

rbcL* sequences support exclusion of *Retzia*, *Desfontainia*, and *Nicodemia* from the *Gentianales

BIRGITTA BREMER, RICHARD G. OLMSTEAD, LENA STRUWE, and JENNIFER A. SWEERE

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Abstract: The taxonomic positions of *Retzia*, *Desfontainia*, and *Nicodemia* have been much discussed, and all three genera have been included in *Loganiaceae* (*Gentianales*). We have made a cladistic analysis of *rbcL* gene sequences to determine the relationships of these taxa to *Gentianales*. Four new *rbcL* sequences are presented; i.e., of *Retzia*, *Desfontainia*, *Diervilla* (*Caprifoliaceae*), and *Euthystachys* (*Stilbaceae*). Our results show that *Retzia*, *Desfontainia*, and *Nicodemia* are not closely related to *Loganiaceae* or the *Gentianales*. *Retzia* is most closely related to *Euthystachys* and is better included in *Stilbaceae*. The positions of *Desfontainia* and *Nicodemia* are not settled, but *Desfontainia* shows affinity for the *Dipsacales* s.l. and *Nicodemia* for the *Lamiales* s.l.

The order *Gentianales* is the second largest and the most distinct and well-supported group within the *Asteridae* s.l. The order is accepted by most systematists and is also strongly supported by molecular data (OLMSTEAD & al. 1992, DOWNIE & PALMER 1992). However, the circumscription and the phylogeny of the order are not settled. The families *Apocynaceae*, *Asclepiadaceae*, *Gentianaceae*, and *Loganiaceae* s.str. are unquestionable members of the *Gentianales*, and most authors also include *Rubiaceae* (not CRONQUIST 1981). The inclusion of other families within the order is less certain, and in several cases the circumscriptions of the families are unclear.

The *Loganiaceae* (LEEUEWENBERG & LEENHOUTS 1980) are paraphyletic (BREMER & STRUWE 1992), and the family has sometimes been circumscribed to include some genera of very uncertain affinity. The monotypic genera *Retzia* THUNB. from the Cape region and *Desfontainia* RUIZ & PAV. from the Andes have been classified as members of the *Loganiaceae* or as monotypic families in both *Gentianales* and distantly related orders. *Retzia*, a bird-pollinated ericoid shrub, has also been suggested to be related to the *Stilbaceae* (cf. DAHLGREN & al. 1979). *Desfontainia*, with spinose leaves and fleshy berries, has been associated with *Solanaceae* (HUMBOLDT & BONPLAND 1808) and *Aquifoliaceae* (LINDLEY 1836), but more recent anatomical studies point toward a position close to *Columelliaceae* (STERN & al. 1969). The *Buddlejaceae* are generally regarded as a separate family of the *Scro-*

Table 1. Sources of living material extracted for cpDNA. Bold: species with newly analysed *rbcL* sequence. Family names according to MABBERLEY (1987)

Species, family	Source/voucher information
<i>Aphelandra sinclariana</i> , <i>Acanthaceae</i>	CHASE & al. 1993
<i>Lepidagathis villosa</i> , <i>Acanthaceae</i>	CHASE & al. 1993
<i>Nelsonia canescens</i> , <i>Acanthaceae</i>	ALBERT & al. 1992
<i>Ruellia graecizans</i> , <i>Acanthaceae</i>	CHASE & al. 1993
<i>Ruttya fruticosa</i> , <i>Acanthaceae</i>	CHASE & al. 1993
<i>Thunbergia usambarica</i> , <i>Acanthaceae</i>	CHASE & al. 1993
<i>Adoxa moschatellina</i> , <i>Adoxaceae</i>	DONOGHUE & al. 1992
<i>Kopsia fruticosa</i> , <i>Apocynaceae</i>	OLMSTEAD & al. 1993
<i>Ilex crenata</i> , <i>Aquifoliaceae</i>	ALBERT & al. 1992
<i>Apium graveolens</i> , <i>Apiaceae</i>	ALBERT & al. 1992
<i>Hedera helix</i> , <i>Araliaceae</i>	OLMSTEAD & al. 1992
<i>Dasyphyllum diacanthoides</i> , <i>Asteraceae</i>	KIM & al. 1992
<i>Helianthus annuus</i> , <i>Asteraceae</i>	KIM & al. 1992
<i>Catalpa spec.</i> , <i>Bignoniaceae</i>	OLMSTEAD & al. 1992
<i>Berzelia lanuginosa</i> , <i>Bruniaceae</i>	OLMSTEAD & al. 1993
<i>Nicodemia diversifolia</i> , <i>Loganiaceae</i>	OLMSTEAD & al. 1993
<i>Buddleja davidii</i> , <i>Loganiaceae</i>	OLMSTEAD & al. 1993
<i>Byblis liniflora</i> , <i>Byblidaceae</i>	ALBERT & al. 1992
<i>Callitriche heterophylla</i> , <i>Callitrichaceae</i>	OLMSTEAD & al. 1992
<i>Diervilla sessilifolia</i> , <i>Caprifoliaceae</i>	DONOGHUE, no voucher, Arnold Arb. 960-79, EMBL z 29672
<i>Viburnum acerifolia</i> , <i>Caprifoliaceae</i>	OLMSTEAD & al. 1992
<i>Sambucus racemosa</i> , <i>Caprifoliaceae</i>	DONOGHUE & al. 1992
<i>Symphoricarpos albus</i> , <i>Caprifoliaceae</i>	OLMSTEAD & al. 1992
<i>Cercidiphyllum japonica</i> , <i>Cercidiphyllaceae</i>	OLMSTEAD & al. 1992
<i>Cornus canadensis</i> , <i>Cornaceae</i>	ALBERT & al. 1992
<i>Corokia cotoneaster</i> , <i>Cornaceae</i>	XIANG & al. 1993
<i>Griselinia lucida</i> , <i>Cornaceae</i>	XIANG & al. 1993
<i>Dipsacus sativus</i> , <i>Dipsacaceae</i>	OLMSTEAD & al. 1992
<i>Eucommia ulmoides</i> , <i>Eucommiaceae</i>	ALBERT & al. 1992
<i>Garrya elliptica</i> , <i>Garryaceae</i>	ALBERT & al. 1992
<i>Gentiana procera</i> , <i>Gentianaceae</i>	OLMSTEAD & al. 1993
<i>Streptocarpus holstii</i> , <i>Gesneriaceae</i>	OLMSTEAD & al. 1993
<i>Escallonia coquimbensis</i> , <i>Grossulariaceae</i>	MORGAN & SOLTIS 1993
<i>Phyllonoma laticuspis</i> , <i>Grossulariaceae</i>	MORGAN & SOLTIS 1993
<i>Lamium purpureum</i> , <i>Lamiaceae</i>	OLMSTEAD & al. 1993
<i>Salvia divinorum</i> , <i>Lamiaceae</i>	OLMSTEAD & al. 1993
<i>Scutellaria bolanderi</i> , <i>Lamiaceae</i>	ALBERT & al. 1993
<i>Teucrium fruticans</i> , <i>Lamiaceae</i>	OLMSTEAD & al. 1993
<i>Pinguicula caerulea</i> , <i>Lentibulariaceae</i>	ALBERT & al. 1992
<i>Utricularia biflora</i> , <i>Lentibulariaceae</i>	ALBERT & al. 1992
<i>Desfontainia spinosa</i> , <i>Loganiaceae</i>	Gothenburg Bot. Gard., BREMER 2739 (UPS), EMBL z 29670
<i>Retzia capensis</i> , <i>Loganiaceae</i>	KÄLLERSJÖ 0401191 (BOL), EMBL z 29669
<i>Anthocleista grandiflora</i> , <i>Loganiaceae</i>	OLMSTEAD & al. 1993
<i>Mostuea brunonis</i> , <i>Loganiaceae</i>	OLMSTEAD & al. 1993
<i>Strychnos nux-vomica</i> , <i>Loganiaceae</i>	OLMSTEAD & al. 1993
<i>Menyanthes trifoliata</i> , <i>Menyanthaceae</i>	OLMSTEAD & al. 1993
<i>Villarsia calthifolia</i> , <i>Menyanthaceae</i>	OLMSTEAD & al. 1992

