MOLECULAR PHYLOGENY AND MICRO CT-SCANNING REVEALED EXTREME CRYPTIC BIODIVERSITY IN KUKRI SNAKE, *Muhtarophis* gen. nov., A NEW GENUS FOR *Rhynchocalamus barani* (SERPENTES: COLUBRIDAE)

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Application of taxonomy exclusively based on external morphology, molecular phylogeny and noninvasive anatomical investigation using micro CT scanning together is effective in resolving systematic problems, such as cryptic species. The external morphology, skull osteology and molecular phylogeny of Baran’s black-headed dwarf snake, *Rhynchocalamus barani*, and a subspecies of the Palestine kukri snake, *Rhynchocalamus melanocephalus*, were examined. Considerable osteological and molecular differences indicate that the genus *Rhynchocalamus* is paraphyletic. As a result, Baran’s black-headed dwarf snake should be referred to a monotypic genus, *Muhtarophis,* gen. nov. Based on morphology and molecular data, *R. satunini*, previously known as a subspecies of *R. melanocephalus*, should be assigned the rank of species.

**Keywords:** *Muhtarophis* gen. nov.; *Rhynchocalamus melanocephalus*; *Rhynchocalamus satunini*; Colubridae; Turkey.

INTRODUCTION

The genus *Rhynchocalamus* is composed of three known species, distributed across the Middle East. *Rhynchocalamus melanocephalus* (Jan, 1862) is distributed over Egypt, Jordan, Lebanon, Syria, Israel, Iran, Iraq, Armenia, Azerbaijan, and Turkey (Nikolsky, 1905; Flower, 1933; Schmidt, 1939; Darevsky, 1970; Eiselt, 1970; Esterbauer, 1985; 1992; Gasperetti, 1988; Werner, 1988; Latifi, 1991; Leviton et al., 1992; Baran and Atatür, 1998; Avci et al., 2007; 2008; Baran et al., 2012). *R. melanocephalus* has two subspecies. The nominate subspecies is distributed over Egypt, Jordan, Lebanon, Syria, Israel and Turkey. The second subspecies, *R. m. satunini*, was described as *Contia satunini* by Nikolsky in 1899, but was later considered as a subspecies of *Oligodon melanocephalus* (Chernov, 1937). Reed and Marx (1959) included this snake in their samples from Iraq as *Rhynchocalamus satunini*, due to its pholidotic characters and color pattern features. Finally, Darevsky (1970) placed *satunini* as a subspecies of *Rhynchocalamus melanocephalus*. This subspecies inhabits Turkey, Iran, Iraq, Armenia, and Azerbaijan (Baran, 1976; Başoğlu and Baran, 1980; Franzen and Bischoff, 1995; Avci et al., 2007). The second species, *R. arabicus* Schmidt, 1933, is only known from Aden (terra typica) in South Yemen (Schmidt, 1933).

The third unusual species of *Rhynchocalamus* is distributed in the Amanos Mountain and Yayladağı, Hatay, Turkey (Olgun et al., 2007; Avci et al., 2009). *R. barani* differs from other *Rhynchocalamus* species in having a higher number of dorsalia (17 instead of 15) and lower number of ventralia (163 – 173 instead of 180 – 240) and upper labials in contact with the eye (1 instead of 2) and by a characteristic color-pattern of the body (Olgun et al., 2007). Comparison of the *cyt b* sequence data of *R. bara-

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ni and subspecies of *R. melanocephalus* with those in the GenBank for other genera of Colubrinae showed that *R. barani* is highly divergent from related taxa (Yılmaz et al., 2009).

The aim of this study is to investigate the phylogenetic position of *R. barani* and subspecies of *R. melanocephalus* in order to examine whether its current classification, based on morphology, is also supported by molecular and osteological data.

**MATERIAL AND METHODS**

The samples examined


The preserved samples were deposited in the Zoology Laboratory of the Department of Biology at the Science and Arts Faculty, Adnan Menderes University, and incorporated into the collection of ZDEU (Zoology Department, Ege University, Turkey).

Color and pattern characteristics of the samples were recorded and color slides taken while the samples were alive. The samples were anaesthetised with ether, fixed with 96% ethanol, and later kept in 70% ethanol.

Measurements (in mm) of specimens were taken using a dial caliper. The ventral plates were counted using the Dowling (1951) system. The terminology used conforms to Olgun et al. (2007) and Avci et al. (2009).

