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ORIGINAL ARTICLE

Of dwarfs and giants: phylogeny of the Petasites-clade (Asteraceae–Senecioneae) and evolution of miniaturization in arctic–alpine environments

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Abstract Decreasing plant size with increasing latitude or altitude is a commonly observed pattern. Among the four genera of the Petasites-clade (Asteraceae–Senecioneae), *Petasites* and *Tussilago*, widespread throughout the Northern Hemisphere, mostly have large leaves and many capitula, whereas *Homogyne* and *Endocellion* from alpine and arctic environments have much smaller leaves and only one or few capitula. We present a comprehensively sampled and dated phylogeny of *Petasites*, *Endocellion*, *Homogyne* and *Tussilago* based on nuclear ribosomal ITS and plastid *ndh*F-*rpl*32 and *rpl*32-*trn*L sequences. The four genera form a well-supported monophyletic group. *Endocellion* was found to be nested in *Petasites*, and relationships among the other three genera remain unresolved. Dwarfism with small leaves and a reduced number of capitula evolved five times in arctic–alpine species of this group. Although all dwarf species of the Petasites-clade grow in arctic or alpine habitats, not all species from such habitats are dwarfs. In the European Alps, *Homogyne alpina*, *H. discolor* and *Petasites paradoxus* occur in (sub-) alpine habitats, but only the species of *Homogyne* are dwarfs with small leaves and only one flowering head, whereas *P. paradoxus* has much larger leaves and numerous capitula. These species differ in ecology: whereas *Homogyne* is found in nutrient-poor and stable habitats, *P. paradoxus* grows in nutrient-rich and often disturbed habitats. We conclude that although decreasing plant size with increasing latitude or altitude is an overall trend in the group, factors such as nutrient availability and/or habitat disturbance can counteract this trend.

Keywords Arctic–alpine plants · Dwarfism · Evolution · *Petasites* · Tussilagininae

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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; (2) tussock-forming graminoids; (3) diminutive, prostrate shrubs and (4) herbaceous and often rosulate perennials. All of these are characterized by a reduction in plant and organ size in comparison to relatives from lower latitudes or altitudes. 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).

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* (Siebold & Zucc.) Maxim. var. *giganteus* F.Schmidt ex Makino (Hind and Kay 2006). The only exceptions in *Petasites* are *P. doerfleri* Hayek, *P. rubellus* (J.F.Gmel.) J.Toman and *P. fominii* Bordz., three species restricted to high altitude habitats which have small basal leaves that are <5 cm in diameter (Toman 1972; Fig. 1). Leaves of *Homogyne* from the Alps and other European high mountain ranges and of *Endocellion* from the Asian Arctic 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* (Scop.) Cass. is the only non-clonal species in this group. *Tussilago* is monospecific containing only *T. farfara* L., a species widely distributed throughout Eurasia and introduced to North America (Hegi 1929; Tutin 1976b; Kuprianova 2000; Barkley 2006). It can be found from the lowlands to high altitude habitats and is a pioneer species, colonizing open habitats such as alluvial drifts, clay pits, moraines, avalanche deposits but also roadsides 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. *Homogyne alpina* (L.) Cass. is widespread across the European Alpine System and grows 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. 2008). *Homogyne discolor* Cass. and *H. sylvestris* are confined to the peripheral 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. 2008). *Petasites* comprises 16 species (Toman 1972; Bayer et al. 2006; Chen et al. 2011) distributed mainly throughout Eurasia, with one species (*P. fragrans* (Vill.) C.Presl) also found in North Africa and one (*P. frigidus* (L.) Fr.) 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 1972; Dingwall 1976; Ellenberg 1996; Cherniawsky and Bayer

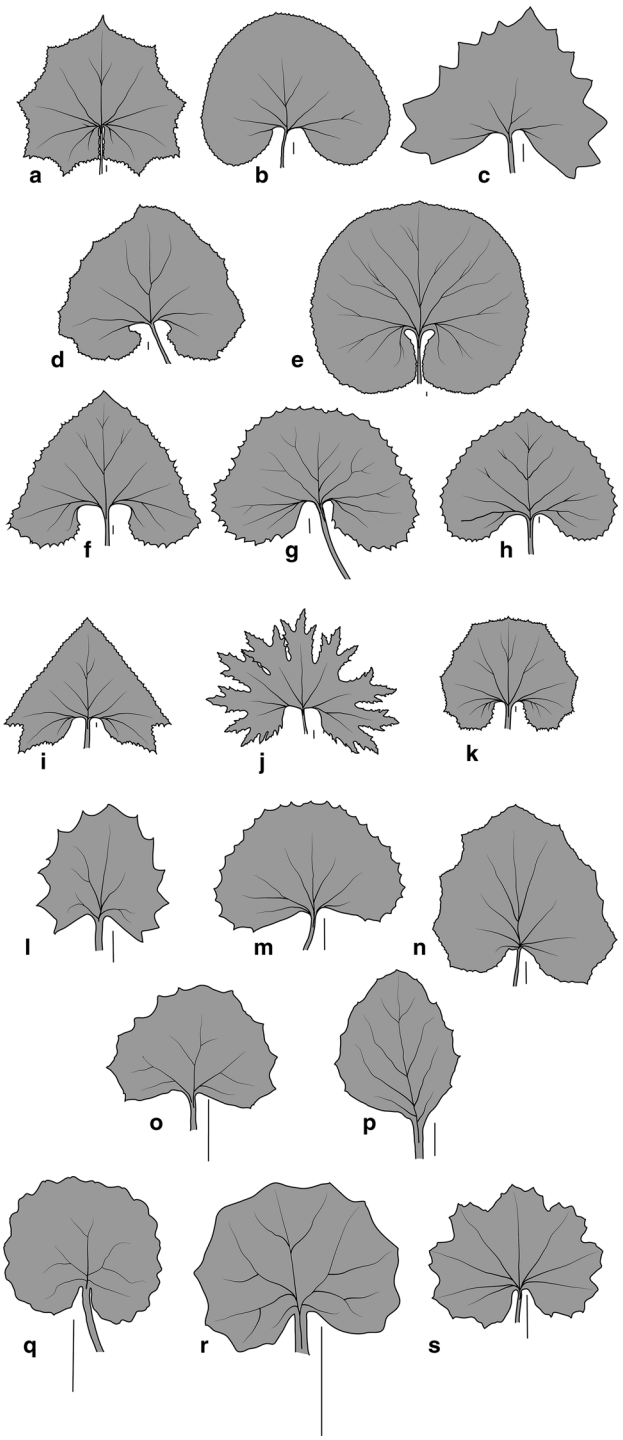

Fig. 1 Leaf size variation in the Petasites-clade. **a** *Petasites albus* (after Toman 1972). **b** *P. fragrans* (drawn from *Steffen 180908/1*). **c** *P. frigidus* (*Boyko s.n.*). **d** *P. hybridus* (*Steffen 130409/1*). **e** *P. japonicus* (Toman 1972). **f** *P. kablikianus* (Toman 1972). **g** *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** *Tussilago farfara* (*Steffen 060608/1*). **o** *Endocellion glaciale* (*Pospepov 00-467*). **p** *E. sibiricum* (*Kharkevich* and *Buch 814b*). **q** *Homogyne alpina* (*Steffen 100809/4*). **r** *H. discolor* (*Steffen 010578*). **s** *H. sylvestris* (*Steffen 010572*). *Scale bar* 1 cm

