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ORIGINAL ARTICLE

A new cryptic species of the *Darevskia parvula* group from NE Anatolia (Squamata, Lacertidae)

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Abstract

In this study, we re-examine the *Darevskia parvula* group comprehensively using morphology, osteology and mitochondrial phylogeny, and describe a new endemic species from Turkey: *Darevskia tuniyevi* **sp. nov.** A total of 257 adult specimens were evaluated for external morphology (scalation and biometry) with univariate (descriptive statistics and ANOVA with post-hoc tests) and multivariate (Discriminant Analysis and ANOSIM) analyses. In parallel, osteological data and molecular analyses using three DNA markers (mitochondrial *16S rRNA* and *Cyt-b*, nuclear *Rag-1*) were used to complete the description of the new taxon. The molecular phylogenetic analyses indicated that the *D. parvula* group is composed of three taxa as *D. parvula*, *D. adjarica* and *D. tuniyevi* **sp. nov.**, and showed that *D. adjarica* and *D. tuniyevi* **sp. nov.** are reciprocal sister taxa. On the other hand, *D. adjarica* is morphologically very different from other two forms, while *D. parvula* is hardly distinguishable externally from *D. tuniyevi* **sp. nov.** Therefore, we can consider that *D. parvula* and *D. tuniyevi* **sp. nov.** are cryptic species. These two cryptic species retain their primitive morphology within the group, while *D. adjarica* has changed, perhaps due to different bioclimatic conditions in its Pleistocene refuge and current area.

Keywords Caucasic rock lizards · Distribution · Morphometry · Phylogenetics · Osteology · Northeastern Anatolia

Introduction

Biodiversity is a fundamental phenomenon that includes all natural units (diversity of species, genetic or ecosystem, etc.) (Rawat & Agarwal, 2015). The main unit in biodiversity is the species. This is because species are not only the main clustering units that form populations, communities, ecosystems, and biodiversity hotspots, but they also form many diversity units within themselves (Carstens et al., 2013). Anatolia is a melting pot of biodiversity influenced by Central Asia, Europe, and Africa, and is itself a hotspot with a high proportion of endemism (Gür, 2016; Şekercioğlu et al., 2011; Tavşanoğlu, 2016). One of the reasons for, and probably the most important cause of, the rich species diversity is the fact that Anatolia is the point of intersection of three biodiversity hotspots (Mediterranean, Iran-Turano and Caucasian ones) (Mittermeier et al., 2004). In addition, the isolation created by the Anatolian diagonal formed by the high mountain ranges in Turkey and the mountain range to the northeast and the south plays an important role in increasing its diversity (Gündüz et al., 2007; Korkmaz et al., 2014; Mutun, 2010; Rokas et al., 2003; Vamberger et al., 2013).

Thanks to many phylogenetic studies which were performed to contribute to the study of this biodiversity, the boundaries of previously morphologically determined groups and their taxonomic positions has changed (Kornilios et al., 2020). Phylogenetic approaches apply various datasets including ecological, geological, genetic, anatomical and morphological when examining processes among current organisms. Phylogenetic analyses examine intra- or interspecific variations and provide information about relationships between recognized species. Also, the possible divergence times between species-groups can be calculated with geographical, geological or ecological events that may cause this separation. Phylogenetic analyses can be used to explain

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all the changes (genetic, morphological and anatomical) that a species has experienced from the moment it appeared until the present and the factors or processes that may have caused these changes (Lemey et al., 2009).

The group of Caucasian rock lizards, *Darevskia* (Arribas, 1999), is a genus represented by numerous species in Anatolia and is also distributed in Caucasus, southern Caspian sea, northern Iran, eastern Europe (Balkans) and the Crimean Peninsula. *Darevskia parvula* was first described by Lantz and Cyrén (1913) with samples collected from Artvin province (around Çoruh Valley, Borçka, and Ardanuç), and was considered to be a variety of *Lacerta saxicola* (var. *parvula* nova) from Turkey. After a long time being considered as a variety, it was adopted as one of five subspecies of *L. saxicola* from Turkey (Bodenheimer, 1944). However, Clark and Clark (1973) stated that two specimens from 15 km east of Artvin were very different from other rock lizards and stated that the "*parvula"* taxon should be considered a species. Later, Darevsky and Eiselt (1980) described a new subspecies (*L. p. adjarica*) from Abastumani in Georgia. Thus, it was accepted that *D. parvula* was represented by two subspecies, namely "*parvula"* and "*adjarica"*. Correspondingly, in a comprehensive morphological study of the *D. parvula* group in Turkey, Ilgaz (2009) provided some information about "*parvula"* and "*adjarica"* samples from 10 populations based on meristic characteristics, morphometric measurements, colour pattern, and ecological and biological features.

Two recent studies have contributed to the taxonomy and systematics of the *D. parvula* group. Arribas et al. (2018) revealed that these two taxa should be considered different species in terms of morphology, osteology and molecular data. Also, the assumption of high genetic divergence between the two taxa in their results further increased the view that they are different species. Ultimately, they reported that the Ardahan (Turkey) specimens (morphologically more similar but not identical to *D. adjarica,* and hence provisionally treated as "inland *adjarica*") had an extra vertebra in both males and females when compared to specimens from other populations. Kurnaz et al. (2019) examined all populations of the species in terms of phylogeographical and ecological niche differences and confirmed the results of Arribas et al. (2018) that "*parvula"* and "*adjarica"* are two different species. In addition, Kurnaz et al. (2019) determined that there were three different taxa they called *parvula*, *adjarica* and an unnamed new taxon, which were also different in terms of ecological niche, and had different ecological needs. Moreover, the geographic distribution of the three genetic lineages and the regions where they are genetically isolated from each other were identified. However, Kurnaz et al. (2019) made an important nomenclatural mistake in their work: they ignored the restriction of the type locality of *D. parvula* to Artvin in Arribas et al. (2018) (since the original description included both "*parvula"* and "*adjarica"* localities among the syntype series) naming the supposed new species as "*parvula*", and considering what was in fact *D. parvula* s. str as new.

As can be seen from the studies carried out so far, the taxonomic status of *D. parvula* sensu lato has changed greatly since it was first described. First, the populations of the species were divided into two different subspecies, and then these two subspecies were considered to be two different species. Ultimately, a third genetically different species was proposed according to results of a recent phylogeographic study (Kurnaz et al., 2019). Based on these premises, the purpose of this study is: i) to update the nomenclature of this species group, ii) to re-examine this group more comprehensively using morphology, osteology and phylogeny views, and iii) most importantly to describe a new endemic species of the *D. parvula* group from Turkey.

Materials and methods

Sampling

A total of 257 adult specimens were used for morphological comparisons (see details in Appendix 1). Acronyms include information of the taxon to which they are ascribed and the name of the nearest locality (e.g., "parHatila" are specimens of *D. parvula* from the surroundings of Hatila). The study area covered the north-eastern and eastern parts of Turkey and Georgia (Fig. 1). The specimens were incorporated into the collection of ZDEU (Zoology Department, Ege University) and kept in the Zoology Lab of the Department of Biology at the Science Faculty, Buca, İzmir, Turkey. Species boundaries are the ones identified by Arribas et al. (2018), together with the presumptive new taxon (Arribas et al., 2018; Kurnaz, 2019; this study).

For molecular phylogenetic approaches, 60 specimens (14 for *D. parvula*, 33 for *D. adjarica*, and 13 for the new taxon) were used (see details in Appendix 2). 11 of them stored as museum materials were used in this study for the first time. In order to create broader comparative datasets, we also included sequences retrieved from GenBank, to complement our sampling for specific analyses. To infer tree phylogeny, we used the following outgroups: *Podarcis muralis* (Laurenti, 1768), *P. siculus* (Rafinesque-Schmaltz, 1810), *Darevskia valentini* (Boettger, 1892), *D. rudis* (Bedriaga, 1886), *D. portschinskii* (Kessler, 1878), *D. praticola* (Eversmann, 1834), *D. brauneri* (Mehely, 1909) and *D. saxicola* (Eversmann, 1834) (see Figs. 3 and S1 for accession numbers). All localities of our specimens used in both molecular and morphological examinations are given in Fig. 1; Appendix 1–2.

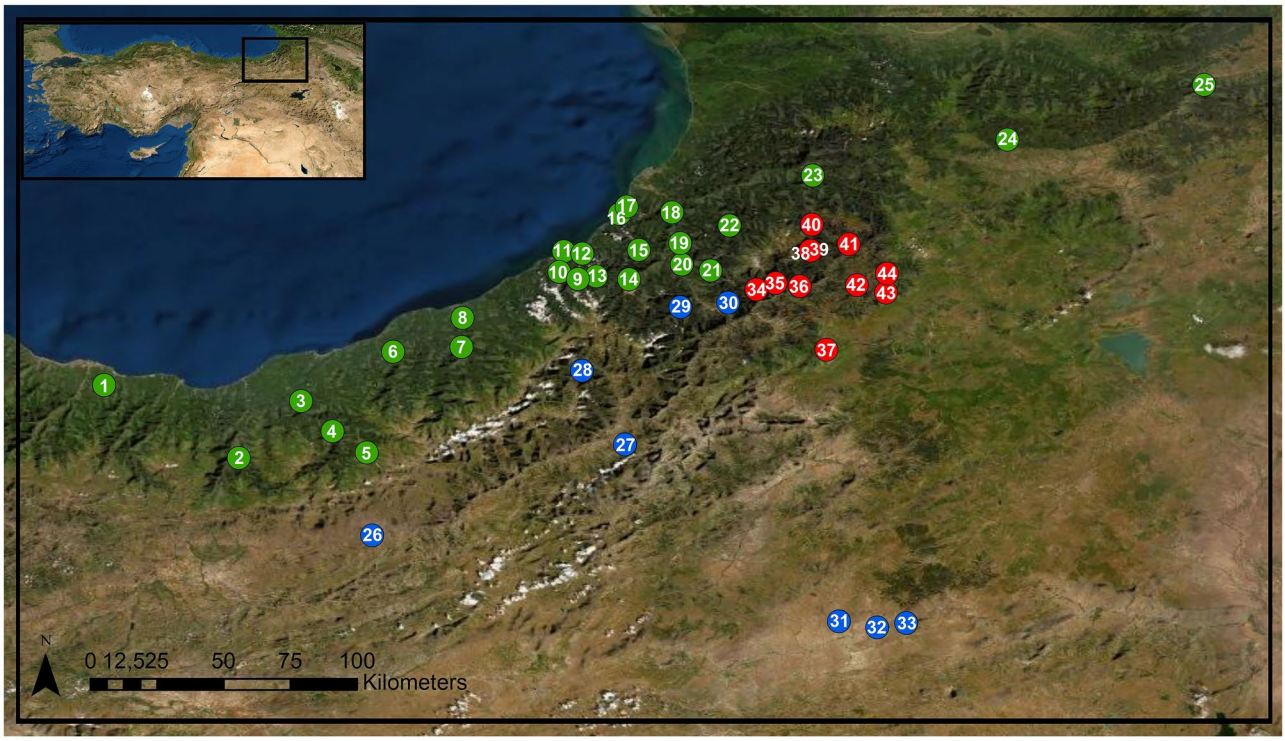

Fig. 1 Map showing the localities of the samples included in the morphological and molecular analyses. Blue: *Darevskia parvula* (Lantz & Cyren, 1913). Green: *Darevskia adjarica* (Darevsky & Eiselt, 1980). Red: *Darevskia tuniyevi* **sp. nov**

Morphological examinations

The following metric dimensions were taken using dial calipers with accuracy to the nearest 0.01 mm: 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 widest point of head. Head length (HL): tip of snout to posterior margin of ear opening. Furthermore, morphometric indexes were calculated, Pileus Index (PI) [PL / PW] and Head Index (HI) [HL / HW].

