

## Hidden Diversity in a Narrow Valley: Description of New Endemic Palearctic Rock Lizard *Darevskia* (Squamata: Lacertidae) Species from Northeastern Turkey

Muammer Kurnaz<sup>1,\*</sup>, Mehmet Kürsat Şahin<sup>2,3</sup>, and Ali İhsan Eroğlu<sup>4</sup>

<sup>1</sup>Gümüşhane University, Kelkit Vocational School of Health Services, Department of Medical Services and Techniques 29600, Kelkit / Gümüşhane, Turkey. \*Correspondence: E-mail: muammerkurnazz@gmail.com (Kurnaz)

<sup>2</sup>Department of Biology, Kamil Özdag Faculty of Science, Karamanoğlu Mehmetbey University, Karaman, Turkey. E-mail: yasambilimci.kursat@gmail.com (Şahin)

<sup>3</sup>Biodiversity Research Center, Karamanoğlu Mehmetbey University, Karaman, Turkey

<sup>4</sup>Universite Neighborhood, Cengaver Street, No:5-C, Ay Yapı Bostancı Zirve Sites, 61080, Ortahisar / Trabzon, Turkey. E-mail: zoolog@hotmail.com.tr (Eroğlu)

### ORCiDs

Muammer Kurnaz: <https://orcid.org/0000-0002-0498-0208>

Mehmet Kürsat Şahin: <https://orcid.org/0000-0003-0834-5081>

Ali İhsan Eroğlu: <https://orcid.org/0000-0001-7642-4086>

(Received 19 October 2021 / Accepted 23 May 2022 / Published -- 2022)

Communicated by Benny K.K. Chan

*Darevskia* is a particularly species-rich radiation of Palearctic rock lizards from the Caucasus region. Thanks to intense systematic and taxonomic research, the knowledge of species – level diversity within this genus has increased over the last quarter century. Here, we described a new species, *Darevskia salihae* sp. nov. from northeastern Turkey. The new taxon is differentiated from other nearby taxon by the low number of dorsal scales in the middle of the body, the shorter body length, and the absence of blue dots both on the lateral region above the forelimbs and on the margin of the ventral plates. In addition to their morphological differences, the new taxon is phylogenetically different from close groups. It is located in a separate subclade from the *rudis-valentini-portschinskii* subclade. This distinction is supported by both a high bootstrap value (100) and a high posterior probability value (1.00). These two subclades are separated from each other by a genetic distance of almost 4%. This separation is supported not only genetically and morphologically, but also geographically. Since the habitat of the new taxon is limited to a high mountain and a narrow valley, it does not provide an opportunity for a different *Darevskia* species to shelter because it creates geographical isolation. However, *Darevskia parvula* that live closest to

the habitat of the new taxon live only at the habitat boundaries and do not enter areas where the new taxon is found. Therefore, it might be possible that while it was separated from the *rudis-valentini-portschinskii* group during the evolutionary transformation, it remained as a refuge and relict in a narrow area as a result of the collapse of the valleys and the partial uplift of the Kaçkar Mountains.

**Key words:** *Darevskia salihae* sp. nov., Caucasian rock lizards, New species, Phylogeny, Morphology, Isolation, Relict endemic, Anatolia.

Citation: Kurnaz M, Şahin MK, Eroğlu Aİ. 2022. Hidden diversity in a narrow valley: Description of new endemic Palearctic rock lizard *Darevskia* (Squamata: Lacertidae) species from northeastern Turkey. Zool Stud 61:44.

## BACKGROUND

The Anatolian Peninsula has a valuable species richness as it interacts with three well-known biodiversity hotspots. One of them, Caucasus hotspot, is located in the Caucasus Region and covers northeastern Anatolia. The genus *Darevskia* (Arribas 1999), is one of the most diverse genera of lizards, and new species are continuously described after expeditions into the Caucasus. Therefore, this genus remarkably shapes the Caucasian biodiversity in terms of herpetology, and as a result of it, a high number of species within this genus live in Turkey. According to the last valid checklist, six members (*D. bendimahiensis*, *D. bithynica*, *D. parvula*, *D. sapphirine*, *D. tuniyevi* and *D. uzzelli*) of this genus, represented by approximately 15 species, are endemic to Turkey (Kurnaz 2020). Considering that there are about 35 *Darevskia* species in the world (Murtskhvaladze et al. 2020; Arribas et al. 2022), these numbers show that Turkey has an important position in terms of the taxonomy of *Darevskia*.

The complex orographic conditions of habitats in the Caucasian biodiversity hotspot have an undeniable impact on ensuring diversification, as it provides significant isolation between species and hinders gene flow. This situation causes the diversification of an important group that has adapted to Caucasian biodiversity, such as the genus *Darevskia*. Furthermore, the formation of the Caucasus provoked the origin of multiple endemic species within valleys and characteristic biotopes (Tarkhnishvili 2012; Tuniev and Petrova 2019; Murtskhvaladze et al. 2020). The aim of this study is to describe a new endemic *Darevskia* species isolated in a very narrow valley on the Anatolian side of the Caucasian biodiversity hotspot.

