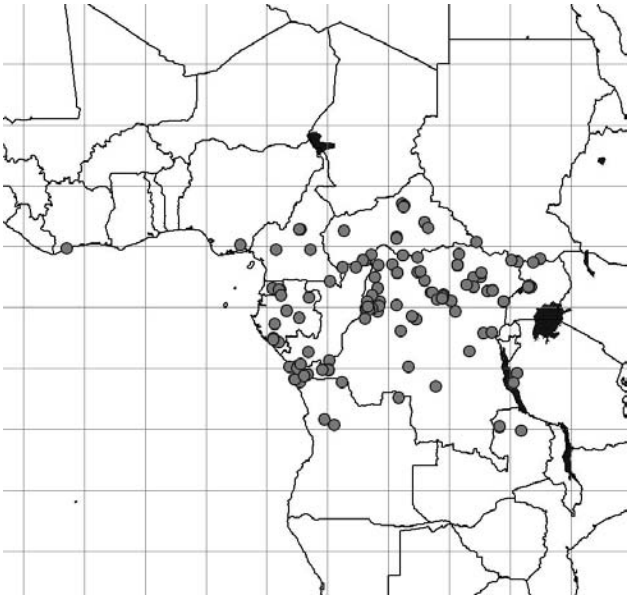


Figure 6.27. *Monodora angolensis*. A. Flower. B. Outer petal. C. Inner petal. (inside vierw). D. Fruit. E. Leaf. *Monodora crispata*.. F. Flower. G.Leaf. H. Inner petal (inside surface). I. Fruit. Modified from La Flore du Gabon, Le Thomas, Fig. 64. 1969. F and H drawn by Hans de Vries.



Map 21. Distribution of *Monodora angolensis*.

ADDITIONAL SPECIMENS EXAMINED:

ANGOLA: Cabinda. Chitumba, Cabinda, November 1921, *M.T. Dawe 301* (K); Cabinda, 3 December 1915, *J. Gossweiler 6064* (BM, COI, K, LISJC, LISU); Maiombe, Pango Mongo, 11 January 1916, *J. Gossweiler 6125* (COI); Maiombe, belize, junto ao rio Luali, 400 m, 9 December 1918, *J. Gossweiler 7607* (COI, K); Maiombe, Hombe pr Caio, 15 January 1919, *J. Gossweiler 7685* (COI, K); Maiombe, Buco Zau, no date, *J. Gossweiler 7198* (COI); Maiombe, Buco Zau, no date, *J. Gossweiler 7302* (COI); **Cuanza Norte**. Cazengo, no date, *J. Gossweiler 735* (BM, K); Golingo Alto, 500 m, December 1855, *F.M.J. Welwitsch 776* (B, BM, C, COI, G, K); **Lunda Norte**. Dundo, riv. Luachimo, 9 October 1946, *J. Gossweiler 13706* (B, K, US); **Malanje**. Pungo Andongo, 600 m, November 1855, *F.M.J. Welwitsch 774* (B, BM, COI, G, K); **Uíge**. Entre Quimbele e Icoca, andados 60 km, 750 m, 26 August 1971, *F. Raimundo 842* (LUA, WAG); **Zaire**. Sumba, Peco, near river Zaire, 30 m, 11 October 1923, *J. Gossweiler 674* (K); Sumba, Peco, 1930, *J. Gossweiler 8674* (US).

CAMEROON: Central Province. Environ de Ndokalendé; 10 km SW de Ndikinimeki, 900 m, 4 March 1984, *B. Satabié 745* (P); **East Province**. 700 m N. of Koundi, 22 km N. of Bertoua, 670 m, 10 July 1978, *H.C. van den Burg 78* (WAG); Molunda Sation. Ngoko. Nginda 21 km north of Molundu, 9 November 1911, *G.W.J. Mildbraed 4199* (B, HBG); **North Province**. km 10 Tibati-Mabouka Road, 1000 m, 29 June 1972, *A.J.M. Leeuwenberg 10034* (BR, FHI, HBG, K, LISC, MO, P, PRE, UPS, WAG, YA); **Unknown.** Tibati, bord de lac, 8 November 1967, *H. Jacques-Félix 9101* (P, WAG); Tibati, 890 m, 1 February 1909, *C.L. Ledermann 2462* (B).

CENTRAL AFRICAN REPUBLIC: Bamingui-Bangoran. Small tributary of Malo creek 2 kmNW of Goubiche creek, 580 m, 20 March 1983, *J.M. Fay 4318* (C, K); Plants of the Manovo Gounda St Floris National Park. WWF inter Elephant Cons. Project. 18 km S of Pende-Koumbala confluence on Pende Creek, 640 m, 13 March 1984, *J.M. Fay 6435* (MO); Koumbala creek at Koumbala W and E confluence, 610 m, 8 April 1983, *J.M. Fay 4394* (K); Manovo-Gounda-St. Floris National park. WWf Inter. Elephant Cons. project. Koumbala creek 1 km douwnstream of Pende-Koumbala confluence, 590 m, 2 September 1983, *J.M. Fay 5629* (MO); **Haute-Kotto**. Haute-Kotto, région de Yalinga, 27 February 1922, *G.M.P.C. Le Testu 3793* (BM, P, WAG); Haute-Kotto, no date, *G.M.P.C. Le Testu 3883* (BM, K, P, US); Haute-Kotto, 9 November 1922, *G.M.P.C. Le Testu 4295* (BM, P, US); **Lobaye**. Foret du Baiki, Oubangui et Boganga, confluent de l Oubangui et du Congo, December 1916, *Fidao s.n.* (A, FHO, K, MO, P); Boukoko, 11 December 1947, *C. Tisserant 528* (P); Bord de la Ouaka, Bambari, 14 March 1922, *C. Tisserant 1600* (P); Oubangui, région de Bukoko, 4 January 1949, *C. Tisserant 1637* (P); Oubangui, région de Bukoko, 30 December 1949, *C. Tisserant 1647* (K, P); 17 km N de Bambiri, 23 March 1929, *C. Tisserant 1858* (P); Boukoko, 26 April 1951, *C. Tisserant 2093* (BR); 60 km E de Babari, 28 January 1928, *C. Tisserant 2393* (P); **Ouaka**. 4 km SE of Bambari on the Akndoa road on the west side of the road alond Bengue creek, 500 m, 27 January 1982, *J.M. Fay 2127* (K); **Ouham Pendé**. Region de Bozoum, Oubangui Chari (A.E.F.), 20 April 1933, *C. Tisserant 3298* (BM, P); **Unknown.** sur la Kémo (notes de Tisserant), 12 February 1892, *J. Dybowski 664* (P); Bessou, 26 December 1917, *C. Tisserant 146* (P).

DEMOCRATIC REPUBLIC OF CONGO: Bandundu. entre Libenge et Zongo, November 1930, *J. Lebrun 1670* (K); **Bas-Congo**. Luki, 6 December 1980, *H. Breyne 4066* (BR); Inkisi (Kisantu), Jardin Botanique, 18 September 1988, *H. Breyne 5685* (BR); Kisantu, 4 September 1948, *H. Callens 1805* (K); Zundu, riviere Mpioka, territoire de Thysville, 14 March 1960, *P. Compère 1678* (BR); Luki, 20 January 1949, *C. Donis 2331* (K); Luki, 28 February 1949, *C. Donis 2429* (MO); Vista, December 1958, *A. Flamigni 10507* (BR, L, WAG); Ineac-Luki en bordure de la road qui va a Nkakala, 31 July 1961, *D. Kuasa 32* (BR); Luki, 11 September 1904, *E. Lescrauwaert 182* (BR); Luki, vallée de la Makeueke, 28 November 1949, *E. Madoux 229* (K); Luki, road bloc 52, 14 February 1957, *J. Matton 33* (BR); Luki, vallée de la N'Kula, 9 December 1947, *L. Toussaint 63* (C, MO); Luki valle de Nkakala, 10 December 1947, *L. Toussaint 64* (BR); Luki, valle de la Minkudu,

9 January 1948, *L. Toussaint 132* (BR); Luki, Mayombe, 7 May 1948, *L. Toussaint 361* (BR, COI); Gimbi, 18 November 1948, *L. Toussaint 655* (BR); Gimbi, vallée de la Mouzi, 8 February 1949, *L. Toussaint 812* (K); Luki, Partie Nord du plateau du Poste, 20 April 1948, *L. Toussaint 2002* (BR); Luki, bordure de chemin du camp, 18 February 1948, *L. Toussaint 2182* (BR); Moanda, 13 November 1930, *H.J.R. Vanderyst 27623* (BR); Moanda, 14 November 1930, *H.J.R. Vanderyst 27670* (BR); Moanda, 14 November 1930, *H.J.R. Vanderyst 27675* (BR); Moanda, 14 November 1930, *H.J.R. Vanderyst 27678* (BR); Moanda, 15 November 1930, *H.J.R. Vanderyst 27784* (BR); Temvo, 4 April 1919, *F. Vermoesen 1936* (BR, FHO, K); Léopoldville, Bsna, Luki, 1 December 1954, *J. Wagemans 909* (BR, S, UPS); **Equateur**. Riv. Ikelemba (cours inf.), May 1913, *Bonnivair 48* (BR); Eala, 20 January 1932, *A. Corbisier-Baland 1408* (BR); Eala, 20 June 1932, *A. Corbisier-Baland 1548* (BR); Eala, 1930, *A. Corbisier-Baland 1588* (B, BR, G, K, WAG); Eala, 2 August 1932, *A. Corbisier-Baland 1664* (BR); Nouvelles Avers, February 1913, *S. De Giorgi 255* (BR); Nouvelles Avers, February 1913, *S. De Giorgi 316* (BR); Nouvelles Avers, July 1913, *S. De Giorgi 1169* (BR); Likimi, November 1913, *S. De Giorgi 1482* (A, BR, US); Bas Uele, 8 March 1935, *A. De Wulf 771* (BR); Wangata, 14 January 1896, *A. Dewèvre 613* (BR); Lac Kwada, 23 March 1955, *C.M. Evrard 537* (BR); Station Ineac Boketa, 29 April 1955, *C.M. Evrard 853* (BR); Emenyeye, Monkoko, 20 October 1957, *C.M. Evrard 2865* (BR, FHO, K); Djoa, 15 October 1958, *C.M. Evrard 5021* (K); Baringa, 30 October 195, *C.M. Evrard 5130* (K); Bokote, 6 April 1959, *C.M. Evrard 6024* (BR); Flandria [Boteka], 25 June 1936, *J.H.P.A. Ghesquière 2834* (A, B, BR, K); Eala, 30 June 1936, *J.H.P.A. Ghesquière 2845* (A, B, BR, K, U); Bokuma, July 1936, *J.H.P.A. Ghesquière 3344* (BR); Moma, November 1952, *S. Gorbatoff 141* (BR); Bokuma, 5 March 1941, *G. Hulstaert 143* (BR); Bokuma, 1 February 1942, *G. Hulstaert 698* (BR); 13 January 1953, *G. Hulstaert 1597* (BR); Yaligimba. Bumba zone, February 1988, *C. le Jeune 28* (BR, K, WAG); Basoko, 27 January 1896, *E. Laurent s.n.* (BR); Jardin Botanique d'Eala, 1 December 1905, *E. Laurent 1149* (BR, US); Eala, June 1906, *E. Laurent 1624* (BR); Coquilhatville, 1906, *E. Laurent 1625* (BR); Eala, September 1930, *J. Lebrun 109* (K); Eala, August 1930, *J. Lebrun 939* (BR, MO, U); Eala, August 1930, *J. Lebrun 1091* (BR, L, WAG); Wendji, August 1930, *J. Lebrun 1123* (BR, G, WAG); between Libenge and Gemena, December 1920, *J. Lebrun 1763* (BR); Banzyville, January 1931, *J. Lebrun 2080* (BR, L, WAG); Eala, January 1933, *J. Lebrun 6801* (BR, WAG); Banzyville, February 1931, *J. Lebrun 2234* (BR, WAG); Eala, November 1936, *S. Leeman 20437* (BR); Mondjo sur Ikelemba, 7 September 1946, *J.J.G. Léonard 537* (COI, K); entre Coq. et Ikefo, environt de Eala, 15 November 1946, *J.J.G. Léonard 1018* (FHO); Ter. Bomboma; village de Bobo's, 30 April 1938, *C. Leontovitch 74* (BR); Entre Lolifa et Bamania, au Sud de Eala, 30 May 1936, *J. Louis 2100* (BR); Environs de Likimi, 10 February 1910, *L. Malchair 34* (BR); Likimi, 10 November 1910, *L. Malchair 464* (K); Dundusana, September 1913, *M.G. Mortehan 498* (BR); Kimbinga (Bandundu), 10 September 1982, *Mwanza Zenga 5* (BR); Lolungu, 1920, *A. Nannan 158* (BR, WAG); Eala, December 1905, *L.A. Pynaert 328* (BR); 15 October 1906, *L.A. Pynaert 561* (BR);1 February 1907, *L.A. Pynaert 1098* (BR); Eala, July 1907, *L.A. Pynaert 1538* (BR); Lac Tumba. Irsac Mabali. Terr. Bikoro, 350 m, 18 September 1958, *J. Thonet 249* (BR, WAG); Monbongo (Mongala), 10 November 1909, *F. Thonner 168* (BR); Jardin botanique d'Eala, 30 April 1919, *F. Vermoesen 2085* (BR, G); Eala, 8 May 1919, *F. Vermoesen 2153* (BR); Eala, 12 May 1919, *F. Vermoesen 2201* (BR); **Kasai**. Bena-Longo, 18 June 1959, *R. Dechamps 147* (BR, WAG); **Katanga (Shaba)**. Muala, 23 September 1981, *Nsimundele 916* (BR); **Maniema**. Zone de Mambasa (Ituri Forest), Epulu Collection Terese Butler Hart, 750 m, 29 January 1993, *T.B. Hart 1448* (MO); **Nord-Kivu**. Entre Beni et la riviere Zori, 1000 m, November 1931, *J. Lebrun 4384* (BR); Beni, January 1908, *G.W.J. Mildbraed 2158* (HBG); **Orientale**. Epulu, Zone de Mambasa, 750 m, 24 February 1997, *Amsini 51* (BR); Panga, 17 December 1913, *J.C.C. Bequaert 1503* (BR); Bafwankei (Bomili), 28 December 1913, *J.C.C. Bequaert 1663* (BR); Penghe, 27 January 1914, *J.C.C. Bequaert 2149* (BR); Penghe, 2 February 1914, *J.C.C. Bequaert 2249* (BR); Stanleyville, 25 February 1915, *J.C.C. Bequaert 6976* (BR); 10 km W of Kisangani, near Lindi River, 19 November 1971, *J. Bokdam 3382* (WAG, YBI); Bambesa et environs, 1933, *H.J.A.E.R. Brédo 772* (BR); 1933, *H.J.A.E.R. Brédo 704* (BR); Yangambi, 11 October 1950, *C. Donis 2905* (BR); Yangambi, 19 October 1951, *C. Donis 3154* (BR); Yangambi, 27 December 1952, *C. Donis 3268* (BR); Yangambi, 31 December 1951, Yangambi, *C. Donis 3277* (BR); Yangambi, 3 January 1952, *C. Donis 3304* (BR); Yangambi, 9 February 1952, *C. Donis 3612* (BR); Yangambi, 14 February 1952, *C. Donis 3652* (BR); Yangambi, 30 January 1952, *C. Donis 3510* (BR); Bambesa, 194, *H. Dubois 993* (BR); Bambesa, 30 January 1952, *P. Gérard 133* (BR); Bambesa, 1 December 1952, *P. Gérard 499* (BR, M); Bambesa, 20 May 1954, *P. Gérard 1422* (BR); Bambesa, 7 November 1956, *P. Gérard 2495* (BR, UPS); Bambesa, 20 November 1956, *P. Gérard 2516* (BR, WAG); Bambesa, 8 January 1957, *P. Gérard 2604* (BR); Kurukwata (Aba), 14 December 1957, *P. Gérard 3656* (BR); Bambesa, 15 December 1959, *P. Gérard 4203* (BR, WAG); Digba-Ango. Foret des Akare entre riviere Bili et Asa, 6 December 1963, *P. Gérard 5608* (BR); Yanbambi, 19 November 1949, *R.G.A. Germain 5381* (BR); Bambesa, 8 July 1942, *G. Gilbert 562* (BR); Yangambi, 18 July 1944, *G. Gilbert 5793* (BR); Yangambi, no date, *G. Gilbert 8009* (BR); Yangambia, February 1950, *G. Gilbert 8153* (BR); Yangambi, 1944, *G. Gilbert 8160* (K); Yangambi, no date, *G. Gilbert 8205* (BR); Yanbambi, 1944, *G. Gilbert 8505* (K); Yangambi, no date, *G. Gilbert 8621* (BR); Yangambi, no date, *G. Gilbert 9815* (BR); Récolté sur l'arbre en observation, No 2918 à Yangambi, no date, *G. Gilbert 10219* (BR, K, WAG); no date, *G. Gilbert 10552* (BR); Barumbu, pres de Basoko, May 1922, *V. Goossens 1699* (BR); Zone de Mambasa (Ituri Forest) about 6 km. W of Epulu, 750 m, 26 January 1994, *T.B. Hart 1593* (MO, WAG); Zone de Mambasa (Ituri), 15 December 1981, *T.B. Hart 181* (K); Bamanga, 12 August 1972, *G. Hulstaert 1682* (BR, WAG); Yangambi, 8 January 1958, *A. Léonard 219* (BR, CAH, M); Yanonghe (environ de Yanbambi), 31 December 1947, *J.J.G. Léonard 1603* (BR); à 6 km de Yangambi, 470 m, 27 January 1937, *J. Louis 3178* (B, BM, BR, C, K, MO, NY, P, US); Yangambi, plateau de l Isalowe, piste de Yaselia, 17 August 1937, *J. Louis 5808* (BR); Yangambi, à 5 km nord du fleuve, 15 November 1937, *J. Louis 6612* (B, BR, C, FHO, MO, NY, P, WAG); Ngazi, au bord des chutes de la Lolembo, 29 January 1938, *J. Louis 7744* (BR, NY); 20 km en amont de Yangambi, 470 m, 21 March 1938, *J. Louis 8573* (BR, US); Yangambi, 470 m, 28 July 1938, *J. Louis 10555* (B, BR, K, MO); Uele, environ de Bambesa, 1936, *R. Pittery 160* (BR); Uele, environ de Bambesa, 1936, *R. Pittery 165* (BR); Uele, environ de Bambesa, 1936, *R. Pittery 170* (BR); Uele, environ de Bambesa, 1936, *R. Pittery 171* (BR); Environ de Stanleyville, February 1932, *A. Robyns 1377* (BR); Stanleyville, 1926, *F.H.E.A.W. Robyns 1377* (BR, FHO, K); Sur Nala, 1907, *F. Seret 761* (BR); Mayumbe, 11 June 1947, *L. Toussaint 2378* (BR, BRLU,

FHO, K, WAG); **Sud-Kivu**. km 83, route Walungu-Shabunda. Terr. Kabare, 10 August 1958, *A.R. Christiaensen 2459* (BR, K, WAG); Kampene, territoire de Pangi, 7 August 1959, *A. Léonard 5619* (BR); Ironga, terr. Kalehe, 950 m, 24 August 1955, *R. Pierlot 774* (BR); **Unknown**. Eala, 30 June 1936, *GhenguiŠre 2845* (U); Evlo, September 1921, *Karmann s.n.* (L); Eala, August 1930, *J. Lebrun 939* (U); **Unknown.** Benite Benite, no date, *J. de Briey 185* (BR); Environs de Dimonika; road de voula, 24 December 1982, *C. Cusset 1303* (WAG); Moamro (?), January 1907, *J. Gillet s.n.* (BR); Ter. Banzyville; Bokusu (Bobusu?), 8 December 1938, *C. Leontovitch 129* (BR); Région de Yanama, Chefferie Lité, 13 April 1939, *C. Leontovitch 144* (BR); Beni, January 1908, *G.W.J. Mildbraed 2208* (HBG); Lusholela (?), 11 July 1906, *L.A. Pynaert 248* (BR); no location, January 1899, *H. Tilman 64* (BR).

EQUATORIAL GUINEA: Unknown. Region d'Anisok, au dessus du village Nzuamayong, 11 September 1997, *S. Lisowski m 715* (BRLU); Inselberg de Piedra Nzas, a 6h de marche du village d'Ascoaseng, a 9 km d'Aconibe, ou celui de Afaanam, 650 m, 27 May 1999, *N.S. Nguema Miyono 506* (BRLU); Inselberg de Bikurga, près du village de Bicurga (face est 120 deg. de l'inselberg), 22 May 2002, *T.O.B.E.B. Stévart 1517* (BRLU).

GABON: Ngounié. Between Yombi and Fougamou, eastern slope of Koumounabouali ridge, 22 September 1997, *F.J. Breteler 14057* (WAG); **Nyanga**. Tchibanga, February 1914, *G.M.P.C. Le Testu 1697* (BM, K, P); Ca 15 km au O.S.O. de Doussala, 545m, 29 May 2000, *M.S.M. Sosef 1576* (LBV, WAG); chantier SFN, 340 m, 2 December 2003, *J.L.C.H. van Valkenburg 2688* (BR, K, LBV, MO, P, WAG); **Ogooué-Ivindo**. 11 km E of Batouala, 15 October 1964, *N. Hallé 2628* (LBV, P); South of Lopé, near SEGC site, in NE of Lopé-Okanda Reserve, 200 m, 11 January 1993, *G.D. McPherson 16025* (B, G, L, LBV, MO, UPS, US, WAG); **Ogooué-Lolo**. Lastoursville, April 1929, *G.M.P.C. Le Testu 7222* (BM, BR, LBV, LISC, P); **Ogooué-Maritime**. Monts Doudou, campagne, 270 m, 19 September 2000, *H.P. Bourobou 322* (BR, LBV, MO, P, WAG); **Woleu-Ntem**. Inselberg de Ntan (Bikougou), à 1h30 de marche du village de La Hollande (à 2 km de Sam), 790 m, 22 January 2000, *I. Parmentier 781* (BRLU, LBV).

IVORY COAST: San Pedro. F.C. Monogaga, just N of Sassandra-San Pedro road, 24 March 2000, *C.C.H. Jongkind 4697* (WAG).

NIGERIA: Akwa-Ibom State. Road from Uyo to Ikot Ekpene. Along the road, about 10 km from Uyo, 7 April 1971, *P.P.C. van Meer 1213* (WAG); **Unknown.** W. boundry of Ojogba-Ugun Forest Reserve, 10 June 1958, *J. Olorunfemi FHI 38063* (BR).

REPUBLIC OF THE CONGO: Lekoumou. Sibiti, foret Tilé, embranchement a gauche sur la road de Sibiti à Mouyondzi (5 km), 10 February 1965, *A. Bouquet 1233* (K, WAG); **Pool**. Djoumouna, 18 November 1990, *F. Malaisse (1990 series) 2635* (BR, WAG); **Sangha**. 8 km ENE of Kabo, 6 February 2007, *D.J. Harris 8798* (E, WAG); **Unknown.** Loango, 20 November 1874, *H. Soyaux L 168* (K, M).

SUDAN: Eastern Equatoria. Lotti, 1219m, 5 February 1929, *T.F. Chipp 16* (K); S.W. Equatorial Province, Aloma Plateau, +- 1 mile from Iwatoka, 23 March 1939, *A.C. Hoyle 829* (FHO, K); **Western Equatoria**. Li Yubu, Zande District, 27 May 1939, *J.G. Myers 11383* (K).

TANZANIA: Rukwa. Tongwe forest, Issa River Ibalampasi Hill, NE of camp, 1300 m, 25 December 2002, *Y.S. Abeid 1419* (MO); Tongwe forest, Issa River Ibalampasi Hill, NE of camp, 1360 m, 25 December 2002, *Y.S. Abeid 1427* (MO); Mwese Hill, 1828m, 21 May 1975, *J. Kahurananga 2623* (EA, M, WAG).

UGANDA: Northern Province. Stream bank between enclaves Otze, 1371m, 30 January 1964, *Oakley, J.S. 28* (EA); **Western Province**. Budongo forest Reserve, Nyakafunjo Block, c.a. 2.5 km N of Nyabyeya Forestry College, 1080 m, 15 June 1998, *African tropical Biodiversity Program 433* (MO); Budongo forest Reserve, Nyakafunjo Block, c.a. 3.0 km N of Nyabyeya Forestry College, 1060 m, 18 June 1998, *African tropical Biodiversity Program 562* (MO); Budongo Foesrt, 1000 m, 15 February 1907, *A.G. Bagshawe 1490* (MO); 100 m east of Coupe 5 of the south Nyakaifunje block, on old road to budongo gombolola, 1650 m, 3 January 1951, *H.C. Dawkins 690* (EA, FHO, K, S); Budongo Forest, Bunyoro, no date, *W.J. Eggeling 3066* (K); Budongo Forest, February 1930, *Gibson, R.D. 16* (K); Bodongo Forest, Masindi, no date, *Gibson, R.D. 98* (FHO); Budongo, 1060 m, February 1933, *Harris, C.M. 179* (A, EA, FHO); Budongo Forest, Bunyoro, behind Kasokwa state, 1939, *J. Jardine S 555* (K, S); Budongo Forest, 18 December 1940, *J.G. Myers 3620* (K); Budongo Forest Reserve, 1000 m, September 1995, *D.N. Nkuutu BN95U 217* (C); Budongo Forest Reserve, Biso block, compt 2, 26 September 1962, *B.T. Styles 81* (K); Budongo Forest, east of Busingire, 1150 m, 10 December 1970, *T.J. Synnot, 495* (K); **Unknown.** no location, 2 May 1910, *M.T. Dawe 993* (K); no location, no date, *W.J. Eggeling 1154* (FHO); no location, 1935, *W.J. Eggeling 1599* (F, FHO).

UNKNOWN: cultivated in National Botanic Garden, Meise, 17 October 2005, *D. Alpin S 4013* (WAG).

ZAMBIA: Luapula. Mukabi, Kawambwa, 6 December 1961, *R.M. Lawton 815* (FHO); **Northern Province**. Kawambwa, 28 February 1957, *D.B. Fanshawe 3499* (EA, K); Chishimba Falls, 10 September 1958, *D.B. Fanshawe 4776* (K).

2. Monodora carolinae Couvreur, Adansonia 28: 247. 2006. — TYPE: TANZANIA. Pwani: Matumbi Hills, Kiwengoma Forest, 08°08´34´´S, 38°59´56´´E, 18 October 1997, *P.B. Phillipson 4940* (holotype: C!; isotypes: P!, MO!). *Figure 6.28*

Tree to 6 m tall; trunk with d.b.h. up to 15 cm; bark grey, striate, with white lenticels; young branches drying black, glabrous or sometimes sparsely covered with short appressed hairs. *Petioles* ca. 4 mm long, ca. 0.8 mm in diameter, glabrous, leaf lamina inserted on the side, broadly grooved adaxially. *Leaf lamina* 8-10 cm long, 4-6 cm wide, length:width ratio 2.5-3, narrowly elliptic to elliptic, base cuneate, apex acuminate, acumen 1-2 cm long, glabrous adaxially, abaxial surface glabrous or sparsely covered with short appressed hairs in younger leaves, papyraceous; midrib glabrous, rarely sparsely hairy when young on both sides; secondary veins 9-11 pairs, uniformally curving upwards, glabrous. *Flowers* single, leaf-opposed, pendulous. *Flowering pedicels* 15-35 mm long, ca. 0.5 mm in diameter, glabrous. *Upper bract* inserted in upper half of pedicel, curving upwards, 4-6 mm long, 6-9 mm wide, length:width ratio 1-2, very broadly ovate to ovate, base auriculate-clasping, apex acute, glabrous on both surfaces, the margins straight with straight white hairs. *Sepals* 6-12 mm long, 4-8 mm wide, length:width ratio ca. 1.5, triangular to ovate, base truncate, apex rounded, glabrous on both surfaces, green, reflexed, margins straight, densely covered with short curly hairs. *Outer petals* 15-25 mm long, 6-12 mm wide, length:width ratio 1.5-2.5, oblong to elliptic, apex acuminate and reflexed upwards when at anthesis, both surfaces glabrous, margins undulate, sometimes very sparsely covered with short curly hairs, creamy yellow with red spots on both sides. *Inner petals* 6-8 mm long, 6-14 mm wide, length:width ratio 0.6-0.5, clawed ovate, base obtuse, apex acuminate, both surfaces glabrous, same color as outer petals, connivent by tip over receptacle, margins straight, densely covered with curly short hairs; claw 3-5 mm long, claw:inner petal ratio 0.6-0.8, glabrous. *Receptacle* ca. 5 mm in diameter, flat to slightly convex. Stamens in 6-7 rows, ca. 0.8, mm long, connective shield ca. 0.1 mm long, covered with short straight white hairs, those of inner whorl not elongated over ovary wall. *Ovary* 1-2 mm long, ca. 1 mm in diameter; stigma ca. 2 mm in diameter, glabrous, light yellow. *Fruits* unknown.

Distribution: South-eastern Tanzania and north-eastern Mozambique (see Map 22); in moist semi-deciduous coastal forests, on deep leached sandy soils; at 50-800 m altitude..

Phenology: Mature flowers collected from October to December.

IUCN conservation status: EN B1ab(iii)+2ab(iii). *Monodora carolinae* is represented by only seven collections, none of them from protected areas. Most of the collections come from the Kiwengoma forest in the Matumbi hills. This region is not protected and suffers from intensive fire wood logging. Some collections were made recently (2006). The category “endangered” is therefore appropriate.

Notes: *Monodora carolinae* is characterized by its small flowers, hairy sepal margins, flat receptacle and glabrous pedicel. It would appear from field observations to grow in sympatry with *M. minor*. However, molecular data indicate that this species is most closely related to *M. stenopetala*.

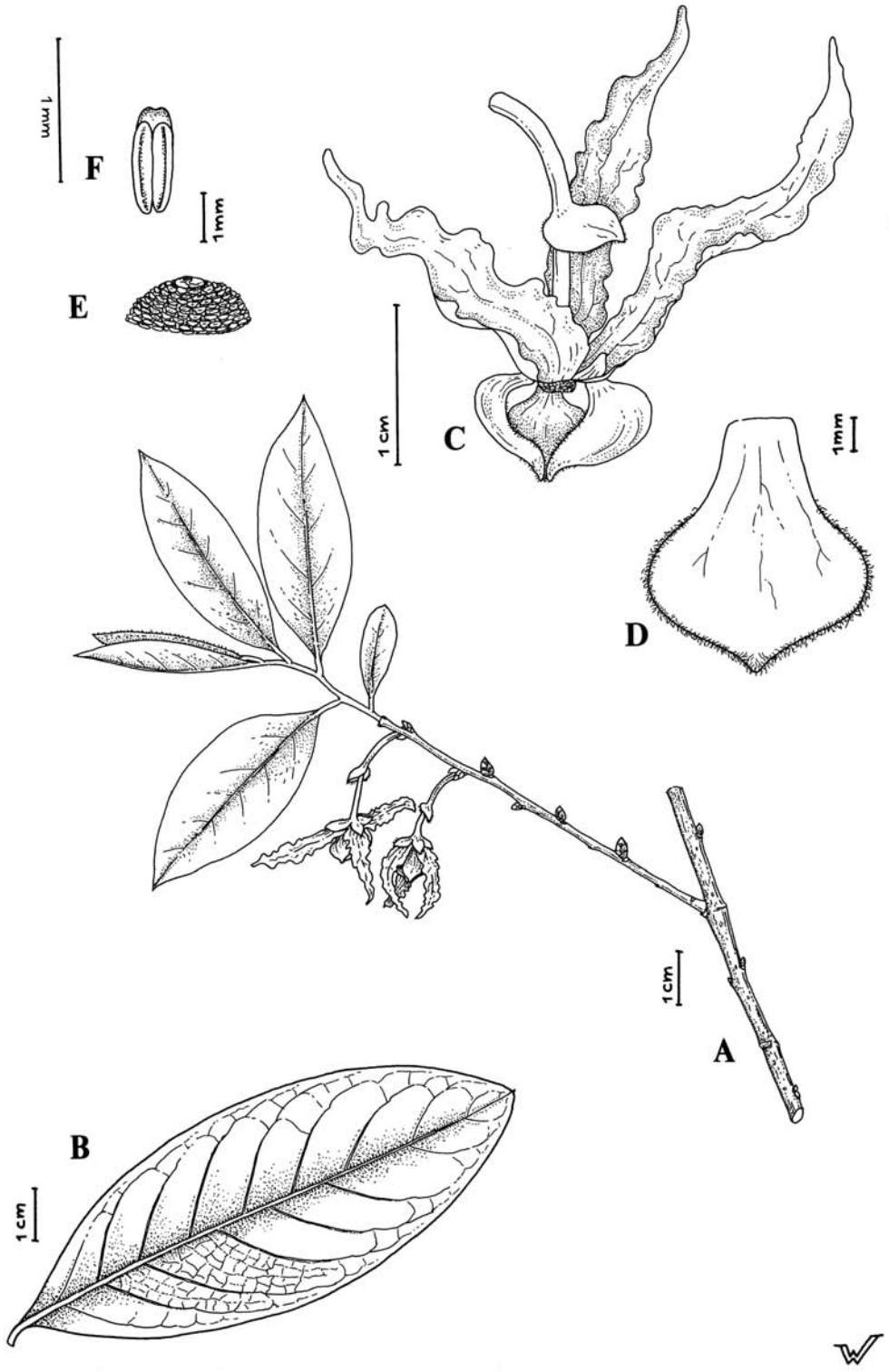


Figure 6.28. *Monodora carolinae.* A. Flowering branch. B. Leaf. C. Mature flower. D. Detail of inner petal (inside surface). E. Androecium (stigma missing). F. Stamen. Drawings by Wil Wessel-Brand.

Map 22. Distribution of *Monodora carolinae.*

ADDITIONAL SPECIMENS EXAMINED:
MOZAMBIQUE: Cabo Delgado. Mueda Plateau, Pt 509, 790 m, 12 December 2003, *W.R.Q. Luke 10054* (EA, K, LMA, MO, NHT).
TANZANIA: Lindi. Mchinjiri, Rondo plateau, 82m, November 1951, *W.J. Eggeling 6406* (FHO, K, S); **Pwani**. Matumbi Hills. Nambunju village. Foret patch under the old WWF house, 260 m, 18 November 2006, *T.L.P. Couvreur 54* (DSM, K, MO, WAG); Matumbi Hills. Nambunju village, on path just before 200 m before arriving to the old WWF house, 250 m, 18 November 2006, *T.L.P. Couvreur 57* (DSM, K, MO, NHT, WAG); Matumbi Hills. On slope just above Kitapi village, 5 km from Mbwara, 230 m, 19 November 2006, *T.L.P. Couvreur 65* (DSM, MO, WAG); Matumbi Hills, along raod to Kiwengoma Forest, 250 m, 18 October 1997, *P.B. Phillipson 4940* (C, MO, P); Rufiji, 375m, 19 October 1997, *P.B. Phillipson 4946* (C, MO).

3. Monodora crispata Engl., Notizbl. Bot. Gart. Berlin-Dahlem 2: 301. 1899. — TYPE: CAMEROON. South-Province: 9 km N. of Kribi, 2 February 1970, *J.J. Bos 6224* (neotype, designated here: WAG!, isotypes: BR!, C!, K!, LD, LISC!, LMA, MO!, P!, PRE, UPS!, WAG-2 sheets!, YA!). *Figure 6.27 F-I*

Monodora klaineana Pierre, manuscript name; Engler, 1901, pro syn.

Monodora crispata var. *klaineana* Engl., Monogr. Afrik. Pflanzen-Fam. 6: 90. 1901. — TYPE: GABON. Estuaire: Libreville, 14 January 1899, *T.J. Klaine 1435* (lectotype, designated here: P!)

Monodora angolensis auct. non Welw.: Oliv., Fl. Trop. Afr. 1 : 38. 1868.

Tree to 20 m high, sometimes leaning and giving a lianescent appearance; trunk with d.b.h. up to 30 cm; outer bark dark brown with vertical lenticels; young branches drying black, glabrous; old branches brown-blackish, glabrous. *Petioles* 3-7 mm long, 1-1.5 mm in diameter, leaf lamina inserted on side, narrowly grooved adaxially. *Leaf lamina* 5-10(-17) cm

long, 2.5-5(-6) cm wide, length:width ratio1.8-3.2, narrowly obovate to obovate or narrowly elliptic to elliptic, base rounded to acute, apex acuminate, acumen 3-10 mm long, membranous when young to coriaceous, glabrous, dark green; midrib adaxially raised becoming sunken towards the base, glabrous, prominent and glabrous abaxially; secondary veins 9-13 pairs. *Flowers* single, leaf opposed, sometimes extra axillary, pendulous. *Flowering pedicels* 20-50 mm long, 0.9-0.7 mm in diameter, glabrous, dark green. *Upper bract* inserted centrally or subapically on the pedicel, 6-15 mm long, 5-9 mm wide, length:width ratio 2-3, ovate to narrowly ovate, base decurrent, apex acute, glabrous on both surfaces, green, margins undulate, sparsely covered with curly hairs to glabrous. *Sepals* (5-)9-18 mm long, 3-6 mm wide, length:width ratio 2-3.6, narrowly ovate to ovate, base truncate, apex acute, glabrous on both surfaces, green, reflexed upwards, falling in fruit, margins undulate to crisped, glabrous. *Outer petals* 35-70 mm long, (6-)10-20 mm wide, length:width ratio 2-6.5, narrowly oblong to oblong, base truncated, apex attenuate, arc-shaped in the lower part, both surfaces glabrous, white or yellow at base then yellow with red-brown tiger markings, strongly crisped, margins strongly crisped, glabrous. *Inner petals* 4-17 mm long, 6-20 mm wide, length:width: ratio 0.5-1.5, clawed triangular to cordate, base cordate, apex acute, connivent by the margins, sparsely to densely covered with 2-3 mm long straight hairs inside, glabrous outside, white to yellow with red streaks towards margins, margins crisped, densely covered with short curly hairs; claw 3-8 mm long, 1-3 mm wide, claw:inner petal ratio, 0.4-1.1, glabrous, bright yellow. *Receptacle* 3-4 mm in diameter, flat to slightly convex. *Stamens* in 9-11 rows, 0.5-1 mm long, connective shield ca. 0.2 mm long, densely covered with short erect hairs, white, those of innermost whorl not elongated over ovary wall. *Ovary* ca. 2 mm long, ca. 1.5 mm wide stigma ca. 1.5-2 mm in diameter, weakly capitate, sparsely covered with short erect hairs. *Fruiting pedicels* 3-5 cm long, 4-10 mm in diameter, woody, glabrous. *Fruits* 6-15 cm long, 3.5-5 cm in diameter, length:width ratio 1.5-2.5, conic, apex acute, conspicuously 6 to 7-ribbed, sometimes ribs separated halfway up the fruit, small secondary ribs present but disappearing upwards, smooth, glabrous, green-grey; pericarp 3-5 mm thick. *Seeds* 10-13 mm long, 5-9 mm wide, broadly ellipsoid, packed in white pulp; testa smooth, light brown; raphe not thickened, brown; hilum 4-5 mm long, 2-2.5 mm wide, elliptical.

Distribution: Sierra Leone to Gabon (see Map 23); in primary and secondary rain forests, along streams, on sandy soil; at 0-400 m altitude.

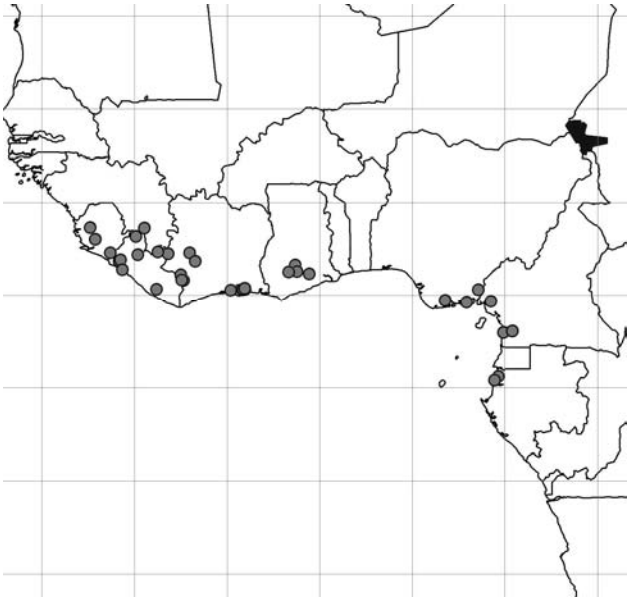
Phenology: Mature flowers collected from September to June. Mature fruits collected from June to September.

Uses: Planted as ornamental tree in botanic gardens. Seeds are used as an alternative to nutmeg (*Myristica fragrans*). The wood is used in construction.

ICUN conservation status: NT. *Monodora crispata* is known from a reasonable number of collections. It has a fairly large distribution in West and Central Africa, occurring in certain protected areas such as national parks (Sapo in Liberia; Banco and Moria in Ivory Coast) and forest reserves (Haut-Sassandra in Ivory Coast; Bobiri in Ghana). However, the area of occupancy is fairly small (370 km^2) and only a few collections were made in the past two decades (5), thus the "near threatened" category is applied.

Notes: *Monodora crispata* is distinguishable by its undulate upper bract, crisped inner and outer petals and conspicuously 6- to 7-ribbed fruit. It closely resembles *M. angolensis* by the shape of the petals, but is distinguished by crisped outer petals and the presence of 2-3 mm long hairs on the inside of the inner petals versus undulate outer petal margins and a glabrous inside surface of the inner petals. However, the molecular phylogeny places it with low support as sister to *M. tenuifolia*.
The type material *Preuss s.n.* from the protologue seems no longer extant in B. There is, however, a spirit collection from *Preuss 11* identified as *Monodora sp.* A scan from the outside of the flask was kindly sent by the Botanischer Garten und Botanisches Museum Berlin-Dahlem. Unfortunately, the content of the flask was not visible, and no identification from Engler nor Diels was seen (there was, however, an identification but dating from 1932 as *Monodora* aff. *crispata*). Because it is impossible to assess, let alone name, the content of this container without opening it, an option not provided by B, and because this specimen does have a collection number, while the holotype explicitly was designated as *s.n.*, I do not consider it possible to regard *Preuss 11* as original type material. Furthermore, it bears no identification by Engler. Thus it would seem no material exists that can be used for lectotypification, hence I designate a neotype: *J.J. Bos 6224*.
Engler and Diels (1901) indicated that the name *Monodora klaineana* was first published by Pierre citing "msc. in herb"(manuscript on herbarium sheet), seen on specimens *Klaine 1435* and *1752*. For many of such Pierre names lithographic drawings were distributed to other herbaria around 1900 (Breteler, 2006). However, no evidence what so ever has been found that such a drawing exists in this case. The P sheet only has pencil drawings and a handwritten manuscript, which apparently was seen by Engler. Engler and Diels (1906) made it a variety of *Monodora crispata*, which was validly published.

