Phylogenetics of *Stelis*

(Orchidaceae: Pleurothallidinae)

and closely related genera

By:
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Based on:

Molecular Data, Morphological Characteristics

and Geographical Distribution

In the Mesoamerican and Andean Cordilleras

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"*Systematics is a lonely voice speaking on behalf of an interest in diversity in Biology*" (Robinson 1901 cited in Constance 1964).

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Summary

The classification of genus *Stelis* Sw. (Orchidaceae) has suffered large modifications in the last decade. Morphological and anatomical characters were traditionally used to infer phylogenetic relationships (Luer 1986), however, after a phylogenetic anaylisis by Pridgeon *et al*. (2001) based on nuclear and plastid sequences, the concept of the genus was broadened (Pridgeon & Chase 2001). The "new" circumscription was not accepted by all authors, Carlyle Luer reknown authority within the Pleurothallidinae (to which Stelis belongs), rejected the broad sense of *Stelis* and published several new genera to accommodate the species involved.

One of the major critiques towards the published molecular phylogeny of the group is that only eight species of *Stelis s.l.* were included in all analyses, whereas more than a thousand species belong to it. The clear need of more sampling and the lack of constant morphological characters that define the genus are the biggest reasons for this reevaluation of the case.

Plant material was collected and individuals were scored morphologically at the living plant collection of the Lankester Botanical Garden´s (LBG), University of Costa Rica. DNA extraction, amplification and sequencing were done at the Plant Sciences laboratorium in Wageningen University. The nuclear region ITS and plastid matK were used for sequencing for their previous use in *Stelis* and their good results in other studies.

Consensus trees were produced using Bayesian analysis on the data sets. Bayesian inference was preferred, because of its good results in other studies and because it gives probability values to the clades (PP). *matK* and ITS data sets were evaluated individually and together in a concatenated matrix. Consensus networks were produced for each data set in order to view areas of conflict between resulting trees. Clades were proposed based on all the results.

A morphological matrix was produced using 26 characters chosen for their high between and low within clade variability. Using a parsimony analysis, ancestral characters states were determined. The data set was not used to contruct a phylogeny, but to explain the evolutionary trends found in the group using the molecular reconstruction. Morphological modifications from ancestral to derived states are discussed for all clades.

Geographical trends were plotted on the resulting phylogeny. Preference of some clades for certain geographical areas became quite evident. The possible Costa Rica – Colombia origin of the *Stelis s.l.* group was determined using a parsimony analysis. The large *Stelis s.s.* clade was clearly more diverse in the Andes than in Central America, making the center of origin and of more diversity different from each other.

Several plausible explanations for the very large number of species of *Stelis s.s.* as compared to other clades in the group are presented. A possible radiation event from Central into South America could explain the tremendous amount of species present in the relatively recently uplifted Andes mountains. The explosive increase in number of species was also accompanied by large morphological modifications, especially of the pollination syndrome.

Molecular, morphological and geographical evidence suggests that *Stelis* in its broad sense is not justified. *Stelis s.s.* is a very well distinguished monophyletic and natural group. Several smaller generic concepts such as *Crocodeilanthe* Rchb.f. & Warsz., *Dracontia* Luer, *Mystacorchis* Szlach & Marg., *Physosiphon* Lindl., *Physothallis* Garay and *Salpistele* Dressler are also accepted, while others are in need of readjustment.

1. Introduction

The diversity of organisms in our planet has long captured the attention and triggered the imagination of mankind. Systematics is the science of that organismal diversity, Judd *et al.* (2008) consider that "the fundamental aim of systematics is to discover all the branches of the evolutionary tree of life, to document changes that have occurred during the evolution of these branches, and to the greatest extent possible describe all species which represent the tip of the branches."

In plants, the backbone of systematic has always been plant structure, morphology and anatomy. Angiosperm classification until relatively recently relied only upon what could be observed in the phenotype without any information about the genetic basis of those characters. Authors could only speculate about how much molecular change was required to alter specific traits, and plant diversity, evolutionary history and phylogeny were quite difficult to explain (Chase *et al*. 2000; Endress *et al.* 2001). Although taxon circumscription had been historically somewhat intuitive, the application of Cladistics in the late 1970s formalized the process. Cladistic methods attempted to reduce complex characters into more simple units, however some plant morphologists such as Cronquist (1987) and Johnson (1989) were hesitant at first, mainly because of the problem of defining morphological characters and states. Computational techniques were introduced in the early 1980s and kept on being refined from then on. The introduction of molecular systematics greatly improved the computational techniques for morphology as well (Chase *et al*. 2000; Endress *et al.* 2001). Molecular systematic studies have come to produce more confident and better supported phylogeny for the Angiosperms. The first molecular studies supported several of the traditional phylogenetic classifications, but suggested many rearrangements as well (APG 1998). The use of DNA sequence data for higher level classification is more advanced in Angiosperms than any other major group of organisms. Large-scale phylogenetic analyses began with Zimmer *et al.* (1989) and Chase *et al*. (1993), and was followed by several others (Chase *et al*. 2000; Soltis *et al*. 2000). For further information on the history, advances and other details about plant systematics refer to the excellent compilations of Stuessy *et al*. (2001) and Judd *et al*. (2008).

Plant systematics at the generic level and higher has advanced greatly since the work of Chase and Soltis, however, as Soltis & Soltis (2001) suggest, there is still much to be done in the frame of systematics. Species-level systematics, for example, has lagged behind, and is not until recently that species phylogenies are being resolved. Now that most familial relationships have been resolved, emphasis on the species level has grown, especially focusing on the processes that drive speciation, extinction or evolutionary radiations, all which can be better learnt from species-level studies

(Bakker *et al*. 2005). Studies on species radiations can give invaluable information on patterns and rates of evolutionary diversification, for example, Schluter (2000) finds that ecological character displacement (or phenotypic evolution in a species generated by resource competition) is one of the most frequent drivers of radiation, but not the only one. Important issues are the relative importance of diversification at the lineage, character, or ecological level, and whether the levels can be coupled, how to test hypothesis of radiation, and to what extent observed radiative patterns are adaptive. Harvey and Rambaut (2000) give good examples on how to test models of evolution. A non-adaptive radiation can be defined as the speciation not triggered by ecological divergence. Adaptive radiations, on the other hand, are characterized by clear ecological and/or morphological diversification in relation to the environment and a possible high species production (Several species-level studies are discussed in a compilation by Bakker *et al.* 2005).

The Orchidaceae (commonly known as Orchids) are one of the largest families of flowering plants. With more than 30.000 species, it is present in all the continents and almost in every known life zone (Dressler 1981). Systematics and classification of Orchidaceae has been traditionally complex, and until recently based exclusively on morphological observations, and especially of the floral morphology (for fundamentals in orchid biology refer to Arditti 1992; for discussion on natural history and classification of Orchids refer to Dressler 1981; for pollination and evolution in Orchid flowers refer to van der Pijl & Dodson 1966). In the last ten years more or less, phylogenetic analyses based on DNA sequence data have been carried out for most of the major groups within the Orchidaceae, for example, Apostasioidae (Kocyan *et al.* 2004) Chloraeinae (Chemisquy & Morrone 2010) Dendrobiinae (Yukawa *et al.* 1996), Laeliinae (van den Berg *et al*. 2009), *Maxillaria* (Whitten *et al.* 2007) Pleurothallidinae (Pridgeon *et al.* 2001), Prescotiinae (Alvarez-Molina & Cameron 2009) and others. Even more recently, phylogenetic relationships within the whole family have been published (Gorniak *et al*. 2010).

Within the Orchidaceae, the subtribe Pleurothallidinae L. has captured the attention of many for the large variation and fantastic diversity of its flowers. Vastly studied by Luer on the basis of its morphological characters, a phylogenetic analysis based on nuclear and plastid DNA was presented by Pridgeon *et al*. in 2001. Modifications in the taxonomy of the group followed, the mammoth genus *Pleurothallis* with more than a thousand species was “chopped-up” into smaller generic concepts, several species were lumped into a broader concept of the other mammoth genus of Pleurothallidinae, *Stelis* Sw. The genus *Stelis*, which is the central topic of this paper, was described in 1799 by Swartz. The genus is lectotypified by *Stelis ophioglossoides* (Jacq.) Sw. (1799) (Green 1929), based on Jaquin's *Epidendrum ophioglossoides* (1760). An alternative proposal by Garay and Sweet to lectotypify *Stelis* with *Humboldtia purpurea* Ruiz & Pav. was not recommended (Taxon 32: 282. 1983). In a strict sense (see discussion further), it includes around 900+ species of epiphytic and

terrestrial orchids found throughout the Neotropics, from Mexico and the West Indies through Central America to Brazil, Bolivia and Peru, with the highest diversity in the Andean regions of South America (Luer 2003, 2009; Solano-Gómez & Salazar 2007). That makes it one of the largest genera within the Orchidaceae only surpassed by *Bulbophyllum* Thouars, *Dendrobium* Sw. *s.l*., *Epidendrum* L. and *Pleurothallis* R.Br. *s.l*., and at the same time one of the largest within the Angiosperms (Govaerts *et al.* 2010).

1.1. Taxonomical History of *Stelis*

The generic circumscription of *Stelis*, based on its morphological characters, was not substantially discussed until Garay (1979) proposed the segregation of several taxa on the basis of their bilobed stigma into the genus *Apatostelis* Garay, a concept that received little acceptance. Species of *Stelis s.s.* can be distinguished from other groups of the subtribe Pleurothallidinae by the terminal, racemose, fascicled, few or multiflowered inflorescences, the flowers with almost identical, diversely connate sepals, much larger than the petals and lip, the very reduced petals usually with a thick margin, the lip that is similar to the petals, and a very short, unwinged column (Luer 2003). In 2001, molecular evidence is obtained by Pridgeon *et al.* using nuclear ribosomal DNA internal transcribed spacers (ITS1 and ITS2) and 5.8S gene supported by plastid sequences from *matK*, the *trnL* intron, and the *trnL-F* intergenic spacer, suggested a different scheme of phylogenetic relationships among the Pleurothallidinae, and therefore the need for a recircumscription of the genus in order to attain monophyly. Consequently, several subgenera of the sister genus *Pleurothallis* [i.e. *Crocodeilanthe* (Rchb.f. & Warsz.) Luer*, Dracontia* Luer*, Effusia* Luer*, Elongatia* Luer*, Mystax* Luer*, Physosiphon* (Lindl.) Luer*,* (Garay) Luer*, Pseudostelis* (Schltr.) Luer, and *Unciferia* (Luer) Luer], as well as the smaller genera *Condylago* Luer and *Salpistele* Dressler were reduced in synonymy under *Stelis s.l*.

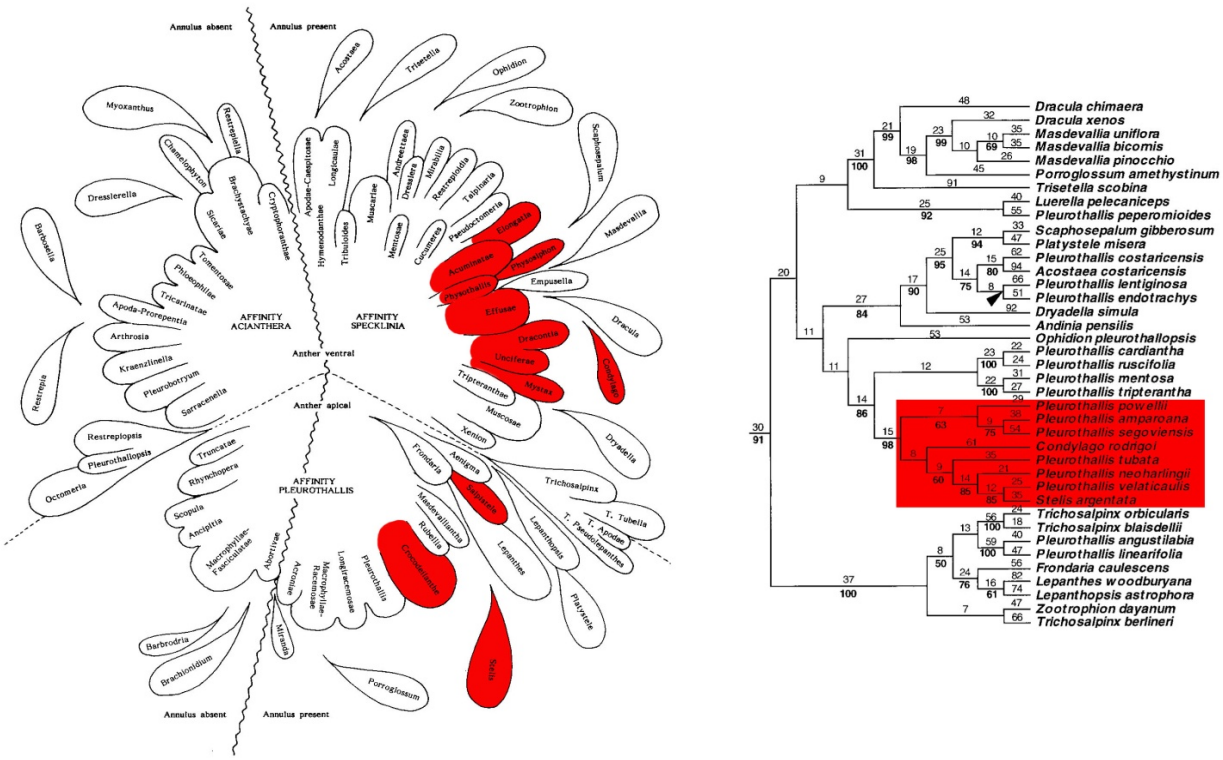

Figure 1. Phylogenetic relationships within the Pleurothallidinae,. *Left* showing Luer´s (1986) based on morphology . *Right* shows Pridgeon *et al.´*s (2001) consensus tree from combined nuclear and plastid data. In red those taxa belonging to *Stelis s.l.* as treated here.

As newly circumscribed, however, the genus *Stelis* Sw. is difficult to define on the basis of any specific set of morphological and anatomical features, as well as from an ecological and geographic perspective. The most recognized taxonomic authority in the Pleurothallidinae, Carlyle Luer, rejected the new circumscription of *Stelis,* and instead recognized the genera *Condylago* Luer, *Crocodeilanthe* Rchb.f. & Warsz., *Mystacorchis* Szlach. & Marg., *Physothallis* Garay, *Physosiphon* Lindl., *Salpistele* Dressler and *Specklinia* Lindl., and elevated to the generic rank four subgenera of *Pleurothallis*, (i.e. *Dracontia*, *Effusia*, *Elongatia* and *Unciferia*) (Luer 2004 & 2007). Additionally, he described the monotypic genera *Lomax* Luer and *Loddigesia* Luer in 2006 and *Niphantha* Luer in 2007 for a few "misfit" species (Luer 2006, 2007) not clearly belonging to any of the previously recognized groups. All of these genera include one or more species treated by Pridgeon *et al.* (2001) as members of *Stelis* in its broader sense. There is general consensus in considering other older generic names, like *Dialissa* Lindl., *Humboldtia* Ruiz & Pavon, *Pseudostelis* Schltr., *Steliopsis* Brieger (*nom. inval*.) and *Apatostelis* Garay (*nom. illeg*.), as synonymous to *Stelis*.

Luer (1986) is the first to try to describe the evolutionary relationship between the Pleurothallidinae (Figure 1, *Left*). His graphical representation is based solely on morphological features, however, he was able to correctly group most of the members of *Stelis s.l.* together. In

2001, Pridgeon *et al.* present a phylogeny of the Pleurothallids based on combined plastid and nuclear data Figure 1, *Right*). The *Stelis s.l.* group is present in the combined analysis, unfortunately, with only eight species. The group, with over 1000 species, is clearly under-sampled.

In order to maintain certain consistency and simplicity throughout the text, I will adopt the nomenclature accepted by Govaerts *et al.* 2010, who has adopted the genus *Stelis* in its broad sense. However, a few species have no combination in *Stelis*, and thus no accepted name, in those cases the basionym is used (indicated further in the text). A general summary of the taxonomical changes in this group is presented in Appendix I.

1.2. Phylogenetic Analyses

The first form of molecular data used in angiosperms was plastid restriction site variation, however, its information was not easily transmitted by computational means. That changed with the advent of DNA sequence data which now has been electronically banked for more than 20 years. DNA sequences are easily transferred electronically and the language of their four constituent bases is universal. The first and most common programs for DNA analyses where Hennig 86, PHYLIP and PAUP, the first is a parsimony programs, while the last two incorporated algorithms such as neighbor-joining and maximum likelihood (Chase & Cox 1998).

Parsimony is (or used to be) the dominant method for phylogenetic analyses of discrete character data. Likelihood methods for phylogenetics were introduced by Edwards and Cavallini-Sforza in 1964. They used the laws of probability in order to come up with prior probabilities and calculates posterior probabilities (Felsenstein 2004). Maximum likelihood (ML) had lagged behind because of its much greater computational cost for evaluating any given candidate trees.

However the increased availability of nucleotide and protein sequences from a diversity of organisms and genes has stimulated the development of stochastic models describing evolutionary changes in molecular sequences over time. Those models are important as the basis for phylogenetic inference using the models of maximum likelihood and Bayesian inference. Felsenstein's 1981 model was the first to allow nucleotide frequencies to differ, further developments led to models that accommodate both transition-transversion substitution bias and unequal nucleotide frequencies, in 1994, Muse and Gaut, and Goldman and Yang independently introduced likelihood methods designed to account for evolutionary dependency among sites within codons. That trend culminated in the general time-reversible (GTR) model, which allows unequal nucleotide frequencies and allows each of the six possible transitions between nucleotide states to occur at different rates (For an deeper discussion on the subject refer to Felsenstein 2004).

It is not until the recent development and application on phylogenetics of Bayesian methods that probabilities have been assigned to the resulting trees (amply discussed by Lewis 2001a & 2001b). These methods offer the prospect of obtaining meaningful nodal support measures without the unreasonable computational burden. Bayesian methods differ from other likelihood methods in the use of a prior distribution of the inferred trees. The use of a prior allows us to interpret the result as the distribution of the trees given the data. The Markov Chain Monte Carlo methods draw a random sample from the posterior distribution of trees and can make probability statements about the true tree (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003; Felsenstein 2004).

First described by Sanger *et al.* in 1977, dideoxynucleotide sequencing of DNA has changed from local business to large scale production enterprise that requires a specialized infrastructure of robotics, bioinformatics, computer databases and instruments. The costs have dropped very much, especially in the last 5-10 years because of efforts necessary to sequence the human genome (Mardis 2008).

In molecular phylogenetics, we want to infer an underlying tree of relatedness from our gene sequence and its close relatives. The same gene sequences are collected from each taxon of interest and are aligned so that columns represent homologous sites. From that alignment a phylogenetic tree can be inferred. We can expect a higher frequency of correct inference from large numbers of variable, independent characters and larger amount of taxa (Givnish & Sytsma 1997). Phylogenetic trees can be inferred from any single gene or for that matter any DNA sequence, however the evolutionary pathway of any given gene is not necessarily equal to that of the species (Edwards *et al.* 2007; Edwards 2009). Whole genome sequencing would then be the most adequate solution, as we would then be able to compare one entire genome against the other, instead of only a handful of genes. Next generation sequencing has the ability to process millions of sequence reads in parallel. The reads are produced from fragment libraries that have not been subjected to the conventional vector-based cloning and *Escherichia coli* based amplification stages used in the capillary sequencing (Mardis 2008). However the cost of sequencing an entire genome is still quite high and, the computational effort of comparing entire genomes for large species matrices would hamper any study at the moment. A review of how phylogenies are inferred from genes, consequences and pitfalls are discussed in a very useful paper by Edwards (2009).

Several genes have been used in phylogenetic reconstructions in the Orchidaceae family, the most common ones are the nuclear ITS (Pridgeon *et al*. 2001; Koehler *et al*. 2002; Whitten *et al.* 2007; Lubinsky *et al.* 2008; Alvarez-Molina & Cameron 2009; Bateman *et al.* 2009; Chase *et al.* 2009; Salazar *et al.* 2009; van den Berg *et al.* 2009; Russel *et al.* 2010), and the plastid *matK* (Pridgeon et al. 2001; Bogarín 2007; Whitten *et al.* 2007; Salazar *et al.* 2009; van den Berg *et al.* 2009), *rbcL* (Pridgeon et al. 2001; Whitten *et al.* 2007; Alvarez-Molina & Cameron 2009) and, *trnL-trnF* (Koehler *et al.* 2002;

Alvarez-Molina & Cameron 2009; Salazar *et al.* 2009; van den Berg *et al.* 2009; Pinheiro *et al.* 2009). More recently used plastid genes include the *psbA-trnH* intergenic spacer (Lubinsky *et al.* 2008; Chase *et al.* 2009) and the *ycf1* (Chase *et al.* 2009), which have been strongly advised by the University of Florida group as being more variable than *matK* (Neubig *et al.* 2009).

matK is one of the most rapidly evolving plastid coding regions and has consistently shown high levels of discrimination among angiosperm species. Mixed reports have been published regarding the universality of *matK* primers ranging from routine success to more patchy recovery (Doyle *et al.* 1992; Cronn *et al.* 2002; Cuénoud *et al.* 2002; Fazeka*s et al.* 2008; Lahaye *et al.* 2008; Hollingsworth *et al.* 2009). It offers higher resolution, but requires primer universality improvement.

Complemented with the rapidly evolving internal transcribed spacers (ITS) of nuclear ribosomal DNA an even better resolution is obtained. Ribosomal DNA (rDNA) is well-suited for a broad range of phylogenetic studies (Baldwin 1992; Hamby & Zimmer 1992; Baldwin *et al*. 1995; von Balthazar 2000) because the different components of rDNA vary in their degree of conservation (Sun *et al.* 1994). ITS2 is advocated by Koetschan *et al.* (2009) as being useful in phylogenetic analyses, especially for species of the same genus. Some advantages of ITS include the availability of several sets of universal PCR primers working with a large diversity of taxonomic groups, the multicopy structure that facilitates PCR amplification even from herbarium specimens, the moderate size of the ITS (below 700bp) that usually allows amplification and sequencing without internal primers, and frequent provision of enough polymorphic sites for evolutionary studies at the species level (Feliner *&* Rosselló, 2007). However, ITS could also be very problematic due to ITS multicopy structure. It is possible to have different ITS sequences in one individual, making contig building quite complicated. This is especially hampering in the case of hybrids, as two quite different ITS sequences could be obtained as mentioned by Alvarez & Wendel (2003), who strongly advise not to use it. Another reason could be that in an extreme case, fungal DNA could be picked up, for the same ITS primers that were developed by White *et al.* (1990) for fungi have been used for ITS in plants. Hard to visualize, endophytic fungi could be present in the leaves prepared for DNA extraction and picking them up is a real possibility (Freek Bakker pers. comm. 2010).

Inquiry into the *trnH-psbA* intergenic spacer began with Aldrich *et al.* (1988) who showed that indels were prevalent in this region, even between closely related species. It has been shown that this region is very valuable to phylogenetics as it is highly variable compared to *matK* and *trnL-trnF* and has been used to study closely related genera and species and in intraspecific studies. Nevertheless it has proven to be unalignable at higher levels. Its average length is about 465 bp, and this short length provides relatively few overall characters. It has the advantage that it sequences easily and needs few primers for most taxa (Shaw *et al.* 2005).

As for DNA barcoding, Hollingsworth *et al.* (2009) propose *rbcL* + *matK* as the most adequate 2-locus barcode combination for the barcoding of land plants. This because high quality sequences of *rbcL* are easily retrievable across phylogenetically divergent lineages, and it performs well in discrimination tests in combination with other loci. The combination represents a pragmatic solution to a complex trade-off between universality, sequence quality, discrimination and cost (Hollingsworth *et al.* 2009). On the other hand, Kress & Erickson (2007), Chase *et al.* (2007), Hollingsworth *et al.* (2009) and van de Wiel *et al.* (2009) advocate *trnH-psbA* for DNA barcoding, because of its high level of sequence variation in studies separating closely related species, but warned about the large sequence length variation which can interfere with amplification. However barcoding differs from phylogenetic inference in that in the first we wish to encounter unique characters that can set a species aside from others while in the second we wish to find characters that group closely related species together and explain their relationship with other such groups. Gigot *et al.* (2007) disccuss the subject of DNA barcoding of Neotropical Orchidaceae. Hajibabaei *et al. (*2007) discuss more in dept how barcoding can complement taxonomy, phylogenetic reconstruction and population genetics.

In a general sense, having more regions to evaluate gives more information, however each region can evolve with its own rate. Some regions might have similar evolutionary paths, such as *matK* and *rbcL*, as they are both from the chloroplast. It is therefore much more informative to have both nuclear and plastid information. A balance between the actual additional information an extra region gives and the costs in money and time must be considered. A big advantage of using the *matK*, *rbcL* and ITS over the other DNA regions, particularly in studies on the family Orchidaceae family, is that there are already many sequences available in GenBank. Those sequences will enrich this study greatly and are unfortunately not available for the *trnH-psbA* intergenic spacer. Doyle and Gaut (2000) discuss the topic of the relationship between the evolution of genes and taxa.

Further information on molecular evolution and phylogeny inference can be found in detail in a compilation by Salemi & Vandamme (2003), “The phylogenetic handbook. A Practical Approach to DNA and Protein Phylogeny”. Further information about phylogenetics in the Angiosperms is given by Soltis *et al*. (2005), “Phylogeny and Evolution of Angiosperms”. A very complete and useful compilation of the available software and online data bases is given by J. Dear (2007) in “Bioinformatics: Methods Express”.

1.3. The Present Study

The alternative taxonomic systems (i.e., the fine generic splitting by Luer and the more conservative approach proposed by Pridgeon; Table 1) are still vigorously debated among taxonomists. While some of the "lumped" genera, like *Condylago* and *Salpistele,* are generally *maintained* in usage (i.e., Pupulin 2003, Dressler & Bogarín, 2007), others, like *Crocodeilanthe*, *Dracontia, Effusiella, Elongatia, Lomax*, *Loddigesia, Mystacorchis, Niphantha, Physothallis, Physosiphon and Unciferia* are not commonly used. The concept of *Stelis,* in a broad sense, is in general more accepted (Pupulin 2002; Soto Arenas 2003; Soto Arenas & Solano 2003a, 2003b, 2003c; Pridgeon 2005; Solano-Gomez & Salazar 2007; Govaerts *et al.* 2010).

The uncertainties in the use of a common systematic model are mainly due to the sampling size used in the previous molecular study. Less than 1% of the species belonging to the broad concept of *Stelis* were actually sequenced, even missing species from some of the segregated genera. Additionally, molecular and morphological data were not coupled, rendering *Stelis* in its broad sense a genus almost impossible to define with no clearly distinguishing morphological characters.

The main purpose of this study is to demonstrate and explain the evolutionary relationships between the diverse species groups (or genera) within the *Stelis s.l.* concept. To do so, not only is the number of species of *Stelis* evaluated larger, but also most of the species groups (several already recognized as separate genera) found in the broad concept of the genus are covered. The importance of this study is that it will combine molecular data (from a larger source than previous analyses) with the morphological and functional characters that are linked to the emerging evolutionary paths.

Secondarily, the data will help to assess the effectiveness of the use of molecular techniques within this particular group. Pertinent taxonomic conclusions are discussed for each of the involved taxa, suggesting modifications where needed, and some little known species of the group were collected, documented, preserved, drawn and photographed. Finally, the results obtained will be correlated to available geographical data.

A phylogentic analysis including a larger number of species of *Stelis s.l.* was presented by Solano-Gomez (2005) in the World Orchid Conference, however, it only included ITS sequences and morphological data, and the phylogeny was reconstructed using parsimony. No data or confidence values were ever published and sequences were never added to GenBank.

1.3.1. Main Hypothesis

> "The classification system presented by Pridgeon *et al.* 2001 for the genus *Stelis* makes it a natural and monophyletic group. It takes into consideration molecular data, it is capable of assigning morphological characteristics to the relationships found and relates the result with geographical evidence."
>
> *Alternative*: "The classification system presented by Pridgeon *et al.* 2001 for the genus *Stelis* does not make it a natural and/or monophyletic group, is not capable of assigning any clear morphological trends within the groups and is not supported by any geographical considerations."

1.3.2. Secondary Hypotheses

1. Hypotheses: "The genera *Dracontia*, *Elongatia*, *Effusiella* and *Unciferia* recently proposed by Luer (2004; 2006) represent monophyletic and natural groups."

   Alternative: "The genera *Dracontia*, *Elongatia*, *Effusiella* and *Unciferia* recently proposed by Luer are paraphyletic or polyphyletic and do not represent natural groups."

2. Hypotheses: "No geographical patterns can be found correlated with the phylogeny of the *Stelis s.l.* group."

   Alternative: "The *Stelis s.l.* group presents clear geographical trends which correlate to the phylogenetic relationships. They are especially related to uplift of large mountain ranges and presence of vast lowlands."

3. Hypotheses: "Lineage size and number among the clades in the *Stelis* s.l. grows in a constant manner."

   Alternative: "A large increase in the number of species is found in the *Stelis* s.s. group, linked to morphological modifications and geographical patterns, suggesting adaptive radiation."

2. Materials and Methods

2.1. Field Work

Field work was carried out during two trips to Costa Rica for a total period of 4 months. Several field trips were done, most were short one day visits to close by areas, but a few remote areas were also visited for longer time periods. The objective of those field trips was the observation, documentation and collection of plant and flower material. Most of the time, however, was dedicated to working with the living plant collection at the Lankester Botanical Gardens where most of the plants of interest were already in cultivation.

2.1.1. Species Selection

Species belonging to the genus *Stelis* (*sensu* Pridgeon *et al.* 2001) were selected among the taxa cultivated in the living collection of Lankester Botanical Garden (LBG), University of Costa Rica, on the basis of availability and inter-specific variability. At least one sample from each of the genera, subgenus or artificial grouping (Table 1 & 2) accepted in the two alternative classification systems was included in the sampling when available. Most of the species included in the sampling are Costa Rican in distribution, reflecting the nature of the LBG collections. Nomenclature follows Govaerts *et al.* (2010). The complete list of accessions is given in Appendix II.

Table 1. List of botanical names applicable to the individuals belonging to *Stelis s.l.* that have been included in the phylogenetic analysis presented here. Showing the two confronting classification systems for the group.

Pridgeon *et al.* 2001/ Govaerts *et al.* 2010	**Luer 2004/ Luer 2006/ Luer 2007**
Anathallis dolichopus (Schltr.) Pridgeon & M.W. Chase	*Specklinia dolichopus (Schltr.) Luer*
Stelis adrianae (Luer & Sijm)	*Salpistele adrianae Luer & Sijm*
Stelis alajuelensis Pridgeon & M.W. Chase	*Dracontia ramonensis (Schltr.) Luer*
Stelis alta Pridgeon & M.W. Chase	*Dracontia grandis (Rolfe) Luer*
Stelis argentata Lindl.	*Stelis argentata Lindl.*
Stelis atroviolacea Rchb.f.	*Stelis atroviolacea Rchb.f.*
Stelis brunnea (Dressler) Pridgeon & M.W. Chase	*Salpistele brunnea Dressler*
Stelis carnosilabia (Heller & Hawkes) Pridgeon & M.W. Chase	*Dracontia carnosilabia(Heller & Hawkes) Luer*
Stelis carpinterae (Schltr.) Pridgeon & M.W. Chase	*Elongatia carpinterae(Schltr.) Pridgeon & M.W. Chase*
Stelis ciliaris Lindl.	*Stelis ciliaris Lindl.*
Stelis cobanensis (Schltr.) Pridgeon & M.W. Chase	*Dracontia cobanensis (Schltr.) Luer*
Stelis convallaria (Schltr.) Pridgeon & M.W. Chase	*Effusiella convallaria (Schltr.) Luer*
Stelis crystallina Ames	*Stelis crystallina Ames*
Stelis despectans Schltr.	*Stelis despectans Schltr.*
Stelis dracontea (Luer) Pridgeon & M.W. Chase	*Dracontia dracontea (Luer) Luer*
Stelis dressleri Luer	*Stelis dressleri Luer*
Stelis emarginata (Lindl.) Soto Arenas & Solano	*Physosiphon emarginatus (Ruiz & Pavon) Lindl.*

Stelis aff. ferrelliae Pridgeon & M.W. Chase	*Dracontia aff. ingramii (Luer) Luer*
Stelis gelida(Lindl.) Pridgeon & M.W. Chase	*Niphantha gelida (Lindl.) Luer*
Stelis gemma Garay	*Stelis gemma Garay*
Stelis glossula Rchb.f.	*Stelis glossula Rchb.f.*
Stelis gigantea Pridgeon & M.W. Chase	*Dracontia powellii (Schltr.) Luer*
Stelis guatemalensis Schltr.	*Stelis guatemalensis Schltr.*
Stelis guttata (Luer) Pridgeon & M.W. Chase	*Elongatia guttata (Luer) Luer*
Stelis harlingii (Garay) Pridgeon & M.W. Chase	*Physothallis harlingii Garay*
Stelis immersa (Linden & Rchb.f.) Pridgeon & M.W. Chase	*Effusiella immersa (Linden & Rchb.f.) Luer*
Stelis imraei (Lindl.) Pridgeon & M.W. Chase	*Effusiella imraei (Lindl.) Luer*
Stelis janetiae (Luer) Pridgeon & M.W. Chase	*Elongatia janetiae (Luer) Luer*
Stelis lanata Lindl.	*Stelis lanata Lindl.*
Stelis listerophora (Schltr.) Pridgeon & M.W. Chase	*Effusiella listerophora (Schltr.) Luer*
Stelis maculata Pridgeon & M.W. Chase	*Salpistele lutea Dressler*
Stelis megachlamys (Schltr.) Pupulin	*Dracontia tuerckheimii (Schltr.) Luer*
Stelis aff. microchila Schltr.	*Stelis aff. microchila Schltr.*
Stelis morae Luer	*Stelis morae Luer*
Stelis mystax (Luer) Pridgeon & M.W. Chase	*Mystacorchis mystax (Luer) Szlach. & Marg.*
Stelis papillifera (Rolfe) Pridgeon & M.W. Chase	*Dracontia papillifera (Rolfe) Luer*
Stelis pilosa Pridgeon & M.W. Chase	*Effusiella amparoana (Schltr.) Luer*
Stelis pompalis (Ames) Pridgeon & M.W. Chase	*Unciferia pompalis (Ames) Luer*
Stelis aff. pulchella Kunth	*Crocodeilanthe aff. pulchella (Kunth) Luer*
Stelis quadrifida (Lex.) Solano & Soto Arenas	*Loddigesia quadrifida (Lex.) Luer*
Stelis resupinata (Ames) Pridgeon & M.W. Chase	*Effusiella resupinata (Ames) Luer*
Stelis rodrigoi (Luer) Pridgeon & M.W. Chase	*Condylago rodrigoi Luer*
Stelis segoviensis (Rchb.f.) Pridgeon & M.W. Chase	*Unciferia segoviensis (Rchb.f.) Luer*
Stelis standleyi Ames	*Stelis standleyi Ames*
Stelis tacanensis Solano & Soto Arenas	*Physosiphon tacanensis (Solano & Soto Arenas)**
Stelis velaticaulis (Rchb.f.) (Rchb.f.) Pridgeon & M.W. Chase	*Crocodeilanthe velaticaulis (Rchb.f.) Luer*

*The combination has not been done, but would apply under the generic concept.

Table 2. Total known species number per genus within *Stelis s.l.*, adding the percentage of species of each of those genera that have been used in the previous and current phylogenetic studies of the group.

Genus*	**Total Species Number****	**Pridgeon *et al.* 2001**	**Karremans 2010**
Crocodeilanthe	70	1%	3%
Niphantha	2	0	50%
Physosiphon	4	25%	50%
Physothallis	2	50%	50%
Ple. Acum. Acuminatia	27	0%	4%
Stelis s.s	900	0.1%	2%
Dracontia	17	6%	60%
Salpistele	5	0%	60%
Ple. Elong. Petiolatae	2	0%	100%
Ple. Elong. Elongatia	8	0%	13%
Mystacorchis	1	0%	100%
Condylago	2	50%	50%
Unciferia	10	10%	20%
Effusiella	41	2.5%	15%
Total (Stelis s.l.)	**1,091**	**0.7%**	**5%**

*Genera names following Luer´s system in order to visualize coverage of possible naturally distinct groups.
**Number of species follows that given by Govaerts *et al.* 2010.

2.1.2. Documentation

The selected species and individuals were documented during the field work period in Costa Rica. Flowers were photographed, drawn with the aid of a stereomicroscope fitted with a drawing tube, and preserved in the spirit collection at LBG (Appendix XX & XXI). Duplicates of several specimens of the spirit collection were deposited in the Herbarium Vandense, branch of the Nationaal Herbarium Nederland. Dried specimens prepared from the vouchers were deposited at the Herbarium of the National Museum of Costa Rica (CR). Photographs and drawings were kept in the LBG´s databases. Field work was done in order to recollect any other species of interest that was not present in the collections. The data collected was stored in the various data bases of the Lankester Botanical Gardens, the information present in most of them has been combined online and is available at *www.epidendra.org*.

2.1.3. Sample Collection

Specimens were collected from plants in cultivation at the LBG in Costa Rica. Fresh leaf and flower cuttings of about 1 cm^2 were taken from all the selected individuals of each species. Each individual sample was put into a polypropylene bag with silica gel to dry for about a week after which the silica was removed and new dry silica was added.

2.1.4. Scoring Morphology

A list of morphological features found in Pleurothallidiniae and potentially useful for phylogenetic reconstruction was compiled by researchers at LBG (Pupulin pers. comm. 2010). It initially included 70 potentially useful characters which were selected based on their previous use in morphological treatments of the group, especially those of Luer (1986; 1987; 1991; 1994; 1998a; 1998b; 1999; 2000; 2007). In the scope of the present study, the character list was modified to fit criteria useful for *Stelis,* and each species was characterized morphologically and scored in a matrix. The full list of used characters and evaluated species is found further in the text when morphology is discussed.

2.2. Laboratory Work

All laboratory work intended for molecular analyses was done at the Plant Sciences Department in the Radix building of Wageningen University and Research Centre. The complete list of accessions used in different stages is given in Appendix II).

2.2.1. DNA Extraction

Before DNA extraction, samples were pulverized by taking 20 mg of each from the polyethylene bags and reallocating them to a small *Eppendorf* container. Three "bullets" were then added to each container and were submerged in a liquid nitrogen solution. After samples had crystallized they were pulverized by shaking them violently in a *Retsch MM* 300 shaker for 5 min. Pulverized material was stored for one night at -80°C in a common freezer.

Extraction was done following the *QUIAGEN* (*DNEasy*) extraction protocol, except for the tissue rupture procedure which was explained above. DNA concentration for each sample was determined using a Nano Drop Spectrophotometer (ND 1000), by making a taration with water followed by one with buffer AE. Analysis of the 2 µl drop of DNA from each sample was done with the ND1000 v3.3.0 program. A concentration of 10 µmol/l of DNA was used from here on. When needed, samples were diluted in MQ water or dehydrated to obtain the required concentration.

2.2.2. Primer Selection

ITS was amplified using the methods and primers described by Sun *et al.* (1994), who used 17SE and 26SE for sequencing and amplification, and 5.811 and 5.812 for sequencing. The 5.8S and 5.8R primers were not used. These primers amplify ITS*1*, ITS*2* and the 5.8S ribosomal gene. *trnH-psbA* was amplified and sequenced using the primers trnH-psbA_1F and trnH-psbA_1R as described by Kress & Erickson (2007) and van de Wiel (2009). *matK* was amplified and sequenced using the Kew *matK* primers 1.1F and 5R (Table 3).

Table 3. PCR primers used for amplification of plastid and nuclear DNA sequences in this study.

Region	Primer Name	Primer Sequence (5´-3´)
ITS	17SE	ACGAATTCATGGTCCGGTGAAGTGTTCG
ITS	26SE	TAGAATTCCCCGGTTCGCTCGCCGTTAC
ITS	5.8I1	GTTGCCGAGAGTCGT
ITS	5.8I2	GCCTGGGCGTCACGC
trnH-psbA	trnH-psbA_1F	ACTGCCTTGATCCACTTGGC
trnH-psbA	trnH-psbA_1R	CGAAGCTCCATCTACAAATGG
matK	2.1aF	ATCCATCTGGAAATCTTAGTTC
matK	5R	GTTCTAGCACAAGAAAGTCG

2.2.3. Amplification

Amplification was done by preparing each sample with a PCR mix composed of DTB, dNTP, both primers (four in the case of ITS), Dream Taq, Water and the extracted DNA (Table 2). Samples were amplified in a *MJ Research PTC-200 Pelthier Thermal Cycler*, using a cycle of 94°C/5 min, followed

by 35 times 94°C/30 s, 55°C/30 s and 72°C/2 min, and finally 72°C/10 min. From the resulting samples of 20 µL, 10 µL were mixed with 1 µl/10 µl of loading buffer and allocated to a slot in a gel. A 1% gel was prepared by dissolving 1 g of agarose in 100 ml of TAE and letting it heat up and then cool down in a gel plate. The gel was run in a *Shu13 Horizon® 11-14* gel box for about 60 min. To determine whether the bands are the ones expected, a ladder (marker) was used. The bands were observed by removing the gel from the bath and illuminating it in the *Ωctopus F* apparatus.

