

Li-Bing Zhang

(张丽兵)

Phylogeny, biogeography and systematics of
Soldanella L. and *Primula* L. sect. *Auricula* Duby
(Primulaceae)

based on molecular and morphological evidence



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Li-Bing Zhang
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Dekan:

Prof. Dr. J. Markl

1. Berichterstatter:

Prof. J. W. Kadereit, Ph.D.

2. Berichterstatter:

Prof. Dr. R. Claßen-Bockhoff

Tag der mündlichen Prüfung:

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SUMMARY

Phylogenetic relationships and biogeographical patterns of the two European high mountain endemics *Soldanella* and *Primula* sect. *Auricula* were inferred from the sequences of internal transcribed spacer region (ITS) of nrDNA and/or the intron sequences of the ribosomal protein L16 (*rpl16*) gene of cpDNA, and amplified fragment length polymorphism (AFLP) markers. The analyses show that both *Soldanella* and *Primula* sect. *Auricula* are monophyletic group of Asian origin, and that their ancestors were montane taxa. The molecular dating implies *Soldanella* diversified in the late Quaternary later than 0.62 mya, and *Primula* sect. *Auricula* originated in the Pliocene ca. 5.34 mya.

Further results and conclusions for *Soldanella* include: (1) intrageneric subdivisions of *Soldanella* were not supported by molecular data which instead suggest that "sect. *Soldanella*" is paraphyletic in relation to "sect. *Tubiflores*", implying that alpine species of the genus evolved from montane taxa; (2) *Soldanella alpina* is possibly derived through hybridization between *S. pusilla* and an unknown taxon from "sect. *Soldanella*"; (3) different patterns of geographic distribution of alpine and montane species resulted from the different cycles of range expansion and contraction they experienced during late Quaternary climatic changes; and (4) the differentiation of alpine species in allopatric regions during glacials and the secondary contact of East European montane taxa at lower elevations were hypothesized to be responsible for the higher genetic divergence between alpine species than among East European montane taxa.

Further results and conclusions for *Primula* sect. *Auricula* include: (1) species of the section were resolved into two well-supported clades of eastern and western distribution. This subdivision was possibly caused by the cool climate near the end of the Tertiary and the late up-lift of the Alps; (2) *Primula allionii*, *P. latifolia*/*P. marginata* and *P. palinuri* were identified as possible early-diverging lineages of the western clade and *P. deorum* as an early-diverging lineage of the eastern clade; (3) cladogenesis in sect. *Auricula* was a pure-birth process; (4) the eastern and western clades show far-reaching temporal congruency in inter- and intraspecific diversification, implying that the two clades, irrespective of their different geographical distribution, and possibly also different species attributes, experienced the same external (e.g. geological or climatic) events.

The comparison of the biogeographical patterns of three co-distributional plant groups (*Soldanella*, *Primula* sect. *Auricula* and *Gentiana* sect. *Ciminalis*) shows no overall similarities. However, the speciation activity of all three groups took place almost exclusively in the Quaternary, suggesting that Quaternary climatic oscillations strongly affected plant evolution.

Based on extensive herbarium surveys, field observations and molecular evidence, *Soldanella* is divided into 16 species and four subspecies, and *Primula* sect. *Auricula* into two subsections, six series, 25 species and eight subspecies. Altogether 11 taxa were treated or described as new and ca. 237 names were synonymized, including many little-known and/or incorrectly synonymized names. Phylogenetic relationships in *Soldanella* and *Primula* sect. *Auricula*, respectively, are discussed based on morphological and molecular evidence.

ZUSAMMENFASSUNG

Phylogenetische Verhältnisse und biogeographische Muster der beiden europäischen Hochgebirgsendemiten *Soldanella* und *Primula* Sekt. *Auricula* wurden mit Hilfe von Sequenzen der Internal-Transcribed-Spacer Region (ITS) der nrDNA und/oder den Intron-Sequenzen des ribosomalen Proteins L16 (*rpl16*) Gens der cpDNA und Amplified-Fragment-Length-Polymorphism (AFLP) Markern analysiert. Die Untersuchungen zeigen, dass sowohl *Soldanella* als auch *Primula* Sekt. *Auricula* monophyletische Gruppen asiatischen Ursprungs sind und dass ihre Vorfahren montane Taxa waren. Die molekulare Datierung deutet an, dass sich *Soldanella* im späten Quartär differenziert hat (< 0,62 Millionen Jahre) und dass *Primula* Sekt. *Auricula* im Pliozän entstanden und ca. 5,34 Millionen Jahre alt ist.

Weitere Resultate und Schlußfolgerungen aus den molekularen Untersuchungen für *Soldanella*: (1) Die intragenerische Unterteilung von *Soldanella* in zwei Sektionen (*Soldanella*, *Tubiflores*) wird nicht durch die molekularen Daten gestützt. Diese implizieren stattdessen, dass Sekt. *Soldanella* paraphyletisch in Bezug auf Sekt. *Tubiflores* ist. Damit sind die alpinen Taxa der Gattung aus den montanen Taxa entstanden; (2) *Soldanella alpina* evolvierte wahrscheinlich durch Hybridisierung zwischen *S. pusilla* und einem unbekannten Taxon aus der Sekt. *Soldanella*; (3) Die unterschiedlichen Muster der geographischen Verbreitung der alpinen und montanen Taxa entstanden durch sich wiederholende Phasen von Areal-Expansionen und -Kontraktionen in Folge der spätquartären Klimaschwankungen; (4) Die allopatrische Differenzierung der alpinen Arten und der Sekundär-Kontakt der osteuropäischen montanen Taxa in niedrigeren Höhenlagen während der Glazialzeiten wurden postuliert, da die genetische Divergenz zwischen den alpinen Arten größer ist als zwischen den osteuropäischen montanen Taxa.

