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Phylogeny of Temperate Gentianaceae: a Morphological Approach

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ABSTRACT. The intrafamilial relationships of the Gentianaceae are investigated by means of a cladistic analysis based on morphological and to a lesser extent on chemical data. The 21 genera that are selected for the analysis represent all tribes and subtribes except Leiphaimeae, Rusbyantheae and Voyrieae. The large genus *Gentiana* is represented by three of its sections. The former loganiaceous genera *Anthocleista* and *Fagraea* are used as outgroups. Standard parsimony analyses and analyses using weights that are based on the cladistic reliability of the characters give congruent results as far as the global relationships are concerned. The best supported clade contains *Eustoma* (Tachiinae) and all included Gentianinae, Erythraeinae and Chironiinae. The basal division in this clade is between *Ixanthus* and the other genera. In this way *Ixanthus*, an endemic of the Canary Islands, connects the mostly woody tropical and the mostly herbaceous temperate taxa. Subtribe Gentianinae (excluding *Ixanthus*) is monophyletic, unlike Erythraeinae and Chironiinae. In most analyses, however, both subtribes together (and including *Eustoma*) are the sister-group of Gentianinae. Possibly Erythraeinae, Chironiinae and *Eustoma* should be merged. The basal parts of the cladograms, involving the woody tropical representatives and *Exacum*, are poorly resolved. More extensive sampling, especially among the tropical representatives, is necessary to elucidate these basal relationships. The tropical ancestry of the family, the switch from a woody to a herbaceous life form, and the position of critical taxa, such as *Swertia* and *Halenia* or *Tripterospermum*, are discussed.

The Gentianaceae is a cosmopolitan family of medium size, with 76 genera (Brummitt 1992) and about 1200 species (Mabberley 1990; see Table 1). Its oldest known fossils are from the Eocene of North and Central America (Crepet and Daghlian 1981; Graham 1984). Recent cladistic analyses based on *rbcL* sequence data (Olmstead et al. 1993; Bremer et al. 1994), restriction site variation of the chloroplast genome (Downie and Palmer 1992) and morphological, anatomical, embryological and chemical data (Struwe et al. 1994) indicate that Gentianaceae are one of the principal families of the monophyletic order Gentianales. Results in Bremer et al. (1994) and Struwe et al. (1994) are consistent with the hypothesis (e.g. Downie and Palmer 1992; Bremer and Struwe 1992) that Loganiaceae sensu Leeuwenberg and Leenhouts (1980) are a paraphyletic assemblage with members showing closest relationships to other families both within and outside of the Gentianales. As far as Gentianaceae is concerned, Struwe et al.'s (1994) main conclusion is to formally include *Potalia* Aubl., *Fagraea* Thunb. and *Anthocleista* Afzel. ex R. Br. (tribe Potalieae of Loganiaceae sensu Leeuwenberg and Leenhouts 1980) in the Gentianaceae. This transfer had already been proposed by Bureau (1856) in the previous century and more recently by Fosberg and Sachet (1980) on the basis of gross morphology (although monographers of the Loganiaceae disagreed, e.g. Leeuwenberg and Leenhouts 1980) and by Jensen (1992) on the basis of the presence of advanced iridoid glucosides. It should be noted that the inclusion of *Anthocleista* and *Fagraea* increases the woody paleotropic representation of the family, that is otherwise restricted to *Gentianothamnus* Humbert (Humbert 1937).

While a consensus seems to be emerging about the monophyly of the Gentianales and the inclusion of Potalieae in Gentianaceae, much work remains to be done concerning the interfamilial relationships within the order, including the relationships of the smaller families often included in Gentianales (e.g. Saccifoliaceae, Dialypetalanthaceae) and concerning the infrafamilial relationships of the bigger Gentianales families. We focus on the Gentianaceae.

Because the broad-based cladistic analyses (e.g. Downie and Palmer 1992; Olmstead et al. 1993; Bremer et al. 1994; Struwe et al. 1994) to date

TABLE 1. Principal genera of Gentianaceae listed according to classification of Gilg (1895). Over 75% of total number of species in Gentianaceae belongs to listed genera (about ¼ of total number of genera recognized by Brummitt 1992). Unless indicated otherwise, the numbers of species (second column) and the distributional data (third column) are from Mabberley (1990; his species estimates are low; e.g. in the *Exacum* monograph of Klackenberg (1985) 65 species are recognized). Genera marked with "*" are included in this study (see text for further details and additional included genera).

Gentianineae		
Exacinae		
Exacum L.*	c. 25	paleotropics
Sebaea Sol. ex R. Br.	60	Africa to India, Australia, New Zealand
Erythraeinae		
Canscora Lam.*	30	paleotropics
Centaurium Hill*	30	northern hemisphere, one extending to Australia, one to Chile
Faroa Welw.	17	tropical Africa
Sabatia Adans.	17	northern America, West Indies
Chironiinae		
Chironia L.*	c. 15	subSaharan Africa, Madagascar
Gentianinae		
Frasera Walter*	15	northern America
Gentiana L.*	c. 300	temperate and arctic, usually montane elsewhere but absent from Africa
Gentianella Moench*	125	temperate, excluding Africa
Gentianopsis Ma*	16–25	northern temperate Asia and America
Halenia Borkh.*	c. 70	Eurasian mountains, America
Lomatogonium A. Braun	18	temperate Eurasia
Swertia L.*	50	northern temperate, African mountains
Tripterospermum Blume*	25	from Japan and South Korea to the Himalayas, Sri Lanka and Indonesia (excl. Borneo) (Murata 1989)
Tachiinae		
Lisianthius P. Brown*	27	tropical America
Macrocarpaea Gilg*	30	tropical America
Helieae		
Schultesia Mart.	20	tropical Africa and America
Symbolanthus G. Don*	15	tropical America
Voyrieae		
Voyria Aubl.	30	tropical America, western Africa

included few representatives of the Gentianaceae, they are not very informative with respect to its problematic and unclear infrafamilial relationships. The three main monographs (Grisebach 1845; Bentham 1876; Gilg 1895) that deal with the systematics of the Gentianaceae all date from the previous century. More recent classifications of the family exist, but these are based on taxa occurring in local floras, and not on a worldwide survey (e.g. Garg 1987; Zuyev 1990). Grisebach (1845) and Bentham (1876) used mainly characters of anthers, styles, stigmas and ovaries, while Gilg (1895) based his classification almost exclusively on pollen features. He distinguished two subfamilies: Gentianoideae and Menyanthoideae; within the subfamily Gentianoideae he recognized five tribes: Gentianeae (with subtribes Exacinae, Erythraeinae, Chironiinae, Gentianinae and Tachiinae), Rusbyantheae, Helieae, Voyrieae and Leiphaimeae. Gilg's classification has been much criticized, major issues being the position of Menyanthoideae and the status of the neotropical (sub)tribes.

