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PHYLOGENETIC RELATIONSHIPS OF AROIDS AND DUCKWEEDS (ARACEAE) INFERRED FROM CODING AND NONCODING PLASTID DNA[1]

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Familial, subfamilial, and tribal monophyly and relationships of aroids and duckweeds were assessed by parsimony and Bayesian phylogenetic analyses of five regions of coding (*rbcL*, *matK*) and noncoding plastid DNA (partial *trnK* intron, *trnL* intron, *trnL–trnF* spacer) for exemplars of nearly all aroid and duckweed genera. Our analyses confirm the position of *Lemna* and its allies (formerly Lemnaceae) within Araceae as the well-supported sister group of all aroids except Gymnostachydoideae and Orontioideae. The last two subfamilies form the sister clade of the rest of the family. Monophyly of subfamilies Orontioideae, Pothoideae, Monsteroideae, and Lasioideae is supported, but Aroideae are paraphyletic if *Calla* is maintained in its own subfamily (Calloideae). Our results suggest expansion of the recently proposed subfamily Zamioculcadoideae (*Zamioculcas*, *Gonatopus*) to include *Stylochaeton* and identify problems in the current delimitation of tribes Anadendreae, Heteropsideae, and Monstereae (Monsteroideae), Caladieae/Zomicarpeae, and Colocasieae (Aroideae). Canalization of traits of the spathe and spadix considered typical of Araceae evolved after the split of Gymnostachydoideae, Orontioideae, and Lemnoideae. An association with aquatic habitats is a plesiomorphic attribute in Araceae, occurring in the helophytic Orontioideae and free-floating Lemnoideae, but evolving independently in various derived aroid lineages including free-floating *Pistia* (Aroideae).

Key words: Araceae; Lemnaceae; molecular phylogenetics; plastid DNA; *rbcL*; subfamilial and tribal classification; *trnK-matK*; *trnL-trnF*.

Over the last decade, DNA sequence data have contributed greatly to improve our understanding of the phylogenetic relationships of flowering plants (reviews in Soltis and Soltis, 2004; Soltis et al., 2005), permitting for the first time independent testing of earlier classifications constructed on the basis of similarities and differences in morphological attributes (e.g., Cronquist, 1981; Takhtajan, 1997). DNA studies have also allowed the systematic placement of groups problematic because of their highly modified vegetative or reproductive organs, such as aquatic family Podostemaceae (Soltis et al., 1999 and references therein) and many parasitic plants (Nickrent et al., 1998). Furthermore, molecular phylogenetic trees provide explicit evolutionary frameworks for assessments of character evolution, biogeography, and many other biological comparative studies (e.g., Brooks and McLennan, 1991; Harvey and Pagel, 1991; Harvey et al., 1996; Givnish and Systma, 1997; Bateman, 1999; Futuyma, 2004). In this work, we use DNA sequence data to assess the phylogenetic relationships of Araceae (aroids) and Lemnaceae (duckweeds), which in spite of obvious morphological differences have long been suspected to be closely related, and to gain insights into evolution of the aquatic habit in these groups.

According to Mayo et al. (1997), Araceae include 105 genera and 3300 species occurring on all continents except Antarctica. About 90% of genera and 95% of species are found in the tropics. Aroids are one of the most ecologically and structurally diverse groups of monocots. They occupy a wide variety of habitats and display a notable diversity of life forms, including geophytes, climbers, epiphytes, helophytes, and free-floating aquatics (Croat, 1988; Grayum, 1990; Boyce, 1995; Mayo et al., 1997; Bown, 2000; Keating, 2002). Their vegetative parts are extremely varied; for instance, stems can be creeping or climbing and form rhizomes or distinct tubers; leaves range from simple to complexly divided, and some such as those of *Amorphophallus titanum* are among the largest produced by a herb (Bown, 2000). The most distinctive features of Araceae are found in their inflorescences, which characteristically consist of a fleshy axis, the spadix, bearing small flowers usually arranged in spirals and subtended by a conspicuous leaf-like or petal-like bract—the spathe. Flowers may be bisexual or unisexual, and a perigone is present in some groups. Unisexual flowers usually are borne in separate female and male zones of the spadix, which often has a sterile apical appendix (Boyce, 1995; Mayo et al., 1997, 1998; Judd et al., 2002; Soltis et al., 2005).

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Historically, several major classifications of Araceae have been proposed (reviews in Nicolson, 1960, 1987; Croat, 1990, 1998; Grayum, 1990; Mayo et al., 1995a, 1997). The earliest modern classification encompassing Araceae was that proposed by Schott (1860), who based his groupings mainly on floral morphology (e.g., he divided the family into two major groups, one with bisexual flowers and the other with unisexual flowers). On the other hand, Engler (e.g., 1876, 1920) relied on a broader spectrum of information sources, including vegetative morphology and anatomy, in addition to floral morphology, and his system explicitly incorporated an evolutionary perspective (Grayum, 1990; Mayo et al., 1997; Govaerts et al., 2002). Hooker (1883) modified Schott's classification and incorporated many of Engler's generic concepts, and subsequently Hutchinson (1973) elaborated on Hooker's system. Most contemporary aroid taxonomists (Bogner, 1979; Bogner and Nicolson, 1991; Mayo et al., 1997, 1998) have been strongly influenced by the Englerian views, and some of them have discussed and modified previous systems by incorporating diverse types of information into classifications on cladistic grounds (Grayum, 1990; Mayo et al., 1997). Recently, several molecular phylogenetic studies have been published that provide independent frameworks for evaluating earlier proposals of relationship among aroids (French et al., 1995; Renner and Zhang, 2004; Renner et al., 2004; Tam et al., 2004; Gonçalves et al., 2007) or evolution of specific traits (e.g., atypical bisexual flowers; Barabé et al., 2002, 2004).

For over 250 years, botanists have been perplexed by the systematic position of duckweeds, a group of five genera and about 35 species of diminutive, specialized free-floating aquatics consisting of minute fronds or thalli that bear only a few roots (*Landoltia*, *Lemna*, *Spirodela*) or none at all (*Wolffia*, *Wolffiella*) and multiply predominantly by asexual means (Hillman, 1961; Landolt, 1986, 1998; Les and Crawford, 1999). A link to aroids has long been suspected (Engler, 1876; Beille, 1935, cited in Lawalrée, 1945; Cronquist, 1981; Takhtajan, 1997), but the extreme morphological reduction in the duckweeds made it difficult to carry out meaningful comparisons for many structural traits routinely used in ascertaining taxonomic limits and relationships among aroids (e.g., Lawalrée, 1945; Landolt, 1986, 1998; Mayo et al., 1995b, 1997; Les et al., 1997, 2002). However, recently published molecular phylogenetic studies have consistently placed the duckweeds among Araceae (French et al., 1995; Barabé et al., 2002; Rothwell et al., 2004), and most contemporary aroid taxonomists now consider the duckweeds to be members of the latter (e.g., Mayo et al., 1995b; Govaerts et al., 2002; Les et al., 2002; Keating, 2002; Renner and Zhang, 2004; Bogner and Petersen, 2007). Phylogenetic classifications such as APG (1998, 2003) also do not recognize Lemnaceae as distinct from Araceae. Nevertheless, evidence for the precise phylogenetic position of the duckweeds within Araceae has remained inconclusive. For instance, in the plastid DNA restriction site analysis of French et al. (1995), *Lemna* is nested in subfamily Aroideae, but the *trnL-trnF* DNA sequence analysis conducted by Barabé et al. (2002) placed *Lemna* as sister to all Araceae sampled except *Lysichiton* and *Symplocarpus*. More recently, Rothwell et al. (2004) obtained a similar result. They analyzed sequences of the intergenic spacer (IGS) between *trnL* and *trnF* of 22 exemplars of Araceae and six species that represented all extant genera of Lemnaceae and found that the duckweeds formed a polytomy with various groups of "true Araceae."

In this study, we assess relationships of aroids and duckweeds using DNA sequence and indel data from coding and noncoding plastid DNA. The regions analyzed include the exons of *rbcL* and *matK* plus the 3′ portion of the *trnK* intron (downstream *matK*) and the *trnL-trnF* region, which consists mostly of the *trnL* intron and the IGS between *trnL* and *trnF*. These plastid regions have been broadly used for phylogenetic estimation in angiosperms, including previous assessments of various aroid lineages (Barabé et al., 2002; Les et al., 2002; Rothwell et al, 2004; Tam et al., 2004; Renner and Zhang, 2004; Renner et al., 2004; Gonçalves et al., 2007). Our study is aimed at attaining a clearer picture of duckweed relationships to other clades of Araceae and evaluating monophyly and relationships for aroid subfamilies and tribes recognized in the classification of Mayo et al. (1997, 1998). We are also interested in the evolution of the aquatic habit in Araceae. Throughout this paper, genera and suprageneric groups follow Mayo et al. (1997) unless otherwise specified.

MATERIALS AND METHODS

Taxonomic sample—Exemplars representing 97 of 105 aroid genera accepted in Mayo et al. (1997) and all five genera of duckweeds recognized by Les et al. (2002) were studied. Representatives of other families of Alismatales, including *Alisma* (Alismataceae), *Tofieldia* (Tofieldiaceae), *Triglochin* (Juncaginaceae), as well as of Acorales (*Acorus*), Chloranthaceae (*Hedyosmum*), Magnoliales (*Magnolia*), and Piperales (*Piper*), were used as outgroups. A list of taxa with voucher information and GenBank accessions is given in Appendix 1; the aligned data matrix was deposited in TreeBase (http://TreeBASE.org; matrix accession number M3914).

DNA extraction, amplification, and sequencing—Genomic DNA was usually extracted from fresh or silica-gel-dried material, but in some instances leaf fragments from herbarium specimens were used. Genomic DNA was extracted using a modified 2× cetyltrimethylammonium bromide (CTAB) procedure based on Doyle and Doyle (1987); DNA extracts were purified with QIAquick minicolumns (Qiagen, Crawley, West Sussex, UK) following the manufacturer's protocol for cleaning PCR products or precipitated with 100% ethanol at –20°C and purified on a cesium chloride/ethidium bromide density gradient (1.55 g/mL).

