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# Lianas in Annonaceae

## A wood anatomical and evolutionary approach

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# Introduction

The diversity in plant growth forms is a topic in which evolutionary perspectives play an important role. Different strategies in the race to light often result in convergent evolution resulting in apparently homologous structures. It is clear that lianescence cannot be explained by a single evolutionary origin of the climbing strategy (Ichihashi and Tateno 2011). A climbing habit exists in more than 130 plant families (Gentry 1991), so it is immediately clear that a climbing habit cannot be a homologous character (Patterson 1988; Scotland 2010). If the climbing strategy is to come from one single origin, this means that it has to be subsequently lost in all no-climbing plants. This is not a very parsimonious scenario, and it has been demonstrated that multiple independent origins towards a climbing habit have occurred. This often results in more species rich clades compared to their sister clades with erect species (Gianoli 2004). In tropical forests, up to 44% of species diversity can be allocated to lianas (Schnitzer and Bongers 2002), with an increasing trend in the last decades (Phillips et al. 2002). Likewise, there is an increase in studies in climbing growth forms, evolution and ecology, possibly also owing to a lack of such studies in the past. Nevertheless, some old standard publications show the long-lasting interest in climbing habits in plants (Schenk 1892). A liana is generally considered as a woody climber that relies on neighbouring plants for support (Gartner 1991a; Schnitzer and Bongers 2002). They are more abundant in tree fall gaps, not so much due to increased light (Ichihashi and Tateno 2011) but more likely due to an increase in trellises (Putz 1984).

Several comparative studies on lianas have focused on wood anatomy. Notable previous results include comparative wood anatomy in Apocynaceae. Most climbing species are found in the four subfamilies Apocynoideae, Periplocoideae, Secamonoideae and Asclepiadoideae (APSA-clade) (Lens et al. 2009), with a few independent origins in the Rauvolfioideae (Lens et al. 2008). Climbing and non-climbing Apocynaceae were found to differ in presence of tracheids, type of axial parenchyma, vessel diameter and density, fibre and vessel element length and multiseriate ray height. In the Apocynaceae two different origins of a climbing habit are recognized based on differences in vessel grouping and type of imperforate tracheary cells present in the ground tissue between the climbing species of the Rauvolfioideae and the climbing species of the APSA-clade (Lens et al. 2009). Similar differences were found in balsaminoid Ericales, where climbing species were found to have vasicentric parenchyma, and wider vessels and higher multiseriate rays (Lens et al. 2005).

The description of cambial variants in lianas may be rare, but shows how different angiosperm families containing lianas have developed different stem architectures. Lianas in Bignoniaceae and Menispermaceae have several cambial variants that drive stem anatomical diversity (Jacques and De Franceschi 2007; Pace et al. 2009; Pace et al. 2011; Angyalossy et al. 2012). Whereas several species of Bignoniaceae and Menispermaceae were shown to have different cambial variants, a study on dendrochronology of Fabaceae lianas showed far fewer species with variant cambium (Brandes et al. 2011). In all cases cambium variants were associated with the ability to form xylem features in climbers that differ greatly from that in trees.

Liana xylem features differ from those in trees because the water transport system in lianas must be very efficient, because water has to flow through stems with a relatively small diameter, while the canopy is often comparable to that of a tall tree. To cope with this efficient water flow, lianas often have wide and long vessels (Ewers et al. 1997). These adaptations are known to cause vulnerability to vessel embolism due to drought stress (Hargrave et al. 1994), for which lianas are traditionally thought to have found a number of anatomical solutions like vasicentric parenchyma. For at least several species from seasonal forests, however, it was found that the risk of embolism was minimized by simply tapping into deeper soil water (Schnitzer 2005). This seems a critical observation, since most of the hypotheses on liana wood anatomy seem derived from limited taxonomic sampling (Angyalossy et al. 2012).

Studying Annonaceae wood anatomy follows the call for more model groups to assess broader taxonomic variation (Spicer and Groover 2010) very well. As an angiosperm plant family with roughly 2400 species of trees, shrubs and lianas they harbour a large variety in plant growth forms. With a single exception, all species have a distribution in eitherNeotropical, Afrotropical or Indomalayan regions with a few exceptions in temperate regions. With commercially interesting species such as ylang-ylang (*Cananga odorata*), custard apple (*Annona reticulata*) or pawpaw (*Asimina triloba*) they have been of long-lasting interest to various disciplines, ranging from biochemical analysis of floral compounds to phylogenetic dating anaylises. The Annonaceae harbour striking characteristics across the whole range of species. From large trees producing edible fruit to shrubs or even lianas, from old rainforest trees to pioneering species indisturbed areas. In the past this has led to various attempts to characterize the family by morphological characters and later with molecular data. Whereas only a handful of the Neotropical species have evolved into a climbing habit, at least 30% of Palaeotropical (Afrotropical and Indomalayan) species are reported to be climbers of some sort.

The recent family-wide molecular phylogeny by Chatrou et al. (2012) provides new insights into intrafamilial relationships and infers the Annonoideae and Malmeoideae subfamilies as the two major clades. These two subfamilies have previously been indicated as the long branch clade (Annonoideae) and short branch clade (Malmeoideae) referring to their differences in sequence divergence (Richardson et al. 2004).

Strikingly, a climbing habit has only evolved in the Annonoideae. Within the Annonoideae, however, five different clades with climbing habits can be distinguished: a species-rich climbing genus Artabotrys. (125 spp.), a small clade with Letestudoxa (three species) and Pseudartabotrys (monotypic), and the large 'climbing clade', the Uvarieae containing 14 genera with approximately 400 species. The Uvarieae also contain several non-climbing species, essentially confined to a single subclade representing the genus Dasymaschalon (Wang et al. 2012). Furthermore there are two isolated cases of lianescence in the tribes Annoneae (Annona scandens) and Guatterieae (Guatteria scandens). These five evolutionary shifts towards the climbing habit in one family make the Annonaceae an ideal model family in studying the evolution of lianas.

As a scandent growth form requires some very specific properties in wood structure and ontogeny (Gartner 1991b), it seems intuitive to look for these properties in Annonaceae. Previously, wood anatomy has been an integral part in comparative studies in Annonaceae (Chatrou and He 1999). Previous work on wood anatomy in Annonaceae has revealed several characteristic characters present throughout the family, like vessels that are arranged either solitary and/or in short radial multiples, and the typical cobweb-like appearance in cross sections due to the tall multiseriate rays combined with numerous narrow parenchyma bands (Koek-Noorman and Westra 2012). Although useful to recognize Annonaceae wood, such features are of no use for intrafamilial comparison. We want to assess whether there are uniquely differing wood characters between scandent and related self-supporting species that could shed interesting light on questions regarding character evolution in lianas.

An interesting feature of *Artabotrys* spp. is their unique climbing mechanism: lignified inflorescences (Posluszny and Fisher 2000). This differs greatly in ontogeny and morphology from all other Annonaceae climbers, which mostly have tendrils,  and already provides some information on the homology of a climbing habit in Annonaceae.

The aim of this study is therefore to identify the wood anatomical characters that describe a climbing habit best and identify correlations between these characters. Correlated characters could be indicative of syndrome-like evolution of wood anatomical characters in lianas. Previous attempts at understanding the evolution of lianas are often accompanied by the notion of phylogenetic relationships between the species studied (e.g. Angyalossy et al. 2012). However, as described by Felsenstein (1985) these phylogenetic relationships should be used to correct for non-independence of the data.

Different methods of accounting for phylogenetic relationships are currently available. Felsenstein's (1985) Phylogenetic Independent Contrasts (PICs) are traditionally underappreciated in wood anatomical surveys. However, in recent years there has been an increase in the use of this method (Preston et al. 2006; Bhaskar et al. 2007; Wright et al. 2007; Willson et al. 2008; Hacke et al. 2009; Martínez-Cabrera et al. 2011; Fu et al. 2012).    Ancestral State Reconstruction is a statistical technique incorporating phylogenetic information that can provide arguments for evolutionary processes as well. Other than the PICs, Ancestral State Reconstruction (ASR) does not focus on trends in current patterns, but attempts to reconstruct character states at nodes in the phylogeny. This allows for the estimation of trends in character change over time.

In addition to identifying wood anatomical characters that describe a climbing habit in plants, this study will infer evolutionary patterns in these characters. The data will be tested for a variety of evolutionary hypotheses in the form of phylogenetic tree transformations (Pagel 1999). This allows for the testing of four alternatives to the standard assumption of Brownian motion in character evolution:

1. Pagel's lambda (Pagel 1999). Due to shared ancestry, character values of closely related species are more similar than those of distantly related species. Lambda tests whether the similarity is larger or smaller than can be expected from the phylogeny.
2. Pagel's kappa (Pagel 1999).  Compares a scenario of gradual character evolution to a scenario of stepwise evolution
3. Pagel's delta (Pagel 1999). Tests whether rates of character evolution have accelerated or decelerated through time.
4. Ornstein-Uehlenbeck (Butler and King 2004). Adaptation in a character deviates from Brownian motion, and a directional pattern can be observed in the character evolution.

Subsequently, if any alternative model is found to be more likely than the Brownian motion model, the branch lengths of the phylogenetic tree can be transformed in such a way that they represent the alternative model. This transformed tree can then be used in the calculation of PICs, so that the PIC method can also account for alternative models of evolution.

# Materials and methods

**Wood anatomical observations**

Mature Annonaceae wood samples from one species was collected in Madagascar, one species was sampled in Thailand and another one from Hong Kong. These samples were added to 11 species using the xylaria in the Naturalis Biodiversity Center (Lw, Uw and WAGw) and in the Royal Museum for Central Africa in Tervuren (Tw; Stern 1988, (Lynch and Gasson 2010). To broaden our sampling with more climbing species and related erect species, we made use of 25 light microscopic slides that were available in the slide collection of the Naturalis Biodiversity Center. In total, we collected 36 Annonoideae species, of which 15 are climbers, based on the species represented in the latest molecular phylogeny of the family (Chatrou et al. 2012).

Observations were carried out following the recommendations made in the IAWA list of microscopic features for hardwood identification (IAWA Committee (1989). For vessel diameter, vessel element length and fibre length, 25 measurements were made, and the mean for height of uniseriate and multiseriate rays were based on 15 measurements. Vessel density and ray width were measured 10 times. Uniseriate and multiseriate ray density was calculated five times. The different numbers of measurements correlate with the amount of variability in our sampling and the number of measurements possible in the given size of a specimen.

Wood sectioning was performed following the standardized protocol described in Lens et al. (2005). For the measurement of individual vessel elements and fibres, maceration slides were made according to an adjusted procedure of Franklin (1945): macerating wood splinters overnight in a 1:1 mix of 30% $H_2O_2$ : 90% acetic acid, followed by rinsing three times with demi water and staining with astra blue. Fibre and vessel element length was always measured from splinters close to the vascular cambium of a specimen, and could not be obtained for species only present as light microscope slides.

## Molecular data

Molecular chloroplast sequences for 131 Annonaceae species were used, with *Persea americana* (Lauraceae) as outgroup. One coding (*ndhF*) and three non-coding (*trnL-trnF, psbA-trnH* and *trnS-trnG*) chloroplast markers were used. For all four markers insertions/deletions were binary coded by hand. See appendix for a full description of availability per marker for all 132 species. Since a climbing habit only occurs in the subfamily Annonoideae, all other subfamilies were reduced to a few species representing the clades. Most sequences are identical to (Chatrou et al. 2012), with original sequences for XX species and five species from GenBank (Benson et al. 2006), mainly from the Uvariae. See appendix for a detailed description.

## MrBayes

A Bayesian phylogenetic analysis was performed using the parallel version of MrBayes 3.2.1 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003; Altekar et al. 2004). The data was divided into three partitions: coding markers (ndhF), non-coding markers (trnL-trnF, psbA-trnH and trnS-trnG) and insertions/deletions. Insertions and deletions were coded as absent/present and the sequence data was excluded from further analysis. The data were analysed applying mixed substitution models to allow for the integration over all available models. For the insertions/deletions a Kimura 2-parameter model was used to allow asymmetrical changes in a binary character. An MCMC was run for $1.5 \times 10^7$ generations with two runs of four chains each, sampling every 1000 generations with the first 25% of trees removed as burn-in. This procedure was repeated resulting in 22,500 trees, which were summarized in a 50% majority rule consensus tree (fig X).

## BEAST

To create a dated phylogeny BEAST 1.7 was used (Drummond et al. 2012). The same matrix with 132 species as for the MrBayes analysis was used. Indels were not coded, and data was not partitioned. The uncorrelated lognormal relaxed clock model (Drummond et al. 2006) was used with a prior lognormal distribution of mean=1.0 and standard deviation=0.33. Three nodes were calibrated to make the resulting chronogram reflect actual evolutionary timescales:

1. The crown node of the tree (Magnoliineae) was fixed with the age of *Endressinia* by means of a uniform prior with minimal bounds: 115.1 – 114.9 mya (Doyle and Endress 2009).
2. The crown node of the Annonaceae with fossil data of *Futubanthus*: exponential prior distribution with mean=5 and offset=89, giving a distribution between 115mya and 89mya which is biased towards the younger age (Couvreur et al. 2011).
3. The crown node of *Duguetia* with was given a lognormal distribution with mean=10 log(stdev)=1 and offset=38 giving a distribution with a minimum age of 38mya, a median of 44mya and the ability to reach approximately 100mya but a strong bias towards a younger age (Pirie and Doyle 2012).