The Menyanthoideae proved to be very different from Gentianoideae, and on the basis of anatomical, embryological and phytochemical evidence it was raised to family level by Wagenitz (1964). Gross floral and vegetative morphology point to a close affinity with either Solanales or Gentianales (cf. Cronquist 1981), but both *rbcL* sequence data (Chase et al. 1993, Olmstead et al. 1992, 1993) and restriction site variation of the chloroplast genome (Downie and Palmer 1992) associate the family with Campanulales/Asterales.

Gilg's mainly or exclusively neotropical (sub)tribes Rusbyantheae, Helieae, Voyrieae, Leiphaimeae and Tachiinae have often been criticized for being artificial or redundant groups. Maas (1983a) noted that the neotropical genus *Lisianthius* P. Browne and a number of related neotropical shrubby genera (the "lisanthoid gentians," Sytsma 1988) are scattered over Helieae, Tachiinae and Rusbyantheae, resulting in a very unnatural grouping of genera. It is now agreed (Weaver 1974; Maas 1983b) that *Rusbyanthus cinchonifolius* Gilg, the only species in Rusbyantheae, is to be included in *Macrocarpaea* Gilg (Tachiinae). *Voyriella* Miq. (Leiphameae) is considered to be related to the genera *Curtia* Cham. & Schltdl. and *Tapeinostemon* Benth. (Erythraeinae), and *Leiphamos* Cham. & Schltdl. (the second genus of Gilg's Leiphameae) is included in *Voyria* Aubl. (Weaver 1974; Maas and Ruyters 1986). In this way, the tribes Rusbyantheae and Leiphameae are redundant (Weaver 1974). Wood and Weaver (1982) proposed merging the tribe Helieae and the subtribe Tachiinae, and Fosberg and Sachet (1980) suggested lumping Tachiinae and Potalieae. Gilg's (1895) subtribes Exacinae, Erythraeinae, Chironiinae and Gentianinae have been less criticized. The criticisms are mainly restricted to transfers from the neotropical subtribes; e.g. *Hockinia* Gardner (Tachiinae) to Erythraeinae (Maas and Ruyters 1986), *Tachiadenus* Griseb. (Tachiinae) to Exacinae (Klackenberg 1987), and *Eustoma* Salisb. (Tachiinae) and *Coutoubea* Aubl. (Helieae) to Erythraeinae (Kaouadji 1990).

Based on morphological, cytological or chemical data, phylogenetic hypotheses or evolutionary trees (without any cladistic methodology) have been published for *Gentiana* L. and *Gentianella* Moench (Scharfetter 1953), *Blackstonia* Huds. and *Centaurium* Hill (Zeltner 1970) and for the subtribe Gentianinae (Toyokuni 1963, 1965; Massias et al. 1982). Cladistic analyses exist for *Exacum* L. (Klackenberg 1985), *Tachiadenus* (Klackenberg 1987), *Lomatogonium* A. Braun (Liu and Ho 1992), and part of *Lisianthius* (Sytsma and Schaal 1985). In order to study xanthone evolution in Gentianaceae, Mészáros (1994) performed a cladistic analysis of a group of 12 genera of Gentianinae, Erythraeinae and Tachiinae.

In this study we extend Mészáros's (1994) data set both in number of characters and number of taxa, and we present a more complete cladistic analysis of the family. We use mainly morphological and anatomical characters and to a lesser extent chemical data. Because of limited availability of the xanthone data, especially for tropical taxa, we focus on temperate representatives of the family.

MATERIALS AND METHODS

Taxa. A total of 21 genera of Gentianaceae (including Potalieae) were selected for our analysis. In addition to the principal genera indicated in Table 1, we included the former loganiaceous genera *Anthocleista* and *Fagraea*, and the following smaller genera (numbers of species and distributional data from Mabberley 1990): *Blackstonia* (Erythraeinae; 5–6; Europe), *Chorisepalum* Gleason & Wodehouse (Tachiinae; 5; Guyana highlands), *Eustoma* (Tachiinae; 3; southern North America to northern South America), *Hoppea* Willd. (Erythraeinae; 2; India), *Ixanthus* Griseb. (Gentianinae; 1; Canary Islands), and *Orphium* E. Mey. (Chironiinae; 1; Southern Africa). Excluding the redundant tribes Rusbyantheae and Leiphameae, the chosen genera represent all of Gilg's tribes and subtribes except the monogeneric Voyrieae. In order to reduce problems with polymorphisms (Nixon and Davis 1991), we have split up the diverse genus *Gentiana* and included three European sections (following Pringle 1978) for which xanthone compounds are well documented: *Gentiana* sect. *Calathianae, Gentiana* sect. *Ciminalis* and *Gentiana* sect. *Gentiana.*

The former loganiaceous genera *Anthocleista* and *Fagraea* were included as outgroups (Nixon and Carpenter 1993). Shared synapomorphies with Gentianaceae s. str. (i.e. Gentianaceae excluding Potalieae) are the presence of bilobed placentas, the presence of xanthones, and the presence of swertiamarin and other unique seco-iridoids (Struwe et al. 1994). With respect to Potalieae, Gentianaceae sensu stricto (the ingroup) is defined by the absence of stipules and the presence of capsular fruits (characters 30 and 31). However, the assumption that Gentianaceae s. str. is monophyletic is contradicted in most broad-based cladistic analyses of molecular and morphological data (e.g. Downie and Palmer 1992, including *Fagraea, Exacum, Gentiana, Lisianthius* and *Obolaria* L.; Olmstead et al. 1993, including *Anthocleista, Fagraea, Exacum,* and *Gentiana;* Struwe et al. 1994, including *Anthocleista, Fagraea, Potalia, Centaurium, Gentiana* and *Tachia* Aublet). In these analyses, however, few representatives of Gentianaceae were included, and therefore they may not be very reliable as far as intrafamilial structure of Gentianaceae is concerned: coarse sampling within a clade may lead to a wrong connection of the clade to the rest of the tree (see e.g. Olmstead et al. 1993: in an analysis of Asteridae with few Gentianales included, they obtained a branching sequence within the order that was almost exactly the reverse of what they found when more Gentianales were included; see also Olmstead et al. 1992: 261–263; Struwe et al. 1994: 188–189). Nevertheless, it would be too easy to dismiss these results a priori as artifacts of taxon sampling, and we are currently extending our data matrix with additional genera of Loganiaceae and other families of Gentianales to address the question of monophyly of Gentianaceae s. str. In the present analysis, we will arbitrarily depict all cladograms as rooted between *Anthocleista* and the other genera and we will shortly discuss the effect of alternative root positions on our results.

