A trnL-F cpDNA sequence study of the Condamineae-Rondeletieae-Sipaneeae complex with implications on the phylogeny of the Rubiaceae

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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 Rondeletiae, Sipaneeae, and Condamineae. Sipaneeae are not close to Rondeletiae and belong in the Ixoroideae. There is no support for a widely distributed Rondeletiae in a broad sense. Instead, Rondeletiae sensu stricto form an almost entirely Antillean clade. Support was found for the separation of Arachnothryx, Rogiera, Roigelia, and Suberanthus from Rondeletia. The Guettardeae as well as Gonzalagunia are found close to a complex formed by Arachnothryx, Juvorkea, and Rogiera. Condamineae, in a strict sense, belongs in the Ixoroideae. A number of Rondeletiae genera should be transferred to Condamineae or other parts of Ixoroideae. Support is found for an emended tribe Nucleaee, comprising several genera with spherical pseudanths. For the first time, tribal or subfamilial affiliation based on molecular sequence data is suggested for Allenanthus, Blepharidium, Chione, Coutaportla, Dolichodelphys, Mazaea, Neobertiera, Neoblakea, Phialanthus, Phyllacanthus, Phyllomelia, Schmidtiota, and Suberanthus.

Key words: cladistics; Condamineae; Rondeletiae; 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), macroevolutics 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, Rondeletiae, and Sipaneeae should, based on morphology, be regarded as very closely related tribes in subfamily Cinchonoideae. Shortly after Robbrecht’s comprehensive study was published, the first molecular systematic studies of Rubiaceae began to appear (Bremer and Jansen, 1991; Bremer, 1992; Bremer and Struve, 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, Andreasen, 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 Catesbaeae and Chiococceae (Bremer, 1992) was established, and a suggestion to divide the tribes Rondeletiae and Condamineae between subfamilies Cinchonoideae and Ixoroideae was made, with some representatives of Rondeletiae found to be closely related to Guettardeae in subfamily Cinchonoideae (e.g., Bremer, Andreasen, and Olsson, 1995; Andersson and Rova, 1999). Further, several molecular systematic studies have focused on particular parts of the family, such as subfamily Ixoroiodeae (e.g., Andreasen and Bremer, 1996, 2000; Andreasen, 1997; Andreasen, Baldwin, and Bremer, 1999), the Alibertia group (Persson, 2000), subfamily Rubioidaeae (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 Rubiaeae (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 Catesbaeaeae and Chiococceae were closely related and several Condamineae genera should be regarded as closely related to Rondeletiae (Delprete, 1996a).
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 (Franzina L. and Jasminium L. of the Oleaceae) were also selected (Bremer and Struve, 1992; Struve, Albert, and Bremer, 1994; Bremer, Andreasen, and Olsson, 1995; Bremer, 1996b; De Laet and Smets, 1996; Struve 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.

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 cetvitrinethalamonomium 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. (Gielly 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 AmpliTag 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 Sequencer (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 Sequencer 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 accordingly 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. Dolichodelphus and Pseudomus-saenda 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. aristeguietiae because of a large autapomorphic deletion in the spacer region of R. spinifex. In the sequence of Bikka and one of the Arachnothrix 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). Data on origin of plant material, vouchers, and GenBank accession numbers are given at http://ajbsupp.botany.org/s89 (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 Rubioideae 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://ajb supp .botany .org/v 89 (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, Andreasen, and Olsson, 1995; Andersson and

