BRILL

Amphibia-Reptilia 40 (2019): 121-127

brill.com/amre

Short Note

Phylogeographic evidence for multiple long-distance introductions of the common wall lizard associated with human trade and transport

Joana L. Santos[1], Anamarija Žagar[1,2,3,*], Katarina Drašler[3], Catarina Rato[1], César Ayres[4], D. James Harris[1], Miguel A. Carretero[1], Daniele Salvi[1,5,*]

Abstract. The common wall lizard has been widely introduced across Europe and overseas. We investigated the origin of putatively introduced *Podarcis muralis* populations from two southern Europe localities: (i) Ljubljana (Slovenia), where uncommon phenotypes were observed near the railway tracks and (ii) the port of Vigo (Spain), where the species was recently found 150 km far from its previously known range. We compared *cytochrome-b* mtDNA sequences of lizards from these populations with published sequences across the native range. Our results support the allochthonous status and multiple, long-distance origins in both populations. In Ljubljana, results support two different origins, Serbia and Italy. In Vigo, at least two separate origins are inferred, from western and eastern France. Such results confirm that human-mediated transport is promoting biological invasion and lineage admixture in this species. Solid knowledge of the origin and invasion pathways, as well as population monitoring, is crucial for management strategies to be successful.

Keywords: biological invasions, human-mediated introduction, *Podarcis muralis*, population admixture, Slovenia, Spain.

Introduction

Biological invasions are one of the main threats to biodiversity and together with habitat destruction and fragmentation are considered the main causes behind recent biodiversity loss (Lee, 2002; Schulte et al., 2012; Simberloff et al., 2013). Species are transported to new locations by different means, the importance of which varies taxonomically, geographically and temporally. Often such introductions pose a risk to native biota, via processes such as competitive exclusion of native populations of ecologically similar species, predation of native taxa, or through the possibility of invaders carrying diseases (Simberloff et al., 2013; Sacks et al., 2011). Consequently, this can lead to a decrease of population size and fitness, promoting partial isolation and strong selective pressure, compromising the continuity and stability of native populations (e.g. Sacks et al., 2011; Schulte et al., 2012). One particular problem is when populations of closely related species or evolutionary lineages are introduced in the range of another, since they can compete with native populations or interfere with reproduction, resulting in hybridization (Allendorf et al. 2001; Lee, 2002; Schulte et al., 2012). In fact, admixture between multiple conspecific lineages, introduced into the same locality, can

---

1 - CIBIO Research Centre in Biodiversity and Genetic Resources, InBIO, Universidade do Porto, Campus de Vairão, Rua Padre Armando Quintas, N° 7, 4485-661 Vairão, Vila do Conde, Portugal
2 - National insitute of Biology, Večna pot 111, 1000 Ljubljana, Slovenia
3 - Herpetological society – Societas herpetological slovenica, Večna pot 111, 1000 Ljubljana, Slovenia
4 - Asociación Herpetológica Española, Museo Nacional de Ciencias Naturales, C/ José Gutiérrez Abascal 2, 28006 Madrid, Spain
5 - Department of Health, Life and Environmental Sciences, University of L'Aquila, Via Vetoio, 67100 Coppito, L'Aquila, Italy
*Corresponding authors;
e-mails: danielesalvi.bio@gmail.com;
anamarija.zagar@gmail.com

DOI:10.1163/15685381-20181040

increase genetic diversity and thus the invasiveness potential of a given species (Wagner et al., 2017). A remarkable number of cases of biological invasion in terrestrial habitats correspond to reptiles (with over 4500 introductions reported since 1850; Kraus, 2009). The overall frequency of species introductions is increasing, and lizards are not exceptions (e.g. Michaelides et al., 2015). Therefore, understanding the origin and pathways of introductions is essential to corroborate the alien status of putative introduced populations, as well as to design effective management strategies to control or prevent invasions.

