PHYLOGENY OF THE RUBIACEAE (CHIOCOCCEAE) BASED ON MOLECULAR AND MORPHOLOGICAL DATA— USEFUL APPROACHES FOR CLASSIFICATION AND COMPARATIVE ECOLOGY[1]

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ABSTRACT

Phylogenies reconstructed with molecular data may provide new hypotheses of relationships. These may serve as a basis for improved morphological analyses and comparative analyses of ecological features. In this study a new phylogenetic hypothesis based on a chloroplast DNA restriction site analysis of the Rubiaceae prompted a critical analysis of morphological characters. Several unique morphological characters were identified that support a large, previously unrecognized monophyletic group, including the tribe Chiococceae. Hence, the tribe Chiococceae is amended to include members of the former Condamineeae (subtribe Portlandiinae) and the genera *Exostema* and *Coutarea* (formerly in the Cinchoneae). The new phylogeny of the Rubiaceae, based on molecular data and the great variety of fruits in the family, makes this family suitable for comparative studies of evolution of dispersal systems and for testing hypotheses of species diversity in connection with the different dispersal systems. In the Rubiaceae, fleshy fruits, adapted to animal dispersal, have originated a limited number of times and have remained unchanged since the time of origin. The hypothesis that animal dispersal should promote species diversity is not supported for the Rubiaceae.

The position of the Rubiaceae in the order Gentianales close to the Loganiaceae was first suggested by Utzschneider (1947) and later established by Wagenitz (1959, 1964). This systematic position is accepted by most systematists dealing with higher-level classification (Dahlgren, 1980; Thorne, 1983; Takhtajan, 1987) and also is supported by phytochemistry (cf. Bisset, 1980) and sequence data of the *rbcL* gene (R. Olmstead & J. Palmer, pers. comm.). Relationships within the order are unresolved and have been proposed to be reticulate (Leeuwenberg & Leenhouts, 1980), with the Loganiaceae as a central or "ancestral" paraphyletic taxon. A morphological study, aimed at identifying the sister group to the Rubiaceae, analyzed representatives of all tribes of the Loganiaceae as well as a few taxa of the other families of the Gentianales (Bremer & Struwe, unpublished data). The results confirmed that the Loganiaceae were paraphyletic. The closest relatives to the Rubiaceae were found to be a part of the Loganiaceae, *viz* the tribe Gelsemieae or at least a part of it.

The Rubiaceae are, with the exception of a few aberrant taxa, an easily circumscribed family, characterized by inferior ovary, opposite leaves with stipules, and absence of internal phloem. Intrafamilial delimitations have always been complicated and uncertain, however. There are two main reasons for this uncertainty. First, "traditional" classification is based on phenetic similarities, and hence several groups are defined by symplesiomorphies or mere absences of characters. Second, much emphasis has been put on fruit structures for sorting genera into subfamilies and tribes. Single structures have been used as cardinal characters. However, in this family comprising about 10,000 species and 600 genera (Mabberley, 1987), many different fruit traits occur. If evolutionary shifts in these traits are common, i.e., highly homoplastic, they may be a source of error in classification. During the past 35 years three important treatments of the family with new classification schemes have been presented (Verdcourt, 1958; Bremekamp, 1966; Robbrecht, 1988). A comparison of these

different schemes (Bremer & Jansen, 1991, table 2) displays many dissimilarities and conflicts, even though there is also much congruency. The merits of the different systems are difficult to evaluate because, in several cases, they are based on a few cardinal characters only, and no strict character analyses are presented. So far, studies of relationships between the subfamilial entities have been rare. Affinities between high-level Rubiaceae taxa have been addressed by serological similarities (Lee & Fairbrothers, 1978). The first phylogenetic analysis of representatives of different tribes of the family is the one by Bremer & Jansen (1991) based on chloroplast DNA (cpDNA) variation.

In order to evaluate different classification schemes, and to use the classification as a framework for evolutionary studies, phylogenetic analyses are necessary. In this context, neither molecular nor morphological data are superior. Both types of data are useful for phylogenetic reconstruction. When a phylogeny based on molecular data is compared with an "accepted" classification, the latter is often rejected because most classifications today are based on phenetic similarity and do not reflect the phylogeny (cf. Sytsma, 1990). However, this does not mean that morphological data should be dismissed as inferior for phylogenetic reconstruction. With cladistic analysis, both molecular and morphological data may be useful and complementary in phylogenetic reconstruction. It is also important to get rid of preconceived notions concerning which characters are "good" or "useless." Character homoplasy and hence usefulness can only be determined a posteriori, following an analysis involving comparison with other characters.

