The LINNEAN SOCIETY of London BOTANICAL Journal of the Linnean Society

Botanical Journal of the Linnean Society, 2016, **181**, 585–609. With 3 figures

Phylogenetic relationships in the *Marcetia* alliance (Melastomeae, Melastomataceae) and implications for generic circumscription

MARIA JOSÉ R. ROCHA[1]*, JOÃO A. N. BATISTA[1], PAULO J. F. GUIMARÃES[2] and FABIÁN A. MICHELANGELI[3]

[1]*Departamento de Botânica, Universidade Federal de Minas Gerais, Av. Antônio Carlos 6627, Pampulha, Belo Horizonte, 31270-901 MG, Brazil*
[2]*Instituto de Pesquisas Jardim Botânico do Rio de Janeiro, Rio de Janeiro, RJ, Brazil*
[3]*The New York Botanical Garden, Bronx, NY 10458, USA*

Received 25 September 2015; revised 13 January 2016; accepted for publication 24 March 2016

The *Marcetia* alliance of Melastomataceae is an exclusively Neotropical group that includes at least 12 genera of mostly herbs and subshrubs, occurring in the cerrado of central Brazil and savannas of the Amazon region and Guayana highlands. This study aimed to test the monophyly of genera in the *Marcetia* alliance, evaluate their phylogenetic relationships and generic boundaries, and investigate morphological characters as potential synapomorphies for delimiting clades or genera. We used nuclear (ITS, ETS) and plastid (*accD-psaI*, *atpH-atpF*, *trnS-trnG*) DNA sequences of 107 terminals in 12 genera from the alliance. *Aciotis*, *Fritzschia*, *Marcetia* and *Siphanthera* were shown to be monophyletic and supported by molecular and morphological characters. Other genera with variable morphology and wider distributions, such as *Acisanthera*, *Comolia*, *Ernestia* and *Macairea*, were recovered as paraphyletic or polyphyletic. Most morphological characters analysed were found to be homoplastic, but when combined they are potentially useful for the diagnosis of genera and infrageneric groups. This study represents a major step in understanding internal relationships and provides the basis for a revision of the generic classification in the *Marcetia* alliance. © 2016 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2016, **181**, 585–609

ADDITIONAL KEYWORDS: cerrado – character reconstruction – molecular markers – Neotropics – taxonomy.

INTRODUCTION

Melastomataceae comprise 150–166 genera and *c*. 4570 species (Renner, 1993) and although they have a worldwide distribution, most species are concentrated in the Neotropics. They are usually recognized by their leaves with acrodomous venation, bisexual, diplostemonous flowers and poricidal anthers (Clausing & Renner, 2001).

Previous studies have shown that Melastomaceae are monophyletic, as supported by morphological and molecular data (Clausing & Renner, 2001). However, many questions remain regarding the limits and relationships of tribes and genera (Fritsch *et al.*, 2004; Penneys *et al.*, 2010; Penneys & Judd, 2011; Goldenberg *et al.*, 2012; Michelangeli *et al.*, 2013), due to the high degree of morphological variability and the lack of evolutionary studies in some tribes. The most recent worldwide classification of the family is that of Renner (1993), based on morphological data; this replaced the tribal classification proposed by Triana (1871), Cogniaux (1891) and van Vliet, Koek-Noorman & Ter Welle (1981). However, morphological (Almeda & Martins, 2001; Stone, 2006; Ionta *et al.*, 2007; Bécquer-Granados, 2008 Goldenberg *et al.*, 2008, 2012; Kriebel, 2008, 2008; Martin *et al.*, 2008; Amorim, Goldenberg & Michelangeli, 2009; Reginato, Michelangeli & Goldenberg, 2010; Almeda & Robinson, 2011;

*Corresponding author.
E-mail: mariajosereisdarocha@gmail.com

Penneys & Judd, 2011; Mendoza-Cifuentes & Fernández-Alonso, 2012) and molecular phylogenetic studies have shown that even this revised classification is inadequate (Clausing & Renner, 2001; Michelangeli *et al.*, 2004, 2011, 2013; Penneys *et al.*, 2010; Goldenberg *et al.*, 2013).

Melastomeae are one of largest tribes in Melastomataceae in generic, and hence morphological, diversity (Michelangeli *et al.*, 2013). They have a pantropical distribution with about 870 species in 47 genera (Michelangeli *et al.*, 2013). The majority of species occur in South America (*c.* 570 species, 30 genera) (Renner, 1993), mainly in the Brazilian cerrado. This tribe is characterized by the presence of a pedoconnective at the base of the anthers, capsular fruits and cochleate seeds with curved embryos (Michelangeli *et al.*, 2013). However, the pedoconnective can be highly reduced or secondarily lost and many taxa have ventral connective appendages. One clade of Melastomeae, *Monochaetum* (DC.) Naudin, also seems to have gained dorsal appendages independently (Michelangeli *et al.*, 2013).

Changes in the taxonomic circumscription of Melastomeae have been common over time. De Candolle (1828) was the first to establish tribes, and he included some current genera of Melastomeae in Lavosiereae, Rhexieae and Osbeckieae. Naudin (1849–1853) recognized four tribes, with the current Melastomeae corresponding more or less to Lasiandrae (Triana, 1865).

Triana (1865) placed most genera of Lasiandreae in Pleromeae and, contrary to previous authors, included continental location as a criterion for organizing genera in tribes. Cogniaux (1891), with an almost identical delimitation, named the tribe Tibouchineae. Just over a century later, Renner (1993) proposed Melastomeae based on morphological and anatomical data. In her concept (Renner, 1993), Melastomeae included tribes Tibouchineae (Cogniaux, 1891) and Osbeckieae (Triana, 1871), disregarding geographical criteria. Renner (1993) also included the genera *Acanthella* Hook.f., *Poteranthera* Bong., *Siphanthera* Pohl. ex DC. and *Monochaetum* in her expanded Melastomeae.

Given the issues surrounding the circumscription of Melastomeae, Michelangeli *et al.* (2013) expanded sampling and investigated the limits of the tribe. The results of this analysis showed that Melastomeae as currently defined are biphyletic, consisting of two non-sister clades, the '*Marcetia* alliance' and 'core Melastomeae', supported by molecular characters and differentiated mainly by seed coat, floral merosity, ovary apical appendages and habit (Michelangeli *et al.*, 2013). Core Melastomeae were characterized by cochleate seeds with a tuberculate surface (Renner, 1993), ovary apex with a crown of hairs or appendages and staminal pedoconnectives with bifurcated dorsal vascular bundles (Wilson, 1950; K. Sosa , P.J.F. Guimaraes & F.A. Michelangeli, unpubl. data). On the other hand, the *Marcetia* alliance was characterized by cochleate, oval or lacrimiform seeds with foveolate cells (except *Siphanthera*; Whiffin & Tomb, 1972; Almeda & Robinson, 2011), ovaries that are either glabrous or with scattered trichomes, flowers often tetramerous, hypanthium glabrous or with glandular trichomes, and two to four ovary locules. In general, species of the *Marcetia* alliance are predominantly herbs or subshrubs, some annual, mainly distributed in Neotropical savannas. Brazil, with > 41% endemic species, is probably the major centre of diversity (Baumgratz *et al.*, 2015). A similar distribution is also found in Microlicieae (Fritsch *et al.*, 2004) and some clades of Melastomeae *s.l.* (Michelangeli *et al.*, 2013). The *Marcetia* alliance putatively comprises *c.* 137 species in 12 genera: *Acanthella* Hook.f. (two species); *Aciotis* D.Don. (13 species); *Acisanthera* P.Browne (14 species); *Appendicularia* DC. (one species); *Comolia* DC. (19 species); *Ernestia* DC. (16 species); *Fritzschia* Cham. (three species); *Macairea* DC. (22 species); *Marcetia* DC. (31 species); *Nepsera* Naudin (one species); *Sandemania* Gleason (one species); and *Siphanthera* Pohl ex DC. (15 species) (Fig. 1). Additionally, based on morphological characters, Michelangeli *et al.* (2013) suggested that *Comoliopsis* Wurdack, *Loricalepis* Brade, *Mallophyton* Wurdack and *Poteranthera* Bong. could potentially be included in the *Marcetia* alliance. However, none of these genera has been included in a molecular phylogenetic analysis.

The *Marcetia* alliance was first recovered by Fritsch *et al.* (2004), but the taxonomic sampling for Melastomeae (ten terminals) and the *Marcetia* alliance (four terminals) in that study was insufficient to recognize these groups. Michelangeli *et al.* (2013), with a broader sampling, confirmed the existence of this group, calling it the *Marcetia* alliance, and suggested that it could be segregated from Melastomeae. These results also hinted that some of the genera in the alliance are polyphyletic. Nevertheless, despite the significant increase in sampling of the *Marcetia* alliance by Michelangeli *et al.* (2013), many questions remain about the monophyly of the genera, their relationships and limits. Additionally, thorough evaluation of different morphological characters is needed to better define genera in the alliance and to produce a workable taxonomy. In view of these questions and the taxonomic problems found in some genera of the *Marcetia* alliance, the main objectives of this study were: (1) to test the monophyly of the genera with an increased sampling of taxa and molecular markers; (2) to assess the generic limits and infrageneric relationships; and (3) to investigate

Figure 1. Representatives of major clades of the Marcetia alliance. A, *Nepsera aquatica.* B, *Appendicularia thymifolia.* C, *Ernestia glandulosa.* D, *Comolia microphylla.* E, *Comolia stenodon.* F, *Fritzschia erecta.* G, *Marcetia macrophylla.* H, *Siphanthera arenaria.* I, *Acisanthera tetraptera.* J, *Acisanthera genliseoides.* K, *Aciotis rubricaulis.* L, *Acisanthera bivalvis.* M, *Macairea multinervia.* N, *Acisanthera* va*iabilis.* O, *Macairea radula.* P, *Acanthella sprucei.* Photographs: A–C, Olivier Gaubert; P, Francisco Fajardo-Gutiérrez; D–O, M. J. Rocha.

selected morphological characters as potential synapomorphies for delimiting clades or genera in the alliance.

MATERIAL AND METHODS

Taxon sampling

We sampled 107 taxa of 27 genera. Of these, 89 accepted species and 12 genera correspond to taxa putatively in the *Marcetia* alliance, representing 64% of the 137 species thought to belong to this group. The numbers of species sampled per genus were as follows: *Acanthella* (1/50% of the accepted species); *Aciotis* (8/61%); *Acisanthera* (12/85%); *Appendicularia* (1/100%); *Comolia* (17/89%); *Ernestia* (10/62%); *Fritzschia* (2/40%); *Macairea* (5/22%); *Marcetia* (18/51%); *Nepsera* (1/100%); *Sandemania* (1/100%); and *Siphanthera* (10/66%). Whenever possible, we tried to cover the greatest morphological variation and geographical distribution for each genus. Most samples were field collected and identified by M. J. Rocha, P. J. Guimarães, F. A. Michelangeli and other specialists in Melastomataceae. We included the type species for most genera of the *Marcetia* alliance, except for *Comolia*, the type of which is *C. berberifolia* (Bonpl.) DC., known only from the type material. Nevertheless, this species is morphologically similar and possibly conspecific with *C. villosa* (Aubl.) Triana (Wurdack, 1973), which was sampled. As outgroups we included 13 representatives of core Melastomeae, Microlicieae and Rhexieae. Trees were rooted with *Cambessedesia hilariana* DC., following the results of Goldenberg *et al.* (2012), Michelangeli *et al.* (2013) and Michelangeli, Ulloa & Sosa (2014).

DNA extraction and markers

Total genomic DNA was extracted from silica-dried leaves collected in the field or from herbarium specimens. Samples were extracted using NucleoSpin 96 Plant II extraction kit (Macherey-Nagel), following the manufacturer's instructions or DNeasy plant mini kit (Qiagen), with the modifications suggested by Alexander *et al.* (2007). Phylogenetic analyses were based on five molecular markers: two nuclear (nrITS, nrETS) and three plastid intergenic spacers (*accD-psaI*, *atpH-atpF*, *trnS-trnG*). The ITS region consisted of the ribosomal internal transcribed spacers (ITS1 and ITS2) and the intervening 5.8S ribosomal gene. The ETS consisted of a section of about 241 bp at the 3′ end of the ribosomal external transcribed spacer (ETS) and 5′ end of the 18S ribosomal gene. Primers used for amplification are listed in Table 1. The ITS region has been used in several studies to elucidate phylogenetic relationships of tribes and genera in Melastomataceae (Fritsch *et al.*, 2004; Michelangeli *et al.*, 2004, 2008, 2013; Ionta *et al.*, 2007; Bécquer-Granados, 2008; Goldenberg *et al.*, 2008; Martin *et al.*, 2008; Reginato *et al.*, 2010; Kriebel, Michelangeli & Kelly, 2015), whereas the ETS region is still little used in Melastomataceae, but has proved to be useful in understanding infrageneric relationships (Stone & Andreasen, 2010; Kriebel *et al.*, 2015) and to be easily amplified. The *accD-psaI*, *atpH-atpF* and *trnS-trnG* plastid intergenic spacers have also been informative for Melastomataceae and have been employed in several studies (Reginato *et al.*, 2010; Michelangeli *et al.*, 2013; Penneys & Judd, 2013).

