



## Molecular phylogenetic analyses identify Alpine differentiation and dysploid chromosome number changes as major forces for the evolution of the European endemic *Phyteuma* (Campanulaceae)

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### ABSTRACT

*Phyteuma* is a chromosomally and ecologically diverse vascular plant genus and constitutes an excellent system for studying both the role of chromosomal change for species diversification and the evolution of high-mountain biota. This kind of research is, however, hampered by the lack of a sound phylogenetic framework exacerbated by the notoriously low predictive power of traditional taxonomy with respect to phylogenetic relationships in Campanulaceae. Based on a comprehensive taxon sampling and analyses of nuclear and plastid sequence and AFLP fingerprint data, *Phyteuma* is confirmed as a monophyletic group sister to the monotypic *Physoplexis*, which is in line with their peculiar flower morphologies. Within *Phyteuma* two clades, largely corresponding to previously recognized sections, are consistently found. The traditional circumscription of taxonomic series is largely rejected. Whereas distinctness of the currently recognized species is mostly corroborated, some interspecific relationships remain ambiguous due to incongruences between nuclear and plastid data. Major forces for diversification and evolution of *Phyteuma* are descending dysploidy (i.e., a decrease in chromosome base number) as well as allopatric and ecological differentiation within the Alps, the genus' center of species diversity.

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### 1. Introduction

In the current circumscription, the Campanulaceae comprise nearly 2400 species in 84 genera (Lammers, 2007a,b). Molecular phylogenetic studies have shown that many of the genera recognized in traditional taxonomic treatments (Boissier, 1875; de Candolle, 1830; Fedorov, 1957; Kolakovsky, 1994) do not constitute monophyletic groups (Antonelli, 2008; Borsch et al., 2009; Cellinese et al., 2009; Eddie et al., 2003; Haberle et al., 2009; Mansion et al., 2012; Park et al., 2006; Roquet et al., 2008, 2009). Instead, large genera such as *Lobelia*, *Wahlenbergia* or *Campanula* are para- or polyphyletic (Antonelli, 2008; Eddie et al., 2003; Haberle et al., 2009; Prebble et al., 2011; Wendling et al., 2011) and appear to be the remainder after exclusion of morphologically conspicuous groups as separate genera. Discomfortingly, many of

the smaller genera, such as *Symphyandra* or *Legousia*, are not monophyletic either (Cellinese et al., 2009; Eddie et al., 2003), emphasizing that current taxonomy is non-predictive with respect to the delimitation of monophyletic groups and their relationships. Suggestions to accommodate the lack of monophyly in *Campanula* range from merging all genera phylogenetically nested in *Campanula* with it (Roquet et al., 2008) to splitting *Campanula* into smaller genera (Haberle et al., 2009; Park et al., 2006). Since our understanding of the phylogenetic relationships at the generic level is, however, still incomplete, the pragmatic approach of retaining traditionally circumscribed genera (as used in the most recent synopses of the entire family: Lammers, 2007a,b) remains the best solution for the time being.

One of the morphologically distinct segregates phylogenetically nested within *Campanula* is *Phyteuma* (Borsch et al., 2009; Eddie et al., 2003; Mansion et al., 2012; Roquet et al., 2008; Wendling et al., 2011). It comprises about 24 species (Castroviejo et al., 2010; Damboldt, 1976) mainly distributed in Europe; only a single species, *P. charmelii*, reaches the Middle Atlas mountains in northern Morocco (Quézel, 1954). *Phyteuma* species are characterized by

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proterandrous flowers with a deeply (4)5-lobed corolla of mostly blue, more rarely dark violet or white color (Damboldt, 1976). In the male anthetic phase, the corolla lobes separate only at their base and remain fused at their tips (Stadler, 1991). In the course of retraction of the thus formed corolla hood, pollen is brushed to the style (secondary pollen presentation: Erbar and Leins, 1995; Leins and Erbar, 2006). Full separation of the corolla lobes is achieved in the female anthetic phase (Stadler, 1991; Wheeler and Hutchings, 2002). Similar flower morphology characterizes *Physoplexis*, a monotypic genus endemic to the southeastern European Alps, but here the corolla lobes do not separate at their tips (Pignatti, 1982). The shared peculiar flower morphology suggests that these two genera may constitute a monophyletic group. This hypothesis gained support from molecular data, but despite morphological and karyological differences between *Phyteuma* and *Physoplexis* (flowers in spikes or capitula, non-sunk stomata, and chromosome base numbers of  $x = 10, 11, 12, 13, 14$  versus flowers in umbels, sunk stomata, and chromosome base number of  $x = 17$ : Damboldt, 1976; Polatschek, 1966; Schulz, 1904), their separation at the molecular level is weak and often poorly supported (Borsch et al., 2009; Eddie et al., 2003; Mansion et al., 2012; Park et al., 2006; Roquet et al., 2008). Furthermore, in previous studies *Phyteuma* is represented by maximally six species, rendering assessment of its monophyly and its relationships to *Physoplexis* uncertain. Similarly, molecular studies arrived at conflicting conclusions whether the monotypic *Petromarula*, endemic to the eastern Mediterranean island of Crete (Akeroyd, 1994), is sister to a clade of *Phyteuma* and *Physoplexis* (Cellinese et al., 2009; Haberle et al., 2009; Park et al., 2006; Roquet et al., 2008) or not (Borsch et al., 2009; Eddie et al., 2003; Mansion et al., 2012; Roquet et al., 2008; Wendling et al., 2011). Evidently, further data are necessary to test these hypotheses.

A detailed taxonomic treatment of *Phyteuma* is available in the monograph by Schulz (1904). He distinguished 29 species, most of which are still recognized in modern floras (e.g., Aeschmann et al., 2004; Damboldt, 1976; Podlech, 2008), and grouped them in an elaborate infrageneric classification including ten series and two sections (Table 1). Sections are characterized by different inflorescence types (spikes and capitula in sects. *Spicata* and *Capitata*, respectively), and series are mostly distinguished by basal leaf shape (e.g., cordate basal leaves in ser. *Cordifolia*). Schulz (1904) provided an explicit hypothesis on the phylogenetic relationships of the species (Fig. 1), but this has never been tested using molecular data.

A sound phylogenetic hypothesis on relationships within *Phyteuma* will form the basis for a better understanding of evolutionary processes in this genus. One of those is dysploidy ( $x = 10, 11, 12, 13, 14$ ; Ochlewska, 1965; Polatschek, 1966), which is an important factor affecting species diversification (Blösch et al., 2009; Hansen et al., 2006), but whose role in *Phyteuma* remains unknown. Another interesting aspect of *Phyteuma* is the displayed habitat diversity (Aeschmann et al., 2004; Podlech, 2008), rendering *Phyteuma* a well-suited system for studying the evolution of high mountain biota also in the context of habitat adaptation. Given its wide distribution in European mountain ranges and temperate lowlands, it provides an excellent system for studying spatio-temporal aspects within and across different habitats, e.g., the temporal framework of the evolution of high mountain taxa and their responses to Pleistocene climatic oscillations (Schönswetter et al., 2002). Currently, following any of those research avenues is hampered by the lack of a sound phylogenetic framework.

The first aim of this study is to test previous hypotheses on the phylogenetic relationships of and within *Phyteuma* thus providing a sound phylogenetic framework for evolutionary studies. To this end, we obtained (1) plastid (*trnK* intron including *matK*; *trnL* intron and *trnL-trnF* intergenic spacer) and nuclear sequence data

(ribosomal ITS) from usually multiple accessions of almost all currently recognized *Phyteuma* species and from putatively closely related genera and analyzed those using parsimony, likelihood and Bayesian methods of phylogenetic inference; (2) AFLP fingerprint data for a smaller taxon set and analyzed those with a distance-based network approach. Specifically, we addressed the following questions: (1) Is *Phyteuma* a monophyletic group distinct from *Physoplexis*, as suggested by morphological and karyological differences? What is the closest relative of *Phyteuma* and *Physoplexis*? (2) What are the phylogenetic relationships within *Phyteuma* and do inferences from molecular data agree with the intuitive phylogenetic hypothesis by Schulz (1904)? Using the thus established phylogenetic framework, a further aim is (3) to investigate chromosome number evolution to assess whether different chromosome numbers correlate with phylogenetic lineages in *Phyteuma*, and (4) to reconstruct the biogeographic history of this genus for identifying patterns of range formation in the context of species differentiation.

## 2. Material and methods

### 2.1. Plant material

Leaf material of 1–13 populations (median three) of 24 *Phyteuma* species (including two subspecies each of *P. globulariifolium* and *P. scheuchzeri*), totaling 106 accessions, as well as of 16 populations of ten outgroup taxa (*Physoplexis*, *Petromarula*, *Asyneuma*, *Campanula uniflora*, *C. persicifolia*) was collected in the field and dried in silica-gel or obtained from herbarium specimens (Table 1).

### 2.2. DNA sequencing

Total genomic DNA was extracted from silica-gel dried or herbarium material following the CTAB extraction protocol (Doyle and Doyle, 1987) with slight modifications (Schönswetter et al., 2002). Amplification and sequencing of the nuclear ITS1–5.8S–ITS2 region and of the plastid *trnL-F* region (*trnL<sub>UAA</sub>* intron and *trnL<sub>UAA</sub>-trnF<sub>GAA</sub>* spacer) was conducted as described in Schönswetter and Schneeweiss (2009). Primers for amplification and sequencing of *matK* were *trnKF5an* (Russell et al., 2010), *matK50Rdi* (5'-CYATTYAAACCATGATCATGAGCAAG-3'), *matK-50Fdi* (Demaio et al., 2011), *matK750Rdi* (5'-GATCGTAAATGAGAGATTGGT TCGG-3'), *matK550Fdi* (Demaio et al., 2011), *matK1300R* (Samuel et al., 2005), *matK1200Fdi* (5'-GTTCCAATTATTCYCCTGATTGGATC-3'), and *trnKR3an* (Demaio et al., 2011; Russell et al., 2010). PCR amplifications of double-stranded DNA were performed as described in Russell et al. (2010) and Demaio et al. (2011). GenBank accession numbers of obtained DNA-sequences are listed in Table 1.

### 2.3. AFLPs

Due to contradicting relationships inferred from different DNA sequence markers (see Results), a complimentary AFLP data set for a selected number of individuals of species, where suitable material was available (all except *P. gallicum*, *P. nigrum*, *P. pseudorbiculare*, *P. tetramerum*, *P. vagneri*, *P. villarsii*), was produced. In most cases, AFLPs were generated for the same accessions as used for sequencing, except for *P. globulariifolium* and *P. hemisphaericum*, where individuals were selected from larger species-wide data sets (Schönswetter et al., 2002; A. Tribsch et al., unpubl.), some of which were not used for sequencing (Table 1). AFLP data were generated following protocols described in Schönswetter et al. (2002).

Table 1

Sampled taxa (supraspecific taxa in *Phyteuma* according to Schulz, 1904) and accession (acc.) numbers, localities and voucher information (herbarium acronyms follow Thiers, 2012; herb. Gutermann refers to the private herbarium of W. Gutermann, deposited at the Faculty Centre of Biodiversity, University of Vienna), GenBank accession numbers (nuclear ribosomal ITS, plastid *trnL-F* region, plastid *trnK* intron containing the *matK* gene) and indication of inclusion in the AFLP data set. Country abbreviations: A, Austria; BiH, Bosnia and Herzegovina; CH, Switzerland; E, Spain; F, France; G, Germany; GR, Greece; HR, Croatia; I, Italy; PL, Poland; RO, Romania; SI, Slovenia; Collectors abbreviations: AT, A. Tribsch; GMS, G.M. Schneeweiss; MT, M. Thiv; PS, P. Schönswetter.

