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TWO MAJOR GROUPS OF CHLOROPLAST DNA HAPLOTYPES IN DIPLOID AND TETRAPLOID *ACONITUM* SUBGEN. *ACONITUM* (RANUNCULACEAE) IN THE CARPATHIANS

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Abstract. *Aconitum* in Europe is represented by ca. 10% of the total number of species and the Carpathian Mts. are the center of the genus variability in the subcontinent. We studied the chloroplast DNA intergenic spacer *trn*L$^{(UAG)}$-*rpl*32-*ndh*F (cpDNA) variability of the *Aconitum* subgen. *Aconitum* in the Carpathians: diploids (2n=16, sect. *Cammarum*), tetraploids (2n=32, sect. *Aconitum*) and triploids (2n=24, nothosect. *Acomarum*). Altogether 25 *Aconitum* accessions representing the whole taxonomic variability of the subgenus were sequenced and subjected to phylogenetic analyses. Both parsimony, Bayesian and character network analyses showed the two distinct types of the cpDNA chloroplast, one typical of the diploid and the second of the tetraploid groups. Some specimens had identical cpDNA sequences (haplotypes) and scattered across the whole mountain arch. In the sect. *Aconitum* 9 specimens shared one haplotype, while in the sect. *Camarum* one haplotype represents 4 accessions and the second – 5 accessions. The diploids and tetraploids were diverged by 6 mutations, while the intrasectional variability amounted maximally to 3 polymorphisms. Taking into consideration different types of cpDNA haplotypes and ecological profiles of the sections (tetraploids – high-mountain species, diploids – species from forest montane belt) we speculate on the different and independent history of the sections in the Carpathians.

Key words: *Aconitum,* Carpathian Mts., cpDNA haplotypes, ploidy levels

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Introduction

The genus *Aconitum* L. comprises approximately 300 species distributed in temperate regions of the N Hemisphere, mostly in eastern Asia (Kadota 1987; Liangqian & Kadota 2001). The subgenus *Aconitum* contains ca. 250 species and around 10% of them can be found in Europe, mainly in the mountain areas (Götz 1967; Seitz 1969). The subgenus *Aconitum* in Europe consists of the sect. *Aconitum*, sect. *Cammarum* DC. and nothosect. *Acomarum* Starmühl. (Starmühler 2001; Mitka 2003). This division goes along ploidy level, where sect. *Cammarum* represents diploids (2n=16) and sect. *Aconitum* – tetraploids (2n=32), while hybridogenic nothosect. *Acomarum* contains plants with 2n=24 (Joachimiak *et al.* 1999; Mitka *et al.* 2007; Ilnicki & Mitka 2009, 2011).

These sections in Europe differ not only morphologically and cytogenetically but also ecologically (Novikoff & Mitka 2011, 2015). Diploids are the lowland and montane-zone species (up to ca. 1150 m above sea level), and tetraploids are high-mountain species found predominantly in the subalpine and alpine zones. Triploids have intermediate position but mostly prefer lower altitudes and open habitats. Generally, diploids are forest and tetraploids an open mountainous area species growing above the upper forest line (Joachimiak *et al.* 1999; Mitka 2003; Novikoff & Mitka 2011).

The aim of this study was to check variability of chloroplast DNA (cpDNA haplotypes) in the sections *Aconitum* and *Cammarum* in the Carpathians. cpDNA is thought to be slowly evolving and thus useful in the phylogenetic reconstructions of the higher taxonomic levels

(Avise 2004). The species are the Carpathian endemics but *A. variegatum* being a European endemic and *A. toxicum* distributed also in the Balkans. Isolated position of the mountain range and high endemicity makes it a promising object of the phylogeographic investigations.

The background hypothesis concerns the origin of the tetraploids in the Carpathians. If they originated directly from the local diploid stock they should share a great deal of the variability. If diploids and tetraploids harbor distinctly different cpDNA haplotypes it can mean that they originated in the mountain range by independent migrations. To check the hypothesis we use maternally inherited *trn*L$^{(UAG)}$-*rpl*32-*ndh*F intergenic spacer sequences of cpDNA.

