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1 **Target sequence capture data shed new light on the infrafamilial classification of Araceae**

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23 **Araceae phylogenomics**

24

25 **ABSTRACT**

26 **PREMISE:** Recent phylogenetic studies of the Araceae have confirmed the position of the
27 duckweeds nested within the aroids, and the monophyly of a clade containing all of the
28 unisexual flowered aroids plus the bisexual-flowered *Calla palustris*. The main objective of
29 this work was to better resolve the deep phylogenetic relationships among the main lineages
30 within the family, particularly the relationships between the eight currently recognized
31 subfamilies. We also aimed to confirm the phylogenetic position of the enigmatic genus *Calla*
32 in relation to the long-debated evolutionary transition between bisexual and unisexual flowers
33 in the family.

34 **METHODS:** Nuclear DNA sequence data were generated for 128 species across 111 genera
35 (78%) of Araceae using target sequence capture and the Angiosperms353 universal probe set.

36 **RESULTS:** The phylogenomic data confirmed the monophyly of the eight Araceae
37 subfamilies but the phylogenetic position of subfamily Lasioideae remains uncertain.
38 Zamiculcadoideae is not retrieved; and we expanded the delimitation of subfamily Aroideae
39 which still includes, as sister to the rest of the group, the genus *Calla* and defined the new
40 *Nepthytis bintuluensis*–*Aglaonema* Clade.

41 **CONCLUSIONS:** Our results strongly suggest that new research on African genera
42 (*Calloopsis*, *Nepthytis* and *Anubias*) and *Calla* will be important to understand the early
43 evolution of the Aroideae. Also, of particular interest is to resolve the phylogenetic positions
44 of the “satellite” genera *Montrichardia*, *Zantedeschia* and *Anchomanes*, the placement of
45 which remains only moderately supported.

46

47 **KEY WORDS**

48 Angiosperms353, nuclear phylogenomic tree, Alismatales, Lemnoideae.

49

50 **INTRODUCTION**

51
52 Araceae is a large, ancient family of monocots comprising 3,667 species assigned to 143
53 genera (Boyce and Croat, 2011 onwards), of which many are globally important in
54 horticulture and agriculture. They make up one of the most ecologically diverse families of
55 flowering plants, from the smallest known species (*Wolffia globosa* (Roxb.) Hartog & Plas) to
56 the largest unbranched inflorescence (*Amorphophallus titanum* (Becc.) Becc.), and with habits
57 ranging from floating aquatics, geophytes and large arborescent herbs to nomadic vines and
58 epiphytes. The family is cosmopolitan, although it is by far the most diverse in the wet
59 tropics, particularly South and Central America and Southeast Asia. There is much active
60 research on the taxonomy of the family with over 100 new species published in 2020 alone
61 (IPNI), mostly assigned to the genus *Anthurium*, and 21 new genera published in the past 10
62 years, mostly from Southeast Asia (IPNI).

63 The family is also notable for its extensive fossil history, which includes some of the
64 earliest known monocots in the fossil record, stretching back to the early Cretaceous (Friis et
65 al., 2004, 2010; Hesse and Zetter, 2007; Bogner, 2009; Iles et al., 2015; Smith, 2013; Mayo et
66 al., 2013; Hoffman, 2021). A recent review of aroid fossils, focusing on the early evolutionary
67 history of Araceae, found that the family was particularly diverse during the late Cretaceous
68 and early Paleocene, exhibiting combinations of morphological characters unknown in extant
69 Araceae (Stockey et al., 2021). The historical biogeography of Araceae has been analysed in
70 detail by Nauheimer et al. (2012), using a molecular analysis combined with fossil data and
71 computational modelling. They concluded that the early evolution of the family was linked to
72 aquatic or sub-aquatic environments and that early diversification within the family, which
73 led to the evolution of subfamilial lineages, took place during the Cretaceous and was
74 associated with the fragmentation of the supercontinent Pangaea.

75 There is a large body of work on the classification, phylogenetics and systematics of
76 Araceae (summarised by Mayo et al., 2013). The first detailed phylogenetic tree of the whole
77 family using computational analysis and including most accepted genera at the time was
78 presented by French et al. (1995), based on chloroplast restriction site data, following an
79 earlier study by Duvall et al. (1993), which included only nine genera. Various other
80 molecular phylogenetic analyses based on DNA restriction sites or sequence data were
81 published, but most used only a limited number of genera and with diverse research aims (e.g.
82 Sriboonma et al., 1993; Wen et al., 1996; Othman, 1997, Barabé et al., 2002; Grob et al.,
83 2002; Renner et al., 2004; Renner and Zhang, 2004; Rothwell et al., 2004; Tam et al., 2004;
84 Kitano et al., 2005; Nie et al., 2006; Gauthier et al., 2008; Mansion et al., 2008). The
85 landmark study of Cabrera et al. (2008), using plastid DNA sequence data and including most
86 genera then recognized, was crucial in establishing for the first time with confidence the
87 phylogenetic position of the duckweeds within Araceae (Lemnoideae or Lemnaceae; see also
88 Tippery et al., 2021). Studies on a similar scale then followed by Cusimano et al. (2011), who
89 focused on a comparison with morphological characters, and Nauheimer et al. (2012), who
90 investigated the historical biogeography of the family including fossil data. These three
91 family-wide studies were all based entirely on plastid sequence data. Chartier et al. (2014)
92 presented a phylogenetic analysis combining a nuclear marker, the gene *PhyC*, to six plastid
93 markers, with a focus on the reconstruction of the ancestral pollination systems in Araceae
94 and several associated floral traits. The first phylogenomic study of Araceae was carried out
95 by Henriquez et al. (2014) using high-throughput sequencing of combined plastid and
96 mitochondrial data, but with a relatively small sample of only 32 genera in addition to five
97 obtained from public repositories. More recently, a phylogenetic tree based on whole plastid
98 genomes of 30 species with *Acorus americanus* (Raf.) Raf. as outgroup taxon, provided
99 additional information on the potential phylogenetic position of *Calla palustris* L., the sole

100 species of a genus whose placement within the family has long been uncertain and which is
101 consider key to understanding floral evolution in the family (Abdullah et al., 2020). Finally,
102 Zhao et al. (2022) investigated the role of whole-genome duplication in the evolution of the
103 family using transcriptome data for 60 species and 57 genera, but their study did not comprise
104 genus *Calla*.

105 The first subfamilial classification of Araceae, proposed by Engler (1876), was also
106 the first based on evolutionary principles (Mayo & Bogner 2013). It provided the framework
107 for studies of aroid systematics and evolution until the 1990s, when the first significant
108 molecular phylogenetic studies of the family were undertaken (Mayo et al. 2013). The most
109 recent studies provide support for the recognition of eight subfamilies (Cusimano et al., 2011;
110 Nauheimer et al., 2012; Chartier et al., 2014; Henriquez et al., 2014): Gymnostachydoideae,
111 Orontioideae, Lemnoideae, Pothoideae, Monsteroideae, Lasioideae, Zamioculcadoideae and
112 Aroideae. Lemnoideae is now widely accepted as a subfamily of Araceae, even if the
113 reinstatement of family Lemnaceae has been recently proposed (Tippery et al., 2021). The
114 monophyly of the clade comprising all the unisexual flowered aroids (i.e. subfamilies
115 Zamioculcadoideae and Aroideae) has reinforced the long-held view that possession of
116 unisexual flowers is a derived condition in Araceae, and may suggest a single evolutionary
117 event leading to monoecy in the family (cf. Hay, 2019; but see below discussion regarding the
118 position of *Stylochaeton* and tribe Zamioculcadeae).

119 The phylogenetic position of the monospecific genus *Calla* is of particular interest and
120 still largely debated. This species has aperigoniata bisexual flowers and is traditionally
121 thought (from a morphological point of view) to be a transition stage between genera with
122 perigoniata bisexual flowers and the large subfamily Aroideae comprising species with
123 unisexual, mainly aperigoniata flowers. Interestingly, molecular phylogenetic analyses to date
124 have placed *Calla palustris* consistently within Aroideae, only sometimes opting for a closer

125 position to the transition node between bisexual and unisexual flowers. Cusimano et al.
126 (2011) placed it within the Aroideae (sensu Mayo et al., 1997), sister to Cryptocoryneae and
127 Schismatoglottideae. One significant difference between Araceae phylogenetic trees based on
128 plastid data only (Cusimano et al., 2011; Nauheimer et al., 2012) and the phylogenetic study
129 based on combined nuclear *PhyC* and six plastid markers (Chartier et al., 2014) is that in the
130 latter, *Calla* was placed closer to the root of subfamily Aroideae with genera *Montrichardia*
131 and *Anubias*. Recently, despite their relatively small sample size, plastid genome-based
132 phylogenetic trees (Abdullah et al., 2020; Henriquez et al., 2020), recovered six monophyletic
133 subfamilies (Gymnostachydoideae and Pothoideae were not sampled), and placed *Calla*
134 within the Aroideae, relatively close to genus *Schismatoglottis*.

135 Here, we present a phylogenomic study of Araceae based on target sequence capture
136 using the universal Angiosperms353 probes (Johnson et al., 2019). We compare the resulting
137 phylogenetic tree to one obtained from the analysis of whole plastid genome data recovered
138 from the off-target reads combined to those available in public sequence repositories. The
139 Angiosperms353 probes were designed to selectively capture a large set (up to 353) of low-
140 copy nuclear orthologs that are expected to have high phylogenetic information content
141 (Baker et al., 2021; McDonnell et al., 2021). This high-throughput sequencing method has
142 been successful in resolving phylogenetic relationships at different taxonomic levels from
143 species up to orders (e.g. Murphy et al. 2020; Maurin et al. 2021), or even all angiosperms
144 (Baker et al., 2022). The present study is unique in terms of generic coverage, comprising 111
145 out of the 143 genera that the family currently includes, with all eight of the previously
146 recognized subfamilies represented. Our main objective was to resolve the deep phylogenetic
147 relationships within the family with strong support, particularly between the eight currently
148 recognised subfamilies and to confirm the phylogenetic position of the enigmatic genus *Calla*

149 in relation to the long-debated evolutionary transition in the family between perigoniate
150 bisexual flowers and aperigoniate unisexual flowers.

151

152 **MATERIALS AND METHODS**

153

154 *Sampling*

155 We selected at least one specimen per genus from a list of genera standardized according to
156 the World Checklist of Vascular Plants (WCVP, 2021) and the Plants of the World Online
157 (POWO, 2020). This initial list was supplemented with recommendations from taxonomic
158 specialists and information from Boyce & Croat (2011 onwards), and recent publications (e.g.
159 Low et al., 2018). In total 128 species in 111 genera were sampled, representing ca 78% of all
160 genera in the family (Appendix S1). Twelve species used as outgroup taxa from orders
161 Acorales and Alismatales were included (Appendix S1). *Acorus gramineus*, the sister group to
162 the remainder of monocotyledons (APG IV, 2016), was used to root all phylogenetic analyses.

163 Selection of plant tissue was based on the best available quality material considering
164 an ideal sample extraction should contain 100–200 ng of DNA with fragment sizes \geq 350bp
165 following the manufacturer recommendations regarding library preparations (New England
166 BioLabs, Ipswich, Massachusetts, USA). Material was selected from, in order of preference,
167 (1) fresh leaf tissue dried in silica gel, which generally gave optimal results; (2) DNA aliquots
168 from the bank at the Royal Botanic Gardens, Kew
169 (<https://dnabank.science.kew.org/homepage.html>), and (3) plant tissue material selected from
170 herbarium specimens, which yielded DNA of variable quality. In addition, we took advantage
171 of public data for three samples available in the Sequence Read Archive (SRA;
172 www.ncbi.nlm.nih.gov/sra; Appendix S1).

173

174 ***DNA Extraction, Library Preparation, Hybridization and Sequencing***

175 We performed DNA extractions using a modified CTAB protocol (Doyle and Doyle, 1987),
176 with samples purified using Mag-Bind TotalPure NGS magnetic beads (Omega Bio-tek,
177 Norcross, Georgia, USA). We ran the purified DNA extracts on a 1.5x agarose gel to assess
178 the average fragment size and then quantified them using a Qubit 3.0 fluorometer (Thermo
179 Fisher Scientific, Waltham, Massachusetts, USA). DNA extracts with fragment sizes > 350
180 bp were sonicated using a M220 Focused-ultrasonicator with microTUBEs AFA Fiber Pre-
181 Slit Snap-Cap (Covaris, Woburn, Massachusetts, USA), following the manufacturer's
182 protocol. Dual-indexed libraries for Illumina sequencing were prepared using the DNA
183 NEBNext Ultra™ II Library Prep Kit at half the recommended volume, with Dual Index
184 Primers Set 1, NEBNext Multiplex Oligos for Illumina (New England BioLabs, Ipswich,
185 Massachusetts, USA). Quality of libraries was evaluated on a 4200 TapeStation System using
186 High Sensitivity D1000 ScreenTape (Agilent Technologies, Santa Clara, California, USA)
187 and quantified using a Qubit fluorometer. Equimolar pools comprising 20-25 libraries for a
188 total of 1 µg of DNA were hybridized using the myBaits Expert Predesigned Panel (Arbor
189 Biosciences, Ann Arbor, Michigan, USA) Angiosperms353 v1 (Catalog #308196; Johnson et
190 al., 2019) following the manufacturer's protocol with v4 chemistry
191 (<http://www.arborbiosci.com/mybaits-manual>). Hybridizations were performed at 65°C for
192 28–32 h in a Hybex Microsample Incubator, using an equal volume of red Chill-out Liquid
193 Wax (Bio-Rad, Hercules, California, USA) to prevent evaporation. Enriched products were
194 amplified with KAPA HiFi (2x) HotStart ReadyMix PCR Kit (Roche, Basel, Switzerland) for
195 10 cycles. We purified the PCR products using the QIAquick PCR purification kit (Qiagen).
196 Products were quantified with the Qubit fluorometer and in some cases re-amplified a second
197 time for 3–8 cycles. Final products were run on a 4200 TapeStation System using High
198 Sensitivity D1000 ScreenTape (Agilent Technologies, Santa Clara, California, USA) to assess

199 quality and average fragment size. Sequencing was performed at Royal Botanic Gardens,
200 Kew on an Illumina MiSeq (Illumina, San Diego, California, USA) with v3 reagent chemistry
201 (2×300 bp paired-end reads) or on an Illumina HiSeq (2×150 bp paired-end reads) at
202 Genewiz (Takeley, UK) or at Macrogen (Geumcheon, Republic of Korea).

