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EVOLUTIONARY TRENDS,  
MAJOR LINEAGES, AND NEW  
GENERIC LIMITS IN THE  
DIOECIOUS GROUP OF THE  
TRIBE VANGUERIEAE  
(RUBIACEAE): INSIGHTS INTO  
THE EVOLUTION OF  
FUNCTIONAL DIOECY<sup>1</sup>

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ABSTRACT

New generic circumscriptions of the Paleotropical tribe Vanguerieae in the subfamily Ixoroideae s.l. (Rubiaceae) have recently been established as a result of a series of phylogenetic studies conducted by Lantz and Bremer. The genus *Canthium* Lam. was shown in their study to be highly polyphyletic, and a largely dioecious group was for the first time identified within Vanguerieae. The dioecious group sensu Lantz and Bremer comprises about 140 species classified in eight genera: *Canthium* (*Canthium* subgenus), *Bullockia* Bridson, *Dinocanthium* Bremek., *Leroya* Cavaco, *Neoleroya* Cavaco, *Peponidium* (Baill.) Arènes, *Pseudopeponidium* Homolles ex Arènes, *Pyrostria* Comm. ex Juss., and *Scyphochlamys* Balf. f. We sequenced 79 Vanguerieae taxa and performed phylogenetic analyses based on sequence data from two nuclear (ETS and ITS) markers to: (1) pinpoint the phylogenetic positions of the Comorean and Indian Ocean *Canthium* and the Southeast Asian *Canthium confertum* Korth. group in Vanguerieae; (2) evaluate the phylogenetic utility of three taxonomic characters (bract type, locule number, and fruit shape) previously and currently used for delimiting genera in the dioecious group; and (3) assess the evolution of functional dioecy in Vanguerieae. The results support a further disintegration of *Canthium* s.l., as the Comorean and Malagasy *Canthium* species are shown for the first time to be closely related to *Peponidium*. Similarly, *C. confertum* appears to have a close affinity with *Cyclophyllum* Hook. f. The combined analyses show that the dioecious group can be subdivided into four morphologically distinct clades formally recognized as genera: *Bullockia* (Bridson) Razafim., Lantz & B. Bremer, here elevated from *Canthium* subgenus. *Bullockia* Bridson, as well as *Cyclophyllum*, *Peponidium* (including all Comorean, Malagasy, and Seychellean *Canthium* species), and *Pyrostria* (including *Dinocanthium*, *Leroya*, *Neoleroya*, *Pseudopeponidium*, and *Scyphochlamys*). All described species of *Canthium* subgenus. *Bullockia* are transferred to *Bullockia*: *B. dyscriton* (Bullock) Razafim., Lantz & B. Bremer, *B. fadenii* (Bridson) Razafim., Lantz & B. Bremer, *B. impressinervis* (Bridson) Razafim., Lantz & B. Bremer, *B. mombazensis* (Baill.) Razafim., Lantz & B. Bremer, *B. pseudosetiflora* (Bridson) Razafim., Lantz & B. Bremer, and *B. setiflora* (Hiern) Razafim., Lantz & B. Bremer. Furthermore, the results seem to point to a single origin of functional dioecy followed by subsequent reversals back to the hermaphroditic conditions at least within the *Cyclophyllum*–*Canthium confertum* clade and *Pyrostria* s.l. The Malagasy *Bullockia* species seem to have an African ancestor, whereas the Comorean *Peponidium* and the African *Pyrostria* appear to have originated from Malagasy progenitors.

**Key words:** Biogeography, *Bullockia*, *Canthium*, *Cyclophyllum*, ETS, functional dioecy, ITS, *Peponidium*, *Pyrostria*, Rubiaceae, Vanguerieae.

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The tribe Vanguerieae comprises between 600 and 700 species of trees, shrubs, geofrutices, and climbers, which are classified in 17 (Lantz & Bremer, 2004, 2005), 27 (Robbrecht, 1988), or 37 (Bridson,

unpublished talk) genera depending on the generic limits used. Vanguerieae belongs to the subfamily Ixoroideae s.l. (Bremer et al., 1999) of the coffee family (Rubiaceae) and is a monophyletic group,

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<sup>1</sup> We thank Aaron Davis (Royal Botanic Gardens, Kew, England), Thierry Pailler (Université de la Réunion, France), and Sylvie Andriambololoner (Missouri Botanical Garden Program, Madagascar) for kindly providing DNA material for this study; Ministère des Eaux et Forêts (MEF) and Association Nationale pour la Gestion des Aires Protégées (ANGAP) for issuing collecting permits for SGR; Missouri Botanical Garden Program, Madagascar (especially Lalao Andriamahafarivo) for arranging collecting permits for SGR; Désiré Ravelonarivo for being an excellent field assistant in Marojejy National Park; the following herbaria for providing loans and access to collections: BR, GB, Herbarium Universitaire de la Réunion, K, MO, P, S, TAN, TEF, and UPS; and Simon Owens (curator of K) for kindly sending us scanned images of the type specimens of five species of *Canthium* subgenus. *Bullockia*. This study was funded by the Bergius Foundation, the Swedish Research Council, and the Knut and Alice Wallenberg Foundation to BB.

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doi: 10.3417/2006191

which can be recognized by a combination of the following eight characters: absence of raphides, axillary inflorescences, valvate corolla-lobe aestivation, a unique type of pollen presenter, uniovulate locules, pendulous ovules attached toward the top of septa, drupaceous fruits, and large embryos with superior radicles (Bridson, 1987, 1992, 1998; Lantz et al., 2002). There is a considerable variation in both flower and fruit sizes and shapes, which attract various pollinators and a range of animal dispersers, respectively. The members of the tribe inhabit various habitats, ranging from evergreen rainforests to dry deciduous forests to xerophyllous thickets. They occupy a wide geographic range extending from tropical Africa and Asia, throughout the Indian Ocean islands, to the Pacific region and northern Australia. The African mainland is the center of diversity with ca. 350 species, and Southeast Asia and Madagascar have ca. 150 and 120 species, respectively. In Madagascar, at least 20 new species of *Peponidium* (Baill.) Arènes (Arènes, 1960) and *Pyrostria* Comm. ex Juss. (Jussieu, 1789) are yet to be described (Razafimandimbison et al., unpublished data).

While a member of Vanguerieae is relatively easy to recognize, the generic limits within the tribe have always been considered to be extremely problematic (e.g., Verdcourt, 1958; Bridson, 1987, 1992; Schatz, 2001). A series of recent phylogenetic studies based on both molecular and morphological data conducted by two of the authors (Lantz et al., 2002; Lantz & Bremer, 2004, 2005) led to the establishment of largely new generic circumscriptions of Vanguerieae. Both *Canthium* Lam. sensu Verdcourt and Bridson (1991) and Bridson (1992) and *Pyrostria* (Bridson, 1987) were among the genera shown to be highly polyphyletic. *Canthium* subgen. *Afrocanthium* Bridson was resolved as monophyletic and was more closely related to *Keetia* E. Phillips than it was to the other three subgenera of *Canthium* described by Bridson (1987, 1992): *Canthium* subgen. *Bullockia* Bridson, *Canthium* subgen. *Canthium*, and *Canthium* subgen. *Lycioserissa* (Roem. & Schultes) Bridson. As a result, Lantz and Bremer (2004) raised *Canthium* subgen. *Afrocanthium* to the generic level and restricted *Canthium* to include only species with paired supra-axillary spines. In addition, Lantz and Bremer's (2004) study identified for the first time a strongly supported and mostly dioecious clade (hereafter the dioecious group), which contained all sequenced species of *Canthium* subgen. *Bullockia*, *Dinocanthium* Bremek. (Bremekamp, 1933), *Leroya* Cavaco (Cavaco, 1970), *Neoleroya* Cavaco (Cavaco, 1971a), *Peponidium*, *Pseudopeponidium* Homolles ex Arènes (Arènes, 1960), *Pyrostria*, and *Scyphochlamys* Balf. f. (Balfour, 1877; Verdcourt, 1983). The genus *Cyclo-*

*phyllum* Hook. f. (Hooker, 1873) was, however, erroneously included as part of the dioecious group in Lantz and Bremer's (2004) figures 1 and 5. In total, the dioecious group sensu Lantz and Bremer (2004) comprises at least 22% (ca. 140 species) of the total species of Vanguerieae and has its center of species diversity in Madagascar with ca. 71% (at least 100 species [Govaerts et al., 2006; Davis et al., 2007; Lantz et al., 2007; Razafimandimbison et al., 2007]). Mainland Africa has ca. 14% (ca. 19 species [Bridson, 1987]), and the Comoro, Mascarene, and Seychelles islands together have ca. 9% (ca. 13 species [Verdcourt, 1983; Friedmann, 1994; Mouly, 2007]). The number of the species of the Southeast Asian *Pyrostria* group B (sensu Bridson, 1987) is currently unknown. The generic limits within the dioecious group were not addressed in Lantz and Bremer (2004) mainly due to lack of internal phylogenetic support and limited sampling of *Pyrostria* and its allied genera. The establishment of the new narrowly circumscribed *Canthium* by Lantz and Bremer (2004) has left *Canthium* subgen. *Bullockia* and the two groups of *Canthium* species (one consisting of the Indian Ocean and Comorean *Canthium* species corresponding to the two-locular species of group V [sensu Bridson, 1987] and the other consisting of the Southeast Asian *C. confertum* Korth. group or group IV [sensu Bridson, 1987]) unplaced within Vanguerieae.

The circumscriptions of *Pyrostria* have always been controversial and unsettled (see Table 1), and there are at least two reasons for this situation. First, the circumscriptions of some of the closely related genera (e.g., *Dinocanthium* [Bremekamp, 1933]; *Pseudopeponidium* [Arènes, 1960]; Table 2) largely overlapped with the early narrow circumscriptions of *Pyrostria* (Jussieu, 1789; Cavaco, 1967) based on the combination of three characters, i.e., presence or absence of persistent, basally fused, and long acuminate paired bracts completely enclosing the young inflorescence, type of sexual systems, and number of locules per ovary (see Table 1). Second, previous authors proposed conflicting morphological concepts of *Pyrostria* as a result of the application of different weightings to the above characters (Tables 1, 2) used in combination (Jussieu, 1789; Cavaco, 1967) or as "cardinals" (Capuron, 1969; Bridson, 1987). The monophyly of these competing circumscriptions of *Pyrostria* has never been tested before using molecular phylogenies. In addition, Capuron (1969) even questioned the identity of *Pyrostria* and viewed the Malagasy *Canthium*, almost all erroneously described as hermaphrodite by Cavaco (1972), as dioecious. He intended to group all other dioecious Malagasy Vanguerieae genera (*Peponidium*, *Pseudopeponidium*,

Table 1. Previous and new circumscriptions of *Pyrostria*.