Material and methods

Sampling and DNA extraction and molecular techniques

25 accessions (Operational Taxonomic Units or OTUs) of *Aconitum* sect. *Aconitum* (ingroup) were sampled from the Carpathians (Tab. 1; Fig. 1). Two additional OTUs: *A. lycoctonum* L. em. Koelle and *A. moldavicum* Hacq. were set as an outgroup. The following species represent the whole taxonomical variability of the ingroup in the Carpathians: *A. degenii* Gáyer, *A. lasiocarpum* (Rchb.) Gáyer, *A. ×pawlowskii* Mitka & Starmühl., *A. toxicum* Rchb., and *A. variegatum* L. (diploids); *A. bucovinense* Zapał., *A. ×czarnohorense* (Zapał.) Mitka, *A. firmum* Rchb., and *A. ×nanum* (Baumg.) Simonk. (tetraploids). The ingroup encompasses additionally the two triploid (2n=24) hybrids found in the Carpathians: *A. ×cammarum* L. em. Fries (*A. napellus* × *A. variegatum*) and *A. ×berdaui* Zapał. (*A. firmum* × *A. variegatum*). Both of them are representatives of the nothosect. *Acomarum*. *A. ×cammarum* is an ornamental plants found in the rural gardens. The nomenclature of *Aconitum* follows www.ipni.org (see also Mitka 2003; Novikoff & Mitka 2011).

For all accessions either recently collected samples (stored as silica-dried leaves) or herbarium specimens were obtained. From this material samples for DNA extractions were prepared using ca. 2 cm^2 of fully developed leaf blade with no symptoms of damage caused by insects or fungal infections (Gawal & Jarret 1991). Samples were then grounded in 2 ml microcentrifuge tubes with 3 stainless steel beads (ø 3mm) by shaking in an oscillation mill (MM 200 – Retsch, Germany) for 4 minutes at 25Hz. Then, DNA was extracted separately for each sample with a Genomic Mini AX Plant DNA extraction kit (A&A Biotechnology, Poland), according to the manufacturer protocol.

The undiluted DNA extracts were used as templates in the amplification of the *trn*L$^{(UAG)}$-*ndh*F region of chloroplast DNA with primers *trn*L$^{(UAG)}$ – 5'-CTGCTTCCTAAGAGCAGCGT-3' and *ndh*F – 5'-GAAAGGTATKATCCAYGMATATT-3' (Shaw *et al.* 2007). The reaction was carried out in a total volume of 50 µl containing: 1× DreamTaq Green buffer (ThermoFisher Scientific), 3.5 mM $MgCl_2$, 0.08mM each of dNTPs, 0.08µM of both primers and 1u of DreamTaq DNA polymerase (ThermoFisher Scientific). Amplifications were run on a T100 Thermal Cycler (Bio-Rad) with the following temperature profile: 5 minutes of initial denaturation at 94°C; 25 touchdown cycles composed of 30 seconds at 94°C; 30 seconds at decreasing annealing temperatures (0.5°C/cycle from 67.5°C in the 1st to 55°C in the 25th cycle); 1 minute at 72°C; and 20 cycles of 30 seconds at 94°C, 30 seconds at 55°C, and 1 minute at 72°C; and 10 minutes at 72°C for the final extension step. The amplification effectiveness was verified by agarose gel electrophoresis, and positive PCR products were purified with the Clean-Up DNA purification kit (A&A Biotechnology, Poland). The purified PCR products were used as template in sequencing reaction.

Entire *trn*L$^{(UAG)}$-*ndh*F region was sequenced for all samples with use of PCR primers and set of internal sequencing primers: V1_F – 5'-AGGTTGAGTTATTGGTGGATGA-3', V2_F – 5'-GTTCGCAAAGAACTGAAGTGAC-3', V3_F – 5'-TGGATGATAGAATAYATATCA AAATCA-3' (forward primers) and V2_R – 5'-TTTCCGGATTCACCAGCTCTT-3', V3_R – 5'-CGAAAAGCCATTACATTCTTAAA-3'

Tab. 1. Localities of twenty five accessions of *Aconitum* subgen. *Aconitum* from the Carpathians (ingroup) and two accessions of an outgroup (*A. moldavicum* and *A. lycoctonum* from subgen. *Lycoctonum*). **JM** – J. Mitka; **MG** – M. Graniszewska; **PB** – P. Bochenek. Taxonomy follows Mitka (2003) and Novikoff & Mitka (2011).

