

COMBINED PHYLOGENETIC ANALYSIS IN THE RUBIACEAE-IXOROIDEAE: MORPHOLOGY, NUCLEAR AND CHLOROPLAST DNA DATA¹

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Parsimony analyses of morphology, restriction sites of the cpDNA, sequences from the nuclear, ribosomal internal transcribed spacer (ITS), and the chloroplast gene *rbcl* were performed to assess tribal and generic relationships in the subfamily Ixoroideae (Rubiaceae). The tribes Vanguerieae and Alberteae (Antirheoideae) are clearly part of Ixoroideae, as are some Cinchonoideae taxa. Pavetteae should exclude *Ixora* and allies, which should be recognized as the tribe Ixoreae. *Heinsen*, representing Aulacocalyceae, is part of Gardenieae, as is *Duperrea*, a genus earlier placed in Pavetteae. *Posoqueria* and *Bertiera* and the taxa in the subtribe Diplosporinae should be excluded from Gardenieae. *Bertiera* and three Diplosporinae taxa are part of Coffeae, while *Crema* (*Diplosporinae*) is best housed in a tribe of its own, Cremasporeae. The mangrove genus *Scyphiphora*, recently placed in Diplosporinae, is closer to Ixoreae and tentatively included there. The combined analysis resulted in higher resolution compared to the separate analyses, exemplifying that combined analyses can remedy the incapability of one data set to resolve portions of a phylogeny. Twenty-four new *rbcl* sequences representing all five Ixoroideae tribes (sensu Robbrecht) are presented.

Key words: cpDNA; Ixoroideae; morphology; nuclear ITS sequences; phylogeny; *rbcl*; Rubiaceae.

Rubiaceae are the fourth largest angiosperm family (10 200 species; Mabberley, 1997), but have until recently received remarkably little attention from phylogenetic systematists compared to other large families like Asteraceae and Orchidaceae. In recent years, however, the number of higher level phylogenetic studies in Rubiaceae has increased considerably (see, e.g., Bremer and Jansen, 1991; Bremer, Andreasen, and Olsson, 1995; Natali, Manen, and Ehrendorfer, 1995; Andersson and Rova, 1999, for molecular studies; Andersson, 1995, 1996; Delprete, 1996; Persson, 1996, for morphological studies; and Andreasen and Bremer, 1996; Bremer, 1997, for combined molecular and morphological analyses). Renewed interest in systematic relationships of Rubiaceae can be attributed in part to a recent survey and classification of the family by Robbrecht (1988). The situation in Rubiaceae exemplifies the importance of phylogenetic hypotheses as a base to stimulate new classifications based on phylogenetic relationships and points to gaps in our systematic knowledge.

In Robbrecht's classification, Rubiaceae are divided into four subfamilies: Rubioideae, Cinchonoideae, Antirheoideae, and Ixoroideae. The major difference compared with the two other classifications of the family presented during the century

(Verdcourt, 1958; Bremekamp, 1966) is the much wider delimitation of the subfamily Antirheoideae. It has now been shown, by different investigators (Bremer, Andreasen, and Olsson, 1995; Rova and Andersson, 1995; Andersson, 1996; Delprete, 1996; Young et al., 1996; Andersson and Rova, 1999), that this subfamily is polyphyletic and, as a consequence, the remaining subfamilies need to be recircumscribed. The subfamily Rubioideae seems to be well supported as a group (Bremer, 1996; Manen and Natali, 1996), but the exact delimitation of Cinchonoideae and Ixoroideae remains to be further explored. In addition, tribal delimitations, especially in the large "Gardenieae-Ixoreae" complex (= Ixoroideae sensu Robbrecht), are still not settled.

The Ixoroideae sensu Robbrecht comprise about one-fifth of all Rubiaceae genera and consist of five tribes: Gardenieae, Octotropideae, Pavetteae, Aulacocalyceae, and Coffeae. Two of the more well-known Rubiaceae genera are members of Ixoroideae: the economically important *Coffea* and the often cultivated *Gardenia*. The distribution of the subfamily is concentrated in the paleotropics, but the Gardenieae-Gardeniinae and the type genus *Ixora* extend to the tropics of the New World. Typical characteristics used in combination to delimit the Ixoroideae include secondary pollen presentation, entire interpetiolar stipules, contorted aestivation, and different kinds of fleshy fruits. Fruit characters have been very important for classification of the entire family and at the end of the nineteenth century (e.g., Schumann, 1891) the number of seeds per carpel served as the base for classification. As ovule number varies from one to numerous in Ixoroideae this approach placed taxa of this group in different tribes and subfamilies. Taxa with contorted aestivation and numerous seeds were placed in Gardenieae, while those with one seed belonged to "Ixoreae" (correct name should be Coffeae; Darwin, 1976). Bremekamp (1934) recognized this inconsistency and moved a group of genera with drupes from Gardenieae to "Ixoreae." He placed these two tribes together with Vanguerieae, Chioceae, Cremasporeae, Coptosapelteae, and Achranthereae, in the subfamily Ixoroideae (Bremekamp, 1966). Ixoroideae were

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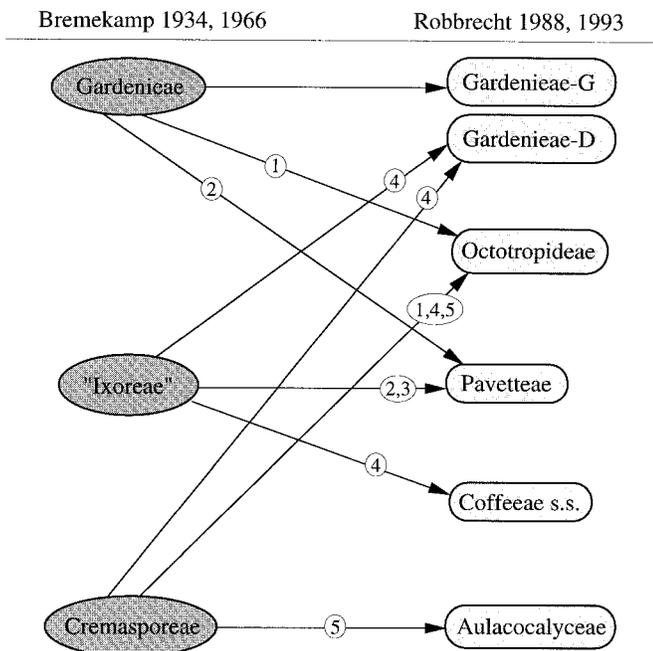


Fig. 1. Tribal classifications of Ixoroideae s.s. (Robbrecht, 1988, 1993) compared to Bremekamp (1934, 1966). Arrows indicate changes in tribal delimitations. Numbers on arrows refer to references where these changes were proposed; 1 = Robbrecht, 1980; 2 = Robbrecht, 1984; 3 = Bridson and Robbrecht, 1985a; 4 = Robbrecht and Puff, 1986. 5 = Robbrecht, Bridson, and Deb, 1993. G = Gardeniinae; D = Diplosporinae.

restricted by Robbrecht (1988) to the genera Bremekamp included in Gardenieae, "Ixoreae," and Cremasporeae. Additionally, Robbrecht moved Vanguerieae and Chiococceae to subfamily Antirrhoideae, while the other two smaller tribes were included in Cinchonoideae.

As for the tribal classification of Ixoroideae several new tribal delimitations have been proposed (Fig. 1). The main part of Bremekamp's "Ixoreae" (1934) is now housed in Pavetteae (Robbrecht, 1984). Genera with axillary inflorescences were transferred to a separate subtribe of the Gardenieae (Diplosporinae), leaving Coffeae restricted to only *Coffea* and *Psilanthus* (Robbrecht and Puff, 1986). Some genera with superior embryo radicles and axillary inflorescences variously treated in Gardenieae (Bremekamp, 1934) or Cremasporeae (Bremekamp, 1966), were merged in tribe Octotropideae (Robbrecht, 1980; Robbrecht, Bridson, and Deb, 1993). In addition, the small tribe Aulacocalyceae was erected for genera with superior embryo radicles but terminal inflorescences (Robbrecht and Puff, 1986).

Recently, cladistic studies concerning generic and tribal arrangements in the subfamily have been published. Persson conducted both a morphological analysis (Persson, 1996) treating Gardenieae, the largest tribe in Ixoroideae, and a molecular analysis (Persson, 1998) using the *rps16* intron and *trnL-trnF* spacer of the chloroplast DNA (cp DNA). Focusing on the whole subfamily, Andreasen and Bremer (1996) used *rbcL* sequence and morphological analyses of four of the five Ixoroideae tribes. Recently, ITS (internal transcribed spacer) data was produced to assess its usefulness in the Ixoroideae (Andreasen, Baldwin, and Bremer, 1999). Despite differences in taxon sampling and results, these cladistic analyses agree in

the exclusion of the Diplosporinae taxa from Gardenieae and the exclusion of the genus *Posoqueria* from the same tribe.

Combined analyses are becoming increasingly more common in phylogenetic studies (see, e.g., Qiu et al., 1999; Soltis, Soltis, and Chase, 1999). Morphological data, as well as molecular data both from the chloroplast and the nuclear DNA, are analyzed simultaneously. Using not only more data, but data from different, independent sources, strengthens the phylogenetic hypothesis and can in some instances reveal inconsistencies between data sets (reviewed in de Queiroz, Donoghue, and Kim, 1995; Huelsenbeck, Bull, and Clifford, 1996; Nixon and Carpenter, 1997). For example, since cpDNA is transferred more readily across lineages than nuclear DNA (see, e.g., Rieseberg and Wendel, 1993), combined cpDNA-nuclear DNA studies have the potential of discovering effects of hybridization events on evolutionary patterns.

In this study of the subfamily Ixoroideae we use data from morphology, restriction site mapping of the cpDNA, and sequences of the chloroplast gene *rbcL* and the ITS region in the nuclear, ribosomal DNA. The present study examines tribal and generic relationships in Ixoroideae and proposes improvements in the present classification.

MATERIALS AND METHODS

Taxa—In the analyses we included taxa representing all the tribes and subtribes of subfamily Ixoroideae sensu Robbrecht (1988, 1993; see Table 1), sampling >40% of the genera. In addition, representatives from the tribes Vanguerieae, Alberteae, Hipptideae, and the genus *Emmenopteryx* (incertae sedis) were included based on results of previous analyses (Bremer and Jansen, 1991; Bremer, Andreasen, and Olsson, 1995; Andreasen, Baldwin, and Bremer, 1999; Bremer et al., 1999). Outgroup taxa used were *Cinchona*, *Luculia*, *Nauclea*, *Sarcocephalus*, and *Hallea*, which all have been found to be outside Ixoroideae s.l. (sensu lato) in earlier analyses (see, e.g., Bremer, Andreasen, and Olsson, 1995). In Robbrecht's classification they belong to the tribes Cinchoneae, Coptosapelteae, and Naucleae and were included in his subfamily Cinchonoideae.

A few terminal taxa are composite taxa (i.e., represented by multiple accessions) because of limited quantities of DNA. For the *rbcL* and restriction enzyme data we used different accessions of *Pouhettia baumanniana*, and different species for *Gardenia* and *Rothmannia* (thought to be closely related; Verdcourt, 1979; Bridson and Verdcourt, 1988; see Table 1). Different accessions of *Nauclea* were used in the *rbcL* and restriction enzyme studies and of *Vangueria* for *rbcL* and ITS. Restriction site information from *Vangueriopsis* was substituted for *Vangueria*.

