



Towards a better understanding of intertribal relationships and stable tribal delimitations within Cinchonoideae s.s. (Rubiaceae)

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ABSTRACT

This study focuses on the subfamily Cinchonoideae s.s. utilizing information from six DNA markers and 206 taxa. The nine tribes (i.e. Cinchoneae, Chiococceae s.l., Guettardeae s.s., Hamelieae, Hillieae, Hymenodictyeae, Isertieae, Naucleaeae s.l., and Rondeletieae s.s.) are resolved in four major lineages, all strongly supported and relationships between them are resolved. The tropical American Cinchoneae and Isertieae constitute the first diverging lineage within the subfamily, followed by the predominantly paleotropical Naucleaeae and Hymenodictyeae. The remaining two lineages primarily include neotropical taxa: Rondeletieae and Guettardeae are sister clades in the first, while the second comprises Chiococceae, Hamelieae, and Hillieae. Additionally, taxonomic placement of several genera, not previously included in molecular analyses, were confirmed: *Acunaeanthus* belongs in Rondeletieae, *Ottoschmidia* in Guettardeae, *Nernstia* in Chiococceae, *Pinarophyllon*, *Plocaniophyllon*, and *Syringantha* in Hamelieae, and *Balmea* in Hillieae. *Colleteria*, of previously unknown taxonomic position, is resolved as sister to *Chione*.

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1. Introduction

Cinchonoideae s.s. (sensu Bremer et al., 1995, 1999) is the smallest of three subfamilies within Rubiaceae, and comprises c. 120 genera. Taxa belonging to this subfamily are primarily distributed in the New World, from North America and the West Indies to Central and South America, but a number of genera have a distribution in (tropical parts of) the Old World. Species within Cinchonoideae are characterized as small trees or shrubs, with imbricate or valvate corolla aestivation and, often, dry, capsular fruits. Many members contain complex indole alkaloids. The most widely known genus is *Cinchona*, whose bark was used to produce “quinine” which was the first effective cure to treat malaria.

Circumscription of Cinchonoideae has varied over time. It was introduced, along with Coffeoidae, by Schumann (1891) as one of

two large groups within Rubiaceae, based on the number of ovules per carpel. Later, Verdcourt (1958) found morphological support to propose three subfamilies: Rubioideae with raphides and albuminous seeds, Guettardoideae without raphides and seeds ± exalbuminous, and Cinchonoideae s.l. without raphides and albuminous seeds. Based on the same characters as Verdcourt, but in combination with aestivation, characteristics of the testa cell walls and secondary pollen presentation, Bremekamp (1966) recognized as many as eight subfamilies, of which one was a more narrowly circumscribed Cinchonoideae. Robbrecht (1988) maintained only three of Bremekamp's subfamilies, namely Rubioideae, Ixorioideae, and Cinchonoideae, and proposed the new subfamily Antirrhoideae. Cinchonoideae represents a well supported clade and is regarded as one of three subfamilies within Rubiaceae (i.e. Rubioideae, Ixorioideae s.l., and Cinchonoideae s.s.), and sister to subfamily Ixorioideae s.l. (Bremer et al., 1995, 1999; Bremer and Thulin, 1998; Andersson and Rova, 1999; Rova et al., 2002; Andersson and Antonelli, 2005; Rydin et al., 2009). However, Cinchonoideae s.s. and Ixorioideae s.l. are

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sometimes considered parts of a wider Cinchonoideae (Robbrecht and Manen, 2006).

Present circumscription of Cinchonoideae, based on molecular data, includes nine tribes: Cinchoneae, Chiococceae s.l., Guettardeae s.l., Hamelieae, Hillieae, Hymenodictyeae, Isertieae, Naucleaeae s.l., and Rondeletieae s.s. However, several tribes of Cinchonoideae have previously been placed in other subfamilies of Rubiaceae (e.g. Verdcourt, 1958; Bremekamp, 1966; Robbrecht, 1998), based on morphological characters. Likewise, tribes previously placed in Cinchonoideae, even in a narrow sense (Robbrecht, 1998), are now placed within other subfamilies, or are placed outside all three subfamilies (e.g. Andersson and Rova, 1999; Rova et al., 2002; Rydin et al., 2009). The different placements indicate that solid relationships even between larger groups within Rubiaceae can be problematic to decide based solely on morphological characters, as they may be highly homoplasious.

Difficulties to assess homology and different views on the importance of certain morphological characters have also led to different delimitations of tribes. Recent analyses of molecular data have, however, further contributed to our present understanding. Analyses of *rbcl* data (Bremer et al., 1995) supported a wider circumscription of Chiococceae (Bremer, 1992), and a few years later, major changes were made in the circumscription of Isertieae (Bremer and Thulin, 1998). Razafimandimbison and Bremer (2001) proposed a wider circumscription of Naucleaeae and described a new tribe, Hymenodictyeae, based on analyses of nrITS, *rbcl*, and morphological data. In analyses of nrITS and chloroplast data (Andersson and Antonelli, 2005), the narrower circumscription of Cinchoneae (Andersson, 1995) was confirmed. Finally, many genera, previously placed in Rondeletieae, have recently shown to be closer related to Guettardeae, or Condamineae (Ixoroideae; Rova et al., 2002, 2009; Delprete and Cortéz-B, 2004), and a new circumscription of Rondeletieae has been proposed (Rova et al., 2009). However, monophyly and delimitation of Hamelieae and Hillieae have not been as thoroughly tested using molecular data, mainly due to limited sampling. Furthermore, a number of genera placed within the other tribes have not yet been included in molecular phylogenetic analyses.

There is some knowledge of intertribal relationships within Cinchonoideae. Cinchoneae is sister to Isertieae (Bremer and Thulin, 1998; Andersson and Rova, 1999; Andersson and Antonelli, 2005; but see Robbrecht and Manen, 2006), Naucleaeae s.l. to Hymenodictyeae (Razafimandimbison and Bremer, 2001; Andersson and Antonelli, 2005; Robbrecht and Manen, 2006; Rydin et al., 2009), Hamelieae to Hillieae (Bremer et al., 1995; Bremer and Thulin, 1998; Rova et al., 2002; Andersson and Antonelli, 2005; but see Robbrecht and Manen, 2006), and Rondeletieae s.s. to Guettardeae s.l. (Bremer and Thulin, 1998; Bremer et al., 1999; Rova et al., 2002, 2009; Robbrecht and Manen, 2006; Rydin et al., 2009). Further deep node relationships within the subfamily are, however, unresolved (Rova et al., 2002; Bremer and Eriksson, 2009), or relationships

are in conflict and have low support (Bremer et al., 1995; Andersson and Antonelli, 2005; Rydin et al., 2009). Previous studies within Cinchonoideae have, however, focused on one or a few tribes, and sampling has primarily been in the tribe(s) of interest.

This study focuses on the entire subfamily Cinchonoideae and aims not only to further resolve intertribal relationships and deep nodes, but also to rigorously assess tribal delimitations. Our intention is to use molecular data from five chloroplast markers and nuclear ribosomal ITS data from as many as possible of genera placed within Cinchonoideae. We also aim to include genera placed within tribes based on morphology (e.g. Robbrecht, 1998, 1993) that have not yet been included in molecular analyses.

2. Material and methods

2.1. Taxon sampling

Our ambition was to include at least one representative, preferably the type species, of all genera placed within Cinchonoideae (sensu Bremer et al., 1995, 1999). Of approx. 120 genera, 107 were sampled, representing all nine tribes. The majority of genera not included are members of Rondeletieae. Several genera were represented by more than one taxon and the complete data set included 200 terminals of the Cinchonoideae. *Calycophyllum candidissimum*, *Coffea arabica*, *Ixora coccinea*, *Sabiceae aspera*, and *Sipanea hispida* of the Ixoroideae, and *Luculia gratissima* of the Luculieae (Rydin et al., 2009) were sampled outside Cinchonoideae. Ixoroideae has been shown as sister clade to Cinchonoideae in previous studies (e.g. Bremer et al., 1995, 1999; Andersson and Rova, 1999; Rova et al., 2002). *Luculia* is placed outside this alliance (Robbrecht and Manen, 2006; Rydin et al., 2009) and therefore *Luculia gratissima* was used as the outgroup in the analyses.

2.2. Laboratory procedures

Leaves dried in silica gel or from herbarium specimens were used to extract total DNA. Extraction, amplification and sequencing of DNA data followed standard procedures previously described (Kårehed and Bremer, 2007). We utilized information from the internal transcribed spacers of the nuclear ribosomal DNA (nrDNA ITS1 and ITS2), including the conservative 5.8 region, and five chloroplast regions (the *atpB-rbcL* spacer, *ndhF*, *rbcl*, the *rps16* intron, and the *trnT-L-F* region). Information of primers used are specified in Table 1. Amplified sequences were proofread and assembled using the Staden package (Staden, 1996).

2.3. Alignments

The obtained sequences were used together with sequences already available at GenBank, and aligned manually using Se-Align

Table 1
List of primers used for sequence amplification.

DNA region	Primer names	Reference/sequence
<i>atpB-rbcL</i> spacer	<i>rbcl</i> 5'R & <i>atpB</i> '5R	Rydin et al. (2009)
<i>atpB-rbcL</i> spacer	oligo 2 <i>atpB</i>	Manen et al. (1994)
<i>ndhF</i>	2F, 1000R, 720F, 1700R & 2280R	Rydin et al. (2009)
<i>rbcl</i>	5'F, 3'R & bs427F	Bremer et al. (2002)
<i>rbcl</i>	z895R	Zurawski, DNAX Research Institute
<i>rps16</i> intron	F & 2R	Oxelman et al. (1997)
<i>trnT-F</i>	aF, bR, cF, dR, eF & fR	Taberlet et al. (1991)
<i>trnT-F</i>	a1F & iR	Bremer et al. (2002)
nrITS	p17 & p26S-82R	Bolmgren and Oxelman in Popp and Oxelman (2001)
nrITS	p25	Oxelman (1996)
nrITS	its2CINr (modified its2; White et al., 1990)	5' GCTRCGTTCTCATCGATGC 3'; this study
nrITS	its3CINr (modified its3b; Baum et al., 1994)	5' GCATCGATGAAGAACYGAGC 3'; this study

Table 2

List of taxa investigated in the study, voucher information, classification, and GenBank accession numbers. CHI = Chiococceae s.l., CIN = Cinchoneae, HAM = Hamelieae, HIL = Hillieae, HYM = Hymenodictyeae, GUE = Guettardeae s.l., ISE = Isertieae, NAU = Naucleaeae s.l., RON = Rondeletieae s.s., IXOR = Ixoroideae, and LUCU = Luculieae.

