

CLADISTICS AND FAMILY LEVEL CLASSIFICATION OF THE GENTIANALES

Lena Struwe,^{1,3} Victor A. Albert^{1,2,3} and Birgitta Bremer¹

¹Department of Systematic Botany, Uppsala University, Villavägen 6, S-752 36 Uppsala, Sweden and ²Department of Physiological Botany, Uppsala University, Villavägen 6, S-752 36 Uppsala, Sweden.

³Present and corresponding address: The New York Botanical Garden, Bronx, New York 10458-5126 USA.

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Abstract — The most recent classification of the angiosperm order Gentianales (Thorne, 1992) includes four principal families: Apocynaceae, Gentianaceae, Loganiaceae, and Rubiaceae. Ever since Bentham (1857) the status of Loganiaceae has been questioned, and several segregates of that family have been proposed both before and after his treatment. In this study we present cladistic results that show Loganiaceae, sensu lato, to be a paraphyletic group definable only by plesiomorphies, with members showing closest relationships to other families of the order. As the impact of different character-state representations of polymorphic terminals remains largely untested, our morphological and phytochemical data were analysed both with restricted polymorphism coding as well as with the monomorphic “subtaxon” recoding method of Nixon and Davis (1991). Both approaches yield highly compatible results, and we here discuss a new classification of the Gentianales based on (i) monophyletic groups identified by outgroup analysis, and (ii) the maximal portrayal of evidence provided by subtaxon polymorphism recoding. Most prominently, the Loganiaceae sensu lato are divided into four segregate families, two previously named (Loganiaceae sensu stricto and Strychnaceae), and two defined as a result of this study (Gelsemiaceae, L. Struwe & V. A. Albert, stat. nov. and Geniostomaceae, L. Struwe & V. A. Albert, fam. nov.). Apocynaceae (incl. Asclepiadaceae), Gentianaceae (incl. Loganiaceae—Potalieae), and Rubiaceae remain as monophyletic families. Outgroup analysis supports both the monophyly of the Gentianales as well as the exclusion from the order of *Buddleja*, *Desfontainia*, *Plocosperma*, *Polyprenum*, and *Retzia* (all Loganiaceae sensu Leeuwenberg and Leenhouts).

Introduction

Although the monophyly of the sympetalous angiosperm order Gentianales (or Rubiales sensu Reveal, 1993a) has received substantial recent support from chloroplast DNA data (Downie and Palmer, 1992; Chase et al., 1993; Olmstead et al., 1993; Bremer et al., 1994), the status and interrelationships of its component families are much less well established. Family level cladistic analysis of the Gentianales is thus desirable, yet the units of comparison (either in terms of taxa or characters) are by no means straightforward. The families Apocynaceae (including Asclepiadaceae; Thorne, 1992), Gentianaceae, Loganiaceae, and Rubiaceae are included in the Gentianales by most modern plant systematists (e.g., Wagenitz, 1964; Takhtajan, 1987; Dahlgren, 1989; Thorne, 1992). Perhaps the greatest impediment to a natural classification of the order is the heterogeneous Loganiaceae, which may comprise several monophyletic groups both within and outside the Gentianales (cf. von Martius, 1827; Leeuwenberg and Leenhouts, 1980; Bremer and Struwe, 1992; Bremer et al., 1994).

The hierarchic level of the present study was designed to address both the Loganiaceae problem and other family level concerns within the Gentianales. In all cladistic studies maximizing information content is desirable (see Farris, 1979,

1983); thus, factors that could generate results not supported by the evidence at hand should be avoided. For example, artifacts may be introduced with the definition of characters as well as with the choice of terminals, their circumscription, assumed monophyly, and representation of polymorphic character states.

Family level studies need not use families and familial characters as their units of comparison. First, traditionally maintained families (like Loganiaceae) may not be monophyletic, and their use as terminal taxa (see, e.g., Donoghue and Doyle, 1989; Anderberg, 1992; Taylor and Hickey, 1992; Hufford, 1992) could lead to gross misrepresentation. Likewise, the use of presumed ancestral states for broadly defined terminal taxa, a coding method that has been used by several authors (e.g., Anderberg, 1992; Bremer and Struwe, 1992; Hufford, 1992; Taylor and Hickey, 1992), will necessarily lead to assumption-laden results and further distortion of the primary evidence (see Nixon et al., 1994).

What practical means may exist for avoiding these difficulties? For example, it might be argued that all accepted species of the Gentianales would be the ideal taxa for an encompassing cladistic study at levels of classification, including familial. However, restrictions on the number on available characters relative to available taxa and the enormous amount of missing data that could be expected make this approach prohibitive.

A derivative concept, the exemplar method, would involve selecting a particular species to represent each gentianalean unit of interest. This approach is commonly used when analysing DNA data, where a sequence from an individual may be assumed to represent a whole genus, tribe, or even family (see, e.g., Albert et al., 1992; Olmstead et al., 1993; Chase et al., 1993). However, representation of diversity may be highly skewed. Although some genera of the Gentianales are monotypic (e.g., *Usteria* [Loganiaceae]), others comprise numerous recognized species (e.g., *Strychnos* [Loganiaceae], *Gentiana* [Gentianaceae], and *Apocynum* [Apocynaceae]).

Ideally, use of presumed monophyletic *genera* could permit a family level classification from information that relates directly to the *species* level; that is, if all species level variation in the terminals is taken into account. This may be accomplished by scoring generic level terminals for as many putative homologies as possible while restricting the number of polymorphic taxa that must be recognized. However, cladograms produced from such a data matrix will contain only genus level information and may contain arbitrary character-state reconstructions unless systematic error introduced by polymorphic taxa (i.e., with character states falsely coded as "missing" or restricted [e.g., "0 or 1", when states 0–3 are available]) is eliminated. Rather than deleting polymorphic taxa (e.g., large genera showing variation in several characters), they may be recoded into monomorphic subunits devoid of inappropriately coded character states (Nixon and Davis, 1991; Platnick et al., 1991).

Cladograms incorporating "subtaxa" (the monomorphic subunits of a polymorphic taxon), which represent all empirically observed variation for every given character showing polymorphism within genera, will have the information content of a species level analysis and therefore the power to address higher order classificatory questions if sufficient topological resolution is obtained. They will also have the potential of suggesting where putatively monophyletic and polymorphic genera could be either paraphyletic or polyphyletic. It must be noted, however, that such suggestions are dependent upon the particular taxon and character sampling. In this study, all genera from the Loganiaceae are included, whereas only a few genera

from the Apocynaceae, Gentianaceae, and Rubiaceae are represented. Therefore, relationship status of subtaxa *within* the last three families could be misleading as many genera and their character variation have been excluded.

Taking these issues into account, we present a new cladistic analysis of the Gentianales, focusing particularly on the status of the various loganiaceous clades. The characters used include morphological, anatomical, and phytochemical hypotheses of taxic homology. Taxonomic sampling reflected ordinal diversity (including taxa previously excluded from the Gentianales) as well as other suggested relatives. Based on the results of (i) an outgroup-oriented analysis tolerating polymorphisms and (ii) a subtaxa analysis of the ingroup devoid of polymorphisms, the Loganiaceae may be split into two previously recognized families (Loganiaceae *sensu stricto* and Strychnaceae) and two new families (Gelsemiaceae, L. Struwe & V. A. Albert and Geniostomaceae, L. Struwe & V. A. Albert). The previous merger of Loganiaceae—Potalieae with Gentianaceae (cf. Bureau, 1856; Fosberg and Sachet, 1974) is also supported. Apocynaceae (incl. Asclepiadaceae), Gentianaceae, and Rubiaceae remain as monophyletic families. Outgroup analysis supports both the monophyly of the Gentianales as well as the exclusion from the order of several taxa formerly assigned to Loganiaceae: *Buddleja*, *Desfontainia*, *Plocosperma*, *Polypre-mum*, and *Retzia*.

Cladistic Analysis

TAXA

For the purposes of taxon selection a strictly defined Gentianales (including the Rubiaceae; Utzschneider, 1951; Wagenitz, 1964) was considered along with rejected loganiaceous genera of dubious affinity. Discriminating characteristics for Gentianales *sensu stricto* include the presence of interpetiolar stipules or stipular lines (Leeuwenberg and Leenhouts, 1980; Wood and Weaver, 1982; Robbrecht, 1988; Rosatti, 1989a,b), colleters (Wagenitz, 1959; Robbrecht, 1988), and vestured pits in the wood (Bailey, 1933; Carlquist, 1992). Gentianaceae, Rubiaceae, Apocynaceae (including the derived Asclepiadaceae; Wanntorp, 1989; Judd et al., 1994), and most Loganiaceae fall under this circumscription. Several taxa that have previously been excluded from Loganiaceae (and Gentianales) are included in the present sampling: *Retzia* Thunb. (Retziaceae; R. Dahlgren et al., 1979; Mennega, 1980; Punt, 1980; Carlquist, 1992; Engell, 1987; Wagstaff et al., 1993; Bremer et al., 1994), *Desfontainia* Ruiz et Pav. (Desfontainiaceae; Mennega, 1980; Maldonado de Magnano, 1986a; Bremer et al., 1994) *Buddleja* L. (Buddlejaceae; Solereder, 1892–95; Hasselberg, 1937; Bendre, 1973; Jensen et al., 1975; Mennega, 1980; Jensen, 1992; Chase et al., 1993; Bremer et al., 1994), *Polypre-mum* L. (uncertain position in the Scrophulariales; Moore, 1948; Punt and Leenhouts, 1967; Bendre, 1973; Mennega, 1980; Rogers, 1986; Jensen, 1992), and *Plocosperma* Benth. (Plocospermataceae; Punt, 1980; Jensen, 1992; cf. Bremer and Struwe, 1992). The relationships of Saccifoliaceae (a monotypic segregate of Gentianaceae; Maguire and Pires, 1978), Dialypetalanthaceae, and Theligonaceae (monotypic and monogeneric segregates of Rubiaceae, respectively; Rizzini and Occhioni, 1949; Wunderlich, 1971; Nowicke and Skvarla, 1979; Cronquist, 1981) are not considered here.

Gentiana L. (subtribe Gentianinae), *Centaurium* Hill (subtribe Erythraeinae), and *Tachia* Aubl. (subtribe Tachiinae) were chosen as representative genera from the

Gentianaceae according to the classification of Gilg (1895). *Plumeria* L. (Plumerioideae), *Apocynum* L. (Apocynoideae), *Periploca* L. (Periplocoideae), and *Asclepias* L. (Asclepiadoideae) were chosen to represent the different subfamilies of Apocynaceae (Thorne, 1992). Within the Rubiaceae, one genus from each of three subfamilies (sensu Robbrecht, 1988) were chosen: *Cinchona* L. from Cinchonoideae, *Pentas* Benth. from Rubioideae, and *Coffea* L. from Ixoroideae. From Loganiaceae (sensu Leeuwenberg and Leenhouts, 1980), all genera of Spigeliaceae (*Spigelia* L., *Mitreola* L., and *Mitrasacme* Labill., but excluding *Polypremum*; see above), Loganieae (*Geniostoma* J.R. Forst. & G. Forst., *Labordia* Gaudich., and *Logania* R.Br.), Strychnaceae (*Strychnos* L., *Gardneria* Wall., and *Neuburgia* Blume), Gelsemieae (*Gelsemium* Juss. and *Mostuea* Didr.), Antonieae (*Antonia* Pohl, *Bonyunia* Schomb. ex Progel, *Norrissia* Gardner, and *Usteria* Willd.), and Potalieae (*Potalia* Aubl., *Anthocleista* Afzel. ex R.Br., and *Fagraea* Thunb.) were sampled.

