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Relationships within the Araceae: comparison of morphological patterns with molecular phylogenies[1]

Natalie Cusimano[2,11], Josef Bogner[3], Simon J. Mayo[4], Peter C. Boyce[5], Sin Y. Wong[6], Michael Hesse[7], Wilbert L. A. Hetterscheid[8], Richard C. Keating[9], and Jim C. French[10]

[2]Department of Biology, LMU Munich, D-80638 Munich, Germany; [3]Augsburger Str. 43a, D-86368, Gersthofen, Germany; [4]Royal Botanic Gardens Kew, Richmond, Surrey, TW9 3AE, U.K.; [5]BRT Research Associate, Forest Herbarium (BKF), The Office of Forest and Plant Conservation Research, National Park, Wildlife and Plant Conservation Department, 61 Phahonyothin Road, Chatuchak, Bangkok 10900, Thailand; [6]Department of Plant Science and Environmental Ecology, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak 94300 Samarahan, Sarawak, Malaysia; [7]Department of Structural and Functional Botany, University of Vienna, 1030 Vienna, Austria; [8]Von Gimborn Arboretum, Velperengh 13, 3941 BZ Doorn, Netherlands; [9]Missouri Botanical Garden, P.O. Box 299, St. Louis, Missouri 63166-0299, USA; and [10]188 San Jose Court, San Luis Obispo, California 93405, USA

- *Premise of the study:* The first family-wide molecular phylogeny of the Araceae, a family of about 3800 published species in 120 genera, became available in 1995, followed by a cladistic analysis of morpho-anatomical data in 1997. The most recent and comprehensive family-wide molecular phylogeny was published in 2008 and included species from 102 genera. We reanalyzed the molecular data with a more complete genus sampling and compared the resulting phylogeny with morphological and anatomical data, with a view to contributing to a new formal classification of the Araceae.
- *Methods:* We analyzed 113 aroid genera and 4494 aligned nucleotides that resulted from adding 11 genera to the 2008 molecular matrix. We also analyzed 81 morphological characters in the context of the molecular phylogeny, using an extended version of the 1997 morpho-anatomical data set.
- *Key results:* The resulting maximum-likelihood phylogeny is well resolved and supported, and most of the 44 larger clades also have morphological or anatomical synapomorphies as well as ecological or geographic cohesion. Of the 44 clades, 16 are here newly circumscribed and informally named. However, some relationships remain poorly supported within the Aroideae subfamily. The most problematic placement is *Calla* within Aroideae, which conflicts with the distribution of morphological, anatomical, and palynological character states.
- *Conclusions:* The comparison of the molecular analysis with morphological and anatomical data presented here represents an important basis for a new formal classification for the Araceae and for the understanding of the evolution of this ancient family, a monocot group known in the fossil record from the early Cretaceous.

Key words: Araceae; *Calla*; character evolution; classification; Lemnoideae; molecular phylogeny; phenotypic characterization.

In recent years, the phylogeny and evolution of the Monocots have come under intense scrutiny with the rapid development of molecular phylogenetic systematics (e.g., Barfod et al., 2010) and these studies have highlighted the position of the Araceae as an early-diverging Monocot clade, within which the duckweeds (Araceae, Lemnoideae) have evolved (Cabrera et al., 2008). During the same period, exciting new fossil discoveries have been made in the Araceae (e.g., Friis et al., 2004). These have pushed back the history of the family to the early Cretaceous and justify an increased focus on the study of phylogeny and character evolution in this family.

Since the landmark study of French et al. (1995), phylogenies based on molecular data have been the primary basis for interpreting patterns of relationships in the Araceae at the suprageneric level (Barabé et al., 2002; Renner and Zhang, 2004; Renner et al., 2004; Rothwell et al., 2004; Tam et al., 2004; Gonçalves et al., 2007; Cabrera et al., 2008, Gauthier et al., 2008; Wong et al., 2010). The most comprehensive molecular analysis to date has been provided by Cabrera et al. (2008), a study that effectively settled the long-standing question of the relationships of the duckweeds (the former Lemnaceae, now Araceae subfamily Lemnoideae), using a matrix of 102 aroid genera (including the duckweeds) and 5188 aligned base pairs of chloroplast DNA. In attempting to transform such phylogenies into a formal classification, it is desirable to compare them with phenotypic data sets so as to highlight clades that are supported by distinctive morphological or anatomical synapomorphies and those that are not, but are nevertheless well supported by molecular synapomorphies. Keating (2002), for example, was able to interpret his vegetative anatomical data using the phylogeny of French et al. (1995), leading him to propose a new formal classification of the family. Bogner and Petersen (2007) presented an updated version of the classification of Mayo et al. (1997), which itself emerged as a result of comparison of morpho-anatomical data with French et al.'s (1995) molecular tree.

The availability of the morpho-anatomical data set of Mayo et al. (1997), molecular sequence data of Cabrera et al. (2008), and restriction-site data of French et al. (1995) prompted us to

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[11] Author for correspondence (e-mail: cusimano@bio.lmu.de)

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carry out a new study with a view to contributing to a new formal classification of the Araceae. Here, we report the results of analyses of these three data sets (augmented versions in the case of the morpho-anatomical and sequence data). Both separate and combined analyses (total evidence methods) were undertaken during this study, but in light of the results, we concluded that the most useful approach for the purposes of a future formal classification would be to trace the morpho-anatomical characters onto the molecular sequence phylogeny. Our aim is to highlight well-supported clades with distinct phenotypic characterization and show those areas of conflict that require further investigation.

The main part of the discussion thus concentrates on 44 robust molecular clades, most of which are also supported by morphological synapomorphies, reinforcing proposals previously made by Cabrera et al. (2008) for modifying the family classification. Sixteen of these clades are newly presented here, but they are currently considered informal taxonomic groups. All early-diverging subfamilies and the relationships between them are well supported. The major subclades within Aroideae are each well supported, but the relationships between them are still not resolved. The only significant case in which molecular and morphological data seem to contradict one another is the location of *Calla* within the Aroideae.

BACKGROUND

The most detailed modern taxonomic study of the genera of the Araceae is the monograph by Mayo et al. (1997; *The Genera of Araceae*, hereafter GoA), which, although it treats the morphology of most of the currently recognized genera, did not include the duckweeds, then still regarded as a separate family. To provide the framework for the GoA classification, Mayo and colleagues carried out a maximum-parsimony (MP) cladistic analysis, using a matrix of 63 morphological and anatomical characters for 107 genera (including three outgroups), assembled from the literature and by examination of living, spirit, and herbarium materials. This cladistic study (the first MP analysis of morpho-anatomical data within Araceae) was motivated by, and based on, the pioneering work of Grayum (1984, 1990, 1992), who made a very wide-ranging revision of the taxonomic literature of the family and conducted a comprehensive SEM pollen study that provided the basis for his classification, itself derived by informal cladistic methodology.

It was French et al. (1995), however, who published the first computer-generated cladistic analysis of the Araceae, and they were also the first authors to present a within-family cladogram based on molecular patterns (restriction-site data from chloroplast DNA), using a matrix of 88 aroid genera and 488 characters. Their cladistic results, along with those of the independent morpho-anatomical GoA study, were first made public at the International Symposium *Monocotyledons: Systematics and Evolution* held at the Royal Botanic Gardens, Kew, in 1993 (Rudall et al., 1995) and led to discussions about combining the two matrices for further studies. This did not come to fruition, but the GoA classification eventually published in 1997 was strongly influenced by French et al.'s (1995) results, as can be seen from the discussion in Mayo et al. (1997, chapter 21). The GoA cladistic analysis was presented orally at the Tokyo International Botanical Congress in 1993 but never published in full, and the morpho-anatomical matrix on which it was based is presented for the first time here, albeit in a new and extended version.

MATERIALS AND METHODS

Character Matrix and Data Analyses—Most of the morpho-anatomical data were gathered during the preparation of the genus descriptions for GoA, when the morphology and anatomy of the stem, leaf, inflorescence, fruits, and seeds were reexamined using existing taxonomic literature supplemented by observations made from specimens in the herbaria, spirit and living collections of the Royal Botanic Gardens Kew, and the Munich Botanical Garden. The morphological and anatomical characters used here are mostly documented by Mayo et al. (1997), Grayum (1984, 1990, 1992), and Keating (2002), together with the literature cited in those works. We have added data sets for the lemnoid genera (*Lemna, Spirodela, Landoltia, Wolffia, Wolffiella* from Landolt, 1986, 1998 and Landolt and Kandler, 1987) and for more recently published genera not included in GoA. The morphological and anatomical characters are described in Appendix S1 (see Supplemental Data online at http://www.amjbot.org/cgi/content/full/ajb.1000158/DC2). Where no references are given, GoA is our primary information source. The resulting matrix consists of 81 characters for 109 genera of Araceae and one outgroup taxon, *Acorus*. In the original matrix, polymorphic characters were coded as ambiguities, but for the present analysis, where possible, we inferred an ancestral character state (IAS) for polymorphic characters because this has been found to yield more reliable results in analyses of higher-level taxa (Simmons, 2001, and references therein). The IAS matrix is presented in Appendix S2 (see Supplemental Data; for editable versions of the IAS matrix as well as the original morpho-anatomical matrices, see http://scratchpad.cate-araceae.org/). The chloroplast restriction site (RFLP) data matrix of French et al. (1995) included 88 aroid genera and 488 characters, with *Acorus* as outgroup (for editable version, see http://scratchpad.cate-araceae.org/).

The alignments of the six chloroplast markers of Cabrera et al. (2008; *rbcL*, *matK*, partial *trnK* intron, partial *tRNA-Leu* gene, *trnL–trnF* spacer, and partial *tRNA-Phe* gene), including 102 aroid genera and seven outgroup taxa, were obtained from TreeBase (for GenBank numbers, see Cabrera et al., 2008: appendix 1). We completed it by adding sequences from the six accepted genera not then included (*Anaphyllum, Croatiella, Furtadoa, Theriophonum, Zomicarpa*, and *Asterostigma* (the single *Asterostigma* species sampled by Cabrera et al. (2008) is now classified as *Incarum pavonii*), two recently published genera, *Bakoa* and *Schottariella* (Boyce and Wong, 2008, 2009), two other genera recently resurrected (*Philonotion,* Wong et al., 2010; *Sauromatum*, Cusimano et al., 2010), and an Australian genus composed of species previously assigned to *Typhonium* (the name *Lazarum* A. Hay is available but cannot yet be applied, pending availability of new material; Cusimano et al., 2010), giving a total of 113 genera. We also added a second accession of *Calla* as a check, and used *Tofieldia* (Tofieldiaceae), *Acorus* (Acoraceae), and *Hedyosmum* (Chloranthaceae) as outgroups. Appendix 1 shows the sources of the sequences added for the additional species. Sequences of two different species (*Typhonium horsfieldii*, *T. hirsutum*) have been combined to represent the genus *Sauromatum*. Several of the sequences of the additional species were available in GenBank, and the missing sequences were generated according to the methods described in Cabrera et al. (2008) and deposited in GenBank (accession nos. HQ687765–HQ687767). Alignment of the new sequences was first done automatically in MacClade 4.08 (Maddison and Maddison, 2005) and afterwards adjusted visually, trying to maximize similarity (Simmons, 2004). Unlike Cabrera et al. (2008), we did not use gap-coding, because it did not increase support or resolution either in the resulting phylogeny from Cabrera et al.'s (2008) MP analysis or from our partitioned MrBayes analyses of the gap-coded molecular data (data not shown). The sequence data matrix and the resulting trees were deposited in TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S11083).

All three data sets were analyzed with a Bayesian Markov-chain Monte Carlo (MCMC) approach (Yang and Rannala, 1997), using MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). Searches for the RFLP and morphological data sets relied on an F81 and a JC model, respectively; transition rates of the binary (RFLP) model relied on the stationary frequencies of 0 and 1; rates of the morphology model were all equal; to correct for the coding bias (i.e., all absent and/or all present characters are not detected), coding was set to "variable". The search for the sequence data relied on the GTR + Γ model with the number of gamma categories set to four (Yang, 1993). We used a flat Dirichlet prior for the relative nucleotide frequencies and rate parameters, a discrete uniform prior for topologies, and an exponential distribution (mean 10) for the γ-shape parameter and all branch lengths.