Characters. Thirty-two morphological and anatomical and eight chemical characters (Tables 2 and 3) were used in the cladistic analysis. Data for morphological character states were compiled mainly from literature, in some cases supplemented with herbarium studies (BP and BR). Literature data were collected either from monographs (mainly Gilg 1985; Kusnezow 1896–1904; Allen 1933; Ewan 1948; Marais and Verdoorn 1963; Weaver 1972; Leeuwenberg 1980; Maguire 1981; Wood and Weaver 1982; Garg 1987; Murata 1989) or from papers dealing with specific characters (mainly Perrot 1898; Hasselberg 1937; Lindsey 1940; Metcalfe and Chalk 1950; Krishna and Puri 1962; Patel et al. 1981; Nishino 1983; Carlquist 1984; Neubauer 1984).

The xanthone data are from the same sources as in Mészáros (1994), to which new information on *Hoppea*, *Chironia* L. and *Orphium* was added (Stout et al. 1969; Rezende and Gottlieb 1973; Chapelle 1974; Okorie 1976; Carbonnier et al. 1977; Gottlieb 1982; Hostettmann and Wagner 1977; Massias et al. 1977; Sullivan et al. 1977; Ghosal et al. 1978; Hostettmann-Kaldas and Jacot-Guillarmod 1978; Luong et al. 1980; Dreyer and Bourell 1981; Hostettmann-Kaldas et al. 1981; Lin et al. 1982a, 1982b; Massias et al. 1982; Sluis 1985; Lin et al. 1987; Ortega et al. 1988; Khetwal et al. 1990; Bennett and Lee 1991; Wolfender et al. 1991; Wolfender and Hostettmann 1992; Roitman et al. 1992). The majority of the flavonoid data are from Kaouadji (1990) and Hegnauer (1989) (but see also the sources of the xanthone data). The sugar data are from Massias et al. (1978).

Most of the palynological, embryological and cytological data that we reviewed were excluded from the matrix because of insufficient coverage. Still, 16% of the data matrix cells are scored as missing or inapplicable.

Coding of the Xanthone Data. Xanthones are yellow-coloured dibenzo-γ-pyron compounds that arise biosynthetically from a benzephenone precursor that is derived from acetate (leading to ring A) and shikimate (ring B). With the exception of the widespread compound mangiferin they occur only in a limited number of tracheophyte families; in the angiosperms, they are found mainly in Guttiferae and Gentianaceae (Gottlieb 1982; Frohne and Jensen 1992). Discussions of xanthone evolution center around the degree of oxygenation of the aromatic rings, and are often based on an a priori designation of the primitive type of oxygenation pattern from which the other observed patterns are deduced. Based on the biosynthetic pathway of xanthones, Rezende and Gottlieb (1973) and Gottlieb (1982) suggested that 1,3-dioxygenation of ring A and 5,6- or 6,7-dioxygenation of ring B is the primitive oxygenation pattern in all families that have xanthones. Gottlieb (1982) derived the other observed oxygenation patterns from this type on the basis of a common-is-primitive argument. Carbonnier et al. (1977) and Massias et al. (1982), working on Gentianinae, assumed that trioxygenation is primitive and higher degrees of oxygenation are increasingly derived. Mészáros (1994) was the first to apply cladistic reasoning to this problem, but his analysis was constrained by assuming Camin-Sokal parsimony, which does not allow reversals.

In his study of xanthone evolution in Gentianaceae, Mészáros (1994) did not directly code the oxygenation patterns (absence/presence of oxygenation at the different C-positions), but he used four characters that are derived from patterns as they are observed: minimal grade of substitution, diversity of substitution, specialization of ring A, and specialization of ring B. However, cladistic characters should represent primary hypotheses of homology (de Pinna 1991) and therefore should reflect certain correspondences of parts. This is problematic for these derived characters. For instance, in the character "specialization of ring A," the states are dioxygenation, trioxygenation and tetraoxygenation, and it is perfectly possible that the state "trioxygenation" refers to different sets of positions in different genera, or even in the same genus (e.g. 1–2–3 or 1–3–4, which would imply the hypothesis that an oxygenated C2 corresponds somehow to an oxygenated C4). For this reason, we choose to code the xanthone data as absence/presence characters describing whether or not each of the different C-positions is oxygenated. Genera in which a

TABLE 2. Characters and character states.

0. Life form: trees or shrubs (0) herbs (1)
1. Xylem rays: multi- and uniseriate (0) only uni-(bi-)seriate (1) rayless (2)
2. Nodal anatomy: unilacunar (in *Swertia* sometimes also trilacunar) (0) multilacunar (1)
3. Leaves: petiolate (0) sessile (1) perfoliate (2)
4. Morphological type of stomata: anomocytic (0) paracytic (1)
5. Leaf venation: penninerved (0) parallel veined (1)
6. Calcium oxalate crystals in mesophyll: absent (0) present (1)
7. Calyx symmetry: actinomorphic (0) zygomorphic (1)
8. Inflorescence: dichasium (0) monochasium (1) flowers in clusters (2) solitary flowers (3)
9. Fusion of sepals: scarcely (0) half (1) almost completely (2)
10. Intracalycine membrane: absent (0) present (1)
11. Calyx lateral traces: free (0) fused at origin (1) fused throughout (2)
12. Corolla mery: polymerous (0) pentamerous (1) tetramerous (2)
13. Petal fusion: scarcely (0) half (1) almost completely (2)
14. Corolla aestivation: contorted (0) plicate (1)
15. Pollen: in tetrads (0) in monads (1)
16. Nectaries: none (0) epipetalous (1) gynoecial (2)
17. Anther fixation: basifixed (0) versatile (1)
18. Anther twisting: none (0) moderately (1) largely (2)
19. Anther abortion: none (0) 1–3 aborted stamens (1) only 1 fertile stamen (2)
20. Anther cohesion: free (0) connate (1)
21. Ovary: 4-locular (0) 2-locular (1) unilocular (2)
22. Ovary shape: globular (0) oval (1) long (2)
23. Ovary: sessile (0) stipitate (1)
24. Placentation: axial (0) parietal (1) superficial (2)
25. Carpel ventral traces: free (0) fused at origin (1) fused throughout (2)
26. Seed shape: angular (cubical) (0) globular (1) oval (2) long (3)
27. Seed wing: absent (0) present (1)
28. Flavonoids: flavonol (O-glycosides) (0) flavones (C- or O-glycosides) (1)
29. Sugars: simple (glucose, primverose, rhamnose, galactose) (0) compound (gentianose, gentiobiose) (1)
30. Stipules: absent (0) present (1)
31. Fruit: capsular (0) baccate (1)
32. Seed testa surface: smooth (0) with reticulum of thickened radial cell walls (1)
33. Seed testa-cell shape: isodiametric (in *Exacum* sometimes also star-shaped) (0) elongated (1)
34. Oxygenation of xanthone position C2: absent (0) present (1)
35. Oxygenation of xanthone position C4: absent (0) present (1)
36. Oxygenation of xanthone position C5: absent (0) present (1)
37. Oxygenation of xanthone position C6: absent (0) present (1)
38. Oxygenation of xanthone position C7: absent (0) present (1)
39. Oxygenation of xanthone position C8: absent (0) present (1)

certain position is oxygenated in some species or in some xanthones but not in others are coded as polymorphic for that position. We did not distinguish between the different substituents (hydroxyl, methoxyl, O-glycosyl) that may occur on the oxygenated C-positions because this variation seems to be subsidiary to the oxygenation pattern (cf. Hostettmann and Wagner 1977 for *Gentiana* and Wolfender and Hostettmann 1992 for *Chironia*). Positions C1 and C3 of ring A are oxygenated throughout, leaving positions C2 and C4 from ring A and positions C5-C8 of ring B as informative xanthone characters.

