

Cryptic diversity in the smooth snake (Coronella austriaca)

Daniel Jablonski^{1,*}, Zoltán T. Nagy², Aziz Avcı³, Kurtuluş Olgun³, Oleg V. Kukushkin^{4,5}, Barbod Safaei-Mahroo⁶, David Jandzik^{1,7,*}

Abstract. The smooth snake, *Coronella austriaca*, is a common snake species widespread in the Western Palearctic region. It does not form conspicuous morphological variants and, although several evolutionary lineages have been distinguished based on the analyses of the mitochondrial DNA sequences, only two subspecies with very limited distribution have been traditionally recognized. Here we present an mtDNA phylogeography of the species using geographically extended sampling while incorporating biogeographically important areas that have not been analyzed before, such as Anatolia, Crimea, and Iran. We find that the smooth snake comprises 14 distinct phylogenetic clades with unclear mutual relationships, characterized by complex genetic structure and relatively deep divergences; some of them presumably of Miocene origin. In general, the biogeographic pattern is similar to other Western Palearctic reptiles and illustrates the importance of the main European peninsulas as well as the Anatolian mountains, Caucasus, and Alborz Mts. in Iran for the evolution of the present-day diversity. Considerable genetic structure present in the smooth snake populations within these large areas indicates the existence of several regional Plio-Pleistocene refugia that served as reservoirs for dispersal and population expansions after the glacial periods. The current taxonomy of *C. austriaca* does not reflect the rich genetic diversity, deep divergences, and overall evolutionary history revealed in our study and requires a thorough revision. This will only be possible with an even higher-resolution sampling and integrative approach, combining analyses of multiple genetic loci with morphology, and possibly other aspects of the smooth snake biology.

Keywords: Asia, Colubridae, Europe, glacial refugia, phylogeny, refugia-within-refugia, Serpentes, speciation.

Introduction

The Western Palearctic is one of the most thoroughly studied biogeographic regions of the world and harbors several hot-spots of biological diversity. The richness of its biodiversity

- 3 Department of Biology, Faculty of Science and Arts, Adnan Menderes University, 09010 Aydın, Turkey
- 4 Department of Herpetology, Zoological Institute of the Russian Academy of Sciences, Universitetskaya emb. 1, 199034 St. Petersburg, Russia
- 5 Department of Biodiversity Studies and Ecological Monitoring, T.I. Vyazemsky Karadag Research Station
 Nature Reserve of the Russian Academy of Sciences, Nauki 24, 298188 Theodosia, Crimea
- 6 Pars Herpetologists Institute, 1st Floor, No. 5, Corner of third Jahad alley, Arash St., Jalal-e Ale-Ahmad Boulevard, Tehran, Iran
- 7 Department of Zoology, Charles University in Prague, Vinicna 7, 128 44 Prague, Czech Republic

*Corresponding author; e-mail: daniel.jablonski@balcanica.cz; david.jandzik@uniba.sk has resulted from enhanced diversification rates in landscapes that are extremely geographically diverse, characterized by a combination of longitudinally and latitudinally oriented mountain ranges separated by deep valleys, large lowlands, and high-altitudinal plateaus. The topography, in combination with the Plio-Pleistocene climatic oscillations that followed the warm Miocene climate, caused repeated retractions of populations surviving the climatic pessima in the refugial areas and subsequent dispersals and expansions to the areas inhabited in present times (Hewitt, 1999). Besides the critical importance of the main European peninsulas and Near Eastern mountain ranges for the evolution of the biotas in the Western Palearctic (Hewitt, 2011), existence of a dynamic array of numerous, smaller glacial refugia has been suggested to exist during the Pliocene and Pleistocene glacials (Kapli et al., 2013; Bellati et al., 2015; Stümpel et al., 2016; Van Riemsdijk et al., 2017). Slower extinction rates and population dispersals from these smaller refugia are

Department of Zoology, Comenius University in Bratislava, Mlynská dolina, Ilkovičova 6, 842 15 Bratislava, Slovakia

^{2 -} Hielscherstraße 25, 13158 Berlin, Germany

reflected in complex phylogeographic patterns and prominent genetic divergences, even within phenotypically very similar taxa (Gvoždík et al., 2010; Mikulíček et al., 2013; Kornilios, 2017). While the existence of refugia in the main European peninsulas, Eastern Mediterranean, and Caucasus is generally recognized as critical for the evolution of recent diversity of reptiles (Tuniyev, 1995; Stewart et al., 2010), the importance of the eastern parts of Western Palearctic, such as Anatolia, Iranian Mountain ranges, and Ponto-Caspian steppes, has only recently become more appreciated (Gvoždík et al., 2012; Jandzik, Avc1 and Gvoždík, 2013, 2018; Stümpel et al., 2016; Javanbakht et al., 2017).

One of the most common and widely distributed reptilian species across the Western Palearctic region, including its eastern parts (fig. 1; Sindaco, Venchi and Grieco, 2013), is

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the smooth snake, Coronella austriaca Laurenti, 1768 (Colubridae). Traditionally, it is considered to have a relatively uniform morphology (Engelmann, 1993), although some differentiation has been described in populations from Iberian Peninsula (Llorente et al., 2012). In contrast to many other reptiles from the same region, only two other subspecies with very limited distribution have been described so far: C. a. fitzingeri (Bonaparte, 1840) from southern Italy and Sicily that has usually been synonymized with the nominotypic subspecies (Speybroeck et al., 2016) and C. a. acutirostris Malkmus, 1995 from the Iberian Peninsula (Santos et al., 2008). The species thus represents an ideal candidate for comparisons of genetic diversity among the main speciation centers and refugial areas, and between the western and eastern parts of the Western Palearctic biogeographic region.



Figure 1. Geographic distribution of samples of the smooth snake (Coronella austriaca) used in our study with colors corresponding to the main recovered phylogenetic clades (for locality details see supplementary table S1). The distribution range of the species is highlighted in dark brown. Type locality of C. austriaca is Vienna, Austria (Laurenti, 1768), of C. a. fitzingeri Sicily, southern Italy (Bonaparte, 1840), and of C. a. acutirostris Lagoa Comprida/Serra da Estrela, Portugal (Malkmus, 1995). The pictured specimen originates from the locality No. 125, Nevesinje, Bosnia and Herzegovina.

So far, three studies analyzing mitochondrial DNA (mtDNA) sequence data of the smooth snake in a biogeographic framework have been published (Santos et al., 2008; Galarza, Mappes and Valkonen, 2015; Sztencel-Jabłonka et al., 2015). In each, the authors addressed particular regional phylogeographic or taxonomic questions, as well as analyzed samples collected from other parts of the range. Our study represents a follow-up of this research and significantly expands the geographic coverage with the ambition to analyze mtDNA diversity from across the entire range, including previously unsampled areas of the Near East. We combined the published data with newly obtained longer sequences of the commonly used molecular marker cytochrome b (cyt b), which allowed us to corroborate regionally recognized genetic diversity. We also reveal that the overall diversity is far more complex than previously thought and propose historical biogeographic scenarios. Our results provide the necessary preliminary data critical for a thorough taxonomic revision of the smooth snake.

Materials and methods

Study species and sampling

We investigated specimens of *C. austriaca* (45 individuals from 40 localities; supplementary table S1) from all biogeographically important parts of its wide range in the western Palearctic (fig. 1). Blood and saliva collected from live specimens and muscle from the dead animals (e.g. road-kills) were used as a DNA source. Tissue samples were either preserved in 96% ethanol or frozen and stored at -25 or -80° C. Few specimens have been collected and they are deposited in the Zoology Laboratory of the Department at Science and Arts Faculty, Adnan Mendres University, Turkey, Zoological Museum of National Museum of Natural History of National Academy of Sciences, Kyiv, Ukraine and Museum of Nature of Karazin Kharkiv National University, Kharkiv, Ukraine.

DNA and alignment preparation

For DNA extraction, we used either a standard phenolchloroform protocol followed by ethanol precipitation or various commercial DNA extraction kits (Qiagen DNeasy[®] Blood and Tissue Kit; NucleoSpin Tissue Kit, Macherey-Nagel). The complete mitochondrial DNA cytochrome *b* gene (cyt *b*) was amplified with primers L14910, L14919, and H16064 (Burbrink, Lawson and Slowinski, 2000; modified by De Queiroz, Lawson and Lemos-Espinal, 2002) using the following PCR program: 7 min denaturing step at 94°C followed by 40 cycles of denaturing for 40 s at 94°C, primer annealing for 30 s at 46-50°C, and elongation for 1 min at 72°C, with a final 7 min elongation step at 72°C. The same primers as for amplification were used for sequencing. Cyt b is the most common marker used in reptilian phylogeographic analyses and has also been successfully analyzed in previous phylogeographic studies of the smooth snake (Santos et al., 2008, 2012; Galarza, Mappes and Valkonen, 2015; Sztencel-Jabłonka et al., 2015). PCR products were purified using the ExoSAP-IT enzymatic clean-up (USB Europe GmbH, Staufen, Germany; manufacturer's protocol). The sequencing was performed by Macrogen Inc. (Seoul, South Korea or Amsterdam, Netherlands; http://www.macrogen.com), and new sequences have been deposited in GenBank under accession numbers MH382907-382950, and MH557091 (supplementary table S1). In our analyses, we combined our newly obtained sequences with those published in Gen-Bank and provided by Dr. Juan A. Galarza (University of Jyväskylä, Finland) that were previously used in Galarza, Mappes and Valkonen (2015) (supplementary table S1). Due to inconsistent lengths of the available sequences, we constructed two datasets: 1) the inclusive dataset, which combined all our newly obtained sequences of cyt b with all previously published sequences of variable length (285-1031 bp), 2) the exclusive dataset comprising all available sequences of the cyt b fragment of the length close to the complete cyt b (924-1031 bp), predominantly used for the first time in this study (supplementary table S1). We used the inclusive dataset in most analyses including phylogenetic tree, haplotype network (fig. 2), and phylogenetic network reconstructions (fig. 3), while both inclusive and exclusive datasets served for polymorphism and p-distance calculations (table 1, supplementary table S2).