As pointed out by Hardy (2006) incomplete taxon sampling can cause several problems. The most severe problem seems to be wrong estimation of divergence times in a phylogenetic dating analysis. To minimize this effect a dating analysis on a larger species matrix comprised of mainly already published species and divergence times was constructed. The resulting phylogeny is then pruned from species for which no character data is available afterwards.

## Ancestral state reconstruction

ASR relies heavily on the assumption that the phylogeny depicts true relationships between species. Since phylogenies often incorporate nodes with low confidence, a means of estimating phylogenetic uncertainty is necessary. The simplest way of doing this is by sampling a post-burnin subset of trees from a Bayesian phylogenetic analysis and reconstructing ancestral states over all trees. Due to the nature of Bayesian phylogeny reconstruction, clades with a high probability, given the prior probabilities and the data used, are represented in more trees than clades with a low degree of certainty. If ancestral states are subsequently summarized over all trees, this provides an excellent approximation of phylogenetic uncertainty in ancestral state reconstruction.

In all cases of ancestral state reconstruction or morphological character evolution analysis, a model of character evolution must be defined. Irrespective of the model definition, one prerequisite must be fulfilled: the branch lengths of a tree used to analyse character evolution must reflect evolutionary time. In other words: the tree used must be a chronogram. This is because of the use of branch lengths in estimating evolutionary patterns. Character change is treated as the amount of change in a character per branch length. If a chronogram is used, this directly translates to amount of change over time, correctly following models of character evolution. If for instance a phylogeny with amount of genetic variation in branch lengths is used, character change is treated as amount of morphological change over amount of genetic change. This incorrectly assumes a direct link between the morphological character studied and the genetic markers used to reconstruct the phylogeny.

Two methods were used to reconstruct ancestral states for the wood anatomical characters. For the summary of these ancestral states to remain informative a selection of nodes to summarize was made. The node representing the most recent common ancestor (MRCA) of the subfamily Annonoideae was selected as representative for all species in the subfamily. The nodes representing the MRCA of the tribes Annoneae, Duguetieae, Guatterieae, Uvarieae and Xylopieae were used for describing ancestral states. These tribes were selected for three reasons: 1) the nodes representing the MRCAs of the tribes are highly supported by Bayesian phylogenetic analysis, 2) these tribes are all represented by more than one species (i.e. since the Monodoreae are represented by only one species they were left out) and 3) these tribes contain different numbers of lianas, allowing for a good comparison.

### Bayesian analysis

To account for phylogenetic uncertainty the stochastic character mapping method implemented in SIMMAP1.5 was used (Bollback 2006). From the BEAST output 5000 trees per repetition were discarded as burn-in. From the remaining 20.000 trees every 40th tree was sampled resulting in 500 independent trees. Because SIMMAP only works for discrete data all characters were discretized in two ways: 1) using the gap weighting method by Thiele (1993) implemented in the software package MorphoCode (Schols et al unpubl. data) and 2) by assigning each sample to one of seven bins of equal size (bin size calculated as max-min/number of bins). Some characters were discretized in such a fashion that not all discrete states were represented (in other words, character states with zero presence). If one or more of the character state bins has zero occurrences, SIMMAP cannot interpret this form of data so these characters were excluded from the analysis. This left the characters lianescence, vessel diameter, height of the multiseriate rays, and density of the multiseriate rays to be analysed. An initial MCMC was run to estimate appropriate priors per character (table X). These parameter values were used as prior and an MCMC with ten prior draws and ten samples per tree was run for 50.000 generations.

### Maximum likelihood analysis

Parallel to the Bayesian analyses performed in SIMMAP a maximum likelihood ASR was performed in the R package APE (Paradis et al. 2004). To account for phylogenetic uncertainty 95% confidence intervals for ancestral values were calculated from the values calculated over 500 trees from the BEAST analysis. The same five nodes representing the tribes Annoneae, Duguetiae, Guatteriae, Uvariae and Xylopiae were used, along with the node representing the subfamily Annonoideae. Since this type of maximum likelihood ASR calculates continuous ancestral states the problem of empty classes that prevented SIMMAP from analysing several characters does not apply. Therefore, ancestral states could be calculated for all eight characters. However, since the analysis of continuous data does not allow for empty fields, several species had to be omitted from the analysis. The remaining consensus tree is in figure .

### Phylogenetic tree transformations

Most comparative methods of continuous data using phylogenies rely heavily on the assumption that the character evolved under Brownian motion. One way of testing this assumption is to impose different models of character evolution by transforming the branch lengths of the phylogeny. This can be done in the R package geiger using the function *fitContinuous*. This function allows for the fitting of five different models:

1. Pagel's lambda (Pagel 1999). Multiplies all internal branches by lambda. Tip branches are left at their original length. Lambda < 1.0 is generally interpreted as less similarity among species than could be expected from their phylogeny, and lambda > 1.0 can be seen as the reverse.
2. Pagel's kappa (Pagel 1999). Raises all branch lengths to the power kappa. As kappa approaches zero, the model becomes speciational.
3. Pagel's delta (Pagel 1999). Raises all node depths to the power delta. Delta < 1.0 indicates evolution is concentrated early in the tree, delta > 1.0 indicates evolution is concentrated more towards the tips.
4. Ornstein-Uehlenbeck (Butler and King 2004). Brownian motion with a central tendency proportional to the parameter alpha. This allows for directional patterns of evolution.

By transforming the branches according to the specifics of each model and then assessing the fit of the altered tree to the data using a likelihood optimization algorithm, a comparison between models can be made using Akaike's Information Criterion corrected for sample size (AICc). From the best fitting model the most likely value of the transformation parameter can be used to transform the branch lengths of the tree. The resulting tree is now an appropriate depiction of the character evolution if it were to follow Brownian motion and can subsequently be used in analyses assuming Brownian motion.

To assess the behaviour of the model found most likely a more in depth approach was followed. For each wood character the optimal model parameter value was estimated on each of the 500 trees used in the SIMMAP analysis.

## Phylogenetic independent contrasts

To calculate the Phylogenetic Independent Contrasts (PIC)(Felsenstein 1985) for each character the R package APE was used (Paradis et al. 2004). The PIC method transforms the tip data to correct for phylogenetic signal under the assumption of Brownian motion using a phylogeny with morphological character data for all species. In order to test the assumption of Brownian Motion, PICs were also calculated using a phylogenetic tree transformed to fit the most likely model of character evolution. This resulted in three datasets: the raw data, the PICs and the PICs corrected to fit the most likely evolutionary model. For each of these three datasets correlations between all characters were calculated, corrected for multiple comparisons with Bonferroni's correction.
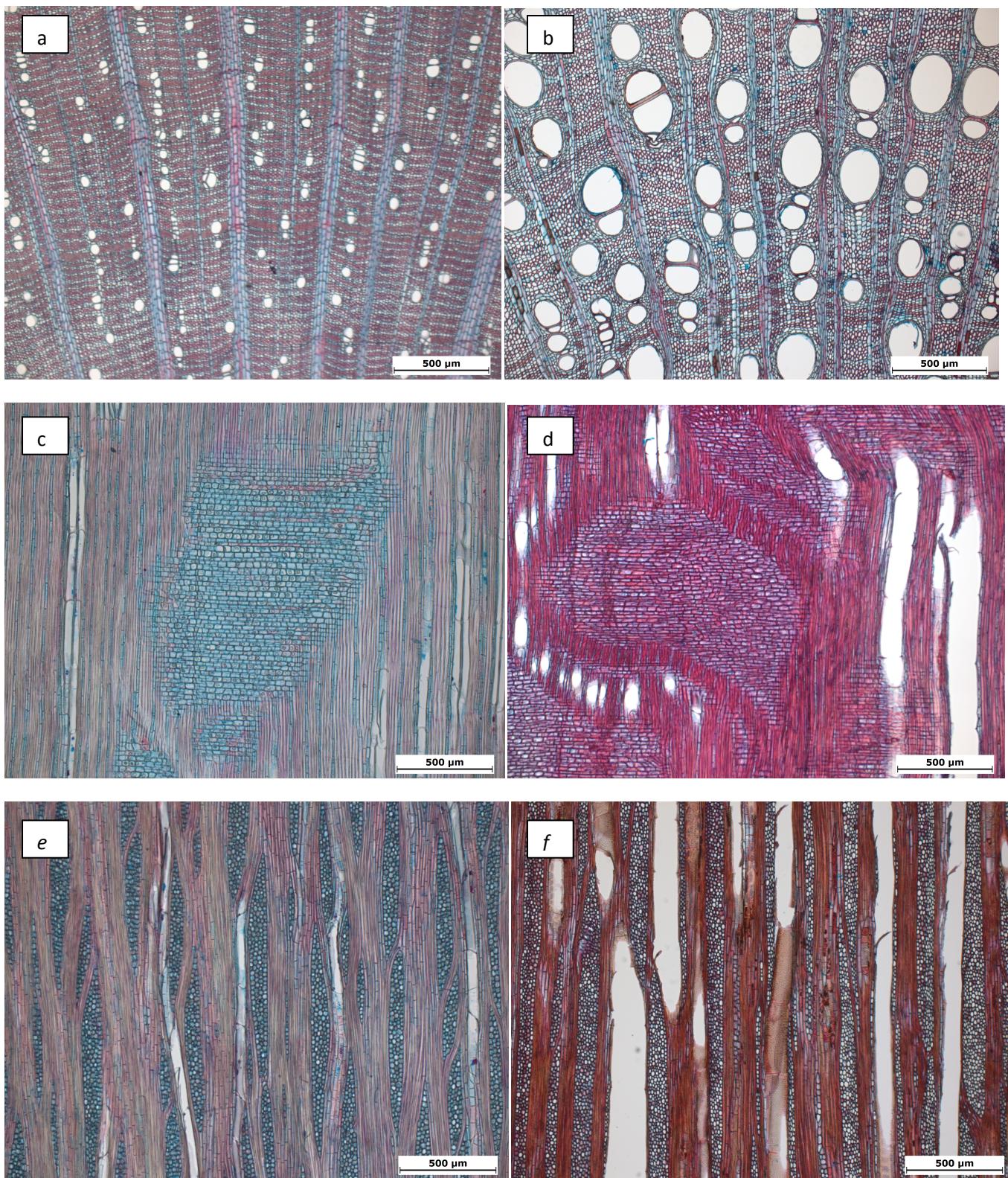
**Figure 1:** Light microscopic pictures, *a, c & e* are trees, *d, e & f* are climbers. a) *Dasymaschalon dasymaschalum* cross section. b) *Fissistigma bicolor* cross section. c) *Goniothalamus griffithii* axial. d) *Friesodielsia gracilis* axial. e) *Goniothalamus griffithii* tangential. f) *Exellia scamnopetala* tangential.

# Results

## Wood anatomical observations

The wood anatomical characters are briefly described for species sampled from the subfamily Annonoideae (fig. 1), for details per species data see appendix. For each genus examined, the numerator presented in the text represents the number of species studied and the denominator includes the total number of species. For wood anatomical descriptions, numbers without parentheses are ranges of means, while numbers between parentheses represent minimum or maximum values. A summary of the species of Annonaceae that were studied and their descriptions is as follows.

*Annona* 4/162, *Artabotrys* 1/102, *Asimina* 1/7, *Cymbopetalum* 1/27, *Dasymaschalon* 1/21, *Duguetia* 2/93, *Exellia* 1/1, *Fissistigma* 2/48, *Friesodielsia* 3/51, *Fusaea* 2/2, *Goniothalamus* 1/134, *Guatteria* 4/210, *Hexalobus* 1/5, *Isolona* 1/20, *Melodorum* 1/10, *Monodora* 1/16, *Pseudartabotrys* 1/1, *Sphaerocoryne* 1/3, *Uvaria* 4/187, *Uvariastrum* 1/8, *Xylopia* 2/187.

Growth ring boundaries usually indistinct. Wood diffuse porous, except semi-ring porous in *Asimina triloba*. Tangential diameter of vessels (10)-30-250-(1150) µm wide and density (1)-3-188-(260) mm$^{-2}$, mostly solitary and in radial multiples of 2-3-(8), except in *Asimina triloba* which mainly forms clusters of 3-10-(15) vessels. Perforation plates exclusively simple. Intervessel pits bordered, alternate, 2.5-12.6µm in horizontal size, except for all *Duguetia* and *Fusaea* studied, where horizontal diameter is around 2µm. Vessel-ray pits similar to intervessel pits in size and shape. Fibres non-septate and thin-to-thick-walled. Axial parenchyma usually both apotracheal and paratracheal, but apotracheal type prevails. Apotracheal parenchyma scalariform, 4 cells per strand, except 2-celled strands in *Annona glabra* and *Annona montana*, and 2-4 celled strands in *Annona muricata*; apotracheal parenchyma absent in *Asimina triloba*. Paratracheal axial parenchyma absent to scanty, except for *Guatteria scandens* (1-2 cell layers of vasicentric parenchyma). 8 cells per strand, but 4-celled strands in *Annona glabra* and *Annona montana*; up to 13 cells or more per strand observed in *Duguetia staudtii*. Uniseriate rays present in all species except *Annona montana*, *Fissistigma bicolor* and *Goniothalamus griffithii*; uniseriate rays extremely rare in *Duguetia staudtii*, *Fissistigma minuticalyx*, *Uvaria flava*, *Uvaria marenteria* (≤ 3 uniseriate rays observed); rays (27)-70-695-(2200) µm in length and 0-3 rays mm$^{-1}$. Multiseriate rays generally 2-9 seriate, and 2-24 seriate in *Friesodielsia gracilis* and *Friesodielsia velutina*, multiseriate rays (100)-305-3665-(8000) µm in length, 2-10 rays mm$^{-1}$; rays consisting of mainly procumbent body ray cells sometimes mixed with square to upright cells and 1-2 rows of square to upright marginal ray cells.