Methods. Standard parsimony analyses with a priori equal weighting of all characters were carried out using NONA (Goloboff 1993b). We also performed analyses using implied weighting (Goloboff 1993a), a method that is based on the concept of cladistic reliability of characters (Farris 1969; cf. Carpenter 1988, 1994). In this approach, characters are non-iteratively weighted during tree search by means of a concave function of their homoplasy. It should be noted that implied character weights are different from the weights that can be assigned to the characters prior to the analysis (these a priori weights were mostly kept equal; see below). We refer to Goloboff (1993a) for further theoretical background. Following Goloboff (1993a), we will call the resulting cladograms the "fittest" cladograms, as opposed to the equally most parsimoni-

TABLE 3. Data matrix. Numbers of characters and character states refer to Table 2. "–" indicates polymorphisms in binary characters; polymorphisms in multistate characters are indicated between square brackets; "?" indicates missing values and inapplicable characters.

	0123	45678	9	111 012	111111122 345678901	2 2	2222 3456	2223333 7890123	333333 456789
Anthocleista	0110	00?00	0	0?0	201??0000	1	00?[02]	0??1110	000—1
Fagraea	0110	?0?00	[12]	0?1	201??000[12]	1	0–?0	0??11??	??????
Symbolanthus	00?0	?0?0[13]	0	0?1	2002?0002	1	01?0	0??00??	??????
Chorisepalum	0??0	?0?0[03]	0	0?0	201200001	2	00?0	0??00??	??????
Lisianthius	–??0	?0100	[01]	020	201210001	2	01?0	0?000??	??????
Chironia	–??1	?1?01	0	001	001002002	0	0101	0?00–10	001111
Orphium	0?01	?1?01	0	0?1	001?01002	1	0101	0??0010	000011
Macrocarpaea	0??0	?0?00	0	001	20–200001	1	00?0	0??00??	100111
Eustoma	1??1	?1101	0	001	001211002	1	0101	0000010	001111
Canscora	1??1	01100	2	002	1010?0102	1	0101	010001?	001111
Hoppea	1??1	01?00	1	012	201??0202	0	0121	0100010	001110
Centaurium	1201	01100	0	001	101002002	2	0101	0000010	–0——
Blackstonia	12?2	?1101	0	0?0	001?01002	1	01??	000001?	000011
Ixanthus	11?2	?1?00	1	0?1	101200002	1	01?[12]	0?0001?	100101
Swertia	1?00	01000	0	001	001110002	[01]	0102	—100–0	——0—
Halenia	1??0	?1001	0	002	001110002	[12]	0102	0?1000?	1110–0
Gentianella	1??1	11000	1	001	101110002	2	1221	010000?	0–10–1
Gentianopsis	1??1	11011	1	122	101110002	2	1213	010001?	000011
Tripterospermum	1??0	?1?01	1	0?1	211200002	2	1201	1?00–??	0–0–1–
GentGentiana	1210	?1112	2	101	211200012	1	1202	1110011	–00010
GentCiminalis	1201	11103	1	101	211200012	2	1203	0110011	000011
GentCalathianae	1201	11003	2	101	111200002	2	1203	0110011	000011
Exacum	–2?[01]	–1?00	[01]	02[12]	001?00001	0	0010	01?0010	??????

ous cladograms of the standard approach. Searches for fittest cladograms were carried out with the computer program Pee-Wee (Goloboff 1993c). In Pee-Wee, the degree of concavity of the weighting function is determined by the concavity constant K (Goloboff 1993c). Beyond the fact that the weighting function should be concave (Farris 1969), it is far from obvious how it should look exactly. For this reason we tried several values of K and compared the results. We varied K between its minimum (1; highest concavity, i.e. strongest differential downweighting of homoplasy) and its maximum (6; lowest concavity, i.e. lowest differential downweighting of homoplasy; this comes closest to the standard approach). In order to avoid confusion we note that the concavity constants K (Goloboff 1993c) and k (Goloboff 1993a) are not equal (K = k+1).

In most analyses we treated all multistate characters as unordered (cf. Hauser 1992). However, in our data set all multistate characters except 8, 16, and 24 (see Table 2) represent fairly straightforward morphoclines and hence can be ordered very well using the similarity criterion (cf. Lipscomb 1992; all morphoclines are linear and the numerical codes of the states of these characters in Table 2 follow the order of the morphoclines). Treating these characters as unordered would imply that some of the observed primary homologies (de Pinna 1991) are dismissed a priori. For this reason we also ran analyses in which these characters were ordered. Carpenter (1988) showed that the way in which ordered multistate characters are coded (additive binary or ordinal) can influence the final stable solution under successive weighting (Farris 1969): using ordinal coding (Mickevich and Weller 1990) distorts the picture because it yields higher weighting of these characters simply because they are coded that way. Although Goloboff (1993a) did not mention it, the situation is similar when using implied weights. To avoid this distortion, we derived a second data set from Table 3 to perform the ordered analyses. In this data set, we coded the linearly ordered multistate characters in a binary additive way. The polymorphisms in the ordered characters involved mostly adjacent states. Only for *Anthocleista* it was necessary to expand an observed subset of states (character 26) to a range of states.

In all analyses, we used subset coding for polymorphisms. Polymorphisms in terminal taxa may indicate that the terminal taxa are nonmonophyletic, a possibility that may not be overlooked when using large and traditionally defined genera. The best way to avoid unwarranted assumptions of monophyly is to split up polymorphic taxa into monomorphic subunits (Nixon and Davis 1991), an approach we informally followed when splitting up *Gentiana* and including three of its sections. Little is known of the effect of using subset coded polymorphic taxa. However, in their analysis of the Gentianales, Struwe et al. (1994) compared subset coding and a version of monomorphic subtaxon recoding and found highly compatible results as far as the global branching pattern is concerned.