Extra references: Engl. & Diels, Monogr. Afrik. Pflanzen-Fam. 6: 89, Fig. 29B. 1901; Pellegrin, Bull. Soc. Bot. France 94: 386. 1947; Keay, Fl. W. Trop. Afr. ed. 2, 1, 1: 54. 1954; Le Thomas, Fl. Gabon 16: 345. 1969; Aubréville, Fl. For. Cote d'Iv. ed. 2, 1: 152. 1959; Irvine, Woody plants of Ghana 12. 1961; Keay, Trees of Nigeria 32. 1989; Aké Assi, Boissiera 57: 102. 2001.



Map 23. Distribution of *Monodora crispata*.

ADDITIONAL SPECIMENS EXAMINED:

CAMEROON: South Province. 9 km N. of Kribi, 2 February 1970, *J.J. Bos 6224* (BR, C, K, LD, LISC, LMA, MO, P, PRE, UPS, WAG, YA); 20 August 1970, *J.J. Bos 7199* (BR, P, WAG); Bipindi, 1909, *G.A. Zenker 3935* (BM, BR, G, K, MO, P, S, US); Bipindi, 1909, *G.A. Zenker 3884 a* (B, US); Bipindi, May 1905, *G.A. Zenker s.n.* (F); **South-West Province**. versant extérieur SSE du cratère Dissoni, 20 km WNW de Kumba, 400-500 m, 20 March 1976, *R. Letouzey 14498* (K, MO, WAG, YA); **Unknown.** no location, 24 February 1905, *W. Busse 3653* (B).

EQUATORIAL GUINEA: Rio Muni. Bata-Senye-Rio Benito: Estrada km 40. Chegada á ponte sobre o río Benito, 15 January 1992, *M.F. de Carvalho 4980* (MA, WAG).

GABON: Estuaire. Environs de Libreville, 1897, *T.-J. Klaine 720* (P); 14 January 1899, *T.-J. Klaine 1435* (P); 1899, *T.-J. Klaine 1752* (P); 1901, *T.-J. Klaine 2230* (P); Ndombo oil-concession area of Conoco ca 4 km SW of No Ayong, 25 February 1991, *J.M. Reitsma 3695* (LBV, WAG).

GHANA: . **Ashanti Region**. Bobiri Forest Reserve, January 1948, *J.E. Andoh 5102* (FHO); Fumso; Ashanti région, 24 March 1950, *K.O. Darko 547* (MO); Wiawso, March 1937, *A. Foggie 4450* (MO, NY); Bamba, March 1930, *C. Vigne 1870* (FHO); **Eastern Region**. Kibi, February 1926, *R. Burnett 48* (FHO); **Unknown.** 1 mile N of Awuto Forest Reserve, 24 May 1953, *J.K. Morton A 1007* (GC, WAG).

GUINEA: Unknown. Environs de Macenta, March 1937, *H. Jacques-Félix 1550* (P).

IVORY COAST: Abidjan. Jardin Botanique d Adiopodoume, November 1969, *L. Aké Assi 10906* (G); Foret du Banco, 6 March 1974, *L. Aké Assi 12500* (G); Foret du Banco, 1 June 1981, *L. Aké Assi 15903* (G); Banco forest, no date, *A. Aubréville (Ivory Coast series) 1908* (P); Jardin Botanique Abidjan, 24 February 1973, *A. Frédoux 5* (G); Banco, 16 March 1976, *Kadio, A. 643* (G); Abidjan. Experimental Station ORSTOM, Adiopodoume, 3 April 1974, *J. de Koning 3684* (WAG); Abidjan. Adiopodoume, Botanic Garden, 27 August 1974, *J. de Koning 3881* (WAG); Abidjan. Banco Forest Reserve, in Arboretum, 13 January 1975, *J. de Koning 5131* (WAG); Abidjan. Adiopodoume. Arboretum of ORSTOM station, 29 January 1975, *J. de Koning 5266* (WAG); Abidjan. Adiopodoume. ORSTOM Station. In Arboretum, 5 February 1975, *J. de Koning 5338* (WAG); Abidjan. Experimental Station ORSTOM, Adiopodoume, 6 April 1975, *J. de Koning 5656* (WAG); 23 April 1975, *J. de Koning 5721* (WAG); Abidjan. Banco Forest Reserve. In Arboretum, 20 June 1975, *J. de Koning 5839* (WAG); Abidjan. Banco Forest Reserve. Botanic Garden, 26 February 1976, *J. de Koning 6584* (WAG); Abidjan. Banco Forest Reserve, 16 March 1976, *J. de Koning 6666* (WAG); 3 June 1976, *J. de Koning 6960* (WAG); Forêt d'I.D.E.R.T., Adiopodoumé, ca. 17 km W of Abidjan, January 1979, *F.M. van der Laan 8* (WAG); Forêt du Banco, ca. 3 km NW of Abidjan, 11 February 1964, *R.A.A. Oldeman 957* (B, BR, WAG); Adiopodoumé, 19 March 1990, *H.G. Téré 1697* (CSRS); 10 km SE of Dabou, direction lagune, 21 May 1969, *C. Versteegh 100* (U, WAG); 50 km W of Dabou, along the road to Grand-Lahou, 23 July 1969, *C. Versteegh 567* (WAG); Forêt d'I.D.E.R.T., Adiopodoumé, ca. 17 km W of Abidjan, 4 September 1963, *W.J.J.O. de Wilde 867* (WAG); Forêt du Banco, 12 January 1961, *H.C.D. de Wit 9022* (WAG); Forêt d'Adiopodoumé, 26 December 1957, *H.C.D. de Wit 7954* (WAG); **Daloa**. 32 km W of Daloa, 20 February 1951, *G. Roberty 13882* (G, MO); **Danané**. Danane, 26 February 1969, *L. Aké Assi 10483* (G); **Guiglo**. in the vicinity of Tienkoula, 28 February 1962, *L. Bernardi 8292* (G, K); 7 km S of Taï, near Diéoula, left bank Cavally river, 120 m, 8 March 1959, *A.J.M. Leeuwenberg 3014* (BR, WAG); Taï, no date, *A. de Rouw 524* (WAG); **Vavoua**. F.C. du Haut-Sassandra, Nord. forêt dégradée, 6 January 1995, *F.N. Kouamé 1624* (CSRS).

LIBERIA: Bong. Peáhtah, 7 October 1926, *D.H. Linder 943* (A); **Lofa**. Pandamai, 29 February 1944, *J.C.C. Bequaert 89* (MO); **Montserrado**. Gola Forest, 12 March 1910, *R.H. Bunting s.n.* (BM); University New Site, 18 miles from Monrovia, 4 March 1967, *W. Goll 26* (WAG); Bomi Hills, 10 February 1969, *J.W.A. Jansen 1448* (BR, NY, WAG); **Nimba**. Saniquelle,

400 m, 3 March 1962, *A.G. Voorhoeve 931* (WAG); **Sino**. Sapo National Park, buffer zone, around Safari Camp on short distance of Sinoe River, 115m, 22 November 2002, *C.C.H. Jongkind 5316* (WAG).
NIGERIA: Akwa-Ibom State. Eket District, 1913, *P.A. Talbot s.n.* (BM); 1913, *P.A. Talbot 3221* (BM); **Cross River State**. Oban, 1912, *P.A. Talbot 1499* (BM, WAG, Z); **Rivers State**. Degema, 1915, *P.A. Talbot s.n.* (BM); 1916, *P.A. Talbot s.n.* (BM).
SIERRA LEONE: . **Eastern Province**. Kamaranka, Magbema, 17 February 1952, *H.D. Jordan 396* (K); **Southern Province**. near Zimi, 2 April 1939, *F.C. Deighton 3632* (K); Mano, 20 March 1928, *F.C. Deighton 1108* (BM, K); 27 May 1938, *F.C. Deighton 3538* (K). **Unknown.** Below lake Sonfon, Moria National Park, 15 February 1966, *D. Gledhill 432* (K); no location, 1915, *N.W. Thomas 9331* (K); no location, 1915, *N.W. Thomas 9400* (K); no location, 1915, *N.W. Thomas 10542* (K).

4. Monodora globiflora Couvreur, Adansonia 28: 248. 2006. — TYPE: TANZANIA. Iringa: Udzungwa Mountains, 07°42´S, 36°37´E, 13 October 2002, *W.R.Q. Luke 9136* (holotype: MO!; isotypes: EA!, K!, NHT). *Figure 6.29*

Tree to 12 m tall; trunk with d.b.h. up to 30 cm; bark grey with white lenticels; young branches drying black, densely covered with short erect hairs (glabrous); old branches grey, striate, glabrous. *Petioles* 6-8 mm long, ca. 1 mm in diameter, covered with short erect hairs, leaf lamina inserted on the side, weakly grooved adaxially. *Leaf lamina* 12-21 cm long, 4-7 cm wide, length:width ratio 2.1-3, narrowly obovate to obovate or narrowly elliptic to elliptic, base cuneate to acute, apex acute to cuspidate, papyraceous, abaxial surface densely covered with short erect hairs when young to sparsely covered with short erect hairs when older, adaxial surface glabrous to sparsely covered with short erect hairs adaxially, rarely glabrous on both surfaces; midrib densely covered with short erect hairs on both sides; secondary veins 14-16 pairs, strongly curved upwards, parallel, covered with short appressed hairs abaxially; tertiary venation conspicuously percurrent. *Flowers* single, leaf opposed, pendulous. *Flowering pedicels* 40-45 mm long, ca. 0.5 mm in diameter, covered with short appressed hairs, light green. *Upper bract* inserted in lower half of pedicel, ca. 8 mm long, ca. 7 mm wide, length:width ratio ca. 1.5, ovate, base auriculate-clasping, apex rounded, glabrous, green; margins straight, densely covered with short erect hairs. *Sepals* 8-10 mm long, 6-8 mm wide, length:width ratio 1.3-1.5, oblong, base truncate, apex rounded, glabrous on both surfaces, reflexed upwards, light green, caducous in fruit; margins entire, densely covered with short erect hairs. *Outer petals* 28-32 mm long, 18-23 mm wide, length:width ratio 1.4-1.8, ovate, apex rounded, slightly reflexed and curved inwards at apex when fully opened, glabrous but sparsely covered with short appressed hairs along the veins, creamy yellow streaked with red slashes and spots; margins undulate, glabrous to sparsely covered with short erect hairs. *Inner petals* 7-8 mm long, 10-12 mm wide, length:width ratio 0.7-1, clawed triangular, base truncate, apex acuminate to rounded, covered with ca. 1 mm long curly hairs becoming shorter towards margins, the whole lamina connivent over receptacle like a globe, bright yellow with red streaks; claw 3-5 mm long, 2-4 mm wide, claw:inner petal ratio ca. 1.5-2.5, glabrous, bright yellow without red streaks. *Receptacle* 4-5 mm in diameter, flat. *Stamens* in 5-6 rows, ca. 0.8 mm long, connective shield ca. 0.1 mm long, glabrous, white, those of innermost row not extended over ovary wall. *Ovary* ca. 1.5 mm long, ca. 1 mm wide; stigma ca. 1 mm diameter, glabrous, white. *Fruiting pedicels* ca. 40 mm long, 3-4 mm in diameter. *Fruits* 4-5 cm long, ca. 5 cm in diameter, irregularly globose, surface reticulate,

glabrous, green-reticulate; pericarp ca. 2 mm thick. *Seeds* 13-15 mm long, 7-8 mm wide, transversely elliptic, in white pulp; testa smooth, light brown; raphe not thickened, brown; hilum not seen.

Distribution: Tanzania (see Map 25); montane rain forest, on well drained brown sandy loams with extensive areas of rock faces; at 1700-2000 m altitude.

Phenology: Mature flowers collected from September to December. Mature fruits collected in February.

Vernacular name:
Tanzania: Mkimi (Kihehe).

IUCN conservation status: EN B1ab(iii) + 2ab(iii). *Monodora globiflora* is represented by eight collections all from the Udzungwa Mountains National Park. With an extent of occurrence of 1000 km^2 and an area of occupancy of 70 km^2, the category "endangered" seems appropriate.

Notes: *Monodora globiflora* is characterized by a clear percurrent tertiary venation, which is unique in *Monodora*, hairy leaves, and an inner petal that is longer than the claw. The species has been photographed by Mr. Quentin Luke and Mrs. Ann Robertson which showed a strong color variation. The specimen *Luke 9178* has green to yellow inner and outer petals with white streaks, while *Luke 9136* has yellow inner and outer petals with red streaks. This variation in color needs further investigation in the field. Molecular data indicate that there is strong support for this species being sister to *M. carolinae* and *M. stenopetala*.

ADDITIONAL SPECIMENS EXAMINED:
TANZANIA: Iringa. Uhimbila, 1850 m, November 1953, *Carmichael 274* (EA, K); Udzungwa Mountains National Park, 1400 m, 27 October 2005, *Festo, L. 2075* (MO, WAG); Udzungwa Mountain, NP Mt Luhoero Pt 131, 1440 m, 27 September 2000, *W.R.Q. Luke 6724* (EA, K, NHT); Udzungwa Mountain, Pt 362, 1800 m, 13 October 2002, *W.R.Q. Luke 9136* (EA, K, MO, NHT); Udzungwa Mountain; Pt 362, 1800 m, 15 October 2002, *W.R.Q. Luke 9178* (EA, K, MO, NHT); Ndundulu, Udzungwa Mountains, West of Kilombero Forest Reserve, 1700 m, 10 February 2000, *Price, D. WK 354* (MO); Iringa district, Image Mt, 2000 m, November 1959, *J. Procter 1541* (EA, K); **Morogoro**. East Udzungwa National Park. In forest south of Mwanihana hill. c. 2 km south of last camping site on Mwanihana trail, 1400 m, 30 November 2006, *T.L.P. Couvreur 99* (DSM, MO, WAG).

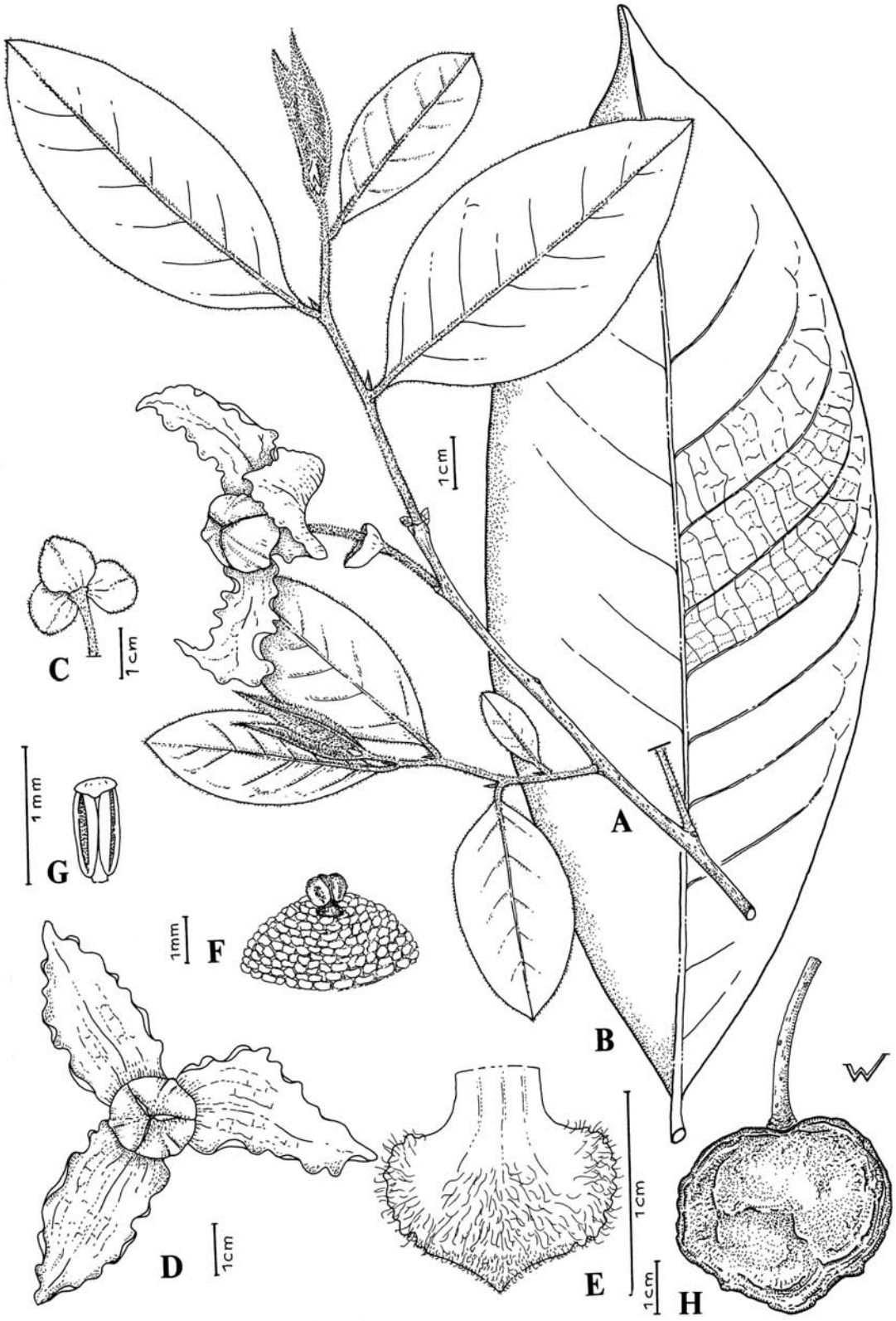


Figure 6.29. *Monodora globiflora*. A. Flowering branch. B. Leaf. C. Pedicel with sepals. D. Mature flower (top view). E. Detail of inner petal (inside view). F. Androecium and stigma. G. Stamen. H. Fruit. Drawings by Wil Wessel-Brand.

5. Monodora grandidieri Baill., Adansonia 8: 301. 1868.— TYPE: TANZANIA. Zanzíbar: Zanzibar Island, 1864, *A. Grandidier 28* (holotype: P!, isotype: K!). *Figure 6.30 D-F*

Monodora veithii Engl.& Diels, in Diels, Bot. Jahrb. Syst. 39: 485. 1907. — TYPE: TANZANIA. Tanga: Usambara Mountains, Mombo, *A. Engler 3268* (holotype: B!; isotype: EA!).

Monodora stocksii Sprague, Kew Bull. 1916: 38. 1916. — TYPE: MOZAMBIQUE, Mocimbua, *Stocks 96* (holotype: K!, isotype: K!).

Monodora somalensis Chiov., Fl. Somalia 2: 2. 1932. — TYPE: SOMALI REPUBLIC. Baddada, *L. Senni 228* (holotype: FI!).

Monodora hirsuta E. Peter, Tanganyika Territory Ch. Lis. 2: 42. 1949. *nomen nudum*.

Monodora taylori Engl., *nomen nudum*.

Tree to 10-12 m tall, much branched; trunk with d.b.h. up to 15 cm; outer bark grey or brown, fairly smooth and faintly longitudinally striate, sometimes pealing off; young branches green, densely covered with short erect hairs to glabrous; old branches black with white lenticels, glabrous to sparsely covered with short erect hairs. *Petioles* 2-6 mm long, 1.5-2.5 mm in diameter, densely covered with short erect hairs, rarely glabrous, leaf lamina inserted on top, broadly grooved adaxially. *Leaf lamina* 4-23(-25) cm long, 3-10 cm wide, length:width ratio 1.5-3.5, narrowly obovate to obovate, base cordate, apex acuminate, acumen ca. 15 mm long, papyraceous when young becoming coriaceous when old, densely to sparsely covered with short appressed hairs to glabrous on both sides, young emerging leaves always densely to very sparsely covered with short appressed hairs; midrib adaxially impressed towards the base, covered with short appressed hairs to glabrous on both sides; secondary veins 9-14(-19) pairs, uniformally curving upwards, covered with short appressed hairs to glabrous. *Flowers* single, leaf-opposed, developing from young shoots before or during leaf flush, pendulous. *Flowering pedicels* (6-)20-50(-80) mm long, 1-1.5 mm in diameter, densely to sparsely covered with short erect hairs, rarely glabrous. *Upper bract* inserted at the centre to subapical to the pedicel, curving upwards, 4-10(-20) mm long, 4-10 mm wide, length:width ratio 0.7-2, broadly ovate to ovate, base decurrent, apex attenuate to acute, densely covered with short erect hairs, green, dark red at base, reflexed upwards, margins undulate, densely covered with short erect hairs. *Sepals* 5-15(-20) mm long, 5-7 mm wide, length:width ratio 1.8-5, elliptic to narrowly elliptic, base truncate, apex acute, densely covered with short appressed hairs outside, sparsely covered with short appressed hairs inside, green to red brown with green veins, margins undulate, densely covered with short appressed hairs. *Outer petals* (10-)15-60(-70) mm long, 3-12(-18) mm wide, length:width ratio 3.3-12, narrowly oblong to linear or spatulate, base truncate, apex acute, sparsely covered with short appressed hairs on both surfaces, green to yellow horizontally streaked with red-purple markings becoming bright

white at base, spreading horizontally and curving downwards towards the end; margins undulate, densely covered with short erect hairs. *Inner petals* (4-)6-10(-12) mm long, 5-15 mm wide, length:width ratio 0.4-1.3, clawed cordate, base cordate, apex acute, densely covered with erect ca. 1.5 mm long hairs around the centre inside, sparsely covered with short appressed hairs outside, white with red-brown veins and yellowish to red margins, connivent by margins; margins undulate, densely covered with short appressed hairs; claw incurved, 5-15 mm long, 2-4 mm wide, claw:inner petal ratio 0.4-1.2(-1.5), sparsely covered with short erect hairs to glabrous, white. *Receptacle* 4-5 mm in diameter, slightly convex. *Stamens* in 5-7 rows, ca. 1.5 mm long, connective shield ca. 0.2 mm long, glabrous, white, those of innermost row not elongate over ovary wall. *Ovary* ca. 2 mm long, ca. 1 mm wide; stigma 1.5-2 mm in diameter, sparsely covered with short erect hairs, yellow. *Fruiting pedicels* 15-30 mm long, 3-4 mm in diameter, glabrous to densely covered with short appressed hairs. *Fruits* 45-65 mm long, 35-45 mm wide, globose to obovoid, minutely rugose, covered with short erect hairs to sparsely covered with short erect hairs; pericarp Ca. 2-4 mm thick. *Seeds*10-18 mm long, 5-11 mm wide, transversely ellipsoid, packed in white pulp; testa smooth, light brown; raphe slightly thickened, brown; hilum 6-7 mm long, 2-3 mm wide, narrowly elliptical.

Distribution: Present throughout East Africa from southern Somalia to southern Mozambique (see Map 24); in lowland rain forests and thicket; at 0-900 m altitude.

Phenology: Mature flowers and fruits collected all year round.

Vernacular name:
Kenya: Mudzala simba, Mcherere (Giriama); Mganda-simba; Mubungo (Swahili); Mkere (Digo).

Uses: In Kenya the wood is sometimes used to make arrows and bows, or used as medicine (but unknown for what it is treated against). The fruit is edible (Maundu and Tengnäs, 2005).

IUCN conservation status: NT. *Monodora grandidieri* is well represented in herbaria and has been collected quite often in recent years. It has been collected in numerous protected areas (Selous Game Reserve, Amani Nature Reserve, Udzungwa National Park and Jozani Forest Reserve in Tanzania; Livulezi Reserve in Malawi, Sokoke Forest Reserve in Kenya). Even though the area of occupancy is less than 2000 km^2, it is known from more than 10 localities and thus is better placed in the “near threatened” category.

Notes: *Monodora grandidieri* is a variable species as noted by Robson (1960) and Verdcourt (1971). The flowers can vary from small to large. However, this variation is continuous, and thus does not merit description of varieties between the two extremes. Hair indument of the leaves and young branches is also very variable, ranging from densely hairy to glabrous sometimes even within the same specimen. However, the very young emerging leaves are

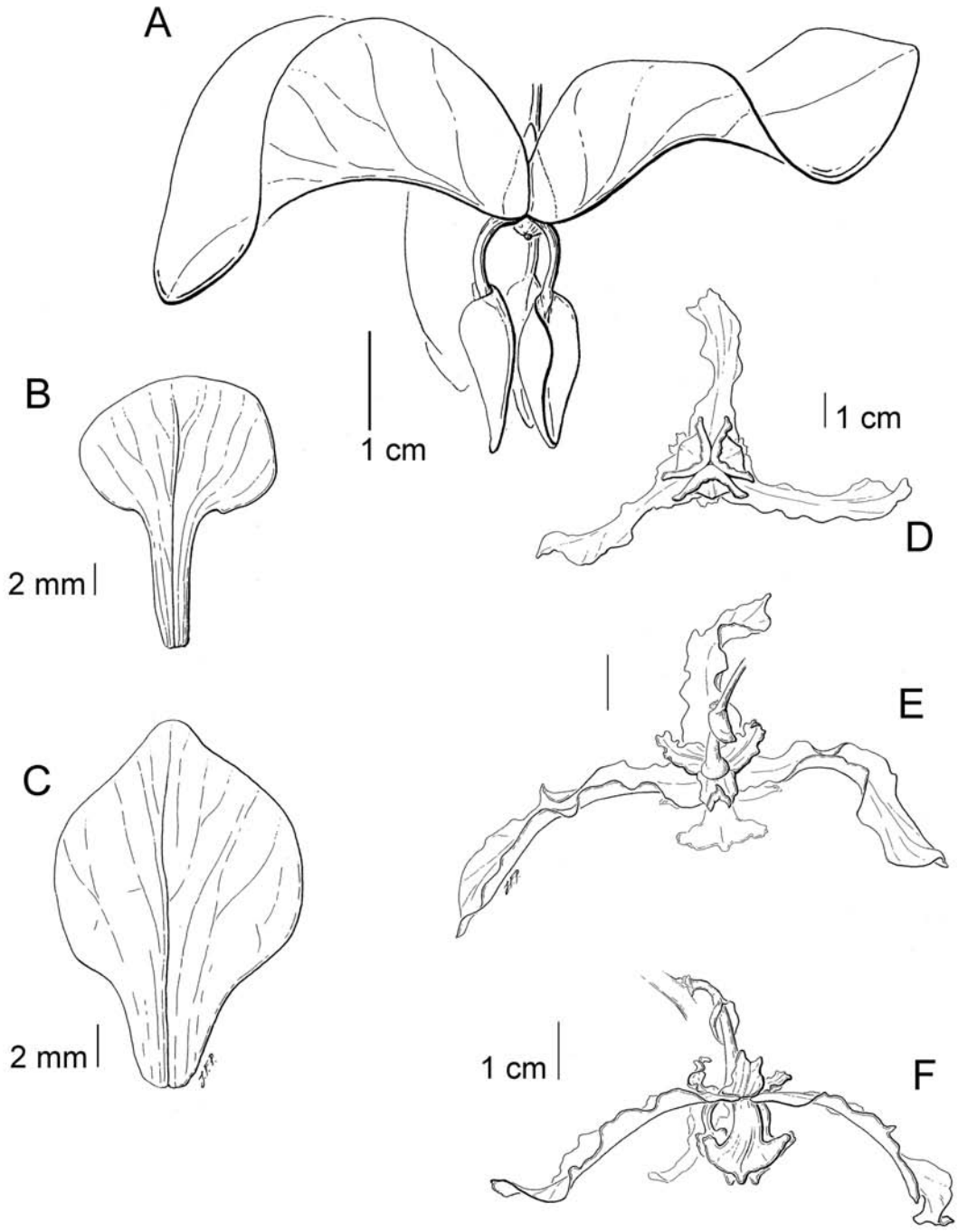
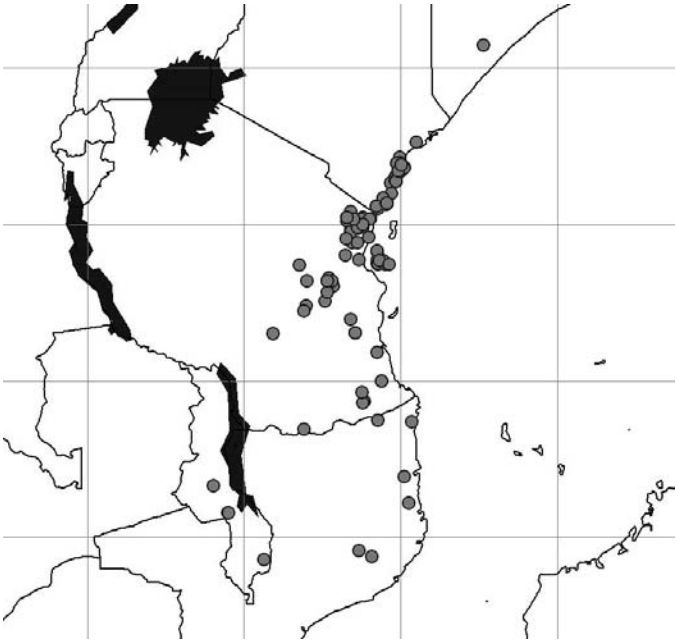


Figure 6.30. *Monodora junodii*. A. Flower. B. Inner petal (outer surface). C. Inner petals (outer surface). *Monodora grandidieri*. D. Flower (bottom view). E. Flower (top view). F. Flower (side view). Drawings by Joanne Porck.

always at least sparsely hairy, mainly along the margins. Petals and sepals are invariably hairy. At lower altitudes, *M. grandidieri* sheds its leaves every year, while at higher altitudes leaves can persist for longer.

Monodora grandidieri is easily recognized by its cordate leaf base, hairy pedicels and narrowly oblong or linear outer petals. The cordate leaf base is only found in one other species, namely *M. myristica*. The narrowly oblong or linear outer petals resembles those of *M. stenopetala*, although the latter are in general much narrower and straight. Molecular data indicate that this species is sister to the rest of the monophyletic East African species.

Extra references: Engl. & Diels in Engl., Monogr. Afrik. Pflanzen-Fam. 6: 85, Fig. 28F. 1901; Diels, Bot. Jahrb. Syst. 39: 485. 1907; Brenan & Greenway, Tanganyika Territory Ch. Lis. 2: 42. 1949; Robson, Fl. Zamb. 1, 1: 146. Fig. 16B. 1960; Dale & Greenway, Kenya Trees and Shrubs 36. 1961; Verdcourt, Fl. Trop. E. Afr., Annonaceae 122. 1971; Beentje, Kenya trees, shrubs and lianas 49. 1994.



Map 24. Distribution of *Monodora grandidieri* in East Africa.

ADDITIONAL SPECIMENS EXAMINED:

KENYA: Coast. Sokoke forest, no date, *E. Battiscombe 798 a* (EA, K); 22 October 1963, *Beecher H 345 634* (EA); Pumwani "forest", 60 m, 21 September 1985, *H.J. Beentje 2347* (EA, WAG); Jadini Forest, July 1962, *W.R. Birch 62/ 255* (K); Shaitani Forest near Diani, 17 November 1978, *J.P.M. Brenan 14501* (C, K, MO, WAG); Adu, N Giriama, January 1937, *I.R. Dale 1074* (FHO); Near Sokoke, 1 December 1962, *I.R. Dale 2030* (EA, K); Mida, 16 miles S of Malindi, January 1937, *I.R. Dale 3653* (K); Mida, October 1930, *G.H. Donald 448* (FHO); Mida Forest Coast, 1924, *C.W. Elliot 1427* (K); Mrima Hill, 5 March 1977, *R.B. Faden 77/ 674* (K, US); Mida forest, no date, *H.M. Gardner 1427* (G); Gede forest, 15m, 9 May 1985, *Gerhardt, K. 88* (S); Diani Forest. Areas NW and NE within 1 km N. of trun off from new road for Jadini hotel, W and E of this road, 11 July 1972, *J.B. Gillett 19861* (K); Arabuko, Kilifi, April 1929, *R.M. Graham 1716* (G, K, MO); Mida Forest, no date, *R.M. Graham 1427* (EA); Jadini, 3m, 6 December 1959, *P.J. Greenway 9632* (K); Witu, June 1957, *P.J. Greenway 11272* (EA, K); D'Oliera drive, N. Beach, Kilifi, 30 May 1958, *P.H. Irwin 401* (K); Sokoke forest near Kilifi, 10 March 1945, *G.W. Jeffrey K 128* (G, K); Kilifi, 13 April 1945, *G.W. Jeffrey k 160* (G, K); Kibarani, 26 November 1945, *G.W. Jeffrey K 408* (G); Borne River, Kwale District, 16 March 1902, *T. Kassner 313* (K); About 30 km S of Mombasa, 26 March 1973, *S.P. Kibuwa 1206* (BR, EA, K, M, WAG); Diani forest 30 km South of Mombasa, 29 March 1973, *S.P. Kibuwa 1226* (BR, FHO, K, M); Godoni Forest, Shimba Hills, 450 m, 8 December 1975, *J.O. Kokwaro 3974* (K); Sokoke forest, August 1965, *W.P. Langridge 26* (EA); August 1965, *W.P. Langridge 84* (EA, K); Kilifi District. Kaya Kivara, on top of Kivara hill, penetrating the forest from the southern exposed forest edge on top of the hill, 8 January 1982, *L.J. Lap 163* (WAG); Dzombo Mountains, 365m, 8 April 1968, *F.C. Magogo 779* (K); Kilifi, June 1936, *G.M. Moggridge 95* (EA); Jadini, 12 January 1956, *D.M. Napper 462* (K); Sabaki, 4 ml N. of Malindi, 2m, 12 November 1961, *R.M. Polhill 747* (B, BR, K, S);

Malindi district, bwtween Marafa and Dagrama, June 1959, *S. Rawlins 711* (EA, K); Diana Forest, 10 m, 5 May 1985, *S.A. Robertson 3873* (K, MO, U, WAG); Arabuko Sokoke Forest, 60 m, 21 April 1985, *S.A. Robertson 3867* (K, MO, U); 3 km E of Wakala, 80 m, 13 April 1989, *S.A. Robertson 5681* (K, MO); Kaya Kinondo, 5m, 12 April 2005, *S.A. Robertson 7545* (EA, WAG); Dzombo Hill, 300 m, 8 February 1989, *S.A. Robertson MDE 251* (K); Kaembeni-dida, NNW of Kilifi, 19 March 1973, *G.W. Sangai 15645* (EA); Fumbini, Kilifi, 15 September 1936, *C.F.M. Swynnerton 89* (K); Kilifi, 10 m, 14 April 1954, *Trump, E.C. 127* (EA, K); Bridge over Galana, N. of Malindi, 15m, July 1959, *D.R. Tweedie 1872* (B, K, P, WAG); Mrima Hill, 9 April 1978, *B. Verdcourt 5267* (BR, K, MO, P); Diani Forest, 15m, 18 April 1972, *J.G. Williams 15111* (K, P); **Kilifi**. Marikebuni to Dagamra, Pumwani, 60 m, 22 September 1985, *S.A. Robertson 4089* (MO); **Unknown.** no location, April 1967, *T. Adamson 58* (EA); no location, no date, *H. Padera B 15* (EA).

MALAWI: Central Province. Livulezi Reserve. North east Essort block. Foot of escarpment, 9 December 1953, *P.G. Adlard 12* (EA, K); Mua, north of Sasola Rest House, 6 January 1965, *E.A.K. Banda 615* (K); below Nchisi forest, 29 February 1964, *E.G. Chapman 2246* (FHO); **Southern Province**. Reg. Mulanje, Machemba Hill, 12 January 1984, *J.L. Balaka 314* (MO).

MOZAMBIQUE: Cabo Delgado. Kilimakito, Nangororo, near Porto Amelia, 15 November 1963, *A. de Figueiredo Gomes e Sousa 4818* (K); Cabo Delgado, Distrito de Ancuabe, localidade de Metoro, Aldeia de namatuco, caminho para Mecaruma à 10 km de Namatuco, 31 January 1984, *E.M.C. Groenendijk 899* (K, WAG); Mueda Plateau, 13 December 2003, *W.R.Q. Luke 10104* (MO); Mocimbua, 1907, *J.E. Stocks 96* (K); **Nampula**. Mozovolas, Calipo, 22 July 1948, *J. Pedrógão de Jesus 4619* (EA); Nampula, Nargens do rio Mutivaze, lugares Humidos Estrada, 8 January 1937, *A.R. da Torre 1324* (MO).

SOMALIA: Jubbada Hoose. Jelid District, Maleenda, near Faanole Dam, 5 August 1984, *Warfa, A.M. 1089* (K, S).

TANZANIA: Kilimanjaro. Below Mlalo, Lushoto Dist, 900 m, December 1966, *J. Procter 3400* (EA); **Lindi**. Ca. 4 km W of Mavuji Village along road track to Mavuji Plateau, 120 m, 21 November 2003, *C.J. Kayombo 4751* (MO); Lindi, 40 km West of Lindi, 250 m, 9 May 1934, *H.J.E. Schlieben 5457* (BR, G, P); 250 m, 9 December 1934, *H.J.E. Schlieben 5702* (BR, G, P); Liwali-Nachingwea road, 26 September 1954, *F.G. Smith 1330* (K); Kingupira, 125m, 22 November 1975, *K. Vollesen MRC 3031* (C, EA, WAG); Kingupira, 125m, 10 September 1976, *K. Vollesen MRC 3983* (C, EA, WAG); Lake Utunge, 50 m, 15 December 1971, *K. Vollesen 1359* (C); **Morogoro**. Ulugurus, Monogora, 5 December 1934, *E.M. Bruce 281* (K, P); Mlali Hills, Mpwapwa, 550 m, 8 December 1933, *B.D. Burtt 5044* (BR, FHO, G, K, WAG); 5 km from Morogoro, on road to Kimboza Forest Reserve On asphalt road down hill, 500 m, 26 November 2006, *T.L.P. Couvreur 83* (DSM, MO, NHT, WAG); East Udzungwa National Park. On trail just c. 2 km m before arriving to Sanje Falls, 500 m, 27 November 2006, *T.L.P. Couvreur 93* (DSM, MO, WAG); Morogoro, November 1957, *W.J. Eggeling 6344* (EA, K); Morogora Rural District. Ruvu Forest Reserve, 250 m, 19 July 2000, *E.B. Mhoro UMBCP 140* (MO); Kisaki, Morogoro, 609m, no date, *N.V. Rounce 496* (K); Kidodi, December 1952, *S.R. Semsei 1072* (BR, K, MO); Mazungungu, 25 October 1961, *S.R. Semsei 3340* (EA, K);, 579m, 27 November 1932, *G.B. Wallace 497* (K); Kingolwira Station, 3 November 1954, *J.R. Welch 265* (EA, K); 8 miles NE of Kingolwira Station, 450 m, 21 December 1956, *J.R. Welch 339* (BR, EA, K); Kiticha mum stream above house 28, Morogoro, 853m, November 1947, *L.T. Wigg 2280* (K); **Mtwara**. near Masasi, Masasi Hill, 500 m, 9 March 1991, *S. Bidgood 1865* (K); Masasi, 23 January 1943, *Gillman, H. 274* (K); **Singida**. Chita, Makutano subvillage, 350 m, 23 August 2000, *H.J Ndangalasi 644* (C); **Tanga**. Kwamkono, 30 km E of Handeni, 520 m, 10 October 1976, *M.E. Archbold 2182* (BR, DSM); in forest about 1 km straight up from Kisiwani village (just before Amani Nature Reserve), 800 m, 11 November 2006, *T.L.P. Couvreur 15* (DSM, MO, WAG); on road 2 km before arriving to Kisiwani village, 280 m, 14 November 2006, *T.L.P. Couvreur 33* (WAG); road 3 km before arriving to Kwedikwazu comming from Dar Es Salaam. At Msangasi river, 300 m, 14 November 2006, *T.L.P. Couvreur 35* (DSM, MO, NHT, WAG); Usambaras Mountains, Mombo, 500 m, no date, *A. Engler 3268* (B, EA); Pangani, 23 March 1950, *H.G. Faulkner 527* (BR, K, S); Sigi, 580 m, 28 January 1931, *P.J. Greenway 2878* (FHO, K); Kwamkuyu Bridge, Sigi, 490 m, 17 February 1939, *P.J. Greenway 5858* (FHO, K); Genda-Genda South, South of hill, 29 July 1982, *W.D. Hawthorne 1004* (DSM, K); Mombo on the road to Lushoto, 560 m, 7 December 1978, *O. Hedberg 6809* (K, S); Amani Research station, Kibosiana, 1610 m, 11 December 1935, *A.J.W. Hornby 727* (K); road Mombo-Lushoto, 4.3 km from Mombo, 30 April 1996, *D.M. Johnson 1933* (OWU); Mahuyuni, 23 March 1936, *H. Koritschoner 1526* (EA, K); Mahuyuni, 23 March 1936, *H. Koritschoner 1534* (EA, K); Amani-Sigital, 21 January 1909, *F.W.L. Kränzlin 2179* (K); Maramba Division, Bwiti Village, east Usambaras mountains, Mgambo Forest Reserve, 460 m, 26 April 2002, *A. Ntemi Sallu 906* (MO); East Usambara Mountains, May 1914, *A. Peter 3574* (B); Useguha, 10 June 1914, *A. Peter 4493* (B); West Usambara, 6 January 1915, *A. Peter 7998* (B, WAG); East Usambara Mountains, Amani, 18 April 1915, *A. Peter 9881* (B); East Usambara Mountains, January 1917, *A. Peter 18935* (B); Usambara, January 1917, *A. Peter 19028 b* (B, K); West Usambara, February 1917, *A. Peter 19636* (B, BR, K, S, WAG); East Usambara Mountains, March 1917, *A. Peter 19835* (B); East Usambara Mountains, February 1917, *A. Peter 19836* (MO); Udigo, 14 April 1926, *A. Peter 39560* (B, K); East Usambara Mountains, May 1926, *A. Peter 40079* (B); Sigi, 24 April 1922, *R. Saleman 5984* (K); Bulwa, 7 April 1922, *R. Saleman 5988* (K); East Usambaras Mountains, 24 April 1909, *Unknown s.n.* (MO); East Usambaras Mountains, 18 January 1906, *N. Zimmermann 1003 A* (MO); **Zanzibar**. no location, 10 m, 6 December 1999, *S.A. Fakih 580* (L); M'kokotoni, 17 February 1961, *H.G. Faulkner 2755* (B, BR, EA, S); M'kokoloni, 14 February 1961, *H.G. Faulkner 2756* (K); Mile 14, Chwaka, 17 February 1965, *H.G. Faulkner 2969* (K);, 1864, *A. Grandidier 28* (K, P); Haitajwa Hill, 28 January 1929, *P.J. Greenway 1207* (EA, K); N. Haitajwa Hill, 4 December 1930, *P.J. Greenway 2649* (K); Jozani Forest Reserve, 29 December 1981, *F.A. Mturi 206* (K); Jozani Forest Reserve, 21 January 1981, *F.A. Mturi 375* (K); Sagara, January 1895, *C. Sacleux 958* (P); South Zanzibar, January 1890, *C. Sacleux 1031* (P); no location, 1927, *K.E. Toms 108* (K); Kombeni Cave wells, 7 September 1930, *R.M Vaugh 1484* (EA); Ufufuma, 23 December 1930, *R.M Vaugh 1755* (EA); 25 December 1933, *R.M Vaugh 2180* (EA); Kombeni, Cave Wells, Hiatajwa, 2 September 1930, *J.H. Vaughan 1484* (K); Ufufuma, 23 December 1930, *J.H. Vaughan 1755* (K); no location, no date, *J.H. Vaughan 2180* (FHO); **Unknown.** Kwamkono, 8 November 1990, *M.E. Archbold 3312* (K, P); Kwa-Ntora, 1900, *W. Busse 1026* (G, P); Planis, 487m, 1 April 1932, *G.B. Wallace 305* (K); Upiji Ravine, 12 February 1971, *R.C. Wingfield 994* (S).

