

PHYLOGENETIC RELATIONSHIPS WITHIN THE GENTIANALES BASED ON *NDHF* AND *RBCL* SEQUENCES, WITH PARTICULAR REFERENCE TO THE LOGANIACEAE¹

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Phylogenetic relationships in the Gentianales with focus on Loganiaceae sensu lato are evaluated using parsimony analyses of nucleotide sequence data from the plastid genes *rbcL* and *ndhF*. Inter- and intrafamilial relationships in the Gentianales, which consist of the families Apocynaceae (including Asclepiadaceae), Gelsemiaceae, Gentianaceae, Loganiaceae, and Rubiaceae, are studied and receive increased support from the combination of *rbcL* and *ndhF* data, which indicate that the family Rubiaceae forms the sister group to the successively nested Gentianaceae, Apocynaceae, and Loganiaceae, all of which are well supported. The family Gelsemiaceae forms a distinct, supported group sister to Apocynaceae. The Loganiaceae sensu stricto form a strongly supported group consisting of 13 genera: *Antonia*, *Bonyunia*, *Gardneria*, *Geniostoma*, *Labordia*, *Logania*, *Mitrasacme*, *Mitreola*, *Neuburgia*, *Norrisia*, *Spigelia*, *Strychnos*, and *Usteria*. These genera form two well-supported lineages. Several members of Loganiaceae sensu Leeuwenberg and Leenhouts, i.e., *Androya*, *Peltanthera*, *Plocosperma*, *Polypremum*, and *Sanango* are clearly not members of the Gentianales. The earlier exclusion of Buddlejaceae (including *Buddleja*, *Emorya*, *Gomphostigma*, and *Nicodemia*) as well as the reclassification of the genera *Nuxia* and *Retzia* to Stilbaceae of the Lamiales are all well supported.

Key words: Apocynaceae; Gelsemiaceae; Gentianales; Loganiaceae; *ndhF*; phylogeny; *rbcL*; Rubiaceae.

Members of the Gentianales (APG, 1998) share several vegetative, floral, and phytochemical traits and range from small alpine herbs to large rain forest trees, including many ornamentals and economically important plants (e.g., *Catharanthus*, *Cinchona*, *Coffea*, and *Strychnos*). In general agreement with most recent classifications, Gentianales consist of the families Apocynaceae, Asclepiadaceae, Gentianaceae, Loganiaceae, and Rubiaceae (e.g., Wagenitz, 1959). A majority of the plants in the order are woody with opposite, entire leaves, often with stipules and colleters. The latter are a special type of multicellular glandular hair, located on the stipules, at the base of the leaves, or inside the calyx. Generally the flowers are regular and pentamerous, and endosperm formation is nuclear (Schumann, 1891, 1895; Gilg, 1895; Hakki, 1980); this in contrast to other euasterid II orders, e.g., Solanales and Lamiales (APG, 1998). The most significant feature of their wood anatomy is internal phloem, the presence of which led to the inclusion of the monotypic family Saccifoliaceae, with its only member *Saccifolium bandeirae* from the Guyana Highlands, in the

order (Cronquist, 1981). Since then this species has been considered as part of or closely allied to Gentianaceae (Metcalf and Chalk, 1983; Takhtajan, 1997), an opinion mainly based on general morphological similarities in wood anatomy and flower morphology (Maguire and Pires, 1978). The absence of internal phloem in the Rubiaceae has occasionally been used as an argument to exclude the latter from the Gentianales (Cronquist, 1981). Furthermore, several chemical constituents, of which indole alkaloids are the most well known, unite Loganiaceae (excluding Retziaceae and Buddlejaceae) with Rubiaceae and Apocynaceae according to Kisakürek and Hesse (1980).

The circumscription of Gentianales has long been debated (de Candolle, 1824–1873; Bartling, 1830; Lindley, 1833; Meisner, 1836–1843; Endlicher, 1841; Bentham and Hooker, 1862–1883; Baillon, 1888, 1889; Engler, 1898; Solereder, 1899; Wilhelm, 1910; Hallier, 1912; Bessey, 1915; Wettstein, 1924; Rendle, 1952; Tournay and Lawalrée, 1952; Hutchinson, 1959, 1973; Wagenitz, 1959, 1964; Cronquist, 1968, 1981, 1983, 1988; Soó, 1975; Thorne, 1976, 1983, 1992a, b; Benson, 1979; Dahlgren, 1980a, b, 1983; Dahlgren, Jensen, and Nielsen, 1981; Takhtajan, 1987, 1997; Dahlgren, 1992; Nicholas and Baijnath, 1994; Struwe and Albert in Struwe, Albert, and Bremer, 1994). Taxa with contorted flower aestivation were grouped by Bartling (1830) in Contortae, later renamed as Gentianales by Lindley (1833). Recognized in *Genera Plantarum* (Bentham and Hooker, 1862–1883), the order consisted of the six families Apocynaceae, Asclepiadaceae, Gentianaceae, Loganiaceae, Oleaceae, and Salvadoraceae. Nearly 100 years later Wagenitz (1959) was the first author to include Rubiaceae, earlier associated with Caprifoliaceae (Jussieu, 1789; Baillon, 1880) in the order. Debate continued, and at the “angiosperm

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TABLE 1. Summary of various classifications from different authors, organized according to the synopsis outlined in Table 5. All genera of the Loganiaceae s.l. by Leeuwenberg and Leenhouts (1980) are included.

Taxon	Leeuwenberg and Leenhouts (1980)	Cronquist (1981)	Thorne (1983)	Struwe and Albert in Struwe, Albert, and Bremer (1994)	Takhtajan (1997)	Backlund, Oxelman, and Bremer (1999)
<i>Gelsemium</i>	Log-Gels	Log	Log-Loga	Gel	Gel	Gel
<i>Mostuea</i>	Log-Gels	Log	Log-Loga	Gel	Gel	Gel
<i>Anthocleista</i>	Log-Pota	Log	Log-Loga	Gen	Gen-Pota	Gen
<i>Fagraea</i>	Log-Pota	Log	Log-Loga	Gen	Gen-Pota	Gen
<i>Potalia</i>	Log-Pota	Log	Log-Loga	Gen	Gen-Pota	Gen
<i>Antonia</i>	Log-Anto	Log	Log-Loga	Str	Ant	Log
<i>Bonyunia</i>	Log-Anto	Log	Log-Loga	Str	Ant	Log
<i>Norrisia</i>	Log-Anto	Log	Log-Loga	Str	Ant	Log
<i>Usteria</i>	Log-Anto	Log	Log-Loga	Str	Ant	Log
<i>Gardneria</i>	Log-Stry	Log	Log-Loga	Str	Str	Log
<i>Neuburgia</i>	Log-Stry	Log	Log-Loga	Str	Str	Log
<i>Spigelia</i>	Log-Spig	Log	Log-Loga	Str	Spi	Log
<i>Strychnos</i>	Log-Stry	Log	Log-Loga	Str	Str	Log
<i>Geniostoma</i>	Log-Loga	Log	Log-Loga	Geo	Geo	Log
<i>Labordia</i>	Log-Loga	Log	Log-Loga	Geo	Geo	Log
<i>Logania</i>	Log-Loga	Log	Log-Loga	Log	Log	Log
<i>Mitrasacme</i>	Log-Spig	Log	Log-Loga	Log	Spi	Log
<i>Mitreola</i>	Log-Spig	Log	Log-Loga	Log	Spi	Log
<i>Buddleja</i>	Log-Budd	Bud ●	Bud	?	Bud ●	Bud ●
<i>Emorya</i>	Log-Budd	Bud	Bud	?	Bud ●	Bud ●
<i>Gomphostigma</i>	Log-Budd	Bud ●	Bud	?	Bud ●	Bud ●
<i>Nicodemia</i>	Log-Budd	Bud ●	Bud	?	Bud ●	Bud ●
<i>Nuxia</i>	Log-Budd	Bud	Bud	?	Bud ●	Sti ●
<i>Retzia</i>	Log-Retz	Ret	Log-Retz	?	Ret ●	Sti ●
<i>Peltanthera</i>	Log-Budd	Bud	Bud	?	Bud ●	Ges ●
<i>Sanango</i>	Log-Budd	Bud	Bud	?	Bud ●	Ges ●
<i>Androya</i>	Log-Budd	Bud	Bud	?	Bud ●	Myo ●
<i>Plocosperma</i>	Log-Ploc	Log	Log-Ploc	?	Plo	Plo ●
<i>Polypremum</i>	Log-Spig	Log	Log-Loga	?	Bud ●	Tet ●
<i>Desfontainia</i>	Log-Desf	Log	Log-Desf	?	Des ●	Col ●

