PHYLOGENY OF THE RUBIACEAE AND THE LOGANIACEAE: CONGRUENCE OR CONFLICT BETWEEN MORPHOLOGICAL AND MOLECULAR DATA?[1]

BIRGITTA BREMER[2] AND LENA STRUWE

Department of Systematic Botany, Uppsala University, Box 541, S-751 21 Uppsala, Sweden

Phylogenetic analyses of 33 genera of Rubiaceae were performed using morphological and a few chemical characters. Parsimony analysis based on 29 characters resulted in eight equally parsimonious trees, with a consistency index of 0.40 and a retention index of 0.69. These results were compared to a phylogenetic analysis of the same genera based on chloroplast DNA restriction site data. There are discrepancies between the two analyses, but if we consider groupings reflected in the present classification there is much congruency. With the exception of four genera, all the genera are positioned in the same group of taxa in the two analyses. Clades of taxa representing three of the four subfamilies (~the Antirheoideae, ~the Rubioideae, and the ~Ixoroideae) are monophyletic, while the fourth subfamily Cinchonoideae is shown to be paraphyletic. Both analyses support a widened tribe Chiococceae, including the former subtribe Portlandiinae (Condamineeae). Furthermore, in both analyses the tribe Hamelieae is placed outside the subfamily Rubioideae where it is now housed. In search for the most plausible sister group to the Rubiaceae, the genus *Cinchona* (Rubiaceae) was analyzed together with 13 genera of the Loganiaceae, *Nerium* (Apocynaceae), and *Exacum* (Gentianaceae). *Cornus* (Cornaceae), *Olea* (Oleaceae), and these two genera together were used as outgroups. The analysis, including 25 characters, 16 taxa, and with *Cornus* and *Olea* together as an outgroup, resulted in four equally parsimonious trees, with a consistency index of 0.53 and a retention index of 0.62. The non-Loganiaceae taxa *Cinchona* (Rubiaceae), *Nerium* (Apocynaceae), and *Exacum* (Gentianaceae) were all found to have their closest relatives within the Loganiaceae indicating that the Loganiaceae are paraphyletic and ought to be reclassified. As a result of the morphological data the most plausible sister group to the Rubiaceae is the tribe Gelsemieae of the Loganiaceae.

Recently the first, limited phylogenetic analysis of the Rubiaceae using cladistic methodology was presented (Bremer and Jansen, 1991). That analysis, based on restriction site data of chloroplast DNA (cpDNA), was congruent in many aspects with earlier proposed classifications, and several new phylogenetic relationships were also proposed. The mainly tropical family Rubiaceae is one of the largest angiosperm families, and exhibits a great array of morphological variability especially in its fruit types. As fruit morphology has had a great impact on its classification, fruit variability may be one reason for the partially unstable intrafamilial classification (Robbrecht, 1988).

The most important question raised by the first phylogenetic analysis of cpDNA data from the Rubiaceae is if the newly indicated relationships resulted from differences between morphological and molecular data or from different methods of analysis (the classification is mainly based on phenetic similarities). To answer that question a cladistic analysis was performed on the same genera that used morphological characters and a few chemical characters.

The Rubiaceae are usually considered as a natural, monophyletic, and easily circumscribed family (Verdcourt, 1958; Bremekamp, 1966; Robbrecht, 1988). How-

ever, its exact position in a larger taxonomic context has not been settled. Most botanical "system builders" (Dahlgren, 1980a, 1983; Thorne, 1983; Takhtajan, 1987) have accepted a position of the family in the order Gentianales close to the Loganiaceae, first indicated by Utzschneider (1947, 1951) and later established by Wagenitz (1959, 1964). This position has also been supported by phytochemistry (cf. Bisset, 1980) and by sequence data of the *rbc*L gene (Bremer and Olmstead, unpublished data). Based on several morphological, anatomical, and chemical characters (Wagenitz, 1959) the Gentianales are probably monophyletic, but the interrelationships in the order are not resolved. Furthermore, the centrally positioned Loganiaceae (Bisset et al., 1980) are probably paraphyletic. Cronquist (1981, 1988), on the other hand, does not include Rubiaceae in the Gentianales but indicates that the Gentianales (Loganiaceae) are near-ancestral to the Rubiaceae (in a separate order) and that Rubiaceae are near-ancestoral to the Caprifoliaceae (Dipsacales). We are convinced that the Rubiaceae and the Gentianales form a monophyletic group because of the several unique morphological (cf. the colleters) and chemical characters, while the similarities to the Dipsacales seem to be just superficial.

The Loganiaceae were described by de Martius (1827), but its circumscription has varied (cf. Table 1). Solereder (1892–1895) classified the Loganiaceae into two subfamilies but did not include the doubtful genera *Plocosperma* and *Desfontainia*. Hutchinson (1973) distributed the genera traditionally included in the Loganiaceae among seven different families, including the Buddlejaceae. All these families together with the Oleaceae were included in the order Loganiales, but the genus *Plocosperma* was placed in a monotypic family in the order Apocynales. In the latest revision of the Loganiaceae (Leeuwenberg and Leen-

TABLE 1. *Comparison of six familial classifications of the Loganiaceae*

Leeuwenberg and Leenhouts[a]	Hutchinson[b]	Cronquist[c]	Dahlgren[d]	Thorne[e]	Takhtajan[f]
Tribe Spigelieae *Polypremum* *Spigelia** *Mitreola** *Mitrasacme*	SPIGE[g,h]	LOGAN	=[i]	=[j]	SPIGE
Tribe Loganieae *Geniostoma** *Labordia* *Logania**	LOGAN	LOGAN	=	=[j]	=
Tribe Gelsemieae *Mostuea** *Gelsemium**	LOGAN	=	=	=[j]	=
Tribe Strychneae *Strychnos** *Gardneria** *Neuburgia*	STRYC	LOGAN	=	=[j]	=
Tribe Antonieae *Bonyunia* *Antonia* *Norrisia** *Usteria**	ANTON	LOGAN	=	=[j]	=
Tribe Potalieae *Potalia* *Anthocleista** *Fagraea*	POTAL	LOGAN	=	=[j]	GENTI
Tribe Desfontainieae *Desfontainia**	POTAL	LOGAN	DESFO	LOGAN[k]	DESFO
Tribe Plocospermeae *Plocosperma**	PLOCO	APOCY	LOGAN	=[l]	PLOCO
Tribe Retzieae *Retzia*	RETZI	=	=[k]	LOGAN[m]	RETZI[o]
Tribe Buddlejeae *Pelthantera* *Sanango* *Nuxia* *Androya* *Gomphostigma* *Buddleja* *Emorya*	BUDDL[n]	BUDDL[o]	=	=	=[o]

[a] Classification according to Leeuwenberg and Leenhouts (1980). Genera investigated in this study are marked with *.
[b] Hutchinson, 1973.
[c] Cronquist, 1981.
[d] Dahlgren, 1983.
[e] Thorne, 1983.
[f] Takhtajan, 1987.
[g] The families are Antoniaceae = ANTON; Apocynaceae = APOCY; Buddlejaceae = BUDDL; Desfontainiaceae = DESFO; Gentianaceae = GENTI; Loganiaceae = LOGAN; Plocospermataceae = PLOCO; Potaliaceae = POTAL; Retziaceae = RETZI; Spigeliaceae = SPIGE; and Strychnaceae = STRYC.
[i] "=" denotes the same family as the previous author; "–" denotes a tribe not mentioned by the author; "?" denotes uncertain position according to the author.
[j] In the subfamily Loganioideae.
[k] In the subfamily Desfontainioideae.
[l] In the subfamily Plocospermatoideae.
[m] In the subfamily Retzioideae.
[n] The genus *Peltanthera* was included in the family Antoniaceae.
[o] Included in the order Scrophulariales.

houts, 1980) the family was circumscribed in its widest sense, classified into ten tribes (Table 1).

The second question asked in this study concerns the most plausible position of the Rubiaceae and its outgroup within the Gentianales-Loganiaceae complex. Outgroup comparison is used for polarizing characters (Watrous and Wheeler, 1981). In searching for the best outgroup (the sister group) to the Rubiaceae, representatives of eight tribes of the Loganiaceae and a few other families of the Gentianales were analyzed using morphological and chemical data.

