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A Molecular Phylogeny of the Species-Rich Neotropical Genus *Anthurium* (Araceae) based on Combined Chloroplast and Nuclear DNA

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Abstract*—Anthurium* is a strictly Neotropical genus of Araceae ranging from southern Mexico to northern Argentina, including approximately 900 accepted species names. Despite its immense diversity, its ecological importance in Neotropical forests, and a long history of botanical collection, cultivation, and taxonomical research, *Anthurium* has been only cursorily sampled in previous molecular phylogenies. This study aims to test the monophyly of *Anthurium*, to understand the evolutionary history of the genus, and to elucidate relationships among its species using maximum parsimony, maximum likelihood and Bayesian analyses on a combined chloroplast (*trnG* intron, *trnH-psbA* and *trnC-ycf6* intergenic spacers) and nuclear (*CHS* first intron and partial flanking coding regions) DNA sequence dataset for 102 *Anthurium* species and closely related outgroups. Despite some limitations (ca. 11% species richness coverage, and lack of nuclear sequences for outgroups), results indicate that *Anthurium* is a strongly supported monophyletic genus and that at least 18 major supported clades are recognizable within it, most of them easily characterized morphologically and/or geographically. This study also suggests that the current sectional classification of *Anthurium* does not accurately represent its evolutionary history since most of the major clades recovered in these analyses do not correspond with the current circumscriptions of infrageneric groups. Despite using the most variable gene regions available, low sequence divergence was found among *Anthurium* species, relatively short branches characterize the core of the *Anthurium* clade, and resolution is still lacking in the deeper nodes of the phylogeny, a pattern consistent with a rapid, and probably recent, radiation of species.

Keywords*—CHS* first intron, geography, rapid radiation, sectional classification.

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 912 accepted names of largely well-differentiated species (Mayo et al. 1997; Govaerts and Frodin 2002; Govaerts et al. 2011; CATE Araceae 2011) and many more are still being described (T. B. Croat, unpubl. data; Boyce and Croat 2012). 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, and the monotypic genera *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, terrestrials 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 in 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 65% of the total), ranks among the five major epiphytic plant clades, surpassed only by a few clades of Epidendroideae–Orchidaceae (Gentry and 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, commonly known as "flamingo flower" or "wax flower") (Bown 2000).

Anthurium is also one of the most morphologically diverse genera in Araceae. It displays remarkable variation in leaf morphology, including entire (linear, lanceolate, ovate, elliptical, rounded, peltate, cordate, sagittate, and hastate), variously lobed (trilobed and palmately-lobed), and compound (trisect and 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 similar, all having bisexual flowers in uniform, mostly cylindrical, tapered spadices, though flowering behavior is quite variable among species (Croat 1980). On the other hand, great diversity is displayed in inflorescence and fruit colors, ranging from inconspicuous green and white, to highly attractive yellow, orange, red, lavender, and purple, with several shades in between.

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 and Sheffer 1983). The currently accepted sectional classification of *Anthurium* (Croat and Sheffer 1983) consists of 18 sections and two series, characterized mainly by differences in habit, conditions of the dried cataphylls, leaf shape, vernation, presence of punctations, cellular inclusions or pale lineations, leaf venation, root distribution, and number of seeds per fruit. A few sections are easily diagnosed by unique characters (e.g. four seeds per fruit in section *Tetraspermium* Schott), while others

have more complicated combinations 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 in their revisions corresponded to some sort of relationship among them.

Phylogenies of species-rich clades, like *Anthurium*, can help assess the roles of ecological diversification, dispersal and vicariance in the evolution of the group, as well as key innovations affecting speciation rates in the Neotropics (Richardson et al. 2001; Kay et al. 2005; Erkens et al. 2007). However, despite its great diversity, its importance in Neotropical forests, and a long history of botanical collection, cultivation, and taxonomical research, previous molecular phylogenetic studies have only sampled a small subset of the diversity in *Anthurium*. Molecular phylogenies of Araceae focusing on large-scale intrafamilial relationships have included only up to seven *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). A preliminary attempt at phylogenetic reconstruction within *Anthurium* includes a phylogeny with 30 species based solely on a parsimony analysis of partial sequences of the chloroplast *trnG-trnS* intergenic spacer (Swart 2001). The resulting phylogeny shows *Anthurium* as a monophyletic genus, but species relationships are mainly unresolved, except for the presence of four strongly supported species-pairs (Swart 2001). Temponi (2006) reconstructed a phylogeny of *Anthurium* using parsimony analysis of three chloroplast markers (*trnG* intron, *trnH-psbA* and *trnC-ycf6* intergenic spacers) with an increased sample of 75 species, mainly focused on Brazilian representatives, and having only five taxa in common with Swart (2001). This expanded phylogeny recovered all species of *Anthurium* section *Urospadix* Engl. as a monophyletic group, however support values for all other relationships within the genus are low, except for three pairs of species (Temponi 2006).

In the current study, maximum parsimony, maximum likelihood and Bayesian phylogenetic analyses of combined chloroplast (*trnG* intron, *trnH-psbA* and *trnC-ycf6* intergenic spacers) and nuclear (*CHS* first intron and partial flanking coding regions) sequence data are used to test the monophyly of *Anthurium* and elucidate relationships among its species. This study includes 73 newly sampled *Anthurium* species, and overlaps in 29 species with Temponi (2006) and in 12 species with Swart (2001). Therefore, it comprises the most comprehensive molecular phylogeny of the genus *Anthurium* to date, 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 suggested.

Materials and Methods

Taxon Sampling—A total of 102 *Anthurium* species were sampled for this study (ca. 11% of the currently recognized species names in the genus), spanning the morphological, taxonomic, and geographic diversity within the group (Appendix 1). Taxonomic sampling included at least one and up to 10 representatives of each of the 18 sections and two series proposed by Croat and Sheffer (1983), except for the monotypic section *Gymnopodium* Engl. endemic to Cuba. Five species of *Pothos*, the monotypic *Pothoidium* (both genera belonging to the sister tribe Potheae), five species of the closely related subfamilies Orontioideae and Monsteroideae, and *Acorus calamus* L. were used as outgroups (Appendix 1).

DNA Sequence Data—Total genomic DNA was extracted from fresh or silica dried leaf material using a modified CTAB protocol (Doyle and Doyle 1987). The PCR amplification reactions contained 2–3 μL of DNA template, 5 μL of 5X reaction buffer, 5 μL of 2.5 mM $MgCl_2$, 3 μL of 2.5 mM dNTPs mix, 2 μL of 10 μM stock for each primer, 0.8 μL of Taq polymerase (5 units/μL) (Promega, Madison, Wisconsin) and water to a final volume of 50 μL. Thermocycling conditions included a 2 min denaturation step at 94°C, followed by 40 cycles of 1 min at 94°C, 1 min at 60°C, 2 min at 72°C, and a final 7 min extension at 72°C. The PCR products were recovered and purified from a 2% agarose gel using QIAquick Gel Extraction Kit (Qiagen Inc., Valencia, California) following the manufacturer's protocols. Sequencing reactions used the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California) 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 (U. S. A.) or Macrogen (Korea). 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 level of 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 (cpDNA) 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 (nDNA) primer sets included the first intron of chalcone synthase (*CHS*) and partial flanking coding regions, amplified using *Anthurium*-specific primers designed here (CHSf: 5′-AGGAGAAGTTCAGGCGCATG-3′ and CHSr: 5′-A(CG)GTGGGTGATCTT(CG)GA(CT)-3′). A set of nuclear ITS primers (Baldwin 1992) was also tried, but *Anthurium* ITS sequences showed a high occurrence of pseudogenes, as inferred by angiosperm 5.8S motifs not being conserved (Harpke and Peterson 2008), and thus the ITS region was not included in this study. In order to determine if there were multiple copies of the *CHS* gene, PCR fragments were cloned using the p-GEM-T Easy Vector System (Promega, Madison, Wisconsin) following the manufacturer's protocol. For each species a maximum of three clones were sequenced. If all clones per taxon clustered together in a single 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 but not multiple copies of the gene. Using these criteria, no taxon in the study had multiple copies of *CHS*, so for all final analyses a single randomly-chosen sequence was used to represent each taxon. Contigs between two sequence strands (forward and reverse) with sequence overlap of more than 75% were created for each taxon using Lasergene-Seqman Pro version 8.0.2 (DNASTAR Inc., Madison, Wisconsin). Species sequences were first aligned using Clustal-X version 1.81 (Thompson et al. 1997) and the alignment was manually edited in MacClade v. 4.08 OS X (Maddison and Maddison 2000). Partial 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 of *CHS* intron and partial flanking coding regions. Species overlap among data partitions is high for chloroplast (98–100%) and *CHS* (87%) datasets. Outgroup 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 (Appendix 1) and the aligned cpDNA-nDNA data matrix is available in TreeBASE (study number 13701).

