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A phylogenetic analysis of Primulaceae s.l. based on internal transcribed spacer (ITS) DNA sequence data

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Abstract. Phylogenetic relationships in Primulaceae were investigated by analysis of nuclear rDNA ITS sequences. Thirty-four species of Primulaceae, two of Myrsinaceae and four outgroup taxa were analyzed. In accordance to the results of recently published papers on the phylogeny of Primulaceae we found the family to be paraphyletic and resolved the positions of some genera. Our results show (a) the rather basal position of *Centunculus* within Lysimachieae, the genus thus being rather distantly related to *Anagallis*, (b) the close relationship between *Lysimachia* sect. *Lerouxia*, *Anagallis*, *Asterolinon*, and *Pelletiera*, (c) the well-supported monophyly of a group consisting of the four genera *Hottonia*, *Omphalogramma*, *Bryocarpum*, and *Soldanella*, and (d) the affinity of *Stimpsonia* to the Myrsinaceae-Lysimachieae-*Ardisiandra* clade. The ITS sequence data do not provide sufficient information to resolve basal relationships within the Primulaceae s.l. There is evidence against the monophyly of the large genera *Primula*, *Androsace*, and *Lysimachia*. In contrast to the phylogenetic reconstructions based on plastid gene sequences, *Cyclamen* does not appear as a member of the Myrsinaceae-Lysimachieae clade, but its position remains unclear.

Key words: Primulales, Primulaceae, phylogeny, ITS.

The latest complete treatment of the family Primulaceae includes nearly 1000 species in 22 genera (Pax and Knuth 1905). Together with Myrsinaceae and Theophrastaceae it constitutes the order Primulales, which was recently merged into a large order Ericales s.l. (APG 1998).

Phylogenetic approaches based on morphology have been undertaken by, for example, Wendelbo (1961b; tribe Primuleae), Røsvik (1969a), and Anderberg and Ståhl (1995). Primulaceae and Myrsinaceae were distinguished by their growth form (mostly herbaceous vs. woody) and fruit morphology (capsular vs. drupaceous), but their separation had already been thought to be artificial by Mez (1902) and Pax and Knuth (1905) because of striking congruence in flower morphology: in both families at least some genera have a connate five-lobed corolla with twisted aestivation, epipetalous stamens fused to the corolla, bilocular introrse anthers usually longer than the filaments, and a superior ovary with free central placenta and few to numerous bitegmic tenuinucellate ovules often immersed in the placenta. This was not considered by later authors until recently, when phylogenetic studies based on plastid molecular data were

made by Anderberg et al. (1998), Källersjö et al. (2000), and Mast et al. (2001). These studies have shown Myrsinaceae (except *Maesa*) to be nested within Primulaceae, thus the latter being paraphyletic, and changes of the circumscription of primuloid families have been proposed by Källersjö et al. (2000) and Anderberg et al. (2000a).

Throughout all classifications of Primulaceae two major groups (tribes or subfamilies) are recognized: Lysimachieae, which are characterised by leafy stems, contort corolla aestivation, and rather short corolla tubes, and Primuleae with leaves usually in basal rosettes, imbricate corolla aestivation, and longer corolla tubes. The positions of the isolated genera *Coris*, *Samolus*, *Cyclamen*, and *Ardisiandra* are uncertain; each of them has been placed in a separate tribe (e.g. Røsvik 1969a).

This study is an attempt to elucidate the phylogeny of major groups of Primulaceae in their traditional circumscription (i.e. including Lysimachieae) and to evaluate the monophyly of the larger genera (i.e. *Androsace, Lysimachia*, and *Primula*) using nuclear ribosomal DNA internal transcribed spacer sequences. ITS rDNA sequences have been widely used in plant molecular phylogenetics of genera, subfamiliar taxa and families, e.g. in Agavaceae (Bogler and Simpson 1996) and Polemoniaceae (Porter 1997).

Material and methods

Taxon sampling

For sequence analysis, at least one species was chosen from each genus of Primulaceae s.l. No suitable material could be obtained of *Kaufmannia* and *Sredinskya*. From the larger genera representatives of major infrageneric groups were chosen: six of the seven subgenera of *Primula* (following the subgeneric classification of Fenderson 1986) are represented by one species each. Additionally, two species from Myrsinaceae were chosen.

Two species from Theophrastaceae, which are the sister family of the Primulaceae-Myrsinaceae complex (Anderberg et al. 1998) and another two species from more distantly related families of the Ericales s.l. in the sense of APG (1998) were selected as outgroup taxa. The investigated species and their accession data are given in Table 1.

