# A REVIEW OF MOLECULAR PHYLOGENETIC STUDIES OF RUBIACEAE[1]

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## ABSTRACT

Rubiaceae is one of the five largest families of flowering plants with over 13,000 species. We have seen a tremendous increase in our understanding of the phylogeny of the family through studies on molecular data during the 15-year period from 1991 to 2005; some new relationships are completely unexpected and different from traditional classification. At the end of 2005, ca. 50 phylogenetic reconstructions from the family had been published based on more than 4400 sequences. Most studies are based on ITS and *rbcL* sequences, but 13 different markers have been used. Most sequences available in GenBank (as of 2005) are from *rps16*, *trn(T)L-F*, *rbcL*, and ITS. We can now see a framework of the family phylogeny with support for three subfamilies and over 43 tribes; subfamily Cinchonoideae (Chiococceae, Cinchoneae, Guettardeae, Hamelieae, Hillieae, Hymenodictyeae, Isertieae, Naucleeae, Rondeletieae), subfamily Ixoroideae (Alberteae, Bertiereae, Coffeeae, Condamineeae, Cremasporeae, Gardenieae, Ixoreae, Mussaendeae, Octotropideae, Pavetteae, Posoquerieae, Retiniphylleae, Sabiceeae, Sipaneeae, Vanguerieae), and subfamily Rubioideae (Anthospermeae, Argostemmateae, Coussareeae, Craterispermeae, Danaideae, Gaertnereae, Knoxieae, Lasiantheae, Morindeae, Ophiorrhizeae, Paederieae, Psychotrieae, Putorieae, Rubieae, Schradereae, Spermacoceae, Theligoneae, Urophylleae), and tribe Coptosapelteae, which is placed outside the three subfamilies. Two of these tribes, Gardenieae and Morindeae, are paraphyletic/polyphyletic. Only about half of the tribes have been the focus of specific investigations. However, we have seen increased interest in using Rubiaceae phylogenies for studies of ecology, evolution, and biogeography, e.g., and also for morphological and anatomical investigations. Evolution of fruit traits, flower types, and myrmecophytism has been investigated, and biogeographic patterns for specific taxa in Africa, the Caribbean, and the Pacific have been studied. In addition, distribution of pollen types, chemical substances, and wood characteristics have been compared with molecular phylogenies.

Key words: Biogeography, classification, ecology, evolution, ITS, morphological characters, phylogeny, *rbcL*, *rps16*, Rubiaceae review, *trn(T)L-F*.

The Rubiaceae family, with more than 13,000 species (Govaerts et al., 2006), has been the subject of many molecular phylogenetic studies during the 15-year period from 1991 to 2005. Here, I review and summarize the main conclusions from these studies. Molecular phylogenetics of Rubiaceae was preceded by a few phylogenetic analyses based on morphology from the late 1970s and early 1990s. In 1979, the first cladogram of *Neurocalyx* Hook. placed the genus in Argostemmateae (Bremer, 1979); in 1990 the first cladogram of *Xanthophytum* Reinw. ex Blume placed the genus in Hedyotideae (Axelius, 1990). Both phylogenies were published in association with minor generic revisions, and the trees were the result of simple parsimony analyses with few morphological characters. Andersson and Persson (1991) published a very early morphological analysis of tribe Cinchoneae and relatives. Their analysis resulted in a new circumscription of Cinchoneae, a description of the new tribe Calycophylleae, and an emended tribe Coptosapelteae. The Cinchoneae tree has a low

resolution with many odd relationships compared to later molecular analyses (Razafimandimbison & Bremer, 2001, 2002; Rova et al., 2002; Andersson & Antonelli, 2005). The relationships in *Neurocalyx* and *Xanthophytum* have not yet been tested by molecular data, but both genera have been transferred to tribe Ophiorrhizeae based on sequence data (Bremer & Manen, 2000). Very soon after the analyses described above, molecular data (from 1991, see below), or combinations of molecular and morphological data, analyzed with computer programs replaced simple manual morphological analyses. There is no evident difference in quality between morphological and molecular data, but because higher numbers of characters can be produced from DNA, it is easier to get better-supported trees (e.g., Bremer et al., 1999).

During 15 years of molecular phylogenetic analyses of Rubiaceae taxa, from the beginning of 1991 to the end of 2005, ca. 50 studies have been published, which cover many parts of the family and address questions at different taxonomic levels, from closely
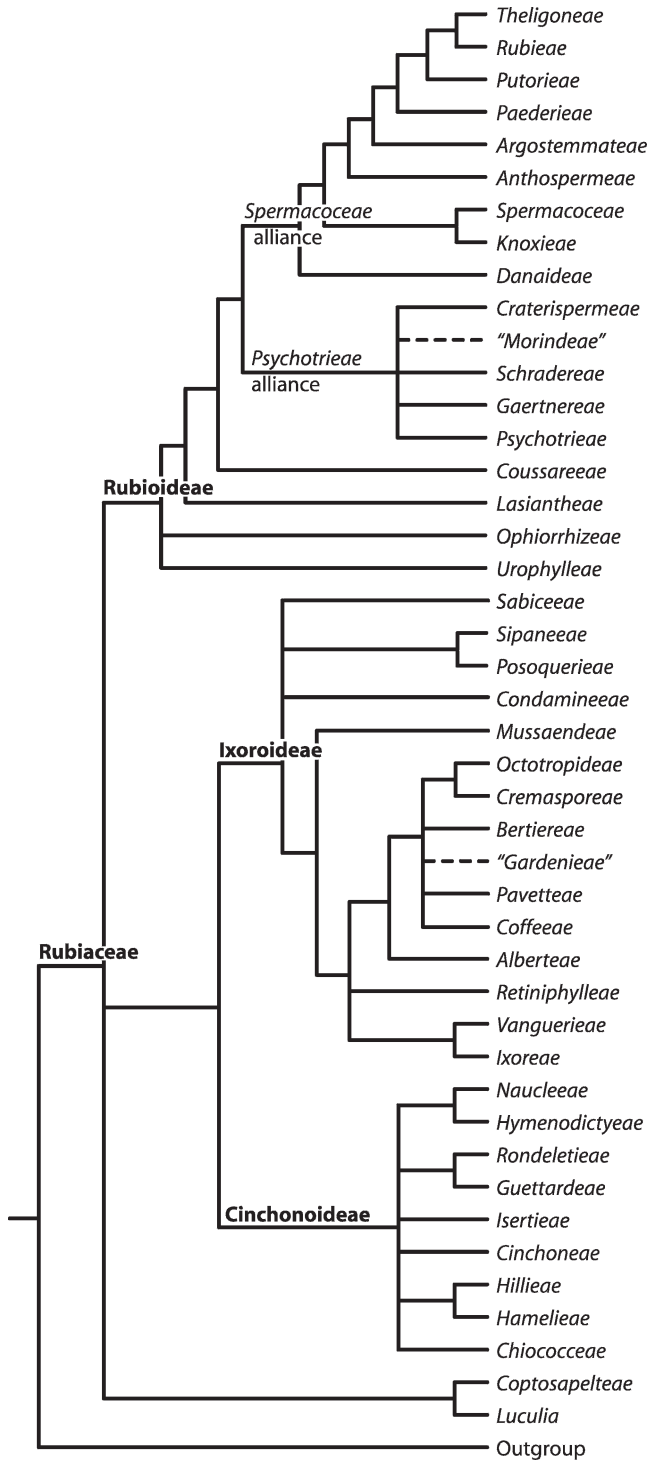
Figure 1. Simplified majority rule consensus tree from MrBayes 3.1.1 analysis, of 538 Rubiaceae taxa and 9420 characters from five chloroplast markers. All resolved nodes and tribes have 0.95 to 1.0 clade credibility (except Guettardeae, with 0.92) and are accepted as monophyletic (Cremasporeae, Retiniphylleae, Schradereae, and Theligoneae are monotypic or represented by single taxa and thus could not be tested for monophyly). Two tribes, Gardenieae and Morindeae, are paraphyletic/polyphyletic. Presented (slightly modified) at the Third International Rubiaceae Conference in Leuven in 2006.

related species to the whole family. Except for the first analysis of restriction site data, all later studies have used sequence data, and the most popular markers (the largest number of studies) have been ITS and *rbcL*. Altogether, 13 different sequence markers have been used, seven from chloroplast DNA (cpDNA) (*atpB-rbcL*, *ndhF*, matK, *rbcL*, *rps16*, *trn(T)L-F*, *trnS-G*) and six nuclear DNA (ETS, ITS, nontranscribed spacer [NTS], *pep-C* large, *pep-V* small, *Tpi*). At the end of 2005, more than 4400 sequences from the family were available from GenBank/European Molecular Biology Laboratory (EMBL) (excluding the double number of *Coffea* L. sequences produced for purposes other than phylogenetics). Of these 4400, most sequences are from *rps16* (719), *trn(T)L-F* (672), *rbcL* (643), and ITS (323). In the future, we will see many more markers used in Rubiaceae, but of the 13 that have been used so far, many are underexplored (e.g., *matK* and *ndhF* for higher taxonomic levels and ETS and NTS for more closely related taxa).

This paper is divided into two main parts. The first part focuses on phylogenetic reconstructions, studies covering the whole family, studies sorted under the three subfamily headings, first tribal studies, and finally genera studies. I have tried to discuss them in chronological order according to the first molecular study of the specific group. Some studies have been difficult to classify according to taxonomic level unless the author(s) had indicated a focus on a specific rank. Studies including substantially new data, not just reanalyzed data sets, have been considered. The second part of this review is a presentation of studies in which a Rubiaceae phylogeny has been used to ask other questions about the family, concerning, e.g., ecology, evolution, biogeography, anatomy/morphology, or chemistry. To assist the reader in navigating among all subfamilial and tribal names, I refer to a phylogeny and classification (Fig. 1) presented at the Third International Rubiaceae Conference in Leuven in 2006 (Bremer & Eriksson, unpublished data). In the tree, three subfamilies and 43 tribes are well supported (all resolved nodes and tribes have 0.95 to 1.0 clade credibility, except Guettardeae, with 0.92; the Bayesian analysis is based on 538 taxa for five molecular markers) and accepted as monophyletic (Cremasporeae, Retiniphylleae, Schradereae, and Theligoneae are monotypic or represented by single taxa and thus could not be tested for monophyly), and two tribes, Gardenieae and Morindeae, are paraphyletic/polyphyletic. Representatives from all 43 of these tribes have been included in some of the analyses, but only 16 tribes have been the focus of specific studies. All genera discussed are listed in Table 1.

## Phylogenetic Reconstructions

### Family Rubiaceae

The first attempt to reconstruct the Rubiaceae phylogeny based on molecular data was published in 1991 by Bremer and Jansen in the *American Journal of Botany*. The data were from restriction site mapping of cpDNA. Included were 161 informative characters for 33 taxa and genera representing 17 different tribes. Unfortunately, no external outgroup was incorporated, which affected the rooting of the family. Several relationships suggested in earlier classifications by Bremekamp (1954, 1966), Verdcourt (1958), Bridson and Verdcourt (1988), and Robbrecht (1988) were corroborated, but many new relationships disagreeing with earlier classifications were also proposed. The subfamily Rubioideae of Verdcourt (1958) was mostly monophyletic (including the tribes Rubieae, Anthospermeae, Coccocypseleae, Hedyotideae, Psychotrieae, but excluding Hamelieae [*Hamelia* Jacq., *Hoffmannia* Sw.] and Ixoroideae fide Robbrecht [1988; including Coffeeae, Gardenieae, Pavetteae, and Vanguerieae but not Chiococceae]). Several taxa earlier classified to Cinchonoideae (e.g., *Calycophyllum* DC., *Mussaenda* L., *Pinckneya* Michx., and *Pogonopus* Klotzsch) were shown to be closer to the subfamily Ixoroideae. It was also shown that the recircumscribed Antirheoideae (Robbrecht, 1988) was highly polyphyletic; the tribes Cephalantheae, Chiococceae, and Vanguerieae were not close to each other or to Guettardeae (*Antirhea* Comm. ex Juss., *Guettarda* L.). The subfamily Cinchonoideae was not supported as a monophyletic group in Bremer and Jansen (1991). New relationships included *Chiococca* P. Browne and *Erithalis* P. Browne of the Chiococceae as close to *Coutarea* Aubl. and *Exostema* (Pers.) Bonpl. of the former Cinchonoideae. It was also shown that Cephalantheae and Vanguerieae are closest to Naucleeae and Ixoroideae, respectively.

