

The possible origin of the common wall lizard, *Podarcis muralis* (Laurenti, 1768) in Ukraine

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Abstract

The phylogenetic relationships and possible origin of a putative non-native population of *Podarcis muralis* in Ukraine were assessed based on sequences of the mitochondrial gene *cytochrome b*. Phylogenetic analysis showed that the Ukrainian lizards belong to two distinct mitochondrial lineages (haplogroups), both occurring within the Central Balkan clade, which includes most of central and south-eastern European populations. From overall three detected Ukrainian haplotypes, one haplotype share same genetic signal with the haplotype from the locality Bjala (Bulgaria), the other two are unique for Ukrainian population. Two of haplotypes correspond with haplogroup covering large geographic region of Bulgaria, Serbia, and Romania. These results reinforce previous findings that the species has the ability to establish new populations out of its native range. While most introductions to Germany and Britain have been deliberate, it appears likely that human transport of goods via the Danube river of goods is responsible for the range expansion into Ukraine.

Key Words

genetic diversity, human-mediated introduction, Lacertidae, natural dispersions

Introduction

Most terrestrial reptiles tend to have poor dispersal abilities (Steinitz et al. 2006; Qian 2009). However, many species are increasingly transported by humans, which promotes both short- and long-distance range expansion (Kraus 2009). Lizards are the most abundant and widespread reptiles (Novosolov et al. 2016) and many species live close to humans or in highly disturbed habitats. Moreover, they can withstand long periods of starvation and desiccation, and many use small cavities as refuge,

increasing their chances of being passively transported (McCue 2010; Silva-Rocha et al. 2019). It is, hence, not surprising that many lizard species have been introduced around the world. In some cases, repeated introductions of the same species increase the probability of admixture, which may enhance the invasive potential of introduced species (Kolbe et al. 2007; Michaelides et al. 2013) or increase the risk of hybridization with native species or lineages (Santos et al. 2019).

The common wall lizard, *Podarcis muralis* (Laurenti, 1768) (Squamata: Lacertidae) has the largest native

distribution of all species of the genus (Arnold et al. 2007; Sillero et al. 2014). The species occupies a great variety of habitats within the range from northern Iberia in the west and to north-western Turkey in the east, and from the Netherlands in the north to southern Italy (Schulte 2008). At its north-eastern limit (Romania), *P. muralis* occurs primarily along the Carpathian Mountains and in several sites in the Danube river valley and near the city of Dobruja (Gruschwitz and Böhme 1986; Schulte 2008). Remarkably, this lizard has successfully established populations in central and north-western Europe far outside its sub-Mediterranean native range (Schulte et al. 2012; Michaelides et al. 2013; Santos et al. 2019). These introductions are either deliberate by releasing individuals in the wild or by unintentional escape from captivity, or passive dispersal via vehicles, boats and railways (Burke and Deichsel 2008; Schulte et al. 2008; Jablonski et al. 2019; Santos et al. 2019). The high phenotypic variability of this species (Bellati et al. 2011) often hampers the detection of such introductions within the native range and makes it nearly impossible to establish the geographic origin of non-native populations or detect hybridization based on morphology alone. However, the high phylogenetic diversity of the species, structured in up to 23 clades (Salvi et al. 2013), allows identifying the source of putative introductions. As such, there is already evidence of cryptic introductions and lineage admixture with these allochthonous populations (Schulte et al. 2012; Michaelides et al. 2015; Beninde et al. 2018; Jablonski et al. 2019; Santos et al. 2019).

Since 2012, *Podarcis muralis* is known from the territory of Ukraine (southern part of Odessa region, Reni city; Matveev et al. 2013). This is 250 km from the natural range of this species (Constanța, Romania) (Schulte 2008). Small populations of *P. muralis* have been reported from northern Dobruja, near the current border of Romania and Ukraine, in the first half of the 20th century (Gruschwitz and Böhme 1986). The reports were confirmed later (Kotenko 1993; Strugariu et al. 2008; Tudor and Cozma 2011). Therefore, the aim of this study is to assess whether i) the Ukrainian populations are introduced or native, ii) discuss possible colonization routes and the origin of the species in Ukraine; and iii) deduce implications of these findings.