Phylogenetic analyses

DNA sequences were manually checked, aligned, and inspected using BioEdit 7.0.9.0 (Hall, 1999). No stop codons were detected when the sequences were translated using the vertebrate mitochondrial genetic code in the program DnaSP 5.10 (Librado and Rozas, 2009). The same program was used to calculate uncorrected *p*-distances among the main clades, and to estimate the number of haplotypes (h), haplotype diversity (h_d) , number of segregating sites (S), nucleotide diversity (π) , and Watterson's theta per site (θ_{W}) . The best-fit codon-partitioning schemes and the best-fit substitution models were selected using PartitionFinder v1.1.1 (Search algorithm: all, branchlengths =linked; Lanfear et al., 2012), according to Bayesian information criterion (BIC). Phylogenetic trees for the long inclusive were inferred using the Bayesian approach (BA) and maximum likelihood (ML) by MrBayes 3.2 (Ronquist et al., 2012) and RAxML 8.0. (Stamatakis, 2014), respectively. The best-fit substitution model with each codon position treated separately for the BA analysis was as follows: 1031 bp GTR+G (1st), HKY+I+G (2nd), HKY+I (3rd) while



it was GTR+G in each codon position in the ML analysis. The ML clade support was assessed by 1000 bootstrap pseudoreplicates. MrBayes analysis was set as follows: two separate runs with four chains for each run, 10 million generations with samples saved every 100th generation. The convergence of the two runs was confirmed by the convergence diagnostics (average standard deviation of split frequencies, potential scale reduction factor). The first 20% of trees were discarded as the burn-in after inspection for stationarity of log-likelihood scores of sampled trees in Tracer 1.6 (Rambaut et al., 2013) (all parameters had effective sample size >200). A majority-rule consensus tree was drawn from the post-burn-in samples and posterior probabilities were calculated as the frequency of samples recovering any particular clade. A sequence of the congeneric species Coronella girondica (Daudin, 1803) (GenBank accession number AF471088; Lawson et al., 2005) was included as an outgroup in tree analyses.

Since network approach can sometimes generate more effective presentation of the intraspecific evolution than the tree-based phylogenetic approaches (Posada and Crandall, 2001), we also constructed median-joining haplotype networks for each mtDNA phylogenetic clade comprising at least two haplotypes in the software PopArt (http://popart. otago.ac.nz). We also built phylogenetic network using the NeighborNet algorithm (Bryant and Moulton, 2004) implemented in the software SplitsTree 4.10 (Huson and Bryant, 2006). This analysis has proven to be a very powerful tool for visualization conflicting and consistent information present in a sequence dataset (Huson and Bryant, 2006).

Results

For our main alignment (built form the inclusive dataset), we obtained a data matrix of 141 DNA sequences of 1031 bp long fragment of cyt b forming 65 separate haplotypes (supplementary table S2). This number might be underestimated given the fact that most of the previously published sequences included in our analyses were considerably shorter than the full alignment length. No signal of contamination or sequence of nuclear genomic origin was detected.

We have observed essentially identical topology of both ML and BA trees, thus only the BA tree is depicted with BA posterior probabilities and ML bootstrap values for each main branch (fig. 2). In total, the sequences clustered in 14 well-supported clades, some of them represented by single samples (Anatolian 2, Iranian 1, and Western 1 clades). Similar split pattern was recovered by SplitsTree analysis and is presented in the form of phylogenetic network (fig. 3).

All clades exhibit clear geographical pattern (fig. 1), and most of them also have conspicuous internal structure as can be seen on the phylogenetic tree, haplotype as well as phylogenetic networks (figs 2, 3). The clades comprising the most divergent phylogenetic lineages (Iberian 2, 3, Iranian 2, Anatolian 1, Balkan, Eastern) are characterized with high numbers of missing or extinct haplotypes (fig 2).

Unlike the main clades, the higher-order branches are generally not very well supported in BA tree and almost not at all in ML tree (fig. 2), presumably due to gaps that still exist in the sampling as well as heterogenous lengthcharacter of our alignment.

The western part of the range is inhabited by six clades (Iberian 1-3, Western 1-2, Sicilian), with the highest diversity observed in Iberian Peninsula. Two clades (Central European and Balkan) dominate in central and northern Europe, while the Eastern clade is distributed in large portions of the species range in the East, north of the Caucasus. Relatively high diversity can be found in Anatolia (Anatolian clades 1-2), Transcaucasia (Transcaucasian clade), and especially Iran, where populations belonging to two divergent clades occur in nearby areas south

Figure 2. Bayesian tree reconstructed from the inclusive dataset of mtDNA cyt b gene sequences (1031 bp; see Materials and methods, supplementary table S1), rooted with the sequence of congeneric sister species *C. girondica*. Numbers above the branches show posterior probabilities/maximum likelihood (ML) bootstrap support values. The scale bar corresponds to one substitution per one hundred nucleotide positions. Each terminal branch represents a sample with locality number, GenBank accession number, and name of the country or region of origin (see also fig. 1 and supplementary table S1). To the right of the main clades are median-joining haplotype networks. Symbol sizes reflect haplotype frequencies, small black circles are missing or extinct node haplotypes, and each internode connecting two haplotypes corresponds to one mutation step. Haplotype colors reflect the main mtDNA clades.



Figure 3. SplitsTree phylogenetic network (Huson and Bryant, 2006) reconstructed using the inclusive sequence dataset of mtDNA cyt b gene sequences (1031 bp) (see Materials and methods; supplementary table S1). Phylogenetic clade colors correspond to those used in figs 1 and 2.

of the Caspian Sea (Iranian clades 1-2; figs 1, 2, 3, supplementary table S2).

The SplitsTree analysis recovered the same clades as BA and ML trees, however, the relationships among lineages both within and among some clades (Eastern, Transcaucasian, and Iranian 2) are quite complex as indicated by high number of reticulation branches (fig. 3).

Relative genetic distances among the most clades of the smooth snake vary around 3-5% as expressed by uncorrected *p*-distances, with the maximum of 9.0% between the Western clade 2 and Iranian clade 1 (table 1). The lowest average intra-clade distance was recorded among the samples of the Western clade 1 (0.3%), however, the distance between the most divergent samples within the Balkan clade was 6.1%, which is far more than among samples of most of the main clades (table 1; see also the haplotype network on fig. 2). The *p*-distance between *C. austriaca* and its sister species *C. girondica* is 7.7%.

Discussion

Diversity and phylogeography

Previous studies of the smooth snake phylogeography (Santos et al., 2008; Galarza, Mappes and Valkonen, 2015; Sztencel-Jabłonka et al., 2015) distinguished between eight and ten phylogenetic clades with unresolved or uncertain relationships within four different parts of the distribution range (supplementary table S1). Santos et al. (2008) speculated that the limited number of higher branch supports might have resulted from low numbers of phylogenetically informative positions in obtained sequences (meaning the used fragments were too short) or from the fact that the split within lineages occurred during a very short period of time. Galarza, Mappes and Valkonen (2015) presented a better supported phylogenetic tree based on a 303 bp long cyt b fragment. However, we were not able to corroborate their results by analyzing a dataset of similar length, supplemented with newly obtained sequences