Table 4. Mix used for DNA amplification with the PCR technique.

	µl/sample
DTB	2.0
dNTP	0.4
F-Primer	2.0
R-Primer	2.0
Taq	0.08
Water (MQ)	11.52
DNA	2.0

2.2.4. Sequencing

To prepare for sequencing, a mega-mix composed of Dett, a single primer, water and the amplified DNA of one sample were prepared (Table 3). Each sample had two mega-mixes, one for the F-primer and another for the R-primer (four for ITS). Samples are allocated to a PCR plate (2 slots per sample, four for ITS) and were once more amplified using the same procedure mentioned before, but using a cycle of 94°C/20 s, 50°C/15 s and 60°C/1 min repeated 25 times. The final steps of sequencing were done by the Greenomics Company at WUR using their protocols.

Table 5. Mega-Mix used for DNA amplification and sequencing.

	µl/sample
Dett	4
Primer	1
Water (MQ)	4
DNA	1

2.3. Building the data sets

2.3.1. Sequence Editing

The *STADEN* (Staden 2003) package was used for editing the sequences. Contig were assembled using the sequence traces from the ABI (one forward and one reverse in the case of *matK* and *trnH-psbA*, and two forward and two reverse in the case of ITS) with the *Pregap* v1.5 program, parameters were kept as preset default, except for, minimum exact match set to 14, percentage of mismatch set to 30%, and interactive clipping, used to clip off the unclear ends of the tracers. When tracers were too short or had too much missing data, mismatch percentage was changed to 75%. The contig was then manually checked using *Gap4* v4.10, template correct directionality was revised (all forwards going from left to right and all reverse from right to left), missing data and misinterpretations were revised by looking at the trace display, modifying them if necessary, but trying to be conservative in deciding the correct base pair. Where more than one base pair was equally probable, the Unicode nomenclature was used.

In a few cases the two tracers for one sample were too short and there was no overlap. In those cases *Pregap* was unable to build a Contig. In order to keep the information, both sequences were opened in the program *Mesquite* v2.72 (Maddison & Maddison 2007) and aligned with a reference sequence (any complete sequence from a close relative). The sequences were then merged and N´s were used to fill in the missing data.

2.3.2. Sequence Alignment

Sequences, for each region independently, were aligned using *Clustal X* in *BioEdit* v7.5.0.3. (Hall 1993). They were then exported as a *.fas* file and opened in the *Mesquite* v2.72 program were they were checked for misalignments and edited manually. The ends of each data set were trimmed to eliminate possible erroneous data and gaps at the ends of sequences were regarded as missing data (filled in with N´s). Each *indel* and possible informative sites were re-checked by going back to the original traces.

After the data sets had been aligned and edited, additional sequences were taken from GenBank. To determine which sequences would be useful to complement the data set, one of the sequences from the original data set was tested against the stored sequences using *BLAST*. The highest scoring sequences determined as *Pleurothallis* or *Stelis* were then included by choosing the "fetch sequence" option in *Mesquite*.

2.4. Analyzing the data Sets

Various analyses were done to the two final data sets (a *trnH-psbA* data set was not produced, as discussed in the results). *Myoxanthus uncinatus* AF265478 (now called *Echinosepala uncinata* (Fawc.) Pridgeon & M.W. Chase) was used as out-group in all cases, as it is suggested to be the furthest related of all included species (Pridgeon *et al.* 2001).

2.4.1. Parsimony

The basis of this type of analysis is looking for the minimum net amount of evolution, in other words seeking the phylogeny which explains the data with least amount of events as possible (Felsenstein 2004), under the assumption that in nature it will also be so. The *TNT* software (Tree Analysis Using New Technology) is one of the programs based on parsimony analysis. *TNT* v1.1 (Goloboff *et al.* 2008) was used here as a complement to other analyses. Jackknifing was done using the resample option to estimate empirically the variability of the estimates.

2.4.2. Maximum Likelihood

An analysis was done with RAxML-III as a complement to the Bayesian, it was used to infer trees for both *matK* and ITS complete data sets. It utilizes a heuristic search method to make processing time shorter for large data sets. Its output is a most likely (best tree) with likelihood values for nodes and branches, it presents an accurate and fast alternative (Stamatakis *et al.* 2005).

2.4.3. Bayesian Analysis

MrBayes version 3.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) was used to get a distribution of possible gene trees which are summarized in a consensus tree with posterior probability values for each node. Parameters chosen were preset, except for lset nst = 6 and temp = 0.05 for all data sets. Both *matK* and ITS were tested without partitions, however, *matK* was also analyzed with a partition based on the codon position, 1, 2 or 3 (Table 4). Gaps were very small and scarce and therefore were treated as missing data. The ITS and *matK* un-partitioned analysis were set to gamma rates and *matK* partitioned to invgamma (GTR substitution matrix plus Gamma distribution). A combined analysis was done where partitions were set for each gene. It is because of the ability of giving probabilities to clades, in a relatively manageable time frame, and with high confidence results, that, Bayesian inference is here preferred over other analyses.

Table 6. Details of the diverse analyses done on each of the individual regions' data sets and their concatenated data set.

Region	Data Set (sequences)*	Analysis	Generations	Partitions	Refer to
ITS	Complete (92)	Parsimony	-	-	Appendix VII
ITS	Complete (92)	Bayesian	5,600,000	No	Figure 3
ITS	Complete (92)	RAxML	-	No	Appendix VI
ITS	No *S. rodrigoi* (91)	Bayesian	-	No	Appendix V
ITS	No *A. dolichopus* (91)	Bayesian	-	No	Appendix V
matK	Complete (58)	Parsimony	-	-	Appendix IV
matK	Complete (58)	Bayesian	15,000,000	No	Appendix III
matK	Complete (58)	Bayesian	8,533,000	Yes (3, one for each codon position)	Figure 2
matK	Complete (58)	RAxML	-	No	Appendix III
ITS+*matK*	Complete (96)	Bayesian	3,066,000	Yes (2, one for each gene)	Figure 4

* Most analyses were carried out on the complete data sets, in order words with all the available sequences, however two analyses for the ITS matrix were done with the removal of one sequence.

2.4.4. Testing the model

Methods of phylogenetic inference depend on their underlying models. In order to trust the results of the analysis the model must be trusted, therefore one must explore which explicit evolution model fits the data best. *MODELTEST* is a program designed to compare different nested models of DNA substitution in a hierarchical hypothesis-testing framework (Posada & Crandall 1998). Both ITS and *matK* complete data sets were analyzed using the *Find Model* web server (available at http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html) which uses *MODELTEST* to calculate the model scores. In both cases the GTR + Γ (gamma) model was the most likely to fit the data best. It was therefore used in all Bayesian analyses.

2.4.5. Concatenation

It is generally considered that concatenating genes can improve the power of an analysis, the assumption being that analysis of a concatenated data set provides a phylogeny that is closer to a species tree. However concatenation can lead to misleading phylogenies, especially when discord is found between the individual gene trees (Edwards *et al.* 2007; Kubatko & Degnan 2007). In the concatenated data set, ITS sequences are directly followed by the *matK* sequence. A Bayesian analysis is done on the data, with a partition for ITS, and another for *matK*, allowing different substitution models for each. Clades were described following all results obtained, but, especially considering those from the Bayesian analysis of the concatenated matrix.

The *.con* files produced by the Bayesian analysis from the concatenated matrix (as well as all others) were loaded into *FigTree* v1.3.1 with which the trees were visualized. PP values wereadded to the nodes of the trees using the labeling option. Branches were reorder for better appreciation. All tree files presented here were exported from *FigTree* (Rambaut 2009).

2.4.6. Consensus Networks

A central task in evolutionary biology is the construction of phylogenetic trees, most of the time a collection of trees is produced rather than a single optimal tree. Normally a consensus tree is built from the collection; however, a consensus tree does not carry the information about conflicting hypotheses. A consensus network in a way summarizes all (or most) of the possible trees resulting from one data set, "it extends the notion of strict and majority consensus trees to allow the display of conflicting evolutionary hypotheses within a collection of trees" (Holland & Moulton 2003; Holland 2005). When calculating the posterior probabilities, in MrBayes for example, the program actually produces a distribution of possible trees with several alternative explanations for the same data. In the consensus network all the alternative explanations above a certain threshold are included in a three dimensional multi branched network, giving much more information than the two dimensional two branched tree.

A number of trees obtained from the Bayesian analyses of the complete ITS, partitioned *matK*, and the combined ITS + *matK*, data sets, were all analyzed separately in the *Splits Tree4 v4.11.3* program in order to produce consensus networks (Huson & Bryant 2006).

2.4.7. Quality of the Analyses

The *Tracer* v1.5 program (Rambaut & Drummond 2007) was used to revise the quality of each of the Bayesian runs. The *.p* files produced by MrBayes were loaded in the program and compared. Log likelihood, for example, can be compared between equal data sets such as the *matK* partitioned and unpartitioned analyses, were a likelihood closer to cero is a "better" one. It can also help to compare between two Bayesian runs of each analyses, which should be more or less equal. ESS (effective sampling size) values are given for sampling size of each run which indicates whether sampling was enough or not (red values are indicate insufficient sampling). It is also possible to view the trace, which looks like a condense brush-like figure when the analysis runs "normally". Bayesian factors can be calculated in *Tracer*, they represent a statistical way of measuring whether the assumptions about probabilities are maintained and if the priors truly correlate to the posteriors. They are the best way of comparing different Bayesian analyses from different data sets (Appendix VIII – X).

2.4.8. Morphological Matrix

Even though it is possible to use the data from the morphological matrix to produce a phylogeny, it has was not done here, the morphological matrix was actually used to explain the evolutionary pathways that were been found in the molecular phylogeny. From the latter it was possible to determine which characters are ancestral and which derived in the group, it was also possible to indicate cases of morphological convergence and parallelisms.

Ancestral states were reconstructed using the *Mesquite* v2.72 program by doing a parsimony analysis on the data. To do so, the morphological matrix was used as input, and character and their states were labeled accordingly. The consensus tree resulting from the Bayesian analysis on the concatenated data was used to plot the character history.

2.4.9. Geographical Trends

Geographical data has was recorded for all the accessions used here, however, general known distribution for all the species in the analysis can be found in Govaerts *et al* (2010). That information was related with the results found in all other analyses in order to spot geographical trends.

Ancestral States were reconstructed using that data for several of the nodes in the produced trees. In order to do so, geographical distribution was added to the morphological matrix as a character, were character states 1 through 5 represent the diverse geographical areas from Mexico through Bolivia. Geographical characters were also analyzed using *Mesquite* by a parsimony analysis. It was assumed that the ancestral character for a node (in this case a place) would be the geographical origin for that node. However, a bias might be present in the data as almost all species have Costa Rica in their distribution.

3. Results

3.2. Phylogenetic Reconstruction

3.1.1. DNA Extraction and Amplification

The concentration of DNA among samples ranged from 5 to 100 ng/µl. Amplification of the selected DNA regions was successful for most samples. In particular, the ITS region was amplified easily and consistently. Samples that failed to amplify were retested several times, some by re-sampling, re-extracting and/or varying concentrations of DNA and Taq. After all the tests, for all three regions only a few species (or accessions) consistently failed to amplify, *Condylago furculifera* (Samples 2A and 1B), *Stelis segoviensis*, *S. aff. segoviensis*, *S. pilostoma* and *S. canae* (all belonging to clade Unciferia) were never successfully amplified. *Stelis standleyi* and *S. imraei* were not successfully amplified for the region *matK*.

3.1.2. Analysis of ITS

ITS gave the largest percentage of informative characters, having also the highest resolution, number of clades and clade support. After clipping the data set was 848 base pairs (bp) long. It was also the most complete in species number, with 92 included sequences from 70 different species. A few very short sequences were present in the data set: *Stelis dracontea* DB616a (206bp) *S. gelida* AK2481 (342bp) *S. pompalis* DB6516a (227bp) and *S. pompalis* DB6516b (227bp), all the other taxa presenting sequences above 500bp. The sequence of *S. dracontea* was taken out because it created great disturbance in the consensus network having no clear relatives and did not group with the other sequence of *S. dracontea* taken from the same sample (DB616b). The other three short sequences did not create great disturbance in the consensus network and *S. gelida* grouped with the other sequence from *S. gelida* DB622), while both *S. pompalis* grouped together and in the same clade as their closest relative *S. segoviensis* (AF262866).

Three different ITS data sets were analyzed with MrBayes and each one of the resulting consensus tree was presented. The reason for the multiple data sets is that when the complete data set (92 sequences) is analyzed, sequences from *Stelis rodrigoi* and from *Anathallis dolichopus* group together in a clade related to *Stelis s.s*. (Figure 3). However when the *A. dolichopus* sequence is removed, *S. rodrigoi* moves to a basal polytomy, next to *S. imraei*. Posterior probabilities (PP) values and several parts of the tree morphology were also modified. When on the other hand *S. rodrigoi* is removed from the data set (both resulting trees are shown in Appendix V), *A. dolichopus*

and *S. imraei* remain in the same position as in the complete data set, but still PP values were modified.

3.1.3. Analysis of *matK*

matK gave a lower resolution than ITS, with fewer and less supported clades. After editing, the data set was 784 bp long. Less species were included than in ITS because a lower number of sequences were obtained and less previously sampled taxa are found in GenBank. 58 sequences were included from 44 different species. Excluded species (accessions) were those that presented too short and/or messy sequences, including all species belonging to the clades *Unciferia* (*S. aff. canae*, *S. pilostoma*, *S. aff. segoviensis* and *S. segoviensis,* but not *S. pompalis*), *Condylago* (*C. furculifera*), Umbraticola (*S. imraei*) and other species such as *S. dracontea*, *S. aff. ferrelliae* and *S. standleyi*.

matK was analyzed in MrBayes using a partitioned data set. As it is a protein coding gene, it is possible that the evolutionary rate of the base pair in the third position is faster as its constraint is more relaxed than the first and second positions. The resulting consensus tree (Figure 2) was compared with one without any partition (Appendix III), and showed more resolution and higher PP values.

3.1.4. Analysis of *trnH-psbA*

The tracers were many times too short or of low quality to be able to build adequate contigs from them. For several species contigs were built successfully, but even so, satisfactory alignment was not possible due to high divergence between some species, the amount of gaps and the difference in the size of the contig.

3.1.5. Concatenated Analysis

The concatenated tree actually has similar topology as that found for the ITS data, and confirms the position of the clade *Condylago,* diverging close to the base of group instead of being closely related to *Stelis s.s*. Even though the topology of *matK* differs much more from the combined tree, many clades can still be found and the ones that are not present are in most cases pretty close to them.

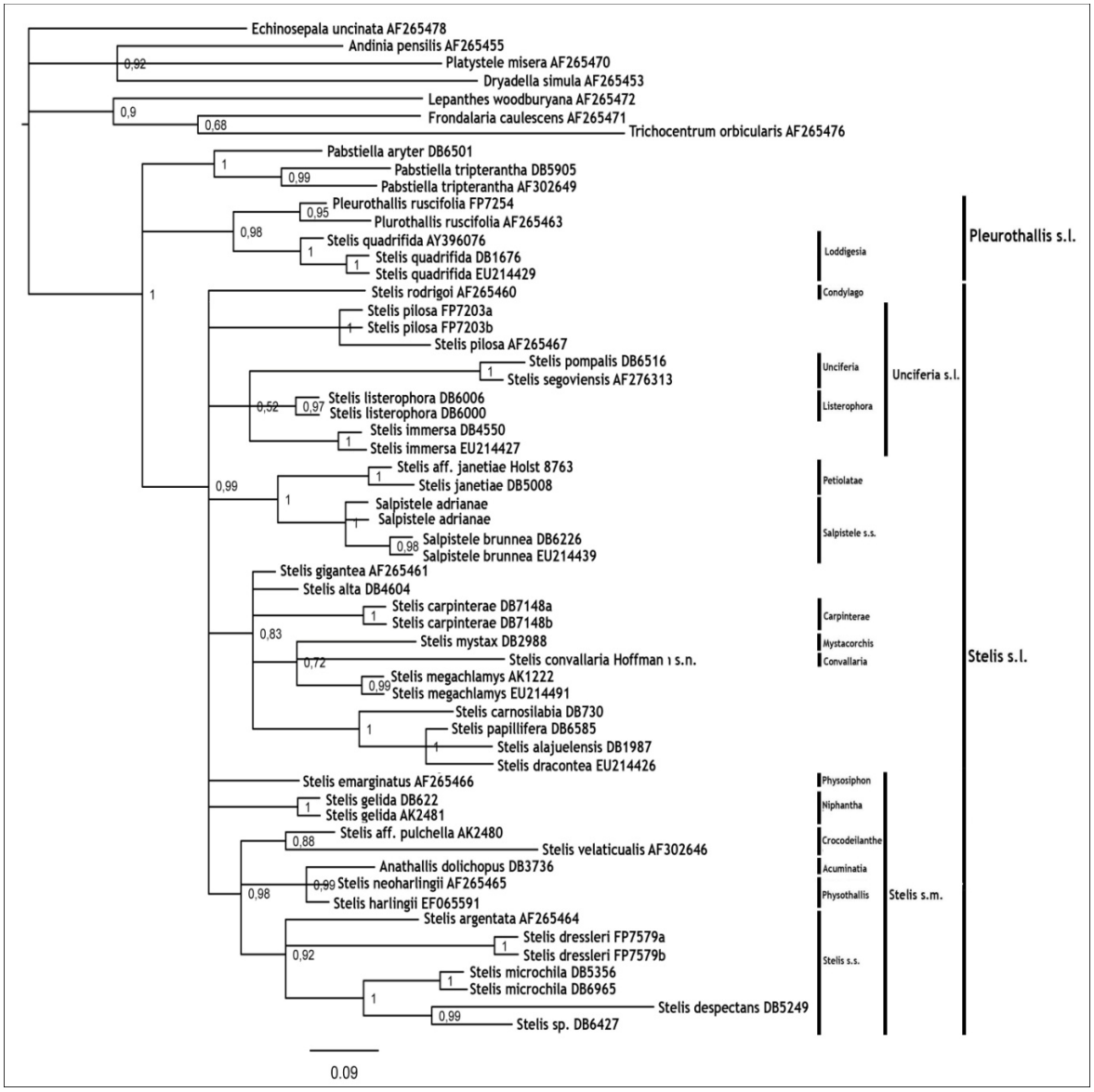

Figure 2. Consensus tree from a Bayesian analysis of a matrix of 58 *matK* sequences after 8,533,000 generations, with three partitions, one for every codon position. Values on the nodes are Posterior Probabilities. The clades proposed in this documented are added to it (clade circumscription is discussed later in the text).

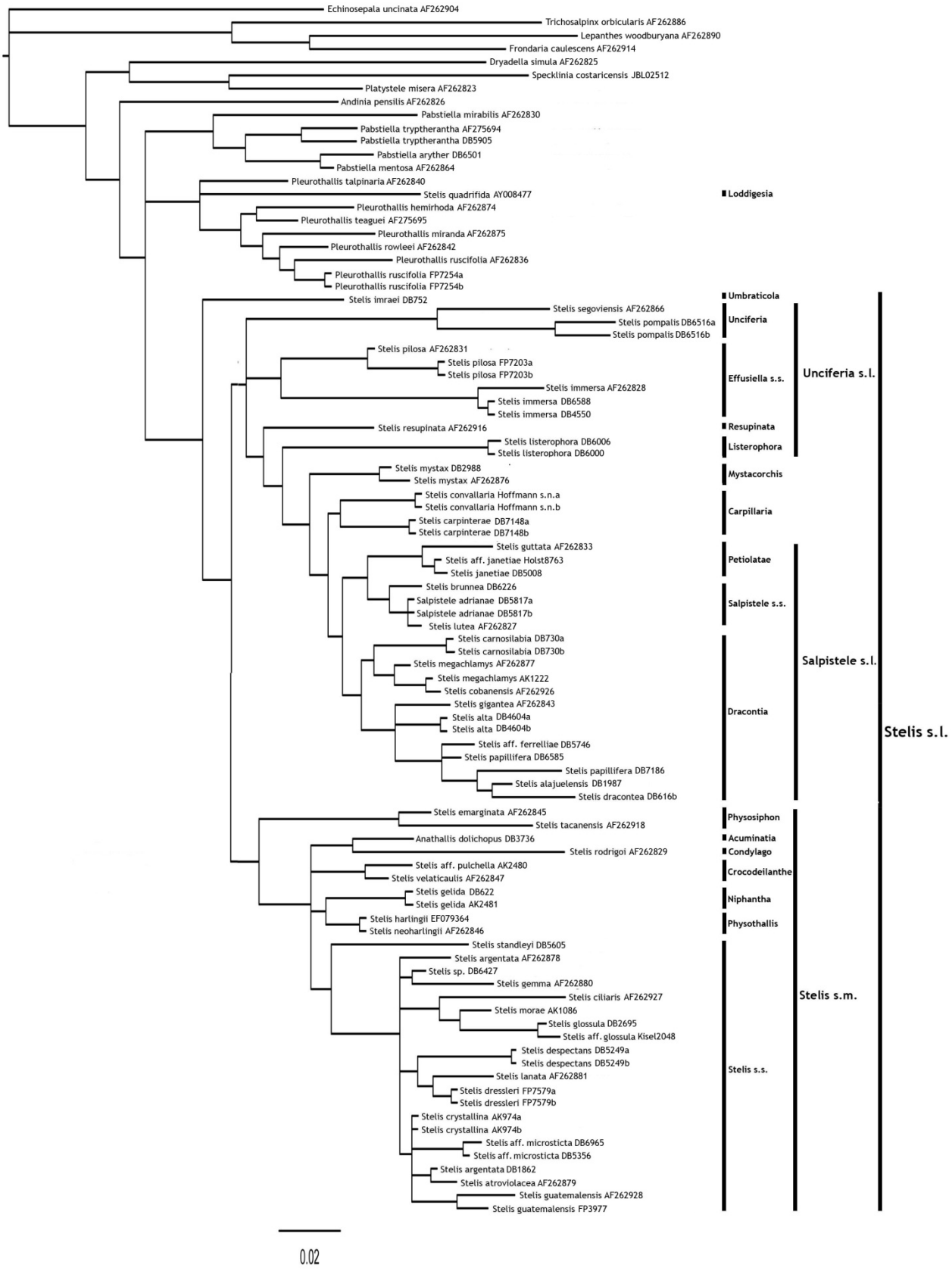

Figure 3. Consensus tree from a Bayesian analysis of a matrix of 92 ITS sequences after 5,600,000 generations. It is unpartitioned. Values on the nodes are Posterior Probabilities. The clades proposed in this documented are added to it (clade circumscription is discussed later in the text).

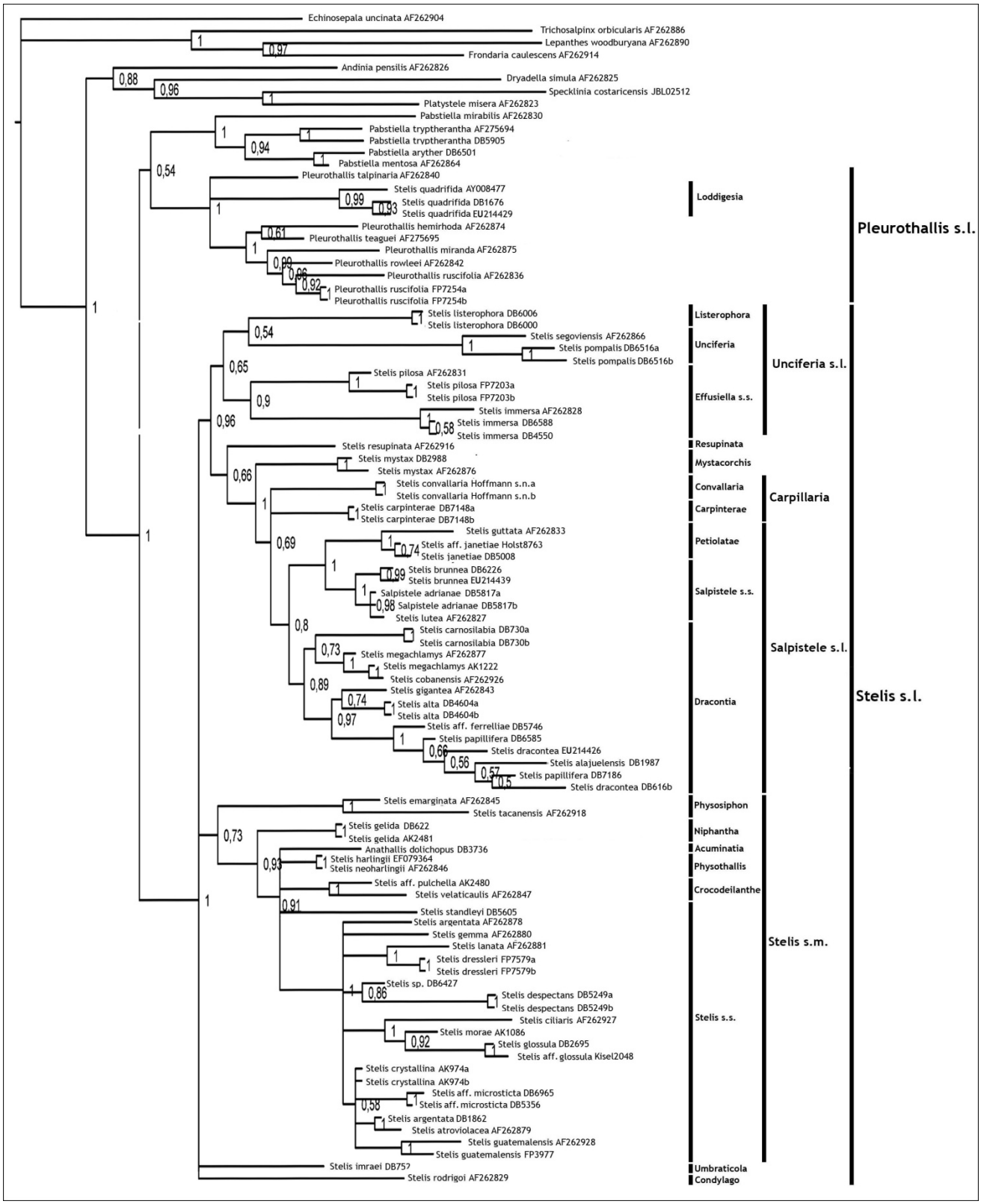

Figure 4. Consensus tree from a Bayesian analysis of a concatenated matrix of 92 ITS and 56 *matK* sequences for a total of 96 combined sequences of which some had both and others had one of the two. Partitions were set for each gene. The analysis ran for 3,066,000 generations. Values on the nodes are Posterior Probabilities. The clades proposed in this documented are mostly based on the morphology of this tree (clade circumscription is discussed later in the text).

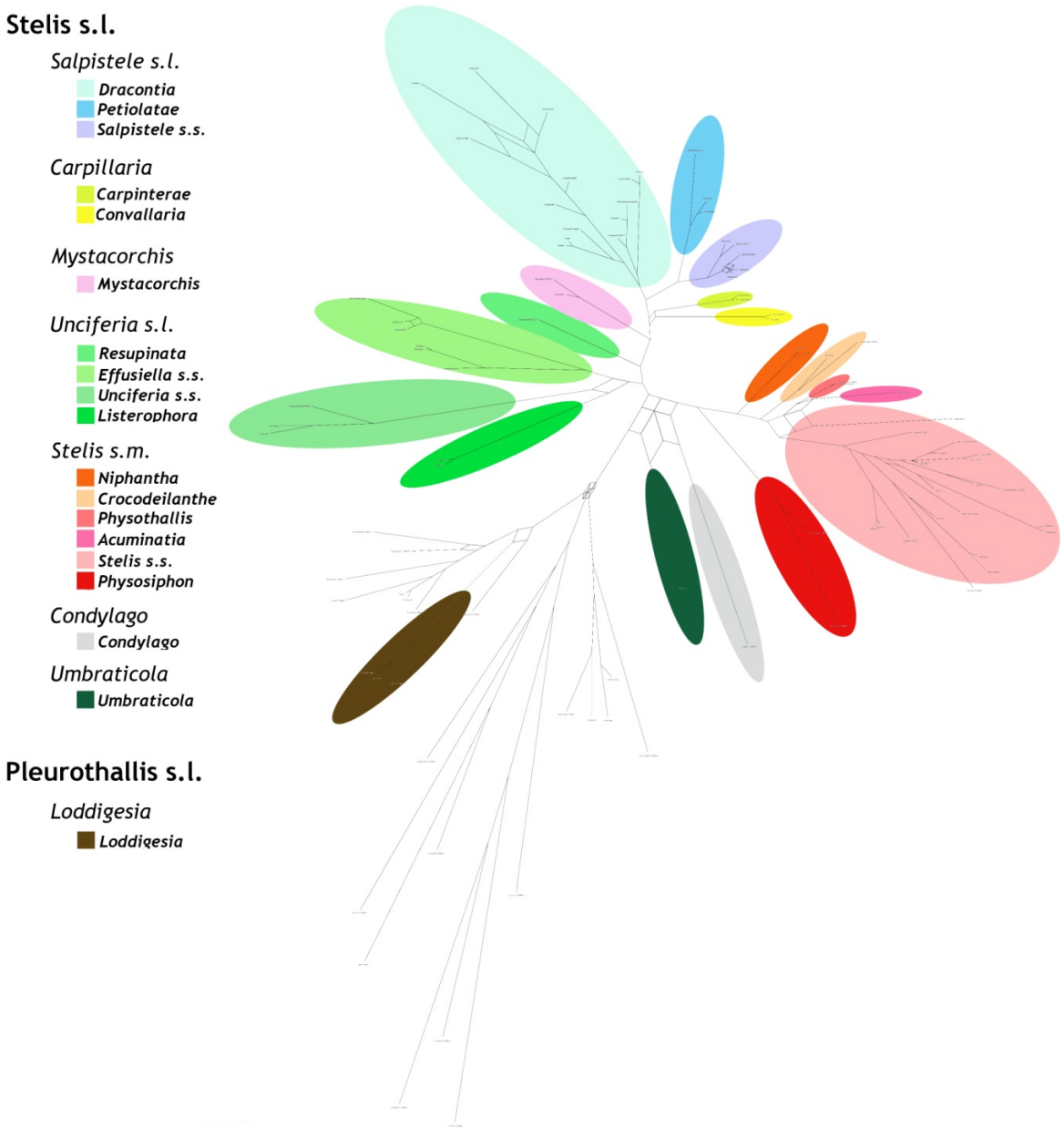

Figure 5. Consensus Network calculated from the last 1500 trees resulting of the Bayesian analysis of the concatenated matrix of ITS and *matK* sequences using a threshold of x = 0.2. The clades proposed in this paper are delimited here by color.

3.3. Clade Descriptions

In order to discuss certain groups of interest, species were allocated to clades. A clade, at least for the effects of this paper, is considered as any series of all the accessions that form a natural group and that include their common ancestor. A clade can be established at any branching point, and so, they can be delimited arbitrarily at any node. Clade recognition in the present work has been

established on the basis of groups previously recognized as separate by other authors and own field experience. The nomenclature used to designate the different clades is based on the earliest taxonomical name assigned to each group of species, independently of their taxonomic rank (genus, subgenus, section, etc). In a few cases, when no older names were found, a new, informal name was selected, trying to reflect some particular synapomorphy of the concerned group. Informal names are written in regular font. Clades were described on the tree produced with a Bayesian analysis of the concatenated matrix (including ITS and *matK*). To establish clade delimitations both tree topology and relationships in the consensus network were considered (Figures 2 through 5). Three levels of clades have been established in order to easily identify a particular group of interest. Support is taken from the PP values of the combined analysis tree. Some clades with only one species have been created mostly because they cannot, under the author's opinion, be treated as part of other clades, or are best kept separate for the sake of easily understanding what is being discussed. In any case, most of them probably represent groups of species, which have not been included in this study, rather than solitary individuals. No PP value is given for clades with only one species or that are polyphyletic and/or paraphyletic.

3.2.1. First Level Clades

Acuminatia: This clade is named after Luer´s *Pleurothallis* subgen. *Acuminatia*, which has two sections, *Acuminatae* and *Alatae*. *Anathallis dolichopus*, of sect. *Acuminatia*, is the only species included in this study.

Carpinterae: The clade Carpinterae groups two sequences from *S. carpinterae*. It has been classified by Luer (2004) in genus *Elongatia*.

Condylago: The monotypic genus *Condylago* was described in 1982 by Luer on the basis of *Condylago rodrigoi.* More recently a second species has been described, *Condylago furculifera* (without combination in *Stelis*), which was unfortunately not included in the phylogenetic analyses presented here.

Convallaria: The clade Convallaria includes two sequences from *S. convallaria*. It has been classified by Luer (2007) in genus *Effusiella*.

Crocodeilanthe (PP 1.0): Genus *Crocodeilanthe* was proposed in 1854 by Reichenbach *filius* and Warszewics. *Stelis aff. pulchella* and *S. velaticaulis* were included in this clade.

Dracontia (PP 0.89): Established in 2004 by Luer, *Dracontia* includes several species related to *S. megachlamys* (Type). Here included are samples of *S*. *megachlamys*, *S*. *papillifera*, *S*. *gigantea*, *S*. *alta*, *S*. *dracontea*, *S. aff. ferelliae*, *S. carnosilabia*, *S. alajuelensis* and *S. cobanensis*.

Effusiella s.s. (PP 0.9): Clade *Effusiella s.s*. derives from Luer´s genus *Effusiella* proposed in 2007 and typified by *Stelis pilosa*. The clade is formed by three samples of *S. pilosa* and three of *S. immersa*.

Listerophora: Clade Listerophora represents two samples of *Stelis listerophora*. Classified by Luer (2007) in the genus *Effusiella*.

Loddigesia: This clade has only one species, *S. quadrifida*, which was segregated by Luer into the monotypic genus *Loddigesia* in 2006.

Mystacorchis: This clade is named after the monotypic genus *Mystacorchis* typified by *Stelis* (*Pleurothallis*) *mystax*. *Stelis mystax* is here represented by two samples.

Niphantha: *Niphantha* was established by Luer in 2007 to accommodate two species, of which *S. gelida* is included here with two samples.

Physothallis (PP 1.0): The genus was proposed by Garay in 1953 to allocate the species *S. harlingii*.

Petiolatae (PP 1.0): The name derives from Luer´s *Pleurothallis* subgen. *Elongatia* sect. *Petiolatae*, a section created for two dwarf Central American species with petiolate leaves, *S. guttata* and *S. janetiae*.

Physosiphon (PP 1.0): Clade *Physosiphon* is named after the genus proposed by Lindley in 1835. Here *S. tacanensis* and *S. emarginata* are included.

Resupinata: Clade Resupinata is made up of a solitary sample from *S. resupinata,* classified by Luer (2007) in the genus *Effusiella*.

Salpistele s.s. (PP 1.0): The clade *Salpistele* is named after the homonym genus described by Dressler in 1979 typified by *S. brunnea*. *Salpistele s.s.* is here represented by *S. brunnea*, *Stelis maculata* and *Salpistele adrianae* (recently described and never transferred to *Stelis*).

Stelis s.s. (PP 1.0): *Stelis s.s*. is the name used for a clade formed by all the species that have always been considered the "true" *Stelis*. *S. standleyi* does not fall into the *Stelis s.s*. clade in the consensus tree of the Bayesian analysis on the concatenated data set, however, in several other trees presented here it does. Knowing that the sequence recovered from this accession was not of optimal length and quality, and having no morphological characters that would suggest the otherwise, the species is included in the *Stelis s.s.* clade under the author´s discretion.

Umbraticola: The name is the specific epithet of a synonym of *S. imraei*, *Pleurothallis umbraticola* Schltr., and makes allusion to the flowers which are born in the shade of a concave leaf. Clade Umbraticola appears in a polytomy in the consensus tree of concatenated and complete ITS data sets (Figures 4 & 5), however in other trees it appears as sister to the whole *Stelis* s.l. clade (Appendix V, VI & VII). The consensus networks (Figures 5 & 6) suggest an intermediate relationship of this clade with *Stelis s.l.* and *Pleurothallis s.l.* It is here represented by a single species, *Stelis imraei* (classified by Luer in the genus *Effusiella*).

Unciferia s.s. (PP 1.0): The name is taken from *Unciferia* Luer, established in 2004, which includes the species related to *S. segoviensis* (type). Three samples are assigned here to *Unciferia s.s*., one of *S. segoviensis* and two of its close relative *S. pompalis*.

3.2.2. Second Level Clades

Carpillaria: The name derives from the combination of the names of the two species that belong to the clade, *S. carpinterae* and *S. convallaria*. The clade groups two species that do not seem to be related to any of the other clades.

Salpistele s.l. (PP 0.8): *Salpistele* is the oldest name for a group formed by species of the clades *Dracontia*, *Salpistele s.s*. and *Petiolatae*.

Stelis s.m. (PP 0.73): *Stelis* is the oldest name for the group that includes the clades *Stelis s.s*., *Acuminatia*, *Niphantha*, *Physosiphon*, *Physothallis* and *Crocodeilanthe*. This is an indermediately broad concept of *Stelis* (*sensu medio*).

Unciferia s.l. (PP 0.65): *Unciferia* is the oldest name for the group that includes the clade *Unciferia*, *Effusiella*, Listerophora and Resupinata.

3.2.3. Third Level Clade

Stelis s.l. (PP 1.0): *Stelis s.l.* (in a broad sense) includes all the species and clades that belong to our in-group, and corresponds to Pridgeon´s *et al.* (2001) concept of *Stelis*, with a few emends: it does not include clade *Loddigesia* and it embraces clade *Acuminatia*, previously excluded from *Stelis*.

3.4. Consensus Networks

The main purpose of a consensus network is to identify areas of conflict, allowing comparison between data from different origins as well as the identification of possible cases of horizontal gene flow. Even when they are not used to reconstruct phylogenies they can shine more light on the relationships between some species groups. A consensus network, with clades plotted on it, is calculated using 10% of the trees produced by the Bayesian analysis of the complete ITS data (Figure 6).

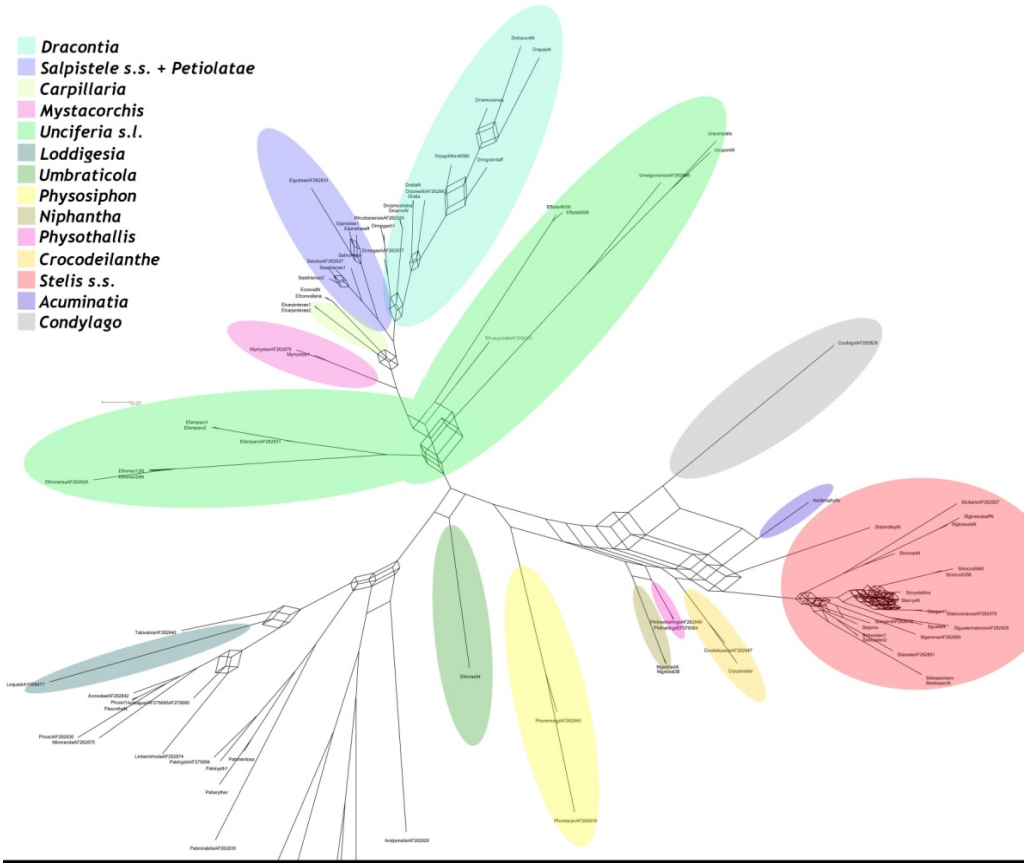

Figure 6. Consensus Network calculated from the last 10% of the trees resulting of the Bayesian analysis of the complete matrix of ITS sequences using a threshold of x = 0.1. The clades proposed in this paper are delimited here by color.