DNA Extraction, PCR and Sequencing

The cytochrome *b* (*cyt b*), and oocyte maturation factor Mos (*c-mos*) genes of six samples belonging to the genus *Rhynchocalamus* were sequenced. Genomic DNA was extracted from liver tissue by the standard method of proteinase *K* digestion in lysis buffer, followed by phenol/chloroform extraction (Burbriumph, 2000).

The mtDNA fragment was amplified: *cyt b* with primers L14910, 5′-GACCTGTTAGTGAAGCACTAC-3′ and S77, 5′-CATGGACTGGGATCAGTTTATG-3′ (~1100 bp, de Queiroz et al., 2002). The nDNA fragment was amplified: *c-mos* with primers S77, 5′-CATGGACTGGGATCAGTTATG-3′ and S78, 5′-CTTTGTTTTACTAAGCAATGCTTTA-3′ (~570 bp, Lawson et al., 2005).

The 50 μl volume for each PCR reaction was contained: 0.5 U of Taq polymerase, 5 μl of 10× reaction buffer (100 mM Tris-HCl, pH 8.8, 500 mM KCl, and 0.8% Nonidet P-40), 10 pmol of forward and reverse primers, 0.2 mM of each of the four dNTPs, 1.5 mM MgCl2, and 1 μl of DNA template (100 ng of DNA). Cycling conditions for *cyt b* were: 7 min at 94°C, 35 cycles of denaturing for 40 sec at 94°C, annealing for 30 sec at 46°C, and extending for 1 min at 72°C, with a final extension at 72°C for 7 min. *c-mos* parameters were: initial denaturing at 95°C for 1 min, 5 cycles of denaturing at 94°C, 35 cycles of denaturing for 40 sec at 94°C, annealing for 30 sec at 46°C, and extending for 1 min at 72°C, with a final extension at 72°C for 7 min.

Amplification products were run at 1.5% agarose gel electrophoresis and cleaned for sequencing using a GenElute PCR Clean-Up Kit (Sigma) according to the manufacturers’ instructions. Sequencing reactions were carried out in both directions using the same primers as in the PCR reactions. The forward and reverse nucleotide sequences were assembled, edited and aligned by eye using the CodonCode Aligner 3.5.6 (CodonCode Corp.). The sequences obtained from each gene fragment were
submitted to GenBank (Accession numbers: cyt b; Accession Number c-mos; Accession Number).

**Phylogenetic analyses**

To assess the phylogeny of the genus *Rhynchocalamus*, the above mentioned sequences of *Rhynchocalamus* were added to a concatenated data set composed of 77 sequences of cyt b, 39 sequences of NADH-Dehydrogenase subunit 4 (ND4) and 79 sequences of c-mos sequences, belonging to 68 genera and 83 species within the subfamily Colubrinae. These genera were selected based on the most recent and comprehensive phylogeny of Colubrinae (Pyron et al., 2013). ND4 gene was added to this dataset to achieve a phylogenetic cladogram with similar topology to those of Pyron et al. (2013). The final dataset contained sequences from 93 specimens and 2743 bp (see Appendix 3). Alignment has been carried out with the MAFFT web server (Katoh et al., 2002; Katoh and Toh, 2008). The alignment was submitted to a test of substitution saturation as implemented in DAMBE v. 5.2.34 (Xia and Lemey, 2009). In addition, transitions and transversions were plotted against Kimura 2-parameter (K2p) divergences to visualize possible saturation at higher divergence level. For likelihood based phylogenetic analyses, we created three data partitions, treating each gene separately. Also, an appropriate model of nucleotide substitution was inferred with PartitionFinder v1.1.0 (Lanfear et al., 2012). We calculated a Bayesian tree and posterior probabilities supporting nodes using MrBayes v3.2.1 (Ronquist et al., 2012). The setup was a dual-head x-ray tube with a transmission-type head, with a focal spot size of 900 nm at a tube voltage of 130 kV for high resolution. In total, 2001 projections were recorded, which covered the whole head and part of the trunk. The raw data were processed and reconstructed using the in-house CT software Octopus (Vlassenbroeck et al., 2007) and rendered using Amira (Mercury Systems of Visage Imaging GmbH). The CT-rendered images were color coded to distinguish separate ossified units, where stiff and rigidly interconnected bones were given a single color. 3D reconstruction of the skulls was generated using Amira v. 5.4.1 (Mercury Systems of Visage Imaging GmbH). For descriptions of skeletal elements, the terminology follows that of Romer (1956).

