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Phylogenetics of *Stelis* and closely related genera (Orchidaceae: Pleurothallidinae)

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Abstract *Stelis*, one of the largest genera within Pleurothallidinae, was recently recircumscribed to include a few hundred more species, most of which had previously been assigned to *Pleurothallis*. Here, a new phylogenetic analysis of *Stelis* and closely related genera based on DNA sequences from nuclear ITS and chloroplast *matK*, based on a much larger sample, is presented; it includes more than 100 species assigned to *Stelis* and covers all proposed groupings within the genus, many of which have not previously been represented. Clades are proposed to enable easier discussion of groups of closely related species; each

clade is characterized morphologically, ecologically, and geographically to explain the evidence found in the molecular analysis. Discussion of the evolutionary trends of character states found in the genus in its broad sense is given. The current taxonomy of the group is given and the possible taxonomical implications of the findings presented here are discussed.

Keywords *Stelis* · Pleurothallidinae · Orchidaceae · Molecular phylogeny · Morphology · Evolutionary trends

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Introduction

The general characteristics of the genus *Stelis* Sw., on the basis of its morphological characters, were 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 was not widely accepted (Pupulin 2002; Luer 2003; Pridgeon 2005; Govaerts et al. 2011). Species of *Stelis* sensu stricto (*Stelis* s. str.) can be distinguished from other groups of the subtribe Pleurothallidinae by the terminal, racemose, fascicled, few or multi-flowered inflorescences, the triangular flowers with almost identical sepals, tending to radial symmetry, diversely connate sepals, much larger than the petals and lip, the very reduced petals usually with a thick margin, the thickened lip that is similar to the petals, and a very short, unwinged column with an apical stigma and anther (Luer 2003).

The analysis of Pridgeon et al. (2001) using DNA sequences 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., *Crocodelanthe* (Rchb.f. & Warsz.) Luer, *Dracontia* Luer, *Effusia* Luer, *Elongatia* Luer, *Mystax* Luer, *Physosiphon* (Lindl.) Luer, *Physothallis* (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* sensu lato (*Stelis* s.l.). Because that phylogenetic inference was based on few species of *Stelis* in its broad sense, extrapolation of the results using previous morphologically inferred relationships (basically those described by Luer 1986) was required to re-accommodate most of the species involved.

Luer, rejected the new circumscription of *Stelis*, and instead recognized it in its narrower delimitation and the genera *Condylago*, *Crocodelanthe* Rchb.f. & Warsz., *Mystacorthis* Szlach. & Marg., *Physothallis* Garay, *Physosiphon* Lindl., *Salpistele*, and *Specklinia* Lindl., and elevated to the generic rank four subgenera of *Pleurothallis* as *Dracontia* (Luer) Luer, *Effusiella* Luer, *Elongatia* (Luer) Luer, and *Unciferia* (Luer) Luer (Luer 2004, 2006). He also described the monotypic genera *Lomax* Luer, *Loddigesia* Luer (an illegitimate name later legitimized as *Lalexia* Luer), and *Niphantha* Luer for a few “misfit” species not clearly belonging to any of the previously recognized groups (Luer 2006, 2007, 2011). All of these genera include one or more species treated by Pridgeon and Chase (2001) as members of *Stelis* in its broader sense. There is general consensus that other older generic names, for example *Dialissa* Lindl. (1845), *Humboldtia* Ruiz & Pavón (1794), *Pseudostelis* Schltr. (1922), *Steliopsis* Brieger (1976; nom. inval.), and *Apatostelis* Garay (1979; nom. illeg.), should be regarded as synonymous with *Stelis* (Pridgeon 2005).

The two contradicting taxonomic systems, i.e., the fine generic splitting proposed by Luer based mostly on morphological grounds, and the more conservative approach proposed by Pridgeon and Chase (2001) on the basis of molecular data, are still debated. Although the concept of *Stelis* in a broad sense is more commonly accepted (Govaerts et al. 2011; Hágsater and Soto 2003; Pridgeon 2005; Pupulin 2002; Solano-Gómez and Salazar 2007), the narrow circumscription has also been used (Dressler and Bogarín 2007; Duque 2008; Karremans 2011, 2012; Karremans & Smith 2012; Luer 2009, 2011; Miller et al. 2011).

With approximately 900 species in its narrower circumscription and over 1100 in its broadest circumscription (Luer 2009), *Stelis* is one of the largest genera in the largest angiosperm family on the planet, accounting for 3–4 % of Orchidaceae species, only rivaled by *Bulbophyllum* Thou., *Dendrobium* Sw., *Epidendrum* L., and *Lepanthes* Sw. Even though they are restricted to the humid environment of American tropics and subtropics, species of *Stelis* are major epiphytic components of forest landscapes, in which many of the taxa occur in large sympatric populations. Although

“mammoth” genera such as *Stelis*, with their astonishing and intricate diversity, have traditionally discouraged systematic botanists, they are unparalleled opportunities enabling evolutionary biologists to speculate on the mechanisms leading to speciation. Irrespective of the taxonomic system used to define *Stelis*, the success of this group of plants in colonizing the American tropics, in terms of ecological diversity, frequency, and species number (i.e. accepted species names), deserves particular attention by botanists.

The objective of this work was to produce an overall picture of phylogenetic relationships within the genus *Stelis* in its broad sense. It includes larger and more balanced sampling, covering all groups involved in *Stelis* s.l. We used the molecular phylogenetic tree based on chloroplast and nuclear sequences as a hypothesis to establish phylogenetic relationships and further investigate congruence with morphological data and geographic distribution patterns.

Although there might be evidence of a need for taxonomic changes, these are not proposed here. To produce a stable system of nomenclature for this complex group, it is necessary:

1. to estimate phylogeny on the basis of maximum taxonomic and character sampling;
2. to conduct a morphological study of each species group in order to characterize it uniquely; and
3. to associate clades and morphological characters with biological and ecological data.

Some of these objectives are beyond the scope of this work, which focuses on the internal relationships among groups of *Stelis* s.l., as suggested by the molecular evidence obtained as a result of improved sampling and the use of updated software for data analysis.

Materials and methods

Most specimens were field collected or obtained from the living collections of Lankester Botanical Garden (JBL), University of Costa Rica; a few were obtained from the private collections of T. Sijm and J. Wubben in the Netherlands. Material was selected on the basis of availability and inter-specific variability (thus preferring species that were not very closely related). At least one sample from each of the genera, subgenera, or artificial groupings accepted in the 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 prevailing nature of the JBL collections. Vouchers of the specimens are kept in the liquid collections at JBL, WAG, or L, unless specified otherwise.

In general DNA sequences of determinate species are obtained from any specimen available and rarely is that

specimen part of the type collection. Although those are equally useful, their determination is interpretative. It is, therefore, noteworthy that in this work the DNA sequences of *Dracontia hydra*, *D. lueriana*, *Pleurothallis sijmii*, *P. silvae-pacis*, *Salpistele adrianae*, *Stelis adrianae*, and *S. tacanensis* were obtained from the actual plants that served as types, and sequences of *Stelis alajuelensis*, *S. atwoodii*, *S. ferrelliae*, *S. kareniae*, and *S. segoviensis* (Karremans 544), even though not part of the type collection, were nonetheless obtained from specimens collected at the type locality.

DNA extraction and sequencing

Fresh leaf and flower cuttings of approximately 1 cm² were obtained from all the selected individuals of each species. Each individual sample was placed in a polypropylene bag with silica gel to dry for approximately a week after which the silica was removed and new dry silica was added. Samples (20 mg) were pulverized in liquid nitrogen by shaking them in a Retsch MM 300 shaker for 5 min using three bullets. Extraction was performed by following the DNEasy extraction procedure (Qiagen). DNA concentration for each sample was adjusted to 10 µmol/l by use of a Nano Drop Spectrophotometer (ND 1000).

The nuclear ribosomal internal transcribed spacer (ITS) region was amplified using the methods and primers, 17SE (ACGAATTCATGGTCCGGTGAAGTGTTTCG) and 26SE (TAGAATTCCTCCGGTTCGCTCGCCGTTAC), for sequencing and amplification described by Sun et al. (1994). The chloroplast gene *matK* was amplified and sequenced using the Kew *matK* primers 2.1aF (ATCCATCTGGAAATCTTAGTTC) and 5R (GTTCTAGCACAAAGAAAGTCG). Amplification was done by preparing each sample with a PCR mix composed of DTB, dNTPs, both primers (four in the case of ITS), Dream Taq, water, and the extracted DNA. Samples were amplified in an MJ Research PTC-200 Pelthier Thermal Cycler, using a temperature profile of 94 °C/5 min, followed by 34 cycles of 94 °C/30 s, 55 °C/30 s, and 72 °C/2 min, and finally 72 °C/10 min. To prepare for sequencing, a DETT kit was used according to the manufacturer's instructions (GE Healthcare). Each sample had two mega-mixes, one for the F-primer and another for the R-primer (four for ITS), and were cycle-sequenced using a standard thermo-profile of 94 °C/20 s, 50 °C/15 s, and 60 °C/1 min, repeated 25 times. Products were analyzed on an ABI 9600 DNA analyzer.

Building the data sets

The Staden (2003) package was used for editing the sequences. When more than one base pair was equally probable, the Unicode nomenclature (IUPAC) was used. In

a few cases the two traces for one sample were too short and there was no overlap so Pregap was unable to build a contig. To keep the information, both sequences were merged by filling in missing positions with Ns. Sequences, for each region independently were aligned by use of Clustal X in BioEdit v.7.5.0.3 (Hall 1999). These were then exported as .fas files and opened in Mesquite v2.72 (Maddison and Maddison 2007) where they were checked for misalignments and adjusted 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 with Ns). Each indel and possible informative sites were re-checked by going back to the original traces.

After the alignments had been edited, additional sequences were obtained from Mark Wilson (unpublished), Rodolfo Solano-Gómez (unpublished), Hagen Stenzel (sequences published in his dissertation thesis, 2004), and from GenBank, the latter using nBLAST. *Myoxanthus uncinatus* AF265478 (now *Echinosepala uncinata* (Fawc.) Pridgeon & M.W. Chase) was used as outgroup in all cases, because it is suggested to be the furthest related of all included species (Pridgeon et al. 2001).

Phylogenetic analysis

Bayesian analysis methods were preferred over parsimony and maximum likelihood because they were found to explain the data better and have overall greater support and resolution. MrBayes version 3.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) was used to obtain a distribution of possible gene trees which are summarized in a consensus tree with posterior probability values for each node. 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 (software designed to compare different nested models of DNA substitution in a hierarchical hypothesis-testing framework (Posada and Crandall 1998) to calculate the model scores, based on the AIC criterion. In both cases the GTR + Γ (gamma) model was the most likely to fit the data best and was therefore used in all subsequent Bayesian analysis. The GTR + Γ model was implemented throughout and the temperature for heated chains was set to 0.05. Both *matK* and ITS were tested without partitions, however, *matK* was also analyzed with a partition based on the codon position 1 + 2 versus 3. Gaps were very small and scarce and were, therefore, treated as missing data or eliminated from the data set. A combined analysis was done where partitions were set for each gene. Methods of phylogenetic inference depend on their underlying models. If the results of the analysis are to be trusted, the model must be trusted; one must, therefore, investigate which explicit evolution model

fits the data best. In all cases 3,500,000 generations were run and results were inspected for convergence and mixing by use of Tracer v.1.5 software (Rambaut and Drummond 2007).

Bayesian evolutionary analysis sampling trees (BEAST; Drummond and Rambaut 2007) were used to analyze the ITS and ITS + *matK* combined matrices. BEAST estimate 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. Substitution and clock models were unlinked. The GTR + Γ model, estimated frequencies, and 10 categories were used for both ITS + *matK*, only *matK* was analyzed using independent codon positions. The relaxed clock model was used for both; however, that of ITS was lognormal, whereas for *matK* the clock model was set to exponential. The tree prior used was speciation—yule birth—and the number of generations was set to 20,000,000. Concatenated gene sequences for phylogenetic analysis can lead to artifacts, especially when discord is found between the individual gene trees (Edwards et al. 2007; Kubatko and Degnan 2007). Therefore we tested whether strongly-supported incongruence existed between our rDNA ITS and *matK*-based trees. In the concatenated data set, ITS sequences are directly followed by the *matK* sequence. Trees were visualized in FigTree v.1.3.1 (Rambaut 2009). Posterior probability (PP) values were added to the branches of the trees by use of the labeling option. Branches were reordered for better visualization. Consensus networks summarize 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 and Moulton 2003; Holland et al. 2005). When calculating the posterior probabilities, in MrBayes for example, the software produces a distribution of possible trees with several alternative explanations for the same data. In the consensus network all the alternative explanations above a specific threshold are included in a three-dimensional multi-branched network, resulting in more information than the two-dimensional two-branched tree.

Trees obtained from BEAST analysis of the combined ITS + *matK* data set were analyzed by use of Splits Tree4 v.4.11.3 (Huson and Bryant 2006). The consensus network was built on the basis of 2800 trees, eliminating the first and using a 0.20 cutoff value. By allowing for different explanations of the data (viewed as branching points), in a consensus network one can detect areas of conflict between a percentage of the resulting trees. This enables comparison of data from different origins and identification of possible cases of horizontal gene flow. Here, the consensus network is not used for phylogenetic reconstruction but as evidence for unclear phylogenetic relationships.

Exclusion and editing of sequences

While handling the sequences matrix, reading mistakes could be seen in the form of repeated insertions (for example 5 times A instead of 4) or similarities at the beginning and/or end of unrelated sequences which share a common sequencing origin. DNA extraction and/or sequencing of *Condylago furculifera* Dressler & Bogarín, *Stelis pilostoma* (Luer) Pridgeon & M.W.Chase, and *Stelis vaginata* (Schltr.) Pridgeon & M.W.Chase failed repeatedly. Sequences obtained from *Stelis aristocratica* (L.O.Williams) Pridgeon & M.W.Chase, *Stelis jalapensis* (Kraenzl.) Pridgeon & M.W.Chase, *Stelis nigriflora* (L.O.Williams) Pridgeon & M.W.Chase, and *Stelis resupinata* (Ames) Pridgeon & M.W.Chase were too short and/or messy, and were therefore omitted. The same criteria were used to exclude GenBank sequences from *Stelis rodrigo* (Luer) Pridgeon & M.W.Chase (type species of genus *Condylago*), for which different affinities were observed in every unique analysis, and *S. resupinata*. Most of these “problematic” sequences in terms of quality belonged to species of genus *Pleurothallis* subgenus *Effusia* and subgenera *Unciferia* and *Condylago*, all probably close relatives. The complete list of sequences used, and their vouchers and origin, are found in Table 1. The aligned ITS and/or *matK* matrices are available from the corresponding author.