MATERIAL AND METHODS

Thirty-three genera of the Rubiaceae (Table 2) representing 18 different tribes (including the same species as studied by Bremer and Jansen, 1991, Table 1), 13 genera of the Loganiaceae (Table 1) representing eight of ten

TABLE 2. *Studied genera of the Rubiaceae and a comparison of their subfamilial classification*

Genera[a]	Verdcourt[b]	Bremekamp[c]	Robbrecht[d]
Tribe Gardenieae	CINC[e]	IXOR	=[f]
Gardenia			
Mitriostigma			
Tribe Pavetteae	CINC	IXOR	=
Ixora			
Enterospermum			
Tribe Coffeeae	—	—	IXOR
Coffea			
Tribe Chiococceae	CINC	IXOR	ANTI
Erithalis			
Chiococca			
Tribe Vanguerieae	CINC	IXOR	ANTI
Vangueria			
Tribe Cinchoneae	CINC	=	=
Calycophyllum			
Cinchona			
Coutarea			
Exostema			
Luculia			
Tribe Naucleeae	CINC	=	=
Haldina			
Cephalanthus[d]			
Tribe Condamineeae	—	CINC	=
Pinckneya			
Pogonopus			
Tribe Rondeletieae	CINC	=	=
Rogiera			
Tribe Catesbaeeae	CINC	IXOR	?
Catesbaea			
Tribe Isertieae	CINC	=	=
Mussaenda			
Tribe Psychotrieae	RUBI	=	=
Hydnophytum			
Myrmecodia			
Psychotria			
Tribe Hamelieae	RUBI	=	=
Hamelia			
Hoffmannia			
Tribe Hedyotideae	RUBI	=	=
Bouvardia			
Pentas			
Tribe Anthospermeae	RUBI	=	=
Coprosma			
Nertera			
Tribe Coccocypseleae	RUBI	=	=
Coccocypselum			
Tribe Rubieae	RUBI	=	=
Galium			
Tribe Guettardeae	ANTI	=	=
Antirhea			
Guettarda			

[a] Genera ordered as in Bremer and Jansen (1991); according to the classification of Bremekamp (1954, 1966) and Bridson and Verdcourt (1988).

[b] Verdcourt, 1958.

[c] Bremekamp, 1966.

[d] Robbrecht, 1988; the monotypic tribe Cephalantheae (genus *Cephalanthus*) is treated as a member of the subfamily Antirheoideae (ANTI).

[e] The subfamilies are Antirheoideae = ANTI; Cinchonoideae = CINC; Ixoroideae = IXOR; and Rubioideae = RUBI. These names are used by the authors, but the circumscriptions of the subfamilies differ.

[f] "=" Denotes the same subfamily as the previous author; "–" denotes a tribe not mentioned by the author or included in another tribe; "?" denotes uncertain position according to the author.

TABLE 3. *Characters and character states used in the cladistic analyses*

Habit

1.[a] 0—Stems woody
      1—Stems herbaceous[b]

2. 0—Without distinct lenticels on stems
    1—With distinct lenticels on stems

3. 0—Without myrmecophily
    1—With myrmecophily

4. 0—External indumentum not of articulate type[c]
    1—External indumentum of articulate type

5. 0—Plants bisexual
    1—Plants unisexual

Leaves

6. 0—Stipules absent
    1—Stipules a ridge or scar
    2—Stipules interpetiolar

7. 0—Colleters absent
    1—Colleters present

8. 0—Domatia absent
    1—Domatia present

9. 0—Leaves without "moiré" striation pattern
    1—Leaves with "moiré" striation pattern

Inflorescences and flowers

10. 0—Inflorescences axillary
    1—Inflorescences terminal

11. 0—Flowers not in dense heads
    1—Flowers in dense heads

12. 0—Without clubshaped bracts between the flowers
    1—With clubshaped bracts between the flowers

13. 0—Flowers homostylous
    1—Flowers heterostylous

14. 0—Ixoroid pollen mechanism absent
    1—Ixoroid pollen mechanism present[d]

15. 0—Without semaphylls
    1—With semaphylls[e]

Corolla

16. 0—Aestivation valvate
    1—Aestivation contorted to the right
    2—Aestivation imbricate
    3—Aestivation contorted to the left

17. 0—Corolla rotate
    1—Corolla salvershaped
    2—Corolla funnelshaped
    3—Corolla campanulate

18. 0—Inside of corolla glabrous
    1—Upper part of corolla hairy
    2—Lower part of corolla hairy
    3—Corolla irregularly hairy

Stamens

19. 0—Stamens inserted at the base of the corolla
    1—Stamens inserted near the mouth of the corolla
    2—Stamens inserted at least one-fifth down from the mouth of the corolla, but not at the base

20. 0—Stamens not fused into a basal ring
    1—Stamens fused into a basal ring[f]

21. 0—Filaments glabrous
    1—Filaments hairy

22. 0—Anthers not linear
    1—Anthers linear

23. 0—Anthers latrorse
    1—Anthers introrse
    2—Anthers extrorse

TABLE 3. *Continued*

Pistil

24. 0—Ovary superior
     1—Ovary inferior
25. 0—Placentation axile
     1—Placentation parietal
26. 0—Style glabrous
     1—Style hairy
27. 0—Stigma entire
     1—Stigma lobate
28. 0—Stigma clavate to scarcely widened
     1—Stigma cylindric to capitate
29. 0—Stigma glabrous
     1—Stigma hairy

Fruit

30. 0—Drupe
     1—Berry
     2—Capsule
     3—Nut[g]
31. 0—Without lenticels on the fruits
     1—With lenticels on the fruits
32. 0—Fruit only syncarpous
     1—Basal parts of capsule syncarpous and the top apocarpous
     2—Capsule bivalved, syncarpous, but mature capsule splitting
        from the middle resulting in two "apocarpous" parts.

Seeds

33. 0—One seed per carpel
     1—Seeds numerous in each caprel
34. 0—Seeds without wing
     1—Seeds with wing
35. 0—Seeds without hair tuft
     1—Seeds with hair tuft
36. 0—Testacells smooth
     1—Testacells with small pits
     2—Testacells with ridges, i.e., very large pits
     3—Testacells granulate to tuberculate
37. 0—Embryo at most one-fourth of the size of the endosperm
     1—Embryo at least one-fourth of the size of the endosperm
38. 0—With distinct endosperm
     1—Without endosperm

Chemistry

39. 0—Iridoids present
     1—Iridoids absent
40. 0—Xanthones absent
     1—Xanthones present[h]
41. 0—Triterpenes present
     1—Triterpenes absent
42. 0—Complex indole-alkaloids absent
     1—Complex indole-alkaloids present
43. 0—C-16 skeleton used as starting center in alkaloid bonds
     1—C-16 skeleton is never used as starting center in alkaloid
        bonds[i]

Anatomy

44. 0—Without raphides
     1—With raphides
45. 0—Without internal phloem
     1—With internal phloem

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[a] The character numbers correspond to those in the data matrix (Table 4).

[b] Also plants with a basal woodiness are included.

[c] Cf. Robbrecht, 1988.

[d] Cf. Bremekamp, 1966.

[e] Cf. Leppik, 1956, 1977.

[f] Cf. Bremer, 1992.

tribes (Leeuwenberg and Leenhouts, 1980), and finally *Nerium* (Apocynaceae), *Exacum* (Gentianaceae), *Cornus* (Cornaceae), and *Olea* (Oleaceae) were investigated. Unless otherwise stated in Bremer and Jansen (1991, Table 1) all specimens were studied on herbarium material at the Swedish Museum of Natural History (S) or at Stockholm University (SUNIV). Genera have been used as the terminal taxa in the analyses.

The circumscription of the Loganiaceae differs significantly between different authors (cf. Table 1). We followed the latest major treatment (Leeuwenberg and Leenhouts, 1980) except that we excluded the tribes Buddlejeae and Retzieae as we found no convincing evidence for their inclusion in the Loganiaceae or Gentianales. It has been suggested that the Buddlejeae form a separate family, the Buddlejaceae, close to the Scrophulariaceae in the order Scrophulariales (Dahlgren, 1980b, 1983; Cronquist, 1981, 1988; Takhtajan, 1987). This is supported by shared morphological features such as the absence of colleters, presence of stellate hairs, and absence of internal phloem, by sequence data of the *rbc*L gene (Olmstead and Palmer, unpublished data), and perhaps even more conspicuously by the chemical similarities such as the presence of verbascoside and iridoides of aucubin type (Jensen, 1992). The monotypic Retzieae have also been accepted as a separate family, the Retziaceae, and have been placed close to the Stilbaceae in the Scrophulariales, because of their chemical constituents (Dahlgren et al., 1979). The position of Retziaceae in the Scrophulariales is further supported by embryological data such as cellular endosperm, the formation of endosperm haustoria and well-developed hypostase (Engell, 1987), and molecular data as *rbc*L sequences (Bremer and Olmstead, unpublished data).