Amplification of DNA was performed using commercial kits, including PCR Master Mix (Advanced Biotechnologies, Epsom, Surrey, UK) and *Taq* PCR Core Kit (Qiagen), according to the manufacturers' protocols. To each PCR reaction tube were added 1% of each primer (100 ng/μL) and 2–4% of a 0.4% aqueous solution of bovine serum albumin (BSA) to neutralize phenolic compounds and other potential inhibitors (Kreader, 1996).

The *rbcL* exon was amplified with primers 1F and 1460R (Asmussen and Chase, 2001) or, in the case of degraded DNA from herbarium specimens, using as well internal primers 636F and 724R (Muasya et al., 1998). The PCR program was: 2 min initial denaturation at 94°C; 28–30 cycles of 1 min at 94°C, 30 s at 48°C, and 1 min at 72°C; 7 min final extension at 72°C. When amplification did not yield enough DNA for sequencing, 0.3–0.5 μL of PCR product was used directly as template for a second PCR with the same parameters as before but performing only 14 cycles.

The *trnK-matK* region, including the *matK* gene and the 3′ portion of the *trnK* intron, was usually amplified as a single segment with primers −19F (Molvray et al., 2000) and *trnK*2R (Steele and Vilgalys, 1994), and the following PCR parameters: 2 min 30 s at 94°C; 28–32 cycles of 1 min at 94°C, 45 s at 52°C, and an initial 2.5-min extension at 72°C, increasing the time by 8 s on each consecutive cycle with a 7 min final extension at 72°C. However, degraded DNA had to be amplified in smaller fragments using additional primers, including 390F (Sun et al., 2001), 731F (Molvray et al., 2000), 1309F (Civeyrel and Rowe, 2001), and 1326R (Sun et al., 2001).

The *trnL-trnF* region, consisting of the intron in *trnL* and the *trnL-trnF* IGS, was amplified either as a single piece with primers c and f or as two fragments with primer combinations c–d and e–f (all from Taberlet et al., 1991). The PCR program was: 2 min at 94°C; 28–35 cycles of 30 s at 94°C; 30 s at 52°C; 2 min at 72°C; final extension of 7 min at 72°C. In some instances, this region could

not be reliably amplified using primer c, and in such cases we used instead primer c2 of Bellstedt et al. (2001). Some samples required reamplification, which was conducted as in the first round of PCR but only for 16 cycles, using 0.5 μL of the product of the first PCR directly as template.

PCR products were purified with QIAquick (Qiagen) or CONCERT (Life Technologies, Paisley, UK) minicolumns following the manufacturers' protocols. The cleaned products were used in cycle-sequencing reactions with the Big Dye Terminator Cycle Sequencing Ready Reaction kit version 3 or 3.1 (Applied Biosystems, ABI, Warrington, Cheshire, UK). Cycle sequencing was carried out in 5.25 μL reactions including 2 μL Big Dye, 0.25 μL primer at the same concentration as for PCR, and 3 μL PCR product. Products of cycle-sequencing were purified by ethanol precipitation or with Centri-Sep Sephadex columns (Princeton Separations, Adelphia, New Jersey, USA). Both DNA strands were sequenced in an ABI 377 automated sequencer or a 3100 Genetic Analyzer. The chromatograms were edited and assembled with Sequencher versions 3.1–4.1 (GeneCodes Corp., Ann Arbor, Michigan, USA).

Sequences of both coding regions (*rbcL* and *matK*) were aligned visually trying to maximize similarity (Simmons, 2004). The *trnK* intron and the *trnL-F* region were initially aligned with Clustal W (Thompson et al., 1994) and subsequently adjusted visually following the recommendations of Kelchner (2000). A 233-bp segment of the *trnL* intron could not be aligned unambiguously and was excluded from the analyses; the excluded portion amounts to about 4.3% of the data cells of the combined data set.

Phylogenetic analyses—Previous phylogenetic analyses of aroids (Gonçalves et al., 2007) and duckweeds (Les et al., 2002) based on plastid DNA sequences, including those studied here, have shown that both resolution and overall bootstrap support for clades improve when multiple DNA regions are analyzed in combination. Parsimony analyses of the separate (*rbcL, matK-trnK, trnL-trnF*) and combined plastid data sets were performed using the program PAUP* version 4.0b10 for Macintosh (Swofford, 2002) and consisted of heuristic searches with 1000 replicates of random sequence addition with the MulTrees option (keeping multiple trees) activated and tree-bisection-reconnection (TBR) branch swapping, saving up to 20 trees per replicate to reduce the time spent in swapping large islands of trees (Maddison, 1991). All characters were unordered and equally weighted. Individual gap positions were treated as missing data, but all nonautapomorphic indels were coded using the simple method of Simmons and Ochoterena (2000) and appended to the sequence matrices as presence/absence characters. Internal support for clades was evaluated by nonparametric bootstrapping (Felsenstein, 1985), performing 500 bootstrap replicates, each with five heuristic replicates and TBR branch swapping, saving up to 20 shortest trees per replicate.

We also conducted a model-based analysis of the combined data set, excluding the indels, using Bayesian Markov chain Monte Carlo (MCMC) inference (Yang and Rannala, 1997) as implemented in the computer program MrBayes version 3.1.2 (Ronquist et al., 2005). A six-parameter model of molecular evolution with gamma distribution (Yang, 1993) and a proportion of invariant characters (Reeves, 1992) fit best each of the three separate data sets (*rbcL*, *trnK-matK*, and *trnL-trnF*) in the program Modeltest 3.7 (Posada and Crandall, 1998) using both the likelihood ratio test (Goldman, 1993) and the Akaike information criterion (Akaike, 1974). Accordingly, the GTR + I + G model was set in MrBayes. Two independent analyses, each running four Markov chains and starting with a random tree, were run simultaneously for 3 200 000 generations, sampling trees every hundredth generation. The temperature of the heated Markov chains was set to 0.2. Values for the rate matrix and proportion of each nucleotide were estimated from the data as part of the analyses. Stationarity of log likelihoods was reached around generation 100 000, and the first 800 000 generations (25% of the trees) of each analysis were discarded as the burn-in (Ronquist et al., 2005). A majority-rule consensus tree of the pooled 48 000 remaining trees (24 000 from each run) was calculated using PAUP*, and inferences about relationships and posterior probabilities (PP) were based on this tree.

RESULTS

Maximum parsimony analyses—Data and statistics for each region analyzed separately (Appendices S1–S5; see Supplemental Data with the online version of this article) and the combined data set are summarized in Table 1. The combined matrix of all five regions consisted of 5188 characters, 1683 (32.4%) of which were parsimony informative. The heuristic search found 1105 shortest trees with a length of 7144 steps, an ensemble consistency index (CI, including parsimony uninformative characters; Kluge and Farris, 1969) = 0.50, and an ensemble retention index (RI; Farris, 1989) = 0.71. Figure 1 shows the strict consensus of the 1105 most parsimonious trees (MPTs), which is more resolved than any of the consensus trees from the three separate analyses (not shown). Likewise, overall clade support is higher than for any of the separate analyses, with 81 clades receiving bootstrap support (BP) greater than 50, and 70.4% of them attaining a BP greater than 85. *Acorus* is sister (BP 100) to Alismatales (BP 100), the latter consisting of a clade with *Tofieldia* moderately supported (BP 82) as sister to *Triglochin-Alisma* (BP 100). Araceae are strongly supported (BP 100) and within them diverge successively the following: Gymnostachydoideae/Orontioideae (BP 91), Lemnoideae (BP 100), Pothoideae/Monsteroideae (BP 100), Lasioideae (BP 100), and paraphyletic Aroideae including *Calla* (Calloideae) (BP 72). Pothoideae (BP 100) comprise Potheae, including *Pothos*, *Pothoidium*, and *Pedicellarum* (BP 100), and monogeneric Anthurieae. Monsteroideae (BP 100) include a trichotomy with strongly supported Spathiphylleae (BP 100) and two further clades in which monotypic tribes Heteropsideae (*Heteropsis*) and Anadendreae (*Anadendrum*) are intermingled with genera of Monstereae. Internal relationships in Lasioideae (BP 100) obtained little support; *Urospatha* is sister to the rest, with *Dracontium*, *Dracontioides*, and *Anaphyllopsis* forming a trichotomy (BP 84) sister to a polytomy consisting of *Lasimorpha* through *Lasia* (BP 57). The group consisting of *Stylochaeton* sister to *Zamioculcas*/*Gonatopus* (BP 92) is sister to the rest of Aroideae (including *Calla* of Calloideae) (BP 84). The next clade to diverge within Aroideae includes *Calla* (Calloideae) as sister (BP < 50) to Cryptocoryneae/Schismatoglottideae (BP 100). Farther up the tree, *Anubias* and *Callopsis* diverge successively, followed by a clade in which Culcasieae are sister to Homolameneae/Philodendreae (BP < 50), and all these are sister to another clade in which Aglaonemateae/Nephthytideae (BP 96) are collective sisters of a weakly supported group (BP < 50) consisting of *Zantedeschia* and a polytomy comprising Dieffenbachieae/Spathicarpeae (BP 100). Culcasieae through Spathicarpeae is sister to a clade in which *Montrichardia* is sister (BP < 50) to Thomsonieae (BP 89) plus Caladieae (including Zomicarpeae) and the "core aroid" clade (BP 100) within which the group comprising Ambrosineae through Arophyteae (BP 97) is sister to a colocasioid grade (BP 100) that includes Pistieae and Arisaemateae/Areae.