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.s. (Pelser et al. 2007). Within Tussilagininae s.s. the phylogenetic position of the Petasites-clade is unclear as 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 by Pelser et al. (2007). However, Pelser et al. (2007) suggested that the Petasites-clade is either sister to the remainder of Tussilagininae s.s. or to the exclusively New World Crocidium-Tetradymia-clade. Pelser et al. (2007) included representatives of all genera traditionally placed in Tussilagininae (Bremer 1994) except for *Digitacalia* Pippen, *Nelsonianthius* H.Rob. & Brettell, *Paracalia* Cuatrec., *Pippenalia* McVaugh, *Psacaliopsis* H.Rob. & Brettell, *Rugelia* Shuttlew. ex Chapm., *Villasenoria* B.L.Clark and *Yermo* Dorn. 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 positions of these genera in the subtribe are unclear, their distribution ranges make it unlikely that they are part of the predominantly northern temperate/boreal Petasites-clade as circumscribed by 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 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.

Materials and methods

Taxon sampling

Of the 22 species currently recognized in *Petasites*, *Endocellion, Homogyne* and *Tussilago* (see Introduction) all except *P. versipilus* Hand.-Mazz. were included in this study. *Ligularia tsangchanensis* (Franch.) Hand.-Mazz., *Nordenstamia kingii* (H.Rob. and Cuatrec.) B.Nord. and *Tetradymia canescens* DC. of the Senecioneae-Tussilagininae and *Adenostyles alpina* (L.) Bluff & Fingerh. of the Senecioneae-Senecioninae were used as outgroups. 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. Additional sequences were obtained from GenBank. 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) was 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 was

Table 1 Materials, voucher information, DNA accession numbers and GenBank accession numbers

Taxon	Country; collector, collection no.; Herbarium, Herbarium no.	DNA accession	GenBank accession no.		
			ITS	*ndh*F-*rpl*32	*rpl*32-*trn*L
Adenostyles alpina (L.) Bluff & Fingerh.	France, Savoie; *Klein s.n.*; MJG 000492	Ac4	KC784503	KC7844610	KC784552
Endocellion glaciale (Ledeb.) J.Toman	Russia, Sakha Republic; *Solstad* and *Elven 04/1062*; O	AST24	KU570801	KU570818	KU570863
	Russia, Chukotka; *Solstad* and *Elven 05/1008*; O	AST25	KU570809	KU570819	KU570864
	Russia, Sakha Republic; *Solstad* and *Elven 04/1049*; O	AST32	KU570810	KU570820	KU570865
Endocellion sibiricum (J.F.Gmel.) J.Toman	Russia, Taymyr; *Schönswetter* and *Tribsch T316*; WU		EF538197		
	Russia, Taymyr; *Schönswetter* and *Tribsch T480*; WU		EF538198		
	Russia, Sakha Republic; *Solstad* and *Elven 04/0330*; O	AST23	KU570811	KU570821	KU570866
	Russia, Magadan; *Boyko s.n.*; MJG 010546	AST71	KU570797	KU570853	KU570867
Homogyne alpina (L.) Cass.	Austria, Carinthia; *Steffen 120807/1*; MJG 012444	AST1	KU570767	KU570823	KU570868
	Switzerland, Schwyz; *Uhink* and *Kadereit s.n.*; MJG	AST3	KU570770	KU570827	KU570870
	Italy, Trentino; *Steffen 190807/2*; MJG 012445	AST15	KU570769	KU570825	KU570869
	Austria, Carinthia; *Steffen 140809/4-2*; MJG 010564	AST413	KU570768	KU570824	KU570871
	Slovakia, Prešovský Kraj; *Comes 11*; MJG	AST582	KU570815	KU570828	KU570872
Homogyne discolor Cass.	Austria, Styria; *Steffen 090807/1*; MJG 012447	AST13	KU570771	KU570830	KU570873
	Austria, Carinthia; *Steffen 130807/2*; MJG 012443	AST14	KU570772	KU570831	KU570874
	Austria, Carinthia; *Steffen 140809/5-1*; MJG 010565	AST197	KU570813	KU570826	KU570875
	Austria, Salzburg; *Steffen 070809/3-10*; MJG 010598	AST86	KU570812	KU570822	KU570876
Homogyne sylvestris (Scop.) Cass.	Austria, Carinthia; *Uhink 98-139*; MJG	AST19	KU570773	KU570832	KU570877
	Austria, Carinthia; *Steffen 140809/2-2*; MJG 010584	AST75	KU570775	KU570834	KU570880
	Austria, Carinthia; *Steffen 130809/5-2*; MJG 010582	AST702	KU570814	KU570829	KU570879
	Austria, Carinthia; *Steffen 290708/4*; MJG 010610	AST62	KU570774	KU570833	KU570878
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 010562	AST28	KU570776	KU570835	KU570881
	Georgia, Abkhazia; *Mikheev s.n.*; PGFA	AST64	KU570777	KU570836	KU570882
Petasites doerfleri Hayek	Albania; *Rakaj* and *Surina NHMR833*; PMR	AST61	KU570779	KU570837	KU570883
Petasites fominii Bordz.	Georgia; *Mikheev 2750*; PGFA	AST65	KU570780		KU570884
Petasites formosanus Kitam.	Taiwan; *Yang 1683*; HAST	AST30	KU570781	KU570838	KU570885