During the First International Conference on Rubiaceae at the Missouri Botanical Garden in 1993, an analysis of *rbcL* sequences from 49 Rubiaceae genera representing 23 tribes was presented (later published in Bremer et al., 1995). That study included outgroups from Gentianales and also Oleaceae. Rubiaceae came out as sister group to the rest of Gentianales in agreement with an *rbcL* study of the Asteridae (Olmstead et al., 1993) and a morphological analysis of Loganiaceae and Gentianales (Bremer & Struwe, 1992). In the 1995 study, the family was classified into three subfamilies: Rubioideae (including Rubieae, Anthospermeae, Hedyotideae, Morindeae, Ophiorrhizeae, Psychotrieae, and Theligoneae), Ixoreae s.l. (including Coffeeae, Gardenieae, Pavetteae, and Vanguerieae, as well as several genera of the

former Cinchonoideae), and Cinchonoideae s. str. (including Cinchoneae, Chiococceae s.l., Guettardeae, Hamelieae, Hillieae, Naucleeae, and Rondeletieae). The genus *Luculia* Sweet was unresolved at the base of the family, and the genus *Hintonia* Bullock was unresolved between Cinchonoideae and Ixoroideae. At about the same time, Ehrendorfer et al. (1994) published the first analysis of the *atpB-rbcL* spacer of cpDNA in a short communication, foregoing a more comprehensive study of the Rubieae (Natali et al., 1995; see below) that was presented at the 1993 meeting in St. Louis. They showed results for eight genera (*Bouvardia* Salisb., *Coffea*, *Galium* L., *Hydnophytum* Jack, *Ixora* L., *Pentas* Benth., *Psychotria* L., and *Rubia* L.) representing five tribes, and the resulting tree was concluded to be in agreement with the relationships based on the restriction site data, with *Ixora* and *Coffea* together as sister group to the rest.

In a study investigating effects of the number of characters, the number of taxa, and the kind of data for bootstrap values within a phylogenetic tree, Bremer et al. (1999) used different data sets of Rubiaceae. In the study, 43 Rubiaceae genera together with 11 outgroups representing the rest of the Gentianales were analyzed for *rbcL* and *ndhF*. It was shown that the percentage of supported nodes within the trees positively correlated to the number of characters, but negatively correlated to the number of taxa. Further, the three subfamilies Rubioideae, Cinchonoideae, and Ixoroideae were all monophyletic and highly supported (100% bootstrap). There were only two investigated genera, *Luculia* and *Coptosapelta* Korth., placed at the base of the Rubiaceae, that were left unclassified to subfamily.

Rova et al. (2002) performed a phylogenetic analysis of *trnL-F* for a large data set including 154 Rubiaceae sequences and 11 outgroups in a study to test what had been suggested to form a tight complex of the tribes Condamineeae, Rondeletieae, and Sipaneeae by Robbrecht (1988). Several earlier molecular studies had indicated that this suggested relationship had no support (e.g., Bremer et al., 1995; Andersson & Rova, 1999). Rova et al. (2002) included taxa from most parts of the family, and the results were very much in agreement with earlier molecular analyses. Their main conclusions were that most former Condamineeae and several Rondeletieae genera (*Aleisanthia* Ridl., *Aleisanthiopsis* Tange, *Augusta* Pohl, *Greenea* Wight & Arn., and *Wendlandia* DC.) are members of the Ixoroideae, as are the Sipaneeae (*Maguireothamnus* Steyerm., *Neobertiera* Wernham, and *Sipanea* Aubl.) and its sister clade (*Gleasonia* Standl., *Molopanthera* Turcz., and *Posoqueria* Aubl., the latter two correspond to the circumscription of tribe Posoquerieae by Delprete et

al. [2004]). Condamineeae (as the first Ixoroideae clade [*Condaminea* DC., *Alseis* Schott, *Bathysa* C. Presl, *Calycophyllum*, *Capirona* Spruce, *Chimarrhis* Jacq., *Dioicodendron* Steyerm., *Dolichodelphys* K. Schum. & K. Krause, *Elaeagia* Wedd., *Emmenopterys* Oliv., *Hippotis* Ruiz & Pav., *Macbrideina* Standl., *Parachimarrhis* Ducke, *Pentagonia* Benth., *Picardaea* Urb., *Pinckneya*, *Pogonopus*, *Rustia* Klotzsch, *Sommera* Schltdl., *Warszewiczia* Klotzsch, and *Wittmackanthus* Kuntze]) formed a supported but almost unresolved clade of Ixoroideae. Rova et al. (2002) found no support for a broad circumscription of the tribe Rondeletieae, and Guettardeae (sensu Robbrecht, 1988, 1993), including several former Rondeletieae taxa, was paraphyletic. Rondeletieae s. str. was almost entirely Antillean in geographic distribution. Furthermore, there was support for separation of several genera from the genus *Rondeletia* L. (*Arachnothryx* Planch., *Rogiera* Planch., *Roigella* Borhidi & M. Fernández Zeq., and *Suberanthus* Borhidi & M. Fernández Zeq.). The *trnL-F* data corroborated the position of *Retiniphyllum* Humb. & Bonpl. (Retinophylleae) in the Ixoroideae (in Antirheoideae fide Robbrecht, 1988) between Mussaendeae and the main part of Ixoroideae as proposed in Andersson and Rova (1999), based on *rps16* data. Rova et al. (2002) also presented new taxonomic positions for several genera sequenced for the first time: *Allenanthus* Standl. (close to Guettardeae/Rondeletieae), *Blepharidium* Standl. (Rondeletieae), *Chione* DC. (close to Hamelieae–Hillieae), *Coutaportla* Urb. (Chiococceae), *Dolichodelphys* (close to *Calycophyllum–Condaminea–Hippotis*), *Mazaea* Krug & Urb. (Rondeletieae), *Neobertiera* (Sipaneeae), *Neoblakea* Standl. (close to Guettardeae–Rondeletieae), *Phialanthus* Griseb. (Chiococceae–Catesbaeeae), *Phyllacanthus* Hook. f. (Chiococceae–Catesbaeeae), *Phyllomelia* Griseb. (Rondeletieae), *Schmidtottia* Urb. (Chiococceae–Catesbaeeae), and *Suberanthus* (Rondeletieae).

The studies discussed above provide strong support for three large supported subclades corresponding to the subfamilies Rubioideae, Ixoroideae, and Cinchonoideae. However, the basalmost nodes in the family are still uncertain or unresolved (but these basal nodes are under investigation by Rydin et al. [2009]). We still do not know how the genus *Luculia* and the tribe Coptosapelteae are related to the three subfamilies, for example. To have a detailed phylogenetic picture of the family and to understand circumscriptions of subgroups, we need sequence data for all described genera, and, so far, more than 200 genera have not been included in published molecular analyses. In most cases, morphological data or traditional classification can indicate a possible phylogenetic position, such as placing genera within tribes, but for some genera this is difficult. Further-

Table 1. List of the 348 Rubiaceae genera discussed in the text, with tribal position.

| Genus | Position | Genus | Position |
|---|---|---|---|
| *Acranthera* Arn. ex Meisn. | no tribe | *Ceratopyxis* Hook. f. | CHI |
| *Adina* Salisb. | NAU | *Ceriscoides* (Hook. f.) Tirveng. | GAR* |
| *Adinauclea* Ridsdale | NAU | *Chalepophyllum* Hook. f. | SIP |
| *Afrocanthium* (Bridson) Lantz | VAN | *Chassalia* Poir. | PSY |
| & B. Bremer | | *Chazaliella* E. M. A. Petit & Verdc. | PSY |
| *Aidia* Lour. | GAR* | *Chimarrhis* Jacq. | CON |
| *Alberta* E. Mey. | ALB | *Chiococca* P. Browne | CHI |
| *Aleisanthia* Ridl. | IXOR, no tribe | *Chione* DC. | c HAM/HIL |
| *Aleisanthiopsis* Tange | IXOR, no tribe | *Ciliosemina* Antonelli | CIN |
| *Alibertia* A. Rich. ex DC. | GAR* | *Cinchona* L. | CIN |
| *Allenanthus* Standl. | c GUE/RON | *Cinchonopsis* L. Andersson | CIN |
| *Alseis* Schott | CON | *Coccocypselum* P. Browne | COU |
| *Amaioua* Aubl. | GAR* | *Coddia* Verdc. | GAR* |
| *Amphiasma* Bremek. | SPE | *Coelospermum* Blume | MOR* |
| *Amphidasya* Standl. | URO | *Coffea* L. | COF |
| *Ancylanthos* Desf. | VAN | *Commitheca* Bremek. | URO |
| *Anthorrhiza* C. R. Huxley & Jebb | PSY | *Condaminea* DC. | CON |
| *Anthospermum* L. | ANT | *Conostomium* (Stapf.) Cufod. | SPE |
| *Antirhea* Comm. ex Juss. | GUE | *Coprosma* J. R. Forst. & G. Forst. | ANT |
| *Aoranthe* Somers | GAR* | *Coptosapelta* Korth. | COP |
| *Aphaenandra* Miq. | MUS | *Corynanthe* Welw. | NAU |
| *Arachnothryx* Planch. | RON | *Cosmibuena* Ruiz & Pav. | HIL |
| *Arcytophyllum* Willd. ex | SPE | *Coussarea* Aubl. | COU |
| Schult. & Schult. f. | | *Coutaportla* Urb. | CHI |
| *Argostemma* Wall. | ARG | *Coutarea* Aubl. | CHI |
| *Asemnantha* Hook. f. | CHI | *Craterispermum* Benth. | CRA |
| *Asperula* L. | RUB | *Cremaspora* Benth. | CRE |
| *Atractocarpus* Schltr. & K. Krause | GAR* | *Crucianella* L. | RUB |
| *Atractogyne* Pierre | GAR* | *Cruciata* Mill. | RUB |
| *Augusta* Pohl | IXOR, no tribe | *Crusea* Cham. & Schltdl. | SPE |
| *Badusa* A. Gray | CHI | *Cubanola* Aiello | CHI |
| *Bathysa* C. Presl | CON | *Cyclophyllum* Hook. f. | VAN |
| *Benkara* Adans. | GAR* | *Damnacanthus* C. F. Gaertn. | MOR* |
| *Bertiera* Aubl. | BER | *Danais* Comm. ex Vent. | DAN |
| *Bikkia* Reinw. | CHI | *Deccania* Tirveng. | GAR* |
| *Blepharidium* Standl. | RON | *Declieuxia* Kunth | COU |
| *Borojoa* Cuatrec. | GAR* | *Dendrosipanea* Ducke | SIP |
| *Borreria* G. Mey. | SPE | *Dentella* J. R. Forst. & G. Forst. | SPE |
| *Bouvardia* Salisb. | SPE | *Dialypetalanthus* Kuhlm. | IXOR, no tribe |
| *Bremeria* Razafim. & Alejandro | MUS | *Dictyandra* Welw. ex Hook. f. | PAV |
| *Breonadia* Ridsdale | NAU | *Didymaea* Hook. f. | RUB |
| *Breonia* A. Rich. ex DC. | NAU | *Didymosalpinx* Keay | GAR* |
| *Burchellia* R. Br. | GAR* | *Diodia* L. | SPE |
| *Burttdavya* Hoyle | NAU | *Dioicodendron* Steyerm. | CON |
| *Calochone* Keay | GAR* | *Diplospora* DC. | COF |
| *Calycophyllum* DC. | CON | *Dolichodelphys* K. Schum. & K. Krause | CON |
| *Canthium* Lam. | VAN | *Duperrea* Pierre ex Pit. | GAR* |
| *Capirona* Spruce | CON | *Duroia* L. f. | GAR* |
| *Carapichea* Aubl. | PSY | *Durringtonia* R. J. F. Hend. & Guymer | ANT |
| *Carpacoce* Sond. | ANT | *Ecpoma* K. Schum. | SAB-tent |
| *Carphalea* Juss. | KNO | *Elaeagia* Wedd. | CON |
| *Carterella* Terrell | SPE | *Emmenopterys* Oliv. | CON |
| *Casasia* A. Rich. | GAR* | *Erithalis* P. Browne | CHI |
| *Catesbaea* L. | CHI | *Ernodea* Sw. | SPE |
| *Catunaregam* Wolf | GAR* | *Euclinia* Salisb. | GAR* |
| *Cephalanthus* L. | NAU | *Exostema* (Pers.) Bonpl. | CHI |

Table 1.  Continued.