Bayesian analysis—The summary Bayesian tree is shown in Fig. 2. In most respects, the relationships recovered by the Bayesian analysis mirror those depicted in the strict consensus of the combined parsimony analysis (Fig. 1). The major differences between them are in the positions of *Stylochaeton*/Zamioculcadeae and *Calla*. Whereas in the parsimony tree *Stylochaeton*/Zamioculcadeae are moderately supported as sister to Aroideae (including *Calla*; BP 72), in the Bayesian results *Stylochaeton*/Zamioculcadeae are sister to a weakly supported clade (PP 0.55) formed by Lasioideae and Aroideae. As in the parsimony results, the Bayesian analysis has *Calla* nested among members of Aroideae sensu Mayo et al. (1997). However, in the parsimony analysis, *Calla* is recovered as sister to Cryptocoryneae/Schismatoglottideae, whereas in the Bayesian analysis, *Calla* is sister to the core Aroideae including *Pseudodracontium* through *Arum* (PP 0.76; Fig. 2). Cryptocoryneae/Schismatoglottideae in turn are sister to the *Calla*/core

TABLE 1. Summary of characteristics for the data sets analyzed in this study (separate analyses not discussed in the text are available as Appendices S1–S5; see Supplemental Data with the online version of this article).

Data set	No. of aligned characters	No. (%) of variable /informative characters	No. of shortest trees	Tree length	CI/RI
rbcL	1391	467 (33.6)/328 (23.6)	360	1465	0.42/0.69
matK	1706	1021 (59.9)/717 (42.3)	11 460	3009	0.51/0.71
3′ portion of the *trnK* intron	296	173 (58.5)/115 (38.9)	8260	431	0.60/0.79
trnL intron	1006	371 (36.9)/219 (21.8)	17 760	869	0.61/0.73
trnL-trnF IGS	745	431 (57.9)/298 (40.0)	4180	1155	0.55/0.77
All data sets combined	5188	2480 (47.8)/1683 (32.4)	1105	7144	0.50/0.71

Aroideae clade with strong support (PP 0.95). Other differences with respect to the parsimony analysis include the positions of *Montrichardia* and *Zantedeschia*, but these alternatives obtained only weak support.

DISCUSSION

Familial, subfamilial, and tribal limits and relationships—Mayo et al. (1997) recognized seven subfamilies in Araceae, namely Gymnostachydoideae, Orontioideae, Pothoideae, Monsteroideae, Lasioideae, Aroideae, and Calloideae. Our results support the recognition of the first five subfamilies, but *Calla palustris*, the sole member of Calloideae, is nested in Aroideae (Figs. 1, 2; see later), whereas the association of *Stylochaeton* and its sister group, Zamioculcadeae, to other Aroideae is only weakly supported by parsimony and was not recovered by the Bayesian analysis. On the other hand, this study provides evidence supporting inclusion of the duckweeds in Araceae, showing that they are sister to the "true Araceae" of Mayo et al. (1997). In the following paragraphs, we discuss each of the major clades recovered by our analyses and argue for recognition of eight subfamilies within Araceae. Our proposal differs from the system of Mayo et al. (1997) in that the duckweeds are included in Araceae as subfamily Lemnoideae, *Calla* is included in Aroideae, and subfamily Zamioculcadoideae is accepted, but its original circumscription (Bogner and Hesse, 2005) is broadened to include also *Stylochaeton*. We also consider issues related to monophyly and delimitation of the tribes recognized by Mayo et al. (1997).

"***Proto-Araceae*"*: Gymnostachydoideae and Orontioideae***—A close relationship between *Gymnostachys*, the sole member of subfamily Gymnostachydoideae, and Orontioideae was unsuspected until the 1990s. Instead, *Gymnostachys* was often associated with *Acorus*, either within Araceae (e.g., Schott, 1860; Engler, 1905, 1920; Hotta, 1970) or as a distinct family (Engler, 1876). Early molecular studies based on plastid DNA restriction site variation and sequence data yielded conflicting hypotheses concerning *Gymnostachys*. On the one hand, Duvall et al.'s (1993a, b) analyses of *rbcL* sequences placed *Gymnostachys* as sister to the other aroids (with *Lemna* nested among them) and identified *Acorus* as the sister of the rest of the monocots, whereas the plastid DNA restriction site analysis of Davis (1995) grouped *Gymnostachys* with *Acorus* as sister to the rest of the monocot clade, with the two other aroids studied by him (*Arisaema* and *Symplocarpus*) either being sister to one another and then to the alismatids or forming a polytomy with the alismatids. Grayum (1987) and Rudall and Furness (1997) provided ample morphological evidence supporting the removal of *Acorus* from Araceae, and their observations have been corroborated by several molecular analyses (e.g., Duvall et al., 1993a, b; Chase et al., 1993, 1995a, b, 2000, 2006; this study).

In recent classifications, *Gymnostachys* has been included in Araceae either as a member of subfamily Pothoideae (Grayum, 1990) or as a subfamily on its own (Bogner and Nicolson, 1991; Mayo et al., 1997, 1998). Our study strongly supports *Gymnostachys* being sister to Orontioideae, in agreement with the plastid DNA restriction site analysis of French et al. (1995), the morphology-based analysis of Mayo et al. (1997), and a cladistic analysis of DNA sequences of plastid *trnL-trnF* by Tam et al. (2004). On morphological grounds, *Gymnostachys* differs greatly from Orontioideae, and from all other Araceae, in having linear, somewhat plicate leaves with serrate margins, undivided into blade and petiole, as well as an inconspicuous spathe and a monopodial synflorescence consisting of several floral sympodia borne on an upright scape (Mayo et al., 1997; Buzgo, 2001). However, Buzgo (2001) noted several structural and developmental similarities between *Gymnostachys* and both *Symplocarpus* and *Lysichiton* (Orontioideae), including perigone tetramery, unidirectional development of the inner whorls of tepals and stamens, and orthotropous, pendant ovules. Therefore, reproductive morphology mirrors our molecular data in pointing to a close relationship between *Gymnostachys* and Orontioideae. Tam et al. (2004) suggested combining *Gymnostachys* and the orontioids in a single subfamily, but in the view of most of us (except MWC), the unique habit and inflorescence morphology of *Gymnostachys* justifies its maintenance as a distinct subfamily (Bogner and Nicolson, 1991; Mayo et al., 1997, 1998; Buzgo, 2001).

Placements of *Orontium*, *Symplocarpus*, and *Lysichiton* have varied among the various aroid classifications. For instance, Engler (1876) placed all these genera in Symplocarpeae (= Orontieae) within subfamily Pothoideae but later (1920) transferred Symplocarpeae to subfamily Calloideae. Hutchinson (1973), largely based on previous proposals by Schott (1860) and Hooker (1883), accommodated *Orontium* and *Lysichiton* in tribe Orontieae but included *Symplocarpus* in tribe Dracontieae. Grayum (1990) considered all these genera as members of Lasioideae, placing *Symplocarpus* and *Lysichiton* in tribe Symplocarpeae but *Orontium* in tribe Orontieae. Bogner and Nicolson (1991) resurrected Engler's (1876) concept of Orontieae to include *Lysichiton*, *Orontium*, and *Symplocarpus* but assigned them to subfamily Lasioideae. Recently, Mayo et al. (1997) raised Orontieae sensu Bogner and Nicolson (1991) to subfamilial rank (as Orontioideae). This group is strongly supported in our analyses (Figs. 1, 2). All three genera consist of helophytes (*Orontium* often immersed), but there are no unambiguous morphological features that diagnose them as a group. Mayo et al. (1997, 1998) listed a set of characters as distinctive of this group: a nonlinear, expanded leaf blade, anatropous or

