Pl. Syst. Evol. 207: 225–254 (1997)

Plant Systematics and Evolution

Phylogeny of the *Asteridae* s. str. based on *rbc*L sequences, with particular reference to the *Dipsacales*

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Received March 19, 1996; in revised version June 13, 1996

Key words: *Asteridae*, *Dipsacales*, *Apiales*, *Asterales*, *Caprifoliaceae*, *Morinaceae*, *Dipsacaceae*, *Valerianaceae*, *Triplostegia*, *Columellia*, *Desfontainia*, *Bruniaceae*, *Apiaceae*, *Alseuosmiaceae*. – Chloroplast DNA, phylogeny, *rbc*L.

Abstract: The *rbc*L gene of 15 taxa was sequenced and analyzed cladistically together with a large sample of genera representing all main clades of the subclass *Asteridae* in order to determine more precisely the delimitation of the order *Dipsacales* and to elucidate the phylogeny of the families within the order. The cladistic analyses show that the Dipsacales comprise the families *Caprifoliaceae*, *Morinaceae*, *Dipsacaceae*, and *Valerianaceae* including *Triplostegia*. The results also provide a basis for the exclusion of a number of taxa previously placed in the *Dipsacales*, such as *Desfontainia*, *Columellia* and *Adoxaceae* s. l. (including *Sambucus* and *Viburnum*). Ever since the order *Dipsacales* was first suggested by DUMORTIER (1829) and the similar *Caprifoliales* by LINDLEY (1833, 1836), there has been confusion concerning the circumscription of the order, the relations between the included families, their circumscriptions, and the position of the order in a larger context.

***Dipsacales* and *Rubiaceae*.** The order *Dipsacales* is built up around a core of families, namely *Dipsacaceae*, *Valerianaceae* and *Caprifoliaceae*, all considered to be related. Due to the superficial morphological similarities between *Caprifoliaceae* (especially the formerly included genera *Sambucus* and *Viburnum*) on one hand and *Rubiaceae* on the other, early theories (LINNAEUS 1738, JUSSIEU 1789, BARTLING 1830) suggested a close relationship between those. This association was retained well into the 20th century (e.g., TIEGHEM 1909), until workers like UTZSCHNEIDER (1947) and WAGENITZ (1959) with more thorough anatomical and chemical analyses instead of suggested a connection between *Rubiaceae* and *Gentianaceae* leaving *Dipsacales* from them isolated.

***Dipsacales* and the *Cornales*.** After the dispatch of the connections between *Dipsacales* and *Rubiaceae* a completely different theory of relationships emerged, also based on the inclusion of the genera *Sambucus* and *Viburnum* in the *Caprifoliaceae*, and leading to a number of problems. The most obvious one concerns the small and enigmatic genus *Adoxa*. Morphological studies had shown that *Adoxa* features a number of traits especially similar to *Sambucus* (BOLLI 1994). *Adoxa* earlier

had been considered a close relative of the family *Saxifragaceae* connected through *Chrysosplenium*. Furthermore, the superficial morphological similarities between *Viburnum* and *Hydrangea* (of the *Hydrangeaceae* in the *Cornales*) were regarded as an argument for close relations between *Dipsacales* and cornalean groups. The theory was further boosted by phytochemical studies, which were interpreted as strongly supportive of this view (DAHLGREN 1975, 1977, 1980).

***Dipsacaceae* and *Calyceraceae*.** Parallel to the ideas described above, a third view advocated by BAILLON (1880) among others suggested that the headlike inflorescences in *Dipsacaceae* and *Calyceraceae* are homologous, thus implying a close relationship between these families. This theory, and the impact on the sister-group relations of the *Asteraceae* to which *Calyceraceae* undoubtly are related, has been dealt with at length in a number of studies (GUSTAFSSON & BREMER 1995, GUSTAFSSON & al. 1996, and references therein). The similarities between *Dipsacaceae* and *Calyceraceae* now are regarded as merely superficial, and consequently, the position of the order *Dipsacales* remains an open question.

Other suggested relatives. Additional taxa that have been suggested to be closely related to the *Dipsacales* are the families *Alseuosmiaceae* (AIRY-SHAW 1965a), *Desfontainiaceae* (BREMER & al. 1994), *Bruniaceae* (DONOGHUE & al. 1992, GUSTAFSSON & al. 1996), *Apiaceae* (DONOGHUE & al. 1992), and *Columelliaceae* (JUSSIEU 1848, HALLIER 1901), all of which will be considered in the study presented here.

***Dipsacales* and *Columelliaceae*.** The small monogeneric family *Columelliaceae* has been suggested to occupy positions in extremely diverse parts of the system. Suggestions of related taxa include: *Gesneriaceae* (REICHENBACH 1828, BAILLON 1888, FRITSCH 1894, MACBRIDE 1961), *Saxifragaceae-Escallonioideae* (SCHNIZLEIN 1849), particularly *Argophyllum*, *Brexia* and *Roussea* (SOLEREDER 1899, HALLIER 1910, HERZOG 1915, WILLIS & SHAW 1966) or closer to *Hydrangeaceae* and *Loganiaceae* (THORNE 1968), or to *Montinioideae* in *Saxifragaceae* (GENTRY 1993). Positions also have been suggested in *Rubiales* (actually in *Cinchonales* ≈ *Rubiales*; LINDLEY 1853), with the families *Vacciniaceae*, *Onagraceae*, and *Cinchonaceae* ≈ *Rubiaceae* very close to *Caprifoliaceae* (JUSSIEU 1848), or even nested between *Caprifoliaceae* and *Valerianaceae* (HALLIER 1901). Other taxonomic placements include: *Oleaceae* (JUSSIEU 1801, REICHENBACH 1837), *Scrophulariaceae* (KUNTH 1818, BARTLING 1830, HALLIER 1903), *Ebenaceae* (ENDLICHER 1839), *Loganiaceae* (MAOUT & DECAISNE 1873, HOOKER 1875), *Lythraceae* (AGARDH 1858– due to the peculiar anthers which appear to be similar to the ones found in *Cucurbitaceae*, which AGARDH considered closely related) and finally also in the assembly *Pittosporaceae-Grossulariaceae* but anyway "definitely in the order *Rosales*" (CRONQUIST 1968). Recently it was suggested that *Columellia* and *Desfontainia* might be related due to significant similarities in wood anatomy, features shared also by some members of "*Saxifragaceae*" s. l. (CARLQUIST 1992).

***Dipsacales* and *Alseuosmiaceae*.** Another small genus–*Alseuosmia*– was formerly believed to be connected to the *Caprifoliaceae* (FRITSCH 1897). Numerous systematic positions have since then been suggested for *Alseuosmia*, including *Saxifragaceae* (STEENIS 1984; DICKISON 1986, 1989), and *Escalloniaceae-Loganiaceae* (AIRY-SHAW 1965b). The family *Alseuosmiaceae* consists of the three genera *Alseuos-*

mia, *Crispiloba* and *Wittsteinia* all of which were recently shown to belong to the *Asterales* s. l. (GUSTAFSSON & al. 1996). There they form a sister-group to *Argophyllaceae* (*Argophyllum* and *Corokia*). A position within the *Asterales* is strongly supported by *rbc*L sequence data (GUSTAFSSON & al. 1996) and morphology and anatomy have been suggestive of a position close to *Argophyllum* (GARDNER 1978a, b). The reason for including members of the *Alseuosmiaceae* also in this analysis is to show the stability of the previously obtained grouping also in the presence of a wider sampling of *Caprifoliaceae*.

Molecular data. In recent years a large number of nucleotide sequences have become available for a wide variety of studies. Within the field of phylogenetic studies of plants, variation in nucleotide sequences of the gene *rbc*L – coding for the enzyme ribulose-1,5-bisphosphate carboxylase and residing in the chloroplast genome – has been the most explored thus far. The large number of available sequences have made possible broad studies and comparisons aiming at a wide variety of problems.

It has been shown (ALBERT & al. 1994a, b) that in some instances – especially at higher taxonomic levels – built-in functional constraints of the variation of the nucleotide sequences may have contributed to erroneous results and conflict between different data sets (cf. ALBERT & al. 1994a, b, and references therein). These problems are due to accumulated convergent mutations, and it follows that the risk of obtaining erroneous results increases significantly when comparing sequences from very distantly related taxa. Two methods of addressing these potential problems are a priori by searching for larger "motifs", i.e. groups consisting of several nucleotides, or a posteriori by evaluating each of the characters contributing to the hierarchical structure of the data – thereby lowering the risk of focusing on superficial similarities and saturated mutations.

Despite the above-mentioned problems, studies of the variation in the nucleotide sequence of the gene *rbc*L nevertheless have contributed new and unique information in a number of cases. The most important property of the nucleotide sequence data is the freedom from preconceived ideas. This is in contrast to morphological data where traditional views may influence character coding and obscure true homologies. These points have been demonstrated in a number of studies (e.g., DONOGHUE & al. 1992, CHASE & al. 1993, BREMER & al. 1994, GUSTAFSSON & al. 1996).

The aims of the present study are to investigate by means of *rbc*L sequences the circumscription and internal relationships of the order *Dipsacales* as well as to identify the closest outgroups to the order *Dipsacales*.

Materials and methods

Taxon sampling. A total of 15 new and previously unpublished *rbc*L sequences (listed in Table 1) have been analysed together with four sequences kindly made available by QIU-YUN XIANG and DOUGLAS E. SOLTIS (listed in Table 2) and 127 sequences obtained from the EMBL and NCBI/GenBank databases (all of which are presented in Table 3). The considerable number of suggested systematic positions of some of the included taxa – especially *Columellia* in the *Columelliaceae* and *Brunia* in *Bruniaceae* – has called for the inclusion of a fairly wide variety of taxa.

A group of prime importance for the understanding of basal relationships in the *Asteridae* is the *Saxifragaceae-Escallonioideae* sensu ENGLER (1930). This diverse and highly unnatural group comprises several taxa which belong in or near the *Asteridae* s. str. ("asterid II") clade. According to phylogenetic analyses based on *rbc*L sequences (CHASE & al. MORGAN & SOLTIS 1993, GUSTAFSSON & al. 1996, XIANG & SOLTIS 1996) the genera *Abrophyllum*, *Argophyllum*, *Corokia*, *Escallonia*, *Polyosma* and *Quintinia* all belong in this assemblage. A number of genera remain to be sampled for DNA sequencing, but another two, *Anopterus* and *Cuttsia*, were included in the present study in the hope that this could improve the understanding of the basal relationships among *Asteridae*. *Anopterus* seems to be a morphologically relatively isolated genus (placed in a tribe of its own by ENGLER 1930), whereas *Cuttsia* shows strong morphological affinity to *Abrophyllum*, which in turn belongs within the *Asterales*, one of the major clades within the *Asteridae* s. str. (GUSTAFSSON & al. 1996).

In a recent study by XIANG & SOLTIS (1996) the genus *Polyosma* was indicated to occupy a position close to *Viburnum*. *Polyosma*, comprising approximately 50 species of trees and shrubs in tropical south-east Asia and Australia, has been placed in the families *Polyosmataceae* (WILLIS & SHAW 1966), *Saxifragaceae–Escallonioideae* (ENGLER 1930) or close to the *Hydrangeaceae* (HUTCHINSON 1959).

We also have included a hitherto unsequenced species of the genus *Hydrostachys*, although this genus has not been associated directly with *Dipsacales*. The reason for the inclusion of this aberrant aquatic genus from Africa and Madagascar is that its traditional taxonomical position has been questioned recently (HEMPEL & al. 1995). *Hydrostachys* was placed close to *Lamiales* and *Scrophulariales* by DAHLGREN (1980) and TAKHTAJAN (1987) or as in CRONQUIST (1981) close to *Callitriche* (the latter by molecular sequence data shown to be close to the *Scrophulariaceae*, e.g., OLMSTEAD & al. 1992). THORNE (1992) proposed that *Hydrostachys* ought to be included in *Bruniales*, which is represented in this study with a new sequence of *Brunia*. However, that position was contradicted by a new hypothesis presented by HEMPEL & al. (1995), based on a study of *rbc*L sequences, which suggested a position for *Hydrostachys* within the family *Hydrangeaceae*. The accuracy of that position–or any placement in the *Cornales* – has been questioned, because *Hydrostachys* lacks any clear morphological synapomorphies with these taxa. Instead it is reputed to possess asterid synapomorphies among its morphological features and it has been suggested that the closest relatives should be sought among African *Asteridae* (L. HUFFORD, pers. comm.). We found it relevant to include *Hydrostachys* in our study because the suggested relative *Hydrangea* has been considered to be close to *Viburnum* (DAHLGREN 1975, 1977, 1980), and because previous *rbc*L studies have suggested a position for *Bruniaceae* (i.e. *Berzelia*) in the vicinity of the *Dipsacales* (e.g., DONOGHUE & al. 1992, GUSTAFSSON & al. 1996). Our idea was to verify the accuracy of the previously published sequence (HEMPEL & al. 1995) by inclusion of another species of *Hydrostachys*, and to explore whether a different taxon sampling would affect the position of the genus.

In order to maximize the sampling in the *Dipsacales* and *Apiales* the previously unpublished sequence of *Steganotaenia araliacea* was included. This species is one of the very few arborescent members of the "Apioid taxa" of the family *Apiaceae*. The genus *Steganotaenia* is entirely African and comprises three species (one perennial herb and two small trees) considered to be very close to the larger genus *Peucedanum* (THULIN 1991).

Laboratory work. Total DNA was extracted from fresh or silica gel dried leaves (CHASE & HILLS 1991), according to the methods by SAGHAI-MAROOF & al. (1984) and DOYLE & DOYLE (1987). Double-stranded DNA of the *rbc*L gene was amplified by the polymerase chain reaction (PCR) using two synthetic primers (OLMSTEAD & al. 1992). The 5′-end primer is identical to the first 26 nucleotides of *rbc*L of tobacco, *Nicotiana tabacum* L., and the 3′-end

primer corresponds to a region approximately 100 nucleotides outside the coding region. For one of the 15 taxa studied (*Columellia oblonga*), PCR amplification with this primer combination proved unsuccessful, in spite of repeated attempts. For this taxon the 3'-primer was replaced by an internal primer attaching at position 1375, near the end of the gene. A second run with asymmetric amplification was performed to obtain single-stranded DNA (KALTENBOECK & al. 1992). The single-stranded DNA was sequenced using internal primers designed by G. ZURAWSKI at the DNAX Research Institute. The 15 new sequences (Table 1) have been submitted to the European Molecular Biology Laboratory (EMBL) archives.