Amplification, editing and alignment

DNA amplification was performed by polymerase chain reaction (PCR) in a final volume of 15 μL with the following reaction components: 0.5–0.7 μL genomic DNA (*c.* 30 ng μL^{-1}), 7.5 μL 2× EconoTaq Plus Green (Lucigen Technologies), 2 μL of each primer (3 μM), 0.75 μL spermidine (4 mM) and 2.5 μL purified water. The PCR conditions were similar for all

Table 1. List of molecular markers and primers used in this study

Locus	Primer	Sequence (5′–3′)	Reference
nrITS	NY 183	CCTTATCATTTAGAGGAAGGAG	Michelangeli *et al.* (2004)
	NY 887	ATTGATGGTTCGCGGGATTCTGC	
nrETS	NY320	AGACAAGCATATGACTACTGGCAGG	Kriebel *et al.* (2015)
	NY1428	ACGTGTCGCGTCTAGCAGGCT	
accD-psa1	NY826	AATYGTACCACGTAATCYTTTAAA	Shaw *et al.* (2005)
	NY827	AGAAGCCATTGCAATTGCCGGAAA	
atpH-atpF	NY822	ACTCGCACACACTCCCTTTCC	Reginato *et al.* (2010)
	NY 823	GCTTTTATGGAAGCTTTAACAAT	
trnS-trnG	NY368	GCCGCTTTAGTCCACTCAGC	Hamilton (1999)
	NY369	GAACGAATCACACTTTTACCAC	

markers, with small adjustments in the annealing temperature and extension time, as follows: initial denaturation at 94 °C for 3 min; 40 cycles of denaturation at 94 °C for 45 s; annealing at 58 °C (ETS), 50 °C (ITS), 57 °C (*accD-psaI*) or 55 °C (*trnS-trnG*, *atpH-atpF*) for 45 s; extension at 72 °C for 1 min (except for *trnS-trnG* and *atpH-atpF* which was 1 min 30 s); and a final extension at 72 °C for 3 min for all markers. All reactions were performed in Eppendorf thermocyclers. Cycle sequencing reactions were carried out with the same amplification primers using the sequencing service at the University of Washington, USA (High Throughput Genomics Center – htSEQ).

Consensus sequences obtained for each marker from bidirectional reads were generated in Sequencher 4.10.1 (GeneCodes Corp.). Sequences of low quality were discarded. Sequence alignments were preliminarily performed using Muscle (http://www.ebi.ac.uk/Tools/msa/muscle/) and manually adjusted in Mega 6 software (Tamura *et al.*, 2013) to maximize homology hypotheses between the sequences (Simmons, 2004). The 5′ and 3′ ends of each matrix were trimmed and internal regions with ambiguous or problematic alignment were excluded. Individual gap positions were treated as missing data.

DNA substitution models for Bayesian inference (BI) and maximum-likelihood (ML) analyses were selected for each marker using jModeltest v.2.1.3 (Darriba *et al.*, 2012), using the five-model scheme with or without four discrete rate categories approximating a gamma distribution (+G) and including models with equal/unequal base frequencies (+F) and a proportion of invariable sites (+I). The likelihoods were calculated using an ML optimized base tree with NNI topology search using phyml (Guindon & Gascuel, 2003) and the models were evaluated using the corrected Akaike's information criterion (AICc).

In total, 273 sequences were generated for this study, and another 96 were obtained from GenBank, mostly from Michelangeli *et al.* (2013). Voucher information and GenBank accession numbers are listed in the Appendix.

Phylogenetic analyses

All data sets were analysed using maximum parsimony (MP), ML and BI. First, phylogenetic analyses were performed individually for each marker and the congruence between the topologies was visibly evaluated. The matrices for each marker were then concatenated and analysed as described below.

Parsimony analyses were performed in PAUP v.4 (Swofford, 2002) using Fitch parsimony as the optimality criterion (Fitch, 1971). Heuristic searches consisted of 10 000 replicates of random taxa addition, using the tree bisection-reconnection algorithm (TBR) and saving up to 15 MP trees per replicate. The strict consensus was then built from all the most-parsimonious trees obtained. All characters were treated as unordered and of equal weight. Internal support was evaluated by non-parametric bootstrapping (Felsenstein, 1985) with 10 000 replicates, random addition and TBR branch swapping, saving up to 15 trees per replicate. For bootstrap support levels, we considered bootstrap percentages (BPs) of 50–70% as weak, 71–85% as moderate and >85% as strong (Kress, Prince & Williams, 2002).

ML analyses were performed with RAxML using default parameters (Stamatakis, 2006; Stamatakis, Hoover & Rougemont, 2008) and run through the CIPRES Science Gateway (http://www.phylo.org/; Miller, Pfeiffer & Schwartz, 2010). Bootstrap values were estimated on the ML tree also using RAxML based on 1000 searches run through the CIPRES Science Gateway (results not shown).

Bayesian analyses were performed using MrBayes 3.1.2 run through the CIPRESS Science Gateway (http://www.phylo.org/; Miller *et al.*, 2010). Analyses were performed with mixed models and independent parameters. The analysis consisted of two independent runs, each with four Markov chains for ten million generations, sampling one tree every 1000 generations. To achieve convergence the temperature parameter for heating the chains was lowered to 0.05. Convergence between the chains was assessed by the average standard deviation of split frequencies (< 0.01) and the stationarity of the chains with the generated graphical outputs. Convergence was achieved after 1 941 000 generations and the first 25% of the resulting trees were discarded as burn-in. The remaining trees were used to assess topology and posterior probabilities (PPs) in a majority-rule consensus. Because PPs in Bayesian analysis are not equivalent to BP, but are generally much higher (Erixon *et al.*, 2003), we used criteria similar to a standard statistical test, considering groups with PP > 95% as strongly supported, PP 90–95% as moderately supported and PP < 90% as weakly supported.

Morphology: evolution of characters

We selected characters that were used for circumscription of sections and genera of the *Marcetia* alliance, including those traditionally cited by Triana (1871), Naudin (1849–1853) and Cogniaux (1885) and characters used in identification keys. The goal was to investigate the evolution of these characters within the clade and to identify putative synapomorphies that might distinguish well-supported groups

in the *Marcetia* alliance. The characters were coded from herbarium collections (ALCB, BHCB, CEPEC, HUEFES, HUFU, INPA, IAN, MG, MIRR, NY, RB, SP, SPF, US) and/or obtained from descriptions and monographs (Renner, 1987, 1989, 1993; Clausing & Renner, 2001; Freire-Fierro, 2002; Seco, 2006; Kriebel, 2008; Almeda & Robinson, 2011; Michelangeli *et al.*, 2013).

The morphological matrix was edited using Xper2 v.2.0 (Ung *et al.*, 2010) and Mesquite v.2.74 (Maddison & Maddison, 2001). Characters were optimized using parsimony in Winclada 1.00.008 (Nixon, 1999). All characters were treated as unordered and assigned equal weight. Multistate characters were coded as non-additive. Both algorithms for optimization [ACCTRAN (accelerated transformation) and DELTRAN (delayed transformation)] were considered. The matrix with all characters and their respective character states coded for the terminals used in the molecular phylogenetic analysis is shown in Appendix.

Characters and coding

Twenty-four discrete characters from life cycle, perianth, androecium, gynoecium, fruit and seed were coded (Table S1). Other characters were not included in the analysis because it was difficult to establish primary homology hypotheses, they were shown to be continuous or would need field data to be coded, and therefore were not available for all species. Examples of these excluded characters include habitat, leaf and hypanthium indumentum, petal and anther colour, inflorescence type, stamen size, shape of the stamen appendages, anther shape, ovary shape and seed testa. The following six characters were recovered as the most useful to diagnose clades in the *Marcetia* alliance.

Floral merosity: tetramerous (0); pentamerous (1). Merosity varies across the family, but the most common numbers are four, five or six (Michelangeli, 2000). The *Marcetia* alliance is commonly tetramerous, although pentamerous flowers are present in a few species (Michelangeli *et al.*, 2013). The combination of floral merosity and the number of ovary locules was used to establish the sections in *Acisanthera* (Triana, 1871) and *Ernestia* (Triana, 1871; Cogniaux, 1885). Although intraspecific variation in petal number was observed in other groups of Melastomataceae, we did not observe it in the species included in this study.

Ovay pubescence: absent (0); glandular (1); eglandular (2). Cogniaux (1885) used this character, associated with other reproductive structures, to delimit genera in Melastomeae and to characterize the sections of *Ernestia*. *Ernestia* section *Ernestia* was characterized by tetramerous flowers and a pubescent, tetralocular ovary, whereas *Ernestia* section *Pseudoernestia* Cogn. comprises species with pentamerous flowers and a glabrous, trilocular ovary. Kriebel (2008) observed that the character state (0) was constant for the three sections of *Acisanthera* proposed by Triana (1871), except for *Acisanthera tetraptera* (Cogn.) Gleason which has glandular pubescence on the ovary apex.

Ovary locule number: 2 (0); 3 (1); 4 (2); 5 (3). Since the 1800s this character has been used in taxonomic studies of Melastomataceae. Based on the number of locules in the ovary, Triana (1871) proposed two sections in *Comolia*. *Comolia* section *Comolia* has bilocular ovaries, whereas *Comolia* section *Tricentrum* (DC.) Triana has tetralocular ovaries. In *Acisanthera*, *A.* section *Dicrananthera* (C. Presl.) Triana has tetramerous flowers and a bilocular ovary; *A.* section *Acisanthera* has tetra- or pentamerous flowers and a trilocular ovary; *A.* section *Dichaetandra* (Naudin) Triana has tetramerous flowers and a tetralocular ovary and *A.* section *Noterophila* (Mart.) Triana has pentamerous flowers and a bilocular ovary.

Filament/anther angle at anthesis: straight or lightly curved (0); curved or arched (1). State 0 includes species with an angle $> 90°$ between the anther base and filament, whereas in species scored as 1 the angle between the pedoconnective and filament is $\leq 90°$. In most species coded as 0 the style is surrounded by the stamens; when the anther is curved or arched the filament tends to bend to one side of the flower, opposite the style.

Pedoconnective in antesepalous stamens: absent (0); present (1). Stamens of Melastomataceae vary widely and therefore provide many morphological characters, including shape, colour and size and the presence or absence of associated structures, among others. The pedoconnective is the extension of the connective between the thecae base and filament insertion. This character was scored as present when the prolongation is visible, regardless of size. In the *Marcetia* alliance this structure can have different modifications, such as appendages, or can sometimes be dorsally thickened and basally enlarged below the thecae. Although the stamens provide many other characters, it is difficult to compare them among different taxa and to establish hypotheses of homology.

Anther fertility: both cycles fertile (0); only the antesepalous cycle fertile (1). Most species of Melastomataceae are diplostemonous and both cycles of stamens are fertile, but in some cases the number of fertile stamens can be equal to the number of petals. The number of stamens was used by Triana (1871) to segregate the sections of *Siphanthera*: *S.* section *Eumeisneria* (DC.) Triana was characterized by having eight stamens, four of them reduced, whereas *S.* section *Siphanthera* was characterized by four

stamens, alternating with rudimentary or reduced ones. In *Acisanthera genliseoides* (Hoehne) Wurdack and many *Siphanthera* spp., only the antesepalous cycle is fertile (character 11, state 0). The antepetalous cycle, if sterile, may be completely absent or staminodial.

RESULTS

Phylogenetic analyses

The aligned matrix with all regions consisted of 5264 characters, 1502 (28%) of which were potentially informative. Consistency index (CI) and retention index (RI) were 0.54 and 0.78, respectively. Other information from the parsimony analyses is shown in Table 2. The models selected for the BI and ML analyses were GTR + I + G for both nuclear markers and GTR + G for all three plastid markers. In general the nuclear markers produced better resolved trees, probably because of the higher polymorphism between the sequences and the greater number of informative characters. However, lower CI and RI values for the nuclear markers indicate a greater proportion of homoplasy.

In all analyses, the *Marcetia* alliance was recovered as monophyletic with high support (PP = 1.00, BP = 100%) (Fig. 2). The same major clades are present in the combined datasets of the BI, MP and ML analyses and the topology is completely congruent, considering the clades with PP ≥ 0.95 and BP ≥ 85%.

There were no significant conflicts between well-supported clades from the BI and parsimony analyses of individual and combined matrices. The main difference was the position of the possible first-branching lineage of the *Marcetia* alliance. In the BI analysis the *Comolia montana* Gleason clade was resolved as sister to the rest of the group, whereas in the MP and ML analyses the *Comolia s.s.* + *Ernestia s.s.* clade was sister to the rest of the group. However, in all three cases support for these sister clades was low (PP = 0.53, BP = 61%), indicating that relationships at the base of the *Marcetia* alliance clade are not well supported. We have chosen the BI analyses for presentation and all further comments on relationships and characters. The majority-rule consensus trees from a Bayesian analyses of the combined nuclear (ETS, ITS) and plastid (*accD-psaI*, *trnS-trnG*, *atpH-atpF*) data sets are shown in Figs. S1 and S2, respectively.