DNA isolation and amplification

Genomic DNA was extracted from silica-dried leaves or from herbarium material following the protocol described by Hellwig et al. (1999). When possible, double-stranded DNA of the complete ITS region was amplified by PCR using the primers "ITS5" and "ITS4"; otherwise ITS-1 and ITS-2 were amplified separately using the primers "ITS5" and "P2" for ITS-1 and "ITS3" and "ITS4" for ITS-2. Primers "ITS3" (5′-GCATCGATGAAGAACGCAGC-3′), "ITS4" (5′-TCCTCCGCTTATTGATATGC-3′), and "ITS5" (5′-GGAAGTAAAAGTCGTAACAAGG-3′) were designed and named by White et al. (1990), primer "P2" (5′-CTCGATGGAACACGGGATTCTGC-3′) by Ochsmann (2000). The amplification profile was 28 cycles of 95 °C for 30 s, 42 °C for 60 s, 72 °C for 60 s, preceded by an initial denaturation at 95 °C for 90 s and followed by a final extension at 72 °C for 180 s. In some cases, where the PCR resulted in multiple bands, the annealing temperature was increased to max. 61 °C and/or Q-solution (QIAGEN) was added to the PCR reactions.

Sequencing

The amplified DNA was sequenced directly using the dideoxy chain termination method. Cycle-sequencing was performed using IRD-labelled primer pairs "ITS5"/"P2" and "ITS3"/"ITS4" (MWG-Biotech AG) and the ThermoSequinase labelled primer cycle sequencing kit (Amersham Pharmacia) following the manufacturer's instructions and using the following cycling program: 95 °C for 120 s, 28 cycles of 95 °C for 15 s, 60 °C (for ITS-1) or 57 °C (for ITS-1) for 15 s, 70 °C for 15 s. The resulting DNA fragments were separated on an acrylamide gel, using an automatic LI-COR DNA sequencer 4000L. Both forward and reverse strands of each ITS region were sequenced. All new sequences were submitted to GenBank (accession numbers in Table 1).

Phylogenetic reconstruction

Sequence alignment was carried out using the software ClustalW (Thompson et al. 1994, 1997),

Table 1. The taxa under study and their accession data. Voucher information is given for new sequences, for the others the original papers are cited. Herbarium abbreviations according to Holmgren et al. (1990). Classification according to Røsvik (1968)

Species	Voucher data	Accession number
Ardisiandreae		
Ardisiandra wettsteinii R. Wagner	Martins 464 (JE), cult. in BG Jena	AJ491426, AJ491670
Corideae		
Coris monspeliensis L.	Renker & Beyer 371 (JE), France, Alpes-Marit.	AJ491447, AJ491688
Cyclamineae		
Cyclamen hederifolium Aiton	Martins 624 (JE), cult. in BG Jena	AJ491440, AJ491684
Lysimachieae		
Anagallis arvensis L.	Martins 549 (JE), Germany, Thüringen	AJ491414, AJ491658
Asterolinon linum-stellatum (L.) Duby	Martins 467 (JE), cult. in BG Jena	AJ491416, AJ491660
Centunculus minimus L.	Martins 468 (JE), Germany, Sachsen-Anhalt	AJ491422, AJ491666
Glaux maritima L.	Martins 628 (JE), cult. in BG Jena	AJ491417, AJ491661
Lysimachia azorica Hornem. ex Hook.	(Anderberg et al. 2000b)	AF164017
Lysimachia nummularia L.	Martins 625 (JE), cult. in BG Jena	AJ491418, AJ491662
Lysimachia thyrsiflora L.	Martins 477 (JE), cult. in BG Jena	AJ491420, AJ491664
Lysimachia vulgaris L.	Martins 481 (JE), Germany, Thüringen	AJ491419, AJ491663
Pelletiera wildpretii Valdés	Martins 398 (JE), Spain, Tenerife	AJ491415, AJ491659
Trientalis europaea L.	Martins 479 (JE), Germany, Thüringen	AJ491421, AJ491665
Primuleae		
Androsace alpina (L.) Lam.	Günther 25. 7. 1998 (JE), Switzerland, Bern	AJ491444, AJ491686
Androsace helvetica (L.) All.	Günther 25. 7. 1998 (JE), Switzerland, Bern	AJ491445
Androsace lanuginosa Wall.	Martins 630 (JE), cult. in BG Jena	AJ491441, AJ491685
Bryocarpum himalaicum Hook. f. & Thoms.	Bartholomew 5. 5. 1984 (E), Bhutan	AJ491428, AJ491672
Cortusa matthioli L.	Martins 627 (JE), cult. in BG Jena	AJ491437, AJ491681
Dionysia aretioides (Lehm.) Boiss.	Martins 480 (JE), cult. in BG Jena	AJ491434, AJ491678
Dodecatheon meadia L.	Martins 626 (JE), cult. in BG Jena	AJ491436, AJ491680
Douglasia montana A. Gray	Lowry 288 (GOET), USA, Wyoming	AJ491446, AJ491687
Hottonia palustris L.	Martins & Müller 476 (JE), cult. in BG Göttingen	AJ491430, AJ491674
Omphalogramma vinciflora Franch.	Chamberlain 6. 7. 1987 (E), China, Yunnan	AJ491427, AJ491671
Pomatosace filicula Maxim.	Dickoré 4553 (GOET), China, Qinghai	AJ491442
Primula farinosa L.	(Anderberg et al. 2000b)	AF164014
Primula forrestii Balf. f.	Martins 473 (JE), cult. in BG Jena	AJ491438, AJ491682
Primula palinuri Petagna	Martins 471 (JE), cult. in BG Jena	AJ491435, AJ491679
Primula petiolaris Wall.	Heider 16. 3. 1892 (B), India, C. Himalaya	AJ491431, AJ491675
Primula veris L.	Martins 470 (JE), cult. in BG Jena	AJ491433, AJ491677
Primula verticillata Forssk. subsp. *simensis* (Hochst.) W. W. Sm. & Forrest	Martins 469 (JE), cult. in BG Jena	AJ491432, AJ491676