## MATERIALS AND METHODS

### Study area and Materials

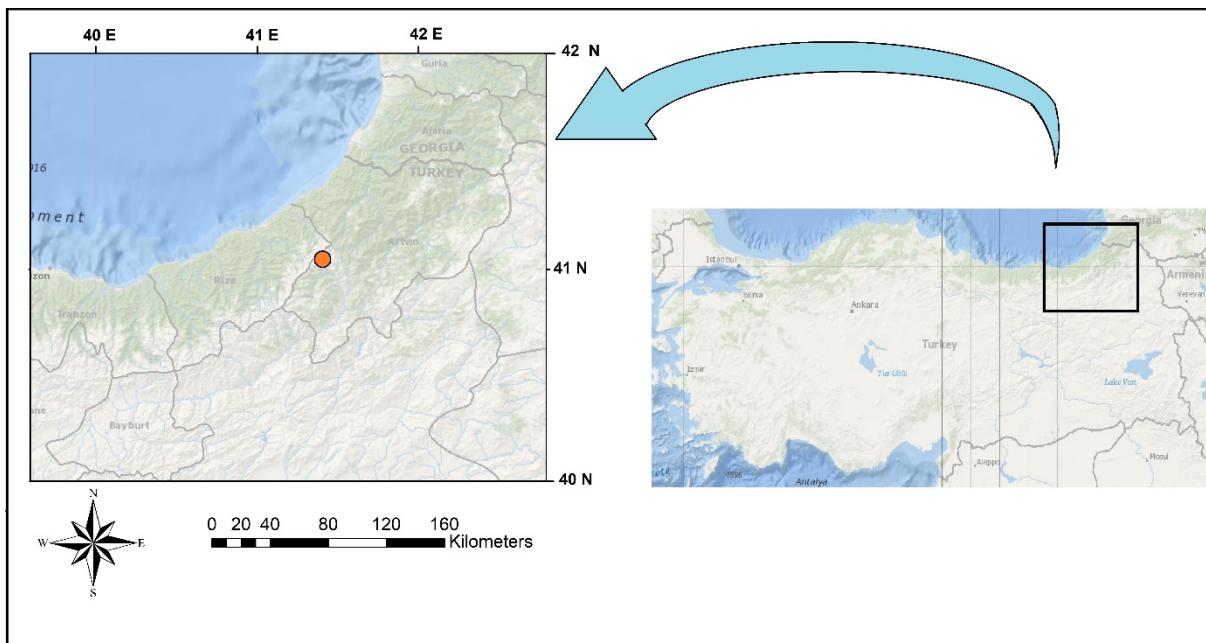
Five lizard specimens (1 ♂, 2 ♀♀, 1 subadult ♂, 1 subadult ♀) were collected from the north of Yusufeli, Altıparmak Mountains in Barhal valley, Artvin Province on the Eastern Black Sea (Lat: 40.957°N–Long: 41.325°E and about 1531 m a.s.l.). The male specimen was found dead (as prey in the mouth of *Zamenis hohenackeri*), thus it could not be preserved as a collection specimen (Fig. 1). However, all characteristics of this male specimen are measurable and quantifiable, therefore, color pattern features, measurement and pholidolial characters were counted. The locality of the expedition area is demonstrated in figure 2. All specimens (except the male one) were anesthetized with ether, fixed with a 96% ethanol and deposited in the Gümüşhane University Kelkit Vocational School of Health Services with collection number: GUK 1/2021 1-4.

1 ♂, 1 subadult ♂, Altıparmak Mountains, Barhal Valley, Yusufeli, Artvin (Lat: 40.957° N – Long: 41.325° E and about 1531 m a.s.l.). Leg. Muammer Kurnaz, Ali İhsan Eroğlu, 21 June 2021.

2 ♀♀, 1 subadult ♀, Altıparmak Mountains, Barhal Valley, Yusufeli, Artvin (Lat: 40.957° N – Long: 41.325° E and about 1531 m a.s.l.). Leg. Muammer Kurnaz, Mehmet Kürşat Şahin, 4 July 2021.



**Fig. 1.** Unpreserved male specimen and its natural predator in the habitat.



**Fig. 2.** The map shows type locality of the *Darevskia salihae* sp. nov. in Turkey.

## Morphological examinations

The metric and meristic characters were examined for each specimen. For morphometric measurements, we used a digital caliper with 0.01 mm sensitivity (the measurements were performed by Muammer Kurnaz). Mensural and meristic data were recorded by the guidance of Arribas et al. (2013) and Arribas et al. (2018).

The studied mensural characters were as follows: supraciliary granules (right-left, SCGa-SCGb), loreal plates behind the postnasal plates and in front of the preocular plates (right-left, LOa-LOb), supraciliar plates (right-left, SCPa-SCPb), supralabial plates (right-left, SRLa-SRLb, number of labials both anterior and posterior to center of eye), sublabial plates (right-left, SLPa-SLPb), inframaxillary plates (right-left, IMa-IMb), transversal series of gular scales between inframaxillary symphysis and collar (MG), collar (C), supratemporals (right-left, STa- STb), temporal plates (T); transversal ventral plates (TVP), femoral pores (right-left, FPa-FPb), subdigital lamellae in the 4th toe (SDL), transversal series of dorsal scales at the midbody (DS), the dorsal scales in contact with 10 ventral plate (VDS) and number of preanal scales surrounding anal (PA1) and all plates surrounding anal plate (PA2). The morphometric measurements in this study are as follows: snout-vent length (SVL), tip of snout to anal cleft; tail length (TL), anal cleft to tip of tail; pileus width (PW), at widest point between parietal plates; pileus length (PL), tip of snout to posterior margins of parietals; head width (HW), at the widest point of head; head length (HL), tip of snout to posterior margin of ear opening; total body length (TBL), tip of snout to tip of tail.

## Molecular analyses

The clipped tips of tails obtained from collected specimens were kept in 96% ethanol at -20°C. Afterwards, the tissues were cut into small pieces for the DNA isolation process which implemented the CTAB protocol (Doyle and Doyle 1990).

Fragment of the mitochondrial cytochrome *b* gene (*cyt b*; 504 bp) was amplified for three specimens using primers L15369 forward and H15915 reverse primers (Fu 2000). Cytochrome *b* gene is commonly used for phylogenetic studies in lizards (Troncoso-Palacios et al. 2018; Fernando et al. 2019; Quiroz et al. 2021). *cyt b* gene amplification involved an initial incubation at 93°C for three minutes, followed by 30 cycles at 93°C for 60 seconds, the appropriate annealing temperature at 53°C for 60 seconds, elongation temperature at 69°C for 120 seconds and final extension temperature at 70°C for ten minutes. PCR amplifications for *cyt b* were conducted as described by Gabelaia et al. (2015). Amplified DNA segments were purified and sequenced by BM Labosis in Ankara, Turkey.