Results

Nomenclature

Clades have been coded to simplify description of some species groups. They have been assigned letters from A to F, and have been chosen among those found in the Bayesian Analysis Consensus Tree, which were more constant and easiest to discuss. They may not be found in all trees and do not necessarily reflect the authors’ opinions about the taxonomy of those particular groups. Taxa names follow Pridgeon (2005) and/or Govaerts et al. (2011).

Analysis of combined ITS/*matK*

Differences between the separate analyses of the plastid *matK* and nuclear ITS matrices were found. The differences were mostly “soft”, however the *matK* analyses being less well resolved may be shading some “hard” incongruencies between both. An explanation for the differences might be their different origin and ancestry. Even so, the combined analyses were preferred for their higher resolution and support, and because they better explained the data than either single analysis. Overall the consensus

Table 1 Complete list of all taxa, their available vouchers, and DNA sequences and their source, used in the different analysis presented in this study

Taxon	Voucher	<i>matK</i>	ITS	Source
<i>Andinia pensilis</i>	–	AF265455	AF262826	GenBank
<i>Anathallis anderssonii 1</i>	A. P. Karremans 2957 (N.V.)	JF934841	JF934777	This Study
<i>Anathallis anderssonii 2</i>	A. P. Karremans 4842 (L-Spirit; Epidendra)	–	JQ995324	This Study
<i>Anathallis angustilabia</i>	–	AF302647	AF262868	GenBank
<i>Anathallis dolichopus 1</i>	A. P. Karremans 2871 (JBL-Spirit)	JF934838	JF934774	This Study
<i>Anathallis dolichopus 2</i>	D. Bogarín 3736 (JBL-Spirit; Epidendra)	JF934840	JF934776	This Study
<i>Anathallis dolichopus 3</i>	F. Pupulin 5301 (JBL-Spirit; WAG-Spirit)	JF934839	JF934775	This Study
<i>Anathallis dolichopus 4</i>	M. Soto 4358 (AMO)	–	JF934755	This Study
<i>Anathallis obovata</i>	H. Stenzel 840 (HAJB)	–	JF934822	Stenzel (2004)
<i>Anathallis rubens</i>	A. P. Karremans 4824 (L-Spirit; Epidendra)	–	JQ995325	This Study
<i>Anathallis sclerophylla 1</i>	A. P. Karremans 4791 (JBL-Spirit)	–	JQ995326	This Study
<i>Anathallis sclerophylla 2</i>	A. P. Karremans 4830 (L-Spirit)	–	JQ995327	This Study
<i>Dracontia hydra</i>	D. Bogarín 5746 (JBL-Spirit; Epidendra)	–	JF934809	This Study
<i>Dracontia lueriana</i>	D. Bogarín 1987 (JBL-Spirit; CR; Epidendra)	JF934870	JF934810	This Study
<i>Dracontia sp. nov.1 1</i>	A. P. Karremans 4604 (A) (JBL-Spirit)	–	JQ995328	This Study
<i>Dracontia sp. nov.1 2</i>	A. P. Karremans 4604 (B) (JBL-Spirit)	–	JQ995329	This Study
<i>Dracontia sp. nov.2</i>	D. Bogarín 7698 (JBL-Spirit; CR)	–	JQ995330	This Study
<i>Dryadella simula</i>	–	AF265453	AF262825	GenBank
<i>Echinosepala uncinata</i>	–	AF265478	AF262904	GenBank
<i>Fronitaria caulescens</i>	–	AF265471	AF262914	GenBank
<i>Lepanthes platysepala</i>	A. P. Karremans 4847 (L-Spirit)	–	JQ995331	This Study
<i>Lepanthes woodburyana</i>	–	AF265470	AF262890	GenBank
<i>Pabstiella aryster</i>	D. Bogarín 6501 (JBL-Spirit; Epidendra)	JF934876	JF934816	This Study
<i>Pabstiella mirabilis</i>	–	–	AF262830	GenBank
<i>Pabstiella tripterantha 1</i>	D. Bogarín 5905 (JBL-Spirit)	JF934875	JF934815	This Study
<i>Pabstiella tripterantha 2</i>	–	AF302649	AF275694	GenBank
<i>Pabstiella wacketii</i>	A. P. Karremans 4832 (L-Spirit; Epidendra)	–	JQ995334	This Study
<i>Pabstiella yauaperyensis</i>	–	–	AF262864	GenBank
<i>Platystele misera</i>	–	AF265470	AF262823	GenBank
<i>Pleurothallis allenii</i>	–	–	AF262844	GenBank
<i>Pleurothallis cardiantha</i>	–	–	AF262832	GenBank
<i>Pleurothallis cardiothallis</i>	–	–	AF262917	GenBank
<i>Pleurothallis excavata</i>	–	–	AF262841	GenBank
<i>Pleurothallis grandiflora</i>	–	–	AF368320	GenBank
<i>Pleurothallis loranthophylla</i>	–	–	AF262837	GenBank
<i>Pleurothallis miranda</i>	–	–	AF262875	GenBank
<i>Pleurothallis niveoglobula</i>	–	–	AF262834	GenBank
<i>Pleurothallis nuda</i>	–	–	AF262874	GenBank
<i>Pleurothallis penicillata</i>	–	–	AF368320	GenBank
<i>Pleurothallis rowleei</i>	–	–	AF262842	GenBank
<i>Pleurothallis ruscifolia 1</i>	–	AF265463	AF262836	GenBank
<i>Pleurothallis ruscifolia 2</i>	F. Pupulin 7254 (B) (JBL-Spirit)	JF934874	JF934814	This Study
<i>Pleurothallis ruscifolia 3</i>	H. Stenzel 635 (HAJB)	–	JF934821	Stenzel (2004)
<i>Pleurothallis ruscifolia 4</i>	F. Pupulin 7254 (A) (JBL-Spirit)	JF934873	JF934813	This Study
<i>Pleurothallis sijmii</i>	A. P. Sijm 200425 (MO; Epidendra)	–	JQ995335	This Study
<i>Pleurothallis silvae-pacis 1</i>	A. P. Karremans 3069 (A) (JBL-Spirit; CR; Epidendra)	–	JQ995336	This Study
<i>Pleurothallis silvae-pacis 2</i>	A. P. Karremans 3069 (B) (JBL-Spirit; CR; Epidendra)	–	JQ995337	This Study
<i>Pleurothallis sp. nov.</i>	D. Bogarín 8775 (JBL-Spirit)	–	JQ995338	This Study

Table 1 continued

Taxon	Voucher	<i>matK</i>	ITS	Source
<i>Pleurothallis talpinaria</i>	–	–	AF262840	GenBank
<i>Pleurothallis teaguei</i>	–	–	AF275695	GenBank
<i>Pleurothallis truncata</i>	–	–	AF262834	GenBank
<i>Pleurothallis viduata</i>	–	–	AF262838	GenBank
<i>Salpistele adrianae 1</i>	D. Bogarín 5917 (A) (JBL-Spirit; Epidendra)	JF934860	JF934799	This Study
<i>Salpistele adrianae 2</i>	D. Bogarín 5917 (B) (JBL-Spirit; Epidendra)	JF934861	JF934800	This Study
<i>Salpistele adrianae 3</i>	A. P. Sijm 220411 (L-Spirit; MO; Epidendra)	–	JQ995339	This Study
<i>Specklinia costaricensis</i>	JBL-02512 (JBL-Spirit)	–	JF934817	This Study
<i>Stelis adrianae</i>	A. P. Sijm 201231 (MO; Epidendra)	–	JQ995340	This Study
<i>Stelis alajuelensis</i>	F. Pupulin 4900 (JBL-Spirit; CR; Epidendra)	–	JQ995341	This Study
<i>Stelis alta 1</i>	D. Bogarín 4604 (A) (JBL-Spirit)	JF934865	JF934804	This Study
<i>Stelis alta 2</i>	D. Bogarín 4604 (B) (JBL-Spirit)	JF934866	JF934805	This Study
<i>Stelis allenii</i>	JBL-03905 (JBL-Spirit)	–	JQ995342	This Study
<i>Stelis antillensis</i>	H. Stenzel 662 (HAJB)	–	JF934818	Stenzel (2004)
<i>Stelis argentata</i>	D. Bogarín 1862 (CR; JBL-Spirit; Epidendra)	–	JF934764	This Study
<i>Stelis atroviolacea</i>	–	–	AF262879	GenBank
<i>Stelis atwoodi</i>	A. P. Karremans 3540 (JBL-Spirit; Epidendra)	–	JQ995343	This Study
<i>Stelis brunnea 1</i>	D. Bogarín 6226 (JBL-Spirit)	JF934859	JF934798	This Study
<i>Stelis brunnea 2</i>	–	EU214439	–	GenBank
<i>Stelis aff. canae 1</i>	D. Bogarín 6805 (JBL-Spirit)	–	JF934793	This Study
<i>Stelis aff. canae 2</i>	D. Bogarín 6790 (JBL-Spirit)	–	JF934782	This Study
<i>Stelis carnosilabia 1</i>	D. Bogarín 730 (A) (JBL-Spirit)	JF934868	JF934807	This Study
<i>Stelis carnosilabia 2</i>	D. Bogarín 730 (B) (JBL-Spirit)	JF934869	JF934808	This Study
<i>Stelis carpintera 1</i>	D. Bogarín 7148 (A) (JBL; WAG-Spirit; Epidendra)	JF934857	JF934796	This Study
<i>Stelis carpintera 2</i>	D. Bogarín 7148 (B) (JBL; WAG-Spirit; Epidendra)	JF934858	JF934797	This Study
<i>Stelis ciliaris</i>	–	–	AF262927	GenBank
<i>Stelis cobanensis 1</i>	D. Bogarín 8884 (JBL-Spirit; Epidendra)	–	JQ995344	This Study
<i>Stelis cobanensis 2</i>	–	–	AF262926	GenBank
<i>Stelis convallaria 1</i>	Hoffmann s.n. (A) (CR; JBL; WAG-Spirit; Epidendra)	JF934851	JF934791	This Study
<i>Stelis convallaria 2</i>	Hoffmann s.n. (B) (CR; JBL; WAG-Spirit; Epidendra)	JF934852	JF934792	This Study
<i>Stelis cylindrata</i>	A. P. Karremans 4025 (JBL-Spirit; Epidendra)	–	JQ995345	This Study
<i>Stelis cypripedoides</i>	A. P. Karremans 2951 (WAG-Spirit)	–	JQ995346	This Study
<i>Stelis deregularis</i>	D. Bogarín 5331 (JBL-Spirit)	–	JF934771	This Study
<i>Stelis despectans 1</i>	D. Bogarín 5249 (A) (JBL-Spirit)	JF934831	JF934761	This Study
<i>Stelis despectans 2</i>	D. Bogarín 5249 (B) (JBL-Spirit)	JF934832	JF934762	This Study
<i>Stelis dracontea</i>	D. Bogarín 616 (JBL-Spirit; Epidendra)	–	EU214426	This Study/GenBank
<i>Stelis dressleri 1</i>	F. Pupulin 7579 (A) (JBL-Spirit)	JF934829	JF934759	This Study
<i>Stelis dressleri 2</i>	F. Pupulin 7579 (B) (JBL-Spirit)	JF934830	JF934760	This Study
<i>Stelis emarginata 1</i>	–	AF265466	AF262845	GenBank
<i>Stelis emarginata 2</i>	A. P. Karremans 2947 (WAG-Spirit)	–	JF934781	This Study
<i>Stelis endresii</i>	M. Soto 4382 (AMO)	–	JF934753	Solano-Gómez (Unp.)
<i>Stelis ephemera</i>	A. P. Karremans 4805 (L-Spirit)	–	JQ995332	This Study
<i>Stelis ferrelliae</i>	A. P. Karremans 4326 (JBL-Spirit; Epidendra)	–	JQ995347	This Study
<i>Stelis galeata</i>	A. P. Karremans 4800 (L-Spirit)	–	JQ995348	This Study
<i>Stelis gelida 1</i>	A. P. Karremans 2481 (JBL-Spirit)	JF934843	JF934779	This Study
<i>Stelis gelida 2</i>	D. Bogarín 622 (JBL-Spirit)	JF934842	JF934778	This Study
<i>Stelis gelida 3</i>	D. Bogarín 7639 (N.V.)	JF934844	JF934780	This Study
<i>Stelis gemma</i>	–	–	AF262880	GenBank