The common wall lizard, *Podarcis muralis* (Laurenti, 1768) has the largest distribution range among species of the genus *Podarcis* (Wagler, 1830), including most of central Europe, Mediterranean peninsulas (Iberia, Italia, Balkans), and NW Anatolia (Sillero et al., 2014). Across this range, up to 23 evolutionary lineages were identified, likely originating from multiple glacial refugia within the three Mediterranean peninsulas, but also in extra Mediterranean regions (Salvi et al., 2013). This species is also widely introduced outside of its native range in Europe and North America (Brown et al., 1995; Schulte et al., 2008; Michaelides et al., 2013, 2015). While many studies have assessed the origin and paths of introductions in northern and central European populations using molecular data (e.g. Schulte et al., 2008, 2012; Michaelides et al., 2013, 2015), to date little is known in the rest of the species range

We used mitochondrial *cytochrome-b* (*cytb*) DNA sequence data to assess the alien status and phylogeographic origin of two possibly introduced populations in urban and heavily humanized landscapes: (i) one from Ljubljana (Slovenia), where lizards showing uncommon phenotypes in terms of colouration were reported; and (ii) the second in Vigo (Spain), which is outside the known species' range. By comparing our new DNA sequences with those from native populations, we aimed to assess their origin and, in case of introduction, to infer their possible pathway.

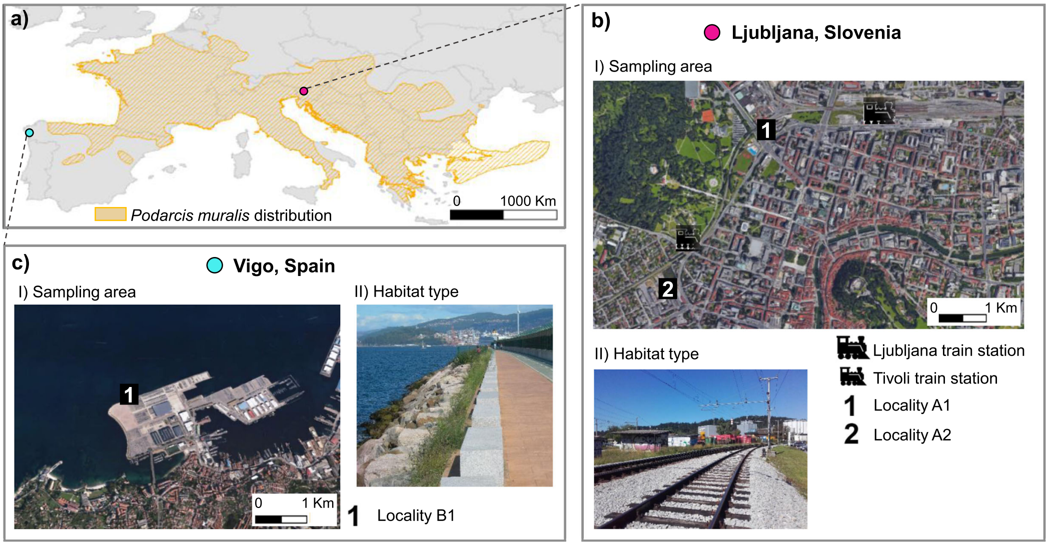

Figure 1. (a) Maps of sampling locations for the two study-cases and representation of the distribution range of *Podarcis muralis*. (b) Satellite images with sampling localities and photographs of the habitat type for the two study-cases: Ljubljana, Slovenia and (c) port of Vigo, Spain.

Materials and methods

Two independent samplings were conducted in this study. In July 2013, nine samples were collected in Ljubljana (Slovenia), in two different places separated by 1.5 km, across transects of approximately 200 m, next to the railway tracks (Location A1: Lat. 46.059°N, Lon. 14.504°E; Location A2: Lat. 46.049°N, Lon. 14.491°E; fig. 1a, b). *Podarcis muralis* is relatively common and widespread in Ljubljana (Krofel et al., 2009), with a typical mostly uniformly brown dorsal colouration, and a red or white ventral colouration in both males and females. However, the observed sampled individuals had uncommonly interconnected dark patches and a light greenish colouration on the dorsal side, with an orange or white ventral colouration.