6. Monodora hastipetala Couvreur, Adansonia 28: 250. 2006.— TYPE: TANZANIA. Pwani: Matumbi Hills, Kiwengoma Forest, 8°19´01´´S, 38°57´07´´E, 19 October 1997, *P.B. Phillipson 4958* (holotype: MO!). *Figure 6.31*

Tree to 8 m tall; trunk with d.b.h. up to 5 cm; bark distinctly striate, grey; young branches drying black, glabrous; old branches grey, striate, glabrous. *Petioles* ca. 2 mm long, ca. 1 mm in diameter, glabrous, leaf lamina inserted on side, grooved adaxially. *Leaf lamina* 10-12 cm long, 3-4 cm wide, length:width ratio 2.5-3, narrowly obovate to spatulate, base acute to rounded, apex acuminate, acumen 1-1.5 cm long, glabrous; midrib glabrous on both sides; secondary veins 10-14 pairs, uniformally curving upwardse, glabrous. *Flowers* single, leaf-opposed, pendulous. *Flowering pedicels* 17-20 mm long, 0.6-0.8 mm in diameter, glabrous. *Upper bract* inserted in upper half of pedicel, 5-9 mm long, 3-5 mm wide, length:width ratio 1.4-1.6, ovate, base auriculate-clasping, apex acute, glabrous on both surfaces, pale green; margins straight, with short straight hairs or glabrous. *Sepals* 6-8 mm long, 3-4 mm wide, length:width ratio ca. 2, elliptic, apex rounded to acute, glabrous on both surfaces, green; persistent in fruit, margins entire, with short straight hairs or glabrous. *Outer petals* 20-26 mm long, 6-8 mm wide, length:width ratio 3-4.3, narrowly elliptic, base truncate, apex acute, glabrous, light green; held horizontally at anthesis, margins undulate, with short straight hairs or glabrous. *Inner petals* 10-17 mm long, 4-7 mm wide, length:width ratio 2-3, clawed ovate, base truncate, apex long-acuminate, densely covered with short straight hairs at base of inside surface and all over outside, white with purple base at anthesis, connivent by the center of lamina over the receptacle; margins straight; claw 4-5 mm long, claw:inner petal ratio 0.3-0.4, glabrous, white to pale green, tinged purple at apex. *Receptacle* ca. 2 mm in diameter, flat. *Stamens* in 3-4 rows, ca. 0.6 mm long, white; connective shield ca. 0.1 long, glabrous, those of innermost whorl extending slightly over ovary wall. *Ovary* ca. 1 mm long, ca. 0.5 mm wide; stigma ca. 0.8 mm in diameter, glabrous, light yellow. *Fruiting pedicels* 18-25 mm long, 3-4 mm in diameter, woody, glabrous, green. *Fruits* ca. 1.5-2.5 cm long, ca. 2-2.5 cm in diameter, length:width ratio 0.75-1, broadly ovoid, constricted around seeds when dried, slightly bumpy when fresh, glabrous, green with white dots; pericarp 1-2 mm thick. *Seeds* 10-12 mm long, 9-10 mm wide, transversely broadly ellipsoid; testa smooth, light brown; raphe slightly thickened, brown; hilum 4-5 mm wide, 2-2.5 mm wide, elliptical.

Distribution: East Tanzania (see Map 25); in dry scrub and riverine coastal forest; at 225-365 m altitude.

Phenology: Mature flowers collected from October to November. Mature fruits found in November.

Vernacular name:
Tanzania: Nnjende (Kimatumbi).

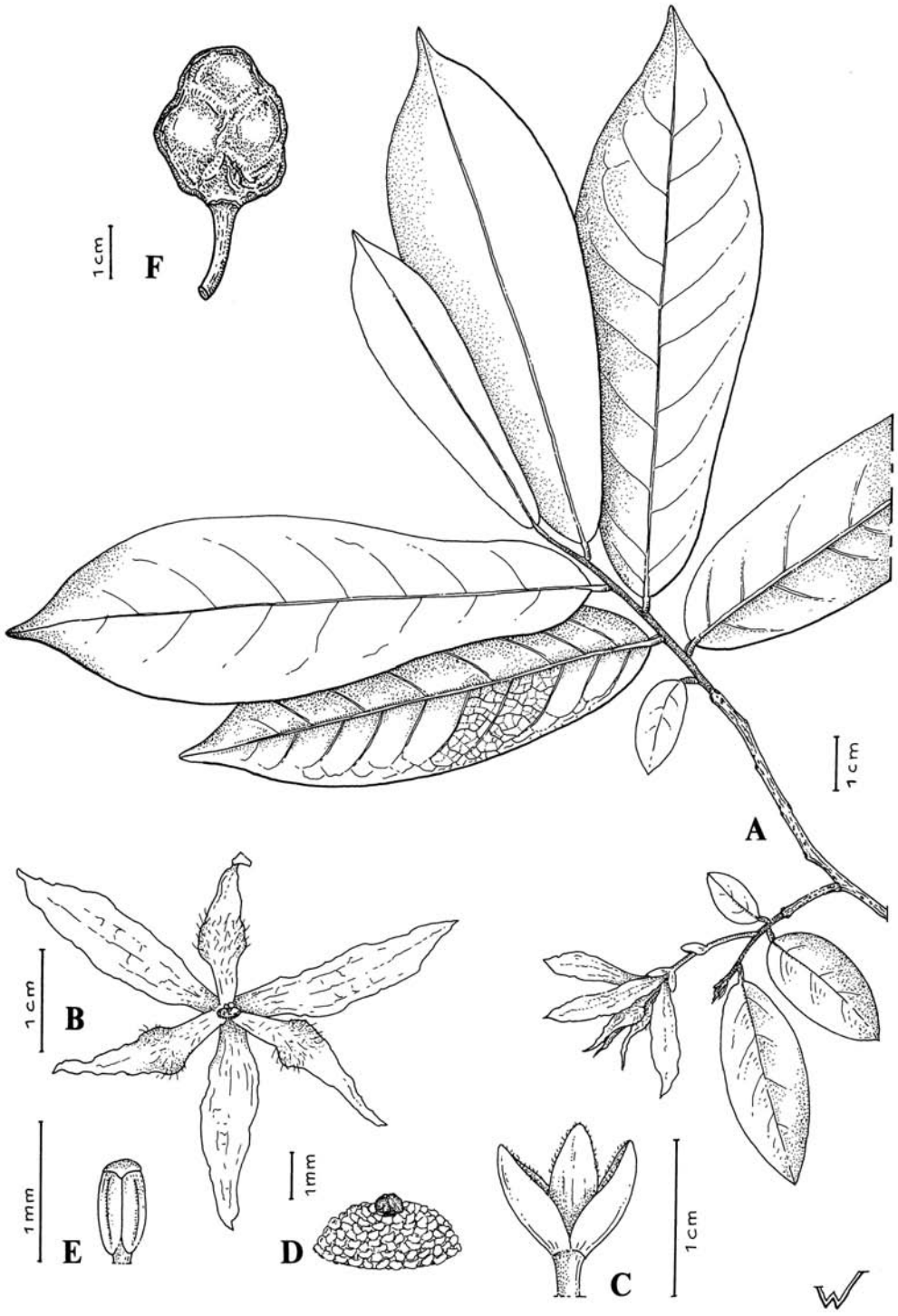
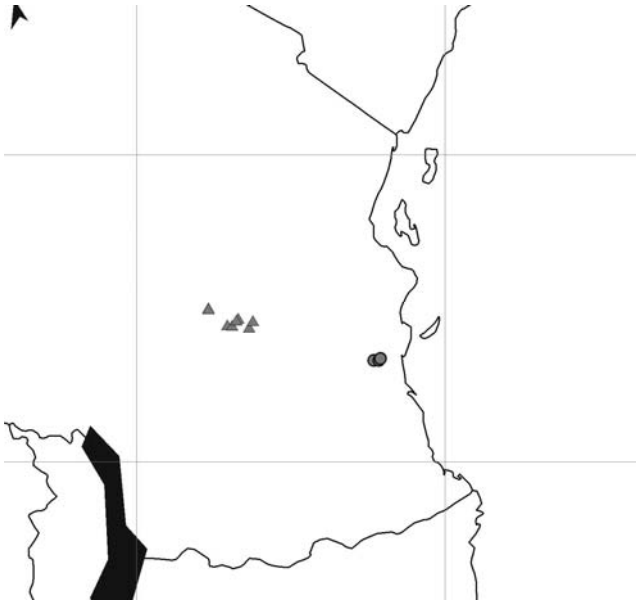


Figure 6.31. *Monodora hastipetala.* **A.** Flowering branch. **B.** Mature flower (top view), outer petals opened. **C**. Pedicel with sepals. **D.** Androecium and stigma. **E.** Stamen. **F.** Fruit. Drawings by Wil Wessel-Brand.

IUCN conservation status: CR B1ab(iii). *Monodora hastipetala* is endemic to the unprotected Kiwengoma Forest in the Matumbi Hills. It is represented by only 6 collections in

herbaria and has an extent of occurrence of 17km^2, justifying the "critically endangered" category.

Notes: *Monodora hastipetala* is distinctive in having long-acuminate inner petals and spatulate leaves. It is very unlike the other species in the region, *M. minor* and *M. carolinae*. It appears close to *M. junodii* by the shape of the inner petals, connivent at the center of the lamina which is corroborated by molecular data.



Map 25. Distibution of *Monodora globiflora* (triangles) and *M. hastipetala* (circles).

ADDITIONAL SPECIMENS EXAMINED:
TANZANIA: Pwani. Matumbi Hills. On small path from Nambunju to the Kiwengoma forest, 200 m, 18 November 2006, *T.L.P. Couvreur 42* (DSM, MO, NHT, WAG); 200 m, 18 November 2006, *T.L.P. Couvreur 44* (DSM, MO, WAG); Kiwengoma Forest, Northern edge of the Matumbi Highlands, 365m, 20 November 1989, *Frontier-Tanzania Coastal Forest Research Programe 22* (C, MO); Kiwengoma Forest, 31 January 1990, *Frontier-Tanzania Coastal Forest Research Programe 696* (MO); Matumbi Hills, Kiwengoma Forest, 19 October 1997, *P.B. Phillipson 4958* (MO); 8 June 1997, *P.B. Phillipson 4803* (MO).

7. Monodora junodii Engl. & Diels, Notizbl. Bot. Gart. Berl. 2: 301. 1899. — TYPE: MOZAMBIQUE. Maputo: Delagoa Bay, 1893, *H.A. Junod 411* (lectotype: B!, isotypes: BM!, COI!, G!, K!, Z!). *Figure 6.30 A-C*

Monodora junodii var. *macrantha* Paiva, Bol. Soc. Brot. 44: 373. 1971. — TYPE: MOZAMBIQUE. Zambezia: Maganja da Coasta, Gobene forest, 10 January 1968, *A.R. da Torre 17059* (holotype: LISC!; isotypes: B!, COI!, K!). ***syn. nov.***

Tree or shrub to 7-8 m high; trunk with d.b.h. up to 10 cm; outer bark light grey to brown, striate; young branches blackish with white lenticels, glabrous; old branches striate, grey, glabrous. *Petioles* 3-12 mm long, ca. 1 mm in diameter, glabrous, leaf lamina inserted on side, broadly grooved adaxially. *Leaf lamina* 5-16 cm long, 2-6 cm wide, length:width ratio 2-3.3,

narrowly obovate to obovate or narrowly elliptic to elliptic, base cuneate to obtuse to rounded, apex acuminate, acute or obtuse, acumen up to 9 mm long, sub-coriaceous or papyraceous, glabrous; midrib glabrous on both sides; secondary veins (7-)9-12(-17) pairs, uniformally curving upwards, glabrous. *Flowers* single, leaf-opposed, developing before or during leaf flush, pendulous. *Flowering pedicels* 5-30(-40) mm long, glabrous to sparsely covered with short appressed hairs. *Upper bract* inserted sub-basally to centrally on the pedicel, 9-14 mm long, 4-10 mm wide, length:width ratio 0.8-1.5, broadly ovate to ovate, base decurrent, apex rounded to obtuse, covered with short erect hairs. *Sepals* 5-15 mm long, 4-11 mm wide, length:width ratio 1.2-1.8, ovate to oblong, base truncate, apex acute, covered on both sides with short appressed hairs, pale green, non persistent in fruit; margins straight, densely covered with short curly hairs. *Outer petals* (18-)25-35(-41) mm long, 13-30 mm wide, length:width ratio 1.2-2, ovate to broadly ovate or elliptic to broadly elliptic, base narrowed, apex acute to rounded, sparsely covered with short erect hairs to glabrous on both surfaces, green with purple base when young, dark reddish-purple at anthesis; spreading horizontally then curving downwards, margins straight, densely covered with short curly hairs to glabrous. *Inner petals* (8-)10-21 mm long, 8-18 mm wide, length:width ratio 1.2-1.6, clawed ovate to rhomboid, base truncate or rounded to acute, apex rounded or acute to shortly acuminate, covered with short appressed hairs on both surfaces except on the proximal part of the lamina or sometimes glabrous on the outside, whitish-green with purple base, the centers of inner petal lamina pressed against each other over receptacle, presence with a bi-lobed gland at base of lamina; margins straight, folded outwards, covered with short erect hairs; claw curved inwards, 4-8 mm long, 2-4 mm long, claw:inner petal ratio 0.3-0.7, glabrous to sparsely covered with short erect hairs, white. *Receptacle* ca. 4 mm in diameter, slightly convex. *Stamens* in 8-10 rows, 0.7-1 mm long; connective shield 0.2 mm long, glabrous, those of inner whorl not extended over ovary wall. *Ovary* ca. 2 mm long, 1-1.2 mm in diameter; stigma ca. 1.5 mm in diameter, covered with short erect hairs. *Fruiting pedicels* 1-5 cm long, 3-5 mm in diameter. *Fruits* 20-55 mm long, 12-55 mm in diameter, length:width ratio 1-1.5, globose to broadly ovoid, wrinkled, constricted over seeds, glabrous, dark green spotted with white; pericarp 1-2 mm thick. *Seeds* 8-16 mm long, 5-8 mm wide, broadly ellipsoid, packed in white pulp; testa smooth, darkish brown; raphe very slightly thickened, dark brown; hilum 3-3.5 mm long, 1.5-2 mm wide, elliptical.

Distribution: Throughout East Africa, from Kenya to northern South Africa (see Map 26); in coastal riverside thicket on sand or in moist lowland forests or in moist evergreen coastal forests; at 10-900 m altitude.

Phenology: Mature flowers collected September to May. Fruits collected from October to May.

Vernacular names:
South-Africa: Green-apple (English); groenappel (Afrikaans).

Zimbabwe: Green-appel (English).

Uses: Wood has been used to make walking sticks.

IUCN conservation status: LC. *Monodora junodii* is well represented in herbaria and has been collected numerous times since 1990. It has been collected in several protected areas (forest reserves, national parks and a game reserve). Therefore, the category "least concern" seems justified.

Notes: *Monodora junodii* is distinct from other East African species by the ovate to broadly ovate outer petals and the straight margins of the inner and outer petals. However, *M. junodii* is a variable species in terms of the dimensions of the leaves, petiole, and pedicel length, as well as the shape of the leaves, which has led to the distinction of two varieties by Paiva (1970). Additionally, the shape of the inner petals can also vary being rounded to acuminate at apex. With significantly more specimens at hand, a continuum from one extreme to another can be observed, and thus the two varieties are no longer recognized. Moreover, as indicated above what unites this species is straight margins of the outer and inner petals as well as the inner petals being connivent by the center of the laminas, a rare character in *Monodora*.
This species resembles the endemic Cameroonian species *M. zenkeri* by the shape of the inner and outer petals but is different by the absence of two small lobes at the base of the outer petals. *Monodora junodii* also resembles the East African species *M. hastipetala* by the inner petals being connivent at the center as well as by the shape of the leaves. However, *M. hastipetala* has narrowly oblong outer petals and a long-acuminate inner petal apex, as well as smaller flowers. Molecular data strongly supports the sister relationship between the two species.

Extra references: Engl. & Diels, Monogr. Afrik. Pflanzen-Fam. 6: 86. Fig. 28D. 1901; Brenan & Greenway, Tanganyika Territory Ch. Lis., 2: 42. 1949; Robson, Fl. Zamb. 1, 1: 149. Fig. 16A. 1960; Verdcourt, Fl. Trop. E. Afr., Annonaceae 121. 1971; Palgrave, Trees of Southern Africa 2 ed., 174. 1981; Beentje, Kenya trees, shrubs and lianas 49. 1994.

ADDITIONAL SPECIMENS EXAMINED:
KENYA: Coast. Shimba Hills National Reserve, Makadara Forest, 380 m, 25 May 1971, *A. Evans 45* (K); Jadini Hotel forest, c. 36 km S of Mombasa, 15 m, 26 May 1971, *A. Evans 49* (K); Diani forest 30 km South of Mombasa, 29 March 1973, *S.P. Kibuwa 1229* (BR, EA, K); Pangani Rocks, 80 m, 1 May 1989, *W.R.Q. Luke 1831 A* (K, MO); Shimoni, 15 m, 17 December 1980, *W.R.Q. Luke 2668* (EA); Diani Forest, 10 m, 20 April 1993, *W.R.Q. Luke 3560* (K, MO); Kaya Bomu, 100 m, 22 April 1999, *W.R.Q. Luke 5741* (EA); Diana Forest, 10 m, 5 May 1985, *S.A. Robertson 3872* (K, MO, U, WAG); 3 km E of Wakala, 80 m, 13 April 1989, *S.A. Robertson 5680* (K); 3 km E of Wakala, 80 m, 22 April 1989, *S.A. Robertson 5708* (K, MO, US); Kaya Diani, 10 m, 23 August 1989, *S.A. Robertson 5889* (EA); Gongoni Forest Reserve, NE side, 30 m, 1 June 1990, *S.A. Robertson 6311* (EA).
MALAWI: Southern Province. Lengwe Reserve, NE corner, 90 m, 5 March 1970, *R.K. Brummitt 8881* (K); Mangochi District. East lake, 21 km N. of Malindi, 500 m, 19 November 1977, *R.K. Brummitt 15104* (K); Lengwe, 106m, 13 December 1970, *Hall-Martin, A.J. 1063* (FHO, K); Sokola, N.W. Nankumba, 19 September 1953, *G. Jackson 1388* (BR, FHO, K); Mulange Dist., Chingozi Hill (Northern side) in Mpinda village, 6 November 1983, *I.H. Patel 1324* (NY); Mulanje, Litchenya Hill north west of Mulanje, 12 January 1984, *I.H. Patel 1409* (K, MO); Nkandwe Hill above Nkopola, 22 December 1984, *I.H. Patel 1722* (MO); Sambani Forest reserve, 6 December 1989, *Tawakali, E.J. 1713* (NY).

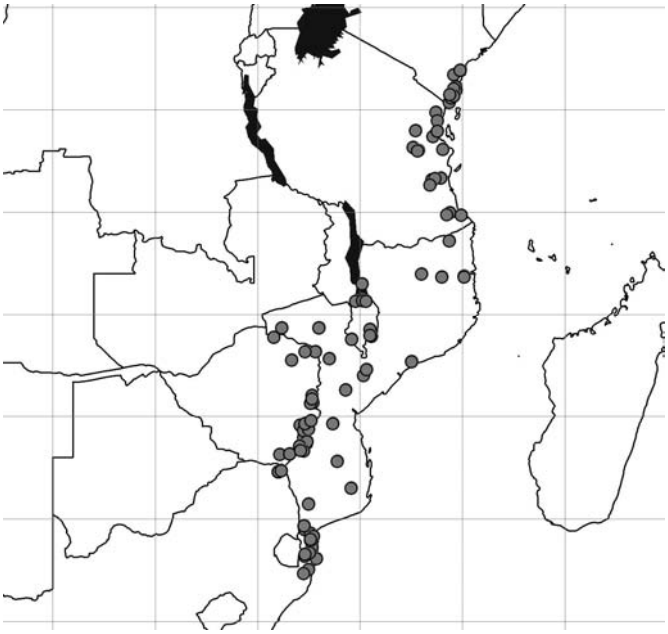
MOZAMBIQUE: Cabo Delgado. Lpiério, estrada para Tehamba, 19 October 1948, *E.C. Andrada 1422* (K); Distrito de Matera, Namacuto, 350 m, 30 January 1984, *E.M.C. Groenendijk 869* (K, MO, WAG); Mueda Plateau, 790 m, 12 December 2003, *W.R.Q. Luke 10053* (MO); Pemba, Ancuabe; Metoro. Namatuca, monte Nametiri, 368m, 1 February 1984, *A. Maite 216* (WAG); Niassa, entre Lurio y Chamba, 19 October 1948, *J. Pedrógáo de Jesus 5568* (EA); Montepues, andados 5 km de Montepues para Nantulo, 530 m, 27 December 1963, *A.R. da Torre 9735* (K, WAG); **Gaza**. Inhambane: Govuro, andados 14 kms da povoaçao Banamana para Machaíla, 25 March 1974, *M.F. Correia 4198* (WAG); Mavume, 10 km N of the official residence, October 1938, *A. de Figueiredo Gomes e Sousa 2164* (K); Limpopo: Guijá, entre os rios Masimechopes e dos Elefantes, 1 December 1944, *F.A. Mendonça 3202* (BR, LISC, WAG); **Manica e Sofala**. Madanda forests, 121 m, 5 December 1906, *C.F.M. Swynnerton 1765* (K); Manica E Sofala: Baruè, Mungari, a 54 km, estrada para Tambara, 300 m, 16 December 1965, *A.R. da Torre 13684 A* (K); **Maputo**. Lourenco Marques: Sabié, ao 29 km de Manhica para Chinhanguanine, 4 February 1969, *M.F. Correia 570* (K, MO); Santaca, région of Maputo, 14 February 1949, *A. de Figueiredo Gomes e Sousa 3875* (K); Junto da estrada Santaca-Catuane, regio da Maputo, 22 September 1948, *A. de Figueiredo Gomes e Sousa 3879* (BR, K, L, MO); Maputo, Matutuine, Reserve florestal Licuati, 27 January 1983, *E.M.C. Groenendijk 213* (MO); Delagoa bay, 1893, *H.A. Junod 411* (B, BM, COI, G, K, Z); Lourenco Marques, 6 December 1897, *H.A. Junod 11630* (K); Lourenco Marques, etre a Costa do Sol e Vila Luiza, 9 November 1958, *L. Macuácua 69* (K); Lourenco-Marques, Marracuene, ao 2.9 km de Vila Luisa, para a Costa do Sol picada a esquerda para os lados do Rio Incomati, 23 October 1969, *A. Marques 2220* (MO); Maputo. Bela Vista, 14 November 1944, *F.A. Mendonça 2838* (LISC, WAG); Lourenço Marques; terras de Guija, entre os rios Mazimechopes e dos Elefantes, 1 December 1944, *F.A. Mendonça 3216* (BR); Lourenco Marques, 54m, 6 December 1897, *F.R.R. Schlechter 11630* (BM, COI, G, HBG, K, S, WAG, Z); 54m, 6 December 1897, *F.R.R. Schlechter 11630* (WAG); Entre bela Vista e Umbeluzi, 20 November 1940, *A.R. da Torre 2093* (A); Maomba, Sàbié, picada em direcçao a Incomanine, 10 October 1978, *D. Zunguze 178* (MO, WAG); Maputo, Matutuine, Catuane, propriedade de Makhandene, 2 December 1982, *D. Zunguze 438* (BR, MO, P); **Niassa**. Kpjes east of Chipinda Pools, Chiredzi district, 14 October 1951, *Mullin, L.J. 51/ 121* (MO); **Sofala**. Manica e Sofala, 36 km NE Inhamitanga, 1 km N of railway, 200 m, 5 December 1971, *T. Müller 1902* (K, MO); Gorongosa, Parque Nacional de Caça, na picada no 4, entre os cruzamentos km a picade no 3 e a picade no 2, 40 m, 4 November 1963, *A.R. da Torre 9035* (WAG); **Tete**. Tete district, Estima-Candodo, 25 January 1972, *J.M. Aguiar Macêdo 4672* (MO); Village Cabora Bassa, 2 km towards the mountains from the river Zambeze, 400 m, 25 December 1973, *M.F. Correia 3830* (M); Village Cabora Bassa, 1.5 km towards the mountains from the river Zambeze, 400 m, 20 November 1973, *M.F. Correia 3863* (M); Tete: Cabora Bassa, ao longo de linha de crista dos Montes con cotas, 330-533m, 19 May 1972, *A. Pereira 2749* (MO); **Zambézia**. River Shire Valley, 12 December 1863, *J. Kirk s.n.* (K); Maganja da Costa, floresta de Gobene. prox. da praia Raraga, ao 40 km de Vila de Maganja, 20 m, 10 January 1968, *A.R. da Torre 17059* (COI, K); Maganja da Costa, floresta de Gobene. prox. da praia Raraga, ao 48 km de Vila de Maganja, 20 m, 12 February 1966, *A.R. da Torre 14579* (K); **Unknown.** no location, 10 February 1921, *G. Coombes s.n.* (K); Likabula River Chikwawa, 3 July 1955, *G. Jackson 1710* (FHO).

SOUTH AFRICA: Kwazulu-Natal. 3/4 mile from Makanes Pont on Kosi Bay road, 90 m, 30 January 1963, *D. Edwards 2979* (K, M, PRE, WAG); Natal, 10 miles Pongola Bridge-Maputa, 8 November 1969, *E.J. Moll 4384* (K); Zululand Distrit. Makatini-ulakte, 17 miles from Jozini to Mbazwane, 8 December 1964 *J. Vahrmeijer, 195* (K); Mkuzi game reserve, 11 December 1959, *C.J. Ward 3371* (K); **Northern Transvaal**. Kruger National Park, Near Panda Maria-Lawveld, 457m, 30 October 1948, *L.E. Codd 4529* (K); Kruger National Park, Punda Maria, 7 January 1950, *W. Lamont 42* (K); 15 November 1932, *H. Lang 32119* (K); Kruger National Park. Punda Maria, 518m, 15 October 1952, *H.P. Schijff van der 930* (K); Kruger National Park, SE of Klopperfontein, 30 April 1953, *Schijff, H.P. van der 2949* (K); **Transvaal**. behind Punda Maria, 31 October 1948, *L.E. Codd 4539* (K, NY); **Unknown.** 4 mile NE of Sihangwa Store, 6 March 1970, *J.H. Ross 2375* (K).

TANZANIA: Dar es Salaam. Pugu Hills, 10 December 1939, *J.H. Vaughan 2922* (EA, K); **Lindi**. Rondo plateau, Rondo forest reserve, below forest Station, 650 m, 13 February 1991, *S. Bidgood MCR 1531* (C, K); On track between Madangwa and Sudi, 3-5 km from Sudi, 50 m, 6 March 1996, *D.M. Johnson 1904* (OWU, WAG); Kingupira forest, Selous km Reserve, 2 December 1969, *Ludanga, R. 837* (EA); Lindi, 40 km West, Lutanba see Hill, 225m, 4 December 1934, *H.J.E. Schlieben 5685* (B, BR, G, K, MO, P, Z); 7 km NNW of Kingupina, Selous km Reserve, 125m, 22 May 1975, *K. Vollesen MRC 2343* (C, EA); About 5 km NNW of Kingupira, 125m, 19 November 1975, *K. Vollesen MRC 3011* (C, EA, K, WAG); Nahilala Valley, Selous km Reserve, 300 m, 14 December 1975, *K. Vollesen MRC 3091* (C, EA, K); About 5 km NNW of Kingupira, 125m, 17 December 1976, *K. Vollesen MRC 4232* (C, EA, K, WAG); About 5 km NNW of Kingupira, 125m, 17 December 1976, *K. Vollesen MRC 4234* (C, EA, K, WAG); **Morogoro**. on slope of big rocky hill, just after field, road to Kimboza Forest Reserve, after Mkuyuni village, 400 m, 26 November 2006, *T.L.P. Couvreur 88* (DSM, MO, WAG); Nguru Mts. Matumle beneath bourge rockwell N of Mhonda Mission, 400-1600 m, 7 November 1991, *M. Manktelow 91/ 220* (BR); Uluguru Mountains. Ruvu Forest Reserve, 250 m, 18 July 2000, *E.B. Mhoro UMBCP 138* (MO); Kanga Mts, North of Morogora town, 3 December 1987, *L.B. Mwasumbi 13871* (MO, WAG); Turiani, November 1953, *S.R. Semsei 1473* (FHO, K); **Pwani**. Bagamoyo, 150 m, 8 September 1999, *Y.S. Abeid 685* (MO); Matumbi Hills., 250 m, 4 February 1998, *O.A. Kibure 175* (MO); Matumbi Hills. Along road to Kiwengoma Forest., 250 m, 18 October 1997, *P.B. Phillipson 4940* (L); Kiono Forest Reserve, 21 August 1989, *Ralangaranga, Z.K. 185* (MO); Kiono Forest Reserve, 21 August 1989, *Z.K. Rulangaranga 185* (MO); **Tanga**. Eastern Usambara Mts., Western slopes of the Mlinga Massif, between Tongwe Mission and Mt. Mlinga, 250-400 m, 31 March 1974, *R.B. Faden 74/ 376* (BR, K, MO, WAG); Msumbugwe Forest Reserve. 20 km NE of Genda Genda Village, 130 m, 22 August 1991, *F.M. Mbago 872* (MO); East Usambaras, 30 January 1917, *A. Peter 19207* (B).

ZIMBABWE: Manicaland. 17 miles south of Umtali/ Umtali district, 915m, 20 November 1952, *N.C. Chase 4732* (COI, K); Road side north of Wengezi River, 600 m, 16 January 1966, *N.C. Chase 8350* (K, MO); Zimunya s Reserve, 3 miles on Bazeley Bridge raod, 600 m, 7 October 1956, *N.C. Chase 6214* (B, BR, K, S); 16 miles S of Utsli, Rowa Reserve, 914m, 30 November 1952, *N.C. Chase 40265* (K); Zimunya Reserve, 914m, 20 March 1955, *N.C. Chase 5504* (K); Between river

Chikuramadziwa and Matekwatekwa hill, Farm 44, Zimunya reserve, 1097m, 12 February 1961, *N.C. Chase 7431* (K); Nyangamba river Valley, March 1962, *B. Goldsmith 62/ 84* (K, MO); near Matobo, near P.E.A. border, Sabi Valley, East Bank, 7 December 1956, *R. Goodier 102* (EA, K); Few hundred yards from Sabi river, Dotts Drift camp, 17 November 1959, *Johnstone, P.A. 67* (MO); Sabi Valley, East bank, 5 December 1956, *Salisbury 102* (COI); **Mashonaland central**. Mtoko district. Near Nyadesi, between Wutu and Nyakasanga rivers. Fungwi Reserve, 18 October 1955, *D.F. Lovemore 450* (K); Chipoli, Mazoe District, 2 November 1958, *D.M. Moubray 59* (K); Gonono area, 4 April 1998, *P. Poilécot 7800* (G); Mkota Reserve, Sekoni, 20 September 1951, *J.A. Whellan 521* (K); **Masvingo (Victoria)**. Tswiza, Gona Re Zho Games Reserve, 500 m, September 1955, *R.M. Davies 1626* (K, MO); District Muanetsi [=Mwenezi], overlooking rapids Lundi River, November 1956, *R.M. Davies 2199* (K); Melangwe river, south west of Mateke Hills, 625m, 6 May 1958, *R.B. Drummond 5638* (M); near Kapateni, 64 km NE of Malvernia, 25 April 1962, *R.B. Drummond 7727* (K); Nuaneksi, 9 November 1951, *D.E. Gibson 51/ 75* (BR); Chipinge district, 13 February 1957, *R. Goodier 120* (K); 30 km W of Fishan, 9 September 1967, *T. Müller 626* (K); Chipinda pools, on hill to the east, 13 October 1951, *G.M. McGregor 86* (B); Chipinge District, E. Sabi. 5 miles of Hippo. Mopane sandveld, 381m, 21 January 1957, *J.B. Phipps 63* (K); 32 km SW of Sabi, Lundi Rivers. Junction on Mozambique-Rhodesia border, 30 November 1968, *B.Y. Sherry SRGH 191977* (K); Lone Star Ranche. Near ranch homestead, 12 January 1971, *P. Taylor 46* (K, SRGH, WAG); Lower Sabi, June 1948, *H. Wild 2369* (NY); **Matabeleland South**. Iswiza, 31 October 1955, *H. Wild 4716* (K); **Unknown.** 1/2 km from the confluence of the Nyamazisi R. and the Rwenija River, 20 October 1982, *Munjoma, S. 18* (MO).
UNKNOWN. Unknown. Rikatla, October 1917, *H.A. Junod 104* (G); no location, 1908, *H.A. Junod 2926* (G).



Map 26. Distribution of *Monodora junodii*.

8. Monodora laurentii De Wild., Ann. Mus. Congo Belge, Bot. Sér. 5, tome 2: 84. 1909. — TYPE: DEMOCRATIC REPUBLIC OF CONGO. Bas Congo: Sabuka-Léopoldville, 27 February 1905, *M. Laurent 498* (holotype: BR!, isotype BR!). *Figure 6.32*

Tree to 4 m high, d.b.h. unknown; young branches drying black, glabrous; old branches dark brown, glabrous. *Petioles* 5-7 mm long, 1-1.5 mm in diameter, glabrous, lamina inserted on side, broadly grooved adaxially. *Leaf lamina* 12-14 cm long, 3.5-4.5 cm wide, length:width ratio 2.5-5.3, narrowly ovate to ovate, base acute to obtuse, apex obtusely acuminate, acumen 15-20 mm long, papyraceous when young becoming coriaceous, glabrous; secondary veins 6-10 pairs, uniformally curving upwards, glabrous. *Flowers* single, leaf-opposed, pendulous, on old branches or on newly developed branches. *Flowering pedicels* 15-20 mm long, ca. 0.8 mm in diameter, glabrous. *Upper bract* attached central to subbasal to the pedicel, 9-10 mm

long, ca. 4 mm, length:width ratio 2.25-2.5, narrowly elliptic, base decurrent, apex acute, glabrous. *Sepals* 8-10 mm long, 3 mm wide, length:width ratio ca. 2.6-3.3, narrowly oblong, base truncate, apex acute, glabrous, green, spreading horizontally, falling when in fruit, margins straight, glabrous. *Outer petals* 35-50 mm long, 9-12 mm wide, length:width ratio 3.8-4, narrowly ovate, base cordate, apex acute, glabrous, white pinkish streaked with yellow and green, spreading horizontally then curving slightly downwards, margins straight, glabrous. *Inner petals* 8-9 mm long, 10-12 mm wide, length:width ratio 0.7-0.8, clawed rhomboid, base cuneate, apex acute, inside covered with linear ribbon-like hairs, glabrous outside, connivent at margins over receptacle; margins straight, with short curly hairs; claw ca. 2 mm long, ca. 3 mm wide, claw:inner petal ratio 0.2-0.3, glabrous. *Receptacle* 3-4 mm in diameter, flat to slightly convex. *Stamens* in 6-7 rows, ca. 0.7 mm long, glabrous; connective shield 0.1 mm long, those on innermost whorl not extending over ovary wall. *Ovary* ca. 2 mm long, ca. 1.5 mm wide; stigma ca. 1.5 mm in diameter, glabrous. *Fruiting pedicels* ca. 15 cm long and ca. 5 mm in diameter, glabrous. *Fruits* 4.5-6.5 cm long, 2-2.5 cm in diameter, length:width ratio 2.25-2.6, conic, apiculate, apicule ca. 5 mm long, smooth but conspicuously 5-6 ribbed, ribs thin, glabrous; pericarp 1-3 mm thick. *Seeds* 12-15 mm long, 10-11 mm wide, transversely ellipsoid, no apparent pulp in dried material; testa smooth, dark brown; raphe thickened, very dark brown; hilum 3-4 mm long, 1.5-2 mm wide, elliptical.

Distribution: Disjunct, Gabon, Republic of Congo (Brazzaville) and the Democratic Republic of Congo (see Map 27); in primary lowland rain forests; at 400-500 m altitude.

Phenology: Mature flowers collected from October to April. Mature fruits collected in October, January to February and June.

Vernacular names:
Democratic Republic of Congo: Emgambuli, Ligala (Yambata); Ifambola (Mongo).

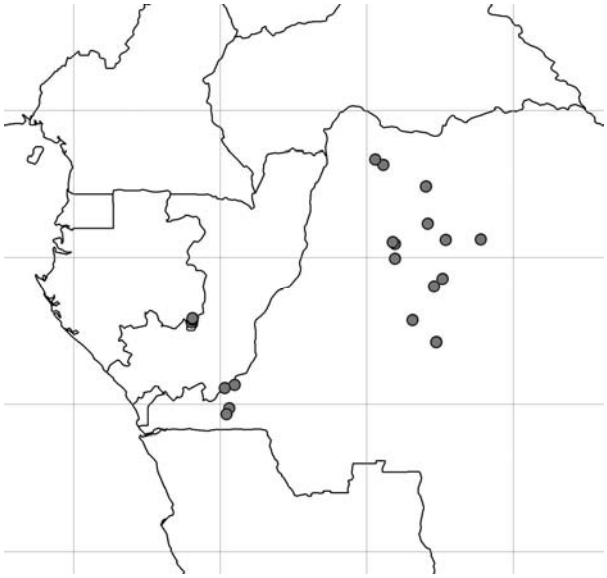
Uses: The wood is very hard and serves for making spears. Bark used as a remedy against diarrhoea. The fruits are eaten by the indigenous people (Boutique, 1971b).

ICUN conservation status: VUB2ab(iii). *Monodora laurentii* is moderately represented in herbaria. It has not been collected since 1960, except in Gabon where it was collected for the first time in 2003. Although there are not many collections of this species, some specimen labels indicate that it is locally abundant. It occurs in one national park, namely the Plateau Batéké in south-eastern Gabon. The area of occupancy is low (200 km^2). There are more than five locations but less than ten, which favors the “vulnerable” category. However, most of the collections come from the North-East of the Democratic Republic of Congo, where conflicts over the past few years have made collections and therefore assessments of current status virtually impossible.

Notes: *M. laurentii* can be distinguished from other species of *Monodora* by the distinctly acuminate leaf lamina, outer petals with straight margins, and presence of ribbon-like hairs on the inner side of the inner petals. The non undulate petals bear a resemblance with those of *M. zenkeri* and the east African *M. junodii*. However, the structure and connivance of the inner petals at the margins places it closer to *M. myristica* and *M. undulata*. This affinity is corroborated by molecular data which moderately supported *M. myristica* as sister to *M. laurentii*.

The presence of the ribbon-like linear hairs in the middle of the inner petal is quite remarkable for the whole genus. A small note from flowers collected at the Laeken Botanical Garden, Belgium, indicates that when the flower opens, the stigmata and/or the whole ovary detaches and falls off being captured by globular and slimy hairs in the inner part of the inner petals. More data are needed to understand the function of these ribbons though they may play some role in pollination.

Extra references: Boutique, Fl. Congo Belge 2: 269. 1951.



Map 27. Distribution of *Monodora laurentii*.

ADDITIONAL SPECIMENS EXAMINED:

DEMOCRATIC REPUBLIC OF CONGO: Bas-Congo. Kinkasi, 8 February 1953, *H. Callens 143* (BR); Kisantu, 23 October 1949, *H. Callens 2126* (K); Sabuka, 27 February 1905, *M. Laurent 498* (BR); Entre Kole et Sekese, October 1932, *J. Lebrun 6373* (BR); **Equateur**. Djombo, January 1913, *A. Collaer13* (BR); Yambata, January 1914, *S. De Giorgi 1617* (BR); Inéac Bongabo, 6 June 1955, *C.M. Evrard 1116* (BR); près de Loile, 13 October 1957, *C.M. Evrard 2819* (BR); Befale, Riviere Ifale, 24 October 1957, *C.M. Evrard 2899* (BR, M); Befale, 19 February 1958, *C.M. Evrard 3510* (BR); Iwama Eandza Yalikungu (Mondombe), 7 January 1959, *C.M. Evrard 5480* (BR, M); Bokota, 13 February 1959, *C.M. Evrard 5695* (BR); road Djolu-Simba, 18 February 1959, *C.M. Evrard 5731* (K); Karawa, 2 April 1924, *A.P.G. Goossens 4428* (BR); Yambata, 2 December 1913, *H. Montchal 131* (BR); Dundusana, November 1913, *M.G. Mortehan 688* (BR); October 1913, *M.G. Mortehan 608* (BR); January 1914, *M.G. Mortehan 1055* (BR); **Kinshasa**. Bongoy, 13 January 1958, *C.M. Evrard 3307* (K); **Orientale**. Yabwesu, 14 March 1957, *C.M. Evrard 2197* (K).

GABON: Haut-Ogooué. Plateaux Batéké National Parc. Station Projet Protection des Gorilles, piste M 2810, 510 m, 1 March 2003, *R. Niangadouma 179* (LBV, MO); Plateaux Batéké National Parc. Galerie forestière avec une canopé haute et fermeé. Sous-bois assez ouvert et dense. 300 m du Canion, 420 m, 2 March 2003, *R. Niangadouma 188* (LBV, MO); à 4,5 km au Nord de la station PPG, 400 m, 30 January 2004, *R. Niangadouma 405* (LBV, MO, WAG).

REPUBLIC OF THE CONGO: Unknown. Foret de Uayama, February 1959, *J. Koechlin 5720* (P).