Aciotis, *Fritzschia*, *Marcetia* and *Siphanthera*, as currently defined, were recovered as monophyletic and had strong support in all analyses. Additionally, *Acisanthera* could easily become monophyletic with the inclusion of *Comolia ayangannae* Wurdack. On

Table 2. Dataset and statistical results from phylogenetic analyses

	nrITS	nrETS	*accD-psaI*	*atpH-atpF*	*trnS-trnG*	Nuclear	Plastid	Total
Taxa	90	92	77	53	66	102	85	107
Aligned characters	1110	730	1218	933	1249	1840	3400	5264
Potentially informative sites	382 (34%)	490 (67%)	233 (19%)	141 (15%)	256 (20%)	872 (47%)	630 (18%)	1561 (29%)
Length of most-parsimonious trees	1657	2467	605	354	755	4258	1786	6109
Number of trees retained	38 820	40 097	78 615	1931	145 995	64 740	57 090	54 616
Consistency index	0.52	0.46	0.76	0.81	0.74	0.47	0.74	0.54
Retention index	0.78	0.79	0.86	0.88	0.85	0.77	0.83	0.78

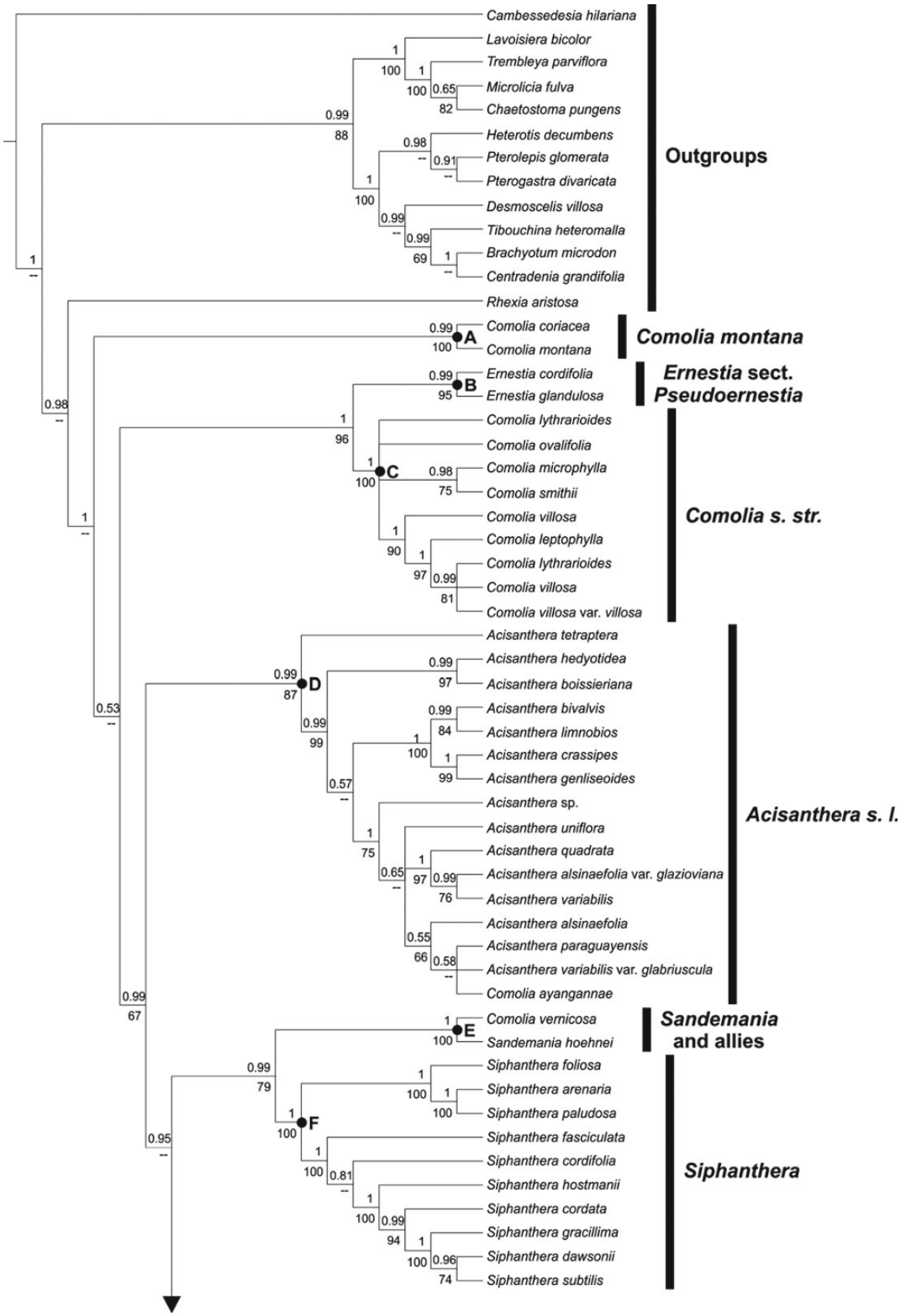

Figure 2. The majority-rule consensus tree from a Bayesian analysis of the combined nuclear (ETS, ITS) and plastid (*accD-psaI*, *trnS-trnG*, *atpH-atpF*) DNA sequences. Numbers above and below the nodes are posterior probabilities and bootstrap percentages from the Bayesian and parsimony analyses, respectively (only for clades with BS ≥ 60%). Well-supported clades discussed in the text are named.

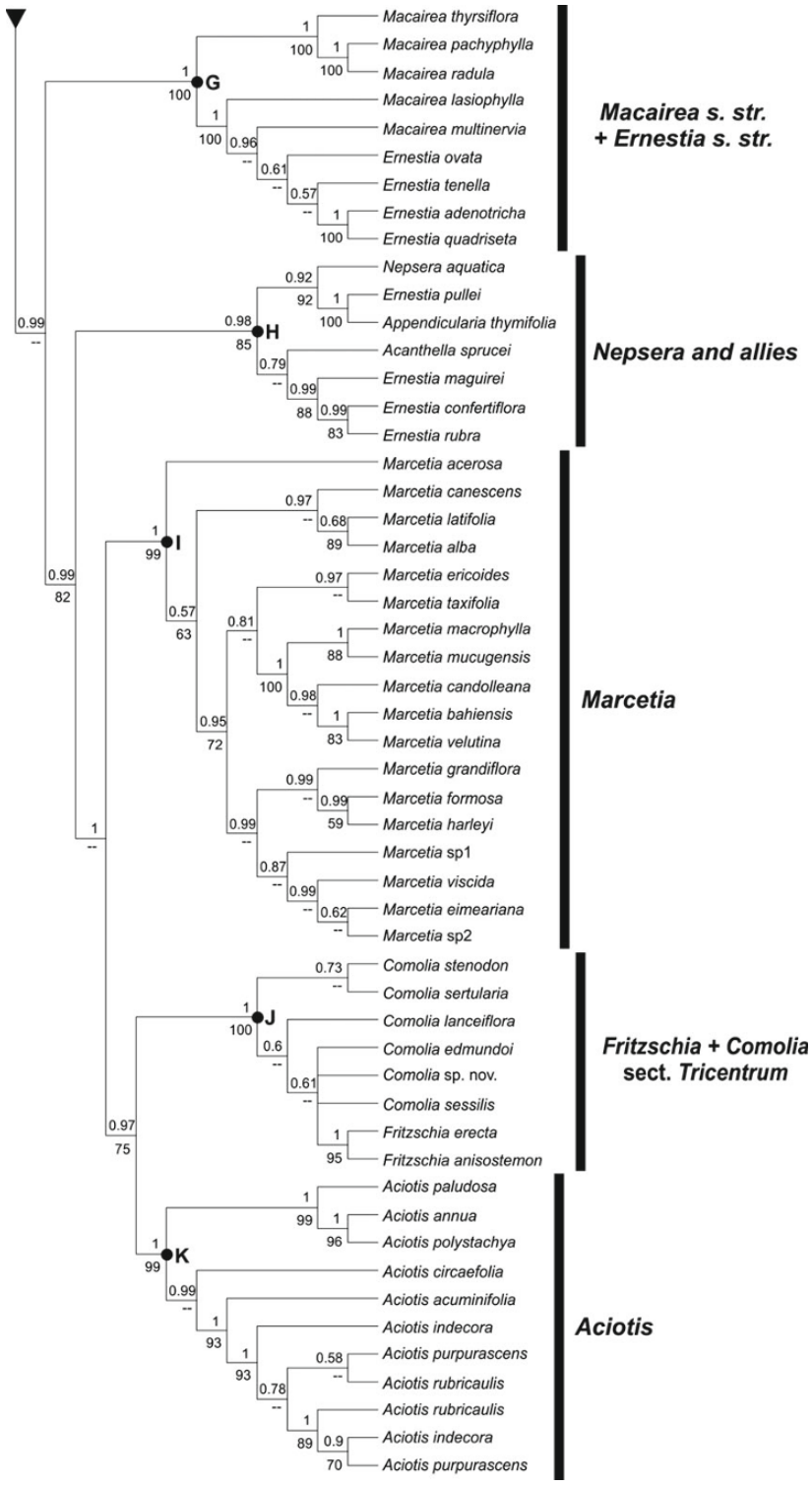

Figure 2. *Continued*

the other hand, *Comolia* and *Ernestia* are polyphyletic and were recovered in four and three unrelated clades, respectively. *Macairea* is resolved as paraphyletic due to *Ernestia s.s.* being nested in it.

In the Bayesian analysis the *Comolia montana* clade (clade A: PP = 0.99; BP = 100%) is sister to the remaining clades of the *Marcetia* alliance. *Ernestia* section *Pseudoernestia* (clade B: PP = 0.99;

BP = 95%) is resolved as sister to *Comolia s.s.* (clade C: PP = 1.00; BP = 100%) with strong support (PP = 1.00; BP = 96%). In *Acisanthera s.l.* (clade D: PP = 0.99; BP = 87%), *A. tetraptera* was recovered as the first divergent branch (PP = 0.99; BP = 87%), sister to a clade with three consistent groups, as follows. The *A. hedyotidea* (C.Presl.) Triana subclade (PP = 0.99; BP = 97%) is constituted by members of *Acisanthera* section *Dicrananthera*. The *A. bivalvis* (Aubl.) Cogn. subclade (PP = 1.00; BP = 100%) includes species currently placed in *Acisanthera* section *Noterophila*. The *Acisanthera s.s.* subclade (PP = 1.00; BP = 75%) is represented by the type species and other species allied to *Acisanthera* section *Acisanthera*.

Sandemania and *Comolia vernicosa* (Benth.) Triana form a strongly supported group (clade E: PP = 1.00; BP = 100%), termed *Sandemania* and allies, that is sister (PP = 0.99; BP = 79%) to a strongly supported *Siphanthera* (clade F: PP = 1.00; BP = 100%). In the Bayesian analysis Clade E+F is sister to the remaining clades G–K (PP = 0.95), whereas in the parsimony analysis this relationship is unresolved.

Macairea and some *Ernestia* spp. form a strongly supported clade (clade G: PP = 1.00; BP = 100%), comprising the subclades *Macairea s.s.* (PP = 1.00; BP = 100%) and *Ernestia s.s.* (PP = 0.61; BP < 50%). Relationships in *Macairea* are not well resolved, and *Macairea lasiophylla* (Benth.) Wurdack and *M. multinervia* Benth. are successively recovered as sisters to *Ernestia s.s.* Clade H (*Nepsera* and allies) (PP = 0.98; BP = 85%) is composed of two major subclades with intermingled genera. The monotypic genus *Nepsera* is sister to (PP = 0.92; BP = 92%) the *Ernestia pullei* Gleason + *Appendicularia thymifolia* (Bonpl.) DC. subclade (PP = 1.00; BP = 100%). The other subclade is formed by *Acanthella* and the *Ernestia maguirei* Wurdack subclade (PP = 0.99; BP = 88%), but support is low (PP = 0.79; BP < 50%).

Marcetia was recovered as monophyletic with strong support in all analyses (clade I: PP = 1.00; BP = 99%). Although the relationships of this clade to other genera is not strongly supported in all analyses, our results suggest that *Marcetia* is related to the *Fritzschia* and *Comolia* section *Tricentrum* clade and *Aciotis*. The *Fritzschia* and *Comolia* section *Tricentrum* clade (clade J: PP = 1.00; BP = 100%) is composed of two geographically related subgroups: the weakly supported and/or paraphyletic *Comolia sertularia* (DC.) Triana subclade, but which is morphologically well defined, and the *Fritzschia* subclade (PP = 1.00; BP = 95%), which is strongly supported in all analyses. Lastly, *Aciotis* (clade K: PP = 1.00; BP = 99%) is resolved as sister to the *Fritzschia* and *Comolia* section *Tricentrum* clade.

Morphological inference

Six morphological characters traditionally used in taxonomy of the genera of the *Marcetia* alliance were reconstructed onto the combined phylogenetic tree (Fig. 3). Although most of them revealed some degree of homoplasy, when combined they can be useful in diagnosing some of the clades recovered in the molecular analyses. In the optimization of floral merosity, the presence of pentamerous flowers was informative for characterizing the *Acisanthera bivalvis* and *Acisanthera s.s.* subclades, except for *Comolia ayangannae* which is tetramerous, and this may be why Wurdack (1964) described it in *Comolia*. The number of ovary locules was highly homoplastic. However, in association with floral merosity it was useful to circumscribe the *Acisanthera bivalvis* (pentamerous; bilocular) and *Acisanthera s.s.* subclades (pentamerous; trilocular). Although *Macairea* and *Ernestia* have not been adequately sampled, our analyses showed that ovary pubescence is an important character to diagnose the *Ernestia s.s.* subclade, which is the only clade of *Ernestia* with trichomes on the ovary. Most species of the alliance have arched or curved anthers; however, straight anthers are common in the monophyletic *Aciotis*, *Marcetia* and *Siphanthera*. Almost all genera have well-developed pedoconnectives on the antesepalous stamens, with the exception of *Marcetia*, in which the connective is not prolonged below the anther. Lastly, in only one species of the *Acisanthera bivalvis* subclade and most *Siphanthera* spp. the antesepalous stamens are fertile.

DISCUSSION

Phylogenetic relationships in the *Marcetia* alliance

Our results confirm the monophyly of the *Marcetia* alliance as previously shown by Michelangeli *et al.* (2013). In that work the *Marcetia* alliance was characterized by the tetramerous flowers, number of ovary locules equal to the number of petals (tetralocular) or reduced to two or three locules, absence of crown hairs on the ovary apex, and the seeds cochleate, ovate or lacrimiform (Michelangeli *et al.*, 2013). Furthermore, in the *Marcetia* alliance the pubescence of the ovary is glandular, whereas in most of members of Melastomeae *s.s.* it is eglandular. A recent study suggested the presence of three layers in the outer integument of the ovule as a possible synapomorphy for the *Marcetia* alliance (Caetano, 2014).