Methods of analysis. To investigate the systematic position of the taxa studied, the obtained sequences were analyzed together with 131 sequences already published. The latter were obtained directly from the authors (QIU-YUN XIANG and DOUGLAS E. SOLTIS, pers. comm.), from the National Center for Biotechnology Information (NCBI) database "GenBank" or from the European Molecular Biology Laboratories (EMBL) "Nucleotide Sequence Database", and are listed in Table 3. The previously published sequences for the first analysis were sampled with the aim to represent most major lineages within the "asterid" and "rosid" groups sensu CHASE & al. (1993). The strategy was further to include all available sequences of *Dipsacales* and *Apiales*. The tree resulting from the first cladistic analysis was oriented with *Cercidiphyllum japonicum* at the base, in agreement with the trees obtained by CHASE & al. (1993), and the second tree in concordance with results from the first analysis.

The data matrices for the phylogenetic analyses comprise characters corresponding to nucleotide positions 27 to 1428 of the *rbc*L sequence. The "C/G-positions" 172, 173, 1132, and 1133, which are known to give ambigous results depending on whether the sequencing is performed with "forward" or "reverse" primers, were excluded from the analysis. For the taxon amplified with an internal primer, 52 of the positions at the end of the gene are missing. In the analyses partial uncertainties (i.e. IUPAC symbols other than A, C, G, or T) were all treated as uncertainty (N) in order to avoid application of the very time-consuming equate-macro accounting for such ambiguities in one of the programs used. All substituted ambiguous codings are listed in Table 4, and a comparison with the entire "large" matrix shows that a very limited amount of information is lost in this procedure, because the majority of these codings would have been interpreted as phylogenetically uninformative (either as invariant or as autapomorphies depending on the alternative chosen by the algorithm) during the analysis. More significant is the necessary introduction of some gaps in order to align four of the sequences obtained from NCBI/EMBL archives. These manipulations are listed at the end of Table 3.

Parsimony analyses were conducted using PAUP versions 3.1.1 (SWOFFORD 1993) and 4.0d45 (SWOFFORD 1996) under the assumptions of Fitch parsimony (FITCH 1971) as well as by Jac 4.4 (FARRIS & al. 1996) and PAUP 4.0d45 performing parsimony jackknifing.

Parsimony analysis using PAUP. The "large" matrix was analyzed in two steps. First 500 repetitive runs with PAUP using random addition sequences of the taxa followed by the "subtree pruning regrafting" (SPR) branch swapping algorithm were performed. From each of these 500 runs one single tree was saved, thus yielding 500 "primary trees" of varying length. All of these 500 trees (regardless of length) were then used as starting-trees for the more efficient, but also more time-consuming "tree bisection reconnection" (TBR) branch swapping algorithm.

On the basis of the results from the analysis of the "large" matrix, a subset of taxa forming a monophyletic group in the strict consensus tree (indicated in Fig. 1) was selected. These taxa, forming the "small" matrix, was further analyzed by 100 repetetive runs with PAUP using random addition sequences of the taxa followed by the TBR branch swapping

algorithm. The results from this analysis was then used as the basis for a character reweighted according to the characters retention index (ri) values using the successive approximations weighting method devised by FARRIS (1969), as implemented in PAUP. The matrix was then reanalyzed repeatedly with the same options as in the first run.

Jackknifing with Jac and PAUP. Two different computer programs, a "prerelease" version of the program Jac 4.4 for Macintosh computers (FARRIS & al. 1996) and the earlier mentioned PAUP 4.0d45 were used to perform a parsimony-jackknifing analysis of both matrices. Discussion about the theoretical background of parsimony jackknifing relates mainly to the paper by FARRIS & al. (1996).

By a jackknifing procedure, a portion ($e^{-1} \approx 36.79\%$ in Jac, adjustable in PAUP) of the characters in the matrix are deleted. By this mechanism the program repeatedly constructs a large number of new matrices, so called "replicates", which then are subject to a fast parsimony analysis. The procedure was repeated a large number of times (10000 for Jac – equalling the maximum number allowed by the program – and 1000 for PAUP) for both matrices. It has been shown (FARRIS & al. 1996) that with a removal probability of $\approx 37\%$, a jackknife value (fraction) of more than 63% corresponds to a node supported by at least one unambiguous character. Naturally this support can also consist of the additive support from a concordant set of several less unambigous characters (which is often the case in nucleotide sequence data). In our interpretations of the results we have regarded groups with jackknife values $\geq 63\%$ as well supported by the data.

Nodes with a jackknife value of more than 50% are indicative of some support for the defined group. Nodes with less than 50% jackknife values, however, may be in conflict with other groupings and are in the versions of Jac and PAUP used here automatically excluded by the programs and not indicated in the presented tree. In jackknife analysis as implemented in PAUP as well as in the windows version of Jac, the "cut-off level" at which branches are collapsed can be manually adjusted, but not to a value below 50%. The results from the jackknife-analyses are shown in Figs. 1 and 3, where "white" nodes correspond to jackknife values between 50 and 63%, and "black" thick nodes have jackknife values exceeding 63%. For designation of "white" and "black" thick nodes the results of jackknife analysis of the large matrix has been used for Fig. 1, and best result from either analysis has been used in Fig. 3, all jackknife values are also listed in Table 5.

Support analysis. In order to further evaluate the stability of different branches in the obtained trees, a Bremer support analysis (BREMER 1988, KÄLLERSJÖ & al. 1992, BREMER 1994) was performed on the "small" matrix, making use of the computer program "Autodecay 3.0" (TORSTEN ERIKSSON & NIKLAS WIKSTRÖM, pers. comm.) in combination with PAUP in the generalized manner described by BREMER (1994). Furthermore, two bootstrap analyses (FELSENSTEIN 1985) with 100 replicates and TBR swapping and 1000 replicates and no swapping respectively was also performed on the "small" matrix using PAUP. Branch lengths, Bremer support values, and bootstrap values are summarized and listed in Table 5, according to the node numbers indicated in the subtree shown in Fig. 3.

Matrix check. The computer program GACT (ROLF STAFLIN & KARL-KÖNIG KÖNIGSSON, pers. comm.) performs a search for larger, randomly generated 'motifs' or 'strings' among the nucleotide sequences; from the latter it constructs a binary matrix according to the method described by ALBERT & al. (1994a, b). This binary matrix was then analyzed using PAUP in the same manner as described for the sequence matrix.

Table 1. Enumeration of previously unpublished sequences. Species are listed alphabetically, with family classification according to the system of TAKHTAJAN (1987)

Species and author	Family	NCBI/EMBL#	Voucher
Anopterus macleayanus F. MUELL.	*Escalloniaceae*	Y10673	TELFORD s.n. (CBG)
Brunia albiflora PHILLIPS	*Bruniaceae*	Y10674	GUSTAFSSON 239 (UPS)
Columellia oblonga RUIZ & PAV.	*Columelliaceae*	Y10675	BREMER 3374 (UPS)
Cuttsia viburnea F. MUELL.	*Escalloniaceae*	Y10676	CARROLL & TELFORD 1191 (CBG)
Hydrostachys cf. *angustisecta* ENGL.	*Hydrostachyaceae*	Y10708	BREMER 3089 (UPS)
Knautia intermedia PERNH. & WETTST.	*Dipsacaceae*	Y10698	BREMER 3317 (UPS)
Morina coulteriana ROYLE.	*Morinaceae*	Y10706	BACKLUND 263 (UPS)
Nardostachys jatamansi DC.	*Valerianaceae*	Y10705	WANG LI-SONG 9364 (UPS)
Patrinia rupestris (PALL.) DUFR.	*Valerianaceae*	Y10704	BREMER 3113 (UPS)
Phyllactis bracteata WEDD.	*Valerianaceae*	Y10703	BREMER 3405 (UPS)
Pterocephalus lasiospermus LINK.	*Dipsacaceae*	Y10702	BACKLUND 254 (UPS)
Steganotaenia araliaceae HOCST.	*Apiaceae*	Y10701	MANKTELOW & al. s.n. (UPS)
Triplostegia glandulifera WALL. ex DC.	*Triplostegiaceae*	Y10700	WANG LI-SONG 93-13327 (UPS)
Valeriana hirtella H. B. & K.	*Valerianaceae*	Y10699	BREMER 3396 (UPS)
Valerianella locusta BETCKE.	*Valerianaceae*	Y10707	BACKLUND 258(UPS)

Table 2. Enumeration of sequences supplied by XIANG & SOLTIS (1996). Species are listed alphabetically, with family classification according to the system of TAKHTAJAN (1987)

Species	Family	NCBI/EMBL#
Aralidium pinnatifidum	*Aralidiaceae*	s.n.
Melanophylla pachypoda	*Melanophyllaceae*	U50254
Polyosma cunninghamii	*Polyosmataceae*	s.n.
Toricellia tilifolia	*Toricelliaceae*	s.n.

Table 3. Enumeration of previously published sequences extracted from NCBI archives that were used in the analyses. Species are listed alphabetically, with family classification according to the system of TAKHTAJAN (1987)

Species	Family	NCBI/EMBL no.
Abrophyllum ornans	*Escalloniaceae*	X87375
Acanthus montanus	*Acanthaceae*	Li2592
Acer saccharum	*Aceraceae*	L13181
Acicarpha tribuloides	*Calyceraceae*	X87376
Adoxa moschatellina	*Adoxaceae*	L01883
Alseuosmia macrophylla	*Alseuosmiaceae*	X87377
Anagallis arvensis	*Primulaceae*	M88343
Anthocleista grandiflora	*Loganiaceae*	L14389
Antirrhinum majus	*Scrophulariaceae*	L11688
Apium graveolens	*Apiaceae*	L01885
Aralia spinosa	*Aliaceae*	L11166
Argophyllum sp.	*Argophyllaceae*	X87379
Aucuba japonica	*Aucubaceae*	L11210
Berzelia lanuginosa	*Bruniaceae*	L14391
Boopis anthemoides	*Calyceraceae*	L13860
Borago officinalis	*Boraginaceae*	L11680
Brassica oleracea	*Brassicaceae*	M88342
Brexia madagascarensis	*Brexiaceae*	L11176
Brunonia australis	*Brunoniaceae*	X87380
Byblis liniflora	*Byblidaceae*	L01891
Byrsonima crassifolia	*Malpighiaceae*	L01892
Callitriche heterophylla	*Callitrichaceae*	L11681
Campanula ramosa	*Campanulaceae*	L13861
Camptotheca acuminata	*Nyssaceae*	L11211
Carthamnus [sic!] *tinctorius*	*Asteraceae*	L13862
Cercidiphyllum japonicum	*Cercidiphyllaceae*	L11673
Chiococca alba	*Rubiaceae*	L14394
Chrysosplenium iowense	*Saxifragaceae*	L19935
Clarkia xantiana	*Onagraceae*	L01896
Clermontia kakeana	*Campanulaceae*	L18789
Clethra alnifolia	*Clethraceae*	L12609

Table 3 (continued)

Codonopsis ovata	*Campanulaceae*	L18797
Conium maculatum	*Apiaceae*	L11167
Convolvulus tricolor	*Convolvulaceae*	L11683
Coriandrum sativum	*Apiaceae*	L11676
Cornus mas	*Cornaceae*	L11216
Corokia cotoneaster	*Argophyllaceae*	L11221
Crispiloba disperma	*Alseuosmiaceae*	X87382
Cucurbita pepo	*Cucurbitaceae*	L21938
Cyphia elata	*Cyphiaceae*	L18796
Cyphocarpus rigescens	*Cyphiaceae*	L18792
Dampiera spicigera	*Goodeniaceae*	X87383
Dasyphyllum dicanthoides	*Asteraceae*	L13863
Davidia involucrata	*Davidiaceae*	L11223
Desfontainia spinosa	*Desfontainiaceae*	Z29670
Diervilla sessilifolia	*Caprifoliaceae*	Z29672
Digitalis purpurea	*Scrophulariaceae*	L01902
Dillenia indica	*Dilleniaceae*	L01903
Diplopanax stachyanthus	*Cornaceae*	L11224
Dipsacus sativus	*Dipsacaceae*	L13864
Donatia fascicularis	*Donatiaceae*	X87385
Eremosyne pectinata	*Eremosynaceae*	L47969
Escallonia coquimbensis	*Escalloniaceae*	L11183
Eucommia ulmoides	*Eucommiaceae*	L01917
Fagus sylvatica	*Fagaceae*	L13340
Fouquieria splendens	*Fouquieriaceae*	L11675
Francoa sonchifolia	*Frankoaceae*	L11184
Gardenia thunbergia	*Rubiaceae*	X83637
Garrya elliptica	*Garryaceae*	L01919
Gentiana procera	*Gentianaceae*	L14398
Geranium grandiflorum	*Geraniaceae*	L01920
Goodenia ovata	*Goodeniaceae*	X87386
Gossypium hirsutum	*Malvaceae*	X15886
Griselinia lucida	*Griseliniaceae*	L11225
Hedera helix	*Araliaceae*	L01924
Heliotropium arborescens	*Boraginaceae*	L14399
Helwingia japonica	*Helwingiaceae*	L11226
Humiria balsaminifera	*Humiriaceae*	L01926
Humulus lupulus	*Cannabaceae*	U02729
Hydrangea macrophylla	*Hydrangeaceae*	L11187
Hydrophyllum virginianum	*Hydrophyllaceae*	L01927
Hydrostachys multifida	*Hydrostachyaceae*	U17879
Ilex crenata	*Aquifoliaceae*	L01928
Itea virginica	*Iteaceae*	L11188
Kopsia fruticosa	*Loganiaceae*	L14402
Lactuca sativa	*Asteraceae*	L14073
Lechenaultia heteromera	*Goodeniaceae*	X87388
Ligustrum vulgare	*Oleaceae*	L11686
Lobelia erinus	*Lobeliaceae*	L13930

Table 3 (continued)

