

Short Note

The distribution and biogeography of slow worms (*Anguis*, Squamata) across the Western Palearctic, with an emphasis on secondary contact zones

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Abstract. The slow-worm lizards (*Anguis*) comprise five species occurring throughout most of the Western Palearctic. Although these species are relatively uniform morphologically – with the exception of *A. cephallonica*, which exhibits a quite unique morphology – they are genetically deeply divergent. Here, we provide detailed distribution maps for each species and discuss their biogeography and conservation based on updated genetic data and a robust distribution database. We pay particular attention to the so called ‘grey zone’, which typically represents secondary contact zones and in some cases confirmed or presumed hybrid zones. Four of the five species live in parapatry, while only two species, *A. cephallonica* and *A. graeca* from the southern Balkans occur in partial sympatry. Further research should focus on the eco-evolutionary interactions between species in contact, including their hybridization rates, to reveal deeper details of the slow-worm evolutionary and natural history.

Keywords: distribution atlas, hybrid zone, lizards, phylogeography, reptiles, taxonomy, UTM grid.

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The widespread use of molecular-genetic analyses has facilitated discoveries of many new species of reptiles even in well studied regions such as Europe (for a review see Speybroeck et al., 2020). Most typically, known populations became elevated to the rank of full species. However, the distributions of individual species within such species complexes often remain insufficiently known due to difficulties in their unambiguous morphological identification. Thus, the knowledge of the distribution ranges of many recently recognized species of European amphibians and reptiles remain insufficient, especially in regions of secondary contacts. The New Atlas of Amphibians and Reptiles of Europe (hereafter the New Atlas) revised the distributions of amphibians and reptiles and identified knowledge gaps in their ranges (Sillero et al., 2014a). Despite the huge increment in the chorological information provided by the New Atlas, distributions of some species groups have remained unresolved, and in need of further updates (Wielstra et al., 2014, 2018; Mizsei et al., 2018; Sillero et al., 2018). The legless lizards known as slow worms (*Anguis*, Anguidae) represent such an example.

Anguis fragilis Linnaeus, 1758 used to comprise two to three subspecies (or morphotypes, depending on the taxonomic authority) of uncertain geographical distributions (e.g., Wermuth, 1950; Voipio, 1962; Džukić, 1987; Cabela and Grillitsch, 1989). The Peloponnese Slow Worm (*A. cephalonica* Werner, 1894;

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formerly known as *A. fragilis peloponnesiacus* Štěpánek, 1937) had been recognized as the only distinct slow-worm species beside *A. fragilis* since its elevation to the species level in the 1990s (Grillitsch and Cabela, 1990; Mayer et al., 1991). *Anguis fragilis* and *A. cephallonica* are genetically distinct and relatively well-diagnosable morphologically: the latter species has a slender body with a characteristic lateral colour pattern formed by a chocolate-brown undulated line in the anterior part of the body, and a higher number of scale rows around the mid-body [30–36 (Štěpánek, 1937; Wermuth, 1950; Grillitsch and Cabela, 1990) vs. 24–30, rarely 22–31 in *A. fragilis* sensu lato (Džukić, 1987; Sos, 2010; Gvoždík et al., 2013)]. *Anguis cephallonica* was the only species mapped separately in the New Atlas as well as in the first version of the Atlas (Gasc et al., 1997). *Anguis fragilis* sensu lato was distinguished as a species complex based on the mitochondrial (mtDNA) and nuclear (nDNA) molecular data by Gvoždík et al. (2010), initially recognizing five taxa with three of them at the species level (names were resurrected from the synonymy of *A. fragilis*). The Common European Slow Worm (*A. fragilis* sensu stricto) occurs in the western part of Europe. This species is characterised by 24–26 (less often 22–28) scale rows around the mid-body (Sos, 2010; Gvoždík et al., 2013; Gvoždík and Moravec, 2015; Benkovský et al., in revision). The Eastern Slow Worm (*A. colchica* (Nordmann, 1840)) occurs in the eastern part of Europe, Anatolia and the Ponto-Caspian region. The south-western populations have 28–30 (less often 26–31) scale rows around the mid-body (Sos, 2010; Gvoždík and Moravec, 2015; Moravec and Gvoždík, 2015; Benkovský et al., in revision), although Baran (1977) found only 26–27 scale rows in the southern populations from northern Turkey. It is presently not clear whether the difference in the scale count reflects environmentally-induced variation or evolutionary differentiation. Currently, three subspecies corresponding to evolutionary lineages are recognized: *A. colchica*