Table 1 (continued)

Species	Voucher data	Accession number
Soldanella montana Willd.	Martins 629 (JE), cult. in BG Jena	AJ491429, AJ491673
Stimpsonia chamaedryoides Wright	Luo 23. 4. 1995 (E), China, Hunan	AJ491425, AJ491669
Vitaliana primuliflora Bertol.	Renker & Beyer 482 (JE), France, Hautes-Alpes	AJ491443
Samoleae		
Samolus valerandi L.	Martins 466 (JE), cult. in BG Jena	AJ491439, AJ491683
Myrsinaceae		
Heberdenia excelsa (Aiton) Banks	Martins 402 (JE), Spain. Tenerife	AJ491424, AJ491668
Pleiomeris canariensis (Willd.) DC.	Martins 392 (JE), Spain. Tenerife	AJ491423, AJ491667
outgroup		
Theophrastaceae		
Clavija grandis Decne.	Martins 622 (JE), cult. in BG Jena	AJ491448, AJ491689
Deherainia smaragdina Decne.	Martins 623 (JE), cult. in BG Jena	AJ491449, AJ491690
Ebenaceae		
Diospyros texana Scheele	(Jackson et al. 1999)	AF174622
Ericaceae		
Vaccinium fuscatum Aiton	(Schultheis & Baldwin 1999)	AF084322, AF084323

and apparent misalignments were corrected manually. Two data sets were analyzed: (1) complete ITS-1 + ITS-2 and (2) ITS-1 + ITS-2 excluding highly variable regions which could not be aligned unambiguously. The exclusion of such regions follows the recommendations of Baldwin (1992) and Baldwin et al. (1995). Gaps were treated as missing data, but phylogenetically informative indels were coded as binary data and added to the data matrix. The data matrices can be obtained from the authors.

Evolutionary distances were calculated and a neighbor joining tree was constructed using Treecon (Van de Peer 1995) on the basis of data set (1), using the Kimura two parameter model (Kimura 1980) and gaps taken into account.

Parsimony analysis was performed using the heuristic search modus in PAUP version 4.0b4a (Swofford 2000) with 100 random addition sequence replicates, TBR branch swapping, and MulTrees options in function. The analysis follows the principles of Fitch parsimony (Fitch 1971), i.e. all character states were equally weighted and treated as unordered. Strict consensus trees were calculated if the analysis resulted in more than one most parsimonious tree.

Branch support was evaluated by bootstrap analysis (Felsenstein 1985), with 100 replicates and the same settings as described above, and Bremer support (Bremer 1988), using the computer program Autodecay version 3.0 (Eriksson and Wikström 1995).