Meristic scalation characters considered here consisted of the following counts: supraciliar granules (left–right) (SCGa-SCGb), supraciliar plates (left–right) (SCPa-SCPb), supralabial plates (left–right) (SRLa-SRLb), sublabial plates (left–right) (SLa-SLb), transversal series of gular scales between inframaxillar symphysis and collar (MG), collaria (Coll), supratemporal scales (Sptmp), temporal scales 1 (transversal rows of temporal scales between masseteric and tympanic) (left–right) (TS1a-TS1b), temporal scales 2 (longitudinal rows of temporal scales between tympanic and parietal) (left–right) (TS2a-TS2b), temporal scales 3 (longitudinal rows of temporal scales between supratemporal and masseteric) (left–right) (TS3a-TS3b), postemporal plates (left–right) (POTa-POTb), ventral plates (transversal and longitudinal) (TVP and LVP), preanals 1 (number of preanals located anterior of anals) (PA1), preanals 2 (number of preanals surrounding anals) (PA2), femoral pores (left–right) (FPa-FPb), longitudinal rows of scales on ventral surface of thigh between the femoral pores and the outer row of enlarged scales (left–right) (LSa-LSb), subdigital lamellae in the 4th toe (left–right) (SDLa-SDLb), tibial scales (scales lying on dorsal surface of ankle between the large scales (TS), and transversal series of dorsal scales at the midtrunk (DS). The bilateral scales were combined in only one, non-redundant variable (the same acronyms, but without "a" and "b"). The shapes of the submaxillary plates were also examined and codified as 1 (narrower, more parallel, smooth and rounded scale: types A, B, C from Eiselt & Darevsky, 1980) or 2 (more angulate, subtriangular scale: types: D, E, F from these same authors; See Arribas et al., 2018, Figs. 2 and 10) (SBXTypx). Rostral-Internasal (R-I) and Postocular-Parietal (Post-Par) contacts were also examined and codified as 0 (no contact) and 1 (contact).

Statistical procedures

As a result of the sexual dimorphism in biometry and scalation presented by all the species of *Darevskia* (see for instance Darevsky, 1967), morphological analyses were

carried out separately for males and females. The genetic results (see below) were used to define the three OTUs.

Statistical analyses used in the morphological study were both univariate (ANOVA) and multivariate (Canonical Discriminant Analysis, CDA). ANOVA was run for SVL, scalation characters and biometric indexes, with post-hoc Tukey–Kramer tests at $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***) to detect differences among samples. In Canonical Discriminant Analysis (CDA), Chi-square and Wilks' Lambda were used to test the significance of each axis of the CDA. If the groups have different scores, then the models discriminating between the groups and axes are significant. Bartlett's Sphericity Tests prove if the variables are uncorrelated, a prerequisite for a successful discriminant analysis (Sokal & Rohlf, 1969; Blackiht & Reyment, 1971; Legendre & Legendre, 1998, and online help in the statistic programs utilized, see below). In this Canonical Discriminant Analysis, each population is represented by a centroid (a hypothetical middle individual).

Analysis of Similarity (ANOSIM) (Clarke, 1988, 1993) was carried out to test the significance of the differences between samples. This tests whether the assigned groups are meaningful, that is, more similar within groups than between samples from different groups (see more details in Arribas, 2010). To check for significance, pseudo-replication tests (1000 randomizations) were run to test if the given results could occur by chance. If the value of R is significant, there is evidence that the samples within groups are more similar than would be expected by random. The most useful feature of this test is that pairwise tests among populations allow significance testing of the differences among the groups concerned and detect which ones are really different from the others.

ANOVA was calculated with NCSS 2007© (Hintze, 2007). CDA and ANOSIM analyses were performed with Community Analysis Package 6© (Seaby & Henderson, 2019). Coloration tones have been established by comparison of photographs with Köhler (2012).

Molecular genetic analyses

Laboratory procedure and sequence analyses

Genomic DNA isolation was performed using PureLink (Invitrogen, Thermo Fisher Scientific, https://www.thermofisher.com/order/catalog/product/K182001). For our study we selected and analyzed three DNA genes: *16S rRNA* and Cytochrome b (*Cyt-b*) located on the mitochondrial DNA (mtDNA), and the recombination activating gene (*Rag-1*) located on the nuclear DNA (nDNA). In order to obtain DNA sequences of these regions, they were amplified by Polymerase Chain Reaction (PCR) and PCR products were sequenced with an ABI PRISM 3700 (PE Applied Biosystems, Forster City, CA, USA). The primer pairs used for each gene and the reaction conditions of each PCR are given in Table S1. All DNA sequences generated in this study have been submitted to GenBank (see Appendix 2 for accession numbers).

Chromatographs were manually edited in Bioedit 7.2.5 (Hall, 1999), and heterozygous positions were coded using ambiguity codes (IUPAC) for the *Rag-1* nuclear gene. Both coding gene sequences (*Cyt-b* and *Rag-1*) were translated into amino acids, and no stop codons were observed. We, however, detected that some of *Cyt-b* sequences showed a pseudo-gene pattern with double peaks despite sequencing twice. To avoid erroneous results, these sequences were removed from the dataset and the phylogenetic analyses were inferred using each mitochondrial gene, separately.

Alignment, genetic divergence, and model selection

DNA sequence data from both the present study and GenBank were aligned with MAFFT v7 (Katoh & Standley, 2013) with default parameters and the FFT-NS-2 algorithm. The number and diversity of haplotypes of both mtDNA and nDNA sequences belonging to all individuals were determined using DnaSP v6 (Rozas et al., 2017). The uncorrected genetic distances (p-distances) among the main lineages considered distinct species in our phylogeny were calculated in MEGA X (Kumar et al., 2018).

ModelFinder (Chernomor et al., 2016; Kalyaanamoorthy et al., 2017) implemented in the IQ-TREE web server (Trifinopoulos et al., 2016; http://iqtree.cibiv.univie.ac.at) was used to determine the most suitable nucleotide evolutionary model for each DNA region, based on the BIC criterion, and to decide the most appropriate partition strategy for *Cyt-b*, which is protein-coding gene. Based on the result from this analysis, *Cyt-b* was considered as a single partition with HKY + G as the most suitable substitution model. For *16S rRNA*, we selected TIM2e + G and SYM + G which were the suitable models for both ML and BI, respectively.

Phylogenetic analyses, species delimitations, and haplotype networks

Maximum Likelihood (ML) analyses were performed separately for the mtDNA markers using IQ-TREE, with the appropriate partition scheme and model. Statistical support of the nodes was tested with SH-aLRT tests with 10,000 replicates (Guindon et al., 2010), 10,000 ultrafast bootstrap alignments (Minh et al., 2013) and 100 standard bootstrap alignments (Felsenstein, 1985).

A Bayesian Inference (BI) analysis was also performed for the two datasets using MrBayes v3.2.6 (Ronquist et al., 2012). The analyses were carried out twice with eight chains and 10^7 generations each, and with tree and log files

recorded every 100 generations. We determined whether the analyses had reached convergence (ESS values ≥ 200) with Tracer v1.7.1 (Rambaut et al., 2018). After a 25% burn-in, a majority-rule consensus tree was created from the remaining trees.

The BI phylogenetic trees obtained for both *16S rRNA* and *Cyt-b* datasets were used as input files in the Bayesian implementation of the Poisson tree processes model (bPTP; Zhang et al., 2013;—https://species.h-its.org/ptp) and the multi-rate PTP (mPTP; Kapli et al., 2017;—https://mptp.h-its.org/#/tree) to determine species boundaries.

Haplotype networks were inferred using *16S rRNA*, *Cyt-b* and the phased haploid dataset for the nuclear marker (*Rag-1*) under the statistical parsimony algorithm of TCS (Clement et al., 2000) using PopART v1.7 (Leigh & Bryant, 2015). Heterozygous positions for the nuclear marker were phased with the Bayesian algorithm of Phase 2.1 (Stephens & Scheet, 2005; Stephens et al., 2001) implemented in DnaSP (Rozas et al., 2017), with 1,000 iterations after a burn-in of 100. All haplotypes were estimated with high probabilities (between 0.5 and 1.0). Haplotype networks were preferred to gene-trees for the nuclear marker, because of its low diversity.

Divergence times estimation

To estimate divergence times, we used BEAST v1.10.4 (Suchard et al., 2018) and a known substitution rate of cytochrome b for the Lacertidae family (mean value 0.0164, S.D. 0.00317; Carranza & Arnold, 2012). For this analysis, a new cytochrome b dataset was created by selecting one *Cyt-b* sequence from each of the different phylo-groups (clusters) obtained from PTP, in order to conform to the Yule (speciation) model of cladogenesis. The analysis was carried out with three independent runs for 2×10^7 generations. Other settings were as follows: Relaxed Uncorrelated Lognormal Clock, random starting tree. Stationarity was again determined with Tracer. The results from all runs were combined with LogCombiner v1.10.4 and a consensus tree was created using TreeAnnotator v1.10.4.

Results

Morphology

Canonical Discriminant Analysis (MALES)

Canonical discriminant analysis (CDA) maximizes the discrimination of samples between the first two significant axes, with Eigenvalues greater than one; Chi-square 351.85 [$P = 1.91 \cdot 10^{-6}$] (Eigenvalue 7.74; 87% of the variability explained) and 89.40 [$P = 9.28.10^{-7}$(1.09; 12% explained)] for the first and second axes, respectively. Both axes together explain 99% of the total variability. Wilks Lambda is 0.0545 ($F_{44} = 16.54$, $P = 2.01 \cdot 10^{-49}$). As $P < 0.05$, this demonstrates the existence of significant differences among the samples analysed. Bartlett's sphericity tests further proved that the variables were uncorrelated, a prerequisite for successful discriminant analysis (Bartlett's Chi-Squared Test Statistic = 353.30, 44 degrees freedom; $P = 2.52 \cdot 10^{-6}$).

As shown in Fig. 2a, the first axis (87% of variability explained) separates *D. adjarica* males in the negative part, characterized by greater values for SbmxTyp (-0.69) and SPTMP (-0.24) and the smaller scores for POT (0.62), LS (0.44) and TVP (0.33), from the new taxon and especially *D. parvula* is characterised by contrary scores. On this first axis, *D. parvula* and *D. adjarica* appear well separated, and the new taxon is in an intermediate position, with sensible overlap with *D. parvula* and almost none with *D. adjarica*. The second axis (12% of variability explained) is the one which separates in great part the new taxon from *D. parvula* especially, but also from *D. adjarica*. This new taxon, in the positive part of the axis, is characterised by higher values for SBMXTyp (0.56), TVP (0.42), TS1 (0.36), DS (0.34), and SDL (0.33), and smaller values for LS (-0.39). This CDA ensures the discrimination of 95.5% of the specimens, which is very high. The best discriminated is *D. adjarica* (100%), followed by *D. parvula* (97.72%), whereas the one discriminated least was the new taxon (however, there was 80.76% correct discrimination).

Mahalanobis distances among centroids derived from CDA show that the new taxon is morphologically closer to *D. parvula* ($D^2 = 3.53$) than to *D. adjarica* ($D^2 = 4.66$), whereas these two are mutually the most different between them ($D^2 = 6.11$).

Analysis of similarity (ANOSIM) of male sample data show good taxa assignation (R-statistic = 0.349618, $P < 0.001$; 1000 permutations). As the general test is significant, different samples vary more between themselves than internally. The greatest discrimination is between *D. parvula* and *D. adjarica* ($R = 0.464633$; $P = 0.001$), with intermediate discrimination between the new taxon and *D. adjarica* ($R = 0.298279$; $P = 0.001$), and the lowest but still significant discrimination between the new taxon and *D. parvula* ($R = 0.221463$; $P = 0.001$).