Table 2 (continued)

Species, family	Source/voucher information
<i>Nyssa ogeche</i> , Nyssaceae	XIANG & al. 1993
<i>Jasminum suavisissimum</i> , Oleaceae	ALBERT & al. 1992
<i>Ligustrum vulgare</i> , Oleaceae	OLMSTEAD & al. 1992
<i>Harpogophytum grandieri</i> , Pedaliaceae	ALBERT & al. 1992
<i>Proboscidea louisianica</i> , Pedaliaceae	ALBERT & al. 1992
<i>Sesamum indicum</i> , Pedaliaceae	OLMSTEAD & al. 1993
<i>Pittosporum japonicum</i> , Pittosporaceae	MORGAN & SOLTIS 1993
<i>Chiococca alba</i> , Rubiaceae	OLMSTEAD & al. 1993
<i>Antirrhinum majus</i> , Scrophulariaceae	OLMSTEAD & al. 1992
<i>Digitalis purpurea</i> , Scrophulariaceae	ALBERT & al. 1992
<i>Nicotiana tabacum</i> , Solanaceae	LIN & al. 1986
<i>Petunia hybrida</i> , Solanaceae	ALDRICH & al. 1986
<i>Tetracentron sinense</i> , Tetracentraceae	CHASE & al. 1993
<i>Valeriana officinalis</i> , Valerianaceae	OLMSTEAD & al. 1992
<i>Euthystachys abbreviata</i>, Stilbaceae	MCDONALD & ROURKE, no voucher, EMBL z29671
<i>Verbena bonariensis</i> , Verbenaceae	OLMSTEAD & al. 1993

phulariales, but in the latest large treatment of *Loganiaceae* (LEEUWENBERG & LEENHOUTS 1980), they are accepted as part of that family. *Nicodemia* (VAHL) TENORE is usually included in *Buddleja*, but it differs from *Buddleja* by having fleshy fruits.

To investigate further if these taxa belong to the *Gentianales* we have sequenced the *rbcL* gene from *Retzia* and *Desfontainia*. We have also included two new sequences, of *Euthystachys* (*Stilbaceae*) and *Diervilla* (*Caprifoliaceae*), as we expected that these taxa should be relevant to our investigation. The *rbcL* sequence of *Nicodemia* (OLMSTEAD & al. 1993) was also included in our analyses.

Material and methods

The *rbcL* gene has been sequenced from *Desfontainia spinosa*, *Diervilla sessilifolia*, *Euthystachys abbreviata*, and *Retzia capensis* (Table 1); sequences are accessioned in EMBL as z29670, z29672, z29671, and z29669. The remaining sequences included in the analyses are from different sources (Table 1).

Total DNA was extracted from fresh leaves (SAGHAI-MAROOF & al. 1984, DOYLE & DOYLE 1987). Double-stranded DNA of the *rbcL* gene was amplified by PCR with two synthetic primers, the 5' primer was identical to the first 26 nucleotides of *rbcL* of tobacco, and the 3' primer corresponds to a region ca. 100 nucleotides downstream from the coding region (OLMSTEAD & al. 1992). This covers the entire coding gene (excluding the first 26 nucleotides). A second run with asymmetric amounts of primers (1 : 100) was performed to get single-stranded DNA (GYLLENSTEN 1989). Single-stranded DNA was sequenced (SANGER & al. 1977) using internal primers designed by G. ZURAWSKI (DNAX Research Institute).

The data matrices in the phylogenetic analyses comprise characters corresponding to each nucleotide position (27 to 1428) of the *rbcL* sequences. Only phylogenetically informative characters (365 positions) were analysed. To evaluate the branch support we have

performed a BREMER support analysis (BREMER 1988, KÄLLERSJÖ & al. 1992) and saved all trees one to six steps longer than the minimal trees. In one of the analyses we had to stop after two extra steps as we reached PAUP's upper limit of saving trees (32,767). Parsimony analyses were conducted using PAUP vers. 3.1.1 (SWOFFORD 1991) on a MacQuadra 840AV, with all character changes weighted equally.

In the first set of analyses a large part of the available sequences of *Asteridae* s.l. (OLMSTEAD & al. 1993) was included (61 species), with two taxa as the outgroup (*Tetracentron-Tetracentraceae* and *Cercidiphyllum-Cercidiphyllaceae*, both of the *Hamamelidae*) (Table 1). The aim was not to find the best supported phylogeny for the *Asteridae*, a problem that has been addressed elsewhere (OLMSTEAD & al. 1993), but to determine whether or not *Desfontainia*, *Nicodemia*, and *Retzia* are closely related to the *Gentianales*. Subsets of taxa from the first analysis were further analysed, e.g., the clade including *Nicodemia* and *Retzia* (26 taxa), and the clade including *Desfontainia* (eight taxa).

The approaches to search for the best trees were different for the different analyses. In the analyses with only eight taxa including *Desfontainia* and one outgroup, we performed an exhaustive search. For the other analyses with 26 or 61 taxa and outgroups, we searched with heuristic methods, with random addition of sequences and 1000, 100, or 10 replications, and TBR branch swapping with MULPARS on and Steepest descent off.

