Testing the monophyly of *Spathiphyllum*, and the relationship between Asian and tropical American species.

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ABSTRACT

As part of a broader phylogenetic study of the subfamily Monsteroideae we used three plastid and one nuclear markers to test the monophyly of the genus *Spathiphyllum*. Our results support the tribe Spathiphylleae as sister of the remaining Monsteroideae, and the monophyly of *Spathiphyllum*. The monotypic genus *Holochlamys* appears nested within *Spathiphyllum* in a clade with the Asian species and *S. cannifolium*. However, support for this clade is low. The section *Spathiphyllum* shows a high support and is sister to the remaining species in the genus. As a consequence *Spathiphyllum* remains as the only disjunct aroid genus between the Old and New World Tropics.

INTRODUCTION

With the increased application of molecular data for phylogenetic analysis, the understanding of relationships within Araceae has been improved considerably (Cabrera, 2008, Cusimano, 2011, Henríquez, 2014). Additionally, new fossils and biogeographical studies have helped to elucidate the historical and current geographic distribution patterns in the family (Nauheimer, 2012a). As a result, aroid classification has changed dramatically; several genera have been resurrected; and new genera have been created (Cabrera, 2008, Cusimano, 2011). Nevertheless, many critical issues are still in need of more data.

Before this molecular era there were thought to be three geographically disjunct genera between the old world and new world tropics. Species in the genera *Homalomena*, *Schismatoglottis* and *Spathiphyllum* were found in both tropical America and tropical Asia, particularly within the Malay Archipelago and Melanesia (van Steenis, 1962, Grayum, 1990, Mayo et al., 1997). However, recent studies (Barabé, 2002, Gauthier, 2008, Wong et al., 2013) have shown evidence challenging the monophyly of disjunct *Schismatoglottis* and *Homalomena* (they are no longer disjunct in their revised circumscriptions), leaving *Spathiphyllum* as the only remaining aroid genus with a tropical disjunction.

The genus *Schismatoglottis*, with only three species in South America, and about 150 in tropical Asia, belongs to the tribe Schismatoglottidae that in its current circumscription comprises 11 genera. Several of these genera are small groups recognized thanks to molecular data (Wong et al., 2010a, 2010b, Low, 2014). The three Neotropical species previously recognized in *Schismattoglotis* are now in the resurrected genus *Philonotion* (Wong, 2010c) that belong to its own tribe Philonotieae. Equally, the genus *Homalomena* is considerable more diverse in Tropical Asia with about 500 species, versus only 10 species in Tropical America (Boyce & Croat, 2013). Unlike *Schismatoglottis*, molecular evidence for the separation of Neotropical *Homalomena* is not conclusive (Barabé, 2002, Gauthier, 2008, Wong et al., 2013). The most plausible options for the assignment of the Neotropical *Homalomena* would be the resurrection of the genus *Adelomena,* or the combination of these species of *Homalomena* with *Philodendron* subgenus *Pterosmischum* in the genus *Elopium* (Wong et al., 2013). Thus, more extensive sampling and more molecular data are necessary to fully understand the relationships among *Homalomena*, and the subgenera of *Philodendron*.

Finally, we have the genus *Spathiphyllum,* which has a different distribution pattern. The genus is more diverse in Tropical America with about 50 species (Cardona, 2004), and only three species in Southeast Asia. *Spathiphyllum commutatum* Schott is widespread in Malesia and reaches Micronesia; *S. solomonense* Nicolson is restricted to the Solomon Islands; and *S. schlechteri* (Engl. & K.Krause) Nicolson is restricted to New Guinea. Neotropical species are concentrated in the northern Andes, with only a few species reaching Central America and Mexico (Cardona, 2004), and one species, *S. leave* reaches Cocos Island in the Pacific Ocean west of Costa Rica. No comprehensive phylogenetic studies, until now, have included more than three *Spathiphyllum* from Tropical America, or any of the Asian species.