Note: The following abbreviations are used: for families, Ant = Antoniaceae, Bud = Buddlejaceae, Col = Columelliaceae, Des = Desfontainiaceae, Gel = Gelsemiaceae, Geo = Geniostomataceae, Gen = Gentianaceae, Ges = Gesneriaceae, Log = Loganiaceae, Myo = Myoporaceae, Plo = Plocospermataceae, Ret = Retziaceae, Spi = Spigeliaceae, Sti = Stilbaceae, Str = Strychnaceae, Tet = Tetrachondraceae, for tribes, Anto = Antonieae, Budd = Buddlejeae, Desf = Desfontainieae/Desfontainioideae, Gels = Gelsemieae, Loga = Loganiaceae/Loganioideae, Ploc = Plocospermeae/Plocospermatoidae, Pota = Potalieae, Retz = Retzieae/Retzioideae, Spig = Spigeliaceae, Stry = Strychnaceae, and for additional entries: ? = *incertae sedis*, and ● = explicitly excluded from the Gentianales.

meeting" in Sydney in 1983, several new different circumscriptions of the Gentianales were proposed, some including Rubiaceae (Dahlgren, 1983; Thorne, 1983) and some not (Cronquist, 1983). In later phylogenetic studies, Gentianales were conceived as a monophyletic group including Apocynaceae, Asclepiadaceae, Gentianaceae, Rubiaceae, and parts of Loganiaceae (Bremer and Struwe, 1992; Downie and Palmer, 1992; Olmstead et al., 1993). Asclepiadaceae have been shown to belong to Apocynaceae according to several recent studies (Endress et al., 1996; Sennblad and Bremer, 1996; Sennblad, 1997). In contrast to the order, Loganiaceae (sensu Leeuwenberg and Leenhouts, 1980) in all these studies was suggested to be polyphyletic, eventually leading to the erection of two new families by Struwe and Albert (in Struwe, Albert, and Bremer, 1994), Gelsemiaceae and Geniostomataceae. In his latest comprehensive classification Takhtajan (1997) recognized nine families in Gentianales: Antoniaceae, Gelsemiaceae, Geniostomataceae, Gentianaceae, Loganiaceae, Plocospermataceae, Saccifoliaceae, Spigeliaceae, and Strychnaceae.

Leenhouts (1962) and Leeuwenberg and Leenhouts (1980) emphasized Loganiaceae as a core family in Gentianales and believed it to constitute a link between the

other families (Leeuwenberg and Leenhouts, 1980, p. 19). A number of studies (Bremer and Struwe, 1992; Downie and Palmer, 1992; Chase et al., 1993; Olmstead et al., 1993; Struwe, Albert, and Bremer, 1994) have indicated polyphyly of Loganiaceae sensu Leeuwenberg and Leenhouts. Several taxa have been demonstrated not to be part of Gentianales, but instead related to other groups, e.g., *Retzia* (Bremer et al., 1994) and Buddlejaceae (Oxelman, Backlund, and Bremer, 1999) to Lamiales and *Desfontainia* to Dipsacales (Bremer et al., 1994; Backlund and Bremer, 1997). No recent previous study, however, has presented a complete analysis of all suggested elements of Loganiaceae. An overview of some previous classifications of Loganiaceae is presented in Table 1.

As sequencing targets for this study the two plastid genes *rbcL* and *ndhF* were selected. The *rbcL* gene codes for the large subunit of the ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) and has been widely used in plant systematic studies (Ritland and Clegg, 1987; Kim et al., 1992; Olmstead et al., 1992; Chase et al., 1993; Morgan and Soltis, 1993; Olmstead et al., 1993). The *ndhF* gene is (on the basis of observed homologies) presumed to code for subunit 6 of NADH-

dehydrogenase (Sugiura, 1992) and due to its different substitution rate has been advocated as an alternative to *rbcL* (Kim and Jansen, 1995).

The combined sequence data from *rbcL* and *ndhF* were analyzed using parsimony methods. This approach of combining data sets has provided important contributions in studies of several other plant groups (Olmstead and Sweere, 1994; Olmstead and Reeves, 1995; Scotland et al., 1995; Chase and Cox, 1998; Soltis et al., 1998; Savolainen et al., 2000).

The aims of this study were threefold: (1) to evaluate the monophyly of Gentianales, (2) to study the inter- and intrafamilial relationships of the order, and (3) to determine in further detail the relationships and systematic positions of the members of Loganiaceae sensu Leeuwenberg and Leenhouts (1980).

MATERIAL AND METHODS

Taxon sampling—Attempts were made to obtain a representative sampling of Gentianales. Additionally, several taxa from other closely related orders (Olmstead et al., 1992, 1993; Chase et al., 1993; Bremer et al., 1994; Gustafsson, Backlund, and Bremer, 1996; Backlund and Bremer, 1997; Oxelman, Backlund, and Bremer, 1999) were included. This was done to permit an extra-ordinal evaluation of the systematic positions of the former “loganiaceous” taxa (Leeuwenberg and Leenhouts, 1980). Taxon sampling in Loganiaceae included all genera recognized by Leeuwenberg and Leenhouts (1980), with the exception of *Norrissia* (due to lack of material). The tribe Antonieae, in which *Norrissia* was placed by Leeuwenberg and Leenhouts, is, however, represented by all the other genera (*Antonia*, *Bonyunia*, and *Usteria*).

A matrix consisting of *rbcL* and *ndhF* sequences of 62 taxa was compiled, in total 124 sequences. Of these, eight *rbcL* and 22 *ndhF* sequences were previously unpublished. All sequences analyzed are listed in Table 2 with their familial affinities according to Takhtajan (1997), EMBL accession numbers, and voucher information or references to their original publication.

Methods—Total DNA was extracted from fresh or silica-gel dried leaves (Chase and Hills, 1991) according to the methods of Doyle and Doyle (1987) or from herbarium material (Oxelman, Backlund, and Bremer, 1999). Double-stranded DNA of the two genes were amplified by the polymerase chain reaction (PCR) using two primers for the *rbcL* gene (Olmstead et al., 1992) and four for the *ndhF* gene (Kim and Jansen, 1995). The positions and sequences of the primers are listed in Table 3. For the manually sequenced DNA, a second run with asymmetric amplification was performed to obtain single-stranded DNA (Kaltenboeck et al., 1992). Amplification products were purified with the QIAquick[®] Gel Extraction Kit according to the manufacturer's instructions (QIAGEN[®]). DNA was sequenced using internal primers designed by G. Zurawski at the DNAX Research Institute (for *rbcL*) and K.-J. Kim, R. Jansen, and B. Oxelman (for *ndhF*, Table 3, including a schematic drawing of the primer positions and directions). Sequencing reactions were performed according to the method originally devised by Sanger, Nicklen, and Coulson (1977). Sequences were produced either manually with S³⁵ radioactively labelled dideoxy nucleotides resulting in autoradiograms or automated with the PRISM[®] Ready Reaction Dye Deoxy Terminator FS kit (Applied Biosystems) and analyzed on a ABI PRISM 377 automated sequencer (PE corporation).

Sequence alignment and matrix compilation—To investigate the systematic position of the taxa studied, the new sequences were analyzed together with other relevant sequences already published. These were obtained from the National Center for Biotechnology Information (NCBI, “GenBank”) database or from the European Bioinformatic In-

stitute (EBI, “EMBL Nucleotide Sequence Database”). The sequences were manually aligned to the reading frame of the corresponding genes in the complete *Nicotiana* plastid genome sequence (Shinozaki et al., 1986; GenBank Z00044). Positions 114168–112096 (reverse complement) were used for the *ndhF* and 57612–59020 for the *rbcL* gene. The *rbcL* sequences have no nucleotide insertions or deletions, whereas the *ndhF* sequences exhibit variation in length. The complete aligned matrices can be obtained from <http://www.botany.org/bsa/ajbsupp/v86/s01-01.html>, or upon request.

Phylogenetic analysis—The combined *rbcL* and *ndhF* matrix was analyzed using parsimony methods with PAUP* 4.0d64 (D. Swofford, personal communication) and xac 1.2 (S. Farris, personal communication) both under the assumptions of Fitch parsimony (Fitch, 1971).

The analyses were made on a Power Macintosh 8600/250 using 85 Mb of RAM. As a starting point 100 random stepwise additions were used for a thorough branch swapping. This was made by the tree bisection-reconnection algorithm of PAUP (Swofford, 1993). To estimate stability of the obtained result, Bremer support values (b) were determined (Bremer, 1988, 1994; Källersjö et al., 1992) using the generalized reversed constraint approach (Eernisse and Kluge, 1993). A batch processing file for calculating the Bremer support values was constructed using the computer program AutoDecay 2.9.5 (Eriksson, 1995). The constrained searches for each of the internal nodes were performed in the same way as the initial analysis but with ten random stepwise additions. PAUP* was also used to perform a bootstrap analysis (Felsenstein, 1985). Here 100 replicate matrices were produced and each of these was analyzed using five random stepwise additions followed by branch swapping as described above. Finally parsimony-jackknife analyses (Farris et al., 1996) were made both with PAUP* and with xac. Equal probability deletion of 37% of the characters were made after recommendation by Farris et al. (1996) and the resulting 100 (PAUP*) or 1000 (xac) replicate matrices were analyzed analogously to the bootstrap analysis.