Results

Analysis of *Asteridae* (Figs. 1, 2). Published sequences of 57 representatives of *Asteridae* s.l. (see under discussion) and four new *rbcL* sequences for *Desfontainia*, *Diervilla*, *Euthystachys*, and *Retzia* were analysed with the distantly related *Tetracentron* (*Tetracentraceae*) and *Cercidiphyllum* (*Cercidiphyllaceae*) as outgroup. This analysis, a heuristic search with random addition of sequences and with 10 replications, including only phylogenetically informative characters, resulted in 89 equally parsimonious trees 1716 steps long (509 minimal possible steps) with a consistency index (ci) of 0.30 and a retention index (ri) of 0.55.

According to this analysis neither *Retzia*, nor *Nicodemia*, nor *Desfontainia*, is related to the *Gentianales*. *Desfontainia* is positioned within the *Dipsacales* clade (cf. OLMSTEAD & al. 1993), *Retzia* is the sister taxon to *Euthystachys* (*Stilbaceae*) in the *Lamiales* s.l. clade, and *Nicodemia* is the sister taxon to *Verbena* (*Verbenaceae*) in a clade with *Pedaliaceae* p.p. and *Lamiaceae*.

Analyses of the “*Desfontainia* clade” or the *Dipsacales* s.l. (Fig. 3). The clade including *Desfontainia* and the *Dipsacales* s.l. from the analysis above (eight species) was first analysed with a large outgroup representing five different clades from the first analysis: *Cornus* (*Cornaceae*) and *Nyssa* (*Nyssaceae*), *Garrya* (*Garryaceae*) and *Eucommia* (*Eucommiaceae*), *Ilex* (*Aquifoliaceae*) and *Phyllonoma* (*Grossulariaceae*), *Pittosporum* (*Pittosporaceae*) and *Hedera* (*Araliaceae*), and *Menyanthes* and *Villarsia* (*Menyanthaceae*). The analysis (a heuristic search with 1000 replications) resulted in six equally parsimonious trees 396 steps long (190 minimal possible steps) with a consistency index (ci) of 0.48 and a retention index (ri) of 0.46. Three of the six trees differ concerning the position of *Desfontainia* while the others differ in relationships among the outgroup taxa. The different positions of *Desfontainia* are at the base of the whole *Dipsacales* s.l. or at the base of one or the other of the two earlier identified subclades of the *Dipsacales* (DONOGHUE & al. 1992) here represented by *Viburnum/Sambucus/Adoxa* and *Symphoricarpus/Dipsacus/Valeriana/Diervilla* (Fig. 2), respectively. The closest sister group to the “*Desfontainia* clade” from this analysis was *Menyanthaceae* represented by *Menyanthes* and *Vil-*

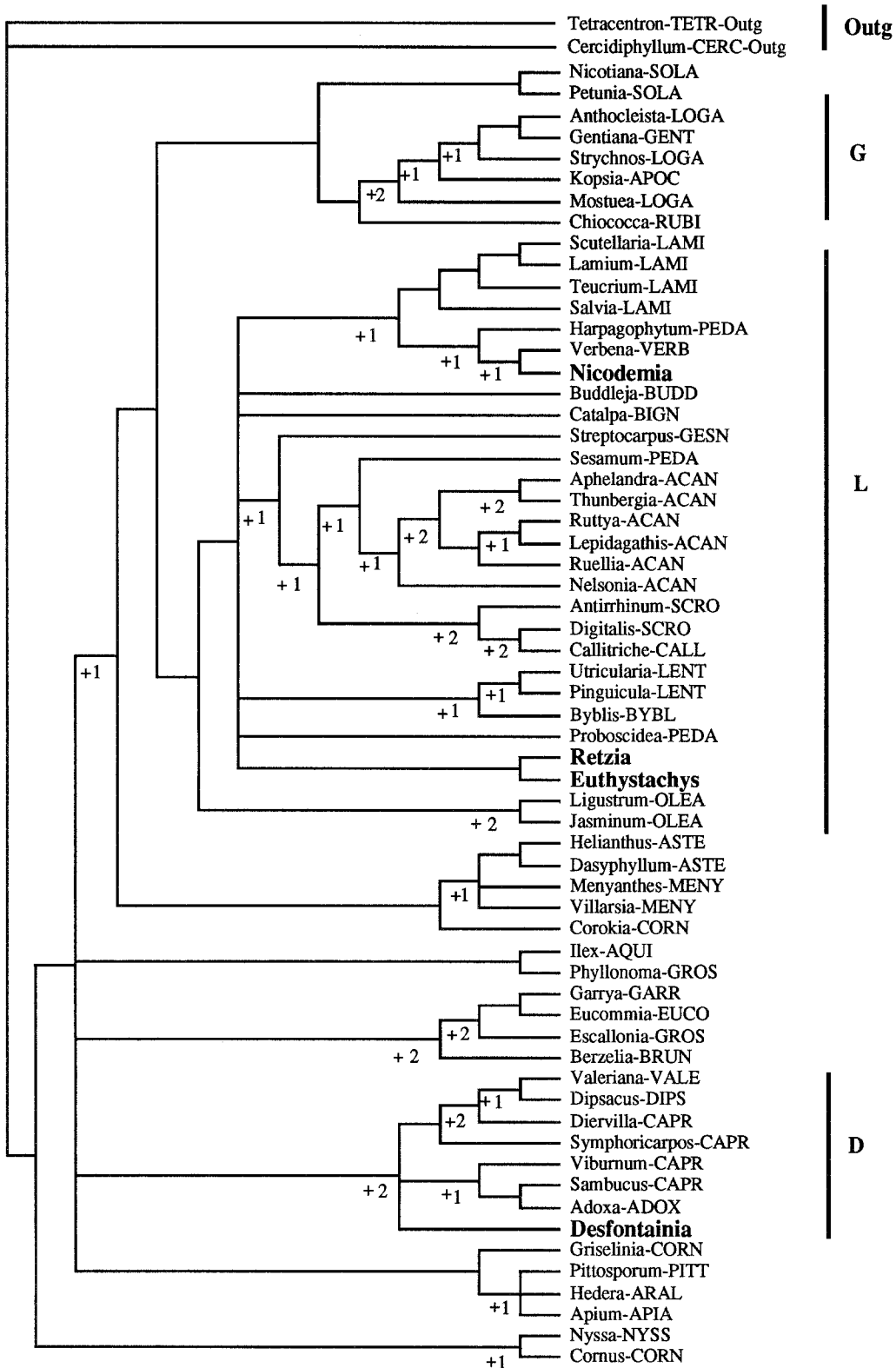


Fig. 1. Strict consensus tree of 89 equally parsimonious trees of *Asteridae* s.l. based on *rbcL* sequences. Families are indicated by a four-letter suffix corresponding to the families in Table 1. Vertical bars and corresponding letters represent: D *Dipsacales* s.l., G *Gentianales*, L *Lamiales* s.l., and Outg outgroups. Numerals indicate number of extra steps required to collapse the particular node. Nodes without numerals require more than 2 extra steps to collapse

