

A *trnL-F* CPDNA SEQUENCE STUDY OF THE CONDAMINEAE-RONDELETIEAE-SIPANEEAE COMPLEX WITH IMPLICATIONS ON THE PHYLOGENY OF THE RUBIACEAE¹

JOHAN H. E. ROVA,^{2,5} PIERO G. DELPRETE,³ LENNART ANDERSSON,²
AND VICTOR A. ALBERT⁴

²Botanical institute, Göteborg University, P.O. Box 461, SE-405 30 Göteborg, Sweden; ³The Lewis B. and Dorothy Cullman Program for Molecular Systematic Studies, and Institute of Systematic Botany, The New York Botanical Garden, Bronx, New York 10458-5126 USA; and ⁴Natural History Museums and Botanical Garden, University of Oslo, Sars' gate1, N-0562 Oslo, Norway

DNA sequences from the chloroplast *trnL-F* region of 154 Rubiaceae and 11 outgroup taxa were analyzed cladistically. An emphasis was placed on the tribes Rondeletieae, Sipaneeae, and Condamineae. Sipaneeae are not close to Rondeletieae and belong in the Ixoroideae. There is no support for a widely distributed Rondeletieae in a broad sense. Instead, Rondeletieae sensu stricto form an almost entirely Antillean clade. Support was found for the separation of *Arachnothryx*, *Rogiera*, *Roigella*, and *Suberanthus* from *Rondeletia*. The Guettardeae as well as *Gonzalagunia* are found close to a complex formed by *Arachnothryx*, *Javorkaea*, and *Rogiera*. Condamineae, in a strict sense, belongs in the Ixoroideae. A number of Rondeletieae genera should be transferred to Condamineae or other parts of Ixoroideae. Support is found for an emended tribe Naucleaeae, comprising several genera with spherical pseudanthia. For the first time, tribal or subfamilial affiliation based on molecular sequence data is suggested for *Allenanthus*, *Blepharidium*, *Chione*, *Coutaportia*, *Dolichodelphys*, *Mazaea*, *Neobertiera*, *Neoblakea*, *Phialanthus*, *Phyllacanthus*, *Phyllomelia*, *Schmidtottia*, and *Suberanthus*.

Key words: cladistics; Condamineae; Rondeletieae; Rubiaceae; Sipaneeae; systematics; *trnL* intron; *trnL-F* spacer.

Although Rubiaceae is one of the largest families of flowering plants, comprising ~650 genera and 13 000 species (P. G. Delprete, personal observation), macrosystematics within the family is still debated. One reason for this debate is the tremendous morphological diversity that is found in the family (Robbrecht, 1988), a condition that makes it difficult for the phylogenist to find homologous morphological characters for tribal and subfamilial level studies. In 1988, Robbrecht published the last mainly morphological overview of tribal and subfamilial delimitations and relationships in the Rubiaceae. He concluded that the tribes Condamineae, Rondeletieae, and Sipaneeae should, based on morphology, be regarded as very closely related tribes in subfamily Cinchonoideae.

Shortly after Robbrecht's comprehensive study was pub-

lished, the first molecular systematic studies of Rubiaceae began to appear (Bremer and Jansen, 1991; Bremer, 1992; Bremer and Struwe, 1992). Since then, molecular techniques have further contributed to the understanding of Rubiaceae systematics from species to family level. In some cases, molecular phylogenies have also reconsidered Robbrecht's apparently stable classification. For example, Robbrecht's (1988) subfamily Antirheoideae was shown to be paraphyletic (Bremer, Andreassen, and Olsson, 1995; Bremer and Thulin, 1998; Andersson and Rova, 1999), a position for the elusive genus *Strumpfia* was indicated (Bremer et al., 1999), support for a close relationship between Condamineae subtribe Portlandiinae and the tribes Catesbaeeae and Chiococceae (Bremer, 1992) was established, and a suggestion to divide the tribes Rondeletieae and Condamineae between subfamilies Cinchonoideae and Ixoroideae was made, with some representatives of Rondeletieae found to be closely related to Guettardeae in subfamily Cinchonoideae (e.g., Bremer, Andreassen, and Olsson, 1995; Andersson and Rova, 1999). Further, several molecular systematic studies have focused on particular parts of the family, such as subfamily Ixoroideae (e.g., Andreassen and Bremer, 1996, 2000; Andreassen, 1997; Andreassen, Baldwin, and Bremer, 1999), the *Alibertia* group (Persson, 2000), subfamily Rubioideae (e.g., Ehrendorfer, Manen, and Natali, 1994; Andersson and Rova, 1999; Bremer and Manen, 2000), the *Psychotria* complex (e.g., Andersson and Rova, 1999; Nepokroeff, Bremer, and Sytsma, 1999), and the tribe Rubieae (e.g., Manen, Natali, and Ehrendorfer, 1994; Manen and Natali, 1995; Natali, Manen, and Ehrendorfer, 1995; Natali et al., 1996). At the same time, several phylogenetic studies using morphological data were performed and showed that Catesbaeeae and Chiococceae were closely related and several Condamineae genera should be regarded as closely related to Rondeletieae (Delprete, 1996a).

¹ Manuscript received 2 February 2001; revision accepted 19 July 2001.

The authors thank the staff at the New York Botanical Garden, the Botanical Institute in Göteborg, and the American Museum of Natural History in New York; Magnus Lidén and Claes Persson for their comments on the data matrix; Attila Borhidi for help with identification of specimens; Mari Källersjö and James Farris for performing the Xac-run; and Per-Ola Karis, Henrik Lantz, and the anonymous reviewers for valuable comments on the manuscript. In addition to the herbaria listed at <http://ajbsupp.botany.org/> (Appendix 1), the following institutions, organizations and their staff are acknowledged for their kind help in accessing material for this study: ORSTOM in Cayenne, French Guyana; herbarium HUA, Fundación Inguedé, and Municipio de Coquí in Colombia; the Smithsonian Tropical Research Institute (STRI), IN-RENARE, and IRHE in Panama; and Instituto de Ecología y Sistemática and Jardín Botánico Nacional in Havana, Cuba. This work was supported by grants from the Lewis B. and Dorothy Cullman program for molecular research to VA, the Swedish Natural Science Research Council (NFR) to LA, and grants to JR from the Royal Swedish Academy of Sciences, Helge Ax:son Johnsons stiftelse, Stiftelsen Wilhelm och Martina Lundgrens vetenskapsfond, Kungliga och Hvitfeldska stipendieinrättningen, Stiftelsen Kulturfonden Botaniskas vänner, Knut och Alice Wallenbergs stiftelse, and Göteborg University. They are all gratefully acknowledged.

⁵ Author for reprint requests (e-mail: jorova@privat.utfors.se).

Through these studies it became apparent that the tribes Condamineae, Rondeletieae, and Sipaneeae do not form such a tight complex as suggested by Robbrecht (1988). Rather, circumscriptions and systematic positions of these tribes needed to be reconsidered in relation to the entire family. In other words, what Robbrecht regarded as a question of finding the circumscriptions of the three closely related tribes changed to the task of finding which genera of this "complex" are actually closely related, which are not, and where in the family these tribal fragments have their closest relatives. To answer these questions, we performed a DNA sequence analysis including as many genera as possible from Robbrecht's Condamineae-Rondeletieae-Sipaneeae complex (from which comparatively few representatives had been included in earlier molecular systematic studies) as well as selected genera from other tribes in the family. This study is based on a cladistic analysis of chloroplast *trnL-F* intron and spacer DNA sequences from 165 taxa (154 Rubiaceae and 11 outgroup taxa) and a comparison of these results with morphological evidence.

MATERIALS AND METHODS

Selection of taxa—When designing the data matrix, our ambition was to get at least one species sequenced from all tribes in the Rubiaceae, based on the Robbrecht (1993) classification. A special effort was also made to select representatives from as many genera as possible from the tribes Rondeletieae, Condamineae, Sipaneeae, Guettardeae, Calycophylleae, Chiococceae, and Catesbaeeae as circumscribed by Delprete (1996a). Our goal was initially to include, when possible, at least two species, including the type species, from each genus in these tribes. The *Rondeletia* complex (Delprete, 1999b) and *Guettarda* were considered to be of special interest because of their size and morphological diversity, and these genera were thus sampled more extensively. An attempt was also made to include as many taxa as possible from the "genera incertae sedis" and "tribus incertae sedis" listed by Robbrecht (1993). Outgroup taxa were sought in families considered close to the Rubiaceae, and two representatives from outside Gentianales (*Fraxinus* L. and *Jasminium* L. of the Oleaceae) were also selected (Bremer and Struwe, 1992; Struwe, Albert, and Bremer, 1994; Bremer, Andreasen, and Olsson, 1995; Bremer, 1996b; De Laet and Smets, 1996; Struwe et al., 1998). An effort was made to use voucher specimens identified by the authors or identified by specialists in the respective parts of the family and confirmed by the second author. Authors of taxa are given in the text only when mentioned for the first time and when not listed at <http://ajbsupp.botany.org/v89> (Appendix 1) or (Appendix 2). An historical summary of the tribal and subfamilial positions of the genera included in this study may be found at <http://ajbsupp.botany.org/v89> (Appendix 2).

Extraction, amplification, and sequencing—Total DNA was extracted from leaves or seeds of silica gel dried plant material (Chase and Hills, 1991 [although leaves were usually kept in silica gel for several months]) or from herbarium specimens. Occasionally, fresh material was used. Plant tissue was ground in 1.5–2 mL microcentrifuge tubes either in a FastPrep homogenizer (BIO 101, Vista, California, USA) or by hand with a plastic pestle. Liquid nitrogen was only used when fresh or nonbrittle material was ground manually. A cetyltrimethylammonium bromide (CTAB) buffer was used for lysis together with 2-mercaptoethanol, mainly following Doyle and Doyle (1987), except that PEG 4000 or PEG 6000 was included (1 g/100 mL buffer). Samples were spun immediately after lysis and cleaned with the GeneClean® III Kit (BIO 101) according to the manufacturer's instructions. The *trnL* (UAA) intron and the intergenic spacer between the *trnL* (UAA) 3' exon and the *trnF* (GAA) gene were chosen for the analysis because they have been shown to evolve more rapidly than a coding region such as *rbcL* (Gjelly and Taberlet, 1994). Polymerase chain reaction (PCR) amplifications were performed with the "c," "d," "e," and "f" primers of Taberlet et al. (1991). DNA extracts made from fresh or silica gel dried material was usually amplified successfully

using only the "c" and "f" primers. Extractions from herbarium material, in which DNA was more likely to be degraded, were generally amplified in two parts, i.e., with primer pairs "c" + "d" (amplifying the intron) and "e" + "f" (amplifying the spacer). When first PCR did not yield enough amplified product to perform sequencing, the PCR product was run on a low melting agarose gel, allowing the first PCR product to be cut out and used as template for a second PCR. Amplification products were purified with QIAquick™ PCR Purification Kit (QIAGEN GmbH, Hilden, Germany) or with GeneClean (BIO 101) according to the manufacturers' instructions. Sequencing was performed on an ABI Prism™ 377 DNA Sequencer (Perkin-Elmer Applied Biosystems, Foster City, California, USA) or on an ALFexpress™ DNA sequencer (Pharmacia Biotech AB, Uppsala, Sweden). Sequencing reactions were done with all four primers (same as for PCR) using the dRhodamine Terminator Cycle Sequencing Ready Reaction DNA Sequencing Kit with AmpliTaq DNA polymerase (Perkin-Elmer Applied Biosystems) when using the ABI 377 or Thermo Sequenase Fluorescent Labeled Primer Cycle Sequencing Kit with 7-deaza-dGTP (Amersham Life Science, Little Chalfont, UK) when sequencing on the ALFexpress. Reactions to be run on the ABI were cleaned with Sephadex G-50 Fine DNA Grade (Pharmacia Biotech) before loading. Sequences were evaluated using the ABI Prism 377 Collection package (Perkin-Elmer Applied Biosystems) or AM v3.02/ALFwin software (Pharmacia Biotech AB) on the ABI and the ALFexpress, respectively. Editing of the sequences was performed with Sequencher (Gene Codes Corporation, Ann Arbor, Michigan, USA).