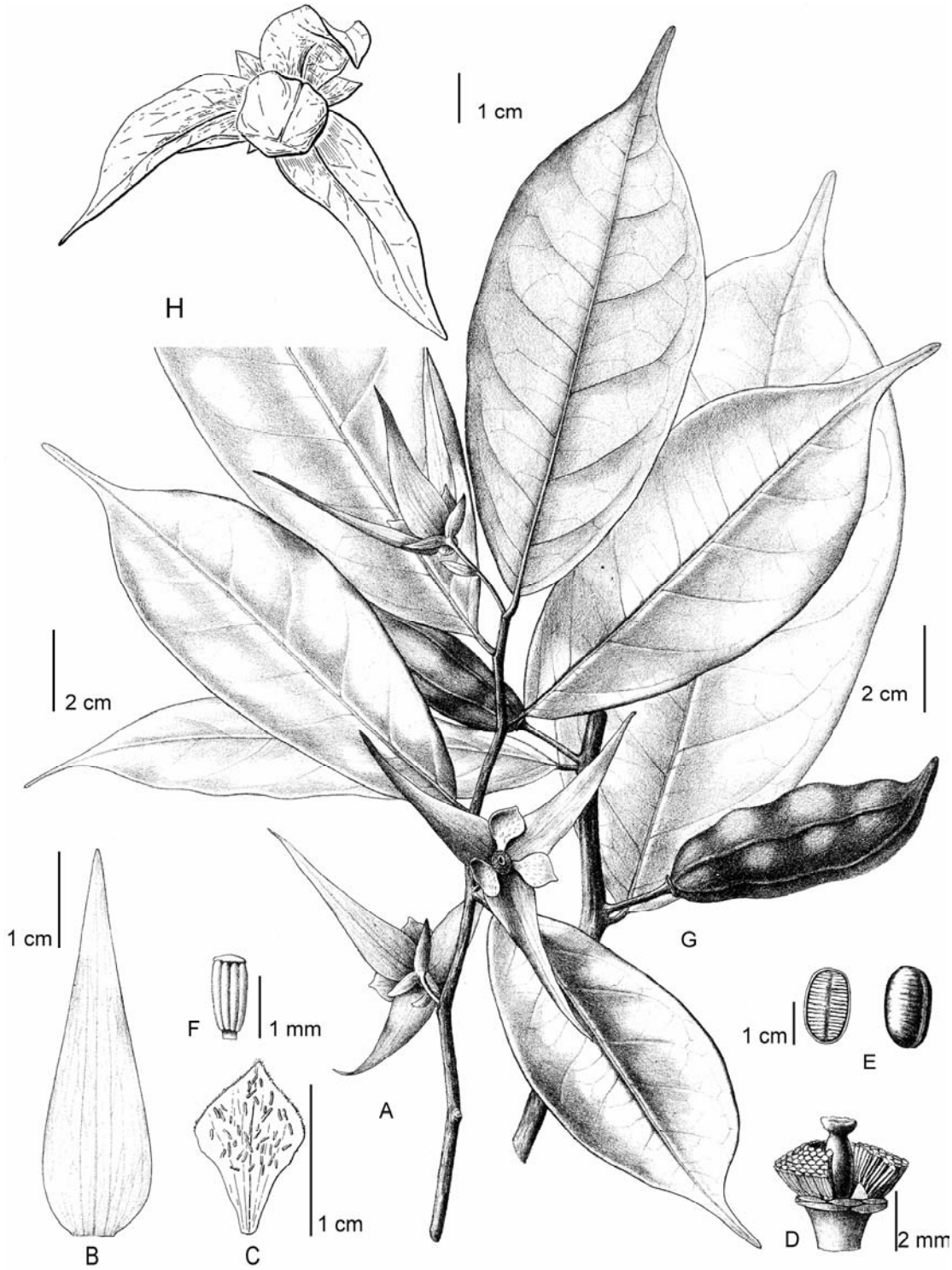


Figure 6.32. *Monodora laurentii*. A. Flowering branch with inner petals opened. B. Outer petal (outside surface). C. Inner petal (inside surface). D. Receptacle with ovary, stigma and stamens. E. Seeds. G. Fruiting branch. H. Flower with natural position of inner petals. Modified from De Wildeman, Fig. 21, 1909. Drawings C and H by Joanne Porck.

9. Monodora minor Engl. & Diels, Monogr. Afrik. Pflanzen-Fam. 6: 88, tab. 28A. 1901. TYPE: TANZANIA. Pwani: Pugu Hills, *W. Goetze 3* (lectotype: B!). *Figure 6.33*

Tree to 6-7 m high; trunk with d.b.h. up to 5 cm; bark smooth, blue-grey; young branches green, smooth, glabrous, with a bluish indument on the dried material; old branches dark grey to brown, striate, glabrous. *Petioles* 4-5(-7) mm long, 1-1.5 mm in diameter, glabrous, lamina inserted on side, broadly grooved adaxially, with grey wax layer. *Leaf lamina* 7-13 cm long, 3.5-6 cm wide, length:width ratio 1.8-2(3), obovate or rarely narrowly obovate, base rounded to acute, apex acuminate, acumen ca. 10 mm, coriaceous, glabrous, dark green glossy above, flaccid polish green below; midrib but sunken towards the base afaxially, glabrous on both sides; secondary veins 8-11 pairs, uniformally curving upwards, glabrous. *Inflorescences* erect above foliage, 2-3-flowered rhipidia, leaf-opposed or supra axillary, born during the leaf flush. *Flowering pedicels* 1-3 cm long, glabrous, with grey wax layer. *Upper bract* inserted around the centre tor sub-apically on the pedicel, 25-30 mm long, ca. 15 mm wide, length:width ratio 1.5-2, ovate, base decurrent, apex slightly acuminate, glabrous, light green with red steaks towards base; margins undulate, glabrous. *Sepals* 6-10 mm long, 3-5 mm long, length:width ratio 1.5-2, ovate, base truncate, apex rounded to acute, glabrous, green-grey with red steaks towards base; strongly curved upwards, falling when in fruit, margins undulate, glabrous. *Outer petals* 11-25 mm long, 5-12 mm wide, length:width ratio 1.8-2.5, ovate to narrowly ovate, base truncate, apex acute to rounded, glabrous, yellow streaked with red; hanging down when immature to spreading upwards at anthesis, margins undulate, glabrous. *Inner petals* 5-8 mm long, 6-13 mm wide, length:width ratio 0.5-1.1, clawed, cordate, base cordate, apex acuminate to obtuse, inside very densely covered with ca. 2 mm long straight hairs, glabrous outside, light yellow striped with purple and red, lamina connivent at margins over receptacle, margins non undulate, densely covered with short curly hairs; claw 3-5 mm long, 2-3 mm wide, claw:inner petal ratio 0.6-0.7, glabrous, bright yellow. *Receptacle* 4-5 mm in diameter, slightly convex. *Stamens* in 6-8 rows, ca. 0.7 mm long; connective shield ca. 0.2 long, densely covered with short erect hairs, yellow whitish, those of innermost row slightly appressed against ovary wall. *Ovary* 0.8-1 mm long, ca. 0.8 mm wide; stigma 1-1.5 mm in diameter, glabrous to sparsely covered with short erect hairs. *Fruiting pedicels* ca. 7 cm long, 2-3 mm in diameter, glabrous. *Fruits* 25-35 mm long, 15-20 mm in diameter, length:width ratio 1.7-2, ellipsoid to ovoid, apiculate, apicule 3-4 mm long, smooth, glabrous, pale green with white dots, covered in places with grey wax layer; pericarp 1-2 mm thick. *Seeds* 10-13 mm long, 8-10 mm wide, transversely to flat ellipsoid, no apparent pulp on dried material; testa smooth, dark brown; raphe thickened, rugose, very dark brown; hilum 4-5 mm long, ca. 2 mm wide, narrowly elliptical.

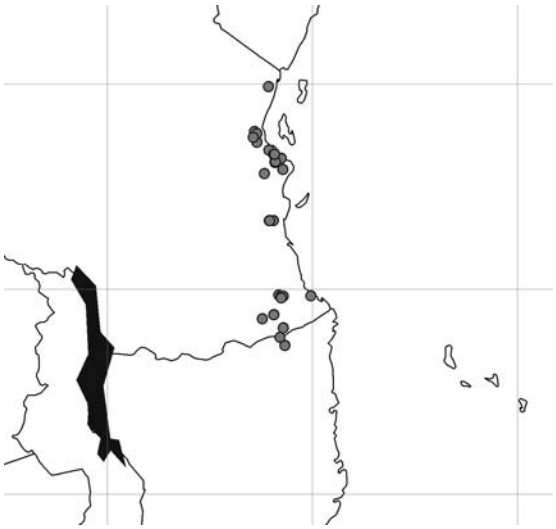
Distribution: Coastal Tanzania and the very North of Mozambique (see Map 28); in woodland, coastal wet forests and thickets; at 100-780 m altitude.

Vernacular names:
Tanzania: Mnjende (Kimatumbi); Komanyuk, Mohdaro or Uvalie (Swahili); Ngamba (Yao).

IUCN conservation status: VU B2ab(ii, iii). *Monodora minor* is moderately represented in herbaria. It is mainly found in small patches of coastal forests near populated areas. It has been collected from several forest reserves (Rondo, Zaraninge, Pande, Pugu and Bane in Tanzania). The area of occupancy is less than 500 km^2 but it is found in about ten locations, so the "vulnerable" category seems appropriate.

Notes: *Monodora minor* is easily recognizable by its many-flowered erect rhipidia, while all other species are strictly one-flowered and pendulous, and by the characteristic grey wax layer found on young leaves, branches, pedicels, and fruits. Based on flower morphology this species closely resembles *M. carolinae* by the connivance of the inner petals and the upward spreading outer petals. The position of *M. minor* in the molecular phylogeny is, however, unresolved within the East African clade. The lectotype of *M. minor* was chosen by Brenan & Greenway in Tanganyika Territory Ch. Lis. 2: 43. 1949.

Extra references: Brenan & Greenway, Tanganyika Territory Ch. Lis. 2: 43. 1949; Verdcourt, Fl. Trop. E. Afr., Annonaceae 117. 1971.



Map 28. Distribution of *Monodora minor*.

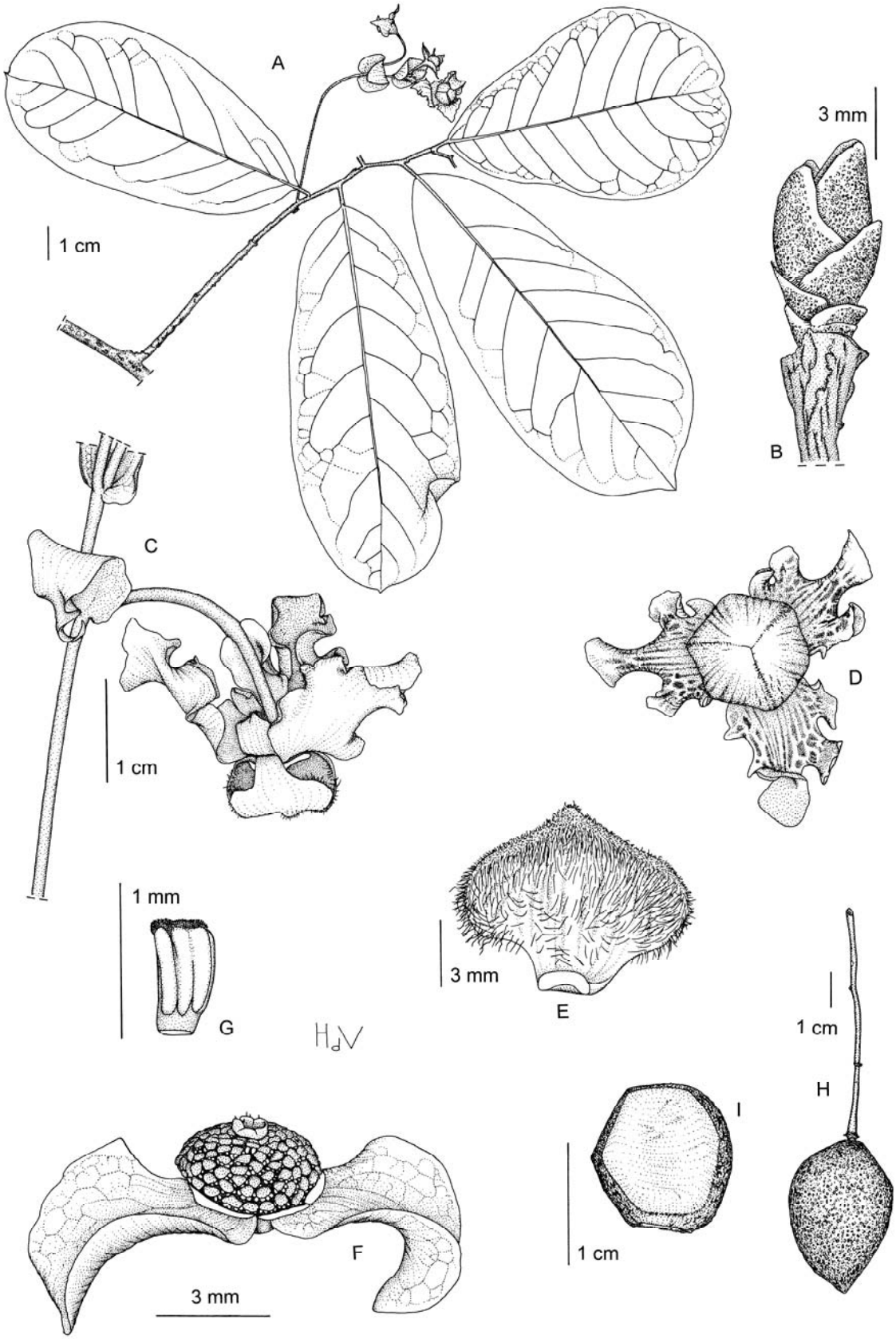


Figure 6.33. *Monodora minor*. A. Flowering branch. B. Leaf bud. C. Flower (side view). D. Flower (top view). E. Inner petal (inside surface). F. Receptacle with stamens, stigma, and two sepals. G. Stamen. H. Fruit. I. Seed. Drawings by Hans de Vries.

ADDITIONAL SPECIMENS EXAMINED:

MOZAMBIQUE: Nampula. Mueda Plateau, 570 m, 13 December 2003, *W.R.Q. Luke 10081* (UPS).
TANZANIA: Dar es Salaam. Pande, W edge, 8 July 1982, *W.D. Hawthorne 1072* (DSM); Pande Forest reserve,.SW of Bunju village on Bagamoyo Road ca. 20 km N. of Dar Es Salaam, 100-120 m, 3 February 1996, *D.M. Johnson 1881* (OWU); Pande Forest reserve, 100-120 m, 3 February 1996, *D.M. Johnson 1880* (OWU); Dar es Salaam, no date, *J. Kirk 4181* (K); Dar es Salaam, no date, *J. Kirk s.n.* (K); Bane Forest Reserve, 29 October 1965, *Mgaza 783* (EA, K); Pande Forest Reserve, 25 km NNW of Dar es Salaam, 150 m, 24 November 1984, *L.B. Mwasumbi 12701* (BR, MO, P, WAG); Pande Forest Reserve, 23 km WNW of Dar Es Salaam, 110-190 m, 8 February 1976, *R.C. Wingfield 3309* (DSM); **Lindi**. Rondo Forest reserve, 700 m, 14 February 1991, *S. Bidgood 1571* (K, NHT); St. Cyprinas College, Rondo Plateau, 600 m, 17 February 1991, *S. Bidgood 1618* (K, NHT); South face of Rondo escarp, Melurijiri, 780 m, December 1951, *W.J. Eggeling 6412* (FHO, K); Rondo Plateau, Mchinjiri, December 1951, *W.J. Eggeling 6413* (K); Rondo, 500-1000 m, 12 May 1985, *T.W. Fison 90* (K); Sudi, 16 December 1942, *Gillman, H. 1140* (K); Chidya, "South Wood", Masasi district, 600 m, 15 December 1966, *J.A.H. Leonhardt11* (DSM, EA); **Morogoro**. Kazimzumbwi Forest, 150 m, March 1991, *Frontier-Tanzania Coastal Forest Research Programe 2033* (MO); **Mtwara**. Newala, 26 December 1958, *W. Hay 16* (K); Chidya, Masasi district, 700 m, December 1969, *J.A.H. Leonhardt 337* (EA); Chidya, "South Wood", 610 m, 17 December 1966, *J.A.H. Leonhardt 17* (DSM); **Pwani**. Zaraninge Forest Reserve. Gongo Village. Forest near WWF office, 260 m, 6 January 1998, *Y.S. Abeid 199* (MO); road between Bagamoyo and Msata at Fukayosi village, 100 m, 14 November 2006, *T.L.P. Couvreur 36* (DSM, MO, NHT, WAG); Matumbi Hills. Nambunju village, on path just before 200 m before arriving to the old WWF house, 250 m, 18 November 2006, *T.L.P. Couvreur 55* (DSM, WAG); Matumbi Hills, on slope just above Kitapi village, 5 km from Mbwara, 230 m, 19 November 2006, *T.L.P. Couvreur 64* (DSM, MO, NHT, WAG); Sachsenwald and Pugu Hills, no date, *W. Goetze 3* (B); 21 mi S of DSM on road to Kilwa, 150 m, 25 January 1970, *B.J. Harris 3974* (DSM); Pugu Forest Rserve, along N raod 0.5 km E of brick factory, trail up from north side of road, 100-200 m, 16 February 1996, *D.M. Johnson 1884 B* (OWU); Pugu Forest Reserve, ridges along north road between Pugu railway station and brick works, 100-200 m, 29 February 1996, *D.M. Johnson 1985* (OWU); Matumbi Hills. Kiwengoma forest., 250 m, 6 October 1998, *O.A. Kibure 297* (MO); Bagamoyo, Zaraninge Forest Reserve, 260 m, 31 December 2000, *F.M. Mbago 1906* (NY); Usaramo, 29 October 1925, *A. Peter 31590* (B, K, WAG); **Tanga**. Kazimzumbwi forest, in the Pugu Hills, south of Kisarawe, 150 m, May 1991, *Frontier-Tanzania Coastal Forest Research Programe 1749* (MO); Pande forest reserve, 19 August 1982, *W.D. Hawthorne 1477* (K); **Unknown.** no location, 1901, *W. Busse 1107* (G, P).

10. Monodora myristica (Gaertn.) Dunal, Monogr. Anon. 3: 80. 1817. *Annona myristica* Gaertn., Fruct. Sem. Pl. 2: 194. 1791. TYPE — JAMAICA. Specimen cultivated in Jamaica obtained from Banks (holotype: BM!). *Figure 6.34*

Monodora borealis Scott-Elliot, Journ. Linn. Soc., Bot. 30: 72. 1895. — TYPE: SIERRA LEONE. Northern Province: Scarcies Rivers, 7 June 1892, *G.F. Scott-Elliot 4716* (holotype: K!).

Monodora claessensii De Wild., Bull. Jard. Bot. État Brux. 3: 263. 1911. — TYPE: DEMOCRATIC REPUBLIC OF CONGO. Maniema: Kindu, 1910, *J. Claessens 504* (holotype: BR!, isotypes: BR-2 sheets!).

Tree to 35-40 m; trunk with d.b.h. up to 40(-100) cm; crown drooping, bole cylindrical, fissured with some flutes present; bark pale grey; young branches drying black sometimes sparsely spotted with white lenticels, with blue-greyish wax layer; old branches ash-grey to brown. *Petioles* 8-14 mm long, 1-2 mm in diameter, leaf lamina inserted side, broadly grooved adaxially, sometimes with blue-greyish wax layer. *Leaf lamina* 11-50 cm long, 4-14 cm wide, length:width ratio 2-3.5, narrowly obovate to obovate or narrowly elliptic to elliptic, base cordate to rounded or rarely cuneate, apex acuminate, acumen 10-15 mm long, membranous to coriaceous, glabrous, green above, pale green below; midrib raised and glabrous adaxially, prominent and glabrous abaxially; secondary veins 13-23 pairs, uniformally curving upwards, glabrous. *Flowers* single, leaf opposed, pendulous. *Flowering*

pedicels 5-27 cm long, ca. 2 mm in diameter, glabrous, light green stained with dark purple patched, with blue-greyish wax layer. *Upper bract* inserted central to subapically on the pedicel, 1.5-4 cm long, 0.8-3 cm wide, length:width ratio 2-2.5, elliptic to obovate, base decurrent, apex acute to attenuate, glabrous, green with dark purple streaks; margins undulate, glabrous. *Sepals* 2-4 cm long, 0.7-1.7 cm wide, length:width 2-2.5, elliptic to ovate, base truncate, apex attenuate, glabrous, pale yellow densely streaked with purple to completely dark red; reflexed upwards, falling when in fruit, margins strongly undulate, glabrous. Inner and outer petals basally fused running down the pedicel for 6-8 mm before reflexing upwards. *Outer petals* 4-10.5 cm long, 2-4 cm wide, length:width ratio 1.8-3, narrowly ovate to ovate, base truncate, apex acute, glabrous, deep yellow at base streaked with dark red, pale yellow spotted dark purple when younger; arc shaped and dropping, margins strongly undulate, glabrous. *Inner petals* 25-35 mm long, 25-30 mm wide, length:width ratio 1-1.5, clawed cordate, base cordate, apex acute to obtuse, glabrous, basal lobes of lamina covered with short erect hairs, white with red central vein on the outside, white and specked with red-yellow on the inside; margins straight, covered with short curly hairs, connivent over receptacle; claw 2-5 mm long, 6-8 mm wide, claw:inner petal ratio 0.08-0.15, glabrous, yellow turning dark red. *Receptacle* 6-9 mm in diameter, strongly convex. *Stamens* in 16-20 rows, 1.8-2 mm long, connective shield ca. 0.2 mm long, elongated, covered with short erect hairs, those of inner whorl not elongated over ovary wall, white. *Ovary* partially overtopping stamens, 4-5 mm long, 4-5 mm in diameter, light green at anthesis; stigma ca. 3 mm in diameter, sparsely covered with short erect hairs, yellow-green. *Fruiting pedicels* to 30-35 cm long, 10-15 mm thick, woody, dark brown, with a clear scar of the bracteole, glabrous. *Fruits* 9-15 cm long, 8-15 cm in diameter, globose, 2-3 mm in diameter, finely longitudinally ribbed-rugose, lenticellate, green with white dots turning pale yellow when mature; pericarp 1-1.5 cm thick. *Seeds* 15-22 mm long, 10-13 mm wide, transversely ellipsoid, packed in white pulp; testa light to dark brown with strong scent of lemon; raphe not thinckened, slightly darker brown; hilum 4-6 mm long, 2.5-3 mm wide, narrowly elliptic to narrowly ovate.

Distribution: Throughout West and Central Africa, east to Uganda and the extreme west of Tanzania and Kenya (see Map 29); in primary and secondary rain forests, along rivers and near marshes, on sandy or rocky soils; at 0-1600 m altitude.

Phenology: Mature flowers and fruits collected all year round.

Vernacular names:
Europe: Muscadier de Calabash, fausse noix de muscade (French), Calabash Nutmeg, African nutmeg, false nutmeg (English); muskatnußduftender Orchideenbaum, Kalabassenmuskat (German).
Angola: Gipepe, Mepepe, N'zingo.
Central African Republic: Lango (Lissongo).

Democratic Republic of Congo: Bende-bende, M'bende-bende, Mombende-bende, Mumbende-mbende (Mayumbe); Boniningo (dial. turumbu); Ifuafa (Eala); Kimbuba (dial. kinande); Musahusa (dial. kimbudi); Mukasa (dial. kinianga); Pinguingu (dial. mogandu), Makúedsa (Kibile); Angbe (Dila).
Gabon: Feup, Tep, Pousa.
Ghana: Awerewa (Twi); Were, Abotokuradua, Kotokorowa, Motukrodua, Asan-menasi, Weriw, Werie-aba (Ashanti); Ayerew-Amba (Fante); Awi Ara, awiadada (Wassaw); Avonba or Avonoba (Nzima); Malai (Ga); Yikwi, Ayiku (Ewe); Efu-aba (Sefwi); Yiku or Ayikui (Krepi); Ayikui (Awuna).
Guinea-Bissau: Sambè (balanta) ; Durétche (biafida) ; Guélé, Quélè-nai (Fulapulaar).
Ivory Coast: Moué (Abé); Efuain (Agni); M'kbo (Attié); Hané (Ebrié).
Kenya: Lubushi (Luhya)
Liberia: Kray-bu (Kru-basa).
Nigeria: Ariwo (the seed), Abo lakoshe, (Yoruba); Ebenoyoba (Edo); Ehuru (Igbo); Ukposa (Benin); Ehuru (Ibo, Awka).
Sierra Leone: Gboite (Mende); Fufui or Funfui (Susu).
Uganda: Nagomola (Luganda) ; Mukoza (Lusoga) ; Mugema (Rutoro).

Uses: Planted as an ornamental tree throughout the tropics and in botanical gardens. The aromatic seeds have a spicy taste and are used in soup, generally as an alternative to nutmeg (*Myristica fragrans*). Seeds eaten grilled against constipation, gastritis, or squashed and rubbed against forehead against migraines. Seeds can also be mixed with palm oil for treating hair lice. Extracts from the bark have been used against hemorrhoids, stomach pain or fever. Used in family circles to bless the loved one by spitting the chewed seeds on his head or in his hands. Used in Uganda as firewood or charcoal.

Notes: *Monodora myristica* is easily recognizable by its very long and pendulous pedicels, with an undulate upper bract, a large globose fruit with a black and smooth but finely ribbed surface. It bears great similarity to *M. undulata* by the shape of the flower and the connivance of the inner petals, but is distinguished because it has a shorter pedicel and a straight upper bract and an ovoid fruit with a rough brownish surface. It also resembles *M. laurentii* by the connivance of the inner petals over the receptacle. This later species was inferred with moderate support as sister to *M. myristica*.

Extra references: A.P. de Candolle, Prod. 1: 87. 1924; Oliv., Fl. Trop. Afr. 1: 38. 1868 ; Engl. & Diels, Monogr. Afrik. Pflanzen-Fam. 6: 86, Fig. 30A. 1901; Exell & Mendonça, Consp. Fl. Angol. 1, 1: 31. 1937; Pellegrin, Bull. Soc. Bot. France 94: 385. 1947; Boutique, Fl. Congo Belge 2: 268. 1951; Keay, Fl. W. Trop. Afr. ed. 2, 1, 1: 54. 1954; Tisserant & Sillans, Not. Syst. 15: 323. 1958; Aubréville, Fl. Forestière Côte d'Ivoire ed. 2, 1: 150, Fig. 45. 1959; Dale & Greenway, Kenya trees and shrubs 36. 1961; Irvine, Woody plants of Ghana 12. 1961; Paiva, Mem. Soc. Brot. 19: 122. 1966; Le Thomas, Fl. Gabon 16: 342. 1969;

Verdcourt, Fl. Trop. E. Afr. Annonaceae: 118. 1971; Keay, Trees of Nigeria 32. 1989; Beentje, Kenya trees, shrubs and lianas 49. 1994; Aké Assi, Boissiera 57: 102. 2001.

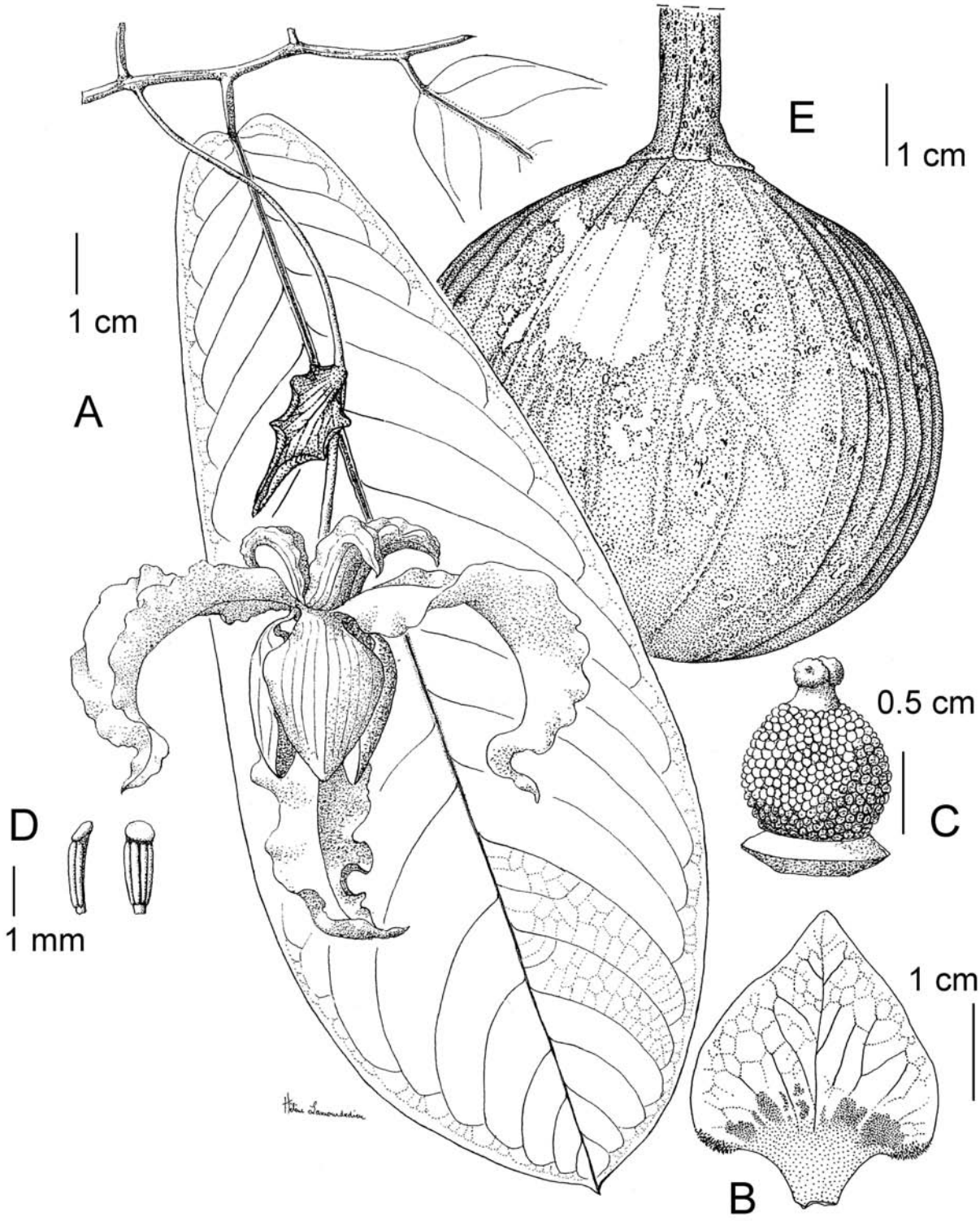
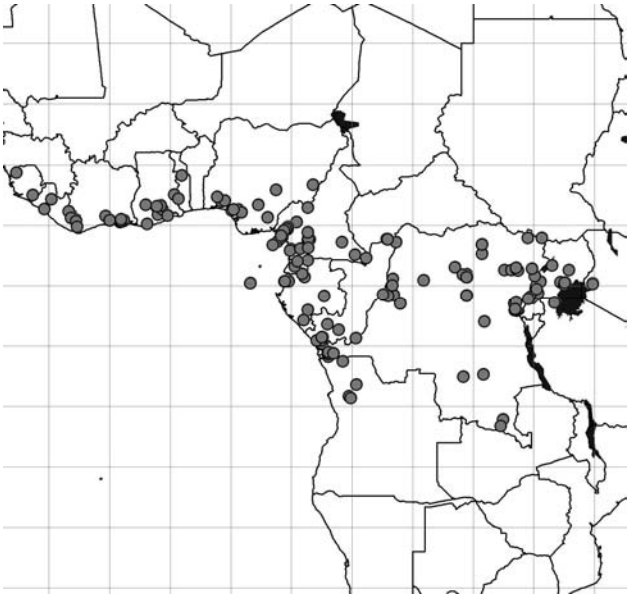


Figure 6.34. *Monodora myristica.* A. Flowering branch. B. Inner petal (inner surface). C. Androecium and stigma. D. Stamens; side view (left), front view (right). Modified from La Flore du Gabon, Le Thomas, Fig. 63. 1969. Drawings A (bract), B and E by Hans de Vries.



Map 29. Distribution of *Monodora myristica*.

ADDITIONAL SPECIMENS EXAMINED:

ANGOLA: Cabinda. Cabinda west, 70 m, 2 May 1952, *F. Càmeira 133 F* (COI); Buco Zau, Maiomhe, Chiluango, 1919, *J. Gossweiler 7203* (K); Cabinba, Buco Zau, Chiaca, 26 August 1958, *Monteiro, R. 243* (COI); **Cuanza Norte**. Cuanza Norte, Ambaca, Roça Lusíadas, 1210 m, 6 July 1953, *F. Càmeira 623* (COI); Salazar, Entrada, derivacao para Santos Dinis, 900 m, 9 January 1968, *M. da Silva 2194* (COI); Golungo Alto, 500 m, 1877, *F.M.J. Welwitsch 773* (BR, C, G); **Zaire**. San Salvador do Congo. Seens along Nkanda plateau, 500-600 m, 18 October 1921, *M.T. Dawe 113* (K); **Unkwon.** Cazengo; Granja de S. Luiz, 750 m, no date, *J. Gossweiler 4651* (K); 750 m, no date, *J. Gossweiler 5990* (K).

BENIN: Unknown. Cascade de Seva (Kpimé), 1974, *J.F. Brunel 138* (B); Dahomey, entre Pobé et Adjaouéré, 3 February 1910, *A.J.B. Chevalier 22948* (P); Près de Allada: Niaouli, 29 March 1910, *A.J.B. Chevalier 23415* (P).

CAMEROON: Central Province. Nkolbisson, 7 km W. of Yaoundé, 700 m, 26 December 1961, *F.J. Breteler 2267* (A, BR, FI, K, LISC, M, P, SL, UC, WAG, YA); Ndounda, 25 February 1959, *R. Letouzey 1527* (P); Nkolandoom pres de Ngoakélé, a 25 km a l W de Ngoulémakong, 12 July 1972, *R. Letouzey 11474* (BR, P); Marché Central, Yaoundé, 4 May 1975, *E. Westphal 8707* (WAG); Bafia market, 27 June 1975, *E. Westphal 8832* (WAG); Messa market, Yaoundé, 30 July 1976, *E. Westphal 9149* (WAG); 14 February 1978, *E. Westphal 9883* (WAG); 1km NW Nouma, Miviami-Zibi Mt, 935m, 4 March 2007, *J.J. Wieringa 5818* (K, WAG, YA); N'Kolbisson, 8 km W. of Yaoundé, 650 m, 23 January 1964, *W.J.J.O. de Wilde 1684* (BR, K, P, WAG); Yaunde station, August 1896, *G.A. Zenker 127 a* (K); **East Province**. Asia, 21 April 1961, *R. Letouzey 3899* (P); Berzik Molundu Bange-Busch. Lokomo, Bumba u Bange, 21 February 1911, *G.W.J. Mildbraed 4530* (HBG); **Littoral Province**. market of Douala (New Bell), 19 July 1975, *E. Westphal 8892* (WAG); **South Province**. Longii, 22 March 1969, *J.J. Bos 4194* (BR, K, LMA, M, MO, P, WAG, YA); Kribi. In native quartier, 14 May 1969, *J.J. Bos 4526* (BR, K, LD, LMA, M, MO, P, POZG, WAG, YA); In the Tropenbos research area. Block I2, 550 m, 20 February 1996, *M. Elad 444* (WAG); About 7 km NE of Ebom. Plot 9, subplot 88, tree 3, 500 m, August 1996, *M.P.E. Parren 136* (KRIBI, WAG); Forest of Kala on Kala Mountain, 20 kmW of Yaoundé on the Douala Road, 700 m, 20 May 1983, *D.W. Thomas 2118* (K); **South-West Province**. Mount Kupe, Nyasoso. Walter's trail, 970 m, 25 October 1995, *M.R. Cheek 7519* (BR, K, MO, P, SCA, US, WAG, YA); Mungo river Forest Reserve, 150 m, 2 December 1999, *M.R. Cheek 10228* (K, MO, P, WAG, YA); Kupe-Muanenguba Division. Ngomboku. Abang road, 800 m, 14 December 1999, *M.R. Cheek 10357* (K, MO, P, WAG, YA); no date, *H. Deistel 97* (B, Z); Mount Kupe, Kupe village. Main trail to the mountain, 990 m, 16 November 1995, *M. Etuge 1497* (K, MO, P, SCA, WAG, YA); Kupe-Muanenguba Division. Nyasoso. Max's Trail, 650 m, 15 January 1996, *M. Etuge 1576* (K, SCA, WAG, YA); Kupe-Muanenguba Division. Kupe Village, Kupe village to Loum State Forest, 29 May 1996, *M. Etuge 2017* (K, MO, P, SCA, WAG, YA); Kupe-Muanenguba Division. Nyasoso. Nyasoso-Bedume road, God-dat trail (opposite Ngusi road.), 850 m, 2 July 1996, *M. Etuge 2516* (K, MO, P, SCA, WAG, YA); Kupe-Muanenguba Division. Ngomboko. Ngabekem, locality 2 km W of Ngomboku, 880 m, 15 December 1999, *J.-P. Ghogue 494* (K, WAG, YA); Moliko, 20 July 1904, *Hubert, H. 21* (G); Bmbuko Forest Reserve, northern part near Kuke Bova, Line 2, 27 January 1957, *R.W.J. Keay 37410* (K); Ambas Bay, written on sheet, January 1861, *G. Mann 27* (K, P); Johann-Albrechtshöhe[=Kumba], 1896, *A. Staudt 583* (G, K, P, S, Z); Johann-Albrechtshöh, no date, *A. Staudt 824* (B, Z); market of Tiko, 20 m, 29 January 1977, *E. Westphal 9473* (WAG); market of Buea, 850 m, 15 April 1977, *E. Westphal 9538* (WAG); market of Victoria, 30 m, 19 April 1977, *E. Westphal 9558* (WAG); **West Province**. Region of Bayemgam, 17 December 1978, *E. Westphal 10173* (P, WAG). Region of Bayemgam, 28 March 1979, *E. Westphal 10204* (P, WAG); **Unknown.** 1938, *H. Jacques-Félix 5149* (K); Kona, 12 January 1960, *R. Letouzey 2616* (P); Entre Okoroba et Mbinda, 20km NW de Nguti, 14 June 1975, *R. Letouzey 13825* (P); no date, *P.R. Preuss 1303* (BR, K, S, US, Z); no location, 1895, *P.R. Preuss 1369* (P, U).