In this study, with a more comprehensive sampling, we tested for the first time the monophyly of all genera of the *Marcetia* alliance and their

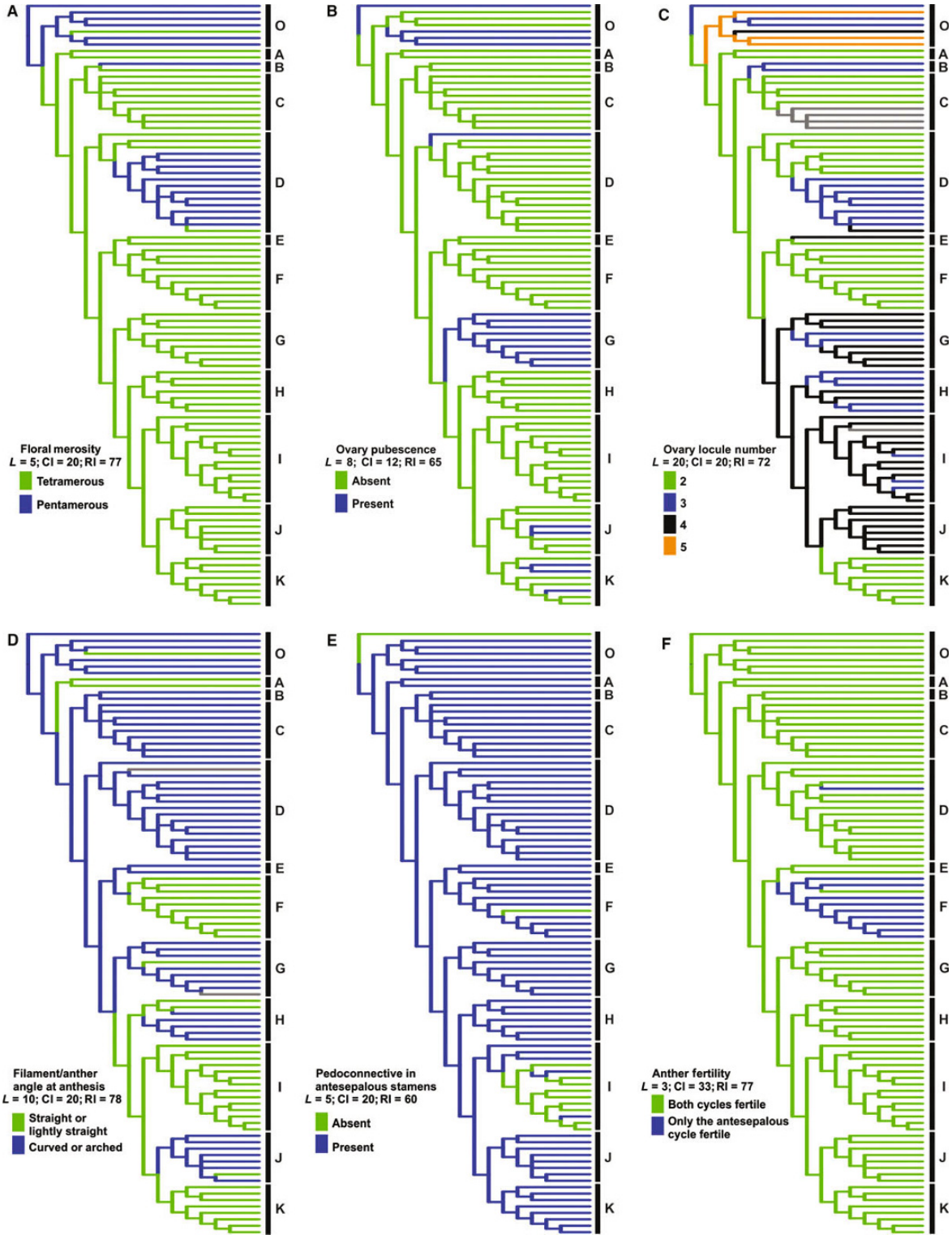

Figure 3. Reconstruction of morphological characters onto a 50% majority-rule Bayesian tree showing the evolution of: A, floral merosity; B, ovary pubescence; C, ovary locule number; D, filament/anther angle at anthesis; E, pedoconnective in antesepalous stamens; and F, anther fertility. CI, consistency index; RI, retention index.

boundaries and relationships. Among the genera with more than two species, *Aciotis*, *Fritzschia*, *Siphanthera* and *Marcetia* were recovered as monophyletic. Also, the monotypic genera *Appendicularia*, *Nepsera* and *Sandemania* are morphologically well characterized. The traditionally problematic genera *Comolia*, *Ernestia* and *Macairea* were shown to be polyphyletic or paraphyletic and *Acisanthera* was recovered as paraphyletic, because it included *Comolia ayangannae*. *Comolia* and *Ernestia* are distributed in four and three distantly related clades, respectively, whereas *Comolia vernicosa* emerged as an isolated lineage. However, the *Comolia* clades are morphologically well defined and/or geographically structured. Few of the morphological characters analysed constituted strict synapomorphies; nonetheless, it is possible to circumscribe most clades morphologically with a specific combination of features. With the aim of supporting and informing a future generic revision of groups in the *Marcetia* alliance, we discuss and characterize each of the clades recovered in our phylogenetic analyses in the following paragraphs.

Comolia montana clade

This lineage includes two species endemic to tepuis in the Guayana highlands. Gleason (1939a; 1952) recognized and distinguished *C. coriacea* Gleason and *C. montana* from other *Comolia* spp. mainly by the coriaceous leaves with ciliate trichomes. Additionally, the leaves are thick, with visible stomatal crypts, a hypanthium with indument in the torus region and almost pyramidal seeds that are larger than in other *Comolia* spp. The monotypic *Comoliopsis* was not sampled in this study, but based on morphology of the leaves, anthers, seeds and geographical distribution it may belong in this clade. However, this needs to be further evaluated because *C. montana* and *C. coriacea* have flowers that are tetramerous and have a glabrous ovary apex and a bilocular ovary, whereas in *Comoliopsis* the flowers are pentamerous, the ovary has glandular trichomes and it is (tri-) tetramerous (Wurdack, 1984). If indeed *Comoliopsis* forms part of this clade, then these *Comolia* spp. may be combined into *Comoliopsis*.

Ernestia section *Pseudoernestia* clade

This clade is composed of *E. cordifolia* O.Berg ex Triana and *E. glandulosa* Gleason. Cogniaux (1885) placed *E. cordifolia* in *Ernestia* section *Pseudoernestia* Cogn. Later, Krasser (1893) elevated this section to generic rank, whereas Wurdack, Renner & Morley (1993) synonymized it in *Ernestia*. These two species differ from other *Ernestia* spp. by their glabrous, trilocular ovary, almost rounded calyx tube with narrowly oblong lobes and tuberculate dorso-basal connective with ventral, simple aristae, but differ from each other in the inflorescence and floral merosity. Commonly, *E. cordifolia* has a terminal inflorescence with pentamerous flowers and *E. glandulosa* an axillary inflorescence and tetramerous flowers. A relationship between these species was suggested by Gleason (1925), based on their trilocular ovary. Despite the differences between these two species, our results revealed they are not closely related to the *Ernestia s.s.* clade and should be segregated as a resurrected *Pseudoernestia* Krasser.

Comolia s.s. clade

The *Comolia s.s.* clade includes almost all species of *Comolia* section *Comolia*, as suggested by Cogniaux (1885). They are mainly characterized by bilocular ovaries and seeds with a tuberculate and costate surface. Except for *C. ovalifolia* Triana, which is tetralocular and occurs in restinga vegetation in northern and north-eastern Brazil, all other species in this clade are found in lowland savannas of northern South America, Trinidad and northern Brazil. Although *Comolia berberifolia*, the type of the genus, was not sampled, we are confident that this clade indeed represents *Comolia s.s*. *Comolia berberifolia* is known only from the type collection. However, it is clear that *C. berberifolia* is part of the same species complex as *C. villosa* (Wurdack, 1973, cited as *C. veronicaefolia* Benth.) and may be the same taxon as *C. lythrarioides* Naudin if the complex is treated as a group of small segregated species.

Acisanthera s.l. clade

Acisanthera was included in previous molecular phylogenetic analyses (Fritsch *et al.*, 2004; Michelangeli *et al.*, 2013), but the limited sampling so far has been insufficient to understand relationships in this morphologically complex group. Kriebel (2008) performed a morphological phylogenetic analysis for this genus, suggesting that *Acisanthera* was not monophyletic because *Acisanthera tetraptera* was more closely related to *Siphanthera*, based on the rostrate anther apex and bilobed ovary apex. However, in our study, *A. tetraptera* was recovered as sister to the remaining *Acisanthera* spp. and not closely related to *Siphanthera*. *Acisanthera tetraptera* is characterized by axillary or terminal inflorescences, the capitate cymes subtended by foliaceous bracts, anthers with a rostrate apex, glandular style, tetralocular ovary with glandular trichomes and seed surface ridged with minute tubercles. The *A. hedyotidea* subclade comprises species currently grouped in

Acisanthera section *Dicrananthera*, characterized by tetramerous flowers. These species resemble *A. tetraptera* in the tetramerous flowers and bilocular ovary, but differ in the morphology of leaves, stamens and seeds: the leaves are fleshy, the thecae are small with broad pores and the connective is prolonged into two upturned, subulate-aristate appendages. Additionally, the seed surface is not covered with tubercles. The *A. bivalvis* subclade is composed of six species of annual herbs, commonly found in swampy areas. It is easily diagnosed by the commonly inflated stems, sessile leaves that can be heterophyllous, pentamerous flowers and bilocular ovary. Lastly, the *Acisanthera s.s.* subclade is the only group in the *Marcetia* alliance with pentamerous flowers and trilocular ovaries. Seco (2006) suggested that *Acisanthera* and *Comolia* could be related based on their seed morphology, ovary locules and stamens. Also, only the *Acisanthera s.s.* clade has anthers that are similar to *Comolia s.s.*, but the floral merosity and the number of ovary locules are different. *Acisanthera s.l.* could become monophyletic with the inclusion of *Comolia ayangannae* in *Acisanthera*. Although the morphological relationship of *C. ayangannae* with this clade is not obvious, this possibility should be studied further, as in the original description of this species, Wurdack (1964) compared its stamens to *Acisanthera alsinaefolia* (DC.) Triana. Although *Acisanthera* could easily become monophyletic with the inclusion of *C. ayangannae*, perhaps it is more appropriate to recognize each of the four clades as separate genera because they are morphologically distinct and well characterized, whereas *Acisanthera* as a whole is morphologically heterogeneous and difficult to characterize. Note that sections *Dicrananthera* and *Noterophila* were previously treated as genera by Presl (1832) and Martius (1831), respectively. Additionally, the four groups of *Acisanthera* are also ecologically distinct.

Sandemania and allies clade

Sandemania has long been a nomenclaturally and taxonomically confusing entity. Its sole species was first described in *Leandra* Raddi (Miconieae) by Cogniaux (1909), using an illegitimate name, and later based on a different type in *Comolia* (Tibouchinae). Later, Gleason (1939b) described the monotypic *Sandemania* (based on *S. lilacina* Gleason), also in tribe Tibouchineae. However, Wurdack (1970) later realized that *Comolia hoehnei* Cogn. and *Sandemania lilacina* were indeed the same species and made the appropriate transfer. Renner (1987) reviewed this genus and argued that it was closely related to *Macairea*, *Comolia* and *Tibouchina* Aubl. However, the phylogenetic studies of Michelangeli *et al.* (2013) revealed that *Sandemania* is not related to *Tibouchina* and does not belong in Melastomeae *s.s.* Our results confirmed that the sole species in the genus, *S. hoehnei* (Cogn.) Wurdack, belongs to the *Marcetia* alliance and is related to *Comolia vernicosa*. These two species share some characters, such as tetramerous flowers, a glabrous ovary, and rostrate and corrugated anthers. Also, both occur in open savannas from the Amazon Basin. Despite these similarities, we agree with Renner (1987) that *S. hoehnei* cannot be accommodated in any other genus, because of its unique combination of characters: paniculate inflorescence with many small flowers, stamens with ventrally bilobed pedoconnectives at filament insertion, corrugated anthers, glabrous ovary and seeds with a shallowly tuberculate surface. Likewise, *C. vernicosa* was first described in *Leiostegia* Benth. (Bentham, 1840) and later synonymized in *Comolia* by Triana (1871). However, it is easily distinguished from all the groups of *Comolia* and from *S. hoehnei* by its glabrous, uninerved leaves with a revolute margin, fasciculate and axillary flowers, and tetralocular ovary.

Siphanthera clade

Siphanthera was long assigned to Microliceae in traditional morphological studies by Naudin (1849–1853), Triana (1871), Cogniaux (1883, 1891) and Krasser (1893), probably based on its staminal ventral appendages and seeds (Almeda & Robinson, 2011). Later, Renner (1993) transferred the genus to a broadly circumscribed Melastomeae and this was corroborated by morphological and molecular analyses (Almeda & Martins, 2001; Clausing & Renner, 2001; Fritsch *et al.*, 2004) and more recently in the *Marcetia* alliance (Michelangeli *et al.*, 2013). The monophyly of *Siphanthera* was previously recovered in morphological analyses (Almeda & Robinson, 2011) and is corroborated in this study. The ellipsoid to lacrimiform seed with elongated cells can be considered an autapomorphy for *Siphanthera*. Beyond the unique seed morphology, *Siphanthera* is also characterized by the herbaceous habit (annual or perennials), usually < 100 cm tall, mostly four fertile stamens, sometimes with one to four staminodes, anthers straight, commonly rostrate or occasionally truncate, connective dorsally thickened, rarely prolonged below the thecae, modified into well-developed ventral appendages and style occasionally enlarged below the stigma. Kriebel (2008) suggested that *Siphanthera* is morphologically related to *Acisanthera tetraptera*. However, Almeda & Robinson (2011) suggested that the similarity of the anther between the two taxa could be due to convergence or parallelism, as it occurs between the seeds of

Siphanthera and Microlicieae. In our study, *Siphanthera* is phylogenetically related to the *Sandemania* and allies clade. However, the two species in this clade are differentiated from *Siphanthera* mainly by the stamens, in which both cycles are fertile, the anthers are larger and the pedoconnective is clearly prolonged below the thecae. Also, the leaves are completely coriaceous, and they are mostly shrubs up to 2 m tall.