Alignment and gap coding—Initial alignment of a few dozen taxa was done with the assembling features included in the Sequencher program. When more taxa were added, numerous or long deletions in some sequences made computer-calculated alignment unwieldy, and the alignment method was then changed to manual editing. When all taxa had been added to the matrix, a preliminary parsimony analysis was performed using PAUP version 3.1.1 (Swofford, 1993). Taxa were then rearranged according to the result of the analysis, alignment was modified, and a new run was made. This set of actions was repeated until a stable alignment was believed to be found. Alignment and gap coding followed mainly the criteria listed in Andersson and Rova (1999). An effort was made to identify repeated regions and arrange indels according to these. When a repeat in which base substitutions had occurred was identified, one of the copies was deleted from the matrix, and an appropriate IUPAC ambiguity code was inserted in the position where bases differed. As an attempt to avoid a capricious alignment, the alignment was evaluated and discussed by several persons before final analysis, and parts of the matrix where doubts existed were excluded. *Dolichodelphys* and *Pseudomussaenda* were only successfully sequenced with the "e" and "f" primers; thus, only the spacer region of these genera are included in the analysis. The *Randia* sequence was compiled from the intron sequence of *R. spinifex* and the spacer sequence of *R. aristeguietae* because of a large autapomorphic deletion in the spacer region of *R. spinifex*. In the sequence of *Bikkia* and one of the *Arachnothyrix leucophylla* sequences, the intron and the spacer are sequenced from two different collections. The *Fraxinus* sequence was obtained as two separate sequences (intron and spacer) from GenBank. All sequences are deposited in GenBank, and the final matrix of aligned sequences with indel codings used in the analysis is available at [http://ajbsupp.botany.org/\(matrix\)](http://ajbsupp.botany.org/(matrix)). Data on origin of plant material, vouchers, and GenBank accession numbers are given at <http://ajbsupp.botany.org/v89> (Appendix 1).

Cladistic procedures—In order to find a phylogenetic hypothesis, a jackknife search was performed using a test version of the program "Xac" (J. S. Farris, Swedish Museum of Natural History, Stockholm, personal communication); 1000 replicates were run, each of them with 20 random addition sequence replicates and branch swapping.

When jackknife support is discussed below, supports of 81–100% are considered "strong," those of 51–80% "weak," and those of 50% or less "non-support."

Morphology—The material available for the molecular analysis directed the selection of taxa for morphological studies. The material studied is listed

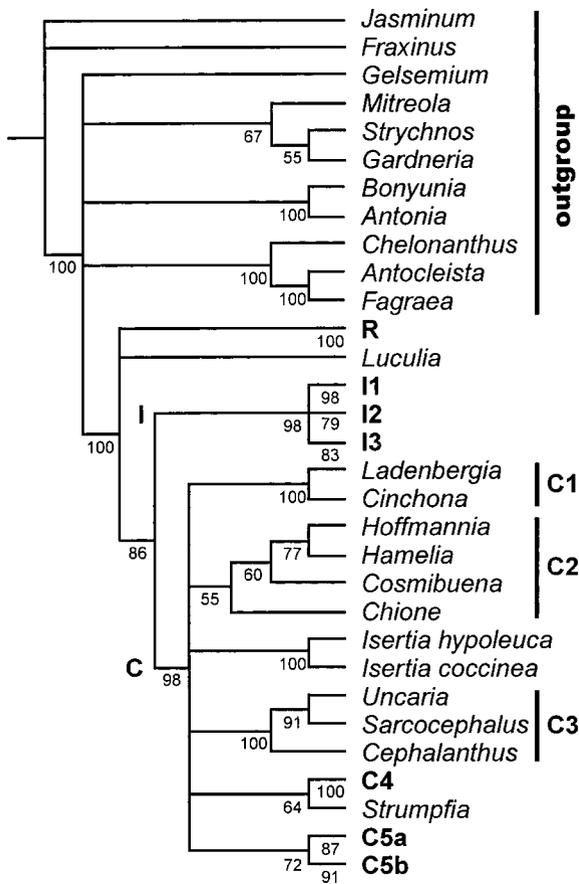


Fig. 1. Tree showing the main groups from the jackknife analysis of *trnL-F* sequence data. Numbers below branches indicate jackknife support. Major clades are shown as abbreviations: R denotes the Rubioidae clade; I1, I2, and I3 the three Ixoroideae (I) clades; and C1 to C4 and C5a–b the different Cinchonoideae (C) clades as discussed in the text.

at <http://ajbsupp.botany.org/v89> (Appendix 1). Macromorphology was studied through direct observation of herbarium material, pickled specimens, and/or fresh material. Pollen was acetolyzed and studied with the aid of scanning electron microscopy (SEM), using the same methods as those described by Andersson (1993a). Observations on the sculpturing of the inside of exotesta cells were made with the aid of SEM as described by Rova and Andersson (1995). When material was unavailable, literature was consulted to complement the morphological observations.

RESULTS

The final matrix comprised 165 taxa and 1045 characters, of which 480 were informative. The number of coded informative insertions/deletions was 67. Trees and jackknife values resulting from the cladistic search are presented in Figs. 1–7 and discussed below.

DISCUSSION

Gentianales and the subfamilies of Rubiaceae (Fig. 1)—Tree topologies among the Gentianales is mainly the same as found in the extensively sampled *trnL* intron study of Struwe et al. (1998) and will not be further discussed here.

Within Rubiaceae, three main clades (R, I, and C) are found in this analysis, just as in other molecular systematic studies (e.g., Bremer, Andreassen, and Olsson, 1995; Andersson and

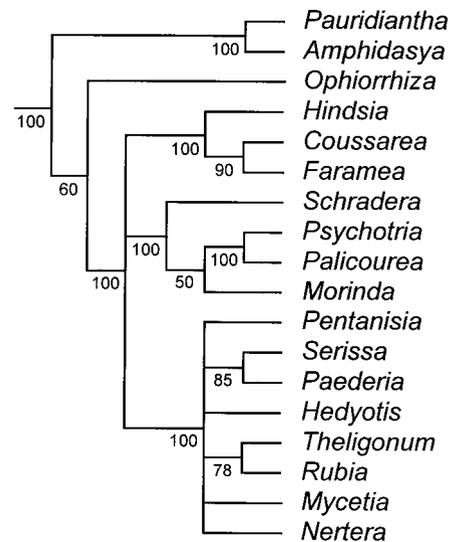


Fig. 2. The Rubioidae clade (corresponds to clade R in Fig. 1). Numbers below branches indicate jackknife support.

Rova, 1999; Bremer et al., 1999). These clades are usually compared to three of the four subfamilies presented by Robbrecht (1988, 1993). The first clade (clade R in Fig. 1) corresponds to the subfamily Rubioidae, the second clade (I) to a recircumscribed and significantly widened “Ixoroideae,” and the third clade (C) to the subfamily “Cinchonoideae” sensu stricto (s.s.).

The subfamily Antirheoideae, as recognized by Robbrecht (1988), has no support in the present analysis, nor in any other of the molecular analyses cited above. Most representatives of the tribe Guettardeae (including *Antirhea*) are found in the vicinity of *Arachnothryx* and its related genera (clade C5a). Further, Chiococceae and Catesbaeae (as defined by Delprete, 1996a) form a monophyletic group (clade C4). According to this study, *Cephalanthus* (Cephalantheae) is placed in the Naucleaeae (clade C3), as also shown by Bremer and Jansen (1991). Further, this study confirms that *Retiniphyllum* (Retiniphyllaeae) and *Vangueria* (represented by *Vangueria*, *Keetia*, and *Psydrax*) are nested within Ixoroideae (clade I2), as previously shown by Bremer, Andreassen, and Olsson (1995) and Andersson and Rova (1999). Based on *rbcl* sequences, Bremer (1996b) placed *Pentanisia* (Knoxieae) in Rubioidae (clade R), and *trnL-F* data confirms this here.

The basal, and elusive, position of *Luculia* is consistent with the results of other authors (e.g., Bremer, Andreassen, and Olsson, 1995; Bremer et al., 1999). Although there is no doubt that the genus belongs within Rubiaceae, it can not be unambiguously assigned to any subfamily based on *trnL* and *trnL-F* data. According to these results, *Luculia* might better be treated in a subfamily of its own.

The Rubioidae clade (Fig. 2)—The internal structure of the Rubioidae clade (clade R; Fig. 2) is by and large in accordance with other recent and more densely sampled DNA phylogenies of the Rubioidae, such as those of Manen and Natali (1996), Bremer and Thulin (1998), Andersson and Rova (1999), and Bremer and Manen (2000). The sparse sampling of Rubioidae taxa in the present study, together with a relatively high DNA sequence mutation rate, made alignment of Rubioidae sequences a difficult task, and because of this dif-

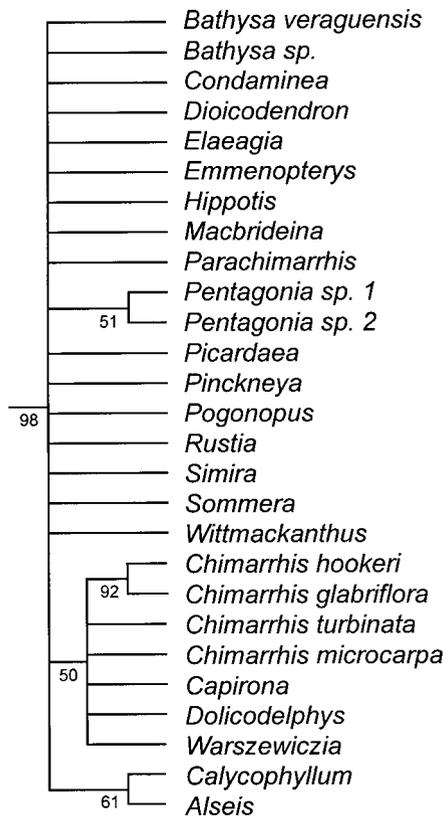


Fig. 3. The first Ixoroideae clade (corresponds to clade I1 in Fig. 1). Numbers below branches indicate jackknife support.

faculty, an insertion/deletion complex unique to the Rubioideae had to be removed from the data matrix before analysis.

