

# Phylogenetic structure and clade circumscriptions in the Gardenieae complex (Rubiaceae)

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**Abstract** In this study we investigate the large and diverse Rubiaceae-Gardenieae and closely related tribes Bertiereae, Coffeae, Cremasporeae, Octotropideae, and Pavetteae. Some of the tribes or groups have been shown to be monophyletic and strongly supported, but the phylogeny of this large complex is still far from being satisfactorily elucidated particularly for Gardenieae, both in terms of intertribal relationships as well as tribal delimitations. We reconstruct the phylogeny of the complex using an extensive sampling of 108 genera and five plastid DNA regions. Phylogenetic relationships demonstrate that Gardenieae sensu Andreasen & Bremer is polyphyletic, as *Burchellia*, *Didymosalpinx*, *Monosalpinx*, and *Mantalania* are closer to Octotropideae-Cremasporeae. In addition, Pavetteae and the investigated members of Aulacocalyceae are nested in a supported but partially unresolved Gardenieae-Pavetteae clade. Within this clade, several strongly supported groups are resolved: an *Aidia* group, an *Alibertia* group, a *Gardenia* group, Pavetteae including *Pelagodendron*, a *Porterandia* group, a *Randia* group, a *Rothmannia* group (including *Aulacocalyx* and *Heinsenia*), a *Sherbournia* group, and the two isolated genera *Massularia* and *Schumanniohyton*. The latter genus presented a high rate of genetic substitutions, which resulted in perturbations of the phylogenetic reconstruction. A revised tribal circumscription is given for Gardenieae, the *Alibertia* group is recognized at tribal level as an emended Cordiereae, and a new tribe, Sherbournieae, is described to accommodate the members of the *Sherbournia* group.

**Keywords** Aulacocalyceae; Cordiereae; Gardenieae; mapping; molecular phylogeny; Octotropideae; Pavetteae; radiation; Rubiaceae; Sherbournieae

**Supplementary Material** Electronic Supplement (Fig. S1) and alignment are available in the Supplementary Data section of the online version of this article at <http://www.ingentaconnect.com/content/iapt/tax>

## ■ INTRODUCTION

Rubiaceae is the fourth-largest flowering plant family (Davis & al., 2009), although only a few species are well known and commonly used, such as coffee and the fragrant ornamental ixoras and gardenias. *Coffea* L., *Ixora* L., and *Gardenia* J.Ellis (respectively) belong to the same Rubiaceae lineage, Ixoroideae, a subfamily with a long and intricate taxonomic history and an unsatisfactorily resolved phylogeny, despite numerous recent studies (e.g., Persson, 1996, 2000a; Andreasen & Bremer, 2000; Rova & al., 2002; Robbrecht & Manen, 2006; Bremer & Eriksson, 2009; Razafimandimbison & al., 2011; Kainulainen & al., 2013). Within Ixoroideae, tribe Gardenieae is a large and morphologically diverse group of ca. 100 genera (Fig. 1), whose circumscription has long been

controversial (e.g., Candolle, 1830; Schumann, 1891; Verdcourt, 1958; Bremekamp, 1966; Robbrecht & Puff, 1986; Andreasen & Bremer, 1996, 2000; Robbrecht & Manen, 2006; see Table 1). Candolle (1830) described Gardenieae to accommodate Rubiaceae genera with indehiscent fleshy bilocular (rarely unilocular) fruits and “remarkable” flowers (the author meant large flowers) with or without pedicel. The original circumscription (Candolle, 1830) included 28 genera: *Sarcocephalus* Afzel. ex R.Br., *Zuccarinia* Blume, *Schradera* Vahl (as *Lucinaea* DC.), *Burchellia* R.Br., *Amaioua* Aubl., *Mussaenda* L. (incl. *Menestoria* DC.), *Kutchubea* Fisch. ex DC., *Isertia* Schreb. (as *Casupa* DC.), *Aidia* Lour. (as *Gynopachis* Blume, *Cupia* DC., and *Stylocoryna* Cav.), *Tocoyena* Aubl., *Posoqueria* Aubl., *Oxyanthus* DC., *Genipa* L., *Gardenia*, *Randia* L. (incl. *Oxyceros* and *Euclinia*), *Chapelieria* A.Rich., *Heinsia* DC., *Timonius* DC. (as



**Fig. 1.** Flowers of Gardenieae and related groups. Gardenieae: **A**, *Gardenia taitensis* DC., cultivated in Tahiti Botanical Garden (credit: C. Ollier, IRD Papeete); **B**, *Gardenia thunbergia* L.f., cultivated in Lisbon Botanical Garden (credit: A. Mouly); **C**, *Duperrea pavettifolia*, cultivated in Stockholm Bergius Botanical Garden (credit: K. Kainulainen); **D**, *Euclinia longiflora*, cultivated in Tahiti Botanical Garden (credit: A. Mouly); **E**, *Catunaregam spinosa*, Vietnam, forest (credit: K. Kainulainen); Sherbournieae: **F**, *Mitriostigma axillare*, cultivated in Stockholm Bergius Botanical Garden (credit: K. Kainulainen); **G**, *Oxyanthus speciosus*, cultivated in Leiden Hortus Botanicus (credit: A. Mouly); Cordiereae: **H**, *Mitriostigma axillare*, cultivated in Stockholm Bergius Botanical Garden (credit: K. Kainulainen); Octotropideae s.l.: **I**, *Burchellia bubalina*, cultivated in Stockholm Bergius Botanical Garden (credit: K. Kainulainen); **J** *Dydimosalpinx norea* (Swynn.) Keay, cultivated in Stockholm Bergius Botanical Garden (credit: K. Kainulainen)

*Helospora* DC.), *Hippotis* Ruiz & Pav., *Bertiera* Aubl. (incl. *Pomatium* DC.), *Pouchetia* A.Rich. ex DC., *Tarenna* Gaertn., *Rondeletia* L. (as *Petesia* P.Browne), *Coccocypselum* P.Browne, *Fernelia* Comm. ex Lam., *Hypobathrum* Blume (as *Petunga* DC.), *Hoffmannia* Sw. (incl. *Higginsia* Pers.), and *Catesbaea* L. Candolle (1830) described another tribe, Cordiereae, for the two genera *Cordia* A.Rich ex DC. and *Tricalysia* A.Rich. ex DC. characterized according to him by multilocular baccate fruits. Since then, several specialists have worked on the group and proposed different classifications. Among them, Schumann (1891) transferred Cordiereae to Gardenieae as an infratribal group.

Robbrecht & Puff (1986) published a comprehensive morphological study of Gardenieae based on several Rubiaceae systematic treatments by Schumann (1891), Verdcourt (1958), Bremekamp (1966), and their own observations and proposed a new classification (Table 1). Tribe Gardenieae was subdivided into two groups, subtribe Diplosporinae with ca. 20 genera and subtribe Gardeniinae including ca. 60 genera. Tribe Aulacocalyceae was described by Robbrecht & Puff (1986) to accommodate several genera previously placed in Gardenieae

(i.e., *Aulacocalyx* Hook.f., *Heinsenia* K.Schum., *Himalrandia* T.Yamaz., and *Alleizettella* Pit.), as these authors considered these genera to be more closely affiliated to subfamily Cinchonoideae of Rubiaceae. The study by Robbrecht & Puff (1986) served as a starting point for subsequent phylogenetic studies dealing with Gardenieae by, e.g., Andreasen & Bremer (1996) and Persson, (1996, 2000a), as reviewed by Bremer (2009).

The study of subfamily Ixoroideae by Andreasen & Bremer (1996), based on morphological and molecular (*rbcL*) data, indicated that subtribe Diplosporinae and *Posoqueria* should be excluded from Gardenieae. Andreasen & Bremer's (2000) extended phylogenetic study proposed the return of *Heinsenia* to Gardenieae from Aulacocalyceae, and the inclusion of *Duperrea* Pierre ex Pit. in Gardenieae. Persson (1996), in a phylogenetic study of Gardenieae s.str., analysed 70 morphological and anatomical characters for 81 taxa, and found support for a clade grouping genera with pollen grains in tetrads and an *Alibertia* A.Rich. ex DC. group. In a subsequent molecular phylogenetic study, Persson (2000a) resolved a core Gardenieae group (excluding subtribe Diplosporinae, *Burchellia*, *Didymosalpinx* Keay, and *Schumanniphyton* Harms), which included a

**Table 1.** Summary of generic placement in the Gardenieae complex according to recent morphological or molecular studies, and proposed new circumscription of tribes.

	Robbrecht & Puff, 1986: morpho-anatomy	Robbrecht, 1988, emend. in 1993: morpho-anatomy	Andreasen & Bremer, 2000: phylogeny	Robbrecht & Manen, 2006: phylogeny	This study: phylogeny
<i>Agouticarpa</i> C.H.Perss.					Cordiereae
<i>Aidia</i> Lour.	Gardenieae	Gardenieae	Gardenieae	Gardenieae	Gardenieae
<i>Alibertia</i> A.Rich. ex DC.	Gardenieae	Gardenieae	Gardenieae	Gardenieae	Cordiereae
<i>Amaioua</i> Aubl.				Gardenieae	Cordiereae
<i>Aorantho</i> Somers		Isertieae?			Gardenieae
<i>Argocoffeopsis</i> Lebrun	Gardenieae	Gardenieae		Coffeae	Coffeae
<i>Atractocarpus</i> Schltr. & K.Krause	Gardenieae?	Gardenieae	Gardenieae	Gardenieae	Gardenieae
<i>Atractogyne</i> Pierre	Gardenieae	Gardenieae	Gardenieae		Sherbournieae
<i>Aulacocalyx</i> Hook.f.	Aulacocalyceae	Aulacocalyceae	Gardenieae		Gardenieae
<i>Belonophora</i> Hook.f.	Aulacocalyceae?	Aulacocalyceae?		Coffeae	Coffeae
<i>Benkara</i> Adans.		Gardenieae	Gardenieae	Gardenieae	Gardenieae
<i>Bertiera</i> Aubl.		Gardenieae	Coffeae	Coffeae	Bertiereae
<i>Botryarrhena</i> Ducke		Retiniphyllaeae?			Cordiereae
<i>Brachytome</i> Hook.f.	Gardenieae	Gardenieae	Gardenieae		Gardenieae
<i>Brenania</i> Keay	Gardenieae	Gardenieae	Gardenieae		Gardenieae
<i>Bungarimba</i> K.M.Wong					Gardenieae
<i>Burchellia</i> R.Br.	Gardenieae	Gardenieae	Gardenieae		
<i>Calochone</i> Keay	Gardenieae	Gardenieae	Gardenieae	Gardenieae	Gardenieae
<i>Calycosiphonia</i> Pierre ex. Robbr.	Gardenieae	Gardenieae	Gardenieae	Gardenieae	Coffeae
<i>Canephora</i> Juss.	Hypobathreae	Octotropideae			Octotropideae
<i>Canthiopsis</i> Seem.					Pavetteae
<i>Casasia</i> A.Rich	Gardenieae	Gardenieae	Gardenieae	Gardenieae	Gardenieae

Table 1. Continued.

