

**The major evolutionary lineages of the coffee family  
(Rubiaceae, angiosperms).  
Combined analysis (nDNA and cpDNA) to infer the position of  
*Coptosapelta* and *Luculia*, and supertree construction based on *rbcL*,  
*rps16*, *trnL-trnF* and *atpB-rbcL* data.  
A new classification in two subfamilies, Cinchonoideae and Rubioideae**

Elmar Robbrecht<sup>a</sup> & Jean-François Manen<sup>b</sup>

<sup>a</sup> National Botanic Garden, Domein van Bouchout, B - 1860 Meise, Belgium  
author for correspondence [robbrecht@br.fgov.be]

<sup>b</sup> Conservatoire et Jardin botaniques de la Ville de Genève,  
1, ch. de l'Impératrice, case postale 60, CH-1292 Chambésy/Genève, Switzerland

**Abstract.** – Two analyses are carried out for a reconstruction of the phylogeny of the large angiosperm family Rubiaceae. The position of *Luculia* and *Coptosapelta*, unresolved or sister to all other Rubiaceae in most of the recently published results, is reanalysed by using five plastid and three nuclear DNA regions sequenced for selected Rubiaceae taxa. A supertree is constructed for more than 500 species in 300 genera, i.e. ca. 50% of the genera known in the family. The supertree is derived from four source trees based on *rbcL*, *rps16*, *trnL-trnF* and *atpB-rbcL*, the latter with 106 newly generated sequences. The results are confronted with the morphological, anatomical and biogeographical knowledge of the family and discussed in the light of other molecular phylogenies. It is proposed to recognize only two subfamilies, Cinchonoideae (raphides mostly absent; secondary pollen presentation common) and Rubioideae (raphides present; heterostyly common). *Luculia* and *Coptosapelta* are found to be basal to the rest of the Cinchonoideae, which are divided in two newly recognized supertribes, *Ixoriidinae* and *Cinchonidinae*. Also in the subfamily Rubioideae, two major clades may be distinguished and are recognized as supertribes *Psychotriidinae* (predominantly woody) and *Rubiidinae* (predominantly herbaceous); the genus *Colletocema* and the tribes *Urophyllaeae*, *Ophiorrhizaeae*, *Lasiantheae* and *Coussareeae* are basal Rubioideae falling outside these two supertribes. Four alliances are spotted as groups for which major problems exist with regard to relationships and tribal delimitation: three neotropical groups, viz. the *Calycophylleae/Condamineae* alliance, the *Catesbaeeae/Chiococceae* complex and the *Rondeletiaeae* alliance, and the pantropical *Spermacocceae* s.lat. The cladogenesis of Rubiaceae depicted by the supertree is strongly correlated with the present geographical distribution of taxa. The results are summarized in a conspectus of the family enumerating the tribes and listing all their genera for which the inclusion is corroborated by DNA sequences. Newly recognized tribes and subtribes are the *Henriquezieae-Posoqueriinae*, the *Palicoureeae* and the *Rubieae-Theligoninae* and *-Kelloggiinae*. Some tribes are reduced to subtribes, viz. *Virectariaeae* (subtribe of *Sabiceaeae*), *Bertiariaeae* (subtribe of *Coffeaeae*) and *Isertiariaeae* (subtribe of *Cinchoneaeae*). Novel positions are proposed for the genera *Aulacocalyx*, *Calycosiphonia*, *Cremaspora*, *Crossopteryx* and *Morelia*.

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## Introduction

The coffee family (Rubiaceae; ca. 11,150 species in ca. 660 genera according to the first author's world database of the family) is the fourth largest family of angiosperms. The family was classically divided (e.g. Schumann 1891) in two major groups, the multiovulate subfamily Cinchonoideae versus the uniovulate Coffeoideae. But Solereder (1893) contemporaneously used anatomical evidence to conclude that this was an artificial division. The common occurrence of placentas with one or more ovules in the same species of *Tarenna* had already been mentioned by Baillon (1878a: 215; as *Webera*), and today we are able to enumerate several such genera, e.g. *Tricalysia* (Robbrecht 1979) and *Neanotis* (Puff & al. 2005). In the 20<sup>th</sup> century Bremekamp and Verdcourt therefore looked for other characters to define subfamilies, such as the occurrence of raphides, absence of endosperm (in fact a poor development of the endosperm), 'pitted testa' (in fact exotestal cells with perforations in the thickened inner tangential wall), 'ixoroid pollination mechanism' (now designated as secondary pollen presentation) and heterostyly. This allowed them to recognize five (Bremekamp 1952), three (Verdcourt 1958) or eight (Bremekamp 1966) subfamilies.

Robbrecht (1988) presented a world survey of the Rubiaceae wherein he defined four subfamilies based on correlated trends between the abovementioned features: Ixoroideae, Cinchonoideae, Antirheoideae and Rubioideae. His system was updated (Robbrecht 1994b) to incorporate modifications at the tribal level resulting from the then advances in Rubiaceae research. Simultaneously the first molecular analyses of the Rubiaceae were published (Bremer & Jansen 1991, Bremer & al. 1995). These and other analyses (the most important ones: Andersson & Rova 1999, Bremer & Manen 2000, Andreasen & Bremer 2000, Rova & al. 2002) showed the polyphyly of one of Robbrecht's subfamilies, the Antirheoideae. His other subfamilies and many tribal concepts have generally withstood the test of comparative DNA sequencing. The Rubioideae were confirmed almost unchanged. The Ixoroideae and Cinchonoideae needed mutual re-delimitation, resulting in

a widening and a narrowing, respectively. These Ixoroideae s.lat. and Cinchonoideae s.str. can no longer be defined morphologically. At lower classification levels also, (partial) answers to long-standing disputes were given, e.g. whether *Psychotria* needs splitting (e.g. Nepokroeff & al. 1999; Andersson 2001, 2002a, 2002b).

The most often used molecular data to reconstruct the phylogeny of the Rubiaceae family come from four plastid sequences: *rbcL* (Bremer & al. 1995; Bremer 1996; Bremer & al. 1999), *rps16* (Andersson & Rova 1999), *trnL-trnF* (Rova & al. 2002) and the *atpB-rbcL* spacer (Manen & Natali 1996 and this work). Despite the fact that taxa analyzed in the phylogenetic studies using these four genes are not totally shared, the evidence from all these data sets supports the monophyly of the family and the recognition of three subfamilies (Rubioidae, Cinchonoideae s.str. and Ixoroideae s.lat.). However, in most of the prior phylogenetic reconstructions including these two genera or one of them, their position remained unresolved or they were sister to all other Rubiaceae.

The new phylogenetic hypotheses supported by these genetic data provide a strong framework for interpretation of morphological and anatomical data. For example, a manually constructed consensus cladogram – representing not much more than a stimulating mental exercise – summarizing all available molecular phylogenies was used to discuss wood anatomical (Jansen & al. 2002: fig. 3) and pollen morphological data (Dessein & al. 2005b: fig. 58) for the entire family, plotting the distribution of many features on the summary tree.

Today, so-called supertree methods and algorithms exist to combine multiple sources of genetic data into a single analysis, despite different sampling (fig. 1). On top of that we judged necessary, as a first step, to undertake a separate investigation of the position of the problematic genera *Luculia* and *Coptosapelta* by augmenting the data, viz. by also using nuclear data instead of chloroplast data alone; this work mainly examines the root of the Rubiaceae phylogenetic tree.

The aims of the present paper are multiple. It firstly reports the results of two sets of analyses:

- (1) The position of *Luculia* and *Coptosapelta* is reanalysed by combining five plastid and three nuclear DNA regions sequenced for selected Rubiaceae taxa.
- (2) To clarify overall Rubiaceae phylogeny, a supertree for more than 500 species in 300 genera is constructed from four source trees based on *rbcL*, *rps16*, *trnL-trnF* and *atpB-rbcL*, with 106 newly obtained sequences in the last one. This supertree provides a formally constructed summary tree of the existing phylogenetic hypotheses and shows the main clades within the family.

These results are then

- (3) compared with the morphological, anatomical and biogeographical knowledge of the family and discussed in the light of other molecular phylogenies, and
- (4) used to propose a new classification of the family, providing a placement for all the genera for which molecular data are now available.

A preliminary version of the present results has been presented as the keynote address at the symposium 'Phylogeny and deep morphology of Rubiaceae' (July 21, 2005) at the seventeenth International Botanical Congress in Vienna (Robbrecht & Manen 2005).

## M Materials and methods

### M.1 Taxa analysed; names and acronyms of taxa; classification

The electronic appendix 1 gives the list of Rubiaceae and outgroup species analysed in this study with the corresponding DNA sequence accession numbers and voucher information for newly determined sequences.

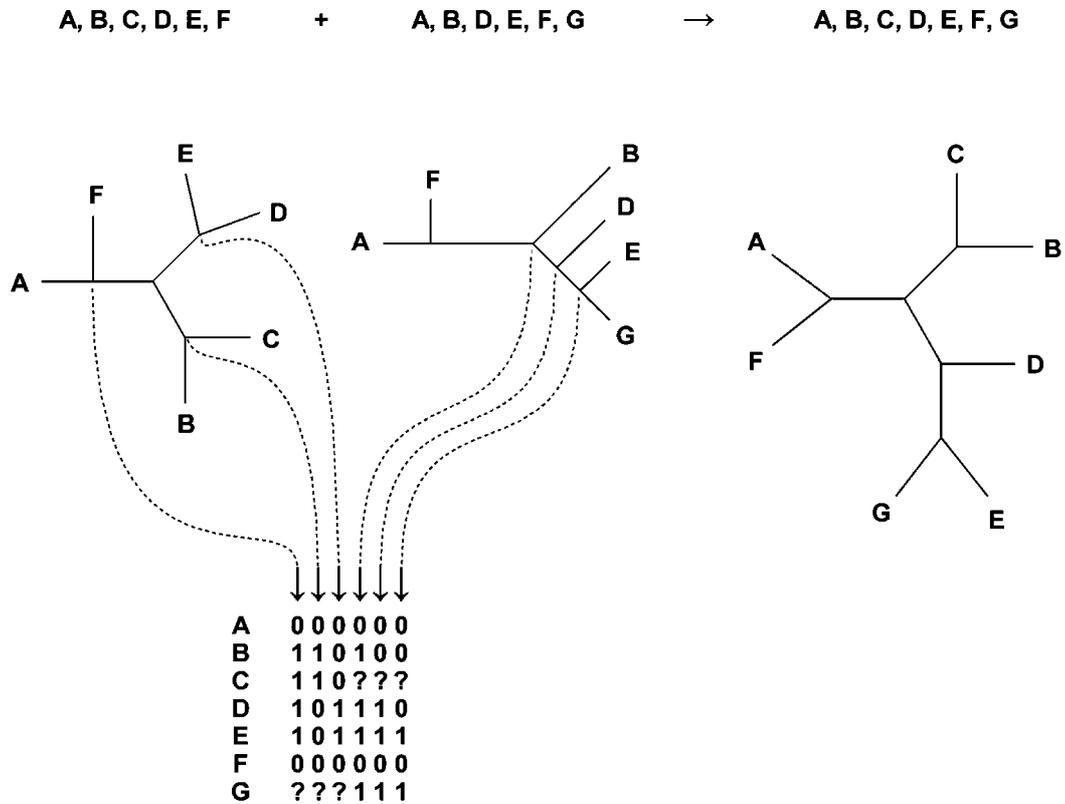


Figure 1. Theoretical representation of a supertree constructed from two source trees with a different sampling. Modified from Daubin & al.

Where needed, e.g. to write out cladograms as formulas, the three letter acronyms for tribes proposed in the Taxonomic conspectus are used. In that case, the tribes given in the formula are meant in their present circumscription.

Generic names are throughout given without author, as explained in Robbrecht (1988: 26).

The Taxonomic conspectus at the end of the paper proposes a new classification translating the topology of the supertree the best possible, and using monophyletic units. In general we have adopted a conservative approach, although we opted for proposing subtribes rather than augmenting the number of tribes. We felt no need to formally name all basal groups or all elements of the basal grades because this would result in an unnecessary inflation of formal names at supertribal and tribal level. E.g., the supertree would support recognition of six monotribal or even monogeneric supertribes in the basal Rubioideae. This would be at best premature because the present work is only the first attempt to reconstruct the phylogeny of the entire family with a very wide molecular sampling.

## M.2 Sequencing

The plastid *atpB-rbcL* spacer, *trnL-trnF* intron and spacer, *rps16* intron and *ndhF* were sequenced according to Manen & Natali (1996), Gielly & Taberlet (1994), Oxelman & al. (1997) and Olmstead & Reeves (1995), respectively. The nuclear ribosomal 5.8S subunit and Intergenic Transcribed Spacer 1 and 2 (*ITS*), and the nuclear encoded plastid glutamine synthetase (*nepGS*) were sequenced according to White & al. (1990) and Emshwiller & Doyle (1999), respectively. For the nuclear paralogous large subunit of RNA polymerase II (*RPB2*), an alignment of published Rubiaceae data (Oxelman & Bremer 2000) was used to design Rubiaceae specific forward primer 5'-TAYACBAASCGTGAYCAYAG and reverse primer 5'-ACRTGRAYYCTRTAYGCATCAC.

### M.3 Phylogenetic analyses

In order to reexamine the phylogenetic position of *Luculia* and *Coptosapelta*, five plastid regions (*rbcL*, *atpB-rbcL*, *rps16* intron, *trnL-trnF* and *ndhF*) and three nuclear regions (*ITS*, *nepGS* and *RPB2*) were analyzed for both of these taxa, and for three representatives each of the current Rubioideae (*Ophiorrhiza*, *Bouvardia* and *Rubia*), current Cinchonoideae (*Cephalanthus*, *Chiococca* and *Guettarda*), and current Ixoroideae (*Calycophyllum*, *Coffea* and *Ixora*) and for four non-Rubiaceae outgroup genera (*Gentiana*, *Alstonia*, *Gelsemium* and *Logania*, see electronic appendix 1). These eight sequences for each taxon were generated during this work or found in GenBank (see electronic appendix 1).

Contrary to the alignment of the five plastid genes and of *RPB2*, the alignment of *ITS* and *nepGS* was not obvious (and impossible for *nepGS* in the outgroup, except for exons). Thus, only selected homologous positions of *ITS* were retained for the analysis and intron positions of *nepGS* were replaced by question marks in the outgroup.

The plastid matrix, the nuclear matrix or the combined plastid and nuclear matrix were analyzed using PAUP 4.0b3a (Swofford 2003). Trees were calculated by heuristic searches with tree bisection-reconnection (TBR) swapping (Multrees option on), with 100 replications and random addition of taxa, saving no more than the first 100 most parsimonious trees. Clade support was estimated by 1000 bootstrap replications with TBR branch swapping, ten trees being kept at each replication.

In the plastid matrix, a simple outgroup sequence was constructed from only those positions that are invariant in the outgroup (*Gentiana*, *Alstonia*, *Gelsemium* and *Logania*), all other positions being recorded as question marks. This decreases the chance for spurious ingroup branches pairing with the outgroup (Smith 1994).

### M.4 Supertree construction

We choose a limited number of source trees, with the aim of having independent data (Bininda-Emonds & al. 2004) and a relatively good sampling of the family. The phylogenetic signal of each source tree used resulted from an identical method of analysis, namely a 50% majority-rule consensus bootstrap tree constructed by us from the source matrices. The following four plastid source trees were used:

- (1) the *atpB-rbcL* spacer tree (169 taxa);
- (2) the *rps16* tree of Andersson & Rova (1999); then *Coptosapelta* and *Luculia* were added, realigned on the matrix and the tree was recalculated (150 taxa);
- (3) the *trnL-trnF* tree recalculated from the matrix of Rova & al. (2002; <http://ajbsupp.botany.org/v89/rova-dna.txt>), with *Coptosapelta* lacking (156 taxa); and
- (4) the *rbcL* tree obtained from selected *rbcL* Rubiaceae sequences (mostly from the team of B. Bremer) downloaded from GenBank (215 taxa).

These four plastid source trees are bootstrap trees (including groups compatible with 50% majority-rule consensus) obtained from 1000 replications (retaining the ten shortest trees with TBR option). The branch lengths of trees shown (fig. 3 & electronic appendix 2) are proportional to the branch bootstrap values and do not represent nucleotide substitution rate.

Supertree construction was done using matrix representation with parsimony (MRP) with the programme SuperTree 0.85b (Salamin & al. 2002; <http://www.tcd.ie/Botany/NS/SuperTree.html>). MRP was chosen because it can be used whether or not the source trees are compatible. A matrix was constructed (Baum/Ragan coding scheme and irreversible characters, following recommendations of Salamin & al. 2002) with weighted characters according to the bootstrap value of each branch of the four source trees (weight of 1 for bootstrap values below 50%, and 50 to 100 for bootstrap values of 50% to 100%, respectively).

Preliminary analyses showed that the resolution of Ixoroideae in the supertree was low, due to the low rate of nucleotide substitution in this subfamily. To overcome this problem, also due to the fact that only a few species are shared between the four source trees, taxa representing Ixoroideae in these trees were considered at the generic level only. When different species of the same Ixoroideae genus were available for a data set, only one was selected, as indicated in the electronic appendix 1. The Ixoroideae genus *Bertiera* is represented by two operational units on the supertree, *Bertiera* B (*Bertiera batesii*) and *Bertiera* A (the three other species sequenced); this is due to our discovery of a wrongly identified voucher in a late stage of our work. Because of sufficient resolution in the two other currently recognized subfamilies, the specific level was retained for Rubioideae and Cinchonoideae.

The supertree matrix (486 taxa and 667 weighted characters) was analyzed with PAUP (200 replications of random addition of taxa) retaining the 100 shortest trees with TBR option (Multrees option on). The analysis stopped at 24,100 most parsimonious trees, by a lack of memory. The supertree shown (fig. 4 & electronic appendix 3) is a majority rule consensus tree.

## R Results

### R.1 Reexamination of the phylogenetic position of *Luculia* and *Coptosapelta*

The phylogenetic position of *Coptosapelta* and *Luculia* was reexamined using five plastid sequences and three nuclear sequences of selected taxa, resulting in a 15-taxa matrix of 8516 characters

(6056 and 2460 characters for plastid and nuclear sequences, respectively, of which 899 and 747 are informative).

The unique most parsimonious tree based on the eight combined plastid and nuclear data sets has the topology shown in fig. 2A, with *Luculia* and *Coptosapelta* monophyletic, nested inside the Rubiaceae family, and sister to the clade (Ixoroideae, Cinchonoideae) which unites all non-Rubioideae. But this position is not supported by bootstrap. In this case the bootstrap value is below 50%.

However, there is a contradiction between the nuclear tree and the plastid trees. The unique most parsimonious nuclear tree has the same topology – with the sister relationship of *Luculia* and *Coptosapelta* to the non-Rubioideae clade supported by a bootstrap of 78% – and is shown in fig. 2B. Analysis of the plastid matrix resulted in two most parsimonious trees (fig. 2C & D) which are incongruent with the most parsimonious tree based on nuclear data. In the two chloroplast trees, *Coptosapelta* and *Luculia* do not form a monophyletic group, *Coptosapelta* forming the sister group to all other Rubiaceae. The chloroplast trees differ from each other in the position of *Luculia*: in fig. 2C the genus is sister to all other Rubiaceae minus *Coptosapelta*, while in fig. 2D *Luculia* forms a clade with the Rubioideae included. This compares to many analyses based on plastid data (e.g. Bremer & al. 1999). Neither Maximum likelihood (HKY85 or GTR models) nor Bayesian analyses (GTR model) were able to define a sustained position for both genera (data not shown).

One way to detect possible anomalies caused by long branch attraction (Smith 1994) is to reconstruct a simple outgroup sequence comprising only positions that are invariant in the outgroup, with all other positions scored as question marks. The plastid tree produced in this variant of our analysis has the topology shown in fig. 2E. This is more similar to the nuclear results, with *Luculia* and *Coptosapelta* forming a clade that is sister to the non-Rubioideae clade; however, this relationship is weakly supported. Note that the *rps16* matrix (Andersson & Rova 1999) to which *Luculia* and *Coptosapelta* were added, produced the same topology (see electronic appendix 2C).

The tree obtained with the combined plastid (with the simple invariant outgroup sequence) and nuclear data has the topology shown in fig. 2F. Thus the Rubiaceae family might comprise only two major clades: (1) Rubioideae, and (2) a large clade uniting current Ixoroideae and Cinchonoideae (bootstrap 79%), wherein a *Luculia* / *Coptosapelta* clade is sister to the entire rest of the clade.

## R.2 Supertree construction

A supertree was constructed from four source trees *atpB-rbcL* (further often called “spacer”), *rps16*, *trnL-trnF* and *rbcL*. For the first source tree, 106 new *atpB-rbcL* sequences, mainly from current Ixoroideae and Cinchonoideae, were generated; the bootstrap tree (fig. 3) includes groups retrieved by 50% or more majority-rule consensus. Branch lengths are proportional to bootstrap values. As already observed with plastid trees (Bremer & al. 1999), *Luculia* and *Coptosapelta* have unresolved positions. The electronic appendix 2 shows the three other source trees, the *rps16* bootstrap tree, *trnL-trnF* bootstrap tree, and *rbcL* bootstrap tree, respectively. As explained in ‘Materials and methods’, Ixoroideae taxa are represented at genus level only. The constructed supertree is shown in fig. 4 and electronic appendix 3.

Contrary to results described in R.1 (fig. 2), but as expected from the observation of individual source trees, *Luculia* and *Coptosapelta* still have an undefined position.

As the source trees are not totally compatible, and despite the fact that characters are weighted according to bootstrap values of each branch in each tree, some parts of the supertree show reiteration of clades of similar composition. An example is observed in the Morindeae (clade *Craterispermum caudatum* to *Morinda moaensis* on the supertree; fig. 4C). The close relationship between the genera *Mitchella* and *Damnacanthus* is confirmed by the supertree, but the two genera

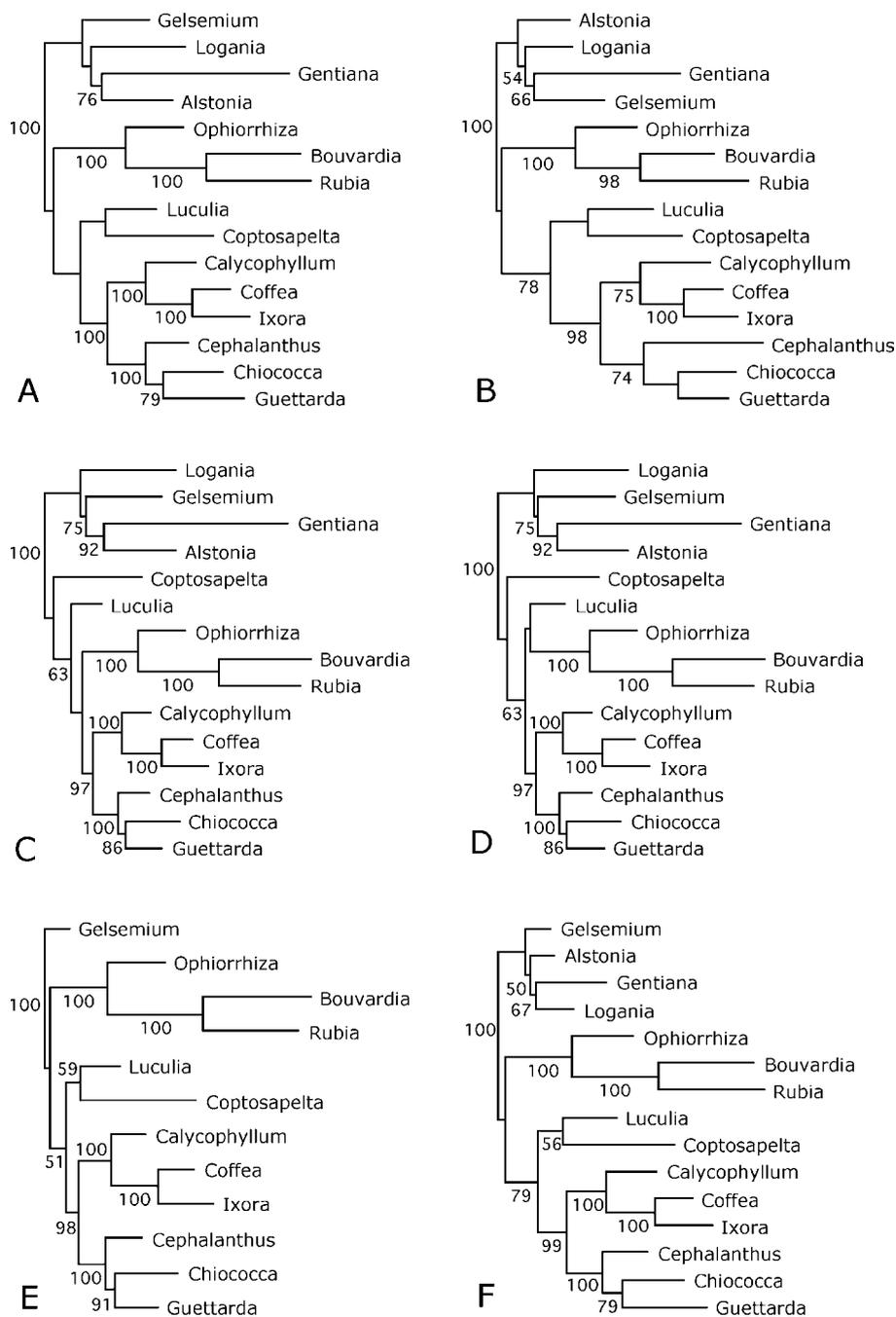


Figure 2. Examination of the phylogenetic position of *Coptosapelta* and *Luculia* using 5 plastid sequences and 3 nuclear sequences on selected taxa. Most parsimonious trees obtained from the combined plastid and nuclear matrices (A), the nuclear matrix (B), the plastid matrix (C & D), the plastid matrix with the simple invariant outgroup sequence (E) and the combined nuclear matrix and plastid matrix with the simple invariant outgroup sequence (F). All analyses (A, B, E and F) produced a single most parsimonious tree, except for the plastid matrix (two most parsimonious trees, shown in C and D). Bootstrap values are indicated on branches.



are (1) in a clade of their own (derived from *rbcL* and spacer source trees) and (2) in another clade with *Coelopyrena* and *Gynochtodes* (derived from *rps16* source tree). Other genera (*Craterispermum*, *Morinda*, ...) similarly 'reiterate' because this part of the supertree is derived from three source trees.

The position of *Gaertnera* is another obvious example of some odd results directly linked to the shortcomings of the supertree technique. The *rbcL* tree postulates that *Gaertnera* (voucher Malcomber 2709) is sister to a clade of Psychotrieae and Morindeae. In the spacer tree *Gaertnera* (voucher Malcomber 999) is sister to a clade of other Psychotrieae. The *rps16* tree finally shows a sister relationship *Gaertnera-Pagamea*, with the two genera in turn basal to a large clade including Schradereae, Morindeae and Psychotrieae. *Pagamea* is not represented in the sampling for *rbcL* and spacer trees. It is thus logical that the supertree repeats the hypothesis based on *rps16*, but puts one *Gaertnera* species in a remote relationship (spacer data with relatively good bootstrap against weak *rbcL* data). In our proposed classification the first hypothesis was retained, especially because it is corroborated by morphological data.

Because of these problems, it is necessary to consider the source trees for a correct understanding of the clades proposed by the supertree. Incompatibility problems do not affect the general topology of the supertree, which allows postulating and understanding the major evolutionary lineages in Rubiaceae, as they can be deduced from the sequencing evidence available in the source trees.

From the two given examples of problems, it is obvious indeed that the supertree approach is a formal method for reconstructing the major lineages of phylogenesis. It is an inadequate technique to address questions at lower taxonomic levels such as the phylogenies inside tribes or the monophyly of genera, for which wider samplings in more restricted study groups are needed.