Taxon	acc.	Locality (voucher-information)	ITS	<i>trnL-F</i>	<i>trnK-matK</i>	AFLPs
<b>Campanula L.</b>						
<i>C. persicifolia</i> L.		A, northeastern Alps (WU: GMS & PS 6288)	DQ304590	KC455648	KC455763	
<i>C. uniflora</i> L.		Norway, Sør-Trøndelag (WU: C. Brochmann & A.C. Sheen 7090)	DQ304588	KC455653	KC455768	
<b>Asyneuma Griseb. &amp; Schenk</b>						
<i>A. campanuloides</i> (M. Bieb. ex Sims) Bornm.		Georgia, Greater Caucasus (WU: GMS 4469)	DQ304586	KC455655	KC455770	
<i>A. canescens</i> (Waldst. & Kit.) Griseb. & Schenk	acc. 1	cultivated at HBV (WU: G. Cufodontis 4.8.1949)	KC455545	KC455656	KC455771	
	acc. 2	BiH, Mt. Čvrtnica (NHMR: B. Surina 448)	KC455546	KC455657	KC455772	
<i>A. limonifolium</i> (L.) Janch.	acc. 1	GR, Ionian Islands, Lefkada (herb. Gutermann: W. Gutermann 35549)	DQ304587	KC455651	KC455766	
	acc. 2	GR, Mount Olympus (STU: A. Wörz 40 24.07.26.01)	KC455543	KC455652	KC455767	
<i>A. lobelioides</i> Hand.-Mazz.		Turkey (A7), from Yelkendirek to Kürtün (WU: D. Albach & F. Özgökce 884)	KC455541	KC455649	KC455764	
<i>A. pichleri</i> (Vis.) D. Lakušić & F. Conti		Montenegro, Prokletije Mtns. (WU: B. Frajman & M. Turjak 11087)	KC455542	KC455650	KC455765	
<i>A. trichocalycinum</i> (Ten.) K. Malý		I, Calabria, Serra Dolcedorme (C.J. Dixon 11179)	KC455544	KC455654	KC455769	
<b>Petromarula Vent. ex R. Hedw.</b>						
<i>Pe. pinnata</i> A. DC.	acc. 1	GR, Crete (WU: N. Sauberer 7821)	DQ304582	KC455658	KC455773	
	acc. 2	GR, Crete (O: A.C. Sheen & H.M. Bendiksby 410)	KC455547	KC455659	KC455774	
<b>Physoplexis Schur</b>						
<i>Ph. comosa</i> (L.) Schur	acc. 1	I, southern Alps, Mte. Raut (no voucher)	KC455604	KC455717	KC455832	
	acc. 2	I, southern Alps, Prealpi Carniche (WU: GMS, PS & AT 3902)	DQ304585	KC455718	KC455833	x
	acc. 3	I, Dolomites, Schlern (STU: MT 4035)	KC455605	KC455719	KC455834	
	acc. 4	I, southern Alps, Prealpi Breschiani (WU: GMS, PS & AT 3923)				x
<b>Phyteuma L.</b>						
<b>sect. Spicata</b>						
<b>ser. Cordifolia</b>						
<i>P. ovatum</i> Honck.	acc. 1	CH, Tessiner Alpen, Airolo (STU: MT 3891)	KC455592	KC455705	KC455820	
	acc. 2	I, Alpi Carniche (WU: PS & AT 4581)	KC455569	KC455682	KC455797	x
<i>P. pyrenaicum</i> Rich. Schulz		F/E, Pyrenees, Col du Pourtalet (WU: GMS, PS & AT 6444)	KC455559	KC455671	KC455786	x
<i>P. spicatum</i> L.	acc. 1	F, eastern Pyrenees (WU: GMS, PS & AT 6375)	KC455566	KC455679	KC455794	x
	acc. 2	F, Massif Central, Puy Mary (WU: GMS & PS 8815)	KC455564	KC455677	KC455792	
	acc. 3	F, Massif Central, Le Gerbier de Jonc (WU: GMS, PS & AT 6511)	KC455567	KC455680	KC455795	x
	acc. 4	G, Baden-Württemberg, Alpenvorland (STU: MT s.n.)	KC455596	KC455709	KC455824	
	acc. 5	F, Alpes Grées (WU: PS & AT 4763)	KC455562	KC455675	KC455790	x
	acc. 6	A, central Alps, Hohe Tauern (herb. Gutermann. W. Gutermann 23478)	KC455561	KC455674	KC455789	x
	acc. 7	A, northeastern Alps (WU: GMS & PS 6280)	KC455560	KC455672	KC455787	x
	acc. 8	PL, Tatry Wysokie (WU: PS & M. Ronikier 10574)	KC455565	KC455678	KC455793	
	acc. 9	HR, Gorski Kotar, Bjelolasica (WU: GMS, PS & AT 6233)	DQ304584	KC455673	KC455788	x
<i>P. vagneri</i> A. Kern.	acc. 1	RO, Carpathians, Munții Bucegii (no voucher)	KC455571	KC455684	KC455799	
	acc. 2	RO, Carpathians, Munții Bucegii, Poiana Stanii (B: P. Anastasiu s.n.)	KC455599	KC455712	KC455827	
	acc. 3	RO, Carpathians, Munții Bucegii, Poiana Stanii (B: P. Anastasiu s.n.)	KC455600	KC455713	KC455828	
	acc. 4	RO, Carpathians, Munții Rodnei (CLA: C. Groza 7474)	KC455556	KC455668	KC455783	
<b>ser. Lanceolata</b>						
<i>P. gallicum</i> Rich. Schulz		F, Massif Central, between Besse-en-Chandesse and Condat-en-Feniers (M: W. Lippert & D. Podlech 17687)	KC455587	KC455700	KC455815	
<i>P. nigrum</i> F.W. Schmidt	acc. 1	G, Baden-Württemberg, Black Forest (STU: MT s.n.)	KC455590	KC455703	KC455818	
	acc. 2	G, Rheinland-Pfalz, Westeifel (WU: R. Hand 7822)	KC455568	KC455681	KC455796	
	acc. 3	Czech Republic (no voucher)	KC455563	KC455676	KC455791	
<b>ser. Tetramera</b>						
<i>P. tetramerum</i> Schur	acc. 1	RO, Carpathians, Munții Apuseni, Munții Bihorului (CLA: C. Groza 7475)	KC455570	KC455683	KC455798	
	acc. 2	RO, Carpathians, Munții Giurgeului (STU: F. Cernoch 47347)	KC455598	KC455711	KC455826	

Table 1 (continued)

Taxon	acc.	Locality (voucher-information)	ITS	<i>trnL-F</i>	<i>trnK-matK</i>	AFLPs
<b>ser. <i>Angustifolia</i></b>						
<i>P. betonicifolium</i> Vill.	acc. 1	I, Alpi Liguri (WU: PS & AT 4673)	KC455572	KC455685	KC455800	x
	acc. 2	CH, Graubünden, Berninapass (STU: MT 3831)	KC455573	KC455686	KC455801	
	acc. 3	A, Allgäuer Alpen (WU: F. Gugerli INTRABIODIV G19)	KC455573	KC455686	KC455801	
<i>P. michelii</i> All.	acc. 1	F, Alpes Maritimes (WU: PS & AT 4691)	KC455578	KC455691	KC455806	x
	acc. 2	F, Alpes Maritimes (STU: MT s.n.)	KC455588	KC455701	KC455816	
<i>P. persicifolium</i> Hoppe	acc. 1	A, Karnische Alpen (STU: A. Wörz 24.06.16.01)	KC455602	KC455715	KC455830	
	acc. 2	A, central Alps, Saualpe (WU: PS & AT 4568)	KC455574	KC455687	KC455802	x
	acc. 3	HR, Gorski Kotar (WU: GMS & PS 6306)	KC455575	KC455688	KC455803	x
<i>P. scorzonrifolium</i> Vill.	acc. 1	I, Apennines, (WU: C.J. Dixon 11028)	KC455577	KC455690	KC455805	
	acc. 2	I, Alpi Liguri (WU: PS & AT 4674)	HF586687	HF586689	HF586688	
	acc. 3	F, Alpes Maritimes (WU: PS & AT 4704)	KC455576	KC455689	KC455804	
	acc. 4	F, Alpes Maritimes (STU: MT s.n.)	KC455589	KC455702	KC455817	
	acc. 5	CH, Alpi Lepontine (STU: MT 4023A)	KC455593	KC455706	KC455821	
	acc. 6	CH, Alpi Lepontine (STU: MT 4023B)	KC455594	KC455707	KC455822	
	acc. 7	CH, Alpi Lepontine (STU: MT 4023C)	KC455595	KC455708	KC455823	
	acc. 8	I, Alpi Liguri (WU: GMS, PS & AT 6370)				x
<b>ser. <i>Fagopyrifolia</i></b>						
<i>P. cordatum</i> Balb.	acc. 1	F, Alpes Maritimes (M: M. Nydegger 30694)	KC455585	KC455698	KC455813	
	acc. 2	I, Alpi Liguri (WU: GMS, PS & AT 6371)	KC455579	KC455692	KC455807	x
	acc. 3	I, Alpi Liguri (M: W. Lippert & H. Merxmüller 20170)	KC455586	KC455699	KC455814	
<b>sect. <i>Capitata</i></b>						
<b>ser. <i>Orbiculata</i></b>						
<i>P. orbiculare</i> L.	acc. 1	E, Pyrenees, Oroel (WU: GMS, PS & AT 6427)	KC455548	KC455660	KC455775	x
	acc. 2	I, Alpi Liguri (WU: PS & AT 4675)	KC455551	KC455663	KC455778	x
	acc. 3	F, Dévoluy, Col du Noyer (MSB: M. Nydegger 30303)	KC455597	KC455710	KC455825	
	acc. 4	CH, Graubünden, Berninapass (STU: MT 3836)	KC455591	KC455704	KC455819	
	acc. 5	I, southern Alps, Mte. Raut (WU: GMS, PS & AT 3889)	KC455550	KC455662	KC455777	x
	acc. 6	A, central Alps, Hohe Tauern (WU: PS & AT 4880)	KC455549	KC455661	KC455776	x
	acc. 7	PL, Tatry Wysokie (WU: PS & M. Ronikier 10579)	KC455554	KC455666	KC455781	
	acc. 8	BiH, Mt. Čvrsnica (WU: B. Frajman & M. Turjak 11083)	KC455552	KC455664	KC455779	
	acc. 9	RO, Carpathians, Munții Apuseni (WU: PS & AT 10742)	KC455555	KC455667	KC455782	
	acc. 10	RO, Carpathians, Munții Bucegi (WU: F. Schumacher 7178)	KC455553	KC455665	KC455780	x
<b>ser. <i>Latifolia</i></b>						
<i>P. pseudorbiculare</i> Pant.	acc. 1	BiH, Mt. Maglić (WU: B. Frajman & M. Turjak 11084)	KC455637	KC455752	KC455867	
	acc. 2	Montenegro, Bijelasica (B: G. Parolly CN 85.61)	KC455642	KC455757	KC455872	
<i>P. sieberi</i> Spreng.	acc. 1	I, Dolomiti (WU: GMS, PS & AT 6208)	KC455638	KC455753	KC455868	x
	acc. 2	I, Dolomiti, Schlern (STU: MT 4038)	KC455647	KC455762	KC455877	
	acc. 3	A, southern Alps, Lienzer Dolomiten (WU: L. Schratt-Ehrendorfer 7032)	KC455639	KC455754	KC455869	x
<b>ser. <i>Saxicola</i></b>						
<i>P. charmelii</i> Vill.	acc. 1	E, Pyrenees, Oroel (WU: GMS, PS & AT 6431)	KC455584	KC455697	KC455812	x
	acc. 2	I, Alpi Maritimi (WU: GMS, PS & AT 5581)	KC455581	KC455694	KC455809	
	acc. 3	F, Clus de Barles (STU: P. Aleksejew s. n.)	KC455558	KC455670	KC455875	
<i>P. scheuchzeri</i> All. subsp. <i>scheuchzeri</i>	acc. 1	I, Alpi Pennine, Mte. Nery (WU: PS & AT 4786)	KC455636	KC455751	KC455866	
	acc. 2	I, Alpi Pennine, Val di Gressoney (WU: GMS, PS & AT 6539)	KC455635	KC455750	KC455865	x
	acc. 3	CH, Alpi Lepontine, Cima dell'Uomo (WU: PS 11033)	KC455634	KC455749	KC455864	
	acc. 4	CH, Alpi Lepontine, Mte. Camoghè (WU: M. Staudinger & S. Knechtel 11037)	KC455630	KC455745	KC455860	
	acc. 5	CH, Alpi Lepontine (STU: MT 4016A)	KC455644	KC455759	KC455874	
	acc. 6	CH, Alpi Lepontine (STU: MT 4016C)	KC455645	KC455760	KC455875	
subsp. <i>columnae</i> (Gaudin) Becherer	acc. 1	I, gruppo del Bernina (WU: PS 4944)	KC455628	KC455743	KC455858	x
	acc. 2	I, Alpi Bergamasche, Val d'Arigna (WU: PS 11038)	KC455603	KC455716	KC455831	

(continued on next page)

Table 1 (continued)