Lonicera orientalis	*Caprifoliaceae*	X87389
Ludwigia peruviana	*Onagraceae*	L10221
Manikara zapota	*Sapotaceae*	L01932
Medicago sativa	*Fabaceae*	X04975
Menyanthes trifoliata	*Menyanthaceae*	L14006
Moschopsis rosulata	*Calyceraceae*	X87390
Nemacladus ramosissmus	*Nemacladaceae*	L18791
Nemopanthus mucronatus	*Aquifoliaceae*	X69747
Nephrophyllidium crista-galli	*Menyanthaceae*	X87391
Nicotiana tabacum	*Solanaceae*	Z00044
Nyssa ogeche	*Nyssaceae*	L11228
Osyris lanceolata	*Santalaceae*	L11196
Oxalis dillenii	*Oxalidaceae*	L01938
Paeonia tenuifolia	*Paeoniaceae*	L13187
Parnassia fimbriata	*Parnassiaceae*	L01939
Pelargonium capitatum	*Geraniaceae*	L14702
Pentaphragma ellipiticum	*Pentaphragmataceae*	L18794
Pentas lanceolata	*Rubiaceae*	L13931
Phelline comosa	*Phellinaceae*	X69748
Phyllachne uliginosa	*Stylidiaceae*	X87393
Phyllonoma laticuspis	*Dulongiaceae*	L11201
Pittosporum japonicum	*Pittosporaceae*	L11202
Polemonium reptans	*Polemoniaceae*	L11687
Prunus laurocerasus	*Rosaceae*	U06809
Pterostemon rotundifolius	*Pterostemonaceae*	L11203
Quintinia verdonii	*Escalloniaceae*	X87394
Rhamnus catharticus	*Rhamnaceae*	L13189
Rhododendron hippophaeoides	*Ericaceae*	L01949
Ribes aureum	*Grossulariaceae*	L11204
Sambucus racemosa	*Sambucaceae*	L14066
Sanicula gregari	*Apiaceae*	L11170
Sarracenia flava	*Sarraceniaceae*	L01952
Saxifraga integrifolia	*Saxifragaceae*	L01953
Scaevola frutescens	*Goodeniaceae*	L13932
Sedum rubrotinctum	*Crassulaceae*	L01956
Sphenoclea zeylanica	*Sphenocleaceae*	L18798
Streptocarpus holstii	*Gesneriaceae*	L14409
Strychnos nux-vomica	*Loganiaceae*	L14410
Stylidium graminifolium	*Stylidiaceae*	L18790
Symphoricarpos albus	*Caprifoliaceae*	L11682
Vahlia capensis	*Vahliaceae*	L11208
Valeriana officinalis	*Valerianaceae*	L13934
Viburnum acerifolia	*Viburnaceae*	L01959
Viburnum rhytidophyllum	*Viburnaceae*	X87398
Villarsia calthifolia	*Menyanthaceae*	L11685
Viola soraria	*Violaceae*	L11674
Vitis aestivalis	*Vitaceae*	L01960
Wittsteinia vacciniacea	*Alseuosmiaceae*	X87399

Table 3 (continued)

Deliberately inserted "N" for "missing data" to obtain sequence alignment:	
Taxon	At position
Cyphia elata	528, 672, 927, 933
Cyphocarpus rigescens	98, 563, 564, 565
Nemacladus ramosissimus	562, 563, 564, 565, 566, 567, 722, 963, 1000, 1001, 1002, 1020, 1021
Sphenoclea zeylanica	133, 134, 135

Results

The large matrix. The first part of the "large" analysis yielded 500 unique trees of varying length to be used as starting trees for the second part. The second part of this analysis, using the more powerful TBR branch swapping, retrieved 23906 equally parsimonious trees with a length of 4789 steps, a consistency index (CI; KLUGE & FARRIS 1969) of 0.2408 and a retention index (RI; FARRIS 1989) of 0.4980. The strict consensus tree is shown in Fig. 1. Included in the same Figure is information obtained by parsimony jackknifing. These results are compatible (viz., no branches supported in the jackknife analyses are lacking in the tree obtained by parsimony analysis with PAUP).

According to the results from the PAUP analysis of the "large" matrix, all the taxa tested for affinity with *Asteridae* s. str. – marked with a bullet (•) in Fig. 1 – proved to belong within this group. The group is monophyletic and marked with an arrow in the strict consensus of the 23906 trees retrieved. The analysis performed with parsimony jackknifing did not recognize a support exceeding 50% for the entire *Asteridae* s. str. clade, as indicated in Fig. 1. and Table 5. Several of the larger groups that belong to the *Asteridae* s. str. according to the PAUP analysis have jackknife values well exceeding 50%, however.

The small matrix. The "small" matrix resulted in 48 equally parsimonious trees of 1850 steps, and with a CI of 0.4141 and a RI of 0.6068 (KLUGE & FARRIS 1969 and FARRIS 1989 respectively). The strict consensus of these 48 trees is shown in Fig. 2. After one round of successive weighting one single most parsimonious tree was obtained, a result thereafter being stable. This single tree had a topology identical to one of the 48 trees retained from the equally weighted matrix. This tree, shown in Fig. 3, will be selected for the further discussions below.

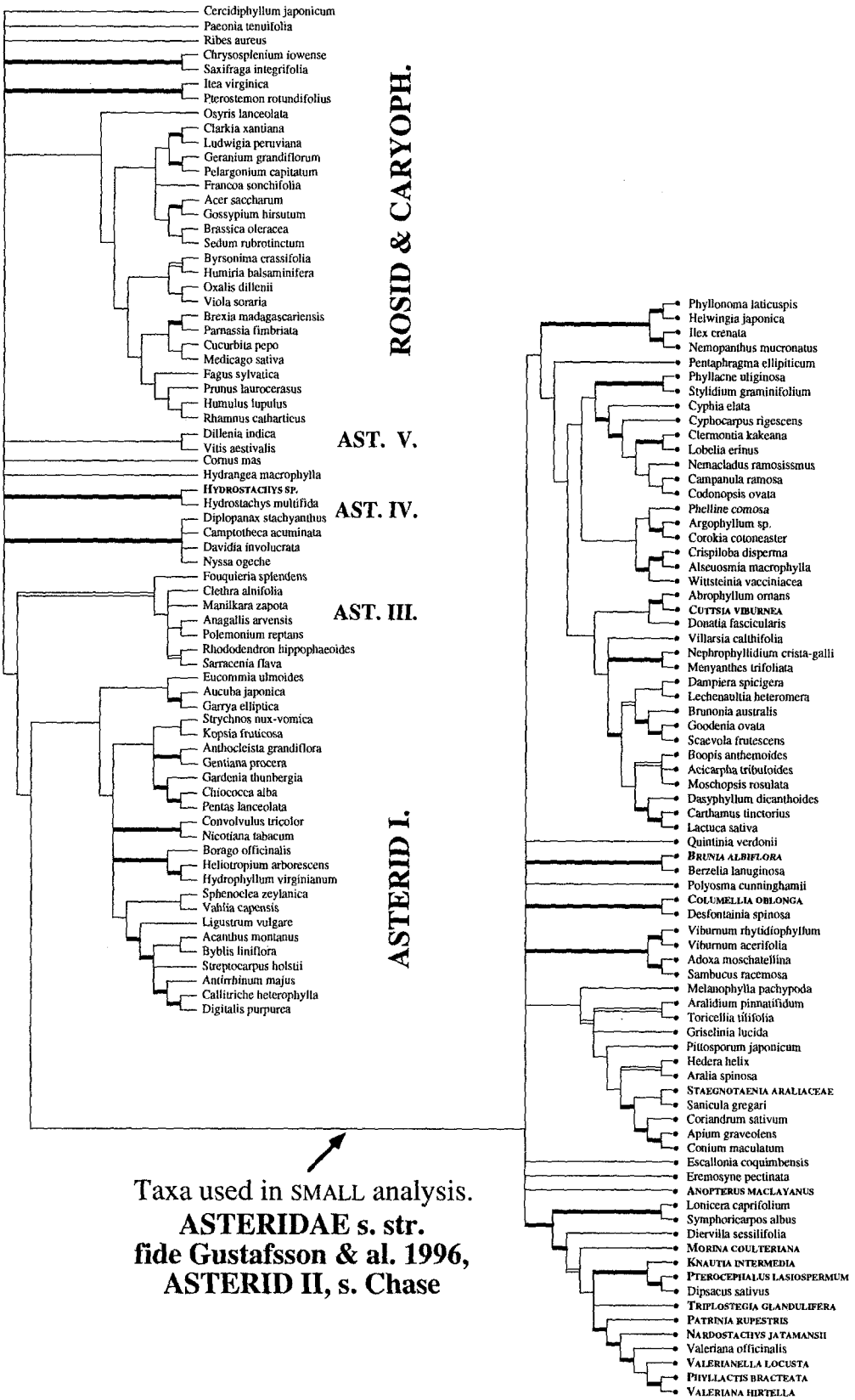
Results with reference to Fig. 3. Basal in the *Asteridae* s. str. a major dichotomy divides the subclass into two clades. One of these consists of the *Ilex*-clade together with the order *Asterales* s. l., and the other of the *Dipsacales-Apiales* complex.

The order *Asterales* s.l. is relatively well supported (branch length 10 steps, bootstrap value < 50%, jackknife value 64.8%, Bremer support 5). One of the taxa sequenced in the present study, *Cuttsia*, has a very strongly supported position within this clade, as the sister-group to *Abrophyllum*. Apart from these genera, the *Asterales* comprise the families *Asteraceae*, *Calyceraceae*, *Goodeniaceae*, *Menyanthaceae*, *Donatiaceae*, *Campanulaceae* s.l., *Stylidiaceae*, *Argophyllaceae*, *Phellinaceae*, *Alseuosmiaceae*, and *Pentaphragmataceae*.

Table 4. Enumeration of changes made in sequences obtained from GenBank/EMBL Sequence Database. All "ambigous codings" following the IUPAC standard found in sequences included in the analysis have been changed to "N" for "unknown" (= A/C/G/T) in the analysed matrices to avoid time-consuming equate-macros. Data in this Table indicate potential loss of information, and subsequent possible implications of alternative interpretations of the "ambigous IUPAC codings". Additional, "N" for unknown have been inserted at 24 places in sequences in order to obtain sequence alignment. The symbol for "unknown/missing data" rather than "gap" is used as no instances are known where deletions have been detected in the *rbc*L-gene

Taxon	Position	Coding	Alts. in matrix	Implications
Unequivocal and thus, in this matrix, uninformative codings				
Eremosyne pectinata	61	R = A/G	**A**	A in all taxa
Eremosyne pectinata	1163	S = C/G	**C**	C in all taxa
Nephrophyllidium crista-galli	37	K = G/T	**T**	T in all taxa
Nephrophyllidium crista-galli	1352	S = C/G	**G**	G in all taxa
Nephrophyllidium crista-galli	1353	S = C/G	**G**	G in all taxa
Gardenia thunbergia	451	S = C/G	**C**	C in all taxa
Gardenia thunbergia	452	S = C/G	**C**	C in all taxa
Gardenia thunbergia	580	H = A/C/T	**C**	C in all taxa
Lechenaultia heteromera	454	S = C/G	**C**	C in all taxa
Lechenaultia heteromera	455	S = C/G	**C**	C in all taxa
Equivocal codings, in this matrix with pronounced tendencies of distribution				
Brunia albiflora	354	Y = C/T	C/G/**T**	T in 140 taxa; G in bot sequences of *Hydrostachys*; C in *Brassica oleracea*, *Medicago sativa* and *Sarracenia flava*
Cercidiphyllum japonicum	341	M = A/C	A/**C**	C in 144 taxa; A in *Medicago*
Cercidiphyllum japonicum	354	Y = C/T	C/G/**T**	T in 140 taxa; G in both sequences of *Hydrostachys*; C in *Brassica oleracea*, *Medicago sativa* and *Sarracenia flava*
Cuttsia viburnea	280	R = A/G	A/C/**G**	G in 140 taxa; A in *Acanthus montanus*, *Convolvulus tricolor*, *Sphenoclea zeylanica*, *Streptocarpus holstii*; C in *Callitriche heterophylla*
Desfontainia spinosa	1341	S = C/G	**G**/A	G in 134 taxa; A in 11 taxa scattered in the system.
Eremosyne pectinata	62	R = A/G	**A**/G	A in 141 taxa; G in *Griselinia lucida*, *Ludwigia peruviana*, *Scaevola frutescens*, *Hedera helix*
Gardenia thunbergia	391	Y = C/T	**C**/G/T	C in 141 taxa; G in *Aralidium pinnatifidum*, *Brassica oleracea*; T in *Nemopanthus mucronatus*
Lechenaultia heteromera	42	R = A/G	**A**/G	A in 130 taxa, G in 15 taxa scattered in the system
Lechenaultia heteromera	453	S = C/G	A/C/**G**/T	G most common; A in 16 taxa scattered in the system; T in *Anthocleista grandiflora*, *Gentiana procera*, *Medicago sativa*, *Paeonia tenuifolia*; C in *Acer saccharum*, *Pentas lanceolata*
Strychnos nux-vomica	943	S = C/G	**G**/C	G in 144 taxa; C in *Desfontainia spinosa*
Strychnos nux-vomica	944	S = C/G	G/**C**	C in 144 taxa. G in *Desfontainia spinosa*
Equivocal codings, in this matrix with diffuse distributions and thus possibly indicating loss of information				
Eremosyne pectinata	168	R = A/G	A/C/G	highly variable position
Eremosyne pectinata	1164	S = C/G	A/C/G/T	highly variable position, T most common; C in all Dipsacales and some other taxa; G in *Borago officinalis*, *Davidia involucrata*, *Dillenia indica*, *Heliotropium arborescens*, *Oxalis dillenii*; A in *Hydrophyllum virginianum*
Gardenia thunbergia	393	Y = C/T	C/G/T	highly variable position

Fig. 1. Strict consensus from the 23906 equally parsimonious trees resulting from parsimony analysis of the "large" *rbc*L sequence data matrix. Taxa in capital letters have been sequenced for this study and are not previously published. Full taxon names and vouchers for these are given in Table 1. Sequences from the other taxa are listed in Tables 2 and 3. Support obtained from jackknife analyses of the "large" matrix are indicated with white, thick nodes for support in the range 50–63%, black thick nodes for support exceeding 63%. The taxa belonging to the *Asteridae* s. str., used also in the "small" matrix, are indicated with a vertical bar and a bullet (•) in front of the taxon names. The node defining the *Asteridae* s. str. is indicated with an arrow