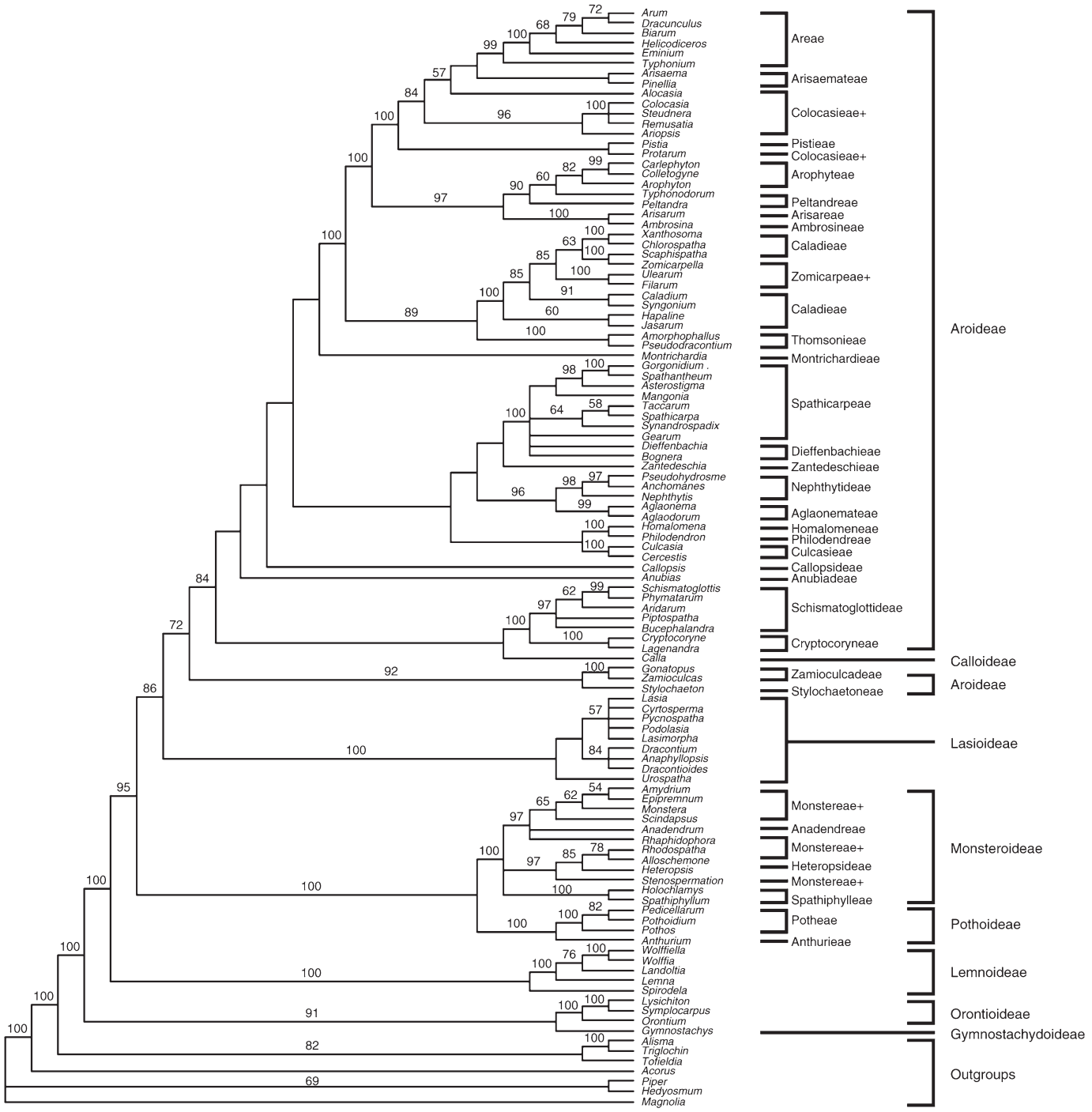
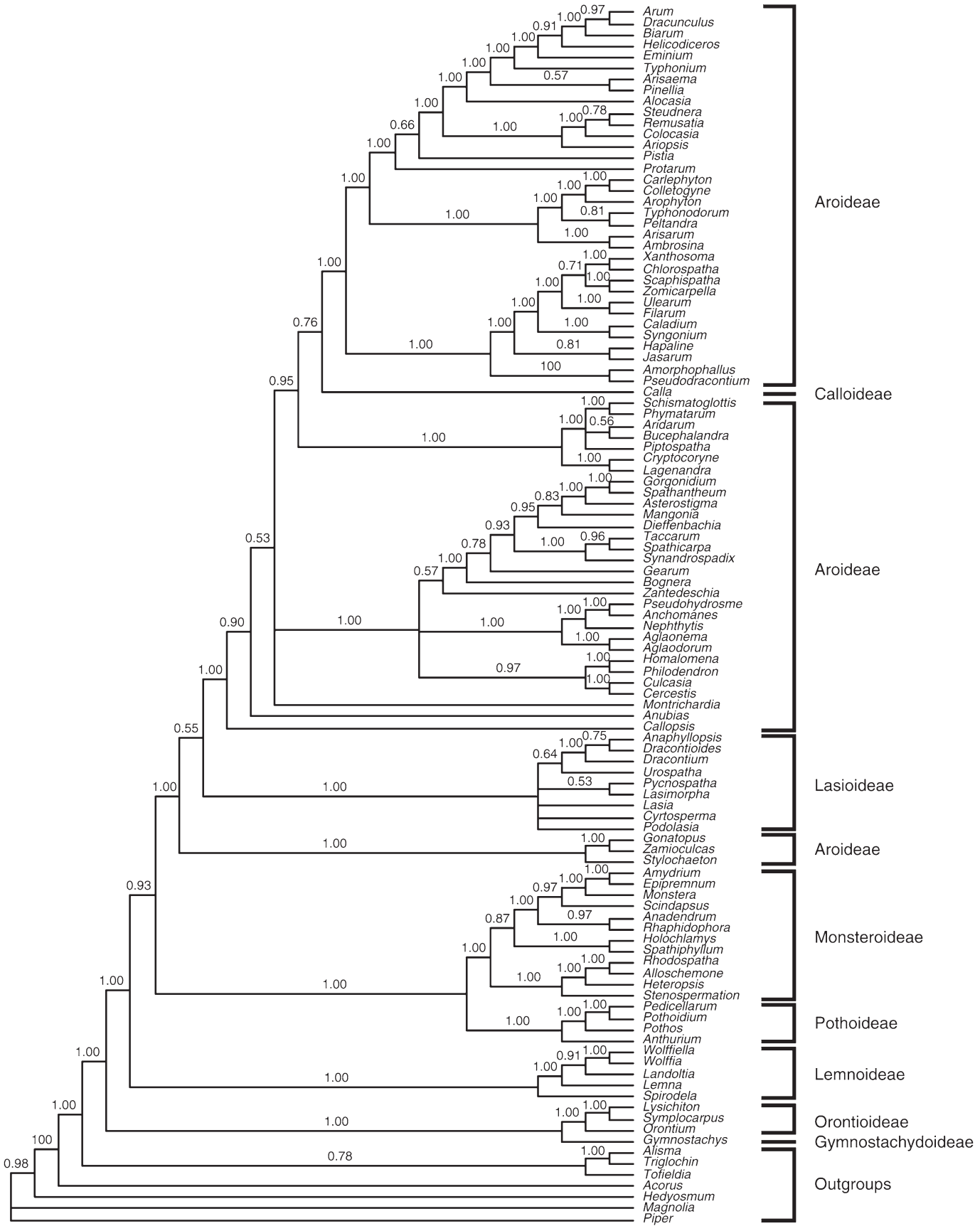


Fig. 1. Strict consensus of 1105 trees from the combined parsimony analysis (length = 7144 steps, CI = 0.50, RI = 0.71). Bootstrap percentages >50 are indicated above the branches. Bars indicate the subfamilies and tribes recognized by Mayo et al. (1997). + = nonmonophyletic tribes in the classification of Mayo et al. (1997).

hemianatropous ovules, and sparse or absent endosperm. However, an expanded leaf blade occurs throughout the aroids except in the highly modified Gymnostachydoideae and Lemnoideae and is also present in many alismatids. Buzgo (2001) found that the ovule in *Lysichiton* is orthotropous, whereas that of *Orontium* is hemianatropous, and in addition he cited differences in gynoecium development. Nevertheless, and in spite of the lack of clear structural diagnostic features, Orontioideae are consistently recovered as monophyletic by plastid DNA sequences, in agreement with their current subfamilial status.

Duckweeds (Lemnoideae)—The duckweeds have long been believed to be closely related to Araceae, but their precise relationships have remained unclear (e.g., Mayo et al. 1997 and


Arum
Dracunculus
Biarum
Helicodiceros
Eminium
Typhonium
Arisaema
Pinellia
Alocasia
Steudnera
Remusatia
Colocasia
Ariopsis
Pistia
Protarum
Carlephyton
Colletogyne
Arophyton
Typhonodorum
Peltandra
Arisarum
Ambrosina
Xanthosoma
Chlorospatha
Scaphispatha
Zomicarpella
Ulearum
Filarum
Caladium
Syngonium
Hapaline
Jasarum
Amorphophallus
Pseudodracontium
Aroideae
Calla
Calloideae
Schismatoglottis
Phymatarum
Aridarum
Bucephalandra
Piptospatha
Cryptocoryne
Lagenandra
Gorgonidium
Spathantheum
Asterostigma
Mangonia
Dieffenbachia
Taccarum
Spathicarpa
Synandrospadix
Gearum
Bognera
Zantedeschia
Pseudohydrosme
Anchomanes
Nephthytis
Aglaonema
Aglaodorum
Homalomena
Philodendron
Culcasia
Cercestis
Montrichardia
Aroideae
Anubias
Callopsis
Anaphyllopsis
Dracontioides
Dracontium
Urospatha
Pycnospatha
Lasimorpha
Lasia
Cyrtosperma
Podolasia
Lasioideae
Gonatopus
Zamioculcas
Stylochaeton
Aroideae
Amydrium
Epipremnum
Monstera
Scindapsus
Anadendrum
Rhaphidophora
Holochlamys
Spathiphyllum
Rhodospatha
Alloschemone
Heteropsis
Stenospermation
Monsteroideae
Pedicellarum
Pothoidium
Pothos
Anthurium
Pothoideae
Wolffiella
Wolffia
Landoltia
Lemna
Spirodela
Lemnoideae
Lysichiton
Symplocarpus
Orontium
Orontioideae
Gymnostachys
Gymnostachydoideae
Alisma
Triglochin
Tofieldia
Acorus
Hedyosmum
Magnolia
Piper
Outgroups

references therein). Recently published molecular phylogenetic studies have placed *Lemna* and its allies in Araceae, although the phylogenetic position of the former varied among these studies or was unresolved. As noted earlier, French et al.'s (1995) plastid DNA restriction site analysis of Araceae placed *Lemna* among representatives of Aroideae, but in the *trnL-trnF* analysis of Barabé et al. (2002) *Lemna* is sister to the "true Araceae" of Mayo et al. (1997). However, in the analysis of the *trnL-trnF* IGS by Rothwell et al. (2004), the duckweeds formed a polytomy with various groups of "true Araceae."

Our data place the duckweeds as the strongly supported sister group of the "true Araceae," in agreement with the findings of Barabé et al. (2002) and with palynological evidence (Hesse, 2006). Therefore, the inclusion of *Lemna* and its allies in Araceae, as suggested by several workers (e.g., Engler, 1876; Mayo et al., 1995b; Govaerts et al., 2002; Keating, 2002; Hesse, 2006; Bogner and Petersen, 2007), is fully justified from a phylogenetic standpoint. Maintaining Lemnaceae as a distinct family results in paraphyly of Araceae. Araceae sensu Mayo et al. (1997) could be split to maintain Lemnaceae, creating a new family for the "proto-aroids" or a new family each for *Gymnostachys* and the orontioids. In this way, Araceae would contain only the "true Araceae" of Mayo et al. (1997). However, we see no advantage in dismembering this natural group, with the ensuing loss of phylogenetic information and inflation of nomenclature.