**RESULTS**

**Molecular phylogeny**

The complete alignment of the c-mos, cyt b and ND4 genes were carried out and the best evolutionary models for each gene, as determined by PartitionFinder v. 1.1.0 (Lanfear et al., 2012), were defined as: HKY + I + G for c-mos, and GTR + I + G for the rest of genes. Saturation analyses revealed that our data set bears little substitution saturation (the observed Iss values are significantly lower than Issc); thus, our data could be well used for phylogenetic analyses (see Appendix 4).

Bayesian and maximum likelihood analyses (Fig. 1) revealed that the genus *Rhynchocalamus* is paraphyletic, indicating that *R. melanocephalus* ssp. and *R. barani* are independent lineages within the subfamily Colubrinae. The clade bearing *R. melanocephalus* ssp. is supported very well as a monophyletic unit (bootstrap value and percent posterior probability of 100%). Nevertheless, within this group there are two distinct and well supported clades, corresponding to both subspecies of *R. me-
The sister group relationship of *R. melanocephalus* ssp. and *Lytorhynchus diadema* is well supported (bootstrap value of 88% and percent posterior probability of 100%). This study failed to distinguish the exact phylogenetic position of *R. barani*.

Calculation of uncorrected pairwise genetic divergence in the *cyt b* gene between *R. melanocephalus* and *R. satunini* revealed that there is 13.1 – 14.2% genetic divergence between these subspecies.

**Skull Comparison of Rhynchocalamus melanocephalus (N = 2), Rhynchocalamus satunini (N = 2), and Rhynchocalamus barani (N = 2)**

There was no clear osteological difference between the skull of *Rhynchocalamus melanocephalus* and *R. satunini* but as described below, the skulls of *R. melanocephalus* and *R. barani* differ from each other in many aspects (Figs. 2 – 4). The snout, composed of bones anterior to the eye, is pointed in *R. melanocephalus*, but beveled anteriorly in *R. barani*. The premaxilla in *R. melanocephalus* is small, thin, directed down and backward, and does not have a dorsal process. In *R. barani*, the premaxilla is wider, thicker, directed forward and its dorsal process is prominent, lying anteriorly against the nasals. In *R. melanocephalus*, the nasals are loosely connected to the premaxilla and prefrontals, but in *R. barani* the nasals separate anteriorly to form a space, which receives the posterior process of the premaxilla. The nasals in *R. melanocephalus* articulate with the prefrontals both ventrally and dorsally. Ventral articulation of the nasals with the septomaxilla in *R. melanocephalus* is less pronounced than in *R. barani*. The thickness and length of the medial septomaxillae in *R. melanocephalus* is shorter than in *R. barani*. The overall size of the vomer in *R. melanocephalus* is smaller than in *R. barani*.

The neurocranium in *R. barani* is longer and broader than in *R. melanocephalus*. The frontals, which form a complete enclosure for the anterior portions of the brain, are larger in *R. barani* than in *R. melanocephalus*. The thickness and length of the medial septomaxillae in *R. melanocephalus* is shorter than in *R. barani*. The overall size of the vomer in *R. melanocephalus* is smaller than in *R. barani*.

In *R. barani*, frontoparietal articulation is through a closed suture, but in *R. melanocephalus* the suture remains open except at its anterolateral edges. In *R. barani* the parietals, which form the roof of the braincase and expand far down along both sides of the brain, are longer and broader than in *R. melanocephalus*.

The postorbitals in *R. barani* are narrow, elongated, flattened bones which articulate with the anterolateral surfaces of the parietal and form the dorsoposterior boundary of each orbit, but in *R. melanocephalus* they are reduced into slender tubular bones.

There is no big difference in the posterior and inferior bones of the neurocranium such as the prootics, supraoccipitals, exoccipitals, basioccipitals, basisphenoid and parasphenoid, except that most of their intersection sutures are denticulated in *R. barani*, while in *R. melanocephalus* the sutures are smooth. The palatines in *R. barani*
are thicker and broader than in *R. melanocephalus*. Also, in *R. melanocephalus* each palatine bears 0–2 teeth while in *R. barani* each bears four similarly sized, posteriorly curved teeth. Each pterygoid in *R. barani* bears six similar size, curved teeth positioned on the anterior 1/3 of pterygoid, while in *R. melanocephalus*, pterygoids bear no teeth. The flat bony processes of the ectopterygoids that connect the maxillae to the pterygoid are deeper bifurcated anteriorly in *R. barani* than in *R. melanocephalus*. Each maxilla is a short and curved bar and bears sockets for maxillary teeth. In *R. melanocephalus*, there are two sets of teeth, separated by a toothless area: an anterior set comprises four curved teeth of similar size, whereas the posterior one comprises three longer teeth (about 3.5 times longer than the anterior teeth). In *R. barani* there are two sets of teeth, separated by a toothless area: an anterior set comprises five curved teeth of similar size, whereas the posterior one comprises one longer tooth (about 2 times longer than the anterior teeth).