Phylogenetic Analyses—Maximum parsimony, maximum likelihood, and Bayesian 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). Topological congruence among different datasets was assessed using the incongruence length difference (ILD) test (Farris et al. 1994) as implemented in PAUP* version 4.0b10 (Swofford 2002). Each ILD test consisted of 100 replicates, with 10 random additions per replicate. 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 and Bryant 2006). Maximum parsimony analyses were performed using the parsimony ratchet (Nixon 1999) with the program

PAUPRat (Sikes and 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. Strict consensus trees from the resulting most parsimonious PAUPRat trees were computed in PAUP* (Swofford 2002), and clade support was estimated using non-parametric bootstrap (Felsenstein 1985). One thousand maximum parsimony 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 and Crandall 1998). 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. Maximum likelihood analyses (Felsenstein 1973) and non-parametric, fast bootstrapping were performed using RAxML v.7.2.7 (Stamatakis 2006; Stamatakis et al. 2008). This program uses the GTRGAMMA model of sequence evolution for complete likelihood evaluation and the GTRCAT approximation of the model for 1,000 bootstrap replicates. Bayesian analyses were carried out using MrBayes v.3.1.2 (Huelsenbeck et al. 2001; Ronquist and Huelsenbeck 2003) 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, and a burn-in of 4,500 trees. In interpreting phylogenetic confidence, nodes are considered "strongly supported" when they received bootstrap values from both the parsimony and likelihood analyses equal to or higher than 90% and Bayesian posterior probability (PP) 0.95 or higher, as "highly supported" when one of the bootstrap values (usually in parsimony analyses) falls between 70–89%, whereas the other two measures of support are still "strong," and as "moderately supported" when Bayesian posterior probability is 0.95 or higher and at least one of the other bootstrap supports is between 70–89%. This confidence system was mostly based on support categories for bootstrap values previously proposed (Hillis and Bull 1993; Huelsenbeck et al. 1996) and slightly modified to account for the combination of different support measures in the same node.

Results

cpDNA Sequence Variation And Phylogenetic Analyses—The three cpDNA regions were sequenced for 113 taxa (102 *Anthurium* species and 11 outgroups), except for *A. sagittatum* (Sims) G. Don and *Symplocarpus foetidus* (L.) W. Salisb. missing from the *trnC-ycf6* dataset (Appendix 1). *Acorus calamus* cpDNA sequences were obtained from the whole chloroplast genome in GenBank (NC-007407.1). In the *trnH-psbA* dataset, five *Anthurium* species were represented by partial sequences missing 28–40 aligned base pair (bp) positions. In the *trnC-ycf6* dataset, *Pothoidium lobbianum* Schott is represented by a partial sequence missing 86 aligned bp and *Lysichiton americanus* Hultén & H. St. John is missing 554 aligned bp, but in both cases the missing portions are part of indels that were 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 662–690 bp. Those of the sequenced portion of the *trnH-psbA* intergenic spacer ranged from 240–472 bp within *Anthurium* and 327–545 bp in outgroups. In general, *trnH-psbA* sequences of outgroups were longer than for most *Anthurium* species (average size 425 bp vs. 287 bp, respectively) due to a 142 bp indel that was excluded from all analyses. Also excluded from the *trnH-psbA* dataset was a long 147 bp indel unique to *A. parvispathum* Hemsl. and a large, size variable, AT-rich region (up to 203 aligned bp). Unaligned sequences of the portion of *trnC-ycf6* intergenic spacer ranged between 515–610 bp in *Anthurium* and 864–989 bp in outgroups, mainly due to a 485 bp indel present in the outgroups but lacking in *Anthurium* (Table 1). In general, parsimony, maximum likelihood, and Bayesian analyses of the individual cpDNA datasets produced topologies largely lacking in resolution but congruent with one another in the few (5–28) shallow nodes supported (Table 2).

nDNA Sequence Variation And Phylogenetic Analyses—The complete first intron of the chalcone synthase gene (*CHS*) and partial flanking coding regions were sequenced for 99 *Anthurium* species, sequences being missing only from *A. longicaudatum* Engl., *A. palenquense* Croat and *A. napaeum* Engl. Due to primer sequence specificity and PCR amplification problems no outgroups could be sequenced for this region. Unaligned sequences of *CHS* included in the analyses varied in length from 583–941 bp (Table 1). *Anthurium* sp. 4 is missing about 280 bp of this region. The *CHS* intron is characterized by long species-specific indels, such as in *A. wendlingeri* G. M. Barroso (145 aligned bp), while a 255 bp indel is present only in *A. clidemioides* Standl. and *A. flexile* Schott. Parsimony, maximum likelihood and Bayesian analyses of the nDNA dataset increased considerably the resolution of nodes within *Anthurium* (Table 2); however, the backbone of the tree still lacks support (Supplemental Fig. 1).

Table 1. Characteristics of cpDNA (*trnG* intron, *trnH-psbA* and *trnC-ycf6* intergenic spacers) and nDNA (first intron and partial flanking coding regions of chalcone synthase – *CHS*) datasets used to reconstruct the molecular phylogeny of *Anthurium*. N/A = not applicable.

	trnG	*trnH-psbA*	*trnC-ycf6*	Combined cpDNA	*CHS*	Combined cpDNA-nDNA
Number of taxa (*Anthurium*/outgroups)	102 / 12	102 / 12	101 / 11	102 / 12	99 / 0	102 / 12
Sequence length (bp) (*Anthurium*)	662–690	240–472	515–610	N/A	583–941	N/A
Sequence length (bp) (outgroups)	671–687	327–545	864–989	N/A	N/A	N/A
Alignment length (bp)	814	1,007	1,328	3,149	1,252	4,401
Number of characters excluded	141	782	734	1657	583	2,240
Number of characters included	673	225	594	1,492	669	2,161
% gaps / % missing data	1.12 / 0	1.34 / 0.37	0.68 / 0.59	0.97 / 0.98	2.40 / 0	1.32 / 4.75
Number of variable sites (%)	203 (30%)	90 (40%)	212 (36%)	505 (34%)	341 (51%)	846 (39%)
Number of parsimony informative sites included (%)	131 (19%)	50 (22%)	103 (17%)	284 (19%)	174 (26%)	458 (21%)
Number of parsimony informative sites within *Anthurium* (%)	46 (7%)	28 (12%)	53 (9%)	127 (8%)	171 (26%)	298 (14%)
Average uncorrected pairwise sequence divergence among *Anthurium* species (%)	0.60	1.23	1.16	N/A	3.33	N/A
Average uncorrected pairwise sequence divergence within tribe Potheae (%)	1.72	2.01	1.61	N/A	N/A	N/A

TABLE 2. Characteristics of the parsimony ratchet, maximum likelihood and Bayesian analyses performed on each of the cpDNA and nDNA datasets used to reconstruct the phylogeny of *Anthurium*. MP = bootstrap values from the parsimony analysis; ML = fast-bootstrap values from the likelihood analysis; PP = Bayesian posterior probability.

	trnG	*trnH-psbA*	*trnC-ycf6*	Combined cpDNA	*CHS*	Combined cpDNA-nDNA
Parsimony ratchet analysis						
Tree length (steps)	321	141	291	768	598	1427
Consistency index (CI)	0.735	0.787	0.818	0.762	0.741	0.734
Retention index (RI)	0.855	0.902	0.915	0.880	0.804	0.819
Number of supported clades within *Anthurium* (MP > 70%)	14	5	11	22	26	41
Maximum likelihood analysis						
Log likelihood score	–2,834.796040	–1,100.194169	–2,525.080809	–6,719.779297	–5,023.405129	–9,710.837407
Number of supported clades within *Anthurium* (ML > 70%)	18	15	23	41	36	51
Bayesian analysis						
Total mean tree length	22.263566	22.237199	21.845756	20.109512	18.263092	1.529200
Variance in tree length	2.082776	2.235785	2.290113	2.179169	1.969983	0.007811
Arithmetic mean of the log-likelihood	–3,167.07	–1,291.26	–2,862.73	–7,333.25	–5,393.63	–12,604.72
Number of supported clades within *Anthurium* (PP > 0.95)	14	12	28	46	29	63

Congruence Among DNA Partitions/Datasets—Results of the ILD 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. 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. 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 13 bp, respectively. Several authors (Sang et al. 1997; Mes et al. 2000; Bain and 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 and Wendel 1996; Ki-Joong and 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* vs. *trnH-psbA* $p = 0.51$; *trnC-ycf6* vs. *trnH-psbA* $p = 0.63$).

Significant phylogenetic conflict was also found among all independent cpDNA regions and the nDNA (*CHS*) dataset (ILD tests with $p = 0.01$). 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. Closer examination of the *CHS* alignment identified a few nucleotides in the third 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* vs. *CHS* $p = 0.79$; *trnC-ycf6* vs. *CHS* $p = 0.61$; *trnH-psbA* vs. *CHS* $p = 0.63$).