	Robbrecht & Puff, 1986: morpho-anatomy	Robbrecht, 1988, emend. in 1993: morpho-anatomy	Andreasen & Bremer, 2000: phylogeny	Robbrecht & Manen, 2006: phylogeny	This study: phylogeny
<i>Catunaregam</i> Wolf	Gardenieae	Gardenieae	Gardenieae	Gardenieae	Gardenieae
<i>Ceriscoides</i> (Hook.f) Tirveng.	Gardenieae	Gardenieae	Gardenieae	Gardenieae	Gardenieae
<i>Chapelieria</i> A.Rich	Hypobathreae	Octotropideae			Octotropideae
<i>Cladoceras</i> Bremek.	Pavetteae	Pavetteae	Pavetteae		Pavetteae
<i>Coddia</i> Verdc.	Gardenieae	Gardenieae	Gardenieae	Gardenieae	Gardenieae
<i>Coffea</i> L.	Coffeae	Coffeae	Coffeae	Coffeae	Coffeae
<i>Coptosperma</i> Hook.f.		Pavetteae		Pavetteae	Pavetteae
<i>Cordia</i> A.Rich. ex DC.					Cordieryae
<i>CremaSpora</i> Benth.	Gardenieae	Gardenieae	CremaSporaeae	Octotropideae	Octotropideae
<i>Deccania</i> Tirveng.	Gardenieae	Gardenieae	Gardenieae	Gardenieae	Gardenieae
<i>Dictyandra</i> Welw. ex Benth. & Hook.f	Pavetteae	Pavetteae	Pavetteae	Pavetteae	Pavetteae
<i>Didymosalpinx</i> Keay	Gardenieae	Gardenieae	Gardenieae		
<i>Dioecrescis</i> Tirveng.	Gardenieae	Gardenieae	Gardenieae		Gardenieae
<i>Discospermum</i> Dalzell		Gardenieae	Coffeae	Coffeae	Coffeae
<i>Duperrea</i> Pierre ex Pit.		Pavetteae	Gardenieae	Gardenieae	Gardenieae
<i>Durioa</i> L.f.	Gardenieae	Gardenieae	Gardenieae	Gardenieae	Cordieryae
<i>Euclinia</i> Salisb.	Gardenieae	Gardenieae	Gardenieae	Gardenieae	Gardenieae
<i>Fagerlindia</i> Tirveng.	Gardenieae	Gardenieae	Gardenieae		Gardenieae
<i>Feretia</i> Delile	Hypobathreae	Octotropideae		Octotropideae	Octotropideae
<i>Fernelia</i> Comm. ex Lam.	Hypobathreae	Octotropideae		Octotropideae	Octotropideae
<i>Flagenium</i> Baill.	Hypobathreae	Octotropideae			Octotropideae
<i>Galiniera</i> Delile	Hypobathreae	Octotropideae			
<i>Gallienia</i> Dubard & Dop		Octotropideae			Octotropideae
<i>Gardenia</i> J.Ellis	Gardenieae	Gardenieae	Gardenieae	Gardenieae	Gardenieae
<i>Genipa</i> L.	Gardenieae	Gardenieae	Gardenieae	Gardenieae	Gardenieae
<i>Glossostipula</i> Lorence		Gardenieae	Gardenieae	Gardenieae	Cordieryae
<i>Heinsenia</i> K.Schum.	Aulacocalyceae	Aulacocalyceae	Gardenieae		Gardenieae
<i>Hyperacanthus</i> E.Mey. ex Bridson	Gardenieae	Gardenieae	Gardenieae		Gardenieae
<i>Hypobathrum</i> Blume	Hypobathreae	Octotropideae		Octotropideae	Octotropideae
<i>Hyptianthera</i> Wight & Arn.	Hypobathreae	Octotropideae			Octotropideae
<i>Jovetia</i> Guédès	Hypobathreae	Octotropideae			Octotropideae
<i>Kailarsenia</i> Tirveng.	Gardenieae	Gardenieae	Gardenieae	Gardenieae	Gardenieae
<i>Kochummenia</i> K.M.Wong	Gardenieae	Gardenieae	Gardenieae		Gardenieae
<i>Kraussia</i> Harv.	Hypobathreae	Octotropideae		Octotropideae	Octotropideae
<i>Kutchubaea</i> Fisch. ex DC.	Gardenieae	Gardenieae	Gardenieae	Gardenieae	Cordieryae
<i>Lamprothamnus</i> Hiern	Hypobathreae	Octotropideae			Octotropideae
<i>Larsenaikia</i> Tirveng.					Gardenieae
<i>Lemyrea</i> (A.Chev.) A.Chev. & Beille		Octotropideae?			Octotropideae
<i>Leptactina</i> Hook.f.	Pavetteae	Pavetteae	Pavetteae	Pavetteae	Pavetteae
<i>Macrosphyra</i> Hook.f.	Gardenieae	Gardenieae	Gardenieae	Gardenieae	Gardenieae
<i>Mantalanina</i> R.Cap. ex J.-F.Leroy	Gardenieae	Gardenieae	Gardenieae		
<i>Massularia</i> (K.Schum.) Hoyle	Gardenieae	Gardenieae	Gardenieae	Gardenieae	Gardenieae
<i>Melanopsidium</i> Colla		Gardenieae	Gardenieae	Gardenieae	Cordieryae

Table 1. Continued.

	Robbrecht & Puff, 1986: morpho-anatomy	Robbrecht, 1988, emend. in 1993: morpho-anatomy	Andreasen & Bremer, 2000: phylogeny	Robbrecht & Manen, 2006: phylogeny	This study: phylogeny
<i>Mitriostigma</i> Hochst.	Gardenieae	Gardenieae	Gardenieae	Gardenieae	Sherbournieae
<i>Monosalpinx</i> N.Hallé	Gardenieae	Gardenieae	Gardenieae		
<i>Morelia</i> A.Rich. ex DC.	Gardenieae	Gardenieae	Gardenieae	Gardenieae	Gardenieae
<i>Neofranciella</i> Guillaumin		Gardenieae	Gardenieae		Gardenieae
<i>Oligocodon</i> Keay	Gardenieae	Gardenieae	Gardenieae	Gardenieae	Gardenieae
<i>Oxyanthus</i> DC.	Gardenieae	Gardenieae	Gardenieae	Gardenieae	Sherbournieae
<i>Oxyceros</i> Lour.	Gardenieae	Gardenieae	Gardenieae	Gardenieae	Gardenieae
<i>Paracephaelis</i> Baill.		Pavetteae?			Pavetteae
<i>Paragenipa</i> Baill.	Hypobathreae	Octotropideae		Octotropideae	Octotropideae
<i>Pavetta</i> L.	Pavetteae	Pavetteae	Pavetteae	Pavetteae	Pavetteae
<i>Pelagodendron</i> Seem.		Gardenieae	Gardenieae		Pavetteae
<i>Petitiocodon</i> Robbr.		Gardenieae			Octotropideae
<i>Phellocalyx</i> Bridson	Gardenieae	Gardenieae	Gardenieae		Gardenieae
<i>Pleiocoryne</i> Rauschert	Gardenieae	Gardenieae	Gardenieae		Gardenieae
<i>Polysphaeria</i> Hiern	Hypobathreae	Octotropideae		Octotropideae	Octotropideae
<i>Porterandia</i> Ridl.		Gardenieae	Gardenieae	Gardenieae	Gardenieae
<i>Pouchetia</i> A.Rich. ex DC.	Hypobathreae	Octotropideae		Octotropideae	Octotropideae
<i>Preussiodora</i> Keay	Gardenieae	Gardenieae	Gardenieae	Gardenieae	Gardenieae
<i>Ramosmania</i> Tirveng.	Hypobathreae	Octotropideae		Octotropideae	Octotropideae
<i>Randia</i> L.	Gardenieae	Gardenieae	Gardenieae	Gardenieae	Gardenieae
<i>Riodocea</i> Delprete					Cordiereae
<i>Robbrechtia</i> De Block					Pavetteae
<i>Rosenbergiodendron</i> Fagerl.			Gardenieae	Gardenieae	Gardenieae
<i>Rothmannia</i> Thunb.	Gardenieae	Gardenieae	Gardenieae	Gardenieae	Gardenieae
<i>Rubovietnamia</i> Tirveng.					Gardenieae
<i>Rutidea</i> DC.	Pavetteae	Pavetteae	Pavetteae	Pavetteae	Pavetteae
<i>Schizenterospermum</i> Homolle ex Arènes		Pavetteae?			Pavetteae
<i>Schumanniohyton</i> Harms	Gardenieae	Gardenieae	Gardenieae		Gardenieae?
<i>Sericanthe</i> Robbr.	Gardenieae	Gardenieae	Coffeae	Coffeae	Coffeae
<i>Sherbournia</i> G.Don	Gardenieae	Gardenieae		Gardenieae	Sherbournieae
<i>Sphinctanthus</i> Benth.	Gardenieae	Gardenieae		Gardenieae	Gardenieae
<i>Stachyarrhena</i> Hook.f.	Gardenieae	Gardenieae		Gardenieae	Cordiereae
<i>Stenosepala</i> C.H.Perss.					Cordiereae
<i>Sukunia</i> A.C.Smith	Gardenieae	Gardenieae	Gardenieae	Gardenieae	Gardenieae
<i>Tamilnadia</i> Tirveng.	Gardenieae	Gardenieae	Gardenieae	Gardenieae	Gardenieae
<i>Tarenna</i> Gaertn.	Pavetteae	Pavetteae	Pavetteae	Pavetteae	Pavetteae
<i>Tarennoidea</i> Tirveng. & Sastre	Gardenieae	Gardenieae	Gardenieae	Gardenieae	Gardenieae
<i>Tennantia</i> Verdc.	Pavetteae	Pavetteae	Pavetteae		Pavetteae
<i>Tocoyena</i> Aubl.	Gardenieae	Gardenieae	Gardenieae	Gardenieae	Gardenieae
<i>Tricalysia</i> A.Rich. ex DC.	Gardenieae	Gardenieae	Coffeae	Coffeae	Coffeae
<i>Trukia</i> Kaneh.		Gardenieae	Gardenieae	Gardenieae	Gardenieae
<i>Vidalasia</i> Tirveng.					Gardenieae
<i>Xantonnea</i> Pierre ex Pit.	Hypobathreae	Octotropideae		Coffeae	Coffeae

“*Gardenia* clade”, a “*Randia* clade” and an *Alibertia* group (for a complete circumscription of clades, see Persson, 2000a: fig. 2). In the most recent large-scale phylogenetic study of Rubiaceae, Bremer & Eriksson (2009) included 534 taxa from 329 genera, of which over 100 taxa were from Gardenieae and relatives. Using the Bayesian (MCMC) method, they found no support for a monophyletic Gardenieae, but good support for several “Gardenieae” groups that correspond to extended circumscriptions of groups presented above: an “*Alibertia* clade” (*Alibertia*, *Amaioua*, *Borojoa* Cuatrec., *Duroia* L.f., *Genipa*, *Glossostipula* Lorence, *Kutchubaea* including *Ibetrulia* Bremek., *Melanopsidium* Colla, *Stachyarrhena* Hook.f., *Stenosepala* C.H.Perss.; cf. Andreasen & Bremer, 1996; Persson, 2000a, b), a “*Randia* clade” (*Calochone* Keay, *Casasia* A.Rich., *Euclinia* Salisb., *Oligocodon* Keay, *Macrosphyra* Hook.f., *Preussiodora* Keay, *Randia*, *Rosenbergiodendron* Fagerl.), a “*Gardenia* clade” (*Aoranthe* Somers p.p., *Ceriscoides* (Hook.f.) Tirveng., *Coddia* Verdc., *Gardenia*, *Genipa*, *Kailarsenia* Tirveng.; cf. Persson, 2000a), and an “*Aidia* clade” (*Aidia*, *Benkara* Adans., *Hyperacanthus* E.Mey. ex Bridson, *Oxyceros* Lour., *Randia* p.p., *Sphinctanthus* Benth.). *Schumanniphyton* was resolved as the most basal lineage of the Gardenieae in Bremer & Eriksson (2009).

Previous phylogenetic studies have indicated the paraphyletic status of Gardenieae with respect to the previously recognized tribe Aulacocalyceae. Even though other major groups (including tribes) have been resolved as monophyletic, e.g., Octotropideae: Andreasen & Bremer (2000), Tosh & al. (2008), Alejandro & al. (2011); Coffeeae and Bertiereae: Davis & al. (2007), Tosh & al. (2009), the circumscriptions of Gardenieae and its relatives remain unsettled. Moreover, many Gardenieae genera have so far not been included in any molecular phylogenetic studies (see Table 1). Most of them are small genera and often narrow endemics, segregated from the traditionally larger *Gardenia*, *Genipa*, *Randia*, or *Rothmannia* Thunb. The lack of phylogenetic resolution in previous phylogenetic studies within the Gardenieae complex (which is here delimited as the clade shown in Bremer & Eriksson, 2009: fig. 4 and including Cremasporae, “Gardenieae”, Octotropideae, and Pavetteae) despite the use of several phylogenetic markers (*rbcl*, *rps16*, *trnLF*, ITS) is very intriguing. The low resolution could be explained by missing data, lack of variation in the markers used, or data incongruence (as with Rubiaceae-Ixoroideae: see Mouly & al., 2009).

To establish a monophyletic Gardenieae and elucidate the placement of satellite genera such as *Burchellia*, we consequently investigated an extensive sampling of relevant genera within Gardenieae and related tribes. Using five plastid gene regions (*rbcl*, *ndhF*, *trnTF*, *rps16*, *atpB-rbcL*), we performed phylogenetic analyses of combined data in order to obtain a well-resolved and supported tree topology for the Gardenieae complex.