Authors	Characters used for circumscribing <i>Pyrostria</i>	Variable characters	Genera included in <i>Pyrostria</i>	Genera not included in <i>Pyrostria</i>
Jussieu (1789)	plurilocular ovary, paired bracts, dioecy	—	—	—
Cavaco (1967) <sup>1</sup>	two-locular ovary, paired bracts, dioecy	—	—	<i>Peponidium</i> and <i>Pseudopeponidium</i>
Capuron (1969) <sup>1</sup>	functional dioecy	two to plurilocular ovary, with or without paired bracts	<i>Peponidium</i> and <i>Pseudopeponidium</i>	—
Cavaco (1972) <sup>1</sup>	functional dioecy	two to plurilocular ovary, with or without paired bracts	<i>Peponidium</i> and <i>Pseudopeponidium</i>	<i>Leroya</i> and <i>Neoleroya</i>
Bridson (1987)	presence of persistent and basally connate paired bracts	two to plurilocular ovary, sexual systems (functionally dioecious or hermaphrodite)	<i>Dinocanthium</i> , <i>Leroya</i> , <i>Neoleroya</i> , and <i>Pseudopeponidium</i>	<i>Cyclophyllum</i> , <i>Peponidium</i> , and <i>Scyphochlamys</i>
Schatz (2001) <sup>1</sup>	functional dioecy	two to plurilocular ovary, with or without paired bracts	<i>Leroya</i> , <i>Neoleroya</i> , <i>Peponidium</i> , and <i>Pseudopeponidium</i>	—
Razafimandimbison et al. (this study)	presence of persistent and basally connate paired bracts	two to plurilocular ovary breeding systems (functionally dioecious or hermaphrodite)	<i>Dinocanthium</i> , <i>Leroya</i> , <i>Neoleroya</i> , <i>Pseudopeponidium</i> , and <i>Scyphochlamys</i>	<i>Cyclophyllum</i> and <i>Peponidium</i>

<sup>1</sup> Taxa from Madagascar and the Comoro Islands are considered herein.

and *Pyrostria*) under *Canthium* based on accumbent (as “accompanants” [Capuron, 1969]) cotyledons (cotyledons lying against the radicle along one edge [Harris & Harris, 1994]). Leroy (1972: 1683), seemingly influenced by Capuron (1969), used the term “*Canthium–Pyrostria sensu lato*” for the Malagasy

species (including both *Leroya* and *Neoleroya*) with the typical paired bracts of *Pyrostria*. Schatz (2001: 339) also recognized the Malagasy *Canthium* as dioecious but treated them as *Canthium* and maintained the generic status of *Pyrostria* (including *Leroya*, *Neoleroya*, *Peponidium*, and *Pseudopeponi-*

Table 2. Comparison between all described genera of the dioecious group sensu Lantz and Bremer (2004), *Cyclophyllum*, the Comorean and Indian Ocean *Canthium*, and the Southeast Asian *Canthium confertum* group.

Taxa	No. of locules per ovary <sup>1</sup>	Type of inflorescence bracts <sup>1</sup>	Type of sexual systems <sup>1</sup>
<i>Canthium</i> (Comoro Islands and Indian Ocean)	2	cupular bracts	functional dioecy
<i>Canthium confertum</i> group	2 to 6	without bracts	hermaphroditism
<i>Canthium</i> subgen. <i>Bullockia</i>	2	without bracts	functional dioecy
<i>Cyclophyllum</i>	2	variable	hermaphroditism and functional dioecy
<i>Dinocanthium</i> <sup>2</sup>	4 to 6	paired bracts	functional dioecy
<i>Leroya</i> <sup>2,3</sup>	4 to 6	paired bracts	functional dioecy
<i>Neoleroya</i> <sup>2,3</sup>	2	paired bracts	functional dioecy
<i>Peponidium</i> <sup>3,4</sup>	2 to 10	cupular bracts	functional dioecy
<i>Pseudopeponidium</i> <sup>2,3,4</sup>	2 to 10	paired bracts	functional dioecy
<i>Pyrostria sensu Cavaco (1967)</i> <sup>1</sup>	2	paired bracts	functional dioecy
<i>Pyrostria sensu Jussieu (1789)</i> <sup>1</sup>	8	paired bracts	functional dioecy
<i>Scyphochlamys</i>	4 to 6	spathe-like bracts	functional dioecy

<sup>1</sup> Characters traditionally and currently used in combination or as cardinals for delimiting *Pyrostria*.

<sup>2</sup> Genera merged by Bridson (1987) with *Pyrostria*.

<sup>3</sup> Genera merged by Schatz (2001) with *Pyrostria*.

<sup>4</sup> Genera considered by Capuron (1969) as merged with *Canthium* based on the presence of accumbent cotyledons.

Table 3. List of the known dioecious Rubiaceae.

Subfamilies (sensu Bremer et al., 1999)	Tribes	Taxa	References
Cinchonoideae	Guettardeae	<i>Antirhea borbonica</i> J. F. Gmel. <sup>1,2</sup>	Litrico et al. (2005)
		<i>Bobeia</i> Gaudich.	Achille et al. (2006)
		<i>Guettarda</i> L. <sup>2</sup>	Achille et al. (2006)
		<i>Tinadendron</i> Achille	Achille (2006)
Ixoroideae s.l.	Bertiereae	<i>Bertiera borbonica</i> A. Rich. <sup>1,2</sup>	Pailler et al. (1998)
	Coffeae	<i>Tricalysia</i> A. Rich. <sup>2</sup>	Robbrecht (1979)
	Condamineae	<i>Diocodendron</i> Steyerem.	Delprete (1999)
		<i>Dolicholobium</i> A. Gray <sup>2</sup>	Skottsberg (1944)
	Gardenieae	<i>Agouticarpa</i> C. H. Perss.	Persson (2003)
		<i>Alibertia</i> A. Rich. group	Persson (2000)
		<i>Atractocarpus</i> Schltr. & K. Krause <sup>2</sup>	Puttock (1999)
		<i>Casasia</i> A. Rich.	Robbrecht (1988)
		<i>Gardenia actinocarpa</i> Puttock <sup>2</sup>	Osunkoya (2003)
		<i>Melanopsidium</i> Colla	Delprete (2000)
		<i>Randia</i> L.	Gustafsson & Persson (2002)
	Ixoreae	<i>Trukia</i> Kanehira	Smith & Darwin (1988)
		<i>Doricera</i> Verdc.	Verdcourt (1983)
	Mussaendeae	<i>Ixora pudica</i> Baker <sup>1,2</sup>	Friedmann (1994)
		<i>Mussaenda parviflora</i> Miq. <sup>1,2</sup>	Burck (1883); Baker (1958); Naiki & Kato (1999)
	Octotropideae	<i>Kraussia</i> Harv.	Skottsberg (1944)
	Vanguerieae	<i>Bullockia</i> (Bridson) Razafim., Lantz & B. Bremer <sup>1</sup> (this study)	Bridson (1987); this study
		<i>Canthium laeve</i> Teijsm. & Binn. <sup>1,2</sup>	Burck (1884)
		<i>Cyclophyllum</i> Hook. f. <sup>1,2</sup>	Mouly et al. (2007)
		<i>Peponidium</i> (Baill.) Arènes s.l. <sup>1</sup> (this study)	Arènes (1960); Bridson (1987); this study
<i>Pyrostria</i> Comm. ex Juss. s.l. <sup>1</sup> (this study)		Bridson (1987); Verdcourt (1983)	
<i>Pyrostria</i> (Bridson) Razafim., Lantz & B. Bremer <sup>1</sup> (this study)		Bridson (1987); Verdcourt (1983)	
Rubioidae	Anthospermeae	<i>Coprosma billardieri</i> Hook. f. <sup>2</sup>	Skottsberg (1922)
		<i>Normandia</i> Hook. f.	Guillaumin (1930)
	Coussareae	<i>Coussarea latifolia</i> Standl. <sup>2</sup>	Burger & Taylor (1993)
		<i>Coussarea talamancana</i> Standl. <sup>2</sup>	Beach & Bawa (1980)
	Gaertnereae	<i>Gaertnera</i> Lam. <sup>2</sup>	van Beusekom (1967)
	Morindeae s. str.	<i>Morinda</i> L. <sup>2</sup>	Burck (1883); Wong (1984)
	Psychotriaceae	<i>Chassalia corallioides</i> (Cordem.) Verdc. <sup>1,2</sup>	Pailler et al. (1995)
		<i>Psychotria officinalis</i> (Aubl.) Raeusch. ex Sandwith <sup>2</sup>	Hamilton (1990)
		<i>Gouldia</i> A. Gray <sup>2</sup>	Burck (1884)
	Spermacoceae	<i>Hedyotis</i> L. <sup>2</sup>	Baker & Cox (1984)
<i>Kadua</i> Cham. & Schldtl. <sup>2</sup>		Wagner & Lorence (1998)	
<i>Nesohedyotis arborea</i> (Roxb.) Bremek.		Percy & Cronk (1997)	

<sup>1</sup> Taxa reported to be functionally dioecious.<sup>2</sup> Genera known to have some or many hermaphroditic species.

*dium*) (see Table 1). No species of the Indian Ocean or Comorean *Canthium* were included in Lantz and Bremer (2004, 2005).

Breeding systems have been shown to have evolved independently numerous times throughout angiosperms (e.g., Renner & Ricklefs, 1995; Sakai et al., 1995; Weiblen et al., 2000). As in most flowering plant families, hermaphroditism is the most common

breeding system in Rubiaceae. Monoecy, separate male and female flowers on each plant (Heilbuth, 2000), has been recorded at least in the monogeneric tribe Theligoneae (Robbrecht, 1988), presently classified in Rubioidae (Bremer & Manen, 2000). Dioecy, male or female flowers on separate individuals of the same species, has been reported in many genera in all three subfamilies (sensu Bremer et al.,

1999) of Rubiaceae (see Table 3). In the functionally dioecious Vanguerieae species, unisexual flowers are morphologically hermaphrodite but functionally dioecious; the functionally female flowers produce functional ovaries and stigmatic lobes but sterile anthers, whereas the functionally male flowers bear reduced or nonfunctional ovaries and stigmatic lobes but fertile anthers. The two forms are usually easy to distinguish because the functionally female plants have flowers with well-developed ovaries and one- to few-flowered inflorescences, rather than small ovaries and many-flowered inflorescences in the functional male plants (Bridson, 1987). In Vanguerieae, functional dioecy (also known as cryptic dioecy [Mayer & Charlesworth, 1991]) is commonly found in *Pyrostria* and its allied genera (Bridson, 1987) and was once reported from the Asian *Canthium laeve* Teijsm. & Binn. (Burck, 1884). The phylogenetic placement of *C. laeve* is currently unknown, and therefore we do not know whether the species represents a separate origin of functional dioecy in the tribe or belongs to the dioecious group. Two African and one Afro-Comorean-Indian Ocean *Pyrostria* species have been reported to be hermaphroditic (Bridson, 1987). In addition, gynodioecy, a regular coexistence of hermaphrodite and female flowers on the same individual (Mayer & Charlesworth, 1991), was reported from *Psydrax odorata* (G. Forst.) A. C. Sm. & S. P. Darwin (as *Canthium odoratum* (G. Forst.) Seem. [Skottsberg, 1945]) and from *Afrocanthium gilfillanii* (N. E. Br.) Lantz (Bridson, 1998: 324) and *A. mundianum* (Cham. & Schltdl.) Lantz (Balkwill et al., 1996).