Relatively many taxa are polymorphic for the xanthone characters. This may be an indication that the xanthone characters we have delineated do not capture the variation that is relevant for this taxonomic level: they may be more useful at lower taxonomic levels. The degree of polymorphism may actually even be higher than apparent from Table 3: in many cases, especially for the bigger genera, the xanthone scores are generalized from only a limited number of species. For these reasons it can be argued that the xanthone characters should not get the same a priori weights as the other characters. Following this line of thought, we also did some analyses in which the xanthone characters were excluded or given lower a priori weights than the other characters.

In all analyses with NONA and Pee-Wee, the most parsimonious cladograms or the fittest cladograms were obtained using the instruction MULT*25. This instruction carries out 25 replications of randomizing the taxa, creating a Wagner tree and submitting it to branch-swapping by means of tree-bisection reconnection. Apart from the setting of the a priori weights, the value of the concavity constant, and the ordering of multistate characters, all other default settings were retained in all analyses. By default, NONA collapses all branches that have no unambiguous synapomorphies (a character provides an unambiguous synapomorphy for a branch if a state transition occurs on that branch under every possible optimization of the character on the tree; Goloboff 1993c; see also Coddington and Scharff 1994). The ensemble consistency indices (CI; Kluge and Farris 1969) and ensemble retention indices (RI; Farris 1989) for the standard parsimony analyses were derived from the "minimum" tables, giving minimum and maximum possible steps for each character. Consistency indices are calculated with autapomorphies included (see Yeates 1992). The distribution of the nine autapomorphic states in the matrix is as follows: one in each of the binary characters 15, 35, and 38; one in each of the multistate characters 1, 8, 11 and 21; two in multistate character 19.

In order to evaluate the relative support of clades, we calculated branch support, i.e. the number of extra steps needed to lose a branch in the strict consensus of near-most-parsimonious trees (Bremer 1994; also called "Bremer support" or "decay index"). For similar reasons a bootstrap analysis was performed (Felsenstein 1985; but see Bremer 1994). The calculation of branch support values ("decay analysis") and the bootstrap analysis were performed with PAUP (Swofford 1993; characters unordered; initial seed = 1; heuristic search by means of simple addition and tree-bisection-reconnection branch swapping; one tree held at each step during stepwise addition). For the bootstrap analysis MAXTREES was set to 1000 and 100 replicates were run.

RESULTS

With equal a priori weighting and all characters unordered, the standard parsimony analysis resulted in eight equally most parsimonious trees (steps 111; CI=0.51; RI=0.64). The strict consensus of these is shown in Fig. 1 (some clades are given a number to ease further discussion). The trichotomy involving clades two, three, and six is present in all of the most parsimonious trees. *Fagraea* and *Symbolanthus* G. Don f. are present in the basal polytomy because *Fagraea* is the sister group of clade one in two of the eight most parsimonious trees, while it branches below the polytomy in the other cases; *Symbolanthus* is the sister group of clade 1 or of clade 1 + *Fagraea* in all trees.

In the decay analysis, PAUP found 1160 trees of length ≤ 112. The strict consensus of these is completely unresolved except for a sister-pair relation between *Swertia* L. and *Halenia* Borkh. The search for trees with a length ≤ 113 was stopped prematurely because of memory limitations when 3100 trees were found. The strict consensus of this partial result already refutes the sister-pair relation between *Swertia* and *Halenia*. This comes down to Bremer branch support 2 for *Swertia-Halenia*, while the other branches of the strict consensus of the equally most parsimonious trees (Fig. 1) have branch support 1. Low branch support values are typical for morphological data sets (Karis 1995); in this particular case the low values are at least

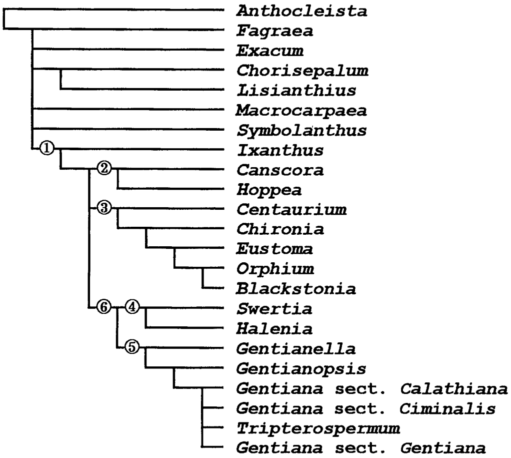


FIG. 1. Strict consensus of the eight most parsimonious trees (steps 111) for the data of Table 3, all characters unordered. *Gella* and *Gesis* are *Gentianella* and *Gentianopsis*; *GenCa, GenCi* and *GenGe* are the sections *Calathianae, Ciminalis* and *Gentiana* of the genus *Gentiana*. Numbered clades are discussed further in text. Bremer branch support value of clade four is 2; all other clades have branch support 1.

partially due to the fact that the positions of some genera, especially *Exacum*, vary greatly within a topology that is otherwise fairly constant (see Wilkinson 1994 for a general discussion of this problem). This is clear when the strict consensus is calculated with *Exacum* excluded. In this case, a clade containing all taxa of clade 1 is still present in the consensus of all trees of length ≤ 112 and in the consensus of the 3100 trees of length ≤ 113. This implies a branch support value of at least 2 for a group that is nested (Adams 1986) within the set of all terminal taxa excluding *Exacum* and that is composed of all taxa of clade one. In terms of monophyly this means that clade one has a branch support of at least 2 when the question whether *Exacum* belongs to it is left open. In order to overcome the memory limitations, we also ran an alternative analysis in which *Exacum* and *Fagraea*, two genera with strongly varying positions, were excluded from the data matrix. As expected, this resulted in fewer equally most parsimonious and near-most-parsimonious trees. In the consensus of the six equally most parsimonious trees (length 104) for this reduced matrix, clade 1 is present and has the same internal structure as in the strict consensus of the eight equally most parsimonious trees of the full analysis (Fig. 1). It survives in trees up to 106 steps long.

On a Performa 450 computer, it took almost 32 hours to complete the bootstrap analysis (MAXTREES was set to 1000 to constrain the duration; in 29 out of the 100 replicates the search for shortest trees was stopped prematurely because of tree-buffer overflow). Only six clades are present in at least 50% of all trees that were found. These are (a) the clade that contains all terminal taxa except *Anthocleista* and *Fagraea* (69%), (b) the sister pair *Chorisepalum-Lisianthius* (50%), (c) the sister pair *Swertia-Halenia* (84%), (d) the three sections of *Gentiana* (unresolved; 51%) (e) the three sections of *Gentiana* + *Tripterospermum* Blume (53%) and (f) the previous clade in trichotomy with *Gentianella* and *Gentianopsis* Ma. (51%). As in the decay analysis, the poor result of the bootstrap analysis is partially due to the varying positions of some genera within a topology that is fairly constant otherwise. This can be illustrated by the following example. PAUP reports that a clade composed of all taxa of clade one appears only in 36% of all trees found during the analysis. Consequently, this clade is not retained in the bootstrap tree. However, a clade composed of all these taxa and *Exacum* appears in 33% of all trees. In combination, this implies that in 69% of all trees a clade composed of all taxa of clade one is nested (Adams 1986) within the set of all terminal taxa excluding *Exacum*. In terms of monophyly this means that the clade is fairly well supported (69%) in the bootstrap analysis, but that we do not know whether *Exacum* belongs to it or not. When the uncertainty of the exact composition of the clade is extended to *Ixanthus*, the support value for this clade becomes 88%.