| Genus | Position | Genus | Position |
|---|---|---|---|
| *Fadogia* Schweinf. | VAN | *Leroya* Cavaco | VAN |
| *Faramea* Aubl. | COU | *Limnosipanea* Hook. f. | SIP |
| *Feretia* Delile | OCT | *Luculia* Sweet | no tribe |
| *Fernelia* Comm. ex Lam. | OCT | *Ludekia* Ridsdale | NAU |
| *Gaertnera* Lam. | GAE | *Macbrideina* Standl. | CON |
| *Galium* L. | RUB | *Macrosphyra* Hook. f. | GAR* |
| *Galopina* Thunb. | ANT | *Maguireothamnus* Steyerm. | SIP |
| *Gardenia* Ellis | GAR* | *Manostachya* Bremek. | SPE |
| *Genipa* L. | GAR* | *Margaritopsis* C. Wright | PSY |
| *Geophila* D. Don | PSY | *Maschalocorymbus* Bremek. | URO |
| *Gleasonia* Standl. | c POS | *Massularia* (K. Schum.) Hoyle | GAR* |
| *Glossostipula* Lorence | GAR* | *Mazaea* Krug & Urb. | RON |
| *Gomphocalyx* Baker | SPE | *Melanopsidium* Colla | GAR* |
| *Greenea* Wight & Arn. | IXOR, no tribe | *Metadina* Bakh. f. | NAU |
| *Guettarda* L. | GUE | *Meyna* Roxb. ex Link | VAN |
| *Gynochthodes* Blume | MOR* | *Mitchella* L. | MOR* |
| *Gyrostipula* J.-F. Leroy | NAU | *Mitracarpus* Zucc. ex Schult. & Schult. f. | SPE |
| *Haldina* Ridsdale | NAU | *Mitragyna* Korth. | NAU |
| *Hamelia* Jacq. | HAM | *Mitriostigma* Hochst. | GAR* |
| *Hedyotis* L. | SPE | *Molopanthera* Turcz. | POS |
| *Heinsia* DC. | MUS | *Morelia* A. Rich. ex DC. | GAR* |
| *Heinsenia* K. Schum. | GAR* | *Morierina* Vieill. | CHI |
| *Hekistocarpa* Hook. f. | SAB | *Morinda* L. | MOR* |
| *Heterophyllaea* Hook. f. | COU | *Multidentia* Gilli | VAN |
| *Hindsia* Benth. ex Lindl. | COU | *Mussaenda* L. | MUS |
| *Hintonia* Bullock | CHI | *Mussaendopsis* Baill. | CON |
| *Hippotis* Ruiz & Pav. | CON | *Mycetia* Reinw. | ARG |
| *Hoffmannia* Sw. | HAM | *Myonima* Comm. ex Juss. | IXO |
| *Houstonia* L. | SPE | *Myrmecodia* Jack | PSY |
| *Hutchinsonia* Robyns | VAN | *Myrmeconauclea* Merr. | NAU |
| *Hydnophytum* Jack | PSY | *Myrmephytum* Becc. | PSY |
| *Hydrophylax* L. f. | SPE | *Nauclea* L. | NAU |
| *Hymenocoleus* Robbr. | PSY | *Neblinathamnus* Steyerm. | SIP-tent |
| *Hymenodictyon* Wall. | HYM | *Nenax* Gaertn. | ANT |
| *Hyperacanthus* E. Mey. ex Bridson | GAR* | *Neobertiera* Wernham | SIP |
| *Ibetralia* Bremek. | GAR* | *Neoblakea* Standl. | c GUE/RON |
| *Isertia* Schreb. | ISE | *Neolamarckia* Bosser | NAU |
| *Isidorea* A. Rich. ex DC. | CHI | *Neolaugeria* Nicolson | GUE |
| *Ixora* L. | IXO | *Neoleroya* Cavaco | VAN |
| *Janotia* J.-F. Leroy | NAU | *Neomussaenda* Tange | MUS-tent |
| *Joosia* H. Karst | CIN | *Neonauclea* Merr. | NAU |
| *Kailarsenia* Tirveng. | GAR* | *Nertera* Banks & Sol. ex Gaertn. | ANT |
| *Keetia* E. Phillips | VAN | *Neurocalyx* Hook. | OPH |
| *Kelloggia* Torr. ex Benth. & Hook. f. | c RUB | *Normandia* Hook. f. | ANT |
| *Kerianthera* J. H. Kirkbr. | ISE | *Notopleura* (Benth. & Hook. f.) Bremek. | PSY |
| *Knoxia* L. | KNO | *Ochreinauclea* Ridsdale & Bakh. f. | NAU |
| *Kraussia* Harv. | OCT | *Oldenlandia* L. | SPE |
| *Kutchubaea* Fisch. ex DC. | GAR* | *Oldenlandiopsis* Terrell & W. H. Lewis | SPE |
| *Ladenbergia* Klotzsch | CIN | *Oligocodon* Keay | GAR* |
| *Lagynias* E. Mey. ex Robyns | VAN | *Opercularia* Gaertn. | ANT |
| *Landiopsis* Bosser | MUS | *Ophiorrhiza* L. | OPH |
| *Lasianthus* Jack | LAS | *Oreopolus* Schltdl. | COU |
| *Leptactina* Hook. f. | PAV | *Osa* Aiello | CHI |
| *Leptodermis* Wall. | PAE | *Otiophora* Zucc. | KNO |
| *Leptostigma* Arn. | ANT | *Otomeria* Benth. | KNO |
| *Lerchea* L. | OPH | *Oxyanthus* DC. | GAR* |

Table 1. Continued.

| Genus | Position | Genus | Position |
|---|---|---|---|
| *Oxyceros* Lour. | GAR* | *Retiniphyllum* Humb. & Bonpl. | RET |
| *Pachystigma* Hochst. | VAN | *Richardia* L. | SPE |
| *Paederia* L. | PAE | *Rogiera* Planch. | RON |
| *Pagamea* Aubl. | GAE | *Roigella* Borhidi & M. Fernández Zeq. | RON |
| *Palicourea* Aubl. | PSY | *Rondeletia* L. | RON |
| *Parachimarrhis* Ducke | CON | *Rosenbergiodendron* Fagerl. | GAR* |
| *Paracoffea* J.-F. Leroy | COF | *Rothmannia* Thunb. | GAR* |
| *Paracorynanthe* Capuron | HYM | *Rubia* L. | RUB |
| *Paragenipa* Baill. | OCT | *Rudgea* Salisb. | PSY |
| *Parapentas* Bremek. | KNO | *Rustia* Klotzsch | CON |
| *Pauridiantha* Hook. f. | URO | *Rutidea* DC. | PAV |
| *Pausinystalia* Pierre ex Beille | NAU | *Rytigynia* Blume | VAN |
| *Pavetta* L. | PAV | *Sabicea* Aubl. | SAB |
| *Pentagonia* Benth. | CON | *Salzmannia* DC. | CHI |
| *Pentaloncha* Hook. f. | SAB-tent | *Sarcocephalus* Azfel. ex R. Br. | NAU |
| *Pentanisia* Harv. | KNO | *Schizomussaenda* H. L. Li | MUS |
| *Pentanopsis* Rendle | SPE | *Schizostigma* Arn. ex Meisn. | SAB-tent |
| *Pentas* Benth. | KNO | *Schmidtottia* Urb. | CHI |
| *Peponidium* (Baill.) Arènes | VAN | *Schradera* Vahl | SCH |
| *Pertusadina* Ridsdale | NAU | *Schumanniophyton* Harms | GAR* |
| *Phialanthus* Griseb. | CHI | *Scolosanthus* Vahl | CHI |
| *Phuopsis* (Griseb.) Hook. f. | RUB | *Scyphiphora* C. F. Gaertn. | c IXO/VAN |
| *Phyllacanthus* Hook. f. | CHI | *Scyphochlamys* Balf. f. | VAN |
| *Phyllis* L. | ANT | *Serissa* Comm. ex A. Juss. | PAE |
| *Phyllomelia* Griseb. | RON | *Sherardia* L. | RUB |
| *Phylohydrax* Puff | SPE | *Sherbournia* G. Don | GAR* |
| *Picardaea* Urb. | CON | *Siemensia* Urb. | CHI |
| *Pimentelia* Wedd. | CIN-tent | *Sinoadina* Ridsdale | NAU |
| *Pinckneya* Michx. | CON | *Sipanea* Aubl. | SIP |
| *Pittierothamnus* Steyerm. | SAB-tent | *Sipaneopsis* Steyerm. | SIP |
| *Placopoda* Balf. f. | KNO | *Solenandra* Hook. f. | CHI |
| *Platycarpum* Humb. & Bonpl. | SIP | *Sommera* Schltdl. | CON |
| *Pogonopus* Klotzsch | CON | *Spermacoce* L. | SPE |
| *Pomax* DC. | ANT | *Spermadictyon* Roxb. | PAE |
| *Porterandia* Ridl. | GAR* | *Sphinctanthus* Benth. | GAR* |
| *Portlandia* P. Browne | CHI | *Squamellaria* Becc. | PSY |
| *Posoqueria* Aubl. | POS | *Stachyarrhena* Hook. f. | GAR* |
| *Pouchetia* DC. | OCT | *Stenaria* (Raf.) Terrell | SPE |
| *Praravinia* Korth. | URO | *Stenostomum* C. F. Gaertn. | GUE |
| *Pravinaria* Bremek. | URO | *Steyermarkia* Standl. | SIP-tent |
| *Preussiodora* Keay | GAR* | *Stilpnophyllum* Hook. f. | CIN |
| *Pseudocinchona* A. Chev. ex Perrot | NAU | *Stipularia* P. Beauv. | SAB-tent |
| *Pseudomussaenda* Wernham | MUS | *Streblosa* Korth. | PSY |
| *Pseudopeponidium* Arènes | VAN | *Strumpfia* Jacq. | c CHI |
| *Pseudosabicea* N. Hallé | SAB | *Suberanthus* Borhidi & M. Fernández Zeq. | RON |
| *Psilanthus* Hook. f. | COF | *Sukunia* A. C. Sm. | GAR* |
| *Psychotria* L. | PSY | *Tamilnadia* Tirveng. & Sastre | GAR* |
| *Psydrax* Gaertn. | VAN | *Tamridaea* Thulin & B. Bremer | SAB |
| *Psyllocarpus* Mart. & Zucc. | SPE | *Tapiphyllum* Robyns | VAN |
| *Pteridocalyx* Wernham | SIP-tent | *Tarenna* Gaertn. | PAV |
| *Putoria* Pers. | PUT | *Tarennoidea* Tirveng. & Sastre | GAR* |
| *Pyrostria* Comm. ex Juss. | VAN | *Temnopteryx* Hook. f. | SAB-tent |
| *Ramosmania* Tirveng. & Verdc. | OCT | *Theligonum* L. | THE |
| *Randia* L. | GAR* | *Timonius* DC. | GUE |
| *Raritebe* Wernham | URO | *Tocoyena* Aubl. | GAR* |
| *Readea* Gillespie | PSY | *Tricalysia* A. Rich. ex DC. | COF |
| *Remijia* DC. | CIN | *Trichostachys* Hook. f. | LAS |

Table 1.   Continued.

| Genus | Position | Genus | Position |
|---|---|---|---|
| *Trukia* Kaneh. | GAR* | *Wendlandia* DC. | IXOR, no tribe |
| *Uncaria* Schreb. | NAU | *Versteegia* Valeton | IXO |
| *Urophyllum* Wall. | URO | *Virectaria* Bremek. | SAB |
| *Valantia* L. | RUB | *Wittmackanthus* Kuntze | CON |
| *Vangueria* Juss. | VAN | *Xanthophytum* Reinw. ex Blume | OPH |
| *Warszewiczia* Klotzsch | CON | *Yutajea* Steyerm. | ISE |

* Paraphyletic/polyphyletic tribes.

Abbreviations: no tribe, without tribal position (taxon has been molecularly investigated, but has not been placed within any described tribe); c, close to (taxon is sister group to or close to one or two tribes); tent, tentatively (taxon is not molecularly investigated but has been suggested to be included in the tribe); ALB, Alberteae; ANT, Anthospermeae; ARG, Argostemmateae; BER, Bertiereae; CHI, Chiococceae; CIN, Cinchoneae; COF, Coffeeae; CON, Condamineeae; COP, Coptosapelteae; COU, Coussareeae; CRA, Craterispermeae; CRE, Cremasporeae; DAN, Danaideae; GAE, Gaertnereae; GAR*, Gardenieae; GUE, Guettardeae; HAM, Hamelieae; HIL, Hillieae; HYM, Hymenodictyeae; ISE, Isertieae; IXO, Ixoreae; IXOR, Ixoroideae; KNO, Knoxieae; LAS, Lasiantheae; MOR*, Morindeae; MUS, Mussaendeae; NAU, Naucleeae; OCT, Octotropideae; OPH, Ophiorrhizeae; PAE, Paederieae; PAV, Pavetteae; POS, Posoquerieae; PSY, Psychotrieae; PUT, Putorieae; RET, Retiniphylleae; RON, Rondeletieae; RUB, Rubieae; SAB, Sabiceeae; SCH, Schradereae; SIP, Sipaneeae; SPE, Spermacoceae; THE, Theligoneae; URO, Urophylleae; VAN, Vanguerieae.

more, if Rubiaceae should become the perfect model family for ecological, evolutionary, biogeographic, or other studies, we must work hard over the coming years with the challenge to sequence all described genera and species.