Table 1. Avera inclusive/exclus value of p-dista	ge percental kive datasets nce between	l uncorrected (see Material : 1 Western 2 an	<i>p</i> -distances and methods d Iranian 1 c	calculated an ; supplementa lades is highl	nong the cyt ary table S1). ighted in bol	<i>b</i> haplotype In diagonal d.	es of the ma (in italics) a	ain clades tre the aver	of <i>Corone</i> age and m	<i>illa austriac</i> aximum (in	a. The dista parentheses)	nces were cal intra-clade <i>p</i>	ulated separately for listances. The highest
<i>p</i> -distances %	Anatolian 1	Anatolian 2	Balkan	Central European	Eastern	Iberian 1	Iberian 2	Iberian 3	Iranian 1	Iranian 2	Sicilian T	ranscaucasian	Western 1 Western 2
Anatolia 1	1.0 (2.0)/ 0.9 (2.0)												
Anatolia 2	1.8/1.7												
Balkan	2.7/2.8	1.9/2.3	2.4 (6.1)/ 2.4 (6.1)										
Central European	4.0/3.0	1.5/1.6	3.7/3.2	0.4 (1.1)/ 0.2 (0.4)									
Eastern	3.2/3.2	2.2/3.4	2.7/3.5	2.8/2.9	1.4 (2.7)/ 1.2 (1.2)								
Iberian 1	3.3/3.0	3.2/4.4	2.4/3.2	3.2/2.9	3.0/4.2	1.3 (1.3)/ 1.1 (1.1)							
Iberian 2	3.3/-	5.4/-	2.9/-	3.0/-	3.9/-	3.9/-	1.9 (3.3)/-						
Iberian 3	3.6/-	4.1/-	3.0/-	3.7/-	3.8/-	3.2/-	5.0/-	1.8 (2.0)/					
Iranian 1	2.6/2.6	7.5/7.5	2.3/3.0	1.8/2.1	2.9/4.0	3.5/5.7	7.2/-	5.4/-					
Iranian 2	3.5/3.1	6.7/4.4	2.6/3.2	2.8/2.8	3.5/3.6	4.3/4.7	6.8/-	5.9/-	4.5/4.9	1.3 (1.3)/ 1.3 (1.3)			
Sicilian	3.1/-	4.6/-	2.4/-	2.7/-	3.1/-	2.5/-	4.9/-	3.5/-	5.1/-	5.3/-	0.7 (0.7)/-		
Transcau- casian	2.6/-	2.4/-	2.5/-	2.5/-	1.9/-	3.2/-	5.0/-	4.4/-	3.9/-	3.9/-	3.6/-	-/(6.1) 0.1	
Western 1	2.9/-	3.0/-	2.6/-	3.1/-	3.2/-	3.0/-	4.3/-	4.1/-	3.8/-	4.6/-	3.1/-	3.7/-	0.3 (0.3)/
Western 2	2.7/2.7	7.1/7.2	2.1/3.2	1.7/2.4	2.8/4.1	3.3/4.7	5.3/-	4.6/-	9.0/0.6	7.9/5.4	4.3/-	3.5/-	2.8/

from other parts of the range (not shown). Our datasets of longer cyt *b* sequences yielded significantly better results, though many nodes still remain unresolved (fig. 2), allowing us to only speculate about relationships among some of the main clades. This stresses the need for even better geographical coverage of the samples, as well as for employment of additional genetic markers, preferably from various loci, in future studies.

We recovered 14 divergent clades with varying internal structure (figs 2, 3). These clades roughly correspond to four geographic regions (i) Western Europe with Iberian and Italian Peninsulas, (ii) Central Europe, Balkans, and Anatolia, (iii) Crimea, Eastern Europe, European part of Russia, and Transcaucasia, and (iv) Iran. Santos et al. (2008), Galarza, Mappes and Valkonen (2015), and Sztencel-Jabłonka et al. (2015) have already identified some of these clades in past studies. For the first time, we report the existence of one Western European clade (2), along with Transcaucasian, Anatolian, and Iranian clades. One of the sequences from the well-supported Anatolian clade (AY486930; Nagy et al., 2004) was previously analyzed by Sztencel-Jabłonka et al. (2015), however, it was a part of a large polytomy unrelated to any other clade in their tree.

Our sampling from Iberian Peninsula does not contribute any new information to the detailed analysis of Santos et al. (2008) – we similarly found three divergent clades (Iberian 1-3), one of which (Iberian 1) corresponds to one of the two described subspecies – *C. a. acutirostris* (figs 2, 3) according to Santos et al. (2008). The other two Iberian clades (Iberian 2, 3) seem to be composed of more diversified populations than the Iberian clade 1 (figs 2, 3).

Similarly, our sparse sampling in Western Europe confirms relatively low structure in most of the range of the large, and widely distributed, western clade (Western 1), with the exception of one sample originating from central Italy (locality 42, MH382919; supplementary table S1). This new sample constitutes a sister lineage to

all remaining samples of the Western clade 1, therefore, we designate it as a separate clade (Western 2). Sicily is supposedly inhabited by another described, though not generally accepted, subspecies, C. a. fitzingeri, and our phylogenetic analyses confirm the previous results of Santos et al. (2008) that the samples from here indeed belong to a distinct clade (Sicilian). Surprisingly, however, it seems that this clade is more closely related to the Iberian clades (Iberian 3, though with very low support, fig. 2; see also fig. 3) than to the Western clade 2 from central Italy. Low sample numbers (two from Sicily, one from central Italy) only allow us to speculate whether the ranges of these lineages meet in southern parts of the Italian peninsula such as in Zamenis longissimus/lineatus and Hierophis viridiflavus/carbonarius (Salvi et al., 2017; Mezzasalma et al., 2018), or whether the split occurred between continental Italy and Sicily as is known in Emys orbicularis/trinacris, or Podarcis siculus/waglerianus (Capula, 1994; Fritz et al., 2005). Better sampling and detailed analysis from this biogeographically and taxonomically important region is needed to address these hypotheses.

All six clades described from Western Europe, i.e. Iberian 1-3, Western 1, 2, and Sicilian, cluster together in a relatively well-supported clade, however, our analyses were not able to reconstruct their mutual relationships with the exception of a well-supported sister relationship of both Western clades (figs 2, 3).

In the remaining parts of the range we corroborated the existence of multiple clades identified by our colleagues in previous studies and, thanks to filling several sampling gaps, we obtained a better picture of their overall distribution (fig. 1), relationships, and genetic structure (figs 2, 3). The Eastern clade is distributed in the Baltic region, European part of Russia, Caucasus, Abkhazia, and Crimea (fig. 1). The phylogenetic network (fig. 3) indicates the possible relationship of the Transcaucasian (and some Iranian) samples with this clade, but the supports for such arrangements in our tree analyses are low (fig. 2). The Transcaucasian clade from Armenia and Georgia shows high diversity with every one of four samples belonging to a distinct haplotype (fig. 2).

A single sample from Greece, which surprisingly clustered with the Western European clade in Sztencel-Jabłonka et al. (2015), belongs to a clade we call Balkan here, since the main portion of its range lies in the Balkan Peninsula; mainly in its eastern and southern parts (figs 1, 2). It is unclear where this clade meets the Eastern clade due to the vast gap in sampling in Ukraine, Belarus, Moldova, eastern Romania, and western Russia (fig. 1). In the North, the Balkan clade extends to eastern Poland and Slovakia, where it abuts the range of the Central-European clade. In the South, these two clades meet in the region of Montenegro and Bosnia and Herzegovina (fig. 1). Similar to the Western clade 1, the Central European clade (North Balkan clade in Sztencel-Jabłonka et al., 2015) is characterized by low diversity despite its large range extending from western Balkans across central Europe to Scandinavia (figs 1, 2). In contrast, the Balkan clade comprises at least three separate phylogenetic lineages with relatively well-supported relationships among them (figs 2, 3). Two samples from Montenegro, from the easternmost part of this clade's range, form a sister lineage to all remaining samples, which further cluster into two lineages. Surprisingly these two lineages do not seem to be separated geographically - samples of both were recorded from Poland, Slovakia, Romania, Bulgaria, and Macedonia (one additional in one of the lineages from southern Greece as well).

Our BA tree suggests that two clades from Anatolia could be related to the Balkan clade, however, this result is not confirmed by ML tree or network analyses (figs 2, 3). The distinction of the separate Anatolian clade 2 consisting of a single sample is somehow arbitrary here and is mainly based on its distance from the remaining samples as suggested by the phylogenetic network (fig. 3). However, it is clear that the relationships among the samples from Anatolia are more complex and the real diversity could be, in fact, significantly higher, especially given the fact that we lack sampling from the (presumably) isolated part of the range in the southern part of the Anatolian Diagonal (fig. 1).

The most interesting and novel results in our study come from Iran, despite the fact that we only had a chance to analyze three samples. Two of these samples form one clade (Iranian 2) presumably related to Transcaucasian and Eastern clades, though still very divergent (3.5-3.9% in *p*-distance; table 1, figs 2, 3). The last sample (Iranian clade 1), originating from the southeastern-most corner of the smooth snake range, is the most distant sample of all, with an unclear relationship to other clades. The phylogenetic network suggests it might be related to the Anatolian clades (fig. 3), but neither BA nor ML trees were able to resolve the polytomy on the very base of the tree, which includes this clade (fig. 2).

Given the wide distribution of the smooth snake that includes several refugial areas and speciation centers, it is not surprising that divergences between some clades are relatively deep. The mean *p*-distances among the clades in cyt b vary in the range of 1.5% to 9.0%(table 1), which in most cases roughly corresponds to the divergences seen in the congeneric species and closest relative Coronella girondica in the Iberian Peninsula (max. 6.82%; Santos et al., 2012). However, the highest genetic distance, found between the sample of the Western clade 2 and the Iranian clade 1, reaches 9.0%, which is equivalent to interspecific distances among some other snake species, i.e. Vipera ursinii/renardi (Gvoždík et al., 2012), species in the genus Zamenis (Jandzik, Avc1 and Gvoždík, 2013), or in the Natrix natrix complex (Kindler et al., 2013, 2017). Particularly, the latter species complex is a good example with similar phylogeographic patterns and levels of genetic divergence, in which more thorough studies involving employment of independent markers have recently resulted into the elevation of several forms to separate species (Pokrant et al., 2016; Kindler et al., 2017).