Nr. of accession	Genus	species	subspecies	Region/Locality	Date of collection	Collector	Haplotype
001	*Aconitum*	×*cammarum*	-	W Carpathians/Vel'ká Fatra	27.07.2007	JM	C
003	*Aconitum*	*variegatum*	*variegatum*	W Carpathians/ Muranska planina	28.07.2007	JM	-
007	*Aconitum*	*degenii*	*degenii*	E Carpathians/Rodna	16.08.1998	JM	A
009	*Aconitum*	*firmum*	*maninense*	W Carpathians/Tatras	15.05.2008	JM	C
017	*Aconitum*	*toxicum*	*toxicum*	S Carpathians/Piatra Craiului	07.08.1999	PB	A
023	*Aconitum*	*firmum*	*maninense*	W Carpathians/ Stražovske vrchy	15.05.2008	JM	C
025	*Aconitum*	*bucovinense*	-	E Carpathians/Rarau	15.05.2008	JM	C
026	*Aconitum*	*variegatum*	*variegatum*	W Carpathians/Pieniny	26.08.2007	JM	B
029	*Aconitum*	*bucovinense*	-	Bihor/Cornul Muntilor	15.07.2006	JM	B
032	*Aconitum*	×*nanum*	-	S Carpathians/Fogaraš	15.05.2008	JM	-
033	*Aconitum*	*lasiocarpum*	*lasiocarpum*	E Carpathians/Ceahlău	08.08.2007	JM	-
034	*Aconitum*	*firmum*	*moravicum*	W Carpathians/W Beskyds	15.05.2008	JM	C
045	*Aconitum*	*degenii*	*degenii*	E Carpathians/Gorgany	11.08.2003	MG	A
047	*Aconitum*	*firmum*	*firmum*	W Carpathians/Tatras	27.08.2007	JM	C
048	*Aconitum*	×*czarnohorense*	-	E Carpathians/Rodna	15.08.1998	JM, PB	C
049	*Aconitum*	*toxicum*	*toxicum*	S Carpathians/Bucegi	07.08.1998	PB	A
054	*Aconitum*	*firmum*	*fissurae*	S Carpathians/Retezat	28.07.2007	JM	B
057	*Aconitum*	×*pawlowskii*	-	W Carpathians/Tatra	27.08.2007	JM	B
059	*Aconitum*	*lasiocarpum*	*kotulae*	W Carpathians/Nízke Tatra	19.08.2007	JM	-
062	*Aconitum*	*moldavicum*	*moldavicum*	Małopolska Upland/ Przeginia	26.07.2006	JM	-
063	*Aconitum*	*lycoctonum*	*lycoctonum*	Montenegro (Balkans)/ Durmitor	13.08.2009	JM	-
065	*Aconitum*	×*berdaui*	-	W Carpathians/Malá Fatra	31.07.2009	JM	-
066	*Aconitum*	*firmum*	*maninense*	W Carpathians/ Stražovske vrchy	01.08.2009	JM	C
072	*Aconitum*	*bucovinense*	-	E Carpathians/W Bieszczady	25.06.1996	JM	C
074	*Aconitum*	*bucovinense*	-	S Carpathians/Piatra Craiului	11.08.1999	JM	-
107	*Aconitum*	×*pawlowskii*	-	W Carpathians/ E Beskyds	24.08.2001	JM	A
50A	*Aconitum*	*degenii*	*degenii*	S Carpathians/Retezat	24.07.2009	JM	B

(revers primers) (Boroń *et al.* – unpublished). Sequencing was performed with a BigDye Terminator v.3.1 Cycle Sequencing Kit (Life technologies, USA) on a T100 thermal cycler (Bio-Rad) and a 3500 Series Genetic Analyzer (Life Technologies, USA) using standard protocols.

Phylogenetic analyses

The resulting sequences were processed and aligned with MEGA 6 software (Tamura *et al.* 2013). The alignment revealed the extensive length variation among sequences resulting from two types of length mutations, namely the indels and mononucleotide SSR loci. For our study we consider an indel all non-loci-specific gaps (all indels observed were at least 3 bp long safe for one 1 bp long indel). Similarly, all polynucleotide sites longer than 5 mononucleotide repeats for which length variation was observed were considered SSR loci. As the clear gaps' and SSR mutations' pattern were observed, these characters were encoded into two matrixes, i.e. binary (present vs. absent) gap matrix and standard characters (no. of mononucleotide repeats) SSR matrix. Than all sites with gaps were deleted from alignment as no point mutations were found for these regions. All three datasets, i.e. gap-free alignment, gap binary matrix and SSR standard matrix were combined into one datafile and analyzed simultaneously.

