Syst. Biol. 53(6):856–876, 2004
Copyright © Society of Systematic Biologists
ISSN: 1063-5157 print / 1076-836X online
DOI: 10.1080/10635150490522566

Complex Biogeographic Patterns in *Androsace* (Primulaceae) and Related Genera: Evidence from Phylogenetic Analyses of Nuclear Internal Transcribed Spacer and Plastid *trnL-F* Sequences

GERALD M. SCHNEEWEISS,[1,2] PETER SCHÖNSWETTER,[1] SYLVIA KELSO,[3] AND HARALD NIKLFELD[1]

[1]*Department of Plant Biogeography and*
[2]*Department of Higher Plant Systematics and Evolution, Institute of Botany, University of Vienna, Rennweg 14, A-1030 Vienna, Austria; E-mail: gerald.schneeweiss@univie.ac.at (G.M.S.)*
[3]*Department of Biology, Colorado College, Colorado Springs, Colorado, USA*

Abstract.—We conducted phylogenetic analyses of *Androsace* and the closely related genera *Douglasia*, *Pomatosace*, and *Vitaliana* using DNA sequences of the nuclear internal transcribed spacer (ITS) and the plastid *trnL-F* region. Analyses using maximum parsimony and Bayesian inference yield congruent relationships among several major lineages found. These lineages largely disagree with previously recognized taxonomic groups. Most notably, (1) *Androsace* sect. *Andraspis*, comprising the short-lived taxa, is highly polyphyletic; (2) *Pomatosace* constitutes a separate phylogenetic lineage within *Androsace*; and (3) *Douglasia* and *Vitaliana* nest within *Androsace* sect. *Aretia*. Our results suggest multiple origins of the short-lived lifeform and a possible reversal from annual or biennial to perennial habit at the base of a group that now contains mostly perennial high mountain or arctic taxa. The group containing *Androsace* sect. *Aretia*, *Douglasia*, and *Vitaliana* includes predominantly high alpine and arctic taxa with an arctic-alpine distribution, but is not found in the European and northeastern American Arctic or in Central and East Asia. This group probably originated in Europe in the Pliocene, from where it reached the amphi-Beringian region in the Pleistocene or late Pliocene. [*Androsace*; biogeography; *Douglasia*; evolution of short-lived life history; phylogeny; *Pomatosace*; Primulaceae; *Vitaliana*.]

The Primulaceae have been subject of diverse interpretations of familial and generic boundaries (Pax, 1897; Pax and Knuth, 1905; Schwarz, 1963). Recent molecular phylogenetic studies utilizing chloroplast markers have helped to clarify the circumscription of this family (Anderberg et al., 1998, 2000, 2001; Källersjö et al., 2000). Several genera traditionally included within the Primulaceae have been assigned either to the Theophrastaceae (*Samolus*) or to the Myrsinaceae (*Anagallis*, *Ardisiandra*, *Asterolinon*, *Coris*, *Cyclamen*, *Lysimachia*, *Pelletiera*, *Stimpsonia*). Primulaceae s. str. is currently considered to be composed of three major clades (Källersjö et al., 2000; Mast et al., 2001; Trift et al., 2002; Martins et al., 2003): (1) the smallest contains the European *Soldanella*, the European and eastern North American *Hottonia,* and the East Asian *Omphalogramma* and *Bryocarpum*; (2) the largest with the most species-rich genus of the family, *Primula*, in which *Dionysia* (Southwest Asia), *Sredinskya* (Caucasus), *Dodecatheon* (western North America), and *Cortusa* (Eurasia) are nested; and (3) the tribe Androsaceae in the circumscription of Takhtajan (1997), with the exception of *Stimpsonia* now included in Myrsinaceae (Anderberg et al., 2001). This latter clade contains the second-largest genus of the family, *Androsace*, the monotypic European *Vitaliana*, the amphi-Beringian *Douglasia*, and the monotypic East Asian *Pomatosace* (Fig. 1). The molecular studies of Mast et al. (2001) and Trift et al. (2002) focussed on the phylogenetic relationships within the clade containing *Primula*. A comparable treatment of the tribe Androsaceae has so far been lacking.

The generic boundaries within Androsaceae have been controversial, in particular the taxonomic treatment of *Douglasia* and *Vitaliana* as separate genera. *Douglasia* differs from *Androsace* in having a higher chromosome base number, multiple short scapes with dense pubescence of branched hairs, shortly pedicillate or sessile umbels with solitary or few flowers, a violet corolla (at least in early anthesis) with a tube as long or longer than the calyx, and irregularly thickened endosperm cell walls (Kelso, 1992; Anderberg and Kelso, 1996). Most North American authors treat *Douglasia* as separate genus (Constance, 1938; Robbins, 1944; Kelso, 1992; Kelso et al., 1994), whereas European authors assign it only sectional status within *Androsace* (Ovchinnikov, 1952; Wendelbo, 1961; Kress, 1965; Smith and Lowe, 1997). *Vitaliana* is morphologically unique within Androsaceae by having heterostylous flowers, and most authors treat it as separate genus (Ferguson, 1972; Smith and Lowe, 1997), whereas Wendelbo (1961) and Kress (1965) include it as a section in *Androsace*. A wider circumscription of *Androsace* is supported by recent molecular investigations of Trift et al. (2002) and Martins et al. (2003), in which *Douglasia* and *Vitaliana* are nested within *Androsace*. Additionally, *Pomatosace*, thought to be closely related to *Soldanella* because of its circumscissile capsules, falls within *Androsace*, rendering *Androsace* paraphyletic (Mast et al., 2001; Trift et al., 2002; Martins et al., 2003). The sampling of *Androsace* in those studies, however, is too scarce for a full understanding of the relationships in this group.

The phylogenetic position of *Douglasia* and *Vitaliana* in relation to *Androsace* has important implications for hypotheses on the biogeography of this group. Kress (1965) noted that no morphological and karyological characters can be found, which would clearly separate amphi-Beringian *Douglasia*, which he considers a section within *Androsace*, and the European *Androsace* sect. *Aretia*. Nevertheless, he rejected a close phylogenetic relationship between *Douglasia* and *Androsace* sect. *Aretia* for two reasons. First, based on the distribution

Downloaded from https://academic.oup.com/sysbio/article/53/6/856/1650198 by guest on 20 April 2024

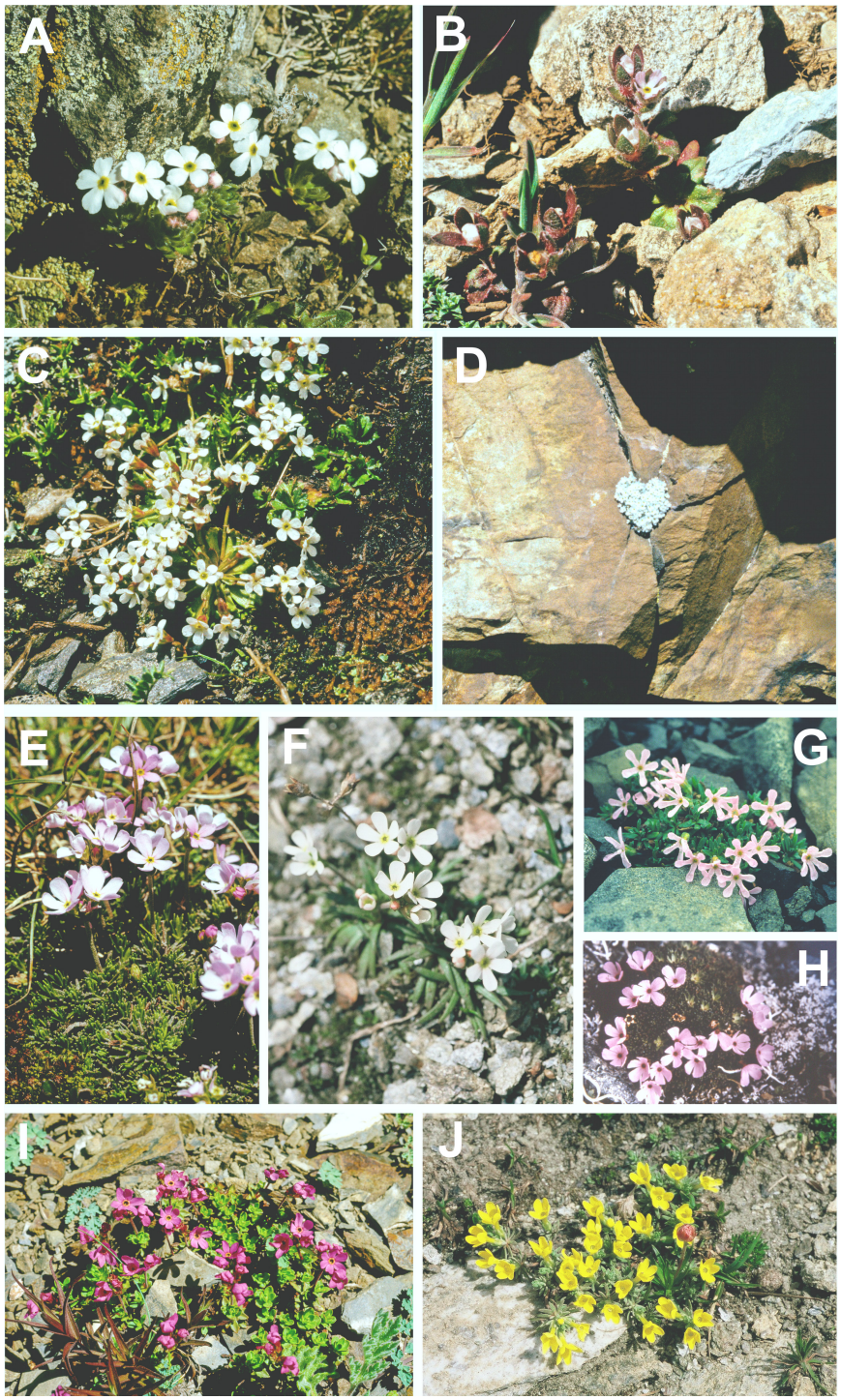

FIGURE 1. Representatives of *Androsace* and related genera. (A) *A. barbulata* is the SW Asian member of sect. *Chamajasme* subsect. *Villosae* (Georgia; photo: G. M. Schneeweiss). (B) *A. maxima* is an annual species in sect. *Andraspis* widely distributed in lowland and mountain steppes in Eurasia (Spain; photo: G. M. Schneeweiss). (C) *A. raddeana* is a biennial member of sect. *Andraspis* confined to the subnival belt of the Greater and Minor Caucasus (Georgia; photo: G. M. Schneeweiss). (D) *A. vandellii* of sect. *Aretia* forms dense cushions and is distributed as disjunct populations in mountain ranges from Southern Spain to the Eastern Alps (Italy; photo: G. M. Schneeweiss). (E) *A. laggeri* of sect. *Aretia* is a frequent species in alpine pastures of the Pyrenees (Spain; photo: G. M. Schneeweiss). (F) *A. adfinis* subsp. *brigantiaca* of sect. *Aretia* is the polyploid member of a group of closely related taxa in the SW Alps (France; photo: M. Wiedermann). (G) *Douglasia beringensis* is an endemic of rocky outcrops in western Alaska (Alaska; photo: S. Kelso). (H) *D. gormanii* occurs in the mountains of central Alaska and the Yukon (Alaska; photo: S. Kelso). (I) *D. laevigata* is an endemic of the Pacific Northwest in Washington and Oregon (Washington; photo: P. Schönswetter). (J) *Vitaliana primuliflora* is widely distributed in mountain ranges from the Iberian Peninsula to the Eastern Alps and the central Appenine mountains (France; photo: M. Wiedermann).

Downloaded from https://academic.oup.com/sysbio/article/53/6/856/1650198 by guest on 20 April 2024

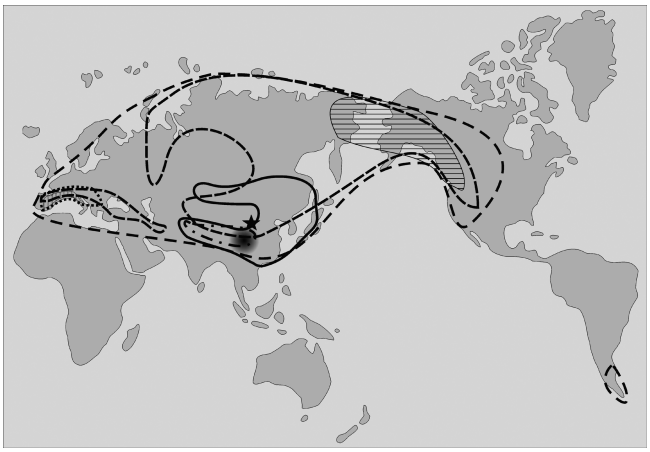

FIGURE 2. Distribution of *Androsace* (sect. *Pseudoprimula*, solid line; sect. *Chamaejasme*, narrowly spaced dashed line; sect. *Aizoidium*, dashed-dotted line; sect. *Andraspis*, widely spaced dashed line; sect. *aretia*, dotted line) and the related genera *Douglasia* (horizontally hatched area), *Pomatosace* (star), and *Vitaliana* (vertically hatched area). The dark spot in East Asia indicates the center of species richness of *Androsace* (>35 species; see Meusel et al., 1978).

of the morphologically and karyologically most primitive group of *Androsace* (sect. *Pseudoprimula*) in Southeast Asia (Fig. 2), Kress suggested centrifugal evolution from Southeast Asia eastwards (*Douglasia*) and westwards (*Androsace* sect. *Aretia*). Second, a close relationship between *Douglasia* and *Androsace* sect. *Aretia* would involve a disjunction between the amphi-Beringian region and Europe (Fig. 2), which Kress considered to be too difficult to be bridged. Later, Anderberg and Kelso (1996) suggested, based on the presence of unusual irregularly thickened endosperm cell walls in *Douglasia* and *Vitaliana*, that these two genera form a monophyletic group. This again implies a disjunction between the amphi-Beringian region and Europe, which the authors consider to be a remnant of a once continuous distribution.

A similar biogeographic pattern to that of *Androsace* and related genera is found in *Primula*. Like *Androsace*, it has its center of diversity in the East Asian mountain ranges, where also the most primitive sections occur (Richards, 1993). A close relationship of the mainly western North American *Primula* sect. *Cuneifolia* and the European sect. *Auricula*, which has been suggested on the basis of morphological and karyological characters (Kress, 1965; Richards, 1993), has been recently confirmed by molecular studies (Mast et al., 2001; Trift et al., 2002). Currently, it is not possible to decide if the biogeographic pattern found in *Primula* is just an idiosyncrasy of this genus, or if it is also present in other groups. Therefore, testing the biogeographic hypothesis of a Beringian-European disjunction in *Androsace* and related genera is desirable.

We present the first detailed molecular phylogenetic study of the genus *Androsace* and the closely related satellite genera *Douglasia*, *Pomatosace*, and *Vitaliana* using nuclear (internal transcribed spacer [ITS]) and plastid markers ($trnL_{(UAA)}$ intron, $trnL_{(UAA)}$ 3′ exon, and $trnL_{(UAA)}$-$trnF_{(GAA)}$ spacer, from here on referred to as *trnLF*) to establish a hypothesis on the phylogenetic relationships among and within these groups. Based on this phylogeny, we test the biogeographic hypothesis of a large scale disjunction between the amphi-Beringian region and western North America on one side and European mountain ranges on the other.

MATERIAL AND METHODS

Taxon Sampling

Names of species and sectional delimitation follow Smith and Lowe (1997) with a few exceptions (Appendix 1). These concern *A. delavayi* and *A. lehmannii*, which are treated as members of *Androsace* sect. *Aretia* by Smith and Lowe (1997), but are included in *A.* sect. *Chamaejasme* by the monographers of Chinese *Androsace* Hu and Yang (1986), and *Douglasia*, which is treated as separate genus, thus allowing better comparability with previous studies of Mast et al. (2001) and Trift et al. (2002).