Pothoideae and Monsteroideae—Both these subfamilies are strongly supported as monophyletic by our data, as is their sister-group relationship (Figs. 1, 2). Within Pothoideae, monogeneric tribe Anthurieae (*Anthurium*) is sister to Potheae. All three genera of Potheae (*Pothos*, *Pedicellarum*, and *Pothoidium*) possess main axes with monopodial growth (a feature only otherwise found among the aroids in the monsteroid genus *Heteropsis*) and flowering shoots always with axillary inflorescences. Potheae are restricted to Southeast Asia, Madagascar, and eastern Australia, whereas *Anthurium*, with sympodial growth, is restricted to the Neotropics (Mayo et al., 1997). In all these genera, the secondary and tertiary veins are reticulate, and the spathe does not enclose the spadix. Although Pothoideae have long been recognized as a subfamily (e.g., Engler, 1876, 1920; Grayum, 1990), some authors have included *Anthurium* in Lasioideae (Bogner and Nicolson, 1991).

Within Monsteroideae, tribe Monstereae is paraphyletic with monogeneric tribe Heteropsideae nested in a strongly supported clade consisting of *Stenospermation*, *Heteropsis*, *Alloschemone*, and *Rhodospatha* (all these belonging in Monstereae sensu Mayo et al., 1997). This result is in agreement with the analysis of Tam et al. (2004) based on sequences of *trnL-trnF*. *Anadendrum* (Anadendreae) is placed in another strongly supported clade in which it forms a trichotomy with *Rhaphidophora* and (*Scindapsus*-(*Monstera*-(*Epipremnum-Amydrium*))). Tribal monophyly would be achieved by expanding Heteropsideae to also include *Stenospermation*, *Alloschemone*, and *Rhodospatha*, as suggested by Tam et al. (2004). All these genera have a basic chromosome number $x = 14$ and are exclusively neotropical. On the other hand, Monstereae might be redefined to include *Anadendrum*, *Rhaphidophora*, *Scindapsus*, *Monstera*, *Epipremnum*, and *Amydrium*, all with a basic chromosome number of $x = 15$ and an Old World tropical distribution, except for neotropical *Monstera*. These two monsteroid clades form a trichotomy with Spathiphylleae, which consist of *Spathiphyllum* and *Holochlamys*. The last two genera share with Monstereae, as defined before, a basic chromosome number $x = 15$ (Mayo et al., 1997; Bogner and Petersen, 2007), which may be an indication of a closer relation of Spathiphylleae with Monsterae than with Heteropsideae.

Lasioideae—Bogner and Nicolson (1991) proposed tribe Lasieae to include 10 genera, and Mayo et al. (1997) recognized this group at subfamilial rank. We analyzed nine of the 10 genera accepted by Mayo et al. (1997), and these form a strongly supported group (BP 100, PP 1). Members of this group share some distinctive features such as no starch in the pollen grains, well-developed basal ribs of primary leaf veins, dracontioid leaf-margin development, basipetal flowering sequence, anthers dehiscing by oblique pore-like slits, and a basic chromosome number of $x = 13$. Relationships within this group are only weakly supported, except for a clade formed by the neotropical genera *Dracontium*, *Dracontioides*, and *Anaphyllopsis* (BP 84, PP 1.00). Another clade consists of the Old World tropical genera (*Lasimorpha*, *Podolasia*, *Pycnospatha*, *Cyrtosperma*, and *Lasia*), but this obtained only weak support (BP 57). On the other hand, *Urospatha* is placed by parsimony as sister to a clade encompassing the two aforementioned monophyletic groups, but in the Bayesian analysis it is sister to the *Dracontium* clade (Fig. 2).

Expanded Zamioculcadoideae, including Stylochaeton—Recently, Bogner and Hesse (2005) proposed a new subfamily, Zamioculcadoideae, to include the distinctive genera *Zamioculcas* and *Gonatopus*. These have been placed in recent classifications either in Pothoideae (Grayum, 1990), Lasioideae (Bogner and Nicolson, 1991), or Aroideae (Mayo et al., 1997, 1998). The new subfamily was diagnosed by the possession of a unique type of pinnatisect or trisect leaves, absence of laticifers and biforines, perigone with four free tepals, zona-aperturate pollen grains with a double tectum, and the capacity to propagate from fallen leaflets (Hesse et al., 2001; Bogner and Hesse, 2005). Bogner and Hesse (2005) also discussed the systematic position of *Stylochaeton*, the only other aroid genus with unisexual flowers that also has perigoniate flowers, but because it lacks the distinctive leaf and pollen features of *Zamioculcas* and *Gonatopus*, those authors concluded that it does not fit into their concept of Zamioculcadoideae. Grayum (1990) considered *Stylochaeton* an isolated and primitive genus without any clear link to Zamioculcadeae (including *Zamioculcas* and *Gonatopus*), placing it in subfamily Lasioideae. On the other hand, in the morphological cladogram of Araceae in Mayo et al. (1997), Zamioculcadeae and monotypic Stylochaetonae branch off successively at the basal nodes of Aroideae. In our study, *Stylochaeton* is strongly supported (BP 92, PP 1.00) as sister to *Zamioculcas-Gonatopus*. Recognizing Zamioculcadoideae but leaving out *Stylochaeton* breaks up this relationship and results in paraphyly of Aroideae, unless a further subfamily is created for *Stylochaeton*. We find this last option unappealing

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Fig. 2. Bayesian summary tree from analysis of all regions combined. Numbers above branches are posterior probabilities. Bars indicate subfamilies recognized by Mayo et al. (1997).

and argue here for the expansion of Zamioculcadoideae to include also *Stylochaeton*. Thus redelimited, Zamioculcadoideae consist of geophytic plants restricted to sub-Saharan Africa, lacking laticifers and having perigoniate, unisexual flowers.

Aroideae, including Calla (formerly Calloideae)—*Calla palustris*, the only member of subfamily Calloideae sensu Bogner and Nicolson (1991; Mayo et al., 1997, 1998), has been referred to as a highly autapomorphic taxon of obscure affinities (Mayo et al., 1997). Indeed, *Calla* is puzzling because of its peculiar combination of features, including bisexual flowers with some staminate flowers at the apex of the spadix (a condition reminiscent of *Orontium*, Orontioideae) and absence of a perigone—which seems to link it to Aroideae but is also typical of many Monsteroideae. In the cladogram of Mayo et al. (1997), *Calla* is the earliest member of the "true Araceae" to diverge, being sister to all the other Araceae except the protoaroids, but the plastid DNA restriction site analysis of French et al. (1995) placed *Calla* as sister to a clade matching subfamily Aroideae sensu Mayo et al. (1997). Our combined parsimony analysis (Fig. 1) places *Calla* within the clade that is sister to the rest of subfamily Aroideae (excluding *Stylochaeton*, *Zamioculcas*, and *Gonatopus*, here placed in an expanded subfamily Zamioculcadoideae; discussed before), with bootstrap support <50; its sister clade is strongly supported and composed of Cryptocoryneae/Schismatoglottideae. Our Bayesian analysis (Fig. 2) also places *Calla* within Aroideae but in a strongly supported clade (PP 0.95) containing Cryptocoryneae and Schismatoglottideae as collective sisters to a weakly supported (PP 0.76) group in which *Calla* is in turn sister to a clade containing various tribes of derived Aroideae (*Pseudodracontium* through *Arum* in Fig. 2; PP 1.00). It is worth noting that in both analyses, *Calla*, a helophyte, is located near tribes Cryptocoryneae and Schismatoglottideae, which consist predominantly of helophytic and rheophytic species. Given its embedded position within Aroideae, *Calla* must be included in that subfamily, and tribal status (as Calleae Schott) seems advisable in view of its peculiar morphology.

Aroideae as understood here (i.e., including *Calla* but excluding *Stylochaeton*, *Zamioculcas*, and *Gonatopus*) obtained strong support (BP 84, PP 1.00) and morphologically is distinguished by several features, such as the aperigoniate, unisexual flowers (except *Calla*) and laticifers (except *Pistia*). Both aperigoniate flowers and laticifers represent putative synapomophies of Aroideae, but unisexual flowers are symplesiomorphic (they are also present in Zamiculcadoideae). The clade including Schismatoglottideae-Cryptocoryneae (and *Calla*, in the combined parsimony analysis) is sister to the rest. Monogeneric Anubiadeae (*Anubias*) and Callopsideae (*Callopsis*) diverge successively. *Callopsis* is weakly supported as sister to the rest of Aroideae by parsimony (BP < 50), but strongly so by the Bayesian analysis (PP 1.00). In the Bayesian analysis, *Montrichardia* (Montrichardieae) formed a polytomy with two strongly supported clades of Aroideae, but in the parsimony strict consensus it was recovered as sister to one of them (BP < 50).

The remaining Aroideae form a weakly supported major clade that in turn contains two groups. The first of them includes, on the one hand, members of Culcasieae, Philodendreae, Homalomeneae, Aglaonemateae, and Nephthytideae, and on the other Zantedeschieae, Dieffenbachieae, and Spathicarpeae. Philodendreae (*Philodendron*) and Homalomeneae (*Homalomena*) form a strongly supported sister to Culcasieae (*Culcasia* and *Cercestis*). The last relationship is weakly supported by the bootstrap (BP < 50) but obtained a high posterior probability (PP 0.97). These three groups—Philodendreae, Homalomeneae, and Culcasieae—consist mainly of climbing hemiepiphytes that possess resin canals in roots, stems, and leaves and sclerotic hypodermis in the roots. Given their close relationship and great morphological similarity, these three tribes might be merged in a more inclusive concept of Philodendreae. Tribe Aglaonemateae, consisting of *Aglaonema* and *Aglaodorum*, are sister to Nephthytideae (*Nephthytis*, *Anchomanes*, and *Pseudohydrosme*). Both of these strongly supported tribes share adjacent male and female flower zones, free stamens, and collenchyma arranged in threads peripheral to the vascular strands of leaf blades and petioles (with the exception of *Nephthytis*, in which collenchyma can form interrupted bands; Keating, 2002). Aglaonemateae and Nephthytideae jointly are sisters to a clade in which *Zantedeschia* is weakly supported as sister to a strongly supported group containing all members of Dieffenbachieae and Spathicarpeae.