Cercidiphyllum japonicum
Paeonia tenuifolia
Ribes aureus
Chrysosplenium iowense
Saxifraga integrifolia
Itea virginica
Pterostemon rotundifolius
Osyris lanceolata
Clarkia xantiana
Ludwigia peruviana
Geranium grandiflorum
Pelargonium capitatum
Francoa sonchifolia
Acer saccharum
Gossypium hirsutum
Brassica oleracea
Sedum rubrotinctum
Byrsonima crassifolia
Humiria balsaminifera
Oxalis dillenii
Viola soraria
Brexia madagascariensis
Parnassia fimbriata
Cucurbita pepo
Medicago sativa
Fagus sylvatica
Prunus laurocerasus
Humulus lupulus
Rhamnus catharticus
ROSID & CARYOPH.
Dillenia indica
Vitis aestivalis
AST. V.
Cornus mas
Hydrangea macrophylla
HYDROSTACHYS SP.
Hydrostachys multifida
AST. IV.
Diplopanax stachyanthus
Camptotheca acuminata
Davidia involucrata
Nyssa ogeche
Fouquieria splendens
Clethra alnifolia
Manilkara zapota
AST. III.
Anagallis arvensis
Polemonium reptans
Rhododendron hippophaeoides
Sarracenia flava
Eucommia ulmoides
Aucuba japonica
Garrya elliptica
Strychnos nux-vomica
Kopsia fruticosa
Anthocleista grandiflora
Gentiana procera
Gardenia thunbergia
Chiococca alba
Pentas lanceolata
Convolvulus tricolor
Nicotiana tabacum
Borago officinalis
Heliotropium arborescens
Hydrophyllum virginianum
Sphenoclea zeylanica
Vahlia capensis
Ligustrum vulgare
Acanthus montanus
Byblis liniflora
Streptocarpus holstii
Antirrhinum majus
Callitriche heterophylla
Digitalis purpurea
ASTERID I.
Phyllonoma laticuspis
Helwingia japonica
Ilex crenata
Nemopanthus mucronatus
Pentaphragma ellipticum
Phyllacne uliginosa
Stylidium graminifolium
Cyphia elata
Cyphocarpus rigescens
Clermontia kakeana
Lobelia erinus
Nemacladus ramosissmus
Campanula ramosa
Codonopsis ovata
Phelline comosa
Argophyllum sp.
Corokia cotoneaster
Crispiloba disperma
Alseuosmia macrophylla
Wittsteinia vacciniacea
Abrophyllum ornans
CUTTSIA VIBURNEA
Donatia fascicularis
Villarsia calthifolia
Nephrophyllidium crista-galli
Menyanthes trifoliata
Dampiera spicigera
Lechenaultia heteromera
Brunonia australis
Goodenia ovata
Scaevola frutescens
Boopis anthemoides
Acicarpha tribuloides
Moschopsis rosulata
Dasyphyllum dicanthoides
Carthamus tinctorius
Lactuca sativa
Quintinia verdonii
BRUNIA ALBIFLORA
Berzelia lanuginosa
Polyosma cunninghamii
COLUMELLIA OBLONGA
Desfontainia spinosa
Viburnum rhytidiophyllum
Viburnum acerifolia
Adoxa moschatellina
Sambucus racemosa
Melanophylla pachypoda
Aralidium pinnatifidum
Toricellia tiliifolia
Griselinia lucida
Pittosporum japonicum
Hedera helix
Aralia spinosa
STAEGNOTAENIA ARALIACEAE
Sanicula gregari
Coriandrum sativum
Apium graveolens
Conium maculatum
Escallonia coquimbensis
Eremosyne pectinata
ANOPTERUS MACLAYANUS
Lonicera caprifolium
Symphoricarpos albus
Diervilla sessilifolia
MORINA COULTERIANA
KNAUTIA INTERMEDIA
PTEROCEPHALUS LASIOSPERMUM
Dipsacus sativus
TRIPLOSTEGIA GLANDULIFERA
PATRINIA RUPESTRIS
NARDOSTACHYS JATAMANSII
Valeriana officinalis
VALERIANELLA LOCUSTA
PHYLLACTIS BRACTEATA
VALERIANA HIRTELLA
Taxa used in SMALL analysis.
ASTERIDAE s. str.
fide Gustafsson & al. 1996,
ASTERID II, s. Chase

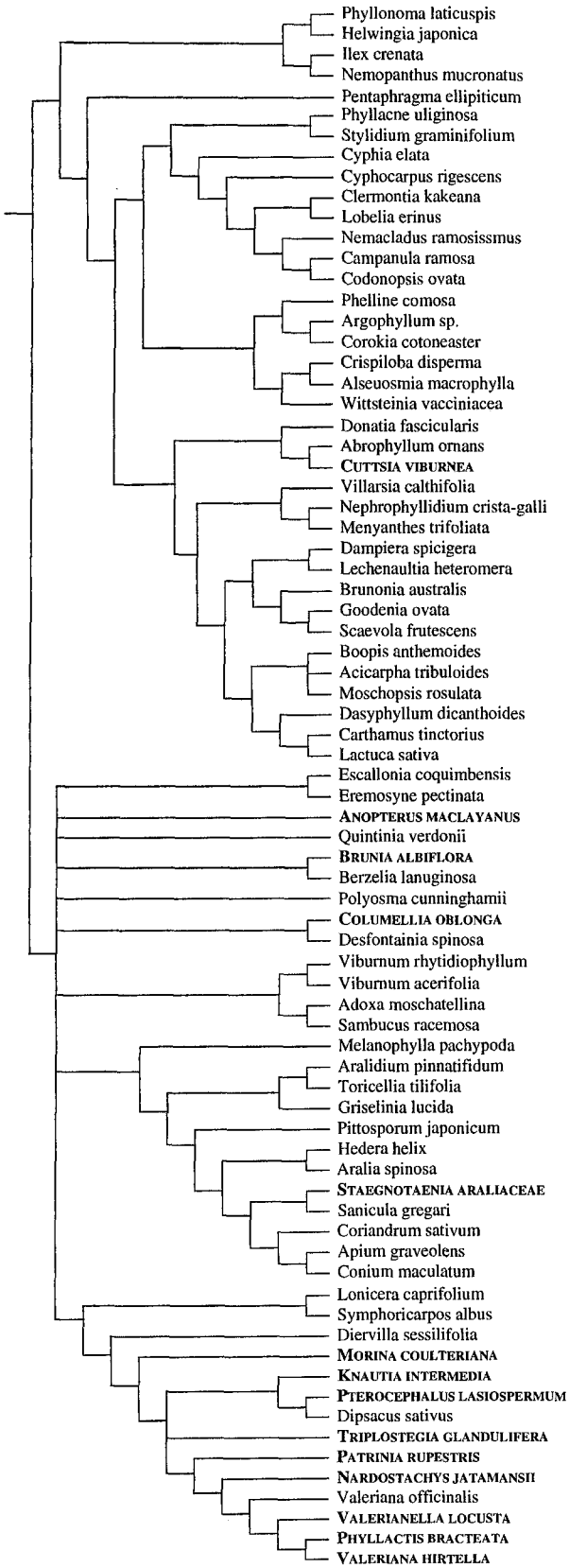


Fig. 2. Strict consensus from the 48 equally parsimonious trees resulting from parsimony analysis of the "small" *rbc*L sequence data matrix

The *Dipsacales-Apiales* complex consists of two main groups here defined as the *Dipsacales* and *Apiales* associations respectively, and basally to this complexsome additional taxa are found forming a grade. The *Dipsacales* association is made up of a strongly supported order *Dipsacales* s. str. (branch length 16 steps, bootstrap value 97.0%, jackknife value 94.7%, Bremer support 8) consisting of the core families *Dipsacaceae*, *Valerianaceae*, *Morinaceae* and *Caprifoliaceae*. In the *Apiales* association there are – except for the moderately supported *Apiaceae* s. l. clade – two additional evolutionary lines forming a grade. From the base up we first encounter a group that comprises the former members of *Caprifoliaceae: Viburnum* and *Sambucus* together with *Adoxaceae*. Above this assemblage we find the genus *Melanophylla* of the *Melanophyllaceae*.

In the grade basal to the two major groups we find several members of the Englerian *Saxifragaceae* as well as the families *Bruniaceae*, *Desfontainiaceae* and *Columelliaceae* represented.

Information on all nodes in the tree in Fig. 3 is summarized in Table 5.

Analysis of the "control matrix" compiled using GACT. Analyzing the strings matrix using PAUP renders a result compatible with those obtained from both PAUP and Jac (parsimony jackknifing) of the original "large" sequence matrix. This serves as a check that the evolutionary span among the included nucleotide sequences is narrow enough not to be severely affected by the problem with functional constraints.

General discussion

With the exception of *Hydrostachys*, the taxa under study all belong in a monophyletic group comprising the *Dipsacales*, *Apiales*, *Asterales* and several additional taxa (Figs. 1, 2 and 3). The group containing the three aforementioned orders corresponds to *Asteridae* s. str., or the "asterid II" as defined by CHASE & al. (1993) in their study of *rbc*L sequences sampling from all angioperms.

There has been some debate over the existence of the *Asteridae* s. str., because no support was found for this group in some of the most parsimonious trees obtained by the analysis of OLMSTEAD & al. (1993). That study – which specifically treated the interrelationships of *Asteridae* s. l. based on *rbc*L sequences – had some major advantages over the one performed by CHASE & al. (1993), due both to a more extensive sampling among presumably related taxa and to the fact that the analysis was run to completion (which was not the case in the study by CHASE & al. 1993). The present study provides further insights into the interrelationships of "higher" *Asteridae*, and with an even more extensive sampling the possible existence of – and support for – *Asteridae* s. str. Numerous preliminary matrices tested during this study also have shown that the support for the node defining *Asteridae* s. str. is dependent on an extensive sampling among the phylogenetically basal taxa of the subclass. This study corroborates the monophyly of the *Asteridae* s. str. with unambiguous results from the PAUP analysis. However, the supportive indices obtained for this branch are low.

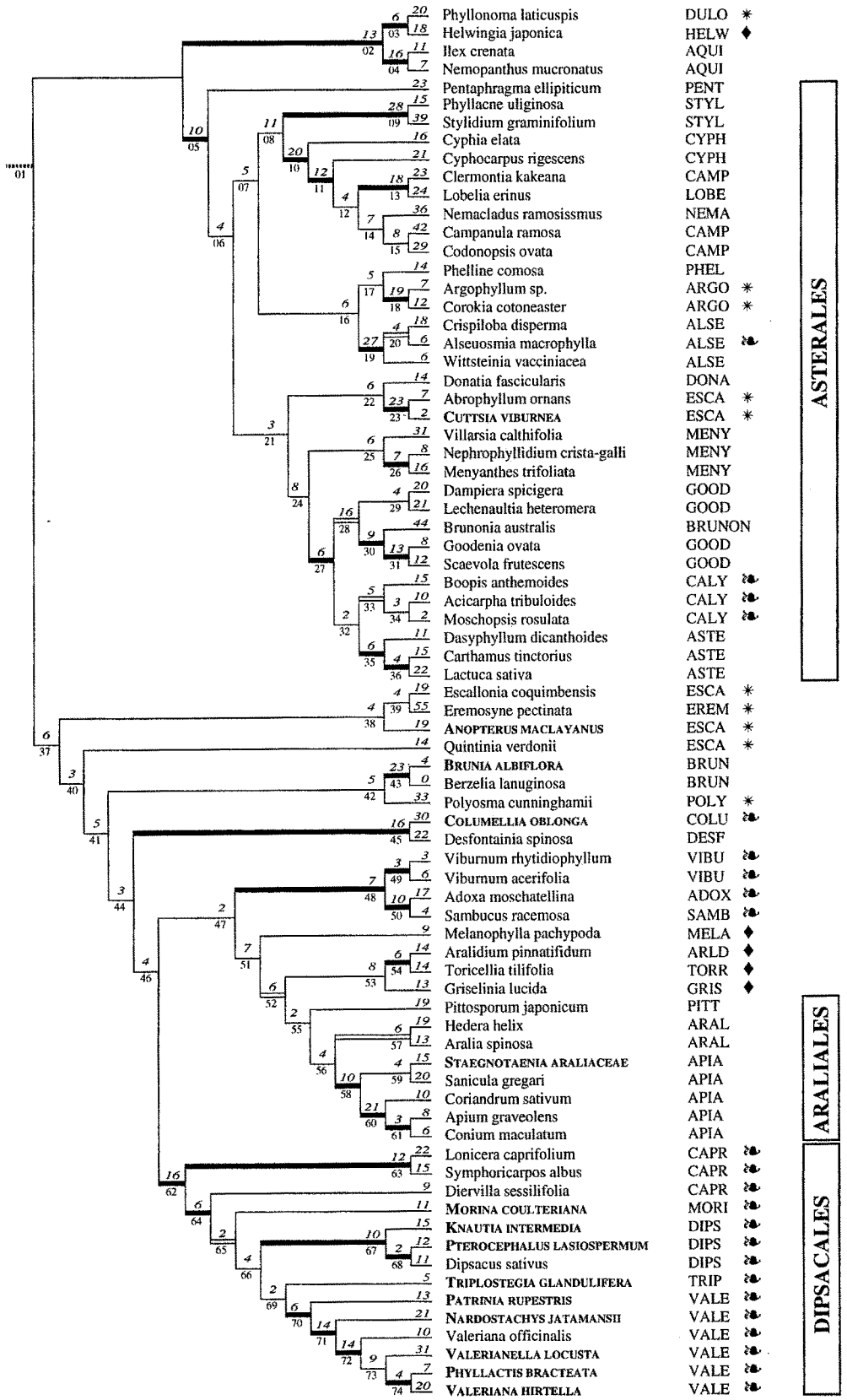
The new sequence of *Hydrostachys* attains a position as sister-group to the previous sequenced species of *Hydrostachys*, the position of this group is, however, ambigous with the present sample of taxa.

Table 5. Tree statistics for the selected tree after successive weighting shown in Fig. 3. Abbreviations used are:

#	= node number in tree
bl	= branch length (unweighted branch lengths). Terminal branch lengths indicated in Fig. 3.
bst100	= bootstrap values from 100 replicates using TBR branch swapping on the "small" matrix.
bst1000	= bootstrap values from 1000 replicates without branch swapping on the small matrix.
jacL	= jackknife values from 10000 replicates on the "large" matrix with Jac.
jacS	= jackknife values from 10000 replicates on the "small" matrix with Jac.
PjacL	= jackknife values from 1000 replicates on the "large" matrix with PAUP.
PjacS	= jackknife values from 1000 replicates on the "small" matrix with PAUP.
Bs	= Bremer support values calculated from the "small" matrix, with exception for node 01.
–	= value below 50%.

