American Journal of Botany 94(3): 313–329. 2007.

SEARCHING FOR THE RELATIVES OF *COFFEA* (RUBIACEAE, IXOROIDEAE): THE CIRCUMSCRIPTION AND PHYLOGENY OF COFFEEAE BASED ON PLASTID SEQUENCE DATA AND MORPHOLOGY[1]

AARON P. DAVIS,[2,6] MICHAEL CHESTER,[3] OLIVIER MAURIN,[4] AND MICHAEL F. FAY[5]

[2]The Herbarium, Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AB, UK; [3]School of Biological and Chemical Sciences, Queen Mary, University of London, Mile End Road, London, E1 4NS, UK; [4]Department of Botany and Plant Biotechnology, University of Johannesburg, PO Box 524, Auckland Park, 2006, Gauteng, South Africa; and [5]The Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3DS, UK

The circumscription of Coffeeae (Rubiaceae) and phylogenetic relationships within the tribe were evaluated using sequence data from four plastid regions (*trnL-F* intron, *trnL-F* intergenic spacer [IGS], *rpl16* intron, and *accD-psa1* IGS) and a morphological data set. Eleven candidates for inclusion in Coffeeae were examined using plastid data, and a further three were investigated using morphology alone. Based on previous phylogenetic analysis of the subfamily Ixoroideae, nine genera representing five tribes were used as outgroups. Our results support an enlarged circumscription for Coffeeae, containing 11 genera, viz. *Argocoffeopsis*, *Belonophora*, *Calycosiphonia*, *Coffea*, *Diplospora*, *Discospermum*, *Nostolachma, Psilanthus*, *Tricalysia*, *Sericanthe*, and *Xantonnea*. The inclusion of *Diplospora* and *Tricalysia* within Coffeeae, based on published molecular data, and the inclusion of *Argocoffeopsis*, *Belonophora*, *Calycosiphonia*, *Discospermum*, and *Sericanthe*, based on morphological evidence, are well supported. *Nostolachma* is newly transferred from Gardenieae subtribe Diplosporinae to Coffeeae, and *Xantonnea* from Octotropideae to Coffeeae. The exclusion of *Bertiera* from Coffeeae and placement in tribe Bertiereae is supported on the basis of molecular and morphological data. The removal of *Diplospora* and all other genera from Gardenieae subtribe Diplosporinae to Coffeeae and Octotropideae renders Diplosporinae superfluous. It is proposed that *Xantonneopsis* be transferred to Octotropideae; *Petitiocodon* is tentatively placed in Gardenieae. The monophyly of seven genera is supported, but *Coffea* is identified as paraphyletic in relation to *Psilanthus* on the basis of molecular and combined molecular and morphological data.

Key words: *accD-psa1*; *Coffea*; coffee; Coffeeae; molecular systematics; morphology; *rpl16*; Rubiaceae; *trnL-F*.

Coffea L. is the genus from which the beverage coffee is produced, with *C. arabica* L. (arabica coffee) the principal commercial species. The importance of coffee as an agricultural commodity is paramount: it is the world's most heavily traded commodity after oil in terms of monetary value and supports at least 20 million coffee farming families in more than 50 countries (Vega et al., 2003). Given the commercial and social importance of the genus, it is alarming that we know so little about the genus and its species. In particular, the systematic relationships between *Coffea* species have not been extensively studied and the systematic position of the genus within Rubiaceae is poorly understood. The resolution of systematic relationships between *Coffea* and *Psilanthus* represents a key objective for the understanding of *Coffea*. Before this can happen, however, it is necessary to investigate the relationship between these two genera and other closely related groups within Rubiaceae.

Coffea comprises 96 species and occurs naturally in Africa, Madagascar, and the Mascarenes (Davis et al., 2006). A close relationship between *Coffea* and *Psilanthus* Hook. f. is evident from studies covering several disciplines. Careful morphological study by Robbrecht and Puff (1986) and Robbrecht (1988b, 1994) has shown that the two genera share several key features and on this basis were considered the component genera of tribe Coffeeae DC. (Robbrecht and Puff, 1986). Couturon et al. (1998) produced a fertile intergeneric hybrid via the crossing of *C. arabica* and *P. ebracteolatus* Hiern, and genetic correspondence has been further revealed by recent cytological studies (Lombello and Pinto-Maglio, 2003, 2004). *Coffea* and *Psilanthus* have also been the focus of several recent phylogenetic studies, using systematic data from various sources, including morphology (Stoffelen, 1998; Davis et al., 2005), random amplified polymorphic DNA (RAPD) (Lashermes et al., 1993), sequences from plastid DNA (Cros, 1994; Lashermes et al., 1996; Cros et al., 1998), and ITS sequences of nuclear ribosomal DNA (Lashermes et al., 1997). At the species level, the studies of Lashermes et al. (1997) and Cros et al. (1998) have provided the most useful data: they were able to separate *Coffea* species into geographical groupings and to begin to examine the relationships between *Coffea* and *Psilanthus* species. Lashermes et al. (1997) found that one *Psilanthus* species (*P. travancorensis*) was nested within *Coffea* and that there was limited sequence divergence

[1] Manuscript received 23 November 2005; revision accepted 17 January 2007.

The authors are grateful to directors and staff at the Herbaria at DSM, K, L, P, and YA (abbreviations after Holmgren et al., 1990) for making herbarium specimens available for study. Permit-issuing authorities in Cameroon, Tanzania, and Madagascar are gratefully acknowledged. They are also indebted to those institutes and individuals who provided DNA material, including D. Harris, IRD-Montpellier, E. F. Mvungi, F. Rakotonasolo, B. Sonké, and P. Stoffelen. They thank D. Bridson at the Royal Botanic Gardens, Kew, for her guidance on Coffeeae and other Rubiaceae, and D. Crayn at the Royal Botanic Gardens, Sydney, for assistance in the preparation of this paper. They are also grateful to Kraft Foods for generous support in funding the laboratory research for this project.

[6] Author for correspondence (e-mail: a.davis@rbgkew.org.uk)

between these two genera. They concluded that their ITS data did not support the recognition of two genera. On the basis of *trnL-trnF* sequence data, Cros et al. (1998) concurred with Lashermes et al. (1997) about the close relationship between *Coffea* and *Psilanthus*, although their tree topology shows an unresolved relationship between the two species of *Psilanthus* that they sampled (*P. mannii* and *P. ebracteolatus*) and *Coffea*. Cros et al. (1998) and Lashermes et al. (1997) did not include representatives of closely related genera in their studies, for example, as outgroups. However, broader studies of Rubiaceae (Ixoroideae) by Andreasen et al. (1999) and Andreasen and Bremer (2000, fig. 3) infer paraphyly of *Coffea*.

Potential relatives of *Coffea* should be found within Coffeeae, but at the present time there is no general agreement on the constituent genera of the tribe (Robbrecht and Puff, 1986; Robbrecht, 1988b, 1994; Andreasen and Bremer, 1996, 2000; Persson, 2000; Bridson and Verdcourt, 2003) and few data on how they are related to one another (Andreasen and Bremer, 2000).

The nomenclatural origin of tribe Coffeeae is De Candolle's Coffeaceae (De Candolle, 1807), one of four tribes recognized by De Candolle in his early classification of Rubiaceae. In a later conspectus of the family by De Candolle (1830), which includes 13 tribes, it is evident that his Coffeeae ('Coffeaceae') was very broadly circumscribed and included a large number of genera, many of which have since been placed in different tribes and subfamilies. De Candolle's characterization of Coffeeae translated and reorganized by us from the Latin diagnosis (De Candolle, 1830, p. 472), is as follows: Trees or shrubs with opposite leaves; stipules interpetiolar, paired, adnate or free. Ovaries 1-locular, each bearing a single ovule. Fruits fleshy, containing two pyrenes, each containing a single seed with a bony-crustaceous surface (endocarp layer of pyrene), endocarp flat on the adaxial surface and usually sulcate on the abaxial surface, or fruits with one pyrene by abortion; endosperm hard/horny.

Richard (1830) provided a similar concept for Coffeeae, although it should be noted that De Candolle (1830) had access to Richard's unpublished manuscript and incorporated parts of the classification in order to avoid nomenclatural conflict (Stern, 1957).

Further attempts to define Coffeeae were not made until much later. Leroy (1980) included three genera in his informal 'Caféiers' group, namely *Coffea*, *Nostolachma* T. Durand, and *Psilanthus*, although no direct reference was made to this tribe by Leroy. Robbrecht and Puff (1986; see also Robbrecht, 1988b, 1994) provided the first rigorous and explicit delimitation for Coffeeae and included just two genera in the tribe, *Coffea* and *Psilanthus*. Their characterization of the tribe was based on the presence of 2-carpellate ovaries, each with a single ovule, axile placentation, a hard (horny/crustaceous) endocarp, seeds with a deep L- or T-shaped ventral groove on the adaxial surface (as seen in transverse section; endocarp and seed coat invaginated), and a seed coat exotesta consisting of thin, elongated parenchymatic cells usually containing many more or less isolated fibers (derived from Robbrecht, 1981; Robbrecht and Puff, 1986). In addition, Stoffelen et al. (1997) showed that the pollen of Coffeeae are (2–)3–5-colporate (zonocolporate). A simple but efficient means of characterizing Coffeeae sensu Robbrecht and Puff (1986) is of a tribe possessing "coffee beans," i.e., seeds with a groove on the flat side of the seed. The groove ramifies within the seed as an invagination through the endosperm into the center and is clearly evident when a coffee bean is cut in transverse section. The pyrene (horny/crustaceous endocarp) also has a deep, ventral groove that follows the invagination within the seed. Robbrecht and Puff (1986) excluded *Nostolachma* (= *Lachnastoma* Korth.), which was associated with *Coffea* and *Psilanthus* by Leroy (1980). *Nostolachma,* together with *Argocoffeopsis* Lebrun, *Calycosiphonia* Pierre ex Robbr., *Cremaspora* Benth., *Diplospora* DC., *Sericanthe* Robbr., and *Tricalysia* A. Rich. ex DC. were transferred to Gardenieae DC. subtribe Diplosporinae Miq. by Robbrecht and Puff (1986). It was not explicitly stated by Robbrecht and Puff (1986) why and how Gardenieae subtribe Diplosporinae was separated from Coffeeae, although it seems that the absence of characters found in Coffeeae (as discussed earlier) coupled with the presence of exotestal cells (with or without thickenings and lacking fibers) separated these two groups. *Petitiocodon* Robbr. and *Xantonneopsis* Pit. were added to this subtribe by Robbrecht (1988b), followed by *Discospermum* Dalz. (Ali and Robbrecht, 1991; Robbrecht, 1994) on the basis that they conformed to the circumscription of the subtribe (after Robbrecht and Puff, 1986).

Molecular studies (Andreasen and Bremer, 1996, 2000; Persson, 2000) have demonstrated that certain genera of Gardenieae subtribe Diplosporinae are closely related to *Coffea* and *Psilanthus*. On the basis of molecular data, Andreasen and Bremer (2000) placed two genera of Gardenieae subtribe Diplosporinae, *Diplospora* and *Tricalysia*, with *Coffea* and *Psilanthus*, in Coffeeae. The genus *Paracoffea* J.-F. Leroy (Leroy, 1967) used in that work is a synonym of *Psilanthus* (Davis, 2003). *Bertiera* Aubl., formerly either a genus of unknown systematic position within Rubiaceae (Robbrecht, 1988b) or placed within Gardenieae subtribe Gardeniinae (Robbrecht et al., 1994), was also shown to be closely related to *Coffea* and *Psilanthus* (Andreasen and Bremer, 1996, 2000; Persson, 2000). Andreasen and Bremer (2000) placed *Bertiera* in Coffeeae, based on a moderately well-supported (BP = 76, *b* = 4) sister relationship to *Coffea*, *Psilanthus*, *Diplospora*, and *Tricalysia*. Andreasen and Bremer (2000) placed two further members of Gardenieae subtribe Diplosporinae, *Discospermum* and *Sericanthe*, into Coffeeae, based on the morphological evidence (Schumann, 1891; Ali and Robbrecht, 1991) that the former was closely related to *Diplospora* and the latter to *Tricalysia*. Andreasen and Bremer (2000) placed *Cremaspora*, formerly included in Gardenieae subtribe Diplosporinae by Robbrecht and Puff (1986), in its own tribe (Cremasporeae Bremek. ex S.P. Darwin).

An enlarged and modified concept of Coffeeae (see Table 1) was followed by Bridson and Verdcourt (2003, p. 387) although further significant changes were made. Based on morphology (discussed later) and on preliminary plastid sequence data that we provided (A. Davis, unpublished data), Bridson and Verdcourt (2003, p. 451) added the genera *Argocoffeopsis*, *Belonophora* Hook. f., and *Calycosiphonia* to Coffeeae. *Argocoffeopsis* and *Calycosiphonia* were formerly members of Gardenieae subtribe Diplosporinae (Robbrecht, 1988b), and *Belonophora* was tentatively included in Aulacocalyceae Robbr. & Puff (Robbrecht, 1988b, 1994). Bridson and Verdcourt (2003, p. 388) excluded *Bertiera* from Coffeeae because they believed that the extension of characters necessary to accommodate this morphologically distinct genus would greatly distort the circumscription of Coffeeae. To this end, *Bertiera* was placed in its own tribe, Bertiereae (K.Schum.) Bridson (Bridson and Verdcourt (2003, p. 386).

TABLE 1. Genera studied including tribal placement, general distribution, and new or confirmed tribal placement. The number of species is taken from Govaerts et al. (2006).

Genus	No. of species	Tribe	Reference for tribal placement	Distribution	Placement following this study
Argocoffeopsis Lebrun	7	GarD/Cof[b]	Robbrecht (1988b)/Bridson and Verdcourt (2003)[b]	Africa	Cof[a]
Belonophora Hook. f.	5	Aul ?/Cof[b]	Robbrecht (1988b, 1994)/Bridson and Verdcourt (2003)[b]	Africa	Cof[a]
Bertiera Aubl.	52	Cof[a]	Andreasen and Bremer (2000)[a]	Africa (incl. Madagascar, Mascarenes), America	Ber[a]
Calycosiphonia Pierre ex Robbr.	2	GarD/Cof[b]	Robbrecht (1988b)/Bridson and Verdcourt (2003)[b]	Africa	Cof[a]
Coffea L.	96	Cof[a]	Type genus	Africa (incl. Madagascar, Mascarenes)	Cof[a]
Diplospora DC.	19	Cof[a]	Andreasen and Bremer (2000)[a]	Asia	Cof[a]
Discospermum Dalz.	6	Cof	Andreasen and Bremer (2000)	Asia	Cof[a]
Nostolachma T. Durand	6	GarD	Robbrecht (1988b)	Asia	Cof
Petitiocodon Robbr.	1	GarD	Robbrecht (1988b)	W. Africa	Gar
Psilanthus Hook. f.	18	Cof	Robbrecht (1986)	Africa, Asia, Australia	Cof[a]
Sericanthe Robbr.	17	Cof	Andreasen and Bremer (2000)	Africa	Cof[a]
Tricalysia A. Rich. ex DC.	98	Cof[a]	Andreasen and Bremer (2000)[a]	Africa (incl. Madagascar)	Cof[a]
Xantonnea Pierre ex Pit.	2	Oct	Robbrecht (1988b)	Asia	Cof[a]
Xantonneopsis Pit.	1	GarD	Robbrecht (1988b)	Asia	Oct
Total no. of species	330				

Notes: Aul = Aulacocalyceae (tentatively included in this tribe by Robbrecht, 1988b); Cof = Coffeeae DC.; Ber = Bertiereae (K. Schum) Bridson; Gar = Gardenieae DC.; GarD = Gardenieae DC. subtribe Diplosporinae Miq.; Oct = Octotropideae Bedd.

