Evolution von Miniaturisierung in arktisch-alpinen Lebensräumen in *Petasites* Mill., *Endocellion* Turcz. ex Herder, *Homogyne* Cass. und *Tussilago* L. (Asteraceae) sowie *Soldanella* L. (Primulaceae)

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Inhaltsverzeichnis

Einleitung

In arktisch-alpinen Lebensräumen leben Pflanzen und Tiere unter wesentlich härteren Umweltbedingungen als im Tiefland der gemäßigten Breiten. Für gleichwarme Tiere wurden bereits im 19. Jahrhundert typische Anpassungen an die vorherrschenden harschen Klimabedingungen beschrieben (Bergmann, 1847; Allen, 1877). Obwohl für Pflanzen keine ähnlichen ökogeographischen Regeln formuliert wurden, ist die Miniaturisierung von Pflanzen in arktisch-alpinen Habitaten ein bekanntes Phänomen. So haben alpine Pflanzen durchschnittlich nur ein Zehntel der Blattfläche von Arten aus tieferen Lagen (Körner, 1999). Auch die vorherrschenden Wuchsformen arktisch-alpiner Floren sind durch Miniaturisierung gekennzeichnet. Typische Wuchsformen sind Polsterpflanzen, horstbildende Grasartige, niederliegende Zwergsträucher und mehrjährige, häufig rosettenbildende Kräuter (Johnson, 1969; Körner, 1999). Miniaturisierung kann als morphologische Anpassung an die ungünstigen Klimabedingungen in arktisch-alpinen Lebensräumen, v.a. niedrige Jahresmitteltemperaturen und kurze Vegetationsdauer, interpretiert werden (Billings und Mooney, 1968; Bliss, 1971; Johnson; 1969; Körner und Larcher, 1988; Körner, 1999). Durch Transplantationsexperimente konnte gezeigt werden, dass Miniaturisierung arktischer und alpiner Arten und entsprechender Ökotypen weitverbreiteter Arten genetisch fixiert ist (z.B. Turreson, 1925, 1930; Körner et al., 1989; Körner, 1999; Shinohara und Murakami, 2006). In der vorliegenden Dissertation wird in zwei Gruppen arktisch-alpiner bzw. alpiner Pflanzen die Evolution der Miniaturisierung untersucht.

Die erste untersuchte Gruppe umfasst *Petasites* Mill., *Endocellion* Turcz. ex Herder, *Homogyne* Cass. und *Tussilago* L. (Asteraceae). Die vier Gattungen bilden eine monophyletische Gruppe (Petasites-Clade; Pelser et al., 2007). Sie sind holarktisch verbreitet und sind rosettenbildende Stauden, die sich durch die Ausbildung langer Rhizome auch vegetativ vermehren können. Die Blätter von *Tussilago* sind 20-30 cm im Durchmesser (Hegi, 1929), und die Blätter der meisten *Petasites*-Arten haben eine ähnliche Größe. Der Durchmesser der Blattspreite kann bei *P. japonicus* subsp. *giganteus* (Sieb. et Zucc.) Maxim. sogar 1,5 m erreichen (Hind und Kay, 2006). In der Gattung sind *P. doerfleri* Hayek, *P. rubellus* (J.F. Gmel.) Toman und *P. fominii* Bords. die einzigen Arten mit Blättern, deren Durchmesser kleiner als 5 cm ist (Toman, 1972). Diese drei Arten sind alpin verbreitet, genauso wie die Vertreter der kleinblättrigen Gattung *Homogyne*, die in den Alpen und anderen europäischen Hochgebirgen vorkommen. *Endocellion* hat ebenfalls kleine, 1-7 cm lange Blätter; die Gattung ist im arktischen Asien verbreitet. Im ersten Teil der Arbeit wird anhand einer molekularen Phylogenie untersucht, wo, wann und wie oft Miniaturisierung im

Petasites-Clade entstanden ist. Zudem wird auf Grundlage der Ökologie der kleinwüchsigen Arten untersucht, welche Umweltfaktoren die Evolution von Miniaturisierung beeinflussen.

Der zweite Teil der Arbeit befasst sich tiefergehend mit einer der Gattungen aus dem Petasites-Clade. *Homogyne* ist eine in den Alpen endemische Gattung mit drei Arten, die sich in ihrer edaphischen Toleranz und somit auch in ihrer Verbreitung unterscheiden. *Homogyne alpina* Cass. wächst sowohl auf kalkigem als auch auf silikatischem Untergrund und ist eine in den gesamten Alpen und angrenzenden Gebirgen vorkommende Art. *Homogyne discolor* Cass. und *H. sylvestris* Cass. hingegen kommen nur auf kalkigem Untergrund vor und unterscheiden sich in ihrer Verbreitung von *H. alpina*. *Homogyne discolor* zeigt mit Vorkommen in den nordöstlichen und südöstlichen Kalkalpen eine disjunkte Verbreitung, *H. silvestris* findet man in den südöstlichen und Dinarischen Alpen. Die im ersten Teil der Arbeit erstellte Phylogenie des Petasites-Clades hat gezeigt, dass die ökologisch variable *H. alpina* Schwester zu dem edaphisch spezialisierten Artenpaar *H. discolor/sylvestris* ist. Fingerprint-Daten implizierten, dass *H. discolor* die Eiszeiten in den Kalkalpen überdauerte (Uhink 1999). Diese Ergebnisse führten zu der Hypothese, dass Arealverschiebungen in Gebiete mit anderen Bodenverhältnissen zu Differenzierung und Artbildung geführt haben. In einem vergleichenden Kulturexperiment wird hier untersucht, ob die edaphische Präferenz von *H. alpina* und *H. discolor* eine genetische Grundlage hat.

Im dritten Teil der Arbeit wird die Gattung *Soldanella* L. (Primulaceae) untersucht, die mit 16 Arten in den europäischen Hochgebirgen verbreitet ist. Die Gattung wurde traditionell in zwei morphologisch gut voneinander abgegrenzte Sektionen geteilt. Der Großteil der Arten findet sich in sect. *Soldanella*. Die Pflanzen sind relativ groß, und die Blüten sind durch ausgeprägte Schlundschuppen, lange Konnektivanhänge sowie einen langen Griffel charakterisiert. Section *Tubiflores* beinhaltet hingegen nur zwei relativ kleine Arten und ist durch Reduktion in der Blütenmorphologie von sect. *Soldanella* abgegrenzt. Die Blüten haben keine oder nur sehr kleine Schlundschuppen, und die Konnektivanhänge sowie der Griffel sind kurz. Die Sektionen unterscheiden sich auch in ihrer Ökologie: die beiden Arten der sect. *Tubiflores* (*S. minima* Hoppe und *S. pusilla* Baumg.) wachsen in alpinen Habitaten oberhalb der Baumgrenze, wohingegen der Großteil der Arten der sect. *Soldanella* in Wäldern der montanen Stufe vorkommt. Eine Ausnahme ist die weitverbreitete *S. alpina* L., die an montanen und alpinen Standorten vorkommt. Morphologisch gehört sie in sect. *Soldanella* (Zhang und Kadereit, 2002). In molekularen Stammbäumen gruppiert *S. alpina* aber mit sect. *Tubiflores* und ist Schwesterart von *S. pusilla* (Zhang et al., 2001), so dass sect. *Tubiflores* nicht monophyletisch ist. Die beschriebenen Verwandtschaftsverhältnisse können durch zwei

alternative Hypothesen erklärt werden: einerseits könnte es sich bei *S. alpina* um eine homoploide Hybridart zwischen *S. pusilla* (sect. *Tubiflores*) und einem Vertreter der sect. *Soldanella* handeln; andererseits könnten *S. minima* und *S. pusilla* parallel entstanden sein (Zhang et al., 2001), und die reduzierte Morphologie der sect. *Tubiflores* kann als Anpassung an den alpinen Lebensraum interpretiert werden. Diese beiden Hypothesen zur Nicht-Monophylie der sect. *Tubiflores* werden mit Sequenz- sowie Fingerprint-Daten untersucht.

Of dwarfs and giants: evolution of miniaturization in arctic-alpine representatives of the *Petasites-Endocellion-Homogyne-Tussilago* clade (Asteraceae-Senecioneae)

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Abstract

We examine the commonly observed pattern of decreasing plant size with increasing latitude or altitude in a phylogenetic context. *Petasites* and *Tussilago* (Asteraceae) are widespread throughout the northern hemisphere and mostly have large leaves and many capitula (not *Tussilago*), while *Homogyne* and *Endocellion* are solely found in alpine and arctic environments, respectively, and have much smaller leaves and only one or few capitula. We present a comprehensively sampled and dated phylogeny of these four genera based on nrDNA and cpDNA sequences (ITS, *ndh*F-*rpl*32 and *rpl*32-*trn*L). The four genera form a well-supported monophyletic group within subtribe Tussilagininae of tribe Senecioneae. *Endocellion* was found to be nested in *Petasites*. Relationships among the three genera remain unresolved as *Homogyne*, *Tussilago* and *Petasites* incl. *Endocellion* form a trichotomy. Dwarfism with small leaves and a reduced number of capitula evolved five times within the group, i.e., in *Homogyne*, in *Endocellion* as a sublineage of *Petasites*, and in *P. doerfleri*, *P. fominii* and *P. rubellus*. The latter two species are restricted to alpine habitats. To better understand the forces that drive the evolution of dwarfism, we took a closer look at the ecology of those species that occur in the Alps. *Homogyne alpina*, *H. discolor* and *P. paradoxus* occur in (sub-) alpine habitats, but only the species of *Homogyne* are true dwarfs with leaves less than 5 cm in diameter and only one flowering head, while *P. paradoxus* has leaves up to 30 cm in diameter and numerous capitula. These species differ in ecology: *Homogyne* is found in nutrient-poor and stable habitats, while *P. paradoxus* grows in nutrient-rich and often disturbed habitats. This pattern can be observed as an overall trend in the entire group: plant size decreases with increasing latitude or altitude, but factors like nutrient availability and habitat disturbance can counteract this trend. Although all dwarf species grow in arctic or alpine habitats, not all species from such habitats are dwarfs.

Keywords

Arctic-alpine plants, dwarfism, ecogeographical rules, evolution, *Petasites*, Tussilagininae

Introduction

Miniaturization of plant size is a phenomenon well known from both arctic and alpine regions. Plants growing at high latitudes or altitudes can often be assigned to one of four major growth forms that constitute large parts of arctic-alpine floras (Johnson, 1969; Körner, 1999). These are (1) cushion plants (e.g., *Silene acaulis* L., *Androsace* L. and *Saxifraga* L. species); (2) tussock-forming graminoids (e.g., *Sesleria caerulea* (L.) Ard., *Carex* L. and *Luzula* DC. species); (3) diminutive, prostrate shrubs (e.g., *Dryas octopetala* L., *Loiseleuria procumbens* (L.) Loisel., *Salix* L. species) and (4) herbaceous and often rosulate perennials (e.g., *Arabis alpina* L., *Primula* L. and *Semperivum* L. species). All of these are characterized by a reduction in plant and organ size. This dwarfism has been interpreted as a morphological adaptation to the severe climatic conditions plants experience in arctic and alpine environments (Billings and Mooney, 1968; Bliss, 1971; Johnson, 1969; Körner and Larcher, 1988; Körner, 1999). Transplant experiments have shown that dwarfism is a genetically fixed character in both alpine and arctic species and in alpine or arctic ecotypes of more widely distributed species (e.g., Turesson, 1925, 1930; Körner et al., 1989; Körner, 1999; Shinohara and Murakami, 2006).

Similar to the observed tendency for plant size to decrease with decreasing environmental temperatures, other ecogeographical principles such as Bergmann's Rule (latitudinal variation in body size; Bergmann, 1847), the Island Rule (gigantism or dwarfism of insular taxa; Foster, 1964) and Cope's Rule (phyletic body size increase; Cope, 1887) have been formulated using mainly animals as examples. Many studies have confirmed the validity of these rules for at least some groups of animals (e.g., Meiri and Dayan, 2003, and refs. therein; Gould and MacFadden, 2004, and refs. therein; Lomolino, 2005, and refs. therein), but understanding of the processes resulting in these patterns is still fragmentary. However, an increasing number of studies have attempted to elucidate the evolution of ecogeographical variation in a phylogenetic framework (e.g., de Queiroz and Ashton, 2004; Diniz-Filho et al., 2007; Pincheira-Donoso et al., 2008; Gür, 2010). Aiming at a more comprehensive understanding of ecogeographical rules, Lomolino et al. (2006) not only pointed out the importance of phylogenetic and phylogeographic analyses in ecogeographic studies, but also suggested to widen the research focus to functionally different groups of organisms, including plants.

We here investigate the evolution of plant size in a clade of four genera of holarctic plants with both lowland and arctic-alpine species, i.e., *Petasites* Mill., *Endocellion* Turcz. ex Herder, *Homogyne* Cass. and *Tussilago* L. (Asteraceae-Senecioneae; Pelser et al. 2007),

henceforth referred to as the Petasites-clade. Fully developed leaves of *Tussilago* are about 20-30 cm in diameter (Hegi, 1929), and most species of *Petasites* have basal leaves of similar or larger size (Fig. 1); they can be up to 1.5 m in diameter in *P. japonicus* subsp. *giganteus* (Sieb. et Zucc.) Maxim. (Hind and Kay, 2006). The only exceptions in *Petasites* are *P. doerfleri* Hayek, *P. rubellus* (J.F. Gmel.) Toman and *P. fominii* Bords., three species restricted to high altitude habitats which have small basal leaves that are less than 5 cm in diameter (Toman, 1972; Fig. 1). Leaves of *Homogyne* from the Alps and other European high mountain ranges and *Endocellion* from the Asian Arctic, both closely related to *Petasites* and *Tussilago* (Pelser et al., 2007), are rather small. Their rounded to cordate or ovate leaves reach only about 1-7 cm in diameter (Toman, 1972; Dingwall, 1976; Tutin, 1976a; Fig. 1).

Members of the entirely holarctic Petasites-clade are perennial, rosette-forming herbs that propagate clonally with long rhizomes; *H. sylvestris* Cass. is the only non-clonal species in this group. *Tussilago* is monospecific containing only *T. farfara*, a species widely distributed throughout Eurasia and introduced to North America (Hegi, 1929; Kuprianova, 2000; Tutin, 1976b; Barkley, 2006). It can be found from the lowlands to high altitude habitats where it is a pioneer species, colonizing open habitats such as roadsides, alluvial drifts, clay pits, moraines and avalanche deposits with the aid of its long, slender rhizomes (Hegi, 1929; Kuprianova, 2000). *Homogyne* is endemic to the European Alpine System (sensu Ozenda 1988) and comprises three species which are separated by their edaphic and altitudinal preferences. *Homogyne alpina* Cass. is widespread across the European Alpine System and grows on both siliceous and calcareous substrates in a wide range of habitats including montane coniferous and deciduous forests, subalpine dwarf-shrub heaths and alpine grasslands (Hegi, 1929; Tutin, 1976a; Aeschimann et al., 2004; Fischer et al., 2005). *Homogyne discolor* Cass. and *H. sylvestris* are more restricted in their soil preferences by growing solely on calcareous substrates. Their distribution is confined to the peripheral limestone and dolomite ranges of the Eastern Alps, where *H. discolor* occurs in subalpine and alpine habitats such as subalpine dwarf-shrub heaths, alpine grasslands and snow beds, while *H. sylvestris* is largely restricted to coniferous and deciduous forests of the montane and subalpine altitudinal belts (Hegi, 1929; Tutin, 1976a; Aeschimann et al., 2004; Fischer et al., 2005). *Petasites* comprises c. 16 species distributed mainly throughout Eurasia, with one species (*P. fragrans* (Vill.) C.Presl) endemic to North Africa and one (*P. frigidus* (L.) Fries) occurring in both northern Eurasia and North America. Species of *Petasites* are commonly found in moist and often disturbed habitats such as stream banks, moist subalpine and alpine slopes and meadows, marshy tundra, peat bogs and wet forest margins (Hegi, 1929; Toman,

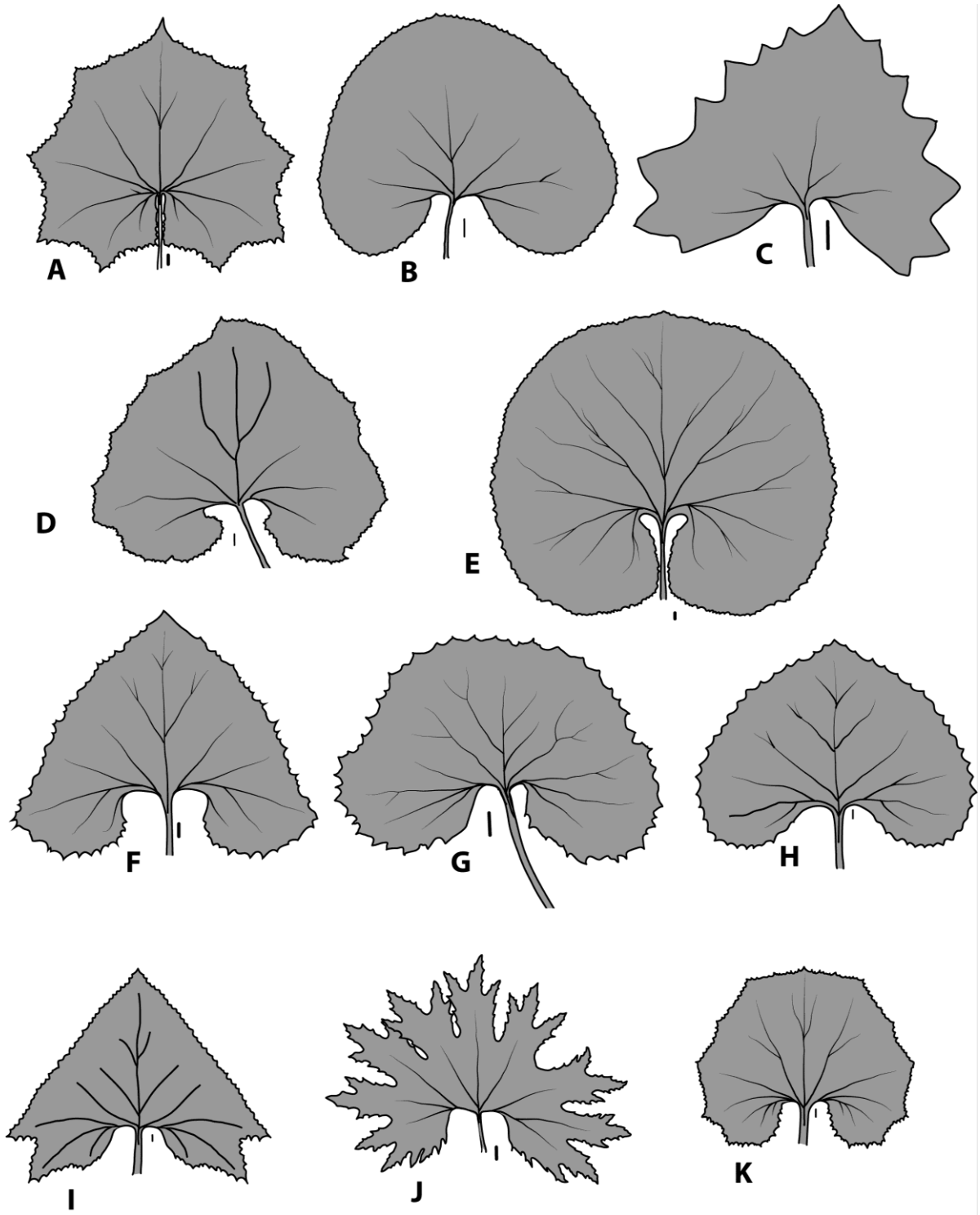

FIG. 1. Leaf size variation in the Petasites-clade. **A** *P. albus* (after Toman 1972). **B** *P. fragrans* (drawn from Steffen 180908/1). **C** *P. frigidus* (Boyko s.n.). **D** *P. hybridus* (Steffen 130409/1). **E** *P. japonicus* (Toman 1972). **F** *P. kablikianus* (Toman 1972). **G** *P. paradoxus* (Steffen 290708/3). **H** *P. radiatus* (Toman 1972). **I** *P. spurius* (Toman 1972). **J** *P. tatewakianus* (Salokhin s.n.). **K** *P. tricholobus* (Toman 1972). **L** *P. doerfleri* (Dörfler 569). **M** *P. rubellus* (Boyko s.n.). **N** *T. farfara* (Steffen 060608/1). **O** *E. glaciale* (Pospepov 00-467). **P** *E. sibiricum* (Kharkevich and Buch 814b). **Q** *H. alpina* (Steffen 100809/4). **R** *H. discolor* (Steffen 010578). **S** *H. sylvestris* (Steffen 010572). Scale bar = 1 cm.