Due to the correlation between large data sets (many taxa) and a high level of homoplasy (Sanderson & Donoghue, 1989; Archie, 1989), it is probably not realistic to expect to resolve all relationships in one analysis of all genera of a large family such as the Rubiaceae. However, if not all taxa are analyzed, character optimization and tree topology may be affected (Donoghue et al., 1989). In Rubiaceae it is not sufficient to sample only a few examples of each tribe in order to resolve tribal relationships, since the tribes in many cases are badly circumscribed (cf. Hallé, 1961; Steyermark & Kirkbride, 1975; Kirkbride, 1979; Ridsdale, 1982; Tirvengadum, 1984; Robbrecht & Puff, 1986; Bremer, 1987; Robbrecht, 1988). One strategy for tackling the sampling problem is to use "common knowledge" of relationships and select a limited number of taxa for a pilot study with molecular data. Results of a pilot study may suggest

new hypotheses of relationships, as in the study by Bremer & Jansen (1991). In that study, a cladistic analysis of cpDNA restriction data was performed for 33 genera representing 18 tribes and four subfamilies. Several monophyletic groups postulated in this analysis were congruent with "traditional" classification, e.g., the subfamilies Rubioideae and the Ixoroideae. However, the large subfamily Cinchonoideae was shown to be paraphyletic. In addition, totally new relationships were indicated.

Following such a molecular pilot study, the postulated new relationships may then be tested by morphological data. If the new groupings are supported, it should be possible to identify larger monophyletic groups defined by particular morphological characters. This morphological study may subsequently suggest suitable taxa for new molecular and morphological analyses that may provide further support for particular intrafamilial taxa. Using a sample of representatives from these larger corroborated monophyletic taxa, it should be possible to analyze and reconstruct the phylogeny and resolve the relationships for the whole family. This is an enormous task for the Rubiaceae, but important for a stable and informative classification.

The first steps in the strategy outlined above are here illustrated by an example in which a new relationship indicated by a cpDNA analysis (Bremer & Jansen, 1991) provides the basis for a morphological analysis, resulting in the identification of a large monophyletic group including the tribe Chiococceae, the subtribe Portlandiinae (of the Condamineeae) and some genera from other tribes.

Another kind of analysis that can be performed is comparative study of ecologically important characters. Such an analysis is founded on the assumption that phylogenetic reconstruction provides information on evolutionary sequences. The usefulness of a phylogenetic reconstruction based on molecular data for testing ecological characters or hypotheses will be illustrated and discussed.

A CASE STUDY—CHIOCOCCEAE

The cpDNA cladogram of the Rubiaceae (Bremer & Jansen, 1991, fig. 2) revealed several hitherto unknown relationships. One of the branches in the cladogram (Bremer & Jansen, 1991, fig. 2) included four genera, Exostema and Coutarea of the tribe Cinchoneae and Erithalis and Chioccocca of the Chiococceae. The members of the tribe Chiococceae (Chioccocca, Fig. 1) have many small flowers in axillary inflorescences, mostly fleshy fruits (drupes), and one seed per carpel, whereas Exo-
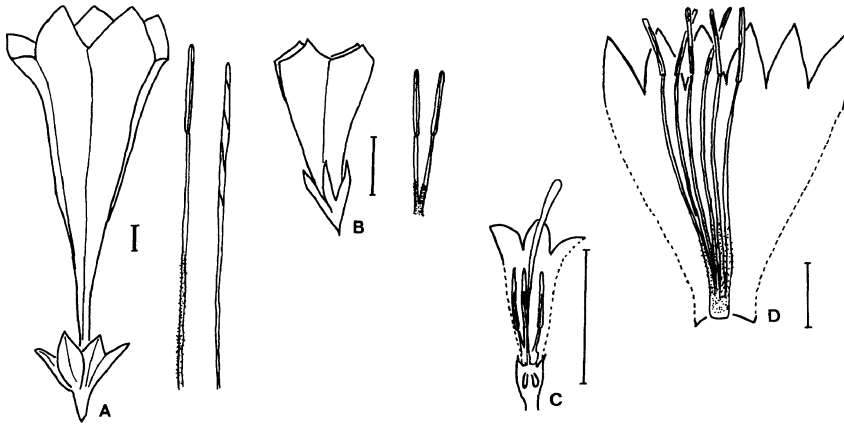
FIGURE 1.   Flower parts.—A. *Portlandia* (redrawn after Aiello, 1979).—B. *Bikkia* (redrawn after Jérémie & Hallé, 1976).—C. *Chiococca* (redrawn after Correll & Correll, 1982).—D. *Coutarea* (redrawn after Steyermark, 1974). Bar scales = 1 cm.

stema and *Coutarea* (Fig. 1) usually have few large flowers and dry, many-seeded capsules. An affinity between these taxa has never been proposed, and in the latest classification (Robbrecht, 1988) they are placed in different subfamilies, with the Chiococceae in the Antirheoideae and the others in the Cinchonoideae.