## Bayesian phylogenetic analysis

The Bayesian phylogenetic analysis largely agrees with Chatrou et al. (2012) (fig.1). All major Annonoideae tribal clades were retrieved. Tribal clades are strongly supported as monophyletic, all with posterior probabilities over 95%. Relationships within tribes are all very similar to those found in Chatrou et al. (2012) with some large exceptions in the Uvariae.

Most species added to the existing alignment are placed in the Uvariae. Placement and structure of the genera Fissistigma and Uvaria is largely congruent with Chatrou et al. (2012). The relation between existing and new sequential data of *Sphaerocoryne gracilis* and *Melodorum fruticosum* however is not. These species together are a clade inside a polytomy consisting of the already published data. A similar pattern arises from the genera Friesodielsia, Monanthotaxis, Dasymaschalon and Desmos, where the newly added species are more similar to each other than to species of the same genus that were published earlier. In general, the newly added species cluster together with moderate posterior probabilities, separating them from the existing data. Apart from that, it was found that the sampled Friesodielsia spp. and Monanthotaxis spp. are indistinguishable as separate genera at the molecular level. The placement of "Uvaria_cordata_SM176" inside a clade of Desmos spp. seems more likely assignable to error or false determination.
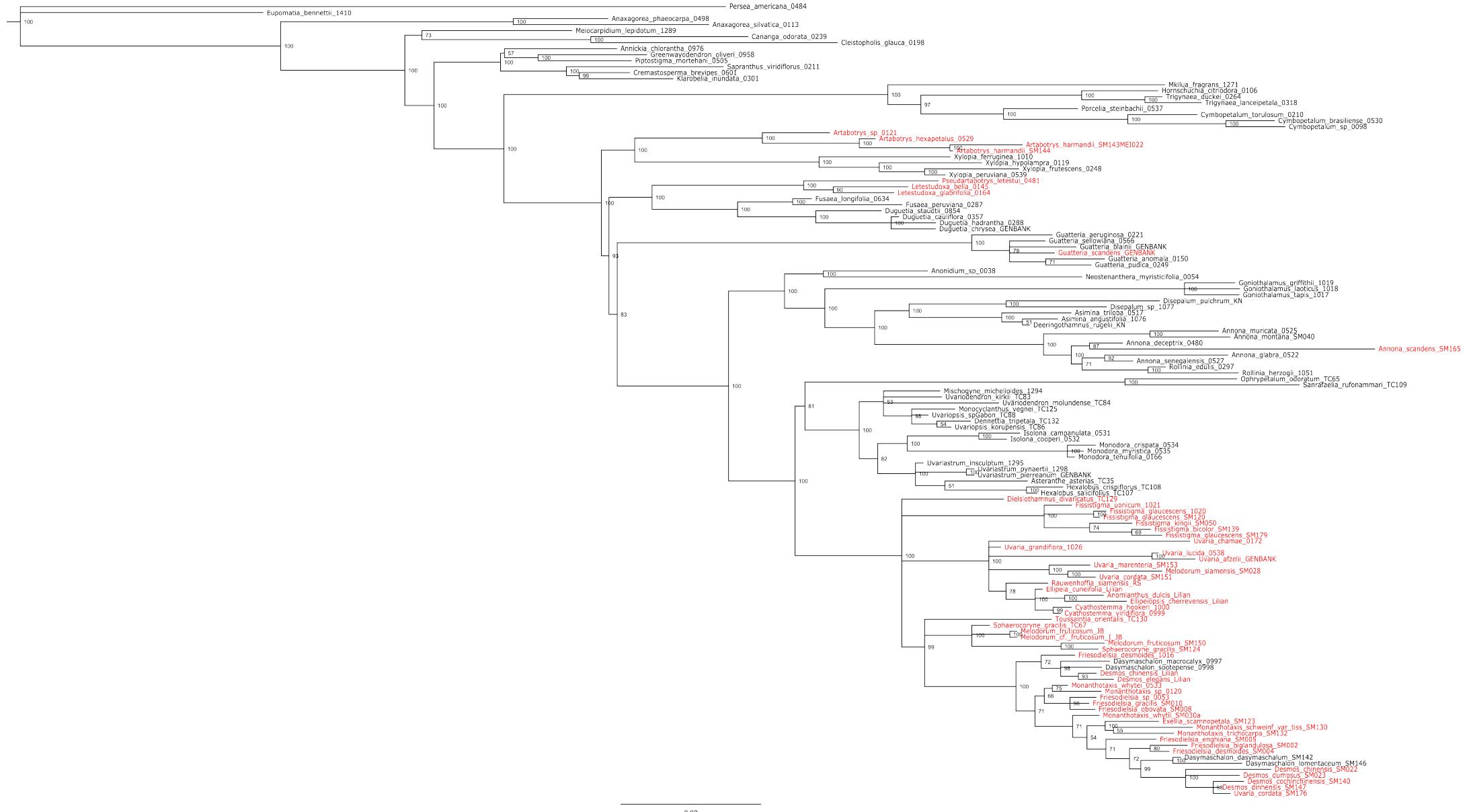
**Figure 1:** Phylogenetic tree representing relationships in the Annonaceae. Branch length represents amount of change. Node numbers represent posterior probabilities from MrBayes. 95% or higher indicates strong support. Climbing species are marked in red.
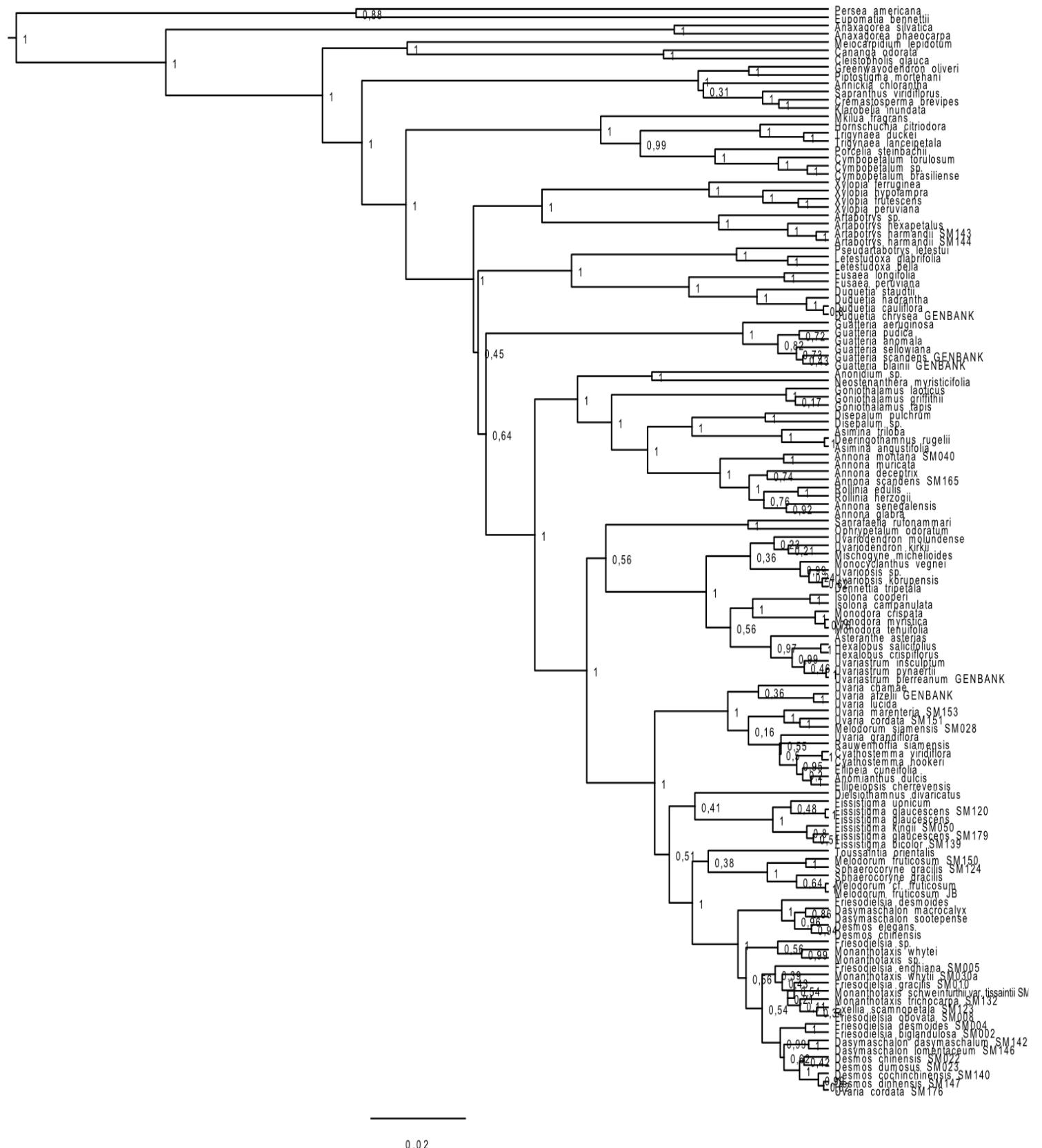
**Figure 2:** Dated phylogenetic tree representing relationships in the Annonaceae. Branch length represents time. Node numbers represent posterior probabilities from BEAST. 95% or higher indicates strong support.

# Ancestral state reconstruction

## Bayesian analysis

SIMMAP calculates the probability of discrete characters at nodes in a tree. Pie charts representing probabilities of character states are placed on the tribal nodes of the Annoneae, Dugueteiae, Guatterieae, Uvarieae and Xylopieae and on the node representing the subfamily Annonoideae (fig. 3).

A lianescent habit appears to be the most likely ancestral states of the clades representing the tribes Annoneae and Uvarieae. On the other hand the ancestral state for the Guatteriae is most likely a tree. For both the Duguetiae and the Xylopiae the ancestral state is effectively unknown since SIMMAP yielded a 50-50 chance of either state. The ancestral state of the subfamily Annonoideae was found to most likely be lianescent.
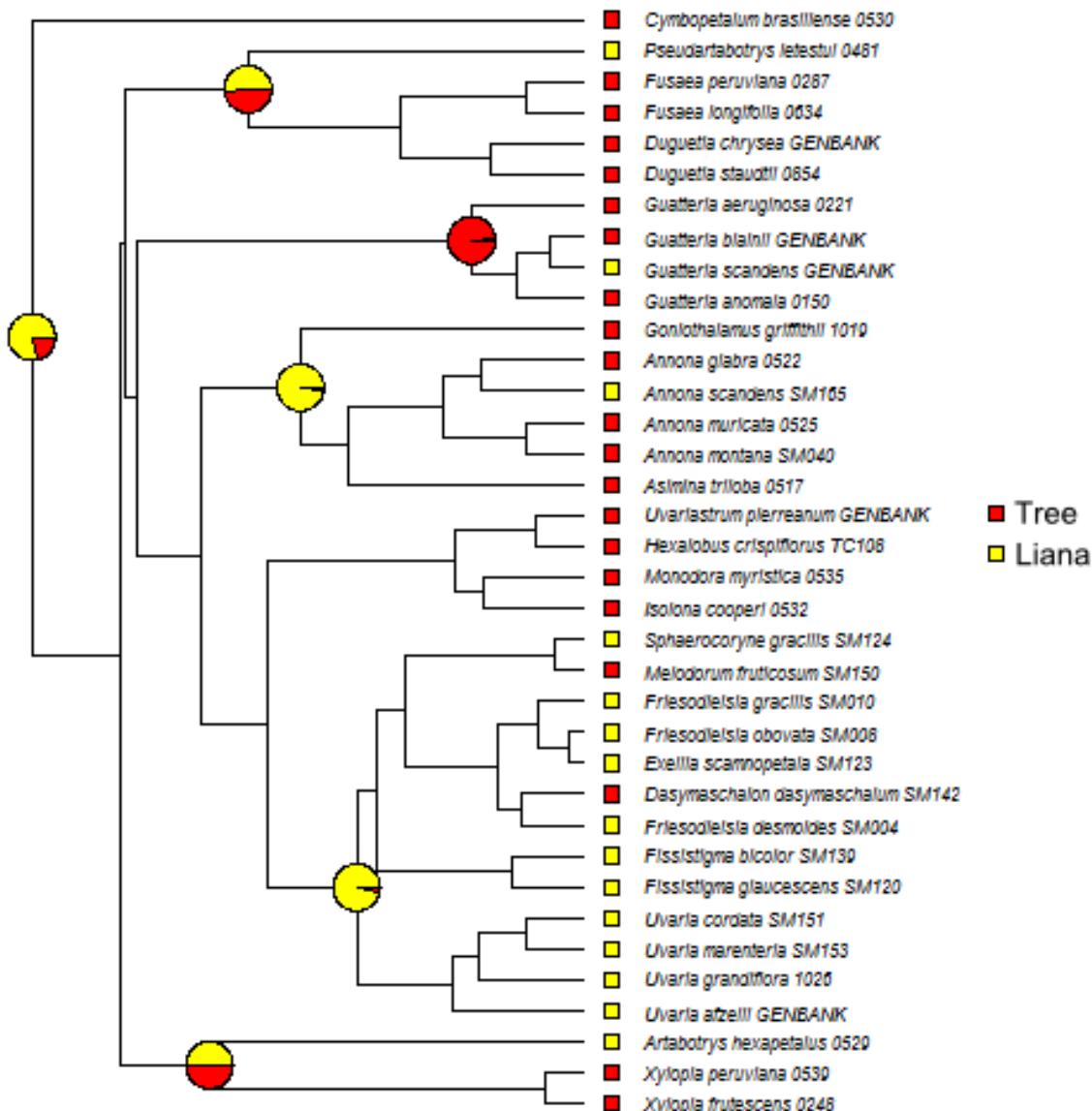