Our analyses failed to provide evidence for the monophyly of Dieffenbachieae and Spathicarpeae as classified by Mayo et al. (1997). Both the parsimony and the Bayesian analyses recovered two clades of genera of Spathicarpeae, namely *Synandrospadix*, *Spathicarpa*, and *Taccarum* (BP 64, PP 1.00) and *Mangonia*, *Asterostigma*, *Spathantheum*, and *Gorgonidium* (BP < 50, PP 0.83). In the parsimony consensus tree these two clades form a polytomy with *Bognera*, *Gearum*, and *Dieffenbachia*, which does not exclude the possibility of monophyly. However, in the Bayesian tree Dieffenbachieae are paraphyletic, with *Bognera* occupying an isolated position and *Dieffenbachia* embedded in Spathicarpeae as sister of the *Mangonia* clade (PP 0.95). Thus, all these closely related genera might best be merged in a single tribe pending further study, with Spathicarpeae having nomenclatural priority. These results are in agreement with a recently published phylogenetic analysis of the genera of Spathicarpeae and Dieffenbachieae by Gonçalves et al. (2007) based on plastid DNA sequences and morphology and a broader taxonomic sample, in which they expand Spathicarpeae to include also *Bognera* and *Dieffenbachia*. The group is nearly exclusively South American, with only *Dieffenbachia* spreading through Central America north to southeastern Mexico.

The last major clade of Aroideae recovered by our analyses is strongly supported (BP 100, PP 1.00) and consists of two groups. In the first group, tribe Thomsonieae (*Amorphophallus* and *Pseudodracontium*) are strongly supported as sister of Caladieae, in which paraphyletic Zomicarpeae are nested. Of the genera included in Zomicarpeae by Mayo et al. (1997), *Ulearum* and *Filarum* are sisters to each other, whereas *Zomicarpella* is strongly supported as sister to *Scaphispatha* (BP 100, PP 1.00) within a weakly supported clade of Caladieae that also includes *Chlorospatha* and *Xanthosoma*. Mayo et al. (1997) grouped tribes Caladieae and Zomicarpeae in their "*Caladium* alliance," and the mingling of their constituent genera in the molecular trees supports the inclusion of all of them in a more broadly defined Caladieae, as in Keating (2002). Thus redefined, Caladieae are supported by two putative morphological synapomorphies, i.e., anastomosing laticifers and at least a partial adnation of the female part of the spadix to the spathe (Mayo et al., 1997; Keating, 2002). Such adnation is more evident in the genera previously assigned to Zomicarpeae but only because of the smaller number of female flowers they produce. Except for Southeast Asian geophytes of the genus *Hapaline*,

the tribe is restricted to the neotropics. In our analyses, *Hapaline* is the weakly supported sister of *Jasarum*, a distinctive genus consisting of a single species that is a submerged aquatic in oligotrophic upland streams (Mayo et al., 1997).

The second group (BP 100, PP 1.00) encompasses a clade with monotypic tribes Ambrosinae (*Ambrosina*) and Arisareae (*Arisarum*) as collective sisters of another clade formed by Peltandreae and Arophyteae. Both *Ambrosina* and *Arisarum* have reticulate higher order venation, basal placentation, and copious endosperm. The two genera of Peltandreae, *Peltandra* and *Typhonodorum*, do not group with each other in the strict consensus of the parsimony analysis, but they form a clade in the Bayesian tree (PP 0.81). Both genera consist of helophytes inhabiting fresh and brackish water, but they have a bizarre disjunction, with *Peltandra* restricted to eastern North America and *Typhonodorum* found in the Comores, Madagascar, Mauritius, and some islands off the coast of Tanzania (Mayo et al., 1997). The Madagascan endemic tribe Arophyteae obtained moderate and strong support in the parsimony and Bayesian analyses, respectively. The clade formed by Ambrosinae, Arisareae, and Peltandreae is sister to the "*Pistia* clade" as defined by of Renner and Zhang (2004), which consists of genera placed by Mayo et al. (1997) in tribes Pistieae, Colocasieae, Arisaemateae, and Areae.

Our results do not support monophyly of Colocasieae, in which a clade formed by Arisaemateae (*Arisaema*, *Pinellia*) and Areae (*Typhonium*, *Eminium*, *Helicodiceros*, *Biarum*, *Dracunculus*, and *Arum*) is nested. *Alocasia* is weakly supported as sister to the Arisaemateae/Areae clade in the parsimony analysis (BP 57) but strongly so in the Bayesian analysis (PP 1.00). The recently published study of the "*Pistia* clade" by Renner and Zhang (2004), based on noncoding plastid and mitochondrial DNA, recovered Colocasieae as paraphyletic to Arisaemateae and Areae, but in their tree the species of *Colocasia* sampled (*C. gigantea*) grouped with two species of *Alocasia* as collective sisters of the Arisaemateae-Areae clade. In our Bayesian tree, *Protarum* (Colocasieae sensu Mayo et al., 1997) is sister to a weakly supported clade (PP 0.66) consisting of *Pistia* (Pistieae) as sister of the remaining Colocasieae plus Arisaemateae and Areae, but in the parsimony strict consensus tree *Protarum* and *Pistia* are sister taxa with BP < 50 (Figs. 1, 2). In the study of Renner and Zhang (2004: Fig. 3), *Protarum* and *Pistia* form a polytomy with a clade that includes other Colocasieae, Areae, and Arisemateae. These results suggest the need to revise the current tribal limits of Colocasieae. Distinctive *Protarum* should be removed from Colocasieae to monotypic Protareae, as proposed by Engler (1920), but resolution of relationships among *Colocasia*, *Alocasia*, and the other Colocasieae requires further study, including more thorough sampling.

Arisaemateae, consisting of *Pinellia* and *Arisaema*, obtained only BP < 50 and PP 0.57, and there are no obvious morphological features permitting diagnosis. On the other hand, *Typhonium*, *Eminium*, *Helicodiceros*, *Biarum*, *Dracunculus*, and *Arum* all belong in the strongly supported (BP 99, PP 1.00) tribe Areae of Mayo et al. (1997). This tribe is predominantly Mediterranean and Asian (with *Typhonium* reaching Australia) and includes plants from temperate, seasonally dry, cold environments.

Evolution of the aquatic habit in Araceae—With the exception of the highly autapomorphic *Gymnostachys*, all of the small clades diverging at the basalmost nodes consist of aquatic plants: Orontioideae are helophytes, and Lemnoideae include only free-floating aquatics. Several additional, independent invasions of the aquatic habitat have occurred in subfamilies Lasioideae (nearly all genera) and Aroideae (mainly *Calla*, Cryptocoryneae, Schismatoglottideae, Peltandreae, *Anubias*, *Montrichardia*, *Jasarum*, and *Pistia*). All these are secondarily aquatic in a morphologically canalized aroid clade and have the characteristic aroid features. However, both orontioids and duckweeds are much simpler and diverged from the main aroid line before morphology became canalized in the manner now considered to be the hallmark of the family. Thus, the early evolution of the aroid lineage, estimated to have occurred more than 120 million years ago as indicated by early Cretaceous pollen assigned to tribe Spathyphylleae (Friis et al., 2004), appears to have involved "experimentation" with a wide range of habits and inflorescence architectures, which were later canalized in the main aroid clade into the typical highly stereotyped syndrome of spathe and spadix. This group then underwent a major radiation in the tropics, more recently evolving again into tropical aquatic habitats (e.g., *Pistia*, *Cryptocoryne*) and temperate environments (Areae). Both protoaroids and duckweeds, lacking the canalized development typical of the family, have not undergone a comparable radiation of taxa. This same pattern—a few, relatively species-poor, morphologically atypical lineages diverging prior to a major morphological canalization—is found in most other large plant families as well, including orchids, grasses, sedges, legumes, and composites (cf. Chase, 2004). Many of these atypical lineages have also been proposed as separate families, as in the case of the duckweeds.

Several earlier workers have pointed to the morphological and ecological similarities between the duckweeds and *Pistia* as evidence of a close relationship (reviewed in Landolt, 1986, 1998; Mayo et al., 1995b, 1997; Stockey et al., 1997; Lemon and Posluszny, 2000b; Les et al., 2002), but both DNA sequence data (Barabé et al., 2002; Rothwell et al., 2004; this paper) and structural studies (Mayo et al., 1997 and references therein; Hesse, 2006) have shown that these two groups are only distantly related. The duckweeds are an early offshoot of the aroid lineage and are represented in the fossil record since the late Cretaceous by the genus *Limnobiophyllum* (Kvaček, 1995; Stockey et al., 1997), whereas the oldest fossils attributable to *Pistia* date back only to late Oligocene/early Miocene (Renner and Zhang, 2004; Wilde et al., 2005). Their similarities are best interpreted as independent evolutionary acquisitions resulting from adaptation to the aquatic environment (Landolt, 1986, 1998; Rothwell et al., 2004; cf. Grace, 1993). However, the notable likeness of *Pistia* in habit and development to the duckweeds (Lemon and Posluszny, 2000a, b) makes *Pistia* a useful living model of the sort of modifications that may have led to the extreme reduction and specialization of the duckweeds.

