

First record of *Podarcis peloponnesiacus* (BIBRON & BORY, 1833) from outside the Peloponnese

The lacertid genus *Podarcis* comprises 23 species that occur in middle and south Europe, westernmost Asia and northwesternmost Africa. Greece hosts nine *Podarcis* species, five of which (*cretensis*, *gaigeae*, *levendis*, *milensis*, *peloponnesiacus*) are endemic to the country (UETZ & HOSEK 2016). The Peloponnesian Wall Lizard *Podarcis peloponnesiacus*, is among the latter and was described by the naturalists Jean Baptiste BORY DE SAINT-VINCENT and Gabriel BIBRON (1833) who participated in the French Morea (the Greek vernacular name for the Peloponnese Peninsula) Expedition in the early 19th century, thereby inaugurating herpetology in Greece (PAFILIS 2010).

Podarcis peloponnesiacus is endemic to the Peloponnese Peninsula, where it occurs in almost all habitats from sea level up to 1,500 m a.s.l. (VALAKOS et al. 2008). The only gap in this otherwise continuous range is located in the northwestern Peloponnese from where the species is absent (BRINGSØE 1986). The Peloponnesian Wall Lizard was also found on the Islet of Psili, in the Argolic Gulf (37°26'15.05"N, 22°59'12.30"E), 2.5 km off the coast of the Peloponnese (CLARK 1972) and on the small Island of Elafonissos

in the south of the Peloponnese, just 0.8 km off the coastline (BROGGI 2016).

On June 22, 2014, during a field survey of undeveloped natural habitat patches located in the greater Athens metropolitan area the authors recorded one specimen of the Peloponnesian Wall Lizard in the District of Nikaia (located in the south-western section of Athens, close to Piraeus, Prefecture of Attica; 37°58'22.84"N, 23°38'07.76"E). The area constitutes an island of natural vegetation, surrounded by an urban matrix of buildings and streets (CLERGEAU et al. 2004). During the survey of the fragment, which contained mostly patches of dense *Avena* sp. (Poaceae) alternating with exposed limestone rockfaces (Fig. 1), the authors consistently searched under possible refugia. Species encountered at the site included *Chalcides ocellatus* (FORSSKÅL, 1775), *Ablepharus kitaibelii* (BIBRON & BORY ST-VINCENT, 1833), *Testudo marginata* SCHOEPPF, 1792, and, unexpectedly, *P. peloponnesiacus* that was hidden under a small stone. The adult female individual (snout-vent-length 62 mm, tail length 102 mm and body mass 6.7 g) was taken to the University of Athens and later added to the Herpetological Collection of the Natural History Museum of Crete, University of Crete (Museum voucher number NHMC 80.3.54.133).

The specimen (Fig. 2) bore the typical color features of the species' females: six longitudinal, clearly marked yellowish stripes in dorsal and lateral position on brown ground and a whitish, unspotted underside and displayed characteristic blue ocelli at the shoulder and the flanks, a trait that is even more pronounced in males of the species (BRINGSØE 1986). The following pholidosis counts were made: 1/1 (left/right) postnasal, 3/3 supraciliary granules, 4/4 supralabials, 6/6 sublabials, 20/21 temporals, 26 gulars and 10 collar scales. All above data fall within the corresponding value ranges previously reported for the species (BRINGSØE 1986; LYMBERAKIS et al. 2008).

Podarcis peloponnesiacus may be confused with *Podarcis erhardii* (BEDRIAGA, 1882), which can be similar in coloration and morphology (ARNOLD & OVENDEN 2002). Both species live in sympatry in cer-



Fig. 1: A general view of the island of natural vegetation in an otherwise urban area at Nikaia, southwest of Athens, Prefecture of Attica, Greece, where *Podarcis peloponnesiacus* (BIBRON & BORY, 1833) was found.



Fig. 2: The specimen of *Podarcis peloponnesiacus* (BIBRON & BORY, 1833), that was found outside of the Peloponnese in Nikaia, southwest of Athens, Prefecture of Attica, Greece (NHMC 80.3.54.133).

Table 1: Primers and conditions used in PCR amplification using single *Taq* DNA polymerase (KAPA BIOSYSTEMS®), and in cycle sequencing reaction.

