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Source: *Annals of the Missouri Botanical Garden*, Vol. 82, No. 3 (1995), pp. 383-397
Published by: Missouri Botanical Garden Press
Stable URL: <http://www.jstor.org/stable/2399889>
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SUBFAMILIAL AND TRIBAL RELATIONSHIPS IN THE RUBIACEAE BASED ON *rbcl* SEQUENCE DATA¹

Birgitta Bremer²,
Katarina Andreassen², and
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ABSTRACT

A parsimony analysis of *rbcl* sequences from 49 Rubiaceae genera (representing 23 tribes) and 7 outgroup taxa was performed. Species representing 48 genera of Rubiaceae were sequenced: *Anthospermum*, *Antirhea*, *Bertiera*, *Bowardia*, *Calycophyllum*, *Catesbaea*, *Cephalanthus*, *Cinchona*, *Coffea*, *Cubanola*, *Deppea*, *Enterospermum*, *Erithalis*, *Exostema*, *Gardenia*, *Guettarda*, *Haldina*, *Hallea*, *Hamelia*, *Hillia*, *Hintonia*, *Hoffmannia*, *Hydnophytum*, *Ixora*, *Keetia*, *Luculia*, *Meyna*, *Mitriostigma*, *Morinda*, *Mussaenda*, *Nauclea*, *Nertera*, *Oldenlandia*, *Ophiorrhiza*, *Parapentas*, *Pentagonia*, *Pentas*, *Pentodon*, *Pinckneya*, *Pogonopus*, *Psychotria*, *Rachicallis*, *Rogiera*, *Rubia*, *Sarcocephalus*, *Theligonum*, *Uncaria*, and *Vangueria*. The cladistic analysis resulted in 24 equally parsimonious trees with a consistency index (C.I.) of 0.38. The results were analyzed to test higher-level classification and reconstruction of Rubiaceae phylogeny, and to place taxa with disputed taxonomic positions. There are three groups of taxa more or less corresponding to three of Robbrecht's four subfamilies: Cinchonoideae s. str., Ixoroideae s.l., and Rubioideae. There is no support for the subfamily Antirheoideae, the taxa of which are nested within Cinchonoideae s. str. and Ixoroideae s.l. The positions of *Luculia* and *Hintonia* are uncertain. The tribal positions (sensu Robbrecht) are supported for a majority of the genera, but other indicated relationships contradict earlier classification. The data, although based on a limited number of taxa, support the monophyly of the tribes Anthospermeae, Chiococceae s.l., Guettardeae, Hamelieae, Hedyotideae, Naucleaeae s.l., Psychotrieae, and Vanguerieae, but there is no support for the positions and/or narrow circumscriptions of Cephalantheae and subtribe Mitragyninae (Cinchoneae), or for a wide circumscription of Cinchoneae. The *rbcl* data also give useful suggestions for taxonomic positions of the following genera with uncertain affiliations: *Bertiera*, *Catesbaea*, *Deppea*, *Hillia*, *Ophiorrhiza*, *Rachicallis*, and *Theligonum*.

The Rubiaceae are one of the most speciose families, especially in the tropics, with about 10,000 species (Mabberley, 1990). Biologically and morphologically they are diverse, with many different life forms and reproductive traits. The life forms vary from tiny herbs, epiphytes, lianas, and shrubs to tall trees, and the various kinds of flowers have different pollination systems, where pollen is spread directly from the stamens by insects, birds, or wind or secondarily from the upper part of the styles (stylar pollen presentation) by insects. There is a great variety of fruits and seeds dispersed by different agents, e.g., dry capsules with wind-dispersed seeds, dry dehiscent or indehiscent mericarps, or fleshy and animal-dispersed berries or drupes. The different fruit traits have been much used for higher-level classification. The Rubiaceae are known to be a family with difficult intrafamilial classification. A persistent problem with the different classification schemes proposed has been that

one or a few characters have been used as absolute markers for the different taxonomic groups, and this has led to unnatural groupings in many cases.

Schumann (1891) divided the Rubiaceae into two subfamilies, Cinchonoideae and Coffeoidae, based on a single character, the number of ovules per locule. This character and Schumann's classification were almost totally rejected by later authors (Table 1 presents a comparison between tribal and subfamilial classification schemes historically used). Bremekamp (1954, 1966) instead emphasized testa structure, occurrence of albumin in the seeds, raphides, and the "ixoroid pollen presentation mechanism" (Ixoroideae), and he recognized eight subfamilies. Three of these subfamilies, the Cinchonoideae, the Rubioideae, and the Guettardoideae (= Antirheoideae), were accepted by Verdcourt (1958). He utilized the same characters as Bremekamp, but rejected the pollen presentation mechanism as a subfamilial character; instead, he

¹ We are grateful for many kinds of help and support; for providing plant material: Y. B. Linhart, L. McDade, T. McDowell, E. Robbrecht, R. Sanders, O. Seberg, and the directors of the botanical gardens listed in Table 2; for comments on the manuscript: K. Bremer, C. Taylor, and one anonymous reviewer; and for technical assistance: P. Jalonen and J. Rönholm. This research was supported by Swedish Natural Science Research Council grant B-BU 1487 to BB.

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Table 1. Previously proposed tribal and subfamilial classification schemes of all analyzed Rubiaceae genera compared to the results of the *rbcl* phylogeny. The tribes are indicated with the first three letters of the tribal names: ANThospermeae, CATesbaeae, CEPhalantheae, CINchoneae, CHIococceae, COFfeeae, CONdamineae, GARdeniae, GUEttardeae, HAMeliae, HEDyotideae, HILLiae, HIPpotieae, ISERTieae, MORindeae, NAUcleeae, OPHiorrhizeae, PAVetteae, PSYchotrieae, RONdeletieae, RUBieae, THEligoneae, VANguerieae; the subfamilies are indicated with the first four letters of the subfamilial names: ANTIrheoideae, CINChonoideae, COFFeoideae, HILLioideae, IXO-Roideae, RUBIoideae, UROPhylloideae.