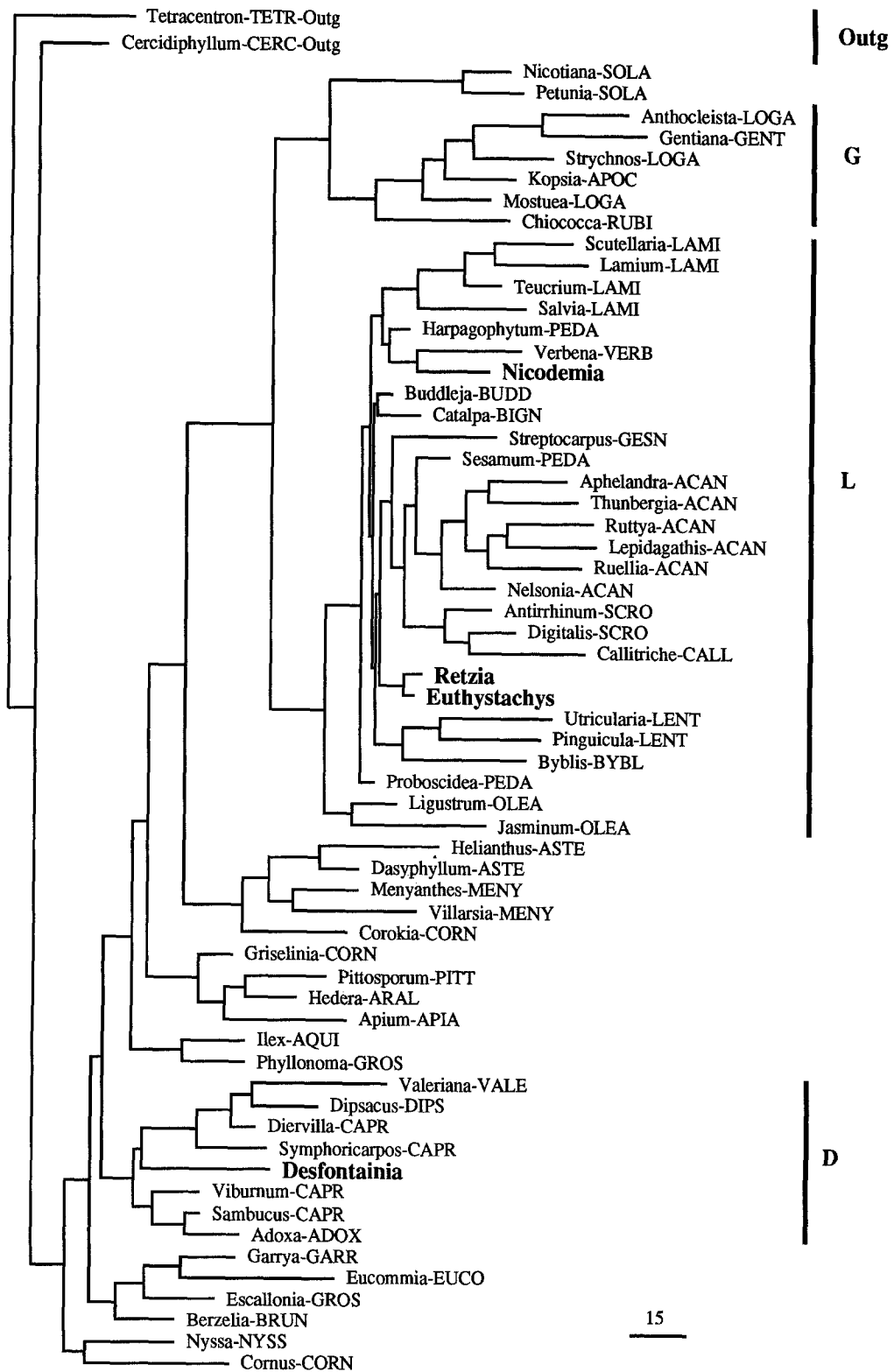


Fig. 2. Sample phylogram of 89 equally parsimonious trees of *Asteridae* s.l. based on *rbcL* sequences, 1716 steps long with a ci of 0.30, and a ri of 0.55 (only phylogenetically informative characters are included). Families are indicated by a four-letter suffix corresponding to the families in Table 1. Vertical bars and corresponding letters represent: D *Dipsacales* s.l., G *Gentianales*, L *Lamiales* s.l., and Outg outgroups. Branch lengths are proportional to the number of substitutions supporting a node (note scale bar equal to 15 substitutions)

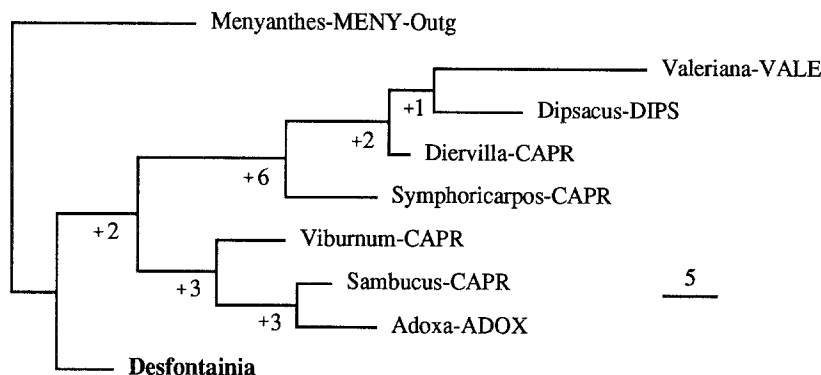


Fig. 3. The single most parsimonious tree of the *Dipsacales* s.l., 125 steps long with a ci of 0.62 and a ri of 0.55 (only phylogenetically informative characters are included). Numerals indicate number of extra steps required to collapse the particular node. Branch lengths are proportional to the number of substitutions supporting a node (note scale bar equal to 5 substitutions)

larsia. An analysis (exhaustive search) with *Menyanthes* as the outgroup resulted in one most parsimonious tree (Fig. 3) 125 steps long (78 minimal possible steps) with a consistency index (ci) of 0.62 and a retention index (ri) of 0.55. The topology of this tree is identical to trees found in two of the analyses above, namely with *Desfontainia* at the base of the whole *Dipsacales*.

