

## INTERGENERIC AND INFRAFAMILIAL PHYLOGENY OF SUBFAMILY MONSTEROIDEAE (ARACEAE) REVEALED BY CHLOROPLAST *trnL-F* SEQUENCES<sup>1</sup>

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The chloroplast *trnL-F* region was used as an independent data set for phylogenetic analysis of 118 aroid taxa. We investigated the intergeneric relationships of subfamily Monsteroideae (Araceae) and used this as a basis for an interspecific phylogenetic study of *Rhaphidophora* Hassk., the largest genus of the Monsteroideae. Results of the molecular tree were useful for inferring subfamilial and tribal circumscription and evolution in Araceae. Our results show that family Araceae consists of five clades that correspond to the subfamilies traditionally recognized. Starting from the most basal clade, these correspond to subfamilies Gymnostachydoideae and Orontioideae (proto aroids), with *Lemna* sp. (Lemnaceae) embedded in the Araceae and sister to the true aroids consisting of Pothoideae sister to Monsteroideae, immediately sister to Lasioideae and Aroideae. There is less agreement with existing tribal classifications. Complex relationships exist between members of the Monsteroideae. Our results show that *Rhaphidophora* and *Epipremnum* are paraphyletic with species of *Rhaphidophora* sampled, forming three informal groups with other genera of the Monstereae. Phylogenetic results may be used to suggest taxonomic changes to the current systematics of the monsteroideae.

**Key words:** Araceae; classification; intergeneric; Monstereae; Monsteroideae; phylogeny; *trnL-F* region.

With over 3300 species and 105 genera of herbs and vines, the Araceae is one of the dominant tropical families. The Araceae is strongly supported as a monophyletic group with the inclusion of Lemnaceae (Mayo et al., 1997). The aroids have a subcosmopolitan distribution and are most abundant and diverse in tropical latitudes (Grayum, 1990; Mayo et al., 1997). Chase et al. (2000) showed that Araceae are found with the Alismatales (alismatid families plus Tofieldiaceae) as the next branching order from *Acorus* L. Tofieldiaceae is sister to Araceae, which in turn, is sister to the rest of the alismatid families. The systematics of the genera of Araceae was comprehensively reviewed by Mayo et al. (1997), who provided a stable and modern framework for Araceae classification.

One of the most taxonomically challenging and important subfamilies of Araceae is the Monsteroideae, being one of the more basal groups in the family and thus providing a picture of the early development in the Araceae. The subfamily Monsteroideae was treated by Mayo et al. (1997) as part of their higher-level study of the entire family Araceae, based largely on nonmolecular data and incorporating results of the molecular study by French et al. (1995). Monsteroideae consists of four tribes: Spathiphyllae Engl., Anadendreae Bogner & French, Heteropsidae Engl., and Monstereae Engl. In Mon-

steroideae, genera of Monstereae, Heteropsidae, and Anadendreae formed a monophyletic clade. Spathiphyllae failed to group consistently with these three tribes. The classification of taxa into tribes within the subfamily Monsteroideae sensu Mayo et al. (1997) is shown in Table 1.

For such a large and diverse family as the Araceae, much remains to be done in Araceae to understand the relationships between the various tribes and subfamilies. The publication of high level phylogenetic studies (based on morphological and molecular data) has allowed further work to be carried out to better understand numerous finer scale questions concerning genera and species. Previous studies have demonstrated a history of confusion in the taxonomy of Monsteroideae, especially in the tribe Monstereae. It was not possible to confidently state the phylogeny of the group or fully comprehend intergeneric relationships because of insufficient data (Tam, 2002).

The first grouping of subfamily Monsteroideae originated from Schott's (1860) classification of the family, which was not based on phylogenetic concepts but on "natural affinities" utilizing mostly floral morphology with a few vegetative characters. In tribe Calleae, subtribe Monsterae (under Monoclines), Schott placed *Rhaphidophora*, *Stenospermaton*, *Atimeta* (= *Rhodospatha*), *Anepsias* (= *Rhodospatha*), *Rhodospatha*, *Tornelia* (= *Monstera*), *Alloschemone*, *Monstera*, *Heteropsis*, *Epipremnum*, *Anadendron*, *Scindapsus*, and *Cuscutaria* (= *Scindapsus*).

The subfamily Monsteroideae was circumscribed by Engler in 1876 and revised by Engler and Krause in 1908. Engler's (1876) classification was based mainly on morphological and anatomical studies and was arranged to reflect phylogenetic principles. In his classification, subfamilies were defined by a combination of floral and vegetative characters. It also embodied an idea about the evolution of the family: genera with bisexual flowers gave rise to unisexual flowers in several independent phylogenetic lines (clades). Monsteroideae was de-

<sup>1</sup> Manuscript received 26 June 2003; revision accepted 7 October 2003.

The authors thank the Royal Botanic Gardens Kew for providing material and the Jodrell Laboratory for allowing use of their facilities. We thank Josef Bogner, Tom Croat, and Nguyen van Du for sending us material and Ruth Kiew and Saw Leng Guan for help with fieldwork in Malaysia and Singapore. We thank Prof. Mark Chase for his help with data analyses and Simon Mayo for his critical review of our manuscript. This research was supported by the Cambridge Commonwealth Trust, the Frank Smart Studentship, and the George Bidder & Thriplow Charitable Trust Fund (Lucy Cavendish College, University of Cambridge).

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TABLE 1. Classification of tribes and genera under Monsteroideae sensu Mayo et al. (1997).

