BRILL

Amphibia-Reptilia 42 (2021): 383-389

brill.com/amre

Short Note

Two species of slow worm (*Anguis fragilis*, *A. colchica*) present in the Baltic region

Václav Gvoždík[1,2,*], Zdeněk Harca[1,3], Alexandra Hánová[1,3], Daniel Jablonski[4], Mihails Pupins[5], Andris Čeirāns[5], Timo Paasikunnas[6]

Abstract. Five European slow worms (*Anguis*) have mostly parapatric distributions. Two species, *A. fragilis* and *A. colchica*, are widely distributed across the western and eastern parts of the genus range. Their contact zone runs from the north-eastern Balkans, through Pannonia to northern Central Europe. In northern Poland, the contact zone has been located approximately between the North and East European Plains. Here, we present the first mitochondrial and nuclear DNA data from Finland and the coastal Baltics. We demonstrate that *A. fragilis* enters the East European Plains, where it is presumably distributed along the Baltic coast. Our data indicate that *A. colchica* is present more inland and to the north of Riga. The genetic structure suggests three independent postglacial colonization events in the Baltics (two by *A. colchica*). The presence of the two species, *A. fragilis* and *A. colchica*, should be considered by the conservation legislations of Lithuania, Latvia and Russia.

Keywords: Anguidae, Baltic Rim, contact zone, Finland, haplotype distribution, Squamata.

Slow-worm lizards (*Anguis*) are represented by five species distributed in the Western Palearctic, with a majority of the range in Europe. Most of the species are hard to distinguish morphologically (Gvoždík et al., 2013), although a detailed morphological study based on a genetic background is yet to be done. The present knowledge on their distributions is based on the molecular data (Gvoždík et al., 2010, 2013; Keskin et al., 2013; Szabó and Vörös, 2014; Thanou, Giokas and Kornilios, 2014; Jablonski et al., 2016, 2017; Mikulíček et al., 2018; Renet et al., 2018). The common European slow worm (*A. fragilis*) is distributed north of the Alps in the western part of Europe, including Great Britain and Scandinavia, to the north-western Balkans. The eastern slow worm (*A. colchica*) is known to continue further east in the north-eastern Balkans, Anatolia, Caucasus and the southern Caspian region, East European Plain, and possibly north into Finland as a morphological survey indicated (Voipio, 1962). The remaining three species are endemics of the Italian Peninsula (*A. veronensis*; near-endemic occurring also in south-eastern France) and Balkan Peninsula (*A. cephallonica*, *A. graeca*). Only the latter two are known to occur partly in sympatry in the northern Peloponnese (Grillitsch and Cabela, 1990; Thanou, Giokas and Kornilios, 2014; Jablonski et al., 2016). All the other species have probably parapatric distributions and form hybrid zones where their ranges meet (Gvoždík et al., 2013, 2015; Szabó and Vörös, 2014). However, little is known about exact distributions of slow-worm species in

1 - Institute of Vertebrate Biology of the Czech Academy of Sciences, Brno, Czech Republic
2 - National Museum, Department of Zoology, Prague, Czech Republic
3 - Masaryk University, Department of Botany and Zoology, Brno, Czech Republic
4 - Comenius University in Bratislava, Department of Zoology, Bratislava, Slovakia
5 - Daugavpils University, Department of Ecology, Daugavpils, Latvia
6 - Helsinki Zoo, Helsinki, Finland
*Corresponding author;
e-mail: vaclav.gvozdik@gmail.com

DOI:10.1163/15685381-bja10055

some areas, especially the Baltic region (Jablonski et al., 2017).

Here, we present the first molecular data from Finland and the coastal Baltics, and demonstrate the presence of two slow-worm species, *A. fragilis* and *A. colchica*, in the region.