Canonical Discriminant Analysis (FEMALES)

For females, discrimination is a bit worse than for males, as is usual in Lacertini (First eigenvalues greater than one, but the second inferior to one; both however have significant canonical correlations (Chi-square 246.46 [$P = 2.14.10^{-6}$] (Eigenvalue 5.16; 89.7% of the variability explained); and 50.05 [$P = 0.00036$] (Eigenv. 0.58; 10.2% explained) for the first and second axes, respectively) (see Fig. 2b) and, as in the male analysis, separates the samples with reduced

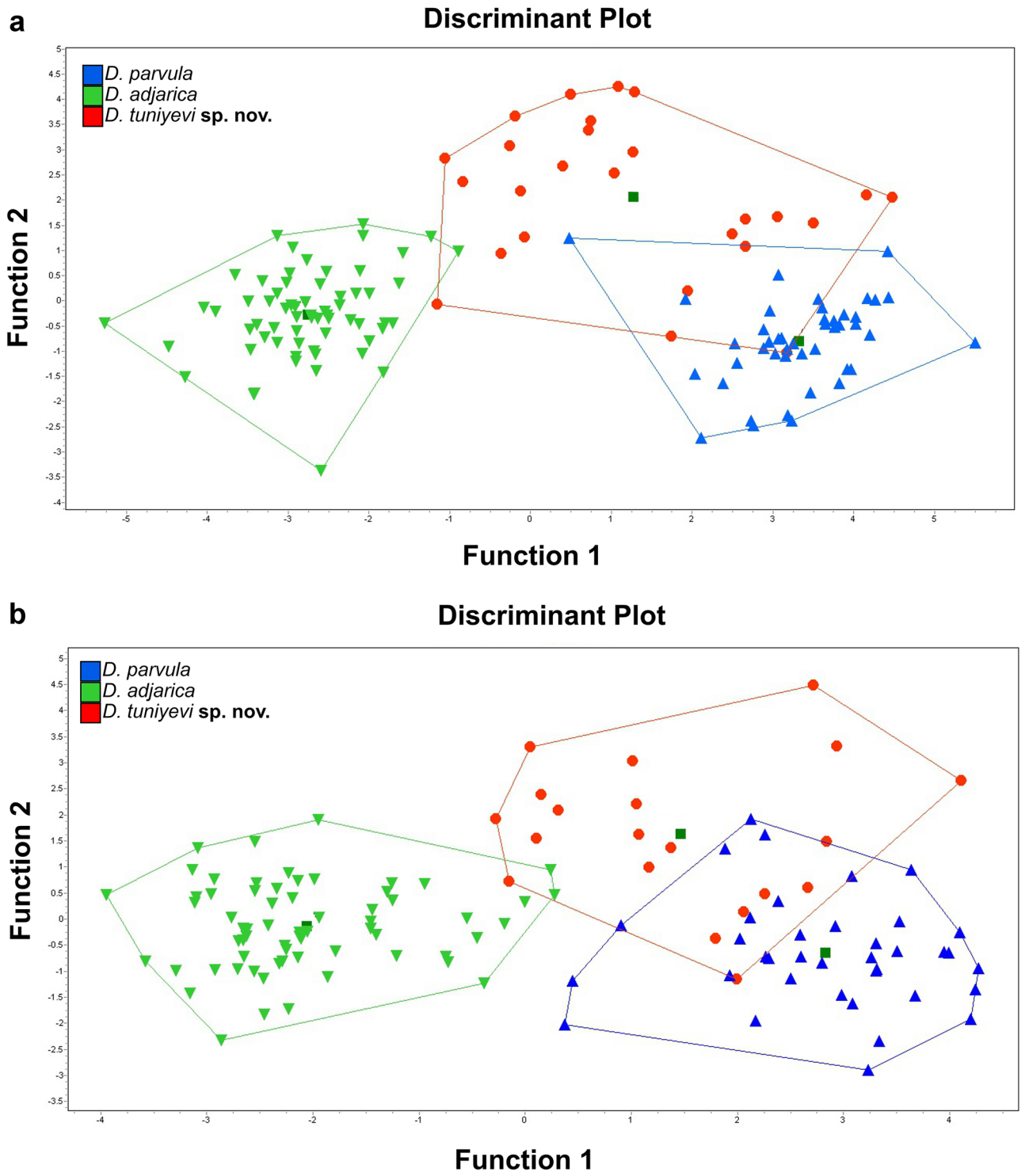

Fig. 2 Canonical Discriminant Analysis (CDA) plot for MALES **a** and FEMALES **b**. Specimens, sample centroids and group perimeters are represented. For details, percentage of variance explained by axes and interpretation, see text

overlap between them. Both axes together explain 99.9% of the total variability. Wilks Lambda is 0.102073 ($F_{44} = 9.48$, $P = 5.41 \cdot 10^{-30}$). As $P < 0.05$, this demonstrates the existence of significant differences among the samples analysed. Bartlett's sphericity tests further proved that the variables were uncorrelated, a prerequisite for successful discriminant analysis (Bartlett's Chi-Squared Test Statistic = 247.60, 44 degrees freedom; $P = 1.9 \cdot 10^{-6}$).

As shown in Fig. 2b, the first axis (89.7% of variability explained) separates *D. adjarica* females in the negative part, characterised by higher values for SbmxTyp (-0.53) and lower scores for POT (0.51), DS (0.34) and TVP (0.30), from the new taxon and especially from *D. parvula* characterised by the contrary scores. On this first axis, *D. parvula* and *D. adjarica* appear well separated, without overlap and the new taxon appears to be more similar to the scores for *D. parvula*, with partial overlap and very few with *D. adjarica*. The second axis (10.2% of variability explained) has considerable overlap between the three taxa but is useful into separate the new taxon from *D. parvula*. This new taxon, in the positive part of axis, is characterised by higher values for TVP (0.49), SBMXTyp (0.38), SL (0.37), DS (0.36), TS1 (0.35) and SDL (0.33), and lower values for TS2 (-0.48), COLL (-0.38) and LS (-0.34). This CDA ensures the discrimination of 90.16% of the specimens, which is also fairly high. The best discriminated is *D. adjarica* (95.38%), followed by *D. parvula* (89.18%), whereas the least discriminated group was the new taxon (however, there was still 75% correct discrimination).

Mahalanobis distances among centroids derived from CDA show that the new taxon is morphologically closer to *D. parvula* ($D^2 = 2.66$) than to *D. adjarica* ($D^2 = 3.95$), whereas these two are mutually the most different ($D^2 = 4.91$). The results are parallel to those for males, but the differences are smaller.

Analysis of similarity (ANOSIM) of the female sample data show good taxa assignation (R-statistic = 0.276373, $P < 0.001$; 1000 permutations). The greatest discrimination is between *D. parvula* and *D. adjarica* (R = 0.407713; $P = 0.001$), with intermediate discrimination between the new taxon and *D. parvula* (R = 0.131815; $P = 0.005$), and the lowest discrimination between the new taxon and *D. adjarica* (R = 0.156961; $P = 0.016$). It should be noted that the partial test that measures the intra versus intergroup affinities (R) shows a slightly better discrimination between the new taxon and *D. adjarica*, but the pseudoreplication test is slightly more significant for *D. parvula*.

ANOVA

Descriptive statistics and ANOVA results can be seen in Table S2. The different taxa do not differ significantly in size (Males: $F_{2, 117} = 2.38$, $P = 0.09$, NS; Females: $F_{2, 114} = 2.06$, $P = 0.13$, NS). The new taxon has comparatively slightly longer pilei in males than *D. parvula* ($p < 0.01$) and especially *D. adjarica* (at $p < 0.001$); or only than *D. adjarica* for females (at $p < 0.001$); and the whole head is also significantly bigger than in *D. adjarica* (in males and females at $p < 0.001$) and *D. parvula* (only in males at $p < 0.05$).

Of the studied characteristics, and only taking into account the very highly significant characteristics ($p < 0.001$), the new taxon differs from *D. parvula* (at $p < 0.001$) in only two characteristics in males [TS1 and SBMXTyp] and one in females [SBMXTyp]. At $p < 0.01$ for PLI (males) and TS2 & POT (in females); and at $p < 0.05$ for TS2, FP, LS and HWI (in males) and LS (females).

Darevskia parvula differs from *D. adjarica* (at $p < 0.001$; for these species comparisons at $p < 0.01$ and 0.05 see Table S2) in 13 and 12 characters variables (in males and females, respectively) [Males: SCP, SRL, MG, TS2, POT, TVP, FP, LS, SDL, DS, SBXTyp, R-I, HWI; and in Females in SRL, MG, Sptmp, TS2, POT, TVP, FP, SDL, DS, SBXTyp, PLI, HWI].

Finally, *D. adjarica* differs from the new taxon (at $p < 0.001$) in 14 and 9 characters variables (males and females, respectively) [Males: SCG, SRL, Sptmp, TS1, POT, TVP, FP, SDL, TS, DS, SBXTyp, R-I, PLI and HWI; and in Females in SRL, POT, TVP, FP, SDL, DS, SBXTyp, PLI and HWI].

Description of *Darevskia tuniyevi* sp. nov.

ZooBank registration (http://zoobank.org): urn:lsid:zoobank.org:pub:DFA82B86-D97A-4A5D-B11D-CDF14CB8489C.

Holotype ZDEU3/2020, ♂, Pırnallı, Artvin, Turkey. leg. Muammer Kurnaz. 11.09.2020.

Paratypes **1-** 10 ♂♂, 6 ♀♀, same locality, date and collectors as holotype. **2-** ZDEU149/2001, 6 ♂♂, 4 ♀♀, 15 km W of Şavşat, Turkey. leg. Yusuf Kumlutaş, Kurtuluş Olgun, Çetin Ilgaz, Aziz Avcı, Fatma İret. 04.07.2001. **3-** ZDEU152/2001, 4 ♂♂, 7 ♀♀, 19 km W of Ardahan, Turkey. leg. Yusuf Kumlutaş, Kurtuluş Olgun, Çetin Ilgaz, Aziz Avcı, Fatma İret. 06.07.2001. **4-** ZDEU218/2014, 5 ♂♂, 3 ♀♀, Çağlıyan, Artvin, Turkey. leg. Yusuf Kumlutaş. 23.07.2014.

Etymology The specific epithet refers to Dr. Boris S. Tuniyev, for his remarkable work about the knowledge of Caucasian herpetofauna and its remarkable diversity.

Diagnosis A medium-small sized *Darevskia*, morphologically very similar to *D. parvula* (cryptic species, are not sister taxa), characterised by greater values of submaxyllary type (SBMXTyp) (nearly half of the specimens attributed to *D. tuniyevi* **sp. nov.** have submaxillary scales of types characteristic of *D. adjarica* and the other half are similar to *D. parvula*), greater ventralia (TVP), greater number of temporal scales among masseteric and tympanic (TS1), higher dorsalia (DS), greater number of 4th digit subdigital lamellae (SDL), and smaller values for ventral side tight scales (LS). Also, slightly longer pilei (PLI). See the "Comparison among species" section below and Table S2 for detailed

differences. Genetically it is reciprocally monophyletic in respect to the other two *D. parvula* group species (*D. parvula* and *D. adjarica*).

Description of the holotype Adult male with an intact tail, preserved in ethanol (Fig. S2). Coloration and pattern (in alcohol): dorsum background brownish (Beige -254- in life). Pattern faintly reticulated, composed of white ocelli surrounded by black, that appears well developed in the sides (costal or temporal bands), a last faint row of clear ocelli just in the upper border (more or less scalloped) of the costal bands (especially in the forebody), leaving the dorsal tract mainly brownish colour (Beige -254- in life), almost without a trace (or barely visible) of these clear ocelli, and dark pattern (derived from the black reticulation) reduced to two paravertebral rows of irregular spots, well visible, that continue and coalesce in the tail base. Legs brownish (Beige -254- in life) tone with faint reticulate black and (less visible) clear spots. Tail with scale rings of alternating width, usually the distal part of the scales are darker than the medium and proximal parts. Pileus with tiny well-defined spots above, the temporal bands faintly extending into the temporal area of the head. No trace of blue axillar ocelli. Supra, infralabials and subocular with more or less well-defined rounded spots. Gular area, throat, belly and underside tail without a dark pattern (white in alcohol, Salmon -83- in life). Blue spots (Light Caribbean Blue -163-) on the outer ventral scales.