Material and methods

We collected a total of 21 *P. muralis* samples from Ukraine (n = 11) and Romania (n = 10) (Table 1). Specimens were collected in Odessa region (the district of Reni) in 2017–2018, Ukraine. *Podarcis muralis* was found in two different locations near the city of Reni; in an abandoned complex opposite the sea harbor and near water canals. Romanian samples (near the border with Ukraine) were collected in 2016–2017. That study area was chosen as the closest for *P. muralis* (Török 2010; Tudor and Cozma 2011), and a poorly sampled region within the native

Table 1. Novel sequences used in this study and their geographic position.

Accession number	Country	Locality	Haplogroup	Coordinates	
				N	E
MN866797, MN866798, MN866799, MN866802, MN866804, MN866805, MN866807	Ukraine	Reni	II	45.43	28.29
MN866800, MN866801, MN866803, MN866806	Ukraine	Reni	V	45.43	28.29
MN866808	Romania	Gaura cu Musca, Banat	IV	44.66	21.70
MN866809, MN866810	Romania	Măru	II	45.45	22.44
MN866811	Romania	Zăvoi	II	45.52	22.39
MN866812, MN866813, MN866814	Romania	Bănița	II	45.45	23.30
MN866815	Romania	Dâmbovicioara	II	45.44	25.22
MN866816	Romania	Coronini	III	44.67	21.68
MN866817	Romania	Voineasa	II	45.43	23.85

range. For further investigations, tail tips were collected from live specimens. Tissue samples were preserved in 96% ethanol.

Total genomic DNA was extracted from tissue samples with Qiagen DNeasy blood and tissue kit or following the standard saline method (Sambrook et al. 1989). A fragment of 656 bp of mitochondrial (mt) marker *cytochrome b* (*cyt-b*) was amplified by PCR using the primers for GluDG-A and cyt-b2 (Kocher et al. 1989; Palumbi et al. 2002). The PCR products were purified and sequenced by an external service (Beckman Coulter Genomics). The amplification conditions followed Jablonski et al. (2019). The sequences were checked and aligned with those from GenBank using ClustalW (Larkin et al. 2007). Sequence alignments were analyzed using the Maximum Likelihood (ML) approach. The first phylogenetic tree was built to infer the relationships between 21 *P. muralis* samples (from Ukraine and Romania) and 254 published sequences from the native species range (Michaelides et al. 2013; Salvi et al. 2013; Jablonski et al. 2019; Santos et al. 2019). The ML analysis was performed in MEGA-X (Tamura et al. 2011) with the heuristic search. The node support was calculated over 1000 bootstrap replicates. HKY+G was chosen as a model of sequence evolution selected for each dataset under the Bayesian Information Criterion (Hasegawa et al. 1985). Then, a second was constructed to get more precision inferring the relationships between study samples and 64 sequences from lizards in Eastern European countries with the accession numbers MG851915–MG851979, HQ652886–HQ652887 (Schulte et al. 2012; Jablonski et al. 2019). The ML analysis for second phylogenetic tree was also performed in MEGA-X but the model of sequence evolution selected was HKY (Hasegawa et al. 1985). *P*-distance between haplogroups II and III was calculated in MEGA-X. In ad-

dition to the tree-building method, we analyzed the genealogical relationships among haplotypes by means of a statistical parsimony network using the program TCS 1.21 and TcsBU-master (Clement et al. 2000; Santos et al. 2016), using only the second tree sequences. All new sequences were deposited in GenBank under the accession numbers present on Table 1.

Results

The final alignment includes 85 sequences with the length 656 bp. According to the haplotype-network analysis of

656 bp-long sequence alignment (Fig. 2), 23 mt haplotypes were detected within the Central Balkan clade structured into five main haplogroups (Figs 2, 3): haplogroup I found in the area from eastern Germany, Czech Republic, Slovakia, Hungary and northern Bosnia and Herzegovina (terminology sensu Jablonski et al. 2019); and four Balkan haplogroups (haplogroups II, III, IV, V) from western Romania, Serbia, Bulgaria and Ukraine. Four mt haplotypes were detected in the haplogroup I, including one slightly distant mt haplotype from northern Bosnia and Herzegovina. Ten mt haplotypes were detected in haplogroup II (western Romania, eastern Serbia, northern Bulgaria and Ukraine), three mt haplotypes in haplogroup