Table 1 continued

Taxon	Voucher	<i>matK</i>	ITS	Source
<i>Stelis gigantea 1</i>	–	AF265461	AF262843	GenBank
<i>Stelis gigantea 2</i>	F. Pupulin 4498 (JBL; WAG-Spirit)	JF934864	JF934803	This Study
<i>Stelis glomerosa</i>	A. P. Karremans 4837 (L-Spirit)	–	JQ995349	This Study
<i>Stelis glossula</i>	D. Bogarín 2695 (JBL-Spirit; Epidendra)	–	JF934766	This Study
<i>Stelis aff. glossula</i>	Y. Kisel 2046 (JBL-Spirit)	–	JF934767	This Study
<i>Stelis guatemalensis 1</i>	–	–	AF262928	GenBank
<i>Stelis guatemalensis 2</i>	F. Pupulin 3977 (JBL-Spirit)	–	JF934765	This Study
<i>Stelis guttata</i>	–	–	AF262833	GenBank
<i>Stelis harlingii 1</i>	–	AF265465	AF262846	GenBank
<i>Stelis harlingii 2</i>	–	EF065591	EF079364	GenBank
<i>Stelis hypnicola</i>	A. P. Karremans 4803 (L-Spirit)	–	JQ995333	This Study
<i>Stelis immersa 1</i>	–	EU214427	AF262828	GenBank
<i>Stelis immersa 2</i>	D. Bogarín 6588 (JBL-Spirit)	JF934850	JF934789	This Study
<i>Stelis immersa 3</i>	D. Bogarín 5450 (JBL-Spirit)	–	JF934790	This Study
<i>Stelis imraei</i>	D. Bogarín 752 (JBL-Spirit; WAG-Spirit-Epidendra)	–	JF934784	This Study
<i>Stelis janetiae 1</i>	D. Bogarín 5008 (JBL-Spirit)	JF934863	JF934802	This Study
<i>Stelis janetiae 2</i>	Holst 8763 (JBL-Spirit)	JF934862	JF934801	This Study
<i>Stelis kareniae</i>	D. Bogarín 7594 (JBL-Spirit)	JF934834	JF934769	This Study
<i>Stelis kefersteiniana 1</i>	A. P. Karremans 4845 (L-Spirit)	–	JQ995350	This Study
<i>Stelis kefersteiniana 2</i>	A. P. Karremans 2948 (A) (WAG-Spirit)	–	JQ995351	This Study
<i>Stelis kefersteiniana 3</i>	A. P. Karremans 2948 (B) (WAG-Spirit)	–	JQ995352	This Study
<i>Stelis lanata</i>	–	–	AF262881	GenBank
<i>Stelis lankesterii</i>	A. P. Karremans 4269 (JBL-Spirit)	–	JQ995353	This Study
<i>Stelis leucopogon</i>	E. Pérez 167 (AMO)	–	JF934750	Solano-Gómez (Unp.)
<i>Stelis listerophora 1</i>	D. Bogarín 6000 (JBL-Spirit)	JF934846	JF934785	This Study
<i>Stelis listerophora 2</i>	D. Bogarín 6006 (JBL-Spirit; Epidendra)	JF934847	JF934786	This Study
<i>Stelis maculata</i>	–	–	AF262827	GenBank
<i>Stelis megachlamys 1</i>	–	EU214491	AF262877	GenBank
<i>Stelis megachlamys 2</i>	A. P. Karremans 1222 (JBL-Spirit; WAG-Spirit)	JF934867	JF934806	This Study
<i>Stelis megachlamys 3</i>	PL296 (COCO)	–	JF934823	Wilson (Unp.)
<i>Stelis aff. microchila 1</i>	D. Bogarín 6965 (JBL-Spirit)	JF934827	JF934757	This Study
<i>Stelis aff. microchila 2</i>	D. Bogarín 5356 (JBL-Spirit)	JF934828	JF934758	This Study
<i>Stelis aff. microchila 3</i>	M. Soto 7222 (AMO)	–	JF934751	Solano (Unp.)
<i>Stelis morae</i>	A. P. Karremans 1088 (JBL-Spirit)	–	JF934768	This Study
<i>Stelis multirostris 1</i>	A. P. Karremans 4826 (L-Spirit)	–	JQ995354	This Study
<i>Stelis multirostris 2</i>	H. Stenzel 643 (HAJB)	–	JQ995368	Stenzel (2004)
<i>Stelis mystax 1</i>	–	–	AF262876	GenBank
<i>Stelis mystax 2</i>	D. Bogarín 2988 (JBL-Spirit; Epidendra)	JF934855	JF934794	This Study
<i>Stelis mystax 3</i>	A. P. Karremans 4868 (L-Spirit)	–	JQ995355	This Study
<i>Stelis nexipous</i>	A. P. Karremans 4874 (L-Spirit)	–	JQ995356	This Study
<i>Stelis ornata 1</i>	M. Soto 4947 (AMO)	–	JF934756	Solano
<i>Stelis ornata 2</i>	A. P. Karremans 4838 (L-Spirit)	–	JQ995357	This Study
<i>Stelis ornata 3</i>	A. P. Karremans 4870 (Epidendra)	–	JQ995358	This Study
<i>Stelis pachyglossa</i>	A. P. Karremans 4822 (L-Spirit; Epidendra)	–	JQ995359	This Study
<i>Stelis papillifera 1</i>	D. Bogarín 6585 (JBL-Spirit)	JF934871	JF934811	This Study
<i>Stelis papillifera 2</i>	D. Bogarín 7186 (JBL-Spirit; WAG-Spirit)	–	JF934812	This Study
<i>Stelis pilosa 1</i>	–	AF265467	AF262831	GenBank
<i>Stelis pilosa 2</i>	F. Pupulin 7203 (A) (JBL-Spirit)	JF934848	JF934787	This Study

Table 1 continued

Taxon	Voucher	<i>matK</i>	ITS	Source
<i>Stelis pilosa</i> 3	F. Pupulin 7203 (B) (JBL-Spirit)	JF934849	JF934788	This Study
<i>Stelis platystylis</i> 1	A. P. Karremans 4819 (L-Spirit)	–	JQ995360	This Study
<i>Stelis platystylis</i> 2	A. P. Karremans 4802 (L-Spirit)	–	JQ995361	This Study
<i>Stelis pompalis</i> 1	D. Bogarín 6516 (A) (JBL-Spirit)	JF934853	–	This Study
<i>Stelis pompalis</i> 2	D. Bogarín 6516 (B) (JBL-Spirit)	JF934854	–	This Study
<i>Stelis aff. pompalis</i>	A. P. Karremans 4076 (JBL-Spirit; Epidendra)	–	JQ995362	This Study
<i>Stelis pulchella</i> 1	A. P. Karremans 2480 (JBL-Spirit)	JF934836	JF934772	This Study
<i>Stelis pulchella</i> 2	A. P. Karremans 2502 (JBL-Spirit; Epidendra)	–	JQ995363	This Study
<i>Stelis punctulata</i> 1	JBL-11487 (JBL-Spirit)	–	JQ995364	This Study
<i>Stelis punctulata</i> 2	A. P. Karremans 2946 (WAG-Spirit)	JF934845	JF934783	This Study
<i>Stelis quadrifida</i> 1	H. Stenzel 1298 (HAJB)	–	JF934819	Stenzel (2004)
<i>Stelis quadrifida</i> 2	H. Stenzel 967 (HAJB)	–	JF934820	Stenzel (2004)
<i>Stelis quadrifida</i> 3	–	AY396076	AY008477	GenBank
<i>Stelis quadrifida</i> 4	D. Bogarín 1676 (JBL-Spirit; Epidendra)	JF934872	–	This Study
<i>Stelis quadrifida</i> 5	–	EU214429	–	GenBank
<i>Stelis quadrifida</i> 6	PL294 (COCO)	–	JF934824	Wilson (Unp.)
<i>Stelis restrepioides</i> 1	A. P. Karremans 2953 (N.V.)	JF934856	JF934795	This Study
<i>Stelis restrepioides</i> 2	PL297 (COCO)	–	JF934825	Wilson (Unp.)
<i>Stelis restrepioides</i> 3	PL362 (COCO)	–	JF934826	Wilson (Unp.)
<i>Stelis rufobrunnea</i>	M. Soto 7816 (AMO)	–	JF934754	Solano-Gómez (Unp.)
<i>Stelis segoviensis</i> 1	–	AF276313	AF262866	GenBank
<i>Stelis segoviensis</i> 2	A. P. Karremans 544 (JBL-Spirit; CR; Epidendra)	–	JQ995365	This Study
<i>Stelis aff. segoviensis</i> 1	D. Bogarín 8099 (JBL-Spirit)	–	JQ995366	This Study
<i>Stelis aff. segoviensis</i> 2	A. P. Karremans 4844 (L-Spirit)	–	JQ995367	This Study
<i>Stelis sp.nov.</i> 1	D. Bogarín 5576 (JBL-Spirit)	JF934835	JF934770	This Study
<i>Stelis sp.nov.</i> 2	D. Bogarín 6427 (JBL-Spirit)	JF934833	JF934763	This Study
<i>Stelis tacanensis</i>	M. Soto 2939 (AMO; K; MO; MEXU)	–	AF262918	GenBank
<i>Stelis tenuissima</i>	E. Hágsater 11722 (AMO)	–	JF934752	Solano-Gómez (Unp.)
<i>Stelis velaticaulis</i>	–	AF302646	AF262847	GenBank
<i>Stelis cf. velaticaulis</i>	A. P. Karremans 2954 (WAG-Spirit)	JF934837	JF934773	This Study
<i>Trichosalpinx orbicularis</i>	–	AF265476	AF262886	GenBank

AMO, COCO, CR, HAJB, JBL, L, and WAG are the herbaria where the material has been deposited. Epidendra refers to the digital voucher data base available online at: <http://www.epidendra.org>

N.V., no voucher; *matK*, ITS, accession numbers assigned by GenBank to those sequences

trees from the Bayesian and BEAST analysis for the clades found similar tree topologies, differing mostly in some support values for those clades and that the first allows for polytomies. Theoretically it is possible to establish an infinite number of clades in the resulting phylogenetic trees; for the purpose of facilitating the discussion, however, six clades were established (A, B, C, D, E, and F). These were chosen for their consistency, support, and the ease of morphological characterization. Concatenation of sequences was not always possible, because fewer *matK* sequences were available. The Bayesian consensus tree (Fig. 1a) is used hereafter for discussion purposes, and only hard topological differences from the BEAST consensus tree (Fig. 1b) are mentioned.

Combined analysis (Fig. 1). The *Stelis* s.l. clade (clades A to F) is highly supported, posterior probability (P.P.) = 1; it excludes *Stelis restrepioides* (Lindl.) Pridgeon & M.W.Chase and *S. quadrifida* (Lex.) Solano & Soto Arenas, both are closer to *Pleurothallis ruscifolia* (type species of *Pleurothallis*). Additionally, *Anathallis anderssonii* (Luer) Pridgeon & M.W.Chase and *Anathallis dolichopus* (Schltr.) Pridgeon & M.W.Chase are embedded in this clade.

Clade A is basal (or sister) to the *Stelis* s.l. and is made up of one sequence of *S. imraei* (Lindl.) Pridgeon & M.W.Chase only. Its inclusion in *Stelis* s.l. is highly supported (P.P. = 1).

Clade B is a weakly supported clade (P.P. = 0.55) that includes *Stelis canae* (Ames) Pridgeon & M.W.Chase,

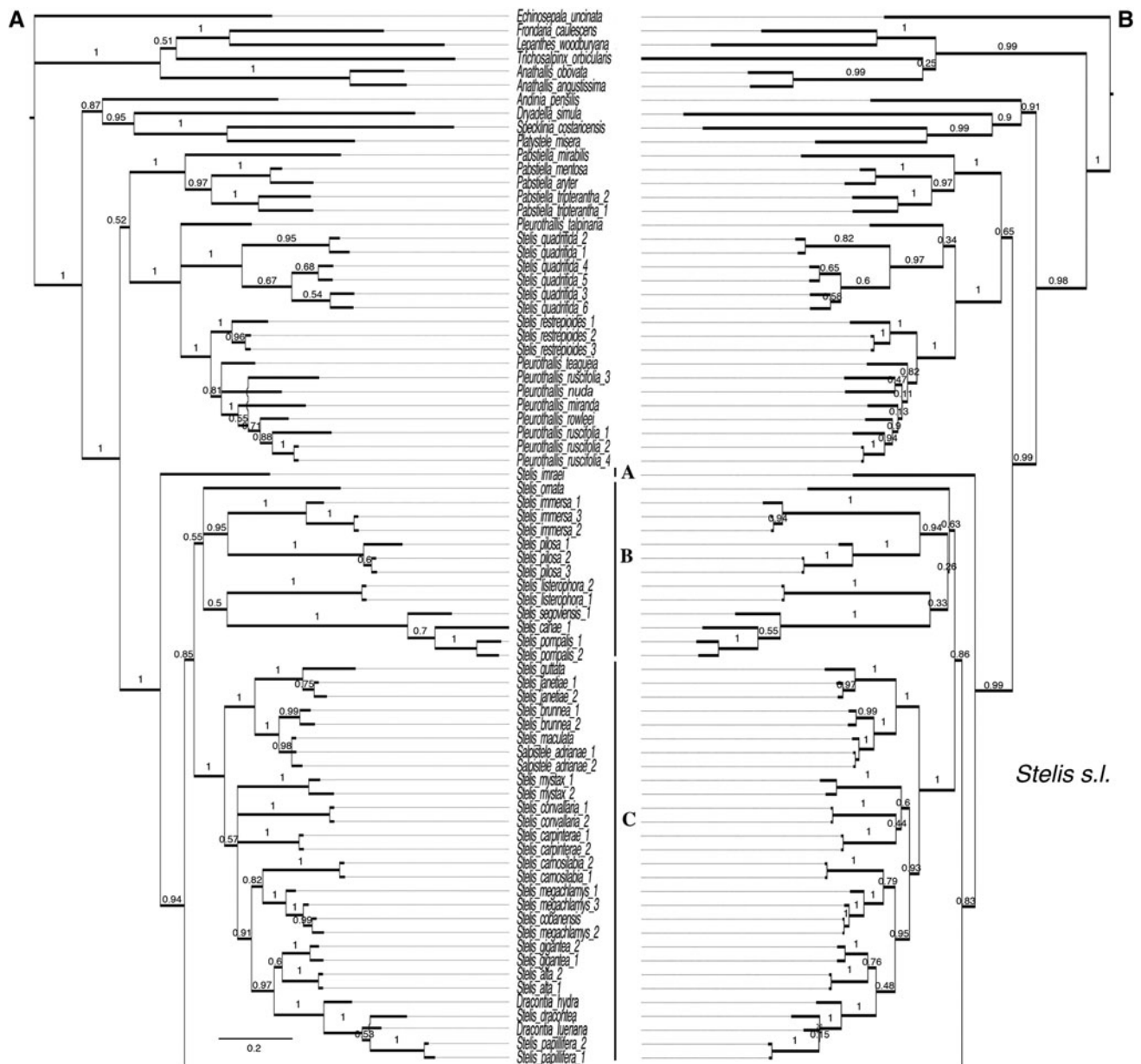


Fig. 1 Mirrored consensus trees obtained from analysis of a concatenated matrix of 117 ITS and 73 *matK* sequences for a total of 120 combined sequences of: **a** a 3,500,000 generation Bayesian analysis,

with partitions set for each gene. **b** A 20,000,000 generation BEAST analysis, with partitions set for each gene. Branch values are posterior probabilities

S. pompalis (Ames) Pridgeon & M.W.Chase, and *S. segoviensis* (Rchb.f.) Pridgeon & M.W.Chase in a well-supported subclade (P.P. = 1), *S. immersa* (Linden & Rchb.f.) Pridgeon & M.W.Chase and *S. pilosa* Pridgeon & M.W.Chase in a second well-supported subclade (P.P. = 0.95), and accessions of *S. listerophora* (Schltr.) Pridgeon & M.W.Chase and *S. ornata* (Rchb.f.) Pridgeon & M.W.Chase.