Tissue samples were collected from road-killed individuals and preserved in 96% ethanol. Eighteen new samples from Finland, Estonia, and Latvia were supplemented by six samples from Lithuania, Russian Kaliningrad Oblast, and Sweden available from previous studies (Gvoždík et al., 2010; Jablonski et al., 2017), see fig. 1A, table 1 (hereafter as the Baltic Rim region). Two molecular markers were targeted. A mitochondrial DNA (mtDNA) fragment of the NADH dehydrogenase subunit 2 gene (*ND2*; 732 bp after trimming) was amplified and sequenced following Jablonski et al. (2016). A nuclear DNA fragment encompassing an exon of the recombination-activating gene 1 (*RAG1*; 1087 bp) was amplified by the R13 and R18 primers (Groth and Barrowclough, 1999), and sequenced by the PCR primers from both sides. The PCR thermal profile was as follows: initial step at 94°C for 15 min, followed by 40 cycles of 94°C for 40 s, 56°C for 40 s, 72°C for 1 min, and final extension at 72°C for 7 min. New sequences were deposited in GenBank (MW595722-MW595760; table 1). Twenty-four *ND2* sequences from the Baltic Rim were supplemented by 20 sequences from GenBank (including *Pseudopus apodus* as an outgroup, FJ666588) representing all five *Anguis* species (Gvoždík et al., 2010, 2013), and lineages and relevant haplotypes present in northern Central Europe (Jablonski et al., 2016, 2017). Twenty-one *RAG1* sequences (PCR failed in three individuals) were supplemented by all available homologous *RAG1* of *Anguis* from GenBank (10 from Hungary, Szabó and Vörös, 2014; one from Spain, Carvalho et al., 2017) and one *Ophisaurus attenuatus* (AY662602, Townsend et al., 2004) as an outgroup. GenBank numbers are given directly in the trees (fig. 1B, C). The *RAG1* alignment was trimmed to the length of 1043 bp to be congruent with the GenBank data (the trimmed ends contained no variation). Gametic haplotypes of the ingroup were inferred by the coalescent-based Bayesian algorithm of Phase 2.1 (Stephens, Smith and Donnelly, 2001; Stephens and Scheet, 2005) to check for a possible sign of hybridization (if gametic haplotypes segregate in different-species parental lineages). Input and output files were processed in SeqPHASE (Flot, 2010). The Phase analysis was repeated three times with different starting seeds to check if the phase estimates are consistent across the runs according to goodness-of-fit values. Phasing was conducted under the parent-independent mutation model with a burn-in period of 100 iterations followed by further 1000 iterations. Altogether, 11 from 32 ingroup individuals contained one to four heterozygous positions (three to four positions in five individuals from the Baltic Rim). All heterozygous positions except one were phased with the full support (probability = 1.00). The only position, which did not receive the full support, was also resolved with a high probability (average 0.86). The final *RAG1* alignment was composed of two sequences (both gametic alleles) in heterozygous individuals and of one sequence in homozygous individuals, resulting in 44 sequences in total (including outgroup).

Assemblies and alignments were done in Geneious 8.1 (Kearse et al., 2012), as well as translations into amino acids, which revealed no stop codons. The best-fit codon position partitions and substitution models were selected by the Akaike information criterion in PartitionFinder2 (Lanfear et al., 2017) as follows: all codon positions treated separately in both markers; *ND2*, HKY + G for position 1, HKY + I for position 2, and TrN + G for position 3; phased *RAG1*, HKY + G for positions 1 and 3, and HKY for position 2. Phylogenetic trees were constructed using maximum likelihood and the best-fit partitions and substitution models in RAxML-NG v0.9 (Kozlov et al., 2019). Nodal support values were received by the bootstrap method with the automatic bootstopping cut-off value 0.03, stopping at 200 pseudoreplicates.

The maximum-likelihood phylogenetic tree based on mitochondrial *ND2* (fig. 1B) inferred the same topology of main clades (all five species) and similar support values as previously published (Gvoždík et al., 2013; Jablonski et al., 2016). The Baltic Rim samples were placed in two main clades corresponding to *A. fragilis* and *A. colchica*, respectively. All samples from Finland, as well as the only available sample from Estonia belonged to the *A. colchica* clade. This is in an agreement with results of a morphological survey (Voipio, 1962). Samples from Latvia were present in both clades. Samples from earlier studies from eastern Lithuania and Sweden/Russian Kaliningrad Oblast were placed in the *A. colchica* and *A. fragilis* clades, respectively. In Latvia, the north-eastern part of the country was found to harbour the *A. colchica* haplotypes, while the south-western part those of *A. fragilis*. All individuals belonging to the *A. fragilis* mtDNA clade bore the same haplotype, corresponding to the most common f1 haplotype, which is known to be widely distributed from the north-western Balkans, across Central Europe to Scandinavia (Gvoždík et al., 2010, 2013; Jablonski et al., 2016, 2017). Specimens belonging to the *A. colchica* clade were grouped into two haplogroups, III and IV of the Carpathian lineage (fig. 1A, B, table 1; terminology follows Jablonski et al., 2016). This finding corresponds to the results found in a previous study from Poland (Jablonski et