Weitere Resultate und Schlußfolgerungen aus den molekularen Analysen für *Primula* Sekt. *Auricula*: (1) Die Arten der *Primula* Sekt. *Auricula* wurden in zwei gut unterstützte Clades mit östlicher und westlicher Verbreitung gruppiert. Diese Unterteilung wurde wahrscheinlich durch das kühle Klima nahe dem Ende des Tertiärs sowie durch die späte Hebung der Alpen verursacht; (2) *Primula allionii*, *P. marginata*/*P. latifolia* und *P. palinuri* wurden als mögliche frühe Entwicklungslinien des westlichen Clades und *P. deorum* als frühe Linie des östlichen Clades identifiziert; (3) Die Kladogenese in Sekt. *Auricula* folgt einem reinen Geburtsmodell ohne Extinktion; (4) Die inter- und intraspezifische Differenzierung des östlichen bzw. westlichen Clades zeigt eine weitreichende zeitliche Kongruenz. Diese impliziert, dass die beiden Clades, unabhängig von ihrer unterschiedlichen geographischen Verbreitung und unabhängig von sich unterscheidenden artspezifischen Eigenschaften, dieselben externen (z.B. geologischen und/oder klimatischen) Ereignisse erfuhren.

Der Vergleich der biogeographischen Muster von drei Gruppen (*Soldanella*, *Primula* Sekt. *Auricula* und *Gentiana* Sekt. *Ciminalis*), die eine ähnliche Verbreitung in den europäischen Hochgebirgen haben, zeigt insgesamt kaum Gemeinsamkeiten. Jedoch fanden Speziationseignisse in allen drei Gruppen fast ausschließlich im Quartär statt. Das deutet darauf hin, dass die quartären Klimaschwankungen die pflanzliche Entwicklungsgeschichte stark beeinflussten.

Basierend auf umfangreichen Herbaruntersuchungen, Feldbeobachtungen und molekularen Analysen wurde die Gattung *Soldanella* in 16 Arten und vier Unterarten und die Sektion *Auricula* der Gattung *Primula* in zwei Subsektionen, sechs Serien, 25 Arten und acht Unterarten eingeteilt. Insgesamt wurden 11 Taxa neu kombiniert oder beschrieben sowie ca. 237 Namen synonymisiert, einschließlich vieler Namen, die wenig bekannt waren und/oder bisher falsch synonymisiert worden waren. Die phylogenetischen Verhältnisse innerhalb von *Soldanella* und *Primula* Sekt. *Auricula* wurden auf der Basis morphologischer und molekularer Merkmale diskutiert.

1. INTRODUCTION

1.1 Quaternary biogeography

Tracing and explaining temporal changes in rates of speciation and extinction represents major challenges in evolutionary biology (Zink & Slowinski, 1995). The Quaternary offers a particular challenge because as the most recent geological period it experienced marked climatic oscillations, which led to extinction and/or speciation, adaptive evolution or changes of geographical distribution of species (Bennett, 1997).

In animals, it is generally agreed that the Quaternary glaciation resulted in very few complete extinctions, in comparison with the K-T (Cretaceous-Tertiary) mass extinctions of e.g. ammonites and dinosaurs (Raup, 1994). In terms of speciation, clear evidence has shown decreased avian diversification during the Quaternary (Zink & Slowinski, 1995), whereas accelerated speciation has been hypothesized for the vertebrates in North America during the Pleistocene epoch (Bermingham et al., 1992).

In plants, it has also been believed that only a small proportion of plant species went extinct during the Quaternary climatic changes (Bennett, 1997). However, it has been controversial whether little or no speciation occurred in this period (Bennett, 1997) or plant taxa diversified in the Quaternary (Hungerer & Kadereit, 1998; Zhang et al., 2001). As a central question of modern biogeography (Cracraft, 1986; Riddle, 1996) it remains unclear whether the reaction of plants to similar but temporally consecutive conditions caused by the Quaternary glaciation was similar in terms of adaptive evolution, speciation and/or extinction, and thus predictable (Carlquist, 1974; Givnish, 1988, 1997), or whether evolutionary change is dominated by historical contingency (Gould, 1989).

Inferences about the impact of the Quaternary climatic changes on the geographical distribution and evolution of plant taxa have traditionally been based only on the fossil record (e.g. Harland et al., 1967; Godwin, 1975; Huntley & Birks, 1983; Benton, 1993; Lang, 1994; Bennett, 1997) and the extant geographical distribution of plant taxa and their variation in chromosome number (e.g., Pampanini, 1903; Brockmann-Jerosch & Brockmann-Jerosch, 1926; Merxmüller, 1952–1954; Ehrendorfer, 1958; Favarger, 1964; Stehlik, 2000).

The application of molecular approaches allows to survey the central question of modern biogeography further by reconstructing phylogenies at various taxonomic levels and across a wide range of geographical scales (Avice, 2000). Although a real phylogeny would require perfect fossil evidence, many properties of the past, present and even the future of a clade leave their signatures in its molecular phylogeny, even if that phylogeny is based solely on

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information from extant organisms (Hey, 1992; Nee et al., 1992, 1994a, b; Harvey et al., 1994; Zink & Slowinski, 1995; Pybus & Harvey, 2000).

In recent years many molecular studies have provided insights into Quaternary plant distribution and evolution in the Northern Hemisphere (reviewed by Hewitt, 1996, 1999, 2000, 2001; Riddle, 1996; Comes & Kadereit, 1998; Taberlet et al., 1998). The majority of published studies were concerned with the genetic variation and DNA patterns at the intraspecific level, or took a "phylogeographical" perspective to detect glacial refugia and postglacial migration routes (Abbott et al., 1995; Konnert & Bergmann, 1995; Brochmann et al., 1996; Demesure et al., 1996; Dumolin-Lapègue et al., 1997; Gabrielsen et al., 1997; Le Corre et al., 1997; Ferris et al., 1998; Tollefsrud et al., 1998; Gugerli et al., 1999, 2001; Abbott et al., 2000; Comps et al., 2001; Mátyás & Sperisen, 2001; Stehlik et al., 2001; Bittkau & Kadereit, 2002; Kropf et al., 2002). In contrast, few studies have dealt with both molecular phylogenetic relationships and range formation at supraspecific level and a broad coverage of geographical areas [e.g., *Gentiana* L. sect. *Ciminalis* (Adans.) Dumort., Hungerer & Kadereit, 1998; *Saxifraga* L. sect. *Ligulatae* Haworth, Conti et al., 1999]. In addition, most of these studies related their findings explicitly or implicitly to the last and best-documented Quaternary glacial period without, however, using a molecular clock approach to absolutely date the genetic divergences found. Only in a few cases (e.g. Hungerer & Kadereit, 1998) a molecular clock approach was used mainly to document that the phylogenetic diversification of the taxa investigated took place in the Quaternary. None of these studies, however, made an effort to trace rates of speciation over time.

