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Generic delimitations, biogeography and evolution in the tribe Coleeae (Bignoniaceae), endemic to Madagascar and the smaller islands of the western Indian Ocean ☆

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ABSTRACT

This study presents the most complete generic phylogenetic framework to date for the tribe Coleeae (Bignoniaceae), which is endemic to Madagascar and the other smaller islands in the western part of the Indian Ocean. The study is based on plastid and nuclear DNA regions and includes 47 species representing the five currently recognized genera (including all the species occurring in the western Indian Ocean region). Bayesian and maximum likelihood analyses supported (i) the monophyly of the tribe, (ii) the monophyly of *Phylloctenium*, *Phyllarthron* and *Rhodocolea* and (iii) the paraphyly of *Colea* due to the inclusion of species of *Ophiocolea*. The latter genus was also recovered paraphyletic due to the inclusion of two species of *Colea* (*C. decora* and *C. labatii*). The taxonomic implications of the mutual paraphyly of these two genera are discussed in light of morphological evidence, and it is concluded that the two genera should be merged, and the necessary new nomenclatural combinations are provided. The phylogenetic framework shows *Phylloctenium*, which is endemic to Madagascar and restricted to dry ecosystems, as basal and sister to the rest of the tribe, suggesting Madagascar to be the centre of origin of this clade. The remaining genera are diversified mostly in humid ecosystems, with evidence of multiple dispersals to the neighboring islands, including at least two to the Comoros, one to Mauritius and one to the Seychelles. Finally, we hypothesize that the ecological success of this tribe might have been triggered by a shift of fruit-dispersal mode from wind to lemur.

1. Introduction

Madagascar is renowned for its unique flora and exceptionally high levels of species richness and endemism (Goodman and Benstead, 2005; Callmander et al., 2011a; Buerki et al., 2013). The island is the second largest centre of Bignoniaceae diversity in the world after South America (Gentry, 1988), currently with 83 accepted species across nine genera, all of which are Malagasy endemics (Madagascar Catalogue, 2015; Table 1). Perrier de la Bâthie (1938a, 1938b) published the first and only comprehensive taxonomic treatment for Madagascar, in which 54 species and numerous varieties and forms were recognized. Subsequently, 29 additional species have been described: five by Capuron (1960, 1970), one by Gentry (1977), 11 by Zjhra (2006) and 12 by MC, PBP and colleagues (Callmander and Phillipson, 2011, 2012; Callmander et al., 2011b, 2012). As part of our on-going work toward a full revision of the family for Madagascar, MC and PBP have evaluated most of the available material at the relevant herbaria, and estimate that no fewer than 35 new species await formal description, which would bring the total number of species to ca. 120 (Madagascar Catalogue, 2015; Table 1).

The nine Bignoniaceae genera present in Madagascar have been divided between two tribes, based primarily on fruit dehiscence: Coleeae (five genera with indehiscent fruits) and Tecomeae (four genera, dehiscent) (Gentry, 1976). The widespread African genus *Kigelia* DC. with indehiscent fruits was included in Coleeae, but Zjhra et al. (2004) excluded this genus from the tribe based on

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Table 1
Account of species richness per genus of Coleeae (Madagascar and neighboring islands).

	Accepted species	Estimated undescribed species	Estimated total species	Accepted species sampled	Undescribed species sampled	Total species sampled
Colea Bojer ex Meisn.	25	9	34	10	2	12
Ophiocolea H. Perrier	9	2	11	6	2	8
Phyllarthron DC	18	6	24	9	4	13
Phylloctenium Baill.	2	6	8	1	2	3
Rhodocolea Baill.	15	5	20	6	5	11

molecular evidence, retaining only the genera from Madagascar. Subsequently, a broader molecular phylogenetic study by Olmstead et al. (2009) provided further evidence of a Coleeae clade reflecting the delimitation of Zjhra et al. (2004), which was strongly supported as sister to a grade of old world genera previously assigned to the tribe Tecomeae s.l., referring to the entire lineage as the "Paleotropical clade", with Tecomeae s.s. restricted to the Neotropics.

As currently delimited, Coleeae comprises five genera, two of which are strictly endemic to Madagascar, *Phylloctenium* Baill. and *Rhodocolea* Baill., which currently include a total of 17 published species, plus three genera endemic to the broader western Indian Ocean region: *Colea* Bojer ex Meisn. (Madagascar, Mauritius and the Seychelles), *Ophiocolea* H. Perrier (Comoros and Madagascar) and *Phyllarthron* DC. (Comoros and Madagascar). These genera comprise a total of 48 published species endemic to Madagascar, two endemic to the Comoros, one endemic to Mauritius and one endemic to the Seychelles. We estimate that the currently available herbarium record represents just short of 100 species in total for the tribe (see Table 1 for a more complete breakdown of these details).