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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 Rubioideae, 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 Catesbaeeae (as defined by Delprete, 1996a) form a monophyletic group (clade C4). According to this study, Cephalanthus (Cephalantheae) is placed in the Nauclaeae (clade C3), as also shown by Bremer and Jansen (1991). Further, this study confirms that Retiniphyllum (Retiniphyl-leae) and Vanguerieae (represented by Vangueria, Keetia, and Psydraax) are nested within Ixoroideae (clade I2), as previously shown by Bremer, Andreasen, and Olsson (1995) and Andersson and Rova (1999). Based on rbcL sequences, Bremer (1996b) placed Pentanisia (Knoxieae) in Rubioideae (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, Andreasen, 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 Rubioideae clade (Fig. 2)—The internal structure of the Rubioideae clade (clade R; Fig. 2) is by and large in accordance with other recent and more densely sampled DNA phylogenies of the Rubioideae, 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 Rubioideae taxa in the present study, together with a relatively high DNA sequence mutation rate, made alignment of Rubioideae sequences a difficult task, and because of this dif-
difficulty, 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): Warszewiczia, Bathysa, Macbideina, and Elaeagia to Bonheureneae; Chimarrhis, Parachimarrhis, Rustia, Pogonopus, Picardaea, Dioecodendron, Condaminea, and Pinckneya to Condamineae; Witmankanthus, Calycophyllum, and Alseis to Calycophyllaeae (as described by Andersson, 1993a, 1995); Simira to Simireae; Hippotis, Sommara, and Pentagonia to Hippoti-deae; Capirona to Cinchoneae; and Dolichodelphys to Gar-deneae. 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, Witmankanthus, 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) proposed an amendment of Calycophyllaeae to include Alseis, Calycophyllum, Capirona, Ferdinandusia, Macrocnemum, Semaphyllanthe (a segregate of Calycophyllum), and Witmankanthus. 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 protogyne in these genera. Protogyne is rare in the Rubiaceae and never before reported outside Antherospermatae (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 Rubioidae (e.g., Andersson and Rova, 1999) and are not included in the present study. Claßen-Bockhoff (1996) also reported calycophylls in the Alberteae (Albetta and Nematosylis, shrubs/trees with 1–2-seeded, dry fruits; Puff, Robbrecht, and Randrianasolo, 1984), Jackieae (Jackioipsis, a tree with one-seeded nutlets; Ridsdale, 1979), the genera Mussaenda and Pseudomussaenda (discussed together with the I2 clade below), and the two genera Pentaconylia (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 Simpsonia (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 glabri¯ora 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 Hippodieae genera (Hippotis, Sommara, 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
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), 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 Rohrhafer (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 Condamineae and Calycophyllum. It is interesting to note the presence of calychoyls 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, Andersen, and Olsson, 1995; Andersen, 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, 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-

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

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-Coffeae-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 Andersen and Bremer (1996), Andersen (1997), and Andersen, Baldwin, and Bremer (1999).
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, 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 Aleiantha 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-Coffeaceae-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-olate with smooth testa in Augusta [Cowan, 1932; Darwin, 1976]), but both genera share fruit type (capsule), exserted stigma (Cowan, 1932; Darwin, 1976), and maybe most interesting, 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 Coffeaeae (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 Coffeaee 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 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 13a in Fig. 5, corresponds to the tribe Sipaneeae (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 μm² (in Limnosipanea, Sipanea, and Neobertiera) down to 0.7 per μm² 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 13b in Fig. 5) is found as sister clade to the Sipaneeae. 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, 1988, 1998).
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 rubiaceous 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; Andreasen, 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 13b 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 (Rubiioideae), Cephalantheae, Guettardaeae, Chiococcceaee (all three Antirrhieoideae), and Catesbaeaeae (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 Rubiioideae, 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 nuclear ribosomal DNA ITS sequence data sets (Bremer, Andresen, and Olson, 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.

Isertiae—One of the Cinchonoideae clades is formed by two Isertia species. Of the traditional Isertiaeae (sensu García Kirkbride, 1979) included in this study (Amphidasyia, Gonzia-lagunia, 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).

Naucleeae (Fig. 1)—The third Cinchonoideae clade, C3, includes the genera Cephalanthus, Sarcocephalus, and Uncaria. All three these 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 Naucleeae in a strict sense was performed by Ridsdale (1976). In this work, he excluded Mitragyna and Uncaria from Naucleeae 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 Naucleeae 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 monogenic tribe Cephalantheae, Sarcocephalus as a member of Naucleeae, 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, Andresen, and Olson (1995), based on rbcL sequence data, in which all three genera are considered to belong in the Naucleeae. A close relation between Cephalanthus and Naucleeae based on cytological data is also proposed by Kiehn (1995), who reports that the type of interphase nuclei is similar in Cephalanthus and Naucleeae.

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 affiliates to other rubiaceous taxa, since Strumpfia possesses a combination of plesiomorphic characters and a number of more or less unique morphological features, such as protogyne, 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
on molecular data, Bremer et al. (1999) found Strumphi a to be “close to the Chiococceae s.l.” but the only representatives from that clade that were included in their study were Exos- tema, Chiococca, and Erithalis. However, the present study confirms that Strumphi a 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, Strumphi a pollen is verrucose (Igersheim, 1993).

