Phylogeography and postglacial colonization of Central Europe by Anguis fragilis and Anguis colchica

Daniel Jablonski¹, Bartłomiej Najbar², Renata Grochowalska³, Václav Gvoždík^{4,5}, Tomasz Strzała^{6,*}

Abstract. Five slow-worm species are distributed in the Palearctic region. Two species, *Anguis fragilis* and *A. colchica*, are native to Central Europe, where only limited information on the phylogeography of the genus exists. Here, we examined the situation in Poland and surrounding regions, where a mitochondrial contact zone between the species is expected. We used new mitochondrial DNA sequences and available published data from Central Europe and the northern Balkans. Haplotypes of both species were recorded in the study area. *Anguis fragilis* is represented by a single haplogroup, while *A. colchica* by three haplogroups. This suggests four independent sources/refugia for postglacial colonization of northern Central European Plain, and the south-eastern Poland uplands, while the Vistula River does not constitute a barrier. The presence of both species, *A. fragilis* and *A. colchica*, in Poland should also be considered for protection by the Polish conservation legislation.

Keywords: Anguidae, contact zone, haplotype distribution, mitochondrial DNA, Poland, slow worms, Squamata.

Slow worms, the genus *Anguis*, represents legless lizards (Squamata: Anguidae) distributed in the Western Palearctic region (Gvoždík et al., 2010, 2013). Recent phylogeographic analyses carried out with both mitochondrial and nuclear loci revealed that the genus is represented by *A. cephallonica* Werner, 1894 from the Peloponnese, and four species previously comprised under the name *A. fragilis*: *A. fragilis* Linnaeus, 1758 from the western part of Europe,

- Department of Zoology, Comenius University in Bratislava, Mlynská dolina, Ilkovičova 6, 842 15 Bratislava, Slovakia
- 2 Faculty of Biological Sciences, University of Zielona Góra, Prof. Z. Szafrana 1, 65-516 Zielona Góra, Poland
- 3 Department of Biochemistry and Bioinformatics, Faculty of Biological Sciences, University of Zielona Góra, Prof. Z. Szafrana 1, 65-001 Zielona Góra, Poland
- 4 Institute of Vertebrate Biology, Czech Academy of Sciences, 603 65 Brno, Czech Republic
- 5 Department of Zoology, National Museum, 193 00 Prague, Czech Republic
- 6 Faculty of Biology and Animal Science, Department of Genetics, Wrocław University of Environmental and Life Sciences, Kożuchowska 7, 51-631 Wrocław, Poland

*Corresponding author; e-mail: tomasz.strzala@upwr.edu.pl A. colchica (Nordmann, 1840) from the eastern part of Europe and western Asia, A. graeca Bedriaga, 1881 from the Balkans, and A. veronensis Pollini, 1818 from the Italian Peninsula and south-eastern France (Gvoždík et al., 2010, 2013). Two species are native to Central Europe: A. fragilis and A. colchica (uncorrected pdistances 8.1% in the mitochondrial ND2 gene; Gvoždík et al., 2010), which were for a long time previously treated as subspecies of A. fragilis (e.g., Arnold, 2002). The phylogeography and postglacial colonization history of the genus Anguis have been studied in the area of southern Europe (Gvoždík et al., 2013; Thanou et al., 2014; Jablonski et al., 2016), where slow-worm populations are represented by haplogroups with restricted distributions and high level of haplotype diversity shaped, among others, by the heterogeneous topography (Jablonski et al., 2016). These populations have not dispersed much from their refugial areas. On the other hand, only limited information is available for the Central or Northern European space (Gvoždík et al., 2010, 2013, 2015a; Szabó and Vörös, 2014).