For outgroup-orientation of unrooted cladograms *Syringa* L. (Oleaceae), *Cestrum* L. (Solanaceae), *Verbascum* L. (Scrophulariaceae), and *Viburnum* L. (Viburnaceae) were also considered. *Syringa*, *Cestrum*, and *Verbascum* were assumed to represent the Lamiales/Solanales clade(s), which have been presented as possible sister group(s) of the Gentianales (Olmstead et al., 1992, 1993; Chase et al., 1993; Bremer et al., 1994; cf. Thorne, 1983, 1992; Takhtajan, 1987). Furthermore, *Retzia*, *Buddleja*, *Polypremum*, and *Plocosperma* have been suggested to belong within the Lamiales clade (see above references). *Viburnum* has been placed in the Dipsacales clade, which is positioned outside the Lamiidae (Donoghue, 1983; Albert et al., 1992; Donoghue et al., 1992; Olmstead et al., 1992, 1993). *Desfontainia* has recently been suggested to be more closely related to the Dipsacales (Bremer et al., 1994).

CHARACTERS

Characters, i.e., individual hypotheses of taxic homology, were compiled both from the literature and from studies of fresh and herbarium material (Uppsala Botaniska Trädgård, K, S, and UPS herbaria). These are listed in Appendix 1, and the matrix is shown in Table 1. All characters were treated as nonadditive except character 39, which is an additive transformation series representing a biosynthetic pathway (see Appendix 1). For embryological and chemical characters, usually only one or a few species in each genus have been investigated; nevertheless, if a given feature has been recorded in any species of a genus, it has been indicated with the state "present" (thus, never yielding polymorphisms from negative evidence).

For the representatives from Solanaceae (*Cestrum*), Scrophulariaceae (*Verbascum*), and Oleaceae (*Syringa*), anatomical, embryological, and chemical information was often very scarce, so available data from other genera in the same families were used for character coding (a practice that has required the recognition of polymorphisms).

MATRIX CONSTRUCTION

During the coding and definition of the characters the following rules were used: inapplicable character states were completely avoided by redefinition of characters (but never by assigning new states for inapplicables), missing data were indicated by question marks, and polymorphic states were placed within parentheses. The last measure was used instead of question marks, which may inflate variation

Table 1

Data matrix for ingroup and outgroup taxa. Missing data are indicated with question marks. Polymorphisms are indicated by alternative states enclosed in parentheses.

Viburnum	00100001000000022000000100021000(01)100001???0?00??
Syringa	00100000000002000001101000000010000001000110000??
Cestrum	00000001000000000001?0?000000000?10100001000000??
Verbascum	00000011000000000001000000000000?10000100010100??
Desfontainia	0010000(12)00000000000000100000000110000100100?000??
Retzia	000000100000000000010020000000001000000101010000??
Buddleja	0(01)(01)000(12)(01)00000000001(03)0(01)0000000000000101010000??
Plocosperma	00100001000000000101010?0000000?????000000010000??
Polypremum	0110000100000000001000000?0000?????0?00?001?00??
Gentiana	0110000200000000(01)001(01)0(01)01110000?1???111001101010??
Centaurium	01100(01)02000000001001(03)000110000?1???11100110100100
Tachia	00100002000000001001300??110?00?????001?0?00000
Anthocleista	00110002100000001000(13)0(01)??2110011111010100?1?100000
Fagraea	00110002(01)0000000100030001(012)110011101010100010?00000
Potalia	00110002100000000000100??21?00?111101?????0000
Logania	(01)010(01)0(12)100000(01)00004000??110000001101(01)100100000000
Labordia	0010100200?00?000002000??21000011000101?0100?00000
Geniostoma	(01)010(01)(01)(012)(12)001000000002000??2100001100010100100000000
Gelsemium	001001(01)100000100010410(01)??110000010001?200?00000000
Mostuea	001001(01)1000001000101100??1100001100010200?0?000010
Antonia	00100020000000000004?01??0100001112111000100000000
Bonyunia	00100010000000000004?01??1100001110111000100000000
Norrisia	00100010000001000004101??0?00001112111000?00000000
Usteria	00100010000000000001101??0100001011010?????00000
Spigelia	00100000000000000003300010100001111011000?00000000
Mitreola	00100021000000110001000??1?0000?????10?1000?0000
Mitrasacme	0010002000000(012)110001(03)000?010000?????1001000?0000
Strychnos	001000(012)0001(01)00000000(0123)0(01)010100001110(01)11300?00000000
Gardneria	00100000001000000000200??010000?????3300?00000000
Neuburgia	001000(12)0001100000000200??01000001111010??0?0000000
Plumeria	00000012001000110001101011100101?00010300100010000
Apocynum	00100002001010100001010012100101?0?0101001?0010000
Periploca	001000020111101000110100121001010?001?000100010000
Asclepias	0010000(01)0110101000111110131001010????1(01)000100010000
Cinchona	0010011000000020001101011101001??000201100000000
Coffea	00100002000000020000000011101001??0000?011?0000000
Pentas	00100(01)1000000020001(03)00011101001??000?011?0000001

beyond that empirically observed (e.g., when $\neq(0123)$ but 0 and 1 are the only states known for a taxon). Data for Gentianales sensu stricto were further recoded into a new subtaxa matrix according to the method of Nixon and Davis (1991).

If a taxon is variable in more than one character, several combinations of the characters and their states will be possible. To avoid distortion of the evidence, it is important to use only those combinations that occur in reality. Unfortunately, most combinations could not be checked for lack of information, so all possible subtaxa were used to derive a "worst-case" representation of polymorphism in the matrix. According to K. Nixon (pers. comm.) it is preferable to include autapomorphies (if they exist) for genera that will be divided into subtaxa; when replicated among the monomorphic subunits, these then form similarities that could support generic monophyly. Autapomorphies were found for four of the polymorphic taxa in this study (*Centaurium*, *Gentiana*, *Mostuea*, and *Pentas*; see Appendix 1). The resulting subtaxa matrix for Gentianales sensu stricto consists of 235 monomorphic taxa instead of the initial 28 genera (Table 2).

Table 2

Subtaxa matrix. Missing data are indicated with question marks. See text for further explanation.

Gentiana.1	011000020000000000100001110000?1???111001101010??
Gentiana.2	0110000200000000100100001110000?1???111001101010??
Gentiana.3	0110000200000000000110001110000?1???111001101010??
Gentiana.4	0110000200000000100110001110000?1???111001101010??
Gentiana.5	011000020000000000100101110000?1???111001101010??
Gentiana.6	0110000200000000100100101110000?1???111001101010??
Gentiana.7	0110000200000000000110101110000?1???111001101010??
Gentiana.8	0110000200000000100110101110000?1???111001101010??
Centaurium.1	0110000200000000100100001110000?1???11100110100100
Centaurium.2	0110010200000000100100001110000?1???11100110100100
Centaurium.3	0110000200000000100130001110000?1???11100110100100
Centaurium.4	0110010200000000100130001110000?1???11100110100100
Tachia	00100002000000001001300??110?00?1???001?0?00000
Anthocleista.1	00110002100000001000100??2110011111010100?1?100000
Anthocleista.2	00110002100000001000300??2110011111010100?1?100000
Anthocleista.3	00110002100000001000101??2110011111010100?1?100000
Anthocleista.4	00110002100000001000301??2110011111010100?1?100000
Fagraea.1	00110002000000001000300010110011101010100010?00000
Fagraea.2	00110002100000001000300010110011101010100010?00000
Fagraea.3	00110002000000001000300011110011101010100010?00000
Fagraea.4	00110002100000001000300011110011101010100010?00000
Fagraea.5	00110002000000001000300012110011101010100010?00000
Fagraea.6	00110002100000001000300012110011101010100010?00000
Potalia	00110002100000000000100??21?00?111101?1???0000
Logania.1	0010001100000000004000??110000001101010010000000
Logania.2	1010001100000000004000??110000001101010010000000
Logania.3	0010101100000000004000??110000001101010010000000
Logania.4	1010101100000000004000??110000001101010010000000
Logania.5	0010002100000000004000??110000001101010010000000
Logania.6	1010002100000000004000??110000001101010010000000
Logania.7	0010102100000000004000??110000001101010010000000
Logania.8	1010102100000000004000??110000001101010010000000
Logania.9	00100011000000100004000??110000001101010010000000
Logania.10	10100011000000100004000??110000001101010010000000
Logania.11	00101011000000100004000??110000001101010010000000
Logania.12	10101011000000100004000??110000001101010010000000
Logania.13	00100021000000100004000??110000001101010010000000
Logania.14	10100021000000100004000??110000001101010010000000
Logania.15	00101021000000100004000??110000001101010010000000
Logania.16	10101021000000100004000??110000001101010010000000
Logania.17	0010001100000000004000??110000001101110010000000
Logania.18	1010001100000000004000??110000001101110010000000
Logania.19	0010101100000000004000??110000001101110010000000
Logania.20	1010101100000000004000??110000001101110010000000
Logania.21	0010002100000000004000??110000001101110010000000
Logania.22	1010002100000000004000??110000001101110010000000
Logania.23	0010102100000000004000??110000001101110010000000
Logania.24	1010102100000000004000??110000001101110010000000
Logania.25	00100011000000100004000??110000001101110010000000
Logania.26	10100011000000100004000??110000001101110010000000
Logania.27	00101011000000100004000??110000001101110010000000
Logania.28	10101011000000100004000??110000001101110010000000
Logania.29	00100021000000100004000??110000001101110010000000
Logania.30	10100021000000100004000??110000001101110010000000
Logania.31	00101021000000100004000??110000001101110010000000
Logania.32	10101021000000100004000??110000001101110010000000
Labordia	0010100200?00?000002000??21000011000101?0100?0000
Geniostoma.1	0010000100100000002000??210000110001010010000000
Geniostoma.2	1010000100100000002000??210000110001010010000000
Geniostoma.3	0010100100100000002000??210000110001010010000000
Geniostoma.4	1010100100100000002000??210000110001010010000000
Geniostoma.5	0010010100100000002000??210000110001010010000000