Analysis of the “*Retzia/Nicodemia* clade” or the *Lamiales* s.l. (Figs. 4, 5). The clade including *Lamiales* s.l. and *Retzia* and *Nicodemia* (26 species) was first analysed with a large outgroup: *Nicotiana* and *Petunia* (*Solanaceae*), *Nyssa* (*Nyssaceae*) and *Dasyphyllum* (*Asteraceae*), *Mostuea* (*Loganiaceae*) and *Kopsia* (*Apocynaceae*), and *Ligustrum* and *Jasminum* (*Oleaceae*). This analysis (a heuristic search with 100 replications) resulted in 49 equally parsimonious trees 803 steps long (318 minimal possible steps) with a consistency index (ci) of 0.40 and a retention index (ri) of 0.43. We also analysed the same ingroup taxa but with each of the outgroup taxa separately. The shortest trees were found with *Ligustrum* used as the outgroup. That analysis (heuristic search with 100 replications) resulted in 7 equally parsimonious trees 506 steps long (221 minimal possible steps) with a consistency index (ci) of 0.44 and a retention index (ri) of 0.40. The strict consensus tree (Fig. 4) is very similar to that of the preceding analyses, using the large outgroup, but slightly less collapsed at the base (a polytomy with 9 branches), and shows the same close relationships for *Retzia* and *Nicodemia*, respectively.

Discussion

The name *Gentianales* was introduced rather early (LINDLEY 1833) but was not generally accepted until WAGENITZ (1959) in a convincing way demonstrated morphological traits that characterize this order. A typical member of the *Gentianales* is a woody plant with opposite, entire leaves with interpetiolar stipules or stipular ridges, and a certain type of glandular hairs, colleters, inside the stipules and/or the calyx lobes. The flowers are usually animal-pollinated, actinomorphic, often showy and fragrant, with isomerous stamens, and often with contorted aestivation.

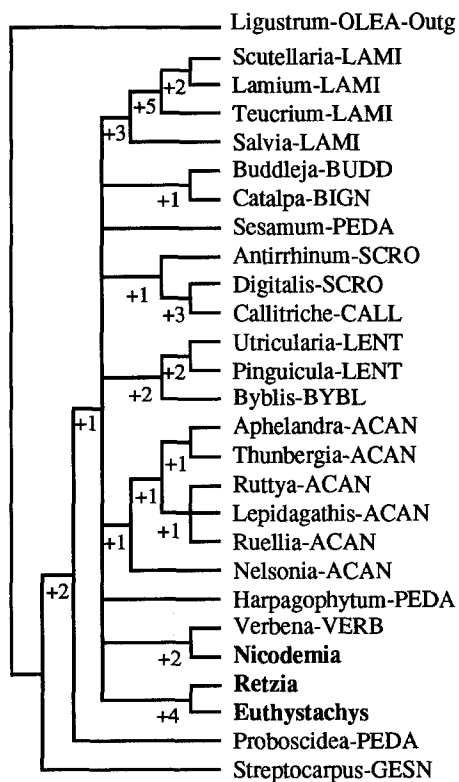


Fig. 4. Strict consensus tree of seven equally parsimonious trees of *Lamiales* s.l. based on *rbcL* sequences. Numerals indicate number of extra steps required to collapse the particular node

Endosperm formation is generally nuclear. Wood features common to the order are simple perforation plates, fibre-tracheids, and banded apotracheal axial parenchyma (CARLQUIST 1992), but the most well known anatomical characteristic of the order, although not present in the *Rubiaceae*, is the occurrence of intraxylar-phloem. Alkaloids and glycosides are very common, and three of the families (*Apocynaceae*, *Loganiaceae*, and *Rubiaceae*) are characterized by a special type of complex indole alkaloids (KISAKÜREK & HESSE 1980). The monophyly of the order is also strongly supported by molecular data, such as *rbcL* sequence data (OLMSTEAD & al. 1992, CHASE & al. 1993) and restriction site data (DOWNIE & PALMER 1992). The positions and circumscriptions of the included taxa vary between different authors, and the taxonomic history for a certain group can be long and complex. This is especially true for some genera or tribes that have been included in the heterogeneous family *Loganiaceae*, i.e., *Retzia*, *Desfontainia*, and the tribe *Buddlejeae* (LEEUEWENBERG & LEENHOUTS 1980), here represented by *Nicodemia*, a genus usually included in *Buddleja*.

Prior to this study there were morphological indications that *Desfontainia*, *Nicodemia*, and *Retzia* do not belong to the *Gentianales* (see below). The intention with this study is to analyse the *rbcL* sequences of these taxa together with some of the available *rbcL* sequences (CHASE & al. 1993, OLMSTEAD & al. 1993) of taxa that should represent possible relatives. The taxa were selected from all clades (CHASE & al. 1993, OLMSTEAD & al. 1993) which represented families or genera that have been proposed to be close to *Desfontainia*, *Nicodemia*, and *Retzia*, respectively. All the selected taxa are part of *Asteridae* s.l. (OLMSTEAD & al. 1993)

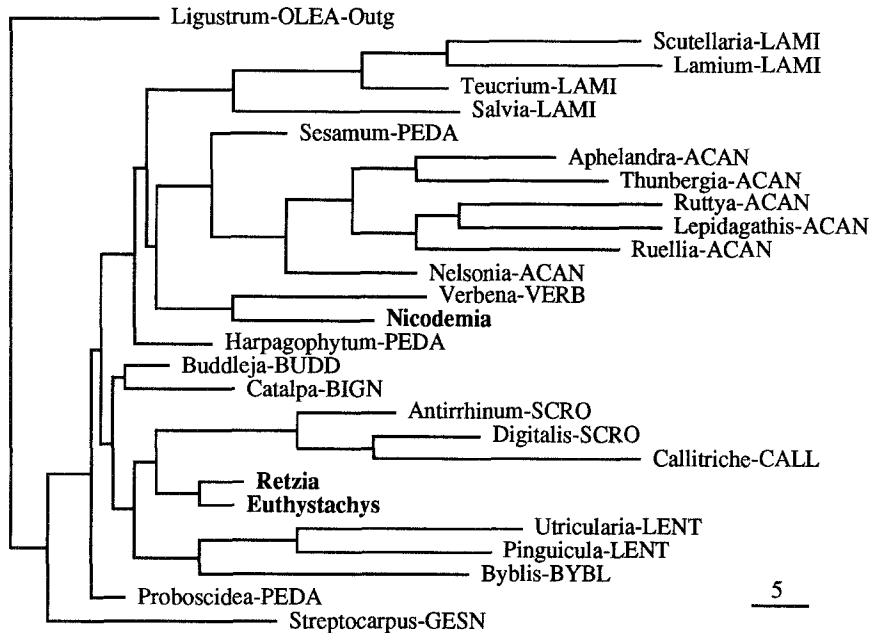


Fig. 5. Sample cladogram of seven equally parsimonious trees of *Lamiales* s.l. based on *rbcL* sequences, 506 steps long with a ci of 0.44 and a ri of 0.40 (only phylogenetically informative characters are included). Branch lengths are proportional to the number of substitutions supporting a node (note scale bar equal to 5 substitutions)