Bayesian runs were started from independent random starting trees and repeated four times. Markov chain Monte Carlo runs extended for 10 million generations for the RFLP and morphological data and 8 million runs for the molecular sequence data, with trees sampled every 100th generation (resulting in 100 000 and 80 000 trees, respectively, for each run). Besides the standard

convergence diagnostic in MrBayes, AWTY (Wilgenbusch et al., 2004; Nylander et al., 2008) was used to assess the convergence of all runs and to determine their burn-in fraction (i.e., the number of generations to be discarded before trees were combined).

Sequence data were additionally analyzed under maximum likelihood (ML; Felsenstein, 1973) with RAxML 7.2.7 ALPHA (Stamatakis et al., 2008); ML bootstrap values were obtained by running 1000 replicates. RAxML uses the GTRCAT approximation of the GTR + Γ model, with the gamma shape parameter having 25 rate categories. In interpreting phylogenetic confidence, we considered nodes "strongly supported" when they received both bootstrap proportion (BP) of 85% and a posterior probability (PP) of 0.97 or higher, and as "well supported" when only the PP is at least 0.97.

For a combined analysis, we examined the trees that resulted from the analyses of the single data sets. This revealed few, though supported, discrepancies between the trees. Because they were few, we combined them and ran a partitioned Bayesian analysis with the models described above for the three partitions. Runs did not converge after nearly 10 million generations within the time limit of 7 d of the Bio High Performance Cluster (BioHPC: Computational Biology Service Unit Web Computing Resources cluster at http://cbsuapps.tc.cornell.edu/). The same was true for an analysis of the combined RFLP and sequence data sets. Consequently, no phylogeny derived by combining all three data sets is shown here.

To explore character evolution, we reconstructed all 81 characters under parsimony in MacClade 4.08. For this we used the ML phylogeny and the morphological data matrix. Genera that did not occur in one or another matrix were cut off by the program, resulting in a tree of 111 genera (*Hedyosmum, Philonotion, Bakoa, Schottariella,* the Australian *Typhonium,* and one *Calla* species are missing). We traced every character on the phylogeny individually without resolving equivocal tracings. We also traced all unambiguous character changes on the ML phylogeny, indicating how the characters behaved elsewhere on the tree so as to detect synapomorphies, homoplasies, plesiomorphies, or ambiguous changes. The consistency, retention, and rescaled consistency indices of every character were also calculated.

Nomenclature—The names we use for previously recognized suprageneric groups refer to taxa as circumscribed by Bogner and Petersen (2007), unless specifically referenced to other published classifications or synopses (e.g., Mayo et al., 1997; Keating, 2002, 2004). Other groups are referred to by clade numbers and informal names given in Table 1, which summarizes clades that emerge from the present analyses (Fig. 1) and includes groups that do not correspond precisely to previously recognized taxa.

RESULTS AND DISCUSSION

Phylogenetic analyses—*Morphological data*—The Bayesian MCMC analysis of the morphological data matrix of 109 taxa and 81 characters was run for 10 million generations. The resulting phylogeny was built from 35 000 trees and is shown in Appendix S3 (see Supplemental Data online). Resolution of the phylogeny as well as clade support is generally low. The phylogeny placed the duckweeds (Lemnoideae) as the first-diverging clade of the Araceae. Somewhat surprisingly, *Calla* was placed as sister to the Lemnoideae. The Orontioideae were recovered as a clade, but *Gymnostachys* did not group with them to form the Proto-Araceae clade detected by French et al. (1995) and recognized by GoA. The unisexual flowered taxa all compose a single clade. The analysis failed to reveal a number of currently accepted groupings (following Bogner and Petersen, 2007), such as (1) Pothoideae (*Anthurium* + Potheae); (2) Monsteroideae, including Spathiphylleae; and (3) Caladieae. The tree also expresses some old higher groupings recognized in earlier, premolecular classifications, such as the Cryptocoryneae with *Ambrosina* and *Arisarum* (Bogner and Nicolson, 1991), and the grouping of the genera of the Caladieae and Colocasieae into a single taxon (Engler, 1920). *Stylochaeton* does not group with the Zamioculcadeae, reflecting their very different morphologies; the Areae form a clade including *Arisaema, Pinellia,* and, surprisingly, *Zomicarpa*.

Restriction-site data—We reanalyzed the chloroplast restriction-site data of French et al. (1995), using a Bayesian MCMC approach instead of parsimony analysis. We ran 10 Mio generations and discarded the first 55 000 trees as burn-in. The resulting phylogeny is presented in Appendix S4 (see Supplemental Data online) and does not differ substantially from the original of French et al. (1995), apart from the fact that it now includes support values. These are high for the True Araceae clade (Mayo et al., 1997: 67) and for many lower clades individually, but much of the backbone has low support. *Pothos* and *Anthurium* are sister to the Monsteroideae. The next branching clade is the Lasioideae, followed by *Calla*, which is sister to the unisexual flowered taxa. As in the original tree (French et al., 1995), Zamioculcadoideae are embedded within the Aroideae, making the latter paraphyletic. Also, *Lemna* is embedded within the Aroideae in a position distant from *Pistia*. The *Dracunculus* clade (37; Table 1) appears and is well supported. As in the molecular sequence data (see below), *Alocasia* does not group with the other genera of the Colocasieae (sensu Bogner and Petersen, 2007).

Sequence data—The matrix of the six combined chloroplast markers consisted of 117 taxa and 4494 aligned characters. Synapomorphic indels that occurred only in one species and therefore gave no phylogenetic information were excluded from the analysis in all markers except for *rbcL* (1391 nt), which did not have any indels, as well as one large, highly variable region in the *trnL–trnF* genes and spacer. Alignment lengths of the single markers before and after gap exclusion were 1122/674 nt (*tRNA–Leu*), 728/585 nt (*trnL-F* spacer, *tRNA-Phe*), 1965/1844 nt (*matK*, partial *trnK* intron). Analyses of the molecular data yielded similar topologies for the ML and Bayesian approaches. Only a few, unsupported differences were found (e.g., *Pistia* and *Protarum* as sister spp. instead of forming a grade; the same is true for *Anubias/Montrichardia* and for relationships within the Schismatoglottideae). None of these differences have been resolved, even in finer-scale analyses (Renner and Zhang, 2004; Boyce and Wong, 2008).

We present the ML tree with support values from both analyses (Fig. 1). Our ML phylogeny is similar to that of Cabrera et al. (2008), but places the Lasioideae (clade 7) as sister to the Unisexual Flowers clade (40), a weakly supported node that unites the *Stylochaeton* clade (25) and the Aroideae (clade 39). Several other weakly supported nodes (ML < 85, PP < 0.97) remain within the Aroideae (clade 39). These include the branchings between *Callopsis, Anubias, Montrichardia,* the *Zantedeschia* clade (32) and the *Philonotion* clade (38). Within the *Zantedeschia* clade, the branching patterns between clades 26 (*Anchomanes* clade) and 27 (*Homalomena* clade), the genus *Zantedeschia* and clade 13 (Spathicarpeae) have low support (Fig. 1, Table 1). This region of the tree has very short branch lengths. However, support within the *Dracunculus* clade (37) is generally high. All the formerly missing, new, and recently described taxa group with their hypothesized relatives. The second accession of *Calla* used to confirm the veracity of the original sequence and its position in the tree forms with the first accession a well-supported clade. As in the result of Cabrera et al. (2008), *Calla* falls well within the Aroideae clade, as sister to the well-supported *Dracunculus* clade (see further discussion below), but this node has only weak support. An additional analysis that excluded

TABLE 1. Suprageneric clades for consideration as taxa in future classifications of the Araceae. The names of the 44 clades are either referenced to previous studies where previously recognized or provided here with an informal name (in bold); the numbers are as in Figures 1 and 2. The generic composition is given for each clade, with an indication of the support (high: ML bootstrap >85 and PP >0.97; good: PP >0.97; low: ML bootstrap <85 and PP <0.97). The synapomorphies and homoplasies that result from optimization of the morpho-anatomical matrix onto the ML tree with a retention index >0.5 or that are considered to have resulted from convergent evolution and, therefore, to be "synapomorphic" are given in the right-hand column (Appendix S5, S6, S7) and numbered as in Appendix S1; the terms used have the following meanings: s*ynapomorphy* ≡ a unique change (strict synapomorphy) occurring only on the stem of the clade in question; *homoplasy: above* ≡ a new state changing again within the clade in question to a state that is also found outside the clade (reversal or convergence); *homoplasy: outside* ≡ the derived state also occurs outside the clade in question (reversal or convergence); *ambiguous* ≡ a change not present in all most-parsimonious reconstructions. The following 11 isolated genera are not included in lower level clades (1-21) *Calla, Callopsis, Montrichardia, Anubias, Zantedeschia, Philonotion, Protarum, Pistia, Alocasia, Pinellia,* and *Arisaema.*

Clade no.	Group name (References)	Genera/clades included	Support	Morphological/anatomical support (computed in MacClade)
1	Orontioideae (Bogner and Petersen, 2007)	*Lysichiton, Orontium, Symplocarpus*	High	55-1: endosperm sparse to absent, embryo relatively large (homoplasy: outside)
2	Lemnoideae (Keating, 2002)	*Landoltia, Lemna, Spirodela, Wolffia, Wolffiella*	High	2-1: perigon absent (homoplasy: outside) 8-7: pollen ulcerate (synapomorphy) 10-2: pollen globose to ellipsoid (homoplasy: outside) 12-2: pollen exine surface spinose (homoplasy: outside) 28-4: fine venation absent, only primary veins present (synapomorphy) 32-3: stem reduced to a minute button, or indistinguishable from the thalloid structure (synapomorphy) 35-2: floating aquatics (homoplasy: outside) 57-5: chromosome base number $x = 10$ (homoplasy: outside)
3	Potheae (Bogner and Petersen, 2007)	*Pedicellarum, Pothoidium, Pothos*	High	6-3: shoot architecture with monopodial scandent structure (homoplasy: outside) 55-1: endosperm sparse to absent, embryo relatively large (homoplasy: outside) 57-0: chromosome base number $x = 12$ (homoplasy: outside)
4	*Heteropsis* clade (Tam et al., 2004; Cabrera et al., 2008)	*Alloschemone, Heteropsis, Rhodospatha, Stenospermation*	High	57-2: chromosome base number $x = 14$ (homoplasy: outside)
5	Spathiphylleae (Bogner and Petersen, 2007)	*Holochlamys, Spathiphyllum*	High	3-2: trichosclereids present in bundles, ± small (synapomorphy) 8-8: pollen multiaperturate (synapomorphy) 12-3: pollen exine surface striate/polyplicate (homoplasy: outside)
6	*Rhaphidophora* clade (Tam et al., 2004; Cabrera et al., 2008)	*Amydrium, Anadendrum, Epipremnum, Monstera, Rhaphidophora, Scindapsus*	High	
7	Lasioideae (Bogner and Petersen, 2007)	*Anaphyllopsis, Anaphyllum, Cyrtosperma, Dracontioides, Dracontium, Lasia, Lasimorpha, Podolasia, Pycnospatha, Urospatha*	High	46-1: flowering sequence of spadix basipetal (synapomorphy) 57-1: chromosome base number $x = 13$ (homoplasy: outside) 64-3: leaf TS spongy aerenchyma type 4 (homoplasy: above) 68-3: petiole TS ground tissue type 4 (homoplasy: outside)
8	Zamioculcadoideae (Bogner and Petersen, 2007)	*Gonatopus, Zamioculcas*	High	8-1: pollen aperture extended monosulcate (homoplasy: outside) 32-1: stem condensed, strongly thickened but not depressed globose (homoplasy: outside) 55-1: endosperm sparse to absent, embryo relatively large (homoplasy: outside) 57-8: chromosome base number $x = 17$ (homoplasy: outside)
9	Aglaonemateae (Bogner and Petersen, 2007)	*Aglaodorum, Aglaonema*	High	38-2: entire spathe usually caducous or marcescent with distinct basal abscission (homoplasy: outside) 65-2: leaf TS collenchyma type Sb (homoplasy: outside)
10	Nephthytideae (Bogner and Petersen, 2007)	*Anchomanes, Nephthytis, Pseudohydrosme*	High	24-1: primary leaf venation with basal ribs of primary veins very well developed, i.e., ± tripartite primary development (homoplasy: outside)
11	Culcasieae (Bogner and Petersen, 2007)	*Cercestis, Culcasia*	High	33-3: mostly aerial and climbing, including hemiepiphytes (homoplasy: outside)
12	***Philodendron* clade**	*Furtadoa, Homalomena, Philodendron*	High	44-2: spadix zonation normally female-sterile-male (homoplasy: outside)
13	Spathicarpeae (Gonçalves, 2002; Gonçalves et al., 2007)	*Asterostigma, Bognera, Croatiella, Dieffenbachia, Gearum, Gorgonidium, Incarum, Mangonia, Spathantheum, Spathicarpa, Synandrospadix, Taccarum*	High	50-1: stamens entirely connate (including connectives) (homoplasy: outside) 57-8: chromosome base number $x = 17$ (homoplasy: outside)

TABLE 1. Continued.