The molecular analysis prompted a more detailed morphological analysis of these taxa. I found that they share several specific characters (Table 1, characters 1–7): corolla funnelform or rotate; corolla aestivation imbricate; stamens inserted at the corolla·base; filaments fused into a basal ring; anthers linear, basifixed; and stigmas entire or indistinctly lobed. The combination of these characters, particularly the staminal arrangement, is rare in the family, but not unique to these four genera, because they also characterize the subtribe Portlandiinae (*Portlandia*, Fig. 1) of the tribe Condamineeae. In addition, members of the Portlandiinae usually have very large flowers; in the genera *Cubanola*, *Osa*, *Portlandia*, and *Thogsennia*, it is not unusual to have 20-cm-long corollas.

The neotropical genus *Portlandia* and associated taxa (≈ Portlandiinae) have recently been revised by Aiello (1979), who identified the same characters (Table 1, characters 1–7) and indicated that *Exostema*, *Coutarea*, and other genera also have identical character states. Aiello did not reconsider the established tribal classification and did not associate *Exostema* and *Coutarea* with *Portlandia*, because the first two genera have vertically arranged winged seeds, characteristic of the Cinchonoideae, while *Portlandia*, of the tribe Condamineeae, has horizontally arranged wingless seeds. However, the orientation of the ovules to the placentas and the occurrence of a seed wing are

characters that can vary even within the same genus (e.g., in *Steenisia* and *Cinchona*). Because these homoplastic characters have been the main criteria for tribal delimitation in the subfamily Cinchonoideae, it is not surprising that the classification seems arbitrary (see Robbrecht, 1988). The paraphyly of the tribe Cinchoneae was also indicated by the cpDNA analysis (Bremer & Jansen, 1991).

Because the tribal classification is uncertain, it seemed possible that other genera could be closely related to *Portlandia*, *Exostema*, and *Coutarea*. Hence I gathered additional morphological information from most genera of the Chiococceae and the Condamineeae (listed in Robbrecht, 1988), as well as from other genera reported to have the same staminal arrangements (Hooker, 1873; Schumann, 1891). A majority of the genera were studied from herbarium material (in S and UPS), and complementary data were taken from the literature (Mueller, 1861; Hooker, 1873; Schumann, 1891; Verdcourt, 1958; Bremekamp, 1966; Steyermark, 1974; Jérémie & Hallé, 1976; Darwin, 1977; Aiello, 1979; Jansen, 1979; Correll & Correll, 1982; Ridsdale, 1982). The following genera of the tribe Condamineeae (Robbrecht, 1988) were studied but rejected as unrelated to *Portlandia*, *Exostema*, and *Coutarea*, because they lack characters 1–7 listed in Table 1, particularly the staminal characters: *Chimarris*, *Condaminea*, *Flexanthera*, *Kerianthera*, *Parachimarris*, *Picardaea*, *Pinckneya*, *Pogonopus*, *Rustia*, and *Tresanthera*. The following genera of the Chiococceae were also rejected: *Allenanthus*, *Chiona*, and *Hodgkinsonia*. *Allenanthus* and *Chiona* have neither the staminal characters nor the same corolla shape, but they have imbricate aestivation. The flowers of

TABLE 1. Character list. Plesiomorphic state = 0. Apomorphic state = 1 or 2. Character 10 is treated as non-additive and character 12 as additive.

1. Corolla: neither funnel-shaped nor rotate, 0; funnel-shaped or rotate, 1.
2. Aestivation: not imbricate, 0; aestivation imbricate, 1.
3. Stamens; not inserted at the corolla base, 0; inserted at the corolla base, 1.
4. Stamens: not fused, 0; stamens fused into a basal ring, 1.
5. Anthers: not linear, 0; linear, 1.
6. Anthers: dorsifixed, 0; basifixed, 1.
7. Stigma: distinctly lobed, 0; indistinctly bilobed or not, 1.
8. Inflorescence: terminal, 0; axillary, 1.
9. Flowers: not yellowish, 0; often yellowish, 1.
10. Corolla: between 1 and 2 cm, 0; < 1 cm, 1; > 10 cm, 2.
11. Corolla lobes: neither recurved nor reflexed, 0; recurved or reflexed, 1.
12. Corolla lobes: ovate, 0; triangular, 1; linear, 2.
13. Cross section of corolla: circular, 0; distinctly angled, 1.
14. Filaments: glabrous, 0; hairy, 1.
15. Anthers: not exserted, 0; exserted, 1.
16. Stigmatic area: not of two twisted lines, 0; of two twisted lines, 1.
17. Mesocarp: dry, 0; fleshy, 1.
18. Endocarp: not compressed, 0; compressed, 1.
19. Number of seeds per carpel: more than one, 0; one, 1.
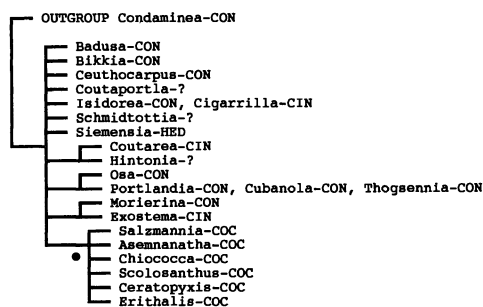20. Seed: not winged, 0; winged. 1.