Under the same conditions as in the above standard analysis (equal a priori weighting and all characters unordered) the search for the fittest trees resulted in a single fittest cladogram with fit=263.1 for the concavity constant K equal to 3 (Fig. 2). Apart from the position of *Lisianthius*, this cladogram is identical to one of the eight fundamental trees of the unordered standard analysis.

Varying the concavity constant (all characters unordered) has little effect for values between 2 and 6: K=6 gives three fittest trees (fit 296.9), K=5 results in two trees (fit 288.9), as does K=4 (fit 278.2). Each time the strict consensus tree is as in Fig. 2, except that *Lisianthius* is the sister genus of *Chorisepalum*. For K=2 the same fittest tree (fit 239.8) is found as for K=3 (Fig. 2). Setting K to 1 (i.e. strong concavity) results in two fittest trees (fit 201.7). The first is similar to Fig. 2 (only the positions of *Gentianopsis* and *Tripterospermum* are

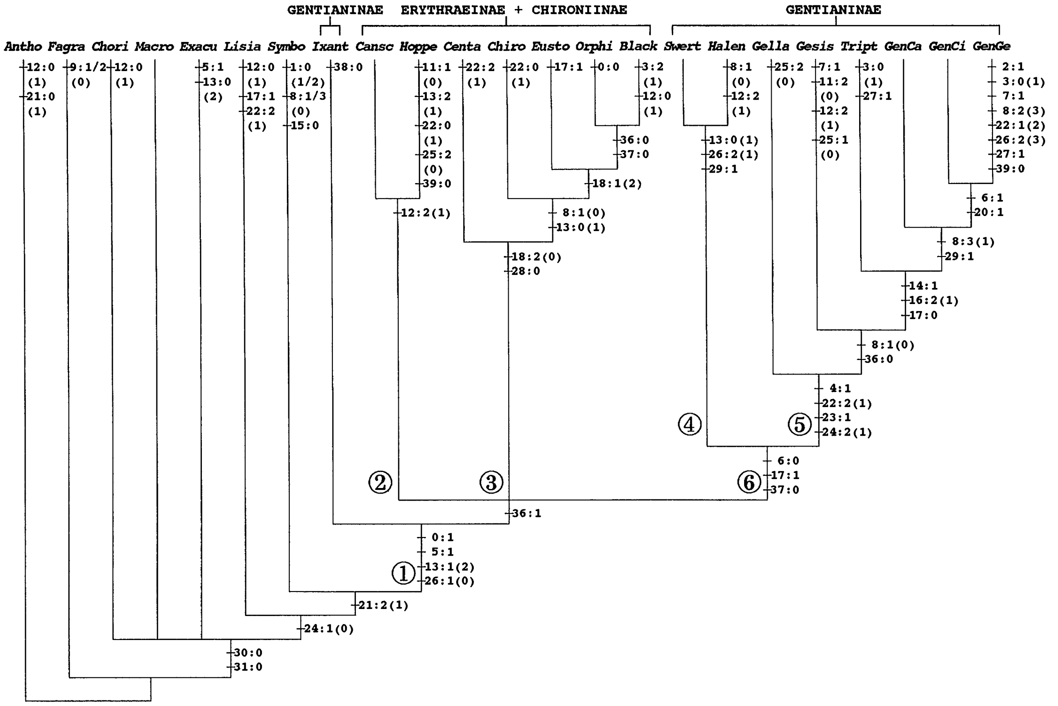


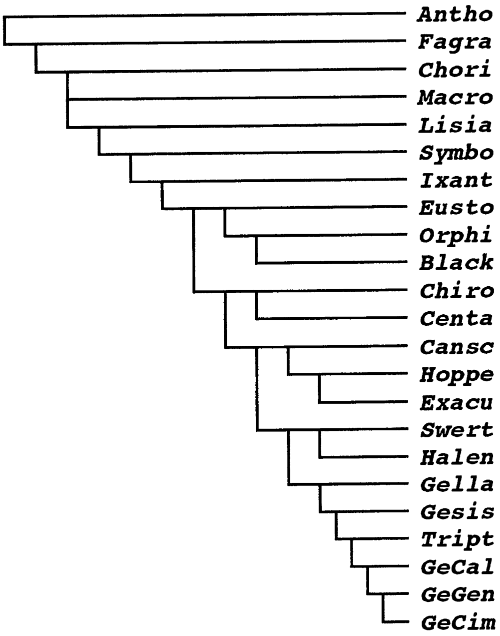
FIG. 2. Single fittest cladogram (fit 263.1; K=3) with equal a priori weighting and all characters unordered. Apart from the position of *Lisianthius*, this cladogram is identical to one of the eight most parsimonious trees of the unordered standard analysis (Fig. 1). Numbers of characters and character states refer to Table 2; analysis is based on the data matrix given in Table 3. Only unambiguous synapomorphies are shown (a:b stands for state b of character a; for multistate characters, number between brackets indicates character state that transforms into the synapomorphic state). *Gella* and *Gesis* are *Gentianella* and *Gentianopsis*; *GenCa*, *GenCi* and *GenGe* are the sections *Calathianae*, *Ciminalis* and *Gentiana* of the genus *Gentiana*; the other genera are indicated by their five first letters. *Eustoma* is included in Erythraeinae + Chironiinae. Clades with encircled numbers are discussed further in text.

switched), but the second (Fig. 3A) has a deviant topology: clade three is disrupted to form two subclades and *Exacum* is the sister group of *Hoppea*.

With equal a priori weighting but all multistate characters except 8, 16, and 24 ordered, the standard parsimony analysis resulted in six equally most parsimonious trees (steps 118; CI=0.48; RI=0.64). In two of these equally most parsimonious cladograms *Exacum* is the sister group of *Hoppea*, while it is a more basal branch in the other four. Because this results in a highly unresolved strict consensus tree, the strict consensus excluding *Exacum* is shown (Fig. 3B). *Fagraea* and *Symbolanthus* join the basal polytomy because *Fagraea* is the sister group of clade one in two of the six equally most parsimonious cladograms. The trichotomy involving clades two, three, and six is present in all of the equally most parsimonious trees. Under the same conditions (equal a priori weighting but all multistate characters except 8, 16, and 24 ordered), three fittest trees (fit 348.6) are obtained for K=3. The strict consensus of these is shown in Fig. 3C.