Our study covers all sections of *Androsace* as well as *Douglasia*, *Pomatosace*, and *Vitaliana*, with some species of *Primula* and *Dionysia* as outgroups (Appendix 1). Initial studies indicated that both *Douglasia* and *Vitaliana* are phylogenetically well nested within *Androsace* sect. *Aretia*. Therefore, our sampling strategy focused on species of these groups, and in the final analyses (see below), all species of *Douglasia* and *Androsace* sect. *Aretia*, except *D. alaskana*, *A. hirtella*, and *A. adfinis* subsp. *adfinis*, are included. A second focus were the short-lived species of *Androsace* sect. *Andraspis*. Species that are split off as separate sections and members of all subsections, which have been recognized (Ovchinnikov, 1952; Kuvajev and Pirozhkova, 1987; Menitsky, 2000), are included. The sampling in the remaining sections is less comprehensive, especially in the species-rich sections *Pseudoprimula* and *Chamaejasme* (with ca. 24 and ca. 77 species, respectively; Smith and Lowe, 1997), and a denser sampling is undoubtedly desirable, but must be left for future studies due to the restricted availability of material. The molecular results (see below) together with morphological and karyological evidence (see Discussion) strongly suggest that these groups constitute phylogenetically independent lineages clearly separated from *A.* sect. *Aretia*, *Douglasia*, and *Vitaliana* (see Discussion for details on the only exception, *A. triflora*, from sect. *Chamaejasme*). Therefore, our relatively weak sampling in *A.* sects. *Pseudoprimula* and *Chamaejasme* should not impair the conclusions on *A.* sect. *Aretia*, *Douglasia*, and *Vitaliana*.

DNA-Amplification and Sequencing

DNA was extracted from silica-gel dried, rarely from air-dried, leaf material following the CTAB extraction protocol (Doyle and Doyle, 1987) with slight modifications (Schönswetter et al., 2002, 2003a). The ITS1-5.8S-ITS2 region of the nuclear ribosomal DNA was amplified using primers 17SE and 26SE (Sun et al., 1994). The *trnLF* region in the plastid genome was amplified using primers c and f from Taberlet et al. (1991). The reaction mix for polymerase chain reaction (PCR) of 50 μl contained 1 $\times$ PCR buffer (Roche Diagnostics,

Downloaded from https://academic.oup.com/sysbio/article/53/6/856/1650198 by guest on 20 April 2024

Germany; Sigma-Aldrich, Vienna, Austria), 200 to 500 ng of DNA template, 0.2 mM of each dNTP (Roche Diagnostics, Mannheim, Germany; Fermentas, St. Leon-Rot, Germany), 0.04 μM of each primer, 0.25 units of Taq DNA polymerase (Roche Diagnostics, Mannheim, Germany) or 0.38 units of Red Taq DNA polymerase (Sigma-Aldrich, Vienna, Austria), and 2.5 mM $MgCl_2$. In cases of poor amplification results, PCR was repeated with 0.02% bovine serum albumin (BSA) (Sigma-Aldrich, Vienna, Austria) and/or 5% DMSO (Sigma-Aldrich, Vienna, Austria). PCR for both regions was conducted with the following conditions: denaturation for 4 min at 94°C; 35 cycles with 1 min at 95°C, 1 min at 48°C, 1 min at 72°C; final elongation for 10 min at 72°C. PCR products were purified from 1% agarose gels using either Qiaquick gel purification kit (Qiagen Germany, Hilden, Germany) or GFX PCR DNA & Gel Band Purification Kit (Amersham Biosciences Europe, Vienna, Austria) according to the manufacturer's instructions. Sequencing was done using BigDye terminator cycle sequencing kit (Applied Biosystems, Foster City, USA) and analyzed on an ABI PRISM 377 DNA autosequencer (Applied Biosystems, Foster City, USA).

Due to more than 1% of ambiguous sites (double-peaks), ITS sequences of *Androsace axillaris*, *A. rigida*, and *A. stenophylla* were cloned. The purified fragment was ligated into the pGEM-T Easy vector (Promega, Madison, Wisconsin) according to manufacturer's instructions. The plasmids were transformed into *Escherichia coli* JM109 competent cells (Promega, Madison, Wisconsin) and blue/white screening was used to identify transformants. Plasmid DNA was isolated using a standard miniprep method (Sambrook et al., 1989). The clones were digested with *Eco*RI (Promega, Madison, Wisconsin) to check the insert length. Positive clones were sequenced using primers 17SE and 26SE and analyzed on an ABI 377 automated sequencer as described above. Of three to four clones checked for *A. axillaris* and *A. rigida*, three different sequences were obtained. These sequences formed in all phylogenetic analyses well supported species-specific monophyletic groups. Sequences of *A. stenophylla*, however, did not form a monophyletic group or formed only a weakly supported one (data not shown). Additionally, of six sequences checked three were chimeras, probably resulting from PCR recombination (Bradley and Hillis, 1997; Cronn et al., 2002). The reasons for the presence of at least two paralogues in this species are unclear. It is possible that the accession of *A. stenophylla*, obtained from garden material, actually is a hybrid. Further investigations using wild material are necessary. The phylogenetic position of the two groups of sequences of *A. stenophylla* concerns only parts of the phylogenetic trees that are in general poorly resolved (see Results), and their position does not affect other parts of the tree. Therefore, for final analyses (see below) as for *A. axillaris* and *A. rigida* only one randomly chosen sequence of *A. stenophylla* was used.

Variability of ITS sequences in polyploid *Androsace* was tested in an accession of the highly polyploid *A. lactea* by cloning PCR products as described above. This species was chosen, because the phylogenetic position inferred from ITS data contradicts morphological and karyological evidence (see Discussion), and this might be caused by the presence of different paralogues. In eight clones sequenced, eight unique base changes and one change shared by three clones were observed in the ITS1-5.8S-ITS2 region. In phylogenetic analyses, all sequences group in a well-supported monophyletic clade (data not shown), indicating that the present paralogues do not change the inferred phylogenetic position. All newly obtained sequences have been deposited in GenBank under accession numbers AY275015 to AY275105 and AY502123 to AY502124 (ITS) and AY274936 to AY275014 (*trnLF*; see Appendix 1).

Phylogenetic Techniques

Alignment.—The boundaries of ITS1 and ITS2 were determined with the sequence of *Senecio vulgaris* (Asteraceae; AF422136) and ITS1; 5.8S and ITS2 were used for further analyses. Similarly, the borders of the $trnL_{(UAA)}$ intron and the spacer region were determined with the sequence of *Nicotiana tabacum* (Solanaceae, NC_001879), and the partial $trnF_{(GAA)}$ sequence was excluded from the analyses. In some sequences, the beginning of the $trnL_{(UAA)}$ intron was not readable due to the presence of multiple peaks, and therefore 20 bp on the 5′ end of the $trnL_{(UAA)}$ intron were excluded from the analyses. The alignment of the ITS sequences was conducted in two steps. The data set was divided into groups according to the sectional boundaries in *Androsace* with the exception of sect. *Andraspis*, which was divided in two groups based on results of preliminary analyses (data not shown). These groups were aligned separately using the multiple alignment mode in ClustalX 1.81 (Thompson et al., 1997) with DNA transition weight of 0.5 and the penalties of 15 for gap-opening and 6.66 for gap extension. The alignments were adjusted manually using BioEdit 5.0.9 (Hall, 1999). Initial attempts to use also information on secondary structure to aid the alignment were discontinued, because these did not appear helpful. These partitions were aligned using the profile alignment mode in ClustalX 1.81 with DNA transition weight of 0.5 and the penalties of 10 for gap-opening and 5 for gap-extension. Manual adjustments were again conducted using BioEdit 5.0.9. The alignment of *trnLF* was computed with ClustalW as implemented in BioEdit 5.0.9 and adjusted manually.

Phylogenetic analyses.—Initial studies with a selected number of taxa indicated that ITS gives better resolution than *trnLF*. The higher sequence divergence in ITS, however, causes alignment problems for more distant taxa. This, together with problems in obtaining sequences for some taxa, resulted in differently sized data sets. The ITS data set includes more taxa from *Androsace*, *Douglasia*, *Pomatosace*, and *Vitaliana* (88 accessions), but only one outgroup taxon from *Primula*, whereas the *trnLF* data set comprises fewer taxa from *Androsace* and related genera (73 accessions) but in total seven outgroup species from several sections of *Primula* and from *Dionysia*.

Downloaded from https://academic.oup.com/sysbio/article/53/6/856/1650198 by guest on 20 April 2024

In order to assess the influence of regions with larger amounts of gaps or missing data on the inferred phylogenetic relationships, initial analyses were conducted on three subsets from each region, differing by inclusion/exclusion of taxa or regions with longer stretches of missing data, which resulted from indels (e.g., in the *trnL-trnF* spacer of *A. strigillosa*; see Results) or the unavailability of parts of sequences (e.g., the *trnL-trnF* spacer of *Pomatosace*). Both maximum parsimony (using PAUP 4.0b10; Swofford, 2001) and Bayesian analyses (using MrBayes 2.01; Huelsenbeck and Ronquist, 2001) of these subsets detected the same major phylogenetic lineages (data not shown). Generally, there was less resolution within these clades from analyses of data sets with fewer taxa included than from the corresponding larger data sets. A few contradictory relationships were inferred, but in no case did both of the two alternatives show high nodal support (bootstrap values >70 or posterior probabilities >0.95). The inferred phylogenetic positions of species having bigger stretches of missing data were identical in the analyses; differences, if present, only concerned the degree of resolution within clades or unsupported alternative relationships (data not shown). Results of simulation studies by Wiens (2003) suggest that the problem for taxa with incomplete data is one of including too few characters rather than one of including too many missing data cells. The issue of too few characters does not appear to be a problem in *Androsace* and related genera for taxa with larger stretches of missing data, in particular for *Pomatosace filicula* and *Androsace strigillosa* with about 45% of missing data in the *trnLF* data set, and accordingly these taxa were included in the final analyses.

For final analyses single data sets per marker were used including only those taxa, for which sequences from both regions are available (in total 69 accessions). Insertions (often duplications) restricted to fewer than seven taxa, i.e., <10% of taxa, were excluded from the analyses (35 and 115 characters for the ITS and the *trnLF* data set, respectively). Maximum parsimony analyses were conducted using PAUP 4.0b10 (Swofford, 2001) with the following settings: random addition sequence with 1000 replicates and no more than 200 (ITS data set) or 1000 trees (*trnLF* data set) saved per replicate, TBR branch swapping, MulTrees on, steepest descent option in effect (ITS data set) or not in effect (*trnLF* data set), swap on best trees only, and collapse branches if minimum length is zero. Characters were treated as unordered and of equal weight, and gaps were treated as missing data. Clade support was assessed using bootstrap with 1000 replicates (heuristic search options as above, except random addition sequence with 10 replicates and no more than 1000 trees saved per replicate). The best-fit substitution models were selected using the hierarchical likelihood ratio test as implemented in ModelTest 3.06 (Posada and Crandall, 1998). Bayesian analyses were performed with MrBayes 3.0b4 (Ronquist and Huelsenbeck, 2003) using models with six substitution types (nst = 6) and a gamma distribution for describing rate heterogeneity across sites as suggested by Modeltest. Values for all parameters, such as the shape of the gamma-distribution, were estimated during the analyses. The settings for the Markov chain Monte Carlo process were four chains were run simultaneously for 2×10^6 generations with trees being sampled every 100th generation starting from uniform priors. The stationarity of the maximum likelihood scores of three independent runs, each starting from a random tree, was checked by comparing means and variances of the model likelihood after the burn-in period of 1000 trees. The posterior probability (PP) of the phylogeny and its branches was determined from 19000 trees.

Combinability of the *trnLF* with the ITS data set was assessed in a parsimony framework using the incongruence length difference (ILD) test (partition homogeneity test in PAUP). Although this test is widely used, its validity as a criterion for combinability is debated. Several properties are known that can upset the test, such as noise, i.e., data that do not reveal any features of shared phyletic history (Dolphin et al., 2000), widely different sizes of data sets (Dowton and Austin, 2002), or very heterogeneous among-site substitution rates (Darlu and Lecointre, 2002). The results of simulation studies by Barker and Lutzoni (2002) indicate that the ILD test might be misleading in a large number of cases, whether to combine data sets or not, suggesting that the ILD test might not be well suited to test for combinability. On the other hand, Cunningham (1997) found that the ILD test predicts combinability better than other parsimony-based tests. However, several studies suggest that the significance threshold of 0.05 is too conservative (Sullivan, 1996; Cunningham, 1997; Darlu and Lecointre, 2002), and Cunningham (1997) suggests a significance threshold of 0.01 to 0.001. The ILD test was conducted on two data sets, one including all characters, the second only including variable characters, using PAUP 4.0b10 (Swofford, 2001) employing 1000 partition replicates, not more than 200 trees saved per replicates, and steepest descent option in effect.

In a mixed-model approach, a combined data set is analyzed using different models for different partitions. We used MrBayes 3.0b4 (Ronquist and Huelsenbeck, 2003) to conduct such an analysis in a Bayesian framework. Two partitions congruent with the ITS and the *trnLF* data set, respectively, were defined, and the same models as for the single marker analysis were applied. Settings for the Markov chain Monte Carlo process, determination of stationarity of the likelihood score, and determination of posterior probabilities of clades were the same as for the single marker analyses described above. The data matrices and the phylogenetic trees are deposited in Treebase study number S1165 (http://www.treebase.org).

Estimation of divergence times.—Androsace has a poor fossil record. The oldest *Androsace* seeds are known from the Miocene (Dorofeev, 1963), but these cannot be assigned to any of the sections within *Androsace*. Hence, the age of these fossils could only be used to suggest a minimum age for *Androsace* as a whole, with a caveat that the fossil might actually be from a member of a derived group within *Androsace*. In order to obtain age estimates

Downloaded from https://academic.oup.com/sysbio/article/53/6/856/1650198 by guest on 20 April 2024

TABLE 1. List of taxa and accession numbers of sequences included in the analyses of divergence times for the Primulaceae data set (see text for details).

Taxon	Genbank accession number		
	*atp*B	*ndh*F	*rbc*L
Primulaceae			
Androsace sp.	AF213775	AF213736	AF213794
Androsace erecta Maxim.	—	AF421115	AF395004
Androsace spinulifera Knuth	AJ235392	—	AJ235772
Androsace septentrionalis L.	—	AF421116	AF394369
Douglasia nivalis Lindl.	AF213766	AF213742	AF213796
Dodecatheon meadia L.	AF213767	AF213741	U96658
Primula palinuri Petagn.	AF213785	AF213756	AF213801
Primula sieboldii E. Morren	AF213787	AF213757	U96657
Primula veitchiana Ptmgn.	AF213788	AF213759	AF213802
Cortusa turkestanica Los.-Losinsk.	AF213769	AF213739	AF213803
Omphalogramma delavayi Franch.	AF213783	AF213752	AF213805
Soldanella montana Willd.	AF213791	AF213761	U96943
Myrsinaceae			
Myrsine africana L.	AF213764	AF213751	U96652
Anagallis arvensis L.	AF213774	AF213735	M88343
Ardisia crenata Roxb.	AF209530	AF207960	LI2599
Coris monspeliensis L.	AF213770	AF213738	U96660
Cyclamen hederifolium Ait.	AF213773	AF213740	U96656
Lysimachia maxima (R. Knuth) R. St. John	AF213777	AF213748	AF213806
Theophrastaceae			
Theophrasta americana L.	AF213792	AF213762	AF213819
Clavija euerganea Macbride	AF213771	AF213737	—
Maesaceae			
Maesa tenera Mez.	AF213781	AF213750	—
Ebenaceae			
Diospyros kaki L. f.	AJ235457	—	Z80185
Polemoniaceae			
Gilia capitata Sims	AJ236220	AJ236269	—

for the nodes of interest, we therefore used an approach similar to that of Goldblatt et al. (2002) in their study of the genus *Moraea* (Iridaceae). They used age estimates provided for most angiosperm families by Wikström et al. (2001) to infer an age estimate for the genus *Moraea* from a phylogeny of the whole family Iridaceae. In a second step, this age estimate was then applied to a more detailed phylogenetic tree of *Moraea* as a new calibration point. A phylogenetic hypothesis for Primulaceae and related families was established using sequences from the three chloroplast genes, *atp*B, *ndh*F, and *rbc*L, as used in the study of Anderberg et al. (2001). In total, 23 taxa were included, covering Primulaceae, Maesaceae, Myrsinaceae, and Theophrastaceae plus one species each of *Diospyros* (Ebenaceae) and *Gilia* (Polemoniaceae) as outgroups (Table 1). Sequences were obtained from Genbank and aligned manually in BioEdit 5.0.9 (Hall, 1999). Regions with missing data for more than one third of taxa were removed prior to analyses. This concerned stretches at the beginning and end of the three genes and two indels in the *ndh*F gene.