Clade C is a well-supported clade (P.P. = 1). It can be subdivided into two subclades, a highly supported (P.P. = 1) subclade which includes, on the one hand, *Salpistele adrianae* Luer & Sijm, *Stelis brunnea* (Dressler)

Pridgeon & M.W.Chase, and *S. maculata* Pridgeon & M.W.Chase, brought together with a support of P.P. = 1 and, on the other hand, *S. guttata* (Luer) Pridgeon & M.W.Chase and *S. janetiae* (Luer) Pridgeon & M.W.Chase, equally well supported. The second subclade is weakly supported (P.P. = 0.57) and includes the accessions of *S. carpintera* (Schltr.) Pridgeon & M.W.Chase, *S. convallaria* (Schltr.) Pridgeon & M.W.Chase, and *S. mystax* (Luer) Pridgeon & M.W.Chase in a polytomy with a highly supported (P.P. = 0.91) clade comprising *Dracontia hydra* Karremans & C.M.Sm., *Dracontia lueriana* Karremans,

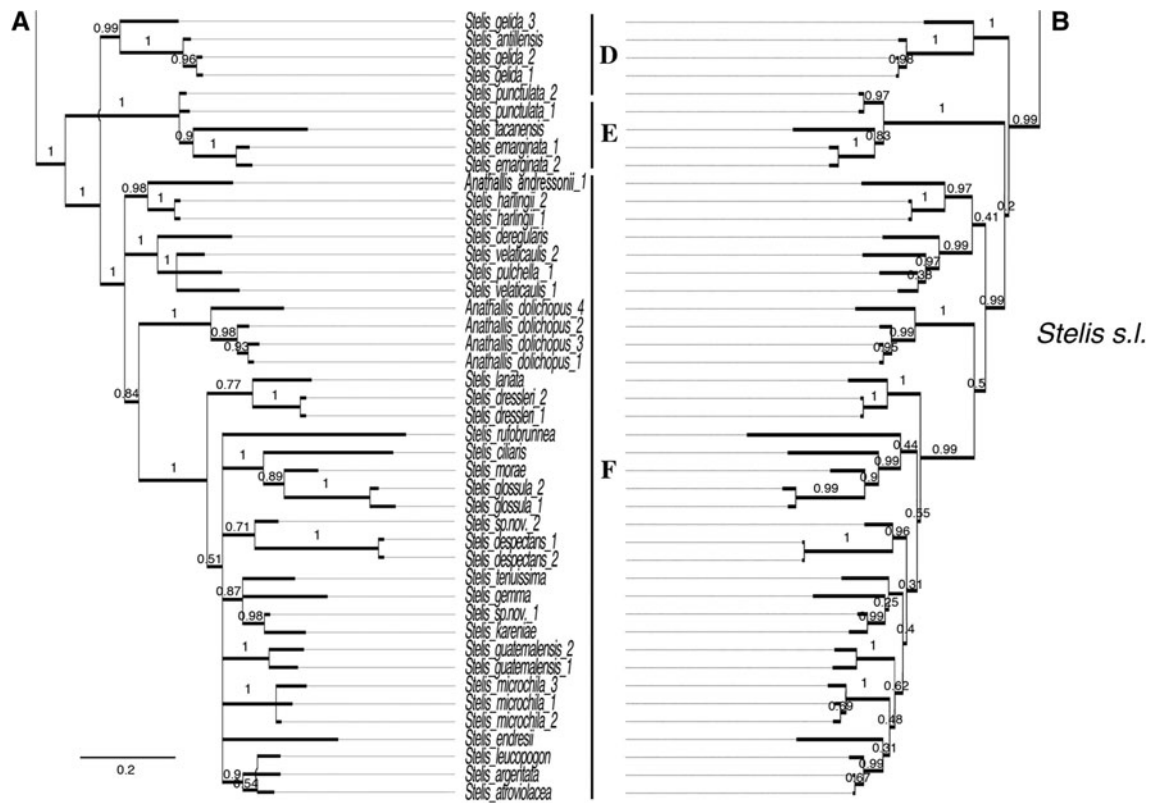


Fig. 1 continued

Stelis alta Pridgeon & M.W.Chase, *S. carnosilabia* (A.H.Heller & A.D.Hawkes) Pridgeon & M.W.Chase, *S. cobanensis* (Schltr.) Pridgeon & M.W.Chase, *S. dracontea* (Luer) Pridgeon & M.W.Chase, *S. gigantea* Pridgeon & M.W.Chase, *S. megachlamys* (Schltr.) Pupulin, and *S. papillifera* (Rolfe) Pridgeon & M.W.Chase.

Clade D is a well-supported clade (P.P. = 0.99) that includes several accessions of *Stelis gelida* (Lindl.) Pridgeon & M.W.Chase and one marked as *S. antillensis* Pridgeon & M.W.Chase. The clade is found basal to clade F, with high support (P.P. = 1), in the Bayesian analysis whereas it is found basal (weakly supported) to clades E and F in the BEAST analysis.

Clade E is a well-supported clade (P.P. = 1) that includes accessions of *Stelis emarginata* Soto Arenas & Solano, *S. tacanensis* (Lindl.) Soto Arenas & Solano, and *S. punctulata* (Rchb.f.) Soto Arenas.

Clade F is a well-supported clade (P.P. = 1) with a basal polytomy indicative of three well supported subclades. The first (P.P. = 0.98) includes accessions of *Stelis harlingii* (Garay) Pridgeon & M.W.Chase and *Anathallis anderssonii*, the second (P.P. = 1) includes *Stelis deregularis* Barb. Rodr., *S. pulchella* Kunth., and *S. velaticaulis* (Rchb.f.) Pridgeon & M.W.Chase, and the third (P.P. = 84) shows in one subclade different accessions of *Anathallis dolichopus* (P.P. = 1) and in another a highly

supported subclade (P.P. = 1), with all species belonging to *Stelis* s. str.

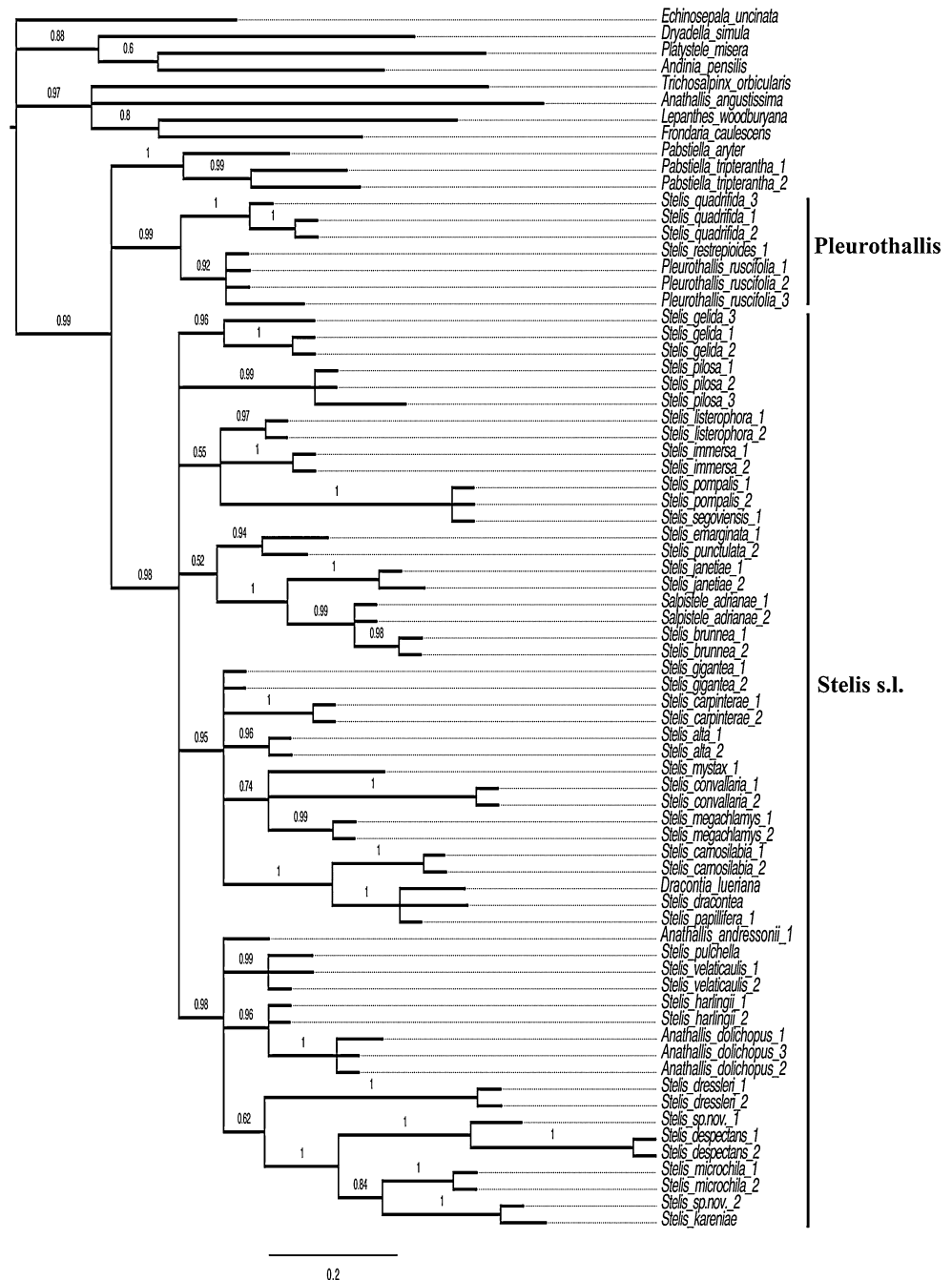
Single analyses

The topology of the Bayesian consensus trees of each of the single dataset's analyses is not discussed in as much detail as were the combined analyses, which have been preferred for their better representation of the data and overall support.

Bayesian analysis of the *matK* dataset (Fig. 2). In the analyses the *Stelis* s.l. clade is still highly supported (P.P. = 0.98), with the exclusion of *Stelis restrepioides* and *S. quadrifida*, and the inclusion of *Anathallis anderssonii* and *A. dolichopus*. Species belonging to the other established clades are not found together in monophyletic groups, except for those of clade F, which includes *Stelis* s. str.

Bayesian analysis of the ITS dataset (Fig. 3). The *Stelis* s.l. clade has very high support (P.P. = 1), with the exclusion of *Stelis restrepioides* and *S. quadrifida*, and the inclusion of *Anathallis anderssonii* and *A. dolichopus*. Clade A is basal (or sister) to the whole *Stelis* s.l. clade. Clade B appears in a moderately supported (P.P. = 0.72) polytomy that includes a well-supported Clade C (P.P. = 0.92). Clade D is highly supported (P.P. = 1) and

Fig. 2 Consensus tree from Bayesian analysis of a matrix of 58 *matK* sequences after 8,533,000 generations, with three partitions, one for each codon position. *Node values* are posterior probabilities



is excluded from clades E and F with high support (P.P. = 1). Clade E is moderately supported (P.P. = 0.76). Clade F includes four highly supported subclades, one (P.P. = 0.99) including *Stelis harlingii* and *Anathallis anderssonii*, another (P.P. = 1) that includes *S. deregularis*, *S. pulchella*, and *S. velaticaulis*. (P.P. = 1), a third subclade (P.P. = 1) formed by different accessions of *Anathallis dolichopus*, and a last one (P.P. = 1) that includes all the species assigned to *Stelis* s. str.

BEAST analysis of the ITS dataset (Fig. 4). The *Stelis* s.l. clade has very high support (P.P. = 1). It excludes

Stelis ephemera (Lindl.) Pridgeon & M.W.Chase, *Stelis hypnicola* (Lindl.) Pridgeon & M.W.Chase, *S. restrepioides*, and *S. quadrifida*, the first two related to *Pabstiella* Brieger & Senghas and the latter two to *Pleurothallis*. *Anathallis anderssonii*, *A. dolichopus*, *A. rubens* (Lindl.) Pridgeon & M.W.Chase, and *A. sclerophylla* (Lindl.) Pridgeon & M.W.Chase are embedded in the clade. *Stelis imraei*, the only member of clade A, was not included, and Clade B and Clade C were only weakly supported. Clade D, however, is a well-supported clade (P.P. = 1) and high support (P.P. = 1) is found to exclude it from clades E and

Fig. 3 Consensus tree from Bayesian analysis of a matrix of 92 ITS sequences after 5,600,000 generations. It is unpartitioned. *Node values* are posterior probabilities



F. Clade E is moderately supported (P.P. = 0.74) with an accession of *Stelis nexipous* Garay weakly supported as basal to it (P.P. = 0.58). Clade F is weakly supported (P.P. = 0.64), but includes four major highly supported and interrelated clades.

Consensus networks

So called “boxes” in the consensus network show areas of phylogenetic uncertainty. Alternative explanations of the data were plotted if they were found in 20 % or more of the

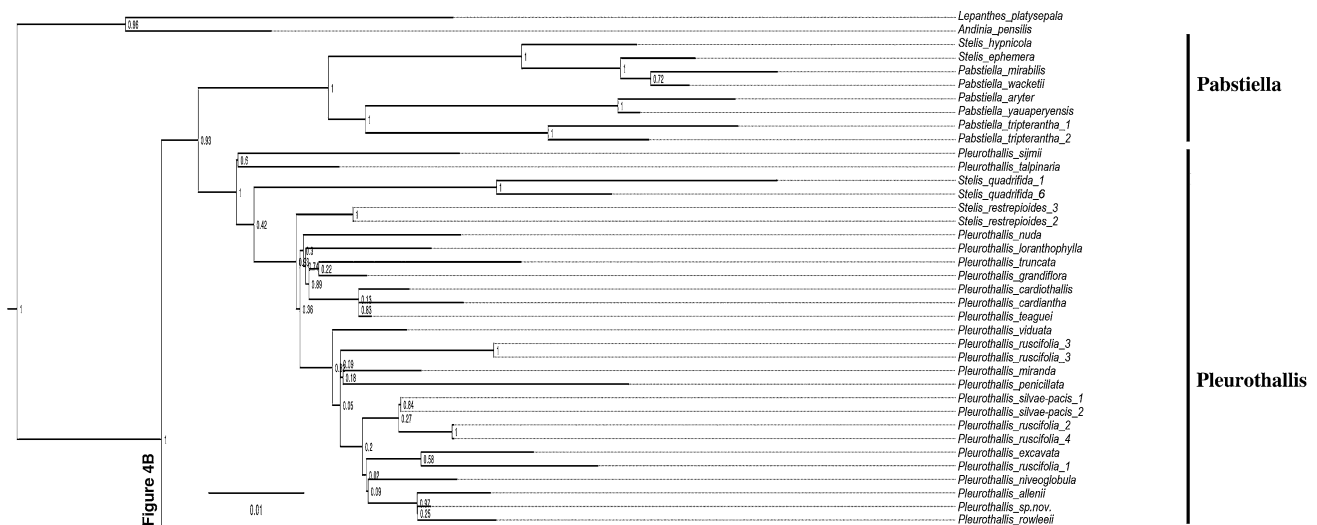


Fig. 4 Consensus tree from BEAST analysis of a matrix of 149 ITS sequences. The analysis ran for 20,000,000 generations. *Node values* are posterior probabilities

trees (0.20 cutoff value used here). The consensus network (Fig. 5) calculated from BEAST analysis of the combined matrix basically suggests the phylogenetic relationships are unclear:

1. between species of clade B;
2. between *Stelis carpinterae*, *S. convallaria*, and *S. mystax* in clade C;
3. between clades D and E; and
4. between species of *Crocodelanthe* and *Pleurothallis* subgen. *Acuminatia* in clade F.