Macairea s.s. + *Ernestia s.s.* clade

According to Renner (1989), *Macairea* is characterized by the presence of a dorso-basal connective anther appendage and glandular trichomes in the vegetative and reproductive structures. In our analysis, *Macairea* was not resolved as monophyletic because *Ernestia s.s.* is resolved in *Macairea*. In *Macairea*, *M. lasiophylla* and *M. multinervia* are resolved as a grade basal to subtending *Ernestia s.s.* (Fig. 2). Although only five *Macairea* spp. were sampled, from a total of 22, making it difficult to infer internal relationships, the characters used by Cogniaux (1891), Gleason (1934) and Renner (1989) as diagnostic are clearly not appropriate for recognizing the genus or its sections, because they also occur in unrelated genera. *Macairea* was divided by Renner (1989) into two groups based on trichome types. Even though our sampling does not allow a clear test of these groups, some trends are apparent. *Macairea thyrsiflora* DC. treated in 'group A' by Renner (1989) was recovered with *M. pachyphylla* Benth. and *M. radula* (Bonpl.) DC. from 'group B', indicating that hair type alone is not an informative character for recognizing species groups in this genus. The *Macairea s.s.* subclade is phylogenetically well resolved and supported by staminal filaments with short-stalked glands, a prolonged and dorso-basally expanded connective, without well-developed appendages, and a glandular style and ovary. On the other hand, *M. lasiophylla* has a set of characters that are not clearly related to any other genera, including straight anthers, with the connective briefly prolonged and modified into two bilobed appendages and a glabrous filament and style, besides other differences in vegetative structure and habit. In general, the flowers of *M. multinervia* are similar to those of the *Macairea s.s.* subclade, except for the presence of glands on the filament and style. Also, its leaves are thinner, basally cordate and seven- to nine-nerved; differing from all other *Macairea* spp. (Renner, 1989). Wurdack (1966) argued that the pubescence and floral features of *M. lasiophylla* indicate that it represents a reduced evolutionary offshoot related to *M. multinervia*, with stamens really quite conformable to other species in the genus. Renner (1989) suggested that the ventral appendages of *M. lasiophylla* could be similar to those of *Acisanthera* and *Ernestia*. However, the ventral appendages of the *Ernestia s.s.* subclade are long with two bifurcated aristae. The *Ernestia s.s.* subclade is constituted by the species of *Ernestia* section *Ernestia*. Although support for this subclade is weak, this group is morphologically well characterized by tetramerous flowers and a tetralocular ovary with a glandular–setose apex (Cogniaux, 1885). Also, this is the only clade of *Ernestia* where the large stamens have aristate and bifurcated ventral appendages and a dorsal spur at the median part of the antesepalous pedoconnective.

Nepsera and allies clade

This strongly supported clade includes two easily recognized monotypic genera: *Nepsera* and *Appendicularia*. It also includes the only species sampled of *Acanthella*, a genus composed of two species, and some *Ernestia* spp. *Nepsera aquatica* (Aubl.) Naudin is a herb or small shrub, commonly found in swampy areas and it differs from *Appendicularia* by its paniculate lax inflorescences, terminal and nearly isomorphic stamens with a briefly prolonged connective and bilobed ventral appendages. In the *Appendicularia* + *Ernestia pullei* subclade the inflorescences are paniculate and terminal, but not lax and slender as in *Nepsera*. Also, the hypanthium is clearly eight-ridged, tubulose–campanulate with semicircular calyx lobes and the connective is prolonged with ventral appendages basally enlarged with long terminal aristae. *Acanthella* was placed in Merianeae by Bentham & Hooker (1867). Although *Acanthella* shares some features with members of this tribe, they are differentiated by seed morphology. All genera of Marianeae have elongate, wingless or narrowly winged seeds, whereas in *Acanthella* they are large, broad and strongly winged (Gleason, 1952). Renner (1993) transferred *Acanthella* to Melastomeae and more recently it was recovered in the *Marcetia* alliance (Michelangeli *et al.*, 2013). Our results did not determine accurately the relationships of *Acanthella sprucei* Benth. & Hook.f. However, this genus is supported by morphological synapomorphies including yellow to orange petals and winged seeds. Although the *Ernestia maguirei* subclade is phylogenetically well supported, this clade consists of three morphologically distinct species. *Ernestia confertiflora* Wurdack + *E. rubra* Pulle have basally inflated ventral appendages with two terminal aristae. In *E. maguirei*, the stamens are not enlarged at the base, the aristae are smaller and the appendages are dorsally auriculate, similar to those of *E. rubra* but different from *E. confertiflora*.

In addition, *E. maguirei* has a glandular style and tetralocular, glabrous ovary. Morphologically, this subclade is particularly difficult to characterize due to the absence of synapomorphies or even a set of exclusive morphological characters. The incomplete sampling of *Ernestia* associated with its morphological diversity precluded a morphological characterization of the *E. maguirei* subclade, as well as resolving generic relationships. Currently, this is the most complex clade in the *Marcetia* alliance, with great diversity of habit, inflorescence, stamen and seed morphology, and the one most in need of further studies.

Marcetia clade

The monophyly of *Marcetia* is well supported and the genus was previously recovered as monophyletic by Santos (2009) using nrITS sequence data. Accordingly, its floral morphology is quite homogeneous, despite being the largest genus in the alliance. Putative morphological synapomorphies for *Marcetia* include usually isomorphic, straight anthers surrounding the style at anthesis and a connective without appendages, not prolonged and thickened at the base of the thecae. *Aciotis*, *Comolia*, *Fritzschia* and *Nepsera* were traditionally associated with *Marcetia* in molecular and/or morphological studies (Martins, 1989; Santos, 2009; Almeda & Robinson, 2011; Michelangeli *et al.*, 2013). In our analysis, *Marcetia* is resoved as sister to the clade formed by *Fritzschia* and *Comolia* section *Tricentrum* clade + *Aciotis*. In terms of morphological relationships, *Aciotis* lacks a pedoconnective and appendages or they are occasionally poorly developed, as in *Marcetia*, but the connective of *Aciotis* is never thickened at the base of thecae. In addition, fruit, leaf morphology and habitat of *Aciotis* spp. are different from *Marcetia*. In *Comolia*, species of *Comolia* section *Tricentrum* (the *Comolia* spp. in clade J) are those phylogenetically more closely related to *Marcetia*. In terms of distribution, *Fritzschia* and *Marcetia* occur predominantly in the campos rupestres (rocky fields) vegetation of the Espinhaço Range in Brazil. However, *Marcetia* is concentrated in the northern part of the Espinhaço, whereas the *Fritzschia* + *Comolia* section *Tricentrum* clade is found in the southern part. This group of *Comolia* is distinguished from *Marcetia* mainly by the well-developed pedoconnective, modified into bilobed ventral appendages. In *Fritzschia*, the pedoconnective can be briefly prolonged and modified at the filament insertion, into conspicuous lobes or auricules, as in *F. erecta* Cham., or have well-developed pedoconnective and ventral appendages. Also, *Fritzschia* has anthers that are purple/dark pink, whereas in *Marcetia* they are always yellow. Lastly, the phylogenetically distantly related genus *Nepsera* has a shortly developed pedoconnective, ventral appendages and a unique inflorescence pattern.

Fritzschia and *Comolia* section *Tricentrum* clade

This clade is strongly supported by molecular data and recognized mainly by its stems and leaves with glandular trichomes, and tetralocular ovary. Additionally, this clade is mostly endemic to the Espinhaço Range in Minas Gerais state, except for *Comolia lanceiflora* (DC.) Triana, which also occurs in Goiás and Distrito Federal. The six *Comolia* spp. recovered here were treated in *Comolia* section *Tricentrum* by Cogniaux (1885). *Comolia vernicosa*, which was also placed in section *Tricentrum*, is actually more closely related to *Sandemania* (see above). *Comolia* section *Tricentrum* is recognized by the curved anthers, pedoconnective well developed below the thecae, normally purple, with two small tuberculate ventral appendages and dorsal appendages not developed. *Fritzschia* consists of three species that also have a well-prolonged pedoconnective modified into auriculate ventral appendages, except for *F. erecta*, in which the pedoconnective is absent or shortly developed with small auriculate appendages. In addition, these species have a prostrate and decumbent habitat, leaf lamina with translucent glands and hypanthium and ovary with secretory structures. Martins (1989) suggested that *Fritzschia* is morphologically related to *Marcetia*, differing by the presence of glands in the leaves and by becoming black when dried. However, these genera also differ in stamen features: in *Fritzschia* they are similar to those of the *Comolia* section *Tricentrum*, except in *F. erecta*. As the species of *Comolia* section *Tricentrum* are paraphyletic, share several morphological characters and geographical distribution with *Fritzschia*, and clade J has strong support in all phylogenetic analyses, the best option to circumscribe a monophyletic genus would be to include everything in this clade in *Fritzschia*.

Aciotis clade

Aciotis is morphologically easily recognized by the presence of tetramerous flowers, < 1.5 cm long, absence of connective appendages, straight anthers and bilocular ovary (rarely trilocular), among other features (Freire-Fierro, 2002). Ecologically, the genus is often found in flooded areas or in waterlogged soils, in open and forested areas. Some studies have suggested that *Aciotis* has affinities with *Nepsera* (Clausing & Renner, 2001; Fritsch *et al.*, 2004; Kriebel, 2008) and/or *Marcetia* (Almeda & Robinson, 2011). However, in our analysis *Aciotis* is moderately

to strongly supported as sister to the *Fritzschia* and *Comolia* section *Tricentrum* clade. In the *Marcetia* alliance, *Aciotis* and *Nepsera* are the only genera that occur mainly in moist areas in forest edges. According to Freire-Fierro (2002), it is possible to recognize two main groups in *Aciotis* based on inflorescence patterns, fruits and presence of sclereids. In our study we recovered these two groups with some intermediate species, as suggested by Freire-Fierro (2002). The first group, which includes *Aciotis annua* (Mart. ex DC.) Triana and *A. polystachya* (Bonpl.) Triana, has filiform sclereids, biparous to double biparous cymose inflorescences and dry capsular fruits. The second group includes *A. circaeifolia* (Bonpl.) Triana, *A. indecora* (Bonpl.) Triana, *A. purpurascens* (Aubl.) Triana and *A. rubricaulis* (Mart. ex DC.) Triana and has thyrsoid to paniculate inflorescences and baccate fruits. *Aciotis paludosa* (Mart. ex DC.) Triana and *A. acuminifolia* (Mart. ex DC.) Triana have characteristics of both groups.

Morphological optimizations

Some morphological characters traditionally used in the taxonomy of the *Marcetia* alliance were reconstructed in light of the molecular phylogenetic analysis, emphasizing their evolution inside the alliance (Fig. 3). Levels of homoplasy for each character were measured by CI and RI (Fig. 3). Individually, none of the studied characters supported the groups recovered in the molecular analyses, but when combined were useful for the diagnosis of clades and genera.

Floral merosity (Fig. 3A)

Pentamerous flowers are the plesiomorphic state and the presence of tetramerous flowers is synapomorphic for the *Marcetia* alliance. This state is widely distributed in almost all genera, but it is not an uncontested synapomorphy as the character is optimized to have two reversals to pentamerous flowers, once in *Acisanthera s.l.* and once in *Ernestia* section *Pseudoernestia*. There was also a secondary shift to tetramerous flowers in *Acisanthera*. In fact, in this genus floral merosity is optimized as changing twice. In the *Marcetia* alliance, pentamerous flowers are diagnostic for the *Acisanthera bivalvis* subclade and the *Acisanthera s.s.* subclade, except for *Comolia ayangannae*.

Ovary pubescence (Fig. 3B)

A glabrous ovary is the ancestral state in the *Marcetia* alliance. Optimization of this character indicated that the plesiomorphic state was retained in almost all branches of the *Marcetia* alliance and that the presence of a glandular ovary in a few members of the alliance arose probably by convergence. Although glandular ovaries have evolved independently in four different lineages, this character state proved to be useful to recognize the *Ernestia s.s.* subclade, which is the only clade of *Ernestia* with a glandular ovary. Also, in *Acisanthera s.l.*, the glandular ovary is an autapomorphy for *A. tetraptera.*

Ovary locule number (Fig. 3C)

The number of ovary locules is reduced to two to four in the *Marcetia* alliance from five in the outgroups. This character has played an important role in the circumscription of genera and sections in the *Marcetia* alliance, but it is highly homoplastic with multiple character state transitions inferred by our reconstructions. In the *Marcetia* alliance and related tribes such as Microlicieae and Rhexieae, the number of ovary locules is commonly equal to or smaller than the number of petals, except in Melatomeae *s.s.* which has a pentalocular ovary and tetra- or pentamerous flowers (Michelangeli *et al.*, 2013). Although homoplastic, the ancestral state (two locules) was retained in the *Comolia montana* clade, *Acisanthera tetraptera*, the *Acisanthera hedyotidea* subclade, the *Acisanthera bivalvis* subclade, *Sandemania hoehnei*, *Siphanthera* and, by reversal, in *Aciotis*. Although the same number of ovary locules evolved independently in unrelated groups, this character is more conserved in monophyletic genera and can be potentially useful as diagnostic when associated with other characters.