Furthermore, parsimony and jackknife analyses were performed in a similar manner as described above for each of the two genes separately.

Character weighting—A series of arguments have been brought forward both promoting and criticizing posteriori character weighting (Farris, 1983; Goloboff, 1993, 1995; Turner and Zandee, 1995; Allard and Carpenter, 1996; Nixon and Carpenter, 1996). In this study we decided to use successive approximation weighting (Farris, 1969). With this method characters are weighted according to their behavior on a (or a set of) tree(s). Different indices such as the consistency index (CI; Farris, 1969), retention index (RI; Farris, 1989), or the rescaled consistency index (RC; Farris, 1989) can be used as basis for the weighting. According to recommendations by Farris (1989) and Swofford (defaults in PAUP) we have used RC. The analysis using Farris' successive approximations weighting procedure were performed with analogous settings and options as described for the unit-weighted analysis.

RESULTS

Separate vs. combined analyses—Results from the separate as well as combined analyses of nucleotide sequence data from the genes *rbcL* and *ndhF* are largely congruent. Apart from minor differences in relationships of taxa outside Gentianales, none of the well-supported groups were in conflict. Hence, only results from more thoroughly analyzed combined matrix will be discussed.

The unit-weighted analysis—The initial analysis using Fitch parsimony yielded 12 equally parsimonious trees with a length of 5941 steps, a consistency index (CI; Kluge and Farris, 1969) of 0.36 and a retention index

TABLE 2. List of investigated taxa. Familial assignments following Takhtajan (1997), abbreviations: Acanthaceae (Aca), Adoxaceae (Ado), Apocynaceae (Apo), Asteraceae (Ast), Boraginaceae (Bor), Buddlejaceae (Bud), Caprifoliaceae (Cap), Columelliaceae (Col), Gesneriaceae (Ges), Gentianaceae (Gen), Gelsemiaceae (Gel), Loganiaceae (Log), Menyanthaceae (Men), Oleaceae (Ole), Pedaliaceae (Ped), Rubiaceae (Rub), Solanaceae (Sol), Scrophulariaceae (Scr), Verbenaceae (Ver). Accession numbers correspond with accessions in EBI or NCBI public databases; sequences with * are previously unpublished. Herbaria abbreviations follow Holmgren, Holmgren, and Barnett (1990). Vouchers are presented for previously unpublished sequences. For previously published sequences a reference is given for the original publication. Methods for new sequences are: A for sequences obtained by automated sequencer, M for manually.

Taxon	Family	<i>rnhF</i>	Voucher	<i>rbcL</i>	Voucher	Method
<i>Ajuga reptans</i>	Lam	L36391	Olmstead and Reeves, 1995	U32163	Wagstaff and Olmstead, 1997	-/-
<i>Alstonia scholaris</i>	Apo	AJ011982	Oxelman, Backlund, and Bremer, 1999	X91760	Sennblad and Bremer, 1996	M/-
<i>Androya decaryi</i>	Log	AF027276	Oxelman, Backlund, and Bremer, 1999	AJ001756	Oxelman, Backlund, and Bremer, 1999	-/-
<i>Anthocheistia grandiflora</i>	Log	AJ235829*	Bremer 3098 (UPS)	L14389	Bremer 3098 (UPS)	A/-
<i>Antirrhinum majus</i>	Scr II	L36392	Olmstead and Reeves, 1995	L11688	Olmstead et al., 1992	-/-
<i>Antonia ovata</i>	Log	AJ235832*	Rova 1964 (GB)	AJ235817*	Rova 1964 (GB)	A/A
<i>Barleria prionitis</i>	Aca	U12653	Scotland et al., 1995	L01886	Albert, Williams, and Chase, 1992	-/-
<i>Barnadesia caryophylla</i>	Ast	L39394	Kim and Jansen, 1995	L13859	Michaels, unpublished data	-/-
<i>Bonyunia minor</i>	Log	AJ235833*	Berry 5522 (MO)	AJ235818*	Berry 5522 (MO)	A/A
<i>Borago officinalis</i>	Bor	L36393	Olmstead and Reeves, 1995	L11680	Olmstead et al., 1992	-/-
<i>Buddleja asiatica</i>	Bud	AF027277	Oxelman, Backlund, and Bremer, 1999	AJ001758	Oxelman, Backlund, and Bremer, 1999	-/-
<i>Chiococca racemosa/alba</i>	Rub	AJ130835	Bremer et al., 1999	L14394	Olmstead et al., 1993	-/-
<i>Cinchona pubescens</i>	Rub	AJ235843*	Bremer 2733 (UPS)	X83630	Bremer, Andreassen, and Olsson, 1995	M/-
<i>Desfontainia spinosa</i>	Col	AJ011988	Oxelman, Backlund, and Bremer, 1999	Z29670	Bremer et al., 1994	A/-
<i>Emorya suaveolens</i>	Bud	AF027278	Oxelman, Backlund, and Bremer, 1999	AJ001759	Oxelman, Backlund, and Bremer, 1999	-/-
<i>Exacum affine</i>	Gen	AJ011983	Oxelman, Backlund, and Bremer, 1999	L11684	Olmstead et al., 1992	M/-
<i>Fagraea</i> sp.	Log	AJ235830*	Bremer, Su-S 900109	L14396	Olmstead et al., 1993	A/-
<i>Gardenia thunbergia</i>	Rub	AJ235844*	Gillis 10913 (FTG)	X83637	Bremer, Andreassen, and Olsson, 1995	M/-
<i>Gardenia angustifolia</i>	Log	AJ235834*	Ludlow et al. 20888 (UPS)	AJ235819*	Ludlow et al. 20888 (UPS)	M/A
<i>Gelsemium sempervirens</i>	Log	AJ011984	Oxelman, Backlund, and Bremer, 1999	L14397	Olmstead et al., 1993	M/-
<i>Gentiana rupestris</i>	Log	AJ235835*	Garnock-Jones 2200 (WELTU)	Z68828	Endress et al., 1996	M/-
<i>Genitana procer</i>	Gen	L36400	Olmstead and Reeves, 1995	L14398	Olmstead et al., 1993	-/-
<i>Gonphostigma scoparioides</i>	Bud	AF027279	Oxelman, Backlund, and Bremer, 1999	AJ001760	Oxelman, Backlund, and Bremer, 1999	-/-
<i>Kopsia fruticosa</i>	Apo	AJ235824*	Bremer 3033 (UPS)	X91763	Sennblad and Bremer, 1996	M/-
<i>Labordia tinifolia</i>	Log	AJ235839*	Takeuchi 2244 (GB)	AJ235820*	Takeuchi 2244 (GB)	M/A
<i>Logania vaginalis</i>	Log	AJ235836*	Bremer 3013 (UPS)	Z68826	Bremer, 1996b	M/-
<i>Lonicera orientalis</i>	Cap	AJ235837*	Oxelman, Backlund, and Bremer, 1999	X87389	Gustafsson, Backlund, and Bremer, 1996	M/-
<i>Menyanthes trifoliata</i>	Men	AF027274	Kim and Jansen, 1995	L14006	Olmstead et al., 1993	-/-
<i>Mitrasacme pilosa</i>	Log	L39388	Muir 1817 (UPS)	AJ235821*	Muir 1817 (UPS)	A/A
<i>Mostuea brunonis</i>	Log	AJ236058*	Miller 7570 (UPS)	AJ235822*	Miller 7570 (UPS)	A/A
<i>Mitreola petiolata</i>	Gel	AJ235828*	Thulin 7831 (UPS)	L14404	Olmstead et al., 1993	M/-
<i>Mussaenda erythrophylla</i>	Rub	AJ130836	Bremer et al., 1999	X83652	Bremer, Andreassen, and Olsson, 1995	-/-
<i>Neuburgia corynocarpum</i>	Log	AF027275	Oxelman, Backlund, and Bremer, 1999	AJ001755	Oxelman, Backlund, and Bremer, 1999	-/-
<i>Nicodemia diversifolia</i>	Bud	L36405	Olmstead and Reeves, 1995	L14413	Olmstead et al., 1993	-/-
<i>Nicotiana tabacum/debneyi</i>	Sol	L14953	Olmstead, Sweete, and Wolfe, 1993	D70815	Shikanai et al., 1996	-/-
<i>Nuxia</i> sp.	Sti	AF027280	Oxelman, Backlund, and Bremer, 1999	AJ001761	Oxelman, Backlund, and Bremer, 1999	-/-
<i>Nyctanthes arbor-tristis</i>	Ole	U78708	Wagstaff et al., 1998	U28877	Wagstaff and Olmstead, 1997	-/-
<i>Oldenlandia</i> cf. <i>corymbosa</i>	Rub	AJ130837	Bremer et al., 1999	X83655	Bremer, Andreassen, and Olsson, 1995	-/-
<i>Ophiorrhiza mungos</i>	Rub	AJ130838	Bremer et al., 1999	X83656	Bremer, Andreassen, and Olsson, 1995	-/-
<i>Peltanthera floribunda</i>	Log	AF027281	Oxelman, Backlund, and Bremer, 1999	AJ001762	Oxelman, Backlund, and Bremer, 1999	-/-
<i>Periploca graeca</i>	Apo	AJ235825*	Sennblad 255 (UPS)	AJ002889	Sennblad, unpublished data	A/-
<i>Pinckneya pubescens</i>	Rub	AJ130839	Bremer et al., 1999	X83661	Bremer, Andreassen, and Olsson, 1995	-/-
<i>Plocosperma buxifolium</i>	Log	AJ011985	Oxelman, Backlund, and Bremer, 1999	Z68829	Endress et al., 1996	M/-
<i>Polypremum procumbens</i>	Log	AJ011986	Oxelman, Backlund, and Bremer, 1999	AJ011989	Oxelman, Backlund, and Bremer, 1999	M/M
<i>Potalia resinifera</i>	Log	AJ235831*	Stahl and Knudsen 3025 (GB)	AJ235816*	Stahl and Knudsen 3025 (GB)	A/A
<i>Retzia capensis</i>	Sti	AF027289	Oxelman, Backlund, and Bremer, 1999	Z29669	Bremer et al., 1994	-/-
<i>Rondeletia odorata</i>	Rub	AJ235845*	Bremer and Andreassen 3504 (UPS)	Y11857	Bremer and Thulin, 1998	A/-
<i>Sanango racemosum</i> /sp.	Log	U62144	Smith et al., 1997	AJ001763	Oxelman, Backlund, and Bremer, 1999	-/-
<i>Schlegelia parviflora</i>	Scr s.lat.	L36410	Olmstead and Reeves, 1995	L36448	Olmstead and Reeves, 1995	-/-

