8-3-2011

Understanding the origin and rapid diversification of the genus Anthurium Schott (Araceae), integrating molecular phylogenetics, morphology and fossils

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A Thesis Submitted to The Graduate School at the University of Missouri – St. Louis in partial fulfillment of the requirements for the degree
Doctor of Philosophy in Biology with emphasis in Ecology, Evolution and Systematics

June 2011

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Peter Stevens, Ph.D. (Advisor)

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ACKNOWLEDGEMENTS

I would like to thank my Advisor, Dr. Peter Stevens (University of Missouri – St. Louis), and co-Advisor, Dr. Thomas Croat (Missouri Botanical Garden) for helping me all the way during my graduate studies. I am also thankful to my committee members, Dr. Elizabeth "Toby" Kellogg (University of Missouri – St. Louis) for letting me work in her molecular lab and for very helpful suggestions to improve grant and manuscript writing, Dr. Mick Richardson (Missouri Botanical Garden) for always finding funds to cover my studies, and Dr. Simon Mayo (Royal Botanic Gardens at Kew, England) for sharing his knowledge of the family Araceae with me.

I am also deeply grateful to the faculty, curators, staff and graduate students at both the University of Missouri – St. Louis and the Missouri Botanical Garden for their help, discussions, suggestions and friendship. And, very specially, the Kellogg's lab members, Jan Barber, Mark Beilstein, Paulo Camara, Andrew Doust, Cynthia Hong-Wa, Meredith Jacques, Elma Kay, David Kenfack, Shelby Kleweis, Lucia Lohmann, Simon Malcomber, Anya Penly, Jill Preston, Renata Reinheimer, Patrick Sweeney, Anthony Verboom, Beto Vicentini, Sarah Youngstrom, Felipe Zapata, all of whom help me discover and enjoy the wonders and problems of producing a molecular phylogeny.

I would also like to thank the many funding sources that made possible this research through their generous financial support: the National Science Foundation through a Doctoral Dissertation Improvement Grant (# 0709851), the Desmond Lee Fund at the Kellogg's Lab, the Missouri Botanical Garden, the W. R. Harris World Ecology Center at the University of Missouri -St. Louis, the Botanical Society of America, the American Society of Plant Taxonomists, the Royal Botanical Gardens in Kew, England

with a Kew Latin American Research Fellowship, the Garden Club of America, and the International Aroid Society.

This study would not have been possible without the help and “green thumb” of Emily Colletti, the Araceae greenhouse manager at the Missouri Botanical Garden, who has taken excellent care of one the best cultivated aroid collections in the world. Approximately 90% of the specimens used in this study came from those greenhouses. Other aroid researchers also contributed to the development of this project with their suggestions, samples and discussions, Anne Swart (while at Washington University – St. Louis), Dr. Lívia Godinho Temponi (Universidade Estadual do Oeste do Paraná, Brazil) and Dr. Natalie Cusimano (Ludwig-Maximilians Universität, München, Germany).

I would also like to thank my field assistants in several countries, Dan Levin and Thomas Croat in Ecuador; Carmen Emilia Benítez, Ismael Capote, Alfonso Cardozo, Dumas Conde and Carlos Reyes in Venezuela; and, Hector Gómez, Jorge Martínez, Nayarit Martínez and Miguel Angel Perez-Ferrara in Mexico.

I am thankful to all my friends and family, in St. Louis and in Venezuela, that have helped in the long road of my graduate studies, especially to Kuo-Fang Chung, Indiana Coronado, Patty Feria, Sara Fuentes, Jason Krause & family, Rosa Ortiz, John Pruski, Ben Torke, Lupita Sanchez, Homero Vargas.

I would love to thank my favorite graphic designer, Rhandus Malpica, for designing my *Anthurium* logo and for being with me even when I was in another country.

And finally, I am in debt with Mauricio Diazgranados for a very important last minute “rescue” when my computer unexpectedly died a week before my Dissertation defense.

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ABSTRACT

Anthurium is a strictly neotropical genus of Araceae ranging from southern Mexico to northern Argentina, including ca. 900 species and displaying an enormous variation in leaf morphology, growth habit, leaf venation pattern and inflorescence and fruit colors. Despite its immense diversity, its ecological importance in Neotropical forests, and a very long history of botanical collection, cultivation, and taxonomical research, *Anthurium* had been almost neglected in molecular phylogenies.

This study combines chloroplast (*trnG* intron, *trnH-psbA* and *trnC-ycf6* intergenic spacers) and nuclear (*CHS* first intron) DNA sequence data for 102 *Anthurium* species and uses maximum parsimony, likelihood and Bayesian phylogenetic analysis to test the monophyly of *Anthurium*, to elucidate relationships among its species, to test statistically the validity of the currently accepted sectional classification and its associated morphological characters, and to study the pattern of *Anthurium* species diversification through time.

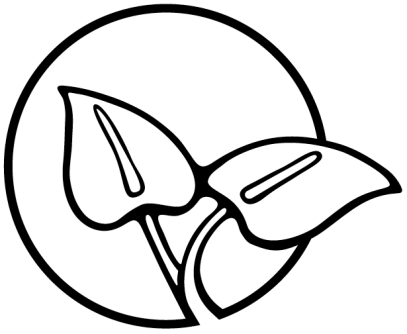
Results show that *Anthurium* is monophyletic and that at least 20 highly supported (bootstrap values > 70% and/or posterior probabilities > 0.90) major clades are recognizable within it, most of them easily characterized morphologically and/or geographically. Despite giving a better idea about the evolutionary history of the genus, resolution is still lacking in the deeper nodes of the phylogeny. This study suggests that the current sectional classification of *Anthurium* does not accurately represent its evolutionary history. Most of the major clades do not correspond with the circumscriptions of infrageneric groups and morphological characters used in the sectional classification are in fact highly homoplasious within *Anthurium*. This study also

reveals that there is very low sequence divergence among *Anthurium* species, and relatively short branches characterize the core of the *Anthurium* clade. Although the stem node of *Anthurium* is quite old (Late Cretaceous), species diversification did not start until quite recently – the crown node age is dated to Late Oligocene, and the bulk of species diversification did not occur until even later, during the Late Miocene (ca. 8-9 Mya). *Anthurium* species diversification through time is indeed consistent with the pattern of a rapid radiation, having a diversification rate of approximately 0.61-0.69 species per million years.

CHAPTER 1

A MOLECULAR PHYLOGENY OF *ANTHURIUM* SCHOTT (ARACEAE) BASED ON COMBINED CHLOROPLAST AND NUCLEAR DNA

Mónica M. Carlsen



INTRODUCTION

The genus *Anthurium* Schott is a strictly Neotropical genus of Araceae ranging from southern Mexico into Central America and the West Indies, to southern Brazil, northern Argentina, and Paraguay. The genus includes approximately 900 largely well-differentiated species (Mayo et al. 1997, Govaerts & Frodin 2002, Govaerts et al. 2011, CATE Araceae 2011) and many more still being discovered (T. Croat, pers. comm.). Previous molecular phylogenies (French et al. 1995, Barabé et al. 2002, Rothwell et al. 2004, Tam et al. 2004, Cabrera et al. 2008, Cusimano et al. 2011) place *Anthurium* in a highly supported monophyletic subfamily, Pothoideae, one of the earliest divergent lineages in Araceae. This subfamily is characterized by genera with fine reticulate venation, geniculate petioles and perfect flowers with a perigone. It also includes the genus *Pothos* L. (approx. 57 species) from Southeast Asia, Australasia and Madagascar, the monotypic *Pothoidium* Schott, from Taiwan and Malaysia, and *Pedicellarum* M. Hotta, from Borneo. These three genera of Old World climbers have monopodial shoots, distichous leaves and flattened petioles (French et al. 1995, Mayo et al. 1997). *Anthurium* is distinguished by being composed of New World tropical climbers or epiphytes with sympodial growth, spirally arranged leaves, rounded petioles, collective veins along the leaf margins and seeds with copious endosperm (Grayum 1990, Mayo et al. 1997).

Within Araceae, *Anthurium* has several outstanding claims to fame. It is the most species-rich genus of aroids, accounting for ca. 27% of the species of the whole family and about half of Araceae in the New World (Bown 2000, CATE Araceae 2011). It is also the most conspicuous representative of the family both in montane cloud forests and lowland Neotropical rain forests (Grayum 1990, Croat 1994, Mayo et al. 1997). In

addition, *Anthurium*, with about 600 epiphytic species (up to 60% of the total), ranks among the five major epiphytic plant clades, surpassed only by a few genera of Epidendroideae – Orchidaceae (Gentry & Dodson 1987). Furthermore, since the 19th century, it has been the source of exciting horticultural discoveries for both ornamental houseplants and cut flowers (e.g. *A. andraeanum* Linden, "flamingo flower" or "wax flower") (Bown 2000).

Anthurium is one of the most morphologically diverse genera in Araceae. It displays a remarkable variation in leaf morphology, including entire (linear, lanceolate, rounded, peltate, cordate, sagittate), variously lobed (trilobed, palmate), and compound (trisect, palmately-compound) leaves. Epiphytic *Anthurium* species have several growth habits: vining, creeping, appressed-climbing, rosulate or "bird's nest", and pendent (Bown 2000). Leaf venation patterns are remarkably variable within the genus, and have long been used in species identification. In terms of reproductive morphology, however, *Anthurium* species are very similar, all having bisexual flowers in uniform, mostly cylindrical, tapered spadices, although great diversity is displayed in inflorescence and fruit colors, which range from inconspicuous green and white, to highly attractive yellow, orange, red, lavender and purple.

Phylogenetic relationships within *Anthurium* are poorly understood. Taxonomists have long tried to partition its extraordinary morphological diversity into several "natural" groups (Schott 1860, Engler 1905, Croat & Sheffer 1983). The currently accepted sectional classification of *Anthurium* (Croat & Sheffer 1983) consists of 18 sections, characterized mainly by differences in habit, leaf shape, vernation, punctation and venation, root distribution and anatomy, cataphylls and number of seeds per fruit. A

few sections are easily diagnosed by unique characters (e.g. 4 seeds per fruit in section *Tetraspermium* Schott), while others need a more complicated combination of characteristics and quite frequently have overlapping limits. Aside from Engler (1905), no author has attempted to propose explicit relationships among sections, although all suggested that the order or placement of each section within their revisions corresponds to some sort of relationship among them.

Phylogenies of species-rich clades, like *Anthurium*, can help assess the roles of ecological diversification, dispersal-vicariance as distribution diversification mechanisms, and of key innovations in speciation rates in the Neotropics (Richardson et al. 2001, Kay et al. 2005, Erkens et al. 2007). However, despite its immense diversity, its importance in Neotropical forests, and a very long history of botanical collection, cultivation, and taxonomical research, *Anthurium* has been almost neglected in molecular phylogenetic studies. Molecular phylogenies of Araceae have focused on large-scale intrafamilial relationships and have included only up to 7 *Anthurium* species (French et al. 1995, Barabé et al. 2002, Rothwell et al. 2004, Tam et al. 2004, Cabrera et al. 2008, Cusimano et al. 2011). However, preliminary attempts at phylogenetic reconstructions within *Anthurium* have included a phylogeny with 30 species based solely on partial sequences of the chloroplast *trnG-trnS* intergenic spacer (Swart 2001) and a phylogeny with an increased sample of 75 species, mainly focused on Brazilian representatives (Temponi 2006), using the same three chloroplast markers as in here.

In the current study, phylogenetic analyses of combined chloroplast (cpDNA) and nuclear (nDNA) sequence data are used to test the monophyly of *Anthurium* and elucidate relationships among its species. This study comprises the most comprehensive

molecular phylogeny of the genus *Anthurium* to date, including ca. 80 more species than previous studies and a combination of three cpDNA (*trnG* intron, *trnH-psbA* and *trnC-ycf6* intergenic spacers) and one nuclear (*CHS* first intron) regions, greatly increasing the sampling of molecular, morphological and geographic diversity within the genus. Morphological variation is discussed in the context of the new phylogenetic framework outlined here and some taxonomic rearrangements are proposed.

MATERIALS AND METHODS

Taxon sampling. A total of 102 *Anthurium* species were sampled for this study, approximately 11% of the currently published species in the genus, spanning the morphological, taxonomic, and geographic diversity within the group (Table 1). Taxonomic sampling included at least one and up to 13 representatives of each of the 18 sections proposed by Croat and Sheffer (1983), except for the monotypic section *Gymnopodium* endemic to Cuba. Within each section, species were selected to maximize the coverage of morphological and geographical diversity. Five species belonging to the genus *Pothos* and the monotypic *Pothoidium*, both of the sister tribe Potheae, were used as outgroups. Each species was represented by a single collection, except *A. clidemioides* Standl. and *A. flexile* Schott, both represented by 3 accessions in order to confirm their position in the phylogeny. However, for all final analyses, each species was represented by only one accession. Leaf samples were obtained from plants growing in research greenhouses at the Missouri Botanical Garden and the Royal Botanic Gardens at Kew, and herbarium and field collections. Their identity was confirmed by comparison of

herbarium specimens and/or by specialists in the family (Dr. T. Croat, Missouri Botanical Garden, and Dr. S. Mayo, Royal Botanic Gardens at Kew).

DNA sequence data. Total genomic DNA was extracted from fresh or silica dried leaf material using a modified CTAB protocol (Doyle & Doyle 1987). For herbarium material, an extra purification step with QIAquick PCR Purification Kit (Qiagen Inc., California, USA) was added before DNA precipitation. PCR amplification reactions contained 2-3µL of DNA template, 5µL of 5X reaction buffer, 5µL of 2.5 mM $MgCl_2$, 3µL of 2.5 mM dNTPs mix, 2µL of 10µM stock for each primer, 0.8µL of Taq polymerase (5 units/µL) (Promega, Wisconsin, USA) and distilled Millipore water to a final volume of 50µL. Thermocycling conditions included a 2 min denaturation step at 94ºC, followed by 40 cycles of 1 min at 94ºC, 1 min at 60ºC, 2 min at 72ºC, and a final 7 min extension at 72ºC. PCR products were recovered and purified from a 2% agarose gel using QIAquick Gel Extraction Kit (Qiagen Inc., California, USA) following the manufacturer's protocols. Sequencing reactions used the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, California, USA) and were analyzed using an ABI Prism 377 automated DNA sequencer at the University of Missouri - St. Louis, or sequencing services of Penn State University (USA) or Macrogen (Korea). Both forward and reverse strands of DNA were sequenced for each taxon. After preliminary testing of chloroplast sequence variation in a subset of 10 species with 21 primer combinations for non-coding chloroplast DNA regions (Shaw et al. 2005), three target regions were chosen for complete analysis based on higher variability among species, number of informative characters, ease of PCR amplification, and ability to carry out straightforward sequencing

without the use of internal primers. These chloroplast regions are the *trnG* intron (Shaw et al. 2005), and the *trnH-psbA* (Hamilton 1999) and *trnC-ycf6* (Shaw et al. 2005) intergenic spacers. Nuclear DNA primer sets included the first intron of chalcone synthase (*CHS*), amplified using *Anthurium*-specific primers designed here (CHSf: 5'-AGG AGA AGT TCA GGC GCA TG-3' and CHSr: 5'-A(CG)G TGG GTG ATC TT(CG) GA(CT)-3'). A set of nuclear ITS primers (Baldwin 1992) was also tried, but ITS sequences showed a high occurrence of pseudogenes based on the lack of conserved angiosperm 5.8S motifs (Harpke & Peterson 2008) and, sequence alignment was ambiguous, and required insertion of many gaps (Carlsen, unpublished data) and so this region was omitted from further analyses. In order to determine the presence of multiple copies of the *CHS* gene, PCR fragments were cloned using the p-GEM-T Easy Vector System (Promega, Wisconsin, USA) following the manufacturer's protocol. For each species a maximum of 3 clones were sequenced. If all clones per taxon clustered together as a monophyletic clade in a preliminary maximum parsimony analysis and the genetic Kimura 2-parameter distance (Kimura 1980) between clones was lower than 1%, then such sequences were considered to be alleles. If genetic variation was higher than 1% and clones did not belong to the same monophyletic clade, then they were considered multiple copies of the gene. Using these criteria, no taxa in the study had multiple copies of *CHS*, so for all final analyses a single randomly-chosen sequence was used to represent each taxon. In order to insure sequence quality, all raw sequences were compared to the GenBank database using BLAST, confidence scores for individual bases were assigned using the program PHRED (Ewing et al., 1998), and sequences were manually edited with 4Peaks version 1.7.2 (Griekspoor & Groothuis,

www.mekentosj.com) to ensure that only sequences with overall PHRED scores ≥20 were kept for further analysis. Contigs between two sequence strands (forward and reverse) and with sequence overlap of more than 75% were created for each taxon using Lasergene-Seqman Pro version 8.0.2 (DNASTAR Inc., Madison, USA). Species sequences were first aligned using Clustal-X version 1.81 (Thompson et al. 1997) and the alignment was later manually edited in MacClade v. 4.08 OS X (Maddison & Maddison 2000). Coding regions of CHS gene sequences were translated to amino acids in order to improve alignment and to check for stop codons. Although protein sequences were used as an aid to alignment, all analyses were done using the nucleotide matrix. *Anthurium* sampling overlap among data partitions is very high for chloroplast (99-100%) and *CHS* (97%) datasets. *Pothos* and *Pothoidium* species did not amplify with the primers designed for *CHS* and thus could not be included in the nuclear partition. Sequences are deposited in GenBank (Table 1) and the aligned data matrix, including chloroplast and nuclear data partitions, is available in TreeBASE (website http://www.treebase.org).

Phylogenetic analyses. Maximum parsimony (MP), maximum likelihood (ML) and Bayesian (MrB) approaches were used to examine phylogenetic relationships among species. Indels, mono- and bi-nucleotide repeats and regions with alignment ambiguity were excluded from all analyses. All included characters were treated as unordered and equally weighted. Analyses were performed using the Beowulf cluster at the University of Missouri – St. Louis and the CIPRES web portal (Miller et al. 2009). Phylogenetic congruence among different datasets was assessed using the partition homogeneity test (Farris et al. 1994) as implemented in PAUP* version 4.0b10 (Swofford 2002).

Incongruence was also further evaluated by visually inspecting tree topologies from the independent analyses. In addition, conflicting datasets were evaluated with a splits-graph (SplitsTree4 software, Huson & Bryant 2006) specifically to detect patterns of incongruence and exclude highly homoplasious incongruent characters from final analyses. In order to explore a larger distribution of possible topologies, Maximum Parsimony (MP) analyses were performed using the parsimony ratchet (Nixon 1999) with the program PAUPRat (Sikes & Lewis 2001) implemented in PAUP* (Swofford 2002). PAUPRat analyses consisted of 20 replicates of 200 iterations with 25% of the characters reweighted for each iteration. Resulting trees were retrieved, filtered to obtain only the most parsimonious PAUPRat trees with the best MP tree scores, and majority rule and strict consensus trees were computed in PAUP* (Swofford, 2002). Support for individual branches or clades was estimated using bootstrap values (Felsenstein 1985). One thousand MP bootstrap replicates were performed, each comprising one random sequence addition, tree-bisection-reconnection (TBR) swapping, and MULTREES=yes. Models of sequence evolution were selected a priori using the Akaike information criterion (AIC) for independent partitions and the entire combined dataset in Modeltest v.3.7 (Posada & Crandall 1998). Maximum Likelihood (ML) analyses (Felsenstein 1973) and ML bootstrapping were performed using RAxML v.7.2.7 (Stamatakis 2006, Stamatakis et al. 2008). In this program, the GTRGAMMA model was used for complete likelihood evaluation and the GTRCAT approximation of the model for 1000 bootstrap replicates. Bayesian analyses (MrB) were carried out using MrBayes v.3.1.2 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003) using selected sequence evolution models. All MrB analyses were conducted with two simultaneous runs comprising six

Markov chains (temperature = 0.1) starting from randomly chosen trees and running for 14,000,000 generations with sampling every 2,000 generations. A burn-in of 4,500 trees to be discarded was estimated by examination of log likelihood scores of sampled trees, final average standard deviation of split frequencies for simultaneous searches approaching zero, and potential scale reduction factor for estimated parameters approaching 1 as runs converge. Posterior probabilities were calculated from 2,501 post-burn trees per run. For both ML and MrB, exploratory analyses of a subset of species in the combined datasets with and without independent models of evolution for each partition yielded very similar results in terms of topology and support values. The only visible difference was the appearance of slightly longer branch lengths when using independent models for each partition (data not shown). Therefore, given the time constraints for runs in computer clusters, all combined analyses were performed using a single model of evolution for the entire combined dataset. In interpreting phylogenetic confidence, nodes are considered "strongly supported" when they received both bootstrap value equal to or higher than 70% and a posterior probability (PP) 0.90 or higher, and as "moderately supported" when only the PP is at least 0.90.

RESULTS

cpDNA sequence variation and phylogenetic analyses

The three cpDNA regions were sequenced for all 108 taxa (102 *Anthurium* species and 6 outgroups), except for *A. sagittatum* (Sims) G. Don, missing from the *trnC-ycf6* dataset. In the *trnH-psbA* dataset, five *Anthurium* species were represented by partial sequences missing 28-40 aligned base pair positions (bp). In the *trnC-ycf6* dataset,

Pothoidium lobbianum Schott is represented by a partial sequence missing 86 aligned bp, but the missing portion is part of an indel that was not included in analyses.

Unaligned sequences (not including partial sequences) of the portion of the *trnG* intron included in the analyses varied in length from 662 to 690 bp. Those of the sequenced portion of the *trnH-psbA* intergenic spacer ranged from 240 to 472 bp within *Anthurium* and 391-489 bp in outgroups. In general, *trnH-psbA* sequences of the outgroups were longer than for *Anthurium* (average size 435 bp vs. 287 bp, respectively) due to a 143 bp indel that was excluded from all analyses. Also excluded from the *trnH-psbA* dataset were a long 147 bp indel unique to *A. parvispathum* Hemsl. and a large AT-rich region common to all *Anthurium* species although variable in size (203 aligned bp maximum). Unaligned sequences of the portion of *trnC-ycf6* intergenic spacer ranged between 515-609 bp in *Anthurium* and 960-989 bp in outgroups, mainly due to a 398 bp indel unique to *Pothos* and *Pothoidium* (Table 2). Aligned length, number of variable characters, number of parsimony informative characters and uncorrected pairwise sequence divergence are summarized in Table 2. Characteristics and results from maximum parsimony, maximum likelihood and Bayesian analyses are compared in Table 3. In general, parsimony, likelihood and Bayesian analyses of the individual cpDNA datasets produced topologies largely lacking in resolution but congruent with one another in the few shallow nodes supported by either PB/LB > 70% or PP > 0.90.

nDNA sequence variation and phylogenetic analyses

The complete first intron of the chalcone synthase gene (*CHS*) was sequenced for 100 *Anthurium* species, sequences being missing only from *A. altotambense* Croat, *A.* cf.

palenquense Croat and *A. napaeum*. Due to primer sequence specificity and PCR amplification problems no outgroups could be sequenced for this region.

Unaligned sequences of the *CHS* intron included in the analyses varied in length from (449-) 583 to 941 bp. *Anthurium muyunense* Croat (unpubl.) is missing about 280 bp of this region. The *CHS* intron is also characterized by long indels, such as in *A. wendlingeri* G. M. Barroso (145 aligned bp), while a 258 bp indel is present only in *A. clidemioides* and *A. flexile*. Aligned length, number of variable characters, number of parsimony informative characters and uncorrected pairwise sequence divergence are summarized in Table 2.

Characteristics of the maximum parsimony, maximum likelihood and Bayesian analyses are compared in Table 3. Parsimony analysis of the nDNA region showed higher resolution of medium and shallow nodes than the independent chloroplast datasets; however, the backbone of the tree still lacks resolution. Likelihood and Bayesian analyses of nDNA increased considerably the resolution of nodes within *Anthurium*, none of which are in conflict with the independent cpDNA trees.

Congruence among DNA partitions/datasets

Results of the partition homogeneity tests performed on the original cpDNA datasets showed a highly significant (P = 0.01) phylogenetic conflict among *trnH-psbA* and all other chloroplast regions. Consensus trees from parsimony analyses of independent datasets showed mostly poorly supported clades and visual examination of these trees yielded no sign of obvious conflict (data not shown). However, a split-graph of the *trnH-psbA* dataset detected a phylogenetically incongruent signal creating a highly

supported split of species not found in any other dataset (Figure 1). The characters supporting such split are in an 8 bp region toward the 5' end of the sequence and another of 9 bp toward the 3' end. These areas within *trnH-psbA* showed sequence inversions flanked on both sides by palindromic sequences of 7 and 14 bp, respectively (Figure 1). Several authors (Sang et al. 1997, Mes et al. 2000, Bain & Jansen 2006, Whitlock et al. 2010) have previously characterized these kinds of small inversions for *trnH-psbA* and they have also been noted as occurring in other chloroplast non-coding regions (Kelchner & Wendel 1996, Ki-Joong & Hae-Lim 2005). These inversion events appear to be frequent and random, with a high level of homoplasy and therefore most authors recommend exclusion of these regions from phylogenetic analyses. When these two small inverted regions (17 bp total) were excluded from the *trnH-psbA* dataset, the conflicting phylogenetic signal in the ILD tests disappeared (*trnG/trnH-psbA*, P = 0.51 and *trnC-ycf6/ trnH-psbA*, P = 0.63).

Significant phylogenetic conflict was also found among all independent cpDNA regions and the nDNA (*CHS*) dataset (ILD tests P = 0.01). Examination of consensus trees from parsimony analyses of independent datasets revealed no well-supported topological conflict, and the same was true for split-graph analysis of *CHS* dataset (data not shown). Closer examination of the *CHS* alignment identified a few nucleotides in the 3rd codon position towards the 3' end of the coding region of *CHS* that varied randomly among species while preserving the amino acid sequence. Because analyses were performed on nucleotides and not amino acids, this homoplasious random noise was included in the dataset and was apparently conflicting and/or obscuring the real phylogenetic signal of the *CHS* dataset. After removal of these five nucleotides, the ILD

test showed that *CHS* and all cpDNA regions were not significantly incongruent (*trnG/CHS*, P = 0.79; *trnC-ycf6/CHS*, P = 0.61; *trnH-psbA/CHS*, P = 0.63).

Combined phylogenetic analyses

The combined cpDNA data matrix contained 108 taxa (6 outgroups and 102 *Anthurium* species) and 2,706 aligned base pair positions. Included characters consist of 1,500 aligned base pair positions, with a total of 275 variable sites and 182 parsimony informative sites of which 127 are exclusively within *Anthurium* (Table 2). Characteristics and results from maximum parsimony, maximum likelihood and Bayesian analyses are compared in Table 3. In general, the parsimony strict consensus tree showed lack of resolution in the deepest nodes within *Anthurium* and only 20 nodes within the ingroup with PB > 70%. Maximum likelihood analysis recovered 38 highly supported clades (LB > 70%) within the ingroup (trees not shown but see Figure 2). The consensus Bayesian tree had 44 highly supported nodes within *Anthurium* (PP > 0.90) and some resolution was achieved along the backbone of the tree (Figure 2). Likelihood and Bayesian trees are characterized by relatively long branches in the outgroup and the first diverging lineages within *Anthurium*, but branch lengths are noticeably shorter inside the core of *Anthurium* with the exception of the branch leading to Clade 10.

The combined cpDNA and nDNA dataset included a total of 102 *Anthurium* species. Because this dataset does not include Potheae outgroups, it was rooted instead with the first diverging lineage in *Anthurium* (*A. clidemioides* and *A. flexile*) as had been recovered in the analysis of combined cpDNA. The combined cpDNA-nDNA data matrix consisted of 3,958 aligned base pair positions, of which 2,166 were included, containing

a total of 614 variable sites and 354 parsimony informative sites within *Anthurium* (Table 2). Characteristics and results from maximum parsimony, maximum likelihood and Bayesian analyses are compared in Table 3. As before, the parsimony strict consensus tree showed poor resolution in the backbone but 38 highly supported nodes (PB > 70%). Maximum likelihood analysis found 43 highly supported clades (LB > 70%) (trees not shown but see Figure 3). There were 65 highly supported nodes in the Bayesian consensus tree (PP > 0.90) (Figure 3). In general, the combined cpDNA-nDNA analyses produced considerably greater resolution and higher support for clades within *Anthurium* than did individual analyses.

The trees obtained in all analyses of combined cpDNA and cpDNA-nDNA datasets are largely congruent with trees recovered from analyses of individual datasets, the differences being in the degree of resolution within the ingroup. Among different phylogenetic methods, the Bayesian consensus tree showed greater resolution than the parsimony strict consensus tree, but the estimated measures of support (PB vs. LB vs. PP) were similar and there was no conflict between highly supported nodes in model-based and parsimony-based analyses.

DISCUSSION

Phylogenetic relationships (Figures 2 and 3)

The phylogenetic analyses presented here confirm the monophyly of *Anthurium* and reveal several species groups within the genus, as well as possible relationships among them. This study demonstrates the utility of the first intron of the low copy nuclear gene chalcone synthase (*CHS*) in resolving relationships among closely related

species in Araceae. Chloroplast markers, although widely used in plant molecular systematics, proved to be much less informative in *Anthurium,* even though the three regions chosen for analyses were the most variable of a total of 21 regions tested (Shaw et al. 2005). Gauthier et al. (2008) found the same pattern in their molecular study of *Philodendron* Schott, the second most species-rich genus in Araceae, and they thus decided to rely only on nuclear genes (ITS and ETS) for their final analyses.

In this study, the combination of chloroplast and nuclear gene sequences with highly congruent phylogenetic signals - after the exclusion of 22 homoplasious base pairs (see above) - increased both confidence in the results and resolution in the phylogeny. Therefore, the following explanation of major clades recovered by the phylogenetic analyses will focus on the results of combined datasets, and uses the phylogeny generated by Bayesian analyses as a framework for subsequent discussion. Any disagreements between these general results with those from analysis of single gene regions or other analytic methods (maximum parsimony and likelihood) will be mentioned when they occur. All measures of support for individual clades cited herein use the following abbreviations: Maximum Parsimony Bootstrap – PB / Maximum Likelihood Bootstrap - LB / Bayesian posterior probability - PP. Major clades are all characterized by being strongly supported (70% or higher PB/LB and 0.90 or higher PP) or moderately supported (only PP higher than 0.90). Clades with less support or species pairs with strong molecular support are generally not discussed. Most of the major clades can also be characterized by morphological or geographical synapomorphies and diagnostic characters, but not always completely so.

The results of the combined cpDNA analyses (Figure 2) revealed that within the outgroup, the monotypic genus *Pothoidium* is strongly supported (100 PB/98 LB/1.00 PP) as embedded within a larger genus *Pothos*. Previous Araceae family phylogenies (Cabrera et al. 2008, Cusimano et al. 2011) have included only one species of each of these genera, and therefore this pattern had not been recovered before. In their taxonomic revision of the tribe Potheae (sensu Mayo et al. 1997), Boyce and Hay (2001) suggested that this group was an assemblage of three very similar, possibly inseparable genera, *Pothoidium*, *Pedicellarum* and *Pothos*. They further noted that "it is tempting to regard *Pothoidium* as a derived offshoot of subgenus *Pothos* in which functional dioecy has arisen" (p. 456). The present study indeed suggests that at least *Pothoidium* should be considered a synonym of *Pothos*.

Monophyly of the genus *Anthurium* is strongly supported in all analyses (100 PB/100 LB/1.00 PP) (Figure 2). This result agrees with previous molecular studies that included a limited sampling of *Anthurium* species diversity (Barabé et al. 2002, Rothwell et al. 2004, Tam et al. 2004, Temponi 2006). The genus *Anthurium* is endemic to the New World tropics and it is easily recognized among Araceae by a combination of morphological characters such as sympodial growth, spirally-arranged leaves, reticulate secondary and tertiary venation, bisexual flowers with four decussate tepals, uniform spadix, persistent open spathe and copious endosperm, although none of these characters is unique to *Anthurium* and several are plesiomorphies within the family (Mayo et al. 1997). This study also reveals 6 indel substitutions in the cpDNA regions sequenced (but not included in the analyses) that support *Anthurium* as a monophyletic clade, 3 bp indel in *trnG*, 6, 7, 10 and 398 bp indels in *trnC-ycf6* and 4 bp indel in *trnH-psbA*.

Within *Anthurium*, 20 monophyletic and highly supported major clades are discussed here (Figures 2 and 3, Clades A-B and 1-18). A few of these clades are congruent with the current sectional classification of the genus (Croat & Sheffer 1983) or previous molecular studies (Temponi 2006), although 14 of them are recognized and circumscribed for the first time in this study. These major clades comprise ca. 90% of the species sampled, leaving only 10% uncharacterized.

There is strong support (99 PB/100 LB/1.00 PP) for an early-diverging lineage within *Anthurium* that includes *A. flexile* and *A. clidemioides* (Figures 2 and 3, Clade A), and this clade corresponds to section *Polyphyllium* Engler. This species pair has long been recognized as having a unique morphology within *Anthurium*, characterized by anisophyllous growth pattern, lack of 1-ribbed cataphylls, presence of internodal adventitious roots, inaperturate pollen and, shiny black-dark brown seeds (Engler 1905, Croat & Baker 1978, Grayum 1990). In fact, Grayum (1990) suggested that section *Polyphyllium* may merit separate generic status based on its atypical morphological features. From the standpoint of molecular data alone, this study shows that the cpDNA average pairwise distance divergence among *A. clidemioides* - *A. flexile* and the rest of *Anthurium* (1.1-2.9%) is lower than the average distance among the genera *Pothos* and *Anthurium* (4-6.8%); this unusual species pair is better kept within *Anthurium*.

Clade B (Figures 2 and 3) includes all other *Anthurium* species and it is strongly supported as monophyletic (100 PB/100 LB/1.00 PP). Although previously not recognized as a clade within *Anthurium*, all these species share a morphological synapomorphy unique within Araceae, the presence of forate pollen (i.e. the pollen has apertures), mostly 3-4 pores, more rarely two (Grayum 1990, Grayum 1992).

The combined cpDNA and nDNA Bayesian phylogeny (Figure 3) shows three main lineages within the core of *Anthurium*, Clade C (0.91 PP), Clade D (0.70 PP) and Clade E (0.78 PP), only the first one being moderately supported. They form part of a basal polytomy within Clade B and thus relationships among these clades, as with most of the other deep nodes within *Anthurium*, are not well resolved. So far, no diagnostic morphological characters have been found to characterize any of these possible clades within *Anthurium*.

In two clades, a strongly supported Clade 1 (92 PB/96 LB/1.00 PP) and a moderately-supported Clade 2 (0.99 PP), that are apparently sister to each other, species are clustered based on geographic affinity, but exclusive unifying morphological features are not apparent. Clade 1 contains, with no exception, all species of *Anthurium* endemic to Brazil. The clade was also recovered in the analyses of Temponi (2006), who suggested that the presence of trichomes on the funicle could be a synapomorphy for this clade, or perhaps for a less inclusive group within it. Clade 2 includes all endemic *Anthurium* species from the Lesser Antilles and Jamaica, as well as the type species for the genus, *A. acaule* (Jacq.) Schott. This clade has not been previously recognized and it also lacks an evident morphological synapomorphy.