SUBFAMILY RUBIOIDEAE

At the Second International Conference on Rubiaceae in Brussels in 1995, Bremer (1996) focused on subfamily Rubioideae; 59 taxa representing most tribes of the subfamily were investigated for *rbcL*. The analysis showed that Anthospermeae, Rubieae, Spermacoceae s.l. (including the *Pentas* group = Knoxieae [*Pentas*, *Carphalea* Juss., *Parapentas* Bremek., *Pentanisia* Harv., and *Placopoda* Balf. f.], Hedyotideae, and Spermacoceae s. str.), and Psychotrieae s.l. (including also Morindeae and Gaertnereae) are monophyletic. Paederieae and Argostemmateae were shown to be polyphyletic. *Lasianthus* Jack and *Gaertnera* Lam. were shown not to belong to Psychotrieae s. str. The following genera from different tribes were represented by single species and thus could not be tested for monophyly, but could be positioned phylogenetically: *Coccocypselum* P. Browne (Coussareeae), *Danais* Comm. ex Vent., *Faramea* Aubl. (Coussareeae), *Mycetia* Reinw., *Ophiorrhiza* L., *Pauridiantha* Hook. f. (Urophylleae), and *Theligonum* L. The genus *Mycetia* was shown to be close to *Argostemma* Wall. and not a member of the Isertieae (Robbrecht, 1988).

A few years later, Andersson and Rova (1999) published an analysis of *rps16* sequences from 143 Rubiaceae taxa and five outgroups, also focusing on subfamily Rubioideae. The results confirmed those based on *rbcL* data (Bremer, 1996) for the main groups of the family, but more taxa were included and the support was stronger for several clades. A few differences between the *rps16* and the *rbcL* results were revealed. In the *rbcL* data, Spermacoceae s.l. forms one monophyletic clade with 76% jackknife support including three of the tribes recognized by Andersson and Rova (1999), Spermacoceae, Heyotideae, and Knoxieae. In the *rps16* analysis, Knoxieae is instead sister to a larger group of Spermacoceae, Heyotideae, and also Paederieae and Rubieae, but without support. Morindeae (80% bootstrap support) is found to be monophyletic, which disagrees with the *rbcL* data. The included and supported tribes of the Rubioideae from the base of the tree were the following: Urophylleae (*Urophyllum* Wall., *Pauridiantha*, *Raritebe* Wernham [100%]), Ophiorrhizeae (single taxon), Coussareeae (*Coussarea* Aubl., *Faramea* [76%]), Coccocypseleae (100%) together with the two unclassified genera *Hindsia* Benth. ex Lindl. and *Declieuxia* Kunth, Cruckshanksieae (*Heterophyllaea* Hook. f., *Oreopolus* Schltdl. [78%]), Gaertnereae (*Gaertnera*, *Pagamea* Aubl. [100%]), Schradereae (*Schradera* Vahl, single taxon), Morindeae (*Morinda* L., *Damnacanthus* C. F. Gaertn., *Mitchella* L., *Coelospermum* Blume, *Gynochthodes* Blume [80%]), Psychotrieae (*Psychotria*, *Chassalia* Poir., *Chazaliella* E. M. A. Petit & Verdc., *Geophila* D. Don, *Hydnophytum*, *Margaritopsis* C. Wright, *Myrmecodia* Jack, *Palicourea* Aubl., *Readea* Gillespie, *Rudgea* Salisb., *Squamellaria* Becc., *Streblosa* Korth. [99%]), Knoxieae (*Knoxia* L., *Otiophora* Zucc., *Otomeria* Benth., *Pentas*, *Pentanisia* Harv. [100%]), Anthospermeae (*Coprosma* J. R. Forst. & G. Forst., *Galopina* Thunb., *Leptostigma* Arn., *Nenax* Gaertn., *Nertera*

Banks & Sol. ex Gaertn., *Opercularia* Gaertn., *Phyllis* L. [53%]), Rubieae (*Rubia*, *Asperula* L., *Crucianella* L., *Galium*, *Sherardia* L., *Valantia* L. [100%]), and Spermacoceae (*Spermacoce* L., *Borreria* G. Mey., *Crusea* Cham. & Schltdl., *Diodia* L., *Ernodea* Sw., *Mitracarpus* Zucc. ex Schult. & Schult. f., *Psyllocarpus* Mart. & Zucc., *Richardia* L. [85%]). The tribes Paederieae and Hedyotideae were paraphyletic as in Bremer (1996). The genus *Psychotria* is paraphyletic in agreement with Nepokroeff et al. (1999).

A new phylogeny and a new comprehensive classification of Rubioideae were presented by Bremer and Manen (2000). They analyzed 151 genera with three different molecular markers, *rbcL*, *atpB-rbcL*, and *rps16* (latter data from Andersson & Rova, 1999). The separate markers and combined analyses gave similar results. The tribes Ophiorrhizeae (*Ophiorrhiza*, *Neurocalyx*, *Lerchea* L., *Xanthophytum*), Urophylleae (*Urophyllum*, *Amphidasya* Standl., *Commitheca* Bremek., *Maschalocorymbus* Bremek., *Praravinia* Korth., *Pravinaria* Bremek., *Pauridiantha*), Lasiantheae (*Lasianthus*, *Trichostachys* Hook. f.), and Coussareeae formed a grade to the rest of the family, which consisted of two newly established but informal groups (with 99% and 100% bootstrap support, respectively): the Psychotrieae alliance (Psychotrieae, Craterispermeae [*Craterispermum* Benth.], Gaertnereae, Morindeae [paraphyletic], Schradereae) and the Spermacoceae alliance (Spermacoceae, Anthospermeae, Argostemmateae, Danaideae, Paederieae [paraphyletic], Rubieae, Theligoneae). Of the accepted 16 Rubioideae tribes, 11 were in agreement with earlier circumscriptions. Ophiorrhizeae, Coussarieae, and Spermacoceae received wider circumscriptions, and Lasiantheae and Danaideae were described as new. All monophyletic tribes received 100% bootstrap support (except for Psychotrieae, with only 81% support).

From the studies outlined above, there is support for most of the Rubioideae tribes and the many relationships between them. However, at the end of 2005, only seven of the tribes had been the subject of detailed studies, presented below. It should be stressed that several tribes and also relationships between tribes (e.g., the basal clades Coussareae, Lasiantheae, Ophiorrihizeae, Urophylleae, and clades within the Psychotrieae alliance) are under investigation. Rubioideae is probably the best understood subfamily phylogenetically, but still only a minority of its species have been investigated. The most important task for the coming years will be to analyze and sequence most species of the large and problematic genera. Rubioideae contains 11 of the 20 largest genera of the family (*Psychotria*, *Galium*, *Ophiorrhiza*, *Palicourea*, *Spermacoceae*, *Oldenlandia* L., *La-*

*sianthus*, *Faramea*, *Asperula*, *Argostemma*, and *Coussarea*). These genera together contain about 40% of all species in the family and, because some of these genera represent much of the Rubiaceae species diversity, understanding of their phylogeny would be an important asset for deeper evolutionary studies.

Tribe Rubieae was investigated by Manen et al. (1994), who used the *atpB-rbcL* spacer of 25 species of the tribe. They found support for a monophyletic Rubieae, and the two investigated species of *Rubia* were found to be sister to the rest of the tribe. Manen and coworkers identified four further clades, but with low or moderate bootstrap support. The highest support (87% bootstrap support) was for the *Sherardia* clade (*Sherardia* together with *Crucianella*, and *Phuopsis* (Griseb.) Hook. f.) and 81% bootstrap support was found for the *Asperula* clade (*Asperula* together with *Galium elongatum* C. Presl and *G. palustre* L.). The relationship between the four clades was unresolved and *Galium* was paraphyletic. Later, Natali et al. (1995) added more sequences to the Manen et al. (1994) data set, for a total of 70 Rubieae species and 25 taxa of 12 other tribes of Rubioideae. They got 100% bootstrap support for tribe Rubieae and subfamily Rubioideae. They excluded Ophiorrhizeae, and, with that circumscription, the subfamily was also characterized by a 204 bp deletion in the *atpB-rbcL* region. Natali et al. (1995) divided the Rubieae into the same five clades as in Manen et al. (1994), but with lower support; *Rubia* is still monophyletic (100% support) and sister to the rest. They showed that the genus *Asperula* is paraphyletic, with all added species instead belonging to their *Sherardia* clade. Manen and Natali (1996), in an article about the deletion in the *atpB-rbcL* region (loss of an *atpB* promoter) in the Rubioideae, investigated the *atpB-rbcL* spacer from representatives of the whole family, but with a main focus on subfamily Rubioideae. They presented a tree for 22 genera (they refer to an analysis of 111 taxa, which was not presented in the article). They rooted the published tree between subfamily Ixoroideae (*Coffea* and *Ixora*) and the rest. The Cinchonoideae, including five genera, was sister to a clade including their Rubioideae and *Ophiorrhiza*. They found strong support for Rubioideae (*Ophiorrhiza* excluded) and Rubieae (including the two genera *Rubia* and *Didymaea* Hook. f.). Rubieae was sister to Theligoneae and *Putoria* Pers. and these are sister to *Paederia* L.; other Rubioideae taxa in the analysis included Anthospermeae, Coccosypseleae, Hedyotideae, Morindeae, Psychotrieae, and Spermacoceae. Their results agree with the *rbcL* data (Bremer & Jansen, 1991; Bremer et al., 1995) that Hamelieae does not belong to Rubioideae but instead to the Cinchonoideae. Their main conclusion is that the lack

of the *atpB* promoter for the Rubioideae excluding the Ophiorrhizeae "gives strong evidences on the boundary between the subfamily Rubiodieae and the other Rubiaceae" (Manen & Natali, 1996: 56). However, they do not suggest any taxonomic position, or to which subfamily Ophiorrhizeae belongs. In another article, Natali et al. (1996) published the same tree based on *atpB-rbcL* data for the 22 genera, but they also analyzed the Rubieae with a denser sampling of 78 Rubieae taxa. The result agrees with their earlier analysis in Natali et al. (1995) but divides the Rubieae into seven clades, now with *Didymaea* as sister to the rest, followed by the clades *Rubia*, *Asperula* sect. *Asperula*, *Asperula* sect. *Glabella*, *Sherardia*, *Cruciata* Mill., and *Galium* sect. *Galium*. Only *Rubia* was highly supported as monophyletic. Despite the extended sampling, the relationships between the different groups were unresolved.

*Kelloggia* Torr. ex Benth. & Hook. f. (Paederieae fide Robbrecht [1988], but in Backlund et al. [2007] without tribal position), a genus of two species with disjunct distribution in western North America and the western part of eastern Asia, was analyzed with three chloroplast markers (*rbcL*, *atpB-rbcL*, *rps16*) by Nie et al. (2005). They showed that the genus is monophyletic and sister to the Rubieae. *Kelloggia* was also included in a Ph.D. thesis by Backlund (2005), and the same position of the genus close to Rubieae was well supported. It was further demonstrated (Backlund, 2005) that the clade of Theligoneae–*Kelloggia*–Rubieae is sister group to a reestablished tribe Putorieae (a position that makes the rest of the Paederieae monophyletic).

The taxonomically complex tribe Psychotrieae and the very large genus *Psychotria* were molecularly investigated for the first time by Nepokroeff et al. (1999). They analyzed 85 taxa for ITS and *rbcL*. The results suggested that *Psychotria* is broadly paraphyletic. Taxa earlier assigned to *Psychotria*, *Psychotria* sect. *Notopleura* Benth. & Hook. f., and subgenus *Heteropsychotria* Steyerm., plus *Palicourea* were closer to other genera of Psychotrieae than to subgenus *Psychotria*. *Psychotria* was suggested to be restricted to a monophyletic group including two subclades. One subclade is Pacific in distribution and includes the myrmecophytic subtribe Hydnophytineae (including *Hydnophytum*, *Anthorrhiza* C. R. Huxley & Jebb, *Myrmecodia*, *Myrmephytum* Becc.) as a subgroup. The other subclade included *Psychotria* subg. *Psychotria* and subgenus *Tetramerae* E. M. A. Petit. It was also shown that the genus *Declieuxia* was not a member of the Psychotrieae but closer to *Coccosypselum*. Later, Andersson (2002a) sequenced *rps16* for 111 species of the *Psychotria* complex. The result was very much in agreement with Nepokroeff et al.

(1999). Andersson also analyzed a combined data set (the ITS sequences of Nepokroeff et al. [1999] and their *rps16* sequences) for 15 taxa that were shared between the two studies. That analysis resulted in a tree with three well-supported clades, the outgroup (including, e.g., *Carapichea* Aubl., *Chassalia*, *Geophila*, *Hymenocoleus* Robbr., *Notopleura* (Benth. & Hook. f.) Bremek., *Rudgea*, *Palicouria*), two *Psychotria* subclades, *Psychotria* s. str. (= subgenus *Psychotria*, and subgenus *Tetramerae* in Nepokroeff et al. [1999]), and a Pacific subclade (including several *Psychotria* species and also the Hydnophytineae). *Psychotria* s. str. is characterized by usually having pyrenes with or without preformed germination slits (Piesschaert, 2001), a plane or shallowly furrowed adaxial surface, and usually numerous distinct ridges on the abaxial side. Other characters are discussed by Davis et al. (2001). The Pacific clade is characterized by pyrenes with distinct marginal preformed germination slits. The main difference between the studies by Nepokroeff et al. (1999) and Andersson (2002a) is that Nepokroeff et al. included the Pacific clade in *Psychotria* s. str. while Andersson excluded it.