#	bl	bst100	bst1000	jacL	jacS	PjacL	PjacS	Bs	#	bl	bst100	bst1000	jacL	jacS	PjacL	PjacS	Bs
01	–	–	–	–	–	–	–	2	**38**	4	–	–	–	–	–	–	0
02	13	72	78.3	80.8	91.2	69.0	81.9	5	**39**	4	–	–	–	–	–	–	3
03	6	81	82.2	80.2	82.7	73.0	72.3	6	**40**	3	–	–	–	–	–	–	0
04	16	99	99.8	99.9	99.8	99.0	98.5	12	**41**	5	–	–	–	–	–	–	0
05	10	–	–	–	64.8	–	57.9	5	**42**	5	–	–	–	–	–	–	0
06	4	–	–	–	–	–	–	2	**43**	23	100	100.0	99.7	100.0	100.0	100.0	15
07	5	–	–	–	–	–	–	1	**44**	3	–	–	–	–	–	–	1
08	11	–	–	–	–	–	–	1	**45**	16	58	60.4	68.1	74.6	60.0	68.8	10
09	28	94	93.5	96.2	98.1	93.0	95.4	18	**46**	4	–	–	–	–	–	–	0
10	20	100	99.6	97.7	97.5	96.0	96.0	12	**47**	2	–	–	–	–	–	–	0
11	12	86	80.3	86.4	87.4	79.0	76.7	6	**48**	7	83	69.6	73.3	71.3	64.0	64.2	2
12	4	–	–	–	–	–	–	2	**49**	3	93	91.9	87.7	88.5	84.0	84.1	2
13	18	98	97.2	99.1	99.1	98.0	96.6	12	**50**	10	90	86.4	90.5	90.2	85.0	83.6	6
14	7	–	–	–	–	–	–	2	**51**	7	–	–	–	–	–	–	4
15	8	–	–	–	–	–	–	3	**52**	6	–	–	51.1	–	–	–	4
16	6	–	–	–	–	–	–	3	**53**	8	–	–	–	–	–	–	2
17	5	55	50.5	–	–	–	–	3	**54**	6	57	62.9	62.7	62.2	59.0	57.6	1
18	19	100	100.0	100.0	100.0	100.0	99.5	19	**55**	2	–	–	–	–	–	–	2
19	27	100	99.8	99.9	99.9	100.0	99.6	24	**56**	4	–	–	–	–	–	–	3
20	4	100	61.7	61.7	62.6	60.0	59.0	2	**57**	6	–	–	55.7	51.4	–	–	2
21	3	–	–	–	–	–	–	1	**58**	10	60	62.1	70.5	68.5	58.0	56.8	6
22	6	–	–	–	–	–	–	1	**59**	4	–	–	–	–	–	–	2
23	23	100	100.0	100.0	100.0	100.0	100.0	23	**60**	21	100	100.0	100.0	100.0	100.0	100.0	16
24	8	54	–	–	–	–	–	3	**61**	3	85	76.9	64.4	64.8	69.0	67.2	3
25	6	53	53.2	–	–	–	–	3	**62**	16	97	95.9	94.8	94.7	90.0	90.0	8
26	7	91	78.7	78.2	78.2	72.0	73.0	6	**63**	12	92	92.5	95.6	95.9	91.0	91.8	8
27	6	84	65.5	71.2	72.4	61.0	65.0	6	**64**	6	73	66.9	63.7	62.4	51.0	50.3	6
28	16	69	54.0	54.0	56.4	–	–	3	**65**	2	66	52.7	52.0	–	–	–	2
29	4	–	–	–	–	–	–	2	**66**	4	53	–	–	–	–	–	4
30	9	82	69.0	78.0	80.0	66.0	69.4	6	**67**	10	95	96.4	97.8	97.8	94.0	94.8	8
31	13	100	100.0	100.0	100.0	100.0	100.0	11	**68**	2	90	89.7	82.4	82.2	80.0	80.0	2
32	2	56	–	–	–	–	–	2	**69**	2	–	–	–	–	–	–	0
33	5	69	55.6	52.2	50.9	–	–	4	**70**	6	89	82.6	82.0	80.7	72.0	72.4	6
34	3	–	–	–	–	–	–	0	**71**	14	99	98.2	98.7	98.7	96.0	96.0	12
35	6	78	71.4	74.5	74.7	65.0	65.8	6	**72**	14	97	94.7	99.4	98.6	96.0	94.5	11
36	4	79	–	85.7	85.4	76.0	74.2	1	**73**	9	–	–	–	–	–	–	1
37	6	–	–	–	–	–	–	2	**74**	4	73	64.5	66.5	66.5	60.0	62.2	2

Fig. 3. Single most parsimonious tree resulting from parsimony analysis of the "small" *rbc*L sequence data matrix after applying the successive weightings approach. Node numbers (below branches) correspond to Table 5, where supportive indices for all nodes are summarized. Branch lengths (italics, above branches) are corresponding to the unit weighted matrix. Familial classification according to TAKHTAJAN (1987) is indicated in abbreviated form after each taxon name. Also symbols indicating taxa at various times suggested to belong to the order *Dipsacales* (❧), taxa by ENGLER (1930) placed in the family *Saxifragaceae* (*) and taxa by WANGERIN (1910) included in the family *Cornaceae* (◆) are supplied in the Figure. Support obtained from jackknife analyses of the "small" matrix is indicated with white, thic nodes for support in the range 50–63%, black thick nodes for support exceeding 63%


Phyllonoma laticuspis DULO
Helwingia japonica HELW
Ilex crenata AQUI
Nemopanthus mucronatus AQUI
Pentaphragma ellipiticum PENT
Phyllacne uliginosa STYL
Stylidium graminifolium STYL
Cyphia elata CYPH
Cyphocarpus rigescens CYPH
Clermontia kakeana CAMP
Lobelia erinus LOBE
Nemacladus ramosissmus NEMA
Campanula ramosa CAMP
Codonopsis ovata CAMP
Phelline comosa PHEL
Argophyllum sp. ARGO
Corokia cotoneaster ARGO
Crispiloba disperma ALSE
Alseuosmia macrophylla ALSE
Wittsteinia vacciniacea ALSE
Donatia fascicularis DONA
Abrophyllum ornans ESCA
CUTTSIA VIBURNEA ESCA
Villarsia calthifolia MENY
Nephrophyllidium crista-galli MENY
Menyanthes trifoliata MENY
Dampiera spicigera GOOD
Lechenaultia heteromera GOOD
Brunonia australis BRUNON
Goodenia ovata GOOD
Scaevola frutescens GOOD
Boopis anthemoides CALY
Acicarpha tribuloides CALY
Moschopsis rosulata CALY
Dasyphyllum dicanthoides ASTE
Carthamus tinctorius ASTE
Lactuca sativa ASTE
Escallonia coquimbensis ESCA
Eremosyne pectinata EREM
ANOPTERUS MACLAYANUS ESCA
Quintinia verdonii ESCA
BRUNIA ALBIFLORA BRUN
Berzelia lanuginosa BRUN
Polyosma cunninghamii POLY
COLUMELLIA OBLONGA COLU
Desfontainia spinosa DESF
Viburnum rhytidiophyllum VIBU
Viburnum acerifolia VIBU
Adoxa moschatellina ADOX
Sambucus racemosa SAMB
Melanophylla pachypoda MELA
Aralidium pinnatifidum ARLD
Toricellia tilifolia TORR
Griselinia lucida GRIS
Pittosporum japonicum PITT
Hedera helix ARAL
Aralia spinosa ARAL
STAEGNOTAENIA ARALIACEAE APIA
Sanicula gregari APIA
Coriandrum sativum APIA
Apium graveolens APIA
Conium maculatum APIA
Lonicera caprifolium CAPR
Symphoricarpos albus CAPR
Diervilla sessilifolia CAPR
MORINA COULTERIANA MORI
KNAUTIA INTERMEDIA DIPS
PTEROCEPHALUS LASIOSPERMUM DIPS
Dipsacus sativus DIPS
TRIPLOSTEGIA GLANDULIFERA TRIP
PATRINIA RUPESTRIS VALE
NARDOSTACHYS JATAMANSII VALE
Valeriana officinalis VALE
VALERIANELLA LOCUSTA VALE
PHYLLACTIS BRACTEATA VALE
VALERIANA HIRTELLA VALE
ASTERALES
ARALIALES
DIPSACALES

Some of the results and groupings, with regard to the basal taxonomic levels in *Asteridae* s. l. and *Rosidae* indicated in this study, should be considered with caution. Our study was not designed to evaluate these areas of the angiosperm system and the results partly disagree with those of more inclusive analyses already published.

The PAUP analysis of the binary matrix retained by GACT with information about larger nucleotide sequence motifs resulted in a tree compatible with the one obtained from the "large" nucleotide sequence analysis. Hence, it can be assumed that the sample of sequences selected for this study are not likely to suffer from major problems with the functional constraints inflicting on the variation and changes in coding nucleotide sequences (ALBERT & al. 1994a, b).

Asteridae s. l.

The sections of the tree above the node defining the asterid III sensu CHASE (1993) obtained in the analysis (Fig. 1) are compatible with a majority of the groups and branches found in the analysis by CHASE & al. (1993). Asterid III form a monophyletic group, as a sister-group to asterid I and *Asteridae* s. str.

In the large branch corresponding to asterid I (bootstrap value < 50%, jackknife value < 50%, Bremer support 2), the five main branches–*Solanales*, *Boraginales*, *Gentianales*, *Lamiales* s. l., and the *Garrya*-clade–can be identified readily. The relationships among these groups are largely unresolved, but not incongruent with either those obtained by OLMSTEAD & al. (1993) or CHASE & al. (1993).

As a sister-group to asterid I, a branch is leading up to the group referred to as *Asteridae* s. str. (GUSTAFSSON & al. 1996) or asterid II (CHASE & al. 1993). This clade is only moderately supported (branch length 6, bootstrap value < 50%, jackknife value < 50%, Bremer support 1), but unambiguously retained in all trees from the large matrix.

Asteridae s. str.

In the *Asteridae* s. str. there are four large clades, roughly corresponding to the three orders *Asterales*, *Apiales* and *Dipsacales*–with several additional taxa as successive sister-groups to the two latter orders–and the *Ilex*-clade. These four clades will be dealt with below, but in short the main novelties of the arrangements suggested from the PAUP analysis are the position of the *Ilex*-clade as sister-group to *Asterales* and the shift of a number of groups–e.g., *Adoxaceae* s. l. (including *Sambucus* and *Viburnum) and Bruniaceae*–from near *Dipsacales* to near *Apiales*.

The *Ilex*-clade. Basal most in the evolutionary lineage and sister-group to the *Asterales* s. l. attaches a very stable and well supported group commonly referred to as the *Ilex*-clade (OLMSTEAD & al. 1993, GUSTAFSSON & al. 1996). This clade comprises *Ilex crenata* and *Nemopanthus mucronatus*–both in the *Aquifoliaceae*–*Helwingia japonica* from *Helwingiaceae* or earlier *Cornaceae*, and *Phyllonoma laticuspis* earlier referred to *Grossulariaceae* (MORI & KALLUNKI 1977) or *Dulongiaceae* (AGARDH 1858). The *Ilex*-clade has been retrieved and placed at or near the base of *Asteridae* s. str. in virtually all larger molecular analyses where two or more of the taxa have been included (i.e. CHASE & al. 1993, MORGAN & SOLTIS 1993, OLMSTEAD & al. 1993, XIANG & al. 1993, GUSTAFSSON & al. 1996).

The *Asterales* clade. The relationships in the *Asterales* s. l. are almost entirely congruent with those found in the recent analysis by GUSTAFSSON & al. (1996). One exception is the sister-group of the *Asteraceae*, which in the present study is the *Calyceraceae* in concordance with the results from KIM & JANSEN (1995), while in the study by GUSTAFSSON & al. (1996) the *Goodeniaceae* hold this position. This difference may well be a result of the more restricted sampling of *Goodeniaceae* and *Asteraceae* in the present study, and it can be noted that the branch shared by *Asteraceae* and *Calyceraceae* is very short (branch length only 2 steps) and the support measures are low (bootstrap 56%, jackknife value 50%, branch length 6 and Bremer support 2). The support for the group formed by all three families, on the other hand, is much stronger (bootstrap 84%, jackknife value 72.4%, branch length 6 and Bremer support 6; Figs. 1 and 2).

One taxon not included by GUSTAFSSON & al. (1996) that now turns up within the *Asterales* is *Phelline*, placed in the monotypic *Phellinaceae* by TAKHTAJAN (1987) but usually as an aberrant member of the *Aquifoliaceae*, in accordance with the monographic treatment of that family by LOESENER (1942). BAAS (1975) found numerous anatomical differences between *Phelline* on one hand and *Ilex* and *Nemophanthus* (*Aquifoliaceae* s. str.) on the other, and ruled out a close relationship to the *Aquifoliaceae* on these grounds. The *rbc*L gene of *Phelline* was sequenced by SAVOLAINEN & al. (1994), in which study *Phelline* and *Ilex* were indicated as sister-groups. As they were the only two members of the class *Asteridae* included in that study, however, the indications from that study are compatible with present results. The sister-group of *Phelline*, according to the present results, is the *Argophyllaceae*. This relationship has not been suggested previously and is only weakly supported (bootstrap 55%, jackknife value < 50%, branch length 5 and Bremer support 3). The position within the *Asterales*, however, is supported very strongly. An evaluation of the morphological similarities between *Phelline* and the families of *Asterales* is yet to be done.

The genera *Cuttsia* and *Abrophyllum* form a well supported group (bootstrap 100%, jackknife value 100%, branch length 23 and Bremer support 23), and indeed the difference in the *rbc*L sequences is quite small. The position of *Abrophyllum* in the *Asterales* was established in the *rbc*L study by GUSTAFSSON & al. (1996), and the many similarities between this genus and *Cuttsia* were discussed. The close relationship between the genera, and their relatively isolated position in the *Asterales* would justify their recognition as a distinct family.

***Saxifragaceae-Escallonioideae* and the *Dipsacales-Apiales* complex.** The subfamily *Escallonioideae* of the *Saxifragaceae* – often referred to as *Escalloniaceae* with family rank – has varied considerably with regard to its circumscription. The most extreme views are probably those taken by ENGLER (1930) and TAKHTAJAN (1987), respectively. In the former system this group is very large and extremely heterogenous, comprising no less than 80 genera. The "unnaturalness" or polyphyly of this grouping has been a subject of debate for a considerable time and is demonstrated not only by molecular studies (e.g., this analysis, and the studies by MORGAN & SOLTIS 1993, GUSTAFSSON & al. 1996, and XIANG & SOLTIS 1996), but also by numerous investigations including morphology and palynology (BENSEL & PALSER 1975, HIDEUX & FERGUSON 1976, AL-SHAMMARY & GORNALL 1994) as earlier pointed out by GUSTAFSSON & al. (1996).