FIGURE 2. A strict consensus tree for 92 equally parsimonious Wagner trees with *Condaminea* as outgroup. The tribal position of each taxon is indicated by a three-letter suffix (CIN = Cinchonoideae, CON = Condamineeae, COC = Chiococceae, and HED = Hedyotideae, according to Robbrecht, 1988). A question mark indicates uncertain tribal position. The dot indicates the tribe Chiococceae sensu Hooker f.

Hodgkinsonia are very different, with pitcher-shaped corollas, valvate aestivation, short filaments inserted at the midlength of the corollas, dorsifixed anthers, and three to four branched stigmas. The fruit of *Hodgkinsonia* is a drupe with a very hard bilocular endocarp and elongated seeds almost devoid of endosperm. These fruit characters clearly demonstrate that the genus belongs to the tribe Guettardeae, which also was indicated in the original description of the genus (Mueller, 1861). The genus *Phialanthus* has also been excluded since the anthers are ovate and the filaments are free, inserted at the corolla base. I have not been able to study material of the genus *Placocarpa*, but the description (Schumann, 1891) does not indicate any close connection to the *Chiococca* group.

Three genera of uncertain position in the Rubiaceae have been mentioned in connection with the Chiococceae or the Condamineeae (Robbrecht, 1988): *Mastixiodendron* (revised by Darwin, 1977); *Kajewskiella* (revised by Jansen, 1979); and *Pseudomussaenda* (treated and illustrated in Bridson & Verdcourt, 1988). None of these genera

have the characteristic staminal arrangements or stigma shape. In addition, the pollen of *Mastixiodendron* (Darwin, 1977) is tectate, while the pollen of the *Portlandia* group is diffusely foveolate with echinate processes (Aiello, 1979).

All taxa with fused filaments and the combination of characters 1-7 (Table 1) were hypothesized to form a new monophyletic group. A character matrix (Table 2) for these taxa was constructed and analyzed with Wagner parsimony (Hennig86; Farris, 1988). As the sister-group relationships within the family are mostly unknown, four functional Rubiaceae outgroups, representing three tribes, were used: *Condaminea* (Condamineeae), *Luculia* (Cinchoneae), *Rondeletia* (Rondeletieae), and these three together. With *Condaminea* as the outgroup the result was 92 equally parsimonious trees, 34 steps long, with a consistency index of 0.41, and a retention index of 0.71 (Fig. 2). With *Luculia* as the outgroup there were 16 equal-
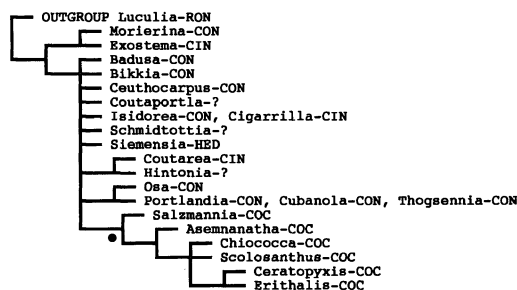

FIGURE 3. A strict consensus tree for 16 equally parsimonious Wagner trees with *Luculia* as outgroup. The tribal position of each taxon is indicated by a three-letter suffix (Robbrecht, 1988). A question mark indicates uncertain tribal position. The dot indicates the tribe Chiococceae sensu Hooker f.
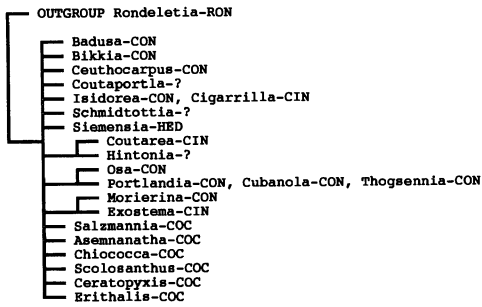
FIGURE 4. A strict consensus tree for 17 equally parsimonious Wagner trees with *Rondeletia* as outgroup. The tribal position of each taxon is indicated by a three-letter suffix (Robbrecht, 1988). A question mark indicates uncertain tribal position.

ly parsimonious trees, 35 steps long, with a consistency index of 0.42, and a retention index of 0.71 (Fig. 3). With *Rondeletia* there were 17 equally parsimonious trees, 34 steps long, with a consistency index of 0.44 and a retention index of 0.72 (Fig. 4). When all three genera were used as the outgroup, the result was 58 equally parsimonious trees, 37 steps long, with a consistency index of 0.40, and a retention index of 0.71 (Fig. 5). Most relationships within the ingroup remain unresolved or uncertain, because of the lack of available characters. There were only 13 phylogenetically informative characters and 22 ingroup taxa. However, all genera with small flowers and one-seeded carpels ($\approx$ Chiococceae sensu Hooker f.) were shown to form a monophyletic group (indicated by a dot in Figs. 2, 3, 5) in all analyses except that with *Rondeletia* as the outgroup. This small-flowered group is supported by several flower and fruit characters (Tables 1, 2). If we retain Chiococceae in the narrow sense, all the remaining
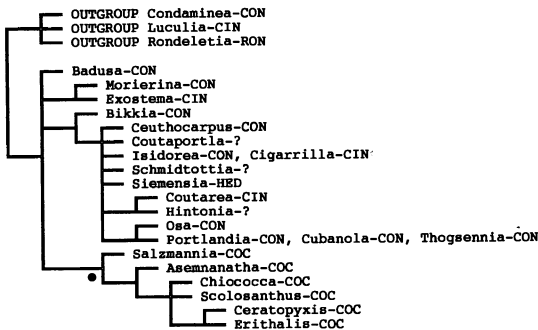