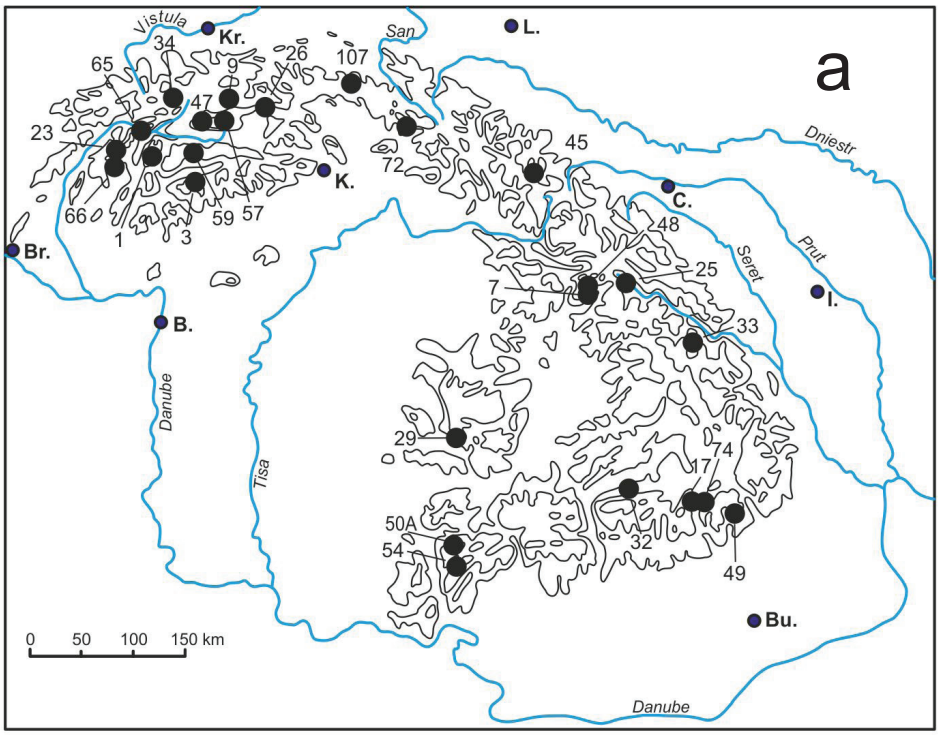
Restriction site data were analyzed using the maximal parsimony (MP) optimality criterion (Felsenstein 2004) using PAUP* (version 4.0.b10, Swofford 2002). Gaps were coded as a new character state. Insertions/deletions (indels) for which at least one sample exhibited polymorphism were coded as interleaved characters, and the corresponding nucleotide characters were excluded (Fergusson *et al.* 1999). Heuristic search was conducted with random addition, tree bisection-reconnection (TBR) branch swapping, and the MULTREES option on. The consistency index (CI; Kluge & Farris 1969), the retention index (RI), and rescaled consistency index (RC; Farris 1989a, 1989b) were calculated with PAUP*. Support for branches was evaluated by bootstrapping (BS; Felsenstein 2004). One thousand bootstrap replicates, each with 10 random stepwise additions were performed using the same settings as above and no more than 100 trees were retained per replicate.

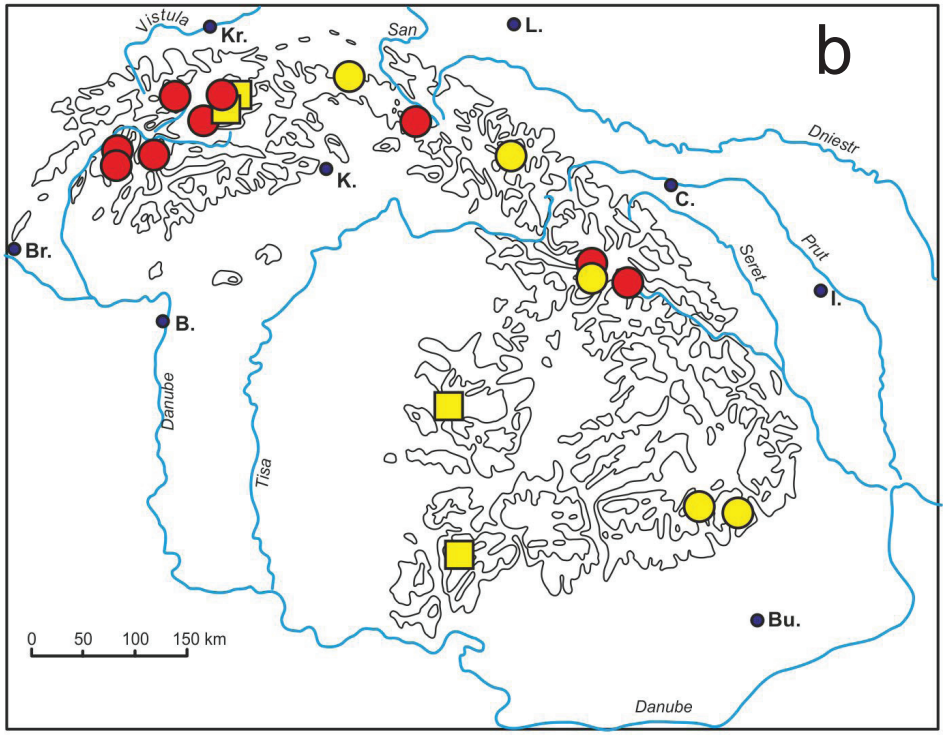
A Bayesian Inference (Rannala & Yang 1996) implemented in the program MrBayes v. 3.1.2 (Huelsenbeck & Ronquist 2001) was used. In the analysis, two independent runs with four Markov chain Monte Carlo (MCMC) runs (three heated and one cold) were run simultaneously, sampling every 100 generations for 10 million generations and starting with a random tree. The first 2 million generations (25% of the total) were excluded as burning after convergence of the chains, which was evaluated by the average standard deviation of splitting frequencies reaching below 0.01. Gaps were coded in the matrix as presence/absence following the method proposed by Simmons & Ochoterena (2000) as implemented in SeqState (Müller 2005). Gaps were assumed to follow the binary model of evolution (Ronquist *et al.* 2005) and were included in the Bayesian analyses following Dwivedi & Gadagkar (2009).