Tribes	Genera	Number of species	Distribution
Spathiphyllae	<i>Spathiphyllum</i> Schott	41	Tropical America, West Indies, eastern Malay Archipelago, Melanesia
	<i>Holochlamys</i> Engl.	1	Indonesia, Papua New Guinea
Anadendreae	<i>Anadendrum</i> Schott	7	Southeast Asia, Malay Archipelago
Heteropsidae	<i>Heteropsis</i> Kunth	13	Tropical America
Monstereae	<i>Amydrium</i> Schott	4–6	Tropical Southeast Asia, Malay Archipelago
	<i>Rhaphidophora</i> Hassk.	100	Tropical Africa, tropical Southeast Asia, Malay Archipelago, Melanesia, Australasia, Pacific
	<i>Epipremnum</i> Schott	20	Tropical Southeast Asia, Australasia, Pacific
	<i>Scindapsus</i> Schott	36	Tropical Asia, Malay Archipelago, Melanesia, Pacific
	<i>Monstera</i> Adanson	40	Tropical America, West Indies
	<i>Allochemone</i> Schott	2	Brazil
	<i>Rhodospata</i> Poeppig	75	Tropical America
	<i>Stenospermation</i> Schott	36	Tropical America

rived from Pothoideae and consisted of three tribes and 10 genera. *Rhaphidophora* was placed in the same tribe as *Epipremnum* (Rhaphidophoreae) and was considered more derived than *Spathiphyllum*, *Amomophyllum* (= *Monstera*), *Anepsias* (= *Rhodospata*), *Rhodospata*, and *Stenospermation* (tribe Anepsiadeae) and less derived than *Scindapsus*, *Cuscuaria* (= *Scindapsus*), *Monstera*, and *Allochemone* (tribe Monstereae). Hooker (1883) adapted and modified Schott's system (according to natural affinities) and took into account the studies of Engler and Brown (Mayo et al., 1997) to propose his classification of the Araceae. Members of the Monsteroideae were placed in tribe Calleae with *Calla* L., *Rhaphidophora*, *Stenospermation*, *Rhodospata*, *Heteropsis*, *Anadendrum*, *Monstera*, *Scindapsus*, *Epipremnum*, and *Amydrium*.

Since then, the monsteroideae have been included in many systematic studies utilizing different methods such as general morphology (Bakhuizen van den Brink, 1958; Birdsey, 1962; Bunting, 1962; Hotta, 1971; Hutchinson, 1973; Nicolson, 1978; Bogner and Nicolson, 1991; Grayum, 1991; Hay and Maberley, 1991; Hay, 1992), cytology (Mookerja, 1955; Petersen, 1989, 1993), anatomy (Nicolson, 1960; Eyde et al., 1967; French and Tomlinson, 1981; French, 1985, 1986a, b, 1987; Ray, 1988; Carvell, 1992; Dietmar-Benhke, 1995) and chemotaxonomy (Dring et al., 1995; Hegnauer, 1997). Generally, these studies have revealed a lack of taxonomically useful characters, albeit different character states were observed between genera. For example, Hay (1992) expressed the situation precisely noting that generic limits of the Monstereae in Asian genera based on gynoecial and seed characters are not only blurred but also cut across suites of complex vegetative characteristics. The main obstacle is a lack of resolution resulting from the small number of morphological characters that separate closely related species and even genera.

There have been four recent high-level systematic studies of the Araceae using cladistics that shed some light on the intergeneric problems of the subfamily: Grayum (1990), French et al. (1995), Mayo et al. (1997), and Barabe et al. (2002). The infrafamilial taxa that were circumscribed by Grayum (1990) using nonmolecular data resulted in several significant points for classification at the tribal level. Subfamily Monsteroideae and Pothoideae were combined. *Spathiphyllum* was considered the most primitive genus of Pothoideae sensu Grayum (1990), while *Anadendrum* was more related to the tribe Monstereae than Potheae Engl. and was as such regarded provisionally to be sister taxon to Monstereae/Zamioculcadeae

Engl. *Heteropsis* was aligned with Monstereae. Genera belonging to the Monstereae (including *Heteropsis*) were strongly united vegetatively, florally, and palynologically, but relationships among these genera could not be fully resolved. For example, *Rhaphidophora*, *Epipremnum*, and *Monstera* were poorly differentiated; *Scindapsus* and *Allochemone* were sometimes united; and *Rhodospata* and *Allochemone* share several unusual characters and are probably sister taxa (Grayum, 1990).

French et al. (1995) studied restriction site variation in the plastomes of 86 genera of Araceae and included *Lemna* L. and *Acorus*. All genera classified under subfamily Monsteroideae sensu Mayo et al. (1997) except *Allochemone* and *Heteropsis* were included. Their cladogram showed a clear delimitation between subfamily Pothoideae (*Pothos* L. and *Anthurium* Schott) and Monsteroideae. Monsteroideae was derived from Pothoideae, and together they formed a monophyletic clade. *Spathiphyllum* and *Holochlamys* are unresolved, sister to a clade consisting of *Stenospermation* and *Rhodospata* and *Scindapsus*, which in turn was sister to *Rhaphidophora* followed by *Anadendrum*, *Monstera*, *Epipremnum*, and *Amydrium*.

Barabe et al. (2002) primarily studied the correlation between development of atypical bisexual flowers and the phylogeny of Aroideae. They performed a phylogenetic analysis of 33 genera of Araceae using the plastid *trnL-F* region and presented the first Araceae phylogeny based on sequence data, albeit with a limited sampling. At the subfamily level, their results generally corroborated the studies of French et al. (1995) and Mayo et al. (1997). *Heteropsis* is sister to *Spathiphyllum*, which in turn is sister to an unresolved clade comprising *Rhaphidophora*, unresolved with *Stenospermation*, *Epipremnum*, and a clade consisting of genera *Amydrium* and *Monstera*.

In the past 10 years, there has been an immense accumulation of phylogenetic evidence based on DNA sequences due to the rapid progress in techniques and the broadening interest in the field of plant molecular systematics. The organization and evolution of the chloroplast genome and the advantages and disadvantages it offers to studies of phylogeny and systematics have received many comprehensive reviews (Palmer, 1985; Clegg and Zurawski, 1990; Clegg et al., 1991, 1994; William-Birky, 1991; Olmstead and Palmer, 1994; Morton et al., 1997; Soltis and Soltis, 1998). The chloroplast *trnL-F* region has proven to be phylogenetically useful from the species level to the family level. The *trnL-F* region is a noncoding

region that includes the *trnL* (UAA) intron ranging from 350 to 600 base pairs (bp) and the intergenic spacer between *trnL* (UAA) 3' exon and the *trnF* (GAA) gene (Taberlet et al., 1991; Gielly and Taberlet, 1996) ranging from 120 to 350 bp (Soltis and Soltis, 1998). Several studies of monocotyledonous groups have used sequence data from the *trnL-F* region such as Themidaceae (Fay et al., 1996), Alliaceae (Fay et al., 1997), Iridaceae (Reeves et al., 1997), and Palmae (Baker et al., 1999).