Scalation Contact between rostral and internasal plates absent; Supraciliary granules in the right side, 12; Supraciliary granules in the left side, 12; Supraciliary plates_right, 6; Supraciliary plates_left, 6; Supralabial plates_right, 4; Supralabial plates_left, 4; Sublabial plates_right, 6; Sublabial plates_left, 6; Type of submaxillars, F (from Darevsky & Eiselt, 1980, Fig. 5); Gulars, 27; Collaria, 9; Supratemporals_right, 1; Supratemporals_left, 1; Scales between tympanic and parietal_right, 2; Scales between tympanic and parietal_left, 1; Scales between masseteric and tympanic_right, 3;Scales between masseteric and tympanic_left, 3; scales between masseteric and supratemporal_right, 2; Scales between masseteric and supratemporal_left, 2; Posttemporals_right, 6; Posttemporals_left, 6; Ventral plates (transversal rows), 25; Ventral plates (longitudinal rows), 6; Contact between Postorbital and Parietal, absent; Preanal 1, one big and enlarged plate; Preanal 2, six scales; Femoral pores_right, 23; Femoral pores_left, 24; Scales between femoral pores and outer plates_right, 3; Scales between femoral pores and outer plates_left, 3; Subdigital lamellae_right, 28; Subdigital lamellae_left, 28; Tibials, 16; Dorsalia, 56. Tail proximal rows of scales fairly keeled and outwards rose.

Biometry Snout-vent length, 52.44 mm; Tail length, 122.82 mm; Total length, 175.26 mm; Pileus length, 12.85 mm; Pileus width: 6.06 mm; Head width, 6.06 mm; Head length: 6.85; Pileus Index, 2.12; Head Index, 1.94.

Intraspecific variation Males can have background tone more greyish than the holotype (from Beige -254 -, Drab Gray -256- to Pale Neutral Grey -296-) (Fig. S3). Paravertebral rows of dark spots can be wider, occupying a good part of the dorsal tract, or less and more centred (as in the holotype), or even substituted by a fine stipple widespread across the entire dorsal tract, without defined paravertebral rows. The white ocelli in the dorsolateral area (upper limit of the costal (temporal) bands can be more marked or almost faint. In some rare specimens the paravertebral and costal bands connect and form a near-reticulated pattern. Underside, out of the reproductive period, the salmon or orange colour is conserved especially in the aged males (bigger ones). Throat, chest, and bellies are completely immaculate in all the specimens. The bigger ones have blue spots in the outer ventral scales.

Females less variable than males, with more or less developed spots in the paravertebral rows. In general, both these paravertebral dots, as with the costal bands, are clearly less developed than in males. Old females conserve the ventral salmon coloration outside the breeding period. No blue spots in outer ventral scales. In all the other aspects, similar to males.

Juveniles have the pattern and coloration of adults, are white bellied, and with big scales of the underside of the hind legs completely pigmented except a clear flange. Tail not brightly coloured, with the same colour and tone as the dorsum.

Osteology *Darevskia tuniyevi* **sp. nov.** (from Ardahan, Turkey) has 7 premaxillary teeth, from 14 to 18 maxillary teeth, from 18 to 23 dentary teeth, 28 presacral vertebrae in males and 29 in females (with 6 and 7 short ones in the lumbar area, respectively). There is no trace of short vertebrae associated with the third presacral vertebrae. Tail autotomic vertebrae are type A (Arnold, 1973; Arnold et al., 2007). All the clavicles studied are open (marginated). The interclavicle is typically cruciform and oval sternal fontanelle. Costal formula is (3 + 2) with one or no inscriptional rib with a similar proportion of occurrence. Postorbital bone is shorter than the postfrontal. Anteromedial process of postorbital is present but small and frequently much reduced and barely discernible. Anterodistal process of the postfrontal is present. Squamosal and postorbital overlap approximately along one third of the length of the latter.

Habitat and ecology Artvin province receives average annual precipitation of approximately 700 mm, the air temperature ranges from -0.2 °C in winter to 26.3 °C in summer (see Fig. S4). The type locality (Pırnallı) is an area with dense forest at an altitude of 1258 m above sea level (coordinates: 41°15′13.66"N; 42° 3′57.16"E; 1258 m) (Fig. S4-a). Other localities like Çağlıyan (Artvin, Turkey) have similar traits for habitat and vegetation (Fig. S4-b).

Lizards were found to be active all day, with air temperatures between 25 and 33 °C. In general, specimens were caught in rocky, stony, or bare soil areas along the edge of the forest road in a mixed forest with *Fagus orientalis* (Oriental Beech), *Picea orientalis* (Caucasian Spruce) and *Pinus sylvestris* (Scots Pine). The range is composed of volcanogenic flysch and sandstones (Fig. S4). Other reptile species living in sympatry in the area are *Mediodactylus* sp., *Darevskia derjugini* (Nikolsky, 1898), *Lacerta media* Lantz and Cyren,1920, *Hemorrhois ravergieri* (Menetries, 1832) and *Zamenis hohenackeri* (Strauch, 1873).

Distribution In the Meskheti and Şavşat Ranges of the Lesser Caucasus (Şavşat, Kedi, Karçal Dağı, Yalnızçam Dağı) [centre of Artvin, northeast of Şavşat, Ardanuç and Ardahan] (see Fig. 1). The area seems to be encompassed in the interfluvium at the right side of the Çoruh (Coroch) river (that separates it from *D. parvula* and the Turkish area of *D. adjarica*), and perhaps the Ajarisckali river at the north (separating from the Georgian area of *D. adjarica*). *D. tuniyevi* **sp. nov.** is only known from Turkey but is very near the Georgian frontier (i.e. Camili, Artvin) and it is possible that enters Georgia in a narrow strip (perhaps up to the Ajarisckali river, as said above).

Comparisons among species Combining CDA and ANOVA results (in **bold CDA characters also p < 0.01 or 0.001 in ANOVA**; see Table S2 for specific values of scalation and indexes of each species):

Darevskia tuniyevi **sp. nov.** males differ from *D. parvula* (cryptic species) with greater values for **SBMXTyp** (near half of the specimens attributed to *D. tuniyevi* **sp. nov.** have submaxillary scale similar to the types typical also in *D. adjarica*), TVP (ventralia), **TS1** (temporal scales among masseteric and tympanic), DS (dorsalia), and SDL (4th digit of subdigital lamellae), and smaller **LS** (ventral side tight scales) [also in PLI (longer pilei) at p < 0.01 in ANOVA, not included in CDA]. In females *D. tuniyevi* **sp. nov.** have greater values for TVP, **SBMXTyp**, SL, DS, TS1 and SDL, and smaller values for **TS2** (longitudinal rows of temporal scales between tympanic and parietal), and LS (-0.34) [also POT (postemporal plates) in ANOVA at p < 0.01)]. The sixth submaxillary morphology is very similar to the *D. parvula* one, but the smaller scale is comparatively greater than in this later (Fig. S2-c). Differences between *D. tuniyevi* **sp. nov.** and *D. parvula* are subtle and it can be considered cryptic species, despite not being a sister taxa.

Darevskia adjarica, the most different taxa in the group, is differentiated from *D. parvula* and *D. tuniyevi* **sp. nov.** by greater values of **SbmxTyp** and **Sptmp**, and smaller scores for **POT**, **LS** and **TVP** in males [also in ANOVA: SCG, SCP, SRL, MG, TS2, TS1, POT, FP, SDL, TS, DS, R-I, PLI and HWI]. In females, there are higher values of **Sbmxtyp** and smaller scores for **POT**, **DS** and **TVP** [also in ANOVA: SRL, FP, SDL, PLI and HWI]. + *D. parvula* (and *D. tuniyevi* **sp. nov.**) have contrary values for these characteristics. The sixth submaxillary has the higher scale subtriangular, more than twice the smaller one (Fig. S2-c). See also Arribas et al. (2018) for a description of *D. adjarica*.

Concerning pattern and pigmentation, *D. adjarica* is also the most different, with a generally more darker and contrasted pattern, a common greenish sheen (physical colour, not pigment) and sometimes axillary blue ocelli in males. Also, the belly is more frequently and more intensely pigmented with reddish tones than in the other two species (*D. parvula* and *D. tuniyevi* **sp. nov.**). Variability in some of these characteristics (extension of black pattern and intensity of pigmentation) is parallel to the lizard's bioclimatic characteristics and are more typical of moist places (own data).

Molecular analyses

In total, 505 bp of *16S rRNA*, 468 bp of *Cyt-b* and 644 bp of *Rag-1* were obtained. Detected haplotypes were 25 for *16S rRNA*, 27 for *Cyt-b* and 15 for *Rag-1*. Haplotype diversity was as follows: 0.97 for *16S rRNA*, 0.99 for *Cyt-b*, and 0.92 for *Rag-1*. Genetic distances, within and among the main lineages, are given in Table S3.

Mitochondrial phylogenetic trees

Each gene region (*16S rRNA* and *Cyt-b*) was used in ML and BI analyses, separately. Due to the topological similarity of the resulting phylogenetic trees, we present the BI tree with bootstrap and posterior probability (pp) values obtained from both analyses indicated on the branches (Figs. 3 and S1). The main finding is that the *D. parvula* and *D. adjarica* populations form two respective monophyletic lineages, as was theoretically expected. Another important result is that some populations closely related to *D. adjarica* appear phylogenetically distinct from all other populations (*D. tuniyevi* **sp. nov.**, Figs. 3 and S1). Both gene trees reveal these distinctions with high statistical support (Figs. 3 and S1).

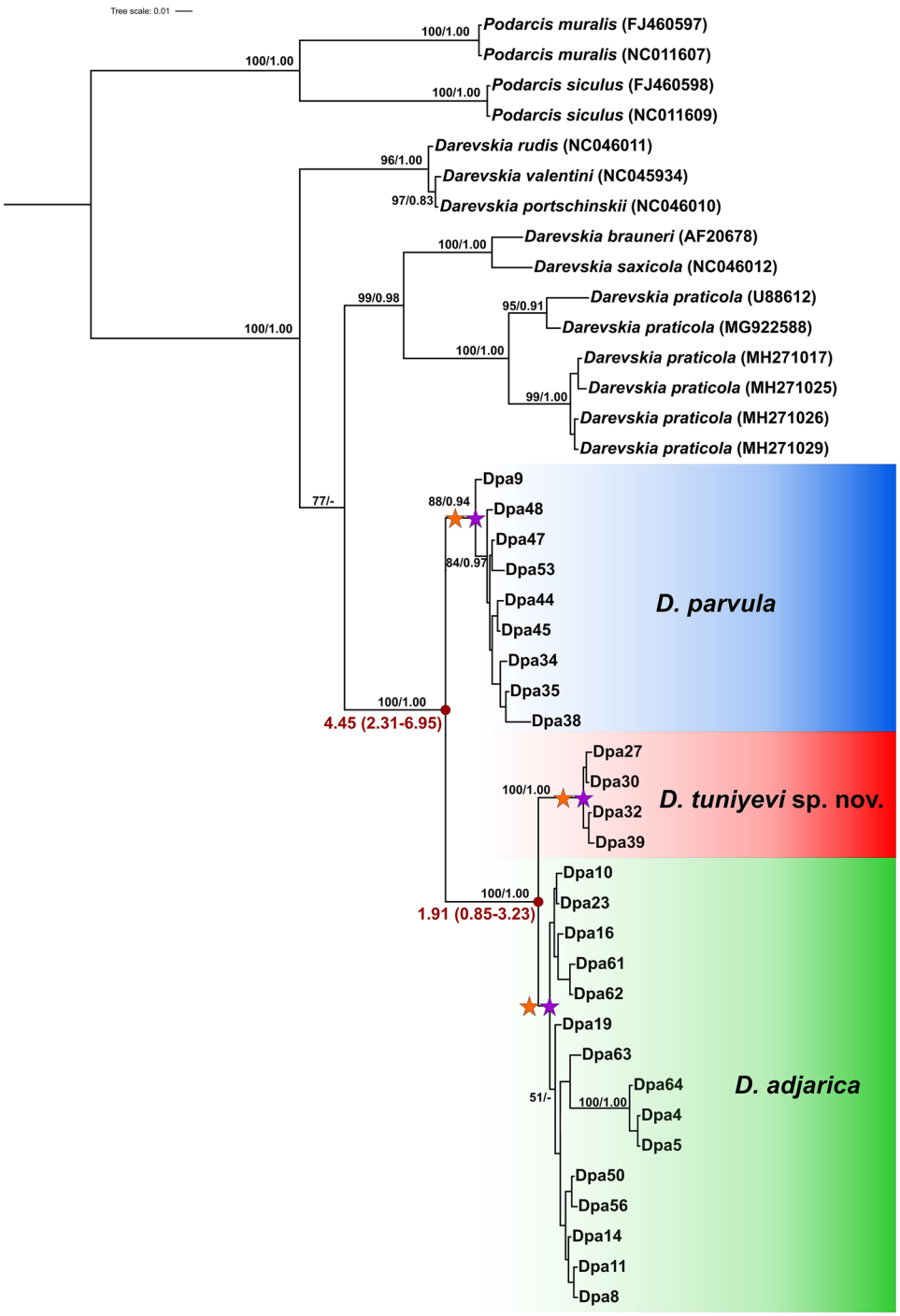

Fig. 3 BI tree inferred from the *Cyt-b* dataset. Numbers on branches indicate the bootstrap and posterior probability (pp) values (ML/BI, values lower than 50/0.70 are not shown). Orange stars represent species boundaries inferred by mPTP, while purple ones the results of bPTP. Divergence times between main clades are shown on the tree