(Nordmann, 1840) is ascribed to populations from the Caucasus; *A. colchica orientalis* Anderson, 1872 to the southern Caspian populations; and *A. colchica incerta* Krynicki, 1837 to the remaining widespread populations, mostly distributed within the European range of *A. colchica*. A yet-unnamed lineage at the subspecies level was identified in south-eastern Bulgaria (Jablonski et al., 2016), distributed further eastward into northern Anatolia (our unpublished data). The Greek Slow Worm (*A. graeca* Bedriaga, 1881) occurs in the southern Balkans and has typically 24–26 (less often up to 28) scale rows around the mid-body (Cabela and Grillitsch, 1989; Grillitsch and Cabela, 1990). Later, based on mtDNA, nDNA, and morphological data (Gvoždík et al., 2013), the Italian Slow Worm (*A. veronensis* Pollini, 1818) was recognized with the distribution in the Italian Peninsula and south-eastern France. This species has typically 24–26 (less often up to 28) scale rows around the mid-body, a relatively longer tail – when complete – than the phenotypically similar *A. fragilis* (Gvoždík et al., 2013), and it seems to have particularities in its colour pattern (P.G., pers. obs.). Morphological variation of the latter four species is yet little known, and morphological identification remains difficult in most cases. Therefore, a comprehensive morphological analysis of the entire genus is needed.

The geographical distribution of what used to be “*A. fragilis*” is well understood in a broad sense (Völkl and Alfermann, 2007; Sillero et al., 2014a). The map in the New Atlas was presented as a single map for “*Anguis colchical/fragilis/graeca*” (at that time the very recently revalidated *A. veronensis* was not listed yet). However, recent phylogeographical studies (Gvoždík et al., 2010, 2013, 2021; Szabó and Vörös, 2014; Thanou, Giokas and Kornilios, 2014; Jablonski et al., 2016, 2017; Thanou et al., 2021) provide further information allowing us to update the distributions of all individual slow-worm species. We delineate the ranges of the five slow-worm species after reviewing

available genetic and phylogeographical data and compiling a new extensive dataset on the distribution of slow worms. Our data update the *Anguis* maps in the New Atlas, and allow us to discuss the biogeography and conservation of slow worms. We also identify areas where further research should be prioritized, in particular to better understand eco-evolutionary interactions in secondary contact zones, including the degree of hybridization.

We compiled a distribution database for each species (supplementary table S1). As a starting point, we collected data from the published studies, where species identities were carefully verified (see below), and from our own unpublished genetic, morphological, and distributional databases for all species. We also sequenced a mtDNA fragment (*ND2*) of eleven samples from regions bordering distributions of two taxa (Jablonski et al., 2016) to refine localizations of contact zones in the Balkans (supplementary table S2) following the methodology of previous studies (Gvoždík et al., 2010; Jablonski et al., 2016). New nucleotide sequences were deposited in GenBank (acc. nos. MZ728058–MZ728068). Additional distribution data were gathered from museum collections, literature, and communications to us by experienced colleagues (examined museum data and data sources given in supplementary table S1). We also included the data from the New Atlas from its online repository NA2RE (<http://na2re.ismai.pt>; Sillero et al., 2014b), and with data from the public online databases – generally available at a high resolution: Global Biodiversity Information Facility (GBIF, accessible via www.gbif.org), Hungarian National Amphibian and Reptile Mapping website (www.herpterkep.mme.hu), iNaturalist (www.inaturalist.org), Nature observation platform of the Latvian Fund for Nature (www.dabasdati.lv), OpenHerpMaps (www.openherpmaps.ro), and Ukrainian Biodiversity Information Network (www.ukrbn.com). We classified the species records based on the geographical origin and our knowledge of