CENTRAL AFRICAN REPUBLIC: Lobaye. Foret de la Moboké, route de Mbaïki-Boda à 18 km de Mbaïki, 12 December 1968, *F. Badré 311* (MO, P, WAG); Boukoko, 27 September 1947, *C. Tisserant 309* (BR, P); Boukoko, 1947, *C. Tisserant 629* (BR, P); **Sangha**. Ndakan, gorilla study area from B500 to B2000, 350 m, 28 April 1988, *D.J. Harris 538* (K); Région Mbalki et Boukoko, 1948, *C. Tisserant 202* (K, P).
DEMOCRATIC REPUBLIC OF CONGO: Bas-Congo. M'vuazi, 29 November 1947, *R. Devred 622* (BR); Luki, 30 April 1948, *E. Madoux 18* (BR, L); Luki, 22 November 1949, *E. Madoux 217* (BR; L); Luki, 6 December 1946, *E. Madoux 2088* (BR); Temvo, 8 February 1919, *F. Vermoesen 1425* (BR); Temvo, 8 February 1919, *F. Vermoesen 1434* (BR); Temvo, 22 February 1919, *F. Vermoesen 1641* (BR); Temvo, 4 April 1919, *F. Vermoesen 1946* (BR); 9 April 1919, *F. Vermoesen 1970* (BR); Temvo, 25 February 1919, *F. Vermoesen 1655* (S); Temvo, 9 April 1919, *F. Vermoesen 1969* (S); Temvo, 1946, *F.M.C. Vermasen s.n.* (A); **Equateur**. Eala, 1930, *A. Corbisier-Baland 1084* (K); Eala, 14 April 1932, *A. Corbisier-Baland 1244* (BR); Eala, 5 May 1933, *A. Corbisier-Baland 1719* (BR, C, K, MO); Bomandjea, Befale, 18 February 1958, *C.M. Evrard 3492* (K); Yalikungu (Mondombe, Terr. Ikela), 6 January 1959, *C.M. Evrard 5471* (BR); Lac Tumba, 16 December 1903, *E. Laurent s.n.* (BR); Kiri (Lac), 5 November 1903, *E. Laurent s.n.* (BR); 6 November 1903, *E. Laurent s.n.* (BR); between Bokatola and Bikoro, Leopold II Lake, September 1930, *J. Lebrun 1442* (B, BR, K, NY); Libenge, 2 October 1913, *E. Mestdagh 65* (BR, Z); près du village Boyeka, 7 September 1914, *A. Nannan 177* (BR); Bolomba, Lulonga, 11 March 1959, *L. Toka 25* (BR, C); Gombe, bloc enrichi, 14 April 1959, *L. Toka 47* (BR); **Katanga (Shaba)**. Bsumba, +- 98 km N E de la ville, 1273m, 26 September 1957, *T. de Caters 125* (BR); Kaniama-Haut Lomani, August 1947, *W. Mullenders 1307* (BR); Vallée de la Kibembe, 20 Km N. NO de Elisabethville, 6 September 1948, *A. Schmitz 1971* (BR); Kamunza, September 1957, *A. Schmitz 5762* (BR); **Kinshasa**. Léopoldville, 15 September 1959, *P. Compère 379* (K); Léopoldville (Kinshasa), 18 December 1959, *P. Compère 1092* (K); Tubalaka, pres de Bunyakiri (terr. Kahele), 1000 m, 18 September 1957, *R. Gutzwiller 1486* (BR); Entre Irumu et Beni, 1020 m, October 1931, *J. Lebrun 4223* (BR); Entre Masisi et Walikale (Kiru), 800-1000 m, March 1932, *J. Lebrun 5187* (BR); **Maniema**. Kindu, 1910, *J. Claessens 504* (BR); **Nord-Kivu**. Ishunga-Kakeyi, km 120 sur Sake-Walikale, 1250 m, 11 June 1958, *R. Pierlot 2301* (BR); Kisharo, km 30 route Rutshuru-Katwe pres de la frontière de l Uganda), 1250 m, 8 June 1959, *R. Pierlot 3041* (BR); Afarama, Zone de Mambasa (Ituri Forest), 800 m, 9 February 1998, *A. Selemani 31* (BR); road Kavumu-Walikale, vers km 110, environ d'Irangi, 860 m, 12 September 1958, *G.M.D.J. Troupin 9166* (BR); road Kavumu-Walikale, vers km 110, environ d'Irangi, reserve IRSAC, 860 m, 9 September 1959, *G.M.D.J. Troupin 10741* (BR); **Orientale**. Avakubi, 13 January 1914, *J.C.C. Bequaert 1911* (BR); Penghe, 26 January 1914, *J.C.C. Bequaert 2107* (BR); Bambesa, 1933, *H.J.A.E.R. Brédo s.n.* (BR); Barumbu, 6 December 1920, *J. Claessens 8* (K, WAG); Yangambi, plateau, 3 January 1952, *C. Donis 3287* (BR, U); Yangambi, fond de vallé dans une ile, 22 January 1952, *C. Donis 3472* (K); Yangambi, 28 January 1952, *C. Donis 3484* (BR); Yangambi, 20 February 1952, *C. Donis 3694* (K); Yangambi, 27 February 1952, *C. Donis 3736* (K); Bambesa, 20 December 1952, *P. Gérard 422* (BR, M); Madabu, 16 February 1956, *P. Gérard 2144* (BR); En amount de Lileko, 28 February 1940, *R.G.A. Germain 201* (K); Bambesa, 9 July 1942, *G. Gilbert 586* (BR); Yangambi, no date, *G. Gilbert 10218* (BR, K, WAG); Epulu, Zone de Mambasa (Ituri Forest), 27 February 1991, *T.B. Hart 1241* (BR); Yangambi, 7 February 1958, *A. Léonard 231* (BR, EA, K, WAG); Yangambi, 24 January 1948, *J.J.G. Léonard 1627* (K); Yangambi, km 5, 24 January 1948, *J.J.G. Léonard 1630* (B, BR); 3 February 1936, *J. Louis 1191* (BR); à 6 km de Yangambi, près del embouchure de la Lusambila, 17 February 1936, *J. Louis 1282* (B, BR, C, K, NY, WAG); Yambao, 30 km NW from Yangambi, 21 February 1936, *J. Louis 1314* (BR, NY, S); Yangambi, 8 May 1936, *J. Louis 1837* (BR); Yaossuka, a 6 km a l E de Yangambi, 2 May 1936, *J. Louis 1871* (BR); Yangambi, 470 m, 31 December 1936, *J. Louis 3066* (BR, FHO); Yangambi, no date, *J. Louis 3178 bis* (K); Yangambi, 15 July 1938, *J. Louis 10358* (BR, EA, S, U); 6 km from Yangambi, ile de Booke wa Mbole, 470 m, 9 August 1938, *J. Louis 10748* (BR, NY, WAG); 25 km NW from Yangambi, Yambao, 470 m, 6 February 1939, *J. Louis 13584* (BR, COI); Yangambi, 14 February 1955, *E. Madoux 882* (BR); Yangambi, 30 August 1955, *E. Madoux 1020* (BR); Kashebere vers Walikale, no date, *A. Michelson 391* (BR); Parc National de la Garamba, piste frontiére Soudan A.. vers km 68, 8 March 1952, *G.M.D.J. Troupin 2145* (BR); Bambesa, 15 January 1940, *J.-M. Vrydagh 78* (BR); **Sud-Kivu**. Mingazi, 950 m, no date, *R. Pierlot 228* (BR); 900 m, 19 August 1955, *R. Pierlot 745* (BR); **Unknown.** Bende Bende, 1911, *J. de Briey 14* (BR); Kimdele, 1100 m, September 1951, *R. Desenfans 2037* (BRLU); no location, no date, *P.J.M.C. Gille 53* (BR); Lnjolo Monene, 20 August 1908, *F. Seret 994* (BR).
EQUATORIAL GUINEA: Unknown. Belebú Balachá-Las palmas, estrada km 2, 11 August 1986, *A.M.V. de Carvalho 2282* (BR, MA); National Park of Monte Alen, sentier pédagogique, 0.5 km a l O de la station Ecofac, 750 m, 2 January 1999, *J. Lejoly 99/ 33 T1* (BRLU); N of National Park of Monte Al'n, sur le sentier pédagogique, 1.5 km au NO de Moca, 900 m, 10 January 2002, *B. Senterre 1750 1* (BRLU); **Bioco (Fernando Poo)**. Malabo-Pico Basilé, estrada km 3, 9 August 1986, *M.F. de Carvalho 2270* (MA, WAG); Bokoko, 1911, *G.W.J. Mildbraed 6849* (HBG); **Rio Muni**. Bata-Pembe: Estrada km 33 chegada á povoacao de Pembe. Rio Otong-Eyang, 7 October 1991, *M.F. de Carvalho 4864* (MA, WAG).
GABON: Estuaire. Sibang, 8 September 1951, *F. Bernard SRFG 266* (LBV); Sibang, 25 January 1961, *N. Hallé 901* (P); Sibangue, 22 January 1935, *H. Heitz 12* (B, FHO, K, NY, P, S); Environs de Libreville, 1895, *T.-J. Klaine 215* (P); Libreville, Sibang Arboretum, 50 m, 17 February 2003, *M.S.M. Sosef 2022* (LBV, WAG); Mission de St. Mave du Gabon, Bassin de la Haute Ndzéme, près des Monts de Cristal, 1899, *H. Trilles 17* (P); **Ngounié**. 4 km SW de Bilengui, 16 December 1984, *C.M. Wilks 1023* (LBV); **Nyanga**. Tchibanga, January 1908, *G.M.P.C. Le Testu 1289* (BR, K, P, Z); no date, *G.M.P.C. Le Testu 2318* (BR, P); **Ogooué-Lolo**. Lastoursville, March 1929, *G.M.P.C. Le Testu 7112* (BR, P); **Woleu-Ntem**. forêt derrière le village Nsimy, à plus ou moins 25 km au Nord Est de Bitam, 5 May 1995, *A.M. Louis 3431* (LBV); c.9 km ESE Medouneu, Efot, côté droit route Efot Nkumadza, 450 m, 25 December 2002, *L. Ngok Banak 1210* (BRLU, LBV, WAG); Chantier Rougier Ocean, Oveng; 40 km NW of Oveng, 20 September 1985, *J.M. Reitsma 1509* (WAG).
GHANA: Ashanti Region. Auobeko, 6 March 1912, *T.F. Chipp 123* (K); near Bompata, Ashanti, 16 December 1921, *J.M. Dalziel 119* (K); Mount Juaso, December 1931, *P.S. Green 2392* (FHO, K); **Central Region**. Atewa Range Forest Reserve, near Bossa, 9 January 1958, *H.C.D. de Wit A 2924* (K); **Eastern Region**. Aburi, September 1937, *F.C. Deighton 3415* (K, P);

Oda, August 1925, *F.N. Howes 962* (FHO, P); Abetifi Kwaha, 609m, January 1932, *F.R. Irvine 1822* (K); just N of Abetifi, 700 m, 6 April 1994, *C.C.H. Jongkind 1414* (P, WAG); Atewa Range Forest Reserve, 500 m, 25 May 1994, *C.C.H. Jongkind 1518* (WAG); Atewa Range Forest Reserve near Boma, 9 January 1958, *J.K. Morton A 2924* (WAG); Atewa Range Forest Reserve near Pran River, 27 March 1960, *J.K. Morton A 3873* (K, WAG); **Volta Region**. east side of Oboghan Hill, 330 m, 17 February 1932, *Hughes, F.E. 2948* (FHO); **Western Region**. Benso, September 1954, *J.E. Andoh 5886* (P); **Unknown.** 1/2 down Princes Rd. W.P., April 1952, *J.K. Morton GC 6646* (K).

IVORY COAST: Abidjan. Foret d'Abobo, 21 February 1974, *L. Aké Assi 12491* (G); Forêt du Banco, 15 February 1980, *L. Aké Assi 15115* (WAG); Foret du banco, 26 March 1980, *L. Aké Assi 15147* (G); Abidjan-Azopé, Km 31, 8 February 1969, *P. Bamps 2023* (BR, P); Banco per iter versus Avodire, 22 February 1962, *L. Bernardi 8141* (G); 1500 m. S. of Centre on R. Martineau, 31 July 1975, *W.J. van der Burg 718* (WAG); Banco, 24 January 1990, *L. Gautier 1567* (CSRS); Near Adiopodoumé, 1958, *P. Gruys s.n.* (WAG); Foret du Banco, 29 July 1954, *P. Jaeger 4393* (G); Banco Forest Reserve. Near the river in centre of the forest, 16 February 1973, *J. de Koning 1146* (WAG); Abidjan.Experimental Station ORSTOM, Adiopodoumé, 18 July 1973, *J. de Koning 1933* (WAG); Abidjan. Banco Forest Reserve. South of Arboretum, near river, 20 July 1973, *J. de Koning 1956* (U, WAG); Abidjan. Experimental station ORSTOM, Adiopodoumé, 24 August 1973, *J. de Koning 2109* (WAG); Abidjan. Anguededou Forest, 28 August 1973, *J. de Koning 2195* (WAG); Abidjan. Experimental Station ORSTOM, Adiopodoume, 18 September 1973, *J. de Koning 2247* (WAG); 22 September 1973, *J. de Koning 2255* (WAG); Abidjan. Experimental Station ORSTOM, Adiopodoumé, 25 October 1973, *J. de Koning 2526* (WAG); 26 October 1973, *J. de Koning 2558* (WAG); 28 October 1973, *J. de Koning 2573* (WAG); 17 December 1973, *J. de Koning 2952* (WAG); 19 December 1973, *J. de Koning 2992* (WAG); 20 December 1973, *J. de Koning 3013* (WAG); Abidjan. Experimental Station ORSTOM, Adiopodoume, 24 January 1974, *J. de Koning 3198* (WAG); 18 February 1974, *J. de Koning 3276* (WAG); 18 February 1974, *J. de Koning 3328* (WAG); 21 February 1974, *J. de Koning 3346* (WAG); Abidjan. Banco Forest Reserve. In Arboretum, 4 September 1974, *J. de Koning 3917* (WAG); Abidjan. Banco Forest Reserve. Near source of Banco river. Clay and loam, 30 September 1974, *J. de Koning 4021* (WAG); Abidjan. Experimental Station ORSTOM, Adiopodoume, 15 October 1974, *J. de Koning 4091* (WAG); 1 November 1974, *J. de Koning 4593* (WAG); 1 November 1974, *J. de Koning 4598* (WAG); 1 November 1974, *J. de Koning 4599* (WAG); 1 November 1974, *J. de Koning 4600* (WAG); 27 November 1974, *J. de Koning 4855* (WAG); 28 November 1974, *J. de Koning 4877* (WAG); 28 November 1974, *J. de Koning 4878* (WAG); 29 November 1974, *J. de Koning 4909* (WAG); 25 March 1975, *J. de Koning 5599* (WAG); 25 March 1975, *J. de Koning 5603* (WAG); Abidjan. Banco Forest Reserve, 5 May 1976, *J. de Koning 6861* (WAG); Adiopodoumé, arbre à l'entrée de la concession, 10 November 1997, *J. Munzinger 1* (P); Adiopodoumé, 7 November 1997, *J. Munzinger 7* (P); Adiopodoumé. bord de lagune, 27 March 1990, *L. Ortéga 3030* (CSRS); 30 km NW of Abidjan, 22 May 1969, *C. Versteegh 113* (WAG); WNW of Blieux, mountain top S of Col de Reibert, 30 m, 8 September 2004, *J.J. Wieringa 5385* (WAG); Jardin botanique de Bingerville, 2 January 1957, *J.J.F.E. de Wilde 1030* (WAG); Forêt du Banco, 6 December 1956, *J.J.F.E. de Wilde 985* (WAG); ca. 3 km N of IFAC, 6 km WNW of Adiopodoumé, 6 July 1963, *W.J.J.O. de Wilde 405* (WAG); **Divo**. Surroundings of IRCC, 10 km SE of Divo, 7 July 1969, *C. Versteegh 410* (U, WAG); **Grand-Lahou**. Djibi, March 1935, *A. Aubréville 2261* (K, P); March 1935, *A. Aubréville 2262* (K, P); March 1935, *A. Aubréville 2249* (K, P); March 1935, *A. Aubréville 2248* (K, P); June 1932, *A. Aubréville (Ivory Coast series) 1328* (P); Moué de la Djibi, June 1932, *A. Aubréville (Ivory Coast series) 1356* (P); no date, *A. Aubréville (Ivory Coast series) 1893* (P); **Unknown**. no location, 14 March 1928, *A. Aubréville (Ivory Coast series) 5* (P); no location, no date, *A. Aubréville 4002* (K, P); Montézo, 2 km du village, 25 February 1907, *A.J.B. Chevalier 16227* (P).

KENYA: Nyanza. Kakamega Forest. Yala River, 1676m, no date, *F.T. Machin 3243* (K); Forest vicinity of Kakamega Forest station, 22 December 1967, *R.E. Perdue 9430* (K); **Western**. Kakamega Forest, 1550 m, 21 January 1970, *R.B. Faden 70/ 33* (EA, K); 10 December 1956, *B. Verdcourt 1690* (EA, K).

LIBERIA: Bong. Peahtah, 7 October 1926, *D.H. Linder 946* (A, K); **Grand Bassa**. Diebla, 4 July 1947, *J.T. Baldwin jr. 6326* (K, MO); **Grand Gedeh**. East slope of the Putu Hills East Range west of Tiama Town, 200-250 m, 21 May 2005, *C.C.H. Jongkind 6267* (WAG); Grebo Forest, 200 m, 8 December 2005, *C.C.H. Jongkind 7197* (WAG); 29 km NW of Chiehn (Zwedru village), 31 March 1962, *J.J.F.E. de Wilde 3724* (A, BR, K, WAG); **Montserrado**. from vicinity of Firestone Plantations along Dukwai River, Monrovia, 1929, *G.P. Cooper 352* (A, K).

NIGERIA: Akwa-Ibom State. Abini Rest House, 23 March 1931, *D.R. Rosevear 31/ 38* (FHO); **Edo State**. Okomu Forest reserve, 23 February 1948, *J.P.M. Brenan 9105* (BR, FHO, K); Along the pipe line raod near Ugo town, in the Sakpoba Forest Reserve, 9 July 1993, *B.O. Daramola 93/ 196* (MO, US); Igboetiti farmland, 17 April 1972, *J.A. Emwiogbon FHI 63927* (K); Sapoba, no date, *J.D. Kennedy 770* (B, BR, FHO, US); Sapoba, no date, *J.D. Kennedy 771* (BR, FHO); between Ora and Udo, 27 March 1958, *C.F.A. Onochie FHI 38301* (FHI, FHO, K, WAG); Okomu Forest Reserve, compartement 70, 23 February 1948, *C.F.A. Onochie 9105* (COI, P); Sapoba, 1 June 1988, *D.A.H. Taylor 150* (FHO); **Ogun State**. Omo forest Reserve, 15 km NE of Sawmill, near Oliji. Border with Oyo State. Transect 4, 150 m, 17 June 1981, *A.H. Gentry 32753* (BR, MO, WAG); **Osun State**. Shasha reserve, January 1935, *P.C. Lancaster 5* (FHO, NY); **Oyo State**. Lokose, Lagos colony, Ibadan Forest Reserve, April 1901, *C. Punch 150* (K); **Taraba State**. Vogel Peak, Below Heppers Camp, 1400 m, 27 February 1977, *J.D. Chapman 4789* (FHO, K); Gongola, Sardauna, Akwaijantar Forest, Foothills of Mambilla Plateau, 31 November 1978, *J.D. Chapman 5170* (FHO, K); **Unknown.** Usonigbe Reserve, compt 52, no date, *Unknown FHI 31933* (K).

REPUBLIC OF THE CONGO: Bouenza. Village de Massia, Road de Tsomono, 18 November 1964, *A. Bouquet 815* (P); **Kouilou**. Niouvou, 16 January 1894, *H. Lecomte c 95* (P); Mamboma, Mayombe, 27 November 1970, *Mabiala 794* (P); **Niari**. Komono, road de M'bila, Monts Abongo, 20 January 1968, *A. Bouquet 2405* (P); Les Saras, piste Cofibois, env. 8 km avant le village, 9 January 1987, *H. de Foresta 1179* (P); 9 January 1987, *H. de Foresta 1178* (P); **Unknown.** Bangui-Road Damara, km 15, 30 November 1967, *H. Breyne 1252* (BR); Environ de Dimonika, 13 December 1982, *C. Cusset 1150* (P); 13 December 1982, *J.-M. Moutsamboté 89* (P); Foret du Mayumbe, January 1891, *F.-R. Thollon 4024* (P); May 1944, *C. Tisserant 3687* (P).

SAO TOMÉ & PRINCIPE: Sao Tomé Island. Island of St Thomas, August 1889, *G. Mann 1100* (A, K, P); Sao Tome, Mt Café, 500 m, September 1885, *A.F. Moller 818* (Z).
SIERRA LEONE: Eastern Province. Levuma (Koya), 19 December 1939, *F.C. Deighton 3843* (BR, P); **Northern Province**. Way to Kukuna (Scarcies River), 7 June 1892, *G.F. Scott Elliot 4716* (K).
SUDAN: Eastern Equatoria. Lado, Yei River [Yei valley close to Congo-border], 23 October 1919, *Sillitoe, F. 281* (K).
TANZANIA: Dar es Salaam. University of Dar Es Salaam, 45m, 18 August 1973, *L.B. Mwasumbi 11191* (WAG); **Kagera**. Kiamawa, October 1935, *Gillman, H. 408* (K).
TOGO: Centre. Sokode to Basari, December 1904, *O. Kersting s.n.* (A); **Unknown**. Plateau de Danyi, between Dzobegan and Bago, 15 February 1978, *H. Ern 3149* (B); Makpame, 19 February 1914, *G.W.J. Mildbraed 7453* (HBG).
UGANDA: Central Province. Nambigirwa Forest, Entebbe, 1173m, no date, *Brasnett, N.V. 376* (K); Kampala, Makerere University college, plant on campus just outside the library building, 10 December 1967, *O. Hedberg 1200* (UPS); Kampala, Makerere University College planted on the campus just outside the libary, 1200 m, 10 December 1967, *O. Hedberg 4567* (K); Kampala-Mityana Road, 25 mile, 1200 m, 15 November 1962, *B.T. Styles 211* (FHO); **Western Province**. Masaka District, Lake Nabugabo. N.W. side, 1140 m, 9 October 1953, *R.B. Drummond 4717* (B, K); Kasala Forest, June 1915, *R.A. Dümmer 1417* (K, MO, US, Z); Kashoya Forest, Ankole, August 1935, *W.J. Eggeling 3212* (K); Kibale, Forest Ngogo, 1370 m, June 1997, *D.L.N. Hafashimana 202* (K); Bushenyi: Kasyoha-Kitomi Forest Reserve NE of Kyambura river, 1400 m, 27 June 1994, *D.N. Nkuutu KK94U 170* (C); Rukungiri: Bwindi Impenetrable National park, on slopes towards the Ishasha R. Lower, 1400 m, 26 February 1995, *D.N. Nkuutu BW95U 29* (C); Masindi: Budongo Forest Reserve, 1100 m, September 1995, *D.N. Nkuutu BW95U 182* (C); Kabarole: Burahya county. Kibale National Park, 1400 m, July 1994, *D.N. Nkuutu KI94U 27* (C); Bwindi National Park, Nothren sector (Kayonza), on slopes near the Ishasha River, 1250 m, 23 February 1995, *D.N. Nkuutu 5 51* (C).
UNKNOWN: Unknown. No location, 29 December 1971, *L. Makany 1955* (P).

11. Monodora stenopetala Oliv., Fl. Trop. Afr. 1: 39. 1868. — TYPE: MOZAMBIQUE. Zambézia: Rapids of the Shire, Kavuma, 4 November 1861, *J. Kirk 338* (lectotype, designated here: K!; isotypes: B!, K!-2 sheets).

Tree to 7 m high; outer bark smooth, grey with black lenticels; young branches dark green, densely covered with short erect or appressed hairs; old branches ash grey, glabrous to sparsely covered with short erect or appressed hairs. *Petioles* 4-5 mm long, 1-2 mm in diameter, densely covered with short erect hairs, leaf lamina inserted on side, weakly grooved adaxially. *Leaf lamina* 5-13 cm long, 2-4 cm wide, length:width ratio 2-3, narrowly elliptic to elliptic, base rounded, apex acute to obtuse, papyraceous when young becoming coriaceous, covered with short appressed hairs on both sides when young, sparsely so abaxially, glabrous adaxially when older; midrib densely to sparsely covered with short appressed hairs abaxially, glabrous to sparsely covered with short appressed hairs adaxially; secondary veins 10-12 pairs, uniformally curving upwards, glabrous to sparsely covered with short appressed hairs. *Flowers* single, leaf-opposed, on old leafless branches, appearing before leaf flush. *Flowering pedicels* 7-15 mm long, ca. 0.5 mm in diameter, densely covered with short appressed hairs. Upper bract inserted halfway up the pedicel, 3-4 mm long, 2-3 mm, length:width ratio 1.2-1.5, broadly ovate to ovate, cup shaped, base decurrent, apex rounded, glabrous; margins with short erect hairs. *Sepals* 4-6 mm long, 2 mm wide, length:width ratio 2-3, narrowly elliptic to elliptic, base truncate, apex rounded, glabrous to sparsely covered with short appressed hairs; reflexed upwards, falling when in fruit, margins undulate, glabrous. *Outer petals* 35-55 mm long, 3-4 mm wide, length:width ratio 10-20, linear, base truncate, apex acute or rounded, sparsely covered with short appressed hairs or glabrous, yellow; margins straight, glabrous. *Inner petals* 3-5 mm long, 3-6 mm wide, length:width ratio 0.5-1.2, clawed triangular to very broadly ovate, base truncate to cordate, apex rounded to shortly acuminate, densely covered with ca. 2 mm long curly hairs inside, glabrous outside, connivent by margins over receptacle;

margins straight, densely covered with short curly hairs; claw 2-4 mm long, 1-3 mm long, claw:inner petal ratio 0.6-0.8, glabrous. *Receptacle* ca. 3 mm in diameter, slightly convex. *Stamens* in 3-4 rows, ca. 0.5 mm long; connective shield ca. 0.1 long, covered with short erect hairs, those of inner whorl not extended over ovary wall. *Ovary* ca. 2 mm long, 1.5 mm wide; stigma ca. 1 mm in diameter, covered with short erect hairs. *Fruiting pedicels* ca. 1 cm long, long, ca. 3 mm in diameter, glabrous. *Fruits* 7-8 cm long, ca. 4 cm in diameter, length:width ratio 1.75-2, ellipsoid, apex apiculate, apicule ca. 3 mm, finely rugose, glabrous; pericarp ca. 3 mm thick. *Seeds* 15-20 mm long, 10-15 mm wide, broadly ellipsoid; testa smooth, light yellow-brown; raphe not seen; hilum not seen.

Distribution: Mozambique and southern Malawi (see Map 30); in dense thickets and woodlands; at 100-500 m altitude.

Phenology: Mature flowers and fruits collected in November.

IUCN conservation status: EN B2ab(iii, iv). *Monodora stenopetala* is only represented by six collections in herbaria, and has a small area of occupence. Moreover, it hasn't been collected for over 30 years. Only one collection comes from a protected area (Lengwe Game Reserve in Malawi). Therefore the "endangered" category seems justified.

Notes: *Monodora stenopetala* is unique within *Monodora* because of its long linear non undulate outer petals and finely rugose fruits. Because of it's characteristic appearance it does not bear any relationship with other species. However, molecular data indicated a close relationship with *M. carolinae*.

Extra references: Engl. & Diels, Monogr. Afrik. Pflanzen-Fam. 6: 85, Fig. 28E. 1901; Robson, Fl. Zamb. 1, 1: 148, fig. 16C. 1960; Palgrave, Trees of Southern Africa, 2 ed., 174. 1981.

ADDITIONAL SPECIMENS EXAMINED:

MALAWI: Southern Province. Chikwawa District, Lengwe Game Reserve, 100 m, 8 March 1970, *R.K. Brummitt 8965* (K); near Nchalo, 7 November 1963, *A.J. Salubeni 130* (K);
MOZAMBIQUE: Sofala. Manica c Sofala, Inhamitanga, 27 November 1946, *J.S. Simaõ 1196* (COI); **Tete**. Village Cabora Bassa, 500 m, 27 November 1973, *M.F. Correia 3840* (M, MO); Mutarara, Baúe no 1, 8 August 1977, *L. Macuácua 456* (WAG); **Zambézia**. Karuma, Shire Rapids, 152m, 4 November 1861, *J. Kirk 338* (B, K).

Map 30. Distribution of *Monodora stenopetala*.

12. Monodora tenuifolia Benth., J. Proc. Linn. Soc., Bot. 5: 72. 1860. — TYPE: NIGERIA. Eppah, *C. Barter 3298* (holotype: K!). *Figure 6.35*

Monodora cabrea De Wild., Compt. Rend. Soc. Bot. Belg. 4: 64. 1901. — TYPE: DEMOCRATIC REPUBLIC OF CONGO. Tchoa, *A.F.F. Cabra 2* (holotype: BR!, isotypes: BR!-2 sheets).

Tree to 20(30) m high; trunk with d.b.h. up to 60 cm; outer bark dark grey to greenish, dark green reticulated striped or white lenticellate; young branches green to brown, glabrous; old branches grey to dark brown sometimes with white elongated lenticels. *Petioles* 2-7 mm long, 1-2 mm in diameter, glabrous, leaf lamina inserted on side, broadly grooved adaxially. *Leaf lamina* (6-)9-21 cm long, 2-7.5 cm wide, length:width ratio 2-3, narrowly elliptic to elliptic or narrowly ovate to ovate, base cuneate, apex acuminate, acumen 5-10 mm long, papyraceous to coriaceous, glabrous, green above, pale green below; midrib raised and glabrous adaxially, prominent and glabrous abaxially; secondary veins 9-15 pairs, strongly curved upwards, glabrous. *Flowers* single, generally leaf-opposed, appearing on old branches just before or during leaf flush, pendulous. *Flowering pedicels* 25-75 mm long, 1-1.5 mm in diameter, glabrous, light green. *Upper bract* inserted halfway up to sub apically on the pedicel, 55-60 mm long, 10-30 mm wide, length:width ratio 2-3, narrowly ovate to ovate, base decurrent, apex rounded, glabrous on both surfaces, green shortly streaked red at apex; margins undulate, with short erect hairs. *Sepals* 10-35 mm long, 4-16 mm wide, length:width ratio 1.5-2.5, narrowly ovate to ovate, base truncate, apex rounded to attenuate, glabrous, green with red-brown markings; reflexed vertically, falling when in fruit, margins undulate, with short erect hairs. *Outer petals* 30-74(-90) mm long, 25-30 mm wide, length:width ratio 2-3,

narrowly ovate to ovate, base truncate, apex acute to rounded, glabrous, yellow-greenish, streaked with red-brown, base shading into bright white; spreading horizontally with tips slightly falling downwards; margins strongly undulate, glabrous. *Inner petals* 10-35 mm long, 6-10 mm wide, length:width ratio 2.8-3, clawed cochleate, base cuneate, apex acute to rounded, both surfaces glabrous, green to white streaked with red brownish, non connivent; margins straight, with short erect hairs, presence of two hairy appendices 3-5 mm long halfway up the lamina. *Receptacle* 4-6 mm in diameter, convex. *Stamens* in 10-13 rows, 0.8-1 mm long, covered with short erect hairs, white turning yellow; connective shield ca. 0.1 mm long, those of inner whorl not extended over ovary wall. *Ovary* 2-3 mm long, ca. 1.5 mm wide; stigma ca. 2 mm in diameter, glabrous, green at anthesis. *Fruiting pedicels* 3-7 cm long, 3-4 mm in diameter, woody, glabrous. *Fruits* 4-7 cm long, 4.5-7 cm in diameter, length:width ratio ca. 1, globose, non apiculate, smooth, glabrous, green with pale spots turning yellow and drying black, partly covered in grey-blue wax layer on dried material; pericarp 5-6 mm thick. *Seeds* 12-17 mm long 10-13 mm wide, transversely broadly ellipsoid, packed in white pulp; testa smooth, light brown; raphe slightly thickened, rugose, brown; hilum 5-6 mm long, 2-2.5 mm wide, narrowly ovate.

Distribution: West and Central tropical Africa, from Guinea to the east of the Democratic Republic of Congo (see Map 31); in evergreen primary and secondary rain forests, fringing and disturbed forests and deciduous forests, sometimes in savanna like vegetation (Benin), on sandy soils; at 0-800 m altitude.

Phenology: Mature flowers collected all year round. Mature fruits collected from January to September.

Vernacular names:
Europe: Orchid tree (English).
Benin: Sasalikum.
Democratic Republic of Congo: Akubisa (Bila), Bunjahukumu (Teturi, Mbuti Pygmies).
Gabon: Andzing (Fang), Munzingou (Punu, Vili), Oudjingo (Gallois).
Ghana: Dubiri (West Ashanti), Abotokuradua (Ashanti), Otutu-bofunnua (Twi), Dubusintim, Bulusintim (Twi, Wassaw), Abokobi (Ga), Gbloti (Ewe), Mleteo (Adanme).
Ivory Coast: Pitimoué; Ghana: Kray-Bu (Bassa).
Nigeria: Lakosin, Aihyo (Yoruba); Ehinawosin (Ikale); Uyenghen (Edo); Ehuru ofia (Igbo); Uyenghen (Benin).

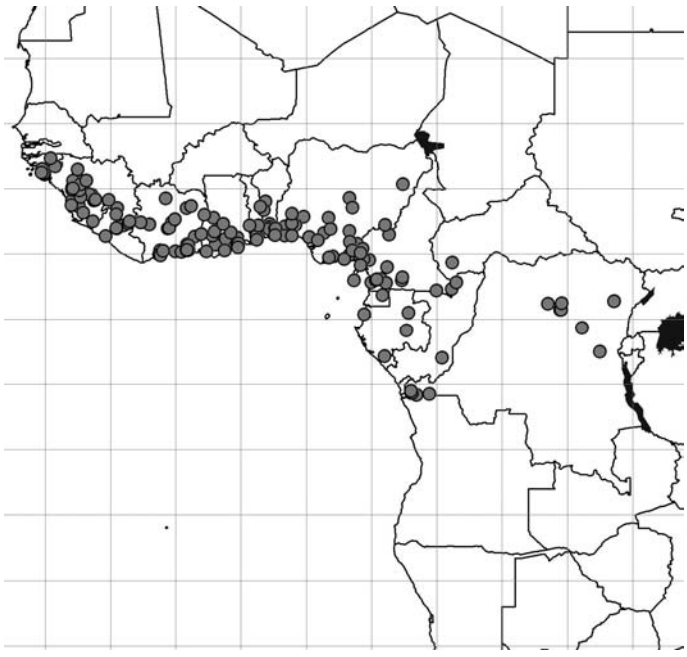
Uses: Planted as ornamental tree in the tropics and in certain botanical gardens. Seeds aromatic, used to prevent haemorrhaging and cicatrisation. Fruits eaten by children. In Democratic Republic of Congo young branches are used as bows, and in the Uturi forest the bark fiber is made into waistbands (Terashima and Ichikawa, 2003). Roots used against

dysentery and toothache (Yorubas, Nigeria). In Sierra Leone, the bark is used as medicine for dogs.

IUCN conservation status: LC. *Monodora tenuifolia* is a common species regarding its extensive representation in herbaria, and has a wide distribution across West and Central Africa. Thus the “Least Concern” category is suitable.

Notes: *Monodora tenuifolia* is easily recognizable by the presence of two small appendages halfway up the inner petals. The young fruits have a grey-blue wax layer, which is also found in *M. minor*, although in the latter it is present on young branches and leaves, and pedicels. Molecular data indicate that this species is most closely related to *M. crispata*, although with low support.
There has been some confusion regarding the type of *M. tenuifolia*, some authors suggesting there are two syntypes (*Mann 111* and *Barter 3298*). However, in the protologue Bentham (1960) only cites a *Barter* collection from Eppah, with precise details on the label information. The number *3298* fits perfectly both the location and label information, and is thus the appropriate and only type.

Extra references: Oliv., Fl. Trop. Afr. 1: 38. 1868; Engl. & Diels, Monogr. Afrik. Pflanzen-Fam. 6: 89, Fig. 28B. 1901; Pellegrin, Bull. Soc. Bot. France 94: 386. 1947; Boutique, Fl. Congo Belge 2: 265. 1951; Keay, Fl. W. Trop. Afr. ed. 2, 1, 1: 54. 1954; Aubréville, Fl. Forestière Côte d'Ivoire, ed. 2, 1: 150, Pl. 44:1. 1959; Irvine, Woody plants of Ghana 12. 1961; Paiva, Mem. Soc. Brot. 19: 120. 1966; Le Thomas, Fl. Gabon 16: 339, Fig. 62. 1969; Keay, Trees of Nigeria 32. 1989; Aké Assi, Boissiera 57: 102. 2001.



Map 31. Distribution of *Monodora tenuifolia*.

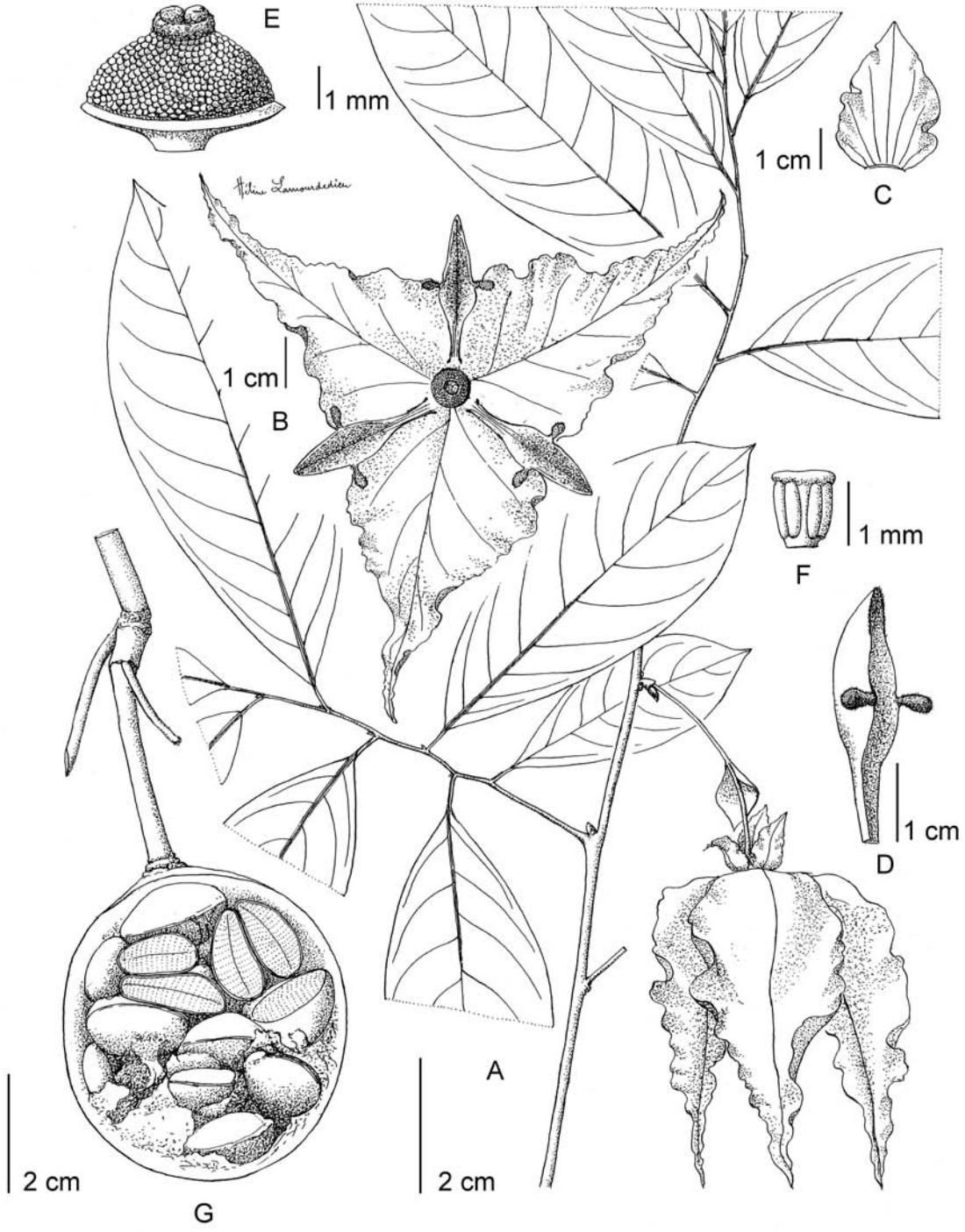


Figure 6.35. *Monodora tenuifolia*. A Flowering branch. B. Opened flower. C. Sepal. D. Inner petal. E. Androecium and stigma. F. Stamen. G. Sectioned fruit showing seeds and rumination. Modified from La Flore du Gabon, Le Thomas, Fig. 62. 1969.