The ITS of the nuclear ribosomal DNA (nrDNA) have been shown to be useful for assessing phylogenetic relationships at the generic level in some rubiaceae groups (e.g., Gardenieae [Persson, 2000]; Naucleaeae s.l. [Razafimandimbison & Bremer, 2001, 2002]; Vanguerieae [Lantz et al., 2002]; Mussaendeae [Alejandro et al., 2005]). The ETS of the nrDNA, in combination with ITS data, have also recently been used in Rubiaceae for assessing phylogenetic relationships of young lineages (e.g., Nepokroeff et al., 2003; Razafimandimbison et al., 2005). The main objective of this study is to reconstruct a robust phylogeny of the dioecious group using sequencing data from two nuclear markers, ETS and ITS regions. The resulting phylogeny will then be used to: (1) pinpoint the phylogenetic positions of the Comorean and Indian Ocean *Canthium* and the Southeast Asian *C. confertum* group in Vanguerieae; (2) evaluate the phylogenetic utility of three taxonomic characters (bract type, locule number, and fruit shape) previously and currently used for delimiting genera in the dioecious group (Table 1); and (3) assess the evolution of functional dioecy in Vanguerieae.

## MATERIAL AND METHODS

### TAXON SAMPLING

We tried to sequence as many representatives as possible of all formally described genera in the dioecious group (see Appendix 1) and the Comorean and Malagasy *Canthium*. We were unable to obtain any sequenceable material of *Pyrostria* group B (Bridson, 1987). As there are currently no available identification keys for the Malagasy *Canthium* and *Pyrostria*, all determinations of the studied collections were made by comparisons to the type specimens received on loan. In total, we sequenced 79 taxa of Vanguerieae, of which 67 taxa were from the dioecious group. Twenty-three of the 67 taxa were new undescribed species of the Malagasy *Canthium*, *Leroya*, *Peponidium*, and *Pyrostria* (see Appendix 1). All formally published generic names in the dioecious group are used in the trees (Figs. 1–3), although we are aware that no single author accepts all of them.

### DNA EXTRACTION, AMPLIFICATION, AND SEQUENCING

The total DNA, extracted from leaves dried in silica gel (Chase & Hills, 1991) and/or herbarium material, was isolated following the mini-prep procedure of Saghai-Marooif et al. (1984), as modified by Doyle and Doyle (1987). We amplified and sequenced parts of the ETS region of all investigated taxa with two primers, 18S-E (5'-GCAGGATCAACCAGGTGAA-3'), designed by Baldwin and Markos (1998) and situated at the 5' border of 18S and ETS, and ETS-HL (5'-GATCACAGCCTGAGC GGTG-3'), designed by H. Lantz and located at the 3' border of ETS, following the protocols described in Razafimandimbison et al. (2005). The entire ITS region (including the 5.8S gene) of all newly studied taxa was amplified and sequenced using the protocols outlined in Razafimandimbison et al. (2004). In addition, we amplified the ETS and ITS regions of the DNA templates of both *Peponidium* sp. indet. 6 and *Pseudopeponidium oleifolium* Arènes using the 97°C/ribosomal RNA primers described in Razafimandimbison et al. (2004). Direct sequencing of their purified PCR products consistently produced multiple sequence signals for both markers, indicating the presence of intraindividual polymorphism. As a result, the PCR products of both taxa were cloned according to the TOPO TA cloning kit (Invitrogen, Paisley, Scotland) (see Razafimandimbison et al., 2004). Four white colonies from both the ETS and ITS cloning reactions were screened and amplified with two universal primers, T7 (5'-AAT ACG CTC ACT ATA G-3') and M13R (5'-CAG GAA ACA GCT ATG AC-3'), which were included in the TOPO TA cloning kit. Their

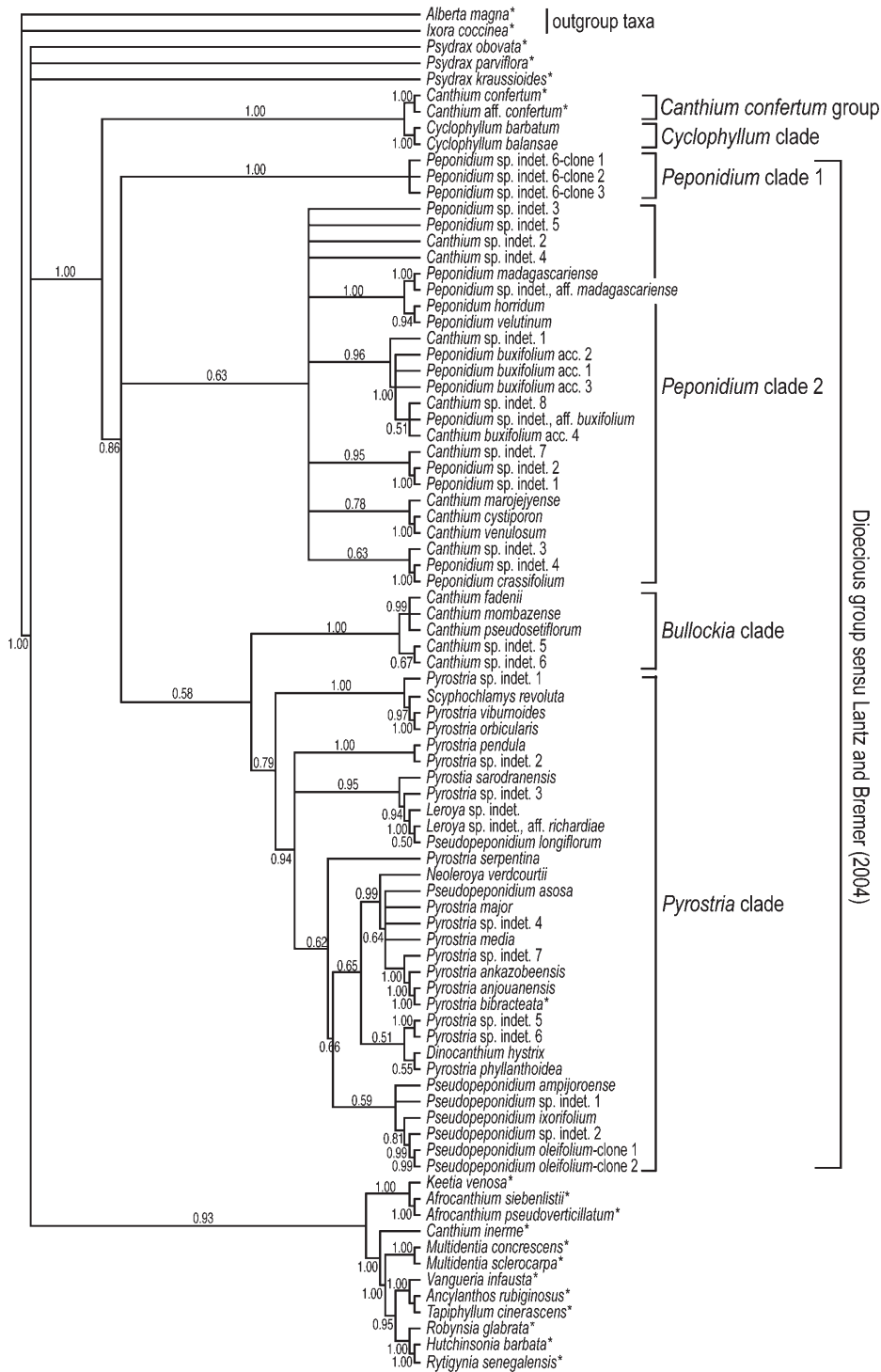


Figure 1. Fifty percent Bayesian majority rule consensus ITS tree under the GTR + I + G model of substitution from a 2-million MCMC generation analysis, showing mean branch lengths. Numbers on internodes indicate PPs. A vertical bar delimits outgroup taxa. Brackets correspond to the major clades and the dioecious group. \*Denotes hermaphroditic taxa.

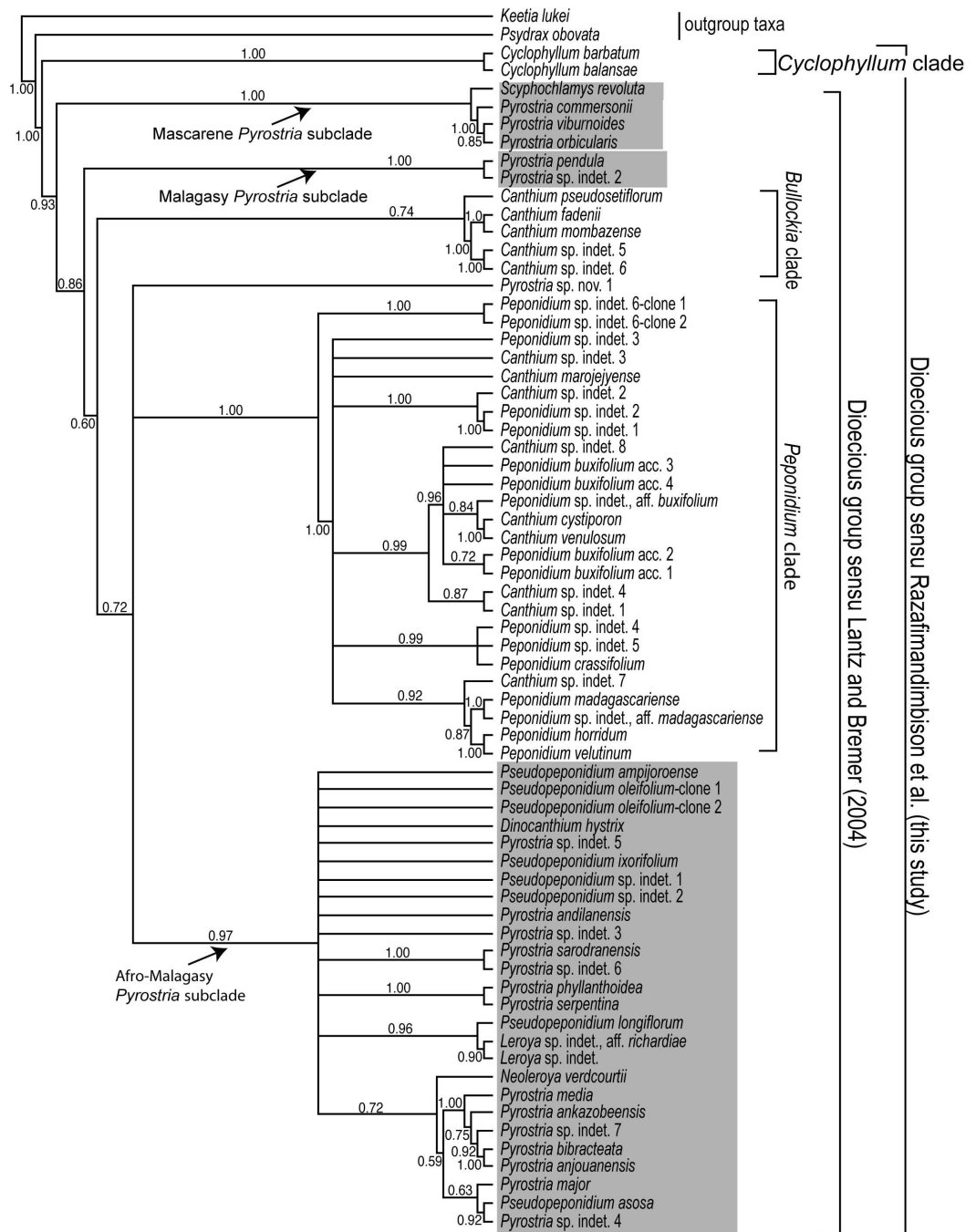


Figure 2. Fifty percent Bayesian majority rule consensus ETS tree under the GTR + G model of substitution from a 2-million MCMC generation analysis, showing mean branch lengths. Numbers on internodes indicate PPs. Taxa highlighted in shaded boxes are subclades belonging to the *Pyrostria* group. The vertical bar delimits outgroup taxa. Brackets correspond to the major clades.