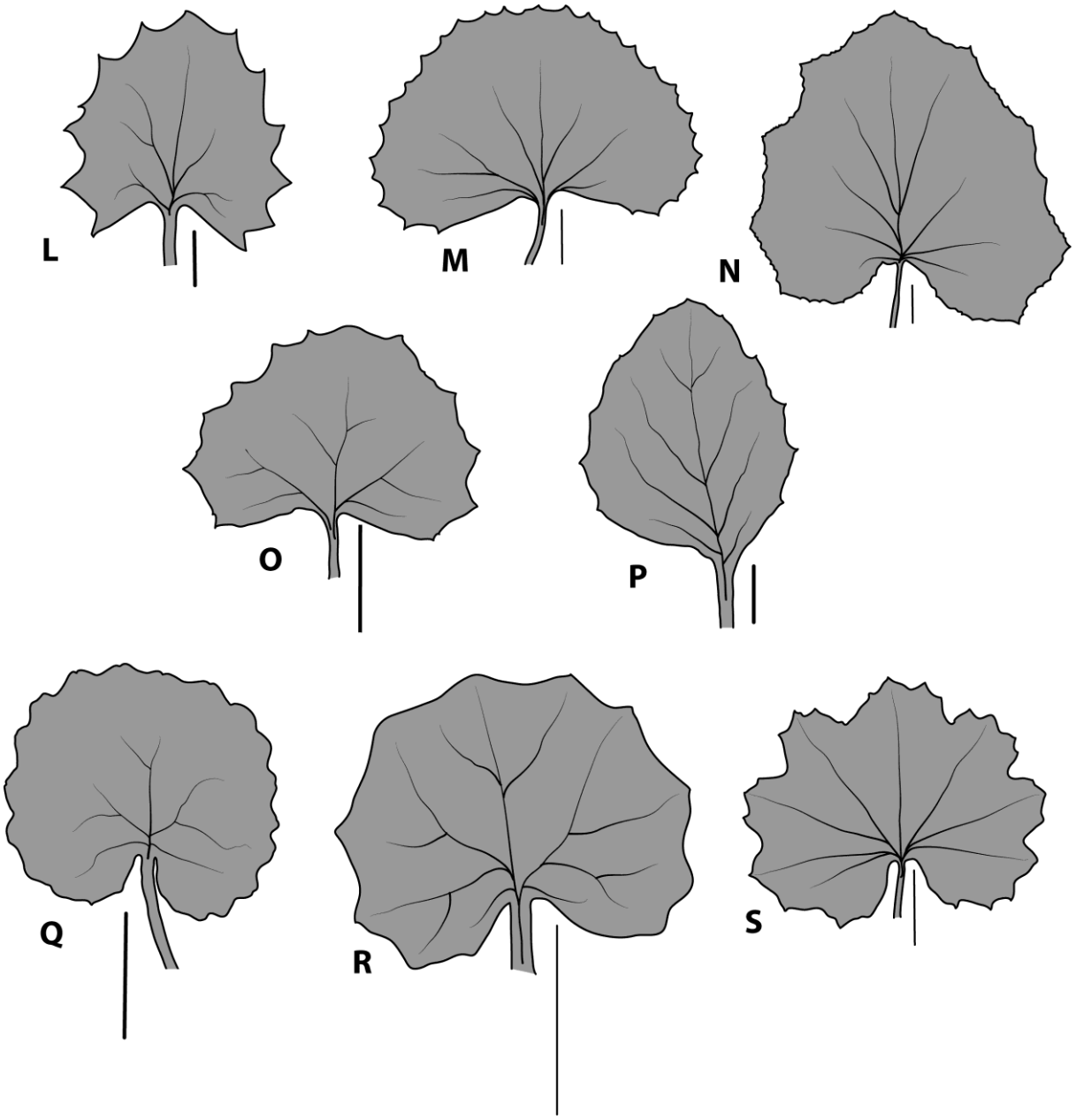

FIG. 1 continued.

1972; Dingwall, 1976; Ellenberg, 1996; Cherniawsky and Bayer, 1998; Kuprianova, 2000; Bayer et al., 2006; Chen et al., 2011). The two species of *Endocellion* can be found in Arctic regions from the Ural Mountains through Siberia to the Far East, where they grow in pebbly, rather dry tundra in lowland and montane regions (Toman, 1972; Dingwall, 1976; Kuprianova, 2000).

Members of the Petasites-clade differ not only in plant size, but also in phenology and breeding system. *Homogyne* is the only genus with evergreen leaves, while the remaining three genera have basal leaves that develop either during (*Endocellion*) or after (*Petasites*, *Tussilago*) anthesis (Nordenstam, 2007). Furthermore, *Petasites* and *Endocellion* are (sub-) dioecious: in *Endocellion* the female capitula are radiate and lack disc florets, and the male

capitula are discoid with functionally male florets. *Petasites* has female capitula with numerous tubular to filiform female and sterile tubular or shortly radiate florets. Its male capitula have numerous tubular, functionally male florets. The latter sometimes can also have marginal female florets which make them hermaphrodite. *Homogyne* and *Tussilago* do not show this dimorphism in reproductive structures. All capitula of *Homogyne* have marginal female florets which are tubular and/or very shortly radiate, and hermaphrodite disc florets. Disc florets of *Tussilago* are tubular and functionally male, while the numerous ray florets are female. Capitula of *Tussilago, Homogyne* and *Endocellion* are either solitary or more rarely in twos or threes on rather long peduncles, whereas capitula of *Petasites* are numerous and form paniculate-racemose synflorescences (Nordenstam, 2007). The flowering shoots arise from rhizomes of the previous year in *Tussilago* and *Petasites* and only differ in the number of capitula they develop (Troll, 1939).

A molecular phylogenetic analysis of tribe Senecioneae using ITS sequence data revealed that *Petasites, Endocellion, Homogyne* and *Tussilago* form one of four monophyletic groups of subtribe Tussilagininae s.str. (Pelser et al., 2007). Relationships among these four monophyletic groups remained largely unresolved, but the data suggested that the Petasites-clade is either sister to the remainder of Tussilagininae s.str. or to an exclusively New World clade comprising, of the genera sampled, *Aequatorium* B.Nord., *Gynoxys* Rchb., *Nordenstamia* Lundin and *Paragynoxys* (Cuatrec.) Cuatrec. (Pelser et al., 2007).

We here present a phylogenetic analysis of the Petasites-clade based on nrDNA and cpDNA sequences (ITS, *ndh*F-*rpl*32 and *rpl*32-*trn*L). We will use this phylogeny mainly to examine the evolution of dwarfism within the group. In particular, we will examine how many times and where and when dwarfism evolved. Also, by considering the ecology of dwarf species, we will identify the environmental variables relevant for the evolution of their dwarfism.

Material and Methods

Taxon sampling. We included all species currently recognized in *Petasites, Endocellion, Homogyne* and *Tussilago* except *P. versipilus* Hand.-Mazz. Most of the DNA sequences produced for this study were obtained from leaf tissue samples taken from herbarium specimens from B, HAST, M, O, PE, PGFA, PMR, VLA and WU. Others were obtained from silica dried leaf tissue. Herbarium vouchers are deposited at MJG. Voucher information for all samples is listed in Table 1.

DNA extraction, amplification and sequencing. Total genomic DNA was extracted using the Qiagen DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany) following the manufacturer's protocol. For some herbarium specimens this method did not yield DNA of sufficient quality for PCR. In these cases total genomic DNA was isolated as follows: mortared leaf material was incubated in 800 µl AP1 buffer (Qiagen), 8 µl RNase A (Qiagen), 60 µl 2-mercaptoethanol and 60 µl proteinase K for 12 h at 42°C. After a second incubation step for 0.5 h at 65°C, 260 µl AP2 buffer (Qiagen) were added to the lysate, followed by 5 min incubation on ice and 5 min centrifugation at 20 000 g. The supernatant was transferred into a new reaction tube and mixed with 500 µl chloroform/isoamyl alcohol (24:1), incubated for 5 min at room temperature and centrifuged for 5 min at 20 000 g. The aqueous phase was transferred into a new tube and 500 µl isopropanol were added. After incubation for 45 min at 4°C this was centrifuged for 5 min at 4°C. After decantation the DNA pellet was diluted in 0.2 ml TE buffer, followed by one washing step with 1 ml ice-cold 100% ethanol and 50 µl 2.5M sodium acetate, and incubation for 1 h at -20°C was followed by 20 min centrifugation at 20 000 g at 4°C. After decantation a second washing step with 0.4 ml algid 70% ethanol was conducted, followed by dissolving the dried DNA pellet in 60 µl elution buffer (Qiagen). PCR amplification of the entire ITS region was performed using primers ITS A (5'-GGA AGG AGA AGT CGTAAC AAG G-3', Blattner, 1999) and ITS 4 (5'-TCC TCC GCT TAT TGA TAT GC-3'; White et al., 1990). In some cases it was necessary to produce two overlapping fragments using primers ITS A and ITS C (5'-GCA ATT CAC ACC AAG TAT CGC-3'; Blattner, 1999), ITS D (5'-CTC TCG GCA ACG GAT ATC TCG-3'; Blattner, 1999) and ITS 4. PCR amplification of ITS was performed using the procedures described in Zhang et al. (2007). The *ndh*F-*rpl*32 intergenic spacer was amplified using primers rpL32-R (5'-CCA ATA TCC CTT YYT TTT CCA A-3'; Shaw et al., 2007) and ndhF (5'-GAA AGG TAT KAT CCA YGM ATA TT-3'; Shaw et al., 2007). Primers trnL$^{(UAG)}$ (5'-CTG CTT CCT AAG AGC AGC GT-3'; Shaw et al., 2007) and rpL32-F (5'-CAG TTC CAA AAA AAC GTA CTT C-3'; Shaw et al., 2007) were used for the *rpl*32-*trn*L intergenic spacer. PCR amplification for both chloroplast regions was carried out following the ITS protocol differing only in the annealing temperature which was set to 56°C. PCR products were purified using either NucleoSpin Extract II (Macherey-Nagel, Düren, Germany) or ExoSAP-IT PCR Product Cleanup (USB Corporation, Cleveland, Ohio, USA) following the manufacturers' protocols. Cycle sequencing was carried out using the ABI Prism Dye Terminator Cycle

TABLE 1. Materials, voucher information, DNA accession numbers and GenBank accession numbers.

Taxon	**Country; collector, collection no.; Herbarium, Herbarium no.**	**DNA accession no.**	**GenBank accession no.**		
			ITS	ndhF-rpl32	rpl32-trnL
Adenostyles alpina DC.	France; Klein s.n.; MJG 000492	AST60	-	+	+
Endocellion glaciale (J.F. Gmel.) Toman	Russia, Sakha Republic; Solstad & Elven 04/1062; O	AST24	+	+	+
	Russia, Chukotka; Solstad & Elven 05/1008; O	AST25	+	+	+
	Russia, Sakha Republic; Solstad & Elven 04/1049; O	AST32	+	+	+
Endocellion sibiricum (Ledeb.) Toman	Russia, Schönswetter & Tribsch T316; WU		EF538197	-	-
	Russia, Schönswetter & Tribsch T480; WU		EF538198	-	-
	Russia, Sakha Republic; Solstad & Elven 04/0330; O	AST23	+	+	+
	Russia, Magadan; Boyko s.n.; MJG	AST71	+	-	+
Homogyne alpina Cass.	Austria, Carinthia; Steffen 120807/1; MJG	AST1	+	+	+
	Switzerland, Schwyz; Uhink & Kadereit s.n.; MJG	AST3	+	+	+
	Italy, Trentino; Steffen 190807/2; MJG	AST15	+	+	+
	Austria, Carinthia; Uhink 98-183; MJG	AST12	+	+	+
Homogyne discolor Cass.	Austria, Styria; Steffen 090807/1; MJG	AST13	+	+	+
	Austria, Carinthia; Steffen 130807/2; MJG	AST14	+	+	+
	Austria, Carinthia; Steffen 110807/3; MJG	AST74	+	-	-
	Austria, Salzburg; Uhink 98-431; MJG	98_431	+	-	-
Homogyne sylvestris Cass.	Austria, Carinthia; Uhink 98-139; MJG	AST19	+	+	+
	Austria, Carinthia; Uhink 98-146; MJG	AST20	+	+	-
	Austria, Carinthia; Uhink 98-187; MJG	98_187	+	-	-
	Austria, Carinthia; Steffen 290708/4; MJG	AST62	+	+	+

TABLE 1 continued.

Ligularia tsangchanensis (Franch.) Hand.-Mazz.	China, Yunnan; Liu 2193; HNWP		AY723264	-	-
Nordenstamia kingii (H.Rob. & Cuatrec.) B.Nord.	Bolivia, Cochabamba; Ståhl 5572A; S		EF538267	-	-
Petasites albus (L.) Gaertn.	Austria, Styria; Steffen 280508/5; MJG	AST28	+	+	+
	Georgia, Abkhazia; Mikheev s.n.; PGFA	AST64	+	-	+
Petasites doerfleri Hayek	Albania; Rakaj & Surina NHMR833; PMR	AST61	+	+	+
Petasites fominii Bordz.	Georgia; Mikheev 2750; PGFA	AST65	+	+	-
Petasites formosanus Kitam.	Taiwan; Yang 1683; HAST	AST30	+	+	+
Petasites fragrans (Vill.) C.Presl	Great Britain; Jury 1039; B	AST45	+	-	-
	New Zealand, South Island; Wagstaff s.n.; CHR541965A		AY554108	-	-
Petasites frigidus (L.) Fries	Russia; Sagspokora 240; M	AST5	+	+	+
	Russia, Taymyr; Schönswetter & Tribsch 4703; WU	AST9	+	+	+
	Norway; Nordenstam 9501; S	1143	+	-	-
Petasites frigidus var. *palmatus* (Aiton) Cronquist	Canada, Québec; Lemieux 21229; B	AST54	+	-	-
	USA, California; Sharsmith 5249; M	AST4	+	+	+
Petasites hybridus (L.) Gaertn., Mey. & Scherb.	Russia, Karachay-Cherkessia; Claßen-Bockhoff s.n.; MJG	AST29	+	+	+
	Germany, Lower Saxony; Steffen 301207/1; MJG	AST2	+	+	+
	Russia, Stavropol; Shilnikow s.n.; PGFA	AST63	+	+	+
Petasites japonicus (Siebold & Zucc.) Maxim.	China, Sichuan; Liu 15232; B	AST53	+	-	+
	Germany, Rhineland-Palatinate; Steffen 100108/2; MJG	AST17	+	+	+
	Russia, Sakhalin; Salokhin s.n.; MJG 010547	AST73	+	+	+
	unknown		AY176152	-	-
Petasites kablikianus Tausch	Romania; Negrean & Anastasiu s.n.; B	AST46	+	-	-
	Slovakia; Schuhwerk 04/100; M	AST6	+	+	+
Petasites paradoxus Baumg.	Germany, Bavaria; Parker s.n.; M	AST7	+	+	+

TABLE 1 continued.

Petasites tatewakianus Kitam.	Russia, Primorsky; Salokhin s.n.; MJG	AST72	+	-	+
	Austria, Tyrol; Vitek 3748; WU	AST10	+	+	+
Petasites radiates (J.F. Gmel.) Toman	Russia, Komi; Alsos & Tribsch 4854; WU	AST11	+	-	-
Petasites rubellus (J.F. Gmel.) Toman	Russia, Primorsky; Boyko s.n.; MJG	AST70	+	-	-
Petasites spurius (Rchb.f.)	Germany, Schleswig-Holstein; Wesener 1; MJG	AST26	+	+	+
	Germany, Lower Saxony; Schimmitat s.n.; M	AST8	+	+	+
Petasites tricholobus Franch.	Nepal; Schwabe s.n.; B	AST35	+	-	-
	unknown		AY176153	-	-
Tetradymia canescens DC.	USA, Colorado; Dunn 15291; U		EF538410	-	-
Tussilago farfara L.	Germany, Rhineland-Palatinate; Steffen 100108/3; MJG	AST18	+	+	+
	Slovakia; Taubmann 2; MJG	AST58	+	+	+
	Pakistan, Nüsser 966; B	AST41	+	+	-
	unknown; Caesar & Loretz 40923276		EU785941	-	-
	unknown		AY176167	-	-

Sequencing ready Reaction Kit (Perkin Elmer/Applied Biosystems, Foster City, California, USA) using the primers listed above and following the manufacturer's protocol. The purified products were analyzed on an ABI 3130XL automated sequencer by ourselves and by StarSeq GmbH (Mainz, Germany).

DNA sequence alignment and phylogenetic analyses. Forward and reverse sequences were manually edited and merged into consensus sequences using Sequencher 4.1.2 (Gene Codes Corporation, Ann Arbor, Michigan, USA), and aligned manually in MacClade 4.1 (Maddison and Maddison, 2000).

Likelihood and Bayesian analyses were performed for the entire ITS region and for the combined cpDNA regions (*ndh*F-*rpl*32-*trn*L) separately. We did not combine the two data sets because the taxon sampling for cpDNA was much smaller than that for ITS, and because preliminary analyses showed that the combined data set resulted in poorly resolved trees. For Maximum Likelihood (ML) and Bayesian Analyses (BI) the appropriate model of DNA substitution for the inference of phylogenetic relationships under ML was estimated using Modeltest 3.06 (Posada and Crandall, 1998). We identified the Tamura-Nei (TrN) model with gamma-distributed rates (G) for ITS and the general time-reversible model (GTR) with gamma-distributed rates (G) and a proportion of invariable characters (I) for *ndh*F-*rpl*32-*trn*L as best-fitting the sequence data under the Akaike Information Criterion (AIC).

Maximum Likelihood tree searches and ML bootstrap searches were performed using the online version of RAxML (Stamatakis et al., 2008; available at http://phylobench.vital-it.ch/raxml-bb/). The GTR+G model was used for ITS and the GTR+G+I model was used for cpDNA analyses, with a total of 100 bootstrap replicates.

Bayesian analysis was performed using MrBayes version 3.1.2 (Huelsenbeck and Ronquist, 2001). For the cpDNA data set parsimony-informative gaps for the ingroup were coded by hand according to the simple indel coding approach (Simmons and Ochoterena, 2000). The ITS data were analyzed with the GTR+G model, and the analysis of the cpDNA data was performed using the GTR+G+I model. The ITS data analysis was run for 5 million Markov Chain Monte Carlo (MCMC) generations, the *ndh*F-*rpl*32-*trn*L data for 10 million generations , with sampling every 1000. Convergence of model parameters was examined using Tracer v.1.5 (Rambaut and Drummond, 2007); a total of 250,000 generations (ITS) and 500,000 generations (cpDNA) were discarded as burn-in.

Molecular clock dating. For the molecular clock dating analysis we constructed a reduced ITS data set with only *Ligularia tsangchanensis* Franch.) Hand.-Mazz. as outgroup. The dating analysis was carried out with BEAUTi/BEAST v.1.5.4 (Drummond and Rambaut,

2007) using the GTR+G model. Due to the lack of internal calibration points, we took a molecular clock dating approach using published ITS substitution rates. For this, we used the minimum and maximum ITS substitution rates in Asteraceae identified by Kay et al. (2006). A uniform distribution (parameter ucld.mean) of these rates between 0.00251 and 0.00783 substitutions per site per million years was used. Starting trees were generated randomly. The BEAST analysis was run for 50 million generations to ensure that all parameters had an effective sampling size >200. Convergence was examined using Tracer v.1.5.

Character evolution. To investigate the evolution of dwarfism, we collected data on plant size from the literature: Hegi (1929), Polunin (1959), Dingwall (1976), Tutin (1976a, b), Toman (1972), Cherniawsky and Bayer (1998), Kuprianova (2000), Bayer et al. (2006), Chen et al. (2011), and Liu and Illarionova (2011). This was supplemented by information collected from material in the following herbaria: B, K, M, MJG, O, US and W. Each taxon was coded for leaf size and number of capitula per synflorescence. Characters were coded as follows: leaves less than 7 cm in diameter, leaves more than 10 cm in diameter, leaves 2-50 cm in diameter; capitula single (rarely 2-3), capitula numerous. Although leaf size and number of capitula are continuous characters, we coded them as discrete characters because the material studied can be clearly assigned to the categories defined. The only exception to this is *P. frigidus* with leaf size ranging from 2-50 cm which we coded as a third character state. To reconstruct the evolution of leaf size and number of capitula under Maximum Parsimony (MP) and Maximum Likelihood criteria, respectively, Mesquite v2.75 (MP; Maddison and Maddison, 2011) and the StochChar module (ML; Maddison and Maddison, 2006) implemented in Mesquite v2.75 were used. Character states were reconstructed using the ITS cladogram obtained from the BI analysis. For optimization on the ITS tree we excluded *E. sibiricum* from the data set without changing the overall topology and chose only *E. glaciale* to represent the genus, as *Endocellion* was shown to be monophyletic (see Results and Discussion).