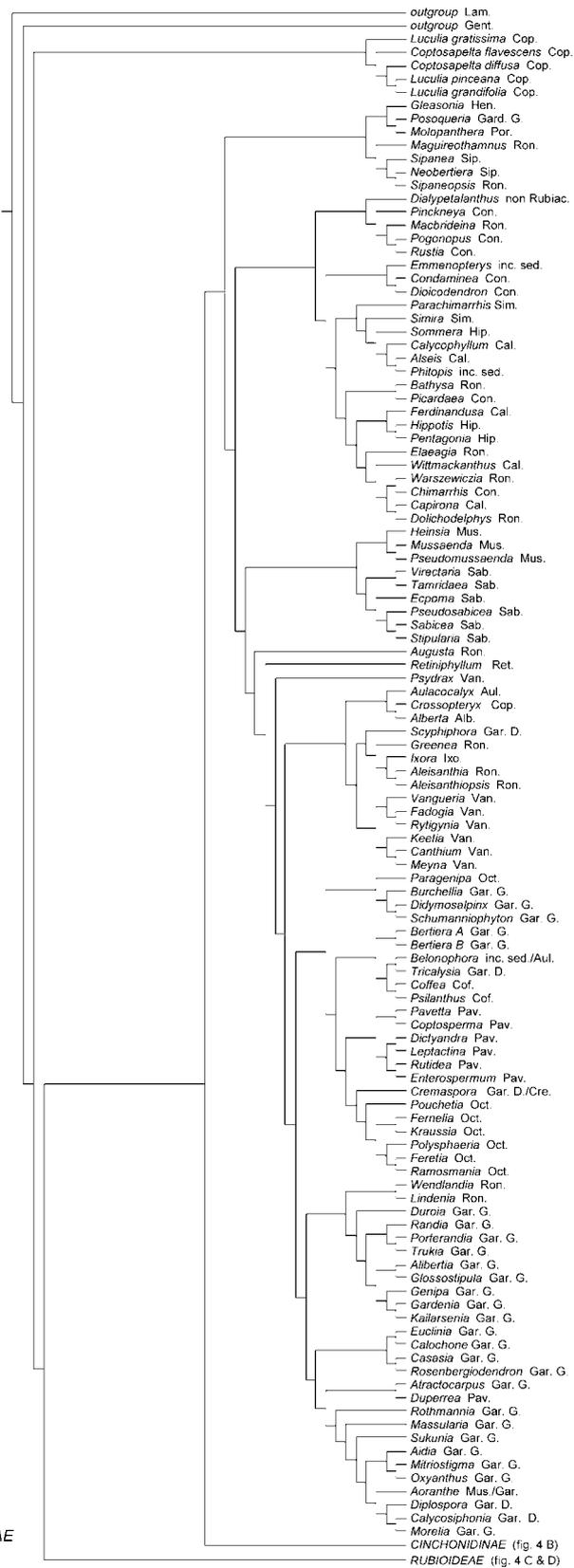
## D Discussion

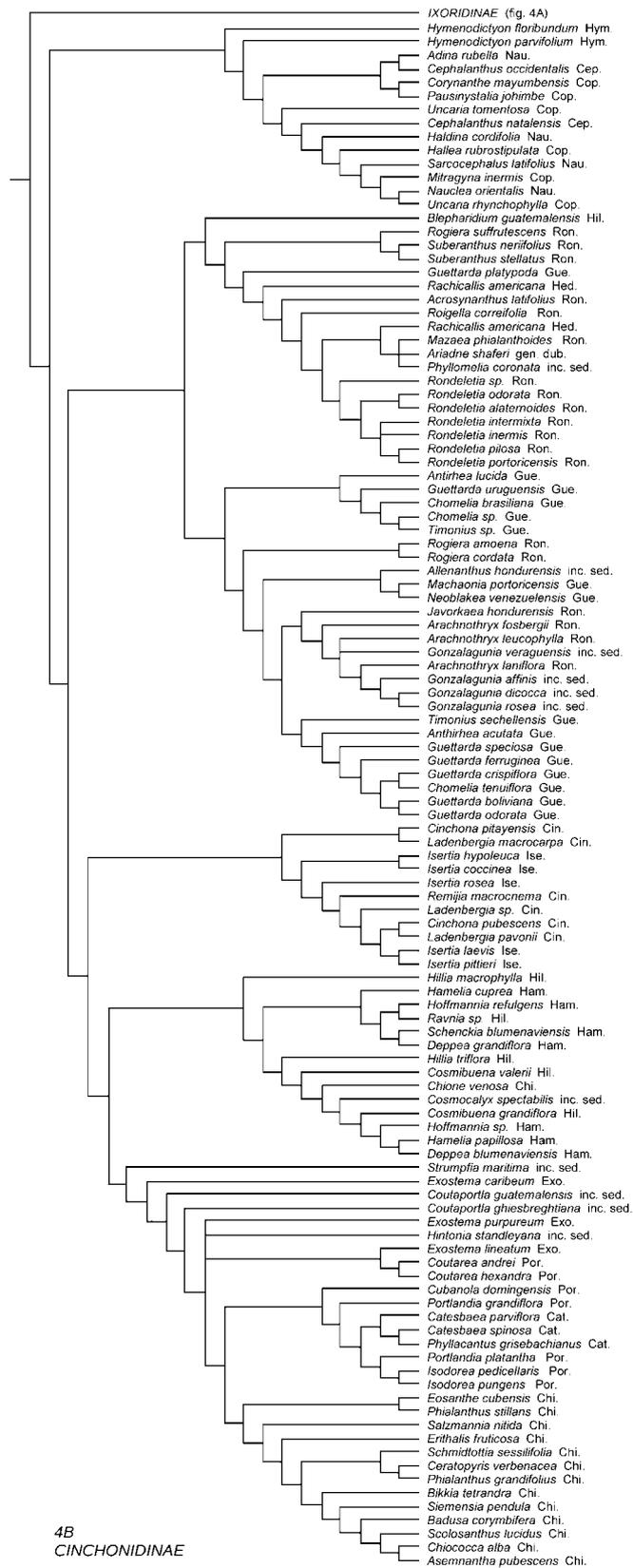
### D.1 Delimitation problems and monophyly

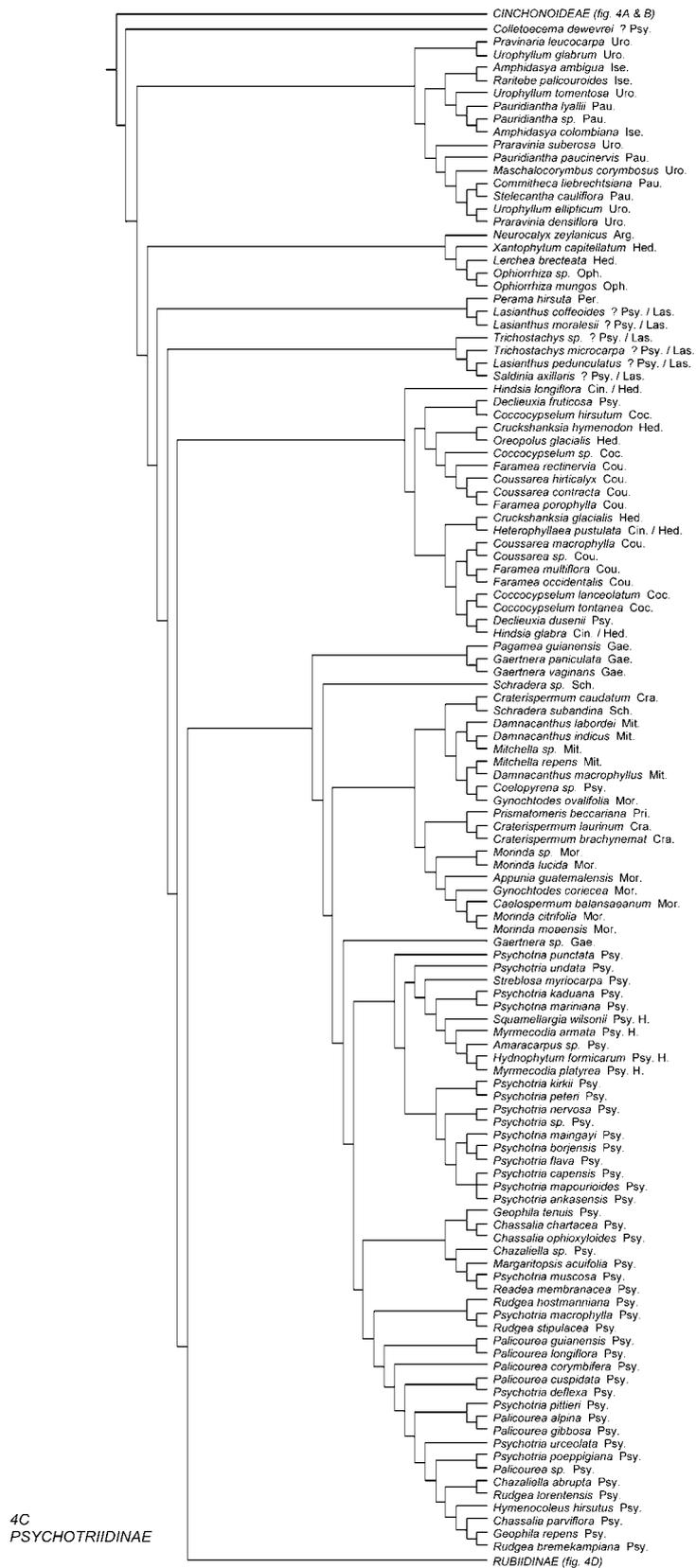
The delimitation of the Rubiaceae has caused very few problems in the past (for a review, see Robbrecht 1994a), and the monophyly of the family was supported in all formal analyses. The outgroup (not shown in fig. 4 but given in electronic appendix 3) corresponds to the clade *Fraxinus* to *Mitreola*, including Oleaceae (Lamiales) and several families of the Gentianales; it corresponds to all outgroups in the four source trees.

▶

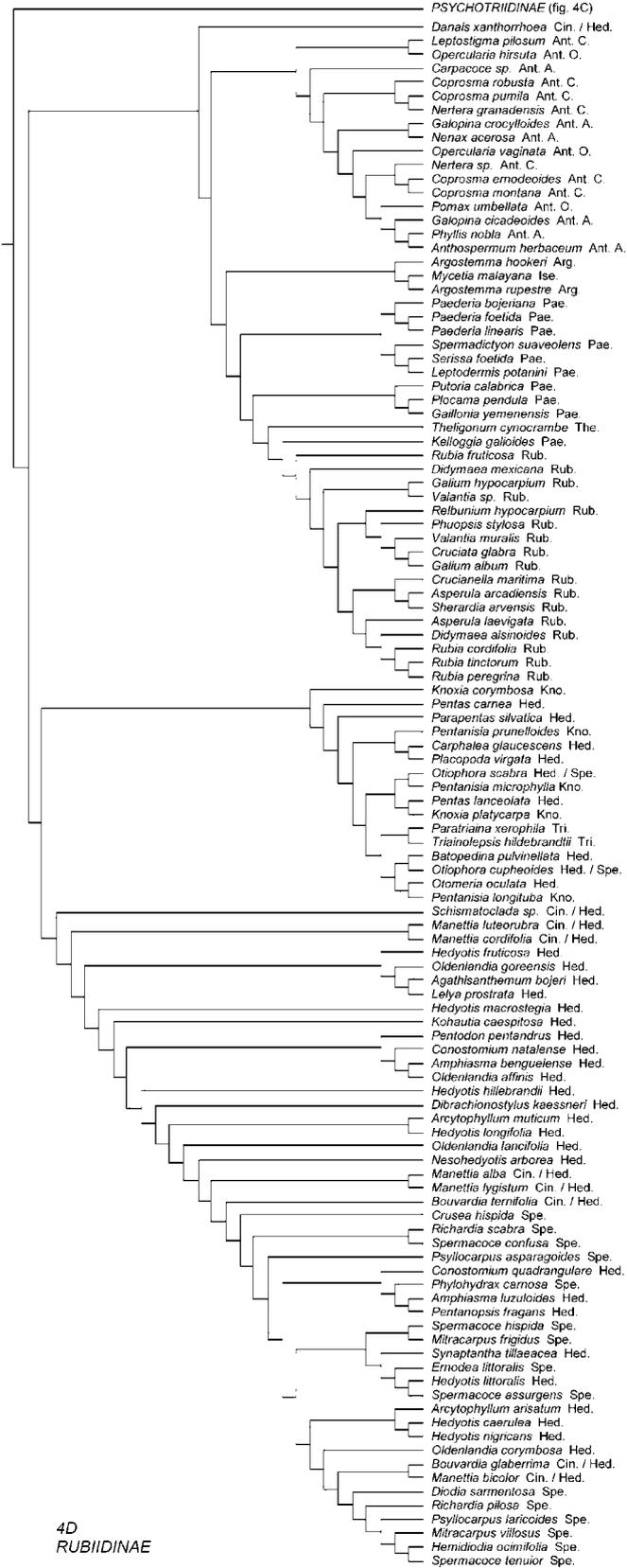
Figure 4. Supertree constructed from the 4 source trees:  
 A, partim tribe *Coptosapelteae* (clade *Luculia gratissima* to *Luculia grandifolia*)  
 and supertribe *Ixoridinae* (clade *Gleasonia* to *Morelia*);  
 B, partim supertribe *Cinchonidinae*;  
 C, partim supertribe *Psychotriidinae*;  
 D, partim supertribe *Rubiidinae*.  
 The taxa are followed by a symbol indicating their tribal position







4C  
PSYCHOTRIDINAE



4D  
RUBIIDINAE

The first firm demonstration of the monophyly of the family has been given by Bremer (1996). She emphasized that earlier molecular studies took the monophyly of the Rubiaceae for granted, and therefore provided an analysis with a large enough outgroup, including all major clades of the then Asteridae. Her analysis resulted in two equally parsimonious trees, which were, however, identical with regard to the monophyly and phylogeny of the Rubiaceae. All later works (e.g. Bremer & al. 1999) have corroborated the monophyly of the Rubiaceae.

The supertree confirms the monophyly of Rubiaceae. It includes several elements sometimes excluded from the family in the past, discussed in detail in section D.3: the Henriquezieae (1.2.1), *Dialypetalanthus* (1.2.2), the Gaertnereae (2.2(a)) and *Theligonum* (2.3.1(e)).

## D.2 The position of *Luculia* and *Coptosapelta*: towards two subfamilies again?

“Within Rubiaceae it seems impossible to achieve a completely resolved subfamilial classification, i.e., to include all genera in one of the ... subfamilies” (Bremer & al. 1999: 433). Because of the problematic position of the two genera in question, *Luculia* and *Coptosapelta*, we carried out a separate in depth investigation (R.1) that indirectly examined the root of the tree of the entire family. It allowed concluding that nuclear data indicate that (i) only two major clades exist in Rubiaceae, Rubioideae and non-Rubioideae, and (ii) point to a position of these two genera within the non-Rubioideae, where they are sister to the rest. This was also confirmed by our novel sequencing of the *rps16* intron for these two genera (electronic appendix 2C). Our analyses also suggest that (iii) the two genera form a monophyletic group. For their taxonomic history, see D.3 (1.1).

Our results thus contradict the basal or unresolved position of the two genera (or one of them) in the family obtained by analyses of cpDNA data (e.g. Bremer & Jansen 1991, Bremer & al. 1995, Bremer 1996, Bremer & Manen 2000, Rova & al. 2002).

However, our two hypotheses (ii) and (iii) should still be taken prudently. Is the bootstrap of 79% high enough to accept the first hypothesis? Are there other arguments for the monophyly of the clade with these two genera? Morphologically they hardly share any characters, and the occurrence of secondary pollen presentation (*Coptosapelta*) versus heterodistyly (*Luculia*) is only one of the several striking differences between them. Pollen features are also widely diverging. *Luculia* possesses the ‘standard’ tricolporate grains common in many Rubiaceae (Huysmans 1993), while *Coptosapelta* has unique pororate grains without columellae (Verellen & al. 2004). Other remarkable differences are the presence of raphides in *Coptosapelta* versus crystal sand in *Luculia* (Jansen pers. comm.), and aluminium accumulation characteristic of *Coptosapelta* but absent from *Luculia* (Jansen & al. 2000a). Note that the heterostyly of *Luculia* and the raphides of *Coptosapelta* are inconsistent with the distinction between Rubioideae and non-Rubioideae (see table 1).

In conclusion, our classification (Taxonomic conspectus) accepts a provisional recognition of the tribe Coptosapelteae for the two genera, and its placement at the base of all the other non-Rubioideae, although further work, morphological-anatomical and molecular, is clearly required. The alternative conclusion, recognizing a separate subfamily at the same hierarchic level of the two other major groups, is not well supported either and less conservative nomenclaturally; subfamilial names based on *Luculia* or *Coptosapelta* are not available.

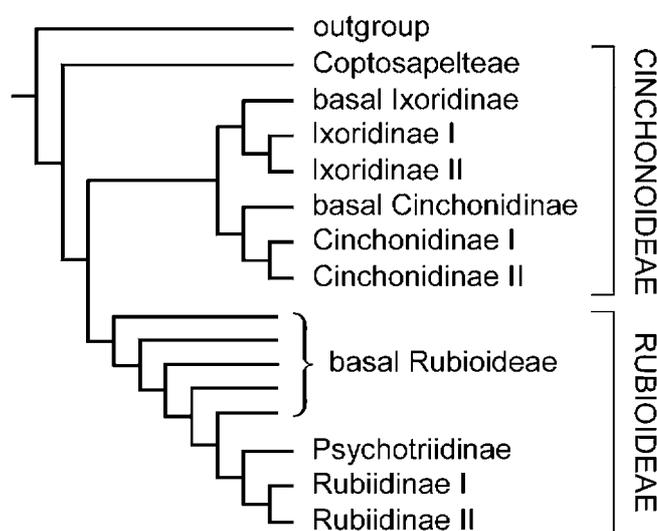
Our examination of the position of *Luculia* and *Coptosapelta* also results in a better rooting of the Rubiaceae tree. Fig. 2F obviously shows that only two major evolutionary lines exist in the family, and suggests a classification in two subfamilies rather than three as proposed in all previous molecular phylogenies. We discuss this further in the next section.

### D.3 The main clades and subclades of the Rubiaceae as derived from the supertree

#### D.3.1 Major lineages

If one bears in mind that the previous paragraph demonstrates that the basal position of *Luculia* and *Coptosapelta* on the supertree is incorrect, the supertree (main structure shown in fig. 5) also supports recognition of only two major evolutionary lineages in the Rubiaceae: the *Gleasonia* to *Asemnantha* (fig. 4A-B), and the clade *Colletoeccema* to *Spermacoce tenuior* (fig. 4C-D). For reasons set out in D.3.3 we designate these clades as Cinchonoideae and Rubioideae.

The two lineages are characterized by distinguishing features (table 1) with regard to morphology, anatomy, flower biology and chemical compounds. Karyological features are also somewhat



#### (1) Cinchonoideae

(1.2.1) basal Ixoridinae: Henriquezieae & Sipaneeae

(1.2.2) Ixoridinae I: Calycophylleae-Condamineae alliance

(1.2.3) Ixoridinae II: Mussaendeae, Sabiceae, Alberteae, Ixoreae, Coffeae, Pavetteae, Octotropideae & Gardenieae

(1.3.1) basal Cinchonidinae: Hymenodictyoneae and Naucleae s.l.

(1.3.2) Cinchonidinae I: Rondeletieae alliance

(1.3.3) Cinchonidinae II: Cinchoneae, Hamelieae, Catesbaeeae, and relatives of these tribes

#### (2) Rubioideae

(2.1) basal Rubioideae: *Colletoeccema*, Urophyllaeae, Ophiorrhizeae, Lasiantheae, Coussareae

(2.2) Psychotriidinae: Gaertnereae, Morindeae, Psychotrieae, Palicoureeae

(2.3.1) Rubiidinae I: Danaeae, Anthospermeae, Argostemmateae, Paederieae, Putorieae, Rubieae

(2.3.2) Rubiidinae II: Knoxieae, Spermacoceae s.l.

Figure 5. Supertree, schematic representation of main structure.

The *Coptosapelteae* are outside the Cinchonoideae clade but probably belong to it according to a separate study (see fig. 2 and text, R.1 & D.2); the position here is derived from the undefined position in the source trees.

The numbering in the box is also used in text section D.3.4 and in the Taxonomic conspectus.

better supported than in the four subfamilies framework used by Kiehn (1995). Basic numbers below or above 10 or 11 are more common in Rubioideae than in Cinchonoideae. Ploidy levels above 6 $\times$  are almost nonexistent in Cinchonoideae (i.e., also 8 $\times$  only in Naucleaeae, Coffeaeae, and Guettardeae; also 8 $\times$  and 12 $\times$  in Hamelieae). High ploidy levels on the contrary (up to 22 $\times$ ) are common in Rubioideae.

**Table 1. Distinguishing features between the subfamilies Cinchonoideae and Rubioideae.**

(a) if herbaceous very rarely annual, only in *Sipanea* and *Limnosipanea*; (b) annuals common; (c) reference pertaining to a survey of wood features, occurrence of crystals in other vegetative and reproductive structures apparently the same, but systematic survey not available.

	<b>Cinchonoideae</b>	<b>Rubioideae</b>
habit	predominantly woody (a)	woody or herbaceous (b)
Ca-oxalate crystals (Jansen & al. 2002: 56 (c))	mostly crystal sand, rarely (Ham.) raphides	raphides
trichomes of the 'external' indumentum (Robbrecht 1988: 61)	mostly cylindrical type	mostly articulate type
corolla lobes aestivation	variable (contorted, imbricate, valvate)	predominantly valvate
flower biology (Puff & al. 1996)	frequently secondary pollen presentation (SPP), rarely heterostyly	frequently heterostyly; very rarely (Rub., <i>Phuopsis</i> ) SPP
aluminium accumulation (Jansen & al. 2000 a, b)	in only a few representatives	frequently strong
chemical compounds	iridoids and indole alkaloids	anthraquinones
ploidy levels (Kiehn 1995)	2x, 4x predominant	high ploidy levels not uncommon

### D.3.2 A new classification in two subfamilies and four supertribes

The supertree thus easily allows a classification in only two, not three, subfamilies: Cinchonoideae and Rubioideae.

A recent system with two subfamilies, Cinchonoideae and Rubioideae, has also been proposed by Bridson & Verdcourt (2003: 383). They concluded that the proposed redelimitation of Cinchonoideae (narrowed) and Ixoroideae (widened) by the molecular phylogenies (as reviewed in the Introduction) resulted in obscuring the morphological distinction between them. They judged it therefore better to return to Verdcourt's wide concept of the Cinchonoideae, a decision here corroborated, although we propose the use of another name (see next paragraph).

The two subfamilies show the evolutionary pattern that is characteristic for many groups, i.e. they comprise some more poorly developed lineages at the base and further a few successful groups, in the Rubiaceae case only two subclades per main clade. We translate this pattern in a new system with two subfamilies, four supertribes and some informal categories ("basal Ixoridinae", etc.). This classification is summarized in fig. 5 and extensively surveyed in a Taxonomic conspectus at the end of the paper. Note that Schumann (1891: 16) is the only previous author having a category in between the subfamilies and tribes of the Rubiaceae. He divided his subfamily Cinchonoideae in Cinchoninae and Gardeninae, and his subfamily Coffeoidae in Guettardinae and

Psychotriinae. These names have been listed by Darwin (1976); we follow his viewpoint that they cannot be considered as validly published because they remained rankless until today.

### D.3.3 Names for the subfamilies and supertribes

While the delimitation of the Rubioideae has remained more or less unchanged since the subfamily was proposed the first time (Bremekamp 1952), the subfamilial name 'Cinchonoideae' now covers an extremely wide array of different concepts. The main ones based on morphological data are, arranged from the narrowest to the widest sense: Bremekamp 1966; Robbrecht 1988; Verdcourt 1958; Bridson & Verdcourt 2003. The molecular phylogenetic publications from the last fifteen years proposed still other circumscriptions. The first molecular results (Bremer & Jansen 1991) suggested elimination of the subfamily, while later contributions (Bremer & al. 1995, Bremer 1996, Bremer & Manen 2000, Rova & al. 2002) delimited the Cinchonoideae more narrowly than Bremekamp's (1966) circumscription, but including the Hillieae and Hamelieae. Moreover, none of the newer delimitations based on either morphological or molecular data is congruent with the classical Cinchonoideae concept (i.e., all the multiovulate Rubiaceae; Hooker 1873, Schumann 1891).

Consequently the name Cinchonoideae cannot be used without carefully specifying which of the various senses is meant. Next to the scientific names for the subfamilies, which cannot be modified, we propose English names which indicate the wide present concept: **Cinchonoideae** or 'cinchona and ixora subfamily', and **Rubioideae** or 'psychotria and madder subfamily'.

We propose four new formal supertribes, **Ixoridinae** and **Cinchonidinae** in the Cinchonoideae, **Psychotriidinae** and **Rubiidinae** in the Rubioideae. They were coined using the proposal for modification of recommendation 16A of the ICBN (McNeill & Turland 2005: 222), i.e. they are formed with the suffix -idinae. We felt no need for formal names, on the contrary, for the basal groups in each of the two subfamilies, or at least judge it too premature to propose them (see M.1).

In the paper (Darwin 1976) detailing the effectively published names of subfamilies, tribes and subtribes of the Rubiaceae, the type genus *Rubia* Linnaeus is followed by the family name Rubiaceae Jussieu and the authorless infrafamilial names Rubioideae, Rubieae and Rubiinae. The Seattle Code (1972) in effect at the time designated these names as autonyms. Rubioideae and Rubieae were still autonyms at the time of Robbrecht's (1988) survey of the family, but the same year the Tokyo Code (art. 19.3) suppressed the autonym rule for ranks between family and genus; yet it prescribed the existence of a subfamilial, tribal and subtribal name derived from the name of the type genus of the family. As a consequence authors should now be attributed to the former autonyms; these are available (Rubioideae Verdcourt and Rubieae Baill.; e.g., Bremer & Manen 2000).

Note also that the here proposed new concepts 'supertribe Ixoridinae' and 'supertribe Cinchonidinae' read as (subfamilies) Ixoroideae and Cinchonoideae in the preceeding parts of the present paper. From this point in the paper onwards, we will consistently use the supertribal names, even when discussing literature that referred to or used the subfamilial names.

### D.3.4 Discussion of the subfamilies, supertribes, tribes and subtribes

The following discussion of the major clades shown by the supertree and their subclades uses the numbering of the box in fig. 5. The numbers are also used in the Taxonomic conspectus.

(1) The **cinchona and ixora subfamily** (Cinchonoideae; fig. 6) unites the Cinchonoideae and Ixoroideae of recent authors (e.g. Bremer & al. 1995, Bremer 1996, Bremer & Manen 2000, Rova & al. 2002). It is morphologically and biologically characterized by the almost universal absence of raphides (present in Hamelieae only) and the common occurrence of secondary pollen presentation.

Chemically it is relatively diverse (iridoids and indole alkaloids; Young & al. 1996)). It consists of two major subclades, in the above-cited molecular phylogenetic works distinguished as subfamilies Ixoroideae and Cinchonoideae; they are here recognized as supertribes Ixoridinae and Cinchonidinae, which are sister to one another. According to the separate combined analysis discussed in section D.2 of this paper, the Coptosapelteae are a small clade at the base of the subfamily.

### (1.1) Coptosapelteae

The Coptosapelteae correspond to the clade with three *Luculia* and two *Coptosapelta* species on the supertree. In the classical systems (Hooker 1873; Schumann 1891) the two genera were placed in Cinchoneae, a tribe then very widely conceived to contain all Rubiaceae having numerous winged seeds. The disintegration of the Cinchoneae inter alia resulted in an emended concept of the Coptosapelteae (Andersson & Persson 1991), a tribe originally conceived for only two genera, *Crossopteryx* and the name-bringing one. Andersson & Persson adopted its name when they proposed tribal level for an emendation of the Mitragyninae of the Cinchoneae, following suggestions by Ridsdale (1978: 44) to assemble capituliferous (*Mitragyne* and *Uncaria*) and non-capituliferous genera (e.g. *Corynanthe*, *Hymenodictyon*) with winged seeds in one alliance.

Previous molecular results (Razifimandimbison & Bremer 2002 a, b) and our supertree experiment have relegated (see D.3 (1.3.1)) most of the concerned genera to the Naucleaeae and Hymenodictyoneae, to which *Coptosapelta* and *Luculia* are not closely related.

(1.2) In the **Ixoridinae** (clade *Gleasonia* to *Morelia* in fig. 4A), two major evolutionary lineages may be distinguished. These are designated in our Taxonomic conspectus and on the supertree summary (fig. 5 & 6) as **Ixoridinae I**, a neotropical lineage, and **Ixoridinae II**, an essentially paleotropical lineage. They are here shown to be sister to one another and have at their base a small clade here designated as **basal Ixoridinae**.

Our Ixoridinae correspond to the clade (I1, I2, I3) of Rova & al. (2002: fig. 1 & 3-5; *trnL-F*); these three clades are the same (with a much wider sampling) in our supertree (fig. 6), where they are resolved at the base, however.

(1.2.1) **Basal Ixoridinae**. This basal group (clade *Gleasonia* to *Sipaneopsis* in fig. 4A) is neotropical and consists of two clades, on the one hand three genera of which the relationships were much disputed in the past, viz. *Gleasonia*, *Posoqueria* and *Molopanthera*, on the other hand the Sipaneae. The same sister alliance has already been postulated by Rova & al. (2002; *trnL-F*; their clade I3 in an unresolved position), and by Delprete & Cortés-B. (2004; *trnL-F*), who also have it in an unresolved position between Ixoridinae and Cinchonidinae. The supertree is consistent with their findings (i) in that *Molopanthera* and *Posoqueria* are sister genera, and (ii) that *Gleasonia* and *Platycarpum* (genus not on the supertree) belong to the same clade as these two.

(a) *Gleasonia* [and *Platycarpum*] represent the small tribe **Henriquezieae**, of which only the name-bringing genus has not yet been sequenced. Bremekamp (1957) gave morphological and anatomical arguments for treating *Henriquezia* and *Platycarpum* as a separate family Henriqueziaceae in the 'Tubiflorae' (Lamiales), while he maintained *Gleasonia* in the Rubiaceae as a monogeneric subfamily Gleasonioideae, characterized inter alia by colpate pollen grains in permanent tetrads, a feature otherwise not existing in Rubiaceae. Rogers (1984) undertook a thorough morphological and anatomical investigation, including wood anatomy and pollen morphology; he was unable to follow Bremekamp's viewpoint and maintained one tribe Henriquezieae for the three genera, kept in Rubiaceae although he did not indicate close relatives in that family.