Phylogenetic analyses were based on *cyt b* gene sequences obtained from the collected specimens from Turkey, and additional sequences of *Darevskia* species retrieved from GenBank. Accession numbers of all sequences used for the phylogenetic analysis are from the studies of Murtskhvaladze et al. (2020). All *cyt b* sequences used in the molecular analysis were aligned using Geneious Prime 2019. The best-fit substitution model was determined with JModelTest v.2.1.8 (Darriba et al. 2012) and the best model was chosen according to the lowest AIC (Akaike's information criteria) degree (Akaike 1974). To reconstruct the phylogenetic tree, we carried out a Bayesian Inference (BI) analysis by using MrBayes v.3.2.6 (Ronquist et al. 2011) and Maximum Likelihood (ML) analysis by using MEGA X (Kumar et al. 2018). In the BI analysis, the following settings were used: number of Markov Chain Monte Carlo (MCMC) generations = ten million; sampling frequency = 100; burn-in = 25%. Maximum likelihood (ML) analyses were carried out using a heuristic search method (10,000 random addition replicates tree-bisection-reconnection, TBR, branch swapping) and bootstrap analyses with 1000 replications ML (Felsenstein 1985) were applied. Transitions and transversions were equally weighted, and gaps were treated as missing data. ML trees were evaluated using bootstrap analyses with 1000 replicates and statistical support of the resultant BI trees was determined based on Bayesian posterior probability (BPP). We considered nodes with a BPP of 95% or greater as significant (Leaché and Reeder 2002). The BI tree topology was determined based on Bayesian posterior probability (BPP). Uncorrected pairwise sequence divergences for the *cyt b* gene were calculated using MEGA X v (Kumar et al. 2018).

## RESULTS

## Morphology

All morphological characteristics of five newly collected specimens of the newly described *Darevskia* species are given in the taxonomy section. These specimens differ from the geographically close *Darevskia* species (*D. portschinskii*, *D. rufus* and *D. valentini*) in terms of having remarkably small body sizes and an absence of a greenish-toned dorsal ground color. Additionally, the absence of blue spots on the outermost plaques, especially in the ventral region, is another differentiated feature. Moreover, the low average number of dorsal scales in the middle of the body makes them different from the *Darevskia* species living in the close vicinity. Lastly, no carinated scale was observed on the tibia in all *D. salihae* sp. nov. specimens. These highlighted features are also given as a comparison in table 1.

**Table 1.** Descriptive characters that distinguish the new taxon from nearby species.

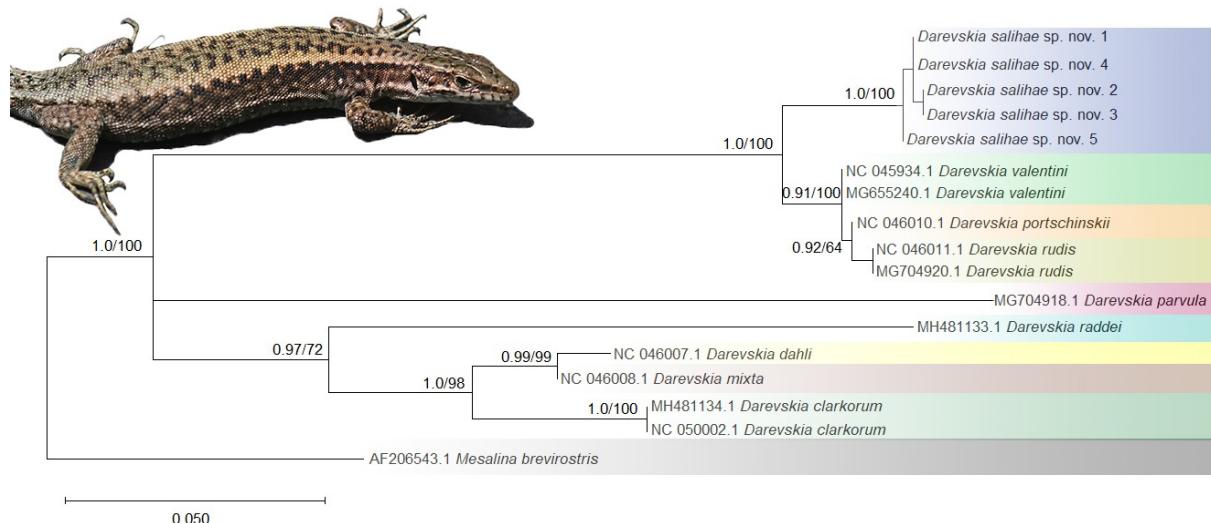
Species Characters	<i>D. salihae</i> sp. nov.	<i>D. rufus</i>	<i>D. valentini</i>	<i>D. parvula</i>
Blue spot in the margin of ventrale	absent	present	present	present
Greenish coloration in the dorsum	absent	present	present	absent
Mean of snout-vent length (mm)	< 60	> 60	> 60	< 60
Ventral coloration of adult	White-cream	Yellow-cream	Yellow-cream	Orange-red
Tibial carinated scales	absent	present	absent	absent

## Phylogeny

A total of 503 bp fragment of *cyt b* gene was obtained for the five newly collected specimens from Turkey. *cyt b*, as a valid biomarker, has been used to identify the newly discovered reptile species alone (Poyarkov Jr. et al. 2019; Liu and Rao 2019; Quiroz et al. 2021).