In Poland, based on morphological data and the former subspecies concept *A. colchica* was suggested to be present by some authors (e.g. Dely, 1972) but only A. fragilis was listed by others (e.g. Juszczyk, 1987). The presence of A. colchica haplotypes was definitively confirmed by molecular analyses by Gvoždík et al. (2010, 2013). Nevertheless, discussions still persist about differentiation of both species and where there is a contact zone between them (Skórzewski et al., 2012; Skórzewski, 2017). The above is even more interesting if we consider that hybridization occurs between A. fragilis and A. colchica (Szabó and Vörös, 2014; Gvoždík et al., 2015a). Current data on slowworm distribution in Poland indicate that both species may occur in close parapatry and show that the genus is widespread all over the country (mostly in forested regions; Skórzewski, 2017). Due to the fact that the two slow-worm species are difficult to distinguish morphologically, the knowledge on distributions of the two slow-worm species has remained scarce and conflicting. Moreover, slow-worm populations are threatened in some parts of Poland and are of conservation concern (Głowaciński and Rafiński, 2003), therefore, it is necessary to understand their taxonomic identification, distributions, and also the area of the contact zone between the species as well as their ecological differentiation.

In the present study, we aim to use new sequences of the mitochondrial DNA (mtDNA) to i) provide information on distributions of *A. fragilis* and *A. colchica* haplotypes in Poland, and to detect an approximate area of the contact zone; ii) find out mtDNA structure in the Central European space; and iii) postulate a scenario of postglacial colonizations of Central and Northern Europe in the context of previously published (Szabó and Vörös, 2014; Jablonski et al., 2016) phylogeographical scenarios in the Balkans and Carpathian Basin.

Tissue samples were obtained from road-killed individuals and preserved in 96% ethanol. A portion, 732 base pairs (bp), of the mitochondrial gene of the NADH dehydrogenase subunit 2 (*ND2*) was targeted. Newly produced nucleotide sequences mainly from the eastern North European Plain and the Lesser Poland Uplands [29 specimens from 29 localities in Poland and samples from adjacent areas in Belarus (1), Germany (4), Russian Kaliningrad Oblast (1), Ukraine (2), and, to present the situation in a wider context of Holocene colonizations also samples from Norway (2) and Sweden (1); table 1] were supplemented to the previously published haplotype datasets from Central Europe and the northern Balkans, including two A. colchica samples from Poland (Gvoždík et al., 2010, 2013; Szabó and Vörös, 2014; Jablonski et al., 2016; fig. 1). The final dataset focused on the area of interest thus contained only sequences of the Illyrian-Central European (ICE) haplogroup of A. fragilis, and four haplogroups of the Carpathian lineage of A. colchica as previously defined by Jablonski et al. (2016). The laboratory procedures, DNA sequence evaluation and haplotype-network constructions followed Jablonski et al. (2016). Haplotype networks were constructed using the 95% limit of parsimony as implemented in TCS 1.21 (Clement et al., 2000) and results visualized with tcsBU (dos Santos et al., 2016). 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 estimate genetic diversity and uncorrected *p*-distances of slow-worms in Poland: number of haplotypes (h), haplotype diversity (h_d) , number of segregating sites (S), nucleotide diversity (π) , parsimony informative sites (P), and Watterson's theta per site ($\theta_{\rm W}$). New nucleotide sequences have been deposited in GenBank under accession numbers MF817454-MF817493.

Anguis fragilis shows low genetic diversity within Central Europe, specifically within the ICE haplogroup, with only two haplotypes found in Poland (out of 27 presently known haplotypes of the ICE haplogroup; fig. 1, table 2) one widespread and one haplotype found only in one specimen from north-western Poland (Szczecin Dąbie). Estimates of genetic diversity for this species in Poland are very low (table 2). Haplotypes of the ICE haplogroup were so far detected through the north-western Balkans, western Hungary, Austria, Germany, Czech Republic, Slovakia, southern Great Britain, Spain and herein for the first time in Russia (exclave Kaliningrad Oblast), southern Sweden and southern Norway (the most common and widespread haplotype f1; fig. 1). In Poland, A. fragilis haplotypes occur in northern, western and central parts of the country with the most south-eastern records from near Prudnik town (fig. 1).