[a] Based or confirmed on published molecular sequence data; others based on morphology.

[b] Partly based on unpublished molecular data (A. P. Davis et al., unpublished data).

The differences between the tribes were not stated, but instead the characterizations for each tribe were given in tribal descriptions (Bridson and Verdcourt, 2003, pp. 387–388). The salient characters of Coffeeae according to Bridson and Verdcourt (2003, p. 388) are as follows (we selected the main characters and the comments in square brackets are ours): inflorescences axillary or less often terminal on short lateral spurs; bracts and bracteoles sometimes free but more often with opposite pairs fused together to form cup-like structures ('cupules' [= calyculi]) and arranged in a series of usually 1–4, each cupule with (0–2–) 4 lobes; secondary pollen presentation mostly present (said to be lacking in *Coffea* [and *Psilanthus*]). Anthers exserted from corolla throat or occasionally included. Pollen presenter/stigmatic lobes divergent or not. Seeds free or wholly or partly covered in arilloid placenta tissue; embryo radicle inferior or rarely superior; testa cells isodiametric or elongated, thickenings mostly absent or more or less weak, smooth along outer tangential walls, or in Coffeeae sensu stricto [*Coffea* and *Psilanthus*] with cells crushed during the development of endosperm, composed of thin elongate parenchymatic cells usually containing many more or less isolated fibers. Pollen grains 3–4-colporate.

In a review of the major lineages of Rubiaceae, using supertree construction based on *rbcL*, *rps16*, *trnL-trnF*, and *atpB-rbcL* data, Robbrecht and Manen (2006) reclassified Coffeeae, splitting it into two subtribes: Coffeeae subtribe Coffeinae (DC.) Robbr. & Manen (*Argocoffeopsis*, *Belonophora*, *Coffea*, *Discospermum*, *Psilanthus*, *Sericanthe*, *Tricalysia*, *Xantonnea* Pierre ex Pitard), and Coffeeae subtribe Bertierinae (K. Schum) Robbr. & Manen (*Bertiera*). The inclusion of component genera was largely based (i.e., all except *Belonophora*) on the work presented here and that given by Andreasen and Bremer (2000). *Diplospora*, a member of Coffeeae according to Andreasen and Bremer (2000), was not placed in Coffeeae but instead within a clade named 'Gardenieae 4'; *Calycosiphonia*, a candidate for inclusion in Coffeeae was also placed in the same clade as *Diplospora*. According to Robbrecht and Manen (2006) subtribe Coffeinae and subtribe Bertierinae are separated by inflorescence position and number of ovules per locule. In subtribe Coffeinae the inflorescences are axillary and paired at nodes (in some genera accompanied by terminal inflorescences) and ovules per locule 1–few (only in some *Tricalysia* spp. more than 20). In subtribe Bertierinae the inflorescences are terminal and ovules per locule numerous.

Following the work of Robbrecht and Puff (1986), Andreasen and Bremer (1996, 2000), Persson (1996), Bridson and Verdcourt (2003), and Robbrecht and Manen (2006), the aims of this study are to further investigate the delimitation and characterization of Coffeeae and to elucidate the relationships within Coffeeae with particular attention to the position of *Coffea* and *Psilanthus*. To achieve our objectives, we used sequence data from four plastid regions (the *trnL* intron, the *trnL-F* intergenic spacer [IGS], the *rpl16* intron, and the *accD-psa1* IGS) and compared and combined these data with morphological data. For the purposes of this study, the *trnL* intron and the *trnL-F* IGS are treated as one region: *trnL-F*. All genera with potential to be placed within Coffeeae, including all members of Gardenieae subtribe Diplosporinae (based on Robbrecht, 1988b, 1994), were included in the sampling, although we were unable to locate DNA material for three genera from Gardenieae subtribe Diplosporinae. Morphological data alone were used to deduce the systematic position of genera not sampled in our plastid sequence analyses.

MATERIALS AND METHODS

Taxon sampling—Species representatives from all genera placed within the current enlarged concept of Coffeeae by Andreasen and Bremer (2000) and Bridson and Verdcourt (2003) were included in our analyses, viz. *Argocoffeopsis*, *Belonophora, Bertiera*, *Calycosiphonia, Coffea*, *Diplospora*, *Disco-*

TABLE 2. Amplification primers for loci.

Locus	Primer	Primer sequence	Reference
trnL intron	Forward (c)	5′-CGAAATCGGTAGACGCTACG-3′	Taberlet et al. (1991)
	Reverse (d)	5′-GGGGATAGAGGGACTTGAAC-3′	
trnL-F IGS	Forward (e)	5′-GGTTCAAGTCCCTCTATCCC-3′	Taberlet et al. (1991)
	Reverse (f)	5′-ATTTGAACTGGTGACACGAG-3′	
*rpl*16	Forward (F71)	5′-GCTATGCTTAGTGTGTGACTCGTTG-3′	Jordan et al. (1996)
	Reverse (R1661)	5′-CGTACCCATATTTTTCCACCECGAC-3′	
	Internal Forward	5′-GTAAGAAGTGATGGGAACGA-3′	Designed at Kew
	Internal Reverse	5′-TCGTTCCCATCACTTCTTAC-3′	
accD-psa1 IGS	Forward (ACCD 769 F)	5′-GGAAGTTTGAGCTTTATGCAAATGG-3′	Mendenhall (1994)
	Reverse (PSA1 75 R)	5′-AGAAGCCATTGCAATTGCCGGAAA-3′	
	Internal Forward Coffeeae	5′-GCTAAAAATCTCTSTTGGTTCGG-3′	Designed at Kew
	Internal Reverse Coffeeae	5′-CCGAACCAASAGAGATTTTTAGC-3′	

spermum, *Psilanthus*, *Sericanthe*, and *Tricalysia*. For the three remaining members of Gardenieae subtribe Diplosporinae (Robbrecht, 1998b), our material of *Nostolachma*, *Petitiocodon,* and *Xantonneopsis* did not yield usable DNA and plastid sequences could not be obtained. *Petitiocodon*, *Xantonneopsis*, and *Nostolachma* were included in the morphological analysis and then in a combined morphological–molecular analysis to determine their systematic position. A review of genera within Rubiaceae (A. Davis and D. Bridson, Royal Botanic Gardens, Kew, personal observations) identified one further genus likely to be placed within Coffeeae, namely *Xantonnea* Pierre ex Pit. This was included in the plastid sequence and morphological analyses. Collections made in 2005 from Cameroon and the Congo (B. Sonké, University of Yaoundé, personal communication) yielded a new taxon that was likely to fall within Coffeeae, although as yet it cannot be placed within a currently accepted genus. This taxon is currently referred to (B. Sonké and A. Davis, personal communication) as *Calycosiphonia* cf. as it superficially resembles species within this genus. All genera, and their respective samples, with the potential to fall with the broader concept of Coffeeae were defined as the ingroup. A selection of genera from subfamily Ixoroideae sensu stricto (i.e., excluding 'basal Ixoroideae', after Andreasen and Bremer (2000), were used as outgroups. In total nine genera from five tribes were included in the outgroup sample, representing the tribes Ixoreae A. Gray, Pavetteae A. Rich ex Dumort, Vanguerieae Dumort, Octotropideae Beddome, and Gardenieae DC. Following the circumscription of Ixoroideae s.s. after Andreasen and Bremer (2000), Cremasporeae (Verdc.) S.P. Darwin, and Alberteae Hook. f. are the only tribes not included in our analyses; these taxa are not close relatives of Coffeeae (Andreasen and Bremer, 2000; Robbrecht and Manen, 2006). Hereafter, we refer to Ixoroideae, less basal groups (after Andreasen and Bremer, 2000), as Ixoroideae s.s. An overview of the genera studied in this contribution, including their current tribal placement and general distribution, is given in Table 1. Taxon names, voucher information and GenBank accession numbers are given in Appendix 1.

DNA extraction, amplification and sequencing—Most of the DNA samples were obtained from silica-dried leaf material (Chase and Hills, 1991) and the remainder from single seeds, flowers, or leaf samples obtained from herbarium specimens. DNA extraction was performed from a maximum of 0.3 g of silica-dried flower or leaf material (or from one seed) using the 2× CTAB method described by Doyle and Doyle (1987). The DNA was purified on cesium chloride/ethidium bromide gradients (1.55 $g \cdot ml^{-1}$ density) and dialyzed before inclusion in the DNA Bank at the Royal Botanic Gardens, Kew (http://www.rbgkew.org.uk/data/dnaBank/homepage.html). To avoid problems of PCR inhibition, all DNA samples were further purified on QIAquick purification columns (QIAgen) following the manufacturer's protocol.

DNA sequences for seven taxa were taken from another phylogenetic study (O. Maurin et al., unpublished data). The remaining 35 taxa were sequenced according to the methods described next.

Amplification of plastid regions was performed using the primers listed in Table 2. Amplification of *trnL-F* was carried out using primers c and f designed by Taberlet et al. (1991). For many taxa, the internal primers d and e also had to be used because of difficulty in amplifying the region as a single piece. Amplification of the *rpl16* region was carried out using primers 71F and 1661R of Jordan et al. (1996). For many taxa, the amplification of DNA using these primers was not satisfactory, so we designed internal primers (Table 2) on the basis of our first sequences in a conserved and GC-rich region suitable for amplification of the *rpl16* region in two fragments for Rubiaceae. The *accD-psa1* IGS region was amplified using the primer ACCD-769 forward and PSA1-75 reverse from Mendenhall (1994). Two internal primers (Table 2) were again designed to obtain satisfactory PCR products for our recalcitrant specimens.

The PCR program for the *trnL-F* region consisted of 2 min at 94°C followed by 28 cycles of 1 min at 94°C (denaturation), 1 min at 50°C (annealing), and 1 min at 72°C (extension); then a final 7-min extension (72°C). For the *rpl16* region, we used the PCR program: 2 min at 94°C followed by 30 cycles of 1 min at 94°C, 1 min at 52°C, and 3 min at 72°C; with a final extension at 72°C for 7 min. The *accD-psa1* region was amplified using the PCR program: a denaturation phase of 3 min (94°C), followed by 30 cycles of 1 min at 94°C, 1 min at 51°C and 1 min at 72°C; and a final extension of 72°C for 5 min.

A PCR mastermix containing 2.5 mM $MgCl_2$ (Advanced Biotechnologies, Epsom, Surrey, UK) was used for *trnL-F* and *rpl16* amplifications. For *accD-psa1*, the commercial mastermix did not give good amplifications, so we prepared our own premix using Biotaq DNA polymerase (Bioline, UK), 10× NH_4 reaction buffer (Bioline, UK), 50 mM $MgCl_2$, and dNTPs (Promega, Southampton, UK). Amplified products were purified using QIAquick purification columns (QIAgen) as described in the manufacturer's protocol. Cycle sequencing reactions were carried out using BigDye Terminator Mix (Applied Biosystems, Warrington, Cheshire, UK). The program consisted of 26 cycles of 10 s denaturation (96°C), 5 s annealing (50°C), and 4 min elongation (60°C). PCR and sequencing reactions were run using a Perkin-Elmer (Warrington, Cheshire, UK) GenAMP model 9600 or 9700 PCR system, and sequencing products were run on either an ABI 3100 Genetic Analyzer or an ABI 377 automated sequencer according to the manufacturer's protocols (Applied Biosystems). Electropherograms were edited and assembled into contigs using Sequencher version 3.2.2. (Gene Codes Corp., Ann Arbor, Michigan, USA). The sequences generated were submitted to GenBank using the Sequin application (version 5.26; available from http://www.ncbi.nlm.nih.gov/Sequin/).

Data matrix composition and parsimony analysis—All sequences were aligned manually in PAUP* (version 4.0b10; Swofford, 2002) without difficulty due to low levels of sequence variation. Areas with ambiguous alignment were excluded from the analysis, as were regions with missing sequences, for example, the beginning and end of sequences and around the internal primer binding sites. In total, 508 characters were excluded from the molecular matrix.

Thirty-one morphological characters were scored using the available literature (given later) and new observations from herbarium specimens, samples preserved in alcohol, and in some cases living material in situ. Selected herbarium material from DSM, K, L, P and YA, and specimens preserved in alcohol at K were consulted (abbreviations after Holmgren et al., 1990). Representatives of all African and Madagascan genera, excluding *Petitiocodon*, were examined in situ, in Cameroon, Tanzania, and Madagascar by A. Davis (personal observation). The literature was consulted for most genera: Bremekamp (1934), Bridson (1978), Robbrecht (1978, 1981, 1988a, b), Puff et al. (1984), Bridson and Verdcourt (1988, 2003), Robbrecht and Puff (1986), Leroy (1989), Ali and Robbrecht (1991), Puff et al. (1996), Robbrecht et al. (1994), Persson (1996), Bridson (1998), Andreasen and Bremer (2000), Cheek and Dawson (2000), Vinckier et al. (2000), Stone and Davis (2004), Davis et al. (2005), and Rakotonasolo and Davis (2006).

TABLE 3. Description of trees for each plastid region, combined plastid region, morphology, and combined molecular and morphology data sets.

Characteristics	*trnL-F*	*rpl16*	*accD-psaI*	Molecular combined	Morphology	Molecular and morphology	
No. of taxa	42	41	42	42	46	42	46 [b]
Total no. of characters	875	1153	1389	3417	31	3448	3448
Invariable characters	746	981	1217	2944	0	2944	2944
Parsimony uninformative characters	64	75	84	223	0	223	223
Parsimony informative characters	65	97	88	250	31	281	281
Tree length	154	241	247	652	101	762	767
Consistency index (CI)[a]	0.78	0.73	0.69	0.71	0.46	0.67	0.65
Retention index (RI)	0.89	0.85	0.82	0.84	0.80	0.81	0.81
No. of trees	39 577	560	84 580	4	2745	31	234

[a] Calculated excluding uninformative characters.

[b] Indicates description of trees for analysis including *Nostolachma* (2 spp.), *Petitiocodon,* and *Xantonneopsis*, which lack sequence data (see Materials and Methods).