Robbrecht's classification (Robbrecht, 1988, 1993) will be followed when discussing the results of our analyses, unless otherwise stated.

Missing taxa—For some taxa we do not have information from all of the four data sets. The *rbcL* sequence data set consists of 82 taxa, and, of these, 75 are represented in the morphological data set, 26 in the restriction enzyme data set, and 21 in the ITS sequence data set. As a consequence of the missing taxa, ~30% of the data entries in the combined data matrix are missing. Combining the data sets despite the lack of overlap in taxa represented was preferred since it allowed us to consider all available information simultaneously, and for all of the taxa. Analyses on a subset of the taxa may result in spurious relationships due to incomplete sampling and long branch attraction (Huelsenbeck and Hillis, 1993).

Morphology—The base for the morphological matrix was a compilation of Ixoroideae characters from Robbrecht and Puff (1986) and investigations of herbarium and fresh material. Additional information was compiled from literature (see Andreasen and Bremer, 1996). For taxa not included in Andreasen and Bremer (1996), the following sources were also consulted: Bentham and Hooker, 1862–1883; Hemsley, 1916; Pitard, 1922–1924; Cuatrecasas, 1953;

Cavaco, 1965; Heine and Hallé, 1970; Ridsdale, van den Brink, and Koek-Noorman, 1972; Verdcourt, 1981, 1983; Tirvengadam, 1983; Puff, Robbrecht, and Randrianasolo, 1984; Robbrecht, 1984; Bridson and Robbrecht, 1985b; Tirvengadam and Robbrecht, 1985; Ali and Robbrecht, 1991; Owens et al., 1993; Puff and Rohrhofer, 1993; Puff et al., 1996; De Block, 1997, 1998; De Block and Robbrecht, 1997). The 49 characters (Appendix 1) used in the parsimony analysis are included in the morphological matrix (Appendix 2). Genera were used as terminal taxa unless the assumption of monophyly could be questioned. In those cases the sequenced species was used as the terminal taxon in the morphological matrix (for further discussion see Andreasen and Bremer, 1996).

Molecular methods—Total DNA was extracted from fresh or silica gel dried leaf material using the standard CTAB procedure (Saghai-Marooft et al., 1984; Doyle and Doyle, 1987). In the case of *Heinsenia*, herbarium material was used. The DNA was further purified with cesium chloride/ethidium bromide gradient centrifugation or QiaQuick's polymerase chain reaction (PCR) purification kit (QIAGEN, Valencia, California, USA).

***rbcL* sequence data**—Of the 82 *rbcL* sequences in this study 24 are new (see Table 1 for EMBL number, voucher information for new sequences, and references for earlier published sequences). The *rbcL* region was amplified using 5' and 3' PCR (polymerase chain reaction) primers designed by Olmstead et al. (1992), or, for some problematic DNAs and for automated sequencing, internal primers designed by G. Zurawski (DNAX Research Institute, Palo Alto, California, USA). For manual sequencing the double-stranded DNA was asymmetrically amplified to obtain single-stranded DNA (Kaltenboeck et al., 1992). Single-stranded DNA was cleaned with QiaQuick's PCR purification kit and sequenced with the standard dideoxy chain termination reaction (Sanger, Nicklen, and Coulson, 1977), using the following internal primers (listed with permission from G. Zurawski), numbered by the corresponding first position of *rbcL* of *Zea mays* (R = reverse): *rbcLz*-1 5'-ATGTCACCACAAACAGAACTAAAGCAAGT, *rbcLz*-234 5'-CGTTACAAAGGACGATGCTACCACATCGA, *rbcLz*-427 5'-GCTTATTCAAAACTTTCCAAGGCCCGCC, *rbcLz*-674 5'-TTTATAAATCACAAGCCGAAACTGGTGAATC, *rbcLz*-895 5'-GCAGTTATTGATAGACAGAAAAATCATGGT, *rbcLz*-1020 5'-ACTTTAGGTTTTGTGATTATTGCGCGATGATT, *rbcLz*-1204 5'-TTTGGTGGAGGAACCTTAGGACACCCTTGGGG, *rbcLz*-346R 5'-AAATACGTTACCCACAATGGAAGTAAATAT, *rbcLz*-674R 5'-GATTTTCGCTGTTTCGGCTTGCTTTATAAAA, *rbcLz*-1020R 5'-ATCATCGCGCAATAAATCAACAAAACCTAAAGT, *rbcLz*-1204R 5'-CCCTAAGGGTGTCTAAAGTTTCTCCACC, *rbcLz*-1375R 5'-AATTTGATCTCTCCATATTTCGCA. Both strands were sequenced to obtain at least partial sequence overlap. Five DNAs were sequenced with automated sequencing: *Emmenopterys*, *Dictyandra*, *Tarenna supra-axillaris*, *Hyperacanthus*, and *Coddia*. For these, double-stranded DNA was cycle sequenced using Perkin-Elmer's FS kit and GeneAmp PCR Systems 9600 (Perkin-Elmer Applied Biosystems, Foster City, California, USA). Ethanol precipitation was used to clean the sequenced products before loading the samples on an ABI Prism 377 Automated Sequencer (Perkin-Elmer Applied Biosystems). Nucleotide positions 27 to 1428 in the resulting *rbcL* sequences were used in the subsequent phylogenetic analyses. The variable 3' end of *rbcL* after position 1428 was aligned manually, and blocks of nucleotides were recoded into present/absent characters (Fig. 2). Using blocks, rather than the actual nucleotides, were preferred since the alignment was difficult between more divergent taxa. For some parts of the matrix, certain blocks of nucleotides were assessed as homologous and used as characters in the analyses. If taxa had just a few nucleotides in the 3' region, the alignment was deemed too uncertain for inclusion of these nucleotides in any of the block characters (e.g., "AAA" in *Gardenia augusta-Genipa*). By not including the actual nucleotides in the analyses we may lose information, but by including them we would run the risk of incorrectly grouping taxa based on uncertain homologies. For *Ixora biflora*, *Duroia*, and *Scyphiphora* we were unable to obtain sequences of the 3' end of *rbcL*.

ITS sequence data—ITS sequences for 21 of the taxa sequenced for *rbcL* were obtained (EMBL numbers in Table 1). The ITS region of nrDNA was amplified using ITS 1 (Andreasen, Baldwin, and Bremer, 1999) and ITS 4

(White et al., 1990) and the PCR ingredients and parameters followed Baldwin (1992). The amplified DNA was sequenced using automated sequencing as for the six *rbcL* sequences. Both strands were sequenced using sequencing primers ITS 5 (White et al., 1990), or ITS 1-TM (McDowell and Bremer, 1998), ITS4, ITS 3, and ITS 2 (White et al., 1990). For details about the sequencing procedure and for criteria used in alignment of the ITS sequences see Andreasen, Baldwin, and Bremer (1999).

Restriction enzyme data—Of the taxa in Table 1, 26 were included in the restriction enzyme study (indicated with * in Table 1). The purified DNA was digested with nine restriction endonucleases (EcoRV, ClaI, AvaI, BclI, DraI, BamHI, NcoI, SacI, and HindIII) following the specifications of the manufacturer. The DNA fragments were separated by agarose electrophoreses (1% gels) and transferred to nylon filters by bidirectional blotting (Palmer, 1982, 1986). The filters were hybridized with 16 cloned chloroplast probes from *Lactuca* (Table 2, see also Table 2 in Jansen and Palmer, 1987). Probes were labelled with α -³²P dATP via nick translation. Hybridizations were carried out at 65°C and the DNA fragments were visualized by autoradiography. Fragments <0.4 kb were not visualized. The lengths of the DNA fragments were estimated by comparing them to lambda DNA fragments with known length. For each restriction enzyme, a restriction map containing all taxa was constructed and the sites of the different taxa were aligned relatively to each other. The overlap hybridization method described in Palmer (1986) was used to order the fragments. Restriction site occurrences and absences were used as characters (Table 3). For *Luculia*, *Vangueria*, *Tarenna*, *Ixora*, *Coffea*, *Mitriostigma*, and *Gardenia* character data from the restriction enzyme study of Bremer and Jansen (1991) were added to the matrix (characters 38–61; data matrix with the 61 informative characters in Appendix 3). No informative sites were identified for SacI and the *rbcL* region was not included in the analyses.

Analyses—Separate and combined parsimony analyses were carried out with PAUP 3.1.1 or PAUP* 4.0b2 (Swofford, 1993, 1998), using heuristic methods and with all character changes weighted equally. The following options were used: TBR branch swapping, Mulpars on, Steepest descent off, random addition sequences (at least 100 replicates). The partition homogeneity test (Farris et al., 1995) (1000 replicates, Mulpars off, five TBR replicates) was used to assess incongruencies between the matrices. Support for the groupings in the trees was evaluated using jackknife analysis (Farris et al., 1996) with 10000 replicates, Mulpars off, and five TBR replicates for the combined and the restriction site analyses. In addition, Bremer support values, *b* (Bremer, 1988, 1994; Källersjö et al., 1992), were obtained using PAUP* 4.0b2 and the program Autodecay 4.0.1 (Eriksson, 1998) with ten replicates of random addition for each constraint tree. The program MacClade (Maddison and Maddison, 1992) was used for ACCTRAN reconstruction of character evolution and MacVector 4.1 (Kodak Scientific Imaging Systems, New Haven, Connecticut, USA) for translation of *rbcL* DNA sequences into amino acids.

RESULTS

For the *rbcL* data, 274 (20%) of the 1402 nucleotide positions included in the analysis were variable and 166 (12%) phylogenetically informative. At amino acid sequence level, a few of the nucleotide mutations have caused unique synapomorphies and nonhomoplastic changes in amino acids (consistency index, CI = 1.0). The four *Ixora* species, *Myonima*, and *Versteegia* share a unique change from alanine to cysteine at nucleotide positions 269–271. Coffeeae s.s. (sensu stricto) and *Diplospora* all have valine at positions 392–394, and *Pavetta* and *Tarenna drummondii* are the only taxa with glycine at positions 230–232.

The *rbcL* analysis (excluding the variable 3' end of the gene) resulted in 134978 equally parsimonious trees, 692 steps long (CI = 0.37, and retention index, RI = 0.67). The strict

TABLE 1. Reference, voucher (in UPS unless otherwise stated) or source information for the sequenced taxa. EMBL accession number (EMBL no.) for sequences. *used for the restriction enzyme data. Tribal classification follows Robbrecht (1988, 1993) except for Mussaendeae (Bremer and Thulin, 1998).

