RESEARCH ARTICLE

Botany
American Journal of

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

Anna L. Haigh[1] | Marc Gibernau[2] | Olivier Maurin[1] | Paul Bailey[1] |
Mónica M. Carlsen[3] | Alistair Hay[4] | Kevin Leempoel[1] | Catherine McGinnie[1] |
Simon Mayo[1] | Sarah Morris[1] | Oscar Alejandro Pérez-Escobar[1] |
Wong Sin Yeng[5] | Alejandro Zuluaga[6] | Alexandre R. Zuntini[1] |
William J. Baker[1]* | Félix Forest[1]*

[1]Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AE, United Kingdom

[2]Laboratory of Sciences for the Environment (UMR 6134), CNRS–University of Corsica, Ajaccio, 20000, France

[3]Missouri Botanical Garden, St. Louis, Missouri 63110, USA

[4]Australian Institute of Botanical Science, Royal Botanic Gardens & Domain Trust, Mrs Macquarie's Road, Sydney, 2000, Australia

[5]Institute of Biodiversity and Environmental Conservation, Universiti Malaysia Sarawak (UNIMAS), 94300, Kota Samarahan, Sarawak, Malaysia

[6]Departamento de Biología, Universidad del Valle, Calle 13 #100-00, Cali, Colombia

Correspondence

Anna L. Haigh and Olivier Maurin, Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AE, United Kingdom
Email: a.haigh@kew.org and o.maurin@kew.org

Abstract

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

Methods: Nuclear DNA sequence data were generated for 128 species across 111 genera (78%) of Araceae using target sequence capture and the Angiosperms 353 universal probe set.

Results: The phylogenomic data confirmed the monophyly of the eight Araceae subfamilies, but the phylogenetic position of subfamily Lasioideae remains uncertain. The genus *Calla* is included in subfamily Aroideae, which has also been expanded to include Zamioculcadoideae. The tribe Aglaonemateae is newly defined to include the genera *Aglaonema* and *Boycea*.

Conclusions: Our results strongly suggest that new research on African genera (*Callopsis*, *Nephthytis*, and *Anubias*) and *Calla* will be important for understanding the early evolution of the Aroideae. Also of particular interest are the phylogenetic positions of the isolated genera *Montrichardia*, *Zantedeschia*, and *Anchomanes*, which remain only moderately supported here.

KEYWORDS

Alismatales, Angiosperms353, Lemnoideae, nuclear phylogenomic tree

The Araceae has a particular significance in the evolution of the monocots, one of the major clades of angiosperms (APG IV). It arises from one of the earliest branching events in Alismatales and is by far the most diverse subclade of the order (Stevens, 2001 onward). It also has a remarkable fossil history, including some of the earliest known records for monocots, stretching back to the early Cretaceous (Friis et al., 2004, 2010; Hesse and Zetter, 2007; Bogner, 2009; Mayo et al., 2013;

Anna L. Haigh, Marc Gibernau and Olivier Maurin have contributed equally to this work.

*Joint senior authors

- -

Smith, 2013; Iles et al., 2015; Hoffman, 2021). A recent review found that the family was particularly diverse during the late Cretaceous and early Paleocene (Stockey et al., 2021). Nauheimer et al. (2012), using a molecular analysis combined with fossil data and computational modeling, concluded that the early evolution of the family was linked to aquatic or subaquatic environments and that early diversification within the family, which led to the evolution of subfamilial lineages, took place during the Cretaceous and was associated with the fragmentation of the supercontinent Pangaea.

Araceae comprises 3667 species assigned to 143 genera (Boyce and Croat, 2011 onward), many of which are globally important in horticulture and agriculture. They make up one of the most ecologically diverse families of flowering plants, from the smallest known species (*Wolffia globosa* (Roxb.) Hartog & Plas) to the largest unbranched inflorescence (*Amorphophallus titanum* (Becc.) Becc.), with habits ranging from floating aquatics, geophytes, and large arborescent herbs to nomadic vines and epiphytes. The family is cosmopolitan, although it is by far most diverse in the wet tropics, particularly in South and Central America and Southeast Asia.

There is a large body of work on the molecular systematics of Araceae (e.g., Duvall et al., 1993; Sriboonma et al., 1993; French et al., 1995; Wen et al., 1996; Othman, 1997; Barabé et al., 2002; Grob et al., 2002; Renner and Zhang, 2004; Renner et al., 2004; Rothwell et al., 2004; Tam et al., 2004; Kitano et al., 2005; Nie et al., 2006; Cabrera et al., 2008; Gauthier et al., 2008; Mansion et al., 2008; Wong et al., 2010; Cusimano et al., 2011; Nauheimer et al., 2012; Carlsen and Croat, 2013; Chartier et al., 2014; Henriquez et al., 2014; Wong et al., 2016; Low et al., 2018; Sakuragui et al., 2018; Vasconcelos et al., 2018; Canal et al., 2019; Zuluaga et al., 2019; Abdullah et al., 2020; Henriquez et al., 2020; Tippery et al., 2021; Zhao et al., 2022; see also the early summary by Mayo et al., 2013). This molecular focus, among many other aspects of Araceae