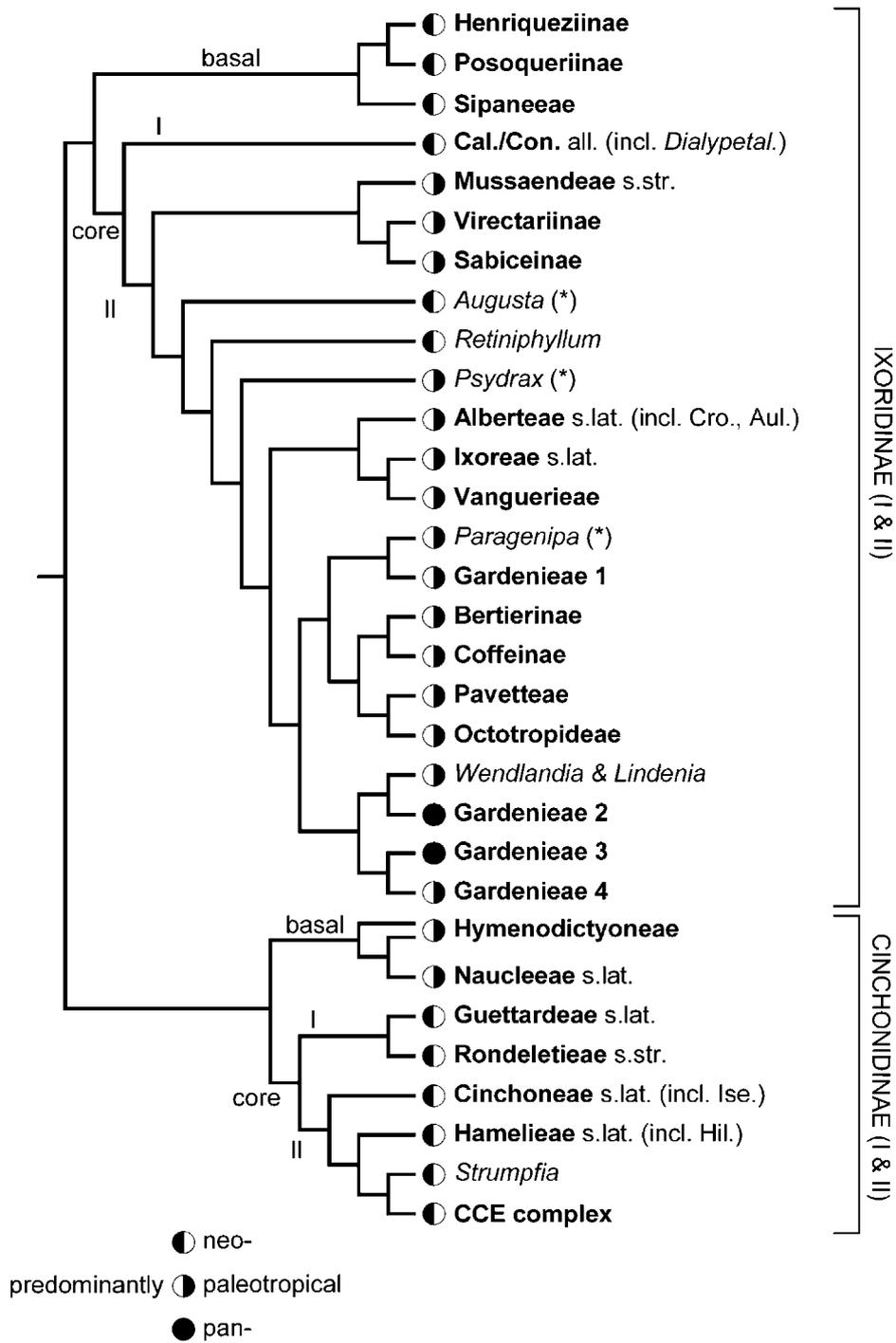


Figure 6. Supertree, schematic representation of structure for subfamily Cinchonoideae.  
 The symbols before the groups indicate their pantropical versus (predominantly or exclusively) neo- or paleotropical distribution.  
 (\*) problematic positions discussed in text.

The position of *Posoqueria* and *Molopanthera* has been controversial. Morphologically, the two genera are extremely different and were previously never considered to be related. *Posoqueria* has large, often zygomorphic flowers and large, fleshy fruits and has often been included in the Gardenieae. Andreasen & Bremer (2000; combined analysis of morphological and molecular data) concluded that the genus should be excluded from Gardenieae and is possibly a member of the Mussaendeae, although they stressed that more inclusive taxon sampling was needed to confirm that relationship. *Molopanthera* has small flowers with rotate corollas and capsular fruits with winged seeds, and was classically placed in the Cinchoneae. Using morphological arguments, Andersson & Persson (1991) transferred the genus to Condamineae, but Delprete (1996a, 1999) included it in the '*Bathysa* group' of his broadly circumscribed Rondeletieae.

Rova & al. (2002) also discussed the taxonomic history of the two genera briefly. From their review, one should stress that the unique mechanism of pollen projection well known from the very large flowers of *Posoqueria* (see Hallé 1967: 131, pl. 40) also exists in the tiny flowers of *Molopanthera* (Delprete 1999: 193). Rova & al. concluded that their clade I3b comprised the Henriquezieae and the two genera in a tentative tribe. As already stated above, Delprete & Cortés-B. (2004; *trnL-F*) corroborated this, and Delprete (in Delprete & al. 2004: 23) formally recognized the tribe Posoquerieae for the two genera. We prefer to widen the exclusively neotropical Henriquezieae (corollas zygomorphic and corolla lobes imbricate for all these genera) and to distinguish two subtribes.

**(b) The Sipaneeae** (clade *Maguireothamnus* to *Sipaneopsis* in fig. 4A) originally were a small group split off (Bremekamp 1934: 232 & 1966: 20) from Rondeletieae for genera with a herbaceous habit and contorted aestivation of the corolla lobes, viz. the neotropical genera *Sipanea* and *Limnosipanea* and the African *Virectaria*. The latter genus was soon excluded (see (1.2.3.1)). The Sipaneeae were not accepted by Delprete (1999), who recognized an informal '*Sipanea* group' within the Rondeletieae, suggesting that *Sipaneopsis* and *Neobertiera* "could be interpreted as the phylogenetic links [he surely intends 'links with regard to habit'] between the Sipaneeae and Rondeletieae s.str." Macromolecular evidence (Rova & al. 2002; *trnL-F*) indicated that Sipaneeae should be segregated and even have no close affinities with Rondeletieae (which are in Cinchonidinae versus Sipaneeae in Ixoridinae). The tribe was significantly extended (to ten genera) based on morphological and molecular evidence (Delprete & Cortés-B. 2004; *trnL-F* and *ITS*); these authors' analysis also endorsed the clade (Hen.,Sip.). Several woody or subwoody genera are now added to the Sipaneeae, originally circumscribed to comprise only herbaceous representatives.

### (1.2.2) Ixoridinae I

This first clade of the supertribe Ixoridinae (clade *Dialypetalanthus* to *Dolichodelphys* in fig. 4A), designated here Ixoridinae I, is almost exclusively neotropical. Genera of the Calycophylleae, Condamineae, Simireae, Hippotideae and some of the former Rondeletieae find a place here, but the clades inside it on the supertree do not correspond with any of these tribes as currently circumscribed. The shortcomings of the supertree technique as well as poor sampling and ill knowledge of this **Condamineae/Calycophylleae** alliance are no doubt an explanation for the poor congruence of the postulated clades with existing classifications. This group obviously is one of the poorest known of the family with regard to phylogeny and tribal delimitation. A discussion of the clades appearing on the supertree would be too provisional. Our Ixoridinae I correspond with the "first Ixoroideae clade" or "I1" of Rova & al. (2002); their cladogram of I1 (op. cit.: fig. 3) was also very poorly resolved. Probably new more informative sequence data will resolve the reconstruction of the phylogeny of this alliance.

*Emmenopterys* is the sole paleotropical representative in Ixoridinae I. This Chinese monospecific genus was included in Schumann's (1891) widely conceived Cinchoneae and maintained in that tribe by Robbrecht (1988). In their cladistic analysis of morphological features of the Cinchoneae, Andersson & Persson (1991) included the genus but had not enough data available for keeping it in all analyses. In two analyses it came out in a clade with *Alseis* and *Calycophyllum*, i.e. in a relationship also obtained by molecular data (Rova & al. 2002 and our supertree; position of the genus here based on the spacer and *trnL-F* source trees). Andersson & Persson (op. cit.; not Robbrecht 1994b as stated by Rova & al. 2002) excluded the genus from the Cinchoneae and considered it of uncertain position. Rova & al. (op. cit.) did not comment on the genus, despite the apparent geographical disjunction from its supposed relatives. It awaits an in-depth study.

Our Ixoridinae I also include *Dialypetalanthus* that has long been excluded from Rubiaceae. Although the vegetative and fruit morphology of this tree from the Amazon Basin are very characteristic for the Rubiaceae, its dimerous, dialypetalous and polyandrous flowers do not match the family at all. Piesschaert & al. (1997) have reviewed in depth its morphology and anatomy and concluded that it belongs in Gentianales where it is closely related to Rubiaceae, but they kept the monogeneric Dialypetalanthaceae separate. Plastid *rbcL* sequence data, however, showed *Dialypetalanthus* to be a member of Rubiaceae (Fay & al. 2000); the relationships shown by this analysis, in a clade with neotropical genera of Calycophylleae and Condamineae, agrees well with the position found for *Dialypetalanthus* on our supertree. Cortés-B. & al.'s (2005) analysis based on *trnL-F*, *rps16* and *ITS* corroborated this relationship. We therefore postulate that the dialypetaly and polyandry of the genus are secondarily derived, a hypothesis to be confirmed by ontogenetic research.

### (1.2.3) Ixoridinae II

In this second and largest group of the core Ixoridinae (clade *Heinsia* to *Morelia* on the supertree, fig. 4A), here called Ixoridinae II, the Mussaendeae-Sabiceae alliance (1.2.3.1) is basal. The rest of the Ixoridinae II consists of two large and successful groups, sister to one another, and designated here as the Vanguerieae alliance (1.2.3.3) and the Gardenieae alliance (1.2.3.4) respectively. At their base the genera *Augusta* (subg. *Augusta*; discussed below under Gardenieae 1) and *Retiniphyllum* (1.2.3.2) finds its place.

Our Ixoridinae II correspond with the "second Ixoroideae clade" or "I2" of Rova & al. (2002); their cladogram of I2 (op. cit.: fig. 4) is well resolved; our supertree (summary fig. 5) with a much wider sampling confirms the relationships postulated by their analysis, viz. (Mus.(Ret.(Ixo.Van.all.,Gar.all.))).

(1.2.3.1) The almost exclusively paleotropical **Mussaendeae-Sabiceae alliance** (clade *Heinsia* to *Stipularia* on the supertree, fig. 4A) is predominantly diversified in tropical Africa where the majority of the genera occur; in the New World it is only represented by the Afro-American genus *Sabicea*. At the time of Verdcourt's (1958) and Bremekamp's (1966) classifications the tribe Mussaendeae was very broadly conceived, although Bremekamp started its dismantling by separating a monogeneric tribe Sabiceae as well as by removing all raphide-bearing genera. Darwin (1976) renamed this Mussaendeae concept as tribe Isertieae for nomenclatural priority reasons. Andersson (1996), in a cladistic analysis of morphological features, confirmed Bremekamp's circumscription and distinguished between Sabiceae (by now including eight genera) and Isertieae s.str. In the latter tribe the neotropical *Isertia* was kept together with paleotropical representatives (e.g. *Mussaenda*, *Pseudomussaenda*), but Andersson stressed that the branch uniting *Isertia* with the *Mussaenda* group was not well supported. Molecular investigations subsequently led to the final

collapse of the Isertieae. Bremer & Thulin (1998; *rbcL*) reestablished the Mussaendeae in a strict sense, excluding *Isertia*; they confirmed its distinction from Sabiceae, to which tribe they also added a newly described Socotran genus *Tamridaea*. Dessein & al. (2001 a & b; *rbcL* & *rps16*) also associated *Virectaria*, of which the relationship was disputed for decades, and *Hekistocarpa*, before always included in Hedyotideae. They adopted the previously monogeneric tribe Virectarieae, emending it to also include *Tamridaea* and *Hekistocarpa*, and thus proposed to narrow the Sabiceae.

The supertree (fig. 6) shows the structure (Mus.(Vir.,Sab.)). The cited analyses of *rbcL* and *rps16* data (Dessein & al. 2001b: fig. 38 & 39) supported the assumption of monophyly of the three groups, but left their relationships with other clades of the Ixoridinae unresolved. The monophyly of the group (Vir.,Sab.) is also corroborated by *ITS* data (Khan & al. 2005). We recognize only two tribes, Mussaendeae and Sabiceae, with a reduction of the Virectarieae to a subtribe of the latter, because this decision is a better translation of the structure of the supertree.

**(1.2.3.2) *Retiniphyllum*.** The neotropical genus *Retiniphyllum* (with one other genus *Botryarrhena*<sup>1</sup> placed in **Retiniphyllae**) has often been suggested to have a relationship with the paleotropical tribe Vanguerieae, and the morphological similarities between these two are impressive (see Robbrecht 1988: 153). Cortés-B. & al.'s (2005) analyses based on *trnL-F*, *rps16* and *ITS* confirmed that the morphological similarity is congruent with the phylogenetic relationship of *Retiniphyllum*, i.e. they obtained it in a position basal to the Vanguerieae plus Gardenieae alliances. The supertree (fig. 6) is congruent with their findings; *Retiniphyllum* is here also found in an isolated position at the base of these two alliances. We maintain the tribe in our classification.

The analysis of Cortés-B. & al. included more than twenty species of *Retiniphyllum* (and corroborated the monophyly of the genus); the apparently related genus *Botryarrhena* was not in the sampling.

**(1.2.3.3) The Vanguerieae alliance** (*Psydrax* and the clade *Aulacocalyx* to *Meyna* on the supertree; fig. 4A) is comprised of three tribes, which correspond to three clades including the name-bringing genera, viz. Alberteae, Ixoreae and Vanguerieae. The basal position of *Psydrax* in the supertree, isolated from other Vanguerieae, is derived from *rps16* data for *Psydrax odorata* (Andersson & Rova 1999); *trnL-F* data are available for the same species<sup>2</sup>. That *Psydrax* (a segregate of *Canthium*, Bridson 1992) belongs to Vanguerieae is beyond any doubt (see comments under (c) below), and this was confirmed by *ITS* and *trnT-F* data (Lantz & Bremer 2004). These authors confirmed the monophyly of *Psydrax* and found it to belong to a Vanguerieae clade with inter alia *Keetia* and *Pyrostria*, two other segregates from the formerly widely conceived genus *Canthium*. Note that the genus *Canthium* s.str. on the supertree is represented by another species, *C. coromandelicum*.

<sup>1</sup> This other neotropical genus is ill-known. Apart from the genus' protologue (Ducke 1932) and the addition of a second species (Steyermark 1983) nothing has been published. Steyermark did not take a personal position with regard to the relationship of *Botryarrhena*, but only summarized, in a somewhat confused way, the views of Ducke (1932; placement of *Botryarrhena* in Eugardenieae, where it is closely related to *Retiniphyllum*). Although the monogeneric tribe Retiniphyllae goes back to the system in *Genera Plantarum* (Hooker 1873; in a small "series B" for Rubiaceae with two collateral ovules in the locules), it was not taken up in *Die natürlichen Pflanzenfamilien* (Schumann 1891; *Retiniphyllum* in Gardenieae Eugardenieae) or in the Rubiaceae treatment in the *Flora Brasiliensis* (Schumann 1888-89) which formed the obvious taxonomic framework for Ducke's comments. In any way, *Botryarrhena* should now be considered of uncertain position and awaits an in depth investigation.

<sup>2</sup> As *Canthium* sp. (Rova & al. 2002), a fact that we discovered in a too late stage of our analyses. A future recalculation of the supertree should consider this.

(a) The **Alberteae** as delimited by Puff & al. (1984) comprise the essentially Madagascan genus pair *Alberta* and *Nematostylis*. Robbrecht (1988) also tentatively included *Octotropis*, *Airosperma* and *Boholia*. The relationship of *Octotropis* was later shown to lie elsewhere (see (1.2.3.4.c)). The two other genera remain ill-known and are not discussed here because they have not yet been included in our and other molecular samplings.

For a survey of the intricate taxonomic history of the Alberteae, we refer to Puff & al. (op. cit.: 354-359). Despite an in-depth morphological and anatomical study, these authors were unable to suggest close relatives for *Alberta* and *Nematostylis*, but they mainly surveyed the subfamily Antirheoideae where the tribe was then supposed to belong. By now, molecular results have shown that the tribe belongs to Ixoridae, a hypothesis first formulated by Andreasen & al. (1999). In a combined analysis of morphological and molecular data (Andreasen & Bremer 2000), the tribe Alberteae was sister to Vanguerieae, whereas our supertree postulates the relationship (Alb.(Ixo.,Van.)).

With regard to the Alberteae, only *Alberta* is represented on the supertree. However, it postulates the novel hypothesis that the afro-tropical *Crossopteryx* and *Aulacocalyx* form a clade with *Alberta*.

A fully appropriate relationship for the afro-tropical monospecific genus *Crossopteryx* has never been postulated until present. The genus features a quite unique combination of characters: left-contorted aestivation of the corolla lobes; secondary pollen presentation; massive placentas bearing only 3-5 ovules;  $\pm$  dry (capsular) fruits with accrescent calyx; grown out placentas showing impressions of the seeds, which are winged and deeply fimbriate all around; large embryos and a limited amount of endosperm. Morphologically and biogeographically, a relationship with *Alberta* should not be ruled out, although an in-depth comparison is needed. The dry fruits and winged seeds explain the classical inclusion in the Cinchoneae. In their examination of the circumscription of this tribe, Andersson & Persson (1991; cladistic analysis of morphological features) referred *Crossopteryx* to the Coptosapelteae (s.lat., see D.2 above); the winged seeds characteristic of this alliance, however, in no way morphologically compare to those of *Crossopteryx*, as shown in a comparative depiction (Stoffelen & al. 1996: fig. 2). White (1962) had emphasized the morphological uniqueness of the African genus by recognizing a monogeneric tribe Crossopteryxideae, a decision followed by Bridson & Verdcourt (2003: 389) by validating the name and correcting its spelling (Crossopterygeae F.White ex Bridson).

The present hypothesis for a relationship with two other afro-tropical genera puts doubts on the need for a monogeneric Crossopterygeae. The position of *Crossopteryx* with *Alberta* goes back to the *rbcL* tree; the genus' sole species *C. febrifuga* has been sequenced for a phylogenetic investigation of Naucleaeae (s.lat., comprising Coptosapelteae and Cephalanteae as then delimited; Razafimandimbison & Bremer 2002a). In this study, it proved to be the only element falling outside the Naucleaeae s.lat., as it came out as a member of the Ixoridae. The very limited sampling of the Ixoridae (as an outgroup) in this study, however, did not allow any further conclusion. The results do not contradict our findings, since *Crossopteryx* was found in a clade with *Vangueria* and *Alberta*.

The genus *Crossopteryx* is also in the sampling for the spacer source tree, where it is in a basal position to the Octotropideae. The numerous striking morphological differences between *Crossopteryx* and *Alberta* (e.g. several ovules  $\pm$  immersed in a peltate placenta versus a solitary pendulous one) await interpretation.

The position of *Aulacocalyx* on the supertree suggests returning the genus to the Alberteae; indeed, in Schumann's (1891) system the genus was included in the then widely conceived Alberteae. It has

been transferred to the Cremasporae by Bremekamp (1966) and to the 'Ixoreae-Heinsiinae' by Verdcourt (1958<sup>3</sup>). Robbrecht & Puff (1986) recognized a separate tribe Aulacocalyceae for *Aulacocalyx*, *Heinsenia* and a few other paleotropical genera combining superior embryo radicles with a totally reduced seed-coat. The supertree's placement of *Aulacocalyx* is derived from our novel sequencing for the spacer tree only. It is contradictory to the results obtained by Andreasen & Bremer (2000; combined morphological-molecular analysis) who included *Heinsenia* in the Gardenieae. The position *Aulacocalyx* needs further investigation. The same holds for three other genera placed in Aulacocalyceae by Robbrecht & Puff (op.cit.), viz. *Alleizettella*, *Himalrandia* and *Gardeniopsis*; for none of them molecular data are available.

(b) The tribal name **Ixoreae** has a long history in Rubiaceae classification and has in the past been used in a very wide sense; in the classical systems of Hooker (1873) and Schumann (1891) the tribe included almost all representatives of the Rubiaceae with contorted aestivation and solitary ovules (in this wide delimitation, including *Coffea*, the correct tribal name is Coffeae; Darwin 1976). The subsequent history, detailed below, is intricate and has the implications that several tribal names involved have entirely different circumscriptions. This is clarified in table 2.

The tribe was taken up but narrowed by removing the genera with inflorescences axillary and paired at the nodes (Robbrecht 1984; Bridson & Robbrecht 1985, Robbrecht & Puff 1986); in that delimitation these authors used the name Pavetteae for it. Robbrecht & Puff (1986), based on a morphological comparison of numerous genera of the "Gardenieae-Ixoreae" complex, recognized next to these Pavetteae s.str. with predominantly terminal inflorescences two other higher taxa with axillary inflorescences: a small tribe Coffeae (*Coffea* and *Psilanthus* only), and a subtribe Diplosporinae in the Gardenieae; these two are discussed below. Andreasen & Bremer's (2000) combined analysis of a broad sampling of Ixoridinae genera brought to light that in fact two clades should be recognized, the Ixoreae in a very restricted sense (*Ixora* and a few close relatives), and a narrowed concept of the Pavetteae. The supertree confirms their findings, placing the Ixoreae s.str. (*Scyphiphora* to *Aleisanthiopsis*; fig. 4A) in the Vanguerieae alliance and the Pavetteae s.str. (clade *Pavetta* to *Enterospermum*; fig. 4A) in the Gardenieae alliance – we give a further discussion of the tribe Pavetteae below, under the Gardenieae alliance. These circumscriptions are largely congruent with two informal groups morphologically recognized in the Pavetteae s.lat., viz. a group of genera related to *Ixora* and another to *Tarenna* (see Robbrecht & Puff 1986: 122).

Rova & al. (2002; *trnL-F*) obtained similar results; their clade I2 (op.cit.: fig. 4) includes Vanguerieae and Ixoreae s.str. in a sister relationship, and the tribe Pavetteae s.str. placed in another clade. Three tropical Asian genera, *Greenea*, *Aleisanthiopsis* and *Aleisanthia*, further called the '**Greenea group**', sequenced by them for the first time, were part of the Ixoreae clade. These three genera previously had a biogeographically odd position in the almost exclusively neotropical Rondeletieae. Recent work on these genera (Tange 1997a, b; taxonomic revisions, morphology and anatomy) preceded these molecular findings and discussed relationships in the framework of Rondeletieae only. However, Tange's documentation reveals similarities to the core Ixoridinae (resemblance to *Bertiera* with regard to habit; seed-coat anatomy as in Gardenieae) but not to *Ixora* in particular. The differences between *Ixora* and the '*Greenea group*' are profound, inter alia in fruit type (fleshy fruits with uniovular locules versus many-seeded capsules), but the supertree does not distinguish it as a clade. More inclusive molecular sampling and deep morphological work have to be undertaken to see whether the *Greenea group* needs to be distinguished.

<sup>3</sup> As the genus *Heinsenia*. At that moment Verdcourt overlooked the very close relationship between *Aulacocalyx* and *Heinsenia*; the latter was sometimes reduced to a subgenus or even a synonym of the former (see Robbrecht & Puff 1986: 124).

The mangrove genus *Scyphiphora*, appearing in this alliance on the supertree, needs to be commented in detail. Puff & Rohrhofer (1994) surveyed the history of the disputed placement of this genus and provided a thorough morphological and anatomical documentation, concluding that it should be placed in or near the Gardenieae-Diplosporinae. In a combined analysis of morphological, nuclear and plastid data by Andreasen & Bremer (2000), *Scyphiphora* was the sister taxon to *Ixora* and allies. These authors stressed that this relationship – never before suggested in the genus' intricate taxonomic history – conflicted with their *rbcL* results where *Scyphiphora* was sister to the Vanguerieae genera *Vangueria* and *Canthium*. On the supertree also, where the position is derived from the *rbcL* source tree, *Scyphiphora* is sister to the rest of the Ixoreae as in the combined analysis of these authors. The incongruence between our and these author's *rbcL* results may be due to our better sampling (four genera) of the Vanguerieae. In the Taxonomic conspectus, we maintain *Scyphiphora* in the Ixoreae.

A deep morphological study of the newly widened Ixoreae, i.e. comprising *Ixora* and close allies plus the 'Greenea group' and *Scyphiphora*, is much needed.

**Table 2. Chronological survey of tribal circumscriptions once comprised by the Ixoreae in their widest sense (s.latiss.), i.e. as conceived by Bremekamp.**

Schumann 1891	<b>Ixoreae s.lat.</b> , incl. only 1-ovulate genera: <i>Coffea</i> , <i>Psilanthus</i> , <i>Ixora</i> , <i>Pavetta</i> , <i>Rutidea</i> , <i>Myonima</i> & cet.		
Bremekamp 1934	<b>Ixoreae s.latiss.</b> , widened to also include pluri-ovulate elements from Gardenieae sensu Schumann: + <i>Tarenna</i> , <i>Tricalysia</i> & cet.		
Darwin 1976	previous concepts include <i>Coffea</i> ; correct name <b>Coffeae (s.latiss.)</b>		
Robbrecht 1984 Robbrecht & Bridson 1985 Robbrecht & Puff 1986	<b>Pavetteae s.lat.</b>		<b>Gardenieae - Diplosporinae</b> <i>Tricalysia</i> & cet.
	<i>Tarenna</i> group	<i>Ixora</i> group	<b>Coffeae s.str.</b> only <i>Coffea</i> & <i>Psilanthus</i>
Andreasen & Bremer 2000	<b>Pavetteae s.str.</b>	<b>Ixoreae s.str.</b>	<b>Coffeae s.lat.</b>
Rova & al. 2002	idem	idem but <i>Greenea</i> group added	idem

(c) The tribe **Vanguerieae** is one of the easiest of the Rubiaceae to define morphologically and its delimitation has hardly caused any trouble in the past, the sole major problem being the former inclusion of *Collettoecema* (see (2.1); now basal Rubioideae). The supertree (clade *Vangueria* to *Meyna*; fig. 4A) fully confirms the tribe's delimitation, but erroneously places *Psydrax* at the base of the Ixoroideae (see comment above). The main taxonomic problems in the Vanguerieae are at the generic level, and much sequencing work has been undertaken recently to clarify the delimitation of the genera (Lantz & al. 2002, Lantz & Bremer 2004, 2005). These works all confirmed the monophyly of the tribe. The inferred results include e.g. the widening of *Vangueria* and the recognition of *Canthium* subg. *Afrocanthium* at the generic level, matters that are not at the issue here.

**(1.2.3.4) The Gardenieae alliance** contains four tribes, the Gardenieae, Coffeae s.lat., Pavetteae s.str. and Octotropideae.

(a) The tribal name **Coffeae** has been used in widely different circumscriptions (see table 2; literature references therein). Its widest delimitation corresponds to the classical concept of Ixoreae

(Schumann) or its modified circumscription (Bremekamp); it is the correct name for these two concepts because they comprise the genus *Coffea* (Darwin 1976). Robbrecht and coauthors narrowed the tribe Coffeae to the genus pair *Coffea* and *Psilanthus* alone, but the molecular investigations of Andreasen & Bremer (2000) proposed to merge the Gardenieae with axillary inflorescences paired at the nodes (the subtribe Diplosporinae) with the Coffeae.

Andreasen's concept of the Coffeae corresponds to the clade *Bertiera* to *Psilanthus* in fig. 4A. The addition of *Belonophora* to this alliance is novel, however, and derived from our spacer source tree. Another investigation (Davis & al. 2006; four plastid regions) of the phylogeny and circumscription of the Coffeae has been carried out at the same time as the analyses here reported; they added other former Diplosporinae to their sampling, viz. *Argocoffeopsis*, *Calycosiphonia*, *Discospermum*, *Sericanthe*, as well as *Xantonnea*, formerly in Octotropideae, and found that also these genera belong to the Coffeae in present-day sense.

The supertree contradicts the molecular findings of other authors (Andreasen & Bremer 2000; Davis & al. 2006) with regard to *Diplospora* and *Calycosiphonia*, which are placed in Gardenieae 4. Even morphologically this result is surprising: with their axillary inflorescences paired at the nodes and bracteoles fused to calyculi, the genera have always been associated with *Tricalysia* and its relatives (see Robbrecht & Puff 1986). The two genera are obviously in need of further investigation to clarify the different results.

***Bertiera*** is a genus with a long disputed taxonomic history. It was placed in four different tribes in publications between 1830 and 1976, and more modern treatments kept it among the genera of uncertain position. References to this history were given in a surveying table by Robbrecht & al. (1994: table 3). These authors documented the morphology and anatomy of *Bertiera*, concluding from this evidence that it is "an in many respects derived element of isolated position" belonging to the "very diverse subtribe Gardeniinae."