It has 143 variable positions. The 486th position of the 503 bases paired *cyt b* fragment was deleted for all five *Darevskia salihae* sp. nov. specimens. Probably, the thymine base, which is in the sister group *rufus-valentini-portschinskii*, has been deleted along the evolutionary process of the new taxon. Because there is a thymine base for the *rufus-valentini-portschinskii* group in the same position. According to model test results, the best-fit substitution model was GTR+G+I. The ML tree of the *cyt b* gene is shown in figure 3. The rooted tree is divided into three well-supported clades with 100 bootstraps. First clade is constituted by four species (*D. raddei*, *D. clarcorum*, *D. mixta* and *D. dahli*). Second clade is represented with only *D. parvula*. The third clade, in which the new taxon is nested, is represented with a total of four species, *D. rufus*, *D. valentini*, *D. portschinskii* and *D. salihae* sp. nov. Phylogenetic trees topologies demonstrate that *Darevskia*

*salihae* sp. nov. has been differentiated from the *rudis-valentini-portschinskii* group (Fig. 3). As a result, this new lineage nested as a sister clade to the *rudis-valentini-portschinskii* group within the genus *Darevskia*. The separation of these two groups from each other was supported by both 1.0 posterior probability and 100 bootstraps value. Thus, the new species is phylogenetically close and a sister to the *rudis-valentini-portschinskii* group (BPP = 1). Furthermore, *D. salihae* sp. nov. is distinct by approximately 4% from the species within this group (Table 2).



**Fig. 3.** Phylogenetic tree based on combined mitochondrial DNA data set. Bootstrap and posterior probability values given by BI/ML.

**Table 2.** Uncorrected genetic distance among some *Darevskia* species based on the 503 base-paired mitochondrial *cyt b* fragment

Species	1	2	3	4	5	6	7	8	9
<i>D. salihae</i> sp. nov.	-								
<i>D. valentini</i>	0.037								
<i>D. portschinskii</i>	0.039	0.001							
<i>D. rufa</i>	0.044	0.006	0.004						
<i>D. dahli</i>	0.152	0.156	0.159	0.161					
<i>D. mixta</i>	0.154	0.154	0.157	0.159	0.010				
<i>D. clarkorum</i>	0.172	0.171	0.173	0.176	0.058	0.046			
<i>D. parvula</i>	0.199	0.195	0.198	0.200	0.176	0.165	0.171		
<i>D. raddei</i>	0.214	0.203	0.200	0.202	0.134	0.126	0.134	0.191	-

## Taxonomy

The results of the present study revealed that five lizard specimens collected from northeastern Anatolia, both morphologically and phylogenetically, belong to a unique group within the genus *Darevskia* and can be characterized as a new species in this group.

**Family: Lacertidae Oppel, 1811**

**Genus: *Darevskia* Arribas, 1999**

***Darevskia salihae* sp. nov. Kurnaz, Şahin and Eroğlu, 2022**

(Figs. 4, 5)

urn:lsid:zoobank.org:act:7FD67D31-1B02-4E82-8FE6-242E57AFD94E

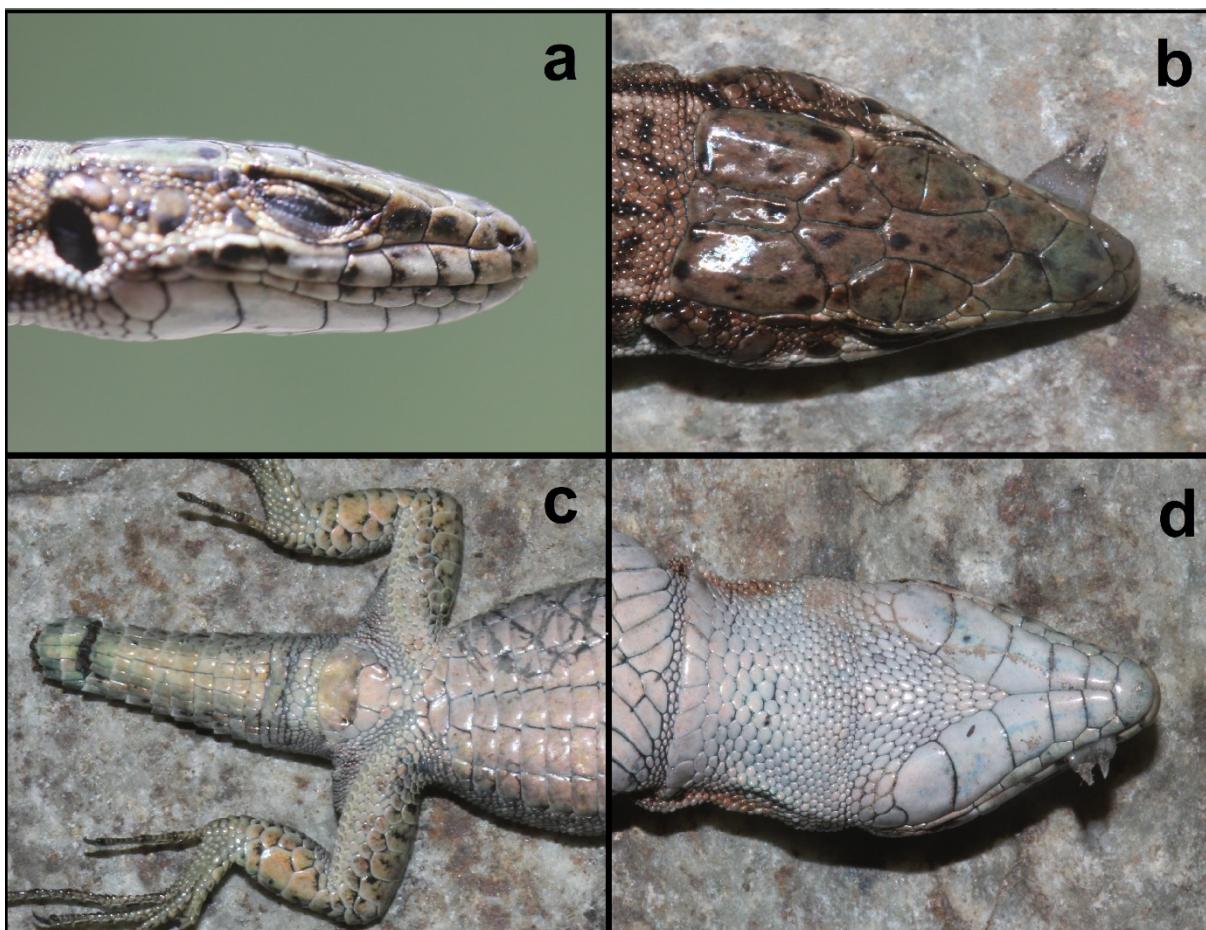
*Holotype:* An adult female specimen (GUK 1/2021-1), collected from north of Yusufeli, Altıparmak Mountains in Barhal valley, Artvin Province in Eastern Black Sea (Lat: 40.957°N–Long: 41.325°E and about 1531 m a.s.l.) during a field study on 21 July 2021, by Muammer Kurnaz and Mehmet Kürşat Şahin (Fig. 6).