Likelihood ratio testing was used to test if substitution rates can be modeled as clock-like. As best-fit substitution model, a general time reversible model with a gamma-distribution accounting for heterogeneity among sites and a proportion of invariable sites (GTR+Γ+I) was suggested by the hierarchical likelihood ratio test as implemented in ModelTest 3.06 (Posada and Crandall, 1998). Using the parameter values suggested by ModelTest, heuristic searches were run with PAUP 4.0b10 (random addition sequence with 50 replicates, TBR branch swapping, MulTrees on, steepest descend option not in effect, swap on best trees only, and collapse branches if minimum length is zero). The likelihood ratio test statistic is calculated as $2([-\ln L_0] - [-\ln L_1])$, in which L_0 and L_1 are the likelihoods under the null (clock) and alternative (nonclock) hypotheses. The significance of this value is judged by comparing it to a χ^2 distribution with $n-2$ degrees of freedom, with n being the number of taxa.

A clock-like evolution of substitution rates was rejected for the Primulaceae data set (data not shown). Hence, we used two other methods as implemented in the program r8s (Sanderson, 2002b) to produce ultrametric trees. Nonparametric rate smoothing (NPRS; Sanderson, 1997) estimates rates and times via a least-squares smoothing criterion that penalizes rapid rate changes from branch to neighboring branch on a tree. In a penalized likelihood approach (PL; Sanderson, 2002a), a saturated model in which each lineage has its own rate is combined with a roughness penalty, which forces rates to change smoothly from branch to branch. The tradeoffs between smoothness and goodness-of-fit of the data to the saturated model is determined by a smoothing parameter. The optimal value for this smoothing parameter is estimated from the data via a cross-validation procedure as described in Sanderson (2002a, 2002b). In practice, several values are tested, and the one resulting in the lowest cross validation-score is chosen for the final run.

Branch lengths were estimated with both parsimony methods (settings as described above, ACCTRAN or DELTRAN optimization) and maximum likelihood methods (settings as for the likelihood ratio testing) using PAUP 4.0b10. Branch lengths estimated from equally weighted parsimony are not corrected for multiple substitutions and their lengths might therefore be underestimated. Despite this possible pitfall for age estimations, we present these estimates to allow comparisons with other studies (e.g., Wikström et al. [2001]) and with branch lengths calculated via the model-corrected maximum likelihood method. In all analyses, ages of two nodes were fixed using age estimates obtained by the respective method of branch length optimization (ACCTRAN, DELTRAN, maximum likelihood) provided by Wikström et al. (2001). These are the most recent common ancestors of Maesaceae, Theophrastaceae, Primulaceae, and Myrsinaceae (49/48/46 Mya; node no. 335 in Wikström et al., 2001) and of Myrsinaceae s. str., respectively (15/24/22 Mya; node no. 338 in Wikström et al., 2001). In order to assess if the global optimum was found, searches started from 10 different starting conditions (by setting num_time_guesses = 10) and perturbations of the solutions in different directions were conducted (by setting num_restarts = 10). Confidence intervals on estimated ages were obtained using bootstrapping (Efron and Tibshirani, 1993). Using the program

Downloaded from https://academic.oup.com/sysbio/article/53/6/856/1650198 by guest on 20 April 2024

SeqBoot from the package PHYLIP 3.5c (Felsenstein, 1999), 100 bootstrap data matrices were generated, and for each of these data sets branch lengths were estimated as described above on the same tree topology as used for the age estimation. Divergence times for these were estimated as described above, and the central 95% of the age distribution of these provide the confidence interval.

The resulting age estimates were then used as new calibration points for a detailed phylogenetic tree of *Androsace* and related genera based on the ITS data. In cases with more than one accession per species, only one randomly chosen sequence was kept. Because *Androsace helvetica* and *A. pubescens* have identical ITS sequences, only one of *A. pubescens* was included. Because we were interested in age estimates of only few nodes and the relatively high number of terminals would just increase the calculation time, especially for the likelihood ratio testing, we excluded several taxa from the analyses. This was done in a way that from terminal species pairs (e.g., *A. integra* and *A. stenophylla* or *A. rioxana* and *A. chaixii*; see Fig. 4), the one species with the longer branch was chosen for the analysis. The final data set thus includes 37 taxa. Again, likelihood ratio testing was conducted using PAUP 4.0b10 as described above, but starting trees were obtained via neighbor joining (instead of random sequence addition) and maxtrees was set to 25. A clocklike evolution of substitution rates was rejected ($2 \times \mathrm{LR} = 85.7318$, $P < 0.001$), and NPRS and PL were used to obtain age estimates.

Evolution of lifeform.—Phylogenetic analyses congruently suggest short-lived lineages as sister taxa to a clade including mostly perennial species of mountain habitats (see Results). To investigate the evolution of the trait lifeform in more detail, a parsimony and a maximum likelihood based approach were applied. Methods based on the principle of parsimony reconstruct the ancestral character states to minimize the number of historical character changes (Maddison et al., 1984). Parsimony reconstructions can be misleading when rates of evolution are rapid, and when the probabilities of gains and losses are not equal (Cunningham et al., 1998). In maximum likelihood methods branch lengths are considered (see Cunningham, 1999, for a discussion of possible pitfalls). Because every possible reconstruction is considered, they can also estimate the relative probability of each character state at every node (Cunningham et al., 1998; Pagel, 1999). For our study, we used equally weighted unordered parsimony and the 1-parameter Markov k-state model with rate of change estimated from the data (Lewis, 2001) as implemented in Mesquite 1.0 (Maddison and Maddison, 2003). The evolution of lifeform was investigated on the phylogenetic hypothesis provided by Bayesian analysis of the combined data set.

RESULTS

Molecular Parameters

The length of ITS1 ranges from 215 bp in *Androsace raddeana* to 251 bp in *Pomatosace*, and that of ITS2 from 217 bp in *A. delavayi* to 235 bp in *A. wulfeniana*. The length of 5.8S rDNA is 162 bp, with the exception of *Androsace* sect. *Aretia*, *A. chaixii*, *A. elongata*, and *A. septentrionalis* of sect. *Andraspis*, *Douglasia*, and *Vitaliana*. In these species, the length is 161 bp due to a deletion at position 129. In the aligned data set, ITS1 has 271 characters and ITS2 has 255 characters, resulting in a total length of 688 characters. For *Pomatosace*, due to sequencing problems in *trnLF*, only the $trnL_{(UAA)}$ intron is available. The length of the $trnL_{(UAA)}$ intron in *Androsace* ranges from 461 bp in *A. strigillosa* to 505 bp in *A. helvetica*, *A. obtusifolia*, and one accession of *A. alpina*, that of the $trnL_{(UAA)}$-$trnF_{(GAA)}$ spacer from 56 bp (!) in *A. strigillosa* to 411 bp in *A. integra*. In the aligned data set, the $trnL_{(UAA)}$ intron has 577 characters and the $trnL_{(UAA)}$-$trnF_{(GAA)}$ spacer has 468 characters resulting in a total length of 1095 characters (including 50 bp of the the $trnL_{(UAA)}$-3′ exon). Numbers of variable characters for the ITS and the *trnLF* data set are 278 and 353, of which 173 and 266, respectively, are parsimony informative.

Phylogenetic Relationships

Androsace is paraphyletic, but forms together with *Douglasia*, *Pomatosace*, and *Vitaliana* a well-supported monophyletic group (data not shown). In the final analyses, only one outgroup taxon, *Primula kitaibeliana*, is included, and therefore the obtained trees are shown with a basal trichotomy.

ITS.—Maximum parsimony and Bayesian inference congruently detect the following monophyletic groups (Fig. 3). One is comprised of *Androsace axillaris* and *A. paxiana* (the latter only included in the initial analyses: BS 100, PP 1.00) of sect. *Pseudoprimula*, and constitutes the basalmost lineage within *Androsace* (BS 75, PP 1.00). The two accessions of *A. maxima* s. l. of sect. *Andraspis* form another clade (BS 100, PP 1.00), which is suggested as sister-group to the remaining species, albeit with only weak support (BS 54, PP 0.64). A third clade includes most species of sect. *Chamaejasme* and *A. integra* from sect. *Aizoidium* (BS 100, PP 1.00) plus *A. erecta* from sect. *Andraspis* (only included in the initial analyses: BS 99, PP 1.00), and groups as sister to the rest (BS 64, PP 1.00). The core group of the remaining taxa includes species of *Androsace* sect. *Aretia*, *A. triflora* of sect. *Chamaejasme*, *A. chaixii* of sect. *Andraspis*, and *Vitaliana* and *Douglasia* (BS 100, PP 1.00), and is sister to *A. elongata* of sect. *Andraspis* (BS 80, PP 1.00). Three lineages arrange as consecutive sister-groups to this main group with high support (BS 93–100, PP 1.00). These are, in descending order, a clade comprising *A. raddeana*, *A. septentrionalis*, and *A. lactiflora* (the latter only included in the initial analyses) of sect. *Andraspis* (BS 100, PP 1.00), *Pomatosace filicula*, and *A. filiformis* (two accessions included in the initial analyses: BS 100, PP 1.00).

The internal relationships within the clades are generally well resolved, although several nodes do not get sufficient support. This is particularly pronounced in the clade including species of *A.* sect. *Chamaejasme*, where, apart from *A. hookeriana*–*A. rigida* (BS 87, PP 1.00), only groups consisting of very closely related species have

Downloaded from https://academic.oup.com/sysbio/article/53/6/856/1650198 by guest on 20 April 2024

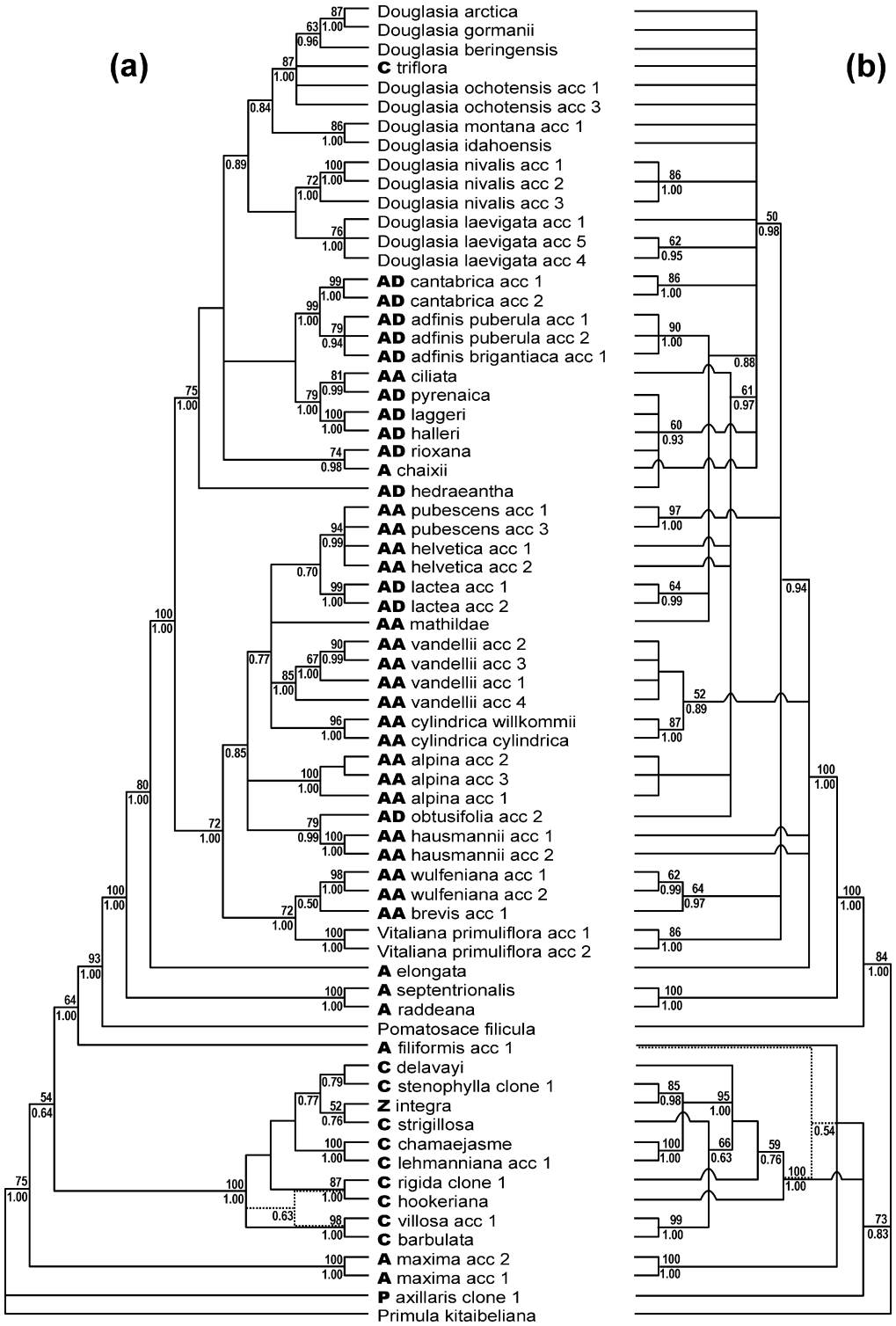

FIGURE 3. Phylogenetic relationships of *Androsace* and related genera inferred from maximum parsimony analyses (solid branches) and Bayesian analyses (identical to the former, exceptions marked as dotted branches) of the nuclear ITS region (a) and the plastid *trnLF* region (b). Numbers above branches are bootstrap values ≥50, numbers below branches represent posterior probability values ≥0.5. (a) 50% majority-rule consensus tree of 35355 equally most parsimonious trees (tree length of 936, consistency index = 0.5962, retention index = 0.8476, rescaled consistency index = 0.5053). (b) 50% majority-rule consensus tree of 777002 most parsimonious trees (tree length of 431, consistency index = 0.8034, retention index = 0.9246, rescaled consistency index = 0.7428). Abbreviations before names indicate assignment of the species to the sections of *Androsace* (see Appendix 1): A = *Andraspis*; AA = *Aretia* subsect. *Aretia*; AD = *Aretia* subsect. *Dicranothrix*; C = *Chamaejasme*; P = *Pseudoprimula*; Z = *Aizoidium*.

Downloaded from https://academic.oup.com/sysbio/article/53/6/856/1650198 by guest on 20 April 2024

high support (BS 98–100, PP 1.00). These are *A. chamaejasme* s. l. (including *A. chamaejasme* and *A. lehmanniana*) and *A. villosa* s. l. (including *A. villosa*, *A. barbulata*, and *A. koso-poljanskii*, the latter only included in the initial analyses). The clade including all species of *A.* sect. *Aretia* falls into two monophyletic groups. One contains *A. chaixii*, *A. triflora*, several species of *A.* sect. *Aretia*, and all species of *Douglasia* (BS 75, PP 1.00), whereas the second includes the remaining species of *A.* sect. *Aretia* and the genus *Vitaliana* (BS 72, PP 1.00). A separation of a monophyletic group containing *Douglasia* and *A. triflora* from the rest is only very weakly supported (BS < 50, PP 0.89). A clade including the arctic species *Douglasia arctica*, *D. beringensis*, *D. gormanii*, *D. ochotensis*, and *Androsace triflora* is well supported (BS 87, PP 1.00).

trnLF region.—Concerning the major lineages, phylogenetic relationships inferred from analyses of the *trnLF* region yield similar results as those from the ITS region with similar support values (BS 100, PP 1.00; Fig. 3). One exception concerns *A. filiformis*, which is suggested either as sister-group to *A. maxima* (maximum parsimony) or to sect. *Chamaejasme* (Bayesian inference), but neither hypothesis gets sufficient support. The second exception is *A. elongata*, which does not group as sister to the clade including species of *A.* sect. *Aretia* and others, but falls into this clade.

Here, the internal relationships within the clades are little resolved, and mostly clades consisting of accessions of the same species (e.g., *Androsace pubescens*, *Douglasia nivalis*) or very closely related taxa (e.g., *A. villosa* and *A. barbulata*, *A. chamaejasme* and *A. lehmanniana*) are retained with high support. A few groups, however, are found consistently: these include *A. chamaejasme/lehmanniana/stenophylla/integra* (BS 95, PP 1.00) and *A. wulfeniana/brevis* (BS 64, PP 0.97).