Table 1 continued

Taxon	Country; collector, collection no.; Herbarium, Herbarium no.	DNA accession	GenBank accession no.		
			ITS	*ndh*F-*rpl*32	*rpl*32-*trn*L
Petasites fragrans (Vill.) C.Presl	Germany, Rhineland-Palatinate; *Steffen 180908/1*; MJG 010602	AST16	KU570817	KU570839	KU570886
	New Zealand, South Island; *Wagstaff s.n.*; CHR541965A		AY554108		
Petasites frigidus (L.) Fr.	Russia, Kotelny Island; *Saffonowa 240*; M	AST5	KU570783	KU570840	KU570887
	Russia, Taymyr; *Schönswetter* and *Tribsch 4703*; WU	AST9	KU570784	KU570842	KU570888
	Norway; *Nordenstam 9501*; S	1143	KU570782		
Petasites frigidus var. *palmatus* (Aiton) Cronquist	Canada, Québec; *Lemieux 21229*; B	AST54	KU570792	KU570841	KU570899
	USA, California; *Sharsmith 5249*; M	AST4	KU570791	KU570850	KU570898
Petasites hybridus (L.) G.Gaertn., B.Mey. & Scherb.	Germany, Lower Saxony; *Steffen 301207/1*; MJG 012441	AST2	KU570786	KU570843	KU570889
	Russia, Stavropol; *Shilnikow s.n.*; PGFA	AST63	KU570785	KU570844	KU570890
	Germany, Hamburg; *Kadereit* and *Steffen 08-36*; MJG 018171	PET1	KU570816	KU570862	KU570891
Petasites japonicus (Siebold & Zucc.) Maxim.	China, Sichuan; *Liu 15232*; B	AST53	KU570788	KU570846	KU570893
	Germany, Rhineland-Palatinate; *Steffen 100108/2*; MJG 012440	AST17	KU570787	KU570845	KU570892
	Russia, Sakhalin; *Salokhin s.n.*; MJG 010547	AST73	KU570778	KU570847	KU570894
	China, Chongqing; *Liu* no. unknown; HNWP		AY176152		
Petasites kablikianus Tausch	Romania; *Negrean* and *Anastasiu s.n.*; B	AST46	KU570789	KU570848	KU570896
	Slovakia; *Schuhwerk 04/100*; M	AST6	KU570790	KU570849	KU570897
	Slovakia; *Taubmann 1*; MJG 010559	AST31	KU570803	KU570861	KU570895
Petasites paradoxus (Retz.) Baumg.	Germany, Bavaria; *Parker s.n.*; M	AST7	KU570794	KU570852	KU570901
	Austria, Tyrol; *Vitek 3748*; WU	AST10	KU570793	KU570851	KU570900
Petasites radiatus (J.F.Gmel.) J.Toman	Russia, Komi; *Alsos* and *Tribsch 4854*; WU	AST11	KU570795		
Petasites rubellus (J.F.Gmel.) J.Toman	Russia, Primorsky; *Boyko s.n.*; MJG 010545	AST70	KU570796		
Petasites spurius (Retz.) Rchb.f.	Germany, Schleswig–Holstein; *Wesener 1*; MJG 010540	AST26	KU570798	KU570854	KU570902
	Germany, Lower Saxony; *Schimmitat s.n.*; M	AST8	KU570799	KU570855	KU570903
Petasites tatewakianus Kitam.	Russia, Primorsky; *Salokhin s.n.*; MJG 010542	AST72	KU570800	KU570856	KU570904
Petasites tricholobus Franch.	Nepal; *Schwabe s.n.*; B	AST35	KU570802		
	Unknown		AY176153		
Tetradymia canescens DC.	USA, Colorado; *Dunn 15291*; U		EF538410		
Tussilago farfara L.	Germany, Rhineland-Palatinate; *Steffen 100108/3*; MJG 012442	AST18	KU570806	KU570857	KU570905
	Slovakia; *Taubmann 2*; MJG 012454	AST58	KU570808	KU570859	KU570908
	Pakistan, *Nüsser 966*; B	AST41	KU570807		KU570907
	Unknown; *Caesar* and *Loretz 40923276*		EU785941		
	China, Qinghai; *Liu* no. unknown; HNWP		AY176167		
	Austria, Styria; *Steffen 280508/4*; MJG 010561	AST27	KU570805	KU570858	KU570906
	Poland; *Taubmann 3*; MJG 012453	AST59	KU570804	KU570860	KU570909

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 2.5 M 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 (Blattner 1999) or ITS 18S (Muir et al. 2001) and ITS 4 (White et al. 1990) or ITS 28S (Muir et al. 2001). In some cases, it was necessary to produce two overlapping fragments using primers ITS A and ITS C (Blattner 1999), ITS D (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 (Shaw et al. 2007) and ndhF (Shaw et al. 2007). Primers trnL$^{(UAG)}$ (Shaw et al. 2007) and rpL32-F (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 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 v.4.1 (Maddison and Maddison 2000).

Likelihood analyses were performed for the entire ITS region and for the combined cpDNA regions (*ndh*F-*rpl*32-*trn*L) separately. These gene trees were inspected for supported conflict between the data sets following Pirie et al. (2008). Supported conflict (bootstrap support for different positions of taxa in both trees >70 %) was found for one sample of *E. glaciale* (Ledeb.) J.Toman (AST24) and for *E. sibiricum* (J.F.Gmel.) J.Toman (see Results). These samples were removed from the dataset before nuclear and plastid DNA data sets were combined and further analyses were performed. For Maximum Likelihood (ML) and Bayesian analyses with BEAST (BB) and MrBayes (BM), respectively, the appropriate models of DNA substitution and the best partitioning of the dataset for the inference of phylogenetic relationships were estimated using PartitionFinder v.1.1.1 (Lanfear et al. 2012). Potential partitions to be tested were ITS1, 5.8S ribosomal gene, ITS2, *ndh*F-*rpl*32 and *rpl*32-*trn*L. Best fitting substitution models were chosen under the Bayesian information criterion. For all analyses we identified three partitions that are shown in Table 2 with the best fitting substitution model for each analysis. Maximum Parsimony (MP) analysis was performed with PAUP* v.4.0b10 (Swofford 2002). In this analysis of the combined data set (ITS+*ndh*F-*rpl*32-*trn*L) we used 1000 random addition replicates and TBR branch swapping. Bootstrap support was also calculated in PAUP* using 1000 replicates and the same settings as in the heuristic search. 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). As the ITS sequences of the ingroup contained only few parsimony-informative gaps, these were not coded.

Maximum Likelihood tree searches and ML bootstrap searches were performed using the online version of RAxML (Stamatakis et al. 2008; available at Cipres Science Gateway, Miller et al. 2010). The substitution models given in Table 2 were used for the different partitions of the data set, with automatic halt of bootstrapping.

Bayesian analysis was performed using MrBayes version 3.2.3 (Huelsenbeck and Ronquist 2001) at Cipres Science Gateway (Miller et al. 2010). Parsimony-informative gaps were coded for the cpDNA data set as for the MP analysis. The partitions of the dataset were analyzed with the appropriate substitution models according to Table 2. The combined data set was run twice independently for 10 million Markov Chain Monte Carlo (MCMC) generations, with sampling every 1000th generation. Convergence of model parameters was examined using Tracer v.1.5 (Rambaut and Drummond 2007); a total of 1 million generations for each run were discarded as burn-in and the maximum clade credibility tree was obtained with TreeAnnotator version 1.5.4 (Rambaut and Drummond 2009) using mean heights.

Molecular clock dating

For the molecular clock dating analysis we used the ITS data set without *Adenostyles alpina* because *Adenostyles* is only distantly related to the ingroup. The dating analysis was carried out with BEAUTi/BEAST v.1.7.5 (Drummond and Rambaut 2007) using the appropriate substitution model according to Table 2. Due to the lack of internal

Table 2 Partitions and substitution models for different analyses found by PartitionFinder

Partition	Best substitution models for analyses		
	RAxML	BEAST	MrBayes
ITS1 + ITS2	GTR[a] + G	GTR + G	GTR + G
5.8S ribosomal gene	GTR + I + G	TrNef[b] + I	K80[c] + I
*ndh*F-*rpl*32-*trn*L	GTR + I + G	GTR + I + G	GTR + I + G

[a] Rodríguez et al. (1990)

[b] Tamura and Nei (1993)

[c] Kimura (1980)

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 three times independently for 25 million generations to ensure that all parameters had an effective sampling size >200 and to ensure convergence of the independent runs to the same optimum. 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), Toman (1972), Dingwall (1976), Tutin (1976a, b), 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 <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 with respect to leaf size is *P. frigidus* with leaf size ranging from 2 to 50 cm which we coded as a third character state. *Petasites doerfleri*, *P. fominii*, *P. frigidus* and *P. rubellus* were coded to have numerous capitula. However, these species usually only have 3–10 flowering heads, while other species of *Petasites* have at least 10, but usually about 40 capitula (up to 130). Although coded as having numerous capitula, the above four species with a reduced number of capitula were highlighted in our character state reconstruction. To reconstruct the evolution of leaf size and number of capitula under Maximum Parsimony (MP) and Maximum Likelihood (ML) criteria, respectively, Mesquite v.2.75 (MP; Maddison and Maddison 2011) and the StochChar module (ML; Maddison and Maddison 2006) implemented in Mesquite v.2.75 were used. Character states were reconstructed using a cladogram based on the combined data set obtained from the BM analysis, with all unsupported nodes collapsed. Phylogenetic uncertainty was considered by reconstructing the evolution of the characters on all 18,000 trees obtained by the BM analysis and mapping the results on the cladogram.

