

## *Podarcis vaucheri* (Sauria: Lacertidae) far away from home: a new invasive species in Greece

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**Abstract.** In this study we aimed to clarify the identity of a wall lizard population that deviates phenotypically from the other *Podarcis* lizards that occur in the broader area (Athens, Greece). To this end we used molecular techniques. Most surprisingly, we identified the focal population as *Podarcis vaucheri*, a species far away from its natural range. Molecular results suggest an Iberian origin of this population. To the best of our knowledge, this is the first report of *P. vaucheri* outside its original range. The new population should be attributed to human-mediated introduction. The future interaction of this introduced species with native lizards, many of which are endemic to Greece, is of critical importance.

**Keywords:** alien species, human introduction, mitochondrial DNA, wall lizards.

With 86 species, Greece hosts one of the richest herpetofaunas in Europe. Thirteen of the species are endemic, whereas for 13 more, Greece hosts the only European populations (Pafilis, 2010). Though reptilian and amphibian species have been moved around Mediterranean Basin for long time, this trend has been considerably accelerated nowadays as a consequence of human activities (on the other hand the more extensive contemporary research efforts might also account for the new records). As such, many species have widened their traditional range, colonizing new locations within the country (Hill and Mayer, 2004; Troidl and Troidl, 2008; Belasen, Li and Foufopoulos, 2012; Spaneli and

Lymberakis, 2014; Itescu et al., 2016; Kornilios and Thanou, 2016; Mizerakis and Strachinis, 2017). Interestingly, the Athens metropolitan area (capital city of Greece) is on the front-line of this trend and new lizard populations of either native or exotic origin have been reported from there (Adamopoulou, 2015; Hedman et al., 2017; Karameta and Pafilis, 2017; Strachinis and Pafilis, 2018).

*Podarcis vaucheri* has a wide range that includes southern Spain, central and northern Morocco, northern Algeria and northern Tunisia, occurring in a variety of habitats such as Mediterranean type vegetation, rocky areas, pastureland, rural gardens and urban areas from sea level up to 3100 m (Mateo et al., 2009). To the best of our knowledge, this species has never been reported outside its range. In a survey conducted at the western outskirts of Athens (suburbs Agioi Anargyroi and Aigaleo), we encountered common wall lizards (*Podarcis muralis*) verifying previous reports (Karameta and Pafilis, 2017), but at Agioi Anargyroi we found numerous lizards that did not resembled *P. muralis*. These individuals had a general morphological appearance closer to *Podarcis tauricus*: dorsal coloration in males bright green with many black spots, head, hind legs and tail brown, whereas flanks had dark and brownish

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spots and blotches in a brown-yellowish background. Females had less or no green coloration dorsally, while both sexes had whitish bellies (Valakos et al., 2008). Nonetheless, the lizards lacked the typical serrated collar of *P. tauricus*.

We captured 17 individuals from Agioi Anargyroi that were deposited in the Herpetological Collection of the Natural History Museum of Crete (NHMC), University of Crete (voucher numbers NHMC: 80.3.183.1-10, 80.3.53.603-604, and 80.3.53.670-674). Our aim was to identify the collected, unknown lizards. To this end we first focused on pholidosis. However pholidotic characters did not indicate any Greek *Podarcis* species. Therefore, we employed molecular techniques to unravel the identity of the focal lizards.

Total genomic DNA was extracted from the 17 specimens with ambiguous assignment from the wider region of Attica using a standard ammonium acetate protocol (Bruford, Hanotte and Burke, 1998). The mitochondrial gene (mtDNA) encoding the cytochrome b (*cyt b*) (~430 bp) was amplified through PCR using primers GLUDG and CB2 (Palumbi, 1996) and conditions described in Psonis et al. (2017).

Single stranded sequencing of the PCR products was performed using the Big-Dye Terminator v.3.1 Cycle Sequencing kit<sup>®</sup> on an ABI3730 automated sequencer following the manufacturer's protocol and using the PCR primers. Sequences were edited using CodonCode Aligner v.3.7.1 (CodonCode Corporation<sup>®</sup>) and the authenticity and homology to the targeted locus was evaluated with a BLAST search in the NCBI genetic database (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). The above search revealed that 10 of the amplified *cyt b* sequences had high similarity with other available *cyt b* sequences of *Podarcis vaucheri* in GenBank (E-value <  $10^{-167}$ ), while the remaining seven sequences had high similarity with *P. muralis* (E-value <  $5 \times 10^{-167}$ ). In both cases, the similarity dropped dramatically when compared to other *Podarcis* sequences.