Varying the concavity constant (all multistate characters except 8, 16, and 24 ordered) has no effect for values between 2 and 6: each time the same three trees are obtained (with a fit of 316.5 for K=2, 348.6 for K=3, 368.4 for K = 4, 382.2 for K = 5, and 392.9 for K=6). Setting K to 1 results in four different fittest trees (fit 263.6). The strict consensus of these is as in Fig. 2, but with clades three and five differently and/or less resolved, and with *Ixanthus* joining the polytomy between clades two, three, and six. The latter is due to the fact that clade two (*Canscora* Lam.-*Hoppea*) is the sister group of *Ixanthus* in three of the fittest trees, while it forms a polytomy with clades three and six in the fourth one.

Changing the a priori weights in order to downweight the xanthone characters affects the topology when they are downweighted fivefold or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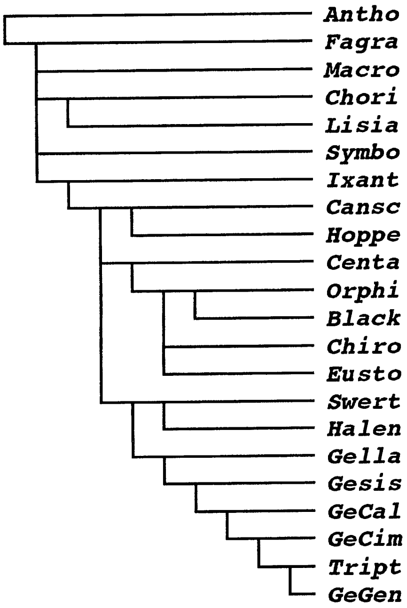
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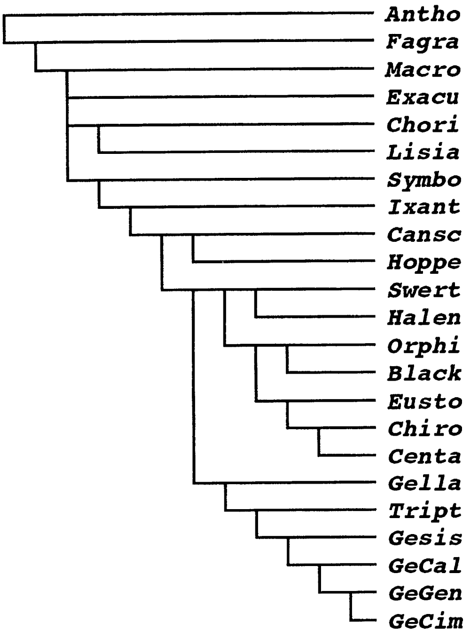
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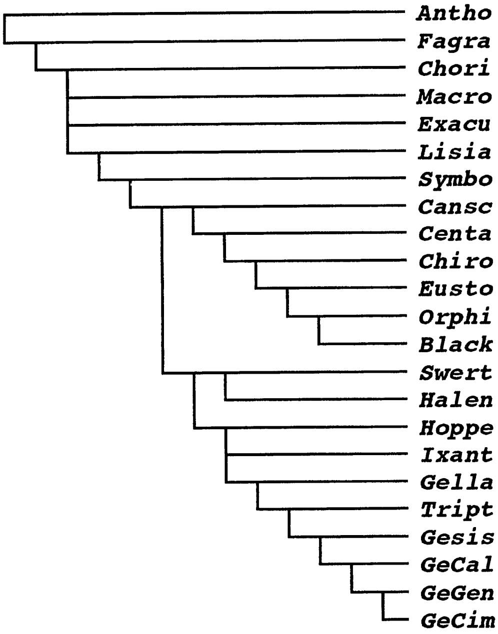
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D

more with respect to the other characters (K=3, all characters unordered). When the a priori weights are set to 1 for the xanthone characters and to 5 for the other characters, a single fittest cladogram is obtained (Fig. 3D; fit 1215.2). The most conspicuous differences with Fig. 2 are the disruption of clade two and the position of *Ixanthus*. Except for an unresolved *Eustoma-Orphium-Blackstonia* clade, the same tree is obtained when the xanthone characters are excluded completely.

DISCUSSION

The best supported clade of this study is clade one (Fig. 2), containing *Eustoma* (Gilg's Tachiinae) and all included Gentianinae, Erythraeinae and Chironiinae. In the unordered analysis using implied weights (Fig. 2), its unambiguous synapomorphies are herbaceous life form, parallel leaf venation, globular seeds and intermediate petal fusion. This result confirms Carlquist's (1984; contra Wood and Weaver 1982: 445) suggestion that a woody habit may be plesiomorphic in the family.

Leaving aside the question whether *Exacum* belongs to it, clade one is present in all analyses. The position of *Exacum* is dubious: it belongs to clade one (as sister genus of *Hoppea*) in two of the six equally most parsimonious trees with ordered characters and in one of the two fittest cladograms with unordered characters and K=1; it falls outside clade one in all other cases. The uncertain position of *Exacum* is also obvious from the decay analysis and the bootstrap analysis, as shown earlier.

The relationships between clade one on the one hand and the woody tropical representatives of Tachiinae and Helieae on the other are not clear: the cladograms are either poorly resolved below clade one or resolved incongruently among different analyses. The only recurring pattern is the position of *Symbolanthus* (Helieae) as the sister group of clade one. The sole exceptions to this are the few cladograms in which *Fagraea* is the sister group of clade one (two of the eight equally most parsimonious trees in the unordered analysis and two of the six in the ordered analysis; in these cladograms, *Symbolanthus* remains the sister group of *Fagraea* + clade one). The fact that *Fagraea* appears as the sister genus of clade one in some cladograms leaves open the possibility that Gentianaceae s. str. (excluding Potalieae) may be paraphyletic, a result that was also obtained in the broad-based analyses mentioned above (Downie and Palmer 1992; Olmstead et al. 1993; Struwe et al. 1994). Clade one, however, is unaffected by the alternative root positions as obtained by Downie and Palmer (1992), Olmstead et al. (1993) or Struwe et al. (1994). Therefore we will concentrate on this clade and its internal structure.

The structure of clade one can be visualized as a basal division between *Ixanthus* and the other genera, that in turn belong to two major clades, clade three and clade six. The position of *Canscora* and *Hoppea* within clade one is not clear: when taking into account only the unambiguous synapomorphies, *Canscora* and *Hoppea* are mostly a sister pair that is in a trichotomy with clades three and six (e.g. Figs. 1, 2, 3C). Under some conditions, or when ambiguous support is considered, *Canscora* + *Hoppea* appear as the sister pair of *Ixanthus*, as the sister group of clade three + clade six, as part of clade three, or as part of clade six. Lastly, in the analyses with strong downweighting or complete exclusion of the xanthone characters (Fig. 3D), the sister group relationship between both genera is disrupted: *Canscora* is in clade three, and *Hoppea* in clade six.