The first Ixoroideae clade (Fig. 3)—The first of the subclades of the Ixoroideae in a wide sense, clade I1 (Fig. 3), comprises mainly genera that have traditionally been considered to be of Cinchonoideae affinity. Robbrecht (1993) referred the genera of the I1 clade to the following tribes (see also [http://ajbsupp.botany.org/\(Appendix 2\)](http://ajbsupp.botany.org/(Appendix 2))): *Warszewiczia*, *Bathysa*, *Macbrideina*, and *Elaeagia* to Rondeletieae; *Chimarrhis*, *Parachimarrhis*, *Rustia*, *Pogonopus*, *Picardaëa*, *Dioicodendron*, *Condaminea*, and *Pinckneya* to Condamineae; *Wittmackanthus*, *Calycophyllum*, and *Alseis* to Calycophylleae (as described by Andersson, 1993a, 1995); *Simira* to Simireae; *Hippotis*, *Sommeria*, and *Pentagonia* to Hippotideae; *Capirona* to Cinchoneae; and *Dolichodelphys* to Gardenieae. *Emmenopterys* had for a long time been treated as a member of the Cinchoneae (Schumann, 1897; implicitly by Bremekamp, 1966) but was transferred to the “genera incertae sedis” by Robbrecht (1993).

Many genera in clade I1 possess calycophylls, i.e., enlarged and usually brightly colored calyx lobes. According to Claßen-Bockhoff (1996), such “calyx-borne semaphylls” are present in at least some species of the clade I1 genera *Pogonopus*, *Pinckneya*, *Warszewiczia*, *Capirona*, *Calycophyllum*, *Wittmackanthus*, and *Schizocalyx* (synonymous with *Bathysa*; Delprete, 1997a). *Chimarrhis* and *Simira* also contain species possessing calycophylls (Delprete, 1996a, p. 176). In addition, *Parachimarrhis* is reported to have small calycophylls of peculiar morphology (Delprete, 1999b). Andersson (1995) pro-

posed an amendment of Calycophylleae to include *Alseis*, *Calycophyllum*, *Capirona*, *Ferdinandusa*, *Macrocnemum*, *Semaphyllanthus* (a segregate of *Calycophyllum*), and *Wittmackanthus*. This is not contradicted by the present analysis, although the last three genera are not included in this study.

Dolichodelphys was placed in Gardenieae by Robbrecht (1988, 1993), but it is reported to have capsular fruits (Delprete, 1996a, 1999b). Therefore, this genus is morphologically similar to the members of the I1 clade and Delprete (1996a, 1999b) accordingly included *Dolichodelphys* in his widely circumscribed Rondeletieae. There is a weak (50%) support in this study for a clade that comprises *Capirona*, *Chimarrhis*, *Dolichodelphys*, and *Warszewiczia* and a 61% support for an *Alseis-Calycophyllum* clade, but these two clades do not necessarily form a monophyletic group based on present data. Of all these genera, *Alseis* and *Dolichodelphys* are the only ones reported to lack calycophylls. It is worth noting that *Alseis*, *Calycophyllum*, *Chimarrhis* (Delprete, 1999b), and *Warszewiczia* all have receptive stigmas protruding above corolla lobes before the anthers are mature, which supports protogyny in these genera. Protogyny is rare in the Rubiaceae and never before reported outside Anthospermeae (Robbrecht, 1988, p. 120), so it is possible that this is a character that could be useful for the study of internal subdivisions in the I1 clade.

With further regard to calycophylls, Claßen-Bockhoff (1996) stated that they can also be found in *Carphalea* (shrubs/trees with two-seeded indehiscent fruits; Puff, 1988), *Cruckshanksia* (herbs/shrubs with few-seeded capsules; Taylor, 1996), *Morinda* (lianas or shrubs/trees with compound fruits composed of connate drupes; Johansson, 1994), *Neurocalyx* (subshrubs with many-seeded, dry, indehiscent fruits; Bremer, 1979), and *Pentas* (herbs/shrubs with many-seeded capsules; Verdcourt, 1976). All these genera have been shown to belong in Rubioideae (e.g., Andersson and Rova, 1999) and are not included in the present study. Claßen-Bockhoff (1996) also reported calycophylls in the Alberteae (*Alberta* and *Nematostylis*, shrubs/trees with 1–2-seeded, dry fruits; Puff, Robbrecht, and Randrianasolo, 1984), Jackieae (*Jackiopsis*, a tree with one-seeded nutlets; Ridsdale, 1979), the genera *Mussaenda* and *Pseudomussaenda* (discussed together with the I2 clade below), and the two genera *Pentaloncha* (of unknown tribal position; Robbrecht, 1993) and *Cosmocalyx* (of the tribe Hamelieae, with pterophylls that develop after anthesis; Delprete, 1998). Calycophylls are also present in *Kerianthera preclara* Kirkbr. of the Isertieae (Delprete, 1996b). It should also be noted that calycophylls are occasionally present in many other genera of the Rubiaceae, such as *Hamelia*, *Rondeletia*, *Pteridocalyx*, and *Sipanea* (P. G. Delprete, personal observation). In assessing the importance of this character, it is also important to remember that there is little or no evidence that all calycophylls are strictly homologous.

Resolution within the I1 clade is generally very poor, and, apparently, variation in *trnL* and *trnL-F* data is not enough to give reasonable resolution. Only the clade formed by *Chimarrhis glabriflora* Ducke and *Ch. hookeri* K. Schum. have support >90%. Thus, *trnL* and *trnL-F* data manage to unite neither the two *Bathysa* species nor the Hippotideae genera (*Hippotis*, *Sommeria*, and *Pentagonia*), although the latter have earlier (Rova and Andersson, 1995) been shown to form a morphologically distinct group.

The second Ixoroideae clade (Fig. 4)—The second Ixoroideae subclade (clade I2; Fig. 4) comprises mainly taxa of the

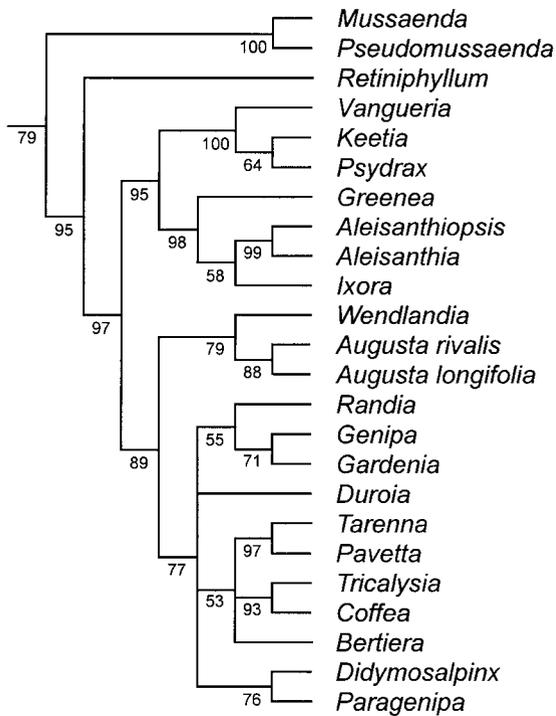


Fig. 4. The second Ixoroideae clade (corresponds to clade I2 in Fig. 1). Numbers below branches indicate jackknife support.

Ixoroids in the strict, traditional sense of Robbrecht (1993), i.e., Gardenieae (*Gardenia*, *Duroia*, *Genipa*, *Randia*, *Tricalysia*, *Bertiera*, and *Didymosalpinx*), Coffeeae (*Coffea*), Octotropideae (*Paragenipa*), and Pavetteae (*Ixora*, *Pavetta*, and *Tarenna*), together with a number of genera thought to belong to Isertieae (*Mussaenda* and *Pseudomussaenda*), Retiniphyllae (*Retiniphyllum*), Vanguerieae (*Keetia*, *Vangueria*, and *Psydrax*), and Rondeletieae (*Greenea*, *Aleisanthia*, *Aleisanthiopsis*, *Wendlandia*, and *Augusta*). The traditional Ixoroideae tribes do not form a monophyletic group. For instance, the Seychellois genus *Paragenipa* (Octotropideae) is found connected to *Didymosalpinx* as part of a basally unresolved Gardenieae-Coffeeae-Pavetteae clade. Furthermore, *Posoqueria* and *Dolichodelphys*, both included in the Gardenieae by Robbrecht (1993), are not closely associated with other Gardenieae. The exclusion of *Posoqueria* from Gardenieae has also been confirmed by morphological data (Persson, 1996). The topology of the clade corroborates results from several other studies (see below). A detailed discussion of subfamily Ixoroideae based on other molecular data can be found in Andreasen and Bremer (1996), Andreasen (1997), and Andreasen, Baldwin, and Bremer (1999).

Mussaenda* and *Pseudomussaenda—*Mussaenda* and *Pseudomussaenda* are found at the base of the I2 clade. These two genera have been considered by some authors to belong in two different tribes, Condamineae and Isertieae (see [http://ajbsupp.botany.org/\[Appendix 2\]](http://ajbsupp.botany.org/[Appendix 2])), because of their differences in fruit morphology (fleshy, indehiscent fruits vs. dry capsules). Along with *Vangueria* and *Psydrax*, *Mussaenda* and *Pseudomussaenda* are the only representatives with valvate corolla aestivation in the I2 clade. This study confirms the conclusions drawn from morphological data by Verdcourt (1958),

Puff, Igersheim, and Rohrhofer (1993), and Andersson (1996), that *Pseudomussaenda* should be treated as a close ally of *Mussaenda*. According to the present study, these genera should be considered of Ixoroideae rather than of Cinchonoidae affiliation, a conclusion also drawn in an *rbcL* study by Bremer and Thulin (1998). In that paper, Bremer and Thulin also reestablished the tribe Mussaendeae because Isertieae had to be circumscribed in a much stricter sense (*Isertia* and *Kerianthera*). Their Mussaendeae s.s. is in accordance with *trnL-F* data. However, Bremer and Thulin failed to find a stable position of Mussaendeae in the Ixoroideae. In the strict consensus tree of their study, Mussaendeae was found as sister group to a number of taxa equivalent to the I1 clade in the present study, but bootstrap support for this position was <50%. The present analysis shows that Mussaendeae is closer to *Ixora* and Gardenieae than they are to *Condaminea* and *Calycophyllum*. It is interesting to note the presence of calycophylls in *Mussaenda* and *Pseudomussaenda*, a feature which is otherwise fairly common (but not necessarily homologous) in the I1 clade.

Retiniphyllum* and *Vanguerieae—*Retiniphyllum* (tribe Retiniphyllae) is found in an isolated position between the Mussaendeae and the rest of clade I2. It is a neotropical genus with secondary pollen presentation and contorted corolla aestivation, and thus it fits morphologically well into the *Ixoroideae* s.s. (Robbrecht, 1988).