Combined dataset.—Both ILD tests, including all sites and including only variable sites, indicated that the ITS data cannot be combined with the *trnLF* data ($P = 0.001$ and $P = 0.002$, respectively). Several contradictory phylogenetic relationships are inferred from the single marker analyses, which probably account for these results. If a cutoff level of 70% bootstrap support is used, these contradicting nodes do not get high support in at least one of the alternatives, with the exception of *A. filiformis* and *A. axillaris* (Fig. 3). Exclusion of those taxa would be a possible way to circumvent this problem, but each of the two species constitutes a major phylogenetic lineage within *Androsace* (see above), and should therefore not be excluded from phylogenetic analyses.

Bayesian analysis using a mixed model approach detects the same major phylogenetic lineages as the analyses of the single marker data sets (Figs. 4 and 5). *Androsace* sect. *Chamaejasme*, *A. axillaris*, *A. filiformis*, and *A. maxima* fall into one clade (PP 0.99). As in analyses of ITS data alone, *Androsace* sect. *Aretia* falls into two clades. One is only weakly supported and additionally includes *Vitaliana* (PP 0.83), whereas the second one is well supported and comprises, among several species of *Androsace*, all species of *Douglasia* (PP 1.00). Within these clades, several well-supported groups are found, e.g, the arctic species of *Douglasia* plus *Androsace triflora* or *A. wulfeniana* and *A. brevis*. Some of these agree with groups found in analyses of the ITS data, e.g., *A. adfinis* s. l. and *A. cantabrica*, whereas others agree with those of the *trnLF* data, e.g., *A. vandellii* and *A. cylindrica*.

Estimated divergence times.—Depending on the method of branch length optimization used, age estimates for Myrsinaceae s. str. differ very much ranging from 15 Mya (ACCTRAN) to 22 Mya (maximum likelihood) and 24 Mya (DELTRAN; Wikström et al., 2001). These differences are reflected in the age estimates for nodes in the phylogeny of *Androsace*, age estimates from ACCTRAN being the youngest and those from DELTRAN optimization being the oldest (Table 2). With a few exceptions, age estimates using one age for the calibration point lay outside confidence intervals obtained when alternative ages as calibration points are used (Table 2). In all cases, age estimates from maximum likelihood lay between those of the two parsimony methods. A second reason for differences of obtained age estimates for the same node(s) arise from using different methods of age estimation (NPRS or PL). In the Primulaceae data set (the first two nodes in Table 2), the use of different methods resulted in nearly identical estimates. This is expected from the low values for the smoothing parameter in PL (≤ 1), which allow much rate variation among branches, a feature also found in NPRS. In the *Androsace* data set (remaining nodes in Table 2), however, the values for the smoothing parameters were higher (between 5 and 18), and accordingly the age estimates differed more strongly. With a few exceptions, the estimated ages of the nodes of interest lay outside the confidence interval of the alternative methods. Age estimates obtained from PL are always younger than those from NPRS. Although age estimates for the same node differ considerably in detail, even if only maximum likelihood branch lengths are used, the differences are not so great as to render any biogeographic conclusions impossible.

Evolution of life history.—A short-lived life history has evolved at least four times independently in *Androsace* (Fig. 5). Two single occurrences of short-lived species within otherwise perennial clades are *A. integra* and *A. chaixii*. Depending on the method used, the common ancestor of *A. filiformis* and *A. maxima* is either reconstructed as short-lived (parsimony) or as ambiguous (maximum likelihood; Fig. 5), and thus the equal life history traits of *A. filiformis* and *A. maxima* might have evolved independently. The most remarkable occurrence of short-lived life history is at the basis of the clade including *Pomatosace* and *A.* sect. *Aretia* among others. Both reconstruction methods used suggest that the common ancestor of this clade was short-lived (with a probability of 64% to 69% in the maximum likelihood method), implying that in *Douglasia*, *Vitaliana*, and *A.* sect. *Aretia*, a perennial life history has evolved secondarily from short-lived ancestors.

Downloaded from https://academic.oup.com/sysbio/article/53/6/856/1650198 by guest on 20 April 2024

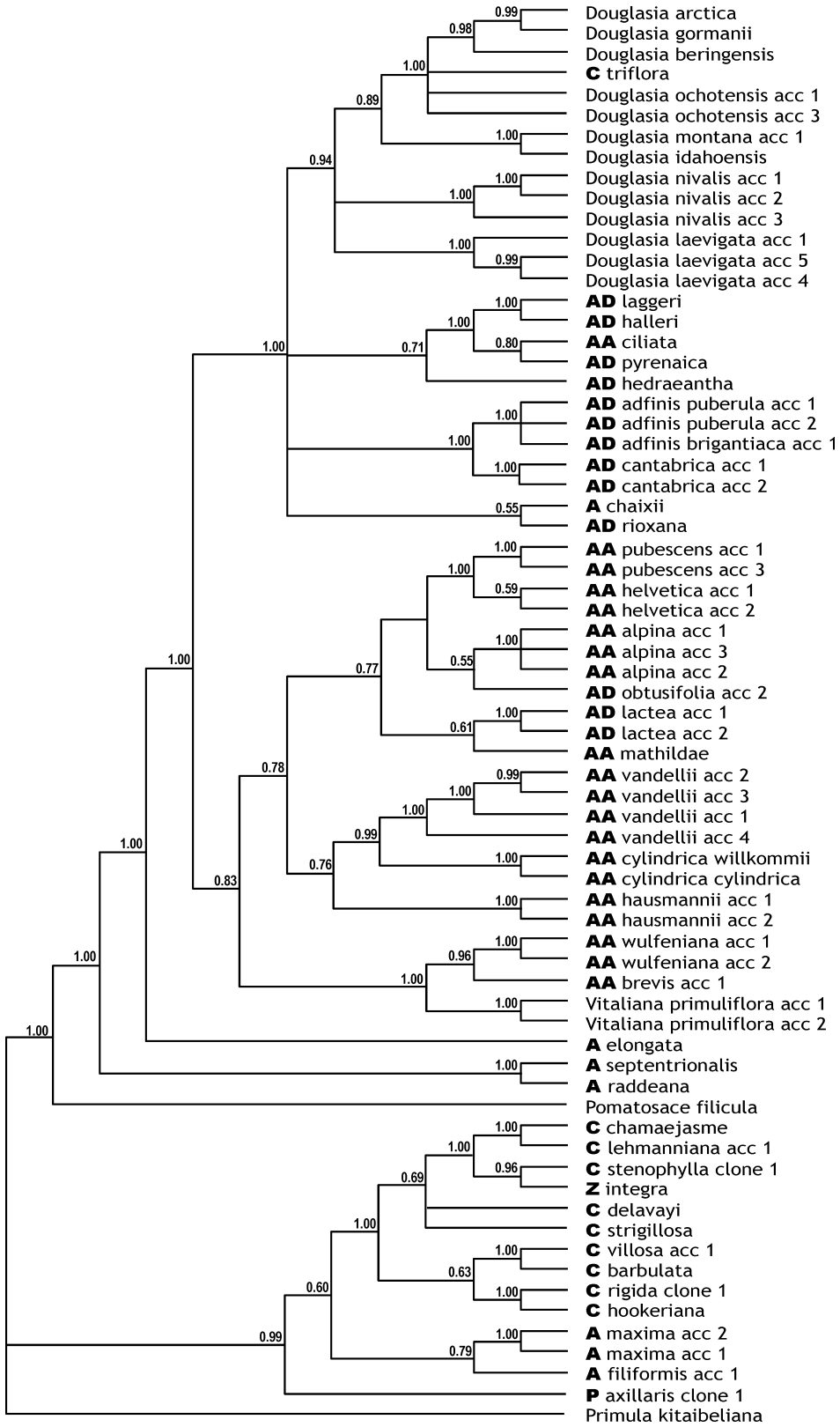

FIGURE 4. Phylogenetic relationships of *Androsace* and related genera inferred from Bayesian analysis of combined nuclear ITS and plastid *trnLF* regions (50% majority-rule consensus tree). Numbers above branches nodes represent posterior probability values ≥0.5. Abbreviations as in Figure 3.

Downloaded from https://academic.oup.com/sysbio/article/53/6/856/1650198 by guest on 20 April 2024

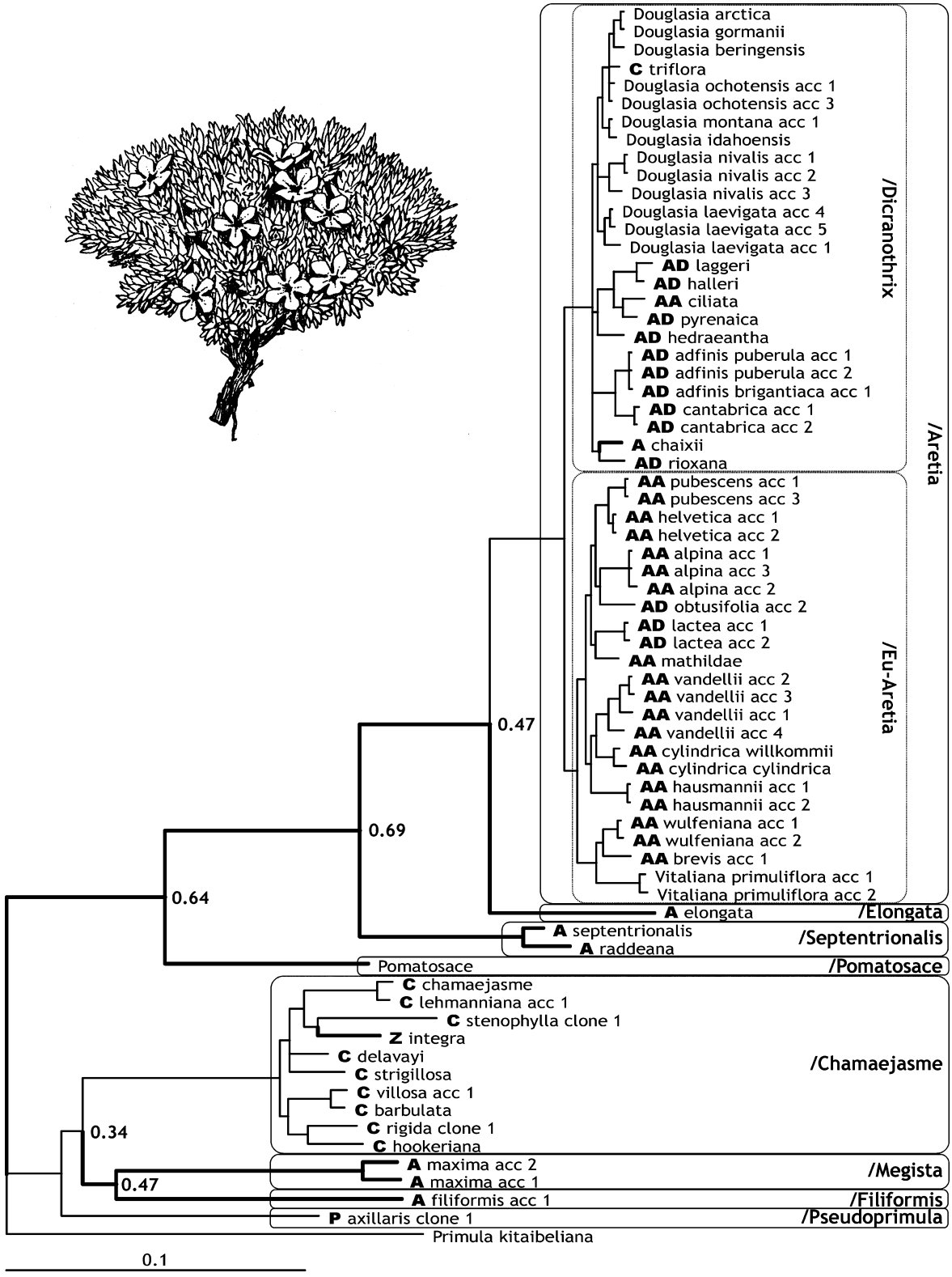

FIGURE 5. Phylogenetic relationships of *Androsace* and related genera inferred from Bayesian analysis of combined nuclear ITS and plastid *trnLF* regions (50% majority-rule consensus tree) showing branch lengths. The circumscription of the clades is indicated (see text for details). Bold branches indicate short-lived (annual to biennial) lineages as reconstructed using parsimony methods. Numbers beside nodes indicate the probability of these ancestors being short-lived (annual to biennial) as reconstructed using maximum likelihood methods. See text for details. Abbreviations as in Figure 3. (Line drawing of *Douglasia beringensis* reprinted with permission [originally published in Novon 4, p. 382].)

Downloaded from https://academic.oup.com/sysbio/article/53/6/856/1650198 by guest on 20 April 2024

TABLE 2. Age estimates for relevant nodes in the phylogeny of *Androsace* and related genera estimated via NPRS and PL, respectively. Ages for the first two nodes were estimated from the Primulaceae data set and then used as calibration points for the *Androsace* data set, from which ages for the remaining nodes were estimated. Numbers in brackets are confidence intervals. Values marked with an asterisk are mean values from ages estimated from five equally most parsimonious trees; confidence for these nodes, however, were estimated only on one randomly chosen tree. See text for further details.

	Most recent common ancestor of . . .							
	Androsace	*/Septentrionalis* and */Aretia*	*/Elongata* and */Aretia*	*/Aretia*	*/Eu-Aretia*	*/Dicranothrix*	*Douglasia*	*Douglasia arctica* and *A. triflora*
MP	17.54	7.08	5.26*	2.96*	2.37*	2.19*	1.60*	0.77*
(ACCTRAN)	(15.26–20.64)	(5.75–8.95)	(4.73–5.93)	(2.44–3.73)	(1.91–3.13)	(1.73–2.94)	(1.17–2.52)	(0.52–1.66)
	17.66	7.11	4.89*	2.26*	1.77*	1.42*	0.91*	0.39*
	(15.45–20.71)	(5.79–8.94)	(4.29–5.56)	(1.69–2.70)	(1.30–2.10)	(1.07–1.85)	(0.62–1.36)	(0.24–0.80)
MP	25.26	16.22	13.56*	7.20*	5.53*	5.27*	3.89*	1.86*
(DELTRAN)	(22.78–27.31)	(14.18–18.35)	(12.29–14.73)	(6.59–9.12)	(4.63–7.71)	(4.10–7.19)	(2.90–6.21)	(1.33–3.81)
	25.19	16.18	12.15*	4.77*	3.56*	2.80*	1.79*	0.76*
	(23.03–27.25)	(14.14–18.29)	(10.60–13.52)	(3.69–5.82)	(2.89–4.40)	(2.05–3.71)	(1.18–2.67)	(0.45–1.50)
ML	23.70	12.75	9.72	4.97	4.17	3.38	3.07	1.69
	(20.98–27.48)	(10.37–15.90)	(8.34–11.43)	(4.06–6.89)	(3.37–5.90)	(2.33–5.05)	(1.98–4.93)	(0.92–3.20)
	23.78	12.81	8.52	3.09	2.44	1.72	1.29	0.55
	(21.12–27.73)	(10.38–16.03)	(6.99–10.32)	(2.34–3.81)	(1.78–3.12)	(1.28–2.27)	(0.87–2.00)	(0.31–1.10)

DISCUSSION

Taxonomy

In accordance with previous molecular phylogenetic studies (Källersjö et al., 2000; Mast et al., 2001; Trift et al., 2002), our results support a monophyletic group largely congruent with tribe Androsaceae (Takhtajan, 1997), which is characterized by a special pollen type ("*Androsace*-Typ") not present elsewhere in Primulaceae (Wendelbo, 1961; Spanowsky, 1962). This group contains *Androsace* and three genera nested within, *Douglasia*, *Pomatosace*, and *Vitaliana*, thus leaving *Androsace* even in its widest circumscription so far (that is, including *Douglasia* and *Vitaliana* as sections; Wendelbo, 1961; Kress, 1965) paraphyletic. Applying a concept of strict monophyly, either *Androsace* would have to be split into several genera or *Douglasia*, *Pomatosace*, and *Vitaliana* would have to be merged with *Androsace*, resulting in monogeneric Androsaceae. A similar situation is found in Primuleae, where the large genus *Primula* can only be retained as monophyletic if small and morphologically distinct genera such as *Cortusa* or *Dodecatheon* are included (Mast et al., 2001; Trift et al., 2002; Martins et al., 2003).