In September 2017, four samples of *P. muralis* were collected at the maritime port of Vigo (NW Spain), along the Bouzas seafront near a carpark for cargo boats (Location B1: Lat. 42.232°N, Lon. −8.761W; fig. 1a, c). This population was found 150 km to the west of the closest previously known population, and outside of the range predicted by ecological models (Cabana et al., 2016). A tissue sample from the tail and photographs were collected for each individual. The sampled lizards were easily identified, showing a morphological pattern distinct from native *P. bocagei*, also present in the area. Namely, *P. muralis* lizards from Vigo showed dark flanks contrasting with the dorsum, and bluish ocelli surrounded by dark colour on the shoulder, dark spots arranged in rows on the submaxilar scales and reddish or white belly.

DNA extraction was performed using a high-saline method (Sambrook et al., 1989) from tail tip tissues. The mitochondrial *cytb* gene was amplified by Polymerase Chain Reaction using the primers CytbG and cb2R (Palumbi et al., 1991). The amplified *cytb* fragment overlaps with the fragment sequenced by Salvi et al. (2013). Amplification conditions were as in Mendes et al. (2017) and sequencing reactions were conducted by an external company, Genewiz (UK).

Electropherograms were checked and consensus sequences were aligned using MUSCLE implemented in Geneious v4.8.5 (Biomatters Ltd). The dataset includes nine sequences of *P. muralis* from Ljubljana and four from Vigo, generated in this study, and 185 sequences downloaded from GenBank used in Salvi et al. (2013). *Podarcis liolepis* (GenBank accession number KF372218) was designated as an outgroup. Phylogenetic relationships were inferred using the Maximum Likelihood (ML) method implemented

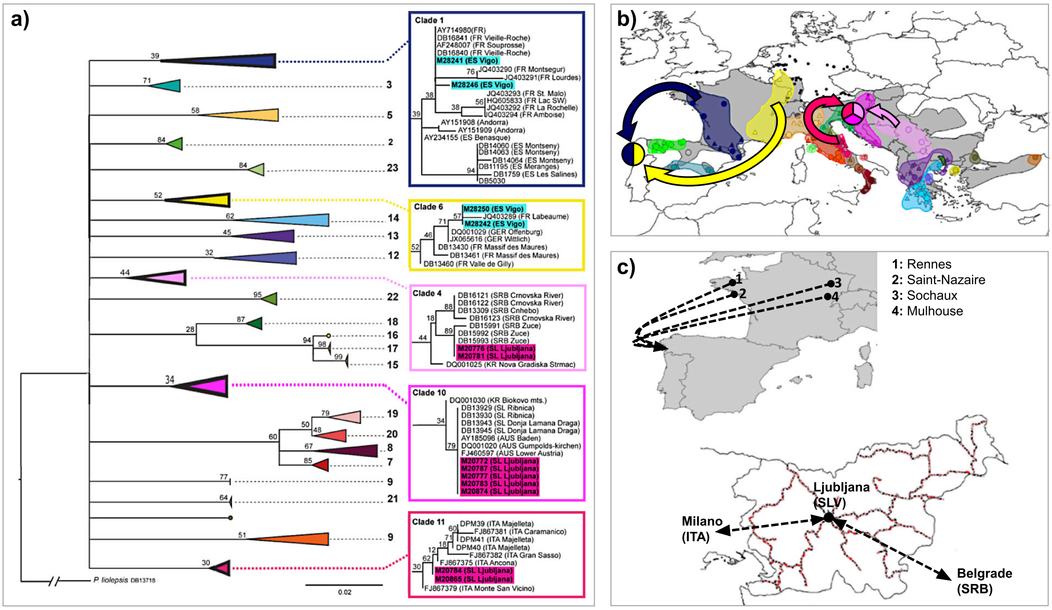

Figure 2. (a) Maximum likelihood phylogenetic tree based on 198 *cytb* mtDNA sequences of *Podarcis muralis* sampled for this study and across its native range. Bootstrap values over 1,000 replicates are reported in correspondence of the nodes; nodes with a support lower than 30% were collapsed. The mitochondrial clades are numbered from 1 to 23, according to Salvi et al. (2013). The clades including the 13 new samples from Ljubljana (highlighted in pink) and Vigo (highlighted in cyan) are expanded in the boxes with detailed information on geographic origin of sequenced individuals (SRB: Serbia; SL: Slovenia; KR: Croatia; ITA: Italy; AUS: Austria; FR: France; ES: Spain; Andorra: Andorra; GER: Germany). (b) Map with the putative distribution range of *P. muralis* lineages (modified from Salvi et al., 2013); grey shade: native range; dots: introduced populations known in Germany and Great Britain; arrows: lineages of origin of introduced populations in Vigo and Ljubljana. (c) Possible introduction routes based on the marine trade of PSA-Citröen factory, in Vigo and the railways tracks of Ljubljana.