Results

Phylogenetic relationships

The ITS data set included the Petasites-clade and *Ligularia tsangchanensis*, *Nordenstamia kingii* and *Tetradymia canescens* of the Tussilagininae as well as *Adenostyles alpina* of the Senecioninae as outgroups. In total, the ITS matrix contained 62 accessions and was 648 bp long, of which 93 were variable but uninformative and 174 were parsimony-informative (alignments see Online Resource 1).

The *ndh*F-*rpl*32-*trn*L data matrix consisted of 48 accessions including *Adenostyles alpina* as outgroup. It was 1986 bp long, with 94 variable and 68 parsimony-informative characters. Indel coding added another 9 parsimony-informative characters.

The ML phylogeny of the ITS data set is largely identical with the phylogeny of the combined data set described below. The ML phylogeny of the plastid data set was poorly resolved but showed supported conflict with the ITS data set in two cases. First, in the ITS phylogeny *E. sibiricum* is part of a clade with *P. frigidus* and *P. tatewakianus* Kitam. (ML bootstrap support 100 %; electronic supplementary Online Resource 2), whereas *E. glaciale* groups with *P. radiatus* (J.F.Gmel.) J.Toman and *P. spurius* (Retz.) Rchb.f. (ML bootstrap support 100 %; Online Resource 2). In the plastid data set (Online Resource 3), one sample of *E. sibiricum* (AST23) groups with *E. glaciale* (74 %) and the other (AST71) as first branch of an unsupported clade including *E. glaciale*, *E. sibiricum*

(AST23), *P. spurius* and *P. tatewakianus*. Second, *E. glaciale* (AST24) is part of a monophyletic *E. glaciale* in the plastid data set (85 %) but supported sister to *P. spurius* and *P. radiatus* in the ITS data set (95 %) which makes *E. glaciale* paraphyletic in relation to these two species. For further analyses, the samples of *E. sibiricum* and *E. glaciale* AST24 were removed from the data set, but this conflict between data sets will be discussed below.

The MP, ML and BM analyses of the combined data resulted in trees of similar topology with respect to well-supported branches; the BM tree is shown in Fig. 2. In the strongly supported Petasites-clade (MP/ML bootstrap support 87 %/97 %/Posterior Probability 1), generic relationships were unresolved as *Homogyne* (100 %/100 %/1), *Tussilago* (100 %/100 %/1) and *Petasites* including *Endocellion* (82 %/99 %/1) formed a trichotomy in all analyses. Within *Homogyne*, all three species were strongly supported, and *H. alpina* was well supported as sister (100 %/100 %/1) to *H. discolor* plus *H. sylvestris* (94 %/95 %/1). The *Petasites*/*Endocellion* clade (82 %/99 %/1) was not well resolved, containing a basal polytomy of seven clades. The weakly supported clade I contained *P. tricholobus* Franch., one accession of *P. japonicus*, *P. formosanus* Kitam. and *P. rubellus* (−/−/0.97), and clade II contained three accessions of *P. japonicus* (100 %/100 %/1). *Petasites kablikianus* Tausch formed a clade of its own (clade III; 100 %/100 %1). Clade IV contained *P. hybridus* (L.) G.Gaertn. et al., *P. fragrans* and *P. fominii* and was well supported (84 %/86 %/1). *Petasites albus* (L.) Gaertn., *P. doerfleri* and *P. paradoxus* (Retz.) Baumg. were part of clade V (97 %/100 %/1). Clade VI was composed of a monophyletic *P. frigidus* (including var.