Combined Phylogenetic Analyses—The combined cpDNA data matrix contained 114 taxa (12 outgroups and 102 *Anthurium* species) and 3,149 aligned base pair positions. Included characters consisted of 1,492 aligned base pair positions (0.98% missing) with a total of 505 variable sites and 284 parsimony informative sites of which 127 are exclusively within *Anthurium* (Table 1). In general, the consensus Bayesian tree has more strongly supported nodes within *Anthurium* than the parsimony strict consensus and maximum likelihood trees (Table 2). When all measures of support are combined for a single node, there are 11 strongly supported nodes, nine highly supported, and 15 moderately supported (Supplemental Fig. 2).

The combined cpDNA and nDNA dataset included the same 114 taxa, but due to PCR amplification problems, nDNA sequences are missing for all outgroups. The combined cpDNA-nDNA data matrix consisted of 4,401 aligned base pair positions, of which 2,161 were included (4.75% missing), containing a total of 846 variable sites and 458 parsimony informative, of which 298 are exclusively within *Anthurium* (Table 1). As before, the Bayesian consensus tree shows higher resolution than the parsimony strict consensus and the maximum likelihood trees (Table 2). When all measures of support are combined, there are 23 strongly supported nodes within *Anthurium*, 11 highly supported and 19 with moderate support (Fig. 1). In general, the combined cpDNA-nDNA analyses produced considerably greater resolution and higher support for clades within *Anthurium* than did individual analyses. Maximum likelihood and Bayesian trees are characterized by relatively long branches in the outgroups and the first diverging lineages within *Anthurium* (Clades A and B), but branch lengths are noticeably shorter inside the core of *Anthurium* (within Clade B), with the exception of the branch leading to Clade 8 (Fig. 2).

DISCUSSION

This is the first molecular phylogenetic study that offers an in-depth insight into the evolutionary history of the extremely

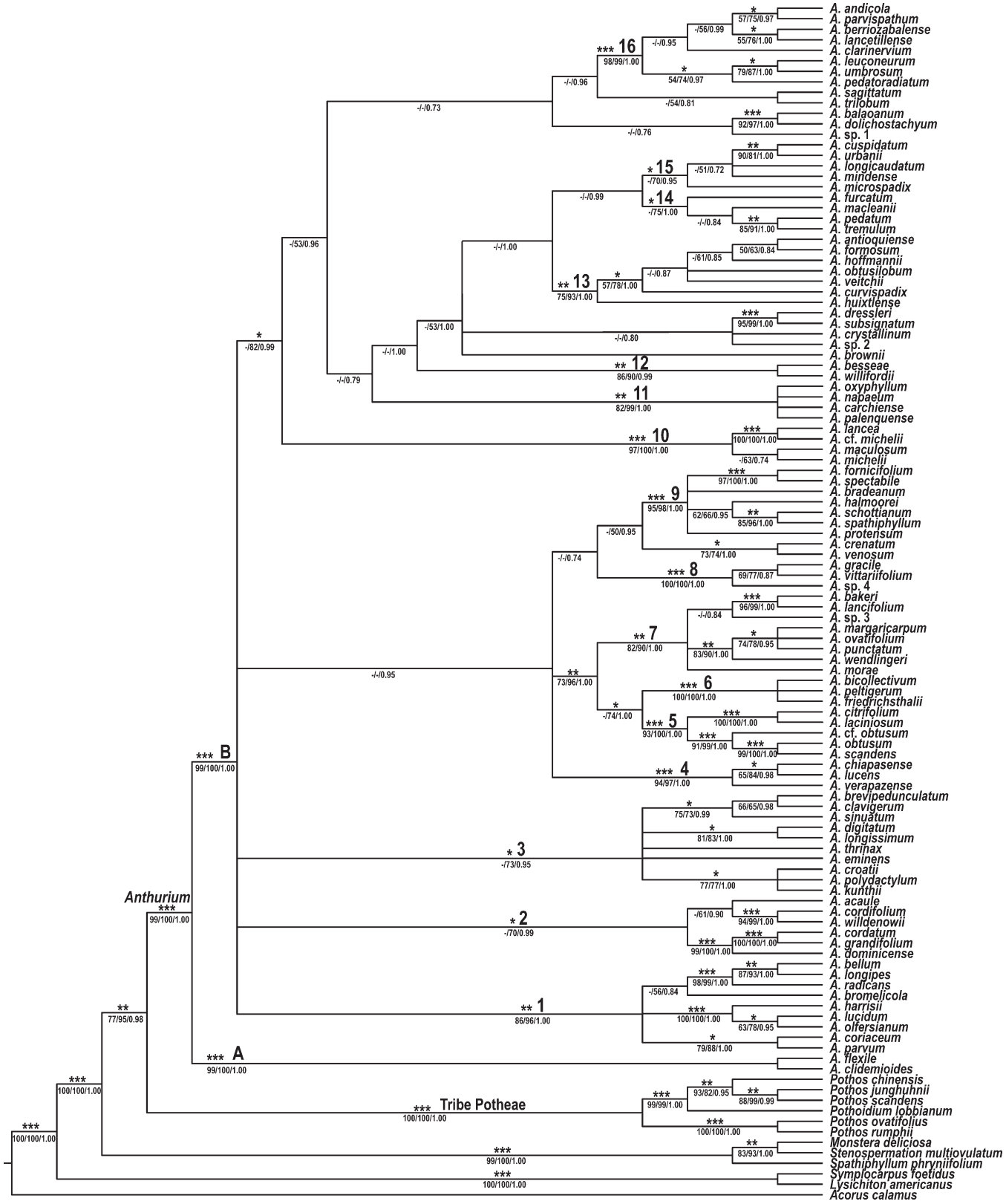

FIG. 1. Bayesian consensus tree from the analysis of the combined cpDNA-nDNA dataset (*trnG* intron, *trnH-psbA* and *trnC-ycf6* intergenic spacers, first intron of *CHS* and partial flanking coding regions) of *Anthurium* and closely related outgroups. Maximum parsimony bootstrap values (MP), maximum likelihood bootstrap values (ML) and Bayesian posterior probabilities (PP) are shown below branches in that order. Bootstrap values < 50% are labeled with a dash (–). Support categories are identified with asterisks above branches, (***) "strongly supported" (MP and ML > 90%, and PP > 0.95), (**) "highly supported" (one bootstrap value 70–89%, other bootstrap value > 90%, and PP > 0.95), and (*) "moderately supported" (MP and/or ML between 70–89% and PP > 0.95). Letters (A–B) and numbers (1–16) above branches identify major clades discussed here (see details in text).