in MEGA 7 (Tamura et al., 2017), applying the nucleotide substitution model K2P+G (Kimura, 1980), selected under the Akaike Information Criterion (Akaike, 1974) and support for nodes was evaluated with 1,000 bootstrap replicates. The obtained phylogenetic trees were visualised and edited using FigTree v1.4.2 (Rambaut, 2014). New sequences were submitted to GenBank (accession numbers MH911656-MH911668).

Results and discussion

For all individuals, we obtained *cytb* sequences of 399 base pairs. The ML phylogenetic tree (fig. 2a, b) showed the same 23 clades previously identified by Salvi et al. (2013). Remarkably, the nine sequences from Ljubljana fell into three distinct clades: 4, 10 and 11, otherwise known from Serbia, Central Italy (Marche region) and Slovenia, respectively (fig. 2a, b). In particular, samples M20776 and M20781 clustered with individuals from Serbia, samples M20772, M20777, M20783, M20787 and M20874 clustered with individuals from Slovenia and, samples M20784 and M20865 clustered with individuals from Marche region. Similarly, the four sequences from Vigo fell into two distinct clades – 1 and 6 (fig. 2a, b). Particularly, samples M28241 and M28246 clustered with individuals from western France and samples M28242 and M28250 clustered with individuals from eastern France.

In Ljubljana, the molecular results confirmed our initial hypothesis based on phenotypic assessment – the presence of non-native lineages. Among the lizards sampled in Ljubljana (fig. 1a, b), our results indicate that, apparently, more than one introduction event of *P. muralis* occurred, since sampled individuals showed a close genetic relationship with populations from two geographically distant sites: Serbia (Clade 4) and Italy (Clade 11). The proximity of two train stations near the collection sites, and the fact that the railway lines are linked to both Serbia and Italy, suggest that transportation by train may be the potential vector for introductions (fig. 2c). However, we cannot exclude the hypotheses that the Serbian lineage may also be distributed in Slovenia, since the sample collected in North-eastern Croatia (DQ001025; Podnar et al., 2007), belongs to the Serbian clade (Salvi et al., 2013), suggesting a widespread distribution of this lineage. Still, since the sampled Croatian location cited above is also close to the same railway line connecting Serbia, Croatia and Slovenia, it may be that along this line multiple introductions of several different populations of *P. muralis* have occurred. Indeed, this is not the first time that range expansion mediated by railways has been proposed (Gherghel et al., 2009; Urosevic et al., 2016).

Inside Vigo's harbour (fig. 2b), our genetic results indicate that the origin of this introduction was not from the Iberian Peninsula, but from two distant and distinct regions within the native range, i.e. the western and eastern France lineages. This then resembles the Ljubljana scenario. The proximity to a large car storage depot (of the PSA Peugeot-Citroën factory) beside a large loading dock may account for the introduction pathway of the lizards. This company uses the port of Vigo as a key node for the trade of cars produced in France – namely in Saint-Nazaire and Rennes, as well as in Sochaux and Mulhouse – towards western Europe (Spain and Portugal), Africa (Nigeria, Ethiopia and Kenia), South America (Brazil and Argentina), and China (www.groupe-psa.com; fig. 2c). If this hypothesis is correct, this also suggests that lizards could also easily be introduced into Africa, Asia or America, following the steps of the car factory trade, if they will find a suitable environment. The multiple origins of the population further suggest that the port may be acting as an invasion hub (Letnic et al., 2015). A neighboring touristic harbor where boats usually depart to the nearby protected area of Atlantic Islands from Galicia National Park (AIGNP), represents a threat for subsequent invasion of these small islands populated by endemic populations of *Podarcis bocagei* and *Podarcis guadarramae* (Galán, 2003).