Genera	Schumann (1891)	Verdcourt (1958)	Bremekamp (1966)	Robbrecht (1988)	<i>rbcl</i> -support*
<i>Anthospermum</i>	ANT-COFF	ANT-RUBI	= ^b	=	=
<i>Antirhea</i>	GUE-COFF	GUE-ANTI ^c	=	=	GUE-CINC*
<i>Bertiera</i>	GAR-CINC	ISE ^d -RUBI	?-UROP	?	?COF-IXOR*
<i>Bouvardia</i>	CIN-CINC	HED-RUBI	=	? ^e	HED-RUBI
<i>Calycophyllum</i>	CIN-CINC	=	—	=	?-IXOR s.l.*
<i>Catesbaea</i>	GAR-CINC	CAT-CINC	—	CAT-?	CHI-CINC*
<i>Cephalanthus</i>	NAU-CINC	=	?	CEP-ANTI	NAU-CINC
<i>Chiococca</i>	CHI-COFF	CHI-CINC	CHI-IXOR ^f	CHI-ANTI	CHI-CINC
<i>Cinchona</i>	CIN-CINC	=	=	=	=
<i>Coffea</i>	PAV ^g -CINC	PAV ^g -CINC	—	COF-IXOR	=
<i>Cubanola</i>	CON-CINC ^h	CON-CINC ^h	—	CON-CINC	CHI-CINC
<i>Deppea</i>	RON-CINC	?HED-RUBI	?	HAM-RUBI	HAM-CINC*
<i>Enterospermum</i>	GAR-CINC	PAV ⁱ -CINC	GAR-IXOR	PAV-IXOR ^j	GAR-IXOR
<i>Erihalis</i>	CHI-COFF	—	—	CHI-ANTI	CHI-CINC*
<i>Exostema</i>	CIN-CINC	—	—	=	CHI-CINC*
<i>Gardenia</i>	GAR-CINC	=	GAR-IXOR	=	=
<i>Guettarda</i>	GUE-COFF	GUE-ANTI ^c	=	=	GUE-CINC*
<i>Haldina</i>	NAU-CINC	= ^j	CIN-CINC ^l	NAU-CINC	NAU-CINC
<i>Hallea</i>	NAU-CINC ^k	= ^k	CIN-CINC ^l	= ^k	NAU-CINC
<i>Hamelia</i>	GAR-CINC	HAM-RUBI	=	=	HAM-CINC*
<i>Hillia</i>	CIN-CINC	?-RUBI	HIL-HILL	HIL-CINC	=
<i>Hintonia</i>	—	—	—	?	?
<i>Hoffmannia</i>	GAR-CINC	HAM-RUBI	=	=	HAM-CINC*
<i>Hydnophytum</i>	PSY-COFF	PSY-RUBI	—	=	=
<i>Ixora</i>	PAV ^g -COFF	PAV ^g -CINC	—	PAV-IXOR	?-IXOR
<i>Keetia</i>	VAN-COFF ^f	VAN-CINC ^l	—	VAN-ANTI	VAN-IXOR*
<i>Luculia</i>	CIN-CINC	—	—	=	?
<i>Meyna</i>	VAN-COFF ^m	VAN-CINC ⁿ	VAN-IXOR ^{m,n}	VAN-ANTI	VAN-IXOR
<i>Mitriostigma</i>	—	—	—	GAR-IXOR	?-IXOR
<i>Morinda</i>	MOR-COFF	MOR-RUBI	= ^o	=	=
<i>Mussaenda</i>	ISE ^d -CINC	=	=	=	ISE-IXOR s.l.*
<i>Nauclera</i>	NAU-CINC	=	=	=	=
<i>Nertera</i>	ANT-COFF	ANT-RUBI	—	=	=
<i>Oldenlandia</i>	HED ^p -CINC	HED-RUBI	=	=	=
<i>Ophiorrhiza</i>	HED ^q -CINC	OPH-RUBI	OPH-UROP	OPH-RUBI	=
<i>Parapentas</i>	—	HED-RUBI	—	=	=
<i>Pentagonia</i>	ISE ^d -CINC	—	?	HIP-?	HIP-IXOR s.l.*
<i>Pentas</i>	HED ^p -CINC	HED-RUBI	—	=	=
<i>Pentodon</i>	HED ^p -CINC	HED-RUBI	—	=	=
<i>Pinckneya</i>	CON-CINC	=	—	=	?-IXOR s.l.*
<i>Pogonopus</i>	CON-CINC	—	—	=	?-IXOR s.l.*
<i>Psychotria</i>	PSY-COFF	PSY-RUBI	=	=	=
<i>Rachicallis</i>	RON-CINC	—	—	HED-RUBI ^r	?-CINC
<i>Rogiera</i>	RON-CINC ^s	= ^r	= ^{r,s}	= ^r	=
<i>Rubia</i>	RUB-COFF	RUB-RUBI	—	=	=
<i>Sarcocephalus</i>	NAU-CINC	NAU-CINC ^t	= ^u	=	=
<i>Theligonum</i>	—	—	—	THE-RUBI	=
<i>Uncaria</i>	NAU-CINC	=	CIN-CINC	=	NAU-CINC
<i>Vangueria</i>	VAN-COFF	VAN-CINC	VAN-IXOR ^v	VAN-ANTI	VAN-IXOR

emphasized external hair types. In the latest classification (Robbrecht, 1988), the Rubiaceae are divided into four subfamilies: Cinchonoideae, Ixoroideae, Rubioideae, and Antirheoideae (Table 1). Of these, the last subfamily differs distinctly from that of the earlier classifications, and includes more tribes than in Verdcourt's (1958) and Bremekamp's (1966) classifications. For example, in Antirheoideae Robbrecht included not only the tribe Guettardeae but also tribes formerly ascribed to Cinchonoideae (sensu Verdcourt, 1958; Cephalantheae, Retinophylleae, Alberteae), Ixoroideae (sensu Bremekamp, 1966; Chiococceae, Vanguerieae), and Rubioideae (sensu Bremekamp, 1966; Knoxieae, Craterospermeae). Most characters for Antirheoideae are variable, but generally taxa have solitary seeds with large embryos.

The use of single key characters in classification is usually very risky (e.g., the use of ovule number per locule, which formed the primary basis for the earlier classifications of the family) and will cause problems if the characters are in conflict; the tribe Vanguerieae has an ixoroid pollen presentation mechanism and fits well into Bremekamp's Ixoroideae, but also has solitary seeds with large embryos that make it a suitable member of Robbrecht's Antirheoideae. Similar problems have also emerged for particular genera, e.g., *Bertiera*, *Catesbaea*, *Deppea*, *Hillia*, *Ophiorrhiza*, *Penta-*

gonia, *Rachicallis*, and *Theligonum*, which are known to be difficult to classify due to lack or contradiction of certain characters. In such a large family as Rubiaceae it is easy to imagine, and it has also been shown (Eriksson & Bremer, 1991; Bremer & Eriksson, 1992), that characters have evolved and/or been lost repeatedly during evolution. Hence, we cannot use absolute markers, or "cardinal characters," for circumscription of taxa. We must consider all characters, not least the molecular, and in the most parsimonious way attempt to identify monophyletic groups that can be circumscribed as taxonomic entities. Currently we do not have the information from all genera of the family to perform a complete analysis of the family based on "all characters," but we can test and search for informative characters that later on can be combined in a large parsimony analysis.

In the past couple of years the ribulose-1,5-bisphosphate carboxylase (*rbcL*) gene of the chloroplast genome has been much used for analyses of angiosperm phylogeny (e.g., Chase et al., 1993; Olmstead et al., 1993), and it has previously been shown to be very useful for family-level analysis (Doebley et al., 1990; Soltis et al., 1990; Conti et al., 1993). The strength of molecular data is that they are independent of morphological characters, and we can discover relationships that might be hidden if only morphological characters are inves-

←

^a Tribal and subfamilial placement supported by the *rbcL* data and all classifications in the different schemes supported by the *rbcL* data are in boldface.

^b "—" Denotes the same tribe and subfamily as previous author that has considered the particular genus.

^c "*" Indicates placement supported by *rbcL* data but not proposed in Schumann (1891), Verdcourt (1958), Bremekamp (1966), or Robbrecht (1988).

^d "—" Denotes a genus (or a commonly used synonym) not considered by the author, "?" denotes uncertain position according to the author.

^e Under the synonym Guettardoideae.

^f Under the synonym Mussaendeae.

^g Uncertain position CIN-CINC or HED-RUBI.

^h The genus *Chiococca* (Meyna) is not mentioned but the tribe Chiococceae is.

ⁱ Under the synonym Ixoreae.

^j Earlier included in the genus *Portlandia*.

^k Included in the genus *Tarenna*.

^l Earlier included in the treated genus *Adina*.

^m Earlier included in the genus *Mitragyna*.

ⁿ Earlier included in the genus *Canthium*.

^o Earlier included in the genera *Vangueria* or *Canthium*.

^p The genus *Vangueria* (*Meyna*) is not mentioned but the tribe Vanguerieae is.

^q The genus *Morinda* is not mentioned but the tribe Morindeae is.

^r Under the synonym Oldenlandieae.

^s In index and classification indicated as HED but in text on p. 39 as RON.

^t Earlier included in the genus *Rondeletia*.

^u The genus *Rondeletia* (*Rogiera*) is not mentioned but the tribe Rondeletieae is.