The strongly supported Clade 3 (97 PB/98 LB/1.00 PP, Figure 3) contains all northern Central American *Anthurium* species (from southern Mexico to Honduras) that possess dark punctations on the lower leaf blade surface and bright red berries. Clade 3 has not been formally recognized before as a section within *Anthurium*, but T.B. Croat (pers. comm.) has long considered this group as “natural”.

Almost all palmately-lobed *Anthurium* species clustered together in a moderately-supported Clade 4 (-/74 LB/0.78 PP, Figure 3). This clade is recovered in the combined cpDNA-nDNA dataset model-based (likelihood and Bayesian) analyses, but not with maximum parsimony. Indeed, Clade 4 is the only slightly conflicting group in the *Anthurium* phylogeny. Combined cpDNA analyses grouped the palmate species pair *A. digitatum* (Jacq.) Schott - *A. longissimum* Pittier, both Caribbean-northern Venezuelan species, with a subset of Caribbean species from Clade 2 (-/-/0.99 PP) (Figure 2). Another pair of palmate species, *A. eminens* Schott – *A. thrinax* Madison, from Amazonia, is weakly grouped with the Brazilian Clade 1 (-/63 LB/-) (Figure 2). On the other hand, the whole Clade 4 appears in all nDNA analyses (61 PB/73 LB/1.00 PP). Given that the clade in the combined cpDNA dataset are not highly supported, and all independent cpDNA datasets place these palmate species in a polytomy, except for the *trnC-ycf6*, it is safe to accept Clade 4 as comprising a cluster of most of the species of *Anthurium* with palmate leaves. *Anthurium* species with palmately-lobed leaves have for a long time been placed in two separate sections, section *Schizoplacium* Schott and section *Dactylophyllium* Engler (Schott 1860, Engler 1905, Croat & Sheffer 1983), but this molecular phylogeny suggests that such division is unnecessary, indeed the newly circumscribed Clade 4 may merit sectional rank instead. Although all members of Clade 4 share palmately-lobed leaves, this leaf form has evolved independently at least two more times, in clades 15 and 18.

Clade 5 (Figure 3), although strongly supported in most analyses (86 PB/-/1.00PP), lacks any apparent morphological synapomorphies, but includes at least three well characterized and highly supported lineages, whose inter-relationships are not well

resolved. Clade 6 (86 PB/98 LB/1.00 PP) (Figure 3) includes the remaining *Anthurium* species with dark punctations on the leaf blade (i.e. all those occurring outside northern Central America). Previous authors (Croat & Sheffer 1983) have placed punctate *Anthurium* species in three separate sections, *Porphyrochitonium* Schott, *Digitinervium* Sodiro and *Tetraspermium*, although recognizing their close affinities. This study provides further evidence for placing most *Anthurium* species with dark punctations on the leaves in a single monophyletic larger clade. However, species of *Anthurium* with cordate leaf blades and punctations are placed in a separate clade (Clade 3, Figure 3). Within Clade 6, Clade 7 was recovered as monophyletic and strongly supported (99 PB/100 LB/1.00 PP), including all *Anthurium* species with 4 seeds per fruit (2 per locule) - section *Tetraspermium*. The two other lineages within Clade 6, Clade 8 (99 PB/100 LB/1.00 PP) and Clade 9 (78 PB/92 LB/1.00 PP), are a mixture of species from sections *Porphyrochitonium* and *Digitinervium*; these clades have no obvious morphological synapomorphies. In contrast with the Bayesian analyses (Figure 3), maximum likelihood results showed that Clades 8 and 9 are more closely related to each other than to Clade 7 (data not shown).

The other two lineages within Clade 5 may be closely related to each other although the relationship is not highly supported (0.63 PP) (Figure 3). *Anthurium gracile* and its relatives are included in the strongly supported Clade 10 (100 PB/100 LB/1.00 PP) characterized by long slender ("strappy") leaves and very thin and long inflorescences with no more than three flowers visible at once in the principal spiral of the spadix. Previous classifications (Schott 1860, Engler 1905, Croat & Sheffer 1983) recognized *A. gracile* as a very distinct species within *Anthurium*, placing it in its own

section *Leptanthurium* Schott, not closely related to any other. Recently, *A. barrieri* Croat, Scherberich & Ferry was added to section *Leptanthurium* (Croat et al. 2006). Results here suggest that *A. gracile* has other very close relatives within the genus that share distinct morphological similarities with it, and therefore this section should be expanded to accommodate all these species.

The moderately-supported Clade 11 (0.99 PP), previously recognized as section *Pachyneurium* series *Pachyneurium* Schott (Croat 1991), includes many species of *Anthurium* with the true "bird's nest" habit, and it is also characterized by involute leaf vernation. The core of the group is always strongly supported in all analyses (91 PB/95 LB/1.00 PP), and includes species growing in continental Central and South America, from Mexico to Argentina. Sister to that core, with only moderate support, is the clade made up of *A. crenatum* (L.) Kunth - the type species for the series - and *A. venosum* Griseb., both with the "bird's nest" habit but endemic to major islands in the Caribbean.

The last large clade within *Anthurium*, Clade E (0.78 PP), is not highly supported but encompasses mostly species with cordate leaves. It also comprises the bulk of the species within the genus, and unfortunately, but perhaps due to the diversity it includes, relationships among most internal clades are not highly supported. However, a few internal clades have strong support in all analyses. The first diverging clade is Clade 12 (98 PB/100 LB/1.00 PP) (Figure 3) and comprises *Anthurium* species with numerous primary lateral veins that are sunken above and prominent below, and that have purple fruits. This clade is recognized here for the first time and may include quite a few more species than those sampled here.

The rest of the species included in Clade E are well supported as a group (1.00 PP) sister to Clade 12. A subset of the previously recognized group of *Anthurium* species with "bird's nest" habit, section *Pachyneurium* series *Multinervia* (Croat 1991) (type species *A. napaeum*), always clustered together and with strong support in Clade 13 (79 PB/98 LB/1.00 PP) (Figure 3). Members of this clade also share involute leaf vernation with the other "bird's nest" *Anthurium* species in Clade 11, but are further distinguished from them by their leaves that dry greenish and with numerous closely spaced primary lateral veins. In contrast, species in Clade 11 generally posses leaves drying dark-brown and fewer primary lateral veins usually spaced more than 3 cm apart (Croat 1991). A unique novel group, strongly supported in all analyses, is Clade 14 (71 PB/84 LB/1.00 PP). Currently, it comprises only the species pair *A. willifordii* Croat and *A. besseae* Croat, both Peruvian-Bolivian species with velvety leaves. *Anthurium willifordii* was previously placed in section *Pachyneurium* due to its involute leaf vernation. Leaf vernation has not been observed in *A. besseae*, but involute vernation may be a synapomorphy for a larger clade uniting both Clade 13 and 14.

The next strongly supported major lineage, Clade 15 (97 PB/100 LB/1.00 PP), includes all *Anthurium* species from northern Central American (Mexico to Honduras) that lack dark punctations on the leaf blade and that possess bright orange berries. Clade 15 has not been previously recognized as a species group within *Anthurium*. Leaf morphology in this clade is quite variable, but reproductive morphology is very uniform. Thus, *Anthurium pedatoradiatum* Schott, a Mexican species with palmately-lobed leaves, is included in Clade 15 even though it shares the leaf form of the palmately-lobed Clade

4. Nonetheless, *A. pedatoradiatum* clearly belongs to Clade 15 based on its bright orange berries.

Another strongly supported lineage in all analyses is Clade 16 (80 PB/91 LB/1.00 PP) (Figure 3) that includes all *Anthurium* species with intact persistent cataphylls. Even independent cpDNA analyses, which were mostly poorly resolved, always recovered a highly supported monophyletic Clade 16. Previous classifications (Schott 1860, Engler 1905, Croat & Sheffer 1983) recognized this clade as section *Calomystrium* Schott emend. Engler, and all agreed in considering this section the most "natural" within *Anthurium*.

The final clades recognized in this study are not so highly supported. Clades 17 (0.92 PP) and 18 (1.00 PP) (Figure 3) may indeed comprise closely related groups of species, but this needs confirmation. Clade 17 includes species characterized by having oblong leaves, a unique morphology within the otherwise largely cordate-leaved Clade E, with numerous primary lateral veins. This set of characters does occur elsewhere within Clade E, in Clade 12, but these two lineages are consistently well separated from each other in all analyses. Clade 18 is not easily characterized morphologically. *Anthurium pedatum* (Kunth) Schott, a species with a highly divided leaf blade, previously recognized as part of the palmately-lobed *Anthurium* group (Schott 1860, Engler 1905, Croat & Sheffer 1983), consistently clustered in Clade 18. Madison (1978) pointed out the possible segregation of *A. pedatum* from all other palmately-lobed *Anthurium* species, and its affinity with the *A. gualeanum* Engler complex. Although the latter species was not sampled here, the results suggest that in fact *A. pedatum* does not belong to the core clade of palmately-lobed *Anthurium* species (Clade 4), but relationships with *A.*

gualeanum are still unclear. The other species in Clade E cannot be assigned to strongly supported clades; this group of *Anthurium* species especially needs further study to provide molecular and morphological characterization of groups within it.

It is interesting to note that the inclusion of *Anthurium* species endemic to the Caribbean islands tends to weaken support for otherwise strongly supported clades, such as the case of the palmately-lobed species (Clade 4) and the "bird's nest" species (Clade 11). More extensive sampling may suggest possible hybridization scenarios that could confuse phylogenetic reconstructions of the evolutionary history of the genus *Anthurium*.

CONCLUSIONS AND FURTHER RESEARCH

Several major conclusions can be drawn from the combined phylogenetic analyses of cpDNA and nDNA datasets. First, among the outgroups, the monotypic genus *Pothoidium* is embedded within the larger genus *Pothos*, and thus it should be recognized as a synonym of the latter. Second, *Anthurium* is strongly supported as a monophyletic genus. Third, the first basal branch within *Anthurium* includes *A. clidemioides* and *A. flexile*, a unique species pair, previously recognized as section *Polyphyllium*. Fourth, there are at least 20 highly supported monophyletic groups within *Anthurium*, most of which can be characterized morphologically and/or geographically. Further study of morphological characteristics in other species not sampled here would help to assign all currently known *Anthurium* species to these major clades.

Taxon sampling and phylogenetic support in this study were sufficient to provide a greatly improved understanding of major clades within *Anthurium* and possible relationships among them. However, despite having a better idea about the evolutionary

history of the genus *Anthurium*, resolution is still lacking in some areas of the phylogeny, especially in the deeper nodes. In future studies, increased genomic sampling with emphasis on low copy nuclear genes will be important for further improving resolution among clades in the phylogeny and for clarifying the composition of the main clades. It will also be interesting to see if the morphological and geographical features that have been identified here as characterizing clades continue to hold even if the species composition of the clades is expanded.

Sequence divergence among *Anthurium* species is generally very low, and relatively short branches characterize the core of *Anthurium* compared to the outgroups. This pattern is expected in cases of rapid diversification, where a clade can be characterized by great morphological diversity but very little molecular variation among species (e.g. *Inga* - Richardson et al. 2001, *Costus* subgenus *Costus* - Kay et al. 2005, Valerianaceae – Bell & Donoghue 2005, *Lupinus* – Hughes & Eastwood 2006, *Guatteria* – Erkens et al. 2007). Grayum (1990) previously suggested that a rapid radiation took place early on the evolutionary history of *Anthurium*, and now molecular data supports this hypothesis. Future dating of the phylogeny will help corroborate this finding and provide a relative date to the major diversification events within *Anthurium*.

The results demonstrate that some morphological characters used in previous sectional classifications of *Anthurium* are homoplasious and therefore such classifications are in need of revision. The major clades recovered by these molecular phylogenetic analyses will serve as the basis for a revised sectional classification of the genus, and more research is needed to further test morphological and anatomical characters not previously studied in *Anthurium*.

Major clades within *Anthurium* share geographical affinities even in the absence of evident morphological diagnostic traits. The lack of relationship between geography and morphology occurs in other plant groups as well (*Cardamine*, Brassicaceae - Carlsen et al. 2009; *Aechmea*, Bromeliaceae - Sass & Specht 2010). These authors suggested that dispersal to new areas was followed by diversification of morphological features and therefore certain morphological characteristics previously thought to be indicative of common ancestry have instead evolved multiple times in parallel. Such a pattern overall supports the hypothesis of ecological conservatism (Peterson et al. 1999, Hadly et al. 2009).

This is the first molecular phylogenetic study that offers an in-depth insight into the evolutionary history of the largest rapid radiation of species within the family Araceae, the extremely diverse genus *Anthurium*. It also provides avenues for further research still needed to better understand the outstanding morphological diversity and complex geographical patterns within the genus.

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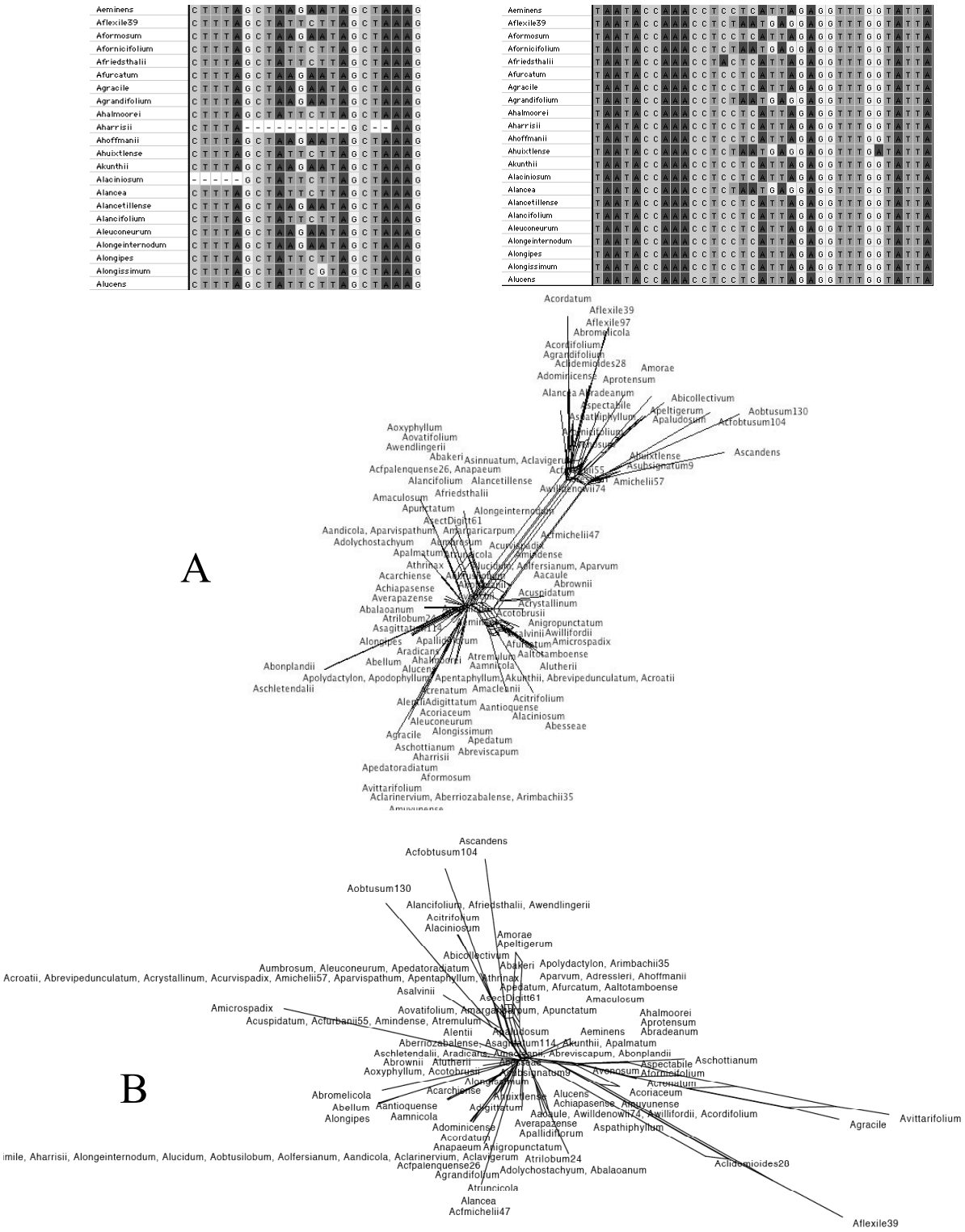


Figure 1. Split-graph of *trnH-psbA* sequences of *Anthurium* showing a highly supported split (A) and the same splitgraph after removal of inversions (B), see text for details. The insets show the regions in the alignment (inversions) responsible for such a split.

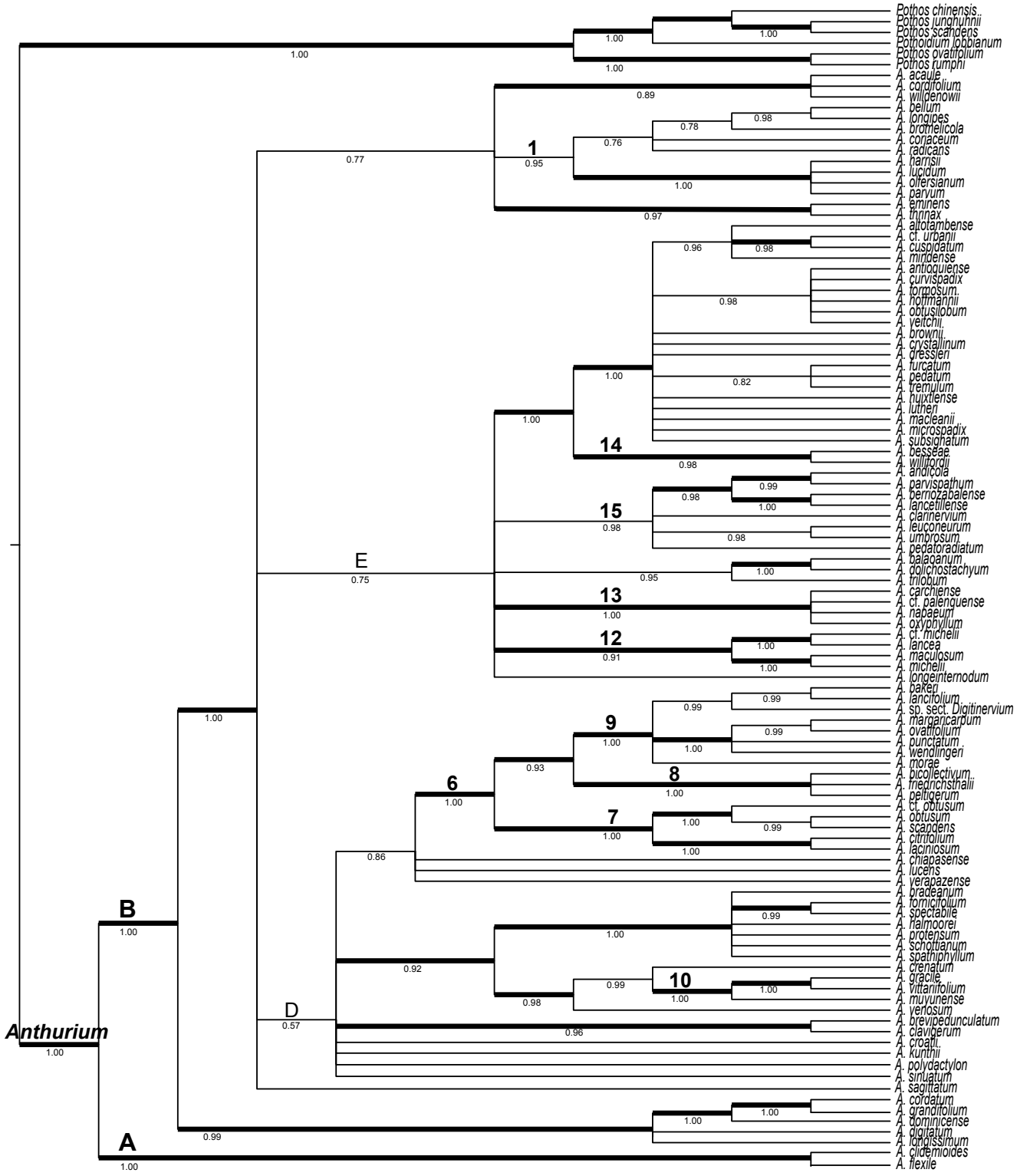


Figure 2. Bayesian consensus tree of combined cpDNA datasets. Thick branches identify parsimony bootstrap (PB) and/or likelihood boostrap (LB) higher than 70%. Posterior probabilities (PP) are shown below branches. Letters and numbers identify major highly supported monophyletic clades (see details in text).

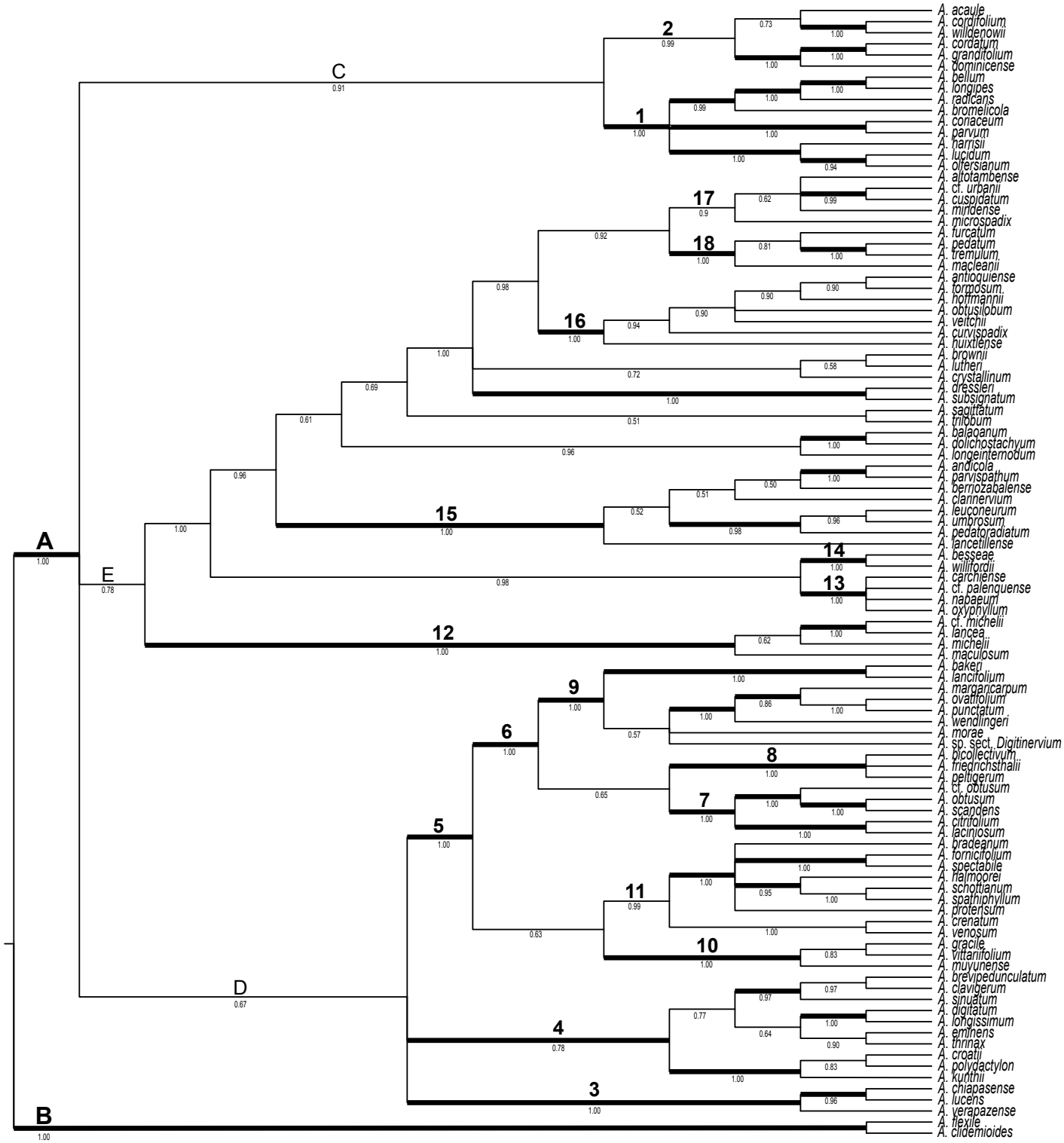


Figure 3. Bayesian consensus tree of combined cpDNA and nDNA datasets. Thick branches identify parsimony bootstrap (PB) and/or likelihood boostrap (LB) higher than 70%. Posterior probabilities (PP) are shown below branches. Letters and numbers identify major highly supported monophyletic clades (see details in text).

Table 1. *Anthurium* species and specimens used in molecular phylogenetic analyses.

Species name	Section	Country of origin	Collection info	Collection origin
Anthurium altotambense	POLYN	Ecuador	Croat 99733	Field – silica
Anthurium andicola	BEL	Guatemala	Croat 90221a	Greenhouse MO
Anthurium antioquiense	CAL	Colombia	Croat 81407	Greenhouse MO
Anthurium bakeri	POR	Costa Rica	Croat 78747	Greenhouse MO
Anthurium balaoanum	CAR	Ecuador	Croat 50706a	Greenhouse MO
Anthurium bellum	URO	Brazil	Croat 82895	Greenhouse MO
Anthurium berriozabalense	BEL	Mexico	Croat 90115	Greenhouse MO
Anthurium besseae	CAR	Bolivia	Croat 71836	Greenhouse MO
Anthurium bicollectivum	POR	Panama	Croat 73978	Greenhouse MO
Anthurium bradeanum	PACpac	Costa Rica	Croat 35751a	Greenhouse MO
Anthurium brevipedunculatum	DAC	Brazil	Croat 62293b	Greenhouse MO
Anthurium bromelicola	Unknown	Brazil	Temponi 343	Herbarium (MO)
Anthurium brownii	BEL	Panama	Croat 76217b	Greenhouse MO
Anthurium carchiense	PACmulti	Ecuador	Croat 90259	Greenhouse MO
Anthurium chiapasense	Unknown	Mexico	Croat 45927	Greenhouse MO
Anthurium citrifolium	TET	Ecuador	Croat 100034	Field – silica
Anthurium clarinervium	CAR	Mexico	VADK 1991-1413 Kew	Greenhouse Kew
Anthurium clavigerum	DAC	Bolivia	Daley 2042a	Greenhouse MO
Anthurium clidemioides	POLYP	Panama	Croat 79567	Greenhouse MO
Anthurium cordatum	Unknown	Virgin Islands, St. Croix	Croat 81387	Greenhouse MO
Anthurium cordifolium	Unknown	Jamaica	Croat 81448	Greenhouse MO
Anthurium coriaceum	URO	Brazil	Croat 67421b	Greenhouse MO
Anthurium crenatum	PACpac	Puerto Rico	Croat 68440	Greenhouse MO
Anthurium croatii	DAC	Peru	Croat 81920	Greenhouse MO
Anthurium crystallinum	CAR	Colombia	Croat 56351	Greenhouse MO
Anthurium curvispadix	CAL	Panama	Croat 33654	Greenhouse MO

Anthurium cuspidatum	POLYN	Panama	Hannon 01-007	Greenhouse MO
Anthurium digitatum	DAC	Venezuela	Croat 54361a	Greenhouse MO
Anthurium dolichostachyum	CAR	Ecuador	Croat 83097a	Greenhouse MO
Anthurium dominicense	Unknown	Unknown	Croat 90070	Greenhouse MO
Anthurium dressleri	CAR	Panama	Folsom 3737	Greenhouse MO
Anthurium eminens	DAC	Peru	van der Werff 10168	Greenhouse MO
Anthurium flexile	POLYP	Mexico	Croat 78692b	Greenhouse MO
Anthurium formosum	CAL	Costa Rica	Croat 79071	Greenhouse MO
Anthurium fornicifolium	DEC	Ecuador	Croat 81400	Greenhouse MO
Anthurium friedrichsthalii	POR	Ecuador	Croat 99861	Field – silica
Anthurium furcatum	SEM	Ecuador	Croat 73249	Greenhouse MO
Anthurium gracile	LEP	Unknown	L.Holy 7-23-99	Greenhouse MO
Anthurium grandifolium	BEL	Dominica	JM 8865	Greenhouse MO
Anthurium halmoorei	PACpac	Mexico	Croat 45337j	Greenhouse MO
Anthurium harrisii	URO	Brazil	Croat 73889c	Greenhouse MO
Anthurium hoffmannii	CAL	Panama	Croat 66203	Greenhouse MO
Anthurium huixtlense	CAL	Mexico	Croat 63309	Greenhouse MO
Anthurium kunthii	DAC	Panama	Croat 38121	Greenhouse MO
Anthurium laciniosum	TET	Ecuador	Cazalet & Pennington 5271	Herbarium (MO)
Anthurium lancea	BEL	Ecuador	Croat 75455	Greenhouse MO
Anthurium lancetillense	BEL	Honduras	Croat 42672	Greenhouse MO
Anthurium lancifolium	POR	Panama	Croat 81520	Greenhouse MO
Anthurium leuconeurum	CAR	Mexico	HOLY 1999-425 Kew	Greenhouse Kew
Anthurium longeinternodum	XIA	Ecuador	Croat 99678	Field – silica
Anthurium longipes	URO	Brazil	Temponi 339	Herbarium (MO)
Anthurium longissimum	SCH	Venezuela	Croat 74497	Greenhouse MO
Anthurium lucens	Unknown	Mexico	Croat 78702	Greenhouse MO
Anthurium lucidum	URO	Brazil	Croat 87586	Greenhouse MO

Anthurium lutheri	DEC	Ecuador	Croat 99766	Field – silica
Anthurium macleanii	BEL	Bolivia	Croat 84725	Greenhouse MO
Anthurium maculosum	POLYN	Ecuador	Croat 73725	Greenhouse MO
Anthurium margaricarpum	TET	Ecuador	Croat 95756b	Greenhouse MO
Anthurium cf. *michelii*	DEC	Unknown	Croat 79381	Greenhouse MO
Anthurium michelii	DEC	Ecuador	Croat 99885	Field – silica
Anthurium microspadix	XIA	Ecuador	Croat 99672	Field – silica
Anthurium mindense	XIA	Ecuador	Croat 99599	Field – silica
Anthurium morae	DIG	Colombia	Croat 83725c	Greenhouse MO
Anthurium muyunense	DEC	Ecuador	Croat 95411a	Greenhouse MO
Anthurium napaeum	PACmulti	Ecuador	Croat 50876	Greenhouse MO
Anthurium obtusilobum	CAL	Costa Rica	Croat 78845c	Greenhouse MO
Anthurium cf. *obtusum*	TET	Ecuador	Croat 90101	Greenhouse MO
Anthurium obtusum	TET	Unknown	Croat 82921a	Greenhouse MO
Anthurium olfersianum	URO	Unknown	Selby 63-75-17	Greenhouse MO
Anthurium ovatifolium	DIG	Ecuador	Croat 95923	Greenhouse MO
Anthurium oxyphyllum	PACmulti	Ecuador	Croat 75325	Greenhouse MO
Anthurium cf. *palenquense*	PACmulti	Ecuador	Croat 72986	Greenhouse MO
Anthurium parvispathum	DEC	Guatemala	Croat 90238	Greenhouse MO
Anthurium parvum	URO	Brazil	Temponi 345	Herbarium (SPF)
Anthurium pedatoradiatum	SCH	Mexico	Fisk 8/15/01	Greenhouse MO
Anthurium pedatum	SCH	Colombia	Croat 62810c	Greenhouse MO
Anthurium peltigerum	Unknown	Ecuador	Croat 99794	Field – silica
Anthurium polydactylum	DAC	Peru	Croat 81643	Greenhouse MO
Anthurium protensum	PACpac	Unknown	Croat 71830	Greenhouse MO
Anthurium punctatum	POR	Ecuador	Croat 73859	Greenhouse MO
Anthurium radicans	CHA	Unknown	Croat 76139	Greenhouse MO
Anthurium sagittatum	CAR	French Guiana	Croat 74285	Greenhouse MO

Anthurium scandens	TET	Costa Rica	Croat 79257	Greenhouse MO
Anthurium schottianum	PACpac	Costa Rica	Croat 43247b	Greenhouse MO
Anthurium sinuatum	DAC	Brazil	Croat 62179	Greenhouse MO
Anthurium sp. sect. *Digitinervium*	DIG	Ecuador	Croat 99803	Field – silica
Anthurium spathiphyllum	PACpac	Costa Rica	Croat 71838	Greenhouse MO
Anthurium spectabile	PACpac	Costa Rica	Croat 69707	Greenhouse MO
Anthurium subsignatum	SEM	Costa Rica	Croat 78774a	Greenhouse MO
Anthurium thrinax	DAC	Unknown	Saul 75175h	Greenhouse MO
Anthurium tremulum	BEL	Ecuador	Croat 100032	Field – silica
Anthurium trilobum	SEM	Colombia	Croat 79726	Greenhouse MO
Anthurium umbrosum	BEL	Mexico	Croat 78706c	Greenhouse MO
Anthurium cf. *urbanii*	POLYN	Ecuador	Croat 99801	Field – silica
Anthurium veitchii	CAL	Unknown	Croat 81530 = 81006	Greenhouse MO
Anthurium venosum	PACpac	Unknown	Croat 69756	Greenhouse MO
Anthurium verapazense	Unknown	Guatemala	Croat 81392	Greenhouse MO
Anthurium vittariifolium	POR	Unknown	Croat 56912c/b	Greenhouse MO
Anthurium wendlingeri	POR	Costa Rica	Croat 71837	Greenhouse MO
Anthurium willdenowii	URO	Unknown	Croat 57219	Greenhouse MO
Anthurium willifordii	PACpac	Peru	Croat 81092	Greenhouse MO
Pothoidium lobbianum	out-Potheae	China	Wen-Pen Leu 2191	Herbarium (HAST)
Pothos chinensis	out-Potheae	Unknown	Kew 1999-3194 / PENG 10037	Greenhouse Kew
Pothos junghuhnii	out-Potheae	Unknown	Kew 1999-3170 / TRO_N8F3	Greenhouse Kew
Pothos ovatifolius	out-Potheae	Unknown	Kew 1996-4425 / FLEC TRO_N8F3	Greenhouse Kew
Pothos rumphii	out-Potheae	Unknown	Kew 2000-3986 / BGNR	Greenhouse Kew
Pothos scandens	out-Potheae	Unknown	Croat 95634	Greenhouse MO

Table 2. Characteristics of cpDNA and nDNA molecular datasets used to reconstruct the phylogeny of *Anthurium*.