Man-made structures, mainly roads and railways, as well as other trade and transportation routes can be seen as corridors for both distribution range expansions and colonization of new areas, especially in cases of habitat degradation (Hedeen and Hedeen, 1999). It has allowed the success of invasive lizard species (Ficetola and Padoa-Schioppa, 2009), not only of *P. muralis* (Gherghel et al., 2009, this study), but also of other species (Kraus, 2009).

Results obtained in our study demonstrate that greater efforts are needed to recognize the source of invasions, and to identify the pathways and timing of introductions. In cases where introduction of non-native lineages is suspected, the molecular approach is the more effective method to identify the origin of populations and provide results for assessment of potential pathways of introduction (e.g. Silva-Rocha et al., 2012, 2014; Salvi et al., 2014). With this study, using a molecular approach, we effectively identified non-native populations of *P. muralis* in Ljubljana and Vigo. The two cases of introductions shown are from multiple population sources belonging to multiple lineages. Consequently, hybridization between closely related individuals is possible. This may cause a disruption of specific gene combinations that results either in a loss of genes adapted to the native habitat of both lineages or in a competitive advantage against native species (Allendorf et al., 2001; Schulte et al., 2012). On the other hand, such hybrid populations can produce genetic pollution if they return to the source population. Many introduced populations develop different behavioral, physiological or parasite resistance adaptations that can allow them to outcompete the native population and endemic species under specific conditions, resulting in a major threat to biodiversity (Ficetola and Padoa-Schioppa, 2009; Simberloff et al., 2013). At this regard, we should distinguish the conservation implications of the two cases of introduction. While in Ljubljana we cannot exclude that the genetic variation that result within hybrids between intra-specific lineages could have conservation value, particularly in the case where adaptive potential in admixed populations would be observed (e.g. Becker et al., 2013); in the case of Vigo the introduced population of *P. muralis* may outcompete with the native species resulting in a loss of biodiversity. Therefore, especially in this latter case, conservation strategies should involve controlling the pathway of these invasions, such as commercial cargo and trade. Recent introduction events, as the case of Vigo, need an urgent intervention to define, together with local authorities, strategies to eradicate the populations. Otherwise, there is a risk of further invading sensitive areas (as the AIGNP, see above). Since they seem to be recent introductions they are concentrated in small and restricted areas, which facilitates the eradication process. Although this is the most efficient strategy and, when applied in the first stages of invasions is also better in both terms of costs and success of probability, it should be combined with periodical monitoring to ensure a total eradication and to prevent or allow early detection of new introductions (Simberloff et al., 2013).

Additionally, resources should be invested for collecting more data concerning the current distribution and demographic status of the introduced populations, to determine the factors limiting and/or promoting the success of their establishment and for identifying the impacts on native populations and on other species (IUCN, 2000). In sum, our study confirmed the human induced dispersal potential of *P. muralis*, showing the importance of investigating sources and pathways of introductions to avoid further possible invasions.

Acknowledgements. The authors would like to thank Francisco Garcia for sharing information regarding the possible presence of *P. muralis* in Vigo. All lizards were collected under the special licenses 35601-14/2013-5 issued by the Slovenian Environment Agency and by licence 07/2015 issued by Consellería de Medio Ambiente e Ordenación do Territorio of Xunta de Galicia (Spain). AŽ, CR and DJH were supported by Fundação para a Ciência e Tecnologia (FCT) under the Programa Operacional Potencial Humano – Quadro de Referência Estratégico Nacional, European Social Fund and Portuguese Ministério da Educação e Ciência (AŽ: PhD grant SFRH/BD/81324/2011; CR: postdoctoral grant SFRH/BPD/92343/2013; DJH: IF-contract IF/01627/2014). MAC is supported by project NORTE-01-0145-FEDER-000007. DS is currently supported by the program 'Rita Levi Montalcini' for the recruitment of young researchers at the University of L'Aquila.

References

Akaike, H. (1974): A new look at the statistical model identification. IEEE Trans. Automat. Contr. **19**: 716-723.