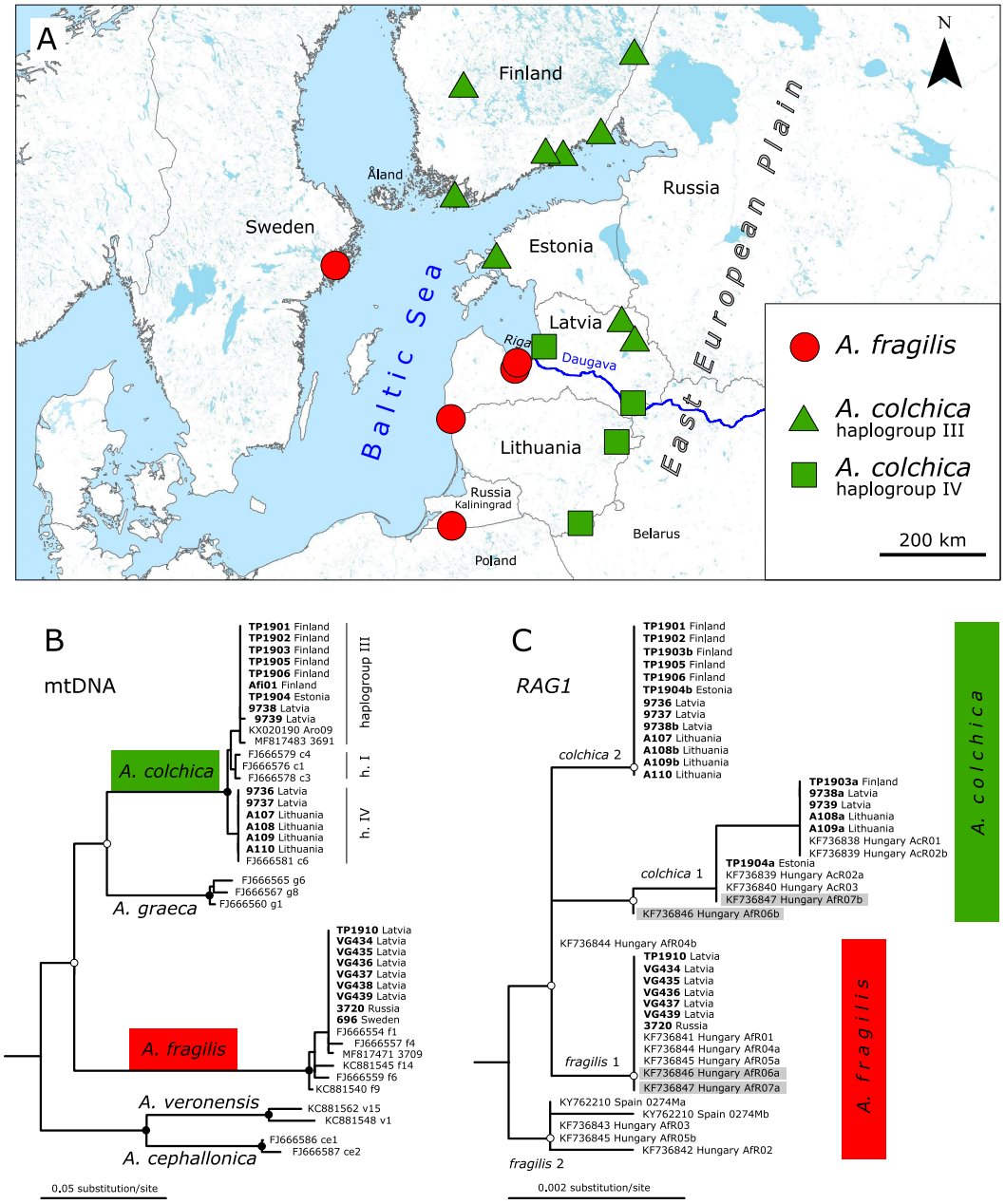

Figure 1. Distribution of *Anguis fragilis* (red) and *A. colchica* (green) in the eastern Baltic region as evidenced by genetic data and phylogenetic trees based on mtDNA and nuclear *RAG1*. (A) Map of sampled *A. fragilis* and *A. colchica*, and two mtDNA haplogroups of the latter. (B) Mitochondrial DNA (*ND2*) phylogeny of all *Anguis* species. (C) Maximum-likelihood tree of the nuclear *RAG1* marker of *A. fragilis* and *A. colchica*. In the phylogenetic trees: samples from the Baltic Rim region are in bold; eight-symbol codes refer to comparative data from GenBank (accession numbers), followed by a haplotype/genotype/isolate code (and country of origin in *RAG1*); filled and open node symbols represent high (⩾95%) and low (<70%) bootstrap support of main clades, respectively; heterozygotes in *RAG1* were phased into gametic alleles, and they are given as a/b suffix at the sample code. Note the two hybrid individuals from Hungary placed in grey boxes (Szabó and Vörös, 2014). No hybrids were detected in the Baltic samples.

Table 1. Samples, localities, coordinates, haplogroups, haplotypes, and GenBank accession numbers. Haplogroups follow Jablonski et al. (2016, 2017), ICE = Illyrian-Central European haplogroup, Roman numerals denote haplogroups of the Carpathian lineage of *A. colchica incerta.*

Anguis	Sample	Country	Locality	°N	°E	Haplogroup (mtDNA)	*ND2* haplotype*	*ND2* GenBank	*RAG1*-phased haplogroup	*RAG1* GenBank
fragilis	TP1910	Latvia	Brušvitu ciems, Nica	56.194	21.098	ICE	f1	MW595722	*fragilis* 1	MW595740
fragilis	VG434	Latvia	Kemeri, site 1	56.953	23.529	ICE	f1	MW595723	*fragilis* 1	MW595741
fragilis	VG435	Latvia	Kemeri, site 2	56.956	23.543	ICE	f1	MW595724	*fragilis* 1	MW595742