*Carapichea* was reestablished as a genus by Andersson (2002b) for three species of the *Psychotria* complex in a study based on *rps16* data. Two of the species, *P. borucana* (Ant. Molina) C. M. Taylor & W. C. Burger (= *Cephaelis affinis* Standl.) and *P. ipecacuanha* (Brot.) Stokes, had been shown by Nepokroeff et al. (1999) to be closely related and sister to *Geophila* and *Hymenocoleus*; Andersson (2002b) found a third species, *P. guianensis* Rusby (described as *Carapichea guianensis* Aubl.), that was distant from the *Psychotria* s. str. but belonged to the same group. These three taxa included in the reestablished genus *Carapichea* were strongly supported as a group, but the exact relationship within the *Palicourea* complex was unsupported. The genus was characterized "by having stipules that are not shed by formation of an abscission layer, leaves that dry greenish or greyish, aperturate pollen, and planoconvex pyrenes with an adaxial furrow and preformed germination slits on abaxial ridges, but not along the margins" (Andersson, 2002b: 363).

Phylogeny of the tribe Anthospermeae was estimated based on ITS and *rps16* data by Anderson et al. (2001). They first analyzed a set of taxa, including Anthospermeae together with representatives of other Rubioideae tribes, to test if the tribe was monophyletic. In a second analysis of 25 Anthospermeae taxa (all except two genera of the tribe), they investigated the internal relationships of the genera. Most genera of Anthospermeae formed a monophyletic but

weakly supported clade, with *Carpacoce* Sond. excluded. The latter was instead sister to the Knoxieae. They found no support for a subdivision of the tribe into three subtribes and no support for a subdivision of *Coprosma* into two subgenera. They found support for a clade corresponding to Puff's (1982) subtribe Anthospermeae (*Anthospermum* L., *Nenax*, *Galopina*, and *Phyllis* with *Carpacoce* excluded) and moderate support for Coprosminae (*Coprosma*, *Durringtonia* R. J. F. Hend. & Guymer, *Leptostigma*, *Nertera*, and *Normandia* Hook. f.— with the latter nested within *Coprosma*), but *Pomax* DC. and *Opercularia* (Puff's subtribe Opercularinae) were placed unresolved in a trichotomy together with the Coprosminae.

Thulin and Bremer (2004) studied parts of the tribe Spermacoceae s.l. to circumscribe the genera *Amphiasma* Bremek. and *Pentanopsis* Rendle and to find the affinity of *Phylohydrax* Puff. They analyzed *rbcL* sequences of 34 tribal members and found that the African genera *Amphiasma*, *Conostomium* (Stapf) Cufod., and *Manostachya* Bremek. together with *Phylohydrax* form a strongly supported clade distant from *Hydrophylax* L. f., which was placed close to *Diodia* and *Spermacoce*. When *Phylohydrax* was established as a new genus (Puff, 1986), it was suggested to have evolved from a different stock than the genus *Hydrophylax*. This was also confirmed in a study by Thulin and Bremer (2004). Furthermore, *Amphiasma* was found to be paraphyletic and a new taxonomy was proposed. *Pentanopsis* was circumscribed as a genus of two species from northeastern tropical Africa, whereas *Amphiasma* was treated in its original sense as a genus of about eight species in south-central tropical Africa.

One year after *Phylohydrax* was positioned in the *Amphiasma–Conostomium* clade by Thulin and Bremer (2004), Dessein et al. (2005) published a study of *Gomphocalyx* Baker and *Phylohydrax*. They investigated morphology and compared it to results from molecular data (mainly sequences from GenBank). They showed that there are many morphological similarities between the genera, and they concluded, based on the molecular results, "that the character states of the two genera are largely consistent with the here-proposed position in Hedyotideae" (Dessein et al., 2005: 91).

The Andean genus *Arcytophyllum* Willd. ex Schult. & Schult. f. was investigated by *rps16* and *trnL-F* sequences by Andersson et al. (2002). They found support for a monophyletic *Arcytophyllum* (with *A. serpyllaceum* (Schltdl.) Terrell excluded, due to its closer relationship to *Bouvardia*) sister to a clade of American *Hedyotis* L. and *Houstonia* L. species. It is further suggested that these latter should be treated as a single genus, under the name of *Houstonia*. It was also suggested that the ancestral area of the *Arcytophyllum–Houstonia* clade is the South American plate.

*Houstonia*, a North American genus, was investigated for nuclear (ITS) and chloroplast (*trnL*) sequence variation (Church, 2003). He analyzed *Houstonia* and other closely related genera (*Carterella* Terrell, *Dentella* J. R. Forst. & G. Forst., *Hedyotis*, *Oldenlandia*, *Oldenlandiopsis* Terrell & W. H. Lewis, *Stenaria* (Raf.) Terrell), 30 taxa altogether. The phylogenetic results were compared to chromosome numbers, breeding systems, and life forms. *Houstonia* was not monophyletic and could not be kept distinct from *Stenaria* and North American *Hedyotis*. Within the North American lineage, it appeared that chromosomal changes have had an important role for history of diversification. The annual habit and a homostylous breeding system have originated several times and have probably not been major factors in the radiation of the species. Later, Church and Taylor (2005) investigated a larger set of species and populations (74 populations from 17 species) of the *Houstonia* lineage for ITS, *trnL*, and *trnS-G*. They found no evidence for hybridization in the ancestral species, but more recently derived species contained a wide degree of morphological and genetic variation both within and among species. They found a clear association between hybridization and polyploidy in the *Houstonia* lineage, supporting the idea that polyploidy may break down species barriers and allow hybridization among lineages.

*Gaertnera* of the tribe Gaertnereae is a Paleotropical genus of regional endemics with its highest diversity on Madagascar (25 species). The genus was investigated by Malcomber (2002; also Malcomber & Davis, 2005). Malcomber (2002) used four usually fast-evolving markers, and the genus was strongly supported as monophyletic. However, the genetic variation among species was insufficient to reconstruct well-supported subgeneric groups "counter to expectations based on the very distinct morphologies and widespread distribution of the genus" (Malcomber, 2002: 42).

The tribe Paederieae was one of the groups studied in a Ph.D. thesis by Backlund (2005). Earlier molecular analyses (Bremer, 1996; Andersson & Rova, 1999) had indicated that the tribe could be polyphyletic, and Backlund (2005) further investigated the tribe in a wide sense and found strong support for Paederieae s. str. (including *Paederia*, *Leptodermis* Wall., *Serissa* Comm. ex Juss., *Spermadictyon* Roxb.) and a reestablished tribe Putorieae.

SUBFAMILY IXOROIDEAE

Andreasen and Bremer (1996) investigated both morphological and molecular (*rbcL*) data of subfamily Ixoroideae s. str. They analyzed 40 ingroup taxa from Gardenieae (*Gardenia* Ellis, *Aidia* Lour., *Alibertia* A. Rich. ex DC., *Burchellia* R. Br., *Calochone* Keay, *Casasia* A. Rich., *Coddia* Verdc., *Didymosalpinx* Keay, *Duperrea* Pierre ex Pit., *Euclinia* Salisb., *Genipa* L., *Glossostipula* Lorence, *Heinsenia* K. Schum., *Hyperacanthus* E. Mey. ex Bridson, *Kailarsenia* Tirveng., *Massularia* (K. Schum.) Hoyle, *Mitriostigma* Hochst., *Oxyanthus* DC., *Oxyceros* Lour., *Porterandia* Ridl., *Randia* L., *Rosenbergiodendron* Fagerl., *Rothmannia* Thunb., *Sukunia* A. C. Sm.), Pavetteae (*Pavetta* L., *Dictyandra* Welw. ex Hook. f., *Leptactina* Hook. f., *Rutidea* DC., *Tarenna* Gaertn.), Octotropideae (*Feretia* Delile, *Fernelia* Comm. ex Lam., *Kraussia* Harv., *Paragenipa* Baill., *Pouchetia* DC., *Ramosmania* Tirveng. & Verdc.), and Coffeeae (*Coffea*, *Diplospora* DC., *Paracoffea* J.-F. Leroy, *Psilanthus* Hook. f., *Tricalysia* A. Rich. ex DC.) with *Mussaenda* as outgroup. They found that Vanguerieae (*Canthium* Lam., *Vangueria* Juss.) should be included in the subfamily. The Octotropideae, Pavetteae, and Coffeeae were monophyletic although with different circumscriptions of the latter two compared to earlier classifications. *Ixora* (together with *Myonima* Comm. ex Juss. and *Versteegia* Valeton) was not part of Pavetteae, and Coffeeae should include *Tricalysia* and probably *Bertiera* Aubl. as well. Subtribe Diplosporineae (*Cremaspora* Benth. and *Tricalysia*) and *Posoqueria* should be excluded from the tribe Gardenieae. Furthermore, they suggested that the informal tetrad group within Gardenieae (Robbrecht & Puff, 1986) is not monophyletic and that the characteristics of the pollen that is released in tetrads may have evolved several times. A few years later, Andreasen et al. (1999) analyzed and compared the utility of the nuclear ITS region with the cpDNA *rbcL* for the Ixoroideae. Variation of ITS was extensive and informative, but the sequences were difficult to align. New phylogenetic positions of taxa (e.g., for *Posoqueria*, *Bertiera*, *Ixora*, and Vanguerieae) that had been reported from the *rbcL* analysis, but contradicted the classification, were corroborated by the ITS data.

Later, Andreasen and Bremer (2000) presented additional analyses of the subfamily based on combinations of *rbcL*, ITS, and restriction fragment length polymorphism (RFLP) data for 77 ingroup taxa. The results agreed with the 1996 and 1999 studies, but many groups received higher support. Further, Alberteae (*Alberta* E. Mey.) was shown to be part of the subfamily, and the mangrove genus *Scyphiphora* C. F. Gaertn. (Antirheoideae fide Robbrecht, 1988; or

Gardenieae s.l. fide Puff & Rohrhofer, 1993) was shown to be close to Ixoreae.

There is strong support for 12 of the 15 investigated tribes of this subfamily as monophyletic (Cremasporeae and Retiniphylleae are monotypic or represented by single taxa and could not be tested for monophyly), but the large tribe Gardenieae is polyphyletic/paraphyletic. Despite strong support for the subfamily and the subgroup including Alberteae, Bertiereae, Coffeeae, Cremasporeae, Gardenieae, Octotropideae, and Pavetteae, most relationships between tribes are unresolved and in need of further research. So far, five Ixoroideae tribes have been studied and are presented below, and several tribes are under investigation. The most important tasks for the future in this subfamily will be to investigate the large complex around the polyphyletic/paraphyletic Gardenieae and to investigate the difficult and large genera *Ixora*, *Pavetta*, and *Tarenna*.

*Coffea* of the tribe Coffeeae has been the focus of several phylogenetic studies (Lashermes et al., 1997; Cros et al., 1998). The phylogeny of *Coffea* was in contradiction to the classification, particularly relative to the genus *Psilanthus*. However, there were correlations between clades and biogeography. It was also shown that *Coffea* has a recent origin and radiation in Africa (Cros et al., 1998).

*Dialypetalanthus* Kuhlm. (without tribal position) is an endemic Amazonian genus that has been treated as a monotypic family Dialypetalanthaceae (Rizzini & Occhioni, 1949), but various affinities have been suggested, e.g., Myrtaceae and Rubiaceae (Kuhlmann, 1925). It is an aberrant genus with free petals and an indefinite, extremely high number of stamens, characters that do not agree with Rubiaceae, but the genus shares many characteristics with taxa of Rubiaceae, e.g., opposite entire leaves with interpetiolar stipules, inferior ovary, bilobed stigma, capsular fruit, and winged seeds. Piesschaert et al. (1997) presented anatomical and morphological data that support an affinity with Gentianales, Rubiaceae in particular. Fay et al. (2000) published the first analysis of molecular (*rbcL*) data in which they showed that the genus belongs to Rubiaceae in the subfamily Ixoroideae s.l., but without tribal position.