Anguis colchica is represented by the Incerta clade (i.e., A. colchica incerta Krynicki, 1837; Gvoždík et al., 2010; Jablonski et

Species	Code	Country	Locality	Coordinates		Haplogroup	GenBank
				N	Е		number
A. fragilis	3684	Poland	Sieniawa Żarska	51.630	15.055	Illyrian-Central European	MF817460
A. fragilis	3685	Poland	Żary	51.629	15.061	Illyrian-Central European	MF817461
A. fragilis	3698	Poland	Szczytno	53.566	20.985	Illyrian-Central European	MF817463
A. fragilis	3699	Poland	Siódmak	53.525	20.971	Illyrian-Central European	MF817464
A. fragilis	3700	Poland	Rudka	53.529	21.039	Illyrian-Central European	MF817465
A. fragilis	3703	Poland	Jarnołtówek	50.285	17.426	Illyrian-Central European	MF817466
A. fragilis	3704	Poland	Pokrzywna	50.285	17.479	Illyrian-Central European	MF817467
A. fragilis	3705	Poland	Żabieniec	52.053	21.041	Illyrian-Central European	MF817468
A. fragilis	3707	Poland	Słotwiny k. Koluszek	51.731	18.821	Illyrian-Central European	MF817469
A. fragilis	3708	Poland	Szczecin	53.395	14.575	Illyrian-Central European	MF817470
A. fragilis	3709	Poland	Szczecin Dąbie	53.402	14.696	Illyrian-Central	MF817471
						European	
A. fragilis	3710	Poland	Iława	53.613	19.529	Illyrian-Central European	MF817472
A. fragilis	3711	Poland	Sarnówek	53.659	19.583	Illyrian-Central European	MF817473
A. fragilis	3712	Poland	Ostromecko	53.148	18.203	Illyrian-Central European	MF817474
A. fragilis	3713	Poland	Sobótka	50.897	16.730	Illyrian-Central European	MF817475
A. fragilis	3719	Poland	Ruda Milicka	51.528	17.341	Illyrian-Central European	MF817476
A. fragilis	1389	Germany	Regenstauf	49.151	12.174	Illyrian-Central European	MF817455
A. fragilis	2069	Germany	Henningsdorf	52.633	13.223	Illyrian-Central European	MF817458
A. fragilis	2422	Germany	Schönsee	49.518	12.538	Illyrian-Central European	MF817459
A. fragilis	3693	Germany	Beeskow	52.209	14.286	Illyrian-Central	MF817462
						European	
A. fragilis	3720	Russia	Bagrationovsk, Kaliningrad Oblast	54.372	20.639	Illyrian-Central European	MF817477
A. fragilis	1977	Norway	Revsvatnet Lake	58.990	6.137	Illyrian-Central European	MF817456
A. fragilis	1978	Norway	Jørpeland	59.006	6.061	Illyrian-Central European	MF817457
A. fragilis	696	Sweden	Mörby Gård, Stockholm	59.120	18.170	Illyrian-Central European	MF817454
A. colchica	443	Poland	Orlica, Pieniny NP	49.417	20.457	Carpathian I	MF817478
A. colchica	446	Poland	Szopka, Pieniny NP	49.418	20.408	Carpathian I	MF817479
A. colchica	3686	Poland	Rajcza	49.501	19.109	Carpathian I	MF817480
A. colchica	3687	Poland	Stary Sącz	49.564	20.624	Carpathian I	MF817481
A. colchica	3690	Poland	Wola Mała	50.545	22.768	Carpathian I	MF817482
A. colchica	3691	Poland	Brodziaki	50.507	22.791	Carpathian III	MF817483
A. colchica	3694	Poland	Sękowiec	49.235	22.557	Carpathian I	MF817484
A. colchica	3695	Poland	Zatwarnica	49.223	22.551	Carpathian I	MF817485
A. colchica	3697	Poland	Krosno	49.686	21.787	Carpathian IV	MF817487
A. colchica	3714	Poland	Racibórz	50.097	18.260	Carpathian I	MF817489
A. colchica	3715	Poland	Kobyła	50.087	18.305	Carpathian I	MF817490
A. colchica	3716	Poland	Polska Cerekiew	50.226	18.125	Carpathian I	MF817491
A. colchica	3717	Poland	Kazimierz Dolny	51.324	21.958	Carpathian IV	MF817492
A. colchica	3706	Belarus	Dobrovolya	52.884	23.996	Carpathian III	MF817488
A. colchica	3696	Ukraine	Boberka	49.206	22.736	Carpathian I	MF817486
A. colchica	4202	Ukraine	Tur'ya Polyana	48.755	22.852	Carpathian I	MF817493