Vangueria is basal in a clade comprising *Vangueria*, *Keetia*, and *Psydrax*, all members of Vanguerieae (Robbrecht, 1988, 1993). Although stylar pollen presentation is present in the Vanguerieae, as is also predicted from its position in clade I2, it should be noted that corolla aestivation in this tribe is valvate, as opposed to most other genera in the I2 clade (Robbrecht, 1988). This close relationship between Vanguerieae and Ixoroideae s.s. has also been reported from restriction site mapping (Bremer and Jansen, 1991) and DNA sequence studies (e.g., Bremer, Andreasen, and Olsson, 1995; Andreasen, 1997).

Aleisanthia*, *Aleisanthiopsis*, *Greenea*, and *Ixora—*Aleisanthia* and *Aleisanthiopsis* are found together with *Ixora* in a clade also including *Greenea*, the four genera thus forming a sister clade to the *Vanguerieae* clade. This study supports the conclusions made by Tange (1996b) that *Greenea distantiflora* Merrill should be removed from *Greenea* and placed closer to *Aleisanthia*. Consequently, Tange also transferred *G. distantiflora* to the new genus *Aleisanthiopsis* at the same time as he suggested that this new genus might be the sister genus to *Aleisanthia*. All three genera (*Aleisanthia*, *Aleisanthiopsis*, and *Greenea*) are part of the Indo-Malayan flora (Mabberley, 1993; Tange, 1996a, b), while *Ixora* (if monophyletic) is a pantropical genus (Bridson and Robbrecht, 1985).

Aleisanthia, *Aleisanthiopsis* (as included in *Greenea*), and *Greenea* have traditionally been regarded as Rondeletieae due to their many-seeded, capsular fruits, in spite of their contorted corolla aestivation (Robbrecht, 1988, 1993; Tange, 1996b). The latter character, however, has been considered a rare feature in the Rondeletieae, and it has mostly been used taxonomically to separate these genera from the "valvate, rarely imbricate" Condamineae (Robbrecht, 1988). As mentioned above, of all taxa found in the I2 clade, most are reported to have contorted corolla aestivation. The only exceptions to this are the valvate genera *Mussaenda*, *Pseudomussaenda*, *Van-*

gueria, and *Psydrax*. This indicates that valvate corolla aestivation might be plesiomorphic at least in the I2 clade and possibly, because valvate corolla aestivation is the prevailing state in Rubioideae, also in the Rubiaceae as a whole. As will be further discussed below, imbricate corolla aestivation was also found to be predominant among taxa associated with the genus *Rondeletia*.

Aleisanthia, *Aleisanthiopsis*, and *Ixora* all have secondary pollen presentation, in which pollen is deposited onto the outside of the stigmatic lobes before they open and the stigmatic surface becomes receptive (Smith and Darwin, 1988; Tange, 1996a, b; De Block, 1998). In *Greenea*, on the other hand, secondary pollen presentation is lacking (Tange, 1996b). The fruit in *Ixora* is a slightly fleshy or coriaceous drupe, usually containing one (by abortion of one ovule) or two thin-walled pyrenes (Bridson, 1988; De Block, 1998), while both *Aleisanthia* and *Aleisanthiopsis* have dry, many-seeded capsules (Tange, 1996a, b).

Wendlandia* and *Augusta—*Wendlandia* and *Augusta* (sensu Kirkbride, 1997) have traditionally been placed in the *Rondeletieae* (see <http://ajbsupp.botany.org/v89> [Appendix 2]), but in this study they form a strongly supported sister group to the *Gardenieae*-*Pavetteae*-*Coffeae*-*Octotropideae* clade, i.e., they are well nested among the classical *Ixoroideae* Rubiaceae. *Wendlandia* is a genus of >50 species ranging from Southeast Asia to northeastern Africa (Cowan, 1932; Robbrecht, 1988; Tange, 1996b), while *Augusta* (including *Lindenia*) is a genus of four species of rheophytic shrubs growing in Brazil, Central America, the Fiji Islands, and New Caledonia (Delprete, 1997b; Kirkbride, 1997). In the present study, *Lindenia* (as *Augusta rivalis*) and *Augusta* are found together. This is in accordance with results from morphological data by Kirkbride (1997) and Delprete (1997b) and supports their common view to merge *Lindenia* into *Augusta*. *Wendlandia* and *Augusta* (including *Lindenia*) differ in corolla size (significantly larger in *Augusta* [Cowan, 1932; Delprete, 1997b]), capsule dehiscence (septicidal in *Augusta* vs. nearly always loculicidal in *Wendlandia* [Cowan, 1932; Darwin, 1976]), and seed characteristics (*Wendlandia* “obscurely winged” vs. non-alate with smooth testa in *Augusta* [Cowan, 1932; Darwin, 1976]), but both genera share fruit type (capsule), exerted stigma (Cowan, 1932; Darwin, 1976), and maybe most interestingly, corolla lobes contorted in bud (Schumann, 1891; Darwin, 1976; J. H. E. Rova, personal observation), a character not compatible with the taxa of the *Rondeletia* group as proposed in the present work.

Bertiera—The taxonomic position of the genus *Bertiera* has long been the cause of debate among Rubiaceae systematists (see Robbrecht, Rohrhofer, and Puff, 1994, for an historical discussion). In recent years, molecular phylogenies have all placed *Bertiera* in the *Ixoroideae* tribe *Coffeae* (Andreasen and Bremer, 1996; Persson, 1998). Although this study is not detailed enough to use for definite tribal affiliations in this part of the family, it clearly shows that *Bertiera* belongs near to *Coffeae* and *Pavetteae* s.s.

***Ixoroideae* in conclusion**—In conclusion, the I2-clade of this study is in general concordant with the results of Andreasen and Bremer (1996), Andreasen (1997), and Andersson and Rova (1999). Andreasen (1997), in her combined morphological and molecular analyses of the *Ixoroideae*, confirmed that

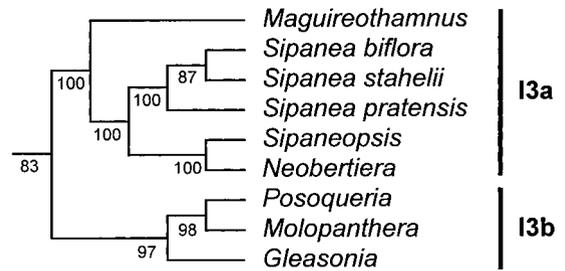


Fig. 5. The third *Ixoroideae* clade (corresponds to clade I3 in Fig. 1). Numbers below branches indicate jackknife support. I3a and I3b are names of subclades within the I3 clade, as discussed in the text.

the *Pavetteae* sensu Robbrecht is paraphyletic and should be divided into *Ixoreae* and *Pavetteae* s.s. However, none of the traditional *Rondeletieae* genera (*Greenea*, *Aleisanthia*, *Aleisanthiopsis*, *Wendlandia*, and *Augusta*), which in this study are shown to belong to the *Ixoroideae*, were included in the studies cited above. In order to clarify the tribal affiliations of these genera, an all-embracing phylogenetic study of the *Ixoroideae* s.s. should be undertaken.

***Sipaneae* (Fig. 5)**—The first of the I3 subclades, clade I3a in Fig. 5, corresponds to the tribe *Sipaneae* (the genera *Sipanea* and *Neobertiera*) supplemented by *Maguireothamnus* and *Sipaneopsis*, both placed in *Rondeletieae* by Robbrecht (1993). *Limnosipanea* (also *Sipaneae*) was not successfully sequenced and thus was not included in the study. *Sipaneopsis* is a genus of shrubs occurring in the Magdalena-Llanos area of Colombia, Venezuela, and the northern Amazon basin, while *Neobertiera* consists of a single species of shrubs restricted to the Guyana shield (Andersson, 1992). *Neobertiera* and *Sipaneopsis* form a clade with strong support, and based on general morphology, they may well be congeneric (J. H. E. Rova, personal observation). *Maguireothamnus*, a shrub of the tepuis of Venezuela (Steyermark, 1964), has thick leaves and a large corolla with a tube length of ~10 cm, and thus it is quite different from the small-flowered *Sipanea*. On the other hand, a number of similarities are found in pollen and exotesta morphology among *Sipanea*, *Maguireothamnus*, and *Neobertiera* (*Sipaneopsis* was not studied). First, all have an exotesta with sculptured inner tangential and radial walls, the sculpturing in form of more (*Limnosipanea*, *Sipanea*, and *Maguireothamnus*) or less (*Neobertiera*) prominent warts (J. H. E. Rova, personal observation). Second, pollen is foveolate (sensu Walker and Doyle, 1975) at least in some species of *Sipanea*, *Limnosipanea*, *Maguireothamnus*, and *Neobertiera* (*Sipaneopsis* not studied), with pits ~0.1–0.3 μm in diameter, although the density of tectal pits varies from as many as 4–9 per μm^2 (in *Limnosipanea*, *Sipanea*, and *Neobertiera*) down to 0.7 per μm^2 in *Maguireothamnus* (J. H. E. Rova, personal observation). Third, aestivation is contorted in all genera of the I3a clade except in *Sipaneopsis*, where both imbricate and contorted aestivation was found (J. H. E. Rova, personal observation).

Henriquezieae*, *Posoqueria*, and *Molopanthera—A group of morphologically specialized genera (clade I3b in Fig. 5) is found as sister clade to the *Sipaneae*. Taxa in this clade have been placed in various positions in the family: *Posoqueria* in *Gardenieae* (see above), the monotypic genus *Molopanthera* in the “*Portlandia*-group” of *Condamineae* (Robbrecht,

1993) or in Rondeletieae (Delprete, 1999b), and *Gleasonia* in Henriquezieae. Henriquezieae is a small tribe of three genera (*Gleasonia*, *Henriquezia*, and *Platycarpum*; Rogers, 1984) that, based on morphological characters (e.g., an inferior ovary that becomes superior or half-superior in fruit), have been treated as non-Rubiaceae by some authors (Verdcourt, 1958; Bremekamp, 1966; see also <http://ajbsupp.botany.org/v89> [Appendix 2]). Robbrecht (1993), following Rogers' (1984) treatment of the tribe, placed Henriquezieae in subfamily Cinchonoideae. The present study confirms that *Gleasonia* belongs to the Rubiaceae, but the tribe Henriquezieae (if monophyletic) is apparently of Ixoroideae rather than of Cinchonoideae affiliation. *Posoqueria* and *Gleasonia* have large and showy flowers (Hallé, 1967; Rogers, 1984), in stark contrast to *Molopanthera*, whose flowers are small with a very short corolla tube (Delprete, 1999b). All three genera have zygomorphic corollas (a few exceptions in *Gleasonia*; Rogers, 1984), a character valid also for the two other genera of Henriquezieae, and only reported from 25 rubiaceae genera (Robbrecht, 1988, p. 81, although note that *Molopanthera* and many others are missing in his list).