203

204 ***Sequence Data Processing***

205 Raw sequence reads (FASTQ files) were trimmed using Trimmomatic (Bolger et al., 2014)
206 with a leading and trailing end trim thresholds of 20 and 10, respectively, and a sliding
207 window trimming threshold of 20, averaged over 4 bp (LEADING: 20; TRAILING: 10;
208 SLIDING WINDOW:4:20). Trimmed reads shorter than 40 bp were then removed (MINLEN:
209 40).

210 Paired reads and combined unpaired reads were used to recover Angiosperms353
211 target genes (Johnson et al., 2019; <https://github.com/mossmatters/Angiosperms353>) using
212 HybPiper v1.3.1 (Johnson et al., 2016). The HybPiper BLASTN option was used to map the
213 reads to each of the target genes. The reads assigned to each target gene were then assembled
214 *de novo* using SPAdes (Bankevich et al., 2012) and coding sequences (hereafter called genes)
215 were extracted using exonerate (Slater and Birney, 2005). Non-coding sequences (i.e., introns
216 and UTRs) flanking the exons were recovered and combined with the exon sequences into so-
217 called supercontigs using the HybPiper intronerate.py script. Sequences from all taxa were
218 combined for each gene and supercontig data set. Each dataset was aligned separately using
219 MAFFT v7 (Katoh and Standley, 2013), with accuracy-oriented methods (--localpair; --
220 maxiterate 1000) and the option to generate reverse complement sequences to align them with
221 the remaining sequences based on 6-mer counting (--adjustdirectionaccurately). Single locus
222 alignments were subsequently trimmed using phyutility
223 (<https://github.com/blackrim/phyutility>) to remove nucleotide sites missing in at least 80% of

224 the taxa (-clean 0.8). Recovery statistics (Appendix S1) and heatmaps (Appendix S2) were
225 generated using scripts from HybPiper v1.3.1 (Johnson et al., 2016). Sequence data from the
226 three samples obtained from the SRA was processed using the approach described in Baker et
227 al. (2022).

228 GetOrganelle 1.7.5 (Jin et al., 2020) was used to assemble the plastid genome from
229 raw reads of target-capture samples. Additional plastid genomes were retrieved from the
230 National Center for Biotechnology Information (NCBI). These supplementary plastid
231 genomes came either from the Organelle Genome Resources
232 (<https://ftp.ncbi.nlm.nih.gov/refseq/release/plastid/>) and Genbank
233 (<https://www.ncbi.nlm.nih.gov/nucleotide/>), or from the Sequence Read Archive
234 (<https://www.ncbi.nlm.nih.gov/sra/>), in which case plastid genomes were also assembled from
235 raw reads using GetOrganelle. Plastid genomes were then queried against a reference set of
236 the 76 most common plastid genes with BLASTN (Camacho et al., 2009) to extract these
237 genes. The dataset was then filtered to keep only samples with a cumulative gene length
238 superior to 5.4kb (10% of the median cumulative gene length for angiosperms; K. Leempoel
239 pers. comm.). The pipeline used to extract plastid genes and build the tree (see below) is
240 available on GitHub (<https://github.com/RBGKew/cpDNA>).

241

242 ***Phylogenetic Reconstructions***

243 Two approaches were used to reconstruct species trees. First, trees were inferred by analyzing
244 all supercontig alignments (i.e., exons and their flanking regions) concatenated into one
245 matrix using FASconCAT-G v1.0 (Kück and Meusemann, 2010). A species tree was
246 generated based on this matrix using RAxML (Stamatakis, 2014), herein referred to as the
247 maximum likelihood (ML) approach. Branches with bootstrap percentages (BP) values > 90%

248 are considered strongly supported, between 90% and 70% as moderately supported and below
249 70% as weakly supported.

250 Second, species trees were inferred using a multispecies-coalescent approach.
251 Individual gene trees were first generated from each trimmed locus alignment using IQ-TREE
252 v2.0 (Minh et al., 2020), with ultrafast bootstrap (1000 replicates, UFBoot2; Chernomor et al.,
253 2016) and model selection (-m MFP). Branches with support values below 10% (Mirarab,
254 2019) were collapsed in each gene tree using Newick Utilities v1.6 (Junier and Zdobnov,
255 2010). A first coalescent analysis was performed after exclusion of genes with less than 25%
256 of the taxonomic sample and with alignments shorter than 100 bp using ASTRAL-III
257 (Mirarab and Warnow, 2015) with extensive branch annotations (-t 2 flag). These annotations
258 allowed recovery of both normalized quartet score (QS) values and local posterior
259 probabilities (LPP). Quartet Score (QS) are considered to indicate high congruence among
260 gene trees if $Q1 \geq 75$, moderate congruence if $Q1$ is ≥ 50 and < 75 and weak congruence if
261 $Q1 < 50$. LPP values above 0.95 are considered to represent strong support, $LPP \geq 0.90$ and
262 < 0.95 represent moderate support, whereas LPP values < 0.90 represent no support.

263 Gene trees were evaluated using TreeShrink (Mai and Mirarab, 2018) to identify
264 outlier taxa that increased the diameter of each gene tree (i.e., the maximum distance between
265 any two tips of the tree) by more than 20%, using centroid re-rooting (-b 20 -c). The second
266 and final coalescent analysis was conducted on this new set of gene trees, using the same
267 method as described above. A quartet-based polytomy test, used to evaluate gene tree
268 discordance in the dataset and identify potential hard polytomies, was conducted using
269 ASTRAL-III as described by Sayyari and Mirarab (2018). The test evaluates the null
270 hypothesis that a given branch is a polytomy.

271 For the plastid data, sequences from each gene were aligned independently in MAFFT
272 7.4 (Kato and Standley, 2013), using the adjustdirection and auto options, then trimmed with

273 Trimal 1.3 (Capella-Gutiérrez et al., 2009) with the gappyout method. The tree was estimated
274 using IQ-TREE 2.1.3 (Minh et al., 2020) with independent GTR+G models for each gene and
275 1000 ultra-fast bootstraps. Simplified versions of the nuclear and plastid trees were compared
276 visually through a tanglegram produced in R with the package phytools (Revell, 2012).

277

278 **RESULTS**

279

280 *Sequence recovery*

281

282 The final nuclear data set comprised 142 accessions, including 128 Araceae representing 111
283 genera, and 13 samples used as outgroup taxa (*Acorus gramineus* and 12 samples from other
284 Alismatales families; Appendix S1). For Araceae accessions, 3.75 million read pairs were
285 sequenced on average, ranging from 0.23 to 57.6 million, while this number was slightly
286 lower for the outgroup taxa (3.01 million read pairs on average, ranging from 0.28 to 20.3
287 million). On the other hand, the percentage of reads on target was lower for Araceae than for
288 the outgroup taxa, on average 0.64% and 2.74%, respectively. The mean number of genes
289 (including flanking regions) with sequences is lower for Araceae than the outgroup taxa (173
290 and 222, respectively, although the range varies considerably, 10 to 347 for Araceae and 58-
291 309 for the outgroup taxa), which is also reflected in the number of genes recovered with a
292 50% target length threshold: mean of 96 for Araceae and 142 for outgroup taxa (Appendix
293 S1).

294

295 We further explore this discrepancy in sequence recovery between Araceae and the rest of
296 Alismatales using a larger sampling of Alismatales obtained from the Kew Tree of Life
297 Explorer (Baker et al., 2022; <https://treeoflife.kew.org/>), as well as comparing it to other

298 monocots and the rest of angiosperms. We found that a similar mean number of genes were
299 recovered for Araceae and Alismatales, 211 and 203, respectively (ranges of 67-322 for
300 Araceae and 91 to 309 for Alismatales). On the other hand, the mean number of genes
301 recovered for other monocots and other angiosperms were 263 (9 to 348) and 308 (28 to 351),
302 respectively, much higher than for Araceae and Alismatales (Figure 1). It is worth noting that
303 the Araceae accessions included in the present study differ slightly from those included in the
304 Kew Tree of Life Explorer (<https://treeoflife.kew.org/>), hence the different values reported for
305 Araceae between the two comparisons presented here. Even though the Tree of Life Explorer
306 data was compiled using a different approach for sequence recovery than the one used here
307 (Baker et al., 2022), it is evident that sequence recovery for the Angiosperms353 probes is
308 much lower in Alismatales than the rest of angiosperms.

309

310 The plastid data set contained 95 samples, 87 of which are Araceae (64 from targeted
311 enrichment sequence data, 23 from public repositories), and covered 79 Araceae genera
312 (Appendix S2). Not all samples processed using targeted enrichment have produced enough
313 off-target reads to allow an adequate recovery of the plastid genome, hence the lower number
314 of genera in the plastid data set. The average length of the plastid genome recovered for all 95
315 samples is 106,082 bp and 107,630 bp for Araceae samples, ranging from 30,527 bp to
316 176,835 bp. The final nuclear and plastid aligned concatenated data sets comprised 119,469
317 and 50,192 bp, respectively (Appendix S3).

318

319 *Phylogenetic relationships*

320 To refer to the clades presented in our trees, we use an informal mixture of naming
321 conventions. Clade names may be single orthodox Latin terms indicating rank, e.g., Aroideae,
322 or terms consisting of two genus names representing the two names encompassing the clade

323 in our Figure 2, e.g., the Stylochaeton-Arum Clade represents subfamily Aroideae (in these
324 terms the genus names are in roman font and “Clade” is capitalized). Other clade names
325 mentioned in the text refer to clades discussed by previous authors. This is comparable but
326 distinct from the naming conventions of Cusimano et al. (2011). Our aim is to provide a
327 clearly defined but provisional vocabulary of clade names which authors can use in discussing
328 aroid phylogenies, and we anticipate that this nomenclature will be updated at some time in
329 the future when a comprehensive formal classification of the family is published. Since
330 studies frequently differ in sampling and clade composition, we believe that the two-genus
331 convention for clade names facilitates the comparison of text and figured trees. However,
332 single term Latin names are more convenient when discussing major taxa such as subfamilies.
333 In all cases, the genus composition of the clades discussed is made clear in our Table 1.

334

335 The polytomy test identifies 32 nodes for which it was not possible to reject the null
336 hypothesis that these nodes may be a polytomy (indicated with asterisks in Figure 2). None of
337 the relationships between subfamilies were identified as such, except for the sister
338 relationship of Lasioideae with Pothoideae-Monteroideae. In addition, most of the nodes
339 identified as putative polytomies are found within subfamily Aroideae (23 out of 32), in
340 particular within the Schismatoglottideae (13).

341

342 The Araceae family, with the inclusion of the duckweeds (subfamily Lemnoideae), is robustly
343 recovered as a monophyletic group (LPP = 1.00 – moderate QS). Perhaps the most significant
344 new feature of the phylogenomic tree, in comparison to previous large-scale analyses of the
345 family, is the strong support found for the crown nodes of major clades. There is generally
346 strong support for all subfamilies and the relationships among these (Figure 2A). The
347 *Gymnostachys*–*Symplocarpus* (*Gymnostachydoideae* and *Orontioideae*), *Spirodela*–

348 Wolffia (Lemnoideae), Dracontium–Podolasia (Lasioideae), Pothos–Anthurium
349 (Pothoideae), and Spathiphyllum–Heteropsis (Monsteroideae) Clades are all strongly
350 supported (LPP = 1.00, QS ranging from moderate to high; Figure 2). Likewise, the
351 relationships between these clades are also strongly supported, except for the sister
352 relationship between Lasioideae and the pair Pothoideae–Monsteroideae, which is only
353 weakly supported (LPP = 0.76, weak QS). The topology of these two clades might be more
354 realistically viewed as a trichotomy with the Stylochaeton–Arum Clade, as indicated by the
355 polytomy test, which in this instance could not reject the null hypothesis that this branch is a
356 polytomy (asterisks on Figure 2). The first divergences within the Lasioideae, Pothoideae and
357 Monsteroideae are also strongly supported. First, the Dracontium–Podolasia Clade is
358 subdivided into the Dracontium–Dracontioides (LPP = 1.00 – high QS) and the Urospatha–
359 Podolasia (LPP = 1.00 – moderate QS) Clades. Second, the Pothos–Anthurium Clade (LPP =
360 1.00 – high QS) is divided into the Pothos (LPP = 1.00 – high QS) and the Anthurium Clades
361 (LPP = 1.00 – moderate QS). Finally, the Spathiphyllum–Heteropsis Clade (LPP = 1.00 –
362 moderate QS) is composed of three groups, the Spathiphyllum–Holo-chlamys (LPP = 1.00 –
363 moderate QS), the Monstera–Anadendrum (LPP = 1.00 – high QS), and the Alloschemone–
364 Heteropsis (LPP = 1.00 – high QS) Clades. Closer to the tips, particularly in the
365 Spathiphyllum–Heteropsis Clade and the Urospatha–Podolasia Clade, most relationships are
366 weakly supported and identified as polytomies by the polytomy test.