	***Pothos* & *Anthurium* dataset**				***Anthurium* only dataset**	
DNA sequence / region	***trnG***	***trnH-psbA***	***trnC-ycf6***	**combined cpDNA**	***CHS***	**combined cpDNA & nDNA**
Number of taxa	109	109	108	109	100	103
Number of taxa (*Anthurium*)	103	103	102	103	100	103
Number of taxa (*Pothos*)	6	6	6	6	0	0
Total length of region (bps)	662-690	240-489	515-989	N/A	(449-)583-941	N/A
Aligned length (bps)	746	860	1100	2706	1252	3958
Number of excluded characters	78	620	508	1206	586	1792
Number of included characters	668	240	592	1500	666	2166
Number of variable characters (% of included)	108 (16%)	54 (22%)	113 (19%)	275 (18%)	339 (51%)	614 (28%)
Number of parsimony informative characters (% of included)	69 (10%)	37 (15%)	76 (13%)	182 (12%)	172 (26%)	354 (16%)
Average uncorrected pairwise sequence divergence among *Anthurium* species	0.6	1.2	1.1	N/A	3.3	N/A
Average uncorrected pairwise sequence divergence among outgroups	1.7	2	1.6	N/A	N/A	N/A
Average uncorrected pairwise sequence divergence between *Anthurium* and outgroups	4.4	7.3	5.7	N/A	N/A	N/A

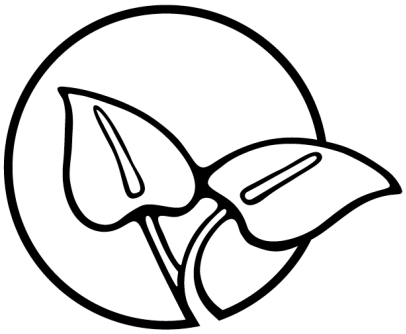
Table 3. Characteristics of the parsimony, likelihood and Bayesian analyses used to reconstruct the phylogeny of *Anthurium*.

	***Pothos* & *Anthurium* dataset**				***Anthurium* only dataset**	
	trnG	***trnH-psbA***	***trnC-ycf6***	**combined cpDNA**	***CHS***	**combined cpDNA & nDNA**
Parsimony Ratchet analysis						
Number of most parsimonious trees	3746	4018	4020	4020	4017	3404
Tree length (in number of steps)	144	77	145	370	583	903
Consistency index (CI)	0.833	0.8331	0.855	0.832	0.758	0.734
Retention index (RI)	0.908	0.933	0.956	0.934	0.81	0.819
Number of highly supported clades (PB > 70%) within *Anthurium*	10	1	8	20	23	38
Maximum Likelihood analysis						
Best fitting evolutionary model (according to the AIC)	K81uf+G	TVM+G	K81uf+G	TVM+G	HKY+G	TVM+I+G
Log likelihood score	-1919.972075	-818.717141	-1806.472910	-4734.081493	-4940.216892	-9710.837407
Number of highly supported clades (LB > 70%) within *Anthurium*	14	11	18	38	33	43
Bayesian analysis						
Total mean tree length	21.45224	21.402905	21.195449	21.454154	18.494112	19.676818
Variance in tree length	2.176028	2.181469	2.124092	2.164774	2.087549	2.235645
Arithmetic mean of the log-likelihood	-2202.72	-966.79	-2070.12	-5271.48	-5308.42	-10284.23
Number of highly supported clades (PP > 0.90) within *Anthurium*	18	16	28	44	50	65

CHAPTER 2

A REVISION OF THE CURRENTLY ACCEPTED SECTIONAL CLASSIFICATION OF *ANTHURIUM* SCHOTT (ARACEAE) AND ITS ASSOCIATED MORPHOLOGY BASED ON A MOLECULAR PHYLOGENY OF THE GENUS

Mónica M. Carlsen



INTRODUCTION

Anthurium Schott is a monophyletic (Carlsen 2011) and strictly Neotropical genus of Araceae ranging from southern Mexico into Central America and the West Indies, to southern Brazil, northern Argentina, and Paraguay. It includes approximately 900 largely well-differentiated species (Mayo et al. 1997, Govaerts & Frodin 2002, Govaerts et al. 2011, CATE Araceae 2011) with many more still being discovered (T. Croat, pers. comm.). It is placed in the subfamily Pothoideae, one of the earliest divergent lineages in Araceae, and is sister to the Old World genus *Pothos* L. (approx. 57 species) from Southeast Asia, Australasia and Madagascar (French et al. 1995, Barabé et al. 2002, Rothwell et al. 2004, Tam et al. 2004, Cabrera et al. 2008, Cusimano et al. 2011, Carlsen 2011). *Anthurium* species are distinguished by being climbers or epiphytes with sympodial growth, spirally arranged leaves, rounded petiole, geniculum at apex of petiole, reticulate minor venation, collective veins along the leaf margins, uniform spadix with open spathe, 4-merous bisexual flowers with tepals and seeds with copious endosperm (Grayum 1990, Mayo et al. 1997). *Anthurium* displays an enormous variation in leaf morphology, growth habit and leaf venation pattern.

Taxonomists have proposed several classifications of groups within *Anthurium* in attempts to partition the extraordinary morphological diversity of this genus (Table 1). The first infrageneric classification of *Anthurium* was that of Schott (1860) in which he grouped the 183 known species of *Anthurium* into 28 “greges”. This arrangement was largely based on vegetative and floral characters, such as leaf venation, leaf shape, spathe color and length, and internode length. In the most recent revision of the genus (Engler 1905), the 486 then known species of *Anthurium* were divided into 18 sections (Table 1),

again mainly using leaf shape characters as well as number of seeds and spadix form. Although most of Schott's and Engler's sections do seem to contain a core of related species, placement of the remaining species included in them seems subjective. In the currently accepted sectional classification of *Anthurium* (Croat & Sheffer 1983) 18 sections - a slightly modified version of Engler's system - are recognized (Tables 1 & 2). In general, these sections were characterized by a combination of characters, mainly differences in leaf shape, vernation, punctation and venation, habit, roots, cataphylls and number of seeds. Only a few groups of species share a distinctive feature not found elsewhere in the genus (e.g. 4 seeds per fruit in section *Tetraspermium* Schott) and are apparently "natural"; most groups have a more complicated combination of diagnostic characters and quite frequently appear to have overlapping limits. It is unclear if groups recognized in previous classifications reflect putative relationships or if the diagnostic characteristics of such sections were used simply to facilitate group recognition, the groups serving to chunk up an otherwise unwieldy genus.

Indeed, phylogenetic relationships within *Anthurium* remained poorly understood until recently. However, a molecular phylogeny of the genus (Carlsen 2011) suggests that some previously recognized infrageneric taxa within *Anthurium* might not be monophyletic, and it provides a strong phylogenetic framework for revising the currently accepted sectional classification of the genus. The study was based on maximum parsimony, likelihood and Bayesian analysis of combined cpDNA and nDNA sequence data from 102 *Anthurium* species representing all but one of Croat and Sheffer's (1983) sections. Within *Anthurium*, the analyses recovered 20 monophyletic and highly

supported major clades with bootstrap values higher than 70% and/or posterior probabilities greater than 0.9 (Carlsen 2011).

The currently accepted sectional classification of *Anthurium* (Croat & Sheffer 1983), and the morphological characters on which it was based, needs to be reevaluated against this new molecular phylogeny of Carlsen (2011). Only a few clades are congruent with the current sectional classification of *Anthurium*, and morphological characters used to delimit sections in previous classifications are clearly homoplasious. Although deeper relationships among clades were largely unresolved in Carlsen (2011), that does not have great consequences for the recognition of monophyletic species groups within *Anthurium*. This study uses topology tests to evaluate support for the monophyly of currently accepted sections within *Anthurium* by statistically comparing the best scoring trees with alternative phylogenetic hypotheses that constrained each currently recognized section or series within *Anthurium* to be monophyletic. Furthermore, morphological characters used to recognize current sections are reconstructed along the phylogeny to determine their usefulness in separating groups within *Anthurium*.

MATERIALS AND METHODS

The phylogenetic hypotheses used in this study were based on the combined DNA sequence data from Carlsen (2011), which included the chloroplast *trnG* intron (Shaw et al. 2005) and *trnH-psbA* (Hamilton 1999) and *trnC-ycf6* (Shaw et al. 2005) intergenic spacers, and the nuclear first intron of the chalcone synthase (*CHS*) gene (Carlsen 2011). A total of 102 *Anthurium* species were included in all analyses, comprising at least one and up to 13 representatives of each of the 18 sections proposed

by Croat and Sheffer (1983), except for the monotypic section *Gymnopodium* endemic to Cuba (Table 2). Unconstrained trees were obtained from maximum parsimony and likelihood analyses of the combined cpDNA-nDNA dataset. Details of taxon sampling, laboratory protocols, data alignment, outgroup selection, and phylogenetic analyses were discussed by Carlsen (2011).

The unconstrained parsimony topology used in this study (Figure 1) is the first of the most parsimonious trees obtained from parsimony ratchet analyses (Nixon 1999, Sikes & Lewis 2001). The unconstrained likelihood topology (Figure 2) is the best scoring maximum likelihood tree from likelihood analyses performed using RAxML v.7.2.7 (Stamatakis 2006, Stamatakis et al. 2008).

Hypothesis testing was performed with Templeton's test (Templeton 1983) under Maximum Parsimony and the Shimodaira–Hasegawa test (Shimodaira and Hasegawa 1999, Goldman et al. 2000) under Maximum Likelihood settings. Seventeen constrained trees were constructed in MacClade v. 4.08 OS X (Maddison & Maddison 2000), each corresponding to one of the sections or series of *Anthurium* being monophyletic, except for the unsampled section *Gymnopodium*, the monotypic section *Chamaerepium* and section *Leptanthurium* that was represented by only one species in this study. Individual constrained trees were loaded in PAUP* version 4.0b10 (Swofford 2002) and evaluated under parsimony in order to obtain the constrained tree length using heuristic search, 10 random addition sequence replicates, TBR branch swapping and MULTREES=yes. Tree length differences between each constrained tree and the unconstrained parsimony topology were compared statistically using the Templeton test as implemented in PAUP* (Swofford 2002). If the constrained parsimony tree was

significantly longer ($P_{TT} \leq 0.05$) than the unconstrained parsimony topology, then the monophyly of the section was rejected. For topology testing under likelihood settings, the most likely topology under the constraint was inferred using RAxML (Stamatakis 2006, Stamatakis et al. 2008) and the GTRGAMMA model of evolution. Lastly, both the unconstrained likelihood topology and the most likely topologies inferred under the constraints for each section or series of *Anthurium* were used as inputs in PAUP* (Swofford 2002), and a Shimodaira-Hasegawa test was performed using 1,000 bootstrap replicates and the RELL (resampling estimated log likelihoods) option. As before, if the constrained likelihood tree was significantly less likely ($P_{SH} \leq 0.05$) than the unconstrained likelihood topology, then the monophyly of the section was rejected.

Twenty-four morphological characters previously used to identify sections within *Anthurium* were scored for all species in the dataset (Tables 3 & 4) and their evolution reconstructed along the unconstrained likelihood topology under parsimony criterion in MacClade (Maddison & Maddison 2000) (Appendix 1). They represent all the characters used in the sectional classification of Croat and Sheffer (1983). The evolution of two other characters, spadix and fruit color, used to further delimit species groups by Madison (1978) was also reconstructed given their correlation with strongly supported clades in the molecular phylogeny of *Anthurium* (Carlsen 2011) (Figure 3). Scoring of morphological characters was based on a combination of species descriptions available in the literature (see Table 4 for details) and study of herbarium specimens and living collections. A number of the characters are quantitative and the limits of the states recognized are arbitrary. Every character was traced individually on the phylogeny

without resolving equivocal tracings. For every character the number of steps, consistency (CI) and retention (RI) indices were also calculated (Farris 1989).

RESULTS

The unconstrained parsimony tree used in all analyses (i.e. the first of all most parsimonious trees from parsimony ratchet analysis) was 903 steps long (CI = 0.734, RI = 0.819) (Figure 1), whereas alternative topologies that constrained each section or series within *Anthurium* as monophyletic ranged between 903 and 974 steps in length (Table 5). The topology represented by the unconstrained parsimony tree closely matched the final results of Carlsen (2011) except for having the group of *Anthurium* species with palmate leaves (Clade 4) as closely related to the clade C instead of grouped with Clades 3 and 5 to form a bigger Clade D (all clade numberings follow Figure 3 in Carlsen 2011). Nonetheless, additional testing suggested that such changes in relationships among clades did not modify the overall results of Templeton's tests (data not shown). The unconstrained likelihood topology used in hypothesis testing has a log-likelihood of -9705.96007 (Figure 2) and matches exactly the final tree of Carlsen (2011). The difference in likelihood scores between unconstrained and constrained topologies ranged from almost zero to 380.25238.

Of the 18 sections and two series of the currently accepted sectional classification of *Anthurium* (Croat & Sheffer 1983), one section (*Gymnopodium*) was not sampled in this study, and two other sections, the monotypic *Chamaerepium* and *Leptanthurium* – represented by only one species in the analyses - clearly could not be tested for monophyly. Results of Templeton's test comparing the parsimony unconstrained tree and

alternative constrained topologies showed that 10 of the 17 sections/series of *Anthurium* included in the analyses are not monophyletic ($P_{TT} < 0.05$) (Table 5). The Shimodaira-Hasegawa test among unconstrained and alternative constrained likelihood topologies also suggested that the majority of the sections/series of *Anthurium*, nine out of 17, are not monophyletic ($P_{SH} < 0.05$) (Table 5). The only discrepancy among parsimony-based and likelihood-based results of hypothesis testing was for section *Urospadix*, for which monophyly could not be rejected by to the Shimodaira-Hasegawa test ($P_{SH} = 0.146$) but could be rejected when the Templeton's test was used ($P_{TT} = 0.026$) (Table 5). Although results of topology tests for sections *Dactylophyllium*, *Digitinervium*, *Tetraspermium* and *Urospadix* show that monophyly cannot be rejected (Table 5), placement of all their representative species in the phylogeny suggests otherwise (Figure 2). In these few cases, small branch length differences among closely related species showed that it is equally statistically probable for the section to be monophyletic as it is to have it including or excluding one or more very closely related species, thus emphasizing the lack of sufficient signal in the data to reject monophyly. In order to render these sections truly monophyletic, one or two species either need to be included (for example, in *Dactylophyllium* and *Digitinervium*) or removed from the section (as in *Tetraspermium* and *Urospadix*).

The twenty-four characters examined here represent all the characters used in the sectional classification of Croat and Sheffer (1983). They include two geographical characters, 20 vegetative characters (six related to stem morphology and 14 to leaf characteristics), and two reproductive characters (one flowering and one fruiting character). Reconstructions of character evolution for each one of these characters

showed widespread patterns of homoplasy and therefore most characters did not track the molecular phylogeny well (Table 6, Appendix 1). Consistency (CI) and retention (RI) indices for each character were in general very low, averaging 0.33 for CI and 0.56 for RI for all characters included (Table 6). Three quarters of the characters showed high levels of homoplasy, with CI from as low as 0.06 (character # 23, spadix length) to 0.29 (character # 16, palmate leaf, number of lobes per leaf), hence the majority of morphological characters previously used in sectional classification do not fit the molecular phylogeny of *Anthurium* very well. Retention indices (RI) are also significantly low for many characters analyzed (50% of the total), with smaller values ranging from 0 (character # 10, petiole shape) to 0.54 (character # 22, venation, number of primary lateral veins), therefore suggesting that most characters used in the current sectional classification of *Anthurium* are not good synapomorphies for the clades recovered in the molecular phylogeny (Table 6).

Among the 24 characters analyzed, four (i.e. characters # 1 geography, endemic to Brazil, # 5 root position, # 7 presence of 1-ribbed cataphyll and # 24 number of seeds per locule) are noticeable for having the highest values for both indices (CI and RI = 1), and two more (characters # 11 leaf vernation and # 18 punctation presence) also ranked higher in both indices, especially in RI (Table 6). These six characters are the least homoplasious of the characters analyzed, they comprise ca. 25% of the characters currently used to separate groups in the sectional classification of *Anthurium*, and correlate well with clades within the molecular phylogeny.

DISCUSSION

In general, reconstructions of the morphological characters currently used to recognize sections and series within *Anthurium* showed high levels of homoplasy, and for the most part, do not correspond well with the strongly supported clades recovered in the molecular phylogeny of the genus (Carlsen 2011). Not surprisingly, many sections and series based on these characters were identified as non-monophyletic according to topological hypothesis testing performed here.

The most homoplasious characters in *Anthurium* are related to leaf characteristics such as petiole length and shape, and leaf texture, including thickness and velvety appearance. Indeed, they have not been used on their own to distinguish groups within *Anthurium*, but instead in combination with other homoplasious leaf characters. For example, thin blades (character # 15 – states 0 or 1) with many close primary lateral veins (character # 22 – state 2) (Tables 3 & 6) are two characters that have been used to distinguish species in section *Polyneurium* (Table 2), a group for which monophyly can be firmly rejected ($P_{SH} = 0.004$, $P_{TT} = 0.0164$) (Table 5). Indeed, 12 of the 14 leaf-related characters ranked the most homoplasious among all characters studied. Only two leaf characteristics, involute vernation (character # 11 - 1, RI = 0.92) and the presence of punctations on the leaf blade (character # 18 - 1, RI = 0.94) (Table 6), can be used to support monophyletic groups, however, both occur in two different strongly supported but not closely phylogenetically related clades in the molecular phylogeny. The first distinguishes both series of section *Pachyneurium* (Clades 11 and 13), and the latter is a characteristic of Clade 3 and of species in sections *Tetraspermium*, *Digitinervium* and *Porphyrochitonium* that together make up Clade 6 (Figure 2, Appendix 1).

Homoplasious characters might imply repetitive adaptive shifts, but it is very difficult to interpret the ecological significance of the leaf characters analyzed. Madison (1978) suggested that shape and venation of leaves were indeed the most useful taxonomic characters in *Anthurium*, but that they had no or at least not a convincing evolutionary or biological meaning. Cusimano et al. (2011) also found that leaf characters used in the intrafamilial classification of Araceae as a whole were highly homoplasious. Thus the leaf characteristics emphasized by previous taxonomists are both difficult to interpret ecologically and certainly do not correlate with the evolutionary history of the genus *Anthurium*.

Stem characteristics used to delimit of sections in *Anthurium* are a combination of homoplasious and highly conserved characters. Root position along nodes or internodes (character # 5) and presence or absence of 1-ribbed cataphylls (character # 7), ranked the best in terms of synapomorphies (Table 6). Adventitious roots arising along the internodes and the absence of 1-ribbed cataphylls are characteristics of section *Polyphyllium* (Table 2), a strongly supported monophyletic clade (P_{SH} = 0.913, P_{TT} = 0.8405) (Table 5) that forms the first divergent lineage within *Anthurium* (Clade A, Figure 2).

The four other stem characters currently used in the sectional classification are homoplasious, but some unique character states might serve as synapomorphies. For example, the presence of cataphylls persisting intact at the nodes (character # 8 - 1) (Table 6) distinguishes very clearly species in section *Calomystrium* (Clade 16) (Figure 2) a strongly supported monophyletic clade ($P_{SH} = P_{TT} = 1$) (Table 5), whereas the other two states (cataphyll persisting as fibers or deciduous) are highly homoplasious in the

phylogeny (Appendix 1). The same is true for stem thickness (character # 4, RI = 0.63), with a higher than average retention index (Table 6). Although very thin stems (< 1 cm in diameter) may have evolved four times in parallel (Appendix 1), they characterize uniquely both monophyletic sections *Tetraspermium* (Clade 7) (P_{SH} = 0.421, P_{TT} = 0.0881) and *Polyphyllium* (Clade A) (Table 5, Figure 2), with a few convergences in unrelated and highly specialized epiphytic species such as *Anthurium bromelicola* (in Clade 1) and *A. microspadix* (in Clade 17) (Figure 2, Appendix 1).

On the other hand, two highly correlated stem characters, habit and internode length, are very homoplasious along the molecular phylogeny, and cannot be used to uniquely characterize any clades in *Anthurium*. The combination of bird's nest habit (character # 3 - 3) and short internodes less than 3 cm long (character # 6 - 0) (Table 6) have been previously used to distinguish section *Pachyneurium*, a group for which monophyly is rejected (P_{SH} = 0.003, P_{TT} = 0.0002) (Table 5) (Figure 2). This general morphology has evolved at least four and up to seven times in *Anthurium* (Appendix 1) probably as the result of repeated adoptions of the epiphytic habit or incursions into seasonally dry environments. The bird's nest habit could help cope with periods of dryness by accumulation of organic matter and water between the leaf bases (Benzing 1987).

Historically, reproductive characteristics have been used to separate species of *Anthurium* but not to unite them into groups. Indeed, the only flowering character used to distinguish groups in the current sectional classification of the genus (Croat & Sheffer 1983), spadix length (character # 23), ranked among the most homoplasious in this study (Table 6). A better circumscription of character states for the spadix length and its

relationship with spadix width and peduncle length could perhaps show that such reproductive characters are indeed useful to recognize clades within *Anthurium* (Carlsen pers. obs.).

Madison (1978) suggested that evolution in *Anthurium* might have comprised two adaptive radiations, one related to the diversification of pollination syndromes, with species producing sweet-spicy fragrances being pollinated by bees and species with rotten fruit-like aroma pollinated by flies. He also argued that pollination syndromes have a loose correlation with spadix color, purple in fly-pollinated species and white-yellow in bee-pollinated ones, but that this character had been employed in classification only to a very limited extent. He thought that in general, characters used in *Anthurium* classification reflected only indirectly, if at all, these biological adaptations (Madison 1978). A reconstruction of the evolution of spadix color in *Anthurium* (Figure 3) shows that purple spadices are plesiomorphic and very common within the genus. On the other hand, cream-colored spadices seem to characterize at least one strongly supported clade in *Anthurium*, Clade 16 (section *Calomystrium*), with independent origins in other species within Clades E, 9 and 11. There is probably a single origin for yellow spadices in Clade E, green-yellow spadices are also only found within this clade, specifically characterizing Clade 12 (Figures 2 & 3). It is possible that either yellow spadices were derived from green-yellow ones, or that they both form a monophyletic group of yellowish-colored spadices (i.e. green-yellow color could be also interpreted as a shade of yellow). However, relationships among clades within Clade E are still poorly understood.

One fruit character, two seeds per locule (character # 24 - 1) (Table 6), has been used to distinguish section *Tetraspermium* (Clade 7) (Figure 2). This section is

monophyletic and the character is indeed a very good synapomorphy. Exploration of other fruit and infructescence characteristics previously overlooked in the sectional classification may yield other characters useful for recognition of clades within *Anthurium*. Indeed, Madison (1978) proposed that color differences in berries could be a good taxonomic character, but that not enough was known about its correlation with dispersal mechanisms. Croat (1991) explored some species groupings within section *Pachyneurium* based on berry color and found that most South American species tend to have purple fruits, whereas orange-red berries were more common among Central American species. However, he did not explicitly recognize the value of such color differences in terms of evolutionary relationships among species groups.

Reconstruction of berry color in the molecular phylogeny of *Anthurium* reveals that it is indeed a good synapomorphic character (Figure 3). Purple fruits are the most common and plesiomorphic condition in *Anthurium*. Although having more than two independent origins, bright orange fruits strongly characterize at least two clades in the genus, Clade 1 (section *Polyphyllium*) and Clade 15 (species from northern Central America). Green berries are synapomorphic for Clade 1 (comprising only Brazilian endemics), however, they also occur outside this clade in several other *Anthurium* species that do not belong to Clade 1 based on their geographic occurrence. These other species were not sampled in the molecular phylogeny, but it is possible that they represent independent origins of green fruits in the genus. Red fruits are only found in Clade D, but they have been lost there in a few species. For example, Clade 7 (section *Tetraspermium*) is characterized by white-lavender berries and Clade 4 (species with palmately-lobed leaves) by purple fruits, both unique colors within this larger red-fruited clade D. In fact,

red fruits could characterize a smaller clade uniting together Clade 3 (northern Central America species with punctations on the leaf blade) and Clade 5, but relationships between these two clades are still unresolved. The red-violet berries characteristic of Clade 16 (section *Calomystrium*) are synapomorphic in the genus. They could be caused by a difference in the proportions of pigments produced within a larger clade of species with purple berries (Clade E) (Figures 2 & 3).

Two non-morphological characters that have been used to reinforce sectional delimitations are geographic location and elevational preference (Tables 2 & 3). The most recent molecular phylogeny of *Anthurium* (Carlsen 2011) identified several monophyletic and strongly supported clades within the genus that could be defined by geographic proximity. For example, whether a species occurs in Brazil or not (character # 1) (Table 6) has helped separate the core of species in section *Urospadix* (Table 2, Clade 1), most with bird's nest habit and short internodes, from species in section *Pachyneurium* with similar morphology (Clade 11) and from other non-Brazilian species previously assigned to section *Urospadix* (Clade 2) (Figure 2). This study reveals that such geographic characterization is indeed a very good synapomorphic character (Appendix 1). Furthermore, species restricted to the Caribbean islands are all grouped together in Clade 2 and species distributed in northern Central America are all included in either Clades 3 or 15. These geographically localized clades within *Anthurium* therefore confirm that geographic location is a good character to distinguish some sections in the genus.

The other non-morphological character, elevational preference (character # 2) (Table 6) is more homoplasious (Appendix 1); elevation ranges described in the literature

are often overlapping (e.g. low-medium 0-1000 m, medium 500-1500 m, medium-high 1200-1600 m) (Table 3), and this may well have had some impact on how this character behaves in the tree. A more detailed circumscription of elevation ranges and its possible correlation with temperature and rainfall could improve our understanding of the evolution of this character along the phylogeny of *Anthurium*.

It is clear that the current sectional classification of *Anthurium* by Croat and Sheffer (1983) is in need of an exhaustive revision since most of the characters on which is based are homoplasious, and consequently, most of the sections, as currently recognized by their morphology, may not be monophyletic. Below, I concentrate on understanding the fate of the accepted sections and series of *Anthurium* in the light of the molecular phylogeny (Carlsen 2011), focusing on individually exploring their monophyly (Table 5) and the level of homoplasy of the characters used to distinguish each group (Tables 2 & 6).

Section *BELOLONCHIUM* Schott emend. Engler. was represented here by 9 species and does not form a monophyletic group ($P_{SH} = 0$, $P_{TT} < 0.0001$) (Table 5). Characters distinguishing this section (Table 2) are indeed highly homoplasious (Appendix 1), cordate leaves (character # 13 - 2), thin blades (character # 15 - 0 or 1) and growing at high elevations (character # 2 - 3 or 4) (Table 6). Species in this section belong to at least four, perhaps up to five, different lineages (Figure 2), the northern Central American epunctate group (Clade 15) with most of the sampled species (*Anthurium andicola*, *A. berriozabalense*, *A. lancetillense* and *A. umbrosum*); *A. macleanii* - *A. tremulum* in Clade 18 and *A. brownii*, all are included in a larger unnamed

clade, but are otherwise not closely related to each other; *A. lancea* within Clade 12; and *A. grandifolium* grouped with the Caribbean Clade 2 (Figure 2).

Croat and Sheffer (1983) acknowledged that section *Belolonchium* might just be a "dumping ground" for several species with cordate leaves that did not fit the other recognizable sections. They also admit that it was the most poorly known of all sections included in Engler's 1905 revision. They suggested that section *Belolonchium* comprises at least two groups of seemingly related species, one with relatively coriaceous leaves that dry brown (e.g. *A. bogotense*) and other with thinner leaf blades that dry green (e.g. *A. lancetillense*). Only the second grouping partially correlates with one of the clades found (Clade 15, Figure 2) (Carlsen 2011).

Section *CALOMYSTRIUM* Schott emend. Engler. Seven species represented this section in the study (*Anthurium antioquiense*, *A. curvispadix*, *A. formosum*, *A. hoffmannii*, *A. huixtlense*, *A. obtusilobum* and *A. veitchii*) and form a monophyletic group (Clade 16, Figure 2) in all analyses ($P_{SH} = P_{TT} = 1$) (Table 5). Monophyly of this section is supported not only by molecular characters but also by the presence of intact persisting cataphylls (character # 8 - 1). Traditionally this section has also been recognized by cordate blades (character # 13 - 2) but this is a highly homoplasious character. (Table 6, Appendix 1).

According to both Engler (1905) and Croat and Sheffer (1983), section *Calomystrium* is one of the most "natural" and recognizable groupings within *Anthurium*. Since its initial circumscription (Schott 1860, Engler 1905), species in this section have also been characterized by their distinctive inflorescences, with erect and broad spathes, and rather thick, glossy spadices that are often variously pastel colored and with exserted

pistils. However, Croat and Sheffer (1983) emphasized the presence of intact cataphylls in distinguishing the section more than any unique reproductive characters.

Section *CARDIOLONCHIUM* Schott. is a non-monophyletic group (P_{SH} = 0.001, P_{TT} = 0.0002) (Table 5) based on the eight species included here. This species group had previously been characterized by its apparently unique leaf morphology (Table 2), with petioles that are often striate or ribbed (character # 10 - 1), velvety blades (character # 14 - 2) and, in some cases, venation much paler than the rest of the blade. The first two are among the most homoplasious characters studied here (Table 6). The velvety appearance of leaves comes from convexly curved outer anticlinal epidermal cell walls, which have been postulated as an ecological adaptation to low-light environments (Vogelmann 1993), high moisture conditions (Brodersen & Vogelmann 2007) or both (Bone et al. 1985). Indeed, in *A. warocqueanum* T. Moore (section *Cardiolonchium*) it has been shown that such epidermal cells are advantageous in increasing the efficiency of energy capture by concentrating light and decreasing leaf reflectance (Bone et al. 1985). Therefore, the presence of velvety leaves may relate more to local ecological characteristics than to the evolutionary history of the genus.

In the current molecular phylogeny (Figure 2), species in section *Cardiolonchium* fall into at least three and perhaps up to seven different lineages, pending clarification of relationships within the bigger Clade E, to which they all belong. *Anthurium balaoanum* and *A. dolichostachyum* clustered together relatively close to *A. sagittatum*, *A. dresslerii* and *A. crystallinum*, but not as a monophyletic group; *A. besseae* stands on its own in a strongly supported group with *A. willifordii* (Clade 14); and the northern Central American species, *A. clarinervium* and *A. leuconeurum*, although not immediately related

to each other, are grouped together in Clade 15, along with other species from this area (Figure 2).

Most of the 30 species that Engler (1905) included in section *Cardiolonchium* were represented at that time by few and incomplete specimens in herbaria, while other species were known to him only in cultivation, but without a specific origin. Thus the characteristics he used to unite these species were very much provisional and in need of further investigation. On the other hand, Croat and Sheffer (1983) described the section as an "apparently natural" group, noting that the species with velvety leaves whose chromosomes had been studied were also distinct, all having B-chromosomes in addition to their regular count of 2n=30 (Sheffer & Kamemoto 1976). The results of the present study suggest that velvety leaves arose in parallel within *Anthurium* (Appendix 1), and that the presence of B-chromosomes also does not reflect evolutionary relationships. Other distantly related species in *Anthurium*, such as *A. crenatum* (in Clade 11) and *A. pentaphyllum* var. *bombacifolium* (related to Clade 4) also have B-chromosomes but they lack velvety leaves (Sheffer & Croat 1983).

Section *CHAMAEREPIUM* Schott. is a monotypic section endemic to Brazil including only *Anthurium radicans*. Because of its monotypic nature, the section cannot be included as a constraint in the analyses, but based on the position of *A. radicans* in the phylogeny, separation of this species in its own section cannot be maintained (Figure 2). This species is included in a strongly supported bigger clade of Brazilian endemic species (Clade 1, Carlsen 2011). Therefore, section *Chamaerepium* should not be recognized on its own, but instead as a synonym of an amended section *Urospadix* (Clade 1). In terms of morphology, *A. radicans* differs greatly from the rest of Brazilian species in having a

creeping habit, cordate leaves with highly impressed venation, and a short ellipsoid spadix with elongated style and stamens which extend to the stigma when ripe, a combination of characteristics unique within the genus. Morphological characters that unite it with the rest of section *Urospadix* are still unknown. Croat and Sheffer (1983) presumed that *A. radicans* was most closely related to section *Urospadix*, but its combination of unique characters prompted them to keep it in its own section.

Section *DACTYLOPHYLLIUM* Schott. This section was here represented by nine species, *Anthurium brevipedunculatum*, *A. clavigerum*, *A. croatii*, *A. digitatum*, *A. eminens*, *A. kunthii*, *A. polydactylon*, *A. sinuatum* and *A. thrinax* and its monophyly could not be rejected ($P_{SH} = 0.332$, $P_{TT} = 0.1551$) (Table 5). The section is characterized (Table 2) by species with palmately divided leaves (character # 12 - 1, character # 13 - 4) with three or more segments (character # 16 - 1 or 2) that are free to the base (character # 17 - 2) (Table 6). However, according to the molecular phylogeny (Figure 2, Clade 4), this group of palmately-lobed species also comprises *A. longissimum*, currently placed in section *Schizoplacium*, and distinguished by having segments united, not free, at the base (Table 2). This is one of the few cases when the molecular data could not reject the monophyly of a section even though the combined molecular phylogeny suggests that one extra closely related species also forms part of the clade. In general, palmately-lobed leaves are not unique to section *Dactylophyllium*, having evolved at least two more times in the genus (Appendix 1) in other distantly related clades, e.g. in *A. pedatoradiatum* (Clade 15) and *A. pedatum* (Clade 18), but both these species, like *A. longissimum*, have the segments united at the base (Figure 2). Therefore, having the segments or lobes of the palmate leaves free at the base is indeed a unique characteristic in *Anthurium* and

synapomorphic for section *Dactylophyllium* (Clade 4); the character is lost in *A. longissimum* (Appendix 1).

In his revision of *Anthurium* species with palmately divided leaves, Madison (1978) suggested that they were an artificial but easily recognizable group, comprising at least four "natural" clades of species with palmately-lobed leaves, which thus evolved independently several times in the genus. Current evidence suggests that the palmate leaf morphology has evolved at least three times within *Anthurium* (Carlsen 2011), but a few species included by Madison (1978) have not been sampled yet.

Section *DECURRENTIA* Croat (section *Oxycarpium* Schott pro parte). This section was proposed (Croat et al. 2005b) to accommodate the remaining species in section *Oxycarpium* after the type species, *Anthurium oxycarpium* Poeppig & Endl., was transferred to section *Pachyneurium* by Croat (1991) (Table 1). Seven species from this section were included in the analyses, and the results suggest that it is not monophyletic ($P_{SH} = 0$, $P_{TT} < 0.0001$) (Table 5). Croat et al. (2005b) characterized this section (Table 2) as having short internodes (character # 6 – state 0) and elongated leaves (character # 13 – states 0 or 1) that lacked punctations (character # 18 – state 0) (Table 6). These characteristics are highly homoplasious (Table 6) or plesiomorphic, as in the case of absence of punctations (Appendix 1), and do not allow a clear distinction of section *Decurrentia* from other sections (e. g. section *Urospadix*, Table 2). Species currently placed in this section belong to at least five different lineages in the molecular phylogeny of *Anthurium* (Figure 2): *A. michelii* and *A.* cf. *michelii* belong to Clade 12, *A. fornicifolium* is grouped with the core of section *Pachyneurium* series *Pachyneurium* (Clade 11), *A. lutheri* is placed with a mixture of other species in Clade E, *A.*

parvispathum, ranging from Mexico to Honduras, clusters together with the northern Central American group (Clade 15), agreeing with its geographic distribution, and *A. muyunense – A. vittariifolium* are paired together with *A. gracile* from section *Leptanthurium* in Clade 10. Unfortunately, some more "typical" species within the section, such as *A. decurrens* and *A. pittieri*, were not sampled; these were part of the section *Oxycarpium* recognized by both Schott (1860) and Engler (1905).