Tribe, species, and reference	Voucher/source	EMBL no. <i>rbcL</i> /ITS
Alberteae		
<i>Alberta magna</i> ^c		AJ224843/AJ224842
Aulacocalyceae		
<i>Heinsenia diervilleoides</i>	Mziray and Pocs 84405 ^h	AJ286690
Cinchoneae		
<i>Cinchona pubescens</i> ^a		X83630/AJ224838
Coffeae		
<i>Coffea arabica</i> ^{c*}		X83631/AJ224846
<i>Coffea</i> sp.	Pettersson and Nilsson 765 (K)	AJ286691
<i>Paracoffea melanocarpa</i> ^{c*}		Z68853
<i>Psilanthus ebracteolatus</i>	Andreasen 223	AJ286692
<i>Psilanthus mannii</i> ^{c*}		Z68852/AJ224822
Coptosapelteae		
<i>Hallea rubrostipulata</i> ^{b*}		X83640
<i>Luculia grandiflora</i> ^{a*}		X83648
Gardenieae-Gardeniinae		
<i>Aidia micrantha</i> ^c		Z68844/AJ224835
<i>Aidia racemosa</i>	Cult. Bogor VD.13	AJ286693
<i>Alibertia edulis</i> ^c		Z68843
<i>Bertiera breviflora</i> ^{b*}		X83625
<i>Bertiera guianensis</i> ^c		AJ224845/AJ224841
<i>Borojoa</i> sp.	Bremer et al. 3336 (QCA, QCNE)	AJ286694
<i>Burchellia bubalina</i> ^{c*}		Z68833
<i>Calochone redingii</i> ^c		Z68845/AJ224830
<i>Casasia clusiifolia</i> ^c		Z68831
<i>Coddia rudis</i>	Bremer 3764	AJ286695
<i>Didymosalpinx norae</i> ^{c*}		Z68834
<i>Duroia hirsuta</i>	Bremer et al. 3335 (QCA, QCNE)	AJ286696
<i>Euclinia longiflora</i> ^{c*}		Z68835
<i>Gardenia augusta</i>	Bremer 2720	AJ286697
<i>Gardenia thunbergia</i> ^a		X83637/AJ224833
<i>Gardenia volkensii</i> [*]	Bremer 2723	
<i>Genipa americana</i> ^c		Z68839
<i>Glossostipula concinna</i> ^c		Z68846
<i>Hyperacanthus amoenus</i>	Bremer 3789	AJ286698
<i>Kailarsenia ochreate</i> ^c		Z68847
<i>Massularia acuminata</i> ^c		Z68841
<i>Mitriostigma axillare</i> ^{a*}		X83650
<i>Oxyanthus pyriformis</i> ^{c*}		Z68836/AJ224837
<i>Oxyanthus</i> cf. <i>zanguebaricus</i> ^c		Z68838
<i>Oxyceros</i> sp.	Ridsdale 2235 (L)	AJ286699
<i>Porterandia crosbyi</i> ^c		Z68840
<i>Posoqueria latifolia</i> ^{c*}		Z68850/AJ224828
<i>Randia aculeata</i> ^c		Z68832/AJ224836
<i>Randia demicostata</i>	Ridsdale 2131 (L)	AJ286700
<i>Randia fitzalanii</i> ^c		Z68848
<i>Randia moorei</i> ^c		Z68849/AJ224831
<i>Randia truncata</i>	Lorence 6631 (NTBG)	AJ286701
<i>Rosenbergiodendron longiflorum</i> ^{c*}		Z68830
<i>Rothmannia longiflora</i> ^c		Z68837
<i>Rothmannia manganjae</i> [*]	Bremer 3138	
<i>Schumanniphyton magnificum</i> [*]	Cult Meise Bot Gard. 85-0094 Robbrecht (BR)	AJ286702
<i>Sukunia longipes</i> ^c		Z68842
Gardenieae-Diplosporinae		
<i>CreMASpora triflora</i> ssp. <i>confluens</i> ^c		Z68856/AJ224824
<i>Diplospora polysperma</i>	Ridsdale s.n. Cult. Bogor IV.E.130 (L)	AJ286703
<i>Scyphiphora hydrophyllacea</i> ^s		Y18717
<i>Tricalysia ovalifolia</i> ^{c*}		Z68855
<i>Tricalysia cryptocalyx</i> ^{c*}		Z68854/AJ224827
Hippotideae		
<i>Pentagonia macrophylla</i> ^{b*}		X83658
Mussaendeae		
<i>Mussaenda erythrophylla</i> ^a		X83652/AJ224823
Naucleae		
<i>Nauclea orientalis</i> ^b		X83653
<i>Nauclea</i> sp.*	Cult. Paris Bot. Gard.	
<i>Sarcocephalus latifolius</i> ^{b*}		X83667

TABLE 1. Continued.

Tribe, species, and reference	Voucher/source	EMBL no. <i>rbcL</i> /ITS
Octotropideae		
<i>Feretia aeruginescens</i> ^c		Z68857
<i>Fernelia buxifolia</i>	De Block s.n. (BR)	AJ286704
<i>Hypobathrum racemosum</i>	Cult. Bogor V.D.81	AJ286705
<i>Kraussia floribunda</i> ^c		Z68858
<i>Kraussia socotrana</i>	Thulin & Gifri 8588 (K)	AJ286706
<i>Paragenipa lanceolata</i>	Persson 156 (GB)	AJ286707
<i>Pouchetia baumanniana</i> ^{c,i}		Z68859
<i>Pouchetia baumanniana</i> [*]	Robbrecht s.n. 1988.27.IV	
<i>Ramosmania rodriguesii</i> ^c		Z68860/AJ224834
Pavetteae		
<i>Dictyandra arborescens</i>	Sonké 1788	AJ286708
<i>Duperrea pavettifolia</i>	Andreasen 220	AJ286709
<i>Ixora coccinea</i> ^c		X83646/AJ224826
<i>Ixora hookeri</i> ^c		Z68864
<i>Ixora biflora</i> ^c		Z68866
<i>Ixora parviflora</i> [*]		AJ224844/AJ224840
<i>Leptactina platyphylla</i> ^c		Z68867/AJ224825
<i>Myonima violacea</i>	Kiehn s.n. 3.10.94 (WU)	AJ286710
<i>Pavetta abyssinica</i> ^c		Z68863
<i>Pavetta lanceolata</i> ^c		Z68865/AJ224832
<i>Rutidea orientalis</i> ^c		Z68862
<i>Tarenna cymosa</i> ^a		X83634
<i>Tarenna drummondii</i>	Andreasen 52 (UPS)	AJ286712
<i>Tarenna neurophylla</i> ^{c*}		Z68861
<i>Tarenna supra-axillaris</i>	Bremer 3810	AJ286711
<i>Versteegia grandifolia</i>	Cult. Bogor XI.B.X.VII.25.	AJ286713
Vanguerieae		
<i>Canthium coromandelicum</i> ^c		Z68851
<i>Vangueria madagascariensis</i>		X83670 ^a /AJ224839 ^c
<i>Vangueriopsis lanciflora</i> [*]	Robbrecht s.n. 1988.27.IV	
Incertae sedis		
<i>Emmenopterys henryi</i> ^{g*}		Y18715
<i>Aoranthe penduliflora</i> ^l		Y11845

^a Bremer and Jansen, 1991; ^b Bremer, Andreasen, and Olsson, 1995; ^c Andreasen and Bremer, 1996; ^d Bremer, 1996; ^e Andreasen, Baldwin, and Bremer, 1999; ^f Bremer and Thulin, 1998; ^g Bremer et al., 1999; ^h Herbarium material, det. Verdcourt; ⁱ under the synonym *Pouchetia gillettii*.

consensus tree of these trees is shown in Fig. 3. The restriction enzyme data analysis gave 3228 trees (CI = 0.59, RI = 0.72) and the consensus is rather unresolved (Fig. 4). The morphological matrix resulted in 8827 trees but because of the low number of characters (49) in comparison to the number of taxa (75) there is little overall support, and the strict consensus tree is very unresolved (not shown). The analysis of the 21 ITS sequences resulted in nine most parsimonious tree (98 informative characters plus 13 gap characters coded as present or absent; trees are 386 steps, CI = 0.48, RI = 0.44; see Andreasen, Baldwin, and Bremer, 1999, for more information). The partition homogeneity test between submatrices ITS, morphology, and chloroplast gave a *P* value of 5%. Combining the data sets (including the recoded 3' end characters of *rbcL*) gave 110 equally parsimonious trees, 1830 steps long (CI = 0.44, RI = 0.60). The strict consensus tree is shown in Fig. 5. The resolution of this combined tree is much higher than when the data sets are analyzed separately.

In the combined analysis the clade containing the subfamily Ixoroideae s.l. (Bremer, Andreasen, and Olsson, 1995) is strongly supported (Fig. 5; jackknife 100%, *b* = 10) and so is the Ixoroideae s.s. taxa (jackknife 100%, *b* = 10). Other major groupings, however, are not strongly supported, except for the clade including Coffeae to Gardenieae (jackknife 99%, *b* = 8).

Mussaenda, *Pentagonia*, and *Emmenopterys* of the Cinchonoideae are basal in Ixoroideae s.l. and genera of the sub-

family Antirheoideae (*Alberta*, *Canthium*, and *Vangueria*) are nested within the Ixoroideae s.s. There are clades corresponding to the tribes Vanguerieae (jackknife 100%), Pavetteae (excluding *Ixora* and allies), Coffeae (including four taxa from Gardenieae-Diplosporinae), and Gardenieae-Gardeniinae. Octotropideae form one monophyletic group with the genus *Cremaspora* (Gardenieae-Diplosporinae) as their sister.

Within the abovementioned clades there is strong support for the grouping of *Myonima*, *Versteegia*, and the *Ixora* species (jackknife 100%, *b* = 9) *Leptactina* and *Dictyandra* (Pavetteae; *b* = 10, jackknife 100%), and *Coffea*, *Paracoffea*, and *Psilanthus* (= Coffeae s.s.; jackknife 99%, *b* = 5). In Gardenieae the African sister genera *Oxyanthus* and *Mitriostigma* have strong support (jackknife 100%, *b* = 10) and the neotropical genera *Glossostipula*, *Duroia*, *Alibertia*, and *Borojoa* form a well-supported group (jackknife 93%, *b* = 5).

DISCUSSION

Missing taxa—Concern may be raised about the effect of combining data sets with substantial “non-overlap” in taxon sampling. The complete *rbcL* sequence data set consists of 82 taxa, while the other three data sets have fewer taxa (the morphological data set consists of 75 taxa, restriction enzyme data were obtained for 26 taxa, and ITS sequences for 21). Wiens and Reeder (1995) investigated how nonrandom missing entries (i.e., taxa lacking certain types of data) affect the accuracy