This dissertation will investigate the evolution and Quaternary history of the two European high mountain endemics *Soldanella* L. and *Primula* L. sect. *Auricula* (Primulaceae) by reconstructing molecular phylogenies. The geographical range of both these two plant groups covers the whole south and central European high mountains, i.e. the Pyrenees (and adjacent Cantabrian Mountains), Alps, Apennines, Balkans and Carpathians (including the Tatra Mountains) and Sudety Mountains. The molecular markers used here are nuclear ribosomal DNA (nrDNA) ITS (internal transcribed spacers; Baldwin, 1992) sequences, intron sequences of the nuclear ribosomal protein L16 gene (*rp16*-intron, Jordan et al., 1996) and highly resolving fingerprint markers AFLPs (amplified fragment length polymorphisms; Vos et al., 1995). The molecular dating technique will be used to date the divergence of the major clades found in the molecular analysis. The questions addressed in this dissertation include: the monophyly, geographical origin, ancestral ecology, geological age, origin of alpine taxa, diversification history of *Soldanella* and *Primula* sect. *Auricula*. For *Primula* sect. *Auricula*, the temporal course of speciation and intraspecific diversification of the various subclades identified will be traced and compared with each other. After this, results of studies of *Soldanella* and *Primula* sect. *Auricula*, together with the previously published study of *Gentiana* sect. *Ciminalis* (Hungerer & Kadereit, 1998) will be compared with each other, because these three plant groups have similar extant geographical ranges. Through such comparison it is expected to learn whether these plants reacted similarly to the Quaternary climatic oscillations and what a role the climatic fluctuations played in the evolution of these plants in European high mountains.

This dissertation further includes a revision of *Soldanella* and the systematics of *Primula* sect. *Auricula*. Over 6300 and ca. 1100 herbarium specimens of *Soldanella* and *Primula* sect. *Auricula*, respectively, were morphologically investigated. The systematic implications of the molecular phylogenetic results obtained will also be used to illustrate phylogenetic relationships among supraspecific and intraspecific taxa and in some cases to delimitate these taxa.

1.2 Study group I — *Soldanella* L.

Species of *Soldanella* are easily recognized by their perennial habit, roundish, evergreen leaves, and violet or, rarely, white flowers. These flowers may have either campanulate (sect. *Tubiflores*) or funnel-shaped (sect. *Soldanella*) corollas, all with lacinate (incised) lobes. Individuals are primarily insect-pollinated (bees, bumble bees, butterflies) and outcrossing (Lüdi, 1927); there is no firm experimental evidence of the self-fertilization in *S. alpina* suggested by several authors (Kerner & Oliver, 1904; Kugler, 1970). The majority of species have a reported chromosome number of $2n = 40$ (Kress, 1969a, 1984b; Peev, 1977), except *S. montana* and *S. villosa* ($2n = 38$) and *S. chrysosticta* ($2n = 38$ or 40).

Table 1. Morphological characters used to distinguish the two sections of *Soldanella*. See also Figs. 1 and 2.

Characters	sect. <i>Soldanella</i>	sect. <i>Tubiflores</i>
Height	13 – 35 cm	<10 cm
Leaf size	>1.5 × 2.1 cm	<1.3 × 1.8 cm
Leaf margin	usually repand	usually entire
No. of flowers per scape	2 – 6	usually 1
Throat scales of corolla	usually present	absent or very small
Corolla shape	funnel-shaped	campanulate
Corolla lobes	long	short
Connective appendages	caudate	acuminate
Glandular hairs on filaments/connectives	present or absent	absent
Style	long	short
No. of capsule teeth	(6–) 10 (–14)	5 (–7)

Based on the most recent taxonomic treatment (Zhang & Kadereit, 2002) the genus is composed of 16 species, four of which contain two or three subspecies. Whereas the two sections are morphologically well-defined (Borbás, 1901; Vierhapper, 1904a, 1926; Knuth, 1905; Honcik, 1963; Pawłowska, 1972; Meyer, 1985; see Figs. 1 and 2, and Table 1), there are only minute differences among taxa at the intrasectional level, including corolla lobe size, leaf dimensions, and glandular hair structure on scapes and stipes (see below). Both species of sect. *Tubiflores* (*S. minima*, *S. pusilla*) grow primarily in high-elevation alpine habitats, where they are largely confined to snow pockets, grassy slopes, and wet meadows above the timberline (Table 2). In contrast, species of sect. *Soldanella* mainly inhabit montane coniferous and/or deciduous forests, and are most common at lower altitudes, at least in the northern part of the genus' range; exceptions are *S. alpina* and *S. rugosa*, both of which have an alpine ecology. Most species within the genus have a broad edaphic tolerance, growing on both base

Table 2. List of *Saxifraga* taxa, together with their simplified geographic distributions, habitat descriptions, substrate preferences, altitudinal distribution, and ecological classification (A = alpine; M = montane).