Interestingly, both Schott (1860) and Engler (1905) emphasized the presence of berries with a prominently acute apex as characteristic of species in their section *Oxycarpium*, hence its name. However, Croat and Sheffer (1983) disregarded this character, instead suggesting that section *Oxycarpium* could be recognized by its early-emergent pistils. Since they also considered this characteristic to cut across sectional lines, the use of this character would make the section not distinct morphologically. Later, Croat et al. (2005b), when transferring the remaining species from section *Oxycarpium* to section *Decurrentia*, noted that the species originally included in this section had little in common overall and were not easily assigned to any other "natural" section of *Anthurium*, but they expanded the section to include a yet more disparate group of species and used highly homoplasious leaf and stem characters to distinguish it.

Section *DIGITINERVIUM* Sodiro. Three species represented the section in all analyses, *Anthurium morae*, *A. ovatifolium* and the undescribed species, *A.* sp. section *Digitinervium*. According to the results of topology tests, the monophyly of the section could not be rejected ($P_{SH} = 0.69$, $P_{TT} = 0.4497$) (Table 5), but the placement of its representative species in the phylogeny might suggest that the section is paraphyletic (Figures 1 & 2). In this case, the lack of resolution, i.e. almost zero-length branches

between the three species sampled here and other closely related taxa, is the reason for the apparently contradicting results. Representatives of section *Digitinervium* are all embedded in a larger group with other punctate *Anthurium* species (Clade 9) that is strongly supported. Species in section *Digitinervium* are distinguished (Table 2) by having thick blades (character # 15 - 4) with glandular punctations (character # 18 - 1), two or more pairs of steeply ascending collective veins (character # 20 - 2), and also numerous closely parallel primary lateral veins (character # 22 - 2) (Table 6). Although most of the characters used to distinguish the section are homoplasious, their combination is unique within *Anthurium*, and was thought to delimit a very "natural" group (Madison 1978, Croat & Sheffer 1983).

Section *GYMNOPODIUM* Engl. was originally based on a single distinctive and rare species from Cuba, *Anthurium gymnopus*, a highly specialized epiphyte restricted to the leaf bases along the stems of palm trees. The species is characterized by its scandent habit, elongated internodes, deciduous cataphylls, subcordate leaf blades, and long inflorescence with a long-stipitate spadix. Croat and Sheffer (1983) argued that the most important character in the species was the berries with up to 4 seeds (Table 2). They suggested that *A. gymnopus* was the only species known to Engler with cordate leaves and more than 2 seeds per berry and that perhaps he placed too much emphasis on its distinct leaf shape. They also thought that the species could provisionally be placed in section *Tetraspermium*, pending the acquisition of living material for further study. Although *A. gymnopus* could not be included in the molecular phylogeny of the genus (Carlsen 2011), there are two possible placements for the section. Based on geography, it could be placed in the Caribbean Clade 2, or, if having 2 seeds per locule is indeed a

stable character in the species, it could belong to section *Tetraspermium* (Clade 7) (Figure 2), but its restricted distribution and other morphological characters, such as deciduous cataphylls and long inflorescences do not match the latter clade well. In any case, it is likely that section *Gymnopodium* will end up embedded in another larger clade within *Anthurium*.

Section *LEPTANTHURIUM* Schott. As currently circumscribed, this section is represented by two species, *Anthurium gracile* and the relatively newly described *A. barrieri* Croat, Scherberich & Ferry. Previously, Croat and Sheffer (1983) emphasized that section *Leptanthurium* was "nevertheless unique and apparently quite natural", and *A. gracile* was perhaps the only species in *Anthurium* with white roots, owing to a layer of velamen (Table 2). They further noted that Sheffer and Kamemoto (1976) were unable to cross *A. gracile* with any other of the 56 species studied, that it has a distinctive polyploid series (2n=20, 40, 60), and that it is apparently self-pollinating or apomictic. More recently, Croat and his collaborators (Croat et al. 2006) in their original description of *A. barrieri* emphasized the presence of white roots, weakly differentiated primary lateral veins and red berries to assign the species to section *Leptanthurium*.

Carlsen (2011) sampled only *A. gracile* as a representative of the section, and found that it occurs in a well-supported clade with at least other two species, *A. vittariifolium* and *A. muyunense* (Clade 10) (Figure 2). If section *Leptanthurium* was expanded to accommodate these other species it would comprise plants with long thin spadices that bear few (up to three per spiral) and relatively large flowers (Carlsen 2011). Both Schott (1860) and Engler (1905) observed that species belonging to *Leptanthurium* – they also included *A. acutangulum*, *A. friedrichsthalii*, *A. guayaquilense* and *A.*

vittariifolium in the section – have the above mentioned inflorescence characteristics. However, Engler (1905) pointed out that several species that he placed in section *Leptanthurium* were only tentatively included there and could be assigned to other sections - he specifically mentioned *A. vittariifolium* as being closely related to section *Urospadix*. However, Engler's (1905) concept of section *Urospadix* was very wide.

Section *PACHYNEURIUM* Schott. This group included 13 species in the molecular phylogeny, four of them in series *Multinervia* (*Anthurium carchiense*, *A*. cf. *palenquense*, *A. napaeum* and *A. oxyphyllum*) and nine in series *Pachyneurium* (*A. bradeanum*, *A. crenatum*, *A. halmoorei*, *A. protensum*, *A. schottianum*, *A. spathiphyllum*, *A. spectabile*, *A. venosum* and *A. willifordii*). In all analyses, monophyly of the section is rejected ($P_{SH} = 0.003$, $P_{TT} = 0.0002$) (Table 5), although when the series are tested separately, monophyly for series *Multinervia* (Clade 13, Figure 2) cannot be rejected ($P_{SH} = 0.992$, $P_{TT} = 1$), whereas it is rejected for series *Pachyneurium* ($P_{SH} = 0.006$, $P_{TT} = 0.0012$) (Table 5). The main morphological character distinguishing section *Pachyneurium* (Table 2) is the distinctly involute leaf vernation (character # 11 - 1) (Table 6), but, as seen in the character reconstructions (Appendix 1), this character has evolved in parallel at least twice in *Anthurium*. Series *Multinervia* (Clade 13) is further recognizable by its numerous and closely spaced primary lateral veins (character # 22 - 2) (Tables 2 & 6). On the other hand, series *Pachyneurium* consists of at least two lineages. *A. willifordii* is placed in Clade 14, which so far comprises only two species with velvety leaves from Peru and Bolivia; Clade 14 is sister to Clade 13 - series *Multinervia*. The other species sampled in the series make up Clade 11, another distantly related group (Figure 2). Within Clade 11, the two species of *Anthurium* section *Pachyneurium*

sampled that are endemic to the Caribbean islands (*A. crenatum* and *A. venosum*) are grouped in a separate clade together, as is the case for other Caribbean endemics in the phylogeny (e.g. Clade 2) (Figure 2). Previous accounts have always suggested that section *Pachyneurium* was a "very natural" one (Croat & Sheffer 1983, Croat 1991), but this study finds that it is the series that are largely monophyletic, but not the section as a whole. In fact other characters used by Croat (1991) to further recognize the section (Table 2), such as the bird's-nest habit (character # 3 – state 3), large blades that are more or less oblanceolate (character # 13 – state 0), typically short petioles (character # 9 – state 0) and the absence of a collective vein (character # 20 – state 0), are highly homoplasious and occur in several other sections (Table 6).

Section *POLYNEURIUM* Engler. Five species of this section were included in this study and there was no support for its monophyly in any test ($P_{SH} = 0.004$, $P_{TT} = 0.0164$) (Table 5). The main morphological characters distinguishing it (Table 2) are highly homoplasious, thin blades (character # 15 - 0 or 1) and numerous closely spaced primary lateral veins (character # 22 - 3) (Table 6). There are at least three separate lineages within this group (Figure 2), *Anthurium altotambense*, *A.* cf. *urbanii* and *A. cuspidatum* cluster together along with other species in Clade 17, *A. longeinternodum* occurs in an unnumbered small clade with *A. dolichostachyum* and *A. balaoanum*, and finally, *A. maculosum* groups with species in the strongly supported Clade 12. All these species are in Clade E, but they are not immediately related. Croat and Sheffer (1983) recognized that this section seemed somewhat "unnatural", but that at least *A. cuspidatum* and its relatives might represent the core of section *Polyneurium*, as highlighted in Croat's online version of The *Anthurium* Primer (www.aroid.org/TAP).

Section *POLYPHYLLIUM* Engler. This section is well distinguished morphologically and molecularly from the rest of *Anthurium* (Clade A, Figure 2) (Carlsen 2011). It was represented by the only two species in the section, *Anthurium clidemioides* and *A. flexile*, and its monophyly (P_{SH} = 0.913, P_{TT} = 0.8405) (Table 5) was supported in all analyses. The section is characterized (Table 2) by having slender, wiry stems (character # 4 - 1) with adventitious roots along the entire internode (character # 5 - 1) and lacking 1-ribbed cataphylls (character # 7 - 1) (Table 6); sheathing petioles protect the new growth instead of cataphylls. These characters are synapomorphic within *Anthurium* (Appendix 1). In addition, these two species have shiny black or dark brown seeds, another unique character in the genus (Croat pers. comm.). All previous authors (Engler 1905, Croat & Baker 1978, Croat & Sheffer 1983) have considered this section to be a "very natural" one.

Section *PORPHYROCHITONIUM* Schott. Six species belonging to this section were included in the analyses, *Anthurium bakeri*, *A. bicollectivum*, *A. friedrichsthalii*, *A. lancifolium*, *A. punctatum* and *A. wendlingeri*. Results of the topology tests suggest that the section is not monophyletic (P_{SH} = 0.046, P_{TT} = 0.0136) (Table 5). Croat and Sheffer (1983) characterized this section almost solely on the presence of punctations on the leaves (character # 18 – state 1) (Tables 2 & 6), although species included also generally had slender stems yet short internodes (character # 6 – state 0), and elongate, non-cordate (i.e. linear or lanceolate) leaf blades (character # 13 – states 0 or 1). In the molecular phylogeny (Carlsen 2011), species belonging to section *Porphyrochitonium* are all loosely related to each other, intermixed with other species in section *Digitinervium*, and embedded within a larger and strongly supported clade (Clade 6, Figure 2). Clade 6

comprises a group of *Anthurium* species all of which have leaves with these punctations and may indeed deserve formal recognition. The original circumscription of the section (Schott 1860, Engler 1905) included only one species, *A. scherzerianum* Schott, which was well known in cultivation at that time. These authors pointed out its close relationship with section *Tetraspermium* (Clade 7) based on leaf punctations and possible presence of two ovules per locule, separating it from the latter by having a stem that was not elongated. On the other hand, Croat and Sheffer (1983) suggested that the characteristic large bright red spathe of *A. scherzerianum* was the reason why previous taxonomists had kept it apart in a separate section.

Section *SCHIZOPLACIUM* Schott. Three species in this section were included in the analyses. Although Croat and Sheffer (1983) suggested that Schott's delimitation of the section was probably "very natural", results of topology tests that constrain the monophyly of the group do not support the section as monophyletic ($P_{SH} = 0$, $P_{TT} < 0.0001$) (Table 5). The main characters used to distinguish the section (Table 2) were the presence of palmately lobed leaves (character # 12 - 1, character # 13 - 4) with more than 3 segments (character # 16 - 2) that are united at the base (character # 17 - 2) (Table 6). In comparison with other sections, the characters used to recognize section *Schizoplacium* are not very homoplasious, but reconstructions of the evolution of these character states on the molecular phylogeny of *Anthurium* (Carlsen 2011) revealed at least three different and distantly related lineages with parallel evolution of this combination of characteristics (Appendix 1). *Anthurium pedatoradiatum* belongs to the strongly supported northern Central American group (Clade 15), sharing with other species in that clade their characteristic bright orange berries. *Anthurium pedatum* is embedded within

Clade 18, a mixture of species with lobed and cordate leaves, and *A. longissimum* is grouped in a larger clade of *Anthurium* with palmate leaves (Clade 4) along with other species from section *Dactylophyllium* (Figure 2).

This pattern suggests that the palmately-lobed morphology with segments united at the base, as in section *Schizoplacium*, is easier to gain and lose than the truly palmate leaves with segments free to the base (see section *Dactylophyllium*, Clade 4, above). Indeed, Madison (1978) had pointed out that the general leaf morphology of some species of section *Schizoplacium*, such as *A. pedatum*, resembles more a highly dissected cordate blade than a truly palmately-divided leaf. Hence, he separated that species from the section, and because of its pendent, not erect, inflorescences he proposed that it would be better grouped with the *A. gualenanum* Engler complex, from Colombia; unfortunately none of the species in this complex were sampled in the molecular phylogeny. Madison (1978) also segregated the Mexican species *A. pedatoradiatum* and *A. podophyllum* from section *Schizoplacium*, placing them in their own clade distinguished by terrestrial, acaulescent habit and elongate peduncle.

Section *SEMAEOPHYLLIUM* Schott. This section is not monophyletic based on the results of topology tests ($P_{SH} = 0.004$, $P_{TT} = 0.001$) (Table 5). In their revision of the section, Carlsen and Croat (2007) had already suggested that section *Semaeophyllium* was probably not monophyletic. However, the section was fairly easy to recognize (Table 2) by its trilobed leaves (character # 12 - 1, character # 13 - 3, character # 16 - 1) with lateral lobes united at the base (character # 17 - 1) (Table 6) and oriented either at 90° from the central lobe (i.e. spreading) or pointing toward the apex of the blade (i.e. falcate), but never toward the base (i.e. cordate). Although the strongly trilobed leaves, as

in *Anthurium trilobum*, found in section *Semaeophyllium* are indeed unique to the section, intermediates between trilobed and cordate species are well known in section *Belolonchium* (e.g. *A. draconopterum* Sodiro and *A. effusilobum* Croat), as well as in *Semaeophyllium* itself (*A. sagittaria*, *A. signatum*, *A. subsignatum*). Such cases may therefore imply that trilobed leaves are more like a special case of cordate leaf morphology.

The three species sampled here all belong to Clade E (Figure 2), a weakly supported group in *Anthurium* comprising mainly species with cordate leaves (Carlsen 2011). *Anthurium furcatum* belongs to the strongly supported Clade 18, which also includes another species with lobed leaves, *A. pedatum*, previously placed in section *Schizoplacium*. *Anthurium subsignatum* and *A. trilobum* are well separate from the first, but are not included in any particular major clade (Figure 2). Carlsen and Croat (2007) had already suggested a similar division, assigning *A. subsignatum* and *A. trilobum* to Group 1 of section *Semaeophyllium* based on their characteristic bright yellow inflorescences and fruits with purple-red tips, while *A. furcatum* was placed in Group 4, which was distinguished by its stipitate, red-purple spadices.

Section *TETRASPERMIUM* Schott. is a monophyletic ($P_{SH} = 0.421$, $P_{TT} = 0.0881$) (Table 5) and strongly supported section (Carlsen 2011), represented here by six species, *Anthurium citrifolium*, *A. laciniosum*, *A. obtusum*, *A.* cf. *obtusum*, *A. scandens* and *A. margaricarpum* (Clade 7, Figure 2). Even though the first five species above belong to this clade, *A. margaricarpum* is grouped in a separate but closely related lineage (Clade 9). Although Engler (1905) had included this species in section *Tetraspermium*, he noted that it was unusual there because the leaves and spadix were

much bigger than those of the rest of species in the section. Sodiro (1903) also pointed out that *A. margaricarpum* was atypical within *Tetraspermium* and that it looked more similar to the *A. andinum* group. The position of *A. margaricarpum* in the molecular phylogeny of *Anthurium* (Clade 9, Figure 2) indeed suggests that the species should be excluded from section *Tetraspermium* (Clade 7). The closest relatives of *A. margaricarpum* are instead found within a larger group of species with punctate leaves that probably includes also the *A. andinum* group. The number of seeds per fruit should be reexamined when material becomes available.

All previous authors (Schott 1860, Engler 1905, Croat & Sheffer 1983) agreed that *Tetraspermium* is a distinct section in *Anthurium* characterized by (Table 2) glandular punctations on the leaves (character # 18 – state 1) and fruits with two seeds per locule (character # 24 – state 1), two of the strongest characters used in the current sectional classification (Table 6). The presence of punctations unites this section with species previously assigned to sections *Digitinervium* and *Porphyrochitonium* in a larger Clade 6 (Figure 2, and Carlsen 2011). Interestingly, Croat and Sheffer (1983) emphasized that none of the numerous interspecific hybridization attempts using *A. scandens* and *A. obtusum* as pollen parents had succeeded, perhaps due to the distinct chromosome numbers of these species, 2n=24, and a polyploid series 2n=24, 48, 84 that is unique in the genus (Sheffer & Kamemoto 1976); is not known if members of section *Digitinervium* and *Porphyrochitonium* were involved in the crosses.

Section *UROSPADIX* Engler. The section was represented by nine species in all analyses and it is the only group with contradictory results in topology tests. The Shimodaira-Hasegawa test showed that monophyly could not be rejected ($P_{SH} = 0.146$),

but according to Templeton's test, the section is not monophyletic ($P_{TT} = 0.026$) (Table 5). From the position of species in the molecular phylogeny (Carlsen 2011), it is evident that sectional limits need some rearrangement (Figure 2). Most of the species sampled (*Anthurium bellum*, *A. coriaceum*, *A. harrisii*, *A. longipes*, *A. lucidum*, *A. olfersianum* and *A. parvum*) cluster together in the strongly supported Clade 1. This clade also includes *A. bromelicola*, a highly specialized epiphyte growing in bromeliad tanks, whose affinities in *Anthurium* were previously unknown (Mayo et al. 2000), and *A. radicans*, a species formerly isolated in its own monotypic section *Chamaerepium*. Although morphologically the two latter species are very distinct, like the rest of Clade 1 they are all Brazilian endemics (character # 1 - 1) (Table 6), indeed, geographic distribution is one of the best characters in *Anthurium*. Carlsen (2011) showed that endemism to Brazil is a better predictor of species relationships in *Anthurium* than morphological characteristics. A new circumscription of section *Urospadix* to include all species in Clade 1, being distinguished on geographic location (i.e. all are Brazilian endemic species), is therefore needed to render this section monophyletic, as previously suggested by the molecular study of Temponi (2006). Engler (1905) and Croat and Sheffer (1983) further recognized the group of species concentrated in Brazil (Table 2) by having typically close, numerous primary lateral veins (character # 22 - 2) that are scarcely more prominent than the interprimary veins (character # 21 - 1) (Table 6). However, these characters are homoplasious in *Anthurium*, although the latter could potentially be a synapomorphy for a larger Clade C that includes both Brazilian (Clade 1) and Caribbean (Clade 2) endemics (Appendix 1, Figure 2). Indeed, the other two species sampled in this section, *A. acaule* and *A. willdenowii*, both restricted to the Caribbean islands, do not group with the

Brazilian species, but rather cluster in Clade 2 with the other Caribbean endemics included. Although these two species superficially resemble other *Urospadix* because of a synapomorphy of the larger Clade C, their primary lateral veins that are poorly differentiated from interprimary veins, they are definitely separable from Brazilian endemics based on their geography, and therefore should be excluded from section *Urospadix*.

Section *XIALOPHYLLIUM* Schott. This species-rich and variable section was represented in the molecular phylogeny by two species, *Anthurium microspadix* and *A. mindense*. So far, based on this limited sampling, it is monophyletic ($P_{SH} = 0.889$, $P_{TT} = 0.8273$) (Table 5) with both species clustering within the highly supported Clade 17 (Figure 2) that also includes *A.* cf. *urbanii*, *A. cuspidatum* and *A. altotambense*, formerly of section *Polyneurium*. Schott (1860) and Engler (1905) characterized section *Xialophyllium* (Table 2) with characters that have turned out to be highly homoplasious, e.g. climbing stems and long internodes (character # 6 - 1) and leaf blades that are typically longer than broad and rarely lobed at the base (i.e. lanceolate or linear) (character # 13 - 0) (Table 6). Croat and Sheffer (1983) argued that Engler's (1905) delimitation of this section was "unnatural", since it included two different types of plants. One included species with thin, "veiny" and somewhat bullate leaf blades and greenish inflorescences (e.g. *A. microspadix* and *A. mindense*, both species sampled here) whereas the other is characterized by having more coriaceous blades that are not markedly "veiny" or bullate (e.g. *A. caucanum* Engler). Results here suggest that at least Clade 17 could be included in *Xialophyllium*; however, sampling should be extended to

include representatives of the other morphology included in the section and then corroborate or not its monophyly.

A few of the species sampled in the molecular study of Carlsen (2011), for example, *A. chiapasense*, *A. lucens* and *A. verapazense*, from northern Central America, could not be unambiguously assigned to only one of the sections recognized by Croat and Sheffer (1983) without modifying their current morphological circumscriptions. These species have both cordate leaves (character # 13 – 2) and punctations on the blades (character # 18 – 1) (Tables 2 & 6). So far, all species in sections characterized by punctate blades have lanceolate or linear leaves, sometimes subcordate but never truly cordate, except for *A. peltigerum* Sodiro, and none of the sections with species having truly cordate leaves posses punctations on the blades. These three distinct species cluster together in Clade 3 (Figure 2), well separated from other northern Central American representatives (in Clade 15) but in the same larger Clade D as other species with punctate leaves (Clade 6). Thus although there are at least two origins of punctations in *Anthurium*, this character is still valuable as synapomorphy for both Clades 3 and 6 (Appendix 1).

Another small group of species, *Anthurium cordatum*, *A. cordifolium* and *A. dominicense*, all endemic to the Caribbean islands, could not be assigned to any of the recognized sections of *Anthurium* with cordate leaves (*Belolonchium*, *Calomystrium* or *Cardiolonchium*) (Table 2) because of their thicker (character # 15 – states 3 or 4) blades that are not velvety (character # 14 – state 0) and cataphylls persisting as fibers (character # 8 – state 0) (Table 6). They group together in the phylogeny with other Caribbean

endemics in Clade 2 (Figure 2), but their cordate leaf morphology is very different from that of the other species in this clade which have lanceolate leaves (Appendix 1).

CONCLUSIONS AND FURTHER RESEARCH

The results of this study suggest that the current sectional classification of *Anthurium* does not accurately represent species relationships or the evolutionary history of the genus. Disagreements between the combined cpDNA-nDNA molecular phylogeny and the morphologically based sectional classification are because the most obvious morphological characters previously used in sectional classifications turned out to be highly homoplasious within *Anthurium*. There have been multiple independent gains or losses of seemingly similar morphologies in distantly related clades. However, it is unknown if the authors of some of these previous classifications may have been emphasizing simple species group recognition over evolutionary history.

This study presents evidence that nine of a total of 18 sections and two series currently recognized in *Anthurium* are not monophyletic (*Belolonchium*, *Cardiolonchium*, *Decurrentia*, *Pachyneurium*, *Pachyneurium* series *Pachyneurium*, *Polyneurium*, *Porphyrochitonium*, *Schizoplacium*, *Semaeophyllium*). Four sections/series are monophyletic (*Calomystrium*, *Pachyneurium* series *Multinervia*, *Polyphyllium*, *Xialophyllium*), however, increased sampling within section *Xialophyllium* would very likely show different results. At least one (*Chamaerepium*) or possibly two (*Gymnopodium*) monotypic sections are included in larger strongly supported monophyletic clades. Section *Leptanthurium* should be expanded to accommodate a few other closely related species. For four sections, their monophyly could not be rejected based on topology tests yet they seem to be paraphyletic or polyphyletic based on the

placement of their representative species in the phylogeny. In these few cases, sections *Dactylophyllium* and *Digitinervium* need to be expanded to include one or two more species, while sections *Tetraspermium* and *Urospadix* need a few species removed in order to produce monophyletic groupings.

A new sectional classification of *Anthurium* that integrates morphological characters better representing evolutionary patterns within the genus is much needed. Despite having a better understanding of the evolutionary history of *Anthurium*, resolution is still lacking in some areas of the phylogeny, and increased taxon sampling may suggest the presence of other monophyletic clades not recovered here. Once monophyletic groups of species are characterized, manageable species-level taxonomic, evolutionary, ecological and biogeographic studies will be more feasible.

Examination of all the characters previously used by Croat and Sheffer (1983) in sectional delimitation, twenty-four in total, showed that the majority of them are highly homoplasious, and therefore not useful for recognizing clades in *Anthurium*. However, a few characters states within an otherwise homoplasious character, e.g. very thin or wiry stems, cataphylls persisting intact, involute leaf vernation, and primary lateral veins poorly differentiated from interprimaries, could be used as synapomorphies for strongly supported clades. Other characters useful for recognizing monophyletic clades in *Anthurium* and with relatively low levels of homoplasy are the presence or absence of punctations on leaves and palmate leaves with segments free or united at the base. The least homoplasious characters revealed by this study are characteristics of the stem, such as position of the roots and presence of 1-ribbed cataphylls; fruiting characters, such as the number of seeds per locule; and geography, mainly Brazilian origin or not. These

characters have been previously used to characterize sections within *Anthurium*. Results here suggest that more research is needed to further test morphological and anatomical characters not previously studied in *Anthurium*.

This study shows that *Anthurium* species endemic to Brazil, the Caribbean islands or northern Central America form strongly supported monophyletic groups, whereas wider ranging species with distributions that reach into such areas are not part of these monophyletic clades. Carlsen (2011) has shown that geographic location is a very good predictor of species relationships in *Anthurium*, sometimes more so than vegetative characteristics. Indeed, given the occurrence of geographically circumscribed clades in *Anthurium*, geographic affinities may help guide further studies.

Inflorescence and infructescence characters, previously neglected in identification of species groups, should be studied in the genus *Anthurium* to better elucidate their role in lineage recognition and diversification. Madison (1978) has been the only author so far to explore the diagnosis of groups based on geographical and reproductive characteristics similar to those that are found most useful in this study. His division of *Anthurium* species with palmately lobed leaves seems to be the most congruent with findings of the molecular phylogeny of the genus (Carlsen 2011), suggesting that this type of leaf evolved independently several times in the genus.

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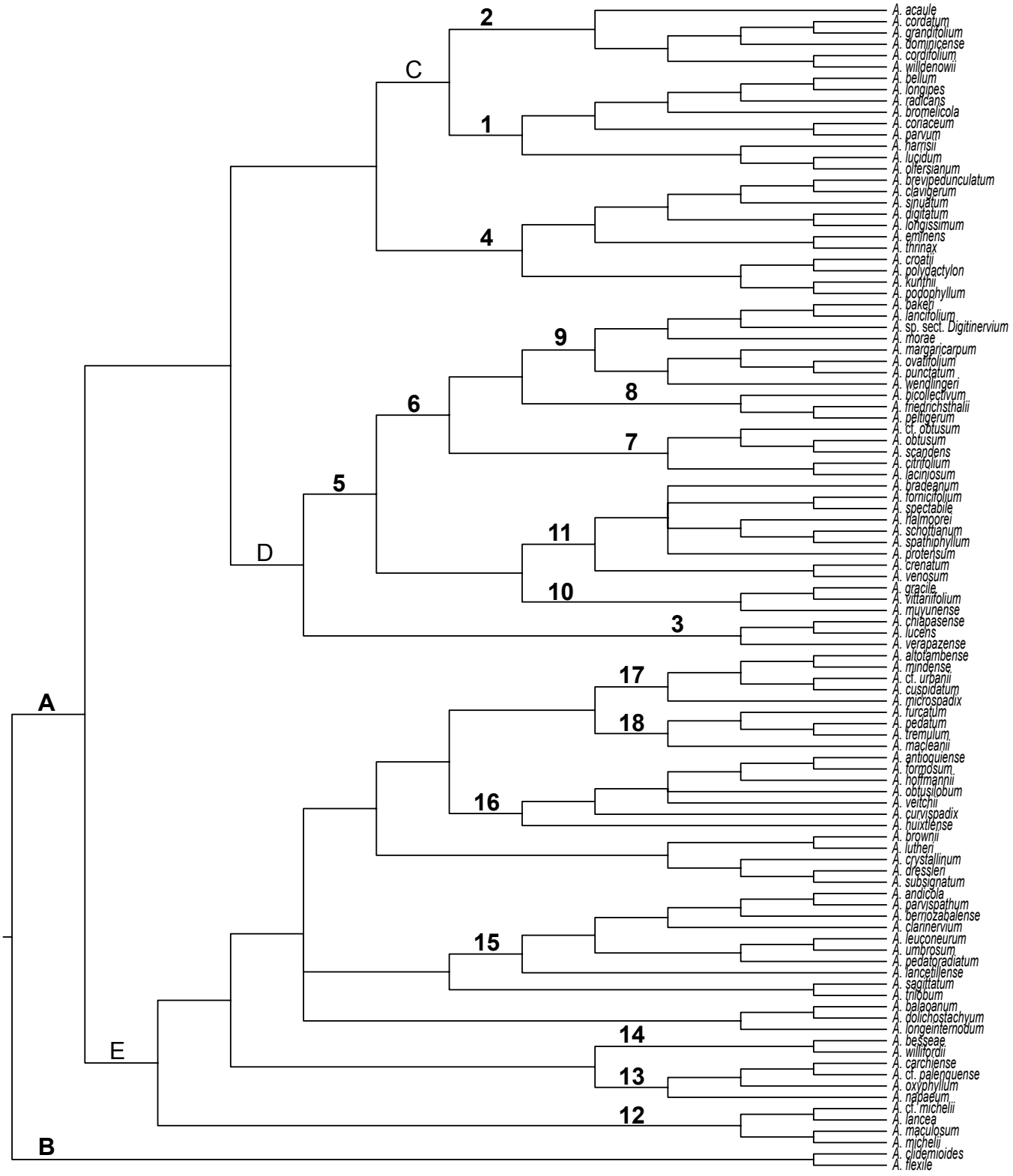


Figure 1. The first of the most-parsimonious trees obtained from parsimony ratchet analyses of combined cpDNA and nDNA sequences of Anthurium. Clade numbering and letters correspond to strongly supported clades in Carlsen (2011).

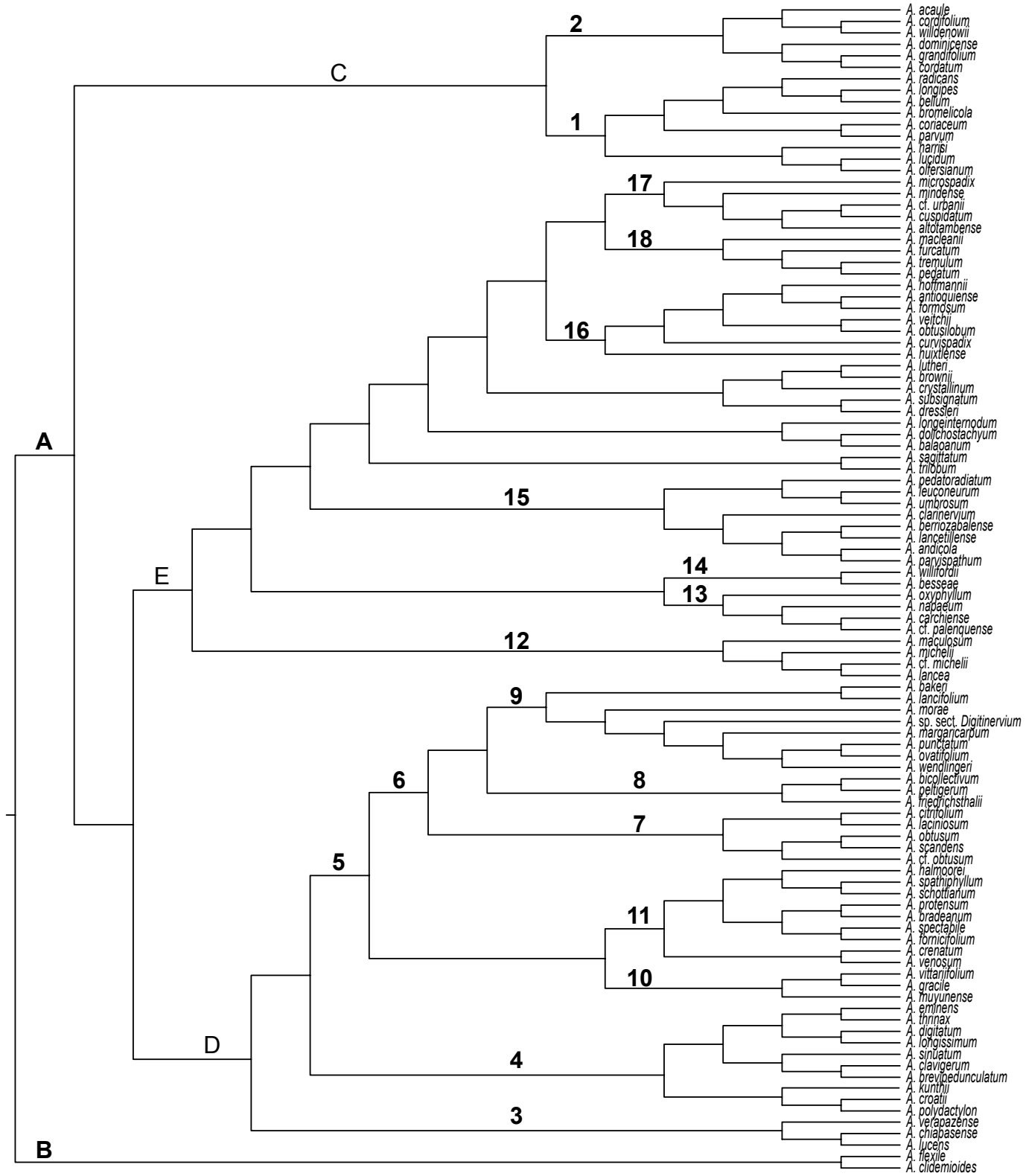


Figure 2. The best scoring maximum likelihood tree obtained from likelihood analyses of combined cpDNA and nDNA sequences of Anthurium. Clade numbering and letters correspond to strongly supported clades in Carlsen (2011).