Short Notes



Figure 1. Distribution of mitochondrial haplogroups and haplotype networks of *Anguis fragilis* and *A. colchica* in Central Europe based on new and published data (Gvoždík et al., 2010, 2013; Szabó and Vörös, 2014; Jablonski et al., 2016; terminology as well as colours of haplogroups follows the latter publication). The dashed orange line denotes approximate location of the mitochondrial contact zone between the species. Whitish transparent shading shows the distribution range of the genus. Question marks denote missing phylogeographic data. Small black dots in the haplotype networks represent unsampled or extinct haplotypes. Bold lines of haplotypes in the networks highlight haplotypes found in the present study (including outside Poland). For clear association of the found haplotype name: f1 - f3, f7, f12, f13 (*A. fragilis*) and c1 - c6, c12 (*A. colchica*); Gvoždík et al. (2010, 2013). "S" in the inset map indicates specimens identified based on the morphology (Skórzewski, 2017).

Table 2. Summary of genetic polymorphism for both slow-worm species in Poland: sample size (*n*), number of haplotypes (*h*), number of polymorphic sites (*S*), parsimony informative sites (*P*), nucleotide diversity (π), haplotype diversity (h_d), Watterson's theta per site (θ_W). SD = standard deviation.

Species	п	h	S	Р	$\pi \pm \text{SD}(\%)$	$h_d \pm SD$	$\theta_{\rm W} \pm { m SD}(\%)$
A. fragilis A. colchica	16 15	2 4	1 8	0 4	$0.02 \pm 0.02 \\ 0.27 \pm 0.08$	$\begin{array}{c} 0.125 \pm 0.106 \\ 0.543 \pm 0.133 \end{array}$	0.04 ± 0.04 0.34 ± 0.16

al., 2016). This clade is distributed in northern Bulgaria, eastern Serbia, in Romania along the Carpathians, and in Eastern Europe. It consists of at least three lineages, with the Carpathian lineage being the only known to be more widespread in Central and Eastern Europe (Jablonski et al., 2016). The samples from Poland are represented by three haplogroups from the Carpathian lineage - I, III, and IV with average uncorrected *p*-distances range from 0.21% (between Haplogroups I and III) to 0.42% (III and IV). Similar to the situation in the Romanian Carpathians, all three haplogroups of A. colchica in Poland have mixed distributions (fig. 1). The westernmost occurrence of an A. colchica haplotype was recorded from Polska Cerekiew (near Kędzierzyn-Koźle town), while 46 km further to the west an A. fragilis haplotype was found. However, Skórzewski (2017) found A. fragilis (based on morphology) also near Kędzierzyn-Koźle (air distance 15 km), which makes this region of particular interest as the two species probably meet in this area (fig. 1). When the genetic polymorphism of the two species is evaluated and compared (table 2), it is evident that A. colchica in Poland has much higher genetic diversity than A. fragilis. A majority of the analysed Polish samples (11 out of 15) carry the most common haplotype of Haplogroup I, which had a probable glacial refugium in Transylvania (region of the Apuseni Mountains; Jablonski et al., 2016). At the same time, this haplogroup is geographically the most widespread in Central Europe being detected in western Ukraine, northern Hungary, Slovakia, and the eastern Czech Republic (Gvoždík et al., 2010, 2013; Szabó and Vörös, 2014; this study). Within Poland, this haplogroup has been found in the southeastern and eastern parts of the country. The other four Polish samples correspond to Haplogroup IV (three samples) and Haplogroup III (one sample from Brodziaki carrying a new haplotype). Besides, another sample from Belarus near the Polish border also carries a haplotype from Haplogroup III.