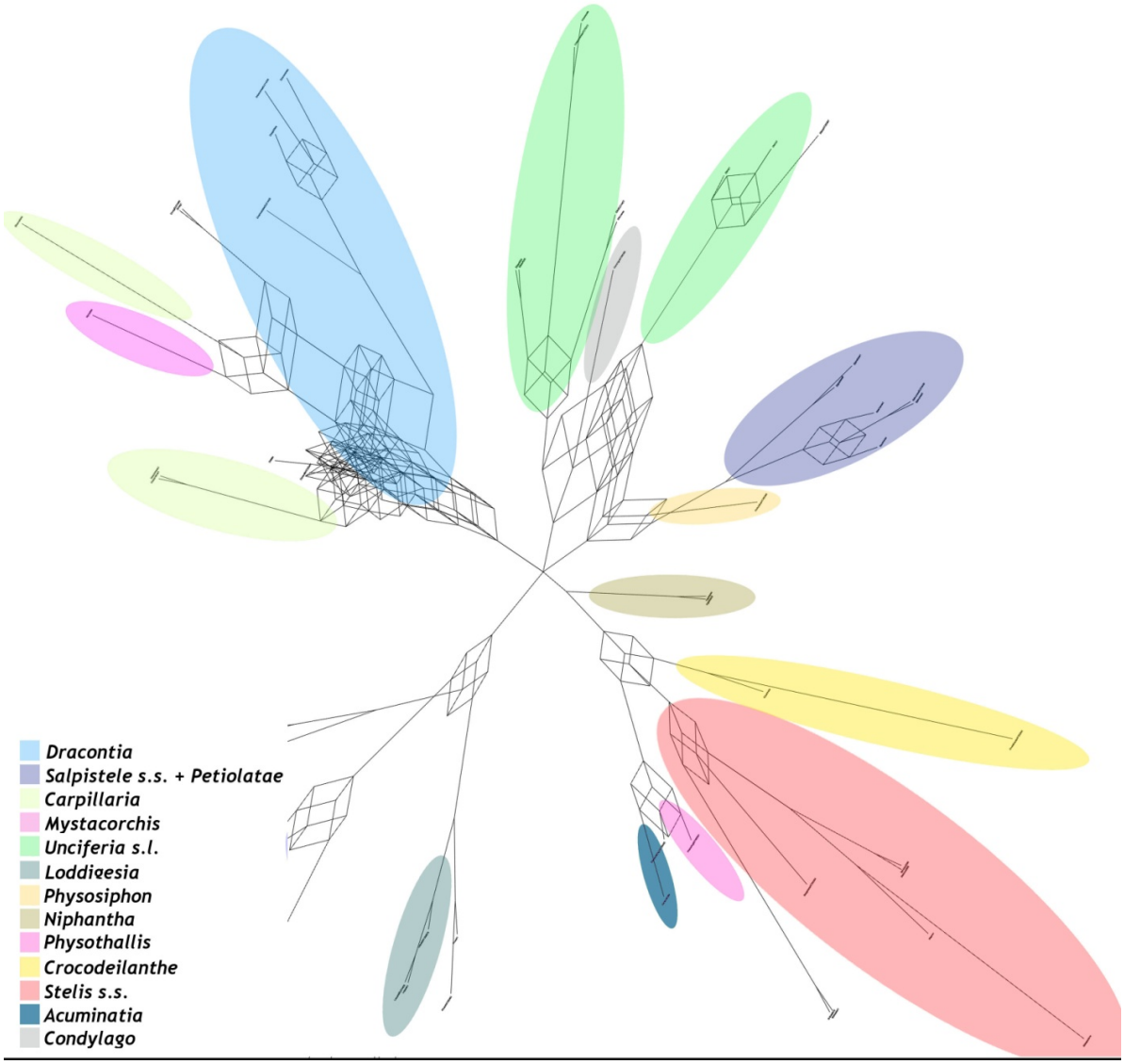

Figure 7. Consensus Network calculated from the last 10% of the trees resulting of the Bayesian analysis of the complete partitioned matrix of *matK* sequences using a threshold of x = 0.1. The clades proposed in this paper are delimited here by color.

The network shows an unclear relationship between the members of clade *Unciferia s.l.* as well as between the members of clade Carpillaria. According to the network topology the clade *Loddigesia* is not related to *Stelis s.l.*, while clade Umbraticola seems to be between *Stelis* and the out-groups. The relationship between *Condylago* and all the other members of *Stelis s.l*. is not quite clear, although finally it lies closest to clade *Acuminatia*. The relationships between *Niphantha*, *Physothallis*, *Crocodeilanthe* and *Stelis s.s*. are not well resolved when all the connecting lines are considered. In particular, *S. standleyi* is the only member of *Stelis s.s*. that does not fall into the very distinct, monophyletic group they form.

The *matK* network (Figure 7) is quite comparable to that generated from ITS data, but with some contradictions. Clade *Condylago* is here related to clade *Unciferia s.l*. and clade *Physosiphon* is

related to clades *Salpistele s.s.* and *Petiolatae*. Additionally, clade *Dracontia* is now quite far from its previous closest relatives, *Salpistele s.s.* and *Petiolatae*, while it remains entangled with the clades *Mystacorchis* and the now fragmented Carpillaria. Once more clade *Loddigesia* is more closely related to the out-group. The relationship between clades *Crocodeilanthe*, *Stelis s.s.*, *Physothallis* and *Acuminatia* remains unresolved.

A consensus network from a combined (concatenated) data set is also presented (Figure 5). It is more resolved than both individual networks, but the relationships within clades *Unciferia s.l.* and the *Stelis s.m*. are still unclear. The position of clades *Condylago* and Umbraticola is also unresolved.

3.5. Morphology

Morphological data were compiled in order to support the phylogenetic analyses. Data are presented in a morphological matrix where characters are scored as states, in most cases being evaluated as presence or absence, in others as alternative character states. The ancestral state (established from its presence at the base of the resulting trees) is normally scored as 0, whereas any modification from that state will be given 1 or 2.

All species of *Stelis s.l.* included in the analyses were evaluated morphologically, as are a few species that have not been transferred to *Stelis* (*Anathallis dolichopus*, *Condylago furculifera* and *Salpistele adrianae*), but that pertain to the group on the basis of both morphological and molecular data. A set of 70 characters was evaluated, of which 26 resulted informative (i.e., presented enough variation among the taxa included in the studied group, and relatively small variation within clades). Each character was assigned a letter from A to Z and they are explained further in the text.

Table 7. Morphological matrix showing the measured characters (from A to Z) and their states (0,1 or 2), for each of the species studied that belongs to the *Stelis s.l.* concept. A description of each character and its states are given in the following sections.

Species*	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
A. *dolichopus* (DB3736)	1	1	0	1	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. (Cond.) *furculifera* (RD6835)	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. (Cond.) *rodrigoi* (AF262829)**	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. (Croc.) *aff. pulchella* (AK2480)	1	1	0	1	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
S. (Croc.) *velaticaulis* (AF262847) **	1	1	0	1	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
S. (Drac.) *alajuelensis* (DB1987)	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	1	0	0	2	1	0	0	0	0	0
S. (Drac.) *alta* (DB4604a & b)	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	2	1	0	0	0	0	0
S. (Drac.) *carnosilabia* (DB730a & b)	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	1	0	0	2	1	0	0	0	0	0
S. (Drac.) *cobanensis* (AF262926) **	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	1	0	0	2	1	0	0	0	0	0
S. (Drac.) *dracontea* (DB616b)	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	2	1	0	0	0	0	0
S. (Drac.) *aff. ferrelliae* (DB5746)	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	1	0	0	2	1	0	0	0	0	0
S. (Drac.) *gigantea* (AF262843)	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	2	1	0	0	0	0	0
S. (Drac.) *megachlamys* (AF262877) **	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	1	0	0	2	1	0	0	0	0	0
S. (Drac.) *megachlamys* (AK1222)	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	1	0	0	2	1	0	0	0	0	0
S. (Drac.) *papillifera* (DB6585)	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	1	0	0	2	1	0	0	0	0	0
S. (Drac.) *papillifera* (DB7186)	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	1	0	0	2	1	0	0	0	0	0
S. (Effu.) *convallaria* (Hoff.s.n. a & b)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. (Effu.) *immersa* (AF262828) **	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. (Effu.) *immersa* (DB4550)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. (Effu.) *immersa* (DB6588)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. (Effu.) *imraei* (DB752)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. (Effu.) *listerophora* (DB6000)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. (Effu.) *listerophora* (DB6006)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

S. (Effu.) *pilosa****	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. (Effu.) *resupinata* (AF262916) **	0	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. (Elon.) *carpinterae* (DB7148a & b)	0	0	0	1	2	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. (Elon.) *guttata* (AF262833) **	0	0	1	1	2	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	1
S. (Elon.) *janetiae* (DB5008)	0	0	1	1	2	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	1
S. (Elon.) *aff. janetiae* (Holst 8763)	0	0	1	1	2	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	1
S. (Myst.) *mystax* (AF262876)	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0
S. (Myst.) *mystax* (DB2988)	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0
S. (Niph.) *gelida* (AK2481)	1	1	0	1	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
S. (Niph.) *gelida* (DB622)	1	1	0	1	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
S. (Phyp.) *emarginata* (AF262845) **	1	0	0	1	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. (Phyp.) *tacanensis* (AF262918) **	1	0	0	1	1	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. (Phyt.) *harlingii* ***	1	1	0	1	1	1	1	0	-	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. (Salp.) *adrianae* (DB5917a & b)	0	0	1	0	2	0	0	0	1	1	0	0	1	0	1	1	2	0	1	1	2	0	0	0	0	0
S. (Salp.) *brunnea* (DB6226)	0	0	1	0	2	0	0	0	1	1	0	0	1	0	1	1	2	0	1	1	2	0	0	0	0	0
S. (Salp.) *maculata* (AF262827)	0	0	1	0	2	0	0	0	1	1	0	0	1	0	1	1	2	0	1	1	2	0	0	0	0	0
S. (Unci.) *pilostoma* (FP7601)	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
S. (Unci.) *pompalis* (DB6516a & b)	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
S. (Unci.) *segoviensis* (AF262866)	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
S. (Unci.) *segoviensis* (AK544)	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
S. (Unci.) *aff. segoviensis* (DB2822)	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
S. *argentata* (AF262878)	1	1	0	0	1	1	1	1	0	2	1	2	0	1	1	1	2	1	1	1	0	1	1	1	1	2
S. *argentata* (DB1862)	1	1	0	0	1	1	1	1	0	2	1	2	0	1	1	1	2	1	1	1	0	1	1	1	1	2
S. *atroviolacea* (AF262879)	1	1	0	0	1	1	1	1	0	2	1	2	0	1	1	1	2	1	1	1	0	1	1	1	1	2
S. *ciliaris* (AF262927)	1	1	0	0	1	1	1	1	0	2	1	2	0	1	1	1	2	1	1	1	0	1	1	1	1	2
S. crystallina (AK974a & b)	1	1	0	0	1	1	1	1	0	1	0	2	0	1	1	1	2	1	1	1	0	1	1	1	1	2
S. *despectans* (DB5249a & b)	1	1	0	0	1	1	1	1	1	2	1	2	0	1	1	1	2	1	1	1	0	1	1	1	1	2
S. *dressleri* (FP7579a & b)	1	1	1	0	1	1	1	1	1	2	1	2	0	1	1	1	2	1	1	1	0	1	1	1	1	2
S. *gemma* (AF262880) **	1	1	0	0	1	1	1	1	0	2	1	2	0	1	1	1	2	1	1	1	0	1	1	1	1	2
S. *glossula* (DB2695)	1	1	0	0	1	1	1	1	0	2	1	2	0	1	1	1	2	1	1	1	0	1	1	1	1	2
S. *aff. glossula* (Kisel 2048)	1	1	0	0	1	1	1	1	0	2	1	2	0	1	1	1	2	1	1	1	0	1	1	1	1	2
S. *guatemalensis* (AF262928)	1	1	0	0	1	1	1	1	0	2	1	2	0	1	1	1	2	1	1	1	0	1	1	1	1	2
S. *guatemalensis* (FP3977)	1	1	0	0	1	1	1	1	0	2	1	2	0	1	1	1	2	1	1	1	0	1	1	1	1	2
S. *lanata* (AF262881) **	1	1	0	0	1	1	1	1	0	2	1	2	0	1	1	1	2	1	1	1	0	1	1	1	1	2
S. *aff. microchila* (DB5356)	1	1	0	0	1	1	1	1	0	2	1	2	0	1	1	1	2	1	1	1	0	1	1	1	1	2
S. *aff. microchila* (DB6965)	1	1	0	0	1	1	1	1	0	2	1	2	0	1	1	1	2	1	1	1	0	1	1	1	1	2
S. *morae* (AK1086)	1	1	0	0	1	1	1	1	0	2	1	2	0	1	1	1	2	1	1	1	0	1	1	1	1	2
S. *sp.* (DB6427)	1	1	1	0	1	1	1	1	0	2	1	2	0	1	1	1	2	1	1	1	0	1	1	1	1	2
S. *standleyi* (DB5605)	1	1	0	0	1	1	1	1	0	2	1	2	0	1	1	1	2	1	1	1	0	1	1	1	1	2

*In parenthesis the genera to which the species have been allocated in the alternative classification system, where Cond.= *Condylago*, Croc.= *Crocodeilanthe*, Drac.= *Dracontia*, Effu.= *Effusiella*, Elon.= *Elongatia*, Myst.= *Mystacorchis*, Niph.= *Niphantha*, Phyp.= *Physosiphon*, Phyt.= *Physothallis*, Salp.= *Salpistele* & Unci.= *Unciferia*. Take into consideration that several specific epithets are modified when transferred to other genera. Missing data is given as "-".

**The author has not personally observed these species, their characters states have been taken from drawings and/or descriptions by other authors (Luer 1987, 1994, 1998, 2000, 2007; Soto Arenas & Solano 2003a, 2003b, 2003c; Dressler & Bogarín 2007; Duque 2008).

***Two or more accessions with this name have been included, but as the author has not seen any plants of the sort. Therefore characters states for all are equal and based on the literature mentioned before.

The morphological matrix was brought into the *Mesquite* program and all characters´ ancestral states were determined at selected node, with a parsimony analysis. This was mainly done to determine the possible ancestral character states for the whole *Stelis s.l.* group. From that

it is possible to discuss how characters evolve from ancestral to derived states within the group. The ancestral states for each character are presented as 0, whereas derived states present 1 or 2. Not all character states were unanimously found by the analysis, a few suggested more than one state as possibly ancestral, in those cases, the ancestral character was selected with the authors discretion (the summarized results of the parsimony analysis on the character set can be found in Appendix XIII).

3.4.1. Inflorescence characters

A = Spathe of the Inflorescence: The spathe (a modified, usually compressed and fibrous bract that protects the inflorescence primordium) is dry, papery and brown-grayish in color in most Pleurothallids, and in the group this is the ancestral condition of the character. The spathe can however turn foliaceous, in other words more leaf-like, fleshy and green. A foliaceous spathe is found in the whole *Stelis s.m.* clade. Spathe papery = 0, foliaceous = 1.

B = Inflorescence Development: The ancestral state is successive flowering, with some flowers open and others still immature at any time. The derived condition is simultaneous flowering, with all the flowers open more or less at the same time. Inflorescence successive = 0, simultaneous = 1. Simultaneous flowering is predominant between the species of *Stelis s.m*., while in the derived clades *Petiolatae* and *Salpistele s.s.* flowers are produced in slow succession appearing to be solitary.

C = Inflorescence Position: An erect or slightly horizontal inflorescence is the ancestral state in the group, however inflorescence can drop and "creep" through the substrate. Inflorescence erect = 0, creeping = 1. Creeping inflorescences can be seen in clade *Petiolatae* and *Salpistele s.s.*

3.4.2. Sepal characters

D = Sepal pigmentation: Ancestrally, the sepals of this group (with some exceptions) are maculate with dark colors. In some groups, the maculae, have converged into one or two large blotches, while in other cases they disappeared completely. Sepals maculate = 0, Concolorous = 1. In derived groups such as *Dracontia*, *Salpistele* and *Mystacorchis*, the maculae have converged into large blotches. In *Stelis s.m.* and related clades, the sepals are never maculate, only in a few cases being suffused with a light color.

E = Lateral Sepals Fusion: The fusion of the lateral sepals is common to all species of *Stelis s.l*. The fusion can be only partial, limited to above the middle of the sepals, with the free segments convergent (= 0), or below the middle with the free segments divergent (= 1), or complete, with the sepals fused up to the apex (= 2). Most clades of *Stelis s.l.* have convergent lateral sepals, but they

are divergent and fused only below the middle in *Stelis s.m*. In *Petiolatae* and *Salpistele s.s*. the sepals are completely fused up to the apex.

F = Dorsal Sepal Fusion: In the species basal to the tree, the dorsal sepal is always free, while it is partially to completely connate to the lateral sepals in some derived clades. Dorsal sepal free = 0, fused to the lateral sepals = 1. In the species belonging to *Stelis s.m*. the lateral and dorsal sepals are fused, forming a trilobed fan-like calyx.

G = Synsepal: A synsepal is defined as a sepal formed by the fusion of the two lateral sepals, which is similar to the dorsal sepal in shape and size. The ancestral state in the group is the presence of a synsepal (= 0), which has been secondarily lost in some species (= 1). Most species of *Stelis s.m.* have completely lost the synsepal.

H = Sepal Length-Width Ratio: In most species of the group, sepal length is at least twice or more the width (= 0), but sub-equal length and width of sepals is also present (= 1). Ancestrally, the sepals of the group are much longer than wide, while in *Stelis s.s.* the sepals are have shortened and most of the time as wide as long.

I = Sepal Indumentum: The ancestral condition of the adaxial surface of the sepals in the group is pubescent. Abaxial surface of the sepals hirsute = 0, glabrous = 1. The character is lost in some derived clades such as in most species of *Mystacorchis and Salpistele s.l*. The clade *Stelis s.m.* predominantly presents hirsute sepals.

3.4.3. Petal characters

J = Petal Apex: Ancestrally petals in the group present apical thickening of the mid-vein (clades Umbraticola, *Effusiella*, Listerophora and Convallaria), but the thickening is lost in all the other clades. A second thickening occurs in clade *Stelis s.s.*, but in this case it is not the mid-vein which is thickened but the entire apical margin. Petal apically thickened along the midvein = 0, un-thickened one = 1, thickened along the margin = 2.

K = Petal Shape: Ancestrally, the petals in the group are linear or elliptic-lanceolate, but in the clade *Stelis s.s*. they are so reduced that they become as wide as long. Petals distinctly longer than wide = 0, as long as wide = 1.

L = Petal Margin: Petal margins in this groupare quite variable, but in general they are straight (= 0), in basal species, while in clade *Dracontia* they are involute (= 1) protecting the column, and in clade *Stelis s.s*. they are revolute (= 2), exposing the column.

M = Petal-Sepal Ratio: In the whole group, sepals are at least twice as long as the petals. Execptions are found in the *Salpistele s.s.* and *Petiolatae* clades where they are equal. Sepals much longer than petals = 0, sub-equal to petals = 1.

3.4.4. Labellum characters

N = Glenion: The presence of a glenion is common in some groups of the Pleurothallids, for example in the "true" *Pleurothallis*. In the *Stelis s.l.* group, the glenion is absent in the basal species, but appears in the derived clade *Stelis s.s.* Glenion absent = 0, present = 1.

O = Lip articulation: In basal species and most clades, the lip is articulated to the apex of the column foot, allowing movement. The articulation of the lip is lost in derived clades such as *Stelis s.s*. and *Salpistele s.s*. Lip articulated, movable = 0, fixed = 1.

3.4.5. Ovary and Column Characters

P = Column Foot: A prominent column foot from which the lip is hinged is present in most clades of this group and in basal species. The column foot is lost in derived clades such as *Stelis s.s.* and *Salpistele s.s*. Column foot present = 0, absent = 1.

Q = Column Foot Position: Ancestrally the column foot is perpendicular to the ovary and column, however in some derived clades such as *Dracontia* the column foot is curved inwards. Column foot straight = 0, curved = 1, absent = 2.

R = Column Position: The column, as well as the lip, is ancestrally somewhat curved, running parallel to each other. A straight column is only found is the clade *Stelis s.s*. where the column length is drastically reduced. Column curved = 0, straight = 1.

S = Column Wings: A winged column is the ancestral state for this group and is kept in most clades. In the derived clades *Petiolatae* and *Salpistele s.s.* the whole androclinium has become very prominent. On the other hand in *Stelis s.s.* the reduced column has lost its wings. Column wings present = 0, absent = 1.

T = Column Shape: A cylindrical column is found in basal clades of this group, however the column apex boradens in the clades *Stelis s.s.* as well as in *Unciferia*, *Petiolatae* and *Salpistele s.s*. On the other hand, in the derived clade *Dracontia* the column becomes narrower towards the apex. Column cylindrical = 0, broader towards the apex = 1, narrower towards the apex = 2.

U = Column – Lip Ratio: Column of the group is ancestrally sub-equal to the length of the lip. However, in the derived clades *Mystacorchis* and *Dracontia,* the lip far exceeds by far the length of the column, while clade *Salpistele s.s*. presents a column that exceeds the length of the lip. Column sub-equal to the lip = 0, shorter than lip = 1, longer than lip = 2.

3.4.6. Anther, Stigma and Pollinaria Characters

V = Anther Position: Anther position in the group is ancestrally incumbent, however in the derived clade *Stelis s.s.* the anther is apical. A tendency of the anther towards being apical is present also in the *Salpistele s.l*. clade, but only in clade *Mystacorchis* is it truly apical. Anther incumbent = 0, apical = 1.

W = Stigma Position: Stigma position in the group is ancestrally ventral, however the derived clades *Stelis s.s*. and *Mystacorchis* present an apical stigma. Stigma ventral = 0, apical = 1.

X = Stigma Opening: Ancestrally in the group, the rostellum is convex, seeming a large "bubble-like" cap that covers the stigma entrance, It is provided with a "glue-like" viscarium (a loose celled, sticky, transformed, perhaps ancestral form of viscidium) on the inner apical margin. However in *Stelis s.s*. the rostellum has been reduced to a triangular hard lobe, very much surpassed by the large stigma, and is provided with a drop-like viscidium at its apex. In *Petiolatae*, the bubble-like rostellum has also been reduced and it is held almost perpendicular to the column axis in order for the viscarium to reach the base of the anther. Stigma covered by rostellum = 0, uncovered = 1.

Y = Pollinarium Shape: A very common trait within the Pleurothallids is that the two pollinia are provided with a pair of unicellular, transparent, dry, flat, "whale-tail" shaped caudicles, which can be seen twisted upwards at the base of the anther when still on the flower. This basic morphology is modified in some groups. In *Stelis s.s*. the caudicles have become cylindrical, narrow and elastic. In *Petiolatae* they have also become narrower, losing the whale-tail shape. Caudicles whale-tail shaped = 0, narrow = 1.

Z = Pollinaria Structure: Ancestral pollinaria in the group are provided only of the, pollen sacs and caudicles, both onthogenetically produced within the anther. On the other hand, in *Stelis s.s*., a hard, sticky and transparent bubble-shaped viscidium, which is formed by the transformation of a stigmatic lobe, is adhered to the caudicles. In clade *Petiolatae*, the glue-like viscarium at the apex of the rostellum is now placed so close to the caudicles that they seem a single organ. Detachable viscidium absent, viscarium not in contact with caudicles = 0, detachable viscidium absent, viscarium adherent to the caudicles = 1, detachable viscidium present = 2.

4. Discussion

4.1. Phylogenetic Reconstruction

Most of the results of the phylogenetic reconstruction are quite straight forward and do not require further discussion, on the other hand the consequences of the results obtained are discussed in later sections. Even so, a few interesting phenomena (if we can call them so) when using molecular data for phylogenetic reconstruction are worthy to mention, the difference between species and gene trees, long branch attraction, and the effect of missing data in the matrices.

4.1.1. Gene Trees vs. Species Trees

Most phylogenetic studies, such as this one, use methodologies that focus not on estimation of species trees but on estimation of gene trees. Concatenation is many times considered to be a solution for that problem, the assumption being that by combining more genes a species tree is obtained. That assumption holds only when no horizontal or other reticulate gene flow is present, when no gene duplication has caused gene lineage splits, and when gene lineages coalesce before divergence of species. The practice of concatenating sequences from genetically separate loci into a simple sequence does not permit heterogeneity among gene trees and is thought to increase the power to make inferences about species history (Edwards *et al.* 2007; Kubatko & Degan 2007). However concatenated sequences can mislead inference of species relationships. ITS and *matK,* the first from the nucleus and the second from the chloroplast, may indeed have quite different evolutionary histories, which if concatenated into a single sequence will average the diverging results. Kubatko & Degnan (2007) suggest that when substantial discord is found between the gene trees estimated independently, potential problems can occur when concatenating them. The effects of reticulate evolution in phylogeny reconstruction, and, how to deal with hybrids and diverging evolutionary hypothesis is discussed amply by Vriesendorp & Bakker (2005) and Weihe *et al.* (2009).

In the present study, when the individual results between ITS and *matK* are compared, several differences become evident. In the *matK* consensus network (Figure 7) a large clade was formed in which clades Carpillaria and *Dracontia* were dissolved, grouping *S. megachlamys* (*Dracontia*), *S. convallaria* (Carpillaria) and *S. mystax* (*Mystacorchis*) on one side, *S. dracontea*, *S. papillifera* and *S. alajuelensis* (*Dracontia*) on the other side, while *S. carpinterae* (Carpillaria), *S. alta* and *S. gigantea* (*Dracontia*) were clustered at the base. A second large clade includes clades

Petiolatae, *Salpistele*, *Condylago*, *Physosiphon* and *Unciferia s.l*. A third large clade includes *Stelis s.s*., *Niphantha*, *Physothallis*, *Acuminatia* and *Crocodeilanthe*.

However using ITS data, *Dracontia* was found to be monophyletic and closely related to *Petiolatae* and *Salpistele*, and *Physosiphon* was found to be closely related to the *Stelis s.m.* clade. The clades found with the ITS data sets are supported by both morphological and geographical evidence, suggesting they could be closer to the actual species tree. The concatenated analysis, which considers both sequences was almost identical topologically to the ITS tree. All the evidence seems to point to the *matK* data as being the one that differs from actual species tree, but how can that be explained?

A crucial issue that arises when multi-gene data are analyzed is that each gene has its own evolutionary history, which may or may not be congruent with the evolutionary history of the species as a whole. Several evolutionary processes lead to incongruence between gene and species phylogenies, including phylogenetic error, hybridization, horizontal gene transfer, incomplete lineage sorting, and gene duplication and extinction (Holland *et al.* 2008; Meng & Kubatko 2008). Lineage sorting is defined by Meng & Kubatko (2008) as the "failure of ancestral gene copies to coalesce into a common ancestral copy until earlier than the previous speciation event". Holland *et al.* (2008) propose the use of supernetworks as useful tools to detect these evolutionary processes and distinguish hybridization from for instance incomplete lineage sorting and phylogenetic error.

Tsitrone *et al.* (2003) suggest that the differences found between chloroplast and nuclear genes can not only be explained by all of the above mentioned evolutionary processes, but also by chloroplast capture, defined as the introgression of a chloroplast from one species into another. It can occur if the cytoplasm substitution provides an advantage in seed production, that capture is promoted by nuclear incompatibilities between two genomes and by partial selfing when inbreeding depression is strong. Acosta & Premoli (2010) associated their phylogenetic incongruences with geographical distribution, assuming that chloroplast capture would only be present in taxa that have distribution overlap. The same could be said about hybridization in general, and both would be true only if no extinction has taken place. Assuming it is so, hybridization and chloroplast capture cannot explain the *matK* results that show close affinity between clade *Physosiphon*, which only grows in Mexico, Guatemala, Ecuador and Peru and clades *Petiolatae* and *Salpistele* which are strict Costa Rica-Panama endemics. However they could explain the entangling of species of clades *Dracontia*, Carpillaria and *Mystacorchis* which are all Central-American endemics.

4.1.2. Missing Data

Missing data has been traditionally considered to be a large problem when trying to reconstruct phylogenies from sequence data. Wiens (2006) on the other hand suggests that taxa with incomplete data sets should not be excluded. Interestingly enough, several sequences here were very short in number of base pairs but were kept in the analyses. Do missing data actually affect the position of an individual sequence? Well the answer is yes and no. In the ITS data set 4 sequences had between 206 and 343 base pairs (of a maximum of 848). But three of the four cases (*Stelis gelida* AK2481, *Stelis pompalis* DB6516a and *Stelis pompalis* DB6516b) behaved "normally" in the tree, being close to their known relatives and not introducing big contradictions in the consensus network. However, the fourth sequence, that of *Stelis dracontea* DB616a created great disruption in the consensus network, and a polytomy with its close relatives at the base of the *Dracontia* clade. When a second *S. dracontea* (DB616b) sequence was added, none of the problems mentioned before were found, it was therefore kept as the "better" sequence.

The reason for this difference might be that the sequence of *S. dracontea* is placed almost completely on a largely conserved region of ITS, on the other hand the other sequences all have non-conserved regions within their sequence. That would suggest that there are just not enough characters to distinguish *S. dracontea* from anything else, whilst it is possible to distinguish *S. gelida* and both the samples of *S. pompalis*.

If that is the case, the conclusion would be that it is possible to use sequences with missing data to reconstruct a phylogeny, but, it depends on what area of the gene was recovered and how much resolution is needed in the tree. More specifically, as Wiens (2006) also suggests, if the characters that determine its "proper" location are present, the fact that other data are missing poses no problem. The other regions then contain conserved regions, non-informative characters, and informative characters that are redundant.

4.1.3. Long Branch Attraction

As mentioned before, three different ITS data sets were analyzed, the first is the complete data set with 92 taxa, the second excludes the sequence from *A. dolichopus*, the third instead excludes *S. rodrigoi*. This has been done because when both *A. dolichopus* and *S. rodrigoi* were present in the analysis they were found together in the resulting trees in a clade sister to *Stelis s.s*. However when the *A. dolichopus* sequence was removed, *S. rodrigoi* moved into a polytomy next to *S. imraei*. On the other hand when the *S. rodrigoi* sequence was removed, *A. dolichopus* remained in the same place, sister to *Stelis s.s.* (Figure 3 shows the complete data set with 92 sequences; Appendix V shows a the consensus trees found when either one of the sequences is removed).

In this curious case, it seems that the *A. dolichopus* sequence somehow "attracts" the long branch in which *S. rodrigoi* was placed, moving it from were it belongs in the tree. An explanation for that could be Long Branch Attraction (LBA) defined by Andersson and Swofford (2004) as ''any situation in which similarity due to convergent or parallel changes produces an artifactual phylogenetic grouping of taxa due to an inherent bias in the estimation procedure". LBA is a phenomenon of molecular data in particular, an "A" at a certain position inherited from a common ancestor in two lineages look identical to an "A" acquired independently (Bergsten 2005). Bergsten suggests that methods to avoid LBA includes the exclusion of long-branched taxa, and faster evolving third codon positions, the use of inference methods that are less sensitive to LBA such as likelihood, the sampling of more taxa to break up long branches and the sampling of more characters. Wiens (2006) mentions that missing data can also cause LBA in some cases.

4.2. Geography

One of the major contributors to speciation, dispersal, and extinction events, without any doubt is geography, and consequently, it plays a very important role in phylogenies. In our area of interest, the cooler mountain belts serve as barriers to gene flow of orchid species adapted to the warm lowlands, as is strongly suggested by the differences in the flora of the Atlantic (Caribbean) and Pacific slopes of Central America and the Atlantic (Amazonian) and Pacific slopes of South America. On the other hand, the warm lowlands in turn also serve as a barrier for the cool mountain adapted species. This is especially true for the tropics were the montane and lowland temperatures frequently do not overlap (Kirby 2010 presents a good review of the topic).

Kirby (2010) suggests that both high mountain ranges and large lowlands serve as isolation barriers, and could explain the large amount of endemism found in the Cordillera de Talamanca between Costa Rica and Panama, which is isolated from the northern cordilleras by the very large Nicaraguan depression and from the southern Andes region by a series of lowlands in the South of Panama followed by the Cordillera Central of Colombia. The same can be said for the Colombian, Ecuadorian and Peruvian Andes, a high and long mountain range close to the Pacific coastline and that remains somewhat isolated by the vast lowlands of the Amazonas. A third case, although not mentioned before due to the fewer number of species, the case of the Mexican Chiapas region similarly has several endemic species of *Stelis s.l.,* and from morphological characters I expect it even has several endemic clades, which was not included in the present study. Chiapas is situated in the South of Mexico and isolated from the Sierra Madre to the north, by the Tehuantepec lowlands, and to the south by the large Nicaraguan depression. Kirby (2010) divided the region in major lowlands that interrupt the Middle-American Cordilleras (From the South of México to the Andes); The major mountain ranges were added (Figure 8, the original image was presented by Kirby (2010), but has been modified by the author for this study).

It is important to point out that the Talamanca and Andean Cordilleras are both relatively young. The natural bridge between North and South America closed up as late as 3 million years ago, in a period of active volcanism and plate movement (Eliécer Duarte pers. comm. 2010). The Andean Cordillera started its uplift about 10 My ago and almost reached its complete high around 7 My ago (Garzione *et al.* 2008). The diversity of life zones, combined with radiation, heat, energy, pressure and other extreme conditions could have triggered speciation events.

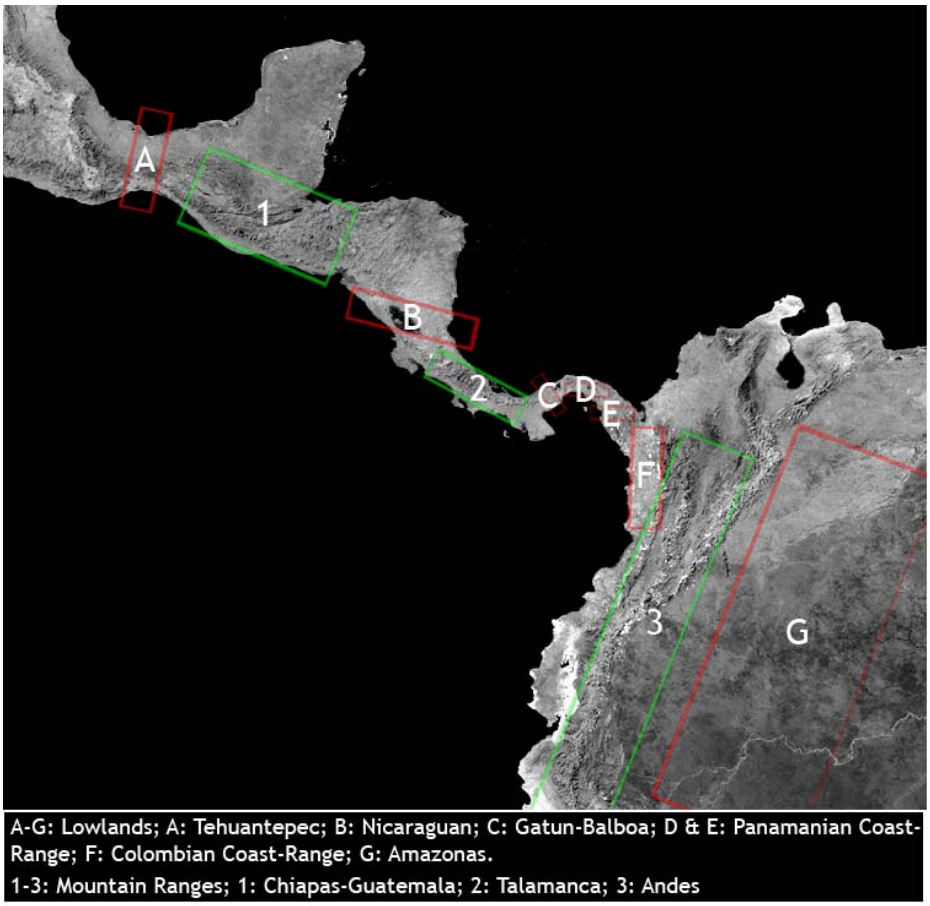

Figure 8. Map of Mesoamerica and Northern South-America showing the most important lowland plains, in red, and high mountain ranges, in green. Taken from Kirby 2010 and modified by the author.

4.2.1. Geographical Trends within *Stelis*

Some very clear geographical patterns are present in the results obtained in the phylogenetic analyses. In a general sense the group mostly belongs to the highlands of tropical America, from Chiapas (South of Mexico), through Central America, to Colombia, Venezuela, Ecuador, Basil, Peru and Bolivia, with some scattered species in Florida, the Caribbean and the Guyanas.

If one takes a closer look, other trends become clear. Clades *Unciferia s.l.*, *Salpistele s.l.*, Carpillaria and *Mystacorchis* present a very distinct pattern (Figure 9A; the total number of species of each clade per country is summarized in Appendix XII). They have much less species, but they also have the highest diversity in the Central American isthmus. Costa Rica is the country with the most species, 34, followed by Panama (23) and Colombia (19). On the other hand, clade *Stelis s.m.* (including *Stelis s.s.*, *Crocodeilanthe*, *Niphantha*, *Acuminatia*, *Physosiphon* and *Physothallis*) is very large in number of species and also very widespread, but if one looks at number of species per country (Figure 9B; Appendix XII) a noteworthy diversity in the Andean Cordillera becomes evident. Ecuador has by far the largest number of species belonging to the group, 476, followed by Colombia (185) and Peru (121), all the other countries having less than 100 species. Even when these

numbers could be transformed to species per area it would beat the purpose of the figure. It is intended to demonstrate that species number is larger in certain areas than others, probably more related to their geographical position and diversity than their actual size. The best way to actually represent species number would be to plot the exact location of every known collection of each species on a map, independently of political delimitations, and then point out the most diverse geographical areas. However, it is not possible with the available data, and would be a tremendous undertaking to do with all +1000 species currently attributed to *Stelis s.l.*

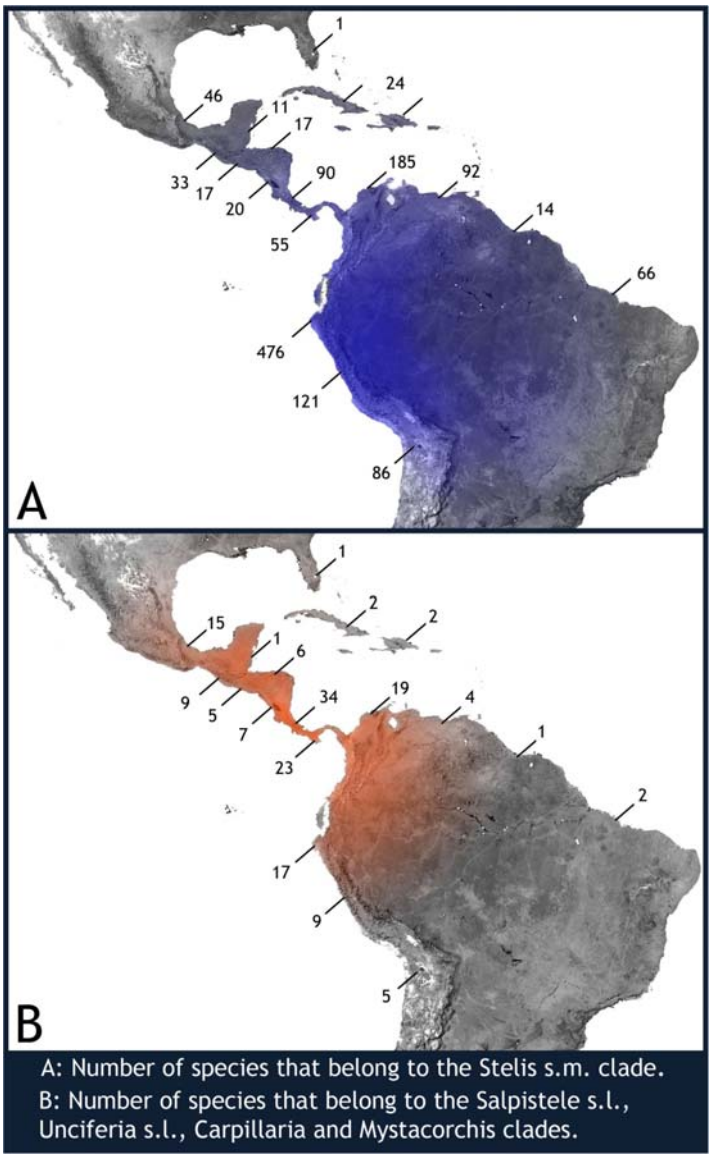

Figure 9. Reported number of species per country belonging to the different clades, showing the trends of relative frequency in each. Species numbers per country follow Govaerts *et al.* 2010. More intense colors represent larger species number while light coloring lower species numbers.