Allendorf, F.W., Leary, R.F., Spruell, P., Wenburg, J.K. (2001): The problems with hybrids: setting conservation guidelines. Trend. Ecol. Evol. **16**: 613-622.

Becker, M., Gruenheit, N., Steel, M., Voelckel, C., Deusch, O., Heenan, P., McLenachan, P.A., Kardailsky, O., Leigh, J.W., Lockhard, P. (2013): Hybridization may facilitate in situ survival of endemic species through periods of climate change. Nature Clim. Change **3**: 1039-1043.

Brown, R.M., Gist, D.H., Taylor, D.H. (1995): Home range ecology of an introduced population of the European wall lizard *Podarcis muralis* (Lacertilia; Lacertidae) in Cincinnati, Ohio. Am. Midland Nat. **1995**: 344-359.

Cabana, M., Vázquez, R., Galán, P. (2016): Distribución y estado de conservación de *Podarcis muralis* en Galicia. Bol. Asoc. Herpetol. Esp. **27**: 107-114.

Ficetola, G.F., Padoa-Schioppa, E. (2009): Human activities alter biogeographical patterns of reptiles on Mediterranean islands. Glob. Ecol. Biogeog. **18**: 214-222.

Galán, P. (2003): Anfibios y Reptiles del Parque Nacional de las Islas Atlanticas de Galicia. Faunística, Biología y Conservación. Organismo Autónomo Parques Nacionales, Madrid.

Gherghel, I., Strugariu, A., Sahlean, T.C., Zamfirescu, O. (2009): Anthropogenic impact or anthropogenic accommodation? Distribution range expansion of the common wall lizard (*Podarcis muralis*) by means of artificial habitats in the north-eastern limits of its distribution range. Acta Herpetol. **4**: 183-189.

Hedeen, S.E., Hedeen, D.L. (1999): Railway-aided dispersal of an introduced *Podarcis muralis* population. Herpetol. Rev. **30**: 57.

IUCN (2000): IUCN Guidelines for the Prevention of Biodiversity Loss Caused by Alien Invasive Species. IUCN – The World Conservation Union, Gland, Switzerland.

Kraus, F. (2009): Alien Reptiles and Amphibians – a Scientific Compendium and Analysis, 1st Edition. Invading Nature: Springer Series in Invasion Ecology, 4. Springer, New York.

Krofel, M., Cafuta, V., Planinc, G., Sopotnik, M., Šalamun, A., Tome, S., Vamberger, M., Žagar, A. (2009): Distribution of reptiles in Slovenia: a review of data collected until 2009. Natura Sloveniae **11**: 61-99.

Lee, C.E. (2002): Evolutionary genetics of invasive species. Trends Ecol. Evol. **17**: 386-391.

Letnic, M.I., Webb, J.K., Jessop, T.S., Dempster, T. (2015): Restricting access to invasion hubs enables sustained control of an invasive vertebrate. J. Appl. Ecol. **52**: 341-347.

Mendes, J., Harris, D.J., Carranza, S., Salvi, D. (2017): Biogeographic crossroad across the Pillars of Hercules: evolutionary history of *Psammodromus* lizards in space and time. J. Biogeogr. **44**: 2877-2890.

Michaelides, S., White, G.M., Bell, C., Uller, T. (2013): Human introductions create opportunities for intra-specific hybridization in an alien lizard. Biol. Invasions. **15**: 1101-1112.

Michaelides, S.N., While, G.M., Zajac, N., Uller, T. (2015): Widespread primary, but geographically restricted secondary, human introductions of wall lizards, *Podarcis muralis*. Mol. Ecol. **24**: 2702-2714.

Palumbi, S., Martin, A., Romano, S., McMillan, W., Stick, L., Grabowski, G. (1991): The Single Tools Guide to PCR. Department of Zoology, University of Hawaii, Honolulu.

Podnar, M., Haring, E., Pinsker, W., Mayer, W. (2007): Unusual origin of a nuclear pseudogene in the Italian wall lizard: intergenomic and interspecific transfer of a large section of the mitochondrial genome in the genus *Podarcis* (Lacertidae). J. Mol. Evol. **64**: 308-320.