Results

DNA sequencing of ITS-1 was successful in all of the species given in Table 1, but sequencing of ITS-2 failed in three species and was partly successful in another six species. In the ingroup the length of ITS-1 varies from 208 in *Asterolinon linum-stellatum* to 256 in *Primula veris*; the minimum and maximum lengths of ITS-2 are 198 in *Anagallis arvensis* and 223 in *Douglasia montana*, respectively.

Total alignment length is 564 positions (311 in ITS-1 and 253 in ITS-2), of which 369 are parsimony-informative, 88 variable but parsimony-uninformative, and 107 constant characters (within ingroup: 324 informative, 88 uninformative, 152 constant). For the data set excluding the regions of ambiguous alignment 112 positions were deleted. Gap coding increased the number of informative characters by 102 (67 when ambiguous regions excluded; within the ingroup 79 and 51, respectively).

Pairwise distances within the ingroup vary from 0.029 between *Asterolinon* and *Pelletiera*

to 0.527 between *Diospyros* and *Bryocarpum* (within the ingroup max. 0.467 between *Vitaliana* and *Bryocarpum*). Very low distances (<0.1) are observed between the two Myrsinaceae s.str., within the group *Vitaliana-Douglasia-Androsace helvetica-A. alpina*, and other pairs of taxa which are supported by bootstrap percentage of 100 in the distance tree (Fig. 1).

The heuristic search in PAUP based on the complete data set resulted in six most parsimonious trees (Fig. 2). The tree length is 2489 steps, retention index (*RI*) 0.56 and consistency index excluding uninformative characters (*CI*) 0.39. The corresponding statistics for the reduced data set are: eight most parsimonious trees with tree length 1853 steps, retention index 0.55 and consistency index 0.39. The consensus tree of the latter analysis is not shown since the topology is only slightly different, all groups with moderate and high support (bootstrap $>60\%$) are the same as in Fig. 2.

When disregarding branches with low support (bootstrap $<60\%$), six basal clades can be recognized in the cladogram (Fig. 2): (1) *Coris*, (2) *Androsace* p. p.-*Vitaliana-Douglasia*, (3) *Cyclamen*, (4) *Samolus*, (5) *Androsace lanuginosa-Pomatosace*, and (6) the remaining Primuleae, Ardisiandreae, Myrsinaceae s.str., and Lysimachieae. The latter clade has moderate support of 62%. It is further split into well-supported clades: (a) *Primula forrestii* + *Cortusa*, (b) *Primula palinuri* + *Dodecatheon*, (c) the rest of *Primula* + *Dionysia*, and (d) the *Hottonia-Soldanella-Bryocarpum-Omphalogramma* clade, and a moderately supported larger clade which contains *Stimpsonia*, *Ardisiandra*, Myrsinaceae s.str., and Lysimachieae. Within Lysimachieae only *Lysimachia vulgaris* + *L. thyrsiflora*, *Pelletiera* + *Asterolinon*, and the latter two plus *Anagallis arvensis* and *Lysimachia azorica* are well-supported groups. In general, the neighbor joining shows the same well-supported groups, but in addition the groups consisting of branches (2) and (5) as well as (a) and (b) and a few smaller groups have good support.

Discussion

Several plastid DNA sequence analyses strengthen the assumption that Myrsinaceae (excluding *Maesa*; Källersjö et al. 2000) are nested within Primulaceae s.l. (Anderberg et al. 1998, Källersjö et al. 2000, Mast et al. 2001). These authors found strong support for a sister group relationship between Myrsinaceae s.str. and the tribe Lysimachieae of Primulaceae. Consequently, Lysimachieae were conferred to Myrsinaceae by Källersjö et al. (2000). This assumption is additionally supported by the results of our investigation.

Contort corolla aestivation can be seen as a synapomorphy of the Myrsinaceae–Lysimachieae clade. Myrsinaceae have been segregated from Primulaceae s.l. because of their woody growth form and drupaceous indehiscent fruits (Cronquist 1981). The herbaceous growth form and capsular many-seeded fruits are plesiomorphic in Primulaceae s.l. (Anderberg et al. 1998) and thus are not suitable features for delimitation of monophyletic groups.

The delimitation of Lysimachieae was undisputed in most classifications and is so in this study, although their monophyly is rather weakly supported. Lysimachieae are characterized by contort corolla aestivation (present also in Myrsinaceae and *Cyclamen*), short or absent corolla tube, entire cauline leaves, and presence of schizogenous resin canals. Most of these characters also occur in Myrsinaceae (Schwarz 1963). Thus, the close relationship between Lysimachieae and Myrsinaceae is corroborated by morphological and anatomical data.