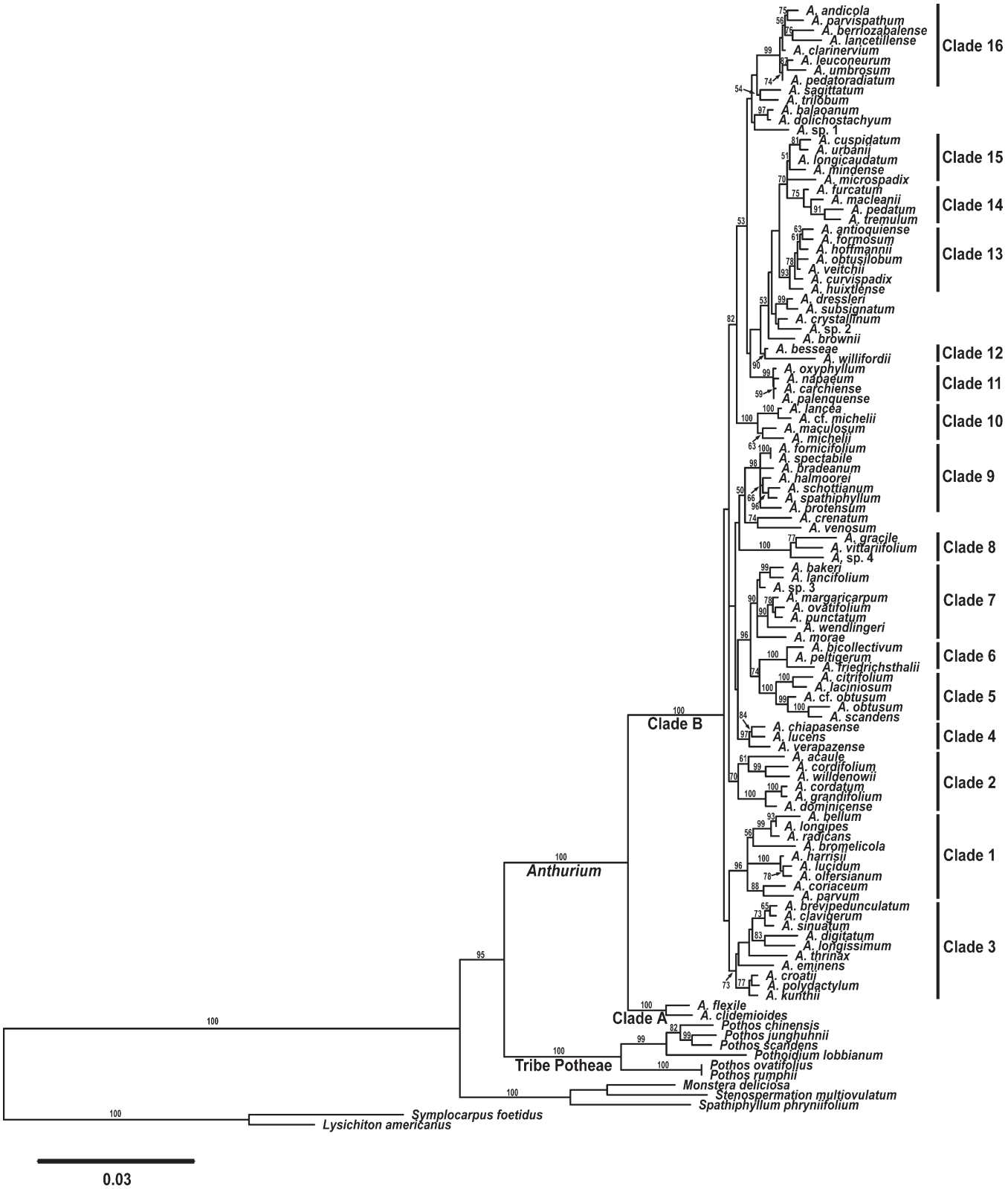

FIG. 2. Phylogram of the most likely tree recovered from the maximum likelihood analysis of the combined cpDNA-nDNA dataset (*trnG* intron, *trnH-psbA* and *trnC-ycf6* intergenic spacers, first intron of *CHS* and partial flanking coding regions) of *Anthurium* and closely related outgroups. Scale bar represents branch length in substitutions per site. *Acorus calamus* L. was deleted from the figure for illustrative purposes. Maximum likelihood bootstrap values (ML) are shown above branches. Bootstrap values < 50% are not included. Major clades are identified below branches inside the tree (Clades A–B) or with bars outside the tree (Clades 1–16).

diverse genus *Anthurium*, which includes approximately 27% of the species in Araceae. Taxon sampling and phylogenetic support in this study were sufficient to provide an improved understanding of major clades within *Anthurium* and possible relationships among them; however, resolution is still lacking in some areas of the phylogeny, especially in the deeper nodes.

This study demonstrates the utility of the first intron and partial flanking coding regions of the low copy nuclear gene chalcone synthase (*CHS*) in resolving relationships among closely related species in *Anthurium*. 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.

The following discussion will focus on the results of Bayesian analyses of the highly congruent combined cpDNA-nDNA dataset (Fig. 1). Any disagreements between these general results with those from analysis of single gene regions or other analytic methods (maximum parsimony and maximum likelihood) will be mentioned when they occur. Most of the major clades recovered can also be characterized by morphological and/or geographical diagnostic characters, but not always completely so. A detailed analysis of morphology and character evolution within the genus is currently underway.

Even though nDNA sequences were not obtained for outgroups, the results of the combined cpDNA-nDNA analyses (Fig. 1) revealed that the monotypic genus *Pothoidium* is strongly supported 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 *Pothos*, 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 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 (Fig. 1). Even though the lack of nuclear outgroup sequences precludes further assessments, a similar outcome is presumed if these data could be included. 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, uniform spadix with persistent open spathe, bisexual flowers with four decussate tepals, and copious endosperm (Mayo et al. 1997). However, none of these characters are unique to *Anthurium* and several are plesiomorphies within the family (Mayo et al. 1997). For the cpDNA regions sequenced, this study also reveals six indel substitutions (not included in the analyses) that support *Anthurium* as being monophyletic. In comparison to the outgroups, all *Anthurium* species present a 3 bp indel in the *trnG* intron, a 5 bp indel in the *trnC-ycf6* intergenic spacer, and a 4 bp indel in the *trnH-psbA* intergenic spacer. *Anthurium* species also lack a 3 bp indel in the *trnG* intron and, 6 and 485 bp indels in the *trnC-ycf6* intergenic spacer characteristic of all outgroups (see DNA matrix in TreeBASE).

Sequence divergence among *Anthurium* species is generally low (Table 1), averaging between 0.6–1.23% pairwise distance divergence for each of the three chloroplast regions sequenced. Comparatively, average pairwise distance divergence among *Pothos* species (including *Pothoidium*) is significantly higher, ranging between 1.61–2.01%. In addition, relatively short branches characterize the core of *Anthurium* compared to the outgroups (Fig. 2). This pattern is expected in cases of rapid diversification or slow-down in the rate of molecular evolution, where a clade can be characterized by great morphological diversity but little molecular variation among species (e.g. *Inga*, Richardson et al. 2001; *Costus* subgenus *Costus*, Kay et al. 2005; Valerianaceae, Bell and Donoghue 2005; *Lupinus*, Hughes and Eastwood 2006; *Guatteria*, Erkens et al. 2007; tree-ferns, Korall et al. 2010). Grayum (1990) previously suggested that a rapid radiation took place early on the evolutionary history of *Anthurium*. Future dating of the phylogeny will help corroborate this assumption and time major diversification events within *Anthurium*, however, this study does suggests the possibility of a rapid radiation of species (Fig. 2).

The phylogenetic analyses presented here reveal 18 major supported clades within *Anthurium* (Fig. 1; Clades A–B and 1–16). All species sampled fall within two major groups, Clade A or B. Clades 1–16 are nested within Clade B, and ca. 88% of the species sampled could be unequivocally assigned to these clades. In addition, most major clades can be diagnosed morphologically and/or geographically. Thirteen of these are recognized and circumscribed for the first time in this study, and only five clades (Clades A, 5, 9, 11, and 13) are congruent with the current sectional classification of the genus (Croat and Sheffer 1983) (Fig. 1; Table 3). However, relationships among major clades and placements of ca. 12% of the sampled species are still unclear due to lack of support along the backbone of the phylogeny. In addition, even though taxon coverage enclosed most of the morphological and geographic diversity within the genus, only ca. 11% of the species richness of *Anthurium* was sampled. Despite limitations, these 18 major clades provide a phylogenetic framework for the entire genus that will anchor future studies and provide guidelines to investigate the more complex clades still in need of work. This approach was also followed in the family Brassicaceae (Al-Shehbaz et al. 2006) characterized by several preliminary phylogenies, none of them fully resolved, that when combined together serve as an initial basis for its classification system and further studies.

There is strong support for an early-diverging lineage within *Anthurium* that includes *A. flexile* and *A. clidemioides* (Fig. 1, Clade A), which corresponds to section *Polyphyllium* Engler (Croat and Sheffer 1983) (Table 3). This species pair is broadly distributed in Central America, from Mexico to Panama. It has long been recognized as having a unique morphology within *Anthurium*, being characterized by the

TABLE 3. A comparison of the current sectional classification system of *Anthurium* (Croat and Sheffer 1983) and the placement of sampled species in major supported clades found in this study. Clade numbering follows Fig 1. [1]This section was mentioned in this classification system, but specifically not recognized due to its "unnatural" character. [2]The sectional name *Oxycarpium* Schott was later synonymized with section *Pachyneurium* due to the transfer of the type species, *A. oxycarpium* Poepp. to the latter by Croat (1991), and a new sectional name, section *Decurrentia* Croat, was proposed to accommodate the remaining species in the group (Croat et al. 2005). *A few of the species sampled in the section (ca. 1–5) do not belong to any of the major supported clades recovered in this study.

Current sectional classification (Croat and Sheffer 1983)	Number of species (total/sampled)	This study (Carlsen and Croat)
Belolonchium	ca. 110 / 10*	Clades 4, 14 and 16
Calomystrium	ca. 85 / 8	Maintained – Clade 13
Cardiolonchium	ca. 90 / 9*	Clades 10, 12 and 16
Chamaerepium	1 / 1	Embedded in Clade 1
Dactylophyllium	19 / 9	Expanded – Clade 3
Digitinervium	ca. 30 / 3	Clade 7
Episeiostenium[1]	7 / 4	Expanded – Clade 2
Gymnopodium	1 / 0	Not sampled
Leptanthurium	2 / 1	Expanded – Clade 8
Oxycarpium[2] / *Decurrentia*	ca. 45 / 7*	Clades 8, 10, and 16
Pachyneurium series *Pachyneurium*	112 / 9*	Clades 9 and 12
Pachyneurium series *Multinervia*	8 / 4	Maintained – Clade 11
Polyneurium	ca. 100 / 5*	Clades 10 and 15
Polyphyllium	2 / 2	Maintained – Clade A
Porphyrochitonium	ca. 110 / 7	Clades 6 and 7
Schizoplacium	8 / 3	Clades 3, 14, and 16
Semaeophyllium	23 / 3*	Clade 14
Tetraspermium	10 / 5	Maintained – Clade 5
Urospadix	79 / 10	Clades 1 and 2
Xialophyllium	ca. 70 / 2	Clade 15

absence of a 1-ribbed cataphyll, the presence of internodal adventitious roots, inaperturate pollen and, shiny black-dark brown seeds (Engler 1905; Croat and Baker 1978; Grayum 1990). For the DNA regions sequenced here, the presence of a 4 bp indel in the *trnH-psbA* intergenic spacer and two indels (5 and 255 bp aligned length) in the first intron of *CHS* also distinguish Clade A from the rest of *Anthurium* (indels not included in analyses; dataset available in TreeBASE).