The supratemporals in *R. melanocephalus* are smaller than in *R. barani*. In *R. melanocephalus* the quadrates are shorter and more vertical than in *R. barani*. In *R. melanocephalus* each dentary bears 8 curved teeth while there are 9 curved teeth on each dentary in *R. barani*. 

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Fig. 2. Dorsal view of the skulls of *Muhtarophis barani* gen. nov. (above), *R. melanocephalus* (middle) and *R. satunini* (below).

Fig. 3. Lateral view of the skulls of *Muhtarophis barani* gen. nov. (above), *R. melanocephalus* (middle) and *R. satunini* (below).
DISCUSSION

Baran’s dwarf snake belongs to the family Colubridae due to lacking teeth on the premaxilla and proteroglyphous or solenoglyphous teeth on the maxilla, having teeth on the dentary, lacking coronoid bone in the mandible and girdle elements, and lacking valvular, crescentic, dorsal nostrils (Zug et al., 2001). This species belongs to the subfamily Colubrinae due to lacking numerous and closely set teeth, having broad articulation between snout bones and lacking an aquatic or semi-aquatic lifestyle (Zug et al., 2001; Zaher et al., 2012).

Dentition characters Baran’s dwarf snake differs from Eirenis (Pediophis) levantinus Schmidtler, 1993 that lives in sympatric in having lower numbers of maxillary (6 instead of 16 – 22) and mandibular teeth (9 instead of 14 – 22) (Mahlow et al., 2013).

Based on molecular phylogenetic research, Baran’s black-headed dwarf snake is an independent lineage, nested in the sister group of African rear fang colubrids with very weak support. Actually, phylogenetic relationships of several clades and lineages among the Colubrinae subfamily are weakly supported in most previous studies (Lawson et al., 2005; Vidal et al., 2007; Pyron et al., 2011; 2013). Sequencing more mitochondrial and nuclear genes may help overcome this weakly supported phylogenetic position (Rokas and Carroll, 2005). Therefore, it is possible that future analysis may reveal that the lineage of Baran’s black-headed dwarf snake is placed elsewhere in the Colubrinae with strong support.

Skull osteology data of Baran’s dwarf snake confirm that this lineage is different than other known lineages within Colubrinae. Although occurrence of a large premaxilla, and broad articulation between frontal, prefrontal and premaxilla bones are representative of a burrowing lifestyle (Romer, 1956; List, 1966), having rear fangs on the posterior maxilla separated from anterior teeth by an interspace, round eye pupils, 17 dorsal scales at mid-body and paired subcaudal scales are observed in none of the known burrowing colubrid snake genera.

Phylogenetic analyses revealed R. melanocephalus and R. satunini are two monophyletic lineages. Studying pairwise genetic divergence in the cyt b gene among valid species of colubrid snakes in the western palearctic reveals that there is an average 10 — 12% DNA divergence (Rajabizadeh, 2013). Since in Colubrid snakes of the western Asia an average 10% pairwise genetic divergence in the cyt b gene delimit different species (Rajabizadeh, 2013), occurrence of 13.1 — 14.2% DNA divergence in the cyt-b gene between R. melanocephalus and R. satunini indicate that they are two different species. Also, this idea is supported by occurrence of certain morphological differences between these two taxa (Werner, 1906; 1917; Reed and Marx, 1959; Darevsky, 1970; Eisel, 1970; Gasperetti, 1988; Franzen and Bischoff, 1995; Avcý et al., 2007; 2008). Following to evolutionary species concept (Wiley, 1978), reproductive isolation must be effective enough to permit the maintenance of an identity from other contemporary lineages. In this paper, data of two species properties, morphologic distinguishability and phylogenetic monophyly (following De Queiroz, 2007), as well as data of pairwise genetic divergence are available that can be used to delimit the evolutionary lineages as separate species. Using integration by cumulation criteria (Padial et al., 2010) to integrate these
species properties and delimitate different species, *R. melanocephalus* and *R. satunini* are two independent monophyletic lineages that are morphologically distinguishable and can be considered as valid species.