Filament/anther angle at anthesis (Fig. 3D)

Straight anthers are uncommon in capsular-fruited Melastomataceae, which are usually characterized by the strongly curved anthers. Among the outgroups, straight anthers appeared only in *Chaetostoma armatum* (Spreng.) Cogn., which is one of the most distinctive members of Microlicieae (Fritsch *et al.*, 2004). The reconstruction of this character in the *Marcetia* alliance suggests that arched or curved anthers were the ancestral condition in the group. However, straight anthers evolved, independently, at least four or five times in unrelated clades of the group. Despite the high level of homoplasy, this character can be useful to characterize some clades of non-monophyletic genera. In *Comolia*, for example, only the *C. montana* clade has slightly straight anthers, whereas the other clades retained the ancestral state. The monophyletic *Siphanthera*, *Marcetia* and *Aciotis* (clades F, I and K, respectively) also have straight anthers. In these genera the pedoconnective is absent or, when present, it is not articulated at the filament insertion.

Pedoconnective in antesepalous stamens (Fig. 3E)

The presence of a pedoconnective was treated by Clausing & Renner (2001) as a possible

synapomorphy for an expanded concept of Melastomeae, Microlicieae and Rhexieae. In the *Marcetia* alliance the pedoconnective varies widely; it may be well developed or reduced. The reconstruction analysis showed that this structure was lost in a few members of the *Marcetia* alliance; it is also absent in Rhexieae, and in some species of Melastomeae *s.s.* Our results suggest that the ancestor of *Marcetia* had pedoconnectives and that it was lost in more derived lineages of *Marcetia*, except in *M. candolleana* A.K.A.Santos & A.B.Martins and *M. grandiflora* Markgr., where it is present and probably represents a reversal. *Marcetia* is mainly characterized by a connective that is not prolonged, but is thickened at the base of the thecae. In future research, the evolution of the androecium should be investigated using morphometric tools to characterize this variation. In *Aciotis*, *Nepsera* and *Siphanthera*, the pedoconnective is poorly developed and briefly prolonged below the thecae.

Anther fertility (Fig. 3F)

Most genera of Melastomataceae are diplostemonous, having two cycles of stamens. The plesiomorphic state in the *Marcetia* alliance is for both cycles to be fertile and this character state was conserved in most genera. A single fertile cycle is an important character to characterize *Siphanthera*, in which only two species (*S. cowanii* Wurdack and *S. paludosa* Cogn.) have diplostemonous flowers. The remaining species can have an antepetalous cycle with one to four staminodia, or it may be absent altogether. According to Almeda & Robinson (2011), the loss of fertile stamens among the species of *Siphanthera* does not seem to have any consistent evolutionary pattern. Heteranthery has occurred to varying degrees in all major clades of the alliance. Our analyses also did not recover any apparent pattern and we agree with Almeda & Robinson (2011) that knowledge about the pollinator spectrum for each species may help in understanding the forces that are driving these losses.

Other characters

Other morphological characters with potential for characterization of clades were also investigated, but were not included in the results because it was difficult to code them and establish homology hypotheses or they were continuous. Nevertheless, some are useful for characterization of genera or groups of species. The stamens of the *Marcetia* alliance, for example, although highly variable, can be used to recognize some genera by the shape of the ventral connective appendages. In *Ernestia s.s.*, the stamens have a long pedoconnective and two aristate ventral appendages. This type of appendage, with developed long caudate projections, is common only in *Ernestia* and related groups, such as *Appendicularia*. In most other genera, the ventral appendages tend to be bilobed or bituberculate, rarely auriculate, with broad variation in size between genera.

CONCLUSIONS AND PERSPECTIVES

This study represents a major step towards understanding generic relationships in the *Marcetia* alliance. Sampling was significantly expanded and now includes 64% of all species putatively assigned to the group. New sequences for five markers was generated in this study. With this expanded data set, we confirmed the monophyly of the *Marcetia* alliance and the genera *Aciotis*, *Fritzschia*, *Marcetia* and *Siphanthera*. The paraphyletic or polyphyletic *Comolia*, *Ernestia* and *Macairea* have been traditionally diagnosed on the basis of a few, broadly distributed, homoplastic characters, and should be re-circumscribed based on well-supported clades and diagnostic morphological characters.

A potential difficulty for taxonomic re-circumscriptions in Melastomataceae is the lack of synapomorphies or even of a set of morphological characters of diagnostic value for well-supported clades identified in molecular phylogenetic analyses. Many characters treated as diagnostic in the past appear to have evolved independently, a problem also seen in Miconieae (Michelangeli *et al.*, 2004; Goldenberg *et al.*, 2008; Martin *et al.*, 2008; Kriebel *et al.*, 2015), Blakeeae (Penneys & Judd, 2013) and Henrietteeae (Penneys *et al.*, 2010). Due to this pervasive presence of homoplasy, character combinations could be more useful to diagnose well-supported clades associated with distributional and ecological data. This approach will be essential to understand and characterize different groups. In our study, we found few uncontested synapomorphies for larger clades. However, our results reveal a strong geographical and ecological structure for several well-supported clades, in which the species tend to occur in the same environment and, hence, biogeographical region.

With these criteria in mind, our results suggest that several taxonomic realignments are necessary in the *Marcetia* alliance. However, additional sampling of taxa and markers could potentially improve resolution and enable a more meaningful interpretation of the morphological and biogeographical patterns for the recovered clades. For example, *Ernestia* and *Comolia* should be segregated into two or more genera. Generic recircumscriptions are also necessary for *Macairea* and *Acisanthera*. All four clades of *Comolia* are morphologically well characterized or geographically structured and our results support the recircumscription of this genus based on the

recognition of monophyletic units. As currently understood, *Macairea* is paraphyletic. However, more taxa and probably also markers are needed to clarify relationships in this genus and with *Ernestia*.

Another approach with potential to be investigated in the *Marcetia* alliance is the evolution of morphological characters, such as androecium and seeds. Understanding the evolution of these structures may provide some clues to the colonization of disjunct habitats such as the campos rupestres of the Espinhaço Range, cerrado of central Brazil, Amazonian savannas, restingas of northern and north-eastern Brazil and the Guayana highlands by the genera of this alliance.

Lastly, three genera that may potentially be part of the *Marcetia* alliance remain unsampled: *Comoliopsis*, *Loricalepis* and *Poteranthera*. *Comoliopsis* is without doubt a member of this clade, and perhaps even closely related to *Comolia montana* (see above). *Loricalepis* is a poorly collected genus from northern Brazil characterized by tetramerous flowers and anthers without appendages or pedoconnectives, which would suggest a relationship with the *Marcetia* alliance (Brade, 1938; Pereira, 1959). Moreover, its leaves are similar to some *Macairea* spp. However, the seeds of *Loricalepis* are typically 'tibouchinoid' and the apex of the ovary is pubescent (Brade, 1938; Pereira, 1959; Whiffin & Tomb, 1972), characters typically associated with core Melastomeae (Michelangeli *et al.*, 2013). *Poteranthera* is a genus of uncertain affinities, with three species of tiny, tetramerous herbs from savannas of Brazil and Venezuela that at times has been suggested to be near *Acisanthera* or *Siphanthera* (Kriebel, 2012).

ACKNOWLEDGEMENTS

We thank the curators and staff of ALCB, BHCB, CEPEC, HUEFES, HUFU, INPA, IAN, MG, MIRR, RB, SP, SPF, NY and US for specimen loans or assistance during visits and the Brazilian conservation bodies ICMBio and IEF Minas Gerais for scientific collection permits. Financial support was provided by Fundação de Amparo a Pesquisa do Estado de Minas Gerais – FAPEMIG (PPM-00226-12), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES (PNADB/UFMG-922/2010), Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq (REFLORA 563541/2010-5) and National Science Foundation – NSF (DEB-1343612 and DEB-1140409). This research was supported by scholarships from CAPES, FAPEMIG and CNPq (sandwich doctorate, 210299/2013-4) to M.J.R.R. J.A.N.B. also acknowledges a scholarship received from CNPq (Pq-2). The first author thanks K. Sosa and M. Sewell for their assistance in the laboratory of the Cullman Program for Molecular Systematics at NYBG and Olivier Gaubert and F. Fajardo-Gutiérrez for the photographs.

REFERENCES

Alexander PJ, Rajanikanth G, Bacon CD, Bailey CD. 2007. Recovery of plant DNA using a reciprocating saw and silica-based columns. *Molecular Ecology Notes* **7:** 5–9.

Almeda F, Martins AB. 2001. New combinations and new names in some Brazilian Microlicieae (Melastomataceae), with notes on the delimitation of *Lavoisiera*, *Microlicia*, and *Trembleya*. *Novon* **11:** 1–7.

Almeda F, Robinson OR. 2011. Systematics and phylogeny of *Siphanthera* (Melastomataceae). *Systematic Botany Monographs* **93:** 1–101.

Amorim AM, Goldenberg R, Michelangeli FA. 2009. A new species of *Physeterostemon* (Melastomataceae) from Bahia, Brazil, with notes on the phylogeny of the genus. *Systematic Botany* **34:** 324–329.

Baumgratz JFA, Caddah MK, Chiavegatto B, Goldenberg R, Guimarães PJF, Koschnitzke C, Kriebel R, Lima LFG, Martins AB, Michelangeli FA, Reginato M, Rocha MJR, Rodrigues KF, Romero R, Rosa P, Silva-Gonçalves KC, Souza MLDR, Woodgyer E. 2015. *Melastomataceae*. In: Forzza, R. C. *et al.*, eds. *Lista de espécies da Flora do Brasil*. Rio de Janeiro: Jardim Botânico do Rio de Janeiro, Available at http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB161. (acessed 2 July 2015).

Bécquer-Granados ER. 2008. *Taxonomía y filogenia del género Pachyanthus (Melastomataceae: Miconieae)*. Ciudad de La Haban: Universidad de la Habana.

Bentham G. 1840. Enumeration of plants collected by Mr. Schomburgk in British Guiana. Melastomaceae. *Journal of Botany (Hooker)* **2:** 286–315.

Bentham G, Hooker JD. 1867. Melastomataceae. *Genera Plantarum* **1:** 748.

Brade AC. 1938. Melastomataceae Novae II. *Archivos do Instituto de Biologia Vegetal* **4:** 71–84.

Caetano AP. 2014. *Contribuição da embriologia na sistemática e na elucidação da apomixia em Melastomataceae Juss*. Campinas, SP: Universidade Estadual de Campinas.

Candolle APD. 1828. Mémoires sur la famille des Mélastomacées. *Prodromus Systematis Naturalis Regny Vegetabilis* **3:** 99–102.

Clausing G, Renner SS. 2001. Molecular phylogenetics of Melastomataceae and Memecylaceae: implications for character evolution. *American Journal of Botany* **88:** 486–498.

Cogniaux CA. 1883. Melastomataceae. *Flora Brasiliensis* **14:** 5–205.

Cogniaux CA. 1885. Melastomataceae. *Flora Brasiliensis* **14:** 1–510.

Cogniaux CA. 1891. Melastomaceae. *Monographiae Phanerogamarum* **7:** 1–1256.

Cogniaux CA. 1909. Melastomataceae peruvianae. *Botanische Jahrbücherfür Systematik*. **42:** 131–148.

Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9:** 772.

Erixon P, Svennblad B, Britton T, Oxelman B. 2003. Reliability of Bayesian posterior probabilities and bootstrap frequencies in phylogenetics. *Systematic Biology* **52:** 665–673.

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39:** 783–791.

Fitch WM. 1971. Toward defining the course of evolution: minimum change for a specific tree topology. *Systematic Zoology* **20:** 406–416.

Freire-Fierro A. 2002. Monograph of *Aciotis* (Melastomataceae). *Systematic Botany Monographs* **62:** 1–129.

Fritsch PW, Almeda F, Renner SS, Martins AB, Cruz BC. 2004. Phylogeny and circumscription of the near-endemic Brazilian tribe Microlicieae (Melastomataceae). *American Journal of Botany* **91:** 1105–1114.

Gleason HA. 1925. Studies on the flora of northern South America-VI. *Bulletin of the Torrey Botanical Club* **52:** 325–340.

Gleason HA. 1934. The genus *Macairea* DC. in northern South America. *Bulletin of the Torrey Botanical Club* **61:** 35–40.

Gleason HA. 1939a. The flora of Mount Auyan-Tepui, Venezuela. *Brittonia* **3:** 141–204.

Gleason HA. 1939b. A new genus of Melastomataceae from Peru. *Kew Bulletin* **8:** 480–481.

Gleason HA. 1952. Contributions to the flora of Venezuela: from Droseraceae through Umbelliferae/Julian A. Steyermark and collaborators. *Fieldiana* **28:** 430–431.

Goldenberg R, Almeda F, Caddah MK, Martins AB, Meirelles J, Michelangeli FA, Weiss M. 2013. Nomenclator botanicus for the Neotropical genus *Miconia* (Melastomataceae: Miconieae). *Phytotaxa* **106**(1)**:** 1–171.

Goldenberg R, Fraga CN, Fontana AP, Nicolas NA, Michelangeli FA. 2012. Taxonomy and phylogeny of *Merianthera* (Melastomataceae). *Taxon* **61:** 1040–1056.

Goldenberg R, Penneys DS, Almeda F, Judd WS, Michelangeli FA. 2008. Phylogeny of *Miconia* (Melastomataceae): patterns of stamen diversification in a megadiverse neotropical genus. *International Journal of Plant Sciences* **24:** 315–327.

Guindon S, Gascuel O. 2003. A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. *Systematic Biology* **52:** 696–704.

Hamilton MB. 1999. Four primer pairs for the amplification of chloroplast intergenic regions with intraspecific variation. *Molecular Ecology* **8:** 521–523.

Ionta GM, Judd WS, Williams NH, Whitten WM. 2007. Phylogenetic relationships in *Rhexia* (Melastomataceae): evidence from DNA sequence data and morphology. *International Journal of Plant Sciences* **168:** 1055–1066.