*Paratypes:* A female (GUK 1/2021-2), one subadult male (GUK 1/2021-3), one subadult female (GUK 1/2021-4) and unpreserved and unnumbered adult male specimens (Figs. 7–9).

*Diagnosis:* *Darevskia salihae* sp. nov. is small sized (SVL: 51.79–58.98 mm) (Fig. 4a, b). Rostral and internasal is rarely in contact. Suboculars on both sides reach the mouth, four supralabials in anterior of suboculars. The first supratemporal plates are bigger than the others on each side of the head. All individuals have 4 supraocular plates on each side of the head. The supranasal plate is separated from anterior loreal plates above nostrils in all specimens. The postnasal plate is single on each side in all specimens. Massetericum is large and present in each side of temporal region. The row of supraciliar granules for all individuals is complete. 23–25 gularia between the third inframaxillary and collars. Generally, 6 longitudinal rows of ventral plates, and 24–30 ventral series in a longitudinal row along the belly between collar and preanal; 39–43 (mean 41) smooth dorsal midbody scales. 17–19 femoral pores exist on the right side. 22–25 lamellae exist beneath the 4th toe. The dorsal body scales in the midbody are tiny and flat. Subdigital lamellae in the 4th toe is smooth. The anal plate is singular in all specimens. Tibial scales are not carinated.



**Fig. 4.** General view of the holotype of *Darevskia salihae* sp. nov. (GUK 1/2021-1) adult female.  
a. dorsal view, and b. ventral view.



**Fig. 5.** Some pholidolial characters of holotype of *Darevskia salihae* sp. nov. (GUK 1/2021-1), adult female. a. lateral view of the head (right view), b. dorsal view of the head, c. anal plates and femoral pores, and d. ventral view of the head.

*Description of Holotype:* An adult female having the following morphological features: Head length (12.81 mm) and head width (7.71 mm); the length-width ratio of the head is 1.66. The SVL is 58.98 mm. The tail is regenerated. The ratio of the pileus length (12.14 mm) to width (5.64 mm) is twice higher. Limbs are relatively slender: forelimbs 17.60 mm, about 30% of snout-vent length; hind limbs 25.93 mm, about 1.5 times of forelimbs and 44% of snout-vent length. The head shields are relatively flat. Rostral and frontonasal are not in contact; supranasals block the connection between them with a deep suture. Nasal region is not swollen. Nostril is bordered by postnasal, supranasal, rostral and first supralabial. There are four intact supraoculars. There is a large tympanicum and massetericum. Seven supratemporals, the anterior big and the posteriors smaller and granular-shaped; postorbital present; 6–6 supraciliaries on each side, the anterior-most is the largest, separated from supraoculars by a complete rows of 10–11; 8–8 supralabials on right and left side, respectively, 4–4 anterior to subocular, respectively; 5 infralabials; five pairs of submaxillary shields, the first three pairs in contact; the last two pairs broadly separated; 24 gular scales in a straight median line between the union of the submaxillaries and the central scale of the collar; collar consist of 11 plates with large scales; 43 dorsal scales at midbody, dorsal scales

smooth and unkeeled); enlarged ventrals in 6 strait longitudinal series (at the level of the widest transversal row) and 30 transverse rows; anal plate present, the ratio of width (4.74 mm) to length (1.48 mm) is 3.2; seven enlarged circumanal plates in a longitudinal row between anterior cloacal margin and the gap between the two series of femoral pores, one preanal developed with one strongly enlarged plates; 18–18 femoral pores; 23–23 lamellae beneath 4th toe.



**Fig. 6.** General view of adult female holotype of *Darevskia salihae* sp. nov. (GUK 1/2021-1).



**Fig. 7.** General view of adult female paratype of *Darevskia salihae* sp. nov. (GUK 1/2021-2).



**Fig. 8.** General view of subadult male paratype of *Darevskia salihae* sp. nov. (GUK 1/2021-3).



**Fig. 9.** General view of subadult female paratype of *Darevskia salihae* sp. nov. (GUK 1/2021-4).

*Color and Pattern:* The coloration of dorsum is generally grayish and light brownish with dark spots (occasionally spotting is reduced). Ventral coloration is white-cream color in adults and not spotted, with a little yellow in subadults with dark spotted margins of ventrales. There are no

blue spots in the lateral of ventral in all specimens. The upper head coloration is light brown and dark spotted. The temporal region is dark brown with less maculation.

*Variation:* The paratypes do not differ substantially from the holotype in the mensural (adult paratypes) or meristic characters (both paratypes), varying slightly in size related measurements (Table 3).