Andreasen & Bremer (1996, 2000) were the first to suggest the here confirmed position of *Bertiera* in the Coffeae. The genus is sister to the rest of the group in their analyses, hence was found by them in a position comparable to that on the supertree, where *Bertiera* is sister to the rest of the Coffeae.

Davis & al. (2006), using two more plastid regions, also found *Bertiera* in a sister position to the core Coffeae. Stressing a morphological difference [inflorescences generally terminal in *Bertiera* versus axillary and paired at nodes (rarely axillary plus terminal) in the core Coffeae], they followed Bridson & Verdcourt (2003) in accepting a monogeneric tribe Bertiereae.

*Bertiera* differs from the core Coffeae in having terminal inflorescences (apart from three peculiar *Bertiera* species; see Robbrecht & al. 1994). We agree with Bridson & Verdcourt (2003: 386) and Davis & al. (2006) that it is useful to distinguish the Bertiereae, but prefer to do so at the subtribal level, thus emphasizing the close relationship with the core Coffeae. Further deep morphological work is needed to provide a better comparison between Coffeinae and Bertierinae.

(b) The birth of a narrow concept for the tribe **Pavetteae** is discussed under Ixoreae above (see table 2). The clade *Pavetta* to *Enterospermum* in fig. 4A corresponds to it. It contains *Rutidea*, the sole element of our sampling that Bridson & Robbrecht (1985) placed in the 'group of genera related to *Ixora*' (= Ixoreae s.str.); the sequencing data indicate that it rather belongs to Pavetteae s.str. *Rutidea* and its close relative *Nichallea* (which was not included in the molecular sampling) are morphologically so derived (one-seeded fruits) that it was not easy indeed to assign them to the groundplan of the Ixoreae or Pavetteae, respectively. The recent reinstatement of *Coptosperma*

(segregated from *Tarenna*; De Block & al. 2002<sup>4</sup>) has, however, provided morphological links between the multiovulate majority of the Pavetteae s.str. and the uniovulate *Rutidea*.

(c) The **Octotropideae** are a tribe set apart from the once widely conceived Gardenieae. This segregation was based on morphology and anatomy, first under the name ‘Hypobathreae’ (Robbrecht 1980; Robbrecht & Puff 1986: 127). A later discovery, demonstrating that the ill-known Indian genus *Octotropis* (previously Alberteae) also belongs here, had the nomenclatural implication that the tribal name Octotropideae had to be reinstated (Robbrecht & al. 1994). Andreasen & Bremer (1996, 2000) delivered DNA data confirming the tribe. Of the thirty genera six are on the supertree. The clade *Pouchetia* to *Ramosmania* (fig. 4A) perfectly matches the earliest delimitation of this tribe, and also includes morphologically derived elements such as *Kraussia* and *Polysphaeria*.

There is good morphological and anatomical evidence (Tirvengadam & Robbrecht 1985) that *Paragenipa*, a genus endemic to the Seychelles, belongs to the Octotropideae, and this relationship is confirmed by *rbcL* sequence data (Andreasen & Bremer 2000; they erroneously cited the voucher as ‘*P. lanceolata*’ for *P. lancifolia*). The same voucher specimen has been sequenced for *trnL-F* (Rova & al. 2002), and these data resulted in a clade *Paragenipa* + *Didymosalpinx*. These analyses are congruent with our *trnL-F* source tree and responsible for the position of *Paragenipa* on the supertree, viz. in our ‘Gardenieae 1.’ *Paragenipa* is also on our *rps16* source tree, in a clade with *Pavetta*. However, these two source trees are poorly sampled for the Octotropideae (in fact only include *Paragenipa*) and for the Ixoridinae in general. Andreasen & Bremer’s study (op. cit.) focused on the Ixoridinae and included a much wider sampling of that group. All their analyses resulted in a placement of *Paragenipa* in the Octotropideae, and we have maintained the genus in that position despite the supertree’s different placement.

Andreasen & Bremer (2000; combined analysis of morphology, cpDNA restriction sites, *rbcL* and *ITS*) found that *Cremaspora* is sister to the Octotropideae, a relationship that is repeated on our supertree. This is corroborated by other data (*rps16* and *trnL-F*) analysed by Persson (2000): he found the genus to be sister to *Paragenipa*, the sole representative of the Octotropideae included in his analysis. The genus’ position has a complex history (see Robbrecht & Puff 1986: 118). *Cremaspora* is morphologically derived, but sufficiently matches the Octotropideae to be included in that tribe. The genus’ most surprising feature is no doubt the combination of a pendulous ovule with an inferior embryo radicle, a fact already stressed by Baillon (1879a). An in depth investigation of the gynoeceum and seed development of *Cremaspora* is needed for a correct understanding of this most unusual character combination. The probable autapomorphies behind it appear, however, not sufficient to recognize a monogeneric tribe Cremasporeae as proposed by Andreasen & Bremer.

(d) The **Gardenieae** are among the first Rubiaceae tribes recognized (Hooker 1873, Schumann 1891). Robbrecht & Puff (1986) have proposed a very narrowed delimitation of that concept, as Gardenieae subtribe Gardeniinae. The latter has largely been confirmed by molecular studies (Andreasen & Bremer 2000), apart from minor differences such as the transfer of *Duperrea*, *Aorantho* and *Heinsenia* to the Gardenieae. On the supertree (fig. 4A), however, the tribe does not appear monophyletic: it falls apart in at least two or possibly four groups, designated Gardenieae 1

<sup>4</sup> Note that this paper reduced *Enterospermum* to a synonym of *Coptosperma*. The name *Enterospermum* on the tree refers to the voucher *E. coriaceum* (*rbcL* source tree), the generic position of which cannot be clarified as long as all the species of the complex are not yet revised (De Block, pers. comm.).

(clade *Burchellia* - *Schumanniohyton*), Gardenieae 2 (clade *Duroia* - *Kailarsenia*), Gardenieae 3 (clade *Euclinia* - *Rosenbergiodendron*) and Gardenieae 4 (clade *Atractocarpus* - *Morelia*). Andreasen & Bremer's (op. cit.: fig. 5) consensus tree for their combined analysis confirmed monophyly of Gardenieae, as already stated, but divided the tribe in ten clades (two of them monogeneric) for which the mutual position remained unresolved.

**Gardenieae 1** (the afrotropical genera *Burchellia*, *Schumanniohyton* and *Didymosalpinx*) corresponds to one of the ten Gardenieae subclades of Andreasen & Bremer (2000: fig. 5; the same three genera); on the supertree it falls totally apart, grouping with the above mentioned other elements of the Ixoridinae II: (Gar.1(Cof.(Pav.,Oct.))). These three genera are morphologically isolated, *Didymosalpinx* e.g. in having axillary inflorescences paired at the nodes. They should probably better be removed from the Gardenieae. In a combined analysis of *rps16* and *trnL-F* data (Persson 2000), the three genera also fall outside the two main groups of this author, core Gardenieae and *Alibertia* group.

The remaining Gardenieae are monophyletic, according to our supertree, if *Wendlandia* and *Lindenia* are included. The supertree postulates indeed a relationship ((*Wendlandia* + *Lindenia*, Gar.2) (Gar.3,Gar.4)).

*Wendlandia* is a predominantly tropical Asian genus hitherto always placed in Rondeletieae on account of the capsular fruits with numerous weakly winged seeds. Its position in that predominantly neotropical tribe has become even more odd, after the recent exclusion of the group of *Greenea* (see above under Ixoreae).

That *Lindenia* is the sister genus of *Wendlandia*, is a fact that needs discussion in the light of the position of *Augusta* in Gardenieae 1. *Lindenia* was reduced to a subgenus of *Augusta* on morphological grounds (Kirkbride 1997). Delprete (1997) followed him and subsequently (Delprete 1999) placed the emended genus in a group of its own in the Rondeletieae. While molecular data (Rova & al. 2002) corroborated the synonymy, the position of *Augusta* in Rondeletieae was not confirmed; the genus pair *Wendlandia/Augusta* was found to have a sister relationship to what we call here the Gardenieae alliance, i.e. in the Ixoridinae, while the Rondeletieae belong to the Cinchonidinae. Our supertree contains the same hypothesis, although the two subgenera of *Augusta* fall apart. *Lindenia* appears as the sister to *Wendlandia* on the *trnL-F* source tree, while *Augusta* is sister to the largest clade of Ixoridinae II on the spacer source tree (clade *Ixora* to *Morelia* in fig. 3). This explains the supertree position of *Augusta*, basal to the clade *Retiniphyllum* - *Morelia*. However, *Wendlandia* does occur on the spacer source tree, where it comes out with *Gardenia* and not with *Augusta*. Consequently the molecular results seem to contradict the reduction of *Lindenia* to synonymy of *Augusta*. Our classification (see Taxonomic conspectus) places *Wendlandia* and the two subgenera of *Augusta* in a provisional group (W.-A.) in the Gardenieae alliance.

The possible transfer of the paleotropical genus *Wendlandia* from the neotropical Rondeletieae to the pantropical Gardenieae is biogeographically plausible. Whether a possible sister relationship between the genus and *Augusta/Lindenia* is also morphologically defensible should be investigated, because they hitherto were never the subject of a detailed morphological and anatomical study.

The remainder of the Gardenieae contains three major clades on the supertree, here numbered 2, 3 and 4. They are only partly congruent with the clades detected in the combined analyses of Andreasen & Bremer (2000) and Persson (2000). Andreasen & Bremer distinguished ten subclades which remained unresolved against one another, two of them monogeneric, *Schumanniohyton* (discussed in Gardenieae 1 above) and *Duperrea*. Persson distinguished an *Alibertia* group next to core Gardenieae, and inside the latter a *Gardenia* clade and a *Randia* clade. The three clades on the

supertree and the clades postulated by these authors show only partial congruence. **Gardenieae 2** on the supertree (clade *Duroia* - *Kailarsenia*) contains three of the ten subclades of Andreasen & Bremer (op. cit.: fig. 5; clade with *Gardenia*, *Genipa*, *Kailarsenia*, clade with the *Alibertia* group, and clade with *Porterandia* and *Sukunia*). Gardenieae 2 seems to support the monophyly of the “*Alibertia* group” proposed by Persson (2000); it is the only one of our Gardenieae clades that comprises elements of that group, viz. the neotropical genera *Duroia*, *Alibertia* and *Glossostipula*.

**Gardenieae 3** (supertree clade *Euclinia* to *Rosenbergiodendron*) and **Gardenieae 4** (supertree clade *Atractocarpus* to *Morelia*) are less congruent with the results of Andreasen & Bremer and Persson and partly rearrange the genera of their clades. This part of the supertree nevertheless corroborates the proposed transfer of *Aorranthe* and *Duperrea* to the Gardenieae. Comments on the inclusion of *Diplospora* and *Calycosiphonia* in Gardenieae 4 are given above [see (1.2.3.4)(a)].

Several mainly paleotropical genera of the Gardenieae possess pollen in tetrads, a feature so unusual in Rubiaceae that Robbrecht & Puff (1986: 133) suggested that they may form a natural group. The dispersal of these genera over Gardenieae 2, 3 and 4 (and over four of the ten clades found by Andreasen & Bremer plus over the groups distinguished by Persson) makes evident that this is not the case.

In conclusion, Gardenieae appear to be paraphyletic, but the exclusion of a few elements (Gardenieae 1 clade) would make it monophyletic. The presently available molecular data comprise a number of contradictory findings. This very large tribe (one of the largest of the family, with more than fifty genera) contains many genera for which sequencing data are not yet available. A better sampling and a “deep morphological” interpretation should be undertaken for a further elucidation of the tribe’s phylogeny.

**(1.3) The Cinchonidinae** are an almost exclusively neotropical supertribe, but the predominantly paleotropical Naucleaeae are sister to the core of the supertribe, which contains two major clades, designated here as Cinchonidinae I and II (see summary fig. 5 & 6).

Our Cinchonidinae correspond to the five ‘Cinchonoideae clades’ (C1, C2 etc.) of Rova & al. (2002); their cladogram (op. cit.: fig. 1) supported the same monophyletic group but was very poorly resolved at the base: (C1,C2,*Isertia*,C3,(C4,*Strumpfia*),(C5a,C5b)). In terms of tribes this reads (Cin.,Ham.,*Isertia*,Nau.,(CCE,*Strumpfia*),(Gue.,Ron.)). The supertree (summary fig. 6) with a much wider sampling has the same groups completely resolved at the base.

### **(1.3.1) Basal Cinchonidinae: Hymenodictyoneae and Naucleaeae**

With their perfectly globular inflorescence heads, Naucleaeae are among the easiest representatives to spot as a group within Rubiaceae; the feature accounts for their familial segregation as Naucleaceae. The gross morphological resemblance between capitulate and other Rubiaceae is, however, so striking that the segregate family was followed by very few botanists only (see Robbrecht 1994a).

When Ridsdale (1975, 1978) undertook a world revision of the group, he followed the only researcher ever arguing that the Naucleaeae in their classical delimitation are not a natural group, and, as Bremekamp (1966), he removed *Uncaria* and *Mitragyna* to a subtribe of the Cinchoneae, the Mitragyninae. He divided the remaining Naucleaeae in three subtribes, Adininae (with three subgroups), Naucleinae and Anthocephalinae, and accepted a monogeneric tribe Cephalantheae next to it. The ‘Mitragyninae’ were later – when Andersson & Persson (1991) adopted the name *Coptosapelteae* for that alliance – widened to a tribe of its own comprehending many genera with non-capitulate inflorescences, e.g. *Hymenodictyon* and *Pausinystalia*. This tribal name has been established before (Bremekamp 1954, 1966) for only two genera, the name-bringing one and

*Crossopteryx*. When the first molecular phylogenies for the Naucleaeae in the old sense were provided (Razafimandimbison & Bremer 2002a, b), the monophyly of the group in its old wide sense was proven. At the same time the results confirmed the need for subdivision and indicated the necessity to also include a number of genera with non-capitulate inflorescences, e.g. *Corynanthe* and *Pausinystalia*. These authors first (op. cit. 2002a; ITS & *rbcL*) showed that *Hymenodictyon* and *Paracorynanthe* are sister to the rest of the alliance, and recognized a tribe Hymenodictyoneae for the genus pair. In their second contribution (op. cit. 2002b) they added *trnT-F* data, which resulted in a better resolution of the core Naucleaeae in their phylogenetic trees. The clades resulting from their analysis were found to be largely congruent with the morphological groups of Ridsdale, which allowed the authors to take up his Naucleinae (including the monogeneric Anthocephalinae, however), and his Adininae (restricted to the second and third group of Ridsdale). For Ridsdale's first group of the Adininae, they recognized a separate subtribe Breoniinae. The elements formerly belonging to Coptosapelteae s.lat. were placed in three other subtribes, Mitragyninae, Corynantheinae and Uncariinae. The monogeneric Cephalantheae were reduced to a subtribe of the Naucleaeae.

In our supertree (clade *Hymenodictyon* to *Uncaria rhynchophylla*; fig. 4B) and its source trees, the Naucleaeae s.lat. are not very well sampled. Our results confirm the monophyly of the alliance; in our sampling, *Hymenodictyon* is represented by two species that do not form a monophyletic group. Our classification in the Taxonomic conspectus adopts the results and classification of Razafimandimbison & Bremer. We have accepted their separate small tribe Hymenodictyoneae next to their resurrection of an enlarged tribe Naucleaeae, although a reduction of the first to a subtribe of the second is equally acceptable. We followed the division of the core of the former Coptosapelteae s.lat. in the subtribes Mitragyninae (head-like inflorescences) and Corynantheinae (branched inflorescences), although the two are morphologically very close (see Stoffelen & al. 1996) and do form a monophyletic group together. We did not follow the subtribal segregation for the genus *Uncaria* alone; it is better referred to the Naucleinae, where it is sister to the rest of the subtribe.

Several elements of the Coptosapelteae were already stated to be morphologically aberrant (Robbrecht 1994b: 175), inter alia *Coptosapelta* and *Crossopteryx*. Molecular results have now shown that these two and *Luculia* belong elsewhere (see (1.1) Coptosapelteae and (1.2.3) Alberteae).

### (1.3.2) Cinchonidinae I

The first clade of the core Cinchonidinae is here designated as Cinchonidinae I. It corresponds to the complex of the tribes **Rondeletieae** and **Guettardeae** (*Blepharidium guatemalensis* to *Guettarda odorata* on the supertree; fig. 3B). This alliance mainly comprises Delprete's (1999) 'Rondeletia complex' (on the supertree represented by *Rondeletia*, *Rogiera*, *Javorkaea* and *Arachnothryx*) and *Mazaea* Group (on the supertree represented by *Acrosynanthus*, *Mazaea*, *Ariadne* and *Phyllomelia*) of the Rondeletieae, which thus should be defined in a quite strict sense. In that delimitation, however, the tribe is paraphyletic because the Guettardeae are deeply nested within them. The reiteration of the Guettardeae on the tree is due to the technique of supertree construction.

The clade also comprises *Blepharidium*, recently placed in the Hillieae (Andersson 1995), the genus *Rachicallis*, a member of the ill defined Hedyotideae, and *Gonzalagunia*. The latter neotropical genus has always been considered as a member of the Isertieae or Mussaendeae; it was excluded from that tribe on morphological and pollen evidence (Andersson 1996: 158) to an uncertain position ("possibly related to the Condamineae-Rondeletieae-Sipaneeae Complex").

An in depth phylogenetic investigation of the complex was undertaken by Rova & al. (2002). Our Cinchonidinae I correspond to their clade C5, which fell apart in two well supported clades in

their study. Their clade C5b corresponds to the clade *Blepharidium guatemalensis* to *Rondeletia portoricensis* in fig. 4B, and exclusively comprises members of the Rondeletieae, including its type genus. It should be investigated morphologically whether this clade could form the base for a new and very narrow concept of the Rondeletieae. Rova & al.'s clade 5a is congruent with the second clade of Cinchonidinae on the supertree (*Antirhea lucida* to *Guettarda odorata*). It comprises a mixture of Guettardeae and other members of the Rondeletieae and advocates for an emendation of the Guettardeae.

For a more extensive discussion of the complex, we refer to Rova & al. (2002). Molecular results have altogether much narrowed the Rondeletieae to comprise now exclusively neotropical representatives [see transfer of the paleotropical elements above: the *Greenea* group to Ixoreae (1.2.3.3.b) and the genus *Wendlandia* to the Gardenieae alliance (1.2.3.4.d)].

### (1.3.3) Cinchonidinae II

Three clades may be distinguished in the second clade of the core Cinchonidinae:

(a) The **Cinchoneae**, represented on the supertree (fig. 4B) by *Cinchona*, *Ladenbergia* and *Remijia*. To the same alliance belongs the neotropical *Isertia*, the name-bringing genus of the tribe **Isertieae**. A few decades ago, this tribal name covered a very wide and pantropical concept (see e.g. Garcia Kirkbride 1979; Robbrecht 1988, 26 genera in the tribe). It corresponded to the Mussaendeae of other authors, the tribal name Isertieae having priority (Darwin 1976). For a survey of recent split offs and the narrowing of the Isertieae, see Andersson (1996). In their strict present-day sense, the Isertieae are neotropical and limited to *Isertia* and its close ally *Kerianthera* (Delprete 1996b). If these two genera were now included in the Cinchoneae, as suggested by the supertree, that latter tribal name (1807) has priority over Isertieae (1830; Darwin 1976).

Andersson & Antonelli (2005) reexamined the phylogeny of this alliance based on five loci (*ITS*, *matK*, *rbcL*, *rps16* and *trnL-F*) and using a somewhat wider sampling than the one here (see Taxonomic conspectus). Their results are congruent with the supertree; however, their more detailed analysis indicates that *Isertia* + *Kerianthera* are sister to the rest of this alliance. It is therefore plausible to recognize here one tribe with two subtribes, Cinchoninae and Isertiinae. These two taxa are much narrower than the once very widely conceived tribes Cinchoneae (see Andersson 1995) and Isertieae (as discussed above).

(b) The **Hamelieae** and **Hillieae**, represented by the clade *Hillia macrophylla* to *Deppea blume-naviensis* on the supertree (fig. 4B). In this clade the Hamelieae (*Hamelia*, *Deppea*, *Schenckia* and *Hoffmannia* on the supertree) mix up with elements of the Hillieae as recently emended [viz. the genera *Hillia*, its synonym *Ravnia* (*Hillia* subg. *Ravnia*) and *Cosmibuena* on the supertree]. The clade also comprises a species of *Chione*<sup>5</sup> and *Cosmocalyx*<sup>6</sup>, whose relationship was recently disputed; further comments on these two genera are given below.

<sup>5</sup> *Chione* was excluded from the Chiococceae by Bremer (1992) but reinserted in that tribe a few years later (Delprete 1996a: 186, 188). Rova & al. (2002; *trnL-F*) have, however, corroborated the genus' belonging to the Hamelieae-Hillieae complex. The position of *Chione* in the Hamelieae on the supertree is based indeed on the *trnL-F* source tree. Pollen morphology too confirms the exclusion from Chiococceae (Huysmans pers. comm. in Jansen 2001: 128). The genus differs from all the representatives of the complex by its solitary pendulous ovules; it is much in need of an in-depth investigation.

<sup>6</sup> The genus *Cosmocalyx* was of uncertain position in the Rubiaceae from its first description onwards (see Bridson & Robbrecht 1985: 104). The genus was recently transferred to the Hamelieae (Delprete 1998; morphology), where it is the sole representative having solitary erect ovules. The position of *Cosmocalyx* in the Hamelieae on the supertree is a novel molecular corroboration and goes back to the spacer source tree.

The neotropical tribes Hillieae and Hamelieae each possess rather unusual combinations of characters and their position consequently often changed. The neotropical epiphytic *Hillia* unusually combines contorted corolla aestivation and raphides; classical authors placed the genus within Cinchoneae on account of its capsules with numerous comose seeds, which compare somewhat with the often bipolar, winged seeds of that alliance (see Robbrecht 1988: 161) but are in fact unique in Rubiaceae (see Puff & Buchner 1998a). Bremekamp (1952, 1966) turned the problem by accepting a monogeneric higher taxon, first a tribe Hillieae and later even a subfamily Hillioideae. Hallé (1967) emphasized the contorted corolla aestivation and transferred *Hillia* to the Gardenieae. Taylor (1989, 1994) was the first to suggest genuine relatives for *Hillia*, viz. the other neotropical genera *Ravnia* (which she reduced to a synonym of *Hillia*), *Cosmibuena* and *Balmea*. She was here-in followed by Andersson (1995) who formally emended the Hillieae after a cladistic analysis of morphological features.

A detailed taxonomic history of the Hamelieae was given by Delprete (1998). The tribe was placed in the Rubioideae by Bremekamp (1952) because of its predominantly herbaceous habit and presence of raphides, and has since mostly been considered in that framework. As a result, Bremer (1987; cladistic analysis of morphological features) concluded that the tribe is the sister group of the paleotropical Argostemmatae. The exclusion of Hamelieae from Rubioideae and its belonging to the Cinchonidinae was for the first time suggested by Bremer & Jansen (1991), and the presently recognized association of Hamelieae and Hillieae by Bremer & al. (1995); their *rbcL* data united *Hillia*, *Hamelia*, *Hoffmannia* and *Deppea* in a single clade. The close relationship has been confirmed by several later studies (e.g. Manen & Natali 1996, Bremer & Manen 2000, Rova & al. 2002). In our classification (see Taxonomic conspectus), we have reduced the Hillieae (1976) to synonymy of the Hamelieae (1830), the latter tribal name having priority (dates given here according to Darwin 1976).

### (c) The “CCE complex”

The classical systems of the Rubiaceae as well as Robbrecht's (1988) survey did not consider a close relationship between the tribes Condamineae and Chiococceae. Bremer (1992; cpDNA restriction site analysis) proved a close relationship between the latter and the subtribe Portlandiinae of the former and emended the Chiococceae to include *Portlandia* and its associates. Delprete (1996a) reevaluated the group based on morphological characters; following Bremer & al. (1995) he brought it also in relation with the till then uncertainly placed tribe Catesbaeeae. He distinguished three groups in the alliance, a strongly enlarged tribe Catesbaeeae (with two informal categories, a ‘*Portlandia* group’ and a ‘*Catesbaea* group’), a tribe Chiococceae, and an informal *Exostema* group associated with these two tribes. Morphologically the complex is characterized by a monadelphous androecium, with hairy filaments fused at their very base. A pollen morphological investigation (Huysmans & al. 1999) of the Catesbaeeae-Chiococceae-*Exostema*-complex (‘CCE complex’) supported its monophyletic nature; the echinate perforate sexine ornamentation is another synapomorphy of the entire group (see also Dessein & al. 2005). The monophyly of the complex was also corroborated by more molecular studies; in Rova & al. (2002) the CCE complex corresponds to their clade C4. Motley & al. (2005; they rebaptized the alliance as CCC) examined the generic relationships within the complex with a broader sampling and reconfirmed its monophyly.

The supertree confirms the monophyly of the CCE complex (fig. 4B: clade *Exostema caribaeum* to *Asemnanthe*; with the genus *Strumpfia* in a sister relationship to the rest of the alliance). Inside it, the monophyly of the Chiococceae s.str. (clade *Eosanthe* to *Asemnanthe*; including

*Phialanthus*<sup>7</sup>) is equally confirmed. The other elements appear as a grade (*Strumpfia* to *Isidorea*) wherein only the monophyly of the *Catesbaea* group (clade *Catesbaea* - *Phyllacanthus*) is confirmed.

The Chiococceae are here confirmed in the tribe's traditional delimitation, but also include, according to the supertree, the extra-neotropical members *Badusa* (Philippines to Fiji and Tonga) and *Bikkia* (E Malesia to Micronesia, New Caledonia and the Pacific Islands). These two genera were formerly included in the Condamineae and differ morphologically from the rest of the Chiococceae in having multi-ovulate placentas. *Badusa* was transferred to the 'Exostema group' by Delprete (1996a: 186), i.e. to a position closer to the one here suggested than the original assignment to Condamineae. The supertree confirms Bremer's (1992) cpDNA results; she included *Bikkia* and *Badusa* in the Chiococceae s.lat. without further comments. Modern revisions (Jérémié & Hallé 1976; Ridsdale 1982) confirm that these two genera have the filaments fused at the base, a character shared with the other Chiococceae.

One paraphyletic group of the supertree (*Exostema caribaeum* to *Isidorea pungens*) is a perfect match for the Catesbaeae sensu Delprete (1996a) plus his 'Exostema group.' His 'Catesbaea group' (*Catesbaea* and *Phyllacanthus*) is deeply nested in this grade. Apart from *Exostema*, all the other taxa (*Coutaportia*, *Coutarea*, *Cubanola*, *Portlandia* and *Isidorea*) appertain to Delprete's *Portlandia* group.

*Schmidtottia* was formerly placed in the Portlandiinae, and retained in the *Portlandia* group by Delprete (1996a), but it falls in the Chi.s.str. on the supertree. This result based on the *trnL-F* source tree was already obtained by Rova & al. (2002) and confirms Bremer's (1992) cpDNA results. Delprete has not given any comments on his decision to return the genus to its original position.

Uncertainty about the affinity of the Caribbean monospecific *Strumpfia* goes far back in taxonomic history, and attempts to find the genus' relatives based on morphology failed to formulate a hypothesis. Bridson & Robbrecht's (1985) morphological and pollen morphological investigation only allowed excluding the genus from the Pavetteae (the then concept for Ixo. + Pav.). Igersheim (1993) provided a detailed morphological-anatomical documentation, but he also could only conclude that relatives were "expected to be 'hidden' amongst neotropical Rubiaceae." A molecular investigation (Rova & al. 2002) finally allowed concluding that the relatives are to be sought in or near the Chiococceae, and that *Strumpfia* is sister to the CCE complex. This is corroborated by the supertree results. Motley & al. (2005: 323) stressed that their analyses support the formerly found sister position of the genus to the rest of the complex, but their statement is contradicted by their three cladograms where *Strumpfia* is in an unresolved position at the base.

The pollen of *Strumpfia* (Bridson & Robbrecht op. cit.: fig. 11; Igersheim op. cit.: fig. 11) has a perforated exine without suprategal elements, unlike all other members of the CCE complex. Also this points to an isolated position within the complex.

<sup>7</sup> The Caribbean genus *Phialanthus*, counting some twenty species, needs further comments. In the classical systems it was housed in the Chiococceae. Bremer (1992) excluded it from that tribe in the framework of the above mentioned paper reconsidering the delimitation of the Chiococceae based on cpDNA restriction site analysis. *Phialanthus* was not investigated molecularly, but excluded based on her observation of the absence of the monadelphous androecium. Delprete (1996a) returned *Phialanthus* to the Chiococceae, without comment; the data matrix for his cladistic analysis of morphological features in the CCE complex did not contain representatives of the genus, however, and he probably simply relied on the genus' placentation for his decision. Modern documentation of this Caribbean genus is entirely lacking. We have confirmed the solitary pendulous ovules in *P. resinifluus* (ER pers. obs.) in young fruits but had no flowers available for an examination of the androecium. This is in line with molecular findings: Rova & al. (2002) postulated that the genus should be maintained in the Chiococceae; the supertree position too confirms the position in Chi. s.str.