^v Under the synonym Galieae.

^w Earlier included in the genus *Nauclea*.

Table 2. Sources of living material extracted for DNA. Species names are followed by a four- or three-letter suffix. In the outgroup taxa the suffix indicates the first four letters of the family names: APOCynaceae, GENTianaceae, and OLEAceae according to Mabberley (1990); GELSemiacae, SPIGeliaceae, STRYchnaceae sensu Struwe et al. (1995). The listed subfamilies of Rubiaceae follow Robbrecht (1988), as do the tribal positions (indicated with a three-letter suffix; cf. Table 1) for most taxa; however, * indicates tribal positions according to Bremer (1992).

Species	Source/voucher information*	New <i>rbcL</i> sequences reported in this paper will appear in the EMBL database under the accession number
Outgroup Taxa		
<i>Anthocleista grandiflora</i> GENT	Olmstead et al., 1993	
<i>Exacum affine</i> GENT	Olmstead et al., 1992	
<i>Kopsia fruticosa</i> APOC	Olmstead et al., 1993	
<i>Ligustrum vulgare</i> OLEA	Olmstead et al., 1992	
<i>Mostuea brunonis</i> GELS	Olmstead et al., 1993	
<i>Spigelia marilandica</i> SPIG	Donoghue et al., 1992	
<i>Strychnos nux-vomica</i> STRY	Olmstead et al., 1993	
Rubiaceae		
Subfamily Antirheoideae		
<i>Antirhea lucida</i> GUE	Bremer & Jansen, 1991	x83624
<i>Cephalanthus occidentalis</i> CEP	Bremer & Jansen, 1991	x83629
<i>Chiococca alba</i> CHI	Olmstead et al., 1993	
<i>Hintonia latiflora</i> CHI* ^b	Bremer & Jansen, 1991	x83643
<i>Cubanola dominguensis</i> CHI*	JBSD, McDowell 4472 (Duke)	x83632
<i>Erithalis fruticosa</i> CHI	Bremer & Jansen, 1991	x83635
<i>Exostema caribaeum</i> CHI*	Bremer & Jansen, 1991	x83636
<i>Guettarda uruguensis</i> GUE	Bremer & Jansen, 1991	x83638
<i>Keetia zanzibarica</i> VAN	Tanzania, Bremer 3069 (UPS)	x83647
<i>Meyna tetraphylla</i> VAN	Tanzania, Bremer 3074 (UPS)	x83649
<i>Vangueria madagascariensis</i> VAN	Bremer & Jansen, 1991	x83670
Subfamily Cinchonoideae		
<i>Calycophyllum candidissimum</i> CIN	Bremer & Jansen, 1991	x83627
<i>Cinchona succirubra</i> CIN	Bremer & Jansen, 1991	x83630
<i>Haldina cordifolia</i> NAU	Bremer & Jansen, 1991	x83639
<i>Hallea (Mitragyna) rubrostipulata</i> CIN	BR 83-1132, Robbrecht s.n. (UPS)	x83640
<i>Hillia triflora</i> HIL ^c	COLO, Bremer 3101 (UPS)	x83642
<i>Luculia grandifolia</i> CIN	Bremer & Jansen, 1991	x83648
<i>Mussaenda erythrophylla</i> ISE	Bremer & Jansen, 1991	x83652
<i>Nauclea orientalis</i> NAU	C, Bremer 3001 (UPS)	x83653
<i>Pinckneya pubens</i> CON	Bremer & Jansen, 1991	x83661
<i>Pogonopus speciosus</i> CON	Bremer & Jansen, 1991	x83662
<i>Rogiera suffrutescens</i> RON	Bremer & Jansen, 1991	x83665
<i>Sarcocephalus latifolius</i> NAU	P, Bremer 2726 (UPS)	x83667
<i>Uncaria rhynophylla</i> CIN	UPS, no voucher	x83669
Subfamily Ixoroideae		
<i>Coffea arabica</i> COF	Bremer & Jansen, 1991	x83631
<i>Enterospermum coriaceum</i> PAV	Bremer & Jansen, 1991	x83634
<i>Gardenia thunbergia</i> GAR	Bremer & Jansen, 1991	x83637
<i>Ixora coccinea</i> PAV	CONN, Birgitta Bremer 2719 (UPS)	x83646
<i>Mitriostigma axillare</i> GAR	Bremer & Jansen, 1991	x83650
Subfamily Rubioideae		
<i>Anthospermum herbaceum</i> ANT	Tanzania, Bremer 3093 (UPS)	x83623
<i>Deppea grandiflora</i> HAM	P, Bremer 2724 (UPS)	x83633
<i>Hamelia cuprea</i> HAM	Bremer & Jansen, 1991	x83641

Table 2. Continued.

Species	Source/voucher information ^a	New <i>rbcl</i> sequences reported in this paper will appear in the EMBL database under the accession number
<i>Hoffmannia refulgens</i> × <i>ghiesbreghtii</i> HAM	Bremer & Jansen, 1991	x83644
<i>Hydnophytum formicarum</i> PSY	Bremer & Jansen, 1991	x83645
<i>Morinda citrifolia</i> MOR	UPS, Bremer 3106 (UPS)	x83651
<i>Nertera granadensis</i> ANT	Bremer & Jansen, 1991	x83654
<i>Oldenlandia cf. corymbosa</i> HED	Tanzania, Bremer 3075 (UPS)	x83655
<i>Ophiorrhiza mungos</i> OPH	BR71-1493; Robbrecht s.n. (UPS)	x83656
<i>Parapentas silvatica</i> HED	Tanzania, Bremer 3091 (UPS)	x83657
<i>Pentas lanceolata</i> HED	Bremer & Jansen, 1991	x83659
<i>Pentodon pentandrus</i> HED	Tanzania, Bremer 3082 (UPS)	x83660
<i>Psychotria kirkii (bacteriophila)</i> PSY	Bremer & Jansen, 1991	x83663
<i>Rachicallis americana</i> HED	FTG 64-266, Fanning KF81	x83664
<i>Rubia tinctorum</i> RUB	UPS, Bremer 3300 (UPS)	x83666
<i>Theligonum cynocrambe</i> THE	UPS, Struwe 1004 (UPS)	x83668
Incertae sedis		
<i>Bouvardia glaberrima</i> CIN/HED	Bremer & Jansen, 1991	x83626
<i>Bertiera breviflora</i> INC	WAG (Cameroun), Setten 713 (WAG)	x83625
<i>Catesbaea spinosa</i> CAT	Bremer & Jansen, 1991	x83628
<i>Pentagonia macrophylla</i> HIP	DUKE, McDade 595A	x83658

^a Reference to literature if the specimen has been used before. For the other taxa the source of the living material is first indicated followed by the collector of the voucher and the herbarium in which it is deposited: BR, C, COLO, CONN, DUKE, FTG, JBSD, P, UPS, WAG.

^b Charlotte Taylor has kindly informed us that this species erroneously was indicated as *Coutarea latiflora* in Bremer & Jansen (1991).

^c The investigated species *Hillia triflora* was described as a *Ravnia* but in a recent revision (Taylor, 1989) that genus is now considered to be a subgenus within *Hillia*.

tigated. In this study we have sequenced the *rbcl* gene of 48 different Rubiaceae species from genera representing 23 tribes and 4 subfamilies. The main aim was to see if the nucleotide variation in the *rbcl* gene was enough to answer the following: (1) Is *rbcl* a useful character set for phylogenetic analysis within the Rubiaceae? (2) Can we use the *rbcl* results to test higher-level classification in the family? To what extent is the result contradicting or corresponding to the classification, or to the earlier phylogenetic analyses? (3) Are analyses of *rbcl* sequences useful to pinpoint taxonomic positions of disputed genera (e.g., *Bertiera*, *Catesbaea*, *Depeea*, *Hillia*, *Ophiorrhiza*, *Pentagonia*, *Rachicallis*, and *Theligonum*)?