**Figure 3:** SIMMAP ancestral state reconstruction for the binary character **lianescence**. Colours at the tip labels indicate the state of the species (see legend for colour chart). Pie charts display the probabilities of the character states on that node.
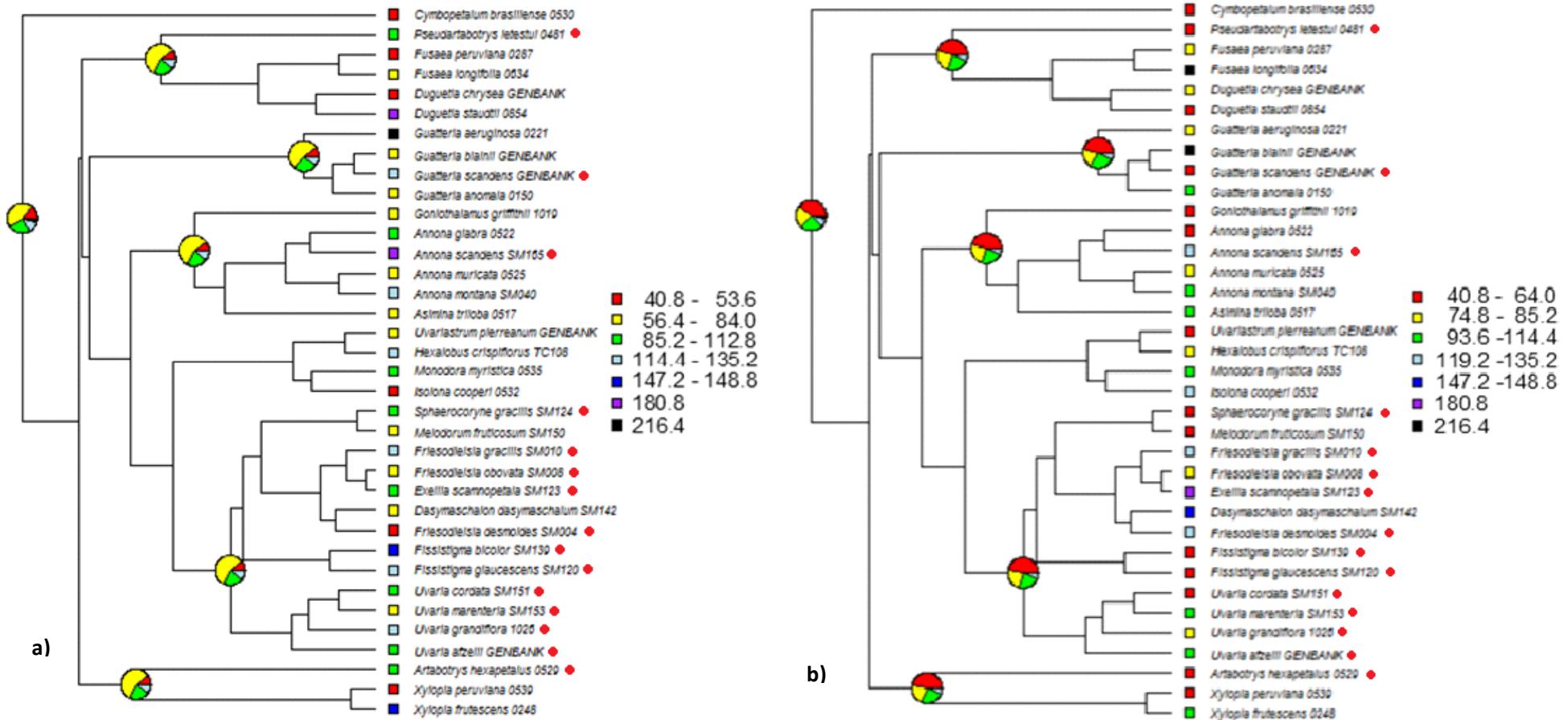
**Figure 4:** SIMMAP ancestral state reconstruction for the character **vessel diameter**. Colours at the tip labels indicate the state of the species (see legend for colour chart). Pie charts display the probabilities of the character states on that node. a) Discretized using Morphocode. b) discretized with equal size classes. Species marked with a red dot are climbers.

The SIMMAP ancestral state reconstruction for vessel diameter yielded similar patterns for all nodes in both Morphocode (fig. 4a) and equally discretized (fig. 4b) data. For the Morphocode discretized data all states are most likely between 56.4µm and 112.8µ. For the equally discretized data all states are most likely between 40.8µ and 85.2µm. The equally discretized data appear to yield smaller values, but there is large overlap between Morphocode and equally discretized data.
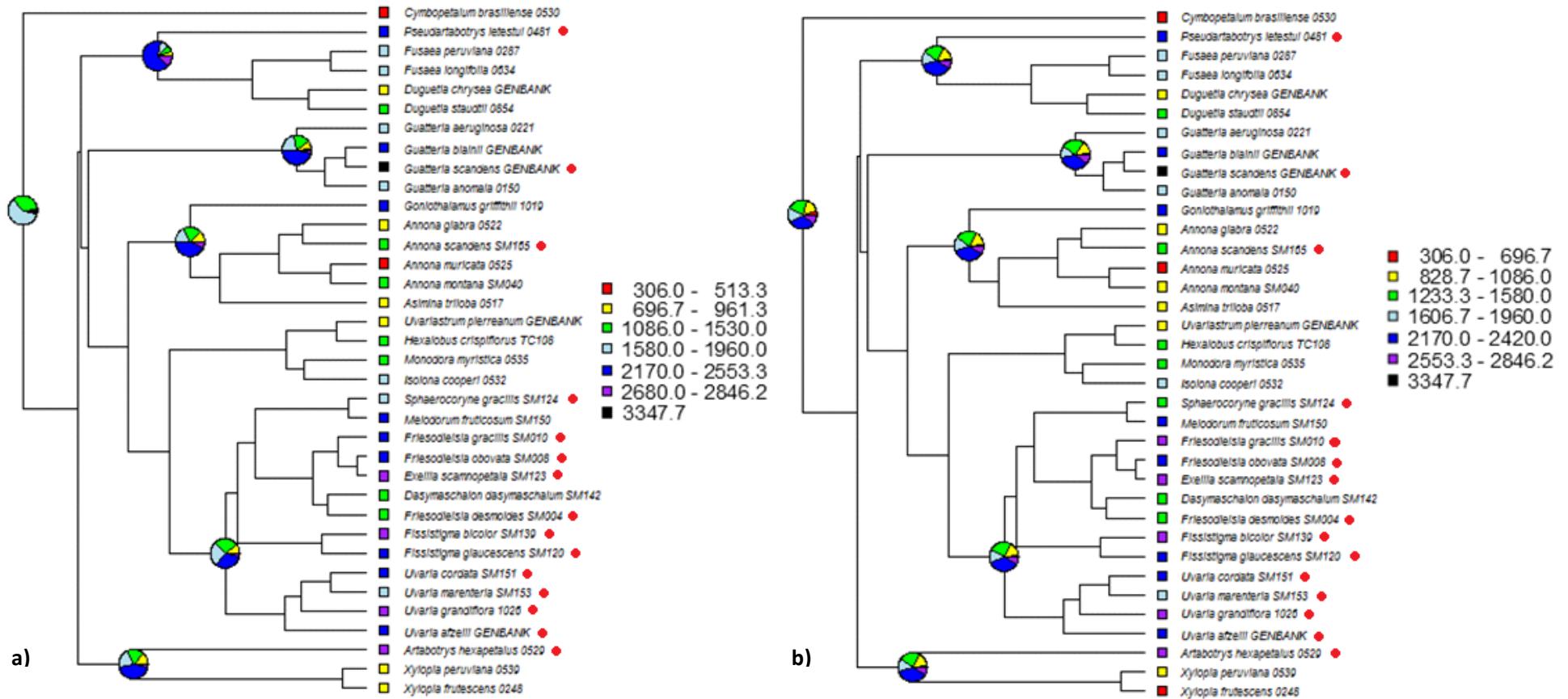
**Figure 5:** SIMMAP ancestral state reconstruction for the character **height of the multiseriate rays**. Colours at the tip labels indicate the state of the species (see legend for colour chart). Pie charts display the probabilities of the character states on that node. a) Discretized using Morphocode. b) discretized with equal size classes. Species marked with a red dot are climbers.

The SIMMAP ancestral state reconstruction for the height of the multiseriate rays for the equally distributed classes (fig. 5b) resulted in almost equal probabilities for all character states except the lowest and two highest classes. This means that all ancestral states most likely lie between 828.7µm and 2420.0µm. For the Morphocode discretized classes (fig. 5a) a similar pattern is found for the Guatterieae, Annoneae, Uvarieae and Xylopieae, which all have values between 1086.0µm and 2553.3µm. Only the Duguetiae and Annonoideae deviate from the values found in the equally distributed classes with 2170.0µm – 2553.3µm for the Annoneae and 1086.0µm – 1960.0µm for the Annonoideae. Again, the equally discretized data seem to yield smaller values, but there is large overlap between morphocode and equally discretized data.
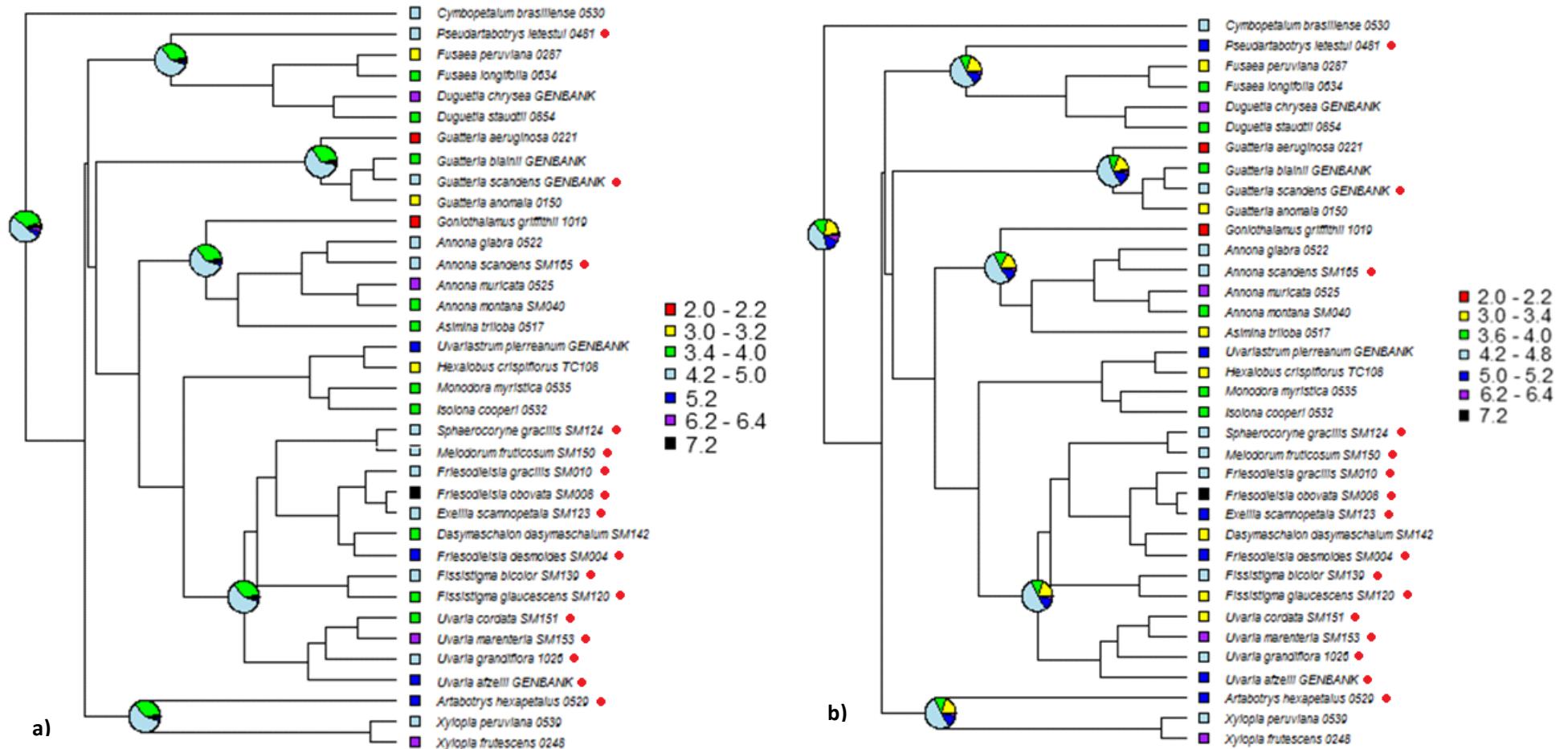
**Figure 6:** SIMMAP ancestral state reconstruction for the character **density of the multiseriate rays** for the six important nodes. Colours at the tip labels indicate the state of the species (see legend for colour chart). Pie charts display the probabilities of the character states on that node.