respective purified PCR products were sequenced with the 18S-E/ETS-HL and P17/26.82R (Popp & Oxelman, 2001). In all PCRs, one reaction was run with water instead of DNA as a negative control to check for

contamination. All sequencing reactions were performed using the Big Dye Terminator v3.1 Cycle Sequencing kit and Big Dye Terminator v1.1 Cycle Sequencing kit (Applied Biosystems, Stockholm,

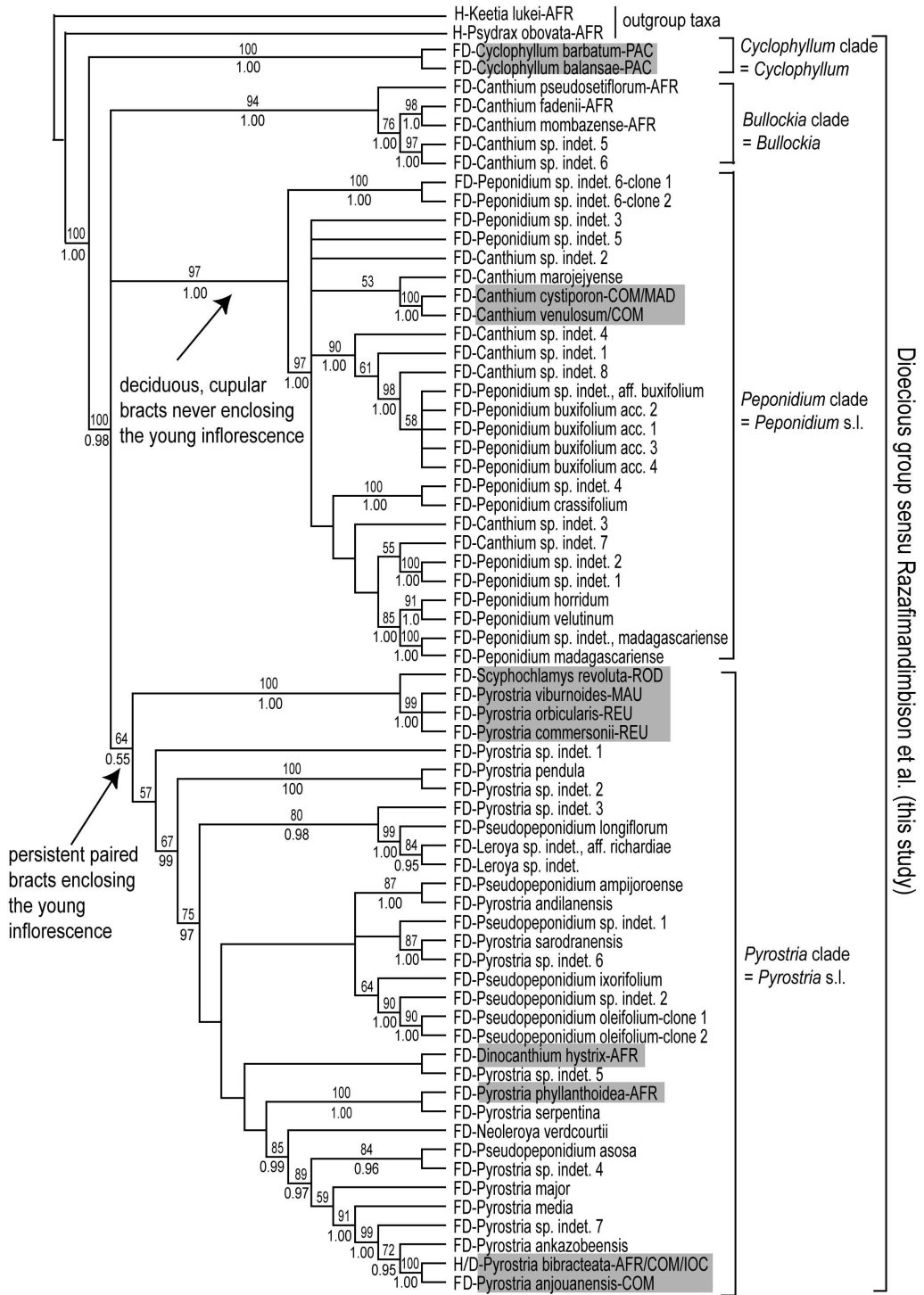


Figure 3. Parsimony strict consensus tree of 1555 MPTs of the combined ITS/ETS data (L = 822; CI = 0.421; RI = 0.735) with the new classification of the dioecious group implemented. Numbers above internodes are JK support (> 50%), and those below internodes correspond to PP values from a Bayesian analysis of the same data sets. The vertical bar delimits outgroup taxa, and brackets denote the major clades. Abbreviations preceding plant names indicate functionally dioecious (FD) taxa and hermaphroditic (H) taxa. Abbreviations after taxa names indicate taxa distribution: AFR = African mainland; COM = Comoro Islands; IOC = Indian Ocean; MAD = Madagascar; MAU = Mauritius; REU = Réunion Island; and ROD = Rodrigues Island; the remaining ingroup taxa are endemic to Madagascar.



Sweden) and subsequently analyzed with the 3100 Genetic Analyzer (Applied Biosystems).

#### DATA ANALYSES

The ETS and ITS sequences were assembled using the Staden Package version 1.6.0 beta-test (Staden, 1996) and aligned using ClustalX (Thompson et al., 1997) to produce an initial alignment and manually aligned using Se-Al (Rambaut, 1996). All newly published sequences have been submitted to the European Molecular Biology Laboratory (EMBL). We initially performed a Bayesian analysis of the ITS data with the computer program MrBayes 3.0b (Huelsenbeck & Ronquist, 2001). The best-fit model of nucleotide substitution (GTR + I + G [Yang, 1994]) was selected using the computer program MrModeltest 2.0 (Nylander, 2004) and Akaike's information criterion (Akaike, 1974). The Bayesian ITS analysis (excluding indels) was conducted with four independent Markov chains run for  $2 \times 10^6$  Metropolis-coupled Markov chain Monte Carlo (MCMC) generations, with tree sampling every  $1 \times 10^3$  generations and burn-in after  $1 \times 10^3$  trees (as detected by plotting the log likelihood scores against generation number). The analyses were repeated four times using different random starting trees to evaluate the convergence of the likelihood values and posterior probabilities (PPs). All saved trees from the four independent runs were pooled for a consensus tree. Groups characterized by PPs more than 95% were regarded as strongly supported.

Based on the results of the Bayesian ITS analysis, we selected two closer outgroup taxa (*Psydrax obovata* (Klotzsch ex Eckl. & Zeyh.) Bridson and *Keetia lukei* Bridson) from within Vanguerieae to root the analyses of the ETS and combined ETS/ITS data from all targeted taxa of the dioecious group and both the Comorean and Malagasy *Canthium*. We subsequently carried out Bayesian analyses of the ETS and the combined ETS/ITS data sets using the same settings as above but with the GTR + G model of nucleotide substitution, selected as the best-fit model for the two ETS and ITS data sets. We performed parsimony analyses of the four data sets (excluding uninformative characters) with PAUP\* version 4.0b (Swofford, 2000), using heuristic searches, with the MULTREES option on, tree bisection-reconnection (TBR) branch swapping, swap on best tree only in effect, and 5000 random addition sequences. In all analyses, characters were given equal weight, gaps were treated as missing data, and phylogenetically informative indels were coded following the simple gap coding method of Simmons and Ochoterena (2000). The consistency index (CI; Kluge & Farris, 1969) and retention index

(RI; Farris, 1989) were calculated to estimate homoplasy. Jackknife (JK; Farris et al., 1995) values were computed using heuristic searches, with MULTREES on, TBR branch swapping, five random additions, and 5000 replicates to assess relative support of the retained clades.

We statistically evaluated the congruence of the ETS and ITS data sets using the incongruence length difference test (ILD test; Farris et al., 1995), as implemented in PAUP\* (Swofford, 2000). The heuristic search was set to 500 replicates with 10 random addition sequence and nearest-neighbor interchange (NNI) branch swapping. If the probability of obtaining a smaller sum of tree lengths from the randomly generated data sets is lower ( $P < 0.05$ ) than that of the original data sets, the null hypothesis that the two data sets are homogenous is rejected and they are interpreted as incongruent (Farris et al., 1995).

#### RESULTS

##### ITS ANALYSES

A total of 84 ITS sequences were analyzed and 58 (ca. 69%) are newly published here. The ITS matrix contained 776 positions and 189 (24.35%) were parsimony informative. Both the parsimony strict consensus (length [L] = 790, CI = 0.439, RI = 0.727; result not shown) and Bayesian ITS trees had similar overall tree topologies, which were not in conflict with those of Lantz and Bremer (2004). In the Bayesian majority rule consensus tree pooled from the Bayesian trees (burn-ins excluded) from the four independent runs shown in Figure 1, all sequenced taxa of the dioecious group sensu Lantz and Bremer (2004) formed a moderately supported monophyletic group (PP = 0.86), which was resolved as sister (PP = 1.00) to a clade containing the two studied species of *Cyclophyllum* and two accessions of *Canthium confertum* (PP = 1.00). The three hermaphroditic *Psydrax* species were unresolved, and the hermaphroditic 12 remaining Vanguerieae taxa formed a moderately supported clade (PP = 0.93). The members of the dioecious group sensu Lantz and Bremer (2004) were resolved in four clades: the *Peponidium* clade 1 (consisting of the three clonal accessions of *Peponidium* sp. indet. 6; PP = 1.00); the *Peponidium* clade 2 (including the remaining sampled *Peponidium* species and all sequenced Comorean and Malagasy *Canthium* species; PP = 0.63); the *Bullockia* clade (including three species of *Canthium* subgen. *Bullockia* and two undescribed new Malagasy species; PP = 1.00); and the *Pyrostria* clade (including all *Pyrostria*, *Scyphochlamys*, *Leroya*, *Neoleroya*, *Dinoanthium*, and *Pseudopeponidium* species; PP =

0.79). The relationships between the four dioecious clades were largely unresolved.