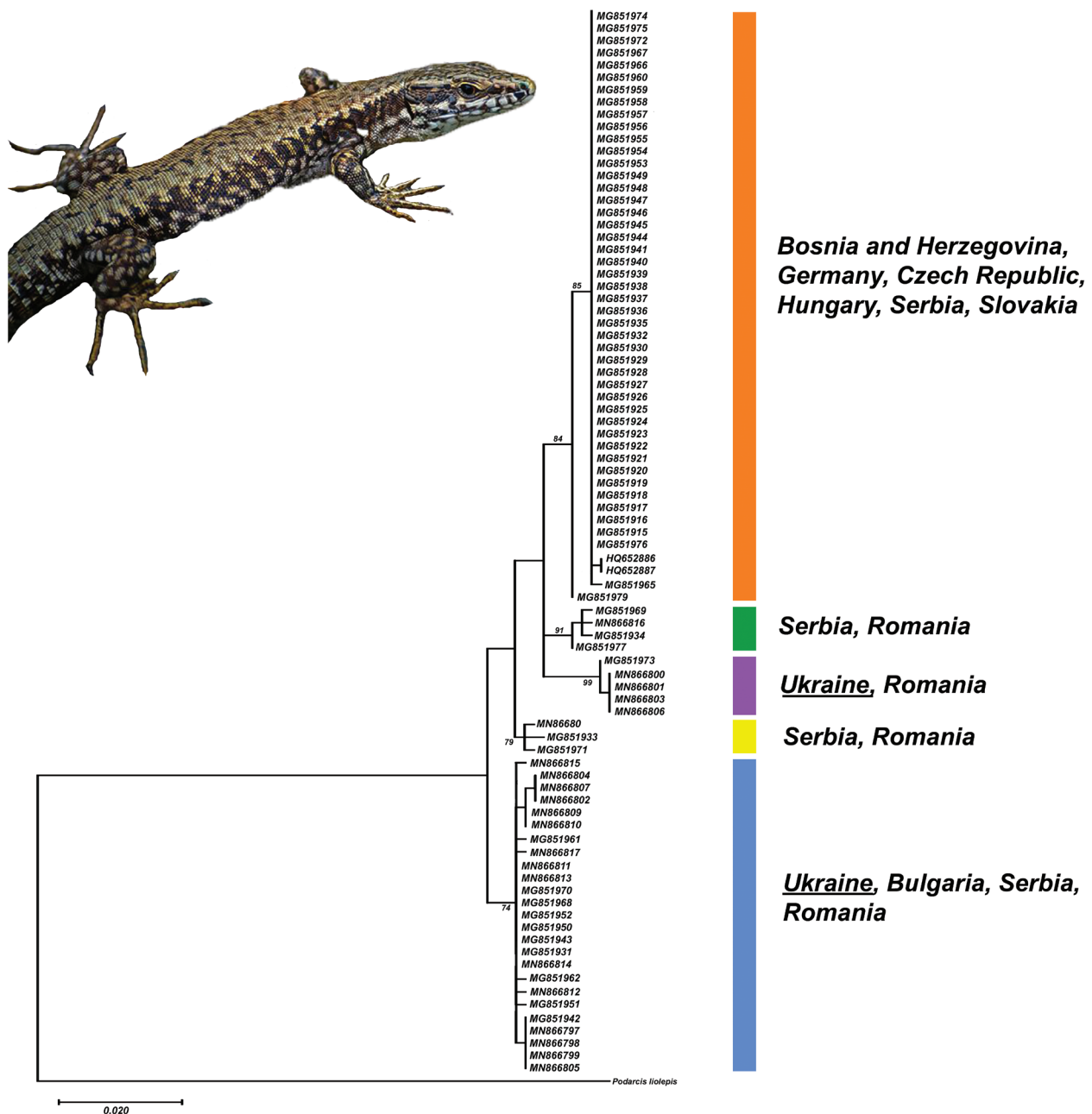


Figure 1. ML phylogenetic tree depicting the relationships between *cytochrome b* sequences haplotypes from Central Balkan clade of *Podarcis muralis* and those from Ukrainian introduced populations. Bootstrap support is indicated next to the nodes of interest. GenBank numbers follow previous phylogeographic studies (Schulte et al. 2012; Jablonski et al. 2019). Coloration of particular lineages corresponding with those used in Jablonski et al. (2019). Inset: an adult male from Reni, Ukraine.