In the classification proposed by TAKHTAJAN (1987), he advocated the elevation of several segregates to family rank, thereby leaving only a small core family *Escalloniaceae* consisting of seven genera. But even in the restricted sense of TAKHTAJAN, the *Escalloniaceae* (in this analysis represented by the genera *Escallonia*, *Anopterus*, *Cuttsia*, *Quintinia* and *Abrophyllum*) remain a highly heterogenous group that is grossly both poly- and paraphyletic according to the present results.

Basal to the two main branches of the *Dipsacales-Apiales* complex we find a grade of smaller groups. Basalmost is a loosely knit group (bootstrap $< 50\%$, jackknife value $< 50\%$, branchlength 4 and Bremer support 1) consisting of *Escallonia* and the two small Australian genera *Eremosyne* and *Anopterus*.

In a comparative palynological study of *Saxifragaceae* in the traditional wide sense by HIDEUX & FERGUSON (1976), pollen from *Anopterus* and *Escallonia* were considered very similar. In their numerical analysis, pollen of *Eremosyne* turns up close to the aforementioned genera but in an intermediate group suggesting a transition from a perforate tectum (found in *Anopterus* and *Escallonia*) towards pollen with a complete tectum.

The pollen of *Quintinia* was, in the study by HIDEAUX & FERGUSON (1976), considered to be similar especially to that of *Escallonia* and *Anopterus*, a conclusion that corresponds well with the position indicated for *Quintinia* in the tree. *Quintinia*, which has been placed in *Escalloniaceae* (or in the *Escallonioideae* of the *Saxifragaceae*, close to *Escallonia*) seems from this study, as well as from the earlier study by GUSTAFSSON & al. (1996), clearly to belong in the *Asteridae* s. str., even if it becomes difficult on the basis of *rbc*L data to comment in detail on its exact position in the subclass.

Above the *Quintinia*-clade in Fig. 3. we find two additional small groups, the first one including the two sequences of the exclusively South African family *Bruniaceae* (*Berzelia lanuginosa* and *Brunia albiflora*). The monophyly of this family is strongly supported by morphology (SAXTON 1910, NIEDENZU & HARMS 1930, PILLANS 1947, CARLQUIST 1991), and now also by molecular data, though the sampling in the family is far from optimal. The sister-group relation to the South Asian genus *Polyosma*, earlier confined to *Escalloniaceae* or *Saxifragaceae-Escallonioideae* (ENGLER 1930), is more difficult to understand in the light of the morphological information available. However, the support for many of these basal branches is low. The calculation of an Adams consensus tree of the 48 trees retained from the "small" matrix reveals that the instability in these groups is largely dependent on the instable positions of *Polyosma* and *Quintinia*.

Continuing upwards in the tree in Fig. 3 the next branch consists of the sequences from *Columellia oblonga* and *Desfontainia spinosa*. The earlier taxonomic positions of *Columellia* have included a wide variety of families from different parts of the system. The new nucleotide sequence for *Columellia* supports a position within *Asteridae* s. str., contradictory to most earlier suggestions. Also the position of *Desfontainia*, earlier considered to be a part of the *Loganiaceae* or the monogeneric family *Desfontainiaceae*, is new. A number of common traits are found for these two taxa, including the Andean cloud-forest distribution, a shrubby habit, comparably large showy pentamerous and sympetalous flowers (*Columellia* clearly shows remains of five stamens although only two are fully developed), epigynous or semi-epigynous ovaries, fruits many-seeded, and features of wood-anatomy such as

tracheary elements being tracheids only (i.e. absence of vessels) and the presence of a pericyclic cork (CARLQUIST 1992).

The *Apiales* association. The large clade including *Apiales* and a series of successive sister-groups is the part of the analysis with the most controversial changes in topology compared with all earlier studies that have included these groups (e.g., DONOGHUE & al. 1992, CHASE & al. 1993, and OLMSTEAD & al. 1993).

Basally in this association we find a group corresponding to *Adoxaceae* s. l. (DONOGHUE 1985, DONOGHUE & al. 1992, JUDD & al. 1994) including four taxa. The two sequenced members of *Viburnum* come out as sister-group to a clade with *Adoxa* and *Sambucus*. The morphological homogeneity of this entire branch is striking compared to the more basal branches discussed earlier; the controversy lies in its position in the *Apiales* association as a sister-group to the *Apiales*-complex (including *Melanophylla*) rather than as a basal clade in the *Dipsacales*. The support for this position of the *Adoxaceae* is weak in the molecular data (bootstrap < 50%, jackknife value < 50%, branch length 2, Bremer support 1), and trees with *Adoxaceae* sister-group to the *Dipsacales* are only one step longer. Similar indications of a closer relationship between *Adoxaceae* and the *Apiales*, however, have been made from various morphological and anatomical investigations. Recently karyosystematic studies (BENKO-ISEPPON 1992) have shown an extreme difference in karyomorphology between *Caprifoliaceae* s. str. on one hand and *Viburnum* and *Sambucus* on the other, strongly supporting the exclusion of the latter from *Caprifoliaceae*. A hypothesis of a position closer to *Cornaceae* or *Hydrangeaceae* for these taxa was explored but could not be confirmed on karyomorphological grounds (BENKO-ISEPPON 1992). Information from anatomical data (METCALFE & CHALK 950), secondary chemistry (HEGNAUER 1969), and serological investigations (HILLEBRAND & FAIRBROTHERS 1970) also have been suggestive of an alternative position for *Viburnum* close to the *Apiaceae*.

In the *Apiales*, i.e. in the group close to *Apiaceae*, we find not only undisputed members of this order such as *Apium*, *Conium*, and *Aralia*, but also a number of additional taxa. The family *Pittosporaceae*, represented in the analysis by *Pittosporum japonicum*, clearly belongs here as sister-group to *Apiaceae*. The close relationship between these families has been shown repeatedly in various studies of both molecular data (e.g., PLUNKETT & al. 1992, PLUNKETT & al. 1996) as well as secondary chemistry (HEGNAUER 1969), and anatomy (RODRÍGUEZ 1971). In complete concordance with the recent study by XIANG & SOLTIS (1996), the genera *Griselinia*, *Melanophylla*, *Aralidium* and *Toricellia*–earlier believed to be parts of the *Cornaceae*–are positioned within or close to this group. The exclusion of these taxa from the *Cornaceae* and placement close to the *Apiaceae* is supported–as pointed out earlier by–by characters of wood anatomy (RODRÍGUEZ 1971) as well as vegetative and floral morphology. The previously unpublished sequence of *Steganotaenia* appears as the sister-group to *Sanicula* in the *Apiaceae*.

The *Dipsacales* association. The order *Dipsacales* in its more restricted sense, i.e. without *Adoxaceae* (including *Sambucus* and *Viburnum*) includes the four families *Caprifoliaceae*, *Morinaceae*, *Dipsacaceae*, and *Valerianaceae*. The branch defining the order *Dipsacales* in the tree in Fig. 3 is strongly supported (bootstrap 97%, jackknife value 94.8%, branch length 16, Bremer support 8) in the *rbc*L data. Within

this group the necessity of several taxonomic rearrangements is indicated, largely opposing the traditional views of the order.

The family *Caprifoliaceae* in its traditional sense (including *Viburnum* and *Sambucus*) seems to be polyphyletic, as earlier suggested by DONOGHUE & al. 1992) and JUDD & al. (1994). These results are supported in all retrieved trees, and are assigned fairly high supportive indices, as can be seen in Fig. 3 and Table 5. The remainder of the family is split between the genera *Lonicera* and *Symphoricarpos* on one hand and *Diervilla* on the other. Morphological characters supporting this partition are among others differences in leaf outline and vernation (CULLEN 1978), fruit type, inflorescence (FUKUOKA 1969), androecial embryology (KAMELINA 1980, 1983), palynology (DONOGHUE 1985), karyomorphology (BENKO-ISEPPON 1992) and rearrangements in the chloroplast genome (DONOGHUE & al. 1992). To investigate further and establish this difference between the two evolutionary branches, a more thorough sampling and sequencing within the *Caprifoliaceae* is called for. In combination with the results from an ongoing extensive morphological study including more than 55 taxa from the *Dipsacales* sensu latissimo (ANDERS BACKLUND & MICHAEL J. DONOGHUE, unpubl. data), indications from this and other studies may necessitate a formal division of the traditional *Caprifoliaceae*.

Next to these branches of the "traditional *Caprifoliaceae*," and sister-group to the rest of the order, we find the sequence of *Morina*, representing the *Morinaceae*. The *Morinaceae* are a small family consisting of three genera mainly from continental south-eastern Asia. The family traditionally has been placed as a sister-group to – or earlier even part of – the *Dipsacaceae* (e.g., CAPUTO & COZZOLINO 1994), a position suggested by the presence in both taxa of an epicalyx. A number of detailed studies from different fields (e.g., VINOKUROVA 1959; VIJAYARAGHAVAN & SARVESHWARI 1968; VERLAQUE 1977; KAMELINA 1980, 1983; BLACKMORE & CANNON 1983; CANNON & CANNON 1984; BENKO-ISEPPON 1992) have pointed to similarities also with parts of the *Caprifoliaceae*. This position basal to both *Dipsacaceae* and *Valerianaceae* and patristically closer to *Caprifoliaceae* s. str. is congruent with preliminary results from the above-mentioned morphological study.

Above *Morinaceae* in the tree in Fig. 3 we find a dichotomy with one branch leading to *Dipsacaceae* and the other to *Valerianaceae*. The family *Valerianaceae* is here taken to include *Triplostegia*. The obtained results indicate that *rbc*L sequence data support, although weakly, the position of this small genus as a part of the *Valerianaceae*. Detailed discussions about the affinities, classification and palynology of *Triplostegia* are given in BACKLUND & BREMER (1996) and BACKLUND & NILSSON (1997), respectively.

The commonly proposed sister-group relation between the families *Dipacaceae* and *Valerianaceae* is supported, but with moderate strength (bootstrap 53%, jackknife value $< 50\%$, branch length and Bremer support both 4). The sampling in each of these families is now becoming large enough to hypothesize cautiously about relationships within the families. The three taxa available in the family *Dipsacaceae* (two previously unpublished) arrange themselves in concordance with most earlier classifications and studies (e.g., DOLL 1927, BAKSAY 1952, EHRENDORFER 1964, NEUBAUER 1978, KAMELINA 1980, CARLQUIST 1982, and CAPUTO & COZZOLINO 1994), with *Knautia* as the sister-group to *Dipsacus* and *Pterocephalus*.

The relationships indicated within *Valerianaceae*, on the other hand, contain some controversial groupings. Apart from *Triplostegia*, the two Asian genera *Patrinia* and *Nardostachys* generally are considered to be the most plesiomorphic taxa in the family and often are placed together in the tribe *Patrinieae*. According to the *rbc*L data they turn out to form a grade and, therefore, provide no support for distinguishing this presumed tribe. Above these taxa we find *Valeriana officinalis*– the only previously published sequence in the *Valerianaceae*. Circumscription of the genus *Valeriana* has been discussed frequently with reference to the South American taxa. Suggestions of lumping most or parts of the South American genera into one large *Valeriana* s. l. have been made (LARSEN 1986, ERIKSEN 1989). The results obtained here show that the differences between the mainly European species *Valeriana officinalis* and the South American Andean species *Valeriana hirtella* are so great that not only the frequently disputed genus *Phyllactis* but also the widely accepted *Valerianella* are grouped between these. These indications, strongly supported in *rbc*L data, as can be seen in Fig. 3 and Table 5, are also partly corroborated by morphological differences, and call for further investigation of the inter- and intrageneric relationships within the *Valerianaceae*.

Summary of morphological traits. Common traits defining the *Asteridae* s. str. are not obvious, because the group is morphologically quite variable, but they include a combination of characters such as epigynous flowers, often with one single ovule per carpel and a corolla initiated by a ring-shaped primordium (ERBAR 1991, 1994; ROELS 1993; ROELS & SMETS 1995). The secondary chemistry of these taxa is partly well known and to the uniting characters may be added the ability to synthezise polyacetylenes and the frequent possession of a wide variety of iridoid compounds.

The *Asterales* s. l. are well supported by molecular data, but they are a highly heterogeneous group morphologically (GUSTAFSSON & BREMER 1995, GUSTAFSSON & al. 1996). To characterize the order one could mention, apart from features common to most *Asteridae*, the apparently universal occurrence of the polyfructan inulin (rare outside the group; POLLARD & AMUTI 1981), the mostly valvate petals and the frequent occurrence of secondary pollen presentation. Polyacetylenes and secoiridoids are common, mostly complemetary to each other in distribution. The families within the order generally are well-defined, and in a few cases they form strong groupings, supported also by morphology, such as the *Asteraceae-Calyceraceae-Goodeniaceae* clade and the *Campanulaceae* s. l. (including *Lobeliaceae*, *Cyphiaceae*, *Nemacladaceae* and *Cyphocarpaceae;* GUSTAFSSON & BREMER 1995, GUSTAFSSON & al. 1996). The basal relationships in the order, on the other hand, still are understood poorly.

Comparable common traits for the *Apiales* include some peculiar features. The leaves are lobed, often deeply so, or even dissected. Flowers are initially sympetalous, but later in ontogeny they become choripetalous in many taxa of the higher *Apiales*. In the group commonly known as *Apiaceae* although disputed (cf. BAUMANN 1946; PHILIPSON 1970; THORNE 1973; PLUNKETT & al. 1992, 1996; and others), all flowers are arranged in umbels, sometimes combined in various larger and more complicated inflorescences. This is true also for the *Apiaceae* s. l. (which should include the *Apiaceae*), but not for some other taxa, which by this study are indicated as related to the *Apiales* (e.g., *Adoxaceae* s. l.). The secondary chemistry of the higher *Apiales* has

been studied extensively, because several well known medical plants and spices are found among these. Most of these taxa have secretory ducts in their vegetative tissue containing ethereal oils of various kinds. The more basal branches in the order are much less well known. It is difficult therefore, to point at any special chemical compounds as diagnostic of the entire *Apiales* as well as for the larger *Apiales* association including also *Adoxaceae* s. l. and several other taxa.