or the group called Asterid I, or II (CHASE & al. 1993). These circumscriptions of the *Asteridae* are much wider than those of CRONQUIST (1981) and TAKHTAJAN (1987). However, our intention was not to address the question on how *Asteridae* should be circumscribed. We have used the name and circumscription in the same way as OLMSTEAD & al. (1993) to get our analysis comparable with that analysis. Our main goal is to define the phylogenetic positions of *Desfontainia*, *Nicodemia*, and *Retzia*.

The South African species *Retzia capensis* represents a monotypic and conspicuous genus in the Cape fynbos flora, where it has a very restricted distribution. *Retzia* is a fire-resistant ericoid shrub with stiff, almost unbranched stems (without intraxylary-phloem) from a root-crown. The densely crowded leaves in whorls, without stipules, are linear, thick-coriaceous, with revolute margins, and they are densely covered with silky hairs as are the branches and flowers. The flowers are solitary and axillary on very short branches. The cylindrical corolla tubes (up to 6 cm long) are red with yellow to white limbs and bird-pollinated, e.g., by the sunbird *Nectarinia violacea* (*Anthobaphes violacea* DAHLGREN & al. 1979, REBELO 1987). There are five epipetalous stamens. The ovary is superior, two-celled at the base, and one-celled at the top. The fruit is a small capsule with 4–6 seeds. Most morphological features are unspecialized within the sympetalous group of families, and no gross morphological features indicate any particular relationship.

Ever since *Retzia* was described by THUNBERG (1776), its affinities have been much debated, and the genus seems systematically isolated.

In this context we note that LEEUWENBERG's (1964) indication of the holotype for *Retzia capensis* in Stockholm (S) is erroneous. Actually, type material is present in Stockholm and in Uppsala (UPS-THUNB), and we hereby choose the Uppsala material as lectotype, and the specimens in Stockholm are isotypes. The specimen in the THUNBERG herbarium is the one illustrated in the protologue (THUNBERG 1776).

Retzia has been assigned to various families, i.e., *Convolvulaceae* (JUSSIEU 1791, PERSOON 1805), *Campanulaceae* (GISEKE 1792), *Solanaceae* (ENDLICHER 1836–1841, BENTHAM & HOOKER 1876, BAILLON 1888), tentatively to *Apocynaceae* (LINDLEY 1836), *Loganiaceae* (THONNER 1908, LEEUWENBERG 1964), *Scrophulariaceae* (HALLIER 1912), or to a monotypic family *Retziaceae* (BARTLING 1830) which has been placed in different orders: *Loganiales* (HUTCHINSON 1959), *Scrophulariales* (TAKHTAJAN 1969), *Gentianales* (CRONQUIST 1981, THORNE 1983), or *Solanales* (CRONQUIST 1988). DAHLGREN & al. (1979) presented many similarities between *Retzia* and *Stilbaceae*, but they were not convinced of the affinity. DAHLGREN (1983) placed *Retziaceae* closest to *Stilbaceae* in the *Scrophulariales*. The supposed affinity to *Stilbaceae* was further supported by CARLQUIST's anatomical study (1986) and in the latest presented angiosperm system, THORNE (1992) included *Retzia* in the *Stilbaceae* (in his *Bignoniales* more or less equivalent to *Scrophulariales* of CRONQUIST 1981 and others).

Recently several detailed investigations of cytology, chemical contents, embryology, and wood anatomy have been performed. GOLDBLATT & KEATING (1976) studied chromosome number and pollen structures. They found no unique features (chromosome base number is $x = 12$; pollen grains are tricolporate) that could settle the position, but based on earlier studies they favoured a position of *Retzia* within the *Loganiaceae*. DAHLGREN & al. (1979) reviewed earlier information of the genus and presented further information on morphology, anatomy, and especially on the iridoid glycoside contents. They found four different iridoids of which two were identified as unedocide, also known from *Ericaceae* (GEISSMAN & al. 1966) and *Stilbaceae* (RIMPLER 1972), and stilbericoside as in *Stilbaceae* (RIMPLER 1972). The conclusion of DAHLGREN & al. (1979) was that *Retzia* is "unrelated to *Gentianiflorae*, but probably allied to *Lamiiflorae*, including *Verbenaceae*, *Scrophulariaceae*, and the endemic Cape family *Stilbaceae*". CARLQUIST (1986) investigated the wood anatomy of *Retzia* and ten species of *Stilbaceae* (*Stilbaceae* are often included in *Verbenaceae*). He concluded that "*Stilbaceae* and *Retziaceae* are alike in wood anatomy but differ from *Verbenaceae* by having scalariform perforation plates with few and wide bordered bars . . . vessel elements clearly fibriform in shape; very scarce axial parenchyma; and long uniseriate wings on multiseriate rays", and he continued: "The wood anatomy of *Retzia* proves astonishingly like that of *Stilbaceae* . . . If the stilboid genera are segregated from *Verbenaceae*, I see little choice but to include *Retzia* in the family . . . also, although the two groups probably deserve subfamilial status". Embryological data (ENGELL 1987), such as the presence of cellular endosperm, formation of endosperm haustoria, and a well-developed hypostase, further supported the affinity of *Scrophulariales*.

The phylogenetic analysis of the *rbcL* data (Figs. 1, 2) clearly shows that *Retzia* is not part of the *Gentianales* but is allied to the *Lamiales* s.l. or the "*Lamiiflorae* including *Verbenaceae*, *Scrophulariaceae* . . . *Stilbaceae*" as first suggested by DAHLGREN & al. (1979). We also agree with CARLQUIST's (1986) conclusions (from wood

anatomy) that *Retzia* is close to *Stilbaceae*. In our *rbcL* analyses *Retzia* always appears as the sister taxon to *Euthystachys* (*Stilbaceae*). The present study supports the inclusion of the South African *Retzia* in the likewise South African family *Stilbaceae*, as proposed by CARLQUIST (1992) and THORNE (1992). The *rbcL* sequences of *Retzia* and *Euthystachys* are very similar to each other, differing by only eight substitutions, the least divergence between any taxa included in this study. To pinpoint the exact position of *Retzia* and *Euthystachys* in the clade with *Lamiales* s.l. is more difficult. The analyses indicate several different possible relationships, e.g., as a sister group to *Gesneriaceae/Scrophulariaceae/Callitrichaceae/Pedaliaceae* p.p./*Acanthaceae* (Fig. 2) or to *Scrophulariaceae/Callitrichaceae* (Fig. 5). However, nothing in our analyses supports a closer relationship to *Verbenaceae*, here represented by *Verbena*. On the other hand there are indications that *Verbenaceae* are paraphyletic (OLMSTEAD & al. 1993) so there may be genera of *Verbenaceae* that could be closely related to *Stilbaceae*.