the species distributions from recent molecular works and, to a lesser extent, morphological studies (Gvoždík et al., 2010, 2013, 2015, 2021; Keskin et al., 2013; Szabó and Vörös, 2014; Thanou, Giokas and Kornilios, 2014; Thanou et al., 2021; Jablonski et al., 2016, 2017; Mikulíček et al., 2018; Renet et al., 2018; Benkovský et al., in revision). This led to five species-based categories. Because the majority of the available molecular studies are biased toward the information gained from mtDNA, we tagged the distribution points from the identified contact zones as the sixth category ‘unidentified’ to account for possible gene flow and/or mtDNA introgression in the contact zone areas [covering approximately two grid cells (see below) or more if contact zone is not well known, e.g., in the Baltics]. Mitochondrial DNA often provides correct species identifications in Eurasian reptiles (Kindler et al., 2013), and generally agrees with the signal of the nDNA markers used in *Anguis* studies (Gvoždík et al., 2010, 2013, 2021; Szabó and Vörös, 2014; Thanou, Giokas and Kornilios, 2014; Thanou et al., 2021). However, the data from the contact zones must be treated with caution, as individuals with discordant information between mtDNA and nDNA (a sign of hybridization) have been detected, specifically: *A. fragilis* and *A. colchica* in the Pannonian Basin (Hungary, Szabó and Vörös, 2014) and the western Outer Subcarpathian depression (Czech Republic, Gvoždík et al., 2015); *A. fragilis* and *A. veronensis* in the south-eastern Alps (Italy, Slovenia, Gvoždík et al., 2013); and *A. fragilis* and *A. graeca* in the southern Dinarides (Montenegro, Mikulíček et al., 2018). There is presently no evidence for hybridization between *A. cephalionica* and *A. graeca*, despite the two species live in partial sympatry in the Peloponnese (Thanou, Giokas and Kornilios, 2014; Thanou et al., 2021). Distribution records of questionable taxonomic identities (evaluated in the context of the overall biogeographical pattern) were also included in the category ‘unidentified’, in particular: some records

from the Peloponnese, north-western Anatolia, wider areas in the Baltics (insufficiently sampled region; Gvoždík et al., 2021), land-bridge islands connecting Finland and Sweden, and a presumably introduced population in Ireland. These unidentified records represent a ‘grey zone’ in our knowledge, i.e., areas deserving further research attention. They are denoted by light grey points in maps (fig. 1).

Finally, we mapped these six slow-worm categories (five species and grey zone) at the official Universal Transverse Mercator (UTM) grid of 50×50 km from the European Environmental Agency (www.eea.europa.eu) following Sillero et al. (2014a). In these standard Atlas maps, we did not consider the distribution data from localities falling outside of Europe as defined by Sillero et al. (2014a). Chorotype affiliations followed the approaches