The SIMMAP ancestral state reconstruction for the density of the multiseriate rays resulted in a range of $3.0mm^{-1} – 5.2mm^{-1}$ for all clades in the equally distributed dataset (fig. 6b). The in Morphocode discretized dataset (fig. 6a) resulted in a range of $3.4mm^{-1} – 5.0mm^{-1}$ for all clades. Thus, the data discretized in Morphocode yield a slightly more narrow estimate than the equally distributed data.

## Maximum likelihood analysis

For vessel density (fig. 7a) the ancestral states for the Duguetieae, Uvarieae and Xylopieae are about 25 vessels per mm, very close to the value for the Annonoideae. The ancestral value for the Guatterieae deviates with an ancestral value of 10 vessels per mm and the ancestral value for the Annoneae deviates with an ancestral value of 33 vessels per mm. Ancestral states for vessel diameter (fig. 7b) show hardly any variation at all, with all values lying between 90µm and 100µm. The Guatteriae are outliers in vessel element length (fig. 7c) and fibre length (fig. 7d) as well. Vessel element length of the Annoneae is roughly equal to the basal value for the Annonoideae (350 µm). For the Duguetiae, Uvariae and Xylopiae all values are calculated to be roughly the same (400µm - 450µm). The Guatteriae deviate from the rest with a vessel element length of 530µm. For fibre length values for all nodes are calculated to be around 1000µm, with the exception of the Guatteriae that have a fibre length of 1200µm. The height of the multiseriate rays (fig. 7e) shows a pattern where the Annoneae are the only tribe with a lower value than the ancestral Annonoideae. The Duguetiae are reconstructed only slightly higher than the Annonoideae, the Guatterieae come out slightly higher than the Duguetieae, and the Uvarieae and Xylopieae are highest. For the height of the multiseriate rays (fig. 7f) the Annoneae are also reconstructed to be lower than the value for the Annonoideae. The Guatteriae have the highest ancestral value for the height of the multiseriate rays. Heights of the Duguetiae, Uvariae and Xylopiae lie in between the heights of the Annonoideae and Guatteriae. The patterns in ray width (fig. 7g) and density of the multiseriate rays (fig. 7h) are approximately mirrored. The Guatterieae have the only deviating ancestral values for both ray width and density of the multiseriate rays, with the widest rays and the lowest density of the multiseriate rays.
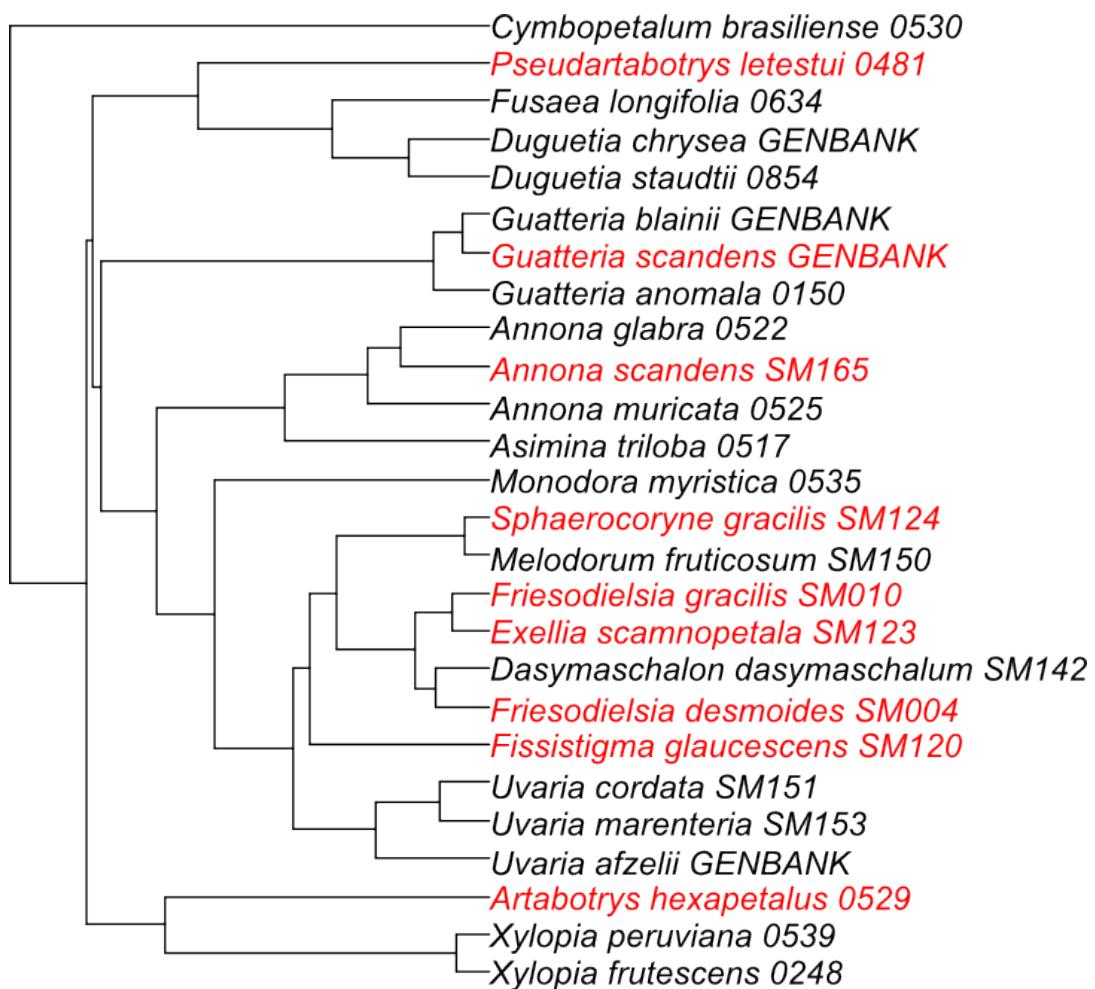


**Figure 7:** Pruned BEAST maximum clade credibility tree with the species used in maximum likelihood ancestral state reconstruction. Climbing species are depicted in red.
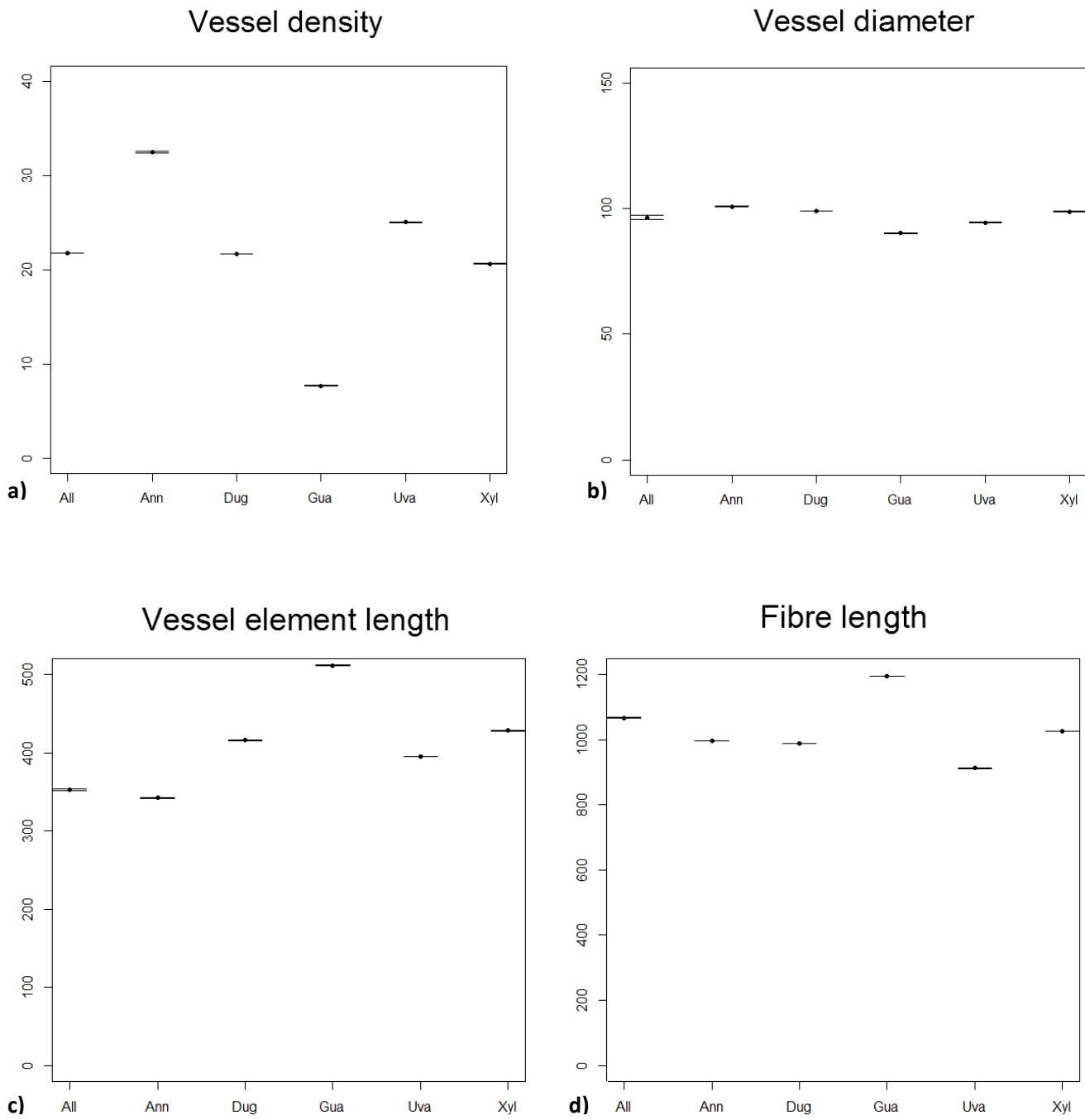
**Figure 8:** Maximum likelihood ancestral state reconstruction over 500 trees. Error bars indicate 95% confidence intervals for phylogenetic uncertainty. X-axis labels indicate the node studied with All = Annonoideae, Ann = Annoneae, Dug = Duguetieae, Gua = Guatterieae, Uva = Uvarieae, Xyl = Xylopieae. a) vessel density, b) vessel diameter, c) vessel element length, d) fibre length, e) height of the uniseriate rays, f) height of the multiseriate rays, g) multiseriate ray width and h) density of the multiseriate rays.