Although a reclassification of *Androsace* and related genera may ultimately be appropriate, we believe that it should be done in concordance with a classification of *Primula* and related genera in order to apply comparable taxonomic concepts within the family. Given the profound differences in cytology, anatomy, floral morphology, and breeding systems, which have shaped previous classifications of this well-known and horticulturally popular plant family, any reclassification will be controversial. Hence, we do not propose a formal system of classification here, but treat the monophyletic groups as rankless phylogenetic taxa and name them using the clademark convention of Baum et al. (1998). We explicitly do not do this in favor of phylogenetic classification or in disfavor of Linnean classification, but consider it a very useful system to communicate monophyletic groups without burdening the discussion with nomenclatural issues.

The only clade that corresponds to a traditionally recognized section is */Pseudoprimula* (Fig. 5). This group is restricted to Asia from Afghanistan to Japan with its center of diversity in the Yunnan and Sichuan provinces of China (Smith and Lowe, 1997). */Pseudoprimula* includes the species of sect. *Pseudoprimula*, considered to be the most primitive group within *Androsace* and a probable link to *Primula* due to its leaf morphology with broad blades and long petioles (Pax and Knuth, 1905; Handel-Mazzetti, 1927; Lüdi, 1927; Kress, 1965). This morphology is otherwise unknown in *Androsace* but closely resembles the leaf morphology in several Asian sections of*Primula* (e.g., sects. *Cortusoides, Obconicolisteri*, and *Malvacea*; Richards, 1993). Schwarz (1963), who emphasized the distinctive leaves of this group, raised it to generic level. Neither an intermediate position between *Androsace* and *Primula* nor a strong distinctness justifying recognition as separate genus is confirmed by our data.

/Chamaejasme includes all investigated species of sect. *Chamaejasme*, which has its center of diversity in Central and East Asian mountain ranges (Meusel et al., 1978). The only exception is *A. triflora*, which belongs to */Aretia* (see below). Based on phytochemical characters, this species was excluded from sect. *Chamaejasme* by Kuvajev and Pirozhkova (1987) and transferred, together with the northeastern Siberian *A. gorodkovii*, to sect. *Andraspis*. The exclusion of *A. triflora* from sect. *Chamaejasme* is also supported by characters of the indumentum. The short bristly hairs on the leaf margins (see the image in Smith and Lowe, 1997: p. 150) are of the same type as those found in species of sect. *Andraspis* or sect. *Aretia*, but are clearly distinct from the long, soft, multicellular hairs present in species of sect. *Chamaejasme*. Obviously, the similarity in overall morphology of *A. triflora* to species of sect. *Chamaejasme* has led to this misclassification. Additionally, two East-Asian lineages of short-lived species fall into */Chamaejasme*. The first lineage contains *A. integra*

Downloaded from https://academic.oup.com/sysbio/article/53/6/856/1650198 by guest on 20 April 2024

belonging to sect. *Aizoidium* characterized by biennial life history and presence of isophyllous rosettes (Handel-Mazzetti, 1927; Hu and Yang, 1986; Fig. 5). The second includes the annual *A. erecta* of sect. *Andraspis* (Pax and Knuth, 1905; Smith and Lowe, 1997; data not shown), whose peculiar morphology (a densely leafy stem and lack of a basal rosette) caused some authors to put it into its own section *Orthocaulon* (Handel-Mazzetti, 1927; Hu and Yang, 1986). A close relationship of *A. erecta* to sect. *Chamaejasme* has already been suggested by Anderberg et al. (2001).

The biggest changes concern *Androsace* sect. *Andraspis*, which is highly polyphyletic and falls into several phylogenetically independent lineages (Figs. 3 to 5). Similar patterns of parallel evolution of short-lived lineages are found in an increasing number of angiosperms (e.g., Liston and Wheeler, 1994; Bena et al., 1998; Albach and Chase, 2001; Andreasen and Baldwin, 2001; Iruela et al., 2002). Apart from *A. erecta*, which belongs to */Chamaejasme* (see above), and *A. chaixii*, which is part of */Aretia* (see below), four clades can be distinguished (Fig. 5). */Filiformis* includes *A. filiformis*, which is widely distributed in Eurasia and western North America. It differs from all other annual taxa by leaves clearly separated in petiole and blade, and has been put into its own section *Filiformis* by Kuvajev and Pirozhkova (1987). The second clade */Megista* comprises *A. maxima* s. l., which has been put into its own section *Megista* due to the postflorally enlarged calyx (Ovchinnikov, 1952; Kuvajev and Pirozhkova, 1987; Menitsky, 2000). The distinctness of *A. maxima* is supported by the presence of pollen differing from those of other species of *Androsace* in its larger size, different shape, and more pronounced structure of the sexine (Punt et al., 1976). This species is widely distributed from northwestern Africa through Europe and Asia. The two accessions included in our study exhibit considerable sequence divergence (Fig. 5). Possible correlations with karyological races (diploid to hexaploid: Kress, 1984; Weiss and Schneeweiss, 2001) or previously recognized taxa such as *A. turczaninovii* (Ovchinnikov, 1952; Měsíček and Soják, 1992) require further study. */Septentrionalis* includes a group of annual lowland taxa of holarctic (*A. septentrionalis*) or Middle Asian (*A. lactiflora*) distribution and mostly biennial taxa from mountain ranges of southwestern Asia (*A. raddeana*). The fourth clade */Elongata* includes the Eurasian *A. elongata*. We are not aware of any morphological feature that would parallel the phylogenetic distinctness of */Elongata* from */Septentrionalis*.

Androsace sect. *Aretia* is paraphyletic and belongs to */Aretia*. Apart from *A. triflora* (sect. *Chamaejasme*) and *A. chaixii* (sect. *Andraspis*), this clade additionally includes *Douglasia* and *Vitaliana* (Fig. 5). A close relationship of *Douglasia* and *Vitaliana* to *Androsace* is already indicated by karyological and palynological data (Favarger, 1958; Wendelbo, 1961; Spanowsky, 1962; Kress, 1965), by a cladistic study using morphological data (Anderberg and Ståhl, 1995), and by recent molecular studies (Källersjö et al., 2000; Anderberg et al., 2001; Trift et al., 2002; Martins et al., 2003). Historically, the separation of *Douglasia* from *Androsace* is mostly due to inadequate comparison. The characters, which discriminate *Douglasia* against American species of *Androsace* (of sect. *Chamaejasme* and sect. *Andraspis*), such as pink flowers, pulvinate growth habit or the presence of branched hairs, connect *Douglasia* with species from sect. *Aretia* (reviewed in Anderberg and Kelso, 1996). This is also true for the presence of distinctly and irregularly thickened endosperm cell walls with numerous narrow constrictions, which also occur in *Vitaliana* (Anderberg and Kelso, 1996) and in less pronounced form in *Androsace helvetica* and other species of sect. *Aretia* (P. Schönswetter, unpublished data). */Aretia* is characterized by a basic chromosome number of $x = 20$ (and, derived from that, $x = 19$ and $x = 17$), which very likely arose by palaeopolyploidization from $x = 10$ found elsewhere in *Androsace* (Favarger, 1958; Kress, 1965, 1984; Sarkar, 1988; Nakata et al., 1997).

Vitaliana is unique within Androsaceae by having yellow heterostylous flowers with extended corolla tubes, and is therefore often treated as separate genus (e.g., Ferguson, 1972; Smith and Lowe, 1997). In *Vitaliana*, however, heterostyly is not accompanied by the dimorphism of pollen grains and stigmatic papillae such as seen in *Primula* (Schaeppi, 1934), and therefore may represent extended herkogamy rather than the classical heterostylous syndrome as described by Dulberger (1992) and Barrett (1992). Schaeppi (1935) has already shown that the dimorphic flowers of *Vitaliana* are derived from monomorphic flowers of the *Androsace* type. In *Primula*, the functionality of heterostyly enforcing cross-pollination is well-studied, whereas reversals to homostyly in species growing in harsh environments, where self-pollination is advantageous, are common (Kelso, 1987; 1991). A similar role for floral dimorphism in *Vitaliana* might be expected, but it remains to be experimentally tested if *Vitaliana* is strictly outcrossing or if self-pollination is successful and to what extent. The evolution of longer corolla tubes in *Vitaliana* may be connected to the evolution of heterostyly in order to enhance the spatial separation of the anthers from the stigma. Although unique in */Aretia*, the third trait, the yellow flower color, is not unique within *Androsace*, because it also occurs in the predominantly white to pink flowered */Chamaejasme*. In *Vitaliana*, it may be related to reproductive ecology as well. The color, in the Primulaceae produced by flavonoid pigments (Harborne, 1968), is attractive to bees (Proctor et al., 1996), which are major pollinators of heterostylous species of *Primula* in both lowland and alpine environments (Christy, 1921; Miller et al., 1994).

Within */Aretia*, two groups can be differentiated (Fig. 5), which roughly correlate with the subsectional structuring proposed by Kress (1963). */Eu-Aretia* includes most species of sect. *Aretia* subsect. *Aretia* plus *Vitaliana*, whereas */Dicranothrix* contains most species of sect. *Aretia* subsect. *Dicranothrix*, *A. triflora*, *A. chaixii*, plus *Douglasia*. An important character in previous classifications of this group (Pax and Knuth, 1905; Kress, 1963)

Downloaded from https://academic.oup.com/sysbio/article/53/6/856/1650198 by guest on 20 April 2024

was inflorescence morphology: single axillary flowers, often combined with pulvinate growth form, versus multiflowered scapose inflorescences, often combined with loose mat-forming growth form. Both types are present in both groups, e.g., the single-flowered pulvinate species *Douglasia gormanii* and *D. ochotensis* appear in */Dicranothrix*, where mat-forming species with scapose multiflowered inflorescences prevail, and the multiflowered scapose *A. obtusifolia* occurs in */Eu-Aretia*, where single-flowered pulvinate species dominate. The split in */Eu-Aretia* and */Dicranothrix* is supported by the distribution of basic chromosome numbers, because $x = 20$ is found in */Eu-Aretia* and $x = 19$ and $x = 17$ in */Dicranothrix*. For some of the species, however, the karyological data are ambiguous: the exact chromosome numbers of *A. ciliata* ($2n =$ ca. 80) and *A. lactea* ($2n =$ ca. 76) are not known (Favarger 1958, Kress 1963, 1984; Skalińska in Skalińska and Pogan, 1966; Kochjarová, 1992), and for *A. obtusifolia* and *Douglasia ochotensis*, both $n = 19$ (Favarger, 1958; Kress, 1965, 1984; Johnson and Packer, 1968; H. Weiss-Schneeweiss et al., unpublished data) and $n = 20$ are reported (Zhukova 1965, 1967; Zhukova and Petrovskij, 1987; Uhriková in Uhriková and Dúbravcová, 1997). A report of $n = 18$ for *Douglasia idahoensis* (Henderson, 1981) is probably due to misinterpration of two overlapping bivalents as one bivalent, as can be seen on the drawings provided by the author. Within */Dicranothrix*, the arctic species of *Douglasia* and *Androsace triflora* share some unique aspects of seed coat morphology (Kovtonyuk, 2002; S. Kelso, unpublished data), supporting the monophyly of this arctic clade.

The third satellite genus of *Androsace*, *Pomatosace*, nests within *Androsace*, where it constitutes a separate lineage */Pomatosace* (Fig. 5). Based on the presence of circumscissile capsules, it was put together with *Bryocarpum* and *Soldanella* into subtribe Soldanellinae by Pax and Knuth (1905). However, Weberbauer (1898) showed that the superficially circumscissile capsules of *Soldanella* and *Bryocarpum* are in fact not operculate but rather open by longitudinal splits as found in other Primulaceae. Spanowsky (1962) and Wendelbo (1961) found in *Pomatosace* the same pollen type as in *Androsace*, and the latter regards *Pomatosace* as a segregate from *Androsace*, suggesting that it could be incorporated into that genus as a section. Similarly, Schwarz (1963) realized the similarity of *Pomatosace* to annual species of *Androsace* sect. *Andraspis*, and accordingly united *Pomatosace* and *Androsace* in subtribe Androsacinae. A close relationship of *Pomatosace* and *Androsace* is also suggested by features of the karyotype ($x = 10$; Kong and Liu, 1999).

Biogeographic Implications

In *Androsace*, similarities between the amphi-Beringian *Douglasia* and some European taxa have been recognized by Kress (1965) and Anderberg and Kelso (1996). The biogeographic interpretation of that large scale disjunction, however, is diametrically different. Whereas Anderberg and Kelso (1996) accept it as a consequence of an inferred sister group relationship between *Douglasia* and *Vitaliana*, Kress (1965) takes it as an argument against a close relationship of *Douglasia* and *Androsace* sect. *Aretia*, despite their morphological and karyological similarities. Instead, he considers them separate lineages independently evolved from an Asian stock. This latter hypothesis is clearly rejected by our results which show that *Douglasia* phylogenetically nests within *Androsace* sect. *Aretia* (see above).

Another reason for Anderberg and Kelso (1996) to accept a disjunction between the amphi-Beringian region and the European mountain ranges was the presence of two similar cases within Primulaceae (then still understood in the circumscription of Pax and Knuth, 1905). One is a sister-group relationship between the western North American *Dodecatheon* and the Mediterranean *Cyclamen* as suggested by Anderberg and Ståhl (1995), which recently has been rejected by molecular phylogenetic analyses (Anderberg et al., 1998, 2001; Källersjö et al., 2000; Martins et al., 2003). The second is the close relationship between the European *Primula* sect. *Auricula* and the two principally western North American sects. *Cuneifoliae* and *Parryi*, which finds support in recent molecular phylogenetic studies (Mast et al, 2001; Trift et al., 2002).

The most striking feature in the distribution of */Aretia* and the above mentioned sections of *Primula* is the obvious distribution gap in the central Asian mountain ranges (Fig. 6), where both genera show a high diversity. Mast et al. (2001) suspected that in *Primula* this gap might be bridged by species of sect. *Amethystina*, but this seems not to be the case (Mast et al., 2004). Due to the relatively weak sampling in the Asian groups, taxa providing a link between the geographically separated groups of */Aretia*

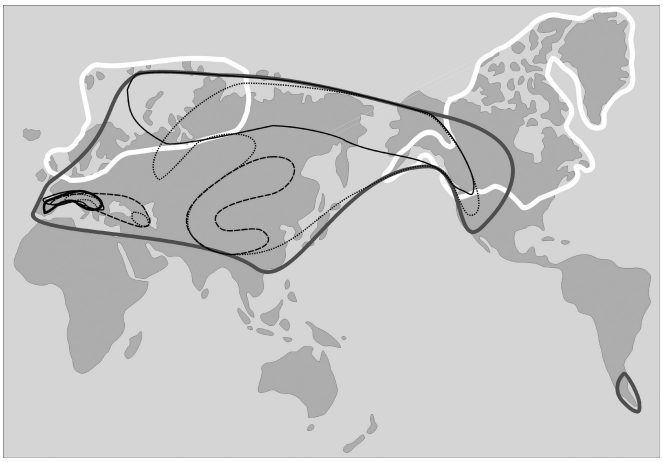

FIGURE 6. Distribution of *Androsace* (thick gray line) and some of the groups identified in this study. */Eu-Aretia* (thick solid line) and */Dicranothrix* (thin solid line) are the two members of */Aretia*, which possibly evolved in Europe (note the largely congruent distribution areas in Europe). In contrast, the distribution areas of *A.*sect. *Chamaejasme* subsect. *Chamaejasmoidea* (dotted line) and subsect. *Villosae* (dashed line) suggest an origin in East Asian mountain ranges and subsequent migration east- and westwards. Together, the distribution areas of *A.* sect. *Chamaejasme* subsects. *Chamaejasmoidea* and *Villosae* cover the whole distribution area of */Chamaejasme*. Thick white lines indicate the maximum extent of the ice-sheets of the last Pleistocene glaciation (local glaciations outside the two major ice-sheets not shown).

Downloaded from https://academic.oup.com/sysbio/article/53/6/856/1650198 by guest on 20 April 2024

could have been missed in our survey. The available morphological and karyological evidence, however, does not suggest any of those species as member of */Aretia*. This is also the case for *A. delavayi* and *A. lehmannii*, which are treated as members of *Androsace* sect. *Aretia* by Smith and Lowe (1997), but are grouped in sect. *Chamaejasme* by Hu and Yang (1986), the latter being in agreement with morphological evidence from the indumentum and, in the case of *A. delavayi*, with molecular data obtained in the present study. A further indication that the gap of */Aretia* in the central and East Asian mountain ranges is not an artifact is given by the lack of species of */Aretia* in the Near Asian mountain ranges, especially in the Caucasus. Admittedly, this is only a weak argument, because in other groups of Eurasian mountain taxa connecting species are also lacking in this region, e.g., in *Leontopodium* (Asteraceae) with the majority of species in central and East Asian mountain ranges (from Afghanistan eastwards) and two species in Europe.