Table 2—*contd*

Geniostoma.6	10100101001000000002000??2100001100010100100000000
Geniostoma.7	00101101001000000002000??2100001100010100100000000
Geniostoma.8	10101101001000000002000??2100001100010100100000000
Geniostoma.9	00100011001000000002000??2100001100010100100000000
Geniostoma.10	10100011001000000002000??2100001100010100100000000
Geniostoma.11	00101011001000000002000??2100001100010100100000000
Geniostoma.12	10101011001000000002000??2100001100010100100000000
Geniostoma.13	00100111001000000002000??2100001100010100100000000
Geniostoma.14	10100111001000000002000??2100001100010100100000000
Geniostoma.15	00101111001000000002000??2100001100010100100000000
Geniostoma.16	10101110010000000002000??2100001100010100100000000
Geniostoma.17	00100021001000000002000??2100001100010100100000000
Geniostoma.18	10100021001000000002000??2100001100010100100000000
Geniostoma.19	00101021001000000002000??2100001100010100100000000
Geniostoma.20	10101021001000000002000??2100001100010100100000000
Geniostoma.21	00100121001000000002000??2100001100010100100000000
Geniostoma.22	10100121001000000002000??2100001100010100100000000
Geniostoma.23	00101121001000000002000??2100001100010100100000000
Geniostoma.24	10101121001000000002000??2100001100010100100000000
Geniostoma.25	00100002001000000002000??2100001100010100100000000
Geniostoma.26	10100002001000000002000??2100001100010100100000000
Geniostoma.27	00101002001000000002000??2100001100010100100000000
Geniostoma.28	10101002001000000002000??2100001100010100100000000
Geniostoma.29	00100102001000000002000??2100001100010100100000000
Geniostoma.30	10100102001000000002000??2100001100010100100000000
Geniostoma.31	00101102001000000002000??2100001100010100100000000
Geniostoma.32	10101102001000000002000??2100001100010100100000000
Geniostoma.33	00100012001000000002000??2100001100010100100000000
Geniostoma.34	10100012001000000002000??2100001100010100100000000
Geniostoma.35	00101012001000000002000??2100001100010100100000000
Geniostoma.36	10101012001000000002000??2100001100010100100000000
Geniostoma.37	00100112001000000002000??2100001100010100100000000
Geniostoma.38	10100112001000000002000??2100001100010100100000000
Geniostoma.39	00101112001000000002000??2100001100010100100000000
Geniostoma.40	10101112001000000002000??2100001100010100100000000
Geniostoma.41	00100022001000000002000??2100001100010100100000000
Geniostoma.42	10100022001000000002000??2100001100010100100000000
Geniostoma.43	00101022001000000002000??2100001100010100100000000
Geniostoma.44	10101022001000000002000??2100001100010100100000000
Geniostoma.45	00100122001000000002000??2100001100010100100000000
Geniostoma.46	10100122001000000002000??2100001100010100100000000
Geniostoma.47	00101122001000000002000??2100001100010100100000000
Geniostoma.48	10101122001000000002000??2100001100010100100000000
Gelsemium.1	00100101000001000104100??110000010001?200?00000000
Gelsemium.2	00100111000001000104100??110000010001?200?00000000
Gelsemium.3	00100101000001000104101??110000010001?200?00000000
Gelsemium.4	00100111000001000104101??110000010001?200?00000000
Mostuea.1	00100101000001000101100??11000001100010200?0?000010
Mostuea.2	00100111000001000101100??11000001100010200?0?000010
Antonia	00100020000000000004?01??0100001112111000100000000
Bonyunia	00100010000000000004?01??1100001110111000100000000
Norrisia	00100010000001000004101??0?00000112111000?00000000
Usteria	0010001000000000001101??0100001011010????????00000
Spigelia	001000000000000000030001010000111011000?000000000
Mitreola	00100021000000110001000??1?0000??????10?100?0000
Mitrasacme.1	001000200000001100010000?010000?????1001000?0000
Mitrasacme.2	0010002000000011100010000?010000?????1001000?0000
Mitrasacme.3	0010002000000021100010000?010000?????1001000?0000
Mitrasacme.4	001000200000001100013000?010000?????1001000?0000
Mitrasacme.5	0010002000000011100013000?010000?????1001000?0000
Mitrasacme.6	0010002000000021100013000?010000?????1001000?0000
Strychnos.1	0010000000100000000000010100001110011300?00000000
Strychnos.2	0010001000100000000000010100001110011300?00000000
Strychnos.3	0010002000100000000000010100001110011300?00000000

Table 2—*contd*

Subtaxa matrix. Missing data are indicated with question marks. See text for further explanation.

Strychnos. 4	001000000011000000000010100001110011300?0000000
Strychnos. 5	00100010001100000000000010100001110011300?0000000
Strychnos. 6	00100020001100000000000010100001110011300?0000000
Strychnos. 7	00100000001000000000100010100001110011300?0000000
Strychnos. 8	00100010001000000000100010100001110011300?0000000
Strychnos. 9	00100020001000000000100010100001110011300?0000000
Strychnos. 10	00100000001100000000100010100001110011300?0000000
Strychnos. 11	00100010001100000000100010100001110011300?0000000
Strychnos. 12	00100020001100000000100010100001110011300?0000000
Strychnos. 13	00100000001000000000200010100001110011300?0000000
Strychnos. 14	00100010001000000000200010100001110011300?0000000
Strychnos. 15	00100020001000000000200010100001110011300?0000000
Strychnos. 16	00100000001100000000200010100001110011300?0000000
Strychnos. 17	00100010001100000000200010100001110011300?0000000
Strychnos. 18	00100020001100000000200010100001110011300?0000000
Strychnos. 19	00100000001000000000300010100001110011300?0000000
Strychnos. 20	00100010001000000000300010100001110011300?0000000
Strychnos. 21	00100020001000000000300010100001110011300?0000000
Strychnos. 22	00100000001100000000300010100001110011300?0000000
Strychnos. 23	00100010001100000000300010100001110011300?0000000
Strychnos. 24	00100020001100000000300010100001110011300?0000000
Strychnos. 25	0010000000100000000001010100001110011300?0000000
Strychnos. 26	0010001000100000000001010100001110011300?0000000
Strychnos. 27	0010002000100000000001010100001110011300?0000000
Strychnos. 28	0010000000110000000001010100001110011300?0000000
Strychnos. 29	0010001000110000000001010100001110011300?0000000
Strychnos. 30	0010002000110000000001010100001110011300?0000000
Strychnos. 31	00100000001000000000101010100001110011300?0000000
Strychnos. 32	00100010001000000000101010100001110011300?0000000
Strychnos. 33	00100020001000000000101010100001110011300?0000000
Strychnos. 34	00100000001100000000101010100001110011300?0000000
Strychnos. 35	00100010001100000000101010100001110011300?0000000
Strychnos. 36	00100020001100000000101010100001110011300?0000000
Strychnos. 37	00100000001000000000201010100001110011300?0000000
Strychnos. 38	00100010001000000000201010100001110011300?0000000
Strychnos. 39	00100020001000000000201010100001110011300?0000000
Strychnos. 40	00100000001100000000201010100001110011300?0000000
Strychnos. 41	00100010001100000000201010100001110011300?0000000
Strychnos. 42	00100020001100000000201010100001110011300?0000000
Strychnos. 43	00100000001000000000301010100001110011300?0000000
Strychnos. 44	00100010001000000000301010100001110011300?0000000
Strychnos. 45	00100020001000000000301010100001110011300?0000000
Strychnos. 46	00100000001100000000301010100001110011300?0000000
Strychnos. 47	00100010001100000000301010100001110011300?0000000
Strychnos. 48	00100020001100000000301010100001110011300?0000000
Strychnos. 49	00100000001000000000010100001110111300?0000000
Strychnos. 50	0010001000100000000000010100001110111300?0000000
Strychnos. 51	0010002000100000000000010100001110111300?0000000
Strychnos. 52	0010000000110000000000010100001110111300?0000000
Strychnos. 53	0010001000110000000000010100001110111300?0000000
Strychnos. 54	0010002000110000000000010100001110111300?0000000
Strychnos. 55	00100000001000000000100010100001110111300?0000000
Strychnos. 56	00100010001000000000100010100001110111300?0000000
Strychnos. 57	00100020001000000000100010100001110111300?0000000
Strychnos. 58	00100000001100000000100010100001110111300?0000000
Strychnos. 59	00100010001100000000100010100001110111300?0000000
Strychnos. 60	00100020001100000000100010100001110111300?0000000
Strychnos. 61	00100000001000000000200010100001110111300?0000000
Strychnos. 62	00100010001000000000200010100001110111300?0000000
Strychnos. 63	00100020001000000000200010100001110111300?0000000
Strychnos. 64	00100000001100000000200010100001110111300?0000000
Strychnos. 65	00100010001100000000200010100001110111300?0000000

Table 2—*contd*

Strychnos. 66	00100020001100000000200010100001110111300?00000000
Strychnos. 67	00100000001000000000300010100001110111300?00000000
Strychnos. 68	00100010001000000000300010100001110111300?00000000
Strychnos. 69	00100020001000000000300010100001110111300?00000000
Strychnos. 70	00100000001100000000300010100001110111300?00000000
Strychnos. 71	00100010001100000000300010100001110111300?00000000
Strychnos. 72	00100020001100000000300010100001110111300?00000000
Strychnos. 73	001000000010000000001010100001110111300?00000000
Strychnos. 74	0010001000100000000001010100001110111300?00000000
Strychnos. 75	0010002000100000000001010100001110111300?00000000
Strychnos. 76	0010000000110000000001010100001110111300?00000000
Strychnos. 77	0010001000110000000001010100001110111300?00000000
Strychnos. 78	0010002000110000000001010100001110111300?00000000
Strychnos. 79	00100000001000000000101010100001110111300?00000000
Strychnos. 80	00100010001000000000101010100001110111300?00000000
Strychnos. 81	00100020001000000000101010100001110111300?00000000
Strychnos. 82	00100000001100000000101010100001110111300?00000000
Strychnos. 83	00100010001100000000101010100001110111300?00000000
Strychnos. 84	00100020001100000000101010100001110111300?00000000
Strychnos. 85	00100000001000000000201010100001110111300?00000000
Strychnos. 86	00100010001000000000201010100001110111300?00000000
Strychnos. 87	00100020001000000000201010100001110111300?00000000
Strychnos. 88	00100000001100000000201010100001110111300?00000000
Strychnos. 89	00100010001100000000201010100001110111300?00000000
Strychnos. 90	00100020001100000000201010100001110111300?00000000
Strychnos. 91	00100000001000000000301010100001110111300?00000000
Strychnos. 92	00100010001000000000301010100001110111300?00000000
Strychnos. 93	00100020001000000000301010100001110111300?00000000
Strychnos. 94	00100000001100000000301010100001110111300?00000000
Strychnos. 95	00100010001100000000301010100001110111300?00000000
Strychnos. 96	00100020001100000000301010100001110111300?00000000
Gardneria	00100000001000000000200??010000??????300?00000000
Neuburgia. 1	00100010001100000000200??0100001111010??0??0000000
Neuburgia. 2	00100020001100000000200??0100001111010??0??0000000
Plumeria	00000012001000110001101011100101?00010300100010000
Apocynum	00100002001010100001010012100101?0?0101001?0010000
Periploca	001000020111101000110100121001010?001?000100010000
Asclepias. 1	001000000110101000111110131001010??01000100010000
Asclepias. 2	001000010110101000111110131001010??010000100010000
Asclepias. 3	001000000110101000111110131001010??011000100010000
Asclepias. 4	001000010110101000111110131001010??011000100010000
Cinchona	00100110000000020001101011101001??000201100000000
Coffea	00100002000000020000000011101001??0000?011?0000000
Pentas. 1	00100010000000020001000011101001??000?011?0000001
Pentas. 2	00100110000000020001000011101001??000?011?0000001
Pentas. 3	00100010000000020001300011101001??000?011?0000001
Pentas. 4	00100110000000020001300011101001??000?011?0000001

METHODS

Cladograms were constructed using PAUP ver. 3.1.1 (Swofford, 1993) and a Macintosh IIfx. The options used were: heuristic search with SIMPLE addition sequence, HOLD = 5, MULPARS, COLLAPSE, and TBR (or TBR followed by NNI) branch swapping.

Tree lengths, consistency indices (*C*; Kluge and Farris, 1969) including all characters (Goloboff, 1991; Yeates, 1992), and retention indices (*R*; Farris, 1989) were calculated automatically by PAUP. Strict (Schuh and Polhemus, 1981) and combinable component (*K. Bremer, 1990*) consensus trees were also calculated using PAUP.