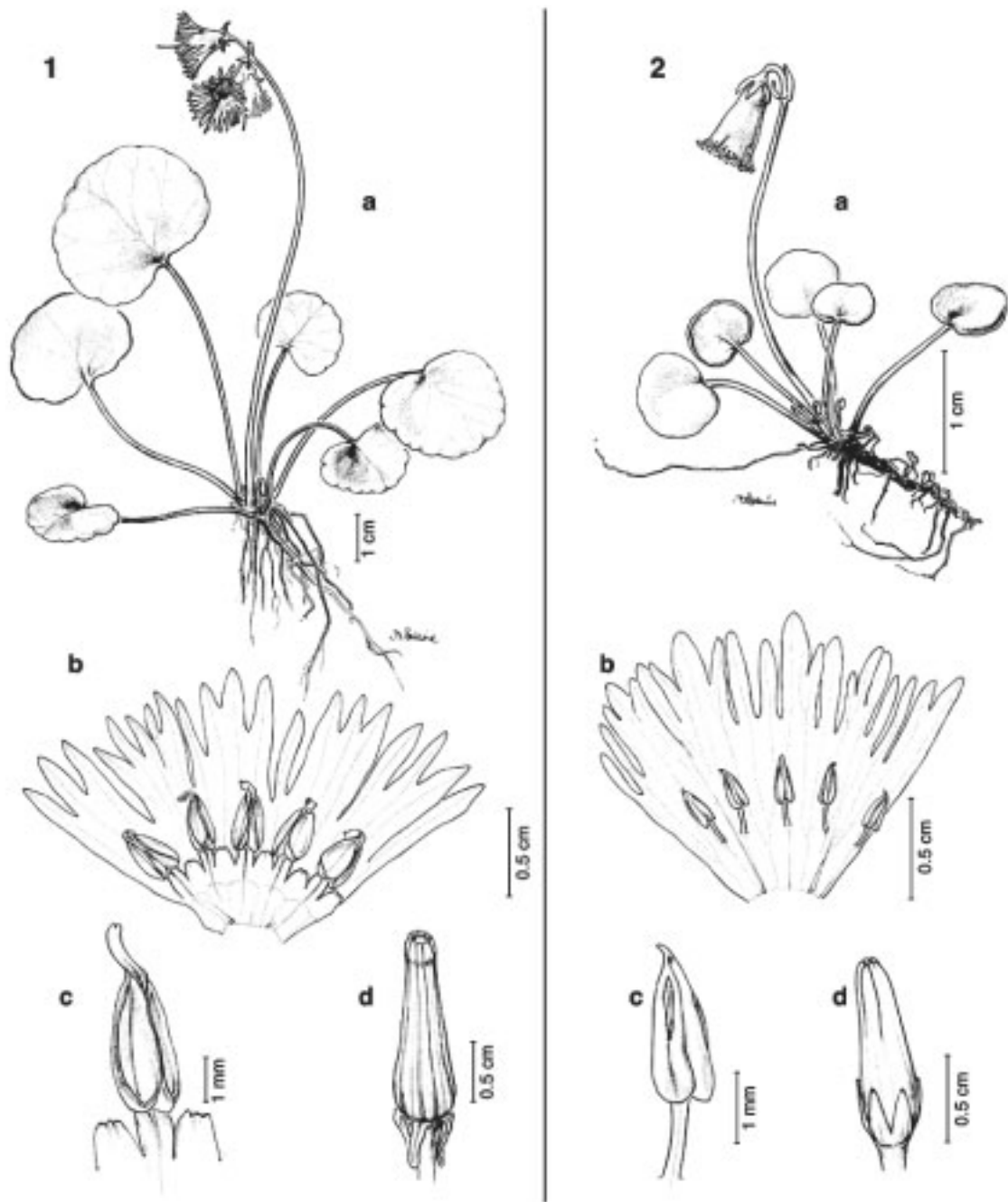
<i>Saxifraga</i> taxon ^a	Distribution ^b	Habitat descriptions ^c	Substrate ^d	Altitude (m) ^e	Ecology
*sect. <i>Saxifraga</i>					
<i>S. alpestris</i> ssp. <i>alpestris</i>	Alps, Pyrenees, Apennines, W Balkans	Grassland, snowpockets; sometimes <i>Abies</i> , <i>Alnus</i> , <i>Caragana</i>	ll, sch, sl, sl	(480) 800–2300 (2800)	A
ssp. <i>caraganae</i> Kness	Carpathians	Alpine pastures; forest margins	ll, sl	1150–1800	A
<i>S. alpestris</i> ssp. <i>zhangii</i>	E Carpathians	<i>Abies</i> , <i>Alnus</i> forests; forest margins	ll, sl	900–1450	M
<i>S. alpestris</i> ssp. <i>caraganae</i>	Carpathians (S Italy)	Wetlands; <i>Abies</i> , <i>Fagus</i> , <i>Alnus</i> forests	acidic soils	1400–1900	M
<i>S. alpestris</i> ssp. <i>alpestris</i>	Tatra	<i>Abies</i> , <i>Alnus</i> , <i>Fagus</i> , <i>Alnus</i> forests	ll, sl	(500) 800–2000	M
<i>S. alpestris</i> ssp. <i>caraganae</i>	S Balkans	<i>Abies</i> , <i>Fagus</i> forests; shrubs; sometimes grassland	sch, sl	1200–2300	M
<i>S. alpestris</i> ssp. <i>caraganae</i>	S Carpathians	<i>Abies</i> , <i>Fagus</i> , mixed forests	ll, sl	1000–1800	M
<i>S. alpestris</i> ssp. <i>alpestris</i>	E Alps, SW Carpathians	<i>Abies</i> , <i>Alnus</i> , <i>Fagus</i> forests; <i>Rhododendron</i> thickets	gn, ll, sl	1200–1800 (2000)	M
<i>S. alpestris</i> ssp. <i>caraganae</i> Krasensky	Tatra, E Carpathians	<i>Abies</i> , <i>Fagus</i> , mixed forests	ll, sl	1000–2000	M
<i>S. alpestris</i> ssp. <i>alpestris</i> Willd.	NE Alps	<i>Abies</i> , <i>Alnus</i> , <i>Alnus</i> , <i>Fagus</i> forests; bushes	gn, ll, sl	300–1000 (1600)	M
<i>S. alpestris</i> ssp. <i>zhangii</i>	C Transylvania	<i>Abies</i> forests	sl	900–1200	M
<i>S. alpestris</i> ssp. <i>zhangii</i>	S Balkan Peninsula	<i>Fagus</i> , <i>Alnus</i> forests; bushes	ll, sa, sch, se	1600–2400	M
<i>S. alpestris</i> ssp. <i>zhangii</i> Hauskn.	Rhodope Mts. (Bulgaria/Greece)	<i>Abies</i> , <i>Alnus</i> , <i>Alnus</i> , <i>Fagus</i> forests	sch, sl	1200–1700	M
<i>S. alpestris</i> ssp. <i>zhangii</i> F. K. Meyer	E Carpathians	Damp rocky meadows; snowpockets; rocky places	?	1600–2300	A
<i>S. alpestris</i> ssp. <i>zhangii</i>	Pyrenees, Carpathian Mts.	<i>Caragana</i> , <i>Rhododendron</i> forests	gr, sl, base-rich soils	(70) 400–600	M
*sect. <i>Taxifraga</i>					
<i>S. minima</i> Hoppe ssp. <i>minima</i>	S & W Alps	Alpine grassland; snowpockets	ll	1400–2800	A
ssp. <i>alpestris</i> (Meth.) Lüd.	NE Alps	Grassy slopes; snowpockets; margins of <i>Abies</i> forests	ll	(530) 1000–2100	A
ssp. <i>caraganae</i> Kness	Apennines (Matera)	Rocky grassland; snowpockets	ll	2000–2400	A
<i>S. minima</i> ssp. <i>minima</i>	S Carpathians, Pirin Mts.	Grassland, snowpockets; sometimes <i>Abies</i> forests	ll, sl	1800–2700	A
ssp. <i>alpestris</i> (F. K. Meyer) J. Chrtek	(Bulgaria) Alps	Wet meadows, snowpockets; sometimes <i>Abies</i> , <i>Caragana</i> , <i>Alnus</i> forests	ll, sch, sl, sl	(530) 1200–2500	A