Species delimitation approaches

The analyses used to detect species boundaries (bPTP and mPTP) showed different outcomes. The former analyses identified four and three "clusters", while the latter revealed one and three clusters for *16S rRNA* and *Cyt-b*, respectively (Figs. 3 and S1). Considering the tree topology of *16S rRNA*, mPTP does not discriminate the studied group and recovered all *D. parvula*, *D. adjarica* and *D. tuniyevi* **sp. nov.** in the same monophyletic lineage (Fig. S1). On the other hand, the bPTP analysis indicates that there are three separate lineages within the *D. parvula* group: 1-Hatila Valley, 2-Pazaryolu, and 3-Horasan, Sarıkamış, Barhal, Salkımlı. The fourth lineage for bPTP includes *D. adjarica* and *D. tuniyevi* **sp. nov.**. The *Cyt-b* tree presents a moderate result considering the analyses. Both showed that there are three "clusters" in our phylogeny: *D. parvula*, *D. adjarica,* and *D. tuniyevi* **sp. nov.** (Fig. 3).

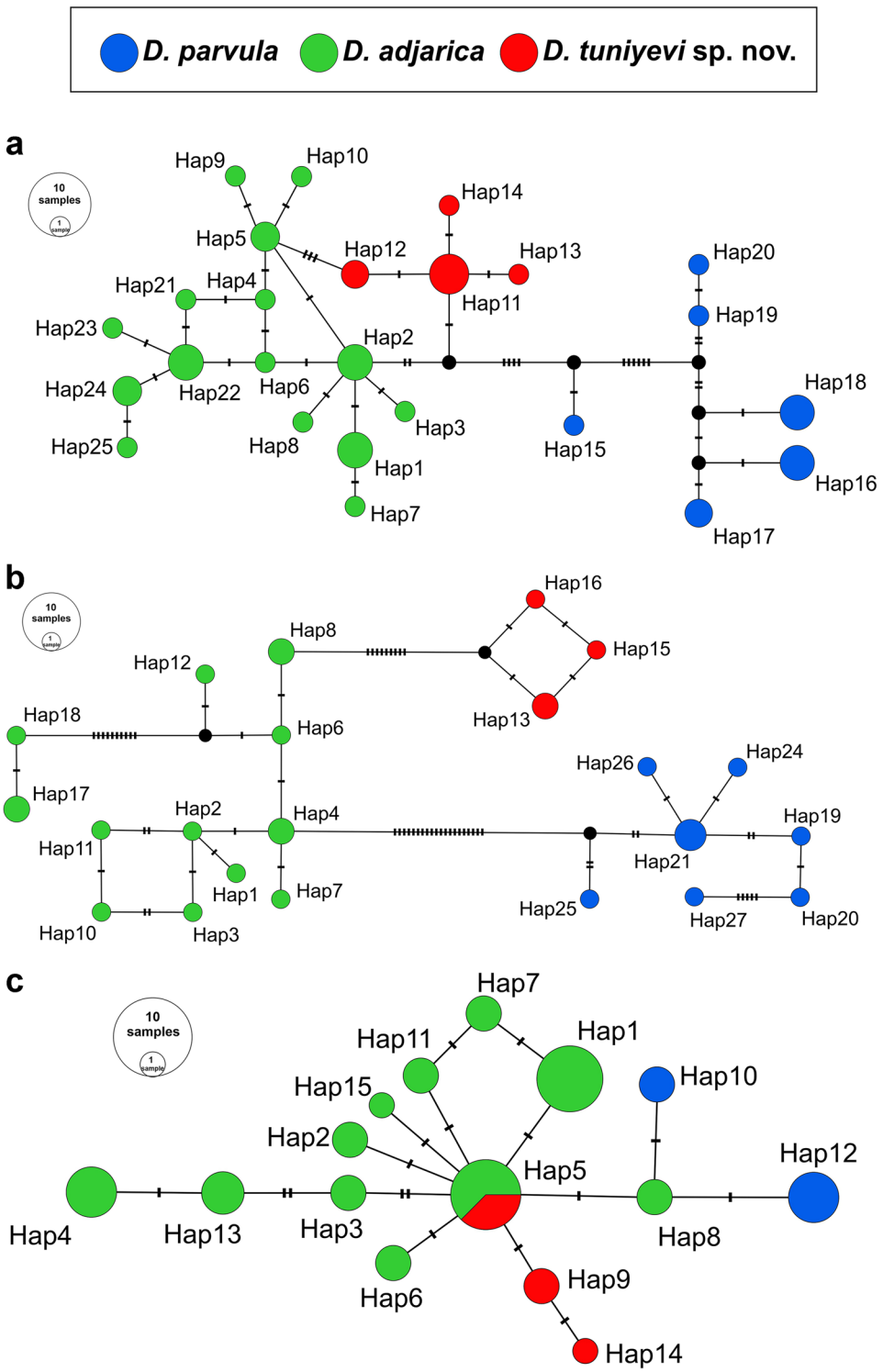

Fig. 4 Haplotype networks corresponding to *16S rRNA* **a**, *Cyt-b* **b** and *Rag-1* **c**. Lines represent a mutational step, black circles missing haplotypes, and coloured circles haplotypes. The circle area is proportional to the number of individuals

Haplotype networks of mitochondrial and nuclear markers

As a result of the analyses, mtDNA markers were more informative compared to nDNA (Fig. 4). Both mitochondrial markers showed that *D. tuniyevi* **sp. nov.** and *D. adjarica* are closely related lineages rather than *D. parvula*. Particularly, *Cyt-b* indicates that the haplotypes of *D. tuniyevi* **sp. nov.** cluster with *D. adjarica* (Hap8) (Fig. 4b). When we consider the *16S rRNA* network, Hatila Valley (Hap15) is placed in a divergent position between *D. parvula* and the remaining taxa (Fig. 4a). This is not supported by *Cyt-b*.

Divergence times

According to the inferred dates, the most recent common ancestor (MRCA) of the group (*D. parvula*, *D. adjarica,* and *D. tuniyevi* **sp. nov.**) dates back to 4.4 mya (95% HPD: 2.3–6.9 mya) in the Late Miocene or Early Pliocene. The split between *D. adjarica* and *D. tuniyevi* **sp. nov.** occurred 1.9 mya (95% HPD: 0.8–3.2 mya) during the Pleistocene (Fig. 3).

Discussion

Taxonomic reconstruction of *Darevskia parvula* group

Darevskia parvula is a rock lizard that is mostly distributed in the Pontic region running across the north-eastern part of Turkey and the west of Georgia. The first scientific studies of the species were morphology-based as for other reptiles, resulting in taxonomic reconstructions (Kurnaz et al., 2019 and references therein). One of them suggested that *D. parvula* consists of two distinct subspecies of *D. p. parvula*, which lived around Artvin, Ardahan, Kars, and Erzurum (Turkey), and *D. p. adjarica*, which was distributed in Trabzon, Rize, the coast of Artvin (Turkey) and the adjoining areas of Georgia (Darevsky & Eiselt, 1980). A recently published study suggested that *D. p. adjarica*, which is one of the subspecies, should be elevated to species level, and accepted that *D. parvula* and *D. adjarica* are distinct species based on the evidence obtained from both morphology and genetic markers (Arribas et al., 2018). To reveal the taxonomic status of both species, elaborate research using molecular phylogenetic approaches identified that there are three genetically distinct lineages, two of which are related to the current species (Kurnaz et al., 2019). The results of our phylogenetic analyses for the *D. parvula* group also showed that the genetically divergent lineage (*D. tuniyevi* **sp. nov.**), which was discovered by Kurnaz et al. (2019) for the first time, is valid (Figs. 3 and S1). These three monophyletic lineages were supported with high or moderate values of bootstrap and posterior probability on main nodes in any of our tree topologies (Figs. 3 and S1). The phenomenon called cryptic species, which means the existence of highly divergent phylogenetic lineages with morphological similarity, is of ubiquitous impact on the taxonomy for reptiles (Candan et al., 2021; Jablonski et al., 2019; Kornilios et al., 2018; Psonis et al., 2017; Simó-Riudalbas et al., 2017; Sindaco et al., 2014; Tamar et al., 2015, 2019). The genus *Darevskia*, particularly, is an extraordinary group due to containing both parthenogenetic and cryptic species, which put it on an eminent position among faunal species of the earth (Ahmadzadeh et al., 2013; Candan et al., 2021; Freitas et al., 2016a). Hence, it is not surprising that a new cryptic lineage was found for the *D. parvula* group as well. Although there is bias in shaping the taxonomic nomenclature with cryptic species, the presence of cryptic species is precious in groups, such as the genus *Darevskia*, where most species have not yet completed their speciation processes and where major hybridization events emerged (Tarkhnishvili et al., 2013, 2020).

The genetic divergence values, which are high for mitochondrial markers and low for the nuclear one, also support this differentiation (Table S3). When *D. tuniyevi* **sp. nov.** compared with *D. adjarica* and *D. parvula*, respectively, the mean levels of mtDNA divergences are 0.8%/2.4% for *16S rRNA* and 4.5%/10.4% for *Cyt-b*. The corresponding values in nuclear DNA (*Rag-1*) are 0.1%/0.3%. Of particular note is the relatively low genetic distance between *D. adjarica* and *D. tuniyevi* **sp. nov.** compared to *D. parvula* for each genetic marker, which helps us to accept that *D. adjarica* and *D. tuniyevi* **sp. nov.** are more closely related. The corresponding values for mitochondrial *Cyt-b* were determined at similar high levels, which is considered sufficient for species discrimination, not only for other species of the genus *Darevskia* but also many of the Lacertids (Ahmadzadeh et al., 2013; Freitas et al., 2016b; Kapli et al., 2013; Karakasi et al., 2021; Psonis et al., 2017). The occurrence of these three divergent monophyletic lineages is confirmed in the gene-tree for mitochondrial *Cyt-b* and they are identified as different "species" by bPTP and mPTP (Fig. 3). Mitochondrial *16S rRNA*, on the other hand, exhibits a dissimilar profile that is at odds with monophyletic lineages. While the result from bPTP of *16S rRNA* suggested that *D. adjarica* and *D. tuniyevi* **sp. nov.** are the same "species", it interestingly identified three different "species" within *D. parvula*. In addition to this, mPTP for the same gene region did not recognize these three genetically different lineages as separate "species" (Fig. S1). According to haplotype networks, all genetic markers used here agree with the genetic distinction of these three lineages (Fig. 4). All showed that the haplotypes are strongly distinguished and that each lineage is isolated from the others. But nuclear *Rag-1* gene, which is less informative due to slower substitution rates compared to the mitochondrial ones, has different situation due to a haplotype (Hap5), which is shared by both *D. adjarica* and *D. tuniyevi* **sp. nov.**, while it supports this pattern within the *D. parvula* group (Fig. 4c).

Darevskia tuniyevi **sp. nov.** includes populations distributed on the eastern side of the intersection between *D. parvula* and *D. adjarica* (Fig. 1). As a consequence of the genetic results in this paper (also supported by Kurnaz et al., 2019), we accept that the *D. parvula* group is composed of three distinct species.