**Table 3.** All mensural and meristic characters for three specimens of *Darevskia salihae* sp. nov. from Yazihan. Character abbreviations are listed in the Material and Methods section. The range and the mean of the mensural characters were calculated for the adult specimens only (i.e. for the holotype and paratype solely)

Characters	GUK 1/2021-1	GUK 1/2021-2	Unpreserved male specimen	GUK 1/2021- 3	GUK 1/2021- 4	Range	Mean
SVL	58.98	51.79	58.23	39.77	40.28	51.79-58.98	56.33
TL	-	-	-	-	82.43	-	-
HW	7.71	7.36	8.81	6.57	6.09	7.36-8.81	7.96
HL	12.81	12.43	13.74	10.11	9.83	12.43-13.74	12.99
PW	5.64	5.74	6.46	4.91	4.96	5.64-6.46	5.95
PL	12.14	11.36	13.84	9.65	9.27	11.36-13.84	12.45
FLL	17.60	16.31	20.90	13.89	12.69	16.31-20.90	18.27
HLL	25.93	27.08	34.12	18.69	19.26	25.93-34.12	29.04
AL	1.48	1.01	2.13	1.30	1.53	1.01-2.13	1.54
AW	4.74	3.84	4.84	2.29	2.87	3.84-4.84	4.47
SCGa	10	10	8	11	11	8-11	10
SCGb	11	10	9	11	11	9-11	10.4
LOa	2	2	2	2	2	2-2	2
LOB	2	2	2	2	2	2-2	2
SCP <sub>a</sub>	6	6	6	6	6	6-6	6
SCP <sub>b</sub>	6	6	6	6	6	6-6	6
SRL <sub>a</sub>	8	8	8	8	8	8-8	8
SRL <sub>b</sub>	8	8	8	8	8	8-8	8
SLP <sub>a</sub>	7	8	7	8	8	7-8	7.6
SLP <sub>b</sub>	8	8	7	8	8	7-8	7.8
IMa	5	5	5	5	5	5-5	5
IMb	5	5	5	5	5	5-5	5
MG	24	23	23	24	25	23-25	23.8
C	11	9	10	7	9	7-11	9.2
STA	7	5	4	6	5	4-7	5.4
STb	7	4	4	5	5	4-7	5
TVP	30	29	26	24	28	24-30	27.4
LVP	6	6	6	6	6	6-6	6
FPa	18	17	18	18	19	17-19	18
FPb	18	19	17	18	19	17-19	18.2
SDL <sub>a</sub>	23	23	24	23	25	23-25	23.6
SDL <sub>b</sub>	23	23	25	22	25	22-25	23.6
DS	43	40	42	40	39	39-43	40.8
PA1	1	1	1	1	1	1-1	1
PA2	7	7	7	8	7	7-8	7.2

**Geographic Distribution and Habitat:** The species is currently known only from the type locality of Yusufeli, Altıparmak Mountains in Barhal valley, Artvin Province in Eastern Black Sea, Turkey. This locality is approximately 40 km north from the known localities of Yusufeli.

*Darevskia salihae* sp. nov. lives in a narrow area in the Barhal valley. The habitat consists of a coniferous forest area (Fig. 10). The lizards were kept on a big stone near the forest road. The dominant species in the area is *Picea orientalis*. The specimens were observed between 11:00–15:00 in all field surveys, and no specimens were encountered before or after this time. One possible reason for this might be that the area starts to receive sunlight around 11:00 a.m. and remains in the shade after 3.00 p.m. as the position of the sun changes in these deep valleys. Since the area is at a high altitude above the sea, the thermal capacity of the shaded areas is not sufficient for lizard populations to inhabit, therefore, lizards cannot be observed. The air temperature during this time fluctuated between 23–25°C in all field surveys. The specimens have been distributed in an area of approximately 150 m width. It is observed that *Darevskia parvula* (Lantz and Cyrén, 1913) populations are present in both edges of the area. However, these two species do not live together in the same rocky zone. *Darevskia salihae* sp. nov. lives in syntopy with the following reptile species: *Zamenis hohenackeri* (Strauch, 1873) and *Platyceps najadum* (Eichwald, 1831).



**Fig. 10.** The type locality and habitat of *Darevskia salihae* sp. nov. from Yusufeli, Altıparmak Mountains in Barhal valley, Artvin Province in Eastern Black Sea, Turkey.

*Comparisons:* The highlighted differences from other close lizard species within this group are as follows: Firstly, while the number of dorsal scales in the middle of the body in *D. salihae* sp. nov. varies between 39 – 43, the closest species have at least 50 dorsal scales. Secondly, SVL of *D. salihae* sp. nov. is smaller than *D. rufus* and *D. valentini*. Finally, the coloration patterns of *D. salihae* sp. nov. are different than the closest species as: i) while blue spots in the margin of ventrale are present in *D. rufus*, *D. valentini* and *D. portschinskii*, *D. salihae* sp.nov. does not have these blue spots, ii) while the greenish coloration in the dorsum is present in *D. rufus*, *D. valentini* and *D. portschinskii*, *D. salihae* sp.nov. does not have this greenish coloration, iii) while ventral coloration of adult specimens of *D. rufus*, *D. valentini* and *D. portschinskii* is yellow-cream, *D. salihae* sp.nov. has white-cream coloration in its ventralia.

*Etymology:* The name of the new taxon was given in honor of Saliha Şahin, who is the mother of one of the authors, Mehmet Kürsat Şahin, who passed away recently.

## DISCUSSION

Genus *Darevskia* is one of the most important and phenomenal terrestrial vertebrate groups in the Caucasian biodiversity hotspot. This genus is one of the most diverse extant tetrapods in the Caucasus, currently it includes 35 species in a relatively limited range along the East Anatolian – Caucasus - Zagros mountainous region (Arribas 1999; Zazanashvili et al. 2004; Murtskhvaladze et al. 2020; Arribas et al. 2022).

The Barhal valley is a unique region in the spruce forests formed by the Altıparmak Mountains and the Barhal Stream, which are a continuation of the Kaçkar Mountains. Due to being located in the Caucasus biodiversity hotspot, this region has many deep valleys and geographic isolation areas along with its high mountain range. This situation is very important in terms of both the divergence of close groups from each other and triggering speciation by terminating gene flow. For example, *D. parvula* was formerly known as a single species. However, Kurnaz et al. (2019) have shown that there is more than one genetic lineage within this group. They expressed that the Kaçkar Mountains are the isolation zone that provides the separation of the two important genetic lineages, *D. adjarica* and *D. parvula*, from each other and interrupting the genetic exchange between them (Kurnaz et. al. 2019). The Kaçkar Mountains are a very important region in the initiation of speciation mechanisms, especially in triggering allopatric speciation.