Aroid workers have long acknowledged the close relationships between genera belonging to Monsteroideae (sensu Mayo et al., 1997), believing Monsteroideae to be a natural grouping, but always stressed that more data was needed. Fundamental questions that remain to be satisfactorily answered include: (1) Are certain large genera that have previously been the source of confusion, such as *Rhaphidophora* and *Monstera*, monophyletic groups? (2) Can we determine a clear delimitation of these genera? (3) How are morphologically diverse (yet extremely similar) Monsteroideae genera related to each other? We present here results of our molecular systematic study of the Monsteroideae based on sequence data in order to address some of these longstanding questions on the phylogenetics relationships of this subfamily and to allow the selection of outgroups for the species-level study of *Rhaphidophora*, the largest genera of Monsteroideae and an evolutionarily diverse palaeotropical climber (S.-M. Tam et al., unpublished manuscript).

## MATERIALS AND METHODS

**Plant material**—All tribes from subfamilies Pothoideae, Monsteroideae, and Lasioideae were sampled. When possible, up to three species per genus were included in the data set. *Gymnostachys* R. Brown, *Symplocarpus* Nuttall, *Orontium* L., and *Lysichiton* Schott were selected as outgroups based on morphological (Mayo et al., 1997) and chloroplast DNA variation studies (French et al., 1995). This study generated 75 new *trnL-F* sequences (34 from different genera and 41 *Rhaphidophora* species). An additional 43 *trnL-F* sequences, which included *Acorus calamus* L., *Lemna* sp., and taxa from subfamilies Lasioideae and Aroideae, were taken from Barabe et al. (2002) and added to improve phylogenetic analysis (higher number of taxa and outgroups), although it is not our main purpose to focus on the molecular phylogeny of the family Araceae. A complete list of investigated taxa, including voucher information, is presented in the Appendix.

**DNA extraction, gene amplification, and sequencing**—Total DNA was extracted using a modified version of the method from Doyle and Doyle (1987) and the Nucleon Phytopure Plant Extraction Kit (Scotlab Bioscience, Coatbridge, UK) according to the manufacturer's protocol.

The *trnL-F* region was amplified from total DNA using primers c, d, and f (Taberlet et al., 1991; Gielly and Taberlet, 1996), which amplified the *trnL* (UAA) intron, 3' exon and *trnL-F* intergenic spacer. Each reaction contained 1  $\mu$ L of DNA template, 0.1  $\mu$ L of *Taq* polymerase (added after initial denaturation step; Biotline, London, UK), 1.2  $\mu$ L of 25 mmol/L  $MgCl_2$ , 1.25  $\mu$ L of 5 mmol/L dNTP (Biotline, London, UK), 0.3  $\mu$ L of each primers at 10  $\mu$ mol/L concentration, 0.5  $\mu$ L of DMSO (dimethyl sulfoxide; SIGMA, Dorset, UK), 1.0  $\mu$ L of 0.1% BSA (bovine serum albumin, New England Biolabs, Hitchin, UK), sterile double deionized water to make up to 25  $\mu$ L final volume.

Amplification used the following PCR profile: initial denaturation at 95°C for 4 min; pause for Hotstart step at 72°C where 0.1  $\mu$ L of *Taq* polymerase was added per sample; denaturation at 92°C for 45 s, annealing at 55°–58°C for 45 s, and extension at 72°C for 2 min for 30 cycles. This was followed by a final extension step at 72°C for 10 min, and samples were held at 4°C until further treatment.

Double-stranded PCR products were viewed on a 0.8% agarose gel and the

expected band excised and purified using QIAquick Gel Extraction Kit (Qiagen, West Sussex, UK) following protocols provided by the manufacturer. Purified products were quantified on 0.8% agarose gel before sequencing. In the case of the aroids, modifications were made in PCR concoctions and profiles where necessary to adapt to the biochemical idiosyncrasies of the material. This included dilution series for template DNA up to 1/1000, reamplifying weak products, reextracting the DNA, and using a different individual of the same species.

Modified dideoxy cycle sequencing with dye terminators was performed on a GeneAmp 9700 Automated Sequencer according to the manufacturer's protocol (Applied Biosystems, Foster City, California, USA) to sequence the amplified products directly. Both strands were sequenced for the fragment with the c, d, and f primers. The cycle sequencing profile was as follows: rapid thermal ramp to 96°C followed by 96°C for 30 s, 50°C for 15 s, and 60°C for 4 min, performed for 25 cycles and held at 4°C.

**Phylogenetic analysis**—*Alignment of sequences*—Forward and reverse sequences were edited using SeqEd version 1.0.3 (Applied Biosystems). An initial alignment of 39 sequences was made using CLUSTAL (Higgins et al., 1992). Subsequent sequences were added to the matrix and aligned manually. Regions where the alignment was ambiguous were excluded from the analysis. Alignment procedures followed the recommendations of Kelchner (2000). Gaps from indels were coded as additional binary characters and appended to the matrix. The final aligned matrix has 1757 characters including 19 coded indels.

**Data analysis**—Parsimony analyses of the aligned sequence matrix was performed using PAUP\* 4.0.8 (Swofford, 1998). Because of an inadequate number of informative characters and the high number of taxa, heuristic searches could not be completed. An alternative strategy was employed to explore tree space (M. Chase, Royal Botanical Gardens, Kew, personal communication). One thousand random addition sequence replicates were conducted using tree bisection-reconnection (TBR) branch swapping, with MULTREES in effect, but holding only one tree per step and saving no more than 10 trees per replicate to minimize time swapping on suboptimal trees. A second round of TBR branch swapping was performed on trees collected in the first analysis saving up to 10000 trees. Once this limit reached, these trees were swapped to completion.