Clade no.	Group name (References)	Genera/clades included	Support	Morphological/anatomical support (computed in MacClade)
14	Cryptocoryneae (Bogner and Petersen, 2007)	*Cryptocoryne, Lagenandra*	High	18-0: laticifers absent (homoplasy: outside) 37-1: intravaginal squamules present (homoplasy: outside) 40-1: spathe and spadix forming two distinct chambers by partial fusion (homoplasy: outside) 41-1: spathe with internal flap covering the spadix apex (synapomorphy) 42-1: spathe margins connate for a distinct distance (homoplasy: outside) 57-9: chromosome base number $x = 18$ (homoplasy: outside) 65-4: leaf blade TS collenchyma with strands discrete, usually circular, type Sv (homoplasy: outside) 67-4: petiole TS collenchyma with strands aligned with vascular bundles, type Sv (homoplasy: outside)
15	Schismatoglottideae (Bogner and Petersen, 2007; Boyce and Wong, 2008)	*Aridarum, Bakoa, Bucephalandra, Phymatarum, Piptospatha, Schismatoglottis, Schottariella*	High	33-1: stem ± erect at least distally, often aerial (homoplasy: outside)) 35-4: rheophytes (most genera) (homoplasy: above and outside) 36-1: petiole sheath long-ligulate apically (most genera) (homoplasy: above and outside) 38-1: spathe tube or lower half persistent, blade marcescent or caducous (homoplasy: outside)
16	Thomsonieae (Bogner and Petersen, 2007)	*Amorphophallus, Pseudodracontium*	High	24-1: basal ribs of primary veins very well developed, i.e., ± tripartite primary development (homoplasy: outside) 27-6: leaf blade margin lobed both pinnately and pedately (decompound) (synapomorphy) 44-3: spadix zonation female-male-sterile (most species) (homoplasy: outside) 45-1: spadix appendix a conspicuous and well developed organ, (sometimes) staminodial (homoplasy: outside)
17	Caladieae (Keating, 2002)	*Caladium, Chlorospatha, Filarum, Hapaline, Jasarum, Scaphispatha, Syngonium, Ulearum, Xanthosoma, Zomicarpa, Zomicarpella*	High	18-2: laticifers present, anastomosing (homoplasy: outside) 38-1: spathe tube or lower half persistent, blade marcescent or caducous (most genera) (homoplasy: outside and above)
18	Arisareae (Keating, 2002)	*Ambrosina, Arisarum*	High	12-3: pollen exine surface striate (homoplasy: outside) 42-1: spathe margins connate for distinct distance (homoplasy: outside) 43-1: spadix female zone adnate to spathe (homoplasy: outside)
19	Arophyteae (Bogner and Petersen, 2007)	*Arophyton, Carlephyton, Colletogyne*	Good	10-1: pollen shape globose (homoplasy: outside) 12-2: pollen exine surface spinose (homoplasy: outside) 23-0: midrib of primary vein ± absent with veins arcuate from the base (homoplasy: outside) 44-1: spadix zonation female-male (homoplasy: outside) 50-1: stamens connate by filaments (most genera) (homoplasy: outside)
20	***Colocasia* clade**	*Ariopsis, Colocasia, Remusatia, Steudnera*	High	34-1: leaves peltate (homoplasy: outside)
21	Areae (Bogner and Petersen, 2007)	*Arum, Biarum, Dracunculus, Eminium, Helicodiceros,* Australian *Typhonium* (≡ *Lazarum*), *Sauromatum, Theriophonum, Typhonium*	High	58-1: staminodes in interfertile zones hair-like, subulate, bristle-like or clavate-elongated (homoplasy: outside; ambiguous)
22	Proto-Araceae (Mayo, Bogner and Boyce, 1997)	*Gymnostachys, Lysichiton, Orontium, Symplocarpus*	High	32-1: stem condensed, strongly thickened but not depressed-globose (homoplasy: outside)
23	Pothoideae (Bogner and Petersen, 2007)	*Anthurium, Pedicellarum, Pothoidium, Pothos*	High	
24	Monsteroideae (Bogner and Petersen, 2007)	clades *4, 5, 6*	High	3-1: trichosclereids present, large, not in bundles (most genera) (homoplasy: above) 8-2: pollen zonate (ring-like aperture; most genera) (homoplasy: above) 28-1: secondaries and tertiaries parallel to primaries, joined by cross veins only (homoplasy: outside and above) 38-2: spathe with no differentiation, entire spathe soon deciduous or marcescent with distinct basal abscission (most genera) (homoplasy: outside; ambiguous)
25	***Stylochaeton* clade**	*Stylochaeton, Gonatopus, Zamioculcas*	Good	11-2: pollen large (homoplasy: outside) 63-1: male flowers with pistillode or vestige, e.g., stigmatoids, present (homoplasy: outside; ambiguous)
26	***Anchomanes* clade**	clades *9, 10*	High	67-2: petiole TS collenchyma: irregular flattened strands, not aligned with vascular bundles, type Sb (most genera) (synapomorphy)
27	***Homalomena* clade**	clades *11, 12*	Good	15-1: sclerotic hypodermis in roots present (homoplasy: outside) 16-1: endothecial thickenings absent (most genera) (homoplasy: above) 17-1: resin canals in roots present (synapomorphy)

TABLE 1. Continued.

Clade no.	Group name (References)	Genera/clades included	Support	Morphological/anatomical support (computed in MacClade)
28	**Rheophytes clade**	*Philonotion,* clades *14, 15*	High	48-1: stamens with horned thecae (most genera) (synapomorphy; ambiguous)
29	***Typhonodorum* clade**	*Peltandra, Typhonodorum,* clade *19*	High	52-1: staminodes present in female flowers (homoplasy: outside) 55-1: endosperm sparse to absent, embryo relatively large (homoplasy: outside) 70-1: storage cotyledon (homoplasy: outside)
30	***Alocasia* clade**	*Alocasia, Arisaema, Pinellia,* clade *21*	Good	
31	**Bisexual Climbers clade**	clades *23, 24*	High	33-3: stem habit aerial and climbing, including hemiepiphytes (most genera) (homoplasy: outside and above)
32	***Zantedeschia* clade**	*Zantedeschia,* clades *13, 26, 27*	Good	
33	***Colletogyne* clade**	clades *18, 29*	High	
34	*Pistia* clade (Renner and Zhang, 2004)	*Pistia, Protarum,* clades *20, 30*	High	
35	***Amorphophallus* clade**	clades *16, 17*	High	
36	***Ambrosina* clade**	clades *33, 34*	High	
37	***Dracunculus* clade**	clades *35, 36*	High	26-2: sympodial inframarginal vein formed by majority of primary veins, lowermost primary veins forming nonsympodial marginal veins (most genera) (homoplasy: outside; ambiguous) 65-4: leaf TS collenchyma strands aligned with vascular bundles, type Sv (homoplasy: outside and above; ambiguous) 67-4: petiole TS collenchyma strands aligned with vascular bundles, type Sv (most genera) (homoplasy: outside and above; ambiguous)
38	*Philonotion* clade	*Calla,* clades *28, 37*	Good	
39	*Aroideae* clade	*Callopsis, Anubias, Montrichardia, Clades 32, 38*	Good	2-1: perigon absent (homoplasy: outside) 18-1: laticifers present, simple, articulated (most genera) (homoplasy: outside and above) 75-1: sporopollenin in ektexine absent (most genera) (homoplasy: above)
40	Unisexual Flowers clade (Mayo, Bogner and Boyce, 1997)	clades *25, 39*	Low	1-1: flowers unisexual (synapomorphy) 8-5: pollen inaperturate (omniaperturate) (homoplasy: outside and above) 44-(1-4): spadix zonation present (homoplasy: above)
41	***Podolasia* clade**	clades *7, 40*	High	7-1: phyllotaxy spiral (most genera) (homoplasy: outside and above)
42	True Araceae clade	clades *31, 41*	High	4-1: spathe modified for attraction and display or specialized in some other way (homoplasy: outside) 5-1: major internode of the inflorescence is the peduncle situated between spathe and next leaf below (homoplasy: outside; ambiguous) 6-1: shoot architecture with continuation shoot in penultimate leaf axil before spathe (n – 1 node) (homoplasy: above; ambiguous) 67-0: petiole TS collenchyma type B (homoplasy: outside and above)
43	***Spirodela* clade**	clades *2, 42*	High	
44	Araceae	clades *22, 43*	High	80-1: raphides present (synapomorphy)

Calla (data not shown) did not improve the support values within the Aroideae.

Reconstruction of Character Evolution—The reconstructions of every character on the ML phylogeny are shown in Appendix S5; a table showing the statistics of all 81 characters, giving number of states, steps and changes, consistency index, retention index, minimum and maximum numbers of steps and the rescaled consistency index is given in Appendix S6; and the ML phylogeny with all unambiguous changes of all characters is shown in Appendix S7 (see Supplemental Data online). All synapomorphic changes are used to support clades found in the phylogeny (Fig.1) and listed in Table 1; homoplasious changes are discussed, since they represent reversal to an ancestral state or hypotheses of convergent evolution and thus may be considered as clade support.

The molecular phylogeny in the context of morphology, anatomy, and ecology—Although, in principle, it might be preferable to base our general discussion on a tree produced by analyzing all three data sets considered in the present study (sequence, restriction-site, and morpho-anatomy), we have preferred instead to select a tree based on sequence data for this purpose. The unsatisfactory results of the combined analyses we carried out were due to conflicts of information between the three data sets (see Material and Methods). Using a nonsequence tree as the main focus for discussion is no longer a credible option, particularly given the much larger matrix and the stronger statistical support obtainable by using model-based methods on molecular sequence data. We therefore focused on the result from ML analysis of the molecular sequence data set as the basis for evaluating the phylogenetic significance of the morpho-anatomical data and for our attempt to provide the major molecular clades with a justifiable phenotypic profile. In the following discussion the term "synapomorphy" refers to both unique and nonunique apomorphic characters that supported clades in the MacClade character optimization, whereas in Table 1 we have used "synapomorphy" to mean strict synapomorphies that occur uniquely on the ML tree.

The results provide a set of clades, mostly supported by phenotypic synapomorphies, that future workers can investigate using both molecular and phenotypic data. New research is needed to test and develop the morphological and anatomical character analyses we have adopted and to strengthen the molecular database using new methodologies.

As our aim was to highlight groups for formal recognition, we focus in this discussion on higher-level clades in the ML phylogeny with good molecular and phenotypic support. These we have numbered and named, using either previously published names or new informal ones. We have not mentioned many lower-level clades with strong molecular support, although these should be taken into account in future studies. We present here 44 clades (Fig. 1, Table 1), of which all but 8 are strongly supported by molecular data, 7 are well supported, and only one has low support. Most of them can additionally be characterized by morpho-anatomical synapomorphies, although not always completely so. Sixteen of these clades are newly circumscribed and named. Twenty-two morpho-anatomical characters are shown on the phylogeny in Figure 2; 16 of these represent synapomorphies, and 6 are shown in their diversity all across the family.

Phenotypic support for the major clades and their bisexual-flowered components—The major clades form the well-supported backbone of the molecular sequence tree (Fig. 1). Clade 44 represents the family Araceae, which in our morphological and anatomical character matrix is supported by the presence of raphides (char. 80-1; Fig. 2). Stevens (2001) gives further details of synapomorphies of the family within a wider phylogenetic context.