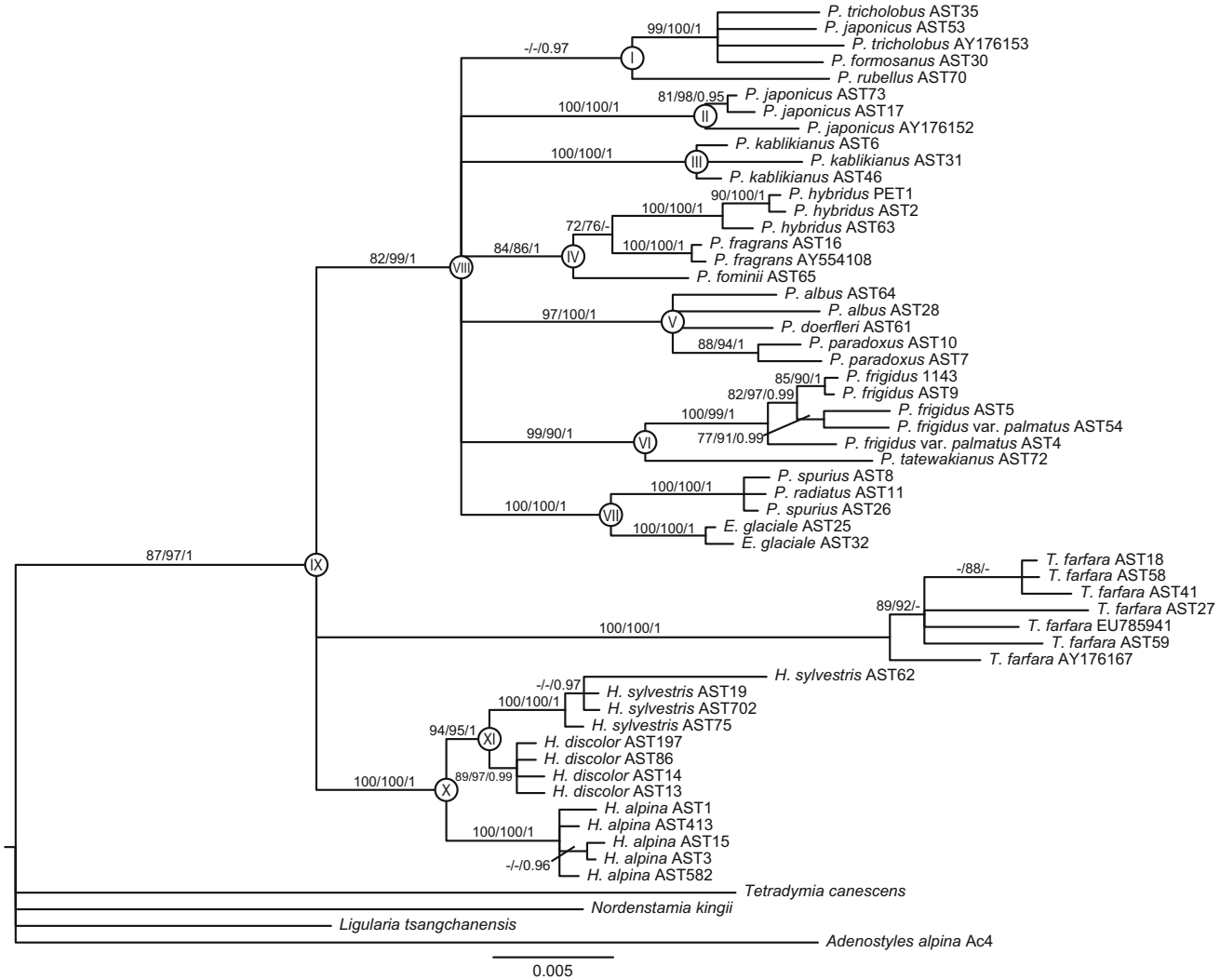

Fig. 2 Phylogenetic relationships in the Petasites-clade. Maximum clade credibility tree of the combined data set (ITS+*ndh*F-*rpl*32-*trn*L), all unsupported branches are collapsed. MP and ML bootstrap values (≥70 %) and Bayesian posterior probabilities (≥0.95) are given at branches. Clades discussed are marked at their nodes

palmatus (Aiton) Cronquist) and *P. tatewakianus* (99 %/90 %/1). *Endocellion glaciale*, *P. spurius* and *P. radiatus* formed clade VII (100 %/100 %/1).

Molecular clock dating

The BEAST analysis revealed a crown group age of the Petasites-clade [clade IX in Fig. 3] of 10.08 (4.77–18.12) million years (Ma; Fig. 3; Table 3). The crown group age of *Petasites* including *Endocellion* (VIII) was 7.91 (3.91–14.26) Ma. The estimated ages of the arctic–alpine species within the *Petasites-Endocellion* clade were 3.37 (1.28–6.31) Ma for *P. frigidus*/*E. sibiricum* (VI), 2.25 (0.65–4.44) Ma for *P. doerfleri* (V), 4.12 (1.35–7.87) Ma for *P. fominii* (IV), 3.28 (0.97–6.46) Ma for *E. glaciale* (VII), and 3.64 (1.30–6.86) Ma for *P. rubellus* (I). The crown group age of *Homogyne* (X) was 5.72 (1.82–10.88) Ma, and the split between *H. discolor* and *H. sylvestris* (XI) was 3.39 (1.05–6.69) Ma.

Evolution of dwarfism

The results of the reconstruction of leaf size and number of capitula using ML over the combined tree are shown in Fig. 4. Both MP and ML reconstructions revealed that large leaves (>10 cm in diameter) were ancestral (ML 97 %) in *Petasites* including *Endocellion*. Maximum Parsimony and ML analyses indicated that leaves smaller than 7 cm arose five times independently, i.e., in *Homogyne* (ML 100 %), *P. rubellus*, *P. fominii*, *P. doerfleri* and *Endocellion*.

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 32 % probability of numerous capitula and 78 % equivocal probability of numerous and single capitula). Single capitula arose in *E. glaciale*, and numerous capitula in the species of *Petasites* of this clade. Within *Petasites*, a reduced number of capitula (3–10) can be observed in the arctic–alpine *P. doerfleri*, *P. fominii*, *P. frigidus*, and *P. rubellus*.

Evolution of sexual systems

Petasites and *Endocellion* show a sexual dimorphism, whereas *Homogyne* and *Tussilago* are hermaphrodite as are 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 *Petasites*/*Endocellion* clade (Fig. 4).

Discussion

Phylogeny, biogeography and classification of Petasites, Endocellion, Homogyne *and* Tussilago

Our phylogenetic analysis (Fig. 2) of the combined data set 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. 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., and 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 by Toman (1972). Toman (1972) considered *Endocellion* a separate genus. On the other hand, Dingwall (1976) in Flora Europaea treated *E. sibiricum* as *P. sibiricus* (J.F.Gmel.) Dingwall. Our phylogeny supports *Petasites* including *Endocellion* as a monophyletic group.

Endocellion

In the phylogeny inferred from the ITS data set (Online Resource 2) *Endocellion* is diphyletic. Whereas *E. sibiricum* forms a clade with *P. frigidus* and *P. tatewakianus*, *E. glaciale* groups with *P. radiatus* and *P. spurius*. In contrast to this, *Endocellion* is (partly) monophyletic in the phylogeny based on the cpDNA data set where one accession of *E. sibiricum* groups with *E. glaciale*. 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 chromosome numbers 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 (and additional aneuploid and/or dysploid chromosome number changes) 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