Successive weighting (Farris, 1969) of resulting trees was carried out according to the rescaled consistency index using the maximum value (best fit) criterion and a base weight of 1.0. This was followed by an heuristic search with 10 random sequence addition replicates, TBR branch swapping, and again saving no more than 10 trees per replicate. All trees from these 10 replicates were then swapped to completion, after which another round of weighting was implemented. This process was repeated until the same tree length/rescaled consistency index was obtained twice in succession. Internal clade support was evaluated both with and without weighting with 1000 bootstrap replicates (Felsenstein, 1985), using simple sequence addition and TBR branch swapping. No more than 15 trees were saved in each replicate and only groups that appeared in >50% of the trees were retained (bootstrap procedure). The tree was rooted with *Acorus calamus* based on morphological (Mayo et al., 1997) and molecular studies (Duvall et al., 1993; Chase et al., 1995, 2000), which indicated strong support for *Acorus* (Acoraceae) as the sister to the rest of the monocots.

## RESULTS

**Range of length of *trnL-F* region**—The aligned matrix consisted of 1757 positions, of which 1202 were included in the analysis. There were 201 autapomorphic and 281 potentially parsimony informative characters. Alignment necessitated the introductions of gaps varying from 1 to 344 bp. There was a long hypervariable repeat region, which reached a maximum of 344 bp in *Arisaema* sp. With the exception of *Acorus calamus* and *Lemna* sp. and the stretch of hypervariable region, the sequences were easily aligned across all taxa.

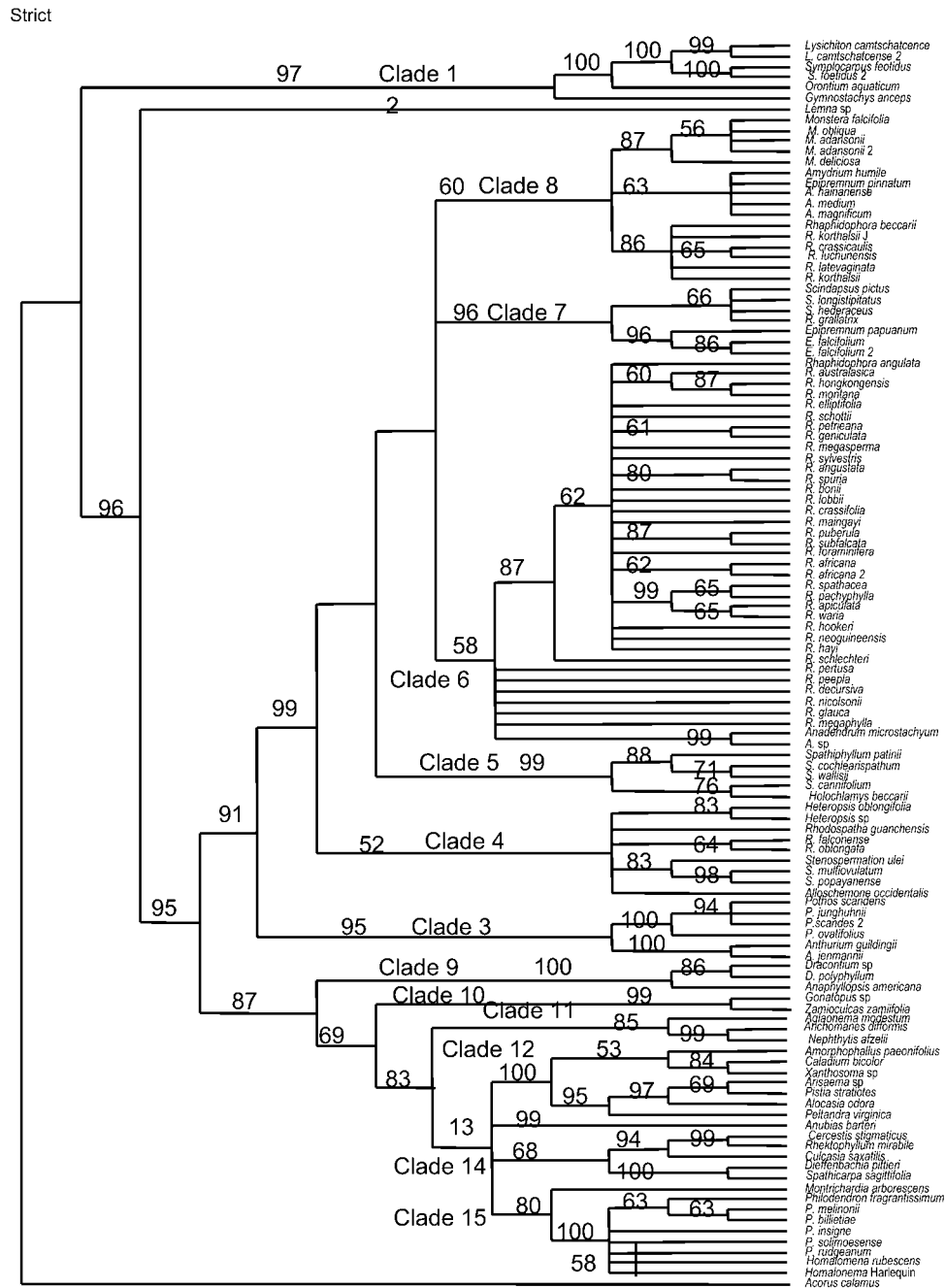


Fig. 1. Strict consensus *trnL-F* tree of >10000 equally parsimonious trees after successive weighting. Numbers above branch lengths indicate bootstrap support. Clades discussed in the text are numbered. Duplicate taxa are labeled “2” at the end of their names. “J” = juvenile sample (*R. korthalsii*).

The >10000 equally parsimonious trees obtained prior to the weighting procedure had 893 steps with a consistency index (CI) = 0.689, retention index (RI) = 0.859, and rescaled consistency index (RC) = 0.592. After successive weighting, the >10000 equally parsimonious trees had 512 steps with a CI = 0.887, RI = 0.949, and RC = 0.842. There were 1040 characters with a weighting of 1, while 162 characters had a weighting other than 1. The strict consensus tree of >10000 equally parsimonious trees was chosen as the working hypothesis and is hereafter referred to as the *trnL-F* tree (Fig. 1).