## ■ MATERIALS AND METHODS

**Taxon sampling and molecular regions.** — We included 137 accessions of Gardenieae and its relatives (the Gardenieae complex) which formed the ingroup (Appendix 1). Attention was given to numerous small genera that are poorly known

and have not previously been included in molecular phylogenetic studies, 21 of which were newly sequenced in the present study. When possible we selected type species. We were unable to extract sequenceable DNA for eight genera currently placed in Gardenieae: *Adenorandia* C.Vermoesen, *Aidiopsis* Tirveng., *Alleizettella* Pit., *Fosbergia* Tirveng., *Himalrandia* T.Yamaz., *Pitardella* Tirveng., *Pseudaidia* Tirveng., and *Sulitia* Merr. Outgroup taxa were mainly representatives of tribes of Ixoroideae (including 18 taxa), plus two taxa of subfamily Cinchonoideae, and one species of *Luculia* Sweet. We utilized five regions in the chloroplast genome, *rbcl*, *ndhF*, *trnTF*, *rps16*, and *atpB-rbcL* as data sources, as they have been shown to provide numerous informative characters within Rubiaceae (e.g., Razafimandimbison & al., 2008; Rydin & al., 2008, 2009). The primers used are listed in Rydin & al. (2008: table 2). DNA was extracted, amplified and sequenced using procedures previously described (Kårehed & Bremer, 2007; Rydin & al., 2008, 2009).

**Alignment and phylogenetic reconstruction.** — Sequences were aligned using Se-Al v.2.0 (Rambaut, 1996) and subsequently manually adjusted with MEGA5 (Tamura & al., 2011). Bayesian analyses were performed in MrBayes v.3.1.2p (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003; Altekar & al., 2004). For each single-marker dataset or partition, the best performing evolutionary models were identified under the Akaike information criterion (AIC; Akaike, 1973). We performed these calculations with the software MrAIC v.1.4.3 (Nylander, 2004). Ambiguous regions of the alignments were excluded from the datasets in the analyses. Each marker was used as one partition, but *trnTF* was decomposed into three partitions, one for the *trnT-trnL* spacer, one for the *trnL* intron, and the third for the *trnL-trnF* spacer. Selected models were: (1) GTR+G for the *trnT-trnL*, *trnL-trnF*, *rps16*, and *atpB-rbcL* partitions; (2) GTR+I for the *trnL* partition; and (3) GTR+I+G for the *ndhF* and *rbcl* partitions. Insertion-deletions were mostly autapomorphic, and were not coded. The single-marker analyses were run for  $10^7$  generations, with a sample frequency of 1000 and using four parallel chains. A flat Dirichlet prior probability (all values are 1.0) was selected for the substitution rates (revmatpr) and the nucleotide frequencies (statefreqpr). The analyses of the combined dataset were done using the same settings, but with an extended number of generations ( $30 \times 10^6$ ). Partitions were unlinked so that each partition was allowed to have its own set of parameters. Due to an apparent high level of substitutions for *Schumanniphyton*, we also performed a combined data analysis without *Schumanniphyton* to test the effect of the genus on the inference of relationships and the estimation of posterior probabilities.

Parsimony analyses were conducted using PAUP\* v.4.0b8b (Swofford, 2002). The maximum parsimony (MP) trees were found by heuristic search, tree bisection-reconnection (TBR) branch swapping, using  $1 \times 10^4$  replicates of random stepwise addition, with the MULTREES on. Characters were given equal weight, gaps were treated as missing data, and only phylogenetically informative indels were coded. Consistency index (CI; Kluge & Farris, 1969) and retention index (RI; Farris, 1989) were calculated to estimate homoplasy. To assess relative support for clades, bootstrap values (BS) were estimated from  $1 \times 10^4$  replicates, with MULTREES off, TBR branch swapping,

and five random addition sequences. Groups characterized by bootstrap support of more than 85% were regarded as strongly supported.

**Evolution of morphological and functional traits.** — Four morphological and functional features previously considered taxonomically important for recognizing infratribal groups in Gardenieae, i.e., pollen grain type and aperture type, exotesta type, and breeding system (Robbrecht & Puff, 1986; Persson, 2000a), were selected in order to see how these correspond to the phylogeny. The information was taken from earlier publications, mainly from Robbrecht & Puff (1986) and Persson (1993, 1996). The mapping was done using the software RASP v.2.0b (Yu & al., 2010, 2011), with one of the topologies inferred from the Bayesian analysis, reduced to a single representative per genus, limited to Gardenieae and Octotropideae, and using *Coffea* as outgroup.

## ■ RESULTS

**Data information.** — The complete cpDNA dataset consisted of 6471 characters (of 7977 aligned sites) of which 2003 were from *ndhF*, 1551 from *trnTF*, 803 from the *rps16*, 702 from *atpB-rbcL*, and 1402 from *rbcL*. The combined dataset contained 2524 informative characters (ca. 39%). The *ndhF* gene alone contained a large part of these informative characters (809; vs. 689 for *trnTF*, 456 for *atpB-rbcL*, 323 for *rps16*, and 247 for *rbcL*). Only 56% of the total of informative characters was informative in the ingroup.

Two short inversions were detected within the datasets, a 4-bp segment (corresponding to positions 55472 to 55475 in the *Coffea arabica* L. chloroplast genome GenBank acc. EF044213, Samson & al., 2007) in the *atpB-rbcL* spacer, and a 8-bp segment (positions 5428 to 5435 in the *C. arabica* chloroplast genome) in the *rps16* intron. These inversions were excluded from the analyses.

The genetic distance of *Schumanniohyton magnificum* (K.Schum.) Harms to other taxa of the Gardenieae complex ranged from 0.021 to 0.035 (0.027 mean), and the terminal branch length of *Schumanniohyton* corresponded to 90 substitutions (Electr. Suppl.: Fig. S1A). Genetic distances within the Gardenieae complex, excluding *Schumanniohyton*, ranged from 0.003 to 0.016 (0.009 in mean) and the mean number of substitutions for ingroup branches was 13.3. A 33-bp insertion (position 48422 in the *Coffea arabica* chloroplast genome) was observed in the three *Schumanniohyton magnificum* samples for the *trnLF* intron segment that did not occur in any other genus within the complete dataset.

**Separate analyses.** — Results of individual markers were mostly congruent and incongruences between analyses, when present, were never supported (not shown). Most of these separate analyses contained phylogenetic information to resolve (but rarely support) some of the groups within Gardenieae but not to resolve relationships between these groups. For a summary of resolution and support for groups see Table 2. Of the five markers used, *ndhF* provided the highest number of resolved and supported phylogenetic relationships.

**Combined analyses.** — The Bayesian analysis of the combined data resulted in a partially supported tree topology (Fig. 2). The MP analyses were congruent with the Bayesian analyses with the exception of *Tocoyena* associating with *Sherbournia* G.Don–*Mitriostigma* Hochst. and *Oxyanthus* in MP, but without support. Minor discrepancies between the two analyses were never supported. However, most of main clades inferred in the Bayesian analyses were not supported by the bootstrap analysis (Fig. 2). The ingroup was monophyletic and well supported (PP = 1.00). Tribes Coffeae and Bertiereae were sister to each (PP = 1.00/BS = 59), and in turn sister to the rest of the ingroup (PP = 0.92). The latter comprised two lineages, henceforth called the Octotropideae s.l. clade (PP = 0.95) and the Gardenieae-Pavetteae clade (PP = 1.00), respectively. A number of genera previously placed in Gardenieae were nested in the Octotropideae s.l. clade: *Burchellia*, *Didymosalpinx*, *Mantalania* R.Cap. ex J.-F.Leroy, and *Monosalpinx* N.Hallé, which together with *Galinierya* Delile formed a grade basal to the Octotropideae-Cremasporeae clade, although without highly supported relationships. The Octotropideae-Cremasporeae clade received good support (PP = 1.00/BS = 88), but was internally poorly resolved. Within this latter clade, a lineage of Malagasy and Seychellois genera (from *Gallientia sclerophylla* Dubard & Dop to *Canephora madagascariensis* J.F.Gmel.; PP = 1.00/BS = 52) and a lineage of African–Indian Ocean–Asian genera (from *Petitiocodon parviflorum* (Keay) Robbr. to *Fernelia buxifolia* Lam.; PP = 1.00/BS = 90) were resolved. The Gardenieae-Pavetteae clade consisted of a polytomy including (1) the *Sherbournia* group (PP = 0.96), (2) the *Alibertia* group (PP = 1.00), (3) the Pavetteae (PP = 1.00/BS = 100), and (4) a large but unsupported clade (PP = 0.85) that consisted of five supported groups, the *Gardenia* group (PP = 1.00/BS = 60), the *Rothmannia* group (PP = 1.00/BS = 92), the *Aidia* group (PP = 1.00), the *Randia* group (PP = 0.96), and the *Porterandia* Ridl. group (PP = 0.98), plus two isolated genera: *Schumanniohyton* as sister to the entire clade, and *Massularia* (K.Schum.) Hoyle as unsupported sister to the *Randia* and *Porterandia* groups. Relationships between these groups were resolved, but not supported by high PP values.

The high level of substitutions in *Schumanniohyton* apparently has an influence on phylogenetic reconstruction, as node support increased considerably when the genus was removed from the analysis (Electr. Suppl.: Fig. S1B). The inclusion or exclusion of the genus did not influence the inferred main relationships within the ingroup, although support for the Octotropideae s.l. clade was reduced (PP = 0.92) with its inclusion.

**Evolution of morphological and functional traits.** — The four features mapped onto the phylogenetic tree (Fig. 3) showed contrasting patterns. The different pollen aggregation types and colpus types appeared homoplastic within the Gardenieae complex. However, pollen grains in monads and colporate apertures seem to be plesiomorphic, and porate grains have evolved several times independently in the lineage associating the *Aidia*, *Porterandia* and *Randia* groups, the *Alibertia* group, and the *Sherbournia* group. Tetrads evolved from monads at least three times in *Gardenia*, the *Randia* group, and the *Sherbournia* group. *Massularia* possesses an autapomorphic state

**Table 2.** Summary of the major clades observed in the combined marker analysis and indication of resolution or lack of resolution of similar clades in each of the individual marker analyses and their posterior probability when resolved.

	<i>trnTF</i>	<i>rps16</i>	<i>atpB-rbcL</i>	<i>ndhF</i>	<i>rbcL</i>	combined data
Ingroup	resolved (incl. <i>Albertia</i> ; PP = 0.91)	resolved (PP = 1.00)	resolved (incl. <i>Boholia</i> )	resolved (PP = 0.76)	resolved (PP = 0.97)	resolved (PP = 1.00)
Octotropideae s.l. clade	resolved (excl. <i>Burchellia</i> & <i>Galiniera</i> )	not resolved	not resolved	not resolved	not resolved	resolved (PP = 0.95)
Gardenieae-Pavetteae clade	not resolved	not resolved	not resolved	not resolved	not resolved	resolved (PP = 1.00)
Octotropideae-Cremasporaeae clade	resolved (PP = 0.95)	not resolved	resolved (PP = 0.83)	resolved (PP = 1.00)	not resolved	resolved (PP = 1.00)
Gardenieae (sensu this study)	not resolved	resolved ( <i>Schumanniophyton</i> excluded; PP = 0.70)	not resolved	not resolved	not resolved	resolved (PP = 0.85)
<i>Aidia</i> group	not resolved	unresolved	not resolved	resolved (PP = 0.94)	resolved (PP = 0.78)	resolved (PP = 1.00)
<i>Alibertia</i> group	resolved (PP = 0.84)	resolved (PP = 1.00)	resolved (PP = 1.00)	resolved (PP = 1.00)	resolved (PP = 1.00)	resolved (PP = 1.00)
<i>Gardenia</i> group	resolved (PP = 0.70)	resolved (PP = 0.82)	not resolved	resolved (PP = 1.00)	resolved (PP = 0.94)	resolved (PP = 1.00)
Pavetteae	resolved (PP = 0.98)	resolved (PP = 1.00)	resolved (PP = 1.00)	resolved (PP = 1.00)	not resolved	resolved (PP = 1.00)
<i>Porterandia</i> group	resolved (excl. <i>Brachytome</i> ; PP = 0.63)	not resolved	resolved (excl. <i>Dioecrescis</i> ; PP = 0.89)	resolved (PP = 0.96)	not resolved	resolved (PP = 1.00)
<i>Randia</i> group	resolved (PP = 1.00)	resolved (PP = 0.66)	resolved (PP = 1.00)	resolved (PP = 1.00)	resolved (PP = 1.00)	resolved (PP = 0.96)
<i>Rothmannia</i> group	not resolved	not resolved	resolved (PP = 1.00)	resolved (PP = 0.99)	not resolved	resolved (PP = 0.98)
<i>Sherbournia</i> group	not resolved	not resolved	not resolved	resolved (PP = 0.99)	not resolved	resolved (PP = 0.96)