Results

Phylogenetic relationships. Preliminary analyses of the ITS data set (results not shown) were run with 64 accessions including representatives from all subtribes of Senecioneae as recognized by Pelser et al. (2007) as outgroups. These were *Abrotanella trilobata* Swenson (Abrotanellinae), *Doronicum pardalianches* L. (Doroniceae or Senecioneae-Doronicinae), *Othonna carnosa* Less. (Othonninae), and *Adenostyles alpina* (L.) Kern. and *Senecio vulgaris* L. (Senecioninae). From Tussilagininae (sensu Bremer, 1994),

Brachyglottis greyi (Hook.f.) B. Nord. was chosen to represent the Senecio medley-woodii-Brachyglottis clade, and *Nordenstamia kingii* (H. Rob. and Cuatrec.) B. Nord. (Aequatorium-Arnoglossum clade), *Tetradymia canescens* DC. (Crocidium-Tetradymia clade) and *Ligularia tsangchanensis* (Ligularia-Cremanthodium-Parasenecio complex) were chosen as representatives of the remaining three clades of Tussilagininae s.str. identified by Pelser et al. (2007). The preliminary analyses showed that Tussilagininae are monophyletic and that *Petasites, Endocellion, Homogyne* and *Tussilago* are highly supported as a monophyletic group (Petasites-clade) with bootstrap (BS)/posterior probability (PP) values of 96%/1. The phylogenetic position of the Petasites-clade within Tussilagininae s.str. remains uncertain as the Petasites-clade forms a polytomy with the taxa representing the other clades of the tribe. For the final analyses, the ITS data set was reduced in order to facilitate alignment, and included the Petasites-clade and *L. tsangchanensis*, *N. kingii* and *T. canescens* of the Tussilagininae as outgroups. In total, the ITS matrix contained 57 accessions and was 647 bp long, of which 87 were variable but uninformative and 167 were parsimony-informative.

The *ndh*F-*rpl*32-*trn*L data matrix consisted of 42 accessions including *Adenostyles glabra* DC. as outgroup. It was 1,999 bp long, with 91 variable and 73 parsimony-informative characters. Indel coding added another ten parsimony-informative characters.

The ML and Bayesian analyses of the ITS data resulted in trees of the same topology with respect to well-supported branches; the BI tree is shown in Fig. 2. In the strongly supported Petasites-clade (99%/1), generic relationships were unresolved as *Homogyne* (93%/1), *Tussilago* (100%/1) and *Petasites* including *Endocellion* (-/0.99) formed a trichotomy. Within *Homogyne*, all three species were strongly supported, and *H. alpina* was well-supported as sister (93%/1) to *H. discolor* plus *H. sylvestris* (98%/1). The *Petasites*/*Endocellion* clade (-/0.99) was not well resolved, containing a basal polytomy with five clades. *Endocellion sibiricum* (Ledeb.) Toman, *P. frigidus* and *P. tatewakianus* Kitam. formed a monophylum (clade I; 100%/1) with one accession of *E. sibiricum* and *P. tatewakianus* (84%/1) as sister to a clade comprising *P. frigidus* and the remaining accessions of *E. sibiricum* (97%/1). The other species of *Endocellion*, *E. glaciale* (J.F. Gmel.) Toman was part of a well-supported clade (85%/1; clade III) with *P. radiatus* (J.F. Gmel.) Toman, *P. spurius* (Retz.) Reichenb. and one accession of *P. fragrans*. The other *P. fragrans* accession formed a clade with *P. fominii* and some accessions of *P. hybridus* (L.) Gaertn., Mey. and Scherb. (75%/1; clade IV). An additional *P. hybridus* accession formed a strongly supported clade (100%/1; clade II) with *P. albus* (L.) Gaertn., *P. doerfleri* and *P. paradoxus* Baumg. A weakly supported clade (-/0.95; clade V) was formed by *P. kablikianus* Tausch (100%/1) and

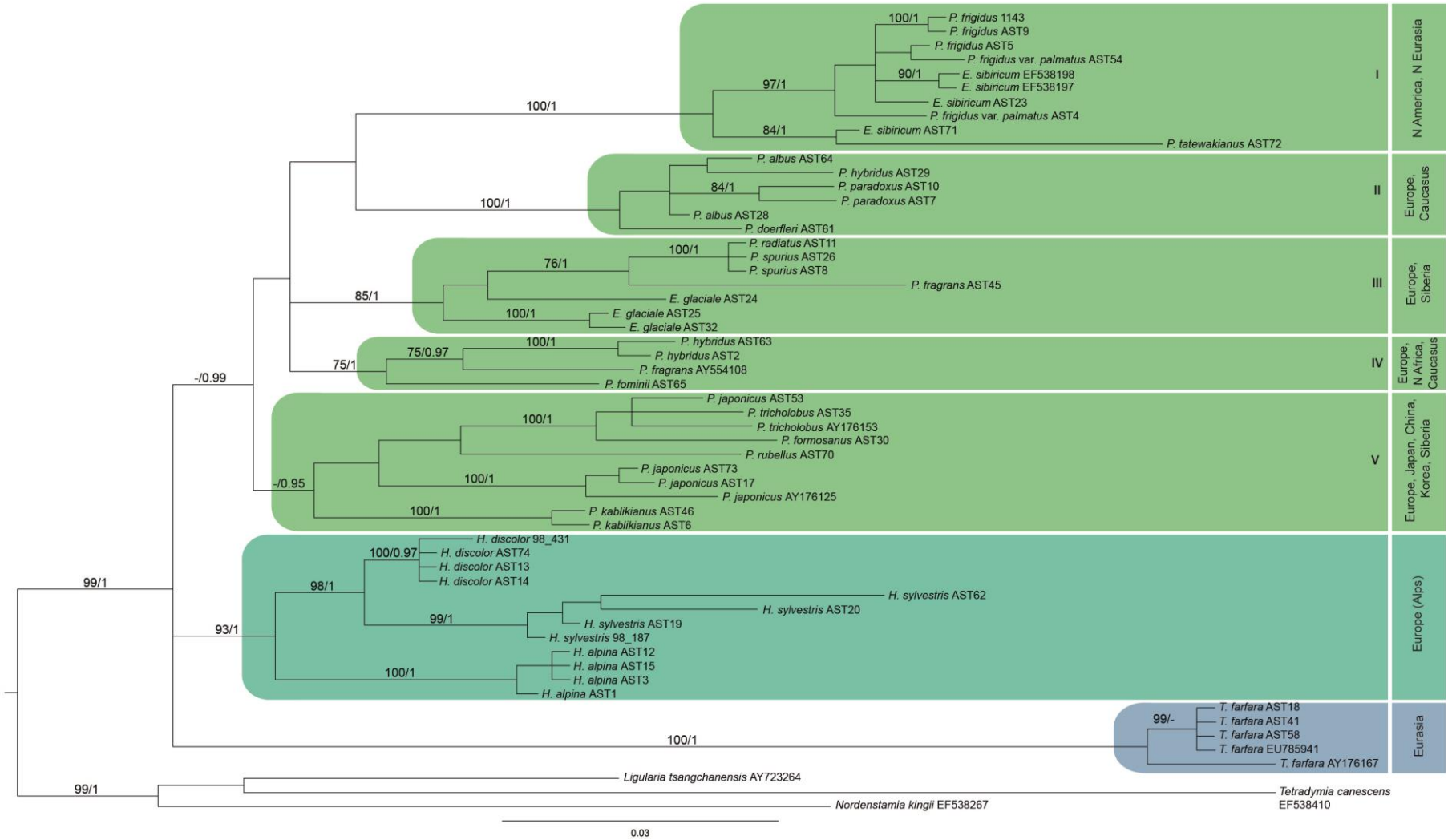

FIG. 2. Phylogenetic relationships in the *Petasites-Endocellion-Homogyne-Tussilago* clade. Phylogram from the Bayesian analysis of the ITS data set. ML bootstrap values (≥ 75%) and Bayesian posterior probabilities (≥ 0.95) are given above branches. The geographic distribution is indicated for each clade. This takes into account the probably correct phylogenetic position of the polyphyletic *P. fragrans* and *P. hybridus* (see text).

Petasites species restricted to East Asia. These species were found in two subclades, one containing all but one accessions of P. japonicus (100%/1) and the other P. tricholobus Franch., *P. formosanus* Kitam. and one accession of *P. japonicus* (100%/1); *P. rubellus* (J.F. Gmel.) Toman also fell into clade V but its relationship to one or the other of the two subclades described was not supported.

Trees obtained from ML and Bayesian analyses of the *ndh*F-*rpl*32-*trn*L data were poorly resolved, with four clades in a basal polytomy (Fig. 3). At least some well-supported branches were congruent with the ITS tree (Fig. 2). *Tussilago* (85%/-), *Homogyne* (82%/-) and *Endocellion* (-/0.96) were each supported as monophyletic, while *Petasites* fell into two weakly supported clades, one of which also contained *Endocellion*. One accession each of *P. albus* and *P. hybridus* formed one clade (78%/0.98), while the remaining accessions of *Petasites* and *Endocellion* formed a second clade (82%/-). Within this clade, *P. doerfleri*, *P. fominii*, *P. fragrans*, *P. kablikianus* and *P. paradoxus* were part of a basal polytomy. The remaining taxa fall into five clades with at least some statistical support. One of these clades comprises additional accessions of *P. albus* and *P. hybridus* (82%/-). *Petasites japonicus* is polyphyletic, and two accessions of this species formed a second clade (86%/0.95), whereas the third accession formed a clade together with *P. formosanus* (-/1). The two *Endocellion* species, *P. spurius* and *P. tatewakianus* formed a moderately supported clade (-/0.97). Within Endocellion (-/0.96) three accessions of *E. glaciale* formed a clade (91%/0.99). The other accessions of *P. frigidus* formed a distinct clade (75%/0.99).

As the phylogeny inferred from the ITS data is far more informative than that deduced from the cpDNA data, major parts of the following discussion will be based on the ITS tree. However, the discussion related to *Endocellion* will refer to the cpDNA data.

Molecular clock dating. The BEAST analysis revealed a crown group age of the Petasites-clade [node 1(N_1) in Fig. 4] of 11.88 (5.52 - 27.81) million years (Ma; Fig. 4 and Table 2). The crown group age of *Homogyne* (N_2) was 4.87 (2.11- 13.93) Ma, and the split between *H. discolor* and *H. sylvestris* (N_3) was 3.27 (1.15 – 8.13) Ma. The crown group age of *Petasites* including *Endocellion* (N_4) was 10.15 (4.34 - 18.42) Ma. The estimated ages of the arctic-alpine species within the *Petasites-Endocellion* clade were 4.75 (1.27 - 7.43) Ma for *P. frigidus*/*E. sibiricum* (N_5), 3.9 (0.77 - 5.49) Ma for *P. doerfleri* (N_6), 4.12 (1.55 - 9.07) Ma for *E. glaciale* (N_7), 4.82 (1.33 - 9.54) Ma for *P. fominii* (N_8), and 4.59 (1.38 - 8.05) Ma for *P. rubellus* (N_9).

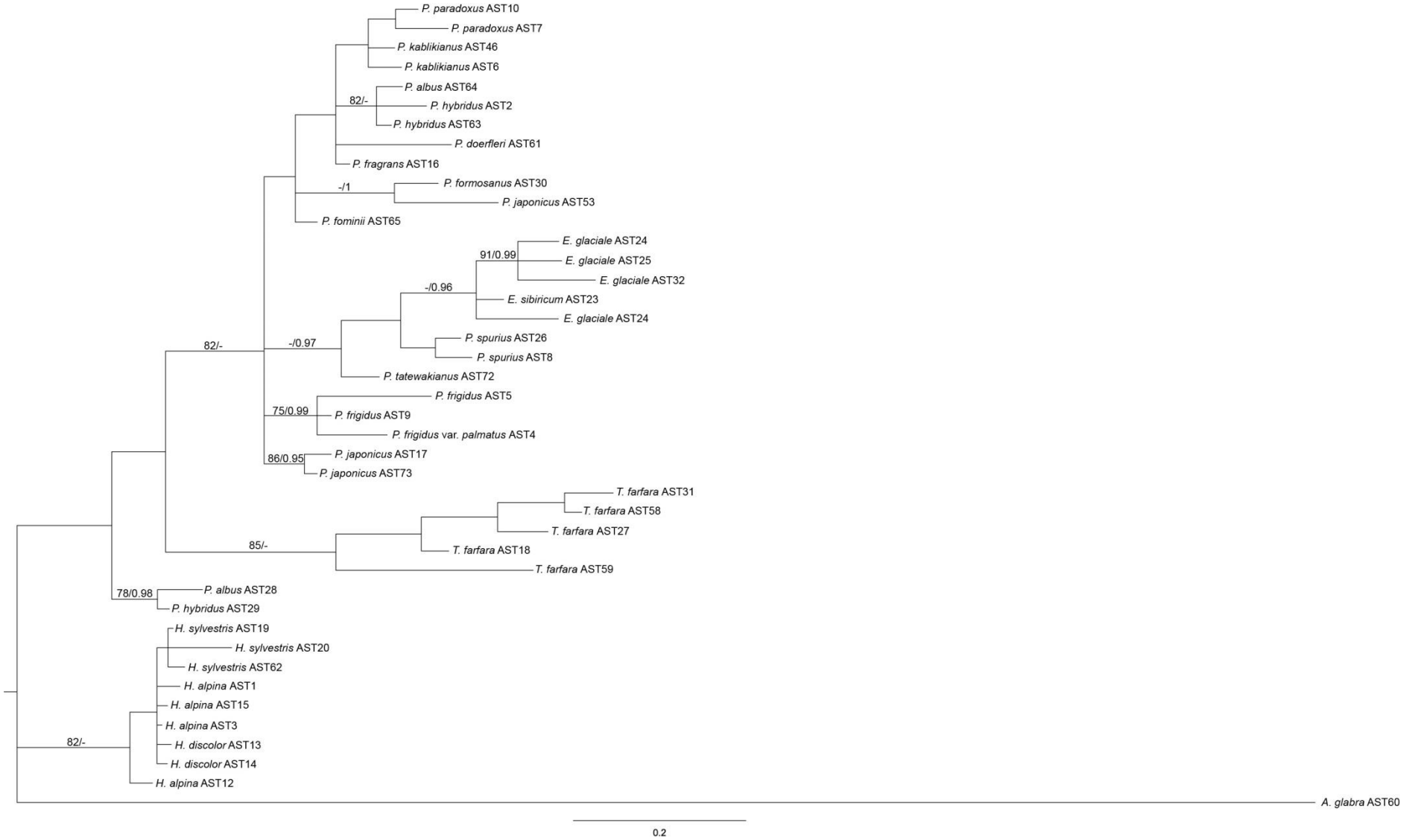

FIG. 3. Phylogenetic relationships in the *Petasites*-*Endocellion*-*Homogyne*-*Tussilago* clade. Phylogram from the Bayesian analysis of the cp DNA (*ndh*F-*rpl*32-*trn*L) data set. ML bootstrap values (≥ 75%) and Bayesian posterior probabilities (≥ 0.95) are given above branches.

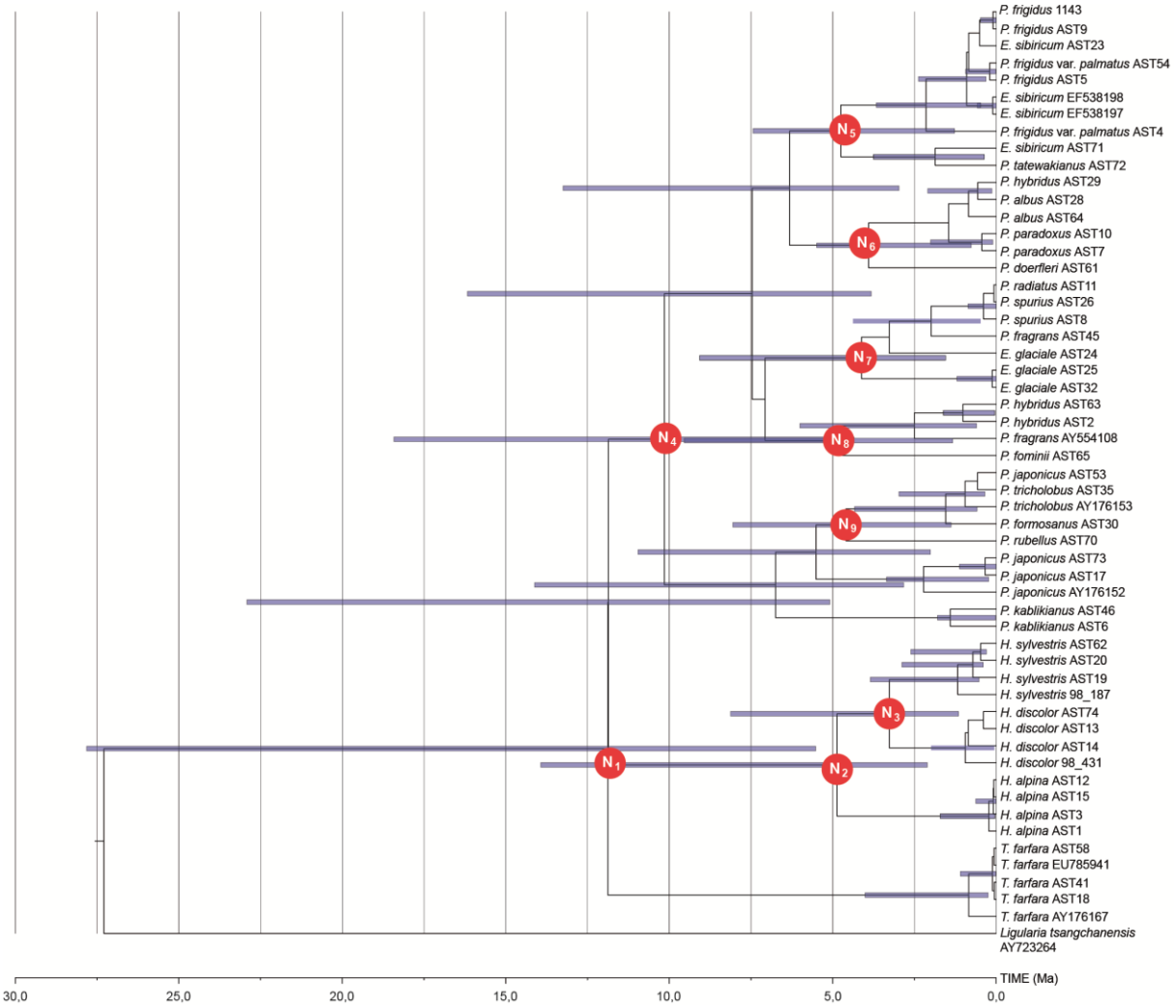

FIG. 4. Chronogram of the *Petasites-Endocellion-Homogyne-Tussilago* clade obtained under a Bayesian relaxed molecular clock using published ITS nucleotide substitution rates (Kay et al. 2006). Grey bars at nodes represent 95% highest posterior densities of node ages. Ages for the nodes marked with red dots are given in Table 2.

TABLE 2. Estimated ages of nodes in Ma revealed from the BEAST analysis based on published ITS nucleotide substitution rates. Given are the estimated ages and the 95%-confidence intervals of the nodes labeled according to Fig. 4.

Node	Age	95%-confidence interval
N1	11.88	5.52 - 27.81
N2	4.87	2.11 - 13.93
N3	3.27	1.15 – 8.13
N4	10.15	4.34 - 18.42
N5	4.75	1.27 - 7.43
N6	3.90	0.77 - 5.49
N7	4.12	1.55 - 9.07
N8	4.82	1.33 - 9.54
N9	4.59	1.38- 8.05

Evolution of dwarfism. The results of the reconstruction of leaf size and number of capitula using ML over the ITS tree after exclusion of *Endocellion sibiricum* is shown in Fig. 5. Both MP and ML reconstructions revealed that large leaves (> 10 cm in diameter) were ancestral (ML 99%) in the Petasites-clade. Maximum Parsimony and ML analyses indicated that leaves smaller than 7 cm arose four times independently, i.e., in *Homogyne* (ML 100%), *P. rubellus*, *P. doerfleri* and *P. fominii*. According to the MP analysis ancestral leaf size was ambiguous for the clade containing *E. glaciale*; the ML analysis indicated large leaves as ancestral with 69% probability. Small leaves evolved in *E. glaciale* and large leaves in the *Petasites* species of this clade. Under MP, the ancestral leaf size was ambiguous in the clade containing *P. frigidus*, and the ML analysis indicated leaves of variable size (2-50 cm, as found in *P. frigidus*) as ancestral with 53% probability. In this clade, large leaves arose in *P. tatewakianus*, while leaf size is variable in *P. frigidus*.