A Minimum Spanning Network (MSN) were used to generate a distance graph with all of the edge sets of all minimum spanning trees (Huson & Bryant 2006) with the use of SplitsTrees software (www.splitstree.org). BS values were calculated based on 1000 replications.

The phylograms were edited in TreeView v.1.6.6 (http://taxonomy.zoology.gla.uc. uk/rod/htlm) and rooted with the two species of the subg. *Lycoctonum*: *A. lycoctonum* and *A. moldavicum*. If the genotypes of accessions were identical, only one of them was included in the analyses.

Fig. 1. Geographical distribution of sampled *Aconitum* subgen. *Aconitum* and haplotypes A, B, and C in the Carpathians. ▸ The numbers are concordant with those in Tab. 1. **Br** – Bratislava; **Bu** – Bucuresti; **B** – Budapest; **C** – Černivce; **I** – Jassy; **K** – Košice; **Kr** – Kraków; **L** – Lviv.





haplotype A haplotype B haplotype C

Tab. 2. Haplotypes of cpDNA in the Carpathians. Haplotype A – accessions nrs. 007, 017, 045, 049, 107; haplotype B – nrs. 026, 029, 50A, 057; haplotype C – nrs. 001, 009, 023, 025, 034, 047, 048, 066, 072 (see Tab. 1 and Fig. 1).

Haplotypes / nr. of site	Ploidy	117	250	322-328	367	545-551	552-555	607-614	635	656-662	794-801	821	871-880
Haplotype A		G	G	AAAAAAA	G	TTTTTI-	ATTT	TTTTTI-	G	-------	TTTTTII--	A	TTTTTTTTT
Haplotype B		G	G	AAAAAAA	G	TTTTTI-	ATTT	TTTTTI-	G	-------	TTTTTTT-	A	TTTTTTTTT
045 *A. d.* ssp. *degenii*		G	G	AAAAAAA	G	TTTTTI-	ATTT	TTTTTI-	G	-------	TTTTTTT-	A	TTTTTTTTT
033 *A. l.* ssp. *lasiocarpum*	2n = 16	T	G	AAAAAA-	G	TTTTTI-	ATTT	TTTTTI-	G	-------	TTTTTTT-	A	TTTTTTTTT
003 *A. v.* ssp. *variegatum*		G	G	AAAAAAA	G	TTTTTI-	ATTT	TTTTTI-	A	-------	TTTTTTT-	A	TTTTTTTTT
065 *A. ×berdaui* (2n = 24)		G	G	AAAAAAA	A	TTTTTI-	ATTT	TTTTTI-	G	-------	TTTTTTT-	A	TTTTTTTTT
059 *A. l.* ssp. *kotulae*		G	G	AAAAAAA	G	TTTTTI-	ATTT	TTTTTI-	G	-------	TTTTTI--	T	TTTTTTTTT
Haplotype C		G	G	AAAAAAA	A	TTTTTTT	----	TTTTTTT	G	AATAATA	TTTTTTTT	T	TTTTTTTT-
054 *A. firmum* ssp. *fissurae*		G	A	AAAAAAC	A	TTTTTTT	----	TTTTTTT	G	AATAATA	TTTTTTTT	T	TTTTTTTT-
074 *A. bucovinense*	2n = 32	G	A	AAAAAAA	A	TTTTTTT	----	TTTTTTT	G	AATAATA	TTTTTTTT	T	TTTTTTTT-
032 *A. ×nanum*		G	G	AAAAAAA	G	TTTTTTT	----	TTTTTTT	G	AATAATA	TTTTTTTT	T	TTTTTTTT-