fragilis	VG436	Latvia	Kemeri, site 3	56.928	23.487	ICE	f1	MW595725	*fragilis* 1	MW595743
fragilis	VG437	Latvia	Kemeri, site 4	56.920	23.470	ICE	f1	MW595726	*fragilis* 1	MW595744
fragilis	VG438	Latvia	Kemeri, site 5	56.919	23.470	ICE	f1	MW595727	-	-
fragilis	VG439	Latvia	Kemeri, site 6	56.917	23.466	ICE	f1	MW595728	*fragilis* 1	MW595745
fragilis	3720	Russia	Bagrationovsk, Kaliningrad Oblast	54.372	20.639	ICE	f1	MF817477	*fragilis* 1	MW595746
fragilis	696	Sweden	Mörby Gård, Stockholm	59.120	18.170	ICE	f1	MF817454	-	-
colchica	A107	Lithuania	Marcinkonys	54.04	24.44	IV	c6	FJ666581	*colchica* 2	MW595747
colchica	A108	Lithuania	Marcinkonys	54.04	24.44	IV	c6	FJ666581	*colchica* 1/2	MW595748
colchica	A109	Lithuania	Paluše	55.33	26.10	IV	c6	FJ666581	*colchica* 1/2	MW595749
colchica	A110	Lithuania	Paluše	55.33	26.10	IV	c6	FJ666581	*colchica* 2	MW595750
colchica	9736	Latvia	Gauja	57.156	24.291	IV	c6	MW595729	*colchica* 2	MW595751
colchica	9737	Latvia	Vecsikeli	55.892	26.846	IV	c6	MW595730	*colchica* 2	MW595752
colchica	9738	Latvia	Kraukleva	57.007	27.288	III	Aro09	MW595731	*colchica* 1/2	MW595753
colchica	9739	Latvia	Vejini	57.374	27.050	III	9739	MW595732	*colchica* 1	MW595754
colchica	TP1904	Estonia	Rohuküla	58.911	23.445	III	Aro09	MW595733	*colchica* 1/2	MW595755
colchica	Afi01	Finland	Seitseminen	61.931	23.437	III	Aro09	MW595734	-	-
colchica	TP1901	Finland	Loviisa	60.411	26.396	III	Aro09	MW595735	*colchica* 2	MW595756
colchica	TP1902	Finland	Pernaja	60.500	25.820	III	Aro09	MW595736	*colchica* 2	MW595757
colchica	TP1903	Finland	Miehikkälä, Laisniementie	60.634	27.884	III	Aro09	MW595737	*colchica* 1/2	MW595758
colchica	TP1905	Finland	Poiksilta	61.789	29.764	III	Aro09	MW595738	*colchica* 2	MW595759
colchica	TP1906	Finland	Kemiönsaari	60.055	22.437	III	Aro09	MW595739	*colchica* 2	MW595760

