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A snake genus new to the reptile list of Lebanon: Genetic affiliation and distribution of *Letheobia simoni* (Boettger, 1879)

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The herpetofauna of Lebanon is rich and diverse with several dozen native species (Hraoui-Bloquet *et al*. 2002) although this inventory has not been recently revised. Moreover, many areas and populations lack both field and genetic assessments, with unknown relationships to other populations of the Middle East and the Western Palearctic. Additionally, the complicated political situation in the Middle East does not allow for smooth comparative assessments based on collaborations between institutions across the region, especially in the Levant. However, several recent studies included Lebanese populations of reptiles and studied their genetic patterns (e.g., Tamar *et al*. 2015; Jablonski & Sadek 2019; Kornilios *et al*. 2020; Tamar & Moravec 2022; Jablonski *et al*. 2023). Based on such research, we know that the reptile fauna of Lebanon displays contrasting patterns of diversity. It includes evolutionary lineages that have dispersed from various areas of the Middle East, as well as lineages unique to the Levant. However, due to the lack of systematic herpetological surveys, Lebanon may harbour species that have previously only been recorded outside its political borders (see Khashab & Jablonski 2022). This is also the case of the genus *Letheobia* Cope, 1868 (family Typhlopidae) described below.

The genus *Letheobia* has its highest species diversity in Africa, including a total of 37 species worldwide. However, recognizing and classifying these snakes is not straightforward due to their morphological similarities. It is expected that the ancestor of two species, *L. episcopa* (Franzen & Wallach, 2002) and *L. simoni* (Boettger, 1879), spread through the present African-Middle Eastern region (when it had a more equatorial climate) and subsequently diverged. However, there is currently no robust biogeographic hypothesis for these snakes, and the issue is generally unresolved (Pyron & Wallach 2014). The first species, *L. episcopa*, is endemic to southern Turkey and northern Syria (Franzen & Wallach 2002; Aidek *et al*. 2023; Murai *et al*. 2023). The second species, *L. simoni*, is found in the southern Levant, specifically in the central and western Negev Mountains to Galilee, and east of the Rift, north of the Jordanian Highlands (Bar *et al*. 2021; Fig. 1A). Although some sources mention the presence of *L. simoni* in southwest Syria (Sivan & Werner 1992; Martens 1997; Werner 2016; Bar *et al*. 2021 but see Franzen 2000 or the specimen in the collection of the Natural History Museum in Vienna, NHMW 15391 from "Syria" without further locality details) and even southern Lebanon (Geniez 2018; Egan 2022), the species is not currently confirmed from these countries (Hraoui-Bloquet *et al*. 2002; Aidek *et al*. 2023).

During field investigations in southern Lebanon on 10 March 2023, we found an adult specimen of *L. simoni* near Kfar Joz/Kfour in Nabatieh Governorate (33.39540°N, 35.46336°E, 432 m) that represents the first vouchered record for the country. During subsequent field surveys (26 March 2023) we found more specimens in the same or nearby locations: 33.39582°N, 35.46636°E, 378 m; 33.40678°N, 35.44533°E, 404 m; 33.40773°N, 35.45477°E, 362 m; 33.40810°N, 35.45453°E, 363 m (one adult specimen on each location). Later, on 15 May 2023 we obtained photographically supported information about the species' presence in Maarake, South Lebanon Governorate (33.27076°N, 35.30640°E, 249 m), about 22 km southwest of Kfar Joz/Kfour (Fig. 1A). Observations were also made in spring 2024 (33.39637°N, 35.46578, 388 m, four individuals on 15 April 2024; 33.40658°N, 35.45505°E, 354 m, one individual, 11 May 2024) and, so far, we have recorded 11 individuals of the species from Mediterranean habitats of southern Lebanon. During the summer and

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autumn of 2023, the species was not recorded from investigated localities, and we expect that snakes move deeper into the soil during hot weather conditions. Both regions represent typical Mediterranean habitats of southern Lebanon, i.e., karst stone areas, with red, soft, wet soil and green spring vegetation (Fig. 1D). Together with *L. simoni*, we observed 25 syntopic species of reptiles in Kfar Joz/Kfour area (see Appendix 1).