Historical biogeography

In general outlines, phylogeography and genetic structure of the smooth snake are consistent with the patterns observed in other Western Palearctic reptiles (Joger et al., 2007; Kindler et al., 2013). The basal split among the main clades is relatively old and presumably occurred around the time of the Messinian Salinity Crisis in late Miocene or in early Pliocene (4.3-5.8 Mya; Santos et al., 2008). Later, during the Pleistocene, the species retracted to all main European peninsular refugia, i.e. Iberian, Italian, and Balkan, as well as refugia in Anatolia, Transcaucasia, and the region south of the Caspian Sea (figs 1, 2). The pronounced genetic structure within populations of these areas (with uncertain situation in the Italian Peninsula, due to insufficient data) suggests the existence of smaller-scale refugia within the main ones. This is concordant with the model of refugia-withinrefugia (Gomez and Lunt, 2007), also proposed for many other Western Palearctic reptiles.

This complex phylogenetic structure and history of the smooth snake has already been described in substantial detail for the populations inhabiting the Iberian Peninsula, where diversification was presumably facilitated by repeated species retreats to higher altitudes surrounding the mountain chains during the warmer interglacials, which subsequently resulted in distribution range fragmentation and isolation of the populations (Santos et al., 2008).

Based on the results of our study, we can now draw a more detailed picture of the biogeographic history of the smooth snake in other parts of its range. The Central European clade, distributed from Scandinavia through central Europe to northwestern Balkans, might be partly formed by descendants of independent extra-Mediterranean refugium presumably located in the north-western Balkans, and partly by descendants from the refugium located south of the Alps, or north of the Italian Peninsula, the region known for its high endemism (Canestrelli et al., 2012; Schmitt and Varga, 2012). The star-like pattern seen in the haplotype network (fig. 2) and relatively low genetic variation within the Central European clade also indicates relatively fast expansion to these recently inhabited areas.

In contrast to relatively low diversity within the Central European clade are the clades from Balkans and Anatolia. They are characterized by higher diversity and deeper divergences reflected in more complex haplotype networks (see table 1, figs 2, 3). This might be partially attributed to more complex topography of the mountainous regions of Balkans and Anatolia, which could have had similar effect on the genetic structure of the smooth snake populations, as it has had on the slow worms of the genus Anguis in the Balkans (Jablonski et al., 2016). The most divergent samples of the Balkan clade are from a locality in Montenegro (126; fig. 1), which indicates the location closest to a potential glacial refugium. The remaining two lineages, with mutual sister relationships, presumably spread along very similar routes northward to Romania, Slovakia, and Poland, and southeastward to Macedonia and Bulgaria (and to Greece in case of one of the lineages; fig. 1). Presence of samples belonging to both lineages was recorded from all of the mentioned countries (except for Greece).

The impact of the topography on the evolutionary history and presence of *C. austriaca* is particularly illustrative in Anatolia, where the smooth snake occurs in the mountains of the Anatolian Diagonal, which also forms the southern limit of its distribution. The mountain ranges within the diagonal pass across Anatolia in northeast-southwestern orientation, and have played a well-recognized role of being an isolating barrier as well as speciation center (Nilson et al., 1990; Bilgin, 2011; Jandzik, Avci and Gvoždík, 2013; Kapli et al., 2013; Bellati et al., 2015; Stümpel et al., 2016). Based on the presence of relatively diversified populations in the mountains of the Anatolian Diagonal and north of it, it is possible that it served as a refugial area for the smooth snake populations with retracted ranges during glacials. Likewise, the high mountains might have prevented the species from expanding to southeastern Anatolia and further southeast towards the distribution ranges of both Iranian clades.

The results of Santos et al. (2008), Galarza, Mappes and Valkonen (2015), Sztencel-Jabłonka et al. (2015), and of our study show that, within the Eastern clade, the Latvian, Crimean, and Russian samples form a sister clade to one sample available from Abkhazia (fig. 2). This implies that large portions of the range of this clade were colonized relatively quickly, presumably from the Caucasian region, which is a wellknown diversity hot-spot (Tuniyev, 1995).

The most intriguing aspects of our study are our findings about the smooth snakes from Iran. The mountains south of the Caspian Sea harbor at least two separate Iranian clades occurring on the localities only about 150 km apart (figs 1-3). Origin of the deeply divergent Iranian clade 1 could possibly be traced back to the time of the basal split of the main lineages during the late Miocene, or close to it. Unfortunately, the unresolved relationships at the base of the smooth snake phylogeny (fig. 2) do not allow us to learn more about the history of this highly interesting clade and test whether its internal genetic structure correlates with its presumed old age. It would be very interesting, and informative for the old evolutionary history of the species, to obtain deeper insights into the relationships of the Iranian populations with those from Transcaucasia and Anatolia.

The second clade (Iranian clade 2) is both phylogenetically and geographically closely related to the Transcaucasian clade (figs 1–3). Since the Transcaucasian clade and the Iranian clade 2 form a common higher-order clade sister to the Eastern clade, it is plausible to hypothesize that the common ancestor of these clades originated from the region of Caucasus or Transcaucasia and subsequently dispersed both northward (Eastern clade) and southward (Iranian clade 1).

Implications for the smooth snake taxonomy

The evolutionary history of a particular species should be reflected in its taxonomy (Burbrink, Lawson and Slowinski, 2000). Translating the obtained picture of genetic diversity, phylogenetic relationships, and divergences into the formal taxonomic arrangement with appropriate nomenclature is a challenging task depending on criteria used for the species or subspecies delimitation (De Queiroz, 2007; Stümpel et al., 2016). In the case of separate species, this is particularly complicated during the transition time when the populations diverge into distinct clades, but before they evolve the isolating reproductive mechanisms (Hey, 2009; Stümpel et al., 2016). Recognition of a subspecies is even more prone to subjective interpretation based on vaguely defined criteria and, as a result, the subspecies do not represent evolutionary entities in many cases, but merely arbitrarily selected sections of clines (Mayr, 1982; Burbrink, Lawson and Slowinski, 2000; Braby et al., 2012). However, in the case of many Western Palearctic reptiles (and amphibians), subspecific taxonomy usually complies with the mtDNA differentiation, and inconsistences can often be explained by low-quality taxonomy or a lack of morphological differentiation (Kindler et al., 2013). If certain criteria are met, such as allopatric distribution, phenotypic distinction, and at least one fixed diagnosable character state, the subspecies might be considered to represent an evolutionary significant unit (Braby et al., 2012).

Identification of multiple separate and deeply divergent phylogenetic lineages with possibly long independent evolutionary histories, challenges the existing taxonomy of *C. austriaca*. However, for the time being, our results should be treated carefully and considered preliminary due to the limited nature of the data our inference is based on. Therefore, we refrain from drawing any taxonomic or nomenclatural conclusions, before different and independent lines of evidence (nuclear DNA data, morphology, ecology etc.) become available, and will only limit ourselves to a brief discussion of possible implications based on our results obtained from mtDNA sequences.

The type locality of the species (Vienna; Laurenti, 1768) presumably lies within the distribution range of the Central European clade, distributed from north-western Balkans in the south through central Europe to Scandinavia in the north (fig. 1). This lineage should thus correspond to the nominotypic subspecies. Subspecies C. a. fitzingeri was described from Sicily, where Santos et al. (2008) indeed identified a distinct mtDNA clade we call "Sicilian" here. The last subspecies, C. a. acutirostris, is an endemic of the Iberian Peninsula (Malkmus, 1995; Santos et al., 2008) and, surprisingly, it seems that two different mtDNA clades (Iberian 1 and 3) occur in the vicinity of the type locality in Portugal (fig. 1). Uncorrected *p*-distances among these three (four if both Iberian clades are considered) clades vary between 2.5-3.5% in the analyzed cyt b fragment, which is very similar to what is known in several other Western Palearctic polytypic snake species, e.g. Ursenbacher et al. (2008) and Kindler et al. (2013). These distances do not stand out among the distances separating other clades, which implies that either they are not sufficient to justify subspecies recognition, or alternatively, most, or all, of the identified mtDNA clades might represent separate subspecies (if further diagnostic criteria are met; see above). Three Iberian clades (1-3) also show clear signs of phenotypic differentiation (Llorente et al., 2012), which offers independent evidence justifying separation of C. a. acutirostris and two other subspecies, presently without assigned names. Whether this is the case for the clades from other parts of the range, including C. a. fitzingeri, still needs to be determined. Interestingly, the *p*-distances from the upper side of their range reach the values of >5.0%, with the maximum of 9.0% (between Western clade 2 and Iranian clade 1), which is

even higher than genetic distance between *C. austriaca* and its congeneric sister species *C. girondica* (7.7%). This opens possibilities that some of the mtDNA lineages, particularly the Iranian clade 1, might in fact represent separate species within the *C. austriaca* complex. Better characterization of the evolutionary clades and disentangling their complicated relationships is the next necessary step in building the solid groundwork critical for robust taxonomy reflecting the true evolutionary history of the smooth snake. This can only be achieved with additional data and further analyses.