MATERIAL AND METHODS

The *rbcl* gene has been analyzed from 56 species representing 7 outgroup genera and 49 Rubiaceae genera. Of these, 48 sequences are new and the sequences are accessioned in the EMBL

database as x83623–x83670 (Table 2). The remaining eight sequences included in the analyses are from Genbank (Table 2). The sampling of Rubiaceae was done to cover as many tribes as possible and represents 23 tribes and 4 subfamilies (according to the classification of Robbrecht, 1988).

Total DNA was extracted (Saghai-Marouf et al., 1984; Doyle & Doyle, 1987) from fresh or silica gel dried (Chase & Hills, 1991) leaves. Double-stranded DNA of the *rbcl* gene was amplified by PCR with two synthetic primers (Olmstead et al., 1992): the 5' primer was identical to the first 26 nucleotides of *rbcl* of tobacco, and the 3' primer corresponds to a region ca. 100 nucleotides downstream from the coding region. This covers the entire coding gene excluding the first 26 nucleotides. A second run with asymmetric amplification was performed to get single-stranded DNA (Kaltenboeck et al., 1992). Single-stranded DNA was sequenced using internal primers designed by G. Zurawski (DNAX Research Institute).

The data matrices in the phylogenetic analyses

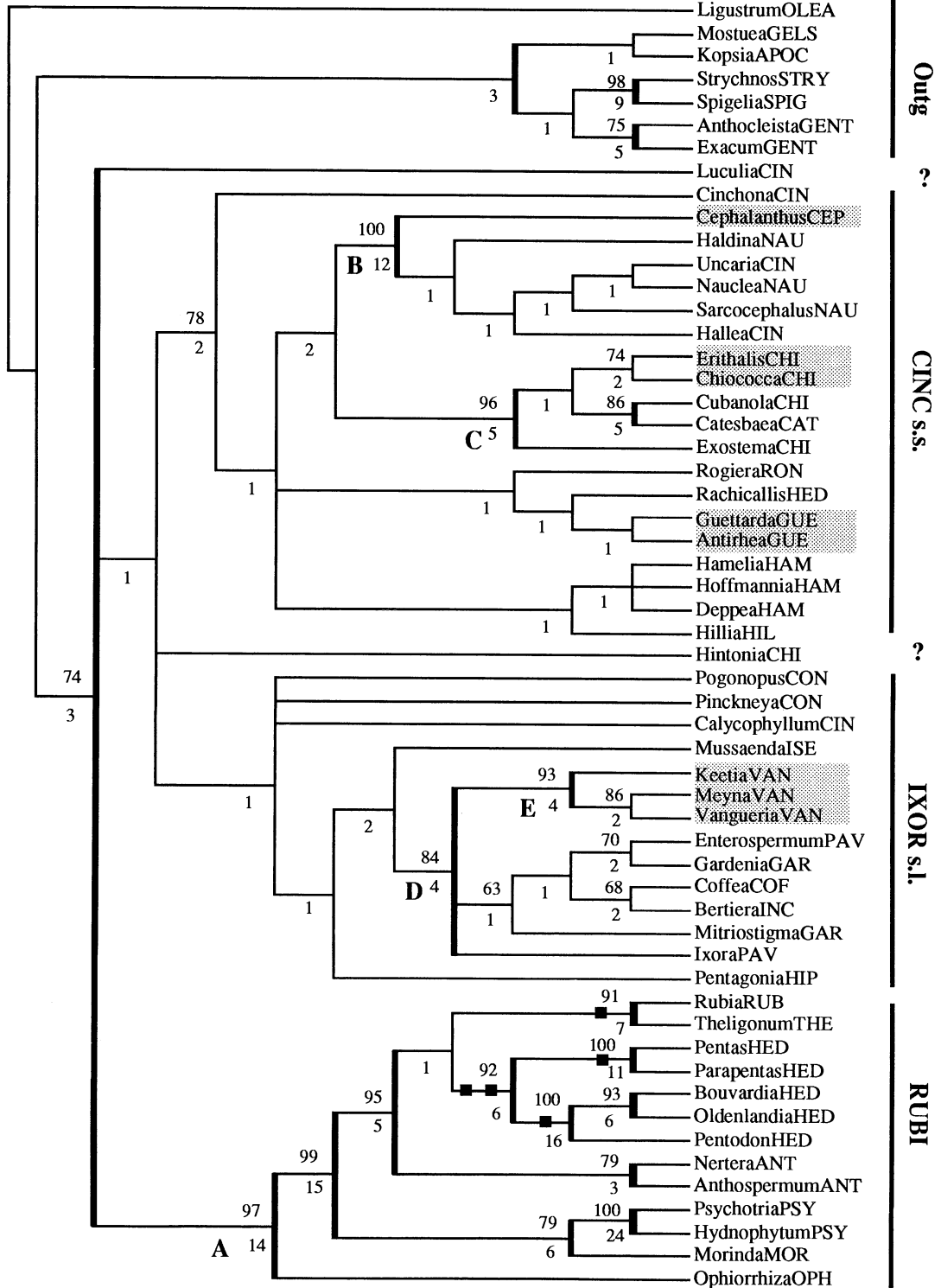


Figure 1. Strict consensus tree of 24 equally parsimonious trees of Rubiaceae based on *rbcL* sequences. The rooting of the tree was accomplished by making the “outgroup paraphyletic with respect to the ingroup” (an option in PAUP). Families are indicated by a four-letter suffix corresponding to the families in Table 2, and tribal positions are indicated by a three-letter suffix corresponding to the tribes in Table 2. Vertical bars and corresponding letters

comprise characters corresponding to each nucleotide position (27 to 1428) of the *rbcl* sequence. Only phylogenetically informative characters (295 positions) were analyzed. Parsimony analyses were conducted using PAUP version 3.1.1 (Swofford, 1991) on a MacQuadra840, under the assumptions of Fitch parsimony (Fitch, 1971). We searched with heuristic methods, with random addition of sequences and 100 replications, and TBR branch swapping. To evaluate the branch support (Fig. 1) we have performed a Bremer support analysis (Bremer, 1988; Källersjö et al., 1992), as well as a bootstrap analysis with 1000 replicates (Felsenstein, 1985).

The outgroups were chosen in agreement with results from earlier investigations; six taxa were chosen from other families of the Gentianales (*Kopsia*—Apocynaceae, *Mostuea*—Gelsemiaceae, *Strychnos*—Strychnaceae, *Spigelia*—Spigeliaceae, and *Anthocleista* and *Exacum*—Gentianaceae) and one taxon *Ligustrum* (Oleaceae) was selected from outside this supposed monophyletic order. The position of Rubiaceae in the order Gentianales was first suggested by Uetzschneider (1947) and later established by Wagenitz (1959, 1964). This position is accepted by most systematists dealing with higher-level classification (Dahlgren, 1980; Thorne, 1983, 1992; Takhtajan, 1987) and is also supported by molecular and morphological data (Downie & Palmer, 1992; Bremer & Struwe, 1992; Chase et al., 1993; Olmstead et al., 1993; Bremer et al., 1994; Struwe et al., in press).