Characters (Table 3) have been compiled from the study of herbarium or living material and from literature studies (Schumann, 1891; Solereder, 1892–1895, 1899; Rendle, 1952; Metcalfe and Chalk, 1957, 1983; Leeuwenberg, 1961a, b, 1963, 1967, 1969a, b, 1975, 1977; Leenhouts, 1963; Cronquist, 1968; Polunin, 1969; Hutchinson, 1973; Corner, 1976; Heywood, 1978; Dahlgren, 1979, 1980b, 1983; Kiew, 1979; Bisset, 1980; Bisset et al., 1980; Conn, 1980; Hakki, 1980; Kisakürek and Hesse, 1980; Leeuwenberg and Leenhouts, 1980; Mennega, 1980; Klackenberg, 1985; Robbrecht and Puff, 1986; Robbrecht, 1988; Hegnauer, 1989). Character states of each taxon are presented in the data matrix in Table 4. In those cases where the characters are variable within a genus, the following principles have been used: in a genus that contains two or more states we have tried to determine the apomorphic state by comparisons to closely related genera and coded the genus under consideration as having the plesiomorphic state; in cases where we have not been able to determine polarity of the characters, the state was coded

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←

[g] Includes all kinds of indehiscent fruits with hard, dry pericarp.

[h] Three different xanthones have been found in *Anthocleista* (Okorie, 1976; Bisset, 1980). The same compounds also occur in the Gentianaceae, e.g., in *Swertzia* and *Gentiana* (Carpenter et al., 1969). *Exacum* is not investigated, but is coded with "1."

[i] Cf. Kisakürek and Hesse, 1980.

TABLE 4. *Data matrix of 45 morphological and chemical characters in the phylogenetic analyses of the Gentianales*

	Character no.[a]				
	0000000001 1234567890	1111111112 1234567890	2222222223 1234567890	3333333334 1234567890	44444 12345
Cornus					
Olea					
	0000010000	0000000000	0001000000	0000000000	01000
	0000000000	0000000000	0020000000	0000000000	00000
Mitreola	100–021001	0000023120	000?000002	0110010000	10001
Spigelia	100?021001	00000020?0	0000000012	01100?0010	10001
Geniostoma	000–021000	00000?3110	0000000002	00100?0000	?0001
Logania	?000001100?	00000?3120	0000000002	0110010000	00001
Gardneria	000–011000	00000000?0	0000000001	0000000000	01001
Strychnos	000–01100?	00000031?0	000000000?1	00?0000000	01001
Plocosperma	0000000000	0000022020	0000101002	02101?101?	?0000
Gelsemium	000001100?	0010022020	0010001002	0011020000	11101
Mostuea	000002100?	0010022320	0010001002	00?1020000	11101
Norrisia	010001?001	0000001110	0010000002	00?10?1010	10001
Usteria	0000011001	0000101210	0000000002	0011021070	1?001
Anthocleista	000–021001	0000012010	0000000101	0010010001	10001
Desfontainia	0000000000	00000?1010	0000000001	001000000?	00000
Exacum	100–010001	0000013000	0000000002	00100?0001	?0001
Nerium	0000001001	0000031320	0000100002	0210111010	00001
Luculia	0100021101	0010021010	0001001002	00110200?0	??100
Cinchona	0000021101	0010001120	0001001002	00110?0000	01100
Cephalanthus	0100021111	1101022010	0001000103	0000021000	01100
Haldina	0100021110	1101002010	0001000102	0011020000	01100
Rogiera	0000021000	0000021110	0001001002	0010020000	?1100
Erithalis	0000021000	0000020001	1101000000	00000000?0	??100
Chiococca	0000021000	0000022001	1101000000	00000000?0	??100
Exostema	0100021100	0000022001	0101000002	00110?00?0	??100
Coutarea	0100021101	0000022001	1101000002	1011000000	??100
Pogonopus	0100021001	0010102220	0001001002	10100010?0	??100
Pinckneya	0100021101	0000102320	0001001002	10110?00?0	??100
Calycophyllum	0100021101	0000102210	0001001002	00110200?0	??100
Mussaenda	0100021001	0010102120	0001001001	0010020000	00100
Guettarda	0100021010	0000021310	0001000000	0000001100	?1100
Antirhea	0100021110	0000021010	0001001000	0000021100	??100
Vangueria	0?00021000	00?1002210	0001000100	0000021000	00100
Enterospermum	0000021101	0001032310	000101?000	0000000000	?0100
Ixora	0000021001	0001031010	0001011000	0000000000	00100
Coffea	0000021100	0001032010	0101001000	00000000?0	??100
Mitriostigma	0000021101	0001032310	0001001001	0010000?0?0	??100
Catesbaea	0?00021001	0000022001	0101000001	00100?00?0	??100
Gardenia	0000021001	0001032010	0101011001	0010020000	00100
Hamelia	0000021001	0000022020	0101000001	0010030000	?1110
Hoffmannia	0000021000	0000022020	0001000001	00100300?0	??110
Pentas	1001021001	0010002110	0001001002	0010000000	?0110
Bouvardia	1001020001	0010002110	0001001002	0011000000	?0110
Galium	100–021001	0010002010	0001001003	0000000000	?0110
Psychotria	0101021001	0010002210	0001001000	0000000000	0?110
Hydnophytum	001–020000	00?0002210	0001001000	0000000000	?0110
Myrmecodia	001–020000	00?0002210	0001001000	00000000?0	??110
Nertera	1000121000	0010002020	0001001010	0000000000	?0110
Coprosma	000–121000	0010002000	0001001010	0000000000	?0110
Coccocypselum	1000021000	1010002?00	0001001001	00100?0000	?0110

[a] The character numbers and their codings correspond to those in Table 3. Characters 2–5, 8, 9, 11, 12, 14, 15, 20–22, 26, 28–31, 38, and 44 were not included in the Loganiaceae analyses with *Cornus,* or *Cornus* and *Olea* together, as the outgroups. In analysis with *Olea* as the outgroup characters 23 and 24 were also excluded. Characters 6, 8, 10, 23–25, 31, 32, 34, 35, 39–43, and 45 were not included in the Rubiaceae analyses.

with a question mark (?). Taxa were coded with a hyphen (-) when the character was inapplicable.

The data matrix is a compilation for all taxa and of all characters. In some analyses only parts of the matrix were used (see footnote *a* of Table 4). Only phylogenetically informative characters were included in the analyses. Au-

tapomorphies were excluded, except in multistate characters where one of the apomorphic states is represented by a single taxon. Characters shared by all ingroup taxa have also been excluded. Characters that were inapplicable or unknown in the outgroup were deleted in the analysis using that specific outgroup. Thus various ele-
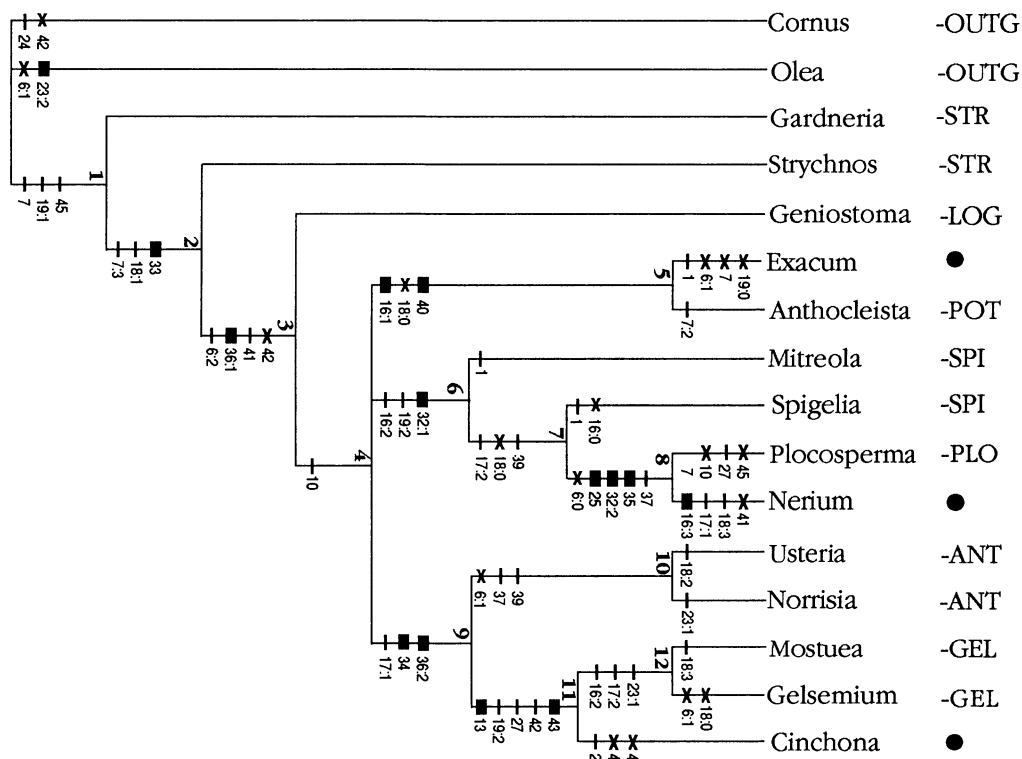
Fig. 1. Sample cladogram of four equally parsimonious trees constructed using morphological characters of the Loganiaceae. The tree is 67 steps long (25 characters of which nine are multistate characters) with a consistency index of 0.53 and a retention index of 0.62. Autapomorphies are excluded, but not multistate characters where one of the states is represented by a single taxon. The tribal position of each taxon is indicated by a three-letter suffix corresponding to the tribes in Table 1. Non-Loganiaceae genera are indicated with a dot. Characters are numbered as in Table 3. Heavy bars indicate a character with a consistency index of 1.0, thin bars indicate a character with a consistency index less than 1.0, and crosses indicate reversals.