Clade B (Fig. 1) includes all other *Anthurium* species and is strongly supported. Although previously not recognized as a clade within *Anthurium*, all these species share a morphological character unique within Araceae, the presence of multi-porate aperturate pollen, mostly with three or four pores, more rarely two. In contrast, biaperturate, zona-aperturate, or sulcate pollen are present in all other Araceae genera with aperturate pollen (Grayum 1990, 1992; Hesse 2006). Clade B is also characterized by the presence of a 5 bp indel in the *trnG* intron and a 5 bp indel in the *trnH-psbA* region, and by the absence of a 6 bp indel in the *trnC-ycf6* intergenic spacer, as well as lacking a 23 bp indel in the *trnH-psbA* region. These last two indels are otherwise present in the outgroups and in Clade A (indels not included in analyses; see matrix in TreeBASE). Most of the major clades identified in this study (i.e. Clades 1–16) are included within the larger Clade B (Fig. 1). In general, lack of support for relationships among clades and relatively short branch lengths are characteristic within this core of *Anthurium* species (Fig. 2).

In two major clades, Clades 1 and 2 (Fig. 1), species are clustered based on geographic affinity, but exclusive unifying morphological features are not apparent. All *Anthurium* species contained in the highly supported Clade 1 are endemic to Brazil. This clade was also recovered in the analyses of Temponi (2006) including a greater sampling of Brazilian endemic species (ca. 20 species). This author also 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, but this characteristic needs further investigation. Likewise, all *Anthurium* species contained in the moderately supported Clade 2 are endemic to the Lesser Antilles and Jamaica, including the type species for the genus, *A. acaule* (Jacq.) Schott, from Martinique. Although a few of these species have been previously suggested as being members of section *Episeiostenum* Schott (Schott 1860; Engler 1905), this group was not recognized in the newest sectional classification of the genus (Croat and Sheffer 1983) (Table 3). Clade 2, as circumscribed in these analyses (Fig. 1), has not been previously recognized and it lacks an evident morphological diagnostic character. This relationship between geography and molecular phylogeny, but not with morphology occurs in other plant groups as well (*Cardamine*, Brassicaceae, Carlsen et al. 2009; *Aechmea*, Bromeliaceae, Sass and Specht 2010). These authors suggested that dispersal to new areas was followed by diversification of morphological features and therefore morphological characteristics previously thought to be indicative of common ancestry have instead evolved multiple times in parallel in different geographic regions.

Almost all *Anthurium* species with palmately-lobed leaves clustered together in a moderately supported Clade 3 (Fig. 1). All species belonging to this clade also share the presence of a 3 bp indel in the *CHS* region sequenced (indels not included in analysis, matrix available in TreeBASE). Palmately-lobed *Anthurium* species have for a long time been placed in two separate sections (section *Schizoplacium* Schott and section *Dactylophyllium* Engler) (Schott 1860; Engler 1905; Croat and Sheffer 1983) (Table 3), but the findings here suggest that such division is unnecessary (Fig. 1)–indeed, the newly circumscribed Clade 3 may merit sectional rank. Although all members of Clade 3 share palmately-lobed leaves, this leaf form has evolved independently at least two more times within *Anthurium*, in Clade 14 (*A. pedatum* (Kunth) Schott) and in Clade 16 (*A. pedatoradiatum* Schott). Combined cpDNA-only analyses (Supplemental Fig. 2) 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. Another pair of palmate species, *A. eminens* Schott, *A. thrinax* Madison, from Amazonia, is grouped with the Brazilian Clade 1 and the rest of species in Clade 2, whereas the remaining palmately-lobed species in Clade 3 are placed in a polytomy (Supplemental Fig. 2). None of these apparently conflicting placements are supported but they indicate the need of additional data to further resolve relationships among Clade 3 and the putatively related Clades 1 and 2.

All *Anthurium* species included in the strongly supported Clade 4 (Fig. 1) occur in northern Central America (southern Mexico to Honduras) and possess cordate leaves with dark punctations on the lower leaf blade surface and bright red berries. This clade has not been formally recognized before as a section within *Anthurium*, but TBC has long considered this group as "natural." The remaining *Anthurium* species with

dark punctations on the leaf blade (i.e. all those not restricted to northern Central America) are included in Clades 5–7. Previous authors (Schott 1860; Engler 1905; Croat and Sheffer 1983) (Table 3) had placed punctate *Anthurium* species in three separate sections, *Porphyrochitonium* Schott, *Digitinervium* Sodiro, and *Tetraspermium*, although recognizing their close affinities. Clade 5 was recovered as strongly supported; it includes *Anthurium* species with four seeds per fruit (two per locule); section *Tetraspermium* (Table 3). On the other hand, Clades 6 and 7 are a mixture of species from sections *Porphyrochitonium* and *Digitinervium* (Fig. 1; Table 3); and neither clade has obvious morphological diagnostic characters.

Anthurium gracile (Rudge) Schott and its relatives are included in the strongly supported Clade 8 (Fig. 1) characterized by long slender ("strappy") leaves and thin and long inflorescences with no more than three flowers visible at once in the principal spiral of the spadix. These species also share a 2 bp indel in the *trnC-ycf6* intergenic spacer not found in the rest of *Anthurium* or in the outgroups (indel not included in analyses, see matrix in TreeBASE). Previous classifications (Schott 1860; Engler 1905; Croat and Sheffer 1983) recognized *A. gracile* as a 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 close relatives within the genus that share the above mentioned morphological similarities, and therefore this section should be expanded to accommodate all these species (Fig. 1; Table 3).

The strongly supported Clade 9 (Fig. 1), previously recognized as section *Pachyneurium* series *Pachyneurium* Schott (Croat 1991) (Table 3), includes many species of *Anthurium* with the true "bird's nest" habit, and it is also characterized by involute leaf vernation (convolute in the rest of the genus, except for section *Pachyneurium* series *Multinervia* Croat, and indeed in all other genera of Araceae, except for *Lagenandra* Dalzell) (Croat 1991). Sister to Clade 9, with only weak support (Fig. 1), is the moderately supported species-pair *A. crenatum* (L.) Kunth and *A. venosum* Griseb., both with the "bird's nest" habit but endemic to major islands in the Caribbean. Additional data will be necessary to prove if these Caribbean species belong to a separate clade on its own or are indeed part of Clade 9. The other set of *Anthurium* species with "bird's nest" habit and involute leaf vernation, section *Pachyneurium* series *Multinervia* (Croat 1991) (type species *A. napaeum*), always clustered together and with high support in Clade 11 (Fig. 1; Table 3). Members of this clade are distinguished by their leaves that dry greenish and with numerous closely spaced primary lateral veins (vs. leaves drying dark-brown and with fewer primary lateral veins, usually spaced more than 3 cm apart, in Clade 9) (Croat 1991).

Clade 10 (Fig. 1) includes *Anthurium* species with numerous primary lateral veins that are sunken above and prominent below, and that have purple fruits. This strongly supported clade is recognized here for the first time and may include quite a few more species which have similar morphology but that were not sampled. A unique novel group, highly supported in the analyses, is Clade 12 (Fig. 1). Currently, it comprises only the species pair *A. willifordii* Croat and *A. besseae* Croat, both Peruvian-Bolivian species with velvety, bullate leaves, with red venation underneath, and purple stubby spadices. *Anthurium willifordii* was previously placed in section *Pachyneurium* series *Pachyneurium* based on its "bird's nest" habit, but the presence of involute leaf vernation, characteristic of that section, has not yet been observed in the species.

The rest of the clades in the *Anthurium* phylogeny (Fig. 1, Clades 13–16) encompass mostly species with cordate leaves and they comprise the bulk of the species diversity within the genus. The highly supported Clade 13 (Fig. 1) includes *Anthurium* species with intact, persistent cataphylls and thick, glossy, colorful spathes and spadices. Previous classifications (Schott 1860; Engler 1905; Croat and Sheffer 1983) recognized this clade as section *Calomystrium* Schott emend. Engler, and all agreed in considering this section the most "natural" within *Anthurium* (Table 3).