RESULTS

In the analysis (Figs. 1 and 2), of the 56 taxa 456 nucleotide positions were variable and of these 295 were phylogenetically informative. Among the variable characters 109 (24%) are first positions, 83 (18%) are second positions, and 264 (58%) are third positions. Of all the nucleotide substitutions only a few resulted in synapomorphic and non-homoplastic changes in amino acid composition. These five unique changes in amino acids occur in subfamily Rubioideae. *Rubia* and *Theligonum* share a change from 2Serine to Glycine (nucleotides 28–

30). All taxa of the Hedyotideae share the changes from Threonine to Asparagine (nucleotides 736–738), and the change from Leucine to Methionine (nucleotides 1111–1113). Within the Hedyotideae *Pentas* and *Parapentas* share a change from Valine to 2Serine (nucleotides 469–471). The last unique change occurs in nucleotide positions 1315–1317; these positions are very variable but *Bouvardia*, *Oldenlandia*, and *Pentodon* are the only taxa with 1Serine in these positions.

The heuristic search with 100 random additions of sequences including only phylogenetically informative characters resulted in 24 equally parsimonious trees 1033 steps long (395 minimal possible steps) with a C.I. of 0.38 and a retention index (R.I.) of 0.66.

All the equally parsimonious trees are very similar concerning positions of most taxa. The Rubiaceae are monophyletic and the sister group to the rest of the Gentianales (Figs. 1 and 2). In all the 24 trees (Fig. 1 = the strict consensus tree of these 24 trees) there are three large clades (CINC s.s., IXOR s.l., and RUBI, which can be seen in the strict consensus tree). These groups of taxa more or less correspond to three of the four subfamilies of the Rubiaceae (Robbrecht, 1982, 1988), namely the Cinchonoideae, the Ixoroideae, and the Rubioideae, although the circumscriptions differ from Robbrecht's assessment. The fourth subfamily, Antirheoideae, receives no support from this analysis. Furthermore, there are two genera (*Luculia* of the tribe Cinchoneae and *Hintonia* of uncertain position) whose positions differ distinctly in the different trees. *Luculia* is positioned at the base of the whole CINC s.s./IXOR s.l. clade or at the base of the RUBI clade, and *Hintonia* is positioned at the base of the IXOR s.l. clade or at the base of the CINC s.s. clade. The most stable, or most supported, part of the trees corresponds to the subfamily Rubioideae (RUBI, Fig. 1: A) where almost all nodes remain in trees two steps longer (Fig. 1; indicated with heavy bars). Other well-supported clades include the tribe Naucleaeae s.l. (Fig. 1: B), the Chiococceae s.l., including *Cubanol*/*Catesbaea* (Fig. 1: C), Ixoroideae s. str./Van-

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represent: Outg = outgroups, CINC s.s. = subfamily Cinchonoideae s. str., IXOR s.l. = subfamily Ixoroideae s.l., RUBI = subfamily Rubioideae. Question marks indicate taxa with uncertain position. Shaded genera are members of Robbrecht's (1988) subfamily Antirheoideae. Numbers below branches indicate number of extra steps required to collapse the particular node. Nodes with heavy bars require more than 2 extra steps to collapse. Numbers above nodes indicate bootstrap fractions above 60%. Black squares indicate synapomorphic and non-homoplastic changes in amino acid composition (C.I. = 1.0). Clade A = subfamily Rubioideae, clade B = tribe Naucleaeae s.l., clade C = tribe Chiococceae s.l., clade D = Ixoroideae s. str./Vanguerieae, clade E = tribe Vanguerieae.

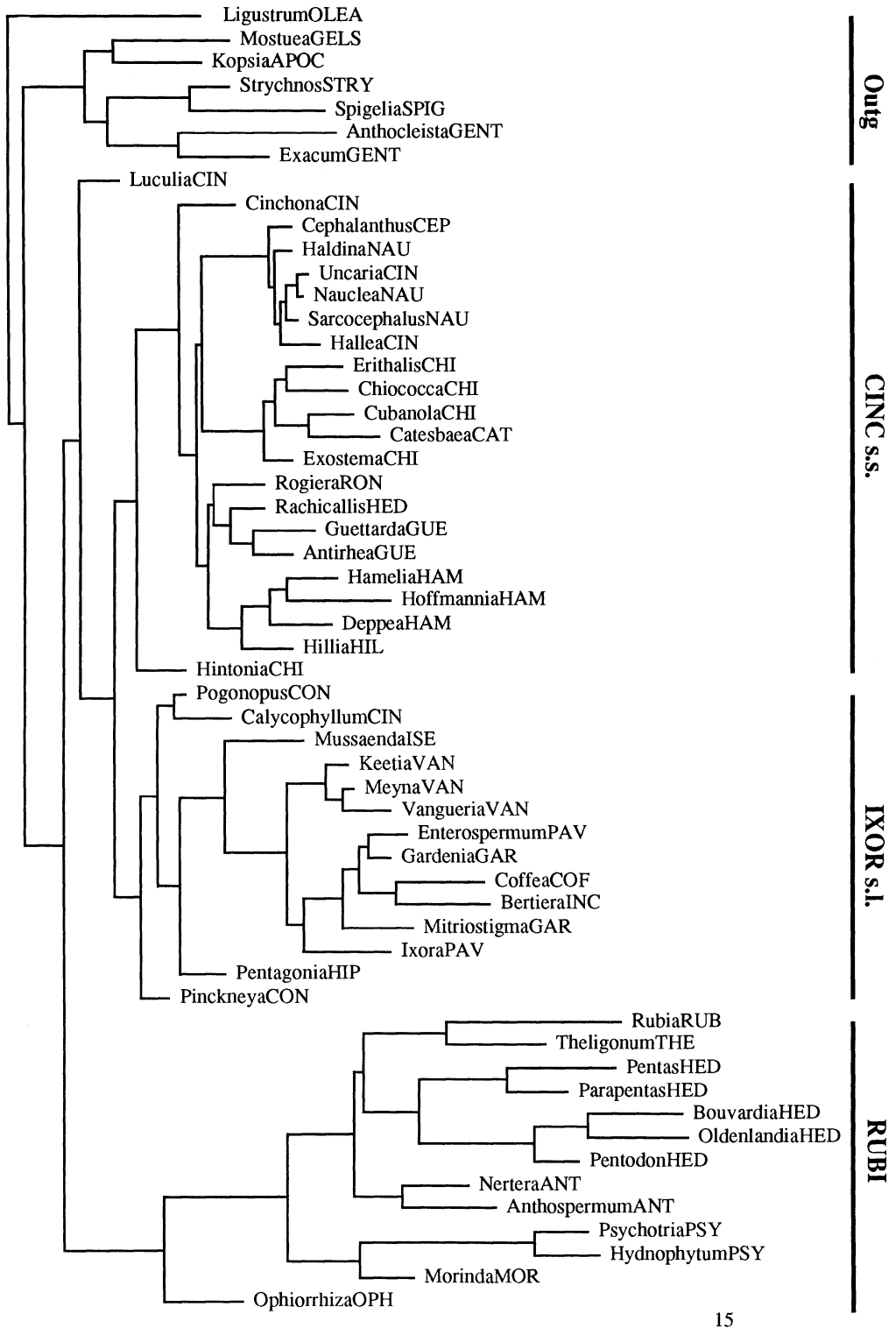


Figure 2. Sample phylogram, one of 24 equally parsimonious trees of Rubiaceae based on *rbcl* sequences, 1033 steps long with a C.I. of 0.38 and an R.I. of 0.66 (only phylogenetically informative characters are included). The rooting of the tree was accomplished by making the “outgroup paraphyletic with respect to the ingroup” (an option

guerieae (Fig. 1: D), and the Vanguerieae (Fig. 1: E).

The Rubioideae (RUBI) clade is supported by 18 characters (Fig. 2) and is one of the strongest in the analysis; the tree topology for this group is the same in all trees. *Ophiorrhiza* is the most basal taxon, followed by the Psychotriaceae/Morindeae clade, which is the sister group to the remaining Rubioideae: the Anthospermeae, Hedyotideae, Theligoneae, and Rubieae. All taxa of the Rubioideae clade belong to the subfamily Rubioideae (sensu Robbrecht, 1988).