To confirm the above results, a phylogenetic tree was built using a dataset with all representatives of the autochthonous Balkan species (Poulakakis et al., 2003, 2005a, 2005b; Psonis et al., 2017) as well as sequences from all main *P. vaucheri* lineages and its sister species *P. hispanicus* (Carranza et al., 2004; Busack et al., 2005; Pinho et al., 2006; Kaliontzopoulou et al., 2011). Two sequences of *Lacerta agilis* were used as outgroup. Sequence alignment was performed using the ClustalW implemented in MEGA v.6 (Tamura et al., 2013) and the nucleotide substitution model selection test was carried out using PartitionFinder (PF) v.2.1 (Guindon et al., 2010; Lanfear et al., 2012, 2016). The dataset was partitioned as specified by PF, with the following parameters: linked branch length; MrBayes models; BIC model selection; greedy search algorithm; each codon as a data block.

Phylogenetic reconstruction was conducted using Bayesian Inference (BI) and the analysis was performed in MrBayes v.3.2.6 (Ronquist et al., 2012), with four runs and eight chains for each run. Each chain run for  $10^7$  generations sampling every  $10^3$  generations. Several MCMC convergence diagnostics were used to check for convergence and stationarity following the manual's instructions. The first 25% trees were discarded as burn-in, as a measure to sample from the stationary distribution and avoid the possibility of including random, sub-optimal trees. A majority rule consensus tree was then produced from the posterior distribution of trees, and the posterior probabilities were calculated as the percentage of samples recovering any particular clade. Posterior probabilities  $\geq 0.95$  indicate statistically significant support (Huelsenbeck and Ronquist, 2001).

In total, 423 base pairs (bp) of *cyt b* sequences were obtained from all examined specimens. The alignment contained 156 variable and 144 parsimony informative sites (160 and 151, respectively when the outgroup was also included). Uncorrected pairwise genetic distances (p-distances) varied from 0 to 18.6%

**Table 1.** Genetic p-distances (%) among the main clades/lineages for *cyt b*.

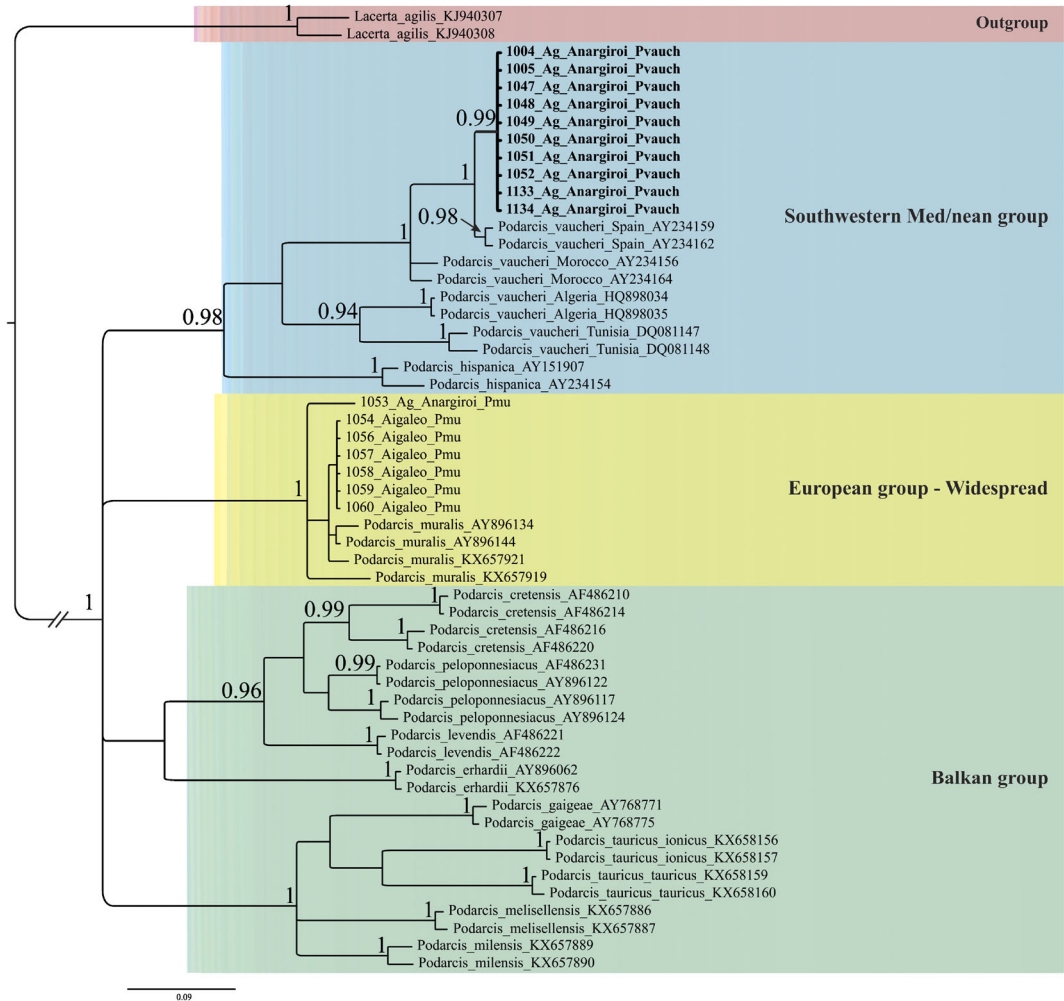
	1	2	3	4	5	6	7	8	9	10	11	12
1. <i>P. vaucheri</i> (Greece)												
2. <i>P. muralis</i>	15.8											
3. <i>P. cretensis</i>	15.4	13.3										
4. <i>P. erhardii</i>	15.4	12.8	12.4									
5. <i>P. gaigeae</i>	15.8	15.2	14.6	14.8								
6. <i>P. hispanicus</i>	12.4	13.4	15.3	14.9	14.3							
7. <i>P. levendis</i>	12.7	13.4	8.9	12.1	15.0	16.4						
8. <i>P. melisellensis</i>	16.2	11.7	14.6	11.8	9.0	15.0	13.3					
9. <i>P. milensis</i>	17.0	13.4	13.6	12.1	9.4	14.0	14.0	9.4				
10. <i>P. peloponnesiacus</i>	13.8	13.5	6.7	11.2	14.7	16.1	6.7	14.3	13.6			
11. <i>P. tauricus</i>	16.2	13.7	13.3	12.9	10.8	16.2	12.8	10.6	10.5	13.5		
12. <i>P. vaucheri</i> (Morocco)	4.9	13.7	14.9	15.0	15.5	11.1	14.8	15.3	15.9	14.7	15.3	
13. <i>P. vaucheri</i> (Spain)	1.3	15.9	15.7	15.8	15.4	12.5	13.3	16.0	16.7	14.1	16.2	4.7