Taxon	Voucher (of previously unpublished seq.)	Classif.	nrITS	atpB-rbcL	ndhF	rbcL	rps16	trnT-F
<i>Acrosynanthus latifolius</i> Standl.	Rova 2208 (GB)	RON	GQ852100	GQ851966	GQ852160	GQ852301	AF242900 ^s	GQ852457
<i>Acrosynanthus minor</i> Urb.	Rova 2230 (GB)	RON	GQ852101	GQ851967	GQ852161	GQ852302	AF242901 ^s	GQ852458
<i>Acrosynanthus revolutus</i> Urb.	Delprete et al. 8818 (UPS)	RON	AY730288 ^a	GQ851968	GQ852162	GQ852303	GQ852364	GQ852459
<i>Acunaeanthus tinifolius</i> (Griseb.) Borhidi	Stähl, Baró & Oviedo s.n. (S)	RON	GQ852102	GQ851969	GQ852163	–	–	GQ852451
<i>Adina pilulifera</i> (Lam.) Franch. ex Drake	SA GBE 1172 (NY)	NAU	AJ346885 ^b	GQ851971	GQ852165	AJ346964 ^b	GQ852366	AJ414548 ^b
<i>Adina rubella</i> Hance		NAU	AJ346856 ^b	DQ131698 ^l	–	AJ346965 ^b	–	AJ346910 ^b
<i>Adinauclea fagifolia</i> (Teijsm. & Binn. ex Havil.) Ridsdale	Cult. Bogor Bot. Gard., Indonesia. No voucher	NAU	AJ346901 ^b	GQ851970	GQ852164	AJ346966 ^b	GQ852365	AJ346911 ^b
<i>Allenanthus erythrocarpus</i> Standl.	R. Espinosa 520 (GB)	GUE	GQ852103	GQ851972	GQ852166	–	–	GQ852452
<i>Allenanthus hondurensis</i> Standl.		GUE	–	–	–	–	AF242904 ^s	AF152734 ^u
<i>Antirhea borbonica</i> J.F.Gmel.		GUE	DQ063666 ^c	–	–	–	–	–
<i>Antirhea chinensis</i> (Champ. ex Benth.) Benth. & Hook.f. ex F.B.Forbes & Hemsl.		GUE	DQ063702 ^c	–	–	–	–	–
<i>Antirhea madagascariensis</i> Chaw	Kårehed et al. 313b (UPS)	GUE	GQ852104	GQ851973	GQ852167	GQ852304	GQ852367	GQ852460
<i>Antirhea megacarpa</i> Merr. & L.M.Perry	CER 2659 (L)	GUE	GQ852105	GQ851974	GQ852168	–	AM117284 ^t	–
<i>Arachnothryx buddleioides</i> (Benth.) Planch.	Rova & Sundbaum 2411 (S)	GUE	AY730299 ^a	GQ851975	GQ852169	–	AF242960 ^s	GQ852461
<i>Arachnothryx chimboracensis</i> (Standl.) Steyerf.	Delprete & Verduga 6398 (NY)	GUE	AY730292 ^a	GQ851976	GQ852170	GQ852305	GQ852368	GQ852462
<i>Arachnothryx leucophylla</i> (Kunth) Planch.	Rova 2287 (GB)	GUE	AY730296 ^a	GQ851977	GQ852171	GQ852306	AF242910 ^a	GQ852453
<i>Asemnantha pubescens</i> Hook.f.	Gaumer & sons 23487 (UPS)	CHI	GQ852106	GQ851978	GQ852172	–	GQ852369	AF152713 ^u
<i>Badusa corymbifera</i> (G.Forst.) A.Gray	Smith 4510 (1947) (P)	CHI	GQ852107	GQ851979	GQ852173	GQ852307	GQ852370	GQ852463
<i>Balmea stormae</i> Martínez	Vazquez & Phillips 825 (MO)	HIL	GQ852108	GQ851980	–	GQ852308	GQ852371	GQ852464
<i>Bikkia artensis</i> (Montrouz.) Guillaumin	Munzinger et al. 2857 (NOU)	CHI	AY763869 ^d	GQ851981	GQ852174	GQ852309	GQ852372	GQ852465
<i>Bikkia macrophylla</i> (Brongn.) K.Schum. in H.G.A.Engler & K.A.E.Prantl	Barabbé et al. 347 (NOU)	CHI	AY763870 ^d	GQ851982	GQ852175	–	GQ852373	GQ852466
<i>Bikkia tetrandra</i> (L.f.) A.Rich.	Curry 1402 (11/1993) (K)	CHI	AY763874 ^d	GQ851983	–	–	GQ852374	GQ852467
<i>Blepharidium guatemalense</i> Standl.	Gustafsson, Fredriksson & Päll 212 (GB)	RON	AY730287 ^a	–	GQ852176	GQ852310	AF242916 ^a	GQ852468
<i>Bobea gaudichaudii</i> (Cham. & Schldt.) H.St.John & Herbt	Fagerlind 7183 (UPS)	GUE	DQ063668 ^c	GQ851984	–	AM117209	–	–
<i>Breonadia salicina</i> (Vahl) Hepper & J.R.I.Wood	SG 277 (P, MO, TAN)	NAU	AJ346857 ^b	GQ851987	GQ852178	AJ346967 ^b	GQ852377	AJ346912 ^b
<i>Breonia chinensis</i> (Lam.) Capuron, Adansonia	Ratsimbazafy 09 (TAN)	NAU	AJ346858 ^b	GQ851985	GQ852291	AJ346968 ^b	GQ852375	AJ346913 ^b
<i>Breonia decaryana</i> Homolle	Razafimandimbison SG 393 (TAN)	NAU	AJ346859 ^b	GQ851986	GQ852177	AJ346969 ^b	GQ852376	AJ346914 ^b
<i>Burttdavaya nyasica</i> Hoyle	Bremer 3075 (UPS)	NAU	AJ346863 ^b	GQ851988	GQ852179	AJ346973 ^b	GQ852378	AJ346918 ^b
<i>Calycophyllum candidissimum</i> (Vahl) DC.		IXOR	–	DQ131708 ^l	AJ236285 ^q	X83627 ^k	AF004030 ^w	AF152646 ^u
<i>Catesbaea holacantha</i> C.Wright ex Griseb.	Areces 6576 (NY)	CHI	AY763878 ^d	GQ851989	GQ852180	GQ852311	AF242920 ^s	GQ852469
<i>Catesbaea parviflora</i> Sw.	Raven 28283 (NY)	CHI	GQ852109	GQ851990	GQ852181	GQ852312	GQ852379	GQ852470
<i>Catesbaea spinosa</i> L.	Gillis 9569 (FTG)	CHI	AY763880 ^d	GQ851991	–	X83628 ^k	AF004032 ^w	GQ852471
<i>Cephalanthus natalensis</i> Oliv.	Bremer & Bremer 3768 (UPS)	NAU	AJ346906 ^b	GQ851992	GQ852182	Y18711 ^q	GQ852380	AJ414549 ^b
<i>Cephalanthus occidentalis</i> L.		NAU	AJ346883 ^b	DQ131710 ^l	AJ236288 ^q	X83629 ^x	AF004033 ^w	AJ346955 ^b
<i>Cephalanthus salicifolius</i> Humb. & Bonpl.	Brant 3989 (MO)	NAU	AJ346886 ^b	GQ851993	GQ852183	AJ346975 ^b	GQ852381	AJ346920 ^b
<i>Ceratopyxis verbenacea</i> (Griseb.) Hook.f.	Rova 2279 (GB)	CHI	AY763881 ^d	GQ851994	GQ852184	GQ852313	AF242921 ^s	GQ852472
<i>Chiococca alba</i> (L.) Hitchc.	Bremer 2703 (UPS)	CHI	GQ852110	DQ131711 ^l	AJ130835 ^q	L14394 ^y	AF004034 ^w	GQ852473
<i>Chiococca pachyphylla</i> Wernham		CHI	AY763884 ^d	–	–	–	–	AY763815 ^d
<i>Chione venosa</i> (Sw.) Urb.	Faivre s.n. (MO, UPS)	No tribe	AY763887 ^d	GQ851995	GQ852185	AM117215 ^f	AF242965 ^s	GQ852474
<i>Chione venosa</i> var. <i>buxifolia</i> (Dwyer & M.V.Hayden) David W.Taylor,	Taylor & Montenegro 230 (MO)	No tribe	GQ852111	GQ851996	GQ852186	GQ852314	GQ852382	GQ852475
<i>Chione venosa</i> var. <i>cubensis</i> (A.Rich.) David W.Taylor	Taylor et al. 260 (MO)	No tribe	GQ852112	–	GQ852187	–	GQ852383	GQ852476
<i>Chione venosa</i> var. <i>venosa</i> (Sw.) Urb.	Taylor 11652 (MO)	No tribe	GQ852113	GQ851997	GQ852188	–	GQ852384	GQ852477
<i>Chomelia angustifolia</i> Benth.	Jansen-Jacobs et al. 4361 (GB)	GUE	GQ852114	GQ851998	GQ852189	GQ852315	GQ852385	GQ852478
<i>Chomelia spinosa</i> Jacq.	Seigler DS-12793 (MO)	GUE	GQ852115	GQ851999	GQ852190	–	GQ852386	GQ852454
<i>Chomelia tenuiflora</i> Benth.	Andersson et al. 2030 (GB, S)	GUE	GQ852116	GQ852000	GQ852191	GQ852316	–	GQ852479
<i>Ciliosemina pedunculata</i> (H.Karst.) Antonelli	Persson et al. 601 (GB)	CIN	AY538373 ^e	GQ852001	GQ852292	AY538506 ^e	AY538444 ^e	GQ852480
<i>Cinchona calisaya</i> Wedd.	Razafimandimbison et al. 471	CIN	AY538352 ^e	GQ852003	GQ852293	AY538478 ^e	AF242927 ^s	GQ852482
<i>Cinchona officinalis</i> L.	Andersson & Nilsson 2551 (GB)	CIN	GQ852117	GQ852004	–	AY538480 ^e	GQ852387	GQ852483

(continued on next page)

Table 2 (continued)

Taxon	Voucher (of previously unpublished seq.)	Classif.	nrITS	atpB-rbcL	ndhF	rbcL	rps16	trnT-F
<i>Cinchona pubescens</i> Vahl		CIN	AY538356 ^e	AJ233990 ^m	AJ235843 ^z	X83630 ^x	AF004035 ^w	AJ346963 ^b
<i>Cinchonopsis amazonica</i> (Standl.) L.Andersson	Antonelli 244 (GB)	CIN	AY538357 ^e	GQ852002	–	AY538482 ^e	AY538428 ^e	GQ852481
<i>Coffea arabica</i> L.		IXOR	–	X70364 ⁿ	AJ236290 ^d	X83631 ^x	AF004038 ^w	DQ153845 ^f
<i>Colleteria seminervis</i> (Urb. & Ekman) David W. Taylor	Taylor DT-329 (MICH)	No tribe	GQ852118	GQ852005	GQ852192	GQ852317	GQ852388	GQ852484
<i>Colleteria seminervis</i> (Urb. & Ekman) David W. Taylor	Liogier 11377 (P)	No tribe	GQ852119	GQ852006	–	GQ852318	GQ852389	GQ852455
<i>Corynanthe paniculata</i> Welw.		NAU	AJ346887 ^b	–	–	AJ346978 ^b	–	AJ346923 ^b
<i>Cosmibuena grandiflora</i> (Ruiz & Pav.) Rusby	Andersson 2075 (GB)	HIL	GQ852120	GQ852007	GQ852193	AY538483 ^e	AM117295 ^t	GQ852485
<i>Cosmibuena valerii</i> (Standl.) C.M.Taylor	Knudsen & Asmussen 666 (GB)	HIL	GQ852121	X81683 ⁿ	GQ852194	GQ852319	GQ852390	GQ852486
<i>Cosmocalyx spectabilis</i> Standl.		HAM	–	DQ131716 ^l	–	–	–	–
<i>Coutaportia ghiesbreghtiana</i> (Baill.) Urb.		CHI	AY763889 ^d	–	–	–	AF242931 ^s	AF152693 ^u
<i>Coutarea hexandra</i> (Jacq.) K.Schum.	Kirkbridge 3931 (2/1981) (K)	CHI	AY763890 ^d	GQ852008	AM117344 ^t	AM117221 ^t	AF242933 ^s	GQ852487
<i>Cuatrecasiodendron</i> sp.	Rova et al. 2093 (GB)	GUE	AY730297 ^a	GQ852009	GQ852195	GQ852320	AF242934 ^a	GQ852488
<i>Cubanola daphnoides</i> (Graham) Aiello	Rova 2281 (GB)	CHI	GQ852122	GQ852010	–	GQ852321	AF242935 ^s	GQ852489
<i>Cubanola domingensis</i> (Britton) Aiello	Bremer 4500 (S)	CHI	AY763891 ^d	DQ131720 ^l	AM117345 ^t	X83632 ^x	AF004044 ^w	GQ852490
<i>Deppea blumenaviensis</i> (K.Schum.) Lorence	Ryding 2359 (C)	HAM	GQ852123	GQ852011	GQ852196	AJ288622 ^m	AF004046 ^w	GQ852491
<i>Deppea erythrorhiza</i> Schldl. & Cham.	Cedillo et al. 1037 (MO)	HAM	GQ852124	GQ852012	GQ852197	GQ852322	GQ852391	GQ852492
<i>Deppea grandiflora</i> Schldl.	Bremer 2724 (UPS)	HAM	GQ852125	GQ852013	GQ852198	X83633 ^x	AM117299 ^t	GQ852493
<i>Deppea splendens</i> Breedlove & Lorence	Taylor (MO)	HAM	GQ852126	GQ852014	GQ852199	GQ852323	GQ852392	GQ852494
<i>Eosanthus cubensis</i> Urb.		CHI	GQ852127	DQ131729 ^l	–	–	GQ852393	GQ852495
<i>Erithalis fruticosa</i> L.	Meagher 990 (FTG)	CHI	AY763892 ^d	DQ131730 ^l	AJ236295 ^d	X83635 ^x	AF242942 ^s	GQ852496
<i>Erithalis harrisii</i> Urb.	Webster, Ellis, Miller 8471 (S)	CHI	AY763893 ^d	–	GQ852200	GQ852324	GQ852394	GQ852497
<i>Exostema acuminatum</i> Urb.	McDowell 4410 (DUKE)	CHI	AY763896 ^d	GQ852015	GQ852201	AY205359 ^v	GQ852395	GQ852498
<i>Exostema caribaeum</i> (Jacq.) Schult. in J.J.Roemer & J.A.Schultes		CHI	AY763897 ^d	AJ233991 ^m	AJ236296 ^d	X83636 ^x	–	–
<i>Exostema lineatum</i> (Vahl) Schult. in J.J.Roemer & J.A.Schultes	Bremer 3311 (UPS)	CHI	AY763902 ^d	DQ131732 ^l	GQ852202	AY205355 ^v	AF242944 ^s	AJ346924 ^b
<i>Exostema longiflorum</i> (Lamb.) Schult. in J.J.Roemer & J.A.Schultes	McDowell 4991 (DUKE)	CHI	AY763903 ^d	GQ852016	GQ852203	AY205352 ^v	AF242945 ^s	GQ852499
<i>Exostema spinosum</i> (Le Vavass.) Krug & Urb.	McDowell 4987 (DUKE)	CHI	AY763899 ^d	GQ852017	GQ852204	AY205350 ^v	AF242947 ^s	GQ852500
<i>Gonzalagunia affinis</i> Standl. ex Steyerl.		GUE	AY730295 ^a	–	–	Y11848 ^q	AM117310 ^t	AJ847405 ^{cc}
<i>Gonzalagunia cornifolia</i> (Kunth) Standl.	Ståhl 1407 (GB)	GUE	GQ852128	–	GQ852205	–	AF242948 ^s	GQ852501
<i>Gonzalagunia dependens</i> Ruiz & Pav.	Persson et al. 461 (GB)	GUE	GQ852129	GQ852018	GQ852206	GQ852325	GQ852396	GQ852502
<i>Gonzalagunia rosea</i> Standl.	Rova 2414 (GB)	GUE	DQ063671 ^c	GQ852019	GQ852207	GQ852326	AF242950 ^s	GQ852503
<i>Guettarda acreana</i> K.Krause	Andersson et al. 2031 (GB)	GUE	DQ063672 ^c	GQ852020	GQ852208	GQ852327	–	GQ852504
<i>Guettarda boliviana</i> Standl.	Persson & Gustafsson 354 (GB)	GUE	GQ852130	GQ852021	GQ852209	GQ852328	AF242962 ^s	GQ852505
<i>Guettarda crispiflora</i> Vahl	Andersson et al. 2081 (GB)	GUE	DQ063674 ^c	GQ852022	GQ852210	GQ852329	AF004052 ^w	GQ852506
<i>Guettarda ferruginea</i> Griseb.	Rova 2206 (GB)	GUE	GQ852131	GQ852023	GQ852211	GQ852330	GQ852397	GQ852507
<i>Guettarda speciosa</i> L.	Rova 2492 (GB)	GUE	DQ063689 ^c	GQ852025	GQ852213	AY538485 ^e	AF242964 ^s	GQ852509
<i>Guettarda splendens</i> Baill.		GUE	DQ063690 ^c	–	–	–	–	–
<i>Guettarda tournefortiopsis</i> Standl.	Andersson et al. 2113 (GB)	GUE	GQ852133	GQ852026	GQ852214	GQ852332	–	GQ852510
<i>Guettarda uruguayensis</i> Cham. & Schldl.		GUE	AY730294 ^a	DQ131739 ^l	AJ236297 ^d	X83638 ^x	EU145489 ^p	EU145533 ^p
<i>Guettardella inconspicua</i> (Seem.) M.E. Jansen	Tuiwana et al. 2999 (P)	GUE	GQ852132	GQ852024	GQ852212	GQ852331	GQ852398	GQ852508
<i>Gyrostipula comorensis</i> J.-F.Leroy	Randrianarivelo 15 (TAN)	NAU	AJ346866 ^b	GQ852027	GQ852215	AJ346979 ^b	AM117312 ^t	AJ346925 ^b
<i>Gyrostipula foveolata</i> (Capuron) J.-F. Leroy	SG 271 (MO,P,TAN)	NAU	AJ346867 ^b	GQ852028	GQ852216	AJ346980 ^b	GQ852399	AJ346926 ^b
<i>Haldina cordifolia</i> (Roxb.) Ridsdale	X2 286 (FTG)	NAU	AJ346884 ^b	GQ852029	GQ852217	X83639 ^x	GQ852400	AJ346956 ^b
<i>Hamelia cuprea</i> Griseb.		HAM	–	–	–	X83641 ^x	AM117313 ^t	AM117361 ^t
<i>Hamelia papillosa</i> Urb.	In cult. Duke Univ. McDowell 4600	HAM	GQ852134	AJ233992 ^m	GQ852218	AY538487 ^e	AF004053 ^w	GQ852511
<i>Hamelia patens</i> Jacq.	Luke 9036	HAM	GQ852135	GQ852030	GQ852219	GQ852333	al046 x	GQ852507
<i>Hillia illustris</i> (Vell.) K. Schum.	Dusén 15539 (S)	HIL	GQ852136	GQ852031	–	–	GQ852401	GQ852565
<i>Hillia macrophylla</i> Standl.		HIL	–	–	–	–	AF004055 ^w	–
<i>Hillia parasitica</i> Jacq.	Taylor 11686 (MO)	HIL	–	–	GQ852220	AM117233 ^t	–	GQ852512
<i>Hillia triflora</i> (Oerst.) C.M.Taylor	Bremer 3101 (UPS)	HIL	GQ852137	AJ233993 ^m	AJ236298 ^m	X83642 ^x	AM117315 ^t	GQ852513
<i>Hintonia latiflora</i> (Sessé & Moc. ex DC.) Bullock	Kufer 308 (MO)	CHI	GQ852138	GQ852032	GQ852221	GQ852334	GQ852402	GQ852514
<i>Hodgkinsonia ovatiflora</i> F.Muell.	Puttock 86022667 (UNSW)	GUE	AY730293 ^a	GQ852033	GQ852222	AM117234 ^t	–	GQ852515
<i>Hoffmannia ghiesbreghtii</i> (Lem.) Hemsl.	CT 499	HAM	GQ852139	GQ852034	GQ852223	GQ852335	GQ852403	GQ852516
<i>Hoffmannia refulgens</i> (Hook.) Hemsl.		HAM	–	X81684 ⁿ	–	X83644 ^x	–	–