**Taxonomic implication**

Skull osteology and molecular phylogeny of *R. barani* and *R. melanocephalus* indicate that Baran’s black-headed dwarf snake is not of the genus *Rhynchocalamus*, but belongs to a new genus within the subfamily Colubrinae. In cladistic theory, it appears that a genus cannot be both monotypic and monophyletic (Platnick, 1976). A genus is a monophyletic group of species and so, monotypic genera are phylogenetically not informative and remain the result of an arbitrary decision. There seem to be two cases that a justification for recognizing monotypic genera is applicable, one in which the sister taxon or taxa of a given species are known and one in which they are unknown. The presence of many Monotypic genera within Colubrinae, even despite comprehensive molecular phylogenetic research (Pyron et al., 2013) may result from occurrence of many unknown or uncollected Colubrin taxa. So, it is possible that in the future works, additional species will be discovered that are sister species of currently single species within the monotypic genera, i.e., genus *Walterinnesia* Lastate, 1887 (Nilson and Rastegar-Pouray, 2007). It is also possible that sister species of currently single species within monotypic genera are extinct. But anyway a new genus cannot be described by a single species, based on assumption of occurrence of one or more uncollected or extinct sister species. So, in this paper we follow the idea of Platnick (1976) to establish a monotypic genus, only in case where a dichotomy cannot be established by synapomorphy and the monotypic genus is not demonstrably paraphyletic. Although the phylogenetic analysis of Colubrinae shade no light on the genealogy of that Baran’s dwarf snake, but it revealed that no dichotomy exist between the Baran’s dwarf snake and any other genera within Colubrinae. So, describing a monotypic genus bearing the Baran’s dwarf snake is phylogenetically acceptable.

**Muhtarophis gen. nov.**

**Type species.** *Rhynchocalamus barani* Olgun, Avci, Ilgaz, Ozum et Yilmaz, 2007.

**Derivatio nominis.** The new genus is named after Prof. Dr. Muhtar Başoğlu, the first Turkish herpetologist, together with ophis, i.e., snake; the gender is masculine (Fig. 5).
two gular scales are in contact with the anterior infra-maxillar. There are 17 dorsal scale rows at mid-body, 17 on the neck one head-length behind the head, and 17 dorsal scale rows on one head-length anterior to anus. The anal plate is divided. Ventrals are 156 – 163 in males; 173 – 177 in females. Subcaudals are 68 – 75 in males; 65 – 66 in females. Snout-vent length is 252 – 286 mm in males; 163 – 313 mm in females. Tail length is 90 – 110 mm in males; 44 – 90 mm in females.

The ground color of the head (from tip of the rostrum to posterior margin of the parietals) is ash gray. This ground color extends to the first upper labial plate at the flanks of the head. A narrow black blotch starting from the lower part of the eyes extends up to the contact zone of the 3rd and 4th upper labials. The 2nd, 3/4 of the 3rd, 4/5 of the 4th and 2/3 of the 5th upper labials are of a white color. The lower labials are white, except for the last lower labial, which is black. A narrow black neck band is present on the upper part of the head, from the margin of the parietals to the top of the posterior margin of the temporals, in the gular region of the head, in contact with dorsal scales over a width of six scales. There is no contact with ventral scales on the lower part of the head. The length of head patterns (from tip of rostrum), proportional to snout-vent length is 4.1 mm. 3/4 of the temporal is white and the rest is black in color. The ground color of the dorsum is reddish brown without maculation. The lower part of the head and ventral area are separated by the black head band, but are otherwise white without maculation. The lower part of the tail is white in color, including the tail tip. See Table 1 for variation in measurements and scale counts.

**Distribution and ecology.** *M. barani* is a species endemic to Turkey. It has only been recorded at Amanos Mountain (altitude 1310 m a.s.l.), 34 km E of Dörtyol (type locality) and Yayladağı (altitude 550 m a.s.l.), Hatay Province, Turkey. The second locality indicates that it could be distributed much more widely within the Mediterranean region of Turkey and possibly northwestern Syria (Avcı et al., 2009).