**Height uniseriate rays**

**Height multiseriate rays**

**e)**

**f)**
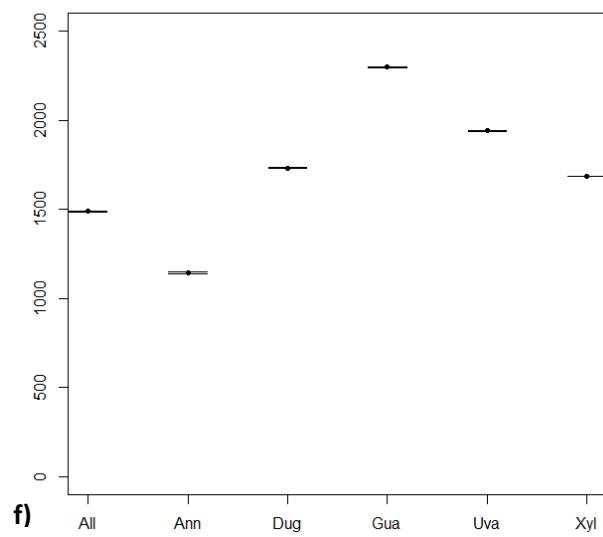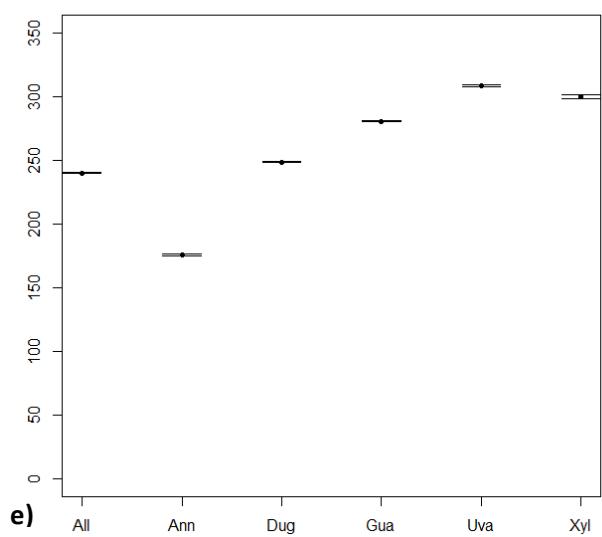
**Ray width**

**Density multiseriate rays**

**g)**

**h)**

**Figure 8.** (cont.)

18
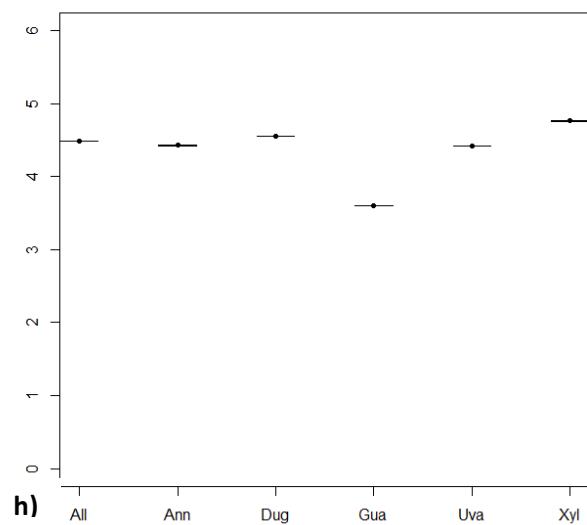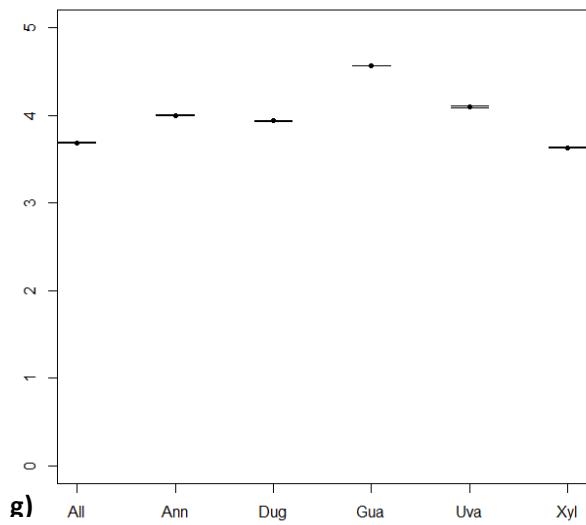
## Fitting models of character evolution

Comparing AICc scores for six different models provides insight in relative performance, where a lower value represents a better fitting model. Relative AICc (ΔAICc) scores were calculated by subtracting the lowest AICc value per character from the AICc values of that character. In this way the best performing model can immediately be identified (ΔAICc=0), as well as the relative performance of all other models. Relative AICc scores resulted in the selection of the Ornstein-Uhlenbeck model as most likely for all wood anatomical characters (table 1).

**Table 1:** ΔAICc values for six evolutionary models.

|  | **Brownian** | **Lambda** | **Delta** | **Kappa** | **OU** |
|---|---|---|---|---|---|
| Ves. diam. | 1.36E+05 | 2.09E+04 | 5.31E+04 | 1.22E+03 | 0 |
| Ves. dens. | 8.36E+03 | 3.51E+03 | 4.09E+03 | 1.09E+02 | 0 |
| Ves. el. Length | 4.00E+05 | 1.80E+05 | 2.21E+05 | 6.88E+03 | 0 |
| Fibre length | 2.21E+06 | 6.33E+05 | 9.64E+05 | 2.99E+04 | 0 |
| Height uni. rays | 6.06E+05 | 2.24E+05 | 2.99E+05 | 1.04E+04 | 0 |
| Height multi. rays | 2.29E+07 | 8.69E+06 | 1.09E+07 | 2.95E+05 | 0 |
| Ray width | 4.25E+01 | 3.40E-04 | 1.22E+01 | 1.44E+01 | 0 |
| Dens. multi. rays | 4.40E+01 | 1.69E+00 | 9.71E+00 | 3.46E+00 | 0 |

Since the fit of these models of character evolution is based on a likelihood optimization of model parameters, optimal parameter results are returned for each model and character. As described in Pagel (1999) and Butler and King (2004) these model parameters are informative on their own. Table 2 states the optimal parameter value for every model and every wood anatomical character.

**Table 2:** Optimal model parameter values.

|  | **lambda** | **delta** | **kappa** | **OU** |
|---|---|---|---|---|
| Ves. diam. | 1.00E-07 | 1.00E-06 | 3.00 | 2.06E-09 |
| Ves. dens. | 1.00E-07 | 1.00E-06 | 3.00 | 29.00 |
| Ves. el. Length | 0.18 | 1.00E-06 | 3.00 | 2.13E-09 |
| Fibre length | 0.10 | 1.00E-06 | 3.00 | 2.18E-09 |
| Height uni. rays | 1.00E-07 | 1.00E-06 | 3.00 | 2.07E-09 |
| Height multi. rays | 0.14 | 1.00E-06 | 3.00 | 1.82E-07 |
| Ray width | 1.00E-07 | 1.03E-06 | 3.00 | 448.60 |
| Dens. multi. rays | 1.00E-07 | 1.14E-06 | 3.00 | 88.87 |

# Character correlations

After applying Bonferroni's correction for multiple comparisons the correlations between the wood anatomical characters yielded several significant results at the α=0.05 level (table 3). Vessel density appeared to be negatively correlated with vessel element length and fibre length, both with a regression coefficient of -0.64 indicating a moderately strong correlation. The height of the multiseriate rays appeared to be positively correlated to the height of the uniseriate rays with a regression coefficient of 0.61 indicating a moderately strong correlation.

**Table 3:** Correlation of wood anatomical characters. Upper triangle shows p-values, lower triangle shows Spearman's rho correlation coefficient. Values in black/bold are significant at the α=0.05 level.

| | VD | D | VEL | FL | HUR | HMR | DMR | RW | |
|---|---|---|---|---|---|---|---|---|---|
| VD | | 1.045 | 0.066 | 2.413 | 1.749 | 0.535 | 13.553 | 1.573 | |
| D | -0.36 | | **0.006** | **0.006** | 8.751 | 14.093 | 4.343 | 10.266 | |
| VEL | 0.54 | **-0.64** | | 0.115 | 0.603 | 0.387 | 6.392 | 4.571 | P-value |
| FL | 0.28 | **-0.64** | 0.51 | | 11.974 | 6.328 | 5.709 | 8.286 | |
| HUR | 0.31 | -0.1 | 0.4 | -0.04 | | **0.013** | 10.623 | 9.145 | |
| HMR | 0.41 | 0 | 0.43 | -0.15 | **0.61** | | 7.974 | 0.546 | |
| DMR | 0.01 | 0.21 | -0.15 | -0.17 | -0.06 | -0.12 | | 0.276 | |
| RW | 0.32 | -0.07 | 0.2 | 0.11 | 0.09 | 0.41 | -0.45 | | |

Spearman's rho

After calculation of the phylogenetic independent contrasts for each character the correlations between characters changed drastically (table 4). Again, several characters were found to be significantly correlated at the α=0.05 level, but all significant correlations between PICs were involved different characters compared to the analyses without phylogenetic correction. Correlation between vessel diameter and vessel element length yielded a correlation coefficient of 0.78 and correlation between vessel diameter and ray width yielded a correlation coefficient of 0.62, both indicating a moderately strong positive correlation. Correlation between vessel element length and fibre length yielded a correlation coefficient of 0.71 indicating a moderately strong positive correlation.

**Table 4:** Correlation of phylogenetically independent contrasts for wood anatomical characters. Upper triangle shows p-values, lower triangle shows Spearman's rho correlation coefficient. Values in black/bold are significant at the α=0.05 level

| | VD | D | VEL | FL | HUR | HMR | DR | RW | |
|---|---|---|---|---|---|---|---|---|---|
| VD | | 0.135 | **0** | 1.329 | 5.535 | 0.207 | 11.409 | **0.019** | |
| D | -0.52 | | 0.119 | 0.91 | 1.579 | 13.73 | 10.799 | 5.697 | |
| VEL | **0.78** | -0.52 | | **0.001** | 3.621 | 0.986 | 10.24 | 0.376 | |
| FL | 0.35 | -0.38 | **0.71** | | 10.325 | 11.365 | 8.346 | 1.941 | P-value |
| HUR | 0.18 | -0.33 | 0.24 | 0.07 | | 6.941 | 9.07 | 6.064 | |
| HMR | 0.49 | -0.01 | 0.38 | -0.05 | 0.15 | | 8.071 | 1.268 | |
| DR | 0.05 | 0.06 | 0.07 | -0.11 | 0.1 | -0.12 | | 5.567 | |
| RW | **0.62** | -0.18 | 0.45 | 0.31 | -0.17 | 0.35 | -0.18 | | |

Spearman's rho

When calculating the correlations for the phylogenetic independent contrasts that were calculated over the tree corrected for an Ornstein-Uhlenbeck model of character evolution a pattern different from the uncorrected and PIC correlations emerges (table 5). Only the vessel diameter and vessel element length remain significantly correlated at the α=0.05 level. A correlation coefficient of 0.72 indicates as moderately strong positive correlation.

**Table 5:** Correlation of phylogenetically independent contrasts corrected for the Ornstein-Uhlenbeck model of character evolution. Upper triangle shows p-values, lower triangle shows Spearman's rho correlation coefficient. Values in black/bold are significant at the α=0.05 level.

|     | VD    | D     | VEL      | FL    | HUR   | HMR   | DMR   | RW    |
| --- | ----- | ----- | -------- | ----- | ----- | ----- | ----- | ----- |
| VD  |       | 1.01  | **0.00** | 0.93  | 3.60  | 1.35  | 9.70  | 1.16  |
| D   | -0.37 |       | 0.09     | 0.40  | 2.59  | 9.14  | 7.57  | 5.80  |
| VEL | **0.72** | -0.53 |       | 0.17  | 3.38  | 0.82  | 12.70 | 0.49  |
| FL  | 0.37  | -0.44 | 0.49     |       | 10.98 | 5.25  | 11.02 | 3.88  |
| HUR | 0.24  | -0.27 | 0.24     | 0.06  |       | 3.63  | 11.45 | 9.78  |
| HMR | 0.34  | 0.09  | 0.38     | -0.19 | 0.23  |       | 3.95  | 1.90  |
| DMR | 0.08  | 0.13  | -0.02    | -0.06 | -0.05 | -0.22 |       | 2.14  |
| RW  | 0.35  | -0.17 | 0.42     | 0.23  | -0.08 | 0.31  | -0.29 |       |

Spearman's rho

## Principal component analysis

Principal component analysis on the wood anatomical data (table 6a, fig. 8b) allows for the distinction of lianas and trees as two separate groups based on the eight wood anatomical characters. As can be seen in figure 8b there is a large spread in the direction of the arrows representing variables. Since the angle between arrows reflects the pair wise correlation between two variables this indicates most variables are uncorrelated. There is however a distinction possible between two groups of variables: 1) height of the multiseriate rays, height of the uniseriate rays and ray width and 2) vessel element length and vessel density.

The PCA for the PICs (table 6b, fig. 8c) does not allow for the distinction between liana or tree, but the grouping of the variables can still be assessed. Two groups consisting of 1) vessel density and vessel element length and 2) density of the multiseriate rays and vessel density can be distinguished. All other variables show large spread. The fact that most contrast values lie closer to the centre of the plot is explained by the fact that PICs are a way of normalizing the data.

The PCA for the PICs calculated over a tree corrected for Ornstein-Uhlenbeck (table 6c, fig. 8a) finally shows no grouping of variables at all. Again, no distinction between tree or liana can be made due to the nature of the PICs.