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Supplementary material. Supplementary material is available online at:

https://figshare.com/s/a0d7d70767158c28de6a

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Gen Ban k Accession Number	Internal id	Locality number	Locality name	Country	z	ш	Gade	Source	Haplotype in published studies	Santos et al., 2008 clade	Sztencel Jabłonka et al., 2015 clade	Galarza et al., 2015 clade	Dataset
EU022662	E2026.1	1	Serra da Estrela	Portugal	40.30	-7.63	Iberian 1	Santos et al., 2008		Clade 1 (C. a. acutirostris)	Clade 1 Santos	Western	inclusive
EU022664	E2026.2	1	Serra da Estrela	Portugal	40.30	-7.63	Iberian 1	Santos et al., 2008		Clade 1 (C. a. acutirostris)	Clade 1 Santos	Western	inclusive
EU022663	E2026.7	2	Northern Portugal	Portugal	41.71	-7.65	Iberian 1	Santos et al., 2008		Clade 1 (C. a. acutirostris)	Clade 1 Santos	Western	inclusive
EU022668	E2026.8	2	Northern Portugal	Portugal	41.71	-7.65	Iberian 1	Santos et al., 2008		Clade 1 (C. a. acutirostris)	Clade 1 Santos	Western	inclusive
E0022669	E27104.1	3	Pitoes	Portugal	41.84	-7.95	Iberian 1	Santos et al., 2008		Clade 1 (C. a. acutirostris)	Clade 1 Santos	Western	inclusive
MU382011	2475	4	Portela de Homen	Portugal	40.52	-7.00	Iberian 1	This study		Clade 3	Clade 1 Santos	Western	inclusive/oxclusivo
MH382915	649 JAB	6	Senhora da Boa Estrela	Portugal	40.32	-7.60	Iberian 1	This study		Clade 1 (C. a. acutirostris)	Clade 1 Santos	Western	inclusive/exclusive
EU022652	E27104.4	7	Sierra Nevada, Granada	Spain	37.09	-3.40	Iberian 3	Santos et al., 2008		Clade 3	Clade 3 Santos	Western	inclusive
EU022653	E2007.1	7	Sierra Nevada, Granada	Spain	37.09	-3.40	Iberian 3	Santos et al., 2008		Clade 3	Clade 3 Santos	Western	inclusive
EU022655	E18124.1	8	Canejan, Lleida	Spain	42.84	0.74	Iberian 3	Santos et al., 2008		Clade 3	Clade 3 Santos	Western	inclusive
EU022656	E2606.1	9	Arguijo, Soria	Spain	41.99	-2.50	Iberian 3	Santos et al., 2008		Clade 3	Clade 3 Santos	Western	inclusive
EU022657	E2606.3	10	Puerto Piqueras, Soria	Spain	42.06	-2.45	Iberian 3	Santos et al., 2008		Clade 3	Clade 3 Santos	Western	inclusive
EU022658	E3010.5	11	Puertos de Beceite, Castellón	Spain	40.82	0.18	Iberian 3	Santos et al., 2008		Clade 3	Clade 3 Santos	Western	inclusive
EU022659	E27014.5	12	Valle de Anson, Navarra	Spain	42.76	-0.83	Iberian 3	Santos et al., 2008		Clade 3	Clade 3 Santos	Western	inclusive
EU022660	E2505.2	13	Lasarte, Alava	Spain	42.83	-2.69	Iberian 3	Santos et al., 2008		Clade 3	Clade 3 Santos	Western	inclusive
EU022661	E2026.4	14	Escoriaza, Alava	Spain	42.86	-2.68	Iberian 3	Santos et al., 2008		Clade 3	Clade 3 Santos	Western	inclusive
EU022665	E3010.3	15	Tendi Valley, Asturias	Spain	43.25	-5.98	Iberian 1	Santos et al., 2008		Clade 1 (C. a. acutirostris)	Clade 1 Santos	Western	inclusive
EU022666	E2026.3	16	Covanera, Burgos	Spain	42.74	-3.80	Iberian 1	Santos et al., 2008		Clade 1 (C. a. acutirostris)	Clade 1 Santos	Western	inclusive
EU022667	E2026.11	17	Sedano, Burgos	Spain	42.72	-3.75	Iberian 1	Santos et al., 2008		Clade 1 (C. a. acutirostris)	Clade 1 Santos	Western	inclusive
EU022672	E1406.30	18	El Espinero, Segovia	Spain	40.72	-4.25	Iberian 2	Santos et al., 2008		Clade 2	Clade 2 Santos	Western	inclusive
EU022673	E2026.5	19	Sierra de Guadarrama, Madrid	Spain	40.67	-4.09	Iberian 2	Santos et al., 2008		Clade 2	Clade 2 Santos	Western	inclusive
EU022674	E2026.6	20	Montes de Toledo, Toledo	Spain	39.55	-4.33	Iberian 2	Santos et al., 2008		Clade 2	Clade 2 Santos	Western	inclusive
EU022675	E2405.2	21	Riopar, Albacete	Spain	38.50	-2.42	Iberian 2	Santos et al., 2008		Clade 2	Clade 2 Santos	Western	inclusive
JQ904297	DB2682	22	Miraflores de la Sierra, Madrid	Spain	40.82	-3.77	Iberian 2	Santos et al., 2012		Clade 2	Clade 2 Santos	Western	inclusive
KM435300	FRROUFFA53-57	23	Bollenberg	France	47.96	7.06	Western 1	Galarza et al., 2015		no name	Western	Western	inclusive
KM435301	FRROUFFA55	23	Bollenberg	France	47.96	7.06	Western 1	Galarza et al., 2015		no name	Western	Western	inclusive
EU022649	E1511.9	24	Tigouleix, Creuse	France	46.04	2.06	Western 1	Santos et al., 2008		no name	Western	Western	inclusive
EU022650	E1110.10	24	Merindal, Creuse	France	46.04	2.06	Western 1	Santos et al., 2008		no name	Western	Western	inclusive
EU022654	E3010.4	25	Canigou	France	42.52	2.46	Iberian 3	Santos et al., 2008		Clade 3	Clade 3 Santos	Western	inclusive