Evolutionary history and biogeography

It seems that *Darevskia* originated in the ancient Anatolian landmass. The *D. parvula* group was probably the first to diverge (perhaps towards 18–23 mya, following the mitogenome analyses of Murtskhvaladze et al., 2020), thus its presence in the area is very old. The climatic deterioration since the end of the Pliocene, perhaps coupled with recent tectonic and river network changes, has led to successive isolations that gave rise to the pattern of isolations that appears in our phylogeny. *Darevskia parvula* s. str. split from the common ancestor of *D. adjarica* and *D. tuniyevi* **sp. nov.** (which we can infer had a very similar morphology to *D. parvula*, today conserved in *D. tuniyevi* **sp. nov.**) approximately 4.4 mya (Late Miocene-Early Pliocene), and *D. adjarica* and *D. tuniyevi* **sp. nov.** diverged in the Lower Pleistocene, approximately 1.9 mya. After this split, *D. adjarica* diverged from the common morphology retained by the other species of the group, perhaps linked to different eco climatic conditions in coastal ranges and lower (more moist and milder) altitudes, probably expanding considerably its area to the east in Georgia more recently.

The Adjara-Şavşat floristic region has 28 endemic plants with narrow distribution. Colchic and Hyrcanic areas are well known as a refuge for thermophilus taxa (Manvelidze et al., 2009), but nearby mountains, not so glaciated as the core of the nearby ranges, probably served as micro-refuges for populations progressively adapting to harsher conditions (altitude, continentality) as is the case of *D. parvula* and *D. tuniyevi* **sp. nov.**, in contrast to *D. adjarica* with more free movements up and down the coastal ranges in response to climatic oscillations.

Conclusions

Darevskia adjarica and *D. tuniyevi* **sp. nov.** are genetically sister species. However, *D. adjarica* is morphologically very different from other two forms, while *D. parvula* is hardly distinguishable externally from *D. tuniyevi* **sp. nov.**. Therefore, we consider that *D. parvula* and *D. tuniyevi* **sp. nov**. are cryptic species. These two cryptic species retain the primitive morphology within the group, while *D. adjarica* has changed, perhaps due to different bioclimatic conditions in its Pleistocene refuge or current range area.

Indeed, while *D. tuniyevi* **sp. nov**. and *D. parvula* remained in inland, continental zones, *D. adjarica* seems to be attracted to the Black Sea coast and adjacent mountains (postglacial recolonisation at altitude) and from there it could have moved into inland Georgia. As it appears today, it closes the area of the group to the north, occupies the lower reaches of the Çoruh river, the Ajarisckali river drainage and nearby areas (Kintrisi river valley), passes east to the Kvabliani river, a tributary of the Kura, without reaching the Rioni lowlands to the north (see map in Darevsky & Eiselt, 1980, Fig. 6; and Arribas et al., 2018, Fig. 1).

The osteological data from Arribas et al. (2018) also support the differences of *D. tuniyevi* **sp. nov.** with respect to *D. parvula* and *D. adjarica*. The studied specimens of *D. tuniyevi* **sp. nov.** (from Ardahan) have an extra vertebra both in males and females, compared to the other studied specimens from *D. parvula* and *D. adjarica*. Also, the females studied had 7 short lumbar ribs (the normal number, which appears also in males, is 6) (see results part for a complete osteological description).

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s13127-022-00540-4.

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Data availability The molecular genetic datasets generated and/or analysed in the present study are available from the GenBank repository (https://www.ncbi.nlm.nih.gov/genbank/).

Declarations

Consent to participate All authors declare that they agree to participate in this research study.

Conflict of interest No potential conflict of interest was reported by the authors.

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Appendix 1. Detailed information for specimens were used for morphological comparisons.

Darevskia parvula (Lantz & Cyren, 1913) (N = 81)

1. **[parYusufeli]** ZDEU 90/2002. 16(M), 12(F), 30 km SW of Yusufeli, Artvin, Turkey, 06.07.2002, Leg. İ. BARAN, Y. KUMLUTAŞ, Ç. ILGAZ, A. ÖZDEMİR [40°59'4.54"N; 41°24'31.99"E; 1600 m; Map ID: 28]

2. **[parKılıçkaya]** ZDEU 98/2002. 7(M), 8(F), between Kılıçkaya and Karadağ, Artvin, Turkey, 07.07.2002, Leg. İ. BARAN, Y. KUMLUTAŞ, Ç. ILGAZ, A. ÖZDEMİR [40°43'28.81" N; 41°33'5.01" E; 1970 m; Map ID: 27]

3. **[parHatila]** ZDEU 101/2002. 13(M), 10(F), Hatila Valley, Artvin, Turkey, 08.07.2002, Leg. İ. BARAN, Y. KUMLUTAŞ, Ç. ILGAZ, A. ÖZDEMİR [41°10'50.24" N; 41°41'57.68" E; 742 m; Map ID: 29]

4. **[parHorasan]** ZDEU 15/2018. 8(M), 7(F), Hızırilyas Village, Horasan, Erzurum, Turkey, 21.07.2013, Leg. Y. KUMLUTAŞ [40° 7'48.22"N; 42°16'11.08"E; 1766 m; Map ID: 31]

Darevskia adjarica (Darevsky & Eiselt, 1980) (N = 130)

1. **[adjBorçka]** ZDEU 157/2001. 7(M), 5(F), 10 km E of Borçka, Artvin, Turkey, 07.07.2001, Leg. Y. KUMLUTAŞ, K. OLGUN, Ç. ILGAZ, A. AVCI, F. İRET [41°23'57.59"N; 41°44'10.36"E; 427 m; Map ID: 19]

2. **[adjÇermik]** ZDEU 115/2002. 19(M), 16(F), 10 km W of Çermik, Artvin, Turkey, 12.07.2002, Leg. İ. BARAN, Y. KUMLUTAŞ, Ç. ILGAZ, A. ÖZDEMİR [41°18'26.1"N; 41°50'19.6"E; 831 m; Map ID: 21]

3. **[adjOrtacalar]** ZDEU 125/2002. 5(M), 5(F), 24 km W of Ortacalar, Artvin, Turkey, 13.07.2002, Leg. İ. BARAN, Y. KUMLUTAŞ, Ç. ILGAZ, A. ÖZDEMİR [41°17'5.10"N; 41°26'41.72"E; 750 m; Map ID: 9]

4. **[adjIkizdere]** ZDEU 140/2002. 13(M), 15(F), 12 km SE of İkizdere, Rize, Turkey, 06.09.2002, Leg. İ. BARAN, Y. KUMLUTAŞ, Ç. ILGAZ, A. AVCI [40°41'50.9"N; 40°41'06.0"E; 1650 m; Map ID: 5]

5. **[adjÇaikara]** ZDEU 152/2002. 13(M), 16(F), 10 km N of Çaykara, Trabzon, Turkey, 08.09.2002, Leg. İ. BARAN, Y. KUMLUTAŞ, Ç. ILGAZ, A. AVCI [40°40'46.11"N; 40°15'17.14"E; 620 m; Map ID: 2]

6. **[adjAbast]** ZDEU 49/2019. 4(M), 1(F), Abastumani, Georgia, 08.09.2019, Leg. D. TARKHNISHVILI [41°44'50.62"N; 42°50'7.46"E; 1300 m; Map ID: 24]

7. **[adjShuak]** ZDEU 50/2019. 4(M), 7(F), Shuakhevi, Georgia, 08.09.2019, Leg. D. TARKHNISHVILI, [41°37'36.46"N; 42°10'55.95"E; 600 m; Map ID: 23]

Darevskia tuniyevi **sp. nov.** (N = 46)

1. **[tunArdahan]** ZDEU 152/2001. 4(M), 7(F), 19 km W of Ardahan, Turkey, 06.07.2001, Leg. Y. KUMLUTAŞ, K. OLGUN, Ç. ILGAZ, A. AVCI, F. İRET [41°03'43.34"N; 42°30'47.99"E; 1850 m; Map ID: 37]

2. [tunPirnalli] ZDEU 3/2020. 11(M), 6(F), Pırnallı, Artvin, Turkey, 11.09.2020, Leg. M. KURNAZ, [41°15'13.66"N; 42°30'57.16"E; 1258 m; Map ID: 35]

3. [tunÇagliyan] ZDEU 218/2014. 5(M), 3(F), Çağlıyan, Artvin, Turkey, 23.07.2014, Leg. Y. KUMLUTAŞ [41°22'29.44"N; 42°10'24.88"E; 1487 m; Map ID: 38]

4. [tunSavsat] ZDEU 149/2001. 6(M), 4(F), 15 km W of Şavşat, Artvin, Turkey 04.07.2001, Leg. Y. KUMLUTAŞ, K. OLGUN, Ç. ILGAZ, A. AVCI, F. İRET [41°18'7.66"N;42°14'13.07"E; 1200 m; Map ID: 44]

Supplementary Figure Files

A new cryptic species of the *Darevskia parvula* group from NE Anatolia (Squamata, Lacertidae)

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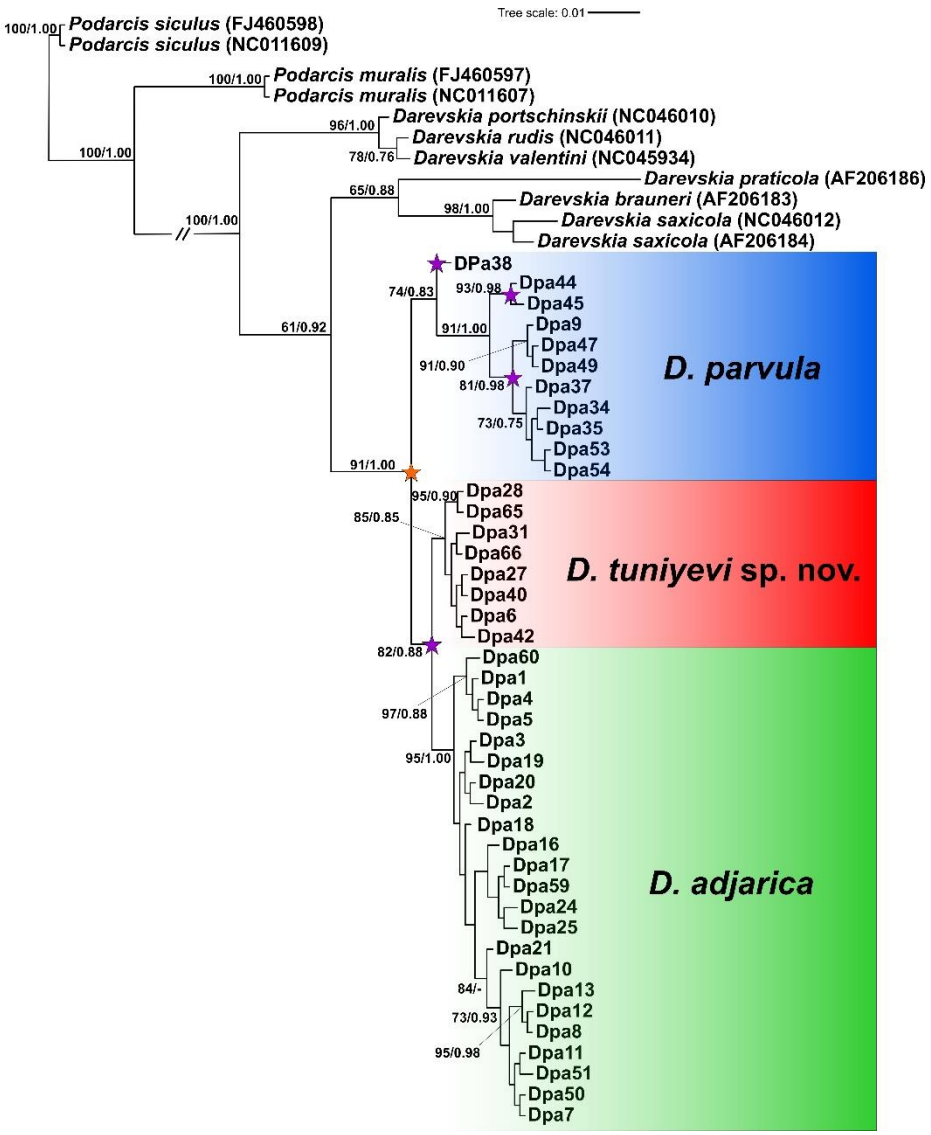

Fig. S1. BI tree reconstructed from the *16S rRNA* dataset. Numbers on branches indicate the bootstrap and posterior probability (pp) values (ML/BI, values lower than 50/0.70 are not shown). Orange star represents species boundaries created by mPTP, while purple ones the results of bPTP.