FIGURE 5. A strict consensus tree for 48 equally parsimonious Wagner trees with *Condaminea, Luculia* and *Rondeletia* as outgroup. The tribal position of each taxon is indicated by a three-letter suffix (Robbrecht, 1988). A question mark indicates uncertain tribal position. The dot indicates the tribe Chiococceae sensu Hooker f.

TABLE 2. Data matrix of 20 morphological characters. Characters 1–7 are synapomorphies defining the study group and not included in the Wagner parsimony analyses. Character numbers are the same as in Table 1. *Condaminea*—CON, *Luculia*—CIN, *Rondeletia*—RON, as well as all three together were used as outgroups. A question mark indicates an unknown or variable state.

		Character number	
			1 2
		1234567	8901234567890
1	*Condaminea*	0000000	0000100100000
2	*Luculia*	0100000	0000000000001
3	*Rondeletia*	0100000	1000000000000
4	*Asemnanatha*	1111111	1111101001010
5	*Ceratopyxis*	1111101	0111101100110
6	*Chiococca*	1111111	1111111001110
7	*Erithalis*	1111111	0011101101110
8	*Salzmannia*	1111111	1?10100001?10
9	*Scolosanthus*	1111111	1111101001110
10	*Badusa*	1111111	1?00010100000?
11	*Bikkia*	1111111	1000111000000
12	*Ceuthocarpus*	1111111	00001?1110000
13	*Cigarrilla*	1111111	1000111010000
14	*Cubanola*	1111111	1020111010000
15	*Coutaportla*	1111111	0000111010100
16	*Coutarea*	1111111	1000111110101
17	*Hintonia*	1111111	1000111110001
18	*Isidorea*	1111111	1000111010000
19	*Morierina*	1?11111	00212?1100001
20	*Osa*	1111111	1020110010000
21	*Portlandia*	1111111	1020111010000
22	*Schmidtottia*	1111111	0100111010000
23	*Siemensia*	1111111	0000111010000
24	*Thogsennia*	1111111	1020111010000
25	*Exostema*	1111111	?101200100001

taxa considered in this analysis ($\approx$ Portlandiinae) become a paraphyletic cluster; so far, no single character has been found to unite the remaining taxa. The best solution to this taxonomic problem is to redefine and widen the tribe Chiococceae, as follows.

Chiococceae J. D. Hooker, Gen. Pl.: 9, 21. 1873. Portlandiinae J. D. Hooker, Gen. Pl: 12. 1873, as "Portlandieae." Type genus: *Chiococca* P. Browne.

The tribe Chiococceae is characterized by funnelform or rotate corollas, imbricate aestivation, stamens inserted at the corolla base, filaments mostly villous and fused into a basal ring, anthers linear and mostly basifixed, stigma entire, carpels one- or many-seeded, and fruits drupes or capsules.

The included genera are *Asemnanatha, Badu-*

sa, *Bikkia* (Fig. 1), *Ceratopyxis*, *Ceuthocarpus*, *Chiococca* (Fig. 1), *Cigarrilla*, *Coutaportla*, *Coutarea* (Fig. 1), *Cubanola*, *Erithalis*, *Exostema*, *Hintonia*, *Isidorea*, *Morierina*, *Osa*, *Portlandia* (Fig. 1), *Salzmannia*, *Schmidtottia*, *Scolosanthus*, *Siemensia*, and *Thogsennia*.