The same nine genera that were used as outgroups for the molecular analysis were scored for the morphological matrix. The total morphological variation within Ixoroideae s.s. and its tribes cannot be covered in a sample of this size, but the objective was to polarize characters for the ingroup only. Likewise, scoring of morphological characters for genera, and particularly large genera such as *Ixora*, by using one terminal, will not encompass all the variation within a genus. However, in the case of the genera we have studied in conjunction with the characters we have used, sampling of variation within genera is sufficiently representative and more than adequate for the purposes of the study. The morphological matrix provided here for Ixoroideae s.s. is the most comprehensive so far undertaken and represents a foundation for further investigation. The general distribution of character states for genera and tribes within Ixoroideae s.s., other than those sampled by us, are given in Appendix 2. The list of characters and character states, with explanatory notes (including potentially informative characters not scored in our analysis), is given in Appendix 2. The morphological matrix is given in Appendix 3. All morphological characters were treated as unordered.

Maximum parsimony was implemented to analyze (1) *trnL-F*, (2) *rpl16,* (3) *accD-psa1*, (4) combined plastid data, (5) morphological data, and (6) combined molecular and morphological data in PAUP* (Swofford, 2002). In all analyses, gaps were treated as missing data and characters were equally weighted and unordered (Fitch, 1971). The data sets were analyzed separately to identify topological conflicts: strict consensus trees were produced from each data set and compared carefully by eye. Almost no incongruence was found in the plastid analyses, as far as each analysis was resolved, and the only anomaly was the placement of *Canephora* sp. (Octotropideae) sister to all *Tricalysia* (Coffeeae) spp. in the *rpl16* analysis (BP 69). In all other analyses *Canephora* is placed with *Polysphaeria* sp. (also Octotropideae). On the basis of this general agreement, the three plastid analyses were combined. The morphological analysis was combined with the combined plastid analysis on the basis that there was no strongly supported incongruence between the two data sets.

An additional combined molecular–morphological analysis was conducted to include the three genera (four taxa) for which we had no DNA material (*Petitiocodon*, *Xantonneopsis*, and *Nostolachma* [2 spp.]). This analysis was performed with the sole objective of elucidating the systematic position of these genera within Ixoroideaes s.s.

Tree searches were conducted using 10 000 replicates of random taxon sequence addition, retaining 10 trees at each step, with tree-bisection-reconnection (TBR) branch swapping, delayed transformation (DELTRAN) optimization, MulTrees in effect, and saving a maximum of 10 trees per replicate. Support for clades in all analyses was estimated using bootstrap analysis (Felsenstein, 1985), with 10 000 replicates of full heuristic search, simple sequence addition, TBR swapping, with MulTrees in effect and saving a maximum of 10 trees per replicate. Bootstrap percentages (BP) are described as high/well-supported (85–100%), moderate (75–84%), or low (50–74%). In addition, Bremer support values (*b*) (Bremer, 1988, 1994; Källersjö et al., 1992), otherwise known as decay values, were obtained using PAUP* (Swofford, 2002), in conjunction with AutoDecay 4.0.2 (Eriksson, 1999), with 100 replicates of random addition for each constraint tree.

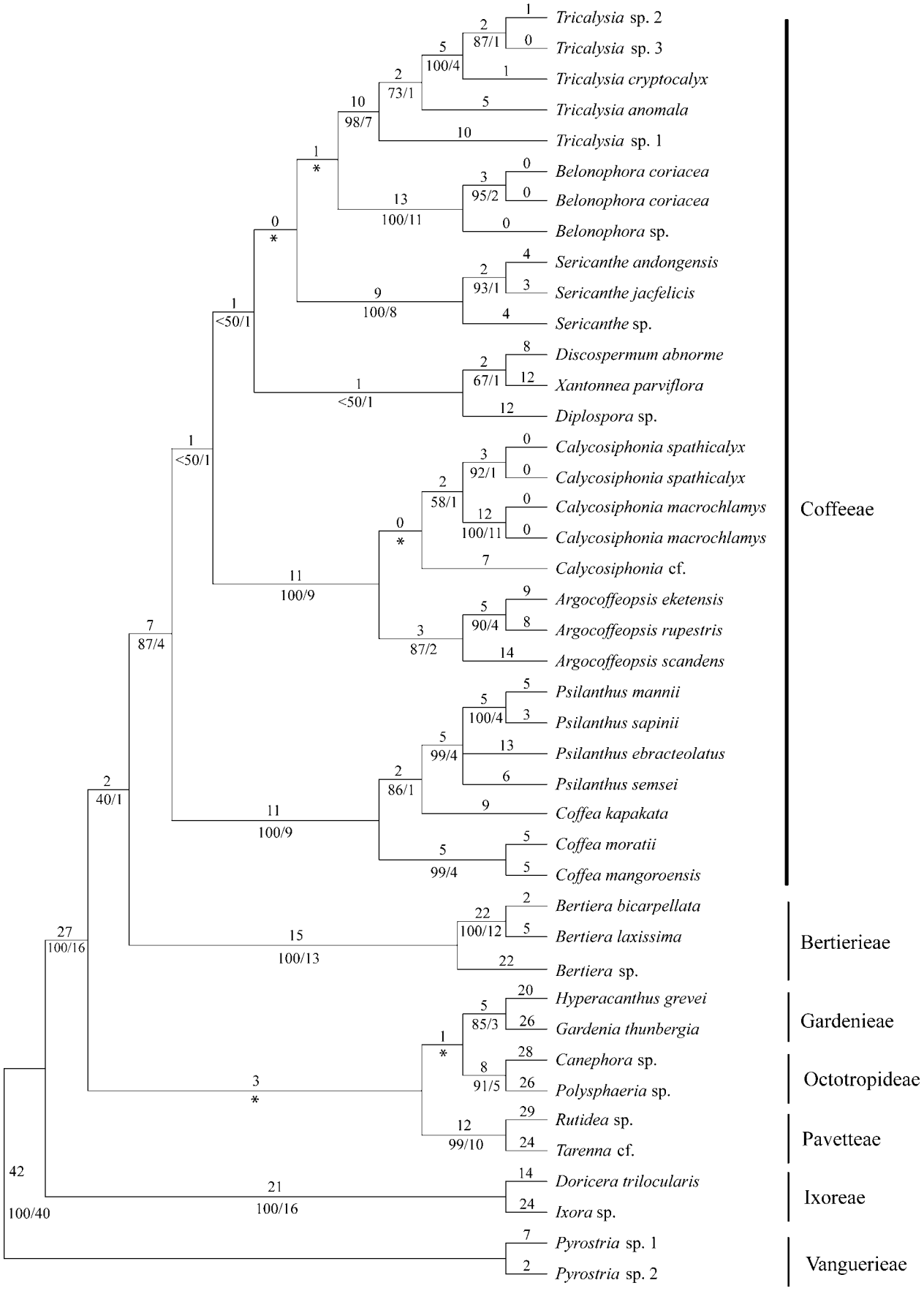
For the morphological, combined molecular analysis, and combined molecular–morphological analyses, two representatives of Vanguerieae (*Pyrostria* spp.) were used as outgroups in the analyses. This decision was based on wider studies of Ixoroideae by Andreasen and Bremer (1996, 2000), Persson (2000), and Robbrecht and Manen (2006). The mapping of morphological characters on the morphological and morphological–molecular trees was undertaken using MacClade (version 4.07 PPC; Maddison and Maddison, 2005). Key morphological characters were mapped onto the strict consensus tree of the morphological–molecular analysis for illustrative purposes (Fig. 3).

RESULTS

Tree data and statistics for individual analyses using (1) *trnL-F*, (2) *rpl16,* (3) *accD-psa1*, (4) combined molecular data, (5) morphological data, and (6) combined molecular and morphological data are given in Table 3. Individual plastid sequence analyses were topologically consistent and, for the purpose of the Results, Discussion, and Conclusions, are here combined and treated as a single analysis. We retrieved a significantly smaller number of trees for the analysis of *rpl16* (Table 3), although we were unable to find the reason for this anomaly. The percentage of parsimony informative characters and the values for the consistency index (CI) and retention index (RI) were similar for all plastid data sets.

Combined analysis of plastid data—The analysis of combined plastid data resulted in four most parsimonious trees. A single most parsimonious tree with bootstrap and decay values and branch lengths is shown in Fig. 1. The genera *Argocoffeopsis*, *Belonophora*, *Calycosiphonia*, *Calycosiphonia* cf. *Coffea*, *Diplospora*, *Discospermum*, *Psilanthus*, *Tricalysia*, *Sericanthe*, and *Xantonnea* are placed within the ingroup (BP 87, $b = 4$). *Bertiera* is consistently placed as sister to all the other genera of the ingroup, but this relationship is very weakly supported (BP 40, $b = 1$). Three suprageneric clades receive bootstrap support within the ingroup: *Coffea* and *Psilanthus* (BP 100, $b = 9$), *Calycosiphonia, Argocoffeopsis*, and *Calycosiphonia* cf. (BP 100, $b = 9$), and *Discospermum* and *Xantonnea* (BP 67, $b = 1$). Genus-level monophyly is supported for all genera with multiple species samples except *Coffea*: *Tricalysia* (BP 98, $b = 7$), *Belonophora* (BP 100, $b = 11$), *Sericanthe* (BP 100, $b = 8$), *Calycosiphonia* (BP = 58, $b = 1$), *Argocoffeopsis* (BP 87, $b = 2$), and *Psilanthus* (BP 99, $b = 4$). *Coffea* is paraphyletic with respect to *Psilanthus* (BP 86, $b = 1$).

Morphological analysis—Analysis of the morphological data set resulted in 2745 most parsimonious trees; the consensus tree is poorly resolved, with few well-supported clades. The morphological analysis exhibits some topological conflict with respect to the combined molecular analysis, but


Tricalysia sp. 2
Tricalysia sp. 3
Tricalysia cryptocalyx
Tricalysia anomala
Tricalysia sp. 1
Belonophora coriacea
Belonophora coriacea
Belonophora sp.
Sericanthe andongensis
Sericanthe jacfelicis
Sericanthe sp.
Discospermum abnorme
Xantonnea parviflora
Diplospora sp.
Calycosiphonia spathicalyx
Calycosiphonia spathicalyx
Calycosiphonia macrochlamys
Calycosiphonia macrochlamys
Calycosiphonia cf.
Argocoffeopsis eketensis
Argocoffeopsis rupestris
Argocoffeopsis scandens
Psilanthus mannii
Psilanthus sapinii
Psilanthus ebracteolatus
Psilanthus semsei
Coffea kapakata
Coffea moratii
Coffea mangoroensis
Bertiera bicarpellata
Bertiera laxissima
Bertiera sp.
Hyperacanthus grevei
Gardenia thunbergia
Canephora sp.
Polysphaeria sp.
Rutidea sp.
Tarenna cf.
Doricera trilocularis
Ixora sp.
Pyrostria sp. 1
Pyrostria sp. 2
Coffeeae
Bertierieae
Gardenieae
Octotropideae
Pavetteae
Ixoreae
Vanguerieae

this is limited and not well supported. The strict consensus tree with bootstrap and decay values is shown in Fig. 2. Moderate to well-supported clades include *Coffea* and *Psilanthus* (BP 79, $b = 3$), *Psilanthus* (BP 94, $b = 3$), *Belonophora* (96, $b = 3$), *Bertiera* (BP 90, $b = 3$), and *Sericanthe* (BP 88, $b = 2$).

Combined molecular and morphological analysis, including taxa with sequence data (42 taxa)—Analysis of the combined molecular and morphological data set resulted in 31 most parsimonious trees. The strict consensus tree of molecular and morphological data is consistent with that of the combined molecular data tree but with less resolution at the internal nodes. Generally support values are higher. The ingroup clade receives increased supported (BP 97, $b = 6$), for example. *Bertiera* is not the sister group of Coffeeae, as in the combined molecular analysis, but is instead placed with the two representatives of Gardenieae (BP 37, $b = 1$). The ingroup is divided into two main clades: *Coffea* and *Psilanthus* (BP 100, $b = 13$), and all other ingroup genera (BP 59, $b = 1$). The only other suprageneric clade receiving strong bootstrap support is that of *Calycosiphonia*, *Argocoffeopsis*, and *Calycosiphonia* cf. (BP 99, $b = 9$). For all genera with multiple species samples, except *Coffea,* monophyly is supported, including *Tricalysia* (BP 100, $b = 10$), *Belonophora* (BP 100, $b = 15$), *Sericanthe* (BP 100, $b = 8$), *Calycosiphonia* (BP 85, $b = 3$), *Argocoffeopsis* (BP 81, $b = 2$), *Psilanthus* (BP 100, $b = 8$), and *Bertiera* (BP 100, $b = 17$). *Coffea* is paraphyletic with respect to *Psilanthus* (BP 87, $b = 2$).

Combined molecular and morphological analysis, including taxa without sequence data (46 taxa)—The objective of this analysis was to deduce the systematic postion for the four taxa lacking sequence data (*Nostolachma densiflora*, *N. khasiana*, *Petitiocodon parviflora*, and *Xantonneopsis robinsonii*). The analysis resulted in 234 most parsimonious trees. The strict consensus tree of 46 taxa is consistent with the combined molecular–morphological data tree based on 42 taxa (Fig. 3) but with some slight changes in topology, slightly lower bootstrap values, and considerably reduced decay values (Appendix S1, see Supplemental Data with online version of this article). The two species of *Nostolachma* are placed with the Asian representatives of Coffeeae, sister to *Xantonnea*; *Petitiocodon* is placed with members of Gardenieae, and *Xantonneopsis* with those of Octotropideae, although these relationships are poorly supported.

Distribution of morphological characters for and within the ingroup—In the combined morphological–molecular analysis, the ingroup is supported by two morphological synapomorphies, the presence of calyculi, and a distinctly lobed style (characters 6, 23, respectively; see Appendices 2 and 3). *Bertiera* does not possess these characters. Within the ingroup, other suprageneric groupings are supported by morphological data. *Coffea* and *Psilanthus* are supported by the apparent loss of secondary pollen presentation, the presence of a hard (horny/crustaceous) endocarp, seeds with a deep ventral groove, and a seed coat consisting of crushed endotestal cells and more or less isolated fibers (characters 9, 26, 28, 29). *Calycosiphonia*, *Argocoffeopsis*, and *Calycosiphonia* cf. by the absence of a seed coat (character 29), and *Diplospora*, *Discospermum*, and *Xantonnea* (and *Nostolachma*) by the universal presence of 4-merous flowers (character 8). The monophyly of several genera is supported by morphology: *Belonophora* by the presence of imbricate calyx lobes, included anthers, and superior radicle (characters 10, 14, 21); *Sericanthe* 7–9-merous flowers, distinctly basifixed anthers, and horizontal micropyle (radicle) orientation (characters 8, 15, 21); *Calycosiphonia* by having anthers with thecae (character 16); *Tricalysia* seeds with a hilar groove or shallow excavation (character 28); and *Psilanthus* by included anthers, supramedifixed anthers, and included style (characters 14, 15, 22). The monophyly of *Bertiera* is supported by a bimorphic corolla tube, a distinctly peltate placenta, and an included style with longitudinal ridges (characters 12, 19, 22, 24). Key characters are mapped on Fig. 3.