Concluding remarks—This is the first molecular phylogenetic study that includes a nearly complete generic sample of aroids and all extant genera of duckweeds, as well as representatives of other alismatids and nonmonocot outgroups, to assess monophyly and relationships of the major lineages of Araceae. The position of the duckweeds (subfamily Lemnoideae) as sister of all other Araceae except the "proto-aroids" is strongly supported. The delimitation of most subfamilies, tribes, and informal alliances proposed by Mayo et al. (1997) is consistent with the phylogenetic trees, with the notable exception of Aroideae, which is paraphyletic unless *Calla*, placed in its own subfamily (Calloideae), is included. The recently proposed subfamily Zamioculcadoideae is supported by our data but should also

include *Stylochaeton* because retaining it in Aroideae, as proposed by Bogner and Hesse (2005), renders the latter nonmonophyletic. The following eight subfamilies might then be recognized in Araceae (in ascending order of their branching in the phylogenetic trees): Gymnostachydoideae, Orontioideae, Lemnoideae, Pothoideae, Monsteroideae, Lasioideae, Zamioculcadoideae, and Aroideae. Our phylogenetic hypotheses are derived from DNA sequence data from a single compartment of the plant genome (plastid DNA), and it would be desirable to compare them with results from other sources of evidence, including, for instance, nuclear markers and structural characters. We hope that the ideas presented here stimulate further research focused on producing a better understanding of the evolution of this highly diverse group of monocots.

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APPENDIX 1. Voucher information and GenBank accession numbers for taxa used in this study. Herbarium acronyms or botanical gardens: Bot. Gard. Osaka City Univ. = Botanical Garden Osaka City University, Osaka, Japan; CHRB = Rutgers University-Cook College, New Brunswick, New Jersey, USA; CONN = University of Connecticut, Storrs, Connecticut, USA; K = Royal Botanic Gardens, Kew, UK; L = Nationaal Herbarium Nederland, Leiden University branch, Leiden, Netherlands; M = Botanische Staatssammlung München, Munich, Germany; MEXU = Herbario Nacional, Universidad Nacional Autónoma de México, Mexico City, Distrito Federal, Mexico; MO = Missouri Botanical Garden, Saint Louis, Missouri, USA; MT = Université de Montréal, Montréal, Québec, Canada; NCU = University of North Carolina, Chapel Hill, North Carolina, USA; SING = Singapore Botanic Gardens, Singapore, Singapore; UPS = Uppsala University, Uppsala, Sweden; — = not available.

FAMILY. ***Species***, *Voucher* (Herbarium), GenBank accessions (+ = sequenced as two separate fragments): *rbcL*, *matK-trnK*, *trnL-F*.

ACORACEAE. ***Acorus calamus*** L., *French 232* (CHRB), M91625, —, —; ***A. calamus*** L., *Tamura & Yamshita 6008* (Bot. Gard. Osaka City Univ.), —, AB040154, —; ***A. calamus*** L., *Joly 226* (MT), —, —, AY054741.

ALISMATACEAE. ***Alisma canaliculatum*** A.Braun & C.D.Bouché, *Tamura & Fuse 10018* (Bot. Gard. Osaka City Univ.), —, AB040179, —; ***A. plantago-aquatica*** L., *Les s.n.* (CONN), L08759, —, —; ***A. plantago aquatica*** L, *Chase 11275* (K), —, —, AM932372 + AM933368,

ARACEAE. ***Aglaodorum griffithii*** (Schott) Schott, *Bogner 1767* (M), AM905758, AM920580, AM932318 + AM933314. ***Aglaonema modestum*** Schott ex Engl., *Chase 10671* (K), AM905757, AM920579, —; ***A. modestum*** Schott ex Engl., *Barabé & Chantha 86* (MT), —, —, AY054700. ***Alloschemone occidentalis*** (Poepp.) Engl. & K.Krause, *Chase 9996* (K), AM905744, AM920566, AM932310 + AM933306. ***Alocasia odora*** (Roxb.) K.Koch., *Chase 10674* (K), AM905802, AM920624, —; ***A. odora*** (Roxb.) K.Koch., *Barabé & Chantha 93* (MT), —, —, AY054705. ***Ambrosina basii*** L., *Chase 12339* (K), AM905798, AM920620, AM932348 + AM933344. ***Amorphophallus hottae*** Bogner & Hett., *Lam s.n.* (L), AM905785, AM920607, —; ***A. paeoniifolius*** (Dennst.) Nicolson, *Barabé & Chantha 98* (MT), —, —, AY054703. ***Amydrium humile*** Schott, *Chase 9974* (K), AM905745, AM920567, —; ***A. zippelianum*** (Schott) Nicolson, *Barabé & Chantha 99* (MT), —, —, AY054735. ***Anadendrum* sp.**, *Chase 9985* (K), AM905740, AM920547, AM932308 + AM933304. ***Anaphyllopsis americana*** (Engl.) A.Hay, *Chase 11914* (K), AM905753, AM920575, —; ***A. americana*** (Engl.) A.Hay, *Barabé 83* (MT), —, —, AY054726. ***Anchomanes difformis*** (Blume) Engl., *Chase 10687* (K), AM905761, AM920583, —; ***A. difformis*** (Blume) Engl., *Barabé 155* (MT), —, —, AY054711. ***Anthurium acaule*** (Jacq.) Shott, *Chase10884* (K), AM905735, AM920557, —; ***A. jenmanii*** Engl., *Barabé & Chantha 92* (MT), —, —, AY054730. ***Anubias barteri*** Schott, *Chase 10997* (K), AM905756, AM920578, —; ***A. barteri*** Schott, *Barabé & Chantha 90* (MT), —, —, AY054710. ***Aridarum nicholsonii*** Bogner, *Bogner 2835* (M), AM905784, AM920606, AM932337 + AM933334. ***Ariopsis peltata*** J.Grah., *Chase 11913* (K), AM905804, AM920626, AM932352 + AM933348. ***Arisaema franchetianum*** Engl., *Chase 10478* (K), AM905806, AM920628, AM932354 + AM933350. ***Arisarum vulgare*** O.Targ-Tozz, *Chase 10992* (K), AM905797, AM920619, AM932347 + AM933343. ***Arophyton buchetii*** Bogner, *Bogner 207* (M), AM905820, AM920642, AM932367 + AF521870. ***Arum hygrophilum*** Boiss., *Chase 10990* (K), AM905809, AM920631, AM932296 + AM933353. ***Asterostigma pavonii*** Schott, *Sizemore 95-062B* (L), AM905768, AM920590, AM932325 + AM933321.

Biarum tenuifolium (L.) Schott, *Chase 282* (K), AM905810, AM920632, AM932357 + AM933354. ***Bognera recondita*** (Madison) Mayo & Nicolson, *Bogner 1995* (M), AM905765, AM920587, AM932322 + AM933318. ***Bucephalandra motleyana*** Schott, *Tomey s.n.* (M), AM905822, AM920644, AM932369 + AM933365.

Caladium bicolor (Aiton) Vent., *Barabé & Chantha 96* (MT), —, —, AY054708; ***C. lindenii*** (André) Madison, *Chase 10670* (K), AM905788, AM920610, —. ***Calla palustris*** L., *Chase 11802* (K), AM905819, AM920641, AM932366 + AM933363. ***Callopsis volkensii*** Engl., *Chase 10668* (K), AM905773, AM920595, AM932330 + AM933325. ***Carlephyton glaucophyllum*** Bogner, *Mangelsdorff 124* (M), AM905821, AM920643, AM932368 + AM933364. ***Cercestis mirabilis*** (N.E.Br.) Bogner, *Chase 11772* (K), AM905817, AM920639, AM932364 + AM933361. ***Chlorospatha* sp.**, *Chase 11912* (K), AM905791, AM920613, AM932341 + AM933339. ***Colletogyne perrieri*** Buchet, *Pronk s.n.* (M), AM905823, AM920645, AM932370 + AM933366. ***Colocasia esculenta*** (L.) Schott, *Chase 10669* (K), AM905800, AM920622, AM932349 + AM933345. ***Cryptocoryne lingua*** Becc. ex Engl., *Chase 10998* (K), AM905779, AM920601, — + AM933329. ***Culcasia liberica*** N.E.Br., *Chase 11777* (K), AM905816, AM920638, AM932363 + AM933360. ***Cyrtosperma macrotum*** Engl., *Chase 11771* (K), AM905750, AM920572, AM932313 + AM933309.

Dieffenbachia aglaonemifolia Engl., *Chase 10678* (K), AM905764, AM920586, —; ***D. pittieri*** Engl. & K.Krause, *Barabé & Chantha 88* (MT), —, —, AY054714. ***Dracontium polyphyllum*** L., *Chase 10688* (K), AM905747, AM920569, —; ***D. polyphyllum*** L., *Barabé 50* (MT), —, —, AY054727. ***Dracontioides desciscens*** Engl., *Chase 11916* (K), AM905754, AM920576, AM932316 + AM933312. ***Dracunulus vulgaris*** Schott, *Chase 11760* (K), AM905812, AM920634, AM932359 + AM933356.

Epipremnum falcifolium Engl., *Barabé & Turcotte 100* (MT), —, —, AY054732; ***E. pinnatum*** (L.) Engl., *Chase 9977* (K), AM905746,

AM920568, —. ***Eminium spiculatum*** (Blume) Kuntze, *Chase 11806* (K), AM905813, AM920635, AM932360 + AM933357.

Filarum manserichense Nicolson, *Sizemore 1996-001* (L), AM905795, AM920617, AM932345 + AM933342.

Gearum brasiliense N.E.Br., *Chase 10693* (K), AM905763, AM920585, AM932321 + AM933317. ***Gonatopus angustus*** N.E.Br., *Chase 10675* (K), AM905777, AM920599, AM932333 + AM933328. ***Gorgonidium* sp.**, *Cultivated* (L), AM905767, AM920589, AM932324 + AM933320. ***Gymnostachys anceps*** R.Brown, *Chase 9473* (K), AM905727, AM920548, AM932297 + AM933293.

Hapaline benthamiana Schott, *Chase 10676* (K), AM905787, AM920609, AM932339 + AM933336. ***Helicodiceros muscivorus*** (L. f.) Engl., *Chase 11759* (K), AM905811, AM920633, AM932358 + AM933355. ***Heteropsis oblongifolia*** Kunth, *Ramírez 11848* (L), AM905737, AM920560, —; ***Heteropsis* sp.**, *Barabé, Forest & Gibernau 147* (MT), —, —, AY054739. ***Homalomena magna*** A. Hay, *Chase 10691* (K), AM905774, AM920596, —; ***Homalomena* sp.**, *Barabé 151* (MT), —, —, AY054724. ***Holochlamys beccarii*** (Engl.) Engl., *Chase 10677* (K), AM905736, AM920558, AM932306 + AM933302.