4.2.2. The origin of *Stelis s.l.*

Even when *Stelis* species seem to grow almost everywhere in tropical America, it is clear that most of the derived clades in the group are quite locally distributed instead of widespread. On one side, clade *Salpistele s.l.* is a Mesoamerican endemic, while clades *Salpistele s.s., Petiolatae*, *Mystacorchis* and Carpillaria are endemic of Costa Rica and Panama. Costa Rica and Panama also present most of the species of clades *Unciferia* (80%) and *Dracontia* (95%). On the other side, *Physothallis* is an Ecuadorian endemic, and more than 75% of the species of *Acuminatia*, *Crocodeilanthe* and *Stelis s.s.*, grow in the Andean region of Bolivia, Colombia, Ecuador and Peru (Figure 10).

If the presence in a specific area is treated as a character state, then just like for the morphological characters one can calculate a geographical ancestral state for the *Stelis s.l.* group. In Appendix XIII, characters 27 and 28 are actually geographical distributions. The difference between both is that in character 27 the most frequent distribution of the clade itself was considered (averaging all the species belonging to the clade, rather than only the species used here), whereas in character 28 the actual distribution of each of the species analyzed here was included. The first will make several assumptions, but, will reflect actual distribution far better than the second. The reason for that is a Costa Rican bias of the data as all (or almost all) species treated here have that country in their distribution. That is not true for an average per clade, were the most common distribution is kept.

In both cases however, after the same parsimony analysis was done as for morphological characters, it is the area between Costa Rica and northern Colombia (labeled South Central in the Analysis) which is indicated as ancestral for the *Stelis s.l.* group (Figure 10 & 11). That striking result is actually quite unexpected as it was never before suggested by any author and was also not to be supposed from previous studies. However, it is possible that the inclusion of more data, especially from the Andean Cordillera, might change the observed results. It is also clear from the data that species of *Stelis s.l.* are almost not present in the Amazonian lowlands.

One might actually suppose that the center of origin of a group is also its center of most diversity, however, it is not always true. In *Stelis s.l.* the largest amount of species is found in the Andes, Ecuador by itself has about 500 species, while the center of origin seems to be the area between Costa Rica and northern Colombia, with around one third of the number of species. What is true on the other hand is that even when less species are present in the Costa Rica – Colombia area, only clades *Physothallis* and Resupinata are not present there, whereas, about halve of the clades are not present in the Andes (like *Condylago*, Convallaria, *Mystacorchis*, *Petiolatae*, Resupinata, *Salpistele,* etc.). Species numbers per area are presented in Appendix XII.

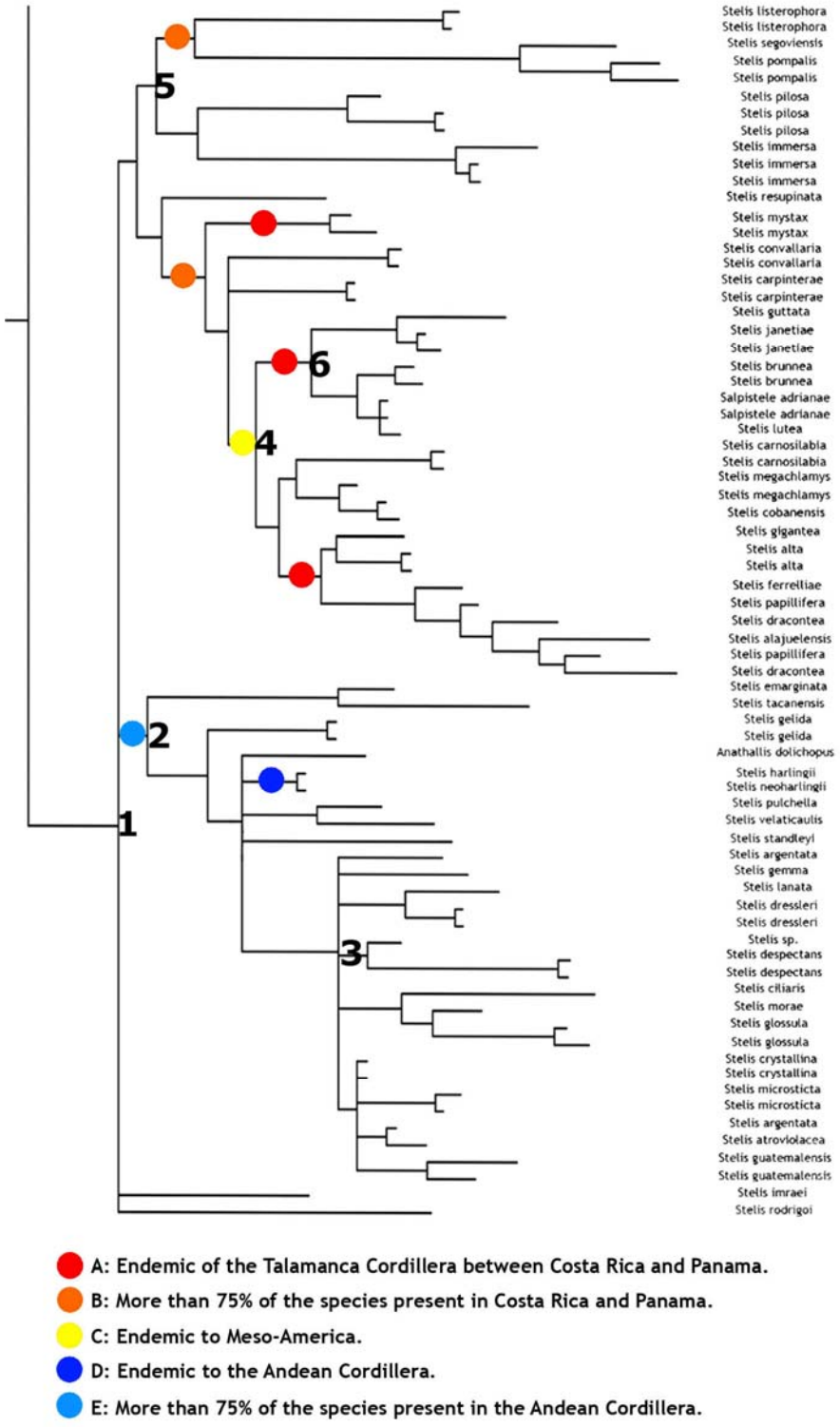

Figure 10. Section of the consensus tree from the Bayesian analysis on the concatenated data set, indicating the geographical distribution within *Stelis s.l.* and several of its clades, reddish or yellow colors indicate Mesoamerican distribution, while bluish colors Andean distribution. Numbers indicate the nodes from which the ancestral states have been calculated. 1: *Stelis s.l.*; 2: *Stelis s.m.*; 3: *Stelis s.s.*; 4: *Salpistele s.l.*; 5: *Unciferia s.l.*; 6: *Salpistele* + *Petiolatae*.

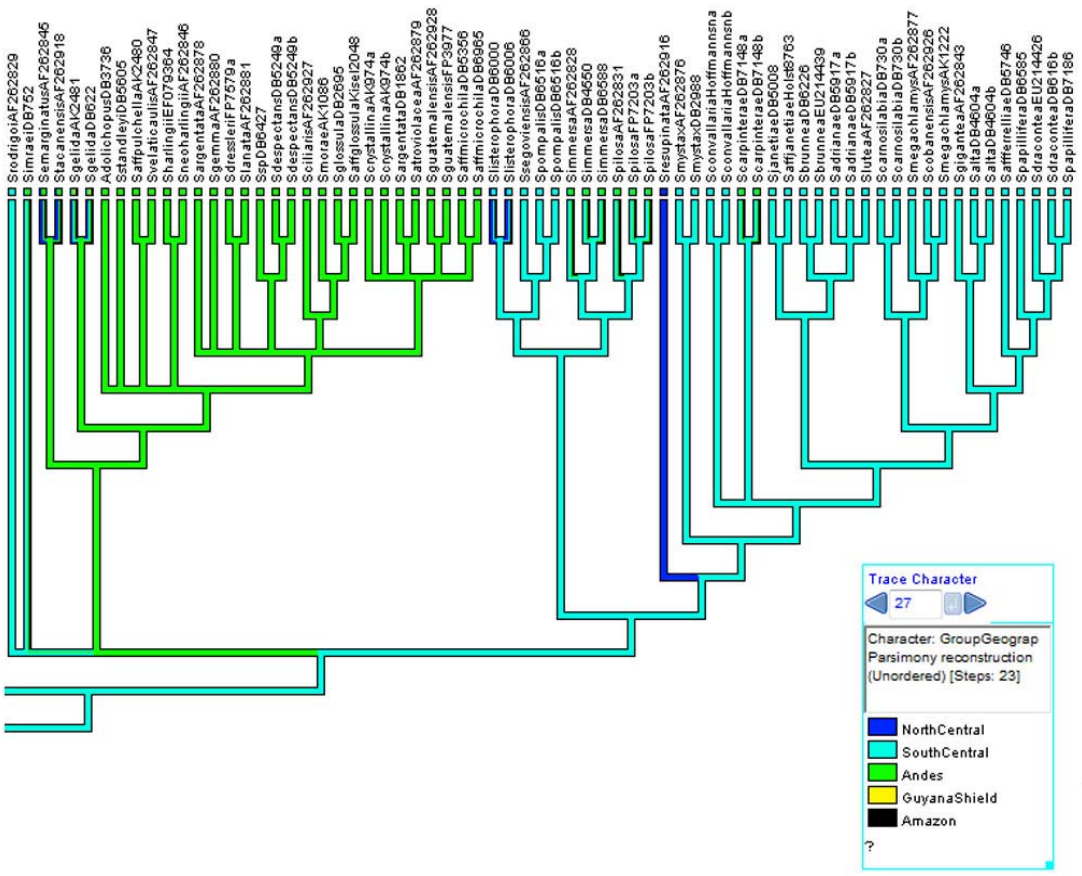

Figure 11. State history of character 27 (Clade Geography) on the consensus tree from the Bayesian analysis on the concatenated data set, calculated by *Mesquite* with a parsimony analysis. The ancestral state is given by the light blue color (South Central).

4.3. Morphology

Morphological data were gathered in order to support and explain the phylogenetic analyses. Some characters proved useful to clarify the tree topology and in turn also the supposedly evolutionary path of those species. Other characters would be present or absent randomly in the tree and were therefore regarded as not informative. A few characters seem to be cases of convergence, where two unrelated taxa end up with the same apomorphy.

Even though each character has the same weight in the analysis (i.e., is not ponderate), from an evolutionary point of view it is perhaps logical to assume that some features, like the structure and shape of the pollinaria, have to be more significant than others (for example the shape of the ramicaul sheaths) in terms of evolutive processes. As it is not the purpose of this study to build a tree based on morphology, but instead to plot morphological trends on the tree topology, most of the characters selected to explain the evolutionary pathway are related to pollination syndromes. It is important to point out that the actual pollinators of this group are not known on the basis of solid evidences, and our assumptions that some morphological modifications are selective for a certain kind of pollinator, have to be considered as working hypotheses.

To determine the ancestral states of each character a parsimony analysis was done in *Mesquite* on all the characters plotted on the consensus tree produced from the Bayesian analysis of the concatenated matrix. Ancestral states were evaluated for a few selected nodes (Shown in Figure 10). The results can be found in Appendix XIII.

Inflorescence Position: The inflorescence in clades *Petiolatae* and especially in clade *Salpistele* "creeps" through the substrate ending up quite far from the plant, making the flower appear as a solitary object erected from the substrate, which could be an adaptation to a certain kind of pollinator.

Sepal Indumentum: Although the indumenta of flowers are not frequently discussed it would seem logical that the high frequency of hairs in such small flowers should be selective as to the attraction and/or orientation of the pollinator. While almost all species of *Stelis s.m.* (more than 700 species) and *Unciferia s.l.* have some kind of hair-like appendages, species of clades *Mystacorchis*, *Dracontia* (with one exception), *Petiolatae*, *Salpistele* and *Stelis carpinterae* have all glabrous flowers.

Glenion: The roundish depression at the base of the lip called glenion, it is said to have a function in the attraction of certain pollinators. Several members of Pleurothallidinae have a glenion, especially in the genus *Pleurothallis s.l*. However in the *Stelis s.l.* clade, only *Stelis s.s*. presents a glenion. This darker, shiny depression in the lip may be important in the pollination of this group.

Column foot and lip movement: In this group the presence of a column foot is quite common. Normally, the column foot extends perpendicularly from the base of the column and the articulate lip holds from its apex. Both column and lip are commonly curved. In the species basal to the tree, the flowers not only present a basal chin (visible on the outer-side of the sepals), that accommodates the column foot, but they also present a second, median chin. This second depression on the sysnsepal, suggested by the author, serves the purpose of giving the movable curved lip a place to be fitted in. While maintained in the basal clades, the second chin is lost in *Unciferia*, *Petiolatae*, *Acuminatia*, *Niphantha* and *Condylago*. In *Dracontia,* the column foot curves forward, eliminating the basal chin, which is no longer needed. On the other hand, lip reduction and complete elimination of the column foot in clades *Salpistele* and *Stelis s.s.* have as an effect loss of lip mobility and of the chin, another good example of convergence. One particular case is worthy to mention. In clade *Dracontia* the lip is articulated to the column foot allowing movement, however *Stelis alta* has no lip mobility. A pair of high keels at the base of each petal blocks the lateral lobes, locking the lip into place. When the petals are removed, the lip moves freely.

Column shape, size and clinandrium and wings: The column of members of the group is in most cases arched. The exception is *Stelis s.s*. which presents very short straight columns. In most species of the group and also ancestrally, the column is subequal to the length of the lip, but in the derived groups *Mystacorchis*, *Elongatia*, *Salpistele* and *Dracontia* the lip is much longer than the column. The ancestral column has a relatively large clinandrium and two wings around the middle. In *Stelis s.s.* the clinandrium has disappeared as well as the column wings, instead the stigma lobes have become larger and widen the column apex. On the contrary, in *Dracontia* and *Mystacorchis* the column becomes narrower towards the apex. In *Salpistele,* the large clinandrium twisted 90 degrees and the wings disappeared, making of the column a long tube with an almost apical anther.

The presence of a long, winged arched column is clearly very important in the pollination of species of this group. In most cases, the lip is articulated and thus mobile, triggering pollination by moving the pollinator to touch the pollinaria and then again to touch the stigma. This would only be possible if both column and lip are curved and more or less equal in length. The column wings help to mechanically keep the pollinator in the "right" place.

In *Salpistele s.s.* and *Stelis s.s.* lip mobility is no longer necessary as the pollinaria and stigma are presented frontally on the flower. Lip and column curvature, lip articulation and column wings have lost their utility in these groups.

A third very curious system was found in *Dracontia* and *Mystacorchis*, where columns are very short and very much exceeded by the lip. Although lips are articulated they have lost the curvature and column wings are very much reduced. There is not much information as to their pollination process and no logical explanation was found for it, but it is clear that several characters

are correlated, the very short column covered by the petals, long thick cylindrical lip and the clear long thin canal present longitudinally from the base of the lip. With this combination of characters the two systems explained before would not be successful (Figure 12).

Anther, Stigma and Pollinaria characteristics: In *Stelis s.s*. the column is short, straight and unwinged. The anther and stigma have become apical and the stigma has now three well developed lobes. Pollinaria have suffered major modifications, they are now provided of two long, narrow, flexible and extremely elastic caudicles. But not only have the caudicles been modified, they are now attached to an oval, hard and sticky viscidium at the apex of the rostellum. The viscidium can be seen clearly as a drop of water placed at the base of the anther when still on the flower. Pollen removal in this case is much simpler, the pollinator just has to touch the frontal viscidium to displace the pollinarium (Figures 13 - 15).

An even more curious case is one of the novelties of this study. In clade *Petiolatae*, the bubble-like rostellum has become shorter, flatter and more perpendicular to the column and the stigmatic cavity. The viscarium only occupies the central, apical portion of the rostellum, instead of the entire distal rim, and is exposed on the puter side of the rostellum margin (instead of the inner rim). Through these modifications the viscarium lies very close to the anther. The pollinia, in turn, have lost their large, flat, whale-tail like caudicles and now present two narrow and short caudicles, which are almost directly in contact with the viscarium. As in the previous case, to remove the pollen, the pollinator needs to make contact only once. The two pollination syndromes differ only in the sense that, in this case, the pollinator must still “enter” the flower and touches the viscarium, and removes the pollen sacks when exiting the flower (Figures 13 - 15).

The last are most probably cases of convergence, where a two-step process is simplified to one step. The simplified pollination system present in *Stelis s.s*. could be one of the explanations for that clade´s explosive speciation.

Information was not found, nor have observations been made on the pollination, anther, stigma, and pollinaria characteristics of *Salpistele s.s.* and *Mystacorchis*, which seem to have apical or pseudoapical anthers and stigmas. They could prove to be more derived such as those of *Petiolatae* and *Stelis s.s*., however if, as suggested before, *Mystacorchis* has the same pollination syndrome as *Dracontia,* then we can expect their pollinaria to be similar.

Plotting Character Changes: As has already been stated, character states were plotted on the consensus tree using parsimony analysis in *Mesquite*. Characters are individually plotted in order to demonstrate their possible linkage. In the first case, between column shape and column/lip ratio, the narrowing short column versus long cylindrical lip present independently in *Dracontia* and *Mystacorchis*, in the second, linkage between apical anther and apical stigma present independently in *Mystacorchis* and *Stelis s.s*. and the linkage between stigma opening, caudicles

shape and pollinaria conformation, stigma being uncovered, caudicles narrow and viscarium/viscidium joint with the caudicles, present independently in *Stelis s.s.* and *Petiolatae* (Figures 12 & 15).

It was not possible, however, to show all character changes on the same tree at once. Instead, the consensus tree (same one as used in all other analyses) was transformed in *Mesquite* so that branch lengths were set to summarize amount of character state changes (Figure 16A), were one can clearly see how for instance, branches such as that of *Stelis s.s.*, *Petiolatae* and *Salpistele* are very long due to their amount of change from ancestral to derived states. It is of course logical to find branches at the base of the tree being shorter as they present most of the ancestral states. To be able to easily visualize which character changes are actually present in each of the branches, all characters were manually added to the tree (Figure 16B), trying to imitate that what was calculated by the software. A consistency index (CI) for the whole matrix is given, however it is not the case for each individual character tested because the cero branch lengths of most species within clades renders very low CI values (see more detailed discussion in the Further Anlyses section).

Finally, several of the most useful characters are visually represented with photographs for the understanding of the reader, they are plotted on the consensus tree (summarizing the nodes by clade), indicating in which point of the tree they were found to change from the ancestral state to a more derived one, and is some cases vice versa (Figure 17).

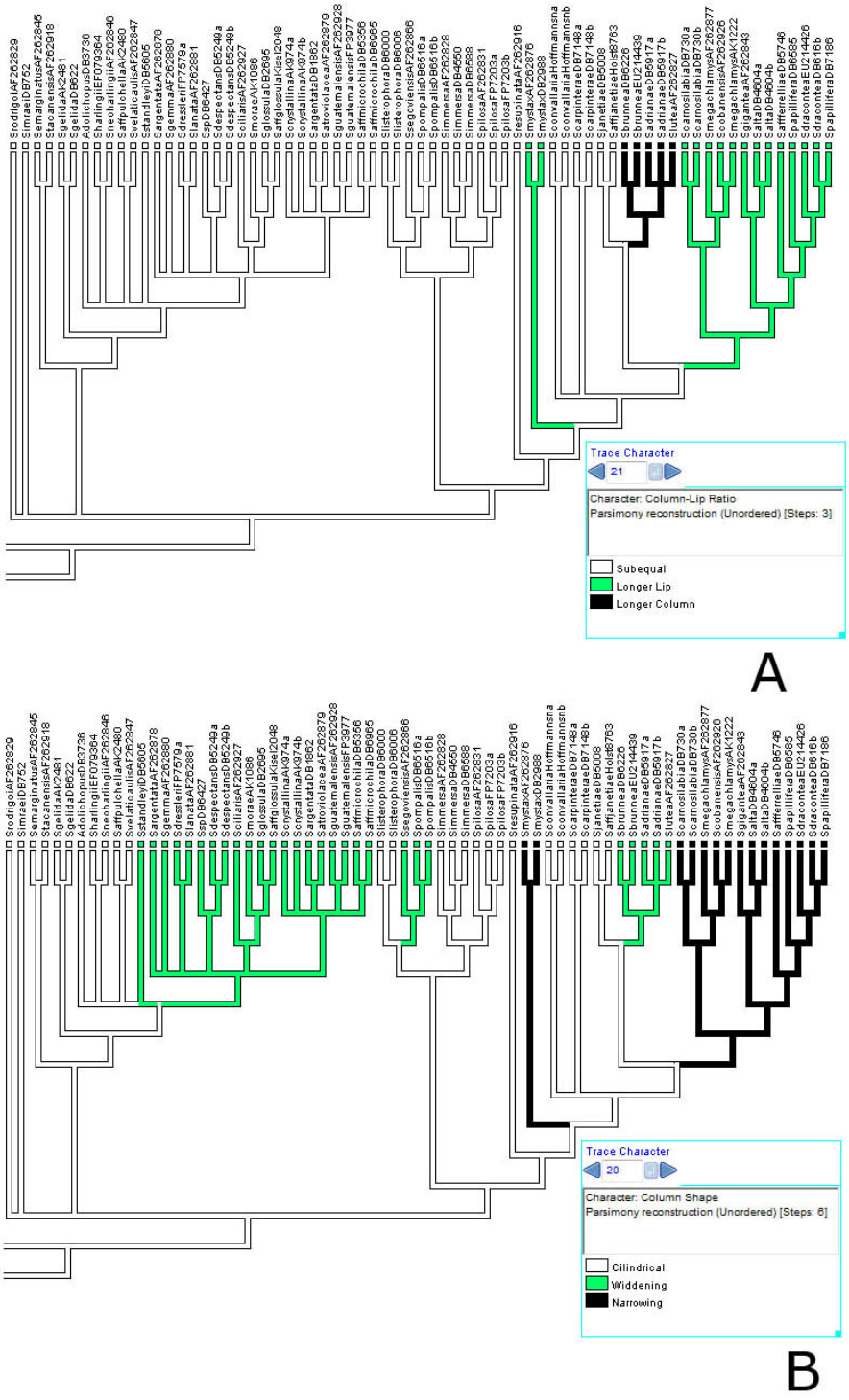

Fig 12. State history of characters 21, Column-Lip ratio (A) and 20, Column shape (B) plotted on the consensus tree from the Bayesian analysis on the concatenated data set, calculated by *Mesquite* with parsimony analysis. Where in both cases, white represents the ancetral state = 0, green = 1 and black = 2.

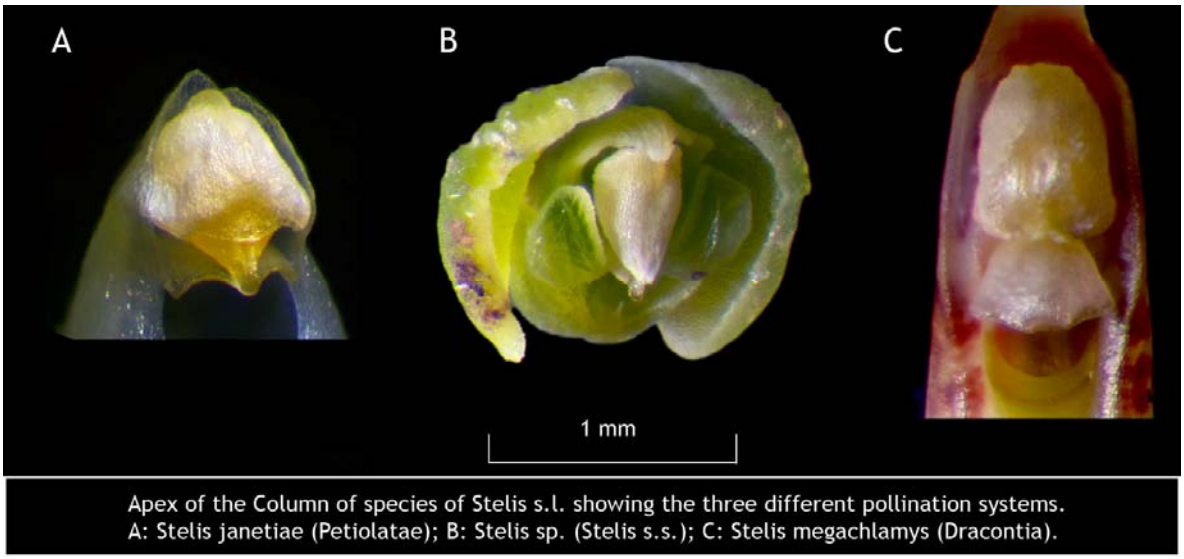

Figure 13: Apex of the column from diverse species of *Stelis s.l.* showing diversity in pollination syndromes and anther and rostellum position. A, shows the derived state were the rostellum is shortened and caudicles reach the translucid, sticky viscarium; B, shows the caudicles fixed to a drop-like, hard, sticky, translucent viscidium; C, shows flat, dry, whale-tail shaped caudicles at the base of the anther, and a buble-like rostellum, which covers the stigma, provided of sticky, loose-celled viscarium in its inner suface (not visible).

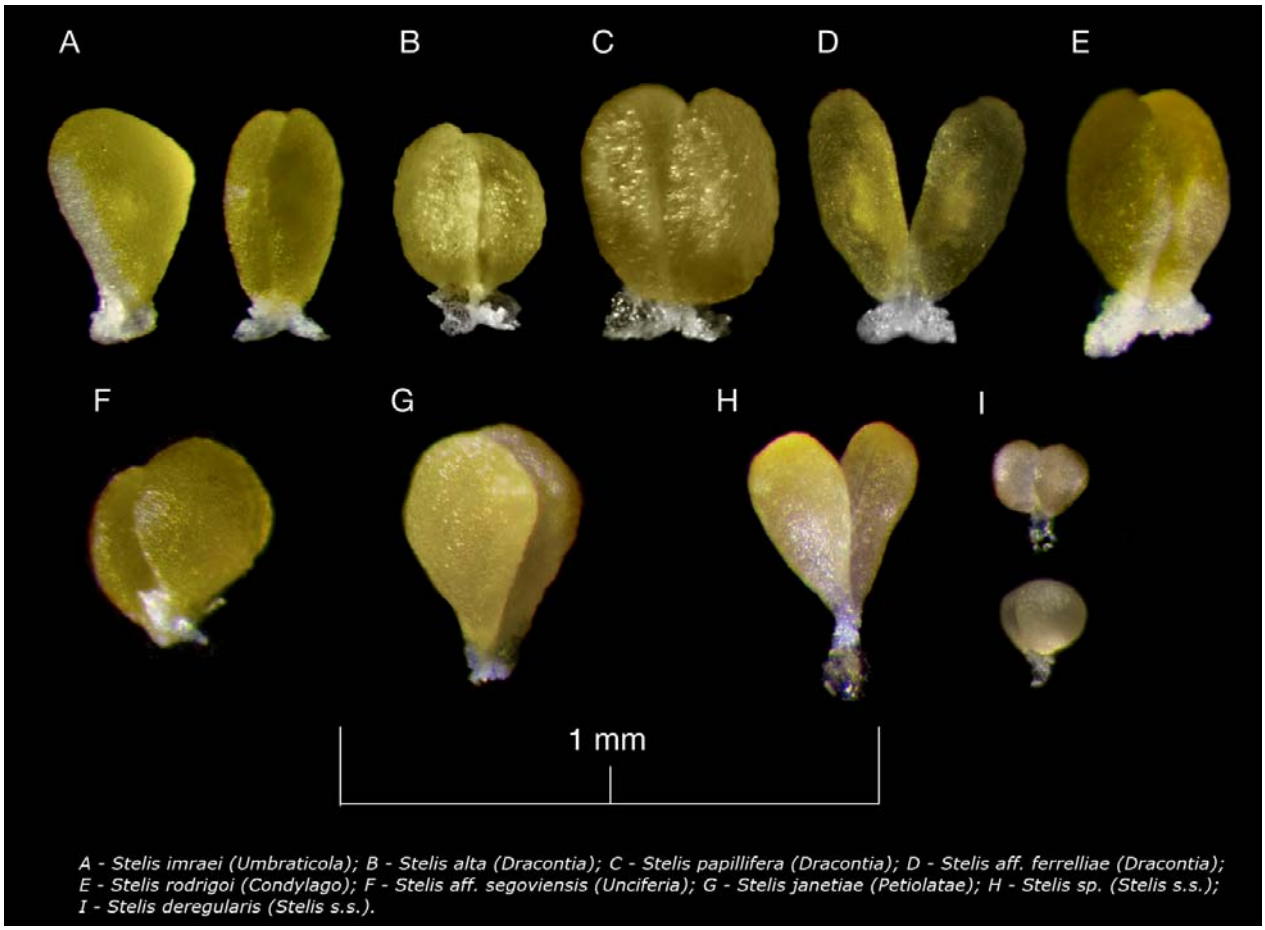

Figure 14: Pollinaria from diverse species of *Stelis s.l.* showing their morphological variability. A to F show the most frequent and ancestral state, “whale-tail” caudicles free from the viscarium (not visible); G shows a more derived state, were the “whale-tail” caudicles have been reduced and not touch the viscarium (not visible); H & I show the derived state were caudicles have become short and narrow and are now fixed to a drop like, sticky, hard and translucent viscidium.

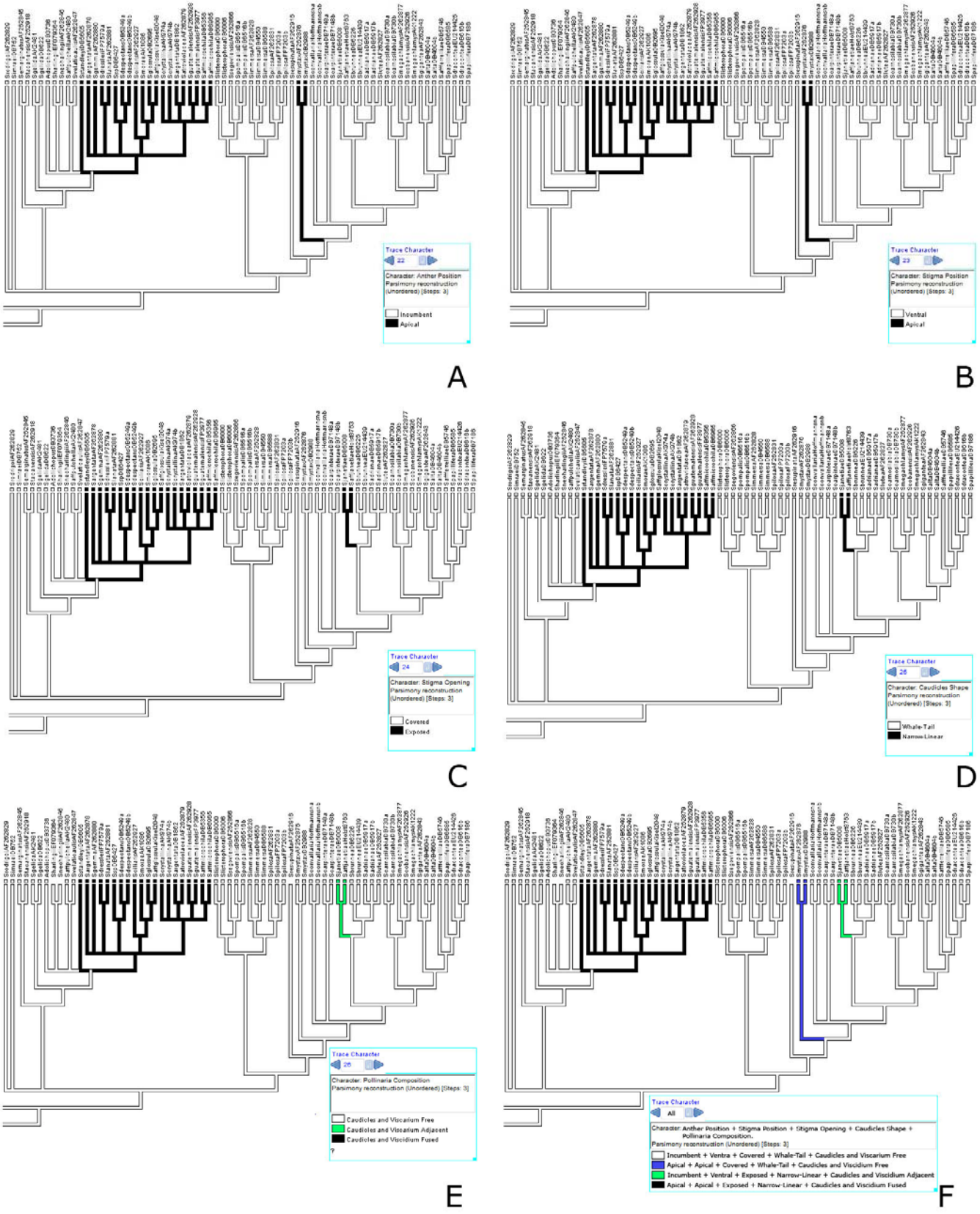

Fig 15. State history of characters 22 to 26 plotted on the consensus tree from the Bayesian analysis on the concatenated data set, calculated by *Mesquite* with a parsimony analysis. *A,* anther position; *B*, stigma postion; *C*, stigma opening; *D*, caudicle shape; *E*, pollinaria composition; *F*, all characters combines. Where white represents the ancetral state = 0, while green and black represent derived states (*A*-*E*). In *F*, white is the ancestral state, blue and green have some derived states and black has all devired states.

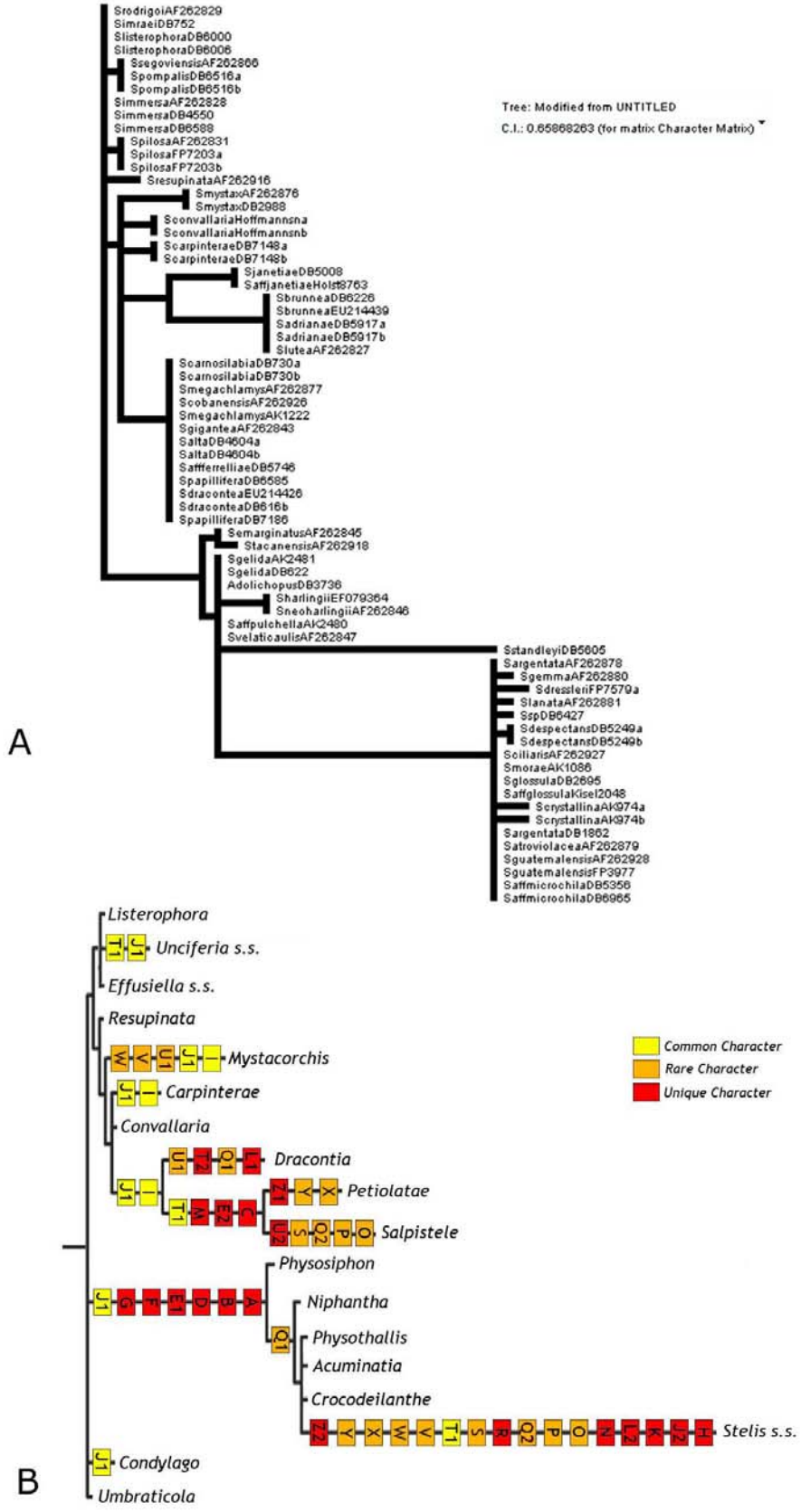

Figure 16: (*A*) Branch lengths in the consensus tree were transformed to represent amount of state changes, calculated with a parsimony analysis in Mesquite. (*B*) All character changes were plotted manually on the branches in order to visualize which character is changing and from which state. Character coding follows that presented in the results.

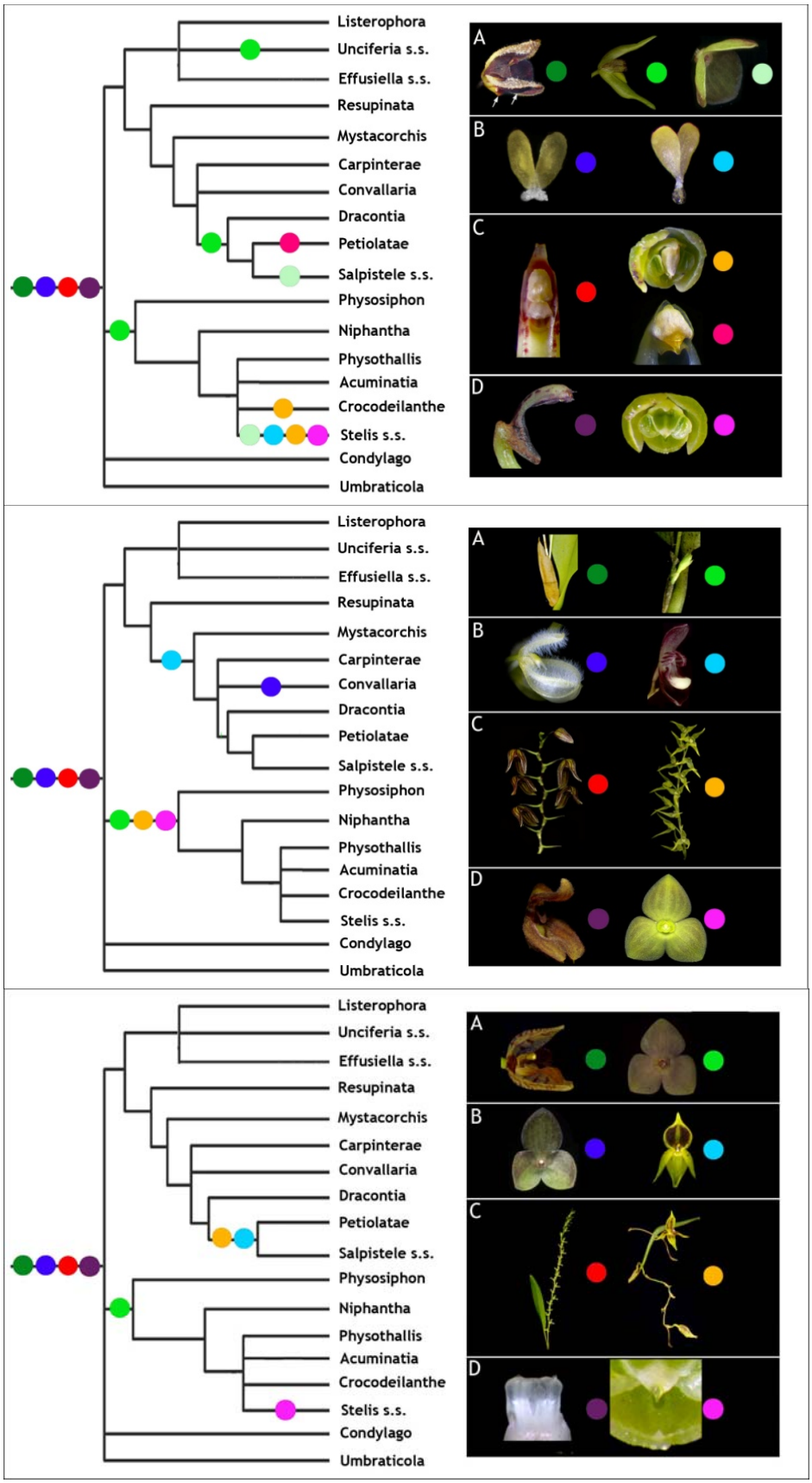

Figure 17. The consensus tree from the Bayesian analysis of the concatenated matrix is summarized keeping the original tree topology but substituting groups of species for clades, and modifying the branch leghts. Several morphological state changes are presented graphically from more ancestral to derived states. Characters are 1, A: chin presence, B: pollinaria composition, C: pollination syndrome, D: column shape; 2, A: spathe, B: indumentums, C: florescence type, D: sepal fusion; 3, A: sepal coloration, B: sepal/petal ratio; C: inflorescence habit; D: glenion presence.