DISCUSSION

Close relatives of Coffea and their inclusion within Coffeeae—On the basis of plastid sequence data and morphology, the genera *Argocoffeopsis*, *Belonophora*, *Calycosiphonia*, *Calycosiphonia* cf. *Coffea*, *Diplospora*, *Discospermum*, *Psilanthus*, *Tricalysia*, *Sericanthe*, and *Xantonnea* form a well-supported clade (Fig. 3: BP 97, $b = 6$). With these genera included, Coffeeae is readily distinguished by the presence of calyculi and a distinctly 2-lobed style (characters 6 and 23). None of these characters are unique synapomorphies for the ingroup, although the presence of calyculi is otherwise so far only known in *Doricera* and perhaps some species of *Ixora*. The presence of a distinctly 2-lobed style (character 23) seems to be restricted to rather few genera within Ixoroideae s.s. (e.g., *Ixora, Robbrechtia De Block*). Paired axillary inflorescences (character 4) are found within several Ixoroideae but serve to provide a further distinguishing character for Coffeeae: paired axillary inflorescences are consistently present in Coffeeae.

Bertiera is sister to the ingroup in the combined plastid sequence analysis (BP 40, $b = 1$) but sister to two representatives of Gardenieae in the combined morphological–molecular analysis (BP 37, $b = 1$). *Bertiera* is morphologically distinct from members of Coffeeae, differing in the following characters (characters for Coffeeae in parentheses): (1) terminal inflorescences (axillary), although in three species of *Bertiera* the inflorescence is distinctly (paired) axillary (Robbrecht et al., 1994); (2) calyculi absent (present); (3) bimorphic corolla (corolla straight); (4) numerous (>30) ovules per locule (usually 1-several, but up to 20 in some species of *Tricalysia*); (5) peltate placenta (various shapes but never peltate); (6) multidirectional micropyle orientation (usually downward but always in one direction); (7) distinctly ridged style (style entire) with (8) adnate lobes (lobes free). The "axillary and then terminal" position of inflorescences in some members of Coffeeae is not homologous with the truly terminal

←

Fig. 1. One tree of four most parsimonious trees from the combined molecular data analysis. Branch lengths are placed above branches. Bootstrap values >50% are placed below branches; Bremer support values (decay values) are given after bootstrap values. Clades not present in the strict consensus tree are marked with an asterisk. See Appendix 1 for species authorities and provenance.

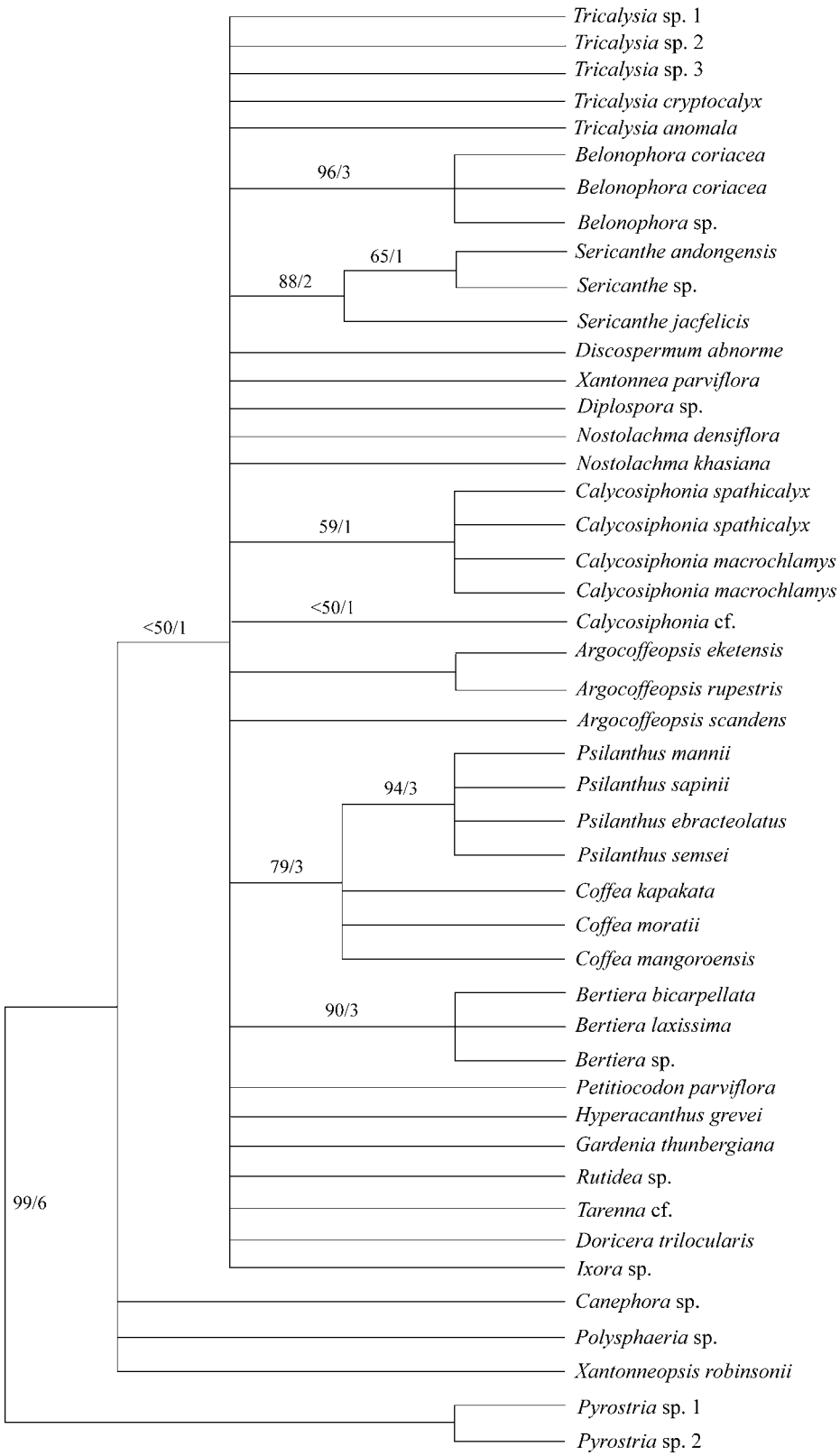


Fig. 2. Strict consensus tree of 2745 most parsimonious trees from the morphological data set. Bootstrap values >50% are placed above the branches; Bremer support values (decay values) are given after bootstrap values. See Appendix 1 for species authorities and provenance.

inflorescences of most *Bertiera* species (Davis et al., 2005). The distinctly peltate placenta in *Bertiera* seems to be unique within Ixoroideae s.s., and the genus possesses a combination of characters that is unique within this part of the subfamily, e.g. peltate placenta, numerous ovules per locule, and distinctly ridged style. In addition, most *Bertiera* species seem to have a type of exotestal thickening not found in other Rubiaceae (Robbrecht et al., 1994), although further critical study is required. *Bertiera* is the only genus of Ixoroideae s.s. that includes herbaceous species. Our findings support the removal of *Bertiera* from Coffeeae and placement in its own tribe, Bertiereae, in agreement with Bridson and Verdcourt (2003). A description and circumscription of *Bertiera* and Bertiereae is given by Bridson and Verdcourt (2003, p. 386). The placement of *Bertiera* within Coffeeae as subtribe Coffeinae as proposed by Robbrecht and Manen (2006) conflicts with the results of our analyses for both molecular and morphological data. Morphological data separate *Bertiera* from Coffeeae, whereas there are no morphological characters supporting the grouping of *Bertiera* and Coffeeae. Robbrecht and Manen (2006) provide no morphological characterization or rationale for Coffeeae including *Bertiera*. We believe that our new circumscription of Coffeeae, with the addition of new genera and the removal of *Bertiera*, is both scientifically coherent and practical.

Three generic candidates for inclusion in Coffeeae (see Table 1), viz. *Nostolachma*, *Petitiocodon*, *and Xantonneopsis* (all currently Gardenieae subtribe Diplosporinae) were not sampled for the plastid sequence analysis, and morphology alone was used to determine their systematic placement: observation of morphological characteristics and position within a combined molecular–morphological analysis (Appendix S1, see Supplemental Data with online version of this article). *Nostolachma* is placed with the Asian representatives of Coffeeae (*Diplospora*, *Discospermum*, and *Xantonnea*), which are characterized within the tribe in having consistently 4-merous flowers. This placement is not well supported, although *Nostolachma* is convincingly placed within Coffeeae. Our observations of floral morphology indicate that *Nostolachma* is dioecious, which is common in *Diplospora* and *Discospermum* but absent in the African genera of the ingroup, although some species of Madagascan *Tricalysia* are dioecious (Ali and Robbrecht, 1991; P. De Block, National Botanic Garden of Belgium, personal communication). Further study of Coffeeae genera occurring in Asia is warranted.

Xantonneopsis consists of a single species, *X. robinsonii* Pit. (Pitard, 1923). The combined molecular–morphological analysis (Appendix S1, see Supplemental Data with online version of this article) places *X. robinsonii* with Octotropideae (BP 69, $b = 1$), which is consistent with basic morphological observation: paired pedunculate, supra-axillary inflorescences and the presence of two distinctly pendulous ovules per locule arranged on a biseriate placenta. The biseriate placenta appears to be unique to Octotropideae (Robbrecht, 1988b; Bridson and Verdcourt, 2003) although it may not occur in all species within the tribe, and some genera (e.g., *Polysphaeria* Hook. f.) have a single ovule per locule. The seed coat of Octotropideae has a very distinct fingerprint-like or sometimes reticulate pattern (Robbrecht, 1988b; Bridson and Verdcourt, 2003; Stone and Davis, 2004) but unfortunately seeds of *X. robinsonii* were not available for study.

Petitiocodon also comprises a single species, *P. parviflorum* (Keay) Robbr.; the morphology of this species is described in detail by Robbrecht (1988b). This species does not have the key characters of Coffeeae but instead shares greater morphological affinity with members of Gardenieae. It has paired pedunculate, supra-axillary inflorescences, and a rather large broad-tubed corolla, and each locule of the bilocular ovary has a placenta attached at its base to the dividing wall of the locule with two more or less immersed ovules. The size and shape of the corolla, the position of the placental attachment, and the position of the ovules within the placenta infer a systematic position close to or within Gardenieae, although further data is required to clearly resolve the tribal placement of *Petitiocodon* within Rubiaceae. The combined molecular–morphological analysis (Appendix S1, Supplemental Data with online version of this article) supports our morphological observations, although the support values for placement within Gardenieae are very low (BP < 50, $b = 1$).

Characterization of Coffeeae—The morphological characters supporting an enlarged circumscription of Coffeeae given here are consistent with the characterization of the tribe given by Bridson and Verdcourt (2003, p. 387). One main point of difference is the nature of the apparently terminal inflorescences in Coffeeae, which has been elucidated by Davis et al. (2005). The position of the inflorescence on a short lateral shoot present in *Coffea* subgen. *Baracoffea* (J.-F.Leroy) J.-F.Leroy, most species of *Psilanthus* subgen. *Afrocoffea* (Moens) Bridson, and most species of *Argocoffeopsis* differs from a true terminal inflorescence. Each inflorescence originates as one of the paired axillary inflorescence and then becomes terminal on a short "shoot" or "spur" following renewed meristematic activity (Davis et al., 2005, p. 410). Thus the inflorescences in Coffeeae are never strictly terminal.

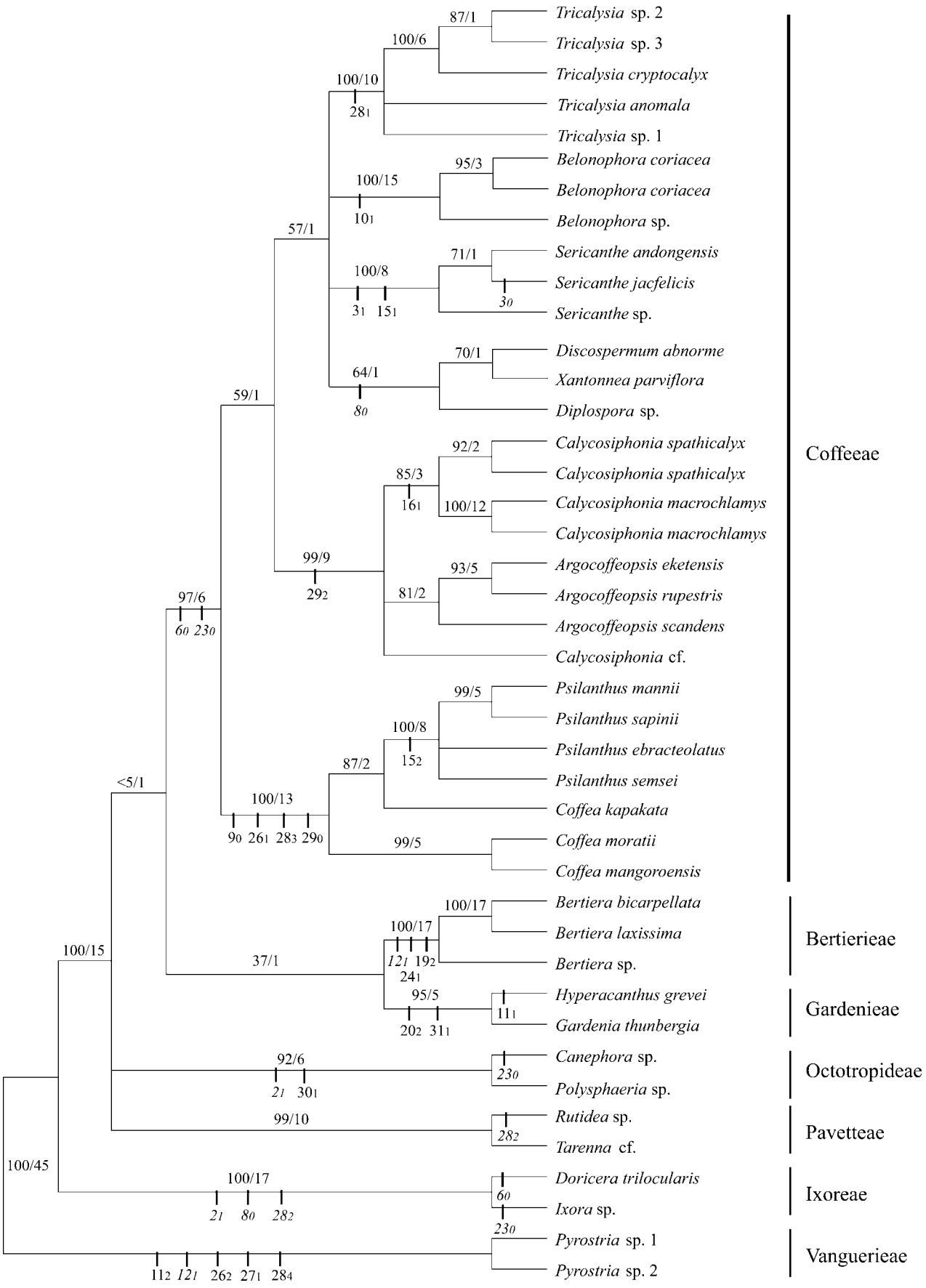
The genus *Doricera*, a member of Ixoreae, is sometimes affiliated with Coffeeae (e.g., Leroy, 1989), as it has paired axillary inflorescences, calyculi, and *Coffea*-like flowers. However, the style is club-shaped, shortly 3-lobed (not simple, and distinctly 2-lobed), the ovary is 3-locular (not 2-locular), and the seeds have a small circular excavation ("hilar cavity") on the ventral surface (not entire or with a longitudinal invagination). Our molecular and combined molecular–morphological analyses indicate the position of *Doricera* within Ixoreae (Fig. 1: BP 100, $b = 16$; Fig. 3: BP 100, $b = 17$).