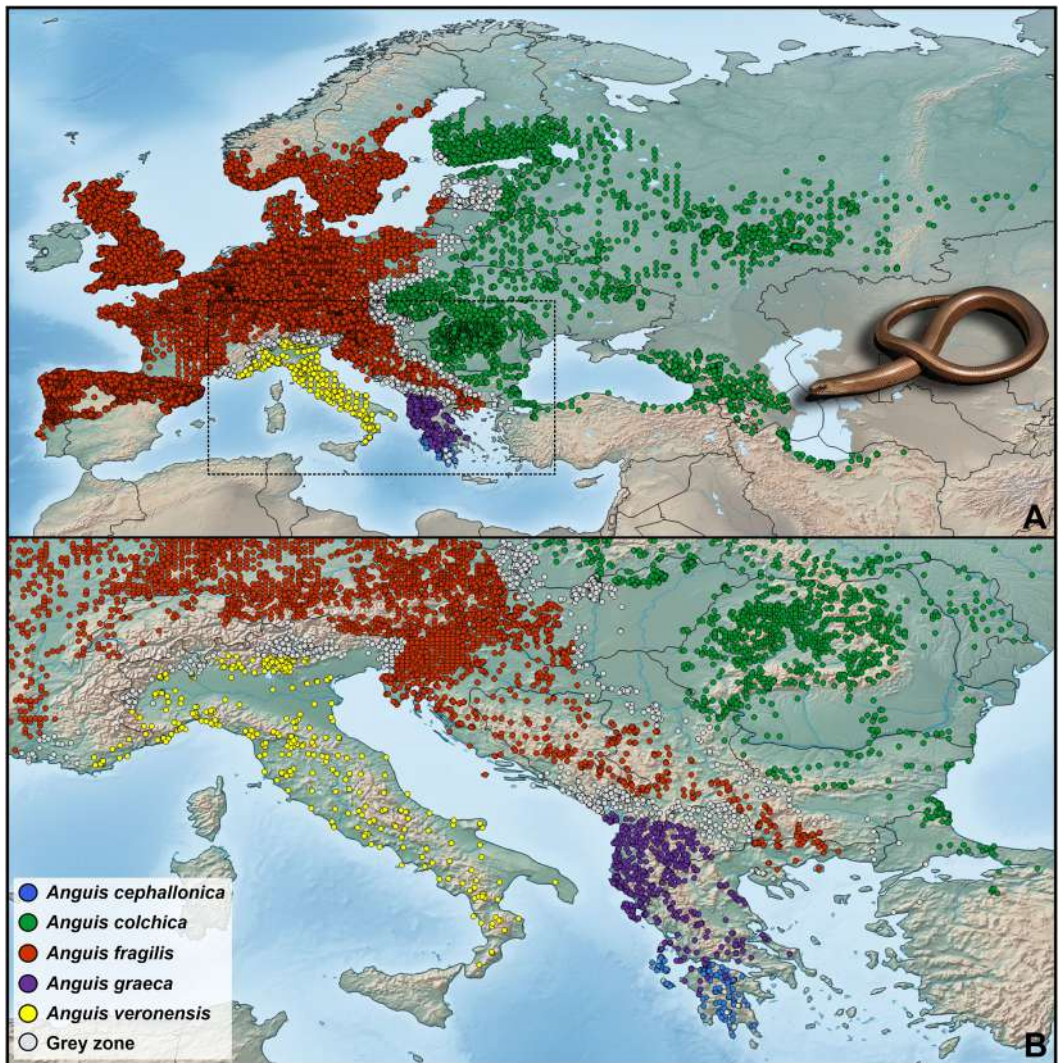


Figure 1. Distribution records of slow worms, including their extra-European ranges: *Anguis cephallonica*, *A. colchica*, *A. fragilis*, *A. graeca*, and *A. veronensis*. Complete raw data are provided in supplementary table S1. Data points are colour-coded according to species following earlier publications (e.g., Gvoždík et al., 2013; Jablonski et al., 2016), light grey points represent taxonomically unassigned distribution records, i.e., ‘grey zone’ where further research is needed, typically secondary contact zones. The boxed area in panel A is shown in more detail in panel B.

by Sindaco and Jeremčenko (2008) and Sindaco et al. (2013). To compare the total extent of occurrence of the species, including the non-European areas, we estimated the distribution ranges by connecting the marginal distribution points in QGIS (QGIS Development Team, 2020).

Our final dataset, which includes the non-European areas, comprised 28 383 distribution data points (fig. 1, supplementary table S1). However, some areas, especially in the east, have still remained poorly represented. Within the European area and compared to Sillero et al. (2014a), our final database provides 15 808 new records and fills 418 additional grid cells (22.8% increment). We gathered and plotted 28 159 distribution records in 2249 grid cells (37.7% of total grid cells), with 185/15 (0.3%) in *A. cephallonica*; 4166/847 (14.2%) in *A. colchica*; 21 232/1069 (17.9%) in *A. fragilis*; 532/49 (0.8%) in *A. graeca*; 367/90 (1.5%) in *A. veronensis*; and 1677/179 (3.0%) in grey zone (supplementary table S3). An overview of the distribution maps following the standards of the New Atlas of the five slow-worm species and grey zone is provided in fig. 2, while the higher resolution maps can be found in supplementary figs. S1-S6. New distribution data of all slow-worm species can now be integrated into the NA2RE database system (<http://na2re.ismai.pt>), the web-based spatial data infrastructure underpinning the New Atlas (Sillero et al., 2014b).

According to the summarized data, the distributions of individual species differ substantially in their extent of occurrence (fig. 1). *Anguis colchica* has the widest range (approx. 3 300 000 km² whole range; 2 900 000 km² within Europe) from Central Europe (Czech Republic, Poland and Slovakia) to the east of the Central Ural Mountains (Russia) and from Finland to Anatolia (Turkey) and the southern Caspian region (Iran). This species biogeographically belongs to the Siberian-European (E European) + Western Asian mountains transition zone chorotype. *Anguis fragilis*