Taxa	Sequences
<i>Luculia</i>	-----CCGT
<i>Emmenopterys</i>	-----CCGT
<i>Hallea</i>	-----CCGT
<i>Nauclea</i>	-----CCGT
<i>Sarcocephalus</i>	-----CCGT
<i>Pentagonia</i>	-----CCGT
<i>Cinchona</i>	-----CCGT
<i>Mussaenda</i>	-----CCGT
<i>Posoqueria</i>	-----CCGT
<i>Canthium</i>	-----CCGA
<i>Vangueria</i>	-----CCGT
<i>Alberta</i>	-----CCGA
<i>Myonima</i>	-----CCGC
<i>Versteegia</i>	-----CCGT
<i>Ixora coc.</i>	-----CCGT
<i>Ixora hoo.</i>	-----CCGC
<i>Ixora par.</i>	-----CCGC
<i>Tricalysia cry.</i>	-----AAAGT
<i>Tricalysia ova.</i>	-----AAAATAAAAATAGATAAAGC
<i>Diplospora</i>	-----AAAGC
<i>Psilanthus man.</i>	-----2 (AAAGAGAAGGAT) AAAGAGAAAGAGT
<i>Psilanthus ebr.</i>	-----AAAGAGAAAAGTT
<i>Paracoffea</i>	-----AAAGAGAAAGAGT
<i>Coffea ara.</i>	-----AAAGAGAAAGATT
<i>Coffea n.sp.</i>	-----AAAGAGAAAGATT
<i>Bertiera gui.</i>	-----AAAGT
<i>Bertiera bre.</i>	-----CCAACGC
<i>Tarenna neu.</i>	-----AAAGTGAT
<i>Tarenna dru.</i>	-----AAAGTGATTGGTAAAGTGAA
<i>Tarenna cym.</i>	-----AAA---TTGGTAAAGTGAA
<i>Tarenna sup.</i>	-----AAAGT---GGTAAAGTGAA
<i>Rutidea</i>	-----AAAGT---AGTGAA
<i>Pavetta aby.</i>	-----AAAGTGATTGATAAAGTGAA
<i>Pavetta lan.</i>	-----AAAGTGATTGATAAAGTGAA
<i>Leptactina</i>	-----AAACCT---AAACC
<i>Dictyandra</i>	-----AAACCTAATAAACC
<i>Cremaspora</i>	-----AAAGTGAT
<i>Paragenipa</i>	-----AAAG---AA
<i>Feretia</i>	-----AAAGTGAA
<i>Kraussia flo.</i>	-----AAAGTGAA
<i>Kraussia soc.</i>	-----AAAGTGAA
<i>Pouchetia</i>	-----AAAGTTAA
<i>Ramosmania</i>	-----AAAGTGAA
<i>Fernelia</i>	-----CAAGTGAA
<i>Hypobathrum</i>	-----AAAGTGAA
<i>Gardenia thu.</i>	-----AAAGT
<i>Gardenia aug.</i>	-----AAA
<i>Kailarsenia</i>	-----AAA
<i>Coddia</i>	-----AAA
<i>Genipa</i>	-----AAA
<i>Heinsenia</i>	-----AAAGTGAA
<i>Rothmannia lon.</i>	-----AAAGTGAA
<i>Rosenbergiod.</i>	-----CCGAC
<i>Oxyanthus pyr.</i>	-----CCGAC
<i>Oxyanthus cf.z.</i>	-----CCGAC
<i>Mitriostigma</i>	-----ccgac
<i>Glossostipula</i>	-----CCA
<i>Borojoa</i>	-----CCA
<i>Casasia</i>	-----AAAAC
<i>Randia tru.</i>	-----AAACC
<i>Randia acu.</i>	-----AAACC
<i>Schumanniophyt.</i>	-----AAACC
<i>Massularia</i>	-----AAACT
<i>Burchellia</i>	-----AA
<i>Didymosalpinx</i>	-----AAAGTGGCTCCGAA
<i>Aidia rac.</i>	-----AAAGCATAAAGCG-ATTGAC
<i>Aidia mic.</i>	-----AAAGCATAAAGCGGATTGAC
<i>Oxyceros</i>	-----AAAGGATAAAGCG-ATTGAC
<i>Randia moo.</i>	-----AAAGGATAAAGCGGATTGAC
<i>Hyperacanthus</i>	-----AAAGCATAAAGCGGATTGAC
<i>Duperrea</i>	-----AACC AAAA
<i>Randia fit.</i>	-----AAACCAAAA GAAGCATAAAGCGGATTGAC
<i>Sukumia</i>	-----AAAGAAAGCCTAAAGCGGATTGAC
<i>Randia dem.</i>	-----AAACCAAAA GAAGCCTAAAGCGGATTGAC
<i>Porterandia</i>	-----AAACCAAAA GAAGCATAAAGCGGATTGAC
<i>Alibertia</i>	-----AAAGAA
<i>Calochone</i>	-----AAAG
<i>Euclinia</i>	-----AAAGGATAAAG
<i>Aoranthe</i>	-----AAAGGATAAAG

Fig. 2. The variable 3' end of *rbcL* with blocks of nucleotides (= boxed) used as characters (block absent, present, or inapplicable) in the combined analysis.

TABLE 2. The *Lactuca* restriction fragments (following Jansen and Palmer, 1987) used to probe for homologous regions in the Rubiaceae DNAs. "a" and "b" = subprobes of fragment 4.

Fragment/region	Fragment size (kb), restriction enzyme(s)	Fragment/region	Fragment size (kb) restriction enzyme(s)
4a	3.8 SacI-XmaI	11	6.3 SacI
4b	8.3 SacI-XmaI	12	5.4 SacI
5	10.6 SacI	13	4.6 SacI
6	9.9 SacI	14	3.8 SacI
7	7.7 SacI	16	1.8 SacI
9	6.9 SacI		

of estimating a true phylogeny or a tree obtained using a complete data set. They found that three factors affect the accuracy (the probability of estimating the true phylogeny) of the analyses: the type of data missing, the total number of characters in the analysis, and the proportion of characters lacking data. The latter parameter had surprisingly little effect on accuracy in Wiens and Reeder's analyses. Accuracy was very similar when taxa were 50, 75, or 100% complete. In comparison, the taxon completeness in our analysis varied within that range: 57–100%. Wiens and Reeder's results suggest that the larger the number of characters in a combined analysis, the smaller the cost in accuracy associated with combining data sets with nonoverlapping taxon sampling. No decrease in accuracy occurred when including 50% incomplete taxa in data matrices with 174 characters. Even if further studies are needed to assess this effect fully, our data set includes a comparatively large number of informative characters (416), so the decrease in accuracy can be assumed to be small, especially given comparatively high taxon overlap between data sets.

Support and incongruence—By using all available information, though incomplete for some taxa, and analyzing it simultaneously we maximize the information content of the analysis (i.e., "simultaneous analysis," Nixon and Carpenter, 1997; also called "total evidence," Kluge, 1989). Our results suggest that combining data results in more robustly supported and resolved topologies (compare Figs. 3 and 4 with Fig. 5). For example, Coffeaeae s.s. are supported by a jackknife fraction of 75% in the *rbcL* analysis and, in the combined analysis, the support is increased to 99%. The partition homogeneity test resulted in a *P* value of 5%, which would indicate that the data matrices are incongruent if $\alpha = 0.05$ is the chosen cut-off level. Inspecting the separate trees, however, reveals no well-supported topological incongruencies, which is not very surprising since the consensus trees from the separate analyses are fairly unresolved. In addition, there is no decrease in support values for any of the clades between the separate and combined trees, indicating that there is no "real" incongruence caused by biological phenomena, e.g., hybridization, but the incongruent topologies are most likely a result of sampling error. Earlier studies (Sullivan, 1996; Cunningham, 1997) have suggested that the 0.05 level is indeed too conservative for real data sets and that $P < 0.001$ is more reasonable. The assumption for the homogeneity test is that the characters are drawn at random from an indefinite universe of characters, an assumption we know is violated, e.g., when using particular DNA regions with possible evolutionary constraints.

Generally, there is least support for intertribal relationships and also for larger clades within Gardenieae-Gardeniinae. The cause for the low support is the low number of characters at

TABLE 3. The cpDNA regions (see Table 2) of the restriction sites, the restriction enzyme used, and the character number (for new characters) for respective site.

Character	Enzyme	Region	Character	Enzyme	Region
1	EcoRV	4b	19	AvaI	4b
2	EcoRV	4b	20	AvaI	6
3	EcoRV	6	21	AvaI	16
4	EcoRV	7	22	AvaI	14
5	EcoRV	5	23	AvaI	5
6	EcoRV	5	24	AvaI	13
7	EcoRV	12	25	AvaI	12
8	EcoRV	11	26	BclI	4a
9	EcoRV	9	27	BclI	13
10	EcoRV	9	28	DraI	4a
11	ClaI	6	29	BamHI	4a
12	ClaI	4a	30	BamHI	4b
13	ClaI	5	31	BamHI	4b
14	ClaI	13	32	BamHI	9
15	ClaI	11	33	BamHI	11
16	ClaI	4a	34	NcoI	9
17	ClaI	4a	35	NcoI	9
18	ClaI	4a	36	NcoI	5
			37	HindIII	12

these nodes, which is especially pertinent when looking at *rbcL* sequence data only (the collapsed consensus tree in Fig. 3). This pattern of few characters at intertribal branches might indicate comparatively fast speciations (branchings per unit of time) at the time when these groups originated or that mutations produced were not fixed but have changed again later on (homoplastic characters).

Phylogenetic implications—Our results suggest that the Cinchonoideae genera (*Pentagonia*, *Mussaenda*, and *Emmenopterys*; Robbrecht, 1988, 1993) are part of the basal branches of the ingroup ("basal Ixoroids," see Fig. 5), thus supporting results of previous analyses (Bremer, Andreasen, and Olsson, 1995; Bremer and Thulin, 1998; Bremer et al., 1999). The relationships of these "basal Ixoroids" will be addressed in future analyses by including more taxa. However, a new and noteworthy relationship with this group is shown by the genus *Posoqueria*, which has been included as an aberrant member in Gardenieae (Robbrecht, 1988). Exclusion of *Posoqueria* from Gardenieae and Ixoroideae s.s. is strongly supported here (jackknife 100%, $b = 10$ steps). The present analysis suggests that *Posoqueria* is a member of or closely related to the recently resurrected tribe Mussaendeae (Bremer and Thulin, 1998), but the exact position must await more inclusive taxon sampling.

Our analysis supports the earlier findings that the subfamily Antirhoeideae is polyphyletic (Bremer, 1996; Rova and Andersson, 1995; Andersson, 1996; Delprete, 1996; Young et al., 1996; Andersson and Rova, 1999) since the Antirhoeideae tribes Vanguerieae and Alberteae are nested in the subfamily Ixoroideae. Initially, Bremekamp (1934) placed Alberteae (including *Cremaspora* and some Octotropideae and Aulacocalyceae genera) in Ixoroideae. Later (1952) he excluded *Alberta* and *Nematostylis* from the subfamily because he thought they lacked the secondary pollen mechanism (see Puff, Robbrecht, and Randrianasolo, 1984). Verdcourt (1958), on the other hand, limited Alberteae to *Alberta* and *Nematostylis* and placed it close to "Ixoreae". In our analysis it is sister to Vanguerieae and clearly part of Ixoroideae. Morphological