Taxon	acc.	Locality (voucher-information)	ITS	trnL-F	trnK-matK	AFLPs
	acc. 3	I, Alpi Bergamasche, Mte. Legnone (WU: M. Staudinger & S. Knechtel 11041)	KC455632	KC455747	KC455862	
	acc. 4	I, Alpi Bergamasche, Aprica (STU: MT 4031)	KC455643	KC455758	KC455873	
	acc. 5	I, Alpi Giudicarie, Val Vestino (WU: GMS, PS & AT 3949)	KC455631	KC455746	KC455861	x
	acc. 6	I, southern Alps, Mte. Grappa (WU: GMS, PS & AT 3914)	KC455633	KC455748	KC455863	x
	acc. 7	SI, Trnovski gozd (WU: PS & B. Frajman 11064)	KC455629	KC455744	KC455859	
<i>P. serratum</i> Viv.	acc. 1	F, Corsica, Gorges de la Restonica (STU: MT 4136)	KC455646	KC455761	KC455876	
	acc. 2	F, Corsica, Mte. d'Oro (WU: C. Dobeš 4475)	KC455623	KC455738	KC455853	x
<i>P. villarsii</i> Rich. Schulz	acc. 1	F, Prealps de Provence (WU: GMS & PS 8911)	KC455583	KC455696	KC455811	
	acc. 2	F, Clue de St. Auban (GAP: L. Vinciguerra 13.07.2000)	KC455580	KC455693	KC455808	
	acc. 3	F, Gorges du Verdon (GAP: s. coll.)	KC455582	KC455695	KC455810	
	acc. 4	F, Gorges du Verdon (M: D. Podlech 50454)	KC455601	KC455714	KC455829	
<b>ser. Alpina</b>						
<i>P. hedraianthifolium</i> Rich. Schulz	acc. 1	CH, Albula-Alpen, Piz Julier (no voucher)	KC455626	KC455741	KC455856	x
	acc. 2	I, gruppo del Bernina (WU: PS 4941)	KC455627	KC455742	KC455857	x
	acc. 3	I, Alpi Giudicarie, Passo di Croce Domini (STU: D. Butter s. n.)	KC455640	KC455755	KC455870	
<i>P. hemisphaericum</i> L.	acc. 1	F, Pyrenees, Col de Pourtalet (WU: GMS, PS & AT 6440)	KC455606	KC455720	KC455835	x
	acc. 2	F, Alpes Grées (WU: PS & AT 4755)	KC455607	KC455721	KC455836	
	acc. 3	CH, Alpes Valaisannes (STU: MT 3910B)	KC455614	KC455728	KC455843	
	acc. 4	CH, Furkapass (STU: MT 3882B)	KC455611	KC455725	KC455840	
	acc. 5	CH, Tessiner Alpen, Nufenenpass (STU: MT 3912A)	KC455610	KC455724	KC455839	
	acc. 6	CH, Graubünden, Albulapass (STU: MT 3851)	KC455613	KC455727	KC455842	
	acc. 7	CH, Graubünden, Berninapass (STU: MT 3817)	KC455612	KC455726	KC455841	
	acc. 8	CH, Graubünden, Berninapass (STU: MT 3875A)	KC455609	KC455723	KC455838	
	acc. 9	I, southern Alps, Alpi Carniche (WU: PS & AT 4569)	KC455608	KC455722	KC455837	
	acc. 10	A, Gurktaler Alpen (WU: PS & AT 3750)				x
	acc. 11	I, Aosta, Alpi Graie (WU: PS & AT 4769)				x
<i>P. humile</i> Gaudin	acc. 1	CH, Alpes Pennines (WU: GMS 4861)	KC455615	KC455729	KC455844	x
	acc. 2	I, Alpi Pennine, Val di Gressoney (WU: GMS, PS & AT 6533)	KC455616	KC455730	KC455845	x
<b>ser. Lingulata</b>						
<i>P. confusum</i> A. Kern.	acc. 1	A, central Alps, Koralpe (WU: PS & AT 3785)	KC455624	KC455739	KC455854	x
	acc. 2	A, central Alps, Koralpe (STU: A. Wörz 24.08.19.01)	KC455641	KC455756	KC455871	
	acc. 3	RO, southern Carpathians, Munții Tarcu (WU: Z. Szeląg INTRABIODIV Q55)	KC455625	KC455740	KC455855	
<i>P. globulariifolium</i> Sternb. & Hoppe subsp. <i>globulariifolium</i>	acc. 1	A, central Alps, Hohe Tauern (WU: PS & AT 4883)	KC455617	KC455732	KC455847	
	acc. 2	A, central Alps, Wölzer Tauern (WU: PS & AT 4551)	DQ304583	KC455731	KC455846	
	acc. 3	A, Niedere Tauern (WU: PS & AT 5241)				x
subsp. <i>pedemontanum</i> (Rich. Schulz) Becherer	acc. 1	F, Pyrenees, Puigmal d'Err (WU: GMS, PS & AT 6508)	KC455618	KC455733	KC455848	
	acc. 2	F, Alpes Maritimes (STU: MT s.n.)	KC455621	KC455736	KC455851	
	acc. 3	CH, Alpes Valaisannes (STU: MT 3908)	KC455620	KC455735	KC455850	
	acc. 4	I, Alpi Pennine, Mte. Nery (WU: PS & AT 4789)	KC455619	KC455734	KC455849	x
	acc. 5	CH, Graubünden, Berninapass (STU: MT 3879)	KC455622	KC455737	KC455852	
	acc. 6	I, Alpi Liguri (WU: PS & AT 4672)				x

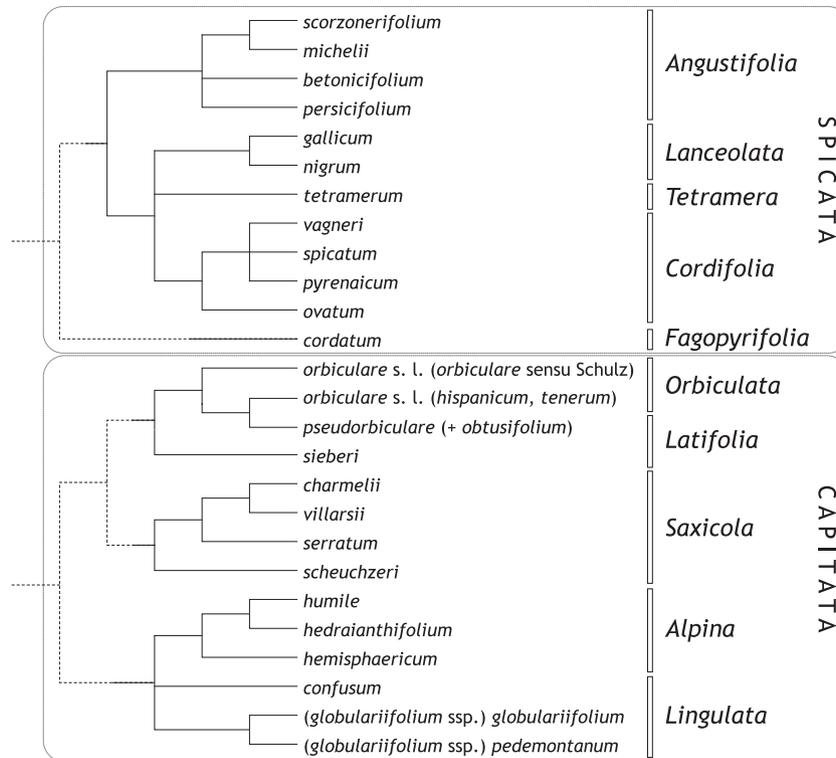


Fig. 1. Genealogy ("Stammbaum") of *Phyteuma* suggested by Schulz (1904: p. 55).

#### 2.4. Phylogenetic analyses

DNA-sequences were edited with SeqMan™ II v. 5.05 (DNASTar Inc., Madison, WI, USA) and aligned manually using BioEdit 7.0.4.1 (Hall, 1999). Phylogenetic analyses were conducted on three data sets: (1) nuclear ribosomal ITS, (2) combined plastid regions, and (3) combined nuclear and plastid regions. The plastid data set was *a priori* partitioned into coding (*matK*) and non-coding regions (*trnK* intron, *trnL-F* region), and the combined data set consequently consisted of three partitions (ITS, coding plastid region, non-coding plastid regions). For the latter data set, a single accession of *P. scorzonerifolium* (accession 2: Table 1) was excluded due to contradicting positions in nuclear versus plastid data set likely due to chloroplast capture. It should be noted, however, that biparental inheritance of plastids is known in other genera of Campanulaceae such as *Campanula* s. str. (Corriveau and Coleman, 1988), but no data is available from *Phyteuma*. As further cases of phylogenetic incongruences affected entire species (see Section 3), we did not formally test for combinability of data sets. Instead we used, in addition to analyses of the combined data set, visualization of the incongruences in a consensus network (Holland and Moulton, 2003), calculated with SplitsTree 4.8 (Huson and Bryant, 2006), using 1000 Bayesian posterior trees (see below) per each nuclear and plastid data set. The alignment of the combined data is available from TreeBASE (study ID 12627 [http://purl.org/phylo/treebase/phylo/treebase/study/TB2:S12627](http://purl.org/phylo/treebase/phylo/treebase/phylo/treebase/study/TB2:S12627)).

For the nuclear, plastid, and combined data sets maximum parsimony analyses were conducted using Nona 2.0 (Goloboff, 1999) within WinClada 1.00.08 (Nixon, 2002). We applied the parsimony ratchet (Nixon, 1999), which allows rapid phylogenetic reconstruction of relationships among large numbers of taxa. Only informative characters were included and gaps were treated as missing data. We used the Multi-Ratchet option with 100 runs. In each run 500 iterations were performed keeping one tree for TBR branch

swapping. The number of characters to which additional weight was given for each data matrix was set to 20% of all informative characters as recommended by Nixon (1999). A strict consensus was calculated. Support was estimated by bootstrap analysis as implemented in WinClada, re-sampling 10,000 times with TBR set to 10 replications holding one tree, using more extensive TBR holding 1,000 trees, and saving the consensus for each re-sampling matrix.

Partitioned maximum likelihood analysis was conducted with RAXML 7.2.2 (Stamatakis et al., 2008). Maximum likelihood tree and model parameters were estimated via searches starting from 20 randomized maximum parsimony starting trees employing the rapid hill-climbing algorithm (option *-f d*) described by Stamatakis et al. (2007) and using the GTRGAMMA model. Where applicable, data partitions were considered, calculating separate model parameters (rate parameters and the shape parameter of the gamma distribution, the latter estimated to an accuracy of 0.1 log-likelihood units), but a common set of branch lengths. The tree from the 20 iterations with the highest likelihood score was considered the maximum likelihood tree. Nodal support was assessed via bootstrap values estimated using a GTRCAT model and 500 replicates.

Bayesian analysis was conducted using MrBayes 3.1 (Ronquist and Huelsenbeck, 2003) run via the Bioportal computer cluster of the University of Oslo ([www.bioportal.uio.no](http://www.bioportal.uio.no)). The best-fit substitution model for each partition was determined using the AIC as implemented in Modeltest 3.6 (Posada and Crandall, 1998). Although different degrees of model uncertainty were found for all data sets, for all partitions the set of models until the cumulative Akaike weights was  $\geq 0.95$  included models with more than two substitution rates and with rate heterogeneity among sites. Therefore, we used for all partitions models with six substitution rates (*nst* = 6) and modeled rate heterogeneity among sites with a gamma distribution (rates = gamma), subsuming a proportion

of invariable sites in the gamma distribution increasing the number of rate categories to 5 (ncat = 5). The overall rate was allowed to differ among partitions (ratepr = variable). As for the ITS data set the two highest-ranked substitution models (symmetrical model), which reached a cumulative Akaike weight of 0.947, entailed equal base frequencies, this was accounted for by setting the state frequency prior for this partition (where present) accordingly (prset statefreqpr = fixed(equal)). In partitioned analyses, the partitions had separate substitution model parameters, but shared topologies and branch lengths. After initial analyses, the heating parameter was decreased to 0.05 to improve mixing among cold and heated chains. Each analysis consisted of 12 parallel runs with four chains each (one cold, three heated), running for  $15 \times 10^6$  generations sampling every 1000th tree. Analyses using default branch length priors (exponential with expectation 0.1: brlenspr = unconstrained:exponential(10)) resulted in runs with different values for the rate heterogeneity parameters in partitioned analyses. For instance, in the plastid data set, the first 4 runs had an about fivefold lower alpha parameter for partition 1 than for partition 2, whereas in the remaining eight runs it was the other way around, causing the two sets of runs to have non-overlapping likelihoods differing by roughly 60 log-units (data not shown). Decreasing the expectations of the exponential distribution alleviated these problems and models with changed branch length priors were also supported by Bayes Factors (up to 70 log-units difference: data not shown). The final analyses were done with an exponential distribution with expectation 0.01 (brlenspr = unconstrained:exponential(100)) as branch length prior. After removal of the initial 10% as burnin, a majority rule consensus tree was constructed from 13,500 trees per run, totaling 162,000 trees.

Alternative phylogenetic hypotheses with regard to monophyly of sections and series of Schulz (1904) were tested in a Bayesian framework using MrBayes with the combined data set and the same settings as for the unconstrained analyses. Bayes factors were calculated using Tracer 1.3 (available from <http://tree.bio.ed.ac.uk/software/tracer/>). As harmonic mean estimators for calculating marginal likelihoods show poor performance (Baele et al., 2012), the thus obtained Bayes Factors are interpreted with appropriate caution. If different runs did not converge on the same likelihoods, deviating runs (those in the minority) were removed. This problem was particularly pronounced for series *Saxicola*, where often more than the usual 10% burnin had to be removed and harmonic means of likelihoods were calculated from  $88.3 \times 10^6$  instead of the usual  $162 \times 10^6$  generations.

AFLP data were analyzed using the NeighborNet algorithm implemented in SplitsTree 4 (Huson and Bryant, 2006) using the Dice coefficient (Dice, 1945); clade support on a Neighbor-Joining tree based on Dice coefficients was estimated employing 2000 bootstrap replicates.