*Following the first published appearance (Gvoždík et al., 2010; Jablonski et al., 2016); 9739 is a new haplotype.

al., 2017), only the haplogroup I (detected in Poland) was not detected in the Baltics. The haplogroup IV corresponded to a single haplotype (c6), which is known to occur widely from the southern Carpathians to Lithuania, and now northern coastal Latvia. The haplogroup III was represented by two haplotypes, one (Aro09) has been found before in the central Carpathians in Romania, western Belarus, and now in Latvia, Estonia and Finland. The second haplotype (9739) originating from north-eastern Latvia was a new haplotype, different by one substitution from Aro09.

In nuclear DNA, when the Baltic Rim *RAG1* sequences were analysed together with ten individuals from Hungary and one from Spain, all together formed two clades in *A. fragilis* and two clades in *A. colchica* (fig. 1C). The two species are not monophyletic in respect to each other in the studied *RAG1* marker, however, the topology did not receive high statistical support (bootstrap values for the four clades 52-67%). This is probably attributable to the overall low variation and incomplete lineage sorting in this marker. The Baltic Rim specimens were placed into three clades, one belonging to *A. fragilis* and two to *A. colchica*. The placements were in congruence to the mtDNA placements. No sign of hybridization between *A. fragilis* and *A. colchica* was detected among the individuals from the Baltic Rim. All individuals from *A. fragilis* were homozygous, bearing the same *RAG1* haplotype, and sharing the clade with some *A. fragilis* from Hungary. All five heterozygous slow worms from the Baltic Rim were *A. colchica*, and their gametic haplotypes originated from the two *A. colchica* clades (fig. 1C, table 1). One *A. colchica* clade contained also individuals from Hungary, while the second *A. colchica* clade was composed solely of the Baltic Rim individuals. In agreement with a previous publication (Szabó and Vörös, 2014), two individuals from Hungary were found to represent hybrids of *A. fragilis* and *A. colchica*. This was evidenced by the placements of each of the two gametic *RAG1* haplotypes into two different lineages, corresponding to the two parental species.

Szabó and Vörös (2014) identified several single nucleotide polymorphism (SNP) sites in *RAG1* that are potentially important to distinguish *A. fragilis* and *A. colchica*. They especially highlighted the positions 72 and 237 in their 1043 bp-long alignment. Nevertheless, we found out that it was not possible to diagnose the two species range-wide on the basis of a single, universal SNP in this marker. On the other hand, based on our inspection of the new and earlier *RAG1* sequences, we conclude that different SNPs might be useful for the species diagnostics in different geographic regions. As Szabó and Vörös (2014) correctly identified the positions 72 and 237 as diagnostic in Hungary, the position 312 works as a diagnostic SNP in the Baltic Rim, with *A. fragilis* bearing adenine (A) and *A. colchica* cytosine (C). No heterozygote, i.e. potential hybrid, was found in our Baltic samples in this SNP.

The widely distributed western and eastern slow-worm species (*A. fragilis*, *A. colchica*) are despite their rather cryptic morphology relatively old species, which diverged probably around the Miocene/Pliocene boundary, 5-6 Mya (Gvoždík et al., 2010; Lavin and Girman, 2019). They probably do not represent a sister-species pair (Gvoždík et al., 2013), but they are capable to form hybrid populations within their contact zones (Gvoždík et al., 2015). However, we did not detect any hybrids in the Baltic Rim, probably due to our limited sampling. The nearest localities of *A. fragilis* and *A. colchica* were found 50 km (by air) away from each other, west and east of Riga, respectively. The Daugava River might serve as a potential biogeographic barrier in the coastal region, however, a detailed population-genetic study is necessary to test this hypothesis. Even though we did not detect *A. fragilis* in Lithuania, its occurrence along the Baltic coast is expected based on the presence of this species in the south-westernmost corner of Latvia and in the Russian Kaliningrad Oblast exclave. It