ments of the data matrix were used in the different analyses.

In a first set of analyses aimed at searching for the most plausible sister group to the Rubiaceae within the Gentianales, all investigated taxa were included (48 genera of which 33 belong to the Rubiaceae), with *Cornus* and *Olea* as the outgroup. In a second set of analyses, focused on the interrelationships and character distribution within the Loganiaceae, the Rubiaceae were represented by a single taxon, the genus *Cinchona*. *Cinchona* was chosen instead of *Luculia* (functional outgroup in the cpDNA analysis by Bremer and Jansen, 1991), because the latter genus has more question marks in the data matrix and differs only in three characters from *Cinchona*. In a third set of analyses only the Rubiaceae genera were included, first with the tribe Gelsemieae (Loganiaceae) as the outgroup and then with *Luculia* as a functional outgroup. The latter analysis was compared to the cpDNA analysis (Bremer and Jansen, 1991) of the same taxa. The morphological and molecular analyses of the Rubiaceae were also tested for incongruency (kindly performed by J. S. Farris; Mickevich and Farris, 1981).

A combined analysis of the morphological and cpDNA data (Bremer and Jansen, 1991) for the Rubiaceae was also performed.

The data matrix (Table 4) was analyzed with Farris's (1988) Hennig86 program, using Wagner parsimony. The initial trees were calculated by the mhennig method, and

the options were mh* and bb*. All multistate characters were treated as nonadditive and all characters were weighted equally in the first analyses. Successive weighting of characters (Farris, 1969) with subsequent analyses in order to choose among equally parsimonious solutions (Carpenter, 1988) was also done with Hennig86.

RESULTS

Analysis of the Gentianales—The Loganiaceae (13 taxa), the Rubiaceae (33 taxa), and one representative each of the Apocynaceae and the Gentianaceae were analyzed with the distantly related taxa *Cornus* (Cornaceae) and *Olea* (Oleaceae) together as the outgroup. The analysis terminated in computer memory overflow when too many equally parsimonious cladograms were found.

Analyses of the Loganiaceae (Figs. 1, 2)—The 13 genera of the Loganiaceae, representatives of the Apocynaceae (*Nerium*), the Gentianaceae (*Exacum*), and the Rubiaceae (*Cinchona*), were analyzed with *Cornus, Olea,* and *Cornus* and *Olea* together, as different outgroups. These analyses resulted in 33, 45, and 79 equally parsimonious trees, respectively. The strict consensus trees from these analyses were much collapsed at the bases because the genera *Desfontainia* and *Logania* had very different positions in the different trees. Thus, in the following analyses they were excluded.

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 ═══ Cornus-OUTG
 ═══ Olea-OUTG
       ═══ Gardneria-STR
        ═══ Strychnos-STR
         ═══ Geniostoma-LOG
           ═══ Anthocleista-POT
           ═══ Exacum ●
             ═══ Mitreola-SPI
              ═══ Spigelia-SPI
                ═══ Plocosperma-PLO
                ═══ Nerium ●
             ═══ Usteria-ANT
             ═══ Norrisia-ANT
              ═══ Cinchona ●
                ═══ Gelsemium-GEL
                ═══ Mostuea-GEL
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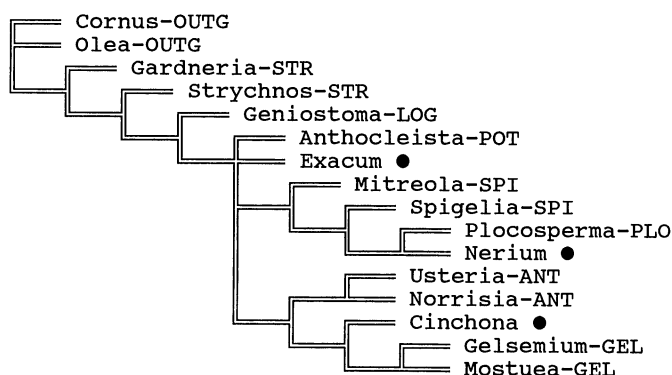
Fig. 2. Strict consensus tree for the four equally parsimonious trees of Loganiaceae constructed using morphological characters. The tribal position of each taxon is indicated by a three-letter suffix corresponding to the tribes in Table 1. Non-Loganiaceae genera are indicated with a dot.

With *Desfontainia* and *Logania* excluded the analyses were again performed with the same outgroups. In the analysis with *Olea* as the outgroup we used 23 characters. There were six equally parsimonious cladograms found. They were 62 steps long with a consistency index of 0.53 and a retention index of 0.59. When *Cornus* or *Cornus* and *Olea* together were used as the outgroups we used 25 characters. The last two analyses resulted in four equally parsimonious trees, which were identical between the two analyses. These four trees were also found among the six trees from the analysis with *Olea*. The four equally parsimonious cladograms, from the analyses with *Cornus* or *Cornus* and *Olea* respectively, were 64 or 67 steps long, with a consistency index of 0.54 or 0.53, respectively, and a retention index of 0.60 or 0.62, respectively. The differences in steps, retention index, and consistency index between the two analyses are mainly due to the different number of outgroup taxa. After successive weighting and subsequent analyses three of the four cladograms remained. We have chosen to illustrate the one with the least resolution (Fig. 1) that was most similar to the strict consensus tree (Fig. 2).

The non-Loganiaceae taxa *Nerium* (Apocynaceae), *Exacum* (Gentianaceae), and *Cinchona* (Rubiaceae) were all found to have their closest relatives within the Loganiaceae, hence indicating that the Loganiaceae (Leeuwenberg and Leenhouts, 1980) are paraphyletic and ought to be reclassified. The Rubiaceae were found to be the sister group to the tribe Gelsemieae (*Gelsemium* and *Mostuea*) of the Loganiaceae. *Anthocleista* (Potalieae) and *Exacum* came out as sister taxa and *Plocosperma* (Plocospermeae) closest to *Nerium*. The analyses also indicated that the tribes Spigelieae and Strychneae are paraphyletic, while the Antonieae and Gelsemieae are monophyletic.

Notes on specific characters on the tree (Fig. 1)—In the Loganiaceae analysis 25 characters were used, seven of which are multistate characters and the remaining binary characters. The consistency index for each character varies between 1.0 and 0.33; nine characters or 36% of the characters are not homoplastic (consistency index 1.0). The following characters are or have been used for classification or are of biological/ecological interest.

Character No. 6—Interpetiolar stipules have long been regarded as a key character for the Rubiaceae, but stipules also occur in parts of the Loganiaceae (node 3). In *Nerium* (Apocynaceae) and *Exacum* (Gentianaceae) no interpetiolar stipules have been found, but interpetiolar stipules or stipular lines occur in other genera of these families, and the absence of this character is probably due to reversions (node 8, and *Exacum*).

Character No. 13—Heterostylous flowers are very common in the Rubiaceae (Robbrecht, 1988) and also characterize the tribe Gelsemieae in the Loganiaceae. This is one of the synapomorphies linking the Gelsemieae and Rubiaceae (node 11).

Character No. 16—Contorted aestivation is a common feature in the Gentianales (former Contortae, cf. Bartling, 1830). However, the aestivation varies within the order and even within the families. In this study, *Exacum* (Gentianaceae) and *Anthocleista* (Loganiaceae) have right-contorted corollas (node 5), in contrast to *Nerium* (Apocynaceae) with left-contorted corollas.