<i>Hymenodictyon floribundum</i> (Hochst. & Steud.) B.L.Rob.	Puff 861109-3/1 (WU)	HYM	AJ346905 ^b	DQ131742 ¹	EU145411 ^P	AJ347015 ^b	AF004058 ^w	GQ852517
<i>Hymenodictyon orixense</i> (Roxb.) Mabb.	Ridsdale IV.E. 107	HYM	–	GQ852035	GQ852224	GQ852336	GQ852404	GQ852518
<i>Isertia coccinea</i> (Aubl.) J.F.Gmel.	Andersson et al. 1912 (GB)	ISE	GQ852140	GQ852036	GQ852225	GQ852337	GQ852405	AF152689 ^u
<i>Isertia laevis</i> (Triana) Boom	Bremer 3364 (UPS)	ISE	AY538359 ^e	GQ852037	GQ852226	Y11852	AM117319 ^t	GQ852519
<i>Isertia parviflora</i> Vahl	Andersson et al. (S)	ISE	GQ852141	GQ852038	GQ852227	GQ852338	GQ852406	GQ852520
<i>Isertia pittieri</i> (Standl.) Standl.	Delprete 6394 (UPS)	ISE	DQ448610 ^o	GQ852039	GQ852228	–	AM117320 ^t	AJ847404 ^{cc}
<i>Isidorea pedicellaris</i> Urb. & Ekman		CHI	AY763908 ^d	–	–	–	AF242968 ^s	AF152703 ^u
<i>Isidorea pungens</i> (Lam.) B.L.Rob.		CHI	AY763910 ^d	–	–	–	–	AF152702 ^u
<i>Ixora coccinea</i> L.		IXOR	–	–	AJ236299 ^q	X83646 ^x	AM117321 ^t	AJ620117 ^{bb}
<i>Janotia macrostipula</i> (Capuron) J.-F. Leroy	Razafimandimbison 336 (TAN, MO, P)	NAU	AJ346869 ^b	–	GQ852229	AJ346982 ^b	AM117322 ^t	AJ346928 ^b
<i>Javorkaea hondurensis</i> (Donn.Sm.) Borhidi & Komlódi		GUE	–	–	–	–	AF243013 ^s	AF152716 ^u
<i>Joosia aequatoria</i> Steyerem.		CIN	AY538360 ^e	–	–	AY538491 ^e	AY538432 ^e	AY538457 ^e
<i>Joosia umbellifera</i> H.Karst.	Rova et al. 2395 (GB)	CIN	AY538361 ^e	GQ852040	GQ852294	AY538492 ^e	AY538433 ^e	GQ852521
<i>Kerianthera preclara</i> J.H.Kirkbr.		ISE	AY538362 ^e	–	–	AY538493 ^e	AF242970 ^s	AY538459 ^e
<i>Ladenbergia amazonensis</i> Ducke		CIN	AY538363 ^e	–	–	AY538494 ^e	AY538434 ^e	AY538460 ^e
<i>Ladenbergia oblongifolia</i> (Humb. ex Mutis) L.Andersson	Persson & Gustafsson 245 (GB)	CIN	AY538462 ^e	GQ852041	GQ852295	AY538497 ^e	AY538436 ^e	AY538462 ^e
<i>Ladenbergia pavonii</i> (Lamb.) Standl.	Delprete 6404 (UPS)	CIN	AY538367 ^e	GQ852042	–	Z68801 ^{dd}	AY538437 ^e	GQ852522
<i>Lorencea guatemalensis</i> (Standl.) Borhidi		–	–	–	–	–	AF242930 ^s	AF152694 ^u
<i>Luculia gratissima</i> (Wall.) Sweet		LUCU	EU145344 ^P	EU145308 ^P	AJ011987 ^{aa}	AM117243 ^t	AJ431036 ^{ee}	AJ430911 ^{ee}
<i>Ludekia borneensis</i> Ridsdale	Cult. Bogor Bot. Gard., Indonesia. No voucher	NAU	AJ346870 ^b	GQ852043	GQ852230	AJ346983 ^b	GQ852407	AJ346962 ^b
<i>Machaonia acuminata</i> Humb. & Bonpl.	Bullock 1432 (MO)	GUE	GQ852142	GQ852044	GQ852231	GQ852339	GQ852408	GQ852523
<i>Machaonia portoricensis</i> Baill.		GUE	–	–	–	–	AF242976 ^s	AF152733 ^u
<i>Machaonia williamsii</i> Standl.	Persson & Grández 700 (GB)	GUE	GQ852143	GQ852045	GQ852232	–	–	GQ852524
<i>Malanea forsteronioides</i> Müll.Arg.	Pirani & Bremer 4910 (SPF)	GUE	GQ852144	–	GQ852233	AM117245 ^t	GQ852409	–
<i>Malanea</i> sp.	Persson 554 (GB)	GUE	–	GQ852046	GQ852234	–	GQ852410	–
<i>Mazaea phialanthoides</i> (Griseb.) Krug & Urb.	Rova et al. 2264 (GB)	RON	AY730302 ^a	GQ852047	GQ852235	GQ852340	AF242980 ^a	GQ852525
<i>Mazaea shaferi</i> (Standl.) Delprete	McDowell 4826 (DUKE)	RON	AY730304 ^a	GQ852048	GQ852236	AM117205 ^t	AF242911 ^a	GQ852526
<i>Metadina trichotoma</i> (Zoll. & Moritz) Bakhf.	Cult. Bogor Bot. Gard., Indonesia. No voucher	NAU	AJ346871 ^b	GQ852049	–	AJ346984 ^b	GQ852411	AJ346930 ^b
<i>Mitragyna inermis</i> (Willd.) Kuntze	Lorence 8376, Cult. Nat. Trop. Bot. Gard. Hawaii (PTBG)	NAU	AJ346873 ^b	DQ131751 ¹	GQ852237	AJ346986 ^b	GQ852412	AJ346932 ^b
<i>Mitragyna rotundifolia</i> (Roxb.) Kuntze	Puff 990811-2/1 (WU)	NAU	AJ346874 ^b	GQ852050	GQ852238	AJ346987 ^b	GQ852413	AJ346933 ^b
<i>Mitragyna rubrostipulata</i> (K.Schum.) Havil.	Robbrecht s.n. (BR, UPS)	NAU	AJ346895 ^b	–	GQ852239	X83640 ^x	AY538429 ^e	AJ346957 ^b
<i>Mitragyna stipulosa</i> (DC.) Kuntze	Razafimandimbison 295 (LBR, MO, P, TAN)	NAU	AJ346868 ^b	–	GQ852240	AJ346981 ^b	–	AJ346927 ^b
<i>Morierina montana</i> Vieill.	Barabbé et al. 321 (NOU)	CHI	AY763912 ^d	GQ852051	GQ852241	GQ852341	GQ852414	GQ852527
<i>Myrmeconuclea strigosa</i> (Korth.) Merr.	Moog AMO- 018 (L)	NAU	AJ821881 ^g	GQ852052	GQ852242	AJ346989 ^b	GQ852415	AJ346934 ^b
<i>Nauclea diderrichii</i> (De Wild.) Merr.	Razafimandimbison 297 (MO, P, TAN)	NAU	AJ346855 ^b	–	GQ852243	AJ346994 ^b	–	AJ346935 ^b
<i>Nauclea orientalis</i> (L.) L.		NAU	AJ346897 ^b	EU145320 ^P	EU145410 ^P	X83653 ^x	AJ320080 ^{ff}	AJ346958 ^b
<i>Neoblakea venezuelensis</i> Standl.		GUE	–	–	–	–	–	AF152732 ^u
<i>Neolamarckia cadamba</i> (Roxb.) Bosser,	Lorence 7608 (NTBG)	NAU	AJ346878 ^b	GQ852054	GQ852245	AJ346990 ^b	AF242985 ^s	AJ346938 ^b
<i>Neolaugeria densiflora</i> (C.Wright ex Griseb.) Nicolson		GUE	AF323057 ^r	–	–	–	–	–
<i>Neolaugeria resinosa</i> (Vahl) Nicolson	Axelrod, Vélez, Axelrod 2723 (NY)	GUE	GQ852145	GQ852056	GQ852247	–	GQ852417	GQ852528
<i>Neonauclea brassii</i> S.Moore	Vogel 2212 (UPS)	NAU	AJ346879 ^b	GQ852053	GQ852244	AJ346991 ^b	GQ852416	AJ346939 ^b
<i>Neonauclea clemensiae</i> Merr. & L.M.Perry	Drozdz & Molem s.n. (L)	NAU	AJ346898 ^b	GQ852055	GQ852246	AJ318450 ^{ff}	AJ320081 ^{ff}	AJ346940 ^b
<i>Nernstia mexicana</i> (Zucc. & Mart. ex DC.) Urb.		CHI	–	GQ852057	GQ852248	GQ852342	GQ852418	GQ852529
<i>Ochreinauclea maingayi</i> (Hook.f.) Ridsdale	Puff 960424-1/2 (WU)	NAU	AJ346882 ^b	GQ852058	GQ852249	AJ346997 ^b	GQ852419	AJ346943 ^b
<i>Omitelia filisepala</i> (Standl.) J.K.Morton	Mexico, Keller 2051 (CAS)	HAM	GQ852146	GQ852059	GQ852250	AM117251 ^t	GQ852420	GQ852530
<i>Osa pulchra</i> (D.R.Simpson) Aiello	Santamaría S-950 (GB)	CHI	AY763913 ^d	GQ852060	GQ852251	GQ852343	GQ852421	GQ852531
<i>Ottoschmidia microphylla</i> (Griseb.) Urb.	Ekman H9433 (S)	GUE	GQ852147	GQ852061	GQ852252	GQ852344	GQ852422	GQ852532
<i>Paracorynanthe antankarana</i> Capuron ex J.-F.Leroy	Gautier 4483 (G, TEF)	HYM	GQ852148	GQ852062	GQ852253	AJ347017 ^b	GQ852423	GQ852533
<i>Pausinystalia johimbe</i> (K.Schum.) Pierre ex Beille	Cult. Bogor Bot. Gard., Indonesia. No voucher	NAU	AJ346888 ^b	DQ131760 ¹	GQ852254	AJ346998 ^b	GQ852424	AJ346945 ^b
<i>Pausinystalia lane-pooei subsp. ituriense</i> (De Wild.) Stoff. & Robbr.		NAU	AJ346889 ^b	–	–	AJ346999 ^b	–	AJ346946 ^b
<i>Pausinystalia macroceras</i> (K.Schum.) Pierre ex Beille	Hart 573 (BR)	NAU	AJ346890 ^b	GQ852063	GQ852255	AJ347000 ^b	GQ852425	AJ346944 ^b
<i>Pertusadina eurhyncha</i> (Miq.) Ridsdale	Cult. Bogor Bot. Gard., Indonesia. No voucher	NAU	AJ346891 ^b	–	GQ852256	AJ347001 ^b	GQ852426	AJ346947 ^b
<i>Pertusadina malaccensis</i> Ridsdale	Uechirachan & Sirirugosa 41058 (A)	NAU	AJ346893 ^b	GQ852064	GQ852257	AJ347003 ^b	GQ852427	AJ346949 ^b

(continued on next page)

Table 2 (continued)