Morphology

Morphological characterization of clades and subclades was done by evaluating the available plant material or, when no specimen was available, by relying on the cited literature, mostly Luer (1986). The characters that proved most consistently distinct between the clades (Table 2) were:

1. the type of inflorescence, which could either have open flowers and undeveloped blossoms on the same inflorescence (successive) and may keep on producing flowers for long periods (indeterminate) or have all or almost all flowers open at a time (simultaneous) and produce a similar amount of flowers per inflorescence (determinate);
2. floral ornamentation, which refers to the fact that even though most species in *Stelis* s.l. have prominently hairy sepals (hirsute), a few groups have virtually no hairs (glabrous);
3. in most Pleurothallidinae the two lateral sepals are fused into a synsepal, a structure similar to the dorsal sepal in shape and size (present), but the synsepal is not found in all clades of *Stelis* s.l. (absent);

4. the glenion is a depression-like, rounded, shiny structure at the base of the lip, of unknown functionality associated with some Pleurothallidinae; some species with apical anthers and stigmas in genera *Pleurothallis* and *Stelis* have a glenion at the base of the lip (present), such a structure is not found in species with an elongated column and lip (absent);
5. the position of the anther in Pleurothallidinae very much depends on the size and shape of the column, in species of *Stelis* s. str. the column is much reduced, placing the anther in an frontal position (apical), whereas species with an elongated column mostly have the anther tending towards the underside (incumbent); and
6. somehow correlated with the position of the anther, the shape and structure of pollinaria can be basically of two kinds in *Stelis* s.l.:
 - (a) pollinaria provided with two pollen sacks brought together by a flat and dry pair of suborbicular caudicles (whale-tail); and
 - (b) pollinaria where the two pollen sacks are brought together by linear caudicles fused to a drop-like viscidium (bubble-like). (Structurally, (a) is made up of male organs only whereas (b) also involves a female organ).

Geographical distribution

Stelis (in the broad sense) is one of the most widespread genera in Pleurothallidinae, found from Florida south to Argentina passing through Central America and the Caribbean (Pridgeon 2005). However, distinct geographical

Figure 4A

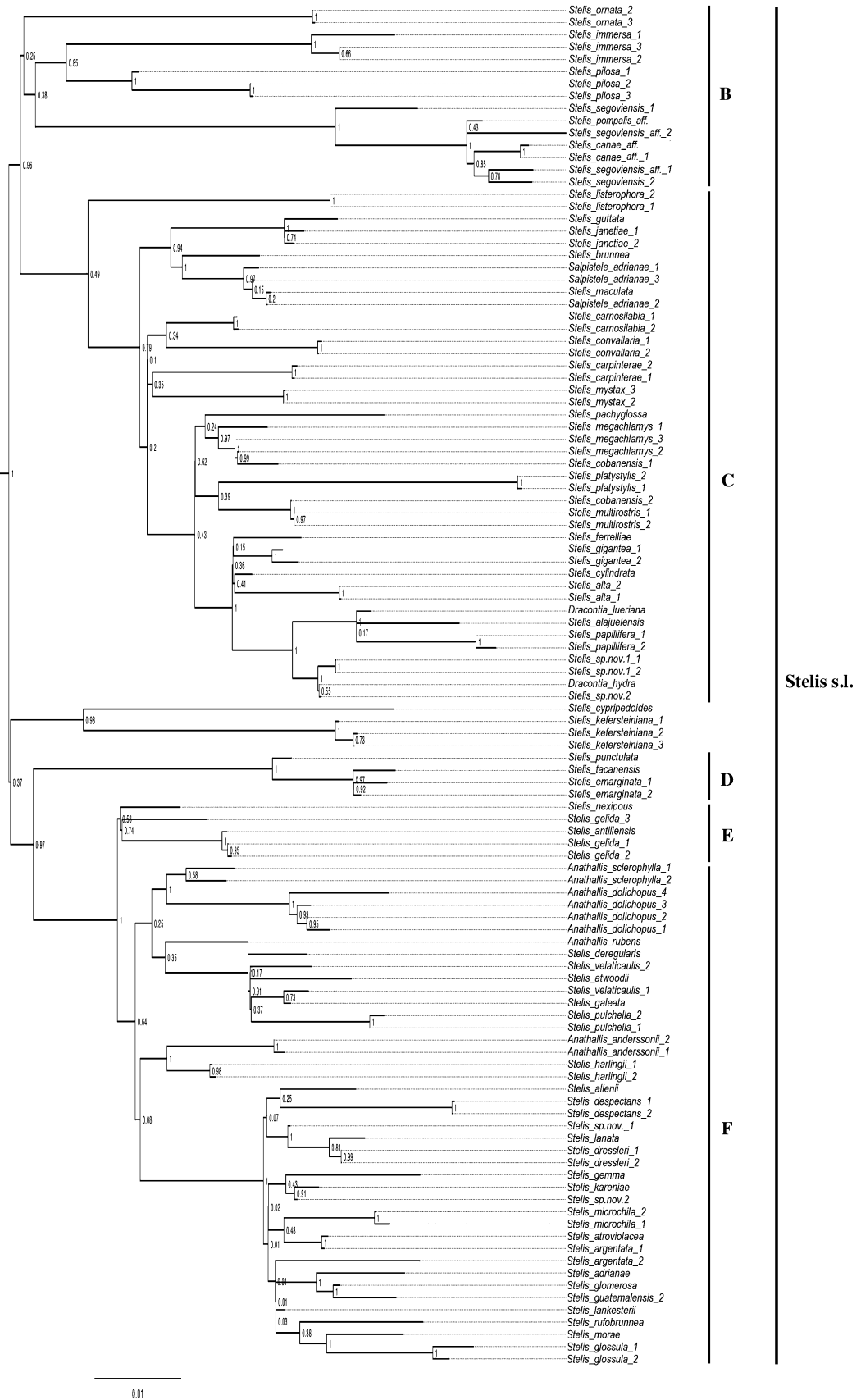


Fig. 4 continued

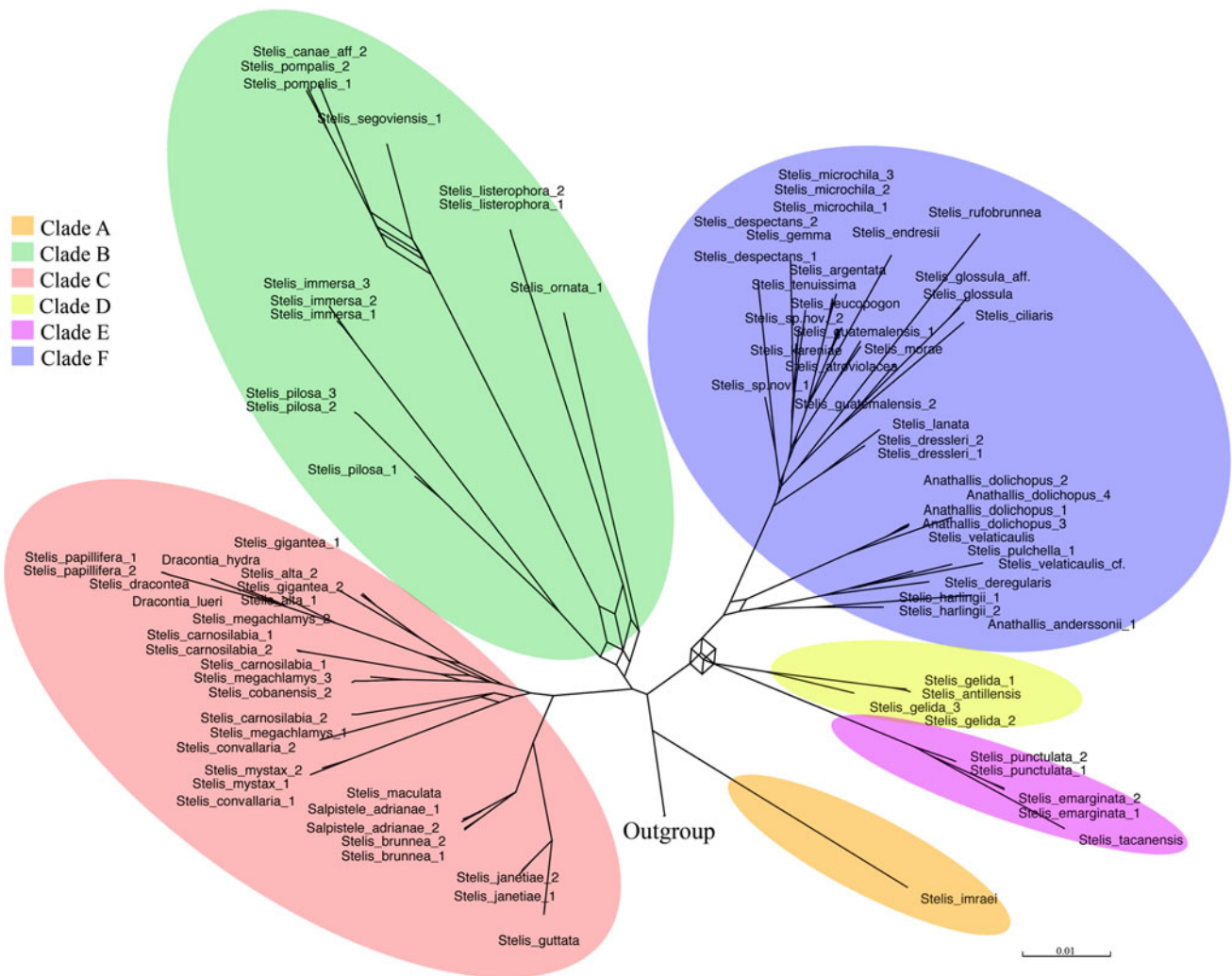


Fig. 5 Consensus Network calculated from the last 2800 trees resulting from BEAST analysis of the concatenated ITS and *matK* sequences. The threshold was set to $x = 0.2$. Areas with boxes

indicate alternative explanations for the data in at least 20 % of the resulting trees as compared with the final consensus tree

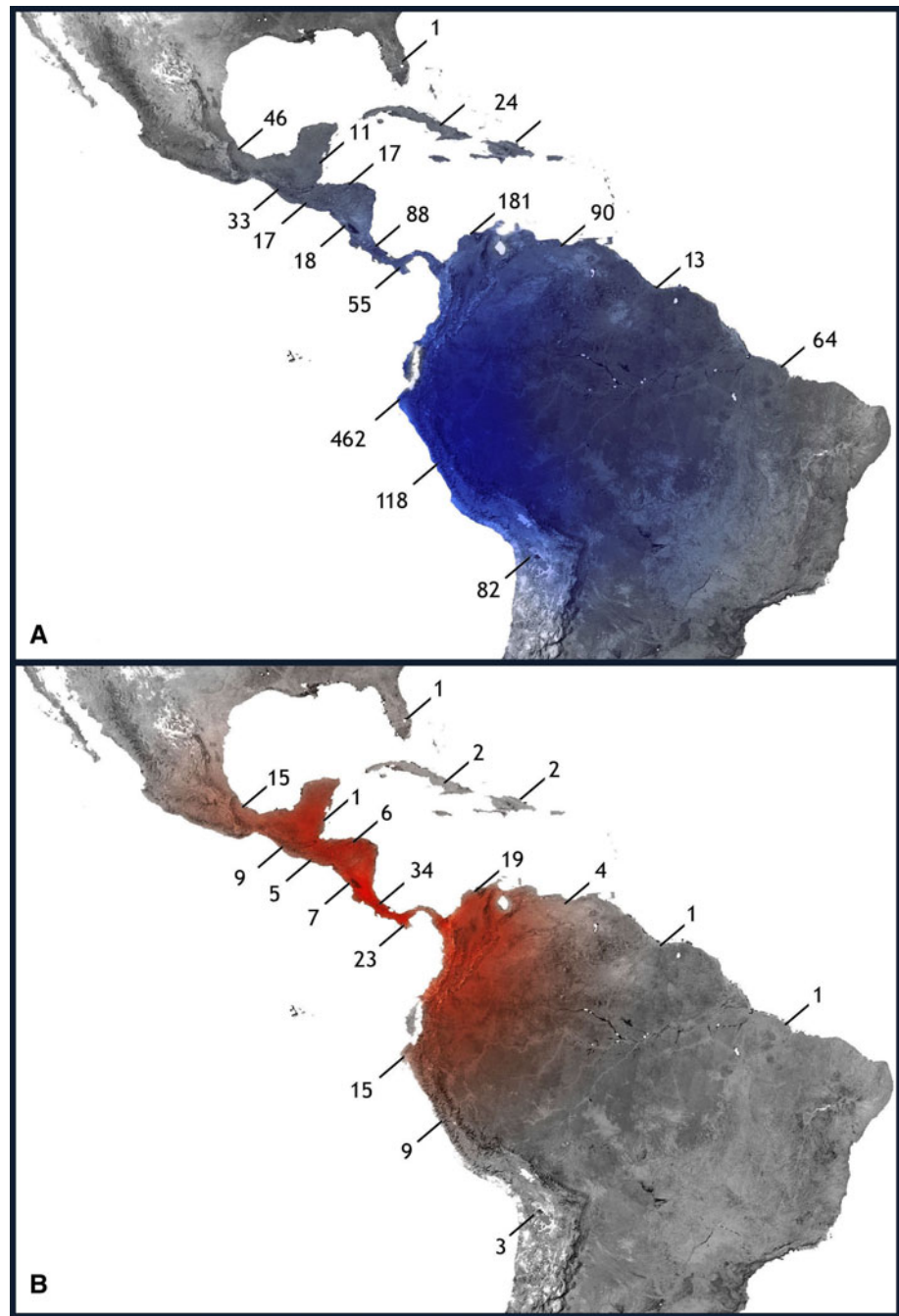
Table 2 Set of diagnostic morphological characters compared among the proposed clades

	Inflorescence	Ornamentation	Synsepal	Glenion	Anther	Pollinaria
Clades A and B	Successive and indeterminate	Hirsute	Present	Absent	Incumbent	Whale-tail
Clade C (<i>Salpistele</i>)	Successive and indeterminate	Glabrous	Present	Absent	Incumbent	Bubble-like
Clade C (<i>Dracontia</i>)	Successive and indeterminate	Glabrous	Present	Absent	Incumbent	Whale-tail
Clades D, E, and F (<i>Acuminatia</i>)	Simultaneous and determinate	Hirsute	Absent	Absent	Incumbent	Whale-tail
Clade F (<i>Crocodelanthus</i> and <i>Stelis</i> s. str.)	Simultaneous and determinate	Hirsute	Absent	Present	Apical	Bubble-like

patterns can be seen in the resulting phylogenetic trees. Following the known distribution given by Govaerts et al. (2011), species closely related to *Stelis imraei*, which are represented here by only one accession, are mostly Colombian, whereas those belonging to clades B and C

have a higher diversity in the south of Central America (especially Costa Rica and Panama) and species of clades D, E, and F are clearly more diverse in the northern Andes (especially Ecuador). A summary of species numbers of the clades reported per country is found in Fig. 6.