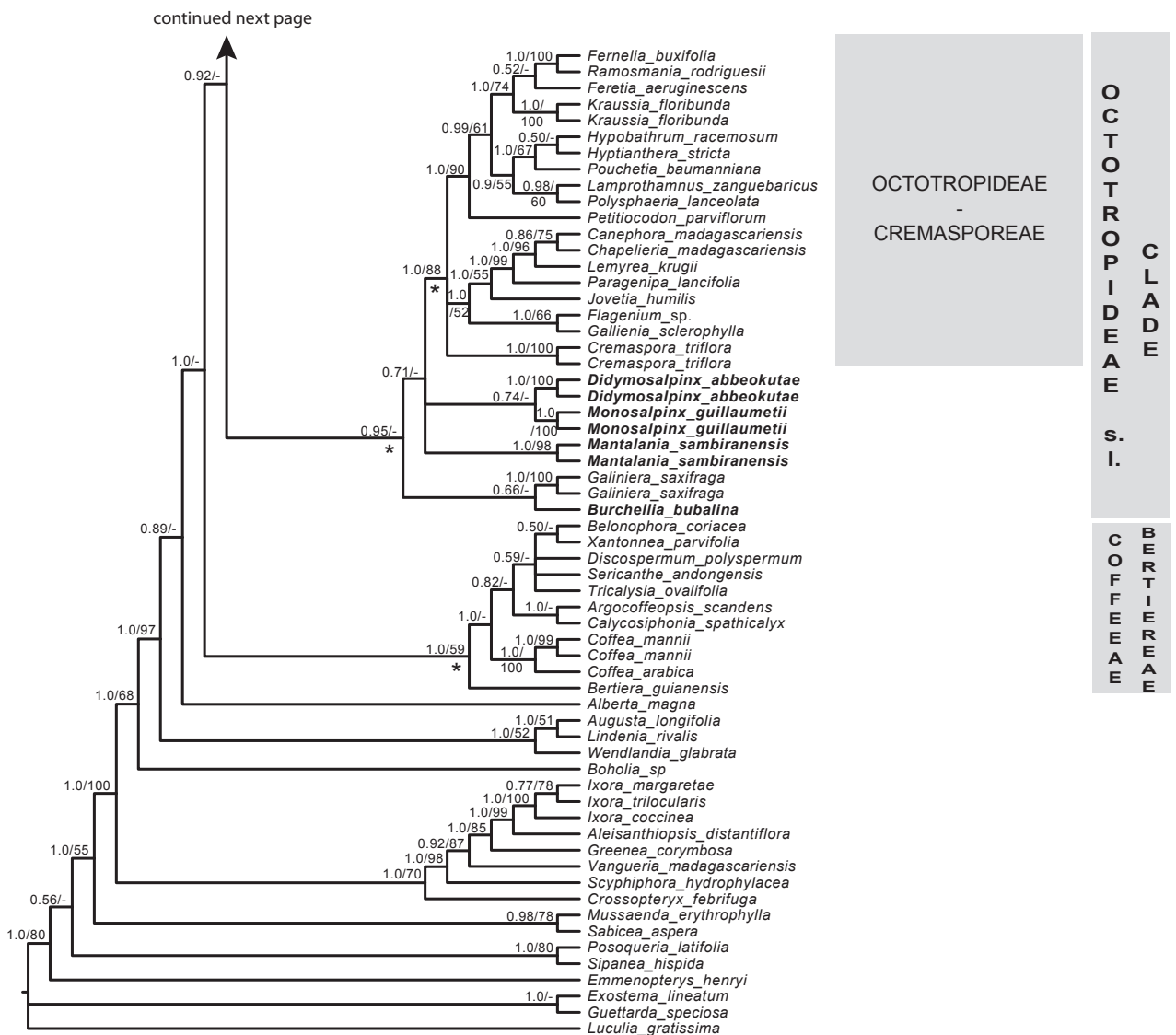


with grains in massulae (polyads). Hermaphrodite flowers were inferred as plesiomorphic with dioecy present in two groups: as a potential synapomorphy of the *Alibertia* group and in several genera of the *Porterandia* and *Randia* groups. The folded testa type appeared to have evolved from an unfolded testa and was characteristic of the *Sherbournia* group, but was homoplasious as it is also present in *Glossostipula*.

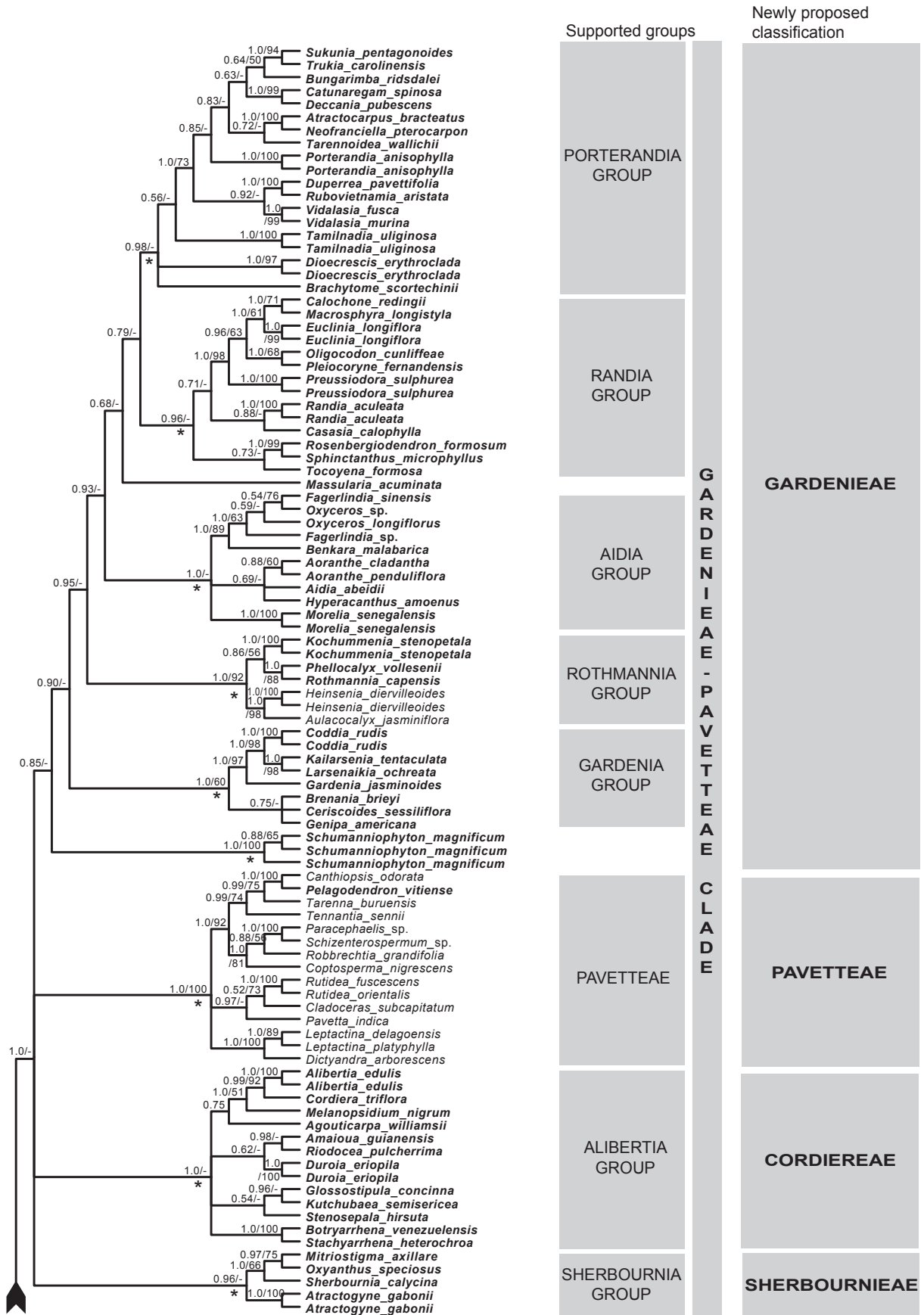
■ DISCUSSION

The ingroup, consisting of the Gardenieae complex plus Coffeae and Bertiereae, is monophyletic with high support. It comprises three major lineages (Fig. 2): the Bertiereae-Coffeae clade, the Octotropideae s.l. clade, and the Gardenieae-Pavetteae

clade. The Gardenieae-Pavetteae and Octotropideae s.l. clades are sisters, with relatively moderate support (PP = 0.92); this species-rich clade is in turn sister to the Bertiereae-Coffeae clade. The latter clade (Fig. 2) includes several members of Gardenieae subtribe Diplosporinae, as shown in earlier studies (Andreasen & Bremer, 2000; Persson, 2000a; Davis & al., 2007; Bremer & Eriksson, 2009). As no other genera of Gardenieae sensu Andreasen & Bremer (2000) investigated in this study belong to the Bertiereae-Coffeae clade, this lineage is not further discussed here. Tribe Gardenieae sensu Robbrecht & Puff (1986, as Gardenieae subtribe Gardeniinae) and Andreasen & Bremer (2000) is polyphyletic as *Burchellia*, *Didymosalpinx*, *Monosalpinx*, and *Mantalania* are more closely related to Octotropideae-Cremasporae (Fig. 2), and Pavetteae and the sampled Aulacocalyceae taxa *Aulacocalyx jasmiflora* Hook.f.



**Fig. 2.** Consensus tree (Bayesian MCMC analysis) of the combined cpDNA sequence data; numbers given at branches are Bayesian posterior probabilities and Bootstrap support (PP/BS) for corresponding nodes. Grey boxes indicate the main groups discussed in the text. Terminals in bold italic indicate taxa currently placed in tribe Gardenieae (as Gardeniinae fide Robbrecht & Puff, 1986; corrected by Andreasen & Bremer, 2000). Asterisks indicate main clades discussed in the text.



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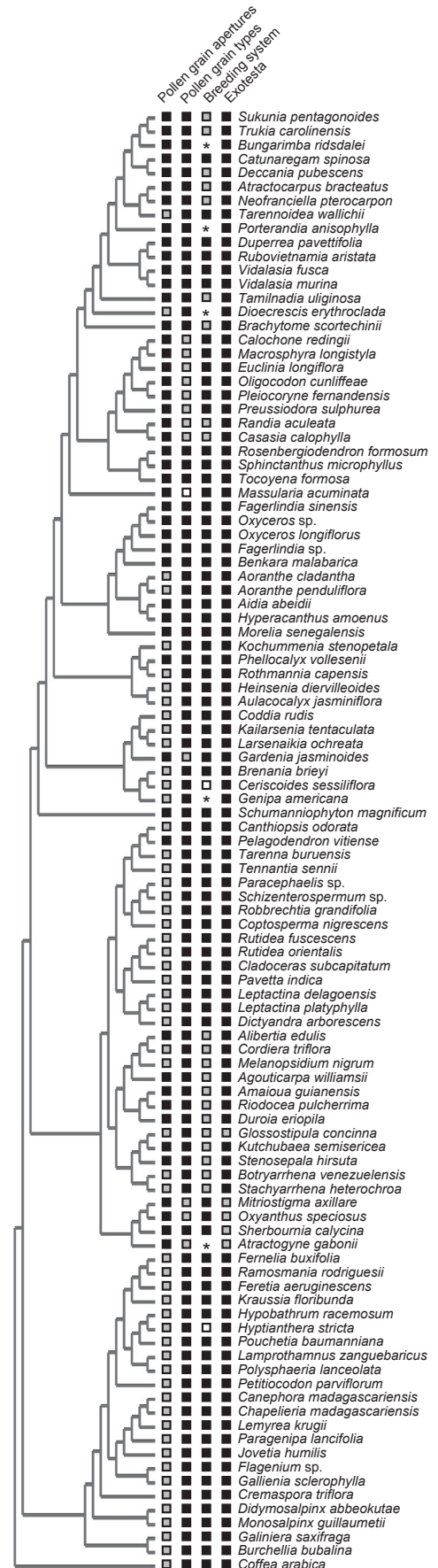
and *Heinsenia diervilleoides* K.Schum. are nested in a supported but partially unresolved Gardenieae-Pavetteae clade (Fig. 2). Our results are congruent with that of Bremer & Eriksson (2009).