367

368 Of particular significance is the strong support (LPP = 1.00 – high QS) for the clade
369 comprising the bisexual-flowered genus *Calla*, all the unisexual-flowered aperi-goniate genera
370 and the perigoniate unisexual-flowered genera *Stylochaeton*, *Gonatopus* and *Zamioculcas*
371 (Figure 2A, Stylochaeton–Arum Clade). It is notable that bisexual-flowered *Calla* and
372 unisexual-flowered aperi-goniate *Anubias* form such a well-supported clade. The weak support

373 (LPP = 0.37 – low QS) for the sister relationship between *Stylochaeton* and the Gonatopus–
374 Zamioculcas Clade indicate that these two lineages essentially form a trichotomy with the
375 strongly supported Anubias–Arum Clade (LPP = 1.00 – weak QS), which comprises all the
376 other genera of subfamily Aroideae. Within the remainder of the Anubias–Arum Clade,
377 several relationships are poorly supported, but we were nevertheless able to identify a number
378 of groups corresponding to definable entities.

379

380 The two largest components of the Anubias–Arum Clade contain a series of highly supported
381 subclades. The Bognera–Galantharum Clade, which is moderately supported, includes three
382 well supported subclades: the Bognera–Gorgonidium Clade (LPP = 1.00 – high QS), the
383 Culcasia–Philodendron Clade (LPP = 1.00 – moderate QS) and the Lagenandra–Galantharum
384 Clade (LPP = 1.00 – high QS). However, the sister relationship of *Zantedeschia* to the
385 Culcasia–Philodendron Clade is relatively weak (LPP = 0.86 – moderate QS) and the position
386 of *Anchomanes* is unclear (LPP = 0.10 – weak QS). These low branch support values
387 therefore suggest that the crown node of the Bognera–Galantharum Clade should be viewed
388 as a polytomy (also supported by the results of the polytomy test). On the other hand, the
389 Amorphophallus–Arum Clade, the second large component in this analysis, is well-resolved
390 into four subclades, all well-supported: the Pistia–Arum Clade (LPP = 1.00 – moderate QS),
391 the Arisarum–Arophyton Clade (LPP = 1.00 – moderate QS), the Hapaline–Chlorospatha
392 Clade (LPP = 1.00 – high QS) and the genus *Amorphophallus* (LPP = 1.00 – high QS).

393

394 Relationships recovered with the plastid data are largely similar to those obtained with the
395 nuclear data among most subfamilies, but the similarity between the two analyses is much
396 reduced within the Aroideae (Figure 3). The Orontioideae are sister to the rest of the family in
397 both analyses, although they form a clade with the Gymnostachydoideae in the nuclear

398 analysis and are not included in the plastid analysis. Lemnoideae are sister to the rest of the
399 family in both analyses. While Pothoideae and Monsteroideae are sister groups in both
400 analyses, their relationship to Lasioideae is different, in one case they are sister, in the other
401 they form a grade subtending subfamily Aroideae. In subfamily Aroideae, except for the sister
402 relationship to the rest of the subfamily of the pair Stylochaeton-Zamioculcadeae, both
403 analyses bear little resemblance, including the position of genus *Calla*, which occupies a more
404 derived position within Aroideae in the plastid analysis. The limited sampling within the
405 plastid analysis compared to the nuclear one is most likely responsible for some of these
406 differences.

407

408 **DISCUSSION**

409

410 The variability of gene number recovery in Araceae and Alismatales is lower than in
411 monocots and angiosperms in general (Figure 1). Within Araceae, the number of genes
412 recovered ranges from 10 to 347 in our data set, but even more so when comparing the length
413 of the genes recovered at 50% of the target file, which averages 96 per sample. Nevertheless,
414 the Angiosperms353 probe set has been efficient for producing a well resolved phylogenetic
415 tree for Araceae. However, it is disappointing to see the probe kit performing poorly in
416 comparison with results obtained for other angiosperm groups. The limited representation of
417 Alismatales (four species representing four families) and Araceae (only one species, *Pistia*
418 *stratiotes*) in the OneKP data set, which was used for the design of the Angiosperms353
419 probes (Johnson et al., 2019), might partly explain the low gene number recovered for
420 Araceae and Alismatales compared to other angiosperms groups. Out of the four samples of
421 Alismatales in OneKP, only two (*Pistia stratiotes* and *Posidonia australis*, Posidoniaceae)
422 contribute with probe sequences to the Angiosperms353 set (50 and 184, respectively), which

423 represents only 0.3% of the 75,451 probes found in the set. Ideally, future versions of the
424 Angiosperms353 probes should try to mitigate this gap.

425 This phylogenetic analysis, based on a large sample of genera (111) and sequence data (130 to
426 347 genes and associated introns), has strengthened some of the findings of recent molecular
427 phylogenetic research on the family and provided clear and well-supported evidence for key
428 relationships within the family. For the first time, all the major clades, including all
429 subfamilies, are fully supported, as well as most of the relationships between them. The
430 results justify the expansion of the major crown group Aroideae to include the perigoniate
431 unisexual-flowered genera *Stylochaeton*, *Gonatopus* and *Zamioculcas* and the aperigoniate,
432 bisexual-flowered *Calla*. The clear support for the inclusion of *Calla* in this expanded
433 Aroideae provide evidence that this enigmatic genus forms part of an early evolutionary phase
434 in which aroid floral structure had not yet stabilized into the aperigoniate unisexual pattern
435 exhibited by the great majority of genera. Another significant issue to which the present study
436 contributes but does not resolve with high levels of confidence is the position of Lasioideae.
437 This subfamily, represented in our study by the Dracontium–Podolasia Clade, is no longer
438 sister to the remainder of Aroideae but forms a weak sister group relationship with the other
439 major bisexual-flowered clades, i.e. subfamilies Pothoideae and Monsteroideae. If this sister
440 grouping was discounted due to its low branch support and the fact that it was not rejected as
441 a polytomy by the polytomy test, Lasioideae would emerge as one of the three lineages
442 forming a polytomy with the Aroideae and the pair Monsteroideae-Pothoideae, thus supporting
443 the conclusions of Hay (1986) and Hay and Mabberley (1991).

444

445 Most major branches in our phylogenetic tree confirm groups retrieved in previous
446 phylogenetic studies but with greater support. In addition, a few of the clades recovered here
447 are also novel. The genera or species belonging to each clade are listed in Table 1. Given that

448 the concatenated analysis of the supercontigs was broadly similar to the one retrieved from
449 the multi-species coalescence approach, we concentrate our discussion on the supercontigs
450 data set analyzed using the multi-species coalescence approach (Figure 2). The result of the
451 concatenated analysis is presented in the supplementary material (Figure S3).

452

453 ***Subfamilies: overview***

454 Our phylogenetic tree (Figures 2A, B) supports the recognition of seven major clades in
455 the family Araceae, all corresponding to previously recognized subfamilies, i.e
456 Gymnostachydoideae, Orontioideae, Lemnoideae, Lasioideae, Pothoideae, Monsteroideae and
457 Aroideae (Cusimano et al., 2011; Chartier et al., 2014); we discuss these below in this order.
458 The eighth subfamily recognized in previous studies, the Zamiculcadoideae, is found in our
459 tree to be included in Aroideae (see below) as tribe Zamiculcadeae.

460 The first supported group (Gymnostachys–Symplocarpus Clade) is composed of
461 subfamilies Gymnostachydoideae (*Gymnostachys anceps*) and Orontioideae (*Orontium*,
462 *Lysichiton*, *Symplocarpus*) (Mayo et al., 1997; Nauheimer et al., 2012). Gymnostachydoideae
463 has long been recognized as a monospecific group (Bogner and Nicolson, 1991), but its sister
464 relationship to the three genera of Orontioideae only became evident following the first
465 studies using molecular data (e.g. French et al., 1995). Since then, *Gymnostachys* has been
466 confirmed in this placement by subsequent molecular analyses (Cabrera et al., 2008;
467 Cusimano et al., 2011; Nauheimer et al., 2012). Tippery et al. (2021) recently confirmed this
468 position, with the inclusion in their analysis of additional species of *Lysichiton* and
469 *Symplocarpus*. Zhao et al. (2022), on the other hand, do not retrieve *Gymnostachys* as sister to
470 the Orontioideae, but as sister to the remainder of the family. Despite the high support we
471 obtained for this clade and for the sister relationship of the two subfamilies, we consider that
472 subfamily rank for each component is taxonomically preferable, given the radically different

473 morphology of *Gymnostachys anceps* from all other Araceae and its isolated geographical
474 location as a subtropical eastern Australian endemic, in contrast to the temperate, boreal
475 Orontioideae (Mayo et al., 1997). In addition, the time of divergence of *Gymnostachys* and
476 Orontioideae is probably very ancient (~60 Ma, Tippery et al., 2021; up to ~ 95 Ma,
477 Nauheimer et al., 2012).

478 The Spirodela–Wolffiella Clade (Figure 2A) is sister to the rest of the family, with the
479 exception of Gymnostachydoideae and Orontioideae, and comprises four genera of subfamily
480 Lemnoideae (*Spirodela*, *Lemna*, *Wolffia*, *Wolffiella*). This group occupies the same position
481 as in previous large-scale analyses (Cabrera et al., 2008; Cusimano et al., 2011; Nauheimer et
482 al., 2012; Zhao et al., 2022), although our study lacks the fifth lemnooid genus, *Landoltia*.
483 Tippery et al. (2021) confirm and reinforce the phylogenetic result found by recent studies,
484 but they also propose that the Lemnoideae should be recognized as family Lemnaceae, as they
485 were in the past before their inclusion in Araceae was confirmed by molecular studies. This
486 change, however, would require to taking out subfamilies Gymnostachydoideae and
487 Orontioideae from the rest of Araceae and recognizing two new families. The re-instatement
488 of Lemnaceae as a family suggested by Tippery et al. (2021) is a matter of historical
489 convention rather than an argument deriving from a distinct new phylogenetic pattern. We
490 prefer to follow the current Angiosperm Phylogeny Group delimitation of Araceae and
491 continue to recognize Lemnoideae as one of its subfamilies (APG IV, 2016). Whatever the
492 taxonomic convention adopted, molecular evidence clearly show that Lemnoideae are derived
493 from a bisexual-flowered ancestor, but their floral structures are so extremely reduced that
494 establishing homologies with aroid flowers and/or inflorescences with confidence is
495 impossible (Bogner, 2009).

496 The Dracontium–Heteropsis Clade is novel, but this grouping is only weakly supported
497 and, as noted above, if disregarded would result in a polytomy comprising the components

498 Lasioideae, Pothoideae-Monsteroideae and Aroideae. In contrast, this clade consists of three
499 highly supported subclades that correspond to the previously recognized subfamilies (clades
500 7, 23, 24 in Cusimano et al., 2011) Lasioideae (Dracontium–Podolasia Clade, our study lacks
501 only *Anaphyllum*), Pothoideae (Pothos–Anthurium Clade) and Monsteroideae
502 (Spathiphyllum–Heteropsis Clade) and a highly supported clade composed of the Pothoideae
503 and Monsteroideae (Pothos–Heteropsis Clade), previously dubbed the Bisexual Climbers
504 clade by Cusimano et al. (2011: clade 31). Nauheimer et al. (2012) also found strong support
505 for these four clades. We nevertheless propose that subfamily rank should be restricted only
506 to the three clades currently recognized as such (Table 1), given the strong and long-
507 established morphological distinctions between them (Mayo et al., 1997; Cusimano et al.,
508 2011; Zuluaga et al., 2019). Hitherto, the most comprehensive molecular analyses have
509 positioned the Lasioideae as sister to the unisexual-flowered subfamilies Aroideae and
510 Zamiculcadoideae with moderate support (Cabrera et al., 2008; Cusimano et al., 2011;
511 Nauheimer et al., 2012), a topology that we also recovered in our plastid data analysis
512 (Figures 3, S4). All plastid analyses to date consistently place Pothoideae and Monsteroideae
513 together as a moderately to well-supported clade sister to a clade comprising Lasioideae,
514 Zamiculcadoideae and Aroideae. In contrast, Chartier et al. (2014), by adding the *PhyC*
515 nuclear gene to the plastid data of Cusimano et al. (2011), weakened the association of the
516 Pothoideae and Monsteroideae recovered in previous studies. Given the weak support in our
517 tree for the sister relationship of Lasioideae and the pair Pothoideae-Monsteroideae, and the
518 fact that the null hypothesis that this node is a polytomy could not be rejected by the
519 polytomy test, the possibility that the Lasioideae is sister to Aroideae cannot be ruled out, a
520 topology that would then support the classification proposed by Hay and Mabberley (1991). It
521 is worth noting that Zhao et al. (2022) found Lasioideae to be sister to a clade in which
522 Pothoideae-Monsteroideae are sister to the Aroideae, but with limited support.