#### ETS ANALYSES

A total of 68 ETS sequences were analyzed together and all are newly published here. The aligned ETS matrix contained 421 positions, and 130 (30.8%), including coded indels, were phylogenetically informative. In the Bayesian ETS tree shown in Figure 2, the *Cyclophyllum* clade was resolved with high support (PP = 1.00) as sister to the moderately supported (PP = 0.93) dioecious group sensu Lantz and Bremer (2004). Within the dioecious group, all deep nodes received only poor (PP = 0.60) to moderate (PP = 0.86) support (Fig. 2). The *Pyrostria* clade, weakly supported in the ITS tree (Fig. 1), collapsed in three highly supported subclades (PP = 0.97–1.00): a Mascarene *Pyrostria* subclade; a subclade containing two Malagasy *Pyrostria* species, *P. pendula* Lantz, Klack. & Razafim. and *Pyrostria* sp. indet. 2; and an Afro-Malagasy *Pyrostria* subclade (including all studied species of *Dinocanthium*, *Leroya*, *Neoleroya*, and *Pseudopeponidium*). The *Peponidium* clades 1 and 2 in the ITS tree (Fig. 1) formed a well-supported monophyletic group (hereafter called the *Peponidium* clade; PP = 1.00). *Pyrostria* sp. indet. 1, the *Peponidium* clade, and the Afro-Malagasy *Pyrostria* subclade were unresolved in a weakly supported clade (PP = 0.72). Two ETS clonal sequences of *Peponidium* sp. indet. 6 formed a highly supported clade (PP = 1.00), which in turn was sister to a well-supported clade corresponding to the *Peponidium* clade in Figure 1. The *Cyclophyllum* clade received high support (PP = 1.00), while the *Bullockia* clade had only weak support (PP = 0.74).

A parsimony analysis of the ETS matrix resulted in 52 equally most parsimonious trees (MPTs; L = 409, CI = 0.430, RI = 0.762). In the strict consensus ETS tree generated from the 52 MPTs (result not shown), the *Pyrostria* clade was resolved in two separate clades: the strongly supported (PP = 1.00) *Mascarene-Pyrostria* clade and the poorly supported (PP < 0.50) Afro-Malagasy *Pyrostria* clade (including *Dinocanthium*, *Leroya*, *Neoleroya*, *Pseudopeponidium*, *Pyrostria* sp. indet. 1, *Pyrostria* sp. indet. 2, and *Pyrostria pendula*). The *Bullockia*, *Cyclophyllum*, and *Peponidium* clades all received the same levels of support as in the Bayesian ETS tree (Fig. 2).

#### COMBINED ANALYSES

The results of the ILD test indicated that the two data sets were not significantly incongruent and therefore

could be combined. Fusion of the ETS and ITS data sets of 68 taxa in one matrix yielded 1122 positions and 238 parsimony-informative characters (including coded indels). Both Bayesian and parsimony analyses of the combined ETS/ITS data produced identical tree topologies. A parsimony analysis of the combined ETS/ITS data resulted in 1555 equally MPTs (L = 822, CI = 0.421, RI = 0.735). In the strict consensus tree shown in Figure 3, the ingroup taxa were resolved in four major clades (Fig. 3): the *Cyclophyllum* clade (= *Cyclophyllum*), the *Bullockia* clade (= *Bullockia* (Bridson) Razafim., Lantz & B. Bremer), and the *Peponidium* clade (= *Peponidium* s.l.), all with high support (JK = 94–100, PP = 1.00), and the *Pyrostria* clade (= *Pyrostria* s.l.; JK = 55, PP = 0.64). The highly supported Mascarene subclade (JK = 100, PP = 1.00) was resolved as sister to the poorly supported (JK = 57) Afro-Malagasy *Pyrostria* subclade. The position of the *Cyclophyllum* clade as sister to the strongly supported (JK = 100, PP = 1.00) dioecious group sensu Lantz and Bremer (2004) was further corroborated. However, the phylogenetic relationships between the *Bullockia*, *Peponidium*, and *Pyrostria* clades remained unresolved. Both *Pseudopeponidium* and *Pyrostria* were shown to be paraphyletic or polyphyletic, while *Leroya*, represented by *Leroya* sp. indet., aff. *richardiae*, and *Leroya* sp. indet., formed a highly monophyletic group. Finally, both *Leroya* and *Neoleroya* were deeply nested within the *Pyrostria* clade.

#### DISCUSSION

##### FURTHER DISINTEGRATION OF *CANTHIUM* S.L.

The Bayesian ITS analysis (Fig. 1) reveals a further disintegration of *Canthium* s.l., as the Southeast Asian *C. confertum* (currently placed in group IV sensu Bridson [1987] and represented here by two accessions of *C. confertum*) is for the first time shown to be closely related to *Cyclophyllum*. However, this does not necessarily apply to the whole group IV (sensu Bridson, 1987), as its monophyly has never been tested before. In ETS and combined ETS/ITS trees (Figs. 2, 3), the sequenced Comorean and Indian Ocean *Canthium* species form a highly supported clade with the sampled *Peponidium* (JK = 97, PP = 1.00). In addition, all our analyses (Figs. 1–3) further corroborate the phylogenetic position of *Canthium* subgen. *Bullockia* in the dioecious group previously demonstrated by Lantz and Bremer (2004).

##### EVOLUTIONARY TRENDS IN THE DIOECIOUS GROUP ACCORDING TO THIS STUDY

Phylogenetic reconstructions can help us understand how morphological variation found within a

monophyletic group has evolved and also highlight synapomorphies for group recognition.

#### *Bract type*

The combined ETS/ITS tree (Fig. 3) points to single origins of both the persistent, basally connate paired bracts of the *Pyrostria* clade (consistent with Lantz & Bremer, 2004) and the deciduous, small cupular bracts of the *Peponidium* clade within the dioecious group (see Fig. 3, Table 2). We consider these characters to be the potential morphological synapomorphies for the two clades. In *Pyrostria*, the connate paired bracts completely enclose the young inflorescences and are located either at the base or at the apex of the peduncles. Many New Caledonian species of *Cyclophyllum* are known to have small and connate paired bracts that never enclose the young inflorescence, but the typical bracts of *Pyrostria* are reported from the New Caledonian *C. calyculatum* Guillaumin (Guillaumin, 1930). We suspect that this species belongs to *Pyrostria*. The *Bullockia* clade and *Canthium confertum* group have no bracts.

#### *Locule number*

The number of locules per ovary, previously used by Jussieu (1789) and Cavaco (1967), in combination with two other characters (e.g., type of breeding systems) for delimiting *Pyrostria* in a narrow sense, is clearly shown to have evolved independently numerous times (i.e., are homoplasious) within the dioecious group (see also Table 2) and in the other parts of Vanguerieae; this is also congruent with Lantz and Bremer (2004).

#### *Ovary/fruit shape*

Leroy (1972: table 1), as well as field observations and herbarium studies conducted by two of the authors (SGR and HL), demonstrate a great and continuous variation of both ovary and fruit shapes and sizes in the *Pyrostria* clade. Cavaco (1970, 1971a) appears to have overlooked or was unaware of the presence of many *Pyrostria* species with intermediate ovary and fruit shapes (Leroy, 1972) between those of *Leroya* and *Neoleroya*. The phylogenetic positions of both *Leroya* and *Neoleroya* deeply nested inside the *Pyrostria* clade are congruent with our field observations and herbarium studies. In addition, the same degree of variation is also found in the *Cyclophyllum* and *Peponidium* clades, further indicating that both fruit shape and size cannot be used alone for recognizing genera in the dioecious

group. The members of the *Bullockia* clade have two-locular and heart-shaped fruits, which are commonly found in the *Peponidium* and *Pyrostria* clades, and many other Vanguerieae genera.

#### EVOLUTION OF FUNCTIONAL DIOECY IN VANGUERIEAE

The present study supports a single origin of functional dioecy in Vanguerieae (Figs. 1–3). The Asian *Canthium laeve* has also been reported by Burck (1884) to be functionally dioecious. For now, we cannot tell whether functional dioecy had single or multiple origins in the tribe, until the phylogenetic placement of this species within Vanguerieae is known. The Southeast Asian species are, in general, poorly understood, and the breeding systems of most species have yet to be studied. The presence of the nonfunctional sexual organs of the opposite sex on both the functionally male and female flowers indicates that the functional dioecy of Vanguerieae may have originated from hermaphroditism. This may also imply that the functionally dioecious flowers in Vanguerieae are probably in transition to morphologically true dioecious ones (see also Mayer & Charlesworth, 1991). On the other hand, the combined analyses (Fig. 3) indicate that reversals back to the hermaphroditic conditions are also possible, at least within *Pyrostria* s.l. (Fig. 3) and the *Cyclophyllum*–*Canthium confertum* clade, according to Bridson (1987) (see also Table 2). Recent herbarium studies and field observations conducted by A. Mouly reveal that the two sequenced *Cyclophyllum* species and seven other New Caledonian species are actually functionally dioecious (Mouly et al., 2007), inconsistent with Bridson (1987), who considers the genus to be hermaphroditic. Similarly, Friedmann (1994) reported functionally male and female and hermaphroditic flowers from the Afro–Comorean–Indian Ocean *Pyrostria bibracteata* (Baker) Cavaco, which is considered by Bridson to be hermaphroditic. Functionally male and female plants of the same species have also recently been seen by Mouly (2007) on Mayotte Island (France). This further suggests that functional dioecy in Vanguerieae is evolutionarily unstable and, accordingly, should not be used alone for diagnosing any genera within the dioecious group, inconsistent with Capuron (1969), Cavaco (1972), and Schatz (2001) (see Table 1). However, it can still be used for characterizing the genera in the newly circumscribed dioecious group (this study) in combination with other characters.

Finally, all described African, Malagasy, and Mascarene dioecious Vanguerieae species, except the Afro–Comorean–Indian Ocean polygamous *Pyros-*

*tria bibracteata* and the functionally dioecious Comorean–Malagasy *Canthium cystiporon* Bynum ex Cavaco, are regional endemics (e.g., Bridson, 1987; Cavaco, 1966, 1967, 1968, 1969a, b; Verdcourt, 1983; Schatz, 2001), indicating an autochthonous evolution of functional dioecy in Vanguerieae throughout the geographic ranges of the dioecious group. On the other hand, we cannot yet rule out the origin of the functionally dioecious Mascarene *Pyrostria* species from a functionally dioecious Malagasy or Southeast Asian colonist.

NEW CIRCUMSCRIPTIONS, MAJOR LINEAGES, AND NEW GENERIC LIMITS OF THE DIOECIOUS GROUP

*New circumscriptions of the dioecious group*

The ITS tree (Fig. 1) resolves the Southeast Asian and hermaphroditic *Canthium confertum* group (sensu Bridson, 1987), represented here by two accessions of *C. confertum*, as sister to the mostly Pacific *Cyclophyllum*. A much larger sampling of *Cyclophyllum* and the *Canthium confertum* group is needed for testing their sister-group relationship. The support for the sister-group relationships between the *Cyclophyllum* clade and the dioecious group sensu Lantz and Bremer (2004) is consistently high in the ETS and combined analyses (Figs. 2, 3) and is also consistent with Mouly et al. (2007). Accordingly, we include *Cyclophyllum* in the dioecious group, which currently contains approximately over 200 species (ca. one third of the total species of Vanguerieae). If the sister-group relationship between *Cyclophyllum* and the *Canthium confertum* group is further confirmed, the *C. confertum* group will have to be considered members of the dioecious group as well. The combined analyses (Fig. 3) indicate that the dioecious group can be subdivided into four morphologically distinct lineages: the *Cyclophyllum* clade, *Bullockia* clade, *Peponidium* clade, and *Pyrostria* clade.