ADDITIONAL SPECIMENS EXAMINED:

BENIN: Borgou. Igbomakoro, 9 April 1999, *A. Akoegninou 2329* (BENIN, WAG); **Ouémé**. Itchèdè, 31 January 1999, *A. Akoegninou 2172* (BENIN, WAG); Pobè, 25 May 2000, *A. Akoegninou 3279* (BENIN, WAG); Pobè, 125m, 14 March 2001, *A. Akoegninou 4365* (BENIN, WAG); Agonwi, 76m, 15 March 2001, *A. Akoegninou 4395* (BENIN, WAG); Avagbodji, 19 September 2001, *A. Akoegninou 5437* (BENIN); Dahomey: Cercle de Porto Novo, reserves forestieres de Bokoulion, 28

January 1910, *A.J.B. Chevalier 22860* (K, P); Village de Tohoué (entre la Lagune de Port0-Novo et la mer), 20 January 1910, *A.J.B. Chevalier 22794* (P); Pobé, 24 September 1991, *A. Dansi tw 50818* (BR); Pobè, 14 July 1988, *P. Houngnon 4523* (BENIN); Dahomey, no date, *G.M.P.C. Le Testu 105* (A, MO, S); Forest around Station de Recherche sur le Palmier à l'Huile FRPH, 14 May 1998, *L.J.G. van der Maesen 6280* (BENIN, WAG); Pobè, 12 November 1988, *N. Sokpon B 5* (BRLU); **Zou**. Mondji-Gangan, 29 April 1998, *V. Adjakidjè 1554* (BENIN); Kèrè, 314m, 17 June 2001, *V. Adjakidjè 4375* (BENIN, WAG); Forêt de la Lama, June 2001, *A.C. Adomou 11* (BENIN); Lougba, 24 July 1998, *A. Akoegninou 1630* (BENIN, WAG); **Unknown.** Pres d'Agon-Nyopbo, February 1974, *J.F. Brunel 394* (B); Nyivé, Kalakala, March 1978, *J.F. Brunel 4771* (B).
CAMEROON: Unknown. Lamouko, 27 March 192, *L. Hédin 249 bis* (BR, P); no location, no date, *P.R. Preuss 35* (BM); **Central Province**. Pres de Mékomo, May 1962, *R. Letouzey 4978* (P); Yanunde, 1896, *G.A. Zenker 795* (COI, G, K, NY, P); **East Province**. 40 km WNW of Moloundou (Ndongo), 17 March 1973, *T.F. Mbenkum 315* (BR, K, P, WAG, YA); **Littoral Province**. Piste Sole-Koum, 20 km NW of Yabassi, 14 March 1976, *R. Letouzey 14426* (K, P, WAG); Etwa, 125 km NO Jaunde, February 1914, *G.W.J. Mildbraed 8301* (K); **South Province**. Bitya, near River Ja, no date, *G.L. Bates 1787* (K); 59 km from Kribi, Lolodorf road, 8 km W. of Bipindi, near Madoungo, 16 February 1970, *J.J. Bos 6365* (B, BR, GENT, HBG, L, M, MO, P, UPS, WAG, YA); About 7 km NE of Ebom. Plot 9, subplot 98, tree 6, 500 m, August 1996, *M.P.E. Parren 156* (KRIBI, WAG); 8 km W of Bipindi, 59 km of Kribi, 16 February 1970, *D. Vuyk 6365* (M); Station du Cacaoyer de N'koemvone, S. of Ebolowa, 14 km on the road to Ambam, 13 December 1974, *J.J.F.E. de Wilde 7836* (B, BR, EA, K, LG, MA, MO, P, PRE, SRGH, U, WAG, YA); 3 July 1975, *J.J.F.E. de Wilde 8399 B* (WAG); 13 km along the road from Kribi to Ebolowa, 70 m, 26 November 1975, *J.J.F.E. de Wilde 8673* (BR, EA, K, LG, MA, MO, P, PRE, SRGH, U, WAG, YA); Mimfia, no date, *G.A. Zenker 64* (B, U, WAG); Bipindi, 1899, *G.A. Zenker 1938* (A, B, BM, BR, G, K, L, S, WAG); Bipindi, 1900, *G.A. Zenker 2251* (A, B, HBG, K, L, S, WAG); Bipindi, 1900, *G.A. Zenker 2251 a* (BM, G, K); Bipindi, 1904, *G.A. Zenker 2727* (BM, BR, K, L, M, S, WAG); Bipindi, 1900, *G.A. Zenker 3667* (BR, G, L, S); Bipindi, 1908, *G.A. Zenker 3668* (BM, G, K); Bipindi, 1908, *G.A. Zenker 3793* (G, K, US); Bipindi, 1912, *G.A. Zenker 4317* (B, BM, BR, G, K, L, MO, S); Bipindi, 1909, *G.A. Zenker 3932* (BM, G, GAB, K); Bipindi, February 1909, *G.A. Zenker s.n.* (F); **South-West Province**. On forest road from Nguti-Kumba Road to Abat, 6 March 1991, *D.J. Harris 2785* (MO, WAG); Few miles away from Ekok. Custom control Post, 3 March 1973, *M.G. Latilo FHI 67772* (FHI, K, WAG); Ambas Bay, February 1861, *G. Mann 3* (K); Johann-Albrechtshöhe[=Kumba], 1896, *A. Staudt 650* (COI, G, K); Forest around Masaka-Batanga, 24 March 1988, *D.W. Thomas 7750* (MO).
CENTRAL AFRICAN REPUBLIC: Haute-Sangha. Bayanga, 20 February 1976, *T. Wraber 49477* (K); **Sangha**. Bai Hokiu Gorilla Study area, 25km E. of Bayanga, 435m, 22 February 1988, *R.W. Caroll 1038* (K); Dzanga-Sangha Reserve. 45 km South of Lidjombo, Dakan gorilla study area, 350 m, 6 November 1988, *D.J. Harris 1553* (K).
DEMOCRATIC REPUBLIC OF CONGO: Bas-Congo. Lovo, 27 November 1959, *P. Compère 903* (K); Matadi, 1 May 1939, *A. Dacremont 444* (K); Luki INEAC, Vallée Kinkoko, 24 January 1949, *C. Donis 2348* (K); Luki, April 1948, *E. Madoux 19* (BR, WAG); Luki. Plateau du poste, 5 November 1948, *E. Maudoux 98* (BR, MO, WAG); Luki, Arboretum, 29 November 1949, *E. Madoux 234* (BR); Vallée de la N'Kula (Luki), 10 December 1947, *L. Toussaint 65* (BR); Luki, 7 May 1948, *L. Toussaint 360* (BR, COI, K); Luki, 3 December 1946, *L. Toussaint 2116* (BR); Vallé de N'kula. Luki, 22 April 1947, *L. Toussaint 2258* (BR); Luki, 22 April 1947, *L. Toussaint 2259* (BR); Temvo, Mayombe, 19 February 1919, *F. Vermoesen 1587* (BR); **Maniema**. Bafwaboli, November 1921, *J. Claessens 254* (BR); Urega (Maniema), July 1932, *J. Lebrun 5799* (MO); **Orientale**. Station de l'Epulu; Mambasa, 750 m, 14 April 1981, *T.B. Hart 33 bis* (BR); 750 m, 14 April 1981, *T.B. Hart 33* (BR); Barumbu, January 1906, *M. Laurent 1623* (BR); Yangambi, ile Tutuku, 3 January 1948, *J.J.G. Léonard 1606* (K); Yangambi, ile Tutuku, 22 March 1948, *J.J.G. Léonard 1677* (BR, M); Yangambi, Ile "Esali II", 7 April 1939, *J. Louis 14516* (BM, K); environ de Yambuya, 1906, *A.F. Solheid 58* (BR); **Unknown.** Tchoa, December 1896, *A.F.F. Cabra 2* (BR).
EQUATORIAL GUINEA: Bioco (Fernando Poo). Fernando Po, 1860, *G. Mann 164* (A, B, K, P); **Unknown.** Nkolentangan, 27 March 1908, *G. Tessmann 314* (K).
GABON: Estuaire. Environs de Libreville, 1903, *T.-J. Klaine 3287* (P); **Nyanga**. Tchibanga, November 1907, *G.M.P.C. Le Testu 1245* (BM, BR, P); **Ogooué-Ivindo**. Route de Loa-Loa, 3 km Sud de Makoukou, 500 m, 24 October 1983, *C. Doumenge 101* (LBV); **Ogooué-Lolo**. Lastoursville, October 1929, *G.M.P.C. Le Testu 7565* (BM, BR, P).
GHANA: Ashanti Region. Auobeko, 6 March 1912, *T.F. Chipp 124* (K); Awura Forest reserve, 12 March 1963, *A.A. Enti FH 7916* (FHO, MO); Abenengi, January 1928, *Mrs Moor 1020* (FHO); Amantia, 150 m, March 1930, *C. Vigne 1850* (FHO); **Brong-Ahafo Region**. Spring next to Wenchi-Bamboi road, 250 m, 7 October 1995, *C.C.H. Jongkind 2365* (LISC, P, WAG); **Central Region**. near Assuantsi, 15 May 1921, *W.C. Fishlock s.n.* (FHO); 17 May 1921, *W.C. Fishlock 31* (K); **Eastern Region**. Aburi, May 1951, *G.K. Akpabla GC 501* (K, P); Aburi Road, 25 February 1927, *J.M. Dalziel 8270* (K); Aburi, no date, *A.E. Evans 1108* (K); Abokobi village, February 1933, *F.R. Irvine 1990* (K); Mayera, Akwapi, March 1931, *F.R. Irvine 1528* (K); Aburi, 4 March 1902, *W.H. Johnson 943* (K); near Abetifi, 21 August 1908, *W.H. Johnson 81* (K); Aiyaola Forest Reserve, near Kade A.R.S, 250 m, 24 March 1994, *C.C.H. Jongkind 1372* (MO, P, WAG); 1/2 up Aburi Scarp, E.R., 19 February 1952, *J.K. Morton 6448* (K); Sutawa, 5 March 1930, *A.S. Thomas D 175* (K); **Western**. Ankasa Forest Resource Reserve. Along foot path bordering in Ankasa River traveling east from the park guard main camp, 50 m, 15 March 1996, *H.H. Schmidt 2104* (MO); **Western Region**. Asenanyo, February 1937, *J.E. Andoh 4299* (FHO); BIA National Park and Production Reserve. Along foot path starting at Adjoafua Park Guard Camp, 1 March 1996, *H.H. Schmidt 2025* (MO, UPS); Mansiso, 150 m, March 1926, *C. Vigne 90* (FHO); **Unknown.** 16 miles from Accra, towards Aburi, 26 February 1927, *Fairchild, D.G. 107* (BR); no location, 1 February 1900, *W.H. Johnson 579* (K); Volta river district, 60-121m, March 1927, *Mrs Moor 250* (FHO); 5 miles south of Adarso on top of rise, November 1951, *J.K. Morton 6149* (K); no location, no date, *C. Vigne 2747* (US); no location, no date, *C. Vigne 2899* (BR).
GUINEA: Kindia. Kinda, March 1905, *A.J.B. Chevalier 13226* (P); Kindia, 9 November 1905, *A.J.B. Chevalier 13374* (P); Friguiagbé, 4 May 1937, *J. Chillou 389* (MO, P); Yacht Utowana, near slopes near Mamou, 10 March 1927, *J.M. Dalziel 8395* (US); Environ de Kindia, no date, *H. Jacques-Félix 315* (P); near Ninia, Talla Hills, February 1917, *G.F. Scott Elliot*

4814 (A); Way to Ninia Talla Hills, 17 February 1892, *G.F. Scott Elliot 4817* (K, P); **Kissidougou**. Cercle de Kissi, Koundiam, 19 February 1909, *A.J.B. Chevalier 20743* (P); **Koumbia**. Telire, 5 September 1990, *Cordonnier 461* (BR); **Macenta**. Macenta, 14 February 1949, *J.G. Adam 3730* (MO, WAG); **Mamou**. Fouta Djallon, Bilima Kanté, 6 March 1907, *A.J.B. Chevalier 18058* (P); Fouta-Djalon Limbo, 16 September 1907, *A.J.B. Chevalier 18342* (P); Mamou, February 1910, *C.-J.M. Pitard 56* (G); road from Timbo to Frigniabé, March 1906, *C.H.O. Pobéguin (Guinea series) 134* (K, P); Fouta Djallon, January 1908, *C.H.O. Pobéguin (Guinea series) 1934* (P); Fouta-Djallon, 27 June 1899, *C. Maclaud 59* (K); Mamou, 10 February 1948, *G. Roberty 10637* (G); C 28 18 Fa Kindia-Foulaga, 30 April 1955, *G. Roberty 17692* (G); **Unknown.** Mont Gangon, 700 m, 4 March 1905, *A.J.B. Chevalier 13227* (P); Vallee de la Santa, March 1905, *A.J.B. Chevalier 12767* (P); Village de N'zo, 27 March 1909, *A.J.B. Chevalier 21039* (P); no location, 15 September 1907, *L. Farmar 26* (K); Environ de Timbo, 2 October 1902, *C. Maclaud s.n.* (BR); Timbo, February 1907, *C.H.O. Pobéguin (Guinea series) 1497* (P); Vallée de la Kaba, 10 September 1954, *R. Schnell 6751* (BR).

GUINEA-BISSAU: **Bafatá**. Chitole, Jangada, 25 June 1946, *J.V.G. do P. Espirito Santo 2270* (K, P); **Tombali**. Cantanhez, Fula-Bolhanei, 5 April 1954, *J.D. D'Orey 375* (K); Catio, 10 July 1945, *J.V.G. do P. Espirito Santo 2125* (K); **Unknown.** Cacine, Campiane, 3 June 1952, *J.V.G. do P. Espirito Santo 2986* (K, LISC, MO, WAG); Boé, Madina, 9 June 1953, *J.V.G. do P. Espirito Santo 3204* (K, LISC, M, MO, WAG); Cubisseco, Empada, 14 August 1945, *J.V.G. do P. Espirito Santo 2167* (FHO); Boe, Madina, 19 February 1951, *J.V.G. do P. Espirito Santo 2888* (B, BR, M, WAG).

IVORY COAST: Abidjan. Adiopodoume, 22 February 1974, *L. Aké Assi 12492* (G); Forest du Banco, 15 February 1980, *L. Aké Assi 15114* (G); Foret de l Anguededou, a 20 Km a l W d Abidjan, 9 January 1969, *P. Bamps 1801* (BR); Abidjan, foret de Banco, February 1970, *P. Bamps 2398* (K); Anonkoa, Abidjan-Adzopé, 18 km, April 1970, *P. Bamps 2621* (K); 17 km west of Abidjan, 13 March 1962, *L. Bernardi 8576* (G, K); Banco Forest Reserve, 17 June 1975, *W.J. van der Burg 572* (WAG); Banco, 30 January 1990, *C. Chatelain 49* (G); Campus, Abidjan, 6 July 1976, *A. Frédoux 682* (G); Adiopodoumé. Ancien jardin botanique, 10 January 1990, *L. Gautier 1533* (CSRS); Banco, 8 March 1988, *L. Gautier & D. Béguin 802* (CSRS); Adiopodoumé, 22 January 1968, *C. Geerling 1938* (BR, MO, WAG); Adiopodoumé, 19 March 1990, *T.H. Gnesio N 1697* (G); Banco Forest Reserve, 22 December 1972, *J. de Koning 982* (WAG); Banco Forest Reserve. Near border with Anguededou forest, 15 February 1973, *J. de Koning 1132* (WAG); Abidjan. Banco Forest Reserve. Along the main road from the entrance, 17 April 1973, *J. de Koning 1527* (WAG); Abidjan. Banco Forest Reserve. Route Reste, 31 December 1973, *J. de Koning 3031* (WAG); Abidjan. Experimental Garden ORSTOM, Adiopodoume, 2 September 1975, *J. de Koning 5954* (WAG); 15 December 1975, *J. de Koning 6268* (WAG); Abidjan. Banco Forest Reserve, near cottage, 27 March 1976, *J. de Koning 6750* (WAG); Abidjan. Banco Forest Reserve, 5 May 1976, *J. de Koning 6859* (WAG); Abidjan. Banco Forest Reserve, 5 May 1976, *J. de Koning 6860* (WAG); About 10 km W of Jacqueville, island Aladian, 3 August 1970, *A.J.M. Leeuwenberg 8085* (K, WAG); Abidjan-Cocody, 10 May 1965, *J. Miège s.n.* (G); Forêt du Banco, ca. 3 km NW of Abidjan, 11 February 1964, *R.A.A. Oldeman 954* (B, WAG); Abidjan. Zoo, 21 March 1990, *P. Poilécot 2733* (G); Forêt du Banco, 6 December 1956, *J.J.F.E. de Wilde 984* (WAG); foret d'Adiopodoumé, 26 July 1957, *H.C.D. de Wit 436* (BR); Forêt d'Adiopodoumé, 26 December 1957, *H.C.D. de Wit 7955* (K, WAG); Adiopodoumé, Botanical Garden, 5 February 1961, *H.C.D. de Wit 9112* (WAG); **Adzopé**. on border of Comoé river, c. 15 km NW of Mbasso, c. 60 km NE of Adzopé, 27 July 1963, *W.J.J.O. de Wilde 583* (BR, K, WAG); **Agboville**. In région Yapo-Nord, 60-70 km ad septentrionem Abidjan, 14 March 1962, *L. Bernardi 8594* (G, K, MO, US, WAG); 4 km N of Abbé, along the road to Abengourou, 100 m, 23 January 1970, *J. de Koning 79* (WAG); **Bondoukou**. Region de Bondoukou, 7 April 1966, *L. Aké Assi 8714* (G); **Bouaké**. Bouaké. IRAT, 15 April 1989, *P. Poilécot 2116* (G); Bouake (sous prefecture), Bouake, 15 April 1989, *P. Poilécot 2116 CI* (G); Kossou, 14 June 1972, *Unknown s.n.* (G); **Bouna**. 4 km N of Kakpin, 6 March 1968, *C. Geerling 2146* (BR, K, WAG); **Dabakala**. 45 km NE of Dabakala, 13 February 1968, *C. Geerling 2021* (BR, MO, WAG); **Grand-Lahou**. Forêt de N'Zida, près de plantation "Ocapana", 3 November 1956, *J.J.F.E. de Wilde 758* (WAG); **Guiglo**. Boka de Titiekro, 21 February 1951, *G. Roberty 13932* (G); **Korhogo**. 25 km SW of Korhogo, along the road to Sirasso, 15 July 1969, *C. Versteegh 520* (MO, WAG); **Man**. Mont Tonkoui, 29 April 1966, *L. Aké Assi 8811* (G, MO); 1976, *J. Miège s.n.* (G); **Sakassou**. Sakassou, 1 October 1971, *R. Spichiger 161* (CSRS); **Sassandra**. road from Dakpadou to Sago, 29 March 1968, *C. Geerling 2320* (BR, K, WAG); 18 km NW of Sassandra, 100 m, 26 February 1959, *A.J.M. Leeuwenberg 2884* (BR, FHO, K, L, MO, U, WAG); 61 km N of Sassandra, W of Niapidou, 100 m, 18 March 1959, *A.J.M. Leeuwenberg 3112* (WAG); Grand Drewin, 5 February 1951, *G. Roberty 13736* (G); **Vavoua.** F.C. du Haut-Sassandra, Nord. bord de piste, Nord-Est, 3 November 1993, *F.N. Kouamé 693* (CSRS); F.C. du Haut-Sassandra, Nord. forêt dégradée, relevé FNK12, 19 May 1994, *F.N. Kouamé 1226* (CSRS); **Unknown.** no location, no date, *A. Aubréville 1024* (B).

LIBERIA: . **Bong**. 3 miles N.E. Suacoco, 1 March 1952, *P.M. Daniel 322 A* (BR, COI, MO); Gbanga, 20 September 1926, *D.H. Linder 737* (A, K); **Lofa**. between Nikabuzu and Zigida, 7 March 1944, *J.C.C. Bequaert 125* (A); **Montserrado**. from vicinity of Firestone Plantations along Dukwai River, Monrovia, 1929, *G.P. Cooper 441* (A, K, NY); **Nimba**. Sanokwele, district Sanokwele, 28 February 1950, *J.T. Baldwin jr. 14185* (K); Ganta, foresthouse, about 160 miles N. of Monrovia, 27 January 1969, *J.W.A. Jansen 1346* (BR, MO, U, WAG); Monts Nimba, March 1942, *R. Schnell 589* (P); **Unknown.** River Cavali, 3 March 1962, *L. Bernardi 8444* (K).

NIGERIA: Adamawa State. In director's Forest Garden, Ibadan, 250 m, 6 January 1924, *Director of forests 9* (FHO); **Akwa-Ibom State**. Eket District, 1913, *P.A. Talbot 3156* (BM); Eket District, 1913, *P.A. Talbot 3157* (BM); **Cross River State**. Iyamoyong Forest Reserve, around the felling area in the high forest near the stream, 16 April 1959, *A. Binuyo FHI 41249* (BR, FHI, FHO, K, WAG); River Calabar, February 1903, *G. Mann 2249* (K, P); Oban, 1912, *P.A. Talbot 1362* (BM); 1912, *P.A. Talbot 1632* (BM); **Edo State**. Compt 86, 17 February 1948, *J.P.M. Brenan 9054* (BM, BR, FHO, K); Okomo Forest reserve, compartement 86, 17 February 1948, *I.J.O. Ebuade 9054* (COI); Sapoba Forest Reserve, Ugo-Ebazogbe-nugu rad near mile 2, 10 June 1967, *J.A. Emwiogbon FHI 60006* (MO); Sapoba, 6 March 1942, *A.P.D. Jones 735* (K); Sapoba, no date, *J.D. Kennedy 164* (FHO); Ubiaja-Olcusesan, 3 October 1946, *D.A. Oyebade FHI 20409* (FHO); Elele, 23 January 1929, *D.R. Rosevear 29/ 1* (K); Nikrowa, 20 March 1935, *R. Ross 116* (BM, MO); Lokoja, 30 March 1909, *B.E.B. Shaw 5* (K);

Kaduna State. Sanga river Forest Reserve, Dogon Kurmi, 12 April 1958, *R.W.J. Keay FHI 37624* (K); **Lagos State**. Eppah, no date, *C. Barter 3298* (K); Lagos, 12 March 1906, *E.W. Foster s.n.* (K); Lagos, May 1911, *Lamborn 315* (K); Lagos, 1893, *Rowland, J.W. s.n.* (K); **Nassarawa**. Shabu, 7 miles N. of Lafia, 22 August 1923, *I.D. Hepburn 52* (K); **Osun State**. Behind Oke-oja quaters in Ibbajo town, 3 May 1993, *B.O. Daramola 93/ 134* (US); **Oyo State**. Moor Plantation, Ibadan, January 1966, *C.L.M. van Eijnatten 1117* (WAG); Gambari, +/- 20 miles SE of Ibadan, 20 March 1966, *C.L.M. van Eijnatten 1279* (WAG); Ibadan, 25 March 1957, *Hambler, D.J. 244* (K); Ibadan, 1958, *F.N. Hepper 2244* (K); Ibadan South Forest Reserve, southern boundary, 31 March 1950, *R.W.J. Keay FHI 25687* (K); University of Ofe Campus, 27 March 1981, *M.G. Latilo s.n.* (K); University of Ibadan, Dept. of Botany, 11 April 1968, *J. Lowe 1305* (FHI, K, WAG); Gambari Forest Reserve, about 18 miles S of Ibadan, 6 March 1968, *P.P.C. van Meer 654* (WAG); near Ibadan, 11 March 1950, *R.D. Meikle 1250* (K); Ibadan, 1935, *R.J. Newberry 43* (K); about 2 miles beyond Govt. College on the Abeokuta road, 19 February 1958, *C.F.A. Onochie 18663* (FHO, K); Forestry hill, base of quartzite ridge. Ibadan town, 12 January 1958, *C.F.A. Onochie 31534* (FHO, K); Forestry hill, base of quartzite ridge, Ibadan village, 7 March 1958, *C.F.A. Onochie 31542* (FHO, K); Arboretum, forest hill, 29 February 1952, *E.U. Ujor FHI 30500* (K); Ag-Owu Forest Reserve, 11 March 1972, *P. Wit 64996* (K); **Rivers State**. About 10 miles from Port Harcourt on the road to Owerri, 1 December 1958, *C.F.A. Onochie 40445* (FHO); Degema, 1916, *P.A. Talbot 3774* (BM); Degema, 1913, *P.A. Talbot 3792* (BM); Degema, 1916, *P.A. Talbot s.n.* (BM); Degema, 1915, *P.A. Talbot 3696* (BM); **Taraba State**. Sardauna Province, Mambilla Plateau, S. West foothills, River Nwum For. Reserve, 9 May 1973, *J.D. Chapman 3791* (FHO); River Nwum forest reserve, Mambilla Plateau, S.West foothills, 780 m, 3 January 1975, *J.D. Chapman 3798* (FHO, K); River Nwum, Mambilla, S. West foothills, Sardauna division, 760 m, 18 April 1977, *J.D. Chapman 4912* (FHO); **Unknown.** Uhiere Forest Reserve, 31 March 1973, *V.E. Eimunjeze 69964* (K); Asu River, 600 m, March 1932, *A.T. Johnstone 295* (FHO); Akpaka, Onitsha, 5 February 1911, *A.E. Kitson s.n.* (BM); no location, 6 May 1975, *R.M. Lawton 1846* (K); Abo-Lakoshin, 12 March 1932, *A.F. Ross R. 111* (K); Okogbo, 16 February 1909, *Unknown 216* (K).
REPUBLIC OF THE CONGO: Pool. Lefini, région de Kindamba, environs de Meya, 3 November 1963, *B. Descoings 11265* (P).
SIERRA LEONE: Northern Province. Mt. Loma, 500 m, 4 February 1966, *J.G. Adam 23546* (MO, WAG); Mt. Loma, Bindekoro, Kabala district, Denkali valley, 550 m, 14 January 1966, *J.G. Adam 23072* (MO, P); near Kamalo, 21 February 1938, *F.C. Deighton 3496* (K); Alangba, N.Province, 21 February 1929, *R.R. Glanville 177* (K); Rokupr to Kambia, 5 April 1958, *F.N. Hepper 2597* (K, MO); Mt Loma, 14 October 1964, *P. Jaeger 8703* (G); foothills W Loma, 500 m, 4 February 1966, *P. Jaeger 9219* (G, K); Rokupr, 13 March 1949, *H.D. Jordan 208* (K); Wara Wara, Yagala Chiefdeni, S4 high crush above Kabala, 23 September 1951, *E.L. King 189 B* (K); Slopes of Loma Mountains above Yifin, 29 March 1964, *J.K. Morton SL 1129* (FHI, IFAN, K, SL, WAG); Port Loko, 29 April 1964, *J.K. Morton SL 1201* (FHI, K, SL, WAG); Port Loko Forest Reserve, 12 March 1954, *D. Small 913* (K, P); **Southern Province**. South Province, 0-121m, March 1923, *M.T. Dawe 444* (K); Taiama, 25 January 1938, *F.C. Deighton 3519* (K); Tiwai Island, on the Moa River, 30 March 1983, *P.G. Waterman 1005* (K); **Unknown.** no location, 25 July 1938, *F.C. Deighton 3579* (K); Luliniania, Halaba, April 1892, *G.F. Scott Elliot 5901* (K); no location, 1915, *N.W. Thomas 10188* (K).
TOGO: Centre. Plateau de Danyi. Weg-abfall zwischen Dzogbégan und Bago, 15 February 1978, *P. Hiepko 3150* (B, K); c. 10 km W of Fazao, Fazao National Park, 620 m, 2 April 1984, *J.M. Lock 84/58* (K); **Maritime**. Mission Tove, pres de Lome, 9 April 1969, *L. Aké Assi 10581* (G); Amedyofwe, February 1926, *F.R. Irvine 156* (K); **Plateaux**. Plateau de Danyi, Zone VI, 15 February 1978, *H. Ern 3150* (MO); **Unknown.** no location, April 1902, *O. Kersting 322* (K); Davie, bosquet sacré sur la station IRAT, 80 m, 3 May 1983, *P.A. Schäfer 7704* (B, BR, K, LMU, MO, MPU, P, WAG).

13. Monodora undulata (P. Beauv.) Couvreur. *Xylopia undulata* P. Beauv., Fl. Owar. 1: 27, Fig. 16. 1804. *Unona undulata* (P. Beauv.) Dunal, Monogr. Anon. 3: 111. 1817. *excluding the fruits.* — TYPE: NIGERIA. *A.M.F. Palisot de Beauvois s.n.* (holotype: G-DC!). ***comb. nov.*** *Figure 6.36 F-J*

Monodora grandiflora Benth., Trans. Linn. Soc. London 23: 474. 1862. *nomen illegitimum*. — TYPE: CAMEROON. South-West Province: Ambas Bay, January 1861, *G. Mann 27* (holotype: K!, isotypes: K-2 sheets!, P!).

Monodora brevipes Benth., Trans. Linn. Soc. London 33: 475. 1862. — TYPE: SAO TOMÉ & PRINCIPE. Principe Island, 1861, *G. Mann 1115* (lectotype, designated here: K!; isotype: P!).

Monodora preussii Engl. & Diels, Notizbl. Bot. Gart. Berlin-Dahlem 2: 301. 1899. — TYPE: CAMEROON. South-West Province: Victoria, 1898, *P.R. Preuss 1314* (holotype: B✝; lectotype, designated here: K!; isotypes: A!, EA!, S!, Z!).

Tree to 20 m high; trunk with d.b.h. to 1 meter; outer bark pale-dark brown to grayish, smooth; young branches drying black, glabrous; old branches ash-grey to pale brown, glabrous. *Petioles* (2-)5-10(-14) mm long, 1-2 mm in diameter, glabrous, leaf lamina inserted on side, very narrowly grooved adaxially. *Leaf lamina* 10-40 cm long, 8-13(-15) cm wide, length:width ratio 1.8-3, narrowly oblong to oblong or narrowly obovate to obovate, base rounded to obtuse, apex acuminate, acumen 3-9 mm long, papery when young becoming coriaceous, glabrous; midrib glabrous on both sides; secondary veins (9-)11-16(-17) pairs, uniformally curving upwards, glabrous. *Flowers* single, leaf-opposed or sometimes extra-axillary, pendulous. *Flowering pedicels* 30-55 mm long, ca. 1 mm in diameter, glabrous, light green. *Upper bract* positioned centrally or sub-apical on the pedicel, 6-10 mm long, 7-11 mm wide, length:width ratio 0.6-1, depressed to very broadly ovate, cup-shaped, base decurrent, apex shortly acuminate, upper and lower surface glabrous, margins straight, glabrous or sparsely to densely covered with erect hairs. *Sepals* reflexed upwards at anthesis, falling in fruit, 7-11 mm long, 5-10 mm wide, length:width ratio 1-1.8, very broadly ovate to ovate, base truncate, apex rounded to obtuse, glabrous, green, margins undulate, glabrous or sparsely to densely covered with short erect hairs. Inner and outer petals fused, flexed down along the pedicel for 5-7 mm before recurving upwards. *Outer petals* (25-)30-45 mm long, 15-30 mm wide, length:width ratio 1.5-2, oblong to ovate, base truncate, apex acute, glabrous on both surfaces, white-cream towards base, specked and streaked yellow purple, straight spreading horizontally, margins strongly undulate, glabrous to sparsely covered with short erect hairs. *Inner petals* (17-)20-27 mm long, (13-)15-20 mm wide, length:width ratio 1-1.6, clawed, rhomboid, base and apex acute, inside sparsely covered with erect 2-2.5 mm long hairs, glabrous on the outside, yellow with brown-purple spots, connivent along the margins over receptacle, margins straight, densely covered with short curly hairs; claw 2-5 mm long, claw:inner petal ratio 0.1-0.3, glabrous, yellow. *Receptacle* 4-6 mm in diameter, strongly convex. *Stamens* in 12-14 rows, ca. 1 mm long, densely covered with short erect hairs; connective shield 0.2 mm long, glabrous, those of innermost whorl not elongated over ovary wall. *Ovary* ca. 4 mm long, ca. 2 mm wide stigma 1.5-2 mm in diameter, sparsely covered with short erect hairs. *Fruiting pedicels* 40-50 mm long, 8-10 mm in diameter, woody, glabrous. *Fruits* 6-12 cm long, 4-6 cm in diameter, length:width ratio 1.3-1.8, ovoid, apex rounded, farinose, smooth, glabrous, pale brown; pericarp 2-4 mm thick. *Seeds* 9-20 mm long, 6-11 mm wide, transversely ellipsoid, packed in white pulp; testa smooth, light brown; raphe not thickened, dark brown; hilum 2-3 mm long, 1.5-2 mm wide, elliptical.

Distribution: Sierra Leone to Equatorial Guinea and the São Tome and Principe islands (see Map 32); in lowland primary and secondary rain forests, along rivers and in swamps, at 0-700 m altitude.

Phenology: Mature flowers found in September to March, June to July. Mature fruits collected in March to April, June to August, December.

Vernacular names:
Europe: Yellow-Flowering Nutmeg (English).
Central African Republic: Nzingo-Dengbwe, Molo-Ingo (lissongo).
Ghana: Abotokurauda (Wassaw).

Uses: Seeds used as flavoring for sauce, to treat coughs and eaten in a mixture with *Scilla oubanghiensis* Hua as an aphrodisiac. A decoction of the roots is taken internally to relieve venereal strictures (Cooper, 1931).

ICUN conservation status: LC. *Monodora* undulata is moderately represented in herbaria. There appears to be no decline in collecting during recent years, with the most recent one in 2006. It was collected in several protected areas, two national parks (Mount Cameroon and Korup in Cameroon) and one wildlife sanctuary (Agumata in Ghana). Thus the "least concern" category seems suitable.

Notes: *Xylopia undulata* was published in 1804 by Palisot de Beauvois and was accompanied by a drawing. This drawing represents one branch with leaves, three flowers, and one fruit. However, the fruit is clearly not from a *Monodora*, being apocarpous with several monocarps. Additionally, the description in the protologue does not describe the fruit. Thus, the interpretation of this name was only based on the flowers, excluding the fruits. In 1817, Dunal made the new combination *Unona undulata* (P. Beauv.) Dunal, the later name also held in the Prodromus of de Candolle (1924). However, in 1867, Bentham describes the new species *Monodora grandiflora* citing *X. undulata* in synonymy, rendering it invalid. A year later, Baillon (1868), not recognizing the invalidity of Bentham's name, synonymized *M. grandiflora* under *M. myristica*. The type specimen of *Xylopia undulata* is located at the historical herbarium of De Candolle in Geneva (*P. de Beauvois s.n.*). A scan of this specimen was kindly provided by G and is composed of three leaves and flower fragments. Although the specimen is fragmentary the size of the flowers appear too small for *M. myristica*. Unfortunately, pedicels and bracts are not present (or not visible from the scan alone). However, in the protologue, Palisot de Beauvois indicates that the bract is "sessile, obtuse, presque ronde, concave" while the bract of *M. myristica* is clearly elliptic with an attenuate apex and undulate margins. The bract described by Palisot de Beauvois corresponds with the species *M. brevipes* described by Bentham (1862) in the same publication as *M. grandiflora*. There is no description of the bract in the protologue of *M. grandiflora*, while the bract is "orbiculata, concava, obtusissima" in the description of *M. brevipes*. The size of the flowers and the description of the bract are sufficient evidence that *X. undulata* is in fact the first description of *M. brevipes*. We thus make a new combination presented here: *M. undulata* (P. Beauv.) Couvreur.
Monodora undulata closely ressembles *M. myristica* both having the uniquely strongly convex receptacles, large leaves, and completely connivent inner petals. They also exhibit the

same pollen tectum surface, smooth with small perforations (see Chapter 5). However, *M. undulata* is distinguished from *M. myristica* by its smaller flowers with shorter flowering and fruiting pedicels, the cup-shaped non undulate upper bract and the ovoid farinose fruits. Molecular data provides little information on the sister species of *M. undulata,* but it is placed within a West African clade containing *M. myristica* and *M. laurentii*.

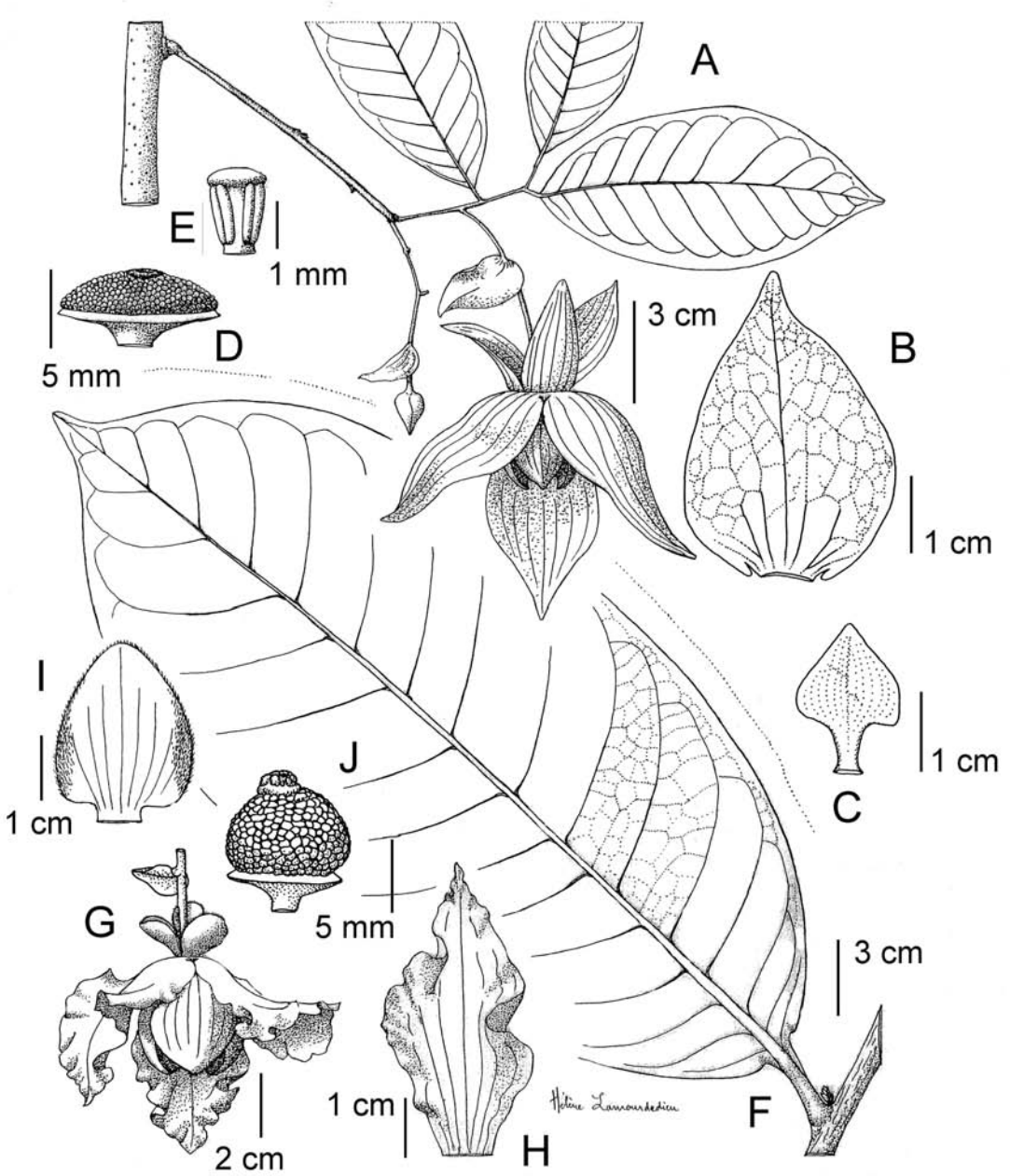
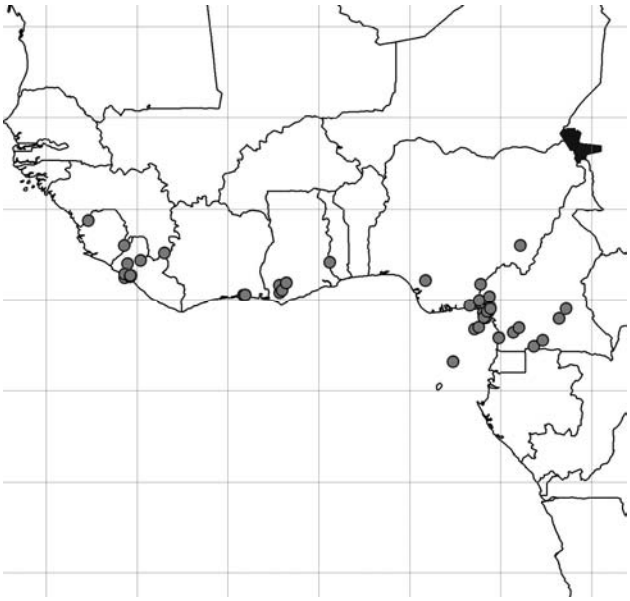


Figure 6.36. *Monodora zenkeri.* A. Flowering branch. B. Outer petal (inside surface). C. Inner petal (inside surface). D. Androcieum with missing stigma. E. Stamen (front view). *Monodora undulata*. F. Leaf. G. Flower. H. Outer petal (inside view). I Inner petal (inside view). J. Androcieum and stigma. Drawings Hélène Lamourdedieux. Drawings B, C and J Hans de Vries.

Extra references: Poiret, Encycl. Method. Bot. 8: 812. 1818. A.P. de Candolle, Prod. 1: 91. 1824; Oliver, Fl. Trop. Afr. 1: 39. 1868; Engler & Diels, Monogr. Afrik. Pflanzen-Fam. 6: 87, Fig. 30B. 1901; Pellegrin, Bull. Soc. Bot. France 94: 386 . 1947; Keay, Fl. W. Trop. Afr. ed.

2, 1, 1: 54. 1954; Tisserant et Sillans, Not. Syst. 15: 325. 1958; Irvine, Woody plants of Ghana 12. 1961; Keay, Trees of Nigeria 32. 1989; Aké Assi, Boissiera 57: 102. 2001.



Map 32. Distribution of *Monodora undulata*.

ADDITIONAL SPECIMENS EXAMINED:

CAMEROON: Central Province. Reserve forestière de Makak, 14 December 1967, *P. Bamps 1449* (BR); **East Province**. About 9 km from Bertoua, E. of the road to Doumé, 660 m, 9 December 1961, *F.J. Breteler 2182* (K, P, WAG, YA); **South Province**. Just outside Kribi, Lolodorf road, 6 November 1968, *J.J. Bos 3227* (BR, K, WAG); Just E. of Kribi, tributary of Kienke river, 31 October 1969, *J.J. Bos 5572* (P, WAG); près Ekowong, 25 km SE de Mvangan, district situé à 40 km SSW se Sangmelima, 8 March 1970, *R. Letouzey 10120* (K); Nkoemvone (12 km S Ebolowa), 26 March 1963, *A. Raynal 10024* (P); Lolodorf, 1896, *A. Staudt 40* (EA, G, K, P); **South-West Province**. by the side of the stream about 1 mile from Baimayan strangers quaters on the path to Barombi new town, 21 February 1956, *A. Binuyo FHI 35555* (FHO, K); Victoria (Limbe), 0-1000 m, no date, *E.W.G. Kalbreyer 88* (K); between Bafia and Likoko, 5 November 1958, *R.W.J. Keay 37521* (K); Korup Forest Dynamics Plot, Korup National Park, 6 February 1998, *D. Kenfack 1027* (MO, WAG); On left bank of Kumba River, the river desending from the Barombi Crater Lake, near Kumba, 350 m, 9 October 1965, *A.J.M. Leeuwenberg 6884* (BR, K, LISC, MO, P, PRE, WAG, YA); 6 km W of Bota, 5m, 31 August 1972, *A.J.M. Leeuwenberg 10295* (BR, MO, P, WAG); Entre Manyemen et Ayong, 15km SSW de Nguti, 9 June 1975, *R. Letouzey 13759* (MO); Victoria (Limbe), February 1929, *T.D. Maitland 408 a* (K); Victoria Botanical garden (Limbe), no date, *P.R. Preuss 1314* (A, EA, K, S, Z); Johann-Albrechtshöhe, 1896, *A. Staudt 495* (A); Johann-Albrechtshöhe, 1896, *A. Staudt 648* (G, K, P); 10 km between Ikata and Munyenge, NE of Muyuka, at the foot of Mont Cameroon, 200 m, 26 August 1983, *D.W. Thomas 2524* (MO, P, WAG); Mount Cameroon, above Batoke, 300 m, 25 January 1984, *D.W. Thomas 3025* (B, BR, MO, P); Around small Koto, 500 m, 7 March 1985, *D.W. Thomas 4516* (K, MO, P); On Western side of Mount Cameroun, around Koto, 500 m, 1 June 1985, *D.W. Thomas 4814* (BR, MO, P, WAG); Slopes of Barombi Mbo Crater, Kumba, 300 m, March 1986, *D.W. Thomas 5710* (BR, K, MO, WAG); Cocoa farms along the road btw Konye and Bakole, 300 m, 25 May 1987, *D.W. Thomas 7026* (F, MO); **Unknown.** no location, 1899, *P.R. Preuss 1364* (B, K, P).

EQUATORIAL GUINEA: Bioco (Fernando Poo). Bioco: Belebu Balachá-Las Palmas, estrada km 2, 11 August 1986, *M.F. de Carvalho 2282* (G, K, MA, S, UPS, WAG); Malabo- Riaba, km 61-62, 150 m, 25 July 1987, *M.F. de Carvalho 3010* (K, MO, WAG); Malabo-Riaba, cerca de Bilelipa, 390 m, 12 February 1989, *F.J. Fernández Casas 11551* (B, BR, K, MA, WAG).

GHANA: Central Region. Dunkoa, 22 November 1950, *G. Roberty 12789* (G, Z); Dunkwa, 22 November 1950, *G. Roberty 12790* (MO); **Volta Region**. Agumata wildife Sanctuary National Park. C. 20km ENE of Hohoe, at the town of Wli-Agorviefe, 320-350 m, 4 June 1995, *H.H. Schmidt 1636* (MO); **Western Region**. near Ancobra River, near Japa, 2 March 1939, *A. Foggie 178* (FHO); between Bogoso and Insu, 4 November 1973, *J.B. Hall GC 44603* (FHO, K); Prestea, 60 m, December 1933, *C. Vigne 3146* (FHO).

GUINEA: Nzérékoré. between Nimba Mountains and Bossou, gallery of Ban River, 560 m, 13 December 2006, *C.C.H. Jongkind 7637* (WAG); **Unknown.** 15 September 1907, *L. Farmar 333* (K).

IVORY COAST: Abidjan. Jardin Botanique Abidjan, 19 October 1984, *L. Aké Assi 16767* (B, G); Jardin Botanique de l'Université d'Abidjan, 29 October 1973, *L. Aké Assi 12140* (G); 11 December 1976, *L. Aké Assi 13599* (G).

LIBERIA: Lofa. Road from Bopolu to Bomi Hills, about 3 miles from Bopolu, 19 January 1978, *A. de Gier 211* (WAG); **Montserrado**. road leading from Gardeniersville to Hydro. White Plains, 8 December 1971, *F. Blyden 2037* (WAG); Firestone Plantation, Division 33, 18 December 1971, *F. Blyden 2052* (WAG); Duport, about 8 miles east of Monrovia, Porroh bush, 10 November 1966, *J.J. Bos 2306* (LIB, WAG); from vicinity of Firestone Plantations along Dukwai River, Monrovia, 26 October 1928, *G.P. Cooper 83* (FHO, NY); Boy Scout's Camp, 23 November 1970, *F.S.C. Stoop-v.d. Kasteele 260* (WAG); **Nimba**. Piatah, 9 December 1947, *J.T. Baldwin jr. 10621* (K); **Unknown.** near Gronyon, 29 October 1910, *R.H. Bunting 53* (MO).
NIGERIA: Akwa-Ibom State. Mile 5 on raod Calabar-Mamfe, no date, *J.T. Baldwin jr. 13789* (MO); **Cross River State**. At Abia-British Obokum road, 25 June 1931, *D.R. Rosevear 31/ 53* (FHO); **Edo State**. Sapoba, no date, *J.D. Kennedy 303* (FHO); Side of Jamieson R. near Sapoba, 15 November 1949, *R.D. Meikle 605* (K, P); **Taraba State**. Gidan-Anju. Kuri Nya, 23 November 1954, *M.G. Latilo 28740* (K); **Unknown.** Km 52 sur le chemin de fer; conssesion de M. Vizées, 19 December 1915, *leg. F. Fleury 33070* (P); Weastern boundary of the Ojogba-Ugun Forest Reserve, 10 June 1958, *J. Olorunfemi 38063* (K, P).
SAO TOMÉ & PRINCIPE: Principe Island. Princes Island, 1861, *G. Mann 1115* (K, P).
SIERRA LEONE: Unknown. near Mesina, 14 April 1939, *F.C. Deighton 3719* (K); **Eastern Province**. Falaba, 20 March 1916, *K.G. Burbridge 472* (K).
UNKNOWN: Unknown. cultivated at the National Botanic Gardens, Meise, 17 October 2005, *D. Alpin S 4012* (WAG).