Taxon	Voucher (of previously unpublished seq.)	Classif.	nrITS	atpB-rbcL	ndhF	rbcL	rps16	trnT-F
<i>Phialanthus ellipticus</i> Urb.	Ekman 15180 (UPS)	CHI	GQ852149	GQ852065	GQ852258	AM117257 ^l	GQ852428	GQ852534
<i>Phyllacanthus grisebachianus</i> Hook.f.	Ekman 17432 (S)	CHI	AY763916 ^d	GQ852067	GQ852260	GQ852345	GQ852430	GQ852536
<i>Phyllomelia coronata</i> Griseb.	Delprete et al. 8913 (UPS)	RON	AY730303 ^a	GQ852066	GQ852259	AM117258 ^l	GQ852429	GQ852535
<i>Pinarophyllon bullatum</i> Standl.	Stevens et al. 25560 (GB)	HAM	–	GQ852068	GQ852261	GQ852346	GQ852431	GQ852456
<i>Pittoniotis trichantha</i> Griseb.		GUE	DQ063695 ^c	–	–	–	–	–
<i>Plocaniophyllon flavum</i> Brandegee	Dwyer 14451 (MO)	HAM	GQ852150	GQ852069	GQ852262	GQ852347	GQ852432	GQ852537
<i>Plocaniophyllon flavum</i> Brandegee	Croat 40853 (MO)	HAM	GQ852151	GQ852070	GQ852263	GQ852348	GQ852433	GQ852538
<i>Portlandia grandiflora</i> L.		CHI	AY763919 ^d	DQ131768 ^l	–	–	–	AY763850 ^d
<i>Portlandia platantha</i> Hook.f.		CHI	AY763922 ^d	–	–	–	AF242997 ^s	AF102469 ^{ss}
<i>Pseudocinchona mayumbensis</i> (R.D.Good) Raym.-Hamet	Razafimandimbison 313 (LBR, MO, P, TAN)	NAU	AJ346864 ^b	DQ131715 ^l	GQ852296	AJ346976 ^b	GQ852434	AJ346921 ^b
<i>Pseudocinchona pachyceras</i> (K.Schum.) A.Chev.		NAU	AJ346865 ^b	–	–	AJ346977 ^b	–	AJ346922 ^b
<i>Remijia chelomaphylla</i> G.A.Sullivan	Persson 517 (GB)	CIN	AY538369 ^e	GQ852071	GQ852297	AY538503 ^e	GQ852435	GQ852539
<i>Remijia macrocnemia</i> (Mart.) Wedd.	Persson & Grández 616 (GB)	CIN	GQ852152	DQ131775 ^l	GQ852298	AY538504 ^e	GQ852436	GQ852540
<i>Remijia ulei</i> K.Krause		CIN	AY538374 ^e	–	–	AY538507 ^e	AY538445 ^e	AY538474 ^e
<i>Rhachicallis americana</i> (Jacq.) Hitchc.	Rova 2259a (GB)	RON	AY730301 ^a	GQ852072	GQ852264	X83664 ^x	AF004073 ^w	GQ852541
<i>Rogiera amoena</i> Planch.	Rova 2409 (1997) (GB)	GUE	AM182205 ^h	GQ852073	GQ852265	GQ852349	AF243000 ^z	GQ852542
<i>Rogiera cordata</i> (Benth.) Planch.	Gustafsson & Fredriksson 126 (GB)	GUE	AY730285 ^a	GQ852074	GQ852266	–	AF242999 ^a	GQ852543
<i>Roigella correifolia</i> (Griseb.) Borhidi & M.Fernández Zeq.	Rova et al. 2262 (GB)	RON	GQ852153	GQ852075	GQ852267	GQ852350	GQ852437	GQ852544
<i>Rondeletia deamii</i> (Donn.Sm.) Standl.	Castillo 1560 (NY)	RON	AM182203 ^h	GQ852076	GQ852268	GQ852351	AJ786765 ^h	GQ852545
<i>Rondeletia hameliifolia</i> Dwyer & M.V.Hayden	Kirkbride & Hayden 164 (NY)	RON	AY730326 ^a	GQ852077	GQ852269	–	GQ852438	GQ852546
<i>Rondeletia nipensis</i> Urb.	Delprete et al. 8651 (UPS)	RON	AY730330 ^a	GQ852078	GQ852270	GQ852352	GQ852439	GQ852547
<i>Rondeletia odorata</i> Jacq.		RON	AY730307 ^a	EU145321 ^p	AJ235845 ^z	Y11857 ^{hh}	EU145490 ^p	AF152741 ⁱⁱ
<i>Rondeletia pitreana</i> Urb. & Ekman	Liogier 13966 (NY)	RON	AY730289 ^a	GQ852079	GQ852299	GQ852353	GQ852440	GQ852548
<i>Rondeletia portoricensis</i> Krug & Urb.	Taylor 11687 (MO)	RON	AY730333 ^a	GQ852080	GQ852271	AM117265 ^l	AF243015 ^a	GQ852549
<i>Rovaeanthus strigosa</i> (Benth.) Borhidi	Lorence 8920 (PTBG)	RON	AY730291 ^a	GQ852081	GQ852272	GQ852354	GQ852441	GQ852550
<i>Rovaeanthus suffrutescens</i> (Brandegee) Borhidi	Bremer 2712 (S)	RON	AY730290 ^a	GQ852082	GQ852273	GQ852355	GQ852442	GQ852551
<i>Sabiceae aspera</i> Aubl.		IXOR	–	–	EU145416 ^p	AY538508 ^e	AF004079 ^w	AY538475 ^e
<i>Salzmannia nitida</i> DC.		CHI	AY763924 ^d	DQ131784 ^l	–	–	–	AY763854 ^d
<i>Sarcocephalus latifolius</i> (Sm.) E.A.Bruce	Bremer 2726 (UPS)	NAU	AJ346899 ^b	DQ131785 ^l	GQ852274	X83667 ^x	AF004080 ^w	AJ346960 ^b
<i>Schmidtottia sessilifolia</i> (Britton) Urb.	Rova 2203 (GB)	CHI	AY763925 ^d	GQ852083	GQ852275	GQ852356	AF243018 ^s	GQ852552
<i>Scolosanthus lucidus</i> Britton	Rova et al. 2209 (GB)	CHI	AY763928 ^d	GQ852084	GQ852276	AM117276 ^l	AF243020 ^s	AF152712 ⁱⁱ
<i>Siemensia pendula</i> (C.Wright ex Griseb.) Urb.	Rova 2278 (1995) (GB)	CHI	AY763930 ^d	GQ852085	GQ852277	GQ852357	AF004083 ^w	GQ852553
<i>Sinoadina racemosa</i> (Siebold & Zucc.) Ridsdale	1350 no collector (MO)	NAU	–	–	GQ852278	AJ347004 ^b	GQ852443	AJ346961 ^b
<i>Sipanea hispida</i> Benth. ex Wernham		IXOR	–	–	EU145322 ^p	EU145458 ^p	EU145492 ^p	AY555107 ^l
<i>Solenandra ixoroides</i> Hook.f.		CHI	Z95530 ^{k+} Z95511 ^k	–	–	AY205355 ^v	AF242943 ^s	AY763862 ^d
<i>Solenandra mexicana</i> (A.Gray) Borhidi	Stone & Stone 4019 (DUKE)	CHI	z95512 ^{k+} z95531 ^k	GQ852086	GQ852279	AY205357 ^v	GQ852444	GQ852554
<i>Solenandra parviflora</i> (A.Rich. ex Humb. & Bonpl.) Borhidi	McDowell 4984 (DUKE)	CHI	Z95529 ^{k+} Z95510 ^k	GQ852087	GQ852280	AY205354 ^v	GQ852445	AY763864 ^d
<i>Stenostomum acutatum</i> DC.		GUE	DQ063696 ^c	–	–	–	AF242907 ^s	AF102378 ^{ss}
<i>Stenostomum lucidum</i> (Sw.) C.F.Gaertn.	Sanders 1801 (FTG)	GUE	DQ063697 ^c	GQ852089	GQ852282	X83624 ^x	GQ852447	GQ852556
<i>Stevensia buxifolia</i> Poit.	Veloz et al. 1868 (MO)	RON	GQ852154	GQ852088	GQ852281	GQ852358	GQ852446	GQ852555
<i>Stevensia minutifolia</i> Alain		RON	AY730309 ^a	–	–	–	–	–
<i>Stilpnophyllum grandifolium</i> L.Andersson	Persson 518 Ecuador (2000) (S)	CIN	AY538375 ^e	GQ852090	GQ852300	AY538510 ^e	AY538446 ^e	GQ852557
<i>Strumpfia maritima</i> Jacq.	Rova 2239 Cuba (1995) (GB)	CHI	AY763935 ^d	GQ852091	AJ236313 ^q	Y18719	AF243027 ^s	GQ852558
<i>Suberanthus brachycarpus</i> (Griseb.) Borhidi & M.Fernández Zeq.	McDowell 4824 (DUKE)	RON	AY730300 ^a	GQ852092	GQ852283	AM117280 ^f	GQ852448	HM045004
<i>Suberanthus nerifolius</i> (A.Rich.) Borhidi & M.Fernández Zeq.	Rova 2273 Cuba (1995) (GB)	RON	GQ852155	GQ852093	GQ852284	GQ852359	AF243030 ^s	GQ852559
<i>Syringantha coulteri</i> (Hook.f.) T.McDowell	Johnston et al. 11173 (MO)	HAM	GQ852156	GQ852094	GQ852285	GQ852360	GQ852449	GQ852560
<i>Timonius celebicus</i> Koord.	Ridsdale V.D.23	GUE	GQ852157	GQ852095	GQ852286	GQ852361	–	GQ852561
<i>Timonius timon</i> (Spreng.) Merr.	Drozd & Molem 1998-11-13	GUE	DQ063701 ^c	GQ852096	GQ852287	AJ318458 ^{ff}	AJ320089 ^{ff}	GQ852562
<i>Timadendron noumeanum</i> (Baill.) Achille	Mouly 859 (P)	GUE	GQ852158	GQ852097	GQ852288	GQ852362	–	GQ852563
<i>Uncaria guianensis</i> (Aubl.) J.F.Gmel.		NAU	AJ414546 ^b	–	–	AJ347007 ^b	–	AJ346952 ^b

<i>Uncaria rhynchophylla</i> (Miq.) Miq. ex Havil.	Bremer 3305 (UPS)	NAU	AJ346900 ^b	GQ852098	GQ852289	X83669 ^x	AB178637 ⁱⁱ	AJ346959 ^b
<i>Uncaria tomentosa</i> (Willd. ex Schult.) DC.	Andersson et al. 2038 (GB)	NAU	GQ852159	GQ852099	GQ852290	GQ852363	GQ852450	GQ852564

- ^a Rova et al. (2009).
^b Razafimandimbison and Bremer (2002).
^c Achille et al. (2006).
^d Motley et al. (2005).
^e Andersson and Antonelli (2005).
^f Maurin et al. (2007).
^g Razafimandimbison et al. (2005).
^h Stranczinger et al. (Genbank unpubl.).
^j Delprete and Cortéz-B (2004).
^k McDowell and Bremer (1998).
^l Robbrecht and Manen (2006).
^m Bremer and Manen (2000).
ⁿ Natali et al. (1995).
^o Antonelli et al. (2009).
^p Rydin et al. (2009).
^q Bremer et al. (1999).
^r Moynihan and Watson (2001).
^s Rova [Genbank unpubl.].
^t Bremer and Eriksson (2009).
^u Rova et al. (2002).
^v McDowell et al. (2003).
^w Andersson and Rova (1999).
^x Bremer et al. (1995).
^y Olmstead et al. (1993).
^z Backlund et al. (2000).
^{aa} Oxelman et al. (1999).
^{bb} Lantz and Bremer (2004).
^{cc} Alejandro et al. (2005).
^{dd} Bremer (1996).
^{ee} Bremer et al. (2002).
^{ff} Novotny et al. (2002).
^{gg} Struwe et al. (1998).
^{hh} Bremer and Thulin (1998).
ⁱⁱ Aoki et al. (2004).

Table 3

Number of included taxa and length of aligned matrices, with information on variable and parsimony informative characters, for the different data sets.

	<i>atpB-rbcL</i>	<i>ndhF</i>	<i>rbcL</i>	<i>rps16</i>	<i>trnT-F</i>	nrITS	Reduced	cpDNA	Complete
Number of taxa	163	159	166	174	191	186	121	121	206
Length of aligned matrices (bp)	1234	2138	1368	999	2472	803	8731	7951	9014
Excluded characters	146	–	–	8	–	–/85	135/218 ^a	135	239 ^a
Variable characters	308	788	377	350	869	479/419	2786/2729	2355	3103
Parsimony informative characters	168	512	224	220	480	372/323	1686/1640	1352	1925
% informative characters of total	15.4	23.9	16.4	22.2	19.4	46.3/45.0	19.6/19.3	17.3	21.9

^a Also excluding problematic ITS1 region.

v.2.0a11 (Rambaut, 1996). GenBank accession numbers for all sequences used are supplied in Table 2.

2.4. Phylogenetic analyses

The six aligned data sets were analyzed with Bayesian inference, separately and in three combined data sets. The combined data sets included: (1) taxa with a representation of all six DNA markers (with the exception of *Rogiera amoena*, deficient of *rbcL* data; hereafter referred to as “the reduced data set”); (2) taxa with a representation of all five chloroplast markers (hereafter referred to as “the cpDNA data set”); and (3) all taxa (i.e. including taxa for which information was missing for some marker(s); hereafter referred to as “the complete data set”). The reduced numbers of taxa in the first two combined data sets aimed to decrease the proportion of missing data as a large proportion of missing data have been shown to, under certain circumstances, decrease resolution and support values (e.g. Wiens and Reeder, 1995; Wiens, 1998; Bremer et al., 1999). Gaps were treated as missing data.

Bayesian analyses of separate as well as combined data sets were performed in MrBayes 3.1 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). The best evolutionary model for each partitioning marker was identified using MrAIC ver 1.4.3 (Nylander, 2004). In the combined analyses, all markers were treated as separate partitions. For each matrix, two parallel analyses were performed starting from random trees. All analyses used four independent MCMC chains for 10–15 millions generations and the temperature set to 0.15. Tree sampling was made every 1000 generation.

The first 1500–2000 trees were excluded (burnin) after which majority rule consensus trees were obtained for the remaining trees sampled. For the complete data set the first 8000 trees (out of 15000) from each analysis were excluded before the majority rule consensus tree was obtained, due to a higher number of generations needed before standard deviation of split frequencies reached an acceptable level.

For the reduced data set, Bootstrap support values were obtained by using Paup* version 4.0b10 for Unix (Swofford, 1998). The search performed 1000 bootstrap replicates, each with 10 random sequence additions. Sampled trees were used to construct a majority rule consensus tree, including nodes with a bootstrap support value above 50% only.

3. Results

3.1. Sequences

A total number of 595 new sequences were obtained from six different DNA regions: five chloroplast markers (*atpB-rbcL* spacer, *ndhF*, *rbcL*, the *rps16* intron, and the *trnT-F* region), and the 5.8S and internal transcribed spacers ITS1 and ITS2 of the nuclear ribosomal DNA region (nrITS). The complete, aligned data set included 9014 characters (*atpB-rbcL* spacer: 1234, *ndhF*: 2138, *rbcL*: 1368, *rps16* intron: 999, *trnT-F* region: 2472, and nrITS: 803 characters).

Information on number of taxa included in all single and combined data sets, as well as variable and informative characters are supplied in Table 3.

3.2. Alignment

Alignment of all chloroplast markers was, generally, unproblematic. However, a TATA/TTTT region of the *atpB-rbcL* spacer was not possible to align unambiguously and, therefore, excluded in all analyses. Furthermore, two inversions, one in the *atpB-rbcL* spacer and one in the *rps16* intron, were identified and excluded from the analyses since they appeared highly homoplasious when matched against supported clades in previous studies (e.g. Rova et al., 2002; Andersson and Antonelli, 2005; Bremer and Eriksson, 2009).

Alignment of nrITS was more time-consuming. Within Cinchonoideae alignment was rather straightforward in all parts except for one short region located in the ITS1 and analyses were conducted both including and excluding this region for the reduced data set. Alignment of Cinchonoideae with outgroup taxa proved to be more problematic, also in the ITS2 region, and, therefore, nrITS data from any outgroup taxa was not included in the combined analyses.

3.3. Model selection

All model selection criteria (AIC, AICc, and BIC) in MrAIC proposed the same model, GTR+ Γ (Yang, 1993), for all five chloroplast markers, while GTR+I+ Γ (Yang, 1993) was proposed for the nrITS region.

3.4. Analyses of the combined data sets

The majority rule consensus trees generated in the Bayesian analyses of the different combined data sets have congruent, or unresolved and thus not contradicting overall topologies. This is also true for the majority rule consensus tree obtained from the Bootstrap search. Support values for some nodes do, however, vary between the different analyses. In the first part, our results will be presented based on the analyses of all combined data sets (Fig. 1). However, phylogenies within the different tribes are described based on the analyses of complete data set (Figs. 2–5), since the reduced data set did not include all genera. Posterior probability values above 0.90 are considered supported and values are supplied within parentheses, if not printed in the text.