Common traits for the *Dipsacales* clade would be features such as opposite leaves without stipules and flowers in complex cymose inflorescences. Flowers in the *Dipsacales* are always sympetalous and vary from actinomorphic to extremely zygomorphic, a trend found in all families of the order and often accompanied by a reduction in stamen number. The ovary is hypogynous with five carpels which are reduced to four, three or two, repeatedly, often with an subsequent reduction in the number of fertile carpels (the others abort) and number of locules to one. Embryology shows a small and straight embryo; in *Dipsacaceae* and *Valerianaceae* also always containing chlorophyll (YAKOVLEV 1980). Pollen grains are tricolporate (in *Morinaceae* pororate), tectate and furnished with spines and in some cases with microperforations.

Conclusions

According to the present results the order *Dipsacales* consists of the core families *Caprifoliaceae* s. str., *Morinaceae*, *Dipsacaceae* and *Valerianaceae* but presumably excluding the *Adoxaceae* s. l. (*Adoxaceae* including *Sambucus* and *Viburnum*) which are indicated to be more closely related to the *Araliales* complex. This is in contrast to the recent systems of angiosperms (CRONQUIST 1981, TAKHTAJAN 1987, DAHLGREN 1989, THORNE 1992), but has been suggested by other studies, particularly analyses of molecular data (DONOGHUE 1985, DONOGHUE & al. 1992, CHASE & al. 1993, BREMER & al. 1994, JUDD & al. 1994). The results presented here from the parsimony analysis conducted with PAUP are unambiguous. The parsimony jackknifing procedure, however, did not find sufficient support for placing *Adoxaceae* s. l. together with either the *Apiales* or *Dipsacales*.

Further indications are that the family *Caprifoliaceae* in its traditional sense might be polyphyletic, as indicated earlier by DONOGHUE & al. (1992) and JUDD & al. (1994). This may necessitate a future division of the family in order to retain the monophyly criterion. The alternative possibility of including all the core families of the *Dipsacales* into a large *Caprifoliaceae* s. l. seems unpractical, lowers the information content in the classification (BACKLUND & BREMER 1996) and would create a morphologically very heterogenous family.

The appearance of numerous members of the Englerian "*Saxifragaceae*" s. l. ENGLER (1930) as basal branches in all the three major lineages of the *Asteridae* s. str. is concordant with several recent studies (DONOGHUE & al. 1992, OLMSTEAD & al. 1993, XIANG & al. 1993, GUSTAFSSON & al. 1996, XIANG & SOLTIS 1996) and further confirms that these taxa originally were assembled merely on the basis of a few plesiomorphic similarities. Not even the division of the *Saxifragaceae* into segregate families suggested by TAKHTAJAN (1987) proved sufficient to describe the morphological variation or to reflect a natural classification of the treated taxa. At the positions held by e.g., *Helwingia*, *Phyllonoma*, *Quintinia*, *Escallonia*, *Anopterus* and

Polyosma, according to the present study, they are of prime importance for the understanding of the relations between the *Dipsacales* and the *Apiales* associations, and of the basal relationships in the *Asteridae* s. str.

This study was financed by Swedish Natural Science Research Council grants to BIRGITTA BREMER and KÅRE BREMER. A number of persons and institutions have contributed significantly to the realization of the present work, for which they are gratefully acknowledged. MATS H. G. GUSTAFSSON sequenced three of the taxa included in the analysis and provided invaluable advice and suggestions on early versions of the manuscript. WANG LI-SONG, RON J. D. MCBEATH, JARMO KUKKA, GEOFF WILLIAMS, MATS THULIN, JOHAN CEJIE and Uppsala Botanic Garden have contributed parts of the plant material. The staff at the Quito Catholic University–particularly LAURA ARCOS–as well as BENTE ERIKSEN-MOLAU from Gothenburg University were most helpful in connection with a field trip in Ecuador, where some of the *Valerianaceae* for the investigation were collected. DANIEL OLSSON and JESSICA RÖNNHOLM helped out with parts of the laboratory work. KÅRE BREMER devoted considerable time and suggested improvements of the manuscript. DAVID L. SWOFFORD generously permitted the use of a test version of his computer program PAUP (4.0d45, 68knoFPU). So did also JAMES S. FARRIS with a "pre-ß-version" (4.4) of his computer program Jac, and ROLF STAFLIN and KARL-KÖNIG KÖNIGSSON with their GACT; the two latter also helped out with parts of the GACT analyses. We are also grateful to RICHARD OLMSTEAD, an additional, anonymous, reviewer, and LENA STRUWE for their valuable comments and suggestions.

References

AGARDH, J. G., 1858: Theoria systematis plantarum; accedit familiarum Phanerogamarum.–Lund: Gleerup.

AIRY-SHAW, H. K., 1965a: Diagnoses of new families, new names, etc., for the 7th edition of Willis's 'Dictionary'.–Kew Bull. **18**: 249–273.

– 1965b: On a new species of the genus *Silvianthus* HOOK.f., and on the family *Carlemanniaceae*.–Kew Bull. **19**: 507–512

ALBERT, V., BACKLUND, A., BREMER, K., 1994a: DNA characters and cladistics: the optimization of functional history.–In SCOTLAND, R. W., SIEBERT, D. J., WILLIAMS, D. M., (Eds): Models in phylogeny reconstruction. Systematics Association Special Volume **52**, pp. 249–272. – Oxford: Clarendon Press.

– BACKLUND, A., BREMER, K., CHASE, M. W., MANHART, J. R., MISHLER, B. D., NIXON, K. C., 1994b: Functional constraints and *rbc*L evidence for land plant phylogeny.–Ann. Missouri Bot. Gard **81**: 534–567.

AL-SHAMMARY, K. I., GORNALL, R. J., 1994: Trichome anatomy of the *Saxifragaceae* s. l. from the southern hemisphere.–Bot. J. Linn. Soc. **114**: 99–131.

BAAS, P., 1975: Vegetative anatomy and the affinities of *Aquifoliaceae*, *Penostemon*, *Phelline*, and *Oncotheca*.–Blumea **22**: 311–407.

BACKLUND, A., BREMER, K., 1996: To be or not to be–principles of classification and monotypic plant families. – In BACKLUNO, A.: Phylogeny of the *Dipsacales*. – Doctoral Thesis, Uppsala University.

– NILSSON, S., 1997: The systematic position of *Triplostegia* WALL. with notes on its pollen. – Taxon (in press).

BAILLON, H., 1880: Rubiacées-Dipsacacées. –In BAILLON, H.: Histoire des plantes **7**, pp. 257 – 546. – Paris: Hachette.

– 1888: Bignoniacées, Gesnériacées.–In BAILLON, H.: Histoire des plantes **10**, pp. 1–112. – Paris: Hachette.

BAKSAY, L., 1952: Monographie der Gattung *Succisa*.–Ann. Hist. Nat. Mus. Natl. Hung. **2**: 237–259.

BARTLING, F. G., 1830: Ordines naturales plantarum eorumque characteres et affinitates.–Göttingen: Dieterichianus.

BAUMANN, G. M., 1946: *Myodocarpus* und die Phylogenie der Umbelliferen Frucht. – Ber. Schweiz. Bot. Ges. **56:** 13–112.

BENKO-ISEPPON, A. M., 1992: Karyologische Untersuchung der *Caprifoliaceae* s.l. und möglicher verwandter Familien. – Doctoral Thesis, University of Wien.

BENSEL, C. R., PALSER, B. F., 1975: Floral anatomy in the *Saxifragaceae* sensu lato. III. *Kirengeshomoideae, Hydrangeoideae* and *Escallonioideae*.–Amer. J. Bot. **62**: 676–687.

BLACKMORE, S., CANNON, M. J., 1983: Palynology and systematics of *Morinaceae*.–Rev. Palaeobot. Palyn. **40**: 207–226.

BOLLI, R., 1994: Revision of the genus *Sambucus*.–Diss. Bot. **223**.

BREMER, B., OLMSTEAD, R. G., STRUWE, L., SWEERE, J. A., 1994: *rbc*L sequences support exclusion of *Retzia, Desfontainia* and *Nicodemia* (*Buddlejaceae*) from the *Gentianales*. –Pl. Syst. Evol. **190**: 213–230.

BREMER, K., 1988: The limits of amino acid sequence data in angiosperm phylogenetic reconstruction.–Evolution **42**: 795–803.

– 1994: Branch support and tree stability.–Cladistics **10**: 295–304.

CANNON, M. J., CANNON, J. F. M., 1984: A revision of the *Morinaceae* (*Magnoliophyta-Dipsacales*).–Bull. Brit. Mus. (Nat. Hist.), Bot. **12**: 1–35.

CAPUTO, G., COZZOLINO, S., 1994: A cladistic analysis of *Dipsacaceae* (*Dipsacales*).–Pl. Syst. Evol. **189**: 41–61.

CARLQUIST, S., 1982: Wood anatomy of *Dipsacaceae*. –Taxon **31**: 443–450.

– 1991: Leaf anatomy of *Bruniaceae*: ecological, systematic and phylogenetic aspects.–Bot. J. Linn. Soc. **107**: 1–34.

– 1992: Wood anatomy of sympetalous dicotyledon families: a summary, with comments on systematic relationships and evolution of the woody habit. – Ann. Missouri Bot. Gard. **79**: 303–332.

CHASE, M. W., HILLS, H. H., 1991: Silica gel: an ideal material for field preservation of leaf samples for DNA studies.–Taxon **40**: 215–220.

– SOLTIS, D. E., OLMSTEAD, R. G., MORGAN, D., LES, D. H., MISHLER, B. D., DUVALL, M. R., PRICE, R. A., HILLS, H. G., QIU, Y.-L., KRON, K. A., RETTIG, J. H., CONTI, E., PALMER, J. D., MANHART, J. R., SYTSMA, K. J., MICHAELS, H. J., KRESS, W. J., KAROL, K. G., CLARK, W. D., HEDRÉN, M., GAUT, B. S., JANSEN, R. K., KIM, K.-J., WIMPEE, C. F., SMITH, J. F., FURNIER, G. R., STRAUSS, S. H., XIANG, Q.-Y., PLUNKETT, G. M., SOLTIS, P. S., SWENSEN, S. M., WILLIAMS, S. E., GADEK, P. A., QUINN, C. J., EGUIARTE, L. E., GOLENBERG, G., LEARN, G. H. Jr., GRAHAM, S. W., BARRETT, S. C. H., DAYANANDAN, S., ALBERT, V. A., 1993: Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbc*L. – Ann. Missouri Bot. Gard. **80**: 528–580.

CRONQUIST, A., 1968: The evolution and classification of flowering plants.–Boston: Houghton Mifflin.

– 1981: An integrated system of classification of flowering plants.–New York: Columbia University Press.

CULLEN, J., 1978: A preliminary survey of ptyxis (vernation) in the angiosperms. – Notes Roy. Bot. Gard. Edinburgh **37**: 161–214.

DAHLGREN, G., 1989: An updated angiosperm classification.–Bot. J. Linn. Soc. **100**: 197–203.

DAHLGREN, R., 1975: A system of classification of the angiosperms to be used to demonstrate the distribution of characters. – Bot. Not. **128**: 119–147.

– 1977: A note of the taxonomy of the "Sympetalae" and related groups.–Publ. Cairo Univ. **7 & 8**: 83–102.
– 1980: Angiospermernes taxonomi, Dicotyledonernes taxonomi: *Fabanae-Lamianae*. 2nd edn.–Kϕpenhavn: Akademisk Forlag.
DICKISON, W. C., 1986: Wood anatomy and affinities of the *Alseuosmiaceae*.–Syst. Bot. **11**: 214–221.
– 1989: Stem and leaf anatomy of the *Alseuosmiaceae*.–Aliso **12**: 567–578.
DOLL, W., 1927: Beitrag zur Kenntnis der Dipsaceen und dipsaceenähnlichen Pflanzen.–Bot. Arch. **17**: 107–146.
DONOGHUE, M. J., 1985: Pollen diversity and exine evolution in *Viburnum* and the *Caprifoliaceae* sensu lato.–J. Arnold Arbor. **66**: 421–469.
– OLMSTEAD, R. G., SMITH, J. F., PALMER, J. D., 1992: Phylogenetic relationships of *Dipsacales* Based on *rbc*L sequences.–Ann. Missouri Bot. Gard. **79**: 333–345.
DOYLE, J. J., DOYLE, J. L., 1987: A rapid DNA isolation procedure for small quantities of fresh leaf tissue.–Phytochem. Bull. Bot. Soc. Amer. **19**: 11–15.
DUMORTIER, B. C. J., 1829: Analyse des familles des plantes.–Tournay: Casterman.
EHRENDORFER, F., 1964: Über stammesgeschichtliche Differenzierungsmuster bei den Dipsacaceen.–Ber. Deutsch. Bot. Ges. **77**: 83–94.
ENDLICHER. S., 1839: *Columelliaceae*.–In ENDLICHER, S: Genera plantarum, p. 745.–Wien: Beck.
ENGLER, A., 1930: *Saxifragaceae*.–In ENGLER, A., (Ed.): Die natürlichen Pflanzenfamilien **18a**, pp. 74–226. 2nd edn. – Leipzig: Engelmann.
ERBAR, C., 1991: Sympetaly–a systematic character?–Bot. Jahrb. Syst. **112**: 417–451.
– 1994: Contributions to the affinities of *Adoxa* from the viewpoint of floral development.–Bot. Jahrb. Syst. **116**: 259–282.
ERIKSEN, B., 1989: Note on generic and infrageneric delimitation in the *Valerianaceae*.–Nordic J. Bot. **9**: 179–187.
FARRIS, J. S., 1969: A successive approximations approach to character weighting.–Syst. Zool. **18**: 374–385.
– 1989: The retention index and the rescaled consistency index.–Cladistics **5**: 417–419.
– ALBERT, V. A., KÄLLERSJÖ, M., LIPSCOMB, D., KLUGE, A. G., 1996: Parsimony jackknifing outperforms neighbor-joining.–Cladistics **12**: 99–124.
FELSENSTEIN, J., 1985: Confidence limits on phylogenies: an approach using the bootstrap.–Evolution **39**: 783–791.
FITCH, W. M., 1971: Toward defining the course of evolution: minimum change for a specific tree topology.–Syst. Zool. **20**: 406–416.
FRITSCH, K., 1894: *Columelliaceae*.–In ENGLER, A., (Ed.): Die natürlichen Pflanzenfamilien, **IV, 3b**, p. 186–188.–Leipzig: Engelmann.
– 1897: *Caprifoliaceae*.–In ENGLER, A., PRANTL K., (Eds): Die natürlichen Pflanzenfamilien, **IV, 4**, pp. 156–169.–Leipzig: Engelmann.
FUKUOKA, N., 1969: Inflorescence of *Linnaeeae* (*Caprifoliaceae*).–Acta Phytotax. Geobot. **23**: 153–162.
GARDNER, R. O., 1978a: Systematic notes on the *Alseuosmiaceae*.–Blumea **24**: 138–142.
– 1978b: The species of *Alseuosmia* (*Alseuosmiaceae*).–New Zealand J. Bot. **16**: 271–277.
GENTRY, A. H., 1993: A field guide to the families and genera of woody plants of Northwest South America.–Washington, DC.: Conservation International.
GUSTAFSSON, M. H. G., BREMER, K., 1995: Morphology and phylogenetic interrelationships of the *Asteraceae*, *Calyceraceae*, *Campanulaceae*, *Goodeniaceae*, and related families (*Asterales*).–Amer. J. Bot. **82**: 250–265.