PHYLOGENETIC ASPECTS OF SEED DISPERSAL

The Rubiaceae display a wide array of seed-dispersal mechanisms (Bremer & Eriksson, 1992). By making outgroup comparisons with different parts of the Loganiaceae, it can be inferred that the ancestral, plesiomorphic fruit condition in the family is likely to be a many-seeded capsule (Bremer & Struwe, unpublished data). This condition has been altered several times in relation to various dispersal agents, both biotic and abiotic. Adaptations for wind dispersal, in the form of winged seeds or pterophyll, occur in many genera. Bird- and mammal-dispersed fleshy fruits are also common in the family. These are basically of three kinds: drupes, berries, and "*Gardenia*-fruits." The "*Gardenia*-fruits" are characterized by a fleshy to leathery or fibrous to woody mesocarp, covering a juicy or fleshy pulp of placental origin (Robbrecht & Puff, 1986). Berries are the predominant fruit type in the tribe Iserteae. Drupes dominate in the Psychotrieae and Guettardeae. The subfamily Ixoroideae has the most variable array of fleshy fruits; in addition to "*Gardenia*-fruits," which occur solely in this subfamily, drupes and berries are common. The great variety of fruits in the Rubiaceae makes this family particularly suitable for comparative studies of the evolution of dispersal systems. Ecological considerations of seed-dispersal evolution have to a large extent focused on coevolutionary relationships between fruit characters and dispersal agents. The "traditional view" of a close adaptative association between plants and animals has recently been challenged (e.g., Howe, 1984, 1986; Herrera, 1986; Wheelwright, 1988). A persistent problem for evaluation of "coevolutionary" hypotheses is the scarcity of phylogenetic studies of fruit evolution incorporating taxa above the genus level. Until recently only a few such studies had been performed (e.g., Tiffney, 1986; Raven, 1988; Stone, 1989). In order to provide some insight into this research field, Bremer and Eriksson (1992) analyzed fruit evolution in the Rubiaceae. The study was based on the phylogenetic relationships of the family derived from cladistic analysis of cpDNA variation (Bremer & Jansen, 1991). The principal results indicated that fleshy fruits, adapted to animal dispersal, have originated only

a limited number of times (approximately 12), and that they evolved mostly during a period from Eocene to Miocene. The inferences on when fleshy fruits appeared were made indirectly from information on pollen fossils (Bremer & Eriksson, 1992). Moreover, optimization of the fleshy fruit character on the cladogram implied that fruit structure in general has remained unchanged since that period. The analysis rested on two assumptions: that capsules with many seeds do not evolve from other fruit types and that seed number reduction is a "fixed" condition. Given these assumptions, the origin events were positioned in the phylogenetic tree on the lowest (oldest) possible branch that was allowed. Since the time of origin of the different fruit types, thousands of species have evolved; there are now ca. 7,000 extant species with fleshy fruits. These results indicate that specific adaptive interpretations of animal-dispersal modes based on contemporary ecological conditions are unwarranted. In contrast to the fleshy fruits, optimization of the wind dispersal by winged seed character on the cladogram suggested a much less conservative evolutionary pattern. Wind-dispersal adaptations may have been changed repeatedly during the course of evolution in several lineages.

Another issue, much debated in recent years, is how ecological features influence taxonomic diversification patterns (e.g., Stanley, 1979, 1989; Kitchell, 1985). For angiosperms, the prevalent view is that aspects of reproduction and dispersal have been responsible for the tremendous diversification of this group since Late Cretaceous (Raven, 1977; Burger, 1981; Stebbins, 1981; Crepet, 1984). One of several hypotheses of angiosperm diversification states that animal dispersal promotes species richness (Regal, 1977; Tiffney, 1984, 1986). Herrera (1989) tested this hypothesis and concluded that it was inconsistent with data on species number in relation to dispersal modes in both angiosperms and "gymnosperms." This conclusion was, however, not based on explicit phylogenetic inferences; since extant species number is directly correlated to diversification rate only if compared taxa are of equal age (cf. Mitter et al., 1988), the conclusion is difficult to evaluate. Eriksson & Bremer (1991) investigated the "animal dispersal hypothesis" in closer detail for the Rubiaceae. We found no consistent support for the hypothesis, but we did find evidence suggesting a positive association between dispersal ability and species diversification. In shrubs, fleshy fruits are likely to enhance seed dispersal, whereas abiotic dispersal modes are more efficient seed dispersers among herbaceous life forms (Eriksson & Bremer,

1991). These two life form/dispersal mode categories were both comparatively species-rich. This conclusion was robust since it was founded on generic and sister-group comparisons. Hence, this study indicated that diversification patterns are influenced by a combination of features. Future hypotheses should be based preferably on several life cycle characteristics, instead of single features.

The use of phylogenetic approaches in ecology has been considered by several authors (e.g., Ridley, 1983; Felsenstein, 1985; Donoghue, 1989; Funk & Brooks, 1990; Wanntorp et al., 1990; Brooks & McLennan, 1991), but empirical studies in botany are still scarce. A synthesis of ecology and phylogenetic inferences based on molecular and morphological data is a promising field for research. As the research on Rubiaceae has shown, data, hypotheses, and conclusions from molecular and morphological phylogeny and ecology, when considered together, have provided new insights that scarcely had been revealed by studies of ecological, morphological, or molecular data in isolation.

