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Discovery of the Black-headed Ground Snake *Rhynchocalamus melanocephalus* (Jan, 1862) in Cyprus (Reptilia: Colubridae)

Karin Tamar[a,*], Hans Jörg Wiedl[b], Erez Maza[a,c], Daniel Jablonski[d] and Shai Meiri[a,c]

[a]The Steinhardt Museum of Natural History, Israel National Center for Biodiversity Studies, Tel Aviv University, Tel Aviv, Israel; [b]Paphos, Cyprus; [c]School of Zoology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel; [d]Department of Zoology, Comenius University in Bratislava, Bratislava, Slovakia

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We identified two snakes from Paphos district in south-western Cyprus as belonging to the secretive genus *Rhynchocalamus*. They represent the first record of these snakes in Cyprus. Morphological features and mitochondrial *16S* DNA sequences suggest that these specimens belong to *R. melanocephalus*, a species widely distributed in the Eastern Mediterranean region. The genetic similarity to a specimen from northern Israel may imply either a human-mediated dispersal or a natural colonization. These observations thus raise the number of snake species known to occur in Cyprus to 12.

Keywords: Distribution; range extension; mitochondrial DNA sequences; Middle East

Introduction

The members of the genus *Rhynchocalamus* are small, non-venomous, semi-fossorial and terrestrial colubrid snakes. They are secretive and relatively little known. These snakes are mostly nocturnal and prefer high humidity, but can also be found active during the day, and underneath rocks (Avcı, Dinçaslan, Ilgaz, & Üzüm, 2008; Baha El Din, 2006; Disi, Modry, Necas, & Rifai, 2001; Gasperetti, 1988; Werner, 2016 and the authors' personal observations). *Rhynchocalamus* snakes are found in mountainous areas in both Mediterranean and Irano-Turanian ecozones (Figure 1) on heavy soils (Baha El Din, 2006; Disi et al., 2001; Werner, 2016). The genus *Rhynchocalamus* comprises five recognised species distributed in the Eastern Mediterranean region (*R. melanocephalus* and *R. dayanae*), southern Arabia (*R. arabicus*), and south-west Asia (*R. satunini* and *R. levitoni*) (Figure 1; see Avcı et al., 2008, 2015; Roll et al., 2017; Tamar, Šmíd, Göçmen, Meiri, & Carranza, 2016; Torki, 2017; Uetz, Freed, & Hošek, 2020).

We report here the first specimens of *Rhynchocalamus* snakes recorded from Cyprus and attempted to clarify their phylogenetic position and relationships by using an integrative approach of morphological characters and mitochondrial DNA sequences.

Material and Methods

The morphological identification included two specimens examined by KT for 17 morphometric, 13 meristic, and two categorical characters as detailed in Tamar et al. (2016) and presented in Table S1. The two individuals, both juveniles, were deposited, one in the Steinhardt Museum of Natural History, Tel Aviv University, Israel (hereafter TAU.R18977), and the other in HJW's private collection, Cyprus (hereafter 2018 snake).

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*Corresponding author. Email: karintmr@gmail.com

For the molecular investigation, we extracted DNA from alcohol-preserved tissue samples using the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA). Molecular identification was possible for specimen TAU.R18977 only; we tried unsuccessfully to amplify DNA from the 2018 specimen for the molecular analyses. We PCR-amplified and bi-directionally sequenced the ribosomal 16S rRNA (*16S*) mitochondrial gene fragment with primers and PCR conditions as detailed in Šmíd et al. (2015). PCR products were purified with ExoSAP-IT PCR Product Cleanup and sequenced using ABI3730XL sequencer (Applied Biosystems). Chromatographs were checked and aligned using Geneious R7 (v.7.1.9; Kearse et al., 2012).