As a result, the present data suggest to recognize only one tribe for the complex, Catesbaeae s.lat. or Chiococceae s.lat. (the two names are from the same date), which should probably also comprise *Strumpfia*. It is perhaps needed to recognize four subtribes (Portlandiinae, *Catesbaea* group, *Exostema* group, Chiococceae s.str.; only one subtribal name available). Uncertainty still exists, however, whether upon their recognition some of these four might turn out to be paraphyletic. For a more extensive discussion of the complex, we refer to the above mentioned study of Motley & al. (2005). These authors did not come up with a division of the complex, but their analyses also point to the need to recognize the *Exostema* group and the Chiococceae s.str. On the other hand, their results are not clear-cut with regard to a possible distinction between Catesbaeae s.str. and the *Portlandia* group.

**(2) The psychotria and madder subfamily** (Rubioidae; fig. 7) is characterized by the universal occurrence of raphides; aestivation is valvate and heterostyly very common. Chemically it is distinguished from the other subfamily by the occurrence of anthraquinones (Young & al. 1996). Aluminium accumulation is almost limited to this subfamily (Jansen & al. 2000a, b); it is limited to a number of representatives but occurs as well in core as in basal Rubioidae. All this evidence in support to the monophyly of the subfamily is corroborated by several molecular analyses.

The subfamily has a core, on the supertree (fig. 4C-D) clade *Pagamea* to *Spermacoce tenuior*, with two major alliances, (1) a predominantly woody one (supertribe Psychotriidinae; clade *Pagamea* to *Rudgea* in fig. 4C) with tribes related to Psychotrieae, and (2) a predominantly herbaceous alliance (supertribe Rubiidinae; clade *Danais* to *Spermacoce tenuior*) with tribes related to Rubieae and Spermacoceae. These two supertribes are sister to one another, and a number of other Rubioidae (grade *Colletocema* to *Hindsia* in fig. 4C) have a position basal to this core.

The subfamily already was subject to several global phylogenetic analyses, using the *rps16* intron (Andersson & Rova 1999 and a modification thereof: Piesschaert & al. 2000a), or a combination of data from *rbcL*, *atpB-rbcL* and *rps16* intron (Bremer & Manen 2000). The findings of all these authors are congruent with the present supertree. They also showed the existence of two major clades, our Psychotriidinae and Rubiidinae, with a grade of basal groups, viz. Ophiorrhizeae, Urophyllaeae, Lasiantheae and Coussareeae. However, the original *rps16* intron study (Andersson & Rova 1999) differs from all the following results and the supertree with regard to that basal grade. In this study it appeared as the monophyletic sister group of the other Rubioidae, so that the authors (op. cit.: 172) argued that the subfamilial name Urophyllloideae might be taken up for it. In the light of the presently available evidence this is no longer justified, since that subfamily should be regarded as paraphyletic.

In two of the papers mentioned (Andersson & Rova 1999, Bremer & Manen 2000), the subfamily Rubioidae and its clades have been discussed at length and we refer to them for extensive comments on previous literature. Our discussion below therefore concentrates on novel viewpoints and main groups.

### **(2.1) Basal clades of Rubioidae**

**(a)** According to the supertree, the African genus *Colletocema*, of which the sole species was originally described in '*Plectronia*' (Vanguerieae; see Petit 1963), is sister to the rest of the Rubioidae. The genus was not included in the analysis of Andersson & Rova (1999) and Bremer & Manen (2000), but was subsequently subject to a detailed morphological and anatomical study, interpreted in the light of another *rps16* intron analysis of the Rubioidae (Piesschaert & al. 2000a). Here the genus came out in a weakly supported basal clade with *Ophiorrhiza*. The authors demonstrat-

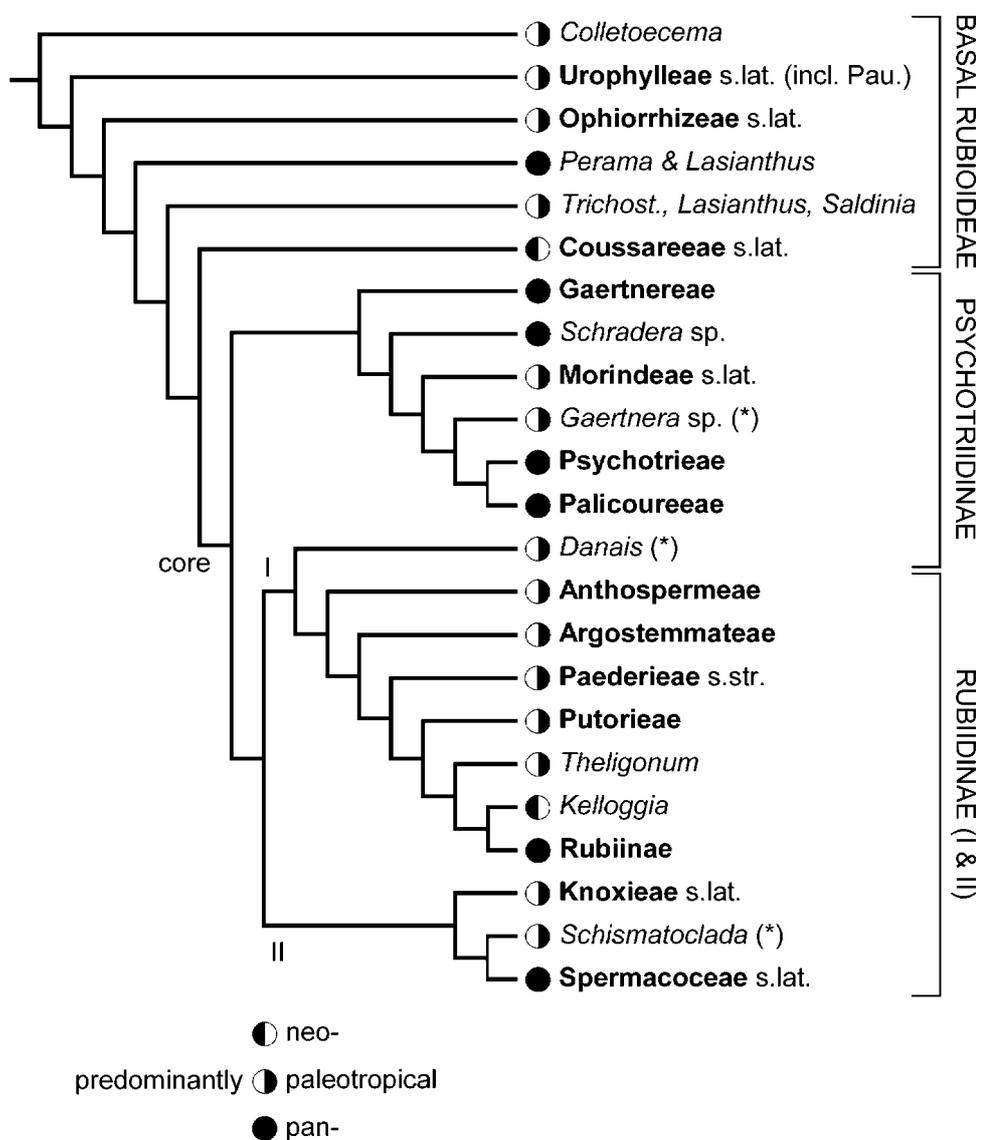


Figure 7. Supertree, schematic representation of structure for subfamily Rubioideae. The symbols before the groups indicate their pantropical versus (predominantly or exclusively) neo- or paleotropical distribution; the symbol pantropical is also used for the cosmopolitan distribution of Rubiinae. (\*) problematic positions discussed in text.

ed that *Colletoecema* exhibits a number of morphological and anatomical features that are unique or not elsewhere combined in the Rubioideae. The supertree (for *Colletoecema* based on our novel sequence of the spacer) places the genus in a sister position to all remaining Rubioideae and thus confirms this morphological isolation.

(b) A next basal clade of the Rubioideae are the **Urophyllaeae** (*Pravinaria* to *Praravinia* on the supertree; fig. 4C) from which Bremekamp (1966) once segregated the Pauridiantheae, on account of evidence from biogeography (tropical Asian versus afrotropical), morphology (plurilocular ovaries versus mostly bilocular ovaries) and flower biology (dioecism versus heterostyly). In depth morphological and anatomical work (Bangoura 1993, Buchner 1995) confirmed these differences and revealed that representatives of Pauridiantheae are diploid while the Urophyllaeae s.str. are polyploid. The supertree does not confirm a distinction between a tropical Asian clade (Urophyllaeae s.str.) and an afrotropical clade (Pauridiantheae). The other available molecular analyses (Andersson & Rova 1999, Bremer & Manen 2000) found no support for two clades. The recently proposed reduction of *Commitheca* to synonymy of *Pauridiantha* (Ntore & al. 2003) was not confirmed either, though the two are represented in the molecular trees. Interpretation of the available morphological data in the light of a more inclusive molecular sampling is obviously needed to reevaluate these and other questions.

The Urophyllaeae s.lat. are paleotropical; the molecular data (the cited works and the supertree) suggest to also include the neotropical genera *Amphidasya* and *Raritebe*<sup>8</sup>.

(c) Upon their first recognition, Bremekamp (1952: 22) included three genera in the Ophiorrhizeae: *Ophiorrhiza* and its close relative *Spiradiclis*, and *Virectaria*. Verdcourt (1958) was very reluctant about the latter genus' belonging here and all later work has shown he was correct; *Virectaria* is now in the Sabiceae (see (1.2.3a)). Molecular work (Bremer 1996, Bremer & Manen 2000 and the supertree) shows that the **Ophiorrhizeae** (clade *Neurocalyx* to *Ophiorrhiza* on the supertree) should be widened to include other elements, mainly from Bremekamp's other tribe Pomazoteae, which he recognized mainly on account of what he considered to be a peculiar kind of exotesta (Bremekamp 1966: 23: "thin-walled cells with a basal wall which is either minutely dotted or more or less coarsely tuberculate"). We have stressed (Robbrecht 1988: 20) that his seed-coat examinations (in surface view only) need reinterpretation using better anatomical techniques. The Ophiorrhizeae s.lat. are therefore awaiting deep morphological work and more inclusive molecular sampling.

The sister group relationship between the Ophiorrhizeae and Urophyllaeae postulated by Andersson & Rova (1999) is not corroborated by later studies nor by the supertree.

(d) Recent molecular investigations almost simultaneously indicated that *Lasianthus* belongs to basal Rubioideae, and not to Morindeae or Psychotrieae where it was traditionally included. Andersson & Rova (1999) and Piesschaert & al. (2000a), using *rps16* intron data, found strong support for a clade including *Trichostachys*, *Saldinia*, *Ronabea* (as *Psychotria erecta*; see Taylor 2004) and *Lasianthus*; the genus *Perama* is sister to this clade. Bremer & Manen (2000) used a wider array of molecular data but only included *Lasianthus* and *Trichostachys* in their sampling; they formally and newly recognized a tribe **Lasiantheae** for these two genera. In the supertree these elements are in a two-clade grade (clade *Perama-Lasianthus* and clade *Trichostachys-Saldinia*) at the base of the Coussareeae.

<sup>8</sup> These two have not been included in the abovementioned morphological investigations of Bangoura and Buchner because they were considered to belong in Isertieae at that time; they are badly in need of thorough work. The gross morphology of the two genera is not contradicting a position here, however. The alleged absence of raphides in *Raritebe* (Bremer & Manen 2000: 57), no doubt based on Kirkbride's (1979) description of the genus, is incorrect. Jansen (2001: 152) described the occurrence of this crystal type in parenchyma cells around the vascular bundles in leaves of *Raritebe*. *Amphidasya* and *Raritebe* are also strong aluminium accumulators (Jansen & al. 2000a: table 1), and in that respect too better match the Urophyllaeae than the Isertieae s.lat., the group to which they were previously assigned; all genera of the Urophyllaeae s.lat. investigated were shown to be strong accumulators.

The recognition of the Lasiantheae in basal Rubioideae removed these genera from the Psychotriaceae-Morindeae alliance in whose context they had always been commented upon since Petit (1963) questioned the mutual delimitation of these two tribes. This brought an end to a discussion that was vivid for decades, as reflected in Igersheim & Robbrecht's (1994) comments on the delimitation of the Prismatomerideae. These latter authors left *Lasianthus* and relatives in an uncertain tentative position in Psychotriaceae.

The sister relationship *Perama* - Lasiantheae, "a surprising relationship strongly supported by the bootstrap" (Andersson & Rova 1999: 173), also needs commenting. The neotropical herbaceous genus *Perama* was traditionally classified with the Spermaceae because of similarities in habit and circumscissile capsules, a feature elsewhere in Rubiaceae only found in *Mitracarpus* (Spe.). That position was doubted by Bremekamp (1934: 248) on account of unusual characters (absence of stipules, bilobed calyx, trimerous corolla and triquetrous seeds) absent from other Rubiaceae; he proposed to exclude the genus from the family, stating that its "real affinity ... remains obscure" and that he could not "allocate its true place in the [angiosperm] system." He later (Bremekamp 1952: 13, footnote 1) no longer saw the need for exclusion, but proposed a monogeneric tribe **Perameae** having an affinity with Morindeae, Coussareeae, Psychotriaceae, Paederieae and Anthospermeae rather than with Spermaceae, a viewpoint maintained in his later classification survey (Bremekamp 1966: 29-30). Piesschaert & al. (2000 a) considered the morphological link between the *Lasianthus* group and the Perameae to be very obscure. The differences are at a first glance impressive (fleshy-fruited woody plants versus perennial and annual herbs with capsules), but the coexistence of such extremes exists in other rubiaceous groups, e.g. the Psychotriaceae. The genus *Perama* hitherto only received floristic attention (Steyermark & Kirkbride 1977 and references therein) and is in need of deep morphological work. The very thin capsules and seeds at first sight have no specialized structures that would a priori rule out a morphological derivation. The triangular seeds have a glossy seed-coat with exotestal cells thickened much like those of the Urophyllaeae (Robbrecht, personal observations in light microscopy). *Perama* is further a strong aluminium hyperaccumulator and in that agrees with *Lasianthus* and *Trichostachys* (Jansen & al. 2000a, b).

If more work should reveal that *Perama* is better included in the *Lasianthus* group, the tribal name Perameae has priority over Lasiantheae.

(e) In contradiction to the previous groups (a-d), the last clade of the basal Rubioideae, the **Coussareeae**, is entirely neotropical<sup>9</sup>. One of the genera in this clade, *Coccocypselum*, was placed in a monogeneric tribe Coccocypseleae (Bremekamp 1952) because of obvious morphological differences (herbaceous habit, raphides, berries) with the Mussaendeae, to which tribe it was originally assigned. This isolated position was uninformative about the genus' relationship, and molecular phylogenetic approaches had to be awaited to find a relationship for this genus with an unusual combination of morphological features. Andersson & Rova (1999) included the genus in their *rps16* intron sampling for a phylogenetic investigation of the Rubioideae. They found *Coccocypselum* in a clade with *Hindsia* (formerly Cinchoneae or Hedyotideae) and *Declieuxia* (formerly Psychotriaceae), two genera so deviant morphologically that their conclusion was very cautious (op. cit.: 179): "*Hindsia* should be removed from the Hedyotideae and *Declieuxia* form the

<sup>9</sup> *Schizocolea*, the sole afro-tropical element ever included in the tribe Coussareeae, was recently excluded based on unpublished sequence data (Bremer & Manen 2000: 65), without assigning it to another place. Bremekamp (1950), when erecting the genus for a former member of the genus *Urophyllum*, found its position 'puzzling'. *Schizocolea* was not sampled for the supertree as material was very scanty; the genus awaits an in depth study.

Psychotriaceae. These two genera appear to be closely related, but no tribal name is available for them and the morphological aspects are obscure.” Piesschaert & al. (2000 b) therefore undertook a deep morphological study of the three genera and were able to find uniting morphological features (the most apparent ones in pollen and stipules) and biogeographical facts. They proposed to amend the **Coccocypseleae** to also include *Hindsia* and *Declieuxia*.

A relationship between *Coccocypselum* and *Faramea* had earlier been detected by Bremer (1996; *rbcl*). This hypothesis was also corroborated by Andersson & Rova (1999), who found the two genera of the Coussareeae, *Faramea* and *Coussarea*, in a sister relationship to *Coccocypselum* / *Hindsia* / *Declieuxia*. Hooker (1873) and all subsequent authors had united *Coussarea* and *Faramea* in a small tribe Coussareeae placed near Psychotriaceae, so its removal to a more basal position was also a novel hypothesis brought in by the molecular work.

Andersson & Rova (1999) also included the genera *Heterophyllaea* and *Oreopolus* in their analysis, and they came out in a sister position to (Cou., Coc.). This was another novel relationship for two genera only difficultly placed before. *Heterophyllaea* was traditionally included in Cinchoneae. The need to transfer it to another place because it possesses raphides was stressed by several authors (Bremekamp 1952, Robbrecht 1988, Andersson & Persson 1991), none of whom, however, could suggest more than a tentative transfer to the tribe Hedyotideae, suspected to be polyphyletic by the last authors. *Oreopolus* is a close relative of *Cruckshanksia* placed both in **Cruckshanksieae** (Hooker 1873). Puff (1988), on the occasion of a revision of *Carphalea*, another genus included in Cruckshanksieae (Baillon 1878b, Bremekamp 1966), discussed the taxonomic status of the tribe and accepted its abolition proposed by Verdcourt (1958). This synonymy of Cruckshanksieae with Hedyotideae was followed in Robbrecht’s (1998) system. Andersson & Rova (op. cit.) concluded that the Cruckshanksieae should be revised and emended to include also *Heterophyllaea*.

The supertree (clade *Hindsia longiflora* to *Hindsia glabra* in fig. 4C) is congruent with the results of Bremer & Manen (2000) and unites all genera of Coussareeae, Cruckshanksieae and Coccocypseleae discussed above; it does not, however, confirm the relationship (Cru.(Coc.,Cou.)) postulated by Andersson & Rova (1999). The first two tribal names are from the same date and have nomenclatural priority over Coccocypseleae (Darwin 1976). We propose to follow Bremer & Manen (2000) and merge the three in an extended delimitation for the Coussareeae. Further work has to be awaited to make out whether Coccocypseleae and/or Cruckshanksieae merit possible subtribal revival, as strongly suggested by morphological evidence (Piesschaert & al. 2000 b).

## (2.2) Supertribe Psychotriidinae

(a) In this predominantly woody alliance of the core Rubioideae, the **Gaertnereae** (clade *Pagamea guianensis* to *Gaertnera vaginans* on the supertree) are sister to the rest of the core. This tribe, comprised of the paleotropical genus *Gaertnera* and the neotropical *Pagamea*, both on the supertree, has a long taxonomic history. The first debates concentrated on the superior ovaries, that were considered in favour of a position in the Loganiaceae. Other arguments, already assembled by Baillon (1879b) and Solereder (1890) proved a position in the Rubiaceae; they pointed to anatomical facts (the most important one the presence of intraxylary phloem) and interpreted the ovary as not truly superior. They suggested a placement near the Psychotriaceae, recently confirmed by a wood anatomical and pollen morphological study (Jansen & al. 1996). These authors interpreted the two genera as morphologically advanced members of the Psychotriaceae alliance, best accommodated in a subtribe Gaertnerinae. A study of the gynoeceal development carried out just before (Igersheim & al. 1994) had already delivered the final explanation for the ovary morphology: “*Gaertnera* ... exhibits the unusual situation that, in the course of development, its ovaries change from basically inferior to

secondarily superior.” This situation is hardly known from the rest of the angiosperms, and at our knowledge only reported from an isolated case in Araliaceae (Eyde & Tseng 1969).

The basal position of the Gaertnereae on the supertree is derived from Andersson & Rova’s (1999) *rps16* data and compares with their results that show the tribe as sister to the rest of their “Psychotriaceae-Morindeae clade.” It is therefore necessary to keep the clade recognized at the tribal, not subtribal level.

An isolated species of *Gaertnera* is sister to the Psychotriaceae (clade *Psychotria punctata* to *Rudgea bremekampiana*) on the supertree. This is in fact one of the poorer results of our supertree construction; we have discussed it in section R.2 and have not considered it for our proposed classification.

**(b)** The clade *Craterispermum caudatum* to *Morinda moaensis* on the supertree (fig. 4C) represents elements that were associated with the **Morindeae** in the past: the Prismatomerideae and the *Mitchella-Damnanthus* group. It also includes the Schradereae and the African genus *Craterispermum*.

Igersheim & Robbrecht (1994) discussed the circumscription of the Morindeae and proposed to restrict the tribe to representatives with a “bicarpellate gynoeceum comprised of a common style with two stigma lobes, and two biovulate locules.” The latter feature distinguishes them from other Psychotriidinae. Other features of the Morindeae s.str. are the massive (septum-like) development of the placentas between the two ovules of a locule, and the four one-seeded pyrenes. The latter originate from two carpels and this equally represents a unique situation. Igersheim & Robbrecht (op. cit.) segregated the Prismatomerideae, and accepted the separation of the *Mitchella* group proposed shortly earlier (Robbrecht & al. 1991). These two groups have uni-ovulate locules. Ovary fusion occurs in all three groups.

The **Schradereae** (*Schradera* and its synonym *Lucinaea*, *Leucocodon* and *Lecananthus*) were subject of an in-depth investigation (Puff & al. 1993; Puff & Buchner 1998b). The first paper carried out a morphological and anatomical study comparing many members of Rubioideae, and analysed the observations with numerical and cladistic methods. The authors were unable to suggest close relatives for the tribe; however, the Morindeae, which on molecular grounds are now indicated as related, were absent from their comparison. Ovary fusion is absent in the Schradereae, but the pseudanthia with the sessile ovaries tightly packed in an involucre result in an inflorescence habit strongly resembling *Morinda* capitules. The ovary locules have a placenta bearing numerous ovules, which on the other hand represents a strongly differentiating feature with the previous group (and all other Psychotriidinae).

Also the Afro-Madagascan genus *Craterispermum* is morphologically so strongly derived that it was placed in a monogeneric tribe of uncertain affinity. A morphological-anatomical investigation (Igersheim 1992) discarded Robbrecht’s (1988: 155) suggestion to place the tribe close to Vanguerieae wherein the genus originally was included.

Andersson & Rova (1999) were the first to suggest the affinity between Schradereae and Morindeae; that relationship was strongly supported in their analysis of *rps16* intron data. It was not fully confirmed in the analysis of Bremer & Manen (2000); their strict consensus tree based on three sequences (op. cit.: fig. 4) found an unresolved relationship (Sch.,Psy.,Mor.). When *Craterispermum* was sequenced for the first time (Bremer & Manen 2000: fig. 3, combined *rbcL/atpB-rbcL* analysis) the genus was found in a basalmost position to their “*Psychotria* alliance” (i.e., our Psychotriidinae).

The supertree is in favour of a return to a wider concept of the Morindeae, including all the above elements, even the Craterispermeae; it, however, shows much reiteration of the taxa included, thus

reflecting the present contradictions in the source trees, as explained in the section R.2 of the paper. The monophyly of the three elements of the Morindeae s.lat. plus the Craterispermeae can nevertheless be considered to be proven. The Schradereae are represented in the molecular sampling by two *Schradera* species. In the supertree, one is with the Gaertnereae in a grade basal to all other Psychotriidinae (a position derived from the *rps16* source tree). The other, *Schradera subandina*, falls inside the Morindeae s.lat., in a sister relationship with *Craterispermum*. The basal position in any event better fits the morphological facts. *Schradera* has placentas with numerous ovules, and a reversal from the 1-2-ovulate condition of the Morindeae s.lat. to pluriovulate placentas is very unlikely. In the angiosperms in general, uniovulate locules or ovaries go hand in hand with a syndrome of adaptations in fruits and/or seeds, which is so advanced that it excludes a reversal to many ovules.

Bremer & Manen (2000) accepted the Schradereae and Craterispermeae at the tribal level and recognized three entities within Morindeae, the *Mitchella* group and the subtribes Morindinae and Prismaticeridinae. Also the supertree constructed indicates that this is at present the most plausible taxonomic conclusion, followed in our classification (Taxonomic conspectus below). A better sampling of the representatives not belonging to Morindeae s.str. is obviously needed to establish a better delimitation of the subtribes and to answer the question whether the Craterispermeae should not be reduced to yet another subtribe of the Morindeae.

The last clade on the supertree (*Psychotria punctata* to *Rudgea bremekampiana*; fig. 4C) contains genera that were never doubted to belong to **Psychotrieae**<sup>10</sup>. Many of the genera included were formerly confused with the genus *Psychotria* itself and reduced to its synonymy. That way *Psychotria* became the sole 'big genus' of the Rubiaceae (Frodin 2004). The wide *Psychotria* concept was refuted as early as the 1960's in classical taxonomic-morphological studies of the afro-tropical representatives (Petit 1964, 1966, Robbrecht 1975, Verdcourt 1975, 1977). These authors segregated several genera or established new criteria to distinguish related genera, mainly using carpological features. The taxonomic application of their findings to other parts of the neotropics had to await the molecular era. In the neotropics, *Psychotria* s.lat. is now disintegrating. *Psychotria* subg. *Heteropsychotria* has been shown to be more closely related to *Palicourea* than to *Psychotria* s.str. *Chazaliella* was found not to be an African endemic genus but a pantropical one with many species, for which the name *Margaritopsis* (previously a Cuban endemic) should be adopted (Andersson 2001, *rps16* intron; Taylor 2005). Several genera were resurrected or demonstrated to deserve continued recognition, e.g. *Notopleura* (Nepokroeff & al. 1999, Andersson & Rova 1999, Taylor 2001), *Carapichea* (Andersson 2002a), *Calycosia*, *Straussia* and *Streblosa* (Andersson 2002b).

These new concepts are reflected on the supertree. Two major clades may be distinguished that correspond to alliances already firmly established by previous authors. The first clade (*Psychotria punctata* to *Psychotria ankasensis* in fig. 4C) corresponds to clade F of the Psychotrieae-Morindeae clade of Andersson & Rova (1999) and to the '*Psychotria sensu stricto*' clade of Nepokroeff & al. (1999). It is an Old-World centred group that contains *Psychotria* in its strictest sense, and long

<sup>10</sup> A remark should be made on the Madagascar-centred Triainolepideae and Madagascan Lathraeocarpeae, according to Bremekamp (1966; followed in Robbrecht's 1988 system) closely related satellite tribes of the Psychotrieae. It now appears that these woody plants belong to the predominantly herbaceous Rubiidae. Sequencing data for the Triainolepideae are available (two genera on the supertree); the tribe is discussed below, under (2.2.2) (a) Knoxiaceae. The monogeneric Lathraeocarpeae remain undocumented with regard to DNA markers; morphologically, a relationship with either *Gomphocalyx* (Spe. s.lat.) or *Triainolepis* is possible (Dessein & al. 2005a).

recognized elements such as *Amaracarpus*, or the myrmecophilous genera of the Psychotriaceae-Hydnohytinae. The latter are deeply nested in the clade in all molecular trees; the recognition of the Hydnohytinae makes the subtribe Psychotriinae (Robbrecht 1994b: 178) for the remainder of the Psychotriaceae paraphyletic. Much work remains to be done on the other Asian and Pacific representatives; morphological work (Piesschaert 2001) indicated a great deal of variation and suggested acceptance of genera such as *Calycosia*, *Chaetostachyidium* and *Streblosa*.

We here propose to redelimit the Psychotriaceae in a stricter sense, to comprise only elements of this first clade.

The second clade (*Geophila tenuis* to *Rudgea bremekampiana* in fig. 4C) corresponds to clade K of the Psychotriaceae-Morindeae clade of Andersson & Rova 1999) and the 'Psychotriaceae + subg. *Heteropsychotria*' clade of Nepokroeff & al. (1999). It is New World centred and morphologically characterized by pyrenes with distinct preformed germination slits and very reduced, pale seed-coats. A tribal name is not available for this clade; it is here recognized as **Palicoureae**.

### (2.3) Supertribe Rubioidinae

This predominantly herbaceous alliance of the core Rubioideae is comprised of two major lineages: on the one hand (clade *Leptostigma* to *Rubia peregrina* in fig. 4D) an assemblage of the tribes Anthospermeae, Argostemmateae, Paederieae, Theligoneae and Rubieae, and on the other hand (clade *Knoxia* to *Spermacoce tenuior* in fig. 4D) a grouping of genera formerly placed in Triainolepideae, Mannetieae, Knoxieae, Hedyotideae and Spermacoceae. These two are here designated Rubioidinae I and II.

#### (2.3.1) Rubioidinae I

(a) On the supertree *Danais* is sister to the Anthospermeae-Rubieae lineage, while its supposed relative *Schismatoclada* is sister to the rest of the Spermacoceae s.lat. clade. They were previously associated with either the Cinchoneae or the Hedyotideae, but a detailed morphological and anatomical documentation of the two genera and *Payera* has shown the distinctness of the Madagascar-centred *Danais-Schismatoclada-Payera* complex (Buchner & Puff 1993). Andersson & Rova (1999) included *Schismatoclada* in their *rps16* intron study of the Rubioideae and found the genus at the base of a clade otherwise comprised of Hedyotideae and Spermacoceae. Bremer & Manen (2000; *rbcL* and other data) on the other hand confirmed an earlier finding of Bremer (1996; *rbcL*), viz. that *Danais* is basal to the herbaceous alliance of the Rubioideae (our Rubioidinae); they also confirmed (*rbcL*) the relationship of *Schismatoclada*. This conflict between *rps16* and other molecular data is also represented by the supertree. The monophyly of the **Danaideae** (formally recognized by Bremer & Manen 2000) and its belonging to Rubioidinae can nevertheless be taken for granted.