We propose an emended characterization of Coffeeae, as given next, with key characters identified with an asterisk *.

Coffeeae DC., Ann. Mus. Hist. Nat. (Paris) 9: 217 (1807), as 'Coffeaceae' (including numerous genera); Robbr. & Puff, Bot. Jahrb. Syst. 108: 122 (1986) (restricted to *Coffea* L. and *Psilanthus* Hook. f.); Andreasen & B. Bremer, Am. J. Bot. 87: 1742 (2000) (also including several genera from Gardenieae DC. subtribe Diplosporinae Miq. and *Bertiera* Aubl.); Coffeeae DC. subtribe Coffeinae (DC.) Robbr. & Manen, Syst. Geogr. Pl. 76: 133 (2006). Type: *Coffea* L.

Synonym: Gardenieae DC. subtribe Diplosporinae Miq., Fl. Ned. Ind. 2: 237 (1857), as 'Diplosporeae'; Robbr. & Puff, Bot. Jahrb. Syst. 108: 114 (1986) (excluding *Cremaspora* Benth.). Type: *Diplospora* DC.

(1) Trees, shrubs, woody climbers, and woody monocauls; (2) stipule pairs adnate at the base and sometimes semi-sheathing, apex entire or apiculate; (3)* inflorescences paired, axillary or axillary and then appearing terminal (by continued meristematic activity of the inflorescence) on short shoots (typically in inflorescences from the previous year); (4)* inflorescences sessile (lacking a peduncle) with lowermost


Tricalysia sp. 2
Tricalysia sp. 3
Tricalysia cryptocalyx
Tricalysia anomala
Tricalysia sp. 1
Belonophora coriacea
Belonophora coriacea
Belonophora sp.
Sericanthe andongensis
Sericanthe jacfelicis
Sericanthe sp.
Discospermum abnorme
Xantonnea parviflora
Diplospora sp.
Calycosiphonia spathicalyx
Calycosiphonia spathicalyx
Calycosiphonia macrochlamys
Calycosiphonia macrochlamys
Argocoffeopsis eketensis
Argocoffeopsis rupestris
Argocoffeopsis scandens
Calycosiphonia cf.
Psilanthus mannii
Psilanthus sapinii
Psilanthus ebracteolatus
Psilanthus semsei
Coffea kapakata
Coffea moratii
Coffea mangoroensis
Bertiera bicarpellata
Bertiera laxissima
Bertiera sp.
Hyperacanthus grevei
Gardenia thunbergia
Canephora sp.
Polysphaeria sp.
Rutidea sp.
Tarenna cf.
Doricera trilocularis
Ixora sp.
Pyrostria sp. 1
Pyrostria sp. 2
87/1
100/6
100/10
281
95/3
100/15
101
57/1
71/1
100/8
31 151
30
70/1
64/1
80
59/1
92/2
85/3
161
100/12
99/9
292
93/5
97/6
60 230
81/2
<5/1
99/5
100/8
152
87/2
100/13
90 261 283 290
99/5
100/17
100/17
121 192
241
100/15
37/1
95/5
111
202 311
92/6
21 301
230
99/10
282
100/45
100/17
21 80 282
60
230
112 121 262 271 284
Coffeeae
Bertierieae
Gardenieae
Octotropideae
Pavetteae
Ixoreae
Vanguerieae

calyculus in the leaf axil; (5)* calyculi (cupule-like structures formed by the contraction of shoot tissue and the reduction and fusion of leaves and stipules) present, usually 4-lobed, but sometimes 2-lobed or lobes lacking; (6) flowers 4–8(−12)-merous; (7)* corolla tube narrow and straight and (8) with lobes overlapping to the left (*Coffea*-like flowers); (9) anthers exserted, rarely included; (10) stigmatic lobes usually excluded but sometimes distinctly included (*Psilanthus*), and included or excluded in dioecious species; (11) ovary 2-locular, placentation axile; (12) each locule with the placenta attached to the septum, at the middle or just above, rarely near the apex (only *Belonophora* and *Sericanthe*); (13)* ovules usually 1 or 2 per locule or up to 10 (rarely c. 20); (14) ovules on a small placenta, or placenta large and ovules either on or semi-embedded within the placental tissue; (15) embryo radicle usually inferior, rarely horizontal (only *Sericanthe*), or superior (only *Belonophora*); (16) style simple (lacking specialized features), glabrous, *distinctly 2-lobed; (17) fruit an indehiscent drupe, with few (1 or 2) to several seeds (rarely up to c. 10); (18) ventral surface of seed more or less entire, (sometimes with a shallow hilar grove or shallow excavation), or with a distinct longitudinal ventral invagination ("coffee-bean" morphology: only *Coffea* and *Psilanthus*); (19) endosperm usually entire but sometimes ruminate (only some species of *Tricalysia* and *Coffea*); (20) testa cells isodiametric or elongated, thickenings present or absent, smooth along outer tangential walls, or (in *Coffea* and *Psilanthus*) with cells crushed during the development of endosperm and composed of thin elongate parenchymatic cells usually containing many more or less isolated fibers; (21) pollen (2–)3–5-colporate (zonocolporate).

Genera included *Argocoffeopsis, Belonophora, Calycosiphonia*, *Coffea*, *Diplospora*, *Discospermum*, *Nostolachma*, *Psilanthus*, *Sericanthe*, *Tricalysia*, and *Xantonnea*. The entity *Calycosiphonia* cf. may represent an additional genus for the tribe; further material and morphological investigations are required.

Further details of salient characters are given in Appendix 2. In addition to the findings presented here, this morphological characterization is based on data derived from Robbrecht (1981), Robbrecht and Puff (1986), Bridson and Verdcourt (2003), Davis et al. (2005), and for pollen, Stoffelen et al. (1997).

Relationships within Coffeeae—Based on combined molecular data (Fig. 1; BP 100, $b = 9$) and combined molecular–morphological data (Fig. 3; BP 100, $b = 13$), there is strong support for a relationship between *Coffea* and *Psilanthus*. This is to be expected, given that they share a carpel morphology that is unique within Ixoroideae s.s. (see Fig. 3; Introduction; Appendices 2 and 3). They are also peculiar within Ixoroideae s.s. in lacking secondary (ixoroid) pollen presentation, which is present in nearly all members of the subfamily (Puff et al., 1996), although the distribution of this character in some monoecious members of Ixoroideae (e.g., *Diplospora* and *Discospermum*) is still unclear (e.g., Ali and Robbrecht, 1991). A close phylogenetic relationship between *Coffea* and *Psilanthus* based on molecular and morphological data is concomitant with close cytological correspondence (Lombello and Pinto-Maglio, 2003, 2004), including the production of a fertile intergeneric hybrid (Couturon et al., 1998). Our results are also in agreement with the phylogenetic studies of Lashermes et al. (1997), Cros et al. (1998), Andreasen et al. (1999), and Andreasen and Bremer (2000, fig. 3), who showed that *Psilanthus* is nested within *Coffea*. Detailed species-level studies are now required to assess the phylogenetic relationship between *Coffea* and *Psilanthus*.

The combined molecular–morphological analysis shows that that there is strong support (Fig. 3: BP 99, $b = 9$) for a clade containing *Argocoffeopsis*, *Calycosiphonia*, and *Calycosiphonia* cf. distinguished by the lack of a testa. Earlier studies did not identify close phylogenetic links between *Argocoffeopsis* and *Calycosiphonia* (Robbrecht, 1981; Robbrecht and Puff, 1996), although it has been known for some time that these genera lack a testa (e.g., Robbrecht and Puff, 1996) and that this feature is all but absent within Coffeeae and Gardenieae subtribe Diplosporinae (Ali and Robbrecht, 1991) and rare in Ixoroideae s.s. (Robbrecht and Puff, 1986). Ali and Robbrecht (1991) report that in *Diplospora* (only *D. wrayi* King & Gamble) the mature exotesta cells are very rarely crushed by the development of the endosperm, so that the seed coat is reduced to a thin pellicle, as in *Argocoffeopsis* and *Calycosiphonia*. The exact systematic position of *Calycosiphonia* cf. has not been elucidated by our study. It generally looks much more like *Calycosiphonia* than *Argocoffeopsis*, although it can be excluded from the former genus on the basis of lacking anther thecae and having 5-merous flowers (7- to 8-merous in *Calycosiphonia*). *Calycosiphonia* cf. also closely resembles one species of *Argocoffeopsis*, *A. lemblinii* (A. Chev.) Robbr. Most species of *Argocoffeopsis* are woody climbers, but *A. lemblinii* [and *A. rupestris* (Hiern) Robbr.] are shrubs or small trees. The only readily discernible difference between *Calycosiphonia* cf. and *A. lemblinii* is that the former has large ellipsoid fruits (seeds elliptic in outline) and the latter has small spheroid fruits (seeds circular in outline). However, further study is required.

The combined molecular–morphological analysis weakly supports a relationship between three of the four Coffeeae genera occurring in Asia: *Diplospora*, *Discospermum*, and *Xantonnea* (Fig. 3; BP 64, $b = 1$), and the fourth, *Nostolachma*, is placed within this group based on morphology alone (Appendix S1, see Supplemental Data with online version of this article). This group is characterized by the presence of 4-merous flowers, a character unique to these four genera within Coffeeae (discussed earlier). This is the first time a relationship between these four genera has been suggested. These results agree with Robbrecht and Puff (1986), who concluded that *Nostolachma* does not have a close relationship with *Coffea* and *Psilanthus* as postulated by Leroy (1980). The recognition

←

Fig. 3. Strict consensus tree of 31 most parsimonious trees generated by combined molecular and morphological data sets (consisting of 42 taxa). Bootstrap values are placed above the branches; Bremer support values (decay values) are given after bootstrap values. Selected characters (Appendix 2) are placed below branches with subscript indicating derived state. Homoplasious characters (i.e., those appearing on more than one branch) are given in italics. Character diagnostics (CI/RI) for morphological characters are mapped onto the consensus tree as follows: 2 (0.5/0.67); 3 (0.5/0); 6 (0.5/0.9); 7 (0.33/0.6); 8 (0.29/0.64); 9 (1/1); 10 (1/1); 11 (1/1); 12 (0.5/0.75); 15 (1/1); 16(1/1); 23 (0.33/0.8); 24 (1/1); 26 (1/1); 27 (1/1); 28 (0.8/0.92); 29 (1/1); 30 (1/1); 31 (1/1). See Appendix 1 for species authorities and provenance.

of *Diplospora* and *Discospermum* (from Asia) as separate from *Tricalysia* (Africa and Madagascar) by Ali and Robbrecht (1991) is supported by our study. According to Ali and Robbrecht (1991), Asian *Diplospora* and *Discospermum* can be separated from African and Madagascan *Tricalysia* (characters for *Tricalysia* in parentheses) by standard type colleters (mostly with modified colleter type); almost consistently 4-merous flowers (predominantly 5-pleiomerous); inconspicuous bracteoles/calyculi (large, sheathing bracteoles resembling the calyx); small flowers (larger flowers); strong tendency toward unisexual flowers (hermaphrodite; unisexual in Madagascan species); secondary pollen presentation possibly absent (generally or universally present); seed coat mostly with thickened exotestal cells (not thickened). Three of these characters are included in our morphological matrix (see Appendices 2 and 3). The other Asian genus, *Xantonnea*, was included in the tribe Octotropideae by Robbrecht (1988b, 1994), but our molecular data and the presence of key morphological features place it with the genera of Coffeeae restricted to Asia (Figs. 1, 3).

Given the low branch support values (BP 57, $b = 1$) and low level of internal resolution for the clade that includes *Tricalysia*, *Belonophora*, *Sericanthe*, and the Asian Coffeeae (*Diplospora*, *Discospermum,* and *Xantonnea*), further data are needed to fully elucidate the systematic relationships for and within this clade. In addition, the relationship of the *Argocoffepsis-Calycosiphonia-Calycosiphonia* cf. clade with all of the above genera needs further investigation.

The monophyly and circumscription of genera of Coffeeae—Combined molecular and morphological data (Fig. 3) are consistent with the assumed monophyly of *Tricalysia* (BP 100, $b = 10$), *Belonophora* (BP 100, $b = 15$), *Sericanthe* (BP 100, $b = 8$), *Calycosiphonia* (BP 85, $b = 3$), *Argocoffeopsis* (BP 81, $b = 2$), and *Psilanthus* (BP 100, $b = 8$). However, for some genera we have only sampled a relatively low number of species per genus. In particular, *Tricalysia* (98 spp.) and *Coffea* (95 spp.) require further sampling. *Coffea* is paraphyletic with respect to *Psilanthus* (see earlier). The monophyly of the Asian Coffeeae genera could not be tested in our study due to a lack of suitable DNA material. Molecular and morphological data are needed to test the monophyly and elucidate the systematic relationships of *Diplospora*, *Discospermum*, *Nostolachma*, and *Xantonnea*. It is likely that some generic synonymy may result from further studies.

Apart from the Asian genera, the monophyly of most genera of Coffeeae is supported by one or more synapomorphies (see earlier). The exceptions are *Argocoffeopsis*, which is based on a combination of characters (e.g., Bridson and Verdcourt, 2003), and *Coffea* (Davis et al., 2005).

Coffea differs from *Psilanthus* in its long, exserted style (short, included in *Psilanthus*) and submedifixed (vs. supramedifixed) excluded (vs. included) anthers. According to Davis et al. (2005), there are two other morphological differences between these two genera [excluding *P. melanocarpus* (Welw. ex Hiern) J.-F. Leroy]. *Coffea* has short to long anther filaments (very short to more or less absent filaments in *Psilanthus*) and pollen with 3 or 4, rarely 2, apertures (vs. 4 or 5, rarely 3). Despite these morphological distinctions, molecular, morphological, and combined analyses all show *Psilanthus* as derived within the *Coffea* clade (Figs. 1, 3), so that the recognition of *Psilanthus* results in a paraphyletic *Coffea*.