For the analysis of the subtaxa matrix the total number of equally parsimonious trees was assumed to be very large because of a terminal/character ratio well above 1.0 (i.e., 235 terminals and 50 characters). Therefore, for estimation purposes it was important to obtain as many equally (and hopefully most) parsimonious trees as possible that could form a representative sampling of the different topologies available. This was done by saving two large sets of trees derived from different branch swapping routines. TBR (tree bisection-reconnection) swapping clips trees along every branch, producing pairs of subtrees that are then reattached at every possible point (Swofford, 1993). In contrast, NNI (nearest neighbour interchange) swapping merely involves rearrangements of internal tree branches. With data such as the subtaxa matrix, the substantial ambiguity caused by conflicting hierarchical evidence will suggest many possible resolutions of that ambiguity. These resolutions are found in different ways by TBR and NNI; the shapes of trees amplified using TBR will tend to reflect different placements of clipped subtrees of the starting topology whereas those of NNI-amplified trees will tend to reflect local rearrangements. Therefore, in finding the first X number of TBR-swapped trees, one may find tree shapes substantially different from the set of Y additional trees found using NNI swapping on a single seed tree borrowed from the TBR round.

This phenomenon was apparent from our treatment of the subtaxa matrix (Table 2). First, 1000 trees were produced using TBR branch swapping and a MAX-TREES specification. In approximately 12 hours of computation, 20 of these trees had been swapped to completion, suggesting that at least a plateau of local optimality had been reached. One of the 1000 trees was then used as a seed tree for a round of NNI branch swapping (MAXTREES set as above). On merging both files of 1000 trees each, 1999 unique trees were retained (the lost 2000th having been the seed tree identical to one of the 1000 TBR trees). As all trees resulted from a single taxon addition sequence, they by definition reside on a single optimality peak (or island, sensu Maddison, 1991) with shape variation ascribable only to swapping behaviour. We take these tree shapes to be a reasonable approximation of component resolutions available from the subtaxa matrix.

Results

The Gentianales sensu stricto were analysed together with nine presumed out-group genera. Parsimony analysis of data coded with restricted polymorphisms resulted in 144 trees of 139 steps ($C = 0.475$, $R = 0.693$). The combinable component consensus (Fig. 1; oriented with *Desfontainia* and *Viburnum* [Viburnaceae] at the base; see Bremer et al., 1994) shows considerable topological resolution among all taxa. As expected, the Gentianales sensu stricto are monophyletic, with *Cestrum* (Solanaceae) as the sister group. The sister group to *Cestrum*/Gentianales is the Scrophulariales/Oleales, including the former loganiaceous genera *Buddleja*, *Plocosperma*, *Polyprenum*, and *Retzia*. *Desfontainia*, also formerly Loganiaceae, is associated with *Viburnum*. Within the Gentianales sensu stricto all families are monophyletic except for Loganiaceae, which is grossly paraphyletic. The genera of Loganiaceae are distributed among five monophyletic groups: (i) *Geniostoma* and *Labordia* sister to Apocynaceae, (ii) Gelsemieae sister to Rubiaceae, (iii) a "Strychnos clade" of eight loganiaceous genera (resolved in 75% of trees, incl. *Strychneae* sister

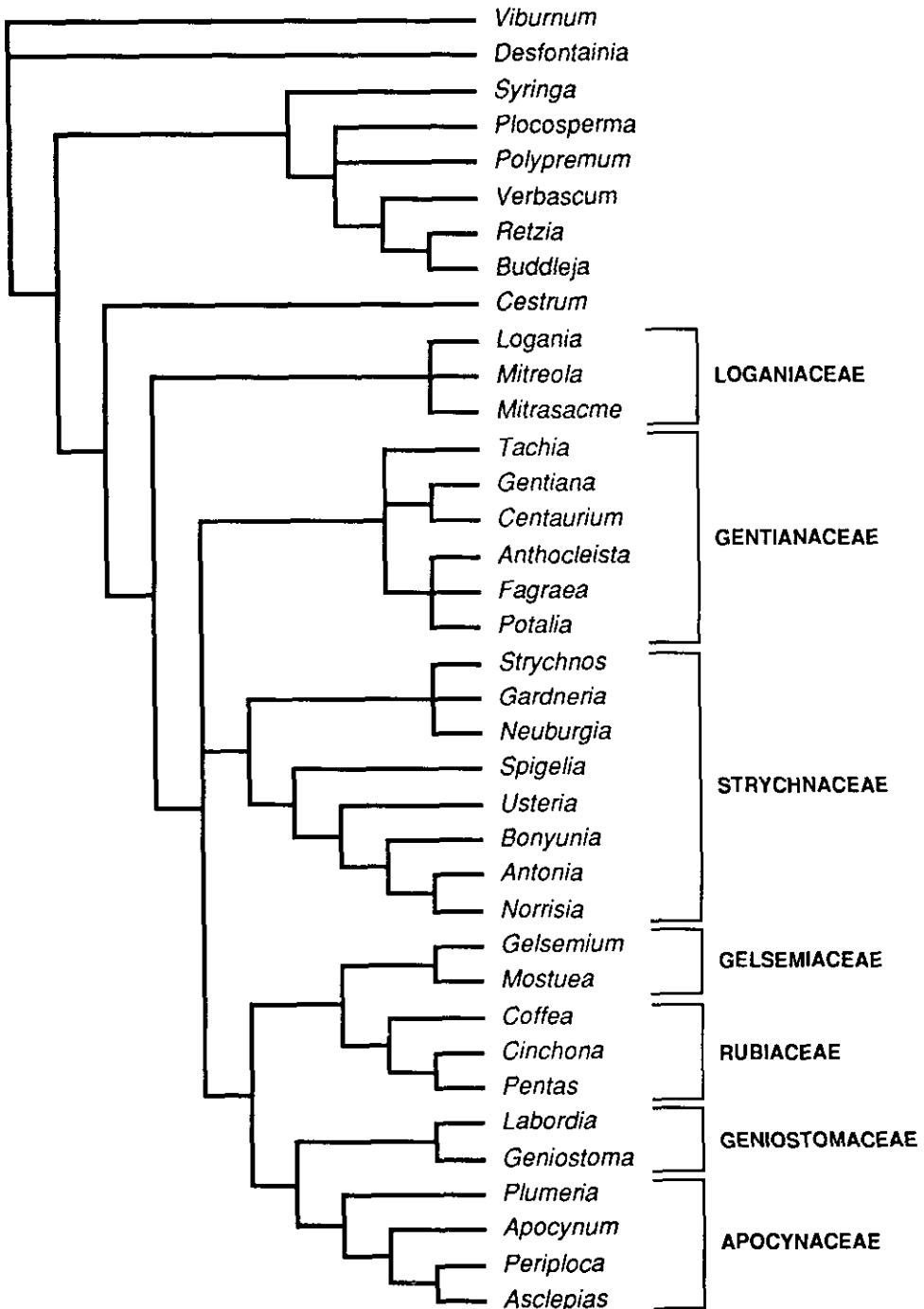


Fig. 1. Relationships of Gentianales. Combinable component consensus of 144 trees. The unrooted consensus tree is oriented with *Viburnum* and *Desfontainia* basally polytymous. The sister group of Gentianales is *Cestrum* of Solanaceae — Solanales. Monophyletic Gentianales are rooted at the Loganiaceae sensu stricto, one of five major clades containing loganiaceous genera. Genera excluded from Loganiaceae (and Gentianales) group with Scrophulariales/Oleales or with Dipsacales. Family names follow the present classification. Note that Strychnaceae are resolved in 75% of trees, and *Antonia* plus *Norrisia* in 50%. All other components are combinable in 100% of trees.

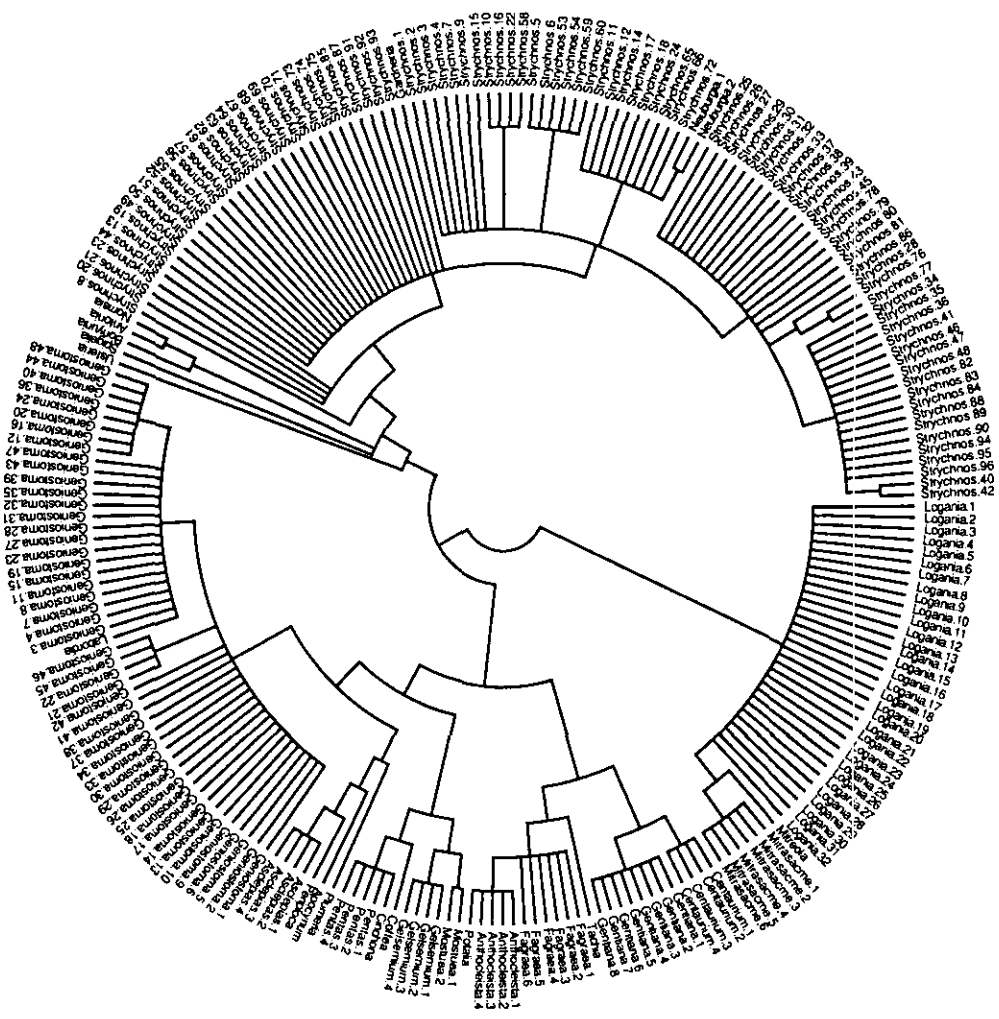


Fig.2. Relationships of subtaxa and genera within the Gentianales. Strict (next page) and combinable component consensus of 1999 trees. The unrooted trees are oriented with a monophyletic Loganiaceae sensu stricto, following from Figure 1. The same five major clades are identified as in Figure 1. Note that *Geniostoma* and *Strychnos* are positively paraphyletic in all trees, whereas *Anthodoleista*, *Fagraea* and *Logania* are only potentially so (being unresolved in the strict consensus). *Pentas* is unresolved in both consensus trees.

to *Spigelia* plus *Antonieae*), (iv) *Potalieae* plus *Gentianaceae*, and (v) a “*Logania* clade” (incl. *Mitreola* and *Mitrasacme*) sister to all of these.