3.5. Major groupings (Fig. 1)

Four major lineages (A–D) are resolved within Cinchonoideae. These four lineages, as all tribes except Hamelieae, have maximum bayesian posterior probability values in all analyses of all combined data sets. In the first diverging lineage (Lineage A), Cinchoneae and Isertieae are sisters, and together they form a sister group to all other lineages (0.96/0.96/1.0/0.96). On the next level,

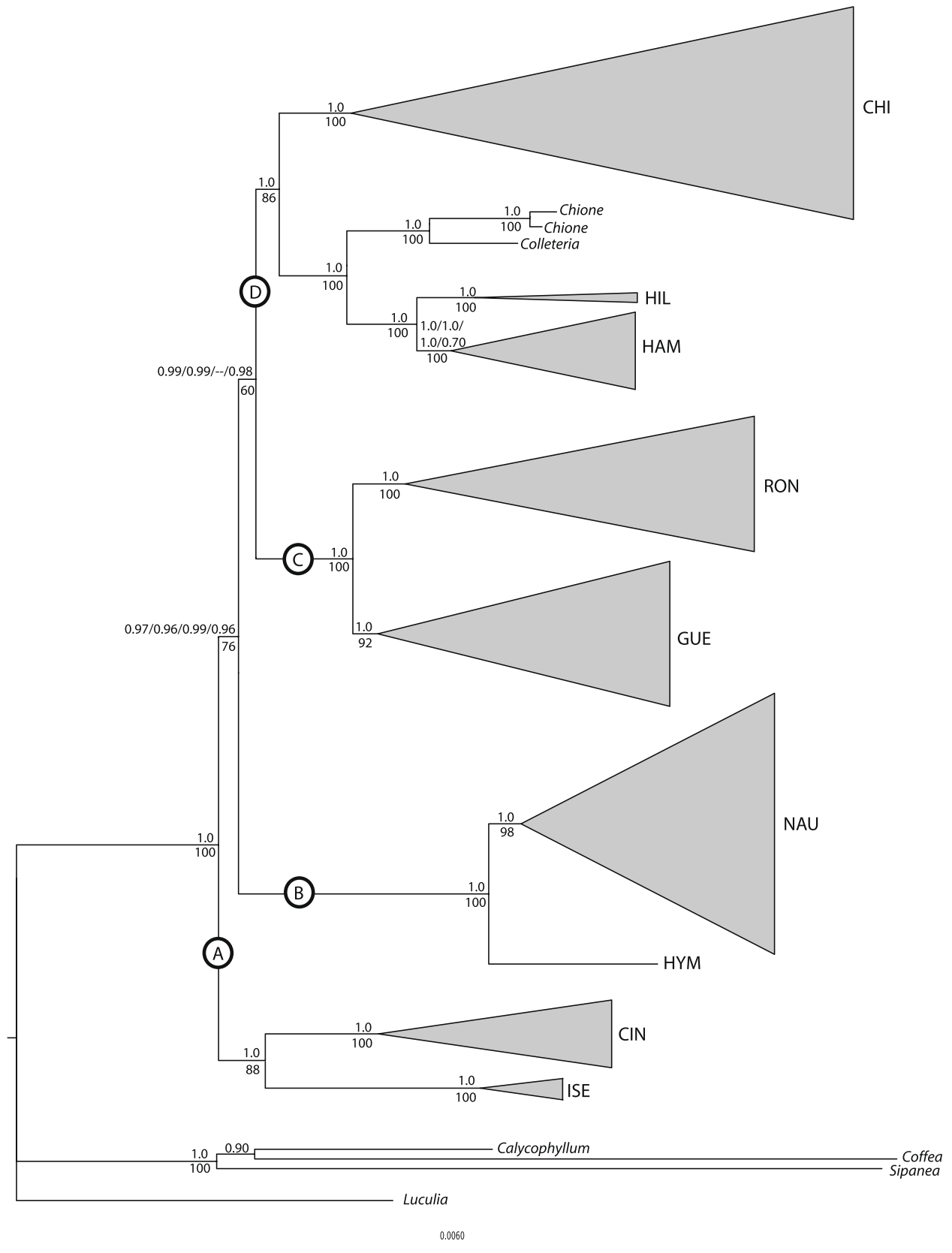


Fig. 1. Simplified majority rule consensus tree, based on the majority rule consensus tree retrieved from the Bayesian analysis of the reduced data set. The tree also shows posterior probability values retrieved in the analyses of the other combined data sets. Numbers given above branches represent posterior probability [reduced data set/reduced data set excluding problematic ITS1 region/cpDNA data set/complete data set], and numbers below branches Parsimony bootstrap value. If only one posterior probability is given, all four Bayesian analyses gave identical posterior probabilities. Circles with A–D indicate Lineages A–D. CHI = Chiococceae, CIN = Cinchoneae, GUE = Guettardeae, HAM = Hamelieae, HIL = Hillieae, HYM = Hymenodictyeae, ISE = Isertieae, NAU = Naucleae, and RON = Rondeletieae. Scale bar = 0.0060 substitutions per site.

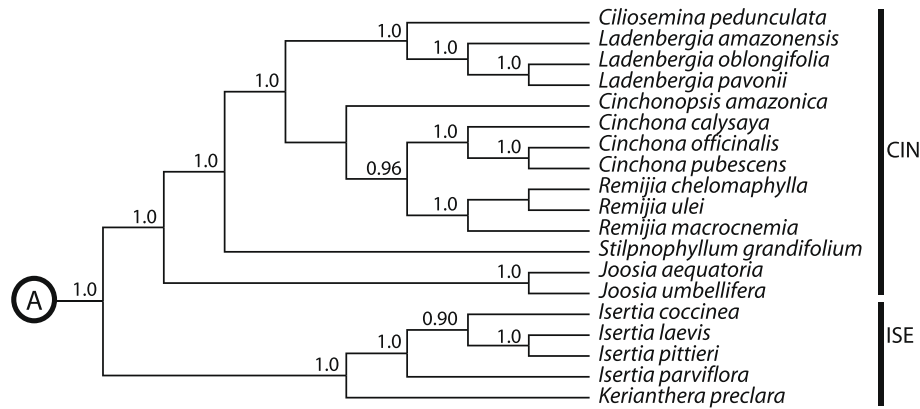


Fig. 2. Detailed phylogeny of Cinchoneae and Isertieae (Lineage A) representing part of the majority rule tree retrieved from the analyses of the complete data set. Numbers given above branches represent posterior probability. CIN = Cinchoneae and ISE = Isertieae.

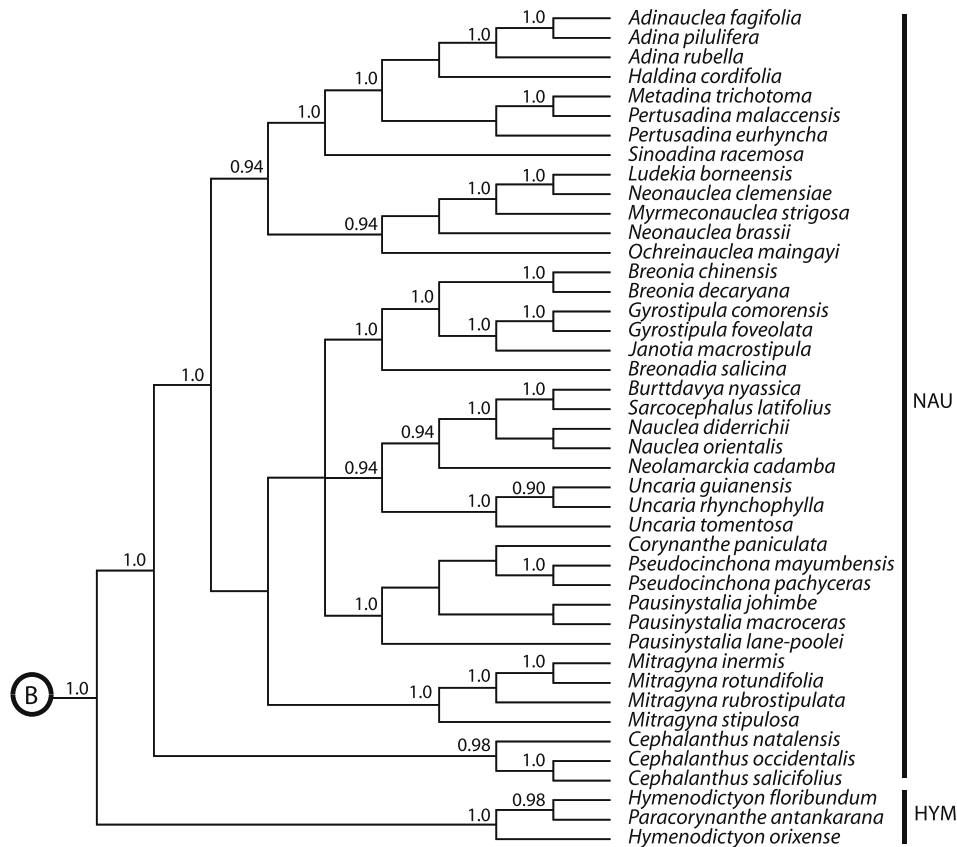


Fig. 3. Detailed phylogeny of Naucleaeae and Hymenodictyeae (Lineage B) representing part of the majority rule tree retrieved from the analyses of the complete data set. Numbers given above branches represent posterior probability. HYM = Hymenodictyeae and NAU = Naucleaeae.

Naucleaeae s.l. and Hymenodictyeae (Lineage B) constitute a sister group to the remaining two lineages (0.99/0.99/-/0.98). Rondeletieae and Guettardeae (Lineage C) are sisters, and form a sister group to Chiococceae s.l., Hamelieae, Hillieae and *Chione* and *Colleteria* (Lineage D). Within Lineage D, Hillieae and Hamelieae are sisters (1.0), with *Chione* and *Colleteria* as their sister group (1.0).

3.5.1. Lineage A – Cinchoneae and Isertieae (Fig. 2)

Cinchoneae is represented by seven genera. The phylogeny is fully resolved, and all nodes but three have maximum support. *Joosia* is the earlier derived, and *Stilpnophyllum* is diverging on the

next level. *Ciliosemina* and *Ladenbergia* form the sister group to a poorly supported clade (0.57), in which *Cinchona* and *Remijia* are sisters (0.96), and *Cinchonopsis* their sister.

The bigeneric Isertieae is also fully resolved and *Kerianthera* is resolved as sister to *Isertia*. For further details of Lineage A, see Fig. 2.

3.5.2. Lineage B – Naucleaeae s.l. and Hymenodictyeae (Fig. 3)

Naucleaeae s.l. is represented by 24 genera and *Cephalanthus* is sister to all other genera (1.0), distributed in two clades. The first clade (0.61) is subdivided in four subclades, with unresolved or

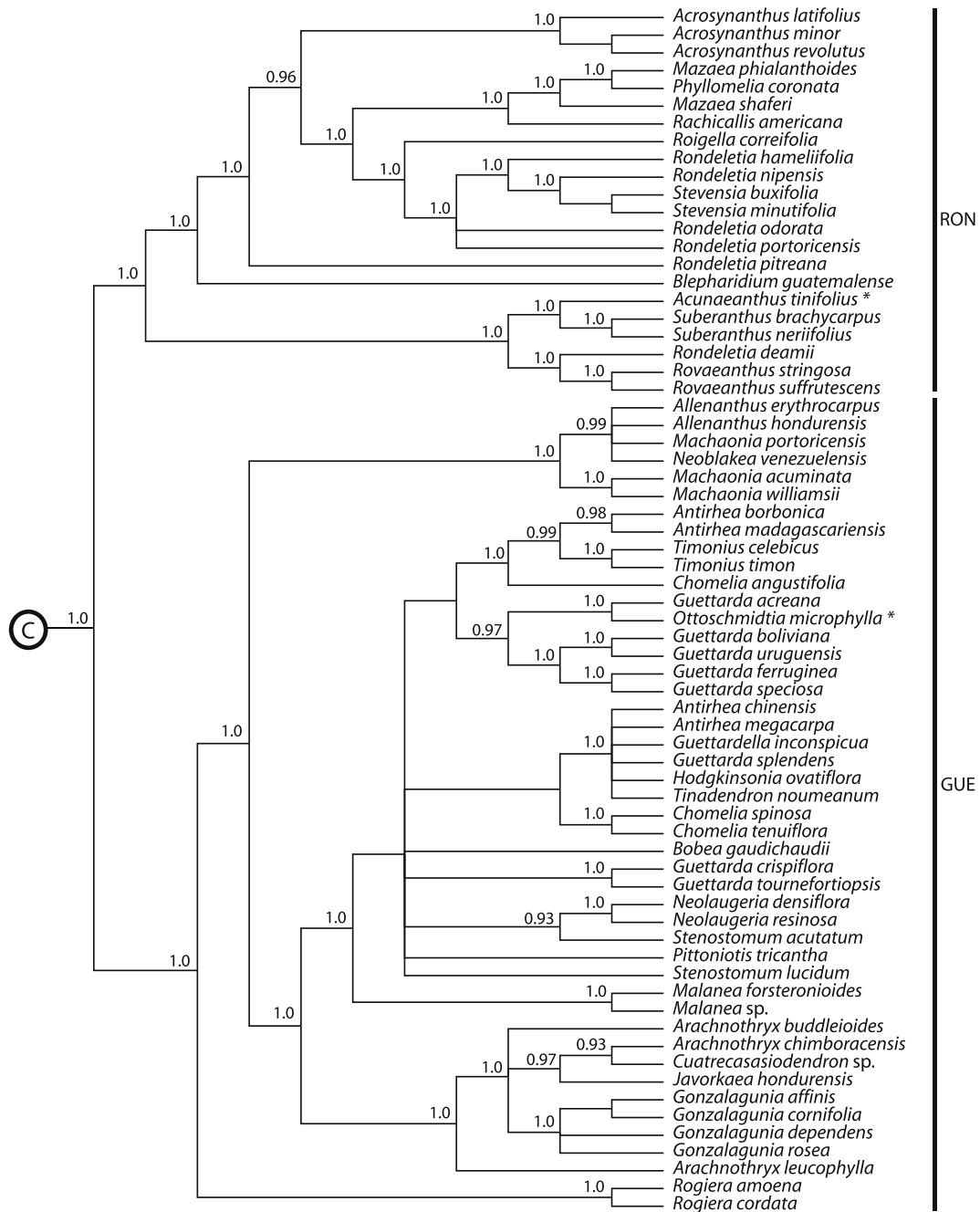


Fig. 4. Detailed phylogeny of Rondeletieae and Guettardeae (Lineage C) representing part of the majority rule tree retrieved from the analyses of the complete data set. Numbers given above branches represent posterior probability. * indicate taxa that have previously not been included in molecular analyses. GUE = Guettardeae and RON = Rondeletieae.

poorly supported relationships. The first subclade comprises *Mitragyna* (1.0). In the second subclade, *Pausinystalia* is found together with *Pseudocinchona* and *Corynanthe* (1.0). The third subclade consists of *Uncaria*, *Neolamarckia*, *Naucllea*, *Sarcocephalus*, and *Burttavya* (0.94). In the fourth subclade (1.0), *Breonadia* is sister to three genera (1.0), *Janotia*, *Gyrostipula*, and *Breonia*. In the second clade (0.94), *Ochreinauclea*, *Neonauclea*, *Myrmeconauclea*, and *Ludekia* group together in a subclade (0.94), while the remaining *Nauclleae* (i.e. *Adinauclea*, *Adina*, *Haldina*, *Metadina*, *Ochreinauclea*, *Pertusadina*, and *Sinoadina*) constitute a second subclade (1.0).

Hymenodictyae is represented by its two genera and *Hymenodictyon orixense* is sister to *Paracorynanthe antankarana* and *Hymenodictyon floribundum* (0.98). For further details of Lineage B, see Fig. 3.

3.5.3. Lineage C – Rondeletieae s.s. and Guettardeae s.l. (Fig. 4)

Ten genera were included from Rondeletieae s.s. The phylogeny is almost fully resolved and all nodes but three have maximum support. There is a basal split in two clades. The first clade consists of *Acunaeanthus*, *Suberanthus*, *Rondeletia deami*, and *Rovaeanthus*. In the second clade several additional *Rondeletia* (including *Stevensia*) are found together with the remaining taxa of Rondeletieae (i.e. *Acrosynanthus*, *Mazaea*, *Phyllomelia*, *Rachicallis*, *Roigella*, and *Blepharidium*).