### Phylogenetic relationships within the Octotropideae s.l. clade

The Octotropideae s.l. clade (Fig. 2) contains the sequenced members of tribes Octotropideae and Cremasporeae and four Gardenieae genera (*Burchellia*, *Didymosalpinx*, *Mantalania*, *Monosalpinx*). Octotropideae s.str. (Andreasen & Bremer, 2000) comprises about 23 genera from the Old World, of which 17 were here included. A close affinity between *Cremaspora* Benth. and Octotropideae as indicated by previous studies (Persson, 1996; Andreasen & Bremer, 2000; Robbrecht & Manen, 2006; Bremer & Eriksson, 2009) is also supported in our analyses, but we found insufficient resolution to distinguish Cremasporeae and Octotropideae as currently circumscribed.

Two Octotropideae clades were retrieved. One includes most of the genera from Madagascar (*Canephora* Juss., *Chapellieria*, *Lemyrea* (A.Chev.) A.Chev. & Beille, *Flagenium* Baill., *Gallienia* Dubard & Dop, *Jovetia* Guédès) together with *Paragenipa* Baill. from the Seychelles, whereas the other clade includes genera distributed in tropical Africa, Madagascar (only *Polysphaeria* Hiern, which is African-Malagasy-Seychellois), and tropical Asia. Unfortunately, we were unable to obtain sequences for the type of the tribe, *Octotropis* Bedd., an endemic Indian genus, as available specimens of this genus are very few and old (Robbrecht & al., 1994). The West African *Petitiocodon* Robbr. was recently placed in Octotropideae (Tosh & al., 2008), and this placement is supported by our results. The East African *Lamprothamnus* Hiern was here sequenced for the first time and fell in the Octotropideae, a phylogenetic position corroborating its placement by Robbrecht (1980). The Indo-Chinese *Hyptianthera* Wight & Arn. was also newly sequenced here, and its placement in Octotropideae supports that by Robbrecht (1980, 1988) based on morphology. *Hyptianthera* is sister to the tropical Asian *Hypobathrum*, of which it is sometimes considered a synonym. *Villaria* Rolfe, recently sequenced by Alejandro & al. (2011), but not included here, was shown to belong to Octotropideae and is associated with *Pouchetia*. According to Tosh & al. (2008) and the salient features these authors give for Octotropideae, *Cremaspora* appears very close to Octotropideae, a reason why several authors have already proposed to merge Cremasporeae and Octotropideae (e.g., Robbrecht & Manen, 2006).

*Burchellia*, *Didymosalpinx*, *Mantalania*, and *Monosalpinx*, currently placed in tribe Gardenieae, are resolved closer to the Octotropideae-Cremasporeae group. The exclusion of *Burchellia* and *Didymosalpinx* from Gardenieae was suggested by Persson (1996, 2000a), and this result is supported here. *Monosalpinx* and *Mantalania* (Hallé, 1968; Leroy, 1973; respectively) were both newly investigated here; and should now also be excluded from Gardenieae (Hallé, 1968; Leroy, 1973; respectively). The African and Malagasy *Galiniera* is related to the South African *Burchellia*, rendering Octotropideae sensu Andreasen & Bremer (2000) polyphyletic. The African *Monosalpinx* and *Didymosalpinx* share membranaceous polyspermous fruits (Hallé, 1968; Persson, 1996). *Mantalania* is endemic to Madagascar and morphologically very similar to *Pseudomantalania* J.-F.Leroy, another Malagasy endemic not successfully sequenced in the present study. An enlargement of Octotropideae



**Fig. 3.** Morphological and functional traits mapping. The tree topology used is one of the topologies inferred from the Bayesian analysis of the combined cpDNA markers. Characteristics coding: pollen grain apertures (black box, porate/pororate; gray box, colporate); pollen grain types (black box, monads; gray box, tetrads; white box, polyads); breeding system (black box, hermaphroditic; gray box, dioecious; white box, monoecious; star, polygamous); exotesta shape (black box, unfolded; gray box, folded).

to include these genera previously placed in Gardenieae would render the tribe very heterogeneous with respect to their very divergent reproductive features, e.g., inflorescence type and position, and fruit type.

### Phylogenetic relationships within the Gardenieae-Pavetteae clade

The members of Pavetteae sensu Andreasen & Bremer (2000) formed a supported clade, which is nested within the Gardenieae complex (Fig. 2). In contrast to Octotropideae s.l., members of this clade are characterized by terminal or pseudo-axillary inflorescences (with the exception of *Burchellia*).

The placement of *Aulacocalyx* and *Heinsenia* within Rubiaceae has long been debated, with various systematic positions postulated. They have been: (1) included together or individually in Alberteae (Bremekamp, 1966; only *Aulacocalyx* in Robbrecht & Manen, 2006); (2) placed in Ixoreae (Verdcourt, 1958); placed in Gardenieae (only *Heinsenia* in Andreasen & Bremer, 2000); or, (3) in their own tribe Aulacocalyceae (Robbrecht & Puff, 1986). Kainulainen & al. (2009) recently refuted the placement of *Aulacocalyx* in Alberteae (see also Mouly & al., 2007: fig. 1). Our present data clearly show that *Aulacocalyx* and *Heinsenia* are sisters, and are resolved with the *Rothmannia* group (Fig. 2).

The Gardenieae-Pavetteae clade consists of 78 genera that were resolved in supported lineages, of which we discuss the ones indicated in Fig. 2: the *Sherbournia* group, the *Alibertia* group, the Pavetteae, *Schumanniphyton*, the *Gardenia* group, the *Rothmannia* group, the *Aidia* group, *Massularia*, the *Randia* group, and *Porterandia* group.

**Sherbournia group.** — This informal group was resolved by Persson (2000a) and Bremer & Eriksson (2009), including *Atractogyne* Pierre, *Mitriostigma*, and *Oxyanthus*. In the present study it also includes *Sherbournia*, a relationship that received high support (Fig. 2). These four genera are quite heterogeneous morphologically, although many species are lianescent, particularly in *Atractogyne*, *Sherbournia*, and also one species of *Oxyanthus*; *Mitriostigma* species are small to medium shrubs, branched or monocaulous (Sonké & al., 2009). *Sherbournia* has pollen grains in monads, whereas the other three genera all have pollen grains in tetrads (Robbrecht & Puff, 1986; Persson, 1993) (Fig. 3). The corolla shape in this group results from different pollination strategies (Robbrecht & Puff, 1986): in *Sherbournia* and *Atractogyne* the corolla tubes are somewhat campanulate and more than 4 cm long, in *Oxyanthus* the corolla is 4 to 25 cm long and narrow, whereas in *Mitriostigma* the corolla tube is less than 3 cm long. Anatomical structures of seeds may provide better morphological support to the group than gross morphology alone. The endosperm is entire, with a more or less folded exotesta (Hallé, 1962a; Bridson, 1979; Robbrecht & Puff, 1986) (Fig. 3).

**Alibertia group.** — This informal group was resolved with strong support by Persson (1996, 2000a), and included *Agouticarpa* C.H.Perss. (as *Genipa williamsii* Standl.), *Alibertia* (incl. *Borojoa*), *Amaioua*, *Duroia*, *Glossostipula*, *Kutchubaea*, *Melanopsidium*, *Stachyarrhena*, and *Stenosepala* (as species

nova). *Botryarrhena* Ducke was shown to belong to the group by Cortés-Ballén & al. (2009). In the present study we found that also *Cordia* and *Riodocea* Delprete are associated with the *Alibertia* group, which appears to be restricted to the Neotropics. The group can be characterized morphologically by the combination: dioecy (Fig. 3) usually with sexually dimorphic inflorescences, heteromerous flowers, and pollen grains shed as monads (Fig. 3). *Cordia* was initially not placed in Gardenieae by Candolle (1830) because of its multilocular fruits. Multilocular fruits, however, are observed in *Alibertia*, *Stenosepala*, and *Agouticarpa*, whereas the other genera of the group have more typical *Gardenia*-like fruits (see Bremer & Eriksson, 1992: berries with a dense fleshy mesocarp and a pulpy placenta embedding the seeds). *Riodocea* (Delprete, 1999) is a monotypic genus from Brazil with numerous corolla lobes (12–15 lobes), compared to the typical 5–6 lobes of Gardenieae (Robbrecht & Puff, 1986). Delprete (1999) suggested a close relationship of the genus with *Kutchubaea*, but from our tree topology, *Riodocea* is closer to *Amaioua* and *Duroia*.

**Pavetteae.** — Pavetteae form a supported clade of 13 genera. The Pacific *Pelagodendron* Seem., which was merged into *Aidia* by Puttock (2001), is here placed unambiguously in the Pavetteae, close to *Tarenna*. A persistent confusion between *Pelagodendron* and *Canthiopsis* Seem. has been noted in literature (Smith & Darwin, 1988; Tirvengadam, 1991; Puttock & Quinn, 2000). The distinction has generally been made based on the post-fertilization placental development in the fruit, present in *Pelagodendron vitiense* Seem. and absent in *Canthiopsis*, justifying the placement in Gardenieae and in Pavetteae, respectively. The new position of *Pelagodendron vitiense* renders the *Pavetta* group non-homogeneous in terms of placental development, but Puttock & Quinn (2000: 196) stressed that in *Aidia* and *Pelagodendron* the placental development is limited, so that it does not look like either Gardenieae or Pavetteae. The position of two New Caledonian species also placed in *Pelagodendron* (Tirvengadam, 1991) remains to be tested. The Fijian *Canthiopsis*, previously synonymized with *Tarenna* (Smith & Darwin, 1988), is here confirmed to belong to tribe Pavetteae, in close association with both *Pelagodendron* and *Tarenna*.

**Schumanniphyton.** — The genus occurs in tropical West Africa and consists of three species only. According to the “simple” habit and the high synchronicity of growth and flowering of *Schumanniphyton problematicum* (A.Chev.) Aubrév., the genus has been suggested to have retained archaic features of Rubiaceae and Gardenieae (Hallé, 1962b, 1970). The tree topologies presented in this study (Fig. 3; Electr. Suppl.: Fig. S1A) instead suggest that these characteristics are derived within Rubiaceae, although the position within the Gardenieae-Pavetteae clade is resolved but not well supported. Moreover, both the coding and the non-coding regions of *Schumanniphyton* cpDNA show numerous autapomorphies and consequently a long branch-length (Electr. Suppl.: Fig. S1A), indicating a higher relative rate of molecular evolution compared to other lineages of the Gardenieae-Pavetteae clade. We found no support for the earlier proposal (e.g., Persson, 1996; Robbrecht & Manen, 2006) to exclude *Schumanniphyton* from Gardenieae, a result in agreement with Bremer & Eriksson (2009).

Some important morphological characteristics clearly relate this genus to Gardenieae. The reduction of a leaf of the terminal leaf-pair supporting an inflorescence, characteristic of *Schumanniohyton*, is present in all members of *Kochummenia* K.M.Wong and *Rothmannia* and is common in the *Aidia* and the *Porterandia* groups (Wong, 2004). This form of leaf reduction and terminal flowering ties in with an apparently linear sympodial branch development (often resulting in the apparently “leaf-opposed inflorescences” found remarkable by morphologists) and is not known for Pavetteae.