is presently not clear if both species occur in the Kaliningrad Oblast and if *A. fragilis* occurs north of Riga. Based on the present sampling, it seems rather implausible that *A. fragilis* is present as north as Estonia and Finland, except for a possibility of colonizing these northern regions from Scandinavia. This hypothesis is open and needs testing based on further sampling. Such a scenario of crossing the Baltic Sea in the Åland region was found in the western and eastern evolutionary lineages of the snakes *Vipera berus* and *Natrix natrix* (Carlsson, Söderberg and Tegelström, 2004; Kindler, Bringsøe and Fritz, 2014). Similarly, the smooth snake (*Coronella austriaca*) is known only from Scandinavia and the Åland Islands, not reaching mainland Finland (Jablonski et al., 2019). A close genetic relationship of populations from Scandinavia (Sweden) and Finland was documented in the pool frog *Pelophylax lessonae* (Zeisset and Hoogesteger, 2018), suggesting also a possibility of the connection via the Åland Islands. Therefore, the Åland region and south-westernmost mainland Finland are of a particular importance to test a possible presence of *A. fragilis*.

Two mtDNA haplogroups in *A. colchica* suggest two postglacial colonization events from two refugial populations of this species. Together with *A. fragilis*, the eastern Baltic region has been colonized by at least three colonization events. Based on a broader framework of previously published studies (Jablonski et al., 2016, 2017), the most plausible scenario is that *A. fragilis* colonized the Baltic coast from the south, form the north-western Balkans via central Europe. On the other hand, *A. colchica* probably colonized the eastern Baltics from the south and east, from the Carpathians and East European Plain, respectively. Both Baltic *A. colchica* haplogroups are present also in the Carpathians, however, the haplogroup IV is so far known by a single haplotype. This suggests that the haplogroup IV reached both the Baltics and Carpathians from a refugium located elsewhere, probably in the East European forest steppes. This scenario needs to be tested with a better sampling from eastern Europe. Interestingly, the two haplogroups seem to have parapatric distributions in the eastern Baltics, with the haplogroup IV present in southern areas (Lithuania, Latvia) and the haplogroup III in northern areas (northern Latvia, Estonia, Finland). This is in contrast to the situation in eastern Poland, where three haplogroups occur within the same region (Jablonski et al., 2017).

Taking into account the presence of two slow-worm species (*A. fragilis*, *A. colchcia*) in the eastern Baltic region and existing threats to herpetofauna (e.g., Čeirāns and Pupins, 2019), the two slow-worm species should also be considered separately in conservation legislations of respective countries. The presence of both species is now confirmed in Latvia, and very plausible in Lithuania. The Kaliningrad Oblast exclave of Russia needs further data to confirm if both species are present there. However, it is evident that Russia harbours two slow-worm species, *A. fragilis* in the Kaliningrad Oblast and *A. colchica* in the main part of Russia (based on the nearby sampling from Finland and Latvia). A denser sampling is needed to clarify the situation in Estonia and westernmost Finland, especially the Åland Islands.

Acknowledgements. The research was supported by the Czech Science Foundation, grant No. 18-24544S, Ministry of Culture of the Czech Republic (DKRVO 2019-2023/6.V.c, National Museum, 00023272), and the Slovak Research and Development Agency under the contract No. APVV-19-0076. We would like to thank L. Choleva, M. Kolařík, J. Lokajová for additional samples, and H. Šifrová for help in the field. Samples were collected under the permits No. VARELY/2260/2018 (Finland) and Nos. 3.6/2019-N, 06/2020-E (Latvia).

References

Carlsson, M., Söderberg, L., Tegelström, H. (2004): The genetic structure of adders (*Vipera berus*) in Fennoscandia: congruence between different kinds of genetic markers. Mol. Ecol. **13**: 3147-3152.

Carvalho, S.B., Velo-Antón, G., Tarroso, P., Portela, A.P., Barata, M., Carranza, S., Moritz, C., Possingham, H.P. (2017): Spatial conservation prioritization of biodiver-

sity spanning the evolutionary continuum. Nat. Ecol. Evol. **1**: 0151. DOI:10.1038/s41559-017-0151.

Čeirāns, A., Pupins, M. (2019): Ongoing shrinkage and fragmentation in the geographic range of the natterjack toad, *Epidalea calamita*, in Latvia and the east Baltic region. Zool. Ecol. **29**: 65-70.