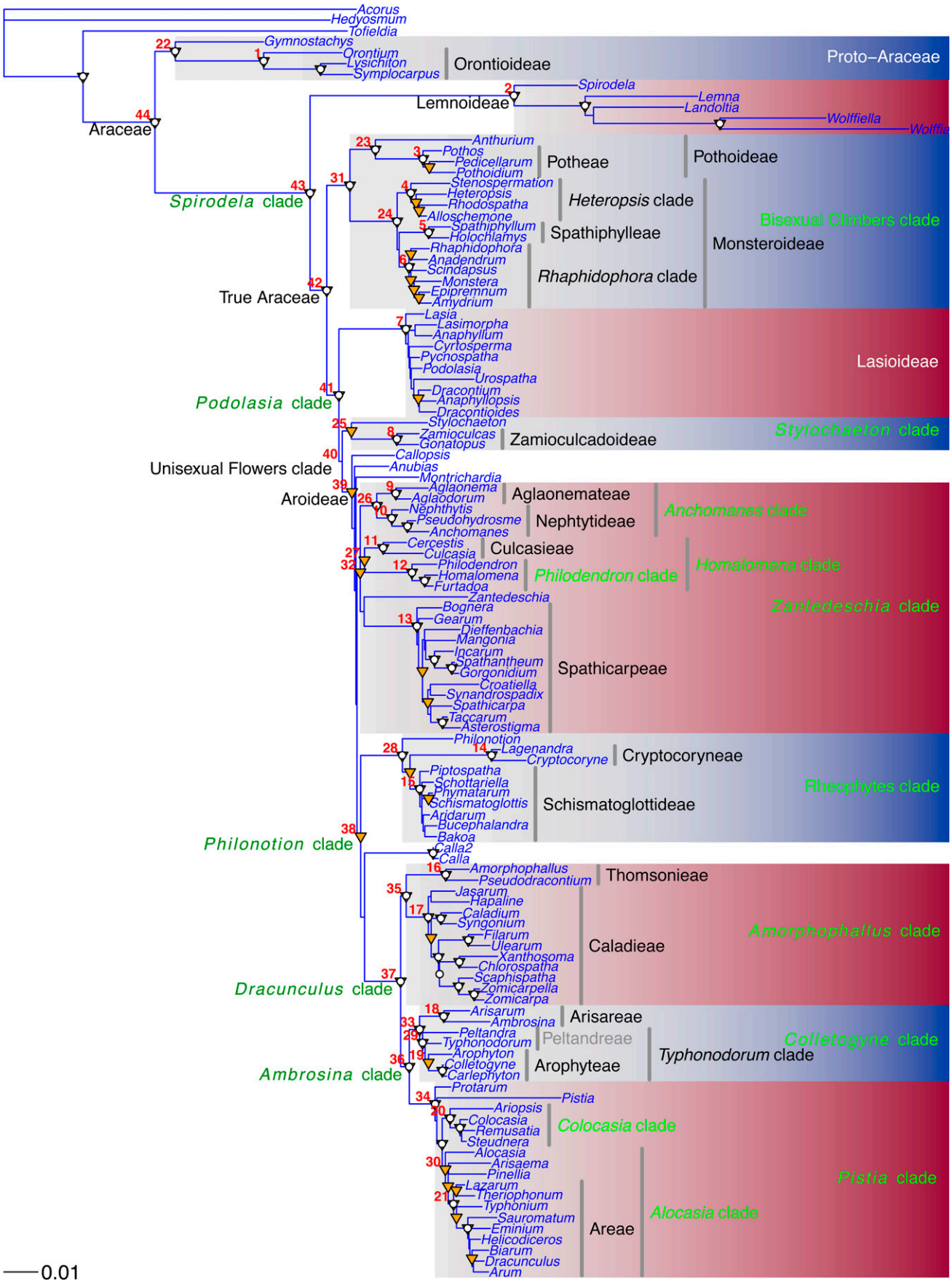
The first division of the aroids separates Proto-Araceae (clade 22) from the *Spirodela* clade (43). Proto-Araceae (clade 22) is only weakly supported by phenotypic synapomorphies (condensed subterranean stems) but is consistently found in molecular analyses (French et al., 1995; Tam et al., 2004; Cabrera et al., 2008). *Gymnostachys* differs radically in its morphology from the other genera of its clade (Orontioideae, clade 1) and indeed from any other aroid, suggesting an early divergence and possibly evolution in a very distinct ecological context.

The *Spirodela* clade (43) lacks morpho-anatomical synapomorphies. Its two component clades are the duckweeds or Lemnoideae (clade 2) and the True Araceae (clade 42). The Lemnoideae are supported by the following synapomorphies: lack of a perigon, ulcerate, globose to ellipsoid and spinose pollen, venation reduced to primary veins only, thalloid stem-leaf, floating aquatic habit, and chromosome base number of $x = 10$. The duckweeds share their free-floating habit with *Pistia* and the fossil lemnoid genera *Limnobiophyllum* and *Cobbania* (Stockey et al., 1997, 2007; Rothwell et al., 2004; Bogner, 2009). The evolution of the genera of the Lemnoideae can be thought to follow a logical sequence according to the following morphological reduction series: in *Spirodela* and *Landoltia* the fronds have veins and many roots, a prophyll is present, the inflorescence has a spathe and is situated at the side of the leaf sheath arising from the plant's growth point, and the anther has two thecae. In *Lemna* the fronds have veins but only a single root; there is no prophyll, but the inflorescence is similar to those of *Spirodela* and *Landoltia*. In *Wolffiella* the fronds lack both veins and roots, there is no prophyll, the inflorescence is situated on the upper side of the flat frond in a cavity, there is no spathe, and the anthers have only a single theca. *Wolffia* differs from *Wolffiella* only in having globular to ellipsoid fronds. Our phylogeny (Fig. 1) places *Spirodela*, with the most ancestral phenotype, as sister to the other Lemnoid genera and groups *Wolffiella* and *Wolffia* as a well-supported subclade (Wolffioideae of Les et al., 2002; Wolffieae of Bogner and Petersen, 2007). In our results (Fig. 1), *Lemna* branches off first, followed by *Landoltia* and then the Wolffieae, but the topology between *Lemna* and *Landoltia* is not well supported in our results, either in the Bayesian (PP ≤ 0.97) or in the ML analysis (Appendix S6). The molecular data presented by Les et al. (2002), based on a comprehensive sampling of Lemnoid species and using *Pistia* as primary outgroup (seven other unnamed aroid genera were used in a preliminary phase of the study), also did not reveal the position of *Landoltia*. Only after adding morphological data to the analysis did it come out well supported as sister to *Lemna, Wolffia,* and *Wolffiella*. So although it seems clear that in general there was evolution from complex to reduced forms, it is possible that this happened twice, once leading to *Lemna* and once to the *Wolffia–Wolffiella* clade. Rothwell et al. (2004) analyzed molecular DNA sequence data from six Lemnoid taxa (all five genera and two species of *Lemna*) and 29 other Araceae genera, using *Aponogeton* as outgroup. They established that the Lemnoids and *Pistia* are not sister clades, and their internal topology of the Lemnoids places *Landoltia* immediately after a basal *Spirodela,* followed successively by *Wolffia* and *Wolffiella,* terminating with *Lemna* as the most derived subclade. The Lemnoids were characterized by much longer branch lengths than other aroid genera, especially in *Wolffia*. As these authors have indicated, a desirable future goal is an analysis of comprehensive sets both of Lemnoid taxa and all other aroid genera to seek a more consistent topology for the Lemnoid genera.

The True Araceae (clade 42) are supported by four synapomorphies: attractive or otherwise conspicuous spathes (char. 4-1), presence of a distinct peduncle (char. 5-1), type B petiole collenchyma (char. 67-0), and shoot architecture (char. 6-1), namely the position of the reiteration of the sympodial unit (continuation shoot) in the axil of the penultimate foliar organ prior to the spathe (Engler, 1877; translated by Ray and Renner, 1990). This is nearly universal in True Araceae, apart from the specialized climbing genera of Potheae and in *Heteropsis,* but sporadic exceptions occur elsewhere (e.g., the *Schismatoglottis calyptrata* group and the *Homalomena bellula* complex). Whether this predominant architectural model is adaptive and mediated some key evolutionary advantage remains to be investigated.

Clade 42 is composed of the *Podolasia* clade (41) and the Bisexual Climbers clade (31). The latter is supported by one phenotypic synapomorphy, the usually hemiepiphytic climbing stems, although terrestrial, rheophytic, and helophytic plants predominate in the two genera of the Spathiphylleae and epiphytes in *Anthurium* and *Stenospermation*. The climbing habit is predominant in 12 of the 16 genera (Fig. 2). The only other climbing plants of Araceae are unisexual-flowered and occur in the *Homalomena* clade (*Philodendron, Culcasia, Cercestis* in clade 27), in the Caladieae (*Syngonium* in clade 17), and in some species of Schismatoglottideae (clade 15).

The Bisexual Climbers are composed of the Pothoideae (clade 23) and the Monsteroideae (clade 24). The Pothoideae, though long-recognized as a group, have little phenotypic support in our matrix, as there is no synapomorphy apparent. However, the three genera of the Potheae (clade 3) are grouped by basic chromosome number ($x = 12$), monopodial shoot architecture (at least the primary climbing axis), and sparse-to-absent endosperm. Monsteroideae (clade 24) is supported by the


Acorus
Hedyosmum
Tofieldia
Gymnostachys
Orontium
Lysichiton
Symplocarpus
Orontioideae
Proto–Araceae
Spirodela
Lemna
Landoltia
Wolffiella
Wolffia
Lemnoideae
Araceae
Anthurium
Pothos
Pedicellarum
Pothoidium
Potheae
Pothoideae
Stenospermation
Heteropsis
Rhodospatha
Alloschemone
Heteropsis clade
Spathiphyllum
Holochlamys
Spathiphylleae
Bisexual Climbers clade
Monsteroideae
Rhaphidophora
Anadendrum
Scindapsus
Monstera
Epipremnum
Amydrium
Rhaphidophora clade
Spirodela clade
True Araceae
Lasia
Lasimorpha
Anaphyllum
Cyrtosperma
Pycnospatha
Podolasia
Urospatha
Dracontium
Anaphyllopsis
Dracontioides
Lasioideae
Podolasia clade
Stylochaeton
Zamioculcas
Gonatopus
Zamioculcadoideae
Stylochaeton clade
Callopsis
Anubias
Montrichardia
Unisexual Flowers clade
Aroideae
Aglaonema
Aglaodorum
Aglaonemateae
Nephthytis
Pseudohydrosme
Anchomanes
Nephthytideae
Anchomanes clade
Cercestis
Culcasia
Culcasieae
Philodendron
Homalomena
Furtadoa
Philodendron clade
Homalomena clade
Zantedeschia
Zantedeschia clade
Bognera
Gearum
Dieffenbachia
Mangonia
Incarum
Spathantheum
Gorgonidium
Croatiella
Synandrospadix
Spathicarpa
Taccarum
Asterostigma
Spathicarpeae
Philonotion
Lagenandra
Cryptocoryne
Cryptocoryneae
Piptospatha
Schottariella
Phymatarum
Schismatoglottis
Aridarum
Bucephalandra
Bakoa
Schismatoglottideae
Rheophytes clade
Philonotion clade
Calla2
Calla
Amorphophallus
Pseudodracontium
Thomsonieae
Jasarum
Hapaline
Caladium
Syngonium
Filarum
Ulearum
Xanthosoma
Chlorospatha
Scaphispatha
Zomicarpella
Zomicarpa
Caladieae
Amorphophallus clade
Dracunculus clade
Arisarum
Ambrosina
Arisareae
Peltandra
Typhonodorum
Peltandreae
Colletogyne clade
Typhonodorum clade
Arophyton
Colletogyne
Carlephyton
Arophyteae
Ambrosina clade
Protarum
Pistia
Ariopsis
Colocasia
Remusatia
Steudnera
Colocasia clade
Alocasia
Arisaema
Pinellia
Lazarum
Theriophonum
Typhonium
Sauromatum
Eminium
Helicodiceros
Biarum
Dracunculus
Arum
Areae
Pistia clade
Alocasia clade
0.01

following phenotypic synapomorphies: trichosclereids, parallel-pinnate fine leaf venation, zonate (ring-like aperture) pollen, and undifferentiated, soon-deciduous spathes, although these characters have exceptions in one or more genera.