Conclusions—The cladistic analyses presented here provide the most inclusive systematic study of Coffeeae to date, with molecular sequence data for all but one of the component genera. The enlargement of Coffeeae by the addition of *Diplospora* and *Tricalysia*, on the basis of molecular sequence data (Andreasen and Bremer, 1996, 2000; Persson, 2000) is supported by our study. The addition of *Discospermum* and *Sericanthe* on the basis of morphological inference (Andreasen and Bremer, 2000) is also confirmed. Preliminary results from this study (A. P. Davis, unpublished data) were used to support the transfer of *Argocoffeopsis*, *Belonophora*, and *Calycosiphonia* to Coffeeae by Bridson and Verdcourt (2003), and these transfers are again confirmed by our completed study. *Xantonnea* is transferred from Octotropideae (Robbrecht, 1988b, 1994) to Coffeeae on the basis of molecular sequence data and morphology. *Nostolachma* is transferred from Gardenieae subtribe Diplosporinae (Robbrecht, 1988b, 1994) to Coffeeae on the basis of morphological data. *Petitiocodon* and *Xantonneopsis* are not members of Coffeeae; based on morphological data, the former is placed tentatively in Gardenieae and the latter Octotropideae.

We propose that Coffeeae should include 11 genera, viz. *Argocoffeopsis*, *Belonophora*, *Calycosiphonia*, *Coffea*, *Diplospora*, *Discospermum*, *Nostolachma,* *Psilanthus*, *Tricalysia*, *Sericanthe*, and *Xantonnea* (see Table 1), and have given an updated morphological characterization of the tribe (see earlier). A poorly known and informal taxon, *Calycosiphonia* cf. is also placed in Coffeeae, although its generic status has yet to be confirmed (A. P. Davis and B. Sonké, personal communication).

Coffea and *Psilanthus* are clearly distinct within Coffeeae, and a sister relationship to the rest of the tribe is inferred but is only weakly supported by our combined molecular–morphological analysis (Fig. 3: BP 59, $b = 1$). Within other Coffeeae, a well-supported phylogenetic relationship is inferred for the species lacking a discernible seed coat (*Argocoffeopsis*, *Calycosiphonia*, and *Calycosiphonia* cf.). The monophyly of *Argocoffeopsis*, *Belonophora*, *Calycosiphonia*, *Psilanthus*, *Tricalysia*, and *Sericanthe* is consistent with our analyses; one or more clear-cut synapomorphies are identified for *Belonophora*, *Calycosiphonia*, *Psilanthus*, and *Sericanthe*. *Coffea* is paraphyletic, as identified in other molecular analyses (Lashermes et al., 1997; Cros et al., 1998; Andreasen et al., 1999; Andreasen and Bremer, 2000, fig. 3). Morphological separation of *Coffea* and *Psilanthus* is possible (Davis et al., 2005; and see Discussion), but further data, including wider sampling in these genera, are now urgently required to ascertain whether two genera can be upheld.

The exclusion of *Bertiera* from Coffeeae and its placement in its own tribe (Bridson and Verdcourt, 2003, p. 386), Bertiereae, are supported by our combined molecular (Fig. 1), combined molecular–morphological data (Fig. 3), and our morphological survey. The inclusion of *Bertiera* within Coffeeae, as subtribe Bertierinae as proposed by Robbrecht and Manen (2006) is not supported by our data. Coffeeae becomes a strictly Old World tribe following the exclusion of *Bertiera*. The decision to place Gardenieae subtribe Diplosporinae into the synonymy of Coffeeae (Bridson and Verdcourt, 2003) is substantiated by our analyses.

LITERATURE CITED

ALI, S. J., AND E. ROBBRECHT. 1991. Remarks on the tropical Asian and Australian taxa included in *Diplospora* or *Tricalysia* (Rubiaceae-Ixoroideae-Gardenieae). *Blumea* 35: 279–305.

ANDREASEN, K., B. G. BALDWIN, AND B. BREMER. 1999. Phylogenetic utility of the nuclear rDNA ITS region in the subfamily Ixoroideae (Rubiaceae): comparisons with cpDNA and *rbcL* sequence data. *Plant Systematics and Evolution* 217: 119–135.

ANDREASEN, K., AND B. BREMER. 1996. Phylogeny of the Ixoroideae (Rubiaceae). *Opera Botanica Belgica* 7: 119–138.

ANDREASEN, K., AND B. BREMER. 2000. Combined phylogenetic analysis in the Rubiaceae-Ixoroideae: morphology, nuclear and chloroplast DNA data. *American Journal of Botany* 87: 1731–1748.

BREMEKAMP, C. E. B. 1934. A monograph of the genus *Pavetta* L. *Repertorium Specierum Novarum Regni Vegetabilis* 37: 1–208.

BREMER, K. 1988. The limits of amino-acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* 42: 795–803.

BREMER, K. 1994. Branch support and tree stability. *Cladistics* 10: 295–304.

BRIDSON, D. M. 1978. Studies in *Pavetta* (Rubiaceae subfamily Cinchonoideae) for part 2 of 'Flora of tropical east Africa: Rubiaceae.' *Kew Bulletin* 32: 609–652.

BRIDSON, D. M. 1998. Rubiaceae. *In* G. V. Pope [ed.], Flora Zambesiaca, vol. 5, part 2, 211–377. Royal Botanic Gardens, Kew, UK.

BRIDSON, D. M., AND B. VERDCOURT. 1988. Rubiaceae, Part 2. *In* R. M. Polhill [ed.], Flora of tropical east Africa, 415–747. Balkema, Rotterdam, Netherlands.

BRIDSON, D. M., AND B. VERDCOURT. 2003. Rubiaceae. *In* G. V. Pope [ed.], Flora Zambesiaca, vol. 5, part 3, 379–720. Royal Botanic Gardens, Kew, UK.

CHASE, M. W., AND H. G. HILLS. 1991. Silica gel: an ideal desiccant for preserving field-collected leaves for use in molecular studies. *Taxon* 41: 215–220.

CHEEK, M., AND S. DAWSON. 2000. A synoptic revision of *Belonophora* (Rubiaceae). *Kew Bulletin* 55: 63–80.

CHINNAPPA, C. C., AND B. G. WARNER. 1981. Pollen morphology in the genus *Coffea* (Rubiaceae) and its taxonomic significance. *Botanical Journal of the Linnean Society* 83: 221–236.

COUTURON, E., P. LASHERMES, AND A. CHARRIER. 1998. First intergeneric hybrids (*Psilanthus ebracteolatus* Hiern × *Coffea arabica* L.) in coffee trees. *Canadian Journal of Botany* 76: 542–546.

CROS, J. 1994. Implications phylogénétiques des variations de l'ADN chloroplastique chez les caféiers (genres *Coffea* L. et *Psilanthus* Hook. f.). Ph.D. dissertation, Université Montpellier II, Montpellier, France.

CROS, J., M. C. COMBES, P. TROUSLOT, F. ANTHONY, S. HAMON, A. CHARRIER, AND P. LASHERMES. 1998. Phylogenetic analysis of chloroplast DNA variation in *Coffea* L. *Molecular Phylogenetics and Evolution* 9: 109–117.

DAVIS, A. P. 2003. A new combination in *Psilanthus* (Rubiaceae) for Australia, and nomenclatural notes on *Paracoffea*. *Novon* 13: 182–184.

DAVIS, A. P., D. M. BRIDSON, AND F. RAKOTONASOLO. 2005. A reexamination of *Coffea* subgenus *Baracoffea* and comments on the morphology and classification of *Coffea* and *Psilanthus* (Rubiaceae-Coffeeae). *In* R. C. Keating, V. C. Hollowell, and T. Croat [eds.], Festschrift for William G. D'Arcy: the legacy of a taxonomist (Monograph in Systematic Botany 104), 398–420. Missouri Botanical Garden Press, St. Louis, Missouri, USA.

DAVIS, A. P., R. GOVAERTS, D. M. BRIDSON, AND P. STOFFELEN. 2006. An annotated taxonomic conspectus of the genus *Coffea* (Rubiaceae). *Botanical Journal of the Linnean Society* 152: 465–512.

DE CANDOLLE, A. P. 1807. Mémoire sur le *Cuviera*, genre noveau de la famille des Rubiacées. *Annales du Muséum d'Histoire Naturelle* 9: 216–222.

DE CANDOLLE, A. P. 1830. Prodromus systematis naturalis regni vegetabilis. Part IV. Saxifragaceae to Dipsacaceae. Treuttel and Würtz, Paris, France.

DOYLE, J. J., AND J. L. DOYLE. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemistry Bulletin* 19: 11–15.

ERIKSSON, T. 1999. AutoDecay, version 4.0.2. Computer program distributed by the author. Bergius Foundation, Royal Swedish Academy of Sciences, Stockholm, Sweden.

FELSENSTEIN, J. 1985. Phylogenies and the comparative method. *American Naturalist* 125: 1–15.

FITCH, W. M. 1971. Toward defining the course of evolution: minimum change for a specific tree topology. *Systematic Zoology* 20: 406–416.

GOVAERTS, R., M. RUHSAM, L. ANDERSSON, E. ROBBRECHT, D. BRIDSON, A. DAVIS, I. SCHANZER, AND B. SONKÉ. 2006. World Checklist of Rubiaceae. Board of Trustees of the Royal Botanic Gardens, Kew. Website http://www.kew.org/wcsp/rubiaceae [accessed 10 October 2006].

HOLMGREN, P. K., N. H. HOLMGREN, AND L. C. BARNETT. 1990. Index Herbariorum, part 1, the herbaria of the world, 8th ed. Regnum Vegetabile [series]. New York Botanical Garden, Bronx, New York, USA.

JORDAN, W. C., M. W. COURTNEY, AND J. E. NEIGEL. 1996. Low levels of intraspecific genetic variation at a rapidly evolving chloroplast DNA locus in North American duckweeds (Lemnaceae). *American Journal of Botany* 83: 430–439.

KÄLLERSJÖ, M., J. S. FARRIS, A. G. KLUGE, AND C. BULT. 1992. Skewness and permutation. *Cladistics* 8: 275–287.

LASHERMES, P., M. C. COMBES, J. ROBERT, P. TROUSLOT, A. D'HONT, F. ANTHONY, AND A. CHARRIER. 1999. Molecular characterisation and origin of the *Coffea arabica* L. genome. *Molecular and General Genetics* 261: 259–266.

LASHERMES, P., M. C. COMBES, P. TROUSLOT, AND A. CHARRIER. 1997. Phylogenetic relationships of coffee-tree species (*Coffea* L.) as inferred from ITS sequences of nuclear ribosomal DNA. *Theoretical and Applied Genetics* 94: 947–955.

LASHERMES, P., J. CROS, M. C. COMBES, P. TROUSLOT, F. ANTHONY, S. HAMON, AND A. CHARRIER. 1996. Inheritance and restriction fragment length polymorphism of chloroplast DNA in the genus *Coffea* L. *Theoretical and Applied Genetics* 93: 626–632.

LASHERMES, P., J. CROS, P. MARMEY, AND A. CHARRIER. 1993. Use of random amplified DNA markers to analyse genetic variability and relationships of *Coffea* species. *Genetic Resources and Crop Evolution* 40: 91–99.

LEROY, J.-F. 1967. Diagnose différentielle du genre *Paracoffea* Leroy. *Journal d'Agriculture Tropicale et de Botanique Appliquée* 14: 276.

LEROY, J.-F. 1980. Evolution et taxogenèse chez les caféiers (*Coffea* L., *Psilanthus* Hook. f. et *Nostolachma* Durand). Hypothèse sur leur origine. *Compte Rendu Hebdomadaire des Séances de l'Académie des Sciences, série D, Sciences Naturelles* 29: 593–596.

LEROY, J.-F. 1989. *Coffea* L. *In* J. Bosser, T. Cadet, J. Guého, and W. Marais [eds.], Flore des Mascareignes La Réunion, Maurice, Rodrigues, 107. Caprifoliacées à 108bis. Valérianacées, 93–99. Mauritius Sugar Industry Research Institute, Reduit, Mauritius; L'Office de la Research Scientifique et Technique Outre-Mer, Paris, France; and Royal Botanic Gardens, Kew, UK.

LOBREAU-CALLEN, D., AND J.-F. LEROY. 1980. Quelques données palynologiques sur le genre *Coffea* et autres genres du cercle des caféiers. *Association Scientifique Internationale du Café, 9th Colloque* 479–506.

LOMBELLO, R. A., AND C. A. F. PINTO-MAGLIO. 2003. Cytogenetic studies in *Psilanthus ebracteolatus* Hiern., a wild diploid coffee species. *Cytologia* 68: 425–429.

LOMBELLO, R. A., AND C. A. F. PINTO-MAGLIO. 2004. Cytogenetic studies in *Coffea* L. and *Psilanthus* Hook. f. using CMA/DAPI and FISH. *Cytologia* 69: 85–91.

MADDISON, D. R., AND W. P. MADDISON. 2005. MacClade 4, version 4.07. Sinauer, Sunderland, Massachusetts, USA.

MENDENHALL, M. 1994. Phylogeny of *Baptisia* and *Thermopsis* (Leguminosae) as inferred from chloroplast DNA and nuclear ribosomal DNA sequences, secondary chemistry, and morphology. Ph.D. dissertation, University of Texas, Austin, Texas, USA.

PERSSON, C. 1996. Phylogeny of the Gardenieae (Rubiaceae). *Botanical Journal of the Linnean Society* 121: 91–109.

PERSSON, C. 2000. Phylogeny of Gardenieae (Rubiaceae) based on

chloroplast DNA sequences from the *rps16* intron and *trnL*(UAA)-F(GAA). *Nordic Journal of Botany* 20: 257–269.

PITARD, C. J. 1923. Flore Générale de l'Indo-Chine. *In* M. H. Lecomte [ed.], Rubiacées, 20–442. Masson et Cie, Paris, France.

PUFF, C., E. ROBBRECHT, R. BUCHNER, AND P. DE BLOCK. 1996. A survey of secondary pollen presentation in the Rubiaceae. *Opera Botanica Belgica* 7: 369–402.

PUFF, C., E. ROBBRECHT, AND V. RANDRIANOSOLO. 1984. Observations on the SE African-Madagascan genus *Alberta* and its ally *Nematosytlis* (Rubiaceae, Alberteae) with a survey of the species and a discussion of its taxonomic position. *Bulletin du Jardin Botanique National de Belgique* 54: 293–366.

RAKOTONASOLO, F., AND A. P. DAVIS. 2006. Six species of Madagascan *Genipa* transferred to *Hyperacanthus* (Rubiaceae-Gardenieae) and new data on general morphology, placentation and ovary structure in *Hyperacanthus*. *Taxon* 55: 387–396.