The amphi-Beringian clade of */Aretia*, i.e., *Douglasia* and *A. triflora*, has evolved in the Pleistocene or late Pliocene (Table 2). At that time the uplift of the Eurasian mountain system was already advanced (Agakhanjanz and Breckle, 1995) and should have allowed the migration of mountain species across Eurasia. The presence of such a migration route is exemplified by members of */Chamaejasme*. *Androsace villosa* s. str. is the westernmost member of subsect. *Villosae* distributed throughout the Eurasian mountain ranges, whereas *A. chamaejasme* s. str. is the European representative of the holarctic arctic-alpine subsect. *Chamaejasmoidea* (Fig. 6; Meusel et al., 1978). Both species obviously arrived independently in the European mountain ranges after centrifugal range expansion from the putative center of origin in the central and East Asian mountain ranges. If a similar route was taken by members of */Aretia*, it is, however, difficult to explain, why occurrences in the Asian mountain ranges should have gone extinct later. This scenario could only be evaluated by fossils, but the fossil record of *Androsace* is scarce and the few fossils cannot be assigned to any of the lineages within *Androsace* and are therefore not conclusive.

The inclusion of *A. triflora* in */Aretia* renders the distribution of this group arctic-alpine (Fig. 6), raising the possibility of a northern Asian connection between the amphi-Beringian region and the European mountain ranges. If so, the gap in the European and the northeastern North American Arctic might be explained as a result of the heavy glaciation of these areas during the Pleistocene (Wilson et al., 2000; see map in Abbott and Brochmann 2003), which could have eradicated occurrences of members of */Aretia*. The Arctic taxa of */Aretia*, however, occupy a phylogenetically terminal position (Figs. 3 to 5) and only evolved in the Pleistocene (Table 2), and thus the distribution area of *A. triflora*, the only Arctic member of */Aretia* occurring outside Beringia, is rather the result of a westward migration after the last glacial maximum than a relict of a connection between the two distribution areas of */Aretia*.

Although the available data do not allow us to determine the route(s) of exchange(s) between the amphi-Beringian region and the European mountain ranges in */Aretia*, they do allow us to infer the direction. */Aretia* evolved in the Pliocene (maybe latest Miocene; Table 2) and subsequently split into the two lineages */Eu-Aretia* and */Dicranothrix*. Climatic oscillations in the Pliocene are recognized as the driving force for speciation events in the bird subfamily Tetraoninae (Drovetski, 2003), and might have played a similar role in the evolution of */Aretia*. The restriction of species of */Eu-Aretia* and ancestral species of */Dicranothrix* to the European mountain ranges together with the phylogenetically derived position and therefore younger age of *Douglasia* and *A. triflora* suggest a European origin. This is in agreement with results from parsimony and maximum likelihood reconstructions of the ancestral areas of */Aretia* and */Dicranothrix* (data not shown).

From Europe, members of */Dicranothrix* would have reached the amphi-Beringian region. A possible scenario includes more contiguous distributions in northern Asia that were later disrupted by Pliocene-Pleistocene glacial and tectonic events. Causes for regional extirpations in northern Asia could have been the rapid glaciations of North Asian mountains in the late Pleistocene (Brigham-Grette, 2001) or late Pleistocene sea level changes resulting in submergence of a large extent of previously emergent shallow continental shelf along the Arctic shoreline (Strelkov, 1982). An alternative scenario involves colonization after long-distance dispersal(s). As previously argued above, the current distribution range of *A. triflora* is probably not a relict of a North Asian connection but the result of westward migration. This together with the scarcity of suitable habitats for mountain species in the West Siberian plain are in favor of the hypothesis of long distance dispersal (but see Weber, 2003, for the opposite view in explaining the similarities between North American and Middle Asian mountain floras). Long-distance dispersal has also been considered to explain comparable disjunctions on the southern hemisphere between South America and New Zealand, e.g, in *Abrotanella* (Asteraceae; Swenson and Bremer, 1997) or *Hebe* (Plantaginaceae sensu Bremer et al., 2002 and APG II, 2003; Wagstaff et al., 2002). Hypotheses employing both scenarios, that is, more contiguous distributions plus some degree of dispersal, in conjunction rather than as mutually exclusive alternatives, may also be considered.

The origin of *Douglasia* falls in the late Pliocene to Pleistocene, a period characterized by alternating cold and warm periods (Wilson et al., 2000; Peizhen et al., 2001, Zachos et al., 2001). The ancestors of *Douglasia* could have reached the amphi-Beringian region in one of the warmer phases, subsequently loosing any intermediate occurrences in cold phases. Rapid range expansions in relatively short phases of more favorable climatic conditions are known from some arctic-alpine species, e.g., *Ranunculus glacialis* (Ranunculaceae), which colonized the Arctic from the Alps only after the last glacial maximum (Schönswetter et al., 2003b), or from

Downloaded from https://academic.oup.com/sysbio/article/53/6/856/1650198 by guest on 20 April 2024

amphiatlantic species, e.g., *Cerastium nigrescens* and *C. arcticum* (Caryophyllaceae), which very likely dispersed over the Atlantic in postglacial times, thus within 18,000 years (see reviews by Abbott and Brochmann, 2003, and Brochmann et al., 2003). Like *Androsace* and *Douglasia*, these species have no particular adaptations in their diaspores for long distance dispersal, and the exact mode of dispersal remains to be investigated.

The center of origin of the ancestors of */Aretia* is unknown. Based on the distribution of */Pseudoprimula*, the most primitive group within *Androsace*, the center of origin of the genus is assumed to be in East Asia (Pax and Knuth, 1905; Kress, 1965). This might also be true for the ancestors of */Aretia*, but the current distribution of the closest relatives of */Aretia*, species of */Elongata* and */Septentrionalis*, suggest an origin outside East Asia. The ancestors of */Aretia* probably were short-lived species (Fig. 5), and might have had similar ecological profiles as species of */Septentrionalis* and */Elongata* today. As such, they could have originated and diversified in open forests or steppes present in northern and central Asia in the Miocene and Pliocene (Tiffney and Manchester, 1999), the time of the split of */Elongata* and */Aretia* (Table 2).

Although the phylogenetic results presented here do not allow us to establish a detailed biogeographic scenario for *Androsace* and related genera, they unambiguously reveal a surprisingly close relationship between taxa of the amphi-Beringian region and the European mountain ranges. It is therefore evident that the similar pattern found in some sections of *Primula* (Mast et al., 2001; Trift et al., 2002) is not just a peculiarity of that genus. Additionally, recent molecular investigations indicate that a similar biogeographic pattern is present in *Ranunculus glacialis* s. l. (Ranunculaceae; Schönswetter et al., unpublished data) as well as *Pulsatilla alpina* s. l. and *P. occidentalis* (Ranunculaceae; Zetzsche and Blattner, 2003; see maps in Meusel et al., 1965 and Hultén and Fries, 1986). Doubtlessly, disjunctions between European mountain ranges and Beringia will continue to be rare in northern hemisphere mountain plants even with increased knowledge of phylogenetic relationships. However, our study illustrates the subtle complexity of biogeographic patterns even in a genus with an apparently continuous distribution in the mountain ranges of the northern hemisphere.

CONCLUSIONS

This study clarifies the relationship of three small satellite genera, *Douglasia*, *Pomatosace*, and *Vitaliana*, to the core genus *Androsace*, and outlines a major revision of the taxonomy of this whole group. A sound molecular phylogeny provides the opportunity to trace the evolution of complex traits such as heterostyly in *Vitaliana*, the multiple independent origin of short-lived life forms, or reversals to perenniality. Finally, these phylogenetic relationships indicate a complex biogeographic history with close connections between the European mountains and northwestern North America without extant linking taxa in central and East Asia, the putative center of origin of *Androsace* and related genera. Overall, this study demonstrates that patterns of evolution in this group of northern hemisphere mountain plants are much more complex than previously believed. In depth studies on additional taxa are likely to provide relevant and fruitful comparisons.

ACKNOWLEDGMENTS

We thank Roberta Mason-Gamer, Chris Simon, and two anonymous reviewers for comments that greatly improved the manuscript. We are thankful to Jeong-Mi Park for her excellent technical assistance. We are grateful to David E. Boufford, Christian Brochmann, Philippe Choler, Alison E. Colwell, Walter Gutermann, Elvira Hörandl, Alarich Kress, Magnus Lidén, Austin Mast, David Mowle and the Alpine Garden Society, Harald Pauli, Ivan Schanzer, Luise Schratt-Ehrendorfer, Markus Staudinger, and Vladimir Vladimirov for providing plant material and to the Olympic National Park administration for issuing a collection permit for *Douglasia laevigata* (OLYM-2001-SCI-0038). Thanks to Dirk Albach for a critical reading of the manuscript. Financial support by the Austrian Science Foundation grant FWF P16104-B03 (to HN) is gratefully acknowledged.

REFERENCES

Abbott, R. J., and C. Brochmann. 2003. History and evolution of the arctic flora: In the footsteps of Eric Hultén. Mol. Ecol. 12:299–313.

Agakhanjanz, O., and S.-W. Breckle. 1995. Origin and evolution of the mountain flora in Middle Asia and neighbouring mountain regions. Pages 63–80 *in* Arctic and Alpine biodiversity: Patterns, causes and ecosystem consequences (F. S. Chapin III and C. Körner, eds.). Ecological studies 113. Springer, Berlin, Germany.

Albach, D. C., and M. W. Chase. 2001. Paraphyly of *Veronica* (Veroniceae; Scrophulariaceae): Evidence from the internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA. J. Plant Res. 114:9–18.

Anderberg, A. A., and S. Kelso. 1996. Phylogenetic implications of endosperm cell wall morphology in *Douglasia*, *Androsace* and *Vitaliana* (Primulaceae). Nord. J. Bot. 16:481–486.

Anderberg, A. A., and B. Ståhl. 1995. Phylogenetic interrelationships in the order Primulales, with special emphasis on the family circumscriptions. Can. J. Bot. 73:1699–1730.

Anderberg, A. A., B. Ståhl, and M. Källersjö. 1998. Phylogenetic relationships in the Primulales inferred from *rbcL* sequence data. Plant Syst. Evol. 211:93–102.

Anderberg, A. A., B. Ståhl, and M. Källersjö. 2000. Maesaceae, a new primuloid family in the order Ericales *s.l.* Taxon 49:183–187.

Anderberg, A. A., C. I. Peng, I. Trift, and M. Källersjö. 2001. The *Stimpsonia* problem: Evidence from DNA sequences of plastid genes *atp*B, *ndh*F and *rbc*L. Bot. Jahrb. Syst. 123:369–376.

Andreasen, K., and B. G. Baldwin. 2001. Unequal evolutionary rates between annual and perennial lineages of checker mallows (*Sidalcea*, Malvaceae): Evidence from 18S-26S rDNA internal and external transcribed spacers. Mol. Biol. Evol. 18:936–944.

APG II. 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. Bot. J. Linn. Soc. 141:399–436.

Barker, F. K., and F. M. Lutzoni. 2002. The utility of the Incongruence Length Difference test. Syst. Biol. 51:625–637.

Barrett, S. C. H. 1992. Heterostylous genetic polymorphisms: Model systems for evolutionary analysis. Pages 1–29 *in* Evolution and function of heterostyly (S. C. H. Barrett, ed.). Springer-Verlag, New York, New York.

Baum, D. A., W. S. Alverson, and R. Nyffeler. 1998. A durian by any other name: Taxonomy and nomenclature of the core Malvales. Harvard Papers Bot. 3:315–330.

Downloaded from https://academic.oup.com/sysbio/article/53/6/856/1650198 by guest on 20 April 2024

Bena, G., B. Lejeune, J.-M. Prosperi, and I. Olivieri. 1998: Molecular phylogenetic approach for studying life-history evolution: The ambiguous example of the genus *Medicago* L. Proc. R. Soc. Lond. [Ser.] B 265:1141–1151.

Bradley, R. D., and D. M. Hillis. 1997. Recombinant DNA sequences generated by PCR amplification. Mol. Biol. Evol. 14:592–593.

Bremer, B., K. Bremer, N. Heidari, P. Erixon, R. G. Olmstead, A. A. Anderberg, M. Källersjö, and E. Barkhordarian. 2002. Phylogenetics of asterids based on 3 coding and 3 non-coding chloroplast markers and the utility of non-coding DNA at higher taxonomic levels. Mol. Phylogenet. Evol. 24:274–301.

Brigham-Grette, J. 2001. New perspectives on Beringian quaternary paleogeography, stratigraphy, and glacial history. Quaternary Sci. Rev. 20:15–24.

Brochmann, C., T. M. Gabrielsen, I. Nordal, J. Y. Landvik, and R. Elven. 2003. Glacial survival or *tabula rasa*? The history of North Atlantic biota revisited. Taxon 52:417–450.

Christy, M. 1921. The pollination of British primulas. Bot. J. Linn. Soc. 46:105–139.

Constance, L. 1938. A revision of the genus *Douglasia* Lindley. Am. Midl. Nat. 19:249–259.

Cronn, R., M. Cedroni, T. Haselkorn, C. Grover, and J. F. Wendel. 2002. PCR-mediated recombination in amplification products derived from polyploid cotton. Theor. Appl. Genet. 104:482–489.

Cunningham, C. W. 1997. Can the incongruence test predict when data should be combined? Mol. Biol. Evol. 14:733–740.

Cunningham, C. W. 1999. Some limitations of ancestral character-state reconstruction when testing evolutionary hypotheses. Syst. Biol. 48:665–674.

Cunningham, C. W., K. E. Omland, and T. H. Oakley. 1998. Reconstructing ancestral character states: A critical reappraisal. Trends Ecol. Evol. 13:361–366.

Darlu, P., and G. Lecointre. 2002. When does the incongruence length difference test fail? Mol. Biol. Evol. 19:432–437.

Dolphin, K., R. Belshaw, C. D. L. Orme, and D. L. J. Quicke. 2000. Noise and incongruence: Interpreting results of the incongruence length difference test. Mol. Phylogenet. Evol. 17:401–406.

Dorofeev, P. I. 1963. Primulaceae. Pages 517–518 *in* Oznovij Paleontologii (A. L. Takhtajan, ed.). Akademia Nauk, Moscow, Russia.

Dowton, M., and A. D. Austin. 2002. Increased congruence does not necessarily indicate increased phylogenetic accuracy—the behavior of the incongruence length difference test in mixed-model analyses. Syst. Biol. 51:19–31.

Doyle, J. J., and J. L. Doyle. 1987. A rapid isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19:11–15.

Drovetski, S. V. 2003. Plio-Pleistocene climatic oscillations, holarctic biogeography and speciation in an avian subfamily. J. Biogeogr. 30:1173–1181.

Dulberger, R. 1992. Floral polymorphisms and their functional significance in the heterostylous syndrome. Pages 41–84 *in* Evolution and function of heterostyly (S. C. H. Barrett, ed.). Springer-Verlag, New York, New York.

Efron, B., and R. J. Tibshirani. 1993. An introduction to the Bootstrap. Chapman and Hall, New York, New York.

Favarger, C. 1958: Contribution à l'étude cytologique des genres *Androsace* et *Gregoria*. Veröff. Geobot. Inst. Rübel Zürich 39:59–80.

Felsenstein, J. 1999. PHYLIP 3.5c. http://evolution.genetics. washington.edu/phylip.html

Ferguson, I. K. 1972. *Vitaliana* Sesler, *Androsace* L. Pages 20–23 *in* Flora Europaea 3 (T. G. Tutin, V. H. Heywood, N. A. Burges, D. M. Moore, D. H. Valentine, S. M. Walters, and D. A. Webb, eds.). Cambridge University Press, Cambridge, U.K.

Goldblatt, P., V. Savolainen, O. Porteous, I. Sostaric, M. Powell, G. Reeves, J. C. Manning, T. G. Barraclough, and M. W. Chase. 2002. Radiation in the Cape flora and the phylogeny of peacock irises *Moraea* (Iridaceae) based on four plastid DNA regions. Mol. Phylogenet. Evol. 25:341–360.

Hall, T. A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids Symp. Ser. 41:95–98.

Handel-Mazzetti, H. 1927. A revision of the Chinese species of *Androsace*, with remarks on other Asiatic species. Notes R. Bot. Gard. Edinburgh 75:259–298.