^a Classification of *Saxifraga* according to Zhang & Kadereit (in press).

^b Data are from Meyer (1985) and extensive herbarium surveys based on over 6300 sheets.

^c Data are from herbarium surveys supplemented by field observations (Zhang & Kadereit, in press).

^d Abbreviations: gn = gneiss, gr = granite, ll = limestone (or dolomite), sa = sandstone, sch = base-rich schists, se = serpentine, sl = silicate rocks, sl = slate.



Figs. 1–2. Morphological characters distinguishing *Soldanella* (Fig. 1) sect. *Soldanella* (here: *S. major*) and (Fig. 2) sect. *Tubiflores* (here: *S. minima*). Section *Soldanella* is characterized by relatively large (13–35 cm) plants with several flowers and a long style (Fig. 1a); long corolla lobes and presence of corolla scales (Fig. 1b); anthers with caudate connective appendages (Fig. 1c); and 10-toothed capsules (Fig. 1d). Section *Tubiflores* has relatively small (<10 cm) plants with only one flower and a short style (Fig. 2a); short corolla lobes and absence of corolla scales (Fig. 2b); acuminate connective appendages (Fig. 2c); and five-toothed capsules (Fig. 2d). See also Table 1.

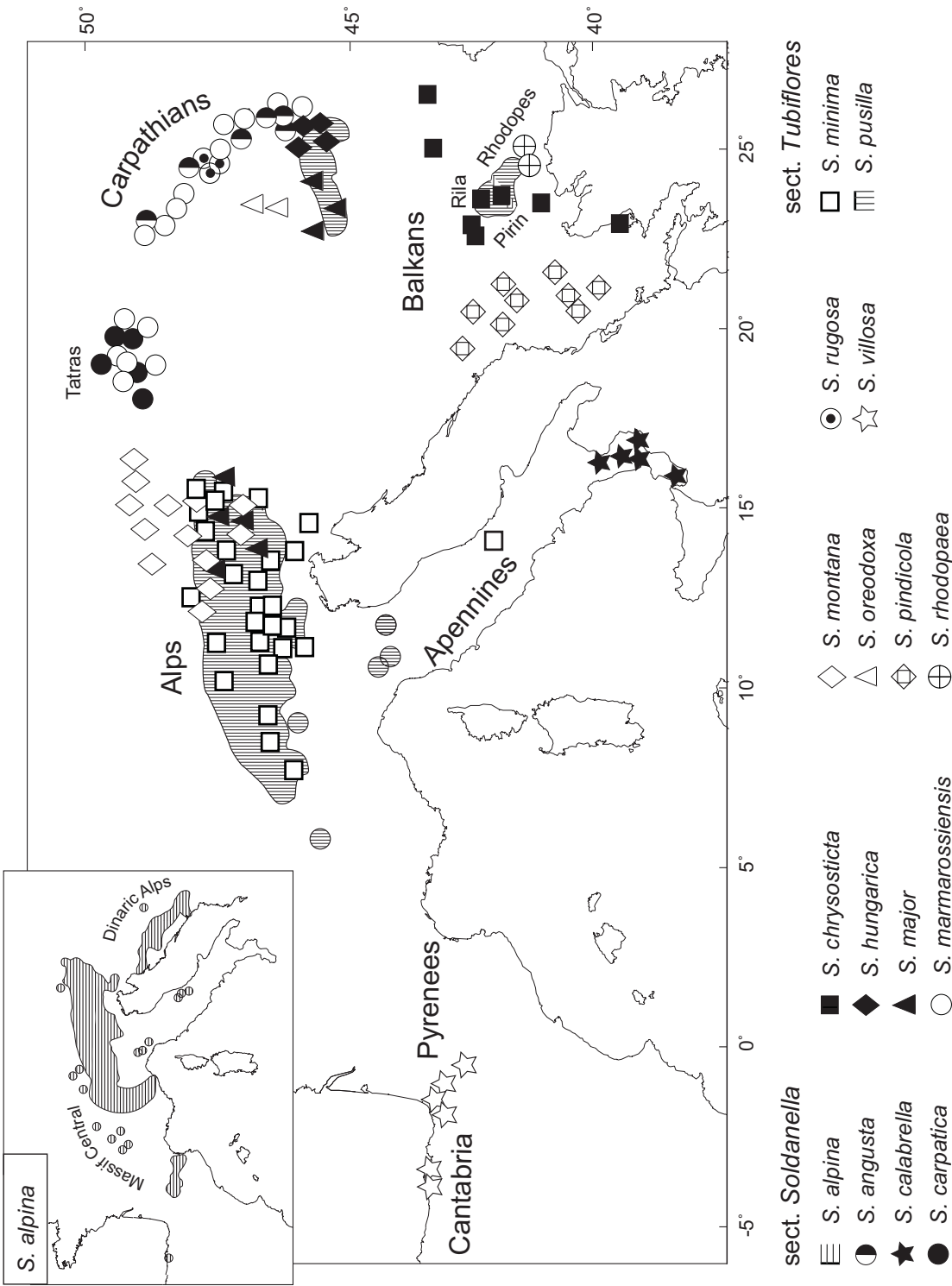


Fig. 3. Distribution of the 16 species of *Soldanella* based on Meyer (1985) and extensive herbarium surveys of over 6300 sheets. Symbols indicate narrowly circumscribed regional occurrences. The overall range of *S. alpina* is shown in the inset. See also Table 2 for the approximate distribution ranges of species and subspecies.

rich and base-poor soils derived from a variety of parent materials (Table 2). Only *S. minima* is entirely restricted to limestone (or dolomite), whereas several species from the Balkans (*S. chrysosticta*, *S. oreodoxa*, *S. rhodopaea*) and southern Italy (*S. calabrella*) exclusively grow on acidic (mostly granitic) soils.

The type species, *S. alpina*, is widely distributed throughout the European Alpine system, including the French Massif Central and the Dinaric Alps but not the Tatra/Carpathians and the Balkan Peninsula (Fig. 3). All other species are less widespread or locally restricted. In the Alps, *S. minima* and *S. pusilla* (sect. *Tubiflores*) are broadly sympatric, whereas *S. montana* and *S. major* (sect. *Soldanella*) are largely parapatric; in this region there is little overlap between members of different sections due to altitudinal displacement. The range distributions