	UKARNE0019-21	26	Dorset	United Kingdom	50.69	-2.04	Western 1	Galarza et al., 2015	no name	Western	Western	inclusive
	UKGODLIN22-25	27	Dorset	United Kingdom	50.65	-2.05	Western 1	Galarza et al., 2015	no name	Western	Western	inclusive
	UKPUDDLE17, 18	28	Dorset	United Kingdom	50.73	-2.37	Western 1	Galarza et al., 2015	no name	Western	Western	inclusive
	UKWAREHA26-32	29	Dorset	United Kingdom	50.72	-2.15	Western 1	Galarza et al., 2015	no name	Western	Western	inclusive
EU022648	E27104.2	30	Hampshire	United Kingdom	51.16	-0.20	Western 1	Santos et al., 2008	no name	Western	Western	inclusive
EU022651	E27104.0	31	Vorarlberg	Austria	47.25	9.98	Western 1	Santos et al., 2008	no name	Western	Western	inclusive
	GERBRACH36	32	Brachttal	Germany	50.20	9.15	Western 1	Galarza et al., 2015	no name	Western	Western	inclusive
	GERPARTE33	33	Partenstein	Germany	50.04	9.52	Western 1	Galarza et al., 2015	no name	Western	Western	inclusive
	GERSCHLI35	34	Schlierbach	Germany	49.31	8.68	Western 1	Galarza et al., 2015	no name	Western	Western	inclusive
	GERSCHWA41	35	Berkhof	Germany	52.41	9.70	Western 1	Galarza et al., 2015	no name	Western	Western	inclusive
	SWMUNCHE39	36	Münchenstein	Switzerland	47.52	7.56	Western 1	Galarza et al., 2015	no name	Western	Western	inclusive
	ITMILANO44	37	Milano	Italy	45.46	9.19	Western 1	Galarza et al., 2015	no name	Western	Western	inclusive
	ITPAVIA037	38	Pavia	Italy	45.19	9.16	Western 1	Galarza et al., 2015	no name	Western	Western	inclusive
EU022646	E3010.1	39	Treviso	Italy	45.67	12.24	Central European	Santos et al., 2008	no name	North Balkan	Central	inclusive
EU022670	E3026.16	40	Etna, Adrano, Sicily	Italy	37.72	14.94	Sicilian	Santos et al., 2008	C. a. fitzingeri (no other name)	no name	Western	inclusive
EU022671	E3026.17	41	Etna, Saifio, Sicily	Italy	37.72	14.94	Sicilian	Santos et al., 2008	<i>C. a. fitzingeri</i> (no other name)	no name	Western	inclusive
MH382919	1067JAB	42	Monte Rufeno	Italy	42.80	11.91	Western 2	This study	not recovered	not recovered	not recovered	inclusive/exclusive
	NORAUREB05	43	Aurebekk	Norway	58.14	7.70	Central European	Galarza et al., 2015	no name	North Balkan	Central	inclusive
	NORHOLME02,03,06	44	Holmesland	Norway	58.13	7.50	Central European	Galarza et al., 2015	no name	North Balkan	Central	inclusive
	NORLANGE04, NORSODEL07	45	Langeland, Sodeland	Norway	58.08	7.51	Central European	Galarza et al., 2015	no name	North Balkan	Central	inclusive
	SWEOREBR09-14	46	Örebro	Sweden	59.38	15.01	Central European	Galarza et al., 2015	no name	North Balkan	Central	inclusive
	SWEOSTER15,16	47	Örebro	Sweden	58.64	16.66	Central European	Galarza et al., 2015	no name	North Balkan	Central	inclusive
KM435298	ALFINSTR01,04,05,08- 13,42,45,46,60, ALHAMMAR39,40,43,44, ALLANGNA43	48	Åland, Finström	Finland	60.22	19.83	Central European	Galarza et al., 2015	no name	North Balkan	Central	inclusive
	ALFINSTR02	48	Åland, Finström	Finland	60.22	19.83	Central European	Galarza et al., 2015	no name	North Balkan	Central	inclusive
	ALFINSTR02 ALFINSTR03	48 48	Åland, Finström Åland, Finström	Finland Finland	60.22 60.22	19.83 19.83	Central European Central European	Galarza et al., 2015 Galarza et al., 2015	no name no name	North Balkan North Balkan	Central Central	inclusive inclusive
	ALFINSTR02 ALFINSTR03 ALHAMMAR14	48 48 49	Åland, Finström Åland, Finström Åland, Finström	Finland Finland Finland	60.22 60.22 60.17	19.83 19.83 19.80	Central European Central European Central European	Galarza et al., 2015 Galarza et al., 2015 Galarza et al., 2015	no name no name no name	North Balkan North Balkan North Balkan	Central Central Central	inclusive inclusive inclusive
	ALFINSTR02 ALFINSTR03 ALHAMMAR14 ALHAMMAR29-31,37,38	48 48 49 50	Åland, Finström Åland, Finström Åland, Finström Åland, Finström	Finland Finland Finland Finland	60.22 60.22 60.17 60.26	19.83 19.83 19.80 19.71	Central European Central European Central European Central European	Galarza et al., 2015 Galarza et al., 2015 Galarza et al., 2015 Galarza et al., 2015	no name no name no name no name	North Balkan North Balkan North Balkan North Balkan	Central Central Central Central	inclusive inclusive inclusive inclusive
	ALFINSTR03 ALFINSTR03 ALHAMMAR14 ALHAMMAR29-31,37,38 ALHAMMAR49	48 48 49 50 51	Åland, Finström Åland, Finström Åland, Finström Åland, Hammarland	Finland Finland Finland Finland Finland	60.22 60.22 60.17 60.26 60.15	19.83 19.83 19.80 19.71 19.78	Central European Central European Central European Central European	Galarza et al., 2015 Galarza et al., 2015 Galarza et al., 2015 Galarza et al., 2015 Galarza et al., 2015	no name no name no name no name no name	North Balkan North Balkan North Balkan North Balkan North Balkan	Central Central Central Central Central	inclusive inclusive inclusive inclusive inclusive
	ALFINSTR02 ALFINSTR03 ALHAMMAR14 ALHAMMAR29-31,37,38 ALHAMMAR49 ALHAMMAR52,57	48 49 50 51 52	Aland, Finström Aland, Finström Aland, Finström Aland, Finström Aland, Hammarland Aland, Hammarland	Finland Finland Finland Finland Finland Finland	60.22 60.22 60.17 60.26 60.15 60.22	19.83 19.83 19.80 19.71 19.78 19.74	Central European Central European Central European Central European Central European	Galarza et al., 2015 Galarza et al., 2015	no name no name no name no name no name	North Balkan North Balkan North Balkan North Balkan North Balkan	Central Central Central Central Central Central	inclusive inclusive inclusive inclusive inclusive inclusive
	ALFINSTR02 ALFINSTR03 ALHAMMAR14 ALHAMMAR29-31,37,38 ALHAMMAR49 ALHAMMAR52,57 ALINGBY006, ALJOMALA32,33	48 49 50 51 52 53	Aland, Finström Aland, Finström Aland, Finström Aland, Finström Aland, Hammarland Aland, Hammarland Aland, Jomala	Finland Finland Finland Finland Finland Finland	60.22 60.22 60.17 60.26 60.15 60.22 60.17	19.83 19.83 19.80 19.71 19.78 19.74 19.94	Central European Central European Central European Central European Central European Central European	Galarza et al., 2015 Galarza et al., 2015	no name no name no name no name no name no name	North Balkan North Balkan North Balkan North Balkan North Balkan North Balkan	Central Central Central Central Central Central Central	inclusive inclusive inclusive inclusive inclusive inclusive
	ALFINSTR02 ALFINSTR03 ALHAMMAR14 ALHAMMAR29-31,37,38 ALHAMMAR49 ALHAMMAR52,57 ALINGBY006, ALJOMALA32,33 ALJOMALA15-18,20-28	48 49 50 51 52 53 54	Aland, Finström Aland, Finström Aland, Finström Aland, Finström Aland, Hammarland Aland, Hammarland Aland, Jomala Aland, Jomala	Finland Finland Finland Finland Finland Finland Finland	60.22 60.17 60.26 60.15 60.22 60.17 60.17	19.83 19.80 19.71 19.78 19.74 19.94 19.92	Central European Central European Central European Central European Central European Central European	Galarza et al., 2015 Galarza et al., 2015	no name	North Balkan North Balkan North Balkan North Balkan North Balkan North Balkan North Balkan	Central Central Central Central Central Central Central Central	inclusive inclusive inclusive inclusive inclusive inclusive inclusive
	ALFINSTR02 ALFINSTR03 ALHAMMAR14 ALHAMMAR29-31,37,38 ALHAMMAR49 ALHAMMAR52,57 ALINGBY006, ALJOMALA32,33 ALJOMALA35,61,62	48 48 50 51 52 53 53 54	Aland, Finström Aland, Finström Aland, Finström Aland, Finström Aland, Hammarland Aland, Hammarland Aland, Jomala Aland, Jomala	Finland Finland Finland Finland Finland Finland Finland Finland	60.22 60.22 60.26 60.26 60.22 60.22 60.17 60.14	19.83 19.83 19.80 19.71 19.78 19.74 19.94 19.92 19.95	Central European Central European Central European Central European Central European Central European Central European	Galarza et al., 2015 Galarza et al., 2015	no name no name no name no name no name no name no name no name	North Balkan North Balkan North Balkan North Balkan North Balkan North Balkan North Balkan North Balkan	Central Central Central Central Central Central Central Central Central	inclusive inclusive inclusive inclusive inclusive inclusive inclusive inclusive
	ALFINSTR02 ALFINSTR03 ALHAMMAR14 ALHAMMAR29-31,37,38 ALHAMMAR49 ALHAMMAR52,57 ALINGBY006, ALJOMALA32,33 ALJOMALA35,61,62 ALJOMALA55,61,62 ALLEMLAN36	48 49 50 51 52 53 54 55 56	Aland, Finström Aland, Finström Aland, Finström Aland, Finström Aland, Hammarland Aland, Hammarland Aland, Jomala Aland, Jomala Aland, Jomala Aland, Jomala	Finland Finland Finland Finland Finland Finland Finland Finland Finland	60.22 60.17 60.26 60.15 60.22 60.17 60.14 60.15 60.07	19.83 19.83 19.80 19.71 19.78 19.74 19.94 19.92 19.95 20.09	Central European Central European Central European Central European Central European Central European Central European Central European	Galarza et al., 2015 Galarza et al., 2015	no name	North Balkan North Balkan North Balkan North Balkan North Balkan North Balkan North Balkan North Balkan North Balkan	Central Central Central Central Central Central Central Central Central Central	inclusive inclusive inclusive inclusive inclusive inclusive inclusive inclusive inclusive