Fig. 6 Map of most of the American countries showing estimated species number per country of: **a** species belonging to clades D, E, and F with high diversity in the northern Andes around Ecuador; **b** species belonging to clades B and C with higher diversity in Costa Rica, Panama, and Colombia



Discussion

The resulting clades

Stelis sensu lato clade. The genus *Stelis* in its broad sense (clade *Stelis* s.l.) is well supported, but as currently defined it is not monophyletic. Excluded are *Stelis restrepioides* and *Stelis quadrifida* (type species of genera *Elongatia* Luer and *Lalexia* Luer, respectively), which are both closer to genus *Pleurothallis*. Also excluded are *Stelis ephemera* and *Stelis hypnicola* (the latter is the type species of

Pleurothallis sect. *Effusae* Lindl.), which are within clade *Pabstiella*. In addition, *Anathallis anderssonii*, *A. dolichopus*, *A. rubens*, and *A. sclerophylla* are embedded in the *Stelis* s.l. clade. All of these belong to *Pleurothallis* subgen. *Acuminatia* section *Acuminatae* (Luer 1999; subsequently referred to as *Pleurothallis* sect. *Acuminatae*).

Clade A. This clade is represented by a single accession of *Stelis imraei* Luer (2007) included this species in his genus *Effusiella*, which is supported by floral morphology but cannot be proved genetically with the evidence provided here. We encountered problems extracting DNA and

sequence species from this group, but the only sequence obtained was placed at the base of *Stelis* s.l. in the combined analysis with high support. Whether that position is correct can only be corroborated by adding sequences from the other species that belong to this group in the future. *Stelis imraei* and close relatives have an uncommon combination of features within *Stelis* s.l. They have a ramicaul that is at least twice as long as the suborbicular leaf, a series of short, racemose, successive inflorescences that are borne from the leaf base and aggregate to its abaxial side, and the flowers are non-resupinate and prominently hirsute. The habit somewhat resembles that of *Pleurothallis* and it may prove to be basal to the whole *Stelis* s.l. group. The *Stelis imraei* group is most diverse in Colombia.

Clade B. One of the least resolved relationships is that of species belonging to genera *Unciferia* (sensu Luer 2004) and *Effusiella* (sensu Luer 2007). Species assigned to those genera weakly group together with several clades depending on:

1. the presence or absence of other related sequences;
2. phylogenetic inference methods and criteria; and
3. the genetic regions analyzed.

Clade B is moderately supported in most of the analysis, with *S. immersa* and *S. pilosa* in one group, sister to *S. canae*, *S. pompalis*, and accessions of *S. segoviensis*; both groups are variously interrelated with *S. listerophora* and *S. ornata*. *Stelis cypripedioides* and *S. kefersteniana*, belonging to *Effusiella*, and *S. rodrigoii*, type of genus *Condylago*, occupy unresolved positions altering from the base of *Stelis* s.l. to anywhere else within this clade. In the ITS tree presented here *S. cypripedioides* and *S. kefersteniana* are placed basal to clades D, E, and F, with low support. It is clear that further sampling is necessary to adequately place all the species related to this clade. On the one hand, *Pleurothallis* subgen. *Effusia* (Luer 2000) was composed of a series of unrelated species that were transferred to *Stelis* by Pridgeon and Chase (2001), but later assigned to several different genera including *Dracontia* (Luer 2004), *Lalexia* (Luer 2011), *Niphantha* (Luer 2010), and *Pabstiella* (Luer 2007), the latter including the type of the subgenus, *Pabstiella hypnicola*. On the other hand, genus *Effusiella* (sensu Luer 2006, typified by *E. amparoana* = *Stelis pilosa*), included a group of much more closely related species, which seem to be intermingled with *Unciferia* (sensu Luer 2004, typified by *U. segoviensis* = *Stelis segoviensis*). Species of *Condylago*, *Effusiella*, and *Unciferia* (with a few exceptions) can be recognized by having ramicauls subequal to shorter (normally much so) than the leaves, long successive inflorescences (mostly exceeding the leaves) and continuing to flower for several weeks, conspicuously hirsute sepals, the lateral ones fused into a concave synsepal, petals that are less than half the

length of the sepals, a winged column and a short (as long as the column) movable lip, pollinaria in pairs, and whale-tail type. They are mostly Mesoamerican. *Condylago* is endemic to Panama and Colombia; most species of *Unciferia* are endemic to Costa Rica and Panama, whereas Mexico and Guatemala share most species of *Effusiella*. A few species of *Effusiella* are reported from the Andean region and from Brazil, but, at least in the latter, most have been misapplied to species of *Pabstiella* (sensu Luer 2007).

Clade C. This clade is composed of:

1. species of genus *Salpistele* (Luer 1991) and *Pleurothallis* subgenus *Elongatia* section *Petiolatae* (Luer 1994);
 2. species belonging to *Dracontia* (sensu Luer 2004); and
 3. *Mystacorchis* (Szlachetko and Margonska 2001) and a few “misfits” from other genera.
1. A highly supported clade includes accessions of *Salpistele adrianae*, *Stelis brunnea*, and *Stelis maculata*, all assigned to genus *Salpistele* (Luer 1991), and *Stelis guttata* and *S. janetiae*, placed in *Pleurothallis* subgen. *Elongatia* sect. *Petiolatae*, later elevated to genus *Elongatia* (Luer 2004). Although the similarities are not immediately apparent, all species of this clade have small plants (less than 10 cm tall), with petiolate leaves that are 3 or more times longer than the ramicauls, creeping successive inflorescences with only one flower open at once, petals and sepals subequal, and a hirsute lip. All the known species are confined to the shared mountain range between Costa Rica and Panama, where they grow at mid-elevations at approximately 1500 m.
 2. *Stelis alajuelensis*, *S. alta*, *S. cobanensis*, *S. cylindrata*, *S. dracontea*, *S. ferrelliae*, *S. gigantea*, *S. megachlamys*, *S. multirostris*, *S. pachyglossa*, and *S. papillifera*, have been placed by Luer (2004) in genus *Dracontia*. Those species, together with *Dracontia hydra*, *D. lueriana*, and *Stelis platystylis*, are mostly found associated in a well-supported clade. Those species can be recognized by the successive inflorescences, fleshy flowers with long, thick, three-lobed, movable lips, convergent sepals forming a synsepal that is similar to the dorsal sepal, concave petals, a triangular column which is apically dentate and much shorter than the lip, an incumbent, helm-like and large anther (exceeding the column), ventral stigma covered by a bubble-like rostellum, and two flat, dry, whale-tail shaped caudicles, to name just a few distinguishing features. Species assigned to genus *Dracontia* range from Mexico to Panama, with one species in the Greater Antilles. The greatest diversity is found in Costa Rica and Panama, whence 16 of the 17 described species have been reported. All are epiphytic herbs or

grow on terrestrial mosses, usually found in humid or seasonally dry forests. Most species grow at mid to high elevations, mostly between 800 and 1800 m.

3. The relationships of the different accessions of *Stelis carpintera*, *S. convallaria*, and *S. mystax* are not clear. They are related somehow to species allocated to *Dracontia* and *Salpistele*; however, they are not placed with support. On the basis of morphology the species do not seem closely related. Sampling of closely related species might help clarify their position; none seems to have known close relatives, however. Luer (2004) placed *Stelis carpintera* in genus *Elongatia*, but the DNA evidence presented here suggests that the other members of that genus (*S. guttata*, *S. janetiae*, and *S. restrepioides*) are not closely related to it. *Stelis mystax* was placed in monotypic genus *Mystacorthis* Szlach. & Marg., and *Stelis convallaria* in genus *Effusiella* (Luer 2006), where it has no close relatives (Luer 2000). The three taxa differ substantially morphologically, both vegetatively and florally. *Stelis carpintera* and *S. mystax* each have a ramicaul that exceeds the suborbicular-cordate leaf for at least twice its length, and *S. convallaria* has a large elliptic leaf that exceeds the ramicaul. They have successive inflorescences, that of *S. convallaria* being multi-flowered and much exceeding the leaf, with several flowers open at once, whereas those of *S. carpintera* (cleistogamous) and *S. mystax* just exceed the leaf and are few-flowered, with only one flower open at a time. Sepals are elliptic and in *S. carpintera* and *S. convallaria* they are fused into a synsepal that is similar to the dorsal sepal, whereas the lateral sepals of *S. mystax* are fused only to the middle and are then divergent. The petals of *S. carpintera* (1:4 sepal length) and *S. mystax* (1:2 sepal length) are elliptic and acute, the petals of *S. convallaria* are subequal to the sepals, spatulate and bilobed. The lip of *S. carpintera* is as long as the synsepal, flat, spatulate, with two lobes in the middle and a suborbicular midlobe, while that of *S. convallaria* is less than half the length of the sepals, inconspicuously bilobed at the base, the midlobe prominently bilobed, and tricallous. The lip of *S. mystax* is half the length of the sepals, thick, spatulate, with an orbicular midlobe, and a depression along the claw. All three have a long claw at the base of the lip. The column of *S. convallaria* (1:1 lip length) is alate and fimbriate, that of *S. carpintera* (1:2 lip length) is narrowed in the middle, alate at the apex and with entire margins. The column of *S. mystax* (1:4 lip length) is cylindrical. The three species have the anther incumbent, the stigma ventral, and the pollinaria whale-tail shaped. All three species are found in Central America, where they are more frequent in Costa Rica and Panama.

Clade D. *Stelis emarginata* (type species of *Physosiphon*), *S. punctulata* (type of the monotypic genus *Lomax*), and *Stelis tacanensis*, are included in a highly supported clade that is constant throughout all analysis. It is noteworthy that this clade seems to be sister to the species of *Salpistele* in the *matK* analysis consensus tree. That relationship is found in no other analysis and is not at all apparent from the morphological characters. Morphologically, species of this clade have ramicauls that are subequal to the elliptic leaf; they have a racemose, simultaneous inflorescence with more than a dozen flowers, which exceeds the leaf by up to twice the length. Sepals are deeply connate into a tube constricted near the middle, with free, spreading, thickened, similar apices. Petals, lip, and column are very much reduced, approximately one-third or less the length of the sepals. Petals are membranous, subspatulate, acute. Lip is linear-elliptic, with callous lateral lobes near the middle and an elliptic, obtuse midlobe. The cylindrical column is as long as the lip, with the apex winged. The anther is incumbent and the stigma is ventral. Pollinaria are whale-tail shaped. The plant and inflorescence morphology remind of species of *Stelis* s. str., but their floral details are unique. Species referable to this clade seem to have their center of diversity in Mexico where *S. emarginata*, *S. greenwoodii*, *S. punctulata*, and *S. tacanensis* are found.

Clade E. Three different accessions of *S. gelida* and one of *S. antillensis* constitute a highly supported clade, that is found in all analysis. Genus *Niphantha* (Luer 2007) was described without Latin description to accommodate *Stelis gelida* (the type species) and *S. pidax*, and later validated by the same author (Luer 2007, 2010). The sequence of *S. antillensis*, taken from Hagen Stenzel's dissertation paper (2004), is embedded in *Stelis gelida*. Rather than considering both species as synonymous, we believe it more likely the samples were mixed up, as both grow in Cuba, are morphologically similar, and the sample of *S. antillensis* was taken from a cleistogamous plant. *Stelis gelida* is characterized by a robust, tall (frequently exceeding 50 cm) habit, with a large elliptical leaf subequal to the ramicaul, which is covered by loose tubular sheaths. The inflorescences are 1–5 (normally a few present), subequal to the leaf, flowers simultaneous, and transparent-white. Sepals are obtuse and pubescent, the dorsal free, lateral sepals fused for the first third, not converged into a synsepal, petals obtuse, glabrous, thin, and margin minutely erose. The lip is thin, subpandurate, arcuate, with a pair parallel calli in the middle third, and apically broadly truncate. The column is semiterete, winged, erose, and exceeds the lip. The anther and stigma are ventral. The pollinaria are whale-tail type. Morphologically, *Stelis gelida* seems to be midway between species of *Crocodelanth* and *Effusiella* (sensu Luer 2006), with the plant and

inflorescence morphology resembling the first, whereas the subpandurate, arcuate lip, the long column that exceeds the lip, the incumbent anther, the ventral stigma, and the whale-tail pollinaria resemble the second. *Stelis gelida* may be the most widely distributed of all Pleurothallidinae, as it is frequent from Florida to the south of Brazil and the Antilles.

An accession of *Stelis nexipous*, the type species of *Stelis* sect. *Nexipous* (Garay) Luer, is related with weak support to the *Stelis gelida* clade in the ITS BEAST analysis. Species of sect. *Nexipous* have an aberrant floral morphology within *Stelis* s. str., and sampling more species of the section may help in clarifying its position. The section includes some 18 species from Colombia, Ecuador, and Peru, with Ecuador by far the center of diversity with 16 endemics. They are characterized by thick ramicauls with loose tubular sheaths, bearing coriaceous leaves, mostly surpassed by a long, thick, racemose, multi-flowered, successive inflorescence, flowers with the lateral sepals connate more deeply to the dorsal sepal than to each other, and stigmatic lobes that overlap the petals. Most species of this section are epiphytic and grow at high elevations, mostly above 2000 m.