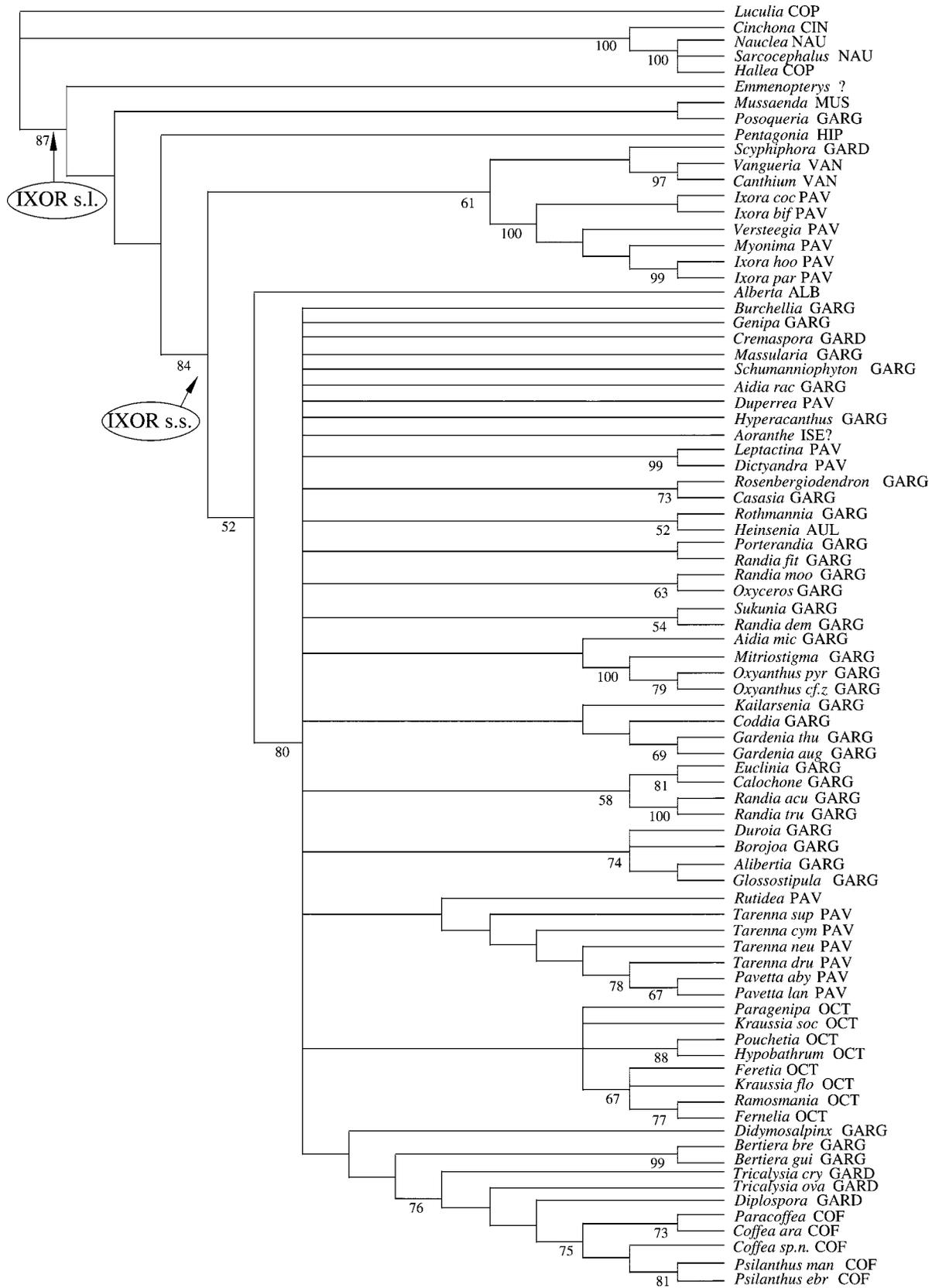


Fig. 3. Strict consensus of 134 978 most parsimonious trees from the analysis of *rbcL* sequence data of Ixoroideae and outgroup taxa. Tribal taxonomic positions (following Robbrecht, 1988, 1993) are abbreviated: ALBerteae; AULacocalyceae; CINchoneae; COFfeeae; COPtosapelteae; CREmasporeae; GARDenieae-Gardeniinae/Diplosporiinae; HIPpotideae; ISErtieae; IXOreae; MUSSaendeae; NAUcleae; OCTotropideae; PAVetteae; VANguerieae; ? = uncertain position. Support for nodes is indicated (Bremer support >1 above branches, jackknife >50% below).

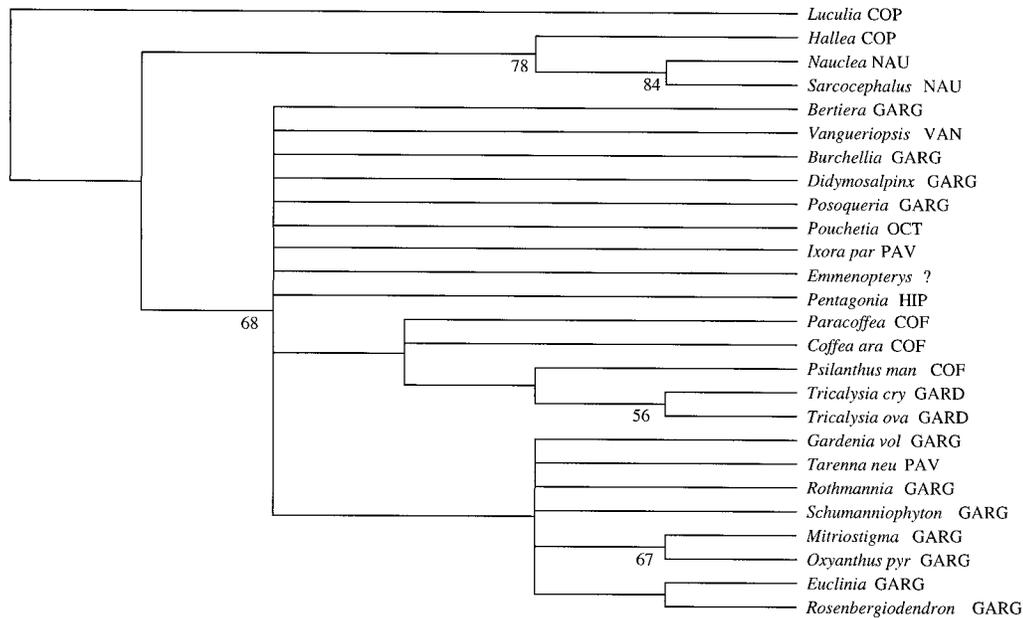


Fig. 4. Strict consensus of 3228 most parsimonious trees from the restriction enzyme analysis of Ixoroideae. Tribal taxonomic positions are abbreviated: COFfeae; COPTosapelteae; GARDenieae-Gardeniinae/Diplosporinae; HIPpotidae; NAUcleeae; OCTotropidae; PAVetteae; VANguerieae; ? = uncertain position. Jackknife values >50% are indicated.

characters supporting this sister relationship are, e.g., the superior embryo radicles and endocarps with apical splits.

The Gardenieae—The exclusion of representatives of Gardenieae-Diplosporinae from Gardenieae is strongly supported (40 extra steps are required to make the taxa of the Diplosporinae a monophyletic group and part of the Gardenieae clade). This was shown for *Tricalysia* and *Cremaspora* in earlier studies (Andreasen and Bremer, 1996; Persson, 1996, 1998) and now for *Diplospora*, the type genus of Diplosporinae and also for *Scyphiphora* (Fig. 5). These Diplosporinae genera are found in three different clades in the trees: in the Coffeae, close to Octotropidae, and the *Ixora* group, respectively (see below).

Three taxa placed in other tribes are part of the Gardenieae clade in our analysis. One is the genus *Duperrea*, which was included in Pavetteae as a genus with unknown affinity (Bridson and Robbrecht, 1985a). De Block and Robbrecht (1997) found that members of this Indo-Chinese genus lack seed coats and also adaxial excavation in the seeds, characteristics that are typical for members of Pavetteae. Instead, the seeds are embedded in placental pulp, a feature typical of Gardenieae-Gardeniinae. The relationship of *Duperrea* with Gardenieae-Gardeniinae is corroborated in our analysis (Fig. 5), although the support is low.

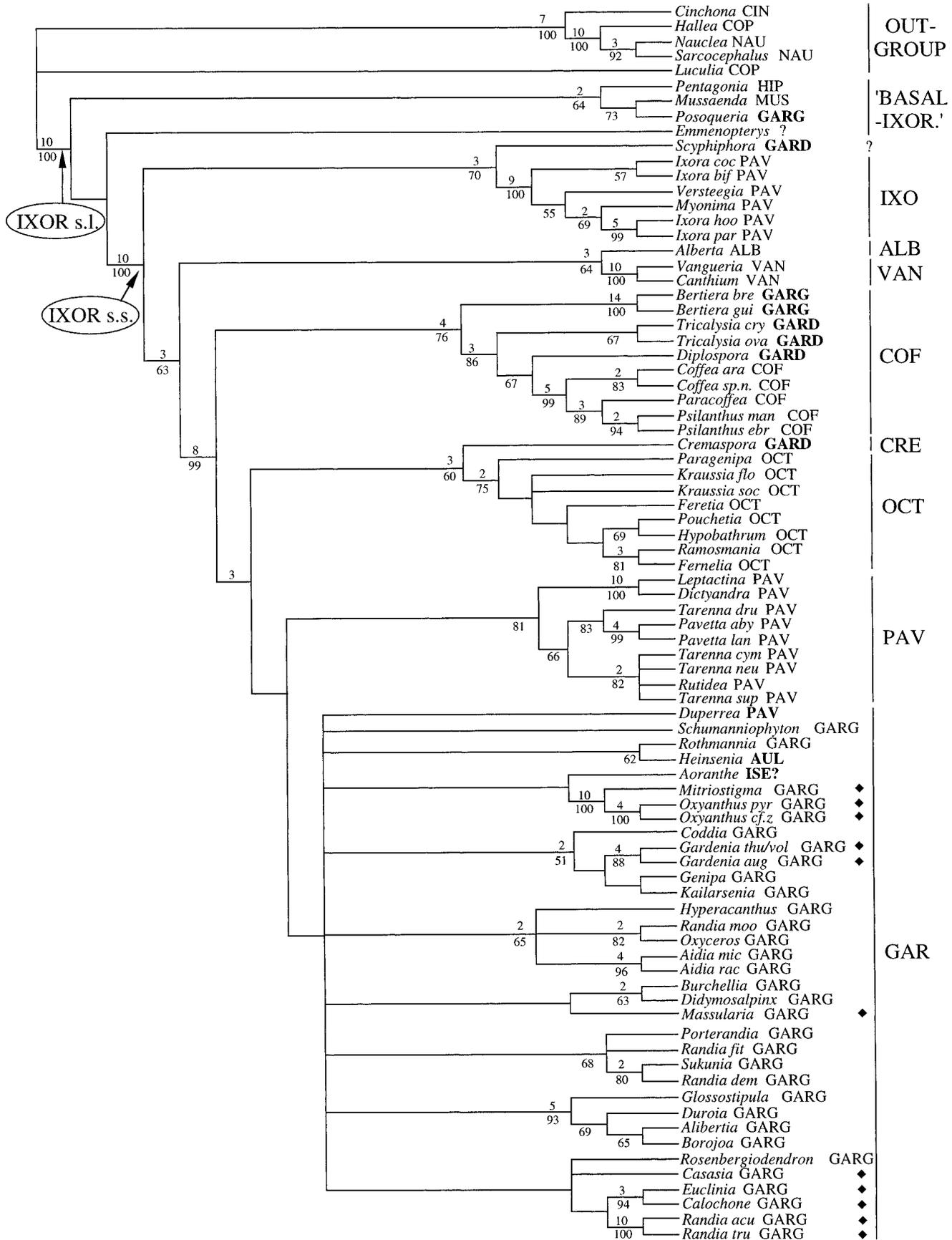
Aoranthe is an African genus which was segregated from the Asian *Porterandia* and placed in the tribe Isertieae (Somers, 1988). This segregation is supported in the present study, although both *Aoranthe* and *Porterandia* are part of the Gardenieae clade. Andersson (1996) and Bremer and Thulin (1998) found it to be part of Ixoroideae s.s., but did not address the question of its position within the subfamily further. Persson (1996, 1998) also found it to be part of Gardenieae, but in the *Gardenia* group and not close to *Oxyanthus* and *Mitriostigma* as in our study. *Aoranthe* shares with *Oxyanthus* and *Mitriostigma* the characters of pseudo-axillary flowers,

presence of domatia, and the sculptured thickenings of the exotesta.