Jasarum steyermarkii G.S.Buting, *Berry 5531* (MO), AM905792, AM920614, AM932342 + AM933339.

Lagenandra ovata Thwaites, *Chase 10991* (K), AM905780, AM920602, — + AM933330. ***Landoltia punctata*** (G. Mey) Les & D.J.Crawford, *Landolt 7248* (—), AY034223, AY034185 + AY034301, —; ***L. punctata*** (G. Mey) Les & D.J.Crawford, *Chase 14451* (K), —, —, AM932301 + AM933297. ***Lasia spinosa*** (L.) Thwaites, *Chase 11779* (K), AM905749, AM920571, AM932312 + AM933308. ***Lasimorpha senegalensis*** Shott, *Bogner s.n.* (M), AM905755, AM920577, AM932317 + AM933313. ***Lemna minor*** L., *Chase 11761* (K), AM905730, AM920552, AM932299 + AM933295. ***Lysichiton americanus*** Hultén & H.St.John, *Chase 11748* (K), AM905728, AM920549, —; ***L. camtschatcense*** (L.) Schoott, *Barabé 153* (MT), —, —, AY054740.

Mangonia tweediana Schott, *Bogner 2376* (L), AM905766, AM920588, AM932323 + AM933319. ***Monstera adansonii*** Schott, *Chase 9980* (K), AM905743, AM920565, —; ***M. adansonii*** Schott, *Barabé & Chantha 94* (MT), —, —, AY054734. ***Montrichardia arborescens*** (L.), Schott, *Cultivated* (SING), AM905818, AM920640, AM932365 + AM933362.

Nephthytis afzellii Schott, *Chase10689* (K), AM905759, AM920581, —; ***N. afzellii*** Schott, *Barabé & Chantha 95* (MT), —, —, AY054702.

Orontium aquaticum L., *Qui 97112* (NCU), AM905729, AM920550, AM932298 + AM933294.

Pedicellarum paiei M.Hotta, *Bogner 2196* (M), AM905733, AM920555, AM932304 + AM933300. ***Peltandra virginica*** (L.) Raf., *Chase 11770* (K), AM905815, AM920637, AM932362 + AM933359. ***Philodendron deltoideum*** Poepp. & Endl., *Chase 10891* (K), AM905775, AM920597, AM932331 + AM933326. ***Phymatarum borneense*** M.Hotta, *Chase 10979* (K), AM905783, AM920605, AM932336 + AM933333. ***Pinellia pedatisecta*** Schott, *Chase 11752* (K), AM905807, AM920629, AM932355 + AM933351. ***Piptospatha ridleyi*** N.E.Br., *Chase 10680* (K), AM905781, AM920603, AM932334 + AM933331. ***Pistia stratiotes*** L., *Chase 10996* (K), AM905799, AM920621, —; ***P. stratiotes*** L., *Barabé 153* (MT), —, —, AY054706. ***Podolasia stipitata*** N.E.Br., *Chase 11915* (K), AM905752, AM920574, AM932315 + AM933311. ***Pothoidium lobbianum*** Schott, *Bogner 1272* (M), AM905734, AM920556, AM932305 + AM933301. ***Pothos scandens*** D.Don, *Chase 9989* (K), AM905732, AM920554, —; ***P. scandens*** D.Don, *Barabé & Lavoie 157*, —, —, AY054731. ***Protarum sechellarum*** Engl., *Bogner s.n.* (M), AM905805, AM920627, AM932353 + AM933349. ***Pseudodracontium lacourii*** (Linden & Andre) N.E.Br., *Chase 10681* (K), AM905786, AM920608, AM932338 + AM933335. ***Pseudohydrosme gabunensis*** Engl., *Wieringa 3308* (L), AM905760, AM920582, AM932319 + AM933315. ***Pycnospatha arietina*** Thorel ex Gagnep., *Sizemore s n.* (L), AM905751, AM920573, AM932314 + AM933310.

Raphidophora africana N.E.Br., *Barabé & Turcotte 110* (MT), —, —, AY054736; ***R. crassifolia*** Aldewer., *Chase 7398* (K), AM905741, AM920563, —. ***Remusatia vivipara*** (Roxb.) Schott, *Chase 11775* (K), AM905803, AM920625, AM932351 + AM933347. ***Rhodospatha oblongata*** Poepp. & Endl., *Croat 53888* (MO), AM905739, AM920562, AM932307 + AM933303.

Scaphispatha gracilis Brongn. ex Shott, Gonçalves 172 (MO), AM905793, AM920615, AM932343 + AM933340. ***Schismatoglottis trifasiata*** Engl., *Chase 10692* (K), AM905782, AM920604, AM932335 + AM933332. ***Scindapsus heredaceus*** Schott, *Chase 9986* (K), AM905742, AM920564, AM932309 + AM933305. ***Spathantheum intermediaum*** Bogner, *Chase 11776* (K), AM905769, AM920591, AM932326 + AM933322. ***Spathicarpa hastifolia*** Hook, *Chase 10995* (K), AM905772, AM920594, AM932329 + AM933324. ***Spathiphyllum wallisii*** Hort, *Chase 210* (NCU), AJ235807, AM920559, —; ***S. wallisii*** Hort, *Barabé & Turcotte 105* (MT), —, —, AY054738. ***Spirodela polirhiza*** (L.) Schleid., *Chase 11096* (K), AM905731, AM920553, AM932300 + AM933296. ***Stenospermation popayanense*** Schott, *Barabé & Lavoie 159* (MT), —, —, AY054737; ***S. ulei*** K.Krause, *Chase 9987* (K), AM905738, AM920561, —. ***Steudnera colocasiifolia*** K.Koch, *Chase 10682* (K), AM905801, AM920623, AM932350 + AM933346. ***Stylochaeton bogneri*** Mayo, *Chase 10685* (K), AM905776, AM920598, AM932332 + AM933327. ***Symplocarpus foetidus*** (L.) Nuttall, *French 219* (CHRB), L10247, —, —; ***S. foetidus*** (L.) Nuttall, *Chase 11749* (K), —, AM920551, —; ***S. foetidus*** (L.) Nuttall, *Barabé 154* (MT), —, —, AY054741. ***Synandrospadix vermitoxicus*** (Griseb.) Engl., *Chase 11774* (K), AM905771, AM920593, AM932328 + AM933292. ***Syngonium auritum*** (L.) Schott, *Chase 10994* (K), AM905789, AM920611, AM932340 + AM933337.

Taccarum weddelianum Brongn. ex Schott, *Hennipman 8315* (L), AM905770, AM920592, AM932327 + AM933323. ***Thyphonium blumei*** Nicolson & Sivad., *Chase 10694* (K), AM905808, AM920630, —; ***T. giganteum*** Engl., *Chase 11803* (K), —, —, AM932356 + AM933352. ***Thyphonodorum lindleyanum*** Schott, *Chase 11780* (K), AM905814, AM920636, AM932361 + AM933358.

Ulearum sagittatum Engl., *Chase 10695* (K), AM905794, AM920616, AM932344 + AM933341. ***Urospatha saggitifolia*** (Rudge) Schott, *Chase 11773* (K), AM905748, AM920570, AM932311 + AM933307.

Wolffia columbiana H.Karts, *Landolt 7467* (—), AY034255, AY034217 + AY034333, —; ***W. columbiana*** H.Karts, *Chase 14447* (K), —, —, AM932303 + AM933299. ***Wolffiella oblonga*** Hegelm., *Landolt 8984* (—), AY034242, AY034204 + AY034320, —; ***W. oblonga*** Hegelm., *Chase 14359* (K), —, —, AM932302 + AM933298.

Xanthosoma helleborifolium (Jacq.) Schott, *Chase 10683* (K), AM905790, AM920612, —; ***Xanthosoma* sp.**, *Barabé & Turcotte 107* (MT), —, —, AY054709.

Zamioculcas zamiifolia (Lodd.) Engl., *Chase 10686* (K), AM905778, AM920600, —; ***Z. zamiifolia*** (Lodd.) Engl., *Barabé & Chantha 84* (MT), —, —, AY054725. ***Zanthedeschia albomaculata*** (Hook. f.) Bail, *Chase 11758* (K), AM905762, AM920584, AM932320 + AM933316. ***Zomicarpella amazonica*** Bogner, *Bogner 1985* (M), AM905796, AM920618, AM932346 + —.

CHLORANTHACEAE. ***Hedyosmum mexicanum*** C.Cordem., *Salazar s.n.* (MEXU), AM905824, AM920646, AM932371 + AM933367.

JUNCAGINACEAE. ***Triglochin maritima*** L., *Les s.n.* (CONN), U80714, —, —; ***T. maritima*** L, *Chase 8279* (K), —, AM920647, AM932373 + AM933369.

MAGNOLIACEAE. ***Magnolia macrophylla*** Michx., BG 790346 (MO), —, —, AF040680; ***M. pseudokobus*** Abe & Akasawa, *Tamura 10015* (Bot. Gard. Osaka City Univ.), —, AB040152, —; ***M. umbrella*** L., — (—), AF206791, —, —.

PIPERACEAE. ***Piper mullesua*** Buch.-Ham., — *s.n.* (—),—,—, AY032651; ***P. nigrum*** L., *Tamura & Fuse s.n.* (Bot. Gard. Osaka City Univ.), —, AB040153, —; ***P. betle*** L., *Qiu 91048* (NCU), L12660, —, —.

TOFIELDIACEAE. ***Tofieldia pusilla*** Pers., *Lundqvist 12935* (UPS), AJ286562, —, —; ***T. pusilla*** Pers., *Chase 1851* (K), —, AM920648, AM932374 + AM933370