The principal goal of this study was to resolve the cladistic relationships within the Gentianales sensu stricto. Left unclear from the highly resolved first analysis (Fig. 1) was the hierarchical content of ingroup-only character information. To eliminate external sources of homoplasy, a parsimony analysis was performed with all outgroup taxa excluded. Using the data coded for restricted polymorphisms (Table 1), 144 trees of 102 steps were found ($C = 0.559$, $R = 0.708$). The combinable component consensus (not shown) depicts an ingroup topology identical to that from the outgroup analysis (Fig. 1) except for one missing component: the

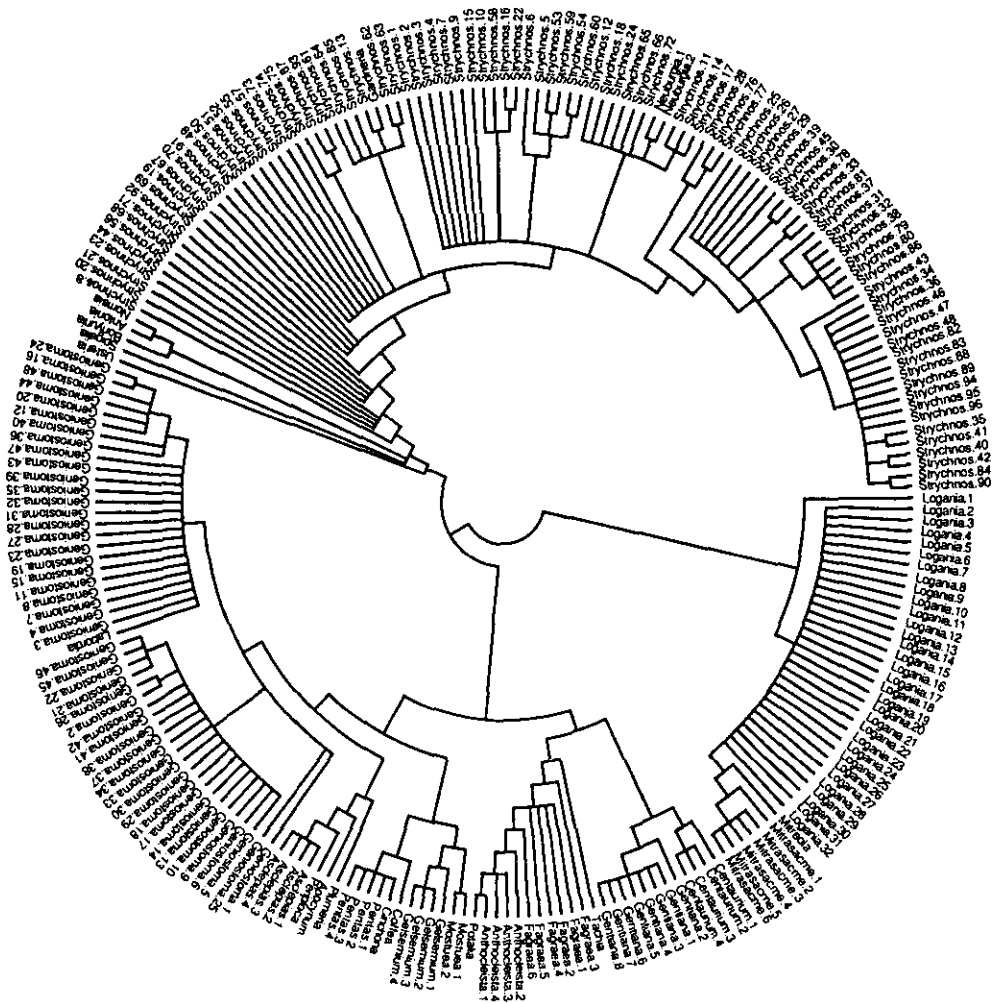


Fig.2. continued

branch connecting Strychneae with *Spigelia* plus Antonieae in 75% of outgroup-oriented trees. Thus, ingroup relationships were not strongly influenced by outgroup character-state distributions.

Accepting the slightly less resolved ingroup result as the baseline estimate of hierarchy within the Gentianales sensu stricto, we investigated the effects of subtaxon polymorphism recoding among genera of the order. To account for all possible combinations of polymorphic character states, the initial 28 genera were sub-divided into 235 monomorphic terminals (most of which referred to *Strychnos* [$n = 96$], *Geniostoma* [$n = 48$], and *Logania* [$n = 32$]). Parsimony analysis of this polymorphism-free data matrix was complicated by the large number of terminals relative to characters (see Methods). Two large sets of trees at 303 steps were found ($C = 0.195$, $R = 0.854$), each set (1000 and 999 trees, respectively) representing

divergent tree shapes. Both sets were nevertheless highly combinable; the strict and combinable component consensus trees (Fig. 2) are well resolved among genera, and the latter is highly resolved among monomorphic terminals. Although the five principal groupings discovered in the outgroup analysis remain intact (the "Strychnos clade" being 100% united in all trees; Fig. 2), subtaxon relationships suggest noteworthy implications for generic instability or paraphyly. *Geniostoma* and *Labordia* remain sister to Apocynaceae, but the embedding of *Labordia* within hierarchically structured *Geniostoma* subtaxa (Fig. 2) renders *Geniostoma* paraphyletic. The rooting of the "Strychnos clade" is altered, splitting basal *Usteria* away from *Antonia*, *Bonyunia*, and *Norrisia*, which leaves Antonieae parasphyetic (Fig. 2). Strychneae remain monophyletic, but *Gardneria* and *Neuburgia* are nested well within considerably hierarchic *Strychnos* subtaxa, making *Strychnos* paraphyletic (Fig. 2). Potalieae are more firmly embedded in Gentianaceae as sister group to the woody genus *Tachia* (Fig. 2). However, *Fagraea* subtaxa are unresolved in the strict consensus tree and are strongly paraphyletic to *Anthocleista* and *Potalia* in the combinable component consensus (Fig. 2). The same pattern holds for *Anthocleista* relative to *Potalia*. *Logania* subtaxa are unresolved with *Mitreola* plus *Mitrasacme* in the strict consensus, but a single combinable component (Fig. 2) suggests that *Logania* may be paraphyletic to this taxon pair.

Discussion

Cladistic analysis of the Gentianales *sensu stricto* identifies five principal lineages, all of which include members of the heterogeneous Loganiaceae. The morphological and phytochemical evidence is in strong agreement across outgroup and ingroup-only studies, including results from monomorphic recoding of polymorphic genera. The Loganiaceae are grossly paraphyletic, although other principal gentianalean families — Apocynaceae, Gentianaceae, and Rubiaceae — remain monophyletic. Outgroup analysis (including taxa formerly placed in Loganiaceae) suggests a possible Solanales/Gentianales relationship, with the Gentianales rooted between the "Logania clade" and the rest of the order. Monomorphic recording of polymorphic taxa suggests that several loganiaceous genera may be paraphyletic. These issues are discussed below with reference to a new classification of Gentianales.

ROOTING ISSUES

The distinction between orienting versus rooting and directing cladograms is frequently ignored in cladistic studies. True rooting may be accomplished using several different methods as well as their assumptions (e.g., specification of ancestral states and use of directed characters). However, an unrooted tree (or network) also shows relationships even if the direction of the tree is not indicated. Outgroup analysis falls under this category, with only orientation being provided (e.g., by including a single outlier taxon; Farris, 1982; Nixon and Carpenter, 1993). However, when a study group is small relative to the number of outgroup taxa, a goal of placing the group within a larger context may be more realizable than a goal of orienting relationships within the group. Our analysis of 37 taxa (including 9 outgroups) is far from inclusive with respect to relationships among Dipsacales, Scrophulariales/Oleales, Solanales, and Gentianales. Nevertheless, the sister group

relationship of Gentianales with Solanales has precedent in the Asteridae-level *rbcL* gene trees of Olmstead et al., (1993) and Bremer et al., (1994). In contrast, our estimate of basal relationships within Gentianales is at odds with several molecular cladistic studies that place Rubiaceae rather than the "*Logania* clade" as sister to the rest of the order (Downie and Palmer, 1992; Olmstead et al., 1993; Bremer et al., 1994). These rooting issues lie at different hierarchical levels, and in keeping with the focus of the present study, we will concentrate on the discrepancy within Gentianales.

Ontogeny as a rooting criterion has been used in several morphological studies (e.g. Kraus, 1988; Wheeler, 1990; see Nelson, 1978). This method directs an ingroup tree according to hypotheses of ontogeny, biosynthetic pathways or other transformation-series criteria for all or some of the characters (Weston, 1988). In this study directed transformation series can be applied to some of the characters, e.g. ch. 15 (apocarpous ovaries are hypothesized to be apomorphic; Endress et al., 1983) and ch. 39 (biosynthesis of seco-iridoids and complex indole alkaloids; Kisakürek and Hesse, 1980). From these characters, it might be argued that a root position could lie anywhere outside the "*Logania* clade" or Apocynaceae (ch. 15) or the Gelsemieae/Rubiaceae, *Plumeria Gardneria*, or *Strychnos* (ch. 39). The second criterion (from ch. 39) directly contradicts information from several molecular studies, which split Gelsemieae from a basally-positioned Rubiaceae. However, other characteristics may support this rooting, e.g. inferior ovaries (ch. 16) and the lack of internal phloem (ch. 37), both of which are common among basal taxa in the Asteridae (e.g., *Aucuba* Thunb. [Aucubaceae or Cornaceae]; cf. Chase et al., 1993; Olmstead et al., 1993). Further studies of these traits and further *rbcL* sequencing within Loganiaceae sensu lato may help to clarify basal gentianalean relationships.

SUBTAXON RELATIONSHIPS

Although our analysis of the subtaxa matrix may have been hampered by computational constraints, there is still good reason to consider its results the most informative about within-Gentianales relationships. Free from the influence of polymorphisms (and, therefore, arbitrary resolutions of node states), the subtaxa trees depict all character variation recognized during data collection. As such, the degree to which subtaxa group within their taxa of origin may be taken as an estimate of the relative support for those larger taxa. For example, *Logania* subtaxa are either entirely or largely unresolved in the strict and combinable component consensus trees (Fig. 2). Including autapomorphies before constructing monomorphic units can insure the monophyly of subtaxa, but that this need not be the case is well illustrated by *Pentas*, which remains unresolved in both consensus trees (Fig. 2). Total support for larger taxa can only come when subtaxa relationships are not in conflict. In cases such as *Logania* or *Pentas*, the polymorphisms inherent in the original matrix must overlap with those of other taxa, producing conflicting patterns among monomorphic subunits. Thus, greater sampling of characters (particularly autapomorphies), rather than greater sampling of taxa, should tend to provide monophyletic resolutions.

Other taxa in our subtaxa analysis are no longer resolved as monophyletic units. *Labordia* is included within *Geniostoma*, and *Gardneria* and *Neuburgia* are included within *Strychnos* (Fig. 2). *Labordia* (endemic to the Hawaiian islands) has in fact been combined with *Geniostoma* (which has a wide Indo-pacific distribution; Conn,

1980). Although both are maintained in Strychnae, *Gardneria* is very similar to *Strychnos* whereas *Neuburgia* differs in its drupaceous fruits and aberrant wood anatomy (Bisset et al., 1980). The resultant paraphyly of *Strychnos* may be best resolved by formal inclusion of its apomorphic derivatives, but this possibility should be studied further using real *Strychnos* species. A clear example of the informativeness of subtaxa resolution is with *Fagraea*, which is unresolved in the strict consensus tree but paraphyletic to *Anthocleista* and *Potalia* in the combinable component tree (Fig. 2). A separate analysis of numerous species of *Pctaliaeae* has reached identical conclusions, namely that *Fagraea* sensu lato is a paraphyletic grade (L. Struwe and V. A. Albert, unpublished data).