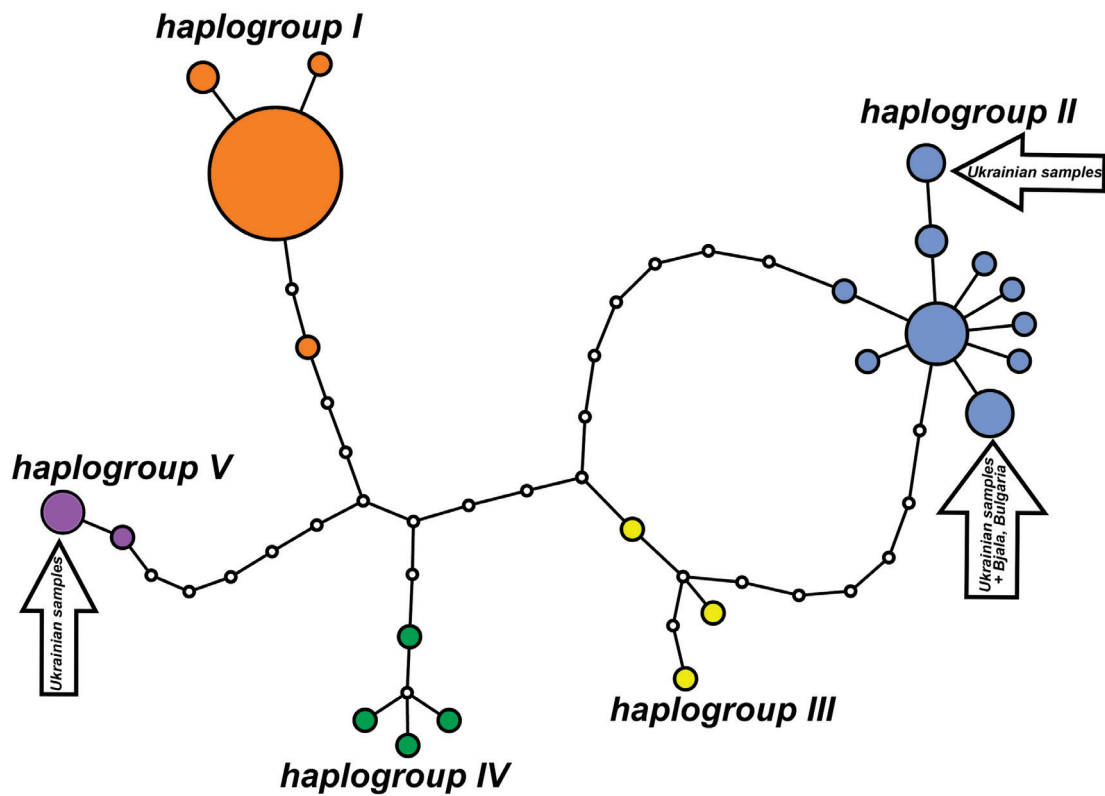


Figure 2. The main haplogroups of the Central Balkan clade. Circle size is proportional to the number of samples under the same *cytochrome b* haplotype. Open circles represent missing haplotypes. Colors of haplotypes follow colors on Fig. 1.