Heinsenia was placed, together with its close ally *Aulacocalyx* and four other genera, in tribe Aulacocalyceae by Robbrecht and Puff (1986). Morphologically, Aulacocalyceae shares characteristics with the Gardenieae-Gardeniinae in the terminal inflorescences and fruits with seeds embedded in placental pulp, but differs in having a superior embryo radicle and lacking a seed coat. Despite these differences, our results support *Heinsenia* as part of the Gardenieae-Gardeniinae. Although support is low, it is interesting to note the sister relationship of *Heinsenia* with *Rothmannia* in the analysis. The branching pattern of *Heinsenia* and *Rothmannia* is very similar, and the corolla of both genera is often white with colored spots inside (Keay, 1958; Bridson and Verdcourt, 1988). As recognition of the tribe Aulacocalyceae would render the Gardenieae-Gardeniinae paraphyletic, the best solution is to sink Aulacocalyceae into Gardenieae.

Within the Gardenieae-Gardeniinae Robbrecht and Puff (1986) suggested the following three informal groups based on morphological similarities: (1) the taxa with pollen grains in tetrads, (2) a group of neotropical genera with unisexual flowers, and (3) *Aidia* and allies with small, spherical fruits.

The first group (1) with pollen in tetrads (character 44, Appendix 1) was supported in a morphological analysis by Persson (1996). Later, based on chloroplast data, he found that this character must have arisen at least three times (Persson, 1998). In the present analysis, taxa with pollen in tetrads are found in essentially the same three groups as found by Persson (1998) (see Fig. 5): in *Gardenia*, which groups with the monad genera *Genipa*, *Kailarsenia*, and *Coddia*; in the *Oxyanthus* group; and in a group consisting of *Euclinia*, *Calochone*, *Randia*, *Casasia*, and *Rosenbergiendendron* (the last genus with pollen in monads). In addition, *Massularia* has pollen in larger clumps, i.e., massulae. The relationships within Gardenieae are rather unresolved and weakly supported, but reconstructing



this character requires three (four with *Massularia*) independent origins in all the equally parsimonious trees.

The genera *Duroia*, *Alibertia*, *Borojoa*, and *Genipa* have unisexual flowers (group 2, character 8, Appendix 1) and were proposed to form a natural, neotropical group in Gardenieae. The three first genera form a clade (jackknife 93%, $b = 5$) including *Glossostipula*. *Glossostipula* also has unisexual flowers and is a newly described neotropical genus for some taxa previously referred to *Genipa* and *Randia* (Lorence, 1986). The sequenced *Genipa* species, *Genipa americana*, is not part of that clade, a result congruent with the analyses presented by Persson (1996, 1998), which also indicated that *Genipa*, as currently circumscribed, may be polyphyletic. *Genipa americana* comprises a clade together with *Gardenia* and *Kailarsenia*.

Aidia and *Oxyceros* are representatives for the third group with small, spherical fruits (the “*Aidia* group” sensu Persson, 1996). They form a clade with the Australian *Randia moorei* and the African genus *Hyperacanthus* but the support is low (jackknife 65%, $b = 2$). Thorns occur in *Randia moorei*, *Oxyceros*, and in *Hyperacanthus*, and *Aidia*, *Oxyceros*, and *Hyperacanthus* all have porate pollen and endotesta with sculptured thickenings.

Randia is today commonly accepted as a strictly neotropical genus (e.g., Fagerlind, 1942; Tirvengadam, 1978) and two of the included species (*R. aculeata* and *R. truncata*) belong to *Randia* s.s. Many of the paleotropical species need further studies, e.g., the New Guinean species included in the analysis, *Randia demicostata*, is sister to *Sukunia*, which is proposed to be close to *Trukia* (Smith and Darwin, 1988). *Randia fitzalanii*, an Australian species that is part of this clade has been placed in *Trukia* (Fosberg, 1987). Recently, Puttock (1999) and Puttock and Quinn (1999) performed morphological analyses of Asian-Australian Gardenieae and considered it appropriate to include *Trukia*, *Sukunia*, and possibly *Porterandia* in *Atractocarpus*. Our results support these relationships, although our analysis includes a more limited sampling compared to Puttock and Quinn’s analysis.

The Coffeae—The tribe Coffeae was restricted to *Coffea* and *Psilanthus* (*Paracoffea* in our analysis is usually included in *Psilanthus*) by Robbrecht and Puff (1986): *Tricalysia* and related genera, which were considered to be closely related to *Coffea* (Bremekamp, 1934), were moved to the subtribe Diplosporinae of Gardenieae by Robbrecht and Puff (1986). According to our results, Coffeae should include not only *Tricalysia* and *Diplospora* (Gardenieae-Diplosporinae) but also *Bertiera* (Gardenieae-Gardeniinae). The position of *Bertiera* in this clade was indicated in previous analyses (Andreasen and Bremer, 1996), and our analyses presented here include the type species, *B. guianensis*, strengthening the inclusion of *Bertiera* in the Coffeae. The Coffeae clade is moderately supported (jackknife 76%, $b = 4$).

The position of *Diplospora* in the Coffeae clade is new, but since *Tricalysia* and *Diplospora* have been considered con-

generic (Schumann, 1891), the position of *Diplospora* close to *Tricalysia* is not surprising. The inclusion of these Diplosporinae taxa in Coffeae is fairly strongly supported (jackknife 86%, $b = 3$). *Diplospora* was recently split into *Diplospora* s.s. and *Discospermum* (Ali and Robbrecht, 1991), and the sequenced species, *Diplospora polysperma*, was considered to be a *Discospermum* by these authors. Ali and Robbrecht (1991) refrained from making a combination under that genus, however, because the origin of the material was unknown. We have coded it as *Discospermum* in the morphological matrix. *Discospermum* has much larger fruits than *Diplospora*, with many seeds frequently embedded in placental tissue, a feature reminiscent of the type of fruit found in many genera of Gardenieae-Gardeniinae. Thus, Ali and Robbrecht (1991) stated that *Discospermum* “links the Diplosporinae with the Gardeniinae and supports the rank (subtribal) given to these.” Our results do not support such a relationship (Fig. 5), since at least some genera of Diplosporinae belong in Coffeae. *Sericanthe*, which was recently separated from *Tricalysia* (Robbrecht, 1978), probably belongs in this group too since the two genera was considered closely related (Robbrecht, 1978).

The Octotropideae—All the included taxa of the Octotropideae form a monophyletic group in the combined tree (Fig. 5). The internal groupings are not well supported, but the presumed “primitive” genera (Robbrecht and Puff, 1986) with many seeds, *Fernelia* and *Ramosmania*, are supported as sister taxa in our results (jackknife 81%, $b = 3$). The sister-group relationship between *Pouchetia* and *Hypobathrum* is supported (jackknife 69%), and they were both placed in the “central group” (Robbrecht and Puff, 1986). This group included genera with small fruits, relatively few ovules, and similar thickenings in the exotesta.

A member of the Gardenieae-Diplosporinae, *CremaSpora*, is sister to tribe Octotropideae, although the support is low (60%, $b = 3$). The taxonomic history of this genus is complex, partly because the orientation of its embryo radicle was misinterpreted (e.g., Schumann, 1891). Bremekamp (1934) placed *CremaSpora* in the tribe Cremasporaeae together with some genera that Robbrecht (1988, 1993) included in Octotropideae and Aulacocalyceae. Verdcourt (1958) included it in “Ixoreae”-Cremasporinae, but excluded the Aulacocalyceae genus *Heinsenia* (discussed above). As noted by Ali and Robbrecht (1991) and others, there are strong similarities between the taxa placed in Gardenieae-Diplosporinae and Octotropideae. While such similarities support inclusion of *CremaSpora* in Octotropideae, the position of *CremaSpora* is not well supported in our analyses, and we suggest a placement in a tribe of its own, and resurrect Bremekamp’s (1934) Cremasporaeae.

The Pavetteae—The Pavetteae in its current circumscription (Robbrecht, 1993) are paraphyletic according to our results (Fig. 5). The included taxa of this tribe are found in two clades (excluding *Duperrea*, which, as discussed above, is part of the Gardenieae-Gardeniinae clade), which conform to the two in-

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Fig. 5. Strict consensus of 110 most parsimonious trees from the combined analysis of Ixoroideae. Taxonomic positions are abbreviated: ALBerteae; AULacocalyceae; CINchoneae; COFFeae; COPTosapelteae; CREmasporeae; “BASAL-IXOR” = basal Ixoroideae; GARDenieae-Gardeniinae/Diplosporinae; HIPpotideae; ISERTieae; IXOreae; MUSsaendeae; NAUCleae; OCTotropideae; OUT = outgroup taxa; PAVetteae; VANguerieae.? = uncertain position. Tribal name in bold for taxa with novel positions. ♦ = taxa with pollen in tetrads. Support for nodes is indicated (Bremer support >1 above branches, jackknife >50% below).

formal groups proposed by Bridson and Robbrecht (1985a): *Ixora*, *Myonima*, and *Versteegia* in one clade (see below) and the rest of the taxa, Pavetteae s.s., higher up in the tree as sister to Gardenieae. The support for the clade consisting of Coffeae to Gardenieae, including Pavetteae s.s. is strong (jackknife 99%, $b = 8$). If monophyly of Pavetteae is included as a constraint in the analysis, 29 extra steps are required, which is fairly high compared to the Bremer support values of up to 14, which were obtained in the analyses. Within Pavetteae s.s., the close relationship between *Leptactina* and *Dicthyandra* (Robbrecht, 1984) is strongly suggested (jackknife 100%, $b = 10$). *Tarenna* s.l. (Bridson, 1979) is a heterogeneous genus as both *Pavetta* and *Rutidea* are nested within it. One of the characters used for delimiting Pavetteae (Robbrecht, 1984), seeds with adaxial excavation and placenta within the seed cavities (character 39) must, according to the analysis, be interpreted as a parallelism occurring in this group and also in *Ixora* and allies.

The grouping of *Ixora* with the genera *Myonima* and *Versteegia*, is strongly supported (bootstrap 100%, $b = 9$), and this clade occupies a basally diverged position in the Ixoroideae s.s. These genera exhibit many common characteristics and are considered to be closely related (Ridsdale, van den Brink, and Koek-Noorman, 1972; Verdcourt, 1983; Jansen, De Block, and Smets, 1997). *Versteegia* is a cauliflorous genus from New Guinea with salmon-pink flowers, a flower color that is uncommon in Rubiaceae but found also in *Ixora*. *Myonima* differs from *Ixora* in having a much shorter corolla tube and frequently three- to seven-locular ovary. Supporting characters in the analysis are: four corolla lobes (character 13), elongated anthers (character 20), and the parallelism shared with *Pavetta* and allies, seeds with adaxial excavation (see above). Some of the other genera included in the group around *Ixora* (Bridson and Robbrecht, 1985a; Table 5 in Robbrecht and Puff, 1986) probably also belong here.