RICHARD, A. 1830. Mémoire sur la Famille des Rubiacées. J. Tastu, Paris, France.

ROBBRECHT, E. 1978. *Sericanthe*, a new African genus of Rubiaceae (Coffeeae). *Bulletin du Jardin Botanique National de Belgique* 48: 3–78.

ROBBRECHT, E. 1981. Studies in tropical African Rubiaceae (II): 5. A survey of *Argocoffeopsis*. 6. A revision of *Calycosiphonia*. *Bulletin du Jardin Botanique National de Belgique* 51: 359–378.

ROBBRECHT, E. 1988a. Studies in tropical African Rubiaceae. 13. *Petitiocodon*, a new genus to accommodate *Didymosalpinx parviflora* (Gardenieae-Diplosporinae). *Bulletin du Jardin Botanique National de Belgique* 58: 109–120.

ROBBRECHT, E. 1988b. Tropical woody Rubiaceae. *Opera Botanica Belgica* 1: 1–271.

ROBBRECHT, E. 1994. Supplement to the 1988 outline of the classification of the Rubiaceae. *Opera Botanica Belgica* 6: 173–196.

ROBBRECHT, E., AND J. F. MANEN. 2006. The major evolutionary lineages of the coffee family (Rubiaceae, angiosperms). Combined analysis (nDNA and cpDNA) to infer the position of *Coptosapelta* and *Luculia*, and supertree construction based on *rbcL*, *rps16*, *trnL-trnF* and *atpB-rbcL* data. A new classification in two subfamilies, Cinchonoideae and Rubioideae. *Systematics and Geography of Plants* 76: 85–146.

ROBBRECHT, E., AND C. PUFF. 1986. A survey of the Gardenieae and related tribes (Rubiaceae). *Botanische Jahrbücher für Systematik* 108: 63–137.

ROBBRECHT, E., U. ROHRHOFER, AND C. PUFF. 1994. A survey of *Bertiera* (Rubiaceae), including a discussion of its taxonomic position. *Opera Botanica Belgica* 6: 101–141.

SCHUMANN, K. 1891. Rubiaceae. *In* A. Engler and K. Prantl [eds.], Die natürlichen Pflanzenfamilien, 1–156. Verlag von Vilhelm Engelmann, Leipzig, Germany.

STERN, W. T. 1957. Achille Richard's "Mémoire sur la Famille des Rubiacées." *Taxon* 6: 186–188.

STOFFELEN, P. 1998. *Coffea* and *Psilanthus* in tropical Africa: a systematic and palynological study, including a revision of the West and Central African species. Ph.D. dissertation, Katholieke Universiteit Leuven, Leuven, Belgium.

STOFFELEN, P., E. ROBBRECHT, AND E. SMETS. 1997. Pollen morphology of *Coffea* and *Psilanthus* (Rubiaceae), mainly from Africa. *Grana* 36: 313–327.

STONE, J. R., AND A. P. DAVIS. 2004. New taxa and nomenclatural notes on the flora of the Marojejy Massif, Madagascar. VI. Rubiaceae: a new species of *Lemyrea*. *Novon* 14: 119–123.

SWOFFORD, D. L. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4.0b10. Sinauer, Sunderland, Massachusetts, USA.

TABERLET, P., L. GIELLY, G. PAUTOU, AND J. BOUVET. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.

VEGA, F. E., E. ROSENQUIST, AND W. COLLINS. 2003. Global project needed to tackle coffee crisis. *Nature* 425: 343.

VINCKIER, S., S. HUYSMANS, AND E. SMETS. 2000. Morphology and ultrastructure of orbicules in the subfamily Ixoroideae (Rubiaceae). *Review of Palaeobotany and Palynology* 108: 151–174.

APPENDIX 1. Voucher information and GenBank accession numbers for taxa used in this study. A dash indicates the region was not sampled. Voucher specimens are deposited in the following herbaria: K = Royal Botanic Gardens, Kew; TAN = Parc de Tsimbazaza, Antananarivo; BR = National Botanic Garden of Belgium, Meise; YA = National Herbarium of Cameroon, Yaoundé.

Taxon*—Voucher* (herbarium), Origin; GenBank accession nos.: *accD-psaI, rpl16, trnL-F*.

Argocoffeopsis eketensis (Wernham) Robbr.—*Davis 3031* (K), Cameroon; DQ180497, DQ180531, DQ180566. ***Argocoffeopsis rupestris*** (Heirn) **subsp. *thonneri*** (Lebrun) Robbr.—*Harris 8168* (K), Central African Republic; DQ180496, DQ180532, DQ180567. ***Argocoffeopsis scandens*** (K.Schum.) Lebrun—*Davis 3016* (K), Cameroon; DQ180498, DQ180533, DQ180568. ***Belonophora coriacea*** Hoyle—*Maurin 5* (K), Cameroon; DQ180499, DQ180534, DQ180569. ***Belonophora coriacea*** Hoyle—*Maurin 19* (K), Cameroon; DQ180500, DQ180535, DQ180570. ***Belonophora*** sp.—*TAF 480* (K), Cameroon; DQ180501, DQ180536, DQ180571. ***Bertiera bicarpellata*** (K.Schum.) N.Hallé—*Davis 3051* (K), Cameroon; DQ180502, DQ180537, DQ180572. ***Bertiera laxissima*** K.Schum.—*Maurin 13* (K), Cameroon; DQ180503, DQ180538, DQ180573. ***Bertiera*** sp.—*Davis 3017* (K), Cameroon; DQ180504, DQ180539, DQ180574. ***Calycosiphonia*** cf.—*Sonké 3783* (K, YA), Cameroon; DQ180505, DQ180540, DQ180565. ***Calycosiphonia macrochlamys*** (K.Schum.) Robbr.—*Davis 3036* (K), Cameroon; DQ180506, DQ180541, DQ180575. ***Calycosiphonia macrochlamys*** (K.Schum.) Robbr.—*Davis 3044* (K), Cameroon; DQ180507, DQ180542, DQ180576. ***Calycosiphonia spathicalyx*** (K.Schum.) Robbr.—*Mvungi 22* (DSM, K), Tanzania; DQ180508, DQ180543, DQ180577. ***Calycosiphonia spathicalyx*** (K.Schum.) Robbr.—*Davis 2925* (K), Tanzania; DQ180509, DQ180544, DQ180578. ***Canephora*** sp.—*Davis 2721* (K), Madagascar; DQ180510, DQ180545 DQ180579. ***Coffea kapakata*** (A.Chev.) Bridson—*IRD-Montpellier OK* (K), Angola; DQ153490, DQ153739, DQ153857. ***Coffea mangoroensis*** Portères—*Rakotonasolo 41* (K, TAN), Madagascar; DQ153503, DQ153752, DQ153870. ***Coffea moratii*** J.-F.Leroy ex A. P.Davis & Rakotonas.—*Davis 2326* (K), Madagascar; DQ153502, DQ153751, DQ153869. ***Diplospora*** sp.—*Bremer 15238* (K), Borneo (Brunei); DQ180511, DQ180546, DQ180580. ***Discospermum abnorme*** (Korth.) S.J.Ali & Robbr.—*DB 87* (K), Borneo; DQ180512, DQ180547, DQ180581. ***Doricera trilocularis*** (Balf. f.) Verdc.—*Friedmann 2939* (K), Mascarenes (Rodrigues); DQ180513, DQ180548, DQ180582. ***Gardenia thunbergia*** L. f.—*1961–29703* (K), SE Africa; DQ180514, DQ180549, DQ180583. ***Hyperacanthus grevei*** Rakotonas. & A.P.Davis—*Davis 2565* (K), Madagascar; DQ180515, DQ180550, DQ180584. ***Ixora*** sp.—*Davis 2297* (K), Madagascar; DQ180516, DQ180551, DQ180585. ***Polysphaeria*** sp.—*Mvungi 15* (DSM, K), Tanzania; DQ180517, DQ180552, DQ180586. ***Psilanthus ebracteolatus*** Heirn—*Davis 3008* (K), Cameroon; DQ153392, DQ153641, DQ153759. ***Psilanthus mannii*** Hook. f.—*Davis 6958* (K), Central African Republic; DQ180518, DQ180553, DQ180587. ***Psilanthus sapinii*** De Wild.—*Sapin s.n. 0856914* (BR), Congo-Kinshasa; DQ153394, DQ153643, DQ153761. ***Psilanthus semsei*** Bridson—*Kisera 1473* (K), Tanzania; DQ153395,

DQ153644, DQ153762. ***Pyrostria*** sp. 1—*Davis 2709* (K), Madagascar; DQ180519, DQ180554, DQ180588. ***Pyrostria*** sp. 2—*OKTAN 171* (K), Madagascar; DQ180520, DQ180555, DQ180589. ***Rutidea*** sp.—*Davis 3056* (K), Cameroon; DQ180521, DQ180556, DQ180590. ***Sericanthe andongensis*** (Hiern) Robbr.—*Bidgood 3490* (K), Tanzania; DQ180522, DQ180557, DQ180591. ***Sericanthe jacfelicis*** (N.Hallé) Robbr.—*Carvalho 4169* (K), Gulf of Guinea Islands (Bioko); DQ180523, —, DQ180592. ***Sericanthe*** sp.—*Sonké 3798* (K, YA), Cameroon; DQ180524, DQ180558, DQ180593. ***Tarenna*** cf.—*Davis 3080* (K), Madagascar; DQ180525, DQ180559, DQ180594. ***Tricalysia anomala*** E.A.Bruce var. ***guineensis*** Robbr.—*Davis 3045* (K), Cameroon; DQ180526, DQ180560, DQ180595. ***Tricalysia cryptocalyx*** Baker—*Davis 2173* (K), Madagascar; DQ153400, DQ153649, DQ153767. ***Tricalysia*** sp. 1—*Mvungi 23* (DSM, K), Tanzania; DQ180527, DQ180561, DQ180596. ***Tricalysia*** sp. 2—*Davis 2325* (K), Madagascar; DQ180528, DQ180562, DQ180597. ***Tricalysia*** sp. 3—*OKTAN 68* (K), Madagascar; DQ180529, DQ180563, DQ180598. ***Xantonnea parvifolia*** (Kuntze) Craib—*Cantaranothai 895* (K), Thailand; DQ180530, DQ180564, DQ180599.

APPENDIX 2. Characters, character states, and explanatory notes on characters used in the cladistic analyses of the morphological data matrix. An asterisk (*) indicates where variation or further variation occurs in other taxa of Ixoroideae s.s. (i.e., sensu Andreasen and Bremer [2000], excluding basal Ixoroideae). See Materials and Methods for data sources.

1. **Habit:** 0 = tree or shrub; 1 = climber (liana, vine); 2 = herb. * *Bertiera* (Bertiereae) is the only member of Ixoroideae s.s. to have herbaceous representatives. Climbing habit is also present in Bertiereae, Cremasporeae, Gardenieae, Pavetteae, and Vanguerieae.
2. **Petioles:** 0 = not articulated; 1 = articulated. * Petioles commonly articulated in Ixoreae and Octotropideae, although this character is not consistent within these tribes and may also occur randomly in Ixoroideae and other Rubiaceae genera.
3. **Bacterial nodules:** 0 = absent; 1 = present. * Bacteria nodules are almost universally present in *Sericanthe* and *Pavetta* (Pavetteae); *Pavetta* was not included in the matrix.
4. **Inflorescence position:** 0 = axillary or axillary and then terminal via continued mersitamtic activity of the inflorescence, paired at nodes; 1 = terminal or terminal-overtopped ("pseudo-axillary"). * Fixed within many tribes: always axillary in Coffeeae, Cremasporeae, Octotropideae, Vanguerieae; terminal in Alberteae (not included in matrix), Bertiereae (three species axillary), Ixoreae, and Pavetteae; variable within Gardenieae. Some of this variation is included within the matrix.
5. **Inflorescence:** 0 = sessile (lacking a peduncle); 1 = pedunculate. * Variable within all tribes, except Coffeeae and Cremasporeae.
6. **Calyculi:** 0 = present; 1 = absent. *Restricted to Coffeeae and *Doricera* (Ixoreae), and one or two species of Asian *Ixora* (Ixoreae).
7. **Flowers:** 0 = monoecious (hermaphrodite); 1 = diocieous (single sexed). * Variable within many tribes (Coffeeae, Gardenieae, Ixoreae, Octotropideae, Vanguerieae).
8. **Flowers (merosity):** 0 = 4-merous (exclusively or predominantly); 1 = 5–6-merous; 2 = 7–9-merous (rarely 12-merous). * Very variable within tribes but fixed, or more or less so, in some genera (e.g., *Diplospora*, *Discospermum*, *Nostolachma, Xantonnea* (Coffeeae), *Ixora* (Ixoreae), which are predominantly or exclusively (respectively) 4-merous).
9. **Secondary (Ixoroid) pollen presentation (SPP):** 0 = absent; 1 = present. * Variable within Coffeeae and Gardenieae and possibly other tribes, but generally predominant in Ixoroideae s.s. Robbrecht and Puff (1986) reported that SPP is present in *Coffea* and *Psilanthus*, but this was contested by Bridson and Verdcourt (1998, 2003), Puff et al. (1996), and Andreasen and Bremer (2000). Robbrecht and Puff (1986) report SPP lacking in *Belonophora*, but this was challenged by Cheek and Dawson (2000).
10. **Calyx lobes:** 0 = absent or free; 1 = imbricate. * Probably restricted to very few genera of Ixoroideae s.s., although no thorough survey has been undertaken. A similar calyx lobe arrangement occurs in some species of *Tricalysia* (for summary, see Bridson and Verdcourt, 1988, 2003), but this was not scored in the morphological matrix (Appendix 3).
11. **Corolla aestivation:** 0 = overlapping (contorted) to left; 1 = overlapping (contorted) to right; 2 = not overlapping (valvate). * Left-contorted corollas are found within nearly all Ixoroideae s.s., apart from Gardenieae, where right-contorted corollas are present in three genera (i.e., *Hyperacanthus*, *Phellocalyx* Bridson, and some species of *Rothmannia* Thunb.) and *Gardeniopsis* Miq. (tribal placement unknown). Vanguerieae have valvate aestivation.
12. **Corolla shape:** 0 = More or less straight, or widening toward apex; 1 = tube bimorphic (circum-constricted: usually above the first half of the tube). * Fixed within most tribes but variable within Gardenieae and perhaps some Vanguerieae.
13. **Corolla (hairs in throat):** 0 = absent; 1 = present. * Variable within all tribes and many genera but always absent in some genera and generic groupings (e.g., *Coffea* and *Psilanthus*). Further study is required.
14. **Anthers:** 0 = emergent; 1 = partially emergent; 2 = included. * Variable within most tribes of Ixoroideae s.s. and some genera (e.g., *Argocoffeopsis*).
15. **Anthers:** 0 = medifixed to submedifixed (at the middle of the anther, or slightly below the midpoint); 1 = basifixed (distinctly fixed at the base); 2 = supramedifixed (fixed well above the midpoint, or near the apex). * Notably fixed within some genera of Coffeeae (*Psilanthus* supramedifixed; *Sericanthe* basifixed), but variable within tribes, and particularly Gardenieae. Medifixed to submedifixed anthers most common state within Ixoroideae s.s. A detailed survey is required.
16. **Anthers:** 0 = simple, lacking thecae; 1 = thecae present. * Within Ixoroideae s.s., this character is only known to occur in *Calycosiphonia* (Coffeeae) and *Dictyandra* Hook. f. (Pavetteae), the latter genus was not included in the morphological matrix.
17. **Gynoecium (number of ovules per locule):** 0 = 1; 1 = 2–10(−15); 2 = 30 or more. * Variable within tribes and genera but few ovules (1–10) and many ovules (> 30) rarely found within genera and closely related genera, except some Gardenieae (Robbrecht and Puff, 1986; Rakotonasolo and Davis, 2006), and Octotropideae (Robbrecht and Puff, 1986; Stone and Davis, 2004).
18. **Gynoecium (placental attachment):** 0 = middle, or very slightly above or slightly below; 1 = apex or near apex (or from apex, downward if more than one ovule: placenta[s] always pendulous); 2 = basal; 3 = along length of placenta or more or less so. * Variable within tribes of Ixoroideae s.s., or fixed (Vanguerieae, Cremasporeae); usually fixed within genera.
19. **Gynoecium (placenta):** 0 = small or inconspicous; 1 = hemi-circular to ± hemi-ellipsoid; 2 = distinctly peltate; 3 = linear; 4 = a soft pulpy matrix. * Variable within Gardenieae, although a pulpy matrix is predominant in this tribe. In Octotropideae the placenta is nearly always linear and biseriate, if there is more than one ovule per locule. Hemi-circular to more or less hemi-ellipsoid placentas predominant in most other Ixoroideae s.s.; peltate placentas are restricted to *Bertiera* (Bertiereae), although study of poorly known genera is required.
20. **Gynoecium (placenta):** 0 = ovules free; 1= ovules semi-embedded; 2 = ovules embedded (within placental matrix). * Variable within tribes of Ixoroideae but usually fixed within genera.
21. **Gynoecium (micopyle orientation):** 0 = downward (radicle inferior); 1 = horizontal; 2 = upward (radicle superior); 3 = variable, in many directions (numerous ovules). * Character states 0, 1 and 2 variable within Coffeeae and Pavetteae, and somewhat