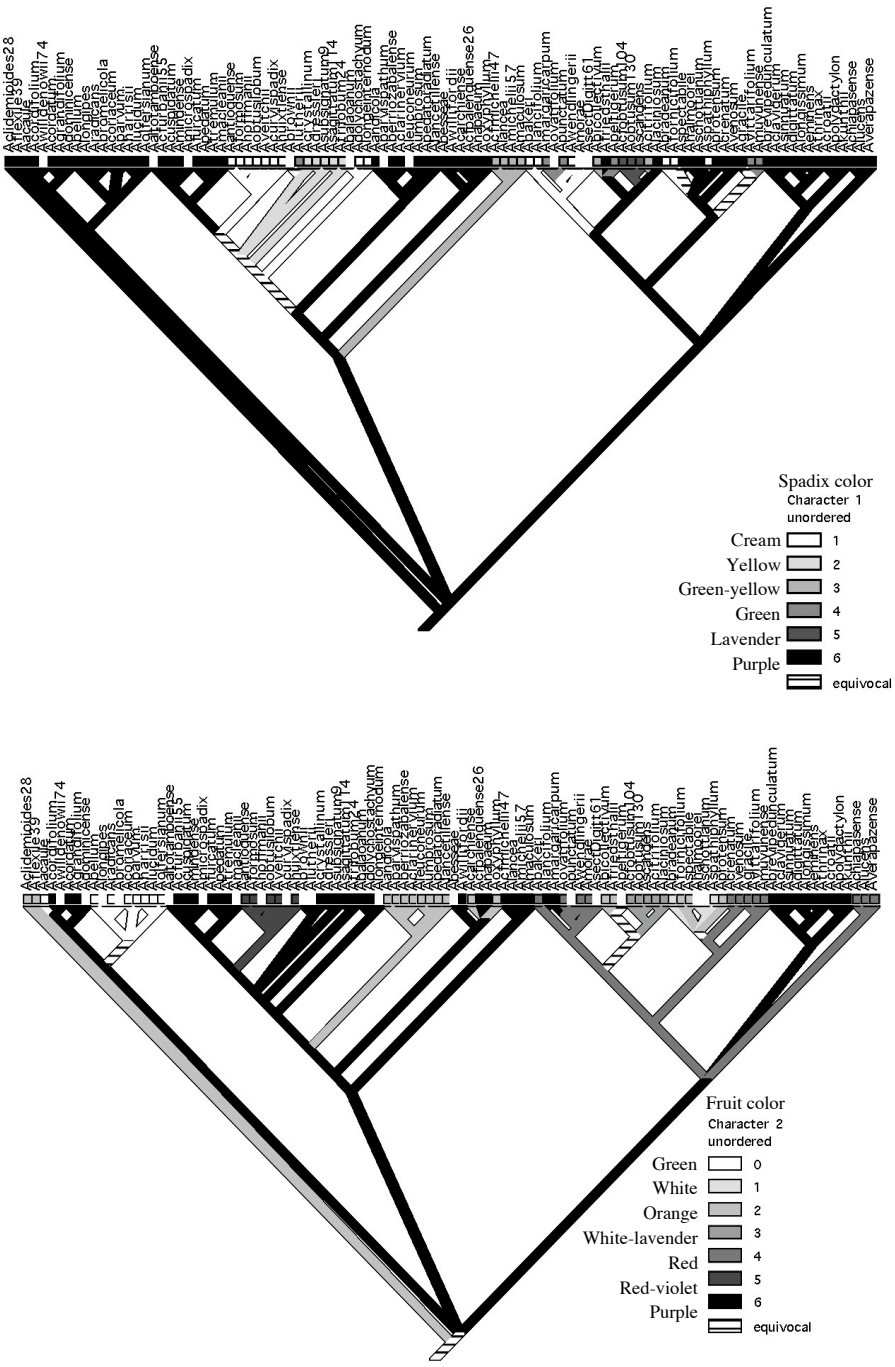


Figure 3. Character state reconstructions for spadix and fruit color along the *Anthurium* molecular phylogeny.

Table 1. A comparison of the main sectional classification systems of *Anthurium* to date.

Schott (1860)	**Engler (1905)**	**Croat and Sheffer (1983)**
Tetraspermium	Tetraspermium	Tetraspermium
	Gymnopodium	Gymnopodium
Porphyrochitonium	Porphyrochitonium	Porphyrochitonium
Pachyneurium	Pachyneurium	Pachyneurium
Eucardium	= Pachyneurium	
Macrophyllium	pro parte Pachyneurium	
Chondrophyllium	pro parte Pachyneurium, pro parte Urospadix	
	Polyphyllium	Polyphyllium
Leptanthurium	Leptanthurium	Leptanthurium
Oxycarpium	Oxycarpium	Oxycarpium [(1)]
Xialophyllium	Xialophyllium	Xialophyllium
	Polyneurium	Polyneurium
	Urospadix	Urospadix
Erythropodium	= Urospadix	
Acamptophyllium	= Urospadix	
Platylonchium	= Urospadix	
Oophyllium	= Urospadix	
Parabasium	= Urospadix	
Episeiostenium	Episeiostenium	Episeiostenium [(2)]
	Digitinervium	Digitinervium
Cardiolonchium	Cardiolonchium	Cardiolonchium
Neurolysium	= Cardiolonchium	
Cosmetophyton	pro parte Cardiolonchium	
Pleonophlebium	pro parte Cardiolonchium, pro parte Belolonchium	
	Chamaerepium	Chamaerepium
Calomystrium	Calomystrium	Calomystrium
Sobaronium	pro parte Calomystrium, pro parte Belolonchium, pro parte Pachyneurium	
Amphineurium	pro parte Calomystrium, pro parte Polyneurium	

Andiphilum	pro parte Calomystrium, pro parte Pachyneurium	
Belolonchium	Belolonchium	Belolonchium
Dorylonchium	pro parte Belolonchium	
Semaeophyllium	Semaeophyllium	Semaeophyllium
Schizoplacium	Schizoplacium series Euschizoplacium	Schizoplacium
Dactylophyllium	Schizoplacium series Dactylophyllium	Dactylophyllium

Note: The circumscriptions of sections vary somewhat among authors. (1) This sectional name was later synonymized with section *Pachyneurium* due to the transfer of the type species, *Anthurium oxycarpium* Poeppig & Endl., to that section by Croat (1991), and a new sectional name, section *Decurrentia*, was proposed to accommodate the remaining species in section *Oxycarpium* (Croat et al. 2005b). (2) This section was mentioned in Croat and Sheffer's classification system, but specifically not discussed or further recognized because it was "the least likely to be a valid section".

Table 2. Currently accepted sectional classification of *Anthurium* (Croat and Sheffer 1983), species diversity, species sampled in this study, and main diagnostic features for each section.

Section	Total species number	Species sampled	Main morphological characters	Geographical distribution
Belolonchium	approx. 130	9	Cordate, thin blades, growing at high elevations	Andes
Calomystrium	approx. 90	7	Cataphylls persisting intact, cordate blades	Costa Rica to Peru
Cardiolonchium	approx. 90	8	Velvety blades with pale venation, short internodes, ribbed petiole	Western slopes of Andes
Chamaerepium	1	1	Creeping habit, short, ellipsoid spadix	Endemic to Brazil
Dactylophyllium	19	9	Palmately divided leaves with 3 or more segments free to the base	Widespread, mostly Amazonian
Decurrentia	approx. 45	7	Short internodes, elongated, epunctate leaf blades, peduncle ridged	Amazonian
Digitinervium	approx. 30	3	Punctations, numerous parallel primary lateral veins, 2 or more pairs of collective veins	Costa Rica to Venezuela
Gymnopodium	1	0	Climber, cordate blades, berries up to 4 seeds	Endemic to Cuba
Leptanthurium	2	1	Roots with velamen	Widespread
Pachyneurium			Involute leaf vernation, "bird's nest habit"	Widespread
series *Multinervia*	8	4	Numerous, conspicuous primary lateral veins	Widespread
series *Pachyneurium*	112	9	Collective vein sometimes absent	Widespread
Polyneurium	approx. 100	5	Thin blades with many, close primary lateral veins	Nicaragua to Venezuela
Polyphyllium	2	2	Adventitious roots along internodes, wiry stems, absence of 1-ribbed cataphylls	Central American
Porphyrochitonium	approx. 130	6	Punctate blades	Panama to South America
Schizoplacium	8	4	Palmately divided leaves with more than 3 segments not free to the base	Mexico, West Indies to Venezuela
Semaeophyllium	23	3	Deeply three-lobed leaves	Central and South America
Tetraspermium	10	6	Scandent, thin stems, long internodes, punctations, 4 seeds per berry	Widespread
Urospadix	79	10	Close, numerous primary veins, short internodes	Brazil and West Indies
Xialophyllium	approx. 70	2	Internodes long, thin blades, longer than broad	Mexico to Bolivia

Table 3. Morphological characters previously used in the sectional classification of *Anthurium*.

Character	Character states
1. Geography, endemic to Brazil	no (0), yes (1)
2. Habitat, elevation	low 0-500 m (0), low-medium 0-1200 m (1), medium 600-1400 m (2), medium-high 1200-1800 m (3), high > 1800 m (4), all (5)
3. Habit	caespitose (0), scandent (1), repent (2), "bird's nest" (3)
4. Stem, thickness	thick > 1 cm (0), thin < 1 cm (1)
5. Root, position	in nodes (0), along internodes (1)
6. Internode, length	short < 3 cm (0), long > 3 cm (1)
7. One-ribbed cataphyll, presence	present (0), absent (1)
8. Cataphyll, texture	persistent fibers (0), persistent intact (1), deciduous (2), not applicable (3)
9. Petiole, length (compared to lamina length)	shorter (0), shorter-equal (1), equal-longer (3), longer (4)
10. Petiole, shape	not ribbed (0), ribbed (1)
11. Leaf, vernation	supervolute (0), involute (1)
12. Leaf, shape 1, lobes	entire (0), lobed – 3 or more lobes (1)
13. Leaf, shape 2, overall shape	lanceolate-elliptic (0), linear (1), cordate (2), trilobed (3), palmate (4)
14. Leaf, texture 1, velvety	not velvety (0), subvelvety (1), velvety (2)
15. Leaf, texture 2, thickness	thin (0), medium-thin (1), medium (2), medium-thick (3), thick (4)
16. Palmate leaf, shape 1, # of segments per leaf	not applicable (0), 3 segments (1), > 3 segments (2)
17. Palmate leaf, shape 2, segment union	not applicable (0), segments united at base (1), segments free at base (2)
18. Punctation, presence	no (0), yes (1)
19. Punctation, position	not applicable (0), abaxial surface only (1), both surfaces (2)
20. Venation, # of collective veins	zero (0), one (1), two (2)
21. Venation, primaries same as interprimaries	no (0), yes (1)
22. Venation, # of primary lateral veins	few-sparse < 5 (0), medium-sparse 5-10 (1), numerous-close > 10 (2)
23. Spadix, length	short 0-5 cm (0), short-medium 5-10 cm (1), medium-long 10-15 cm (2), long > 15 cm (3)
24. Seeds, number per locule	one (0), two (1), up to three (2)

Table 4. Morphological matrix of *Anthurium* species sampled in this study. Character number and states same as in Table 3. Sectional classification follows Croat and Sheffer (1983) (*). Species morphology was based on available literature, herbarium specimens and living collections (**).

Species	Section	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Anthurium acaule	URO	0	0	3	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	1	1	2	2	0
Anthurium altotambense	POLYN	0	1	0	0	0	0	0	0	4	0	0	0	2	0	2	0	0	0	0	1	0	2	3	0
Anthurium andicola	BEL	0	3	0	0	0	0	0	0	4	1	0	0	2	0	3	0	0	0	0	1	0	0	2	0
Anthurium antioquiense	CAL	0	3	0	0	0	0	0	1	4	0	0	0	0	0	3	0	0	0	0	1	0	1	0	0
Anthurium bakeri	POR	0	5	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	1	1	1	1	2	2	0
Anthurium balaoanum	CAR	0	3	1	0	0	0	0	2	4	0	0	0	2	0	1	0	0	0	0	0	0	1	2	0
Anthurium bellum	URO	1	0	2	0	0	0	0	2	4	0	0	0	0	0	2	0	0	0	0	1	1	2	2	0
Anthurium berriozabalense	BEL	0	2	0	0	0	0	0	0	4	0	0	0	2	0	3	0	0	0	0	1	0	0	1	0
Anthurium besseae	CAR	0	0	0	0	0	0	0	0	4	0	?	0	2	2	3	0	0	0	0	1	0	0	0	0
Anthurium bicollectivum	POR	0	5	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	1	2	2	1	2	2	0
Anthurium bradeanum	PACpac	0	0	3	0	0	0	0	0	3	0	1	0	0	0	3	0	0	0	0	1	0	1	1	0
Anthurium brevipedunculatum	DAC	0	1	1	0	0	1	0	2	4	0	0	1	4	0	2	2	2	0	0	1	0	0	0	0
Anthurium bromelicola	???	1	0	1	1	0	1	0	2	4	0	0	0	0	0	3	0	0	0	0	1	1	2	0	0
Anthurium brownii	BEL	0	1	0	0	0	0	0	0	4	0	0	0	2	0	4	0	0	0	0	1	0	1	2	0
Anthurium carchiense	PACmulti	0	3	3	0	0	0	0	0	3	0	1	0	0	0	3	0	0	0	0	1	0	2	0	0
Anthurium chiapasense	???	0	3	0	0	0	0	0	0	4	0	0	0	2	0	3	0	0	1	1	1	0	0	1	0
Anthurium citrifolium	TET	0	5	1	1	0	1	0	0	0	0	0	0	0	2	3	0	0	1	2	1	1	1	0	1
Anthurium clarinervium	CAR	0	2	0	0	0	0	0	0	3	0	0	0	2	2	3	0	0	0	0	1	0	0	1	0
Anthurium clavigerum	DAC	0	5	1	0	0	1	0	2	4	0	0	1	4	0	2	2	2	0	0	1	0	0	3	0
Anthurium clidemioides	POLYP	0	1	1	1	1	1	1	3	1	0	0	0	2	1	1	0	0	0	0	1	0	2	1	2
Anthurium cordatum	???	0	0	0	0	0	0	0	0	4	0	0	0	2	0	3	0	0	0	0	1	0	0	3	0
Anthurium cordifolium	???	0	0	0	0	0	0	0	2	4	0	0	0	2	0	1	0	0	0	0	1	0	1	3	0
Anthurium coriaceum	URO	1	0	3	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	1	1	2	1	0
Anthurium crenatum	PACpac	0	1	3	0	0	0	0	0	3	1	1	0	0	0	4	0	0	0	0	1	0	1	3	0
Anthurium croatii	DAC	0	5	0	0	0	0	0	2	4	0	0	1	4	0	1	2	2	0	0	1	0	1	2	0
Anthurium crystallinum	CAR	0	1	0	0	0	0	0	0	4	0	0	0	2	2	1	0	0	0	0	1	0	0	2	0

Species	Section	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Anthurium curvispadix	CAL	0	1	0	0	0	0	0	1	4	0	0	0	2	0	3	0	0	0	0	1	0	0	2	0
Anthurium cuspidatum	POLYN	0	4	0	0	0	0	0	2	4	0	0	0	1	0	2	0	0	0	0	1	0	2	3	0
Anthurium digitatum	DAC	0	5	1	0	0	0	0	0	4	0	0	1	4	0	2	2	2	0	0	1	0	1	2	0
Anthurium dolichostachyum	CAR	0	5	0	0	0	0	0	0	4	0	0	0	2	1	4	0	0	0	0	1	0	2	3	0
Anthurium dominicense	???	0	0	0	0	0	0	0	0	4	0	0	0	2	0	3	0	0	0	0	1	1	2	2	0
Anthurium dressleri	CAR	0	0	0	0	0	0	0	2	4	1	0	0	2	2	3	0	0	0	0	1	0	0	0	0
Anthurium eminens	DAC	0	5	1	0	0	1	0	0	4	0	0	1	4	0	3	2	2	0	0	1	0	1	3	0
Anthurium flexile	POLYP	0	1	1	1	1	1	1	3	4	0	0	0	0	0	1	0	0	0	0	1	0	2	1	2
Anthurium formosum	CAL	0	3	0	0	0	0	0	1	4	0	0	0	2	0	3	0	0	0	0	1	0	1	1	0
Anthurium fornicifolium	DEC	0	3	0	0	0	0	0	0	3	0	1	0	1	2	4	0	0	0	0	1	0	2	1	0
Anthurium friedrichsthalii	POR	0	5	0	0	0	0	0	0	1	0	0	0	1	0	4	0	0	1	1	1	1	2	2	0
Anthurium furcatum	SEM	0	1	0	0	0	0	0	0	4	0	0	1	3	0	3	1	1	0	0	1	0	2	2	0
Anthurium gracile	LEP	0	0	0	0	0	0	0	2	4	0	0	0	1	0	1	0	0	0	0	1	1	2	3	0
Anthurium grandifolium	BEL	0	0	0	0	0	0	0	0	4	0	0	0	2	0	3	0	0	0	0	1	0	0	3	0
Anthurium halmoorei	PACpac	0	1	3	0	0	0	0	0	3	1	1	0	0	0	4	0	0	0	0	1	0	1	3	0
Anthurium harrisii	URO	1	0	0	0	0	0	0	2	0	0	0	0	1	0	1	0	0	0	0	1	1	2	1	0
Anthurium hoffmannii	CAL	0	4	0	0	0	0	0	1	4	0	0	0	2	0	3	0	0	0	0	1	0	0	1	0
Anthurium huixtlense	CAL	0	3	0	0	0	0	0	1	4	0	0	0	2	0	3	0	0	0	0	1	0	0	0	0
Anthurium kunthii	DAC	0	5	1	0	0	1	0	0	4	0	0	1	4	0	1	2	2	0	0	1	0	1	3	0
Anthurium laciniosum	TET	0	5	1	1	0	1	0	0	0	0	0	0	0	1	3	0	0	1	2	1	1	1	1	1
Anthurium lancea	BEL	0	4	0	0	0	0	0	0	4	0	0	0	2	0	3	0	0	0	0	1	0	1	3	0
Anthurium lancetillense	BEL	0	0	0	0	0	0	0	0	4	0	0	0	2	0	3	0	0	0	0	1	0	0	1	0
Anthurium lancifolium	POR	0	5	0	0	0	0	0	0	4	0	0	0	0	0	3	0	0	1	2	1	0	2	3	0
Anthurium leuconeurum	CAR	0	?	0	0	0	0	0	0	4	0	0	0	2	2	2	0	0	0	0	1	0	0	1	0
Anthurium longeinternodum	POLYN	0	3	1	0	0	1	0	0	4	0	0	0	0	0	3	0	0	0	0	1	0	2	1	0
Anthurium longipes	URO	1	0	0	0	0	0	0	0	4	0	0	0	1	0	4	0	0	0	0	1	1	2	2	0
Anthurium longissimum	SCH	0	3	1	0	0	1	0	2	4	0	0	1	4	0	4	2	1	0	0	1	0	1	3	0
Anthurium lucens	???	0	3	0	0	0	0	0	0	4	0	0	0	2	0	3	0	0	1	1	1	0	1	2	0
Anthurium lucidum	URO	1	0	0	0	0	0	0	2	4	0	0	0	0	0	3	0	0	0	0	1	1	2	1	0
Anthurium lutheri	DEC	0	1	1	0	0	1	0	2	4	0	0	0	1	1	3	0	0	0	0	1	0	2	1	0

Species	Section	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Anthurium macleanii	BEL	0	4	0	0	0	0	0	0	4	0	0	0	2	0	3	0	0	0	0	1	0	1	2	0
Anthurium maculosum	POLYN	0	4	0	0	0	0	0	2	4	0	0	0	0	0	3	0	0	0	0	1	0	1	1	0
Anthurium margaricarpum	TET	0	5	0	0	0	0	0	0	4	0	0	0	0	0	3	0	0	1	1	1	0	2	0	0
Anthurium michelii	DEC	0	3	0	0	0	0	0	0	4	0	0	0	0	0	1	0	0	0	0	1	0	1	1	0
Anthurium cf. *michelii*	DEC	0	3	0	0	0	0	0	2	0	1	0	0	0	0	1	0	0	0	0	1	0	1	1	0
Anthurium microspadix	XIA	0	4	1	1	0	1	0	2	4	0	0	0	1	0	2	0	0	0	0	1	0	2	1	0
Anthurium mindense	XIA	0	4	1	0	0	1	0	2	1	0	0	0	0	0	1	0	0	0	0	1	0	2	2	0
Anthurium morae	DIG	0	1	0	0	0	0	0	0	4	0	0	0	0	0	3	0	0	1	2	2	0	2	2	0
Anthurium muyunense	DEC	0	1	0	0	0	0	0	0	4	0	0	0	0	1	3	0	0	0	0	1	1	2	3	0
Anthurium napaeum	PACmulti	0	3	0	0	0	0	0	0	4	0	1	0	0	0	3	0	0	0	0	1	0	2	2	0
Anthurium obtusilobum	CAL	0	4	0	0	0	0	0	1	4	0	0	0	2	0	3	0	0	0	0	1	0	0	0	0
Anthurium obtusum	TET	0	5	1	1	0	1	0	0	0	0	0	0	0	0	3	0	0	1	2	1	0	1	0	1
Anthurium cf. *obtusum*	TET	0	5	1	1	0	1	0	0	0	0	0	0	0	0	3	0	0	1	2	1	0	1	0	1
Anthurium olfersianum	URO	1	0	2	0	0	0	0	0	4	0	0	0	0	0	3	0	0	0	0	1	1	2	0	0
Anthurium ovatifolium	DIG	0	3	0	0	0	0	0	0	4	0	0	0	2	0	3	0	0	1	2	2	1	2	2	0
Anthurium oxyphyllum	PACmulti	0	1	3	0	0	0	0	0	3	0	1	0	1	0	4	0	0	0	0	1	0	2	1	0
Anthurium cf. *palenquense*	PACmulti	0	0	0	0	0	0	0	0	4	0	1	0	0	1	3	0	0	0	0	1	0	2	1	0
Anthurium parvispathum	DEC	0	3	0	0	0	0	0	0	4	0	0	0	0	0	3	0	0	0	0	1	0	1	1	0
Anthurium parvum	URO	1	0	0	0	0	0	0	0	4	0	0	0	1	0	1	0	0	0	0	1	1	2	0	0
Anthurium pedatoradiatum	SCH	0	1	0	0	0	0	0	0	4	0	0	1	4	0	3	2	1	0	0	1	0	0	1	0
Anthurium pedatum	SCH	0	4	0	0	0	0	0	0	4	0	0	1	4	0	3	2	1	0	0	1	0	1	2	0
Anthurium peltigerum	???	0	5	0	0	0	0	0	0	4	0	0	0	2	1	3	0	0	1	2	2	1	2	2	0
Anthurium polydactylum	DAC	0	3	1	0	0	1	0	0	4	0	0	1	4	0	3	2	2	0	0	1	0	1	3	0
Anthurium protensum	PACpac	0	3	0	0	0	0	0	2	1	0	1	0	1	0	3	0	0	0	0	1	0	2	3	0
Anthurium punctatum	POR	0	1	0	0	0	0	0	0	3	0	0	0	0	1	3	0	0	1	1	1	1	2	3	0
Anthurium radicans	CHA	1	0	2	0	0	0	0	1	4	0	0	0	2	0	4	0	0	0	0	0	0	0	0	0
Anthurium sagittatum	CAR	0	0	0	0	0	0	0	2	4	0	0	0	2	0	2	0	0	0	0	1	0	0	2	0
Anthurium scandens	TET	0	5	1	1	0	1	0	0	0	0	0	0	0	0	3	0	0	1	1	1	1	1	0	1
Anthurium schottianum	PACpac	0	0	0	0	0	0	0	2	4	0	1	0	2	0	3	0	0	0	0	1	0	2	2	0
Anthurium sp. sect. *Digitinervium*	DIG	0	5	0	0	0	0	0	0	4	0	0	0	0	0	3	0	0	1	1	2	0	2	1	0

Species	Section	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Anthurium sinuatum	DAC	0	1	1	0	0	1	0	0	4	0	0	1	4	0	2	2	2	0	0	1	0	0	3	0
Anthurium spathiphyllum	PACpac	0	1	3	0	0	0	0	0	1	1	1	0	1	0	3	0	0	0	0	1	0	2	0	0
Anthurium spectabile	PACpac	0	1	0	0	0	0	0	0	4	1	1	0	0	0	4	0	0	0	0	1	0	2	3	0
Anthurium subsignatum	SEM	0	0	1	0	0	1	0	0	4	0	0	1	3	0	1	1	1	0	0	1	0	1	3	0
Anthurium thrinax	DAC	0	1	1	0	0	0	0	0	4	0	0	1	3	0	2	1	2	0	0	1	0	1	2	0
Anthurium tremulum	BEL	0	4	0	0	0	0	0	0	4	0	0	0	2	0	3	0	0	0	0	1	0	2	3	0
Anthurium trilobum	SEM	0	1	1	0	0	1	0	0	4	0	0	1	3	0	3	1	1	0	0	1	0	1	3	0
Anthurium umbrosum	BEL	0	3	0	0	0	0	0	0	4	0	0	0	2	0	3	0	0	0	0	1	0	0	2	0
Anthurium cf. *urbanii*	POLYN	0	3	0	0	0	0	0	0	4	0	0	0	2	0	1	0	0	0	0	1	0	2	3	0
Anthurium veitchii	CAL	0	3	0	0	0	0	0	1	4	0	0	0	2	0	3	0	0	0	0	1	0	2	2	0
Anthurium venosum	PACpac	0	0	0	0	0	0	0	0	4	0	1	0	0	0	3	0	0	0	0	1	0	1	1	0
Anthurium verapazense	???	0	1	0	0	0	0	0	0	4	0	0	0	2	0	3	0	0	1	1	1	0	1	3	0
Anthurium vittariifolium	DEC	0	1	0	0	0	0	0	0	4	0	0	0	1	1	3	0	0	0	0	1	1	2	3	0
Anthurium wendlingeri	POR	0	5	0	0	0	0	0	0	3	0	0	0	1	2	3	0	0	1	2	1	0	2	3	0
Anthurium willdenowii	URO	0	0	0	0	0	0	0	2	4	0	0	0	0	0	3	0	0	0	0	1	1	2	2	0
Anthurium willifordii	PACpac	0	0	3	0	0	0	0	2	0	1	1	0	0	2	4	0	0	0	0	1	0	1	0	0

Notes: (*) Sectional names in *Anthurium* are abbreviated as follows: *Belolonchium* - BEL, *Calomystrium* - CAL, *Cardiolonchium* - CAR, *Chamaerepium* - CHA, *Dactylophyllium* - DAC, *Decurrentia* - DEC, *Digitinervium* - DIG, *Leptanthurium* - LEP, *Pachyneurium* series *Multinervia* - PACmulti, *Pachyneurium* series *Pachyneurium* - PACpac, *Polyneurium* - POLYN, *Polyphyllium* - POLYP, *Porphyrochitonium* - POR, *Schizoplacium* - SCH, *Semaeophyllium* - SEM, *Tetraspermium* - TET, *Urospadix* - URO, *Xialophyllium* - XIA. Species with unknown affinities in Croat and Sheffer (1983) classification system are noted with "???"
(**) The following references were used in compilation of morphological characters: Croat 1978, Croat 1983, Croat 1986, Croat & Acebey 2005, Croat & Mora 2004, Croat & Rodríguez 1995, Croat et al. 2005b, Mayo 1982, Nadruz et al. 2009.

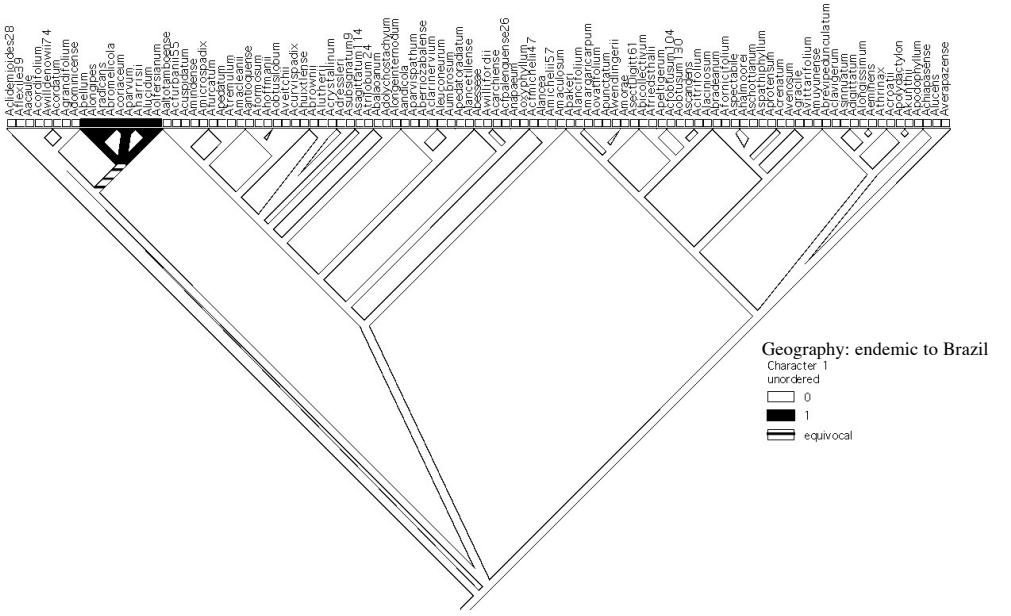
Table 5. Results of hypothesis testing of topological differences among likelihood and parsimony phylogenetic hypothesis using Shimodaira-Hasegawa and Templeton' tests. Constraint trees test the monophyly of the currently recognized sections of Croat and Sheffer (1983). P_{SH} is the P value for Shimodaira-Hasegawa test and P_{TT} is the P value for Templeton' test. Statistically worse trees as compared to the best tree are marked with an asterisk (*), and P values < 0.05.

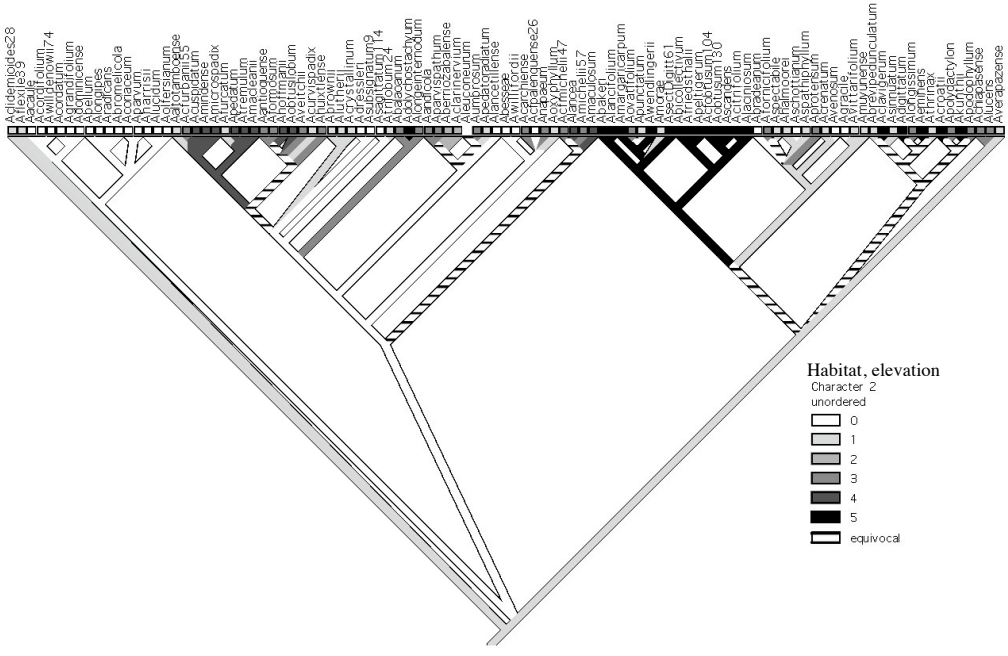
		Shimodaira-Hasegawa test		**Templeton's test**		
Phylogenetic hypothesis of monophyletic section		**Maximum Likelihood tree -ln Likelihood: 9705.96007**		**First most parsimonious tree from parsimony ratchet analysis Tree length: 903**		
Sections	**# spp sampled**	**Likelihood difference from best tree**	**P_{SH} value**	**Constrained tree length**	**P_{TT} value**	**Overall result**
Belolonchium	9	341.56097	0*	967	<0.0001*	non-monophyletic
Calomystrium	7	0.08781	1	903	1	monophyletic
Cardiolonchium	8	147.9498	0.001*	929	0.0002*	non-monophyletic
Chamaerepium	1	N/A	N/A	N/A	N/A	N/A
Dactylophyllium	9	54.65235	0.332	913	0.1551	monophyletic
Decurrentia	7	380.25238	0*	974	<0.0001*	non-monophyletic
Digitinervium	3	30.84055	0.69	907	0.4497	monophyletic
Gymnopodium	0	N/A	N/A	N/A	N/A	N/A
Leptanthurium	1	N/A	N/A	N/A	N/A	N/A
Pachyneurium		125.11551	0.003*	926	0.0002*	non-monophyletic
series *Multinervia*	4	5.59836	0.992	903	1	monophyletic
series *Pachyneurium*	9	111.50427	0.006*	925	0.0012*	non-monophyletic
Polyneurium	5	82.13048	0.004*	917	0.0164*	non-monophyletic
Polyphyllium	2	6.33472	0.913	904	0.8405	monophyletic
Porphyrochitonium	6	108.48379	0.046*	919	0.0136*	non-monophyletic
Schizoplacium	3	194.37107	0*	940	<0.0001*	non-monophyletic
Semaeophyllium	3	87.30137	0.004*	921	0.001*	non-monophyletic
Tetraspermium	6	47.53016	0.421	911	0.0881	monophyletic
Urospadix	9	81.26785	0.146	919	0.026*	?
Xialophyllium	2	17.08595	0.889	904	0.8273	monophyletic

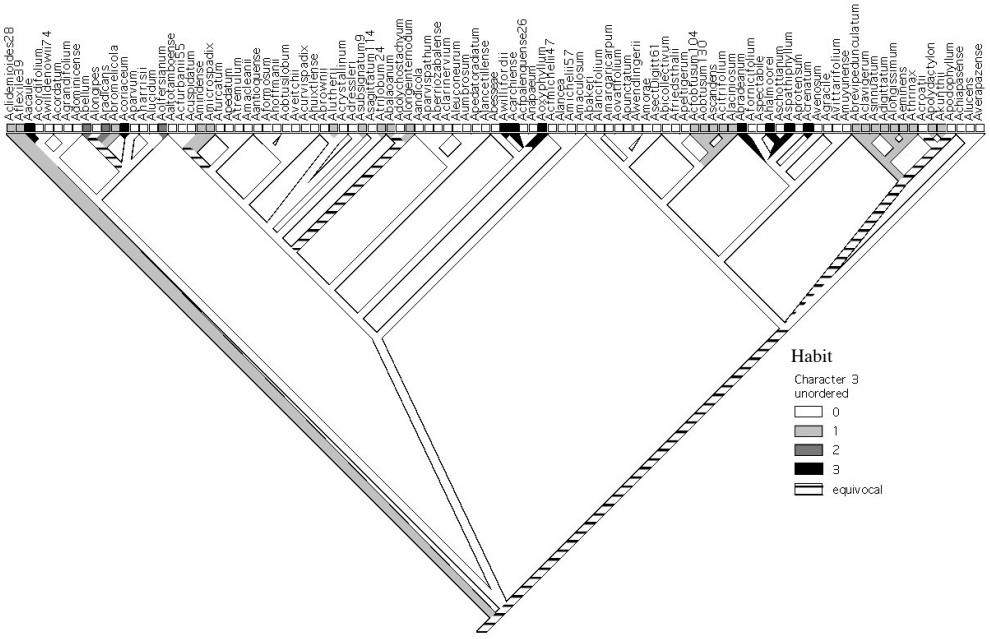
Table 6. Parameters of the reconstruction of morphological characters in *Anthurium*. Consistency index (CI) and retention index (RI) according to Farris (1989).