The sister taxon to *Ixora* and allies (the *Ixora* group) in the combined analysis is the mangrove genus *Scyphiphora*. Puff and Rohrhofer (1993) investigated the morphology in this problematic taxon and suggested a position in Ixoroideae s.s., most probably in the Gardenieae-Diplosporinae group based on the axillary inflorescences and pauciovulate carpels. Recent analyses by Bremer et al. (1999) confirm the position of *Scyphiphora* in the Ixoroideae, although few taxa from the subfamily were included. The position of *Scyphiphora* as sister to the *Ixora* group in the trees resulting from the combined analysis conflicts with the position close to Vanguerieae inferred in the *rbcL* trees. The support for the relationship with the *Ixora* group is not very strong (jackknife 70%, $b = 3$), and the support in the separate *rbcL* analysis even lower. A character supporting the position of *Scyphiphora* as sister to the *Ixora* group are the number of corolla lobes (four; character 13, Appendix 1), an uncommon feature in the subfamily. A position close to any of the Gardenieae-Diplosporinae taxa is strongly rejected as the support is high (jackknife 99%, $b = 8$) for the upper clade including the Gardenieae-Diplosporinae taxa and Coffeae, Octotropideae, Pavetteae s.s., and Gardenieae s.s. We tentatively include *Scyphiphora* in Ixoreae.

The subfamily Ixoroideae and the tribes included in it are in need of new circumscriptions based on the results of our analyses. The exact delimitation of the subfamily (i.e., Ixoroideae s.l., see, e.g., Bremer and Thulin, 1998; Bremer et al., 1999) will not be dealt with here, as more taxa must be investigated before stable conclusions can be drawn. However,

the results of the present study suggest the following new tribal delimitations in the Ixoroideae s.s. based on the included taxa.

Coffeae DC., Ann. Mus. Hist. Nat. (Paris) 9: 217 (1807).
Type: *Coffea* L.

Shrubs, treelets, rarely trees, or geofrutices. Stipules interpetiolar, entire. Raphides absent. Inflorescences axillary and paired at nodes, or rarely terminal. Corolla lobes contorted to the left. Secondary pollen presentation absent (*Coffea*, *Psilanthus*, probably also in *Diplospora* and *Discospermum*) or present (*Tricalysia* and *Bertiera*). Stigmatic lobes free over most of their length (except in *Bertiera*). Ovary two-locular, placenta axile with one to many ovules. Fruit berry-like or with more or less dry wall and placental outgrowths. Embryo radicle inferior or lateral. Endosperm entire (ruminant in some species of *Tricalysia*). Exotesta cells without thickenings, with thickenings along outer tangential walls, or along radial and inner tangential walls (*Bertiera*). Pollen grains in monads, three-to-four colpi.

Genera included: *Coffea*, *Psilanthus* (including *Paracoffea*), *Diplospora*, *Discospermum*, *Tricalysia*, *Bertiera*. Genus that probably belongs here: *Sericanthe*.

Cremalesporeae Bremek. ex S. P. Darwin, Taxon 25: 601 (1976). Type: *Cremalespora* Benth.

Shrubs, treelets, or sometimes lianas. Stipules interpetiolar, entire, deciduous. Raphides absent. Inflorescences axillary and paired at nodes. Corolla lobes contorted to the left. Secondary pollen presentation present. Stigmatic lobes fused over most of their length. Ovary two-locular, axile placenta with one ovule in each locule. Fruit with leathery wall and (1)–2 seeds. Embryo radicle inferior. Endosperm entire. Exotesta with thickenings in radial walls. Pollen grains in monads, three-colpate.

Genus included: *Cremalespora*.

Gardenieae A. Rich. ex DC., Prodr. 4: 342, 367 (1830).
Type: *Gardenia* Ellis.

Shrubs, trees, or lianas. Stipules interpetiolar, entire. Raphides absent. Inflorescences terminal, rarely pseudo-axillary, very rarely truly axillary and paired at nodes. Aestivation contorted to the left, rarely to the right. Secondary pollen presentation present. Stigma club shaped (dividing lobes rare). Ovary with 2(–9) carpels, axile or parietal placentas with one to many ovules. Fruit with more or less dry wall, or berry-like, with seeds imbedded in the fleshy placenta. Embryo radicle orientation variable. Endosperm entire. Seeds mostly exotestal (seed-coat reduced in *Duperrea*, and *Heinsenina*), usually with thickenings along radial and inner tangential wall. Pollen grains in monads or sometimes in tetrads, three-colpate or three-colporate.

Genera included:

From Gardenieae-Gardeniinae: *Aidia*, *Alibertia*, *Borojoa*, *Burchellia*, *Calochone*, *Casasia*, *Coddia*, *Didymosalpinx*, *Duroia*, *Euclinia*, *Gardenia*, *Genipa*, *Glossostipula*, *Hyperacanthus*, *Kailarsenia*, *Massularia*, *Mitriostigma*, *Oxyanthus*, *Oxyceros*, *Porterandia*, *Randia*, *Rosenbergiodendron*, *Rothmannia*, *Schumanniphyton*, *Sukunia*.

From Aulacocalyceae: *Heinsenina*.

From Pavetteae: *Duperrea*.

Genera that probably should be included: most of Robbrecht's Gardenieae-Gardeniinae (1988, 1993) except those mentioned below, and *Aulacocalyx*.

Genera excluded: Gardenieae-Gardeniinae: *Posoqueria*, exact position still uncertain, in vicinity of Mussaendeae. *Bertierra* should be moved to Coffeae.

Gardenieae-Diplosporinae: *Tricalysia*, *Diplospora*, and *Discospermum* to Coffeae. *Cremaspora* to Cremasporeae, *Scyphiphora* to be tentatively accommodated in Ixoreae.

Ixoreae A. Gray, Proc. Amer. Acad. Arts 4: 39 (1858). Type: *Ixora* L.

Shrubs, or small trees. Stipules interpetiolar, entire. Petioles articulate. Raphides absent. Inflorescences terminal. Aestivation contorted to the left. Secondary pollen presentation usually present. Stigmatic lobes free over most of their length. Ovary 2(–7) locular, axile placentas with one ovule per carpel. Fruits fleshy. Seeds with adaxial excavation. Embryo radicle inferior. Endosperm entire. Seeds exotestal, with thickenings (as anastomosing ribs) along outer tangential wall, or rarely absent. Pollen grains in monads, three-colporate.

Genera included: *Ixora*, *Myonima*, *Versteegia*.

Genera that probably belong here: *Captaincookia*, *Doricera*. *Scyphiphora* is tentatively included here although the description is not altered to include its characteristics.

Pavetteae A. Rich. ex Dumort., Anal. Fam. Pl. (Rubiaceae): 33 (1829). Type *Pavetta* L.

Shrubs, small trees, or lianas. Stipules interpetiolar, entire (fimbriate in *Rutidea*). Raphides absent. Inflorescences terminal or sometimes pseudo-axillary. Aestivation contorted to the left. Secondary pollen presentation usually present. Stigmatic lobes fused over most of their length. Ovary with two locular, axile placentas with one to many ovules. Fruits fleshy. Seeds with adaxial excavation. Embryo-radicle inferior or lateral. Endosperm entire or slightly to deeply ruminant. Seeds exotestal, with thickenings (as a continuous plate) along outer tangential wall, or absent. Pollen grains in monads, 3–4(–5)-colporate.

Genera included: *Pavetta*, *Rutidea*, *Tarenna*, *Leptactina*, *Dictyandra*.

Genera that probably belong here: *Cladoceras*, *Tennantia*, *Coleactina*.

Genera excluded: *Ixora*, *Myonima*, and *Versteegia* to Ixoreae. *Duperrea* to Gardenieae.

Genera that probably should be excluded: *Captaincookia*, *Doricera*.

In conclusion, the combined analysis resulted in higher degree of resolution than when the data sets were analyzed alone, supporting the view that, when possible, all available data should be analyzed simultaneously to maximize explanatory power (see, e.g., Nixon and Carpenter, 1997). By adding other, independent data sets (even if taxon sampling is not complete) greater resolution is achieved, exemplifying that combined analyses can remedy the incapacity of one data set to resolve a phylogeny.

The results of the combined analyses suggest several new and important relationships in the subfamily Ixoroideae, and, as a result, new circumscriptions at tribal level are needed. The tribe Ixoreae should be resurrected for *Ixora* and allies, which are excluded from Pavetteae. *Scyphiphora* is probably related to Ixoreae and we tentatively include it there. *Heinseniania*, representing Aulacocalyceae, is part of Gardenieae, as is *Duperrea* (earlier placed in Pavetteae) and *Aoranthe* (Iseriteae). There is strong support for the inclusion of the Gardenieae-Diplosporinae taxa *Diplospora*, *Discospermum*, *Tri-*

calysia, and also *Bertierra* (Gardenieae-Gardeniinae) in Coffeae. *Cremaspora* is sister to Octotropideae but is best housed in a tribe of its own, Cremasporeae. The inclusion of the two Antirheoideae tribes Vanguerieae and Alberteae in Ixoroideae is strongly supported, as is the exclusion of *Posoqueria* from Gardenieae and Ixoroideae s.s.

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APPENDIX 1. Characters and character states used in the morphological matrix.

Character	Character states
1. Habit	0: tree 1: treelet 2: shrub 3: liana 4: hemi-epiphyte, strangler 5: geofrutex
2. Long and short shoots	0: absent 1: present
3. Thorns	0: absent 1: present
4. Leaf arrangement	0: opposite 1: in whorls of 3 2; in whorls of >3
5. Anisophylly	0: absent 1: present
6. Heterophylly	0: absent 1: present
7. Cauliflory	0: absent 1: present
8. Sexual distribution (in flowers)	0: hermaphrodite 1: unisexual
Leaves	
9. Domatia	0: absent 1: present
Inflorescence and flowers	
10. Inflorescence position	0: axillary 1: terminal 2: terminal, pseudo-axillary 3: terminal, leaf-opposed
11. Calyx in fruit	0: absent 1: present
12. Secondary pollen presentation	0: absent 1: present
13. Number of corolla lobes	0: five 1: four 2: six 3: seven-twelve
14. Total corolla size	0: <1 cm 1: 1-2 cm 2: 2-10 cm 3: >10 cm
Corolla	
15. Aestivation	0: contorted 1: valvate 2: imbricate
16. Hairs in tube	0: absent to irregularly hairy 1: upper part 2: lower part
17. Shape	0: salver-shaped 1: funnel-shaped 2: campanulate
Stamens	
18. Staminal insertion	0: near corolla mouth 1: >1:5 down in corolla mouth
19. Filament	0: distinct 1: extremely short
20. Anther attachment	0: dorsifix very near base 1: dorsifix around middle
21. Anther shape	0: button-shaped 1: linear 2: elongated, L: W <5
22. Anther protrusion	0: included 1: exerted
Pistil	
23. Number of carpels	0: two 1: > two
24. Placentation	0: axile 1: axile to axile-parietal 2: axile-parietal 3: axile-parietal to parietal 4: parietal
25. Number of ovules/locule	0: one 1: 2-10 2: >10
26. Micropyle orientation	0: upwards 1: horizontal 2: downwards 3: variable: numerous ovules
27. Stigma shape	0: entire, clavate-scarcely widened 1: lobate 2: entire, cylindric-capitate or spindle-shaped
28. Style hairs	0: absent 1: present
29. Style furrowed	0: absent 1: present
30. Style protrusion	0: included 1: exerted
Fruit	
31. Diameter	0: <1 cm 1: 1-2 cm 2: 2-10 cm 3: >10 cm
32. Endocarp	0: membranous 1: papery 2: chartaceous 3: sclerified

APPENDIX 1. Continued.