523 Our result differs significantly from previous molecular analyses by providing a more
524 solid basis for the delimitation of the large clade composed of all the unisexual-flowered
525 genera (Figure 2B: Stylochaeton–Arum Clade; termed Unisexual Flowers clade by Cusimano
526 et al., 2011), comprising 81 (72%) of the 111 genera sampled here (Table 1). As in other
527 family-level studies (Cabrera et al., 2008; Cusimano et al., 2011; Nauheimer et al., 2012;
528 Chartier et al., 2014), this clade includes the bisexual-flowered *Calla palustris*. In our
529 analysis, however, in contrast to the plastid-based results of previous work, *Calla* is strongly
530 supported as sister to the remainder of Aroideae, with the exception of the Zamioculcadeae
531 (Figure 2A). Therefore, we propose that the Stylochaeton–Arum Clade is recognized as
532 subfamily Aroideae, enlarging its earlier delimitation to include taxa previously assigned to
533 subfamily Zamioculcadoideae (Table 1, compare Cusimano et al., 2011; Nauheimer et al.,
534 2012; Chartier et al., 2014).

535

536 ***Three derived bisexual-flowered subfamilies***

537 Each of the clades corresponding to these subfamilies is divided into highly supported
538 subclades, warranting recognition as tribes, as discussed below.

539

540 ***Lasioideae***

541 Subfamily Lasioideae is composed of two tribes: Lasieae and Dracontieae. Although Cabrera
542 et al. (2008) and Cusimano et al. (2011) recovered a well-supported grouping of the three
543 genera of Dracontieae (*Anaphyllopsis*, *Dracontium* and *Dracontioides*) they did not formally
544 name it, nor did they recover a monophyletic Lasieae, so our finding splitting the Lasioideae
545 into two strongly-supported subclades is novel. This result should be tempered by noting that
546 *Anaphyllum* is missing from our sampling of Lasieae (*Urospatha*, *Lasia*, *Lasimorpha*,
547 *Pycnospatha*, *Cyrtosperma* and *Podolasia*). However, since all recent phylogenetic studies

548 have placed *Anaphyllum* as sister to *Lasimorpha* within the Lasieae (Cusimano et al., 2011;
549 Nauheimer et al., 2012; Tippery et al., 2021), it seems unlikely that its inclusion here would
550 have greatly altered the separation of Lasioideae into two distinct groups.

551

552 ***Pothoideae***

553 Subfamily Pothoideae is composed of two tribes, the Anthurieae and Potheae (Mayo et al.,
554 1997). The first only comprises the hyperdiverse genus *Anthurium*, with an estimated 3,000
555 species (Boyce and Croat, 2011 onwards). The second has long consisted of three genera,
556 *Pothos*, *Pothoidium* and *Pedicellarum*. However, *Pedicellarum* has recently been subsumed
557 into *Pothos* by Wong et al. (2020), a change supported by the present study despite our
558 limited sampling. Furthermore, our very limited sampling also seems to indicate that
559 *Pothoidium* might also be nested in *Pothos*, as it appears to group within *Pothos* with
560 subgenus *Pothos*. The status of *Pothoidium* as a genus separate from *Pothos* is also
561 questioned by a previous study (Carlsen and Croat, 2013). This group is clearly in need of
562 more comprehensive phylogenetic analyses in order to clarify its generic boundaries. .

563

564 ***Monsteroideae***

565 In our phylogenetic tree, subfamily Monsteroideae is composed of three strongly supported
566 tribes: Spathiphyllae, Monstereae and Anepsiadeae. This subdivision, but with different
567 names (Spathiphyllae, and Heteropsis and Rhaphidophora clades), has already been observed
568 in previous molecular analyses (Cusimano et al., 2011; Chartier et al., 2014; Henriquez et al.,
569 2014; Zuluaga et al., 2019). Tribe Spathiphyllae, consisting of *Spathiphyllum* and
570 *Holochamys* (Table 1), is strongly supported (although its QS is moderate) as sister to the two
571 other tribes, both also strongly supported, as previously found by Zuluaga et al. (2019). In our
572 sampling, tribe Anepsiadeae Engler 1876 (Nicolson 1984) comprises the four genera

573 *Alloschemone*, *Rhodospatha*, *Stenospermatum*, and *Heteropsis*. Tribe Monstereae is
574 represented by the six genera *Monstera*, *Amydrium*, *Scindapsus*, *Epipremnum*,
575 *Rhaphidophora* and *Anadendrum*. Our limited sampling at the species level does not allow us
576 to evaluate several phylogenetic hypotheses proposed by Zuluaga et al. (2019), such as the
577 merging of *Holochlamys* within the Asian *Spathiphyllum* species, the paraphyly of
578 *Epipremnum* (one species groups as sister to *Amydrium*, the other to *Scindapsus*), or the
579 probable polyphyly of *Rhaphidophora*.

580

581 ***Subfamily Aroideae (unisexual-flowered genera plus Calla palustris)***

582

583 ***Stylochaetoneae & Zamioculcadeae***

584 The Stylochaeton-Gonatopus Clade (Figure 2B) was recovered with good support by
585 Cabrera et al. (2008), Cusimano et al. (2011) and Zhao et al. (2022), but with less support by
586 Nauheimer et al. (2012, 70%) and by Chartier et al. (2014), with no support in their nuclear
587 tree and > 70% support in their combined nuclear and plastid tree. Our result is similar to that
588 of Chartier et al. (2014); the support for this clade is negligible and considered a polytomy by
589 the polytomy test (Figure 2B). Thus, for the time being, these two groups (*Stylochaeton* and
590 *Zamioculcas* + *Gonatopus*) are probably better represented as a polytomy at the crown node
591 of Aroideae (Figure 2B). The taxonomic consequence is that the Zamioculcadoideae,
592 recognized by Bogner and Hesse (2005) as sufficiently distinct to merit subfamily rank,
593 should be reduced to tribe Zamioculcadeae (Table 1). Our topology supports an evolutionary
594 scenario in which *Stylochaeton* and the Zamioculcadeae are distinct elements of a disparate
595 assemblage of genera at the crown node of Aroideae which led eventually to the aperigoniata,
596 unisexual-flowered lineages. These two elements, although both tropical African geophytes,
597 are very distinct morphologically from other Araceae as well as from each other, so we also

598 recognize the monogeneric tribe Stylochaetoneae (Mayo et al., 1997). Hesse et al. (2001) and
599 Ulrich et al. (2013) showed that there are important palynological differences between
600 *Stylochaeton* and Zamioculcadeae: although both have the plesiomorphous characters of
601 sporopolleninous thick tectate-columellate exine, the former has inaperturate, foveolate
602 and microreticulate pollen and the latter ring-like apertures and psilate, perforate
603 ornamentation. The similarity between the two groups, which has perhaps most influenced
604 researchers in the past (apart from their geography and ecology), is the combination of a
605 perigon with unisexual flowers. However, the presence of a perigon is a symplesiomorphy,
606 and its morphology differs strongly between the two tribes (i.e., free in Zamioculcadeae,
607 urceolate in *Stylochaeton*), as does leaf structure (Mayo et al., 1997; Bogner and Hesse,
608 2005). Morphologically, the impression may be of distinct lineages which have independently
609 evolved different types of unisexual flowers. Such a scenario differs from the view that
610 unisexual flowers evolved only once in Araceae (French et al., 1995; Mayo et al., 1997) with
611 an evolutionary sequence resulting in fused petals in *Stylochaeton*, free petals in
612 Zamioculcadeae and aperigoniata flowers in the derived clade (see hereafter). The current
613 molecular evidence is not strong enough to favor either hypothesis. On the other hand, our
614 result clearly shows a robust placement of Zamioculcadeae and *Stylochaeton* as sister to the
615 Anubias–Arum Clade, which contains all the other unisexual genera of Araceae in addition to
616 *Calla palustris*.

617

618 ***Anubias–Arum Clade***

619 Within the expanded subfamily Aroideae, this is the major clade with strongest support,
620 and it includes all the genera with aperigoniata unisexual flowers plus *Calla palustris*
621 (Aroideae subfamily *sensu* Cusimano et al., 2011: clade 39). The support assigned to the
622 backbone of this clade is mostly poor, but several clades are however well-supported. These

623 include the small groups *Calla*+*Anubias*, *Calloopsis*+*Nephtytis afzelii*, *Nephtytis*
624 *bintuluensis*+*Aglaonema*, as well as two major clades, the strongly supported
625 Amorphophallus–Arum Clade (Dracunculus clade *sensu* Cusimano et al., 2011: clade 37) and
626 the somewhat less supported Bognera–Galantharum Clade. The poorly supported relationship
627 between these six clades resembles the findings of previous studies.

628

629 ***The position of Calla palustris and the genera Anubias and Montrichardia***

630 Ulrich et al. (2013) considered the phylogenetic position of *Calla* in detail and
631 suggested that it (like *Stylochaeton* and the Zamioculcadeae) represents a transitional form in
632 the phylogenetic development of Araceae, from a plesiomorphic syndrome of bisexual
633 flowers, aperturate pollen with a thick, sporopolleninous ektexine and absence of laticifers, to
634 an apomorphic syndrome of unisexual flowers, inaperturate pollen with thin ektexine and
635 little or no sporopollenin and well developed laticifer systems. They argued that pollen data
636 and other morphological and anatomical evidence supports a position for *Calla* either sister to
637 Stylochaeton–Gonatopus Clade plus the rest of Aroideae or just sister to the rest of the
638 Aroideae, commenting that such a position would not require an evolutionary reversal of so
639 many significant structural characters. Molecular analyses have been inconsistent in the
640 phylogenetic position assigned to *Calla*. French et al. (1995) placed it outside the otherwise
641 entirely unisexual-flowered clade we are proposing as subfamily Aroideae, but in Cabrera et
642 al. (2008) its position is just distal to the Stylochaeton–Gonatopus Clade (the apparent
643 association with the Schismatoglottideae is unsupported in their tree). Later studies based
644 largely on plastid data have found *Calla* embedded deeper in the Aroideae but in different
645 positions with widely different support levels (Cusimano et al., 2011; Nauheimer et al., 2012;
646 Henriquez et al., 2014; Abdullah et al., 2020). Chartier et al. (2014), however, associated
647 *Calla* with *Anubias* and *Montrichardia* in an unsupported early branch of the Aroideae (our

648 sense), the latter also unsupported. Our tree proposes a similar position, but with much
649 stronger support. The branch support for including *Calla* in the Aroideae is very strong, and
650 so is its association with *Anubias*. *Montrichardia* then appears immediately above, but its
651 sister relation with the rest of the Aroideae (Callopsis–Arum Clade) is weak. We conclude
652 that our result confirms the inclusion of *Calla* in the Aroideae clade (as delimited here) and
653 supports a position for it at or near the crown node of the subfamily. However, the underlying
654 evolutionary scenario remains obscure. Ulrich et al. (2013) propose that the dissonant
655 characters of *Calla* should be regarded as plesiomorphies, but a possible evolutionary re-
656 emergence of bisexual florets (an atavism) also cannot be ruled out. The extinction of
657 informative lineages at this crucial phase of aroid evolution may have played its part in
658 confounding decisive interpretations at present.

659 Morphological comparison suggests caution in accepting too uncritically an
660 association of the currently temperate *Calla* and tropical African *Anubias*; the former has
661 bisexual (aperigoniata) flowers, free stamens with slender connectives and unilocular ovaries
662 with basal placentation, and the latter has unisexual flowers, stamens connate into synandria
663 with thickened connectives, and usually 2–3-locular ovaries with axile placentation. The two
664 genera share simple articulated laticifers, striate leaf venation, creeping stems and emersed
665 aquatic habit. In previous analyses neither genus has shown any consistent pattern of
666 relationships with other genera, and this is even true of pre-molecular classifications. One
667 hypothesis may be that *Calla* and *Anubias* are remnants of an earlier (sub)tropical clade (both
668 genera are estimated to have differentiated ~74 and 80 Ma ago respectively; Nauheimer et al.,
669 2012), as there are no potential temperate relatives. A surprising feature of our result is the
670 strength of the association between the two genera; perhaps some explanation for this may
671 emerge from a future interpretation of the molecular patterns which are not synapomorphic.
672 Much the same can be said of *Montrichardia*, which in all molecular analyses to date shows

673 no consistent grouping tendency apart from inclusion in Aroideae, and its sister relations
674 typically have low branch support. At a minimum it could be said that our result is consistent
675 with the view that these three genera lack convincing relationships with other genera and
676 occupy phylogenetic positions early in the evolutionary history of subfamily Aroideae.