The IXOR s.l. clade includes all taxa of the Ixoroideae s. str. (Robbrecht, 1988), namely the members of the tribes Gardenieae, Pavetteae, and Coffeae, but also the Vanguerieae (subfamily Antirhoeideae, sensu Robbrecht) and some genera usually included in the Cinchonoideae: *Mussaenda* (Iserteae), *Calycophyllum* (Cinchoneae), and *Pinckneya* and *Pogonopus* (Condamineae). The Vanguerieae/Ixoroideae s. str. clade (Fig. 1: D) is well supported and remains in trees four steps longer than the most parsimonious trees.

The CINC s.s. clade corresponds to parts of the Cinchonoideae, namely the tribes Cinchoneae s. str., Naucleae s.l., Chiococceae s.l., Rondeletieae, Hillieae, but also Hamelieae, which usually is placed in the Rubioideae (Verdcourt, 1958; Bremekamp, 1966; Robbrecht, 1988), and the Guettardeae, which used to be accepted as, or a part of, the subfamily Antirhoeideae (Verdcourt, 1958; Bremekamp, 1966; Robbrecht, 1988).

Of the taxa with disputed taxonomic positions, *Theligonum* is in this analysis the sister taxon to *Rubia* within the Rubioideae, *Ophiorrhiza* is sister taxon to all other Rubioideae, *Bertiera* is nested within Ixoroideae s. str., *Pentagonia* is more basal in that same clade, *Hillia* is close to Hamelieae in the Cinchonoideae, *Catesbaea* is close to *Cubanola* in the Chiococceae s. str. (Cinchonoideae), and *Rachicallis* belongs to Cinchonoideae.

DISCUSSION

The present analysis demonstrates that *rbcL* data are informative for phylogenetic studies within Rubiaceae. The resolution of the strict consensus tree is high, with most branches resolved (35 of

41 within Rubiaceae), and thus most taxa have the same position in all trees. Compared to *rbcL* analyses of other angiosperm families, e.g., Onagraceae (Conti et al., 1993) and Asteraceae (Kim et al., 1992), our analysis results in more trees (24 compared to 1 and 8, respectively) and a lower consistency index (0.38 compared to 0.63 and 0.47, respectively). However, in these studies many fewer taxa (12 and 28 compared to 56) were analyzed, and at least the consistency index is sensitive to the number of taxa (Sanderson & Donoghue, 1989). If we compare the present analysis with similar phylogenetic analyses of Rubiaceae, the resolution of the consensus trees and the consistency indices are about the same as in the restriction site analysis (Bremer & Jansen, 1991) and the morphological analyses (Bremer & Struwe, 1992). The stability in terms of branch support is high but varies between the different branches (Fig. 1).

The answer to the second question, whether we can use *rbcL* to test higher-level classification within Rubiaceae, is yes. With this restricted study, including only about 8% of all the genera of the family, we can test the alleged monophyly of 12 tribes, and also the relationships postulated between these. In a comparison with the latest classification of Rubiaceae (Robbrecht, 1988), we find that our data support the tribal classification for 65% of the genera, and for about 16% the results are contradictory (Table 1); for the remaining part the *rbcL* data are not informative due to incomplete sampling of taxa or homoplasy in data.

The phylogeny and the subfamilial classification are more problematic. The *rbcL* phylogeny shows three basal groups of taxa, more or less corresponding to three of the four subfamilies (Robbrecht, 1988), Cinchonoideae s. str., Ixoroideae s.l., and Rubioideae (Figs. 1 and 2). The fourth subfamily, Antirhoeideae, is scattered within the Cinchonoideae s. str. and Ixoroideae s.l. and thus is not supported. Of the three clades, the Rubioideae are the most stable part of the tree, supported by 18 characters, and all branches except one remain in trees three steps longer. The branch support for the other two basal clades (CINC s.s. and IXOR s.l.) is much weaker and for some investigated taxa (*Luculia* and *Hintonia*) the position is equivocal. However, within the two clades some

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in PAUP). Families are indicated by a four-letter suffix corresponding to the families in Table 2, and tribal positions are indicated by a three-letter suffix corresponding to the tribes in Table 2. Vertical bars and corresponding letters represent: Outg = outgroups, CINC s.s. = subfamily Cinchonoideae s. str., IXOR s.l. = subfamily Ixoroideae s.l., RUBI = subfamily Rubioideae. Branch lengths are proportional to the number of substitutions supporting a node; scale bar equals 15 substitutions.

nodes are stable and strengthen various relationships. The most stable nodes correspond to Naucleaeae s.l. (Fig. 1: B), Chiococceae s.l. (Fig. 1: C), Vanguerieae (Fig. 1: E), and Ixoroideae s. str./Vanguerieae (Fig. 1: D). It is obvious that the molecular data in many parts are congruent with earlier classifications or phylogenetic analyses, but in other parts there are contradictions. In the following we will discuss each of the major groups of the Rubiaceae supported by our analysis.

THE CINCHONOIDEAE S. STR. (CINC S.S.) CLADE (Figs. 1 and 2)

The Cinchonoideae s. str. clade includes a majority of the Cinchonoideae, but not the genera *Pogonopus*, *Pinckneya*, *Calycophyllum*, and *Mussaenda*. Nested within the Cinchonoideae clade are parts of Antirheoideae (Robbrecht, 1988), and thus Antirheoideae is polyphyletic. Some of Robbrecht's (1988) Cinchonoideae tribes are supported by our data. An interesting exception is that the tribe Cinchoneae sensu Robbrecht is not supported. We studied *Cinchona* and five other genera of Cinchoneae (*Calycophyllum*, *Exostema*, *Hallea*, *Luculia*, and *Nauclea*), but none of these five genera came out closely related to *Cinchona*, a result in agreement with a cladistic analysis of Cinchonoideae by Andersson & Persson (1991). Two of the genera we studied, *Uncaria* and *Hallea*, were moved to a tribe Coptosapelteae by Andersson & Persson (1991). However, there was no cladistic support for that grouping in their data, and their tribe is more an example of lumping all paleotropical taxa that earlier were part of the Cinchoneae. Our data definitely point to a position of *Uncaria* and *Hallea* within the tribe Naucleaeae, where they were placed earlier (Verdcourt, 1958). The position within the Naucleaeae is well supported by morphological data (e.g., capituliform inflorescences, and pollen presentation from the "soon-to-be-receptive stigmatic surface"; Imbert & Richards, 1993). *Exostema* belongs to the Chiococceae s.l. and *Calycophyllum* belongs to the Ixoroideae s.l. clade, a position also supported by restriction site data (Bremer & Jansen, 1991).

Hintonia has an uncertain position at the base of the Cinchonoideae clade or at the base of the Ixoroideae s.l. clade. *Luculia* has an even more uncertain position either at the base of Cinchonoideae s. str., or at the base of Cinchonoideae s. str./Ixoroideae s.l., or at the base of the Rubioideae. Based on restriction site data *Luculia* is placed at the base of the family (Bremer & Jansen, 1991);

however, if that tree is rerooted with the root in-between the Rubioideae and the rest of the family, the genus becomes closely related to *Cinchona* and *Rogiara*; based on morphological data the genus is placed at the base of the family (Bremer, 1992; Bremer & Struwe, 1992). We have at the moment no suggestions for a tribal position for *Luculia*. Andersson & Persson (1991) moved *Luculia*, together with other paleotropical taxa, to the Coptosapelteae, but according to our results *Luculia* is at least not close to *Uncaria* and *Hallea*.