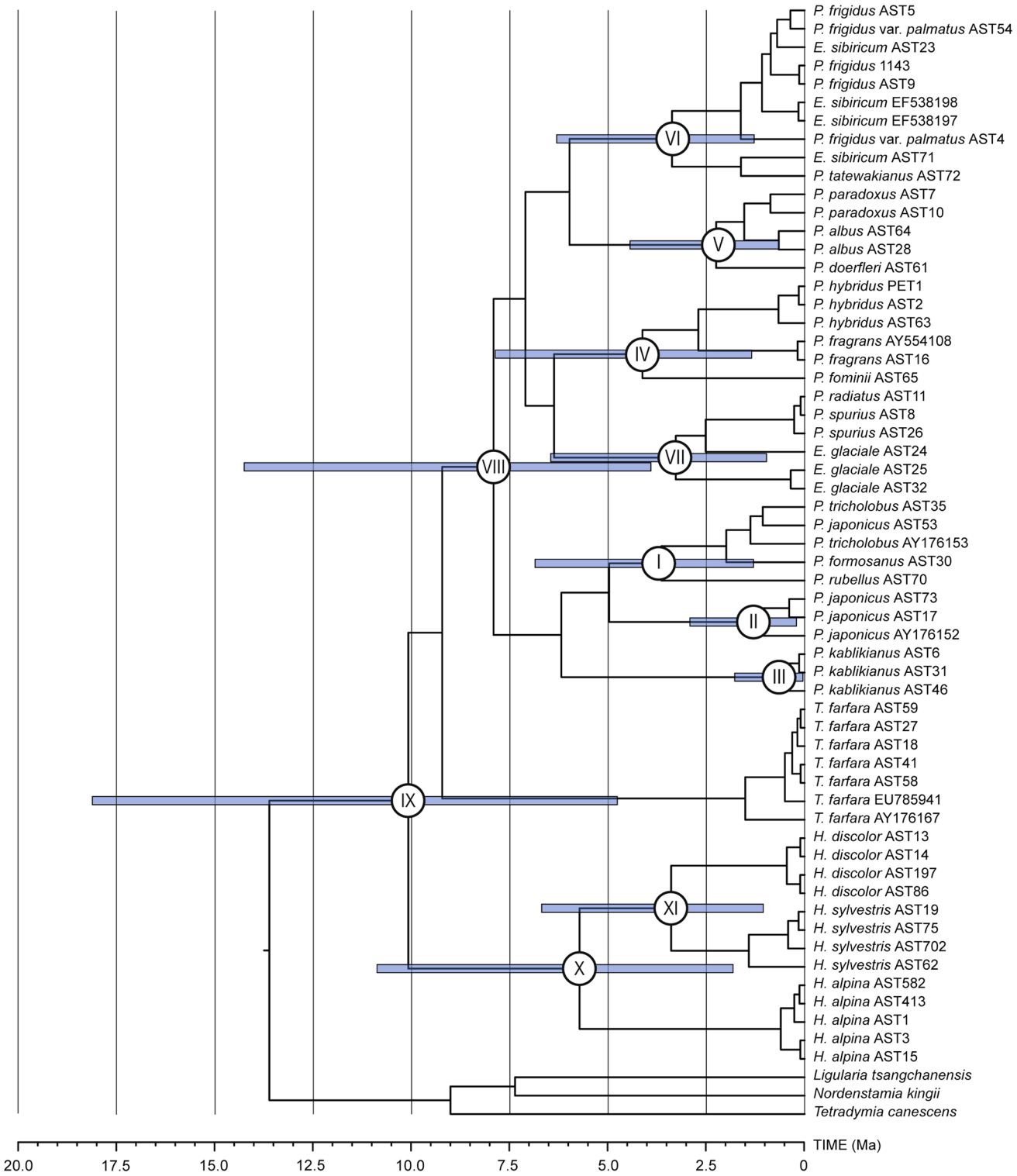

Fig. 3 Chronogram of the Petasites-clade obtained under a Bayesian relaxed molecular clock using published ITS nucleotide substitution rates (Kay et al. 2006). *Bars* at nodes represent 95 % highest posterior densities of node ages discussed. Ages for the clades discussed (I–XI) are given in Table 3

and the Russian Far East (Kuprianova 2000), and some of our samples of the two species were collected in the same region. 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

Table 3 Estimated ages of nodes in million years found in the BEAST analysis (BB) based on published ITS nucleotide substitution rates

Clade	Age	95 % CI
I	3.64	1.30–6.86
II	1.31	0.21–2.92
III	0.69	0.04–1.78
IV	4.12	1.35–7.87
V	2.25	0.65–4.44
VI	3.37	1.28–6.31
VII	3.28	0.97–6.46
VIII	7.91	3.91–14.26
IX	10.08	4.77–18.12
X	3.39	1.05–6.69
XI	5.72	1.82–10.88

Given are estimated ages and their 95 % highest posterior density intervals of the nodes labeled in Fig. 3

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 as concluded from the cpDNA data. Monophyly of *Endocellion* would be supported by several morphological characters. *Endocellion* is 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 hypothesize that the two species of *Endocellion* represent a monophyletic group. However, *Endocellion*, whether monophyletic or not, 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* (Turcz. ex 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 subdioecy/dioecy took place only once in the last common ancestor of *Petasites* incl. *Endocellion* (Fig. 4).

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* J.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, III, IV, V and VI. We could not identify any morphological characters in support of these clades, and they do not differ consistently in geographical distribution.

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 as 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.

Tussilago

Our phylogeny supports *Tussilago* in its current circumscription as a monospecific genus. *Tussilago farfara* is widely distributed throughout Eurasia (Hegi 1929; Tutin 1976b; Kuprianova 2000). 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). Frequent long-distance dispersal and colonization of new habitats together with clonal reproduction might explain low genetic variation in this species.

Evolution of dwarfism

Conforming to the tendency for reduction in plant size with decreasing average temperature, as observed across altitudinal and latitudinal transects (Billings and Mooney 1968; Johnson 1969; Bliss 1971; Körner and Larcher 1988; Körner 1999), most species of the Petasites-clade occurring in high mountain areas and in the Arctic are 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 evolved several times in parallel in this lineage. Parallel evolution of dwarfism has also been reported in arctic *Artemisia* L. (Tkach et al. 2008) and in alpine *Lysimachia* L.