Clade 14, with moderate support (Fig. 1), is not easily characterized morphologically, although most of its species have hooded spathes and pendent spadices. *Anthurium pedatum* (Kunth) Schott, a species with a highly divided leaf blade, previously recognized as part of the palmately-lobed *Anthurium* group in section *Schizoplacium* (Table 3; Schott 1860; Engler 1905; Croat and Sheffer 1983), consistently clustered in Clade 14. 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 3), but relationships with *A. gualeanum* are still unclear. The moderately supported Clade 15 (Fig. 1) includes species characterized by having oblong leaves, a unique morphology within an otherwise largely cordate-leaved group, with numerous primary lateral veins. This set of characters does occur elsewhere within *Anthurium*, in Clade 10, but these two lineages are consistently well separated from each other in all analyses.

The final strongly supported major lineage, Clade 16 (Fig. 1), includes *Anthurium* species from northern Central America (Mexico to Honduras) that lack dark punctations on the leaf blade and that possess bright orange berries with a mealy mesocarp. Clade 16 has not been previously recognized as a species group within *Anthurium*, but the ability of its species to cross and produce viable offspring has long been known (J. Banta, pers. comm.). Leaf morphology in this clade is quite variable, but reproductive morphology is uniform. Thus, *A. pedatoradiatum*, a Mexican species with palmately-lobed leaves, is included in Clade 16 even though it shares the leaf form of the palmately-lobed species in Clade 3 (Fig. 1). Nonetheless, *A. pedatoradiatum* clearly belongs to Clade 16 based on its bright orange berries.

Despite some limitations (i.e. ca. 11% of *Anthurium* species richness coverage and lack of nuclear sequences for outgroups), the overall results of this study show that *Anthurium* remains monophyletic (Fig. 1) and includes a core of species with relatively short branches, low sequence divergence, and poor resolution in deeper nodes along the phylogeny (Fig. 2), a pattern characteristic of rapid radiations. The genus comprises at least 18 supported clades (Fig. 1) that in most cases do not correspond well with the currently accepted sectional classification (Table 3). These major clades in the phylogeny show that some morphological characters used in previous sectional classifications of

Anthurium are homoplasious (e.g. overall leaf shape and texture), and hence these classifications are in need of revision. On the other hand, reproductive characters (e.g. inflorescence color and shape, fruit color, number of seeds) seem to be more reliable to characterize clades within *Anthurium*. Likewise, biogeography has played an important role in the evolutionary history of *Anthurium*, especially in three geographical regions (i.e. Caribbean Islands, Brazil, and northern Central America), where colonization of the area was later followed by morphological radiation of species within the region. The recognition of additional clades within *Anthurium* or relationships among them, and the placement of unassigned or unsampled species will have to rely on future molecular studies. But the major clades recovered here summarize the progress made to date and will serve as the basis for a revised sectional classification of the genus as well as future evolutionary studies.

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Appendix 1. Plant material information and GenBank accession numbers of vouchered specimens used to examine phylogenetic relationships in Anthurium. For each sample the following is given: taxon name, current sectional classification (according to Croat and Sheffer 1983) or outgroup placement, specimen original collection location, specimen collection voucher (herbarium where deposited), and GenBank accession numbers for trnG intron, trnH-psbA intergenic spacer, trnC-ycf6 intergenic spacer, and CHS first intron and partial flanking coding regions.

Anthurium acaule (Jacq.) Schott (sect. *Urospadix*), WINDWARD ISLANDS. Martinique. *T. Croat 74368* (MO), JX894576, JX894791, JX894684, JX894473. ***Anthurium andicola*** Liebm. (sect. *Belolonchium*). GUATEMALA. Alta Verapaz. *T. Croat and J. Vannini 90221* (MO), JX894577, JX894792, JX894685, JX894474. ***Anthurium antioquiense*** Engl. (sect. *Calomystrium*). COLOMBIA. Antioquia. *T. Croat 81407* (MO), JX894578, JX894793, JX894686, JX894475. ***Anthurium bakeri*** Hook. (sect. *Porphyrochitonium*). COSTA RICA. Limón. *T. Croat 78747* (MO), JX894579, JX894794, JX894687, JX894476. ***Anthurium balaoanum*** Engl. (sect. *Cardiolonchium*). ECUADOR. Manabí. *T. Croat 50706* (MO), JX894580, JX894795, JX894688, JX894477. ***Anthurium bellum*** Schott (sect. *Urospadix*). BRAZIL. Bahia. *T. Croat 82895* (MO), JX894581, JX894796, JX894689, JX894478. ***Anthurium berriozabalense*** Matuda (sect. *Belolonchium*). MEXICO. cultivated specimen of unknown locality, *T. Croat 90115* (MO), JX894582, JX894797, JX894690, JX894479. ***Anthurium besseae*** Croat (sect. *Cardiolonchium*). BOLIVIA, Cochabamba. *T. Croat 71836* (MO), JX894583, JX894798, JX894691, JX894480. ***Anthurium bicollectivum*** Croat (sect. *Porphyrochitonium*). PANAMA. Panamá. *T. Croat 73978* (MO), JX894584, JX894799, JX894692, JX894481. ***Anthurium bradeanum*** Croat & Grayum (sect. *Pachyneurium* series *Pachyneurium*). COSTA RICA. Heredia. *T. Croat 35751* (MO), JX894585, JX894800, JX894693, JX894482. ***Anthurium brevipedunculatum*** Madison (sect. *Dactylophyllium*). BRAZIL. Acre. *T. Croat and A. Rosas Jr. 62293* (MO), JX894586, JX894801, JX894694, JX894483. ***Anthurium bromelicola*** subsp. ***bahiense*** S. J. Mayo, J. G. Jardim & A. M. Carvalho (sect. *Urospadix*). BRAZIL. Bahia. *L. Temponi 343* (MO), JX894587, JX894802, JX894695, JX894484. ***Anthurium brownii*** Mast. (sect. *Belolonchium*). PANAMA. Panamá. *T. Croat and G. H. Zhu 76217* (MO), JX894588, JX894803, JX894696, JX894485. ***Anthurium carchiense*** Croat (sect. *Pachyneurium* series *Multinervia*). ECUADOR. Esmeraldas. *T. Croat 90259* (MO), JX894589, JX894804, JX894697, JX894486. ***Anthurium chiapasense*** subsp. ***tlaxiacense*** (Matuda) Croat (sect. *Belolonchium*). MEXICO. Oaxaca. *T. Croat 45927* (MO), JX894592, JX894807, JX894700, JX894489. ***Anthurium citrifolium*** Sodiro (sect. *Tetraspermium*). ECUADOR. Pichincha. *T. Croat et al. 100034* (MO), JX894593, JX894808, JX894701, JX894490. ***Anthurium clarinervium*** Matuda (sect. *Cardiolonchium*). MEXICO. cultivated specimen of unknown locality. *Vadk 1991–1413* (K), JX894594, JX894809, JX894702, JX894491. ***Anthurium clavigerum*** Poepp. (sect. *Dactylophyllium*). BOLIVIA. Pando. *D. C. Daly 2042A* (MO), JX894595, JX894810, JX894703, JX894492. ***Anthurium clidemioides*** Standl. (sect. *Polyphyllium*). PANAMA. Coclé. *T. Croat 79567* (MO), JX894596, JX894811, JX894704, JX894471. ***Anthurium cordatum*** (L.) Schott (sect. *Episeiostenium*). VIRGIN ISLANDS. St. Croix. *T. Croat 81387* (MO), JX894597, JX894812, JX894705, JX894493. ***Anthurium cordifolium*** (Raf.) Kunth (sect. *Episeiostenium*). JAMAICA. Manchester. *T. Croat 81448* (MO), JX894598, JX894813, JX894706, JX894494. ***Anthurium coriaceum***