Within the Monsteroideae, there are three subgroups. Although not supported as sister to the other two clades within the Monsteroideae and only weakly supported as sister to the *Rhaphidophora* clade (6), the strongly supported Spathiphylleae (clade 5) are phenotypically very distinct, being characterized by smaller trichosclereids in bundles and a polyplicate-multiaperturate pollen type (Tarasevich, 1988; Hesse and Zetter, 2007). The *Heteropsis* clade (4) has the phenotypic synapomorphy of chromosome base number $x = 14$ while the *Rhaphidophora* clade (6) lacks phenotypic synapomorphies. Tam et al. (2004), in their molecular study of a much larger sample of species, also found the Spathiphylleae embedded within the Monsteroideae, but with low support.

The next major clade in the tree is the *Podolasia* clade (41), supported by the phenotypic synapomorphy of spiral phyllotaxy in our morphological data set. It is composed of two groups, the strongly supported Lasioideae (clade 7) and the Unisexual Flowers clade (40). The latter clade has weak molecular support and consequently weak support for the branching pattern between the Lasioideae (clade 7), the *Stylochaeton* clade (25), and the well-supported Aroideae (clade 39). However, the cohesion between the latter two clades, placing them as sister to the Lasioideae, is enhanced by the presence of unisexual flowers (except for *Calla*) and inaperturate (omniaperturate) pollen (except for the Zamioculcadoideae). If *Calla* is excluded (discussed later), clade 40 corresponds to the concept of subfamily Aroideae published by GoA, characterized by unisexual flowers.

Lasioideae (clade 7) is composed of tropical bisexual-flowered plants that are predominantly helophytes. Phenotypic synapomorphies of the group are the basipetal flowering sequence in the spadix, a base chromosome number of $x = 13$, type 4 spongy aerenchyma in the leaf, and type 4 petiole ground tissue (Fig. 2). Hesse (2002) reported a unique pollen-aperture structure. Lasioideae are also notable for the common occurrence of prickles on the petiole, peduncle, and undersurface of the main leaf veins. This group has been extensively studied by Hay (1986, 1988, 1992; Hay and Mabberley, 1991).

The *Stylochaeton* clade (25) is composed of rhizomatous geophytes, centered in tropical Africa, and although it expresses a unique combination of unisexual, perigoniate flowers, these characters are plesiomorphic in our result; synapomorphies for this clade are large pollen and male flowers with vestigial pistillodes. *Stylochaeton* differs from its sister group, the Zamioculcadoideae (clade 8), in its inaperturate (= omniaperturate) pollen, chromosome base number $x = 14$, and much thinner, undifferentiated sporopollenin ektexine (Hesse et al., 2001), the latter character also differentiating *Stylochaeton* from the Aroideae (clade 39). The Zamioculcadoideae are phenotypically highly distinctive, supported by the synapomorphies of extended-monosulcate pollen, chromosome base number $x = 17$, condensed, thickened stem, and lack of endosperm.

Phenotypic support for the Aroideae clade: the evolution of unisexual flowered, aperigoniate aroids—The remaining 75 genera form Aroideae (clade 39), which is well supported on both molecular and, when *Calla* is excluded (for discussion, see below), on phenotypic evidence by the following morpho-anatomical synapomorphies: loss of the perigon, presence of laticifers, and loss of sporopollenin in the ektexine. Hesse (2006b, c) has argued that taken together, these changes in morpho-anatomical character patterns seem to imply a major adaptive shift in the evolution of aroids. In this clade, the omniaperturate pollen grains have a thick, spongy endexine and a highly reduced ektexine with either a very thin sporopollenin lamella or a nonsporopollenin outer exine layer (Hesse, 2006b). Almost all genera (*Stylochaeton* is somewhat anomalous) that diverged before the Aroideae clade have aperturate pollen with a well-developed tectate-columellate sporopollenin ektexine and a thin endexine (Hesse, 2006b). The correlation with the presence of laticifers and possibly biforines suggests a connection to chemical defense: this clade includes nearly all genera with laticifers, either simple and articulated or anastomosing. Exceptions are the presence of simple laticifers in *Orontium* (clade 1, Orontioideae) and their absence in the unisexual genera *Cryptocoryne, Culcasia, Gearum, Lagenandra, Mangonia, Pistia, Pseudohydrosme* (not detected by Keating, 2002), and *Spathantheum*. Biforines are found almost exclusively in the Aroideae but with numerous exceptions; outside clade 39, they are present only in *Stylochaeton*.

The anatomy of vascular and support tissues in the leaf and petiole in the family, reported and discussed by Keating (2000, 2002, 2004) and Gonçalves et al. (2004), are interesting in relation to the Aroideae clade and suggest correlations between changes in vascular bundle and collenchyma characters and the appearance and further evolution of the unisexual-flowered, aperigoniate aroids in clade 39.

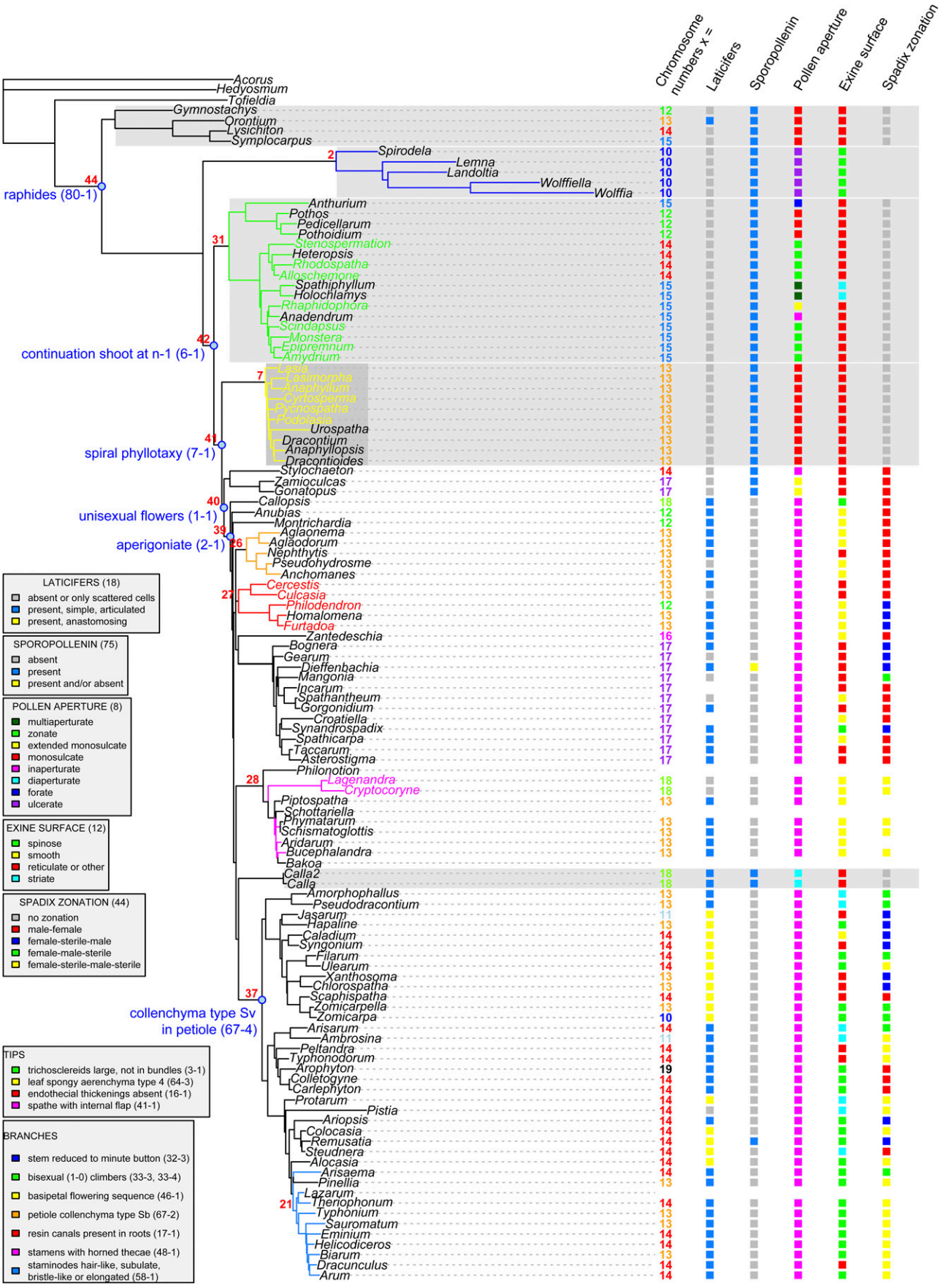
Another possibly significant morphological change is the occurrence of smooth pollen (char. 12-1) in the early-diverging clades of the Aroideae, mostly in the *Zantedeschia* clade (32), *Anubias, Montrichardia,* and the Rheophytes clade (28). This suggests that exine surface patterns evolved away from the reticulate pattern predominant in bisexual-flowered genera at around the same time as the shift from bisexual to unisexual flowers took place (Fig. 2; Appendix S5, S7). In the clades distal to the Rheophytes, spinose pollen becomes much more frequent (Hesse 2006b).

The Aroideae clade (39) is composed of clades 32 and 38 together with the genera *Callopsis, Montrichardia,* and *Anubias*. Contrary to previously published analyses (GoA), *Callopsis, Anubias,* and *Montrichardia* form a grade, followed by the *Zantedeschia* clade (32) and the *Philonotion* clade (38), both well-supported by molecular data but without phenotypic synapomorphies.

The pairing of genus *Zantedeschia* with the Spathicarpeae (clade 13) in clade 32 (*Zantedeschia* clade) is only weakly supported, as in the previous study by Cabrera et al. (2008). The other major subgroups of clade 32 are the *Anchomanes* clade (26) and the *Homalomena* clade (27). Clade 26 is supported

←

Fig. 1. Phylogeny obtained from ML analysis of a molecular data set of 117 species and 4494 nucleotides from four chloroplast markers based on Cabrera et al. (2008). Support is mapped directly onto the nodes as follows. Orange triangles: posterior probability from Bayesian analysis > 0.97. White dots: ML bootstrap support (1000 reps.) > 85. All 44 clades discussed in the text are labeled with clade numbers and names. Green clade names indicate the newly defined clades (cf. Table 1).


Chromosome numbers x =
Laticifers
Sporopollenin
Pollen aperture
Exine surface
Spadix zonation
Acorus
Hedyosmum
Tofieldia
Gymnostachys
Orontium
Lysichiton
Symplocarpus
Spirodela
Lemna
Landoltia
Wolffiella
Wolffia
raphides (80-1)
Anthurium
Pothos
Pedicellarum
Pothoidium
Stenospermation
Heteropsis
Rhodospatha
Alloschemone
Spathiphyllum
Holochlamys
Rhaphidophora
Anadendrum
Scindapsus
Monstera
Epipremnum
Amydrium
continuation shoot at n-1 (6-1)
Lasia
Lasimorpha
Anaphyllum
Cyrtosperma
Pycnospatha
Podolasia
Urospatha
Dracontium
Anaphyllopsis
Dracontioides
spiral phyllotaxy (7-1)
Stylochaeton
Zamioculcas
Gonatopus
Callopsis
Anubias
Montrichardia
unisexual flowers (1-1)
Aglaonema
Aglaodorum
Nephthytis
Pseudohydrosme
Anchomanes
aperigoniate (2-1)
Cercestis
Culcasia
Philodendron
Homalomena
Furtadoa
Zantedeschia
Bognera
Gearum
Dieffenbachia
Mangonia
Incarum
Spathantheum
Gorgonidium
Croatiella
Synandrospadix
Spathicarpa
Taccarum
Asterostigma
Philonotion
Lagenandra
Cryptocoryne
Piptospatha
Schottariella
Rhymatarum
Schismatoglottis
Aridarum
Bucephalandra
Bakoa
Calla2
Calla
Amorphophallus
Pseudodracontium
Jasarum
Hapaline
Caladium
Syngonium
Filarum
Ulearum
Xanthosoma
Chlorospatha
Scaphispatha
Zomicarpella
Zomicarpa
collenchyma type Sv in petiole (67-4)
Arisarum
Ambrosina
Peltandra
Typhonodorum
Arophyton
Colletogyne
Carlephyton
Protarum
Pistia
Ariopsis
Colocasia
Remusatia
Steudnera
Alocasia
Arisaema
Pinellia
Lazarum
Theriophonum
Typhonium
Sauromatum
Eminium
Helicodiceros
Biarum
Dracunculus
Arum
LATICIFERS (18)
absent or only scattered cells
present, simple, articulated
present, anastomosing
SPOROPOLLENIN (75)
absent
present
present and/or absent
POLLEN APERTURE (8)
multiaperturate
zonate
extended monosulcate
monosulcate
inaperturate
diaperturate
forate
ulcerate
EXINE SURFACE (12)
spinose
smooth
reticulate or other
striate
SPADIX ZONATION (44)
no zonation
male-female
female-sterile-male
female-male-sterile
female-sterile-male-sterile
TIPS
trichosclereids large, not in bundles (3-1)
leaf spongy aerenchyma type 4 (64-3)
endothecial thickenings absent (16-1)
spathe with internal flap (41-1)
BRANCHES
stem reduced to minute button (32-3)
bisexual (1-0) climbers (33-3, 33-4)
basipetal flowering sequence (46-1)
petiole collenchyma type Sb (67-2)
resin canals present in roots (17-1)
stamens with horned thecae (48-1)
staminodes hair-like, subulate, bristle-like or elongated (58-1)