Desfontainia spinosa represents a monotypic genus from South America (LEEUWENBERG 1969). The species is morphologically variable, and some authors recognize several species (SCHULTES 1989). It occurs in mountain rain forests along the entire Andes. It is an almost glabrous shrub or a small tree with opposite leaves without stipules but with stipular lines. The leaves are very much like *Ilex aquifolium* (holly) with sharply pointed apices and lobes. The flowers are solitary and axillary. The cylindrical corolla tubes (up to 6.5 cm) are fleshy, and orange to red-coloured with a pale yellow limb. The flowers have five stamens with short filaments inserted near the mouth and the anthers are basifixed. The ovary is superior, five-celled at the base and one-celled at the top. The fruit is a yellow or white berry with several seeds. The leaves are known to be hallucinogenic (LEEUWENBERG 1969, SCHULTES 1977). Gross morphology gives no certain indication of relationship.

The taxonomic position of *Desfontainia* has always been debated. It was first assigned to the *Solanaceae* (HUMBOLDT & BONPLAND 1808), a treatment followed by several authors (cf. BARTLING 1830). A position within the *Gentianaceae* (DON 1831, 1837–1838) or *Aquifoliaceae* (LINDLEY 1836, MEISNER 1836–1843) was also proposed rather early, but ENDLICHER (1836–1841) assigned *Desfontainia* to the monotypic *Desfontainiaceae*, positioned after the *Solanaceae*. A relationship to *Columellia* (*Columelliaceae* or *Saxifragaceae*), another genus with uncertain systematic position (STERN & al. 1969, HUFFORD 1992) has also been suggested (HALLIER 1910, 1912). More recently, however, it has often been connected to *Gentianales*, and in particular the family *Loganiaceae*. A close relationship to the tribe *Potalieae* of the *Loganiaceae* was proposed by BENTHAM (1857). HUTCHINSON (1959) accepted this relationship and included the genus in the family *Potaliaceae*, a treatment followed by AIRY SHAW (1973). In ENGLER'S "Syllabus der Pflanzenfamilien" WAGENITZ (1964) included *Desfontainiaceae* in *Gentianales*, and in many recent systems that position has been accepted, with *Desfontainia* either as a separate family or included in the *Loganiaceae* (TAKHTAJAN 1969, CRONQUIST 1981, DAHLGREN 1983, THORNE 1983, TAKHTAJAN 1987). THORNE (1992), however, removed *Desfontainiaceae* to the *Hydrangeales* close to *Columelliaceae*.

Morphological, palynological, embryological, anatomical, and cytological data contradicted the position of *Desfontainia* within the *Loganiaceae* or the *Gentianales*. Such contradictions were presented early by SOLEREDER (1892–1895) who showed

that *Desfontainia* lacks intraxylary-phloem and colleters, which are characteristic of *Gentianales*. Nevertheless, he retained *Desfontainia* in *Loganiaceae*, but as a genus with doubtful systematic position. In a study of pollen morphology of the *Loganiaceae* (PUNT & LEENHOUTS 1967, PUNT 1980), the authors concluded that *Desfontainia* differs very much from the tribe *Potalieae*, where it was included (HUTCHINSON 1959, AIRY SHAW 1973), but is very similar to *Spigelia anthelmia* (the pollen description, in PUNT, 1980, of *Spigelia* also covers the genus *Desfontainia*). However, these authors have taken the circumscription of *Loganiaceae* for granted and they do not make any comparisons with other families. The pollen grains are described as “3-zonocolporate or may be interpreted as 3-zonocolpate” and with “tectum with perforation of varying size and outline, usually circular”. MENNEGA (1980) referred to personal communications from PUNT saying that palynology supports the affinity between *Desfontainia* and *Columellia* (*Columelliaceae*). Embryological investigations of *Desfontainia* have revealed new interesting features that also contradict a position in the *Gentianales* (MALDONADO DE MAGNANO 1986): the unitegmic ovules are crassinucellate, not tenuinucellate as in most sympetalous plants, and an endothelium is formed, which do not occur in *Loganiaceae*. Furthermore, the endosperm development is of the cellular type, which is found in, e.g., *Solanales*, *Scrophulariales*, and *Dipsacales*, but not in *Gentianales*. Wood anatomy does not support a relationship to the *Loganiaceae* (MENNEGA 1980); long scalariform perforation-plates and scalariform intervessel pitting do not occur in the *Loganiaceae*. On the other hand, a relationship between *Desfontainia* and *Columellia* is supported also by the wood anatomy. The only anatomical differences between them are larger bordered fibre pits in *Columellia*. MENNEGA (1980) pointed to similarities in wood anatomy between *Desfontainia* and *Viburnum* (*Caprifoliaceae*), but this relationship was rejected because of gross morphological differences.

In our analyses of *rbcL* data (Fig. 1), *Desfontainia* is always placed close to the *Dipsacales* and not to the *Gentianales*. The analyses present two different plausible positions of *Desfontainia* in the *Dipsacales* s.l. clade: at the base of the whole *Dipsacales* s.l. (Fig. 3), or at the base of *Lonicera/Symphoricarpos/Valeriana/Dipsacus/Diervilla* (Fig. 2). These results show that *Desfontainia* definitely should be included in further and more extensive analyses of the *Dipsacales* and closely related taxa. Many similarities in anatomical/embryological/cytological characters may support a close relationship. Such similarities occur in wood anatomy (MENNEGA 1980) and embryology (crassinucellate nucellus) between *Desfontainia* and *Viburnum*, and the chromosome base number ($x = 7$) of *Desfontainia* occurs also in *Valerianaceae* and *Dipsacaceae* (GADELLA 1980).