The incredible diversity of Bignoniaceae in the western Indian Ocean islands, and in particular of Coleeae in Madagascar, remains to be adequately documented, and a fully revised account of the family is urgently needed to inform conservation priorities, especially in Madagascar, where most of the species are threatened and narrowly distributed (Buerki et al., 2015; Good et al., 2006). Recent taxonomic work on the Malagasy Bignoniaceae led by MC and PBP has enabled us to refine the delimitation of numerous species and to publish ten new taxa in three of the genera of Coleeae, but these studies have also raised questions about generic delimitations and intergeneric relationships within the tribe, especially following the description of *Colea labatii* Callm. & Phillipson (see below) (Fig. 1H).

The most recent phylogenetic study of Coleeae (Zjhra et al., 2004) included a limited taxon sampling: 13 species in total, and with no representatives of *Phylloctenium* included. It was based on three plastid DNA regions (*ndh*F, *trn*T-L and *trn*L-F). The study yielded limited phylogenetic resolution and was not fully conclusive in assessing the monophyly of the tribe and its genera, nor did the study elucidate the phylogenetic relationships of the genera. A prior study published by Zjhra (2003) based on the nuclear ITS region and including representatives of *Phylloctenium* suggested the latter genus to be congeneric with *Phyllarthron*. However, the DNA sequences at the basis of this study were never submitted to GenBank, preventing further investigations on the phylogenetic status of *Phylloctenium* and its position within the tribe. In this context, this study aims at obtaining a plastid (*psbA-trnH*, *rps16-trnK*$^{(UUU)}$, *trnD-trnT*) and nuclear (ETS and ITS) based phylogenetic framework of the tribe Coleeae with representatives of all the genera, and considerably more comprehensive coverage of the known species (47 species in total). The primary objectives of the study are to: (i) confirm the monophyly of the tribe by including representatives of *Phylloctenium* and *Perichlaena* Baill., a monotypic genus endemic to Madagascar which was previously placed in Tecomeae s.l. close to *Fernandoa* (Gentry, 1988) and which we believe has never been sequenced before; (ii) test the monophyly of each genus and assess these results in light of morphological information; iii) infer phylogenetic relationships within the tribe and finally (iv) provide a biogeographic hypothesis on the diversification of this group in the western Indian Ocean and unravel its major evolutionary trends.

2. Material and methods

2.1. Sampling, DNA extraction, amplification and sequencing

Our study included representatives from all genera of Coleeae, representing 47 species (48% of the estimated total species of Coleeae), including all four species known from the smaller islands (see Table 1 for more details). The choice of outgroup taxa followed that of Olmstead et al. (2009) by selecting species from genera of the Paleotropical clade that is sister to Coleeae, including four species of *Fernandoa* and two species of *Stereospermum* (a species of this latter genus, *S. hildebrandtii* (Baill.) H. Perrier, was used as most external outgroup taxon to root the phylogenetic analyses as required by MrBayes; see below). In addition, we have sequenced *Perichlaena richardii* Baill. Accessions of Coleeae and the outgroup taxa used in this study are listed in Appendix 1.

Genomic DNA was extracted from both silica-gel dried and herbarium material. Extractions of total DNA were performed using a modified version of the DNeasy Plant Mini kit (QIAGEN Inc.) following the procedure applied at the New York Botanical Garden (USA). Two nuclear (ETS and ITS) and three plastid (*psbA-trnH*, *rps16-trnK*$^{(UUU)}$, *trnD-trnT*) DNA regions were amplified. Information on primers and PCR protocols are available in Baldwin and Markos (1998) for ETS, Buerki et al. (2009) for ITS and *trnD-trnT*, Papadopoulou et al. (2015) for *psbA-trnH* and Shaw et al. (2007) for *rps16-trnK*. All PCR products were purified using the ExoSAP DNA purification kit according to the manufacturers' protocols (USB Corp., USA). Dideoxy cycle sequencing was then performed using the chain termination method and ABI Prism Big Dye version 3.1 reaction kit, following the manufacturers' protocols, but using 0.5 μl of reaction mix (Applied Biosystems Inc., USA). The products were prepared for sequencing using the ethanol-precipitation method and visualized on an ABI 3730 Genetic Analyser, also according to the manufacturers' protocols.

2.2. Alignment and Phylogenetic analyses

The program Geneious 8.1.3 (Biomatters) was used to assemble complementary strands and verify software base-calling as well as conducting the alignments (using MUSCLE). Single-gene and partitioned phylogenetic inferences were carried out employing both maximum likelihood (ML) and Bayesian Markov chain Monte Carlo (MCMC) analyses. In the case of the partitioned analyses the dataset was divided into five partitions and each locus was allowed to