4.4. Nomenclature

It would be almost impossible not to mention the nomenclatural aspects of this study. Having already two quite different systems, the advantages and limitations of each one will be discussed here, and the necessary modifications will be done.

The simplest way to deal with the group would be to apply to large concept of *Stelis* (*Stelis s.l.*) proposed by Pridgeon *et al.* in 2001. However, it is currently both paraphyletic and polyphyletic. *Stelis quadrifida* (here clade *Loddigesia*) is certainly not part of the group and should be therefore moved to another genus, on the other hand, some 27 species transferred by Pridgeon *et al.* to the genus *Anathallis* Barb. Rodr. are actually very closely related to *Stelis s.s.* and are surely part of *Stelis s.l.* They are all related to *A. acuminata*, *A. dolichopus* and *A. sclerophyla* (here clade *Acuminatia*). Several species have also since 2001 been published in genera considered synonyms of *Stelis* and have therefore no combination in *Stelis*, the species include *Condylago furculifera*, *Effusiella hamiltonii*, *Effusiella skolnickiae*, *Niphantha pidax* (for some reason transferred to *Anathallis* by Pridgeon *et al.*) and *Salpistele adrianae*.

On the other hand, the alternative fine splitting done mostly be Luer, has several advantages, species of several of his genera concepts are easily recognized as such, making determination efforts much easier both in the field as in herbaria and living collections. *Stelis s.s*. is easily recognized morphologically, while almost no character distinguishes all members of *Stelis s.l.* from all other Pleurothallids. However although several proposed genera seem monophyletic, *Dracontia, Salpistele, Unciferia, Niphantha, Physothallis, Physosiphon, Condylago and Mystacorchis*, several others are not, *Elongatia* and *Effusiella* are both paraphyletic and polyphyletic. To maintain genera such as *Salpistele* and *Dracontia*, several new genera would have to be proposed for some members of *Effusiella* and *Elongatia*, and a new genus should be proposed for the members of *Pleurothallis* subgen. *Acuminatia* sect. *Acuminatia*. Additionally, new generic names have to be proposed for *Loddigesia* and *Unciferia*, both already taken, while genus *Niphantha* was invalidly published.

In contrast, several of the genera are supported by molecular and morphological data, and supported by geographical evidence, some of them being quite strong and enough to segregate to group in smaller genera. Several species of *Elongatia* and *Effusiella* are missing in the study, and their inclusion could clarify the relationships between species groups. For now, each clade formed in the phylogenetic tree is discussed, including its morphological characteristics and geographical distribution.

It is noteworthy to point out that Neyland *et al.* (1995) proposed a phylogeny of the Pleurothallidinae based on morphological characters; however it completely contradicts the molecular phylogeny proposed by Pridgeon *et al.* (2001) and the findings in this study. One reason could probably be that in such a large and diverse group, most characters have evolved several times throughout the whole group, and so several species groups share characters without being necessarily related to each other. Neyland and his coworkers selected characters such as differentiated leaf mesophyll, anther position, flower resupination, velamen cells, hypodermal layers in the leaf and sensitive labellum to produce their clades. However they failed to relate the genera *Condylago*, *Physosiphon*, *Physothallis*, *Salpistele*, *Stelis* and species *Pleurothallis dolichopus*, here treated as clade *Acuminatia*), and actually allocated all of them to different clades. Whitten *et al.* (2007), in their phylogeny of mammoth genus *Maxillaria*, found many characters that had evolved several times throughout their trees. They were therefore not useful as single distinguishing characters, but when combined with others proved to be quite useful to separate clades, as is done for the *Stelis s.l*. clades hereafter.

Acuminatia, Crocodeilanthe and *Niphantha*: Clade *Acuminatia* is more the confirmation of a theory than a surprise. In 1999 Luer proposes *Pleurothallis* subgen. *Acuminatia*, with two sections, *Acuminatae* and *Alatae*. In 2001, after his molecular findings, Pridgeon *et al.* transfers all of the species of the subgenus to *Anathallis*, the lectotype of which is *Anathallis fasciculata* Barb. Rodr. (synonym of *A. obovata* Lindl.), part of sect. *Alatae*. In one of those cases of bad luck, the four species he uses in his analysis all belong to sect. *Alatae*. *Anathallis dolichopus*, of sect. *Acuminatae*, is the only species included in this study and as already expected for its morphological affinities to *Stelis*, it is not a close relative of *Anathallis*. From morphological characteristics, it can be suggested that a bit less than one fourth of the species of subgen. *Acuminatia* belong to *Anathallis*, the rest are more related to *Stelis*. Most Brazilian species are probably closer to *Anathallis* than to *Stelis* if the geographical trends of *Stelis s.l.* are considered.

Genus *Crocodeilanthe* was described by Rchb.f. & Warsz. in 1854 to accommodate *Crocodeilanthe xiphizusa* Rchb.f. & Warsz., but was after that never used again, until in Luer (2004) transferred dozens of species to the genus. *Niphantha* was proposed by Luer in 2007 to accommodate two “misfit” species. He cites an unpublished molecular phylogeny as the evidence to separate the species from genus *Effusiella* (also clearly supported by the findings here), but fails to publish a Latin description, therefore invalidating the genus.

Morphologically, species from this group are quite similar to *Stelis s.s.* in general plant habit and floral morphology. Inflorescences are mostly densely flowered and simultaneous. Flowers start to have a fan-like calyx (especially in *Acuminatia*), were a synsepal is not clearly present, and sepals

are divergent. Sepals are hirsute and never maculate, both characters shared with *Stelis s.s.* They differ, however, from *Stelis s.s.*, in the elongated column and lip, which as in most other clades of *Stelis s.l.* are curved, parallel and subequal to each other. They also do not have glenion at the base of the lip, have an incumbent anther and a ventral stigma covered by the bubble shaped rostellum. The rostellum is provided by a sticky loose celled viscarium at its apex, it is well separated from the whale-tail shaped, dry and flat, caudicles. All suggesting although general morphology is similar to *Stelis s.s.* they keep a more ancestral reproduction system.

Carpillaria: Clade Carpillaria is here represented by two species, *Stelis* Carpillaria and *Stelis convallaria*. The first belongs to Luer´s *Elongatia*, while the second has been placed in *Effusiella*. The two species are quite different from each other morphologically and probably do not belong together. The clade is only weakly supported with ITS data, while it is dissolved using *matK*. The inclusion of several species allocated to *Elongatia* and *Effusiella* would probably split these two taxa. They are both Central-American endemics.

Condylago: Clade *Condylago* is one that has been a bit of a mystery. The genus was described in 1982 by Luer on the basis of one species, *Stelis rodrigoi*. More recently a second species was described, *Condylago furculifera* (without combination in *Stelis*). During DNA amplification, samples never successfully amplified, even after various re-sampling, re-extracting, and modifying concentrations of DNA, buffers, TAQ and others. Even more surprisingly, the same specimen was sent to the laboratory of the University of Costa Rica, and even then, with a different protocol and technicians, it was the only species that would never amplify. *If anyone doubted whether the two species were different, now we know for sure they are, one has DNA and the other one not!*

Dracontia: *Dracontia* was established in 2004 by Luer, it includes several species related to *Stelis megachlamys* (Type). Several species attributed to that genus have been included here, *S. alta, S. carnosilabia, S. cobanensis,* S. *dracontea*, *S. aff. ferrelliae*, *S. gigantea*, *S. megachlamys*, *S. papillifera*, and *S. alajuelensis*. *Dracontia* is well supported and probably also includes the other species of *Stelis* allocated by Luer to the genus that have not been included here. *S. cobanensis* and *S. oblongifolia* (Lindl.) Pridgeon & M.W. Chase were transferred to the genus *Rhynchopera* by Luer after having placed them in *Dracontia*. The molecular and morphological evidence presented here suggest it is a mistake, as *S. cobanensis* is the closest species to the type of the genus *Dracontia*, *S. megachlamys*.

Species of *Dracontia* can be easily recognized by several morphological characteristics, plants are medium to large (except *S. carnosilabia*), with successive inflorescences that exceed the

leaf, frequently curving because of the length and weight of flowers. Flowers are among the largest of the whole *Stelis* s.l. group and are glabrous (except for *S. dracontea*). Most are purplish in color and several seem immaculate, however, most flowers are actually greenish-yellow, covered by very large purplish blotches. Sepals are long and acuminate, the lateral sepals are fused at least to the middle, forming a synsepal which is very similar to the free dorsal sepal. Petals are much shorter, in most cases they are suborbicular and obtuse, they have involute margins, colored veins and papillae on the outer surface (exceptions are S. *alta* and S. *gigantea*n which have linear, straight and glabrous petals). The fleshy cylindrical lip is very much ornate, and is provided with narrow channel like depression crossing it from base to apex. The lip is hinged to a very reduced and inward curved column foot, however, in some (all?) cases the lip is immobile in its natural position. The reason seems to be a pair of tall carina at the base of the petals that block the lip basal lobes and therefore its movement. The column is much shorter than the lip, it narrows towards the apex, the anther is incumbent and stigma is ventral. The stigma is covered by a bubble like rostellum. Pollinaria are provided with two whale-tail shaped, flat and dry caudicles.

Effusiella s.s., Resupinata and Listerophora: These clades all belong to Luer´s generic concept of *Effusiella*, the type species being *Stelis pilosa*. *Effusiella s.s*. includes that species and *Stelis immersa*, however several other species seem to be morphologically closely related to these and would probably be part of this clade, they are *S. cypripedioides* (Luer) Pridgeon & Chase, *S. kefersteiniana* (Rchb.f.) Pridgeon & Chase, *S. lehmanneptis* (Luer & Escobar) Pridgeon & Chase and *S. trichostoma* (Luer) Pridgeon & Chase. Clade Resupinata is here represented by *Stelis resupinata*, which seems to have no clear close relatives. Clade Listerophora is made up by two samples of the species *Stelis listerophora*, which is probably very close to *Stelis retusa* (Lex.) Pridgeon & Chase and *Stelis sotoarenasii* Solano.

Morphologically, species of these clades also keep most of the “ancestral” characters. Plants are similar to those of *Unciferia s.s*., with narrow leaves, much longer than the ramicaul. Flowers mostly have maculate and hirsute sepals (*Stelis pilosa* is immaculate). Sepals are elliptic, longer than wide and much larger than the petals. The two lateral sepals are fused into a concave synsepal, the fusion being above the middle of the synsepal. The dorsal sepal is free. Two chins can be seen on the underside of the synsepal, one basally and another close to the middle. Petals are straight, but the mid-vein is not thickened making the petals flat. The convex (curved downward) lip is attached to the column foot and can move freely, in natural position it rests on the synsepal. The column is parallel to the lip, and is provided with a pair of wings. It becomes wider towards the apex and is at the base provided with a prominent straight column foot. The incumbent anther is placed at the apex and is covered by a prominent androclinium. The stigma is covered by a bubble-

like rostellum. The rostellum is provided by a glue-like loose celled viscarium impregnating the whole inner margin. The two pollinaria are provided of two whale-tail like caudicles, which are one celled and dry. The caudicles can be seen twisted upwards at the apex of the anther.

Mystacorchis: The species is on its own in the clade, and seems to have no close relatives. This curious taxon presents small plants (below 5cm tall), the ramicaul is equal to or longer than the broadly elliptic leaf. The multiflowered inflorescence is clearly successive, with only one flower developed at a time. The relatively large flower is greenish-white largely tainted with dark purple. Sepals are long and elliptic, the two lateral sepals are fused until around the middle, the free portions are strongly divergent. The much shorter petals are somewhat covering the column. The very long lip is provided with a long, almost cylindrical, channeled claw attached to a suborbicular blade. The column and reproductive organs of this species have not been studied, however, Luer (1986) suggests a very short column with apical anther and stigma.

Loddigesia: This clade has only one species, *Stelis quadrifida*, which was segregated by Luer into the monotypic genus *Loddigesia* in 2006. The name is however invalid as it is already taken in Fabaceae. As can be quickly seen in all the trees, the species is not part of *Stelis* in any of its senses, it is actually related to the *Pleurothallis* group, and should be moved there. An unpublished phylogeny produced by Mark Wilson suggests it is quite isolated within the *Pleurothallis* group, if so, it would need a new generic name.

Petiolatae: This clade is one of the main novelties of this study, allocated to the genus *Elongatia* by Luer (2004), clade *Petiolatae* includes two species endemic of Costa Rica and Panama, *Stelis guttata* and *Stelis janetiae*. They are certainly not closely related to the other species of that genus, and make a very strong sister clade to *Salpistele s.s.* Even though it is the first time that a relationship between those groups is noted it is not so farfetched when general plant and flower morphology, and geographical distribution are considered.

Plants from this clade have a rather small habit (below 10 cm tall), ramicauls are much shorter than the elliptic leaf. Inflorescences are creeping and successive, having only one mature flower at a time. Flowers are greenish-white frequently maculate with dark purple or brown. Sepals are ovate, the lateral one fused completely into a synsepal which is almost identical to the dorsal sepal. Petals are subequal to the sepals and serrulate/dentate. The lip is articulated to a straight column foot. The column is subequal to the lip, curved and provided with prominent wings and androclinium. The anther is incumbent and the stigma ventral. The pollinaria are provided with a pair of “whale-tail” like caudicles, which are quite reduced and narrow compared to other groups.

The stigma presents a “bubble” like rostellum, which instead of covering the stigma (as in most cases) has been reduced, flattened and erected in order to reach the anther. The viscarium has been accumulated at the apex of the rostellum and now reaches the caudicles.

Salpistele s.s.: Clade *Salpistele s.s.* includes five species endemic of Costa Rica and Panama, which have all been recognized as species of the genus with the same name. They are closely related to clade *Petiolatae*.

Plants from this clade have a rather small habit (below 5 cm tall, most not even 2 cm tall), ramicauls are much shorter than the elliptic leaf. Inflorescences are creeping and successive, having only one mature flower at a time. Flowers are frequently maculate with dark purple or brown. Sepals are ovate, the lateral one fused completely into a synsepal which is almost identical to the dorsal sepal. Petals are subequal to the sepals. The trilobed hirsute lip seems to be attached directly to the column, therefore no column foot nor is lip mobility is present. The column is much longer than the lip, cylindrical, wingless and with its apex twisted 90º making the anther and stigma seem apical. However it was impossible to study the column and pollinaria characteristics in detail; Luer (1991) suggests that anther and stigma are apical and that the pollinaria are provided with a pair of caudicles attached to a microscopical viscidium. It is possible that a similar syndrome as that of *Petiolatae* is actually the case, where the rostellum apex is very close to the caudicles, making the viscarium touch the caudicles.

Physosiphon and *Physothallis*: Genus *Physosiphon* was described by Lindl. in 1835 and had harbored several species from distinct genera over the years. In its recent treatment of the genus, Luer (2007), considers that only two species belong in the genus, the highly variable *Physosiphon emarginatus* and *P. asperrimus* Luer. The first having about a dozen synonyms described over its wide distribution. *Stelis greenwoodi* Soto Arenas & Solano and *Stelis tacanensis* Soto Arenas & Solano clearly belong to the *Physosiphon* concept and are quite different from *P. emarginatus*. Genus *Physothallis* Garay (1953) is an Ecuadorian endemic with two species, *P. cylindrica* Luer and *P. harlingii*.

It was not possible to study living specimens of member of these clades, morphological characteristics can only be taken from literature. They are superficially similar to *Stelis s.s*., but differ in several floral details. *Physosiphon*, has all three sepals fused until at least the middle (unique within *Stelis s.l.),* forming a tubular flower which is constricted at the apex of the tube. The free portions of sepals are divergent and in some (all?) cases pubescent. *Physothallis* on the other hand presents the two lateral sepals almost completely fused to the dorsal (also unique in *Stelis s.l*.). In both, the reduced column and lip still present the “classic” curved shape and are subequal

to each other. The somewhat trilobed lip is hinged, from a straight column foot, and can probably be moved. The anther seems incumbent and stigma ventral. Pollinaria were not seen, but from the other characteristics one might suggest they have “whale-tail” shaped caudicles.

Stelis s.s.: Refers to the narrow concept of *Stelis*. It includes about 900 species more or less with the same basic floral morphology.

Plant morphology is very variable in this large group, the habit can be caespitose or repent, tall (up to 30 cm or more) or short (below 5cm), the ramicaul can be longer, subequal or shorter than the linear, elliptic or suborbicular leaves. The erect inflorescence is borne from a foliaceous spathe and is simultaneous (all or most flowers open at once). Flowers are resupinate and with an almost perfect horizontal disposition perpendicular to the inflorescence (an almost unique character within *Stelis s.l.*). The ovate sepals are in most cases variously hirsute and suffused (never maculate) with a light color. All three are mostly equally fused below the middle, with spreading free portions, forming a fan-like calyx. The equally long as wide petals are much shorter than the sepals and have a recurved, thickened apex. The lip is similar to the petals, very short and thick, provided with a basal glenion, and immobile. The column is straight and short, stout, cylindrical, widening towards the apex, wing-less, with an apical anther and stigma. A column foot which is suggested by Luer (2009) was not seen, if present, it would be very much reduced and with no apparent functionality. The stigma is trilobed with one lobe transformed into a triangular rostellum positioned just below the anther. The ovate acute anther covers two rounded pollinaria. The pollinaria are provided with a pair of cylindrical (linear?) caudicles which are attached to a sticky, hard viscidium. The viscidium looks like a drop of water put on the apex of the column.

In this very large group many species have diverged from the typical morphological character states, several species have successive inflorescences, glabrous sepals, convergent lateral sepals, elliptic and flat petals, and a curved, elongated column. These divergent states are never found all together in one species. Garay (1979) tried to segregate a group of species from *Stelis* on the basis of an unlobed stigma, however throughout the group, the stigma is variable sometimes seeming clearly lobed and others not at all, without any clear phylogenetic pattern.

Umbraticola: Clade Umbraticola is one of the unexpected results of this study, it was included by Luer in the genus *Effusiella*, but as far as ITS goes, it is basal for the whole *Stelis s.l.* group. Its most common species and only representative in this study was *Stelis imraei*. But it has several close relatives, which will surely sit next to it in the clade, they are *S. cocornaensis* (Luer & R. Escobar) Pridgeon & Chase, *S. erucosa* (Luer & R. Escobar) Pridgeon & Chase, *S. tarantula* (Luer & Hirtz) Pridgeon & Chase and *S. vaginata* (Schltr.) Pridgeon & Chase, the group can be found from Costa

Rica to Peru and Brazil, but most species are present in Colombia and Ecuador. This is a very interesting case, as the consensus network suggests some intermediate relationships between Umbraticola and both *Stelis s.l.* and *Pleurothallis s.l.,* and could be a sort of "ancestral *Stelis*".

Morphologically, species of Umbraticola have all the characters considered basal for the *Stelis s.l*. group. Leaves are round or ovate, shorter than the peduncle and almost horizontally placed. The successive, erect, inflorescences are born at the apex of the ramicaul in fascicles from a papery spathe. The variously maculate (some forms are immaculate) flowers are mostly found covered by the somewhat convex leaf (hence the name umbraticola, which means "in the shade"). The elliptic sepals are longer than wide and much larger than the petals. Petals are straight, and have a very much thickened mid-vein. The two lateral sepals are fused into a concave synsepal, the fusion being up to about the middle of the synsepal. The dorsal sepal is free. Two chins can be seen on the underside of the synsepal, one at the base and the other at the middle. The convex (curved downward) lip is attached to the column foot and can move freely. The column runs parallel to the lip and has a prominent straight column foot. The incumbent anther is placed at the apex and is covered by a prominent androclinium. The stigma is covered by a bubble-like rostellum. The rostellum is provided by a glue-like loose celled viscarium impregnating the whole inner margin. The two pollinaria are provided of two whale-tail like caudicles, which are one celled and dry. The caudicles can be seen twisted upwards at the apex of the anther.

Unciferia s.s.: Lindley had already proposed *Uncifera* in 1859 also in Orchidaceae, and even though the names are not identical, the botanical code regards Luer´s *Unciferia* as invalid for being too similar. Although several samples of Luer´s concept of *Unciferia* were initially included, *Stelis aff. canae*, *Stelis pilostoma, Stelis pompalis*, *Stelis segoviensis* and *Stelis aff. segoviensis*, but only from *Stelis pompalis* was it possible to obtain sequences (and very short ones). All species are quite closely related and, without a doubt, will all turn up to be next to each other in the *Unciferia s.s*. clade.

Morphologically, species of clade *Unciferia s.s.* keep most of the "basal" characters. Plants are shorter than 20cm, the narrow leaves are much longer than the ramicaul, and the successive inflorescence exceeds the leaf. Flowers have maculate and hirsute sepals (in some cases they could be immaculate and seem glabrous). Sepals are elliptic, longer than wide, and much larger than the petals. The two lateral sepals are fused into a concave synsepal, the fusion being above the middle of the synsepal. The synsepal is in most cases ornate with a large carpet of papillae from the base to the apex, along the fusion point. The dorsal sepal is free. Only one basal chin can be seen on the underside of the synsepal. Petals are straight, but the mid-vein is not thickened, making the petals flat. The convex (curved downward) lip is attached to the column foot and can move freely, in

natural position it rests on the synsepal and can therefore only move “upwards”, it is provided with a pair of horn-like lobes at the base. The column is parallel to the lip, and is provided with a pair of wings. It becomes wider towards the apex and is, at the base, provided with a prominent straight column foot. The incumbent anther is placed at the apex and is covered by a prominent androclinium. The stigma is covered by a bubble-like rostellum. The rostellum is has a glue-like loose celled viscarium impregnating the whole inner margin. *Unciferia* with two whale-tail like caudicles, which are one celled and dry. The caudicles can be seen twisted upwards at the apex of the anther.

5. Conclusions

Stelis in its strict sense had been generally accepted as a monophyletic natural genus up to 2001 when Pridgeon *et al.* (2001) redefined the genus by adding several small genera and subgenera and sections of *Pleurothallis* to it. Their phylogenetic analysis showed a monophyletic *Stelis s.l.* group, sister to *Pleurothallis*. However, only a handful of species were included in those studies, excluding several species clades.

The broad concept of the genus has proven very difficult to characterize morphologically. Plants and flowers are very varied, leaving almost no distinguishing features for the genus. Therefore, the broader concept of the genus is not universally accepted and several authors consider that some smaller genera can be distinguished within *Stelis*.

Making smaller groupings (genera or clades or any other) makes it easier for taxonomists to determine plants belonging to the genus in the field and, herbaria as there are less species per group as has been stated by several authors. However, if groups are too small, the contrary occurs, instead of easily finding the species, not even the correct genus can be found, which hampers identification and species description. Chase *et al.* (2000) suggest that it is their philosophy to reduce the number of families and genera to a necessary minimum, and defend their position mostly based on the easiness of remembering less names and maximizing the information content of the system. In both cases taxonomic opinion plays a big role, and systems may be followed for their advantages rather than actual evolutionary relationships. One must take care of not exceeding in splitting closely related species into very small groups, but also not in lumping little related species into very large ones, just for the sake of having less names. It is evolutionary relationships which we finally want to use to group species.

New molecular, morphological and geographical evidence presented here suggests that the broad concept of *Stelis* is both paraphyletic and polyphyletic. It also suggests that several of the smaller genera proposed by various authors within *Stelis* are supported as natural groups. The older narrower concept of *Stelis*, is found to be monophyletic, with several “heavy weighted” morphological modifications, which affect floral morphology and especially its reproductive system which is even structurally different, and by itself is already enough to justify generic distinction. Even though it has the largest amount of species, its floral morphology is almost unvaried and unique among all other clades of the group. There is high molecular support for the clade and it is strengthened by geographical data.

Genus *Salpistele* proposed by R.L. Dressler is highly supported by molecular data from all sources. It also presents several “heavy weighted” morphological modifications which make it

unique within the group, especially those concerning floral morphology; such as, very small plants, creeping inflorescences, no column foot, a long cylindrical column, a very short hirsute tri-lobed lip, to name a few of the unique characters of this genus. All the species are endemic to Costa Rica and Panama, and more specifically to the Talamanca Cordillera shared by both countries. The clades *Dracontia* and *Petiolatae,* similarly, are well supported as distinct groups, they have several morphologically unique traits and have clearly Central-American distributions.

The same discussion can be held about some of the other clades which represent natural groups, and which supported by all three evidence sources, DNA sequences, morphology and geographical distribution, could be justifiably considered distinct genera. Those clades include *Acuminatia*, *Crocodeilanthe*, *Mystacorchis*, *Niphantha*, *Physosiphon* and *Physothallis*, which have all at some point already been recognized as separate genera, except for *Acuminatia*.

On the other hand, the relationships between some of the other clades are not quite resolved, especially those more basal to the group. Luer´s genera *Effusiella* and *Elongatia* are both paraphyletic in the molecular analysis, and are very varied morphologically and have no clear geographical pattern (both supporting they are paraphyletic). The first includes clades Convallaria, *Effusiella s.s.,* Listerophora, Resupinata and Umbraticola, which all have unclear relationships with each other and with other clades. The second includes clades Carpinterae and *Petiolatae* which are not sisters of each other. Luer´s genus *Unciferia* is monophyletic, but is entangled with a few clades of genus *Effusiella*, clade Listerophora is morphologically very similar to the species of *Unciferia*. Its species can be well recognized morphologically and its center of diversity is the Talamancan Cordillera between Costa Rica and Panama. *Condylago* has several unique morphological characters, but also presents similarities with the clades of genera *Effusiella* and *Unciferia*. It is an endemic of Colombia and Panama. In the molecular analysis it was found basally in the group and somehow entangled with the clades of genus *Effusiella*.

Even when there is strong evidence to support the disarticulation of the broad concept of *Stelis* into various smaller generic concepts, it is not possible to adequately define the basal clades (genera) of the group. In order to do so, more species of the clades pertaining to Luer´s genera *Condylago*, *Effusiella*, *Elongatia* and *Unciferia* have to be included in the study. The relationships between *Niphantha*, *Crocodeilanthe*, *Physothallis* and *Acuminatia* are also not completely resolved and also there are more samples required.

This study has provided a very strong starting point for any reconsideration of the generic classification of the genus *Stelis*, presenting evidence from many more species and groups than previously included and considering evidence from other sources such as morphology and geography. In order to attain monophyly, several changes have to be made to the actual classification systems of this group. If the narrow genus concept is accepted, new generic names

should be proposed at least for clades *Acuminatia*, *Petiolatae* and Umbraticola, also for the invalid names *Loddigesia* and *Unciferia* and genus *Niphantha* has to be re-published validly, additionally several new combinations would have to be made to accommodate all the species to their adequate genus. On the other hand, if the broad concept is maintained, it should not include clade *Loddigesia*, which is closer to *Pleurothallis*, and species of Luer´s *Pleurothallis* subgen. *Acuminatia* sect. *Acuminatea* which had been transferred to *Anathallis* would have to be included in *Stelis*.

5.1. New Genera and Combinations

Strong evidence was provided in the previous chapters to support the disarticulation of *Stelis* in its broad sense. Genera have already been proposed for almost all of the clades that I have discussed, but some remain name-less. In order for all groups to attain monophyly and to make them as natural as possible, several new genera and combination have to be made. I will not attempt to rearrange the *Unciferia s.l.* (which includes clades *Effusiella s.s*., Listerophora and Resupinata, excluding clade *Unciferia s.s*. which is monophyletic) and Carpillaria clades (with clades Carpinterae and Convallaria), as there is not enough information to make any changes responsibly. The species in those clades have all been allocated by Luer to the genera *Effusiella* and *Elongatia*, which are probably polyphyletic, but for now will have to do.

Fraternaria Karremans, gen. nov.

Ety.: From the Latin "*Frater*", brother, as it is endemic to the shared mountain range between brother nations Costa Rica and Panama.
Type : *Pleurothallis guttata* Luer, Selbyana 3. 116 (1976).
Syn.: *Pleurothallis* R. Br. subgen. *Elongatia* Luer sect. *Petiolatae* Luer

Planta epiphytica parva. Foliis petiolatis. Inflorescencia racemosa laxa repente. Sepalis ovatis concavis glabris. Sepalo dorsali liberi, lateralibus connatis. Petalis quam sepalis aequilongis ellipticis margine crenulato. Labello elliptico margine crenulato. Columna subterete. Pollinia duobus.

Genus *Fraternaria* Karremans represents what has been called throughout this document clade Petiolatae. Species of the clade were previously classified as part of genus *Elongatia* by Luer, however, from all evidence sources they are not closely related to the other species of that genus. *Fraternaria* is sister to *Salpistele* having a similar distribution (endemic of Costa Rica and Panama), plant habit and inflorescence characteristics. Floral structure is however quite different, it has a well developed column foot, and an elliptic lip with a crenulated margin. Its pollination syndrome is that which sets it aside the most within the *Stelis s.l.* The ancestral bubble like rostellum has shortened and become perpendicular to the column permitting the viscarium to make contact with the narrow caudicles of the pollinaria. Two species are known, but some populations do not seem to fit the criteria for either species and could prove to be different species.

Fraternaria guttata (Luer) Karremans, comb. nov.

Bas. *Pleurothallis guttata* Luer, Selbyana 3. 116 (1976).

Fraternaria janetiae (Luer) Karremans, comb. nov.

Bas. *Pleurothallis janetiae* Luer, Selbyana 5: 169 (1979).

Niphantha Luer ex Karremans, gen. nov.

Ety.: From the Greek "*snow-flake flower*" in reference to the frosty appearance of its flowers.
Type: *Pleurothallis gelida* Lindl., Edwards`s Bot. Reg. 27: Misc. 91, 1841.

Planta epiphytica grande. Foliis latis suborbicularis. Inflorescencia racemosa erecta. Sepalis ovatis concavis pubescentibus. Sepalo dorsali liberi, lateralibus subconnatis. Petalis parvis obtusis. Labello unguiculado obtuso. Columna parva subterete. Pollinia duobus.

Genus *Niphantha* was actually published by Luer in 2007 but no Latin description was given, therefore the genus was not published validly. The evidence presented suggests that *Niphantha* is a distinct genus and therefore is validated it here.

Niphantha gelida (Lindl.) Luer ex Karremans, comb. nov.

Bas*. Pleurothallis gelida* Lindl., Edwards`s Bot. Reg. 27: Misc. 91, 1841.

Niphantha pidax (Luer) Luer ex Karremans, comb. nov.

Bas*. Pleurothallis pidax* Luer, Selbyana 5(2): 174, 1979.

Physosiphon Lindl.

Two species belonging to the concept of *Physosiphon* have been classified in *Stelis* and are here transferred to the first.

Physosiphon greenwoodii (Soto Arenas & Solano) Karremans, comb. nov.

Bas*. Stelis greenwoodii* Soto Arenas & Solano, *Icones Orchidacearum* 5-6: t. 682 (2002 publ. 2003).

Physosiphon tacanensis (Solano & Soto Arenas) Karremans, comb. nov.

Bas*. Stelis tacanensis* Solano & Soto Arenas, *Icones Orchidacearum* 5-6: t. 693 (2002 publ. 2003).

Pupulinia Karremans, nom. nov.

Bas. *Pleurothallis segoviensis* Rchb.f., Bomplandia (Hannover) 3: 223 (1855).
Ety.: In honor of Franco Pupulin, one of the most influential contemporary students of Neo-tropical Orchidaceae.
Syn : *Unciferia* (Luer) Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 95: 265 (2004). Non *Uncifera* Lindl., J. Proc. Linn. Soc., Bot. 3: 39 (1859).

Genus *Unciferia* published by Luer (2004) is illegitimate as the name is too similar to the genus *Uncifera* Lindl. (1859), which has priority. Genus *Pupulinia* Karremans is here proposed to substitute it. *Pupulinia* has been treated in this document as the *Unciferia s.s.* clade.

Pupulinia amaliae (Luer & R. Escobar) Karremans, comb. nov.

Bas. *Pleurothallis amaliae* Luer & R. Escobar, Orquidelogía 14: 124 (1981).

Pupulinia ancistra (Luer & Hirtz) Karremans, comb. nov.

Bas. *Pleurothallis ancistra* Luer & Hirtz, Lindleyana 11: 144 (1996).

Pupulinia bifalcis (Schltr.) Karremans, comb. nov.

Bas. *Pleurothallis bifalcis* Schltr., Beih. Bot. Centralbl. 26(2): 395 (1918).

Pupulinia canae (Ames) Karremans, comb. nov.
Bas. *Pleurothallis canae* Ames, Schedul. Orchid. 2: 18 (1923).

Pupulinia kareniae (Luer) Karremans, comb. nov.
Bas. *Pleurothallis kareniae* Luer, Lindleyana 11: 83 (1996).

Pupulinia pilostoma (Luer) Karremans, comb. nov.
Bas. *Pleurothallis pilostoma* Luer, Lindleyana 11: 89 (1996).

Pupulinia pompalis (Ames) Karremans, comb. nov.
Bas. *Pleurothallis pompalis* Ames, Schedul. Orchi. 7: 23 (1924).

Pupulinia psilantha (Luer) Karremans, comb. nov.
Bas. *Pleurothallis psilantha* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 72: 95 (1998).

Pupulinia segoviensis (Rchb.f.) Karremans, comb. nov.
Bas. *Pleurothallis segoviensis* Rchb.f., Bomplandia (Hannover) 3: 223 (1855).

Pupulinia wagneri (Schltr.) Karremans, comb. nov.
Bas. *Pleurothallis wagneri* Schltr., Repert. Spec. Nov. Regni Veg. 17: 141 (1921).

Triuria Karremans, gen. nov.

Ety.: From the Greek "*triura*" meaning three-tailed, in reference to the free, long acuminate, sepals of most of its species.
Type. *Dendrobium acuminatum* H.B.K., Nov. Gen. Sp. 1: 357, 1816.
Syn.: *Pleurothallis* R.Br. subgen. *Acuminatia* Luer sect. *Acuminatae* Lindl.

Habitu caespitoso. Racemo plurifloro quam folio elliptico longiore. Sepalis liberis ovatis acuminatis pubescentibus. Petalis parvis obtusis apiculatis. Labello lineare. Columna semiteretes. Pollinia duobus.

Genus *Triuria* Karremans is described in order to accommodate a group of species previously classified as *Pleurothallis* subgen. Acuminatia sect. *Acuminatea*, which had been transferred to *Anathallis* by Pridgeon *et al.* (2001). However, this group of species is morphologically very similar to *Stelis s.s.* and *Crocodeilanthe*, relationship which is confirmed by DNA evidence and supported by its geographical trends. Clearly *Pleurothallis* subgen. *Acuminatia* sect. *Alatae* is not closely related and is adequately classified in *Anathallis*. Most of the species of the genus are found in the Andes, especially in Bolivia. Plant morphology is similar to that of *Stelis s.s.* however, it can be distinguished by its larger flower with long acuminate sepals, the elongated and curved lip and column, the incumbent anther, ventral stigma and whale-tail like caudicles. From *Crocodeilanthe* it can be distinguished by plants not having imbricating sheaths at the base, the free, long, and narrow sepals, the elongated column and lip, and the elliptic unlobed lip.

Triuria acuminata (Luer) Karremans, comb. nov.
Bas. *Dendrobium acuminatum* H.B.K., Nov. Gen. Sp. 1: 357, 1816.

Triuria anderssonii (Luer) Karremans, comb. nov.
Bas. *Pleurothallis anderssonii* Luer, Lindleyana 11: 145, 1996.

Triuria ariasii (Luer & Hirtz) Karremans, comb. nov.
Bas. *Pleurothallis ariasii* Luer & Hirtz, Lindleyana 12: 42, 1997.

Triuria asperiliguis (Rchb.f. & Warsc.) Karremans, comb. nov.
Bas. *Pleurothallis asperilinguis* Rchb.f. & Warsc., Bonplandia 2: 114, 1854.

Triuria candida (Luer & Hirtz) Karremans, comb. nov.
Bas. *Pleurothallis candida* Luer & Hirtz, Lindleyana 12: 42, 1997.

Triuria concinna (Luer & R. Vásquez) Karremans, comb. nov.
Bas. *Pleurothallis concinna* Luer & R. Vásquez, Rev. Soc. Bol. Bot. 3: 1999.

Triuria coripatae (Luer & R. Vásquez) Karremans, comb. nov.
Bas. *Pleurothallis coripatae* Luer & R. Vásquez, Phytologia 46: 362, 1980.

Triuria dimidia (Luer) Karremans, comb. nov.
Bas. *Pleurothallis dimidia* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 76: 109, 1999.

Triuria dolichopus (Schltr.) Karremans, comb. nov.
Bas. *Pleurothallis dolichopus* Schltr., Repert. Spec. Nov. Regni Veg. 10: 394, 1912.

Triuria jesupiorum (Luer & Hirtz) Karremans, comb. nov.
Bas. *Pleurothallis jesupiorum* Luer & Hirtz, Lindleyana 11: 64, 1996.

Triuria lagarophyta (Luer) Karremans, comb. nov.
Bas. *Pleurothallis lagarophyta* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 76 : 112, 1999.

Triuria maguirei (Luer) Karremans, comb. nov.
Bas. *Pleurothallis maguirei* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 76: 113, 1999.

Triuria mediocarinata (C.Schweinf.) Karremans, comb. nov.
Bas. *Pleurothallis mediocarinata* C.Schweinf., Fieldiana Bot. 33: 26, 1970.

Triuria meridiana (Rchb.f.) Karremans, comb. nov.
Bas. *Pleurothallis meridiana* Rchb.f., Linnaea 22: 826, 1849.

Triuria papuligera (Schltr.) Karremans, comb. nov.
Bas. *Pleurothallis papuligera* Schltr., Repert. Spec. Nov. Regni Veg. 10: 453, 1912.

Triuria ramulosa (Lindl.) Karremans, comb. nov.
Bas. *Pleurothallis ramulosa* Lindl., Folia Orch. *Pleurothallis* 33, 1859.

Triuria regalis (Luer) Karremans, comb. nov.
Bas. *Pleurothallis regalis* Luer, Selbyana 5: 178, 1979.

Triuria rubens (Lindl.) Karremans, comb. nov.
Bas. *Pleurothallis rubens* Lindl., Bot. Reg. 21, sub. T. 1797, 1836.

Triuria scariosa (La Llave) Karremans, comb. nov.
Bas. *Dendrobium scariosum* La Llave, Nov. Veg. Descr. fasc. 2: Orch. Opusc. 39, 1825.

Triuria schlimii (Luer) Karremans, comb. nov.
Bas. *Pleurothallis schlimii* Luer, Monogr. Syst. Bot. Missouri Bot. Gard. 76 : 120, 1999.

Triuria sclerophylla (Lindl.) Karremans, comb. nov.
Bas. *Pleurothallis sclerophylla* Lindl., Bot. Reg. 21: sub t. 1797, 1835.

Triuria soratana (Rchb.f.) Karremans, comb. nov.
Bas. *Pleurothallis soratana* Rchb.f., Xenia Orch. 3: 25, 1900.

Triuria spathilabia (Schltr.) Karremans, comb. nov.
Bas. *Pleurothallis spathilabia* Schltr., Repert. Spec. Nov. Regni Veg. Beih. 2: 56, 1924.

Triuria spathuliformis (Luer & R. Váquez) Karremans, comb. nov.
Bas. *Pleurothallis spathuliformis* Luer & R.Váquez, Revista Soc. Boliv. Bot. 2 :137, 1999.