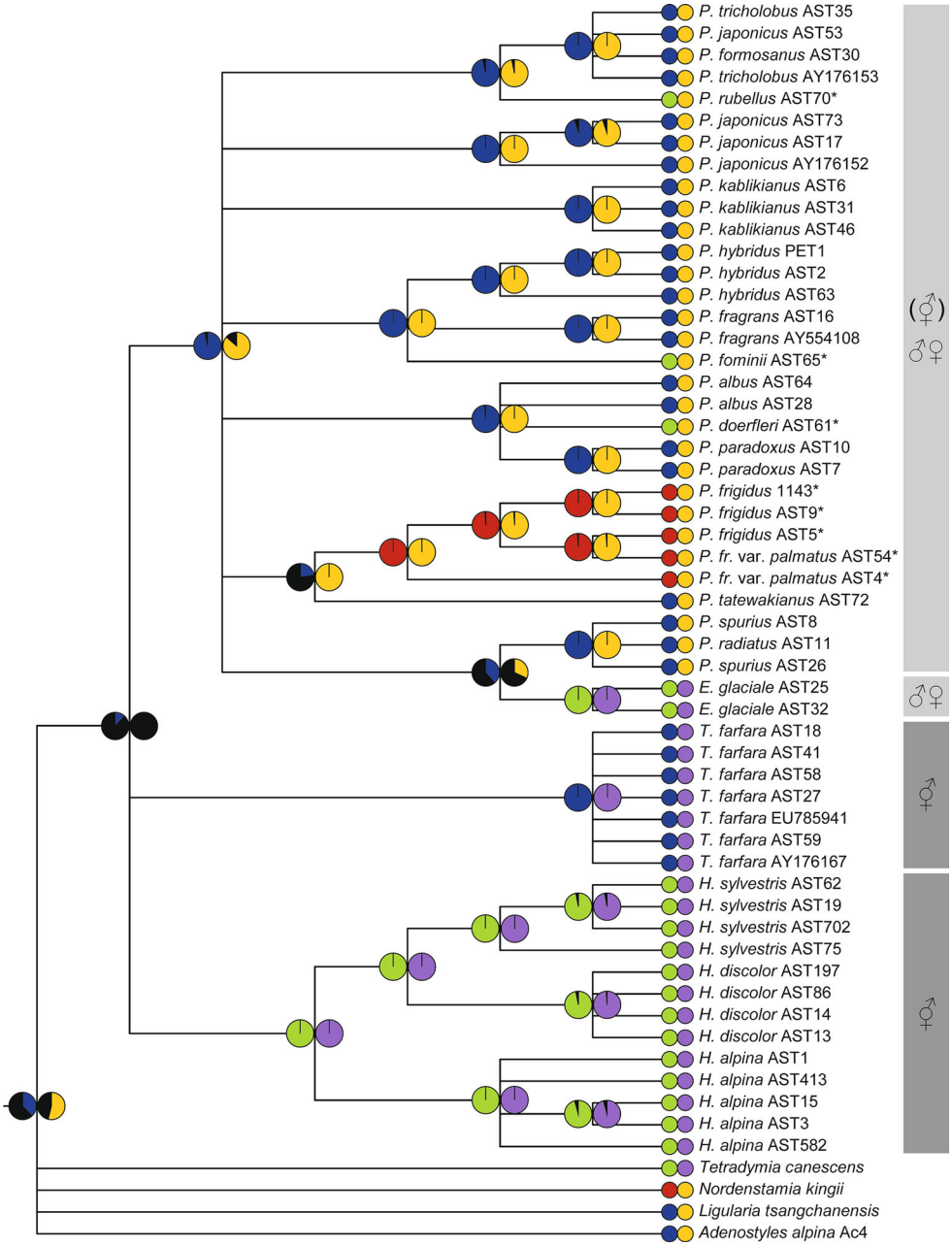

Fig. 4 Cladogram of the Petasites-clade obtained from the Bayesian analysis of the combined data set collapsing unsupported branches. *Pie charts* show the results of the Maximum Likelihood reconstruction of leaf size and number of capitula. *Left chart blue* = leaves more than 10 cm in diameter, *green* = leaves <7 cm in diameter, *red* = leaves 2–50 cm in diameter, *black* = equivocal probability for all three categories or node absent; *right chart violet* = capitula single (rarely 2–3), *yellow* = capitula numerous (more than 3, up to 130), *black* = equivocal probability for both categories or node absent. Taxa with only 3–10 capitula are marked with an *asterisk*. The distribution of sexes is mapped for each clade (♀ = female, ♂ = male, ⚥ = hermaphrodite)

sect. *Nummularia* (Hill) Klatt (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 7 cm 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 often has more than one capitulum (i.e., 2–3) per synflorescence. In conclusion, 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 <2 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. It is unknown whether leaf size variation in *P. frigidus* is the result of either phenotypic plasticity or genetic variation.

It is noteworthy that the arctic and alpine representatives of the Petasites-clade with small leaves are of somewhat similar age (2.25–5.72 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 age estimates are based on ITS substitution rates, and that our mean ages have large confidence intervals, any statement about similarities in age of the different lineages must be viewed very critically.

Although all species (except *H. sylvestris*) 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 also 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* grows at the same altitudes in the European Alps as *H. alpina* and *H. discolor*, which are true dwarfs with leaves <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 are characterized by a higher soil nutrient content (Landolt et al. 2010). This comparison suggests that factors such as nutrient availability, habitat disturbance and/or 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.

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. 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 concluded 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 to conclusively identify the factors governing the evolution of the characters discussed here.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Information on Electronic Supplementary Material

Online Resource 1. The alignments of the different markers.

Online Resource 2. Maximum likelihood tree obtained with RAxML of the nuclear ITS data set.

Online Resource 3. Maximum likelihood tree obtained with RAxML of the plastid *ndh*F-*rpl*32-*trn*L data set.

References

Aeschimann D, Lauber K, Moser DM, Theurillat J-P (2004) Flora Alpina. Haupt, Bern

Barkley TM (2006) *Tussilago*. In: Flora of North America Editorial Committee (eds) Flora of North America vol. 20, Oxford University Press, New York, p 635

Bayer RJ, Bogle AL, Cherniawsky DM (2006) *Petasites*. In: Flora of North America Editorial Committee (eds) Flora of North America vol. 20, Oxford University Press, New York, pp 635–640

Billings WD, Mooney HA (1968) The ecology of arctic and alpine plants. Biol Rev 43:481–529

Blattner F (1999) Direct amplification of the entire ITS region from poorly preserved plant material using recombinant PCR. BioTechniques 27:1180–1186

Bliss LC (1971) Arctic and alpine plant life cycles. Annual Rev Ecol Syst 2:405–438

Bogle AL (1968) Evidence for the hybrid origin of *Petasites warrenii* and *P. vitifolius*. Rhodora 70:533–551

Bremer K (1994) Asteraceae: cladistics and classification. Timber Press, Portland, Oregon

Chen YL, Nordenstam B, Jeffrey C, Koyama HH, Funston M, Vincent L (2011) Asteraceae tribe Senecioneae. In: Wu ZY, Raven PH, Hong DY (eds) Flora of China vols. 20–21, Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis

Cherniawsky DM, Bayer RJ (1998) Systematics of North American *Petasites* (Asteraceae: Senecioneae). III. A taxonomic revision. Canad J Botany 76:2061–2075. doi:10.1139/b98-151

Dingwall I (1976) *Petasites*. In: Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walter SM, Webb DA (eds) Flora Europaea, vol 4. Cambridge University Press, Cambridge, pp 186–188

Drummond AJ, Rambaut A (2007) BEAST: bayesian evolutionary analysis by sampling trees. BMC Evol Biol 7:214. doi:10.1186/1471-2148-7-214

Ellenberg H (1996) Vegetation Mitteleuropas mit den Alpen in ökologischer, dynamischer und historischer Sicht, 5th edn. Ulmer, Stuttgart

Fischer MA, Oswald K, Adler W (2008) Exkursionsflora für Österreich, Liechtenstein, Südtirol, 3rd edn. Biologiezentrum der Oberösterreichischen Landesmuseen, Linz

Hegi G (1929) Illustrierte Flora von Mitteleuropa. Mit besonderer Berücksichtigung von Deutschland, Oesterreich und der Schweiz. Zum Gebrauche in den Schulen und zum Selbstunterricht, VI. Band, 2. Hälfte. Lehmanns Verlag, München

Hind N, Kay J (2006) A nature print of *Petasites japonicus* subsp. *giganteus*. Curtis's Bot Mag 23:325–341. doi:10.1111/j.1467-8748.2006.00547.x

Huelsenbeck JP, Ronquist F (2001) MrBayes: bayesian inference of phylogeny. Bioinformatics 17:754–755

Johnson PL (1969) Arctic plants, ecosystems and strategies. Arctic 22:341–355

Kay K, Whittall J, Hodges S (2006) A survey of nuclear ribosomal internal transcribed spacer substitution rates across angiosperms: an approximate molecular clock with life history effects. BMC Evol Biol 6:36. doi:10.1186/1471-2148-6-36

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J Molec Evol 16:111–120

Kokubugata G, Nakamura K, Shinohara W, Saito Y, Peng C-I, Yokota M (2010) Evidence of three parallel evolutions of leaf dwarfism and phytogeography in *Lysimachia* sect. *Nummularia* in Japan and Taiwan. Molec Phylogen Evol 54:657–663. doi:10.1016/j.ympev.2009.09.004

Körner C (1999) Alpine plant life. Functional plant ecology of high mountain ecosystems, 2nd edn. Springer, Berlin

Körner C, Larcher W (1988) Plant life in cold climates. Plants and temperature. In: Long SF, Woodward FI (eds) Symposia of the Society for Experimental Biology, vol 42. The Company of Biologists Ltd, Cambridge, pp 25–57

Körner C, Neumayer M, Menendez-Riedl SP, Smeets-Scheel A (1989) Functional morphology of mountain plants. Flora 182:353–383