TABLE 2. Continued.

Taxon	Family	<i>ndhF</i>	Voucher	<i>rbcL</i>	Voucher	Method
<i>Sesamum indicum</i>	Ped	L36413	Olmstead and Reeves, 1995	L14408	Olmstead et al., 1993	—/—
<i>Spigelia antheimia</i>	Log	AJ235840*	Bremer 3377 (UPS)	Y11863	Bremer and Thulin, 1998	M/—
<i>Stephanotis floribunda</i>	Apo	AJ235826*	Sennblad 256 (UPS)	SSSSSS	Sennblad, unpublished data	A/—
<i>Stilbe albiflora/vestita</i>	Sti	AF027287	Oxelman, Backlund, and Bremer, 1999	Z68827	Bremer, 1996b	—/—
<i>Streptocarpus holstii</i>	Ges	L36415	Olmstead and Reeves, 1995	L14409	Olmstead et al., 1993	—/—
<i>Strychnos potatorum/nux-vomica</i>	Log	AJ235841*	Bremer 3022 (UPS)	L14410	Olmstead et al., 1993	M/—
<i>Usteria guineensis</i>	Log	AJ235842*	Adams 666 (UPS)	AJ235823*	Adams 666 (UPS)	A/A
<i>Vangueria madagascariensis</i>	Rub	AJ130840	Bremer et al., 1999	X83670	Bremer, Andreassen, and Olsson, 1995	—/—
<i>Verbascum thapsus</i>	Scr I	L36417	Olmstead and Reeves, 1995	L36452	Olmstead and Reeves, 1995	—/—
<i>Verbena bracteata/bonariensis</i>	Ver	L36418	Olmstead and Reeves, 1995	L14412	Olmstead et al., 1993	—/—
<i>Veronica catenata</i>	Scr II	L36419	Olmstead and Reeves, 1995	L36453	Olmstead and Reeves, 1995	—/—
<i>Viburnum rhytidophyllum</i>	Ado	AF027273	Oxelman, Backlund, and Bremer, 1999	X87398	Gustafsson, Backlund, and Bremer, 1996	—/—
<i>Wrightia arborea</i>	Apo	AJ235827*	Leeuwenberg 14225 (WAG)	AJ002891	Sennblad, unpublished data	A/—

(RI; Farris, 1989) of 0.63. The strict consensus tree is shown in Fig. 1.

The successively weighted analysis—The analysis using the successive approximation weighting (Farris, 1969) produced one most parsimonious tree. The topology of this was identical to one of the 12 equally parsimonious trees obtained from the unit-weighted analysis and is selected for further discussions (Fig. 2). Branch lengths and support indices are given in Table 4.

Tree topology and implied relationships—The trees were oriented using an outgroup consisting of five taxa belonging to the euasterid II clade (Chase et al., 1993; Bremer et al., 1994; Backlund and Bremer, 1997; Oxelman, Backlund, and Bremer, 1999; APG, 1998).

Euasterid I—The ingroup corresponds to the euasterid I (Chase et al., 1993; APG, 1998) and is divided into two major lineages, Gentianales and Lamiales. The sole representative for the order Solanales, *Nicotiana*, is found sister to this major dichotomy. The support for euasterid I is high (branch length [bl] = 83, Bremer support [b] = 32, jackknife value according to PAUP* [j] and xac [x] as well as bootstrap value [bt] all = 100%). This indicates that none of the members of Loganiaceae, sensu Leeuwenberg and Leenhouts, has a relationship to euasterid II with the exception of *Desfontainia*, which has previously been placed as a member of Dipsacales (Bremer et al., 1994; Gustafsson, Backlund, and Bremer, 1996; Backlund and Bremer, 1997).

Lamiales—The support for this group is high (bl = 52, b = 17, j = x = bt = 100%). In this lineage, a number of genera sometimes previously placed in the Loganiaceae are found, in agreement with the more detailed recent works (e.g., Oxelman, Backlund, and Bremer, 1999). The topology in this part of the tree differs slightly from the topologies obtained solely from *rbcL* data (Chase et al., 1993; Olmstead et al., 1993). The consensus tree presented by Olmstead and Reeves (1995) is, however, congruent with the results by Oxelman, Backlund, and Bremer (1999) as well as the results from this study. It is shown, both in this and in the study by Oxelman, Backlund, and Bremer (1999), that several of the genera previously included in Loganiaceae sensu Leeuwenberg and Leenhouts belong instead in Lamiales sensu Takhtajan.

Gentianales—The other major lineage within euasterid I (node 28) corresponds to Gentianales, which have high support indices (bl = 58, b = 27, j = x = bt = 100%). Within Gentianales, we find a basal dichotomy between a well-supported Rubiaceae (node 29, bl = 39, b = 18, j = x = bt = 100%) and a lineage (node 37, bl = 39, b = 6, j = 98%, x = 96%, bt = 95%) composed of the Gentianaceae, Apocynaceae, Gelsemiaceae, and a large portion of Loganiaceae. Gentianaceae (bl = 145, b = 96, j = x = bt = 100%) include the three former loganiaceous genera *Anthocleista*, *Fagraea*, and *Potalia*.

Loganiaceae—The core Loganiaceae is well supported (bl = 40, b = 23, j = x = bt = 100%), and this node (49) is used to define which genera are included in Table

TABLE 3. Primers used for PCR amplification for the *rbcL* and *ndhF* genes of the chloroplast genome. All primers are given in their 5' to 3' directions. Position numbers refer to the published strand of the chloroplast genome of *Nicotiana tabacum* (Shinozaki et al., 1986). Symbols in front of nucleotide sequences indicate: ● = published sequence, * = complementary strand, + = actual primer sequence.

<i>rbcL</i> – 5' primer	57587	● ATGTCACCACAAACAGAACTAAAGCAAGT + atgtcaccacaacagagactaaagcaagt	57616
<i>rbcL</i> – 3' primer	59146	* CTTTTAGTAAAAGATTGGGCCGAG + cttttagtaaaagattggcccgag	59123
<i>ndhF</i> – 5' primer 1	114250	* AGGTAAGATCCGGTGAATCGGAAAC + aggtaagatccgggtaatcggaac	114226
<i>ndhF</i> – 5' primer 2	112999	* AGGTACACTTCTCTTTGTGGTATTCC + aggtacacttctctttgctggtattcc	112973
<i>ndhF</i> – 3' primer 1	112849	● ATAGATCCGAAACATATAAAATGCGGTT + atagatccgacacatataaaatcggtt	112876
<i>ndhF</i> – 3' primer 2	111459	● ACCAAGTTCAATGTTAGCCAGATTAGTG + accaagtccaatgttagccagattagtg	111486

5. At the base Loganiaceae divides into two well-supported lineages, one including *Usteria*, *Antonia*, and *Bonyunia*, and the other (node 52) further divided into two groups. One of these (node 53), including *Gardneria*, *Neuburgia*, *Spigelia*, and *Strychnos*, is weakly supported. The remaining group (node 56), on the other hand, is well supported and consists of *Mitreola*, *Logania*, *Mitrasacme*, *Geniostoma*, and *Labordia*.