Both *Posoqueria* and *Molopanthera* share a pollen presentation mechanism, apparently unique in the Rubiaceae, in which pollen of a flower is formed into a ball that is hurled onto the visiting insect by one of the stamens (Müller, 1866; Schumann, 1891; Hallé, 1967; Robbrecht, 1988). The mechanism has been verified in *Posoqueria* in the field (Beach, 1983; P. G. Delprete, personal observation), and the similarity to the mechanism in *Molopanthera* has also been confirmed (P. G. Delprete, personal observation). One plausible explanation of the difference in corolla size (up to 100 times) between *Posoqueria* and *Molopanthera* could be a differentiation in pollinator specialization towards hawk-moths in *Posoqueria* and bees in *Molopanthera* (P. G. Delprete, personal observation) after the evolution of the staminal pollen presentation complex. Fruit size and morphology have also evolved differently in the two genera, with *Posoqueria* having fairly large leathery berries of *Gardenia* type and *Molopanthera* possessing minute loculicidal capsules.

Posoqueria has imbricate corolla aestivation, while other members of the Gardenieae have contorted aestivation, and the placement of *Posoqueria* in the Gardenieae has also lately been questioned (Persson, 1996; Andreassen, 1997, and references therein). Both *Posoqueria* (Persson, 1996) and the Henriquezieae, with the exception of *Gleasonia* (Rogers, 1984), have imbricate corollas, and therefore the systematic position of *Posoqueria* as indicated in this study is more plausible than the traditional view, that it should belong in the Gardenieae (Robbrecht, 1988, 1993). By considering *Gleasonia* aberrant and assuming that Rogers (1984) was correct when he stated the close relationship between *Gleasonia*, *Henriquezia*, and *Platycarpum*, the I3b clade can thus in general be described as having imbricate, zygomorphic corollas, and comprising the tribe Henriquezieae and a tentative new tribe comprising the genera *Posoqueria* and *Molopanthera*.

The Cinchonoideae clade—The six subclades of clade C (C1 to C5 and *Isertia* in Fig. 1) correspond to the subfamily Cinchonoideae in a more strict sense than that of Robbrecht (1988, 1993) but includes also representatives from several tribes that Robbrecht placed in other subfamilies: Hamelieae (Rubioidae), Cephalantheae, Guettardeae, Chiococceae (all three Antirheoideae), and Catesbaeae (of uncertain position).

The first Cinchonoideae subclade, clade C1, comprises *Lad-*

enbergia and *Cinchona*. Jackknife support for this clade is strong, and the clade corresponds to the tribe Cinchoneae in the restricted sense of Andersson (1995).

Clade C2 comprises *Hamelia*, *Hoffmannia*, *Cosmibuena*, and *Chione*. The former two clades have been placed in tribe Hamelieae of subfamily Rubioideae, while *Cosmibuena* is a Hillieae representative, and *Chione* is of uncertain tribal position (Robbrecht, 1988, 1993). A close relationship between Hamelieae and Hillieae has been proposed based on other chloroplast sequence data sets (Bremer, Andreassen, and Olsson, 1995; Andersson and Rova, 1999), although morphological analyses of comparable sample size (Rova and Andersson, 1995; Andersson, 1996) do not suggest that they may be closely related.

Isertiaeae—One of the Cinchonoideae clades is formed by two *Isertia* species. Of the traditional Isertiae (sensu Garcia Kirkbride, 1979) included in this study (*Amphidasya*, *Gonzalagunia*, *Isertia*, *Mussaenda*, *Mycetia*, and *Pseudomussaenda*), none is found to be associated with *Isertia*; instead, these genera are distributed among the clades R, I2, and C5a (Fig. 1). This study thus definitely supports the conclusions drawn by Andersson (1996) and Bremer and Thulin (1998), that Isertiaeae as traditionally circumscribed is polyphyletic.

Naucleaeae (Fig. 1)—The third Cinchonoideae clade, C3, includes the genera *Cephalanthus*, *Sarcocephalus*, and *Uncaria*. All these three genera are characterized by globose pseudanthia. *Cephalanthus* has historically been placed in various tribes with different circumscriptions (Ridsdale, 1976). A morphological comparison between *Cephalanthus* and Naucleaeae in a strict sense was performed by Ridsdale (1976). In this work, he excluded *Mitragyna* and *Uncaria* from Naucleaeae because of their type of fruit dehiscence and their pendulous, multi-ovulate placentas. He also stated that a pendulous, solitary ovule is a character common to *Cephalanthus* and Naucleaeae s.s. On the other hand, Ridsdale (1976) reported *Cephalanthus*, *Uncaria*, and *Mitragyna* to have a common phytochemistry and growth form. Robbrecht (1993) treated *Cephalanthus* in the monogeneric tribe Cephalantheae, *Sarcocephalus* as a member of Naucleaeae, and *Uncaria* as a member of the Cinchonoideae tribe Coptosapeltae. In the latter case, he followed morphological cladistic results of Andersson and Persson (1991). Results from the present study contradict both Ridsdale and Robbrecht and are instead consistent with the phylogenetic implications of Bremer, Andreassen, and Olsson (1995), based on *rbcL* sequence data, in which all three genera are considered to belong in the Naucleaeae. A close relation between *Cephalanthus* and Naucleaeae based on cytological data is also proposed by Kiehn (1995), who reports that the type of interphase nuclei is similar in *Cephalanthus* and Naucleaeae.

Strumpfia—The Antillean monotypic genus *Strumpfia* has been known for a long time as an elusive member of the Rubiaceae (<http://ajbsupp.botany.org/v89> [Appendix 2]). Albeit carefully studied (Igersheim, 1993), morphology has failed to indicate its proper affinities to other rubiaceae taxa, since *Strumpfia* possesses a combination of plesiomorphic characters and a number of more or less unique morphological features, such as protogyny, a "true" anther tube, and plurilocular pyrenes. Igersheim (1993) thus raised the question of whether putting *Strumpfia* in a tribe of its own was justifiable. Based

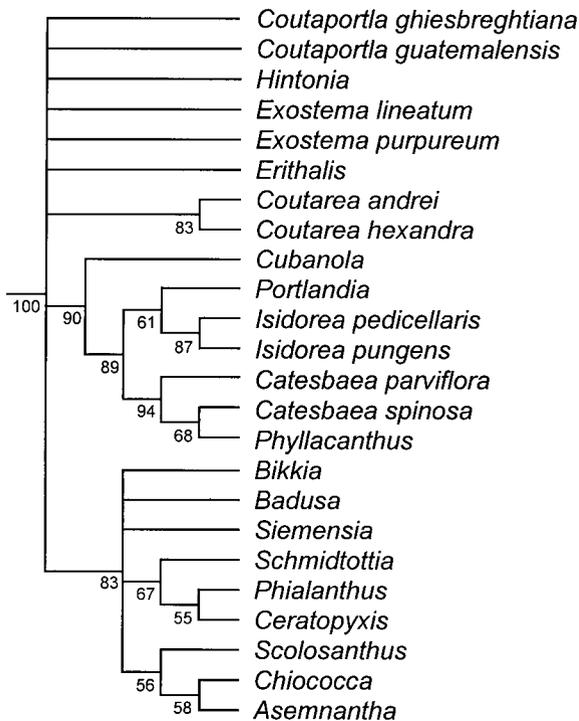


Fig. 6. The fourth Cinchonoideae clade (corresponds to clade C4 in Fig. 1). Numbers below branches indicate jackknife support.

on molecular data, Bremer et al. (1999) found *Strumpfia* to be “close to the Chiococceae s.l.,” but the only representatives from that clade that were included in their study were *Exostema*, *Chiococca*, and *Erithalis*. However, the present study confirms that *Strumpfia* holds an isolated position in the Cinchonoideae, and seemingly should be treated as sister to the C4 clade, although jackknife support for this is weak. Morphological evidence for this affiliation is moreover unclear; whereas members of the C4 clade, e.g., typically have echinate perforate pollen, *Strumpfia* pollen is verrucose (Igersheim, 1993).

Catesbaeae, Chiococceae, and the “Exostema-group” (Fig. 6)—Clade C4 (Fig. 6) comprises the genera *Asemnantha*, *Badusa*, *Bikkia*, *Catesbaea*, *Ceratopyxis*, *Chiococca*, *Coutarea*, *Cubanola*, *Erithalis*, *Exostema*, *Hintonia*, *Isidorea*, *Phialanthus*, *Phyllacanthus*, *Portlandia*, *Schmidtottia*, *Scolosanthus*, and *Siemensia*. These genera are characterized by imbricate corolla aestivation and echinate-perforate pollen (Delprete, 1996a; Huysmans et al., 1999; J. H. E. Rova, personal observation). The C4 clade thus corresponds to the tribes Catesbaeae, Chiococceae, and the “Exostema group” as circumscribed by Delprete (1996a), although this analysis does not support Delprete’s separation of the genera into three groups. However, *Siemensia*, *Asemnantha*, *Phialanthus*, and *Ceratopyxis* were not included in Delprete’s analysis, while *Nernstia*, *Thogsennia*, *Ceuthocarpus*, *Morierina*, and *Placocarpa* were not available for the present study. The recognition of any of Delprete’s groups with their present delimitations would make the other two groups polyphyletic. Instead, and especially if the “Exostema group” of Delprete is treated as part of his Catesbaeae, the C4 group of the present study is closer to the Chiococceae as broadly delimited by Bremer (1992) (but see

below concerning *Phialanthus*). Later, Bremer also suggested an inclusion of *Catesbaea* in the Chiococceae (Bremer, Andreasen, and Olsson, 1995), and this is also concordant with the present study. Kiehn (1995) noted that *Portlandia* and *Cubanola* could be closely related based on their similarities in chromosome morphology, while *Chiococca* differs in chromosome structure and is more similar to *Exostema*. The latter suggestion was based on chromosome appearance and shared unusual basic numbers, $x = 13$ or 14 (although $x = 11$ and $x = 12$ were also reported from *Chiococca* and *Exostema*, respectively). An affinity between *Portlandia* and *Cubanola* is supported by the present study, but the close relationship between *Exostema* and *Chiococca* proposed by Kiehn (1995) was not confirmed by *trnL-F* sequence data.

The monotypic *Phyllacanthus* is a highly specialized Cuban segregate of *Catesbaea*. It is now most probably extinct, as it has not been seen for half a century, even though specially designed expeditions have been sent out to find it (Delprete, 1996a; A. Borhidi, Janos Pannonius University, Pécs, personal communication). This study indicate that *Phyllacanthus* should be included in *Catesbaea*.

Three of the taxa in the C4 group were treated as “incertae sedis” by Robbrecht (1993), i.e., *Hintonia*, *Phialanthus*, and *Schmidtottia*. In Bremer, Andreasen, and Olsson (1995), the Mesoamerican genus *Hintonia* was found at a tricotomy between the Ixoroideae s.l. (sensu lato) and the Cinchonoideae s.s. The present study, as well as the studies of Delprete (1996a), Bremer (1996a), and Ochoterena-Booth (2000), indicate, however, that *Hintonia* is part of the Catesbaeae-Chiococceae complex. Bremer’s (1992) exclusion of the almost entirely West Indian genus *Phialanthus* from the Chiococceae is not supported by this study nor by Delprete’s (1996a); if Chiococceae is defined as broadly as by Bremer (1992), *Phialanthus* should definitely be included in this tribe.