**Principal groupings derived from *trn L-F* tree (Fig. 2A and B)**—The less-derived clades on the *trnL-F* tree are well

supported, with subfamilies Gymnostachyoideae, Orontioideae, and *Lemna* having relatively long branches separating them from the rest of the aroids. Though the genera of other subfamilies were not fairly represented in our analysis, we still included them in the following discussion. The bootstrap percentage for a clade is hereafter given in brackets and shown above the branches on the *trnL-F* tree where the clades are numbered.

Subfamilies Gymnostachyoideae and Orontioideae (97%, clade 1) are resolved as sister clade to the rest of the aroids. *Lemna* sp. (Lemnaceae; labeled as 2) is sister to the remaining aroids (96%). There is 95% bootstrap support for the division

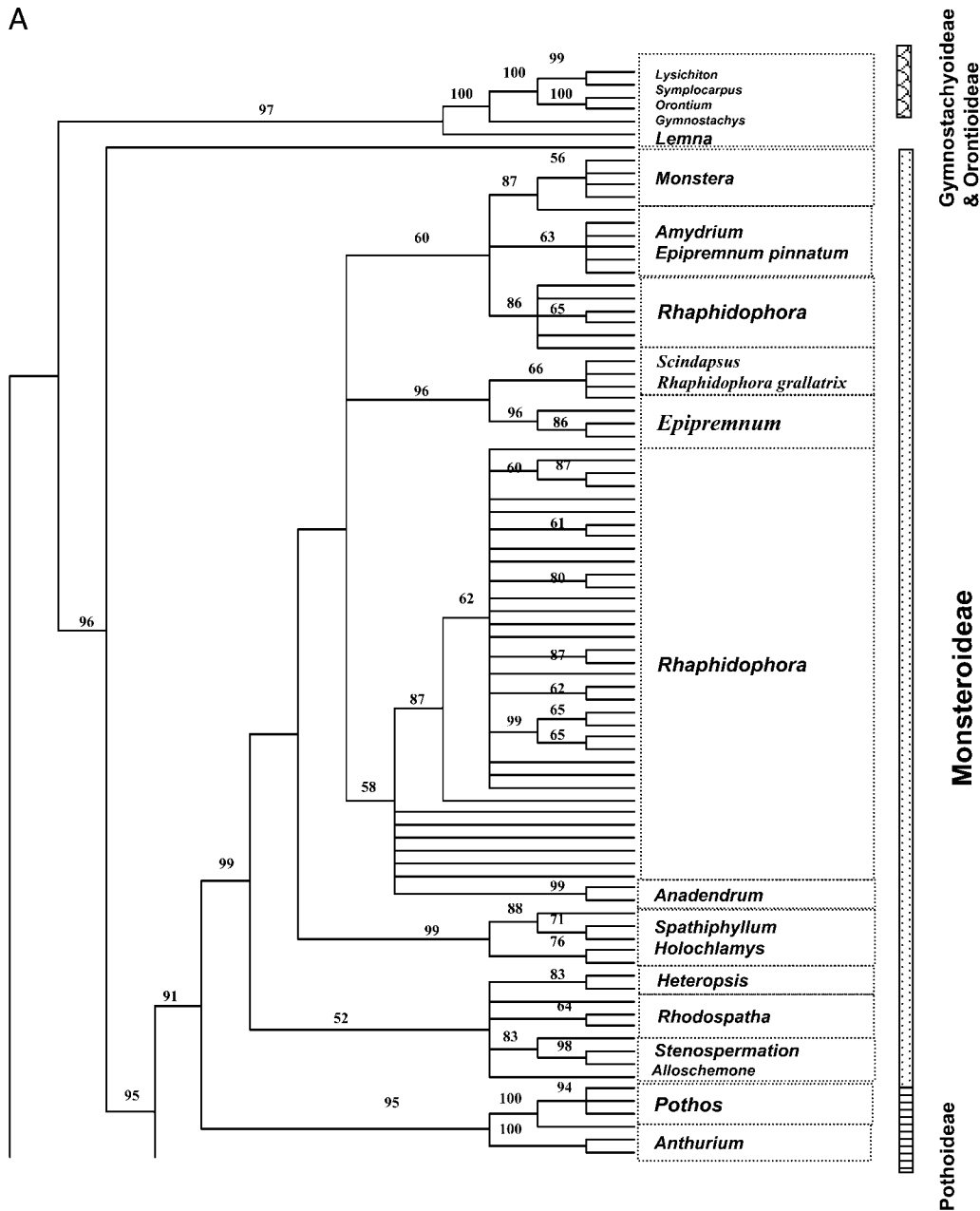


Fig. 2. *trnL-F* tree depicting intergeneric relationships of the Araceae.

of the Araceae into two major clades, one consisting of subfamilies Pothoideae and Monsteroideae (91%), sister to a clade (87%) comprising subfamilies Lasioideae and Aroideae.

Subfamily Pothoideae (clade 3, 95%) is sister to Monsteroideae. Tribes Potheae and Anthurieae form the monophyletic Pothoideae clade each with 100% bootstrap support. Subfamily Monsteroideae is monophyletic (clades 4–9, 99%). Within the Monsteroideae, the genera *Alloschemone*, *Stenospermation*, and *Rhodospatha* (tribe Monstereae) with *Heteropsis* (tribe Heteropsidae) constitute a weakly supported group (clade 4, 52%) that is sister to the remaining monsteroids. In clade 4, there is a polytomy, consisting of two clades, *Stenospermation* and *Heteropsis* are monophyletic (with 83% support) with *Rhodospatha* and *Alloschemone*. Clade 5 corre-

sponds to tribe Spathiphyllae (99%) with *Spathiphyllum* and *Holochlamys* in which *Spathiphyllum* is paraphyletic due to the position of *Holochlamys beccarii* sister to *S. canniifolium*. The next branch is a large polytomous group, consisting of three clades that include the tribes Anadendreae and Monstereae (clades 6, 7, and 8) but with weak bootstrap support (<50%).