DISCUSSION

Monophyly of Gentianales—We find strong support for Gentianales (node 28 in Fig. 2). The inclusion of the families Apocynaceae, Gelsemiaceae, Gentianaceae, Loganiaceae, and Rubiaceae is congruent with several previous molecular studies of the group (Downie and Palmer, 1992; Chase et al., 1993; Olmstead et al., 1993; Bremer et al., 1994), as well as morphological (Struwe, Albert, and Bremer, 1994) and phytochemical (Jensen, 1991, 1992) patterns of variability. Notable among the morphological traits is the combination of opposite and entire leaves with stipules (with some exceptions), colletes, and nuclear endosperm formation (typically cellular in the Lamiales and Solanales; cf. Johri, Ambegao, and Srivastava, 1992). The phytochemical distinction is most evident in the widespread and prominent occurrence of complex indole alkaloids and seco-iridoids synthesized solely along the iridoid biosynthesis route I, as compared to route II compounds, which are encountered in Solanales and Lamiales (Jensen, 1991, 1992).

Inter- and intrafamilial relationships of Gentianales—Recent phylogenetic studies indicate that within the order there are two major evolutionary lineages, one comprising the families Gentianaceae, Apocynaceae, Gelsemiaceae, and Loganiaceae sensu stricto and the other consisting entirely of the Rubiaceae, which is the sister group to the rest of the order (Bremer, 1996b). In this study, the support for both of these lineages is robust. This dichotomy is also supported by the occurrence of superior ovaries and internal phloem in the Gentianaceae-Apocynaceae-Gelsemiaceae-Loganiaceae lineage as well as by differences in biosynthesis of iridoid and indole alkaloid compounds (Jensen, 1991, 1992).

Rubiaceae—The mainly tropical Rubiaceae, with

10 200 species (Mabberley, 1997), is usually considered as a monophyletic group, but the systematic position of the Rubiaceae has been discussed (Verdcourt, 1958; Bremekamp, 1966; Robbrecht, 1988; Bremer, 1996b). Lack of internal phloem (otherwise ubiquitous in the order) and presence of an inferior ovary (otherwise superior in Gentianales with few exceptions), obturator, Casparian thickenings, and early sympetaly (Erbar, 1991) distinguish the family from the rest of the order. Bentham and Hooker (1862–1883) separated Rubiaceae due to its inferior ovary into a monofamilial Rubiales. There it was retained by Cronquist (1981, 1988) together with the small monogeneric family Theligonaceae (usually included in Rubiaceae; Wunderlich, 1971; Robbrecht, 1988, 1993; Bremer, Andreasen, and Olsson, 1995; Natali, Manen, and Ehrendorfer, 1995), as well as by Takhtajan (1997), who also appended the families Dialypetalanthaceae and Carlemanniaceae. Uetzschneider (1947, 1951), on the other hand, was the first to indicate that Rubiaceae are part of the Gentianales. This was later supported by Wagenitz (1959, 1964), but the proposal did not gain wide acceptance until the 1980s after which it was included in Gentianales in most systematic schemes (Dahlgren, 1980a, b, 1983; Thorne, 1983, 1992a, b; Takhtajan, 1987). Many recent works based on both molecular and morphological data indicate that Rubiaceae are part of Gentianales (Bisset, 1980; Bremer and Struwe, 1992; Struwe, Albert, and Bremer, 1994; Bremer, 1996a, b). The topology within Rubiaceae retrieved from this analysis is largely congruent with previous studies, which have more thorough sampling (Bremer, Andreasen, and Olsson, 1995; Bremer, 1996b) and so will not be discussed further here.

Gentianaceae—The cosmopolitan family Gentianaceae mostly consist of herbs, rarely shrubs or trees and comprise 1200 species (Mabberley, 1997). The inclusion of the woody genera *Potalia*, *Fagraea*, and *Anthocleista* (former tribe Potalieae of Loganiaceae, sometimes recognized as the family Potaliaceae; Watson and Dallwitz, 1992) in Gentianaceae, has long been argued (Bureau, 1856; Fosberg and Sachet, 1980; Bremer and Struwe, 1992; Struwe, Albert, and Bremer, 1994; Mészáros, Laet, and Smets, 1996). Morphological features that have been interpreted as synapomorphies and support of this place-

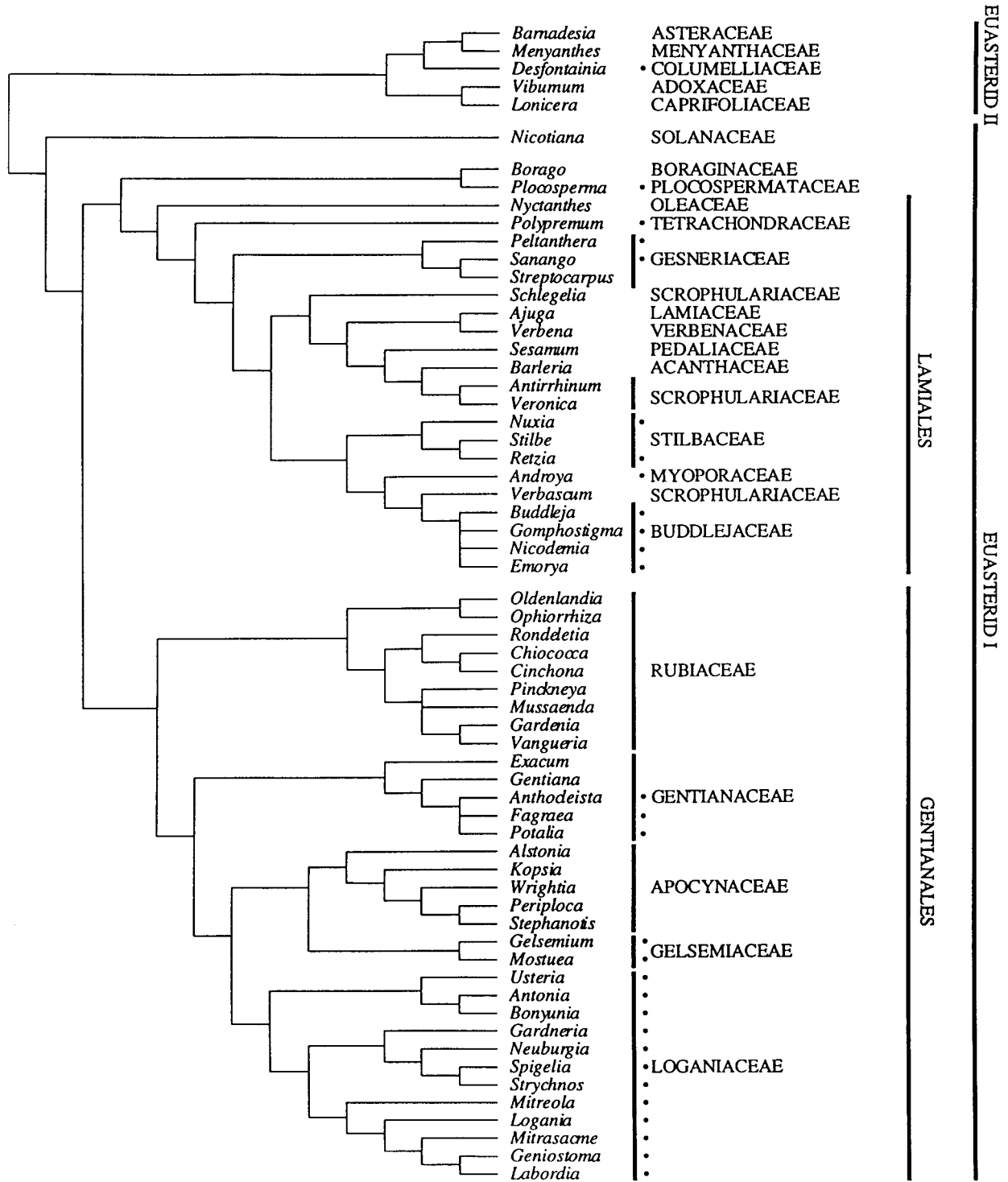


Fig. 1. Consensus tree of 12 equally parsimonious trees resulting from the unit-weighted analysis. Familial classification follows results from this and previous studies (Olmstead and Reeves, 1995; Oxelman, Backlund, and Bremer, 1999). Taxa indicated by dots (•) have previously been assigned to Loganiaceae by Leeuwenberg and Leenhouts (1980).

ment are the monadelphous filaments (not seen in *Fagraea*), dextral-contorted buds, septate parenchyma, and several phytochemical features such as presence of gentianine (Bisset, 1980). These conclusions are also supported from molecular data (Downie and Palmer, 1992;

Olmstead et al., 1993) and appear here to be well supported. The phylogenetically derived position of the woody genera *Potalia*, *Fagraea*, and *Anthocleista*, compared to the mainly herbaceous genera *Exacum* and *Gentiana*, is also well supported.