uniquely by Sb type petiole collenchyma in most genera. It is composed of two previously recognized groups, the Aglaonemateae (clade 9) and the Nephthytideae (clade 10). The Aglaonemateae are supported synapomorphically by boat-shaped spathes, which are soon deciduous or marcescent, and Sb leaf collenchyma. The latter could be a synapomorphy of the *Anchomanes* clade (26), as it also occurs in *Pseudohydrosme*. The phenotypic synapomorphy of the Nephthytideae consists of the very strongly developed basal ribs of the leaves (char. 24-1), which have dracontioid lobing in two genera.

The *Homalomena* clade (27) is supported by synapomorphic anatomical character states observed by French (1985, 1987a, b), including the occurrence of sclerotic hypodermis and resin canals in the roots and absence of endothecial thickenings in the anthers (except in *Homalomena* itself). It is composed of the Culcasieae (clade 11) and the *Philodendron* clade (12), the former supported synapomorphically by hemiepiphytic climbing habit, the latter by female-sterile-male spadix floral zonation. The recent molecular phylogeny of *Philodendron* by Gauthier et al. (2008) has confirmed its close relationship with Neotropical *Homalomena* species.

The Spathicarpeae (clade 13) have been thoroughly studied by Gonçalves and colleagues, whose work provided strong molecular support for the inclusion of *Bognera* and *Dieffenbachia* (formerly in tribe Dieffenbachieae), which are vegetatively distinct from the other genera of the tribe. The Spathicarpeae are supported synapomorphically by connate stamens and a chromosome base number of $x = 17$ (Gonçalves, 2002; Gonçalves et al., 2007).

The *Philonotion* clade (38) lacks phenotypic support and is made up essentially of a trichotomy of the genus *Calla* with two robust groups, the Rheophytes clade (28) and the *Dracunculus* clade (37). In our ML tree (Fig. 1), *Calla* is sister to the *Dracunculus* clade but with only weak support (see below).

The Rheophytes clade (28) corresponds closely to subfamily Schismatoglottidoideae as recognized by Keating (2002, 2004) but now includes the genus *Philonotion,* which was resurrected by Wong et al. (2010). Clade 28 has emerged consistently in molecular analyses (French et al., 1995; Cabrera et al., 2008) and includes a high concentration of rheophytes and aquatics (char. 35-3 or -4). Stamens with horned thecae represent the only (but equivocal) phenotypic synapomorphy for the clade. The two main subclades are the Cryptocoryneae (clade 14) and the Schismatoglottideae (clade 15). Clade 14 is composed of helophytes or submerged aquatics, and is well supported by the phenotypic synapomorphies of absence of laticifers, presence of intravaginal squamules, the spathe and spadix forming two distinct chambers by partial fusion, the spathe with an internal flap and with connate margins, chromosome base number $x = 18$ and type Sv leaf blade and petiole collenchyma. The Schismatoglottideae are supported phenotypically by the synapomorphies rheophytic habit (most genera), long ligules at the apex of the leaf sheath, more-or-less erect aerial stems and the spathe with a persistent basal half and soon-deciduous or marcescent upper blade.

The *Dracunculus* clade (37) may represent another important adaptive shift in the evolution of the aroids. It corresponds to subfamily Aroideae as circumscribed by Keating (2002, 2004) and is supported by the following phenotypic synapomorphies: presence in most genera of a sympodial inframarginal vein on each side of the leaf and type Sv collenchyma (≡colocasioid collenchyma of Gonçalves et al., 2004) in the leaf blade and petiole. This clade also includes most genera with spinose pollen exine (char. 12-2; see also remarks by Hesse, 2006c), and while various patterns are present, spinose exine is otherwise typical only in the Lemnoideae. Although the clade is notable for the occurrence of osmophoric spadix appendices (Vogel, 1963), these are absent in 14 of the 37 genera and also occur elsewhere in several genera of Schismatoglottideae (clade 15, *Aridarum, Bakoa, Bucephalandra, Schismatoglottis, Phymatarum*) and in a few species of *Homalomena* and *Philodendron*. Given that type Sv collenchyma also occurs in a minority of genera in the Rheophytes clade (28), it is possible that future studies will show both spadix appendix and Sv collenchyma to be synapomorphies of the higher *Philonotion* clade (38).

Clade 37 is composed of two subgroups, the *Amorphophallus* clade (35) and the *Ambrosina* clade (36), both of which are poorly characterized phenotypically, with no phenotypic synapomorphies in our result. Clade 35 is composed of two subgroups, the Thomsonieae (clade 16) and the Caladieae (clade 17). The Thomsonieae are supported by the following phenotypic synapomorphies: tripartite primary-leaf development with decompound subdivision that make the leaf together with the involute venation synapomorphic for the group, the absence of a sterile zone between male and female zones in many of the species, and presence of a spadix appendix, which may be smooth or staminodial. Clade 17, the Caladieae in the expanded sense of Keating (2002, 2004), is supported synapomorphically by presence of anastomosing laticifers and a persistent spathe tube with soon-deciduous or marcescent spathe blade.

The *Ambrosina* clade (36) is composed of the *Colletogyne* clade (33) and the *Pistia* clade (34), neither of which has support from phenotypic synapomorphies. Clade 33 is a group of plants with subterranean stems, which brings together two well-characterized and previously recognized tribes, Arisareae (clade 18) and Arophyteae (clade 19). The Mediterranean Arisareae are supported by striate pollen exine, connate spathe margins, and adnation of the spadix female zone to the spathe. Clade 19 forms part of the *Typhonodorum* clade (29), the latter supported by presence of staminodes in the female zone, endosperm sparse to absent with a relatively large embryo and a storage cotyledon. The Arophyteae (clade 19), endemic to Madagascar, are supported by globose pollen with spinose exine, female-male spadix zonation, and stamens connate by their filaments. The previously recognized tribe Peltandreae (e.g., Mayo et al., 1997) did not emerge in our result, although it has in some previous analyses (e.g., Renner and Zhang, 2004; Cusimano et al., 2008). The two other components of clade 29, the genera *Peltandra* and *Typhonodorum*, are helophytes with parallel-pinnate fine venation, spathes with a persistent tube and deciduous or

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Fig. 2. Selected morphological/anatomical synapomorphic character states mapped on the ML phylogeny by labeling nodes (blue dots), by coloring branch stems or branch tips, or by plotting columns of squares corresponding to each genus with color-coded states (see legend; associated numbers in brackets are character numbers with respective character state as in Appendix S1). Gray boxes comprise all bisexual-flowered taxa. Nodes of interest are numbered according to the 44 clades defined in Figure 1 and Table 1.

marcescent blade, female-sterile-male-sterile spadix zonation, staminodial spadix appendices, and entirely connate stamens.

The *Pistia* clade (34) is supported by no phenotypic synapomorphies in our result. It is nevertheless strongly supported by molecular data and was previously recognized by Renner and Zhang (2004). Its two main subgroups are the *Colocasia* clade (20) and the *Alocasia* clade (30). Clade 20 is supported synapomorphically only by peltate leaves, although most genera also have anastomosing laticifers (not *Ariopsis*), "colocasioid" fine leaf venation (char. 28-2), and entirely connate stamens. This topology separates *Alocasia* from *Colocasia,* although in most past classifications (e.g., GoA) these two genera have been considered closely related. There are many characters separating the two genera, including shoot architecture. The *Alocasia* clade (30) has no phenotypic synapomorphies.

The most distinctive subgroup of the *Alocasia* clade is the Areae (clade 21), supported synapomorphically, however, only by its bristle- or hair-like staminodes that otherwise appear only in the closely related genus *Arisaema*. Taxonomically the Areae are notable for spinose pollen exine, reticulate fine leaf venation, mostly depressed-globose, subterranean stems, smooth spadix appendices (exceptions in *Helicodiceros* and some *Eminium*) and strictly orthotropous ovules. This is a long-recognized group, stretching from Europe and the Canary Islands to tropical Southeast Asia and Australia.

In the *Dracunculus* clade (37), synandria (char. 50, a single structure formed by a partially or completely fused androecium) are common, predominating in clades 17, 20, 33, *Protarum, Pistia* and the genus *Alocasia*. Nearly all *Arisaema* species have at least partially fused stamens, whereas *Pinellia* and the Areae (clade 21) have free stamens. In view of this, it would be interesting to investigate these structures from a developmental standpoint.

Adaptation to water-associated life forms—As previously noted by Cabrera et al. (2008), water-associated life forms of various kinds occur throughout the Araceae in all major clades, even in the Areae (clade 21, *Typhonium flagelliforme*). Clades that are entirely or mostly water-associated are the Orontioideae (clade 1), the Lemnoideae (clade 2), the Lasioideae (clade 7), and the Rheophytes (clade 28). Individual aquatic or helophytic genera are often found embedded in otherwise nonaquatic clades, such as *Jasarum* (Caladieae, clade 17), *Aglaodorum* (clade 9), some species of *Dieffenbachia* (Spathicarpeae, clade 13), *Homalomena* in the *Philodendron* clade (12), *Peltandra* and *Typhonodorum* in the *Colletogyne* clade (33), and *Pistia* in the *Pistia* clade (34). Even in the Bisexual Climbers clade (31), dominated by hemiepiphytes and epiphytes, the Spathiphylleae (clade 5) stand out as a largely helophytic group. The genera *Anubias, Montrichardia,* and *Calla* are also helophytic and rather isolated, failing to group consistently in most analyses hitherto. It thus seems possible that a major theme in the phylogeny of the Araceae has been a complex evolution to and from water-associated life forms, either to become more extreme aquatics like *Pistia, Jasarum,* or the strictly rheophytic Schismatoglottids, or toward dry-land geophytes and epiphytism of various kinds. This is supported by our tree in which the helophytic habit (char. 35-1) is plesimorphic from the root to clade 37.

Homoplasious characters suggest repeated adaptative shifts—Some characters that have been used taxonomically in the past for grouping genera into higher taxa are revealed to be very homoplasious, but as yet they are hard to interpret adaptively. For example, Engler (1920) relied on parallel-pinnate (striate) venation (char. 28-1) to group the genera of his subfamily Philodendroideae. Leaf fenestration (char. 29-1), an even more conspicuous character, appears to have evolved independently at least six times and aculeate petioles and stems (char. 30-1) at least five times. Other characters, easier to interpret adaptively, show interesting patterns of homoplasy. Depressed-globose tubers (char. 32-2), while common in the *Dracunculus* clade, have also evolved elsewhere in the phylogeny, which suggests repeated episodes of adaptation to highly seasonal habitats. Other patterns suggest that more work is needed, especially in developmental morphology, to correctly identify homologous states of certain characters, for example, the homoplasious evolution of sterile spadix zones (char. 44), spadix appendices (char. 45), thickened (osmophoric) stamen connectives (char. 49), and stamen connation (char. 50) in the Unisexual Flowers clade.