The ancestral state for number of capitula could not be reconstructed unambiguously; the ML analysis indicated numerous capitula as ancestral (ML 77%), but the MP result was ambiguous for the *Petasites* clade. Maximum Parsimony and ML analyses indicated that single capitula evolved in *Tussilago* (ML 100%) and *Homogyne* (ML 100%). Ancestral capitulum number is ambiguous under MP for the clade containing *E. glaciale*, and the ML analysis resulted in numerous capitula as ancestral (ML 70%). Single capitula arose in *E. glaciale*, and numerous capitula in the species of *Petasites* of this clade.

Evolution of sexual systems. *Petasites* and *Endocellion* show a sexual dimorphism whereas *Homogyne* and *Tussilago* are hermaphrodite as all other members of Tussilagininae (Nordenstam, 2007). Within the *Petasites* clade, a single evolutionary transition from hermaphrodite to (sub)dioecious capitula took place in the in the *Petasites*/*Endocellion* clade (Fig. 5).

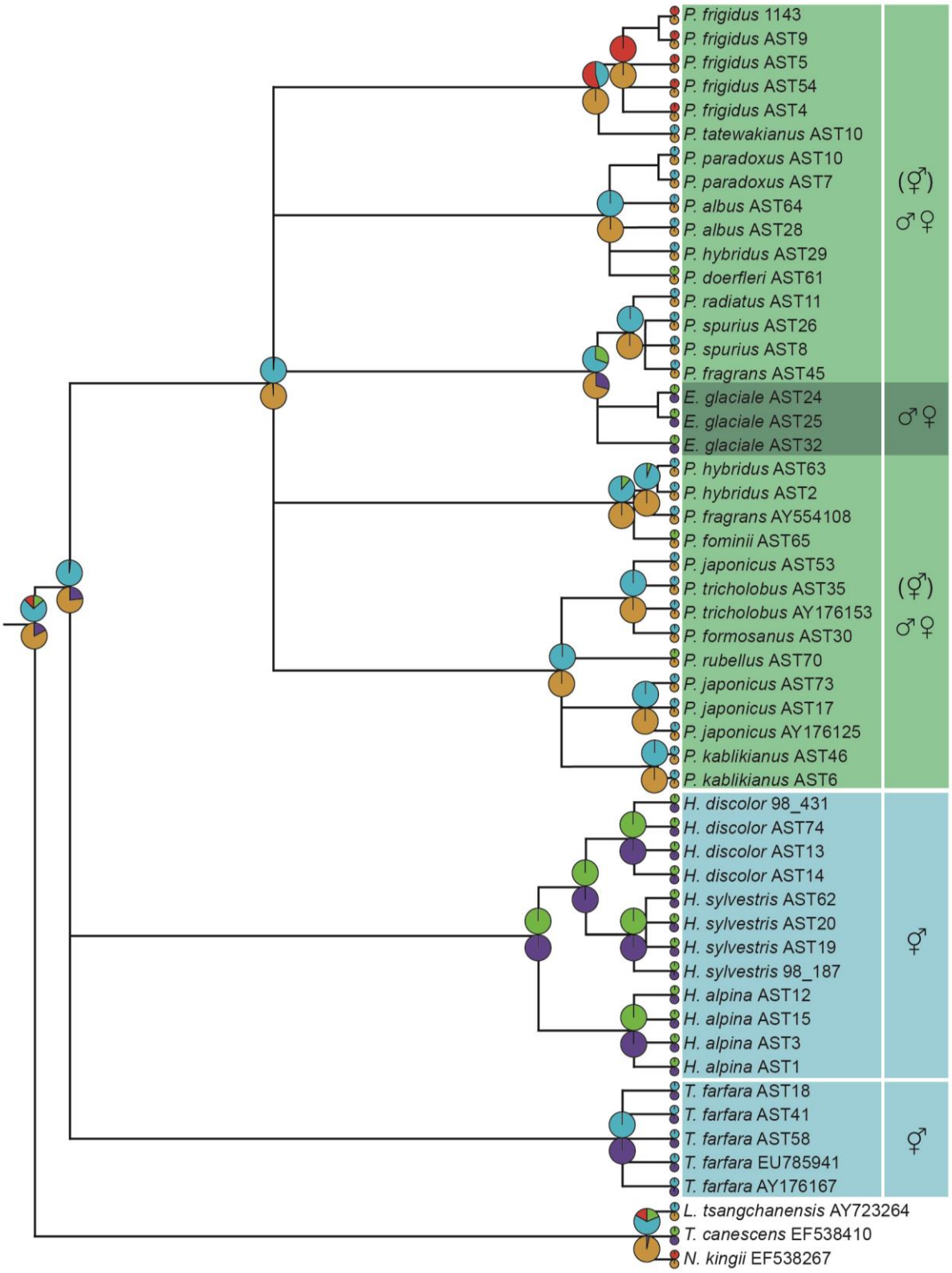

FIG. 5. Cladogram of the *Petasites-Endocellion-Homogyne-Tussilago* clade obtained from the Bayesian analysis of the ITS data set after exclusion of *E. sibiricum*. Circles show the results of the Maximum Likelihood reconstruction of leaf size and number of capitula. Upper circle: blue = leaves more than 10 cm in diameter, green = leaves less than 7 cm in diameter, red = leaves 2-50 cm in diameter; lower circle: violet = capitula single (rarely 2-3), yellow = capitula numerous. The distribution of sexes is depicted for each clade (♀ = female, ♂ = male, ⚥ = hermaphrodite).

Discussion

Monophyly and phylogenetic position of the Petasites-clade in Senecioneae subtribe Tussilagininae. The monophyly of the Petasites-clade, comprising *Petasites*, *Endocellion*, *Homogyne* and *Tussilago*, in the Tussilagininae s.str. had already been recognized by Pelser et al. (2007) using ITS sequences, but only few of the species currently recognized in these four genera were included in that analysis. Based on our extended (and almost complete) taxon sample, we can confirm the results of Pelser et al. (2007) that *Petasites*, *Endocellion*, *Homogyne* and *Tussilago* constitute a highly supported clade. A close relationship of the four genera had already been proposed by Vierhapper (1923) on the basis of morphological characters. Within Tussilagininae s.str. the phylogenetic position of the Petasites-clade is unclear. Relationships among the four monophyletic groups (Petasites-clade: Eurasia and North America, Crocidium-Tetradymia-clade: North America, Aequatorium-Arnoglossum-clade: North and South America, Ligularia-Cremanthodium-Parasenecio complex: Asia) remained largely unresolved in the ITS phylogeny (Pelser et al., 2007), and our own results revealed a basal polytomy in Tussilagininae s.str. However, Pelser et al. (2007) suggested that the Petasites-clade is either sister to the remainder of Tussilagininae s.str. or to an exclusively New World Crocidium-Tetradymia-clade. The first hypothesis was supported by the Maximum Parsimony analysis of the ITS data set, whereas the BI analysis supported the second hypothesis (Pelser et al., 2007). Pelser et al. (2007) included representatives of all genera traditionally placed in Tussilagininae (Bremer, 1994) except for *Digitacalia* Pippen, *Nelsonianthius* H. Rob. and Brettell, *Paracalia* Cuatrec., *Pippenalia* McVaugh, *Psacaliopsis* H. Rob. and Brettell, *Rugelia* Shuttlew ex Chapm., *Villasenoria* B.L. Clark and *Yermo* Dorn. It does not seem likely that these members of Tussilagininae not yet sampled in molecular analyses belong to the Petasites-clade. All these genera with either one or only few species grow in South, Central and North America (Bolivia, Peru, Guatemala, Mexico, southern and western USA; Nordenstam, 2007). Although the phylogenetic position of these taxa in the subtribe is unclear, their distributional range makes it unlikely that they are part of the predominantly northern temperate/boreal Petasites-clade as circumscribed by Pelser et al. (2007). Instead it seems more likely that they are part of one of the two New World clades in Tussilagininae, *viz.* the Crocidium-Tetradymia-clade or the Aequatorium-Arnoglossum-clade. At least *Paracalia* has been assigned to the South American "Gynoxioid group" which is part of the latter clade (Pelser et al., 2007).

Phylogeny, biogeography and classification of Petasites, Endocellion, Homogyne and Tussilago. Our phylogenetic analysis (Fig. 2) of ITS sequence data supports recognition of *Homogyne*, *Tussilago* and *Petasites* including *Endocellion* as monophyletic units which should be assigned generic rank. This conclusion is not contradicted by the cpDNA-based phylogeny (Fig. 3). However, support for intergeneric relationships is lacking. While *Homogyne* has always been treated as a well-circumscribed and distinct genus, species of *Petasites* and *Endocellion* have a more complicated taxonomic history in terms of generic assignment. Species of these two genera have been described in *Tussilago*, *Petasites* or *Nardosmia* Cass. . Even today generic circumscriptions are used inconsistently. For example, Flora of the USSR (Kuprianova, 2000) treats most species of *Petasites* and *Endocellion* as *Nardosmia*, a taxon included in *Petasites* at subgeneric rank according to Toman (1972). Toman (1972) considered *Endocellion* a separate genus. On the other hand, Dingwall in Flora Europaea (1976) treated *E. sibiricum* as *P. sibiricus* (J.F. Gmelin) Dingwall. Our phylogeny supports *Petasites* including *Endocellion* as a monophyletic group.

Endocellion. In the phylogeny inferred from the ITS data set (Fig. 2) *Endocellion* is diphyletic. Whereas *E. sibiricum* forms a clade with *P. frigidus* and *P. tatewakianus*, *E. glaciale* groups with *P. fragrans*, *P. radiatus* and *P. spurius*. In contrast to this, *Endocellion* is monophyletic in the phylogeny based on the cpDNA data set (Fig. 3). This incongruence is probably the result of hybridization between the two genera (Wendel and Doyle, 1998). Hybridization between species of *Petasites* is well documented. Several hybrid taxa have been described (Hegi, 1929; Bogle, 1968; Toman, 1972; Dingwall, 1976; Cherniawsky and Bayer, 1998; Kuprianova, 2000; Bayer et al., 2006), and the wide range of ploidy levels found in *Endocellion* (n = 28, 29, 30, c. 50+, 56) and *Petasites* (n = 10, 14, 16, 26, 28, 29, 30, 40, c. 44, 45, 60; Nordenstam, 2007) probably indicates a high frequency of polyploid hybrid formation in the two groups. *Petasites frigidus*, the species with which *E. sibiricum* groups in the ITS phylogeny, and *E. sibiricum* occur sympatrically in parts of Arctic Siberia and the Russian Far East(Kuprianova, 2000). As the flowering period of *P. frigidus* spans from May to August, and *E. sibiricum* flowers from June to July (Kuprianova, 2000), it is conceivable that the two species could hybridize when growing in close proximity. If this hypothesis were the correct explanation for the incongruence in our data, *P. frigidus* ITS would have introgressed into *E. sibiricum*, which functioned as the maternal parent.

Monophyly of *Endocellion* is supported by several morphological characters. *Endocellion* separated from the rest of *Petasites* by solitary (rarely two or three) capitula in female plants (vs. numerous capitula in *Petasites*), very thin rhizomes (vs. thick and

sometimes tuber-like rhizomes in *Petasites*; Toman, 1972; Kuprianova, 2000), and foliage that develops during flowering (vs. after flowering in *Petasites*). In conclusion, we postulate that the two species of *Endocellion* represent a monophyletic group. Even so, *Endocellion* is clearly nested in *Petasites* and should not be treated as a separate genus. When included in *Petasites*, *E. sibiricum* must be treated as *Petasites gmelinii* (DC.) Polunin and *E. glaciale* as *Petasites glacialis* (Ledeb.) Polunin. The nestedness of *Endocellion* in *Petasites* also implies that the evolutionary transition from hermaphrodite capitula to subdioeciously distributed capitula took place once in the last common ancestor of *Petasites* incl. *Endocellion*. The transition from subdioecy as found in *Petasites* to complete dioecy took place on the branch leading to *Endocellion*.

Petasites. Based on variation in corolla tube shape of pistillate flowers, Toman (1972) divided *Petasites* into three subgenera, namely *Petasites* (comprising *P. albus*, *P. hybridus*, *P. japonicus*, *P. kablikianus*, *P. paradoxus* and *P. tatewakianus*), *Capillopetalum* Toman (*P. tricholobus*, *P. versipilus*) and *Nardosmia* (Cass.) Petermann (*P. doerfleri*, *P. fominii*, *P. fragrans*, *P. frigidus*, *P. radiatus*, *P. rubellus* and *P. spurius*). However, this subgeneric classification is not supported by our phylogeny as these subgenera are not monophyletic. Instead, species of all three subgenera are found in different supported clades. For example, species of subg. *Petasites* can be found in clades I, II, IV and V. The five clades identified by us are instead more geographically congruent. Clade I is largely arctic (northern Eurasia and North America), with *P. tatewakianus* having a Far Eastern distribution. Species of clade III can be found in northern Eurasia, and clades II and IV are western Eurasian with an eastern limit in the Caucasus region. Species of clade V occur in the Far East except *P. kablikianus*, which has a European distribution.

Petasites fragrans and *P. hybridus* were found to be polyphyletic. *Petasites fragrans* is native to northern Africa (Libya, Tunisia, and Algeria) but is naturalized in Western Europe (Dingwall, 1976), Australia (Csurches and Edwards, 1998) and New Zealand (Esler and Astridge, 1987). Accessions from Great Britain (*P. fragrans* AST45) and New Zealand (*P. fragrans* AY554103) were included in our phylogeny, but no material from the native range was available. Whereas the British accession falls into clade III, the accession from New Zealand is part of clade IV. We assume that the New Zealand accession represents the correct phylogenetic position of the species for two reasons. First, *P. fragrans* is the only species of *Petasites* found in New Zealand which makes genetic contamination by hybridization unlikely unless such contamination took place before introduction to New Zealand. Second, the species of clade IV are distributed in Europe, Asia Minor and the Caucasus, and the

distribution of *P. fragrans* fits better here than in the northern Eurasian clade III. In fact, disjunctions between the Caucasus area and northern Africa have been documented for other taxa (Davis and Hedge, 1971, Kadereit, 1996, Zhang et al., 2007). The accessions of *P. hybridus* are found in clades II and IV, which both are western Eurasian. We hypothesize that the accessions in clade IV (*P. hybridus* AST2, AST63) represent the correct position of the species, while the accession in clade II (*P. hybridus* AST29) may represent hybrid material between *P. hybridus* and *P. albus* (*P.* x *rechingeri* Hayek). Accession AST29 was collected in the Caucasus where both *P. hybridus* and *P. albus* occur.

Homogyne. *Homogyne* is a well-defined endemic of the central European high mountains, and the genus has been considered a representative of the old 'Arcto-tertiary stock' of the indigenous Alpine flora (Vierhapper, 1923). Due to its distribution at montane altitudes, *H. sylvestris* was regarded the most basal lineage of the genus by Meusel and Jäger (1992), and Merxmüller (1952) considered *H. discolor* to be the closest relative of *H. alpina*, as they mainly differ in the presence vs. absence of a dense indumentum on the lower leaf surface. In contrast, our phylogeny clearly shows that *H. discolor* and *H. sylvestris* are sister to each other, and that *H. alpina* is sister to these two species.

The three species differ in habitat preference. *Homogyne alpina* is polymorphic both for altitudinal distribution (montane to alpine) and substrate preference (siliceous/calcareous), whereas *H. discolor* and *H. sylvestris* grow solely on calcareous substrates and are restricted to (sub-)alpine and montane to subalpine habitats, respectively. As our phylogeny did not resolve the basal polytomy in the Petasites-clade, character optimization for altitudinal and edaphic preference in *Homogyne* is impossible. However, taking into account that the widespread *Tussilago* and *Petasites* are polymorphic for both altitudinal distribution and substrate preference (Hegi, 1929; Toman, 1972; Dingwall, 1976; Ellenberg, 1996; Cherniawsky and Bayer, 1998; Kuprianova, 2000; Bayer et al., 2006; Landolt et al., 2010; Chen et al., 2011), and that these two properties are also polymorphic in *H. alpina*, it is likely that this ecological polymorphism is ancestral in *Homogyne*. This assumption implies that a fixation of altitudinal (*H. discolor*: (sub-)alpine; *H. sylvestris*: montane to subalpine) and edaphic preference (both species calcareous) took place in the evolution of the genus.

Tussilago. Our phylogeny supports *Tussilago* in its current circumscription as a monospecific genus. *Tussilago farfara* is widely distributed throughout Eurasia (Hegi, 1929; Kuprianova, 2000; Tutin, 1976). Interestingly, we found very little sequence variation within *T. farfara* although we included accessions from Europe (e.g., *T. farfara* AST58) and Asia (e.g., *T. farfara* AST41) in the analysis. *Tussilago farfara* is a typical pioneer species from

open and ruderal habitats. It can form extensive clonal populations by vegetative propagation through rhizomes in a short period of time after wind-dispersal of its achenes (Pfeiffer et al., 2008). Long-distance dispersal together with clonal reproduction might explain low genetic variation in this species.

The age of the Petasites-clade. In the absence of suitable fossils, the molecular dating of the phylogeny had to rely on published ITS substitution rates (Kay et al., 2006). Following this approach, the Petasites-clade started diversification during the Oligocene/Miocene (27.81 – 5.52 My). The onset of diversification of *Homogyne* and the *Endocellion/Petasites* clade took place in the Miocene/Pliocene (13.9 – 2.1/18.2 – 4.34 My), and the arctic/alpine representatives of the *Endocellion/Petasites* clade evolved in Miocene to Pleistocene (9.54 – 0.77 Ma). Although rates of molecular evolution are well known to be taxon-specific (e.g. Sanderson, 2002) and despite the fact that our molecular dating approach resulted in rather broad age estimates, these age estimates do not seem unlikely. For *Homogyne* and the alpine species of *Petasites* it dates diversification to the period of the major uplift of the European Alpine System during the Miocene to Pleistocene (Ager, 1975). The formation of the central European high mountains was accompanied by the evolution of the Alpine flora *in situ* from a 'temperate component' of an otherwise (sub)tropical lowland flora (Ozenda, 1988; Zhang et al., 2001). Our findings support the earlier assumption that *Homogyne* represents the Arcto-tertiary element of the European Alpine flora (Vierhapper, 1923), and we hypothesize that the alpine species of *Petasites* evolved from this Arcto-tertiary element as well. Interestingly, the estimated divergence times of these alpine lineages are substantially older than those of other alpine lineages such as *Anthyllis montana*, *Pritzelago alpina*, *Soldanella*, *Primula* sect. *Auricula*, and *Adenostyles alpina* (Comes and Kadereit, 2003; Dillenberger and Kadereit, in press). These diversified more recently during the Pleistocene and thus support the hypothesis that the Quaternary was a period of very active plant diversification (Kadereit et al., 2004).

The rather scarce fossil record dates the origin of the arctic flora to the Pliocene (5.3 – 1.8 My; Matthews and Ovenden, 1990; Mai, 1995). However, little is known about the time of origin of arctic plants (Abbott and Brochmann, 2003). Comparable to the Arcto-tertiary stock of the Alpine flora, parts of the recent arctic flora may be descended from arctic forests of the late Tertiary (Mai, 1995; Murray, 1995) while other elements may be derived from high mountain taxa in Asia and North America which migrated northwards during the Quaternary (Hultén, 1937; Tolmachev, 1960; Weber, 1965; Hedberg, 1992; Murray, 1995; as reviewed in Abbott and Brochmann, 2003). Our estimated ages date the origin of *Endocellion* (9.07 – 1.55 Ma) and *P. frigidus* (7.43 – 1.27) to the Miocene to Pleistocene. This finding does not support

one or the other of the hypotheses on the origin of the Arctic flora unambiguously. Interestingly, our rather old age estimates of the arctic lineages are in accordance with the results of a molecular dating analysis of *Artemisia* L. (Tkach et al., 2008a) that revealed that some arctic lineages of this genus might also be older than five million years. These age estimates may indicate that Arctic biomes could be older than expected from the fossil record. This hypothesis is supported by oceanographic findings from the Arctic Sea. Sea floor sediments provided evidence of seasonal but regular sea ice and icebergs, calved from glaciers, from about 14 million years ago onwards (Moran et al., 2006). These data make an age older than five million years of arctic lineages at least conceivable.