*Major lineages of the dioecious group*

*Cyclophyllum clade.* The ETS and combined ETS/ITS analyses (Figs. 2, 3) further corroborate with strong support (JK = 100, PP = 1.00) the sister-group relationships between the *Cyclophyllum* clade and the dioecious group sensu Lantz and Bremer (2004). According to Bridson (1987), *Cyclophyllum* can be characterized by the combination of the following characters: inflorescences without paired bracts; fasciculate inflorescences or occasionally with rudimentary inflorescence branches; large hypocrateriform corollas; hermaphrodite flowers with two-locular ovaries and style widening at apex; and dorsal face of

anthers (except margins) covered with dark connective. However, as we mentioned above, at least nine *Cyclophyllum* species found on New Caledonia are functionally dioecious (Mouly et al., 2007). In addition, at least one New Caledonian species is reported by Guillaumin (1930) to have connate paired bracts that enclose the young inflorescences. This indicates that the monophyly of *Cyclophyllum* as presently delimited needs to be tested. We find no support for Baillon's (1879) suggestion of merging *Cyclophyllum* with *Canthium*, which is presently restricted to the African and Asian spiny species (see Lantz & Bremer, 2004).

*Bullockia clade.* The *Bullockia* clade, retained in all analyses (Figs. 1–3), comprises three of the six species of *Canthium* subgen. *Bullockia* (Bridson, 1987) and two undescribed new Malagasy species, consistent with Bridson's (1987) prediction. Bridson (1987) used the combination of several characters to distinguish *Canthium* subgen. *Bullockia* from the other subgenera of *Canthium* and *Pyrostria* and its allied genera: persistent leaves; inflorescences unsubtended by the typical *Pyrostria* paired bracts; umbellate or fasciculate inflorescences bearing functionally unisexual flowers; inside of the corolla tube with a well-defined ring of deflected hairs; corollathroat pubescent but not congested with hairs, rarely fleshy corollas; dorsal face of the anthers with only the central area with dark connective or entirely without dark connective; and hollow stigmatic knob (at least near the base) with recessed style. Our close observations of the sequenced Malagasy species, however, show that both of the undescribed new Malagasy species (*Canthium* sp. indet. 5 and 6) have deciduous leaves.

*Peponidium clade.* In the ETS (Fig. 2) and combined trees (Fig. 3), all sequenced species of *Peponidium* and the Comorean and Malagasy *Canthium* form a strongly supported (JK = 95–97, PP = 1.00) clade. The Comorean *P. comorense* Arènes (Arènes, 1960) and the Seychellean *C. carinatum* (Baker) Summerh. (Friedmann, 1994) have recently been shown to belong to this clade (Avino et al., ETS, ITS, and *trnT-F* unpubl. data). This finding indicates that *Peponidium* sensu Arènes (1960) is not monophyletic, unless the Comorean and Indian Ocean *Canthium* are also included. The *Peponidium* clade (Fig. 3) corresponds to Bridson's (1987) group V, a group she considered to be artificial. In fact, the *Peponidium* clade is supported by at least one potential morphological synapomorphy. The members of the clade can be easily diagnosed by having deciduous, small cupular bracts that are always located at the bases of the inflorescence peduncles

and never enclose the young inflorescences (see also Table 2). Arènes (1960) referred to this type of bract as a false involucre. While the bracts are cup-shaped in most of the species, some species appear to have two to four basally fused and imbricate bracts. This indicates that the cupular bracts in the *Peponidium* clade are most likely to have derived from a complete fusion of more than two bracts. The cupular bracts are conspicuous and often remain intact in species with subsessile and pedunculate inflorescences bearing up to three flowers. In contrast, they are difficult to see and are often crushed in species with sessile inflorescences with four or more flowers, or with both sessile inflorescences and flowers. Finally, neither Lantz and Bremer (2004) nor the present study find support for Capuron's (1969) attempt to merge *Pyrostria* s.l. (including *Peponidium* and *Pseudopeponidium*) in *Canthium* based on accumbent cotyledons.

*Pyrostria* clade. One of the main goals of the present study is to test the monophyly of the different circumscriptions of *Pyrostria*. The combined analyses (Fig. 3) weakly to moderately support a broad circumscription of *Pyrostria*, which includes all sequenced functionally dioecious species of *Dinocanthium*, *Leroya*, *Neoleroya*, *Pseudopeponidium*, *Pyrostria*, and *Scyphochlamys* and the polygamous *Pyrostria bibracteata*. This broadly circumscribed *Pyrostria* is easy to recognize and distinct from the other genera in the dioecious group (sensu Razafimandimbison et al., this study) and the rest of Vanguerieae in having persistent and basally connate, long acuminate paired bracts (see also Table 2). There is no support for the monophyly of the narrowly delimited *Pyrostria* sensu Jussieu (1789) or Cavaco (1967), as the two-ocular or plurilocular species (see Table 2) never form separate clades (Fig. 3). In addition, *Pyrostria* sensu Capuron (1969), sensu Cavaco (1972), and sensu Schatz (2001), all defined by (functional) dioecy (see Table 1), are shown to be paraphyletic because the polygamous *Pyrostria* species (*P. bibracteata*) is deeply nested in the almost functional dioecious *Pyrostria* clade, consistent with Bridson (1987). *Pyrostria* sensu Bridson (1987), diagnosed by the presence of paired bracts, is paraphyletic as it excludes *Scyphochlamys*. No members of the Asian *Pyrostria* group B (Bridson, 1987) are included in our analyses due to lack of material; on the other hand, the fact that they all bear the typical paired bracts of *Pyrostria* indicates that they belong to this genus. The present study further supports the decision of Bridson (1987) to merge the African genus *Dinocanthium* in *Pyrostria*. Bridson (1987: 614) stressed that both *Leroya* and *Neoleroya* “appeared to be little more than *Pyrostria* with marked

elaboration of the fruit” and therefore were “marginally worth generic rank.” Bridson's statement is further supported by our analyses, as *Leroya* and *Neoleroya* are both nested in the *Pyrostria* clade. In addition, the inclusion of *Canthium* sect. *Psydracium* Baill. (Baillon, 1879), represented here by *Pyrostria media* (A. Rich.) Cavaco and *P. major* (A. Rich.) Cavaco, in *Pyrostria* by Cavaco (1967) is supported by our results. Furthermore, our results reveal no support for the two sections of *Pyrostria*, *Pyrostria* sect. *Pyrostria* and *Pyrostria* sect. *Involucratae* Cavaco, described by Cavaco (1971b). *Pyrostria andilanensis* Cavaco, one of the two species of *Pyrostria* sect. *Involucratae* (Cavaco, 1971b), is deeply nested in the members of *Pyrostria* sect. *Pyrostria*, consistent with morphological data. Finally, Bridson's (1987) consideration of grouping *Pyrostria* s. str., *Leroya*, *Neoleroya*, *Pyrostria* group A, *Pyrostria* group B, *Scyphochlamys*, *Cyclophyllum*, her group IV (Bridson, 1987), and group V (*Peponidium* and the Comorean, Malagasy, and Seychellean *Canthium*) under *Pyrostria* is not supported as monophyletic unless *Bullockia* is also included. *Pseudopeponidium* (Bridson, 1987; Schatz, 2001), *Leroya*, and *Neoleroya* have been synonymized under *Pyrostria* by Schatz (2001).

#### *New generic limits of the dioecious group*

The present analyses indicate that the generic limits in the dioecious group sensu Razafimandimbison et al. need to be recircumscribed. Our intention is to recognize both monophyletic and morphologically distinct genera and to minimize nomenclatural changes. At least three alternative solutions are possible. The first solution is to maintain *Cyclophyllum* at the generic level and recognize a broader circumscription of *Pyrostria* (the oldest name) including all species of the *Bullockia*, *Peponidium*, and *Pyrostria* clades without infrageneric subdivision. The second solution is to merge the four clades with *Pyrostria*. Both alternatives would make *Pyrostria* rather heterogenous morphologically and require a large number of new combinations. The third possibility is to recognize the *Cyclophyllum*, *Bullockia*, *Peponidium*, and *Pyrostria* clades all at the generic level. We favor this last option because it reflects the morphological distinctness of the four clades and would minimize nomenclatural changes in the last three clades. In addition, the present study clearly shows that the generic status of *Dinocanthium*, *Leroya*, *Neoleroya*, and *Pseudopeponidium* is untenable. We disregard the possibility of maintaining the current generic status of *Scyphochlamys* because this would force us not only to recognize *Pyrostria* in a narrow sense (i.e., including only the Mascarene species), but also to raise *Dinocanthium* to accommodate

all African and Malagasy *Pyrostria* species. There is no distinctive morphologic character for separating the Mascarene *Pyrostria* from the Afro-Malagasy or Asian ones.

#### PRELIMINARY BIOGEOGRAPHIC HYPOTHESES OF THE DIOECIOUS GROUP

We are unable to perform a proper biogeographic analysis, as the relationships between the major lineages of the dioecious group as delimited here are largely unresolved. On the other hand, some biogeographic facts can still be discussed for *Bullockia*, *Peponidium* s.l., and *Pyrostria* s.l. A well-resolved phylogeny of the dioecious group based on a much larger sampling is needed to test all biogeographic hypotheses put forward below.

The newly delimited dioecious group is distributed throughout eastern and southern Africa, Southeast Asia, the Indian Ocean islands, the Pacific region, and northern Australia (see also Bridson, 1987). *Bullockia* is an Afro-Malagasy genus, consistent with Bridson's (1987) prediction, which seems to have had an African origin and may well have reached Madagascar via a single long dispersal event. The African species are restricted to eastern and southern Africa (Bridson, 1987) and the two studied Malagasy species are confined, respectively, to the dry deciduous forests of southwestern and northwestern Madagascar. In contrast, our circumscribed *Peponidium* is almost completely Malagasy, with only two to four species restricted to the Comoro Islands (Arènes, 1960; Cavaco, 1972) and two species confined to the Seychelles (Friedmann, 1994). The Comorean and Seychellean species appear to have had Malagasy origins (Fig. 3; Avino et al., unpublished data). In addition, the newly delimited *Pyrostria* is predominantly Malagasy, with 14 species confined to both eastern and southern Africa (Bridson, 1987), eight Mascarene-endemic species (one restricted to Rodrigues Island, five restricted to Mauritius, and two endemic to Réunion Island [Verdcourt, 1983]), and one, *P. bibracteata*, shared between the African mainland, the Comoro Islands, Madagascar, and the Seychelles. The number of species of *Pyrostria* in Southeast Asia is currently unknown. The results further indicate that *P. bibracteata* and the African *Pyrostria*, represented here by *P. hystrix* (Bremek.) Bridson and *P. phyllanthoidea* (Baill.) Bridson, appear to have originated from more than one Malagasy ancestor, as the two sequenced African species do not form a clade (see Fig. 3). Furthermore, the highly supported sister-group relationship (JK = 100, PP = 1.00) between the strongly supported

Mascarene *Pyrostria* (JK = 99, PP = 1.00) and the Rodrigues Island's *Scyphochlamys* indicates that they had a common ancestor (Fig. 3). Geologic evidence suggests that Mauritius emerged as a result of a series of volcanic events, the earliest of which occurred ca. 6.8–7.8 million years ago (Ma). Rodrigues Island did not emerge until 1.5 Ma (McDougall & Chamalaun, 1969), suggesting that the common ancestor of both *Scyphochlamys* and the Mascarene *Pyrostria* should be at least 1.5 Ma. Based on the evidence presented here, it can be concluded that the Mascarene *Pyrostria* and possibly *Pyrostria* s.l. had a relatively recent diversification. This may well be one plausible explanation for the low to moderate support for *Pyrostria* s.l. compared to that of *Bullockia*, *Cyclophyllum*, and *Peponidium* s.l. (Fig. 3).