when the outgroup was not included. The mean distance between the 10 *P. vaucheri* specimens from Agioi Anargyroi and the ones from Spain, Algeria, Tunisia, Morocco varied from 1.3% to 12.4% with the Spanish lineage being the closest. Furthermore, the distances among the specimens in question and the Balkan *Podarcis* species varied between 12.4% and 17% (table 1).

The best-fit partitioning scheme and the nucleotide substitution model selected by PF was K80 +  $\gamma$  for the 1st codon position, HKY + I for the 2nd codon position and GTR +  $\gamma$  for the third codon position. In the MrBayes analysis (arithmetic mean  $-\ln L = 2749.53$ ), the MCMC convergence diagnostics did not provide any clues of non-convergence and indicated stationarity. Considering the *P. vaucheri* specimens from Attiki, they form a highly supported monophyletic group [posterior probability (p.p.) = 0.99], which, in turn, form another highly monophyletic group (p.p. = 0.98) when coupled with the *P. vaucheri* and *P. hispanicus* sequences retrieved from GenBank. Additionally, the Greek *P. vaucheri* lineage seems to be more closely related to the Spanish lineage as they form a very well supported clade (p.p. = 1) (fig. 1).

*Podarcis vaucheri* is a highly diverse species (Pinho, Ferrand and Harris, 2006; Lima et al., 2009) that has been raised to specific level from

the *Podarcis hispanicus* species complex (Olivero et al., 2000; Busack, Lawson and Arjo, 2005; Arnold et al., 2007). Though it was considered to be a North African species, new research provided evidence that *P. vaucheri* invaded Africa from the Iberian Peninsula from where it originates (Kaliontzopoulou et al., 2011). To the best of our knowledge, until now *P. vaucheri* has never been recorded outside its original range. Thus, our finding was quite unexpected, particularly due to the remoteness: distance between the Athens population and the easternmost native Spanish population in Almeria (Rivera, Simón and Arribas, 2009; Fernández Guiberteau and González de la Vega, 2012) is some 2315 km. Apparently, the establishment of the new population should be attributed to human transportation. Certain *Podarcis* species (e.g. *P. muralis*, *P. pityusensis*, *P. siculus*) expand their distribution thanks to direct or indirect anthropogenic means of dispersal: railways, pet trade, cargo, merchant or touristic vessels, timber trade, plant trade, building materials (Valdeón et al., 2010; Rivera et al., 2011; Hodgkins, Davis and Foster, 2012; Silva-Rocha et al., 2014). In at least one case, *P. vaucheri* has been reported to form a new population as a consequence of human-mediated introduction (Renoult et al., 2010). The thriving Athenian population (we counted over 60 individuals of different age classes) might have followed a similar way.



**Figure 1.** Bayesian Inference tree based on cyt b sequences. The posterior probabilities (>0.95) are given near the branches. No values means low statistical support.

During the last years several exotic species invaded the country (Adamopoulou and Legakis, 2016). The proven negative consequences that some of them (e.g. *P. siculus*, *Lithobates catesbeianus*) may induce to native species (Kiesecker and Blaustein, 1998; Downes and Bauwens, 2002), underscore the need for high alert. We do not know yet whether *P. vaucheri* represents a threat for the seven endemic lacertids or the other lizards of Greece. A continuous monitoring protocol will provide valuable information on the potential of *P. vaucheri* to establish new populations, occupy new habitats and outcompete other lizards.

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