It is noticeable that the arctic and alpine representatives of the Petasites-clade with small leaves are of almost identical age (4.87 - 3.9 Ma) when mean ages are considered. This result may imply that dwarfism in the Petasites-clade not only evolved several times independently, but that its evolution was possibly driven by the same external trigger. This could have been the dramatic global climatic cooling in the late Pliocene that resulted in the origin of arctic and alpine habitats. However, considering that our mean ages have large confidence intervals, any statement about identical age of the different lineages must be viewed very critically.

Evolution of dwarfism. Based on the tendency for reduction in plant size with decreasing average temperature, as observed across altitudinal and latitudinal transsects (Billings and Mooney, 1968; Bliss, 1971; Johnson, 1969; Körner and Larcher, 1988; Körner, 1999), we expected that the species occurring in the Alps and in the Arctic should be small compared to those from the lowlands or from temperate climates. The reconstruction of ancestral character states for leaf size and capitulum number as measures of dwarfism revealed that decrease in both characters indeed can be observed in arctic/alpine habitats, and evolved several times in the Petasites-clade. Parallel evolution of dwarfism has also been reported in arctic *Artemisia* (Tkach et al., 2008b) and in alpine *Lysimachia* L. sect. *Nummularia* (Kokubugata et al., 2010). In the Petasites-clade, those species living in alpine or arctic environments have both much smaller leaves than other representatives of the group and a reduced number of capitula. This is not only true for the arctic *Endocellion* and the alpine *Homogyne*, but also for the (sub-)alpine *P. doerfleri*, *P. fominii* and *P. rubellus*. These three species also have leaves that are smaller than seven centimeters in diameter and show reduction in capitulum number. They only have three to ten capitula whereas other *Petasites* species have more than ten (up to 130 in *P. hybridus*) capitula (Dingwall, 1976; Cherniawsky and Bayer, 1998; Kuprianova, 2000; Chen et al., 2011). The only exception to the general rule

that all species of the Petasites-clade with both small leaves and a reduced number of capitula grow in arctic or alpine environments is *H. sylvestris*, which grows at montane to subalpine altitudes but has small leaves. As leaf size in *Homogyne* is likely to have been ancestrally small, the small leaves of *H. sylvestris* must be interpreted as a retention of an ancestral character. However, the leaves of *H. sylvestris*, although categorized as small, are larger (3 - 6 cm) than those of *H. alpina* and *H. discolor* (1 - 3 cm), and the species mostly has more than one capitulum per synflorescence. Evolution of leaf size and capitulum number are not completely linked, as is evident in *Tussilago* which has large leaves but only one capitulum per stem.

The pattern of plant size reduction with increasing latitude can also be observed at the intraspecific level. *Petasites frigidus* is widespread throughout northern Europe, northern Asia and North America, where it is found in a wide range of habitats such as wet forests, marshy tundra, peat bogs, alpine and subalpine slopes and disturbed sites like stream banks and roadsides (Dingwall, 1976; Kuprianova, 2000; Bayer et al., 2006). The species shows substantial morphological variation, and leaf diameter can vary from less than 2 cm to 50 cm, and the number of capitula ranges from four to about 40 (Cherniawsky and Bayer, 1998). Within *P. frigidus* size variation seems to be determined by latitude as individuals from the arctic have smaller leaves and a smaller number of capitula than those from lower latitudes.

Although, with the exception of *H. sylvestris*, all species with small leaves plus a reduced number of capitula grow at high altitudes or latitudes, not all species of the *Petasites* clade growing at high altitudes or latitudes have small leaves and a reduced number of capitula, implying that other factors than temperature must influence leaf size and number of capitula. An example for this is *P. paradoxus* from the European Alps. Although this species grows at (sub-)alpine altitudes, it has leaves measuring up to 30 cm in diameter and numerous capitula. *Petasites paradoxus*, which grows at the same altitudes in the European Alps as *H. alpina* and *H. discolor*, which are true dwarfs with leaves less than 5 cm in diameter and only one, rarely two or three capitula. Whereas the two species of *Homogyne* grow in stable habitats with a low soil nutrient content and a dense vegetation cover, such as (sub-)alpine swards, dwarf shrub stands and coniferous forests, *P. paradoxus* mostly grows in often disturbed habitats with an open vegetation cover such as river banks, alluvia and scree as well as nitrophilous and tall herb communities, all of which have a higher soil nutrient content (Landolt et al., 2010). This comparison suggests that factors such as nutrient availability, habitat disturbance and competition can counteract the trend for decrease of leaf size and capitulum number. However, the three species of *Petasites* with small leaves and a small

number of capitula only partly fit this pattern. Whereas *P. fominii* like *H. alpina* and *H. discolor* grows in alpine meadows, *P. doerfleri* is restricted to wet screes and *P. rubellus* grows on stony slopes, along stream banks and at forest margins.

Conclusions

Although our study allows us to conclude that, with the exception of *H. sylvestris*, all species with small leaves and a small number of capitula grow at high altitudes or latitudes, the reverse conclusion is unjustified. This illustrates that reduction of size with increasing altitude and latitude is a rule but not a law. Factors governing the evolution and geographical distribution of the species investigated may be, besides temperature, nutrient availability, habitat disturbance and competition. For nutrient availability, this finding is in accordance with the conclusions drawn by Ordoñez et al. (2009), who in a global study of relationships between leaf traits, climate and soil fertility showed that "plants with leaf traits that allow a fast use of nutrients and growth but for shorter times, like high SLA (specific leaf area) and high LNC (leaf N concentration), were found at high nutrient supply, while the reverse occurred at low nutrient supply where conservation of nutrients is arguably more important". Detailed ecological studies of the Petasites-clade are required in order to conclusively identify the factors governing the evolution of the characters discussed here.

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Genetic basis of edaphic preference in *Homogyne*: a cultivation experiment

Introduction

Homogyne Cass. is endemic to the European Alpine System (sensu Ozenda, 1988). It comprises three species with rounded, evergreen leaves and single, rarely two or three capitula. The three species show pronounced differences in their edaphic and altitudinal preference. *Homogyne alpina* Cass. is widespread throughout the Central European high mountains, with a more or less continuous distribution in the Alps and adjacent mountain ranges (Sudety Mountains, Ore Mountains, Bohemian Massif, Tatras, Carpathians) and rarer occurrences in the Pyreness/Cantabria and the Balkans (Meusel & Jäger, 1992; Fig. 1). The species is ecologically variable with a broad edaphic and altitudinal tolerance growing on both siliceous and calcareous substrates in a wide range of habitats including montane coniferous and deciduous forests, subalpine dwarf-shrub heaths and alpine grasslands (Hegi, 1929; Tutin, 1976; Aeschimann et al., 2004; Fischer et al., 2005). In contrast, *H. discolor* Cass. and *H. sylvestris* Cass. are more restricted in their edaphic preference by growing exclusively on calcareous substrates. Their distribution is confined to the peripheral limestone and dolomite ranges of the Eastern Alps (Meusel & Jäger, 1992; Fig. 1). The two species also differ in their altitudinal preference: *H. discolor* occurs in subalpine and alpine habitats such as subalpine dwarf-shrub heaths, alpine grasslands and snow beds, while *H. sylvestris* is largely restricted to coniferous and deciduous forests of the montane and subalpine altitudinal belts (Hegi, 1929; Tutin, 1976; Aeschimann et al., 2004; Fischer et al., 2005).

A dated molecular phylogeny of the genus (Steffen & Kadereit, submitted) revealed the evolution of habitat preference. *Homogyne* was monophyletic, and the ecologically variable *H. alpina* was sister to the ecologically more restricted *H. discolor*/*H. sylvestris*. The widespread *Tussilago* L. and *Petasites* Mill. formed a polytomy together with *Homogyne*. Like *H. alpina*, these genera are polymorphic for both altitudinal and edaphic preference indicating that ecological variability probably is ancestral in *Homogyne* and that a fixation of altitudinal and edaphic preference took place in the evolution of the genus. Speciation in *Homogyne* was dated to the Miocene to Pleistocene (13.93 - 2.11 million years ago).

Random amplified polymorphic DNA (RAPD) variation among populations of *H. alpina* and *H. discolor* in the Eastern Alps provided insight into the different biogeographical histories of these species (Uhink, 1999). *Homogyne alpina* showed no phylogeographical structure in this area, and the data indicated high recent gene flow and/or postglacial migration into the area sampled. In contrast to this, *H. discolor* showed distinct regional

genetic sub-structuring, indicating lower levels of gene flow. The data pointed towards displacement of this species into one northeastern and two southeastern refugia. These three areas coincide with the distribution of glacial refugia as reconstructed by Schönswetter et al. (2005).

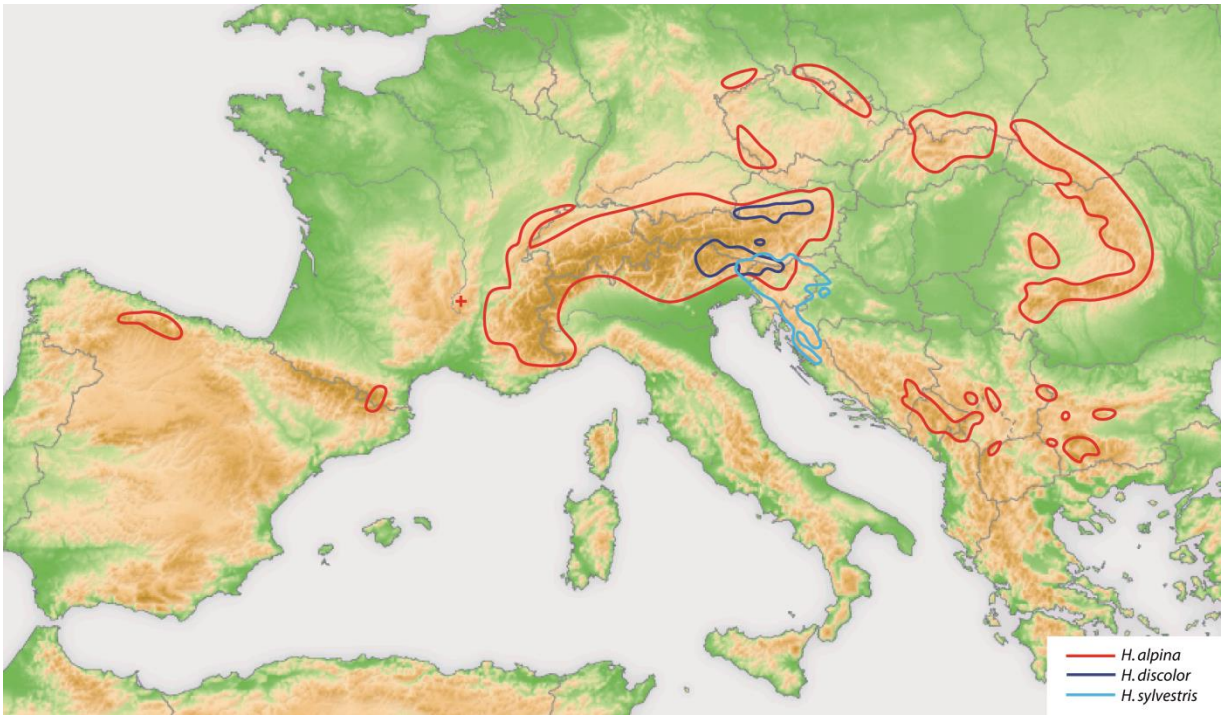

FIG. 1. Distribution of *Homogyne* (after Meusel and Jäger, 1992).

The finding of 1) the evolutionary change of edaphic (and altitudinal) preferences in the Miocene to Pleistocene, 2) last glacial refugia of *H. discolor* in areas with calcareous substrate, and 3) the absence of *H. alpina* from these refugia led to the hypothesis that range shifts into areas with different ecological conditions were the driving force for differentiation and speciation in *Homogyne*.

We conducted a common garden experiment to test for genetic fixation of edaphic preference in *H. alpina* and *H. discolor*. In a first approach, we investigated germination behaviour of the two species on both calcareous and siliceous substrates. In a second step we carried out a cultivation experiment by transplanting adult plants from natural habitats into calcareous and siliceous substrates, respectively.

Material and Methods

Soil analysis. Soils for the cultivation experiments were collected in four different localities, two from calcareous and two from siliceous bedrock (Table 1). The soils were taken in the rhizosphere of alpine mats containing *Homogyne*. Soil reaction was determined

following the protocol of Emde and Szöcs (2009). To measure soil pH, 5 g of air-dried soil samples were suspended in a 12.5 ml 0.01 M $CaCl_2$ solution. After an incubation time of 0.5 h, the solution was resuspended and the pH was measured with a pH meter (MultiCal® pH 526; WTW, Weilheim, Germany). The pH was read after 30 s of stability. The carbonate content of the two soils from calcareous bedrock (C1 and C2) was determined using the Scheibler-apparatus. For the measurement 0.3-3.3 g air-dried soil sample was moistened with distilled water, ca. 10 ml 10% HCl were added, and the amount of CO_2 produced was measured 5 min after volume increase stopped.

TABLE 1. Soil samples, locality information, pH and carbonate content.

Substrate	Locality	pH ($CaCl_2$)	Carbonate content
S1	Austria, Styria, Stuhleck	3.37	
S2	Italy, Friuli-Venezia, Ravascletto	4.16	
C1	Austria, Styria, Schießlingsalm	6.96	54.38%
C2	Italy, Veneto, Passo Mt. Tre Croci	7.27	57.03%

Germination experiment. Seeds of *H. alpina* and *H. discolor* were collected in August 2009 (Table 2). They were taken directly from plants, air-dried and kept at room temperature in paper bags. All seeds collected were examined under a dissecting microscope and all damaged or apparently sterile seeds were discarded. In total, 1775 seeds from 84 individuals from seven populations (3 from siliceous, 4 from calcareous bedrock) of *H. alpina* and 928 seeds from 61 individuals from nine populations of *H. discolor* were used for the germination experiment conducted in May/June 2010. For the germination experiment petri dishes filled with one of the collected soils (C1, C2, S1, S2; referred to as plots hereafter) were used. All seeds collected from one parental individual were divided into four portions and sown on one of the four different substrates in the petri dishes; the maximum number was five seeds per petri dish. The experiment was conducted indoors at room temperature; seeds and seedlings were watered with distilled water and ventilated daily. Germination was observed daily over a period of 21 days, and the appearance of new seedlings was recorded.

TABLE 2. Seed material for germination experiment.

	Population/Locality	Individual	No. of seeds				
			total	S1	S2	C1	C2
H. alpina	Austria, Salzburg, Tauerntunnel, Zederhaus	100809/2-1	16	4	4	4	4
		100809/2-2	16	4	4	4	4
		100809/2-4	25	7	6	6	6
	Italy, Friuli-Venezia, Ravascletto, Passo Tualis-Mt. Crostis	110809/7-1	44	11	11	11	11
		110809/7-2	37	9	10	9	9
		110809/7-3	27	6	7	7	7
		110809/7-4	40	10	10	10	10
		110809/7-5	47	11	12	12	12
		110809/7-6	18	4	4	5	5
		110809/7-7	28	7	7	7	7
		110809/7-8	36	9	9	9	9
	Austria, Salzburg, Tauernpass between Obertauern and Tweng (B99)	090809/1-1	24	6	6	6	6
		090809/1-2	14	4	3	3	4
		090809/1-3	11	3	3	3	2
		090809/1-4	14	4	3	3	4
		090809/1-5	12	3	3	3	3
		090809/1-6	21	5	6	5	5
		090809/1-7	38	9	10	9	10
		090809/1-8	25	7	6	6	6
		090809/1-9	12	3	3	3	3
		090809/1-10	30	7	8	8	7
		090809/1-11	23	6	6	6	5
		090809/1-12	22	5	5	6	6
		090809/1-13	14	3	4	3	4
	Austria, Styria, Donnersbach, Planneralm	080809/5-1	15	4	4	4	3
		080809/5-2	23	6	5	6	6
		080809/5-3	15	4	4	3	4
		080809/5-4	33	8	8	9	8
		080809/5-5	42	10	11	10	11
		080809/5-7	36	9	9	9	9
		080809/5-8	32	8	8	8	8
		080809/5-9	31	8	7	8	8
		080809/5-10	15	3	4	4	4
		080809/5-11	13	3	3	3	4
		080809/5-13	19	4	5	5	5
		080809/5-14	15	4	3	4	4
		080809/5-15	30	8	7	8	7
		080809/5-17	18	5	5	4	4
		080809/5-18	13	3	4	3	3
		080809/5-19	26	6	7	7	6

TABLE 2 continued.

		080809/5-20	22	6	5	5	6
		080809/5-21	19	5	5	5	4
		080809/5-22	16	4	4	4	4
		080809/5-23	12	3	3	3	3
		080809/5-24	39	10	9	10	10
		080809/5-25	25	6	6	6	7
	Austria, Salzburg, Obertauern, Grünwaldkopf	090809/3-1	22	5	6	6	5
		090809/3-2	18	4	5	4	5
		090809/3-3	47	12	12	12	11
		090809/3-4	44	11	11	11	11
		090809/3-5	29	8	7	7	7
		090809/3-6	44	11	11	11	11
	Germany, Bavaria, Hoher Göll	060809/2-2	23	5	6	6	6
		060809/2-3	6	2	1	1	2
		060809/2-4	22	5	6	6	5
		060809/2-5	26	7	6	6	7
		060809/2-6	12	3	3	3	3
		060809/2-7	6	1	2	1	2
		060809/2-8	17	4	4	5	4
		060809/2-9	27	7	7	6	7
		060809/2-10	9	2	2	3	2
		060809/2-11	20	5	5	5	5
		060809/2-12	5	2	1	1	1
		060809/2-13	4	1	1	1	1
		060809/2-15	6	2	1	2	1
		060809/2-16	25	6	7	6	6
		060809/2-17	22	5	6	6	5
		060809/2-18	4	1	1	1	1
		060809/2-19	17	4	4	4	5
		060809/2-20	6	1	2	2	1
		060809/2-22	20	5	5	5	5
		060809/2-23	22	6	5	5	6
		060809/2-24	4	1	1	1	1
		060809/2-25	19	5	5	4	5
		060809/2-26	13	3	3	3	4
		060809/2-27	3	1	0	1	1
		060809/2-28	6	2	1	2	1
		060809/2-29	18	4	5	5	4
	Austria, Salzburg, Schneibstein	070809/2-1	22	6	5	5	6
		070809/2-2	7	2	2	1	2
		070809/2-3	37	9	10	9	9
		070809/2-4	13	3	3	4	3
		070809/2-5	9	2	2	2	3
		070809/2-6	4	1	1	1	1
H. discolor	Austria, Carinthia, Gartnerkofel	120809/3-1	14	3	4	4	3
		120809/3-2	20	5	5	5	5

TABLE 2 continued.

	120809/3-3	1	0	0	1	0
	120809/3-4	30	8	7	7	8
	120809/3-6	48	12	12	12	12
	120809/3-8	23	6	6	5	6
	120809/3-9	29	6	8	7	8
	120809/3-10	15	4	3	4	4
	120809/3-11	30	7	7	8	8
	120809/3-12	22	5	6	6	5
	120809/3-13	34	9	9	8	8
	120809/3-14	28	7	7	7	7
Italy, Veneto, Strada 619	110809/6-2	2	0	1	1	0
	110809/6-3	26	7	6	6	7
Austria, Salzburg, Tauerntunnel, Zederhaus	100809/3-1	22	6	6	5	5
	100809/3-4	5	1	2	1	1
	100809/3-5	5	1	1	2	1
	100809/3-6	8	2	2	2	2
	100809/3-7	19	5	5	4	5
	100809/3-8	8	2	2	2	2
	100809/3-9	6	2	1	2	1
Austria, Carinthia, Villacher Alpe	1300809/1-1	29	7	7	7	8
	130809/1-2	15	4	4	4	3
	130809/1-3	19	5	4	5	5
	130809/1-4	22	5	6	5	6
Austria, Styria, Tauplitzalm	080809/8-7	8	2	2	2	2
	080809/8-8	3	1	1	1	0
	080809/8-12	11	3	3	3	2
Austria, Salzburg, Dachstein	090809/6-1	14	3	4	3	4
	090809/6-2	21	6	5	5	5
	090809/6-3	8	2	2	2	2
	090809/6-4	7	2	1	2	2
	090809/6-5	29	7	8	7	7
	090809/6-7	17	4	4	4	5
	090809/6-8	4	1	1	1	1
	090809/6-9	4	1	1	1	1
	090809/6-10	10	2	3	2	3
	090809/6-11	19	5	5	5	4
	090809/6-12	2	1	0	1	0
Austria, Carinthia, Feistritz/Bleiburg, Petzen	140809/1-1	20	5	5	5	5
	140809/1-3	14	4	3	3	4
	140809/1-4	11	2	3	3	3
	140809/1-5	16	4	4	4	4
	140809/1-6	8	2	2	2	2
	140809/1-7	20	5	5	5	5
	140809/1-8	22	6	6	5	5
	040809/1-9	8	2	2	2	2

TABLE 2 continued.