## 2.5. Chromosome number evolution and biogeographic analysis

Chromosome number data were taken from literature and supplemented with newly obtained chromosome counts (part of ongoing

karyological investigations in *Phyteuma* to be published elsewhere: H. Weiss-Schneeweiss et al., unpubl. data). Chromosome number evolution in *Phyteuma* and *Physoplexis* was explored via ordered parsimony reconstructions on a set of 32,400 posterior trees (i.e., a fivefold thinned set of posterior trees from the Bayesian analysis of the combined sequence data) using Mesquite 2.74 (available from: <http://mesquiteproject.org/mesquite/mesquite.html>). The phylogenetically and taxonomically insufficiently supported (see Section 4) species *P. villarsii* and *P. ovatum* were merged with their closest relatives *P. charmelii* and *P. spicatum*, respectively. All species and the two subspecies of *P. globulariifolium* were reduced to single terminals. We analyzed two data sets: in the first all originally reported chromosome numbers were included, whereas in an edited data set counts deemed doubtful (concerning *P. hedraianthifolium*, *P. humile*, and *P. vagneri*: see Section 4 for details) were removed.

Ancestral area reconstruction was done using a statistical Dispersal-Vicariance Analysis (DIVA). Statistical DIVA extends the original DIVA implementation (Ronquist, 1997) by taking phylogenetic uncertainty and uncertainty in DIVA optimizations into account, where the occurrence of an ancestral area  $x$  at a given node of a given tree is calculated as the actual frequency of  $x$  over all optimizations (Harris and Xiang, 2009). Statistical DIVA was conducted on the same set of trees as used above using RASP 2.0 (Reconstruct Ancestral State in Phylogenies, available from: <http://mnh.scu.edu.cn/soft/blog/RASP/index.html>). The following biogeographic regions were distinguished: North Africa; Iberian Peninsula (outside the Pyrenees); Pyrenees; Massif Central; Corsica; Alps; Apennines; Balkan Peninsula; Carpathians; temperate Europe outside the major mountain ranges. Species distribution data were compiled from Schulz (1904) and from relevant floras or distribution atlases (e.g., Damboldt, 1976; Hayek, 1931; Meusel and Jäger, 1992; Pignatti, 1982; Podlech, 2008). Reconstructions were done allowing maximally two areas per node.

## 3. Results

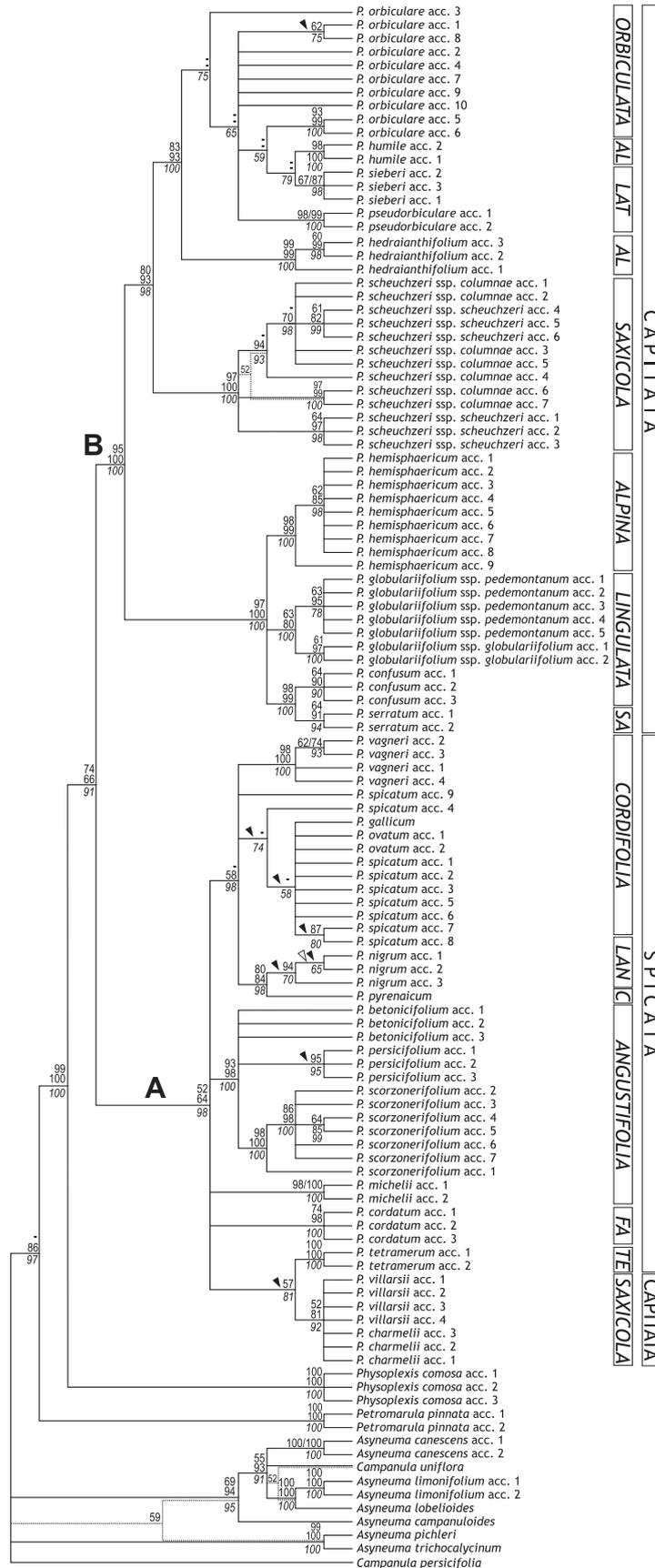
### 3.1. Phylogenetic relationships inferred from sequence data

Sequence and tree statistics are summarized in Table 2. Different methods of phylogenetic inference resulted in largely identical phylogenetic trees, differences being restricted to usually insufficiently supported nodes (the few exceptions with bootstrap values [BS] above 50 are indicated in Figs. 2–4). Hereinafter, support values are reported as: BS maximum parsimony/BS maximum likelihood/Bayesian posterior probability [PP] for nuclear; plastid; combined data.

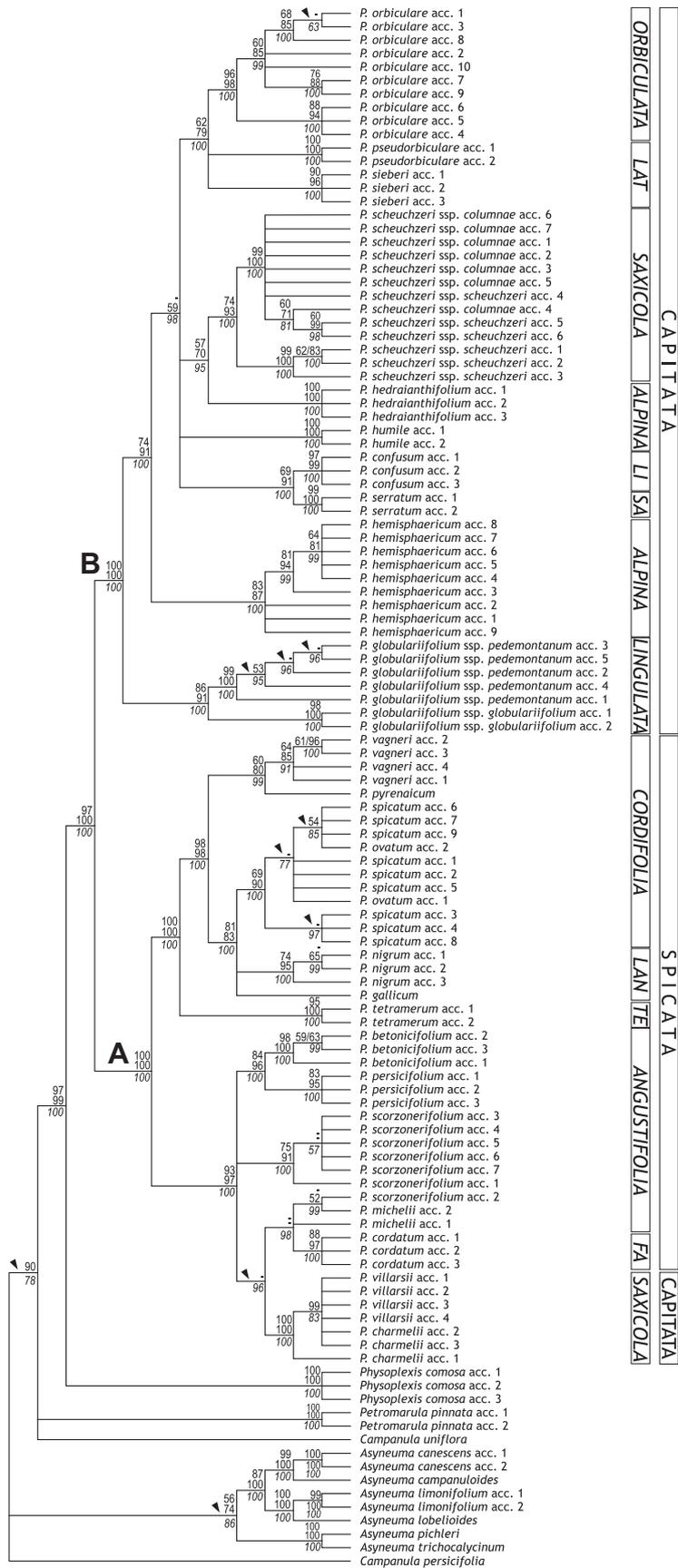
*Phyteuma* constituted a monophyletic group (74/66/91; 97/100/100; 100/99/100) that was sister to *Physoplexis* (99/100/100; 97/99/100; 100/100/100) and consecutively to *Petromarula* (supported only in model-based analyses of the nuclear and the combined data: <50/86/97; n.a./98/100). Of the outgroup species, only *Asyneuma pichleri* and *A. trichocalycinum* (99/100/100; 100/100/100;

**Table 2**  
Sequence descriptives and tree statistics.

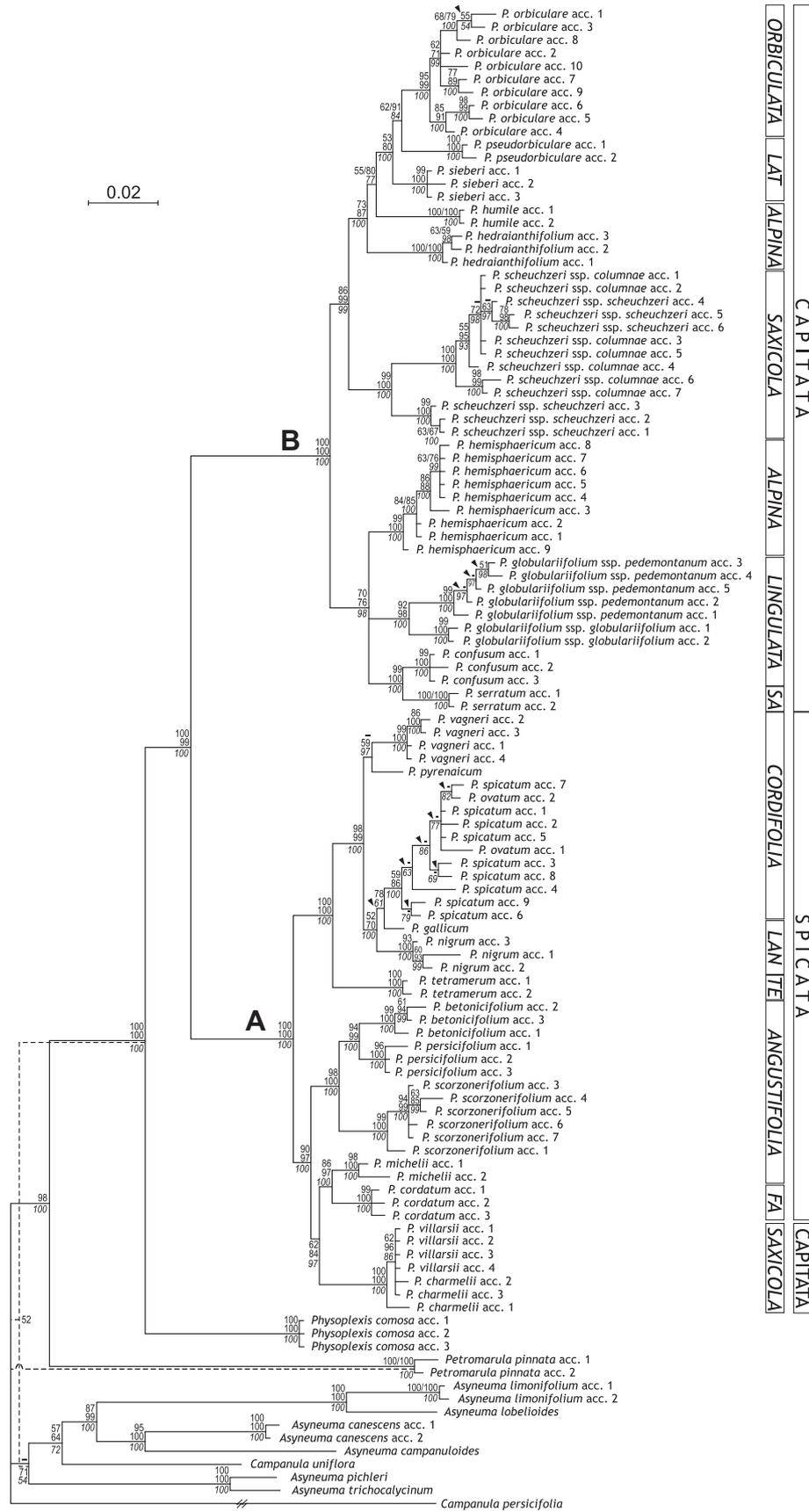
	ITS	Plastid DNA		Combined
		Non-coding	Coding	
Number of characters	730	1987	1557	4274
Number of variable/parsimony-informative characters	218/183	285/201	211/135	714/518
Tree length	402	397	254	1078
C.I./R.I.	0.68/0.92	0.73/0.95	0.89/0.98	0.75/0.94
Maximum log-likelihood score	−3455.70		−9541.91	−13247.31
Harmonic mean of log-likelihood score	−3673.47		−10036.80	−13733.56