*Darevskia salihae* sp. nov. represents a monophyletic lineage within the Kaçkar Mountains formation. This location provides a very narrow inhabiting area for this species. Hence, the

population prefers the deep valley between two high and vertically sharp mountains as its habitat. This condition enabled the species to be isolated from other closely related species in the *rudis-valentini-portschinskii* group, interrupting the gene flow with this group, and thus becoming a unique new species.

*Darevskia salihae* sp. nov. is typically similar to other *Darevskia* species. It even shares many common characteristics with species in the *rudis-valentini-portschinskii* sub-clade within the same clade. However, there are some characters that make the new taxon unique in this group. The highlighted differences from other closest lizard species within this group are as follows: the low number of dorsal scales in the middle of the body, the tinier body length, the absence of blue dots on the margin of the outermost ventral plates and on the lateral region above the forelimbs.

The sub-clade, in which the *Darevskia salihae* sp. nov. has been nested, and the *rudis-valentini-portschinskii* sub-clade moved away from each other due to geographical isolation, even though the phylogenetic tree was constructed with the closer populations of the *rudis-valentini-portschinskii* sub-clade. Consequently, not only are they differentiated by their genetic structures, but have also had to change many morphological characters in order to adapt to their environment. These circumstances also lead to the ecological niche of the new taxon and enabled the formation of unique features that would distinguish it from other species. Because reduction in body size and pale dorsal coloration are some characteristics of relict and isolated species (Case 1978; Sweet and Jockusch 2021). Besides, ~4% differentiation from the closest *Darevskia* species supports the speciation process of this clade in molecular level (Tarkhnishvili 2012; Kurnaz et al. 2019)

When it comes to introducing a new species to the zoological scientific society, one of the important issues is to support the descriptive characteristics with a relatively small sample size, only if an integrative approach of molecular data and morphology is to be used. This approach has been taken into account in numerous studies in recent years (Patel and Vyas 2020; Baptista et al. 2020; Rajabizadeh et al. 2020). Moreover, the impact of scientific pressure on the over-collection of species has been started to be discussed in conservation action plans in the last decade (Hitchmough et al. 2016; Hope et al. 2018). Therefore, this study is based on these principles. As a result, we avoided the maximum sampling effort to solely define a new species - *Darevskia salihae* sp. nov. - due to the endangered status of the species in its habitat. This situation reveals that the species is endangered before it is identified and needs to be protected. The newly identified taxon has an estimated distribution of less than 10 square kilometers. Therefore, it should be classified in the CR category. It is probable that while it was separated from the *rudis-valentini-portschinskii* group during the evolutionary transformation, it is positioned as a refuge and relict in a narrow area as a result of the collapse of the valleys and the partial uplift of the Kaçkar Mountains.

## CONCLUSIONS

In conclusion, with this study, a new endemic lizard species has been introduced to the biodiversity of Turkey and the Caucasian hotspot. This species is unique in both morphology and genetics and is isolated from its close relatives by geographic barriers. Additionally, even the *Darevskia parvula* that has the closest rock lizard to the new species habitat, does not have a niche overlap with the main population of the new species within its entire horizontal range.

**Acknowledgements:** This work and the new species name were registered with ZooBank under urn:lsid:zoobank.org:pub:EEAAA286-B34F-4B4D-9452-D01D5E7DFF63. We wish to thank Yunus Korkmaz and Çağrı Bekircan for assisting in sample collection in the field studies. We also thank anonymous reviewers for reviewing the paper and making valuable suggestions. We also would like to thank Sarah North for proofreading.

**Authors' contributions:** Muammer Kurnaz, Mehmet Kürsat Şahin designed the study. Muammer Kurnaz, Mehmet Kürsat Şahin, Ali İhsan Eroğlu performed the field work. Muammer Kurnaz performed all analyses and Muammer Kurnaz and Mehmet Kürsat Şahin wrote the manuscript. All authors participated in revising the manuscript. All authors read and approved the final manuscript.

**Competing interests:** No potential conflict of interest was reported by the authors.

**Availability of data and materials:** The voucher specimens used in this study have been deposited in the Kelkit Vocational School of Health Services, Gümüşhane University.

**Consent for publication:** Not applicable.

**Ethics approval consent to participate:** Not applicable.

## REFERENCES

- Arribas OJ. 1999. Phylogeny and relationships of the mountain lizards of Europe and Near East (*Archaeolacerta* Mertens, (1921, sensu lato) and their relationships among the Eurasian lacertid radiation. Russ J Herpetol **16**:1–22. doi:10.30906/1026-2296-1999-6-1-1-22.