Toward a new formal classification—Most of the 44 clades we have discussed should, in our opinion, be considered elements of a future formal classification, given that they have strong molecular support and mostly distinct phenotypes. The majority have been named and described earlier, but 16 clades are circumcribed informally here for the first time. Our analyses support most of the taxonomic proposals made by Cabrera et al. (2008), who discussed in detail the probable evolutionary pathways of the Araceae, especially in relation to aquatic adaptation. Our tree differs in placing the Lasioideae (clade 7) as sister to the Unisexual Flowers clade (clade 40), with the *Stylochaeton* clade (25) sister to the Aroideae (clade 39).

In our molecular ML tree, only the Unisexual Flowers clade (40) has poor statistical support, even though the distribution of unisexual flowers (if *Calla* is excluded) and several other morphological synapomorphies seem to make it a distinct taxonomic entity. Despite the weakness of the *Calla–Dracunculus* clade, support for clades 38 and 39 is good, resulting in a strong molecular argument for including *Calla* in the Aroideae. But there is reason to remain skeptical of the position of *Calla,* at least pending further confirmatory evidence. The topology jars with the distinct phenotype of the unisexual-flowered aperigoniate Araceae, whose evolution appears to be a key event in aroid phylogeny (Hesse 2006a, b, c). This is clearly a problem of great interest. Accepting the inclusion of *Calla* within the Aroideae appears to require bisexual flowers, aperturate pollen, and a massive tectate sporopollenin ektexine to have re-evolved from unisexual-flowered, omniaperturate, sporopollenin-less ancestors. This seems highly unlikely because it would require the reversal of a whole "character package" to the character states of earlier-diverging taxa. Additionally, the evolution of bisexual flowers from unisexual ones has, to our knowledge, not yet been reported. Although sporopollenin occurs in the exine of a few genera in the Aroideae (e.g., in the spines of *Remusatia* and *Zomicarpella*), it takes a form quite different from the tectate ektexine of bisexual-flowered genera, which *Calla* most resembles. The absence of collenchyma in *Calla* (Keating, 2000, 2002) also argues against its placement in the Aroideae, since the Unisexual Flowers clade is characterized by possession of type B, Bi, Sb, or Sv collenchyma. Biforines are also absent in *Calla*—although, because they are only patchily present in the Aroideae, this is weak evidence. Possession of simple articulated laticifers is the one important morpho-anatomical character state supporting the inclusion of *Calla* in the Aroideae,

but even this is weakened by the presence of laticifers in *Orontium* in the Proto-Araceae.

In addition, *Calla* emerged in quite different positions in the three main analyses we carried out. In the morpho-anatomical analysis, *Calla* is sister to the duckweeds (Appendix S3), whereas in the restriction-site analysis (Appendix S4) it is sister to the Unisexual Flowers clade (40). All the phylogenies based on sequence data—MP (Cabrera et al., 2008), ML, and Bayesian inference—place *Calla* within the Aroideae (Fig. 1). This lack of agreement between the three data sets regarding *Calla* is striking, given that all the data sources used reflect, directly or indirectly, different sampling of the genomic diversity. In our opinion, the phenotype of *Calla* suggests a position in the "transition zone" between the bisexual taxa and unisexual clades similar to that of the *Stylochaeton* clade (25).

There are some other outstanding questions. It has so far proved impossible with molecular markers to clarify the branching pattern in the following cases: (1) *Protarum, Pistia,* and the rest of the *Pistia* clade; (2) the relationships of *Arisaema, Pinellia,* and the Areae (clade 21), which Renner and Zhang (2004) and Renner et al. (2004) also highlighted as problematic; contrary to recent classifications (Keating, 2004; Bogner and Petersen, 2007), *Arisaema* and *Pinellia* do not group into a unique clade; and (3) the branching between the genera *Callopsis, Anubias,* and *Montrichardia* and the rest of the Aroideae. It seems that the genera *Callopsis, Anubias, Montrichardia, Calla, Alocasia, Protarum,* and *Pistia* might best be treated as monogeneric higher taxa, because the strong support for most major clades leaves them rather isolated phylogenetically.

Although our study is the most comprehensive yet presented in its use of different classes of data, there is still a need to resolve the questions highlighted here before presenting a new formal classification. A consistent position needs to be established for *Calla,* and new work is needed on the relationships of clades within the Aroideae (clade 39). Analyses of phylogenetic relationships within clades at a finer taxonomic scale may reveal further genera or suppress others, but given the generally high level of agreement between the earliest molecular analysis (French et al., 1995) and the one presented here, based largely on Cabrera et al. (2008), it would be surprising if new work contradicted the general cladistic patterns of aroid phylogeny as now understood. These questions, among others, will be the subject of the recently initiated Genera of Araceae 2 (GoA2) project, one of the outputs of which will be a new formal classification for the Araceae.

The most probable source of real phylogenetic novelties is likely to be the discovery of new aroid fossils. In recent years, this has become an exciting field, yielding a number of remarkable finds with important implications not only for Araceae but for the evolution of monocots as a whole (e.g., Smith and Stockey, 2003; Friis et al., 2004; Bogner et al., 2005; , Wilde et al., 2005; Stockey et al., 2007; Herrera et al., 2008). The importance of fossils further emphasizes the need for greater activity in the comparative study and classification of phenotypic character data of extant species, to be able to analyze the phylogenetic position of fossils with greater sophistication. Particularly important fields of study involve leaf venation, seed structure, and pollen structure. While the latter two areas have been the foci of important studies in recent years (e.g., Grayum, 1992; Seubert, 1993; Hesse et al., 2001; Hesse, 2002, 2006a, b, c; Tillich, 2003), comparative leaf venation of the Araceae has generally been neglected since the monograph by Ertl (1932).

The morpho-anatomical matrix presented here (Appendices S1, S2) is a compilation from many different sources but expresses only very approximately the structural variability of the family. The spectrum of character variability and the character analyses employed are likely to change considerably with new research. New initiatives on the Internet (e.g., http://www.morphobank.org/) make it possible to build a more complete database as a collective enterprise, with entries fully documented to specimens and images and fully credited to every contributor. We hope that the compilation and electronic publication of such mega-matrix resources will increasingly become a major focus for collaborative taxonomic work and, thus, provide a more comprehensive foundation for understanding the phylogeny and evolution of the aroids.

LITERATURE CITED

BARABÉ, D., A. BRUNEAU, F. FOREST, AND C. LACROIX. 2002. The correlation between development of atypical bisexual flowers and phylogeny in the Aroideae (Araceae). *Plant Systematics and Evolution* 232: 1–19.

BARFOD, A., J. I. DAVIS, G. PETERSEN, AND O. SEBERG. 2010. Diversity, phylogeny and evolution in the monocotyledons. Aarhus University Press, Aarhus, Denmark.

BOGNER, J. 2009. The free-floating Aroids (Araceae)—living and fossil. *Zitteliana* A48: 113–128.

BOGNER, J., AND E. G. GONÇALVES. 2005. Two new species of *Xanthosoma (Araceae)* from South America and notes on the tribe Caladieae. *Willdenowia* 35: 333–344.

BOGNER, J., G. L. HOFFMAN, AND K. R. AULENBACK. 2005. A fossilized aroid infructescence, *Albertarum pueri* gen. nov. et sp. nov. of Late Cretaceous (Late Campanian) age from the Horseshoe Canyon Formation of southern Alberta, Canada. *Canadian Journal of Botany* 83: 591–598.

BOGNER, J., AND D. H. NICOLSON. 1991. A revised classification of Araceae with dichotomous keys. *Willdenowia* 21: 35–50.

BOGNER, J., AND G. PETERSEN. 2007. The chromosome numbers of the aroid genera. *Aroideana* 30: 82–90.

BOYCE, P. C., AND S. Y. WONG. 2008. Studies on Schismatoglottideae (Araceae) of Borneo VII: *Schottarum* and *Bakoa,* two new genera from Sarawak, Malaysian Borneo. *Botanical Studies (Taipei, Taiwan)* 49: 393–404.

BOYCE, P. C., AND S. Y. WONG. 2009. *Schottariella mirifica* P.C. Boyce and S.Y. Wong: a new name for *Schottarum sarikeense* (Araceae: Schismatoglottideae). *Botanical Studies (Taipei, Taiwan)* 50: 269–271.

CABRERA, L. I., G. A. SALAZAR, M. W. CHASE, S. J. MAYO, J. BOGNER, AND P. DAVILA. 2008. Phylogenetic relationships of aroids and duckweeds (Araceae) inferred from coding and noncoding plastid DNA. *American Journal of Botany* 95: 1153–1165.

CUSIMANO, N., M. BARRETT, W. L. A. HETTERSCHEID, AND S. S. RENNER. 2010. A phylogeny of the Areae (Araceae) implies that *Typhonium, Sauromatum* and the Australian species of *Typhonium* are distinct clades. *Taxon* 59: 439–447.

CUSIMANO, N., L.-B. ZHANG, AND S. S. RENNER. 2008. Reevaluation of the *cox1* group I intron in Araceae and angiosperms indicates a history dominated by loss rather than horizontal transfer. *Molecular Biology and Evolution* 25: 265–276.

ENGLER, A. 1877. Vergleichende Untersuchungen über die morphologischen Verhältnisse der Araceae. II. Theil. Über Blattstellung und Sprossverhältnisse der Araceae. *Nova Acta Leopoldina. Abhandlungen der Kaiserlich Leopoldinisch-Carolinisch Deutschen Akademie der Naturforscher* 39: 158–232.

ENGLER, A. 1920. *Araceae—Pars generalis et index familiae generalis. In* A. Engler [ed.], Das Pflanzenreich, vol. 74 (IV. 23A), 1–71. Wilhelm Engelmann, Leipzig, Berlin, Germany.

ERTL, P. 1932. Vergleichende Untersuchungen über die Entwicklung der Blattnervatur der Araceen. *Flora* 126: 115–248.

FELSENSTEIN, J. 1973. Maximum likelihood and minimum-steps methods for estimating evolutionary trees from data on discrete characters. *Systematic Zoology* 22: 240–249.

FRENCH, J. C. 1985. Patterns of endothecial wall thickenings in Araceae: subfamilies Calloideae, Lasioideae and Philodendroideae. *Botanical Gazette (Chicago)* 146: 521–533.

FRENCH, J. C. 1987a. Systematic occurrence of a sclerotic hypodermis in roots of Araceae. *American Journal of Botany* 74: 891–903.

FRENCH, J. C. 1987b. Systematic survey of resin canals in roots of Araceae. *Botanical Gazette (Chicago)* 148: 360–371.

FRENCH, J. C., M. G. CHUNG, AND Y. K. HUR. 1995. Chloroplast DNA phylogeny of the Ariflorae. *In* P. J. Rudall, P. Cribb, D. F. Cutler, and C. J. Humphries [eds.], Monocotyledons: Systematics and evolution, vol. 1, 255–275. Royal Botanic Gardens, Kew, U.K.

FRIIS, E. M., K. R. PEDERSEN, AND P. R. CRANE. 2004. Araceae from the Early Cretaceous of Portugal: evidence on the emergence of monocotyledons. *Proceedings of the National Academy of Sciences, USA* 101: 16565–16570.

GAUTHIER, M. P. L., D. BARABÉ, AND A. BRUNEAU. 2008. Molecular phylogeny of the genus *Philodendron* (Araceae): Delimitation and infrageneric classification. *Botanical Journal of the Linnean Society* 156: 13–27.

GONÇALVES, E. G. 2002. Sistemática e evolução da tribo Spathicarpeae (Araceae). Ph.D. dissertation, Universidade de São Paulo, São Paulo, Brazil.

GONÇALVES, E. G., S. J. MAYO, M.-A. VAN SLUYS, AND A. SALATINO. 2007. Combined genotypic-phenotypic phylogeny of the tribe Spathicarpeae (Araceae) with reference to independent events of invasion to Andean regions. *Molecular Phylogenetics and Evolution* 43: 1023–1039.

GONÇALVES, E. G., E. A. S. PAIVA, AND M. A. NADRUZ COELHO. 2004. A preliminary survey of petiolar collenchyma in the Araceae. *Annals of the Missouri Botanical Garden* 91: 473–484.

GRAYUM, M. H. 1984. Palynology and phylogeny of the Araceae. Ph.D. dissertation, University of Massachusetts, Amherst, Massachusetts, USA.