Clade F. This highly supported clade has four major subclades, basically formed by the species assigned to:

1. *Stelis* s. str. (Luer 2009);
 2. *Crocodeilanthe* (Luer 2004);
 3. *Pleurothallis* sect. *Acuminatae* (Luer 1999); and
 4. *Physothallis* (Garay 1953).
1. High support is found for a monophyletic and natural clade that includes all species traditionally regarded as part of *Stelis* s. str., and it is found in all analysis. It includes the type species of *Stelis* (*S. ophioglossoides*, results not presented here), and excludes *Stelis nexipous*, from *Stelis* subgen. *Nexipous* Garay. 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 5 cm), 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 horizontal disposition perpendicular to the inflorescence. The flowers of many species have temporal activity, opening and closing in apparent response to environmental conditions. Sepals are ovate, mostly 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 toward the apex, wing-less, with an apical anther and stigma. A column foot which is suggested by Luer (2009) but was not seen, if present, 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 pollinia. The pollinaria are provided with a pair of cylindrical caudicles, which are attached to a sticky, hard viscidium (subsequently referred to as bubble-like pollinaria). The viscidium looks like a droplet on the apex of the column. In this large group many species have diverged from the typical morphological character states, several species have successive inflorescences (instead of the more common simultaneous), glabrous sepals (instead of hirsute), convergent lateral sepals (instead of spreading), elliptic and flat petals (instead of ovate and transversely thickened), and a curved, elongated column (instead of short and straight). However, even if it is common for species of *Stelis* s. str. to have one of these alternative states, they are never all found together in one species. Garay (1979) segregated a group of species from *Stelis* on the basis of an unlobed stigma. Throughout the whole group, however, the stigma is variable, sometimes seeming clearly lobed and others not at all, without any clear phylogenetic pattern. Species of *Stelis* s. str. can be found from Florida to Bolivia and Argentina and in the Antilles, from sea level to above 3000 m elevation and in almost any kind of life zone. The highest diversity is found in the central Andes, almost 500 (more than half the known species) are found in Ecuador, whereas only a few dozen are found in each of the Central American countries (except Costa Rica) and the Antilles.
 2. *Stelis atwoodii*, *S. deregularis*, *S. galeata*, *S. pulchella*, and *S. velaticaulis*, are grouped together (when present) with high support in all the analysis. All these species have been assigned to *Pleurothallis* subgen. *Crocodeilanthe* (Luer 1986, 1998), and were later placed in genus *Crocodeilanthe* (Luer 2004). *Stelis deregularis* was included in *Pleurothallis* subgen. *Crocodeilanthe* (Luer 1986), but not transferred to genus *Crocodeilanthe*. It was instead selected as type species of *Pleurothallis* subgen. *Pseudostelis* (Luer 1999), and genus *Pseudostelis*. The type species of genus *Crocodeilanthe* has not been sequenced, but, because of morphological affinity, is expected to be close to *Stelis galeata* and *S. velaticaulis*. Species of *Crocodeilanthe* (sensu Luer 2004) can be recognized by the relatively large plants with long ramicauls (normally much longer than the leaves), the loose

conspicuous bracts that enclose the bases of the ramicauls, and the erect racemose multiflowered simultaneous inflorescences, borne from a normally conspicuous spathe, bearing relatively small, mostly whitish or yellowish resupinate flowers. Sepals are similar to each other, never caudate, the lateral ones connate to around the middle. The petals are obtuse and 1 to 3 veined. The lip is short and simple, commonly with two small lateral lobes, attached to the bulbous base of the usually short and straight column, the anther is apical to subapical, and the pollinia are bubble-like type. Some 80 species belong to this clade, almost exclusively from the Northern Andes in Colombia to Perú. The only species known from the Antilles is *S. antillensis*, whereas *S. deregularis* has a wide distribution, from Mexico south to Brazil. With the exception of *S. deregularis*, all *Crocodelanthe* are found above 1500 m elevation, more commonly between 2000 and 3000 m.

3. *Pleurothallis* subgen. *Acuminata* (Luer 1999) was divided into two sections, sect. *Acuminatae*, with *Pleurothallis acuminata* as type, and sect. *Alatae* Luer, typified by *Pleurothallis obovata* (Luer 1999). Pridgeon et al. (2001) found that *Pleurothallis obovata* (= *Pleurothallis fasciculata*, lectotype of genus *Anathallis*) and other species of sect. *Alatae* occupied a basal position in the phylogeny of the Pleurothallidinae, but no species of sect. *Acuminatae* were included in their phylogenetic analysis. *Anathallis anderssonii*, *A. dolichopus*, *A. sclerophylla*, and *A. rubens*, all belong to sect. *Acuminatae*, and are close relatives but do not group together into a monophyletic clade; instead, they are all somehow interrelated with species of *Physothallis* (Garay 1953) and *Crocodelanthe* (Luer 2004). Species of this group can be recognized by the ramicauls longer than the elliptic leaf, and the erect, racemose, multiflowered, simultaneous inflorescences that are longer than the leaf, borne from an inconspicuous spathe, with yellowish, resupinate flowers. Sepals are similar to each other, mostly long caudate, and spreading; thus most species appear star-like, pubescent within, the petals broadly obtuse to rounded at the apex, the column is long and slender, with an incumbent anther, the lip is entire to shallowly lobed. Approximately two dozen species belong to this clade, mostly found in the central Andes of Peru and Bolivia, usually above 2000 m elevation. *Anathallis dolichopus* and *A. scariosa* are the only two species to occur north of Panama, and *A. acuminata* and *A. rubens* are the only two species found in Brazil.
4. Contradictory relationships are found between two accessions of *Stelis harlingii*, (type species of genus *Physothallis*), and members of *Pleurothallis* sect.

Acuminatae (Luer 1999). In the ITS and combined consensus trees, *S. harlingii* is found together with *A. anderssonii*, with high support, whereas in the *matK* analysis consensus tree it associates with *A. dolichopus*. The second species assigned to genus *Physothallis*, *Stelis cylindrica* (Luer 1977), was not sequenced. In the strict sense, members of *Physothallis* can be recognized by the long, successive, racemose, multi-flowered inflorescences, the lateral sepals completely fused with the dorsal, and the apex thickened and recurved. The two species known to belong to this clade are endemic to Ecuador, are terrestrial (probably lithophytic), and grow at approximately 2000 m elevation.

Not all species assigned to *Stelis* s. str., *Pleurothallis* sect. *Acuminatae*, *Crocodelanthe* (including *Pseudostelis*), *Physothallis*, *Niphantha*, and *Physosiphon* (including *Lomax*) have been included here; additional sampling may be required to accommodate the remaining species into one of the groupings. Further sampling could possibly resolve how species of *Pleurothallis* sect. *Acuminatae* are related to species of *Physothallis* and *Crocodelanthe*, where the remaining species allocated to *Pleurothallis* subgen. *Pseudostelis* (Luer 1999) should be placed, whether all species in the variable *Crocodelanthe* (Luer 2004) actually belong together, and whether all species of *Stelis* sect. *Nexipous* should be excluded from *Stelis* s. str. and where they should be actually placed.

Evolutionary trends

Several ancestral and derived character states have been suggested for orchids in the past, together with hypotheses about evolutionary trends in some lineages. However, determining state polarization for groups belonging to different phyletic lines has always been a difficult task for systemacists. DNA-based phylogenies enable us, with a greater certainty, to determine which character state is more basal and which more derived in the strict framework of the studied groups. As for *Stelis*, the computer-generated phylogenetic trees reveal distinct tendencies among the sampled taxa and the groups to which they belong (Fig. 7).

Reproductive organs (Fig. 8). Most groups of Pleurothallidinae have what has here been called “whale-tail” type pollinaria, where two pollinia are brought together by a pair of flattened, dry, suborbicular, bifid caudicles, provided with irregular margins and perhaps at least partly formed by sterile pollen grains (as in other groups of the Epidendroideae, e.g., Laeliinae). The non-detachable viscidium (a drop or line of viscid liquid, for which the term viscarium has been proposed) is well separate, and found on the apex of the rostellum. However, in several unrelated

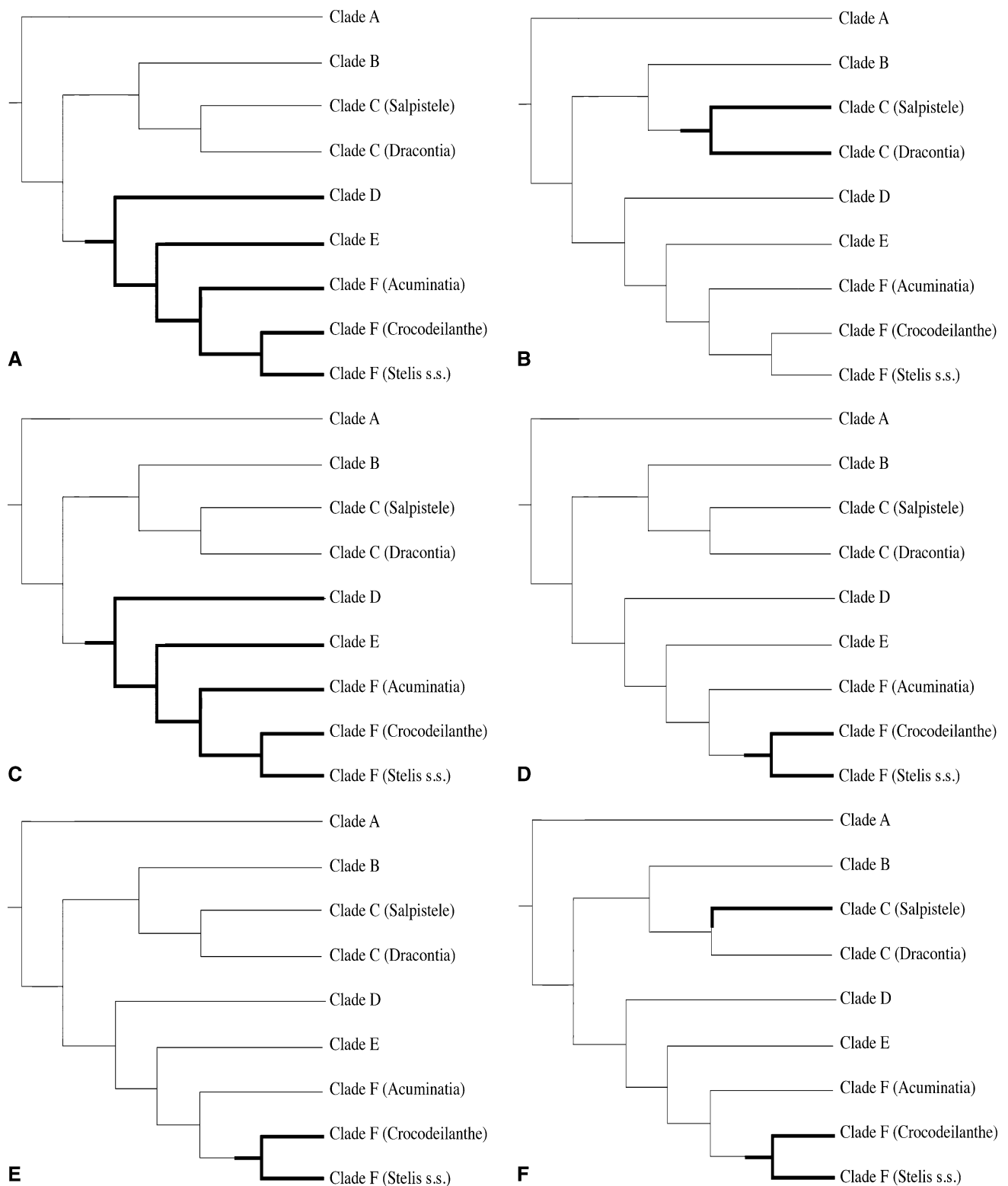


Fig. 7 Morphological characters patterns plotted on per clade summarized trees. *Thickened branches* indicate clades in which: **a** the inflorescence is simultaneous and determinate (vs. successive and indeterminate); **b** the inflorescence is creeping (vs. erect);

c flowers have the lateral sepals fused into a synsepal (vs. no synsepal); **d** flowers have a glenion at the base of the lip (vs. no glenion); **e** flowers have an apical anther (vs. incumbent); **f** flowers have a bubble-like type pollinaria (vs. whale-tail type pollinaria)

clades the distance between the apex of the rostellum and the base of the anther is shorter (proximity achieved by reduction in column length and the apical position of the anther and stigma), and the caudicles are united to the viscid liquid to form a “bubble-like” type pollinaria. Bubble-like type (B) pollinaria are found in derived groups whereas the ancestral state has a whale-tail type (W). Examples are *Pleurothallis quadrifida* (Lex.) Lindl. (W), which is basal to genus *Pleurothallis* (B), and *Pleurothallis rubella* Luer (W) which is basal to genus *Platystele* (B). As discussed here, species of clades A, B, and C (excluding those assigned to genus *Salpistele*) all have W-type pollinaria, whereas species assigned to *Salpistele* in clade C, have B-type; and species of clades D, E, and those assigned

to *Acuminatia* in clade F, all have W-type pollinaria but those assigned to *Crocodeilanthus* and *Stelis* s. str. in clade F are B-type.