Phylogeny within Guettardeae s.l., represented by 20 genera, is to a large extent unresolved or resolution is poorly supported. However, some basal nodes are resolved with maximum support. *Rogiera* is diverging on the first level and *Machaonia*, *Neoblakea*, and *Allenanthus* form a clade on the next level. Remaining taxa

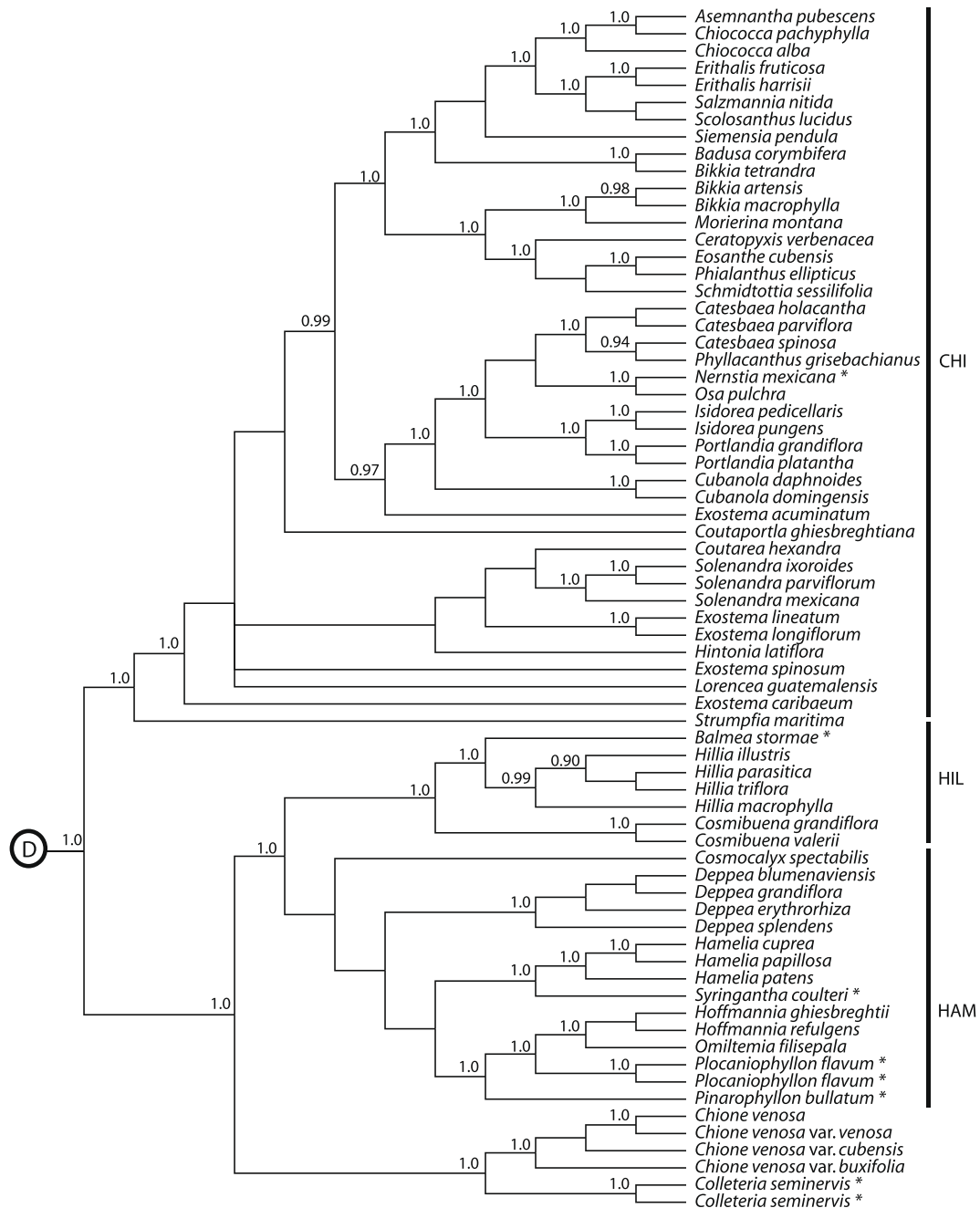


Fig. 5. Detailed phylogeny of Chiococceae, Hamelieae, Hillieae, *Chione*, and *Colleteria* (Lineage D) representing part of the majority rule tree retrieved from the analyses of the complete data set. Numbers given above branches represent posterior probability. * indicate taxa that have previously not been included in molecular analyses. CHI = Chiococceae, HAM = Hamelieae and HIL = Hillieae.

are divided into two highly supported clades. The first clade consists of *Arachnothryx* (including *Cuatrecasiodendron*), *Javorkea*, and *Gonzalagunia*, while the second clade includes all other Guettardeae (i.e. *Antirhea*, *Timonius*, *Chomelia*, *Guettarda*, *Guettardella*, *Ottoschmidtia*, *Hodgkinsonia*, *Tinadendron*, *Bobea*, *Neolaugeria*, *Stenostomum*, *Pittoniotis*, and *Malanea*). For more detailed resolution of Lineage C, see Fig. 4.

3.5.4. Lineage D – Chiococceae s.l., Hamelieae, Hillieae, *Chione* and *Colleteria* (Fig. 5)

Chiococceae s.l. is represented by 26 genera and the phylogeny is almost fully resolved. However, support values vary along the tree. *Strumpfia* is sister to all other taxa (1.0). *Exostema* is not recov-

ered as monophyletic, forming a grade together with *Lorencea*, *Hintonia*, *Solenandra*, *Coutarea*, and *Coutaportla*. Relationships within the grade are unresolved or poorly supported. Remaining taxa form a large clade, which is divided in two subclades (0.99). In the first subclade, *Exostema acuminatum*, *Cubanola*, *Portlandia*, *Isidorea*, *Nernstia*, *Osa*, *Catesbaea*, and *Phyllacanthus* are placed (0.97). The remaining Chiococceae form the second subclade (1.0): i.e. *Chiococca* (including *Asemnantha*), *Erithalis*, *Salzmannia*, *Scolosanthus*, *Siemensia*, *Badusa*, *Bikkia*, *Morierina*, *Ceratopyxis*, *Eosanthus*, *Phialanthus*, and *Schmidtottia*.

The analyses included eight genera from Hamelieae, whose phylogeny is fully resolved, although the tribe and basal nodes are poorly supported. *Cosmocalyx* has a basal position, and *Deppea*

is found on the next level. *Hamelia* and *Syringantha* are sisters (1.0), and *Pinarophyllon*, *Plocaniophyllon*, *Omitelia*, and *Hoffmannia* are strongly supported, in a sister clade to *Hamelia* and *Syringantha*.

All three genera of Hillieae were included and relationships within the tribe are fully resolved. *Cosmibuena* is sister to a clade including group to *Balmea* and *Hillia* (1.0).

Two genera, *Chione* and *Colleteria*, without tribal assignation, are found in a clade, forming sister group to Hamelieae and Hillieae (1.0). For further resolution and support values of Lineage D, see Fig. 5.

3.6. Discrepancies among analyses of the different data sets

Analyses of the single marker data sets generated majority rule consensus trees in which some nodes were resolved differently to the combined analyses. Only a few of these conflicting resolutions were, however, considered supported.

Analyses of nrITS data shows Hillieae nested within Hamelieae (0.92). Analyses of *rps16* resolves two members of Guettardeae, *Guettardella inconspicua* and *Chomelia spinosa*, in a clade of their own (1.0). This clade is placed outside the Rondeletieae – Guettardeae clade. Visual analysis of the alignment identified several, however not identical, regions of missing data (deletions) in the *Guettardella inconspicua* and *Chomelia spinosa* sequences, and not present in other taxa.

Discrepancies found in the analyses of single marker data sets within the separate tribes are not addressed since it is not the main focus of this paper.

4. Discussion

The aim of this study was to resolve deep node relationships and rigorously assess tribal delimitations within Cinchonoideae. To address these issues, extensive sampling was made throughout all tribes within the subfamily, and information from five chloroplast DNA markers and the nrITS region utilized. The complete data set included 206 taxa representing 107 of approx. 120 genera placed within Cinchonoideae, and information from all DNA markers were present in 120 taxa.

Apart from analyses of single markers, our data was combined in three different data sets and analyzed (see Section 2 for more details). Analyses of the combined data sets gave majority rule consensus trees with congruent, or at least not conflicting, overall topologies regardless of sampling. Support values in the different analyses were, however, variable for some of the nodes. Analyses of single data sets presented majority rule trees with somewhat different topologies, although, conflicting nodes had posterior probability values below 90% and were not considered supported. The first part of our discussion will be based on the analyses of all combined data sets (Fig. 1). The second part of the discussion, dealing with tribal delimitation, will be based on the analyses of the complete data set which includes all taxa (Figs. 2–5).

4.1. Subfamilial level – intertribal relationships and tribal support

Cinchoneae and Isertieae, with a distribution in tropical America, is the earlier derived lineage within the subfamily (Fig. 1; Lineage A). This position agrees with previous analyses of *rbcl* data (Bremer et al., 1995 [Isertieae not included]; Andersson and Rova, 1999; Razafimandimbison and Bremer, 2001 [Isertieae not included]), but contradicts other molecular studies (e.g. Andersson and Antonelli, 2005; Rydin et al., 2009). All our analyses of the combined data sets resolve this position of Cinchoneae and Isertieae (Fig. 1). The bayesian posterior probability value was only marginally changed if the problematic region in ITS1 was excluded,

or if a lot of missing data was added to the matrix by including taxa for which information was missing for some marker(s). Bootstrap support value (75) is, however, moderate for the node.

A sister group relationship between Cinchoneae and Isertieae has been shown in several previous studies (e.g. Bremer and Thulin, 1998; Andersson and Rova, 1999; Andersson and Antonelli, 2005). Robbrecht and Manen (2006), on the other hand, found Isertieae nested inside Cinchoneae, and decided to merge Isertieae with Cinchoneae. In addition, they proposed that two subtribes (Cinchoninae and Isertiinae) should be recognized. However, Cinchoneae and Isertieae are both well established tribal names and the two tribes can be distinguished morphologically, e.g. by the septate anthers found in Isertieae but not in Cinchoneae (Bremer and Thulin, 1998). Since our analyses give strong support to Cinchoneae and Isertieae we, therefore, prefer to treat them as separate tribes.

Distribution of Naucleaeae s.l. ranges from tropical Asia, Madagascar, tropical Africa, and extends to the Neotropics and North America, while its sister clade Hymenodictyeae has a paleotropical distribution. These two tribes have previously been shown as sister to all other Cinchonoideae (Andersson and Antonelli, 2005; Robbrecht and Manen, 2006; Antonelli et al., 2009), indicating an Old World (boreotropical) ancestor of the subfamily. Our result, with Naucleaeae and Hymenodictyeae in the second diverging lineage (Fig. 1; Lineage B), however, points to a dispersal of this lineage from the New World to the Old World. This hypothesis is further supported by a distribution in the New World of basal Ixorioideae (Robbrecht and Manen, 2006), indicating a common New World ancestor of the two subfamilies. It must be stressed that, although found in all analyses of combined cpDNA and nrITS data, bootstrap support value (64) is poor. Furthermore, analyses of cpDNA alone fail to resolve this relationship and place Lineages B, C, and D at a trichotomy (Fig. 1). This result has no significance for the here presumed area of origin of the subfamily. Further studies are, however, needed to test the hypothesis of a common New World ancestor of Cinchonoideae and Ixorioideae with subsequent dispersal to the Old World.

Hymenodictyeae and Naucleaeae s.l. are strongly supported, congruent to the analysis of combined molecular and morphological data by Razafimandimbison and Bremer (2001), and to other studies with a more limited sampling (e.g. Andersson and Antonelli, 2005; Rydin et al., 2009; Bremer and Eriksson, 2009), but contradicting Robbrecht and Manen (2006), who did not find support for a monophyletic Hymenodictyeae.

Distribution of the two last lineages (Fig. 1; Lineages C and D) is primarily in the Neotropics, although Chiococceae s.l. and Guettardeae s.l. also have members with a distribution in Southeast Asia and/or the Pacific Islands. The two strongly supported clades in Lineage C correspond to clade C5a (Guettardeae s.l.) and C5b (Rondeletieae s.s.) in Rova et al. (2002). Members of these two tribes have also been shown closely related in other molecular analyses (e.g. Bremer and Thulin, 1998; Bremer et al., 1999; Bremer and Eriksson, 2009; Rova et al., 2009; Rydin et al., 2009).

A close relationship between Chiococceae s.l., Hamelieae, and Hillieae was indicated by Andersson and Rova (1999), and later also by Andersson and Antonelli (2005). Our result strongly supports this relationship, but also finds *Chione* and *Colleteria* in the alliance (Fig. 1; Lineage D). Chiococceae s.l. is strongly supported and Hamelieae and Hillieae are sister clades, corroborating previous phylogenetic analyses (e.g. Bremer et al., 1995; Bremer and Thulin, 1998; Rova et al., 2002; Andersson and Antonelli, 2005). However, as many previous molecular analyses included only one or a few representatives of the Hamelieae (Bremer and Jansen, 1991; Bremer et al., 1995; Bremer, 1996; Manen and Natali, 1996; Bremer and Thulin, 1998; Bremer and Manen, 2000; Rova et al., 2002; Andersson and Antonelli, 2005), and only one representative

of Hillieae (Bremer et al., 1995; Andersson and Rova, 1999; Andersson and Antonelli, 2005), monophyly of these tribes were not actually confirmed. Robbrecht and Manen (2006) had a broader sampling and found Hamelieae and Hillieae representatives mixed in a clade together with *Chione*, and decided to include Hillieae and *Chione* in Hamelieae. Recently, Bremer and Eriksson (2009), on the other hand, showed both Hamelieae and Hillieae as monophyletic. Our result, based on five genera from Hamelieae and two from Hillieae, is congruent to Bremer and Eriksson (2009) but contradicts Robbrecht and Manen (2006). We find Hamelieae and Hillieae strongly supported as separate tribes and as sister clades (Fig. 1). However, in the analyses of the complete data set (Fig. 5), including eight genera of Hamelieae, the tribe has low posterior probability (0.70). This could possibly be explained by conflicting signals in the nrITS data set and the cpDNA data sets, as the analyses of nrITS data alone place Hillieae nested within Hamelieae. A large amount of missing data in *Cosmocalyx*, for which only *atpB-rbcL* data was available may also have contributed to the low posterior probability for Hamelieae. The analyses of single *atpB-rbcL* matrix generated majority rule trees (not shown) with a mainly unresolved topology for the Hamelieae-Hillieae alliance, and excluding *Cosmocalyx* from the complete data set results in a higher, although still not significant posterior probability (0.82; not shown). Placement of *Chione* within the sister group to Hamelieae and Hillieae (Fig. 1) supports previous studies by Rova et al. (2002) and Bremer and Eriksson (2009).

4.2. Tribal level

4.2.1. Cinchoneae DC. (sensu Andersson, 1995)

Cinchoneae was characterized by the presence of winged seeds and included 14 genera (Candolle, 1830). Later, other morphological characters were used to define the tribe and its delimitation was altered by different authors (for a summary, see Andersson and Persson, 1991). At the most, Cinchoneae included as many as almost 50 genera, of which 12 were included with reservation (Robbrecht, 1998). However, after cladistic analyses of detailed morphological data, Andersson and Persson (1991) excluded all but 13 genera, and, later, Andersson (1995) decreased the number of genera even further to eight, also including the new genus *Cinchonopsis*. This circumscription, based on morphology, was confirmed by analyses of nrITS and cpDNA data (Andersson and Antonelli, 2005), and at the same time an additional genus, *Ciliosemia*, was described increasing the number of genera to nine. Our study (Fig. 2) confirms present tribal delimitation and resolves the topology within Cinchoneae mainly congruent to Andersson and Antonelli (2005). However, Andersson and Antonelli (2005) found alternative placements of *Ciliosemia* among the most parsimonious trees. Our result supports a placement of *Ciliosemia* as sister to *Ladenbergia*.