LITERATURE CITED

AIELLO, A. 1979. A reexamination of *Portlandia* (Rubiaceae) and associated taxa. J. Arnold Arbor. 60: 38–126.
ARCHIE, J. W. 1989. Homoplasy excess ratios: new indices for measuring levels of homoplasy in phylogenetic systematics and a critique of the consistency index. Syst. Zool 38: 253–269.
BISSET, N. G. 1980. Phytochemistry. Pp. 211–233 *in* A. J. M. Leeuwenberg (editor), Engler & Prantl's Die Natürlichen Pflanzenfamilien, Fam. Loganiaceae, 28b (1).
BREMEKAMP, C. E. B. 1966. Remarks on the position, the delimitation and the subdivision of the Rubiaceae. Acta Bot. Neerl. 15: 1–33.
BREMER, B. 1987. The sister group of the paleotropical tribe Argostemmateae: a redefined neotropical tribe Hamelieae (Rubiaceae, Rubioideae). Cladistics 3: 35–51.
———— & O. ERIKSSON. 1992. Evolution of fruit characters and dispersal modes in the tropical family Rubiaceae. Biol. J. Linn. Soc. (in press).
———— & R. K. JANSEN. 1991. Comparative restriction site mapping of chloroplast DNA implies new phylogenetic relationships within Rubiaceae. Amer. J. Bot. 78: 198–213.
BRIDSON, D. & B. VERDCOURT. 1988. Rubiaceae. Part 2 *in* R. M. Polhill (editor), Flora of Tropical East Africa. Balkema, Rotterdam.
BROOKS, D. R. & D. H. MCLENNAN. 1991. Phylogeny, Ecology, and Behavior. Univ. of Chicago Press, Chicago.
BURGER, W. C. 1981. Why are there so many kinds of flowering plants? BioScience 31: 572, 577–581.
CORRELL, D. S. & H. B. CORRELL. 1982. Flora of the Bahama Archipelago. J. Cramer, Vaduz.
CREPET, W. L. 1984. Advanced (constant) insect pol-

lination mechanisms: pattern of evolution and implications vis-à-vis angiosperm diversity. Ann. Missouri Bot. Gard. 71: 607–630.
DAHLGREN, R. M. T. 1980. A revised system of classification of the angiosperms. Bot. J. Linn. Soc. 80: 91–124.
DARWIN, S. P. 1977. The genus *Mastixiodendron* (Rubiaceae). J. Arnold Arbor. 58: 349–381.
DONOGHUE, M. J. 1989. Phylogenies and the analysis of evolutionary sequences with examples from seed plants. Evolution 43: 1137–1156.
————, J. A. DOYLE, J. A. GAUTHIER, A. G. KLUGE & T. ROWE. 1989. The importance of fossils in phylogenetic reconstruction. Annual Rev. Ecol. Syst. 20: 431–460.
ERIKSSON, O. & B. BREMER. 1991. Fruit characteristics, life forms, and species richness in the plant family Rubiaceae. Amer. Naturalist 138: 751–761.
FARRIS, J. S. 1988. Hennig86. Version 1.5. James S. Farris, Port Jefferson Station, New York [Computer program and manual].
FELSENSTEIN, J. 1985. Phylogenies and the comparative method. Amer. Naturalist 125: 1–15.
FUNK, V. A. & D. R. BROOKS. 1990. Phylogenetic systematics as the basis of comparative biology. Smithsonian Contrib. Bot. 73: 1–45.
HALLÉ, F. 1961. Contribution à l'étude biologique et taxonomique des Mussaendeae (Rubiaceae) d'Afrique Tropicale. Adansonia 1: 266–296.
HERRERA, C. M. 1986. Vertebrate-dispersed plants: why they don't behave the way they should. Pp. 5–18 *in* A. Estrada & T. H. Fleming (editors), Frugivores and Seed Dispersal. Junk, Dordrecht.
————. 1989. Seed dispersal by animals: a role in angiosperm diversification. Amer. Naturalist 133: 309–322.
HOOKER, J. D. 1873. Rubiaceae. *In:* G. Bentham & J. D. Hooker (editors), Genera Plantarum 2: 7–151.
HOWE, H. F. 1984. Constraints on the evolution of mutualism. Amer. Naturalist 123: 764–777.
————. 1986. Seed dispersal by fruit-eating birds and mammals. Pp. 123–189 *in* D. R. Murray (editor), Seed Dispersal. Academic Press, Sydney.
JANSEN, M. E. 1979. A revision of the genus *Kajewskiella* (Rubiaceae). Blumea 25: 283–294.
JÉRÉMIE, J. & N. HALLÉ. 1976. Le genre *Bikkia* (Rubiaceae–Condamineae) en Nouvelle-Calédonie. Adansonia 15: 341–355.
KIRKBRIDE, M. C. G. 1979. Review of the neotropical Isertieae (Rubiaceae). Brittonia 31: 313–332.
KITCHELL, J. A. 1985. Evolutionary paleobiology: recent contributions to evolutionary theory. Paleobiology 11: 91–104.
LEE, Y. S. & D. E. FAIRBROTHERS. 1978. Serological approaches to systematics of the Rubiaceae and related families. Taxon 27: 159–185.
LEEUWENBERG, A. J. M. & P. W. LEENHOUTS. 1980. Taxonomy. Pp. 8–96 *in* A. J. M. Leeuwenberg (editor), Engler & Prantl's Die Natürlichen Pflanzenfamilien, Fam. Loganiaceae, 28b (1).
MABBERLEY, D. J. 1987. The Plant-Book. A Portable Dictionary of the Higher Plants. Cambridge Univ. Press, Cambridge.
MITTER, C., B. FARRELL & B. WIEGMANN. 1988. The phylogenetic study of adaptive zones: has phytophagy promoted insect diversification? Amer. Naturalist 132: 107–128.