The basal division between *Ixanthus* and the other genera is present in most of the analyses. Only under extreme conditions of strong concavity or strong differential a priori weighting other results are obtained (in the ordered analysis with K=1, the basal division is between [*Ixanthus* (*Canscora Hoppea*)] and the other genera in three of the four fittest trees; with strong downweighting or complete exclusion of the xanthone characters, *Ixanthus* is the sister genus of *Gentianopsis* + the three sections of *Gentiana*). *Ixanthus*, a perennial herbaceous plant that becomes woody only at the

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FIG. 3. Results of some alternative analyses of the data in Table 3; A. One of two fittest cladograms with equal a priori weighting, all characters unordered, and K=1 (fit 201.7); the other fittest tree is as in Fig. 2, but with *Gentianopsis* and *Tripterospermum* switched. B. Strict consensus (excluding *Exacum*) of six most parsimonious trees (steps 118), multistate characters (except 8, 16, and 24) ordered. In two of the most parsimonious cladograms *Exacum* is the sister group of *Hoppea*, while it is a more basal branch in the other four. C. Strict consensus of the three fittest trees (fit 348.6; K=3) with multistate characters (except 8, 16, and 24) ordered. D. Single fittest tree (fit 1215.2; K=3) when the a priori weights for the xanthone characters are set to 1 and for the other characters to 5; all characters unordered.

base of the stem, is an endemic of the laurel forests of the Canary Islands (Bramwell 1972). Taking into account the strong asymmetry of the basal division of clade one and the limited amount of character change on the branch leading to *Ixanthus*, this genus can be interpreted as a kind of living fossil with a character distribution that is intermediate between the mostly woody tropical genera below clade one and the mostly herbaceous temperate taxa within clade one. Indeed, even if the herbaceous life form does occur in the tropical genera, e.g. in *Lisianthius*, it is mostly connected with the Mediterranean or temperate climate that prevails in clade one. The absence of temperate representatives below clade one points to a tropical ancestry of the Gentianaceae. Other, more circumstantial, indications for tropical ancestry are that two thirds of the genera are tropical or have species in the tropical regions (Nilsson 1970; Favarger 1987), that all the other families of the order Gentianales are tropical, and that the oldest fossils represent tropical genera (*Macrocarpaea* and *Lisianthius*; Crepet and Daghlian 1981; Graham 1984). Carlquist (1984) interpreted the presence of interxylary phloem and the lack of rays in the juvenile wood of *Ixanthus* as advanced features which were probably acquired through the adaptation to "winter cold as well as a fluctuation between winter rainfall and summer drought."

Clade three is composed of a mixture of Erythraeinae (*Canscora* and *Hoppea* dubious), Chironiinae and the genus *Eustoma* of Tachiinae. This clade is present in all but one of the reported cladograms, in which it is paraphyletic (one of the two fittest cladograms of the unordered analysis with K=1; Fig. 3A). The unambiguous synapomorphies of this clade are twisted anthers and the presence of flavonols (instead of flavones). The inner structure of clade three is not clear. The sister group relationship of *Chironia* and *Centaurium* in some of the analyses (Figs. 3A, 3C) is in line with their supplementary geographical areas. However, if *Centaurium* originated in the European paleomediterranean area where its extant diploid species exist (Zeltner 1970), this could also mean that *Centaurium* is paraphyletic.

Clade six, the second major subclade of clade one, is composed of the representatives of subtribe Gentianinae, excluding *Ixanthus*. The unambiguous synapomorphies are the absence of calcium oxalate crystals in the mesophyll, versatile anthers and absence of oxygenetion of C6. A problematic point is the position of the sister pair *Swertia* and *Halenia*, which belongs to clade three in the ordered analyses with K between 2 and 6 (cf. Fig. 3C), but to clade six in all other analyses. In clade six, *Swertia-Halenia* (clade four) and the other Gentianinae (clade five) are sister groups. This relationship is in agreement with the recent treatment of Liu and Ho (1992). Garg (1987) and Zuyev (1990), on the other hand, considered this group as a separate tribe.

Apart from the position of *Tripterospermum*, the structure of clade five is as depicted in Fig. 2 in all analyses. Its unambiguous synapomorphies include three well documented features of the ovary, namely superficial placentation, elongate shape, and presence of a stipe. Traditionally *Tripterospermum* is mostly included in *Gentiana* (e.g. Marquand 1937) or, mainly since the revision of Smith (1965), treated as a separate genus closely related to *Gentiana*. In our analyses its position varied: in some cladograms it was the sister group of *Gentianopsis* plus the three sections of *Gentiana*, in others it was the sister group of the three sections of *Gentiana*, and lastly it sometimes appeared as the sister group of *Gentiana* sect. *Gentiana*. *Tripterospermum* is generally considered to be related to *Gentiana* sect. *Stenogyne*, which is either regarded as "the primitive type of the genus" (Ho and Liu, 1990: 186) or as a "more advanced group than the other sections of the genus" (Yuan and Küpfer 1993: 72; based on chromosome number and karyotype asymmetry). As *Gentiana* sect. *Stenogyne* is not included in our data set, and as the position of *Tripterospermum* was variable in our analysis, it is premature to draw any conclusions concerning the status of *Tripterospermum*.

Many questions remain regarding infrafamilial classification. Nevertheless, some tentative proposals can be made. Our results corroborate the traditional composition of the subtribe Gentianinae (probably with the exception of *Ixanthus*). On the other hand, Gilg's (1895) subtribes Erythraeinae and Chironiinae are clearly not monophyletic and we propose merging them, with inclusion of the genus *Eustoma* (Tachiinae). Because this clade (including *Eustoma*) is the sister group of subtribe Gentianinae, it is better to retain subtribal rank (Chironiinae) within the tribe Gentianeae. Following the sequencing convention (Nelson 1972; cf. Wiley 1981), the genus *Ixanthus* can be accomodated in a third subtribe Ixanthinae of a narrowly defined tribe Gentianeae (clade one). *Swertia* and *Halenia* probably belong to subtribe Gentianinae, while the positions of the genera *Canscora* and *Hoppea* within this tribe are not clear. The possibility that they constitute a separate subtribe cannot be excluded. Subtribe Tachiinae (excluding *Eustoma*) falls outside the tribe Gentianeae as defined here, while the status and the position of subtribe Exacinae is not clear. The relationships between Tachiinae, Exacineae, Gentianeae, and Gilg's (1895) other tribes are not clear. An analysis of a broader array of taxa, including a wider array of outgroup taxa, will be necessary to elucidate these relationships.

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