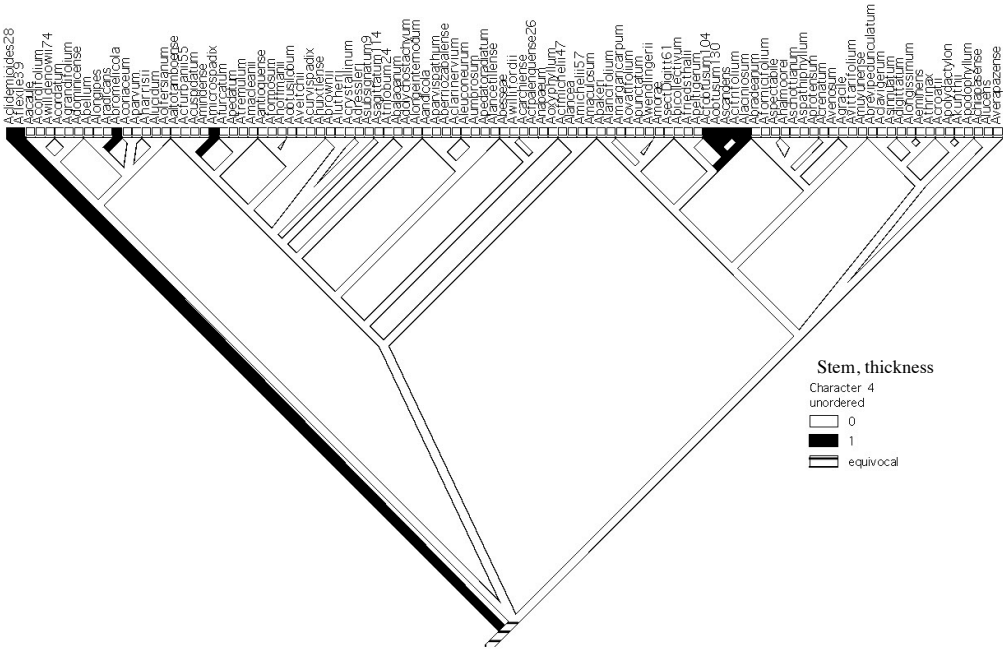
#	Character	# of states	# of steps	Consistency index (CI)	Retention index (RI)
1	Geography, endemic to Brazil	2	1	1	1
2	Habitat, elevation	6	40	0.13	0.51
3	Habit	4	23	0.13	0.39
4	Stem, thickness	2	4	0.25	0.63
5	Root, position	2	1	1	1
6	Internode, length	2	14	0.07	0.35
7	One-ribbed cataphyll, presence	2	1	1	1
8	Cataphyll, texture	4	23	0.13	0.33
9	Petiole, length	4	21	0.14	0.22
10	Petiole, shape	2	8	0.13	0
11	Leaf, vernation	2	2	0.5	0.92
12	Leaf, shape 1	2	6	0.17	0.67
13	Leaf, shape 2	5	35	0.11	0.5
14	Leaf, texture 1	3	17	0.12	0.06
15	Leaf, texture 2	4	32	0.09	0.24
16	Palmate leaf, shape 1	3	7	0.29	0.64
17	Palmate leaf, shape 2	3	8	0.25	0.57
18	Punctation, presence	2	2	0.5	0.94
19	Punctation, position	3	8	0.25	0.65
20	Venation, # of collective veins	3	5	0.4	0.4
21	Venation, primaries same as interprimaries	2	10	0.1	0.59
22	Venation, # of primary lateral veins	3	27	0.07	0.54
23	Spadix, length	4	48	0.06	0.36
24	Seeds, number per locule	3	2	1	1
			Average	0.33	0.56

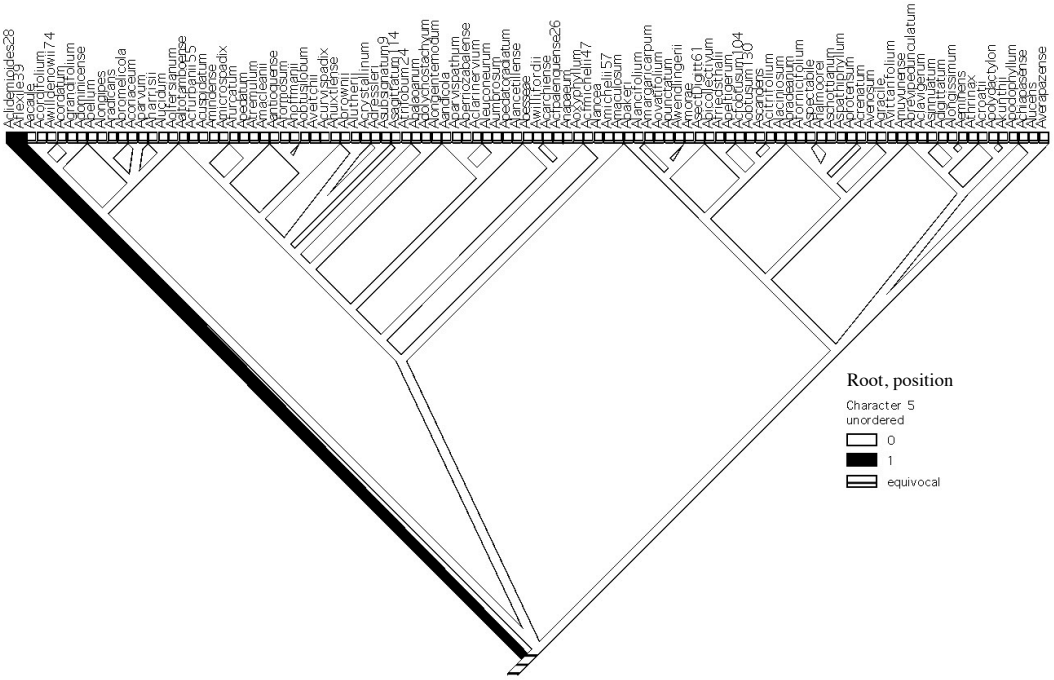
Appendix 1
Reconstruction of morphological characters along the phylogeny of *Anthurium*