Two genera are placed very differently in Delprete’s (1996a) and in the present analyses: *Chione* and *Allenanthus*. In Delprete’s study, they form a clade that is part of the Chiococceae, but in the present study, *Chione* is found to be basal in the C2 clade together with *Cosmibuena*, *Hamelia*, and *Hoffmannia*, while *Allenanthus* is part of the C5a clade together with *Neoblakea* and *Machaonia*. Delprete (1996a) reported that both *Chione* and *Allenanthus* have “echinate” pollen, a synapomorphy of the Chiococceae and Catesbaeae (both sensu Delprete), and one pendulous ovule per locule that develop into a laterally flattened seed. The latter characters were synapomorphies of the entire Chiococceae in Delprete’s study, and they helped to place *Chione* and *Allenanthus* within that clade, but a single pendulous ovule is also characteristic of, e.g., Guettardeae (Robbrecht, 1988, p. 147), which might support the position of *Allenanthus* in the vicinity of *Guettarda* in the C5a clade of this study. During the present study, *Chione sylvicola* was found not to have echinate pollen. A closer study of pollen is needed in order to investigate homologies in pollen morphology between *Allenanthus*, *Chione*, the members of the *Hoffmannia-Hamelia-Hillia* taxon group, and other taxa reported to have “echinate” pollen. Although pollen of *Hillia* and *Cosmibuena* is not echinate, at least pollen of *Hoffmannia gesnerioides* is so, but the spinules of this species are much smaller than those found in the C4 clade (J. H. E. Rova, personal observation).

Guettardeae and Rondeletieae s.s. (Fig. 7)—Clade C5 has only weak jackknife support and includes mainly representa-

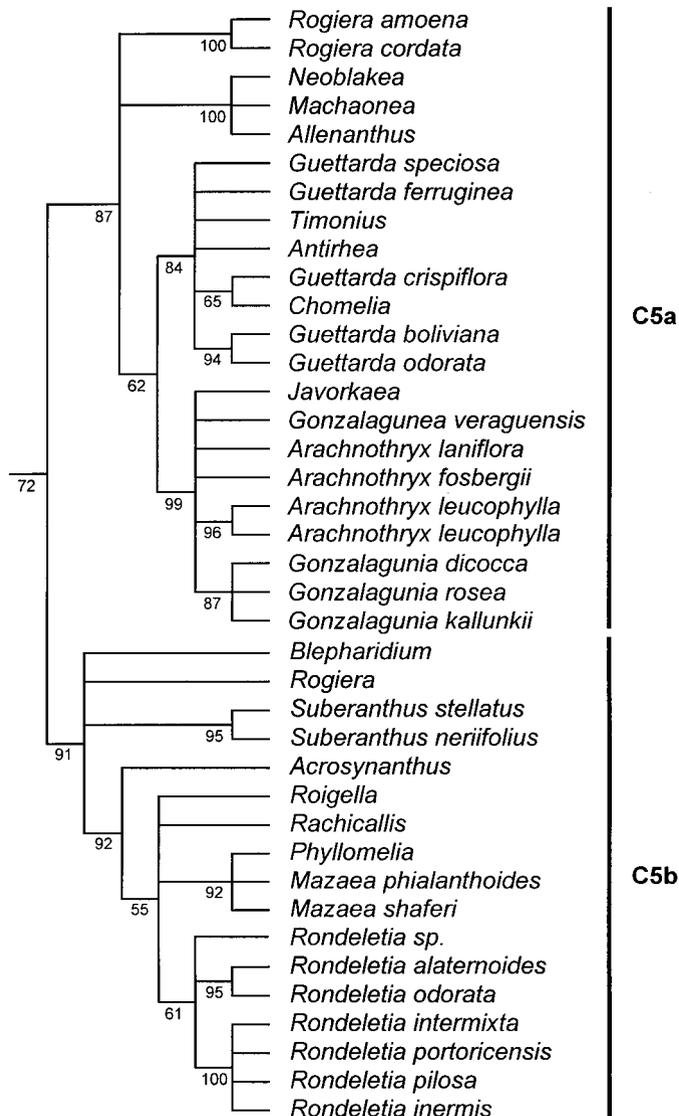


Fig. 7. The Cinchonoideae clades C5a and C5b from Fig. 1. Numbers below branches indicate jackknife support.

tives of the tribes Rondeletieae and Guettardeae. A number of genera that until recently have had a more or less doubtful position are also found in this clade, such as *Gonzalagunia*, *Mazaea* (syn. *Ariadne* and *Neomazaea*; Delprete, 1999a), *Neoblakea*, *Phyllomelia*, *Rachicallis*, and *Suberanthus*.

Genera in the C5 clade are generally shrubs or small trees with entire, triangular, or ovate stipules. Flower merosity and pubescence vary, as do ovary, fruit, pollen, and exotesta morphology. The prevailing basic chromosome number in the C5 clade (as well as in most Rubiaceae) is $x = 11$ (Kiehn, 1986, 1995), although only about half of the genera have been investigated. The only known exceptions are from *Rogiera* ($x = 10$) and *Arachnothryx* ($x = 9$) (see further discussion in Kiehn, 1995).

Circumscription of *Rondeletia*—*Rondeletia* in a broad sense forms an important part of the C5 clade. Circumscription of this genus has been debated for a long time. The main issues in dispute have been whether *Arachnothryx*, *Javorkaea*, and

Rogiera should be treated as separate genera or just as subgenera of *Rondeletia*, largely following the “species groups” of Standley (1918). If treated in its broadest sense, *Rondeletia* is a genus with >250 species distributed over central and northwestern South America, Mexico, and the Antilles (Andersson, 1992). Steyermark (1967) listed a number of diagnostic characters to separate South American *Arachnothryx* from *Rondeletia*. Kirkbride (1968) and Lorence (1991) found these characters less useful in Central America. Lorence (1991) argued that *Arachnothryx*, *Javorkaea*, *Rogiera*, and *Rondeletia* are best treated as subgroups of a broadly defined genus *Rondeletia*, probably at subgeneric level, because of the minute morphological differences between them. Delprete (1999b) included all these taxa into a *Rondeletia* complex without inferring any taxonomic significance of the group. Steyermark’s treatment of *Rondeletia* and *Arachnothryx* as separate genera has been insisted on and further developed by Borhidi and collaborators who, in a number of papers, have defended a segregation of *Arachnothryx*, *Javorkaea*, *Rogiera*, *Roigella*, and *Suberanthus* from *Rondeletia* in “the original Linnean sense” (Borhidi and Fernández, 1981a, b; Borhidi, 1982, 1989, 1993–1994; Borhidi and Járαι-Komlódi, 1983; Fernández, 1993–1994). In these studies, *Arachnothryx* was characterized by four-merous flowers, an externally arachnoid tomentose or pubescent corolla tube, naked annular disc, and a glabrous corolla throat without faucial ring, i.e., an annular thickening below corolla lobe bases (Borhidi, 1982). *Javorkaea* was distinguished by having a broad, connate stipular ring, a glabrous corolla throat without faucial ring, and corolla lobes pubescent at base inside (Borhidi and Járαι-Komlódi, 1983). *Rogiera* was separated from *Rondeletia* by its five-merous flowers with a bearded corolla throat (Borhidi, 1982), and *Roigella* by a combination of large five- to six-merous flowers, a different placentation, and septicial capsules (Borhidi and Fernández, 1981a). *Suberanthus* was recognized by its septicial capsules, reticulate pollen, and a coriaceous corolla with thickened, angustate throat (Borhidi and Fernández, 1981b). *Rondeletia* would thus be a genus almost entirely restricted to the Antilles, and characterized by pubescent annular disc, four- to five-merous flowers, loculicidal capsules, and glabrous corolla throat with a more or less distinct annular thickening (Fernández, 1993–1994).

While Steyermark worked on the generic complex in a predominantly South American context, Kirkbride and Lorence focused on the Mexican and Central American taxa, and Borhidi with collaborators studied *Rondeletia* (s.l.) mostly from a Cuban/Antillean point of view. Possibly, these different ways to approach Rondeletian taxonomy have led to the different ways of handling the generic delimitations of this complex.

In the present study, taxa have been named according to Borhidi’s (and collaborators) narrow circumscription of *Rondeletia* and its segregates, since his view was supported by the phylogenetic analyses. For a complete discussion of the history and taxonomy of the species complex, see Borhidi (1982), Lorence (1991), Fernández (1993–1994), Delprete (1999b), and references therein.

***Arachnothryx*, *Gonzalagunia*, and *Javorkaea* (Fig. 7)**—The *Arachnothryx*-*Gonzalagunia*-*Javorkaea* clade (part of clade C5a in Fig. 7) has a very strong support in all analyses, although it is internally not well resolved. *Javorkaea* is a monotypic Honduran genus characterized by basally connate stipules, five- to six-merous, slightly zygomorphic flowers, a

glabrous corolla throat without annular thickening, and terminal, racemose-cymose inflorescences (Borhidi and Járαι-Komlódi, 1983). Inflorescences are also terminal in *Gonzalagunia* and *Arachnothryx*. In *Gonzalagunia*, as well as in a number of *Arachnothryx* species, inflorescences are elongated, spike-like or thyriform, but in *Arachnothryx* they may also be shortly paniculate. In both genera, flowers are normally four-merous and always without faucial ring. If only representatives with elongated inflorescences are considered, the two genera are very similar, and it is sometimes extremely difficult to tell them apart if no fruits are present. While *Arachnothryx* has septicial capsules, *Gonzalagunia* is characterized by spongy, berrylike fruits. However, this may be a doubtful generic character since fruit dimorphism (where both spongy and dry fruits are mixed in the same infructescence) is reported in *Gonzalagunia* (Ståhl, 1999), and its dry fruits often resemble unripe capsules of *Arachnothryx*. Because of these overall morphological similarities between the two genera, it is not surprising to find them close together in the cladogram. Bremer and Thulin (1998) noted that *rbcl* data also place *Gonzalagunia* near *Rondeletia* s.l. and *Guettarda*. The affinity between *Arachnothryx* and *Gonzalagunia* is also supported by karyological data: Kiehn (1995) reports that both *Arachnothryx* and *Gonzalagunia* are tetraploid with a basic number of $x = 9$, an unusual, and most probably apomorphic, basic number in the Rubiaceae.

Javorkaea, *Arachnothryx*, *Rogiera*, and *Gonzalagunia* all have three-colporate pollen (as do most genera of the Rubiaceae) with a foveolate, smooth surface (Borhidi and Járαι-Komlódi, 1983; Andersson, 1996; J. H. E. Rova, personal observation). Exotesta cells of *Arachnothryx* and *Gonzalagunia* have a secondarily thickened inner tangential wall perforated by large pores. In the studied representatives of *Arachnothryx*, secondary thickening has an even surface, in contrast to the investigated *Gonzalagunia* specimens where secondary thickenings are covered with distinct knobs. Exotesta cells of *Rogiera cordata* have netlike secondary thickenings in the inner tangential walls covered with knobs, in the same manner as in *Gonzalagunia*.