Cinchoneae, as circumscribed today, is supported by morphological characters such as externally sericeous corolla, corolla lobes valvate in bud, and os (the inner part) of the pollen aperture poorly defined (Andersson, 1995), the latter two being consistent for the group. Two genera, *Maguireocharis* and *Pimentelia*, are placed within the tribe based solely on morphology, and were found in a clade together with *Stilpnophyllum* and *Cinchonopsis* in a cladistic analysis based on morphological data (Andersson, 1995). Unfortunately, *Maguireocharis* and *Pimentelia* were not available to us, and *Stilpnophyllum* and *Cinchonopsis* do not seem to be sisters (Andersson and Antonelli, 2005; this study), and the positions of *Maguireocharis* and *Pimentelia* within Cinchoneae are uncertain.

Genera included: *Ciliosemia*, *Cinchona*, *Cinchonopsis*, *Joosia*, *Ladenbergia*, *Remijia*, and *Stilpnophyllum*.

Tentatively included: *Maguireocharis* and *Pimentelia*.

4.2.2. Isertieae A.Rich., ex DC. (sensu Bremer and Thulin, 1998)

When Iseriteae was first described it comprised three genera, of which *Isertia* is the only one still placed within the tribe. Circumscription has varied over time and genera have been added or excluded from the tribe by different authors (for a summary, see Andersson, 1996). In its latest circumscription based on morphological data (Andersson, 1996), the tribe comprised seven genera. Circumscription was, however, drastically changed by Bremer and Thulin (1998), who used *rbcL* data to show that a number of genera, placed within the tribe based on morphology (Andersson, 1996; Delprete, 1996; Robbrecht, 1998, 1993) belong in Ixoroideae s.l. and Rubioideae, leaving Isertieae as a bigeneric tribe.

The tribe shares several morphological and chemical characteristics with Cinchoneae (Bremer and Thulin, 1998). However, the two genera, *Isertia* and *Kerianthera*, can be distinguished by the presence of septate anthers with many small chambers, and are similar in other respects (Delprete, 1996; Bremer and Thulin, 1998). Our result (Fig. 2), supports *Isertia* and *Kerianthera* as sisters, and Isertieae as sister clade to Cinchoneae.

Genera included: *Isertia* and *Kerianthera*.

4.2.3. Naucleae DC ex Miq. (sensu Razafimandimbison and Bremer, 2001)

Present delimitation of Naucleae s.l. (Razafimandimbison and Bremer, 2001) is based on a combination of molecular and morphological data. In addition to previous circumscriptions of Naucleae (i.e. Ridsdale, 1978; Robbrecht, 1993), the tribe also includes four members of Coptosapelteae (sensu Andersson and Persson, 1991) and the former tribe Cephalantheae (sensu Ridsdale, 1976), giving a total of 26 genera. Synapomorphic characters of Naucleae are globose inflorescences, presence of epigynous nectaries that are deeply embedded in hypanthia, and a secondary pollen presentation mechanism where the pollen is presented on immature stigmatic lobes (Razafimandimbison and Bremer, 2001). Our result (Fig. 3) agrees with the present delimitation of Naucleae, and is mainly congruent to the combined molecular tree in Razafimandimbison and Bremer (2002). However, in difference to Razafimandimbison and Bremer (2002), *Adinauclea* seems to be nested within, and may even by congeneric with, *Adina*. This supports the view of Ridsdale (1978) that *Adinauclea* is closer related to *Adina* than to *Neonauclea*. Furthermore, *Haldina* and *Sinoadina* are supported to belong in subtribe *Adininae* (Fig. 3).

There are two genera, *Diyaminauclea* and *Khasiaclunea*, placed within Naucleae s.l. that have not yet been included in molecular analyses. These two genera were previously part of a heterogeneous *Neonauclea* (sensu Merrill, 1915), but excluded by Ridsdale (1978) along with *Ludekia*. *Ludekia* is in our analyses placed in a clade together with *Myrmeconuclea* (another former *Neonauclea* member; Merrill, 1915) and *Neonauclea* (Fig. 3). As pointed out by Razafimandimbison and Bremer (2002) relationships between *Neonauclea* and allied genera are not clear. However, *Diyaminauclea* and *Khasiaclunea* most likely belong in this alliance.

Genera included: *Adina*, *Adinauclea*, *Breonadia*, *Breonia*, *Burttavya*, *Cephalanthus*, *Corynanthe*, *Grostipula*, *Haldina*, *Janotia*, *Ludekia*, *Metadina*, *Mitragyna*, *Myrmeconuclea*, *Nauclea*, *Neolamarckia*, *Neonauclea*, *Ochreinauclea*, *Pausinystalia*, *Pertusadina*, *Pseudo-cinchona*, *Sarcocephalus*, *Sinoadina*, and *Uncaria*.

Tentatively included: *Diyaminauclea* and *Khasiaclunea*.

4.2.4. Hymenodictyeae Razafim. & B.Bremer

Members of Hymenodictyeae were previously placed in Coptosapelteae (sensu Andersson and Persson, 1991) but shown to be closely related to Naucleae s.l. by Razafimandimbison and Bremer (2001), who found molecular and morphological support to place them in a tribe of their own. The tribe is bigeneric and distinguished from Naucleae s.l. by inflorescences with determinate

thyrses, reduced to spicoids (Claßen-Bockhoff, 1996), stipules with large deciduous colleters on the margins, flowers with free epigynous nectaries, corollas with a narrowly cylindrical base and wider towards apex, and lenticellate, woody, capsular fruits (Razafimanimbison and Bremer, 2001, 2006). They also seem to lack indole alkaloids, present in Naucleaeae s.l. (Razafimanimbison and Bremer, 2001). However, although our study supports Hymenodictyae, analyses of the complete data set (Fig. 3) as well as analyses of single markers (i.e. *atpB-rbcL* spacer, *ndhF*, and *rbcL*; not shown) fail to support *Hymenodictyon* as monophyletic.

Genera included: *Hymenodictyon* and *Paracorynanthe*.

4.2.5. *Rondeletieae* DC ex Miq. (sensu Rova et al., 2009)

Delimitation of *Rondeletieae* has shifted heavily, partly due to its morphological resemblance to members of *Condamineae* (*Ixoroideae*), but also to difficulties to evaluate the importance of single morphological characters (For a review of the taxonomy, see Delprete, 1996). In the latest morphological study of *Rondeletieae*, Delprete (1996) found support to suggest that two subtribes of *Condamineae* (i.e. *Condamineinae* and *Pinckneyinae*) should be placed within *Rondeletieae*. This circumscription was, however, not supported by molecular data (Rova et al., 2002). Instead, *Condamineinae* and *Pinckneyinae* were found within *Ixoroideae*. Furthermore, several genera, traditionally placed in *Rondeletieae* (Robbrecht, 1998, 1993; Delprete, 1996), have been placed in *Ixoroideae* or found nested within, or close to *Guettardeae* (Rova et al., 2002; Delprete and Cortéz-B, 2004).

Recently, Rova et al. (2009) proposed a new circumscription of *Rondeletieae* based on molecular data. In this circumscription, *Rondeletieae* is delimited to 14 genera, of which four are tentatively included. Our result (Fig. 4) is congruent to Rova et al. (2002), and to supported nodes in Rova et al. (2009). Our result also mainly supports the narrow circumscription of *Rondeletieae*, however, Rova et al. (2009) decided to maintain *Rogiera* in *Rondeletieae*, which is not supported here. We find *Rogiera* closer related to *Guettardeae* s.l., congruent to Rova et al. (2002).

Rondeletia s.s. (also including *Stevensia*; Rova et al., 2009) appears to be polyphyletic in its present circumscription, as *R. deamii* and *R. pitreana* are found in positions elsewhere in *Rondeletieae*. Position of *Rondeletia deamii* has previously been questioned, and Borhidi (1982), when treating *Rondeletia* in a narrow sense, placed *R. deamii* in *Arachnothryx*. In our study, *Rondeletia deamii* seems to be closely related to *Rovaeanthus*, while *R. pitreana* is placed as sister to a clade consisting of *Acrosynanthus*, the *Mazaea*-alliance, and *Rondeletia* s.s. (Fig. 4). Rova et al. (2009) also found these two taxa outside *Rondeletia*, however, chose to consider *R. deamii* as *Rondeletia* and did not trust their result regarding *R. pitreana*.

Rova et al. (2009) reported conflicting, but not supported, placements of *Acrosynanthus* in their analyses of nrITS data to previous studies of *trnL-F* data (Rova et al., 2002) and proposed that the genus may not be monophyletic. Our study, including three *Acrosynanthus*, supports monophyly of the genus, and it is found monophyletic in analyses of single (not shown), as well as combined data sets (Figs. 1 and 4). The conflicting placement when analyzing nuclear and chloroplast data may, therefore, indicate a hybrid origin of the genus.

The monotypic, Cuban endemic *Acunaeanthus* is among genera tentatively included in *Rondeletieae* (Rova et al., 2009). The genus was separated from *Mazaea* by Borhidi et al. (1980), and maintained as a separate genus after detailed morphological studies by Delprete (1999). Our result supports *Acunaeanthus* as a distinct genus and places it as sister to *Suberanthus*, rather distant from *Mazaea* (Fig. 4).

We were unable to amplify DNA from several monotypic genera, or genera comprising a low number of species, thus tentative placement within *Rondeletieae* are in these cases solely based on

morphology. Considering recent transfer of genera from *Rondeletieae* to *Guettardeae* s.l., or even *Ixoroideae* based on molecular analyses (Rova et al., 2002; Delprete and Cortéz-B, 2004), and the obvious difficulties to identify reliable informative morphological characters, further discussion on their taxonomic position will have to await future molecular studies. Furthermore, some of the genera transferred to *Guettardeae* (Rova et al., 2002, 2009) have even been considered part of a broadly defined *Rondeletia* (Kirkbride, 1969; Lorence, 1991), emphasizing the morphological similarity between members of the two tribes. Subsequently, previously defined characters to recognize the two tribes are no longer informative, and new morphological studies in view of present circumscriptions are needed.

Genera included: *Acrosynanthus*, *Acunaeanthus*, *Blepharidium*, *Mazaea* (including *Ariadne*), *Phyllomelia*, *Roigella*, *Rondeletia* (including *Stevensia*), *Rovaeanthus*, *Rachicallis*, and *Suberanthus*.

Tentatively included: *Acrobotrys*, *Glionnetia*, *Habroneuron*, *Holstianthus*, *Spathichlamys*, and *Standleya*.

4.2.6. *Guettardeae* DC (sensu Rova et al., 2002; clade C5a)

Guettardeae is here treated in a wider sense, according to the result in Rova et al. (2002), where several genera traditionally placed in *Rondeletieae* (i.e. *Arachnothryx*, *Gonzalagunia*, *Javorkaea*) were shown to be closer related to *Guettardeae*. Our result supports a wide circumscription of *Guettardeae*, however, places *Rogiera* as sister to *Guettardeae* s.l. (Fig. 4), indicating that it should also be included in the tribe. *Machaonia*, *Allenanthus*, and *Neoblakea* form one out of two clades comprising traditional *Guettardeae* members, and *Machaonia* appears to be paraphyletic with regard to the latter two genera. The second traditional *Guettardeae* clade is sister to *Arachnothryx* (including *Cuatrecasiodendron*; Rova et al., 2009), *Gonzalagunia*, *Javorkaea*, as in Rova et al. (2002). Our study indicates that *Arachnothryx* (despite inclusion of *Cuatrecasiodendron*), *Antirhea*, *Chomelia*, *Guettarda*, and possibly *Stenostomum* may not to be monophyletic. Polyphyly of *Antirhea*, *Guettarda*, and *Stenostomum* was previously indicated by Achille et al. (2006).

In addition to Rova et al. (2002), several genera considered as *Guettardeae* members (Robbrecht, 1998; Schumann, 1891) have been included in molecular analyses. Moynihan and Watson (2001) indicated *Neolaugeria* and *Stenostomum* to be closely related to *Guettarda* and *Timonius*, however, based on a rather limited sampling. Achille et al. (2006) added *Bobea*, *Malanea*, and *Pittoniotis* to genera closely related to *Guettarda* and allied taxa, in a study restricted to *Guettardeae*. Finally, Rova et al. (2009) showed that *Hodgkinsonia* belongs in *Guettardeae*, and not in *Chiococceae* as has sometimes been suggested (Robbrecht, 1998; Delprete, 1996). Our result is congruent to these studies and clearly place *Bobea*, *Hodgkinsonia*, *Malanea*, *Neolaugeria*, *Pittoniotis* and *Stenostomum* in *Guettardeae* s.l. Furthermore, our result also places the neotropical, monotypic genus *Ottoschmidtia* among these genera (Fig. 4), confirming previous placement based on morphology (Robbrecht, 1998).

The Southeast Asian *Dichilanthe* has strongly zygomorphic flowers and the genus was first placed in *Caprifoliaceae* (Hooker, 1856), however, later transferred to *Rubiaceae* (Hooker, 1873). Its placement within *Guettardeae* is primarily supported by fruit characters (Puff et al., 1996), and despite being convinced about its taxonomic position, they stated that “*Dichilanthe*, although, undoubtedly a member of the *Guettardeae*, in general appears to stand alone and isolated”. No material of *Dichilanthe* was available to us and it is left as a member of *Guettardeae*, pending future molecular studies.

Genera included: *Allenanthus*, *Antirhea*, *Arachnothryx* (including *Cuatrecasiodendron*), *Bobea*, *Chomelia*, *Gonzalagunia*, *Guettarda*, *Guettardella*, *Hodgkinsonia*, *Javorkaea*, *Machaonia*, *Malanea*,

Neoblakea, *Neolaugeria*, *Ottoschmidia*, *Pittoniotis*, *Rogiera*, *Stenostomum*, *Timonius*, and *Tinadendron*.

Tentatively included: *Dichilanthe*.

4.2.7. *Chiococceae* Hook. f.; s.l. (sensu Bremer, 1992; Bremer et al., 1995)

Triggered by a phylogenetic restriction site analysis (Bremer and Jansen, 1991), Bremer (1992) analyzed morphological data and found support to propose a widened *Chiococceae*, comprising *Chiococceae* sensu Hooker, *Portlandiinae* (one of three subtribes of *Condamineae*), *Exostema* and *Hintona*. Later, Bremer et al. (1995) included *Catesbaea* in *Chiococceae* s.l., and recently also *Strumpfia* (Bremer and Eriksson, 2009). Delprete (1996), on the other hand, proposed an alternative solution after a detailed morphological study of members placed in *Chiococceae*, *Condamineae*, *Catesbaeae* and *Rondeletiae*. He maintained *Chiococceae* sensu Hooker, included *Portlandiinae* in *Catesbaeae*, and formed an informal *Exostema*-group. Any of the two alternative circumscriptions of *Chiococceae* are congruent with later analyses of molecular data (Rova et al., 2002; Motley et al., 2005; Bremer and Eriksson, 2009; this study).