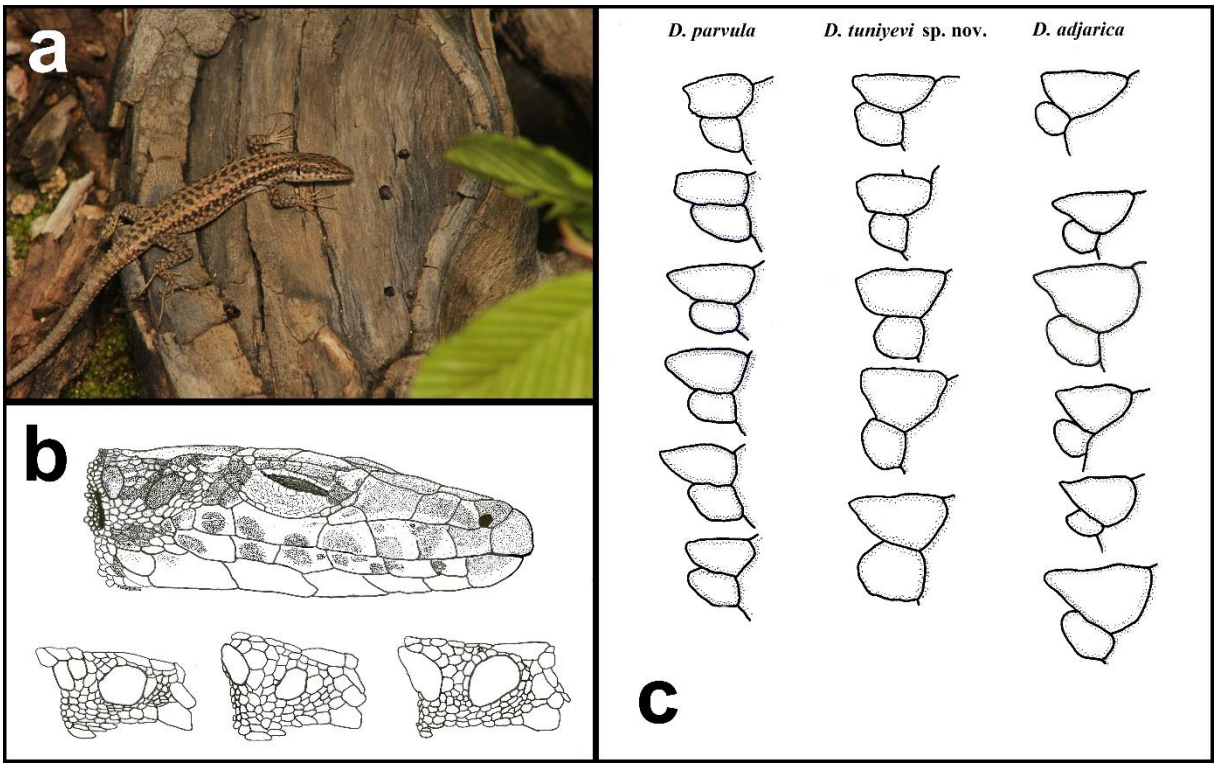

Fig. S2. (a) A living specimen of *Darevskia tuniyevi* **sp. nov.** from Pırnallı, Artvin, Turkey. 11/09/2020. Leg. M. Kurnaz. **(b)** Head profile of the Holotype (represented with closed mouth), ZDEU 3/2020 (n.1). Pırnallı, Artvin, Turkey. 11/09/2020. Leg. M. Kurnaz. (TS1/TS2: 3/2). Head profiles of *D. parvula* (Lantz & Cyrén 1913), *Darevskia adjarica* (Darevsky & Eiselt, 1980), and a *D. tuniyevi* **sp. nov.** (from "9 km West of Ardahan (Turkey)" are represented in Arribas et al. (2018; Fig. 10) (TS1/TS2: 2/3, 2/0, 3/2, respectively). Variability in the temporal area of *D. tuniyevi* **sp. nov.** from (left to right) Pırnallı (Artvin, Turkey), Geçitli (Artvin, Turkey) and Şavşat (Artvin, Turkey) (TS1/TS2: 3/1, 3/2, 3/1, respectively). **(c)** Sixth submaxillary plates of *Darevskia parvula* (left column), *D. tuniyevi* **sp. nov.** (central column) and *D. adjarica* (right column). The first and the later column from Darevsky & Eiselt (1980). *Darevskia tuniyevi* **sp. nov.** (from top to bottom) "between Meydancık and Papart", idem, "Geçitli Village, 19 km W of Ardahan", "Pırnallı Village" and "Çağlıyan" (all from Artvin, Turkey). In the position of this sixth submaxillary, usually appear two plates of different size. In *D. parvula*, the first one is more or less irregularly trapezoidal, and a few greater that the second one (clearly less than its double). In *D. tuniyevi* **sp. nov.** is fairly similar, but the smaller scale is comparatively greater than in *D. parvula*. In *D. adjarica*, the big scale is subtriangular and clearly almost double sized than the smaller one.

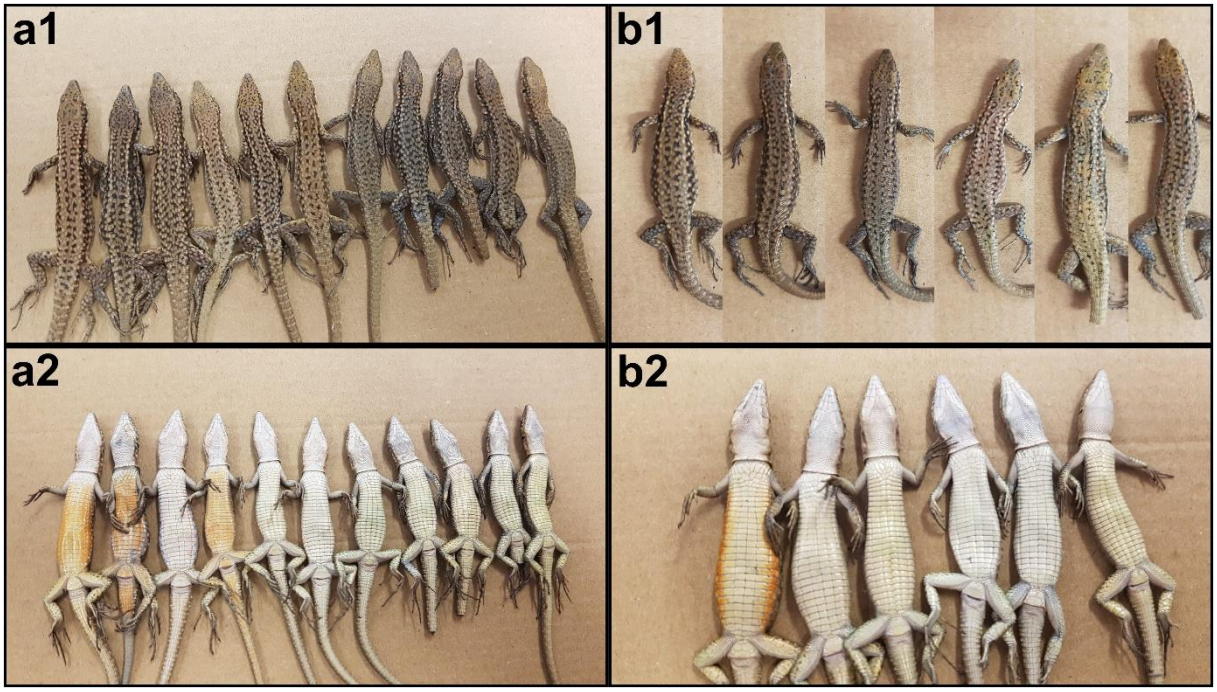

Fig. S3. Freshly euthanized MALES **(a)** and FEMALES **(b)** of *Darevskia tuniyevi* **sp. nov.** from Pırnallı showing color and pattern variability. Specimen at the left is the Holotype, others are paratypes. **(a1-b1)** dorsal view. **(a2-b2)** ventral view. Note that reddish ventral pigmentation fades and disappears towards the end of the summer.

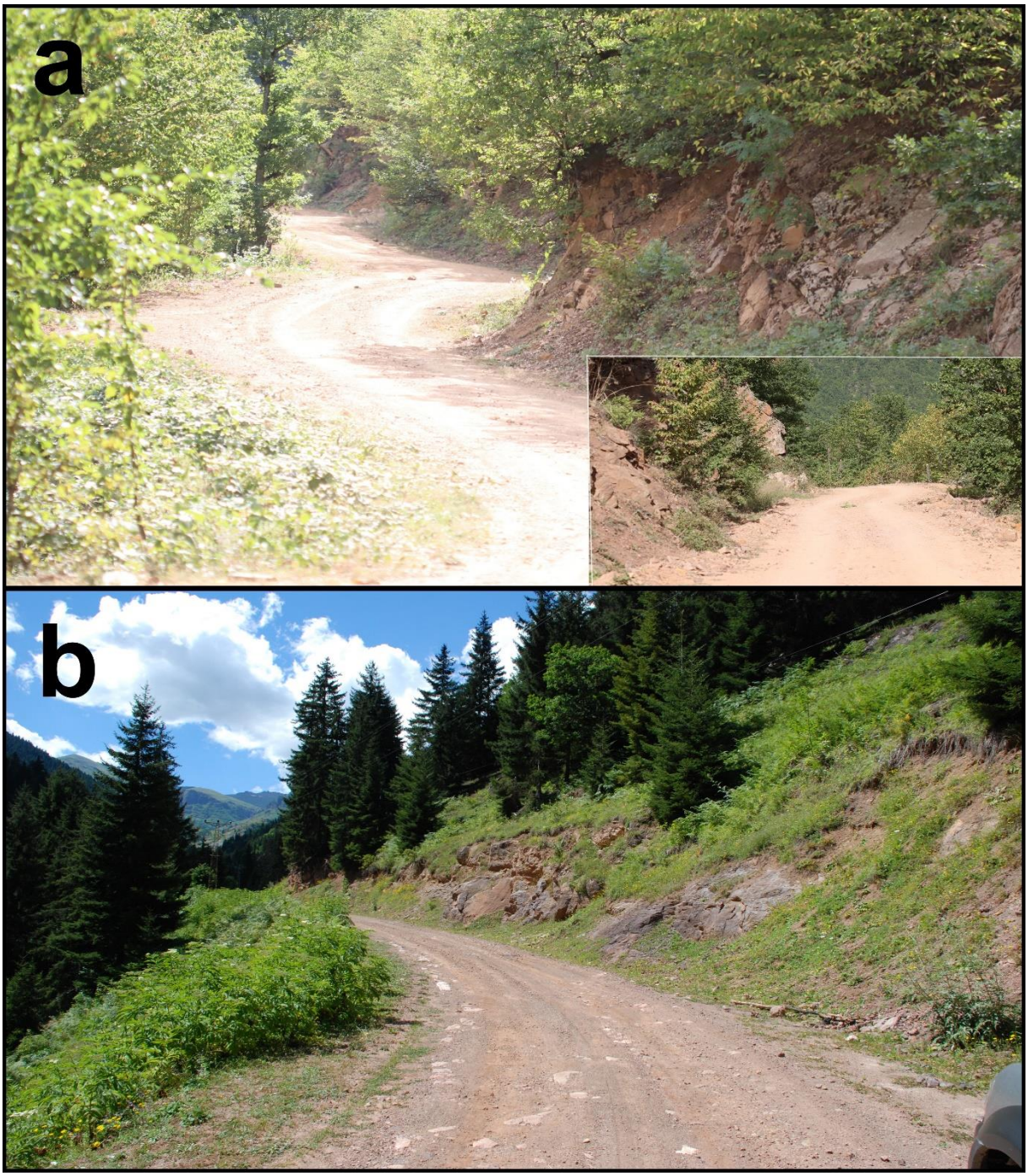

Fig. S4. Habitat of *Darevskia tuniyevi* **sp. nov. (a)** Pırnallı (Artvin, Turkey) forest tracks where the type series was captured. General aspect, substrates and vegetation can be appreciated (see text) in the main photo and the insert. **(b)** Çağlıyan (Artvin, Turkey). Another place where paratype specimens of *D. tuniyevi* **sp. nov.** were collected.