Persson (1996) started his studies of tribe Gardenieae with an analysis of 70 morphological and anatomical characters for 81 taxa. Many nodes were unresolved or unsupported, but he found support for several of Robbrecht's and Puff's (1986) informal groups of the Gardenieae (tetrad group and *Alibertia* group, but *Aidia* group and Gardenieae were not supported). Later, Persson (2000a) continued his study of *rps16* and *trnL-F* data for 57 taxa of Gardenieae s.l. to try to resolve the more or less

unresolved phylogeny of the group; he also wanted to evaluate the conflicts between his morphological study (Persson, 1996) and the results from the *rbcL* data (Andreasen & Bremer, 1996). Persson's molecular tree (2000a) was still unresolved, with few supported groups. However, the informal *Alibertia* group (in the study including *Alibertia*, *Amaioua* Aubl., *Borojoa* Cuatrec., *Duroia* L. f., *Glossostipula*, *Ibetralia* Bremek., *Kutchubaea* Fisch. ex DC., *Melanopsidium* Colla, and *Stachyarrhena* Hook. f.) was well supported (97% bootstrap) and agreed with earlier results (Andreasen & Bremer, 1996; Persson, 1996; Andreasen, 1997). He further identified a core Gardenieae group (*Atractocarpus* Schltr. & K. Krause, *Benkara* Adans., *Catunaregam* Wolf, *Deccania* Tirveng., *Morelia* A. Rich. ex DC., *Sherbournia* G. Don, *Tamilnadia* Tirveng. & Sastre, *Trukia* Kaneh., and *Tarennoidea* Tirveng. & Sastre, among others, but excluding subtribe Diplosporinae, *Burchellia*, *Didymosalpinx*, *Schumanniophyton* Harms, and several taxa belonging to other Ixoroideae tribes) with two subgroups, the *Gardenia* clade and the *Randia* clade. On the other hand, there was no support for an *Aidia* group or for a monophyletic tetrad group (Robbrecht & Puff, 1986), both proposed from morphological data (Persson, 1996). It was further concluded from Persson's molecular data that the pollen release in tetrads had originated several times. It occurs in the large genus *Gardenia*, but not in its close relatives *Aoranthe* Somers, *Ceriscoides* (Hook. f.) Tirveng., *Genipa*, and *Kailarsenia* (a clade with 83% bootstrap support); most genera with tetrad pollen occur in a clade of Neotropical genera around *Randia* in which several genera also have monad pollen, e.g., *Rosenbergiodendron*, *Sphinctanthus* Benth., and *Tocoyena* Aubl. Furthermore, outside the core Gardenieae there was also a clade of the genera *Atractogyne* Pierre, *Mitriostigma*, and *Oxyanthus* (86% bootstrap support) with tetrad pollen.

Persson later (2000b) extended his study of the *Alibertia* group (Gardenieae), the group of taxa that "comprises neotropical, dioecious taxa with heteromerous flowers, and monad pollen grains" (Persson, 2000b: 1018). He sequenced two nuclear spacers (ITS and 5S-NTS) for 38 species (of the ca. 120) and found several strongly supported clades in the group. However, *Borojoa* was paraphyletic and nested within *Alibertia* (in a group close to the type species *A. edulis* A. Rich. ex DC.), with *Borojoa* included and *A. hispida* Ducke excluded. *Alibertia* was monophyletic and distinctly divided into two main clades, one including the type species and one around *A. sessilis* (Vell.) K. Schum. In the combined analysis, *Alibertia* was sister to a clade of *Duroia*, with the genus *Amaioua* nested within *Duroia*. *Ibetralia*, *Kutchubaea*,

and *A. hispida* formed a well-supported clade at the unresolved base of the tree together with the rest of the taxa.

*Randia*, a genus of ca. 90 Neotropical species, was investigated by Gustafsson and Persson (2002). They studied 38 taxa of the genus together with representatives of eight other Gardenieae genera and analyzed molecular (ITS and 5S-NTS) and morphological data. The molecular data do not support a monophyletic *Randia* but with morphological data added, *Randia*, together with *Casasia*, formed a weakly supported (less than 50%) monophyletic group. Basal to the *Randia–Casasia* group is an African clade (*Calochone*, *Macrosphyra* Hook. f., *Oligocodon* Keay, *Preussiodora* Keay) and a *Rosenbergiodendron* clade (*Rosenbergiodendron*, *Sphinctanthus*, *Tocoyena*). Within the *Randia* group, there are three geographically distinct clades: an Andean clade (less than 50% support), Central American *Randia* (58%), and South American *Randia* (85%).

The first attempt to construct a molecular phylogeny of the morphologically distinct tribe Vanguerieae was published by Lantz et al. (2002). They investigated the nuclear spacer ITS for 41 Vanguerieae species representing 19 genera. The taxa fall into several well-supported clades, of which they discussed three informal groups: spiny group (*Canthium*, *Meyna* Roxb. ex Link), *Vangueria* group, and *Fadogia–Rytigynia* group. Based on the investigated taxa, *Keetia* E. Phillips, *Lagynias* E. Mey. ex Robyns, *Multidentia* Gilli, and *Pyrostria* Comm. ex Juss. were monophyletic units, but *Canthium*, *Fadogia* Schweinf., *Rytigynia* Blume, *Tapiphyllum* Robyns, and *Vangueria* were found to be polyphyletic or paraphyletic. The analysis clearly demonstrated that several genera are in need of new circumscriptions. Later, Lantz and Bremer (2004) analyzed data for 69 ingroup taxa representing 23 of the 27 genera of the tribe (ITS, *trnT-F*, and morphology). They found strong support for many groups, but these rarely coincided with traditional genera in accordance with their earlier study (Lantz et al., 2002). Of the investigated taxa, *Keetia*, *Lagynias*, and *Multidentia* were monophyletic with strong support and *Psydrax* Gaertn. was monophyletic with weak support. *Canthium* subg. *Afrocanthium* Bridson was given generic status as *Afrocanthium* (Bridson) Lantz & B. Bremer, and also new combinations were made for *Canthium* s. str. Another identified, well-supported clade was the dioecious group, including *Pyrostria* and *Cyclophyllum* Hook. f. and several genera restricted to Madagascar (*Leroya* Cavaco, *Neoleroya* Cavaco, *Peponidium* (Baill.) Arènes, *Pseudopeponidium* Arènes), *Canthium* subg. *Bullockia* Bridson and *Scyphochlamys* Balf. f. The relationships between the

taxa are not well understood and are in need of more study. The earlier proposed spiny group (Lantz et al., 2002) identified by supra-axillary spines was found identical to *Canthium* s. str., and the large-flowered group including *Vangueria* group and *Fadogia–Rytigynia* group were further investigated in a later study (Lantz & Bremer, 2005). Sixty-six of the estimated ca. 180 species were analyzed for the nuclear ITS and the chloroplast markers *trnT-F* and *rps16*. The data were analyzed in combination and separately. Several taxa (*Ancylanthos rubiginosus* Desf., *Hutchinsonia barbata* Robyns, *R. beniensis* (De Wild.) Robyns, *R. decussata* (K. Schum.) Robyns, and *R. eickii* (K. Schum. & K. Krause) Bullock) had incongruent positions in the different analyses and hybridization, and introgression was proposed as an explanation for the incongruence. These taxa were excluded from the taxonomic discussions. Both the *Vangueria* and *Fadogia–Rytigynia* groups were supported as monophyletic entities. Most of the taxa of the *Vangueria* group were merged into *Vangueria* (the genera *Ancylanthos* Desf., *Lagynias*, *Pachystigma* Hochst., *Tapiphyllum*, and a few investigated species of *Fadogia* and *Rytigynia*). The genus is characterized in the tribe by domatia rarely present, inflorescences usually borne at nodes from which the leaves have fallen, smooth retrorse hairs in the corolla, and large fruits (more than 1 cm long) with three to five locules. The relationships within the *Fadogia* and *Rytigynia* group could not be resolved and are in need of further study. However, the whole group could be distinguished from the *Vangueria* group by presence of domatia and a calyx with or without poorly developed calyx lobes (with exceptions).

Taxa of the tribes Mussaendeae, Isertieae (see under Cinchonoideae), and Sabiceeae have been understood as a complex even before molecular data came into use, but are treated differently by different authors (e.g., Bremekamp, 1966; Robbrecht, 1988; Andersson, 1996). In a study of *rbcL* data from Cinchonoideae and Ixoroideae taxa by Bremer and Thulin (1998), Isertieae was found to be a small tribe close to Cinchoneae of the subfamily Cinchonoideae; however, Sabiceeae and Mussaendeae are two tribes that belong to subfamily Ixoroideae. A new aberrant endemic genus from Socotra, *Tamridaea* Thulin & B. Bremer, was shown to be a sister genus to *Virectaria* Bremek. and placed in Sabiceeae together with *Sabicea* Aubl. and *Pseudosabicea* N. Hallé. The tribe Mussaendeae was reestablished, and *Mussaenda*, *Aphaenandra* Miq., *Heinsia* DC., and *Pseudomussaenda* Wernham were included as the component genera.

Alejandro et al. (2005) later investigated tribe Mussaendeae and tested the monophyly of the genus *Mussaenda* and the circumscription of Mussaendeae

sensu Bremer and Thulin (1998; see under Isertieae–Cinchonoideae). Alejandro et al. included 25 species (of ca. 160) of *Mussaenda* and representatives of all genera of the tribe, except for *Neomussaenda* Tange, plus outgroups (the genus *Mussaendopsis* Baill. was also included, which was shown to belong to the Condamineeae clade). They analyzed *trnT-F* and ITS data and demonstrated that the tribe Mussaendeae (including *Mussaenda*, *Aphaenandra*, *Bremeria* Razafim. & Alejandro, *Heinsia*, *Landiopsis* Bosser, *Pseudomussaenda*, and *Schizomussaenda* H. L. Li) is monophyletic, but the genus *Mussaenda* s.l. is polyphyletic. The Malagasy species were found to be more closely related to *Landiopsis* than to the African and Asian *Mussaenda*. They described a new genus *Bremeria* to accommodate 19 Indian Ocean species. The recircumscribed *Mussaenda* is characterized by reduplicate valvate aestivation and glabrous styles, in contrast to the reduplicate and induplicate aestivation and densely pubescent styles in *Bremeria*.

Dessein at al. (2001) published a study of *Hekistocarpa* Hook. f. and showed that it belongs in the vicinity of *Virectaria*. They also performed jackknife analyses of two molecular data sets, one of *rbcL* and one of *rps16* (mainly sequences from GenBank). Their conclusions from the molecular analysis and the morphological investigation were that the emended tribe Sabiceeae of Bremer and Thulin (1998) could not be morphologically characterized and is better treated as two distinct tribes: (1) Sabiceeae (*Sabicea* and *Pseudosabicea* and also, although not included in the analyses, *Ecpoma* K. Schum., *Pentaloncha* Hook. f., and *Stipularia* P. Beauv.); and (2) Virectarieae (including *Virectaria*, *Hekistocarpa*, and *Tamridaea*). In a sense, the Sabiceeae is characterized by entire stipules, medium to large flowers, valvate aestivation, berries, and small angular seeds with thickened radial walls. According to Dessein et al. (2001: 75), it is more difficult "to diagnose the tribe Virecatrieae emended to include *Hekistocarpa* and *Tamridaea*."

Stimulated by the results from Rova et al. (2002), Delprete and Cortés-B. (2004) carried out a more detailed molecular analysis (*trnT-F* and ITS) of tribe Sipaneeae with *Platycarpum* Humb. & Bonpl. as the outgroup and evaluated relationships and delimitations of genera. They confirmed that the tribe is monophyletic and belongs within the Ixoroideae. In the tribe, they included *Sipanea*, *Chalepophyllum* Hook. f., *Dendrosipanea* Ducke, *Limnosipanea* Hook. f., *Maguireothamnus*, *Neobertiera*, and *Sipaneopsis* Steyerm. All genera investigated were found to be monophyletic. It was inferred that the herbaceous habit of *Sipanea* and *Limnosipanea* had evolved twice in the tribe as these two genera are not sister groups. Delprete and Cortés-B.

(2004) had no material of *Neblinathamnus* Steyerm., *Pteridocalyx* Wernham, and *Steyermarkia* Standl., but, due to morphological similarities, they tentatively included these in the Sipaneeae.

### SUBFAMILY CINCHONOIDEAE

No study has focused explicitly on the entire subfamily Cinchonoideae, but several studies on the whole family (Bremer et al., 1995; Rova et al., 2002) or of specific groups (Bremer & Thulin, 1998; Razafimandimbison & Bremer, 2001; Andersson & Antonelli, 2005) have contributed to the knowledge of the subfamily. Based on these studies, there is support for nine tribes: Cinchoneae, Chiococceae, Guettardeae, Hamelieae, Hillieae, Hymenodictyeae, Isertieae, Naucleeae, and Rondeletieae, six of which are discussed below. The relationships between the tribes in this subfamily are very poorly understood, except for a few sister group relations between Guettardeae and Rondeletieae, Hamelieae and Hillieae, and Hymenodictyeae and Naucleeae, respectively. Most species of *Rondeletia*, the largest genus of this subfamily, have not been investigated so far. It would be interesting to analyze all species in this mainly South American subfamily, particularly because there are several interesting biogeographic patterns of relations between South America and the Old World tropics, the Pacific, and the Caribbean.

Early molecular data (Bremer & Jansen, 1991) indicated the tribe Chiococceae (Antirheoideae fide Robbrecht, 1988) to be close to parts of Condamineeae and Cinchoneae. Based on that indication, Bremer (1992) analyzed 20 morphological characters for 22 genera of Chiococceae and the *Portlandia* P. Browne group, and, as a result, the tribe Chiococceae was emended to include also subtribe Portlandiinae (Condamineeae) and some taxa of Cinchoneae, as there was no resolution or support for two distinct clades corresponding to Chioceeae s. str. and a *Portlandia* group.