(2 100 000 km²) is distributed from the westernmost point of continental Europe (Portugal) to the Baltics (Latvia), and from Scandinavia (Norway, Sweden) to Chalkidiki and Western Thrace (Greece), and possibly to north-western Turkey (not yet confirmed) and belongs to the Siberian-European (W European) chorotype. The remaining three species have much smaller ranges. *Anguis veronensis* (183 000 km²) has the western margin of its range in south-eastern France, while most of the range is located in the Apennine Peninsula (Italy), and belongs to the Mediterranean (Italian) chorotype. It remains yet to be confirmed whether this species enters the Balkan countries. A sign of gene flow from *A. veronensis* was documented in a slow-worm population in north-western Slovenia while *A. fragilis* is expected more to the south (Gvoždík et al., 2013; but see the discussion below). *Anguis graeca* (65 000 km²) has the core of its distribution range in Greece and Albania, while it is partially present also in North Macedonia and Montenegro. The southern and northern limits are unclear, probably located in central Peloponnese and at least in Montenegro (in the north, but possibly going further to southern Bosnia and Herzegovina, and Croatia; see below). *Anguis cephallonica* has the smallest range (15 000 km²) limited to the Peloponnese Peninsula and the islands of Kefalonia, Ithaca, and Zakynthos. Both endemic Balkan species belong to the Mediterranean (Balkanian) chorotype.

All slow-worm species are similarly euryecious in terms of altitudinal distribution. They occur from sea level or even from depressions below sea level to high elevations. The highest recorded elevations correspond to mountainous habitats: *A. cephallonica* 1340 m a.s.l., *A. colchica* around 2700 m a.s.l. (within the Asiatic range; around 2000 m a.s.l. within the European range), *A. fragilis* around 2700 m a.s.l., *A. graeca* around 2300 m a.s.l., and *A. veronensis* 2050 m a.s.l. In the north, slow worms occupy mainly lowlands, while they are present both at sea level and in high mountains in the