CLASSIFICATION

Based on the cladistic analyses presented, Struwe and Albert propose a new familial classification of the order Gentianales (Appendix 2). The primary goal of this classification is to reduce the heterogeneity and paraphyly of *Loganiaceae*, which are split into several smaller families. Although most families in the Gentianales have strong individual support from these data, their interrelationships are only weakly supported by character evidence. The classification of Struwe and Albert is based on the most inclusive monophyletic groups that have distinct characteristics. The issue of rooting the Gentianales will of course affect hypotheses of monophyly. Nevertheless, if future cladograms are rooted not between but within one of the accepted families it will destroy the monophyly of only that taxon.

All families recognized by Struwe and Albert (Appendix 2) are monophyletic units in the outgroup-oriented and subtaxa trees (Figs 1–2). Note, however, that the “*Strychnos* clade” is monophyletic only as a combinable component in the outgroup-oriented analysis and is not completely resolved in the ingroup-only analysis. The relationships, characteristics, and circumscriptions of the families are presented below in abstract form.

Apocynaceae Juss.

This family includes the more derived family *Asclepiadaceae*, so as to avoid a paraphyletic *Apocynaceae* (Wanntorp, 1989; Downie and Palmer, 1992; Olmstead et al., 1993; Judd et al., 1994; Appendix 2). With the present circumscription the number of species reaches nearly 5000, distributed among more than 550 genera (Mabberley, 1987). *Apocynaceae* occur on all continents except Antarctica, and the herbaceous species are mainly temperate. In *Apocynaceae* elaborate gynoeceal and androeceal structures have evolved forming a complex gynostegium (ch. 10, 13, 19). Unique characteristics for the family include laticifers in the vegetative parts (ch. 30) and the follicular fruits borne by most genera. A sister-group relationship to the *Geniostomaceae* is supported in this study by the shared presence of anthers with apical appendages (ch. 11; data for *Geniostoma* only) and porate pollen grains (ch. 26; *Apocynum* and *Periploca*) in both families.

Gelsemiaceae (G. Don) L. Struwe & V. A. Albert

This family (formerly tribe *Gelsemieae*; Leeuwenberg and Leenhouts, 1980; Appendix 2) consists of the two genera *Gelsemium* (3 spp. in East Asia, North and Central America; Leenhouts, 1963; Wyatt et al., 1993) and *Mostuea* (8 spp. in Africa,

Madagascar, and northern South America; Leeuwenberg, 1961c). The plants are shrubs or vines bearing heterostylous flowers with twice dichotomously divided stigmas (ch. 18) and latrorse anthers (ch. 14). The family is also characterized by imbricate corolla aestivation (ch. 8) and flattened seeds (ch. 21). The genus *Gelsemium* had earlier been placed in Apocynaceae (Jussieu, 1789) or Gentianaceae (Bartling, 1830).

This family is positioned as the sister group to the Rubiaceae in both this and an earlier study (Bremer and Struwe, 1992; Figs. 1, 2), sharing heterostylous flowers (ch. 6) and the presence of complex indole alkaloids (ch. 39). On the other hand, other taxonomic views have been proposed. Palynological characters are homogeneous in the family and show similarities with *Antonia*, *Bonyunia*, *Norrisia*, *Usteria*, and the Gentianaceae (especially tribe Potalieae; Punt, 1980).

Geniostomaceae L. Struwe & V. A. Albert

The family Geniostomaceae was described as a result of this study (Appendix 2). It includes *Geniostoma* and *Labordia*, and the latter genus is sometimes included in the former (Conn, 1980; cf. Leeuwenberg, 1977). *Geniostoma* (c. 20 spp.) is confined to eastern Asia, Australia, and the Pacific Islands, and *Labordia* (20 spp.) is a derived Hawaiian genus. They have persistent and fleshy placentas in capsules with deciduous valves (ch. 20; Gray, 1859; Conn, 1980), traits that also characterize some genera of Apocynaceae. *Geniostoma* and *Labordia* have formerly been included in tribe Loganieae. Similarities with *Logania* include the presence of unisexual flowers (ch. 5) and dioecy (ch. 1) in some species (Conn, 1980, 1994). However, these appear to be parallelisms between these taxa. In this study Geniostomaceae is positioned as the sister group to the Apocynaceae, which is supported by the presence of anthers with apical appendages (ch. 11; data for *Geniostoma* only) and porate pollen grains (ch. 26; *Apocynum* and *Periploca*). This relationship had been suggested earlier (Bentham, 1857; Baillon, 1889).

Gentianaceae Juss.

Most species of this family are temperate herbs, but tropical shrubs and trees are included as well. The number of species is over 1000 and the majority of these are alpine, blue-flowering gentians (e.g., most *Gentiana* species). The circumscription of the family has been rather stable during the last 200 years, with the exception of the Menyanthaceae, which was excluded originally by Dumortier (1829) and since then included and excluded alternately. Furthermore, the former loganiaceous tribe Potalieae (incl. *Potalia*, *Anthocleista*, and *Fagraea*) was joined with the tribe Tachiineae of Gentianaceae by Fosberg and Sacht (1974, 1980). In fact, the transfer to the Gentianaceae had already been accomplished by Bureau (1856) over 100 years before. This view was followed by Takhtajan (1987) and is supported in our study (Figs. 1, 2) as well as by molecular data (Downie and Palmer, 1992; Olmstead et al., 1993). The Potalieae and Gentianaceae sensu stricto share synapomorphies such as bilobed placentas (ch. 17; not *Potalia*) and the presence of xanthonoids (ch. 45) as well as swertiamarin and other unique seco-iridoids (ch. 43). The relationships of the Gentianaceae are uncertain in our outgroup and ingroup trees (Fig. 1). However, in our subtaxa results, Gentianaceae are sister to ((Gelsemiaceae Rubiaceae)(Apocynaceae Geniostomaceae)). The last comprehensive classification was published by Gilg (1895), based mainly on palynological characters. The generic and tribal delimitations are still uncertain, and the possibility of a woody

versus herbaceous ancestral state for the family has been argued as well (e.g., Wood and Weaver, 1982).

Loganiaceae Mart. sensu stricto

The circumscription of this family has varied with different authors and the one presented by Struwe and Albert is the most restrictive ever (including only *Logania*, *Mitreola*, and *Mitrasacme*, Appendix 2). The family has been regarded as a heterogeneous artificial assemblage for some time and Bentham predicted in 1857 (p. 54) that "some of these genera will be found even more closely allied to certain others of the above orders [Rubiaceae, Apocynaceae, Gentianaceae, Scrophulariaceae] respectively than they are to each other". This has certainly become true. Even if some authors still keep the Loganiaceae sensu lato together (cf. Leeuwenberg and Leenhouts, 1980; Rogers, 1986), it has become more and more obvious that several genera have been misplaced in Gentianales (*Retzia*, *Desfontainia*, *Plocosperma*, *Buddleja*; Jensen, 1992; Bremer et al., 1994) and that the remaining genera should be segregated into smaller monophyletic units or included in other families.

The three genera of the Loganiaceae sensu stricto are herbs or suffrutescent shrubs. *Logania* (c. 33 spp; B. J. Conn, pers comm.) occurs in Australia, New Caledonia and possibly New Zealand, while *Mitrasacme* (40 spp.) and *Mitreola* (6 spp.) have a wider distribution covering tropical areas in Asia, Africa, and America. According to our results Loganiaceae is basally positioned in the Gentianales. The vessels of *Logania* are lacking spiral thickenings (ch. 33) and vested pits (ch. 32) in contrast to the other families of the Gentianales. The family Loganiaceae in this restricted sense is supported by the presence of a ring of hairs in the corolla mouth (ch. 7; polymorphic in *Logania*) and by partly apocarpous (ch. 15; polymorphic in *Logania*) and semi-inferior gynoecia (ch. 16; *Mitreola* and *Mitrasacme*).

The circumscription of Loganiaceae proposed by Struwe and Alber: (Appendix 2) conflicts with earlier tribal classifications. *Mitreola* and *Mitrasacme* have earlier been placed in the tribe Spigeliaceae, and *Logania* in the tribe Loganieae together with *Geniostoma* and *Labordia* (Leeuwenberg and Leenhouts, 1980). However, the relationships of *Logania* have been unclear for some time (cf. Bentham, 1857; Punt and Leenhouts, 1967; Mennega, 1980). The genera *Mitreola* and *Mitrasacme* have alternatively been proposed as close relatives to the Rubiaceae, especially the Hedyotideae (Bureau, 1856; Bentham, 1857; Thorne, 1976; Metcalfe and Chalk, 1983).

Rubiaceae Juss.

This family is the largest in the order, and is well characterized by the presence of inferior ovaries (ch. 16), isoquinoline alkaloids (ch. 41), and non-laticifer secretory elements (ch. 29), as well as the absence of internal phloem (ch. 37). The members of this family are mostly tropical woody plants, but herbs are not uncommon. The number of genera exceeds 600 and more than 10 000 species are accepted (Mabberley, 1987).

Morphological as well as molecular data have been used to help clarify the relationships within the family (Bremer and Jansen, 1991; Bremer, 1992; Bremer and Struwe, 1992). According to this study the Rubiaceae are the sister group to the Gelsemiaceae (Figs. 1, 2), but this is not supported by molecular cladograms, where Rubiaceae form the basalmost clade in the Gentianales (Downie and Palmer, 1992; Olmstead et al., 1993; Bremer et al., 1994).

Strychnaceae DC. ex Perleb

As circumscribed by Struwe and Albert (Appendix 2), the Strychnaceae includes the former loganiaceous tribes Strychnae and Antonieae (Leeuwenberg and Leenhouts, 1980) as well as *Spigelia*. The Strychnaceae were described by Perleb (1826) and have not been accepted since Hutchinson (1959). In the outgroup-oriented analysis (Fig. 1), this group is supported by the presence of valvate corolla aestivation (ch. 8), colpate pollen without lateral extensions at the endocolpus (ch. 26; not *Bonyunia*), and included phloem (ch. 38; not *Neuburgia* and *Usteria*).

Strychnaceae are monophyletic in the subtaxa analysis (Fig. 2), but they are collapsed into Strychnae alone and *Spigelia* plus Antonieae in the ingroup analysis that tolerated restricted polymorphisms. Although support for this broadly defined Strychnaceae is less than that for other families recognized by Struwe and Albert, these authors have chosen to maintain the present circumscription until further studies can address the problem. The names Spigeliaceae (von Martius, 1827) and Antoniaceae (Hutchinson, 1959) are available should further segregation become necessary.

Strychnos (c. 200 spp. with circumtropical distribution) and the smaller genera *Gardneria* Wall. (5 spp. in Southeast and East Asia) and *Neuburgia* Blume (10–12 spp. in East Malesia, Melanesia and Fiji; Leenhouts, 1963; Leeuwenberg and Leenhouts, 1980) form a monophyletic group supported by anthers with apical appendages (ch. 11), indehiscent fruits (ch. 20), cup-shaped seeds (ch. 21; polymorphic in *Strychnos*) and the presence of complex indole alkaloids (ch. 39). The latter two genera are probably derived within *Strychnos*, which is suggested by the subtaxa analysis (Fig. 2). *Neuburgia* is distinguished from *Strychnos* by drupaceous fruits, and *Gardneria* differs from the latter in having flattened staminal filaments (Leeuwenberg and Leenhouts, 1980).