Motley et al. (2005) stated that *Chiococceae* s.l. could only be identified by a combination of two characters: stamens inserted at the base of the corolla and presence of spinulose pollen. Spinulose pollen is, however, not present in *Strumpfia* (Igersheim, 1993), a morphologically apart and monotypic genus shown to be closely related to the tribe (Rova et al., 2002; Bremer and Eriksson, 2009; this study), and recently included in *Chiococceae* s.l. (Bremer and Eriksson, 2009). When including *Strumpfia*, Bremer and Eriksson (2009) stated that stamens inserted at the base of the corolla is a synapomorphy for an extended *Chiococceae*. This character is, however, also found within *Chione* (Taylor, 2003a) and *Hamelieae* (Bremer, 1987), while lacking in *Colleteria* (Taylor, 2003b) and *Hillieae* (Andersson, 1995), all placed within the sister clade to *Chiococceae* s.l. in our study (Fig. 5). Stamens inserted at the base of the corolla thus seems to be a (homoplastic) synapomorphy for the *Chiococceae*–*Hamelieae*–*Hillieae* alliance. Subsequently, spinulose pollen is the sole identified synapomorphy of *Chiococceae* s.l., but only if *Strumpfia* is again excluded from the tribe.

The overall resolution within the tribe is congruent to previous studies (e.g. Rova et al., 2002; Motley et al., 2005), with two strongly supported clades (i.e. *Chiococceae* s.s. and the *Catesbaea*–*Portlandia* lineage), here placed as sister groups, and a number of taxa placed outside these two lineages in a polytomy. Although information from five chloroplast markers has been used in the present study, resolving relationships of *Exostema* and closely related genera has not been possible. As in the combined *trnL-F* and *nrITS* analysis by Motley et al. (2005), *Exostema acuminatum* is found as sister to the *Catesbaea*–*Portlandia* lineage, while all other *Exostema* are placed outside the two lineages, corroborating earlier indications of a polyphyletic of *Exostema* (McDowell et al., 2003; Motley et al., 2005). However, relationships are, generally, poorly supported or unresolved (McDowell et al., 2003; Motley et al., 2005; this study).

Relationships within the *Catesbaea*–*Portlandia* lineage are congruent to the combined *trnL-F* and *nrITS* analysis in Motley et al. (2005), with the exception of *Phyllacanthus*, which is found nested within *Catesbaea* (Fig. 5). Although not supported by their combined analysis, Motley et al. (2005), suggested that *Phyllacanthus* should be included in *Catesbaea*, based on morphology and an analysis of *trnL-F* data. This had previously been shown in Rova et al. (2002), and is also supported in our study.

The monotypic Mexican genus *Nernstia* was separated from *Portlandia* by Aiello (1979) based on fruit and seed characters, and suggested to belong in *Hedyotideae* or *Cinchoneae*. Later, Andersson and Persson (1991) referred to other morphological

characters and suggested *Nernstia* to be closely related to *Coutarea*, *Exostema*, and *Portlandia*, a view shared with Delprete (1996) who placed it in his “*Portlandia*-group”. Our result (Fig. 5) clearly supports a placement within the *Catesbaea*–*Portlandia* lineage, and *Nernstia* is placed as sister to the monotypic, Costa Rican genus *Osa*.

In his detailed morphological study, Delprete (1996) also found morphological support to place *Ceuthocarpus* and *Thogsennia* within his “*Portlandia*-group”. Unfortunately, we were not been able to amplify sequences from these two genera to confirm this placement using molecular data.

Relationships within *Chiococceae* s.s. are also mainly congruent to Motley et al. (2005). *Bikkia* seems polyphyletic in its present circumscription. The New Caledonian species (*Bikkia artensis* and *B. macrophylla*) are found in a clade together with the monotypic *Morierina*, while the widespread *B. tetrandra*, type species the genus, is found in another clade as sister to *Badusa* (Fig. 5). This was also shown by Motley et al. (2005). However, *Morierina* is here placed as sister to the New Caledonian *Bikkia*, a relationship not resolved in the combined analysis in Motley et al. (2005). Furthermore, the New Caledonian *Bikkia*–*Morierina* clade is in our study placed as sister clade to a Cuban clade consisting of *Ceratopyxis*, *Eosanthus*, *Phialanthus*, and *Schmidtottia*, while Motley et al. (2005) indicated another Cuban genus, *Siemensia*, as sister to the New Caledonian *Bikkia*–*Morierina* clade. *Siemensia* is in our study placed together with the other *Bikkia* clade, *Chiococca* (including *Asemnantha*), *Erithalis*, *Salzmannia*, and *Scolosanthus*, congruent to the *trnL-F* analysis of Motley et al. (2005).

Delprete (1996) also included *Placocarpa* Hook. f. in his narrowly circumscribed *Chiococceae*. Although, as *Placocarpa* does not have the anthers attached to the corolla in the same way as the majority of *Chiococceae* members (Delprete, 1996), or the spinulose pollen present in the other members of the tribe (Huysmans et al., 1999), we agree with Huysmans et al. (1999), who excluded *Placocarpa* from *Chiococceae*.

Genera included: *Asemnantha*, *Badusa*, *Bikkia*, *Catesbaea* (including *Phyllacanthus*), *Ceratopyxis*, *Chiococca*, *Coutaportia*, *Coutarea*, *Cubanola*, *Eosanthus*, *Erithalis*, *Exostema*, *Hintonia*, *Isidorea*, *Lorencea*, *Morierina*, *Nernstia*, *Osa*, *Phialanthus*, *Portlandia*, *Salzmannia*, *Schmidtottia*, *Scolosanthus*, *Siemensia*, *Solenandra*, and *Strumpfia*.

Tentatively included: *Ceuthocarpus* and *Thogsennia*.

4.2.8. *Hamelieae* DC. (sensu Bremer, 1987)

Hamelieae was previously placed in *Rubioideae* due to presence of raphides, but is now considered part of *Cinchonoideae* based on molecular phylogenetic analyses (Bremer and Jansen, 1991; Bremer and Thulin, 1998; Rova et al., 2002). In its current circumscription *Hamelieae* includes ten genera (Bremer, 1987; Lorence, 1990; McDowell, 1996; Delprete, 1998; Delprete et al., 2005), and the tribe is recognized by “almost ebracteolate inflorescences, often yellow or yellowish flowers, [...] and granulate to tuberculate outer testa walls” (Bremer, 1987).

Knowledge of relationships within *Hamelieae* is poor, mainly due to sampling of one or a few genera in previous analyses (e.g. Bremer and Jansen, 1991; Bremer et al., 1995; Bremer, 1996; Manen and Natali, 1996; Bremer and Thulin, 1998; Bremer and Manen, 2000; Rova et al., 2002; Andersson and Antonelli, 2005). However, in the supertree over *Rubiaceae* (Robbrecht and Manen, 2006) four genera of *Hamelieae* were included. Three genera, *Hamelia*, *Hoffmannia* and *Deppea*, were represented by more than one species, and none were found monophyletic. In difference to Robbrecht and Manen (2006), our analyses, using almost identical sampling of the three genera, and three additional genera of *Hamelieae*, find *Hamelia*, *Hoffmannia*, and *Deppea* monophyletic (Fig. 5).

Our analyses also place *Cosmocalyx*, *Syringantha*, *Pinarophyllon* (*P. bullatum*) and *Plocaniophyllon* in *Hamelieae* (Fig. 5). *Cosmocalyx* was long seen as genus of uncertain position until it was placed in

Hamelieae by [Delprete \(1998\)](#), based on morphology. This placement was later supported by [Robbrecht and Manen \(2006\)](#) and is also indicated in our analyses. Systematic position of *Syringantha*, *Pinarophyllon*, and *Plocaniophyllon* have not previously been tested using molecular data, but they have all been placed in Hamelieae based morphological characters. The monotypic *Syringantha* Standl. was first considered closely related to *Exostema* ([Standley, 1930](#)), a view shared with [Andersson and Persson \(1991\)](#). Later, [McDowell \(1996\)](#), suggested *Syringantha* to have affinities with Hamelieae based on leaf, flower, fruit, and pollen characters, but also by the presence of raphides. This view was shared with [Delprete \(1996\)](#) and [Martínez-Cabrera et al. \(2007, 2009\)](#), and is also supported in our study, placing *Syringantha* as sister to *Hamelia* ([Fig. 5](#)). Likewise, the presence of raphides got [Bremekamp \(1966\)](#) to question the placement of *Pinarophyllum* in Rondeletieae ([Verdcourt, 1958](#)), and later, *Pinarophyllum* was included in Hamelieae by [Bremer \(1987\)](#). Raphides were also found in *Plocaniophyllon* and [Lorence and Dwyer \(1988\)](#) placed it in Hamelieae, and at the same time commented its morphological similarity to *Omitelia* and *Hoffmannia*. This view was supported by [Martínez-Cabrera et al. \(2008\)](#), who also found morphological similarities between *Plocaniophyllon* and *Pinarophyllum*. Our study supports a placement of *Pinarophyllon* and *Plocaniophyllon* within Hamelieae, and they are both placed the same clade as *Omitelia* and *Hoffmannia* ([Fig. 5](#)).

There are still genera placed in Hamelieae for which we were not able to amplify molecular data (i.e. *Eizia*, and *Patima*). *Eizia* was considered closely related to *Lindenia* (= *Augusta*, Ixoroideae; [Standley, 1940](#)), and placed in a widely circumscribed Rondeletieae ([Robbrecht, 1998](#)), however, transferred to Hamelieae by [Lorence and Dwyer \(1988\)](#), based on the same morphological characters as found in *Plocaniophyllon*. The taxonomic history of *Patima* is complicated and confusing and it was long considered a synonym of *Sabicea* ([Delprete et al., 2005](#)). *Patima* was, however, transferred to Hamelieae by [Delprete et al. \(2005\)](#), after a thorough morphological analysis where presence of raphides was confirmed, but also due to an overall morphological similarity with other members of the tribe.

Genera included: *Cosmocalyx*, *Deppea* (including *Bellizina*, *Csapodya*, *Edithea*, and *Schenckia*), *Hamelia*, *Hoffmannia*, *Omitelia*, *Pinarophyllon*, *Plocaniophyllon*, and *Syringantha*.

Tentatively included: *Eizia* and *Patima*.

4.2.9. Hillieae Bremek. ex Darwin (sensu Andersson, 1995)

Placement of *Hillia*, type genus of Hillieae, has varied over time. [Verdcourt \(1958\)](#) placed *Hillia* in Rubioideae, based on presence of raphides, [Bremekamp \(1966\)](#) suggested that *Hillia* should be treated as a subfamily of its own, and [Robbrecht \(1998\)](#) proposed that *Hillia* belonged in its own tribe, Hillieae within Cinchonoideae. More recently, based on detailed morphological studies, [Taylor \(1994\)](#) considered *Hillia* a member of Cinchoneae, while [Andersson \(1995\)](#) concluded that Hillieae should comprise *Balmea*, *Blepharidium*, *Cosmibuena*, and *Hillia*. The former three (*Balmea*, *Blepharidium*, and *Cosmibuena*) had all previously been placed in Cinchoneae.

[Rova et al. \(2002\)](#), however, showed that *Blepharidium* was closely related to Rondeletieae s.s. and did not belong in Hillieae. This is also confirmed in our analyses ([Fig. 5](#)). However, although *Hillia* or *Cosmibuena* have been placed as sister group to Hamelieae in several analyses (e.g. [Bremer et al., 1995](#); [Andersson and Rova, 1999](#); [Andersson and Antonelli, 2005](#)), monophyly of Hillieae has not been confirmed since the analyses included only one of them. [Robbrecht and Manen \(2006\)](#), on the other hand, included *Hillia* and *Cosmibuena* in their supertree which showed that they were mixed with members of Hamelieae, and they decided to include Hillieae in Hamelieae. Here we show Hillieae strongly supported as monophyletic, contradicting the result of [Robbrecht and Manen](#)

(2006). Furthermore, the third member of Hillieae, the mexican “Christmas tree” *Balmea stormae*, is placed within the tribe with strong support. *Balmea*, *Hillia*, and *Cosmibuena* are all rather succulent trees or (generally) epiphytic, and have capsules with a beak-like appendage ([Taylor, 1994](#); [Andersson, 1995](#)).

Genera included: *Balmea*, *Cosmibuena*, and *Hillia*.

4.2.10. Chione and Colleteria

The neotropical *Chione* has a distribution in wet forests in North and South America and most of the Greater and Lesser Antilles. The vegetative parts of *Chione* is highly variable ([Taylor, 2003a](#)), and the genus was previously assumed to contain c. 15 species ([Mabberley, 1997](#)). Following a morphological study of *Chione*, [Taylor \(2003a\)](#), however, concluded that the genus was monotypic, if two morphologically very divergent species were excluded. In a subsequent paper, [Taylor \(2003b\)](#), assigned these two former *Chione* species to a new genus, *Colleteria*. He also vaguely indicated a relationship of *Colleteria* to Psychotrieae, based on fruit characteristics.

Our study included three different varieties of *Chione*, and two collections of *Colleteria seminervis*, and despite the many morphological differences identified between *Chione* and *Colleteria* ([Taylor, 2003b](#)), molecular data support the two as closely related ([Fig. 5](#)). *Colleteria seminervis* is placed outside the *Chione* clade and, considering the morphological differences, its treatment in a separate genus seems motivated, although *Colleteria exserta*, type species of *Colleteria*, was not available to us.

Chione and *Colleteria* are left without tribal affinity. [Robbrecht and Manen \(2006\)](#), on the other hand, decided to include *Chione* in Hamelieae along with Hillieae, and stated presence of raphides as a distinct character diagnosing their amended Hamelieae. However, although raphides are present in Hamelieae and Hillieae, they have not been found in *Chione* or *Colleteria*. Pending further morphological studies, we choose not to assign *Chione* and *Colleteria* to any existing tribe, and also to treat Hamelieae and Hillieae as separate tribes supported by molecular and morphological data.

4.3. Conclusions

A broad sampling throughout all tribes within Cinchonoideae, and information from both nuclear and chloroplast data contributed to resolve deep node relationships within the subfamily.

Interestingly, the tropical American clades Cinchoneae and Iseriidae are resolved as sisters, sister in turn to all other tribes of Cinchonoideae, indicating that early ancestors of the subfamily were already present in the New World. By adding nrITS data, further resolution was achieved and Naucleae s.l. and Hymenodictyae form the next diverging lineage, representing a probable dispersal from the New World to the Old World. Remaining tribes, i.e. Rondeletieae s.s., Guettardeae s.l., Chiococceae s.l., Hamelieae, and Hillieae, include predominantly neotropical taxa and are resolved within two sister clades. The two former tribes are found in the first clade, and the three latter ones in the second.

Generally, our analyses found strong support for all nine tribes and confirmed previous tribal affiliations based on molecular data. However, *Rogiera* is not a part of Rondeletieae s.s., and should instead be included in Guettardeae s.l. In addition, our study confirms that *Acunaeanthus* belongs in Rondeletieae s.s., *Ottoschmidia* in Guettardeae s.l., *Nernstia* in Chiococceae s.l., *Pinarophyllon*, *Plocaniophyllon*, and *Syringantha* in Hamelieae, and *Balmea* in Hillieae. Furthermore, since some of the recent tribal re-circumscriptions have invalidated the morphological characteristics used to distinguish the tribes, there is a need of morphological studies in the view of these new circumscriptions. Possibly, new tribal circumscriptions may have to be suggested, if we require the tribes

to be recognized not only by molecular but also morphological characteristics.

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