

Molecular assessment of *Podarcis sicula* populations in Britain, Greece and Turkey reinforces a multiple-origin invasion pattern in this species

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Submitted on 2014, 2nd September, revised on 2014, 17th October, accepted on 2014, 21st October
Editor: Gentile Francesco Ficetola

Abstract. Biological invasions are a challenge to conservation and constitute a threat to biodiversity worldwide. The Italian wall lizard *Podarcis sicula* has been widely introduced, and seems capable of adapting to most of the regions where it is established and to impact on native biota. Here we construct a phylogenetic framework to assess the origin of the introduced populations in the United Kingdom, Greece and Turkey comparing cytochrome-*b* gene sequences of lizards from five locations to published sequences from the native range and other non-native locations. The results support an origin from central Italy for the United Kingdom population, from the Adriatic region for the Greek population and from Calabria for the population from Turkey. These results emphasise the multiple-source pattern of introduction of this species identified in previous studies. The improvement in the knowledge of the origin and pathways by which invaders arrive in new areas, as well as the monitoring of their populations, are crucial for successful strategies to deal with exotic species.

Keywords. Biological Invasions, Italian wall lizard, cytochrome *b*, human-mediated introductions.

Biological invasions are a major concern to biodiversity conservation due to the threat to native biota (Simberloff et al., 2013). The Italian wall lizard, *Podarcis sicula*, is one such reptile species that has been widely introduced (Kraus, 2009). From its native distribution in the Italian Peninsula, Sicily and the north Adriatic coast, this species is considered to have been introduced in several other places, such as the Tyrrhenian Islands, Corsica and Sardinia, Menorca in the Balearics and in islands and coastal areas of the eastern Adriatic Sea (Corti, 2006). Besides these regions, scattered introduced populations are also known from the Iberian Peninsula, in Cantabria (Meijide, 1981), Almería (Mertens and Wermuth, 1960), Lisbon (González de la Vega et al., 2001), La Rioja (Valdeón et al., 2010) and near Barcelona (Rivera et

al., 2011); in Southern France, in Toulon and Château d'If Island (Morgue, 1924; Orsini, 1984); in Switzerland (Schulte and Gebhart, 2011); in Turkey, in Istanbul surroundings and the Marmara Islands (Mollov, 2012; Ilgaz et al., 2013); in North Africa, in Tunisia and Tripoli (Arnold and Ovenden, 2002); and in the United States, in Philadelphia, Kansas, New York and California (Deichsel et al., 2010; Kolbe et al., 2013). Very recently, two additional introductions have been reported in the United Kingdom (Hodgkins et al., 2012) and in Greece (Adamopoulou, 2014).

This Mediterranean lizard is very eclectic regarding habitat choice, being found both in natural areas, agricultural environments and in urban areas (Capula, 1994; Corti 2006). Exotic populations of *P. sicula* have become

established and undergone expansion in different regions encompassing a wide spectrum of environmental conditions, namely in North America (Burke et al., 2002; Burke and Ner, 2005). Therefore, this lizard appears to be an effective and successful coloniser. The ecological and behavioural traits of this species also contribute to the success in the new area, causing a great impact on native lizards with which *P. sicula* is able to compete. Behavioural interference with native *Podarcis* species have been reported, namely with *P. melisellensis* in the Adriatic coast (Downes and Bauwens, 2002) which can result in the extinction of the latter when the introduction of *P. sicula* takes place in small islets (Nevo et al., 1972). Moreover, hybridisation with other *Podarcis* species is also documented, namely with the endemic *P. tiliguerta* in Sardinia (Capula, 2002), with *P. raffonei* in the Aeolian islands (Capula et al., 2002) and with *P. wagneriana* in Sicily (Capula, 1994). These negative effects on native biota, hence, qualify this species as a problematic invader (Kraus, 2009).