	ALSALTVI48, 51, 59	58	Åland, Saltvik	Finland	60.28	20.06	Central European	Galarza et al., 2015		no name	North Balkan	Central	inclusive
	ALSUND0007, 35, 47	59	Åland, Sund	Finland	60.25	20.11	Central European	Galarza et al., 2015		no name	North Balkan	Central	inclusive
	ALSUND0019	60	Åland, Sund	Finland	60.27	20.24	Central European	Galarza et al., 2015		no name	North Balkan	Central	inclusive
	ALSUND0034, 50, 53, 54, 58	61	Åland, Sund	Finland	60.25	20.11	Central European	Galarza et al., 2015		no name	North Balkan	Central	inclusive
KM435299	ALJOMALA56	62	Åland, Jomala	Finland	60.15	19.95	Central European	Galarza et al., 2015		no name	North Balkan	Central	inclusive
	POLKRAKO43	63	Krakow	Poland	50.06	19.94	Central European	Galarza et al., 2015		no name	North Balkan	Central	inclusive
KP756615	5923, 5924, 7399	64		Poland	52.55	19.23	Central European	Sztencel-Jabłonka et al., 2015	า1	no name	North Balkan	Central	-
KP756615	7392	65		Poland	52.55	19.23	Central European	Sztencel-Jabłonka et al., 2015	า1	no name	North Balkan	Central	-
KP756615	7393	66		Poland	52.60	19.09	Central European	Sztencel-Jabłonka et al., 2015	า1	no name	North Balkan	Central	
KP756615	7448, 7450, 7451, 8269	67		Poland	52.56	19.21	Central European	Sztencel-Jabłonka et al., 2015	า1	no name	North Balkan	Central	-
KP756615	7449	68		Poland	52.59	19.19	Central European	Sztencel-Jabłonka et al., 2015	า1	no name	North Balkan	Central	
KP756615	5493	69		Poland	52.77	14.30	Central European	Sztencel-Jabłonka et al., 2015	า1	no name	North Balkan	Central	-
KP756615	6606-6608	70		Poland	53.37	14.86	Central European	Sztencel-Jabłonka et al., 2015	า1	no name	North Balkan	Central	
KP756615	6611	71		Poland	53.39	14.70	Central European	Sztencel-Jabłonka et al., 2015	า1	no name	North Balkan	Central	-
KP756615	7430	72		Poland	53.41	14.71	Central European	Sztencel-Jabłonka et al., 2015	า1	no name	North Balkan	Central	
KP756615	6604, 6605	73		Poland	51.71	14.86	Central European	Sztencel-Jabłonka et al., 2015	า1	no name	North Balkan	Central	-
KP756615	6591-6593	74		Poland	51.48	15.36	Central European	Sztencel-Jabłonka et al., 2015	า1	no name	North Balkan	Central	
KP756615	7412-7418	75		Poland	51.48	15.38	Central European	Sztencel-Jabłonka et al., 2015	า1	no name	North Balkan	Central	-
KP756615	5838, 5912, 6573-6582	76		Poland	49.41	20.40	Central European	Sztencel-Jabłonka et al., 2015	า1	no name	North Balkan	Central	
KP756615	6572, 7481	77		Poland	49.54	20.40	Central European	Sztencel-Jabłonka et al., 2015	า1	no name	North Balkan	Central	-
KP756615	6601	78		Poland	49.55	20.66	Central European	Sztencel-Jabłonka et al., 2015	า1	no name	North Balkan	Central	
KP756615, KP756618	7457-7480	79		Poland	49.41	20.39	Central European	Sztencel-Jabłonka et al., 2015 7	n1, h8 (7457, 7458)	no name	North Balkan	Central	
KP756622	7457, 7458	79		Poland	49.41	20.39	Central European	Sztencel-Jabłonka et al., 2015	า8	no name	North Balkan	Central	inclusive
KP756615	5007	80		Poland	50.01	19.81	Central European	Sztencel-Jabłonka et al., 2015	า1	no name	North Balkan	Central	-
KP756615	5250	81		Poland	50.04	19.91	Central European	Sztencel-Jabłonka et al., 2015	า1	no name	North Balkan	Central	
KP756615	5922	82		Poland	50.66	17.92	Central European	Sztencel-Jabłonka et al., 2015	า1	no name	North Balkan	Central	-
KP756615	6597	83		Poland	50.57	19.54	Central European	Sztencel-Jabłonka et al., 2015	า1	no name	North Balkan	Central	-

KP756615	6610	84	Poland	50.74	18.09	Central European	Sztencel-Jabłonka et al., 2015	h1	no name	North Balkan	Central	-
KP756615	8127	85	Poland	50.04	19.90	Central European	Sztencel-Jabłonka et al., 2015	h1	no name	North Balkan	Central	-
KP756615	8268	86	Poland	50.04	19.91	Central European	Sztencel-Jabłonka et al., 2015	h1	no name	North Balkan	Central	-
KP756615	8128, 8343	87	Poland	50.56	16.43	Central European	Sztencel-Jabłonka et al., 2015	h1	no name	North Balkan	Central	-
KP756616	5496-5499, 5916, 5919, 5920	88	Poland	49.42	22.44	Balkan	Sztencel-Jabłonka et al., 2015	h2	no name	no name	Central	inclusive
KP756617	5008, 5009, 5635, 5636, 5643, 5917, 5918, 6823	89	Poland	50.51	22.79	Balkan	Sztencel-Jabłonka et al., 2015	h3	no name	no name	Central	inclusive
KP756617	5494, 5495	90	Poland	51.65	21.85	Balkan	Sztencel-Jabłonka et al., 2015	h3	no name	no name	Central	-
KP756617	5634	91	Poland	50.48	22.95	Balkan	Sztencel-Jabłonka et al., 2015	h3	no name	no name	Central	-
KP756617	5637	92	Poland	50.51	22.79	Balkan	Sztencel-Jabłonka et al., 2015	h3	no name	no name	Central	-
KP756617	5638-5640, 5839, 5841, 5842, 5844, 5845, 6825- 6827, 6829-6833	93	Poland	50.47	22.99	Balkan	Sztencel-Jabłonka et al., 2015	h3	no name	no name	Central	-
KP756617	5641, 5642	94	Poland	50.37	23.08	Balkan	Sztencel-Jabłonka et al., 2015	h3	no name	no name	Central	-
KP756617	5840	95	Poland	50.46	22.95	Balkan	Sztencel-Jabłonka et al., 2015	h3	no name	no name	Central	-
KP756617	5843	96	Poland	50.48	23.05	Balkan	Sztencel-Jabłonka et al., 2015	h3	no name	no name	Central	-
KP756617	6824	97	Poland	50.52	22.88	Balkan	Sztencel-Jabłonka et al., 2015	h3	no name	no name	Central	-
KP756618	6281	98	Poland	50.85	17.87	Central European	Sztencel-Jabłonka et al., 2015	h4	no name	North Balkan	Central	inclusive
KP756620	6609	99	Poland	53.37	14.86	Central European	Sztencel-Jabłonka et al., 2015	h6	no name	North Balkan	Central	inclusive
KP756621	6587	100	Poland	52.28	14.63	Central European	Sztencel-Jabłonka et al., 2015	h7	no name	North Balkan	Central	inclusive
KP756621	6588	101	Poland	52.28	14.63	Central European	Sztencel-Jabłonka et al., 2015	h7	no name	North Balkan	Central	-
KP756621	6589	102	Poland	52.28	14.63	Central European	Sztencel-Jabłonka et al., 2015	h7	no name	North Balkan	Central	-
KP756621	6590	103	Poland	52.49	14.82	Central European	Sztencel-Jabłonka et al., 2015	h7	no name	North Balkan	Central	-
KP756621	6603	104	Poland	52.74	14.71	Central European	Sztencel-Jabłonka et al., 2015	h7	no name	North Balkan	Central	-
KP756621	7419-7421	105	Poland	52.26	14.72	Central European	Sztencel-Jabłonka et al., 2015	h7, h8 (7420)	no name	North Balkan	Central	-
KP756621	7423	106	Poland	52.27	14.75	Central European	Sztencel-Jabłonka et al., 2015	h7	no name	North Balkan	Central	-
KP756621	7424-7426, 7428	107	Poland	52.26	14.68	Central European	Sztencel-Jabłonka et al., 2015	h7	no name	North Balkan	Central	-
KP756621	7429	108	Poland	52.26	14.69	Central European	Sztencel-Jabłonka et al., 2015	h7	no name	North Balkan	Central	-
KP756619	6598-6600	109	Czech Republic	50.35	13.02	Central European	Sztencel-Jabłonka et al., 2015	h5	no name	North Balkan	Central	inclusive