	140809/1-10	17	4	4	5	4
	140809/1-11	13	3	4	3	3
	140809/1-12	3	1	1	1	0
	140809/1-13	10	3	2	2	3
	140809/1-14	1	0	0	1	0
	140809/1-15	14	3	4	3	4
	140809/1-16	9	2	2	2	3
	140809/1-17	17	5	4	4	4
	140809/1-x	12	3	3	3	3
Austria, Upper Austria, Pryhnpass, Wurzelalm	080809/4-1	11	2	3	3	3
	080809/4-2	5	1	2	1	1
	080809/4-3	14	3	4	4	3
	080809/4-4	6	2	1	2	1
	080809/4-5	17	4	5	4	4
	080809/4-6	18	5	4	4	5
Austria, Styria, Seewiesen	150807/3	25	7	6	6	6

Differences in germination behaviour were statistically analyzed in GenStat 10.1 (VSN International, 2011) using a linear mixed model with restricted maximum likelihood (REML). The model was: germination rate = species + soil type + soil(plots) + soil(individuals). Germination rate was considered as dependent variable. Species and soil type were fixed factors, and plots and individuals were nested within soil and considered random effects.

Cultivation experiment. In June 2010, seedlings obtained from the germination experiment were planted into pots with the same substrate as used for their germination. In total, 781 *H. alpina* seedlings (427 on siliceous, 354 on calcareous substrate), and 133 *H. discolor* seedlings (82 on siliceous, 51 on calcareous substrate) were potted. Pots were 7 cm in diameter and contained one individual each. The experiment was conducted in Mainz Botanic Garden, and plants were watered with rain water when necessary. Survival of individuals was observed.

In a second step, a reciprocal transplantation experiment with adult plants was conducted. Fourty individuals of *H. alpina* and *H. discolor*, respectively, from the northern limestone range of the Alps (Austria, Styria, Tauplitzalm) and 40 individuals of *H. alpina* from the central siliceous Alps (Austria, Styria, Planneralm) were collected in September 2010. Only healthy plants with well-developed rosettes were chosen. Plots of soil containing plants were dug out without destroying the roots. The roots were carefully washed to remove soil and organic matter and single plants were planted in 10x10 cm pots. These were filled

with a 3 cm drainage layer of coarse quartz sand, a drainage mat and either soil S2 or C2. To improve water and air permeability, the soils were mixed with ca. 20% polystyrene (diameter 0.2 – 2.0 cm). In total, 40 individuals of *H. alpina* (20 from siliceous, 20 from calcareous bedrock) and 20 individuals of *H. discolor* were planted on siliceous and calcareous soil, respectively. Plants were kept outdoors in Mainz Botanic Garden, and were watered with rain water as necessary. Leaf growth and survival were observed from March to August 2011.

Results and Discussion

Soil analysis. Table 1 shows the soil reaction for each substrate sampled. The soils from calcareous and siliceous bedrock had a clearly different pH. The substrates from calcareous bedrock (C1, C2) were neutral to basic (pH 6.96 – 7.27), while soils collected from siliceous bedrock (S1, S2) were strongly acidic (pH 3.37 – 4.18). The two soils from calcareous bedrock were extremely rich in carbonate (54.38 – 57.03%).

Germination experiment. Table 3 summarizes germination rates of *H. alpina* and *H. discolor* on the four different substrates. Germination rates differ significantly between the two species (Wald-test, $P < 0.001$). The overall germination rate of *H. alpina* was 65.59%, while it was only 22.1% for *H. discolor*. Soil type had no significant effect on germination ($P = 0.88$), and the estimated effect for soil type was only 2.07%. The latter finding is consistent with the results of Gigon (1971) and Fossati (1980), who found that chemical soil properties had no effect on germination behaviour of calcicole and calcifuge plants. Instead, these authors emphasized that germination of many alpine plants is independent of the substrate, and chemical soil properties affect the emergence of plants only after the nutritive tissue is depleted.

TABLE 3. Germination rates of *H. alpina* and *H. discolor* on the four different substrates (S1, S2: siliceous substrate; C1, C2: calcareous substrate; Ø: average on siliceous/calcareous/all substrates).

	S1	**S2**	**Ø S**	**C1**	**C2**	**Ø C**	**Ø total**
H. alpina	59.13%	75.28%	**67.21%**	72.73%	55.20%	**63.97%**	**65.59%**
H. discolor	13.50%	37.50%	**25.50%**	16.95%	20.43%	**18.69%**	**22.1%**

Cultivation experiment. Table 4 summarizes survival of seedlings of *H. alpina* and *H. discolor* two weeks after the plants had been transplanted into pots. On average, 46.22% of *H. alpina* survived, and there was no difference between the different substrates. Survival of *H. discolor* was lower and 26.73% on average. Survival differed on the different substrates: it was 34.48% on the calcareous soils, while it was only 18.97% on the siliceous substrates. This

finding might indicate a difference in seedling establishment: seedlings of *H. discolor* seem to have better abilities to establish on calcareous than on siliceous soils. However, due to very low total numbers of surviving individuals this observation cannot be statistically tested. In consequence, the experiment was terminated.

TABLE 4. Survival of *H. alpina* and *H. discolor* seedlings two weeks after transplantation on the four different substrates (S1, S2: siliceous substrate; C1, C2: calcareous substrate; Ø: average on siliceous/calcareous/all substrates).

	S1	**S2**	**Ø S**	**C1**	**C2**	**Ø C**	**Ø total**
H. alpina	83.66%	7.11%	**45.39%**	57.29%	36.78%	**47.04%**	**46.22%**
H. discolor	31.58%	6.35%	**18.97%**	42.86%	26.09%	**34.48%**	**26.73%**

Performance of adult plants on the different substrates was observed over a period of five months. Table 5 summarizes leaf growth and mortality. Both factors indicate that *H. alpina* performed better on calcareous than on siliceous soil, and individuals collected from silicate showed higher leaf growth than those originating from limestone. Compared to *H. discolor*, mortality was slightly lower in *H. alpina*. *Homogyne discolor* clearly performed better on the calcareous substrate: plants developed on average three new leaves, while they only developed 0.545 new leaves when grown on the siliceous substrate. Also mortality was higher on siliceous soil (45% vs. 30%). However, the findings can only be carefully interpreted as a trend as the sample size is too small to allow rigorous statistical tests. Nonetheless, all results (plant establishment and adult plant growth) indicate that the preference for calcareous substrate of *H. discolor* is indeed genetically fixed. This interpretation would support the hypothesis that ecological factors were important for speciation in *Homogyne*.

Table 5. Leaf growth and mortality of *H. alpina* and *H. discolor* on siliceous and calcareous substrate. Leaf growth is given as average number of new leaves developed from March to August 2011; mortality over that period is given as percentage.

	Siliceous soil		**Calcareous soil**	
	Leaf growth	mortality	Leaf growth	mortality
***H. alpina* (silicate)**	1,75	40%	2,733	20%
***H. alpina* (limestone)**	1,167	40%	1,562	25%
H. discolor	0,545	45%	3	30%

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Parallel evolution of flower reduction in two alpine species of *Soldanella* L. (Primulaceae)

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Abstract

The European endemic *Soldanella* traditionally has been divided into two morphologically well-defined sections. Section *Tubiflores* contains two species growing in high-elevation habitats, whereas most of the fourteen species of sect. *Soldanella* inhabit montane forests. Section *Tubiflores* has a reduced flower morphology compared to sect. *Soldanella*. A previous phylogenetic study based on ITS and AFLP data revealed, although the genus *Soldanella* itself is monophyletic, both sect. *Soldanella* and sect. *Tubiflores* are paraphyletic. *Soldanella alpina* (sect. *Soldanella*) formed a clade with *S. minima* and *S. pusilla* (sect. *Tubiflores*), and the grouping of *S. alpina* with *S. pusilla* had been hypothesized to be the result of hybridization between *S. pusilla* and an unidentified species of sect. *Soldanella*. We re-examined phylogenetic relationships among the above species by using additional sequence data (cpDNA) and by increasing the sample for ITS and AFLP data. Our new data confirmed that *S. alpina* is most closely related to *S. pusilla*. However, our data do not provide any evidence in support of the hypothesis that *S. alpina* is a hybrid species. In consequence, we consider it likely that the two species of sect. *Tubiflores* originated independently. The reduced flower morphology of these two alpine species probably is evidence for increased levels of self-pollination assuring reproductive success in a high-altitude habitat.

Additional keywords

AFLP – European Alpine System – cpDNA sequences - genetic structure – hybrid speciation – ITS – outbreeding – phylogeny – self-pollination

Introduction

At the beginning of the growing season, flowers of *Soldanella* L. (Primulaceae) constitute a conspicuous element of the Alpine flora. They start flowering immediately after snow-melt, when the soil is still saturated with melt-water, with the scapes often even melting their way through the remaining snow cover (Schröter, 1908; Hegi, 1927). Flower bud formation in the previous growing season is a prerequisite for early anthesis (Lona, 1968; Körner, 1999). The campanulate to funnel-shaped flowers are usually violet and melittophilous (Müller, 1881; Hegi, 1927).

Soldanella is a well-defined genus endemic to the European Alpine System (sensu Ozenda, 1988) and comprises 16 species and four additional subspecies of perennial herbs (Zhang & Kadereit, 2002). The genus has traditionally been divided into two morphologically well-defined sections, mainly on the basis of floral characters (Borbás, 1901; Vierhapper, 1904a; Knuth, 1905; Pawlowska, 1972; Meyer, 1985). Section *Soldanella* contains relatively large plants (13-35 cm) with several flowers per scape. The corolla is funnel-shaped with long lobes and distinct throat scales (Fig. 1). Connective appendages are long and caudate, and the style is long, placing the stigma outside the corolla tube. The capsule opens with ten teeth. Species assigned to sect. *Tubiflores* are relatively small plants (<10 cm) with only one flower per scape. The campanulate corolla has short lobes with nectar guides, throat scales are usually absent or, if present, they are very small (Fig. 1). The short connective appendages are acuminate, and the style is short placing the stigma inside the corolla tube. The capsule has five teeth (Zhang, Comes & Kadereit,2001; Zhang & Kadereit 2002).

Fourteen of the 16 species of *Soldanella* belong to sect. *Soldanella,* and only two to sect. *Tubiflores*, namely *S. minima* Hoppe and *S. pusilla* Baumg. The two sections do not only differ morphologically, but also ecologically. Both species of sect. *Tubiflores* grow in alpine habitats above timberline, where they inhabit snowbeds, grassy slopes and wet meadows. In contrast, the species of sect. *Soldanella* are largely confined to montane coniferous and/or deciduous forests. The only exceptions to this in sect. *Soldanella* are *S. rugosa* L.B. Zhang, an endemic of alpine habitats in the eastern Carpathians, and *S. alpina* L. This latter species is widespread throughout the European Alpine System where it can be found in both montane and alpine habitats (Zhang *et al.*, 2001; Zhang & Kadereit 2002).

A phylogenetic study of *Soldanella* using internal transcribed spacer sequence data (ITS) and Amplified Fragment Length Polymorphisms (AFLPs) showed that *Soldanella* is monophyletic and originated in the Quaternary (Zhang *et al.*, 2001). The data did not support the morphologically well-founded subdivision of the genus into two sections. Instead, it was

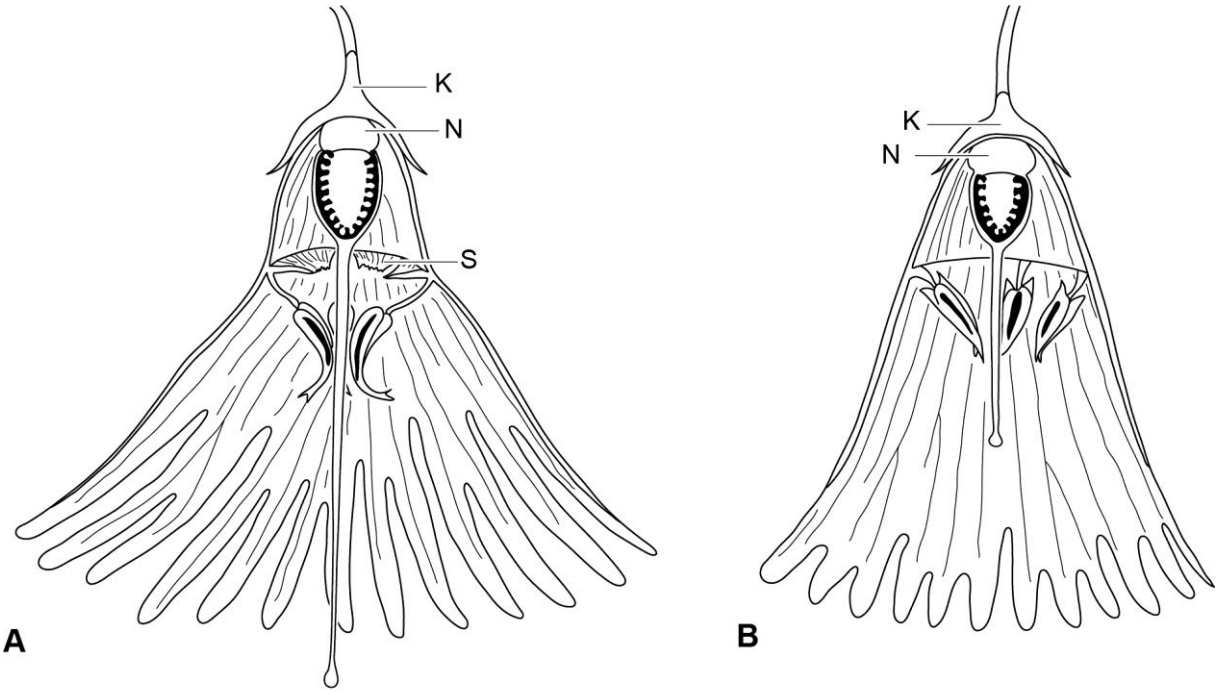

FIG. 1. Differences in floral morphology between "sect. *Soldanella*" and "sect. *Tubiflores*". Longitudinal sections through flowers of A. *S. alpina* and B. *S. pusilla.* K = calyx; N = nectary; S = corolla throat scales (after Müller 1881 and Schroeter 1908).

found that sect. *Soldanella* is paraphyletic in relation to a non-monophyletic sect. *Tubiflores*. Specifically, *S. pusilla* (sect. *Tubiflores*) was found to be more closely related to *S. alpina* (sect. *Soldanella*) than to *S. minima* (sect. *Tubiflores*), but these three species formed one of two major (but unsupported) clades of the genus. The other major clade was formed by the remaining species of sect. *Soldanella*. Zhang *et al.* (2001) explained this unexpected finding by hypothesizing that *S. alpina* represents a homoploid hybrid species with *S. pusilla* as one parent and an unknown species of sect. *Soldanella* as the second parent. If *S. alpina* should indeed be a hybrid species, this would be of particular interest because its morphology is in no way intermediate between the putative parents'. As an alternative explanation, Zhang *et al.* (2001) discussed the possibility of a parallel origin of *S. minima* and *S. pusilla*. Zhang *et al*.'s (2001) data further implied that the alpine species of the genus originated from montane taxa. Based on this molecular evidence, the subdivision of *Soldanella* into two sections was not accepted in the latest taxonomic treatment of the genus (Zhang & Kadereit, 2002).

In this study, we will re-investigate phylogenetic relationships in *Soldanella* in order to confirm or not the non-monophyly of sect. *Tubiflores* shown in both the ITS and AFLP data sets of Zhang *et al.* (2001). We will further examine the two competing hypotheses

explaining the non-monophyly of sect. *Tubiflores*, i.e., parallel origin of *S. minima* and *S. pusilla* vs. hybrid origin of *S. alpina*. We will do this by extending the intraspecific sample of ITS sequences in comparison to Zhang *et al.* (2001), by using sequences from three different chloroplast regions, and by conducting an AFLP analysis of a large sample of individuals. This will be done for *S. alpina* (sect. *Soldanella*) and *S. minima* and *S. pusilla* (sect. *Tubiflores*) as one of the two major clades of the genus identified by Zhang *et al.* (2001), as well as for *S. major* (Neilr.) Vierh. and *S. montana* Willd. The latter two species were chosen as representatives of the two major subclades of the second major clade of *Soldanella* identified by Zhang *et al.* (2001). Also, *S. major* and *S. montana*, besides *S. alpina*, are the only two species of sect. *Soldanella* in the Alps. Given that the Alps are the only area of overlap between *S. alpina* and *S. pusilla* and (except for a small area in the central Apennines) *S. minima*, if the hybridization hypothesis is correct, *S. major* and *S. montana* would be good candidates for the sect. *Soldanella* parent of *S. alpina*.

Material and Methods

Taxon sampling. Our phylogenetic analysis of ITS is based on the published ITS phylogeny of *Soldanella* (Zhang *et al.*, 2001) which included all recognized species. ITS sequences from that study were obtained from GenBank, and were extended by adding newly generated sequences for *S. alpina* (5 sequences), *S. major* (2), *S. montana* (2; all sect. *Soldanella*), *S. minima* (4) and *S. pusilla* (5; both sect. *Tubiflores*). To further investigate relationships among these five species we reconstructed their phylogeny using cpDNA sequences (*psb*J-*pet*A–*ndh*J-*trn*F–*psb*D-*trn*T). Relationships among individuals and genetic structure of species were also examined in an AFLP study. For the latter, we sampled 2-4 individuals per population from 20 populations. In total, 71 *Soldanella* individuals were examined, including 24 individuals from six populations of *S. alpina*, 17 individuals from five populations from *S. pusilla*, 13 individuals from four populations of *S. minima* subsp. *minima*, three individuals from one population of *S. minima* subsp. *austriaca* (Vierh.) Lüdi, eight individuals from two populations of *S. major* and six individuals from two populations of *S. montana*. Leaf material of all plants was collected in 2007 and preserved in silica gel. Herbarium vouchers are deposited at MJG. Voucher and location information is given in Table 1.

TABLE 1. Materials, voucher information, DNA accession numbers (* only DNA accessions used for sequencing), GenBank accession numbers, and numbers of individuals sampled per population for AFLP.

Taxon	**Country; collector, collection no.; Herbarium, Herbarium no.**	**DNA accession no.***	**GenBank accession no.**				**AFLP no. of ind.**
			ITS	psbJ-petA	ndhJ-trnF	psbD-trnT	
Douglasia alaskana (Coville & Standl. ex Hultén) S.Kelso	USA, Alaska; collector and herbarium unknown		AF260774	-	-	-	-
Douglasia beringensis S.Kelso, Jurtzev & D.F.Murray	USA, Alaska; collector and herbarium unknown		AF260773	-	-	-	-
Primula bulleyana Forrest	China, Yunnan, cult. (Mainz Botanical Garden); Zhang 010499; MJG		AJ306362	-	-	-	-
Primula cuneifolia Ledeb.	Japan, Hokkaido; Fujii 290792 (KANA)		EU887002	-	-	-	-
Primula farinosa L.	Cult. (Royal Botanic Gardens, Kew)		AF260772	-	-	-	-
Primula hirsuta	Switzerland, Graubünden; Schorr 40-7; MJG		-	+	+	+	-
	France, C Pyrenees; Kropf 270799; MJG		AJ427779	-	-	-	-
Primula latifolia Lapeyr.	France, Alpes Maritimes; Zhang 060699; MJG		AJ306363	-	-	-	-
Primula parryi A.Gray	USA, Colorado; Hogan 2493; OSC		AF306364	-	-	-	-
Primula tschuktschorum Kjellm.	USA, Alaska; collector and herbarium unknown		EF538267	-	-		
Soldanella alpina L. subsp. *alpina*	Spain, W Pyrenees; Zhang 160399; MJG		AJ306322	-	-	-	-
	Austria, Carinthia; Zhang 010499; MJG		AJ306323	-	-	-	-
	Switzerland, Waadt; Kropf 010698; MJG		AJ306321	-	-	-	-
	Montenegro; Juva 1970; H		AJ306324	-	-	-	-
	Austria, Salzburg; Steffen 080807/1; MJG	P11	+	+	+	+	4
	Austria, Styria; Steffen 090807/3; MJG	P6	+	+	+	+	4
	Austria, Carinthia; Steffen 110807/1; MJG	P12	+	+	+	+	4
	Austria, Carinthia; Steffen 130807/1; MJG		-	-	-	-	4

TABLE 1 continued.