There is evidence from both nuclear rDNA data (this study) and plastid DNA sequence data (Källersjö et al. 2000) for a sister group relationship between Lysimachieae and Ardisiandreae, the latter comprising the single genus *Ardisiandra*. Nevertheless the position of *Ardisiandra* remains questionable (50% bootstrap support for Lysimachieae-*Ardisiandra*-clade). Even if we assumed that the topology (Fig. 2) is correct, no decision can be made

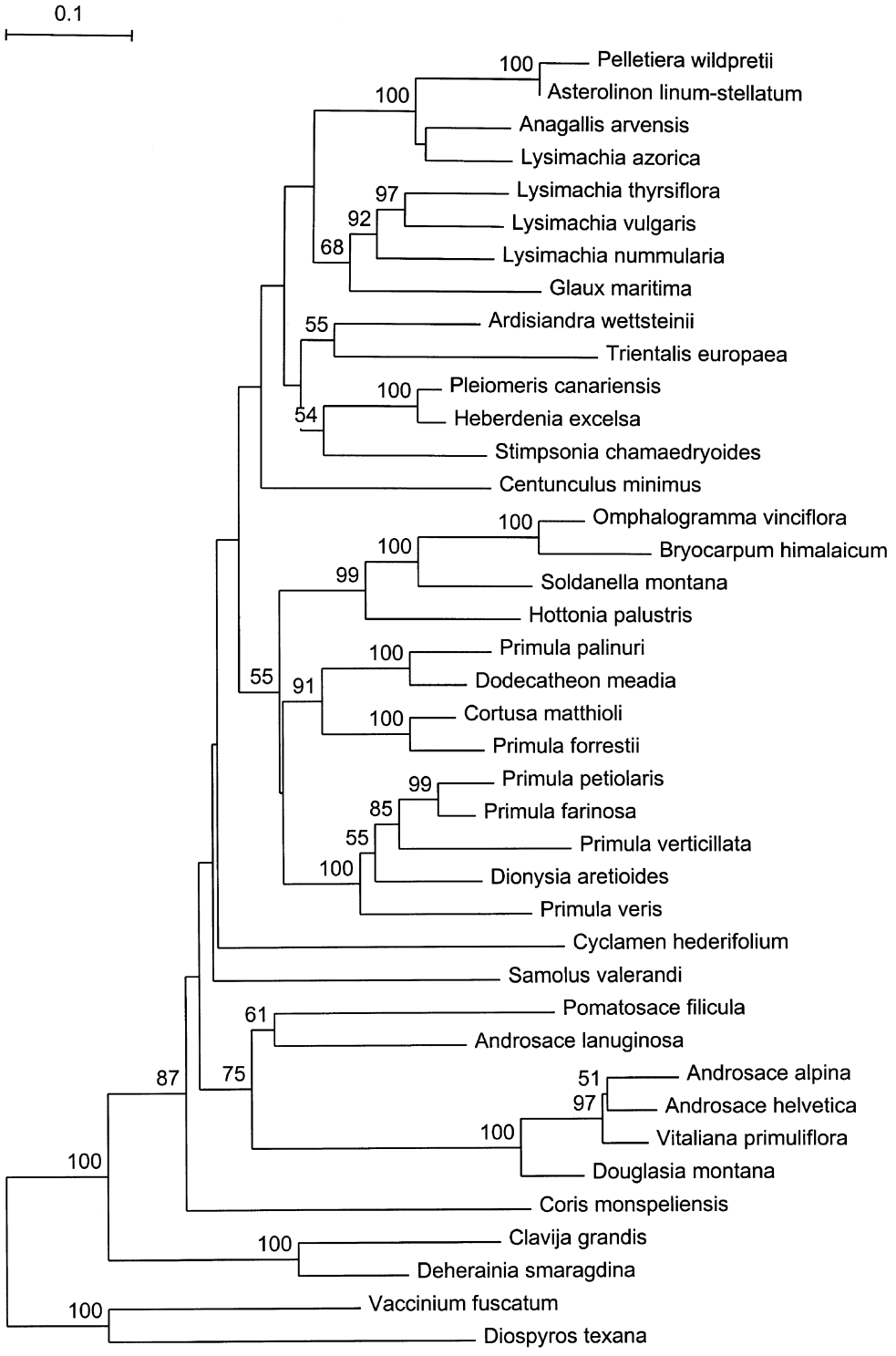

Fig. 1. Neighbor joining tree constructed with the two parameter model of Kimura (1980). Insertions and deletions are taken into account. A bootstrap analysis was conducted with 1000 replicates, bootstrap percentages > 50 are given above branches