**Gardenia group.** — An informally named *Gardenia* clade comprising *Aorantho*, *Ceriscooides*, *Gardenia*, *Genipa*, and *Kailarsenia* was resolved in the study by Persson (2000a). In Bremer & Eriksson (2009) the same group was supported (also including *Coddia*). The clade was also well supported in our analyses, although *Aorantho* (two specimens) is nested within the *Aidia* group. In addition, the newly investigated genera *Brenania* Keay and *Larsenaikia* Tirveng. are also part of the *Gardenia* group. *Larsenaikia* is endemic to northern Australia and is sister group to the South East Asian *Kailarsenia*, the former being a segregate of the latter (Tirvengadam, 1993). The *Gardenia* group appears very heterogeneous morphologically, and is one of two pantropical lineages together with the *Randia* group (see also Persson, 2000a; Gustafsson & Persson, 2002). Among the genera present in this group, *Gardenia* is the only one that produces pollen grains in tetrads (Fig. 3), all the others have monads (Persson, 1993; Dessein & al., 2005). Tetrads are found elsewhere in the Gardenieae-Pavetteae clade, namely in the *Randia* and the *Sherbournia* groups (Fig. 3).

**Rothmannia group.** — The *Rothmannia* group includes five genera, of which *Rothmannia* is the most widely distributed with ca. 40 species in the Old World. However, the monophyly of the genus has never been tested. In the phylogenetic tree presented here (Fig. 2), representatives of *Aulacocalyx* and *Heinsenia* form a clade, which in turn is sister to a clade comprising *Phellocalyx* Bridson, *Rothmannia*, and the newly investigated genus *Kochummenia*. Within this clade *Phellocalyx* and *Rothmannia* form a clade that is sister to *Kochummenia*. *Kochummenia* was described by Wong (1984) and includes three tropical Asian species, of which one was segregated from *Rothmannia* based on morphological characters. Indeed, in *Kochummenia*, the corolla shape is tubular and the lobes are much longer than the tube, a feature unique to that genus (Wong, 1984; Robbrecht & Puff, 1986). In *Rothmannia* the corolla can be campanulate, infundibular or salverform, with broad lobes shorter than the tube. *Phellocalyx* (Bridson & al., 1980), an East African monotypic genus, also has longer lobes than tubes, but differs from *Kochummenia* and *Rothmannia* by an increased number of corolla lobes (8–11 vs. 5 or rarely 8 in *Rothmannia*) and a spathiform calyx (vs. lobed). Interestingly, *Rothmannia* in its current circumscription includes both species with corolla lobes strictly contorted to the left (including African and Asian species) and species with a strict right contortion (exclusively African and Indian Ocean species), *Phellocalyx* has right-contorted corolla lobes and *Kochummenia* has left-contorted corolla lobes. *Aulacocalyx* and *Heinsenia* mainly differ from the three other genera of the group by their axial placentation,

and their reduced or absent seed-coat (Robbrecht & Puff, 1986). As *Kochummenia* and several *Rothmannia* species, *Aulacocalyx* and *Heinsenia* species present a left-contorted aestivation.

**Aidia group.** — A group including *Aidia*, *Benkara* (as *Griffithia*), *Fagerlindia* Tirveng., and *Oxyceros* was suggested by Robbrecht & Puff (1986) based on morphology (shape and size of fruits, seeds, and exotesta cells). Recently, in Bremer & Eriksson (2009), the group was supported, and included *Aidia*, *Benkara*, *Oxyceros*, *Hyperacanthus* and few non-Neotropical *Randia* species (see *Randia* group below). In the present analysis, the group is highly supported and also includes *Aorantho* and *Morelia*. We found no support for a broad *Aidia* group, as suggested by Persson (1996) based on morphology, which includes genera such as *Bertiera*, *Atractocarpus* Schltr. & K.Krause, and *Pelagodendron*. *Fagerlindia* was merged into *Benkara* (Ridsdale, 2008) based on morphology, but our present molecular data show that *Oxyceros* is paraphyletic with respect to *Fagerlindia*, and the species representing *Benkara* is sister to the *Fagerlindia*-*Oxyceros* complex (Fig. 2). We retrieved good support for the segregation of *Aorantho* from *Porterandia* proposed by Somers (1988; see above). In the group, *Aidia* and *Hyperacanthus* are most diverse in terms of number of species, with ca. 60 (Ridsdale, 1996) and an estimated ca. 50 species (Rakotonasolo & Davis, 2006), respectively. *Hyperacanthus* shares the unusual feature of right-contorted aestivation, which in Rubiaceae is only recorded for several *Rothmannia* species, *Phellocalyx* (Bridson & al., 1980), and *Gardeniopsis* Miq. (A.P. Davis, pers. obs.), the last of which was not sampled in this study.

**Massularia.** — This is a tropical African monotypic genus, and its species was previously classified as *Gardenia*. It differs from the other Gardenieae by having inflorescences with scorpioid terminal axes and pollen grains arranged in massulae (polyads; see Dessein & al., 2005: fig. 3), a feature unique in Gardenieae. It is here resolved as sister lineage to the *Randia* and the *Porterandia* groups, although with poor support.

**Randia group.** — This group comprises three distinct lineages, one of which consists of the African genera *Calochone*, *Euclinia*, *Macrosphyra*, *Oligocodon*, *Pleiocoryne* Rauschert, and *Preussiodora*, which is sister to a Neotropical lineage comprising *Casasia* and *Randia* (sensu Gustafsson & Persson, 2002). Members of these two lineages of the *Randia* group have pollen grains in tetrads (Fig. 3). The third lineage contains the Neotropical *Rosenbergiodendron*, *Sphinctanthus*, and *Tocoyena*, all with pollen grains in monads (Persson, 2000a) (Fig. 3). The newly investigated African *Pleiocoryne* was resolved as sister to another African genus, *Oligocodon*. As discussed by Persson (1995, 2000a), the genera of his *Randia* group share smooth exotesta cells (with the exception of *Casasia*), a feature also present in *Pleiocoryne*.

**Porterandia group.** — Several of the newly investigated genera, i.e., *Brachytome* Hook.f., *Bungarimba* K.M.Wong, *Dioecrescis* Tirveng., *Rubovietnamia* Tirveng., *Sukunia* A.C.Smith, *Trukia* Kaneh., and *Vidalasia* Tirveng., appear to be associated with *Porterandia*. The *Porterandia* group comprises here 15 genera that are mainly distributed in tropical Asia, Australia, and the Pacific Islands. Robbrecht & Puff (1986) considered *Atractocarpus* best placed in their Gardeniinae,

but noted that it also resembles some members of Pavetteae in a number of characters (pauciflorous terminal inflorescences on lateral branches, often with leafy bracts, and exotesta cells with delicate thickenings). Puttock (2000) reduced *Neofranciella* Guillaumin, *Sukunia*, *Sulitia*, and *Trukia* to synonymy of *Atractocarpus*, according to the results of a phylogeny by Puttock & Quinn (2000) based on morphological characters. This broad concept of *Atractocarpus* was criticized by Wong (2004) when he described *Bungarimba* following a morphology-based phylogenetic analysis. He showed that *Atractocarpus* sensu Puttock was paraphyletic, with *Porterandia* nested within it (Wong, 2004: fig. 2). Our molecular phylogenetic results also question the circumscription of *Atractocarpus* proposed by Puttock (2000), considering evidence (though not supported) for close relationships of *Sukunia*, *Trukia*, and *Bungarimba* to *Catunaregam* and *Deccania* Tirveng. rather than to *Atractocarpus* and *Neofranciella*. Our analyses confirm the close relationship of *Rubovietnamia* to *Duperrea* recently proposed by Mou & Zhang (2010). Furthermore, the continental Asian *Vidalasia* described as a close relative of *Rubovietnamia* by Tirvengadum (1998), was retrieved, in our study, as sister to the clade of *Duperrea* and *Rubovietnamia*.

Most of the taxa associated with *Atractocarpus* and *Porterandia* share polygamous or unisexual flowers (Wong, 2004), a feature relatively rare in the Gardenieae outside the *Alibertia* group (Fig. 3). The fruits of both *Brachytome* and *Duperrea* lack an arilloidal placenta and can be interpreted as drupes for *Duperrea*, characteristics which have been used to separate Gardenieae and Pavetteae. However, the lack of developed placentas in these genera appears to be autapomorphic within the *Porterandia* clade.

### Implications of the phylogenetic results for Gardenieae complex classification

The initial delimitation of Gardenieae by Candolle (1830) is unnatural as it includes genera here placed within each of the three clades of the ingroup. The phylogenetic support values for the Octotropideae s.l. clade (Fig. 2; Electr. Suppl.: Fig. S1) do not confer enough confidence to include *Burchellia*, *Mantalania*, *Didymosalpinx*, and *Monosalpinx*, in the so far well-defined Octotropideae-Cremasporaeae group. The Gardenieae-Pavetteae clade members (Fig. 2) also contain considerable morphological diversity, and, when considered a single tribe, would be highly heterogeneous. Two main taxonomic strategies can be considered. One is to accommodate the members of the Gardenieae-Pavetteae clade (Aulacocalyceae, Pavetteae, Gardenieae) in one tribe; the name of this clade would have to be Pavetteae (Dumortier, 1829), which is older than Gardenieae (Candolle, 1830) and Aulacocalyceae (Robbrecht & Puff, 1986). The second option is to recognize the lineage of *Massularia* and five of the supported groups as tribe Gardenieae (Aulacocalyceae as a synonym). This clade received only moderate support (PP = 0.90) in the Bayesian analysis (Fig. 2), but was well supported in the Bayesian analysis when *Schumannio-phyton* with a high amount of autapomorphic characters was excluded from the dataset (PP = 0.99; Electr. Suppl.: Fig. S1B).

This option allows the conservation of tribe Pavetteae as currently delimited (though including *Pelagodendron*). On the other hand, it necessitates the recognition at tribal level of the two remaining supported groups: the *Alibertia* group and the *Sherbournia* group. Under this second scenario, the *Alibertia* group has to be called Cordiereae, the only tribal level name available, and a new tribe needs to be described for the *Sherbournia* group. This alternative stabilizes the well-established name Gardenieae and the current circumscription of Pavetteae (plus *Pelagodendron vitiense*), both firmly used in Rubiaceae literature. The placement of *Schumannio-phyton* is not solved yet. We provide here a new circumscription for Gardenieae (Fig. 1); recognize the *Alibertia* group at a tribal level, as Cordiereae; and describe a new tribe for the *Sherbournia* group. Pavetteae, though enlarged to include *Pelagodendron*, does not differ significantly from the concept proposed by Andreasen & Bremer (2000), and therefore does not require a new circumscription.

**Sherbournieae** Mouly & B. Bremer, **tr. nov.** – Type: *Sherbournia* G. Don in Loudon, *Encycl. Pl., Suppl.* 2: 1303. 1855.

Diagnosis: Sherbournieae differ from Gardenieae by their seeds possessing a folded testa (vs. unfolded).

Monococious subshrubs, shrubs, or lianas, with hermaphroditic or gynomonococious flowers. Stipules interpetiolar, entire. Raphides absent. Inflorescences always pseudo-terminal, pauciflorous to multiflorous. Aestivation contorted to the left. Secondary pollen presentation present. Stigma club-shaped or capitate, stigmatic lobes fused over most of their length. Ovary with 2 carpels, unilocular, placentas parietal with many ovules. Fruit a fleshy berry with seeds imbedded in the pulpy placenta. Seeds without adaxial excavation, exotestal, with secondary thickenings and folded testa. Pollen grains in monads or in tetrads, 3(–4)-aperturate, porate or pororate.

Genera included: *Atractogyne*, *Mitriostigma*, *Oxyanthus*, *Sherbournia*.

**Cordiereae** A. Rich. ex DC. emend. Mouly – Type: *Cordia* A. Rich. ex DC., *Prodr.* 4: 342, 445. 1830.

Diococious shrubs or trees. Stipules interpetiolar, entire. Raphides absent. Inflorescence terminal, solitary flower or rarely pauciflorous inflorescence in females and usually multiflorous in males. Flowers heteromorphic. Aestivation contorted to the left. Secondary pollen presentation absent. Stigma linear in male flowers and fusiform in female flowers, stigmatic lobes divided. Ovary of 2–7 carpels, uni- to plurilocular, placentas axile or parietal with (1–)3 to many ovules. Fruit a berry with more or less dry or fleshy walls (*Cordia*). Seeds usually imbedded in a pulpy placenta. Seeds without adaxial excavation, exotestal, with thickenings. Pollen grains in monads, 3(–7)-aperturate, porate, pororate or colporate.