Kuprianova LA (2000) Genera *Tussilago*, *Petasites*, *Nardosmia*, *Homogyne*. In: Schischkin BK, Bobrov EG (eds) Flora of the USSR vol. 26, Smithsonian Institution Libraries, Washington, pp 609–624

Landolt E, Bäumler B, Erhardt A, Hegg O, Klötzli F, Lämmler W, Nobis M, Rudmann-Maurer K, Schweingruber FH, Theurillat J-P, Urmi E, Vust M, Wohlgemuth T (2010) Flora indicativa: Ökologische Zeigerwerte und biologische Kennzeichen zur Flora der Schweiz und der Alpen. Haupt, Bern

Lanfear R, Calcott R, Ho SYW, Guindon S (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Molec Biol Evol 29:1695–1701. doi:10.1093/molbev/mss020

Liu S, Illarionova ID (2011) Asteraceae tribe Senecioneae. In: Wu ZY, Raven PH, Hong DY (eds) Flora of China vols. 20–21, Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis

Maddison DR, Maddison WP (2000) MacClade 4.0: analysis of phylogeny and character evolution. Sinauer Assoc., Sunderland

Maddison WP, Maddison DR (2006) StochChar: a package of Mesquite modules for stochastic models of character evolution. Version 1.1

Maddison WP, Maddison DR (2011) Mesquite: a modular system for evolutionary analysis. Version 2.75. Available at: http://mesquiteproject.org. Accessed 14 March 2013

Merxmüller H (1952) Untersuchungen zur Sippengliederung und Arealbildung in den Alpen. Teil 1. Jahrb Vereins Schutze Alpenpfl 17:96–133

Meusel H, Jäger E (1992) Vergleichende Chorologie der zentraleuropäischen Flora, vol 3. Fischer, Jena

Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the gateway computing environments workshop (GCE), 14 Nov. 2010, New Orleans, pp 1–8

Muir G, Fleming CC, Schlötterer C (2001) Three divergent rDNA clusters predate the species divergence in *Quercus petraea* (Matt.) Liebl. and *Quercus robur* L. Molec Biol Evol 18:112–119

Nordenstam B (2007) Tribe Senecioneae. In: Kubitzki K, Kadereit JW, Jeffrey C (eds) The families and genera of vascular plants, vol 8. Springer, Berlin, pp 208–241

Ordoñez JC, van Bodegom PM, Witte J-PM, Wright IJ, Reich PB, Aerts R (2009) A global study of relationships between leaf traits, climate and soil measures of nutrient fertility. Global Ecol Biogeogr 18:137–149. doi:10.1111/j.1466-8238.2008.00441.x

Ozenda P (1988) Die Vegetation der Alpen im europäischen Gebirgsraum. Fischer, Stuttgart

Pelser PB, Nordenstam B, Kadereit JW, Watson LE (2007) An ITS phylogeny of tribe Senecioneae (Asteraceae) and a new delimitation of *Senecio* L. Taxon 56:1077–1104

Pfeiffer T, Günzel C, Frey W (2008) Clonal reproduction, vegetative multiplication and habitat colonisation in *Tussilago farfara* (Asteraceae): a combined morpho-ecological and molecular study. Flora 203:281–291. doi:10.1016/j.flora.2007.02.008

Pirie MD, Humphreys AM, Galley C, Barker NP, Verboom GA, Orlovich D, Draffin SJ, Lloyd K, Baeza CM, Negritto M, Ruiz E,

Cota Sanchez JH, Reimer E, Linder HP (2008) A novel supermatrix approach improves resolution of phylogenetic relationships in a comprehensive sample of danthonioid grasses. Molec Phylogen Evol 48:1106–1119. doi:10.1016/j.ympev.2008.05.030

Polunin N (1959) Circumpolar arctic flora. Oxford University Press, Oxford

Rambaut A, Drummond AJ (2007) Tracer, version 1.5. MCMC Trace File Analyser. Computer program and documentation distributed by the authors. Available at: http://beast.bio.ed.ac.uk/Tracer. Accessed 22 Nov 2012

Rambaut A, Drummond AJ (2009) TreeAnnotator, version 1.5.4. Available at: http://beast.bio.ed.ac.uk/TreeAnnotator. Accessed 23 Nov 2015

Rodríguez F, Oliver JL, Marín A, Medina JR (1990) The general stochastic model of nucleotide substitution. J Theoret Biol 42:485–501. doi:10.1016/S0022-5193(05)80104-3

Shaw J, Lickey EB, Schilling EE, Smith RL (2007) Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. Amer J Bot 94:275–288. doi:10.3732/ajb.94.3.275

Shinohara W, Murakami N (2006) How have the alpine dwarf plants in Yakushima been miniaturized? A comparative study of two alpine dwarf species in Yakushima, *Blechnum nipponicum* (Blechnaceae) and *Lysimachia japonica* (Primulaceae). J Pl Res 119:571–580. doi:10.1007/s10265-006-0016-5

Simmons MP, Ochoterena H (2000) Gaps as characters in sequence-based phylogenetic analyses. Syst Biol 49:369–381

Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap algorithm for the RAxML web-servers. Syst Biol 75:758–771. doi:10.1080/10635150802429642

Swofford DL (2002) PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4.0. Sinauer, Sunderland, Massachusetts

Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molec Biol Evol 10:512–526

Tkach NV, Hoffmann MH, Röser M, Korobkov AA, von Hagen KB (2008) Parallel evolutionary patterns in multiple lineages of arctic *Artemisia* L. (Asteraceae). Evolution 62:184–198. doi:10.1111/j.1558-5646.2007.00270.x

Toman J (1972) A taxonomic survey of the genera *Petasites* and *Endocellion*. Folia Geobot Phytotax 7:381–406

Troll W (1939) Vergleichende Morphologie der höheren Pflanzen vol. 1(1), Vegetationsorgane. Borntraeger, Berlin

Turesson G (1925) The plant species in relation to habitat and climate. Hereditas (Lund) 6:147–236

Turesson G (1930) The selective effect of climate upon the plant species. Hereditas (Lund) 14:99–152

Tutin TG (1976a) *Homogyne*. In: Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walter SM, Webb DA (eds) Flora Europaea, vol 4. Cambridge University Press, Cambridge, pp 188–189

Tutin TG (1976b) *Tussilago*. In: Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walter SM, Webb DA (eds) Flora Europaea, vol 4. Cambridge University Press, Cambridge, p 186

Vierhapper F (1923) Über Verwandtschaft und Herkunft der Gattungen *Homogyne* und *Adenostyles*. Österr Bot Z 73:150–164

Wendel JF, Doyle JJ (1998) Phylogenetic incongruence: window into genome history and molecular evolution. In: Soltis DE, Soltis P, Doyle JJ (eds) Molecular systematics of plants II. Kluwer Academic Publishers, Boston, pp 265–296

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic Press, San Diego, pp 315–322

Zhang M-L, Uhink CH, Kadereit JW (2007) Phylogeny and biogeography of *Epimedium/Vancouveria* (Berberidaceae): western North American—East Asian disjunctions, the origin of European mountain plant taxa, and East Asian species diversity. Syst Bot 32:81–92. doi:10.1600/036364407780360265