677

678 ***Calloopsis–Nephtytis afzelii* Clade**

679 The moderately supported *Calloopsis–Nephtytis afzelii* Clade is tropical African and novel
680 although the two genera have been associated previously on morphological grounds by
681 Bogner (1969, 1980) and Hay et al. (1994), and Bogner and Nicolson (1991) placed *Calloopsis*
682 close to their tribe Nephtytideae. As with *Montrichardia*, *Anubias*, *Zantedeschia* and *Calla*,
683 *Calloopsis* has never revealed any well-supported sister-group relationships in previous
684 molecular studies but has always appeared as lineage part of a polytomy near the root node of
685 Aroideae (Cusimano et al., 2011; Nauheimer et al., 2012; Chartier et al., 2014). On the other
686 hand, *Nephtytis* (when considered exclusively as an African group) has been consistently
687 associated in both pre-molecular classifications and DNA-based analyses with other tropical
688 African genera such as *Culcasia*, *Cercestis*, *Anchomanes* and *Pseudohydrosme*. In the major
689 plastid analyses (Cabrera et al., 2008; Cusimano et al., 2011; Nauheimer et al., 2012) there is
690 also a fairly consistently recovered clade consisting of the Culcasieae, Aglaonemateae,
691 Nephtytideae (*Nephtytis*, *Anchomanes*, *Pseudohydrosme*), Philodendron clade,
692 *Zantedeschia* and the Spathicarpeae (see Table 1 and Cusimano et al., 2011 for genera
693 included under these names). This clade was well supported in the analysis of Cusimano et al.
694 (2011: *Zantedeschia* clade, clade 32) but not in the other two studies, implying that it is not
695 especially stable. On the other hand, branch support for the Aglaonemateae, Nephtytideae
696 and their common node (*Anchomanes* clade, *sensu* Cusimano et al., 2011: clade 26) is robust
697 in all three of these plastid analyses. The present study (which does not include

698 *Pseudohydrosme*) unexpectedly breaks up the Anchomanes clade, leaving *Anchomanes* itself
699 negligibly associated with *Zantedeschia*, the Culcasieae and the Philodendron clade, and
700 moving Aglaonemateae and African *Nepthytis* to disparate positions on the crown node of
701 the Aroideae.

702

703 ***Nepthytis bintuluensis*–*Aglaonema* Clade**

704 The Asian and Malesian tribe Aglaonemateae is grouped with the Bornean *Nepthytis*
705 *bintuluensis*. This strongly supported clade is associated neither with African *Nepthytis* nor
706 with *Anchomanes* as in the previous plastid trees but is instead one element of the large
707 polytomy surrounding the crown node of our expanded subfamily Aroideae. This clade was
708 previously recovered as a well-supported group (≥ 95 BS) by Nauheimer et al. (2012); our
709 result thus further confirms the distinctness of *Nepthytis bintuluensis* from the African
710 *Nepthytis afzelii*, as proposed by Boyce and Wong (2015) and Boyce and Croat (2011
711 onwards).

712

713 ***Bognera*–*Galantharum* Clade**

714 This major subclade of subfamily Aroideae has moderate support as a whole, but many nodes
715 internally are poorly supported made up of the genera *Zantedeschia* and *Anchomanes* and
716 three strongly supported and previously recognized clades (Figure 2B). The first of these is
717 tribe Spathicarpeae, also recognized by Cusimano et al. (2011: clade 13) and Nauheimer et al.
718 (2012), although in our analysis the following genera were not included: *Asterostigma*,
719 *Mangonia*, *Spathantheum*, *Spathicarpa* and *Synandrospadix*. The second is the Culcasia–
720 Philodendron Clade, which corresponds to the Homalomena clade of Cusimano et al. (2011:
721 clade 27). Our analysis shows this clade to be highly resolved and composed of the tribes
722 Culcasieae (*Culcasia* and *Cercestis*) and Philodendreae (= Philodendron clade, Cusimano et

723 al., 2011), also with strong branch support. According to our new delimitation, tribe
724 Philodendreae encompasses *Homalomena* and *Furtadoa*, *Adelonema* and *Philodendron*. A
725 subdivision seems to clearly differentiate the Asian subclade from the Neotropical one, but
726 our species sampling is too limited to further discuss it. However, in more densely sampled
727 studies of the Philodendreae, *Furtadoa* appears nested in *Homalomena* (Wong et al., 2016;
728 Vasconcelos et al., 2018). Similarly, only one species representing each of the three
729 subgenera of *Philodendron* (*Meconostigma*, *Philodendron*, *Pteromischum*) have been
730 sequenced, and thus it is not possible to contribute to the view that subgenus *Meconostigma*
731 should be recognized as the distinct genus *Thaumatophyllum*, as proposed by Vasconcelos et
732 al. (2018) and Sakuragui et al. (2018), but not taken up by other authors (Canal et al., 2019).

733 The relationships of genus *Zantedeschia* show only weak support, as in previous
734 plastid studies. In our tree it appears as sister to the *Culcasia*–*Philodendron* Clade but with
735 weak support (LPP = 0.86 – moderate QS), whereas in Cusimano et al. (2011) and Nauheimer
736 et al. (2012) it is the unsupported sister to the *Spathicarpeae*.

737 The last group is the strongly supported *Lagenandra*–*Galantharum* Clade which
738 corresponds to the *Rheophytes* clade of Cusimano et al. (2011: clade 28). As also presented
739 by Nauheimer et al. (2012), these plastid trees included the three well supported components
740 *Schismatoglottideae* (clade 15), *Cryptocoryneae* (clade 14) and *Philonotion* (Wong et al.,
741 2010). Our analysis also robustly recovers these three components, but with the difference
742 that *Philonotion* is now sister to the *Schismatoglottideae*, and many more genera are included
743 in the latter clade (*Apoballis*, *Colobogynium*, *Galantharum*, *Gamogyne*, *Hera*,
744 *Heteroaridarum*, *Hottarum*, *Kiewia*, *Nabalu*, *Naiadia*, *Ooia*, *Pichinia*, *Piptospatha*,
745 *Pursegloveia*, *Rhynchopyle*, *Schottarum*, *Tawaia*, *Toga*, *Vesta*). For a detailed study of the
746 *Schismatoglottideae* with the description of its 30 constituent genera supported by a complete
747 molecular phylogenetic analysis, we refer the reader to Low et al. (2018). In Cusimano et al.

748 (2011), *Philonotion* is the sister group (> 85% BS) of the combined clades
749 Schismatoglottideae and Cryptocoryneae (each >85% BS). Nauheimer et al. (2012) found the
750 same but with less support (respectively $\geq 70\%$ BS and $\geq 95\%$ BS). Wong et al. (2010) and
751 Wong (2013) found high support (all nodes posterior probabilities ≥ 0.97) and Low et al.
752 (2018) found a polytomy, but their outgroup sampling was not appropriate for resolving the
753 crown node of this group. In our analysis, Cryptocoryneae is the sister group of the clade
754 combining Schismatoglottideae and tropical American *Philonotion*. We thus propose to
755 include the genus *Philonotion* within the tribe Schismatoglottideae according to our new
756 delimitation. Only Chartier et al. (2014) have previously found a similar topology
757 (respectively $\geq 95\%$ BS, $\geq 85\%$ BS and ≥ 95 BS).

758

759 *Amorphophallus–Arum Clade*

760 The second strongly supported major Aroideae subgroup is the Amorphophallus–Arum
761 Clade, corresponding closely to the Dracunculus clade of Cusimano et al. (2011: clade 37),
762 also discussed by Mayo et al. (2013). Nauheimer et al. (2012) also recovered this as a well-
763 supported clade but left it unnamed. It consists of the genus *Amorphophallus*, tribe Caladieae
764 (*sensu* Cusimano et al., 2011: clade 17), the Arisarum–Arophyton Clade (= Colletogyne
765 clade, Cusimano et al., 2011: clade 33) and the Pistia–Arum Clade (= Pistia clade, Cusimano
766 et al., 2011: clade 34), each of these subgroups having strong support (LPP = 1.00 – high QS,
767 but moderate QS for the Pistia–Arum clade). The overall structure of the Amorphophallus–
768 Arum Clade is thus very similar to that obtained by the previous plastid analyses.

769

770 **CONCLUSIONS**

771

772 The present phylogenomic data has provided significant strengthening of the phylogenetic
773 backbone of Araceae, which is now highly supported throughout. Using this robust
774 framework, the classification of monophyletic subfamilies can be set on a firmer foundation.
775 The expanded delimitation of subfamily Aroideae, including the bisexual-flowered genus
776 *Calla*, provides a sounder basis for future research on the evolution of the aroid phenotypic
777 syndrome, which combines unisexual flowers, highly adapted spathes, zoned spadices, rapid
778 anthesis, inaperturate pollen with little or no sporopollenin and enhanced chemical defense in
779 the tissues.

780 Our results also strongly suggest that new research on African genera will be of particular
781 significance to understanding the evolution of the Aroideae as a whole, since *Calloopsis*,
782 *Nepthytis* and *Anubias* all display significantly different relationships from those recovered
783 in previous, plastid-based studies; similarly, the proximity of *Stylochaeton* and
784 *Zamioculcadeae* emerges as much less convincing. It seems likely that future resolution of the
785 relationships of these taxa will throw light on how the "unisexual syndrome" evolved in
786 aroids. It also now seems clearer that *Calla* will have a key role in this scenario, since the
787 relationship with *Anubias* recovered here is novel as well as moderately robust, and an early
788 divergence of *Calla* within Aroideae can now be viewed with more confidence.

789 Another significant finding of the study is to call into question the position of Lasioideae, a
790 group of tropical, bisexual-flowered geophytes and aquatic macrophytes, previously placed as
791 sister to the Aroideae. Our phylogenomic results group the Lasioideae as sister to the
792 Monsteroideae+Pothoideae clade, but with low support and identified as a polytomy by the
793 polytomy test. This topology can thus be viewed as a trichotomy including Aroideae,
794 Monsteroideae+Pothoideae and Lasioideae, thereby leaving open the possibility that
795 Lasioideae are sister to a clade in which Monsteroideae+Pothoideae are sister to Aroideae,
796 subsequent to the divergence of Lemnoideae, as found by Zhao et al. (2022). This topology is

797 consistent with a dominant feature of the evolution of Araceae, the repeated divergence of
798 clades from ancestors adapted to a greater or lesser extent to freshwater wet habitats.

799 There are a few limits as our phylogenomic analysis includes 111 of the current total of 143
800 recognised genera. However, many of the unsampled genera belong to the tribes
801 Schismatoglottideae and Spathicarpeae and their inclusion is unlikely to change greatly the
802 high-level topology as presented here. Nevertheless, future phylogenomic studies should aim
803 to be complete at generic level, and also to sample more species in the larger genera. It is also
804 of particular interest to resolve some of the key moderately-supported nodes such as the
805 phylogenetic positions of the subfamily Lasioideae, of the genus *Montrichardia*, of the two
806 genera *Zantedeschia* and *Anchomanes*; or finally of the new *Nephtytis bintuluensis*–
807 *Aglaonema* Clade. The possibility cannot be excluded that some of the moderately-supported
808 nodes cited above may be a consequence of missing data rather than sampling gaps. It appears
809 that the number of recovered genes with the Angiosperms353 probe set was significantly
810 lower for Araceae genera (and Alismatales) compared to other angiosperms. So, obtaining
811 sequences from a higher number of genes, either through an updated version of
812 Angiosperms353 or a Araceae specific probe set, may strengthen some of the relationships
813 still in debate.

814

815

816

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829

830 **AUTHOR CONTRIBUTIONS**

831 ALH, MG, OM, SM, WJB and FF conceived the project; CM carried out laboratory work;
832 OM, PB, ARZ and KL performed the analyses; MMC, AH, SYW, AZ provided plant
833 material; ALH, MG, OM, SM, and FF drafted the manuscript; all authors
834 commented/edited the manuscript and approved the final version; WJB and FF acquired
835 funding and supervised the project.

836

837 **Data availability**

838 All Sequences files (fastq) generated for this study are deposited in the European Nucleotide
839 Archive (ENA accession PRJEB35285; Appendix S1). All alignments and Newick tree files
840 generated are available at Zenodo (<https://doi.org/10.5281/zenodo.6539622>).