	Austria, Carinthia; Steffen 150807/2; MJG	P5	+	-	-	-	4
	Austria, Tyrol; Steffen 150807/4; MJG	P13	+	+	+	-	4
Soldanalle alpina subsp. *cantabrica* Kress	Spain, Cantabria; López & Váldes 4148 EV; MA		AJ306325	-	-	-	-
Soldanella angusta L.B.Zhang	Romania, E Carpathians; Groza 110889; LI		AJ306326	-	-	-	-
Soldanella calabrella Kress	Italy, Calabria; Poelt 110788; GZU		AJ306327	-	-	-	-
Soldanella carpatica Vierh.	Poland, High Tatra; Drescher 090793; GZU		AJ306328	-	-	-	-
	Slovakia, Velka Fatra; Raus 090887; B		AJ306329	-	-	-	-
Soldanella chrysosticta Kress	Bulgaria, Rila Mts.; Schwerdtfeger 19735; B		AJ306330	+	-	-	-
	Greece, E Thessalia; Schwerdtfeger 19736; B		AJ306331	-	-	-	-
Soldanella hungarica Simk.	Romania, S Carpathians; Ehrendorfer & Ehrendorfer 060798; MJG		AJ306332	-	-	-	-
	Romania, S Carpathians; Ehrendorfer & Ehrendorfer 080798; MJG		AJ306333	-	-	-	-
Soldanella major (Neilr.) Vierh.	Romania, S Carpathians; Groza 120795; LI		AJ306334	-	-	-	-
	Romania, S Carpathians; Meyer & Meyer 9895; JE		AJ306335	-	-	-	-
	Austria, Styria; Melzer 170892; LI		AJ306336	-	-	-	-
	Austria, Lower Austria; Comes & Kropf 090799; MJG		AJ306337	-	-	-	-
	Austria, Styria; Steffen100807/3; MJG	P3	+	+	+	+	4
	Austria, Lower Austria; Steffen 100807/4; MJG	P4	+	+	+	+	4
Soldanella marmarossiensis Klášterský	Slovakia, High Tatra; Zhang 220899; MJG		AJ306338	-	-	-	-
	Slovakia, Nizke Tatra; Kump 060691; LI		AJ306339	-	-	-	-
	Romania, E Carpathians; Comes & Kropf 060799; MJG		AJ306340	-	-	-	-
Soldanella minima Hoppe subsp. *minima*	Austria, Carinthia; Uhink 98-246; MJG		AJ306341	-	-	-	-
	Germany, Bavaria; Mayer 28; M		AJ306342	-	-	-	-
	Austria, Carinthia; Steffen 120807/3	P16	+	+	-	+	4
	Slovenia; Steffen 140807/1; MJG	P14	+	+	+	+	4
	Austria, Carinthia; Steffen 150807/1	P10	+	+	+	-	2
	Italy, Trentino-Alto Adige; Steffen 170807/1; MJG	P15	+	+	+	+	3
Soldanella minima subsp. *austriaca* (Vierh.) Lüdi	Austria, Styria; Ernet 8555/1; GJO		AJ306343	-	-	-	-
	Germany, Bavaria; Urban 240692; M		AJ306344	-	-	-	-

TABLE 1 continued.

	Austria, Styria; Steffen 090807/4; MJG		-	-	-	-	3
Soldanella minima subsp. *samnitica* Cristofolini & Pignatti	Italy, Abruzzi; Zhang 120699; MJG		AJ306345	-	-	-	-
Soldanella montana Willd.	Austria, Upper Austria; Kleesadl 615; LI		AJ306346	-	-	-	-
	Czech Republic, Bohemian Massif; Manitz & Manitz 030882; JE		AJ306347	-	-	-	-
	Poland, N Carpathians; Pancer 090955; KRAW		AJ306348	-	-	-	-
	Austria, Upper Austria; Steffen 070807/1; MJG	P1	+	+	+	+	3
	Austria, Styria; Steffen 100807/1; MJG	P2	+	+	+	+	3
Soldanella oreodoxa L.B.Zhang	Romania, C Transylvania; Groza 280594; LI		AJ306349	-	-	-	-
Soldanella pindicola Hausskn.	Greece, Epirus: Thiv s.n.; priv. coll. M. Thiv		AJ306350	-	-	-	-
	Macedonia. Tetovo; Baltisberger & Lenherr 80/1172; Z		AJ306351	-	-	-	-
	Serbia; Seidl 0688; M		AJ306352	-	-	-	-
Soldanella pusilla Baumg. subsp. *pusilla*	Bulgaria, Pirin Mts.; Kuzmanov 802338; B		AJ306353	-	-	-	-
	Romania, S Carpathians; Comes & Kropf 010799; MJG		AJ306354	-	-	-	-
	Romania, S Carpathians; Comes & Kropf 040799; MJG		AJ306355	-	-	-	-
Soldanella pusilla subsp. *alpicola* (F.K.Meyer) J.Chrtek	Austria, Carinthia; Zhang 160399; MJG		AJ306356	-	-	-	-
	Austria, Tyrol; Kapuskar 230788; MJG		AJ306357	-	-	-	-
	Italy, W Alps; Wittmann & Wittmann 140795; LI		AJ306358	-	-	-	-
	Austria, Salzburg; Steffen 080807/4; MJG	P7	+	+	+	+	4
	Austria, Tyrol; Steffen 160807/2; MJG	P17	+	-	-	-	3
	Austria, Tyrol; Steffen 160807/3; MJG	P18	+	+	+	-	3
	Italy, Trentino-Alto Adige; Steffen 180807/1; MJG	P8	+	+	+	+	3
	Italy, Trentino-Alto Adige; Steffen 190807/1; MJG	P19	+	+	+	+	4
Soldanella rhodopaea F.K.Meyer	Greece, Makedhonia; Farsakoglou, Franzén & Strid 19470; B		AJ306359	-	-	-	-
Soldanella rugosa L.B.Zhang	Romania, E Carpathians; Comes & Kropf 060799; MJG		AJ306360	-	-	-	-
Soldanella villosa Darracq	Spain, W Pyrenees; Aizpuru 16330; RO		AJ306361	-	-	-	-

DNA extraction, amplification and sequencing. Total genomic DNA was extracted using the Qiagen DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany) following the manufacturer's protocol. PCR amplification of the entire ITS region was performed using primers ITS A (5'-GGA AGG AGA AGT CGTAAC AAG G-3', Blattner, 1999) and ITS 4 (5'-TCC TCC GCT TAT TGA TAT GC-3'; White *et al.*, 1990) and the protocol described in Zhang, Uhink & Kadereit. (2007). The *psb*J-*pet*A intergenic spacer was amplified using primers psbJ (5'-ATA GGT ACT GTA RCY GGT ARR-3'; Shaw *et al.*, 2007) and petA (5'-AAC ART TYG ARA AGG TTC AAT T-3'; Shaw *et al.*, 2007). Primers ndhJ (5'-ATG CCY GAA AGT TGG ATA GG-3'; Shaw *et al.*, 2007) and TabE (5'-GGT TCA AGT CCC TCT ATC CC-3'; Taberlet *et al.*, 1991) were used for the *ndh*J-*trn*F intergenic spacer. The *psb*D-*trn*T intergenic spacer was amplified with primers psbD (5'-CTC CGT ARC CAG TCA TCC ATA-3'; Shaw *et al.*, 2007) and trnT$^{(GGU)}$-R (5'-CCC TTT TAA CTC AGT GGT AG-3'; Shaw *et al.*, 2007). PCR amplifications of all chloroplast regions was carried out following this protocol: a 25 µl PCR reaction mix consisted of 4.25 mM $MgCl_2$, 1% BSA, 200 µM dNTPs, 0.5 µM forward and reverse primer, 0.5 U/25 µL Phusion High-Fidelity DNA polymerase (New England Biolabs GmbH, Frankfurt, Germany) Taq polymerase, and 1–2 µL of DNA extract in the reaction buffer provided by the manufacturer of the polymerase. PCR reactions were carried out in a Biometra T3 or a PTC 100TM MJ Research thermocycler using the program: 30 sec at 98°C, followed by 30 cycles of 10 sec at 98°C, 30 sec at 56°C, 30 sec at 72°C and a post-treatment of 10 min at 72°C. PCR products were purified using NucleoSpin Extract II (Macherey-Nagel, Düren, Germany) following the manufacturers' protocol. Cycle sequencing was carried out using the ABI Prism Dye Terminator Cycle Sequencing ready Reaction Kit (Perkin Elmer/Applied Biosystems, Foster City, California, USA) using the primers listed above and following the manufacturer's protocol. The purified products were analyzed on an ABI 3130XL automated sequencer by StarSeq GmbH (Mainz, Germany).

DNA sequence alignment and phylogenetic analyses. Forward and reverse sequences were manually edited, merged into consensus sequences using Sequencher 4.1.2 (Gene Codes Corporation, Ann Arbor, Michigan, USA), and aligned manually in MacClade 4.1 (Maddison & Maddison, 2000).

Likelihood and Bayesian analyses were performed for the entire ITS region, for the combined chloroplast regions (*psb*J-*pet*A–*ndh*J-*trn*F–*psb*D-*trn*T) and for the combined ITS and cpDNA data set separately. For Maximum Likelihood (ML) and Bayesian Analyses (BI) the appropriate models of DNA substitution were estimated using Modeltest 3.06 (Posada &

Crandall, 1998). We identified the equal-frequency Tamura-Nei (TrNef+G) model with gamma-distributed rates (G) for ITS and the Kimura-3-Parameter with unequal frequencies (K81uf) model with gamma-distributed rates (G) for the cpDNA and the combined data set (ITS and cpDNA) as best-fitting the sequence data under the Akaike Information Criterion (AIC).

Maximum Likelihood tree searches and ML bootstrap searches were performed using the online version of RAxML (Stamatakis, Hoover & Rougemont, 2008; available at http://phylobench.vital-it.ch/raxml-bb/). The GTR+G model was used for all analyses, with a total of 100 bootstrap replicates performed.

Bayesian analysis was performed as implemented in MrBayes version 3.1.2 (Huelsenbeck & Ronquist, 2001). The ITS and cpDNA data were analyzed with the GTR+G model. Both analyses were run with 5 million generations of Markov Chain Monte Carlo (MCMC) searches and a sample frequency of 1000. Sampling of model parameters was estimated using Tracer v.1.5 (Rambaut & Drummond 2007); a total of 250,000 generations were discarded as burn-in.

AFLP fingerprinting. Restriction-ligation was carried out following the protocol of Kropf, Kadereit & Comes(2002). Approximately 150 ng of DNA was simultaneously restricted and ligated to adaptors (EcoRI, 5′-CTCGTAGACTGCGTACC-3′/5′-AATTGGTACGCAGTC-3′; MseI, 5′-GACGATG AGTCCTGAG-3′/5′-TACTCAGGACTCT-3′) at 23 °C for 14 h. The digested and ligated DNA was diluted 2.3-fold. Pre-selective and selective amplifications followed the protocol of Trybush *et al.* (2006). Pre-selective amplification was performed using primers E01 (5′-GACTGCGTACCAATTCA-3′) and M02 (5′-GATGAGTCCTGAGTAAC-3′). For the selective amplifications, primer combinations E38/M49 (E01 + CT/M02 + AG), E39/M49 (E01 + GA) and E45/M49 (E01 + TG) were used in a multiplex PCR, and primers were labeled with fluorescent dyes (6-FAM, NED, and HEX; Applied Biosystems). For electrophoresis, selective amplification PCR products were run together with an internal size standard labelled with ROX (ROX 500; ABI) on an ABI 3130xl Genetic analyzer (ABI).

Amplified fragment length polymorphism fragments were scored manually with GeneMarker 1.5 (GeneMarker, SoftGenetics, LLC, State College, PA, USA) for the presence/absence of fragments between 70 and 370 bp in size. Fragments that could not be scored unambiguously were excluded. The results of the scoring were exported as a presence/absence (i.e. 0/1) matrix and used for further analysis. Genotyping error rates were

estimated with eleven replicated samples by dividing the number of mismatches between replicates by the total number of phenotypic comparisons (Bonin *et al.*, 2004).

AFLP data analysis. The overall genetic structure and relationships among studied individuals were explored using neighbor-net, Bayesian nonhierarchical clustering and principal coordinates (PCoA) analyses. A neighbor-net diagram was based on the population matrix of Nei & Li distances and bootstrapped using 1000 replicates with SplitsTree 4.11.3 software (Huson & Bryant, 2006). Bayesian clustering was performed using Bayesian Analysis of Population Structure (BAPS 5.1; Corander *et al*., 2008). The procedure was run 10 times for each number of clusters ($1 \leq K \leq 6$) with six chosen based on number of taxa present in the sample. Based on the results of the previous analyses, admixture analyses (Corander & Marttinen, 2006) were run with 100 iterations to estimate admixture coefficients for individuals. Two hundred reference individuals from each population and 20 iterations were used to estimate admixture coefficients for reference individuals. The PCoA analysis, a non-hierarchical grouping technique without prior knowledge of the source location of the sampled individuals, was performed with GenAlEx 6.5 (Peakall & Smouse, 2012) using Euclidian distances.

Results

Phylogenetic relationships. The ITS data matrix consisted of 65 accessions including *Douglasia alaskana* (Coville & Standl. ex Hultén) S. Kelso, *D. beringensis* S. Kelso, Jurtzev & D.F. Murray, *Primula bulleyana* Forrest, *P. cuneifolia* Ledeb., *P. farinosa* L., *P. latifolia* Lapeyr., *P. parryi* A. Gray and *P. tschuktschorum* Kjellm. as outgroup. It was 679 bp long, with 279 variable and 211 parsimony-informative characters. The cpDNA data matrix (*psb*J-*pet*A–*ndh*J-*trn*F–*psb*D-*trn*T) comprised 17 accessions including *Primula hirsuta* All. as outgroup. It was 2391 bp long, of which 142 were variable but uninformative and 25 were parsimony-informative. The combination of the ITS and cpDNA data resulted in a data set consisting of the same 17 accessions as the cpDNA data set. It was 3043 bp long, with 291 variable and 40 parsimony-informative characters. Indel-coding did not result in better resolved trees in either data set, and therefore indels were treated as missing data in the final analyses.

The ML and Bayesian analyses of the ITS data set resulted in trees of the same topology; the BI tree is shown in Fig. 2. *Soldanella* is strongly supported as monophyletic with bootstrap (BS)/posterior probability (PP) values of 100%/1. Within *Soldanella*, relationships among the 16 species remain largely unresolved. All accessions of *S. alpina*, *S.*

pusilla and *S. villosa* Darracq fall into a basal polytomy, together with two clades. Clade A (90%/0.98) comprises all accessions of *S. minima*, and all other *Soldanella* species fall into clade B (88%/-). Within clade B two moderately supported subclades and *S. calabrella* Kress can be found in a basal polytomy. The first subclade comprises *S. angusta* L.B.Zhang, *S. carpatica* Vierh., *S. marmarossiensis* Klášterský , *S. montana*, *S. oreodoxa* L.B.Zhang and *S. rugosa* (79%/0.97), and the second is formed by *S. chrysosticta* Kress, *S. hungarica* Simk., *S. major*, *S. pindicola* Hausskn. and *S. rhodopaea* F.K.Meyer (93%/-).

The tree obtained from the Bayesian analysis of the cpDNA data set is shown in Fig. 3; the ML analysis resulted in a less resolved tree. The two accession of *S. major* form a basal polytomy with a clade comprising *S. alpina*, *S. minima*, *S. montana* and *S. pusilla (*-/1). Within that clade *S. montana* is strongly supported as monophyletic (99%/1), and one accession of *S. alpina* (P12) is sister to one accession of *S. minima* (P16; 98%/1); however, these two subclades are found in a polytomy with all other accessions of *S. alpina*, *S. minima* and *S. pusilla*.

The ML and Bayesian analyses of the combined data set (ITS and cpDNA) resulted in trees of the same topology; Fig. 4 shows the BI tree. The two accessions of *S. major* fall into a basal polytomy. *Soldanella alpina*, *S. minima*, *S. montana* and *S. pusilla* are strongly supported as monophyletic (99%/1). Within that clade, *S. montana* forms one subclade (100%/1), and a second subclade contains *S. alpina*, *S. minima* and *S. pusilla* (100%/1). In this clade, *S. minima* is supported as monophyletic (75%/1) and as sister to a clade comprising *S. alpina* and *S. pusilla* (82%/-) which, however, were not resolved as reciprocally monophyletic.

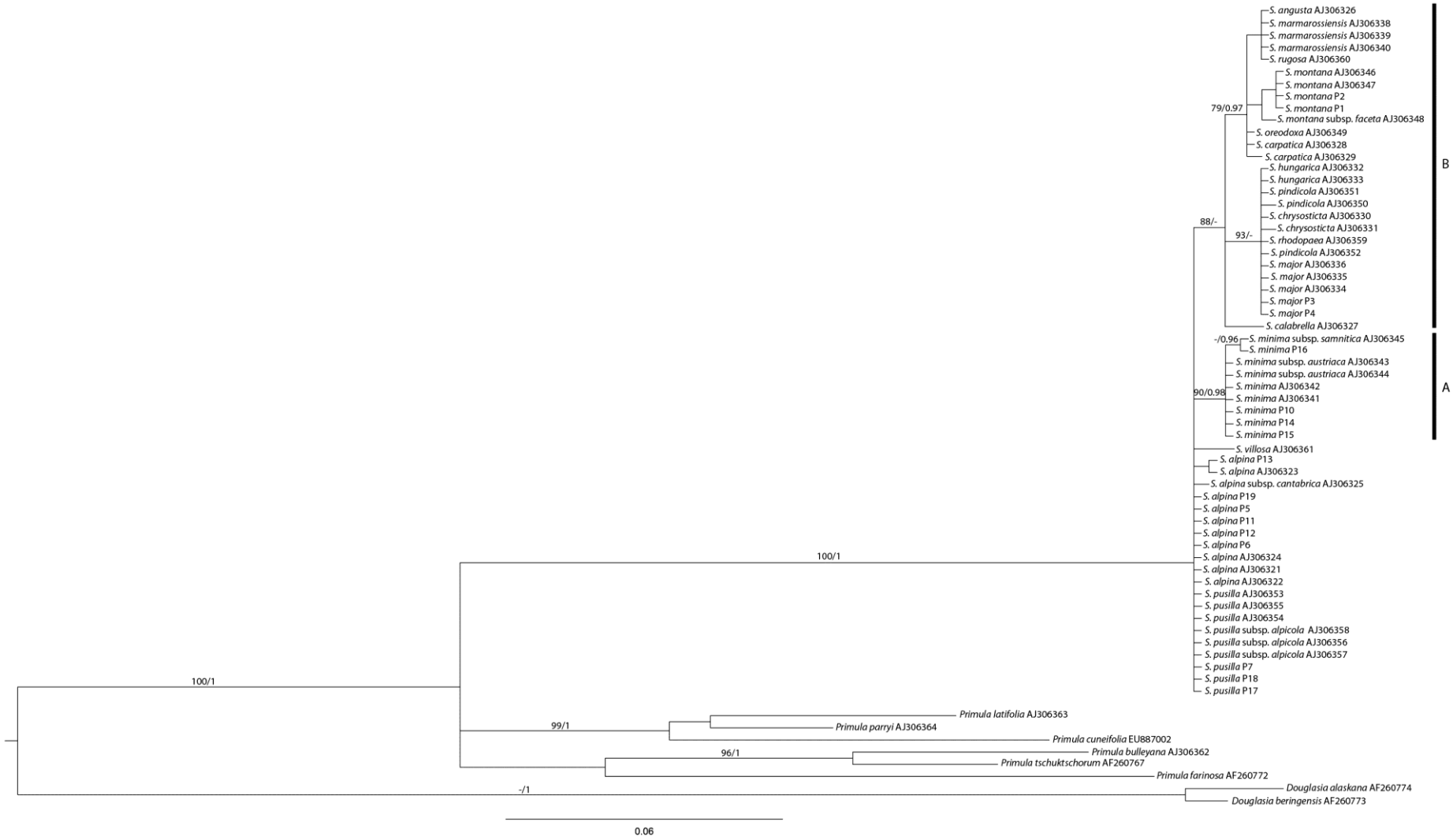

FIG. 2. Phylogenetic relationships in *Soldanella*. Phylogram from the Bayesian analysis of the ITS data set. ML bootstrap values (≥ 75%) and Bayesian posterior probabilities (≥ 0.95) are given above branches.