Given the potential adverse effects of *P. sicula* out of its native range, it becomes crucial to develop effective management strategies. Understanding the colonisation patterns of this successful invader provides the basis for delineating more effective preventive measures (Dorcas et al., 2010). A single source of introduction might facilitate the introduction of control measures on target populations, regions and invasion pathways, while multiple sources would indicate a general invasive character of the species requiring more global measures at a species level. Molecular evidence supporting the origin of *P. sicula* is still lacking for many introduced populations. Recently, taking advantage of the available phylogeographic information for the native range of the species Podnar et al. (2005), Silva-Rocha et al. (2012) and Kolbe et al. (2013) revealed multiple sources for the populations of the Iberian Peninsula and Menorca, and of the United States. Both studies concluded that the pathways by which the species is being introduced are well distinct between cases, ranging from the pet trade, to cargo and the nursery trade of olive trees (Valdeón et al., 2010; Rivera et al. 2011) as well as escaping from captivity and deliberate release (Deichsel et al., 2010).

In this study, we use phylogenetic analyses to assess the putative origin of three other recently introduced populations, occurring in the United Kingdom, Greece and Turkey. The UK population was introduced in 2010 and has already been eradicated (Hodgkins et al., 2012). In Greece, the population was described as a very recent introduction and was detected in 2014 (Adamopoulou, 2014). On the other hand, the population from Turkey was introduced historically and is in apparent expansion

(Mollov, 2012; Ilgaz et al., 2013). The identification of the origin of these three additional introduced populations is expected to improve the picture of the colonization pattern revealed by the previous studies.

One sample of *P. sicula* was collected from Mudanya (locality reported by Mollov 2009; 40°22'5.844"N, 28°54'7.56"E), one sample from Güzelyah (40°21'52.416"N, 28°54'7.56"E) and one sample from Iznik (40°25'43.6434"N, 29° 43' 45.7674"E) in Turkey, three samples from Palaio Faliro (Athens; 37°55'9.38"N, 23°42'0.50"E) in Greece and one sample from Buckinghamshire (52°1'47.604"N, 1°1'10.308" W) in the United Kingdom.

Total genomic DNA was extracted from tail tissue and a fragment of 687 base pairs of the mitochondrial gene cytochrome *b* (*cyt-b*) was amplified by PCR using the same primers and procedures described in Silva-Rocha et al. (2012). The sequences generated in the present study (GenBank accession numbers: KP036396-KP036402) were aligned with 116 sequences downloaded from GenBank: 39 sequences from individuals of *P. sicula* from the native range (Podnar et al., 2005), accession numbers: AY185095, AY185094, AY770869–AY77090; 16 sequences from the Iberia Peninsula and 7 from Menorca introduced populations (Silva-Rocha et al., 2012), accession numbers: JX072938–JX072960; one sequence from Switzerland (Schulte and Gebhart, 2011); 1 sequence from California (Deichsel et al., 2010), accession number: HQ154646; and 52 sequences from Kolbe et al. (2013), from both the introduced populations of the United States (nine sequences) and from the native populations (43 sequences), accession numbers: JX186516–JX186568. Three sequences from *Podarcis muralis* and *P. melisellensis* were also downloaded from GenBank and used as outgroup (accession numbers AY185096, AY185029 and AY185057), following Podnar et al. (2005). We performed a Maximum Likelihood (ML) phylogenetic analysis to infer the relationships between the *cyt-b* haplotypes using the software Mega 6 (Tamura et al., 2013). The model HKY + Gamma was selected as the best model of sequence evolution under the Bayesian Information Criterion (BIC), chosen using Mega 6. Tree searches were performed using the heuristic search mode. Node support was calculated over 1000 bootstrap replicates. In addition to the tree-building approach, we analysed the genealogical relationships among the native and non-native haplotypes clustered in the 'Sicula' clade (Podnar et al., 2005) by means of a statistical parsimony network using the software TCS 1.21 (Clement et al., 2000), in order to get a better resolution on relationships between closely related haplotypes.