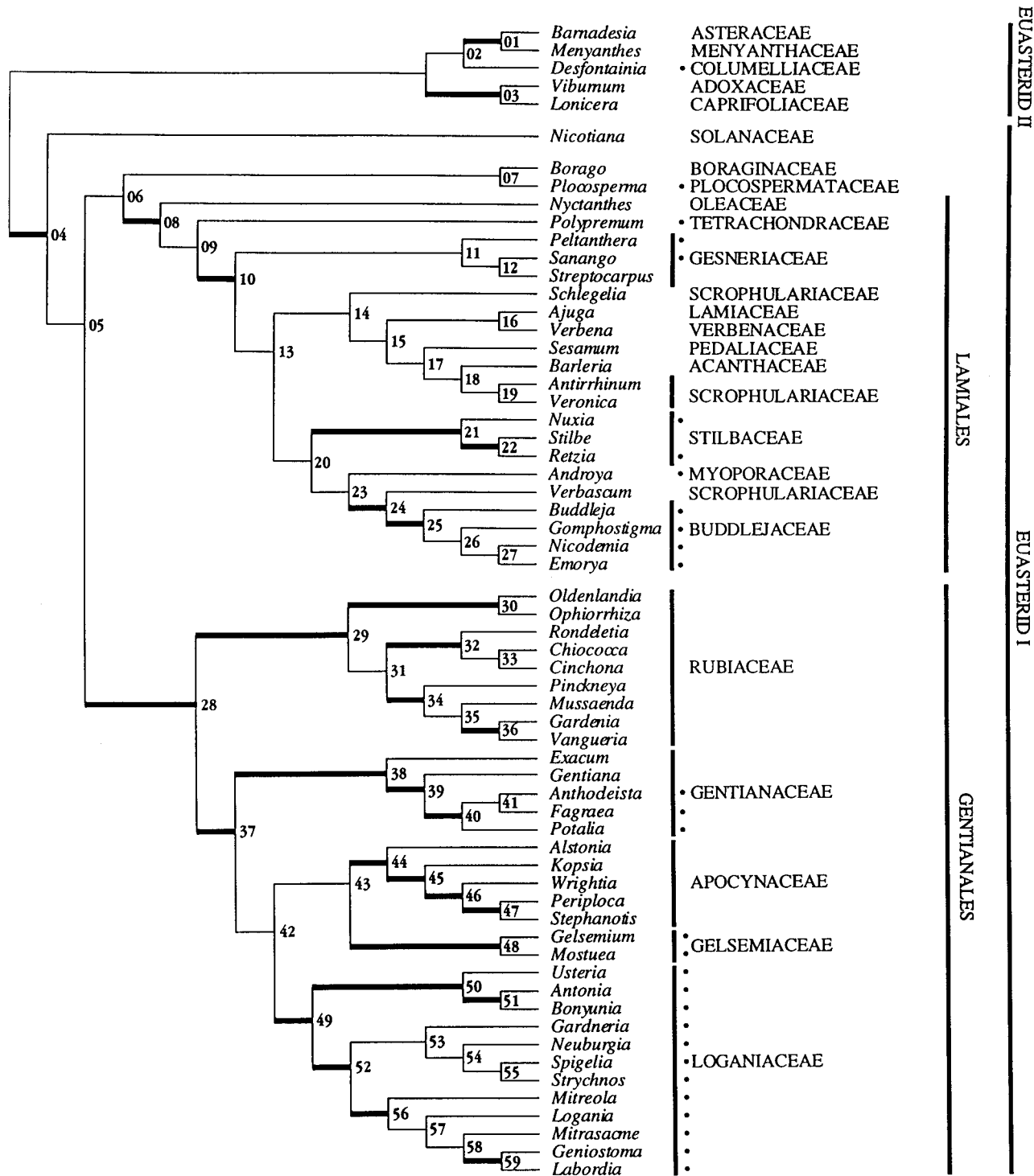


Fig. 2. One of the most parsimonious trees from the unit-weighted analysis with the topology identical to the single most parsimonious tree from the successively reweighted analysis. Familial classification according to results from this and previous studies (Olmstead and Reeves, 1995; Oxelman, Backlund, and Bremer, 1999). Nodes with jackknife and bootstrap values >90% are indicated with thick lines. Taxa indicated by dots (•) have previously been assigned to Loganiaceae by Leeuwenberg and Leenhouts (1980).

The family Menyanthaceae, sometimes included as the tribe Menyantheae in the family Gentianaceae (Bentham and Hooker, 1862–1883) or considered as a close relative (Leeuwenberg and Leenhouts, 1980) have been used as one of the outgroups here. The placement of the Menyan-

thaceae in Asterales has been indicated by *rbcL* analyses (Downie and Palmer, 1992; Chase et al., 1993; Olmstead et al., 1993; Gustafsson, Backlund, and Bremer, 1996; Backlund and Bremer, 1997) and corroborated by studies of morphological data (Gustafsson and Bremer, 1995).

TABLE 4. Support indices for the results from analysis shown in Fig. 2.

no	bl	b	j (%)	x (%)	bt (%)
01.	77	37	100	100	100
02.	41	2	56	—	—
03.	54	37	100	100	100
04.	83	32	100	100	100
05.	29	1	—	—	—
06.	45	1	—	—	—
07.	45	1	—	—	—
08.	52	17	100	100	100
09.	29	1	67	63	75
10.	50	10	100	98	98
11.	19	3	80	76	69
12.	27	5	88	88	76
13.	11	2	54	51	—
14.	7	3	—	—	—
15.	8	2	—	—	—
16.	34	2	—	—	—
17.	12	1	—	—	—
18.	35	2	—	—	—
19.	46	7	98	95	92
20.	6	1	—	—	—
21.	17	14	100	100	100
22.	26	24	100	100	100
23.	17	5	71	66	51
24.	24	16	100	100	100
25.	22	15	100	100	100
26.	7	0	53	—	—
27.	4	0	—	—	—
28.	58	27	100	100	100
29.	39	18	100	100	100
30.	58	30	100	100	100
31.	18	5	80	80	75
32.	44	25	100	100	100
33.	13	2	73	72	77
34.	37	23	100	100	100
35.	15	0	—	—	—
36.	51	33	100	100	100
37.	39	6	98	96	95
38.	145	96	100	100	100
39.	70	25	100	100	100
40.	32	14	99	100	96
41.	13	0	—	—	51
42.	16	3	83	65	60
43.	17	3	78	71	60
44.	44	27	100	100	100
45.	26	9	99	99	97
46.	44	25	100	100	100
47.	56	34	100	100	100
48.	43	25	100	100	100
49.	40	23	100	100	100
50.	96	70	100	100	100
51.	47	40	100	100	100
52.	24	9	99	97	96
53.	10	2	—	—	—
54.	12	2	50	—	—
55.	18	3	68	56	—
56.	44	29	100	100	100
57.	10	2	76	59	55
58.	16	2	64	57	61
59.	37	27	100	100	100

Note: Abbreviations follow the denotations used in the text: no = node number as indicated in Fig. 2, bl = branch length with unit-weighted characters, b = Bremer support calculated with the generalized method by Bremer (1988), j = jackknife values obtained with PAUP from 100 replicates with SPR branch swapping, x = jackknife values from xac from 1000 replicates with SPR branch swapping, bt = bootstrap values obtained with PAUP from 100 replicates with SPR branch swapping. — = indicated j-, x-, or bt-value below 50%.

In total, 12 equally parsimonious trees with a unit-weight length of 5941 steps, CI = 0.356, RI = 0.631, and RC = 0.225 were retrieved.

Apocynaceae—Most members in the Apocynaceae are tropical or subtropical, and the family consists of 4800 species (including Asclepiadaceae; Mabberley, 1997). Many systematists have argued that Asclepiadoideae, sensu Jussieu, should be recognized as the family Asclepiadaceae (Brown, 1810; Schumann, 1895; Cronquist, 1981; Takhtajan, 1987; Rosatti, 1989a, b; Nicholas and Bajnath, 1994), but it has been shown that this group of taxa is clearly nested within Apocynaceae, as one of its morphologically most derived groups. This placement is supported by several recent studies, which have included data from morphology, palynology, phytochemistry, and DNA sequences (Sennblad and Bremer, 1996; Sennblad, 1997). In this combined analysis of *rbcl* and *ndhF* data, we find a strongly supported Apocynaceae (bl = 44, b = 27, j = x = bt = 100%) with a well-supported internal structure congruent with the results from the studies mentioned above.