Geographically, most of Poland is inhabited by A. fragilis, which is distributed throughout lowlands of the North European Plain and in the Bohemian Massif (Sudetes Mts.) in the south. Its range margin in the north-east is not yet well known with A. fragilis haplotypes recorded from near Szczytno town in Poland and the south-western Kaliningrad Oblast of Russia, which indicates a presence of A. fragilis northward along the coast of the Baltics. Anguis colchica haplotypes are distributed in the lesser Poland uplands and the East European Plain of south-eastern and eastern Poland. Thus, the contact zone between the species in Poland seems to mirror the borders between lowlands of the North European Plain and East European Plain, and the south-eastern Poland uplands, while the Vistula River does not constitute a barrier.

The ICE haplogroup of *A. fragilis* and the three haplogroups of the Carpathian lineage of *A. colchica* colonized broad areas of temperate Europe probably from their northern extra-Mediterranean refugia, presumably localized in the Dinarides or the western Pannonian Plain in *A. fragilis*, and the Carpathians in *A. colchica*, respectively (Jablonski et al., 2016). This was possible because most mountain ranges remained ice-free during the Last Glacial Maximum (Reuther et al., 2007).

The star-like topology of the haplotype network in the ICE A. fragilis, along with the inferred historical demographic model (Jablonski et al., 2016), suggests a population expansion of this haplogroup, including a spatial expansion into more northerly located regions. This haplogroup reached Scandinavia probably in the early Holocene as this northern peninsula was connected with the mainland via Jutland (Björck, 1995). On the contrary, the history of A. colchica in northern Central Europe has had a different pattern because the region was obviously colonized by at least three different population sources. One haplogroup (Carpathian I), which also shows a star-like pattern in the haplotype network, almost certainly colonized the northern Central Europe and surrounding regions from the central or southern Carpathians, while the geographic origin of the other two haplogroups present in northern Central and Eastern Europe is not yet known. Their refugia were either located in the Carpathians (Jablonski et al., 2016) or possibly somewhere in the East European Plain. To obtain a clearer picture of the phylogeography and colonization routes in the north-eastern part of the slowworm range, a better sampling from Eastern Europe is needed. As mentioned above, a questionable situation still remains in the region along the coast of the Baltic countries and Finland, which might have been colonized by both species, but data are still lacking.

Similar colonization patterns of northern Central and Northern Europe were also observed in the European pond turtle (*Emys orbicularis*; Sommer et al., 2009) or the grass snake (*Natrix natrix*; Kindler et al., 2013, 2014). Three haplogroups of the grass snake found in Poland were sourced from three distinct glacial refugia with one located in the east (probably the Caucasus region), and two in the south, probably south of the Danube Basin (Kindler et al., 2013). Another similar phylogeographic scheme, although with a different location of the mitochondrial contact zone located along the Vistula River, was found in tree frogs (*Hyla*; Stöck et al., 2012; Gvoždík et al., 2015b). *Hyla arborea* is distributed in the western part of Poland and its glacial refugium was located in the Balkans (Dufresnes et al., 2013), while *H. orientalis* occurs in eastern Poland and the most probable refugium for this species was in the Black Sea area (Dufresnes et al., 2016).

We acknowledge that the distribution patterns revealed here may not fully represent species distributions due to the specific characteristics of mtDNA (maternal and clonal inheritance, reduced effective population size, sexspecific dispersal, relatively common interspecific introgression). The mitochondrial contact zone in northern Central Europe (Poland) between A. fragilis and A. colchica was probably formed by geomorphology of the landscape and ecological specifics of the two species. It is also worth noting that the extent of distribution of the most common haplotype of the ICE haplogroup of A. fragilis and the Carpathian Haplogroup I of A. colchica is very different. The first is distributed from the north-western Balkans to Scandinavia (fig. 1) and the British Isles (Gvoždík et al., 2010), while the latter only from the Romanian Apuseni Mts. to eastern Poland (fig. 1). These different distributions could have been caused by different levels of plasticity to adapt to different environmental conditions. Therefore, slow-worm populations from areas without strong geographic barriers might be a good model to study the causality between genomic and environmental variation and the speed of dispersal.

