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## Isolated population of the Middle Eastern *Phoenicolacerta laevis* from the Georgian Black Sea Coast, and its genetic closeness to populations from southern Turkey

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The Lebanon Lizard (*Phoenicolacerta laevis*) occurs on the Levantine coast and scattered populations are found in isolated coastal habitats along the southern and south-western coast of Turkey. We found an isolated but dense population of this species at and around the castle of Anaklia on Georgia's Black Sea Coast, near the mouth of the river Enguri. The analysis of mitochondrial cytochrome b sequence suggests closeness of this population to the populations in the provinces of Kahramanmaraş, Adana and İçel in southern Turkey, rather than to those in Lebanon and Israel. It is thought that the species was introduced from a Turkish region to Anaklia, which was an important trade location at the eastern Black Sea coast until the late 18<sup>th</sup> century. This is the first established location of this species outside the eastern Mediterranean area. Climate and competition with rock lizards of the genus *Darevskia* are the most likely reasons preventing expansion of the species into the neighboring areas of Western Georgia.

**Keywords:** Black Sea; Caucasus; cytochrome b; enclave populations

### Introduction

The Lebanon Lizard (*Phoenicolacerta laevis*) is a small-bodied lizard species from the eastern Mediterranean, whose range extends from southern Turkey (provinces of Hatay, Adana, Kahramanmaraş, and İçel) over western Syria and Lebanon to northern Israel and north-western Jordan (Crochet et al., 2016; Limberakis, P & Kalionzopoulou, 2013; Karış & Göçmen, 2014). Besides the main range of the species, approximately eleven isolated populations are found along the Mediterranean and Aegean coasts of Turkey (Karış & Göçmen, 2014; Ilgaz, Kumlutaş & Candan, 2016). Aside from the Lebanon Lizard, the genus *Phoenicolacerta* has two recently described species from the Levant, and a population from Cyprus whose taxonomic status was recently elevated to species level. None of the *Phoenicolacerta* species has ever been recorded outside the eastern Mediterranean. We found a population of *Phoenicolacerta laevis* on Georgia's Black sea Coast and validated species identification by mitochondrial DNA sequencing. This helped clarify the origin of the population.

### Methodology

Three adult males and three adult females were caught at the study site on 6 May 2017 and transported to Tbilisi, where they are kept alive in the Institute of Ecology, Ilia State University. DNA was extracted from tissue samples (tail tips) of three lizards using a Qiagen DNeasy tissue kit,

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Figure 1. Distribution map of *P. laevis* (after Crochet et al., 2009; Karış & Göçmen, 2014; Ilgaz et al., 2016; this paper). Numbers on the map indicate locations of the specimens whose sequences deposited to GenBank were used in this paper (see Figure 2). 3\* - location of *P. cyanosparsa* whose sequence was used in the analysis. The star shows new location of *P. laevis* in Anaklia, Georgian Black Sea coast.

according to the manufacturer's instructions (Qiagen, 2007). A *cyt b* fragment (693 bp) was amplified using primer pairs H15915-L15369 and H15488-L15153 (Fu, Murphy, & Darevsky, 1997; Murphy et al., 2000). PCR conditions are described in Gabelaia, Tarkhnishvili, and Murtskhvaladze (2015). The amplicons were sequenced on an ABI 3130 capillary sequencer. Single-stranded sequencing was performed with PCR primers, using Big-Dye v3.1. PCR fragments were sequenced in both directions to assure sequence accuracy. mtDNA sequences were edited using SEQSCAPE 2.5 (Applied Biosystems Inc., Foster City, CA, USA) and compared with the extant sequences using BLAST (Altschul, Gish, Miller, Myers, & Lipman, 1990). After finding the closest sequences from Genbank (*Phoenicolacerta laevis*), the sequences were aligned along with this and the other sequences of *Phoenicolacerta spp*. The latter were downloaded from the GenBank (Table 1). The Neighbor-Joining tree was built using MEGA v7 (Kumar, Stecher & Tamura, 2016). Because all three novel sequences were identical, only one has been deposited to GenBank (Accession Number MF438266).

### Location

The discovered location is about one hectare of the meadow around the Anaklia castle on the Georgian Black Sea coast, on the south bank of the river Enguri (Figure 1; 42.40N, 41.57E). A recently renovated hotel is currently operating in the same area. The walls of the castle where the lizards were recorded, were built of large stones back during the 18<sup>th</sup> century, although the area was has been populated since at least Antic times (Baddeley, 1908; Jibladze, 2007). The landscape dominating in the area is comprised of inundated meadows and sand dunes at the Black Sea coast and banks of the Enguri River; the lizards were found on the old walls of the castle, together with the adjacent plot of meadow with fragmentary shrubs of *Rubus* spp. Besides the Lebanon Lizard, the area is habitat for six species of squamates: Ring Snake (*Natrix natrix scutata*), Dice Snake (*Natrix tessellata*), Colchic Slow Worm (*Anguis colchica*), Sand Lizard (*Lacerta agilis*