14. Monodora zenkeri Engl., Notizbl. Bot. Gart. Berlin-Dahlem 2: 301. 1899. — TYPE: CAMEROON. Central Province: Yaoundé, 1896, *G.A. Zenker 776* (holotype: B!; isotypes: COI!, G!, K!, NY!, P!). *Figure 6.36 A-E*

Lianescent tree to 6 m tall; d.b.h. unknown; outer bark dark greyish-brown with pale brown lenticels; young branches drying black, glabrous, rarely sparsely covered with short appressed hairs; old branches greyish to brown, with sometimes sparsely distributed white lenticels, glabrous. *Petioles* 2-4 mm long, 0.9-1.2 mm in diameter, glabrous, leaf lamina inserted on side, grooved adaxially. *Leaf lamina* 10-15 mm long, 8-14 cm long, length:width ratio 2.5-3, narrowly obovate to obovate or narrowly elliptic to elliptic, base rounded to obtuse, apex acuminate, acumen 3-6 cm long, papyraceous when young becoming coriaceous, glabrous; midrib raised but sunken towards the base, glabrous on both sides; secondary veins 10-13 pairs, uniformally curving upwards, glabrous. *Flowers* single, extra axillary or sometimes leaf-opposed, pendulous. *Flowering pedicels* 28-50 mm long, 0.5-1 mm in diameter, sparsely covered with short erect hairs. *Upper bract* inserted halfway up to subapically on the pedicel, 11-20 mm long; 10-13 mm wide, length:width ratio 1-1.4, broadly ovate to ovate, base decurrent, apex rounded to acute, glabrous, pale green; margins straight to slightly undulate, sparsely covered with short erect hairs. *Sepals* 11-17 mm long; 7-10 mm long, length:width ratio 1.5-2, ovate, base truncate-cordate, apex acute, covered with short erect hairs all over and on both sides, pale green; margins straight, covered with short erect hairs. *Outer petals* 35-45 mm long, 20-28 mm wide, length:width ratio 1.5-2, ovate, base truncate with two small lobes, apex acute to obtuse, glabrous to sparsely covered with short erect hairs on both sides, white or pale green tinged dark red; horizontally spreading, margins straight, covered with short erect hairs. *Inner petals* 9-13 mm long, 13-16 mm wide, length:width ratio 0.7-0.9, clawed triangular, base truncate to cordate, apex acute, glabrous inside, covered with short erect hairs in center outside, pale green with red spots; central parts of lamina appressed over the receptacle, distal parts reflexed outwards; margins straight, folded outwards, densely covered with short curly hairs; claw 4-7 mm long; 3-5 mm long, claw:inner petal ratio 0.3-0.5, glabrous, arched inwards. *Receptacle* ca. 4 mm in diameter, convex. *Stamens* in 9-10

rows, 0.9-1.1 mm long; connective shield ca. 0.2 mm long, covered with short erect hairs, those of innermost whorl not elongated over ovary wall. *Ovary* ca. 2 mm long, ca. 1 mm wide; stigma 1.5-2 mm in diameter, sparsely covered with short erect hairs. *Fruits* unknown.

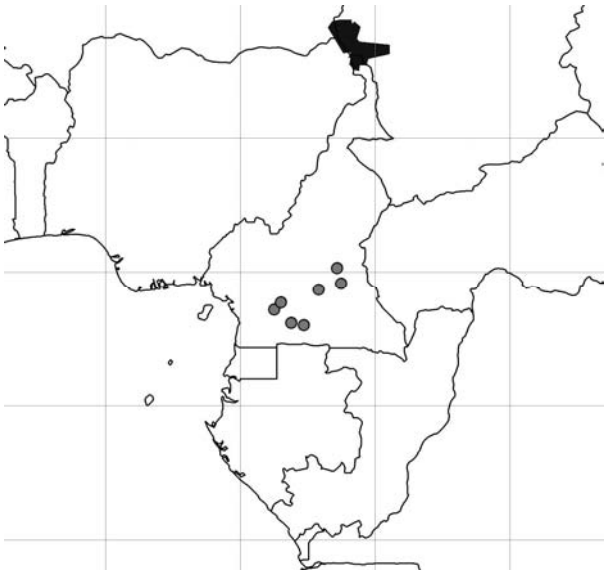
Distribution: Cameroon (see Map 33); in secondary and disturbed lowland rain forests; at 600-700 m altitude.

Phenology: Mature flowers collected March to April.

IUCN conservation status: EN B2ab(iv, iii). *Monodora zenkeri* is represented by only 8 collections in herbaria, and hasn't been collected for 30 years (last collection in 1978). It is not found in any protected areas, and local logging threatens its habitat. The "endangered" status seems justified. However, given the lack of recent collections, it could quickly be upgraded to the "critically endangered" category.

Notes: *Monodora zenkeri* is distinct from other West-Central African species by the straight margins of the outer and inner petals, as well as the inner petals that are connivent at the center and not at the margins. In this respect it closely resembles the East African species *M. junodii*, from which it can be distinguished by the presence of the two minute lobes at the base of the outer petals, as well as differences in leaf shape and longer flowering pedicels.

Extra references: Engl. & Diels, Monogr. Afrik. Pflanzen-Fam. 6: 85, Fig. 28C. 1901; Pellegrin, Bull. Soc. Bot. France 94: 386. 1947.



Map 33. Distribution of *Monodora zenkeri*.

ADDITIONAL SPECIMENS EXAMINED:
CAMEROON: Central Province. Ngoro, 38km N de Bafia, 1 May 1978, *B. Ngameni Kamga 113* (P); c. 4 km NE of Otélé, near the road to Yaoundé, 700 m, 28 March 1964, *W.J.J.O. de Wilde 2249* (BR, P, WAG, YA); Yaoundé, 1896, *G.A. Zenker 776* (B, COI, G, K, NY, P); **East Province**. Bertoua, 5 km along road to Batouri, before junction of road to Bétaré Oya, 670

m, 27 April 1961, *F.J. Breteler 1319* (WAG); 5 km S. of Nguélémendouka, road to Doumé, 700 m, 10 April 1962, *F.J. Breteler 2747* (BR, K, LISC, P, WAG, YA); Dangreng, 700 m, April 1914, *G.W.J. Mildbraed 8850* (K); Subdivision Bertoua, village Deng Deng, 27 May 1955, *P. Nana 98* (P); **South Province**. Bitye river, 1920, *G.L. Bates 1244* (COI); Near Oveng, 27 km NW. of Sangmélima, along road to Yaoundé, 600 m, 21 March 1962, *F.J. Breteler 2683* (BR, K, P, PRE, WAG, YA).

ACKNOWLEDGEMENTS: Marc Sosef, James Richardson, Paul Maas and Lars Chatrou are thanked for critically reading early versions of the manuscript as well as providing important suggestions to improve it. Jan Wieringa is deeply thanked for help in databasing the specimens. I am grateful to Frans Breteler and and Jan Wieringa for useful nomenclatural discussions and help. I also deeply thank Hans de Vries, Wil Wessel-Brand, and Joanne Pork for the botanical illustrations and the Muséum National d'Histoire Naturelle of Paris for allowing me to reproduce some figures from la Flore du Gabon. The curators of the following herbaria are thanked for loans or access to the specimens: A, B, BM, BR, BRLU, C, COI, DSM, E, EA, F, FHI, FHO, G, H, HBG, K, L, LBV, LISC, M, MO, NY, OWU, P, UPS, US, WAG, YA and Z. I am grateful to F. M. Mbago, R. E. Gereau, L. Ngok Balak, Y. Issembe, R. Niangadouma and M. Botermans for their excellent help and assistance in the field. The governmental authorities Gabon and Tanzania (COSTECH) as well as national park directors are thanked for granting collection permits. Funding for fieldwork Gabon and Tanzania came from the Netherlands Organization for Scientific Research (N.W.O., R85-389), National Geographic Society, Alberta Mennega Stichting, Hugo de Vries Fonds, Stichting Fonds Landbouw Export Bureau 1916/1918 Fund of Wageningen University, and Air France-KLM. The following funding bodies are deeply thanked for funding the visits to various herbaria: European Commission's Research Infrastructure Action via the SYNTHESYS Project (FR-TAF-14 and BE-TAF-1146) and N.W.O. (R85-375).

7 General Discussion

The goal of this PhD was to investigate the evolution of the two syncarpous African genera *Isolona* and *Monodora*. Five years ago the University of Wageningen Biosystematics Group embarked on a program of research into the systematics of African Annonaceae. Generic limits and relationships of many African taxa were at that stage unclear. However, the genera *Isolona* and *Monodora* had been demonstrated to be closely related on the basis of morphology and molecular data. They have similar distributions across tropical Africa and were thus ideal candidates for a comparative study of pan-African biogeography. The genera were in need of revision and were of a size, in terms of the estimated numbers of species, that was thought reasonable for such an undertaking during the four year period of a PhD. The logical and necessary first step was to undertake this thorough taxonomic revision of both genera in order to have a precise idea of the total number of species, their geographical distribution and ecological preferences as well as the main features that might have played a role during their evolution. This revision is presented in Chapter 6 of this thesis. With a clear idea of species limits and distributions the second step was to answer a number of questions related to African Annonaceae systematics and evolution within *Isolona* and *Monodora*. However, as often in science, providing answers always leads to many more questions which in turn should lead to new and exciting Masters and PhD projects.

PRIORS IN POSTERIOR MAPPING OF DISCRETE MORPHOLOGICAL CHARACTERS

In order to study the evolution of syncarpy and of other morphological characters in African Annonaceae we adopted the Bayesian-based posterior mapping approach to character evolution. However, as for all Bayesian methods, one needs to select prior values for the various parameters of the model. Prior selection can be problematic and has led to a lot of controversy. It is sometimes said that priors will have little or no effect on the results if the data provides a strong and clear signal (Alfaro and Holder, 2006). An alternative opinion is that one should avoid any subjectivity in the analyses and therefore should choose priors that minimize their influence during the analysis, e.g. uninformative priors. Because posterior mapping is a relatively new method, it was unclear what effects the priors actually have on the results. One of the two parameters for which we need to apply a prior value is the rate of substitution between the morphological character states which is modelled as a gamma distribution. This type of distribution is infinite at the positive end and thus a flat prior cannot be used (i.e. a prior that assigns equal probability to the entire parameter space, see introduction of this thesis). The main question addressed in Chapter 2 is about whether the

prior value placed on the substitution rate influences the results or not. Our results clearly show that different priors will always return different results, meaning that priors do influence the results. Thus, different researchers could arrive at alternative conclusions when analyzing the same data. Moreover, these alternative results were not caused by a weak signal of the data (if anything the signal provided by the analysed data was strong) but were related to a computational "short cut" called *discretization*. Discretization is a widely used method in phylogenetic reconstruction. It allows values to be drawn from continuous distributions in an acceptable amount of time by breaking the distribution into several discrete, equally probable, categories. The ranges of these different generated intervals are defined directly by the values of the priors. Thus, and even though the problem appears more methodological than data-related, when applying posterior mapping the choice of the prior value of the rate of substitution must be carefully selected even if the data present a strong signal. Different methods exist to sample from continuous distributions but this becomes more of a mathematical problem and is out of the scope of this thesis. Ideally you would want the discrete intervals created by discretization to be updated based on the resulting posterior distribution, so that the result depends solely on the resulting posterior distribution. Alternative methods for sampling continuous distributions should be explored and implemented in the near future. In conclusion, priors are part of the Bayesian "world" and should not be ignored. However, this is not a reason not to use Bayesian inference. Instead, one should deal with the problem by assessing if priors do interfere with the analysis. If they do, it would be wise to try and understand how and why, and look for the best possible solution, or at the very least recognize their potential influence in the publication. Clearly specifying what prior values were used and how or why they were chosen should be systematically included in publications, which is far from being the case today. In the specific case of posterior mapping of discrete morphological characters, the results from Chapter 2 show that using the data to inform the researcher of the appropriate prior values appears to be the best solution, because priors that did not agree with the data and the posterior distribution led to highly biased results.

EVOLUTION OF SYNCARPY

Isolona and *Monodora* are both characterized by having a syncarpous gynoecium which is unique in Annonaceae and rare within the order of Magnoliales in general. Syncarpy, however, characterizes over 80% of all flowering plants and is very common in the large eudicot clade (APGII, 2003). The rare occurrence of syncarpy in Magnoliales provided an ideal framework to study the origin of syncarpy in flowering plants. Within Annonaceae two contrasting hypotheses were suggested: 1) syncarpy arose by *fusion* of an originally moderate number of carpels (2-20; Deroin, 1997) or 2) arose via *multiplication* of an initially single carpel (unicarpellate; Endress, 1990). Anatomical studies in *Isolona* and *Monodora* revealed that the gynoecium of both genera is composed of several fused carpels (Deroin, 1997), and is not unicarpellate as suggested by others (Leins and Erbar, 1982; van Heusden, 1992). The

results presented in Chapter 3 provide strong phylogenetic evidence in favour of the fusion hypothesis. It was highly probable that the ancestral taxa of *Isolona* and *Monodora* as well as all the nodes in the African long-branch clade (ALBC, see Chapter 3) had a moderate amount of freely arranged carpels, while the unicarpellate state always received a near-zero probability. Moreover, unicarpellate taxa did not present any close phylogenetic relationships to both syncarpous genera. Thus, both from an anatomical and evolutionary point of view, syncarpy in *Isolona* and *Monodora* appears to have originated by the fusion of a moderate number of carpels.

Interestingly, although a larger number of genera of Annonaceae are also characterized by a moderate number of freely arranged carpels they have not evolved a syncarpous gynoecium. Though the evolutionary advantages of syncarpy are clear (Stebbins, 1974; Endress, 1982; Armbruster et al., 2002), the question remains as to what triggers the evolution of syncarpy. A hint towards the answer comes from a series of simulated studies by Armbruster et al. (2002). They show that syncarpy can increase the quantity (number of seeds) and quality (more viable and fitter) of offspring over apocarpy in response to different combinations of pollen load, number of carpels, number of ovules, thresholds of pollen germination and coefficients of variation for pollen fitness. Basically two scenarios arise from this study in which the evolution of syncarpy would be favoured:

- If selection for the *quantity* of offspring was favoured (total number of seeds), then syncarpy would be favoured *if* the number of arriving pollen grains was low (e.g. rarity of pollen grains).
- If selection favours the *quality* or fitness of offspring (independent of the number of seeds) then syncarpy would be selected *if* a lot of pollen would arrive, encouraging competition and selection of fitter pollen grains.

It will be hard or even impossible to understand why syncarpy evolved just once within Annonaceae. Given the conclusion of Armbruster et al. (2002), one hypothesis could involve long periods of low or high population density of the apocarpic ancestors of *Isolona* and *Monodora*, enough time at least to allow the evolution and fixation of syncarpy. A high number of individuals would favour quality over quantity of offspring in contrast to a small number of individuals (a prolonged bottleneck for example) that would have favoured quality over quantity of offspring.

The genetic basis of the evolution of this major innovation within flowering plants has yet to be determined (Scutt et al., 2006). We do not know which genes are involved in the fusion of carpels. The Annonaceae family provides an ideal setting to isolate and identify the gene(s) responsible for syncarpy. Unfortunately, the life cycle is quite long in *Isolona* and *Monodora* (probably a few years), which hinders the use of gene silencing techniques. Nevertheless, alternative methods for gene identification and isolation have been developed such as the “candidate gene approach” (Pflieger et al., 2001) or via virus induced gene silencing (VIGS; Scutt et al., 2006).

Identification of the genes responsible for syncarpy would be of particular interest in the more economical species within the family such as the cherimoya (*Annona cherimola*). In

cherimoya the carpels are numerous (>20) and free in the flower. However, after pollination and during fructification the carpels start to fuse to produce a large syncarpous fruit. The seeds are embedded in a sweet white pulp which is edible. Cherimoyas have considerable commercial potential and are cultivated in numerous countries, including Spain. Unfortunately, production is limited by inefficient pollination which results in poor fruit set or small and asymmetrical fruits. In theory, if the genetic basis of syncarpy would be revealed in Annonaceae, it should be possible to produce syncarpous cherimoya flowers. This would significantly enhance successful pollination and thus fruit production as well as produce more symmetrical fruits. These improvements would be of tremendous benefit to the development of the commercial potential of this crop.

At a more theoretical level, the identification of the genes responsible for syncarpy in Annonaceae might help us to understand how syncarpy evolved in other angiosperms. Are the genes involved in Magnoliales syncarpy the same as the ones involved in eudicots where syncarpy has apparently been much more successful from an evolutionary perspective? If yes, how different or similar are they? Can we find a genetic reason for the success of syncarpy within eudicots compared to Magnoliales? In a recent comparative study between *Arabidopsis* and the early diverging angiosperm lineage *Amborella*, Fourquin et al. (2007) concluded that the protein function involved in the development of the carpel has conserved a common activity in the control of the establishment of outside–inside polarity in the carpel since the radiation of angiosperms, over 160 Myr ago. This indicates that genes and resulting proteins involved in carpel development can be conserved even between widely divergent organisms. Thus, we could suspect that genes involved in carpel fusion, even in unrelated groups, are also comparable. Unraveling the genetic basis of syncarpy would significantly enhance our knowledge of this fundamental evolutionary innovation in angiosperms.

MOLECULAR PHYLOGENETICS OF AFRICAN ANNONACEAE

A prerequisite to study the evolution of *Isolona* and *Monodora* was to provide a phylogenetic framework that would reveal the closest relatives of both genera. However, prior to this thesis, a large number of African genera of Annonaceae had not been included in previous molecular phylogenies of the family. In Chapter 3, relevant DNA marker regions of 31 out of the ca. 40 African genera were sequenced. The phylogenetic analysis revealed the presence of an African clade nested within the long-branch clade (LBC) called the African long branch clade[1] (ALBC).

[1] I am in agreement with numerous researchers that calling clades after their molecular characteristics (such as having long or short molecular branches) is not appropriate and should be avoided in the systematic community. However, in Annonaceae molecular data provide a fairly new addition to the understanding of the classification within this family (Richardson et al., 2004). Indeed, the generic limits as well as larger clade delimitations have always been problematic. It will take time before a comprehensive synthesis of all the data (molecular and morphological) gathered over the past 25 years of research will allow the naming of clades in a more traditional taxonomic fashion. In the mean time, adopting constant unofficial names for each well-supported clade will help this near future synthesis.

This clade contains 11 genera[2] and about 80 species, and represents the largest and most species rich clade of African genera for the family identified to date. Moreover, it is very diverse at the morphological and palynological levels (see Chapter 5) making this clade hard to define morphologically. Flower structure is strikingly diverse within the ALBC. Some genera are characterized by clearly fused petals while others have completely free ones. The shape of the flowers as well as variation in pollen morphology is also very diverse. Why has such a large diversity of shapes and forms arose in this 30 Myr old clade? It could be that pollination played a vital role in this diversification. Adaptation to different pollinator vectors can influence floral morphology in very dramatic ways (e.g. the case of orchid flowers). In general, Annonaceae flowers are pollinated by a wide array of pollinators such as large and small beetles, thrips, flies, and even bees (Silberbauer-Gottsberger et al., 2003). Unfortunately, little is known about the pollination biology of African Annonaceae. A recent study (Meinke, 2008) already suggests that pollinators within the ALBC could be diverse. Two species of *Uvariodendron* were found to be pollinated by larges beetles, while flowers of *Monodora* and *Uvariopsis* were thought to be fly-pollinated, although the latter still needs confirmation. The large morphological divergence between *Isolona* and *Monodora* could also be the result of diverging pollinating systems and would be well worth investigation in the near future.

BIOGEOGRAPHY OF AFRICAN ANNONACEAE

The pan-African distribution of most of the ALBC genera provided an interesting framework to investigate the origin of the endemic lineages of Annonaceae within the East African rain forest hotspot. Based on fossil evidence, both the West-Central and East African rain forests were postulated to be united into a large pan-African rain forest during the Eocene (ca. 50-55 Myr). Chapter 4 of this thesis provides significant support for the hypothesis that the large number of endemic species found in East African rain forests today originated via multiple (at least three) biogeographic events dating back to the middle of the Oligocene (ca. 33 Myr). The divergence dates of East African sub-clades were correlated with palaeoclimatic and geological data which provided evidence that the origins of these lineages might well be associated with periods of renewed African aridity. Such aridity would have been responsible for the retraction of the rain forests, thus breaking up the pan-African rain forest and leading to the observed divergence and origin of the East African lineages. Numerous recent studies have shown that the high diversity within various hotspot regions was the result of recent and rapid speciation events (e.g. Richardson et al., 2001; Klak et al., 2004). Our results, however, indicate that such high levels of endemicity can also be achieved via a more ancient and slower process. Thus, even though regions characterized by extremely high levels of biodiversity under threat are grouped under the same concept of hotspots, they do not necessarily have originated via the same evolutionary process. Acknowledging these

[2] The monotypic genus *Dennettia* was sunken into *Uvariopsis* (Kenfack et al., 2003).

contrasting processes provides important additional information relevant for the effective conservation of hotspots. Furthermore, our results provide no evidence of Pleistocene speciation supporting the hypotheses that the East African rain forests have remained ecologically stable during the last 2.5 Myr. This is in contrast to the supposed situation in West-Central African rain forests for which Pleistocene speciation is generally invoked to explain the high levels of species richness (Sosef, 1994; Leal, 2004; Plana et al., 2004).

In this study Annonaceae was used as a model to understand the origins of the East African rain forest endemic lineages. There is an additional smaller strictly African clade within Annonaceae found to be sister to the short branch clade (SBC) and composed of five genera called the African *short* branch clade (see footnote one). This clade is *not* closely related to the ALBC and thus provides an ideal situation to compare the obtained dates in an independent fashion within the same molecular dating and calibration framework. Three of the genera, *Annickia*, *Greenwayodendron* and *Polyceratocarpus,* are disjunct between East and West-Central Africa while *Piptostigma* is endemic to West-Central Africa and a new undescribed monotypic genus (gen. nov. in Fig. 7.1) endemic to the coastal forests of Tanzania. Although species-level sampling within this clade is still poor, preliminary results suggest a similar pattern of divergence to that within the ALBC, with all the East African species adopting sister positions with respect to West African congeners. The new genus is also in an early diverging position being sister to *Polyceratocarpus* and *Piptostigma*. Additionally, *Greenwayodendron suaveolens* var. *suaveolens* is disjunct at the subspecies level with *Greenwayodendron suaveolens* var. *usambaricum* restricted to East Africa. Each of the nodes highlighted in grey in Figure 7.1 could be dated and compared with the dates obtained within the ALBC. Interestingly, the taxonomy of these genera is almost complete. *Annickia* was revised recently (Versteegh and Sosef, 2007) while the revision of *Polyceratocarpus* is almost complete (David Johnson, pers. comm.). *Greenwayodendron* is only composed of two (or three) species and could be revised without much effort. *Piptostigma* is the genus in this clade that is in most need of taxonomic revision.

Finally, undertaking phylogenetic and molecular dating analyses within the numerous other rain forest restricted families presenting a disjunct distribution between East and West-Central Africa (such as *Begonia,* Leguminosae, etc.) may also confirm the generality of the patterns we uncovered here, although the results might not be immediately comparable because of uncertainty related to the calibration of the trees. If the calibration of a node within a different family is uncertain or wrong then the generated dates will not match. Analysing divergence dates with the same calibrated tree would prove to be a better and more robust approach as suggested above. It should be possible to determine whether multiple splits have occurred that would support the fact that multiple events had taken place at different times even without provision of an absolute time for these events.

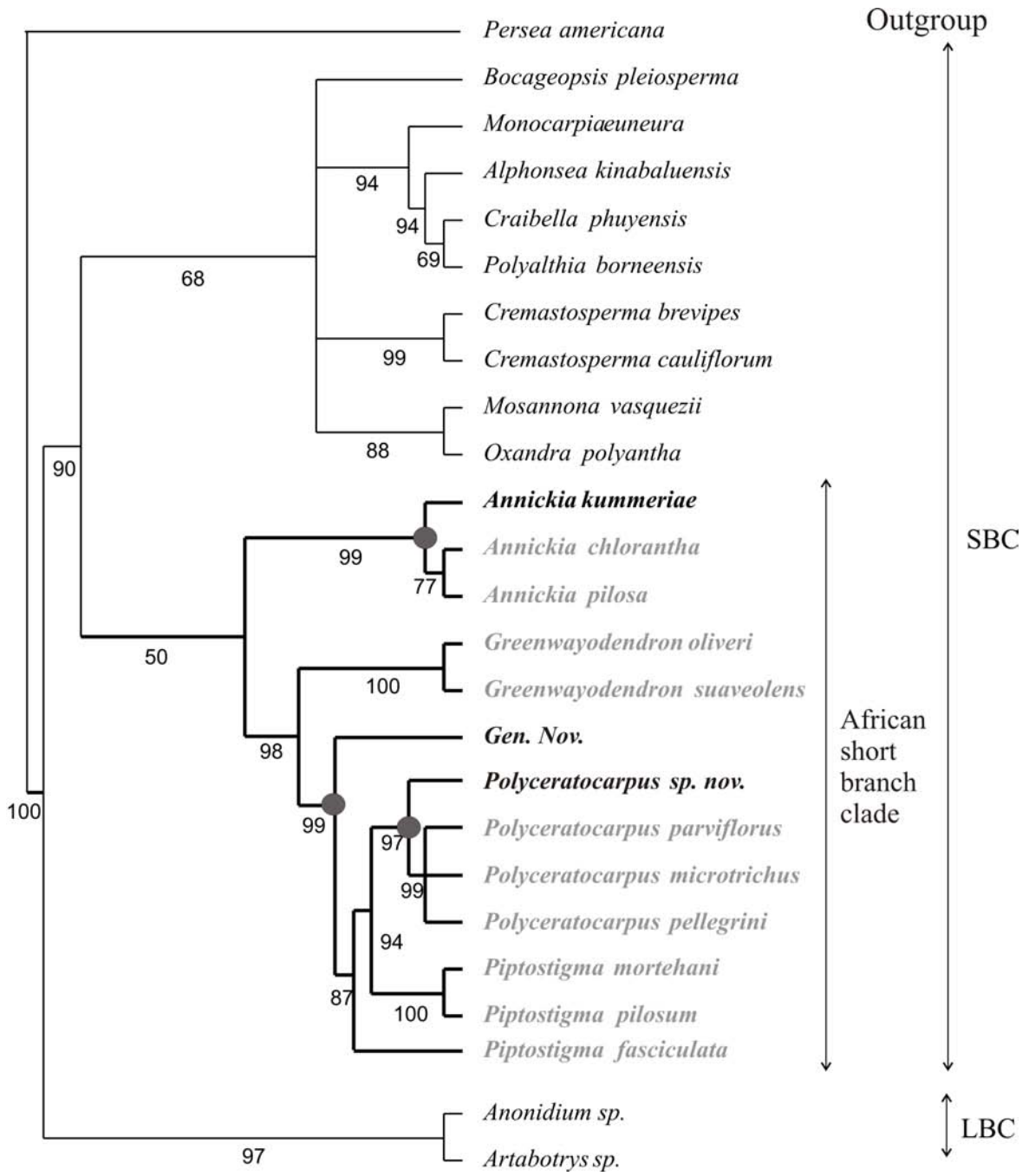


Figure 7.1. Phylogenetic relationships within the African short-branch clade. Two plastid marker (*rbcL* and *trnL-trnF*) bootstrap consensus tree based on 1000 replicates. Bootstrap values are indicated below the nodes. Note the nested position of *Polyceratocarpus* within *Piptostigma*. Grey: West-Central African taxa; Black: East African taxa. Dark grey circles indicate divergence nodes between East and West-Central clades. SBC: short branch clade; LBC: long branch clade (Couvreur et al., in prep).

THE FUTURE OF AFRICAN ANNONACEAE RESEARCH

This was the first PhD project within the newly established African Annonaceae program at the Nationaal Herbarium Nederland - Wageningen branch (NHN-WAG). One important result was the clarification of the phylogenetic relationships between most of the African genera of Annonaceae. This provides a solid framework to stimulate future research on the processes of evolution and systematics of African Annonaceae. Because Annonaceae is a morphologically diverse family that is widespread across tropical Africa, it has the potential to be developed into an ideal model group for testing numerous hypotheses at both the biogeographic and evolutionary levels. A few examples of such studies can be found in this PhD. It is now important to build on this first step. Numerous questions remain to be answered and a few hints towards future research have been provided above.

Annonaceae research in the NHN in general seems to be slowly grinding to a halt now that the NHN-Utrecht branch has been closed due to budgetary decisions of Utrecht University and some NHN-Leiden branch Annonaceae specialists were delocalized to other fields. It is undeniable that the NHN has amassed a huge quantity of data over the past 25 years. By doing so it has established itself as the worldwide leader in Annonaceae systematics both in terms of number of collections and in systematic expertise. However, since my arrival in 2004, I have witnessed a huge decline in interest in Annonaceae research within the NHN. Ironically, I also witnessed and participated in a huge increase of Annonaceae molecular data (Mols et al., 2004; Richardson et al., 2004; Pirie et al., 2006; Erkens et al., 2007; Couvreur et al., in press). Such data bring Annonaceae research to a whole new and exciting level. Indeed, with the near completion of a robust family level phylogeny we can now tackle more in-depth and precise questions about the *processes of evolution* within this important tropical family, such as evo-devo or phylogeographic studies. Furthermore, Annonaceae has the potential to hold up as a model for studies on other groups of plants that are similarly distributed in tropical regions and are some of the most diverse on the planet. The NHN, especially with the prospect of the ambitious National Biodiversity Institute (NCB), should not discard this hard-earned and unique expertise but should stimulate future research with the appointment of new PhD students and post-doctoral researchers.

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Appendices

		GenBank accession numbers							Morphological characters						
Taxon	**Herbarium accession**	***rbcL***	***trnLF***	***matK***	***psbA-trnH***	***ndhF***	***trnSG***	**Locality**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Coelocaryon preussi	Wieringa, J.J. 3640 (WAG)	AY743437	AY743456	AY743475	---		---	Gabon	0	2	0	?	?	0	2
Eupomatia bennettii	Chatrou, L.W. (U)	DQ861790	DQ861842	---	---	--	---	UUBC	?	2	0	?	?	0	0
Persea americana	Chatrou, L.W. 279 (U)	AY841592	AY841669	---	---	--	---	UUBC	0	2	0	?	?	0	0
Anaxagorea phaeocarpa	Maas, P.J.M. 8592 (U)	AY238952	EF179316 AY231284 AY238944	AY238960	AY841426	EF179279	EF179321	Ecuador	0	2	0	1	0	0	0
Anaxagorea silvatica	Maas, P.J.M. 8836 (U)	AY743439	AY743458	AY743477	AY841427	--	EF179322	Brazil	0	2	0	0	0	0	0
ambavioid															
Cananga odorata	Chatrou, L.W. 93 (U)	AY841602	AY841680	AY841394	AY841431	AY841403	AY841548	Costa Rica	0	2	0	1	0	1	0
Cleistopholis glauca	Wieringa, J.J. 3278 (WAG)	AY841603	AY841681	AY841395	AY841432	AY841404	AY841549	Gabon	0	2	0	0	0	0	0
Lettowianthus stellatus	Robertson 7505 (WAG)	EU169775	EU169753	EU169686	EU169730	--	EU169797	Kenya	0	2 [a, h]	0	0 [a]	0	0 [f]	0 [f]
Meiocarpidium lepidotum	Breteler 13947	EU169776	EU169754	EU169686	EU169731	--	EU169798	Gabon	0 [b]	1 [b, h]	0 [b]	1 [b]	0 [b]	0 [f]	0 [f]
Short branch clade															
Annickia chlorantha	Sosef, M.S.M. 1877 (WAG)	AY841594	AY841671	AY841393	AY841442	AY841401	AY841550	Gabon	0	2	0	1	0	0	2
Cremastosperma brevipes	Scharf, U. 76 (U)"	AY743527	AY743573	AY743550	AY841447	AY841405	AY841552	French Guiana	0 [c]	2 [c, h]	0 [c]	0 [c]	0 [c]	0 [c]	?
Greenwayodendron oliveri	Jongkind, C.C.H. 1795 (WAG)	AY743451	AY743470	AY743489	AY841465	AY841408	AY841555	Ghana	0	2	0	1	0	0	0
Monocarpia euneura	Slik, F. 2931 (L)	AY318998	AY319111	AY518865	AY841477	AY841412	AY841559	Indonesia	0 [d]	1 [d, h]	0 [d]	1 [d]	0 [d]	0 [d]	?
Mosannona costaricensis	Chatrou, L.W. 90 (U)	AY743510	AY743496	AY743503	AY841479	AY841413	AY841560	Costa Rica	0 [e]	2 [e, h]	0 [e]	0 [e]	0 [e]	0 [e]	2 [e]
Piptostigma mortehani	Wieringa, J.J. 2779 (WAG)	AY743454	AY743473	AY743492	AY841498	AY841415	AY841562	Gabon	0	1	0	1	0	0	0
Unonopsis stipitata	Chatrou, L.W. 253 (U)	AY841662	AY841740	AY841400	AY841519	Y841423	AY841570	Peru	0	2	0	1	0	0	2
Long branch clade															
Annona glabra	Chatrou, L.W. 467 (U)	AY841596	AY841673	DQ125050	DQ125116	EF179281	EF179323	USA - Florida	0	2	0	1	0	1	2
Annona muricata	Chatrou, L.W. 468 (U)	AY743440	AY743459	AY743478	AY841428	EF179282	EF179324	UUBC	0	2	0	1	0	1	2
Anonidium sp	Cheek, M. 7896 (K)	AY841598	AY841675	DQ125051	DQ125117	EF179283	EF179325	Cameroon	0 [b]	2 [b, h]	0 [b]	1 [b]	0 [b]	1 [f]	2 [f]
Artabotrys hexapetalus	Chatrou, L.W. 470 (U)	AY238953	AY231286	AY238962	AY841429	EF179284	EF179326	India	0	1	1	1	0	0	?

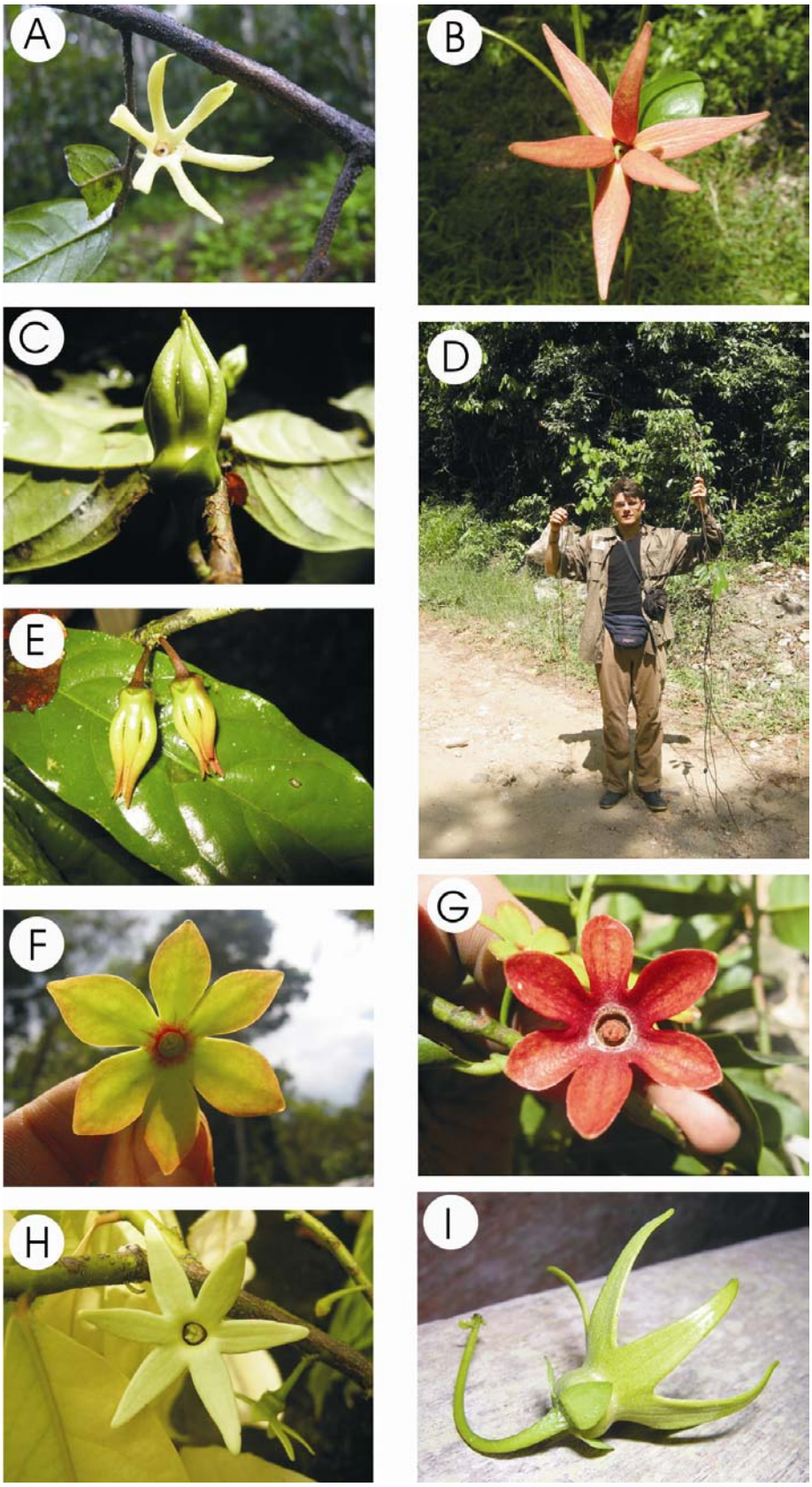
Asimina triloba	Chatrou, L.W. 276 (U)	AY743441	AY743460	AY743479	AY841430	EF179287	EF179329	North America	0	1	0	0	0	1	2
Cymbopetalum brasiliense	Chatrou, L.W. 471 (U)	AY841608	AY841686	DQ125055	DQ125121	EF179289	EF179331	Brazil	0	1	0	?	0	1	2
Disepalum platypetalum	Takeuchi & Sambas 18201 (L)	AY841612	AY841690	DQ125057	DQ125122	EF179292	EF179334	Indonesia	0[g]	2[g,h]	0[g]	?	1[g]	1[g]	2[g]
Duguetia hadrantha	Chatrou, L.W. 181 (U)	AY738161	AY740573	AY740541	DQ125123	EF179293	EF179335	Peru	0	2	0	0	0	0	?
Duguetia staudtii	Andel, T.R. van 3290 (U)	AY738178	AY740590	AY740558	DQ125124	EF179294	EF179336	Cameroon	0	2	0	0	0	0	?
Fusaea peruviana	Chatrou, L.W. 179 (U)	AY743445	AY743464	AY743483	AY841436	EF179295	EF179337	Peru	0	2	0	0	?	1	0
Goniothalamus griffithii	Kessler, P.J.A. 3188 (L)	AY743446	AY743465	AY743484	DQ125125	EF179296	EF179338	Thailand	0	2[h]	0	?	0	1	?
Goniothalamus tapis	Kessler, P.J.A. 3193 (L)	AY841622	AY841700	DQ125058	DQ125126	EF179297	EF179339	Thailand	0	2[h]	0	1	0	1	?
Guatteria aeruginosa	Chatrou, L.W. 66 (U)	AY740958	AY741007	AY740909	DQ125136	EF179299	EF179341	Costa Rica	0	2	0	0	0	0	?
Guatteria anomala	Ishiki, M. 2233 (U)	AY740962	AY741011	AY740913	AY841437	EF179298	EF179340	Mexico	0	2	0	0	0	0	?
Guatteria pudica	Chatrou, L.W. 107 (U)	AY740994	AY741043	AY740945	DQ125197	--	---	Costa Rica	0	2	0	0	0	0	?
Letestudoxa bella	Wieringa, J.J. 2797 (WAG)	AY841629	AY841707	DQ125059	DQ125128	EF179302	EF179344	Gabon	0	2	1	0	0	0	0
Mkilua fragrans	Chatrou, L.W. 474 (U)	AY841634	AY841712	DQ125060	DQ861696	EF179303	EF179345	East Africa	0	2	0	0	0	1	?
Neostenanthera myristicifolia	Wieringa, J.J. 3566 (WAG)	AY743448	AY743467	AY743486	DQ125130	EF179306	EF179348	Gabon	0	2	0	1	0	1	0
Pseudartabotrys letestui	Wieringa, J.J. 3273 (WAG)	AY841650	AY841728	DQ125061	DQ125131	EF179307	EF179349	Gabon	0[e]	2[,h]	0[a]	1[e]	0[e]	0[f]	0[f]
Trigynaea lanceipetala	Chatrou, L.W. 234 (U)	AY743449	AY743468	AY743487	---	EF179309	EF179351	Peru	0	2[h]	0	1	0	1	2
Xylopia ferruginea	Slik, F. s.n. (L)	AY841666	AY841744	DQ125063	DQ125133	EF179311	---	Indonesia	0	?	0	1	0	1	0
Xylopia peruviana	Chatrou, L.W. 483 (U)	AY238958	AY231291	AY238967	DQ125134	EF179312	EF179353	Peru	0	?	0	1	0	1	0
uvarioids															
Dasymaschalon macrocalyx	Kessler, P.J.A. 3199 (L)	AY841610	AY841688	EF179277	EF179313	EF179290	EF179332	Thailand	0	2[h]	0	?	0	0	?
Dielsiothamnus divaricatus	Johnson 1903 (OWU)	EU169781	EU169759	EU169692	EU169736	--	EU169803	Tanzania	0	0[,a,h]	0	?	0	0	0
Monanthotaxis whytei	Chatrou, L.W. 475 (U)	AY841635	AY841713	EF179278	EF179315	EF179304	EF179346	UUBC	0	?	1	1	0	0	0
Sphaerocoryne gracilis	Robertson, A. 7554 (WAG)	EU169777	EU169755	EU169688	EU169732	--	EU169799	Kenya	0	2[,h]	0[a]	1	0	0	1
Toussaintia orientalis	Jonhson 1957 (OWU)	EU169778	EU169756	EU169689	EU169733	EU169710	EU169800	Tanzania	0	2	1	1	0	1	?
Uvaria lucida	Botanische Tuinen 84GR00334 (U)	AY238957	AY231290	AY238966	AY841440	EF179310	EF179352	West African	0	2	1	0	0	0	0
African long branch clade															
Asteranthe asterias	Robertson, A. 7548 (WAG)	EU169779	EU169757	EU169690	EU169734	EU169711	EU169801	Kenya	0	1[a,h]	0	0[a]	0[a]	1[f]	2[f]
Dennettia tripetala	Jongkind 4356 (WAG)	EU169780	EU169758	EU169691	EU169735	EU169712	EU169802	Ivory	0	2[h,h]	0	0[a]	0	1[f]	2[f]

Hexalobus crispiflorus	Sosef, M.S.M. 2287 (WAG)	EU169782	EU169760	EU169693	EU169737	EU169713	EU169804	Gabon	0	1	0	1	1	1	1
Hexalobus salicifolius	Sosef, M.S.M. 2376 (WAG)	EU169783	EU169761	EU169694	EU169738	EU169714	EU169805	Gabon	0	1	0	1	1	1	1
Isolona campanulata	Chatrou, L.W. 472 (U)	AY238954	AY231287	AY238963	DQ125127	EF179301	EF179343	UUCB	1	1	0	1	1	0	1
Isolona cauliflora	Robertson, A. 7555 (WAG)	EU169784	EU169762	EU169695	EU169739	EU169716	EU169807	Kenya	1	1	0	1	1	0	1
Isolona hexaloba	Burgt, X.M. van der 791 (WAG)	EU169785	EU169763	EU169696	EU169740	EU169717	EU169808	Cameroon	1	1	0	1	1	0	1
Mischogyne micheloides	Bamps, P. 4459 (WAG)	EU169786	EU169764	EU169697	EU169741	EU169718	EU169809	Angola	0 [b]	1 [h,h]	0	1 [b]	0 [b]	1 [f]	1 [f]
Monocyclanthus vegnei	Jongkind, C.C.H. 6992 (WAG)	EU169787	EU169765	EU169698	EU169742	EU169719	EU169810	Liberia	0	1 [h]	0	1	0	1 [j]	?
Monodora undulata	Alpin, D. 4012 (WAG)	EU169788	EU169766	EU169701	EU169744	EU169722	EU169813	NBGM	1	1	0	1	1	1	1
Monodora crispata	Chatrou, L.W. 476 (U)	AY841637	AY841715	EU169699	EU169743	EU169720	EU169811	Ivory Coast	1	1	0	1	1	1	1
Monodora myristica	Chatrou, L.W. 477 (U)	AY743447	AY743466	AY743485	DQ125129	EF179305	EF179347	Ivory Coast	1	1	0	1	1	1	1
Ophrypetalum odoratum	Robertson, A. 7547 (WAG)	EU169789	EU169767	EU169702	EU169745	EU169723	EU169814	Kenya	0	1 [a,h]	0	0	0	1 [f]	2 [f]
Sanrafaelia rufonammari	Kayombo 3027 (MO)	EU169790	EU169768	EU169703	EU169746	EU169724	EU169815	Tanzania	0 [i]	0 [i]	0 [i]	1 [i]	1 [i]	1 [i]	?
Uvariastrum insculptum	Jongkind, C.C.H. 4707 (WAG)	EU169791	EU169769	EU169704	EU169747	EU169725	EU169816	Ivory Coast	0	1	0	1	0	1	1
Uvariastrum pierreanum	Wieringa 2620 (WAG)	EU169792	EU169770	EU169705	EU169748	--	EU169817	Gabon	0	1	0	1	0	1	1
Uvariodendron kirkii	Robertson, A. 7550 (WAG)	EU169793	EU169771	EU169706	EU169749	EU169726	EU169818	Kenya	0	2	0	1	0	1	2
Uvariodendron molundense var. *citrata*	Sosef, M.S.M. 2219 (WAG)	EU169794	EU169772	EU169707	EU169750	EU169727	EU169819	Gabon	0	2	0	1	0	1	2
Uvariopsis korupensis	Richardson, J.E. 212 (WAG)	EU169796	EU169774	EU169709	EU169751	EU169729	EU169820	Cameroon	0	2	0	1	0	1	?
Uvariopsis vanderystii	Sosef, M.S.M. 2241 (WAG)	EU169795	EU169773	EU169708	EU169752	EU169728	EU169821	Gabon	0	2	0	1	0	1	?