systematics, resulted in two major advances in our phylogenetic understanding of the group: (1) the embedding of the duckweeds within the early clades of the Araceae and (2) the recognition of a major clade consisting almost entirely of genera with unisexual flowers and lacking a perigon. A plausible picture emerges of the evolution of an early monocot lineage associated with swampy habitats (Nauheimer et al., 2012) to exploit both fully aquatic and terrestrial environments (Zhao et al., 2022), where a major radiation then took place that coincided with a change from bisexual to unisexual flowers, loss of the perigon, and radical changes in pollen structure (Grayum, 1992; Ulrich et al., 2013) and inflorescence morphology (Hay and Mabberley, 1991; Hay, 2019). Why this happened is one of the major prevailing mysteries of the evolution of this ancient family of plants.

However, a closer examination of the phylogenetic studies so far produced reveals awkward topological inconsistencies and weak support in the part of the tree that represents the shift from floral bisexuality to unisexuality (Figure 1), and new investigations are needed to test the current topologies. On the positive side, the intermediate morphology of the three genera *Stylochaeton*, *Gonatopus*, and *Zamioculcas* (comprising the *Stylochaeton* clade) fits well with their consistent position as sister to the remainder of the unisexual clade (Figure 1), because they are the only genera in which the flowers are both unisexual and perigoniate (Hesse et al., 2001; Bogner and Hesse, 2005). On the other hand, the unisexual clade is itself unsupported in the most comprehensive analyses so far published (Cabrera et al., 2008; Cusimano et al., 2011; Nauheimer et al., 2012; Chartier et al., 2014). Further ambiguity is provided by the genus *Calla*, which combines bisexual flowers and pollen structure more typical of bisexual-flowered genera (Ulrich et al., 2013), but which is consistently placed within the unisexual clade by molecular analyses and in very variable positions (Figure 1).
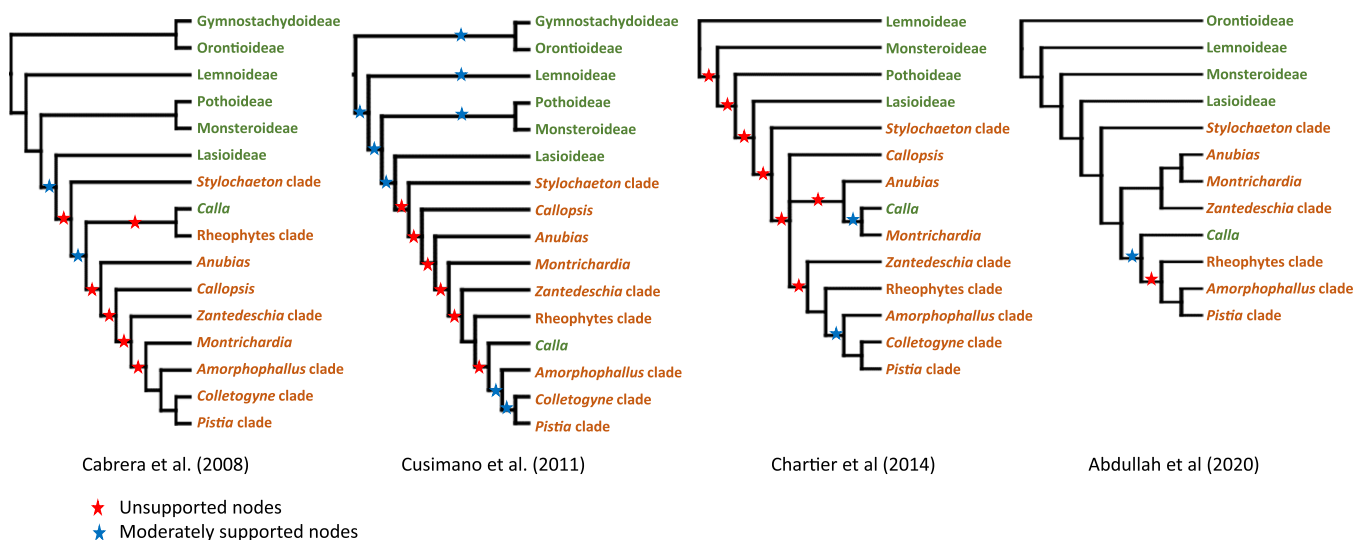

FIGURE 1 Simplified cladograms of previous phylogenetic analyses showing the position of *Calla* in relation to clades with bisexual (in green) and unisexual (in orange) flowers and their relative support. Clade names follow Cusimano et al. (2011). (A) Cabrera et al. (2008: fig. 1, strict consensus of the combined parsimony analysis). (B) Cusimano et al. (2011: fig.1, maximum likelihood analysis). (C) Chartier et al. (2014: fig.1, maximum likelihood analysis). (D) Abdullah et al (2020: fig. 6, maximum likelihood analysis).