In clade 6, two species of *Anadendrum* grouped together (99%) and are unresolved with 34 species of *Rhaphidophora*. *Scindapsus* and *Rhaphidophora grallatrix* are grouped together (66%), sister to three taxa of *Epipremnum* (Clade 7, 96%). Clade 8 (60%) consists of a polytomy involving four genera: a monophyletic *Monstera* clade (87%), *Epipremnum pinnatum* unresolved with *Amydrium*, thus rendering *Epipremnum* par-

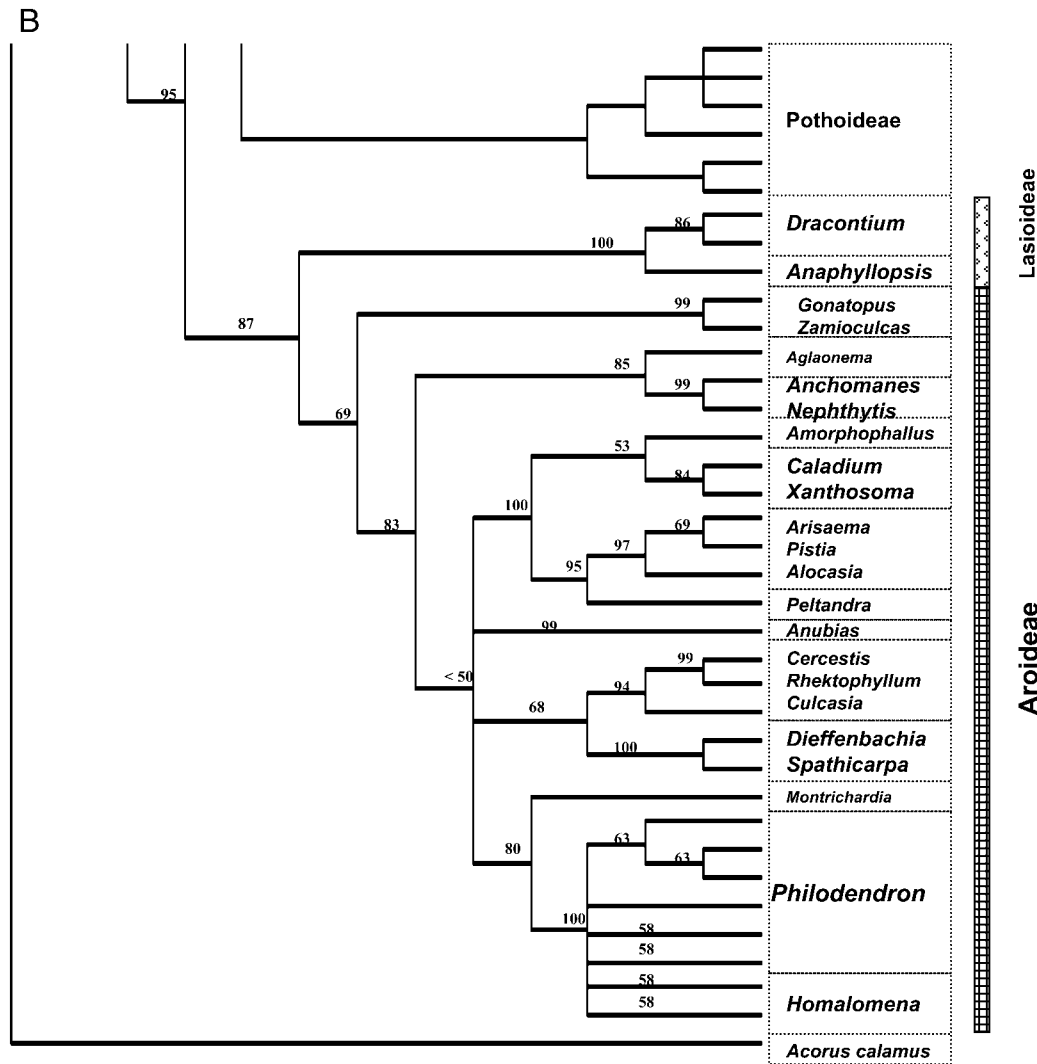


Fig. 2. Continued.

aphyletic (63%) and a third clade consisting of six species of *Rhaphidophora* (86%).

Subfamily Lasioideae is sister to the Aroideae (clade 9, 100%). In Aroideae, tribe Zamioculcadeae (clade 10, 99%) is sister to the remainder of the subfamily. The following clade (clade 11, 85%) comprises of a mixture of tribes: *Aglaonema* (Aglaonemateae) and *Anchomanes* and *Nephthytis* (Nephthytideae). The next group (less than 50%) involves a polytomy of three clades (clades 12, 14, and 15) and one species, *Anubias barteri* (tribe Anubiadeae, labeled 13, 99%).

Clade 12 (100%) consists of two clades: the first clade (53%) comprises tribe Thomsonieae (*Amorphophallus*) and tribe Caladieae (*Caladium* and *Xanthosoma*); the second clade (95%) comprises tribes Arisaemateae (*Arisaema*), Pistieae (*Pistia*), Colocasieae (*Alocasia*), and Peltandreae (*Peltandra*). Clade 14 (68%) comprises tribes Culcasieae (*Cercestis*, *Rhektophyllum*, and *Culcasia*), Dieffenbachieae (*Dieffenbachia*), and Spathicarpeae (*Spathicarpa*). Within clade 14, Culcasieae (94%) is sister to the latter two tribes (100%). Clade 15 (80%) consists of tribe Montrichardieae (*Montrichardia*), sister to a clade comprising tribes Homalomeneae (*Homalomena*) and Philodendreae (*Philodendron*) (100%).

DISCUSSION

**Subfamily circumscription**—The *trnL-F* tree is compared with previous major Araceae studies: Mayo et al. (1997), French et al. (1995), Bogner and Nicolson (1991; based on Engler’s classification system), Hay and Mabberley (1991; based on Grayum’s system), and Grayum (1990).

The *trnL-F* tree recognizes seven clades that represent either recognized subfamilies or groups that could be eventually be given the rank of subfamily in Araceae: Gymnostachydoideae, Orontioideae, *Lemna* sp., Pothoideae, Monsteroideae, Lasioideae, and Aroideae. This is in agreement with Mayo et al. (1997) and French et al. (1995), if we consider Philodendroideae and Aroideae to be one subfamily. All subfamilies sensu Mayo et al. (1997) that were sampled are monophyletic clades on the *trnL-F* tree. However, our tree does not distinguish Philodendroideae and Aroideae as separate clades, as was reported by French et al. (1995) and proposed by Hay and Mabberley (1991). Similarly, the *trnL-F* tree does not recognize the division for Philodendroideae, Colocasioideae, and Aroideae sensu Bogner and Nicolson (1991) or Colocasioideae and Aroideae sensu Grayum (1990).