Guettardeae (Fig. 7)—The present study is the first molecular analysis to include a large number of representatives from both *Rondeletia* s.l. and the tribe *Guettardeae*. In this study, the members of the *Guettardeae* are found in two subclades nested within the C5a clade (Fig. 7). The first comprises *Guettarda*, *Chomelia*, *Timonius*, and *Antirhea*, and the other comprises *Machaonia* and *Neoblakea*. The *Guettardeae* thus appears to be paraphyletic with regard to *Rogiera*, *Javorkaea*, *Arachnothryx*, and *Gonzalagunia*. *Guettardeae* as a whole is pantropical, although almost absent from Africa (Robbrecht, 1988). Genera of the *Guettardeae* are characterized by two- to multi-locular ovaries, single, apically attached ovules, more or less fleshy fruits, and the possession of stylar pollen presentation; inflorescences may be terminal or axillary, and corolla aestivation is reported to be valvate or imbricate (Robbrecht, 1988). The uniovulate loculi of the *Guettardeae* are unique in the C5a clade, but not to the C5 clade as a whole, where one-seeded loculi are also reported from *Phyllomelia* and *Mazaea* (Delprete, 1999a). A phylogenetic affinity between *Guettardeae* and *Rondeletieae* has been suggested also by earlier authors, e.g., Bremer, Andreasen, and Olsson (1995), in whose studies *rbcl* sequence data resulted in a clade comprising *Rogiera*, *Rachicallis*, *Guettarda*, and *Antirhea*.

Guettarda forms a strongly supported clade together with *Timonius*, *Antirhea*, and *Chomelia*. The result shows that *Guettarda* is paraphyletic because *Chomelia tenuiflora* Benth. forms a clade together with *Guettarda crispiflora* Vahl. *Guettarda* is a genus of neotropical and pacific distribution, with ~80 species. The highest species diversity is found in the Antilles but one species, *Guettarda speciosa* L., is found throughout the Pacific and as far west as the Seychelles and the coasts of East Africa in the Indian Ocean (Darwin, 1979). The representatives of *Guettarda*, *Timonius*, *Antirhea*, and *Chomelia* included in this analysis have axillary inflorescences, cupular calyces, and imbricate corolla lobes. Flower merosity ranges between four and seven, and no faucial ring was found in any of the specimens studied. Features of the internal indumentum in *Timonius* are discussed below in connection with *Rogiera*.

Neoblakea and *Machaonia*, both *Guettardeae*, together with *Allenanthus*, form a strongly supported but internally unresolved subclade at the base of the C5a clade. Bremer (1992) excluded *Allenanthus* from the Chiococceae, without suggesting any other position, and Robbrecht (1993) listed the genus as "incertae sedis." *Allenanthus* has dry fruits bordered by a broad wing, this samaroid unit being an adaptation to wind dispersal. *Allenanthus* comprises three species restricted to Central America, while *Neoblakea* is a monotypic Antillean genus, and *Machaonia* is a genus of ~25 species occurring in Mexico and central and northern South America (Andersson, 1992). In contrast to the mainly axillary inflorescences of the *Guettarda* clade, *Neoblakea*, *Machaonia*, and *Allenanthus* are reported to have primarily terminal inflorescences (Liogier, 1963; Steyermark, 1974; Andersson, 1993b; J. H. E. Rova, personal observation).

The colporate and foveolate pollen of *Arachnothryx*, *Rogiera*, and *Gonzalagunia* contrasts with the pollen of the *Guettardeae*, which is more or less uniformly porate (occasionally colporate) and semitectate-perforate (tectate-imperforate in *Guettarda speciosa*), with relatively long collumellae (Darwin, 1979, 1993, 1994; J. H. E. Rova, personal observation), although pollen data are lacking from *Neoblakea*, *Machaonia*, and *Allenanthus*.

Rogiera—The two *Rogiera* species in clade C5a (*R. amoena* Planch. and *R. cordata* (Benth.) Planch.) form a strongly supported subclade (Fig. 7). *Rogiera* is characterized by large foliaceous stipules and a densely yellow-bearded corolla throat (Borhidi, 1982). The results from the present study show *Rogiera* as polyphyletic, while *R. amoena* (the type of *Rogiera*) and *R. cordata* are found in the C5a clade, and *R. suffrutescens* (Brandeg.) Borhidi is found as a basal member of the C5b clade. *Rogiera suffrutescens* differs from the other studied species in stipule morphology (small, narrow ascending stipules in *R. suffrutescens* vs. larger, broader, and reflexed stipules in *R. amoena* and *R. cordata*) and external pubescens of corolla (almost glabrous vs. pubescent).

The genera related to *Rondeletia* s.s. (Fig. 7)—The C5b clade is composed of the genera *Acrosynanthus*, *Blepharidium*, *Mazaea*, *Phyllomelia*, *Rachicallis*, *Roigella*, *Rondeletia* s.s., and *Suberanthus*. With the exception of *Blepharidium* and *Acrosynanthus*, corolla throats were consistently glabrous in the examined representatives of this clade. Capsules in this group are generally loculicidal and inflorescences axillary, but there are exceptions in both of these characters. Seeds in members

of this clade are angular and wingless or winged with concentric or bipolar wings.

Blepharidium, Rogiera, and Suberanthus—*Blepharidium* is a genus of two species occurring in Mexico and Guatemala. It was included in Cinchoneae by Robbrecht (1988) and later was transferred to Hillieae by Andersson (1995), although Andersson noted that “it differs from other members of the tribe in the absence of raphides, in having clasping stipules, corolla with imbricate aestivation, and anthers dorsifixed around the middle.” No other taxon from the C5 clade of the present study was included in Andersson’s analysis.

The genus *Suberanthus* was separated from *Rondeletia* by Borhidi and Fernández (1981b) because of its glabrous annular disc, oblong-pyriform ovary, and roughly reticulate pollen grains. The genus corresponds more or less to the *Rondeletia* section “*Stellatae*” of Standley (1918). Based on several morphological characters (e.g., ascending placenta, winged seeds, and a blackish-purple corolla), Borhidi and Fernández transferred *Suberanthus* from Rondeletieae to Cinchoneae, even though corolla morphology suggests an affinity to *Rondeletia* because of its *Rondeletia*-like annular thickening in the corolla throat. In congruence with the conclusions drawn by Borhidi and Fernández, *Suberanthus* was tentatively treated as member of the Cinchoneae by Robbrecht (1988, 1993). According to the results of the present analysis, corolla morphology is more important than Borhidi and Fernández anticipated, as *Suberanthus* is found to be one of the sister clades to *Rondeletia* s.s. *Suberanthus* consist of about half a dozen species restricted to Cuba and Hispaniola (Borhidi and Fernández, 1981b), and the two representatives included in this study form a clade with a very strong jackknife support.

As discussed above in connection to the C5a clade, this study shows *Rogiera* to be a polyphyletic taxon, recognized morphologically by a densely yellow-barbate corolla throat. Since *Rogiera amoena* (found in clade C5a) is the type of *Rogiera*, *R. suffrutescens* obviously has to be transferred to another genus. It should, however, not be included in *Rondeletia* s.s. according to the results of the present study, as this would make *Rondeletia* s.s. paraphyletic.

Mazaea, Phyllomelia, Rachicallis, and Roigella—*Roigella* was separated from *Rondeletia* by Borhidi and Fernández (1981a) based on ovary, calyx, and pollen characters. However, already Standley (1918) considered *Roigella* (as *Rondeletia correifolia* Griseb.) unique in *Rondeletia*, which he showed by making it the sole member of his section “*Correifoliae*.” According to the present results, *trnL-F* data also support the isolated position of *Roigella* in the close vicinity of *Rondeletia* s.s.

The Cuban endemic genera *Phyllomelia* and *Mazaea* (the latter often referred to *Ariadne* or *Neomazaea*) form a monophyletic, although internally unresolved, part of the C5b clade. Jackknife support is very strong for this clade, and the results of the current study are consistent with the conclusions drawn from morphological characters by Delprete (1999a), that *Ariadne shaferi* (Standl.) Urb. and *Mazaea phialanthoides* should be referred to the same genus, *Mazaea*, and that *Phyllomelia* is closely related to this genus. However, pollen morphology differs between the two *Mazaea* species: while pollen of *M. phialanthoides* is reticulate-foveolate without external ornamentation, pollen of *M. shaferi* is more foveolate and covered with minute verrucae (J. H. E. Rova, personal observation).

For a long time, both *Ariadne* and *Phyllomelia* have been treated as of uncertain position in the Rubiaceae (Robbrecht, 1988, 1993). An interesting feature of the latter is the possession of peculiar pterophyllous calycophylls and indehiscent, two-seeded fruits (Delprete, 1999a).

Rachicallis, a monotypic genus present in Mexico and the Caribbean, was originally included in the Rondeletieae by Hooker (1873), followed by Schumann (1891), and later implicitly by Bremekamp (1966) (see also [http://ajbsupp.botany.org/\[Appendix 2\]](http://ajbsupp.botany.org/[Appendix 2])). Robbrecht (1988) transferred, for unclear reasons, *Rachicallis* to Hedyotideae but later (1993) agreed that this was perhaps dubious. Based on *rbcl* data, Bremer, Andreasen, and Olsson (1995) found that *Rachicallis* should be placed near to *Rogiera* and *Guettarda*, and a similar result was also found based on *rps16* sequence data (Andersson and Rova, 1999). The present study strongly confirms the inclusion of *Rachicallis* in Rondeletieae and tentatively places *Rachicallis* as sister taxon to *Phyllomelia* and *Mazaea*. However, an inclusion of *Rachicallis* in the same clade as *Mazaea* and *Phyllomelia* has no jackknife support.

The *Rondeletia* s.s. clade (Fig. 7)—The largest of the C5b subclades comprises *Rondeletia alaternoides* A. Rich., *R. inermis* (Spreng.) Kr. & Urb., *R. intermixta* Britt., *R. odorata* Jacq., *R. pilosa* Sw., *R. portoricensis* Kr. & Urb., and an unidentified *Rondeletia* sp. from Cuba. According to Fernández (1993–1994), *Rondeletia portoricensis* is supposed to be closely related to *R. americana* L., the type of *Rondeletia*, and this clade may thus be named *Rondeletia* s.s. All these species possess a swollen faucial ring (the unidentified species not studied) and fit well into the description of *Rondeletia* in the strict sense of Borhidi and Fernández (1981a). All species in this clade except *R. odorata* (see below) are entirely Cuban/Puerto Rican, which supports the view of *Rondeletia* s.s. as an Antillean genus (Fernández, 1993–1994; A. Borhidi, Janos Pannonius University, Pécs, personal communication). Pollen in this group is surprisingly diverse, and the present study cannot confirm the picture of uniformity in pollen morphology put forward by Fernández (1993–1994, p. 67). During the present study, *R. alaternoides* was found to have tectate-rugulate pollen covered with minute warts, while *R. inermis* has reticulate to almost semitectate pollen without supratectal sculpturing, and *R. odorata* has “typical *Rondeletia*” tectate foveolate pollen with a smooth surface.

While *Gonzalagunia* and *Arachnothryx* are generally heterostylous (Lorenz, 1991; Ståhl, 1999), no heterostyly that could indicate pollen dimorphism has been found in *Rondeletia*. However, this could be an artifact from poor sampling, and it is thus still an open question if further studies of pollen morphology within *Rondeletia* s.s. might give more characters for determination of infrageneric relationships. Exotesta morphology within Rondeletieae also seems to be variable. In this study, secondarily thickened inner tangential walls of *R. inermis* were found to be covered with large pores, while thickenings in *R. odorata* were in the form of netlike bands. Sample density in the present study is unfortunately too low to test the *Rondeletia* sections proposed by Fernández (1993–1994).