- Arribas O, Candan K, Kurnaz M, Kumlutaş Y, Yıldırım-Caynak E, Ilgaz Ç. 2022. A new cryptic species of the *Darevskia parvula* group from NE Anatolia (Squamata, Lacertidae). *Org Divers Evol* **22**:475–490. doi:10.1007/s13127-022-00540-4.
- Arribas O, Ilgaz Ç, Kumlutas Y. 2018. Reevaluation of the intraspecific variability in *Darevskia parvula* (Lantz and Cyren, 1913): an integrated approach using morphology, osteology and genetics (Squamata: Lacertidae). *Zootaxa* **4472**:71–99. doi:10.11646/zootaxa.4472.1.3.
- Arribas O, Ilgaz Ç, Kumlutas Y, Durmus SH, Avcı A, Üzüm N. 2013. External morphology and osteology of *Darevskia rufa* (Bedriaga, 1886), with a taxonomic revision of the Pontic and Small-Caucasus populations (Squamata: Lacertidae). *Zootaxa* **3626**:401–428. doi:10.11646/zootaxa.3626.4.1.
- Baptista NL, Tolley KA, Bluhm M, Finckh M, Branch WR. 2020. Rediscovery, range extension, habitat and phylogenetic relation of the endemic Scaled Sandveld Lizard *Nucras scalaris* Laurent, 1964 (Sauria: Lacertidae) in the central Angolan plateau. *African J Herpetol* **69**(1):12–28. doi:10.1080/21564574.2020.1778108.
- Case TJ. 1978. A general explanation for insular body size trends in terrestrial vertebrates. *Ecology* **59**(1):1–18
- Darriba D, Taboada GL, Doallo R, Posada D. 2012. JModelTest 2: Moremodels, new heuristics and parallel computing. *Nature Methods* **9**:772. doi:10.1038/nmeth.2109.
- Doyle JJ, Doyle JL. 1990. Isolation of plant DNA from fresh tissue. *Focus* **12**(13):39–40.
- Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**:783–791.
- Fernando L, Hibbard T, Quipildor M, Valdecantos S. 2019. A new species of lizard endemic of Sierra de Fiambalá, northwestern Argentina (Iguania: Liolaemidae: *Phymaturus*). Integrated taxonomy using morphology and DNA sequences: reporting variation within the *antofagastensis* lineage. *Zool Stud* **58**:20. doi:10.6620/ZS.2019.58-20.
- Fu J. 2000. Toward the phylogeny of the family Lacertidae. Why 4708 base pair of mtDNA sequences cannot draw the picture. *Biol J Linn Soc* **71**:203–217. doi:10.1111/j.1095-8312.2000.tb01254.x
- Gabelaia M, Gabelaia M, Tarkhnishvili D, Gabelaia M, Tarkhnishvili D, Murtskhvaladze M. 2015. Phylogeography and morphological variation in a narrowly distributed Caucasian rock lizard, *Darevskia mixta*. *Amphibia-Reptilia* **36**:45–54. doi:10.1163/15685381-00002975.
- Hitchmough RA, Adams LK, Reardon JT, Monks JM. 2016. Current challenges and future directions in lizard conservation in New Zealand. *J Royal Soc New Zealand* **46**(1):29–39. doi:10.1080/03036758.2015.1108923.

- Hope AG, Sandercock BK, Malaney JL. 2018. Collection of scientific specimens: benefits for biodiversity sciences and limited impacts on communities of small mammals. BioScience **68**(1):35–42. doi:10.1093/biosci/bix141.
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Mol Biol Evol **35**:1547–1549. doi:10.1093/molbev/msy096.
- Kurnaz M. 2020 Species list of Amphibians and Reptiles from Turkey. J Anim Divers **2**(4):10–32. doi:10.29252/JAD.2020.2.4.2.
- Kurnaz M, Kutrup B, Hosseiniyan Yousefkhani SS, Koc H, Bülbül U, Eroğlu Aİ. 2019. Phylogeography of the red-bellied lizard, *Darevskia parvula* in Turkey. Mitochondrial DNA Part A **30**(3):556–566. doi:10.1080/24701394.2019.1580270.
- Leaché AD, Reeder TW. 2002. Molecular systematics of the eastern fence lizard (*Sceloporus undulatus*): a comparison of parsimony, likelihood, and Bayesian approaches. Syst Biol **51**:44–68. doi:10.1080/106351502753475871.
- Liu S, Hou M, Mo M, Rao D. 2020. A new species of the genus *Acanthosaura* (Squamata, Agamidae) from Yunnan, China, with comments on its conservation status. ZooKeys **959**:113–135. doi:10.3897/zookeys.959.54601.
- Patel H, Vyas R. 2020. Lost before being recognized? A new species of the genus *Ophisops* (Squamata: Lacertidae) from Gujarat, India. Ecol Montenegrina **35**:31–44. doi:10.37828/em.2020.35.4.
- Poyarkov Jr NA, Nguyen TV, Vogel G. 2019. A new species of the genus *Liopeltis* Fitzinger, 1843 from Vietnam (Squamata: Colubridae). J Nat Hist **53**(27–28):1647–1672. doi:10.1080/00222933.2019.1656784.
- Quiroz AJ, Huamaní-Valderrama L, Gutiérrez RC, Aguilar-Kirigin AJ, López-Tejeda E, Lazo-Rivera A, Huanca-Mamani W, ValladaresFaúndez P, Morrone JJ, Cerdeña J, Chaparro JC, Abdala CS. 2021. An endemic and endangered new species of the lizard *Liolaemus montanus* group from southwestern Peru (Iguania: Liolaemidae), with a key for the species of the *L. reichei* clade. Zool Stud **60**:23. doi:10.6620/ZS.2021.60-23.
- Rajabizadeh M, Pyron RA, Nazarov R, Poyarkov NA, Adriaens D, Herrel A. 2020. Additions to the phylogeny of colubrine snakes in Southwestern Asia, with description of a new genus and species (Serpentes: Colubridae: Colubrinae). PeerJ **8**:e9016. doi:10.7717/peerj.9016.
- Ronquist F, Huelsenbeck J, Teslenko M. 2011. Draft MrBayes version 3.2 Manual: Tutorials and Model Summaries, website. Available at: <http://mrbayes.sourceforge.net/manual.php>.

- Sweet SS, Jockusch EL. 2021. A New Relict Species of Slender Salamander (Plethodontidae: Batrachoseps) with a Tiny Range from Point Arguello, California. *Ichthyol Herpetol* **109(3)**:836–850. doi:10.1643/h2020027.
- Tarkhnishvili DN. 2012. Evolutionary history, habitats, diversification, and speciation in Caucasian rock lizards. *Adv Zool Res* **2**:79–120.
- Troncoso-Palacios J, Esquerré D, Urra FA, Díaz HA, Castro-Pastene C, Ruiz MS. 2018. The true identity of the new world iguanid lizard *Liolaemus chillanensis* Müller and Hellmich 1932 (Iguania: Liolaemidae) and description of a new species in the *Liolaemus longatus* group. *Zool Stud* **57**:22. doi:10.6620/ZS.2018.57-22.
- Zazanashvili N, Sanadiradze G, Bukhnikashvili A, Kandaurov A, Tarknishvili D. 2004. Caucasus in Hotspots Revisited: Earth's Biologically Richest and Most Endangered Terrestrial Ecoregions. Mexico city, pp. 148–153.