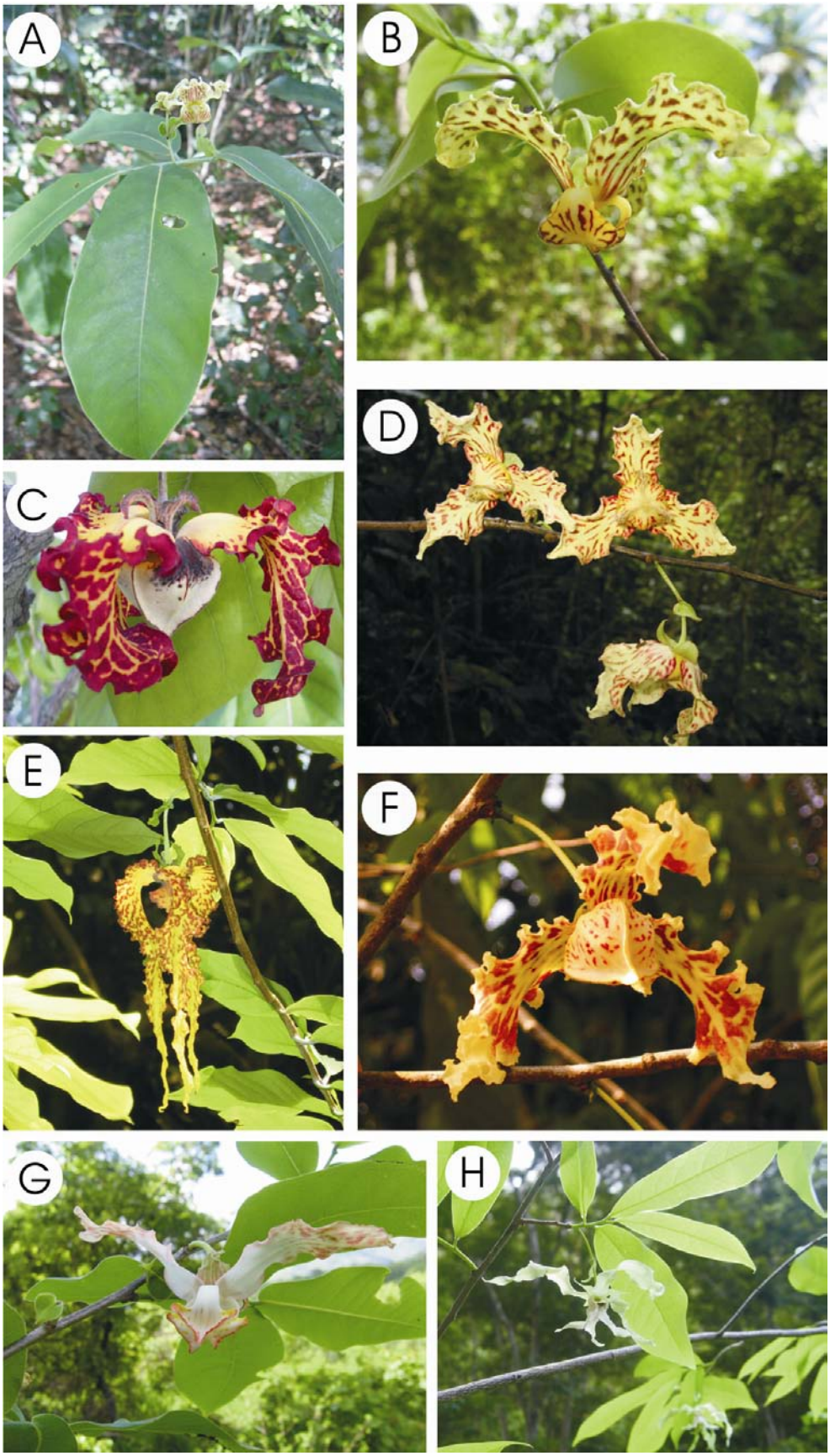
Appendix A. Taxon sampling, voucher information, GenBank accession numbers for each of the six chloroplast markers used in Chapter 3 and in Chapter 4 (dataset A), and matrix of the seven morphological characters defined in Chapter 3, Table 3 scored for all specimens. List of literature used to score the different genera not sampled in Doyle and Le Thomas (1996): [a] (Verdcourt, 1971); [b] (Le Thomas, 1969); [c] (Pirie, 2005); [d] (Mols, 2004a) ; [e] (Chatrou, 1998) ; [f] (Le Thomas, 1980, 1981) ; [g] (Johnson, 1989) ; [h] (van Heusden, 1992); [i] (Verdcourt, 1996); [j] (Walker, 1972). UUCB: University Utrecht Botanical Garden; NBGB: National Botanic Gardens Belgium

Taxon	Herbarium voucher	Collection locality	*trnL-trnF*	*trnSG*	*psbA-trnH*	*ndhF*	*trnD-trnT*
Isolona campanulata	Chatrou, L.W. 472 (U)	UUBC	EF179318 AY231287 AY238947	EF179343	DQ125127	EF179301	EU216689
Isolona capuronii	Service Forestier de Madagascar 8941 (P)	Madagascar	EU216708	EU216617	EU216662	––	---
Isolona cauliflora	Robertson, A. 7555 (WAG)	Kenya	EU169762	EU169807	EU169739	EU169716	EU216682
Isolona congolona	Liben, L. 3852 (WAG)	DRC	EU216704	EU216613	EU216658	EU216637	---
Isolona cooperi	Botanische Tuinen Utrecht, 473 (U)	Cultivated	AY841704	EU216612	EU216657	EU216636	EU216681
Isolona dewevrei	Merello, M.C. 1346 (MO)	Ghana	EU216715	EU216624	EU216669	EU216645	EU216690
Isolona ghesquierei	Schatz, G.E. 3364 (MO)	Madagascar	EU216709	EU216618	EU216663		---
Isolona heinsenii	Couvreur, T.L.P. 10 (WAG)	Tanzania	EU216710	EU216619	EU216664	EU216640	EU216686
Isolona hexaloba	Burgt, X.M. van der 791 (WAG)	Cameroon	EU169763	EU169808	EU169740	EU169717	EU216683
Isolona linearis	Couvreur, T.L.P. 102 (WAG)	Tanzania	EU216711	EU216620	EU216665	EU216641	EU216687
Isolona maitlandii	Leeuwenberg, A.J.M. 9550 (WAG)	Cameroon	EU216714	EU216623	EU216668	EU216644	EU216688
Isolona perrieri	Carlson, B. 48 (K)	Madagascar	EU216707	EU216616	EU216661	EU216639	EU216685
Isolona pleurocarpa	van Andel, T. 4177 (WAG)	Cameroon	EU216712	EU216621	EU216666	EU216642	---
Isolona thonneri	Letouzey, R. 10205 (P)	Cameroon	EU216713	EU216622	EU216667	EU216643	---
Isolona zenkeri (1)	Sosef, M.S.M. 2250 (WAG)	Gabon	EU216705	EU216614	EU216659	EU216638	EU216684
Isolona zenkeri (2)	Sosef, M.S.M. 2322 (WAG)	Gabon	EU216706	EU216615	EU216660	––	---
Monodora angolensis	Alpen, D. S4013 (WAG)	West Africa	EU216718	EU216627	EU216672	EU216648	EU216696
Monodora carolinae	Couvreur, T.L.P. 54 (WAG)	Tanzania	EU216723	EU216632	EU216677	EU216653	EU216700
Monodora crispata	Chatrou, L.W. 476 (U)	UUBC	AY841715	EU169811	EU169743	EU169720	EU216691
Monodora globiflora	Couvreur, T.L.P. 99 (WAG)	Tanzania	EU216724	EU216633	EU216678	EU216654	EU216701
Monodora grandidieri	Vollesen, K. 3031 (WAG)	Tanzania	EU216719	EU216628	EU216673	EU216649	EU216697
Monodora hastipetala	Couvreur, T.L.P. 42 (WAG)	Tanzania	EU216725	EU216634	EU216679	EU216655	EU216702
Monodora junodii	Couvreur, T.L.P. 88 (WAG)	Tanzania	EU216721	EU216630	EU216675	EU216651	EU216699
Monodora laurentii	Niangadouma, R. 179 (WAG)	Gabon	EU216720	EU216629	EU216674	EU216650	EU216698
Monodora minor	Couvreur, T.L.P. 36 (WAG)	Tanzania	EU216726	EU216635	U216680	U216656	U216703
Monodora myristica (1)	Chatrou, L.W. 477 (U)	UUBC	AY743466	EF179347	DQ125129	EF179305	EU216692
Monodora myristica (2)	Richardson, J.E. 191 (WAG)	Cameroon	EU216716	EU216625	EU216670	EU216646	EU216693
Monodora stenopetala	Correia, M.F. 3840 (M)	Mozambique	EU216722	EU216631	EU216676	EU216652	---
Monodora tenuifolia	Schmidt, R. 2025 (MO)	Ghana	EU216717	EU216626	EU216671	EU216647	EU216694
Monodora undulata	Alpin, D. 4012 (WAG)	Cultivated	EU169766	EU169813	EU169744	EU169722	EU216695
Uvariopsis vanderystii	Sosef, M.S.M. 2241 (WAG)	Gabon	EU169773	EU169821	EU169752	EU169728	---

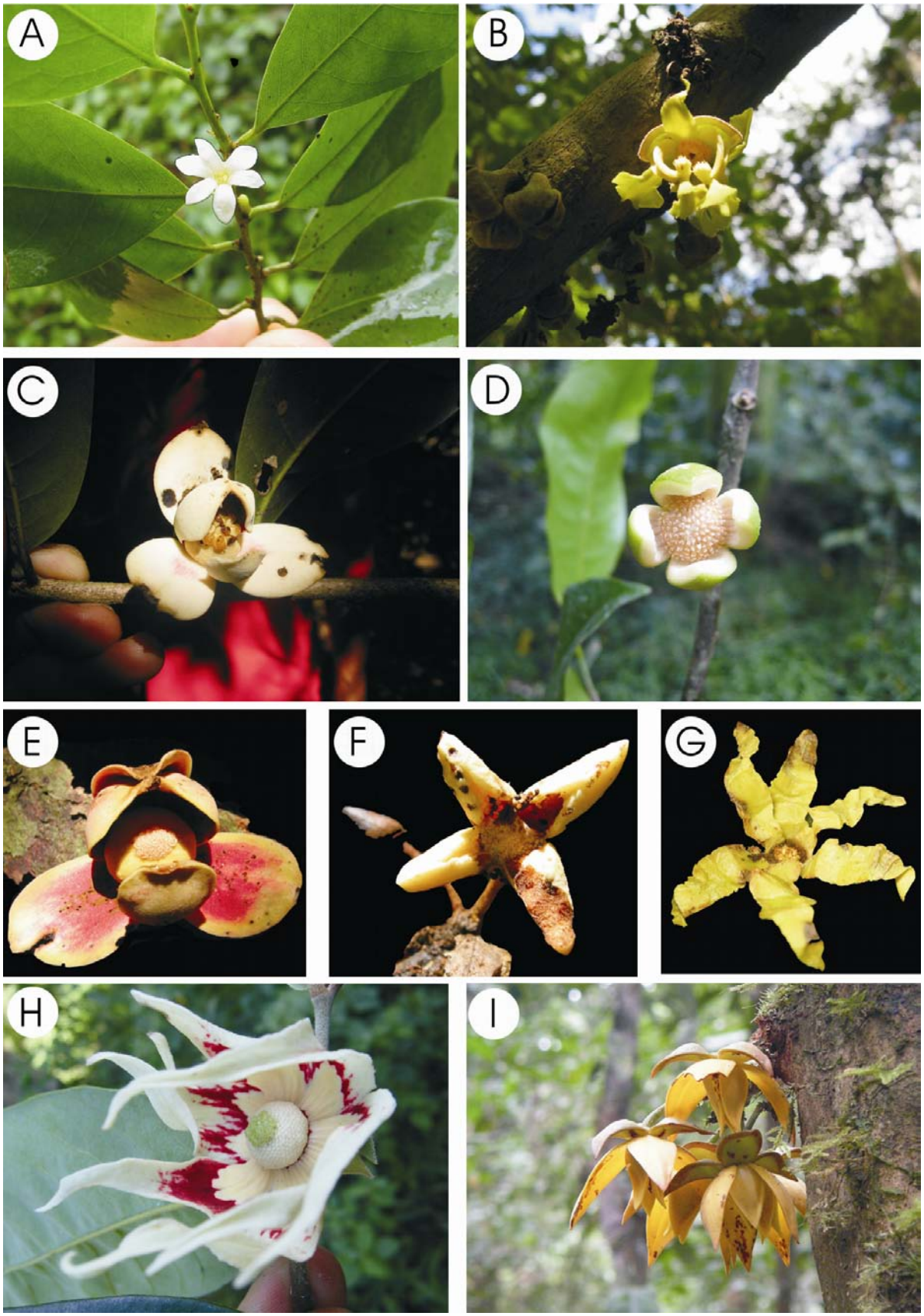
Appendix B. Taxon sampling, voucher information and GenBank accession numbers for each of the five chloroplast markers used in Chapter 5, Figure 5.2b, dataset B. UUCB: University Utrecht Botanical Garden; DRC: Democratic Republic of Congo.



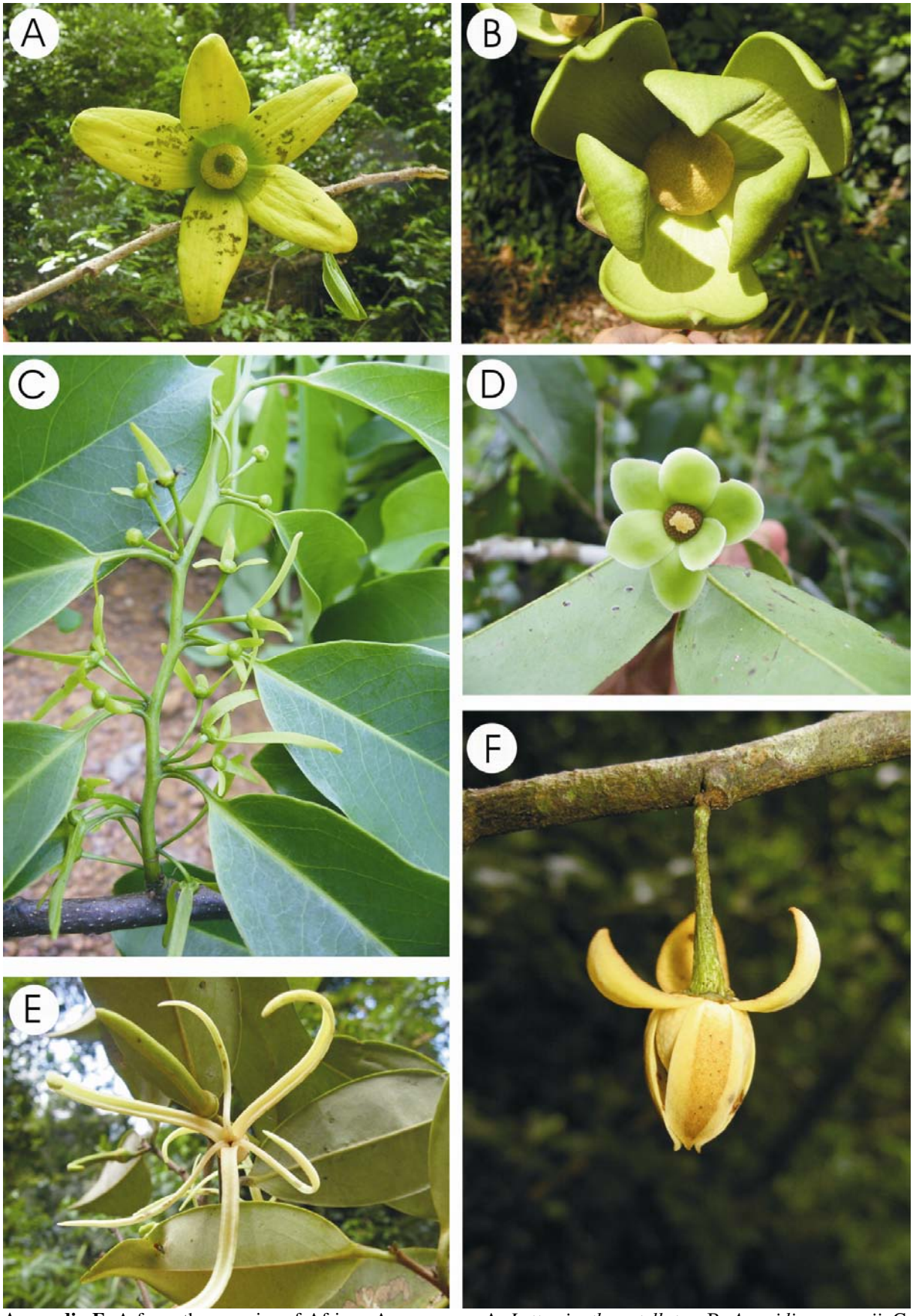
Appendix C: Floral morphology in *Isolona*. A. *I. heinsenii.* B. *I. cauliflora*. C. *I. zenkei*. D. Author holding *I. cauliflora* inflorescences. E. *I. zenkeri*. F. *I. hexaloba* (Young flower). G. *I. hexaloba* (old flower). H. *I. cooperi*. *I. campanulata* (Photo Svenja Meinke).



Appendix D: Floral morphology in *Monodora*. A. *M. minor*. B. *M. carolinae*. C. *M. myristica*. D. *M. globiflora*. E. *M. crispata*. F. *M. undulate* (Photo Carel Jongkind). G. *M. grandidieri*. H. *M. hastipetala*.



Appendix E. A. *Sanrafaelia ruffonammari*. B. *Ophrypetalum odoratum*. C. *Uvariodendron kirkii*. D. *Uvariopsis sp*. E. *Uvariodendron pycnophyllum* F. *Uvariopsis vaderystii*. G. *Hexalobus crispiflorus*. H. *Asteranthe asterias*. I. *Uvariopsis zenkeri* (Photo Xander van der Burgt).



Appendix F. A few other species of African Annonaceae. A. *Lettowianthus stellatus*. B. *Anonidium mannii*. C. *Cleistopholis staudii* C. *Uvaria leptocladon*. E. *Xylopia aethiopica*. F. *Polyceratocarpus sp.* (Tanzania).

Appendix G. A. Gabonese child near Lope. B. Tanzanian children near Amani. C. Marc Sosef, Yves Issembe and Thomas Couvreur in Gabon. D. Trip to Mwanihana-Tanzania; right to left: Frank Mbago, Ranger, Chris, Granted, Lyssa and Thomas. E. Pressing specimens with Davy in Tanzania. F. Collecting *M. globiflora* in Tanzania. G. Collecting flowers using clipping poles in Gabon with Jean-Noel Boussiengui. H. Late afternoon pressing collections near Bambidie-Gabon.; Yves Yssembe, Marleen Botermans, Jean-Noel and Marc Sosef.

Appendices

Abstract

The goal of this PhD project was to study the evolution, systematics and biogeography of two African genera from the pan-tropical Annonaceae family: *Isolona* and *Monodora*. Both genera are unique within the family in that the female reproductive parts (or carpels) are fused into a single unit. All other Annonaceae have freely arranged carpels. We investigated the phylogenetic relationships of *Isolona* and *Monodora* at the intra-familial and intra-generic levels.

In Chapter 2, we explore the influence of priors when using the novel Bayesian based posterior mapping to study the evolution of morphological characters. Up to now, it was unclear if these priors had any influence on the results. Using a family level molecular phylogeny of the Annonaceae, we study the evolution of two morphological characters under different prior values. We show that different prior values will return different results. Thus, inadequate prior values can lead to erroneous conclusions over the evolution of the studied morphological characters. We also indicate a practical way to choose the prior values when using the posterior mapping approach to study morphological character evolution.

In Chapter 3, using the posterior mapping approach, we study the evolutionary origins of syncarpy in Annonaceae. The closest relatives of *Isolona* and *Monodora* are elucidated. We generate a well resolved phylogeny which included for the first time the majority of African Annonaceae genera. We also study additional morphological and palynological characters relevant to Annonaceae classification in general. Our phylogenetic analyses recover a fully resolved clade comprising twelve endemic African genera, including *Isolona* and *Monodora*, which was nested within the so-called long-branch clade. This is the largest and most species-rich clade of African genera identified to date within Annonaceae. Our results indicate that syncarpy arose by fusion of a moderate number of carpels. The alternative hypothesis that syncarpy arose by multiplication of an initial single carpel receives no support.

In Chapter 4 we use African Annonaceae as a model family to study the biogeographical aspects of the evolutionary origins of African rain forests. It is generally thought that the large West-Central rain forest blocks was continuous during the Eocene with the now fragmented and smaller forests of East Africa, explaining the strong floristic affinities between both areas. Using dated molecular phylogenies we provide evidence of the recurring break-up and reconnection of this pan-African rain forest during the Oligocene and Miocene. The reconnections allowed for biotic exchange while the break-ups induced speciation enhancing the levels of endemicity, thus providing an explanation for present-day patterns in the distribution and diversity of plants in African rain forests.

In Chapter 5, we perform a detailed analysis of pollen morphology within a strongly supported monophyletic group of five African genera, including *Isolona* and *Monodora*. We specifically assess if pollen characters are useful for classification purposes within *Isolona*

and *Monodora* using a species-level molecular phylogeny. The results show a wide pollen morphological diversity. The pollen types defined within *Isolona* and *Monodora* provide little taxonomic information for major clades within both genera. However, pollen variation proves useful as a support of phylogenetic relatedness between groups of closely related species.

Finally in Chapter 6, a monographic revision of both *Isolona* and *Monodora* is presented. *Isolona* consists of 20 species with five endemic to Madagascar and one newly described species. *Monodora* has a total of 14 species, three of which were described during this PhD project from Tanzania. Detailed descriptions as well as keys are provided. The conservation status of each species is assessed following the IUCN recommendations. Just under half of the total number of species from both genera is assigned to some level of threat (12 species or 60% in *Isolona* and four species or 28% in *Monodora*).

Samenvatting

Het doel van dit PhD project was de evolutie, systematiek en biogeografie te bestuderen van twee Afrikaanse geslachten van de pantropische Annonaceae familie: *Isolona* en *Monodora.* Beide geslachten zijn uniek binnen de familie vanwege de vrouwelijke reproductieve organen (of carpellen) die vergroeid zijn tot één enkele eenheid. Alle andere Annonaceae hebben vrije carpellen. We onderzochten de fylogenetische relaties van *Isolona* en *Monodora* op het intrafamiliale en intragenerische niveau.

In Hoofdstuk 2 verkennen we de invloed van 'priors' bij de toepassing van moderne op Bayesiaanse methodes gebaseerde 'posterior mapping' om de evolutie van morfologische kenmerken te bestuderen. Tot nu toe was het onduidelijk of deze priors enige invloed op de resultaten hadden. Middels een moleculaire fylogenie van de Annonaceae bestuderen we de evolutie van twee morfologische kenmerken met verschillende prior-waarden. We tonen aan dat verschillende waarden tot verschillende resultaten leiden. Dus, inadequate prior-waarden kunnen leiden to foutieve conclusies m.b.t. de evolutie van de betreffende morfologische kenmerken. We geven ook een praktische weg aan om prior-waarden te kiezen bij een posterior mapping benadering om morfologische kenmerkevolutie te bestuderen.

In Hoofdstuk 3 bestuderen we, met behulp van de posterior mapping benadering, de evolutionaire oorsprong van een vergroeidbladig vruchtbeginsel binnen de Annonaceae. De nauwste verwanten van *Isolona* en *Monodora* worden aangeduid. Een goed opgeloste fylogenie wordt geproduceerd waarin voor de eerste maal de meerderheid van Afrikaanse Annonaceae geslachten zijn meegenomen. We bestuderen ook een aantal extra morfologische en palynologische kenmerken die relevant zijn voor de classificatie van Annonaceae in het algemeen. Onze fylogenetische analyses leiden tot een volledig opgeloste 'clade' met twaalf endemische Afrikaanse geslachten, waaronder *Isolona* en *Monodora*, die valt binnen de zogenoemde 'long-branch clade'. Dit is de tot op heden grootste en meest soortenrijke clade van Afrikaanse geslachten binnen de Annonaceae. Onze resultaten tonen aan dat vergroeidbladige vruchtbeginsels ontstaan zijn uit een fusie van een relatief klein aantal vrije carpellen. De alternatieve hypothese dat deze voortkomen uit de vermeerdering van één enkel carpel wordt niet ondersteund.

In Hoofdstuk 4 gebruiken we Afrikaanse Annonaceae als modelfamilie om de biogeografische aspecten van de evolutionaire oorsprong van Afrikaans regenbossen te bestuderen. Het wordt algemeen aangenomen dat de grote blokken West-Centraal regenbos en de nu gefragmenteerde en kleinere Oost-Afrikaanse regenbossen gedurende het Eoceen een continue strook regenbos vormden, wat de sterke floristische affiniteiten tussen beide gebieden verklaart. Via gedateerde moleculaire fylogeniëen leveren we bewijs voor het regelmatig opbreken en weer versmelten van dit pan-Afrikaanse regenbos gedurende het Oligoceen en Mioceen. De versmelting maakte biotische uitwisseling mogelijk terwijl het opbreken tot soortvorming leidde en het endemisme-niveau verhoogde, wat een verklaring

oplevert voor de huidige patronen in verspreiding en diversiteit van planten in het Afrikaanse regenbos.

In Hoofdstuk 5 voeren we een gedetailleerde pollenmorfologische analyse uit binnen een sterk ondersteunde monofyletische groep van vijf Afrikaanse geslachten, waaronder *Isolona* en *Monodora*. Met behulp van een soort-niveau moleculaire fylogenie bepalen we met name of pollenkenmerken nuttig zijn voor classificatiedoeleinden binnen *Isolona* en *Monodora*. De resultaten laten een brede pollenmorfologische variatie zien. De binnen *Isolona* en *Monodora* gedefinieerde pollentypen geven weinig taxonomische informatie voor de grotere clades binnen beide geslachten. Echter, pollenvariatie blijkt nuttig als ondersteuning van fylogenetische verwantschap tussen groepen van nauw verwante soorten.

Tot slot presenteren we in Hoofdstuk 6 een monografische revisie van zowel *Isolona* als *Monodora*. *Isolona* bevat 20 soorten waarvan vijf endemisch voor Madagaskar en één nieuw beschreven soort. *Monodora* heeft in totaal 14 soorten, waarvan er drie uit Tanzania die gedurende dit PhD project werden beschreven. Gedetailleerde beschrijvingen en sleutels worden gegeven. Van elke soort is de beschermingsstatus bepaald via de IUCN aanbevelingen. Net iets minder dan de helft van het totaal aantal soorten van beide geslachten is toegewezen aan een bepaald niveau van bedreiging (12 soorten ofwel 60% bij *Isolona* en vier soorten ofwel 28% bij *Monodora*).

Acknowledgments

Although a PhD dissertation is very personal undertaking, none of the work presented here would have be possible without the collaboration and help of numerous people at many different levels. The list of people I am in debt to for helping and advising me during these four years will be long! Each one of them has helped in one way or another and I am very grateful to all of them.

I first want to thank my promotor Marc Sosef, director of the Biosystematic group of the Wageningen University, for giving me the opportunity to undertake my PhD at the Nationaal Herbarium Nerderland in Wageningen. I hope he doesn't regret hiring me for the job! My two co-promotors, James Richardson and Lars Chatrou, have been great too. I thank them deeply for bearing with me during my times of doubt and long emails about my thoughts and fears. Even after his departure to Edinburgh, James provided strong and reliable help via lengthy emails and long telephone calls. I am also very grateful to Lars who, without a moment of hesitation, became my third supervisor at a time I really needed it. To all three of my supervisors, Marc, James and Lars, I thank you for all your scientific and personal advice. I must say that it was a perfect contrast of points of view. Sometimes it might have been hard to make a clear consensus, but it definitely brought variety of thought and interpretation, and that was a great addition to my professional experience.

I owe a lot to Jan Wieringa of the NHN Wageningen. He has spent days with me loading specimen information and geographical coordinates into the herbarium database, meticulously checking every entry. He has also been my main help for all the taxonomical and nomenclatural questions I had. He always made time for me and always went to the end of each question. We also had a great time in the field in Cameroon, even though it was just for one day. I wish I could have had an opportunity to accompany you for a proper field trip. I am sure I would have learned a lot!

I wish to thank Roy Gereau, who, from the start, has been involved in my project at many different levels. The first one was for the description of the new species of *Isolona* and *Monodora*. This collaboration taught me a lot about taxonomic consistency. In addition, he has played a major role in my field trip to Tanzania, one of the highlights of my PhD years. He provided important contacts, advice and help in stages leading up to the trip, especially when dealing with work permits. He arrived in Tanzania at the same time as I, and once again provided much needed help and assistance. I will never forget the afternoons we spent in Dar es Salaam going over Annonaceae conservation statuses! I learned a lot about conservation and methods of conservation assessments. Thank you Roy for this wonderful help and inspiration you have given me!

The Museum National d'Histoire Naturelle in Paris has played a major role these last decades in African Annonaceae systematics. Thierry Deroin is deeply thanked for his great help and support. Form the first email we exchanged he showed a great interest in my project. He helped me prepare my application for a SYNTHESYS grant to Paris in 2005 for five weeks. I doubt that without his support I would have been able to go there so long. This trip was vital for my PhD. He also introduced me to floral anatomy studies that he specializes in, which provided a great inspiration for several parts of my PhD. Finally, Thierry always

helped when I needed urgent loans and material from Paris, making my life in that respect very easy! During my visit to Paris, I also met with Annick Le Thomas. She was also very interested in my project and answered the many questions I had about African Annonaceae. She kindly allowed me to use all her archives, from pollen photos to unpublished species descriptions and drawings. Merci à tous les deux pour votre aide généreuse et spontanée.

Raymond van der Ham and Berthie van Heuven from NHN-Leiden are deeply thanked. They provided much assistance and help when I decided to undertake a large pollen survey a few genera of Annonaceae. They were always available for help and assistance during the scanning of the pollen grains, as well as interpretation of the results and in the writing of a chapter in my PhD.

Timo van der Niet is deeply thanked for his help during the short year he spent at Wageningen as a Post doctoral researcher. He really provided a huge boost to my PhD by critically reading my articles, as well as making the long laboratory day's very fun!

I thank also Wil Wessel-Brand, Hans de Vries and Joanne Porck for their wonderful botanical drawings. Wil helped with the ones published in the Adansonia article, while Hans and Joanne drew the ones that shall be published in the monograph. I think I was privileged to have three artists working for me on this project. This provided an exceptional diversity of drawings all with there unique and beautiful style. After all this is a PhD on diversity, so why not include some artistic diversity?

During my PhD I undertook two one-month field trips. For the trip to Gabon, I thank Ludovic Ngok Banak, director of the Herbier National du Gabon, for his help in organizing and coordinating our trip and welcoming us so warmly to his country. I thank Yves Issembe for his great field assistance. Without his cunning eye for spotting Annonaceae, I think we would have gone home empty handed. I learned a lot from him in general! Merci! Raoul Niangadouma is also thanked for assistance in the field.

For the field trip to Tanzania, I owe a huge amount of gratitude to Frank Mbago, curator of the herbarium at the Dar es Salaam University. His experience in the field and his knowledge of plant distribution in Tanzania made the trip extremely successful. I collected all the plants I needed for my PhD project and so much more. A large part of the PhD would not have been possible without his help. He really took care of me during my stay making the trip very pleasant. Asante sana!

Jan Maas from NHN-Utrecht has also helped me significantly. At a time when my lab work was not really working he helped me get back on track thanks to his extensive molecular laboratory knowledge. Hartelijk bedankt!

I thank Jos van der Maesen from NHN Wageningen who provided a roof for me the first couple of weeks I was in the Netherlands. In those first weeks, I learned a bit about the Dutch way of life, such as making your sandwiches in the morning and cycling to work. He also taught me how to cook basmati rice, something I use almost everyday! Frans Breteler is also thanked for his help with taxonomic problems I encountered as well as interpretation of botanical descriptions. His input was really appreciated!

I also thank Gaby, my work neighbor for the past two years and a half. I really enjoyed those long talks we had about our kids, and what to do when they don't want to sleep or eat or go to the potty!

Ik wil ook Folkert Aleva, Ties Smaling, Jan Jansens, Rene Siep en Koen van Setten

hartelijk bedanken voor al de hulp dat ze me hebben gegeven. Ik denk dat zonder hun het herbarium kun niet draaien en dus mijn onderzoek ook niet! Bedankt en hup Holland!

Ria Vrielink is bedankt voor de hulp in het laboratorium met de bestelling van primers en alle dat ik nodig had voor mijn werk. Ria Fluit en Wilma Twigt hebben heel veel gedaan voor mij ook, met de organisatie van de zakken van geld en reis. Hartelijk bedankt!

Pete Lowry and George Schatz from the Missouri Botanical Garden are thanked for helping me during my visit to Paris when I was revising the Malagasy *Isolona'*s. Pete is also thanked for his important input when I wrote a grant proposal for the National Geographic Society to undertake field work in Madagascar.

I also need to thank all the different funding bodies that made this project possible: the Netherlands Organization for Scientific Research (N.W.O.), National Geographic Society, Alberta Mennega Stichting (3 times thank you!), Hugo de Vries Fonds, Air France-KLM, SYNTHESYS and "LEB fonds" at Wageningen University.

Je remercie aussi mon ami Camille Riegel qui m'a hébergé pendant ma visite de 5 semaines à Paris.

I wish to thank Jean-Louis Pham and Jean-Christophe Pintaud of the "Institut de Recherche pour le Développement" (IRD) in Montpellier, for their great support while I was searching for a PhD position in the latter part of 2003. Without that support I might have never landed in Wageningen. They encouraged me to stay scientifically active, by providing short term contracts to write up my articles and also financed a trip for an international conference at Cape Town in early 2004. Additionally, Vincent Savolainen of the Imperial College in London spontaneously helped me during that difficult time by suggesting different options for PhD projects and serving as a reference for my applications. He also encouraged me to subscribe to an online group Taxacom, an email information service for taxonomists. No sooner I did that, I saw the advert for the Wageningen PhD. Without his advice I might have never seen that posting!

As a primary and high school student my teachers used to say to me "you can do better!" Yes, I was kind of sloppy, that is, until I met my wife Carolina. Thanks to her wonderful and everlasting support, encouragements and advice, I was able to focus on my studies and indeed "do better". She also made a huge leap of faith when she accompanied me to Wageningen. Nothing would have been possible without her, entonces gracias gracias gracias mil veces. Creo que no hay como decírtelo suficientemente! The extra source of inspiration came from the birth of my son Luka in 2005. Wow! Even though you didn't realize it at the time, you provided as much motivation as Carolina. Those early (very early!) mornings we spent together before going to work gave me a lot of energy and willpower to work hard and do my best to succeed.

Last but certainly not least, I would like to thank my parents to whom I dedicate this work. Without their constant support throughout my life and the faith they have had in me (even though I did give them lots of reasons to doubt!), I would not be where I am now. This support was especially important during my first year of university. It was a difficult time and I really think that their support was the decisive factor for the years to come! Thanks for being such great parents, and I hope you will enjoy this piece of work!

Curriculum Vitae

Thomas Louis Peter Couvreur was born on the 21st of May 1979 in Canberra, Australia. As a boy he often moved from country to country. At age six he integrated the French school in Bern, Switzerland. At the beginning of 1994 he moved to Quito in Ecuador and discovered a passion for tropical rain forests and botany. In 1997 he completed his secondary school education at the French school "La Condamine" in Quito in general sciences. In September 1998 he started his university studies in General Sciences option Biology at the University of Montpellier II, France. He then integrated the three year B.Sc. program "Biology of Populations and Ecosystems". During the summer of 2001 he undertook an internship in an international Ecological NGO in Quito, Ecuador called Ecociencas. He undertook a survey of palm biodiversity in an Amazonian tropical rain forest in the national park Napo-Galeras. For his bachelor thesis he worked on a project entitled "Chloroplast phylogeny of *Bactris gasipaes* and related species" resulting in the publication Couvreur et al., 2007. In 2002 he was accepted in the Masters Degree course Biology of Evolution and Ecology at the University of Montpellier II. He spent two months undertaking a population level sampling of *Bactris gasipaes* and related species across western Ecuador. He gained his degree in Master of Science in September 2003 with a thesis entitled "Genetic structure of the crop-wild complex in the Peach palm (*Bactris gasipaes* Kunth) in Western Ecuador". He started his PhD project in April 2004 at the Nationaal Herbarium Nederland at the University of Wageningen in The Netherlands on the systematics of African Annonaceae. He followed doctoral courses at the Universities of Utrecht (Neotropical Plant families) and Wageningen (Molecular Phylogenies: Reconstruction and Interpretation). Early 2005 he spent five weeks at the Muséum Nationale d'Histoire Naturelle in Paris, France, with a SYNTHESYS grant learning techniques of floral anatomy. He also undertook two field trips: one to Gabon in November 2005 with Marc Sosef, and one to Tanzania in November 2006 by himself. In April 2008 he will start a seven month postdoctoral position at the University of Osnabrück, Germany on phylogenetics and evolution within the Brassicaceae family.

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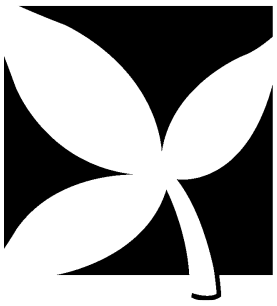
Couvreur, T.L.P., J.E Richardson, M.S.M. Sosef, & L.W. Chatrou. *In prep*. Substitution rate prior influences posterior mapping of discrete morphological characters: an unconventional remedy.

Training and education within the Graduate School Biodiversity

Name PhD student: Thomas L.P. Couvreur
Institute: National Herbarium of the Netherlands – Wageningen branch, Biosystematics Group, Wageningen University

	Credit hours
1. PhD Courses	
Neotropical Plant Families (May Jun 2004)	128
Molecular phylogenies: reconstruction and interpretation (Nov 2004)	32
Supervision and organisation MSc projects (2006)	32
2. Annual PhD meetings	40
3. Essay and seminar on the background and framework of the project	100
4. Literature study resulting in written report	60
5. Presentation of results at international conferences	
International Botanical Congress, Vienna (Jul 2005)	40
AETFAT, Yaoundé (Feb 2007)	40
Biannual meeting of the Systematics Association, Edinburgh (Aug 2007)	40
Evolution and Biogeography of Mediterranean ecosystems, Zurich (Jul 2007)	16
6. Reading course / Seminar series	
Activities within the NHN Task Force Molecular Systematics Phylogenetics and Biogeography	86
7. Facultative elements	
Activities within the Scientific Discussion Group of the Biosystematics Group, Wageningen University	100
Training in various laboratory techniques (palynology and floral anatomy)	48
Organizing PhD Day 2005	8
Total credit hours	770



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Printed by: Wöhrmann Print Service, CPI Group, Zutphen, The Netherlands.
Line illustrations: Joanne Porck
Cover design & layout: Carolina Morales and Thomas Couvreur