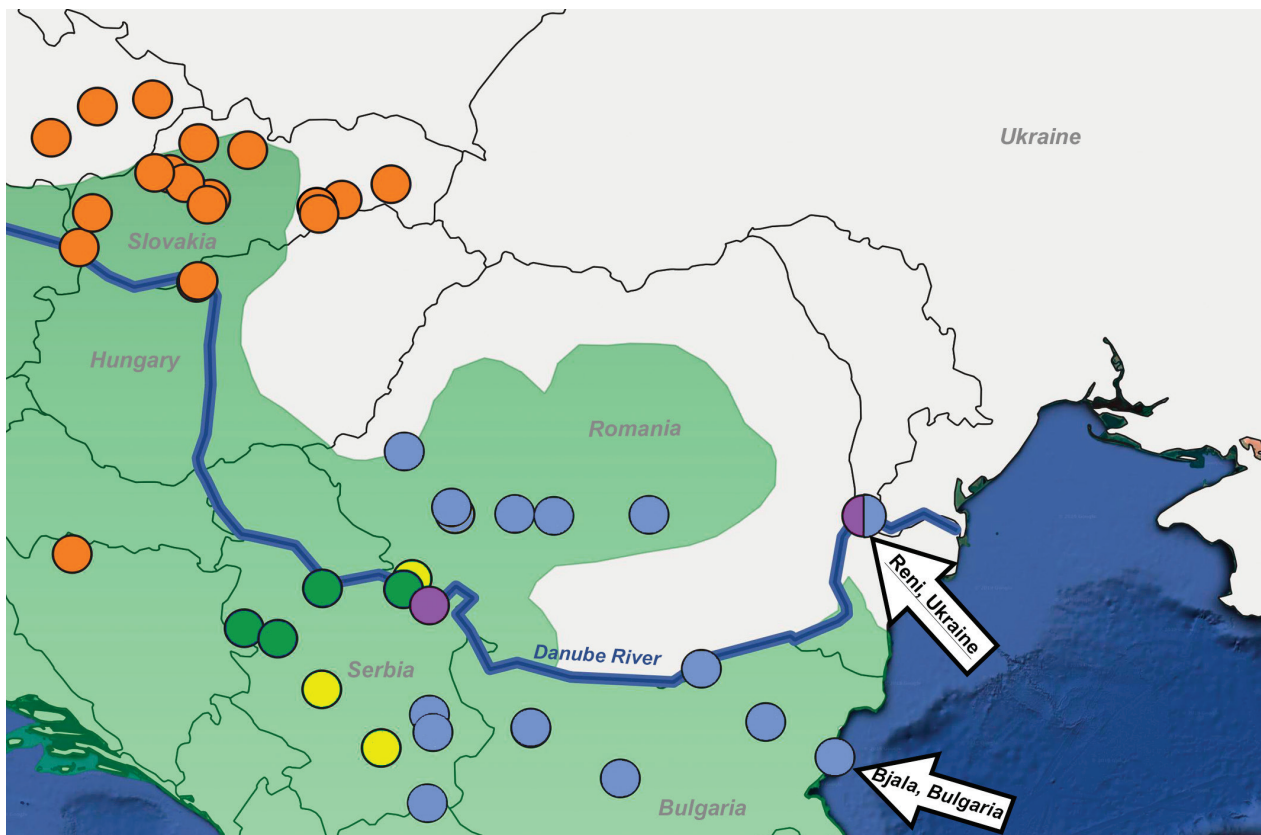


Figure 3. Geographical position of the main *cytochrome b* haplogroups of the Central Balkan clade in the studied area. Approximate species distribution is given in green shading (modified according to Jablonski et al. 2019).

III (Serbia and western Romania), four haplotypes in mt haplogroup IV (Serbian and western Romania), and two mt haplotypes in haplogroup V (near the Danube River in Romania and Ukraine).

The ML phylogenetic tree indicates that the Ukrainian lizards belong to two distinct mt lineages, both occurring within the officially called Central Balkan clade, which includes most of the lizards from Eastern Europe. Using only 85 sequences from Eastern Europe (only Central Balkan clade) it was revealed that the samples from Ukraine fall into two different mt haplotypes (Fig. 1). One mt haplotype corresponds to one lineage in Romania and the other corresponds to a mitochondrial lineage that is present across a large geographical range covering regions in Bulgaria, Serbia and Romania. The Romanian samples collected in this study are included in three different mt lineages. All samples from Ukraine form unique haplotypes except samples MN866797, MN866798, MN866799, that share haplotype with population from Bjala, Bulgaria (Fig. 2).

The most common mt haplotype of the haplogroup I is widely distributed across the Czech Republic, western, central, and eastern Slovakia, eastern Germany, Hungary and northern Bosnia and Herzegovina (20 localities, 48 individuals). Haplogroup II is composed of specimens from Romania, Serbia and Bulgaria. This group also includes some Ukrainian samples. The Ukrainian samples from Haplogroup V are very similar to haplotypes found in Romania (from the village of Svinita). Haplogroups II and V are separated from each other by 2% of uncorrected *p*-distance in their *cyt-b* sequences. Overall, Ukrainian samples belong at least to two different mt haplogroups with distinct locations within the native range (Fig. 3): haplogroup III is composed of samples from south-western Romania and southern Serbia and haplogroup IV is formed by samples from south-western Romania and central-western Serbia. The haplotype diversity is low in Ukraine (three mt haplotypes among 11 individuals) compared to the most likely populations of their source (17 mt haplotypes among 26 individuals from Romania, Serbia and Bulgaria).