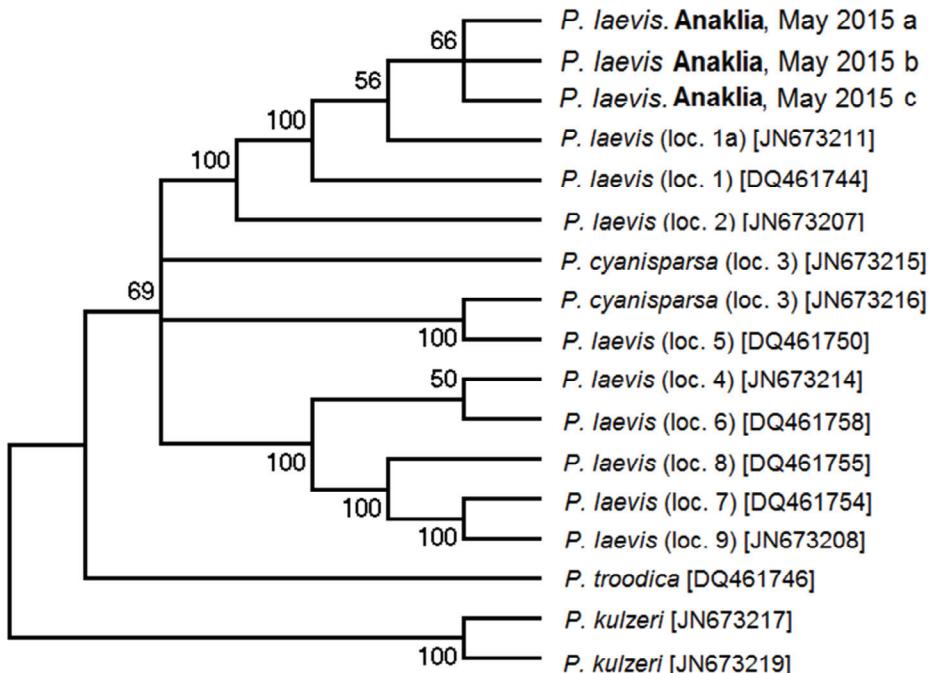


Figure 2. Neighborhood-joining trees (condensed, with bootstrap threshold level 50) including three specimens from Anaklia and sequences from GenBank representing four species of *Phoenicolacerta* from the Middle East. The numbers of the locations correspond to those shown in Figure 1. The numbers in square brackets are GenBank accession numbers.

*gruzinica*), and Artwin Lizard (*Darevskia derjugini*) (Muskhelishvili, 1984; our field survey); presence of the Smooth Snake (*Coronella austriaca*) is possible. None of these reptiles has stony walls as main habitat.

The climate of the study site is Mediterranean-like (although annual mean precipitation is higher, approximately 1800 mm), the mean temperature of the hottest month (July) is 27.7°C, and that of the coldest month (January) is 2.1°C (Museibov, Nazarian, Gabrielian, & Jakeli, 1986), slightly warmer and drier than the Black Sea coastal areas south and east of the site.

## Results and Discussion

Externally, the individuals collected at the Anaklia castle correspond to the description of *Phoenicolacerta laevis* (Schmidtler & Bischoff, 1999). The analysis of the sequences of our specimens and 13 sequences of *P. laevis* (Gray, 1838), *P. kulzeri* (Müller & Wettstein, 1932), *P. cyanisparsa* (Schmidtler & Bischoff, 1999), and *P. troodica* (Werner, 1936) downloaded from Genbank confirmed species identification, and placed the specimens from Anaklia close to the *P. laevis* specimens from the provinces of Kahramanmaraş, Adana and Içel in Turkey (Figure 2). There was one substitution specific for the three sequences from Anaklia (no ingroup variation) and separating it from the closest sequence from Andırın Kahramanmaraş province, Turkey (GenBank accession # JN673211). Moreover, the tree showed that *P. cyanisparsa* is a matrilineal ingroup with respect to different geographic populations of *P. laevis*.

During one hour of observation (6 May 2017, 12:00-13:00 p.m.), more than 100 individual lizards, adults and young, were recorded at the walls and in grass along the walls. Hence, the local population is dense and probably long-lasting, although spatially limited. It is most likely that the lizards from which the Anaklia population descended were unintentionally brought to Anaklia by humans. The questions remain: (1) when was the population established? (2) why did the population not expand into surrounding areas of the Black Sea coast? (3) is the population viable? (4) does it require conservation measures?

Karış and Göçmen (2014) and Ilgaz, Kumlutaş, and Candan (2016) reviewed the distribution of *P. laevis* and counted altogether eleven exclave populations along the Mediterranean coast of Turkey, and one on the Turkish Aegean coast. It is supposed that these exclaves have their origin in unintentional relocations by man, for example in ships loaded with construction materials.

The settlement of Anaklia in Ancient Colchis dates at least to the middle Bronze Age (Jibladze, 2007). In the early 18<sup>th</sup> century, the current Anaklia castle was built (or re-built? Allen, 1953). In that time, the location was an important port/trade area. Anaklia most likely remained an important port from classical Greek times through the 18<sup>th</sup> century, although the trade in the area declined in the 19<sup>th</sup> century. Consequently, ancestral individuals of the population of *Phoenicolacerta laevis* could have been transported into the area with vessels from Eastern Mediterranean ports. The genetic links suggest that the lizards were most likely transported with cargo coming from Andirin area, approximately 150 km north-east of the port of Mersin in the southern Turkey.

A broader geographic area where the established population is located hosts rock lizards from genus *Darevskia*, including *D. derjugini*, *D. praticola* (lowland area), *D. rufa*, *D. brauneri*, *D. mixta* (rocks 10-30km away from the location). Competition with these species, together with climate, which becomes harsher away from the sea coast and more humid south of the mouth of the River Enguri, has most likely prevented further expansion of *P. laevis* in Georgia. However, the local population survived, most probably for over two centuries. Within its natural range *P. laevis* is found in a broad range of natural and man-made habitats, including meadows, gardens, walls of houses and orchards (Crochet et al., 2009). The described location falls within this range. Further genetic analysis can help to infer more specific age of the establishment of the population.

Due to active construction activity in the area of Anaklia, the habitat of *P. laevis* may be transformed and this may cause the extinction of the exclave. To avoid this, urgent measures are necessary for protecting the walls of the Anaklia Castle and adjacent vegetation.

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## Disclosure Statement

No potential conflict of interest was reported by the authors.

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