Anatolia is a predominantly mountainous area whose diverse geomorphology produces many different climatic regions and vegetation types. Based on its position and geology, Anatolia acted in the past as a bridge or as a barrier for species’ dispersal between Asia, Europe, and the Ethiopian region via the Middle East, providing a natural pathway or acting as a vicariant agent (Tchernov, 1992). It also played an important role as a refugium during the Quaternary ice ages, holding populations during glacial periods that could move out during the interglacial to Europe via Thrace and the Caucasus (Kornilious et al. 2011). Accordingly, mountains provide important refuges for fauna (Kosswig, 1955). The term “refugia within refugia” (Gomez and Lunt, 2007) is also applied to the Anatolian peninsula, which includes smaller refugial areas resulting in high levels of cryptic genetic diversity (Bellata et al., 2015). Several recently published analyses of Anatolian herpetofauna have revealed high levels of endemism and cryptic species (Poulakakis et al., 2005; Kyriazi et al., 2008; Akşin et al., 2010; Plötner et al., 2001; Kornilios et al., 2011; Kornilios et al., 2012; Bellati et al., 2014; Sindaco et al., 2014). Anatolian mountains have played an important role in speciation. These mountains harbor both endemic species and also important subspecific genetic diversity (Çıplak, 2004; Kornilios et al., 2011).

*M. barani* inhabits forestland areas under stones (Fig. 8). The distribution areas lie within the Mediterranean Phytogeographical Region (Türkmen and Düzlen, 1998). The common tree species in the localities of *M. barani* are as follows: *Pinus brutia, Pinus nigra pallasiana, Fagus orientalis, Quercus cerris cerris, Carpinus orientalis, Cedrus libani*, and *Abies cilicica* (Türkmen and Düzlen, 1998).
The specimens of *M. barani* were captured from 10:30 to 18:30, under stones along a stream. The altitudes where the samples were collected are 550 and 1310 m a.s.l. The specimens were collected during sunny environmental conditions and the temperatures were 15–24°C. The other amphibian and reptilian species inhabiting the environment were *Salamandra infraimmaculata* (Martens, 1885), *Ablepharus budaki* (Göçmen, Kumlutaş et Tosunoğlu, 1996), *Stellagama stellio* (Linnaeus, 1758), *Phoenicolacerta laevis* (Gray, 1838), *Apathya cappadocica* (Werner, 1902), *Lacerta media* (Lantz et Cyren, 1920), *Ophisops elegans* (Menetries, 1832), and *Eirenis (Pediophis) levantinus* Schmidtler, 1993.

**Conservation.** *M. barani* has only been found in a small area including Amanos Mountain and Yayladağ in Hatay, Turkey. The IUCN Red list of threatened species lists *M. barani* as “Data Deficient” because the threats to this recently described species are poorly known. The type locality of species at 1300 m a.s.l. is a plateau and does not include any negative effects such as tourism, settlement etc. However, the second known locality, Yayladağ, is not far away from settlement. In order to make proper decisions on population status and conservation efforts, firstly the known locality of the species should be determined.

**Rhyynchocalamus melanocephalus** (Jan, 1862)

**Synonyms**

*Homalosoma melanocephalum* Jan, 1862;
*Rhyynchocalamus melanocephalus* — Günther, 1864;
*Oligodon melanocephalus* var. *septentrionalis* Werner, 1906;
Rhynchocalamus melanocephalus — Engelmann, Fritsche, Günther et Obst, 1993;

Rhynchocalamus melanocephalus — Franzen et Bischoff, 1995;

Rhynchocalamus melanocephalus — Baran et Atatur, 1998;

Rhynchocalamus melanocephalus — Venchi et Sindaco, 2006;

Rhynchocalamus melanocephalus — Avcı, Dinçaslan, Ilgaz et Üzüm, 2008;


**Description** (Fig. 9). The body of *R. melanocephalus* is cylindrical; its head is small, not distinct from the neck; the rostral is enlarged and extends backwards between the internasals. Dorsal scales are smooth in 15 rows at mid-body. The internasal is trapezoid-shaped and the suture length of this plate is the same as the prefrontal suture. Parietals are shorter than the distance from the posterior tip of the rostral to the posterior tip of the frontals; ventrals are 180 – 198 in males, 182 – 226 in females. Subcaudals are 54 – 69 pairs in males, 47 – 66 pairs in females. Supralabials are 5 – 6; sublabials are