Character No. 32—Within the Gentianales there is a successive development from syncarpy to secondary apocarpy (cf. Endress, Jenny, and Fallen, 1983). The most common feature is syncarpy, but the Apocynaceae and Asclepiadaceae are characterized by secondary apocarpy with fused stigmas. In the tribe Spigelieae (Loganiaceae) there is an intermediate state with partly apocarpous gynoecium which will result in a bilobed capsule (node 6). In the Apocynaceae and Asclepiadaceae the gynoecium often develops into two dry follicles. Nevertheless, *Plocosperma* (Loganiaceae) and *Nerium* (Apocynaceae) have bivalved, syncarpous capsules (node 8).

Character No. 35—Seeds with a hairtuft are a synapomorphy for capsule-bearing species of the Apocynaceae and *Plocosperma* (Loganiaceae, node 8). However, this character occurs also in one genus of the Rubiaceae (*Hillia* sensu lato).

Character No. 40—Xanthones occur in the Gentianaceae, Guttiferae, Moraceae, and Polygalaceae, families usually regarded as very distantly related. Carpenter, Locksley, and Scheinmann (1969) regarded 29 of the xanthones as unique for the Gentianaceae, but three of them (gentianacaulin, decussatin, and methyl-swertianin) have now been found also in *Anthocleista* (Loganiaceae; Okorie, 1976; Bisset, 1980). *Exacum* (Gentianaceae) has not been investigated, but we assume that xanthones occur also in *Exacum* (node 5) as in many other Gentianaceae genera.

Character No. 43—In the synthesis of indole-alkaloids from seco-loganin, a carbon skeleton, C-16 or C-17, serves as the starting center for the new bond (Kisakürek and Hesse, 1980). Gelsemieae and Rubiaceae are the only taxa that produce alkaloids that never start from C-16, only from C-17 (node 11). The other tribes of the Loganiaceae and Apocynaceae use either C-16 or C-16 together with C-17 as the starting center.

Analyses of the Rubiaceae (Figs. 3–6) — In the first analysis of the Rubiaceae the tribe Gelsemieae, represented by *Gelsemium* and *Mostuea,* was used as the outgroup. This tribe was the most plausible sister group according to the prior analyses (Figs. 1, 2). With this outgroup, however, the analysis terminated in computer memory overflow because too many trees were found. So the genus *Luculia* was used as the functional outgroup, as in the cpDNA analysis (Bremer and Jansen, 1991). There were 29 phylogenetically informative characters, and the analysis resulted in eight equally parsimonious cladograms 91 steps long, with a consistency index of 0.40 and a retention index of 0.69. After successive weighting and additional analyses we found two trees that were identical to two of the initial eight trees. We have chosen to illustate one of the eight trees (Fig. 3), which is identical to one of the two trees retained after successive weighting. A strict consensus tree based on the eight trees is also presented (Fig. 4).

In the analysis with the combined data sets from cpDNA and morphology and using equal weighting for each character there were four equally parsimonious trees found. The resulting trees resemble the cpDNA trees (indicated in Fig. 5). The four trees based on 161 + 29 characters were 453 steps long with a consistency index of 0.43 and a retention index of 0.75.

The morphological and molecular trees were not found to be significantly incongruent; the error rate was 203/1,000 and 3.1% (14 of the 453 steps) of the tree length in the combined analysis was due to incongruence between the two analyses.

A strict and combinable component consensus (Bremer, 1990) tree of the morphological trees and the cpDNA trees is also presented (Fig. 6).

Notes on specific characters on the tree (Fig. 3) — In the Rubiaceae analyses 29 characters were used, six of which are multistate and the remaining binary. The consistency index for each character varies between 1.0 and 0.2; ten characters or 34% of the characters are not homoplastic (consistency index 1.0). The following characters are or have been used for classification or are of biological/ecological interest.

Character No. 15 — One very conspicuous morphological character occurring in a few genera of the Rubiaceae is the "calycophyll." In the inflorescences, some flowers have one or more enlarged, more or less brightly colored calyx lobes. It has been rejected as a diagnostic character above generic level as this is supposed to be an ecological character, but also because it is so widely distributed in the family (occurring in ten tribes). However, Leppik (1956, 1977) suggested that the character should be split into two different characters: "pterophyll" with a function for fruit and seed distribution and a (calyx-born) "semaphyll" (a calyx lobe) with a function as an optical attractant for nectar- or pollen-seeking insects or other animals. If we consider the semaphyll, it occurs in seven tribes within the family (Robbrecht, 1988). However, the morphological structures differ between the groups and are thus probably not homologous. In Rondeletieae and in Alberteae they are narrow in outline, coriacious, and probably also

function as a pterophyll in the fruit stage. In Hedyotideae (*Carpalea*) the whole calyx is enlarged and thus not homologous to the single enlarged calyx lobe of the other taxa. The semaphylls of *Mussaenda* (Iserteae), *Calycophyllum* (Cinchonoideae), *Pogonopus* (Condamineeae), and *Pinckneya* (Condamineeae) are all very similar, soft, white, pink, or red colored and very broad in outline. These four genera from three different tribes have never been considered as a monophyletic group. The same grouping was also encountered in the cpDNA analysis (Bremer and Jansen, 1991). In the genus *Morinda* (Morindeae) the semaphylls are similar to those four genera (node 4).

Character No. 20 — The full merit of basally fused stamens as a diagnostic character in the Rubiaceae has just been recognized (Bremer, 1992). Formerly it was mentioned as a character in the Chiococceae (sensu Hooker f.; cf. Schumann, 1891), in the subtribe Portlandiinae of the Condamineeae, and in various taxa of the Cinchonoideae (Aiello, 1979). These taxa were even placed in different subfamilies (Robbrecht, 1988). In the present study the fused stamens are shown to be an unique character (node 28). Further support for this group (a widened Chiococceae, Bremer, 1992) comes from the cpDNA analysis (Bremer and Jansen, 1991). However, the position of the *Catesbaea* is not settled, as the cpDNA data support a position outside the Chiococceae.

Characters No. 30 and 33 — The different fruit types, whether fleshy or dry, and the number of seeds per carpel have had a great impact on the Rubiaceae classification. To the middle of this century, fruit morphology was one of the main criteria for distinguishing tribes and subfamilies (Schumann, 1891). Verdcourt (1958) and Bremekamp (1966) rejected these characters, as they were supposed to be "ecological and not morphological," and as they sometimes could vary within a single genus. In this study seed number reduction (nodes 10, 24, 31) and origin of fleshy fruits (in *Mussaenda,* nodes 10, 16, 31) are not unique characters, although they have evolved only a limited number of times. In the Rubiaceae most large groups are characterized by the same fruit types.

Character No. 36 — The use of testa (seed-coat) structures in Rubiaceae classification was introduced by Bremekamp (1947, 1952, 1966), who characterized the whole subfamily Cinchonoideae as having testa cells with large pits. More detailed studies (cf. Robbrecht, 1988) have shown that the testa structures are more complex and diverse than depicted by Bremekamp. As indicated before (Bremer, 1987) and shown in this study, the "pitted" or ridged testa cells are not unique characters for the Cinchonoideae but plesiomorphic in the Rubiaceae, as they occur also in the Gelsemieae and Antonieae in the Loganiaceae (Fig. 1, node 9).