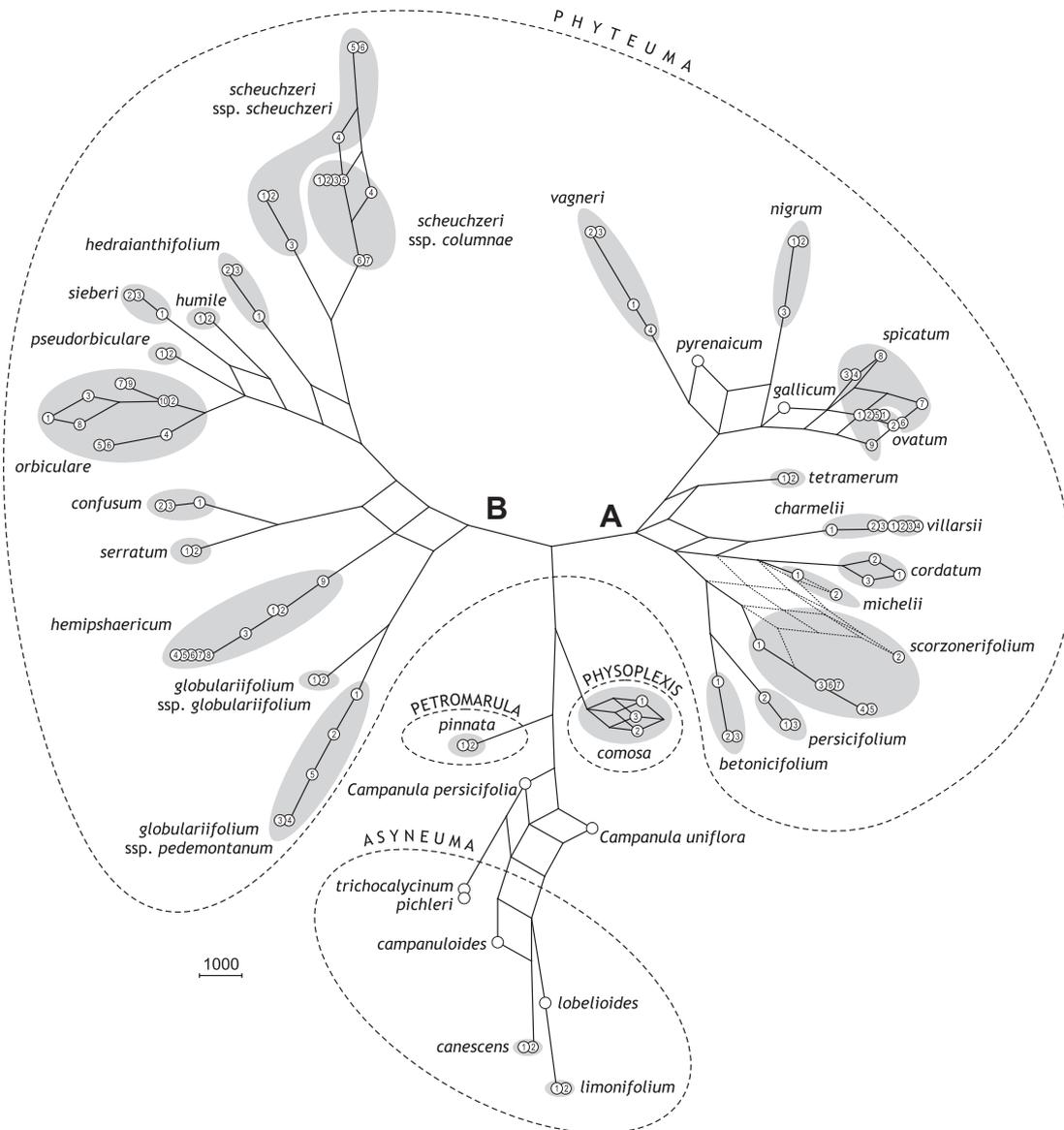
**Fig. 2.** Phylogenetic relationships of *Phyteuma* and relatives inferred from Bayesian analysis of nuclear ribosomal ITS data. Alternative topologies inferred from maximum likelihood analysis are indicated by dotted lines. Nodes collapsing in the strict consensus tree from maximum parsimony analysis and in the maximum likelihood tree are indicated by black and white arrowheads, respectively. Values at nodes are bootstrap support values ( $\geq 50$ , otherwise ‘-’) from maximum parsimony and maximum likelihood analyses and, in italics, Bayesian posterior probabilities. Series and section circumscriptions following Schulz (1904) are indicated on the right (AL, series *Alpina*; C, series *Cordifolia*; FA, series *Fagopyrifolia*; LAN, series *Lanceolata*; LAT, series *Latifolia*; SA, series *Saxicola*; TE, series *Tetramera*).



**Fig. 3.** Phylogenetic relationships of *Pyteuma* and relatives inferred from Bayesian analysis of plastid data. Nodes collapsing in the strict consensus tree from maximum parsimony analysis are indicated by black arrowheads. Values at nodes are bootstrap support values ( $\geq 50$ , otherwise ‘-’) from maximum parsimony and maximum likelihood analyses and, in italics, Bayesian posterior probabilities. Series and section circumscriptions following Schulz (1904) are indicated on the right (FA, series *Fagopyrifolia*; LAN, series *Lanceolata*; LAT, series *Latifolia*; LI, series *Lingulata*; SA, series *Saxicola*; TE, series *Tetramera*).



**Fig. 4.** Phylogenetic relationships of *Phyteuma* and relatives and branch lengths (in substitutions per site) inferred from Bayesian analysis of combined nuclear and plastid data. Alternative topologies inferred from maximum parsimony analysis are indicated by dashed lines. Nodes collapsing in the strict consensus tree from maximum parsimony analysis are indicated by black arrowheads. Values at nodes are bootstrap support values ( $\geq 50$ , otherwise ‘-’) from maximum parsimony and maximum likelihood analyses and, in italics, Bayesian posterior probabilities. Series and section circumscriptions following Schulz (1904) are indicated on the right (FA, series *Fagopyrifolia*; LAN, series *Lanceolata*; LAT, series *Latifolia*; SA, series *Saxicola*; TE, series *Tetramera*).



**Fig. 5.** Consensus network from 1000 trees each of the set of posterior trees from the nuclear ribosomal ITS and the plastid data set, respectively. Splits introduced by *P. scorzonifolium* acc. 2, which does not group with other accessions from this species in the plastid data, are indicated by dotted lines. Circumscription of genera (except *Campanula*) indicated by dashed lines, circumscription of species and subspecies indicated by shaded areas, accessions numbered as in Table 1.

100/100/100) and *A. limoniifolium* and *A. lobelioides* (all 100/100/100) were consistently retrieved as sisters (Figs. 2–5), whereas the relationships between those two species pairs and the other lineages were burdened with incongruences (Fig. 5). This also affected the position of *Campanula uniflora*, which in the nuclear data was nested within *Asyneuma* (excluding *A. pichleri* and *A. trichocalycinum*; 69/94/95), but in the plastid data was together with *Petromarula* sister to the clade of *Physoplexis* and *Phyteuma* (n.a./90/78), rendering *Asyneuma* monophyletic (56/74/86).

Most *Phyteuma* species constituted distinct monophyletic lineages (Figs. 2–5). Exceptions were *P. charmelii* and *P. villarsii* (together forming a clade: 52/81/92; 100/100/100; 100/100/100) and *P. spicatum* and *P. ovatum* (n.a./<50/74: excluding one accession of *P. spicatum* and including *P. gallicum*; 69/90/100; 59/86/100). Although in the nuclear data set *P. orbiculare* was not monophyletic either, its paraphyly with respect to *P. humile*, *P. pseudorbiculare* and *P. sieberi* found little support (<50/<50/75) and was, therefore, not considered any further. In the plastid data, a single accession of *P. scorzonifolium* grouped with *P. michelii* (<50/52/99); this accession was excluded from the combined analysis.

*Phyteuma* species were arranged into two major clades (Figs. 2–5). The first clade (52/64/98; 100/100/100; 100/100/100), hereinafter referred to as clade A, corresponded to sect. *Spicata*, but additionally included *P. charmelii* and *P. villarsii* of sect. *Capitata*. Relationships within the congruently identified clade of *P. gallicum*, *P. nigrum*, *P. pyrenaicum*, *P. spicatum* plus *P. ovatum*, and *P. vagneri* (<50/58/98; 98/98/100; 98/99/100) were unclear (Figs. 2–5). This was due to incongruent positions of *P. gallicum* and of *P. pyrenaicum*. Disregarding one accession of *P. scorzonifolium*, *P. betonicifolium* and *P. persicifolium* (plastid and combined data only: 84/96/100; 94/99/100) and their sister species *P. scorzonifolium* formed a clade (nuclear and combined data only: 93/98/100; 98/100/100). Plastid and combined, but not nuclear data suggested a close relationship of this clade to a clade of *P. cordatum* and *P. michelii* (plastid and combined data only: <50/<50/98; 86/97/100) and to *P. charmelii* plus *P. villarsii* (93/97/100; 90/97/100) as well as a sister group relationship of *P. tetramerum* to the clade including, among others, *P. nigrum* and *P. spicatum* (both 100/100/100).

The second major clade (95/100/100; 100/100/100; 100/100/100), hereinafter referred to as clade B, corresponded to the

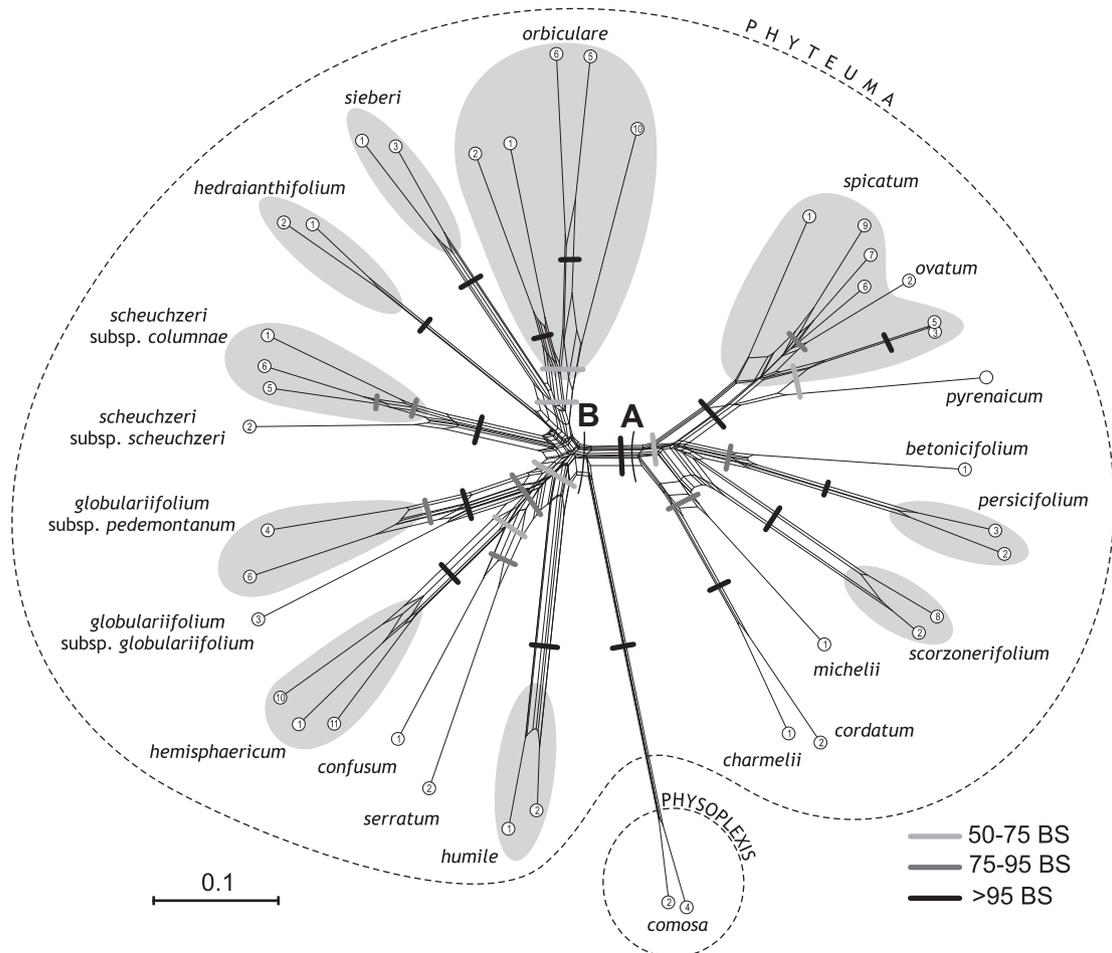
remainder of sect. *Capitata*, i.e., excluding *P. charmelii* and *P. villarsii* (Figs. 2–5). Relationships within the clade of *P. hedraianthifolium*, *P. humile*, *P. orbiculare*, *P. pseudorbiculare*, *P. scheuchzeri* and *P. sieberi* (80/93/98; unresolved; 86/99/99) were uncertain due to incongruent positions of *P. hedraianthifolium* and *P. humile* (Figs. 2–5). In the plastid data set the congruently inferred sister species *P. confusum* and *P. serratum* (98/99/100; 69/91/100; 99/100/100) were loosely tied to the above species (<50/59/98) with *P. hemisphaericum* and *P. globulariifolium* as consecutive sister groups (74/91/100 and 100/100/100, respectively; Fig. 3). In contrast, in the nuclear and combined data sets *P. confusum* and *P. serratum*, *P. globulariifolium*, and *P. hemisphaericum* constituted a clade (97/100/100; 70/76/98) sister to the clade including *P. orbiculare* and others (Figs. 2 and 4). Whereas the subspecies of *P. globulariifolium* were supported by molecular data (subsp. *globulariifolium* 61/97/100; 98/100/100; 99/100/100; subsp. *pedemontanum* 63/95/78; 99/100/100; 99/100/100), those of *P. scheuchzeri* (subsp. *scheuchzeri* and *columnae*) were not. Instead, only the three westernmost accessions of subsp. *scheuchzeri* constituted a clade (64/97/98; 99/100/100; 99/100/100), while the others intermixed with those of subsp. *columnae* (<50/94/93 [excluding two accessions of subsp. *columnae*]; 99/100/100; 100/100/100).