The tribe Naucleaeae has in recent years been split into different tribes and subfamilies (Bremekamp, 1966; Ridsdale, 1975, 1976, 1978; Robbrecht, 1988), but our results support a broad circumscription of the tribe in accordance with Verdcourt's (1958) classification. *Hallea* (*Mitragyna*) and *Uncaria* are united into subtribe *Mitragyninae* of the Cinchoneae in Robbrecht's (1988) classification. Such a grouping is paraphyletic according to our results (Figs. 1 and 2). *Cephalanthus* is placed in a different subfamily (Antirheoideae) by Robbrecht (1988), but our analysis shows it to be very close to the other members of the Naucleaeae (Figs. 1 and 2). In the broad Naucleaeae (Fig. 1: B) all investigated taxa, namely *Cephalanthus*, *Haldina*, *Hallea*, *Sarcocephalus*, *Nauclea*, and *Uncaria*, have capituliform inflorescences and a secondary pollen presentation mechanism.

A few years ago it was shown that the tribe Chiococceae, based on a phylogenetic restriction site analysis (Bremer & Jansen, 1991), is closely related to *Exostema* and *Hintonia*. Later, Bremer (1992) presented morphological data to support a monophyletic unit with Chiococceae, *Exostema* and *Hintonia*, and the subtribe Portlandiinae of the Condamineae. Within this unit there was support for the monophyly of the Chiococceae s. str., e.g., fleshy fruits with compressed endocarps and one-seeded carpels, but no such support was found for the remaining taxa (subtribe Portlandiinae, *Exostema*, *Hintonia*), and the solution was to include these taxa in a widened Chiococceae. Our data do not contradict a wide Chiococceae, but the inclusion of *Hintonia* is not supported. Delprete has made a more thorough cladistic morphological analysis of the group (presented at the International Conference on the Systematics of the Rubiaceae, 4–6 Oct. 1993, in St. Louis), and he found support for the former subtribe Portlandiinae as the sister taxon to Chiococceae, and suggested it forms a separate tribe. If that is correct, both alternatives (a wide Chiococceae or two separate tribes) are

acceptable and the choice is a matter of taste. An additional result that confirms our earlier findings with morphological data (Bremer & Struwe, 1992) is the inclusion of *Catesbaea*.

The tribe Guettardeae, formerly referred to the subfamily Antirhoeidae, is in our analysis related to *Rachicallis* (Hedyotideae) and *Rogiera* (Rondeletieae), and there is no support for a wide circumscription of the subfamily Antirhoeidae (Fig. 1). The justification for Robbrecht's circumscription of Antirhoeidae seems to be "endosperm soft and oily: embryo frequently very large" (Robbrecht, 1988: 186), but according to our study these characters must have evolved several times in the family. If we force Robbrecht's classification of Antirhoeidae (including Vanguerieae, Guettardeae, Chiococceae, Cephalantheae), that grouping requires 50 extra steps (1033 + 50 steps) compared to the most parsimonious trees (1033 steps), and such a solution is thus not very plausible.

The tribe Hamelieae is usually included in the Rubioideae (Verdcourt, 1958; Bremekamp, 1966; Bremer, 1987; Robbrecht, 1988) due to the presence of raphides, a character used to define the subfamily Rubioideae. However, that position was contradicted by an earlier molecular analysis (Bremer & Jansen, 1991) and our data suggest a position within the subfamily Cinchonoideae. The earlier suggestion of Hamelieae as a sister taxon to Argostemmatae (Bremer, 1987) was based on the erroneous assumption that Hamelieae should be part of Rubioideae. Our data support the monophyly of Hamelieae including *Deppea* (Bremer, 1987), also accepted by Robbrecht (1988).

The present analysis also indicates that the neotropical genus *Hillia* is closely related to the Hamelieae and could as well be included in that tribe. It is a genus of often succulent epiphytic shrubs with an interesting taxonomic history. Earlier, *Hillia* was included in the tribe Cinchoneae of the Cinchonoideae (de Candolle, 1830; Schumann, 1891). The seeds of *Hillia* are unique in the family: they have a hair tuft at one end of the seeds, analogous to the hair tuft in many Apocynaceae. *Hillia* also has raphides and right-contorted corolla aestivation, characters that contradict a position in Cinchoneae. Because of these "cardinal characters" Bremekamp moved the genus to Rubioideae as a separate tribe Hillieae (Bremekamp, 1954), and later Verdcourt (1958) accepted the subfamilial position, although he was uncertain about its tribal position. A few years later Bremekamp (1966) even raised it to a new subfamily. In a discussion of testa structures of *Hillia*, in relation

to Cinchoneae and Naucleaeae, Robbrecht (1988) came to the conclusion that the genus should form a separate tribe within Cinchonoideae. Our results support a position within the Cinchonoideae s. str. clade close to the Hamelieae. This position is in agreement with many morphological characters. All members of the Hamelieae (Bremer, 1987) and *Hillia* have raphides; *Hillia* and the Hamelieae genera *Deppea* and *Omittemia* also have right-contorted aestivation, which is otherwise rare in the family (Robbrecht, 1988: 83). Bird pollination occurs in *Hillia* subg. *Ravnia* and in *Hamelia*, and succulence is common in *Hillia* and *Hoffmannia*.

THE IXOROIDEAE S.L. (IXOR S.L.) CLADE
(Figs. 1 and 2)

The core of this clade is the subfamily Ixoroideae (sensu Robbrecht) but also the tribe Vanguerieae. This close relationship is congruent with the restriction site data (Bremer & Jansen, 1991) but incongruent with an earlier morphological analysis (Bremer & Struwe, 1992). The position of Vanguerieae is controversial, since the morphological data support a relationship to Naucleaeae but the molecular data, and also a combination of these data (Bremer, unpublished), strongly support a close relationship to the Ixoroideae s. str. The reason for this incongruency is so far unclear. Within the Ixoroideae s. str. only five genera from three of the five tribes are studied; nevertheless, our data contradict the circumscriptions of the Pavetteae and Gardenieae, respectively. If many more taxa are analyzed, further problems with tribal delimitations appear (Andreasen & Bremer, unpublished). At the base of the Ixoroideae clade we find taxa representing tribes usually included in the Cinchonoideae: *Pogonopus* and *Pinckneya* (Condamineae), *Calycophyllum* (Cinchoneae), *Pentagonia* (Hippotideae, uncertain subfamily in Robbrecht, 1988), and *Mussaenda* (Iserteae). This association with the Ixoroideae was also shown in earlier phylogenetic analyses based on both restriction site data and morphology (*Pentagonia* was not included) (Bremer & Jansen, 1991; Bremer & Struwe, 1992). It is not possible to say from the present analysis how these taxa are related to each other and to the Ixoroideae; there are several equally parsimonious alternatives from this part of the tree, as indicated by the lack of resolution in the strict consensus tree, and the support for the various branches is low, so many more taxa have to be investigated.

THE RUBIOIDEAE CLADE
(Figs. 1 and 2)

The number of taxa used in this analysis is not representative of the entire subfamily Rubioideae (only seven of sixteen tribes are represented), but the phylogeny corresponds in most parts with the tribes of the Rubioideae (sensu Robbrecht, 1988). However, as previously mentioned, Hamelieae and the genus *Rachicallis* (Hedyotideae) are not parts of the clade, but rather members of the Cinchonoideae s. str. (see above). Robbrecht (1988), in an evaluation of Bremekamp's subfamilies, identified two major groups within Rubioideae: "(i) a number of tribes which have numerous ovules on each placenta (mainly the Hedyotideae, and associated to them: Ophiorrhizeae, Hamelieae, Argostemmateae, Coccocypseleae and Scradereae), and (ii) tribes with solitary erect ovules (numerous tribes, inter alia Psychotriaceae and associated tribes) . . . the Anthospermeae, Paederieae, Theligoneae, Spermaceae and Rubieae." This arrangement of taxa is not congruent with our results. Neither of the two groups is monophyletic according to our data. Within our Rubioideae we can identify two larger clades, one including taxa with few-seeded fleshy fruits (Psychotriaceae and Morindeae, but not Anthospermeae, Theligoneae, and Rubieae) and the other group mainly with dry fruits and either numerous (Hedyotideae) or few seeds (Anthospermeae, Rubieae, Theligoneae).