The other clade in the Strychnaceae (or, in the subtaxa analysis, its basal components) consists of four woody tropical genera from Loganiaceae—Antonieae, *Antonia* (1 sp. in northern South America), *Bonyunia* Schomb. ex Progel (4 spp. in South America), *Norrisia* Gardner (2 spp. in the Malesia region), and *Usteria* (1 sp. in West and Central Africa), together with the tropical and subtropical American genus *Spigelia* with about 50 species (Leeuwenberg and Leenhouts, 1980). *Spigelia* differs from the characteristic habit of the genera of Strychnaceae in being herbs, often with the leaves in pseudo-whorls, and the inflorescences are cincinnous. This clade lacks seco-iridoids (ch. 39) and there is also a tendency towards unequal sepal lobes. The presence of calycophylls in *Usteria* as well as in several genera of the Rubiaceae has been thought to indicate relatively close relationship between these two taxa (Bentham, 1857). This view is not supported here, but earlier results have indicated a closer relationship (Bremer and Struwe, 1992). *Spigelia* has also been suggested as a close relative to the Rubiaceae (Bentham, 1857; Thorne, 1976; Mercalfe and Chalk, 1983).

Two molecular studies support a close relationship between *Spigelia* and *Strychnos* (Downie and Palmer, 1992; Olmstead et al., 1993). Strychnaceae forms one of the major clades in this study, and in the subtaxa trees, is sister to all families of Gentianales except Loganiaceae *sensu stricto*.

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Appendix 1. Characters used for cladistic analysis of the Gentianales

MORPHOLOGY

Principal sources for each taxon were: *Verbascum* (Murbeck, 1933), *Polyprenum* (Holm, 1924), Gentianaceae (Maguire and Weaver, 1975, Wood and Weaver, 1982), Loganiaceae sensu lato (Solereider, 1892–95; Van Raalte, 1932; Sherff, 1939; Leeuwenberg 1961a,b,c, 1963, 1967, 1969a,b, 1975, 1977, 1979; Leenhouts, 1963; Conn, 1980, 1994; Leeuwenberg and Leenhouts, 1980; Rogers, 1936), Apocynaceae (Woodson, 1938, 1954; Browicz, 1966; Rosatti, 1989a,b), and Rubiaceae (Verdcourt, 1953; Bridson, 1982; Robbrecht, 1988).

1. Sexuality of plants: (0) monoecious; (1) dioecious or gynodioecious. *Logania* sect. *Logania* is characterized by dioecious plants, while sect. *Stomandra* usually has monoecious plants with bisexual flowers (Conn, 1994). The pattern is similar in *Geniostoma* where both gynodioecious and hermaphroditic plants occur (Smith and Stone, 1962; Conn, 1980). Both of these genera are therefore coded as polymorphic. Dioecy and gynodioecy are closely related genetically, and the latter is typically a precursor of the former (see Dellaporta and Calderon-Urrea, 1993).
2. Annual shoots: (0) cylindrical in cross-section; (1) quadrangular in cross-section or 4-winged. Many genera (cf. *Gentiana*, *Centaurium*) in the Gentianaceae are characterized by 4-winged or quadrangular shoots (Wood and Weaver, 1982). This feature also occurs in *Polyprenum* (Holm, 1924), and in some species of *Buddleja* (coded as polymorphic; Leeuwenberg, 1979).
3. Leaf arrangement: (0) alternate; (1) opposite. Opposite leaves are typical for the order Gentianales but occur in other taxa of the Asteridae as well (cf. Oleaceae, Caprifoliaceae). *Buddleja* is variable with respect to this character as some species have alternate leaves (Leeuwenberg and Leenhouts, 1980).
4. Leaf bases: (0) distinct; (1) confluent and fused. The petioles in *Fagraea*, *Anthocleista*, and *Potalia* (Gentianaceae) are basally fused around the stem and the basal parts of the lamina are often auriculate (Hasselberg, 1937; Leeuwenberg, 1961a; Leenhouts, 1963).
5. Flowers: (0) bisexual; (1) unisexual. Unisexual flowers occurs in *Labordia*, *Logania* sect. *Logania*, and some species of *Geniostoma* that have unisexual female flowers on some plants (Sherff, 1939; Conn, 1980, 1994; Leeuwenberg and Leenhouts, 1980).

6. Flowers: (0) homostylous; (1) heterostylous. All species in *Mostuea*, *Gelsemium* and *Cinchona* are heterostylous (Schumann, 1891; Duncan and Dejong, 1964; Leeuwenberg and Leenhouts, 1980). This also characterizes most species of *Centaureum* (Gilg, 1895), and occurs in *Pentas* and *Geniostoma* as well (these three genera are coded as polymorphic; Schumann, 1891; Smith and Stone, 1962; Robbrecht, 1988).
7. Corolla, internal indumentum: (0) absent; (1) irregularly hairy; (2) with a ring of hairs in mouth. Genera with a distinct zone of hairs in the upper part of the corolla are coded with state 2. Several genera show polymorphism in this character.
8. Corolla aestivation: (0) valvate; (1) imbricate; (2) contorted. This character is very variable in the Gentianales, but only a few genera show polymorphism (*Desfontainia*, *Buddleja*, *Geniostoma*, and *Asclepias*). Contorted corolla aestivation gave rise to the old name for the Gentianales, the *Contortae*, and is present in the taxa representing Gentianaceae, Geniostomaceae and Apocynaceae, *Desfontainia* and *Coffea*.
9. Filaments and bases of anthers: (0) not united; (1) united by a thin membrane or a thickened ring in the corolla mouth. This character occurs in *Potalia*, *Anthocleista*, and *Fagraea* (a few spp.; Leenhouts, 1963, here coded as polymorphic).
10. Filament appendages: (0) absent; (1) present. These appendages are also known as a staminal corona and are only present in *Periploca* and *Asclepias* (Browicz, 1966; Kunze, 1990), and some species of *Geniostoma* (Smith and Stone, 1962).
11. Apical appendages on anthers: (0) absent; (1) present. The apical appendages are sterile tissue from the connective varying from a small sterile tip in *Strychnos*, *Neuburgia*, *Gardneria*, and *Geniostoma* to larger and more elaborated appendages in the Apocynaceae (Conn, 1980; Leeuwenberg and Leenhouts, 1980; Fallen, 1986; Kunze, 1990).
12. Anthers: (0) glabrous; (1) hairy. Anthers with basal hairs (also called bearded anthers) are present in *Strychnos* (some sections), *Neuburgia*, and in *Periploca* (Browicz, 1966; Leeuwenberg, 1969a).
13. Anthers: (0) free from the stigma; (1) adnate to or united with the stigma. In the Apocynaceae a successive transformation of the complex gynostegium can be discerned. The anthers are either free from the stigma (*Plumeria*), adnate and sometimes glued to the stigma (*Apocynum*, *Periploca*), or united with the stigma (*Asclepias*; Woodson, 1938, 1954; Rosatti, 1989a, 1989b). In the Apocynaceae, however, there is no clear distinction between the last two features so they are here treated as one state (Fallen, 1986; Kunze, 1990).
14. Anther dehiscence: (0) introrse; (1) latrorse; (2) extrorse. The anther dehiscence is usually introrse with a few exceptions; Gelsemiaceae and *Norrisia* have latrorse anthers (Leeuwenberg and Leenhouts, 1980) and Oleaceae have extrorse anthers. The subfamily Asclepiadoideae (Apocynaceae, here represented by *Asclepias*) usually have apical dehiscence and are considered strongly introrse, and thus coded as homologous to introrse dehiscence, which also occurs in this subfamily (Endress and Stumpf, 1990). *Mitrasacme* shows polymorphism (Leeuwenberg and Leenhouts, 1980).
15. Gynoecium: (0) syncarpous; (1) partly or totally apocarpous, stigmas united. The ontogeny of the apocarpous and semi-apocarpous ovaries of *Mitreola* and

- Mitrasacme* and most Apocynaceae has been shown to follow the same pattern (Endress et al., 1983; Fallen, 1986). Semi-apocarpous ovaries reportedly occur in some species of *Logania* (Leeuwenberg and Leenhouts, 1980).
16. Ovary: (0) superior; (1) semi-inferior; (2) inferior. Partly inferior ovaries (semi-inferior) occur in *Plumeria* (Woodson, 1938), *Mitreola* and *Mitrasacme* (Leeuwenberg and Leenhouts, 1980). Rubiaceae are the only family in Gentianales with an inferior ovary.
 17. Placentas: (0) 2, not bilobed; (1) 2, bilobed; (2) 3, but 2 reduced. The two placentas of the Gentianaceae are often bilobed or sometimes even totally divided, and are usually enrolled. The common states in *Gentiana* are 2 placentas (or 4 groups of placental tissue) on the inside of the ovary wall (Wood and Weaver, 1982). The latter is considered as a feature derived from 2 bilobed placentas (Krishna and Puri, 1962). Three placentas occur in *Viburnum*, but only one develops in the fruit (Ferguson, 1966).
 18. Stigma branching: (0) entire or bilobate; (1) twice dichotomously divided. Twice dichotomously divided stigmas occur only in Gelsemiaceae and *Plocosperma* (Leeuwenberg, 1961c, 1967).
 19. Stigma in cross-section: (0) rounded; (1) pentagonal. Pentagonal stigmas occur in *Asclepias* and *Periploca* and are produced by the 5 stamens that grow together with the stigma to form a gynostegium (Kunze, 1990).
 20. Fruit dehiscence: (0) indehiscent; (1) dehiscent, valves persistent without torn apices; (2) valves deciduous, septae persistent; (3) valves and septae deciduous; (4) dehiscent, valves persistent with torn apices. Capsules with deciduous septae and valves occurs only in *Spigelia*, and persistent septae (state 2) are characteristic for Geniostomaceae (Leeuwenberg and Leenhouts, 1980).
 21. Seed shape: (0) rounded in transection; (1) flattened in transection; (2) cup-shaped in transection; (3) polyhedral. Several genera show polymorphism in this character.
 22. Seeds: (0) without hair-tuft; (1) with a tuft of hairs. The seeds of *Plocosperma* have a hair-tuft similar to the ones occurring in Apocynaceae (Leeuwenberg, 1967; Rosatti, 1989a,b).
 23. Seeds: (0) wingless; (1) one wing present, partly or completely circumferential; (2) multiple wings or ridges present. Several genera are variable (Leeuwenberg, 1961c, 1979; Wood and Weaver, 1982).

EMBRYOLOGY

Principal sources were: Moore, 1948; Maheswari Devi, 1962; Davis, 1966; Vijayaraghavan and Padmanabhan, 1969; Bendre, 1975; Corner, 1976; Maheswari Devi and Lakshminarayana, 1977; Hakki, 1980; Cronquist, 1981; Kamelina, 1980; Maldonado de Magnano, 1986a,b; Maldonado, 1989; Johri et al., 1992.

24. Ovules: (0) tenuinucellate; (1) crassinucellate. Crassinucellate ovules are only present in *Desfontainia* and *Viburnum*, but several genera in the Gentianales have not been investigated yet. The crassinucellate condition is formed by a primary parietal cell that is cut off from the archesporial cell, and the parietal cell gives rise to several parietal layers (Davis, 1966; Maldonado de Magnano, 1986a).