Triuria stenophylla (Lehm. & Kraenzl.) Karremans, comb. nov.
Bas. *Pleurothallis stenophylla* Lehm. & Kraenzl., Bot. Jahrb. Syst. 26: 442, 1899.

Triuria unduavica (Luer & R. Vásquez) Karremans, comb. nov.
Bas. *Pleurothallis unduavica* Luer & R. Vásquez, Phytologia 46: 372, 1980.

Triuria vasquezii (Luer) Karremans, comb. nov.
Bas. *Pleurothallis vasquezii* Luer, Phytologia 49: 220, 1981.

Umbraticola Karremans, gen. nov.

Ety.: From the Latin "*Umbra"*, shade, in reference to the short twisted inflorescence hidden under the shade of the convex leaf.
Type. *Pleurothallis imraei* Lindl., Fol. Orchid. 9: 9 (1859).

Habitu caespitoso. Racemo laxo quam folio semiorbiculare minore. Sepalis ovatis concavis pubescentibus. Sepalo dorsali liberi, lateralibus subconnatis. Petalis parvis spatulatis obtusis. Labello unguiculado obtuso. Columna semiteretes. Pollinia duobus.

Genus *Umbraticola* Karremans was one of the surprises of this study. *Stelis imraei* clearly does not group with other member of Luer´s generic concept *Effusiella*. In a few of the phylogenetic trees presented it appears as the first species to branch out of the *Stelis s.l.* group. The consensus networks places it as having intermediate relationships between *Stelis s.l.* and *Pleurothallis* s.l. It certainly presents several morphological characteristics shared with both, parsimony analysis of the morphological data shows that *Umbraticola* posses all the ancestral states for the evaluated characters in *Stelis s.l.* Although it is probably not a solitary species, having a few close relatives, it could be considered as a sort of ancestral *Stelis*. On the basis of the evidence presented and the very non-*Stelis* like plant habit and inflorescence it is here described as a distinct genus.

Umbraticola cocornaensis (Luer & Escobar) Karremans, comb. nov.
Bas. *Pleurothallis cocornaensis* Luer & Escobar, Orquideología 20: 45 (1996).

Umbraticola erucosa (Luer & R. Escobar) Karremans, comb. nov.
Bas. *Pleurothallis erucosa* Luer & R. Escobar, Orquideología 21: 88 (1998).

Umbraticola imraei (Lindl.) Karremans, comb. nov.
Bas. *Pleurothallis imraei* Lindl., Fol. Orchid. 9: 9 (1859).

Umbraticola tarantula (Luer & Hirtz) Karremans, comb. nov.
Bas. *Pleurothallis tarantula* Luer & Hirtz, Lindleyana 11: 186 (1996).

Umbraticola vaginata (Schltr.) Karremans, comb. nov.

Bas. *Pleurothallis vaginata* Schltr., Repert. Spec. Nov. Regni Veg. Beih. 19: 197 (1923).

5.2. Recommendations for Further Research

5.2.1. Sampling of additional Genes and Species: Even though this phylogenetic study has already quite improved the number of species of *Stelis s.l*. sequenced and analyzed, it include no more than 5% of all the known species. Certainly more sampling is in order, especially from some of the under sampled clades such as *Stelis s.s., Acuminatia* and *Crocodeilanthe* but also those that have quite a complicated and unresolved taxonomy, such as *Elongatia* and *Effusiella*. The last two are probably polyphyletic from the evidence that has been found here. It is of outmost importance to collect samples especially from countries like Mexico, Colombia and Ecuador which have many species that have not been included and seem to posses species with the most extreme variations.

Only two genes were used to reconstruct the phylogeny of *Stelis s.l*. However, it would be interesting to add a few other genes to the same study in order to (1) further support the evidence from the two genes used here, to (2) suggest other possible evolutionary pathways in the group, and even to (3) assess whether phenomena such as reticulate evolution, horizontal gene transfer or chloroplast capture where ever present in the evolutionary pathways of this group (as was found by Russell *et al.* in (2010) in genus *Polystachya* Hook (Orchidaceae).

5.2.2. Infrageneric classification of *Stelis s.s.*: This study is certainly a good starting point to reconsider sections within the genus *Stelis*. Luer has accepted three sections (Luer 2009), sect. *Humboldtia* (Ruiz & Pav.) Pers., sect. *Nexipus* (Garay) Luer, and sect. *Stelis* (Ruiz & Pav.) Pers. Although most of *Stelis s.s*. species form a polytomy in the combined tree, at least, sect. *Humboldtia* is paraphyletic while sect. *Stelis* is polyphyletic. Inclusion of more species from all sections may bring forth a clearer picture of the sub-generic relationships within *Stelis s.s*. Garay´s genus *Apatostelis* included *S. standleyi*, *S. glossula*, *S. aff. glossula* and *S. ciliaris,* and is here both paraphyletic and polyphyletic. No species of Schltr.´s genus *Pseudostelis* were included, but would be interesting to evaluate as some species present mixed characters between *Stelis s.s*. and members of *Stelis s.l*.

It is noteworthy that before the publication of the molecular results obtained by Pridgeon *et al.* (2001) the status of *Stelis s.s*. as a distinct and monophyletic genus was generally accepted (Luer 1986), even Pridgeon himself suggested that it appeared to be a natural genus (in Neyland *et al.* 1995).

5.2.3. Intergeneric Hybrids: It was the idea at first to also evaluate whether species from the different genera within the *Stelis s.l.* could be crossed in order to produce viable hybrids. It has

been suggested in literature that several genera within the Pleurothallidinae cannot cross with sister genera. Evidence of incompatibility could also help to tell genera apart from each other. There was no time to do this experiment, but it would be of great value, not only for its taxonomical importance, but also to explain the reproductive biology of these organisms, to explain their evolutionary paths, and even for horticultural reasons if breeding of species of *Stelis s.l.* would ever be considered. Self incompatibility on the other hand is found in species of *Acuminatia* and *Anathallis* (Gontijo *et al.* 2010) and *Octomeria* R. Br. (Barbosa *et al.* 2009).

5.2.4. Image Recognition: The first time the author heard about image recognition being used in plants was in July 2010, in a presentation by Dr Alastair Culham from University of Reading. He mentioned it as being the future for plant identification, being less intrusive, easier, cheaper and faster than DNA Barcoding. In fact, Belhumeur *et al.* (2008) have already developed a working computer vision system that aids in plant species identification. A user takes a photograph of a leaf on a blank background and the system is capable of recognizing the shape and matches it to a series of species which are displayed in order of best score.

Although the system has only (as far as has been found) been used by a few botanists and for a few restricted plant groups it certainly could be used by many more people and be extended to other groups. In very large families such as Orchidaceae and were species can be easily (in most cases) distinguished by floral morphology (if one is an expert) such a system would be of great use. Not only will it make it easier for botanists to identify species, but it could aid in conservation efforts of non-taxonomists and it could certainly help authorities in the control of illegal traffic of plants. However the biggest market would without any doubt, be the thousands of orchid enthusiasts around the world, who are always struggling with the correct identification and the constant name changing of their beloved plants.

5.2.5. Speciation of Stelis s.s.: *Stelis s.s.* is a very large clade as compared to all the others treated here and it is clearly differentiated molecularly, morphologically and geographically. It is also flanked by clades *Crocodeilanthe* and *Acuminatia*, which in turn are the second and third largest clades in the group. A very interesting follow up study would be to try to explain that sudden increase in species number, found to be correlated to morphological modifications, such as pollination syndrome, but also to geographical trends, all the groups mentioned being most diverse in the Andes region rather than in their supposed center of origin, between Costa Rica and northern Colombia. The fact that the center of most species diversity does not overlap with the center of origin is also explained by the fact that clade diversity and endemism is clearly larger in the latter.

The radiation of *Stelis s.s.* into the Andean region from its origin accompanied by morphological modifications seems to be a plausible explanation. However, several different explanations are possible, (1) the uplift of the Andes acted as natural barrier separating the Central American from the South American populations, after which *Stelis s.s.* speciated, or (2) the slow uplift of the Andean Cordillera allowed species of *Stelis s.s*. to fill in all the newly created niches and they speciated from there.

Research by Erkens *et al.* (2007) on the genus *Guatteria* (Annonaceae) found similar results, where an originally Central American lineage radiated into South America and then became isolated by the Andean uplift. Pirie et al. (2006), again in Annonaceae, found that the uplift on the most northern part of the Andean Cordillera separated populations and drove to speciation within several genera. Kirby (2010) found geographical patterns within the Maxillariinae (Orchidaceae), some genera being clearly more diverse in Central America, others in Brazil and yet other in the Andes (Whitten *et al.* 2007). Hughes & Eastwood (2006) found that genus *Lupinus* (Fabaceae) radiated into the island like Andes mountain range very recently from North America. The large impact of the rise of the Andes and its effect of plant evolution has been recently discussed amply by Antonelli *et al.* (2009).

5.2.6. Molecular Dating: One way of trying to explain the large difference in species number between clades and number of clades themselves could be using molecular dating. Although the name suggests some kind of twisted reality show, molecular dating actually refers to capacity of estimating the time of origin of any biological lineage (Welch & Bromham 2005). If one could set a date on one of the nodes of the phylogenetic tree, it would be possible to calculate the ages of all other nodes, using a molecular clock, supposing that the substitution rates are constant, and knowing the number of substitutions. Welch & Bromham (2005) mention that rates are not always constant in time, they might actually be an exception rather than the rule, thus rejecting the molecular clock. New methods have appeared, enabling the incorporation of variable rates into molecular dating. An example might be the use of unconstrained substitution models in a Bayesian analysis (discussed in the Additional Analyses section).

It might be quite interesting in this case, to test the theories of geographical modifications as a driver of speciation. The age of a fossil (Paleobotany) is frequently used to calibrate the age of nodes, however, in Orchidaceae very few fossils are known. On the other hand, if one sets the Andean uplift, which started some 10 My ago (Garzione 2008), as the age for the Stelis s.s. clade, then it would be possible to calculate the ages of all other nodes, and even try to tell what the influence of the closing of the Central American isthmus, about 3 My ago (E. Duarte pers. comm. 2010), and the effect it had over speciation in the group.

5.2.7. Pollination within *Stelis s.l.*: Unfortunately not much is known about the pollination of most Pleurothallidinae, including the species of *Stelis s.l.* It can speculated however, that pollination in most of the clades of the group occurs when the pollinator lands on the hinged and curved lip, and the weight of the insect makes the lip move upwards into a position where it is pressed against the also curved column. As the insect exits the flower it touches the apical margin of the bubble shaped rostellum and gets covered in the loose-celled sticky substance called viscarium, it then, reaches up to the base of the anther and touches the dry and flat whale-tail shaped caudicles. It proceeds to remove the pollinaria and repeats the process in the next flower were it deposited the pollinaria onto the stigma.

However, in the *Stelis s.s.* clade, the column and lip are very much reduced and straight, therefore this curved movement of the lip is not possible. Instead, it is presumable, the insect flies against the now apically placed pollinaria and touches the viscidium, which has become solid and drop like in shape, and which is attached to the now narrow and elastic caudicles. In *Petiolatae,* on the other hand, the viscarium has been accumulated at the apex of the now erect and shortened rostellum and is placed adjacent to the now shorter and narrower whale-tail caudicles. In this case, also in one step, the pollinator touches the viscarium and that permits pollinaria removal, however, the column and lip are elongated and curved, suggesting the pollinator does enter the space between them.

In another noteworthy case, the lips of *Dracontia* and *Mystacorchis* have evolved to be quite fleshy and more or less cylindrical in shape. In both cases a narrow channel crosses the middle of the lip from the base to the apex. These characters are found in correlation with a very short, and narrowing column, and incurved petals that cover the column. Pollination in these two clades must be different from others based on those characters, but it is not clear how it works.

It can speculated that the one touch system is also present in clade *Salpistele,* were the column is so far in front of the lip, and with the anther placed pseudo-apically, it is hard to believe that the pollinator has to place itself on the lip for pollination to take place. It is more likely that just like in *Stelis s.s*., pollinators of *Salpistele* somehow make frontal contact with the column and so, removes the pollinaria.

The presence of a glenion (roundish, darker surface at the base of lip) in the *Stelis s.s*. clade must have some kind of influence in pollination. If seen with the naked eye or with a stereoscope the glenion surface seems to be wet or oily. However, a photograph taken by Mark Wilson with an electronic microscope suggests that there is no liquid at all, it is actually a visual effect made by a series of papillae like cells. van der Pijl & Dodson (1966) noted that pollinators of some Pleurothallidinae, like *Stelis,* were attracted by the floral nectar, however, there is no nectar in the

flowers of *Stelis s.s*. It is possible that the glenion did not only fool the authors, but also the pollinators. Getting to the glenion might be the whole reason behind the pollination of *Stelis s.s.* In *Acronia*, exactly as in *Stelis s.s*., the column and lip are very much reduced, the anther and stigma are apical and a glenion is present at the base of the lip. van der Pijl & Dodson observed pollination by Sciaridae flies in both genera, supporting the theory of correlation between reduced column and lip, the apical anther and stigma and the presence of a glenion. Blanco & Barboza (2005) found Sciaridae flies pollinating *Lepanthes* Sw. (Pleurothallidinae) by pseudo-copulation, Barbosa *et al.* (2009) found Scaridae flies entering the flowers of genus *Octomeria* (Pleurothallidinae) and then taking the pollinaria with then when retreating.

All of these cases would be very interesting to study in detail and with more refinement than was done in this study. Some seem clear evidence for convergence, which could make sense since it is suggested that pollinators are one of the biggest drivers of speciation within the Orchidaceae, and could also explain the difficulty of trying to classify the Pleurothallidinae morphologically, as reflected by the failed attempts by Garay (1979), Pridgeon (1982) and Neyland *et al.* (1995) and the moderate success of Luer (1986).

5.2.8. Additional Analyses: Several analyses were not done because of lack of time, but have come up during the study, and could be interesting alternatives for the interpretation of the data.

BEAST (Drummond & Rambaut 2007): The name derives from **B**ayesian **E**volutionary **A**nalysis **S**ampling **T**rees, and, it is a package for evolutionary inference from molecular sequences. It is entirely orientated towards rooted, time-measured phylogenies inferred using strict or relaxed molecular clock models. It is also a framework for testing evolutionary hypotheses without conditioning on a single tree topology. It uses a complex and powerful input format to describe the evolutionary model, the analysis is much more flexible, allowing the user to construct models that do not perform well under the Markov chain Monte Carlo (MCMC).

PRANK (Loytynoja & Goldman 2005): Is a probabilistic multiple alignment program for DNA, codon and amino-acid sequences. It uses a novel algorithm to treat insertions correctly and avoids over-estimation of the number of deletion event. It takes evolutionary distance between sequences into consideration when aligning, additionally, it allows definition of potential structure for sequences and redirects the locations of structural units in the sequences.

Character Correlation Analysis: It is possible to do correlation analyses between one morphological character and each of the others. A correlation analysis would identify which characters are actually dependent or independent from each other. Useful if one wants to analyze the characters as best as possible, taking into consideration that we might want to consider strongly correlated characters as one and not two or more. The analysis can be done in *Mesquite*,

but is only possible if not missing data is present in the matrix (reason for which I was not able to do it).

Consistency Index (CI): The CI value is a statistical way of evaluating the quality of the morphological data. CI values can be calculated for each character using *Mesquite*. Most characters in the data set presented very low CI values because of low within clade variation (cero branch lengths within clades). That result was expected as characters that differ between clades, but remain constant within the clades were purposely selected in this study. However, if more variable characters are added to the matrix CI values might be very useful to statistically support the finding and conclusions.

SIMMAP (Bollback 2006): Ancestral character states were reconstructed using a parsimony analysis in *Mesquite*. However, alternatives such as the program *SIMMAP*, which uses a Bayesian mutational mapping approach (Huelsenbeck *et al.* 2003) can estimate PP values for the ancestral states of characters, have been used to couple molecular phylogenies with morphological data, as has been done with *Pelargonium* by Jones *et al.* (2009). This approach might be very useful as it calculates actual probabilities for the data.

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Glossary

Acuminate
Tapering to a point.

Anther
The pollen bearing portion of the stamen (column in Orchids).

Androclinium
Refers to that part of the column that supports and/or covers the anther cap.

Caespitose
Said of stems that grow very closely together.

Caudicle
A portion of the pollinium which is usually slender and is composed of viscin with some pollen grains.

Clade
For the effects of this paper a clade is a natural grouping at any level which includes all the related species and their common ancestor.

Claw
The narrow basal portion of a lip or petal.

Codon
Sequence of three nucleotides in a DNA or mRNA molecule that represents the instruction for incorporation of a specific amino acid into a growing polypeptide chain.

Column
The central portion of the orchid flower, which is formed by the partial or complete union of the male and female parts (stamens and styles).

Column Foot
A ventral extension of the base of the column which has the lip attached at its tip.

Column Wing
A lateral projection on each side of the column; they may represent sterile anthers.

Gene
Region of DNA that is transcribed as a single unit and carries information for a discrete hereditary characteristic, usually corresponding to a single protein or a single RNA.

Genetics
The study of the genes of an organism on the basis of heredity and variation.

Genome
The totality of genetic information belonging to a cell or an organism; in particular, the DNA that carries this information.

Indel
Any insertion or deletion of base pairs in a sequence.

Labellum
A petal formed into a lip.

Lamina
The broad flattened portion of a lip, petal or leaf.

Monophyletic
Said of organisms having a common ancestor.

Pedicel
The stalk of each individual flower, normally attached to the rachis of an inflorescence.

Peduncle
The stalk of an inflorescence which holds the rachis.

Petiole
The stalk of a leaf, which in Pleurothallidinae attaches the leaf lamina to the ramicaul.

Phylogeny
The evolution of a group of related individuals.

Plastid
Cytoplasmic organelle in plants, bounded by a double membrane, that carries its own DNA and is often pigmented. Chloroplasts are plastids.

Primer
Oligonucleotide that pairs with a template DNA or RNA strand and promotes the synthesis of a new complementary strand by a polymerase.

Pollinaria
Plural of pollinarium.

Pollinarium
The male reproductive part of an orchid flower, consisting of the pollinia from an anther with the associated parts, the viscidium and the stipes.

Pollination
The transfer of pollen from the anthers to the stigma of a flower.

Polyphyletic
Having origin from several lines of descent.

Rachis
The part of an inflorescence which is attached to the peduncle and holds all the pedicels.

Ramicaul
The stem of the Pleurothallids.

Rostellum

A projection of the stigma of an orchid separating the fertile part of the stigma from the anther. Specialized in pollen transfer.

Spathe

A modified leaf at the base of the actual leaf, from which the inflorescence emerges.

Stigma

The terminal, receptive portion of the pistil.

Synsepal

Organ composed of fused or united sepals.

Viscarium

A transformed viscidium, being loose-celled and sticky. Found spread out near the apex of the rostellum.

Viscidium

A viscid part of the rostellum which is removed with the pollinia as a unit and serves to attach the pollinia to the pollinator.

Viscin

An elastic or somewhat viscid material which binds together the pollen grains in the pollinia and caudicles.

Appendix

Appendix I: Different classifications for the *Stelis s.l.* group since 1986, showing to several splitting and lumping events by authors. In the column Karremans 2010, the clades used for discussion during the paper are as unofficial names.

Appendix II: List of all the accessions (or species names) used in this paper, DNA extraction and/or sequencing has not been successful in all cases, those species are not included in the phylogenetic analyses and therefore have no coding in the trees, but have a lab code. Several sequences have been taken from GenBank, for those accessions no collector and number are given.

Appendix III: Two *matK* trees are presented here. The first shows a Bayesian analysis where the partition for codon position is not considered. The second is a best tree obtained from a RAxmL analysis of the data set.

Appendix IV: A tree produced in *TNT* with Jacknifing from the *matK* data set. Using 100 replicates and P=36.

Appendix V: Two incomplete ITS trees are presented here. The first shows a Bayesian analysis where the *Anathallis dolichopus* sequence has been removed. The second is a Bayesian analysis where the *Stelis rodrigoi* sequence has been removed.

Appendix VI: The best tree obtained from a *RAxML* analysis of the complete ITS data set is presented.

Appendix VII: A tree produced in *TNT* with Jackknifing from the ITS data set.

Appendix VIII-X: Output from *Tracer* are shown for the Bayesian runs for the *matK* partitioned and unpartitioned, and complete ITS and the concatenated data sets. Estimates, marginal density, joint-marginal and trace are shown as they have been produced and presented by the Tracer v 1.5 program. Bayesian Factors calculated by Tracer are given comparing the complete ITS data set, partitioned *matK* and concatenated data set.

Appendix XI: Mirrored trees resulting from the combined analysis showing the two different actual classification systems for the *Stelis s.l*. group, demonstrating their flaws if applied on the trees.

Appendix XII: Number of species of each group within *Stelis s.l.* per country. The total number of species per group is also indicated. Two subtotals are calculated to demonstrate their geographical differences. The Subt2/Total value indicates the relative amount of species belonging to that group, showing a strong Mesoamerican preference. Species number has been taken from Govaerts *et al.* (2010).

Appendix XIII: Table showing the summarized results of the reconstruction of the ancestral states of all morphological characters (1 to 26) plus two geographical characters (27 & 28), analyzed in *Mesquite* using parsimony. Ancestral states were reconstructed for six nodes, *Stelis s.l., Stelis s.m*., *Stelis s.s*., *Salpistele s.l*., *Unciferia s.l.* and *Salpistele* + *Petiolatae*. Character were reconstructed on the consensus tree and on a group of 10 last trees taken from the first run of the Bayesian analysis of the concatenated data set. Results between both were compared in order to select the most probable ancestral states for each character that would be used throughout the paper.

Appendix IXX: Graphical representation of the major clades within *Stelis s.l.: Stelis s.s*., *Acuminatia* + *Crocodeilanthe*, *Salpistele s.l.* and *Unciferia s.l*. Flower size has been modified to the relative number of species in each clade, demonstrating the very large difference in species number and therefore evolutionary success between the *Stelis s.s*. and its sister clades and all the other clades.

Appendix XX: Photographs of several of the studied groups including most genera within *Stelis s.l*. and various outgroups. Photographs taken by Franco Pupulin, Diego Bogarín, Melania Muñoz, Christina Smith and Adam Karremans for the Lankester Botanical Gardens. Collector coding = AK: Adam Karremans, AR: Alexander

Rojas, CL: Carlyle Luer, CO: Carlos Ossenbach, DB: Diego Bogarín, FP: Franco Pupulin, GR: Gustavo Rojas, MB: Mario Blanco, RD: Robert Dressler, RG: Reinaldo Gomez, YK: Y. Kisel.

Appendix XXI: Diverse drawings of species of *Stelis s.l*. done by the author, using a stereomicroscope provided with a drawing tube, during the field work period in Costa Rica.

Appendix I

Luer 1986	Luer 1994-2000	Pridgeon 2001	Luer 2004 - 2007	Karremans 2010*
Pleurothallis subgen. Specklinia sect. *Acuminatae*	*Pleurothallis* subgen. *Acuminatia* sect. *Acuminatae*	*Anathallis*	*Anathallis*	*Acuminatia*
Condylago	*Condylago*		*Condylago*	*Condylago*
Pleurothallis subgen. *Crocodeilanthe*	*Pleurothallis* subgen. *Crocodeilanthe*		*Crocodeilanthe*	*Crocodeilanthe*
Pleurothallis subgen. *Dracontia*	*Pleurothallis* subgen. *Dracontia*		*Dracontia*	*Dracontia*
				Convallaria
				Effusiella s.s.
			Effusiella	Listerophora
Pleurothallis subgen. *Specklinia* sect. *Effusae*	*Pleurothallis* subgen. *Effusia*			Resupinata
				Umbraticola
		Stelis		
			Loddigesia	*Loddigesia*
			Niphantha	*Niphantha*
Pleurothallis subgen. *Specklinia* sect. *Unciferae*	*Pleurothallis* subgen. *Unciferia*		*Unciferia*	*Unciferia*
Pleurothallis subgen. *Elongatia*	*Pleurothallis* subgen. *Elongatia* sect. *Elongatae*		*Elongatia*	Carpinterae
Pleurothallis subgen. *Elongatia*	*Pleurothallis* subgen. *Elongatia* sect. *Petiolatae*		*Elongatia*	*Petiolatae*
Pleurothallis subgen. *Mystax*	*Pleurothallis* subgen. *Mystax*		*Mystacorchis*	*Mystacorchis*
Pleurothallis subgen. *Physosiphon*	*Pleurothallis* subgen. *Physosiphon*		*Physosiphon*	*Physosiphon*
Pleurothallis subgen. *Physothallis*	*Pleurothallis* subgen. *Physothallis*		*Physothallis*	*Physothallis*
Salpistele	*Salpistele*		*Salpistele*	*Salpistele*
Stelis	*Stelis*		*Stelis*	*Stelis*

*These are clade names proposed here and not the generic names later proposed.

Appendix II

		GenBank Code			Coding in Trees in this Document***	
Accessions	**Collector and Number**	***matK***	**ITS**	**Lab Numbering****	***matK***	**ITS**
Andinia pensilis	-	AF265455	AF262826	-	AnpensilisAF265455	AndpensilisAF262826
Dryadella simula	-	AF265453	AF262825	-	DrsimulaAF265453	DrysimuAF262825
Echinosepala uncinata	-	AF265478	AF262904	-	MyouncinatusAF265478	MyounciAF262904
Frondaria caulescens	-	AF265471	AF262914	-	FrocaulescensAF265471	FroncauleAF262914
Lepanthes woodburyana	-	AF265470	AF262890	-	LepwoodburyanaAF265470	LewoodbuAF262890
Pabstiella aryter	D. Bogarin 6501	-	-	*3a*	*Paarythe*r	*Pabarythe*r
Pabstiella mentosa	-	-	AF262864	-	-	*Pabmentosa*
Pabstiella tripterantha	D. Bogarin 5905	-	-	*4a*	*Patripter1*	*Pabtrypth1*
Pabstiella tripterantha	-	*AF302649*	AF275694	-	*PatripteranthaAF302649*	PabtrypthAF275694
Platystele misera	-	*AF265470*	AF262823	-	*PlmiseraAF265470*	PlamiseAF262823
Pleurothallis cardiothallis	F. Pupulin 6414	-	-	*2b*	-	-
Pleurothallis hemirhoda	-	-	*AF262874*	-	-	*LinhemirhodaAF262874*
Pleurothallis lentiginosa	F. Pupulin 4113	-	-	*3b*	-	-
Pleurothallis miranda	-	-	*A*F262875	-	-	*Mimiranda*AF262875
Pleurothallis rowleei	-	-	AF262842	-	-	*Acrowleei*AF262842
Pleurothallis ruscifolia	F. Pupulin 7254 *(1)*	-	-	*4b*	-	*PleurothaN*
Pleurothallis ruscifolia	F. Pupulin 7254 *(2)*	-	-	*5a*	*Plruscif1*	*Plrusci1*
Pleurothallis ruscifolia	-	*AF265463*	AF262836	-	*PlruscifAF265463*	*Plrusci*AF262836
Pleurothallis teaguei	-	-	AF275695	-	-	*Acteaguei*AF275695
Specklinia costaricensis	-	-	-	*6a*	-	*Speccostaric*
*Stelis (Salp.) adrianae**	D. Bogarin 5917 *(1)*	-	-	*7a*	*Saadrianae*	*Saadrianae1*
*Stelis (Salp.) adrianae**	D. Bogarin 5917 *(2)*	-	-	*7a*	*Saadrianae2*	*Saadrianae2*
Stelis alajuelensis	D. Bogarin 1987	-	-	*39a*	-	*Drramonensis*
Stelis alta	D. Bogarin 4604 *(1)*	-	-	*8a*	*Dralata*	*Dralta*
Stelis alta	D. Bogarin 4604 *(2)*	-	-	*8a*	-	*DraltaN*
Stelis argentata	D. Bogarin 1862	-	-	*9a*	-	*Stargent1*
Stelis argentata	-	*AF265464*	AF262878	-	*StargentataAF265464*	*Stargent*AF262878

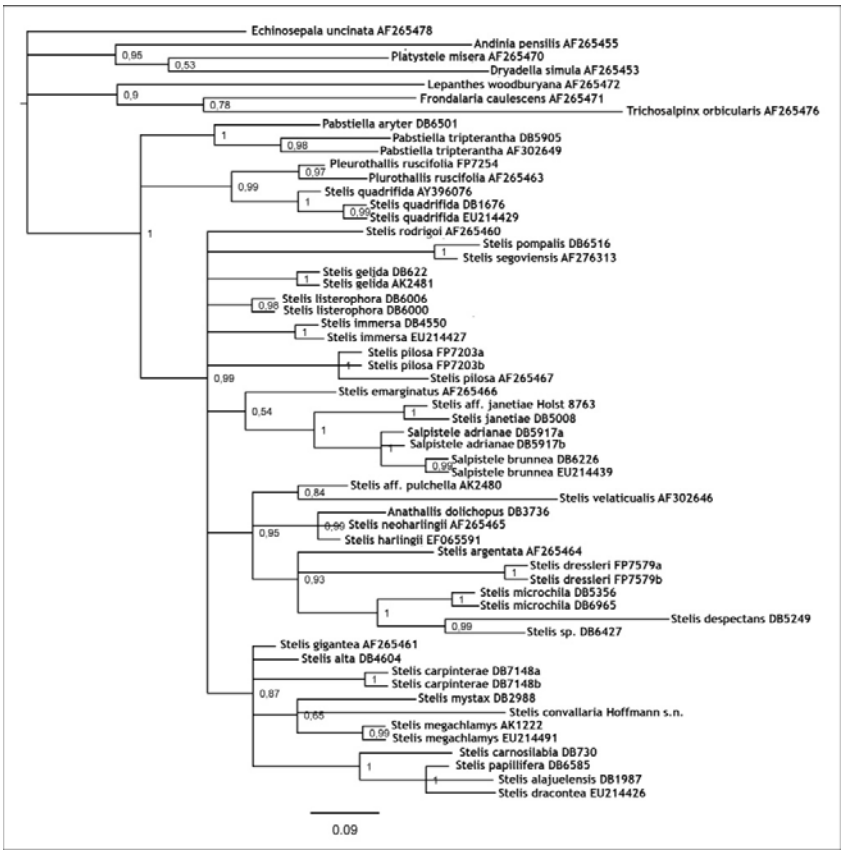
Stelis atroviolacea	-	-	AF262879	-	-	*Statroviolacea*AF262879
Stelis brunnea	D. Bogarin 6226	-	-	*10a*	*Sabrunnea1*	*Sabrunnea*
Stelis brunnea	-	*EU214439*	-	-	*SabrunneaEU214439*	-
Stelis aff, canae	D. Bogarín 6805	-	-	*26b*	-	-
Stelis carnosilabia	D. Bogarin 730	-	-	*11a*	-	*Drcarnosilabia*
Stelis carnosilabia	D. Bogarin 730	-	-	*5b*	-	*DrcarnoN*
Stelis carnosilabia	-	-	*EU214425*	-	-	*DrcanosilabiaEU214425*
Stelis carpinterae	D. Bogarin 7148 *(1)*	-	-	*12a*	*Elcarpintearae1*	*Elcarpintearae1*
Stelis carpinterae	D. Bogarin 7148 *(2)*	-	-	*12a*	*Elcarpintearae2*	*Elcarpintearae2*
Stelis ciliaris	-	-	AF262927	-	-	AF262927
Stelis cobanensis	-	-	*AF262926*	-	-	*RhcobanensisAF262926*
Stelis convallaria	Hoffmann s.n. (1)	-	-	*13a*	*Efconvallaria*	*Efconvallaria*
Stelis convallaria	Hoffmann s.n. (2)	-	-	*6b*	-	*EfconvalN*
Stelis crystallina	A. Karremans 974 (1)	-	-	*14a*	-	*Stcrystallina*
Stelis crystallina	A. Karremans 974 (2)	-	-	*14a*	-	*StecrysN*
Stelis despectans	D. Bogarin 5249 (1)	-	-	*15a*	*Stdespectans*	*Stdespectans*
Stelis despectans	D. Bogarin 5249 (2)	-	-	*15a*	-	*StedespecN*
*Stelis (Anat.) dolichopus**	D. Bogarin 3736	-	-	*1a*	*Asclerophylla*	*Asclreophylla*
Stelis dracontea	D. Bogarin 616	-	-	*16a & 8b*	-	*DrdracontN*
Stelis dracontea	-	-	*EU214426*	-	-	*DrdraconteaEU214426*
Stelis dressleri	F. Pupulin 7579 (1)	-	-	*17a*	*Stdressleri1*	*Stdressleri1*
Stelis dressleri	F. Pupulin 7579 (2)	-	-	*17a*	*Stdressleri2*	*Stdressleri2*
Stelis emarginata	A. Karremans s.n.	-	-	*44a*	-	-
Stelis emarginata	-	*AF265466*	*AF262845*	-	*PhntubatusAF265466*	*PhonemargiAF262845*
Stelis aff. ferrelliae	D. Bogarin 5746	-	-	*31a*	-	*Dringramiiaff*
*Stelis (Cond.) furculifera**	R. Dressler 6835	-	-	*2a & 1b*	-	-
Stelis gelida	A. Karremans 2481	-	-	*18a*	*NigelidaAK*	*NigelidaAK*
Stelis gelida	D. Bogarin 622	-	-	*19a*	*NigelidaDB*	*NigelidaDB*
Stelis gemma	-	-	AF262880	-	-	*Stgemma*AF262880
Stelis glossula	D. Bogarín 2695	-	-	*9b*	-	*StglossulaN*
Stelis aff. glossula	Y. Kisel 2048	-	-	*10b*	-	*StglossulaaffN*

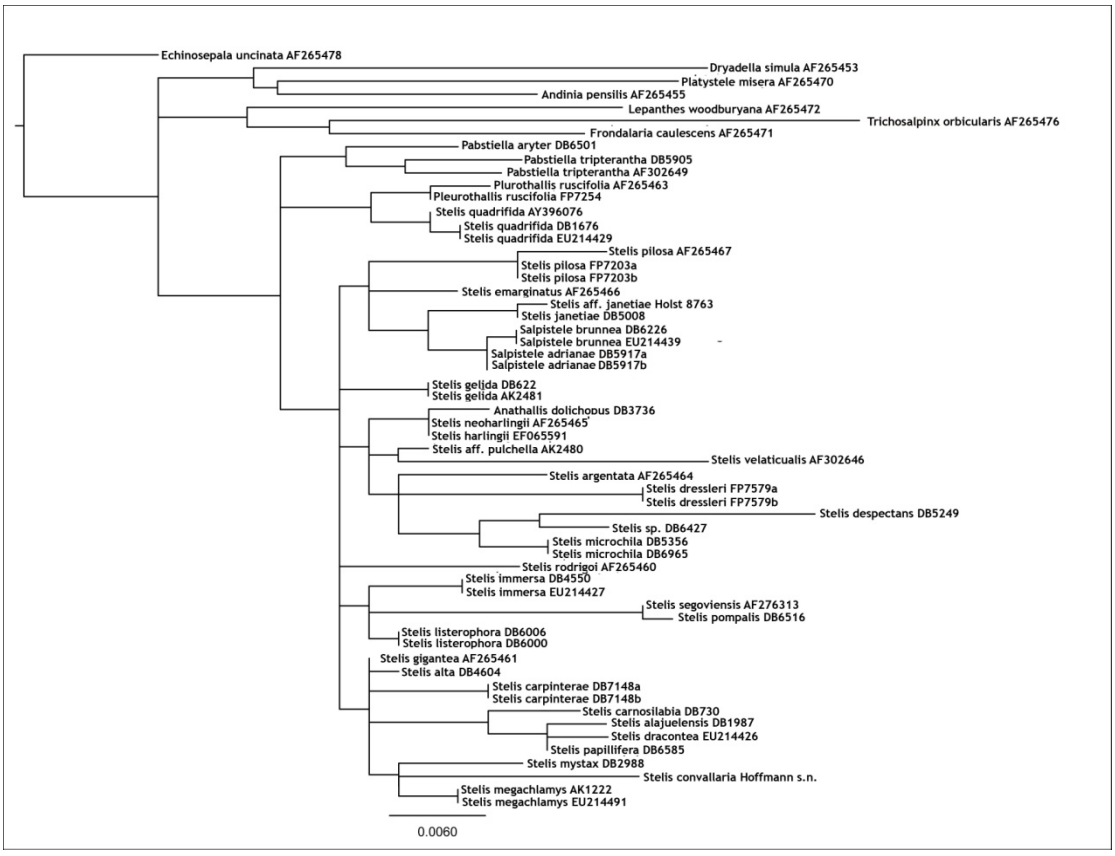
Stelis gigantea	-	*AF265461*	*AF262843*	-	*DrpowelliiAF265461*	*DrpowelliiAF262843*
Stelis guatemalensis	F. Pupulin 3977	-	-	*20a*	-	-
Stelis guatemalensis	F. Pupulin 3865	-	-	*11b*	-	*SguateN*
Stelis guatemalensis	-	-	*AF262928*	-	-	*StguatemalensisAF262928*
Stelis guttata	-	-	*AF262833*	-	-	*ElguttutaAF262833*
Stelis harlingii	-	*EF065591*	*EF079364*	-	*PhsharlingiiEF065591*	*PhilharlingiiEF079364*
Stelis harlingii	-	*AF265465*	AF262846	-	*PhsneoharlingiiAF265465*	*PhilneoharlingiiAF262846*
Stelis immersa	D. Bogarin 4550	-	-	*29a & 13b*	*Efimmersa*	*Efimmers29N*
Stelis immersa	D. Bogarín 6588	-	-	*12b*	-	*Efimmers12N*
Stelis immersa	-	*EU214427*	*AF262828*	-	*EfimEU214427*	*EfimmersaAF262828*
Stelis imraei	D. Bogarin 752	-	-	*30a & 14b*	-	*EfimraeiN*
Stelis janetiae	D. Bogarin 5008	-	-	*21a*	*Eljanetiaea1*	*Eljanetiae*
Stelis aff. janetiae	Holst 8763	-	-	*22a*	*Eljanetiaeaff*	*Eljanetiaeaff*
Stelis lanata	-	-	*AF262881*	-	-	*StlanataAF262881*
Stelis listerophora	D. Bogarin 6000	-	-	*23a*	*Efliste6000*	*Efliste6000*
Stelis listerophora	D. Bogarin 6006	-	-	*24a*	*Efliste6006*	*Efliste6006*
Stelis maculata	-	-	*AF262827*	-	-	*SamaculataAF262827*
Stelis megachlamys	A. Karremans 1222	-	-	*25a*	*Drmeg1*	*Drmegach1*
Stelis megachlamys	-	*EU214491*	*AF262877*	-	*DrmegEU214491*	*Drmegach AF262877*
Stelis aff. microchila	D. Bogarin 5356	-	-	*26a*	*Stmicroc5356*	*Stmicro5356*
Stelis aff. microchila	D. Bogarin 6965	-	-	*27a*	*Stmicroc6965*	*Stmicro6965*
Stelis morae	A. Karremans 1086	-	-	*16b*	-	*StmoraeN*
Stelis mystax	D. Bogarin 2988	-	-	*28a*	*Mymystax*	*Mymystax1*
Stelis mystax	-	-	*AF262876*	-		*MymystaxAF262876*
Stelis papillifera	D. Bogarin 7186	-	-	*32a & 18b*	-	-
Stelis papillifera	D. Bogarin 6585 (1)	-	-	*33a*	*Drpapillifera*	*Drpapillifera6586*
Stelis papillifera	D. Bogarín 6585 (2)	-	-	*17b*	-	*DrapapN*
Stelis pilosa	F. Pupulin 7203 (1)	-	-	*34a*	*Efamp1*	*Efamparo1*
Stelis pilosa	F. Pupulin 7203 (2)	-	-	*34a*	*Efamp2*	*Efamparo2*
Stelis pilosa	-	*AF265467*	*AF262831*	*34a*	*EfampAF265467*	*EfamparoAF262831*
Stelis pilostoma	F. Pupulin 7601	-	-	*35a & 20b*	-	-

Stelis pompalis	D. Bogarin 6516 (1)	-	-	*36a*	-	*Unpompalis*
Stelis pompalis	D. Bogarin 6516 (2)	-	-	*19b*	-	*UncpomN*
Stelis aff. pulchella	A. Karremans 2480	-	-	*37a*	*Crpulchella*	*Crpulchellaf*
Stelis quadrifida	D. Bogarin 1676	-	-	*38a & 21b*	*Loquadri1*	-
Stelis quadrifida	-	*AY396076*	AY008477	-	*LoquadriAY396076*	*Loquadr*AY008477
Stelis quadrifida	-	*EU214429*	-	-	*LoquadriEU214429*	-
Stelis resupinata	-	-	*AF262916*	-	-	*EfresupinataAF262916*
Stelis rodrigoi	-	*AF265460*	*AF262829*	-	*CorodrigoiAF265460*	*CrodrigoiAF262829*
Stelis segoviensis	A. Karremans 544	-	-	*40a & 22b*	-	-
Stelis segoviensis	-	*AF276313*	*AF262866*	-	*UnsegoviensisAF276313*	*UnsegoviensisAF262866*
Stelis aff. segoviensis	D. Bogarin 2822	-	-	*41a & 23b*	-	-
Stelis standleyi	D. Bogarin 5605	-	-	*42a & 24b*	-	*StstandleyiN*
Stelis sp.	D. Bogarin 6427	-	-	*43a*	*Stspnov*	*Stspnov*
Stelis tacanensis	-	-	AF262918	-	-	*Phontacan*AF262918
Stelis velaticaulis	-	*AF302646*	*A*F262847	-	*CrvelaticaulisAF302646*	*Crvelaticaulis*AF262847
Trichosalpinx orbicularis	-	*AF265476*	*AF262886*	-	*TriorbicularistronAF265476*	*TricorbicAF262886*

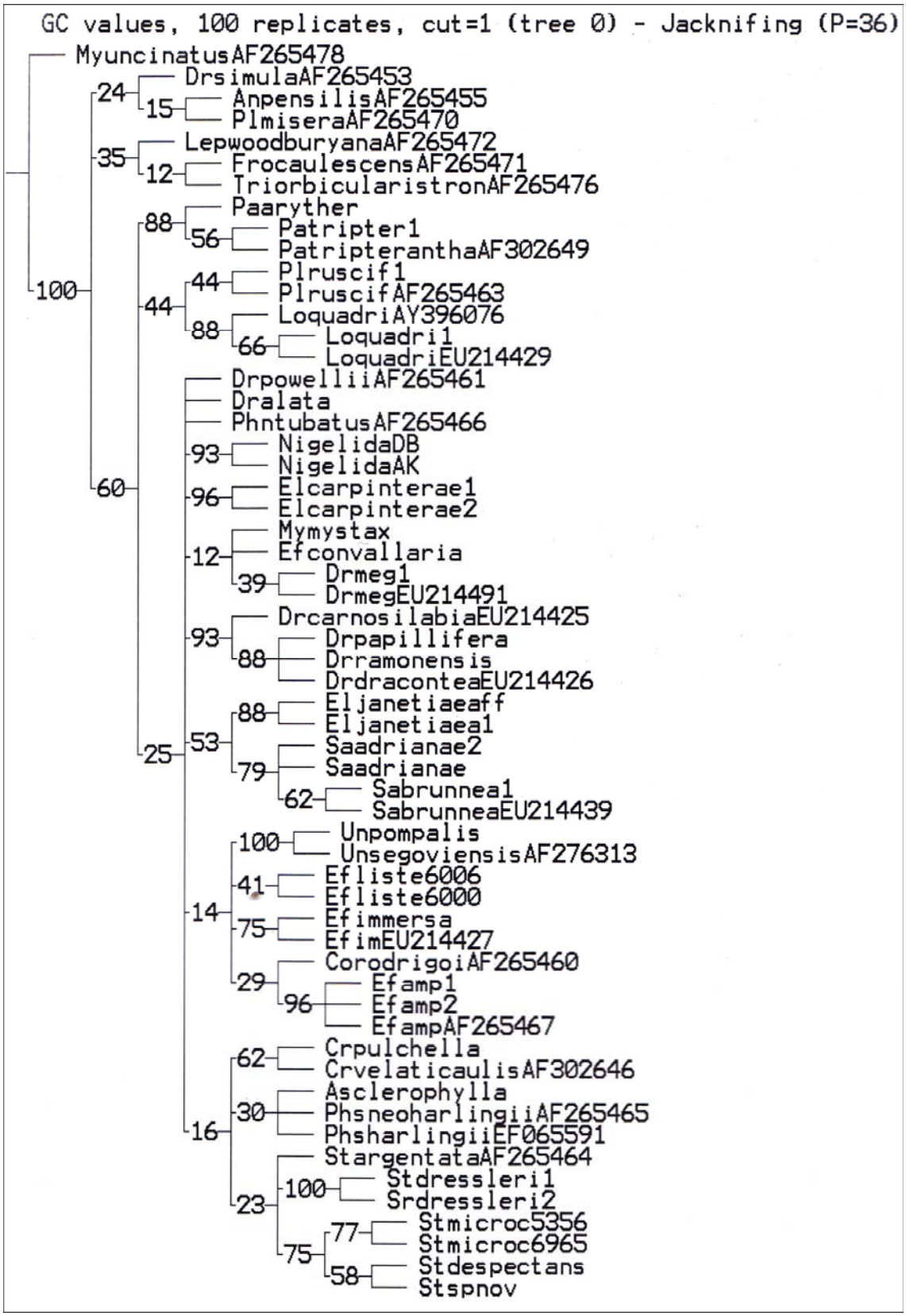
*The combination in *Stelis* has not been published for these species, but they surely fit in the *Stelis s.l.* concept.
**Lab numbering is nothing more than just a reference of the coding that each sample was given during DNA extraction, amplification and sequencing.
***Not all species in this list have finally been used in the reconstruction of the phylogenetic trees.