The molecular phylogenetic position of specimen TAU.R18977 (GenBank accession number MT229307) was assessed by comparing it to 32 sequences of other species of *Rhynchocalamus* retrieved from GenBank of *16S* and Cytochrome b (*Cytb*). These sequences are from the studies of Šmíd et al. (2015) and Tamar at al. (2016), with the addition of a specimen of *R. melanocephalus*, code DJ6851, from Daychounieh, Lebanon (collected by DJ; GenBank accession numbers MT229306 for *16S* and MT239350 for *Cytb*). Three specimens of *Lytorhynchus diadema* were used as an outgroup (data from Tamar et al., 2016). We carried out concatenated maximum likelihood (ML) and Bayesian inference (BI) phylogenetic analyses. We performed the ML analysis in RAxML v.8.1.2 as implemented in raxmlGUI v.1.5 (Silvestro & Michalak, 2012), with the GTR-GAMMA model and 100 ML inferences. Node support was assessed with bootstrap analysis of 1,000 replicates. For the Bayesian analysis we used jModelTest v.2.1.7 (Guindon & Gascuel, 2003; Darriba, Taboada, Doallo, & Posada, 2012) to select the best model of nucleotide substitution under the Bayesian Information Criterion (BIC). The BI analysis was performed with MrBayes v.3.2.6 (Ronquist et al., 2012) with the substitution model TrN+I (nst=6, rate=equal). Two simultaneous parallel runs were performed for one million generations, with sampling frequency of every 100 generations, discarding the first 25% of trees as burn-in. We calculated inter- and intraspecific uncorrected *p*-distance between and within *Rhynchocalamus* species, with pairwise deletion, in MEGA v.7 (Kumar, Stecher, & Tamura, 2016).

Results

Three observations of *Rhynchocalamus* snakes were made in Cyprus in the vicinity of Peyia village, Akamas Peninsula, Paphos district (~34.88N, 32.38E; Figure 1). The first snake was discovered near the village of Peyia in late November 2013, and was identified by HJW (TAU.R18977). The second snake was found alive in October 2017 by a tourist around the area of Coral Bay (~34.86N, 32.36E). The third snake was observed in late September 2018 near Peyia and was identified by HJW (the 2018 snake). All three snakes were found in a rocky area, on a hillside approximately 100 m a.s.l., with carob trees, many bushes, stones and short grass. Within this area, the snakes were found hiding in rock cracks and crevices, and underneath stones. This area is dry and hot, deserted, and used mainly by sheep herders.

The results of the phylogenetic analyses place specimen TAU.R18977 as a member of the genus *Rhynchocalamus*. The BI and ML phylogenetic trees resulted in identical topologies with the five species of *Rhynchocalamus* recovered as monophyletic (Figure 2). The Cypriot specimen clusters with samples of *R. melanocephalus* from Israel, Jordan and Lebanon, and the *16S* sequence was identical to that of a sample (HUJ.R20967) from Mt. Hermon in Israel. The uncorrected genetic distance in the *16S* mitochondrial fragment ranged between 3.7 to 5.4% among *Rhynchocalamus* species. The genetic divergence within *R. melanocephalus*, including the Cypriot specimen, was 0.5%.

The morphological comparisons of the two Cypriot specimens also support their identification as *R. melanocephalus*. We performed all morphological examinations on specimen TAU.R18977 (Figure 3A–F), but could not do so for the 2018 snake as its anterior ventral region is damaged (Figure 3G–L). The two Cypriot specimens have the following characters (see Table S1 for descriptive variables): cylindrical body with 15

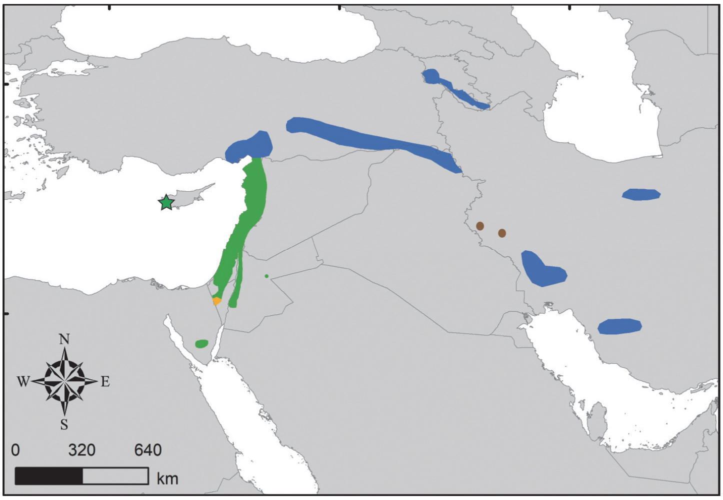

Figure 1. Distribution of *Rhynchocalamus* species (except *R. arabicus*) with the new locality in Cyprus indicated by a star. Colouration of the distribution ranges corresponds to the species in the phylogenetic tree in Figure 2.

smooth dorsal scale rows; head small, not distinct from the neck; rostral shield enlarged, pointed backwards and wedged between the internasals; nostril situated in an undivided elongated nasal scale; small eyes with round pupils; a single loreal scale; anal and subcaudal scales divided. The following details refer to specimen TAU.R18977 (the 2018 snake is too damaged): 3rd and 4th upper labial scales larger than the 1st and 2nd and they are in contact with the eye; shape of the 1st upper labial scale trapezoid, and that of the 2nd scale square; six upper labial scales; seven lower labial scales; three lower labial scales in contact with the anterior inframaxillars.