Character No. 44 — Raphides are found as bundles of needlelike Ca-oxalate crystals. In this study the character occurs in all genera of the Rubioideae (node 7), but also in the tribe Hamelieae (node 22). The occurrence of raphides has been used as a cardinal character for the subfamily Rubioideae (Verdcourt, 1958; Bremekamp, 1966), and is
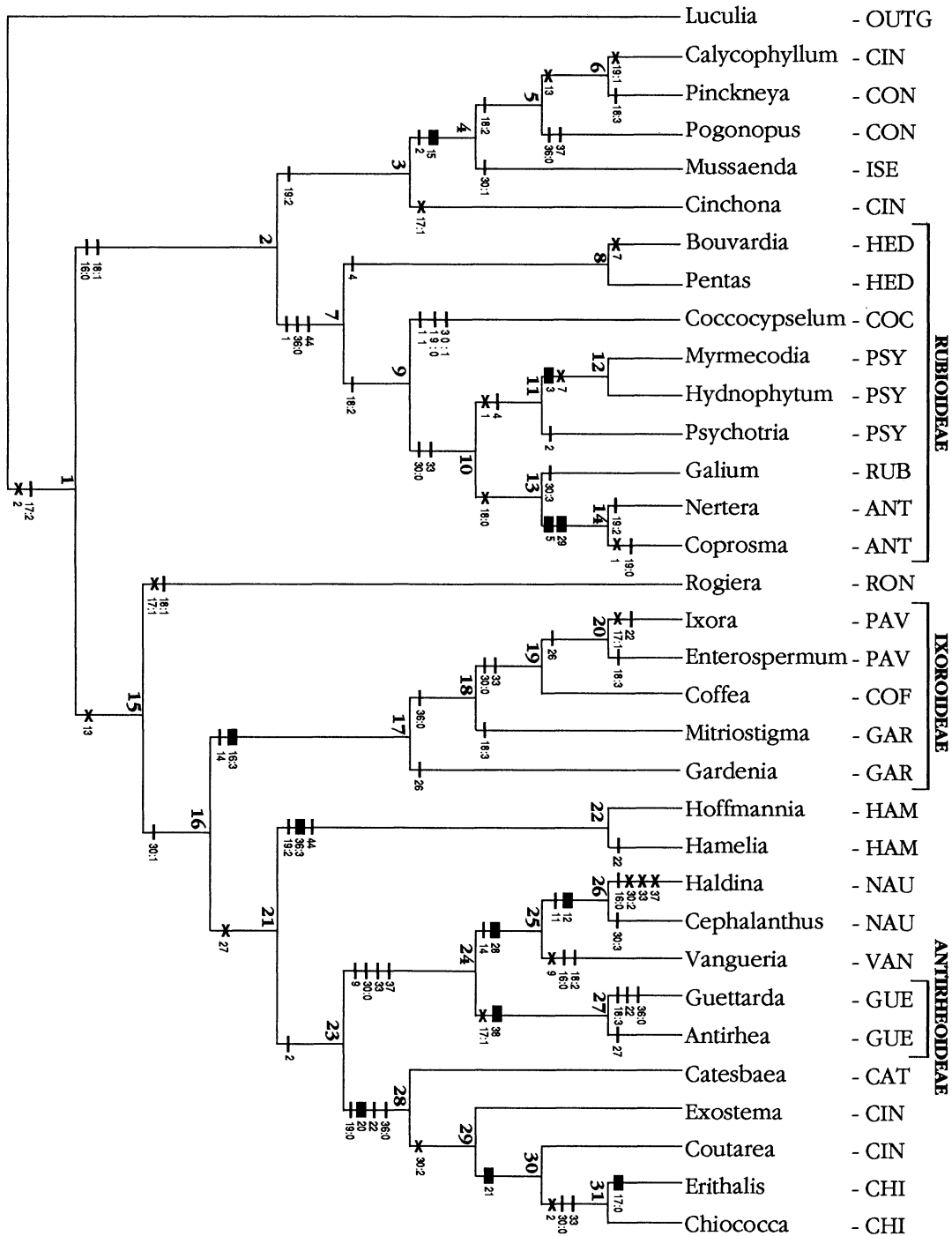
Fig. 3.   Sample cladogram of eight equally parsimonious trees constructed using morphological characters of the Rubiaceae. The tree is 91 steps long (29 characters of which six are multistate characters) with a consistency index of 0.40 and a retention index of 0.69. Autapomorphies are excluded, but not multistate characters where one of the states is represented by a single taxon. The tribal position of each taxon is indicated by a three-letter suffix corresponding to the tribes in Table 2. Characters are numbered as in Table 3. Heavy bars indicate a character with consistency index of 1.0, thin bars indicate a character with a consistency index less than 1.0, and crosses indicate reversals.

the main reason why the Hamelieae were included in that subfamily (Bremekamp, 1952). However, it is documented that this character occurs sporadically outside the Rubioideae (in the Rubiaceae; for review see Robbrecht, 1988).

Comparisons between the molecular and morphological analyses—If we compare the morphological (Figs. 3, 4) and cpDNA analysis (Bremer and Jansen, 1991) of the Rubiaceae, the differences are striking. The strict consensus trees show that the morphological tree is much less

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┌── Luculia-OUTG
│      ┌── Cinchona-CIN ?
│      │   ┌── Mussaenda-ISE
│      │   │   ┌── Pogonopus-CON        ┐ 1
│      │   │   │   ┌── Pinckneya-CON     │
│      │   │   └───┴── Calycophyllum-CIN ┘
│      │   │       ┌── Bouvardia-HED       ┐
│      │   │       ├── Pentas-HED          │
│      │   │       ├── Coccocypselum-COC   │
│      │   │       │       ┌── Myrmecodia-PSY  ┐    │ 4
│      │   │       │       └── Hydnophytum-PSY ┘ 2  │
│      │   │       │   ┌── Psychotria-PSY       ┘   │
│      │   │       │   ├── Galium-RUB              │
│      │   │       │   │   ┌── Nertera-ANT    ┐ 3  │
│      │   │       └───┴───┴── Coprosma-ANT   ┘    ┘
│   ┌── Rogiera-RON ?
│   │   ┌── Gardenia-GAR             ┐
│   │   │   ┌── Mitriostigma-GAR     │
│   │   │   │   ┌── Coffea-COF        │ 5
│   │   │   │   ├── Enterospermum-PAV │
│   │   └───┴───┴── Ixora-PAV        ┘
│   ┌── Hamelia-HAM     ┐ 6
│   └── Hoffmannia-HAM  ┘
│       ┌── Antihea-GUE    ┐ 7
│       │── Guettarda-GUE  ┘
│       ├── Vangueria-VAN ?
│       │   ┌── Cephalanthus-NAU ┐ 8
│       └───┴── Haldina-NAU       ┘
│           ┌── Chiococca-CHI  ┐
│           ├── Erithalis-CHI  │ 9
│           ├── Coutarea-CIN   ┘
└───────────┴── Exostema-CIN
                └── Catesbaea-CAT ?
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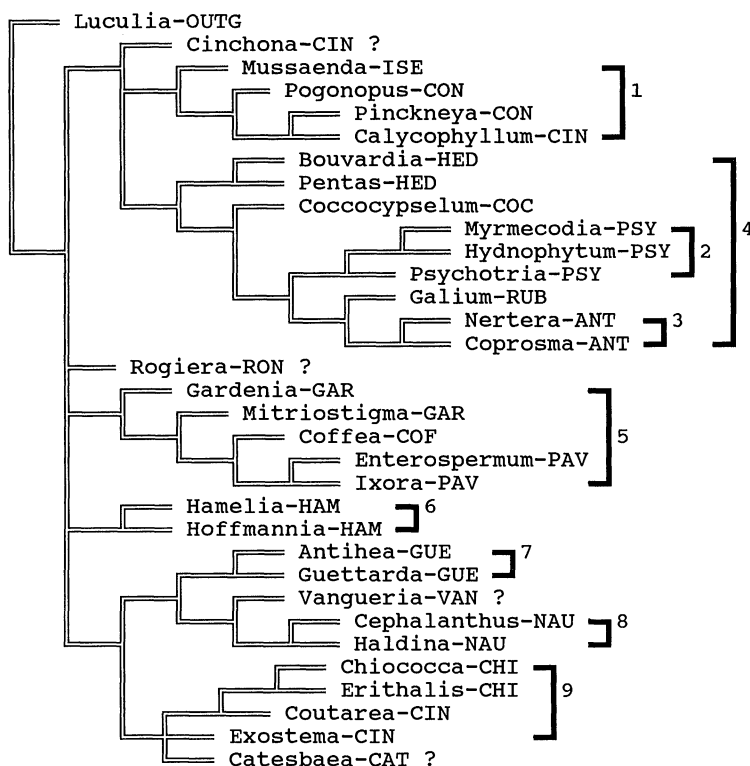
Fig. 4. Strict consensus tree for the eight equally parsimonious trees of Rubiaceae, obtained using morphological characters. Brackets and numbers denote congruent groupings of taxa between this analysis and an analysis using cpDNA from the same taxa (Bremer and Jansen, 1991). Group 1 = taxa with calycophyll; 2 = tribe Psychotrieae; 3 = tribe Anthospermeae; 4 = subfamily Rubioideae; 5 = subfamily Ixoroideae; 6 = tribe Hamelieae; 7 = tribe Guettardeae; 8 = tribe Naucleeae; 9 = a widened tribe Chiococceae (Bremer, 1992). Question marks indicate taxa with uncertain position. For further explanation refer to Results and Discussion.