**Tabel 6:** Proportion of explained variance per principal component.

**a)**

Importance of components:  **PCA**

|  | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 | PC7 | PC8 |
|---|---|---|---|---|---|---|---|---|
| Standard deviation | 1.69 | 1.26 | 1.13 | 0.93 | 0.73 | 0.60 | 0.55 | 0.49 |
| Proportion of Variance | 0.36 | 0.20 | 0.16 | 0.11 | 0.07 | 0.04 | 0.04 | 0.03 |
| Cumulative Proportion | 0.36 | 0.55 | 0.71 | 0.82 | 0.89 | 0.93 | 0.97 | 1.00 |

**b)**

Importance of components:  **PIC**

|  | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 | PC7 | PC8 |
|---|---|---|---|---|---|---|---|---|
| Standard deviation | 1.79 | 1.18 | 1.05 | 0.98 | 0.69 | 0.63 | 0.55 | 0.40 |
| Proportion of Variance | 0.40 | 0.17 | 0.14 | 0.12 | 0.06 | 0.05 | 0.04 | 0.02 |
| Cumulative Proportion | 0.40 | 0.57 | 0.71 | 0.83 | 0.89 | 0.94 | 0.98 | 1.00 |

**c)**

Importance of components:  **OU**

|  | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 | PC7 | PC8 |
|---|---|---|---|---|---|---|---|---|
| Standard deviation | 1.7 | 1.19 | 1.03 | 0.96 | 0.76 | 0.67 | 0.58 | 0.4 |
| Proportion of Variance | 0.3 | 0.18 | 0.13 | 0.11 | 0.07 | 0.06 | 0.04 | 0.02 |
| Cumulative Proportion | 0.3 | 0.56 | 0.70 | 0.81 | 0.88 | 0.94 | 0.98 | 1.00 |
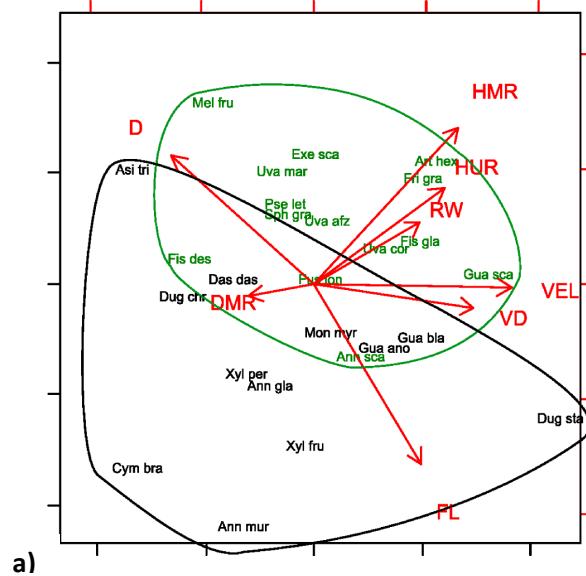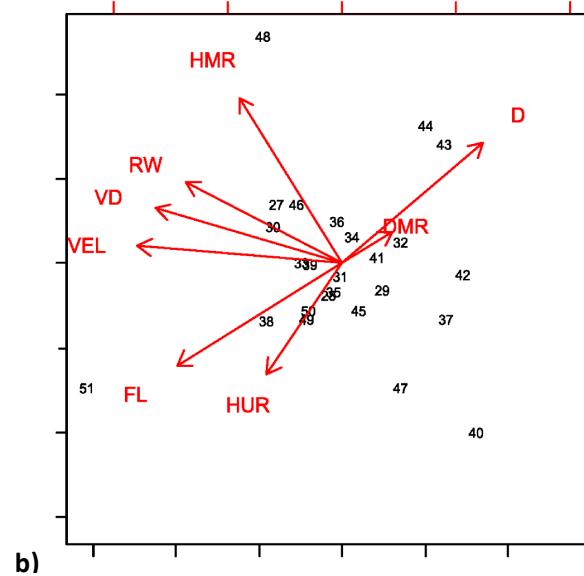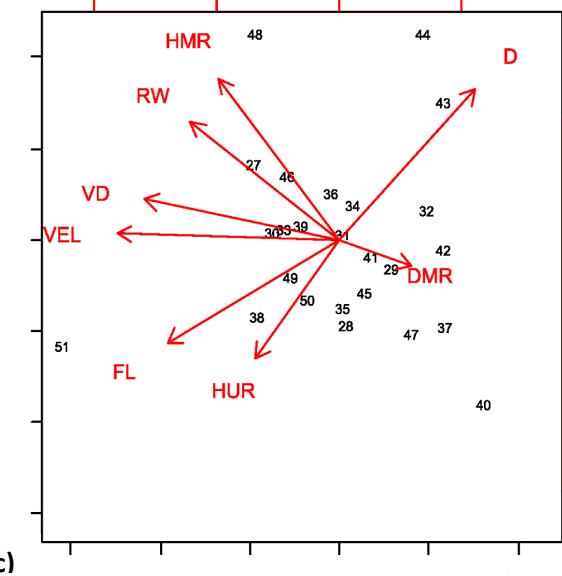
**Figure 8:** PCA biplots of the two components that together maximize explained variance in the data. Arrows represent wood anatomical characters. a) PCA of the wood anatomical data with abbreviations of species names, green indicates climbing species, black indicates trees. b) PCA of PICs calculated over the tree as in fig. X. c) PCA of PICs calculated over the tree corrected for an Ornstein-Uhlenbeck model of character evolution.

# Discussion

Phylogenetic relationships among tribes in the Annonoideae are in agreement with those in Chatrou et al. (2012). Posterior probabilities (PP) for the tribal splits from the MrBayes analysis are above 95%, except for two deep nodes (with PPs of 93% and 83%, see fig. 1) Since the credibility in the node with PP=93% is only marginally below PP=95% there is still ample evidence to support this node, but the support for the node with PP=83% is substantially lower. Following existing literature (Chatrou et al. 2012; Pirie and Doyle 2012) and the fact that this node is supported by repeated phylogenetic analyses provides enough evidence to validate this node. However, relationships below the tribal level prove to be problematic. For example, relationships with low PPs from the MrBayes analysis are sometimes reconstructed differently in BEAST. This is especially the case in the Uvarieae, for which relationships are generally poorly supported. Where MrBayes places the genus Uvaria in a large polytomy with other Uvariae species, BEAST places the genus Uvaria as sister to all other Uvariae species. Current knowledge on Uvariae phylogenetic relationships is insufficient to rule out one of the two hypotheses (Zhou et al. 2009; Zhou et al. 2010). Since the hypothesis that the genus Uvaria is sister to all other Uvariae species was easiest to implement in subsequent analysis we adhere to this hypothesis.

Careful consideration should be taken when interpreting all character analyses incorporating phylogenetic information in this study. Because only wood anatomical data from species that are represented in the phylogeny could be used, we obtained a very limited sampling. Whereas a sampling that covers all tribes of the Annonoideae is obtained, at some points there is not enough data to accurately reflect variability within the tribes. Given that we only sampled X genera of the 51 genera in the Annonoideae, it seems likely that we have missed some of the variation. Another problem that comes with this limited sampling is that several species have very long terminal branches in the pruned phylogeny (fig. 7). In reality these species belong to clades with shorter terminal branches (fig.2). This potentially forces wrong rates of character change over the branch, since the rate of character change is calculated relative to the branch length. Although these limitations place serious limitations on the interpretation of the results, the results obtained will be treated as if these limitations are not too severe.

It becomes clear from figure 3 that a mere distinction between trees and lianas is not enough to describe the evolution of a climbing habit. Whereas a very likely character state reconstruction is available for the MRCA of the Uvariae (most likely a liana, see fig. 3), this is not the case for several other clades. For instance it remains unclear whether the genera Artabotrys and Xylopia are derived from an MRCA that was a tree or a liana. On the other hand, the ancestral state for the Annoneae is reconstructed as being a liana. When taking a closer look at the species in this clade it becomes clear that *Annona scandens* is the only extant species in the Annoneae with a climbing habit. This means that from the MRCA of the Annoneae several independent origins and reversals of a climbing habit should have taken place. Since this hardly makes any biological sense and is not very parsimonious, the explanation should be sought in the evolutionary model used in SIMMAP. Given the priors calculated for character state change **[APPENDIX]** it becomes clear that a very high rate of change has to be accommodated somewhere in the tree. The stochastic process of the analysis has subsequently found the Annoneae to be the most likely place for this to happen, possibly due to some long branches leading to *Asimina triloba* and *Goniothalamus griffithii*.

Discretizing the wood anatomical data into seven possible states per character allows for a good comparison to the analysis of the binary trait lianescence. Where lianescence yielded explicit but dubious results in SIMMAP, the wood anatomical characters were all completely uncertain. Again, the estimated prior probabilities force the use of very high rates of character change. Along with sufficient variation in the clades studied this results in completely unknown ancestral states. When allowing for very high rates of character change, anything could have happened. Since this is a very unsatisfactory explanation, and given the fact that only three wood anatomical characters could be analysed, SIMMAP seems to be unsuitable for evolutionary analysis of this dataset.

Treating the continuous characters as such allowed for the ancestral state reconstruction of all wood anatomical characters. Although a slightly different way of accounting for phylogenetic uncertainty was used compared to SIMMAP, the narrow 95% confidence intervals provide evidence for the minimal influence of phylogenetic uncertainty. The large difference in ancestral reconstructions between the SIMMAP analyses and the continuous reconstruction is striking. This further supports the notion that SIMMAP is unsuitable for this dataset. When observing the continuous reconstruction careful consideration should be given to the magnitude of the difference between clades. For instance ancestral vessel diameters (Fig...) lie between 95μm and 110μm, a difference too small to be interpreted in a biological fashion. For all other characters differences between reconstructed values are considered to be large enough to be of biological meaning. This is also the case for the characters that could be analysed in SIMMAP and showed no significant pattern in that analysis. Again, this leaves no other conclusion than the unsuitability of SIMMAP for analysing these data.

If wood anatomical characters are to evolve as a syndrome it is to be expected that ancestral state reconstructions for different characters show a similar pattern. The ML reconstruction of continuous characters proves this hypothesis to be wrong. The only characters in which a similar pattern is observed are density of the

multiseriate rays and width of the multiseriate rays. The pattern observed shows an inverse relationship, indicating that species with wider multiseriate rays have fewer of them per millimetre.

Since  ML ancestral state reconstruction is based on the assumption of Brownian motion, this assumption has to be verified. This was done by testing alternative evolutionary hypotheses in the form of adaptations to the Brownian motion model. Unfortunately, the results were inconclusive. Although model selection based on AICc values resulted in the OU model as best fit for all characters, the actual model parameters mostly make no biological sense. The alpha parameter for the OU model is near zero in all but three characters. When observing the formulation of the OU model, an alpha value of zero effectively is a Brownian motion model of character evolution. Still, the OU model has by far the best AICc score for all characters. Clearly this is a computational error of some kind, possibly due to flat likelihood surfaces as described in the function's manual. Knowing that parameter values for the most likely model are not trustworthy, all other parameter values become highly conspicuous. With a few exceptions, values for lambda, kappa and delta are zero, three and zero respectively. It is not theoretically impossible to find the same parameter values for every character, but given the performance of the OU model in this case it is highly conspicuous. The exceptions are found for the lambda parameter of fibre length, vessel element length and fibre length. Values between zero and one indicate less similarity between species than can be expected from the phylogeny (Pagel 1999).

The effect of different evolutionary models in this study cannot be properly assessed due to unreliable model optimizations, but for demonstrative purposes they were included in the character correlation analysis and PCA. Where the PICs test correlated evolution, the PICs corrected for an OU model of evolution test the characters for deviation of an evolutionary process following Brownian motion. Both effects of correcting the data with PICs can be observed in the correlation analyses. The significant correlations between vessel density and vessel element length, vessel density and fibre length, and height of the multiseriate rays and height of the uniseriate rays are lost in the PICs. Instead, significant correlations between vessel diameter and vessel element length, vessel diameter and ray width, and vessel element length and fibre length are observed. When correcting for an Ornstein Uhlenbeck model of character evolution, the moderately strong positive correlation (Spearman's rho=0.72, p=0.00) between vessel diameter and vessel element length is the only correlation that remains statistically significant. This correlation is much more likely since the assumption of independent taxa is no longer violated and it has accounted for deviation from Brownian motion in several characters' evolutionary history. The fact that only one out of 28 possible character combinations turns out as significantly correlated refutes the hypothesis that these characters have evolved as a syndrome in Annonaceae.

A clear grouping of variables and clustering of scandent species is observed in the PCA on the wood anatomical characters (fig. 8b, table 6a). However, at least four principal components are needed to reasonably describe the variation in the data. This indicates that although based on these characters a distinction between climbers and trees can be made, there is no single character indicative of a climbing habit in Annonaceae. Since the angle between the vectors of the wood anatomical characters represent the correlation between them, it is clear that several characters are reasonably correlated. The characters derived from the vessels all point in the same direction, perpendicular to the characters derived from the rays. This means that the characters from the vessels are correlated to each other, and the characters from the rays are correlated to each other, but that characteristics of vessels are independent from characteristics of rays. However, due to the nature of principal component analysis the significance of these correlations cannot be tested. The ability to distinguish between species is lost in the PICs (fig. 8c, table 6b), but it does allow for a good multivariate analysis of the wood anatomical characters. Normally, closely related species are more likely to resemble each other, so PICs tend to be small values. The PCA on the PICs substantiates the results found in the character correlations. The fact that the arrows representing variables in the biplot all point in a different direction means there is very little correlation between them. Furthermore, the first two principal components only represent 50-60% of the variance found in the data (table 6). If 95% of the variance is to be explained six orthogonal components are required. Compared to the eight original variables, this can hardly be seen as a reduction in complexity. This means that the PCA also rejects the hypothesis that wood anatomical characters evolved as a syndrome in Annonaceae. The PCA on the Ornstein Uhlenbeck transformed PICs are only marginally different from the PCA on the PICs, although a slightly larger spread in vectors is observed.