Appendix III

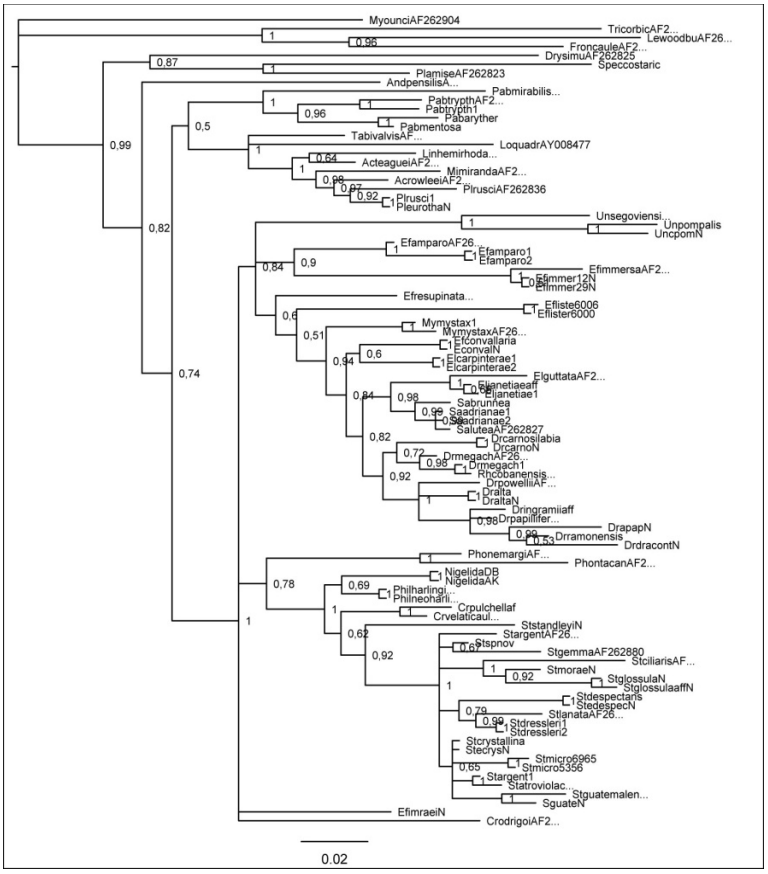




Appendix IV


GC values, 100 replicates, cut=1 (tree 0) - Jacknifing (P=36)
MyuncinatusAF265478
DrsimulaAF265453
AnpensilisAF265455
PlmiseraAF265470
LepwoodburyanaAF265472
FrocaulescensAF265471
TriorbicularistronAF265476
Paarycther
Patripter1
PatripteranthaAF302649
Plruscif1
PlruscifAF265463
LoquadriAY396076
Loquadri1
LoquadriEU214429
DrpowelliiAF265461
Dralata
PhntubatusAF265466
NigelidaDB
NigelidaAK
Elcarpinterae1
Elcarpinterae2
Mymystax
Efconvallaria
Drmeg1
DrmegEU214491
DrcarnosilabiaEU214425
Drpapillifera
Drramonensis
DrdraconteaEU214426
Eljanetiaeaff
Eljanetiaea1
Saadrianae2
Saadrianae
Sabrunnea1
SabrunneaEU214439
Unpompalis
UnsegoviensisAF276313
Efliste6006
Efliste6000
Efimmersa
EfimEU214427
CorodrigoiAF265460
Efamp1
Efamp2
EfampAF265467
Crpulchella
CrvelaticaulisAF302646
Asclerophylla
PhsneoharlingiiAF265465
PhsharlingiiEF065591
StargentataAF265464
Stdressleri1
Srdressleri2
Stmicroc5356
Stmicroc6965
Stdespectans
Stspnov

Appendix V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TricorbicAF2...
LewoodbuAF26...
FroncauleAF2...
DrysimuAF262825
Speccostaric
PlamiseAF262823
AndpensilisA...
Pabmirabilis...
PabtrypthAF2...
Pabtrypth1
Pabaryther
Pabmentosa
TabivalvisAF...
LoquadrAY008477
Linhemirhoda...
ActeagueiAF2...
MimirandaAF2...
AcrowleeiAF2...
PlrusciAF262836
Plrusci1
PleurothaN
Unsegoviensi...
Unpompalis
UncpomN
EfamparoAF26...
Efamparo1
Efamparo2
EfimmersaAF2...
Efimmer12N
Efimmer29N
Efresupinata...
Efliste6006
Eflister6000
Mymystax1
MymystaxAF26...
Elconvallaria
EconvalN
Elcarpinterae1
Elcarpinterae2
ElguttataAF2...
Elanetiaeaff
Elanetiae1
Sabrunnea
Saadrianae1
Saadrianae2
SaluteaAF262827
Drcarnosilabia
DrcarnoN
DrmegachAF26...
Drmegach1
Rhcobbanensis...
DrpowelliiAF...
Dralta
DraltaN
Dringramiiaff
Drpapillifer...
DrapapN
Drramonensis
DrdracontN
PhonemargiAF...
PhontacanAF2...
NigelidaDB
NigelidaAK
Philharlingi...
Philneoharli...
Crpulchellaf
Crvelaticaul...
StstandleyiN
StargentAF26...
Stspnov
StgemmaAF262880
StciliarisAF...
StmoraeN
StglossulaN
StglossulaaffN
Stdespectans
StedespecN
StlanataAF26...
Stdressleri1
Stdressleri2
Stcrystallina
StecrysN
Stmicro6965
Stmicro5356
Stargent1
Statroviolac...
Stguatemalen...
SguateN
EfimraeiN
CrodrigoiAF2...
0.02


MyounciAF262904
TricorbicAF2...
LewoodbuAF26...
FroncauleAF2...
DrysimuAF262825
Speccostaric
PlamiseAF262823
AndpensilisA...
Pabmirabilis...
PabtrypthAF2...
Pabtrypth1
Pabarythe
Pabmentosa
TabivalvisAF...
LoquadrAY008477
Linhemirhoda...
ActeagueiAF2...
MimirandaAF2...
AcrowleeiAF2...
PlrusciAF262836
Plrusci1
PleurothaN
EfimraeiN
Unsegoviensi...
Unpompalis
UncpomN
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Efamparo1
Efamparo2
EfimmersaAF2...
Efimmer12N
Efimmer29N
Efresupinata...
Efliste6006
Eflister6000
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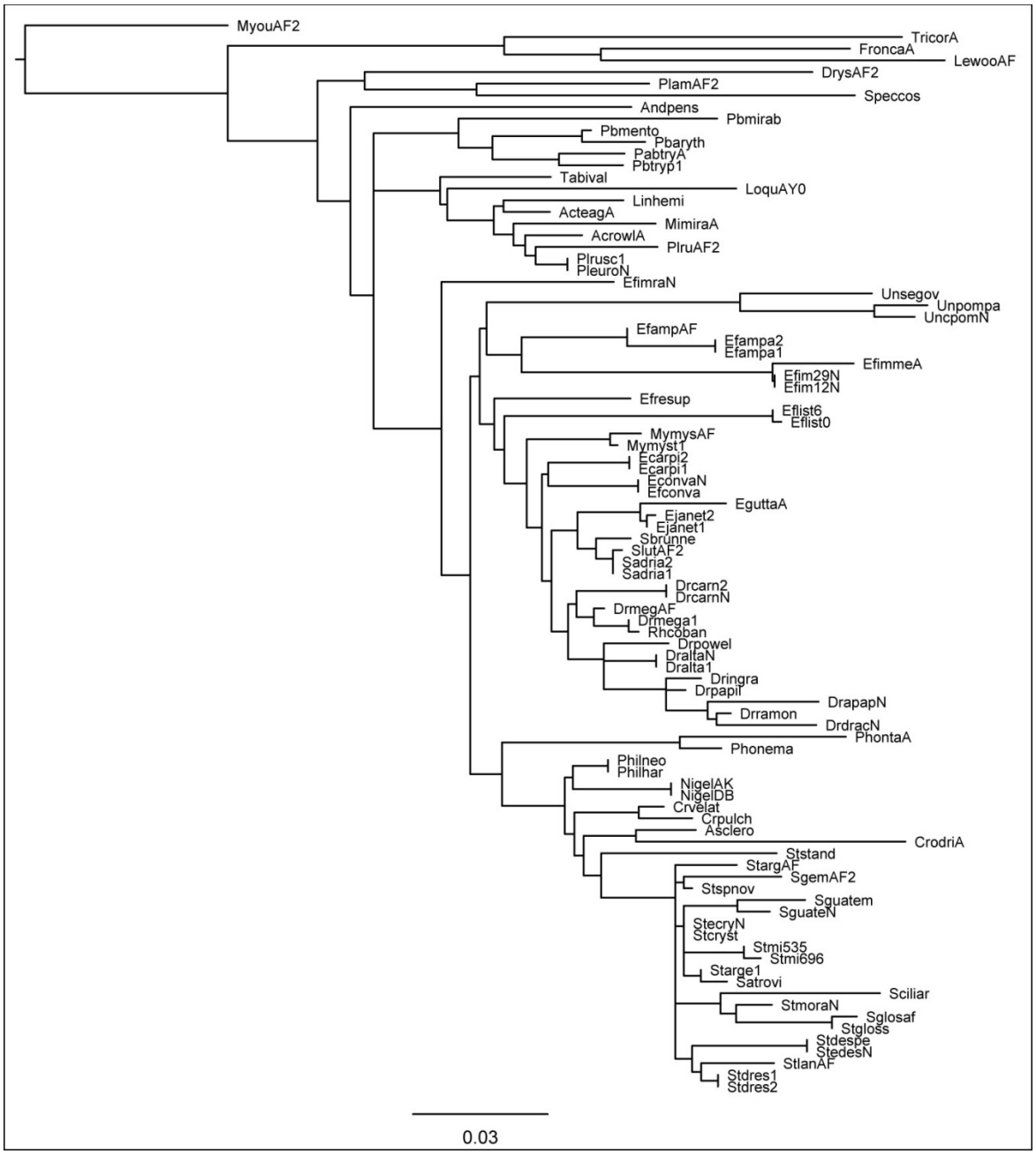
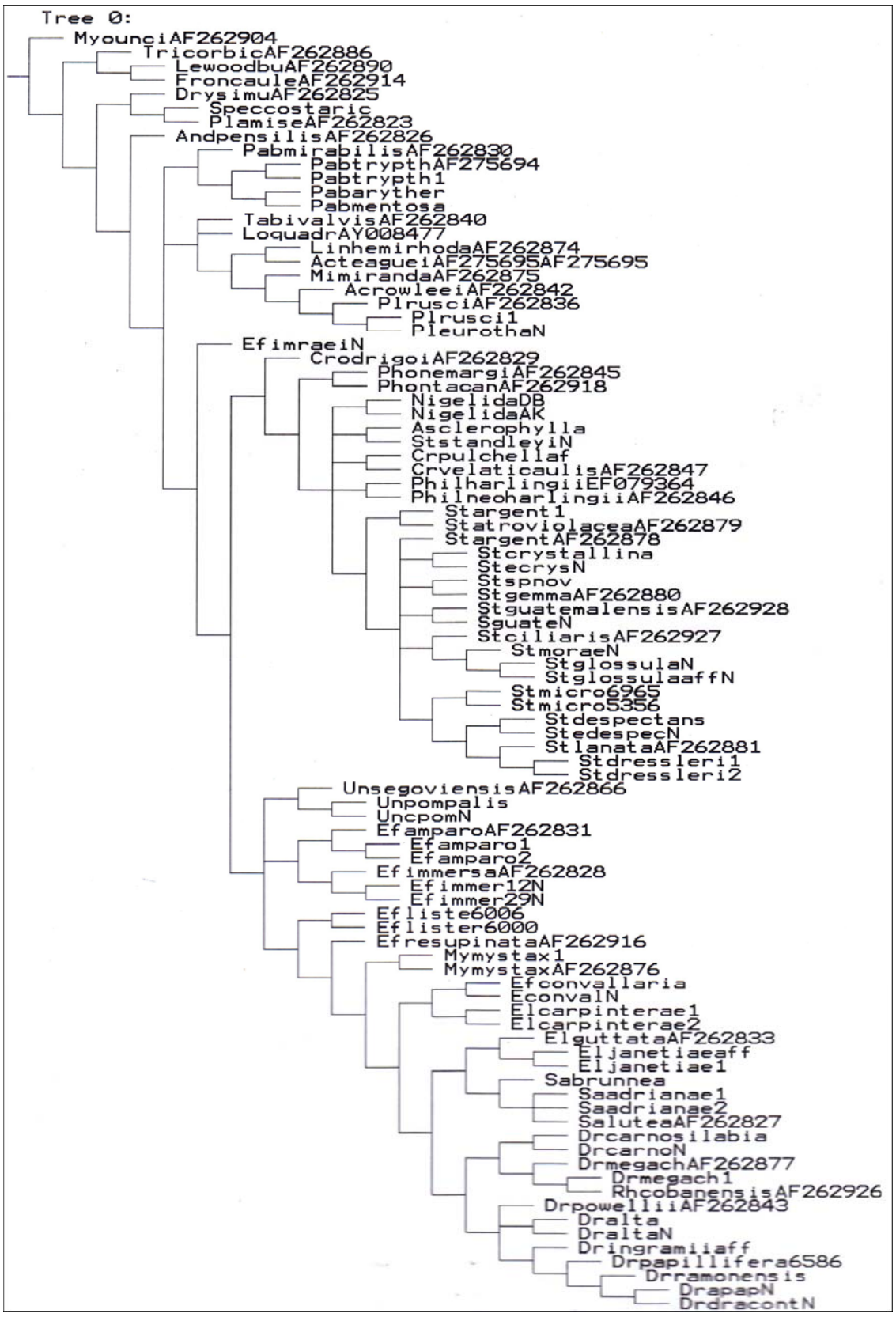
MymystaxAF26...
Elconvallaria
EconvalN
Elcarpinterae1
Elcarpinterae2
ElguttataAF2...
Elanetiaeaff
Elanetiae1
Sabrunnea
Saadrianae1
Saadrianae2
SaluteaAF262827
Drcarnosilabia
DrcarnoN
DrmegachAF26...
Drmegach1
Rhcobbanensis...
DrpowelliiAF...
Dralta
DraltaN
Dringramiiaff
Drpapillifer...
DrapapN
Drramonensis
DrdracontN
PhonemargiAF...
PhontacanAF2...
NigelidaDB
NigelidaAK
Philharlingi...
Philneoharli...
Asclerophylla
Crpulchellaf
Crvelaticaul...
StstandleyiN
StargentAF26...
Stspnov
StgemmaAF262880
StciliarisAF...
StmoraeN
StglossulaN
StglossulaaffN
Stdespectans
StedespecN
StlanataAF26...
Stdressleri1
Stdressleri2
Stcrystallina
StecrysN
Stmicro6965
Stmicro5356
Stargent1
Statroviolac...
Stguatemalen...
SguateN
0.04

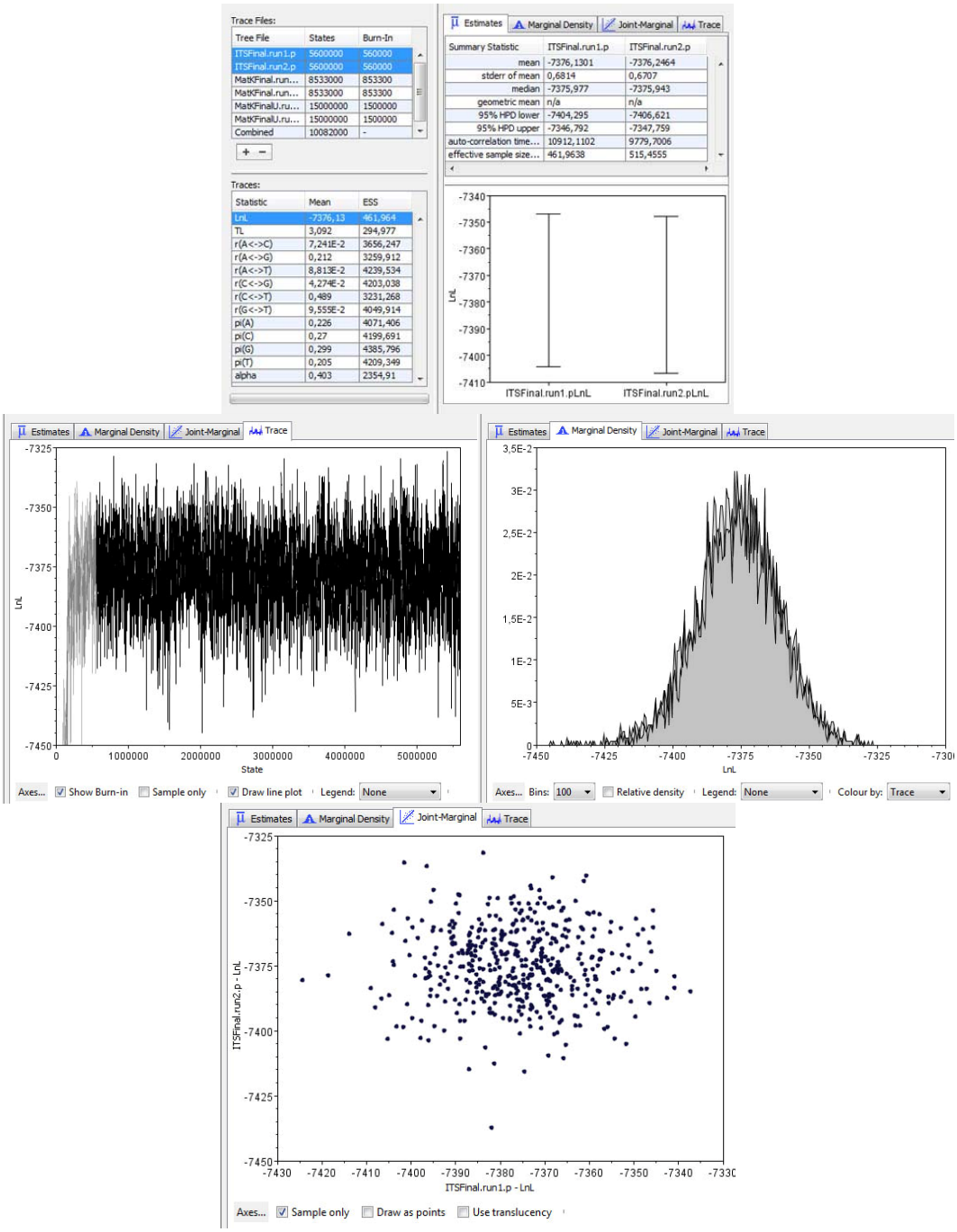
Appendix VI


MyouAF2
TricorA
FroncaA
LewooAF
DrysAF2
PlamAF2
Speccos
Andpens
Pbmirab
Pbmento
Pbaryth
PabtryA
Pbtryp1
Tabival
LoquAY0
Linhemi
ActeagA
MimiraA
AcrowlA
PlruAF2
Plrusc1
PleuroN
EfimraN
Unsegov
Unpompa
UncpomN
EfampAF
Efampa2
Efampa1
EfimmeA
Efim29N
Efim12N
Efresup
Eflist6
Eflist0
MymysAF
Mymyst1
Ecarpi2
Ecarpi1
EconvaN
Efconva
EguttaA
Ejanet2
Ejanet1
Sbrunne
SlutAF2
Sadria2
Sadria1
Drcarn2
DrcarnN
DrmegAF
Drmega1
Rhcoban
Drpowel
DraltaN
Dralta1
Dringra
Drpapil
DrapapN
Drramon
DrdracN
PhontaA
Phonema
Philneo
Philhar
NigelAK
NigelDB
Crvelat
Crpulch
Asclero
CrodriA
Ststand
StargAF
SgemAF2
Stspnov
Sguatem
SguateN
StecryN
Stcryst
Stmi535
Stmi696
Starge1
Satrovi
Sciliar
StmoraN
Sglosaf
Stgloss
Stdespe
StedesN
StlanAF
Stdres1
Stdres2
0.03

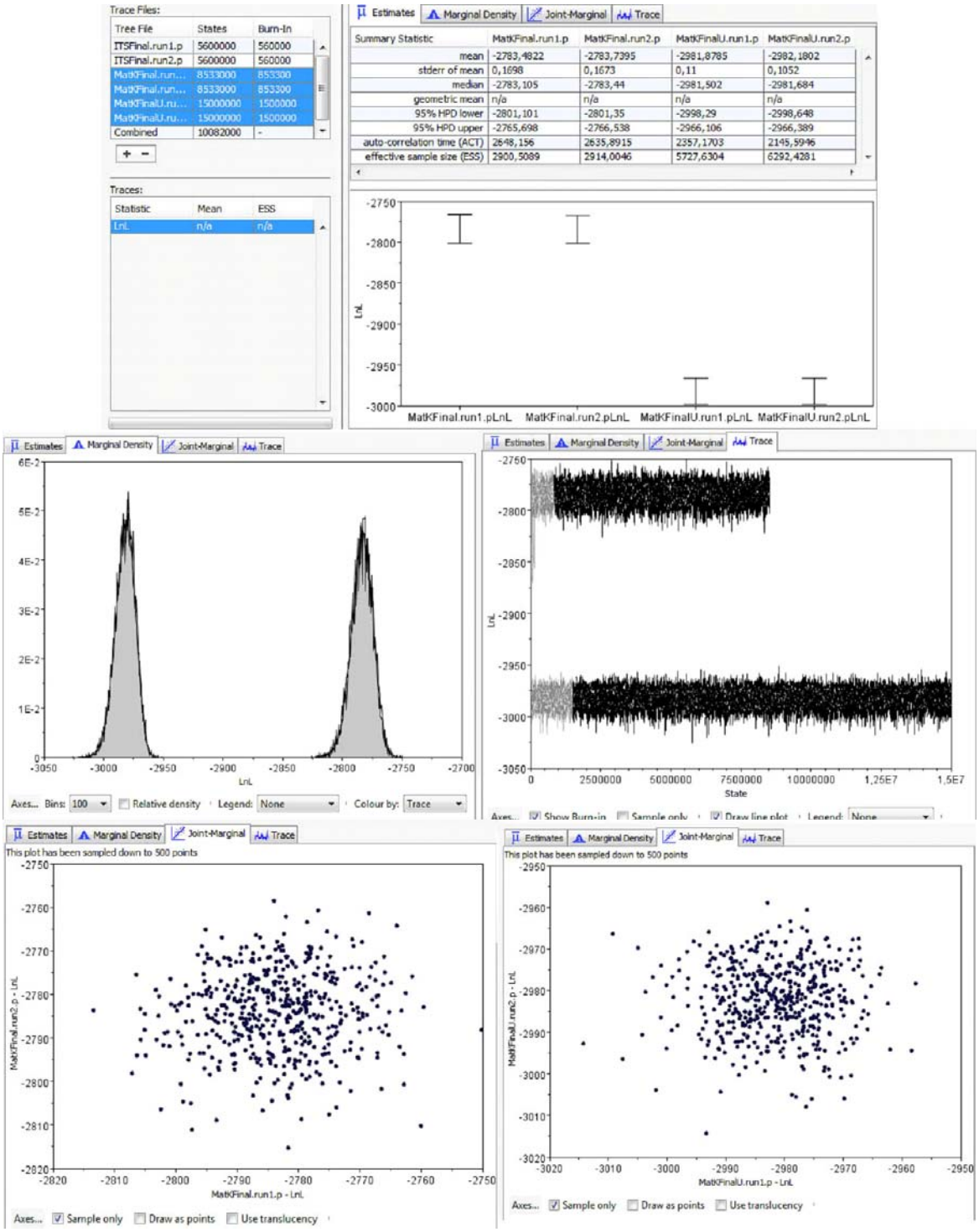
Appendix VII



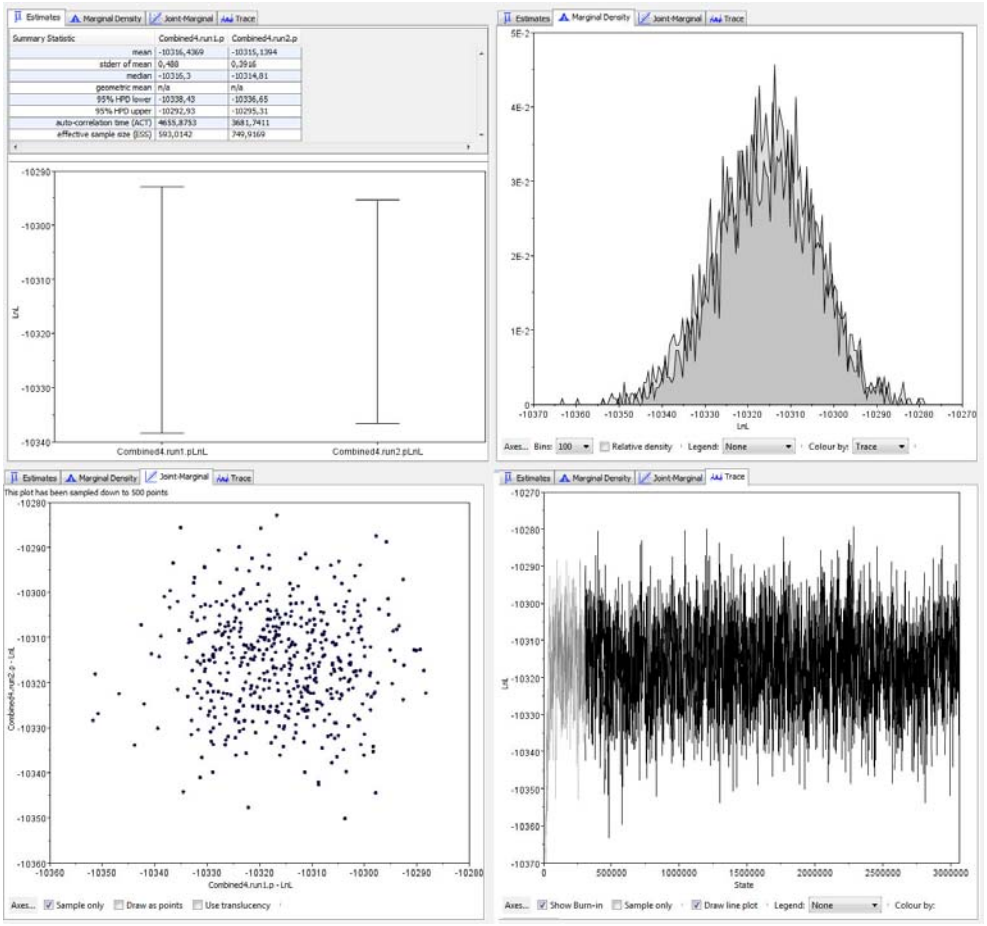
Appendix VIII

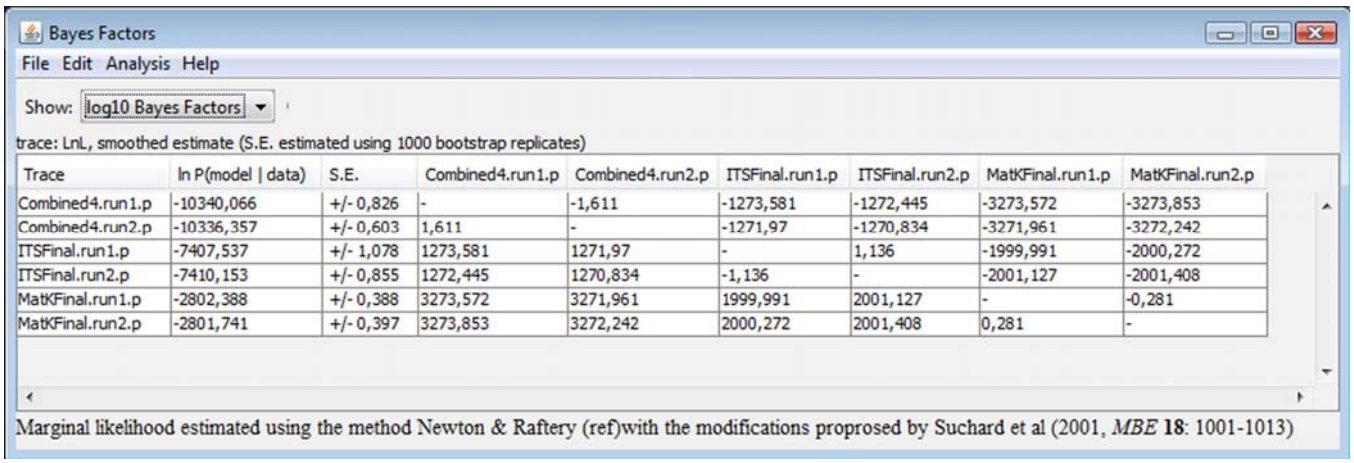


Appendix IX


Trace Files:
Tree File States Burn-In
ITSFinal.run1.p 5600000 560000
ITSFinal.run2.p 5600000 560000
MatKFinal.run... 8533000 853300
MatKFinal.run... 8533000 853300
MatKFinalU.ru... 15000000 1500000
MatKFinalU.ru... 15000000 1500000
Combined 10082000 -
Traces:
Statistic Mean ESS
LnL n/a n/a
Estimates
Marginal Density
Joint-Marginal
Trace
Summary Statistic MatKFinal.run1.p MatKFinal.run2.p MatKFinalU.run1.p MatKFinalU.run2.p
mean -2783,4822 -2783,7395 -2981,8785 -2982,1802
stderr of mean 0,1698 0,1673 0,11 0,1052
median -2783,105 -2783,44 -2981,502 -2981,684
geometric mean n/a n/a n/a n/a
95% HPD lower -2801,101 -2801,35 -2998,29 -2998,648
95% HPD upper -2765,698 -2766,538 -2966,106 -2966,389
auto-correlation time (ACT) 2648,156 2635,8915 2357,1703 2145,5946
effective sample size (ESS) 2900,5089 2914,0046 5727,6304 6292,4281
LnL
MatKFinal.run1.pLnL
MatKFinal.run2.pLnL
MatKFinalU.run1.pLnL
MatKFinalU.run2.pLnL
Axes... Bins: 100 Relative density Legend: None Colour by: Trace
State
Show Burn-in
Sample only
Draw line plot
This plot has been sampled down to 500 points
MatKFinal.run2.p - LnL
MatKFinal.run1.p - LnL
MatKFinalU.run2.p - LnL
MatKFinalU.run1.p - LnL
Axes... Sample only Draw as points Use translucency

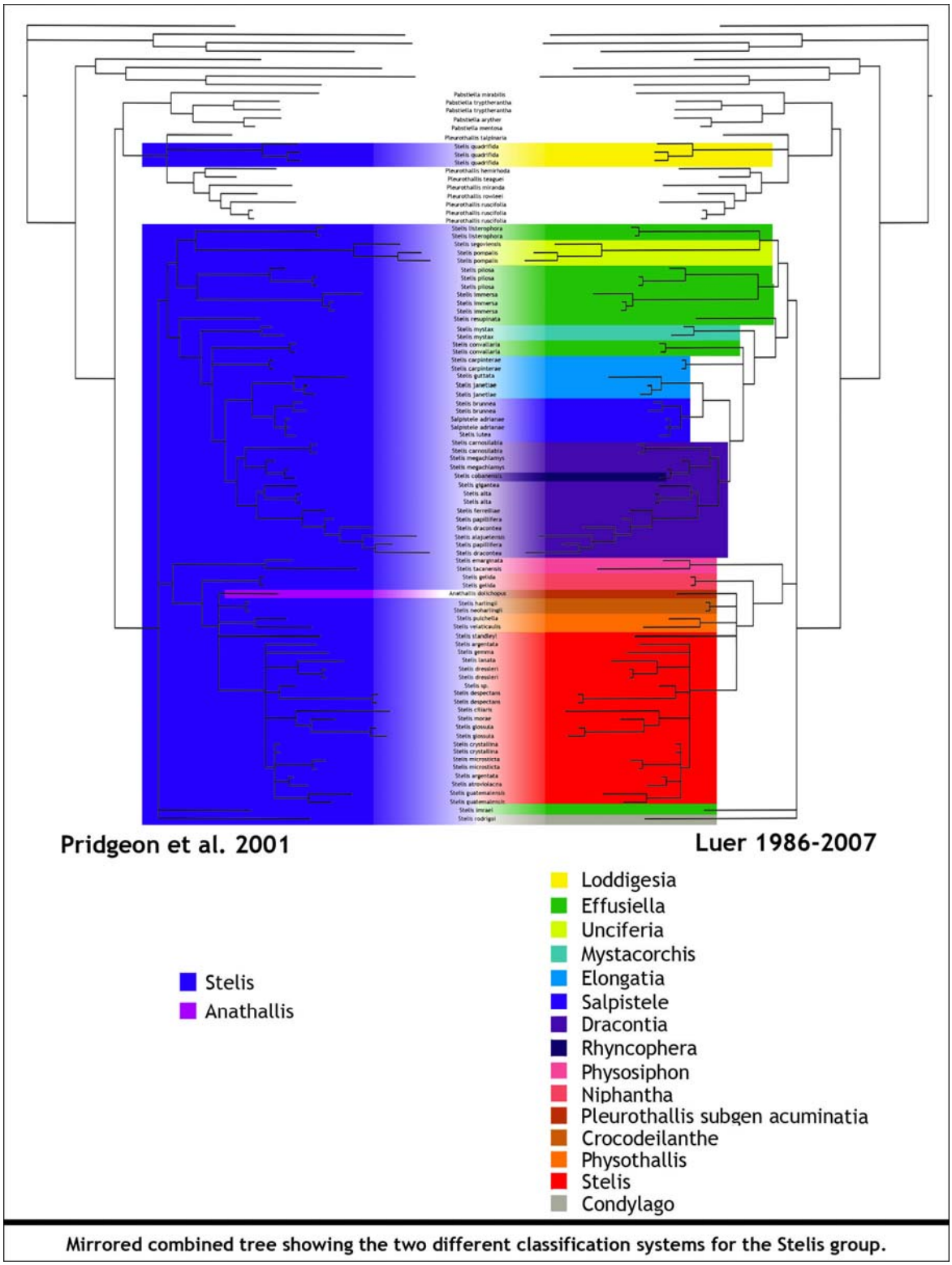
Appendix X





Trace	ln P(model | data)	S.E.	Combined4.run1.p	Combined4.run2.p	ITSFinal.run1.p	ITSFinal.run2.p	MatKFinal.run1.p	MatKFinal.run2.p
Combined4.run1.p	-10340,066	+/- 0,826	-	-1,611	-1273,581	-1272,445	-3273,572	-3273,853
Combined4.run2.p	-10336,357	+/- 0,603	1,611	-	-1271,97	-1270,834	-3271,961	-3272,242
ITSFinal.run1.p	-7407,537	+/- 1,078	1273,581	1271,97	-	1,136	-1999,991	-2000,272
ITSFinal.run2.p	-7410,153	+/- 0,855	1272,445	1270,834	-1,136	-	-2001,127	-2001,408
MatKFinal.run1.p	-2802,388	+/- 0,388	3273,572	3271,961	1999,991	2001,127	-	-0,281
MatKFinal.run2.p	-2801,741	+/- 0,397	3273,853	3272,242	2000,272	2001,408	0,281	-

Appendix XI



Mirrored combined tree showing the two different classification systems for the Stelis group.