(b) With in depth studies of the African representatives in the field (Puff 1986) and a thorough morphological and anatomical documentation (Puff 1982) showed the need of mutual redelimitation of **Anthospermeae** and Paederieae, two tribes already well established in the classical systems (Hooker 1873, Schumann 1891) of the Rubiaceae. He restricted the Anthospermeae to wind-pollinated taxa and divided it in three subtribes, which also have a solid foundation in biogeography.

Later molecular findings corroborated his view on the tribe, and at first also confirmed the subtribes; the tree subclades in clade B of Andersson & Rova (1999: fig. 4) correspond to Coprosminae, Anthosperminae and Operculariinae. Bremer & Manen (2000) examined more sequences and found less support; they did not pay attention to the recognition of subtribes.

But a reinvestigation (Anderson & al. 2001) based on *rps16* and novel data (*ITS*) and with a more inclusive sampling resulted in slightly different hypotheses. It putted some doubt as to the belonging of *Carpacoce* here; if this Cape genus is a member of the tribe, it should be the sister group of the rest. The tribe should also consist of two subclades, the African Anthosperminae (without *Carpacoce*) and the Australasian Operculariinae plus Coprosminae. The former appeared as a grade at the base of the latter.

The supertree (clade *Leptostigma* to *Anthospermum* in fig. 4D) confirms the monophyly of the Anthospermeae and the basal position of *Carpacoce* within the tribe; its subclades largely correspond with the subtribes but show some reiteration, due to slight contradictions in the source trees.

**Table 3. The tribe Paederieae as delimited and divided by Puff, compared to results of molecular phylogeny.**

\* especially Backlund & al. (2005); see also text

Puff 1982 (4 groups)		Puff 1989		sequencing studies *
1	<i>Neogaillonia</i> (now <i>Gaillonia</i> )	no further comments		<b>Putorieae</b>
	<i>Putoria</i>			<b>Putorieae</b>
	<i>Plocama</i>			<b>Putorieae</b>
	<i>Crocyllis</i>			<b>Putorieae</b>
	<i>Kelloggia</i>			sister to Rubieae
2	<i>Mitchella</i>	misplaced, aff. Morindeae, referring to a paper then in preparation (Robbrecht & al. 1991)		Morindeae s.lat.
	<i>Pseudopyxis</i>	linking up with <i>Spermadictyon</i> & <i>Serissa</i> , & the most derived member of that alliance	forming a natural group (from the Himalaya to Japan)	not investigated
3	<i>Spermadictyon</i>	most closely allied, perhaps to be united		<b>Paederieae s.str.</b>
	<i>Leptodermis</i>			<b>Paederieae s.str.</b>
	<i>Serissa</i>	closely related to <i>Leptodermis</i>		<b>Paederieae s.str.</b>
	<i>Aitchisonia</i>	standing apart; forms a morphological, anatomical and geographical link between the <i>Gaillonia</i> group 1 and the <i>Spermadictyon</i> group 3		not investigated
4	<i>Paederia</i>	loosely associated with the <i>Spermadictyon</i> group 3 (morphology, much wider dsitribution ...)		<b>Paederieae s.str.</b>

(c) The **Argostemmataeae** are one of the small tribes segregated when Bremekamp (1952) considered the delimitation and subdivision of the Hedyotideae. It was accepted by Verdcourt (1958) and Bremer (1987). The last author's cladistic analysis of morphological features restricted the tribe to *Argostemma* and *Neurocalyx* and postulated a sister relationship with the Hamelieae. That latter hypothesis did not withstand the test of molecular phylogenies, and the Hamelieae are now considered to belong to Cinchonidinae. Andersson & Rova (1999) found strong support for the belonging of *Mycetia* here. This implies that a number of genera sometimes considered to be related with *Mycetia* are candidate for an inclusion in the Argostemmataeae, e.g. *Myrioneuron* and *Keenania* (Deb 1996), *Mouretia* (Tange 1997a) and *Cyanoneuron* (Tange 1998). All these genera are in need

of further work. On the supertree, this alliance is only represented by *Argostemma* and *Mycetia*, which form a clade at the base of Paederieae and Rubieae.

(d) When Puff (1982) proposed the abovementioned redelimitation of the **Paederieae**, he distinguished four groups. On the occasion of a thorough morphological-anatomical investigation of the Japanese *Pseudopyxis*, he felt the need to postulate certain realignments. Table 3 summarizes his 1982 and 1989 viewpoints and compares them with the subsequent results of molecular phylogenetic investigations.

Two clades (clade *Spermadictyon*, *Serissa*, *Leptodermis*, *Paederia* and clade *Putoria*, *Gaillonia*, *Plocama*) on the supertree (fig. 4D) are quite congruent with Puff's groupings, especially the 1989 version. The first clade comprises three of the four genera of Puff's group 3, plus *Paederia* (monogeneric group 4 of Puff). The second clade consists of three of the five genera of Puff's group 1 (his *Neogaillonia* is now *Gaillonia*; Léonard 1984). However, the Paederieae sensu Puff as a whole form a two-clade grade at the base of the clade *Theligonum* to *Rubia peregrina* in fig. 4D. The paraphyletic nature of the Paederieae has already been postulated by Andersson & Rova (1999: 175). Bremer & Manen (2000: 66) confirmed that the tribe is biphyletic, but refrained from taking decisions because of ongoing work by Backlund & al. These authors recently concluded (Backlund & al. 2005; *rbcL*, *rps16* and *trnT-F*) concluded that the Paederieae should be restricted to the four genera of our first clade. They proposed (i) a restricted definition of the Paederieae for our first clade, and (ii) a monogeneric **Putorieae** for our second clade, reducing all other genera (*Gaillonia*, *Plocama* etc.) to synonymy of *Putoria*. This is in line with an earlier suggestion (Thulin 1998) to reduce *Crocyllis* to a synonym of *Gaillonia*.

As already stated, Puff's (1982) Paederieae group 2 (*Mitchella* and *Pseudopyxis*) later proved to be artificial; Robbrecht & al. (1991) have demonstrated that *Mitchella* is closely related to *Damnacanthus* and that the genus pair is related to the Morindeae, a hypothesis confirmed by the supertree (see above). The Japanese genus *Pseudopyxis* is absent from the sampling of our four source trees and other sequencing investigations; the genus awaits sequencing to confirm its placement in the Paederieae s.str., postulated by Puff on morphological grounds. Sequences of the Irano-Turanian genus *Aitchisonia* (in group 3 of Puff) are also unavailable. Bremekamp (1958: 153) even excluded the genus from the Rubiaceae on account of its glandular emergences not existing in other members of the family, but this was not accepted by Puff (see table 3). *Kelloggia*, another genus of Puff's group 1, falls in a distant clade in fig. 4D, viz. is sister to the Rubieae. It is discussed below. Puff pointed to morphological similarities with the other genera of group 1, but judged *Kelloggia*'s position "more-or-less isolated" on account of its peculiar disjunct distribution (western USA and SW China).

(e) The **Rubieae** (*Rubia fruticosa* to *Rubia peregrina*, fig. 4D) agree with all former concepts; the delimitation of this tribe has never been debated and has been confirmed by all sequencing studies (e.g. Natali & al. 1995). Morphologically it is well defined indeed, especially by the "whorled" leaves that are in fact whorls of opposite leaves and leaf-like stipules or stipule segments (Rutishauser 1999). Pollen features confirm the monophyly of the tribe (Huysmans & al. 2003). The two taxa at the base of the Rubieae clade on the supertree, *Theligonum* and *Kelloggia*, need further comment, however.

*Kelloggia* was found to be sister to the Rubieae rather than a member of the Paederieae (Andersson & Rova 1999; Backlund & al. 2005) and the genus has the same position on the supertree. The questions evoked by the genus' intercontinental disjunction were investigated with sequence data from three cpDNA regions (Nie & al. 2005). These authors confirmed the

monophyly of *Kelloggia* and estimated the divergence between the North American and the East Asian species to be ca. 5.5 million years ago. They also corroborated the genus' sister relationship with the Rubieae, observing that a separate tribe is probably needed, although they judged a more comprehensive analysis of the Paederieae is needed before doing so formally. Morphologically *Kelloggia* remains poorly documented, but it very closely resembles the Rubieae, except in having 'true' interpetiolar stipules and 3-colpate pollen. It can best be interpreted as having kept the ancestral features of what became the Rubieae through the stipules becoming leaf-like and the apertures augmenting in number.

The peculiar genus *Theligonum* has long been considered to represent a separate family Theligonaceae, until Wunderlich (1971) thoroughly documented the genus and found that "almost all peculiar features ... can be explained ... in connection with the development trends and variations within the Rubiaceae." She concluded that it deserves only a tribe Theligoneae within the family, closely related to the Anthospermeae (with which tribe it shares anemophily), but also to Spermaceae and Rubieae. Molecular investigations have shown that the adaptation to wind pollination occurred twice in Rubiaceae and that *Theligonum*'s sister relationship lies with the Rubieae; this fact was recorded independently in an investigation of the Rubioideae (Natali & al. 1995; *atpB-rbcL* intergene region) and a study of the entire Rubiaceae family (Bremer & al. 1995; *rbcL*). Not all later studies have confirmed this, Andersson & Rova (1999) e.g. found *Theligonum* in a sister relationship with *Plocama*, but only on a moderately supported branch.

The clade (Pae.(Put.(The.(*Kelloggia*,Rub.))) (*Paederia bojeriana* to *Rubia peregrina* in fig. 4D) is strongly supported by pollen morphology, viz. by the loss of endoapertures that are otherwise very common in Rubiaceae (Robbrecht 1982, Huysmans & al. 2003, Dessein & al. 2005b). The most plausible scenario suggested by the presently available sequencing evidence reads that Paederieae, Putorieae and *Kelloggia* have kept the ancestral 3-colpate pollen, while shifts have taken place in *Theligonum* (the number of apertures augmented to 4-8 and the colpi transformed in pores) and the Rubieae (where the pollen grains became pluricolpate). The 50bp deletion that characterizes the Rubieae (Natali & al. 1995: 431) is absent from *Kelloggia* (Nie & al. 2005: 649) and *Theligonum* (Natali & al. op. cit.). All this seems best translated in a classification with three tribes, Paederieae and Putorieae as delimited above, and Rubieae in a widened circumscription to include the monogeneric subtribes Theligoninae and Kelloggiinae.

### (2.3.2) Rubiidinae II

The last clade on the supertree (*Knoxia* to *Spermacoce tenuior*; fig. 4D) contains a grouping of genera formerly placed in Triainolepideae, Manettieae, Knoxieae, Hedyotideae and Spermaceae. Of those tribes, especially the Hedyotideae have been the subject of much debate, starting with Bremekamp's (1952) considerations on their delimitation and division. The former unnatural delimitation of the Hedyotideae to include almost all the herbaceous multi-ovulate representatives of the family is highlighted again by our abovegiven discussion of genera to be placed in other alliances, to repeat a few *Hekistocarpa*, *Rachicallis*, and *Hindsia*.

In the context of the present work it is not needed to survey all discussions since 1952. Andersson's & Rova's (1999: 174) comments on an earlier decision of Bremer (1996; *rbcL* study) to recognize only one tribe, Spermaceae s.latiss. incorporating most elements of the other tribes may suffice to start the comments on our supertree. They considered merging the tribes as a rash decision, remarking that an extended definition of the Knoxieae, next to a less enlarged concept of the Spermaceae is supported by Bremer's tree as well. Andersson & Rova (op. cit.: fig. 4) found the Knoxieae s.lat. to be sister to the remaining elements of their 'Hedyotideae-Rubieae clade'

(= our Rubiidinae). This is contradicted by Bremer & Manen (2000) and the supertree. Nevertheless, it is not prudent to follow Bremer & Manen, who maintained the widest possible delimitation of the Spermaceae, while a recognition of two monophyletic entities (Knoxieae s.lat. and Spermaceae s.lat.) is supported as well.

(a) The **Knoxieae** were originally restricted to representatives having solitary pendulous ovules; as such the group was morphologically documented and taxonomically surveyed by Puff & Robbrecht (1989); these original Knoxieae are represented by *Knoxia*, *Pentania* and *Carphalea* on the supertree. The molecular results (inter alia Piesschaert & al. 2000a, Bremer & Manen 2000, Dessein & al. 2005a and the supertree) indicate that three other elements belong to the same clade:

(i) A group of former Hedyotideae designated by Bremer (1987: 39) as the *Pentas* group, on the supertree represented by *Carphalea*, *Batopedina*, *Pentas* and *Parapentas*. Bremer's (1996) *rbcL* data were the first to associate one member of the Knoxieae (*Pentania*) with the *Pentas* group.

(ii) The genus *Otiophora*<sup>11</sup>. It represents a nice illustration how old debates based on morphological and anatomical facts can finally be settled by sequencing results. Note, however, that Verdcourt (1950) already had stated that "*Otiophora* shows affinities to *Otomeria*, *Pentas* and *Pentania*"; the four genera are now brought together in the Knoxieae s.lat. Comparative morphological developmental investigations of the entire Knoxieae s.lat. are obviously needed for a reinterpretation of the gynoecium characteristics in the alliance.

(iii) *Paratriainia* and *Triainolepis*, two Madagascan or Madagascar-centred genera. *Triainolepis* was classically assigned to Psychotrieae (e.g. Schumann 1891), but Bremekamp (1956) felt the need to place it in a tribe of its own, the Triainolepideae, mainly because of the divided stipules and peculiarities of the gynoecium (many locules, each with two collateral ovules); he also recognized two genera next to the tribe's name-bringing one, *Paratriainia* and *Thyridocalyx*. Bremekamp kept the tribe close to the Psychotrieae, no doubt because of the woody habit of the representatives. It is interesting to note that carpological similarities between *Triainolepis* and subgenus *Holocarpa* of *Pentania* (Knoxieae s.str.) has already been remarked by Verdcourt (1958). Dessein & al. (2005a; *rps16* intron and *rbcL*) found *Triainolepis* in a sister relationship to the rest of their [limited, as an outgroup] sampling of the Knoxieae. This finding is nevertheless supported by some morphological facts (woody habit; pluricarpellous ovaries), which is in favour of a revival of the Triainolepideae as a subtribe of the Knoxieae. The two genera *Paratriainia* and *Triainolepis* represent a monophyletic group nested in Knoxieae on the supertree; this is derived from their position on the spacer source tree, where they are in a grade at the base of the Knoxieae. More work is needed before one can decide whether recognition of a subtribe for these genera would not make the remainder paraphyletic.

(b) The large clade (*Manettia luteorubra* to *Spermacoce tenuior*; fig. 4D) at the end of the supertree exclusively comprises representatives formerly included in Hedyotideae, Manettieae and **Spermaceae**. It corresponds to Spermaceae s.latiss. (Bremer 1996, Bremer & Manen 2000) minus the Knoxieae s.lat. (i.e. in the delimitation proposed above). Results of other authors

<sup>11</sup> Puff (1982) excluded the entomophilous genus from the exclusively wind pollinated Anthospermeae, where it was originally assigned to. He suggested (Puff 1983) a placement in the Hedyotideae subsequently criticized by Verdcourt (1989), who preferred to keep it in an uncertain position. A reinvestigation of gynoecium structure and development (Igersheim & Rohrhofer 1993) concluded that *Otiophora* in no single detail differs from the gynoecium development in *Spermacoce* and *Diodia*; they suggested a position in the Spermaceae. The sequencing data now propose an hitherto never formally considered relationship.

(Andersson & Rova 1999, Piesschaert & al. 2000a, Dessein & al. 2005a) are similar, i.e. elements from the Hedyotideae form a grade basal to the Spermaceae s.str. This is not entirely so in our supertree where the Spermaceae s.str. show some reiteration, what is due to differences in the four source trees. In our classification we maintain this clade as Spermaceae s.lat. Several monophyletic entities might exist within the clade. A 'Pentanopsis clade', e.g., was recently detected (Thulin & Bremer 2004; Dessein & al. 2005a). It appears on the supertree, although with the 'reiteration problem' also observed in other groups (fig. 4D: clades *Conostomium natalense* to *Oldenlandia affinis* & *Conostomium quadrangulare* to *Pentanopsis fragrans*). Detailed comments on the Spermaceae s.lat. are premature, because the group is in need of much more work, morphological as well as genetic. A complication is the fact that two large genera, i.e. *Hedyotis* and *Oldenlandia*, are polyphyletic. Ongoing work is in progress (see e.g. Motley 2005), and better phylogenies for the Spermaceae s.lat. are to be expected in the coming years.

## Conclusion

"It is better to analyze more characters than to investigate more taxa if the purpose is to get a strongly supported tree" (Bremer & al. 1999: 433). Our separate investigation of the position of *Luculia* and *Coptosapelta*, based on the investigation of supplementary genetic data, confirms this statement. It results in a hypothesis classifying these problem genera in one of the subfamilies. Supertree construction, on the other hand, proved to be a strong technique for combining several characters with many taxa. It allows to postulating a coherent classification for the entire Rubiaceae family, in translating the two major clades in two subfamilies.

Each subfamily consists of two presently 'successful'<sup>12</sup> lineages designated here as supertribes, but also has a number of less successful alliances or 'evolutionary dead ends' at its base. For the cinchona and ixora subfamily (Cinchonoideae) this regards only *Coptosapelta* and *Luculia*. Morphologically these two paleotropical genera are very different, and further studies are needed to confirm whether they really belong to one monophyletic group. The woody habit predominates in the entire Cinchonoideae. Herbaceousness is rare and annuals are almost unexisting (only some spp. in *Sipanea* and *Limnosipanea*; Taylor pers. comm.).

The psychotria and madder subfamily (Rubioidae) has a whole suite of basal groups, *Colletocema*, Coussareae, Lasiantheae, Ophiorrhizeae, and Urophyllaeae. The core of this second subfamily is comprised of one predominantly woody and one predominantly herbaceous lineage, here designated as Psychotriidinae and Rubiidinae, respectively. In the herbaceous lineage annuals are common.

The herbaceous alliance of the Rubioidae contains very few woody representatives, viz. some Anthospermeae, some Paederieae, the former Triainolepideae (now in Kno.), some Spermaceae (*Tobagoa*, *Emmeorrhiza*, *Scandentia*, ...) and some Rubieae. This has been studied in detail in only a very limited number of cases, but results point to secondary woodiness for Rubieae (Koek-Noorman 1976) and Anthospermeae (Koek-Noorman & Puff 1983). The woody alliance on the contrary contains a few herbaceous groups, e.g. the well-known genus *Geophila*, classically placed in Psychotrieae, now in the segregated Palicoureeae.

The single feature habit (woody versus herbaceous) shows a pattern of predominance in many higher taxa, but the pattern is blurred by exceptions, and reversals seem to have taken place. This

<sup>12</sup> In so far that species richness can be used to measure evolutionary success.

certainly also applies to other characteristics. The occurrence of capsular fruits e.g. in groups where fleshy fruits are predominant is more common than we accepted until now. Examples are *Wendlandia*, here found to be related with Gardenieae, and *Greenea* here corroborated to be related with Ixoreae. These two tribes were previously thought to be exclusively fleshy-fruited.

From this, one can conclude that the phylogenetic pathways of Rubiaceae follow intricate patterns only in part translated by morphology. In the introduction to this paper we cited two examples, wood and pollen, of recent confrontations of morphological with molecular data. Similar 'deep morphological' investigations – in depth comparisons of morphological and anatomical facts with the new phylogenetic hypotheses – need to be undertaken for most features of Rubiaceae. Biogeographical factors on the contrary appear to have had a major impact on Rubiaceae evolution, and many of the newer clades here proposed or confirmed are biogeographically better supported than the old concepts. We intend to examine this further using the DIVA algorithm (Ronquist 1997).

Understanding the phylogeny was secondly hampered previously by the existence of the enumerated less successful lineages or 'basal groups.' Many of the disputes of the past are related to these groups. Several taxonomic problems were the direct result of attempts to attach these deviating taxa to the obvious, well established alliances. Among the basal Rubioideae the genus *Lasianthus* and its supposed relatives form a typical example. These genera have shifted to and from between Psychotrieae and Morindeae (see Igersheim & Robbrecht 1994 for a discussion) until molecular results made clear that they belong elsewhere.

The evolutionary pattern detected here for the Rubiaceae was also found in many other groups. The phylogeny of the angiosperms as a whole (e.g. APG 2003) also shows basal groups next to major successful lineages. The resulting skewness in Gentianaceae classification, e.g., was discussed by Albert & Struwe (2002). In Rubiaceae the model repeats itself when moving towards lower taxonomic levels, e.g. it reappears in the two subfamilies and in many of the tribes. This kind of pattern reflects Jonker's (1977) tendency theory, formulated long before the molecular era in phylogeny reconstruction.

Our investigation, finally, allowed to locate the major gaps in our knowledge of the phylogeny of Rubiaceae, viz. the pantropical predominantly herbaceous Spermaceae s.lat., and three neotropical predominantly woody groups, the Condamineae/Calycophylleae alliance, the CCE complex, and the Rondeletieae and Guettardeae and their mutual delimitation.

### Taxonomic conspectus

Classification of the Rubiaceae corresponding to the phylogeny postulated by the analysis of the position of *Luculia* and *Coptosapelta* (R.1; fig. 2) and the constructed supertree (R.2; fig. 4). The numbering follows the numbering of text section D.3.

A placement is provided for all the genera for which molecular data are now available.

Underlined genera are included in the sampling for the supertree; other genera are added if they have been subject to a molecular phylogenetic study (which is then referred to; those references are preceded by an asterisk). Generic names in **bold** correspond to novel relationships postulated here or corroborated here for the first time by molecular data. Evident synapomorphies are mentioned, but completeness is not attempted.

The three or four letter mnemonic abbreviations for tribes and subtribes are taken from Robbrecht (1988: 22) or newly proposed. Generic names are without author, as explained in Robbrecht (1988: 26), but types are given with their author.

**Rubiaceae** Jussieu ('**gardenia and coffee family**'; 'madder family')

**1. Subfamily Cinchonoideae Raf. ('cinchona and ixora subfamily').**

Raphides mostly absent but present in *Coptosapelta* and Ham.; corolla lobe aestivation variable, contorted, imbricate or valvate; secondary pollen presentation common (Puff & al. 1996); iridoids and indole alkaloids; aluminium accumulation in only a few representatives.

**1.1. Basal Cinchonoideae**

**Cop.** **Coptosapelteae** Bremek. ex Darwin; s.str. [position in subfamily Cinchonoideae and sister relationship to entire rest of it postulated by the separate study R.1 (fig. 2) of the position of these two genera; monophyly in need of confirmation]  
*Coptosapelta*, *Luculia*

**1.2. Supertribe Ixoridinae Robbr. & Manen supertrib. nov.;** Cinchonoidearum supertribus cum fructibus plerumque carnosus. *Typus: Ixora L.*

Not easily defined morphologically, but fleshy fruits are predominant; number of ovules varying from numerous to one per locule; raphides absent.

**1.2.1. Basal Ixoridinae**

**Hen** **Henriquezieae** Bremek., Acta Bot. Neerl. 6: 371 (1957) – corollas mostly zygomorphic

**Hen.H** subtribe **Henriqueziinae** (Bremek.) Robbr. & Manen **stat. nov.** – corolla lobe aestivation imbricate

*Gleasonia*, *Henriquezia* Spruce ex Benth. (tribuum subtribuumque *typus*), *Platycarpum*  
(\* Rova & al. 2002; \* Cortés-B. & al. 2005)

**Hen.P** subtribe **Posoqueriinae** (Delprete) Robbr. & Manen **stat. nov.** [Posoquerieae Delprete, in Delprete, Smith & Klein, Flora ilustrada Catarinense, Rubiaceae. I Parte, Volume I - Géneros de A-G: 1. Alseis até 19. Galium: 23 (2004)] – corolla lobe aestivation contorted; pollen projection mechanism

*Molopanthera*, *Posoqueria* Aubl. (subtribuum *typus*)  
(\* Rova & al. 2002; \* Cortés-B. & al. 2005)

**Sip.** **Sipaneae** Bremek. – corolla lobe aestivation mostly contorted

*Chalepophyllum*, *Dendrosipanea*, *Limnosipanea*, *Maguireothammus*, *Neobertiera*, *Sipanea*, *Sipaneopsis* (\* Rova & al. 2002; \* Delprete & Cortés-B. 2004; \* Cortés-B. & al. 2005)

**1.2.2 Ixoridinae I**

**Cal/Con Calycophylleae/Condamineae alliance**, including also Simireae, Hippotideae and elements some time included in Rondeletieae (tribal delimitation of alliance in need of further investigation)

formerly Con.: *Chimarrhis*, *Condaminea*, *Dioicodendron*, *Picardaea*, *Pinckneya*, *Pogonopus*, *Rustia*

formerly Ron.: *Bathysa*, *Dolichodelphys*, *Elaeagia*, *Macbrideina*, *Phitopis*, *Warszewiczia*

formerly Sim.: *Parachimarrhis*, *Simira*

formerly Hip.: *Sommeria*, *Hippotis*, *Pentagonia*

formerly Cal.: *Alseis*, *Calycophyllum*, *Capirona*, *Ferdinandusa*, *Wittmackanthus*,

formerly inc. sed.: *Emmenopteris*

formerly Dialypetalanthaceae: *Dialypetalanthus*

### 1.2.3 Ixoridinae II

**1.2.3.1 Mussaendeae alliance** (\* Bremer & Thulin 1998) – exotestal cells with pitted thickenings along inner tangential walls; corolla lobe aestivation mostly reduplicate-valvate

**Mus.** **Mussaendeae** Hook.f. – woody plants; inflorescences terminal; some of the calyx lobes frequently changed into semaphylls  
*Heinsia, Mussaenda, Pseudomussaenda*

**Sab.** **Sabiceae** Bremek., Rec. Trav. Bot. Néerl. 31: 253 (1934)

**Sab.S** subtribe **Sabiceinae** (Bremek.) Robbr. & Manen **stat. nov.** – inflorescences axillary and paired at nodes  
*Ecpoma, Pseudosabicea, Sabicea* Aubl. (tribuum subtribuumque *typus*), *Schizostigma, Stipularia* (\* Khan & al. 2005)

**Sab.V** subtribe **Virectariinae** (Verdc.) Robbr. & Manen **stat. nov.** [Virectariae Verdc., Kew Bull. 30: 366 (1975)] – mostly herbaceous to slightly woody, but *Tamridaea* woody; inflorescences terminal  
*Hekistocarpa, Tamridaea, Virectaria* Bremek. (subtribuum *typus*) (\* Dessein et al. 2001b)

### 1.2.3.2 Tribe Retiniphyllae

**Ret.** **Retiniphyllae** Hook.f. – inflorescences terminal; corolla lobe aestivation contorted; ovary plurilocular, two collateral pendulous ovules per locule; pyrenes with apical preformed germination slit  
**Retiniphyllum**

### 1.2.3.3 Vanguerieae alliance

**Alb.** **Alberteae** Hook.f. – corolla lobe aestivation contorted; ovary 2-locular; endosperm reduced; embryos large, with superior radicle  
*Crossopteryx, Alberta, Aulacocalyx*

**Ixo.** **Ixoreae** A.Gray; s.str. – corolla lobe aestivation contorted  
*Aleisanthia, Aleisanthiopsis, Greenea, Ixora, Myonima, Scyphiphora, Versteegia*  
(\* Andreasen & Bremer 2000)

**Van.** **Vanguerieae** Dumort. – corolla lobe aestivation valvate; ovary 2-plurilocular, one pendulous ovule per locule; pyrenes with apical preformed germination slit  
*Afrocanthium, Canthium, Cuviera, Cyclophyllum, Hutchinsonia, Fadogia, Fadogiella, Keetia, Leroya, Meyna, Multidentia, Neoleroia, Peponidium, Pseudopeponidium, Psydrax* (with basal position in the alliance in the supertree), *Pygmaeothamnus, Pyrostria, Robynsia, Rytigynia, Scyphochlamys, Vangueria* (*syn. Ancyranthos, Lagynias, Pachystigma, Tapiphyllum*)  
(\* Lantz et al. 2002, \* Lantz & Bremer 2004, 2005)

### 1.2.3.3 Gardenieae alliance – corolla lobe aestivation contorted

**Cof.** **Coffeae** DC., Ann. Mus. Hist. Nat. 9: 217 (1807), as Coffeaceae.

**Cof.C** subtribe **Coffeinae** (DC.) Robbr. & Manen **stat. nov.** – inflorescences axillary and paired at nodes, in some genera accompanied by terminal inflorescences; ovules per locule 1-few, only in some *Tricalysia* spp. more than 20  
*Argocoffeopsis, Belonophora, Coffea* L. (tribuum subtribuumque *typus*), *Discospermum, Psilanthus, Sericanthe, Tricalysia, Xantonnea* (\* Andreasen & Bremer 2000; these authors have *Paracoffea*, synonym of *Psilanthus*, in the sampling; \* Davis & al. 2006)

**Cof.B** subtribe **Bertierinae** (K.Schum.) Robbr. & Manen **comb. nov.** [Gardenieae subtr. Bertierinae K.Schum., Nat. Pflanzenfam. 4(4): 73 (1891), as Bertiereae; Bertiereae

(K.Schum.) Bridson, Flora Zambes. 5(3): 386 (2003)] – inflorescences terminal; ovules per locule numerous  
*Bertiera* Aubl. (solum genus et subtribuum *typus*)

**Pav.** **Pavetteae** Dumort.; s.str. – ovules many, rarely 1; exotestal cells mostly with thickened outer tangential wall (continuous plate)  
*Coptosperma*, *Dictyandra*, *Leptactina*, *Pavetta*, *Rutidea*, *Tarenna*  
(\* Andreassen & Bremer 2000)

**Oct.** **Octotropideae** Bedd.  
*Cremaspora*, *Feretia*, *Fernelia*, *Hypobathrum*, *Kraussia*, *Paragenipa*, *Polysphaeria*, *Pouchetia*, *Ramosmania* (\* Andreassen & Bremer 2000; see our text for arguments to refute the supertree position of *Paragenipa* in Gar.1)

**Gar.** **Gardenieae** DC. (paraphyletic, monophyletic if Gar. 1 excluded)  
Also belonging to Gardenieae, unplaced in our informal groups: *Amaioua*, *Benkara*, *Borojoa*, *Catunaregam*, *Ceriscoides*, *Coddia*, *Deccania*, *Ibetrulia*, *Kutchubaea*, *Macrosphyra*, *Melanopsidium*, *Oligocodon*, *Oxyceros*, *Preussiodora*, *Sherbournia*, *Sphinctanthus*, *Stachyarrhena*, *Tamilnadia*, *Tarennoidea*, *Tocoyena* (\* Andreassen & Bremer 2000; \* Persson 2000; \* Gustafsson & Persson 2002)

**Gar.1** Gardenieae 1 (basal to a clade with Coffeae, Pavetteae and Octotropideae):  
*Burchellia*, *Didymosalpinx*, *Schumanniophyton*

**W.-A.** *Wendlandia* / *Augusta* group – sister to Gardenieae 2 (see remarks on subg. *Augusta* in text)  
*Wendlandia*, *Augusta* (subgenera *Augusta* & *Lindenia*)

**Gar.2** Gardenieae 2  
*Alibertia*, *Duroia*, *Gardenia*, *Genipa*, *Glossostipula*, *Kailarsenia*, *Porterandia*, *Randia*, *Trukia*

**Gar.3** Gardenieae 3  
*Calochone*, *Casasia*, *Euclinia*, *Rosenbergiodendron*

**Gar.4** Gardenieae 4  
*Aidia*, *Atractocarpus*, °*Calycosiphonia*, *Duperrea*, °*Diplospora*, *Massularia*, *Mitriostigma*, *Morelia*, *Oxyanthus*, *Rothmannia*, *Sukumia* (° supertree position contradicted by \* Andreassen & Bremer 2000 and \* Davis & al. 2006)

**1.3. Supertribe Cinchonidinae** Robbr. & Manen **supertrib. nov.**, Cinchonoidearum supertribus cum fructibus plerumque siccis. *Typus*: *Cinchona* L.  
Not easily defined morphologically, but dry fruits are predominant; numerous ovules except in Nau.Ce & Chi.; raphides absent except in Ham.