Supplementary Table Files

A new cryptic species of the *Darevskia parvula* group from NE Anatolia (Squamata, Lacertidae)

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Table S1-a. Used primers to amplify each gene sequences.

Primer Codes	Gene Region	Source	References
16SarL 16SbrH	*16S rRNA*	mtDNA	Palumbi et al. (1991)
L15369 H15915	*Cyt-b*	mtDNA	Fu (2000)
RAG-fo RAG-re	*Rag-1*	nDNA	Mayer and Pavlicev (2007)

Palumbi, S. R., and J. Benzie. 1991. Large mitochondrial differences between morphologically similar penaeid shrimp. Molecular Marine Biology and Biotechnology 1: 27-34

Fu, J. (2000). Toward the phylogeny of the family Lacertidae - Why 4708 base pairs of mtDNA sequences cannot draw the picture. Biol. J. Linn. Soc. 71: 203-217

Mayer, W., and M. Pavlicev. 2007. The phylogeny of the family Lacertidae (Reptilia) based on nuclear DNA sequences: convergent adaptations to arid habitats within the subfamily Eremiainae. Molecular Phylogenetics and Evolution 44: 1155-1163.

Table S1-b PCR conditions for each gene region (m: minute; s: second).

Gene Region	Initial Denaturation	Denaturation	Annealing	Extension	Cycle	Final Extension
16S rRNA	3m. 95^0C	30 s. 95^0C	30 s. 52^0C	60 s. 72^0C	35	7 m. 72^0C
Cyt-b	3m. 95^0C	30 s. 95^0C	40 s. 55^0C	45 s. 72^0C	35	5 m. 72^0C
Rag-1	3m. 95^0C	30 s. 95^0C	20 s. 52^0C	45 s. 72^0C	35	7 m. 72^0C

Table S2-a. Descriptive statistics and ANOVA from males. Descriptive statistics of each taxa: mean, standard error, minimum and maximum scores from scalation and biometrical characters and indexes. ANOVA and pairwise comparisons among the taxa (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

MALES	***D. parvula*** (n = 44)	***D. adjarica*** (n = 65)	***D. tuniyevi* sp. nov.** (n = 26)	***F***	***P***	***par-adj 1-2***	***par-tun 1-3***	***adj-tun 2-3***
-SCG Supraciliar granulae	23.80±0.34 20-32	22.10±0.45 8-30	25.73±0.71 22-38	11.99	0.000016	*		***
-SCP Supraciliar plates	12.36±0.09 12-14	11.86±0.11 7-13	12.23±0.18 11-14	4.92	0.008695	***		
-SRL Supralabial plates	8.79±0.12 8-10	7.98±0.04 6-9	8.78±0.15 8-10	26.08	0.000000	***		***
-SL Sublabial scales	12.22±0.07 12-14	12.09±0.08 10-14	12.42±0.14 11-14	2.57	0.080705			
-MG Gular scales	26.72±0.23 23-30	25.4±0.24 21-30	26.5±0.33 23-30	8.18	0.000447	***		*
-Coll Collar scales	8.5±0.13 7-10	8.6±0.09 7-10	8.42±0.18 7-10	0.44	0.647459			
-Sptmp Supratemporal scales	2.65±0.08 2-4	2.96±0.07 2-4	2.42±0.11 2-4	9.77	0.000110	*		***
-TS1 Temp(btw. mas-tymp)	5.27±0.21 2-8	5.15±0.22 2-10	6.65±0.27 4-10	8.54	0.000326		***	***
-TS2 Temp(btw. tymp-par)	5.25±1.15 4-8	4.09±0.13 2-6	4.5±0.2 2-6	16.83	0.000000	***	*	
-TS3 Temp(btw.suprt-mas)	3.15±0.18 0-6	2.67±0.16 0-6	3.46±0.20 2-6	4.16	0.017653			*
-POT Postemporal plates	11.79±0.22 8-14	6.78±0.2 3-10	10.96±0.3 8-14	150.74	0.000000	***		***
-TVP Transv ventral rows	24.93±0.21 23-29	23.26±0.14 21-26	25.46±0.28 22-28	36.00	0.000000	***		***
-PA1 Nr plates before anal	1.84±0.05 1-2	1.66±0.06 1-2	1.84±0.07 1-2	3.07	0.049712			
-PA2 scales around anal pl.	6.27±0.15 4-9	6.41±0.15 5-8	6.73±0.17 5-8	2.12	0.124315			
-FP Nr femoral pores	40.40±0.56 33-50	36.47±0.5 19-45	43.07±0.75 34-49	30.27	0-000000	***	*	***
-LS Scales tight vent. Side	10.11±0.17 8-12	9.10±0.17 6-12	9.30±0.24 6-12	8.15	0.000459	***	*	
-SDL 4th toe lamellae	59.04±0.54 52-66	55.27±0.52 44-66	60.92±0.6 44-66	24.92	0.000000	***		***

-TS Tibial sc.(dorsal face)	15.75±0.21 13-19	15.46±0.16 13-20	16.5±0.17 15-18	5.83	0.003761			***
-DS Scales across midbody	59.02±0.57 52-71	54.47±0.40 47-62	60.23±0.53 56-66	38.46	0.000000	***		***
-SBXTyp Submaxyllary sc. type	1.02±0.02 1-2	2±0 2	1.61±0.09 1-2	231.95	0.000000	***	***	***
-R-I Contact Rost.-Int.	0.09±0.04 0-1	0.4±0.06 0-1	0.03±0.03 0-1	12.04	0.000016	***		***
-Post-Par Contact Postoc.-Par.	0.48±0.07 0-1	0.40±0.06 0-1	0.32±0.09 0-1	0.97	0.382763			
-SVL (Body Length) Snout-Vent legth	53.22±0.59 41.2-59.92	52.52±0.45 45.12-62.28	51.15±0.85 43-58.08	2.38	0.096968			
-PLI (Pileus Index) Pileus Length/width	1.94±0.01 1.77-2.2	1.89±0.01 1.72-2.12	2.00±0.01 1.81-2.14	15.23	0.000001	*	**	***
-HWI (Head Index) Head length/width	1.79±0.01 1.65-1.91	1.71±0.01 1.48-1.9	1.84±0.01 1.71-2.0	30.34	0.000000	***	*	***

Table S2-b. Descriptive statistics and ANOVA from females. Descriptive statistics of each taxa: mean, standard error, minimum and maximum scores from scalation and biometrical characters and indexes. ANOVA and pairwise comparisons among the taxa (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

FEMALES	***D. parvula*** (n = 37)	***D. adjarica*** (n = 65)	***D. tuniyevi* sp. nov.** (n = 20)	***F***	***P***	***par-adj 1-2***	***par-tun 1-3***	***adj-tun 2-3***
-SCG Supraciliar granulae	23.64±0.3 21-28	22.46±0.31 17-28	24.4±0.87 19-35	5.20	0.006846			*
-SCP Supraciliar plates	12.10±0.1 10-14	12.01±0.12 8-14	11.95±0.22 10-13	0.22	0.803437			
-SRL Supralabial plates	9.0±0.14 8-10	7.96±0.04 7-9	8.7±0.20 8-10	31.02	0.000000	***		***
-SL Sublabial scales	12.08±0.09 11-14	12.04±0.07 10-14	12.3±0.16 12-14	1.36	0.260684			
-MG Gular scales	27.45±0.34 24-31	25.4±0.21 21-29	26.85±0.3 24-29	17.35	0.000000	***		**
-Coll Collar scales	8.62±0.14 7-11	8.63±0.13 6-11	8.35±0.23 6-11	0.64	0.530600			
-Sptmp Supratemporal scales	2.43±0.08 2-3	2.96±0.07 2-4	2.6±0.13 2-4	10.91	0.000045	***		*
-TS1 Temp(btw. mas-tymp)	5.51±0.25 2-8	5.66±0.23 2-10	6.65±0.31 4-8	3.14	0.047059			
-TS2 Temp(btw. tymp-par)	5.45±0.21 3-8	3.89±0.16 2-10	4.4±0.19 3-6	17.94	0.000000	***	**	
-TS3 Temp(btw.suprt-mas)	3.29±0.22 1-6	2.83±0.20 0-8	3.55±0.18 2-4	2.35	0.099620			
-POT Postemporal plates	11.83±0.28 8-16	7.15±0.19 4-13	10.3±0.46 8-13	93.44	0.000000	***	**	***
-TVP Transv ventral rows	26.89±0.26 23-30	25.50±0.14 23-27	27.55±0.25 26-30	24.18	0.000000	***		***
-PA1 Nr plates before anal	1.94±0.03 1-2	1.73±0.05 1-2	1.95±0.05 1-2	5.12	0.007341	*		
-PA2 scales around anal pl.	6.32±0.11 5-8	6.53±0.11 4-8	6.7±0.20 6-8	1.31	0.273281			
-FP Nr femoral pores	34.67±0.43 34-46	36.93±0.38 28-44	41.2±0.91 36-49	17.93	0.000000	***		***
-LS Scales tight vent. Side	9,75±0.23 8-12	9.41±0.15 6-12	8.8±0.22 8-10	3.69	0.027951		*	
-SDL 4th toe lamellae	58.81±0.8 47-70	54.53±0.46 48-62	59.65±0.45 56-64	20.81	0.000000	***		***

-TS Tibial sc.(dorsal face)	15.48±0.18 14-17	15.35±0.14 13-19	15.95±0.29 13-18	1.94	0.148724			
-DS Scales across midbody	58.45±0.56 54-66	53.92±0.35 47-51	59.05±0.76 53-67	35.14	0.000000	***		***
-SBXTyp Submaxyllary sc. type	1.10±0.05 1-2	1.95±0.02 1-2	1.45±0.11 1-2	91.58	0.000000	***	***	***
-R-I Contact Rost.-Int.	0.10±0.05 0-1	0.30±0.05 0-1	0.15±0.08 0-1	3.16	0.045928			
-Post-Par Contact Postoc.-Par.	0.33±0.07 0-1	0.37±0.06 0-1	0.15±0.07 0-1	2.00	0.139923			
-SVL (Body Length) Snout-Vent length	50.45±0.69 41.66-56.6	50.69±0.50 41.52-60.92	48.68±0.68 43.18-55.06	2.06	0.131898			
-PLI (Pileus Index) Pileus Length/width	1.97±0.01 1.77-2.39	1.87±0.01 1.57-2.32	1.98±0.02 1.84-2.16	12.72	0.000010	***		***
-HWI (Head Index) Head length/width	1.82±0.01 1.68-1.95	1.71±0.01 1.45-1.93	1.84±0.01 1.73-2.02	29.10	0.000000	***		***

Table S3. Sequence divergences, as % uncorrected genetic distance values (p-distances), among the main clades of *D. parvula*-group for mitochondrial *16S rRNA*/*Cyt-b* (below diagonal) and nuclear *Rag-1* (above diagonal). Values in diagonal are genetic divergences within each clade (*16S rRNA*/*Cyt-b*/*Rag-1*).

Main Clades	1	2	3
1. *D. parvula*	**0.8/1.2/0.2**	0.4	0.3
2. *D. adjarica*	2.6/9.0	**0.5/2.2/0.2**	0.1
3. *D. tuniyevi* **sp. nov.**	2.4/10.4	0.8/4.5	**0.2/0.4/0.0**