Character	Character states
33. Mesocarp	0: fleshy 1: leathery 2: fibrous 3: woody
34. Placenta in fruit	0: fleshy, embedding the seeds 1: fleshy, not or partially covering the seeds 2: \pm absent, not detectable 3: \pm elongate, adnate to septa
Seeds	
	Seed coat is lacking in <i>Du-perrea</i> and <i>Heinsenia</i> , so characters 36-37, 40-43 are inapplicable for these taxa.
35. Rumination	0: absent 1: present
36. Exotesta cells	0: isodiametric 1: strongly elongated
37. Isolated fibers in seed coat	0: absent 1: present
38. Vertical groove	0: absent 1: present
39. Adaxial excavation	0: absent 1: with basal groove 2: without basal groove
40. Thickenings in outer tangential wall	0: absent 1: continuous 2: continuous with luminal intrusions 3: anastomosing ribs
41. Thickenings in radial wall	0: absent 1: covering the whole wall 2: not covering the whole wall 3: anastomosing
42. Thickenings in inner tangential wall	0: absent 1: continuous pitted plate 2: anastomosing ribs 3: simple, elongate free ribs 4: transverse rib-like 5: ring-like
43. Thickenings sculptured	0: absent 1: present
Pollen	
44. Dispersal unit	0: monads 1: tetrads
45. Aperture type	0: colporate 1: pororate 2: porate
46. Number of apertures	0: three 1: > three
47. Exine surface	0: reticulate 1: foveolate 2: rugulate 3: perforate 4: psilate
48. Tectal excrescences	0: absent 1: present
Cytology	
49. Chromosome number	0: $x = 11$ 1: $x = 17$

APPENDIX 2. Data matrix of the morphological characters used in the analyses of Ixoroideae. Polymorphic taxa are abbreviated: A: 0,1; B: 0,2; C:0,3; D: 1,2; E: 1,3; F: 2,3; G: 0,1,2; H: 0,1,3; J: 0,2,3; K: 0,2,4; L: 0,2,5; M: 1,2,3; N: 1,2,5; P: 0,1,4; Q: 0,1,2,3. ? indicates unknown state; -inapplicable. *Randia* s.s. = *R. aculeata* and *R. truncata*.

Taxa	Character number			
	10	20	30	40
<i>Aidia</i>	K0001000A30101011000?10023101103A0000000111020000			
<i>Alberta</i>	G000000001110D0B10A1100000000103?20?001221000300			
<i>Alibertia</i>	200000011110Q10A0101?0A3B?1?10D?100000011B00BA000			
<i>Aorantho</i>	C00000A0121?0D01D011?0002?0010D00100?003111000C0?			
<i>Bertiera</i>	N00000000A110A01100010002?1010A20100?00011000A000			
<i>Borojoa</i>	1000000?110Q100011110132?1?10F?00?000113002000?			
<i>Burchellia</i>	D000000001110D02111110002201111000010000200000000			
<i>Calochone</i>	30000000011102021011?0032?1111200000000100120000			
<i>Canthium</i>	D1A000001011101110000100002001A3020?00???0?0?0?0?			
<i>Casasia</i>	B000000A01A10D000A1110032211102000000001111A0EA0			
<i>Cinchona</i>	B00000001110011101001A002?100021230?003110000301			
<i>Coddia</i>	2100000?01101021011?100020011000100000001J0000000			
<i>Coffea</i>	D000000A010QD00A0011100021001A20200110000-00A100			
<i>Cremanospora</i>	M00001000011B10A001?1100020101020D0100002C000001?			
<i>Dictyandra</i>	D000000011110201100011002F101100100002200000130?			
<i>Didymosalpinx</i>	20100000A01102021110D003120A00221001000?2F0000000			
<i>Diplospora</i>	D000000?10A01A0A10011100D21?01DD1001000A10000000?			
<i>Duperrea</i>	D00010000F1101010010210002211100000--00---000300			
<i>Duroia</i>	B00G10010111JD0G011110132?00102?D00000001J002000?			
<i>Euclinia</i>	200000001111JF0200111100B300112100000000100120010			
<i>Feretia</i>	2000000A001010110101100100101A00201000022000?0?0			
<i>Fernelia</i>	100001011011100A00012100201?10AB0200000120000?0?0?			
<i>Gardenia ang.</i>	00000000211?201000011032?011222000?00???120310			
<i>Gardenia thu.</i>	B00D000000111F200000111132?01112?00?00?00???12?0?0			
<i>Genipa</i>	000A00010111B10G0011D1022300112300010003140000000			
<i>Glossostipula</i>	B0000001A11121000010?1100?1110D?011?0010000010?			
<i>Heinsenia</i>	B0000000A11101022011110010101110000--00---00010?			
<i>Hyperacanthus</i>	D01010000A1102300011102221011D20000000011102000?			
<i>Hypobathrum</i>	B000000?01110011011D0000011010202010000100000300			
<i>Ixora</i>	B00000A001111Q0G00002100021A01AF000A001C3B0000C00			
<i>Kailarsenia</i>	B000000A1111B10G0011?103231A11A30000000011000000?			
<i>Kraussia</i>	200000001011010110001100100011020200000000-00?0?0			
<i>Leptactina</i>	D0000000111A0F01001110002F110AD001000022000001300			
<i>Massularia</i>	2000100002110200011110002F1011320001000013012A000			
<i>Mitriostigma</i>	2000000012110201101021001?00111100010000221120200			
<i>Mussaenda</i>	F000A00001A00D111100D0A02?100AG?010?00?00?0010?0			
<i>Myonima</i>	2000010001111000100021A0021001A3B00000013320000000			
<i>Oxyanthus</i>	D0000000A2A1020A00A0210F23000112110100001F1110100			
<i>Oxyceros</i>	M010000?D010D010010?100B?1?11A210000?00111020000			
<i>Paracoffea</i>	D000000010?02010011100002?0?0A2020?010?0?0010?0			
<i>Paragenipa</i>	D00000000011A1021?012100201101A10200000010000000?			
<i>Pavetta</i>	B0000000A1A1110A00001100020111000000022000000H10			

APPENDIX 2. Continued.

Taxa	Character number			
	10	20	30	40
<i>Porterandia</i>	0000100A02110100010110002?011023?000?0011002000?			
<i>Posoqueria</i>	K000000001100F2GA00021002?0A002??000?00000-0A0001			
<i>Pouchetia</i>	B00A0000A011A00010102101100A11120201000120000?0?0?			
<i>Psilanthus</i>	20000000AAA0JD00001110000210?0120200110000-00A0?0			
<i>Ramosmania</i>	2000010000?002010011?100200?002?0?01000?0?00?0?0?			
<i>Randia</i> s.s.	BAAA000A0AA10Q0AA0A12A002?AA0AD2100000001A0ADAP00			
<i>Randia fit.</i>	D?000?0?0?11?02021100100?2?1?102?000?0005002000?			
<i>Randia moo.</i>	21100000?1110000000?110?2?10?10?00?00?0?0?0?0?0?0?			
<i>Rosenbergiodendron</i>	D100000001110F010011210?2?0110D?000?000110021400			
<i>Rothmannia</i>	B0000000A1A10F3GGAl1DA032300AAF2A00A0000AJ0000000			
<i>Rutidea</i>	30000000A1A1AA0B000AG100010A01020010001000-00020?			
<i>Schumanniphyton</i>	B00D1000?111JF00001110A0231?002110000?00101020300			
<i>Scyphiphora</i>	100000000011001000121001?10010322000000210000000			
<i>Sukumia</i>	10001000?0A10202010?1000221?102?20000000100020E00			
<i>Tarenna cym.</i>	B0000000?111000100001100120101020010001000-00000?			
<i>Tarenna dru.</i>	D000000011?1000100001100020101000000?02100?00000?			
<i>Tarenna neu.</i>	B00000000111000100001100120101020010001000-00000?			
<i>Tarenna sup.</i>	D0000000011100010000110012010102001?01000-000?0?			
<i>Tricalysia</i>	L00000A0A011QG0G00A11A00G21AA10BEGAA000A000000000			
<i>Vangueria</i>	D0A0000000A10011100001100020A1D30200?00000-000300			
<i>Versteegia</i>	A00000100111100000002100021011230000001332000001?			

APPENDIX 3. Data matrix of the 61 phylogenetically informative restriction sites for 26 Ixoroideae taxa. Presence of a site is coded as "1," absence as "0." "?" indicates uncertainty in mapping or absence of data. Characters 38–61 correspond to characters 13, 14, 20, 26, 40, 41, 44, 48, 49, 52, 61, 63–65, 69, 76, 100, 110, 111, 123, 124, 136, 144, and 150 in Bremer and Jansen (1991).

Taxa	Character number					
	1	2	3	4	5	6
	0	0	0	0	0	0
<i>Hallea</i>	00101?0011111?101000111100??0??0??0??0????????????????????1??01???					
<i>Luculia</i>	10101?000?11011?001?1?01001011110000000100110000001010??01101					
<i>Nauclea</i>	001010101111000100011110001000000001????????????????010??01???					
<i>Sarcocephalus</i>	001010101111?001000111100??00000000?1??010??01???					
<i>Bertiara</i>	1010110100010101000?????110101?001010????????????????110??01???					
<i>Burchellia</i>	10?0110100010110000?0??1??01100?110????????????????111??01???					
<i>Coffea</i>	10?????????01011100?000001001?11011110110010001101111010101011					
<i>Didymosalpinx</i>	1010010100010?10101000?0110001100111??0??0??0??0??111??01???					
<i>Emmenopterys</i>	10101?0100000101001?1?101110?11101010????????????????11???????					
<i>Euclinia</i>	1000000100010110001?0??01??111100??0????????????????1?01???					
<i>Gardenia</i>	100001010001011000100000110101100111001010001111101110101111					
<i>Ixora</i>	10?????????0?0??0??0??0??0??0??1001010001001111001100011010000					
<i>Mitriostigma</i>	10?????????00011000?000000101?11001110110010010111101110101011					
<i>Oxyanthus</i>	1000110100000110001000000?0?01000??0????????????????1?01???					
<i>Paracoffea</i>	1010110100010111001000001001011?11110????????????????111??01???					
<i>Pentagonia</i>	?0?????????01011001?0?????0??0??101????????????????11???????					
<i>Posoqueria</i>	101?100100010110011?1??111001110?010????????????????010??01???					
<i>Pouchetia</i>	1010110100010100000?0??0?1?0?11?0111????????????????111???????					
<i>Psilanthus</i>	1011110100011?11001000001001111011110????????????????1?1??01???					
<i>Rosenbergiodendron</i>	10000101000?0110101000001101111001110????????????????111??01???					
<i>Rothmannia</i>	1000010100010110001?0??0?01011?0????????????????????1???????					
<i>Schumanniohyton</i>	1000010100011110001?0??01????01????0????????????????????01???					
<i>Tarenna</i>	1000010100010?10001?00001101011001110110011011100001100110001					
<i>Tricalysia cry.</i>	1111110100011111001000011?0?0100011?0????????????????111??01???					
<i>Tricalysia ova.</i>	11?????????0??10100?000011?1?????????????????????????????????					
<i>Vangueriopsis</i>	101010100010?0?001?10001?01011001010011110111101100111001010					