Conflict exists with Bogner and Nicolson (1991) because of their inclusion of Orontioideae, Anthurieae, Zamioculcadeae, Nephthytideae, and Montrichardieae as part of Lasioideae. Conflict also exists with Hay and Mabberley (1991) due to their placement of Symplocarpeae in Lasioideae and Zamioculcadeae in Pothoideae. There would be less conflict if their Philodendroideae and Aroideae were merged. The *trnL-F* tree shows conflict with Grayum's (1990) system in his placement of *Gymnostachys* in Pothoideae and Symplocarpeae with Orontieae in Lasioideae. Otherwise, there is little difference in the groupings of taxa if Colocasioideae, Calloideae, and Aroideae are considered as one large clade.

Occupying the basal position in the Araceae is the clade consisting of the monogeneric subfamily Gymnostachydoideae sister with subfamily Orontioideae. *Gymnostachys* is sister to a clade in which *Orontium* is sister to *Symplocarpus* and *Lysichiton*. Gymnostachydoideae and Orontioideae (clade 1) constitute the major group proto Araceae sensu Mayo et al. (1997). Relationships found within this clade are in complete agreement with those proposed by Mayo et al. (1997) and French et al. (1995). Grayum (1990) proposed that Pothoideae was the most primitive subfamily in Araceae but he included *Gymnostachys*, a genus he considered to contain the most primitive characters.

Hay and Mabberley (1991) excluded *Gymnostachys* from Araceae and placed Symplocarpeae in Lasioideae, which they considered to be the most basal subfamily in Araceae, which is in partial agreement with our results. The *trnL-F* tree also shows partial agreement with Bogner and Nicolson (1991) in whose classification Gymnostachydoideae (*Gymnostachys*) is the most basal group of the family. However, Orontioideae was placed within Lasioideae, between Calloideae and Philodendroideae (both being more advanced groups).

The *trnL-F* tree placed *Lemna* sp. within Araceae (96%). *Lemna* sp. occupies a position between the proto aroids and the true aroids (sensu Mayo et al., 1997) as sister to the true aroids (95%), which supports the view that Araceae is paraphyletic. *Lemna* is sister to Pothoideae and Monsteroideae (Pothoideae basal to Monsteroideae), Lasioideae and Aroideae (subfamily Calloideae was not sampled). This pattern agrees with the results of French et al. (1995). A general agreement is detected with the studies of Mayo et al. (1997) and Bogner and Nicolson (1991). The only difference is in the position of Monsteroideae, which they considered to be derived from Pothoideae and sister to Lasioideae or Calloideae, where the *trnL-F* tree shows Monsteroideae to be a clade derived within Pothoideae. Conflict exists with Grayum (1990) in his considering Lasioideae as being more derived than Colocasioideae and Calloideae but sister to Aroideae. The *trnL-F* tree generally disagrees with Hay and Mabberley's (1991) basal placement of Lasioideae because they considered extant members of this subfamily as having the most primitive aroid characters.

**Tribal circumscription**—The general evolution of tribes is determined by their positions in subfamilies and by the genera composing them. The *trnL-F* tree shows clades in agreement only with French et al. (1995) except for the position of *Lemna* (resolved within Aroideae on their tree). Compared with Mayo et al. (1997), there is less agreement, albeit many genera in Aroideae were not sampled in our study. The *trnL-F* tree supports the tribes Zamioculcadeae, Nephthytideae, Culcasieae, Caladieae, and the *Dieffenbachia* alliance. The *trnL-F* tree does not support the distinction of tribes Heteropsidae and

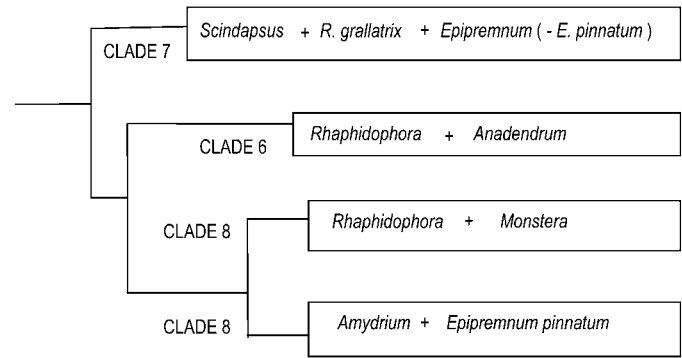


Fig. 3. A hypothesis of intergeneric relationships in the Monstereae based on consensus evidence of the *trnL-F* tree and cpDNA variation tree (French et al., 1995).

Monstereae because their positions are interspersed between members of other tribes. The *trnL-F* tree does not support the composition of tribes Monstereae, Heteropsidae, and Nephthytideae sensu Bogner and Nicolson (1991) and sensu Hay and Mabberley (1991). In comparison with Grayum's (1990) classification, a difference is seen in his circumscription of tribe Monstereae. All these tribes consisted of members that did not form monophyletic clades on the *trnL-F* tree.

**Tribal evolution**—Tribe relationships are most similar to the cpDNA variation tree of French et al. (1995), with few differences caused by Spathiphyllaeae being sister to *Rhodospatha* and *Stenospermatum* on their cpDNA variation tree and the placement of *Lemna*. The *trnL-F* tree disagrees with the position of Spathiphyllaeae sensu Mayo et al. (1997). They positioned Spathiphyllaeae as the basal tribe in Monsteroideae, while the *trnL-F* tree has a clade consisting of *Alloschemone*, *Heteropsis*, *Rhodospatha*, and *Stenospermatum* as the basal group in this subfamily. In Aroideae, agreement is only detected in the positions of Zamioculcadeae being less derived than Aglaonemateae and Peltandreae being less derived than Colocasieae. Compared to Hay and Mabberley (1991), agreement is detected in Culcasieae being sister to Nephthytideae (excluding their position of *Nepthytis*).