In his study, Delprete (1996) reexamined the circumscription of the Condamineeae, Chiococceae, and Catesbaeeae (Delprete, 1996: 165), with the purpose "to test the tribal redefinition of Chiococceae proposed by Bremer (1992)." He analyzed 170 species of 44 genera for 44 morphological characters. His conclusion was that the *Portlandia* group (former Condamineeae) is closer to the Chiococceae s. str. (as suggested by Bremer, 1992) than to the rest of the Condamineeae. Because Chiococceae s. str. was monophyletic without the *Portlandia* group, he retained Chiococceae as a restricted tribe and instead included the *Portlandia* group in the tribe Catesbaeeae. Therefore, the rest of the Condamineeae

(Condamineinae and Pinckneyinae) was merged with the Rondeletieae s.l.

In several later molecular studies, the circumscription of the two tribes by Delprete (1996) was contradicted, and it has instead been shown that all taxa are intermixed in one group approximately corresponding to an emended Chiococceae (Bremer et al., 1995; Andersson & Rova, 1999; Rova et al., 2002). Motley et al. (2005) investigated most of the genera from the Catesbaeeae–Chiococceae complex to reevaluate the generic relationships. They found strong support for a group with *Strumpfia* Jacq. as sister to the complex, but there was no support to separate the taxa into two clades or tribes. They found *Catesbaea* L., *Erithalis*, *Hintonia*, *Isidorea* A. Rich. ex DC., *Phialanthus*, *Portlandia*, and *Scolosanthus* Vahl to be monophyletic genera, but *Bikkia* Reinw., *Chiococca*, *Exostema*, and *Solenandra* Hook. f. are paraphyletic/polyphyletic, and for several taxa, monophyly could not be tested (monotypic genera or single species investigated; *Asemnantha* Hook. f., *Badusa* A. Gray, *Ceratopyxis* Hook. f., *Coutaportla*, *Coutarea*, *Cubanola* Aiello, *Morierina* Vieill., *Osa* Aiello, *Phyllacanthus*, *Salzmannia* DC., *Schmidtottia*, and *Siemensia* Urb.).

*Exostema*, a genus of 25 species that occurs from Bolivia to Mexico throughout the West Indies, represents one of the first molecular analyses of a genus within Rubiaceae. McDowell and Bremer (1998) investigated all species for 37 morphological characters and ITS sequences of 18 species. All data sets (morphology, molecular, and combined) resolved three main species groups corresponding to sections earlier proposed by McDowell (1996). However, the ITS and combined trees placed the two South American species (*E. corymbosum* (Ruiz & Pav.) Spreng. and *E. maynense* Poepp. & Endl.) basal to the three retrieved clades. The genus was later reinvestigated by McDowell et al. (2003), who used *rbcL*, ITS, and combined data in order to understand the biogeographic pattern of the genus in the Caribbean region. The analyses were based on 14 *Exostema* species and nine species from eight related genera. The data did not support *Exostema* as monophyletic. In the ITS analysis, which showed the best resolved trees, *Coutarea*, *Chiococca*, and *Erithalis* were nested within *Exostema*, making *Exostema* highly polyphyletic or paraphyletic. *Coutarea* (from South or Central America) was placed close to the two South American species of *Exostema* (*E. corymbosum* and *E. maynense*).

*Erithalis* is an endemic Caribbean genus, the phylogeny and biogeography of which were studied by Negrón-Ortiz and Watson (2002). They investigated seven of the eight to 10 species with two nuclear

markers, ITS and ETS. They found the genus to be monophyletic relative to the genus *Chiococca* and *Exostema longiflorum* (Lamb.) Roem. & Schult. Surprisingly, there was no support for monophyly for any of those species (*Erithalis fruticosa* L., *E. salmeoides* Correll, *E. odorifera* Jacq.) that were sampled from more than one specimen. Due to low variation in the molecular markers, they hypothesized that the genus radiated rapidly within the Caribbean islands and that an initial colonization may have been from Central America.

Tribe Cinchoneae and the complex around this tribe were first analyzed with morphological characters by Andersson and Persson (1991) and Andersson (1995). They found the tribes Cinchoneae, Hillieae, and Calycophylleae to be monophyletic, and they proposed new circumscriptions of these tribes. However, the morphological tree showed many incongruent relationships compared to later molecular analyses (Razafimandimbison & Bremer, 2001, 2002; Rova et al., 2002). More recently, Andersson and Antonelli (2005) reinvestigated the relationships of the Cinchoneae, making a thorough analysis based on five molecular markers for 51 Rubiaceae taxa sampled from the Cinchoneae and closely allied tribes (Chiococceae, Guettardeae [*Guettarda*], Hamelieae, Hillieae [*Cosmibuena* Ruiz & Pav.], Isertieae [*Isertia* Schreb., *Kerianthera* J. H. Kirkbr.], Naucleeae, Rondeletieae) as well as other representatives of the family. They found the tribe to be strongly supported as monophyletic including the monophyletic genera *Cinchona* L., *Cinchonopsis* L. Andersson (monotypic), *Joosia* H. Karst, *Ladenbergia* Klotzsch, *Remijia* DC., and *Stilpnophyllum* Hook. f. The monotypic *Pimentelia* Wedd. was not investigated, but due to morphological similarities, it was suggested to be close to *Stilpnophyllum*. Further, Antonelli (in Andersson & Antonelli, 2005) described a new genus *Ciliosemina* Antonelli within the tribe, including two species (former species of *Cinchona/Ladenbergia/Remijia*) characterized by "long-pedunculate, corymbose or subcorymbose inflorescences (fig. 3A), and the ciliate to fimbriate wing margins of its seeds" (Andersson & Antonelli, 2005: 26).

Tribe Isertieae was first analyzed by Andersson (1996) with morphological data. He investigated all except one of the Isertieae genera enumerated by Robbrecht (1988), 26 genera total with representatives of other tribes. The analyses resulted in a new circumscription of the tribe including only seven genera: *Isertia* (including *Yutajea* Steyerm.), *Aphaenandra*, *Heinsia*, *Mussaenda*, *Neomussaenda*, *Pseudomussaenda*, and *Schizomussaenda*. Andersson recircumscribed tribe Sabiceeae to include *Sabicea*, *Acranthera* Arn. ex Meisn., *Amphidasya*, *Ecpoma*,

*Pentaloncha*, *Pittierothamnus* Steyerm., *Pseudosabicea*, *Schizostigma* Arn. ex Meisn., and *Temnopteryx* Hook. f.

Molecular data showed contradicting circumscriptions of Isertieae, tested the phylogeny presented by Andersson (1996), and also pinpointed the position of an aberrant endemic species from Socotra. Bremer and Thulin (1998) investigated *rbcL* for Cinchonoideae and Ixoroideae taxa plus seven outgroups. Their conclusion was that Isertieae belongs to the Cinchonoideae but should be restricted to *Isertia* (including *Yutajea*) and *Kerianthera*, and that Sabiceeae and Mussaendeae instead belong to Ixoroideae.

Tribe Naucleeae s.l. was investigated by Razafimandimbison and Bremer (2001, 2002). They investigated molecular (ITS, *rbcL*, *trnT-F*) and morphological characters for a total of ca. 50 taxa of the tribe in the different analyses that represented most genera. They showed that a broader circumscription of the tribes, including not only Naucleeae sensu Ridsdale but also *Cephalanthus* L. (of Antirheoideae fide Robbrecht, 1988) and *Mitragyna* Korth. and *Uncaria* Schreb. (of Cinchoneae fide Robbrecht, 1988), belong to the group. They also showed that Coptosapelteae sensu Andersson and Persson (1991) is paraphyletic. Twenty-four genera were accepted in Naucleeae, which was divided into six highly supported and morphologically distinct subtribes (Breoniinae: *Breonadia* Ridsdale, *Breonia* A. Rich. ex DC., *Gyrostipula* J.-F. Leroy, *Janotia* J.-F. Leroy; Cephalanthinae: *Cephalanthus*; Corynantheinae: *Corynanthe* Welw., *Pausinystalia* Pierre ex Beille, *Pseudocinchona* A. Chev. ex Perrot; Naucleinae: *Nauclea* L., *Burttdavya* Hoyle, *Ochreinauclea* Ridsdale & Bakh. f., *Neolamarckia* Bosser, *Sarcocephalus* Afzel. ex R. Br.; Mitragyninae: *Mitragyna*; and Uncarinae: *Uncaria*) and one paraphyletic or poorly supported subtribe Adininae (*Adina* Salisb., *Adinauclea* Ridsdale, *Haldina* Ridsdale, *Ludekia* Ridsdale, *Metadina* Bakh. f., *Myrmeconauclea* Merr., *Neonauclea* Merr., *Pertusadina* Ridsdale, *Sinoadina* Ridsdale). The *Neonauclea* clade, part of the subtribe Adinae, with many myrmecophytic taxa (see below) was further investigated in a study by Razafimandimbison et al. (2005). They analyzed ITS and ETS and found the *Neonauclea* clade well resolved and supported; *Ludekia* is sister to the two monophyletic genera *Myrmeconauclea* and *Neonauclea* (the latter were earlier suggested to be paraphyletic; Razafimandimbison & Bremer, 2002).

A new tribe Hymenodictyeae, sister group to the Naucleeae, was described for *Hymenodictyon* Wall. and *Paracorynanthe* Capuron (Razafimandimbison & Bremer, 2001). The two genera *Paracorynanthe* (two species) and *Hymenodictyon* (22 species) are distrib-

uted in Madagascar, and in Madagascar, mainland Africa, and tropical Asia, respectively. The sister group relationship to Naucleeae is highly supported (Razafimandimbison & Bremer, 2001).

*Neolaugeria* Nicolson of the tribe Guettardeae, endemic to the West Indies, was studied by Moynihan and Watson (2001). Their data supported the genus of three species as monophyletic, but it was found to be only distantly related to *Stenostomum* C. F. Gaertn., a genus with which *Neolaugeria* sometimes has been merged as a section. Instead, it was closer to *Timonius* DC., although the support was very low. Moynihan and Watson (2001) also tested an earlier hypothesis regarding the origin of the genus in the Lesser Antilles by comparing vicariance with long-distance dispersal. The conclusion, albeit also with low support, was that *N. resinosa* (Vahl) Nicolson may occupy a basal phylogenetic position, supporting a pattern of speciation and colonization in a northwesterly direction from Lesser Antilles to the Greater Antilles and the Bahamas.

## APPLIED STUDIES BASED ON RUBIACEAE PHYLOGENIES

The power of a phylogenetic tree is not only that it can be used for classification and systematics, but that it can be used for studies of diversity, anatomy, morphology, biogeography, ecology, etc., in which evolution of taxa, genes, and characters can be used in a comparative context. With this species-rich and diverse family and with more and better phylogenetic trees from the family, we can probably foresee a strong increase in studies based on phylogenetic trees. So far, we have only seen a limited number of such studies, with interesting evolutionary questions being addressed.

### PHYLOGENETIC TREES FOR ECOLOGICAL, EVOLUTIONARY, OR BIOGEOGRAPHICAL QUESTIONS

In 1991 and 1992, the first phylogenetic ecology papers were published (Eriksson & Bremer, 1991; Bremer & Eriksson, 1992) in which a Rubiaceae phylogeny was used. These studies addressed hypotheses about evolution of fruit traits, animal versus abiotic modes of dispersal, life forms, and species richness. It was shown that fleshy fruits have evolved several times and that in many lineages the animal-dispersed fruits (drupes and berries) have remained largely unaltered since the time of origin. This is in contrast to the evolution of lineages with wind-dispersed seeds in capsules, or with pterophylls promoting wind dispersal of fruits, where traits have shifted more frequently during evolution. Animal dispersal was widespread among shrubs, whereas abiotic dispersal was most prevalent among herbs. Drupes were common in transoceanic taxa and on islands, indicating dispersal over long distances, probably by birds, but no evidence supported the view that animal dispersal in general enhances long-distance dispersal. No single trait explained variation in species richness. Instead, certain combinations of dispersal mode or life forms were shown to be associated with species richness. Genera with herbs and with abiotic dispersal, or with shrubs and with animal dispersal, or with shrubs and trees with winged seeds were all characterized by large species numbers, a result that implies association between seed dispersability and rate of species diversification.

High host specificity of herbivorous insects and global estimates of diversity have been much discussed (cf. Erwin, 1982; Stork, 1993; Odegaard, 2000). Novotny et al. (2002) compared a plant phylogeny of 51 tree species, including Rubiaceae, from New Guinea with more than 900 leaf-chewing insects found on these plants. Compared to earlier, more theoretical studies, they found low host specificity of the tropical herbivorous insects, and, as a consequence, a global estimate on arthropod diversity was reduced from 31 million to 4 to 6 million species.

Razafimandimbison et al. (2004) identified high polymorphism of the ITS region in three Naucleeae species (*Adinauclea fagifolia* (Teijsm. & Binn. ex Havil.) Ridsdale, *Haldina cordifolia* (Roxb.) Ridsdale, and *Mitragyna rubrostipulata* (K. Schum.) Havil.). They found both intra-individual and intraspecific polymorphism in the three species, but no such variation in the other 22 investigated species of the same tribe. Most of the variants were putative pseudogenes. They explored the potential utility of pseudogenes in a phylogenetic analysis and found that the polymorphism does not transcend species boundaries in this group (all variants within a species come together in the tree), so any of the pseudogenes could be of use in a phylogenetic analysis without contradicting the phylogenetic signal.