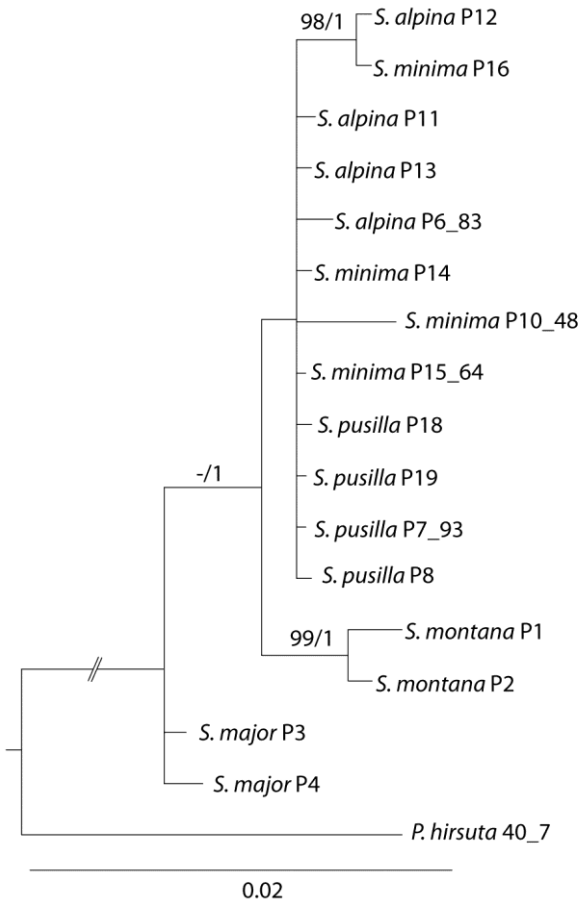

FIG. 3. Phylogenetic relationships in *Soldanella*. Phylogram from the Bayesian analysis of the cpDNA (*psb*J-*pet*A–*ndh*J-*trn*F–*psb*D-*trn*T) data set. ML bootstrap values (≥ 75%) and Bayesian posterior probabilities (≥ 0.95) are given above branches.

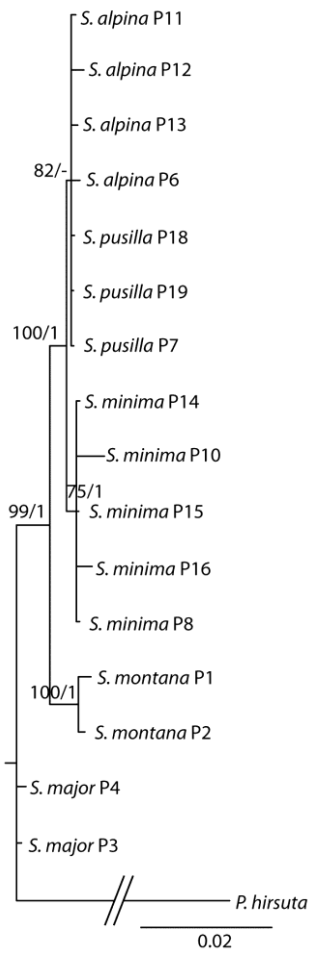

FIG. 4. Phylogenetic relationships in *Soldanella*. Phylogram from the Bayesian analysis of the combined data set (ITS and cpDNA). ML bootstrap values (≥ 75%) and Bayesian posterior probabilities (≥ 0.95) are given above branches.

AFLP analysis. A total of 142 polymorphic loci was detected in *S. alpina*, *S. major*, *S. minima*, *S. montana* and *S. pusilla*. The mismatch error was 5.2% using eleven replicate pairs. The neighbor-net diagram recovered five groups representing the five species included (Fig. 5A). *Soldanella major* (95.6% BS) and *S. montana* (93.6%) are each well-supported and together form a highly supported group (99.2%). *Soldanella pusilla* is well supported (98.4%), and accessions of *S. alpina* and *S. minima* are divided into two separated groups that lack BS support. *Soldanella minima* subsp. *austriaca* is found as a subgroup (96%) nested within *S. minima*.

BAPS divided the samples into five major groups, with a probability of 1 for the number of clusters (Fig. 5B). These five clusters represent the five species; accessions of *S. minima* subsp. *austriaca* belong to the *S. minima* cluster. In the admixture analysis, all individuals but one were unambiguously assigned to their respective group without any probability of being misplaced. The only exception that shows significant admixture ($p = 0$) with *S. minima* is one individual of *S. alpina* (P5). This accession is from Gartnerkofel in Carinthia, where both populations of *S. alpina* and *S. minima* are found.

The PCoA produced results largely congruent with the BAPS analysis. The first two axes (Fig. 5C) explain 31.71% (axis 1) and 27.57% (axis 2) of the variation and separate the individuals into four groups. *Soldanella major* and *S. montana* form one cluster. Accessions of S. *pusilla* fall into a distinct group, and *S. alpina* and *S. minima* including subsp. *austriaca* form two groups, which are clearly separated from each other on the third axis, which explains 17.22% of the total variation.

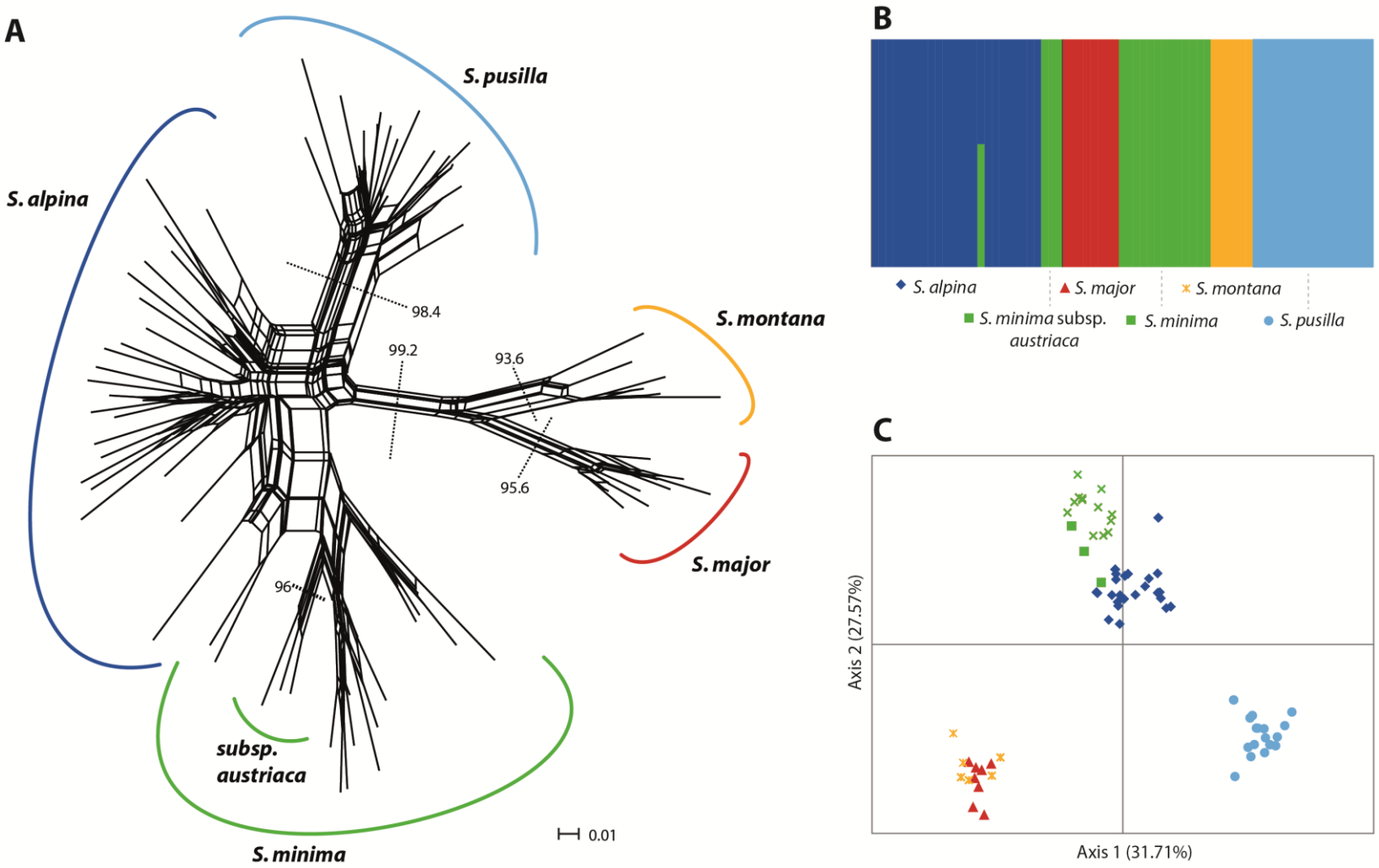

FIG. 5. AFLP analyses of *Soldanella*. A. Neighbor-net of AFLP data based on Nei-Li distances. Numbers at splits are bootstrap values. B. BAPS admixture analysis of the AFLP data set with K = 5. C. PCoA analysis of the AFLP data set based on genetic distances. Percentages of total variance explained by the first two coordinates are shown on the respective axes. Different colours indicate the different taxa studied.

Discussion

Taken together, our molecular results confirm the findings of Zhang *et al.* (2001), that 1) *S. alpina* (sect. *Soldanella*), *S. minima* and *S. pusilla* (sect. *Tubiflores*) fall into one clade, and that 2) the latter two species are not sister to each other. In contrast to Zhang *et al.* (2001), a clade of *S. alpina*, *S. minima* and *S. pusilla* was not found in our expanded ITS analysis (Fig. 2). A clade comprising these three species was recovered in our analysis of cpDNA sequences, but relationships between the three species were unresolved and the clade as a whole was unsupported (Fig. 3). In the combined ITS and cpDNA data set (Fig. 4), however, this clade was found to be supported, and *S. alpina* and *S. pusilla* were found to form a clade together, but were not resolved as reciprocally monophyletic, as also found by Zhang *et al.* (2001). The clearest signal was obtained in our AFLP analyses. In the neighbor-net diagram (Fig. 5A), *S. alpina*, *S. minima* and *S. pusilla* form a well-supported group distinct from *S. major* and *S. montana*, and in the PCoA analysis of the AFLP data (Fig. 5C) *S. alpina* plus *S. minima*, *S. pusilla* and *S. major* plus *S. montana* form three distinct groups. It is worth noting that the alpine *S. minima* and *S. pusilla* are most closely related to *S. alpina* as (apart from the Carpathian *S. rugosa*, see above) the only species of sect. *Soldanella* which grows from montane to alpine altitudes.

The observed discordance between the molecular data and the sectional classification of *S. alpina* were interpreted as a result of hybridization by Zhang *et al.* (2001). The authors hypothesized that *S. alpina* originated through homoploid hybrid speciation (Arnold, 1997; Rieseberg & Carney, 1998) between *S. pusilla* and an unknown member of sect. *Soldanella*. *Soldanella major* and *S. montana* are the only species of sect. *Soldanella* (except *S. alpina*) that grow in the Alps, which is the only area of overlap between *S. alpina* and *S. pusilla* and *S. minima* (except for another small area of overlap in the central Apennines). *Soldanella major* and *S. montana* were included as potential second parents in our analysis, assuming an origin of *S. alpina*, should this species indeed be a hybrid, in the Alps. Admittedly this reasoning is based on the extant distributions of the species involved and does not consider past changes in distribution. Hybridization between species of *Soldanella* has frequently been reported (Huter, 1873; Kerner von Marilaun, 1875; Vierhapper, 1904a, b; Hegi, 1927; Kress, 1984), and in our BAPS analysis, one *S. alpina* accession showed admixture with *S. minima* (Fig. 5B). Nevertheless, our molecular data make a hybrid origin of *S. alpina* seem highly unlikely. First, there is no supported incongruence between the ITS and cpDNA phylogenies. If a member of sect. *Soldanella* were the maternal parent, *S. alpina* should group with *S. major* or *S. montana* in the cpDNA phylogeny. Second, our neighbor-net diagram (Fig. 5A) provides

no evidence for an involvement of *S. major* and *S. montana* in the origin of *S. alpina* as there are no contradictory splits for *S. alpina* with *S. major*/*S. montana* and *S. pusilla* indicating reticulate relationships.. Finally, the admixture analysis of our AFLP data (Fig. 5B) did not identify any admixture between *S. alpina* and *S. major* or *S. montana*. We feel that it is therefore safe to reject the hypothesis that *S. alpina* is a hybrid species.

Based on a phylogeny in which *S. alpina* is nested within sect. *Tubiflores* and in which this group is nested within the remaining species of sect. *Soldanella* (Fig. 3; Figs. 4 and 5 in Zhang *et al.* (2001)), the pattern of morphological variation can either be explained as a reversal to the morphology of sect. *Soldanella* in *S. alpina* or as parallel evolution in *S. minima* and *S. pusilla*. *Soldanella minima* and *S. pusilla* are morphologically very similar to each other and mainly differ in the anatomy of glandular hairs on their petioles and scapes (Zhang & Kadereit, 2002). By having only 1-flowered inflorescences, reduced or absent throat scales, smaller connective appendages, shorter styles and fewer capsule teeth (Fig. 1), the two species differ from *S. alpina* and other species of sect. *Soldanella* in their reduced flower morphology. *Soldanella minima* and *S. pusilla* also have smaller leaves than most species of sect. *Soldanella*. We hypothesize that the reduction in floral size is an adaptation to conditions during sexual reproduction in their alpine habitats. It has been shown that insect-pollinated plants growing in high alpine areas have to cope with lower pollinator visitation rates compared to those growing at lower altitudes (Bingham & Orthner, 1998). Molau (1993) pointed out that early-flowering alpine species such as *S. minima* and *S. pusilla* risk pollen loss when it is too cold for successful cross-pollination. Unfavourable conditions for insect pollination have been suggested to promote self-pollination (reproductive assurance hypothesis; Stebbins, 1957; Jain, 1976; Lloyd & Schoen, 1992). Although *Soldanella* is predominantly outbreeding, self-pollination in the homogamous to protogynous flowers has been described by several authors (Müller, 1881; Schroeter, 1908; Hegi, 1927), and the reduced flower morphology of *S. minima* and *S. pusilla* can be interpreted as evidence for increased levels of self-pollination. In particular, the lower degree of herkogamy in *S. minima* and *S. pusilla* caused by the shorter physical distance between stigma and anthers (Fig. 1) points at autogamous pollination. Furthermore, pollinator observations (Müller, 1881) showed that flowers of *S. pusilla* are far less frequently visited than flowers of *S. alpina.* However, nectar production and the presence of nectar guides in *S. minima* and *S. pusilla* indicate that, even if self-pollination is possible and possibly more frequent than in species of sect. *Soldanella*, these species also attract insects. Indeed, *S. pusilla* is predominantly pollinated by bumblebees (Müller, 1881), which are very effective pollinators in arctic and alpine

environments, as they can carry large amounts of pollen (Bingham & Orthner, 1998) and fly even under cold (minimum temperature 4° C) and windy conditions (Bergmann et al., 1996). The above interpretation of parallel floral reduction in *S. minima* and *S. pusilla* as evidence for increased levels of self-pollination, which clearly requires experimental testing, seems more plausible to us than a reversal to the typical floral morphology of sect. *Soldanella* in *S. alpina*, which would require restoration of a large number of flower characters (flower number, corolla throat scales, connective appendages, style length, capsule teeth) in which this species does not differ from other species of sect. *Soldanella*.

Finally, the reduction of flower number and leaf size in the alpine *S. minima* and *S. pusilla* in comparison to the largely montane species of sect. *Soldanella* fits the general observation of dwarfism in arctic and alpine environments (Billings & Mooney, 1968; Bliss, 1971; Johnson, 1969; Körner & Larcher, 1988; Körner, 1999; Steffen & Kadereit, submitted).

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Zusammenfassung

Hintergrund: Miniaturisierung ist ein häufig beobachtetes Phänomen bei Pflanzen in arktisch-alpinen Lebensräumen und wird als Anpassung an niedrige Jahresmitteltemperaturen und eine kurze Vegetationsperiode interpretiert.

Ziele: In der vorliegenden Arbeit wird im Petasites-Clade (*Petasites* Mill., *Endocellion* Turcz. ex Herder, *Homogyne* Cass., *Tussilago* L.; Asteraceae) und in *Soldanella* (Primulaceae) die Evolution der Miniaturisierung arktisch-alpiner Arten untersucht. Zudem wird innerhalb von *Homogyne* untersucht, ob unterschiedliche edaphische Präferenz von *H. alpina* (variabel) und *H. discolor* (kalkliebend) genetisch fixiert ist.

Methoden: Molekulare Phylogenien des Petasites-Clades und von *Soldanella* wurden mit nukleären und plastidären Markern erstellt, und mit den in den Alpen vorkommenden *Soldanella*-Arten wurde zudem eine Fingerprint-Studie (AFLPs) gemacht. Zur Datierung der Diversifizierungsereignisse im Petasites-Clade diente eine molekulare Uhr, und die Evolution von Miniaturisierung wurde rekonstruiert. Mit *H. alpina* und *H. discolor* wurde ein vergleichendes Kulturexperiment durchgeführt.

Ergebnisse: Miniaturisierung entstand mehrere Male unabhängig voneinander in den arktisch-alpinen Vertretern des Petasites-Clade, aber nicht alle arktisch-alpinen Arten sind klein. Das Alter der arktisch-alpinen Arten deutet darauf hin, dass diese Taxa ihren Ursprung in der arkto-tertiären Flora haben. In *Soldanella* sind reduzierte Blütenmorphologie sowie Kleinwüchsigkeit der beiden alpinen Arten zweimal parallel entstanden. *Homogyne alpina* und *H. discolor* zeigen keine edaphischen Unterschiede hinsichtlich des Keimverhaltens, aber in Kultur zeigt sich, dass die Präferenz von *H. discolor* für Kalk wahrscheinlich genetisch fixiert ist.

Schlussfolgerungen: Miniaturisierung von Pflanzen in größerer Höhe und höherer geographischer Breite kann in der Regel beobachtet werden. Allerdings kann die Evolution arktisch-alpiner Arten auch durch Faktoren wie Nährstoffverfügbarkeit, Konkurrenz und Störung beeinflusst werden, die dem Effekt der Temperatur entgegenwirken, so dass nicht alle Pflanzen in arktisch-alpinen Habitaten klein sind. Blütenmorphologische Reduktion in *Soldanella* kann als Anpassung an einen höheren Grad an Selbstbestäubung interpretiert werden, um eine geringere Bestäuberaktivität im alpinen Lebensraum zu kompensieren.

Summary

Background: Miniaturization of plant size is a phenomenon well-known from arctic and alpine habitats and has been interpreted as an adaptation to low mean annual temperature and short vegetation period.

Aims: This study investigates the evolution of miniaturization in arctic-alpine representatives of the Petasites-clade (*Petasites* Mill., *Endocellion* Turcz. ex Herder, *Homogyne* Cass., *Tussilago* L.; Asteraceae) and in *Soldanella* (Primulaceae). Furthermore, it is tested if different edaphic preferences in *H. alpina* (variable) and *H. discolor* (calcicole) are genetically fixed.

Methods: For the Petasites-clade and for *Soldanella* molecular phylogenies based on nuclear and chloroplast markers were reconstructed. An additional fingerprint study (AFLPs) was conducted for the alpine *Soldanella* species. To date diversification in the Petasites-clade, a molecular clock approach was used, and the evolution of miniaturization was reconstructed. A common garden trial was conducted with *H. alpina* and *H. discolor*.

Results: Miniaturization evolved several times in parallel in the arctic-alpine representatives of the Petasites-clade, but not all arctic-alpine species are dwarfs. The age of the arctic-alpine taxa indicates that these species originated from the Arcto-tertiary stock of todays' arctic and alpine flora. Reduced floral morphology and dwarfism of the two alpine *Soldanella* species evolved two times in parallel. *Homogyne alpina* and *H. discolor* do not display edaphic differences in their germination behaviour, but the cultivation experiment showed that the preference for calcareous substrate found in *H. discolor* is probably genetically fixed.

Conclusions: Miniaturization of plants with increasing altitude and latitude can be observed as a rule. However, factors like nutrient availability, competition and habitat disturbance can counteract the effect of temperature, so that not all plants found in arctic-alpine habitats are dwarfs. Reductions in floral morphology can be interpreted as an adaptation to increased levels of self-pollination to compensate for lower pollinator activities in alpine habitats.

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