#### 1.3.1. Basal Cinchonidinae

**Hym.** **Hymenodictyoneae** Razafim. & B.Bremer – seeds with wing two-forked at base  
*Hymenodictyon*, *Paracorynanthe* (\* Razafimandimbison & Bremer 2002a)

**Nau.** **Naucleae** (DC.) Miq.; s.lat. – inflorescences head-like except in Nau.Co

**Nau.Ce** subtribe **Cephalantinae** DC. – ovules solitary, pendulous  
*Cephalanthus* (\* Razafimandimbison & Bremer 2002b)

**Nau.M** subtribe **Mitragyninae** Havil. – seeds with wing two-forked at base  
*Hallea*, *Mitragyna* (\* Razafimandimbison & Bremer 2002b)

**Nau.Co** subtribe **Corynantheinae** Razafim. & B.Bremer – seeds with wing two-forked at base  
*Corynanthe*, *Pausinystalia* (\* Razafimandimbison & Bremer 2002b)

**Nau.B** subtribe **Breoniinae** Razafim. & B.Bremer (1st group of Adininae sensu Ridsdale 1978)  
*Breonadia*, *Breonia*, *Gyrostipula*, *Janotia*, *Neobreonia*  
(\* Razafimandimbison & Bremer 2002b)

**Nau.M** subtribe **Naucleinae** DC. (incl. subtribe **Uncariinae**)

*Burttidavya*, *Nauclea*, *Neolamarckia*, *Sarcocephalus*, *Uncaria*  
(\* Razafimandimbison & Bremer 2002b)

**Nau.A** subtribe **Adininae** Ridsd. (2nd & 3d group of Adininae sensu Ridsdale 1978)

*Adina*, *Adinauclea*, *Haldina*, *Ludekia*, *Metadina*, *Myrmeconuclea*, *Neonuclea*, *Pertusadina*, *Sinoadina* (\* Razafimandimbison & Bremer 2002b)

**1.3.2. Cinchonidinae I**

**Ron.** **Rondeletieae** (DC.) Miq.; in a strict sense as advocated by \* Rova & al. (2002: clade C5b) and the supertree

*Blepharidium* (formely Hil.), *Roigella*, *Rondeletia*, *Suberanthus*; includes the *Mazaea* group (of Delprete 1999): *Acrosynanthus*, *Ariadne*, *Mazaea*, *Phyllomelia*; ?also *Rachicallis* (formerly Hed.)

**Gue.** **Guettardeae** DC.; probably to be recognized in an enlarged sense to include some former members of Ron.

= clade C5a of \* Rova & al. (2002)

*Antirhea*, *Chomelia*, *Guettarda*, *Machaonia*, *Neoblakea*, *Timonius* (also *Allenanthus*, formerly Chi. and *Gonzalagunia*, formerly inc. sed.)

Former Ron.: *Arachnothryx*, *Javorkaea*, *Rogiera*

**1.3.3. Cinchonidinae II**

**Cin.** **Cinchoneae** DC.

**Cin.C** subtribe **Cinchoninae** DC. – seeds winged

*Ciliosemina*, *Cinchona*, *Cinchonopsis*, *Joosia*, *Ladenbergia*, *Remija*, *Stilpnophyllum*  
(\* Andersson & Antonelli 2005)

**Cin.I** subtribe **Isertiinae** (A.Rich.) Robbr. & Manen **stat. nov.** [Isertiaceae A.Rich., in De Candolle, Prodr. 4: 342, 435 (1830)] – seeds angular; exotestal cells with perforate internal thickenings

*Isertia* Schreber (subtribuum *typus*), *Kerianthera* (\* Andersson & Antonelli 2005)

**Ham.** **Hamelieae** DC. (incl. Hillieae) – epiphytism and succulence common; right-contorted and imbricate corolla aestivation types common; raphides present

*Cosmocalyx*, *Deppea*, *Hamelia*, *Hoffmannia*  
formerly Hil.: *Cosmibuena*, *Hillia* (and its subg. *Ravnia*), *Schenckia*  
formerly Chi.: *Chione*

**CCE** **CCE complex** (Catesbaeeae/Chiococceae alliance; in need of further study: Chi. s.str. monophyletic but having all other elements as a grade at their base) [clade C4 of \* Rova & al. 2002] – monadelphous androecium with hairy filaments; placentas adnate to septum and bearing numerous ovules, but solitary pendulous ovules in Chi.; pollen grains with supracteal echinae

*Strumpfia* sister to rest of alliance

*Exostema* group: *Exostema*, *Solenandra* (\* Motley & al. 2005)

Catesbaeeae s.str.: *Catesbaea*, *Phyllacanthus*

*Portlandia* group: *Coutaportia*, *Coutarea*, *Cubanola*, *Hintonia*, *Isidorea*, *Portlandia*

Cat. s.str. or *Portlandia* group: *Osa* (\* Motley & al. 2005)

Chiococceae (s.str.): *Asemmantha*, *Badusa*, *Bikkia*, *Ceratopyxis*, *Chiococca*, *Eosantha*, *Erithalis*, *Morierina*, *Phialanthus* (see comments in text), *Salzmannia*, *Schmidottia* (formerly *Portlandia* group), *Siemensia*, *Scolosanthus* (\* Motley & al. 2005)

## 2. Subfamily Rubioideae Verdc. ('psychotria and madder subfamily')

Raphides present; corolla lobe aestivation valvate; heterodistyly common; anthraquinones; strong hyperaccumulation of aluminium common (Jansen et al. 2000).

### 2.1. Basal Rubioideae

**Col.** genus *Collettoecema* – woody plants; inflorescences axillary; uniovulate locules; ovules attached to middle of septum; bilocular pyrenes; exotestal cells with thickenings on inner tangential wall; strong aluminium accumulator. Sister to rest of Rubioideae (\* Piesschaert et al. 2000a: also in basal Rubioideae, but in a clade with *Ophiorrhiza*)

**Uro.** **Urophyllaeae** Bremekamp ex Verdc. (incl. Pauridiantheae) – habit woody; multiovulate locules; fleshy fruits; exotestal cells with thickened pitted radial and inner tangential walls; strong aluminium accumulation  
*Maschalocorymbus*, *Praravinia*, *Pravinaria*, *Urophyllum*  
formerly Pau.: *Commitheca*, *Pauridiantha*, *Stelecantha*  
formerly Ise.: *Amphidasya*, *Raritebe*  
(\* Andersson & Rova 1999, \* Bremer & Manen 2000)

**Oph.** **Ophiorrhizeae** Bremek. ex Verdc. – habit woody or herbaceous; multiovulate locules; dry to fleshy fruits; exotestal cells with thickened pitted radial and inner tangential walls  
*Lerchea*, *Neurocalyx*, *Ophiorrhiza*, *Xanthophytum* (\* Bremer 1996, \* Bremer & Manen 2000)

**Per.** **Perameae** Bremek. ex Darwin – perennial or annual herbs; trimerous flowers; circumscissile capsules; exotestal cells with thickened pitted radial and inner tangential walls; strong aluminium accumulators  
*Perama*

**Las.** **Lasiantheae** B. Bremer & Manen (inclusion in Perameae to be considered; monophyly of here proposed delimitation in need of further investigation) – woody plants; drupes; pyrenes with preformed opening mechanism; strong aluminium accumulators  
*Lasianthus*, *Ronabea*, *Saldinia*, *Trichostachys*  
(\* Andersson & Rova 1999, \* Piesschaert & al. 2000a, \* Bremer & Manen 2000)

**Cou.** **Coussareae** Hook.f. [s.lat., incl. Coccocypseleae and Cruckshanksiae, to be revived as subtribes? (\* Andersson & Rova 1999)] – woody and herbaceous representatives; strong aluminium accumulators  
*Coussarea*, *Faramea*  
formerly Coc.: *Coccocypselum*, *Declieuxia*, *Hindsia*  
formerly Cru.: *Cruckshanksia*, *Heterophyllaea*, *Oreopolus*

**2.2. Supertribe Psychotriidinae** Robbr. & Manen **supertrib. nov.**, Rubioidearum supertribus cum habitu lignosa; ovula in loculo solitaria, sed multa in Schradereis et 2 in aliquis Morindeis; fructus saepius carnosus. *Typus*: *Psychotria* L.

= 'Psychotrieae-Morindeae clade' of \* Andersson & Rova (1999)

= 'Psychotria alliance' of \* Bremer & Manen (2000)

Predominantly woody; inflorescences mostly terminal; ovules (2-)1 per locule, but numerous in Sch.; fruits predominantly fleshy, mostly drupes.

**Gae.** **Gaertnereae** Bremek. ex Darwin [syn. Psychotrieae subtribe Gaertnerinae] – long sheathing stipules; secondarily superior ovaries; xylem with parenchyma bands;

compound pollen apertures with crescent-shaped costae; see Igersheim & al. 1994; Jansen & al. 1996)  
*Gaertnera*, *Pagamea*

**Sch.** **Schraderae** Bremek. [to be considered as subtribe in Mor.?] – involucrate heads  
*Schradera*

**Mor.** **Morindeae** Miq., s.lat. – locules 1-ovulate, 2-ovulate in Mor. s.str.; ovary fusion formerly Mor. s.str.: *Appunia*, *Coelopyrena*, *Caelospermum*, *Gynochtodes*, *Morinda* formerly Pri.: *Prismatomeris* formerly *Mitchella* group: *Damnacanthus*, *Mitchella*

**Cra.** **Craterispermeae** Verdc. [rather a subtribe of Mor.?] – inflorescences axillary and paired at nodes, peduncle short but obvious; one-seeded fruits through abortion of ovule in second locule; strong aluminium accumulation  
*Craterispermum*

**Psy.** **Psychotrieae** Cham. & Schtdl., s.str. – stipules caducous; preformed germination slits mostly absent (except in Pacific representatives); red seed coat pigment mostly present = clade F of \* Andersson & Rova 1999 = *Psychotria* complex of \* Andersson 2002b  
*Amaracarpus*, *Calycosia*, *Dolianthus*, *Gillespiea*, *Hydnophytum*, *Myrmecodia*, *Psychotria*, *Squamellaria*, *Straussia*, *Streblosa* (\* Nepokroeff & al. 1999, clade “*Psychotria* sensu stricto”); \* Andersson & Rova 1999: fig. 5, clade F; \* Andersson 2002b)

**Pal.** **Palicoureae** Robbr. & Manen **trib. nov.**, *Psychotrieis* affinis sed testa tenuissima et pyrenis cum fissuris aperientibus. – stipules non-caducous; preformed germination slits mostly present; red seed coat pigment absent = clade K of \* Andersson & Rova 1999 = *Palicourea* complex of \* Andersson 2002b  
*Carapichea*, *Chassalia*, *Geophila*, *Hymenocoleus*, *Palicourea* Aubl. (tribuum *typus*; incl. elements of *Psychotria* subg. *Heteropsychotria*), *Margaritopsis* (syn. *Chazaliella*, *Readea*), *Notopleura*, *Rudgea* (\* Nepokroeff & al. 1999, clade “*Psychotrieae* + subg. *Heteropsychotria*”; \* Andersson & Rova 1999: fig. 5, clade K ; \* Andersson 2002b)

**2.3. Supertribe Rubioidinae** Robbr. & Manen **supertrib. nov.**, Rubioidearum supertribus cum habitu praesertim herbacea; ovula pro loculo multa vel solitaria; fructus saepius sicci. *Typus*: *Rubia* L.

= ‘Hedyotideae-Rubieae clade’ of \* Andersson & Rova 1999

= ‘Spermacoceae alliance’ of \* Bremer & Manen 2000

Predominantly herbaceous; inflorescence position very variable; ovules many-1 per locule.

### 2.3.1. Rubioidinae I

**Dan.** **Danaideae** B. Bremer & Manen – seeds winged  
*Danais*, *Schismatoclada* (\* Bremer & Manen 2000)

**Ant.** **Anthospermeae** Cham. & Schtdl. – adaptations to wind pollination (\* Bremer & Manen 2000):

basal genus *Carpacoce* (\* Anderson & al. 2001)

**Ant.A** subtribe **Anthosperminae** Benth.  
*Anthospermum*, *Galopina*, *Nenax*, *Phyllis* (\* Andersson & Rova 2000)

- Ant.C** subtribe **Coprosminae** Puff  
*Coprosma, Durringtonia, Leptostigma, Nertera, Normandia*  
(\* Andersson & Rova 2000; \* Anderson & al. 2001)
- Ant.O** subtribe **Operculariinae** Benth.  
*Leptostigma, Pomax, Opercularia* (\* Andersson & Rova 2000; \* Anderson & al. 2001)
- Arg.** **Argostemmatae** Bremek. ex Verdc.  
*Argostemma, Mycetia* (\* Andersson & Rova 1999, \* Bremer & Manen 2000)
- Pae.** **Paederieae** DC. – pollen endoapertures absent  
*Leptodermis, Paederia, Spermadictyon, Serissa* (\* Backlund & al. 2005)
- Put.** **Putorieae** (DC.) Sweet – pollen endoapertures absent  
*Putoria, Gaillonia, Plocama* [\* Backlund & al. (2005, proposing to reduce all genera to synonymy of *Putoria*), \* Nie & al. 2005]
- Rub.** **Rubieae** Baill., Hist. Pl. 7: 365, 390 (1880) – pollen endoapertures absent
- Rub.T** subtribe **Theligoninae** Robbr. & Manen **stat. nov.** – extreme anisophylly in upper stem region; flowers unisexual and showing extreme sexual dimorphism; ovary monocarpellous; pollen 4-8-porate  
= Theligoneae Benth., in Benth. & Hook., Gen. Pl. 3: 343 (1880), as ‘Thelygoneae’ under Urticaceae  
*Theligonum* (solum genus et subtribuum *typus*), as Theligoneae: \* Bremer & al. 1995, \* Natali & al. 1995
- Rub.K** subtribe **Kelloggiinae** Robbr. & Manen **subtrib. nov.**; ab alteris Rubieis granis pollinis 3-colpatis differt; ab Rubiinis propter colorem in sicco atrum et stipulas interpetiolares multifidas differt. – pollen 3-colpate  
*Kelloggia* Torr. ex Benth. (solum genus et subtribuum *typus*); as genus sister to Rub.R: also \* Andersson & Rova 1999, \* Nie & al. 2005
- Rub.R** subtribe **Rubiinae** (Baill.) Robbr. & Manen **stat. nov.** – stipules leaf-like; pollen pluricolpate  
*Asperula, Cruciana, Crucianella, Didymaea, Galium, Phuopsis, Rubia* (tribuum subtribuumque *typus*), *Sherardia, Valantia* (as Rubieae: \* Manen & al. 1994, \* Natali & al. 1995, \* Nie & al. 2005)

### 2.3.2. Rubiidae II

- Kno.** **Knoxieae** Hook.f.; s.lat. Not obvious whether the following three groups inside Kno. s.lat. are monophyletic or whether recognition of one of them makes the rest paraphyletic (see also \* Piesschaert & al. 2000a, \* Bremer & Manen 2000, \* Dessein & al. 2005) formerly Triainolepideae (to be recognized as a subtribe?): *Paratriainia, Triainolepis* formerly Knoxieae s.str.: *Knoxia, Pentanisia* formerly *Pentas* group of the Hedyotideae: *Batopedina, Carphalea, Otiophora, Otomeria, Parapentas, Pentas*
- Spe.** **Spermacoceae** Bercht. & J.Presl.; s.lat.  
Tribal recognition of Spermacoceae s.str. makes remainder of the clade paraphyletic; further studies needed to see whether subtribes can be distinguished.  
formerly Hedyotideae: *Agathisanthemum, Amphiasma, Arcytophyllum, Bouvardia, Conostomium, Dentella, Dibrachionostylus, Gomphocalyx, Hedyotis, Houstonia, Kohautia, Lelya, Manostachya, Manettia, Mitrasacmopsis, Nesohedyotis, Oldenlandia, Pentanopsis, Pentodon, Synaptantha* (\* Thulin & Bremer 2004; \* Dessein & al. 2005)  
formerly Spermacoceae s.str.: *Crusea, Diodia, Emmeorhiza, Ernodea, Hemidiodia, Hydrophylax, Galianthe, Gomphocalyx, Hemidiodia, Mitracarpus, Phyllohydrax, Psyllocarpus, Richardia, Spermacoce* (\* Thulin & Bremer 2004; \* Dessein & al. 2005)

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## References

- Albert V.A. & Struwe L. (2002) Gentianaceae in context. In: Struwe L. & Albert V.A. (eds.), *Gentianaceae. Systematics and Natural History*: 1-20. Cambridge, Cambridge University Press.
- Anderson C.L., Rova H.E. & Andersson L. (2001) **Molecular phylogeny of the tribe Anthospermeae (Rubiaceae): systematic and biogeographic implications.** *Austral. J. Bot.* **14**: 231-244.
- Andersson L. (1995) Tribes and genera of the Cinchoneae complex. *Ann. Missouri Bot. Garden* **82**: 409-427.
- Andersson L. (1996) Circumscription of the tribe Isertieae (Rubiaceae). *Opera Bot. Belg.* **7**: 139-164.
- Andersson L. (2001) Margaritopsis (Rubiaceae, Psychotriaceae) is a pantropical genus. *Syst. Geogr. Pl.* **71**: 73-85.
- Andersson L. (2002a) Re-establishment of Carapichea (Rubiaceae, Psychotriaceae). *Kew Bull.* **57**: 363-374.
- Andersson L. (2002b) Relationships and generic circumscriptions in the Psychotria complex (Rubiaceae, Psychotriaceae). *Syst. Geogr. Pl.* **72**: 167-202.
- Andersson L. & Antonelli A. (2005) Phylogeny of the tribe Cinchoneae (Rubiaceae), its position in Cinchonoideae, and description of a new genus, Ciliosemina. *Taxon* **54**: 17-28.
- Andersson L. & Persson C. (1991) Circumscription of the tribe Cinchoneae (Rubiaceae) - a cladistic approach. *Pl. Syst. Evol.* **178**: 65-94.
- Andersson L. & Rova H.E. (1999) The rps16 intron and the phylogeny of the Rubioideae (Rubiaceae). *Pl. Syst. Evol.* **214**: 161-186.
- Andreasen K., Baldwin B.G. & Bremer B. (1999) Phylogenetic utility of the nuclear rDNA ITS region in subfamily Ixoroideae (Rubiaceae): comparisons with cpDNA rbcL sequence data. *Pl. Syst. Evol.* **217**: 119-135.
- Andreasen K. & Bremer B. (1996) Phylogeny of the subfamily Ixoroideae (Rubiaceae). *Opera Bot. Belg.* **7**: 119-138.
- Andreasen K. & Bremer B. (2000) Combined phylogenetic analysis in the Rubiaceae-Ixoroideae: morphology, nuclear and chloroplast DNA data. *Amer. J. Bot.* **87**: 1731-1748.
- APG (2003) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. *Bot. J. Linn Soc.* **141**: 399-436.
- Backlund M., Bremer B. & Thulin M. (2005) Paraphyly of Paederieae, and recognition of Putorieae, *XVII International Botanical Congress, Abstracts*: 438. Vienna.
- Baillon H. (1878a) Sur les limites du genre Ixora. *Adansonia* **12**: 213-219.
- Baillon H. (1878b) Sur l'organisation et les affinités du Jackia. *Bull. Mens. Soc. Linn. Paris* **1**: 185-188.
- Baillon H. (1879a) Sur des racicules d'embryon à direction anormale. *Adansonia* **12**: 320-323.
- Baillon H. (1879b) Sur les Gaertnera et sur la valeur du groupe des Gaertnerées. *Bull. Mens. Soc. Linn. Paris* **1**: 209-210.
- Bangoura D. (1993) Ovary structure and placentation in the tribe Pauridiantheae (Rubiaceae). *Bull. Jard. Bot. Nat. Belg.* **62**: 419-428.
- Bininda-Emonds O.R.P., Jones K.E., Price S.A., Cardillo M., Grenyer R. & Purvis A. (2004) Garbage in, garbage out. In: Bininda-Emonds O.R.P. (ed.) *Phylogenetic supertrees. Combining information to reveal the tree of life*. Kluwer Academic Publishers, Dordrecht, Boston, London, pp. 1-13.
- Bremekamp C.E.B. (1934) Notes on the Rubiaceae of Surinam. *Recueil Trav. Bot. Néerl.* **31**: 248-308.
- Bremekamp C.E.B. (1950) Schizocolea linderi (Hutch. et Dalz.) Brem. Rubiaceae. Tribe Coussareae [sic]. *Hooker's Icon. Pl. ser. 5*, **5**(4): tab. 3482.
- Bremekamp C.E.B. (1952) The African species of Oldenlandia L. sensu Hiern et K. Schumann. *Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., Tweede Sect.* **48**(2): 297 pp.
- Bremekamp C.E.B. (1954) Les sous-familles et les tribus des Rubiacées. Huit. Congr. Int. Bot., Paris 1954, Rapp. Comm. parvenus avant Congr. sect. 2, 4, 5 & 6 : 113-114.

- Bremekamp C.E.B.** (1956) Monographie des Triainolépidées, tribu nouvelle des Rubioidees (Rubiacees). *Proc. Kon. Ned. Akad. Wet. Amsterdam, ser. C* **59**: 1-21.
- Bremekamp C.E.B.** (1957) On the position of *Platyarpum* Humb. et Bonpl., *Henriquezia* Spruce and *Gleasonia* Standl. *Acta Bot. Neerl.* **6**: 351-377.
- Bremekamp C.E.B.** (1958) Monographie des genres *Cremocarpon* Boiv. ex Baill. et *Pyragra* Brem. (Rubiacees). *Candollea* **16**: 147-177.
- Bremekamp C.E.B.** (1966) Remarks on the position, the delimitation and the subdivision of the Rubiaceae. *Acta Bot. Neerl.* **15**: 1-33.
- Bremer B.** (1987) The sister group of the paleotropical tribe Argostemmatae: a redefined neotropical tribe Hamelieae (Rubiaceae, Rubioideae). *Cladistics* **3**(1): 35-51.
- Bremer B.** (1992) Phylogeny of the Rubiaceae (Chiococceae) based on molecular and morphological data. Useful approaches for classification and comparative ecology. *Ann. Missouri Bot. Gard.* **79**(2): 380-387.
- Bremer B.** (1996) Phylogenetic studies within Rubiaceae and relationships to other families based on molecular data. *Opera Bot. Belg.* **7**: 33-50.
- Bremer B., Andreassen K. & Olsson D.** (1995) Subfamilial and tribal relationships in the Rubiaceae based on rbcL sequence data. *Ann. Missouri Bot. Gard.* **82**(3): 383-397.
- Bremer B. & Jansen R.K.** (1991) Comparative restriction site mapping of chloroplast DNA implies new phylogenetic relationships within Rubiaceae. *Amer. J. Bot.* **78**: 198-213.
- Bremer B., Jansen R.K., Oxelman B., Backlund M., Lantz H. & Kim K.-J.** (1999) More characters or more taxa for a robust phylogeny – case study from the Coffee family (Rubiaceae). *Syst. Biol.* **48**: 413-435.
- Bremer B. & Manen J.-F.** (2000) Phylogeny and classification of the subfamily Rubioideae (Rubiaceae). *Pl. Syst. Evol.* **225**: 43-72.
- Bremer B. & Thulin M.** (1998) Collapse of Isertieae, re-establishment of Mussaendeae, and a new genus of Sabiceae (Rubiaceae); phylogenetic relationships based on rbcL data. *Pl. Syst. Evol.* **211**: 71-92.
- Bridson D.M.** (1992) The genus *Canthium* in tropical Africa. *Kew Bull.* **47**: 353-401.
- Bridson D.M. & Robbrecht E.** (1985) Further notes on the tribe Pavetteae (Rubiaceae). *Bull. Jard. Bot. Nat. Belg.* **55**(1/2): 83-115.
- Bridson D.M. & Verdcourt B.** (2003) 94. Rubiaceae (part 3). *Flora Zambesiaca*, **5**(3): 379-720. Royal Botanic Gardens, Kew.
- Buchner R.** (1995) Biosystematische Untersuchungen an ausgewählten Gattungen der Tribus Urophylleae (Rubiaceae). Thesis Univ. Vienna, Vienna, 222 pp.
- Buchner R. & Puff C.** (1993) The genus complex *Danais-Schismatoclada-Payera* (Rubiaceae). Character states, generic delimitation and taxonomic position. *Bull. Mus. Nat. Hist. Nat., 4ème sér.* **15** (section B, *Adansonia* n° 1-4): 23-74.
- Cortés-B. R., Delprete P. & Motley T.J.** (2005) Phylogenetic analysis of the subfamily Ixoroideae (Rubiaceae), with a focus on the controversial systematic position of some genera. *XVII International Botanical Congress - Abstracts*: 152.
- Darwin S.P.** (1976) The subfamilial, tribal and subtribal nomenclature of the Rubiaceae. *Taxon* **25**: 595-610.
- Daubin V., Perrière G. & Gouy M.** (our consultation March 2005) Genome scale phylogenetic analysis of bacteria. <http://www.ii.uib.no/bio2002/programme/ppt/gouy/sld001.htm>
- Davis A.P., Chester M., Maurin O. & Fay M.F.** (2006) **Searching for the relatives of Coffea (Rubiaceae, Ixoroideae): the circumscription and phylogeny of Coffeae based on plastid sequence data and morphology.** *Amer. J. Bot.*, submitted.
- De Block P.** (2002; "2001") Reinstatement of the Afro-Malagasy genus *Coptosperma* (Rubiaceae, Ixoroideae, Pavetteae). *Syst. Geogr. Pl.* **71**: 455-492.
- Deb D.B.** (1996) Taxonomic and nomenclatural status of *Myrioneuron* R. Br. ex Hook. F. (Rubiaceae). *J. Bombay Nat. Hist. Soc.* **93**: 30-33.
- Delprete P.G.** (1996a) Evaluation of the tribes Chiococceae, Condamineae and Catesbaeae (Rubiaceae) based on morphological characters. *Opera Bot. Belg.* **7**: 165-192.
- Delprete P.G.** (1996b) Notes on the taxonomic position of the monotypic Brazilian genus *Kerianthera* (Rubiaceae). *Opera Bot. Belg.* **7**: 271-275.
- Delprete P.G.** (1997) Revision and typification of Brazilian *Augusta* (Rubiaceae, Rondeletieae), with ecological observations on the riverine vegetation of the cerrado and Atlantic forests. *Brittonia* **49**: 487-497.
- Delprete P.G.** (1998) Notes on calycophyllous Rubiaceae. Part III. Systematic position of the monotypic Mexican genus *Cosmocalyx* and notes on the calycophyll development. *Brittonia* **50**: 309-317.
- Delprete P.G.** (1999) Rondeletieae (Rubiaceae) - Part I (*Rustia*, *Tresanthera*, *Condaminea*, *Picardaea*, *Pogonopus*, *Chimarrhis*, *Dioicodendron*, *Molopanthera*, *Dolichodelphys*, and *Parachimarrhis*). *Fl. Neotrop. Monogr.* **77**: 226 pp.
- Delprete P.G. & Cortés-B. R.** (2004) A phylogenetic study of the tribe Sipaneeae (Rubiaceae, Ixoroideae), using trnL-F and ITS sequence data. *Taxon* **53**: 347-356.