MUELLER, F. 1861. Fragmenta Phytographiae Australiae 2. Joannis Ferres, Melbourne.

RAVEN, P. H. 1977. A suggestion concerning the Cretaceous rise to dominance of the angiosperms. Evolution 31: 451–452.

———. 1988. Onagraceae as a model of plant evolution. Pp. 85–107 in L. D. Gottlieb & S. K. Jain (editors), Plant Evolutionary Biology. Chapman & Hall, London.

REGAL, P. J. 1977. Ecology and evolution of flowering plant dominance. Science 196: 622–629.

RIDLEY, M. 1983. The Explanation of Organic Diversity. Oxford Univ. Press, Oxford.

RIDSDALE, C. E. 1982. A revision of Badusa (Rubiaceae, Condamineae, Portlandiinae). Blumea 28: 145–150.

ROBBRECHT, E. 1988. Tropical woody Rubiaceae. Opera Bot. Belgica 1: 1–271.

——— & C. PUFF. 1986. A survey of the Gardenieae and related tribes (Rubiaceae). Bot. Jahrb. Syst. 108: 63–137.

SANDERSON, M. J. & M. J. DONOGHUE. 1989. Patterns of variation in levels of homoplasy. Evolution 43: 1781–1795.

SCHUMANN, K. 1891. Rubiaceae. Pp. 1–156 in A. Engler & K. Prantl (editors), Die Natürlichen Pflanzenfamilien 4 (4–5).

STANLEY, S. M. 1979. Macroevolution—Pattern and Process. Freeman, San Francisco.

———. 1989. Fossils, macroevolution and theoretical ecology. Pp. 125–134 in J. Roughgarden, R. E. May & S. A. Levin (editors), Perspectives in Ecological Theory. Princeton Univ. Press, Princeton, New Jersey.

STEBBINS, G. L. 1981. Why are there so many species of flowering plants? BioScience 31: 573–577.

STEYERMARK, J. A. 1974. Rubiaceae. In Flora de Venezuela 9(1–3). Edición Especial del Instituto Botánico, Caracas.

——— & J. H. Kirkbride. 1975. The genus Wittmackanthus (Rubiaceae). Ann. Missouri Bot. Gard. 62: 504–509.

STONE, D. E. 1989. Biology and evolution of temperate and tropical Juglandaceae. Pp. 117–145 in P. R. Crane & S. Blackmore (editors), Evolution, Systematics, and Fossil History of the Hamamelidae, volume 2, "Higher" Hamamelidae. Clarendon Press, Oxford.

SYTSMA, K. J. 1990. DNA and morphology: inference of plant phylogeny. Trends Ecol. Evol. 5: 104–110.

TAKHTAJAN, A. 1987. Systema Magnoliophytorum. Nauka, Leningrad.

THORNE, A. 1983. Proposed new realignments in the angiosperms. Nordic J. Bot. 3: 85–117.

TIFFNEY, B. H. 1984. Seed size, dispersal syndromes, and the rise of the angiosperms: evidence and hypothesis. Ann. Missouri Bot. Gard. 71: 551–576.

———. 1986. Fruit and seed dispersal and the evolution of the Hamamelidae. Ann. Missouri Bot. Gard. 73: 394–416.

TIRVENGADUM, D. D. 1984. Glionnetia, nouveau genre de Rubiaceés (Rondélétiées) des Seychelles. Adansonia 2: 197–205.

UTZSCHNEIDER, R. 1947. Der Fruchtknotenbau der Rubiaceen mit besonderer Berücksichtigung der Cinchonoideen. PhD. Dissertation. University of Munich.

VERDCOURT, B. 1958. Remarks on the classification of the Rubiaceae. Bull. Jard. Bot. État 28: 209–290.

WAGENITZ, G. 1959. Die systematische Stellung der Rubiaceae. Bot. Jahrb. Syst. 79: 17–35.

———. 1964. Oleales and Gentianales. Pp. 403–424 in H. Melchior (editor), A. Engler's Syllabus der Pflanzenfamilien, ed. 12, 2. Gebrüder Borntraeger, Berlin.

WANNTORP, H. E., D. R. BROOKS, T. NILSSON, S. NYLIN, F. RONQUIST, S. C. STEARNS & N. WEDELL. 1990. Phylogenetic approaches in ecology. Oikos 57: 119–132.

WHEELWRIGHT, N. T. 1988. Fruit-eating birds and bird-dispersed plants in the tropics and temperate zone. Trends Ecol. Evol. 3: 270–274.