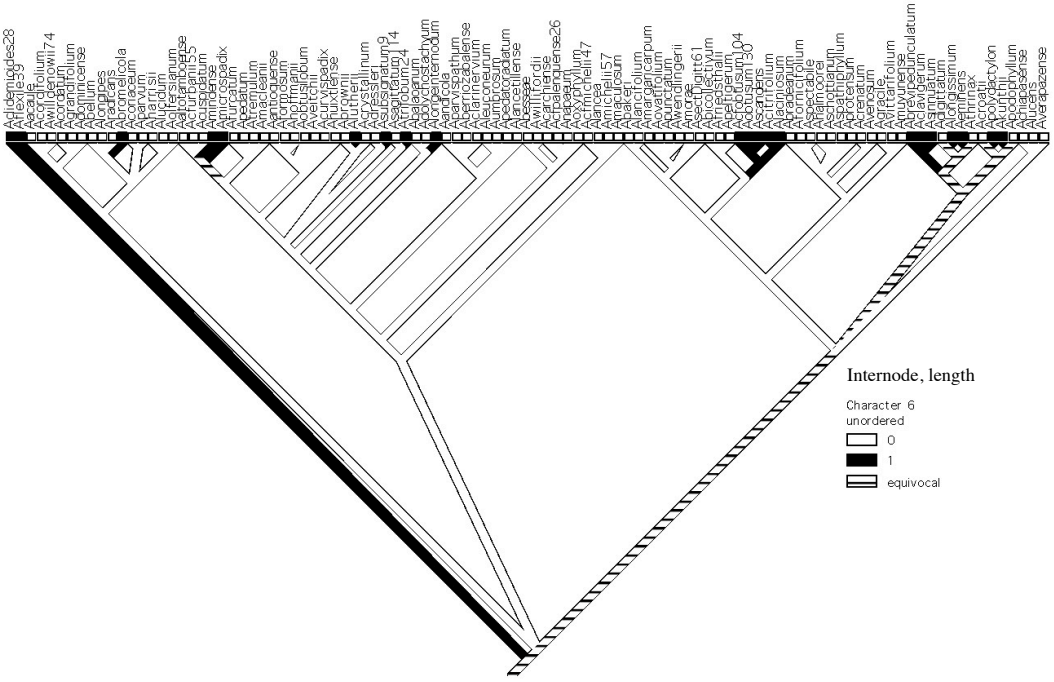

Geography: endemic to Brazil
Character 1
unordered
0
1
equivocal

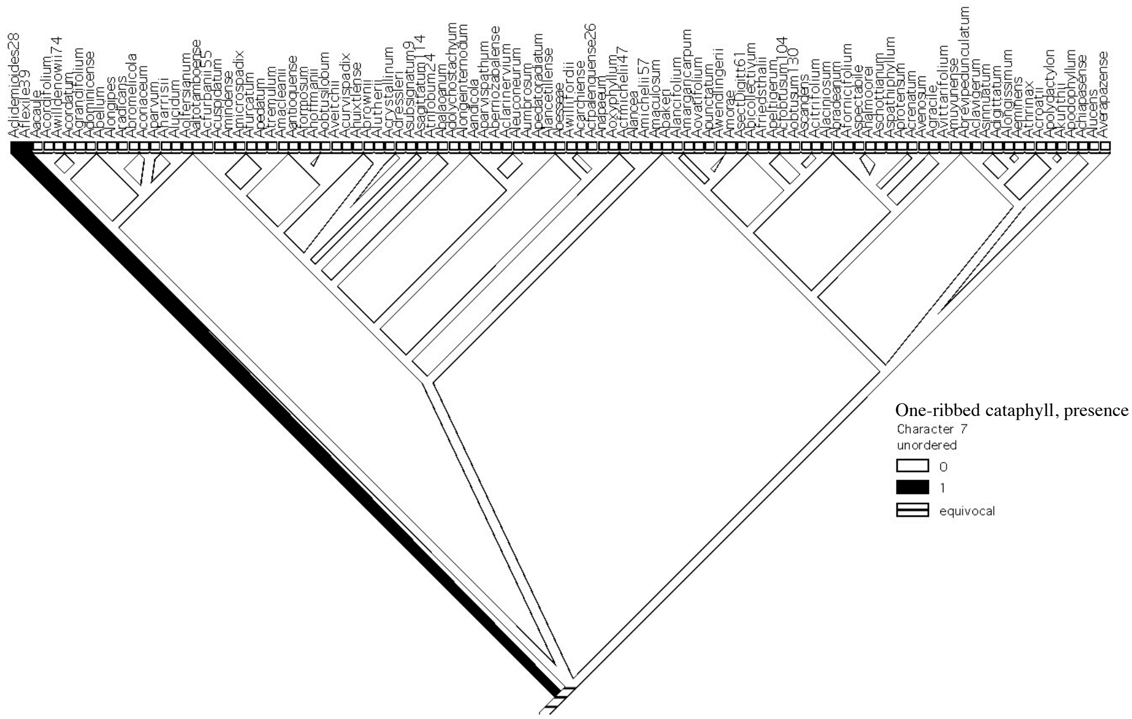

Habitat, elevation
Character 2
unordered
0
1
2
3
4
5
equivocal

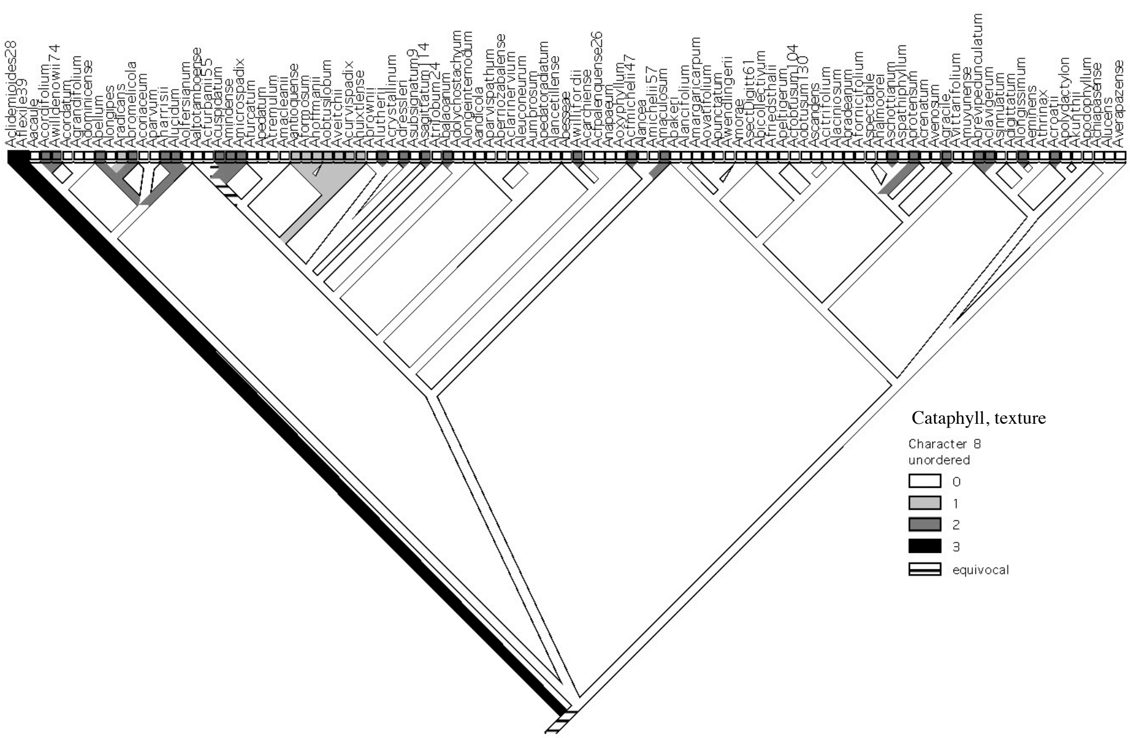

Habit
Character 3
unordered
0
1
2
3
equivocal

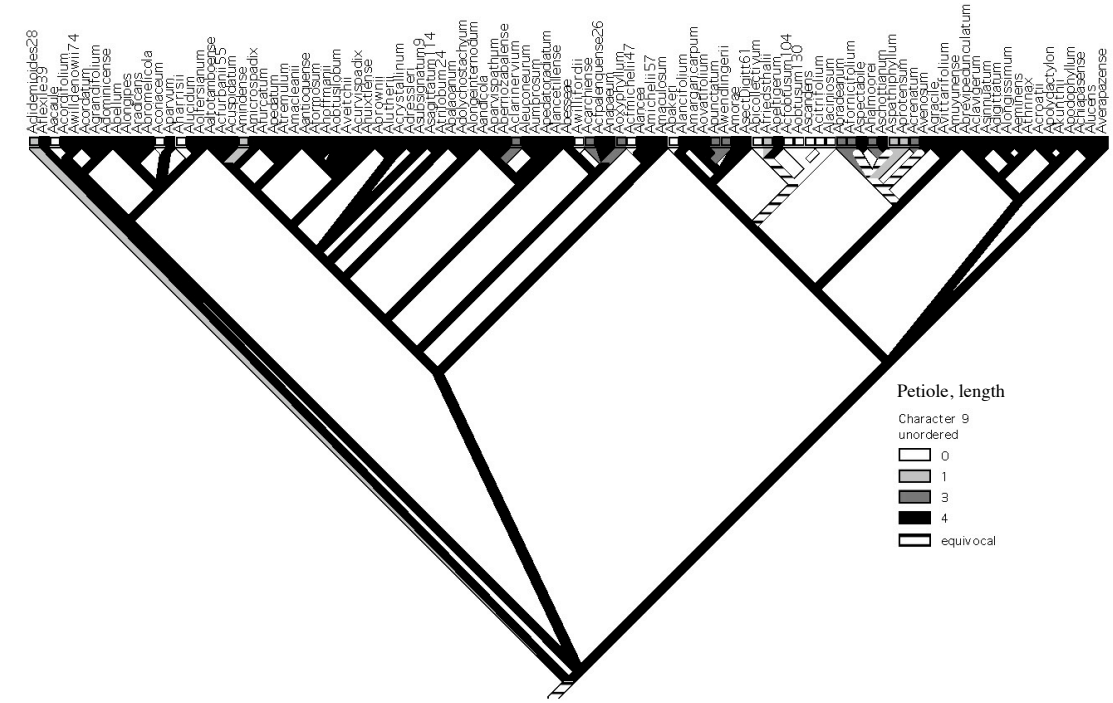

Stem, thickness
Character 4
unordered
0
1
equivocal

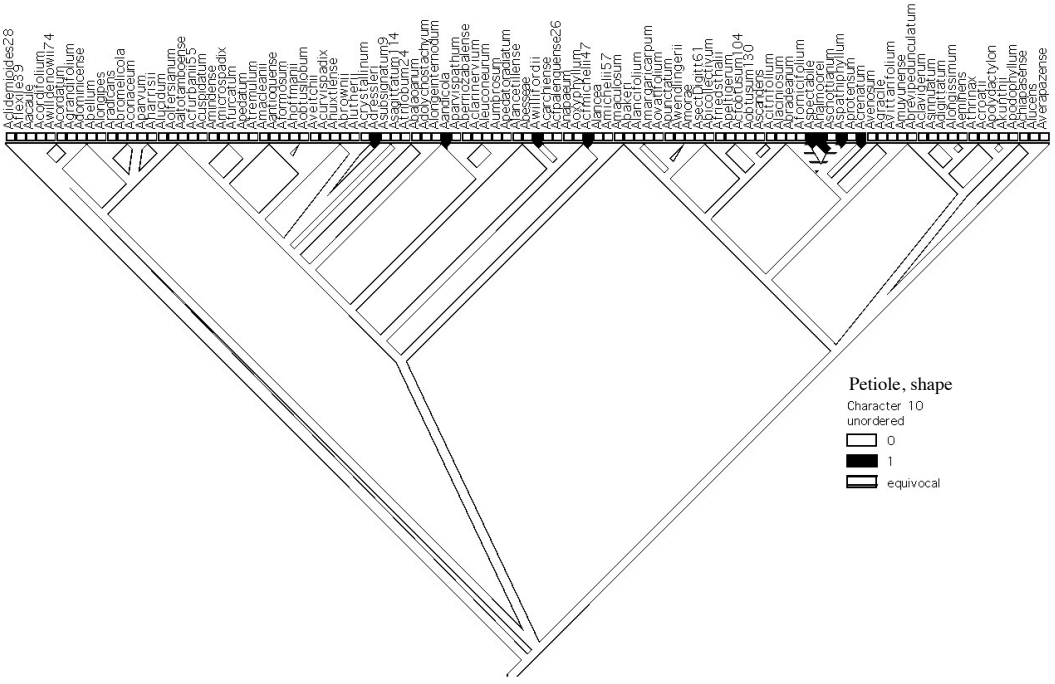

Root, position
Character 5
unordered
0
1
equivocal

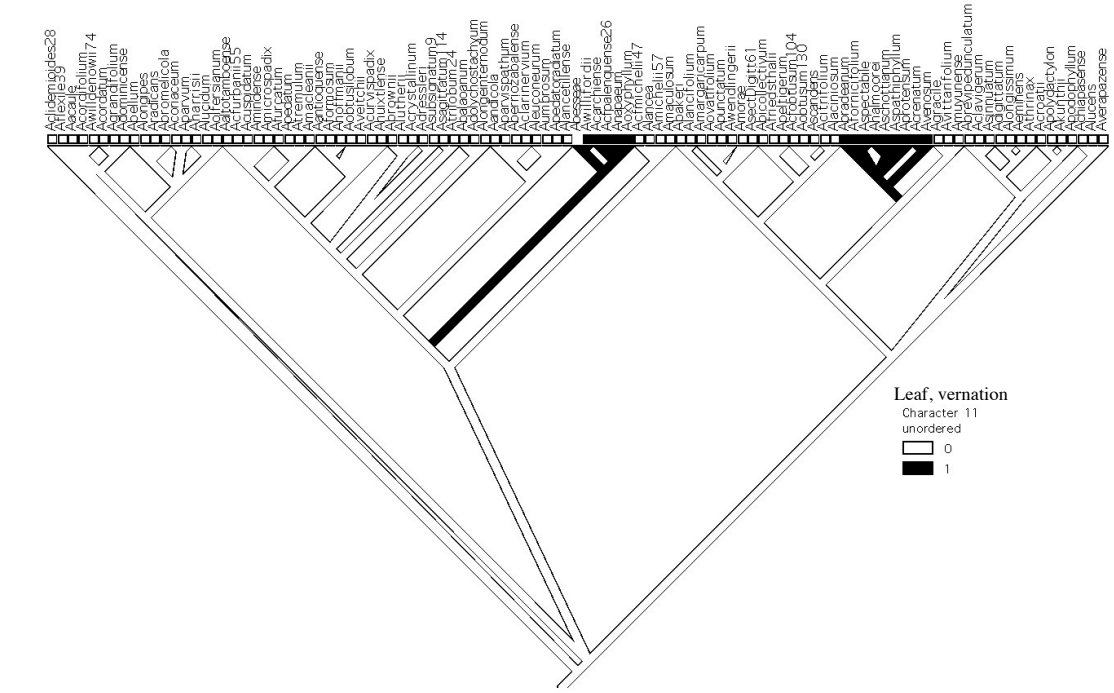

Internode, length
Character 6
unordered
0
1
equivocal

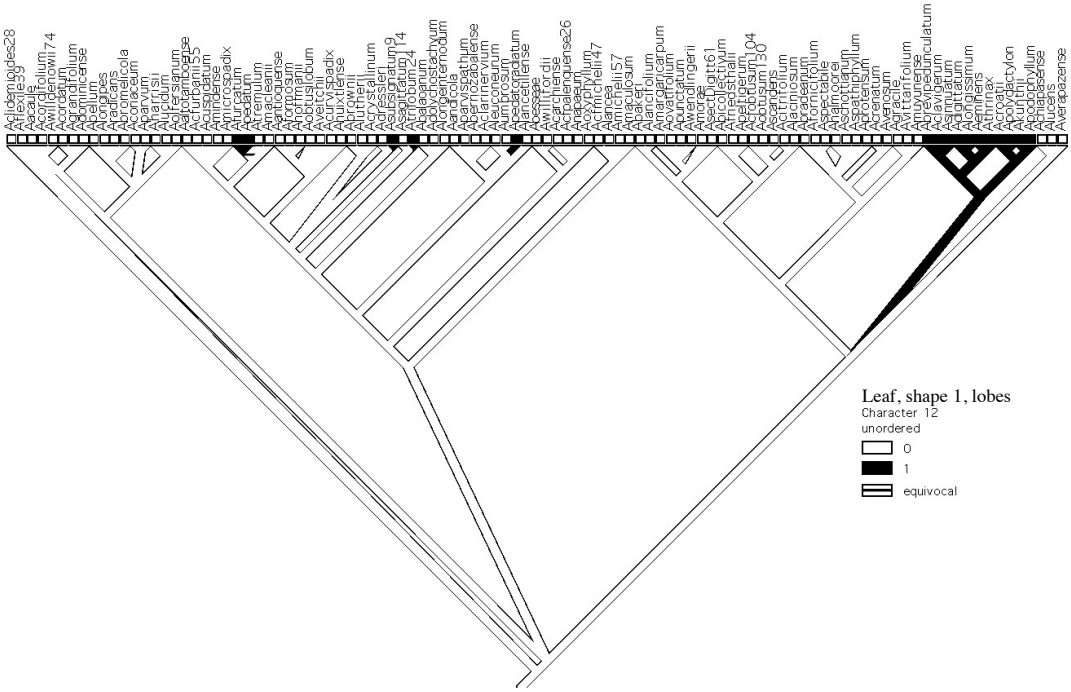

One-ribbed cataphyll, presence
Character 7
unordered
0
1
equivocal

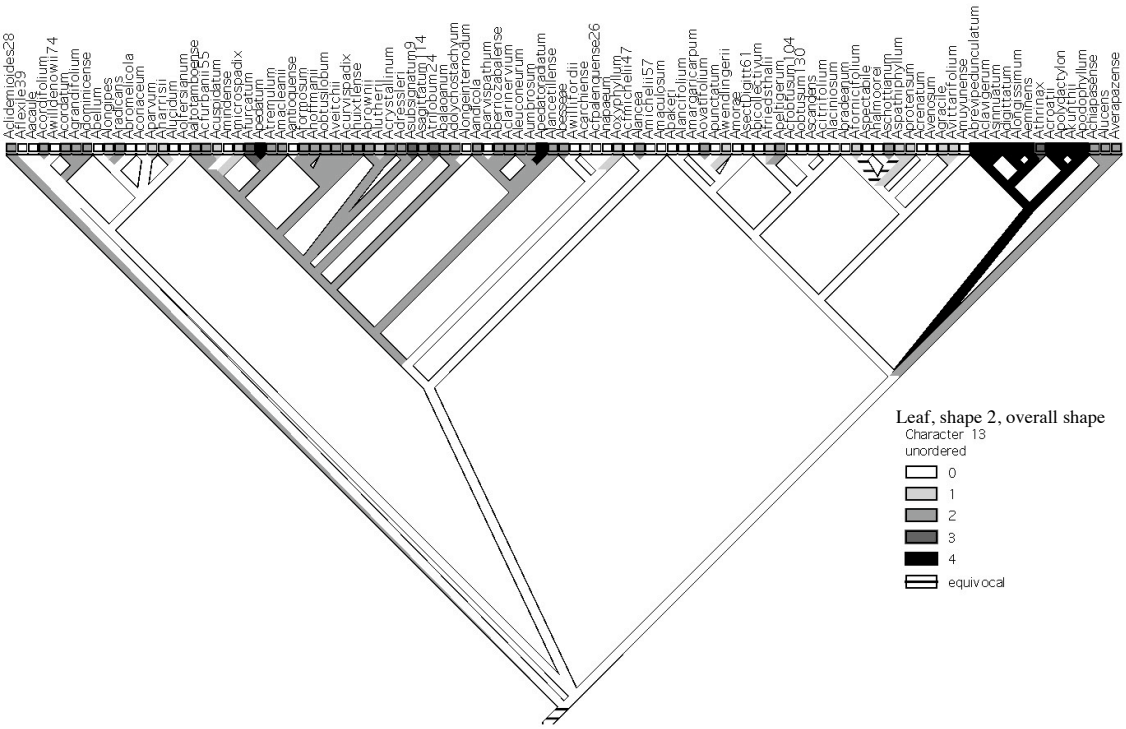

Cataphyll, texture
Character 8
unordered
0
1
2
3
equivocal

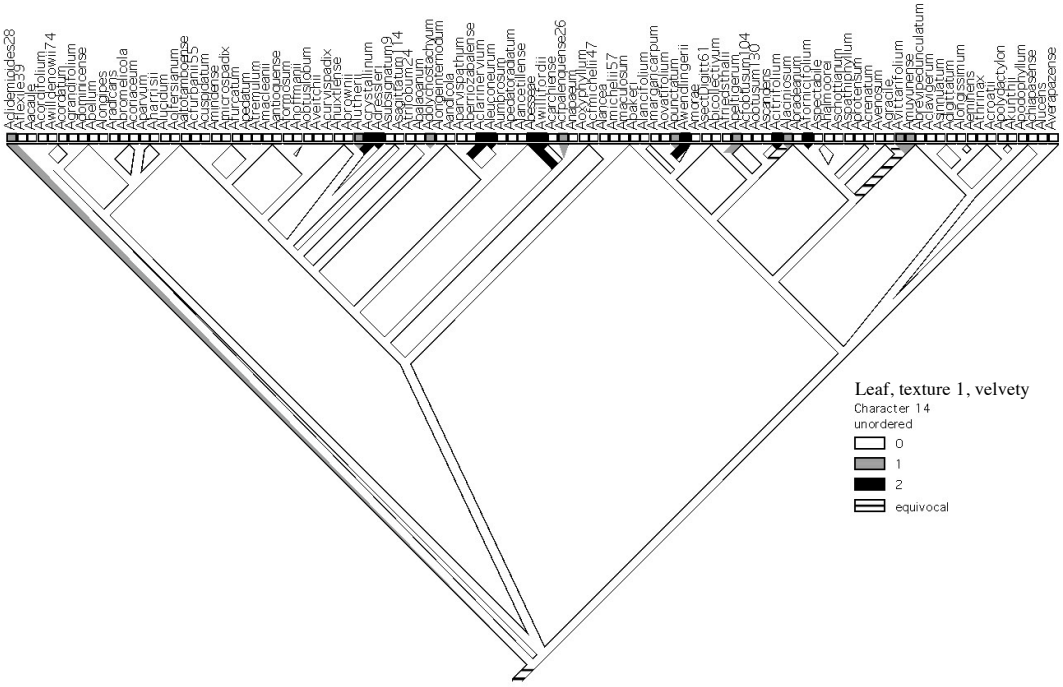

Petiole, length
Character 9
unordered
0
1
3
4
equivocal

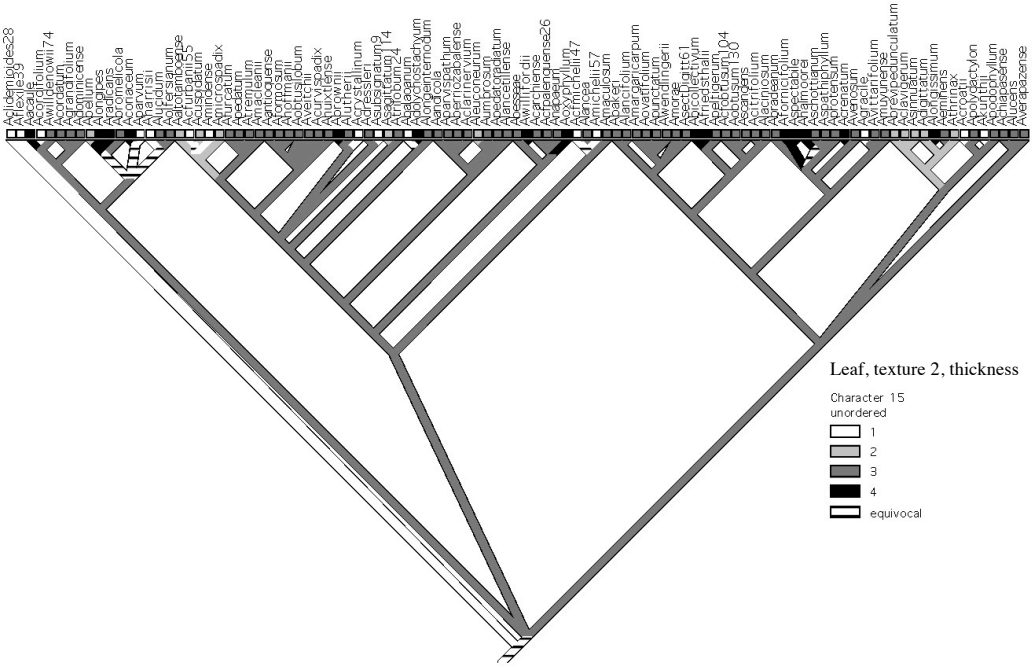

Petiole, shape
Character 10
unordered
0
1
equivocal

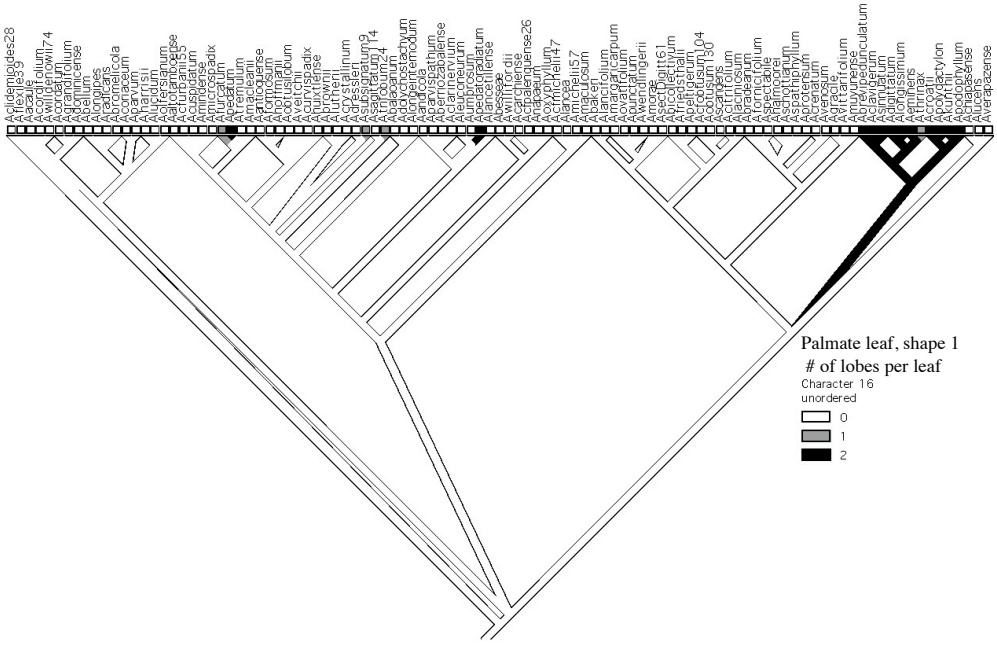

Leaf, vernation
Character 11
unordered
0
1

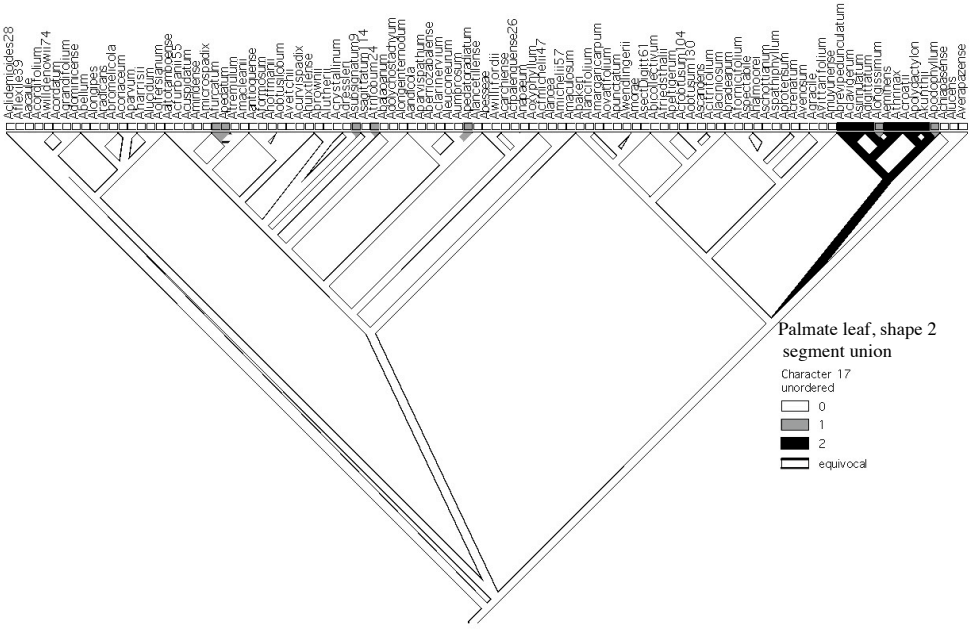

Leaf, shape 1, lobes
Character 12
unordered
0
1
equivocal

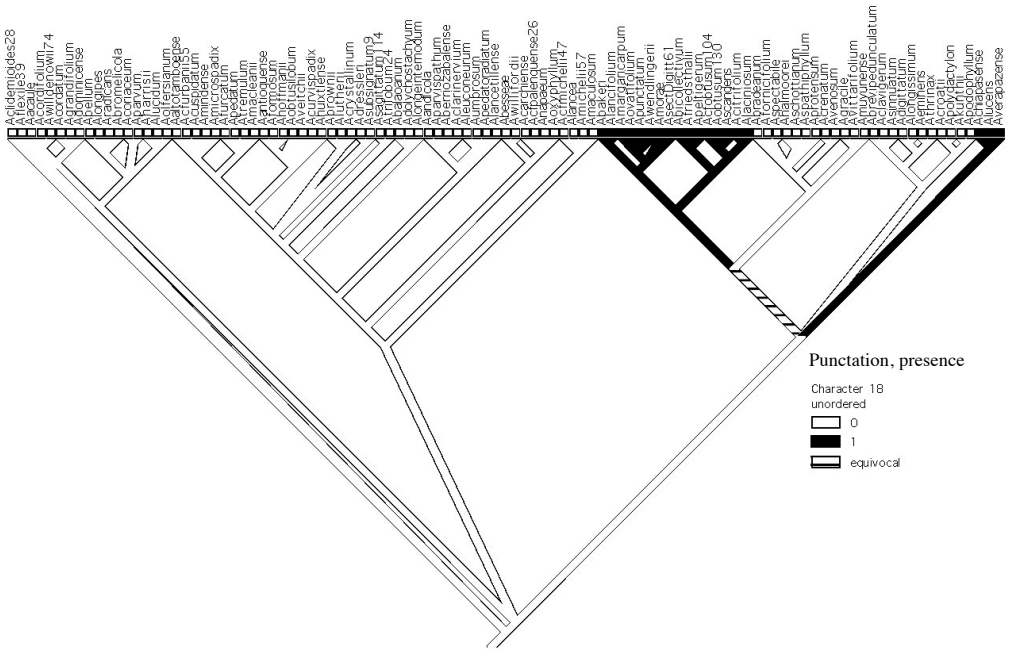

Leaf, shape 2, overall shape
Character 13
unordered
0
1
2
3
4
equivocal

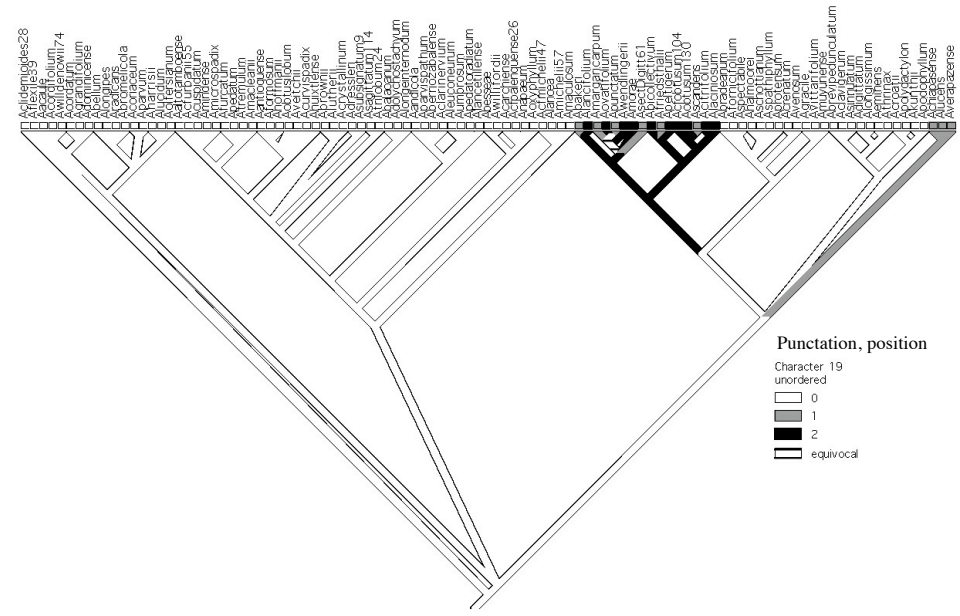

Leaf, texture 1, velvety
Character 14
unordered
0
1
2
equivocal

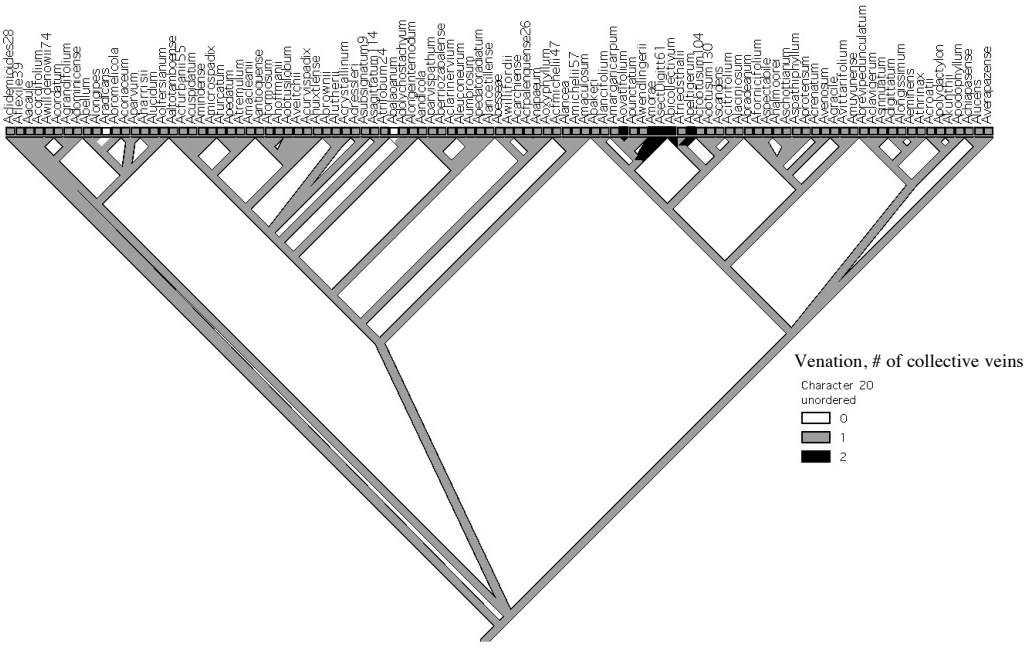

Leaf, texture 2, thickness
Character 15
unordered
1
2
3
4
equivocal

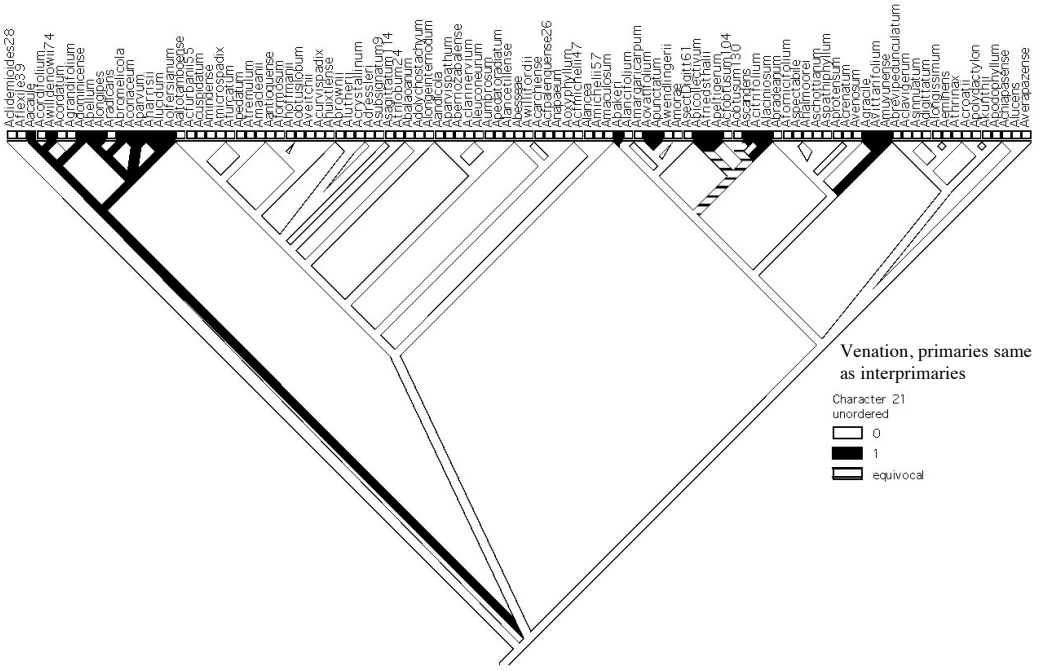

Palmate leaf, shape 1
of lobes per leaf
Character 16
unordered
0
1
2

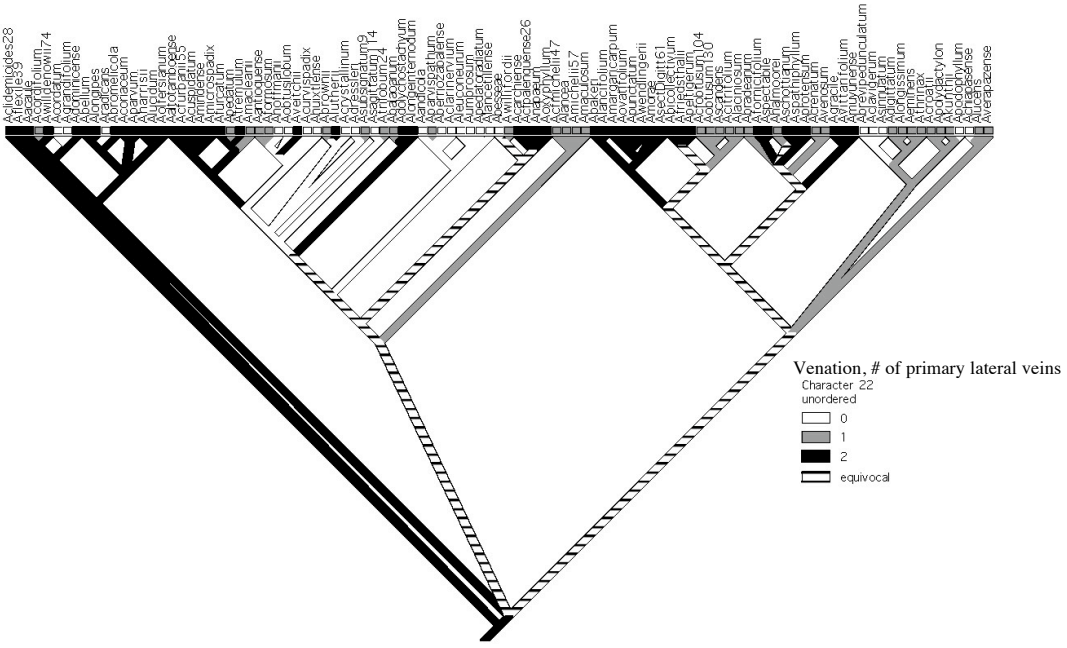

Palmate leaf, shape 2
segment union
Character 17
unordered
0
1
2
equivocal

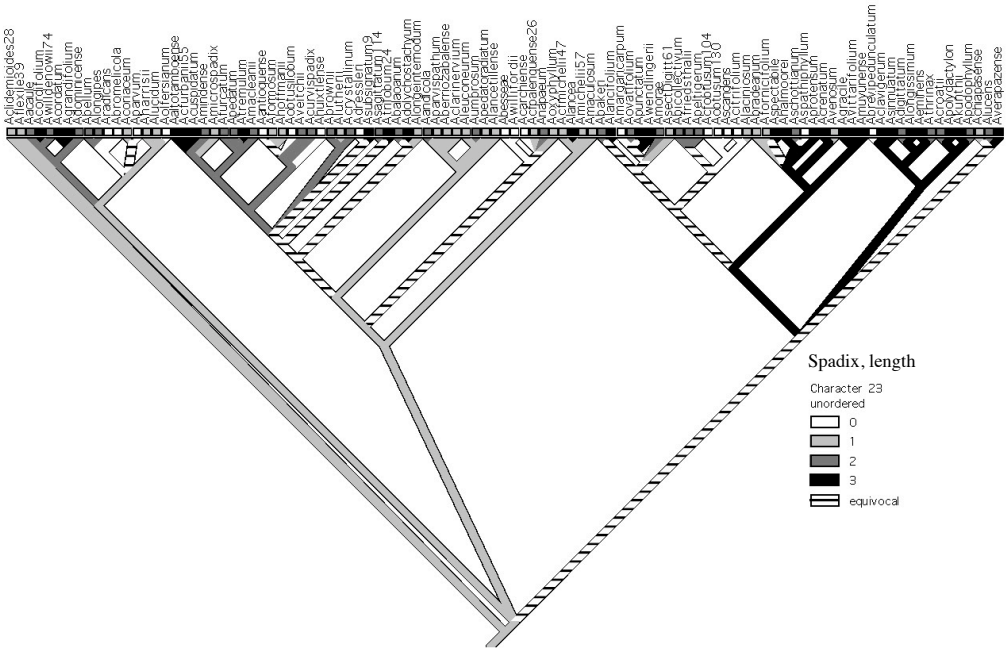

Punctation, presence
Character 18
unordered
0
1
equivocal

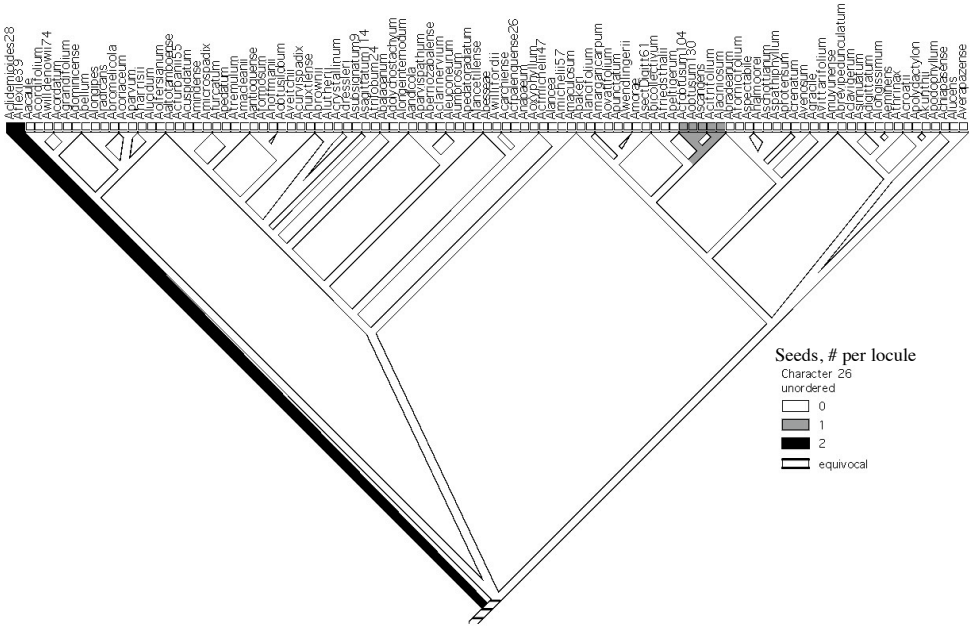

Punctation, position
Character 19
unordered
0
1
2
equivocal


Venation, # of collective veins
Character 20
unordered
0
1
2


Venation, primaries same as interprimaries
Character 21
unordered
0
1
equivocal


Venation, # of primary lateral veins
Character 22
unordered
0
1
2
equivocal

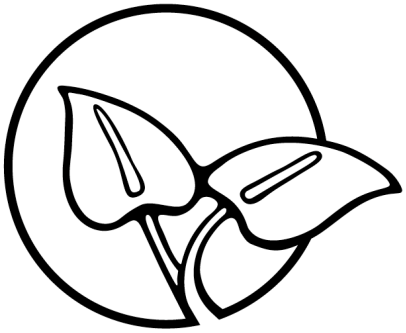

Spadix, length
Character 23
unordered
0
1
2
3
equivocal


Seeds, # per locule
Character 26
unordered
0
1
2
equivocal

CHAPTER 3

ORIGIN AND DIVERSIFICATION OF THE GENUS *ANTHURIUM* SCHOTT (ARACEAE) THROUGH TIME

Mónica M. Carlsen



INTRODUCTION

Anthurium Schott is a monophyletic (Carlsen 2011) and strictly Neotropical genus of Araceae ranging from southern Mexico into Central America and the West Indies, to southern Brazil, northern Argentina, and Paraguay. It includes approximately 900-1,000 largely well-differentiated species (Mayo et al. 1997, Govaerts & Frodin 2002, Govaerts et al. 2011, CATE Araceae 2011) and many more are still being discovered (T. Croat, pers. comm.). *Anthurium* belongs to one of the earliest divergent lineages in Araceae, the subfamily Pothoideae, and is sister to the genus *Pothos* L. (approx. 57 species) from the Old World tropics in Southeast Asia, Australasia and Madagascar (Cabrera et al. 2008, Cusimano et al. 2011, Carlsen 2011). It is a distinctive genus of climbers or epiphytes with sympodial growth, spirally arranged leaves, rounded petiole, geniculum at apex of petiole, reticulate minor venation, collective veins along the leaf margins, uniform spadix with open spathe, tetramerous bisexual flowers with tepals and seeds with copious endosperm (Grayum 1990, Mayo et al. 1997). *Anthurium* is the most species-rich genus of aroids, accounting for ca. 25% of the species of the whole family and about half of Araceae in the New World. Furthermore, it is also considered the most morphologically diverse genus in Araceae, displaying enormous variation in leaf morphology, growth habit and leaf venation pattern, and inflorescence and fruit colors (Bown 2000).

Despite the great diversity both in terms of species number and morphological variation in the genus, it was not until recently that a strongly supported molecular phylogeny of *Anthurium* became available (Carlsen 2011). That study can now be used to provide a solid framework for a more detailed analysis of the pattern of species diversification in *Anthurium* through time. Especially interesting are the apparently

contradictory facts that suggest both a very old and a very recent origin for the genus. On one hand, *Anthurium* belongs to one of the earliest divergent clades in Araceae (Cabrera et al. 2008, Cusimano et al. 2011), it has striking morphological diversity (Bown 2000, Mayo et al. 2007), and it is quite widespread within the Neotropics (Mayo et al. 1997). All these facts suggest that *Anthurium* must have originated a long time ago and through time acquired all that morphological variability and dispersed throughout tropical America. On the other hand, many *Anthurium* species are relatively easy to hybridize in greenhouse trials (Sheffer & Kamemoto 1976, T. Croat pers. comm.) and they show very low molecular differentiation in both the chloroplast and nuclear gene regions examined (Carlsen 2011). This suggests that the species are not yet well differentiated or reproductively isolated, and thus the genus is relatively young.

This study uses a combination of chloroplast and nuclear sequence data to examine the timing of the origin and diversification of the genus *Anthurium*, as well as some closely related subfamilies within Araceae. The approach used for clade age estimation is two-fold. First, a subset of a previously published chloroplast phylogeny of Araceae (Cabrera et al. 2008) is calibrated based on several known fossils and age estimations are obtained for several subfamilies and deep nodes in the Pothoideae. Then, the most recent combined chloroplast and nuclear DNA phylogeny of *Anthurium* (Carlsen 2011) is calibrated based on the range of age estimates for the subfamily Pothoideae obtained above, and significant clades are dated. This study uses a maximum likelihood framework for phylogenetic analyses and a penalized likelihood approach for age estimation (Sanderson 2002, 2004), with confidence intervals for age estimates based on a bootstrap procedure (Sanderson & Doyle 2001).

MATERIALS AND METHODS

Taxon sampling. Two separate datasets were used to estimate the time of origin and diversification of the genus *Anthurium*. The first dataset (hereafter, Araceae-dataset) was downloaded as an aligned data matrix from TreeBase (http://TreeBASE.org; study ID S2078) and corresponds to the dataset used by Cabrera et al. (2008); this included three concatenated chloroplast genes (*rbcL*, *matK* and *trnL-F*). From the original Cabrera et al. (2008) dataset, a total of 31 taxa were included in this study, 26 Araceae genera, with special emphasis on the subfamilies Pothoideae and Monsteroideae, and 5 outgroups, including one species with affinities within Alismatales (*Tofieldia pusilla* Pers.), one representative of Acoraceae (*Acorus calamus* L.) and three basal dicotyledons – Magnoliids (*Magnolia*, *Piper* and *Hedyosmum*).

The second dataset used in this study (hereafter, *Anthurium*-dataset) was based on the combined DNA sequence data from Carlsen (2011), which included the chloroplast *trnG* intron and *trnH-psbA* and *trnC-ycf6* intergenic spacers, and the nuclear first intron of chalcone synthase (*CHS*) gene, for a total of 102 *Anthurium* species and 6 Potheae outgroups sampled. However, nuclear *CHS* sequences are missing for all Potheae representatives. One representative of the subfamily Orontioideae (*Lysichiton americanus*) was sequenced for all chloroplast gene regions and added to this dataset for rooting purposes. Details of taxon sampling, laboratory protocols, data alignment, outgroup selection and GenBank accession numbers are found in Cabrera et al. (2008) and Carlsen (2011).

Phylogenetic analysis. Indels, gaps and ambiguous sequence alignments were excluded from both datasets in all analyses. Phylogenetic trees were obtained from

maximum likelihood analyses (Felsenstein 1973) performed using RAxML v.7.2.7 (Stamatakis 2006, Stamatakis et al. 2008) in the CIPRES web portal (Miller et al. 2009). This program uses the GTRGAMMA model for complete likelihood evaluation, and each gene region was coded as a separate partition with an independent model of evolution. Branch lengths were obtained as "per site" values by adding the branch lengths of individual partitions. *Magnolia* and *Lysichiton* were specified as outgroups for the Araceae- and *Anthurium*-dataset, respectively. The resulting trees obtained from maximum likelihood analysis, Araceae-phylogram and *Anthurium*-phylogram, were used to estimate clade diversification times.

Age and fossil calibration. Two types of age calibrations were used in the analyses of the Araceae-dataset. First, the age of the monocot-*Ceratophyllum*-eudicot split from the Magnoliids (hereafter monocot-eudicot split) was fixed at three different ages, spanning the range of age estimates in the literature: 137 million years ago – Mya (minimum age estimated by Chaw et al. 2004), 154 Mya (age estimate from Wikstrom et al. 2001, and also close to the Chaw et al. 2004 range of estimates), and 220 Mya (maximum age estimate from Chaw et al. 2004).

Second, a set of araceous fossils was used as age calibration points along the family phylogeny. In order to minimize the effects of poor taxon sampling, fossil constraints were placed on strongly supported internal nodes along the Araceae phylogeny. Placement of fossil constraints on terminal and/or weakly supported nodes could lead to contrasting age estimates dependent on different tree topologies. In order to avoid possible calibration errors, the fossil taxon should undisputedly be part of the group defined by the selected node (i.e. the fossil should have at least one or ideally all of the

synapomorphies that characterize the clade to which the fossil is assigned) (Wikstrom et al. 2001). However, the exact point in time along the phylogeny when a synapomorphy or synapomorphies arose is usually unclear. The presence of a given characteristic in a fossil does not necessarily means that such morphology instantaneously appeared at that time, it could have arisen earlier in time but due to fossilization problems and/or the intrinsically sparse nature of the fossil record, it has only been recorded at a later time. Furthermore, recovered fossils do not usually represent complete plants, so fossils could have one of the synapomorphies for the group to which they are assigned, but perhaps not all the current synapomorphies for that crown group. Therefore the most conservative approach to avoid possible calibration errors due to the placement of fossil calibration points is to place all fossil constraints on the stem nodes of the clades where their affinities are known. Errors associated with the age estimates of the fossils themselves may be minimized by specifying an age for the fossil taxon that represents as closely as possible the actual divergence time for the selected node (Wikstrom et al. 2001). Given the uncertainty of the exact time when a synapomorphy arose, when a fossil was described as pertaining to a given stratigraphic position, the upper (younger) boundary of the interval, based on the geological time scale of Gradstein and Ogg (2004), was chosen to represent the minimum possible age of the fossil. Moreover, when several fossils could be assigned to the same node in the phylogeny, then the oldest fossil was chosen as the minimum age constraint for that node. This conservative approach to fossil and node age calibration is designed to minimize calibration errors, yet ages estimated with this method may still be an underestimate of the "real" age of clades.

Based on the criteria above, three fossils were chosen for the analyses of the Araceae-dataset as minimum age constraints for several stem nodes within the family (Figure 1). An infructescence of *Albertarum pueri* from the Late Cretaceous (Late Campanian, 72 Mya) in the Horseshoe Canyon Formation of Alberta, Canada, with affinities to the genus *Symplocarpus* was used to calibrate the stem node of the subfamily Orontioideae (Bogner et al. 2005, Bogner et al. 2007, Herrera et al. 2008). A recently mentioned, but still not formally published araceous inflorescence fossil (Araceae fossil sp. B of Friis et al. 2010) from the Early Cretaceous, Late Aptian-Early Albian (ca. 112 Mya) in Villa Verde 2 and Valle de Agua, Portugal, with affinities within Pothoideae, was used as the minimum age calibration point for the stem node of subfamily Pothoideae. Finally, leaves of *Petrocardium cerrejonense* from the Middle to Late Paleocene, (58-60-63 Mya) in the Cerrejón Formation, Colombia, with affinities to *Anthurium* (subfamily Pothoideae) itself were used to calibrate the stem node of *Anthurium* (Herrera et al. 2008). Despite being the oldest fossil assigned to Araceae (ca. 117-124 Mya), the fossil pollen of *Mayoa portugallica* (Friis et al. 2004), previously affiliated with the tribe Spathiphylleae in subfamily Monsteroideae, was not included in this study as a calibration point because its affinities to Araceae have been recently questioned by Hofmann and Zetter (2010) (see Discussion for details).

Due to the lack of fossil representatives within the tribes Potheae and Anthurieae, analyses of the *Anthurium*-dataset used the range of age estimations resulting from the analyses based on the Araceae-dataset to fix the age of the root (i.e. the subfamily Pothoideae crown group). The only two fossils known pertaining to these tribes, and previously used as minimum age constraints in the analyses of the Araceae-phylogram,

could not be used for the *Anthurium*-phylogram. First, *Petrocardium cerrejonense*, used as calibration point of the stem node for the genus *Anthurium* (or conversely, the crown node of subfamily Pothoideae) could not be used because the program used in age estimation, r8s version 1.71 (Sanderson 2003, 2004), needs to have a fixed age somewhere in a non-terminal clade in the tree and this fossil is only a minimum age constraint. The second Pothoideae related fossil (Araceae fossil sp. B, Friis et al. 2010), formerly assigned as a minimum age constraint to the stem node of subfamily Pothoideae in the Araceae-dataset, was not useful in the *Anthurium*-dataset analyses because a calibration point based on it would disappear when *Lysichiton americanus*, the most distant outgroup in *Anthurium*-dataset, was pruned from the analyses, as recommended in the r8s program user manual (Sanderson 2004, pp. 23-24).

Age estimation. Penalized likelihood (Sanderson 2002) was used to estimate clade ages with the software r8s version 1.71 (Sanderson 2004). To improve optimization of model parameters and avoid technical issues in r8s, the most distant outgroups (*Magnolia* in the Araceae-phylogram and *Lysichiton americanus* in the *Anthurium*-phylogram) had to be deleted (Sanderson 2004, pp. 23-24), and all zero-length internal and terminal branches were collapsed or pruned from the phylogram before performing the analyses (Sanderson 2004, pp. 27).

Analyses in r8s required the use of a phylogram and a set of fixed-age and minimum-age constraints along the phylogeny. A molecular clock test, as implemented in r8s with the Langley-Fitch optimization method (Langley & Fitch 1974), was conducted in order to statistically test the hypothesis of a molecular clock for both datasets. To detect the optimal level of rate smoothing in penalized likelihood analyses, a cross-

validation procedure was performed in r8s, for a total of 16 smoothing magnitudes ranging from log10 = -2 to 5.5 at 0.5 intervals, for each dataset. The best cross-validation smoothing value (λ) is the one with the lowest chi-square error (Sanderson 2004). A fossil-based model cross-validation procedure was used in the Araceae-dataset to determine the predictive error derived from sequentially removing minimum age fossil constraints. Predictive errors were calculated if after the removal of a constraint in a given node, the estimated age for that node violates the original constraint (Sanderson 2004). This procedure was helpful in detecting potential conflicts among fossil calibrations in the phylogeny, and the results were used to separate fossil constraints into two groups, "young fossils" (*Albertarum pueri* and *Petrocardium cerrejonense*) and "old fossil" (Araceae fossil sp. B) that seem to show contradictory age estimates.

Penalized likelihood analyses were conducted on the Araceae- and *Anthurium*-phylograms implementing in each case the optimal smoothing value found in the respective cross-validation, and using a TN algorithm with five initial starts and five perturbed restarts with perturbations of magnitude 0.05, and the option gradientcheck=yes. Penalized likelihood analyses of the Araceae-phylogram were performed for a total of nine combinations of fixed and constrained calibration ages that cover the range of estimated ages for the monocot-eudicot split in the literature (137-154-220 Mya, fixed age) and the ages of fossil constraints (i.e. all fossils included, only "young fossils" included, and only "old fossil" included). Age estimates for nodes within the Araceae-phylogram are discussed after removal of outlying values (see Table 1), specifying as outliers those age estimations that fall in an entirely different geological period (according to the geological time scale of Gradstein & Ogg 2004) than the other

age estimates for the same node. The calibration of the *Anthurium*-phylogram used the age estimated for the crown group of subfamily Pothoideae obtained from the analyses based on the Araceae-phylogram.

In order to obtain confidence intervals for age estimates in the tree, a series of phylograms were generated from bootstrapped sequence data for each dataset while keeping the tree fixed (i.e. maintaining the original topology but allowing different sets of branch lengths) following Torsten Eriksson's r8s bootstrap kit (from http://www.bergianska.se/index_kontaktaoss_torsten.html). These phylograms were uploaded into r8s, ages were estimated for all trees, and age distributions for a particular node summarized; the central 95% of the age distribution provides a confidence interval (Sanderson & Doyle 2001).

RESULTS

Phylogenetic analyses. The Araceae-dataset matrix of three concatenated chloroplast gene regions (*rbcL-matK-trnL-F*) contained 31 taxa and 3943 base pairs after exclusion of gaps and indels, and the Araceae-phylogram obtained from maximum likelihood analyses of that matrix was highly resolved with strongly supported clades (Figure 1, see Cabrera et al. 2008 for details). The *Anthurium*-dataset with three chloroplast (*trnG*, *trnH-psbA* and *trnC-ycf6*) and one nuclear (*CHS* first intron) concatenated gene regions comprised 109 taxa and 2166 nucleotides after removal of all indels, gaps and ambiguously aligned regions. All outgroups in this dataset were missing sequences for the nuclear partition, therefore estimated branch lengths along the outgroup internal and terminal nodes were underestimated. The *Anthurium*-phylogram is

moderately well resolved, and in order to avoid calibration errors associated with possible topological inconsistency, age estimates were calculated only for strongly supported clades within the phylogeny (Figure 2, see Carlsen 2011 for details).

Age estimations. Given the range of ages (137-220 Mya) found in the literature (Chaw et al. 2004, Magallón & Castillo 2009, Wikstrom et al. 2001) for the split of monocotyledons from its closest relatives in the angiosperms, several possible fixed ages for that node had to be evaluated and the age estimations compared to each other in order to provide a better understanding of diversification times within the Araceae-phylogram. The youngest age found in the literature search (128 Mya, Magallón & Castillo 2009) was not used as a fixed age for the monocot-eudicot split because it was calculated constraining the maximum age of the angiosperms to 130 Mya, and maximum age constraints highly underestimate node ages.

Fossils used as calibration points along the Araceae-phylogram also ranged broadly in age, from 58 million years old - Myo (*Petrocardium cerrejonense* - Herrera et al. 2008) to 112 Myo (Araceae fossil sp. B - Friis et al. 2010). Furthermore, fossil cross-validation procedures of the Araceae-dataset with all three originally constrained nodes (see Figure 1, Table 1) showed potential conflicts among the fossils used as constraints. Repeatedly, when the node with the older fossil constraint was unfixed during the cross-validation procedure, the estimated age for such node dropped between 45 and 96 million years (approximately an entire geological epoch), therefore old and younger fossils were contradicting the constraints imposed by each other. These results suggested that the fossils used as minimum age calibration points could be divided in two categories separating fossils that are congruent with each other in terms of cross-validation and age

estimates. *Albertarum pueri* (72 Myo – minimum age constraint of the stem node of subfamily Orontioideae) and *Petrocardium cerrejonense* (58 Myo - minimum age constraint of the stem node of *Anthurium*) represented the "Young fossils". And Araceae fossil sp. B (112 Myo - minimum age constraint for the stem node of subfamily Pothoideae) comprised the "Old fossil".

A molecular clock test of the Araceae-phylogram under Langley-Fitch optimization rejected the hypothesis of a molecular clock ($X^2 = 1629.51$, df = 29, $P = 0.01$). The best cross-validation score for this dataset was found to be $\lambda = 100$, and it was used, along with a total of all possible nine combinations of age ranges for fixed (137, 154, 220 Mya) and constrained nodes (all fossils, "old fossil", "young fossils"), to estimate node ages under penalized likelihood in r8s.

In general, fixing the monocot-eudicot split at 220 Mya provided age estimates older than for the other two fixed ages (137 and 154 Mya) (Table 1). Similarly, regardless of the age fixed for the monocot-eudicot split, age estimates generated using only "young fossils" as minimum age calibration points are several million years younger than when either "old fossil" alone or all fossils ("young" and "old" together) were used (Table 1). In the most basal nodes (i.e. the root, monocots and Alismatales crown groups) all age estimations based on the monocot-eudicot fixed age of 220 Mya were considered outliers (Table 1). Four outliers were identified in the crown nodes of the family Araceae, the Proto-Araceae and the subfamily Orontioideae; two were under- and two over-estimates, and they were all removed (Table 1). For the other nodes in the tree, severe underestimates were found when only the "young fossils" were used in the analyses, regardless of the age fixed for the monocot-eudicot split, and therefore all those age

estimations were considered outliers (Table 1). The broadest range of age estimates corresponds to the most basal nodes in the phylogram (e.g. the root, the monocots, and the Alismatales crown groups) (Table 1).