Results

The complete aligned *trn*L$^{(UAG)}$-*rpl*32-*ndh*F spacer was 996 bp long, 938 bp long after gaps and SSR loci were removed. The gap matrix consisted of 9 characters and SSR of 7 characters. 899 of the characters were constant, 10 variable characters were parsimony-uninformative and 29 characters were parsimony informative. Unweighted parsimony found 1 most parsimony tree of 47 steps, with a CI = 0.9362, RI = 0.9434, and RC = 0.8832. Analysis of the complete cpDNA restriction data set (25 + 2 outgroup OTUs) revealed the same sequences in some OTUs. Among diploids: OTUs no. 007, 017, 049, 057, and 107 (cpDNA haplotype A); 026, 029, 50A, and 057 (cpDNA haplotype B); and among tetraploids: 001, 009, 023, 025, 034, 047, 048, 066, and 072 (cpDNA haplotype C). They are distributed across the whole Carpathians (Fig. 1). The phylogenetic analyses were performed on the reduced data set, with only one representative of the each cpDNA haplotype A, B and C, i.e. with 10 (ingroup) and 2 outgroup accessions.

The differences among accessions within the diploids and tetraploids did not exceed 3 polymorphisms (substitutions and/or SSR mutations) and the differences between the diploids and tetraploids amounted to 6 steps (2 unique indels and 4 SSR mutations – Tab. 2; Fig. 2 c).

A Bayesian Inference (MrBayes) and MP (PAUP*) splits the OTUs into the diploid and tetraploid groups (Fig. 2 a, b). Both diploids and tetraploids had high support (0.99 posterior probability, PP and 61% BS). Among diploids *A. degenii* and *A. lasiocarpum* formed highly supported sister group (0.94 PP, 61% BS). The triploid *A. ×berdaui* joined with the diploids. The tetraploids: *A. firmum* subsp. *fissurae* and *A. bucovinense*, and the diploids: *A. degenii* and *A. lasiocarpum*, formed weakly (61% and 59% BS, respectively) supported sister groups.

The MSN clearly divides the *Aconitum* in the Carpathians into the two ploidy groups with a BS of 100% (Fig. 2 c). The diploid-type haplotype A was placed with haplotype B together. They differ only by one indel (Tab. 2). In the diploid