Biogeographical correlation (Fig. 8)—While C5a is a predominantly Central American clade, C5b is predominantly Antillean (Fig. 8). Separation of the “mainland” genera *Rogiera*, *Javorkaea*, and *Arachnothryx* from the “Antillean” *Rondeletia* s.s. is thus both phylogenetically supported and geographically

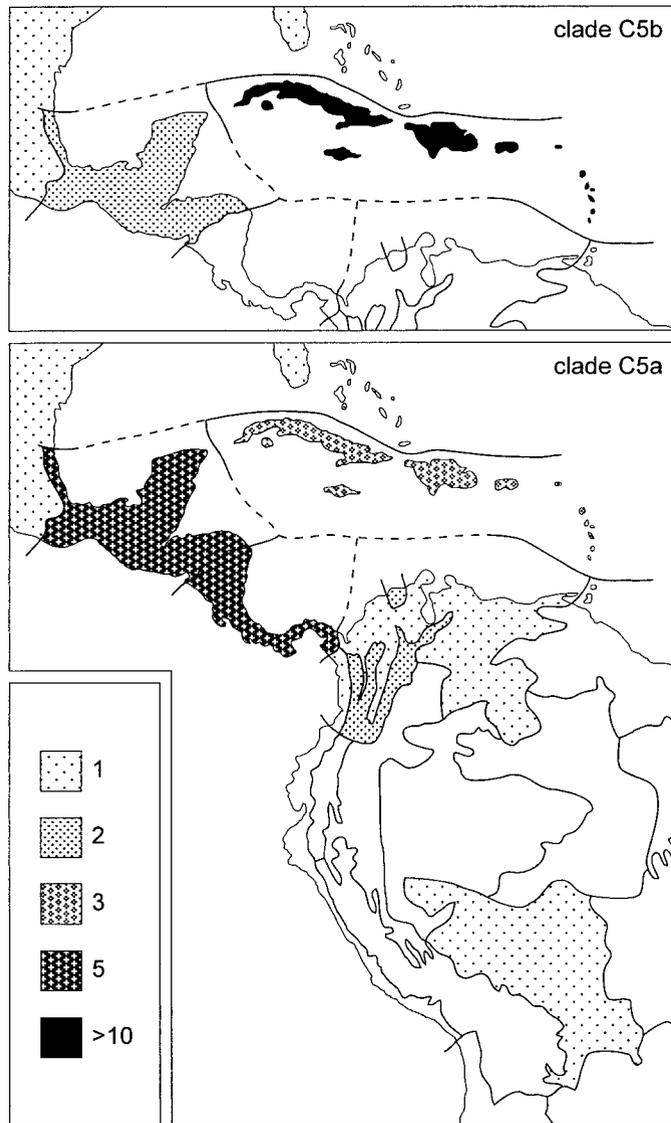


Fig. 8. Distribution of taxa from the C5a and C5b clades in Fig. 7. Numbers indicate number of taxa within each region. This map only includes taxa with restricted distribution, here defined as taxa occurring in no more than two adjacent regions. *Guettarda speciosa* and *Timonius* sp., occurring outside the Americas, are not included. *Rondeletia odorata* is assumed to be introduced by humans in southern Central America (see text) and it is because of this treated as Antillean in the figure. Regions and distribution data as reported by Andersson (1992).

correlated. In clade C5a, a number of species with a wide American distribution (Andersson, 1992) are found, such as *Rogiera amoena* (Central and Northern South America), *Gonzalagunia dicocca* (widespread in tropical South America), *Guettarda crispiflora* (northwestern South America and the Antilles), *Chomelia tenuiflora* (northwestern South America), and *Guettarda odorata* (the Antilles and the Caribbean coast of Central and South America). The Guettardeae are also widespread in the Pacific and Southeast Asian tropics. On the other hand, the northern Central American species *Rogiera suffrutescens* and *Blepharidium guatemalense* Standl. are the only taxa included in clade C5b that are endemic to a region other than the Antilles. *Rachicallis* is found in the Antilles and

in extratropical North America, and *Rondeletia odorata* in Cuba and southern Central America, but the existence of the latter on the mainland is probably due to human dispersal, since the species is a widely cultivated ornamental (Lorence, 1991). When biogeography is discussed based on the results from the present study, it is, however, important to remember that only a fraction of all species of *Rondeletia* s.l. is represented here. An increased sampling among Mexican/Central American species might show also other mainland taxa to belong in clade C5b. One possible mainland member of clade C5b is *Rondeletia panamensis* DC., a species endemic to Panama but treated as part of *Rondeletia* s.s. by Fernández (1993–1994).

Nevertheless, the present data suggest that the Antillean Rondeletieae are more closely related to the Holarctic than to the Gondwanian flora.

Fruit diversity in clade C5—The C5 clade includes an interesting diversity of fruit types, regarding general morphology, number of seeds per fruit, and modes of dispersal. Capsules and indehiscent dry fruits are the most common, but *Gonzalagunia* has spongy berries or indehiscent dry fruits (Ståhl, 1999), and Guettardeae have mostly drupaceous fruits. In *Phyllomelia* and *Allenanthus*, the entire, unopened fruit is wind dispersed. In *Phyllomelia*, the calyx is expanded to form a flight unit (Delprete, 1999a), and in *Allenanthus* the fruit is laterally flattened and surrounded by a ringlike wing perpendicular to the equator of the fruit and originating from the exocarp. Pluriovulate ovaries are predominant in the clade, but Guettardeae, *Phyllomelia*, and *Mazaea* include species with one-seeded loculi.

Classification of clade C5—The formal classification of the C5 clade may be approached in several ways. One possibility is to treat the whole clade as one large tribe “Rondeletieae” with a very modified circumscription. Another alternative would be to separate the clade into two tribes, “Rondeletieae” in a narrow sense (C5b) and “Guettardeae” in a broad sense (C5a). However, before any final decisions are made, taxon sampling within the clade should be denser, and morphological characters must be studied in greater depth. All representatives of the C5 clade available for morphological studies (see [http://ajbsupp.botany.org/\[Appendix 1\]](http://ajbsupp.botany.org/[Appendix 1])) have imbricate corolla aestivation, with the exception of one *Guettarda* species (*G. crispiflora*), in which it is valvate-induplicate. Because genera such as *Greenea*, *Aleisanthia*, *Augusta*, and *Wendlandia* have been shown to have affinities to the Ixoroideae rather than to the Rondeletieae, Rondeletieae may, whatever circumscription is chosen, thus be regarded as almost entirely imbricate.

Conclusions—It is important to include a wide sampling of taxa when performing molecular systematic analyses in the Rubiaceae because relationships within the family are still obscure. Overall relationships within the family are much more complex than previously predicted, as groups usually assumed to be monophyletic or phylogenetically close may prove not to be so. Traditional groups may split into portions belonging to different subfamilies and other unexpected relationships may still be revealed. Great care should be taken when relying only on morphological data, especially when just a limited number of morphological characters are used, or when homologies are not fully understood. On the other hand, corolla aestivation patterns seem to be of taxonomic importance when

considered in the perspective of this molecular study. This study shows that the chloroplast *trnL-F* intron and spacer region works well for inferring phylogeny and higher classification of the Rubiaceae, and, for example, in the case of *Strumpfia*, it has shown how powerful sequence data can be in finding affinities of taxa where morphology alone has failed to do so (similar experiences founded on *rbcL* sequence data in Bremer, Andreasen, and Olsson, 1995). However, in order to propose a more resolved molecular phylogenetic hypothesis of relationships within the II clade, the *trnL-F* region has turned out to be too slowly evolving to be informative. This study also shows that properly collected, well-kept herbarium specimens still have cardinal scientific value even though studies now range from traditional morphology to DNA sequencing projects. Most DNA extractions used in this study were made from herbarium material, and in the case of *Phyllacanthus*, DNA for sequencing was obtained from an Ekman collection made in 1923—one of the two collections ever made of this species, which is now possibly extinct.

This study has revealed several new relationships within Rubiaceae, as well as it has corroborated conclusions drawn from other molecular and morphological studies. The most important results can be summarized as follows.

Subfamily-level conclusions—(1) The former Rondeletieae genera *Aleisanthia*, *Aleisanthiopsis*, *Augusta*, *Greenea*, and *Wendlandia* are members of the Ixoroideae. (2) Sipaneeae are not close to Rondeletieae but is a tribe of the Ixoroideae. (3) *Gleasonia* (Henriquezieae) belongs in the subfamily Ixoroideae. (4) *Molopanthera* and *Posoqueria* are closely related in spite of the huge difference in corolla size and largely different fruit types. They belong in the Ixoroideae, but not in Gardenieae. (5) Results from other molecular studies are corroborated in that *Luculia* holds an isolated position basally in the family and that there is no support for subfamily Antirheoideae.

Tribal-level conclusions—(1) There is no support for a broad circumscription of the tribe Rondeletieae. (2) There is support for a tribe Sipaneeae, including at least *Maguireothamnus*, *Neobertiera*, and *Sipanea*. (3) The Guettardeae (sensu Robbrecht, 1988, 1993) are paraphyletic and their members are found close to *Gonzalagunia* and the *Rondeletia* segregates *Arachnothryx*, *Javorkaea*, and *Rogiera*. (4) There is support for an emended tribe Naucleaeae that should include *Cephalanthus* and *Uncaria*. (5) Rondeletieae s.s. are almost entirely Antillean.

Generic level conclusions—(1) Geographically correlated molecular support is found for the separation of *Arachnothryx*, *Rogiera*, *Roigella*, and *Suberanthus* from *Rondeletia*. (2) *Arachnothryx*, *Gonzalagunia*, and *Javorkaea* are possibly congeneric and need a special study. (3) *Rogiera* is a polyphyletic genus. (4) Based on morphological data, *Neobertiera* and *Sipaneaopsis* could well be congeneric, and this is also supported by *trnL-F* sequence data. (5) *Guettarda* is paraphyletic.

In addition to this, tribal or subfamilial affiliation based on molecular sequence data is for the first time suggested for the following genera: *Allenanthus* (close to *Guettardeae/Rondeletieae*), *Blepharidium* (Rondeletieae), *Chione* (close to Hamelieae/Hillieae in Cinchonoideae), *Coutaportla* (Chiococceae), *Dolichodelphys* (close to *Calycophyllum/Condaminea/Hippotis* in Ixoroideae), *Mazaea* (Rondeletieae), *Neobertiera* (Sipaneeae in Ixoroideae), *Neoblakea* (close to *Guettardeae/*

Rondeletieae), *Phialanthus* (Chiococceae-Catesbaeeae complex), *Phyllacanthus* (Chiococceae-Catesbaeeae complex), *Phyllomelia* (Rondeletieae), *Schmidtottia* (Chiococceae-Catesbaeeae complex), and *Suberanthus* (Rondeletieae).

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