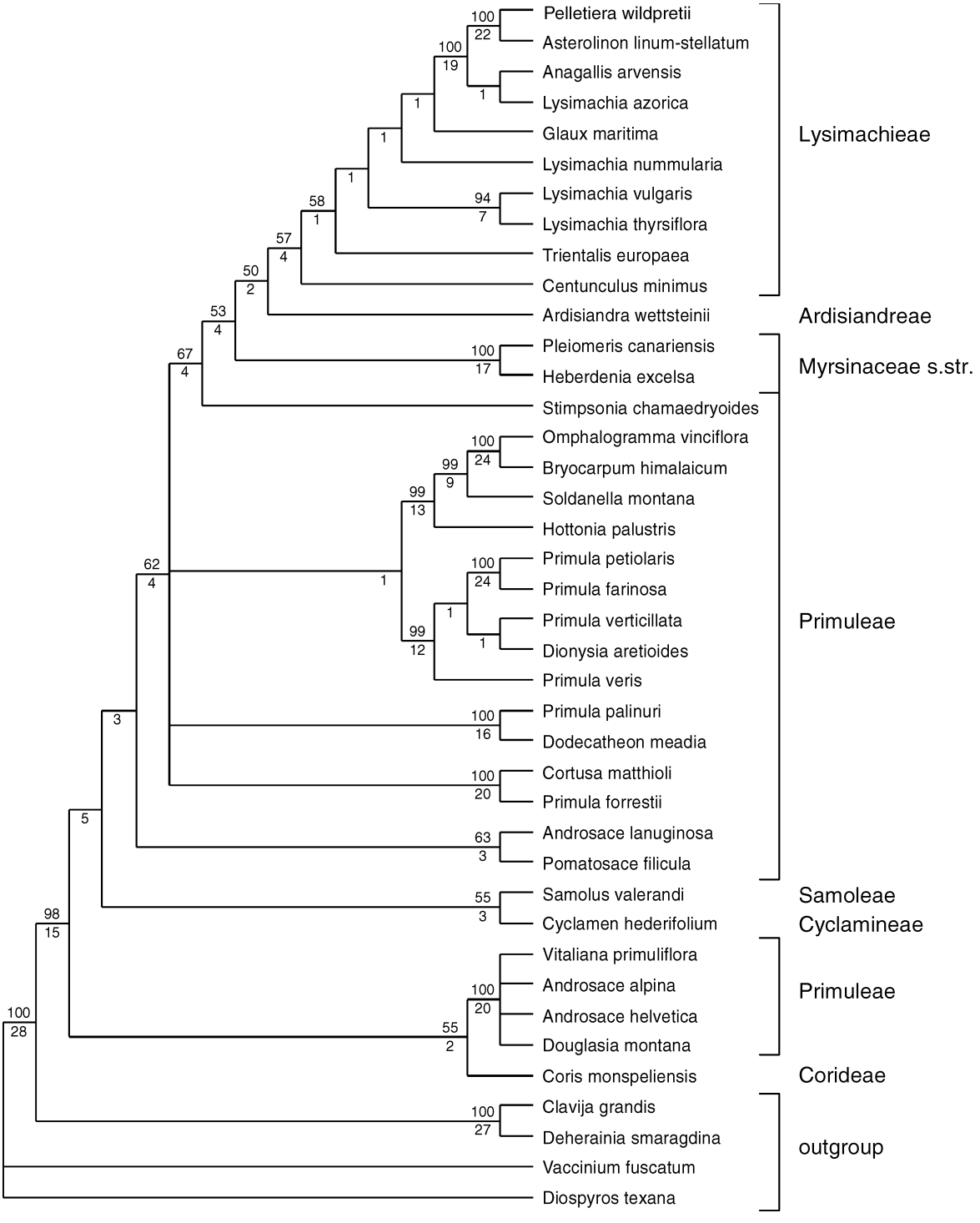

Fig. 2. Strict consensus tree of six equally most parsimonious trees based on ITS sequence data. Gaps are treated as missing data and additionally coded in a binary data matrix. Tree length = 2489; $CI = 0.39$; $RI = 0.56$. A bootstrap analysis was conducted with 100 replicates. Bootstrap percentages >50 are indicated above, Bremer support below branches

at this point whether contort corolla aestivation is synapomorphic for Myrsinaceae and Lysimachieae, which would require a reversal in *Ardisiandra*, or originated twice, whereas *Ardisiandra* retained the plesiomorphic imbricate aestivation.