#### PHYLOGENETIC CONCLUSIONS

The present study supports a further disintegration of *Canthium* s.l., as the Southeast Asian *C. confertum* and the Comorean and Malagasy *Canthium* species group are distantly related to *Canthium* s. str. (Lantz & Bremer, 2004) and are for the first time shown to belong to the new circumscribed dioecious group. The combined analyses show that the dioecious group can be subdivided into four morphologically distinct clades. According to the rules of priority, the following generic names are applicable to the clades: *Cyclophyllum*, *Bullockia*, *Peponidium*, and *Pyrostria*. *Cyclophyllum* is resolved with high support as sister to the dioecious group sensu Lantz and Bremer (2004). The results further point to a single origin of functional dioecy from hermaphroditism for the newly circumscribed dioecious group followed by subsequent reversals back to the hermaphroditic conditions at least in both *Cyclophyllum* and *Pyrostria* s.l. Furthermore, the Malagasy *Bullockia* species seem to have a common African ancestor, which is most likely to have reached Madagascar via a long dispersal event and subsequently radiated there. In contrast, the Comorean *Peponidium* s.l. appears to have originated from a Malagasy common ancestor. Similarly, our results further support the Malagasy origins of the African *Pyrostria* and a single origin of the Mascarene *Pyrostria*. Finally, we conclude that *Pyrostria* s.l. is a relatively young group that seems to have had a recent diversification.

#### TAXONOMIC IMPLICATIONS

Below, we formally describe *Bullockia* (Bridson) Razafim., Lantz & B. Bremer to accommodate all six described African species of *Canthium* subgen. *Bullockia* and the two undescribed new Malagasy

species (*Canthium* sp. indet. 5 and 6). We synonymize *Scyphochlamys* with *Pyrostria*. *Dinocanthium*, *Leroya*, *Neoleroya*, and *Pseudopeponidium* all have been synonymized under *Pyrostria* before (Bridson, 1987; Schatz, 2001). Razafimandimbison et al. (2007) have recently transferred 20 species of the Indian Ocean and Comorean *Canthium* to *Peponidium* and all described species of *Leroya*, *Neoleroya*, *Pseudopeponidium*, and *Scyphochlamys* to *Pyrostria*. The transfer of the two Seychellean *Canthium* to *Peponidium* will be published elsewhere.

**I. *Bullockia*** (Bridson) Razafim., Lantz & B. Bremer, stat. nov. Basionym: *Canthium* subgen. *Bullockia* Bridson, Kew Bull. 42: 630. 1987. TYPE: *Bullockia setiflora* (Hiern) Razafim., Lantz & B. Bremer.

For a description, see Bridson (1987: 630). The only new information we add is that species with persistent (all African members) and deciduous leaves (the two new undescribed Malagasy species; see Figs. 1–3) are found in *Bullockia*.

*Number of species.* There are at least eight species (six species in mainland Africa [Bridson, 1987] and two in Madagascar).

*Distribution and habitat.* The genus is distributed in eastern and southern Africa and Madagascar in bushland, woodland, savannas, and dry, deciduous forests.

*Diagnostic features.* *Bullockia* is distinct from the other functionally dioecious genera of Vanguerieae by the combination of the following characters: the inside of the corolla tube with a well-defined ring of deflected hairs; corolla-throat pubescent but not congested with hairs, rarely fleshy corollas; dorsal face of the anthers with only the central area with a dark connective or entirely without a dark connective; and a hollow stigmatic knob (at least near the base) with a recessed style (Bridson, 1987).

*New combinations.* Here, we transfer all described species of *Canthium* subgen. *Bullockia*.

**1. *Bullockia dyscriton*** (Bullock) Razafim., Lantz & B. Bremer, comb. nov. Basionym: *Canthium dyscriton* Bullock, Bull. Misc. Inform. Kew 1936: 478. 1936. TYPE: Kenya. Teita Distr.: Teita Hills, 2500–3500 ft., s.d., *H. M. Gardner 3000* (holotype, K not seen, photo!).

**2. *Bullockia fadenii*** (Bridson) Razafim., Lantz & B. Bremer, comb. nov. Basionym: *Canthium fadenii* Bridson, Kew Bull. 42: 632. 1987. TYPE: Kenya. Kiambu Distr.: “behind Blue Ports Hotel,

Thika,” 4900 ft., 23 Mar. 1968, *R. B. Faden 68/012* (holotype, K not seen, photo!).

**3. *Bullockia impressinervia*** (Bridson) Razafim., Lantz & B. Bremer, comb. nov. Basionym: *Canthium impressinervium* Bridson, Kew Bull. 42: 633. 1987. TYPE: Tanzania. Lindi Distr., “Noto-Plateau,” ca. 450 m, 9 Mar. 1937, *H. J. Schieben 6109* (holotype, K not seen, photo!).

**4. *Bullockia mombazensis*** (Baill.) Razafim., Lantz & B. Bremer, comb. nov. Basionym: *Canthium mombazense* Baill., Adansonia 12: 188. 1879. TYPE: Kenya. “Côte orientale d’Afrique: Zanzibar,” 1847–1852, *L. H. Boivin s.n.* (holotype, P!).

**5. *Bullockia pseudosetiflora*** (Bridson) Razafim., Lantz & B. Bremer, comb. nov. Basionym: *Canthium pseudosetiflorum* Bridson, Kew Bull. 42: 635. 1987. TYPE: Kenya. “Among granite rocks by watercourse,” 3400 ft., 1952–1953, *J. B. Gillett 14096* (holotype, K not seen, photo!).

**6. *Bullockia setiflora*** (Hiern) Razafim., Lantz & B. Bremer, comb. nov. Basionym: *Canthium setiflorum* Hiern, Fl. Trop. Afr. [Oliver et al.] 3: 134. 1877. TYPE: Mozambique. “Between Tete and the sea coast,” Mar.–Apr. 1860, *J. Kirk s.n.* (holotype, K not seen, photo!).

**II. *Peponidium*** (Baill.) Arènes, Notul. Syst. (Paris) 16: 25. 1960. Basionym: *Canthium* sect. *Peponidium* Baill., Adansonia 12: 197. 1879. TYPE: *Peponidium horridum* (Baill.) Arènes.

For a description, see Arènes (1960).

*Number of species.* There are at least 45 species, all (except two to four Comorean species [Arène, 1960] and two Seychellean species [Friedmann, 1994]) endemic to Madagascar (Arènes, 1960; Razafimandimbison et al., 2007).

*Distribution and habitat.* This genus occurs in Madagascar (e.g., Arènes, 1960; Cavaco, 1969b), the Comoro Islands (Arènes, 1960; Mouly, 2007), and the Seychelles (Friedmann, 1994) in dry, deciduous forests, lowland rainforests, and mid- and high-altitude humid forests.

*Diagnostic features.* Our newly circumscribed *Peponidium* is diagnosed by the presence of deciduous, cupular bracts at the bases of the inflorescence peduncles that never enclose the young inflorescence.

**III. *Pyrostria*** Comm. ex. Juss., Gen. Pl. 206. 1789. TYPE: *Pyrostria commersonii* J. F. Gmel.

*Scyphochlamys* Balf. f., J. Linn. Bot. 16: 14–15. 1877, syn. nov. TYPE: *Scyphochlamys revoluta* Balf. f.

- Canthium* sect. *Psydracium* Baill., *Adansonia* 12: 199. 1879. TYPE: *Pyrostria major* (A. Rich.) Cavaco [= *Psydrax major* A. Rich.].
- Dinocanthium* Bremek., *Ann. Transvaal Mus.* 15: 259. 1933. TYPE: *Dinocanthium hystrix* Bremek.
- Pseudopeponidium* Homolle ex Arènes, *Notul. Syst. (Paris)* 16: 19. 1960. TYPE: *Pseudopeponidium nerifolium* Homolle ex Arènes.
- Leroya* Cavaco, *Adansonia*, n.s., 10: 335. 1970. TYPE: *Leroya madagascariensis* Cavaco.
- Neoleroya* Cavaco, *Adansonia*, n.s., 11: 122. 1971. TYPE: *Neoleroya verdcourtii* Cavaco.
- Pyrostria* sect. *Involucratae* Cavaco, *Adansonia*, n.s., 11: 393. 1971. TYPE: *Pyrostria amporeforensis* Cavaco.

For a description, see Bridson (1987).

*Number of species.* There are at least 80 species (55 species in Madagascar [Govaert et al., 2006; Davis et al., 2007; Lantz et al., 2007; Razafimandimbison et al., 2007], plus at least eight new undescribed species there, with 14 species on mainland Africa [Bridson, 1987; Verdcourt & Bridson, 1991], and eight on the Mascarenes [Verdcourt, 1983]). The number of species in Southeast Asia is currently unknown.

*Distribution and habitat.* The genus occurs on the Comoro Islands, Madagascar, the Mascarenes, the Seychelles, eastern and southern Africa, and Southeast Asia in dry, deciduous forests, thicket xerophyllous forests, lowland rainforests, and both mid- and high-altitude and humid forests.

*Diagnostic features.* Our newly circumscribed *Pyrostria* can be easily distinguished from the other genera of the dioecious group by its persistent, basally connate, and long acuminate paired bracts, which are relatively large and spathe-like in *Scyphochlamys revoluta*.

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Appendix 1. List of the investigated sequenced taxa, voucher information, country origins, and accession numbers.