841

842

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1085
- 1086 Table 1. Comparison of Clades in this study with previous studies.
1087

| Clades named in text and figures containing > 1 genus (bold: names used in this paper; non-bold: names for similar groups in previous publications) | Nuclear (this study) | Plastid (this study) | Cusimano et al. 2011 | Nauheimer et al. 2012 |
|---|--|--|--|--|
| Aglaonemateae | <i>Aglaonema</i> (including <i>Aglaodorum</i>) | | <i>Aglaodorum</i> , <i>Aglaonema</i> | <i>Aglaodorum</i> , <i>Aglaonema</i> , <i>Nepthytis bintuluensis</i> |
| Alloschemone-Heteropsis Clade | See Anepsiadeae | | | |
| Alocasia clade | | | <i>Alocasia</i> , <i>Areae</i> , <i>Arisaema</i> , <i>Pinellia</i> | |
| Ambrosina clade | | Colletogyne clade, Pistia clade | Colletogyne clade, Pistia clade | <i>Arisareae</i> , Typhonodorum clade, Pistia clade |
| Amorphophallus clade | | <i>Amorphophallus</i> , <i>Caladium</i> , <i>Filarum</i> , <i>Syngonium</i> , <i>Xanthosoma</i> , <i>Zomicarpella</i> | Thomsonieae, Caladieae | |
| Amorphophallus-Arum Clade | <i>Alocasia</i> , <i>Ambrosinia</i> , <i>Amorphophallus</i> , <i>Ariopsis</i> , <i>Arisaema</i> , <i>Arisarum</i> , <i>Arophyton</i> , <i>Arum</i> , <i>Biarum</i> , <i>Caladium</i> , <i>Chlorospatha</i> , <i>Colletogyne</i> , <i>Colocasia</i> , <i>Eminium</i> , <i>Englerarum</i> , <i>Filarum</i> , <i>Hapaline</i> , | | | |

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|--|---|----------------------------------|-----------------------------------|--|
| | <i>Helicodiceros, Jasarum,</i> <i>Peltandra, Pistia, Remusatia,</i> <i>Sauromatum, Steudnera,</i> <i>Syngonium, Typhonium,</i> <i>Typhonodorum, Ulearum,</i> <i>Xanthosoma, Zomicarpa</i> | | | |
| Anchomanes clade | | | Aglaonemateae, Nephtytideae | |
| Anchomanes- Galantharum Clade | <i>Adelonema, Anchomanes,</i> <i>Apoballis, Bognera, Cercestis,</i> <i>Colobogynium, Croatiella,</i> <i>Cryptocoryne, Culcasia,</i> <i>Dieffenbachia, Furtadoa,</i> <i>Galantharum, Gamogyne,</i> <i>Gearum, Gorgonidium, Hera,</i> <i>Heteroaridarum, Homalomena,</i> <i>Hottarum, Incarum, Kiewia,</i> <i>Lagenandra, Nabalua, Naiadia,</i> <i>Ooia, Philodendron (sens.lat.),</i> <i>Philonotion, Phymatarum,</i> <i>Pichinia, Piptospatha,</i> <i>Pursegloveia, Rhynchopyle,</i> <i>Schismatoglottis, Schottariella,</i> <i>Schottarum, Taccharum, Tawaia,</i> <i>Toga, Vesta, Zantedeschia</i> | | | |
| Anthurieae | <i>Anthurium</i> | | | |
| Anthurium Clade | <i>See Anthurieae</i> | | | |
| Anubias-Arum Clade | Aroideae without <i>Gonatopus,</i> <i>Stylochaeton, Zamioculcas</i> | | | |
| Anubias-Calla Clade | <i>Anubias, Calla</i> | | | |
| Araceae | <i>Adelonema, Aglaonema</i> (including <i>Aglaodorum</i>), <i>Alloschemone, Alocasia,</i> | Orontioideae, Spirodela clade | Proto-Araceae, Spirodela clade | Proto-Araceae, Lemnoideae, Pothoideae, |

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|--|---|--|--|---|
| | <p><i>Ambrosinia, Amorphophallus,</i> <i>Amydrium, Anadendrum,</i> <i>Anaphyllopsis, Anchomanes,</i> <i>Anthurium, Anubias, Apoballis,</i> <i>Ariopsis, Arisaema, Arisarum,</i> <i>Arophyton, Arum, Biarum,</i> <i>Bognera, Caladium, Calla,</i> <i>Calloopsis, Cercestis,</i> <i>Chlorospatha, Colletogyne,</i> <i>Colobogynium, Colocasia,</i> <i>Croatiella, Cryptocoryne,</i> <i>Culcasia, Cyrtosperma,</i> <i>Dieffenbachia, Dracontioides,</i> <i>Dracontium, Eminium,</i> <i>Englerarum, Epipremnum,</i> <i>Filarum, Furtadoa,</i> <i>Galantharum, Gamogyne,</i> <i>Gearum, Gonatopus,</i> <i>Gorgonidium, Gymnostachys,</i> <i>Hapaline, Helicodicerus, Hera,</i> <i>Heteroaridarum, Heteropsis,</i> <i>Holochlamys, Homalomena,</i> <i>Hottarum, Incarum, Jasarum,</i> <i>Kiewia, Lagenandra, Lasia,</i> <i>Lasimorpha, Lemna, Lysichiton,</i> <i>Monstera, Montrichardia,</i> <i>Nabalu, Naiadia, Nephthytis</i> <i>(Africa), Nephthytis bintuluensis,</i> <i>Ooia, Orontium, Peltandra,</i> <i>Philodendron (sens.lat.),</i> <i>Philonotion, Podolasia, Pothos</i> <i>(including Pedicellarum),</i> <i>Phymatarum, Pichinia,</i></p> | | | <p>Monsteroideae, Lasioideae, Stylochaeton clade, Aroideae</p> |
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|---------------------------------|--|--|--|--|
| | <i>Piptospatha, Pistia, Pothoidium, Pursegloveia, Pycnospatha, Remusatia, Rhaphidophora, Rhodospatha, Rhynchopyle, Sauromatum, Schismatoglottis, Schottariella, Schottarum, Scindapsus, Spathiphyllum, Spirodela, Stenospermatum, Steudnera, Stylochaeton, Symplocarpus, Syngonium, Tacca, Tawaia, Toga, Typhonium, Typhonodorum, Ulearum, Urospatha, Vesta, Wolfia, Wolffia, Xanthosoma, Zamioculcas, Zantedeschia, Zomicarpa</i> | | | |
| Areae | | | <i>Arum, Biarum, Dracunculus, Eminium, Helicodiceros, Lazarum, Sauromatum, Theriophonum, Typhonium</i> | <i>Arum, Biarum, Dracunculus, Eminium, Helicodiceros, Lazarum, Sauromatum, Theriophonum, Typhonium</i> |
| Arisareae | | | <i>Ambrosina, Arisarum</i> | <i>Ambrosina, Arisarum</i> |
| Arisarum-Arophyton Clade | <i>Ambrosinia, Arisarum, Arophyton, Colletogyne, Peltandra, Typhonodorum</i> | | | |
| Aroideae | <i>Adelonema, Aglaonema (including Aglaodorum), Alocasia, Ambrosinia, Amorphophallus, Anchomanes,</i> | | | |

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| | <p><i>Anubias, Apoballis, Ariopsis,</i> <i>Arisaema, Arisarum, Arophyton,</i> <i>Arum, Biarum, Bognera,</i> <i>Caladium, Calla, Calloopsis,</i> <i>Cercestis, Chlorospatha,</i> <i>Colletogyne, Colobogynium,</i> <i>Colocasia, Croatiella,</i> <i>Cryptocoryne, Culcasia,</i> <i>Dieffenbachia, Eminium,</i> <i>Englerarum, Filarum, Furtadoa,</i> <i>Galantharum, Gamogyne,</i> <i>Gearum, Gonatopus,</i> <i>Gorgonidium, Hapaline,</i> <i>Helicodiceros, Hera,</i> <i>Heteroaridarum, Homalomena,</i> <i>Hottarum, Incarum, Jasarum,</i> <i>Kiewia, Lagenandra,</i> <i>Montrichardia, Nabalu, Naiadia,</i> <i>Nephtytis (Africa), Nephtytis</i> <i>binuluensis, Ooia, Peltandra,</i> <i>Philodendron (sens.lat.),</i> <i>Philonotion, Phymatarum,</i> <i>Pichinia, Piptospatha, Pistia,</i> <i>Pursegloveia, Remusatia,</i> <i>Rhynchopyle, Sauromatum,</i> <i>Schismatoglottis, Schottariella,</i> <i>Schottarum, Steudnera,</i> <i>Stylochaeton, Syngonium,</i> <i>Taccarum, Tawaia, Toga,</i> <i>Typhonium, Typhonodorum,</i> <i>Ulearum, Vesta, Xanthosoma,</i> <i>Zamioculcas, Zantedeschia,</i> <i>Zomicarpa</i></p> | | | |
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| Aroideae clade | | <i>Anubias</i> , <i>Montrichardia</i> , Philonotion clade, <i>Stylochaeton</i> , <i>Zamioculcas</i> , <i>Zantedeschia</i> clade | <i>Anubias</i> , <i>Calloopsis</i> , <i>Montrichardia</i> , Philonotion clade, <i>Zantedeschia</i> clade | <i>Anubias</i> , <i>Calloopsis</i> , Culcasieae, Philodendron clade, Nephtytideae, Aglaonemateae, Spathicarpeae, Cryptocoryneae, Schismatoglottid eae, Dracunculus clade |
| Arophyteae | | | <i>Arophyton</i> , <i>Carlephyton</i> , <i>Colletogyne</i> | <i>Arophyton</i> , <i>Carlephyton</i> , <i>Colletogyne</i> |
| Bisexual climbers clade | | | Monsteroideae, Pothoideae | |
| Bognera-Galantharum Clade | <i>Anchomanes</i> , Cryptocoryneae , Culcasieae-Philodendreae Clade , Schismatoglottideae , Spathicarpeae , <i>Zantedeschia</i> | | | |
| Bognera-Gorgonidium Clade | <i>See Spathicarpeae</i> | | | |
| Caladieae | <i>Caladium</i> , <i>Chlorospatha</i> , <i>Filarum</i> , <i>Hapaline</i> , <i>Jasarum</i> , <i>Syngonium</i> , <i>Ulearum</i> , <i>Xanthosoma</i> , <i>Zomicarpa</i> | | <i>Caladium</i> , <i>Chlorospatha</i> , <i>Filarum</i> , <i>Hapaline</i> , <i>Jasarum</i> , <i>Scaphispatha</i> , <i>Syngonium</i> , <i>Ulearum</i> , <i>Xanthosoma</i> , | <i>Caladium</i> , <i>Chlorospatha</i> , <i>Filarum</i> , <i>Hapaline</i> , <i>Jasarum</i> , <i>Scaphispatha</i> , <i>Syngonium</i> , <i>Ulearum</i> , <i>Xanthosoma</i> , |

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| | | | <i>Zomicarpa,</i> <i>Zomicarpella</i> | <i>Zomicarpa,</i> <i>Zomicarpella</i> |
| Calloopsis-Arum Clade | Calloopsis-Nephthytis afzelii Clade, Bognera-Galantharum Clade, Nephthytis bintuluensis-Arum Clade | | | |
| Calloopsis-Nephthytis afzelii Clade | <i>Calloopsis, Nephthytis afzelii</i> | | | |
| Colletogyne clade | See Arisarum-Arophyton Clade | <i>Ambrosinia,</i> <i>Arisarum,</i> <i>Arophyton,</i> <i>Carlephyton,</i> <i>Colletogyne,</i> <i>Typhonodorum</i> | <i>Arisareae,</i> Typhonodorum clade | |
| Colocasia clade | | | <i>Ariopsis,</i> <i>Colocasia,</i> <i>Remusatia,</i> <i>Steudnera</i> | <i>Ariopsis,</i> <i>Colocasia,</i> <i>Remusatia,</i> <i>Steudnera</i> |
| Cryptocoryneae | <i>Cryptocoryne, Lagenandra</i> | | <i>Cryptocoryne,</i> <i>Lagenandra</i> | <i>Cryptocoryne,</i> <i>Lagenandra</i> |
| Cryptocoryneae-Schismatoglottideae Clade | See Lagenandra-Galantharum Clade | | | |
| Culcasieae | <i>Cercestis, Culcasia</i> | | <i>Cercestis,</i> <i>Culcasia</i> | <i>Cercestis,</i> <i>Culcasia</i> |
| Culcasia-Philodendron Clade | <i>Adelonema, Cercestis, Culcasia, Furtadoa, Homalomena, Philodendron (including Thaumatophyllum)</i> | | | |
| Culcasieae-Philodendreae Clade | See Culcasia-Philodendron Clade | | | |
| Dracontieae | <i>Anaphyllopsis, Dracontioides, Dracontium</i> | | | |

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| Dracontium- Dracontioides Clade | <i>See Dracontieae</i> | | | |
| Dracontium-Heteropsis Clade | Lasioideae, Monsteroideae, Pothoideae | | | |
| Dracontium-Podolasia Clade | <i>See Lasioideae</i> | | | |
| Dracunculus clade | <i>See Amorphophallus-Arum Clade</i> | Ambrosina clade, Amorphophallus clade | Ambrosina clade, Amorphophallus clade | Thomsonieae, Caladieae, Ambrosina clade |
| Gonatopus-Zamioculcas Clade | <i>Gonatopus, Zamioculcas</i> | | | |
| Gymnostachydoideae | <i>Gymnostachys</i> | | | <i>Gymnostachys</i> |
| Gymnostachys- Symplocarpus Clade | <i>Gymnostachys,, Lysichiton, Orontium, Symplocarpus</i> | | | |
| Hapaline-Chlorospatha Clade | <i>See Caladieae</i> | | | |
| Anepsiadeae | <i>Alloschemone, Heteropsis, Rhodospatha, Stenospermatum</i> | | | |
| Heteropsis clade | | | | <i>Alloschemone, Heteropsis, Rhodospatha, Stenospermatum</i> |
| Homalomena clade | <i>See Culcasia-Philodendron Clade</i> | | Culcasieae, Philodendron clade | |
| Lagenandra- Galantharum Clade | <i>Apoballis, Colobogynium, Cryptocoryne, Galantherum, Gamogyne, Hera, Heteroaridarum, Hottarum, Kiewia, Lagenandra, Nabalu, Naiadia, Ooia, Philonotion, Phymatarum, Pichinia, Piptospatha, Pursegloveia,</i> | | | |