resolved, with several basal polytomies, while the cpDNA tree shows only two polytomies, both due to uncertainty within the subfamily Ixoroideae (Bremer and Jansen, 1991, Fig. 3). We must remember here that the number of characters differs in the two analyses—in the morphological analysis there are 29 while there are 161 in the molecular analysis—and that the numbers probably have an effect on the resolution. The consistency index is lower for the morphological analysis (0.40, but 0.46 for the cpDNA data), and the retention index is also lower (0.69, but 0.78 for the cpDNA data). However, a detailed inspection of the consensus trees from the two analyses reveals the same groupings for most of the taxa (illustrated by brackets and numbers in Fig. 4). In both analyses three of the four subfamilies, the Rubioideae (4), Antirheoideae (6), and Ixoroideae (5) are monophyletic, although differently circumscribed than in Robbrecht (1988), while the subfamily Cinchonoideae is shown to be paraphyletic in both analyses. With the exception of the genera *Cinchona, Rogiera, Vangueria,* and *Catesbaea* with uncertain positions (indicated by a question mark in Fig. 4), all other genera are positioned in the same groups of taxa (1–9) in the two analyses. Of these nine groups, six (2–7) conform to the latest classification of the Rubiaceae (Robbrecht, 1988), while three groups of taxa (1, 8, 9) are incongruent with that classification. Of these groups numbers 1 and 9 are new groupings (Bremer and Jansen, 1991; Bremer, 1992), while number 8 (*Haldina* and *Cephalanthus,* tribe Nau-

cleeae) agrees with the earlier classifications of Verdcourt (1958) and Bremekamp (1966).

The basal branchings, or how the indicated groups of taxa are mutually related, differ between the analyses and must, so far, be interpreted as unsettled. However, one group of taxa, tribe Hamelieae (number 6), is in both analyses placed outside the subfamily Rubioideae where it is now housed (Robbrecht, 1988).

DISCUSSION

The most powerful approach in systematics available at present is to analyze both molecular and morphological data. However, different opinions occur whether we should pool the data and run one combined analysis (Barrett, Donoghue, and Sober, in press) or run separate analyses and compare the results, by consensus or other techniques (see below). Recently an interesting approach has been presented by Doyle (1992). He suggests that molecular characters should be included in a combined analysis together with morphological data, but that the molecular data should be coded as one multistate character. We recommend a mixed strategy as suggested by Hillis (1987). Most molecular analyses of plants, so far, are based on organelle DNA (as in this study), and the resulting phylogeny might not be the same as the species phylogeny; if we use only a combined analysis we can never identify discrepancies between the two data sets.
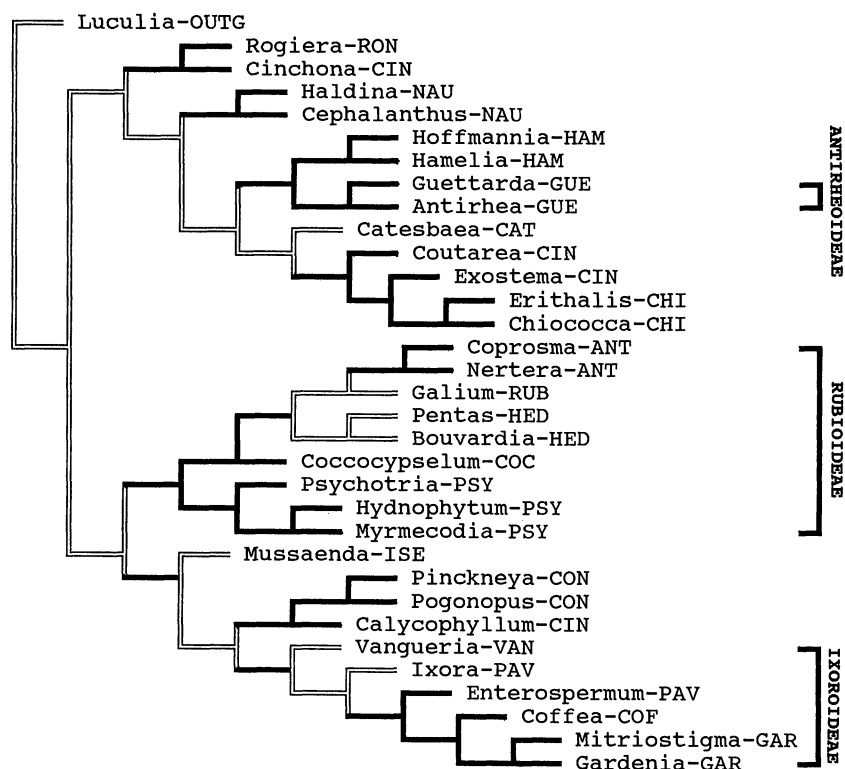
Fig. 5.   One of four equally parsimonious cladograms constructed using a combined data set of morphological (Tables 3, 4) and cpDNA characters (Bremer and Jansen, 1991) with equal weighting for each character. The tree is 453 steps long (29 + 161 characters), with a consistency index of 0.43 and a retention index of 0.75. Heavy lines denote identical branches as in the cpDNA analysis (Bremer and Jansen, 1991).

When we compare data sets it is important to consider what is meant by congruency and discrepancy. Sytsma (1990) examined discrepancies between different analyses (DNA and morphology) and found that except for real discrepancies (due to biological attributes of the organisms) many are due to procedural problems (apparent problems). In the present study the question posed also concerned this problem, i.e., if new relationships indicated from cpDNA are due to real differences. We found that the most important discrepancies occur between the results of cladistic analyses (both molecular and morphological) and the "accepted" classification, which is mainly based on phenetic similarities. The discrepancies are thus of the procedural kind. An important example is the subfamily Cinchonoideae, which is shown to be paraphyletic on both molecular and morphological data, when these were cladistically analyzed. The paraphyly of the Cinchonoideae is also indicated in a cladistic analysis of the tribe Cinchoneae (Andersson and Persson, 1991). Real discrepancies between morphological and molecular analyses seem to be of minor importance in this study.

Several methods for dealing with different data sets have been proposed and discussed. In Hillis (1987) and particularly in Sytsma (1990), different possibilities for comparing morphological and molecular analyses are presented: 1) combining the two data sets with equal weighting for each character; 2) analyzing the two sets independently and constructing a consensus tree; 3) generating a cladogram based on molecular characters and secondarily overlaying the morphological characters; and finally 4) generating a cladogram based on morphological characters and secondarily overlaying the molecular characters. If tree comparisons are chosen there are also other possibilities than just generating consensus trees (Mickevich and Farris, 1981; for review of methods see Page, 1989).

In this study we have applied the procedures with the combined data sets with equal weighting and the consensus method. We also advocate another very simple, but less rigorous, method for comparing results from different data sets. In a tree based on one data set, all branches that are identical or at least that include the same taxa as a tree based on another data set are denoted with brackets and numbers (Fig. 4). In systematics and in dealing with classification in particular, the grouping of taxa is sometimes more important than knowing all the interrelationships, so for that purpose the simplified comparison method (cf. Fig. 4) can be used. If we consider the results from this study it is clear that the different kinds of analyses (molecular, morphological, and the combined) show many congruent groupings. The discrepancies are of minor importance and would, in most cases, not yield a different classification.

Within this study both the molecular and the morphological analyses as well as the combined analysis support monophyly of the subfamilies Rubioideae, Ixoroideae, and Antirheoideae (using a narrow circumscription of the Antirheoideae including only the tribe Guettardeae; Verdcourt, 1958; Bremekamp, 1966). Robbrecht (1988) includes several more tribes in the subfamily Antirheoideae, here represented by the Chiococceae (*Chiococca* and

Erithalis) and Cephalantheae (*Cephalanthus*), and there is morphological support for such a circumscription. However, the molecular data (Bremer and Jansen, 1991) contradict such a circumscription. Robbrecht (1988) also included the tribe Vanguerieae (*Vangueria*) in the Antirheoideae. *Vangueria* is one of the few genera where the morphological and molecular data (Bremer and Jansen, 1991) are in conflict. The morphological data support a position close to *Cephalanthus* and *Haldina,* while the molecular data (and the combined data) support a position in the Ixoroideae where the genus was placed earlier (Bremekamp, 1966). With these conflicting results we must, at the moment, accept an unsettled position for the Vanguerieae and the narrow circumscription of the Antirheoideae.

The paraphyletic nature of the Cinchonoideae implied by the molecular analysis (Bremer and Jansen, 1991) is also corroborated by the morphological data. Within the paraphyletic subfamily several groupings are congruent between the two analyses. Most interesting are the two clades "number 1" (= taxa with semaphyll) and "number 9" (= the new circumscription of Chiococceae; Bremer, 1992).