Taxa	Voucher information	Country of origin	ETS	ITS
<i>Afoacanthium pseudonerticillatum</i> (S. Moore) Lantz	Lantz & Bremer (2004)			AJ617758
<i>Afoacanthium siebenlistii</i> (K. Krause) Lantz	Lantz & Bremer (2004)			AJ617759
<i>Alberta magna</i> E. Mey.	Andreassen et al. (1999)			AJ224842
<i>Arcylanthos rubiginosus</i> Desf. (specimen A)	Lantz & Bremer (2004)	Thailand		AJ617747
<i>Canthium confertum</i> Korth.	<i>Mold Shad &amp; Maxwell 3962</i> (AAU)			EU584288
<i>Canthium cystiporon</i> Bynum ex Cavaco	<i>Labat et al. 3280</i> (P)	Mayotte Island	EU584363	EU584299
<i>Canthium fadenii</i> Bridson	<i>Luke 9014</i> (UPS)	Kenya	EU584319	EU584260
<i>Canthium inerme</i> Kuntze	Lantz et al. (2002); Lantz & Bremer (2004)			AJ315120
<i>Canthium marojjense</i> Cavaco	<i>Razafimandimison &amp; Ravelonarivo 589</i> (S)	Madagascar	EU584377	EU584308
<i>Canthium mombazense</i> Baill.	<i>Muangoka et al. 3375</i> (S)	Kenya	EU584261	EU584261
<i>Canthium pseudoseiflorum</i> Bridson	Lantz & Bremer (2004)			AJ617757
<i>Canthium</i> Lam. sp. indet. 1	<i>Eriksson et al. 1020</i> (S)	Madagascar	EU584307	EU584307
<i>Canthium</i> sp. indet. 2	<i>Kårehed et al. 210</i> (UPS)	Madagascar	EU584358	EU584292
<i>Canthium</i> sp. indet. 3	<i>Noyes et al. 1063</i> (MO)	Madagascar	EU584342	EU584278
<i>Canthium</i> sp. indet. 4	<i>Kårehed et al. 204</i> (UPS)	Madagascar	EU584359	EU584293
<i>Canthium</i> sp. indet. 5	<i>Davis &amp; Rakotonasolo 2576</i> (K)	Madagascar	EU584324	EU584254
<i>Canthium</i> sp. indet. 6	<i>De Block et al. 1100</i> (BR)	Madagascar	EU584325	EU584265
<i>Canthium</i> sp. indet. 7	<i>Davis 1193</i> (K)	Madagascar	EU584323	EU584263
<i>Canthium</i> sp. indet. 8	<i>Davis 1020</i> (K)	Madagascar	EU584321	EU584262
<i>Canthium</i> sp. indet., aff. <i>confertum</i>	<i>Maxwell 77-128</i> (AAU)	Thailand	EU584289	EU584289
<i>Canthium venulosum</i> Boivin ex Baill.	<i>Pignal 1825</i> (P)	Mayotte Island	EU584368	EU584301
<i>Cyclophyllum balansae</i> (Baill.) Guillaumin	<i>Mouly 181</i> (P)	New Caledonia	EU584357	EU584290
<i>Cyclophyllum barbatum</i> (G. Forst.) N. Hallé & J. Florence	Lantz & Bremer (2004)		EU584356	AJ617760
<i>Hutchinsonia barbata</i> Robyns	Lantz et al. (2002); Lantz & Bremer (2004)			AJ315102
<i>Ixora coccinea</i> L.	Andreassen et al. (1999)			AJ224826
<i>Keetia venosa</i> (Oliv.) Bridson	Lantz & Bremer (2004)			AJ617762
<i>Leroyia</i> Cavaco sp. indet.	<i>De Block et al. 1143</i> (BR)			EU584266
<i>Leroyia</i> sp. indet., aff. <i>richardiae</i> Cavaco	Lantz & Bremer (2004)	Madagascar	EU584326	AJ617763
<i>Multidentia concrescens</i> (Bullock) Bridson & Verdc.	Lantz et al. (2002)			AJ315086
<i>Multidentia sclerocarpa</i> (K. Schum.) Bridson	Lantz & Bremer (2004)			AJ874986
<i>Neoleroya verdicourtii</i> Cavaco	Lantz & Bremer (2004)			AJ617764
<i>Peponidium busifolium</i> (Baker) Razafim., Lantz & B. Bremer, acc. 1	<i>Eriksson et al. 908</i> (S)	Madagascar	EU584328	EU584303
<i>Peponidium busifolium</i> (Baker) Razafim., Lantz & B. Bremer, acc. 2	<i>Eriksson et al. 909</i> (S)	Madagascar	EU584371	EU584303
<i>Peponidium busifolium</i> (Baker) Razafim., Lantz & B. Bremer, acc. 3	<i>Eriksson et al. 919</i> (S)	Madagascar	EU584370	EU584302
<i>Peponidium busifolium</i> (Baker) Razafim., Lantz & B. Bremer, acc. 4	<i>Eriksson et al. 920</i> (S)	Madagascar	EU584373	EU584305
		Madagascar	EU584374	EU584311

## Appendix 1. Continued.

Taxa	Voucher information	Country of origin	ETS	ITS
<i>Peponidium crassifolium</i> Lantz, Klack. & Razafim.	<i>Razafimandimbison &amp; Ravelonarivo 628</i> (S)	Madagascar	EU584381	EU584313
<i>Peponidium horridum</i> (Baill.) Arènes	<i>Razafimandimbison &amp; Razafimanansoa 470</i> (UPS)	Madagascar	EU584329	EU584267
<i>Peponidium madagascariense</i> Cavaco	<i>Razafimandimbison &amp; Andrianatoanina 431</i> (UPS)	Madagascar	EU584330	EU584268
<i>Peponidium</i> Arènes sp. indet. 1	<i>Eriksso et al. 1009</i> (S)	Madagascar	EU584375	EU584306
<i>Peponidium</i> sp. indet. 2	<i>Kârehed et al. 260</i> (UPS)	Madagascar	EU584362	EU584296
<i>Peponidium</i> sp. indet. 3	<i>Bremer et al. 4046-B46</i> (UPS)	Madagascar	EU584332	EU584269
<i>Peponidium</i> sp. indet. 4	<i>Davis 1133</i> (K)	Madagascar	EU584333	EU584270
<i>Peponidium</i> sp. indet. 5	<i>Davis 1028</i> (K)	Madagascar	EU584334	EU584271
<i>Peponidium</i> sp. indet. 6 clone 1	<i>Razafimandimbison &amp; Andrianatoanina 458</i> (TAN)	Madagascar	EU584338	EU584273
clone 2			EU584339	EU584274
clone 3			EU584275	EU584275
<i>Peponidium</i> sp. indet., aff. <i>buxifolium</i> (Baker) Razafim., Lantz & B. Bremer	<i>Razafimandimbison 561</i> (TAN)	Madagascar	EU584367	EU584300
<i>Peponidium</i> sp. indet., aff. <i>madagascariense</i>	<i>Kârehed et al. 223</i> (UPS)	Madagascar	EU584317	EU584384
<i>Peponidium velutinum</i> Arènes	<i>Callmander 331</i> (MO)	Madagascar	EU584331	EU584314
<i>Pseudopeponidium ampjoroense</i> Arènes	Lantz & Bremer (2004)		EU584335	AI617766
<i>Pseudopeponidium asosa</i> Arènes	Lantz & Bremer (2004)		EU584337	EU584272
<i>Pseudopeponidium ivorifolium</i> Homolle ex Arènes	<i>Kârehed et al. 266</i> (UPS)	Madagascar	EU584355	EU584291
<i>Pseudopeponidium longiflorum</i> Cavaco	<i>Razafimandimbison 522</i> (UPS)	Madagascar	EU584361	EU584294
<i>Pseudopeponidium oleifolium</i> Homolle ex Arènes clone 1	<i>Razafimandimbison et al. 422</i> (TAN)	Madagascar	EU584340	EU584276
clone 2			EU584341	EU584277
<i>Pseudopeponidium</i> Homolle ex Arènes sp. indet. 1	<i>Kârehed et al. 222</i> (UPS)	Madagascar	EU584361	EU584295
<i>Pseudopeponidium</i> sp. indet. 2	<i>Razafimandimbison 514</i> (UPS)	Madagascar	EU584364	EU584298
<i>Psyrax kraussoides</i> (Hiern) Bridson	Lantz & Bremer (2004)		EU584318	AI617768
<i>Psyrax obovata</i> (Klotzsch ex Eckl. & Zeyh.) Bridson	Lantz & Bremer (2004)		AI315109	AI315109
<i>Psyrax parviflora</i> (Alzel.) Bridson subsp. <i>parviflora</i>	Lantz & Bremer (2004)		EU584364	AI315110
<i>Pyrostria andilanensis</i> Cavaco	<i>Razafimandimbison &amp; Razafimanansoa 467</i> (UPS)	Madagascar	EU584369	EU584297
<i>Pyrostria anjouanensis</i> Arènes ex Cavaco	<i>Pignat et al. 1808</i> (P)	Mayotte Island (France)	EU584366	EU584297
<i>Pyrostria ankazobeensis</i> Arènes ex Cavaco	<i>Davis 1022</i> (K)	Madagascar	EU584349	EU584284
<i>Pyrostria bibracteata</i> (Baker) Cavaco	Lantz et al. (2002); Lantz & Bremer (2004)		EU584365	AI315113
<i>Pyrostria commersonii</i> J. F. Gmel.	<i>Cadet 728</i> (Herbier Universitaire de La Réunion)	La Réunion Island	EU584353	AI315114
<i>Pyrostria hystrix</i> (Bremek.) Bridson	Lantz & Bremer (2004)		EU584343	AI315114
<i>Pyrostria major</i> (A. Rich.) Cavaco	<i>Eriksso et al. 918</i> (S)	Madagascar	EU584372	EU584304
<i>Pyrostria media</i> (A. Rich.) Cavaco	<i>Razafimandimbison 511</i> (UPS)	Madagascar	EU584383	EU584316
<i>Pyrostria orbicularis</i> A. Rich.	<i>Paillet 180</i> (Herbier Universitaire de La Réunion)	La Réunion Island	EU584352	EU584287
<i>Pyrostria pendula</i> Lantz, Klack. & Razafim.	<i>Razafimandimbison &amp; Ravelonarivo 626</i> (S)	Madagascar	EU584379	EU584310

Appendix 1. Continued.

Taxa	Voucher information	Country of origin	ETS	ITS
<i>Pyrostria phyllanthoidea</i> (Baill.) Bridson	Lantz & Bremer (2004)	Madagascar	EU584344	AI315115
<i>Pyrostria sarodranensis</i> Cavaco	<i>Phillipson &amp; Rabeshanaka 3177</i> (MO)	Madagascar	EU584336	EU584280
<i>Pyrostria serpentina</i> Lantz, Klack. & Razafim.	<i>Davis 1162</i> (K)	Madagascar	EU584348	EU584283
<i>Pyrostria</i> Comm. ex Juss. sp. indet. 1	<i>Rabenantoandro et al. 770</i> (MO)	Madagascar	EU584345	EU584279
<i>Pyrostria</i> sp. indet. 2	<i>Razafimandimbison &amp; Ravelonarivo 648</i> (S)	Madagascar	EU584382	EU584315
<i>Pyrostria</i> sp. indet. 3	<i>Eriksson et al. 947</i> (S)	Madagascar	EU584378	EU584309
<i>Pyrostria</i> sp. indet. 4	<i>Razafimandimbison &amp; Ravelonarivo 594</i> (S)	Madagascar	EU584380	EU584312
<i>Pyrostria</i> sp. indet. 5	<i>De Block et al. 1117</i> (BR)	Madagascar	EU584346	EU584281
<i>Pyrostria</i> sp. indet. 6	<i>Razafimandimbison et al. 412</i> (BR)	Madagascar	EU584347	EU584282
<i>Pyrostria</i> sp. indet. 7	<i>Razafimandimbison &amp; Andrianatoanina 451</i> (UPS)	Madagascar	EU584350	EU584287
<i>Pyrostria viburnoides</i> (Baker) Verdc.	<i>Andriambololona s.n.</i> (TAN)	Mauritius	EU584351	EU584286
<i>Robynsia glabrata</i> Hutch.	Lantz & Bremer (2004)			AI617774
<i>Rytigynia senegalensis</i> Blume	Lantz et al. (2002); Lantz & Bremer (2004)			AI315104
<i>Scyphochlamys revoluta</i> Balf. f.	Lantz & Bremer (2004)		EU584354	AI617776
<i>Tapiphyllum cinerascens</i> (Hiern) Robyns	Lantz et al. (2002); Lantz & Bremer (2004)			AI315096
<i>Vangueria infausta</i> Burch.	Lantz et al. (2002)			AI315093