Alternative phylogenetic hypotheses implied by the classification of Schulz (1904; Fig. 1) were tested via Bayes factors (their natural logarithm: lnBF). These indicated that with the exception of series *Lanceolata* (lnBF 0.017) and *Latifolia* (lnBF –2.173) monophyly of series *Alpina*, *Angustifolia*, *Cordifolia*, *Lingulata*, and *Saxicola* (lnBFs

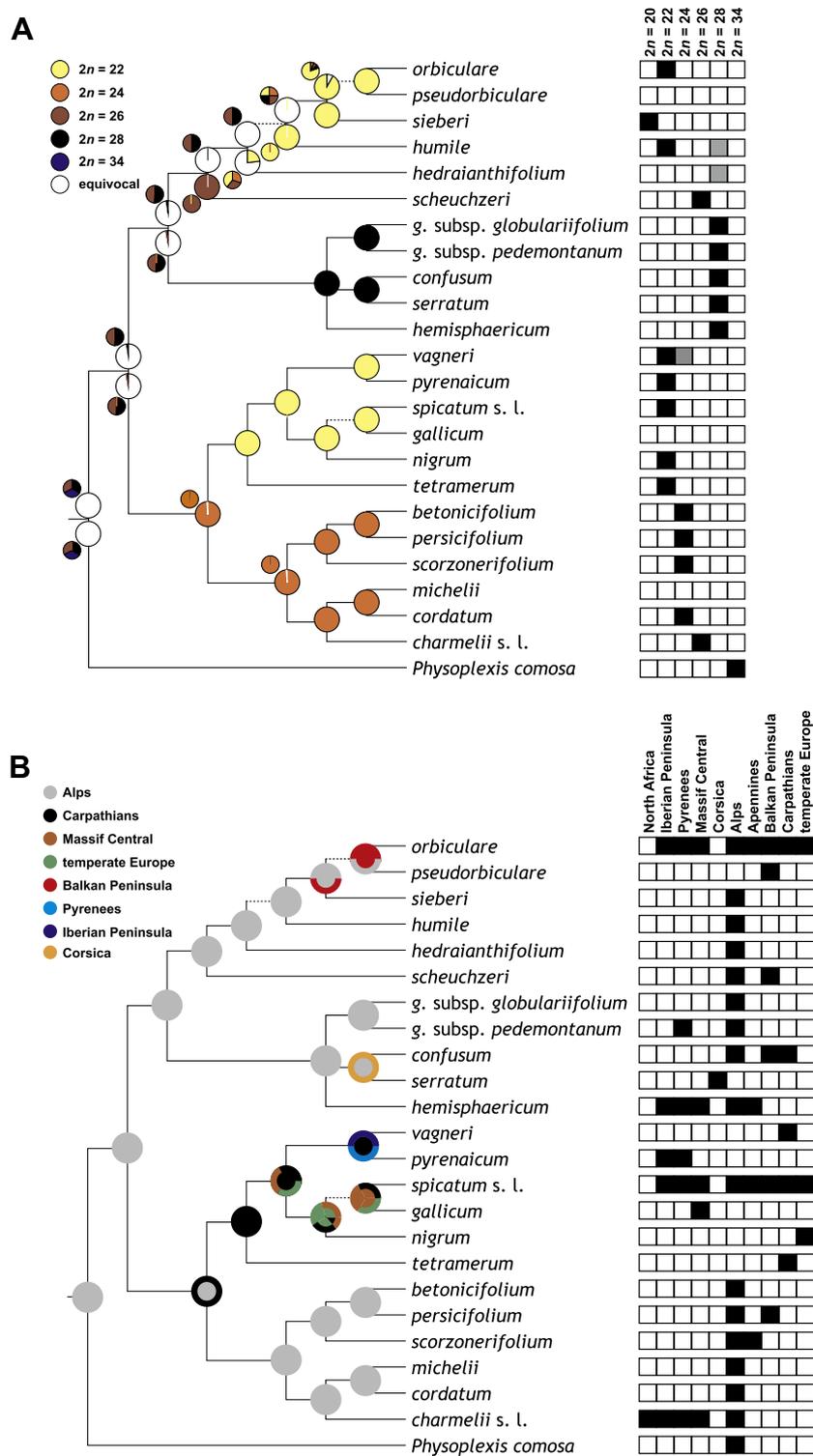
–53.657, –13.005, –15.402, –27.417, –171.185) and of sections *Phyteuma* and *Capitata* (lnBFs –15.659, –89.786) was rejected.

### 3.2. Phylogenetic relationships inferred from AFLPs

Rooting the tree with *Physoplexis*, *Phyteuma* fell into the same two clades as identified with sequence data, but only clade A received significant support (Fig. 6). Even though many of the basal nodes were remarkably poorly supported, inferred species relationships were remarkably similar to those inferred from the combined data set. Specifically, within clade A, *P. michelii* grouped with *P. charmelii* and *P. cordatum* (BS 89); the sister species *P. betonicifolium* and *P. persicifolium* (BS 94) grouped with *P. scorzonrifolium* (BS <50); *P. ovatum* and *P. pyrenaicum* were nested within a clade containing multiple accessions of *P. spicatum* (BS 98). Differences concerned the relationships among those three clades, because the clade including *P. betonicifolium* was inferred as sister to *P. spicatum* and relatives (BS 55; Fig. 6) instead of the clade including *P. charmelii* (Fig. 4). Within clade B, the sister species *P. confusum* and *P. serratum* (BS 86) grouped with *P. hemisphaericum* (BS 72) and, as consecutive sister-group, *P. globulariifolium* (BS 81); the sister-species *P. orbiculare* and *P. sieberi* (BS 52) grouped with *P. hedraianthifolium* (BS <50) and, as consecutive sister-group, *P. scheuchzeri* (BS <50). The only difference concerned the position of *P. humile* as sister to the clade including *P. globulariifolium* (BS 52; Fig. 6) instead of the one including *P. sieberi* (Fig. 4).



**Fig. 6.** Phylogenetic network of *Phyteuma* and *Physoplexis* based on NeighborNet analyses of AFLP data from 41 accessions. Bootstrap values, obtained from a NeighborJoining analysis and given in three classes, are plotted onto the network.



**Fig. 7.** Chromosome number evolution (a) and biogeographic history (b) of *Phyteuma* and *Physoplexis*. In (a), chromosome numbers reported for a given taxon are indicated in the right panel by black and grey boxes, the latter indicating counts deemed doubtful (see text for details); reconstructed chromosome number are shown at nodes either showing only uniquely best states (large circles) or all equally optimal states (small circles); unless identical, reconstructions based on the complete data set and on the data set excluding potentially erroneous counts are displayed in the upper and lower circle, respectively. In (b), occurrences in a specific area are indicated in the right panel by black boxes; reconstructed distribution areas (which can include maximally two regions, indicated by different colors for the outline and for the filling) are shown at nodes, alternative areas are indicated as segments proportional to their probabilities. – Branches with posterior probability <0.95 are indicated by dashed lines.

### 3.3. Chromosome number evolution and biogeographic history

New counts were obtained for *P. charmelii* ( $2n = 26$ ), *P. humile* ( $2n = 22$ ), *P. pyrenaicum* ( $2n = 22$ ), *P. scorzonerifolium* ( $2n = 24$ ), *P. tetramerum* ( $2n = 22$ ), and *P. vagneri* ( $2n = 22$ ). Chromosome num-

ber reconstruction at several nodes, especially the basal ones and those along the backbone of clade B, was ambiguous with usually two possible numbers (Fig. 7a). Using the data set without putatively erroneous numbers affected reconstructions at two nodes in clade B. Specifically, the ancestor of the clade including, among

others, *P. scheuchzeri* and *P. orbiculare* was nearly unambiguously reconstructed as  $2n = 26$  (instead of  $2n = 26$  or 28), and the ancestor of the nested clade including, among others, *P. hedraianthifolium* and *P. orbiculare* was reconstructed as  $2n = 24$ , 26 or 28 (instead of  $2n = 26$  or 28). Generally, numbers at many nodes were usually lower if reconstructed using the data set without putatively erroneous numbers than those from the complete data set (e.g.,  $2n = 26$  versus  $2n = 26$  or 28; Fig. 7a). Irrespective of the data set used, chromosome number changes agreed with a pattern of descending dysploidy, even if due to ambiguous reconstruction at some nodes local ascending dysploidy cannot be entirely ruled out.

The Alps were identified as the ancestral area for *Phyteuma* (Fig. 7b). In clade B, inferred range extensions were restricted mostly to the terminal nodes, wider distributions being inferred only for the ancestors of *P. confusum* and *P. serratum* (Alps and Corsica) and of *P. orbiculare*, *P. pseudorbiculare* and possibly *P. sieberi* (Alps and Balkan Peninsula). In clade A, biogeographic patterns were more complex. For the subclade including, among others, *P. betonicifolium* and *P. charmelii*, again the Alps were identified as ancestral area, geographic extensions being confined to terminal nodes. In contrast, for the subclade including *P. spicatum* and relatives, the Carpathians were suggested as ancestral area. Reconstructions involving taxa outside the high mountain ranges (*P. nigrum*, *P. gallicum*, *P. spicatum*) were burdened with considerable uncertainty, but ancestral areas likely included lowland Central Europe and the French Massif Central.

## 4. Discussion

### 4.1. Intergeneric relationships

A close relationship of *Phyteuma* to *Physoplexis* is suggested by the shared flower morphology, which is unique within Campanulaceae. This hypothesis is supported by molecular data that actually fail to provide convincing evidence for their clear separation (Borsch et al., 2009; Eddie et al., 2003; Park et al., 2006; Roquet et al., 2008), rendering their generic distinction arguable. Employing a comprehensive sampling focused on *Phyteuma* and previously identified closely related lineages, *Physoplexis* and *Phyteuma* are confirmed as closely related, but clearly distinct sister lineages (Figs. 2–5). Although taxonomically this might be accounted for by merging *Physoplexis* (then *P. comosum* L.) and *Phyteuma*, thereby avoiding a monotypic genus, morphological and karyological differences between *Phyteuma* and *Physoplexis* support their generic segregation, as widely exercised (e.g., Aeschmann et al., 2004; Castroviejo et al., 2010; Damboldt, 1976; Pignatti, 1982; Podlech, 2008).

Identification of the closest relative of *Phyteuma* and *Physoplexis* is more problematic. Previous molecular data are ambiguous and suggest *Petromarula* (Cellinese et al., 2009; Haberle et al., 2009; Park et al., 2006; Roquet et al., 2008; Wendling et al., 2011), species of *Asyneuma* (Borsch et al., 2009; Eddie et al., 2003), *Campanula uniflora* (Wendling et al., 2011) or a clade of *Legousia* and *Campanulastrum* (Roquet et al., 2008; Stefanović and Lakušić, 2009), although often with insufficient support. The present data support *Petromarula* as closest relative, although not entirely unequivocally due to insufficient resolution in the plastid data set (Fig. 3) and a deviating phylogenetic position (*Petromarula* as sister to all species except *Campanula persicifolia*) in parsimony analysis of the combined data set (Fig. 4), the latter probably a result of long-branch attraction. Morphologically, a closer relationship of *Petromarula* to *Phyteuma* and *Physoplexis* has been suggested by some resemblance in flower morphology (Roquet et al., 2008). Further studies will be necessary to corroborate the phylogenetic position of *Petromarula*, also with respect to the morphologically similar *Asyneuma*, whose

internal relationships and monophyly, even after the exclusion of *A. comosiforme* (Frajman and Schneeweiss, 2009; Stefanović and Lakušić, 2009), remain uncertain (Fig. 5).