Genera included: *Agouticarpa*, *Alibertia*, *Amaioua*, *Botryarrhena*, *Cordia*, *Duroia*, *Glossostipula*, *Kutchubaea*, *Melanopsidium*, *Riodocea*, *Stachyarrhena*, *Stenosepala*.

**Gardenieae** A. Rich. ex DC., *Prodr.* 4: 342, 367. 1830 – Type: *Gardenia* J. Ellis in *Philos. Trans.* 51: 935. 1761, nom. cons.

= Aulacocalyceae Robbr. & C.Puff – Type: *Aulacocalyx* Hook.f., Hooker's Icon. Pl. 12: t. 1126. 1873.

Hermaphrodite, monoecious or dioecious shrubs, trees, lianas, or rarely (hemi-)epiphytic shrubs. Stipules interpetiolar, entire. Raphides absent. Inflorescences terminal or pseudo-axillary. Aestivation contorted to the left, rarely to the right. Secondary pollen presentation predominantly present. Stigma club-shaped, capitate or linear, stigmatic lobes fused over most of their length (divided lobes rare). Ovary of 2–9(–16) carpels, uni- to plurilocular, placentas axile or parietal with one to many ovules. Fruit a berry with a dense fleshy mesocarp and a pulpy placenta embedding the seeds (known as *Gardenia*-type) or rarely a drupe (*Duperrea*). Seeds with or without adaxial excavation, mostly exotestal (seed-coat reduced in *Duperrea*, and *Heinsenia*), usually with thickenings. Pollen grains in monads, tetrads, or massulae, 3(–4)-porate, 3–4(–5)-colporate, or rarely pantoporate.

Genera included: *Aidia*, *Aoranthe*, *Atractocarpus*, *Aulacocalyx*, *Benkara*, *Brachytome*, *Brenania*, *Bungarimba*, *Calochone*, *Casasia*, *Catunaregam* Wolf, *Ceriscoides*, *Cordia*, *Deccania*, *Dioecrescis*, *Duperrea*, *Euclinia*, *Fagerlindia*, *Gardenia*, *Genipa*, *Heinsenia*, *Hyperacanthus*, *Kailarsenia*, *Kochummenia*, *Larsenaikia*, *Macrosphyra*, *Massularia*, *Morelia* A.Rich. ex DC., *Neofranciella*, *Oligocodon*, *Oxyceros*, *Phellocalyx*, *Pleiocoryne*, *Porterandia*, *Preussiodora*, *Randia*, *Rosenbergiodendron*, *Rothmannia*, *Rubovietnamia*, *Sphinctanthus*, *Sukunia*, *Tamilnadia*, *Tarennoidea* Tirveng. & Sastre, *Tocoyena*, *Trukia*, *Vidalasia*.

Genus possibly associated: *Schumanniphyton*.

Genera previously associated with Gardenieae, based on the above morphological characteristics, but not included in a molecular phylogeny so far: *Adenorandia*, *Aidiopsis*, *Alleizettella*, *Fosbergia*, *Himalrandia*, *Pseudaidia*, *Pseudomantalanina*, *Sulitia*.

Genera excluded: *Burchellia*, *Mantalanina*, *Didymosalpinx*, *Monosalpinx*, and members of Sherbournieae and Cordiereae as listed above.

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**Appendix 1.** Accession numbers for included nucleotide sequences, presenting species name, voucher, and cpDNA markers in the following order: *rbcl*, *rps16*, *trnTF*, *ndhF*, *atpB-rbcl*.

*Agouticarpa williamsii* (Standl.) C.H.Perss., *Persson & Nodernhäll 441* (GB), KF964807, KF964894, KF965153, KF965008, KF964676. *Aidia abeiddii* S.E.Dawson & Guereau, *Luke 8317* (UPS), –, KF964895, KF965154, –, –, *Alberta magna* E.Mey., *Bremer & Bremer 3773* (UPS), Y18708, FM204702, FM207110, KF965009, KF964677. *Aleisanthopsis distantiflora* (Mer.) Tange, *Kessler & al. 41* (P), EU817411, EU817434, EU817453, –, –, *Alibertia edulis* (A.Rich.) A.Rich. 1, M.K. 880325-1/1 (UPS), KF964808, KF964896, KF965155, KF965010, KF964678, 2, M.K. s.n. (GB), –, KF964897, KF965156, KF965011, KF964679. *Amauhoa guianensis* Aubl., *Persson & al. 2061* (GB), AM117202, AF200976, KF965157, KF965012, KF964680. *Aoranthe cladantha* (K.Schum.) Somers., *McPherson 16283a* (P), KF964809, –, KF965158, KF965013, KF964681. *Aoranthe penduliflora* (K.Schum.) Somers., *Iverssen & Steiner 86776* (UPS), Y11845, KF964898, KF965159, KF965014, KF964682. *Argocoffeopsis scandens* (K.Schum.) Robbr., *Davis 3016* (K), –, –, DQ1805668, –, –, *Atractocarpus bracteatus* Schltr. & K.Krause, *Mouly 149* (P), KF964810, KF964899, KF965160, KF965015, KF964683. *Atractogyne gabonii* Pierre 1, *Hladik 2323A* (P), KF964811, KF964900, KF965161, KF965016, KF964684; 2, *Hladik 2035A* (P), KF964812, KF964901, KF965162, KF965017, KF964685. *Augusta longifolia* (Sprng.) Rehder, *Macias 96-348* (NY), –, AF242913, KF965163, –, –, *Aulacocalyx jasminiflora* Hook.f., *Schmidt & al. 1672* (MO), KF964813, KF964902, KF965164, HM164354, KF964686. *Belonophora coriacea* Hoyle, *Maurin 19* (K), –, –, DQ180570, –, –, *Benkara malabarica* (Lam.) Tirveng., *Meebold 13753* (S), AM117207, KF964903, KF965165, KF965018, KF964687. *Bertiera guianensis* Aubl., *Bremer & al. 3363* (UPS), AJ224845, KF964904, KF965166, KF965019, –, *Boholia* sp., *Bicknell 1516A* (SUNIV) AM117210, FM204711, HM164312, KF965020, KF964688. *Botryarrhena venezuelensis* Steyerl., *Persson & al. 625* (GB), KF964814, KF964905, KF965167, KF965021, KF964689. *Brachytome scortechinii* King & Gamble 1, *David 136* (P), –, –, KF964906, KF965168, –, –, 2, *Geesink & Santisuk 5154* (P), –, –, –, KF965022, –, *Brenania brieyi* (De Wild) E.E.A.Petit, *Hallé 4214* (P), –, –, KF964907, KF965169, –, –, *Bungarimba ridsdalei* K.M.Wong, *Wong 2875* (KLU), KF964815, KF964908, KF965170, KF965023, KF964690. *Burchellia bubalina* (L.f.) Sims, *Bremer 3129* (UPS-HORT), Z68833, KF964909, KF965171, KF965024, KF964691. *Calochone redingii* (De Wild.) Keay 1, *Chase 3355* (K), –, AF200986, AF201036, –, –, 2, coll. unknown (K 820315), Z68845, KF964910, KF965172, KF965025, KF964692. *Calycosiphonia spathicalyx* (K.Schum.) Robbr., *Luke 8330* (UPS), AM117211, KF964911, KF965173, KF965026, KF964693. *Canephora madagascariensis* J.F.Gmel., *Razafimanambison 468* (UPS), KF964816, KF964912, KF965174, KF965027, KF964694. *Canthiopsis odorata* Seem., *Smith 6560* (P), KF964817, KF964913, KF965175, KF965028, KF964695. *Casasia calophylla* A.Rich., *Rova & al. 2259B* (S), KF964818, KF964914, KF965177, KF965030, KF964697. *Catunaregam spinosa* (Thunb.) Tirveng., *Luke 8332A* (UPS), AM117214, KF964915, KF965178, KF965031, KF964698. *Ceriscoides sessiliflora* (Wall. ex C.B.Clarke) Tirveng., *Maxwell 87-967* (AAU), KF964819, KF964916, KF965179, KF965032, KF964699. *Chapelieria madagascariensis* A.Rich. ex DC., *Pettersson & Nilsson 20* (S), KF964820, KF964917, KF965180, KF965033, KF964700. *Cladoceras sucapitum* (K.Schum. & K.Krause) Bremek., *Luke 8351* (UPS), AM117216, AM117290, KF965181, KF965034, KF964701. *Coddia rudis* (E.Mey. ex Harv.) Verdc. 1, *Bremer 3764* (UPS), AJ286695, KF964918, KF965182, KF965036, KF964703; 2, *Bremer & al. 4279* (UPS), KF964821, AM117291, AM117353, KF965035, KF964702. *Coffea arabica* L., cultivated (UPS), X83631, KF964919, KF965183, KF965037, KF964704. *Coffea mannii* (Hook.f.) A.P.Davis 1, *Andreasen 223* (UPS), –, –, KF965248, KF965117, –, 2, coll. unknown (UPS 71PT000839), KF964872, KF964980, KF965249, KF965118, –, *Coptosperma nigrescens* Hook.f., *Razafimanambison 442* (UPS), KF964822, KF964920, KF965184, KF965038, KF964705. *Cordia triflora* A.Rich. ex DC., *Persson & Gustafsson 302* (GB), KF964823, KF964921, KF965185, KF965039, KF964706. *Cremaspora triflora* K.Schum. 1, *Andreasen 51* (UPS), KF964824, KF964922, KF965186, KF965040, KF964712; 2, *Andreasen 69* (UPS), Z68856, KF964923, KF965187, KF965041, KF964708. *Crossopteryx febrifuga* Benth., *Bremer 3097* (UPS), AM117223, FM204717, FM207123, KF965042, KF964709. *Deccania pubescens* (Roth) Tirveng. 1, *Fisher s.n.* (K), –, AF200991, –, –, –, 2, *Tirvengadam s.n.* (P), KF964825, KF964923, KF965188, KF965043, KF964710. *Dictyandra arborescens* Welw. ex Benth. & Hook.f., *Sonké 1788* (BR), AJ286708, AM117300, KF965189, KF965044, KF964711. *Didymosalpinx abbeokutae* (Hiern) Keay 1, *Adam 26309* (P), KF964826, KF964925, KF965190, KF965045, KF964712; 2, *Verdcourt & Hesse 268* (S), KF964827, KF964926, KF965196, KF965053, KF965046, KF964713. *Diocresciss erythroclada* (Kurz) Tirveng. 1, *Wong s.n.* (KLU), KF964828, KF964927, KF965192, KF965047, KF964714; 2, *Wong s.n.* (KLU), –, KF964928, –, KF965048, –, *Discospermum polyspermum* (Valeton) Ruhsam, *Ridsdale IV.E:130* (S), AJ286703, AM117301, EU145538, KF965049, –, *Duperrea pavettifolia* (Kurz) Pit., *Bremer s.n.*, cult. Bergianska (S), KF964829, KF964929, KF965194, KF965051, KF964715. *Duroia eriophila* L.f. 1, *Persson & al. 1970* (GB), KF964830, KF964930, KF965195, KF965052, KF964716; 2, *Persson & al. 2012* (S), KF964831, KF964931, KF965196, KF965053, KF964717. *Emmenopterys henryi* Oliv., *Robbrecht s.n.* (UPS), Y18715, FM204719, FM207125, KF965054, KF964718. *Euclinia longiflora* Salisb. 1, *De Block 27* (BR), Z68835, KF964932, AJ847399, KF965055, KF964719; 2, *Mouly 346* (P), KF964832, KF964933, KF965197, KF965056, KF964720. *Exostema lineatum* Roem. & Schult., *Andersson 2208* (GB)/*McDowall 4353D* (UPS), –, AF242944, AJ346924, KF965057, –, *Fagerlindia sinensis* (Lour.) Tirveng., *Gressitt 956* (S), –, –, KF965198, KF965058, KF964721. *Fagerlindia* sp., *ST 1818* (K), KF964833, KF964934, KF965199, KF965059, KF964722. *Feretia aeruginensis* Stapf., *Bremer 3137* (UPS), Z68857, AM117305, EU145539, –