**Fig. 8.** Habitat of *Muhtarophis barani* gen. nov. at Amanos Mountain, Hatay Province, Turkey.

**Fig. 9.** General view of *Rhynchocalamus melanocephalus* from Sofular Village/Harbiye, Hatay Province, Turkey.
7 – 9 in known samples. The top of the head and neck are glossy black, and the upper labials and rostral shield are ivory white (Boulenger, 1894; Barbour, 1914; Gasperetti, 1988; Franzen and Bischoff, 1995; Avcı et al., 2008). The black nuchal band reaches the ventral scales ventrally. Franzen and Bischoff (1995) observed in R. melanocephalus that the black neckband reaches the ventral scales in six specimens out of 17 (35.3%), or only just reaches the ventral scales (35.3%) or not at all (29.4%). The ground color of the dorsum is yellowish-brown without maculation while the ventral side is yellowish-white without any maculation (Avcı et al., 2008).

**Distribution.** Type locality of R. melanocephalus (Jan, 1862) is Beirut, Lebanon. It is distributed over Egypt, Jordan, Israel, Lebanon, Western Syria, and South Turkey (Gasperetti, 1988; Werner, 1988; Engelmann et al., 1993; Franzen and Bischoff, 1995; Avcı et al., 2008).

**Rhynchocalamus satunini** (Nikolsky, 1899)

**Synonyms**

Contia satunini Nikolsky, 1899;

Contia satunini — Werner, 1917;

Rhynchocalamus satunini — Reed and Marx, 1959;

Rhynchocalamus melanocephalus satunini — Darevsky, 1970;

Rhynchocalamus satunini — Baran, 1976;

Rhynchocalamus melanocephalus satunini — Baran and Atattür, 1998;


**Description** (Fig. 10). The body of R. melanocephalus is cylindrical; its head is small, not distinct from the neck; the eyes are small with round pupils; the rostral is enlarged, extended backwards between the internasals; dorsal scales are smooth in 15 rows at mid-trunk. Ventral scales are 201 – 208 in males, 215 – 232 in females. Subcaudals are 57 – 64 pairs in males, 52 – 64 pairs in females. Supralabials are 7 – 8 and sublabials 7 – 9 in known samples. The color of the top of the head is not uniformly black, with two black blotches on top of the head and a bigger black band on the neck. Also, the color of the rostral shield and supraoculars are ivory white. The ground color of the dorsum is pinkish without maculation. The black colored neck band does not reach the ventral scales. The color of the ventral side is more pinkish than the dorsum. (Werner, 1906; 1917; Reed and Marx, 1959; Darevsky, 1970; Eiselt, 1970; Baran, 1976; Başoğlu and Baran, 1980; Gasperetti, 1988; Franzen and Bischoff, 1995; Avcı et al., 2007).

**Distribution.** Type locality of R. satunini is Megri, Armenia (Nikolsky, 1899). It is distributed over South-
east Turkey, Iraq, Western Iran, Armenia and Azerbaijan (Reed and Marx, 1959; Eiselt, 1970; Başoğlu and Baran, 1980; Gasperetti, 1988; Leviton et al., 1992; Engelmann et al., 1993; Avci et al., 2007).

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REFERENCES


Baran İ. (1976), The Taxonomic Revision of Turkey Snakes and Their Geographical Distribution, TÜBİTAK Yayınları, Ankara.


Başoğlu M. and Baran İ. (1980), Türkiye Sürüngenleri Kısm II. Yılanlar [Turkish Reptiles. Part II. Snakes ], Ege Üniversitesi Fen Fakültesi Kitaplar Serisi, Bornova-Izmir [in Turkish].


Appendix 1. Bayesian inference tree of the Colubrinae, based on c-mos gene. The tree was generated based on the method that explained for phylogenetic analyses, using three million generations. Branch support measures are Bayesian posterior probabilities (×100). Arrows indicate the locations of Rhynchocalamus melanocophalus ssp. and Rhynchocalamus barani in the tree.


Appendix 2. Bayesian inference tree of the Colubrinae, based on cyt b gene. The tree was generated based on the method that explained for phylogenetic analyses, using five million generations. Branch support measures are Bayesian posterior probabilities (×100). Arrows indicate the locations of Rhynchocalamus melanocephalus ssp. and Rhynchocalamus barani in the tree.


Appendix 4. Transversions (v)/transitions (s) plotted against Kimura 2-parameter (K2p) divergences of colubrinae concatenated dataset, composed of c-mos, cyt b and ND4 genes.
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