Nicodemia diversifolia [described as *Buddleja indica* (LAMARCK 1785)] is cultivated all over the world, often in greenhouses. It is indigenous in Madagascar, Comores, and the Mascarene Islands, where it occurs in bush land or in open places from the coast to the mountains (LEEUWENBERG 1979). It is a shrub with stellate and glandular hairs and with opposite more or less orbicular, entire to lobed leaves. The inconspicuous flowers are small, yellow to greenish, and they are sessile in terminal or axillary cymes. The five stamens with sessile anthers are included in the corolla tube. The ovary is superior and bicarpellate, and the fruit is a white to yellowish berry with several seeds. This species is generally included in the genus

Buddleja under the section *Nicodemia* (LEEUEWENBERG 1979), the only section of *Buddleja* that possesses baccate fruits. *Buddleja* comprises over 100 species from tropical, subtropical, and temperate regions, and it is the largest of seven genera included in the sympetalous family *Buddlejaceae* (TAKHTAJAN 1987).

The *Buddlejaceae* have been connected to the *Scrophulariaceae* or the *Loganiaceae*, respectively. Originally, the genera were included in *Scrophulariaceae* as a separate tribe, the *Buddlejeae* (JUSSIEU 1789). The affinity between the *Buddlejeae* and *Loganiaceae* was first mentioned by BENTHAM (1857), and in BENTHAM & HOOKER (1876) the tribe was transferred to the *Loganiaceae*. Later it was raised to a subfamily in the *Loganiaceae*, by SOLEREDER (1892–1895), and to a separate family in the *Gentianales* (WILHELM 1910). Nevertheless, a transfer of the *Buddlejaceae* back to the *Scrophulariales* was proposed by WAGENITZ (1959), a treatment that has been followed in recent systems (MELCHIOR 1964, CRONQUIST 1981, DAHLGREN 1983, TAKHTAJAN 1987, CRONQUIST 1988). THORNE (1976) treated the *Buddlejaceae* as part of the *Gentianales*, but later he proposed a position close to the *Stilbaceae* in the *Scrophulariales* (THORNE 1992). LEEUEWENBERG & LEENHOUTS (1980) did not accept the *Buddlejaceae* as a distinct family separated from the *Loganiaceae* but treated it as a tribe of the *Loganiaceae*. They admitted, however, the affinities between the *Buddlejaceae* and the *Scrophulariales*.

The *Buddlejaceae* differ from the *Loganiaceae* and other families of *Gentianales* in several morphological, cytological, embryological, chemical, palynological, and anatomical characters. They have no intraxylary-phloem, no colleters, and the rarely occurring stipules are not homologous to those of the *Gentianales* (HASSELBERG 1937). The typical stellate multicellular hairs of the *Buddlejaceae*, four-armed and two-celled with a short uniseriate stalk, are not present in any taxa of the *Loganiaceae* (SOLEREDER 1892–1895, METCALFE & CHALK 1957, BENDRE 1973). There are also complex glandular hairs with laterally adjacent cells, making up broadened heads or tiers, in the *Buddlejaceae* (BENDRE 1973). These differences were first noticed by SOLEREDER (1892–1895) and led him to recognize the subfamily *Buddlejoideae* in the *Loganiaceae*. GADELLA (1980) reported the basic chromosome number of the *Buddlejaceae* to be $x = 19$, differing from that of the *Gentianales*. Embryology has shown striking dissimilarities between the *Buddlejaceae* and the *Gentianales*, such as cellular endosperm, presence of endothelium, and endosperm haustoria in *Buddlejaceae* (HAKKI 1980). The chemical contents of the *Buddlejaceae* differ also from the *Loganiaceae*. *Buddlejaceae* contain no seco-iridoids but carbocyclic iridoids that link them to the *Scrophulariales* (JENSEN & al. 1975, GERSHENZON & MABRY 1983, JENSEN 1992); i.e., aucubin has been found in the *Buddlejaceae* and *Scrophulariaceae* but does not occur in the *Gentianales* (DANIEL & SABNIS 1979). Palynological investigations reveal a close relationship to the *Scrophulariaceae* but hardly any to the *Loganiaceae* (PUNT & LEENHOUTS 1967, PUNT 1980). Wood anatomy also points to a position in *Scrophulariales* or *Lamiales* (MENNEGA 1980, CARLQUIST 1992). The axial parenchyma (scanty vasicentric) in *Buddlejaceae* is of the same type as in *Scrophulariaceae*, and the imperforate tracheary elements are libriform fibres that do not occur in *Gentianales*.

In earlier molecular analyses (DOWNIE & PALMER 1992, OLMSTEAD & al. 1993) and in our analysis (Figs. 1, 2), *Buddleja* (*Buddlejaceae*) is not closely related to *Gentianales*, but to *Lamiales* s.l. The same is true for *Nicodemia*. It is placed as the

sister taxon to *Verbena* (*Verbenaceae*) in the *Lamiales* s.l. clade, and these two genera are related to *Pedaliaceae* p.p./*Lamiaceae* (Figs. 1, 2) or to *Pedaliaceae* p.p./*Acanthaceae* (Fig. 5). An odd result is that *Nicodemia* is not closest to *Buddleja*, a genus in which it is usually included (LEEUEWENBERG & LEENHOUTS 1980). More sequences of other *Buddlejaceae* are needed to determine if *Nicodemia* truly differs from the rest of this family. *Nicodemia* differs from *Buddleja* by having baccate fruits, a possible autapomorphy for *Nicodemia*; otherwise no important morphological features differentiate between the two genera or support an exclusion of *Nicodemia* from the *Buddlejaceae*.

In conclusion, there is strong molecular support for exclusion of *Desfontainia*, *Nicodemia*, and *Retzia* from the *Gentianales*. *Desfontainia* seems to be close to the *Dipsacales*, but the exact position is not resolved, and *Nicodemia* appears as the sister taxon to *Verbena* (*Verbenaceae*) within the *Lamiales* s.l. clade. *Retzia* belongs in the *Lamiales* s.l. also, very close to *Euthystachys* (*Stilbaceae*). As indicated by DAHLGREN & al. (1979) and CARLQUIST (1986) the best position of *Retzia* seems to be in *Stilbaceae*, where it has been included formally (CARLQUIST 1992, THORNE 1992). To pinpoint the exact positions of *Retzia*/*Euthystachys* and *Nicodemia* within the *Lamiales* s.l. clade and *Desfontainia* within or outside the *Dipsacales* s.l. we must wait for further investigations of these problematic groups.

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Addresses of the authors: BIRGITTA BREMER and LENA STRUWE, Department of Systematic Botany, Uppsala University, Villav. 6, S-752 36 Uppsala, Sweden. — RICHARD G. OLMSTEAD, Department of E.P.O. Biology, University of Colorado, Boulder, Colorado 80309, USA. — JENNIFER A. SWEERE, Laboratory of Molecular Systematics, National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20560, USA.

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