McDowell and Bremer (1996) used a tree of *Exostema* (see above under *Exostema*) to optimize and investigate major trends in morphological diversification of the genus, e.g., attributes for specializations to a xeric environment and for pollination biology. Xeromorphic traits had evolved in all three lineages, e.g., reduction of vegetative characters, and also reduction of reproduction traits such as seed size and seed numbers. In the genus, two different major pollination syndromes occur, a long-flowered moth (*Lepidoptera*) type and a short-flowered bee pollination type. According to the analyses, both of these pollination

types (with characteristic flower lengths, flower numbers, and corolla color) have evolved more than once.

Evolution of myrmecophytism was investigated in a study by Razafimandimbison et al. (2005). This biologically interesting ant-plant association occurs in 22 genera and ca. 140 species of Rubiaceae, most of these in Southeast Asia, especially in the Malesian region. Razafimandimbison et al. investigated the *Neonauclea* clade of Naucleeae, including 25 taxa with myrmecophytism. Based on the molecular phylogeny, they concluded that multiple origins of myrmecophytism occurred in Borneo and that the low level of genetic variation indicates a rapid radiation in the *Neonauclea* (65 species); low radiation in *Myrmeconauclea* (3 species) was explained by the different fruit and seed types and the ability to colonize different habitats.

In their study of the Catesbaeeae–Chiococceae complex, Motley et al. (2005) reconstructed flower and fruit evolution and discussed biogeographic hypotheses for the disjunction between the Caribbean and Pacific genera. According to their optimization on the tree, the ancestral fruit type for the group seems to be capsular; drupaceous fruits seem to have evolved twice and baccate fruits once or twice. The three types of flowers more or less correspond to hypothesized pollinators: *Exostema* type by moths and butterflies, *Chiococca* type by bees, and *Portlandia* type by birds and bats. All types have evolved three or more times. Motley et al. (2005) also concluded that fleshy fruits have been very successful in dispersing between the Caribbean islands, and wind-dispersed seeds of the capsular-fruited taxa have been more successful for long-distance dispersal over the Pacific Ocean.

The first biogeographic analysis of the family was based on a phylogeny of Anthospermeae (Anderson et al., 2001). The biogeographic implications were that the ancestral area of the tribe is Africa (including Madagascar) and that the genus spread by long-distance dispersal to northeastern Antarctica. It was also suggested that the occurrences in America, Hawaii, and Tristan da Cunha are due to long-distance dispersal.

Other publications that discuss the biogeography of Africa are Malcolmer's (2002) *Gaertnera* study and Alejandro et al.'s (2005) study of *Mussaenda*. Malcolmer (2002) proposed that *Gaertnera* migrated to Africa during the early Tertiary, possibly via a boreotropical land bridge, and he further suggested that the genus started to radiate about 5.2 million years ago (Ma). The range of distribution is explained by a number of long-distance dispersal events. The molecular clock estimate gave a rapid diversification rate of 0.717 to 0.832 species/million years, which is comparable to estimates of radiation on Oceanic islands. Alejandro et al. (2005) concluded that *Mussaenda* s. str. has an African origin and that the Asian *Mussaenda* species descended from an African species that migrated to Asia, where the major radiation has occurred (now 97 of 132 species). Despite the close phylogenetic relationship between the African and Asian clades, not one species occurs on both continents. One of the most widespread African *Mussaenda* species, *M. arcuata* Poir., has reached the Comoro Islands, Madagascar, and Mascarenes as suggested probably via stepping-stone dispersal.

Nepokroeff at al. (2003) investigated the phylogeny and biogeography of the Hawaiian species of *Psychotria* to reconstruct the ancestral pattern of colonization and dispersal. Both parsimony and likelihood analysis gave highly congruent results, except for one internal node. They investigated all 11 species from Hawaii together with eight extra-Hawaiian species. The analysis strongly supported the Hawaiian taxa as monophyletic and descended from a single introduction to the islands. The genus *Kelloggia*, with disjunct distribution in western North America and the western part of eastern Asia, was investigated by Nie et al. (2005), who found that the two species diverged from each other about 5.4 Ma; dispersal-vicariance analysis (DIVA) suggested an Asian origin of *Kelloggia*. Nie et al. (2005) further suggested that the disjunct distribution is a result of long-distance dispersal from Asia into western North America.

From the Caribbean region, Negrón-Ortiz and Watson (2003) used the phylogenies of the two endemic genera *Erithalis* (Negrón-Ortiz & Watson, 2002) and *Ernodea* (Negrón-Ortiz & Watson, unpubl. data) in a biogeographic study using Brooks Parsimony Analysis (BPA) and Fitch parsimony methods. They found a biogeographic association between Cuba and the Dominican Republic, but the two countries of Hispaniola (Dominican Republic and Haiti) were found in two places in the cladogram, suggesting Hispaniola to be a composite of geologic areas. The Fitch analyses also supported a Greater Antillean origin for *Erithalis*, in contrast to the Negrón-Ortiz and Watson (2002) article in which they suggest colonization of the genus from Central America. The present distribution of the two genera was explained as a product of dispersal for *Ernodea* and by a combination of vicariance and dispersal events for *Erithalis*. The mainly Caribbean genus *Exostema* (McDowell et al., 2003) has also been analyzed biogeographically, but its distribution pattern was found to be far more complex than anticipated and no clear conclusions could be drawn except for a close affinity between the Cuban and Hispaniolan groups.

UNDERSTANDING DISTRIBUTION AND EVOLUTION OF
MORPHOLOGICAL, ANATOMICAL, AND CHEMICAL CHARACTERS
THROUGH PHYLOGENETIC TREES

Molecular phylogenies have also been very useful for understanding morphological, anatomical, or chemical traits in various parts of the family. Jansen et al. (2001) performed a large survey of anatomical characters of woody Rubioideae taxa and compared the characters with recent phylogenetic insights in the study group on the basis of molecular data. The idea of the study was based on results from molecular phylogenetic analysis, even if this was not stated explicitly. Jansen et al. (2001) presented anatomical data in illustrations and in a table of 26 different characters for 23 genera (and ca. 70 species) representing woody taxa of Coccocypseleae, Coussareeae, *Lasianthus* group, Morindeae s.l., Pauridiantheae, Trianolepideae, and Urophylleae. It would have been even more interesting with a phylogenetic analysis of the morphological data or a combined morphological-molecular analysis, but their results nevertheless seem to be in agreement with most phylogenetic hypotheses presented from molecular data. Soon thereafter, Jansen et al. (2002) presented a survey of wood anatomy of the whole family. They optimized the characters on a hypothetical supertree and found that the wood characters agreed with the phylogeny. Furthermore, they found that fiber types and axial parenchyma distribution, for example, indeed had good taxonomic values in the family, but they concluded that wood anatomical data in Rubiaceae is more useful in confirming or negating already proposed relationships rather than postulating new affinities for problematic taxa (Jansen et al., 2002).

Pollen morphology was investigated in 29 species of northwestern European representatives of Rubieae (*Rubia*, *Asperula*, *Crucianella*, *Cruciata*, *Galium*, *Sherardia*) by Huysmans et al. (2003). They found the combination of pollen characteristics to be unique within the family: several colpate apertures, a perforate and microechinate tectum, a relatively small size, absence of endoapertures, a coarse nexine area beneath the ectocolpi, and absence of orbicules. The tribe Gardenieae also lacks orbicules (Huysmans et al., 1998, 2000). Huysmans et al. (2003) further optimized presence and absence of endoapertures on a Rubioideae tree from Bremer and Manen (2000) and showed that only the Paederieae/Theligoneae/Rubieae totally lack the endoapertures, while the character is variable in Argostemmateae.

Jansen et al. (2003) measured concentration of several metals in Rubiaceae. The most characteristic pattern was for aluminium, and there was also a correlation with occurrence of silicon but not with any other metals. The aluminium accumulation was optimized on a molecular phylogenetic tree, and it was most characteristic of Rubioideae but occurs also in *Coptosapelta* and is partly present in taxa of Vanguerieae and Alberteae.

There are a few examples of surveys of various traits from the family, chemical and morphological data, in which no tree approach has been used but for which analyses in relation to a phylogenetic tree would be very interesting. At the first Rubiaceae conference, Kiehn presented (1995) a survey of chromosome numbers of the family. Although he did not optimize his characters on a molecular phylogeny, many interesting results corroborate the molecular hypothesis about relationships, e.g., a close association of Hedyotideae and Spermacoceae (as in Bremer et al., 1995; Natali et al., 1995).

Wichman et al. (2002) investigated a set of 50 individuals representing 36 taxa of *Coprosma* from New Zealand. They investigated patterns of hybridization and genotype mixing in ITS and ETS sequences. They found high intra-individual heterogeneity, and the conclusion was that the widespread occurrence of sequence mixture was a result of frequent hybridization in the genus. They also suggested that concerted evolution in the genus is depressed and that the mechanisms evolved to maintain a high level of heterogeneity as an adaptive value for *Coprosma* in the climatically unstable and physically complex New Zealand landscape. The authors have sequenced many taxa, but they have not performed any phylogenetic analysis. It would be very interesting to investigate patterns of suggested hybridization in a phylogenetic framework.

Mitova et al. (2002) analyzed iridoid patterns within *Galium* with some phylogenetic considerations. They found differences in iridoid compounds and identified three lines of evolution: one that led to *G. rivale* (Sibth. & Sm.) Griseb., a second that included *G. mollugo* L. and the *G. incurvum* group, and a third that included the rest of the studied species (e.g., *G. palustre* L., *G. odoratum* (L.) Scop.). The study could be much improved if sampling and discussion are based on available phylogenetic data of the group (e.g., Natali et al., 1996).

Recently, Mongrand et al. (2005) investigated 107 Rubiaceae species for composition of leaf fatty acids. They used principal component analysis (PCA) and identified the tribes Coffeeae, Psychotrieae, and Rubieae from their data. It is difficult to see how informative these chemical characters are as the PCA only shows similarities between species, which can completely contradict a phylogenetic relationship; furthermore, the results are compared to a nonphylo-

genetic tribal classification (Robbrecht, 1993), so it is unfortunately very difficult to draw any conclusions about evolution and relationships of the fatty acids.

Since the present review of molecular phylogenetic studies of Rubiaceae was presented at the Third International Rubiaceae Conference in 2006, ca. 10 more molecular phylogenetic studies of Rubiaceae have been published. They are not reviewed in this article, but the most important are as follows. Robbrecht and Manen (2006) have presented a supertree construction of the family Rubiaceae. Several detailed studies of tribes have been published, e.g., Coffeeae (Davis et al., 2007), Knoxieae (Kårehed & Bremer, 2007), Paederieae, Putorieae (Backlund et al., 2007), and Urophylleae (Smedmark et al., 2008). Further, molecular studies of enigmatic or problematic genera have been presented, e.g., *Acranthera* (Rydin et al., 2009), *Coffea* (Maurin et al., 2007), *Guettarda* (Achille et al., 2006), *Psychotria* (Sohmer & Davis, 2007), and *Ropalobrachium* (Mouly et al., 2007).

From the ca. 50 molecular studies of the family reviewed in this article, we now have a good framework of the phylogeny of the family. We know that Rubiaceae are monophyletic and there is high support for three subfamilies (Cinchonoideae, Ixoroideae, Rubioideae) and over 40 tribes. Of these tribes, four are monogeneric (Cremasporeae, Retiniphylleae, Schraradereae, and Theligoneae). Two tribes, Gardenieae and Morindeae, are paraphyletic/polyphyletic. At the base of Rubiaceae, there is a trichotomy between *Luculia* and Coptosapelteae, a clade including subfamilies Cinchonoideae and Ixoroideae, and a third clade including subfamily Rubioideae. These three clades and the two clades corresponding to Cinchonoideae and Ixoroideae are highly supported. Subfamily Cinchonoideae includes nine tribes. Most interrelationships between these are unresolved. Subfamily Ixoroideae includes two monogeneric tribes (Retiniphylleae, Cremasporeae), 12 well-supported clades corresponding to tribes, and also several taxa referred to as a polyphyletic/paraphyletic tribe Gardenieae. Subfamily Rubioideae includes two monogeneric tribes (Schraradereae, Theligoneae), 15 supported clades corresponding to tribes, and also taxa of a paraphyletic/polyphyletic tribe Morindeae. Despite all these studies, there are still many problems to be investigated in Rubiaceae phylogeny. Only half of the tribes have been the focus of specific studies, and the large problematic genera are still in need of much investigation, e.g., *Psychotria*, *Galium*, *Ixora*, *Pavetta*, *Ophiorrhiza*, and *Palicourea*. Evolutionary investigations, biogeography, species richness, morphological traits, and other studies in Rubiaceae have just started, and with the diversity and disparity of the family, we can foresee an increased interest in the family and its phylogeny.

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