## Appendix 1. Continued.

KF965060, KF964723. *Fernelia buxifolia* Lam., *De Block s.n.* (BR), AJ286704, AM117306, EU145540, KF965061, KF964724. *Flagenium sp.*, Davis 1210 (K), KF964834, KF964935, KF965200, KF965062, KF964725. *Galineria saxifraga* (Hochst.) Bridson. 1, *Hylander s.n.* (S), KF964835, KF964936, KF965201, KF965063, KF964726; 2, *Luke 8289* (S), KF964836, KF964937, KF965202, KF965064, KF964727. *Galliena sclerophylla* Dubard & Dop., Davis 2192 (K), KF964837, KF964938, KF965203, KF965065, KF964728. *Gardenia jasminoides* J.Ellis, *Bremer 2720* (UPS), AJ286697, KF964939, KF965204, KF965066, KF964729. *Genipa americana* L., *Kiehn*, HBV sub RR-420 (WU, UPS), Z68839, KF964940, KF965205, KF965067, KF964730. *Glossostipula cinnina* (Standl.) Lorence, *Keller 1901* (CAS), Z68846, KF964941, KF965206, KF965068, KF964731. *Greenea corymbosa* Voigt., *Larsen & al. 44102* (AAU)/*Larsen & al. 43140* (P), –, AF242961, EU817461, –, –, *Guettarda speciosa* L., *Rova 2492* (GB), KF964838, GQ852509, –, KF965069, –, *Heinsenia diervilleoides* K.Schum. 1, *Mizray & Pocs 84405* (UPS), KF964839, KF964942, KF965207, KF965070, KF964732; 2, *Andreasen 316* (UPS), –, KF964943, KF965208, KF965071, KF964733. *Hyperacanthus amoenus* (Sims) Bridson. *Bremer 3789* (UPS), KF964840, KF964944, KF965209, KF965072, KF964734. *Hypobathrum racemosum* Kurz, *Ridsdale s.n.*, cult. Bogor Bot. Gard. V.D. 81 (UPS), AJ286705, AM117318, KF965210, KF965073, KF964735. *Hyptianthera stricta* (Roxb. ex Schult.) Wight & Arn., *Van Beusekom 3031* (P), KF964841, KF964945, KF965211, KF965074, KF964736. *Ixora coccinea* L., *Bremer 3104* (S), KF964842, EF205641, EU817464, KF965075, KF964737. *Ixora margaretae* (N.Hallé) Mouly & B.Bremer, *Mouly & Innocent 222* (P), EU817415, EU817436, KF965176, KF965029, KF964696. *Ixora trilocularis* (Balf.f.) Mouly & B.Bremer, *Lesouef 31* (TAN), EU817417, EU817437, KF965193, KF965050, –, *Jovetia humilis* Guédès, *Davis 1197* (K), KF964843, KF964946, KF965212, KF965076, KF964738. *Kailarsenia tentaculata* (Hook.f.) Tirveng., *Larsen 41627* (AAU), KF964844, KF965029, KF965213, KF965077, KF964739. *Kochummenia stenopetala* (King & Gamble) K.-M.Wong. 1, *Low 247* (KLU), KF964845, KF964948, KF965214, KF965078, KF964740; 2, *Low 247 bis* (KLU), KF964846, KF964949, KF965215, KF965079, KF964741. *Kraussia floribunda* Harv. 1, *Bremer & al. 4280* (UPS), KF964847, KF964950, AM117368, KF965080, KF964742; 2, *Kiehn*, HBV sub RR-742 (WU), KF964848, AM117325, KF965216, KF965081, KF964743. *Kutchubaea semisericea* Ducke, *Stahl & al. 4418* (GB), AM117235, KF964951, KF965217, –, KF964744. *Lamprothamnium zanguebaricum* Hiern, *Luke 8335* (S), KF964849, KF964952, KF965218, KF965082, KF964745. *Larsenakia ochreata* (F.Muell.) Tirveng., *Puttock 722510* (OP), Z68847, KF964953, –, KF965083, –, *Lemryrea krugii* (A.Chev.) A.Chev., *Razafimanambison & al. 410* (UPS), KF964850, KF964954, KF965219, KF965084, KF964746. *Leptactina delagoensis* K.Schum., *Bremer & al. 4284* (UPS), AM117240, KF964955, AM117370, KF965085, KF964747. *Leptactina platyphylla* (Hiern) Wernham, *Bremer 3064* (UPS), Z68867, KF964956, KF965220, KF965086, KF964748. *Lindenia rivalis* Benth., *McDowall 5017* (ETSU), –, AM117242, HM164194, KF965087, KF964749. *Luculia gratissima* Sweet, Cult in Univ. Connecticut 700684 (no voucher), EU817429, EU817448, EU817472, KF965088, KF964750. *Macrosphyra longistyla* Hook.f. 1, *Ern 3120* (P), KF964851, –, KF965221, KF965089, KF964751; 2, *Bagshawe 1457* (BM), –, AF201004, –, –, *Mantalania sambiranensis* R.Cap. ex J.-F.Leroy 1, *Rakotonasolo 815* (K), KF964853, KF964958, KF965223, –, –, 2, *Randrianaivo 807* (MO), KF964852, KF964957, KF965222, KF965090, KF964752. *Massularia acuminata* (G.Don) Bullock ex Hoyle, *De Block 7 BR 64-0484* (BR), Z68841, AF201005, KF965224, KF965091, KF964753. *Melanopsidium nigrum* Colla, *Sant'Ana & al. 703* (GB), KF964854, –, KF965225, KF965092, KF964754. *Mitrostigma axillare* Hochst., *Bremer 2705* (UPS), X83650, KF964959, KF965226, KF965093, KF964755. *Monosalpinx guillaumetii* N.Hallé 1, *Aké Assi 9449* (P), –, KF964960, KF965227, KF965094, –, 2, *Cremers 822A* (P), –, KF964961, KF965228, KF965095, –, *Morelia senegalensis* A.Rich. ex DC. 1, *Ern 2974* (P), KF964855, KF964962, KF965229, KF965096, KF964756; 2, *Ervik 335* (GB)/*Thomas 1915* (S), KF964856, KF964963, KF965230, KF965097, KF964757. *Mussaenda erythrophylla* Schumaker, *Gillis 10838* (FTG), X83652, FJ884628, AJ620116, KF965098, –, *Neofranciella pterocarpon* Guillaumin, *Munzinger 2314* (P), KF964857, KF964964, KF965231, KF965099, KF964758. *Oligocodon cuniliferae* (Wernham) Keay, coll. unknown 348 (P), KF964858, KF964965, KF965232, KF965100, KF964759. *Oxyanthus speciosus* DC., *Bremer 4348* (S), KF964859, KF964966, KF965233, KF965101, KF964760. *Oxyceros longiflorus* (Lam.) Yamazaki, *Maxwell 78-8* (AAU), –, AF201010, AF201058, –, –, *Oxyceros sp.*, *Ridsdale 2235* (L), KF964860, KF964967, KF965234, KF965102, KF964761. *Paracephaelis sp.*, *De Block 1174* (BR), KF964861, KF964968, KF965235, KF965103, KF964762. *Paragenipa lancifolia* (Boj. ex Bak.) Tirveng. & Robbr., *Persson 156* (GB), AJ286707, KF964969, KF965115, KF964774; 2, *Martin & Ducan 71* (BR), KF964871, KF964979, KF965247, KF965116, KF964775. *Ramosmania rodriguesii* Tirveng., cult. Kew 881019 & 890925 (K), Z68860, KF964981, KF965250, KF965119, KF964776. *Randia aculeata* L. 1, *Killip 10070* (S), KF964873, KF964983, KF965251, KF965121, KF964778; 2, *Meagher 881* (FTG), Z68832, KF964982, HM164334, KF965120, KF964777. *Riodocera pulcherrima* Delprete, *De Souza 245* (NY), KF964874, KF964984, KF965252, KF965122, KF964779. *Robbrechia grandifolia* De Block, *Karehd & al. 311* (UPS), AM117263, AM117339, AM117383, KF965123, KF964780. *Rosenbergiodendron formosum* (Jacq.) Fagelr., *Mouly & Florencia 343* (P), KF964875, KF964985, KF965253, KF965124, KF964781. *Rothmannia capensis* Thunb., *Bremer & al. 4346* (S), AM117266, AM117340, AM117384, KF965125, KF964782. *Rubovietnamia aristata* Tirveng., *Bastien VN352* (P), –, KF964986, KF965254, KF965126, KF964783. *Rutidea fuscescens* Hiern, *Lantz 120* (UPS), KF964876, KF964987, KF965255, KF965127, KF964784. *Rutidea orientalis* Bridson, *Andreasen 68* (S), KF964877, KF964988, KF965256, KF965127, KF964785. *Sabicea aspera* Aubl., *Andersson & al. 1941* (GB)/*Andersson & al. 2003* (NY), KF964878, AF004079, AM409143, EU145416, –, *Schizenterospermum sp.*, *Davis 2580* (K), KF964879, KF964989, KF965257, KF965129, KF964786. *Schumannophyton magnificum* (K.Schum.) Harms. 1, *Sembé 64* (P), –, KF964990, KF965258, KF965130, KF964787; 2, *Bourobou Bourobou 619* (P), KF964880, KF964991, KF965259, KF965131, KF964788; 3, *Robbrecht, Meise 85-0094* (BR), AJ286702, –, KF965260, KF965132, –, *Scyphiphora hydrophyllacea* C.F.Gaertn., *Bremer & al. 99* (S), EU817432, EU817450, EU817475, KF965133, KF964789. *Sericanthe andongensis* (Hiern) Robbr., *Bremer & al. 4322* (S), KF964881, KF964992, KF965261, KF965134, –, *Sherbournia calycina* (G.Don) Hua, *Bremer 3132* (UPS), KF964882, KF964993, KF965262, KF965135, KF964790. *Sipanea hispida* Benth., *Irwin & al. 34756* (UPS), EU145458, EU145492, HM164336, KF965136, KF964791. *Sphinctanthus microphyllus* K.Schum., *Persson & Gustavsson 353* (S), KF964883, KF964994, KF965263, KF965137, KF964792. *Stachyarrhena heterocroa* Standl., *Persson & al. 821* (GB), KF964884, KF964995, KF965264, KF965138, KF964793. *Stachyarrhena sp.*, *Jansen-Jacobs & al. 4707* (GB), –, AF201021, –, –, *Stenosepala hirsuta* C.H.Perss., *Fonnegra & al. 2358* (GB), KF964885, KF964996, KF965265, KF965139, KF964794. *Sukunia pentagonoides* (Seem.) A.C.Sm., *Smith 8376* (S), KF964886, KF964997, KF965266, KF965140, KF964795. *Tamlandia utiginosa* (Retz.) Tirveng. & Sastre 1, *Bremer & Bremer 920* (S), KF964888, KF964999, KF965268, KF965142, KF964797. 2, Tirveng. & Sastre, *Tirvengadam 2006* (AAU), KF964887, KF964998, KF965267, KF965141, KF964796. *Tarenna buruensis* Merr., *Drozd & Molem s.n.* (UPS), AJ318457, AJ320088, KF965269, KF965143, KF964798. *Tarennoidea wallichii* (Hook.f.) Tirveng. & Sastre, *Larsen 43720* (AAU), KF964889, KF965000, KF965270, KF965144, KF964799. *Tennantia sennii* (Chiov.) Verdc. & Bridson, *Abdalla & al. 96/166* (P), KF964890, KF965001, KF965271, KF965145, KF964800. *Tocoyena formosa* (Cham. & Schltdl.) K.Schum., coll. unknown (P), KF964891, KF965002, KF965272, KF965146, KF964801. *Tricalysia ovalifolia* Hiern, *Bremer C-88-33* (UPS), Z68855, KF965003, KF965273, KF965147, KF964802. *Trukia carolinensis* (Valeton) Kaneh. & Hatus., *Fosberg 60242* (P), –, KF965004, KF965274, KF965148, KF964803. *Vangueria madagascariensis* J.F.Gmel., *Sanders 1798*, (FTG), X83670, HM164229, AM117386, KF965149, –, *Vidalasia fusca* (Craib) Tirveng., *Larsen 43277* (P), KF964892, KF965005, KF965275, KF965150, KF964804. *Vidalasia murina* (Craib) Tirveng., *Tirvengadam 2004* (P), KF964893, KF965006, KF965276, KF965151, KF964805. *Wendlandia glabrata* DC., *Low 239* (KLU), –, KF965007, KF965277, –, KF964806. *Xantonnea parvifolia* (Kuntze) Craib, *Cantharanotai 895* (K), –, –, DQ180599, KF965152, –.