Gelsemiaceae—The two genera *Gelsemium* and *Mos-tuea*, with three and eight species, respectively, are tropical and subtropical shrubs and lianas (Mabberley, 1997). They have long been regarded as closely related (Bentham and Hooker, 1862–1883; Solereder, 1892–1895; Leeuwenberg and Leenhouts, 1980). The placement of this group has varied, in Apocynaceae (Persoon, 1805), as a separate tribe within Apocynaceae (Don, 1837–1838; Endlicher, 1841; Bureau, 1856), in Antonieae of Loganiaceae (Bentham, 1856), or later in a tribe of its own as Gelsemieae in Loganiaceae (Bentham and Hooker, 1862–1883). As such they remained until the classification of Struwe and Albert (in Struwe, Albert, and Bremer, 1994), when the former tribe Gelsemieae was elevated to familial rank.

The conflicts among systematists regarding the affinities of Gelsemiaceae are due to several morphological features that have appeared to be contradictory and indicative of different systematic positions. Features indicating a close relationship to Apocynaceae include anther anatomy (number of tapetum layers; Johri, Ambegaokar, and Srivastava, 1992), phytochemistry (a special form of C-17 indole alkaloids; Jensen, Nielsen, and Dahlgren, 1975; Jensen, 1992) and cytotaxonomy (Moore, 1947).

Other features appear instead to unite Gelsemiaceae with Loganiaceae (especially the tribe Antonieae). Among these characters we find imbricate corollas (always contorted in Apocynaceae; Leeuwenberg and Leenhouts, 1980), late sympetaly (instead of an “intermediate” stage in Apocynaceae; Erbar, 1991), absence of laticifers (ubiquitous in the Apocynaceae; Sennblad and Bremer, 1996), features of seed anatomy (horny endosperm; Leeuwenberg and Leenhouts, 1980), and thin thyloses and wide multiseriate rays (giving a wood anatomy particularly resembling Antonieae; Mennega, 1980) as well as phytochemistry (presence of scopoletin, kaempferol, and quercetin; Bisset, 1980). Furthermore, a large number of easily detectable autapomorphic features such as heterostylous flowers, twice-dichotomously divided stigmas, and latrorse anthers tend to set Gelsemiaceae apart from both groups of potential closest relatives.

This analysis indicates a sister-group relationship to Apocynaceae. This relationship is not strongly supported, but there is strong support for Gelsemiaceae as a mono-

TABLE 5. Synopsis of the Gentianales. Suggested realignments according to these results.

Gentianales		
Apocynaceae (including (Asclepiadaceae).		
Gelsemiaceae (<i>Gelsemium</i> and <i>Mostuea</i>)		
Gentianaceae To also include Potalieae of Loganiaceae (<i>Anthocleista</i> , <i>Fagraea</i> , and <i>Potalia</i>).		
Loganiaceae (<i>Antonia</i> , <i>Bonyunia</i> , <i>Norrisia</i> , <i>Usteria</i> , <i>Gardneria</i> , <i>Neuburgia</i> , <i>Spigelia</i> , <i>Strychnos</i> , <i>Geniostoma</i> , <i>Labordia</i> , <i>Logania</i> , <i>Mitrasacme</i> , <i>Mitreola</i>)		
Rubiaceae		
Taxa excluded from Gentianales		
Genus	Suggested position	Reference
<i>Buddleja</i>	Buddlejaceae, Lamiales	(Oxelman, Backlund, and Bremer, 1999)
<i>Emorya</i>	Buddlejaceae, Lamiales	(Oxelman, Backlund, and Bremer, 1999)
<i>Gomphostigma</i>	Buddlejaceae, Lamiales	(Oxelman, Backlund, and Bremer, 1999)
<i>Nicodemia</i>	Buddlejaceae, Lamiales	(Oxelman, Backlund, and Bremer, 1999)
<i>Nuxia</i>	Stilbaceae, Lamiales	(Oxelman, Backlund, and Bremer, 1999)
<i>Retzia</i>	Stilbaceae, Lamiales	(Bremer et al., 1994)
<i>Peltanthera</i>	Gesneriaceae, Lamiales	(Oxelman, Backlund, and Bremer, 1999)
<i>Sanango</i>	Gesneriaceae, Lamiales	(Oxelman, Backlund, and Bremer, 1999)
<i>Androya</i>	Myoporaceae, Lamiales	(Oxelman, Backlund, and Bremer, 1999)
<i>Plocosperma</i>	Plocospermataceae, Euasterid I	
<i>Polypremum</i>	Tetrachondraceae, Lamiales	(Oxelman, Backlund, and Bremer, 1999)
<i>Desfontainia</i>	Columelliaceae, Dipsacales	(Backlund, 1996)

phyletic group. Because Gelsemiaceae already exist (retaining stability), form an easily distinguishable and well-supported lineage (ease of identification), and constitute more than one genus, this avoids redundancy. Albeit with weakly supported sister-group relationship (phylogenetic information) we find that all guidelines suggested by Backlund and Bremer (1998) are fulfilled by retaining the family as it presently stands.

Loganiaceae—In the circumscription of Leeuwenberg and Leenhouts (1980) Loganiaceae consisted of 600 species in 30 genera (Mabberley, 1997) and included predominantly tropical, woody plants. Since the description by Martius (1827), both the circumscription of the family and the intrafamilial relationships have been a matter of debate. Some authors accept one large (600 species) family (Leeuwenberg and Leenhouts, 1980), whereas others prefer Loganiaceae split into 12 different families allocated to several distantly related orders (Takhtajan, 1997).

Bentham and Hooker (1862–1883) divided the family into seven tribes (Antonieae, Buddlejeae, Desfontainieae, Euloganieae including *Strychnos* and relatives, Gelsemieae, Potalieae, and Spigelieae). Solereder (1882–1895) recognized two subfamilies (Loganioideae with six tribes, and Buddlejoideae with one tribe) within the family, but he also excluded the two genera *Plocosperma* (of the Gelsemieae sensu Bentham and Hooker) and *Desfontainia* (of the Desfontainieae sensu Bentham and Hooker). Almost 100 years later Hutchinson (1973) further divided the Loganiaceae sensu Bentham and Hooker into seven distinct families and established a new order (Loganiales) for these families together with the family Oleaceae. The morphologically enigmatic genus *Plocosperma* was simultaneously placed as a monotypic family in the new order Apocynales. Nevertheless, Gentianales with a variously circumscribed Loganiaceae as part of its core was

retained by most systematists. Among these, Loganiaceae was considered to occupy a central evolutionarily position (Bisset, 1980; Leeuwenberg and Leenhouts, 1980).

According to many recent studies, Loganiaceae sensu Leeuwenberg and Leenhouts are polyphyletic (Downie and Palmer, 1992; Olmstead et al., 1993; Struwe, Albert, and Bremer, 1994; Takhtajan, 1997; Oxelman, Backlund, and Bremer, 1999). This heterogeneity is also reflected in the classification by Leeuwenberg and Leenhouts (1980) in which the Loganiaceae were divided into ten tribes, some of which consisted of one or only a few species.

In this study, we find a strongly supported group corresponding to the tribe Antonieae (node 50) sensu Leeuwenberg and Leenhouts. These genera share several characters such as valvate aestivation, coriaceous leaves, ability to accumulate aluminium (Leeuwenberg and Leenhouts, 1980), and a homogeneous wood anatomy featuring absence of continuous rays, interxylary phloem of foraminat type, large cavities in rays, and vessels in tangential pairs or small clusters (Mennega, 1980). Also the internal node (51) in this group is strongly supported, and the taxa (*Antonia* and *Bonyunia*) share a venation pattern, as well as the same geographical distribution (South America).

The branch supporting the remaining taxa (52) is also well supported. This relationship, however, has not been encountered among previous studies using morphological data (Bremer and Struwe, 1992; Struwe, Albert, and Bremer, 1994). As would be expected, few or no obvious morphological features are easily identified as diagnostic of this large group. This group further divides into two lineages. The first (53) comprises the two heterogeneous genera *Spigelia* and *Strychnos* together with *Gardneria* and *Neuburgia*. This group, apart from *Spigelia*, corresponds to the tribe Strychneae of Leeuwenberg and Leenhouts (1980). Neither this lineage, nor its internal struc-

ture, is particularly well supported by the molecular data. Additional features that could be interpreted as supporting this hypothesis are valvate aestivation (also found in *Mitrasacme*), some indole alkaloid derivatives, aluminum accumulation (also in the *Antonia* group), and logani-type iridoids. The former Strychnaeae were united on the basis of their anther appendages and indehiscent fruits, features not reported for *Spigelia* by Leeuwenberg and Leenhouts (1980). The other lineage (node 56), by contrast, is well supported both on morphological and molecular grounds. Characters include partly apocarpous carpels (or possibly homologous, early from apex-splitting fruits), ochrea instead of stipules (Leeuwenberg and Leenhouts, 1980), a general change from the presumed plesiomorphic basal chromosome number of $x = 11$ to $x = 10$ (Gadella, 1980), and a general absence of alkaloids (Bisset, 1980). Here we encounter the type genus *Logania*, together with *Mitrasacme* and *Mitreola* (two members of Spigeliaceae sensu Leeuwenberg and Leenhouts) as well as *Geniostoma* and *Labordia* (Geniostomataceae sensu Struwe and Albert in Struwe, Albert, and Bremer, 1994).