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| | <i>Rhynchopyle, Schismatoglottis, Schottariella, Schottarum, Tawaia, Toga, Vesta</i> | | | |
| Lasiaceae | <i>Cyrtosperma, Lasia, Lasimorpha, Podolasia, Pycnospatha, Urospatha</i> | | | |
| Lasioideae | <i>Anaphyllopsis, Cyrtosperma, Dracontioides, Dracontium, Lasia, Lasimorpha, Podolasia, Pycnospatha, Urospatha</i> | <i>Anaphyllopsis, Cyrtosperma, Lasia, Podolasia, Pycnospatha, Urospatha</i> | | <i>Anaphyllum, Anaphyllopsis, Cyrtosperma, Dracontioides, Dracontium, Lasia, Lasimorpha, Podolasia, Pycnospatha, Urospatha</i> |
| Lemnoideae | <i>Lemna, Spirodela, Wolffia, Wolffiaella</i> | <i>Lemna, Spirodela, Wolffia, Wolffiaella</i> | <i>Landoltia, Lemna, Spirodela, Wolffia, Wolffiaella</i> | <i>Landoltia, Lemna, Spirodela, Wolffia, Wolffiaella</i> |
| Monstera-Anadendrum Clade | See Monstereae | | | |
| Monstereae | <i>Amydrium, Anadendrum, Epipremnum, Monstera, Rhapsidophora, Scindapsus</i> | | | |
| Monsteroideae | <i>Alloschemone, Amydrium, Anadendrum, Epipremnum, Heteropsis, Holochlamys, Monstera, Rhapsidophora, Rhodospatha, Scindapsus, Spathiphyllum, Stenospermation</i> | <i>Amydrium, Epipremnum, Heteropsis, Monstera, Rhapsidophora, Rhodospatha, Scindapsus,</i> | Heteropsis clade, Spathiphyllae, Rhapsidophora clade | Heteropsis clade, Spathiphyllae, Rhapsidophora clade |

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| | | <i>Spathiphyllum</i> , <i>Stenospermation</i> | | |
| Nephtytideae | | | <i>Anchomanes</i> , <i>Nephtytis</i> , <i>Pseudohydrosme</i> | <i>Anchomanes</i> , <i>Nephtytis</i> , <i>Pseudohydrosme</i> |
| Nephtytis bintuluensis-Aglaonema Clade | <i>Aglaonema</i> (including <i>Aglaodorum</i>), <i>Nephtytis</i> <i>bintuluensis</i> | | | |
| Nephtytis bintuluensis-Arum Clade | | | | |
| Orontioideae | <i>Lysichiton</i> , <i>Orontium</i> , <i>Symplocarpus</i> | <i>Lysichiton</i> , <i>Orontium</i> , <i>Symplocarpus</i> | <i>Lysichiton</i> , <i>Orontium</i> , <i>Symplocarpus</i> | <i>Lysichiton</i> , <i>Orontium</i> , <i>Symplocarpus</i> |
| Orontium-Symplocarpus Clade | See Orontioideae | | | |
| Peltandreae | | | <i>Peltandra</i> , <i>Typhonodorum</i> | <i>Peltandra</i> , <i>Typhonodorum</i> |
| Philodendreae | <i>Adelonema</i> , <i>Furtadoa</i> , <i>Homalomena</i> , <i>Philodendron</i> (including <i>Thaumatophyllum</i>) | | | |
| Philodendron clade | See Philodendreae | | <i>Philodendron</i> , <i>Furtadoa</i> , <i>Homalomena</i> | <i>Adelonema</i> (<i>Homalomena</i> <i>speariaae</i>), <i>Philodendron</i> , <i>Furtadoa</i> , <i>Homalomena</i> |
| Philonotion clade | | <i>Calla</i> , Dracunculus clade, Rheophytes clade | <i>Calla</i> , Dracunculus clade, Rheophytes clade | |
| Pistia clade | See Pistia-Arum Clade | | <i>Pistia</i> , <i>Colocasia</i> clade, <i>Alocasia</i> clade | <i>Pistia</i> , <i>Colocasia</i> clade, <i>Englerarum</i> |

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| | | | | <i>(Alocasia hypnosa), Leucocasia (Colocasia gigantea), Alocasia, Areac, Arisaema</i> |
| Pistia-Arum Clade | <i>Alocasia, Ariopsis, Arisaema, Arum, Biarum, Colocasia, Eminium, Englerarum, Helicodiceros, Pistia, Remusatia, Sauromatum, Steudnera, Typhonium</i> | <i>Alocasia, Ariopsis, Arisaema, Arum, Colocasia, Dracunculus, Eminium, Englerarum, Helicodiceros, Leucocasia, Pinellia, Pistia, Remusatia, Sauromatum, Steudnera, Typhonium</i> | | |
| Podolasia clade | | Aroideae, Lasioideae | Lasioideae, Unisexual flowers clade | Aroideae, Lasioideae, Stylochaeton clade, |
| Potheae | <i>Pothoidium, Pothos (including Pedicellarum)</i> | | | <i>Pothos, Pedicellarum, Pothoidium</i> |
| Pothoideae | <i>Anthurium, Pothoidium, Pothos (including Pedicellarum)</i> | <i>Anthurium, Pothoidium, Pothos (including Pedicellarum)</i> | | <i>Anthurium, Potheae</i> |

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| Pothos-Anthurium Clade | <i>See Pothoideae</i> | | | |
| Pothos dolichophyllus–chinensis Clade | <i>See Potheae</i> | | | |
| Pothos-Heteropsis Clade | Monsteroideae, Pothoideae | | | |
| Proto-Araceae | | | <i>Gymnostachys,</i> <i>Orontioideae</i> | Gymnostachydoideae, <i>Orontioideae</i> |
| Rhaphiodphora clade | | | | <i>Amydrium,</i> <i>Anadendrum,</i> <i>Epipremnum,</i> <i>Monstera,</i> <i>Rhaphiodphora,</i> <i>Scindapsus</i> |
| Rheophytes clade | <i>See Lagenandra-Galantharum Clade</i> | <i>Cryptocoryne,</i> <i>Hottarum,</i> <i>Kiewia, Ooia,</i> <i>Philonotion,</i> <i>Schismatoglottis,</i> <i>Toga</i> | Cryptocoryneae, Schismatoglottideae | |
| Schismatoglottideae | <i>Apoballis, Colobogynium,</i> <i>Galantharum, Gamogyne, Hera,</i> <i>Heteroaridarum, Hottarum,</i> <i>Kiewia, Nabalua, Naiadia, Ooia,</i> <i>Philonotion, Phymatarum,</i> <i>Pichinia, Piptospatha,</i> <i>Pursegloveia, Rhynchopyle,</i> <i>Schismatoglottis, Schottariella,</i> <i>Schottarum, Tawaia, Toga, Vesta</i> | | <i>Aridarum, Bakoa,</i> <i>Bucephalandra,</i> <i>Phymatarum,</i> <i>Piptospatha,</i> <i>Schismatoglottis,</i> <i>Schottariella</i> | <i>Apoballis,</i> <i>Aridarum,</i> <i>Bakoa,</i> <i>Bucephalandra,</i> <i>Hestia, Ooia,</i> <i>Phymatarum,</i> <i>Pichinia,</i> <i>Piptospatha,</i> <i>Schottariella,</i> <i>Schismatoglottis</i> |
| Spathicarpeae | <i>Bognera, Croatiella,</i> <i>Dieffenbachia, Gearum,</i> | | <i>Asterostigma,</i> <i>Bognera,</i> <i>Croatiella,</i> | <i>Asterostigma,</i> <i>Bognera,</i> <i>Croatiella,</i> |

| | | | | |
|---|---|---------------------------------------|--|--|
| | <i>Gorgonidium, Incarum, Taccarum</i> | | <i>Dieffenbachia, Gearum, Gorgonidium, Incarum, Mangonia, Spathantheum, Spathicarpa, Synandropadix, Taccarum</i> | <i>Dieffenbachia, Gearum, Gorgonidium, Incarum, Mangonia, Spathantheum, Spathicarpa, Synandropadix, Taccarum</i> |
| Spathiphyllae | <i>Holochlamys, Spathiphyllum</i> | | | <i>Holochlamys, Spathiphyllum</i> |
| Spathiphyllum– Heteropsis Clade | <i>See Monsteroideae</i> | | | |
| Spathiphyllum– Holochlamys Clade | <i>See Spathiphyllae</i> | | | |
| Spirodela clade | Crown Araceae clade, Lemnoideae | Crown Araceae clade, Lemnoideae | Lemnoideae, True Araceae | |
| Spirodela-Wolffiella Clade | <i>See Lemnoideae</i> | | | |
| Stylochaeton clade | | | <i>Stylochaeton, Zamioculcadoidea</i> | <i>Stylochaeton, Zamioculcadoideae</i> |
| Stylochaeton-Arum Clade | <i>See Aroideae</i> | | | |
| Stylochaeton-Gonatopus Clade | <i>Gonatopus, Stylochaeton, Zamioculcas</i> | | | |
| Stylochaetoneae | <i>Stylochaeton</i> | | | |
| Thomsonieae | | | <i>Amorphophallus, Pseudodracontium (= Amorphophallus)</i> | <i>Amorphophallus, Pseudodracontium (= Amorphophallus)</i> |

| | | | | |
|--------------------------------------|-------------------------------|---|---|---|
| | | | | <i>Amorphophallus</i>) |
| True Araceae | | | Bisexual climbers clade, Podolasia clade | |
| Typhonodorum clade | | | Arophyteae, Peltandreae | Arophyteae, Peltandreae |
| Unisexual flowers clade | | | Aroideae, Stylochaeton clade | |
| Urospatha-Podolasia Clade | <i>See Lasieae</i> | | | |
| Zamioculcadoideae | <i>See Zamioculcadeae</i> | | | <i>Gonatopus,</i> <i>Zamioculcas</i> |
| Zamioculcadeae | <i>Gonatopus, Zamioculcas</i> | | | |
| Zantedeschia clade | | Anchomanes clade, Homalomena clade, Spathicarpeae, <i>Zantedeschia</i> | Anchomanes clade, Homalomena clade, Spathicarpeae, <i>Zantedeschia</i> | |

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1091 FIGURE 1. Comparison of DNA sequence recovery (in base pairs) between Araceae, other
1092 Alismatales, other monocots (excluding Alismatales), and other angiosperms (excluding
1093 monocots), based on data obtained from the Kew Tree of Life Explorer
1094 (<https://treeoflife.kew.org/>; Baker et al 2022).

1095

1096 FIGURE 2. Multi-species coalescent phylogenetic tree of family Araceae based on
1097 supercontigs sequence data inferred using ASTRAL. (A) Subfamilies Gymnostachioideae,
1098 Orontioideae, Lemnoideae, Lasioideae, Pothoideae and Monsteroideae; (B) Subfamily
1099 Aroideae. Pie charts above branches display quartet score (QS) values for each node (blue:
1100 species tree topology QS; orange: first alternative topology QS; gray: second alternative
1101 topology QS). Local posterior probability values (LPP) are indicated below branches. Red
1102 asterisks indicate branches for which the null hypothesis that a given branch is a polytomy
1103 could not be rejected ($p > 0.05$; polytomy test, Sayyari and Mirarab 2018). Photo credits: from
1104 top to bottom and right to left; (a.) R. Zabeau; ((b.) A. Haigh (AH); (c.) L. Bouttier; (d.)
1105 Unknown; (e.) E. Lucas; (f.; g.) AH; (h.): P. Boyce (PB); (i.) AH; (j.) P. Wilkin; (k.; l.) AH;
1106 (m.) J. Bogner; (n.; o.) AH; (p.) D. Bown; (q.; r.; s.) AH ; (t.) RBGKew ©; (u.) PB; (v.) M.
1107 Gibernau; (w.) M. Check.

1108 FIGURE 3. Comparison of the topologies obtained with the nuclear target enrichment (left)
1109 versus the plastid sequence data (right), analyzed using the multi-species coalescent and
1110 concatenated maximum likelihood approaches, respectively. Colors correspond to those
1111 shown in Fig 2. Values on nodes are local posterior probability values (nuclear) and bootstrap
1112 percentages (plastid); only those below 1.0/100% are shown.

1113 SUPPLEMENTARY FIGURE S1: Voucher specimens and ENA accessions; collection

1114 information and nuclear recovery statistics.

1115

1116 SUPPLEMENTARY FIGURE S2: Heatmap of gene recovery efficiency plotted according to
1117 the multi-species coalescent phylogenetic tree based on the nuclear data in which each row
1118 represents a terminal on the tree and each column a gene. Higher percentage of target length
1119 recovered is indicated by darker shade of grey.

1120

1121 SUPPLEMENTARY FIGURE S3: Source of plastid data and recovery length of the plastid
1122 contig

1123

1124 SUPPLEMENTARY FIGURE S4: (A) Number of genes recovered per sample according to
1125 different target length percentages and (B) overall number of genes per percentage category.

1126

1127 SUPPLEMENTARY FIGURE S4: Maximum likelihood analysis using IQ-TREE of the
1128 nuclear concatenated dataset. Values on branches are bootstrap percentages.

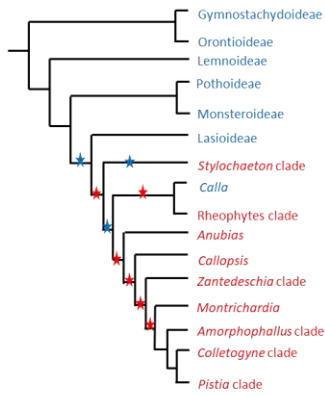
1129

1130 SUPPLEMENTARY FIGURE S5: Maximum likelihood analysis using IQ-TREE of the
1131 plastid concatenated dataset. Values on branches are bootstrap percentages.