of the remaining taxa of sect. *Soldanella* overlap to varying degrees. For example, *S. villosa* and *S. calabrella*, *S. chrysosticta*, *S. pindicola*, and *S. rhodopaea* are restricted to the western and southeastern periphery of the genus' range, respectively. They show little or no overlap with other taxa. In contrast, *S. angusta*, *S. carpatica*, *S. hungarica*, *S. marmarossiensis*, *S. oreodoxa*, and *S. rugosa* are all restricted to the Tatra/Carpathians and quite often overlap with each other. Three species (*S. minima*, *S. pusilla*, *S. major*) whose distribution is largely limited to the Alps have disjunct populations in the South Carpathians (*S. major*, *S. pusilla*), the Balkan mountains (*S. pusilla*), and central Italy (*S. minima*).

1.2 Study group II — *Primula* L. sect. *Auricula* Duby

As another plant group studied in this doctoral work, *Primula* L. sect. *Auricula* Duby is one of the 37 sections traditionally recognized in the genus *Primula* (Richards, 1993) and one of three [or four, see Mast et al. (2001)] sections of subg. *Auriculastrum*. This section is characterized by having smooth and fleshy or coriaceous leaves with involute vernation, bracts which are shorter than the pedicels, obtuse calyx teeth, and by having colporate pollen grains. Like most members of *Primula*, all species of sect. *Auricula* are heterostylous and self-incompatible (Richards, 1993; Kress, 1998). They are hexaploid or hypohexaploid or hexaploid/hypohexaploid-derived based on $x = 11$, and chromosome numbers mostly vary between $2n = 62$ and $2n = 66$. Higher chromosome numbers of $2n = 126$ and $2n = 198$ are limited to *P. marginata* and *P. clusiana*, respectively (Kress, 1963, 1989).

Section *Auricula* has been divided into seven subsections (Pax, 1905; Smith & Fletcher, 1949; Fenderson, 1986; Halda, 1992; Richards, 1993). These are subsections *Auricula*, *Arthritica*, *Brevibracteae*, *Chamaecallis*, *Cyanopsis*, *Erythrodrosom* and *Rhopsidium* (Table 3). Following the most recent systematic treatment of the section by Zhang & Kadereit (in prep.), it contains 25 species with eight subspecies (Table 4). The most important characters for the distinction of species in the section include the anatomy of glandular hairs, leaf texture, leaf form, and the presence or absence of a farinose flavonoid exudate (Blasdale, 1948) on the leaves (Zhang & Kadereit, in prep.).