variable within Octotropideae (A. Davis, personal observation); fixed in Cremasporeae (inferior), Ixoreae, Vanguerieae (Appendix 3); usually fixed within genera of Ixoroideae s.s. Taxa with multiple ovules per locule (*Bertiera* (Bertiereae) and most Gardenieae) have variable micropyle orientation.

22. **Style:** 0 = emergent (exserted); 1 = included. * Variable within tribes, although almost always emergent to some degree in Ixoreae, Cremasporeae, Bertiereae, Octotropideae, Pavetteae, and Vanguerieae. Notably variable in Gardenieae and to a lesser extent Coffeeae.
23. **Style:** 0 = distinctly 2-lobed; 1 = lobes adnate for their entire length, or free at apex (2-, 3- or many-lobed). * Fixed within most tribes but variable within Gardenieae and Pavetteae (e.g., see Bridson, 1988) and Ixoreae.
24. **Style:** 0 = simple; 1 = with distinct longitudinal ridges. * Consistent within *Bertiera* (Bertiereae) (Robbrecht et al., 1994), also found in some genera of Octotropideae (Bridson, 1988; A. Davis, personal observation for Madagascan taxa), Gardenieae and Pavetteae (e.g., see Bridson, 1988), but probably absent in all other Ixoroideae s.s. Variation within the outgroup was not recorded in the morphological matrix (Appendix 3).
25. **Style:** 0 = glabrous; 1 = hairy. * Variable within Ixoroideae s.s., present but not universal in Cremasporeae, Gardenieae, Ixoreae, and Pavetteae. Common or perhaps universal in Octotropideae; always glabrous in Coffeeae, and perhaps Alberteae. Possibly universally glabrous in Vanguerieae. Further critical study is required.
26. **Pyrene:** 0 = endocarp weakly developed, thin or soft; 1 = endocarp hard (horny/crustaceous); 2 = endocarp woody. Character state 1 appears to be restricted to *Coffea* and *Psilanthus*; all other genera of Ixoroideae s.s. with endocarp weakly developed, thin or soft, apart from Vanguerieae, which is woody and tough.
27. **Pyrene:** 0 = lacking preformed germination slits; 1 = apical preformed germination slits present. Present in Vanguerieae; absent in all other Ixoroideae s.s. apart from some members of Alberteae.
28. **Seeds: (ventral [adaxial] surface):** 0 = entire or more or less so; 1 = with a hilar groove or shallow excavation; 2 = with a distinct hilar cavity; 3 = with a deep ventral groove (i.e., ''coffee-bean morphology); 4 = with a notch, or distinct lateral groove (or similar) at or above the midpoint. * More or less consistent within tribes, except Coffeeae: *Coffea* and *Psilanthus* have a deep ventral groove, *Tricalysia* has either a shallow hilar groove or shallow excavation or an entire ventral face, and other Coffeeae are entire. Vanguerieae seed morphology is consistent and highly characteristic.
29. **Seed coat:** 0 = consisting of crushed endotestal cells and more or less isolated fibers (sclereids); 1 = consisting of exotesta cells, without fibers (sclereids); 2 = absent. * State 0 apparently restricted to *Coffea* and *Psilanthus*; state 1 found in most Ixoroideae s.s. Within Ixoroideae s.s., state 2 is restricted to *Argocoffeopsis, Calycosiphonia*, *Calycosiphonia* cf., and one species of *Diplospora* (only *D. wrayi*; Ali and Robbrecht, 1991). Further study is required.
30. **Seed coat:** 0 = testa appearing ± smooth at low magnification (× 10); 1 = testa appearing fibrous with an obvious fingerprint-like pattern at low magnification (×10). * State 1 restricted to Octotropideae. Preliminary study indicates that this feature may be universal in Octotropideae after recircumscription of the tribe (A. Davis, personal observation).
31. **Pollen:** 0 = colporate; 1 = porate. * All Ixoroideae s.s. have colporate pollen grains, except Gardenieae (which may be colporate, colpoidorate, or porate). Most Ixoroideae have 3-colporate pollen, but see following notes for *Coffea* and *Psilanthus*.

Notes: Other characters of potential systematic importance were reviewed but omitted from the data matrix for various reasons. The number of pollen apertures (Lobreau-Callen and Leroy, 1980; Chinnappa and Warner, 1981; Stoffelen et al., 1997) may have systematic utility, but this character was not scored in our morphological matrix due to high levels of polymorphism, overlap between *Coffea* and *Psilanthus*, and missing data. The nature of thickening in exotestal walls in Gardenieae and related tribes has been investigated in detail by Robbrecht and Puff (1986). We could not devise a satisfactory method for scoring these characters and the character states; moreover, there seems to be considerable polymorphism within genera, and there is much missing data. Despite these concerns, we believe that further study of thickening in exotestal cell walls is warranted. The presence and type of pollen orbicules may also provide systematic data, because Vinckier et al. (2000) have shown that the same pollen orbicule type (type VI) was found in *Coffea*, *Psilanthus*, and *Bertiera*, but no other member of Ixoroideae. The incompleteness of their data meant that we could not include this character in the morphological matrix. Stipule characters (e.g., distinctly awned vs. slightly awned or entire, and degree of sheathing), may have systematic utility within Coffeeae and Ixorideae, but so far a detailed investigation of stipule morphology has not been undertaken. Colleter type, i.e., standard vs. modified (Robbrecht, 1988b; Ali and Robbrecht, 1991), also requires further sampling and study.

Some characters are relatively well known as having systematic utility in Ixoroideae although they were not scored in our analysis because the restricted sample size means they would be autapomorphic for some terminals. These characters include placentation, which is axile in all members of Ixoroideae apart from the genera of Gardenieae that are parietal (e.g., *Gardenia*), and placentation also may be variable within Gardenieae genera (Rakotonasolo and Davis, 2006). Another example is pollen arrangement in Gardenieae, in which pollen can be in monads, tetrads and polyads. In our analysis tetrads would be autapomorphic for *Gardenia*.

APPENDIX 3. Morphological matrix of 31 selected characters for 46 operational taxonomic units (OTUs) of potential Coffeeae and outgroups.

Taxon	Character states																														
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*	*27*	*28*	*29*	*30*	*31*
Coffea kapakata	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	3	0	0	0
Coffea mangoroensis	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	3	0	0	0
Coffea moratii	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	3	0	0	0
Psilanthus ebracteolatus	0	0	0	0	0	0	0	1	0	0	0	0	0	2	2	0	0	0	0	0	0	1	0	0	0	1	0	3	0	0	0
Psilanthus mannii	0	0	0	0	0	0	0	1	0	0	0	0	0	2	2	0	0	0	0	0	0	1	0	0	0	1	0	3	0	0	0
Psilanthus sapinii	0	0	0	0	0	0	0	1	0	0	0	0	0	2	2	0	0	0	0	0	0	1	0	0	0	1	0	3	0	0	0
Psilanthus semsei	0	0	0	0	0	0	0	1	0	0	0	0	0	2	2	0	0	0	0	0	0	1	0	0	0	1	0	3	0	0	0
Tricalysia cryptocalyx	0	0	0	0	0	0	1	1	?	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	1	1	0	0
Tricalysia sp. 1	0	0	0	0	0	0	0	1	1	0	0	0	1	0	0	0	1	0	1	1	0	0	0	0	0	0	0	1	?	0	0
Tricalysia anomala	0	0	0	0	0	0	0	1	1	0	0	0	1	0	0	0	1	0	1	1	0	0	0	0	0	0	0	1	1	0	0
Tricalysia sp. 2	0	0	0	0	0	0	1	1	?	0	0	0	?	0	0	0	1	0	1	1	0	?	0	0	0	0	0	?	?	0	0
Tricalysia sp. 3	0	0	0	0	0	0	1	1	?	0	0	0	0,1	0	0	0	1	0	1	1	0	0	0	0	0	0	0	1	1	0	0
Belonophora coriacea	0	0	0	0	0	0	0	1	1	1	0	0	0	2	0	0	1	1	1	1	2	1	0	0	0	0	0	0	1	0	0
Belonophora coriacea	0	0	0	0	0	0	0	1	1	1	0	0	0	2	0	0	1	1	1	1	2	1	0	0	0	0	0	0	1	0	0
Belonophora sp.	0	0	0	0	0	0	0	1	1	1	0	0	0	2	0	0	1	1	1	1	2	1	0	0	0	0	0	0	1	0	0
Sericanthe andongensis	0	0	1	0	0	0	0	2	1	0	0	0	0	0	1	0	1	1	1	1	1	0	0	0	0	0	0	0	1	0	0
Sericanthe jacfelicis	0	0	0	0	0	0	0	2	1	0	0	0	0	0	1	0	1	1	1	1	1	0	0	0	0	0	0	0	1	0	0
Sericanthe sp.	0	0	1	0	0	0	0	2	1	0	0	0	0	0	1	0	1	1	1	1	1	0	0	0	0	0	0	0	1	0	0
Diplospora sp.	0	0	0	0	0	0	?	0	?	0	0	0	?	0	0	0	0	0	1	?	0	?	0	0	0	0	0	0	1	0	0
Discospermum abnorme	0	0	0	0	0	0	1	0	?	0	0	0	1	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	1	0	0
Xantonnea parviflora	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0,1	0	0	0	0	0	0	1	0	0
Nostolachma khasiana	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0,1	0	0	0	0	0	0	1	0	0
Nostolachma densiflora	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0,1	0	0	0	0	0	0	1	0	0
Calycosiphonia macrochlamys	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0	0	0	2	0	0
Calycosiphonia macrochlamys	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0	0	0	2	0	0
Calycosiphonia spathicalyx	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	2	0	0
Calycosiphonia spathicalyx	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	2	0	0
Calycosiphonia cf.	0	0	0	0	0	0	0	1	?	0	0	0	0	0	0	0	0	0	?	?	0	0	0	0	0	0	0	0	2	0	0
Argocoffeopsis eketensis	1	0	0	0	0	0	0	2	1	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
Argocoffeopsis rupestris	0	0	0	0	0	0	0	2	1	0	0	0	0	2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	2	0	0
Argocoffeopsis scandens	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
Bertiera bicarpellata	2	0	0	0	0	1	0	1	1	0	0	1	0	2	0	0	2	0	2	0	3	1	1	1	0	0	0	0	1	0	0
Bertiera laxissima	0	0	0	1	1	1	0	1	1	0	0	1	0	2	0	0	2	0	2	0	3	1	1	1	0	0	0	0	1	0	0
Bertiera sp.	0	0	0	1	1	1	0	1	1	0	0	1	0	2	0	0	2	0	2	0	3	1	1	1	0	0	0	0	1	0	0
Hyperacanthus grevei	0	0	0	1	0	1	0	1	1	0	1	0	0	1	0	0	1	3	4	2	3	0	0	1	0	0	0	0	1	0	1
Gardenia thunbergia	0	0	0	1	1	1	0	2	1	0	0	0	0	2	0	0	2	3	4	2	3	0	1	0	0	0	0	0	1	0	1
Petitiocodon parviflora	0	0	0	0	1	1	0	1	1	0	0	0	1	1	0	0	1	2,3	?	2	0	0	1	0	0	0	0	0	1	0	?
Canephora sp.	0	1	0	0	1	1	0	1	1	0	0	0	1	0	0	0	1	3	3	0	2	0	0	0	1	0	0	0	1	1	0
Polysphaeria sp.	0	1	0	0	0	1	0	1	1	0	0	0	1	1	0	0	0	1	0	0	2	0	1	0	1	0	0	0	1	1	0
Xantonneopsis robinsonii	0	0	0	0	1	1	0	1	1	1	0	0	1	1	0	0	1	1	3	0	2	0	1	0	1	0	0	?	?	?	?
Rutidea sp.	1	0	0	1	1	1	0	1	1	0	0	0	1	0	0	0	0	2	0	0	1	0	1	0	0	0	0	2	0	1	0
Tarenna cf.	0	0	0	1	0	1	0	1	1	0	0	0	1	0	0	0	1	0	1	1	?	0	1	0	1	0	0	0	?	0	0
Pyrostria sp. 1	0	0	0	0	0	1	0	1	1	0	2	1	1	1	0	0	0	1	0	0	2	0	1	0	0	2	1	4	?	0	0
Pyrostria sp. 2	0	0	0	0	0	1	0	1	1	0	2	1	1	1	0	0	0	1	0	0	2	0	1	0	0	2	1	4	?	0	0
Doricera trilocularis	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	2	1	0	0
Ixora sp.	0	1	0	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	1	0