In our analysis (Figs. 1 and 2) *Ophiorrhiza*, a genus with many-seeded capsules, is basal in the tree and is the sister group to all the other Rubioideae. Bremekamp placed *Ophiorrhiza* in a different subfamily (Urophyloideae) because he thought it lacked raphides and also because the testa structures are different (very thick-walled cells vs. thin-walled in Rubioideae). The differences in testa structure can easily be understood if the genus is basal in the subfamily and if the ancestor had the same thick-walled testa cells as in many taxa of the Cinchonoideae.

The next split in our tree goes between the group of taxa with fleshy fruits and few seeds, Psychotriaceae and Morindeae, and the remaining taxa. The Psychotriaceae and Morindeae branch is supported by a bootstrap fraction of 79% (Fig. 2) and is in agreement with the classification and earlier phylogenetic ideas.

In the large branch including the rest of the subfamily, *Rubia* (Rubieae) and *Theligonum* (Theligoneae) are sister taxa and they together are the sister group to the tribe Hedyotideae. The Hedyotideae form a monophyletic unit, supported by two of the five unique amino acid changes and a

bootstrap fraction of 92%. However, in a more extensive analysis of the Rubioideae (Bremer, unpublished) the Hedyotideae become polyphyletic with members of the Spermaceae nested within the tribe. Within Hedyotideae there are two branches corresponding to the two morphologically identified groups earlier discussed by Bremer (1987). Both of these branches, *Pentas/Parapentas* and *Bouvardia/Oldenlandia/Pentodon*, respectively, are supported by unique amino acid changes and both have 100% bootstrap values. The close affinity between *Rubia* and *Theligonum* is also supported by a unique change of amino acids. *Theligonum* is an aberrant genus with mostly alternate leaves, whereas most Rubiaceae have opposite leaves, and a variable number of stamens (6–30) in the male flowers. Although formerly treated as a separate family, Theligonaceae, it was recently included in the Rubiaceae based on embryological characters (Wunderlich, 1971). In earlier classifications *Theligonum* has been associated with various parts of the angiosperms, e.g., as a separate family within Centrospermae (Eichler, 1878) or Myrtiflorae (Melchior, 1964), or it has been included in Urticaceae (Endlicher, 1836–1841) or Rosaceae (Reichenbach, 1837). Close to the Hedyotideae/Rubieae/Theligoneae in our analysis is the tribe Anthospermeae. This tribe is characterized by unisexual and wind-pollinated flowers; they are unusual characters in the family shared by *Theligonum*. These similarities prompted Wunderlich (1971) to propose a relationship of the Theligoneae to Anthospermeae, but she also pointed to affinities with other tribes of the subfamily Rubioideae, viz. Spermaceae and Galieae.

For positioning of taxa with uncertain or disputed taxonomic positions, the information provided by *rbcL* sequence data is very useful. In this investigation we included a few genera with uncertain positions. For most of these the positions were the same in all trees and could be positioned with high certainty. *Hillia* (Cinchonoideae) and *Theligonum* (Rubioideae) have already been discussed. Another taxon with disputed tribal position is *Catesbaea* (Catesbaeeae), a West Indian genus of thorny shrubs with single-flowered inflorescences and berries with fleshy to leathery pericarps. Due to its specific kind of fruit it was included in the Gardenieae by Schumann (1891), although Verdcourt (1958) kept it as a separate tribe close to the Gardenieae. Robbrecht & Puff (1986) later removed the tribe from the Gardenieae-related taxa and left it as a "tribus incertae" (Robbrecht, 1988). In our analysis *Catesbaea* becomes the sister taxon to *Cubanola* within the Chiococceae (where it should

be included) clade, a strongly supported branch with a bootstrap value of 96%. This result is congruent with an analysis based on morphology (Bremer & Struwe, 1992), but in conflict with the restriction site analysis, where it was placed on a separate branch close to but outside the Chiococceae s.l. (Bremer & Jansen, 1991).

Another member of Schumann's Gardenieae whose position has been disputed is the large tropical genus *Bertiera*. Verdcourt (1958) was doubtful but kept it close to the Gardenieae (Ixoreae = Pavetteae); Bremekamp (1966) removed it to the subfamily Urophylloideae; and Robbrecht & Puff (1986) excluded it from the Gardenieae-related taxa. The genus is probably not monophyletic (Kirkbride, 1979) but the African species we have investigated in the molecular analysis, *Bertiera breviflora*, is definitely a member of the subfamily Ixoroideae. This relationship is also supported by morphological characters, such as the ixoroid pollen presentation mechanism, left-contorted aestivation, and fleshy fruits.

The genus *Rachicallis* is also of controversial tribal position. It was included in Schumann's (1891) Rondeletieae, but was not mentioned by either Verdcourt (1958) or Bremekamp (1966). However, Robbrecht (1988) listed it under Hedyotideae, perhaps by mistake because in the text it is once mentioned under Rondeletieae (1988: 39). Our results point to a position close to Guettardeae and Rondeletieae. Rondeletieae, however, is not monophyletic and the tribe is in need of revision, as earlier proposed (Bremekamp, 1966).

Pentagonia is a Central and South American genus with large, sometimes pinnatifid-lobed leaves and fleshy fruits with numerous seeds. In Schumann's (1891) classification it was a part of the Mussaendeae (= Isertieae), but Bremekamp (1966) doubted the position and Kirkbride (1979) excluded it from the tribe. In Robbrecht's (1988) classification it was included in the Hippotieae and treated as a "tribus incertae." In our analysis it is placed at the base of the Ixoroideae clade next to the Isertieae (= *Mussaenda*). However, the branches in this part of the tree are weak, with little support, and we should not put much trust in relationships of these branches; perhaps a better sampling or information from another gene would help.

CONCLUSION

The Rubiaceae are monophyletic and are a sister group to the rest of the Gentianales. With this restricted analysis, representing about eight per-

cent of all genera, we have found that *rbcL* sequencing is very useful for phylogenetic analysis within the family. However, too few taxa have been sequenced to get a complete picture of the family phylogeny. The next goal for our project is to sequence a majority of all genera (so far 165 genera have been sequenced, Bremer & Andreassen, unpublished). Despite the limits in this restricted analysis we can say that the *rbcL* data can be used to test subfamilial and tribal classification in the Rubiaceae and in most parts our results support the present classification based on traditional morphological assessment (Robbrecht, 1988). There is support for the subfamilies Cinchonoideae, Ixoroideae, and Rubioideae, although differently circumscribed. Our data do not support a subfamily Antirtheoideae; rather, it is polyphyletic. There is strong support in our data for the following tribes: Nauceae s.l., Chiococceae s.l., Guettardeae, Hamelieae, Vanguerieae, Hedyotideae, Anthospermeae, and Psychotrieae, but we find no reason to maintain Cephalantheae and subtribe Mitragyninae of the Cinchoneae. We have also found *rbcL* analysis useful in pinpointing genera with uncertain positions, and our results have unequivocally indicated positions for *Bertiera*, *Catesbaea*, *Hillia*, *Ophiorrhiza*, *Rachicallis*, and *Theligonum*.

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