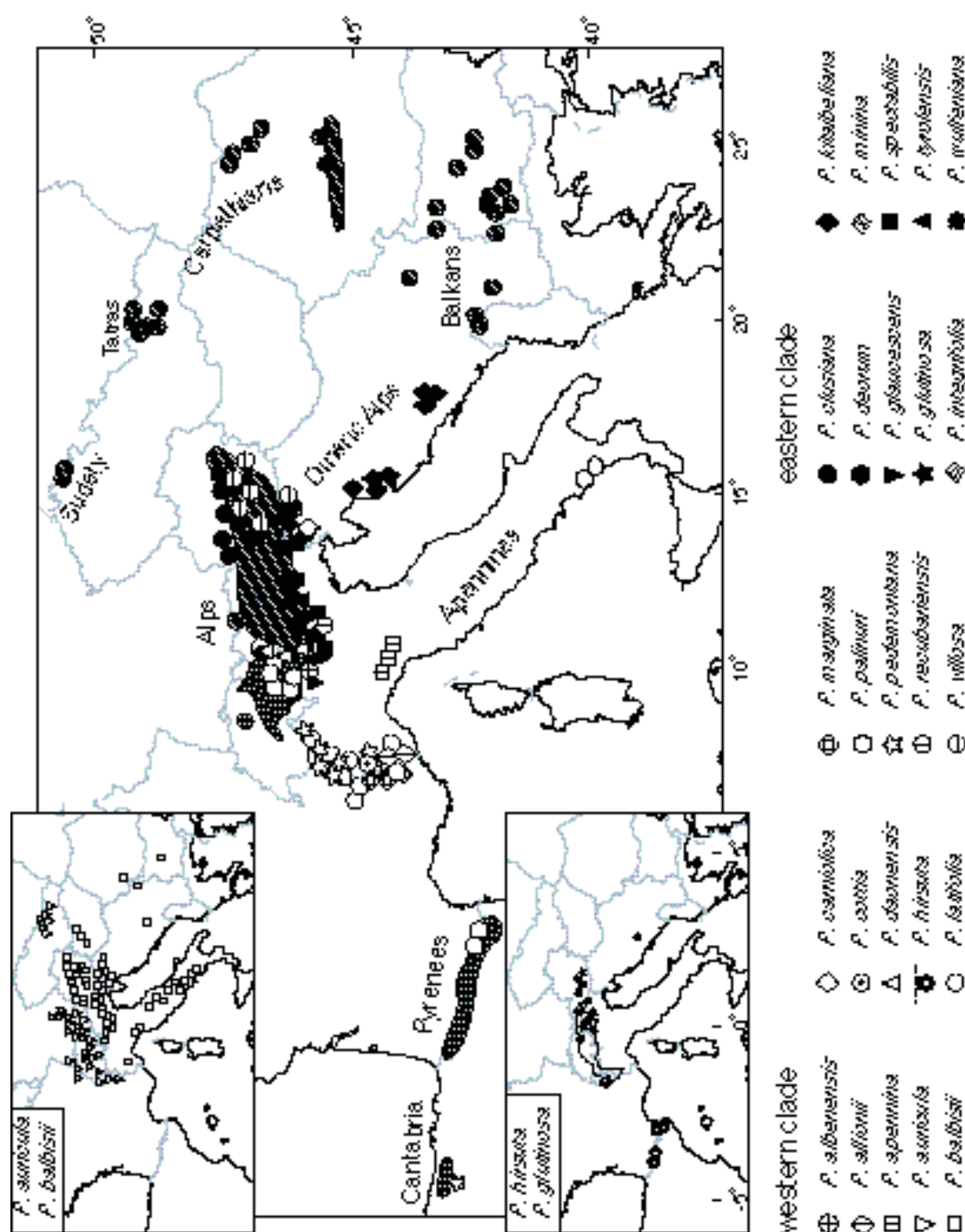


Fig. 4. Distribution of the 25 species of *Psammisia* sect. *Auricula* based on Fenderson (1986) and Richards (1993) and extensive herbarium surveys of about 1100 sheets. Symbols indicate narrowly circumscribed regional occurrences. The overall range of *P. auricula*, *P. balbisii* and *P. glaucescens*/*P. hiszuta* is shown in the two insets, respectively. The dark symbols refer to the taxa of the eastern clade resolved in the ITS phylogeny (Fig. 8), and the light symbols refer to the taxa of the western clade.

Table 3. Main morphological, ecological and karyological differences among seven traditional subsections of *Primula* sect. *Auriucla*. Intrasectional classification following Pax (1905) and Smith & Fletcher (1949).

Subsection	<i>Arthritica</i>	<i>Auricula</i>	<i>Brevibracteae</i>	<i>Chamaecallis</i>	<i>Cyanopsis</i>	<i>Erythrodrosom</i>	<i>Rhopsidium</i>
Habitus	big	very big	very big	very small	small	small to big	small
Bract form	foliose	bracteose, foliose	bracteose	foliose	foliose	bracteose	foliose, bracteose
Calyx size	long	short, long	short	long	long	short	long, short
Farina	absent	present	present	absent	absent	absent	absent
Flower colour	pink, red, rose	yellow	blue, pink, purple	pink	blue, purple	pink, red	rose to violet
Glandular hairs	absent, present	present	present	absent	absent	present	present
Leaf persistence	persistent	persistent	non-persistent	persistent	persistent	persistent	persistent, non-persistent
Leaf margin	entire	toothed, entire	toothed	toothed	toothed, entire	toothed	more or less entire
Leaf texture	leathery	leathery	herbaceous	leathery	leathery	herbaceous	herbaceous
Ecology	montane	montane, alpine	montane, (alpine)	alpine	alpine	alpine (montane)	alpine, montane
Substrate	limestone	limestone	silicate, limestone	silicate	silicate	silicate	silicate, limestone
Chromosome number	66, 198	62, 66	62, 64, 126	66	66	62	66

Section *Auricula* is one of only few endemics of the European alpine system with a comparatively large number of species (Ozenda, 1995). It is distributed throughout the Cantabrian Mountains, Pyrenees, Alps, Apennines, Carpathians (including Tatra Mountains), Sudety Mountains, and the Balkans, but the highest specific diversity is clearly found in the Alps (Fig. 4). Here, altogether 21 of the 25 species occur, and 12 are endemic. Species vary markedly in the size of their geographical ranges. Whereas nine species of the section are relatively widely distributed and geographically disjunct (*P. auricula*, *P. balbisii*, *P. glutinosa*, *P. hirsuta*, *P. integrifolia*, *P. latifolia*, *P. minima*, *P. pedemontana* and *P. wulfeniana*), eight have a locally restricted distribution (*P. albenensis*, *P. allionii*, *P. apennina*, *P. carniolica*, *P. cottia*, *P. deorum*, *P. palinuri* and *P. recubariensis*). The remaining eight species are of regional distribution (Fig. 4).

The species of sect. *Auricula* have rather strict edaphic requirements and most of them grow only in either basic or acid substrates. All species of subsect. *Arthritica*, most species of subsects. *Brevibracteae* and *Rhopsidium* and one species of subsect. *Auricula* require basic soils, and all species of subsects. *Chamaecallis*, *Cyanopsis* and *Erythrodrosom*, and one species each of subsects. *Auricula*, *Brevibracteae* and *Rhopsidium* grow only in acid soils (Table 4). The majority of species are saxicolous, growing directly on calcareous or siliceous rocks, in rock crevices or in stony pastures, and only *P. deorum*, *P. integrifolia* and *P. latifolia* are grassland taxa (Table 4). Many species of the section have a relatively broad altitudinal range and grow in montane to alpine habitats between (50–) 300–3000 m altitude. This applies particularly to members of subsects. *Auricula*, *Brevibracteae* and *Rhopsidium*. In contrast, the species of subsects. *Chamaecallis*, *Cyanopsis* and *Erythrodrosom* occur mainly at higher altitudes ranging from (1000–)1400–3000 m (Table 4).