### 4.2. Infrageneric relationships

*Phyteuma* consists of two clades (clade A and B; Figs. 2–6), which largely correspond to sect. *Spicata* and sect. *Capitata*, respectively, defined by Schulz (1904) on the basis of inflorescence characters (spikes versus heads). The only exceptions are *P. charmelii* plus *P. villarsii*, which have been classified within sect. *Capitata* based on their head-like inflorescences, but phylogenetically clearly tie with species of sect. *Spicata*. A trend towards shortened inflorescences is also evident in the closely related *P. cordatum*, which has almost globose inflorescences, yet without a distinct involucre (Damboldt, 1976; Noble and Diadema, 2011). Well defined morphological characters supporting the phylogenetic position of *P. charmelii* and *P. villarsii* still have to be identified. In any case, the sectional circumscriptions suggested by Schulz (1904) can be maintained with the modification of transferring *P. charmelii* and *P. villarsii* from sect. *Capitata* to sect. *Spicata*.

In contrast to the sections, where traditional morphology-based delimitation and molecular-phylogenetic circumscription are in good congruence, the majority of the taxonomic series distinguished by Schulz (1904) are not supported by molecular data. Beyond the trivial cases of monospecific series (ser. *Fagopyrifolia* with *P. cordatum* and ser. *Tetramera* with *P. tetramerum*) and of ser. *Orbiculata* supported by all analyses (Figs. 2–5), a hypothesis of monophyly cannot be rejected for ser. *Lanceolata* (*P. gallicum*, *P. nigrum*) and for ser. *Latifolia* (*P. pseudorbiculare*, *P. sieberi*). However, in both cases none of the analyses provided positive evidence for their monophyly, rendering their phylogenetic circumscription ambiguous. All other series are polyphyletic (Figs. 2–4). For example, members of series *Saxicola* belong to sect. *Spicata* (*P. charmelii* plus *P. villarsii*) and to sect. *Capitata* (*P. scheuchzeri*, *P. serratum*; (Figs. 2–6). In his classification, Schulz (1904) heavily relied on characters of the leaves and the involucre bracts (e.g., shape or length relative to the inflorescence), but these characters are evidently homoplastic and, therefore, only of limited phylogenetic information above the species level.

Even if most series of Schulz (1904) may be abandoned, several clades, potentially recognizable as taxonomic series, can be distinguished. Within sect. *Spicata*, the group of *P. gallicum*, *P. nigrum*, *P. ovatum*, *P. pyrenaicum*, *P. spicatum*, and *P. vagneri* (i.e., ser. *Lanceolata* plus ser. *Cordifolia*) receives high support. It is characterized by mostly cordate to ovate basal leaves that are 1–2 times as long as wide, conspicuous bracts, a corolla curved in bud, and a chromosome number of  $2n = 22 + 0 - 4B$ . Deviating counts of  $2n = 24$  have been reported for *P. vagneri* (Pashuk, 1983, 1987), yet without drawings or photographs. As new counts show  $2n = 22$  (H. Weiss-Schneeweiss et al., unpubl. data), the earlier counts likely are due to misinterpretation of B chromosomes as part of the A-complement. This clade probably additionally includes *P. tetramerum*, the only member of *Phyteuma* with tetramerous (instead of pentamerous) flowers (Damboldt, 1976; Schulz, 1904). Schulz (1904: p. 44) suggested that this Carpathian endemic were an early derivative of *P. spicatum*. A closer relationship to *P. spicatum* and relatives is in line with the shared chromosome number of  $2n = 22$  (H. Weiss-Schneeweiss et al., unpubl. data) and finds some support in the plastid and the combined data (Figs. 3 and 4), but not the ITS data (Fig. 2).

A second well-supported clade within sect. *Spicata* includes *P. betonicifolium*, *P. persicifolium* (syn. *P. zahlbruckneri*) and *P. scorzonifolium* (Figs. 2–6). This clade is characterized by narrow basal leaves (more than twice as long as wide), long cylindrical inflorescences with inconspicuous bracts, and suberect, hardly curved flowers with blue corollas. A close relationship among these

species is widely accepted (Damboldt, 1976; Heß et al., 1980; Schulz, 1904) and reflected in Schulz' (1904) treatment as members of a single series, ser. *Angustifolia*. Against previous assertions (Damboldt, 1976; Schulz, 1904), *P. michelii* from the southwestern Alps, which was considered a derivative of *P. scorzonerifolium* by Schulz (1904: p. 47), does not belong here, but instead may be more closely related to *P. cordatum* and possibly *P. charmelii* (Figs. 2–6). Despite similar inflorescence shape, *P. michelii* and *P. cordatum* differ markedly in vegetative characters (reflected in Schulz' treatment of *P. cordatum* as sole member of ser. *Fagopyrifolia*), and a closer relationship of these two species has not been suggested before. Chromosome data, where available, are not informative, because both *P. cordatum* (Favarger, 1997; A. Polatschek, unpubl. data) as well as *P. betonicifolium*, *P. persicifolium* and *P. scorzonerifolium* have  $2n = 24$  (Contandriopoulos, 1962; Favarger, 1953; Kovanda, 1983; Polatschek, 1966; H. Weiss-Schneeweiss et al., unpubl. data; no chromosomal data are available for *P. michelii*).

Within sect. *Capitata*, *P. serratum*, an endemic of Corsica (Damboldt, 1976), is consistently inferred as sister-species to *P. confusum* (Figs. 2–6), distributed in the Eastern Alps, the Carpathians and mountain ranges of the Balkan Peninsula (Meusel and Jäger, 1992). Although both species have narrow, but usually non-linear basal leaves and lanceolate to ovate bracts that do not or only slightly exceed the inflorescence, they have never been considered closely related. Schulz (1904: p. 40) hypothesized that *P. serratum* shares a common ancestor with *P. charmelii*, but this scenario is clearly rejected by our data. *Phyteuma confusum* occupies a morphologically somewhat intermediate position between *P. globulariifolium* s. l. and *P. hemisphaericum* (Kerner, 1870) and Schulz (1904: pp. 50–51) hypothesized that these three taxa share a common ancestor. This hypothesis (with modification concerning *P. serratum*) is supported by all data except the plastid markers (Figs. 2–6).

A second clade within sect. *Capitata* includes *P. orbiculare* s. l. (ser. *Orbiculata*), *P. pseudorbiculare* and *P. sieberi* (ser. *Latifolia*), *P. humile* and *P. hedraianthifolium* (ser. *Alpina*) as well as *P. scheuchzeri* (ser. *Saxicola*; Figs. 2–5; the phylogenetic position of *P. humile* inferred from AFLP data is uncertain: Fig. 6). A close relationship of ser. *Orbiculata* and ser. *Latifolia*, which share similarly shaped basal leaves and involucre bracts, has already been proposed by Schulz (1904: pp. 48–49). He suggested that *P. pseudorbiculare* and *P. sieberi* were derived from *P. orbiculare* or its ancestor, respectively. Similarly, Pampanini (1903) suggested that *P. sieberi* may be a recent derivative of *P. orbiculare*. There is, however, no evidence for anagenetic differentiation of either *P. pseudorbiculare* or *P. sieberi* from *P. orbiculare*. Due to their distinctive morphologies, a close relationship of *P. humile*, *P. hedraianthifolium* and *P. scheuchzeri* to ser. *Orbiculata* and/or ser. *Latifolia* has never been proposed. Instead, *P. humile* and *P. hedraianthifolium* were considered derivatives of the widespread *P. hemisphaericum* (Schulz, 1904: p. 52) and *P. scheuchzeri* was grouped with *P. charmelii* (they share the chromosome number of  $2n = 26$ ; Contandriopoulos, 1962) and *P. serratum* (Fig. 1), but these hypotheses are clearly rejected by our data (Figs. 2–6). Phylogenetic affinity of *P. humile* to *P. orbiculare* is supported by a shared chromosome number of  $2n = 22$  (Contandriopoulos, 1962; Kovanda, 1971, 1981; Ochlewska, 1965; Polatschek, 1966; H. Weiss-Schneeweiss et al., unpubl. data; no data available for *P. pseudorbiculare*; *P. sieberi* has the derived and unique number of  $2n = 20$ ; Contandriopoulos, 1962; Polatschek, 1966). Contandriopoulos (1962) additionally reported  $2n = 28$  for *P. humile* and also for *P. hedraianthifolium*, but in the absence of voucher specimens these numbers need to be considered with caution. Both species often grow in close proximity to and can be easily confused with *P. hemisphaericum* (Käsermann, 1999), which has  $2n = 28$  chromosomes (Contandriopoulos, 1962; Favarger, 1953; Polatschek,

1966). Further data will be necessary to establish chromosome numbers in *P. hedraianthifolium* and *P. pseudorbiculare*. Furthermore, morphological synapomorphies for this clade remain yet to be identified.

#### 4.3. Species differentiation

Phylogenetic distinctness of the currently recognized species (Aeschmann et al., 2004; Castroviejo et al., 2010; Damboldt, 1976; Podlech, 2008) is corroborated except for *P. ovatum* and *P. villarsii* (Figs. 2–6). *Phyteuma ovatum* phylogenetically nests in *P. spicatum* and differs from it by dark violet (instead of white or blue) flowers, ovoid (instead of cylindrical) inflorescences, more strongly curved flower buds, and longer styles, but delimitation is considered problematic (Brunerye, 1989; Huber, 1988; Podlech, 2008; Polatschek in Fischer et al., 2008). As the distribution area of *P. ovatum* entirely falls into that of *P. spicatum* (Meusel and Jäger, 1992) and as both species produce hybrid swarms with intermediate flower colors (blue, pale purple, whitish-purple and all transitions: Huber, 1988; Polatschek, 1999; treated as *P. spicatum* subsp. *alpestre* by Brunerye, 1989), *P. ovatum* may be a geographically and elevationally restricted variant (montane to subalpine zone of the Alps, presumably lacking in the Pyrenees: Bolòs and Vigo, 1995; Brunerye, 1989) of the more widespread *P. spicatum* (from lowlands to the subalpine zone in temperate Europe). A derivation of *P. ovatum* from *P. spicatum* has already been suggested by Schulz (1904: p. 43).

*Phyteuma villarsii* is a narrow endemic from the foothills of the French and Italian southwestern Alps, whose morphological distinction from the widespread *P. charmelii* is often difficult (Bono, 1967; Noble and Diadema, 2011) and whose rank as species has been repeatedly questioned (Castroviejo et al., 2010; Damboldt, 1976; Guinochet, 1982; Pignatti, 1982). Furthermore, for both species a chromosome number of  $2n = 26$  has been reported (Contandriopoulos, 1962), even if these must be treated with some caution because of considerable discrepancy between chromosome numbers given in the table and those indicated in the figure legend (Huber, 1988: p. 53; Kovanda, 1971: p. 387; Polatschek, 1966: p. 113;  $2n = 26$  could, however, be confirmed for *P. charmelii*: H. Weiss-Schneeweiss et al., unpubl. data). Together with the lack of any sequence differentiation between the two taxa (Figs. 2–4), recognition of *P. villarsii* as separate species is no longer tenable.

Problems of species discrimination in *Phyteuma* likely are caused by several not mutually exclusive factors, such as high morphological variability (especially in widespread taxa: see Section 4.4) or hybridization. The presumed lack of clear morphological separation from a highly variable species prompted Sales and Hedge (2000) to merge *P. pyrenaicum*, an endemic from northern Iberian mountain ranges (Damboldt, 1976), with the widespread *P. spicatum*. This is supported by AFLP data (Fig. 6), but because of the lack of data for several closely related species (*P. gallicum*, *P. nigrum*, *P. tetramerum*, *P. vagneri*) it must be interpreted with caution. Sequence data, however, clearly suggest that *P. pyrenaicum* constitutes a phylogenetically distinct entity (Figs. 2–5). Furthermore, there is no evidence that *P. pyrenaicum* and the Carpathian *P. vagneri*, its morphologically and ecologically similar putative sister species (Figs. 3–5), are derivatives of *P. spicatum* as hypothesized by Schulz (1904: p. 43; Fig. 1).

Hybridization is common in *Phyteuma* and Schulz (1904) described numerous hybrids (including triple hybrids). Although *P. spicatum* and *P. nigrum* form naturally occurring hybrid swarms (*P. ×adulterinum*) in the Czech Republic, the Netherlands and in Germany (Buttler and Hand, 2008; Kovanda, 1981; Weeda, 1989), hybridization does not erode the phylogenetic distinctness of *P. nigrum* (Figs. 2–5), rejecting proposals to merge it with *P. spicatum* (Weeda, 1989). Ongoing hybridization likely is responsible

for blurring morphological differences between *P. persicifolium* and *P. betonicifolium* (stigma number, a polymorphic trait in other *Phyteuma* species, and seed size; Damboldt, 1976) around their contact zone in the Eastern Alps (Martini, 1978; Melzer, 1960). Probably, these species, whose distinctness has been confirmed earlier by phylogeographic studies (Alvarez et al., 2009; Thiel-Egenter et al., 2011), diverged in allopatry, possibly enforced by Pleistocene glaciations and restriction to different refugia in the western and the eastern Alps (Schönschwetter et al., 2005; Tribsch and Schönschwetter, 2003), from where they gained secondary contact after postglacial range expansion.