1132

1133

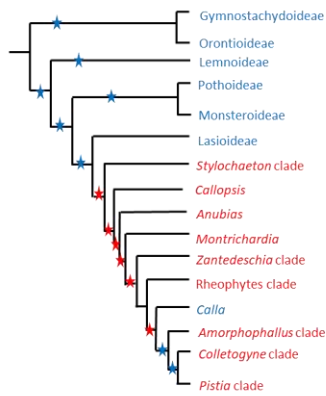
1134



Cabrera & al. (2008 – Fig.1 : strict consensus of the combined parsimony analysis)

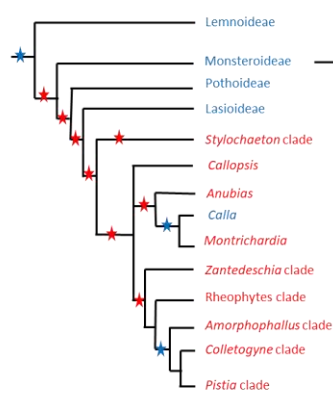
★ < 72
 ★ > 84
 ★ ≥ 95

★ Not supported node
 ★ Moderately supported node



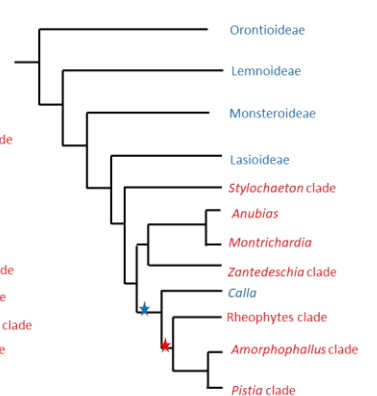
Cusimano & al. (2011 Fig.1: maximum likelihood analysis)

★ (< 0.85)
 ★ > 0.85
 ★ ≥ 0.97



Chartier et al (2014 Fig.1 : maximum likelihood analysis)

★ (< 85)
 ★ ≥ 85
 ★ ≥ 95

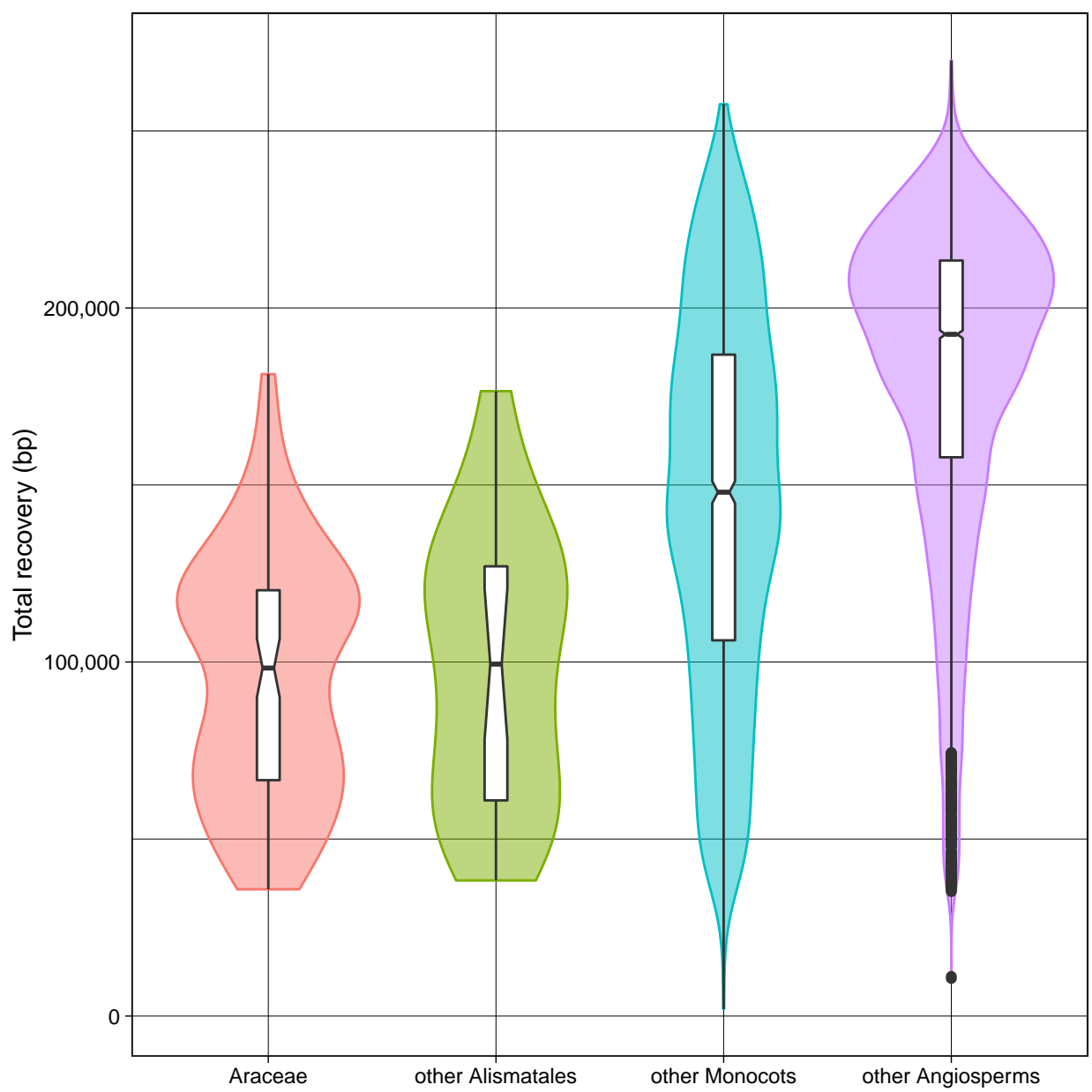


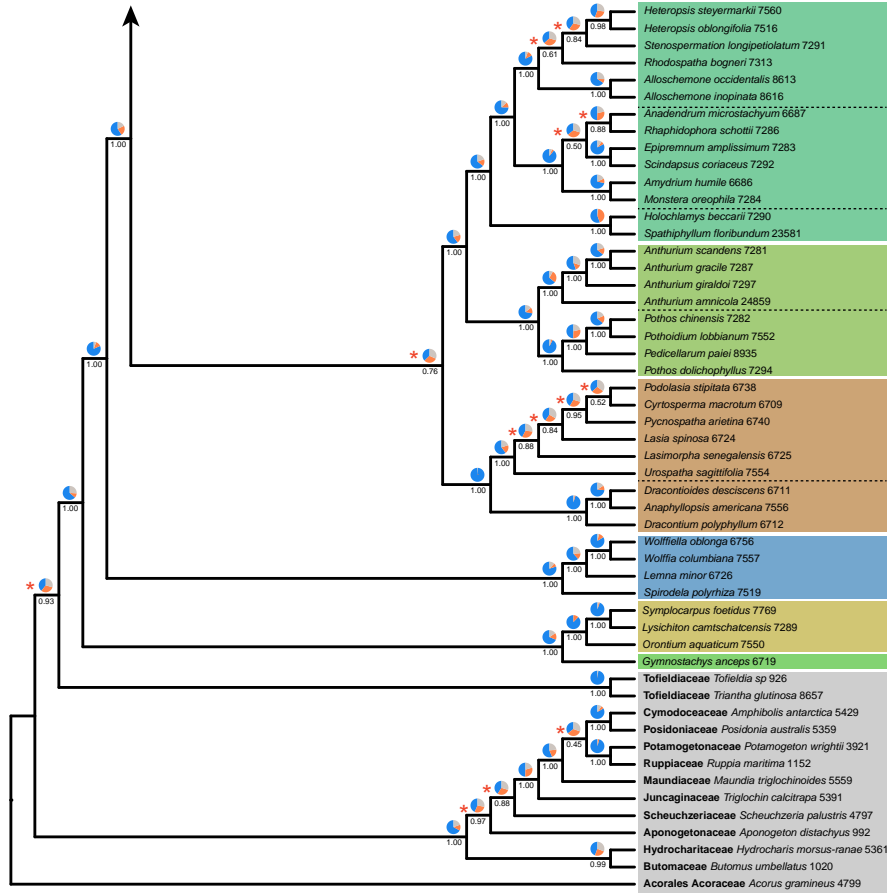
Abdullah et al (2020 Fig.6 : maximum likelihood analysis)

★ 67
 ★ 92
 ★ 100

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1136





Monsteroideae - *Spathiphyllum floribundum*



Monsteroideae - *Heteropsis oblongifolia*



Pothoideae - *Anthurium gracile*



Lasioidae - *Anaphyllopsis americana*



Orentioidae - *Symplocarpus foetidus*



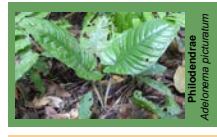
Lemnoideae - *Lemna minor*



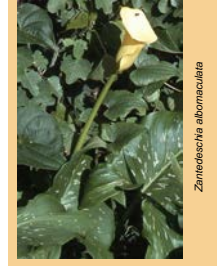
Gymnostachyoidae
Gymnostachys anceps



Schlematoglotteae
Heterandrium nicolsonii



Philodendron
Adelorena picturatum



Zantedeschia albomaculata

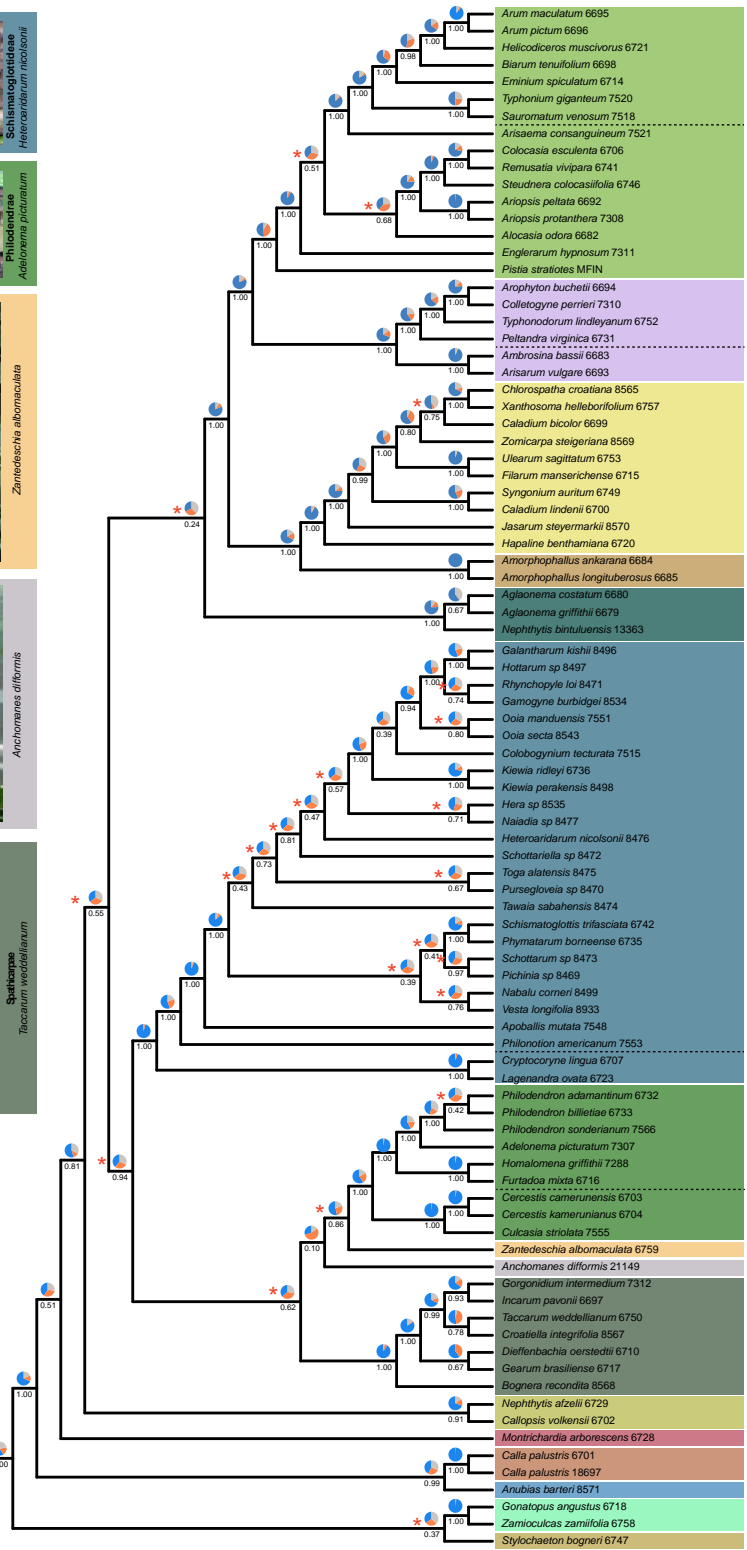


Anchomanes difformis



Staehkeae
Taccarum weddellianum

Aroideae



Arisarium-Arophyton Clade
Colletogyne perrieri



Pistia-Arum Clade
Heliconia muscivora



Caladaceae
Ureum sagittatum



Caladaceae
Ureum sagittatum



Aglonomeae + Nephthytis
Callipopsis volkensii



Aglonomeae + Nephthytis
Aglaonema griffithii



Calla palustris



Calla palustris
Montichardia arborescens



Zamioculcaee
Zamioculcas zamiifolia



Zamioculcaee
Anubias barteri



Stylochaetonaeae
Stylochaeton bogneri

Araceae Clades - A353

Plastid