Gene	Primers	Sequence (5' – 3')	Size (bp)	Conditions	Reference
<i>Cyt b</i>	GLUDG	TGACTTGAARAACCAAYCGTTG	~510	3mM MgCl, 94 °C, 1 min 42-48.6 °C, 1 min 72 °C, 1 min 35 cycles	(PALUMBI 1996)
	CB2	CCCTCAGAATGATATTTGTCCTCA			

tain areas of the Peloponnese (MAYER et al. 1990; VALAKOS et al. 2008), and *P. erhardii* occurs in Attica within 20 km from the study site (PAFILIS & SIMOU 2006). To avoid misidentification with *P. erhardii*, molecular analysis was employed in support of the morphological assessment.

Total genomic DNA was isolated using a standard ammonium acetate extraction protocol from tail tissue of the specimen. Double-stranded PCR was used to

amplify partial sequence of the mitochondrial gene (mtDNA) encoding the cytochrome *b* (*cyt b*). Primers and conditions used in PCR amplification and in cycle sequencing reaction are given in Table 1. Single-stranded sequencing of the PCR product was performed using the BigDye® Terminator (v. 3.1) Cycle Sequencing Kit on an ABI 3730 automated sequencer (Applied Biosystems - Thermo Fisher Scientific) following the manufacturer's protocol and using

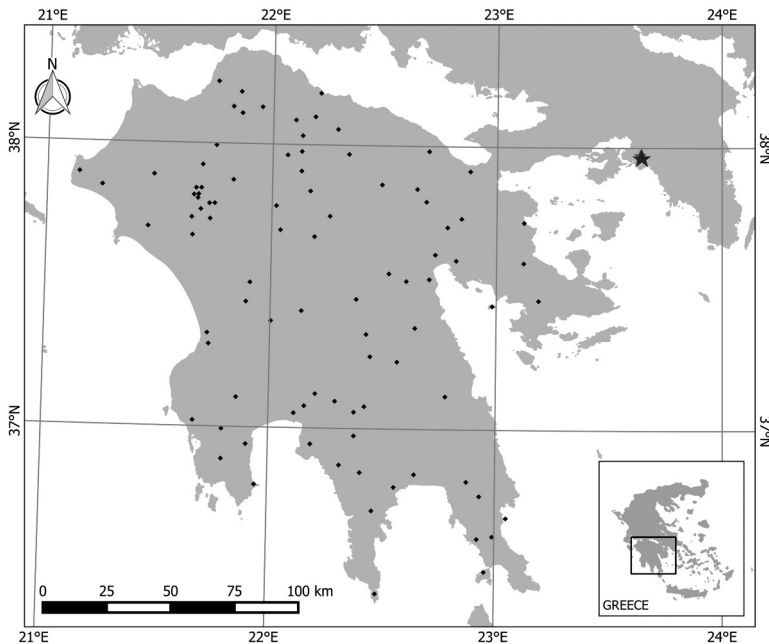


Fig. 3: The known range of *Podarcis peloponnesiacus* (BIBRON & BORY, 1833), endemic to Greece. Black dots denote the known distribution points (data from BRINGSØE 1986; VALAKOS et al. 2008; BROGGI 2016). The star corresponds to the new record locality in Attica.

the primers of PCR. The sequence was viewed and edited in CodonCode Aligner® (v. 3.7.1) (CodonCode Corporation). The authenticity of the sequence and the homology to the targeted gene was evaluated with a BLAST (Basic Local Alignment Search Tool) (v. 2.3.0) (ALTSCHUL et al. 1997) search in the NCBI genetic database (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). The above search revealed that the amplified cyt *b* sequence of 412 bp in length (KU530148) has high similarity (Max score > 654, Total Score > 654, Query Cover = 100 %, and E-value = 0) with all the available cyt *b* sequences of *P. peloponnesiacus* in GenBank (AY896116, AY896117, AY896120, AY896122, AY896124, AF486231, and KF003360), whereas the max score is lower than 636 and the E-value lower than 10^{-178} when it was compared with *P. erhardii* sequences. These results confirm the morphological data and demonstrate that the specimen from Nikaia (NHMC 80.3.54.133) belongs to *P. peloponnesiacus*.

This is the first reliable report of the occurrence of the Peloponnesian Wall Lizard outside the Peloponnese (BRINGSØE 1986; SOFIANIDOU 1997; VALAKOS et al. 2008) (Fig. 3). Although the habitat at the study site corresponds well with the habitat preferences of the species in the core of its range, further studies are needed to verify whether or not the finding refers to an autochthonous or human secondarily introduced specimen or even population.

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