Speciation (Fig. 9). Number of species traditionally has been associated one way or another with evolutionary success. Even though most of the genera within Pleurothallidinae have an elongated column and incumbent anther, the most diverse genera have compact columns and apical anthers. *Lepanthes*, *Pleurothallis*, and *Stelis* are the most species rich genera of Pleurothallidinae, altogether accounting for more than 50 % of the species of the subtribe, and they are predominantly characterized by short columns and apical anthers. On a smaller scale, the clades of *Stelis* s.l. commonly have only a few to a couple dozen

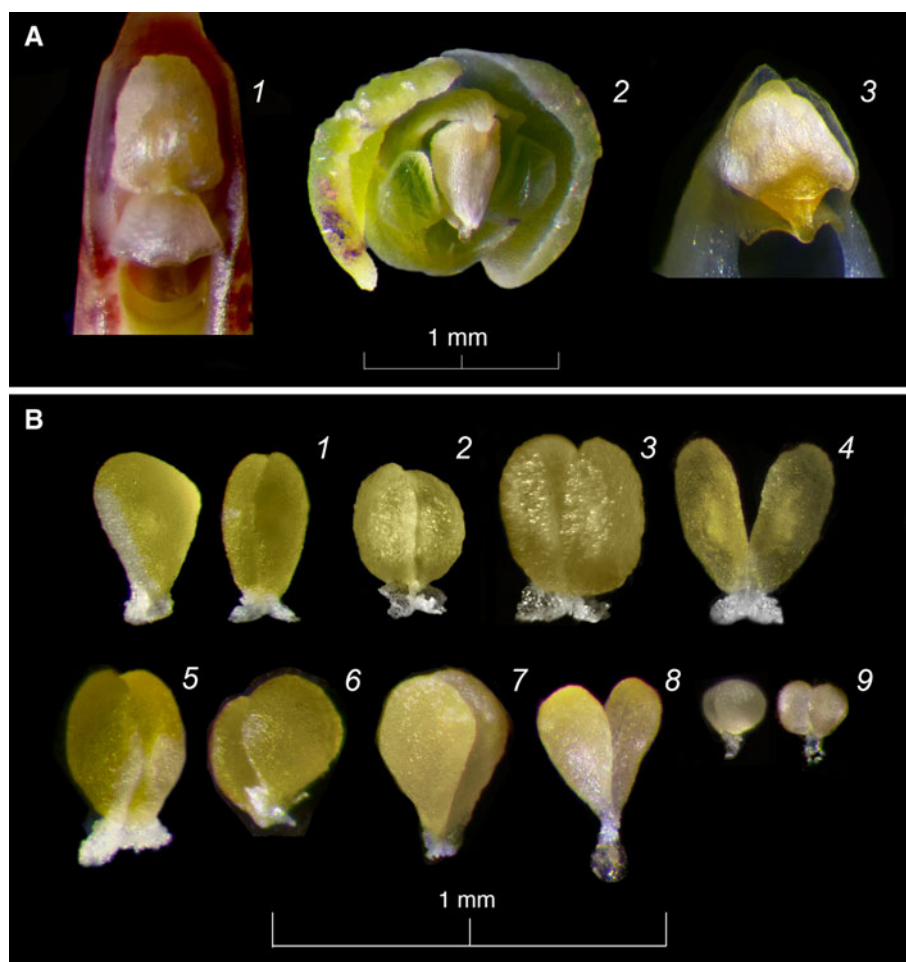
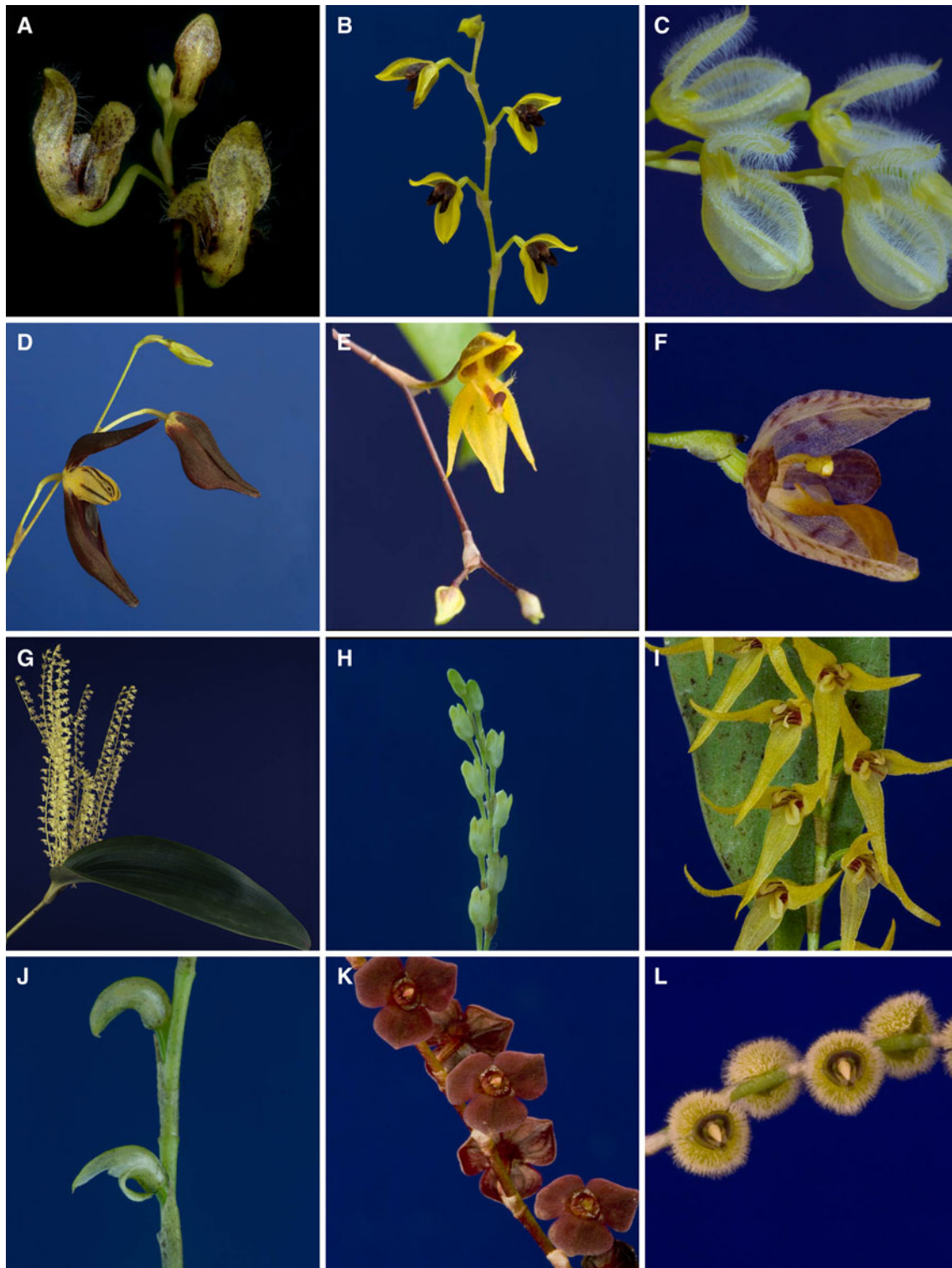


Fig. 8 Variation in the morphology, structure, and function of the reproductive organs within *Stelis* s.l. **a** Column apices showing 1, *S. megachlamys*, ventral view showing an incumbent anther at the base of which the dry pollinaria’s caudicles are visible, well separate from the ventral stigma covered by a bubble-like rostellum (whale-tail type pollinaria); 2, *S. janetiae*, frontal view showing the apical anther, embraced the apical stigma’s lobes, at the base of the anther a drop-like viscidium unites the pollinaria’s caudicles with the apex of the rostellum (bubble-like type pollinaria); 3, *Stelis* s. str., ventral view

showing an intermediate structure, with an incumbent anther and ventral stigma, but a rostellum shortened to enable contact between its viscid apex and the pollinaria’s caudicles. **b** Pollinaria of *Stelis imraei* (1), *S. alta* (2), *S. papillifera* (3), *S. ramonensis* (4), *Condylogo furculifera* (5), *Stelis segoviensis* (6), *S. janetiae* (7), *Stelis* s. str. (8) and *S. deregularis*. 1–6 are whale-tail type, and lack a viscidium; 7–9 are the bubble-like type, with the drop like viscidium still attached in 8 and 9. Photographs by A.P. Karremans and F. Pupulin



A - *Stelis imraei* (A)
 B - *Stelis* aff. *segoviensis* (B,
 C - *Stelis pilosa* (B)
 D - *Stelis alajuelensis* (C)

E - *Salpistele adrianae* (C)
 F - *Stelis carpintera* (C)
 G - *Stelis gelida* (D)
 H - *Stelis punctulata* (E)

I - *Stelis dolichopus* (F)
 J - *Stelis atwoodi* (F)
 K - *Stelis* (F)
 L - *Stelis* aff. *microchila* (F)

Fig. 9 Representative members of *Stelis* s.l. showing variation in flower morphology; with the here assigned clade each species belongs to indicated in *brackets*. Photographs by A.P. Karremans, D. Bogarín and F. Pupulin

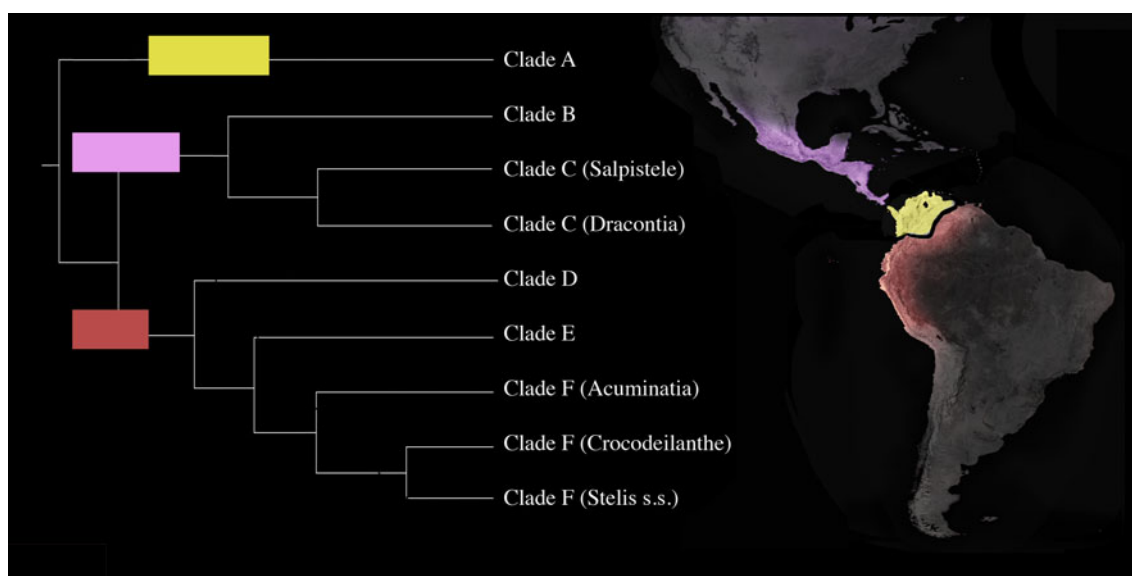


Fig. 10 Geographical patterns plotted on a per clade summarized tree. Latin America has been tentatively divided into three representative geographical regions, Mesoamerica, Colombia, and the

species (clades A, B, C, D, and E), whilst clade F, which includes subclades *Crocodeilanthe* (with almost 100 species) and *Stelis* s. str. (with several hundred species), has about ten times more species than all other clades combined, and both with a compact column and apical anther. It is perhaps not irrational to suggest that the apical position of the rostellum and the detachable viscidium, paired with a distinct shortening of the column, were particularly functional to improving pollination efficiency and broadening the spectrum of effective pollinators.

Geographical distribution (Fig. 10). Species of *Stelis* (in its broad sense) and Pleurothallidinae in general are widely distributed, growing as epiphytes or as terrestrials in humus throughout most of tropical and subtropical America and at almost every elevation available. Nonetheless, there are noticeable patterns in their distribution. *Dilomilis* Raf. and *Neocogniauxia* Schltr., basal to the whole subtribe, are only found in the Antilles, whereas *Acianthera* Scheidw., *Anathallis* Barb. Rodr., and *Octomeria* R.Br., the largest basal genera, have their centers of diversity in the Brazilian lowlands. The overall most species-rich genera (*Lepanthes*, *Pleurothallis*, and *Stelis*) are both more derived and predominantly Andean in distribution. In the clades studied here a similar pattern is found, the more basal clades (species of clades A, B, C, D, and E) being composed of species that are most diverse in Central America south to Colombia and mostly found at mid elevations, from 1000 to 2000 m, whereas clade F includes species which are most predominantly Andean in distribution and more diverse at highest elevations (above 2000 m).

northern Andes. Colors on the tree indicate the region in which each clade(s) is most diverse following their known distributions

Conclusions

Much has been said to defend or reject molecular evidence as the key to classifying organisms. The reality is that DNA-based phylogenies may well be the least subjective in inferring species evolutionary relationships and, therefore, a powerful starting point. DNA-based phylogenetic trees enable us, for the first time, to identify the ancestral and derived states of characters in a group context; they are, thus, a significant tool enabling understanding of evolutionary trends in character states and their systematic relevance in related species groups.

Taxonomic implications

On the basis of DNA alone it is not possible to establish whether genus *Stelis* should include all species of clade *Stelis* s.l. or only those of *Stelis* s. str. (or for that matter any other clade along the way). Both clades are clear, monophyletic, constantly and highly supported, and include a large number of species, and so either is equally justifiable on a genetic basis. *Stelis* s. str. is, however, easier to circumscribe on morphological terms, which seemingly reflect evolutionary trends. It is important to mention that *Stelis nexipous* did not group together with the other members of *Stelis* s. str., this species is the only member of the morphologically aberrant *Stelis* subgen. *Nexipous* (Garay) Luer that was included here. Further research on its floral morphology and inclusion of other species of the group might reveal they should be excluded from *Stelis* s. str.

Elongatia (Luer 2004) and *Effusiella* (Luer 2007) are both paraphyletic. Although the type species of the first is closer to *Pleurothallis* than to *Stelis*, at least three other species assigned to the genus belong to what has here been denoted clade C. Genus *Effusiella* has members spread out among clades A, B, and C. Species assigned to genus *Uncifera* (Luer 2004) seem to form a monophyletic group, but they are variously related to several species of *Effusiella*, including its type, and other taxa placed in clade B. Additionally, that generic name is too similar to *Uncifera* Lindl., another genus in Orchidaceae, and should therefore not be used.

The type species of genera *Pseudostelis* Schltr. (1922) and *Lomax* Luer (2006) are embedded in the generic concepts of *Crocodeilanthe* Rchb.f. (clade F) and *Physosiphon* Lindl. (clade D), respectively. Genus *Lalexia* (Luer 2011) is not synonymous with *Stelis* in any of its circumscriptions; it forms a quite distinctive clade, allied to *Pleurothallis*.

Anathallis, *Pabstiella*, *Pleurothallis*, and *Stelis*, as circumscribed by Pridgeon and Chase (2001) and Pridgeon (2005), are non-monophyletic or paraphyletic according to our results. Several species assigned to *Anathallis* are closer to *Stelis*, including *Anathallis anderssonii*, *A. dolichopus*, *A. rubens*, and *A. sclerophylla*, and, on the basis of morphology, it is highly likely that Luer's whole *Pleurothallis* subgen. *Acuminatia* sect. *Acuminatiae* belongs in *Stelis* s.l. Several species assigned to *Stelis* s.l. are actually closely allied to *Pabstiella* and *Pleurothallis*. *Stelis ephemera* and *S. hypnicola* belong in genus *Pabstiella*, possibly together with all other species placed by Luer in *Pleurothallis* subgen. *Effusia* (2000) and later transferred to *Pabstiella* (Luer 2007). *Stelis quadrifida* and *S. restrepioides* are clearly related to *Pleurothallis*, the first seems to have no close relatives whereas the second, placed in genus *Elongatia* (Luer 2004), is morphologically similar to *Stelis excelsa* Garay, *Stelis holtonii* Luer, *Stelis macrophylla* H.B.K., and *Stelis superbiens*, which we suspect should all be excluded from *Stelis* s.l.

However, it is premature at this point to establish new schemes of classification for this group as a whole. The results of genetic sampling must be coupled with morphological characters and geographical distributions to enable understanding of evolutionary patterns, and, to place them adequately, additional sequencing of several species groups should be conducted.

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