(Graham) G. Don (sect. *Urospadix*). BRAZIL. cultivated specimen of unknown locality. *T. Croat 67421* (MO), JX894599, JX894814, JX894707, JX894495. ***Anthurium crenatum*** (L.) Kunth (sect. *Pachyneurium* series *Pachyneurium*). GREATER ANTILLES. Puerto Rico. *T. Croat 68440* (MO), JX894600, JX894815, JX894708, JX894496. ***Anthurium croatii*** Madison (sect. *Dactylophyllium*). PERU. Junín. *T. Croat and M. Sizemore 81920* (MO), JX894601, JX894816, JX894709, JX894497. ***Anthurium crystallinum*** Linden & André (sect. *Cardiolonchium*). COLOMBIA. Risaralda. *T. Croat 56351* (MO), JX894602, JX894817, JX894710, JX894498. ***Anthurium curvispadix*** Croat (sect. *Calomystrium*). PANAMA. Colón. *T. Croat 33654* (MO), JX894603, JX894818, JX894711, JX894499. ***Anthurium cuspidatum*** Mast. (sect. *Polyneurium*). PANAMA. unknown locality. *L. Hannon 01–007* (MO), JX894604, JX894819, JX894712, JX894500. ***Anthurium digitatum*** (Jacq.) Schott (sect. *Dactylophyllium*). VENEZUELA. Sucre. *T. Croat 54361* (MO), JX894605, JX894820, JX894713, JX894501. ***Anthurium dolichostachyum*** Sodiro (sect. *Cardiolonchium*). ECUADOR. Esmeraldas. *T. Croat et al. 83097* (MO), JX894606, JX894821, JX894714, JX894502. ***Anthurium dominicense*** Schott (sect. *Episeiostenium*). cultivated specimen of unknown locality. *T. Croat 90070* (MO), JX894607, JX894822, JX894715, JX894503. ***Anthurium dressleri*** Croat (sect. *Cardiolonchium*). PANAMA. Colón. *J. P. Folsom 3737* (MO), JX894608, JX894823, JX894716, JX894504. ***Anthurium eminens*** Schott (sect. *Dactylophyllium*). PERU. Loreto. *H. van der Werff et al. 10168* (MO), JX894609, JX894824, JX894717, JX894505. ***Anthurium flexile*** Schott subsp. ***flexile*** (sect. *Polyphyllium*). MEXICO. Veracruz. *T. Croat 78692* (MO), JX894610, JX894825, JX894718, JX894472. ***Anthurium formosum*** Schott (sect. *Calomystrium*). COSTA RICA. Cartago. *T. Croat 79071* (MO), JX894611, JX894826, JX894719, JX894506. ***Anthurium fornicifolium*** Croat (sect. *Decurrentia*). ECUADOR. Morona-Santiago. *T. Croat 81400* (MO), JX894612, JX894827, JX894720, JX894507. ***Anthurium friedrichsthalii*** Schott (sect. *Porphyrochitonium*). ECUADOR. Esmeraldas. *T. Croat et al. 99861* (MO), JX894613, JX894828, JX894721, JX894508. ***Anthurium furcatum*** Sodiro (sect. *Semaeophyllium*). ECUADOR. Cotopaxi. *T. Croat 73249* (MO), JX894614, JX894829, JX894722, JX894509. ***Anthurium gracile*** (Rudge) Schott (sect. *Leptanthurium*). cultivated specimen of unknown locality. *L. Holy 7–23–99* (MO), JX894615, JX894830, JX894723, JX894510. ***Anthurium grandifolium*** (Jacq.) Kunth (sect. *Episeiostenium*). DOMINICA. St. John. *J. S. Miller and M. Merello 8865* (MO), JX894617, JX894832, JX894725, JX894512. ***Anthurium halmoorei*** Croat (sect. *Pachyneurium* series *Pachyneurium*). MEXICO. Nayarit. *T. Croat 45337* (MO), JX894618, JX894833, JX894726, JX894513. ***Anthurium harrisii*** (Graham) G. Don (sect. *Urospadix*). BRAZIL. Espírito Santo. *T. Croat 73889* (MO), JX894619, JX894834, JX894727, JX894514. ***Anthurium hoffmannii*** Schott (sect. *Calomystrium*). PANAMA. Chiriquí. *T. Croat 66203* (MO), JX894620, JX894835, JX894728, JX894515. ***Anthurium huixtlense*** Matuda (sect. *Calomystrium*). MEXICO. Oaxaca. *T. Croat and D. P. Hannon 63309* (MO), JX894621, JX894836, JX894729, JX894516. ***Anthurium kunthii*** Poepp. (sect. *Dactylophyllium*). PANAMA. Bocas del Toro. *T. Croat 38121* (MO), JX894622, JX894837, JX894730, JX894517. ***Anthurium laciniosum*** Sodiro (sect. *Tetraspermium*). ECUADOR. Pichincha. *P. Cazalet and T. Pennington 5271* (MO), JX894623, JX894838, JX894731, JX894518. ***Anthurium lancea*** Sodiro (sect. *Cardiolonchium*). ECUADOR. Pichincha. *T. Croat 75455* (MO), JX894624, JX894839, JX894732, JX894519. ***Anthurium lancetillense*** Croat (sect. *Belolonchium*). HONDURAS. Atlántida. *T. Croat 42672* (MO), JX894625, JX894840, JX894733, JX894520. ***Anthurium lancifolium*** Schott (sect. *Porphyrochitonium*). PANAMA. Panamá. *T. Croat 81520* (MO), JX894626, JX894841, JX894734, JX894521. ***Anthurium leuconeurum*** Lem. (sect. *Cardiolonchium*). MEXICO. cultivated specimen of unknown locality. *L. Holy 1999–425* (K), JX894627, JX894842, JX894735, JX894522. ***Anthurium longicaudatum*** Engl. (sect. *Polyneurium*). ECUADOR. Esmeraldas. *T. Croat et al. 99733* (MO), JX894629, JX894844, JX894737, NA. ***Anthurium longipes*** N. E. Br. (sect. *Urospadix*). BRAZIL. Bahia. *L. Temponi 339* (MO), JX894630, JX894845, JX894738, JX894524. ***Anthurium longissimum*** Pittier (sect. *Schizoplacium*). VENEZUELA. Falcón. *T. Croat 74497* (MO), JX894631, JX894846, JX894739, JX894525. ***Anthurium lucens*** Standl. (sect. *Belolonchium*). MEXICO. Oaxaca. *T. Croat 78702* (MO), JX894632, JX894847, JX894740, JX894526. ***Anthurium lucidum*** Baker (sect. *Urospadix*). BRAZIL. cultivated specimen of unknown locality. *T. Croat 87586* (MO), JX894633, JX894848, JX894741, JX894527. ***Anthurium macleanii*** Schott (sect. *Belolonchium*). BOLIVIA. La Paz. *T. Croat et al. 84725* (MO), JX894635, JX894850, JX894743, JX894529. ***Anthurium maculosum*** Sodiro (sect. *Polyneurium*). ECUADOR. Cotopaxi. *T. Croat 73725* (MO), JX894636, JX894851, JX894744, JX894530. ***Anthurium margaricarpum*** Sodiro (sect. *Porphyrochitonium*). ECUADOR. Pichincha. *T. Croat et al. 95756* (MO), JX894637, JX894852, JX894745, JX894531. ***Anthurium*** cf. ***michelii*** Guillaumin (sect. *Decurrentia*). cultivated specimen of unknown locality. *T. Croat 79381* (MO), JX894590, JX894805, JX894698, JX894487. ***Anthurium michelii*** Guillaumin (sect. *Decurrentia*). ECUADOR. Esmeraldas. *T. Croat et al. 99885* (MO), JX894638, JX894853, JX894746, JX894532. ***Anthurium microspadix*** Schott (sect. *Xialophyllium*). ECUADOR. Esmeraldas. *T. Croat et al. 99672* (MO), JX894639, JX894854, JX894747, JX894533. ***Anthurium mindense*** Sodiro (sect. *Xialophyllium*). ECUADOR. Napo. *T. Croat et al. 99599* (MO), JX894640, JX894855, JX894748, JX894534. ***Anthurium morae*** Croat (sect. *Digitinervium*). COLOMBIA. Chocó. *T. Croat and M. Mora 83725* (MO), JX894641, JX894856, JX894749, JX894535. ***Anthurium napaeum*** Engl. (sect. *Pachyneurium* series *Multinervia*). ECUADOR. Cañar. *T. Croat 50876* (MO), JX894643, JX894858, JX894751, NA. ***Anthurium obtusilobum*** Schott (sect. *Calomystrium*). COSTA RICA. Alajuela. *T. Croat 78845* (MO), JX894644, JX894859, JX894752, JX894537. ***Anthurium*** cf. ***obtusum*** (Engl.) Grayum (sect. *Tetraspermium*). ECUADOR. Morona-Santiago. *T. Croat 90101* (MO), JX894591, JX894806, JX894699, JX894488. ***Anthurium obtusum*** (Engl.) Grayum (sect. *Tetraspermium*). cultivated specimen of unknown locality. *T. Croat 82921* (MO), JX894645, JX894860, JX894753, JX894538. ***Anthurium olfersianum*** Kunth (sect. *Urospadix*). cultivated specimen of unknown locality. *Selby 63–75–17* (MO), JX894646, JX894861, JX894754, JX894539. ***Anthurium ovatifolium*** Engl. (sect. *Digitinervium*). ECUADOR. Pichincha. *T. Croat et al. 95923* (MO), JX894647, JX894862, JX894755, JX894540. ***Anthurium oxyphyllum*** Sodiro (sect. *Pachyneurium* series *Multinervia*). ECUADOR. Esmeraldas. *T. Croat 75325* (MO), JX894648, JX894863, JX894756, JX894541. ***Anthurium palenquense*** Croat (sect. *Pachyneurium* series *Multinervia*). ECUADOR. Pichincha. *T. Croat 72986* (MO), JX894649, JX894864, JX894757, NA. ***Anthurium parvispathum*** Hemsl. (sect. *Decurrentia*). GUATEMALA. Alta Verapaz. *T. Croat and J. Vannini 90238* (MO), JX894650, JX894865, JX894758, JX894542. ***Anthurium parvum*** N. E. Br. (sect. *Urospadix*). BRAZIL. Rio de Janeiro. *L. Temponi 345* (SPF), JX894651, JX894866, JX894759, JX894543. ***Anthurium pedatoradiatum*** Schott (sect. *Schizoplacium*). MEXICO. cultivated specimen of unknown locality. *D. Fisk 8/15/01* (MO), JX894652, JX894867, JX894760, JX894544. ***Anthurium pedatum*** (Kunth) Schott (sect. *Schizoplacium*). COLOMBIA. Valle del Cauca. *T. Croat 62810* (MO), JX894653, JX894868, JX894761, JX894545. ***Anthurium peltigerum*** Sodiro (sect. *Calomystrium*). ECUADOR. Esmeraldas. *T. Croat et al. 99794* (MO), JX894654, JX894869, JX894762, JX894546. ***Anthurium polydactylum*** Madison (sect. *Dactylophyllium*). PERU. Huanuco. *T. Croat and M. Sizemore 81643* (MO), JX894655, JX894870, JX894763, JX894547. ***Anthurium protensum*** Schott (sect. *Pachyneurium* series *Pachyneurium*). cultivated specimen of unknown locality. *T. Croat 71830* (MO), JX894656, JX894871, JX894764, JX894548. ***Anthurium punctatum*** N. E. Br. (sect. *Porphyrochitonium*). ECUADOR. Los Ríos. *T. Croat 73859* (MO), JX894657, JX894872, JX894765, JX894549. ***Anthurium radicans*** K. Koch & Haage (sect. *Chamaerepium*). BRAZIL. cultivated specimen of unknown locality. *T. Croat 76139* (MO), JX894658, JX894873, JX894766, JX894550. ***Anthurium sagittatum*** (Sims) G. Don (sect. *Cardiolonchium*). FRENCH GUIANA. *T. Croat 74285* (MO), JX894659, JX894874, NA, JX894551. ***Anthurium scandens*** (Aubl.) Engl. (sect. *Tetraspermium*). COSTA RICA. Puntarenas. *T. Croat and D. Hannon 79257* (MO), JX894660, JX894875, JX894767, JX894552. ***Anthurium schottianum*** Croat & R. A. Baker (sect. *Pachyneurium* series *Pachyneurium*). COSTA RICA. Limón. *T. Croat 43247* (MO), JX894661, JX894876, JX894768, JX894553. ***Anthurium sinuatum*** Benth ex. Schott (sect. *Dactylophyllium*). BRAZIL. Pará. *T. Croat 62179* (MO), JX894662, JX894877, JX894769, JX894554. ***Anthurium* sp. 1** (sect. *Polyneurium*). ECUADOR. Esmeraldas. *T. Croat et al. 99678* (MO), JX894628, JX894843, JX894736, JX894523. ***Anthurium* sp. 2** (sect. *Decurrentia*). ECUADOR. Esmeraldas. *T. Croat et al. 99766* (MO), JX894634, JX894849, JX894742, JX894528. ***Anthurium* sp. 3** (sect. *Digitinervium*). ECUADOR. Esmeraldas. *T. Croat et al. 99803* (MO), JX894616, JX894831, JX894724, JX894511. ***Anthurium* sp. 4** (sect. *Decurrentia*). ECUADOR. Morona-Santiago. *T. Croat 95411* (MO), JX894642, JX894857, JX894750, JX894536. ***Anthurium spathiphyllum*** N. E. Br. (sect. *Pachyneurium* series *Pachyneurium*). COSTA RICA. Limón. *T. Croat 71838* (MO), JX894663, JX894878, JX894770, JX894555. ***Anthurium spectabile*** Schott (sect. *Pachyneurium* series *Pachyneurium*). COSTA RICA. Cartago. *T. Croat 69707* (MO), JX894664, JX894879, JX894771, JX894556. ***Anthurium subsignatum*** Schott (sect. *Semaeophyllium*). COSTA RICA. San José. *T. Croat 78774* (MO), JX894665, JX894880, JX894772, JX894557. ***Anthurium thrinax*** Madison (sect. *Dactylophyllium*). FRENCH GUIANA. *T. Croat 74175* (MO), JX894666, JX894881, JX894773, JX894558. ***Anthurium tremulum*** Sodiro (sect. *Belolonchium*). ECUADOR. Pichincha. *T. Croat et al. 100032* (MO), JX894667, JX894882, JX894774, JX894559. ***Anthurium trilobum*** hort. ex André (sect. *Semaeophyllium*). COLOMBIA. Valle del Cauca. *T. Croat and J. Gaskin 79726* (MO), JX894668, JX894883, JX894775, JX894560. ***Anthurium umbrosum*** Liebm. (sect. *Belolonchium*). MEXICO. Oaxaca. *T. Croat 78706* (MO), JX894669, JX894884, JX894776, JX894561. ***Anthurium urbanii*** Sodiro (sect. *Polyneurium*). ECUADOR. Esmeraldas. *T. Croat et al. 99801* (MO), JX894670, JX894885, JX894777, JX894562. ***Anthurium veitchii*** Mast. (sect. *Calomystrium*). cultivated specimen of unknown locality. *T. Croat 81530* (MO), JX894671, JX894886,