There is little resolution within Lysimachieae, but apparently *Lysimachia* is not monophyletic. *Lysimachia azorica* groups together with *Anagallis*, *Pelletiera*, and *Asterolinon* rather than with the other representatives of *Lysimachia*. The monophyly of this group is confirmed by a synapomorphic deletion of 20 nucleotides in ITS-1, which is not present in any other of the taxa examined. Despite the differences in capsule dehiscence (circumscissile vs. valvate) and petal colour there is an apparent resemblance (sessile entire leaves, solitary axillary flowers, curving of the fruit pedicel) between *Anagallis arvensis* on the one hand and *L. azorica* and the closely related *L. nemorum* (Heubl and Vogt 1988) on the other. Resurrection of the genus *Lerouxia* Mérat (*Lysimachia* sect. *Lerouxia* (Mérat) Endl.), comprising among others *L. azorica* and *L. nemorum*) might solve the problem, if one is willing to maintain *Anagallis* and *Asterolinon* as separate genera. A decision on the monophyly of the rest of *Lysimachia* requires additional data.

Our results show that the previous infrageneric classification of *Lysimachia* is doubtful. The separation of the subgenera *Naumburgia* and *Lysimachia* is not supported here because the two representatives of subg. *Lysimachia* (i. e. *L. vulgaris* and *L. nummularia*) are more distantly related to each other than *L. vulgaris* is to *L. thyrsiflora* (subg. *Naumburgia*). The latter two have the same basic chromosome number ($x = 7$) and the same pollen type (Heubl 1989). These characters are not shared by the other representatives of the genus. The opinion of Heubl (1989) that the subgeneric rank of *Naumburgia* is doubtful is in agreement with our results.

The ITS sequences of *Asterolinon* and *Pelletiera* show the shortest observed distance. In the cladistic analyses they form a well supported monophyletic group, which is additionally supported by a 15 bp deletion in ITS-1 and moreover by strong reduction in corolla size compared to *Anagallis* and *Lysimachia*. They resemble each other in their habit, the main difference being the reduction of corolla segments, stamens, and carpels from five to three in *Pelletiera* (Valdés 1980).

Centunculus and *Anagallis* form the subtribe Anagallidinae (Pax and Knuth 1905) or are even merged into one genus *Anagallis* (e. g. Røsvik 1969a) due to the presence of pyxidia. In contrast to this, all cladograms show a well-supported close relationship of *Anagallis* to *Pelletiera*, *Asterolinon*, and *Lysimachia* sect. *Lerouxia*, whereas *Centunculus* has a basal position within Lysimachieae. Thus, the circumscissile capsule dehiscence type has evolved independently in *Anagallis* and *Centunculus*. None of the plastid DNA investigations included *Centunculus*.

The systematic position of the monotypic East Asian genus *Stimpsonia* has been controversial. Most authors emphasized its affinities to *Androsace* (Wendelbo 1961b, Røsvik 1969b, Mast et al. 2001), which is widely distributed in Eurasia (circumpolar, if *Douglasia* is included). Schwarz (1963) conferred *Stimpsonia* to Ardisiandreae, whereas Takhtajan (1987) treated it as a member of Primuleae (and not Androsaceae). However, in our analyses *Stimpsonia* appears as a basal member of the Myrsinaceae-Lysimachieae-*Ardisiandra* clade (i.e. Myrsinaceae sensu Källersjö et al. 2000). This is corroborated by morphological characters such as alternate cauline leaves and flowers solitary in leaf axils.

In the genus *Primula* three groups can be distinguished, each of them having high internal support: *P. forrestii,* representing subg. *Auganthus* (Link) Wendelbo, forms a clade together with *Cortusa*; *P. palinuri,* representing subg. *Auriculastrum* Schott, is most closely related to *Dodecatheon*; all other subgenera (except *P.* subg. *Carolinella,* which was not sampled) form a strongly supported monophyletic group including *Dionysia*. *Sredinskya* (Stein) Fedorov with the single species

S. grandis (Trautv.) Fedorov is probably closely related to *Primula* subg. *Primula* (Smith and Fletcher 1946, Wendelbo 1961b, Richards 1993, Trift et al. 2002), and thus it is expected to join this clade when sampled. *Sredinskya* is distinguished from *Primula* by narrow erect corolla-lobes, but shares many characters with *Primula* subg. *Primula*; most striking is the very similar structure of the 6-colpate pollen grains (Wendelbo 1961b).