We conclude that neither Geniostomataceae nor Strychnaceae (sensu Struwe and Albert in Struwe, Albert, and Bremer, 1994) can be retained. Geniostomataceae with very strong support form a clade with Loganiaceae (node 56, and sensu Struwe and Albert in Struwe, Albert, and Bremer, 1994). The Geniostomataceae are thus deeply nested within Loganiaceae with high support. Spigeliaceae (sensu Struwe and Albert in Struwe, Albert, and Bremer, 1994) are by the combined *rbcL* and *ndhF* data indicated to consist of at least two lineages. One of these corresponds, as discussed above, to the former Antonieae of Leeuwenberg and Leenhouts and is strongly supported in our study. The remaining taxa of Strychnaceae (sensu Struwe and Albert in Struwe, Albert, and Bremer, 1994) correspond to the former Strychnaeae of Leeuwenberg and Leenhouts with the addition of *Spigelia*. This lineage is also present in our results and with high support forms a monophyletic group with the Loganiaceae–Geniostomataceae clade.

In summary, Loganiaceae s.s. form a strongly supported monophyletic group consisting of 13 genera: *Antonia*, *Bonyunia*, *Gardneria*, *Geniostoma*, *Labordia*, *Logania*, *Mitrasacme*, *Mitreola*, *Neuburgia*, *Norrisia*, *Spigelia*, *Strychnos*, and *Usteria*.

Systematic positions of genera formerly included in Loganiaceae—The majority of loganiaceous species belong in Gentianales in the three closely related families Gesneriaceae, Gentianaceae, and Loganiaceae s.s. However, a number of genera previously regarded as members of the Loganiaceae s.l. obviously belong in Lamiales. The positions of these genera here are congruent with the results of Olmstead and Reeves (1995) and Oxelman, Backlund, and Bremer (1999).

Buddlejaceae—*Buddleja* was first placed in the Scrophulariaceae by Jussieu (1789) but later moved to Loganiaceae by Bentham (1856) and recently regarded as a tribe within Loganiaceae by Leeuwenberg and Leenhouts (1980). Buddlejaceae were separated by Dahlgren (1975) from the rest of Gentianales and placed in Lamiales because of their seco-iridoids (biosynthesis route II; Jensen,

1991). This issue has attracted attention, and several features from wood anatomy, including lack of borders on pits of imperforate tracheary elements, and embryological features such as cellular endosperm, are characteristic of Lamiales (Bendre, 1973, 1975; Mennega, 1980; Carlquist, 1986, 1992; Maldonado de Magnano, 1986; Engell, 1987; Hegnauer, 1989; Dahlgren, 1992). For a further treatment of *Buddleja* and Buddlejaceae see Oxelman, Backlund, and Bremer (1999).

Retzia and Nuxia—Several recent studies indicate that South African *Retzia* of the monotypic tribe Retzieae, sometimes recognized as the distinct family Retziaceae (Bartling, 1830), differs in many characters from Loganiaceae (Dahlgren et al., 1979; Bremer et al., 1994; Struwe, Albert, and Bremer, 1994). *Retzia*, on phytochemical and anatomical grounds, had been associated with the genus *Stilbe* (Dahlgren et al., 1979; Dahlgren, 1980a; Carlquist, 1986), originally placed in the Verbenaceae.

The genus *Nuxia*, by Leeuwenberg and Leenhouts (1980) included in the tribe Buddlejeae, has been here and in a previous molecular study (Oxelman, Backlund, and Bremer, 1999) strongly supported as a close relative of *Retzia* and *Stilbe*. These genera are now considered to form the family Stilbaceae of Lamiales (Thorne, 1992a, b; Bremer et al., 1994; Oxelman, Backlund, and Bremer, 1999).

Plocosperma, Polypremum, Peltanthera, and Sanango—Apart from the taxa discussed above, these four genera should also be excluded from Loganiaceae s.s. as well as from Gentianales (Jensen, 1992; Oxelman, Backlund, and Bremer, 1999). *Plocosperma* is placed next to *Borago*, and the other three genera are with high support placed in Lamiales in our results.

The monotypic Central American genus *Plocosperma* was earlier the sole member of the tribe Plocospermeae of Loganiaceae (Leeuwenberg and Leenhouts, 1980). In some classifications, *Plocosperma* has been suggested to show a close relationship to Apocynaceae (Hutchinson, 1973; Cronquist, 1981; Takhtajan, 1987). Both here and in other recent phylogenetic studies, both these placements have been refuted (Struwe, Albert, and Bremer, 1994; Endress and Albert, 1995; Oxelman, Backlund, and Bremer, 1999). According to this study, as well as in Oxelman, Backlund, and Bremer (1999), *Plocosperma* is supported as belonging to euasterid I. The sampling in this part of the tree is sparse, and the support indices for this placement are low. The position of *Plocosperma* therefore may still be regarded as uncertain.

The likewise monotypic American genus *Polypremum*, former member of the tribe Spigeliaceae of Loganiaceae, is in this analysis indicated to occupy a relatively early branch within Lamiales. This placement is completely congruent with the strongly supported placement together with the genus *Tetrachondra* in Oxelman, Backlund, and Bremer (1999).

Peltanthera and *Sanango*, two other monotypic American genera, were earlier placed in the tribe Buddlejeae of the Loganiaceae (Leeuwenberg and Leenhouts, 1980). Our study indicates a well supported and close relationship between both *Streptocarpus* and *Sanango* to Gesneriaceae, as well as between these and *Peltanthera*.

Desfontainieae with the single genus *Desfontainia* have previously been placed in the order Dipsacales (Bremer et al., 1994; Backlund and Bremer, 1997) and more specifically in the family Columelliaceae (Backlund, 1996).

Conclusions—Combined sequence data from the plastid genes *rbcL* and *ndhF* resolve (with minor exceptions) inter- and intrafamilial relationships within the Gentianales, in most cases with high levels of internal support. Gentianales, as here defined, consist of the families Apocynaceae, Gelsemiaceae, Gentianaceae, Loganiaceae, and Rubiaceae. As previously indicated in several studies (Sennblad and Bremer, 1996; Sennblad, 1997) Asclepiadaceae must be reduced to Apocynaceae. The recently proposed family Geniostomataceae cannot according to the monophyly criterion be retained without describing several additional small families. It together with Loganiaceae (sensu Struwe and Albert in Struwe, Albert, and Bremer, 1994) forms a well-supported group for which the name Loganiaceae has priority (Reveal, 1993). Nor can Strychnaceae (sensu Struwe and Albert, in Struwe, Albert, and Bremer, 1994) be retained as they become paraphyletic due to the successive nesting of Loganiaceae and Geniostomataceae within them. The other, in our opinion less appealing, possibility would be to recognize several additional small families. Of the 30 genera included in Loganiaceae by Leeuwenberg and Leenhouts (1980), 17 are, according to these results, excluded from the remaining Loganiaceae s.s. Twelve of these genera are indicated to belong to other orders, in one case even to another subclass than Loganiaceae sensu stricto and with affinities to several different large families (e.g., Boraginaceae, Gesneriaceae, Scrophulariaceae s.l.). The remaining five genera excluded from Loganiaceae are placed in Gentianaceae (*Anthocleista*, *Fagraea*, and *Potalia*) and Gelsemiaceae (*Gelsemium* and *Mostuea*). Loganiaceae therefore consists of 13 genera: *Antonia*, *Bonyunia*, *Gardneria*, *Geniostoma*, *Labordia*, *Logania*, *Mitrasacme*, *Mitreola*, *Neuburgia*, *Norrisia*, *Spigelia*, *Strychnos*, and *Usteria*.

The formerly recognized tribes Spigeliaceae and Strychnaceae as delimited by Leeuwenberg and Leenhouts (1980) are all invalidated by the monophyly criterion applied to our results. Retziaceae, Desfontainieae, and Plocospermeae as suggested by Leeuwenberg and Leenhouts (1980) are monotypic and may therefore remain as tribes, albeit not in Loganiaceae according to these, as well as earlier results (Bremer et al., 1994; Backlund and Bremer, 1997; Oxelman, Backlund, and Bremer, 1999). Groups corresponding to the tribes Antonieae, Gelsemieae, and Potalieae, sensu Leeuwenberg and Leenhouts (1980) are retrieved from molecular data, but their interrelationships are different than previously suggested. One of these groups remains as a distinct entity of Loganiaceae (Antonieae), one forms a family of its own (Gelsemiaceae), and one is nested within Gentianaceae (Potalieae). To retain the latter as a family, as suggested by Watson and Dallwitz (1992), thus becomes impossible.

The molecular data at hand, in combination with results from previous studies, provide sufficient information for a preliminary synopsis and realignments, summarized in Table 5. We refrain at this point from attempt-

ing a tribal classification of Loganiaceae in its new circumscription.

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