Krasser F. 1893. Melastomataceae. In: Engler A, Prantl K, eds. *Die natiirlichen Pflanzenfamilien*. Vol. **3**. Leipzig**:** Verlag von Wilhelm Engelmann, 149.

Kress WJ, Prince LM, Williams KJ. 2002. The phylogeny and a new classification of the gingers (Zingiberaceae): evidence from molecular data. *American Journal of Botany* **89:** 1682–1696.

Kriebel R. 2008. *Systematics and biogeography of the Neotropical genus Acisanthera (Melastomataceae)*. San Francisco: San Francisco State University.

Kriebel R. 2012. A synopsis of the genus *Poteranthera* (Melastomeae: Melastomataceae) with the description of a new, apparently pollinator deceiving species. *Brittonia* **62:** 6–14.

Kriebel R, Michelangeli FA, Kelly LM. 2015. Discovery of unusual anatomical and continuous characters in the evolutionary history of *Conostegia* (Miconieae: Melastomataceae). *Molecular Phylogenetics and Evolution* **82:** 289–313.

Maddison WP, Maddison DR. 2001. MESQUITE: a modular system for evolutionary analysis, Version 2.75. Available at: http://mesquiteproject.org

Martin CV, Little DP, Goldenberg R, Michelangeli FA. 2008. A phylogenetic evaluation of *Leandra* (Miconieae, Melastomataceae): a polyphyletic genus where the seeds tell the story, not the petals. *Cladistics* **24:** 315–327.

Martins AB. 1989. *Revisão taxonômica do gênero* Marcetia *DC. (Melastomataceae).* Tese (doutorado), Universidade Estadual de Campinas, Intituto de Biologia, 1–288.

Martius CFP. 1831. *Novae genera et species plantarum 3*. Munich: Caroli Wolf, 189.

Mendoza-Cifuentes H, Fernández-Alonso JL. 2012. Novedades em *Centronia* y *Meriania* (Merianieae, Melastomataceae) y revisión taxonômica de *Meriania* grupo brachycera. *Anales del Jardín Botánico de Madrid* **69:** 259–294.

Michelangeli FA. 2000. A cladistic analysis of the genus *Tococa* (Melastomataceae) based on morphological data. *Systematic Botany* **25:** 211–234.

Michelangeli FA, Guimaraes PJF, Penneys DS, Almeda F, Kriebel R. 2013. Phylogenetic relationships and distribution of New World Melastomeae (Melastomataceae). *Botanical Journal of the Linnean Society* **171:** 38–60.

Michelangeli FA, Judd WS, Penneys DS, Skean JD, Bécquer-Granados ER, Goldenberg R, Martin CV. 2008. Multiple events of dispersal and radiation of the tribe Miconieae (Melastomataceae) in the Caribbean. *Botanical Review* **74:** 53–77.

Michelangeli FA, Nicolas A, Morales-P MA, David H. 2011. Phylogenetic relationships of *Allomaieta*, *Alloneuron*, *Cyphostyla*, and *Wurdastom* (Melastomataceae) and the resurrection of the tribe Cyphostyleae. *International Journal of Plant Sciences* **172:** 1165–1178.

Michelangeli FA, Penneys DS, Giza J, Soltis D, Hils MH, Skean JD. 2004. A preliminary phylogeny of the tribe Miconieae (Melastomataceae) based on nrITS sequence data and its implications on inflorescence position. *Taxon* **53:** 279–290.

Michelangeli FA, Ulloa CA, Sosa K. 2014. *Quipuanthus*, a new genus of Melastomataceae from the foothills of the Andes in Ecuador and Peru. *Systematic Botany* **39:** 533–540.

Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large

phylogenetic trees in Proceedings of the Gateway Computing Environments Workshop (GCE) New Orleans, LA. pp 1–8. Available at: http://www.phylo.org/

Naudin CV. 1849–1853. *Melastomacearum monographicae descriptiones*. Annales des Sciences Naturelles; Botanique. Series III. Consolidated reprint, 1–719.

Nixon KC. 1999. The parsimony ratchet, a new method for rapid parsimony analysis. *Cladistics* **15:** 407–414.

Penneys DS, Judd WS. 2011. Phylogenetics and morphology in the Blakeeae (Melastomataceae). *International Journal of Plant Sciences* **172:** 78–106.

Penneys DS, Judd WS. 2013. Combined molecular and morphological phylogenetic analyses of the Blakeeae (Melastomataceae). *International Journal of Plant Sciences* **174:** 802–817.

Penneys DS, Michelangeli FA, Judd WS, Almeda F. 2010. Henrietteeae (Melastomataceae): a new Neotropical berry-fruited tribe. *Systematic Botany* **35:** 783–800.

Pereira E. 1959. Contribuição ao conhecimento das Melastomataceae brasileiras. *Arquivos Jardim Botânico Rio Janeiro* **17:** 125–160.

Presl CB. 1832. Descriptiones et icones plantarum novarum aut minus cognitarum. *Symbolae Botanicae* **1:** 75.

Reginato M, Michelangeli FA, Goldenberg R. 2010. Phylogeny of *Pleiochiton* (Melastomataceae, Miconieae): total evidence. *Botanical Journal of the Linnean Society* **162:** 423–434.

Renner SS. 1987. *Sandemania hoehnei* (Melastomataceae: Tibouchineae): taxonomy, distribution, and biology. *Brittonia* **39:** 441–446.

Renner SS. 1989. Systematic studies in the Melastomataceae *Bellucia*, *Loreya* and *Macairea*. *Memoirs of the New York Botanical Garden* **50:** 1–112.

Renner SS. 1993. Phylogeny and classification of the Melastomataceae and Memecylaceae. *Nordic Journal of Botany* **13:** 519–540.

Santos AK. 2009. *Estudos filogenéticos e biossistemáticos no gênero* Marcetia *DC. (Melastomataceae)*. Feira de Santana: Universidade Estadual de Feira de Santana.

Seco RS. 2006. *Estudos taxonômicos no gênero Comolia DC (Melastomataceae-Melastomeae) no Brasil*. Campinas: Universidade Estadual de Campinas.

Shaw J, Lickey EB, Beck JT, Farmer SB, Liu W, Miller J, Siripun KC, Winder CT, Schilling EE, Small RL. 2005. The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany* **92:** 142–166.

Simmons MP. 2004. Independence of alignment and tree search. *Molecular Phylogenetics and Evolution* **31:** 874–879.

Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22:** 2688–2690.

Stamatakis A, Hoover P, Rougemont J. 2008. A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology* **57:** 758–771.

Stone RD. 2006. Phylogeny of major lineages in Melastomataceae, subfamily Olisbeoideae: utility of nuclear glyceraldehyde 3-phosphate dehydrogenase (*GapC*) gene sequences. *Systematic Botany* **31:** 107–121.

Stone RD, Andreasen K. 2010. The Afro-Madagascan genus *Warneckea* (Melastomataceae): molecular systematics and revised infrageneric classification. *Taxon* **59:** 83–92.

Swofford DL. 2002. *PAUP*. Phylogenetic analysis using parsimony (*and other methods)*. Version 4. Sunderland, MA: Sinauer Associates.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* **30:** 2725–2729.

Thiers B. 2015. *Index herbariorum: a global directory of public herbaria and associated staff*. New York Botanical Garden's Virtual Herbarium. Avaliable at: http://sweetgum.nybg.org/ih/. (accessed 6 June 2015).

Triana J. 1865. *Dispositio Melastomacearum*. Bulletin du Congres International de Botanique et d'Horticulture reuni à Amsterdam, 457–461.

Triana J. 1871. Les Melastomacées. *Transactions of the Linnean Society of London* **28:** 1–188.

Ung V, Dubus G, Zaragüeta-Bagils R, Vignes-Lebbe R. 2010. Xper2: introducing e-taxonomy. *Bioinformatics Applications Note* **26:** 703–704.

van Vliet G, Koek-Noorman J, Ter Welle BJH. 1981. Wood anatomy, classification and phylogeny of the Melastomataceae. *Blumea* **27:** 463–473.

Whiffin T, Tomb AS. 1972. The systematic significance of seed morphology in the neotropical capsular-fruited Melastomataceae. *American Journal of Botany* **59:** 411–422.

Wilson CL. 1950. Vasculation of the stamen in the Melastomaceae, with some phyletic implications. *American Journal of Botany* **37:** 431–444.

Wurdack JJ. 1964. Melastomataceae. Botany of the Guayana Highlands-part V. *Memoirs of the New York Botanical Garden*, **10:** 135–186.

Wurdack JJ. 1966. Certamen Melastomataceis X. *Phytologia* **13:** 65.

Wurdack JJ. 1970. Certamen Melastomataceis XV. *Phytologia* **20:** 369–389.

Wurdack JJ. 1973. Melastomataceae. In: Lasser T, (ed.). *Flora de Venezuela*, Vol. **8**. Caracas**:** Instituto Botanico, 1–819.

Wurdack JJ. 1984. Certamen Melastomataceis XXXVII. *Phytologia* **55:** 131–147.

Wurdack JJ, Renner SS, Morley T. 1993. Melastomataceae. In: Górts-Van Rijn ARA, ed. *Flora of the Guianas Series A: phanerogams 13*. Königstein: Koeltz Scientific Books, 83–84.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Majority-rule consensus tree from a Bayesian analysis of the combined nuclear (ETS, ITS) data sets.

Figure S2. Majority-rule consensus tree from a Bayesian analysis of the combined plastid (*accD-psaI*, *trnS-trnG*, *atpH-atpF*) data sets.

Table S1. Morphological characters and character states scored in this study as primary homology hypotheses.

Appendix: Voucher information and GenBank accession numbers for taxa used in this study. A dash (–) indicates that the DNA region was not sequenced. Herbarium acronyms according to Thiers (2015).

Species	Voucher (herbarium)	Provenance	nrITS	nrETS	accD-psaI	atpH-atpF	trnS-trnG
Acanthella sprucei Benth. & Hook.f.	*Diaz, W., 4538* (NY)	Brazil	–	–	JQ730247	–	–
Aciotis acuminifolia (Mart. ex DC.) Triana	*Rocha, M. J. R., 801* (BHCB)	Brazil	–	–	KU501160	–	KU500922
Aciotis annua (Mart. ex DC.) Triana	*Rocha, M. J. R., 304* (BH)	Brazil	KU501052	KU500989	KU501161	KU501106	KU500923
Aciotis circaeifolia (Bonpl.) Triana	*Caddah, M. K., 621* (NY)	Brazil	JQ730038	KF462812	JQ730249	KU501108	KU500926
Aciotis indecora (Bonpl.) Triana	*Martin, C. V., 411* (NY)	French Guyana	JQ730039	KF462813	JQ730250	KU501109	KU500927
Aciotis paludosa (Mart. ex DC.) Triana	*Guimarães, P., 317* (RB)	Brazil	JQ730040	KF462814	JQ730251	–	KU500925
Aciotis polystachya (Bonpl.) Triana	*Rocha, M. J. R., 858* (BHCB)	Brazil	KU501053	KU500990	KU501162	KU501107	KU500924
Aciotis purpurascens (Aubl.) Triana	*Martin, C. V., 422* (NY)	French Guiana	JQ730041	KF462815	JQ730252	KU501110	KU500928
Aciotis rubricaulis (Mart. ex DC.) Triana	*Goldenberg, R., 850* (NY)	Brazil	JQ730042	KF462816	JQ730253	KU501111	KU500929
Aciotis rubricaulis (Mart. ex DC.) Triana	*Michelangeli, F. A., 2452* (NY)	Guyana	KU501057	KU500993	KU501165	–	–
Aciotis purpurascens (Aubl.) Triana	*Michelangeli, F. A., 2454* (NY)	Guyana	KU501055	KU500992	KU501164	–	–
Aciotis indecora (Bonpl.) Triana	*Michelangeli, F. A., 2456* (NY)	Guyana	KU501056	KU500991	KU501163	–	–
Acisanthera alsinaefolia var. *glazioviana* Cogn.	*Macedo, A., 5539* (NY)	Brazil	KU501059	KU500995	KU501166	KU501114	KU500932
Acisanthera alsinaefolia (DC.) Triana	*Goldenberg, R., 826* (NY)	Brazil	JQ730043	KF462817	JQ730254	KU501112	KU500930
Acisanthera bivalvis (Aubl.) Cogn.	*Rocha, M. J. R., 871* (BHCB)	Brazil	KU501064	KU501003	KU501172	KU501117	KU500936
Acisanthera boissieriana Cogn. (synonym)	*Maguire, B., 35919* (NY)	Venezuela	–	KU501002	–	–	–
Acisanthera crassipes (Naudin) Wurdack	*Rocha, M. J. R., 833* (BHCB)	Brazil	KU501065	KU501004	–	KU501118	KU500937
Acisanthera genliseoides (Hoehne) Wurdack	*Rocha, M. J. R., 942* (BHCB)	Brazil	KU501066	KU501005	KU501173	KU501119	KU500938
Acisanthera hedyotidea (C.Presl.) Triana	*Popovick, A., s.n.* (BHCB)	Brazil	–	KU501001	–	–	–
Acisanthera limnobios (Schrank & Mart. ex DC.) Triana	*Moreira, S. N., 312* (BHCB)	Brazil	–	KU501006	KU501174	–	–
Acisanthera paraguayensis (Hook.f.) Cogn	*Krapovickas, A., 45640* (NY)	Paraguay	KU501058	KU500994	–	KU501113	KU500931
Acisanthera quadrata Pers.	*Rocha, M. J. R., 729* (BHCB)	Brazil	KU501060	KU500996	KU501167	–	–
Acisanthera sp.	*Rocha, M. J. R., 830* (BHCB)	Brazil	KU501061	KU500997	KU501168	–	–
Acisanthera tetraptera (Cogn.) Gleason	*Rocha, M. J. R., 757* (BHCB)	Brazil	KU501067	KU501007	KU501175	KU501120	KU500939
Acisanthera uniflora (Vahl) Gleason	*Pedraza, P., 2281* (NY)	Colombia	KU501062	–	KU501169	–	KU500933
Acisanthera va*iabilis* var. *gabriuscula* Cogn.	*Irwin, H. S., 19593* (NY)	Brazil	–	KU500999	–	–	–
Acisanthera va*iabilis* (DC.) Triana	*Rocha, M. J. R., 611* (BHCB)	Brazil	–	KU500998	KU501170	KU501115	KU500934
Appendicularia thymifolia (Bonpl.) DC.	*Martin, C. V., 441*	French Guyana	JQ730049	KF462820	JQ730260	KU501130	KU500951
Brachyotum microdon (Naudin) Triana	*Nee, M., 55327* (NY)	Bolivia	JQ730059	KF462828	JQ730270	–	KU500984
Cambessedesia hilariana (A.St.Hil. ex Bonpl.) DC.	*Guimarães, P., 405* (RB)	Brazil	JQ730063	KF462835	JQ730274	–	KU500987