GRAYUM, M. H. 1990. Evolution and phylogeny of the Araceae. *Annals of the Missouri Botanical Garden* 77: 628–697.

GRAYUM, M. H. 1992. Comparative external pollen ultrastructure of the Araceae and putatively related taxa. *Monographs in Systematic Botany from the Missouri Botanical Garden* 43: 1–167.

HAY, A. 1986. *Cyrtosperma* Griff. and the origin of the aroids. Ph.D dissertation, Oxford University, Oxford, UK.

HAY, A. 1988. *Cyrtosperma* (Araceae) and its old world allies. *Blumea* 33: 427–469.

HAY, A. 1992. Tribal and subtribal delimitation and circumscription of the genera of Araceae tribe Lasieae. *Annals of the Missouri Botanical Garden* 79: 184–205.

HAY, A., AND D. J. MABBERLEY. 1991. 'Transference of function' and the origin of aroids: Their significance in early angiosperm evolution. *Botanische Jahrbucher fur Systematik, Pflanzengeschichte und Pflanzengeographie* 113: 339–428.

HERRERA, F. A., C. A. JARAMILLO, D. L. DILCHER, S. L. WING, AND C. GÓMEZ-N. 2008. Fossil Araceae from a Paleocene Neotropical rainforest in Colombia. *American Journal of Botany* 95: 1569–1583.

HESSE, M. 2002. The uniquely designed pollen aperture in Lasioideae (Araceae). *Aroideana* 25: 51–59.

HESSE, M. 2006a. Conventional and novel modes of exine patterning in members of the Araceae—the consequences of ecological paradigm shifts? *Protoplasma* 228: 145–149.

HESSE, M. 2006b. Pollen wall ultrastructure of Araceae and Lemnaceae in relation to molecular classifications. *Aliso* 22: 204–208.

HESSE, M. 2006c. Reasons and consequences of the lack of a sporopollenin ektexine in Aroideae (Araceae). *Flora* 201: 421–428.

HESSE, M., J. BOGNER, H. HALBRITTER, AND M. WEBER. 2001. Palynology of the perigoniate Aroideae: *Zamioculcas, Gonatopus* and *Stylochaeton* (Araceae). *Grana* 40: 26–34.

HESSE, M., AND R. ZETTER. 2007. The fossil pollen record of Araceae. *Plant Systematics and Evolution* 263: 93–115.

HUELSENBECK, J. P., AND F. RONQUIST. 2001. MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics (Oxford, U.K.)* 17: 754–755.

KEATING, R. C. 2000. Collenchyma in Araceae: Trends and relation to classification. *Botanical Journal of the Linnean Society* 134: 203–214.

KEATING, R. C. 2002. Acoraceae and Araceae. *In* M. Gregory and D. F. Cutler [eds.], Anatomy of the monocotyledons, vol. 9. Oxford University Press, Oxford, U.K.

KEATING, R. C. 2004. Vegetative anatomical data and its relationship to a revised classification of the genera of Araceae. *Annals of the Missouri Botanical Garden* 91: 485–494.

LANDOLT, E. 1986. Biosystematic investigations in the family of duckweeds (Lemnaceae) (vol. 2). *In* E. Landolt [ed.], The family of Lemnaceae—a monographic study, vol. 1, 566. Veröffentlichungen des Geobotanischen Instituts der ETH, Stiftung Rübel, Heft 71, Zürich, Switzerland.

LANDOLT, E. 1998. Lemnaceae. *In* K. Kubitzki [ed.], The families and genera of vascular plants. IV. Flowering plants. Monocotyledons. Alismatanae and Commelinanae (except Gramineae), 264–270. Springer, Berlin, Germany.

LANDOLT, E., AND R. KANDLER. 1987. Biosystematic investigations in the family of duckweeds (Lemnaceae) (vol.4). *In* E. Landolt [ed.], The family of Lemnaceae—a monographic study. vol. 2, 638. Veröffentlichungen des Geobotanischen Instituts der ETH, Stiftung Rübel, Heft 95, Zürich, Switzerland.

LES, D. H., D. J. CRAWFORD, E. LANDOLT, J. D. GABEL, AND R. T. KIMBALL. 2002. Phylogeny and systematics of Lemnaceae, the duckweed family. *Systematic Botany* 27: 221–240.

MADDISON, W. P., AND D. R. MADDISON. 2005. MacClade: Analysis of phylogeny and character evolution, version 4.08. Sinauer, Sunderland, Massachusetts, USA.

MANSION, G., G. ROSENBAUM, N. SCHOENENBERGER, G. BACCHETTA, J. A. ROSSELLO, AND E. CONTI. 2008. Phylogenetic analysis informed by geological history supports multiple, sequential invasions of the Mediterranean basin by the angiosperm family Araceae. *Systematic Biology* 57: 269–285.

MAYO, S. J., J. BOGNER, AND P. C. BOYCE. 1997. The genera of Araceae. The Trustees, Royal Botanic Gardens, Kew, U.K.

NYLANDER, J. A. A., J. C. WILGENBUSCH, D. L. WARREN, AND D. L. SWOFFORD. 2008. AMTY (are we there yet?): A system for graphical exploration of MCMC convergence in Bayesian phylogenetics. *Bioinformatics (Oxford, U.K.)* 24: 581–583.

RAY, T., AND S. S. RENNER. 1990. Comparative studies on the morphology of the Araceae. Transl. from A. Engler, 1877, with an introduction, updated nomenclature and a glossary. *Englera* 12: 1–140.

RENNER, S. S., AND L.-B. ZHANG. 2004. Biogeography of the *Pistia* clade (Araceae): Based on chloroplast and mitochondrial DNA sequences and Bayesian divergence time inference. *Systematic Biology* 53: 422–432.

RENNER, S. S., L.-B. ZHANG, AND J. MURATA. 2004. A chloroplast phylogeny of *Arisaema* (Araceae) illustrates tertiary floristic links between Asia, North America, and East Africa. *American Journal of Botany* 91: 881–888.

RONQUIST, F., AND J. P. HUELSENBECK. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics (Oxford, U.K.)* 19: 1572–1574.

ROTHWELL, G. W., M. R. VAN ATTA, H. W. BALLARD JR., AND R. A. STOCKEY. 2004. Molecular phylogenetic relationships among Lemnaceae and Araceae using the chloroplast *trnL–trnF* spacer. *Molecular Phylogenetics and Evolution* 30: 378–385.

RUDALL, P. J., P. J. CRIBB, D. F. CUTLER, AND C. J. HUMPHRIES. 1995. Monocotyledons: Systematics and evolution. 2 vols. Royal Botanic Gardens, Kew, U.K.

SEUBERT, E. 1993. Die Samen der Araceen: Die Samenmerkmale der Araceen und ihre Bedeutung für die Gliederung der Familie. Ph.D. dissertation, University of Kaiserslautern, Kaiserslautern, Germany. Koeltz Scientific Books, Königstein, Germany.

SIMMONS, M. P. 2004. Independence of alignment and tree search. *Molecular Phylogenetics and Evolution* 31: 874–879.

SIMMONS, N. B. 2001. Misleading results from the use of ambiguity coding to score polymorphisms in higher-level taxa. *Systematic Biology* 50: 613–620.

SMITH, S. Y., AND R. A. STOCKEY. 2003. Aroid seeds from the Middle Eocene Princeton Chert (*Keratosperma allenbyense*, Araceae): Comparisons with extant Lasioideae. *International Journal of Plant Sciences* 164: 239–250.

STAMATAKIS, A., P. HOOVER, AND J. ROUGEMONT. 2008. A fast bootstrapping algorithm for the RAxML web-servers. *Systematic Biology* 57: 758–771.

STEVENS, P. F. 2001. Angiosperm Phylogeny Website, version 10. [More or less continuously updated.] Available at http://www. mobot.org/MOBOT/research/APweb/.

STOCKEY, R. A., G. L. HOFFMAN, AND G. W. ROTHWELL. 1997. The fossil *Limnobiophyllum scutatum*: resolving the phylogeny of Lemnaceae. *American Journal of Botany* 84: 355–368.

STOCKEY, R. A., G. W. ROTHWELL, AND K. R. JOHNSON. 2007. *Cobbania corrrugata* gen. et comb. nov. (Araceae): A floating aquatic monocot from the Upper Cretaceous of western North America. *American Journal of Botany* 94: 609–624.

TAM, S., P. BOYCE, T. UPSON, D. BARABÉ, A. BRUNEAU, F. FOREST, AND J. PARKER. 2004. Intergeneric and infrafamilial phylogeny of subfamily Monsteroideae (Araceae) revealed by chloroplast *trnL-F* sequences. *American Journal of Botany* 91: 490–498.

TARASEVICH, V. F. 1988. Peculiarities of morphology in ridged pollen grains in some representatives of Araceae. *In* A. F. Chlonova [ed.], Palynology in the USSR, papers of the Soviet palynologists to the VII International Palynological Congress Brisbane, Australia, 58–61. Nauka Novosibirsk, Russia.

TILLICH, H.-J. 2003. Seedling diversity in Araceae and its systematic implications. *Feddes Repertorium* 114: 454–487.

VOGEL, S. 1963. Duftdrüsen im Dienste der Bestäubung: Über Bau und Funktion der Osmophoren. *Abhandl. Math. -Naturwiss. Kl. Akad. Wiss. Mainz* 1962: 599–763. [English Edition translated and edited by Renner, S. S. 1990. The role of scent glands in pollination: On the structure and function of osmophores. Smithsonian Institution Libraries, Washington, D.C., USA.]

WILDE, V., Z. KVAČEK, AND J. BOGNER. 2005. Fossil leaves of the Araceae from the European Eocene and notes on other aroid fossils. *International Journal of Plant Sciences* 166: 157–183.

WILGENBUSCH, J. C., D. L. WARREN, AND D. L. SWOFFORD. 2004. AWTY: A system for graphical exploration of MCMC convergence in Bayesian phylogenetic inference. Available online at http://ceb.csit.fsu.edu/awty.

WONG, S. Y., P. C. BOYCE, A. SOFIMAN OTHMAN, AND C. P. LEAW. 2010. Molecular phylogeny of tribe Schismatoglottideae (Araceae) based on two plastid markers and recognition of a new tribe, Philonotieae, from the Neotropics. *Taxon* 59: 117–124.

YANG, Z. 1993. Maximum-likelihood estimation of phylogeny from DNA sequences when substitution rates differ over sites. *Molecular Biology and Evolution* 10: 1396–1401.

YANG, Z., AND B. RANNALA. 1997. Bayesian phylogenetic inference using DNA sequences: A Markov chain Monte Carlo method. *Molecular Biology and Evolution* 14: 717–724.

YOUNG, H. 1986. Beetle pollination in *Dieffenbachia longispatha* (Araceae). *American Journal of Botany* 73: 931–944.

APPENDIX 1. GenBank accession numbers and sources of the sequences of the newly added Araceae taxa.

Species	*matK*	*rbcL*	*tRNA-LEU*	*trnL-F* spacer, *tRNA-PHE*	Source
Anaphyllum wightii	HQ687766	—	—	—	Bogner 1833 (M)
Asterostigma cubense	EF173531	—	EF173566	—	Gonçalves et al., 2007
Bakoa lucens	GQ220894	—	—	GQ220962	Wong et al., 2010
Calla palustris	HQ687765				Bogner 2117 (M)
Croatiella integrifolia	EF173538	—	EF173573	—	Gonçalves et al., 2007
Furtadoa sumatrensis	HQ687767	—	—	—	M.Hotta s.n. (M)
Incarum pavonii	EF173548	—	—	—	Gonçalves et al., 2007
Philonotion americanum	GQ220908	—	—	GQ220978	Wong et al., 2010
Schottariella sarikeense	GQ220912	—	—	GQ221009	Wong et al., 2010
Theriophonum dalzelii	EU886534	—	AY249011	AY248973	Cusimano et al., 2010; Renner and Zhang, 2004
Typhonium brownii	EU886538	—	—	—	Cusimano et al., 2010
Typhonium hirsutum			AY249014	AY248976	Renner and Zhang, 2004
Typhonium horsfieldii	EU193593	EU193202			Mansion et al., 2008
Zomicarpa steigeriana	EU542592	—	—	—	Batista et al., DS