Furthermore, our results should also be used as a first stepping stone for conservation legislation in Poland, because *A. fragilis* is the only species of the genus listed in the last regulation (16 December 2016) issued by the Ministry of the Environment. The different range sizes in Poland of *A. fragilis* (most of the country) and *A. colchica* (restricted to south-eastern and eastern regions) and different levels of genetic diversity in mtDNA (*A. fragilis* relatively low diversity, *A. colchica* diverse) must also be considered when conservation status is discussed. However, a more detailed sampling and further analyses, including investigations of nuclear genomes, remain necessary, as well as to confirm a possible presence of hybrids.

Acknowledgements. We would like to thank Andrzej Czylok, Tomáš Husák, Anna Najbar, Ewa Szuszkiewicz and A. Westerstrom for collecting samples for analysis, Jana Pollakova for technical support, Stephen R. Goldberg for English corrections and two anonymous reviewers for their valuable comments and suggestions. DJ was supported by the Slovak Research and Development Agency under the contract no. APVV-15-0147 and by a grant of the Scientific Grant Agency of the Slovak Republic VEGA 1/0073/14. VG was supported by the Institute of Vertebrate Biology, Czech Academy of Sciences (RVO 68081766), and the Ministry of Culture of the Czech Republic (DKRVO 2017/15, National Museum, 00023272).

References

- Arnold, E.N. (2002): Reptiles and Amphibians of Europe. Princeton University Press, Princeton and Oxford.
- Björck, S. (1995): A review of the history of the Baltic Sea, 13.0-8.0 ka BP. Quatern. Int. **27**: 19-40.
- Clement, M., Posada, D., Crandall, K.A. (2000): TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9: 1657-1659.
- Dely, O.G. (1972): Beiträge zur Verbreitung und Systematik der Blindschleiche (*Anguis fragilis* Linnaeus) im Karpatenbecken. Vertebr. Hungarica **13**: 39-79.
- dos Santos, A.M., Cabezas, M.P., Tavares, A.I., Xavier, R., Branco, M. (2016): tcsBU: a tool to extend TCS network layout and visualization. Bioinformatics 32: 627-628.
- Dufresnes, C., Wassef, J., Ghali, K., Brelsford, A., Stöck, M., Lymberakis, P., Crnobrnja-Isailovic, J., Perrin, N. (2013): Conservation phylogeography: does historical diversity contribute to regional vulnerability in European tree frogs (*Hyla arborea*)? Mol. Ecol. 22: 5669-5684.
- Dufresnes, C., Litvinchuk, S.N., Leuenberger, J., Ghali, K., Zinenko, O., Stöck, M., Perrin, N. (2016): Evolutionary melting pots: a biodiversity hotspot shaped by ring diversification around Black Sea in the eastern tree frog (*Hyla orientalis*). Mol. Ecol. 25: 4285-4300.
- Głowaciński, Z., Rafiński, J. (2003): Atlas of Amphibians and Reptiles in Poland (in Polish: Atlas płazów i gadów Polski). Biblioteka Monitoringu Środowiska, Warszawa/Kraków.
- Gvoždík, V., Jandzik, D., Lymberakis, P., Jablonski, D., Moravec, J. (2010): Slow worm, *Anguis fragilis* (Reptilia: Anguidae) as a species complex: genetic structure reveals deep divergences. Mol. Phylogenet. Evol. 55: 460-472.