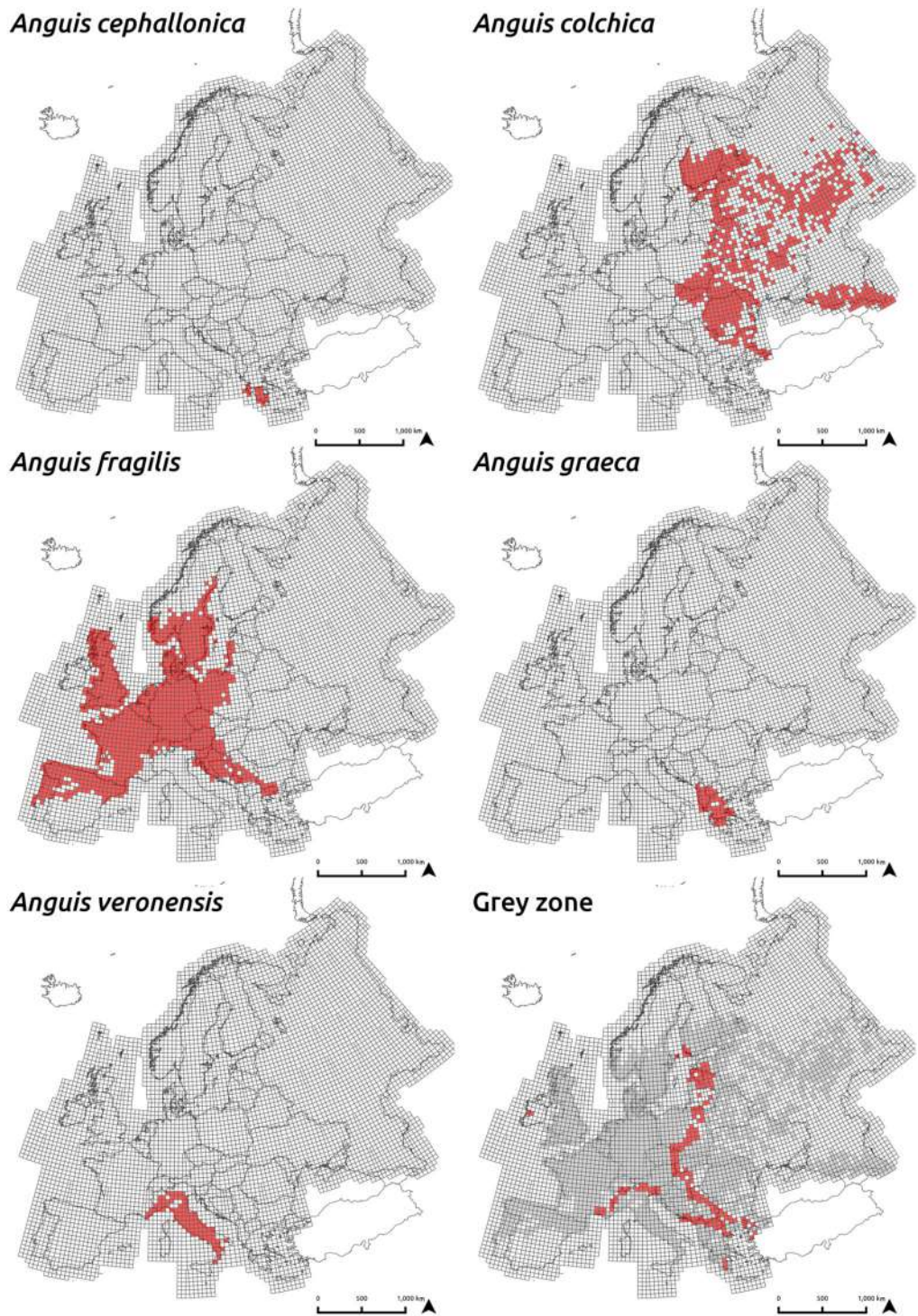


Figure 2. Distribution maps for the five slow-worm species in Europe, in the format of the New Atlas of Amphibians and Reptiles of Europe (grid cell 50×50 km; Sillero et al., 2014a). The 'grey zone' map shows approximate locations of secondary contact zones between parapatric species, and areas deserving further research. Individual maps in a higher resolution can be found in supplementary figs. S1-S6.

south. Thus, it seems that they are more limited by the microclimate in the habitat, with a preference for rather wet and relatively cold conditions, than by general regional climatic conditions.

The newly sequenced samples from the Balkans refined the known distributions in north-eastern Greece, north-eastern Albania, and western Slovenia. Three samples from the Evros region and the Chalkidiki Peninsula corresponded to the South Balkan mtDNA haplogroup of *A. fragilis* (sensu Jablonski et al., 2016), and bore the earlier documented *ND2* haplotypes. Seven samples from western Slovenia, where a possible presence of *A. veronensis* haplotypes was expected (an admixed nDNA genotype was found near Lake Bohinj; Gvoždík et al., 2013), bore only *A. fragilis* haplotypes (of which four were detected here for the first time). However, they belonged to three different haplogroups (supplementary table S2). This provides further evidence for an earlier hypothesis (Jablonski et al., 2016) that the region served as a Pleistocene refugium for *A. fragilis*. The presence of *A. veronensis* in western Slovenia cannot be excluded yet and deserves further attention. Several mostly Italian vertebrates, including reptiles and amphibians, are present in western Slovenia, e.g., *Vipera aspis* (Vogrin, 1997), and we expect that this can be the case for *A. veronensis* too. Some other predominately Italian species are distributed even more to the south-east along the Adriatic coast in Croatia as the Adriatic Sea was approximately 150 m below the present level during the last glacial period, allowing a direct land connection between the Italian and Balkan Peninsulas (e.g., *Hierophis viridiflavus*, Rato et al., 2009; *Rana latastei*, Ficetola et al., 2007). This biogeographical history could explain a possible presence of *A. veronensis* or its genomic footprint (sensu Wielstra, 2019) even in north-western Croatia. The sample from north-eastern Albania carried a haplotype of *A. graeca* (a newly detected haplotype) and allowed placement of the contact zone between *A. graeca* and

A. fragilis further north compared to Jablonski et al. (2016). The new haplotype showed a close relationship to the haplotypes from northern-central Albania and North Macedonia (supplementary table S2). However, the whole region from southernmost Bosnia and Herzegovina, and Croatia, through Montenegro, northern Albania, North Macedonia, to the Chalkidiki Peninsula and Thrace needs further investigation to obtain a better characterization of the contact zone between *A. graeca* and *A. fragilis*. The northern areas deserve special attention as incongruence between mtDNA and nDNA was documented from Montenegro (Mikulíček et al., 2018) and morphological data suggest possible occurrence of *A. graeca* in the southern tips of Bosnia and Herzegovina, and Croatia, including Mljet Island (Džukić, 1987; as *A. fragilis colchicus* at that time). Interestingly, the newt *Lissotriton graecus* has a similar distribution in this region (Wielstra et al., 2018).