Discussion

Our results confirm previous reports of several haplogroups that are not deeply diverged among *P. muralis* populations within the mtDNA clades in Eastern Europe. In a previous study (Jablonski et al. 2019), 24 mt haplotypes (1143 bp-long sequence alignment) were detected within the Central Balkan clade structured into five main haplogroups. We also confirmed five haplogroups (Fig. 2) and 23 mt haplotypes were detected within the Central Balkan clade. Regarding the putative allochthonous population in Ukraine, our results provide evidence of two different *cyt-b* haplogroups (Figs 2, 3): haplotype III closely related to one Romanian sample from Svinita and haplotype II related to a mitochondrial lineage wide-

spread across Bulgaria, Serbia and Romania. These two lineages from Ukraine are quite distant (~2% divergence) suggesting two separate introductions. However, it cannot be ruled out that the Ukrainian lizards originate from a location that harbors both haplotypes. More extensive samplings in other areas, near the Danube or in other localities in Romania, as well as additional genetic markers, are needed to discriminate between the two hypotheses.

These results confirm previous findings that common wall lizards are able to establish stable populations outside their native range. In Ukraine, this species was found only in the south-west of Odessa region (near the border with Romania). *Podarcis muralis* has saxicolous habits which helps the lizards to easily adapt to habitats constructed by humans, such as walls (Fig. 1). The rapid introduction of lizards on the European continent and beyond was not only the result of release of captive animals, but may have been facilitated by railways, roads and other means of transport of goods (Covaciu-Markov et al. 2006; Gherghel et al. 2009; Santos et al. 2019). Finding lizards near the port of Reni is consistent with transport by cargo boats on the Danube river. Network analysis supports the hypothesis that *P. muralis* from Ukraine were introduced from Romania. The hypothesis of passive transportation by cargo via the Danube river is here favored over a deliberate introduction. More intensive sampling along the Danube River is needed to better confirm this hypothesis. In any case, introduction from mid-short-distance, seems the most possible colonization pathway.

A considerable number of wall lizard introductions have already been identified, namely in Romania (Covaciu-Markov et al. 2006; Strugariu et al. 2008; Gherghel et al. 2009), Germany, Austria, Switzerland, France, Belgium, the Netherlands and Croatia (Schulte et al. 2012), Great Britain (Smith 1951; Stafford 1989; Michaelides et al. 2013) and even in the USA (Brown et al. 1995) and Canada (Allan et al. 2006). Evidence of repeated introductions has been found in some cases. For example, populations in southern England originate from at least nine separate native sources in France and Italy, but secondary introductions are common (Michaelides et al. 2015), while lineage admixture have been reported from NW Iberian Peninsula and Slovenia (Santos et al. 2019). In the case of Ukraine, the port of Reni was one of the most important ports of the Danube during the 20th Century, and the introduction of *P. muralis* with trees or stones seems quite possible. Granites were mined, for example, near the town of Măcin near the Danube, located just 25 km south of Reni (Matveev et al. 2013). Therefore, Ukrainian lizards could have been introduced in this way. It also appears likely that lizards were introduced via boats from other parts of Romania. Most Romanian towns and villages near the Danube have ports where boats have stop points, which can favor lizard transport to Reni without active or deliberate introduction.

Currently, *P. muralis* is restricted to the vicinity of Reni but we cannot exclude closer undiscovered populations on the Slovak-Ukraine or Hungarian-Ukraine border

where the species has native range (Sillero et al. 2014). Climate seems likely to play the key role in determining the northern range limit of *P. muralis*. Adaptation to cool environments has been predicted to be a primary driver of lacertid evolution, which will have repercussions on their performance in future warmer scenarios (Garcia-Porta et al. 2019). The northeast boundary of the species can reach through southern Slovakia, Romania, southern Moldova, southern regions Ukraine and Crimea, and the western Ciscaucasia (Wirga and Majtyka 2015). For the moment, *P. muralis* has the status of introduced species in Ukraine but if deleterious effects on native biota were detected it should be classified as invasive (Kraus 2009). Meanwhile, monitoring this population is recommended in Ukraine to prevent further expansion and potential negative effects on native biota.

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