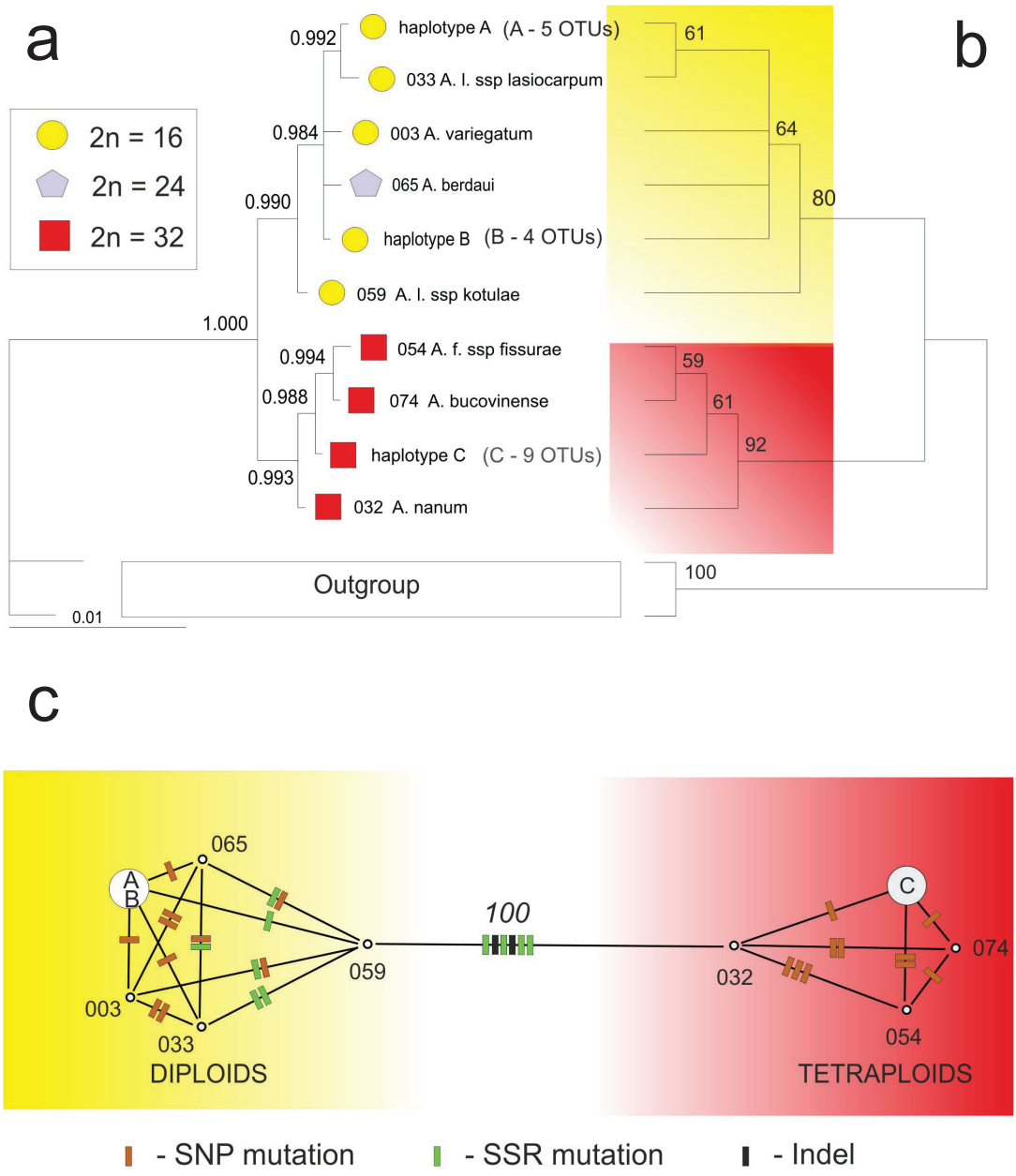


Fig. 2. a – Bayesian Inference (MrBayes) of cpDNA haplotypes of *Aconitum* subgen. *Aconitum* in the Carpathians (above nodes posterior probabilities are given); **b** – maximum parsimony analysis (PAUP*) and majority consensus tree (above nodes bootstrap values are given); **c** – minimum spanning network (SplitsTree). The split between the diploid-type and tetraploid-type cpDNA haplotypes is supported with 100% bootstrap. Bootstrap support for (b) and (c) is based on 1000 random runs. Haplotypes A, B and C are characterized in Tab. 2. **A. f.** – *Aconitum firmum*; **A. l.** – *Aconitum lasiocarpum*.

group both SSR and SNP mutations occurred and in the tetraploid group only SNP mutations were found.

Discussion

The studied chloroplast region in the subgen. *Aconitum* in the Carpathians is clearly different in the diploid *Cammarum* and tetraploid *Aconitum* groups. All the phylogenetic analyses showed the split between these cytogenetic/taxonomic groups. Three cpDNA haplotypes: A, B, and C were represented by more than one accession. cpDNA haplotypes A and B were characteristic of the diploids and cpDNA haplotype C of tetraploids. However, all three haplotypes were not the area or species specific. The remaining cpDNA haplotypes were singular and genetically very close to one the cpDNA haplotypes A, B and C, differing by up to two polymorphisms in diploids and up to three polymorphisms in tetraploids. The genetic affinity of *A. degenii* and *A. lasiocarpum* is weakly (51%) supported in MP.

Nucleotide variation of cpDNA is extremely low in the genus *Aconitum*. In the study on *Aconitum* subgen. *Lycoctonum* in Europe Utelli *et al.* (2000) found 1-2 point mutations in the *psb*A-*trn*H spacer. Similarly, some cpDNA sequences were identical and formed species-specific cpDNA haplotypes. Their specificity could be a result of the geographic isolation of the species. Authors claimed that minute differences in sequence data of the ITS and *psb*A-*trn*H intergenic spacers between *A. lycoctonum* (Alps, Carpathians, Pyrenees, Sierra Nevada), *A. moldavicum* (Carpathians), *A. septentrionale* (Northern Europe), and *A. orientale* (Cacucasus) put in question their taxonomic status as a separate species. According to our results the same cpDNA haplotype occurs in various species within the same ploidy level and rarely, in the effect of hybridization, among the ploidy levels. In this line of reasoning the taxonomical sections (diploids *vs.* tetraploids) in the Carpathians should gain a status of the singular species. In order to maintain a rational taxonomic *status quo* a solution is the morpho-geographical species concept. It attains a logical basis when the pattern of the morphological variability can be arranged in a geographical pattern (Cain 1974). The distribution of the same cpDNA haplotype in various species could be the result of the interspecific hybridization.