JX894778, JX894563. ***Anthurium venosum*** Griseb. (sect. *Pachyneurium* series *Pachyneurium*). CUBA. cultivated specimen of unknown locality. *T. Croat 69756* (MO), JX894672, JX894887, JX894779, JX894564. ***Anthurium verapazense*** Engl. (sect. *Belolonchium*). GUATEMALA. Alta Verapaz. *T. Croat 81392* (MO), JX894673, JX894888, JX894780, JX894565. ***Anthurium vittariifolium*** Engl. (sect. *Decurrentia*). cultivated specimen of unknown locality. *T. Croat 56912* (MO), JX894674, JX894889, JX894781, JX894566. ***Anthurium wendlingeri*** G. M. Barroso (sect. *Porphyrochitonium*). COSTA RICA. Limón. *T. Croat 71837* (MO), JX894675, JX894890, JX894782, JX894567. ***Anthurium willdenowii*** Kunth (sect. *Urospadix*). BARBADOS. cultivated specimen of unknown locality. *T. Croat 57219* (MO), JX894676, JX894891, JX894783, JX894568. ***Anthurium willifordii*** Croat (sect. *Pachyneurium* series *Pachyneurium*). PERU. cultivated specimen of unknown locality. *T. Croat 81092* (MO), JX894677, JX894892, JX894784, JX894569. ***Lysichiton americanus*** Hultén & H. St. John (subfamily Orontioideae). U. S. A. unknown locality. *S. Carboni 07/31/1972* (MO), KC241908, KC241913, KC241917, NA. ***Monstera deliciosa*** Liebm. (subfamily Monsteroideae). MEXICO. Veracruz. *T. Croat 39528* (MO), KC241909, KC241914, KC241918, NA. ***Pothoidium lobbianum*** Schott (tribe Potheae). CHINA. Taiwan. *L. Wen-Pen et al. 2191* (HAST), JX894575, JX894790, JX894683, NA. ***Pothos chinensis*** (Raf.) Merr. (tribe Potheae). cultivated specimen of unknown locality. *L. Peng 10037 (Kew 1999–3194)* (K), JX894570, JX894785, JX894678, NA. ***Pothos junghuhnii*** de Vriese (tribe Potheae). cultivated specimen of unknown locality. *Kew 1999–3170* (K), JX894571, JX894786, JX894679, NA. ***Pothos ovatifolius*** Engl. (tribe Potheae). cultivated specimen of unknown locality, *Kew 1996–4425* (K), JX894572, JX894787, JX894680, NA. ***Pothos rumphii*** Schott (tribe Potheae). cultivated specimen of unknown locality. *J. Bogner s. n. (Kew 2000–3986)* (K), JX894573, JX894788, JX894681, NA. ***Pothos scandens*** L. (tribe Potheae). cultivated specimen of unknown locality. *T. Croat 95634* (MO), JX894574, JX894789, JX894682, NA. ***Spathiphyllum phryniifolium*** Schott (subfamily Monsteroideae). PANAMA. Colón. *T. Croat 79323* (MO), KC241911, KC241916, KC241920, NA. ***Stenospermation multiovulatum*** (Engl.) N.E. Brown (subfamily Monsteroideae). COLOMBIA. Valle del Cauca. *D. Bay 255* (MO), KC241910, KC241915, KC241919, NA. ***Symplocarpus foetidus*** (L.) W. Salisb. (subfamily Orontioideae). JAPAN. Nagano. unknown collection and herbarium, AB206656.1, NA, NA, NA. ***Symplocarpus foetidus*** (L.) W. Salisb. (subfamily Orontioideae). U. S. A. unknown locality. *C. Reed 118344* (MO), NA, KC241912, NA, NA.