The final alignment includes 127 sequences of 687 base pairs. Four new haplotypes were identified from the five introduced locations here studied, and 78 haplotypes

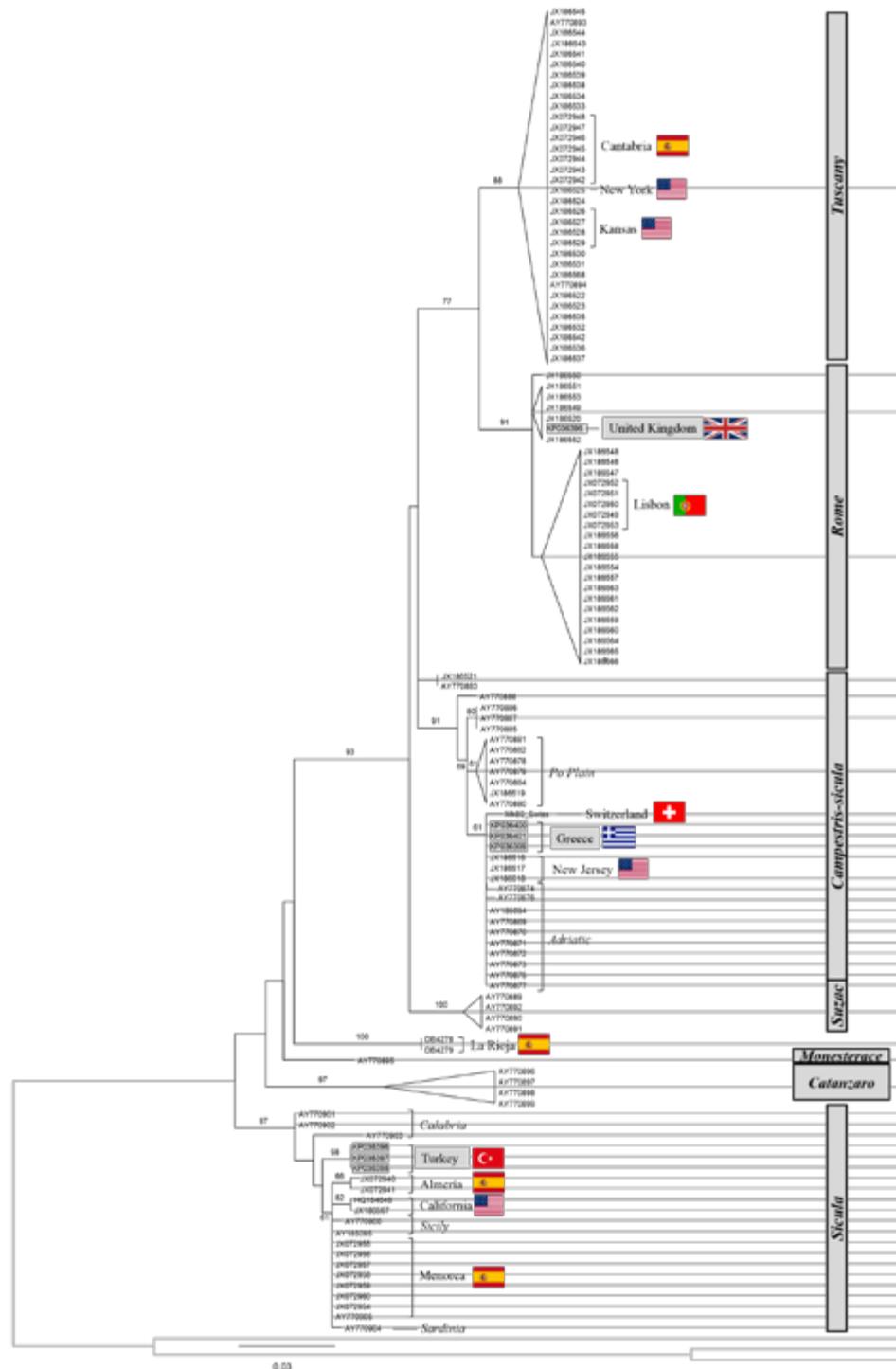


Fig. 1. ML Phylogenetic estimate of relationships between *cytochrome b* (*cyt-b*) haplotypes from native *Podarcis sicula* populations (Podnar et al. 2005, Kolbe et al. 2013) and those from introduced populations generated in this study (Turkey and United Kingdom) and by Silva-Rocha et al. 2012 and Kolbe et al. 2013 (Almeria, Cantabria, La Rioja, Lisbon, Menorca, New Jersey, California, New York and Kansas). *P. muralis* and *P. meliselenis* were used as outgroups (not shown). Sequences downloaded from Genbank are named according to their accession number. Main *P. sicula* haploclades are indicated by grey boxes and subclades are named according to the geographic origin of haplotypes (native samples in italic). Samples from United Kingdom, Greece and Turkey are highlighted in grey and underlined. Bootstrap support values are indicated above the nodes of interest.