Following our review of available data, we highlight all areas that deserve more detailed investigation – ‘grey zone’ (fig. 2F). Grey zone typically represents presumed contact zones, and at least in some regions also hybrid zones (e.g., Gvoždík et al., 2013; Szabó and Vörös, 2014), between two slow-worm species, or areas harbouring slow-worm populations of unknown taxonomic identity. The longest contact zone (of more than 2000 km) is found between *A. fragilis* and *A. colchica*, running from the Baltic region, southward through Central Europe to the north-eastern Balkans (briefly, from the Baltic to Marmara Sea). The second-longest contact zone (about 800 km) is the one between *A. fragilis* and *A. veronensis*, running from western Slovenia to southern France along the southern slopes of the Alps. The third secondary contact zone is found between *A. fragilis* and *A. graeca* (approximately 500 km long), extending from Montenegro (possibly southern Bosnia and Herzegovina, and Croatia)

through northern Albania and North Macedonia to northern Greece. We also identified several areas harbouring populations of unknown taxonomic identities (or phylogeographical origins). This is particularly the case of the Åland Islands (and possibly the Turku archipelago and nearby mainland Finland), the region between Sweden and Finland in the Baltic Sea, where the occurrence of *A. fragilis* is anticipated based on the phylogeographical patterns of other species of herpetofauna (Gvoždík et al., 2021). Ireland is another area of potential biogeographical and/or conservation importance. Traditionally, slow worms were thought to be absent from Ireland. However, a supposedly introduced population of *A. fragilis* of unknown origin occurs in the Burren (western Ireland; McGuire and Marnell, 2000). In view of more recent observations of slow worms from various places in Ireland, and along with a lack of evidence of human-mediated introductions, a refugial character of the Burren population cannot be ruled out (Parry, 2020). The taxonomic identity of the populations in the southern Marmara region is also unclear, with the possibility of *A. fragilis* reaching Turkish Thrace or even north-western Anatolia (based on the biogeographical patterns in other herpetofauna, e.g., newts of the *Lissotriton vulgaris* complex, *Triturus cristatus* complex, or *Bombina* toads; Fijarczyk et al., 2011; Wielstra et al., 2014, 2017, 2018; Pabijan et al., 2015, 2017; Kariş et al., 2017; Dufresnes et al., 2021). In the northern Peloponnese, *A. graeca* was confirmed to live in sympatry with *A. cephallonica*. However, the possible occurrence of *A. graeca* (or “stout *A. fragilis*” as stated in the past) has been reported deep from the *A. cephallonica* range in the central and even southern Peloponnese (Koppitz, 2018). *Anguis cephallonica* and *A. graeca* apparently do not hybridize, or only rarely. Thanou, Giokas and Kornilios (2014) and Thanou et al. (2021) found no genetic evidence of hybridization in the northern Peloponnese when comparing mtDNA and nDNA, including genome-wide data, and concluded

that the two species have a long and complete reproductive isolation. However, surprisingly, they experienced difficulties in morphologically distinguishing some individuals of *A. cephallonica* from *A. graeca* (Thanou, Giokas and Kornilios, 2014). Thanou et al. (2021) also identified certain differences in the ecological niche of the two species, with *A. cephallonica* being better acclimatized to high temperatures. Understanding the extent of the sympatry of *A. cephallonica* and *A. graeca* in the central and possibly southern Peloponnese requires further research.

Slow worms are semi-fossorial lizards, and consequently their ecology, behaviour, niche preferences, and distribution dynamics still remain poorly known. The same is true for the threat level in the majority of the populations. Conservation programs should consider not only the taxonomy of the five slow-worm species but also levels of their genetic variation. For example, the highest genetic diversity in *A. fragilis* populations can be found in the extreme north-western Balkans and surrounding areas (Slovenia and bordering regions in Croatia, Hungary, Italy), whereas in *A. colchica* these areas occur in the southern Carpathians and north-eastern Balkans/Balkanides in Romania and Bulgaria (Jablonski et al., 2016). Moreover, two deeply divergent evolutionary lineages of *A. colchica* with allopatric (or parapatric?) distributions are found in the Balkans, specifically in Bulgaria (Jablonski et al., 2016). This calls for further evolutionary and taxonomic research in these areas. Finally, several European countries should update their national red lists to reflect the newly established taxonomy, and our distribution database and maps can provide them with the knowledge needed to update their national conservation legislation.

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