Harborne, J. B. 1968. Comparative biochemistry of the flavonoids VII. Correlations between flavonoid pigmentation and systematics in the family Primulaceae. Phytochemistry 7:1215–1230.

Henderson, D. M. 1981. A new *Douglasia* (Primulaceae) from Idaho. Brittonia 33:52–56.

Holmgren, P. K., N. H. Holmgren, and L. C. Barnett. 1990. Index Herbariorum. Part I: The Herbaria of the World. Regnum Veg. 120.

Hu, C. M., and Y. C. Yang. 1986. A revision of the genus *Androsace* L. in China. Acta Phytotax. Sin. 24:215–232. (In Chinese.)

Huelsenbeck, J. P., and F. R. Ronquist. 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics 17:754–755.

Hultén, E., and M. Fries. 1986. Atlas of North European vascular plants I. Koeltz Scientific Books, Königstein, Germany.

Iruela, M., J. Rubio, J. I. Cubero, J. Gil, and T. Millán. 2002. Phylogenetic analysis in the genus *Cicer* and cultivated chickpea using RAPD and ISSR markers. Theor. Appl. Genet. 104:643–651.

Johnson, A. W., and J. G. Packer. 1968. Chromosome numbers in the flora of Ogotoruk Creek, N.W. Alaska. Bot. Notiser 121:403–456.

Källersjö, M., G. Bergqvist, and A. A. Anderberg. 2000. Generic realignment in primuloid families of the Ericales *s.l.*: A phylogenetic analysis based on DNA sequences from three chloroplast genes and morphology. Amer. J. Bot. 87:1325–1341.

Kelso, S. 1987. *Primula tschuktschorum* and *Primula eximia* (Primulaceae, section *Crystallophlomis*); a distylous species and its homostylous derivative from the Bering Strait region, Alaska. Brittonia 39:63–72.

Kelso, S. 1991. Taxonomy and biogeography of *Primula* sect. Cuneifoliae (Primulaceae) in North America. Madroño 38:37–44.

Kelso, S. 1992. Conspectus of the genus *Douglasia* (Primulaceae) with comments on *Douglasia alaskana*, an Alaska-Yukon alpine endemic. Can. J. Bot. 70:593–596.

Kelso, S., B. A. Yurtsev, and D. F. Murray. 1994. *Douglasia beringensis* (Primulaceae): A new species from northwestern Alaska. Novon 4:381–385.

Kochjarová, J. 1992. Karyological study of the Slovak flora XXVIII. Acta Fac. Rer. Nat. Univ. Comenianae Bot. 39:67–74.

Kong, H.-Z., and J.-Q. Liu. 1999. Karyomorpholgy of the genus *Pomatosace* Maxim. (Primulaceae). Acta Phytotax. Sin. 37:445–450. (In Chinese.)

Kovtonyuk, N. K. 2002. Seed surface sculpture in relation to taxonomy of the genera *Androsace* and *Douglasia* (Primulaceae) of the northern Asia. Bot. Zh. 87:57–63. (In Russian.)

Kress, A. 1963. Zytotaxonomische Untersuchungen an den *Androsace*-Sippen der Sektion Aretia (L.) Koch. Ber. Bayer. Bot. Ges. 36:33–39.

Kress, A. 1965. Zur Zytotaxonomie der *Androsace-Vitaliana-Douglasia*-Verwandtschaft. Mitt. Bot. Staatssamml. München 5:653–674.

Kress, A. 1984. Chromosomenzählungen an verschiedenen Primulaceen, Teil A, *Androsace*. Primulaceen-Studien 3:1–32.

Kuvajev, W. B., and N. M. Pirozhkova. 1987. Some problems of the systematics of the genus *Androsace* (Primulaceae). Bot. Zh. 72:948–959. (In Russian.)

Lewis, P. O. 2001. A likelihood approach to estimating phylogeny from discrete morphological character data. Syst. Biol. 50:913–925.

Liston, A., and A. J. Wheeler. 1994. The phylogenetic position of the genus *Astragalus* (Fabaceae): Evidence from the chloroplast gene *rpo*C1 and *rpo*C2. Biochem. Syst. Ecol. 22:377–388.

Lüdi, W. 1927. Primulaceae. Pages 1715–1877 *in* Illustrierte Flora von Mitteleuropa V/3 (G. Hegi, ed.). A. Pichlers Witwe & Sohn, Vienna, Austria.

Maddison, W. P., M. J. Donoghue, and D. R. Maddison. 1984. Outgroup analysis and parsimony. Syst. Zool. 33:83–103.

Maddison, W. P., and D. R. Maddison. 2003. Mesquite: A modular system for evolutionary analysis. Version 1.0. http://mesquiteproject.org

Martins, L., C. Oberprieler, and F. H. Hellwig. 2003. A phylogenetic analysis of Primulaceae s.l. based on internal transcribed spacer (ITS) DNA sequence data. Plant Syst. Evol. 237:75–85.

Mast, A. R., D. M. S. Feller, S. Kelso, and E. Conti. 2004. Buzz-pollinated *Dodecatheon* originated from within the heterostylous *Primula* subgenus *Auriculastrum* (Primulaceae): A seven-region

Downloaded from https://academic.oup.com/sysbio/article/53/6/856/1650198 by guest on 20 April 2024

cpDNA phylogeny and its implications for floral evolution. Am. J. Bot. 91:926–942.
Mast, A. R., S. Kelso, A. J. Richards, D. J. Lang, D. M. S. Feller, and E. Conti. 2001. Phylogenetic relationships in *Primula* L. and related genera (Primulaceae) based on noncoding chloroplast DNA. Int. J. Plant Sci. 162:1381–1400.
Menitsky, G. L. 2000. Conspectus of Caucasian Primulaceae species. Bot. Zh. 85:152–167. (In Russian.)
Měsíček, J., and J. Soják. 1992. Chromosome numbers of Mongolian angiosperms. I. Preslia 64:193–206.
Meusel, H., E. Jäger, S. Rauschert, and E. Weinert. 1978. Vergleichende Chorologie der zentraleuropäischen Flora II. Gustav Fischer, Jena, Germany.
Meusel, H., E. Jäger, and E. Weinert. 1965. Vergleichende Chorologie der zentraleuropäischen Flora I. Gustav Fischer, Jena, Germany.
Miller, J., M. Litvak, S. Kelso, and A. Vargo. 1994. Comparative reproductive biology of two alpine species of primrose. Arct. Alp. Res. 26:297–303.
Nakata, M., Q. Wu, and S. Kurokawa. 1997. Cytological studies on Chinese plants introduced from Yunnan Province. I. Karyomorphology of some species of *Primula* and *Androsace*. Bull. Bot. Gard. Toyama 2:1–15.
Ovchinnikov, P. N. 1952. *Androsace* L. Pages 217–242 *in* Flora SSSR 18 (B. K. Schischkin and E. G. Bobrov, eds.). Akademij Nauk SSSR, Moscow, Russia. (In Russian.)
Pagel, M. 1999. The maximum likelihood approach to reconstructing ancestral character states of discrete characters on phylogenies. Syst. Biol. 48:612–622.
Pax, F. 1897. Primulaceae. Pages 98–116 *in* Die natürlichen Pflanzenfamilien IV/1 (A. Engler and K. Prantl, eds.). Wilhelm Engelmann, Leipzig, Germany.
Pax, F., and R. Knuth. 1905. Primulaceae. Pages 1–386 *in* Das Pflanzenreich. Regni vegetabilis conspectus IV.237 (A. Engler, ed.). Wilhelm Engelmann, Leipzig, Germany.
Peizhen, Z., P. Molnar, and W. R. Downs. 2001. Increased sedimentation rates and grain sizes 2–4 Myr ago due to the influence of climate change on erosion rates. Nature 410:891–897.
Posada, D., and K. A. Crandall. 1998. Modeltest: Testing the model of DNA substitution. Bioinformatics 14:817–818.
Proctor, M., P. Yeo, and A. Lack. 1996. The natural history of pollination. Timber Press, Portland, Oregon.
Punt, W., J. S. de Leeuw van Weenen, and W. A. P. van Oostrum. 1976. Primulaceae. Pages 31–70 *in* The Northwest European pollen flora, I (W. Punt, ed.). Elsevier, Amsterdam, The Netherlands.
Richards, J. 1993. *Primula*. Timber Press, Portland, Oregon.
Robbins, G. T. 1944. North American species of *Androsace*. Am. Midl. Nat. 32:137–163.
Ronquist, F., and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.
Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: A laboratory manual, 2nd edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
Sanderson, M. J. 1997. A nonparametric approach to estimating divergence times in the absence of rate constancy. Mol. Biol. Evol. 14:1218–1231.
Sanderson, M. J. 2002a. Estimating absolute rates of molecular evolution and divergence times: A penalized likelihood approach. Mol. Biol. Evol. 19:101–109.
Sanderson, M. J. 2002b. r8s Version 1.60. http://ginger.ucdavis.edu/r8s/
Sarkar, A. K. 1988. Primulaceae – its evolution and assessment in status as judged through cytotaxonomy. Feddes Repert. 99:113–132.
Schaeppi, H. 1934. Untersuchungen über die Narben- und Antherenstellung in den Blüten der Primulaceen. Archiv Julius-Klaus-Stiftung Vererbungsforschung, Sozialanthropologie Rassenhygiene 10:133–236.
Schaeppi, H. 1935. Zur Kenntnis der Heterostylie von *Gregoria vitaliana* Duby. Ber. Schweiz. Bot. Ges. 44:109–132.
Schönswetter, P., A. Tribsch, M. Barfuss, and H. Niklfeld. 2002. Several Pleistocene refugia detected in the high alpine plant *Phyteuma globulariifolium* Sternb. & Hoppe (Campanulaceae) in the European Alps. Mol. Ecol. 11:2637–2647.
Schönswetter, P., A. Tribsch, G. M. Schneeweiss, and H. Niklfeld. 2003a. Disjunctions in relict alpine plants: Phylogeography of *Androsace brevis* and *A. wulfeniana* (Primulaceae). Bot. J. Linn. Soc. 141:437–446.
Schönswetter, P., O. Paun, A. Tribsch, and H. Niklfeld. 2003b. Out of the Alps: Colonization of Northern Europe by East Alpine populations of the glacier buttercup *Ranunculus glacialis* L. (Ranunculaeae). Mol. Ecol. 12:3373–3381.
Schwarz, O. 1963. Die Gattung *Vitaliana* Sesl. und ihre Stellung innerhalb der Primulaceen. Feddes Repert. 67:16–41.
Skalińska, M., and E. Pogan. 1966. Dalsze badania nad kariologia flory poskiej Cz. VI. [Further studies in chromosome numbers of Polish angiosperms]. Sixth contribution. Acta Biol. Cracov. Ser. Bot. 9:31–58.
Smith, G., and D. Lowe. 1997. The Genus *Androsace*. Alpine Garden Society, Avon Bank, U.K.
Spanowsky, W. 1962. Die Bedeutung der Pollenmorphologie für die Taxonomie der Primulaceae-Primuloideae. Feddes Repert. 65:149–213.
Strelkov, S. A. 1982. On the history of the migration of the Arctic Basin shoreline in the Cenozoic Era. Pages 219–232 *in* The Arctic Ocean and its Coast in the Cenozoic Era (A. I. Tolmachev, ed.). Smithsonian Institution and the National Science Foundation, Washington, D.C.
Sullivan, J. 1996. Combining data with different distributions of among-site rate variation. Syst. Biol. 45:375–380.
Sun, Y., D. Z. Skinner, G. H. Liang, and S. H. Hulbert. 1994. Phylogenetic analysis of *Sorghum* and related taxa using internal transcribed spacers of nuclear ribosomal DNA. Theor. Appl. Genet. 89:26–32.
Swenson, U., and K. Bremer. 1997. Pacific biogeography of the Asteraceae genus *Abrotanella* (Senecioneae, Blennospermatinae). Syst. Bot. 22:493–508.
Swofford, D. L. 2001. PAUP*: Phylogenetic Analysis Using Parsimony (* and other methods). Version 4.0b.10 for 32-bit Microsoft Windows. Sinauer Associates, Sunderland, Massachusetts.
Taberlet, P., L. Gielly, G. Pautou, and J. Bouvet. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol. Biol. 17:1105–1109.
Takhtajan, A. 1997. Diversity and classification of flowering plants. Columbia University Press, New York, New York.
Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins. 1997. The ClustalX windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 24:4876–4882.
Tiffney, B. H., and S. R. Manchester. 1999. The use of geological and paleontological evidence in evaluating plant phylogeographic hypotheses in the northern hemisphere tertiary. Int. J. Plant Sci. 162 (Suppl.):S3–S17.
Trift, I., M. Källersjö, and A. A. Anderberg. 2002. The monophyly of *Primula* (Primulaceae) evaluated by analysis of sequences from the chloroplast gene *rbcL*. Syst. Bot. 27:396–407.
Uhríková, A., and Z. Dúbravcová. 1997. The chromosome numbers of some selected plant species of flora in Slovakia. Oecol. Montana 6:1–3.
Wagstaff, S. J., M. J. Bayly, P. J. Garnock-Jones, and D. C. Albach. 2002. Classification, origin, and diversification of the New Zealand Hebes (Scrophulariaceae). Ann. Missouri Bot. Gard. 89:38–63.
Weber W. A. 2003. The Middle Asian element in the southern Rocky Mountain flora of the western United States: A critical biogeographic review. J. Biogeogr. 30:649–685.
Weberbauer, A. 1898. Beiträge zur Anatomie der Kapselfrüchte. Bot. Centralbl. 78:1–52.
Weiss, H., and G. M. Schneeweiss. 2001. Chromosome number and ploidy level of *Androsace maxima* (Primulaceae) in Austria. Neilreichia 1:177–180.
Wendelbo, P. 1961. Studies in Primulaceae III. On the related genera to *Primula* with special reference to their pollen morphology. Årbok Univ. Bergen Mat.-Naturv. Ser. 1961 (19):1–31.
Wiens, J. J. 2003. Missing data, incomplete taxa, and phylogenetic accuracy. Syst. Biol. 52:528–538.
Wikström, N., V. Savolainen, and M. W. Chase. 2001. Evolution of the angiosperms: Calibrating the family tree. Proc. R. Soc. Lond. Ser. B 268:2211–2220.

Downloaded from https://academic.oup.com/sysbio/article/53/6/856/1650198 by guest on 20 April 2024

Wilson, R. C. L., S. A. Drury, and J. L. Chapman. 2000. The Great Ice-age. The Open University, London, U.K.
Zachos, J., M. Pagani, L. Sloan, E. Thomas, and K. Billups. 2001. Trends, rhythms, and aberrations in global climate 65 Ma to present. Science 292:686–693.
Zetzsche, H., and F. Blattner. 2003. The evolution of the *Pulsatilla alpina* (L.) Delarbre species complex. Abstract 16th International Symposium Biodiversity and Evolutionary Biology, 21–27 Sept. 2003, Frankfurt/M. Palmarum Hort. Francofurt. 7:123.
Zhukova, P. G. 1965. [Karyological characteristics of some plant species of Wrangel Island.] Bot. Zh. 50:1320–1322. (In Russian.)
Zhukova, P. G. 1967. Chromosome numbers in some species of plants of the north-eastern part of the U.S.S.R., II. Bot. Zh. 52:983–987. (In Russian.)
Zhukova, P. G., and V. V. Petrovskij. 1987. Chromosome numbers and taxonomy of some plant species from the northern Asia regions. Bot. Zh. 72:1617–1624. (In Russian.)

First submitted 14 May 2003; reviews returned 3 October 2003; final acceptance 23 June 2004
Associate Editor: Roberta Mason-Gamer

APPENDIX 1. List of taxa included in the analyses. Taxonomic grouping according to Smith and Lowe (1997) with minor modifications (see text for details). Acronyms for herbaria following Holmgren et al. (1990). Abbreviations: GMS = herbarium Schneeweiss; S&T = herbarium Schönswetter & Tribsch.