Spathiphyllum together with *Holochlamys becarii* form the tribe Spathiphylleae in the subfamily Monsteroideae (Cabrera, 2008, Cusimano, 2011). The relationships of the tribe Spathiphylleae within Monsteroideae have been controversial, but recent studies strongly support Spathiphylleae as sister to the remaining Monsteroideae (Chartier, 2014, Henriquez, 2014). These two genera are very similar morphologically and ecologically, differing mainly in a few characteristics of the flowers. Whereas *Spathiphyllum* species have two to three locules and ovules with axillar placentation, *Holochamys* has one locule and ovules with basal placentation (Bunting, 1960, Mayoet al.,1997). Nevertheless, anatomical studies suggest that the *Holochlamys* ovary could actually have two locules, and the placentation may not be basal (Eyde, 1967, Carvell,1989).

Currently, *Spathiphyllum* is divided into four sections based the fusion of the perianth segments, the form and length of the pistils relative to the perianth, and the attachment the spathe to the peduncle (Bunting, 1960). Asian species together with *S. cannifolium* and *S. leave* form the section *Massowia,* which is recognized by having the perianth segments completely connate forming a cup around the pistil. This trait led Bunting (1960) to propose the monophyly of this group. However, Williams & Dressler (1967) and Grayum (1984, 1990) suggested this could be an artificial group with *Holochlamys* nested within it. Sections *Amomophyllum, Dysspathiphyllum, and Spathiphyllum* have free perianth segments.

As part of a broader systematic study of the subfamily Monsteroideae (Zuluaga, unpublished data) we tested the monophyly of *Spathiphyllum* and *Holochlamys,* and investigated the relationships of the Asian and American species of *Spathiphyllum.*

METHODS

Taxon Sampling and DNA sequencing. During the sampling for the study of systematics of the subfamily Monsteroideae we sequenced a total of seven accessions of the genus *Spathiphyllum* from Tropical America, *S. commutatum* and *S. solomonense* from Tropical Asia, and one accession of *Holochlamys becarii*. Additional sequences for *S. phryniifolium* were obtained from GENBANK. For this study we used representatives from all ten additional Monsteroideae genera, plus six other aroids as outgroups.

DNA was extracted from silica-dried plant material using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's specifications. Some samples were extracted using a CTAB method adapted from Doyle & Doyle (1987). We sequenced four plastid markers, *matK*, *rbcL*, *trnC-petN* spacer, and partial *ycf1*. PCR products were purified using ExoSap-It (Cleveland, OH). Sequencing reactions used the BigDye Terminator Cycle Sequencing Kit, and cleaned using Agencourt CleanSeq (Beverly, MA) magnetic beads. BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA). Samples were sequenced using PE-Biosystems version3.7 of Sequencing Analysis at the University Wisconsin–Madison Biotechnology Center.

Molecular analysis. All sequences were manually edited in Geneious6.0 (Biomatters, http://www.geneious.com). Sequences were aligned in Geneious6.0 using a combination of Muscle (Edgar 2004) and MAFFT (Katoh 2002). Finally, all alignments were checked manually. We performed individual and combined phylogenetic analyses. Analyses for each marker/dataset were performed under Maximum Likelihood using RaxML 7.0 (Statamakis, 2006), and MrBayes 3.1.2 on XSEDE, as implemented in the CIPRES portal. For RaxML, we used the GTRGAMMA model in all cases. For Bayesian analysis models were calculated using Jmodeltest2 (Darriba, 2012).

RESULTS.

So far at least one of the four molecular markers has been successfully sequenced for *S. cannifolium* (two accessions), *S cochlearispathum*, *S. commutatum*, *S. floribundum*, *S. minor*, *S. patulinervum, S. solomonense, S. wallisii*, and *Holochlamys becarii*. Only the *trnC-petN* spacer was sequenced for the Asian *Spathiphyllum*. The combined alignment length is 4970 bp with 36% missing data. Despite using four molecular markers the identical sites between pairs of species within *Spathiphyllum* is more than 97%. This low variation is common within the genera of the subfamily Monsteroideae (Unpublished data).