In alcohol the colouration of the two individuals also agrees with *R. melanocephalus*. Dorsal area of head, neck and first dorsal scales is glossy black. Most of the ventral side of the head and the lower side of the upper labials white, with the first lower labials and anterior inframaxillars black. Dorsum grey-yellowish-brown without maculation, ventrum white.

Discussion

Our inferred molecular phylogenetic position and morphological examinations of two *Rhynchocalamus* specimens from the Akamas Peninsula of Cyprus were congruent and support their identification as belonging to the Eastern Mediterranean species *R. melanocephalus*. Although both specimens are juveniles, preventing a reliance on the morphometric variables, the meristic characters and colouration pattern range within the variation of the species (Tamar et al., 2016).

This is the first known occurrence of this genus on the island (Baier, Sparrow, & Wiedl, 2009; Uetz et al., 2020). Eleven species of snakes are known in Cyprus (Uetz et al., 2020): One belongs to the Typhlopidae (*Xerotyphlops*), one to the Viperidae (*Macrovipera*), one to the Psammophiidae (*Malpolon*) and eight to the Colubridae (two *Natrix* and one in each of *Dolichophis*, *Eirenis*, *Hemorrhois*, *Hierophis, Platyceps*, and *Telescopus*). *Rhynchocalamus melanocephalus* is thus the 9th colubrid species and the 12th snake in the Cypriot herpetofauna.

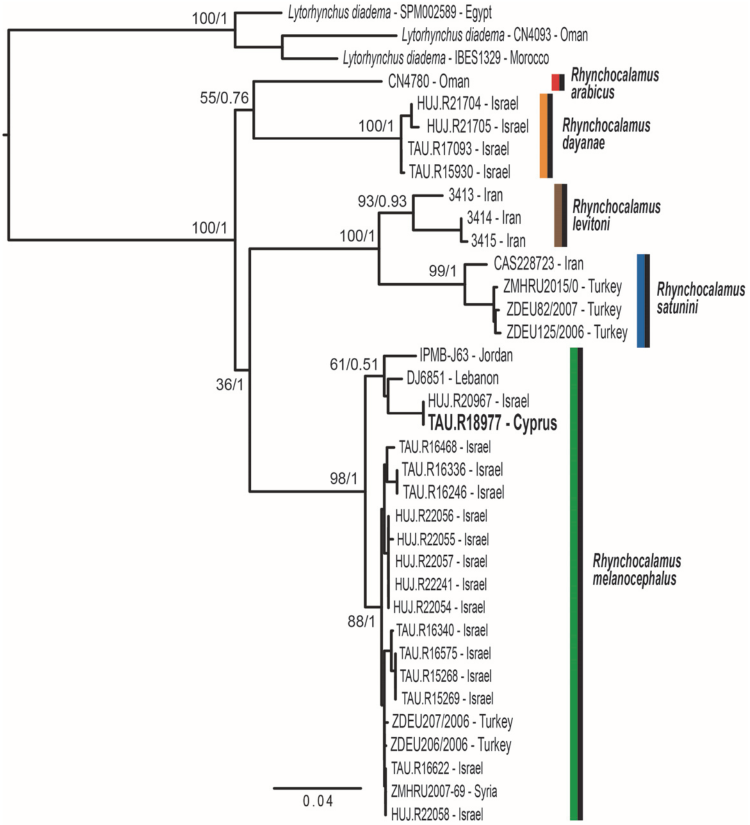

Figure 2. Mitochondrial ML phylogenetic tree with colours corresponding to species. The new sample from Cyprus is in bold. Support values are indicated near the nodes (ML bootstrap/BI posterior probabilities).