MH382913	2487	110	Řadovy	Czech Republic	49.63	14.28	Central European	This study	no name	North Balkan	Central	inclusive/exclusive
MH382918	1056JAB	111	Studenec	Czech Republic	49.22	16.06	Central European	This study	no name	North Balkan	Central	inclusive/exclusive
MH382930	2404JAB	112	Hoštětín	Czech Republic	49.05	17.87	Central European	This study	no name	North Balkan	Central	inclusive/exclusive
KP756617	SLOH3	113	SE Slovakia	Slovakia	48.96	22.02	Balkan	Sztencel-Jabłonka et al., 2015 h3	no name	no name	Central	-
MH382907	1515	114	Borský Mikuláš	Slovakia	48.63	17.21	Central European	This study	no name	North Balkan	Central	inclusive/exclusive
MH382908	1605	115	Jasenie	Slovakia	48.89	19.43	Central European	This study	no name	North Balkan	Central	inclusive/exclusive
MH557091	1709	116	Svetlice	Slovakia	49.19	22.05	Balkan	This study	no name	no name	Central	inclusive/exclusive
MH382909	1713	117	Divina	Slovakia	49.27	18.70	Central European	This study	no name	North Balkan	Central	inclusive/exclusive
MH382910	2393	118	Beňuš-Gašparovo	Slovakia	48.83	19.74	Central European	This study	no name	North Balkan	Central	inclusive/exclusive
MH382921	1450JAB	119	NPR Šúr	Slovakia	48.23	17.21	Central European	This study	no name	North Balkan	Central	inclusive/exclusive
	SLOSLEN038	120	Selnica	Slovenia	46.55	15.49	Central European	Galarza et al., 2015	no name	North Balkan	Central	inclusive
MH382925	2073JAB	121	NP Triglav	Slovenia	46.44	13.77	Central European	This study	no name	North Balkan	Central	inclusive/exclusive
MH382926	2075JAB	122	Trenta	Slovenia	46.39	13.75	Central European	This study	no name	North Balkan	Central	inclusive/exclusive
MH382927	2076JAB	122	Trenta	Slovenia	46.39	13.75	Central European	This study	no name	North Balkan	Central	inclusive/exclusive
	YUGZARA034	123	Zadar	Croatia	44.12	15.25	Central European	Galarza et al., 2015	no name	North Balkan	Central	inclusive
KP756615	6594-6596	124		Croatia	45.33	17.02	Central European	Sztencel-Jabłonka et al., 2015	no name	North Balkan	Central	inclusive
MH382912	2479	125	Nevesinje	Bosnia and Herzegovina	43.24	18.09	Central European	This study	no name	North Balkan	Central	inclusive/exclusive
MH382923	2037JAB	126	Durmitor Mts.	Montenegro	43.13	18.95	Balkan	This study	no name	no name	Central	inclusive/exclusive
MH382924	2038JAB	126	Durmitor Mts.	Montenegro	43.13	18.95	Balkan	This study	no name	no name	Central	inclusive/exclusive
KM435304	MACEDONI42	127	Resen	Macedonia	41.09	21.01	Balkan	Galarza et al., 2015	no name	no name	Central	inclusive
MH382917	1014JAB	128	Korab Mts.	Macedonia	41.68	20.53	Balkan	This study	no name	no name	Central	inclusive/exclusive
MH382922	1647JAB	129	Galichica Mts.	Macedonia	40.96	20.81	Balkan	This study	no name	no name	Central	inclusive/exclusive
EU022647	E27104.3	130	Kazarma, Kardhista	Greece	38.97	22.38	Balkan	Santos et al., 2008	no name	no name	Central	inclusive
MH382916	741JAB	131	Asenovgrad	Bulgaria	40.98	24.88	Balkan	This study	no name	no name	Central	inclusive/exclusive
MH382942	2481JAB	132	Zhilentsi	Bulgaria	42.25	22.64	Balkan	This study	no name	no name	Central	inclusive/exclusive
MH382943	2484JAB	133	Maglenishki rid	Bulgaria	41.34	25.85	Balkan	This study	no name	no name	Central	inclusive/exclusive
MH382944	2485JAB	134	Petrohan	Bulgaria	43.11	23.15	Balkan	This study	no name	no name	Central	inclusive/exclusive
MH382945	2489JAB	135	Senokos	Bulgaria	41.83	23.22	Balkan	This study	no name	no name	Central	inclusive/exclusive
MH382946	2492JAB	136	Zdravets	Bulgaria	42.00	24.73	Balkan	This study	no name	no name	Central	inclusive/exclusive
MH382947	2493JAB	137	Vitata	Bulgaria	42.94	25.22	Balkan	This study	no name	no name	Central	inclusive/exclusive
MH382931	2443JAB	138	Gernik	Romania	44.75	21.79	Balkan	This study	no name	no name	Central	inclusive/exclusive
MH382914	497JAB	139	Ciuguzel - Cicard	Romania	46.27	23.90	Balkan	This study	no name	no name	Central	inclusive/exclusive
KM435303	LATJELGA58	140	Kameru NP	Latvia	56.87	23.47	Eastern	Galarza et al., 2015	no name	Eastern (part)	Eastern	inclusive
	LATSLITE01	141	Slitere NP	Latvia	57.60	22.33	Eastern	Galarza et al., 2015	no name	Eastern (part)	Eastern	inclusive
MH382920	1182JAB	142	Batal'noe	Crimea	45.15	35.61	Eastern	This study	no name	Eastern (part)	Eastern	inclusive/exclusive
MH382928	2379JAB	143	Pchelinoe	Crimea	44.89	34.60	Eastern	This study	no name	Eastern (part)	Eastern	inclusive/exclusive

MH382929	2380JAB	144	Simferopol	Crimea	44.94	34.13	Eastern	This study	no name	Eastern (part)	Eastern	inclusive/exclusive
KM435305	RUSSTAVR51, RUSSTAVR52	145	Stavropol region	Russia	43.88	42.84	Eastern	Galarza et al., 2015	no name	Eastern (part)	Eastern	inclusive
	RUSADUGE49	146	Adygea	Russia	44.32	40.25	Eastern	Galarza et al., 2015	no name	Eastern (part)	Eastern	inclusive
	RUSKRASN08, RUSKRASN48	147	Krasnodar	Russia	45.03	38.96	Eastern	Galarza et al., 2015	no name	Eastern (part)	Eastern	inclusive
	RUSRORTO47	148	Rostov	Russia	48.25	41.03	Eastern	Galarza et al., 2015	no name	Eastern (part)	Eastern	inclusive
	RUSROSTO46	149	Rostov	Russia	47.23	39.30	Eastern	Galarza et al., 2015	no name	Eastern (part)	Eastern	inclusive
EU022642	E17116.3	150	Kalininskiy, Rostov	Russia	49.57	41.85	Eastern	Santos et al., 2008	no name	Eastern (part)	Eastern	inclusive
EU022643	E17116.5	151	Volkonschino, Penza	Russia	53.20	44.99	Eastern	Santos et al., 2008	no name	Eastern (part)	Eastern	inclusive
EU022644	E17116.6	152	Matveyevskiy, Rostov	Russia	49.59	42.08	Eastern	Santos et al., 2008	no name	Eastern (part)	Eastern	inclusive
EU022645	E17116.2	153	Vysha, Republic of Mordovia	Russia	54.24	44.07	Eastern	Santos et al., 2008	no name	Eastern (part)	Eastern	inclusive
KM435297	ABKAZIA050	154	Aguripsta	Abkhazia	43.39	40.81	Eastern	Galarza et al., 2015	no name	Eastern (part)	Eastern	inclusive
KX694868	ROM 26536	155	Tbilisi	Georgia	41.80	44.71	Tran scau casi an	Alencar et al. 2016	not recovered	Eastern (part)	Eastern	inclusive
KM435302	GEORGIA045	156	Ajaria	Georgia	41.65	42.15	Transcaucasian	Galarza et al., 2015	not recovered	Eastern (part)	Eastern	inclusive
KP756623	5320	157		Armenia	40.80	44.89	Transcaucasian	Sztencel-Jabłonka et al., 2015	not recovered	Eastern (part)	Eastern	inclusive
KP756623	6545	158		Armenia	40.58	44.67	Transcaucasian	Sztencel-Jabłonka et al., 2015	not recovered	Eastern (part)	Eastern	
KP756623	6559	159		Armenia	40.58	44.69	Transcaucasian	Sztencel-Jabłonka et al., 2015	not recovered	Eastern (part)	Eastern	
KP756623	8345	160		Armenia	40.74	44.86	Transcaucasian	Sztencel-Jabłonka et al., 2015	not recovered	Eastern (part)	Eastern	
KP756624	6564	161		Armenia	40.68	45.17	Transcaucasian	Sztencel-Jabłonka et al., 2015	not recovered	Eastern (part)	Eastern	inclusive
AY486930	HLMD RA-2608	162	Ilgaz	Turkey	40.93	33.62	Anatolian 1	Nagy et al., 2004	not recovered	no name	not recovered	inclusive
MH382932	2459JAB	163	Ortaçımağıl	Turkey	40.11	40.60	Anatolian 1	This study	not recovered	no name	not recovered	inclusive/exclusive
MH382933	2460JAB	164	Küçükotlukbeli	Turkey	40.02	39.98	Anatolian 2	This study	not recovered	not recovered	not recovered	inclusive/exclusive
MH382934	2464JAB	165	Between Niksar and Başçiftlik	Turkey	40.53	37.15	Anatolian 1	This study	not recovered	no name	not recovered	inclusive/exclusive
MH382935	2467JAB	166	Güneyce	Turkey	40.82	40.48	Anatolian 1	This study	not recovered	no name	not recovered	inclusive/exclusive
MH382936	2468JAB	167	Söğüteli	Turkey	40.54	38.98	Anatolian 1	This study	not recovered	no name	not recovered	inclusive/exclusive
MH382937	2469JAB	168	Between Mecidiye and Yarbaşı	Turkey	39.89	39.58	Anatolian 1	This study	not recovered	no name	not recovered	inclusive/exclusive
MH382938	2470JAB	168	Between Mecidiye and Yarbaşı	Turkey	39.89	39.58	Anatolian 1	This study	not recovered	no name	not recovered	inclusive/exclusive
MH382939	2471JAB	169	Çilhoroz	Turkey	39.88	39.72	Anatolian 1	This study	not recovered	no name	not recovered	inclusive/exclusive
MH382940	2472JAB	169	Çilhoroz	Turkey	39.88	39.72	Anatolian 1	This study	not recovered	no name	not recovered	inclusive/exclusive
MH382941	2473JAB	169	Çilhoroz	Turkey	39.88	39.72	Anatolian 1	This study	not recovered	no name	not recovered	inclusive/exclusive
MH382948	Callran	170	Varewasht Mts.	Iran	36.27	51.40	Iranian 2	This study	not recovered	not recovered	not recovered	inclusive/exclusive
MH382949	Ca2Iran	171	Damash	Iran	36.76	49.81	Iranian 2	This study	not recovered	not recovered	not recovered	inclusive/exclusive
MH382950	Ca3Iran	172	Veresk	Iran	35.84	52.95	Iranian 1	This study	not recovered	not recovered	not recovered	inclusive/exclusive