Although ancient hybridization might be (at least partly) responsible for substantial incongruence between nuclear and plastid markers (Figs. 2–5), its role for the evolution of *Phyteuma* is ambiguous. The hypothesis of Popov (1949) that *P. vagneri* may be of hybridogenic origin involving *P. spicatum* and *P. orbiculare*, finds no support in the molecular data (Figs. 2–5). On the other hand, *P. gallicum*, an endemic of the French Massif Central (Brunerye, 1989) initially associated with *P. nigrum* (Schulz, 1904), occupies a phylogenetically intermediate position between *P. spicatum* and *P. nigrum* (Figs. 2–5). This together with the combination of morphological features found in *Ph. nigrum* (non-cordate basal leaves) and in *P. spicatum* (blue corolla) might indicate a hybridogenic origin of *P. gallicum*, although *P. nigrum* is considered lacking in the French Massif Central (Brunerye, 1989).

#### 4.4. Intraspecific relationships

Following the tradition of his time, Schulz (1904) provided a very detailed intraspecific taxonomic system often distinguishing up to three nested taxonomic levels (subspecies, varietas, forma). Whereas most of his intraspecific taxa are considered taxonomically irrelevant and are no longer in use, high morphological variability in some species is acknowledged by recognizing subspecies (Aeschimann et al., 2004; Bolòs and Vigo, 1995; Damboldt, 1976; Kovanda, 2000; Pignatti, 1982; Podlech, 2008). This is particularly the case in the widespread *P. spicatum* and *P. orbiculare*. In *P. spicatum*, blue flowering individuals commonly found in the southern and central part of the species' distribution (Damboldt, 1976; Hayek, 1931) are usually treated as subsp. *coeruleum* (Damboldt, 1976); reports from the Alps (Aeschimann et al., 2004; Podlech, 2008) and Central European lowlands (Rosenbauer, 1996) mostly refer to hybrid swarms with *P. ovatum* and *P. nigrum*, respectively (Buttler and Hand, 2008; Kovanda, 1981; Polatschek, 1999). A certain distinction of subsp. *coeruleum* is suggested by its predominant occurrence in potential Pleistocene forest refugia, for instance in the southeasternmost Alps or the Balkan Peninsula (Magri et al., 2006; Willner et al., 2009). The blue-flowered accessions in our analysis (accessions 2, 3, 7 and 8) do not form a cohesive group (Figs. 2–5), which may indicate that they represent a paraphyletic ancestral stock, which gave rise to the more widespread and more derived whitish-flowered forms.

*Phyteuma orbiculare* is morphologically and ecologically highly variable, which is reflected in Schulz' (1904) extensive taxonomic treatment. Although some phylogeographic signal in *P. orbiculare* is evident (most notably, accessions 4–6 from the Eastern Alps group together: Figs. 3 and 4), the available data do not allow drawing conclusions concerning phylogeographic history or separation of morphologically and ecologically differentiated (sub)species (Schulz, 1904; Bolòs and Vigo, 1995; Kovanda, 1971, 2000; Müller, 2011). Given the exceptionally broad elevational distribution of *P. orbiculare*, occurring from lowlands to Alpine grasslands, a better understanding of morphological and phylogeographic patterns in this species may contribute significantly to our understanding of the evolution of high mountain plants and the accompanying adaptations.

*Phyteuma globulariifolium* includes two geographically separated subspecies (treated as species by Schulz, 1904), subsp. *globulariifolium* in the Eastern Alps and subsp. *pedemontanum* occurring from the Pyrenees to the westernmost Eastern Alps (Damboldt, 1976; Pignatti, 1982; Podlech, 2008). Using AFLPs, Schönschwetter et al. (2002) identified a major genetic split in the Eastern Alps along the river Etsch/Adige, which is remarkably close to the limit between both taxa identified by Schulz (1904). This split is confirmed by both nuclear and plastid sequence data (Figs. 2–5). Despite considerable and consistent genetic differentiation, the presence of morphologically intermediate forms in the contact area renders treating these lineages on the subspecific level the most reasonable approach. Within *P. scheuchzeri*, two subspecies are distinguished (Damboldt, 1976; Schulz, 1904) that differ in ecology and distribution (subsp. *scheuchzeri* is predominantly calcifuge and restricted to the Western Alps, subsp. *columnae* is mostly calcicole and distributed from the Southern Alps eastwards to Slovenia and northern Croatia). Neither the major split within *P. scheuchzeri* (Figs. 2–5), separating the three westernmost accessions from others, nor a minor split, separating the two southeasternmost accessions from those further west, agree with the taxonomic designations. Instead, they suggest a strong phylogeographic structure, possibly connected to differentiation in separate Pleistocene refugia along the southern margin of the Alps (Schönschwetter et al., 2005; Tribsch and Schönschwetter, 2003). The western Southern Alps might be a secondary contact zone, which could explain the blurred morphological and ecological distinction in this area evident from the phylogenetic position of several accessions of subsp. *scheuchzeri* within a clade of subsp. *columnae* (Figs. 2–5).

#### 4.5. Chromosome number evolution

*Phyteuma* shows a dysploid series of chromosome numbers ranging from  $n = 10$  to  $n = 14$  (Polatschek, 1966) with no polyploid numbers known (Kovanda, 1981). Although our knowledge on chromosome numbers of single species is still incomplete and partly ambiguous due to the high incidence of B chromosomes (Ochlewska, 1965; Polatschek, 1966) there is clear evidence for a strong phylogenetic signal in chromosome numbers. Taking the chromosome number of *Physoplexis* ( $2n = 34$ ) into account (Damboldt, 1966; Favarger, 1965; Polatschek, 1966), the ancestral chromosome numbers of *Phyteuma* are  $2n = 28$  or  $2n = 26$  (Fig. 7a). From there, descending dysploidy progressed independently in the two main lineages. In sect. *Spicata*, after a further reduction to  $2n = 24$  probably already prior to the diversification of this clade, chromosome numbers got reduced to  $2n = 22$  in *P. spicatum* and relatives. In sect. *Capitata*, starting from  $2n = 28$  in *P. globulariifolium* and others, descending dysploidy progressed via  $2n = 26$  (*P. scheuchzeri*) to  $2n = 22$  (*P. orbiculare* and relatives) and eventually  $2n = 20$  (*P. sieberi*). Alternatively, starting from  $2n = 26$  an ascending dysploid change to  $2n = 28$  in *P. globulariifolium* and others would have to be invoked. Disregarding the possibly paleopolyploid chromosome number of *Physoplexis*, the ancestral chromosome numbers of *Phyteuma* are inferred as  $2n = 26$  or  $2n = 24$  and that of sect. *Capitata* as  $2n = 26$  (data not shown). In any case, descending dysploidy predominates, which disagrees with the hypothesis of Ehrendorfer in Polatschek (1966: p. 118), who proposed an ascending series from  $x = 10$  to  $x = 14$ . In both sections, reduced chromosome numbers are characteristic for monophyletic clades or for single species, suggesting that dysploidy contributed significantly to lineage differentiation, as has been shown for other Campanulaceae (Lobelioideae: Schultheis, 2001; Stace and James, 1996; *Campanula* s. lat.: Contandriopoulos, 1971; Roquet et al., 2008) and other angiosperms (Blösch et al., 2009; Guerra, 2008; Hansen et al., 2006; Vallès et al., 2011).

#### 4.6. Biogeography

Considering the distribution of *Physoplexis* (southeastern Alps), *Petromarula* (Crete) and *Asyneuma* (northern Africa and temperate Eurasia), a European origin of *Phyteuma* is likely. The highest species diversity of *Phyteuma* is found in European high mountain ranges in general (only *P. orbiculare* of sect. *Capitata* and *P. spicatum* and *P. nigrum* of sect. *Spicata* occur also in lowlands) and in the Alps in particular (only *P. serratum* and *P. pseudorbiculare* of sect. *Capitata*, and *P. gallicum*, *P. nigrum*, *P. pyrenaicum*, *P. tetramerum* and *P. vagneri* of sect. *Spicata* are lacking from the Alps; Meusel and Jäger, 1992; Fig. 7b). In line with these patterns of species diversity and the restriction of *Physoplexis* to the southeastern Alps, an Alpine origin of *Phyteuma*, as already suggested by Schulz (1904), is best supported (Fig. 7b). Available divergence times for *Physoplexis* and *Phyteuma* vary widely and range from 19–18 Ma (Cellinese et al., 2009) to 8.4–6 Ma (Roquet et al., 2009; this is actually the crown node age for a clade including *Phyteuma*, *Petromarula*, and *Asyneuma*). Accordingly, the differentiation of *Physoplexis* and *Phyteuma* might already have occurred in the early Miocene, although younger ages (late Miocene to Pliocene) as suggested by estimates from Roquet et al. (2009; these are based on four calibration points and thus may be more reliable) appear more likely. This scenario fits well with early hypotheses by Jerosch (1903) and Schulz (1904), who suggested a Miocene origin in the Alps, and rejects a late Pleistocene to postglacial origin, as suggested by Engler (1879) based on the comparatively small distribution areas of the Alpine to subnival species.

Range dynamics in sect. *Spicata* are characterized by an early expansion into the Carpathians (Fig. 7b). From here, diversification into the Central European lowlands (*P. nigrum*, *P. spicatum*) as well as into high mountain ranges outside the Alps, most notably the Pyrenees and adjacent mountain ranges, occurred (*P. pyrenaicum*). Whereas *P. spicatum* reached the Alps secondarily, the lineage leading to, among others, *P. betonicifolium* and *P. charmelii*, diversified primarily in the Alps (Fig. 7b). This pattern is repeated in sect. *Capitata*, where range dynamics are characterized by ancestral distributions mainly restricted to the Alps (Fig. 7b). Range extensions are usually species-specific, such as those from the Alps to the Pyrenees in *P. globulariifolium* subsp. *pedemontanum* (Schönswetter et al., 2002). A notable exception is the ancestor of *P. confusum* and *P. serratum*, inferred to have been widespread in the Alps plus Corsica. Schulz (1904) suggested that the ancestor of *P. serratum* colonized Corsica from the SW Alps via land-bridges, known to have existed at different time periods, such as the Oligocene or during the Messinian salinity crisis (Salvo et al., 2010; Speranza et al., 2002). Such a scenario is unlikely, because the sister species of *P. serratum*, *P. confusum*, is absent from the Western Alps (Meusel and Jäger, 1992). Probably, the ancestor of *P. serratum* has reached Corsica after long-distance dispersal from the Eastern Alps, as has also been suggested for *Bupleurum stellatum* (Apiaceae; Schönswetter and Tribsch, 2005). As *Phyteuma* has no adaptations for long distance dispersal (wind tunnel experiments indicate short-distance dispersal capabilities of seeds of up to 10 m; Maier et al., 1999), the precise dispersal mode remains elusive, but likely involved non-standard vectors (Nathan, 2006). At least some of these range shifts might have been caused by Pleistocene climate fluctuations. These were identified as shaping factors in range formation of *P. betonicifolium* and *P. persicifolium* (Alvarez et al., 2009; Thiel-Egenter et al., 2011), *P. globulariifolium* (Schönswetter et al., 2002) and likely also *P. humile* and *P. hedraianthifolium*, whose distribution areas are associated with Pleistocene refugia (Schönswetter et al., 2005; Tribsch and Schönswetter, 2003).

Our data indicate an enormous plasticity in ecological adaptations in *Phyteuma* with several ecological shifts with respect to vegetation structure and density (woodland versus open habitats),

elevation (below versus above the timber line) and edaphic preferences (calcareous versus siliceous bedrock). For instance, all species of sect. *Capitata* grow above the timberline, but *P. orbiculare* and *P. scheuchzeri* also occur at lower elevations, *P. scheuchzeri* preferentially so. As such, the whole group may have evolved as a high Alpine lineage (Comes and Kadereit, 2003; Zhang et al., 2001) and at least *P. orbiculare* may have secondarily reached lower elevations outside the Alps, possibly in connection with lowland refugia during Pleistocene glaciations (Holderegger and Thiel-Egenter, 2009). In contrast, species occurring above the timberline are rare in sect. *Spicata*. Whereas the majority of species is found in woodlands and semi-open habitats well below the timberline, only *P. cordatum*, *P. michelii*, *P. betonicifolium*, and *P. scorzonrifolium* ascend to the Alpine zone (Aeschmann et al., 2004). Another example concerns shifts in edaphic specialization in sect. *Capitata*, where *P. orbiculare*, *P. pseudorbiculare*, and *P. sieberi* are mostly or exclusively calcicolous, whereas the other species are found on siliceous bedrock (*P. humile* on serpentinites; Käsermann, 1999) or are indifferent (*P. scheuchzeri*). Evidently, *Phyteuma* is an excellent system to study ecological differentiation in the context of diversification in general and the evolution of high mountain taxa in particular.

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