The secretive nature of *Rhynchocalamus* snakes and their often nocturnal activity make their finding difficult – especially where they occur at low abundance. Finding new localities of *R. melanocephalus* is not surprising (e.g., Avcı et al., 2008), and two new species were recently described for the genus (*R. dayanae*, Tamar et al., 2016; *R. levitoni,* Torki, 2017; Rajabizadeh, 2017). Therefore, the fact that there were no earlier records of *Rhynchocalamus* snakes from Cyprus is not good evidence against its continuous, but cryptic, occurrence on the island.

The *16S* mitochondrial sequence we amplified was identical to a specimen from Mt. Hermon, northern Israel. This suggests an alternative scenario, whereby the Cypriot population may have originated in a recent introduction from that general area. In the time-calibrated phylogeny by Tamar et al. (2016) the divergence within *R. melanocephalus* was estimated around 3.5 Mya, well after the Messinian salinity crisis (5.96–5.33 Mya; Krijgsman, Hilgen, Raffi, Sierro, & Wilson, 1999). As post-Messinian sea level changes are unlikely to have formed connections between Cyprus and the mainland (Jolivet, Augier, Robin, Suc, & Rouchy, 2006; Steininger & Rögl, 1984), and as *Rhynchocalamus* species are restricted to Asia (Tamar et al., 2016), it is possible that the Cypriot population has originated from unintentional human-mediated dispersal rather

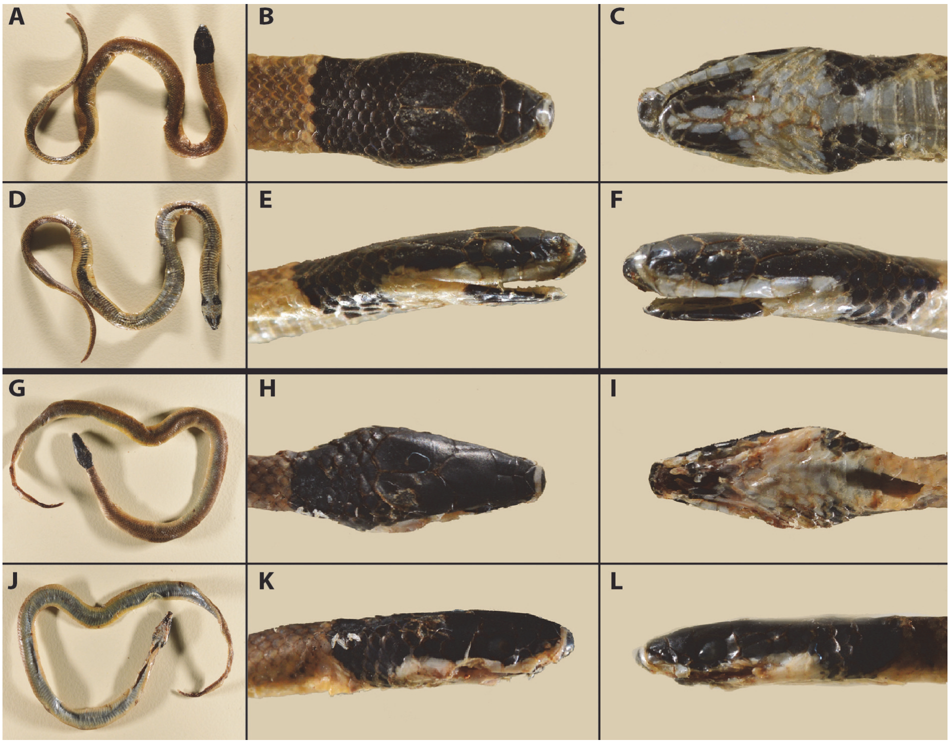

Figure 3. General appearance of the two *Rhynchocalamus* specimens from Cyprus. (A–F) General appearance of specimen TAU.R18977. (G–L) General appearance of the 2018 snake. Photographs: Alex Slavenko.

than from natural overseas dispersal. However, with the data at hand we cannot infer which of the scenarios is more likely. Comprehensive phylogeographical assessment of *R. melanocephalus* necessitates further sampling in Cyprus and in the eastern Mediterranean region.

Supplementary Material

Supplementary material (Table S1) is given as a Supplementary Annex, which is available via the "Supplementary" tab on the article's online page (http://dx.doi.org/10.1080/09397140.2020.1757914).

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Disclosure Statement

No potential conflict of interest was reported by the authors.

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