The level of homoplasy in the molecular and morphological analyses did not differ distinctly. This is a general pattern as has been shown by Sanderson and Donoghue (1989). The main difference between the two data sets is the much higher number of characters in the cpDNA analysis and also a general trend for molecular analyses of plants. The number of characters does not influence the level of homoplasy (Sanderson and Donoghue, 1989) but it will influence tree stability. In a small data matrix each character will have a much higher impact on the resulting tree, and thus only minor changes in character number or coding can give a different topology (Felsenstein, 1985). In our morphological analyses a few changes may result in a different tree, while in the cpDNA analysis with 161 characters almost the same topology was achieved when only 75 or 100 of the characters were used.

This study illustrates that the most important thing for doing classification is not whether phylogenetic reconstruction is based on a molecular, a morphological, or a combined data set. Instead the most important guideline when doing classification is to base it on phylogenetic reconstruction (Eldredge and Cracraft, 1980; Nelson and Platnick, 1981; Wiley, 1981). If most characters are homoplastic, then single characters cannot be used as a strict criterion for group membership, but rather, only for group characterization. Hence, even homoplastic characters could or should be used in phylogenetic analyses. It is the most parsimonious solution to all character distributions that implies the evolutionary pattern on which group classification should be founded and not whether a particular taxon has a specific character. If we consider the "modern" classification schemes of the Rubiaceae (Verdcourt, 1958; Bremekamp, 1966; Robbrecht, 1988), most subfamilies and tribes are based on only a few characters, and most genera have been sorted into tribes and subfamilies according to these few cardinal characters. The tribe Hamelieae (Fig. 3, node 22) has been accepted as a member of the subfamily Rubioideae (cf. Table 2; Bremer, 1987) because of the presence of raphides in its tissues, although other characters such as corolla aestivation and fruit type

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┌── Luculia-OUTG
│  ┌── Cinchona-CIN
│  │      ┌── Pinckneya-CON
│  │      ├── Pogonopus-CON
│  │   ┌──┴── Calycophyllum-CIN
│  │ ┌─┴── Mussaenda-ISE
│  │ │  ┌── Bouvardia-HED
│  │ │  ├── Pentas-HED
│  │ │  ├── Coccocypselum-COC
│  │ │  │      ┌── Myrmecodia-PSY
│  │ │  │   ┌──┴── Hydnophytum-PSY
│  │ │  │ ┌─┴── Psychotria-PSY
│  │ │  ├── Galium-RUB
│  │ │  │  ┌── Nertera-ANT
│  │ └──┴──┴── Coprosma-ANT
│  ├── Rogiera-RON
│  ├── Gardenia-GAR
│  ├── Mitriostigma-GAR
│  ├── Coffea-COF
│  ├── Enterospermum-PAV
│  ├── Ixora-PAV
│  ├── Vangueria-VAN
│  │   ┌── Hamelia-HAM
│  ├──┴── Hoffmannia-HAM
│  │   ┌── Antihea-GUE
│  ├──┴── Guettarda-GUE
│  │   ┌── Cephalanthus-NAU
│  ├──┴── Haldina-NAU
│  │   ┌── Chiococca-CHI
│  ├──┴── Erithalis-CHI
│  ├── Coutarea-CIN
│  ├── Exostema-CIN
└──┴── Catesbaea-CAT
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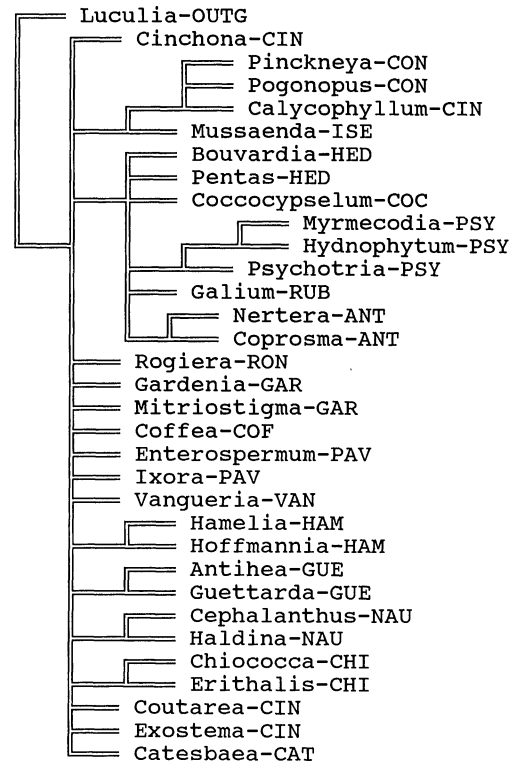
Fig. 6. Strict and also a combinable consensus (Bremer, 1990) tree of the morphological trees and the cpDNA trees (Bremer and Jansen, 1990).

contradict such a position. Both the molecular and the morphological analyses support a position outside the Rubioideae.

The different types of fruits in the Rubiaceae have been very important in the classification. Initially it was the most important character (cf. Schumann, 1891), and later it was almost completely rejected (Verdcourt, 1958; Bremekamp, 1966). Reduction of seed number per locule and the origin of fleshy fruits (Fig. 4) are not unique events, but have occurred only a limited number of times in the family (Bremer and Eriksson, in press). If fruit traits are evolutionarily conservative (Stebbins, 1974) these characters would be suitable as diagnostic characters for larger groups of taxa, even though reductions or parallel evolution do occur. In this context it is appropriate to repeat that only apomorphic characters should be used. The subfamily Cinchonoideae, which in this study has been found to be paraphyletic, was solely "defined" on plesiomorphic characters such as the "pitted" testa structure, elongated placentas, multiovular ovary, absence of raphides, and absence of septate hairs (Verdcourt, 1958; Bremekamp, 1966). All these characters (or absence of characters) are also found outside the Rubiaceae within the Loganiaceae (cf. Figs. 1, 3).

Our results also clearly demonstrate that the family Loganiaceae is paraphyletic. Further analyses, including all genera of the Loganiaceae as well as more representatives of the other families of the Gentianales, are needed to elucidate all phylogenetic relationships within the Loganiaceae.

The paraphyly of the Loganiaceae is demonstrated by the sister group relationships between tribes of the Loganiaceae and the other families of the Gentianales.

The most plausible sister group to the Rubiaceae, according to the morphological analysis, is the Gelsemieae, a relationship supported by several characters, plesiomorphic within the Rubiaceae, but shared between these groups, i.e., heterostyly, lobated stigma, and chemical characters. This relationship is not a new idea. Kisakürek and Hesse (1980) suggested, for chemical reasons, that the Gelsemieae could be the closest relatives of the Rubiaceae. However, the genus *Mitreola* has also been suggested as the member of the Loganiaceae closest to the Rubiaceae (Rendle, 1952; Metcalfe and Chalk, 1983), and has even been included in this family (Torrey and Gray, 1838–1843; Bentham and Hooker, 1873–1876). Such a relationship is not supported by this study. Molecular data (restriction site data, Downie and Palmer, 1992; and *rbc*L sequence data, Bremer and Olmstead, unpublished data) indicate that the family Rubiaceae is the sister group to the remaining part of the Gentianales.

The sister group of *Exacum* (Gentianaceae) is *Anthocleista* (tribe Potalieae), and according to Fosberg and Sachet (1980) the Potalieae should be part of the Gentianaceae. This position is also supported by molecular data (restriction site data, Downie and Palmer, 1992; *rbc*L sequence data, Bremer and Olmstead, unpublished data). The connection between *Plocosperma* and the Apocynaceae was also noticed earlier (e.g., Hutchinson, 1959) and is supported by several characters in our analysis.

As the interrelationships between most angiosperm orders are so poorly understood, there is no obvious choice of outgroup for the Gentianales. We used *Olea* as an outgroup because the Oleaceae have been suggested as close relatives of the Loganiaceae (Hutchinson, 1959, 1973; Dahlgren, 1980a, b, 1983) and *Cornus* because serological data have indicated connections between Cornales and Rubiaceae (Lee and Fairbrothers, 1978). The choice of outgroup is important (Maddison, Donoghue, and Maddison, 1984), and a change of outgroup will usually change tree topology. In our morphological study, however, even though several characters were differently polarized with different outgroups, the resulting trees were very similar.

In conclusion, there is much congruency between the molecular and the morphological data in the Rubiaceae; the subfamilies Antirheoideae, Rubioideae, and Ixoroideae are monophyletic, while the subfamily Cinchonoideae is paraphyletic. The family Loganiaceae is also paraphyletic, but the most plausible sister group to the Rubiaceae is still not settled although the morphological analyses indicate that the closest relatives are the tribe Gelsemieae of the Loganiaceae.

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