PSA group (2017, December 21). Retrieved from www.groupe-psa.com.

Rambaut, A. (2014): FigTree 1.4.2 software. Edinburgh, Institute of Evolutionary Biology, University of Edinburgh.

Sacks, B.N., Moore, M., Statham, M.J., Wittmer, H.U. (2011): A restricted hybrid zone between native and introduced red fox (*Vulpes vulpes*) populations suggests reproductive barriers and competitive exclusion. Mol. Ecol. **20**: 326-341.

Salvi, D., Harris, D.J., Kaliontzopoulou, A., Carretero, M.A., Pinho, C. (2013): Persistence across Pleistocene ice ages in Mediterranean and extra-Mediterranean refugia: phylogeographic insights from the common wall lizard. BMC Evol. Biol. **13**: 147.

Salvi, D., Schembri, P.J., Sciberras, A., Harris, D.J. (2014): Evolutionary history of the Maltese wall lizard *Podarcis filfolensis*: insights on the 'Expansion-Contraction' model of the Pleistocene biogeography. Mol. Ecol. **23**: 1167-1187.

Sambrook, J., Fritsch, E.F., Maniatis, T. (1989): Molecular Cloning: a Laboratory Manual, 2nd Edition. Cold Spring Harbor Laboratory Press, New York.

Schulte, U., Veith, M., Hochkirch, A. (2012): Rapid genetic assimilation of native wall lizard populations (*Podarcis muralis*) through extensive hybridization with introduced lineages. Mol. Ecol. **21**: 4313-4326.

Schulte, U., Thiesmeier, B., Mayer, W., Schweiger, S. (2008): Allochthone Vorkommen der Mauereidechse (*Podarcis muralis*) in Deutschland. Z. Feldherpetol. **15**: 139-156.

Sillero, N., Campos, J., Bonardi, A., Corti, C., Creemers, R., Crochet, P.-A., Crnobrnja Isailovi, J., Denoël, M., Ficetola, G.F., Gonçalves, J., Kuzmin, S., Lymberakis, P., de Pous, P., Rodríguez, A., Sindaco, R., Speybroeck, J., Toxopeus, B., Vieites, D.R., Vences, M. (2014): Updated distribution and biogeography of amphibians and reptiles of Europe. Amphibia-Reptilia **35**: 1-31.

Silva-Rocha, I., Salvi, D., Carretero, M.A. (2012): Genetic data reveal a multiple origin for the populations of the Italian wall lizard *Podarcis sicula* (Squamata: Lacertidae) introduced in the Iberian Peninsula and Balearic Islands. Ital. J. Zool. **79**: 502-510.

Silva-Rocha, I., Salvi, D., Harris, D.J., Freitas, S., Davis, C., Foster, J., Deichsel, G., Adamopoulou, C., Carretero, M.A. (2014): Molecular assessment of *Podarcis sicula* populations in Britain, Greece and Turkey reinforces a multiple-origin invasion pattern in this species. Acta Herpetol. **9**: 253-258.

Simberloff, D., Martin, J.L., Genovesi, P., Maris, V., Wardle, D.A., Aronson, J., Courchamp, F., Galil, B., García-Berthou, E., Pascal, M., Pyšek, P., Sousa, R., Tabacchi, E., Vilà, M. (2013): Impacts of biological invasions: what's what and the way forward. Tren. Ecol. Evol. **28**: 58-66.

Tamura, K., Dudley, J., Nei, M., Kumar, S. (2007): MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol. **24**: 1596-1599.

Urosevic, A., Tomovic, L., Ajtic, R., Simovic, A., Dzukic, G. (2016): Alterations in the reptilian fauna of Serbia: introduction of exotic and anthropogenic range expansion of native species. Herpetozoa **28**: 115-132.

Wagner, N.K., Ochocki, B.M., Crawford, K.M., Compagnoni, A., Miller, T.E.X. (2017): Genetic mixture of multiple source populations accelerates invasive range expansion. J. Anim. Ecol. **86**: 21-34.

Submitted: March 19, 2018. Final revision received: August 14, 2018. Accepted: August 28, 2018.
Associate Editor: Jonathon Marshall.