Trees derived from individual gene analyses are congruent, however the resolution is low except for a clade comprising the species of the section *Spathiphyllum*. Thus, a combined gene analysis is presented (**Figure 1)**. All individual and combined analyses show the tribe Spathiphylleae as monophyletic and sister to the remaining Monsteroideae, thus supporting the topology presented by Chartier (2014) and Henríquez (2014). Section *Spathiphyllum* represented by *S. cochlearispathum*, *S. phryniifolium*, and *S. wallisii* shows strong support, and is sister to the remaining Spathiphylleae. The genus *Holochlamys* appears nested within *Spathiphyllum* in a clade with the Asian species and one accession of *S. cannifolium* (section *Massowia*). Section *Amomophyllum* (*S. minor*, *S. floribundum*, *S. patulinervum*) and one accession of *S. cannifolium* are sister to the clade containing *Holochlamys*. However support for the last two clades is low (Figure1).

DISCUSSION.

A monophyletic tribe Spathiphylleae has been supported since the first molecular studies in Araceae, based on only a few genes, were published (Tam, 2004, Cabrera, 2008). However, the position of Spathiphyllleae as sister to rest of Monsteroideae is only recovered in the most recent studies with more data included

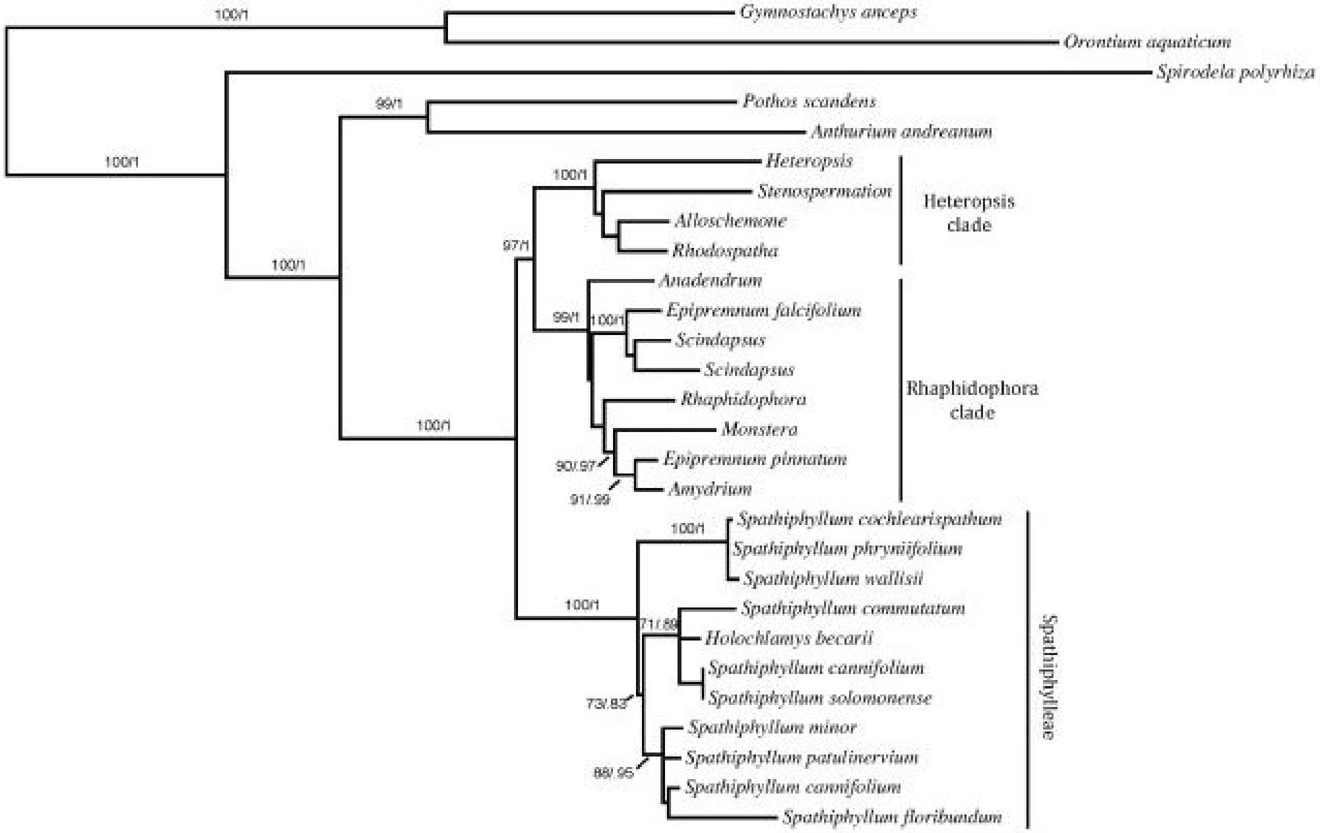

Figure 1. Maximum likelihood tree from RaxML using four plastid markers (*matK*, *rbcL, trnC-petN*spacer, and partial *ycf1*). Bootstrap supports >75 and Bayesian posterior probabilities >0.85 are shown on the branches.

(Chartier, 2014, Henriquez, 2014). Our results strongly support this topology (**Figure 1)**. Most of the synapomorphies defining Monsteroideae as presented in Cusimano (2011) seem to be more appropriate to be assigned to the clade containing all Monsteroideae but Spathiphylleae. These synapomorphies and the alternative characters in Spathiphylleae are: pollen zonate (ring-like) (vs. pollen multiaperturate with exine surface striate/polyplicate); spathe soon deciduous with a distinct basal abscission (vs. Spathe marcescent), large trichosclereids not in bundles (vs. smaller trichosclereids in bundles). The only synapomorphy remaining for Monsteroideae is the presence of trichosclereids.