Our results show that cpDNA markers discriminated *Aconitum* at the sectional/ploidy level. Probably none of the diploids in the Carpathians was ancestral to any tetraploid species from the region. So, both sections could represent here various migratory and historical elements. A wider sampling, including the Asian representatives, could enlighten the problem. The tetraploid group of *Aconitum* in Europe belongs to the oligothermic, high-mountain flora. It represents currently the group of Arctic-Alpine plants (Hultén 1937) covering the boreal and arctic portions of northern hemisphere and the mountains of Europe and Asia. In the Carpathians this group (alongside with *Aconitum* sect. *Aconitum*) includes for example: *Athyrium distentifolium, Cerastium alpinum, Dryas octopetala, Hieracium alpinum, Juncus triglumis, Lloydia serotina, Oxyria digina*, and other (Zając & Zając 2009).

In studies on Asian *Aconitum* with the use of cpDNA and RFLP markers the two main clades were found, one of the eastern Asian diploids and the other of the so called Japenese tetraploid complex (Kita *et al.* 1995). The authors put forward a hypothesis that the entire complex had originated from one of the tetraploid species which populations, more or less isolated, adopted to alpine environmental conditions. It was because the authors were not able to find any genetic affinities of the tetraploid group to any diploid species studied. In another, supplementary studies Kita & Ito (2000) found that the missing link was a diploid *A. volubile* Koelle growing in Russia (the Ural Mts., E and W Siberia), Mongolia, China, Korea and Japan (see Mitka 2003). It suggests that this taxon could be ancestral to the tetraploids.

Similarly, if Carpathian diploids were ancestral to the tetraploids they should form a monophyletic group. It was not a case suggesting their different origins. One of the explanations could be that the diploids formed

the oldest element, and their genetic roots could have been placed in pre-Pleistocene taking their different ecological profile as forest species. They could be linked genetically with temperate Central-Eastern Asian geoflora which might reach Europe as early as in the Early Oligocene. This is also traced in the similarities between European and Asian fossil floras of that period (Tiffney 1985; Mai 1995). Another hypothesis claims that the Arctic-Alpine flora originated in the late Tertiary in Eastern Siberia and Central Asia (Hultén 1937). Current studies corroborate this scenario, as for example phylogeography of *Ranunculus pygmeus* (Schönswetter *et al.* 2006), *Dryas octopetala* (Skrede *et al.* 2006), and *Saxifraga oppositifolia* (Winkler *et al.* 2012). Unexpectedly, the phylogeographic analysis revealed that the latter species has two ancestral areas: in Europe (the Alps) and in Central Asia. This infers that most likely there were other Early Tertiary genetic lineages in the Northern Hemisphere, in addition to the Central Asian. If this was the case, Central Asian Arctic-Alpine flora could not be regarded as the "cradle" but rather "museum". The other explanation is that both the diploids and tetraploids in the Carpathians represent the remnants of an old genetic European line and in fact both they are of the ancient, the pre-Pleistocene age (the result similar to *S. oppositifolia* mentioned above). It is a postulate of the complementary to the Central Asian genetic center of *Aconitum* in Europe. The third possibility is that the Carpathian (in wider context – the European) *Aconitum* had roots in the Asian genetic centre and the common descendant lineage extinct.

The present results showed that the tetraploid *A. bucovinense* was placed within both the diploid (haplotype B) and tetraploid groups. In fact, the species is considered as an old hybrid between *A. degenii* and *A. firmum* (Mitka 2003). On the other hand, it could be the effect of the recent transfer of the plastid gene. It has been often noted incongruence between the morphological-cytogenetic classification, nrDNA inferred phylogeny *vs.* chloroplast-based phylogeny that could be a sign of chloroplast capture (Rieseberg & Soltis 1991). The examples are *Aconitum* (Kita *et al.* 1995; Utelli *et al.* 2000), *Anthoxanthum* (Pimentel *et al.* 2013), *Hedyosmum* (Zhang *et al.* 2015), *Heuchera* (Soltis & Kuzoff 1995), *Meehania* (Deng *et al.* 2015), and *Paeonia* (Sang *et al.* 1997). The occurrence of the diploid-type cpDNA in the tetraploid species could be the effect of its hybridization with a diploid line.

The triploid species *A.* ×*berdaui*, a hybrid between the tetraploid *A. firmum* and diploid A. *variegatum*, was placed in the cpDNA diploid-type group. This hybrid was studied by Zieliński (1982a, 1982b) in the Tatra Mts. The author found the introgression of DNA loci of the nuclear genome from a diploid into a tetraploid line. This mechanism of introgression, from the diploid into the tetraploid species is also known as a triploid bridge (Husband 2004).

In conclusion, the two different types of haplotypes of cpDNA in the diploids and tetraploids in the Carpathians advocates for their independent origin in the mountain area. Probably this pattern of cpDNA diversity is typical for the whole Europe, a hypothesis to be checked based on a wider sampling. It makes *Aconitum* a promising object of the studies on the hybridization between the section *Aconitum* and section *Cammarum*.

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