To analyze the *Anthurium*-dataset, eight terminal branches and four internal branches with length < 0.0001 substitutions per site (effectively zero-length branches for the software) were either pruned or collapsed in the phylogram. After removal of a distant outgroup (*Lysichiton americanus*), the age of the root (subfamily Pothoideae crown group) was fixed to 86 Mya, the age estimate obtained from the previous analyses of the Araceae-phylogram. There are no old fossil records within tribes Potheae and Anthurieae. A test for molecular rate consistency in the *Anthurium*-phylogram, under Langley-Fitch optimization, also rejected the hypothesis of a molecular clock (X^2 = 4856.22, df = 100, P = 0.01) and $\lambda = 3.2$ was found to be the best cross-validation score for this dataset. Estimated ages for internal nodes within *Anthurium* suggest that most of the diversification in the genus occurred between 1 Mya (Holocene – Pleistocene) and approximately 12 Mya (Miocene).

DISCUSSION

Several sources of error are present at different levels when trying to estimate the age of clades using computer programs, phylograms and fossil constraints. One of the most conspicuous is, of course, the age assigned to a calibration point within the phylogenetic tree (Near et al. 2005, Sanderson & Doyle 2001, Soltis et al. 2002, Wikstrom et al. 2001). Such age constraints present at least two intrinsic problems, first, the reliability of the fossil dating itself and second, the placement of such fossil in the

phylogeny. In both instances, conservative approaches are preferred, therefore this study always assumed minimum ages for the fossils used (i.e. the younger age limit within a given geological epoch). In most cases, fossil affinities are not always clear because of missing plant pieces or structures or problems with the interpretation of the fossils. A fossil can only be certainly placed as calibration point of a crown group if it presents all the currently known synapomorphies of such clade. Moreover, the development of any individual morphological structure that is currently recognized as one of the synapomorphies for a given clade could have appeared along the stem of the clade sometime before the main diversification of the group (i.e. the crown node). Therefore, for a given node, the oldest known fossil was used as a minimum age constraint, and fossil calibration points were always placed along stem nodes, thus, all age estimates reported here should be considered underestimates of "real" clade ages. If the fossils used in this study were complete and were used to constrain the crown nodes of their representative clades, then the ages for most clades might have suffered less from underestimation, but it is not known if overestimation could be a problem at that level.

Age estimates in the Araceae-phylogram with variable fixed ages for the monocot-eudicot split were highly congruent with each other, except for those based on a 220 Mya fixed age for the split (Table 1). In fact, Chaw et al. (2004) suggested that this value was an overestimation of the real age because a group of fast evolving sequences from grasses predominated in their analyses. Removal of such sequences resulted in the two younger estimated ages used in this study to fix the monocot-eudicot split. Therefore, it was better to consider most, if not all, age estimates based on 220 Mya fixed age as overestimates and possible outliers in the Araceae-phylogram calibration. The effect of

using an overestimated value to fix the monocot-eudicot split is less evident the farther the node is with respect to the root of the tree.

Although the Araceae fossil record is not sparse (see Bogner et al. 2005 & 2007, Herrera et al. 2008, Hesse & Zetter 2007, for summaries), most of the fossils found to date are quite young (i.e. dating to Paleocene to Miocene, ca. 65 to 15 Mya) when compared to the age estimated for the stem and crown nodes of the family (ca. 128 and 117 Mya, Janssen & Bremer 2004, Anderson 2009). So far, one fossil far pre-dates all others, the still unpublished, Araceae fossil B (Friis et al. 2010) from the Early Cretaceous (ca. 112 Mya). Although several Late Cretaceous (65 to 80 Mya) fossils with affinities to subfamilies Orontioideae and Monsteroideae have been found (Bogner et al. 2007, Bonde 2000, Hesse & Zetter 2007), suggesting diversification of such clades around that period, stem node ages could still be a lot older than those so far represented by the fossil record.

Pollen grains of *Mayoa portugallica* represented the oldest fossil (Early Cretaceous, Late Barremian-Early Aptian, 117-124 Mya) reportedly having affinities within Araceae, specifically to the genus *Holochlamys* in the tribe Spathiphylleae, subfamily Monsteroideae (Friis et al. 2004). However, this fossil was not included in the analyses as a calibration point because its affinities within Araceae have been questioned recently. Hofmann and Zetter (2010) suggest that *M. portugallica* is instead very similar in shape, size, and exine pattern to *Lagenella martinii* (Leschik) Klaus, an euglenoid algae. They further propose that the affiliation of *M. portugallica* with the Monsteroideae requires further investigation before it can be confirmed.

The age estimates based on only "young fossil" calibration points (i.e. *Albertarum pueri* - 72 Myo and *Petrocardium cerrejonense* - 58 Myo) were several million years younger than all others, regardless of the age used to fix the monocot-eudicot split. In all such cases, these "young fossils" severely underestimate the real ages of most clades in the Araceae-phylogram, a pattern that was more evident at nodes further away from the root of the tree (Table 1).

Another potential error in age estimation occurs especially along the most basal nodes in the phylogram (e.g. the root, monocots and Alismatales). In all these nodes, age estimates from different analyses varied widely between 10 and 20 million years. Either their close proximity to the node with varying fixed ages (i.e. the monocot-eudicot split, varying from 137 to 220 Mya) is influencing age estimation, or there are problems in optimizing models to find ages for nodes that do not have a reliable fossil constraint attached (i.e. a minimum age calibration point).

Despite all these problems, this study suggests that the stem node of the family Araceae can be dated to 126.58±0.24 to 136.31±0.22 Mya during the Early Cretaceous Hauterivian-Barremian, and diversification within the family (i.e. crown node) started ca. 122.71±0.21 to 129.80±0.22 Mya during the Early Cretaceous – Barremian-Aptian (Figure 1, Table 1). Previous studies by Anderson (2009) and Janssen and Bremer (2004) suggested a slightly younger but still Early Cretaceous ages for the origin - ca. 123-128 Mya - and diversification - ca. 117 Mya - of Araceae.

Both main groups within Araceae, the Proto-Araceae and all other aroids ("Spirodela clade") (Cusimano et al. 2011) (Figure 1), started diversifying in the Early Cretaceous at least 107.97±0.38 to 115.07±0.36 Mya – Aptian-Albian and 114.72±0.28

to 124.08±0.31 Mya - Aptian, respectively (Table 1). It is interesting to note that a group of morphologically very "primitive" aroids such as the Proto-Araceae (Mayo et al. 1997, Cabrera et al. 2008, Cusimano et al. 2011) diversified about 7-9 million years after more "advanced" traits such as the climbing habit (in subfamilies Pothoideae and Monsteroideae) appeared in its sister group. The inclusion of a "young fossil" as calibration point in the stem node of the Proto-Araceae could not be the explanation for this younger age estimate, because analyses with different calibrations also show the same pattern (Table 1).

According to results here, within the Proto-Araceae, diversification of the subfamily Orontioideae started around 50.14±0.51 to 53.97±0.26 Mya – Early Eocene, Ypresian (Figure 1, Table 1). Once again, such a relatively young age estimate is doubtful, this time because it contrasts with known fossil records of representatives of this subfamily. In addition to *Albertarum pueri* - 72 Myo, used to calibrate the stem node of the subfamily Orontioideae - there are at least three more leaf fossils of species belonging to this subfamily that date to the Late Cretaceous (older than 65 Mya), and are very closely related to extant genera. They are: *Symplocarpus hoffmaniae*, late Maastrichtian, ca. 65.5 Mya, of the Hell Creek Formation, SW Dakota, United States (Bogner et al. 2007, Herrera et al. 2008), *Lysichiton austriacus*, ca. 83.5-70.6 Mya, in central Europe (Bogner et al. 2007), and *Orontium mackii*, Maastrichtian, ca. 70.6-65.5 Mya, in New Mexico, Jose Creek member of the McRae Formation (Bogner et al. 2007). Although they do not contradict the age estimate for the stem node of the subfamily Orontioideae (107.97±0.38 to 115.07±0.36 Mya), if these fossils indeed belong to the genera to which they have been assigned, the ages of all these fossils are between 14-19

and up to 33 million years older than the age estimated here for the crown node of this clade.

The "True-Araceae" (fide Cusimano et al. 2011) started to diversify in the Early Cretaceous approximately 113.67±0.20 to 119.40±0.32 Mya - Aptian (Figure 1, Table 1). In contrast, its sister group, the subfamily Lemnoideae, although having the same stem node age of ca. 114.72±0.28 to 124.08±0.31 Mya, did not start to diversify until more recently, about 48.81±0.21 to 49.97±0.34 Mya during the Early Eocene - Ypresian, some 65-75 million years later. This long lapse in time between stem and crown node ages perhaps reflects that extreme aquatic adaptation and reduction of most of the characteristic "araceous" morphology in the subfamily Lemnoideae was achieved after a very long period; only then did species diversification in aquatic environments occur. There are at least two North American fossils from the Late Cretaceous-early Paleocene (ca. 65 Mya) of complete plants with aquatic adaptations that have previously been associated with the subfamily Lemnoideae, *Cobbania corrugata* (Stockey et al. 2007) and *Limnobiophyllum scutatum* (Stockey et al. 1997, Bogner et al. 2007). However, their characteristic morphology may be a result of convergent aquatic adaptation, and none of these fossils could be unequivocally assigned to the Lemnoideae; *C. corrugata* is placed in the subfamily Aroideae (doubtfully, J. Bogner, pers. comm.) and *L. scutatum* in its own subfamily. The presence of several fossil and extant aquatic lineages in Araceae suggests more than one and probably up to four different origins of the aquatic life form in the family, and roughly within the same time period (i.e. Early Paleocene to Early Eocene).

The split between the two major subfamilies containing species with climbing habit, Pothoideae and Monsteroideae, also occurred during the Early Cretaceous – Aptian-Albian, approximately 112±0 Mya (Figure 1, Table 1). The subfamily Pothoideae started to diversify (i.e. crown group age) 86.06±0.32 to 86.74±0.21 Mya – during the Late Cretaceous, Coniacian. Later on, the subfamily Monsteroideae did so - crown group age of 58.04±0.22 to 58.20±0.23 Mya. Between 23.51±0.23 and 40.11±0.22 Mya, all tribes and main clades within subfamily Monsteroideae started to diversify (Figure 1, Table 1). On the other hand, diversification within the tribe Potheae, in subfamily Pothoideae began only 28.74±0.32 Mya according to this analysis. One fossil plant, *Rhodospathodendron tomlinsonii* (Bonde 2000), from the late Cretaceous, late Maastrichtian, minimum age of 65.5 Mya, in India, with affinities within Monsteroideae, agrees with the stem group ages found in this study for both the subfamily. If the pollen fossil of *Spathiphyllum elsikii*, from middle to Late Cretaceous, Cenomanian to Santonian, between 96 and 84 Mya, in Brazil (Hesse & Zetter 2007) indeed has affinities to the genus *Spathiphyllum* and therefore could be assigned to the tribe Spathiphylleae, this fossil strongly contradicts both the ages estimated for the crown group of the tribe – approximately 23.51±0.23 to 23.54±0.30 Mya, and for its stem (ca. 55.86±0.22 to 56±0.24 Mya) (Figure 1, Table 1).

In a second set of analyses, the age estimated for the crown node of the subfamily Pothoideae (86.06±0.32 to 86.74±0.21 Mya) was used to calibrate the *Anthurium*-phylogram (Table 2). The only two known fossils pertaining to this subfamily, *Petrocardium cerrejonense* (Herrera et al. 2008) and Araceae fossil sp. B (Friis et al. 2010), previously used as minimum age constraints in the analyses of the Araceae-

phylogram, could not be used for the *Anthurium*-phylogram (see Materials and Methods above). Moreover, results of the analyses of the Araceae-phylogram suggest that indeed *P. cerrejonense* - ca. 58 Myo- is younger than the origin (i.e. stem node) of the genus *Anthurium* (i.e. the crown node of subfamily Pothoideae) estimated to be between 86.06±0.32 to 86.74±0.21 Mya (Figure 1, Table 1).

An error related to the use of incomplete sequences to estimate branch lengths could have been introduced in the analyses of the *Anthurium*-dataset, because none of the outgroups (tribe Potheae and *Lysichiton americanus)* had complete sequences for the nuclear *CHS* region. Therefore, internal and terminal branch lengths for taxa belonging to those clades are indeed underestimated, and in turn, it has to be assumed that their age estimates are also lower than for the rest of the taxa.

Analyses of the *Anthurium*-phylogram suggest that the genus started to diversify (*Anthurium* crown node) relatively recently in comparison with other early divergent lineages in Araceae, during the Late Oligocene – Chattian, approximately 27.2±0.18 Mya (Figure 2, Table 2). However, the main onset of species origination (Clade B, core *Anthurium*) took place still more recently, only 8.93±0.22 Mya during the Late Miocene. Indeed, nine of the 18 newly recognized clades in *Anthurium* (Carlsen 2011) diversified in that same geological epoch, nine more clades diversified later, during the Pliocene (ca. 2 to 5 Mya), and a few even more recently during the Pleistocene-Holocene (less than 2 Mya) (Table 2). Although the stem node of *Anthurium* is quite old, the appearance of the majority of extant *Anthurium* species took place quite recently, during the Pliocene, ca. 5 Mya and onwards. This age estimate for the diversification of *Anthurium* species is congruent with the timing of the uplift of the Andes, a main geological event in the

Neotropics that created a great number of new ecological niches potentially available for colonization by *Anthurium* species.

This pattern of diversification in *Anthurium* suggests that about 900-1000 species originated in a 9-10 million year period. Using a simple and common estimator (i.e. pure-birth model, not extinction) of per-lineage rates of diversification calculated as [ln(N) – ln(N0)]/T, with N0 = initial diversity (2, for crown group), N = standing diversity, and T = inferred crown-group age (Baldwin & Sanderson 1998, Baldwin 2007), then diversification rates of the genus *Anthurium* as a whole are estimated to be 0.22-0.23 species per million years (N = 900-1000 spp., T = 27 My) and 0.61-0.69 species per million years for the core *Anthurium* radiation (Clade B, N = 898-998 spp., T = 9-10 My). These diversification rates for the core *Anthurium* radiation are comparable to those found in other recent plant radiations, such as *Costus* subgenus *Costus* (Costaceae), 0.46 – 0.7 species per million years (recalculated from Kay et al. 2005), the paleotropical shrub genus *Gaertnera* (Rubiaceae) at ca. 0.68 species per million years (recalculated from Malcomber 2002), the South African ice plants (Aizoaceae) at 0.77-1.75 species per million years (Klak et al. 2004), and the Hawaiian silversword alliance (Asteraceae) estimated to be 0.56 species per million years (Baldwin & Sanderson 1998).

Gentry (1982) proposed that the herbs, epiphytes and shrubs currently inhabiting the lower slopes of the Andes have been subject to explosive speciation and adaptive radiation in response to dramatic changes in geology and climate in the area. The results of this study agree with this hypothesis both in terms of timing (i.e. *Anthurium* is a young clade with main species diversification ca. 5 Mya and onwards, during the onset of Andes

uplift in the Pliocene) and rate of species diversification (i.e. rapid radiation of 0.61-0.69 species per million year for the core *Anthurium* radiation).

CONCLUSIONS AND FURTHER RESEARCH

Despite the lack of a strong fossil record for either Araceae as a whole or the genus *Anthurium*, the caveats and assumptions of the methodology used, and having missing sequences in a few taxa in the analysis, this is the first study that has been able to obtain age estimates for the basal clades within the family and for the *Anthurium* species radiation. Further research should try to integrate biogeographical data within the dated phylogenies of Araceae and *Anthurium* in order to determine patterns of dispersal to new areas and vicariance between related clades. It is indeed notable that the sister genera *Pothos* and *Anthurium* are geographically quite restricted and non-overlapping in distribution. Biogeographical studies would help to elucidate the "space" component of Araceae and *Anthurium* species diversification. Indeed, a number of monophyletic clades in *Anthurium* inhabit restricted geographical areas.

These age estimates obtained here are likely to be underestimates, but they are still a representative range of possible ages for clade diversification and they agree, in most cases, with the ages of the few known fossils of the family. Unfortunately, these fossils represent only a small sample of the family's diversity in geological time, and in order to get a better understanding of clade diversification through time and a more accurate estimate of the age of the group more fossils are needed, along with more detailed studies of their affinities and better age estimates for the fossils themselves. Assuming that the geological dating used to estimate the ages of fossils is correct, the

obvious age underestimates in analyses when only "young fossils" were used as calibration points suggest that they might not be the oldest representative fossils for either subfamily Orontioideae or Pothoideae. It is likely that there are older fossils of these and several other subfamilies within Araceae to be found.

Araceae diversification started in the Early Cretaceous, ca. 122.71±0.21 to 129.80±0.22 Mya, and all of the stem nodes of the basal subfamilies (Orontioideae, Lemnoideae, Pothoideae and Monsteroideae) were already present during that geological epoch. The subfamilies Pothoideae and Monsteroideae, with the majority of extant genera that are climbers, started to diversify first, the crown nodes being dated to the Late Cretaceous - 86.06±0.32 to 86.74±0.21 Mya and Late Paleocene - 58.04±0.22 to 58.20±0.23 Mya, respectively. The main diversification event (i.e. crown node) of both subfamilies Orontioideae and Lemnoideae occurred later on, during the Early Eocene (ca. 50.14±0.51 to 53.97±0.26 Mya and 48.81±0.21 to 49.97±0.34 Mya, respectively). This study focused on dating the basal subfamilies within Araceae due to their close affinity with the genus *Anthurium*, yet a more complete family-wide analysis including more distantly related subfamilies and their known fossil record would greatly improve our understanding of Araceae diversification.

This study revealed that although the stem node of *Anthurium* is quite old (ca. 86.06±0.32 to 86.74±0.21 Mya, Late Cretaceous), species diversification did not start until quite recently - crown node age dated to ca. 27.2±0.18 Mya - during the Late Oligocene. Moreover, the bulk of species diversification, i.e. "the core *Anthurium*" clade (ca. 900 species), did not occur until even later on, during the Late Miocene (ca. 8.93±0.22 Mya). All extant *Anthurium* species seem to be even younger, having

originated during or after the Pliocene, ca. 5 Mya. This age estimate for the diversification of *Anthurium* species is congruent with the uplift of the Andes and, *Anthurium* species diversification through time is indeed consistent with the pattern of a recent and rapid radiation perhaps spurred by that uplift. The diversification rate of approximately 0.61-0.69 species per million years is comparable with that of other rapid radiations, including other Andean plants.

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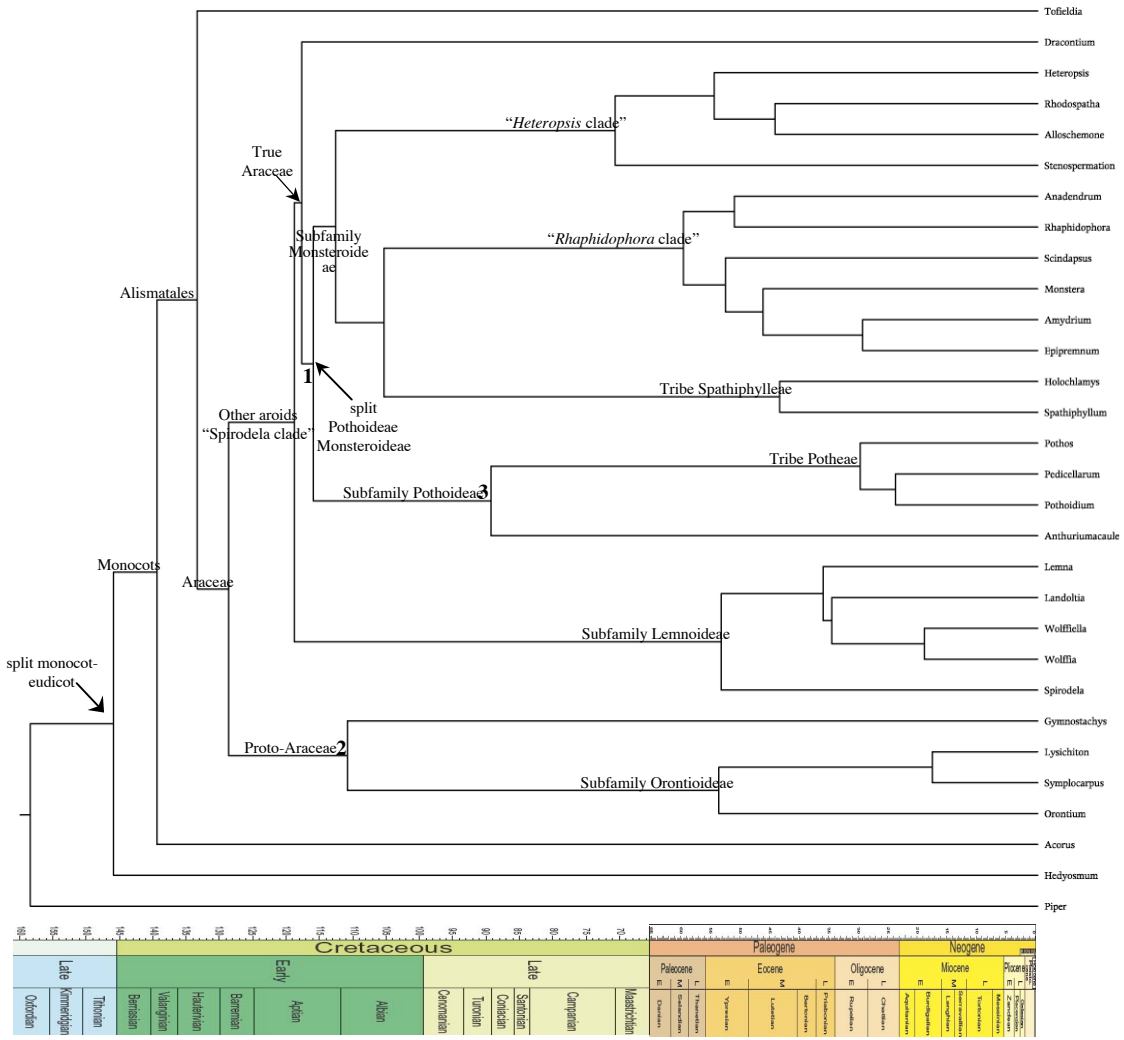

Figure 1. Araceae phylogeny with age estimates for relevant clades. Calibration points are numbered and correspond to the following fossils: (1) Araceae fossil sp. B - 112 Myo, (2) *Albertarum pueri* - 72 Myo, (3) *Petrocardium cerrejonense* - 58 Myo. The node for the monocot-eudicot split was fixed at the following variable ages, 137, 154 and 220 Mya. Geological time scale from Gradstein and Ogg (2004).

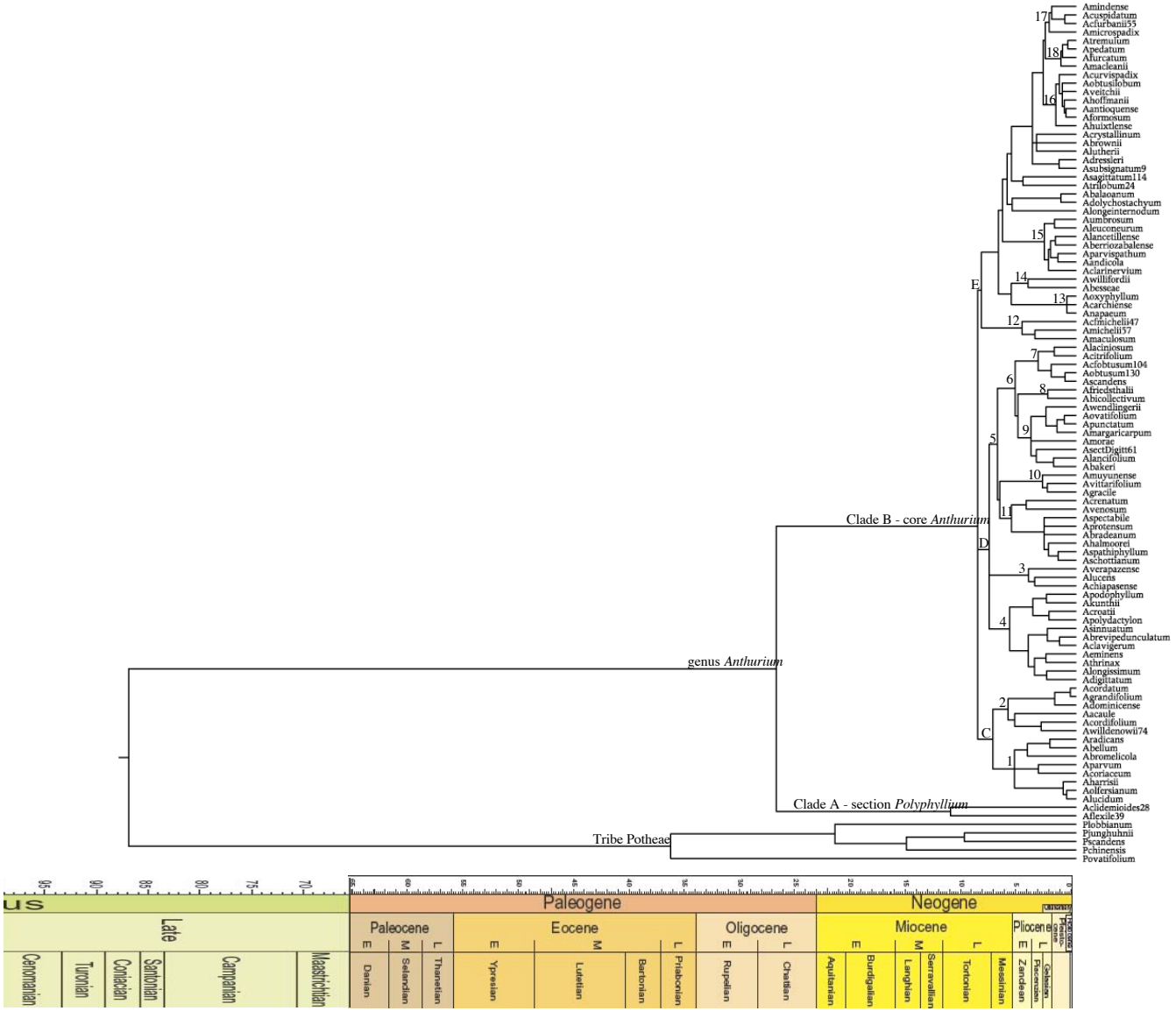

Figure 2. *Anthurium* phylogeny with age estimates for relevant clades. The root of the tree was fixed at 86 Mya. Geological time scale from Gradstein and Ogg (2004). Numbers and letters correspond to the major clades in the phylogeny of Carlsen (2011).

Table 1. Estimated ages (in million years ago - Mya) of nodes in the Araceae-phylogram (i.e. crown group ages).

	Split monocot-eudicot fixed age 137 Mya			**Split monocot-eudicot fixed age 154 Mya**			**Split monocot-eudicot fixed age 220 Mya**			**Geological time [4]**
Node in Araceae phylogram (**)	**All fossils[1]**	**"Old fossil"[2]**	**"Young fossils"[3]**	**All fossils[1]**	**"Old fossil" [2]**	**"Young fossils" [3]**	**All fossils[1]**	**"Old fossil" [2]**	**"Young fossils" [3]**	
Root	146.88 ±0.32	146.88 ±0.32	149.24 ±0.22	165.89 ±0.25	165.89 ±0.25	168.04 ±0.18	239.23 ±0.56	239.23 ±0.56	241.03 ±0.64	Middle to Late Jurassic
Monocots	131.52 ±0.28	131.52 ±0.28	119.79 ±0.30	144.67 ±0.25	144.67 ±0.25	132.79 ±0.29	195.01 ±0.32	195.01 ±0.32	182.63 ±0.42	Early Cretaceous
Alismatales	126.58 ±0.24	126.58 ±0.24	104.5 ±0.28	136.31 ±0.22	136.31 ±0.22	113.97 ±0.29	172.77 ±0.30	172.77 ±0.30	149.54 ±0.40	Early Cretaceous Hauterivian-Barremian
Araceae	122.71 ±0.21	122.71 ±0.21	92.87 ±0.26	129.80 ±0.22	129.80 ±0.22	99.73 ±0.27	155.76 ±0.30	155.76 ±0.30	124.98 ±0.38	Early Cretaceous Barremian-Aptian
Proto-Araceae (min. age 72 Mya)	109.59 ±0.38	109.59 ±0.38	80.94 ±0.42	115.07 ±0.36	115.07 ±0.36	86.70 ±0.40	136.11 ±0.42	136.11 ±0.42	107.97 ±0.49	Early Cretaceous Aptian-Albian
Subfamily Orontioideae	51.58 ±0.28	51.58 ±0.28	37.71 ±0.34	53.97 ±0.26	53.97 ±0.26	40.35 ±0.31	63.47 ±0.42	63.47 ±0.42	50.14 ±0.51	Early Eocene Ypresian
Other aroids ("*Spirodela* clade")	114.72 ±0.28	114.72 ±0.28	71.66 ±0.32	116.71 ±0.26	116.71 ±0.26	74.32 ±0.29	124.08 ±0.31	124.08 ±0.31	83.88 ±0.40	Early Cretaceous Aptian
Subfamily Lemnoideae	49.02 ±0.20	49.02 ±0.20	28.67 ±0.29	48.81 ±0.21	48.81 ±0.21	29.55 ±0.29	49.97 ±0.34	49.97 ±0.34	35.85 ±0.36	Early Eocene Ypresian
True Araceae	113.67 ±0.20	113.67 ±0.20	68.51 ±0.24	114.89 ±0.22	114.89 ±0.22	70.53 ±0.26	119.40 ±0.32	119.40 ±0.32	77.65 ±0.39	Early Cretaceous Aptian
Split Pothoideae-Monsteroideae (min. age 112 Mya)	112 ±0	112 ±0	63.6 ±0.26	112 ±0	112 ±0	64.66 ±0.24	112 ±0	112 ±0	68.31 ±0.38	Early Cretaceous Aptian-Albian
Subfamily Pothoideae‡ (min. age 58 Mya)	86.74 ±0.21	86.74 ±0.21	58 ±0	86.53 ±0.22	86.53 ±0.22	58 ±0	86.06 ±0.32	86.06 ±0.32	58 ±0	Late Cretaceous Coniacian
Tribe Pothoeae	28.76 ±0.20	28.76 ±0.20	19.05 ±0.25	28.75 ±0.23	28.75 ±0.23	19.11 ±0.29	28.74 ±0.32	28.74 ±0.32	19.29 ±0.37	Early Oligocene Rupelian
Subfamily Monsteroideae (min. age 117 Mya)	58.2 ±0.23	58.2 ±0.23	32.96 ±0.27	58.13 ±0.21	58.13 ±0.21	33.50 ±0.29	58.04 ±0.22	58.04 ±0.22	35.40 ±0.38	Late Paleocene Thanetian
"*Rhaphidophora* clade"	38.44 ±0.21	38.44 ±0.21	21.86 ±0.26	38.44 ±0.26	38.44 ±0.26	22.23 ±0.29	38.48 ±0.33	38.48 ±0.33	23.54 ±0.38	Middle Eocene Bartonian

Node in Araceae phylogram (**)	Split monocot-eudicot fixed age 137 Mya			Split monocot-eudicot fixed age 154 Mya			Split monocot-eudicot fixed age 220 Mya			Geological time [4]
	All fossils[1]	"Old fossil"[2]	"Young fossils"[3]	All fossils[1]	"Old fossil" [2]	"Young fossils" [3]	All fossils[1]	"Old fossil" [2]	"Young fossils" [3]	
Tribe Spathiphylleae	23.52 ±0.20	23.52 ±0.20	13.37 ±0.27	23.51 ±0.23	23.51 ±0.23	13.60 ±0.29	23.54 ±0.30	23.54 ±0.30	14.40 ±0.37	Late Oligocene Chattian
"*Heteropsis* clade"	40.11 ±0.22	40.11 ±0.22	22.77 ±0.27	40.09 ±0.23	40.09 ±0.23	23.16 ±0.28	40.10 ±0.32	40.10 ±0.32	24.51 ±0.37	Middle Eocene Bartonian

Notes: (*) Geological time estimates were derived including only values within the same geological period and mostly the same geological epoch, values not included in calculations (outliers) are shaded in the table. (**) Node names within the Araceae are taken from the most recent molecular phylogenies of the family (Cabrera et al. 2008, Cusimano et al. 2011). (‡) The crown node of subfamily Pothoideae is also the stem node of the genus *Anthurium*, and its age estimates were used as calibration point in the *Anthurium* phylogram. (1) Using all four fossils as minimum age constraints along the phylogram (see Materials and Methods - Age and fossil calibration for details). (2) Using only "Old fossil" (Araceae fossil sp. B – 112 Myo) as minimum age constraints along the phylogram. (3) Using only "Young fossils" (*Albertarum pueri* – 72 Myo and *Petrocardium cerrejonense* – 58 Myo) as minimum age constraints along the phylogram. (4) According to the Geological Time Scale of Gradstein & Ogg (2004).

Table 2. Estimated ages (in million years ago - Mya) of nodes in the *Anthurium*-phylogram (i.e. crown group ages).

Node in *Anthurium*-phylogram**	Root – Subfamily Pothoideae crown group (fixed age)* 86 Mya	Geological time(1)
Tribe Pothoeae	36.79±0.24	Middle Eocene – Bartonian
Genus *Anthurium*	27.2±0.18	Late Oligocene – Chattian
Clade A (section *Polyphyllium*)	11.37±0.32	Middle Miocene – Serravalian
Clade B (core *Anthurium*)	8.93±0.22	Late Miocene – Tortonian
Clade C	7.54±0.21	Late Miocene – Tortonian
Clade D	7.87±0.15	Late Miocene – Tortonian
Clade E	8.57±0.24	Late Miocene – Tortonian
Clade 1 (Brazilian clade)	5.57±0.22	Late Miocene – Messinian
Clade 2 (Caribbean clade)	6.16±0.14	Late Miocene – Messinian
Clade 3 (Mexican punctate clade)	4.30±0.21	Early Pliocene – Zanclean
Clade 4 (Palmate-leaves clade)	6.01±0.11	Late Miocene – Messinian
Clade 5	7.13±0.14	Late Miocene – Messinian
Clade 6 (Punctate clade)	5.51±0.15	Late Miocene – Messinian
Clade 7 (section *Tetraspermium*)	3.43±0.14	Early / Late Pliocene
Clade 8	2.55±0.22	Late Pliocene
Clade 9	4.08±0.21	Early Pliocene
Clade 10 (section *Leptanthurium*)	3.04±0.13	Early / Late Pliocene
Clade 11 (section *Pachyneurium* series *Pachyneurium*)	5.84±0.22	Late Miocene – Messinian
Clade 12	4.89±0.21	Early Pliocene - Zanclean
Clade 13 (section *Pachyneurium* series *Multinervia*)	0.82±0.13	Holocene
Clade 14	4.34±0.15	Early Pliocene – Zanclean
Clade 15 (Mexican epunctate clade)	2.87±0.15	Early / Late Pliocene
Clade 16 (section *Calomystrium*)	1.51±0.22	Pleistocene
Clade 17	2.20±0.21	Late Pliocene
Clade 18	1.36±0.21	Pleistocene

Note: (*) The root of the tree was fixed with the age estimated for the crown node of the Subfamily Pothoideae in the analyses of the Araceae-phylogram (see Table 1). (**) Node names within *Anthurium* follow the most recent molecular phylogeny of the genus (Carlsen 2011). (1) According to the Geological Time Scale of Gradstein & Ogg (2004).