Flot, J.-F. (2010): SeqPHASE: a web tool for interconverting PHASE input/output files and FASTA sequence alignments. Mol. Ecol. Resour. **10**: 162-166.

Grillitsch, H., Cabela, A. (1990): Zum systematischen Status der Blindschleichen (Squamata: Anguidae) der Peloponnes und der südlichen Ionischen Inseln (Griechenland). Herpetozoa **2**: 131-153.

Groth, J.G., Barrowclough, G.F. (1999): Basal divergences in birds and the phylogenetic utility of the nuclear RAG-1 gene. Mol. Phylogenet. Evol. **12**: 115-123.

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., 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., Moravec, J., Zavadil, V., Jeřábková, L. (2015): Slepýš křehký a slepýš východní – výskyt v České republice [Common European Slow Worm and Eastern Slow Worm – distributions in the Czech Republic]. In: Fauna ČR, Plazi – Reptilia [Fauna of the Czech Republic, Reptiles – Reptilia], p. 275-278. Moravec, J., Ed., Academia, Praha (in Czech with English summary).

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.

Jablonski, D., Nagy, Z.T., Avcı, A., Olgun, K., Kukushkin, O.V., Safaei-Mahroo, B., Jandzik, D. (2019): Cryptic diversity in the smooth snake (*Coronella austriaca*). Amphibia-Reptilia **40**: 179-192.

Jablonski, D., Najbar, B., Grochowalska, R., Gvoždík, V., Strzała, T. (2017): Phylogeography and postglacial colonization of Central Europe by *Anguis fragilis* and *Anguis colchica*. Amphibia-Reptilia **38**: 562-569.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., Drummond, A. (2012): Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics **28**: 1647-1649.

Keskin, E., Hayretdağ, S., Çiçek, K., Ayaz, D., Tok, C.V. (2013): Genetic structuring of *Anguis fragilis* (L., 1758) inhabiting in the north of 40° north latitude in Turkey. Mitochondrial DNA **24**: 565-576.

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.

Kozlov, A.M., Darriba, D., Flouri, T., Morel, B., Stamatakis, A. (2019): RAxML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. Bioinformatics **35**: 4453-4455.

Lanfear, R., Frandsen, P.B., Wright, A.M., Senfeld, T., Calcott, B. (2017): PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Mol. Biol. Evol. **34**: 772-773.

Lavin, B.R., Girman, D.J. (2019): Phylogenetic relationships and divergence dating in the glass lizards (Anguinae). Mol. Phylogenet. Evol. **133**: 128-140.

Mikulíček, P., Jablonski, D., Paleník, M., Gvoždík, V., Jandzik, D. (2018): Characterization of microsatellite markers in the genera *Anguis* and *Pseudopus* (Reptilia: Anguidae). Salamandra **54**: 158-162.

Renet, J., Lucente, D., Delaugerre, M., Gerriet, O., Deso, G., Abbattista, C., Cimmaruta, R. (2018): Discovery of an Italian slow worm (*Anguis veronensis* Pollini, 1818) population on a Western Mediterranean Island confirmed by genetic analysis. Acta Herpetol. **13**: 165-169.

Stephens, M., Scheet, P. (2005): Accounting for decay of linkage disequilibrium in haplotype inference and missing-data imputation. Am. J. Hum. Genet. **76**: 449-462.

Stephens, M., Smith, J.N., Donnelly, P. (2001): A new statistical method for haplotype reconstruction from population data. Am. J. Hum. Genet. **68**: 978-989.

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.

Townsend, T.M., Larson, A., Louis, E., Macey, J.R. (2004): Molecular phylogenetics of Squamata: the position of snakes, amphisbaenians, and dibamids, and the root of the squamate tree. Syst. Biol. **53**: 735-757.

Voipio, P. (1962): Multiple phaneromorphism in the European slow-worm (*Anguis fragilis*) and the distributional and evolutionary history of the species. Ann. Zool. Soc. Zool. Bot. Fenn. 'Vanamo' **23**: 1-20.

Zeisset, I., Hoogesteger, T. (2018): A reassessment of the biogeographic range of northern clade pool frogs (*Pelophylax lessonae*). Herpetol. J. **28**: 63-72.

Submitted: December 8, 2020. Final revision received: February 16, 2021. Accepted: March 3, 2021.
Associate Editor: Salvador Carranza.