In the analysis of the origin, ancestral ecology and history of European high mountain plants, sect. *Auricula* has always been of great interest to systematists and biogeographers. Although

Table 4. List of *Primula* sect. *Auricula* taxa, together with their simplified geographic distributions, habitat description and altitudinal distribution.

Taxon ^a	Distribution ^b	Habitat ^b	Soil (substrate) ^b	Altitude (m) ^b	Ecology ^b
subsect. <i>Auricula</i>					
<i>P. auricula</i> L.	N Alps, Jura Mts., Tatras	rocks or sometimes grassland	basic (limestone etc.)	250-2900	montane, alpina
<i>P. balisii</i> Lehm.	S Alps, Apennines, Hungary, Balkans, SW Carpathians	rocks or sometimes grassland	basic (limestone etc.)	250-2900	montane, alpina
<i>P. palinuri</i> Petagna	Calabria (Italy)	rocky slopes	acid (sandstone)	50-300	seaside
subsect. <i>Arthrica</i>					
<i>P. clusiana</i> Tausch.	NE Alps (Austria, Germany)	grassy slopes, pastures, scree, crevices	basic (limestone)	(600-) 1700-2500	alpine
<i>P. glaucescens</i> Moretti	S Alps (N Italy)	rocks, moist & shady forests (<i>Picea</i>)	basic (limestone)	450-2400	montane-alpine
<i>P. spectabilis</i> Tratt.	S Alps (N Italy)	rocks, grasslands, rocky ridges	basic (limestone)	500-2500	montane-alpine
<i>P. wulfeniana</i> Schott	SE Alps (Gallia & Karawanken Alps, NE Italy), S Carpathians	grassy slopes, scree, rocks	basic (limestone)	1700-2100	alpine
subsect. <i>Brevibracteae</i>					
<i>P. albanensis</i> Banfi et Ferlinghetti	S Alps (N Italy)	stones, crevices	basic (limestone)	1150-2000	montane-alpine
<i>P. carnioolica</i> Jacq.	Julian Alps	pastures, forests, shady rocks	basic (limestone)	500-1100	montane
<i>P. latifolia</i> Lapeyr.	S & W/C Alps, E Pyrenees	pastures, damp ledges, forests (<i>Picea</i>)	acid (granite), seldom basic	650-3050	montane-alpine
<i>P. marginata</i> Curtis	SW Alps (Maritime & Cottian Alps) (France & Italy)	grassy & stony slopes, cliffs	basic (limestone), seldom acid (slate, granite)	(500-) 1000-3000	montane-alpine
<i>P. recubariensis</i> Posser et Scortegagna	SE Carega Massif, N Italy (among Trento, Vicenza and Verona)	N facing and shady cliffs	basic (triassic dolomite)	1400-2030	montane-subalpine
subsect. <i>Chamaecallis</i>					
<i>P. mirina</i> L.	E Alps, Pirin/Rila Mts., S Carpathians, Tatras, Sudety Mts.	pastures	acid (granite, schist)	1200-3000	alpine
subsect. <i>Cyanopsis</i>					
<i>P. deorum</i> Velen.	Rila Mts.	streamside, very wet boggy ground, often growing in running water, bare peats etc.	acid area	1900-2900	alpine
<i>P. glutinosa</i> Wulfen	Alps (Austria, Italy, Switzerland), Bosnia & Herzegovina	pastures, rock crevices, bushes	acid (granite, schist, slate, syenite sand)	1500-3100	alpine
subsect. <i>Erythrodorum</i>					
<i>P. apennina</i> Widmer	N Apennine Mts	ledges, earthy crevices	acid (sandstone)	1400-2000	montane-alpine
<i>P. cotia</i> Widmer	Cottian Alps (SE France, NW Italy)	stone crevices, pastures	acid (granite)	(400-) 1600-2000	alpine
<i>P. daonensis</i> (Leyb.) Leyb.	S Austria, N Italy, E Switzerland	rocks, stony pastures	acid (granite), rarely basic	1500-3000	alpine
<i>P. hirsuta</i> All.	C & W Alps, C Pyrenees	rocks, stony pastures	acid (granite, schist, limestone/slate border, slate, quartzporphyry)	(500-) 1400-2800	(montane-) alpine
<i>P. pedemontana</i> E. Thomas ex Gaudin	Cottian & Graian Alps (France & Italy)	rocks, moors	acid (granite)	1400-3000	alpine
<i>P. villosa</i> Wulfen	S Austria, NE Italy, Slovenia (Karawanken Alps)	stone crevices, pastures	acid (gneis, schist, slate, trachyt, granite), seldom basic	1400-2900	alpine
subsect. <i>Rhopsidium</i>					
<i>P. allionii</i> Lois.	Maritime Alps (France & Italy)	strictly saxicoline on moist, shady stone cliff faces	basal (limestone)	650-1900	montane-alpine
<i>P. integrifolia</i> L.	C Alps (Austria, N Italy, C & E Switzerland), Pyrenees, Cantabria	pastures (wet, peaty lime-free turf), seldom rocks	acid (granite, slate), seldom basic (calcareous slate)	1500-3000	alpine
<i>P. kitaibeliana</i> Schott	W Balkans (Croatia, Bosnia & Herzegovina)	stony pastures, stone crevices	basic (limestone)	350-2300	montane-alpine
<i>P. tyrolensis</i> Schott	SE Dolomites (Italy)	shady crevices	basic (limestone)	1000-2300	montane-alpine

^a Classification follows Pax (1905) and Smith & Fletcher (1949).^b Data are based on herbarium surveys, field observations and literature (e.g. Fenderson, 1986; Richards, 1993).