were identified from the sequences generated by the previous studies (Podnar et al., 2005; Deischsel et al., 2010; Schulte and Gebhart, 2011; Silva-Rocha et al., 2012; Kolbe et al. 2013). In particular, one different haplotype was found in the British and Greek locations each, while two different haplotypes occurred in the Turkish populations.

The estimate of relationships based on ML indicates that lizards from the United Kingdom belong to the “Rome” clade found by Kolbe et al. (2013) (Fig. 1). This result supports the hypothesis of Hodgkins et al. (2012) that the origin of the lizards introduced in the United Kingdom was from a locality close to Rome. These authors stated that the lizards were found in June 2010 on a consignment of tufa (a type of soft, porous limestone) imported from Italy in March 2010 for a restoration project of an 18th century landscape garden in Stowe, Buckinghamshire. This hypothesis was also supported by J. Foster (pers. comm.) who indicated Tivoli (ca. 30 km east of Rome) as the origin of the building materials.

Lizards from Greece are included in the “*Campestris-sicula*” clade, sharing the haplotype with lizards sampled in the Adriatic region by Podnar et al. (2005) and with lizards sampled in New Jersey by Kolbe et al. (2013). Therefore, the most probable origin for the Greek population is the Adriatic region, which is geographically close to Greece. The population was found in a narrow zone of sand (dimensions approx. 90 × 15 m) between an overcrowded beach and the tram station on the main avenue. The occupied area is a small artificial “park” of various trees planted on bare sand. The park officer noticed the presence of the lizards since he started to plant the trees, so this could be a probable vector by which the animals arrived. Unfortunately, it is not possible to know the origin of the trees since they were collected from the garbage. Furthermore, we cannot exclude other potential introduction pathways, since there is a big yacht marina less than 500 meters away from the colony and a large port, Port of Piraeus, approximately 8km away (Adamopoulou, pers. comm.).