Within the tribe Spathiphylleae, the support for the position of *H. becarii* is not conclusive, but some markers suggest this species may belong to the genus *Spathiphyllum*. *Holochlamys* appears consistently embedded in a clade with section *Massowia*. These two genera are very similar in habit and morphology (Bunting,1960), stem vasculature (French & Tomlison, 1981), palynological features (Grayum,1992), and floral anatomy (Carvell, 1989). Moreover, *Holochlamys* shares with section *Massowia* the presence of fused perianth parts forming a cup around the pistil (Bunting, 1960, Carvell, 1989). The proposed differences between these two genera - a unilocular ovary, and ovules with basal placentation - have been challenged by more detailed anatomical studies (Eyde, 1967). Carvell (1989) undertook a very detailed anatomical study of Monsteroideae and Pothoideae, and reported unilocular ovaries, and basal ovules for *H. becarii*. However, he also reported the presence of an incomplete septum in several flowers, and the attachment of some the ovules to that septum. Additionally, Carvell (1989) found considerable intra- and interspecific variation in locule, ovules, tepals, and stamen numbers in *Spathiphyllum*. Despite the fact that we consider there to be no strong morphological evidence to separate *Holochlamys* from *Spathiphyllum* (especially from the section *Massowia*), our analysis currently lacks adequate resolution within this clade, and so we prefer to be cautious at this point in time, necessarily gathering more data before proposing any taxonomic changes for these species.

Our sampling did not allow a complete assessment of the sectional classification in *Spathiphyllum* as proposed by Bunting (1960). The only section with strong support through all our analysis is the section *Spathiphyllum*, which is also supported by the morphology of their flowers with a long and conical pistil exceeding the perianth (Bunting, 1960). Williams and Dressler (1967), and Grayum (1984, 1990), discussed hypotheses for the origin and biogeography of *Spathiphyllum* and *Holochlamys*. We are undertaking additional molecular and biogeographical analyses of the subfamily Monsteroideae, and more detailed discussion on this topic will be presented in subsequent papers.

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