There is a lack of agreement between the *trnL-F* tree and Bogner and Nicolson (1991). Agreements are detected only in Zamioculcadeae being sister to Nephthytideae (except their position of *Cercestis*) and Culcasieae and the close relationship between Philodendreae and Anubiadeae. When compared with Grayum (1990), agreement is seen in the close relationships between Spathicarpeae and Dieffenbachieae, Philodendreae and Homalomeneae, and Cercestideae and Culcasieae. Our results have highlighted the many differences in tribal circumscription and placement that exist between the *trnL-F* tree and previous classifications with the exception of the cpDNA variation tree of French et al. (1995) and among previous classifications. Much work remains to be done to sort out tribal classification.

**The subfamily Monsteroideae: tribal classification and evolution**—Monsteroideae was found to be a strongly monophyletic clade sister to Pothoideae. We sampled all genera (with three represented taxa per genus when possible) within the subfamily with particularly higher sampling of the largest genus in the subfamily, *Rhabdiphora*, which is a major fo-

cus of our ongoing study. The *trnL-F* tree has three distinct clades corresponding to tribal groupings, some of which are in conflict with existing tribal circumscriptions. The tribe Monstereae is paraphyletic. The least derived clade consists of *Alloschemone*, *Rhodospatha*, *Stenospermatum*, and *Heteropsis*, immediately sister to a clade consisting of *Spathiphyllum* and *Holochlamys*, which are sister to a large clade consisting of *Anadendrum*, *Rhaphidophora*, *Scindapsus*, *Epipremnum*, *Amydrium*, and *Monstera*. The monophyletic tribe Heteropsiadeae could be extended to include *Alloschemone*, *Rhodospatha*, and *Stenospermatum* in order to represent the first clade. The second clade corresponds to tribe Spathiphyllaeae, while the third clade could be circumscribed as tribe Monstereae. We refrain from making any formal taxonomic changes in preference to retaining taxonomic stability until further congruent information is available to support these conflicting schemes.

**Generic delimitation in the tribe Monstereae**—Results from the *trnL-F* tree place in question the circumscription of certain genera. *Rhaphidophora* is found to be strongly paraphyletic (paraphyletic relationships at generic and tribal levels) with species scattered among three clades—clade 6 (with *Anadendrum*), clade 7 (with *Scindapsus* and *Epipremnum*), and clade 8 (with *Monstera*, *Amydrium*, and *Epipremnum pinnatum*). Our results have clarified somewhat the complicated intergeneric relationships of the Monstereae. The generic description of *Rhaphidophora* was problematic since the beginning (solved by Nicolson, 1978; Boyce, 1999) with the generic status of *Rhaphidophora* being questioned several times in the past, closely linked with *Scindapsus*, *Monstera*, and *Epipremnum* (Miquel, 1856; Bakhuizen van der Brink, 1958; Birdsey, 1962; Bunting, 1962; Grayum, 1990). For the moment, we recognize three informal groups of *Rhaphidophora* species, corresponding to clades 6, 7, and 8 on the *trnL-F* tree. The interspecific phylogeny of *Rhaphidophora* will be explored in more detail with additional data from sequences of a nuclear gene (Adh 1) and AFLP (S.-M. Tam et al., unpublished manuscript).

**Intergeneric phylogeny of the tribe Monstereae based on consensus evidence**—The clade sister to the clade comprising the paraphyletic *Rhaphidophora* species found on the *trnL-F* tree consists of *Spathiphyllum* and *Holochlamys* and can be useful as the closest outgroup. Unfortunately, the *trnL-F* region does not permit us to fully elucidate the relationships between closely related interspecific taxa. In clade 6, *Anadendrum* forms a monophyletic clade unresolved with most of the *Rhaphidophora* species. Similarly, in clade 8, *Rhaphidophora* is closely related to the monophyletic *Monstera*, *Amydrium*, and *Epipremnum pinnatum*. The strong similarity between the *trnL-F* tree and the cpDNA variation tree (French et al., 1995) provide additional confidence for the intergeneric relationships of *Rhaphidophora*. On the cpDNA variation tree, *Scindapsus* is sister to *Rhaphidophora*, *Anadendrum*, *Monstera*, *Epipremnum pinnatum*, and *Amydrium* (French et al., 1995). Therefore, we hypothesize that on the *trnL-F* tree, clade 7, which consists of *R. grallatrix*, *Scindapsus*, and *Epipremnum*, is sister to clade 6, which comprises most of the *Rhaphidophora* species and *Anadendrum* and clade 8, which is comprised of *Amydrium* and *E. pinnatum* being sister to a few species of *Rhaphidophora* and *Monstera* (Fig. 3).

**Concluding remarks**—In order to reflect the clades shown by the *trnL-F* tree, the subfamilies Gymnostachydoideae and Orontioideae (proto aroids) should be treated as a single subfamily. In addition, Pothoideae and Monsteroideae could also be considered as a single subfamily, as was suggested by Grayum (1990) and French et al. (1995). Many disagreements were found between the *trnL-F* tree and previous classifications and among previous classifications in tribal classification, and a new working consensus is much needed. Congruent results obtained from the *trnL-F* tree and cpDNA variation tree of French et al. (1995) indicate that both these studies can provide a useful basis for a new tribal agreement.

Our results have highlighted the need for further studies on the generic delimitations in the Monstereae. At the same time, it serves as an alert to us and other aroid workers working on the implicated genera (and species) to take into consideration the complex relationships involved amongst the five genera, and possibly *Anadendrum*. *Rhaphidophora* has approximately 100 species. *Anadendrum* and *Amydrium* are small genera with seven species and 4–6 species, respectively. *Epipremnum* has 20 species, *Scindapsus* has about 36 species, while *Monstera* has about 40 species. A revision of the tribe would be a major study but is much needed and should be undertaken using a molecular phylogenetics framework. Our results confirm what has perplexed aroid taxonomists about the monsterooids: that paraphyletic and complex relationships exist between these genera. More importantly, the *trnL-F* tree contributes to the phylogeographical study of the Araceae and has generated certain hypotheses that can be tested to improve our understanding of these morphologically and geographically diverse groups of lianas.

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