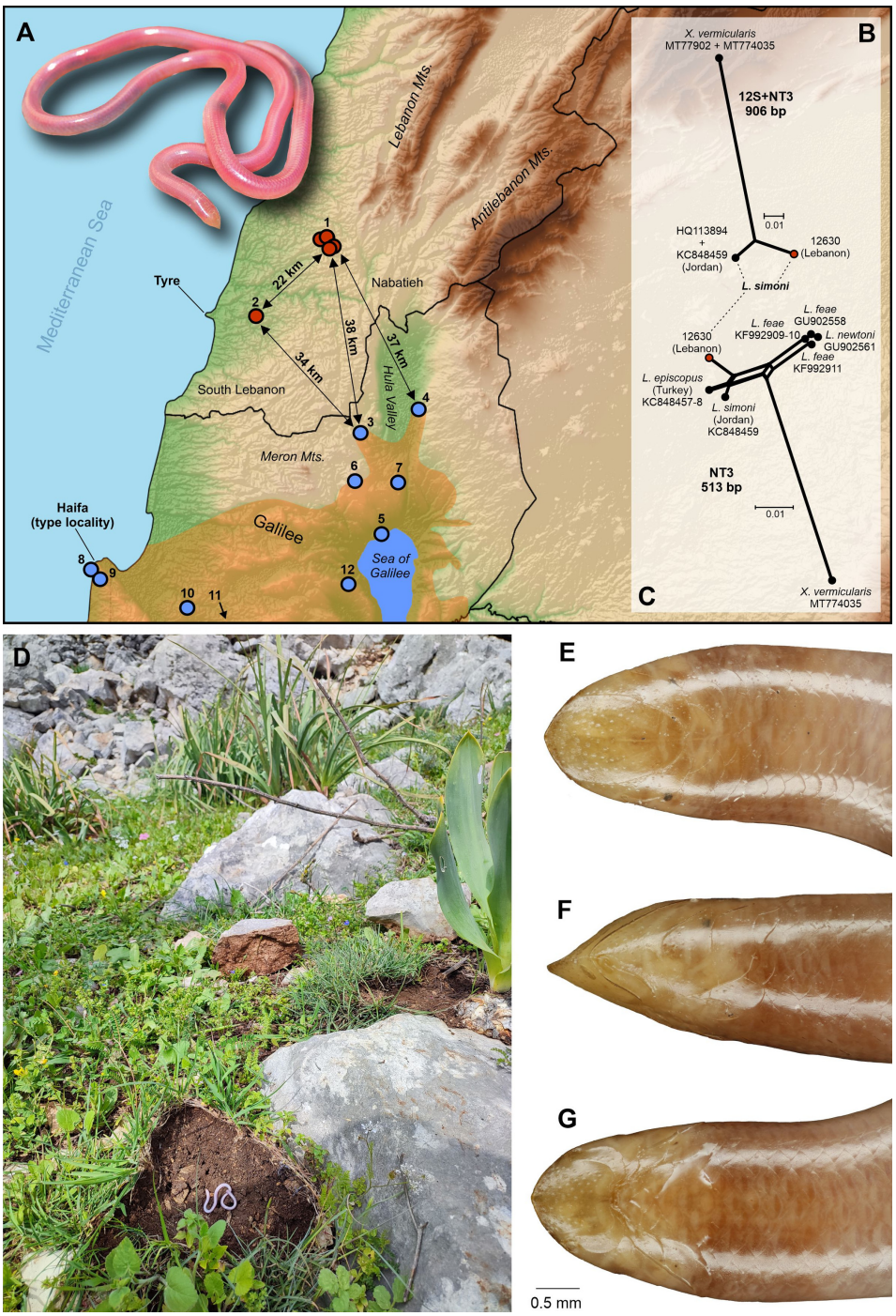

FIGURE 1. (A) Distribution data for *Letheobia simoni* from Nabatieh and South Lebanon and Galilee and the Hula/Jordan Valley: **1**—Kfar Joz/Kfour, **2**—Maarake, **3**—Nahal Dishon (ZFMK 052243; 33.062°N, 35.526°E), **4**—the Jordan Valley, approximately 25 km north of Lake Galilee (Sivan & Werner 1992; 33.123°N, 35.651°E). Other localities are: **5**—Bar *et al*. (2021), **6**—https://www.inaturalist.org/observations/190242290, **7**—https://www.inaturalist.org/observations/31283396, **8**—https://www.inaturalist.org/observations/175493806, **9**—Boettger (1879, 1880), **10**—Warburg (1978), **11**—Franzen (2000), **12**—Haas (1951). The orange shading represents the expected range polygon for the species extralimital to South Lebanon. (B, C) Phylogenetic networks (NeighborNet) from concatenated 12S and NT3 dataset (B) and NT3 only (C), including specimen from Lebanon and other congeners. The microhabitat of the species in Nabatieh Governorate (D) and dorsal (E), lateral (F) and ventral (G) views of the head of *Letheobia simoni* (CUHC 12630) from Kfar Joz/Kfour, Nabatieh Governorate.

Following Franzen & Wallach (2002) we took basic morphometric data from the collected specimen of *L. simoni*: total length in mm (TTL): 131; head length (HL): 2.74; tail length (TL): 1.72; midbody diameter (MBD): 1.99. Photographic details on the head morphology of the specimen from Kfar Joz/Kfour are presented on Fig. 1E-G.

To confirm the genetic affiliation of the newly reported population from Lebanon, we collected one specimen (Comenius University Herpetological Collection [CUHC] 12630) that we preserved in 70% ethanol. From this specimen, a drop of blood from the tail was taken and stored in 96% ethanol. DNA was extracted using the E.Z.N.A.