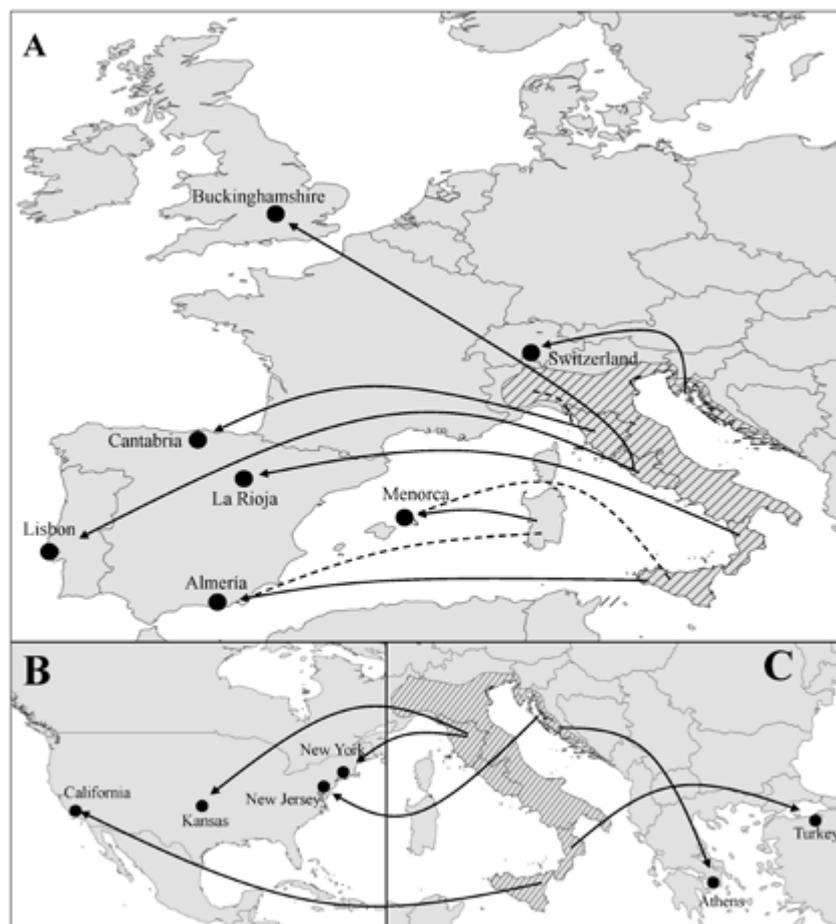


Fig. 2. Map of the introduced populations of *Podarcis sicula* analysed. (A) populations in Iberian Peninsula, United Kingdom and Switzerland, (B) populations in United States, (C) populations in Greece and Turkey.

Regarding the Turkish population, their *cyt-b* haplotype clusters in the “Sicula” clade of Podnar et al. (2005) (Fig. 1). The haplotype from Turkey seems to correspond to the Calabrian stock, since is closely related to the ones found in the Calabrian region. Accidental historical introductions by people or merchant vessels are possible pathways through which this population arrived in Turkey from southern Italy (Mollov, 2009).

Our ML results are in accordance with previous studies (Silva-Rocha et al., 2012; Kolbe et al., 2013), as can be seen both in the ML tree (Fig. 1) and in the summary map of Fig. 2.

Based on the additional sequences generated by Kolbe et al. (2013) from lizards sampled in the native range, it is possible to refine the inferred origin for the populations of Lisbon and Cantabria (northern Spain). Indeed, our ML tree supports an origin from northwestern Tuscany for the Cantabria population in and from central Italy around Rome for the Lisbon population which clustered within the “Rome” clade.

The results of this study reinforce the multiple source and pathways pattern suggested by Silva-Rocha et al. (2012) and Kolbe et al. (2013) and confirm the invasive potential of this species as a whole (Fig. 2). This reveals once more the tendency of *P. sicula* to use man-made objects as refuges and the role of these as an effective vector for the introductions of this lizard. Regarding the British population, the early detection and fast collection of the individuals was the key to preventing the expansion of this species from the formal garden where it was first observed (see details of the eradication in Hodgkins et al. 2012). The Greek population is established and already has at least 50-60 individuals (Adamopoulou, 2014). Early eradication of this population is recommended. On the other hand, the Turkish populations seem to be of more longstanding origin and are currently in expansion towards the south of the Marmara Sea (Mollov, 2009; Tok et al., 2014). A specific monitoring program would be needed to assess in detail the extent and progress of this expansion.

Overall, results obtained here accumulated to the previous evidence demonstrating that the Italian wall lizard *P. sicula* can be an effective invader. Its successful acclimatization to environmental conditions different for those prevailing in its original Mediterranean range such as those in Switzerland or Central USA increases conservation concern, since the probability to become invasive is boosted by the adaptability of the species. Certainly, documenting the origin and pathways of introduced populations and monitoring the expansion of the populations are needed to define effective management strategies (Kraus, 2009; Simberloff et al., 2013).

ACKNOWLEDGMENTS

DS is supported by the FCT post-doctoral grant SFRH/BPD/66592/2009 and IS-R by the FCT PhD grant SFRH/BD/95745/2013 under the Programa Operacional Potencial Humano – Quadro de Referência Estratégico Nacional funds from the European Social Fund and Portuguese Ministério da Educação e Ciência. The molecular work of this study was funded by FCOMP-01-0124-FED-ER-007062 FCT project PTDC/BIA-BEC/102179/2008, PTDC/BIA-BEC/101256/2008, and partially financed by the project “Biodiversity, Ecology and Global Change” co-financed by North Portugal Regional Operational Programme 2007/2013 (ON.2 – O Novo Norte), under the National Strategic Reference Framework (NSRF), through the European Regional Development Fund (ERDF) and by the project “Biodiversity Conservation in a Changing World” financed by the Portuguese Integrated Program of IC&DT Call N° 1/SAESCTN/ALENT-07-0224-FED-ER-001755.

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