- Gvoždík, V., Benkovský, N., Crottini, A., Bellati, A., Moravec, J., Romano, A., Sacchi, R., Jandzik, D. (2013): An ancient lineage of slow worms, genus *Anguis* (Squamata: Anguidae), survived in the Italian Peninsula. Mol. Phylogenet. Evol. **69**: 1077-1092.
- Gvoždík, V., Moravec, J., Zavadil, V., Jeřábková, L. (2015a): Anguis fragilis and A. colchica – distribution in the Czech Republic (in Czech: Slepýš křehký a slepýš východní – výskyt v České republice). In: Fauna ČR. Plazi – Reptilia, p. 275-278. Moravec, J., Ed., Academia, Praha.
- Gvoždík, V., Canestrelli, D., García-París, M., Moravec, J., Nascetti, G., Recuero, E., Teixeira, J., Kotlík, P. (2015b): Speciation history and widespread introgression in the European short-call tree frogs (*Hyla arborea* sensu lato, *H. intermedia* and *H. sarda*). Mol. Phylogenet. Evol. 83: 143-155.
- Jablonski, D., Jandzik, D., Mikulíček, P., Džukić, G., Ljubisavljević, K., Tzankov, N., Jelić, D., Thanou, E., Moravec, J., Gvoždík, V. (2016): Contrasting evolutionary histories of the legless lizards slow worms (*Anguis*) shaped by the topography of the Balkan Peninsula. BMC Evol. Biol. 16: 99. DOI:10.1186/s12862-016-0669-1.
- Juszczyk, W. (1987): Polish Amphibians and Retiles. Part 3: Reptiles (in Polish: Płazy i gady krajowe. Część 3: Gady). PWN, Warszawa.
- Kindler, C., Böhme, W., Corti, C., Gvoždík, V., Jablonski, D., Jandzik, D., Metallinou, M., Široký, P., Fritz, U. (2013): Mitochondrial phylogeography, contact zones and taxonomy of grass snakes (*Natrix natrix*, *N. megalocephala*). Zool. Scripta **42**: 458-472.
- Kindler, C., Bringsøe, H., Fritz, U. (2014): Phylogeography of grass snakes (*Natrix natrix*) all around the Baltic Sea: implications for the Holocene colonization of Fennoscandia. Amphibia-Reptilia 35: 413-424.
- Librado, P., Rozas, J. (2009): DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25: 1451-1452.
- Reuther, A.U., Urdea, P., Geiger, C., Ivy-Ochs, S., Niller, H.P., Kubik, P.W., Heine, K. (2007): Late Pleistocene glacial chronology of the Pietrele Valley, Retezat Mountains, Southern Carpathians constrained by 10Be exposure ages and pedological investigations. Quat. Int. 164-165: 151-169.
- Skórzewski, G. (2017): Remarks on the studies and occurrence of the eastern slow worm Anguis colchica incerta in Poland (in Polish: Uwagi na temat badań i występowania padalca kolchidzkiego Anguis colchica incerta w Polsce). Chrońmy Przyr. Ojczystą 73: 57-63.
- Skórzewski, G., Borczyk, B., Najbar, B. (2012): Morphological differentiation of Anguidae Gray, 1825 in Poland: one or two species? (in Polish: Zróżnicowanie morfologiczne padalcowatych Anguidae Gray. 1825 w Polsce: jeden czy dwa gatunki?). In: Biologia Płazów I Gadów – Ochrona Herpetofauny, p. 117-120. Zamachowski, W., Ed., Wydawnictwo Naukowe Uniwersytetu Pedagogicznego w Krakowie, Kraków.
- Sommer, R.S., Lindqvist, C., Persson, A., Bringsøe, H., Rhodin, A.G.J., Schneeweiß, N., Široký, P., Bachmann, L., Fritz, U. (2009): Unexpected early extinction of the

European pond turtle (*Emys orbicularis*) in Sweden and climatic impact on its Holocene range. Mol. Ecol. **18**: 1252-1262.

- Stöck, M., Dufresnes, C., Litvinchuk, S.N., Lymberakis, P., Biollay, S., Berroneau, M., Borzée, A., Ghali, K., Ogielska, M., Perrin, N. (2012): Cryptic diversity among Western Palearctic tree frogs: postglacial range expansion, range limits, and secondary contacts of three European tree frog lineages (*Hyla arborea* group). Mol. Phylogenet. Evol. 65: 1-9.
- Szabó, K., Vörös, J. (2014): Distribution and hybridization of Anguis fragilis and A. colchica in Hungary. Amphibia-Reptilia 35: 135-140.
- Thanou, E., Giokas, S., Kornilios, P. (2014): Phylogeography and genetic structure of the slow worms *Anguis cephallonica* and *Anguis graeca* (Squamata: Anguidae) from the southern Balkan Peninsula. Amphibia-Reptilia **35**: 263-269.

Submitted: May 4, 2017. Final revision received: September 28, 2017. Accepted: October 15, 2017. Associate Editor: Judit Vörös.