® Tissue DNA Kit (Omega Bio-tek, Inc., Norcross, GA, USA). Sequences of two mitochondrial (mtDNA) and one nuclear (nuDNA) gene were targeted: 12S rRNA (12S), cytochrome *b* (cyt *b*), and the neurotrophin-3 (NT3). For the laboratory procedures and primers of 12S and NT3, we followed the methodology of Vidal *et al*. (2010) and Kornilios *et al*. (2011, 2012). For cyt *b* we used primers Cytb_WWF (AAAYCAYCGTTGTWATTCAACTAC) and CYTB_R2 (GGGTGRAAKGGRATTTTATC) of Broadley *et al*. (2006) and Whiting *et al*. (2003) with an annealing temperature 50°C. For the PCR, we used Red Taq 2X Master Mix 2 mM MgCl. PCR products were purified with ExoSAP-ITTM PCR Product Cleanup Reagent (USB Europe GmbH, Staufen, Germany, following the manufacturer's protocol). Sequencing was performed by Macrogen Inc. (Amsterdam, Netherlands; https://www.macrogen-europe.com/).

New raw sequences were checked visually and aligned using Seaview 5 (Gouy *et al*. 2021). We performed a BLAST search in GenBank to confirm that the targeted loci and genus/species were amplified. The translation of protein-coding sequences into amino acids was checked using DnaSP 6.00 (Rozas *et al*. 2017), confirming the absence of stop codons. This program was also used to estimate uncorrected *p* distances among new and published sequences of *L. simoni*. Then, we combined new sequences with GenBank data (see Fig. 1B,C). Due to limited and unequally represented sequences/genes for the genus *Letheobia*, we constructed two final alignments (concatenated 12S and NT3 and NT3 separately) that we analysed through phylogenetic networks (NeighborNet) implemented in SplitsTree 4.18.3 (Huson & Bryant 2006; default settings). For *L. simoni* only two sequences of 12S and NT3 originating from Jordan ("4 km S of Al Mazar, al Janubi"; ca 31.08°N, 35.75°E) are available: HQ113894 (Kornilios *et al*. 2011) and KC848459 (Kornilios *et al*. 2013), respectively. The sequence JQ045136 (12S) is identical to HQ113894 and thus was not analysed. The sequence of cyt *b* was not compared here due to missing comparative material but it may be used in research. The three novel sequences were deposited in GenBank under accession numbers: PQ363685 (12S), PQ374440 (cyt *b*), and PQ374441 (NT3).