Taxon	Locality; voucher	GB accession numbers	
		trnLF	ITS
Primula			
algida Adam ex Weber & Mohr	Georgia: Minor Caucasus, near Bakuriani; WU: S&T 6660	AY274943	
heterochroma Stapf	Azerbaidjan: Talysh; WU: S&T 6777	AY274940	
juliae Kusnez.	Georgia: Great Caucasus, Lagodechi;WU: S&T 6868	AY274938	
kitaibeliana Schott.	Croatia: Velebit; WU: S&T 6296	AY274942	AY275015
scandinavica Bruun	Norway: Oppdal; WU: S&T 7091	AY274941	
sinolisteri L. B. Balf. var. *sinolisteri*	China:Yunnan, Canshan Mts.; WU: S&T 6581	AY274937	
veris L. subsp.*veris*	Austria: Ebenwaldhöhe; WU: S&T 6561	AY274939	
Dionysia			
curviflora Bunge	Iran	AY274936	
Androsace			
sect. Pseudoprimula			
axillaris Franch.	China: Yunnan, Western Hills Forest Park; E (cultivated plants): D. Mowle s. n.	AY274949	AY275103–AY275105
paxiana Knuth			AF323705[a]
sect. Chamaejasme			
barbulata Ovcz.	Georgia: Great Caucasus, Kazbegi; WU: S&T 4483	AY274945	AY275082
chamaejasme Wulfen	Switzerland: Passo Lucomagno; WU: S&T 4830	AY274947	AY275090
delavayi Franch.	India: Rhotang Pass; E (cultivated plants): D. Mowle s. n.	AY274950	AY275101
hookeriana Klatt	Nepal: Jaljale Himal; E (cultivated plants): D. Mowle s. n.	AY274954	AY275096
koso-poljanskii Ovcz. (acc. 1)	Russia: Belgorod, W Osadcheye; WU: Schanzer & Majorov s. n.		AY275081
koso-poljanskii Ovcz. (acc. 2)	Russia: Belgorod, E Novy Oskol; WU: Schanzer & Majorov s. n.		AY275080
lehmanniana Spreng. (acc. 1)	Georgia: Great Caucasus, Kazbeg; WU: S&T 4480	AY274948	AY275088
lehmanniana Spreng. (acc. 2)	USA: Alaska, near Donnelly Dome; Z: D. S. Feller & A. R. Mast 539		AY275087
lehmanniana Spreng. (acc. 3)	USA: Colorado, Sangre De Christo Mts.; COCO: Conlin 13		AY275089
lehmanniana Spreng. (acc. 4)	USA: Alaska, Kotzebue; COCO: Murray & al. 11242		AY275086
rigida Hand.-Mazz.	China: Yunnan, Lijiang; E (cultivated plants): D. Mowle s. n.	AY274951	AY275093– AY275095
squarrulosa Maxim.	China: Qinghai, Qingshui Xiang;GH: Ho & al. 1350		AY275102
stenophylla (Ptmgn.) Hand.-Mazz.	China: Xizang(Tibet), Lhasa; E (cultivated plants): D. Mowle s. n.	AY274952	AY275085, AY502123–AY502124
strigillosa Franch.	Nepal: Dolpo area; E (cultivated plants): D. Mowle s. n.	AY274956	AY275092
studiosorum Kress	Cultivated in Jardin Botanique Alpin du Lautaret (France)	AY274946	
triflora Adams	Russia: Franz Joseph Land; WU: H. Pauli v-73	AY274992	AY275020
villosa L. (acc. 1)	Italy: Alpi Cozie; WU: S&T 4722	AY274944	AY275084
villosa L. (acc. 2)	Croatia: Velebit; WU: S&T 6252		AY275083
zambalensis (Ptmgn.) Hand.-Mazz.	China: Qinghai,Yushu Xian; A: Boufford & al. 26744		AY275091

Downloaded from https://academic.oup.com/sysbio/article/53/6/856/1650198 by guest on 20 April 2024

APPENDIX 1. *(Continued).*

Taxon	Locality; voucher	GB accession numbers *trnLF*	GB accession numbers ITS
sect. Aizoidium			
integra (Maxim.) Hand.-Mazz.	China: Sichuan, Minya Konka; E (cultivated plants): P. Boardman 98-08-09	AY274953	AY275098
sect. Aretia			
subsect. Aretia			
alpina (L.) Lam. (acc. 1)	Italy: Dolomiten; WU:S&T 3641	AY274975	AY275053
alpina (L.) Lam. (acc. 2)	France: Alpes Graies; WU:S&T 5141	AY274974	AY275052
alpina (L.) Lam. (acc. 3)	Austria: Silvretta; WU:S&T 5201	AY274973	AY275054
brevis (Hegetschw.) Cesati (acc. 1)	Italy: Alpi Orobie, Mte. Legnone; WU: S&T 4961	AY274963	AY275049
brevis (Hegetschw.) Cesati (acc. 2)	Italy: Alpi Orobie, Mte Rotondo; WU: S&T 5004	AY274964	
ciliata DC.	Spain: Pyrenees, Ordizeta; WU: S&T 6495	AY274982	AY275034
cylindrica DC. subsp. *cylindrica*	Spain: Pyrenees, Ordesa; WU: S&T 6487	AY274988	AY275068
cylindrica DC. subsp. *willkommii* P. Monts.	Spain: Pyrenees, Oroel; WU: S&T 6430	AY274987	AY275067
hausmannii Leybold (acc. 1)	Austria: Totes Gebirge; Herb. E. Hörandl: Hö 5467	AY274983	AY275056
hausmannii Leybold (acc. 2)	Austria: Lienzer Dolomiten; WU: S&T 7083	AY274984	AY275055
helvetica (L.) All. (acc. 1)	Switzerland: Glarner Alpen; WU: S&T 4855	AY274980	AY275065
helvetica (L.) All. (acc. 2)	Switzerland/Italy: Ortler Alpen; WU: S&T 5079	AY274981	AY275066
mathildae Levier	Cultivated in Jardin Botanique Alpin du Lautaret (France); —	AY274976	AY275071
pubescens DC. (acc. 1)	Italy/France: Alpi Cozie; WU: S&T 4719	AY274979	AY275064
pubescens DC. (acc. 2)	Italy/France: Alpi Cozie; WU: S&T 4719		AY275063
pubescens DC. (acc. 3)	France: Alpes Cottiennes; WU: S&T 4750	AY274978	AY275062
vandellii (Turra) Chiov. (acc. 1)	Italy: Dolomiti; WU: S&T 4663	AY274970	AY275060
vandellii (Turra) Chiov. (acc. 2)	Italy: Bernina; WU: S&T 5439	AY274969	AY275059
vandellii (Turra) Chiov. (acc. 3)	France: Eastern Pyrenees; WU: S&T 6390	AY274968	AY275058
vandellii (Turra) Chiov. (acc. 4)	Spain: Sierra Nevada; WU: S&T 6560	AY274967	AY275061
wulfeniana Sieber ex Koch (acc. 1)	Italy: Dolomiti; WU: S&T 4657	AY274961	AY275047
wulfeniana Sieber ex Koch (acc. 2)	Austria: Rottenmanner Tauern; WU: S&T 5213	AY274962	AY275048
subsect. Dicranothrix			
adfinis Biroli subsp. *adfinis*	Italy: Alpi Cozie; WU: S&T 5617	AY275008	
adfinis Biroli subsp. *brigantica* (Jord. & Fourr.) Kress (acc. 1)	France: Dauphiné; WU: S&T 5135	AY275005	AY275043
adfinis Biroli subsp. *brigantiaca* (Jord. & Fourr.) Kress (acc. 2)	France: Alpes Cottiennes; WU: S&T 6343		AY275041
adfinis Biroli subsp. *puberula* (Jord. & Fourr.) Kress (acc. 1)	Italy: Alpi Graie; WU: S&T 4766	AY275007	AY275040
adfinis Biroli subsp. *puberula* (Jord. & Fourr.) Kress (acc. 2)	Italy: Alpi Ligurie; WU: S&T 6366	AY275006	AY275042
cantabrica (Losa & P. Monts.) Kress (acc. 1)	Spain: Cordillera Cantabrica; Herb. A. Kress: Kress s. n.	AY275009	AY275038
cantabrica (Losa & P. Monts.) Kress (acc. 2)	Spain: Cordillera Cantabrica; E (cultivated plants): D. Mowle s. n.	AY275011	AY275039
halleri L.	France: Pyrenees, Canigou; WU: S&T 6416	AY275013	AY275037
hedraeantha Griseb.	Bulgaria: Stara Planina; WU: S&T 7174	AY275010	AY275046
lactea L. (acc. 1)	Austria: Grünauer Berge; Herb. W. Gutermann: Gu 26171	AY274985	AY275069
lactea L. (acc. 2)	Slovacia: Nizké Tatry; Herb. W. Gutermann: Gu 36691	AY274986	AY275070
laggeri Huet	Spain: Pyrenees, Pourtalet; WU: S&T 6442	AY275012	AY275036
obtusifolia All. (acc. 1)	Italy: Alpi Carnie; WU: S&T 4582	AY274971	
obtusifolia All. (acc. 2)	France: Alpes Graies; WU: S&T 4746	AY274972	AY275057
pyrenaica Lam.	Spain: Pyrenees, Ordizeta; WU: S&T 6493	AY274977	AY275035
rioxana A. Segura	Spain: Sierra de la Demanda; Herb. A. Kress: Kress s. n.	AY275004	AY275045
sect. Andraspis			
chaixii Gren. & Godron	France: Hautes Alpes, W of Gap; WU: S&T 8780	AY275003	AY275044
elongata L.	Austria: Leithagebirge; WU: S&T 4547	AY275014	AY275072
erecta Maxim.	China: Sichuan, Xiangcheng Xian; A: Boufford & al. 28947		AY275097
filiformis Retz. (acc. 1)	Russia: Altai, N Aktasch; WU: M. Staudinger: Staud 5520	AY274955	AY275078
filiformis Retz. (acc. 2)	USA: Idaho, Clark Co.; COCO: Markow 9707		AY275079
lactiflora Pall. (acc. 1)	Russia: Altai, E Aktasch; WU: M. Staudinger: Staud 5459		AY275075
lactiflora Pall. (acc. 2)	Russia: Altai; WU: M. Staudinger: Staud 5726		AY275076
maxima L. (acc. 1)	Jordan: near Shaubak; WU: GMS 4337	AY274958	AY275099
maxima L. (acc. 2)	Georgia: Minor Caucasus, Borjomi; WU: S&T 6988	AY274957	AY275100
raddeana Somm. & Lev.	Georgia: Great Caucasus, Kazbeg; WU: S&T s. n. (It-Nr. 48)	AY274960	AY275073
septentrionalis L.	Austria: Wienerwald; WU: S&T 4544	AY274959	AY275074
Douglasia			
alaskana (Coville & Standl. ex Hultén) S. Kelso			AF260774[a]
arctica Hook.	USA: Alaska, Eagle Quad; COCO: Lipkin & Cook 93/04	AY274998	AY275016

(*Continued on next page*)

Downloaded from https://academic.oup.com/sysbio/article/53/6/856/1650198 by guest on 20 April 2024

APPENDIX 1. *(Continued).*

Taxon	Locality; voucher	GB accession numbers *trnLF*	GB accession numbers ITS
beringensis S. Kelso, Jurtzev & D. F. Murray	USA: Alaska, Nulato Hills; COCO: Parkert & al. 8017	AY274999	AY275018
gormanii Constance	USA: Alaska, Charley River; COCO: Parkert & Hasslbach 4268	AY275000	AY275017
idahoensis D. Henderson	USA: Idaho, Idaho Co., Gospel Peak; COCO: Henderson & al. 3403	AY275001	AY275025
laevigata Gray (acc. 1)	USA: Oregon, Cone Peak; COCO: Kelso & Levin 00-200	AY274995	AY275029
laevigata Gray (acc. 2)	USA: Oregon, Cone Park; COCO: Kelso & Levin 00-200		AY275030
laevigata Gray (acc. 3)	USA: Washington, Olympic Mts.; WTU: OLYM-453		AY275031
laevigata Gray (acc. 4)	USA: Washington, S Mt. Rainier; E (cultivated plants): D. Mowle s. n.	AY274993	AY275032
laevigata Gray (acc. 5)	USA: Washington, Olympic Mts.; E (cultivated plants): D. Mowle s. n.	AY274994	AY275033
montana Gray (acc. 1)	USA: Montana; E (cultivated plants): D. Mowle s. n.	AY275002	AY275023
montana Gray (acc. 2)	USA: Montana, Missoula; COCO: Kelso 21.7.2000		AY275024
nivalis Lindl. (acc. 1)	USA: Washington, Kittitas Co.; WTU: Olmstead 2001-77	AY274989	AY275026
nivalis Lindl. (acc. 2)	USA: Washington, Kittitas Co.; COCO: Sondenna 336	AY274991	AY275027
nivalis Lindl. (acc. 3)	USA: Washington, Entiat Mts.; E (cultivated plants): R. Ratco NNS 97-79	AY274990	AY275028
ochotensis (Willd. ex Roemer & J. A. Schultes) Hultén (acc. 1)	USA: Alaska, Delang Mts.; COCO: Parkert & Meyers 10676	AY274997	AY275019
ochotensis (Willd. ex Roemer & J. A. Schultes) Hultén (acc. 2)	USA: Alaska, Delang Mts.; COCO: Parkert & Meyers 10676		AY275021
ochotensis (Willd. ex Roemer & J. A. Schultes) Hultén (acc. 3)	USA: Alaska, Hawand Pass; COCO: Parker & Meyers 7574	AY274996	AY275022
Pomatosace			
filicula Maxim.	China: Qinghai, Yushu Xian; A: Boufford & al. 26735	AF402440[a,b]	AY275077
Vitaliana			
primuliflora Bertol. (acc. 1)	France: Alpes Maritimes; WU: S&T 4700	AY274966	AY275050
primuliflora Bertol. (acc. 2)	Italy: Alpi Graie; WU: S&T 5125	AY274965	AY275051

[a]Sequences obtained from GenBank.
[b]*trnL* intron.

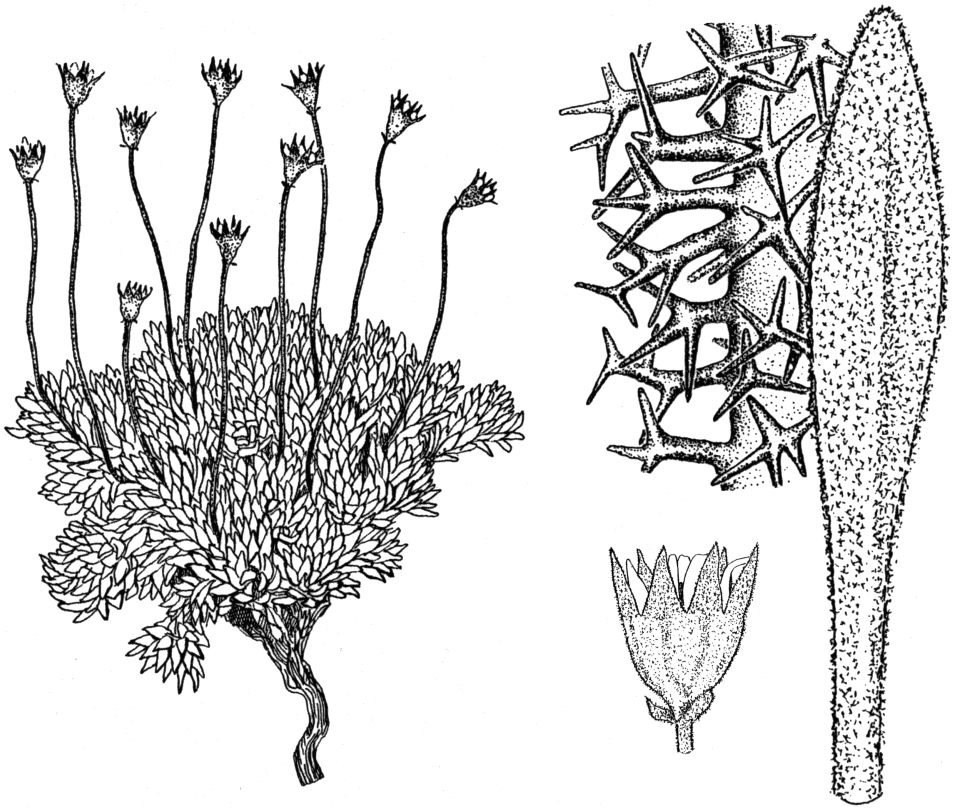

FIGURE. Leaves, scapes, and calyces of the Alasken endemic *Douglasia beringensis* are covered with irregularly branched hairs, a hair-type characteristic for */Aretia*. (Drawing not to scale. Reprinted with permission [originally published in *Novon* 4, p. 382].)

Downloaded from https://academic.oup.com/sysbio/article/53/6/856/1650198 by guest on 20 April 2024