Appendix . *Continued*

Species	Voucher (herbarium)	Provenance	nrITS	nrETS	accD-psaI	atpH-atpF	trnS-trnG
Centradenia grandiflora (Schltdl.) Endl. ex Walp.	*Penneys, D. S., 1544* (FLAS)	Costa Rica	JQ730065	KF462837	–	–	–
Chaetostoma armatum (Spreng.) Cogn.	*Guimarães, P., 396* (RB)	Brazil	JQ730069	KF462840	–	–	–
Comolia ayangannae Wurdack	*Hoffman, B., 2943* (US)	Guyana	KU501063	KU501000	KU501171	KU501116	KU500935
Comolia coriacea Gleason	*Steyermark, J.A., 129919* (US)	Venezuela	–	KU501022	–	–	–
Comolia edmundoi Brade	*Rocha, M. J. R., 975* (BHCB)	Brazil	–	–	KU501176	KU501121	KU500940
Comolia lanceiflora (DC.) Triana	*Rocha, M. J. R., 905* (BHCB)	Brazil	KU501068	KU501009	KU501177	KU501123	KU500941
Comolia leptophylla (Bonpl.) Naudin	*Wurdack, J. J., 39943* (US)	Venezuela	KU501072	KU501015	–	–	–
Comolia lythrarioides Naudin (synonym)	*Maguire, B., 27351* (NY)	Venezuela	–	KU501013	–	–	–
Comolia lythrarioides Naudin (synonym)	*Michelangeli, F. M., 2201* (NY)	Suriname	KU501071	KU501014	KU501180	KU501124	KU500944
Comolia microphylla Benth.	*Redden, K. M., 1454* (NY)	Guyana	JQ730070	KF462841	JQ730281	KU501125	KU500945
Comolia montana Wurdack	*Huber, O., 8851* (US)	Venezuela	KU501078	KU501023	–	–	–
Comolia ovalifolia Triana	*Rocha, M. J. R., 719* (BHCB)	Brazil	KU501077	KU501020	–	–	–
Comolia sertularia (DC.) Triana	*Almeda, F., 7724* (CAS)	Brazil	JQ730071	KF462842	–	KU501122	KU500988
Comolia sessilis (Spreng.) Triana	*Rocha, M. J. R. 531* (BHCB)	Brazil	–	KU501011	KU501178	–	–
Comolia smithii Wurdack	*Jasen-Jacobs, M. J., 4461* (US)	Guyana	KU501075	KU501018	–	–	–
***Comolia* sp. nov.**	*Rocha, M. J. R., 984* (BHCB)	Brazil	KU501069	KU501010	–	–	–
Comolia stenodon (Naudin) Triana	*Rocha, M. J. R., 696* (BHCB)	Brazil	–	KU501008	–	–	–
Comolia vernicosa (Benth.) Triana	*Wurdack, K. J., 4181* (NY)	Guyana	JQ730072	KF462843	JQ730283	KU501149	KU500971
Comolia villosa (Aubl.) Triana var. *villosa*	*Harley, R. M., 29779* (US)	French Guyana	KU501074	KU501017	–	–	–
Comolia villosa (Aubl.)Triana	*Rocha, M. J. R., 739* (BHCB)	Brazil	KU501073	KU501019	–	KU501126	KU500946
Comolia villosa (Aubl.)Triana	*Michelangeli, F. A., 2200* (NY)	Suriname	KU501076	KU501016	KU501181	–	KU500943
Desmoscelis villosa (Aubl.) Naudin	*Zenteno, F., 8902* (NY)	Bolivia	JQ730073	KF462844	–	–	–
Ernestia adenotricha L.Uribe	*Uribe, L., 3662* (NY)	Colombia	KU501083	KU501026	KU501186	KU501136	–
Ernestia confertiflora Wurdack	*Penneys, D. S., 1913* (FLAS)	French Guyana	–	–	JQ730292	–	–
Ernestia cordifolia O.Berg ex Triana	*Groger, A., 975* (US)	Venezuela	–	KU501021	–	KU501127	KU500947
Ernestia ovata Cogn.	*Garcia-Barriga, H.,18006* (US)	Colombia	–	KU501028	–	KU501137	KU500958
Ernestia glandulosa Gleason	*Martin, C. V., 471* (NY)	French Guyana	JQ730080	KF462847	JQ730293	KU501128	KU500948
Ernestia pullei Gleason	*Martin, C. V., 460* (NY)	French Guyana	JQ730081	KF462848	JQ730294	KU501129	KU500950
Ernestia quadriseta O.Berg ex Triana	*Rimachi, M., 11642* (NY)	Peru	–	KU501029	KU501187	–	–
Ernestia maguirei Wurdack	*Michelangeli, F. A., 707* (NY)	Venezuela	KU501079	–	KU501182	–	–
Ernestia rubra Pulle	*Granville, J. J. de, 9722* (US)	French Guyana	KU501080	–	KU501183	–	KU500949
Ernestia tenella (Bonpl.) DC.	*Michelangeli, F. A., 386* (BH)	Venezuela	JQ730082	KU501027	JQ730295	–	KU500957
Fritzschia erecta Cham.	*Guimarães, P., 406* (RB)	Brazil	JQ730083	KF462849	JQ730296	–	–

Appendix . *Continued*

Species	Voucher (herbarium)	Provenance	nrITS	nrETS	accD-psaI	atpH-atpF	trnS-trnG
Fritzschia anisostemon Cham.	*Mota, N., 2694* (BHCB)	Brazil	KU501070	KU501012	KU501179	–	KU500942
Heterotis decumbens (P.Beauv.) Triana	*Smith, S., 1705* (US)	Brazil	JQ730088	KF462853	JQ730302	–	KU500985
Lavoisiera bicolor Naudin	*Guimaraes, P. J., 345* (RB)	Brazil	KF463033	KF462855	KF407958	KU501157	KU500981
Macairea lasiophylla (Benth.) Wurdack	*Rocha, M. J. R., 873* (BHCB)	Brazil	KU501081	KU501024	–	KU501134	KU500955
Macairea multinervia Benth.	*Rocha, M. J. R., 876* (BHCB)	Brazil	KU501082	KU501025	KU501185	KU501135	KU500956
Macairea pachyphylla Benth.	*Redden, K. M., 3869* (NY)	Guyana	JQ730094	KF462858	–	–	–
Macairea radula (Bonpl.) DC.	*Lima, J., 715* (UPCB)	Brazil	JQ730095	KF462859	JQ730307	KU501133	KU500954
Macairea thyrsiflora DC.	*Wurdack, J. J., 4153* (NY)	Guyana	JQ730096	KF462860	KU501184	KU501132	KU500953
Marcetia acerosa DC.	*Santos, A. K. A., 681* (UFB)	Brazil	JQ730097	–	–	–	–
Marcetia alba Ule.	*Goldenberg, R. 2085* (UPCB)	Brazil	KU501095	KU501040	KU501200	–	–
Marcetia bahiensis (Brade & Markgr.) Wurdack	*Rocha, M. J. R., 308* (BHCB)	Brazil	KU501086	–	KU501190	KU501140	KU500961
Marcetia candolleana A.K.A.Santos & A.B.Martins	*Bunger, M. O., 615* (BHCB)	Brazil	KU501085	KU501031	KU501189	KU501139	KU500960
Marcetia canescens Naudin	*Rocha, M. J. R., 295* (BHCB)	Brazil	KU501089	KU501034	KU501193	KU501143	KU500964
Marcetia eimeariana A.B.Martins & Woodgyer	*Santos, A. K. A., 832* (UFB)	Brazil	JQ730098	–	KU501194	–	–
Marcetia ericoides (Spreng.) O.Berg ex Cogn.	*Santos, A. K. A., 532* (UFB)	Brazil	JQ730099	–	–	–	–
Marcetia formosa Wurdack	*Rocha, M. J. R., 345* (BHCB)	Brazil	KU501090	KU501035	KU501195	KU501144	KU500965
Marcetia grandiflora Markgr.	*Rocha, M. J. R., 319* (BHCB)	Brazil	KU501091	KU501036	KU501196	KU501145	KU500966
Marcetia harleyi Wurdack	*Santos, A. K. A., 558* (UFB)	Brazil	JQ730100	–	–	–	–
Marcetia latifolia Naudin	*Santos, A.K.A., 336* (UFB)	Brazil	JQ730101	–	–	–	–
Marcetia macrophylla Wurdack	*Rocha, M. J. R., 297* (BHCB)	Brazil	KU501084	KU501030	KU501188	KU501138	KU500959
Marcetia mucugensis Wurdack	*Rocha, M. J. R., 285* (BHCB)	Brazil	KU501088	KU501033	KU501192	KU501142	KU500963
Marcetia sp1	*Rocha, M. J. R., 318* (BHCB)	Brazil	KU501093	KU501038	KU501198	KU501146	KU500967
Marcetia sp2	*Rocha, M. J. R., 335* (BHCB)	Brazil	KU501092	KU501037	KU501197	–	KU500968
Marcetia taxifolia (A.St.Hil.) DC.	*Michelangeli, F. A., 680* (BH)	Venezuela	JQ730102	KU501041	JQ730311	KU501148	KU500970
Marcetia viscida Wurdack	*Rocha, M. J. R., 334* (BHCB)	Brazil	KU501094	KU501039	KU501199	KU501147	KU500969
Marcetia velutina Markgr.	*Rocha, M. J. R., 293* (BHCB)	Brazil	KU501087	KU501032	KU501191	KU501141	KU500962
Microlicia fulva (Spreng.) Cham.	*Michelangeli, F. A., 1576* (UPCB)	Brazil	KU501105	KU501051	KU501205	KU501156	KU500980
Nepsera aquatica (Aubl.) Naudin	*Struwe, L., 1158* (NY)	Porto Rico	JQ730115	–	JQ730327	KU501131	KU500952
Pterogastra divaicata (Bonpl.) Naudin	*Michelangeli, F. A., 540* (BH)	Venezuela	JQ730126	KF462875	JQ730337	KU501159	–
Pterolepis glomerata (Rottb.) Miq.	*Martin, C. V., 419* (NY)	French Guyana	JQ730129	KF462876	JQ730340	KU501158	KU500982
Rhexia aristosa Britton	*Naczi, R. F. C., 12065* (NY)	USA	JQ730134	KF462878	–	–	KU500986
Sandemania hoehnei (Cogn.) Wurdack	*Goldenberg, R., 1007* (NY)	Brazil	JQ730141	KF462882	JQ730352	KU501150	KU500972
Siphanthera arenaria (DC.) Cogn.	*Mota, N., 2656* (BHCB)	Brazil	KU501099	KU501045	KU501203	–	KU500974

Appendix . *Continued*

Species	Voucher (herbarium)	Provenance	nrITS	nrETS	accD-psaI	atpH-atpF	trnS-trnG
Siphanthera cordata Pohl ex DC.	*Rocha, M. J. R., 964* (BHCB)	Brazil	KU501102	KU501048	KU501208 KU501212	–	KU500977
Siphanthera cordifolia (Benth.) Gleason	*Rocha, M. J. R., 882* (BHCB)	Brazil	KU501096	KU501042	KU501201	KU501151	KU500973
Siphanthera dawsonii Wurdack	*Versiane, A. F., 641* (HUFU)	Brazil	KU501103	KU501049	KU501207 KU501211	–	–
Siphanthera fasciculata (Gleason) Almeda & O.R.Rob.	*Wurdack, J. J., 1959* (NY)	Venezuela	KU501097	KU501043	–	–	–
Siphanthera foliosa (Naudin) Wurdack	*Rocha, M. J. R., 728* (BHCB)	Brazil	KU501098	KU501044	KU501202	KU501152	–
Siphanthera gracillima (Naudin) Wurdack	*Rocha, M. J. R., 941* (BHCB)	Brazil	KU501101	KU501047	KU501206 KU501210	KU501154	KU500976
Siphanthera hostmannii Cogn.	*Wurdack, K. J., 4142* (NY)	Guyana	JQ730142	KF462883	JQ730353	KU501155	KU500978
Siphanthera paludosa Cogn.	*Rocha, M. J. R., 621* (BHCB)	Brazil	KU501100	KU501046	KU501204	KU501153	KU500975
Siphanthera subtilis Pohl ex DC.	*Moreira, S. N., 878* (BHCB)	Brazil	KU501104	KU501050	KU501209 KU501213	–	KU500979
Tibouchina heteromalla (D.Don) Cogn.	*Guimarães, P., 339* (RB)	Brazil	JQ730193	KF462936	JQ730401	–	KU500983
Trembleya parviflora Cogn.	*Goldenberg, R., 824* (NY)	Brazil	JQ730242	KF462987	JQ730451	–	–