Appendix XII

Clades	*Genus*	Mexico	Guatemala	Belize	Honduras	El Salvador	Nicaragua	Costa Rica	Panama	Colombia	Ecuador	Peru	Bolivia	Venezuela	Guyana	Brasil	Caribbean	Species Number
Stelis s.m.	*Crocodeilanthe*	0	0	0	0	0	0	3	3	29	43	21	10	7	0	0	1	70
	Niphantha	1	1	0	1	1	1	1	1	1	2	1	1	1	1	1	0	2
	Physosiphon	3	1	0	0	0	0	0	0	1	2	1	0	0	0	0	0	4
	Physothallis	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	2
	Ple. Acum. *Acuminatia*	2	1	0	2	1	2	3	2	8	9	9	13	4	1	3	0	27
	Stelis s.s	40	30	11	14	15	15	81	49	142	404	86	58	78	11	60	22	900
Subtotal1		**46**	**33**	**11**	**17**	**17**	**18**	**88**	**55**	**181**	**462**	**118**	**82**	**90**	**13**	**64**	**23**	**1005**
Salpistele s.l.	*Dracontia*	3	3	1	1	1	4	16	6	0	0	0	0	0	0	0	1	17
	Salpistele	0	0	0	0	0	0	1	4	0	0	0	0	0	0	0	0	5
	Ple. Elong. *Petiolatae*	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	2
Carpinterae	Ple. Elong. *Elongatia*	0	0	0	0	0	0	1	1	4	2	1	0	0	0	0	0	8
Mystacorchis	*Mystacorchis*	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
Condylago	*Condylago*	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0	2
Unciferia s.l. + Umbraticola	*Unciferia*	0	1	0	1	1	1	8	4	3	1	0	0	0	0	0	0	10
	Effusiella	12	5	0	4	3	2	7	4	11	12	8	3	4	1	1	1	41
Subtotal2		**15**	**9**	**1**	**6**	**5**	**7**	**34**	**23**	**19**	**15**	**9**	**3**	**4**	**1**	**1**	**2**	**86**
TOTAL (Sub1+Sub2)		**61**	**42**	**12**	**23**	**22**	**25**	**122**	**78**	**200**	**477**	**127**	**85**	**94**	**14**	**65**	**25**	**1091**
Subt2/Total (%)*		25	21	8	26	23	28	28	29	10	3	7	4	4	7	2	8	8

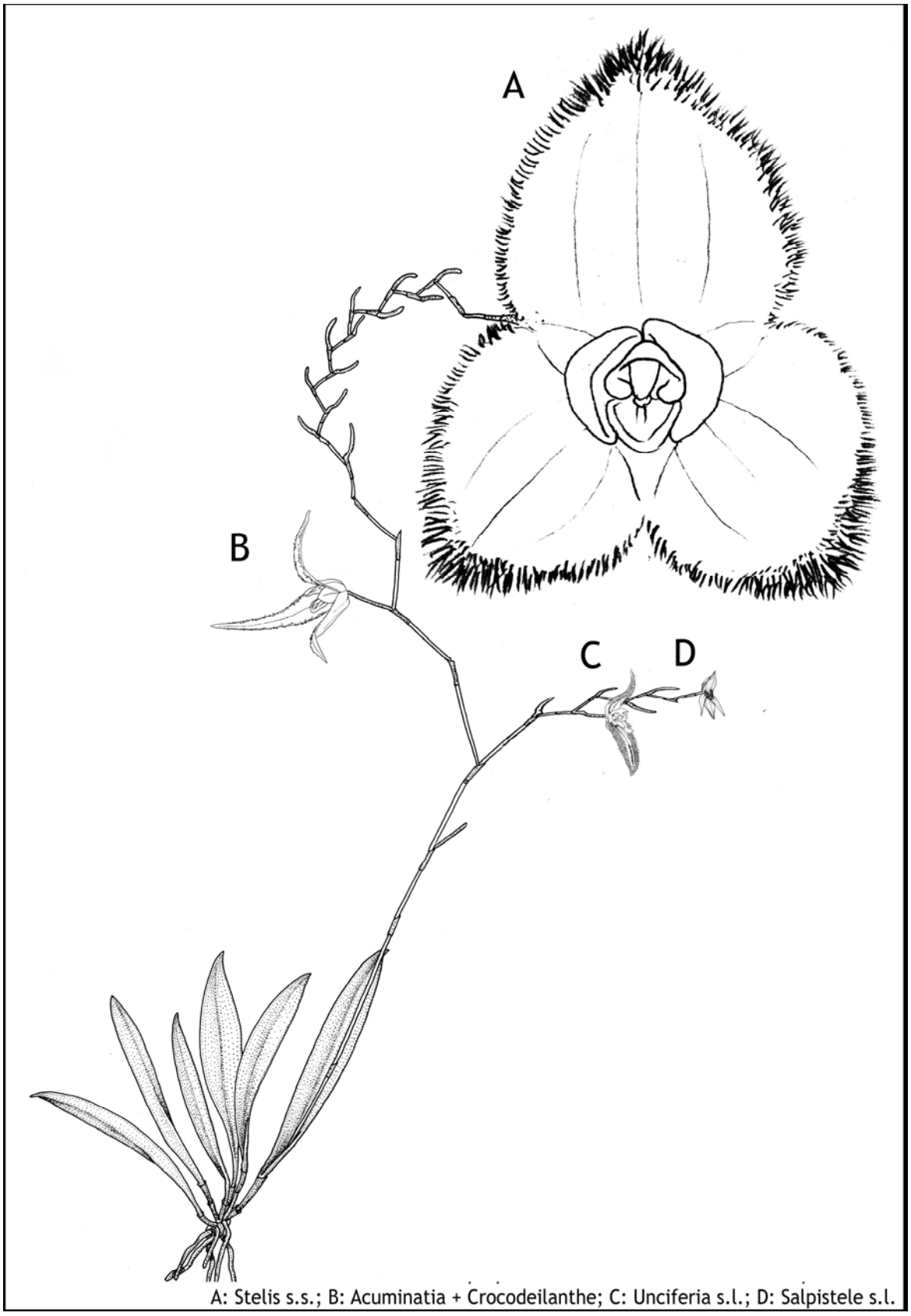
*Numers in red indicate very high relative frequencies of Subt2.

Appendix XIII

Characters	Consensus Tree						10 Trees (Frequency)						Selected	
	Stelis s.l.	*Stelis* s.m.	*Stelis s.s.*	*Salpistele s.l.*	*Unciferia s.l.*	*Salpistele + Petiolatae*	*Stelis s.l.*	*Stelis* s.m.	*Stelis s.s.*	*Salpistele s.l.*	*Unciferia s.l.*	*Salpistele + Petiolatae*	*Stelis s.l.*	
Spathe Substance	0 or 1	0	0	1	0	0	0 (80%) 0 or 1 (20%)	0 (80%) 0 or 1 (20%)	1	0	0	0	Papery	0
Inflorescence Development	0	0	1	0	0	0	0	0	1	0	0	0	Succesive	0
Inflorescence Position	0	0	0	0	0	1	0	0	0	0	0	1	Erect	0
Sepals Maculation	0	1	1	0	0	0	0 (80%) 0 or 1 (20%)	0 (80%) 0 or 1 (20%)	0	0	0	0	Present	0
Lateral Sepal Fusion	0	1	1	0	0	2	0 (80%) 0 or 1 (20%)	0 (80%) 0 or 1 (20%)	1	0 (90%) 0 or 2 (10%)	0	2	Superior Convergent	0
Dorsal Sepal Fusion	0	1	1	0	0	0	0 (80%) 0 or 1 (20%)	0 (80%) 0 or 1 (20%)	1	0	0	0	Absent	0
Synsepal	0	1	1	0	0	0	0 (80%) 0 or 1 (20%)	0 (80%) 0 or 1 (20%)	1	0	0	0	Present	0
Sepal Length-Width Ratio	0	0	1	0	0	0	0	0	1	0	0	0	Longer than Wide	0
Sepal Pubescence	0	0	0	1	0	1	0	0	0	1	0	1	Present	0
Petal Apex	0 or 1	1	2	1	0 or 1	1	0 (30%) 1 (20%) 0 or 1 (50%)	0 (30%) 1 (20%) 0 or 1 (50%)	2	1	0 (30%) 1 (20%) 0 or 1 (50%)	1	Fleshy Midvein	0
Petal Shape	0	0	1	0	0	0	0	0	1	0	0	0	Linear	0
Petal Margin	0	0	2	0	0	0	0	0	2	0 (90%) 0 or 1(10%)	0	0	Straight	0
Petal-Sepal Ratio	0	0	0	0	0	1	0	0	0	0	0	1	Much Longer Sepal	0
Glenion	0	0	1	0	0	0	0	0	1	0	0	0	Absent	0

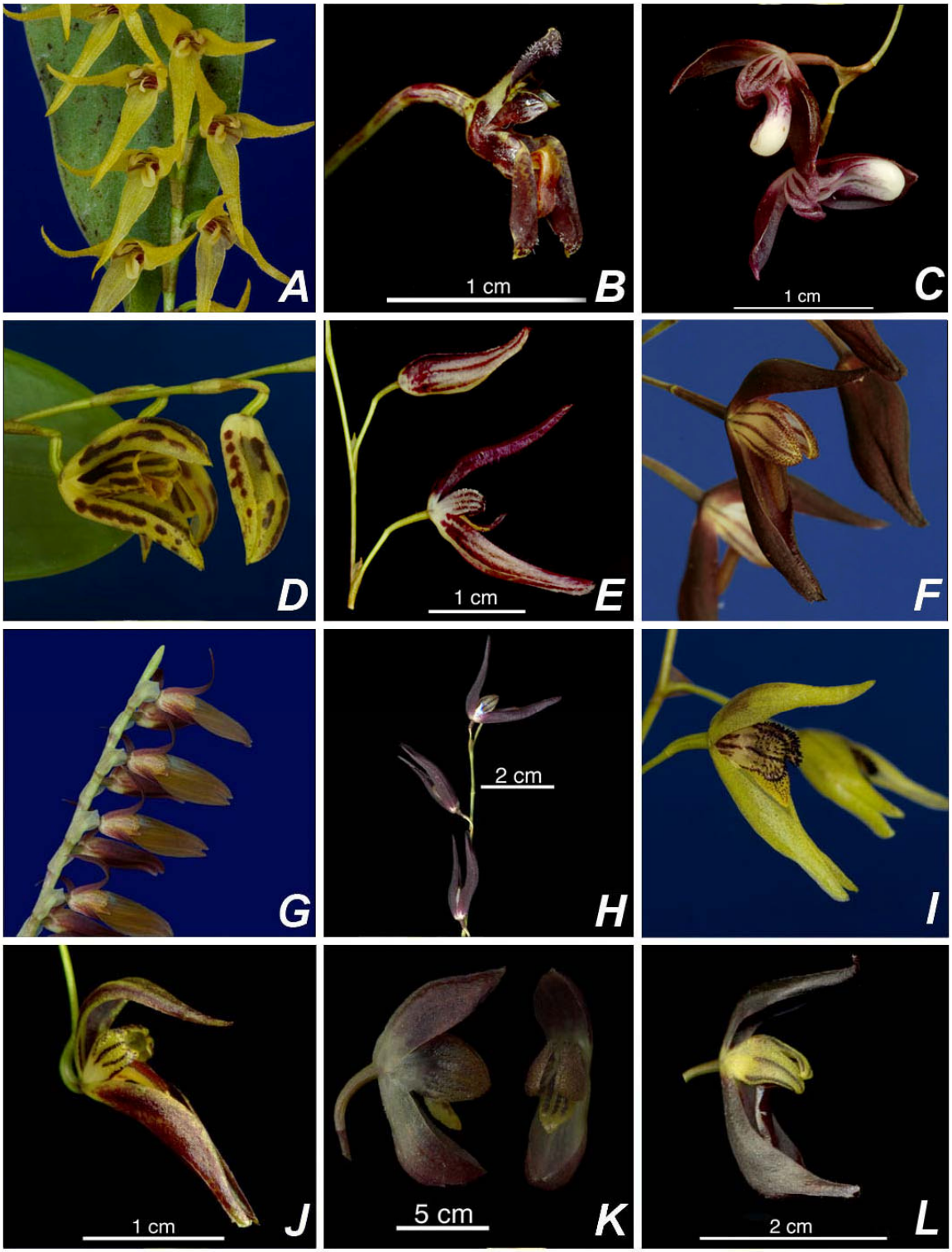
Lip Articulation	0	0	1	0	0	0	0	0	1	0	0	0	Present	0
Column Foot	0	0	1	0	0	0	0	0	1	0	0	0	Present	0
Column Foot Position	0	0	2	Straight	Straight	Straight	0	0	2	0 (90%) 0 or 1 (10%)	0	0 (90%) 0 or 1 (10%)	Straight	0
Column Position	0	0	1	0	0	0	0	0	1	0	0	0	Curved	0
Column Wings	0	0	1	0	0	0	0	0	1	0	0	0	Present	0
Column Shape	0	0	1	Cilindrical	Cilindrical	Cilindrical	0	0	1	0 (90%) 0 or 2 (10%)	0	0 (90%) 0 or 1 or 2(10%)	Cilindrical	0
Column-Lip Ratio	0	0	0	0	0	0	0	0	0	0 (90%) 0 or 1 (10%)	0	0 (90%) 0 or 1 or 2(10%)	Subequal	0
Anther Position	0	0	1	0	0	0	0	0	1	0	0	0	Incumbent	0
Stigma Position	0	0	1	0	0	0	0	0	1	0	0	0	Ventral	0
Stigma Opening	0	0	1	0	0	0	0	0	1	0	0	0	Covered	0
Pollinaria Shape	0	0	1	0	0	0	0	0	1	0	0	0	Whale-Tail	0
Pollinaria Composition	0	0	2	0	0	0	0	0	2	0	0	0	Caudicles and Viscarium Free	0
Clade Geography	2	3	3	2	2	2	2 (80%) 2 or 3 (20&)	2 (80%) 2 or 3 (20&)	3	2	2	2	Costa Rica - Colombia	2
Species Geography	2	2	2	2	2	2	2	2	2	2	2	2	Costa Rica - Colombia	2

Appendix IXX

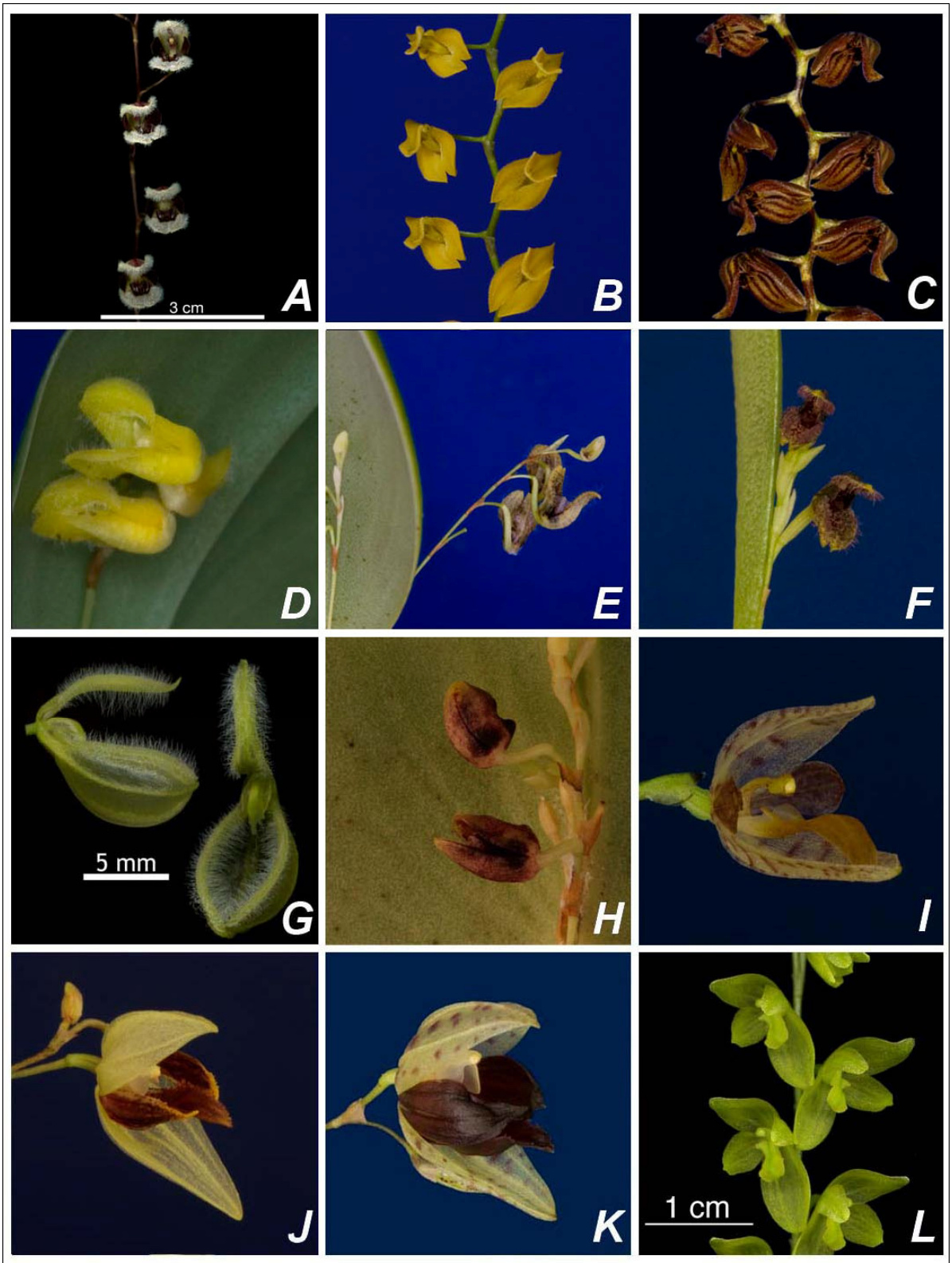


A: Stelis s.s.; B: Acuminatia + Crocodeilanthe; C: Unciferia s.l.; D: Salpistele s.l.

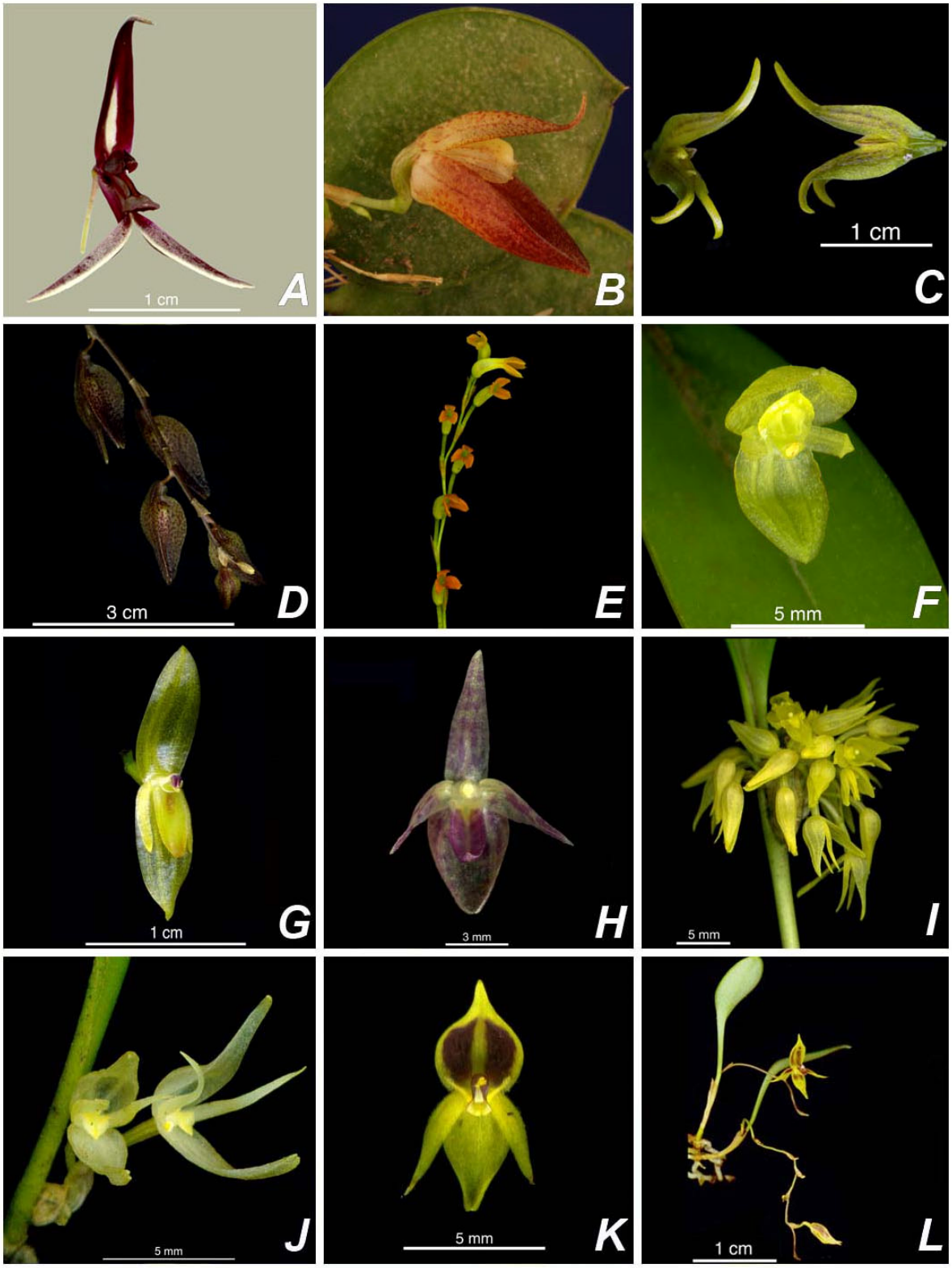
Appendix XX



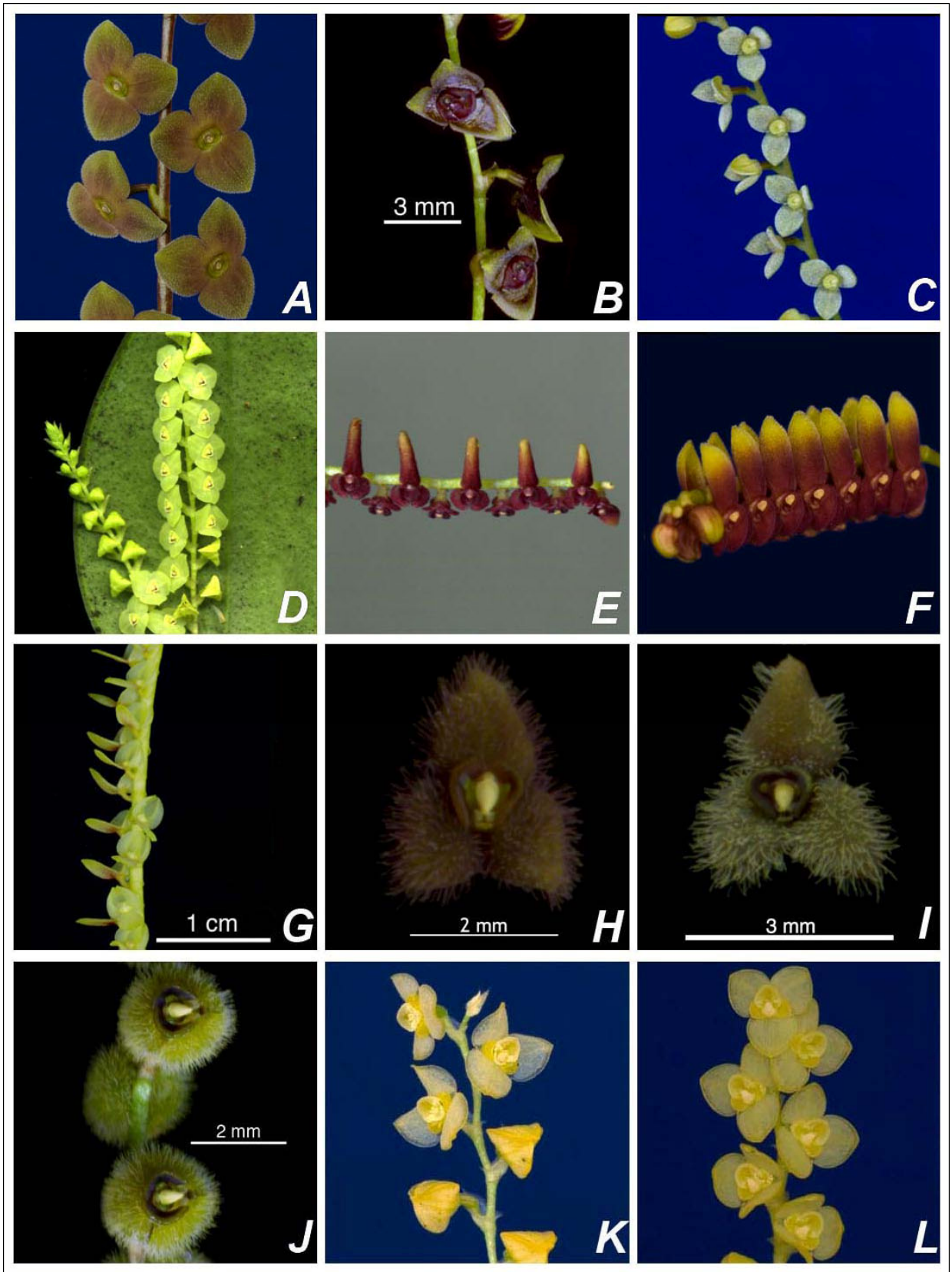
A: Anathallis dolichopus - JBL 05512
B: Stelis (Condylago) furculifera - RD 6835
C: Stelis (Dracontia) carnosilabia - JBL 00976
D: Stelis (Dracontia) cylindrata - DB 7734
E: Stelis (Dracontia) dracontea - DB 616
F: Stelis (Dracontia) aff. ferrelliae - DB 5543
G: Stelis (Dracontia) gigantea - FP 4498
H: Stelis (Dracontia) megachlamys - DB 5106
I: Stelis (Dracontia) papillifera - DB 7215
J: Stelis (Dracontia) alajuelensis - MB 1052
K: Stelis (Dracontia) sp. - DB 7152
L: Stelis (Dracontia) sp. - FP 4900



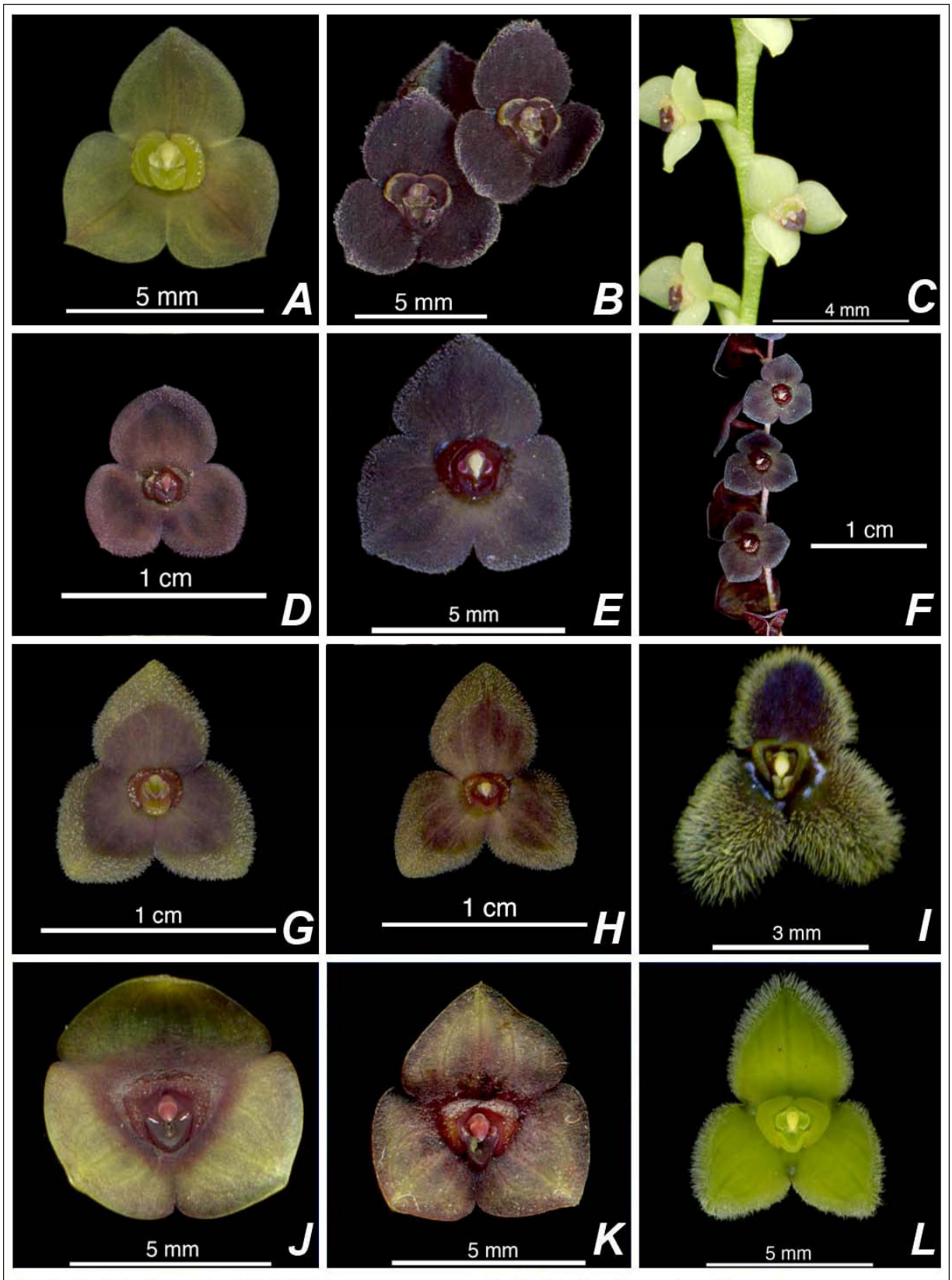
A: Stelis (Effusiella) convallaria - DB 3940
B: Stelis (Effusiella) immersa - JBL 10792
C: Stelis (Effusiella) immersa - AK 2501
D: Stelis (Effusiella) imraei - JBL 02007
E: Stelis (Effusiella) imraei - JBL s.n.
F: Stelis (Effusiella) listerophora - FP 7691
G: Stelis (Effusiella) pilosa - DB 5142
H: Stelis (Effusiella) vaginata - GR 90
I: Stelis (Elongatia) carpinterae - DB 7159
J: Stelis (Elongatia) janetiae - DB 5008
K: Stelis (Elongatia) aff. janetiae - DB 7286
L: Stelis (Loddigesia) quadrifida - DB 3012



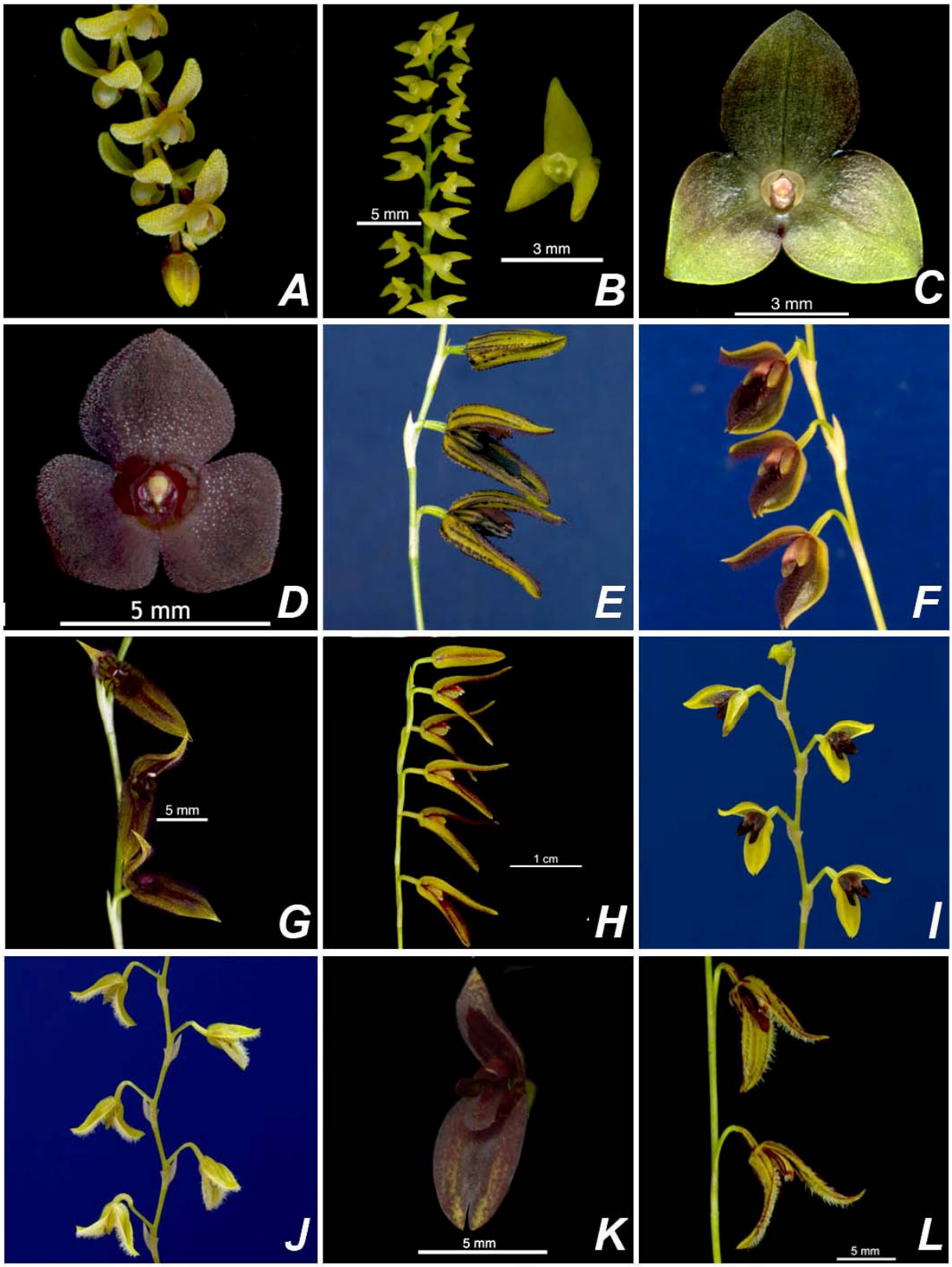
A: Stelis (Mystacorchis) mystax - DB 2988
B: Pabstiella aryter - DB 6501
C: Pabstiella tripterantha - JBL 21087
D: Pabstiella tripterantha - DB 5994
E: Stelis (Physosiphon) emarginata - JBL 01621
F: Pleurothallis aurita CL 17357
G: Pleurothallis isthmii - JBL 05149
H: Pleurothallis loranthophylla - JBL 02079
I: Pleurothallis ruscifolia - RG 26
J: Pleurothallis ruscifolia - JBL 02149
K: Salpistele adrianae - DB 5917
L: Salpistele brunnea - FP 5153



A: Stelis (Stelis s.s.) argentata - FP 5051
B: Stelis (Stelis s.s.) chasei - DB 7380
C: Stelis (Stelis s.s.) crystallina - DB 5619
D: Stelis (Stelis s.s.) dressleri - AR 7029
E: Stelis (Stelis s.s.) glossula - DB 2703
F: Stelis (Stelis s.s.) aff. glossula - YK 2046
G: Stelis (Stelis s.s.) guatemalensis - FP 3977
H: Stelis (Stelis s.s.) aff. microchila - DB 1516
I: Stelis (Stelis s.s.) aff. microchila - DB 2915
J: Stelis (Stelis s.s.) aff. microchila - DB 5356
K: Stelis (Stelis s.s.) parvula - AK 2373
L: Stelis (Stelis s.s.) parvula - DB 4411

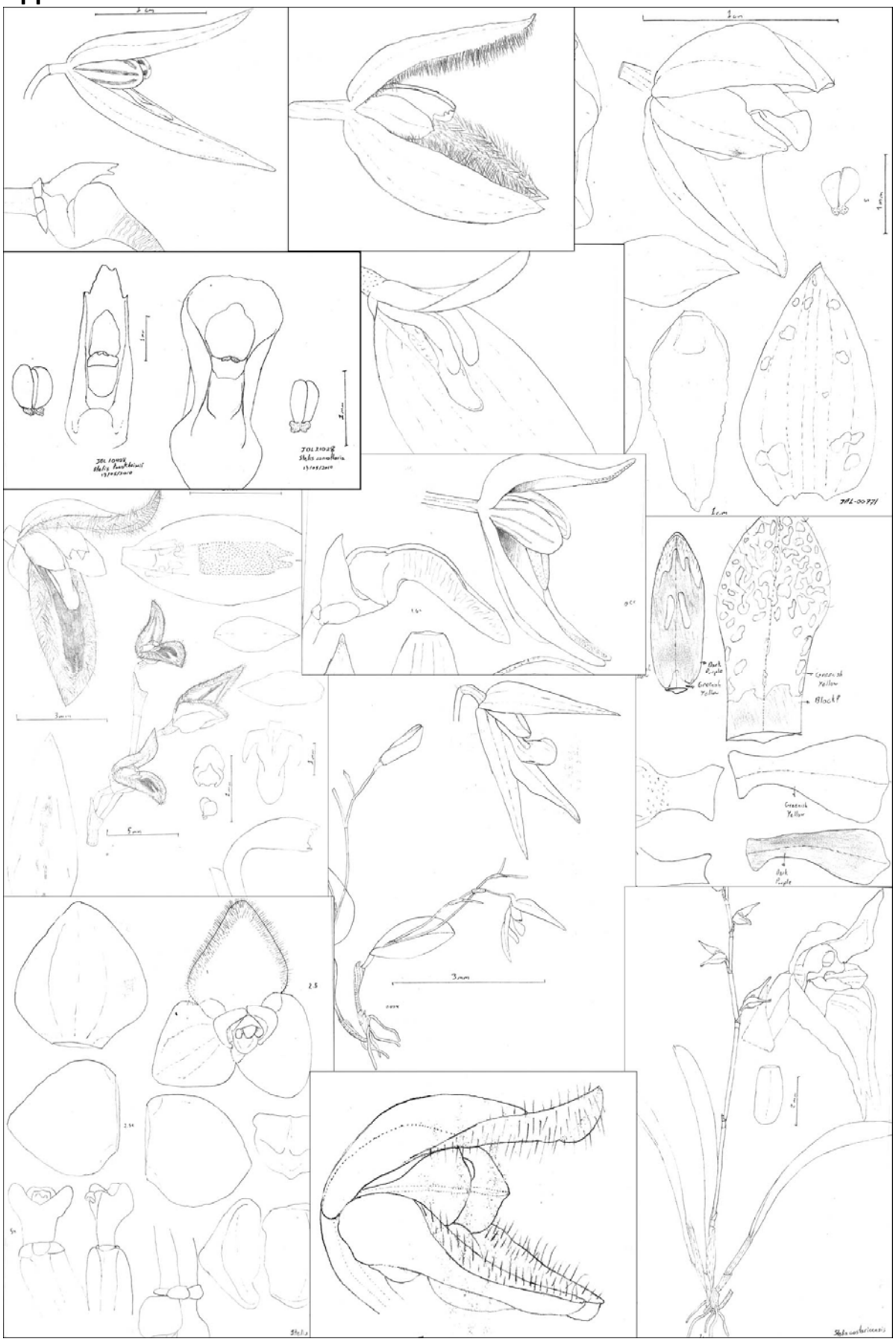


A: Stelis (Stelis s.s.) - JBL 01004
B: Stelis (Stelis s.s.) - AK 2397
C: Stelis (Stelis s.s.) - FP 1990
D: Stelis (Stelis s.s.) - DB 7200
E: Stelis (Stelis s.s.) - JBL 01481
F: Stelis (Stelis s.s.) - JBL 01481
G: Stelis (Stelis s.s.) - JBL s.n.
H: Stelis (Stelis s.s.) - FP 3959
I: Stelis (Stelis s.s.) - DB 5006
J: Stelis (Stelis s.s.) - CO 617
K: Stelis (Stelis s.s.) - AR 6452
L: Stelis (Stelis s.s.) - DB 6480



A: Stelis (Stelis s.s.) - JBL s.n.
B: Stelis (Stelis s.s.) - FP 7503
C: Stelis (Stelis s.s.) - DB 3054
D: Stelis (Stelis s.s.) - FP 4769
E: Stelis (Unciferia) aff. canae - DB 6812
F: Stelis (Unciferia) pilostoma - FP 7601
G: Stelis (Unciferia) pompalis - DB 4548
H: Stelis (Unciferia) segoviensis - AK 1183
I: Stelis (Unciferia) aff. segoviensis - FP 6495
J: Stelis (Unciferia) - JBL 11589
K: Stelis (Unciferia) - JBL 02408
L: Stelis (Unciferia) - JBL 01825

Appendix XXI



"*There are fashions in science, and some scientist climb on the band wagon almost as readily as do some painters and musicians. But although fashions and band wagons may attract the weak, they should be resisted rather than encouraged*" (Popper 1979 cited in Cronquist 1987).