- Delprete P.G., Smith L.B. & Klein R.M.** (2004) Rubiaceae. I Parte, Volume I - Géneros de A-G: 1. Aiseis até 19. Galium. In: Reis A. (ed.), *Flora ilustrada Catarinense*.
- Dessein S., Andersson L., Geuten K., Smets E. & Robbrecht E.** (2005a) Gomphocalyx and Phylohydrax (Rubiaceae): sister taxa excluded from Spermaceae s.s., featuring a remarkable case of convergent evolution. *Taxon* **54**(1): 91-107.
- Dessein S., Andersson L., Robbrecht E. & Smets E.** (2001a) Hekistocarpa (Rubiaceae): a member of an emended tribe Virectarieae. *Pl. Syst. Evol.* **229**: 59-78.
- Dessein S., Jansen S., Huysmans S., Robbrecht E. & Smets E.** (2001b) A morphological and anatomical survey of Virectaria (African Rubiaceae), with a discussion of its taxonomic position. *Bot. J. Linn. Soc.* **137**: 1-29.
- Dessein S., Ochoterena H., De Block P., Lens F., Robbrecht E., Schols P., Smets E., Vinckier S. & Huysmans S.** (2005b) Palynological characters and their phylogenetic signal in Rubiaceae. *Bot. Rev.* **71**: 354-414.
- Ducke A.** (1932) Neue Arten aus der Hylaea Brasiliens. *Notizbl. Kön. bot. Gart. Mus. Berlin* **11**: 471-483.
- Emshwiller E. & Doyle J.J.** (1999) Chloroplast-expressed glutamine synthetase (npsGS): potential utility for phylogenetic studies with an example from Oxalis (Oxalidaceae). *Mol. Phylog. Evol.* **12**: 310-319.
- Exell A.W.** (1944) Catalogue of the vascular plants of S. Tomé (with Principe and Annobon), London.
- Eyde R.H. & Tseng C.C.** (1969) Flower of Tetraplasandra gymnocarpa. Hypogyny with Epigynous Ancestry. *Science* **166**: 506-508.
- Fay M.F., Bremer B., Prance G.T., van der Bank M., Bridson D. & Chase M.W.** (2000) Plastid rbcL sequence data show Dialypetalanthus to be a member of Rubiaceae. *Kew Bull.* **55**(4): 853-864.
- Frodin D.G.** (2004) History and concepts of big plant genera. *Taxon* **53**: 753-776.
- Garcia Kirkbride M.C.** (1979) Review of the neotropical Iseriteae (Rubiaceae). *Brittonia* **31**(3): 313-332.
- Gielly L. & Taberlet P.** (1994) The use of chloroplast DNA to resolve plant phylogenies: noncoding versus rbcL sequences. *Mol. Biol. Evol.* **11**: 769-777.
- Gustafsson C. & Persson C.** (2002) Phylogenetic relationships among species of the neotropical genus Randia (Rubiaceae, Gardenieae) inferred from molecular and morphological data. *Taxon* **51**: 661-674.
- Hallé F.** (1961) Contribution à l'étude biologique et taxonomique des Mussaendeae (Rubiaceae) d'Afrique tropicale. *Adansonia*, sér. 2, **1**: 266-298.
- Hallé F.** (1967) Étude biologique et morphologique de la tribu des Gardéniées (Rubiaceae). *Mém. O.R.S.T.O.M.* **22**: 146 pp., 5 pl.
- Hallé N.** (1966) Familles des Rubiacées, 1ère partie. *Flore du Gabon* **12**: 278 pp.
- Hooker J.D.** (1873) Ordo LXXXIV: Rubiaceae. In: Bentham G. & Hooker J.D. (Editors), *Genera Plantarum* 2: 7-151. London.
- Huysmans S.** (1993) De pollenmorfologie van de Coptosapelteae (Rubiaceae-Cinchonoideae). Thesis, K.U.L., Leuven, 132 pp.
- Huysmans S., Dessein S., Smets E. & Robbrecht E.** (2003) Pollen morphology of NW European representatives confirms monophily of Rubiaceae (Rubiaceae). *Rev. Palaeobot. Palynol.* **127**: 219-240.
- Huysmans S., Robbrecht E., Delprete P. & Smets E.** (2000; "1999") Pollen morphological support for the Catesbaeae-Chiococceae-Exostema-complex (Rubiaceae). *Grana* **38**: 325-338.
- Igersheim A.** (1992) The ovary, fruit and seed development of Craterispermum (Rubiaceae). *Belg. J. Bot.* **125**(1): 101-113.
- Igersheim A.** (1993) The character states of the Caribbean monotypic endemic Strumpfia (Rubiaceae). *Nordic J. Bot.* **13**(5): 545-559.
- Igersheim A., Puff C., Leins P. & Erbar C.** (1994) Gynoecial development of Gaertnera Lam. and of presumably allied taxa of the Psychotrieae (Rubiaceae): secondarily 'superior' vs. inferior ovaries. *Bot. Jahrb. Syst.* **116**(3): 401-414.
- Igersheim A. & Robbrecht E.** (1994; '1993') The character states and relationships of the Prismatomerideae (Rubiaceae-Rubioideae). Comparisons with Morinda and comments on the circumscription of the Morindeae s.str. *Opera Bot. Belg.* **6**: 61-79.
- Igersheim A. & Rohrhofer U.** (1993) The tribal position of Otiophora Zucc. (Rubiaceae): new evidence from gynoecium structure and development. *S. African J. Bot.* **59**(4): 431-441.
- Jansen S.** (2001) Comparative wood anatomy of the Rubiaceae and phylogenetic implications of aluminium hyperaccumulation and vested pits in angiosperms. Thesis, K.U.Leuven, Leuven, 377 pp.
- Jansen S., Dessein S., Piesschaert F., Robbrecht E. & Smets E.** (2000a) Aluminium accumulation in leaves of Rubiaceae: Systematic and phylogenetic implications. *Ann. Bot.* **85**: 91-101.
- Jansen S., Robbrecht E., Beekman H. & Smets E.** (1996) Gaertnera and Pagamea: genera within the Psychotrieae or constituting the tribe Gaertnerae? A wood anatomical and palynological approach. *Bot. Acta* **109**: 466-476.
- Jansen S., Robbrecht E., Beekman H. & Smets E.** (2000b) Aluminium accumulation in Rubiaceae: an additional character for the delimitation of the subfamily Rubioideae? *IAWA Journal* **21**: 197-212.

- Jansen S., Robbrecht E., Beekman H. & Smets E.** (2002) A survey of the systematic wood anatomy of the Rubiaceae. *IAWA Journal* **23**: 1-67.
- Jérémie J. & Hallé N.** (1976) Le genre *Bikkia* (Rubiaceae-Condamineae) en Nouvelle Calédonie. *Adansonia*, sér. 2, **15**: 341-355.
- Jonker F.P.** (1977) Der Tendenzbegriff: eine Anschauung gerichtet auf die Evolution im Pflanzenreich. *Ber. Deutsch. Bot. Ges.* **90**: 391-396.
- Khan S.A., Razafimandimbison S.G., Bremer B., Meve U. & Liede-Schumann S.** (2005) First phylogeny of Sabiceae (Rubiaceae - Sabiceae) and its allied genera inferred from ITS data, *XVII International Botanical Congress. Abstracts*: 438.
- Kiehn M.** (1995) Chromosome survey of the Rubiaceae. *Ann Missouri Bot. Garden* **82**: 398-408.
- Kirkbride J.H.** (1997) Manipulus rubiacearum - VI. *Brittonia* **49**(3): 354-379.
- Kirkbride J.H.** (1979) *Raritebe*, an overlooked genus of Rubiaceae. *Brittonia* **31**: 299-312.
- Koek-Noorman J.** (1976) Juvenile characters in the wood of certain Rubiaceae with special reference to *Rubia fruticosa*. *I.A.W.A. Bull.* **3**: 38-42.
- Koek-Noorman J. & Puff C.** (1983) The wood anatomy of Rubiaceae tribes Anthospermeae and Paederieae. *Pl. Syst. Evol.* **143**: 17-45.
- Lantz H., Andreassen K. & Bremer B.** (2002) Nuclear rDNA ITS sequence data used to construct the first phylogeny of Vanguerieae (Rubiaceae). *Pl. Syst. Evol.* **230**: 173-187.
- Lantz H. & Bremer B.** (2004) Phylogeny inferred from morphology and DNA data: characterizing well-supported groups in Vanguerieae (Rubiaceae). *Bot. J. Linn Soc.* **146**: 257-283.
- Lantz H. & Bremer B.** (2005) Phylogeny of the complex Vanguerieae (Rubiaceae) genera *Fadogia*, *Rytigynia*, and *Vangueria* with close relatives and a new circumscription of *Vangueria*. *Pl. Syst. Evol.* **253**: 159-183.
- Léonard J.** (1984) Contribution à la connaissance de la flore de l'Iran. - VI. Le "complexe *Gaillonia* A. Rich. ex DC." Rubiaceae. *Bull. Jard. Bot. Nat. Belg.* **54**: 493-497.
- Maddison W.P. & Maddison D.R.** (1992) *MacClade: Analysis of Phylogeny and Character Evolution*. Version 3.0. Sinauer Associates, Sunderland, Massachusetts.
- Manen J.-F. & Natali A.** (1996) The chloroplast atpB-rbcL spacer in Rubiaceae. *Opera Bot. Belg.* **7**: 51-57.
- Manen J.-F., Natali A. & Ehrendorfer F.** (1994) Phylogeny of Rubiaceae-Rubieae inferred from the sequence of a cpDNA intergene region. *Pl. Syst. Evol.* **190**: 195-211.
- McNeil J. & Turland N.** (2005) Synopsis of proposals on botanical nomenclature - Vienna 2005. A review of the proposals concerning the International Code of Botanical Nomenclature submitted to the XVII International Botanical Congress. *Taxon* **54**: 215-250.
- Motley T.J.** (2005) Phylogeny of the "Hedyotis" group (Rubiaceae), *XVII International Botanical Conference, Vienna, Abstracts*: 438.
- Motley T.J., Wurdack K.J. & Delprete P.G.** (2005) Molecular systematics of the Catesbaeeae-Chiococceae complex (Rubiaceae): flower and fruit evolution and biogeographic implications. *Amer. J. Bot.* **92**(2): 316-329.
- Natali A., Manen J.F. & Ehrendorfer F.** (1995) Phylogeny of the Rubiaceae - Rubioideae, in particular the tribe Rubieae: Evidence from a non-coding chloroplast DNA sequence. *Ann. Missouri Bot. Gard.* **82**: 428-439.
- Nepokroeff M., Bremer B. & Sytsma K.J.** (1999) Reorganization of the genus *Psychotria* and tribe Psychotrieae (Rubiaceae) inferred from ITS and rbcL sequence data. *Syst. Bot.* **24**: 5-27.
- Nie Z.-L., Wen J., Sun H. & Bartholomew B.** (2005) Monophyly of *Kelloggia* Torrey ex Benth. (Rubiaceae) and evolution of its intercontinental disjunction between western North America and eastern Asia. *Amer. J. Bot.* **92**(4): 642-652.
- Ntore S., De Block P., Huysmans S., Robbrecht E. & Dessein S.** (2003) Two new species from Gabon show the need to reduce *Commitheca* to the synonymy of *Pauridiantha* (Rubiaceae, Pauridiantheae). *Bot. J. Linn Soc.* **141**: 105-117.
- Olmstead R.G. & Reeves P.A.** (1995) Evidence for the polyphyly of the Srophulariaceae based on chloroplast rbcL and ndhF sequences. *Ann. Missouri Bot. Gard.* **82**: 176-193.
- Oxelman B. & Bremer B.** (2000) Discovery of paralogous nuclear gene sequences coding for the second-largest subunit of RNA polymerase II (RPB2) and their utility in Gentianales of the Asterids. *Mol. Biol. Evol.* **17**: 1131-1145.
- Oxelman B., Lidén M. & Berglund D.** (1997) Chloroplast rps16 intron phylogeny of the tribe Sileneae (Caryophyllaceae). *Pl. Syst. Evol.* **206**: 393-410.
- Persson C.** (2000) Phylogeny of the Neotropical *Alibertia* group (Rubiaceae), with emphasis on the genus *Alibertia*, inferred from ITS and 5S ribosomal DNA sequences. *Amer. J. Bot.* **87**(7): 1018-1028.
- Petit E.** (1963) Rubiaceae africanae X. *Colletocema* genre nouveau de Rubiaceae d'Afrique. *Bull. Jard. Bot. Etat, Brux.* **33**: 375-380.
- Petit E.** (1964) Les espèces africaines du genre *Psychotria* L. (Rubiaceae) - I. *Bull. Jard. Bot. Etat, Brux.* **34**: 1-228.
- Petit E.** (1966) Les espèces africaines du genre *Psychotria* L. - II. *Bull. Jard. Bot. Etat, Brux.* **36**: 65-189.
- Piesschaert F.** (2001) Carpology and pollen morphology of the Psychotrieae. Thesis K.U.L., Leuven.

- Piesschaert F., Andersson L., Jansen S., Dessein S., Robbrecht E. & Smets E.** (2000a) Searching for the taxonomic position of the African genus *Colletocema* (Rubiaceae): morphology and anatomy compared to an rps16-intron analysis of the Rubioideae. *Canad. J. Bot.* **78**: 288-304.
- Piesschaert F., Huysmans S., Jaimes I., Robbrecht E. & Smets E.** (2000b) Morphological evidence for an extended tribe - Coccocypseleae (Rubiaceae-Rubioideae). *Plant Biol.* **2**: 536-546.
- Piesschaert F., Robbrecht E. & Smets E.** (1997) *Dialypetalanthus fuscescens* Kuhl. (Dialypetalanthaceae): the problematic taxonomic position of an Amazonian endemic. *Ann. Missouri Bot. Gard.* **84**: 201-223.
- Puff C.** (1982) The delimitation of the tribe Anthospermeae and its affinities to the Paederieae. *Bot. J. Linn. Soc.* **84**: 355-377.
- Puff C.** (1983) Studies in Otiophora Zucc. (Rubiaceae): 4. The taxonomic position of the genus. *Bothalia* **14**: 185-188.
- Puff C.** (1986) A biosystematic study of the African and Madagascan Rubiaceae-Anthospermeae. *Pl. Syst. Evol.*, Suppl., 3: ix + 535 pp.
- Puff C.** (1988) Observations on *Carphalea* Juss. (Rubiaceae, Hedyotideae), with particular reference to the Madagascan taxa and its taxonomic position. *Bull. Jard. Bot. Nat. Belg.* **58**(3/4): 271-323.
- Puff C.** (1989) The affinities and relationships of the Japanese endemic *Pseudopyxis* (Rubiaceae - Paederieae). *Plant Spec. Biol.* **4**: 145-155.
- Puff C., Andersson L., Rohrhofer U. & Igersheim A.** (1993) The tribe Schradereae (Rubiaceae) reexamined. *Bot. Jahrb. Syst.* **114**(4): 449-479.
- Puff C. & Buchner R.** (1998a) Development and structure of the comose seeds of *Hillia* (Rubiaceae). *Pl. Syst. Evol.* **210**: 147-157.
- Puff C. & Buchner R.** (1998b) *Lecananthus* and *Leucocodon*, two genera to be added to the tribe Schradereae (Rubiaceae). *Blumea* **43**: 265-286.
- Puff C., Chayamarit K. & Chamchumroon V.** (2005) Rubiaceae of Thailand. A pictorial guide to indigenous and cultivated genera: viii + 245 pp. Bangkok, Forest Herbarium.
- Puff C. & Robbrecht E.** (1989) A survey of the Knoxieae (Rubiaceae - Antirheoideae). *Bot. Jahrb. Syst.* **110**(4): 511-558.
- Puff C., Robbrecht E., Buchner R. & De Block P.** (1996) A survey of secondary pollen presentation in the Rubiaceae. *Opera Bot. Belg.* **7**: 369-402.
- Puff C., Robbrecht E. & Randrianasolo V.** (1984) Observations on the SE African-Madagascan genus *Alberta* and its ally *Nematostylis* (Rubiaceae, Alberteae), with a survey of the species and a discussion of the taxonomic position. *Bull. Jard. Bot. Nat. Belg.* **54**: 293-366.
- Puff C. & Rohrhofer U.** (1994; '1993') The character states and taxonomic position of the monotypic mangrove genus *Scyphiphora* (Rubiaceae). *Opera Bot. Belg.* **6**: 143-172.
- Razafimandimbison S.G. & Bremer B.** (2002a; "2001") Tribal delimitation of Naucleaeae (Cinchonoideae, Rubiaceae): inference from molecular and morphological data. *Syst. Geogr. Pl.* **71**: 515-538.
- Razafimandimbison S.G. & Bremer B.** (2002b) Phylogeny and classification of Naucleaeae s.l. (Rubiaceae) inferred from molecular (ITS, rbcL, and trnT-F) and morphological data. *Amer. J. Bot.* **89**(7): 1027-1041.
- Ridsdale C.E.** (1975) A synopsis of the African and Madagascan Rubiaceae-Naucleaeae. *Blumea* **22**: 541-553.
- Ridsdale C.E.** (1978) A revision of the tribe Naucleaeae s.s. (Rubiaceae). *Blumea* **24**: 307-366.
- Ridsdale C.E.** (1982) A revision of *Badusa* (Rubiaceae, Condamineae, Portlandiinae). *Blumea* **28**: 145-150.
- Robbrecht E.** (1975) *Hymenocoleus*, a new genus of Psychotrieae (Rubiaceae). *Bull. Jard. Bot. Nat. Belg.* **45**: 273-300.
- Robbrecht E.** (1979) The African genus *Tricalysia* A. Rich. (Rubiaceae-Coffeae). 1. A revision of the species of subgenus *Empogona*. *Bull. Jard. Bot. Nat. Belg.* **49**(3/4): 239-360.
- Robbrecht E.** (1980) The Hypobathreae (Rubiaceae-Ixoroideae). 1. Delimitation and division of a new tribe. *Bull. Jard. Bot. Nat. Belg.* **50**: 69-77.
- Robbrecht E.** (1982) Pollen morphology of the tribes Anthospermeae and Paederieae (Rubiaceae) in relation to taxonomy. *Bull. Jard. Bot. Nat. Belg.* **52**: 349-366.
- Robbrecht E.** (1984) The delimitation and taxonomic position of the tropical African genera *Leptactinia* and *Dictyandra* (Rubiaceae). *Pl. Syst. Evol.* **145**: 105-118.
- Robbrecht E.** (1988) Tropical woody Rubiaceae. Characteristic features and progressions. Contributions to a new subfamilial classification. *Opera Bot. Belg.* **1**: 1-272.
- Robbrecht E.** (1994a; '1993'). On the delimitation of the Rubiaceae. A review. *Opera Bot. Belg.* **6**: 19-30.
- Robbrecht E.** (1994b; '1993'). Supplement to the 1988 outline of the classification of the Rubiaceae. Index to genera. *Opera Bot. Belg.* **6**: 173-196.
- Robbrecht E., Bridson D.M. & Deb D.B.** (1993) The South Indian genus *Octotropis* (Rubiaceae). An investigation of its characters and reinstatement of the tribal name Octotropideae. *Opera Bot. Belg.* **6**: 81-91.
- Robbrecht E. & Manen J.-F.** (2005) Phylogeny and biogeography of the angiosperm family Rubiaceae. *XVII Intern. Bot. Congress, Vienna, Abstracts*: 151.

- Robbrecht E. & Puff C.** (1986) A survey of the Gardenieae and related tribes (Rubiaceae). *Bot. Jahrb. Syst.* **108**(1): 63-137.
- Robbrecht E., Puff C. & Igersheim A.** (1991) The genera *Mitchella* and *Damnacanthus*. Evidence for their close alliance, comments on the campylotropy in the Rubiaceae and the circumscription of the Morindeae. *Blumea* **35**: 307-345.
- Robbrecht E., Rohrhofer U. & Puff C.** (1994; '1993') A survey of *Bertiera* (Rubiaceae), including a discussion of its taxonomic position. *Opera Bot. Belg.* **6**: 101-141.
- Rogers G.K.** (1984) *Gleasonia*, *Henriquezia* and *Platycarpum* (Rubiaceae). *Fl. Neotrop. Monogr.* **39**: 135 pp.
- Ronquist F.** (1997) Dispersal-Vicariance analysis: a new approach to the quantification of historical biogeography. *Syst. Biol.* **195**: 195-203.
- Rova H.E., Delprete P.G., Andersson L. & Albert V.A.** (2002) A trnL-F cpDNA sequence study of the Condamineeae-Rondeletieae-Sipaneeae complex with implications on the phylogeny of the Rubiaceae. *Amer. J. Bot.* **89**(1): 145-159.
- Rutishauser R.** (1999) Polymerous leaf whorls in vascular plants: developmental morphology and fuzziness of organ identities. *Int. J. Pl. Sci.* **160** (6 suppl.): S81-S103.
- Salamin N., Hodkinson T.R. & Savolainen V.** (2002) Building supertrees: an empirical assessment using the grass family (Poaceae). *Syst. Biol.* **51**: 136-150.
- Schumann C.** (1888-89) Rubiaceae. Tribus VII. Paederieae, ... - Tribus XIX. Gardenieae. In: de Martius C.F.P., Eichler A.G. & Urban I. (eds.) *Flora Brasiliensis* 6(6).
- Schumann K.** (1891) Rubiaceae. In: Engler A. & Prantl K. (eds.) *Die natürlichen Pflanzenfamilien*. Engelmann, Leipzig, pp. 1-156.
- Smith A.B.** (1994) Rooting molecular trees: problems and strategies. *Biol. J. Linn. Soc.* **51**: 279-292.
- Solereder H.** (1890) Studien über die Tribus der Gaertnereen. *Ber. Deutsch. Bot. Gesellsch.* **8**: (70)-(100).
- Solereder H.** (1893) Ein Beitrag zur anatomischen Charakteristik und zur Systematik der Rubiaceen. *Bull. Herb. Boissier* **1**: 269-286 & 309-326.
- Steyermark J.A.** (1983) The genus *Botryarrhena* in Venezuela. *Ann. Missouri Bot. Gard.* **70**: 207-208.
- Steyermark J.A. & Kirkbride J.H.** (1977) Review of the genus *Perama*. *Brittonia* **29**: 191-198.
- Stoffelen P., Robbrecht E. & Smets E.** (1996) A revision of *Corynanthe* and *Pausinystalia* (African Rubiaceae - Coptosapelteae). *Bot. J. Linn. Soc.* **120**: 287-326.
- Swofford D.L. (2003) **PAUP\*. Phylogenetic analysis using parsimony (\*and other methods). Version 4.** Sunderland, Sinauer.
- Tange C.** (1996) Studies in SE Asiatic Rondeletieae I: The West Malaysian endemic genus *Aleisanthia* (Rubiaceae). *Nordic J. Bot.* **16**(6): 563-570.
- Tange C.** (1997a) A revision of the genus *Mouretia* (Rubiaceae). *Nordic J. Bot.* **17**: 123-132.
- Tange C.** (1997b) Studies in SE Asiatic Rondeletieae II: *Aleisanthiopsis* (Rubiaceae), a new genus from Borneo. *Nordic J. Bot.* **16**(6): 571-578.
- Tange C.** (1998) *Cyanoneuron* (Rubiaceae), a new genus from Borneo and Sulawesi. *Nordic J. Bot.* **18**(2): 147-158.
- Taylor C.M.** (1989) Revision of *Hillia* subg. *Ravnia* (Rubiaceae: Cinchonoideae). *Selbyana* **11**: 26-34.
- Taylor C.M.** (1994) Revision of *Hillia* (Rubiaceae). *Ann. Missouri Bot. Gard.* **81**(4): 571-609.
- Taylor C.M.** (2001) Overview of the neotropical genus *Notopleura* (Rubiaceae: Psychotrieae), with the description of some new species. *Ann. Missouri Bot. Gard.* **88**: 478-515.
- Taylor C.M.** (2004) The neotropical genus *Ronabea* (Rubiaceae, Lasiantheae). *Syst. Geogr. Pl.* **74**: 35-42.
- Taylor C.M.** (2005) *Margaritopsis* (Rubiaceae, Psychotrieae) in the Neotropics. *Syst. Geogr. Pl.* **75**: 161-177.
- Thulin M.** (1998) *Gaillonia* (Rubiaceae-Paederieae) in Africa and Arabia. *Nordic J. Bot.* **18**(1): 31-38.
- Thulin M. & Bremer B.** (2004) Studies in the tribe Spermaceae (Rubiaceae-Rubioideae): the circumscriptions of *Amphiasma* and *Pentanopsis* and the affinities of *Phylohydrax*. *Pl. Syst. Evol.* **247**: 233-239.
- Tirvengadam D.D. & Robbrecht E.** (1985) Remarks on three *Hypobathreae* (Rubiaceae) from Rodrigues, Seychelles and Sri Lanka. *Nordic J. Bot.* **5**(5): 455-461.
- Verdcourt B.** (1950) A revision of the genus *Otiophora* Zucc. (Rubiaceae). *J. Linn. Soc., Bot.* **53**: 383-412.
- Verdcourt B.** (1958) Remarks on the classification of the Rubiaceae. *Bull. Jard. Bot. Etat, Brux.* **28**(3): 209-281.
- Verdcourt B.** (1975) Studies in the Rubiaceae-Rubioideae for the "Flora of Tropical East Africa": I. *Kew Bull.* **30**(2): 247-326.
- Verdcourt B.** (1977) A synopsis of the genus *Chazaliella* (Rubiaceae- Psychotrieae). *Kew Bull.* **31**: 785-818.
- Verdcourt B.** (1989) Rubiaceae (part 1). *Flora Zambesiaca* 5(1).
- Verellen J., Smets E. & Huysmans S.** (2004) The remarkable genus *Coptosapelta* (Rubiaceae): pollen and orbicule morphology and systematic implications. *J. Plant Res.* **117**: 57-68.
- White F.** (1962) *Forest Flora of Northern Rhodesia*. Oxford University Press, Oxford, xxvi + 455 pp.

**White T.J., Bruns T., Lee S and Taylor J.** (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M., Gelfand D., Sninsky J. & White T.J. (eds.) PCR protocols: a guide to methods and applications. Academic Press, San Diego California USA, pp. 315-322.

**Wunderlich R.** (1971) Die systematische Stellung von *Theligonum*. *Oesterr. Bot. Z.* **119**: 329-394.

**Young M.C.M., Braga M.R., Dietrich S.M.C., Bolzani V.S., Trevisan L.M.V. & Gottlieb O.R.** (1996) Chemosystematic markers of Rubiaceae. *Opera Bot. Belg.* **7**: 205-212.

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#### Electronic appendices

See Systematics and Geography of Plants, Supplementary Data Site

<http://www.nbgpublisher.be>

**Appendix 1. Specimen voucher and GenBank data.** List of sequences analysed in this paper, with GenBank accession numbers and voucher information for original sequences generated during this work (accession number in bold characters). Species are under the name used in the present paper (e.g. '*Randia fitzalanii*' as *Trukia fitzalanii*). Asterisks with sequence accession numbers indicate "Ixoroideae" (Ixoridinae) species removed from the analysis because only one Ixoridinae species of the same genus was selected from each data set (see Material and Methods). C.J.B. = Conservatoire et Jardin botaniques de la Ville de Genève; N.B.G. = National Botanic Garden of Belgium, with living collection acquisition number. [Excel worksheet]

**Appendix 2. Bootstrap trees obtained from (A) the *rbcL* matrix** [Powerpoint document], **(B) the *trnL-trnF* matrix** [Powerpoint document] **and (C) the *rps16* matrix** [Powerpoint document]. The branch lengths are proportional to the corresponding bootstrap values and do not represent substitution rate.

**Appendix 3. Supertree constructed from the 4 source trees** given in text figure 2 and electronic appendix 2 A-C [Word document].