Our data document the first recorded presence of *Letheobia* in Lebanon. This extends the known northernmost distribution of *L. simoni* in the North Galilee, specifically Nahal Dishon, ZFMK 052243, and "the Jordan Valley, approximately 25 km north of Lake Galilee" (Sivan & Werner 1992; Franzen 2000; Fig. 1A), by more than 30 km to the north. This report adds a new genus and species to the list of reptiles in the country and represents the smallest Lebanese snake. However, the presence of this genus in southern Lebanon is not surprising from a biogeographic perspective. There are several (fossorial or semi-fossorial) reptiles that are endemic to the southern Levant that are present or expected in southern Lebanon, following the thermo-Mediterranean and eu-Mediterranean phyto-association zones (Abi Saleh & Safi 1988). These include *Ablepharus rueppellii* (Gray, 1839), *Chalcides guentheri* Boulenger, 1887, *Ophiomorus latastii* Boulenger, 1887 (all Scincidae), and *Micrelaps muelleri* Boettger, 1880 (Micrelapidae). Their evolutionary lineages probably arrived in the region before the formation the Saharo-Arabian deserts and now primarily represent West Asian and African elements that have evolved in the southern Levant.

Letheobia simoni is not the only fossorial snake species present in Lebanon. Another species from the same family, *Xerotyphlops syriacus* (Jan, 1864), is common and can be misidentified in the field as a *Letheobia*. This is why the new member of the Lebanese herpetofauna could have gone unnoticed for a long time. In the future, under better political and safety conditions, which affect biodiversity research, further investigations will be needed in southern Lebanon to find new locations of occurrence for the species and to understand the distribution patterns between the genera *Xerotyphlops* and *Letheobia*. Field investigations are also necessary to determine the potential presence of another fossorial snake, morphologically similar to the family Typhlopidae but belonging to the family Leptotyphlopidae, *Myriopholis macrorhyncha* (Jan, 1860). Although this species has never been recorded in Lebanon, it is known to inhabit surrounding countries with similar environments to those found in southern and eastern parts of the country.

The genetically investigated specimen of *L. simoni* from Kfar Joz/Kfour forms a different evolutionary lineage in phylogenetic networks (Fig. 1B,C) compared to sequences available for the species from Jordan. The average uncorrected *p* distance between them is high—6.0% for 12S and 1.4% for NT3, suggesting an ancient divergence. Similarly, there are high levels of divergence (1.6%) for NT3 (12S data for *L. episcopus* is not available) between *L. simoni* from Lebanon and *L. episcopus* from southern Turkey (KC848457-8).

Because Haifa, the type locality of *L. simoni*, is geographically close to the population genetically investigated in this study (Fig. 1A), we expect that both populations are conspecific, which has taxonomic implications for the genus in the

region. This highlights the need for further phylogeographic investigations, as the diversity of the genus *Letheobia* in the Levant and the Middle East is likely higher than currently recognized.

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Appendix 1: List of reptiles observed during the search for *Letheobia simoni* in the Kfar Joz/Kfour area: *Testudo graeca*, *Hemidactylus turcicus*, *Ptyodactylus puiseuxi*, *Laudakia vulgaris*, *Ablepharus rueppellii*, *Chalcides guentheri*, *Eumeces schneiderii*, *Heremites vittatus*, *Ophisops elegans*, *Phoenicolacerta laevis*, *Pseudopus apodus levantinus*, *Xerotyphlops syriacus*, *Eryx jaculus, Eirenis decemlineatus*, *E. lineomaculatus*, *E. rothii*, *Dolichophis jugularis*, *Hemorrhois nummifer*, *Platyceps collaris*, *Rhynchocalamus melanocephalus*, *Telescopus fallax*, *Natrix tessellata*, *Malpolon insignitus*, *Micrelaps muelleri*, and *Daboia palaestinae*.