Primula palinuri forms a clade with *Dodecatheon meadia* with 100% support. Both *Primula* sect. *Auricula* (represented by *P. palinuri*) and *Dodecatheon* possess pollen of the *Auricula*-type, the same base chromosome number of $x = 11$ (Spanowsky 1962), flowers arranged in umbels, and more or less fleshy leaves with involute leaf vernation. Moreover, the neighbor-joining tree (Fig. 1) shows a short distance between these two taxa, indicating high sequence similarity. Thus, it seems likely that *Dodecatheon* has its closest relatives among *P.* subg. *Auriculastrum*.

The close relationship between *Cortusa* and members of *Primula* subg. *Auganthus* is supported by characters such as the presence of articulated hairs, lobed leaves with a distinct petiole (e.g. Decrock 1901, Wendelbo 1961a), and a common chromosome base number of $x = 12$ (Spanowsky 1962). Richards (1993) proposed the presence of a rather recent common ancestor of the genus *Cortusa* and *Primula* subg. *Auganthus* sect. *Cortusoides*, which is supported by both analyses. *Kaufmannia* probably also belongs to this group since it strongly resembles *Cortusa* and often even is included in it (Decrock 1901, Wendelbo 1961b).

The analyses resulted in a clade combining the genera *Omphalogramma*, *Bryocarpum*, and *Soldanella*, the former two forming a well-supported monophyletic group. This is contradictory to Anderberg and Ståhl (1995), who suggested the closest relatives of *Omphalogramma* within the genus *Primula*, but is a confirmation of the supposition of Mast et al. (2001) who expected *Bryocarpum* to join *Omphalogramma* when sampled. Diels (1910) supposed *Soldanella* to be a European parallel trait to the East Asian *Omphalogramma-Bryocarpum* group. The present results are in agreement with this opinion.

Hottonia appears as the sister of the above mentioned group. There are no or only few apparent common features of *Hottonia* and the *Soldanella-Omphalogramma-Bryocarpum* group. Instead, *Hottonia* shares many characters with some species of *Primula*, as for example heterostyly and verticillate flower arrangement. Therefore it was considered to be a descendant of *Primula* by Richards (1993) and Anderberg and Ståhl (1995). However, there is also support for the assumption of a common ancestry of *Hottonia*, *Soldanella*, *Omphalogramma*, and *Bryocarpum* from plastid DNA sequence data (Mast et al. 2001). The conflict between morphological and molecular data may be resolved by further investigations.

The *Androsace* group (i.e. *Androsace*, *Douglasia*, *Vitaliana*, and *Pomatosace*) is probably monophyletic, based on the neighbor-joining result (still weakly supported), plastid DNA (Mast et al. 2001), and morphological features (short corolla tube with constricted annular throat, pollen morphology, see Wendelbo 1961b), although not supported in the parsimony analysis. The genus *Androsace* is clearly not monophyletic. *Androsace* sect. *Aretia* (L.) Duby (*A. alpina* and *A. helvetica* in this study), *Vitaliana*, and *Douglasia* constitute a strongly supported clade. These four taxa share a deletion of 21 nucleotides in ITS-1, which is not observed in *A. lanuginosa* (sect. *Chamaejasme* Koch) and its putative close relative *Pomatosace*.

The transfer of *Samolus* to Theophrastaceae by Källersjö et al. (2000) is based on a weakly supported topology inferred from plastid molecular data but was confirmed by additional data by Anderberg et al. (2002). The results of our ITS sequence analysis are not in agreement with this. However, the position of *Samolus* close to *Cyclamen* in the parsimony analysis must be looked at with caution. It is weakly supported (55 %) and is not supported by morphology at all. A more

complete sampling and more data are necessary to solve this problem.

Several studies on the systematics of Primulaceae published in the last decade resulted in partly conflicting phylogenies. Analysis of ITS sequences provides new insights in the phylogeny of the family with respect to the systematic position of some genera, but the present sampling and the hitherto analysed molecular markers could not fully resolve intergeneric relationships in Primulaceae.

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