Combining all of the analyses mentioned above, the hypothesis of a syndrome of wood anatomical characters describing a climbing habit is supported in Annonaceae. However the evolutionary process that resulted in this syndrome differs per character and per clade. The conclusions are in support of a hypothesis posed by Carlquist (2012), namely that "wood characters evolve independently from each other and are explained by ecological forces". This means that lianescence cannot be seen as a character, rendering any statements on homology of lianescence invalid per definition. Interestingly, this hints at independent regulation of wood anatomical characters. Given that all the characters analyzed in this study are quantitative, the genetic regulation of these characters probably consists of different QTLs per character.

Unfortunately this study was unable to properly identify the evolutionary models that best describe the evolutionary process followed by each character. This is something that would be a step further in identifying the forces that drive the evolution of lianas in Annonaceae and is possible with an increased sampling of species.

# References

Altekar, G., S. Dwarkadas, J. P. Huelsenbeck and F. Ronquist (2004). "Parallel Metropolis coupled Markov chain Monte Carlo for Bayesian phylogenetic inference." Bioinformatics **20**(3): 407-415.

Angyalossy, V., G. Angeles, M. R. Pace, A. C. Lima, C. L. Dias-Leme, L. G. Lohmann and C. Madero-Vega (2012). "An overview of the anatomy, development and evolution of the vascular system of lianas." Plant Ecology and Diversity **5**(2): 167-182.

Benson, D. A., I. Karsch-Mizrachi, D. J. Lipman, J. Ostell and D. L. Wheeler (2006). "GenBank." Nucleic acids research. **34**(Database issue): D16-20.

Bhaskar, R., A. Valiente-Banuet and D. D. Ackerly (2007). "Evolution of hydraulic traits in closely related species pairs from mediterranean and nonmediterranean environments of North America." New Phytologist **176**(3): 718-726.

Bollback, J. P. (2006). "SIMMAP: Stochastic character mapping of discrete traits on phylogenies." BMC Bioinformatics **7**.

Brandes, A. F. N., C. S. Lisi and C. F. Barros (2011). "Dendrochronology of lianas of the Leguminosae family from the Atlantic Forest, Brazil." Trees - Structure and Function **25**(1): 133-144.

Butler, M. A. and A. A. King (2004). "Phylogenetic comparative analysis: A modeling approach for adaptive evolution." American Naturalist **164**(6): 683-695.

Carlquist, S. (2012). "How wood evolves: A new synthesis." Botany **90**(10): 901-940.

Chatrou, L. W. and P. He (1999). "Studies in Annonaceae XXXIII. A revision of Fusaea (Baill.) Saff." Brittonia **51**(2): 181-203.

Chatrou, L. W., M. D. Pirie, R. H. J. Erkens, T. L. P. Couvreur, K. M. Neubig, J. R. Abbott, J. B. Mols, J. W. Maas, R. M. K. Saunders and M. W. Chase (2012). "A new subfamilial and tribal classification of the pantropical flowering plant family Annonaceae informed by molecular phylogenetics." Botanical Journal of the Linnean Society **169**(1): 5-40.

Committee, I. (1989). IAWA list of microscopic features for hardwood identification. International Association of Wood Anatomists Bulletin, new series. **10**: 219-232.

Couvreur, T. L. P., M. D. Pirie, L. W. Chatrou, R. M. K. Saunders, Y. C. F. Su, J. E. Richardson and R. H. J. Erkens (2011). "Early evolutionary history of the flowering plant family Annonaceae: Steady diversification and boreotropical geodispersal." Journal of Biogeography **38**(4): 664-680.

Doyle, J. A. and P. K. Endress (2009). "Integrating early cretaceous fossils into the phylogeny of living angiosperms: Magnoliidae and eudicots." Journal of Systematics and Evolution **48**(1): 1-35.

Drummond, A. J., S. Y. Ho, M. J. Phillips and A. Rambaut (2006). "Relaxed phylogenetics and dating with confidence." PLoS biology **4**(5).

Drummond, A. J., M. A. Suchard, D. Xie and A. Rambaut (2012). "Bayesian phylogenetics with BEAUti and the BEAST 1.7." Molecular Biology and Evolution **29**(8): 1969-1973.

Ewers, F. W., M. R. Carlton, J. B. Fisher, K. J. Kolb and M. T. Tyree (1997). "Vessel diameters in roots versus stems of tropical lianas and other growth forms." IAWA Journal **18**(3): 261-279.

Felsenstein, J. (1985). "Phylogenies and the comparative method." American Naturalist **125**(1): 1-15.

Franklin, G. L. (1945). "Preparation of thin sections of synthetic resins and wood-resin composites, and a new macerating method for wood [10]." Nature **155**(3924): 51.

Fu, P. L., Y. J. Jiang, A. Y. Wang, T. J. Brodribb, J. L. Zhang, S. D. Zhu and K. F. Cao (2012). "Stem hydraulic traits and leaf water-stress tolerance are co-ordinated with the leaf phenology of angiosperm trees in an Asian tropical dry karst forest." Annals of Botany **110**(1): 189-199.

Gartner, B. L. (1991a). "Is the climbing habit of poison oak ecotypic?" Functional Ecology **5**(5): 696-704.

Gartner, B. L. (1991b). "Structural stability and architecture of vines vs. shrubs of poison oak, *Toxicodendron diversilobum*." Ecology **72**(6): 2005-2015.

Gentry, A. H. (1991). The distribution and evolution of climbing plants. The biology of vines. F. E. Putz and H. A. Mooney, Cambridge university press**:** 3-49.

Gianoli, E. (2004). "Evolution of a climbing habit promotes diversification in flowering plants." Proceedings of the Royal Society B: Biological Sciences **271**(1552): 2011-2015.

Hacke, U. G., A. L. Jacobsen and R. B. Pratt (2009). "Xylem function of arid-land shrubs from California, USA: An ecological and evolutionary analysis." Plant, Cell and Environment **32**(10): 1324-1333.

Hardy, C. R. (2006). "Reconstructing ancestral ecologies: Challenges and possible solutions." Diversity and Distributions **12**(1): 7-19.

Hargrave, K. R., K. J. Kolb, F. W. Ewers and S. D. Davis (1994). "Conduit diameter and drought-induced embolism in Salvia mellifera Greene (Labiatae)." New Phytologist **126**(4): 695-705.

Huelsenbeck, J. P. and F. Ronquist (2001). "MRBAYES: Bayesian inference of phylogenetic trees." Bioinformatics **17**(8): 754-755.

Ichihashi, R. and M. Tateno (2011). "Strategies to balance between light acquisition and the risk of falls of four temperate liana species: To overtop host canopies or not?" Journal of Ecology **99**(4): 1071-1080.

Jacques, F. M. B. and D. De Franceschi (2007). "Menispermaceae wood anatomy and cambial variants." IAWA Journal **28**(2): 139-172.

Koek-Noorman, J. and L. Y. T. Westra (2012). "Macrophotographic wood atlas of Annonaceae." Botanical Journal of the Linnean Society **169**(1): 135-189.

Lens, F., S. Dressler, S. Jansen, L. Van Evelghem and E. Smets (2005). "Relationships within balsaminoid Ericales: A wood anatomical approach." American Journal of Botany **92**(6): 941-953.

Lens, F., M. E. Endress, P. Baas, S. Jansen and E. Smets (2008). "Wood anatomy of Rauvolfioideae (Apocynaceae): A search for meaningful non-DNA characters at the tribal level." American Journal of Botany **95**(10): 1199-1215.

Lens, F., M. E. Endress, P. Baas, S. Jansen and E. Smets (2009). "Vessel grouping patterns in subfamilies apocynoideae and periplocoideae confirm phylogenetic value of wood structure within apocynaceae." American Journal of Botany **96**(12): 2168-2183.

Lynch, A. H. and P. E. Gasson (2010). "Index xylariorum - Edition 4.". Retrieved 27-8, 2012.

Martínez-Cabrera, H. I., H. Jochen Schenk, S. R. S. Cevallos-Ferriz and C. S. Jones (2011). "Integration of vessel traits, wood density, and height in angiosperm shrubs and trees." American Journal of Botany **98**(5): 915-922.

Pace, M. R., L. G. Lohmann and V. Angyalossy (2009). "The rise and evolution of the cambial variant in Bignonieae (Bignoniaceae)." Evolution and Development **11**(5): 465-479.

Pace, M. R., L. G. Lohmann and V. Angyalossy (2011). "Evolution of disparity between the regular and variant phloem in Bignonieae (Bignoniaceae)." American Journal of Botany **98**(4): 602-618.

Pagel, M. (1999). "Inferring the historical patterns of biological evolution." Nature **401**(6756): 877-884.

Paradis, E., J. Claude and K. Strimmer (2004). "APE: Analyses of phylogenetics and evolution in R language." Bioinformatics **20**(2): 289-290.

Patterson, C. (1988). "Homology in classical and molecular biology." Molecular Biology and Evolution **5**(6): 603-625.

Phillips, O. L., R. Vásquez Martínez, L. Arroyo, T. R. Baker, T. Killeen, S. L. Lewis, Y. Malhi, A. Monteagudo Mendoza, D. Neill, P. Núñez Vargas, M. Alexiades, C. Cerón, A. Di Flore, T. Erwin, A. Jardim, W. Palacios, M. Saldias and B. Vinceti (2002). "Increasing dominance of large lianas in Amazonian forests." Nature **418**(6899): 770-774.

Pirie, M. D. and J. A. Doyle (2012). "Dating clades with fossils and molecules: The case of Annonaceae." Botanical Journal of the Linnean Society **169**(1): 84-116.

Posluszny, U. and J. B. Fisher (2000). "Thorn and hook ontogeny in Artabotrys hexapetalus (Annonaceae)." American Journal of Botany **87**(11): 1561-1570.

Preston, K. A., W. K. Cornwell and J. L. DeNoyer (2006). "Wood density and vessel traits as distinct correlates of ecological strategy in 51 California coast range angiosperms." New Phytologist **170**(4): 807-818.

Putz, F. E. (1984). "The natural history of lianas on Barro Colorado Island, Panama." Ecology **65**(6): 1713-1724.

Richardson, J. E., L. W. Chatrou, J. B. Mols, R. H. J. Erkens and M. D. Pirie (2004). "Historical biogeography of two cosmopolitan families of flowering plants: Annonaceae and Rhamnaceae." Philosophical Transactions of the Royal Society B: Biological Sciences **359**(1450): 1495-1508.

Ronquist, F. and J. P. Huelsenbeck (2003). "MrBayes 3: Bayesian phylogenetic inference under mixed models." Bioinformatics **19**(12): 1572-1574.

Schenk, H. (1892). Beiträge zur Biologie und Anatomie der Lianen, Verlag von Gustav Fischer.

Schnitzer, S. A. (2005). "A mechanistic explanation for global patterns of liana abundance and distribution." American Naturalist **166**(2): 262-276.

Schnitzer, S. A. and F. Bongers (2002). "The ecology of lianas and their role in forests." Trends in Ecology and Evolution **17**(5): 223-230.

Scotland, R. W. (2010). "Deep homology: A view from systematics." BioEssays **32**(5): 438-449.

Spicer, R. and A. Groover (2010). "Evolution of development of vascular cambia and secondary growth." New Phytologist **186**(3): 577-592.

Thiele, K. (1993). "The Holy Grail of the Perfect Character: the Cladistic Treatment of Morphometric Data." Cladistics **9**(3): 275-304.

Wang, J., D. C. Thomas, C. F. Yvonne, S. Meinke, L. W. Chatrou and R. M. K. Saunders (2012). "A plastid DNA phylogeny of Dasymaschalon (Annonaceae) and allied genera: Evidence for generic non-monophyly and the parallel evolutionary loss of inner petals." Taxon **61**(3): 545-558.

Willson, C. J., P. S. Manos and R. B. Jackson (2008). "Hydraulic traits are influenced by phylogenetic history in the drought-resistant, invasive genus Juniperus (Cupressaceae)." American Journal of Botany **95**(3): 299-314.

Wright, I. J., D. D. Ackerly, F. Bongers, K. E. Harms, G. Ibarra-Manriquez, M. Martinez-Ramos, S. J. Mazer, H. C. Muller-Landau, H. Paz, N. C. A. Pitman, L. Poorter, M. R. Silman, C. F. Vriesendorp, C. O. Webb, M. Westoby and S. J. Wright (2007). "Relationships among ecologically important dimensions of plant trait variation in seven neotropical forests." Annals of Botany **99**(5): 1003-1015.

Zhou, L., Y. C. F. Su, P. Chalermglin and R. M. K. Saunders (2010). "Molecular phylogenetics of *Uvaria* (Annonaceae): Relationships with *Balonga*, *Dasoclema* and Australian species of *Melodorum*." Botanical Journal of the Linnean Society **163**(1): 33-43.

Zhou, L., Y. C. F. Su and R. M. K. Saunders (2009). "Molecular phylogenetic support for a broader delimitation of *Uvaria* (Annonaceae), inclusive of *Anomianthus*, *Cyathostemma*, *Ellipeia*, *Ellipeiopsis* and *Rauwenhoffia*." Systematics and Biodiversity **7**(3): 249-258.