25. Endosperm formation: (0) cellular; (1) nuclear. All investigated genera in the order Gentianales have nuclear endosperm, with the following exceptions: *Mitrasacme* and a few saprophytic genera in the Gentianaceae. In *Mitrasacme* the endosperm formation follows an intermediate development (Yamazaki, 1963), but Johri et al., (1992) considered this a variant of the cellular type and questioned the previous results.
26. Pollen: (0) colpate, endoporus without two lateral extensions; (1) colpate, endoporus with two lateral extensions; (2) porate, endoporus without two lateral extensions; (3) inaperturate (Nilsson, 1967; Punt, 1978, 1980; Punt and Leenhouts, 1967; Punt and Nienhuis, 1976; Hoc and Bravo, 1984). The lateral extensions on both sides of the endoporus have been noted in some genera in Gentianaceae, Gelsemiaceae, *Bonyunia*, *Logania*, *Plumeria*, *Mitreola* and Rubiaceae (Punt, 1980; S. Nilsson, pers. comm.). In some species of *Viburnum* a bridge occurs over the colpus (Donoghue, 1983), and the lateral extensions of the Gentianales could be a reduction of this bridge or perhaps a non-homologous structure. In this analysis *Viburnum* is coded as having no extensions.

ANATOMY

Principal sources were: Solereder, 1899; Mennega, 1980; Cronquist, 1981; Metcalfe and Chalk, 1983; Ogata, 1988.

27. Colleters: (0) absent; (1) present. Colleters are glandular trichomes that occur inside or at the stipules, inside leaf bases, or inside calyx lobes in the Gentianales (Metcalf and Chalk, 1953; Vijayaraghavan and Padmanabhan, 1969; Robbrecht 1988; Kunze, 1990; Thomas and Dave, 1991). They are often present only in young shoots; in older shoots they die and fall off, and are easily overlooked in herbarium material. Therefore, even if colleters have not always been reported from a taxon, at least one note of presence of colleters has been regarded as significant.
28. Extrafloral epidermal nectaries: (0) absent; (1) present at the petiole as a nectariferous area; (2) present between the lamina and petiole as distinctive glands. The genera *Anthocleista* and *Fagraea* have extrafloral nectariferous areas on both sides of the petiole (B. Pettersson, pers. comm.; Leenhouts, 1963). This character is interpreted as non-homologous to the sometimes stalked nectar glands that often are present on the petioles of *Viburnum*, which are suggested to be reduced leaf lobes with glands (Weberling, 1957).
29. Secretory elements: (0) absent; (1) present. Secretory cells and cavities, mostly with unidentified contents, have been found in many Rubiaceae (Metcalf and Chalk, 1950, 1983; Robbrecht, 1988). Elongated mucilage sacs external to phloem elements are found in the petioles of *Cinchona*, and in *Coffea* and *Pentas*, secretory cells (with unidentified contents) are similarly associated with phloem elements of petioles (Metcalf and Chalk, 1950).
30. Non-articulated laticifers: (0) absent; (1) present. Laticifers of this type, branched or unbranched, are characteristic for the Apocynaceae.
31. Rubber in the fruits: (0) absent; (1) present. Latex canals in the fruits are present in *Fagraea* (Leenhouts, 1963) and *Anthocleista amplexicaulis* Bak. (L. Struwe, pers. obs.).

32. Vestured pits: (0) absent; (1) present. Vestured pits in the wood are present in all investigated taxa in the Gentianales with the exception of *Gelsemium* and *Logania* (Bailey, 1933; Mennega, 1980; Metcalfe and Chalk, 1983). They have not been reported from the Gentianaceae (but are present in *Anthocleista*, *Fagraea*, and *Potalia*), but this may be an artifact caused by the herbaceous habit in most genera and the very few investigations that have been made (Carlquist, 1984, 1992). Vestured pits are also reported from other families in the Asteridae, e.g., Oleaceae and Boraginaceae (Miller, 1977).
33. Spiral thickening of vessels: (0) present; (1) absent. *Viburnum* is variable.
34. Septate fibers in wood: (0) present; (1) absent.
35. Serial thickness of rays: (0) multiseriate and uniseriate, heterocellular; (1) only uniseriate, with square cells; (2) only uniseriate, with procumbent cells. These character states are described by Mennega (1980: 115–117).
36. Continuity of rays: (0) continuous; (1) discontinuous (with cavities from radial extensions of included phloem). This character is described by Mennega (1980: 116).
37. Internal phloem: (0) absent; (1) present. This feature is also called intraxylary phloem and all investigated members of the Gentianales except for the Rubiaceae have this feature (Metcalfe and Chalk, 1983). Hence, the representative genera of the Gentianaceae and Apocynaceae are coded as “present” and the genera from Rubiaceae as “absent” in this analysis, although they have not been investigated.
38. Included phloem: (0) absent; (1) present. Included phloem (also named interxylary phloem) of the foraminate type is present in *Strychnos*, *Logania* (some spp.), *Antonia*, *Bonyunia*, *Norrisia*, and some species of *Asclepias*. In the roots of *Gentiana*, *Centaurium*, and several genera of Solanaceae included phloem is present but the type is unknown (Metcalfe and Chalk, 1983).

CHEMISTRY

Principal sources were: Hegnauer, 1964, 1989; Bisset, 1980; Cronquist, 1981; Jensen, 1992.

39. Seco-iridoids and complex indole alkaloids: (0) absent; (1) seco-iridoids present, but complex indole alkaloids absent; (2) seco-iridoids present together with the derivate: complex indole alkaloids of the corynanthean-type, formed from a C-17 carbon ring only; (3) seco-iridoids present, together with the derivate: complex indole alkaloids of the corynanthean-type, formed from C-16 or C-17 carbon ring, and derivatives of the strychnan-type and aspidospermatan-type. (Jensen et al., 1975; Norn, 1978; Kisakürek and Hesse, 1980; Kisakürek et al., 1983; Jensen, 1992). This character is ordered according to the biosynthetic pathway presented by Kisakürek and Hesse (1980).
40. Carbocyclic iridoids: (0) absent; (1) present. These iridoid compounds have been known to occur mainly in the Scrophulariales and Lamiales. Their biosynthesis is different from the seco-iridoids (Jensen et al., 1975; Jensen, 1992).
41. Isoquinoline alkaloids, emetine-type: (0) absent; (1) present. These alkaloids are present in *Cinchona* and in the subfamilies represented by *Pentas* and

Coffea in the Rubiaceae, and are not reported from other taxa (Kooiman, 1969; Kisakürek et al., 1983).

42. Cornoside: (0) present; (1) absent. The absence or presence of cornoside has recently been used for discussions of systematic positions of genera by Jensen (1992). The compound is found in several taxa in the Scrophulariales, but not in the Gentianales.
43. Sweroside, swertiamarin and/or gentiopicroside: (0) absent; (1) present. These compounds are specific seco-iridoids that occur in the Gentianaceae only (Jensen et al., 1975; Hegnauer and Kooiman, 1978; Bisset, 1980; Jensen, 1992).
44. Verbascosides: (0) absent; (1) present. The presence of verbascosides in *Plocosperma* and *Polypremum* as well as in the Scrophulariaceae and Oleaceae was noted by Jensen (1992).
45. Xanthones: (0) absent; (1) present. Xanthones, secondary metabolites related to flavonoids, are only found in a few angiosperm families, e.g., Gentianaceae, Guttiferae, Moraceae and Polygalaceae (Carpenter et al., 1969; Rezende and Gottlieb, 1973; Okorie, 1976; van der Sluis, 1985a,b; Bisset, 1980). The only exception is the compound mangiferin which occurs widely among the angiosperms and has been proposed not to be biogenetically related to the other xanthones (Hostettmann and Hostettmann, 1989). Therefore, the presence of mangiferin has not been taken into account in the coding of this character.
46. Cardenolids: (0) absent; (1) present. This is a group of cardiotonic glycosides occurring in the Apocynaceae (Hegnauer, 1964).

AUTAPOMORPHIES

47. Plicate appendages between the corolla lobes: (0) absent; (1) present. On the corollas of the genus *Gentiana* plicae or folds are present between the lobes (Wood and Weaver, 1982).
48. Spirally twisted anthers: (0) absent; (1) present. The anthers of *Centaureum* are spirally twisted after anthesis (Wood and Weaver, 1982).
49. Heart-shaped capsules: (0) absent; (1) present. The capsules of *Mostuea* have a truncate to bilobed characteristic shape (Leeuwenberg and Leenhouts, 1980).
50. Raphides: (0) absent; (1) present. Raphides are needle-like crystals of calcium oxalate that are characteristic for some Rubiaceae, e.g. *Pentas* (Robbrecht, 1988).

Appendix 2. Classification of the Gentianales (Rubiales)

(by Lena Struwe and Victor A. Albert)

According to the results presented in this paper the following classification of the order is proposed (references to authors and protologs follow Reveal [1993b]):

1. Rubiaceae Juss., Gen. Pl.: 196 (1789), *nom. cons.*
2. Gelsemiaceae (G. Don) L. Struwe & V. A. Albert, stat. nov.
Basionym: Gelsemieae G. Don, Gen. Hist. 4: 70, 87 (1837).
Type: *Gelsemium* Juss.
Included genera: *Gelsemium* Juss., *Mostuea* Didr.
3. Gentianaceae Juss., Gen. Pl.: 141 (1789, as Gentianae), *nom. cons.* Including Potaliaceae Mart., Nov. Gen. Sp. Pl. 2: 89, 133 (127, as Potalieae): *Potalia* Aubl., *Fagraea* Thunb., *Anthocleista* Afzel. ex R. Br.
4. Apocynaceae Juss., Gen. Pl.: 143 (1789, as Apocineae), *nom. cons.* Including Asclepiadaceae R. Br., Asclepiadeae: 19 (1810, as Asclepiadeae), *nom. cons.*
5. Loganiaceae R. Br. ex Mart., Nov. Gen. Sp. Pl. 2: 133 (1827, as Loganieae), *nom. cons.*
Included genera: *Logania* R. Br., *Mitreola* L., *Mitrasacme* Labill.
6. Strychnaceae DC. ex Perleb, Lehrb. Naturgesch. Pflanzenr.: 220 (126, as Strychneae). Included genera: *Strychnos* L., *Neuburgia* Blume, *Gardneria* Wall., *Spigelia* L., *Antonia* Pohl, *Bonyunia* Schomb. ex Progel, *Norrisia* Gardner, *Usteria* Willd.
7. Geniostomaceae L. Struwe & V. A. Albert, fam. nov.
Apocynacearum Juss. affinium, a quibus latice carente differunt. Circumscriptio eadem est ac Loganiacearum Mart. tribus Loganiarum R. Br. sensu Leeuwenberg et Leenhouts, sed *Logania* R. Br., differente placentis siccis, exclusa.
Small trees or shrubs. Leaves opposite, simple and entire; bases connected by interpetolar stipules or a short ochrea. Flowers solitary or in cymes, either axillary, cauline, or terminal, unisexual or gynodioecious, pentamerous. Sepals free or connate, with colleters at the adaxial bases. Corolla rotate, campanulate or salver-shaped, usually hairy at the mouth, sometimes glabrous; lobes acute. Stamens inserted at the corolla mouth, partly or totally exerted; anthers acute and often with distinct apical appendages formed by extended connective, with longitudinal slits, introrse. Ovary superior, bilocular (rarely trilocular), placentas axile; stigmas usually united into a globose or clavate head. Capsule ellipsoid to subglobose, with thick and woody exocarp, dehiscent from the apex, base or both, with 2 (or 3) valves breaking away from and exposing the persistent and pulpy placentas. Seeds many, embedded in the pulp.
Type: *Geniostoma* J. R. Forst. & G. Forst.
Included genera: *Geniostoma* J. R. Forst. & G. Forst., *Labordia* Gaudich.