– BACKLUND, A., BREMER, B., 1996: Phylogeny of the *Asterales* sensu lato based on *rbc*L sequences with particular reference to the *Goodeniaceae*.– Pl. Syst. Evol. **199**: 217–242.

HALLIER, H., 1901: Über die Verwandtschaftverhältnisse der Tubifloren und Ebenalen.– Abh. Verh. Naturwiss. Vereins Hamburg **16**: 1–112.

– 1903: Über die Abgrenzung und Verwandtschaft der einzelnen Sippen bei den Scrophularineen.–Bull. Herb. Boissier, sér. 2, **3**: 181–207.

– 1910: Über Phanerogamen von unsicherer oder unrichtiger Stellung.–Meded. Rijks-Herb. **1.**

HEGNAUER, R., 1969: Chemical evidence for the classification of some plant taxa. – In HARBORNE, J. B., SWAIN, T., (Eds): Perspectives in phytochemistry, pp. 121–138. – London, New York: Academic Press.

HEMPEL, A. L., REEVES, P. A., OLMSTEAD, R. G., JANSEN, R. J., 1995: Implications of *rbc*L sequence data for higher order relationships of the *Loasaceae* and the anomalous aquatic plant *Hydrostachys* (*Hydrostachyaceae*). –Pl. Syst. Evol. **194**: 25–37.

HERZOG, T., 1915: Die von Dr. TH. HERZOG auf seiner zweiten Reise durch Bolivien in den Jahren 1910 und 1911 gesammelten Pflanzen. II Teil.–Meded. Rijks-Herb. **27**: 1–90.

HIDEUX, M. J., FERGUSON, I. K., 1976: The stereo-structure of the exine and its evolutionary significance in *Saxifragaceae* sensu lato.–In FERGUSON, I. K., MULLER, J., (Eds): The evolutionary significance of the exine, pp. 327–377.–London: Academic Press.

HILLEBRAND, G. R., FAIRBROTHERS, D. E., 1970: Serological investigation of the systematic position of the *Caprifoliaceae*. I. Correspondence with selected *Rubiaceae* and *Cornaceae*. – Amer. J. Bot. **57**: 810–815.

HOOKER, J. D., 1875: *Columellia oblonga*.–Bot. Mag. **101**: table 6183.

HUTCHINSON, J., 1959: The families of flowering plants. **1.** Dicotyledons arranged according to a new system based on their probable phylogeny. 2nd edn.–Oxford: Clarendon Press.

JUDD, W. S., SANDERS, R. W., DONOGHUE, M. J., 1994: Angiosperm family pairs: preliminary phylogenetic analyses.– Harvard Pap. Bot. **5**: 1–51.

JUSSIEU, A.-L. DE, 1789: Genera plantarum. – Paris: Vidaum Herissant.

– 1801: Responsa ad dubia clar (*Columellia*, 147). – In RUIZ, H., PAVÓN, J., (Eds): Suplemento á la Quinologia.–Madrid: Imprenta de la Viuda e Hijo de Marin.

– 1848: Taxonomie. – In D'ORBIGNY, A. C. V. D., (Ed.): Dictionnaire universel d'histoire naturelle, pp. 368–434.–Paris: Renard, Martinet.

KALTENBOECK, B., SPATAFORA, J. W., ZHANG, X., KOUSOULAS, K. G., BLACKWELL, M., STORZ, J., 1992: Efficient production of single-stranded DNA as long as 2 kb for sequencing of PCR-amplified DNA. – Biofeedb. Self-Regulat. **12**: 164–171.

KAMELINA, O. P., 1980: Comparative embryology in the families *Dipsacaceae* and *Morinaceae*. 1st edn. – Leningrad: Nauka (in Russian).

– 1983: Basic results of the comparative embryological investigation of *Dipsacaceae* and *Morinaceae*.–In ERDELSKÁ, O., (Ed.): Fertilization and embryogenesis in ovulated plants. Proceedings of the VII. International cytoembryological symposium, High Tatra, June 14–17, 1982, pp. 343–346.–Bratislawa: Slovak Academy of Sciences.

KIM, K-J., JANSEN, R. K., 1995: *ndh*F sequence evolution and the major clades in the sunflower family.–Proc. Natl. Acad. Sci. USA **92**: 10379–10383.

KLUGE, A. G., FARRIS, J. S., 1969: Quantitative phyletics and the evolution of the anurans. – Syst. Zool. **18**: 1–32.

KUNTH, C. S., 1818: *Columellia* RUIZ et PAV. – In HUMBOLDT, A. VON, BONPLAND, A., KUNTH, C. S., (Eds): Nova genera et species plantarum, pp. 388–389.–Paris: Fol. & Qu.

KÄLLERSJÖ, M., FARRIS, J. S., KLUGE, A. G., BULT, C., 1992: Skewness and permutation. – Cladistics **8**: 275–287.

LARSEN, B. B., 1986: A taxonomic revision of *Phyllactis* and *Valeriana* sect. *Bracteata* (*Valerianaceae*).–Nordic J. Bot. **6**: 427–446.

LINDLEY, J., 1833: Nixus plantarum.–London: Ridgway.

– 1836: A natural system of botany; or, a systematic view of the organization, natural affinities, and geographical distribution, of the whole vegetable kingdom. 2nd edn.–London: Longman, Rees, Orme, Brown, Green, and Longman.

– 1853: The vegetable kingdom. 3rd edn.–London: Bradbury & Evans.

LINNAEUS, C., 1738: Classes plantarum.–Leyden: Wishoff.

LOESENER, T., 1942: *Aquifoliaceae.*–In ENGLER, A., (Ed.): Die natürlichen Pflanzenfamilien, **20b**, pp. 36–68. 2nd edn. –Leipzig: Engelmann.

MACBRIDE, J. F., 1961: Flora of Peru. – Chicago: Field Museum of Natural History.

MAOUT, E. L., DECAISNE, A., 1873: in "Editors' note" by J. D. HOOKER.–In HOOKER, J. D., (Ed.): A general system of botany, p. 594.–London: Longman, Rees, Orme, Brown, Green, and Longman.

METCALFE, D. R., CHALK, L., 1950: Anatomy of the Dicotyledons. – Oxford: Clarendon Press.

MORGAN, D. R., SOLTIS, D. E., 1993: Phylogenetic relationships among members of *Saxifragaceae* sensu lato based on *rbc*L sequence data.–Ann. Missouri Bot. Gard. **80**: 631–660.

MORI, S. A., KALLUNKI, J. A., 1977: A revision of the genus *Phyllonoma* (*Grossulariaceae*).–Brittonia **29**: 69–84.

NEUBAUER, H. F., 1978: On nodal anatomy and petiolar vascularization of some *Valerianaceae* and *Dipsacaceae.*–Phytomorphology **28**: 431–436.

NIEDENZU, F., HARMS, H., 1930: *Bruniaceae.*–In ENGLER, A., (Ed.): Die natürlichen Pflanzenfamilien **18a**, p. 288. 2nd edn.–Leipzig: Engelmann.

OLMSTEAD, R. G., MICHAELS, H. J., SCOTT, K. M., PALMER, J. D., 1992: Monophyly of the *Asteridae* and identification of their major lineages inferred from DNA sequences of *rbc*L. – Ann. Missouri Bot. Gard. **79**: 349–265.

– BREMER, B., SCOTT, K. M., PALMER, J. D., 1993: A parsimony analysis of the *Asteridae* sensu lato based on *rbc*L sequences.–Ann. Missouri Bot. Gard. **80**: 700–722.

PHILIPSON, W. R., 1970: Constant and variable features of the *Araliaceae.* – In ROBSON, N. K. B., CULTER, D. F., GREGORY, M., (Eds): New research in plant anatomy, pp. 87–100. – London: Academic Press.

PILLANS, N. S., 1947: A revision of *Bruniaceae.* – S. African J. Bot. **13**: 121–207.

PLUNKETT, G. M., SOLTIS, D. E., SOLTIS, P. S., 1992: Molecular phylogenetic study of *Apiales* (*Apiaceae, Araliaceae*, and *Pittosporaceae*). – Amer. J. Bot. **79**: 158.

– – – 1996: Higher level relationships of *Apiales* (*Apiaceae* and *Araliaceae*) based on phylogenetic analysis of *rbc*L sequences. – Amer. J. Bot. **83**: 499–515.

POLLARD, C. J., AMUTI, R. S., 1981: Fructose oligosaccharides: possible markers of phylogenetic relationships among dicotyledonous plant families. – Biochem. Syst. Ecol. **9**: 69–78.

REICHENBACH, H. G. L., 1828: Conspectus regni vegetabilis per gradus naturales evoluti.–Leipzig: Cnobloch.

– 1837: Handbuch des natürlichen Pflanzensystems.–Dresden, Leipzig: Arnold.

RODRÍGUEZ, R. L., 1971: The relationships of the *Umbellales.* – In HEYWOOD, V. H., (Ed.): The biology and chemistry of the *Umbelliferae*, pp. 63–91. – Reading: Academic Press.

ROELS, P., 1993: Lengtepolymorfisme van chloroplast-DNA restrictiefragmenten en bloemontogenie in de *Dipsacales.* – Degree Thesis, Katholieke Universiteit Leuven.

– SMETS, E., 1995: A comparative floral ontogenetical study between *Adoxa moschatellina* and *Sambucus ebulus.*–Belg. J. Bot. **127**: 157–170.

SAGHAI-MAROOF, M. A., SOLIMAN, K. M., JORGENSEN, R. A., WALLARD, R. W., 1984: Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. – Proc. Natl. Acad. Sci. USA **81**: 1769–1778.

SAVOLAINEN, V., MANEN, J. F., DOUZERY, E., SPICHIGER, R., 1994: Molecular phylogeny of families related to *Celastrales* based on *rbc*L 5′ flanking sequences. – Molec. Phylogenet. Evol. **3**: 27–37.

SAXTON, W. T., 1910: The ovule of the *Bruniaceae*. – Trans. Roy. Soc. S. Africa **2**: 27–31.

SCHNIZLEIN, A., 1849: Iconographia familiarum naturalium regni vegetabilis. – Bonn: Cohen.

SOLEREDER, H., 1899: Systematische Anatomie der Dicotyledonen. – Stuttgart: Enke.

STEENIS, C. G. G. J. VAN, 1984: A synopsis of *Alseuosmiaceae* in New Zealand, New Caledonia, Australia, and New Guinea. – Blumea **29**: 387–394.

SWOFFORD, D. L., 1993: PAUP: phylogenetic analysis using parsimony, version 3.1.1. Computer program. – Champaign, Illinois: Illinois Natural History Survey.

– 1996: PAUP: phylogenetic analysis using parsimony, version 4.0d45. Computer program. – Swofford, personal distribution.

TAKHTAJAN, A. L., 1987: Systema Magnoliophytorum. – Leningrad: Nauka.

THORNE, R. F., 1968: Synopsis of a putatively phylogenetic classification of the flowering plants. – Aliso **6**: 57–66.

– 1973: Inclusion of the *Apiaceae* (*Umbelliferae*) in the *Araliaceae*. – Notes Roy. Bot. Gard. Edinburgh **32**: 161–165.

– 1992: Classification and geography of the flowering plants. – Bot. Rev. **58**: 225–348.

THULIN, M., 1991: Another arborescent umbellifer: a new species of *Steganotaenia* from north-east tropical Africa. – Bot. J. Linn. Soc. **107**: 164.

TIEGHEM, P. VAN, 1909: Remarques sur les Dipsacacées. – Ann. Sci. Nat. Bot. **10**: 148–200.

UTZSCHNEIDER, R., 1947: Der Fruchtknotenbau der Rubiaceen mit besonderer Berücksichtigung der Cinchonoideen. – Thesis, University of München.

VERLAQUE, R., 1977: Rapports entre les *Valerianaceae*, les *Morinaceae* et les *Dipsacaceae*. – Bull. Soc. Bot. France **124**: 475–482.

VIJAYARAGHAVAN, M. R., SARVESHWARI, G. S., 1968: Embryology and systematic position of *Morina longifolia* WALL. – Bot. Not. **121**: 383–402.

VINOKUROVA, L. V., 1959: Palynological data on the systematic position of *Dipsacaceae* and *Morinaceae*. – Probl. Bot. **4**: 51–67 (in Russian).

WAGENITZ, G., 1959: Die systematische Stellung der *Rubiaceae* – Ein Beitrag zum System der Sympetalen. – Bot. Jahrb. **79**: 17–35.

WILLIS, J. C., revised by AIRY SHAW, H. K., 1966: A dictionary of the flowering plants and ferns. 7th edn. – London: Cambridge University Press.

XIANG, Q.-Y., SOLTIS, D. E., 1996: *rbc*L sequence divergence and phylogenetic relationships of *Cornaceae* sensu lato. – In BOUFFORD, D. E., OHBA, H., (Eds): Sino-Japanese flora – its characteristics and diversification. – Tokyo: University of Tokyo Press.

– – MORGAN, D. R., SOLTIS, P. S., 1993: Phylogenetic relationships of *Cornus* sensu lato and putative relatives inferred from *rbc*L sequence data. – Ann. Missouri Bot. Gard. **80**: 723–734.

YAKOVLEV, M. S., ZHUKOVA, G. Y., 1980: Chlorophyll in embryos of angiosperm seeds, a review. – Bot. Not. **133**: 323–336.

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Accepted June 18, 1996 by I. KRISAI-GREILHUBER