Catesbaeeae, Chiococceae, and the “Exostema-group” (Fig. 6)—Clade C4 (Fig. 6) comprises the genera Asemnantha, Badusa, Bikkia, Catesbaea, Ceratopyxis, Chiococca, Couta- rea, Cubanola, Erithalis, Exostema, Hintonia, Isidorea, Phialanthus, Phyllacanthus, Portlandia, Schmidottia, Scolosan- thus, 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 Catesbaeeae, 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 Cerato- pyxis were not included in Delprete’s analysis, while Nernstia, Thogsennia, Clathrocarpus, 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 Catesbaeeae, 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, Andrew, 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 indicates 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 Schmidottia. In Bremer, Andrew, 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), Phialan- thus 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 Neob- lakea and Machaonia. Delprete (1996a) reported that both Chione and Allenanthus have “echinate” pollen, a synapo- morphy of the Chiococceae and Catesbaeae (both sensu Del- prete), and one pendulous ovule per locule that develop into a laterally flattened seed. The latter characters were synapo-morphies 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 syl- vicola 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 re- ported to have “echinate” pollen. Although pollen of Hillia and Cosmibuena is not echinate, at least pollen of Hoffmannia gesnerioides is so, but the spines 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-
The prevailing basic chromosome number in the C5 clade is 11. Genera in the C5 clade are generally shrubs or small trees with entire, triangular, or ovate stipules. Flower merosity and position are also found in this clade, such as genera that until recently have had a more or less doubtful status. Arachnothryx, Gonzalagunia, and Javorkaea (Fig. 7) 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 (Anderson, 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; Borhidi, 1982, 1989, 1993–1994; Borhidi and Járai-Komlodi, 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 staminal ring, a glabrous corolla throat without faucial ring, and corolla lobes pubescent at base inside (Borhidi and Járai-Komlodi, 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 placenta, and septicidal capsules (Borhidi and Fernández, 1981a). Suberanthus was recognized by its septicidal 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 conuate stipules, five- to six-merous, slightly zygomorphic flowers, a
glabrous corolla throat without annular thickening, and terminal, racemose-cymose inflorescences (Borhidi and Járai-Komlodi, 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 thyrsiform, but in Arachnothryx they may also be shorty 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 septicidal 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 infrutescence) is reported in Gonzalagunia (Stähl, 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 surprising to find them close together in the cladogram. Bremer and Thulin (1998) noted that rbcL data also place Gonzalagunia near Rondeletia and Guettarda, the affinity between Arachnothryx and Gonzalagunia is also supported by karyological data: Kiehn (1995) reports that both Arachnothryx and Gonzalagunia are tetrploid 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-colorporate pollen (as do most genera of the Rubiaceae) with a foveolate, smooth surface (Borhidi and Járai-Komlodi, 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.

**Guettarda** (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, Timonis, 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 Phyllolomelia and Mazaee (Delprete, 1999a). A phylogenetic affinity between Guettardeae and Rondeletiae has been suggested also by earlier authors, e.g., Bremer, Andreassen, 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 Timonis, 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, Timonis, 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 Timonis 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 clade (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 pubescence 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, Phyllolomelia, 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 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 Borhid 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 “Sstellatae” 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 polyphylectic taxon, recognized morphologically by a densely yellow-barbarate 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 (Robbrech, 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 Bremerkamp (1966) (see also http://ab supp. botany.org/Appendix 2). Robbrecht (1988) transferred, for unclear reasons, *Rachicallis* to Hedyotieae 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 *Guetta dura*, and a similar result was also found based on rpl16 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* Brit., *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 Antilllean 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-ru gulate pollen covered with minute warts, while *R. inermis* has reticulate to almost semitectate pollen without supratectal sculpture, and *R. odorata* has “typical Rondeletia” tectate foveolate pollen with a smooth surface.

While *Gonzalaguinia* and *Arachnothryx* are generally heterostylos (Lorence, 1991; Stähl, 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
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 (Lorenze, 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://ajb supp. botany.org/[Appendix 1]) have imbricate corolla aestivation, with the exception of one Guettarda species (G. crispi flora), 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 found 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 I I 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, Augustoa, 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 this study. 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.

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, Neobertiaria, and Sipanea. (3) The Guettardae (sensu Robbrecht, 1988, 1993) are paraphyletic and their members are found close to Gonzalezaguna and the Rondeletia segregates Arachnothryx, Javorkaea, and Rogiera. (4) There is support for an emended tribe Naucleae 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, Gonzalezaguna, and Javorkaea are possibly cogenic and need a special study. (3) Rogiera is a polyphyletic genus. (4) Based on morphological data, Neobertiaria and Sipaneopsis could well be cogenic, 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 Guettardae/Rondeletieae), Blepharidium (Rondeletieae), Chione (close to Hamelleae/Hilleiae in Cinchonoideae), Coutaportia (Chiococceae), Dolichodelphys (close to Calycophyllium/Condaminede Hippotis in Ixoroideae), Mazaea (Rondeletieae), Neobertiaria (Sipaneae in Ixoroideae), Neoblakea (close to Guettardae/Rondeletieae), Phialanthus (Chiococceae-Catesbaeae complex), Phyllacanthus (Chiococceae-Catesbaeae complex), Phyllemelia (Rondeletieae), Schmidiotria (Chiococceae-Catesbaeae complex), and Suberanthus (Rondeletieae).

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