



## Multivariate analysis of geographic variation in *Darevskia clarkorum* (Darevsky & Vedmederja, 1977), correlation with geographic and climatic parameters, and true status of *Darevskia dryada* (Darevsky & Tuniyev, 1997)

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### Abstract

All the Turkish populations studied, both those previously assigned to *D. dryada* (Subaşı and Yoldere villages, near Hopa) and those attributed to *D. clarkorum* (the largest sample studied so far, 177 specimens in total), are indistinguishable from each other and therefore must all be ascribed to the natural variability of a monotypic *D. clarkorum*. The Georgian specimens from the Type Locality of *D. dryada* (Charnaly river gorge, Chevachauri district) are clearly different, so that taxon cannot be considered a simple synonym for *D. clarkorum*, but as a valid taxon, although its proper status (more probably as a subspecies of *D. clarkorum*), is yet to be clarified. It is a highly threatened population, so studies should be done in vivo or with as low intrusiveness as possible.

*Darevskia dryada* is clearly larger (SVL) than any *D. clarkorum* studied, with strongly longer heads and pilei in adult males (and hence more teeth in dentary bone), and higher dorsalia counts. There also seem to be (but need to be studied in a larger sample) more longitudinal rows of temporal scales between tympanic and parietal plates, a tendency to have more supralabial scales; comparatively smaller values for longitudinal rows of scales on the ventral surface of the thigh between the femoral pores and the outer row of enlarged scales, and higher collaria, and circumanalia scales. Other differences in femoralia and gularia are also reflected in Darevsky & Tuniyev's (1997) tables and should also be investigated with more Georgian specimens.

Two supposed discriminant characters, the frontonasal index and the presence of developed masseteric, are not valid. The frontonasal index does not discriminate both taxa; *D. dryada* specimens fall inside the variation of *D. clarkorum* for this character. Also the presence of a developed masseteric plate is supposed to be rare if at all in *D. clarkorum* but always present in *D. dryada*; however, it appears in nearly 75% of *D. clarkorum* studied and in all *D. dryada*, so is also no longer valid for taxa discrimination.

Although very similar, *D. clarkorum* and *D. dryada* are morphologically different, and genetic studies (as the unpublished results mentioned by Fu, 1999) do not make the provenance of the specimens clear, and hence the correct identification of the supposed specimens of *D. dryada* used.

There are no geographical clines in *D. clarkorum*. However, as stated by Schmidtler et al. (2002), there is an inverse relationship between altitude and dorsalia values in *D. clarkorum*. Both the general differentiation between populations and the scalation (dorsalia) appear statistically correlated with the altitude and also with latitude (being both factors not strictly the same). The correlation seems to be stronger with morphology in general (multiple scalation characters and head biometry) than only with dorsalia. In the case of the general differentiation among samples, it is also significantly correlated with temperatures during the activity period (April-September) and with precipitation during incubation (July-August). As these climatic parameters of temperature and precipitation are not directly correlated with the dorsalia variation, the relation with altitude (and perhaps latitude) must be linked to some other climatic parameter not studied here, perhaps solar radiation or evapotranspiration.

**Key words:** *Darevskia clarkorum*, *D. dryada*, Caucasian Rock Lizards, distribution, morphometry, meristic scalation characters, Multivariate analyses, CDA, PCA, ANOSIM, MST, UPGMA, osteology, northeastern Anatolia, Adzharia, Turkey, Georgia

## INTRODUCTION

*Darevskia clarkorum* was described under the name of *Lacerta clarkorum* by Darevsky and Vedmederja (1977) in honour of the first collectors, Richard and Erica Clark. These authors based their study on three specimens collected 20 km west of Borçka (Artvin, Turkey), which were previously identified as *Lacerta saxicola* [= *Darevskia saxicola*] (Clark & Clark, 1973), as well as four other specimens captured in adjacent parts of nearby Adzharia (Georgia). *Darevskia clarkorum* is currently known from southwest Georgia (Adzharia) and part of northeastern Turkey, from Artvin to Giresun (Darevsky and Vedmederja, 1977; Engelmann et al, 1993; Darevsky and Tuniyev, 1997; Baran and Atatür, 1998; Sindaco et al., 2000). Considered as very rare and threatened, Franzen (1991) published the observation of the occasional intake of plant foods (elderberry and carmine herb), although clarifying that they prefer mainly animal food.

Twenty years later, Darevsky and Tuniyev (1997) described a second very similar species from Adzharia (Charnaly River gorge, in Georgia) and Subashi-kaj (= Subaşı Village) (near Hopa, Turkey), under the name of *Lacerta dryada* (= *Darevskia dryada*). The authors split this species from *D. clarkorum*, although the diagnosis certainly did not specify too much: "... some metric and meristic characters of pholidosis, in particular by the scutellation of the temporal head area", especially the body size is emphasized to be really bigger (shown in the photos of the original publication) and a frontonasal scale longer than wide (while in *D. clarkorum* it is clearly shorter than wide, although this important feature is only mentioned at the bottom of Fig 1; by Darevsky & Tuniyev, 1997). They also indicated that the developed masseteric scale is often present in *D. dryada* and frequently absent in *D. clarkorum*. Observing the table of Darevsky & Tuniyev (1997), significant differences between the two species were observed in dorsalia, dorsalia / 10 ventral scales, femoral pores, longitudinal and transverse gularia and tibialia. It should be noted that the characteristics of *D. clarkorum* in the original description (Darevsky & Vedmederja, 1977) were "contaminated" by the presence of specimens of *D. dryada* in the sample, so the characteristics of *D. clarkorum* s. str. are more accurate in the description of *D. dryada* (Darevsky and Tuniyev, 1997), where both species are distinguished. The new species extends from 50 to 300 m, while *D. clarkorum* inhabits 900 to 1700 m asl.

Arribas (1999a) described the genus *Darevskia* based on morphological characteristics, osteological, cariological and behavioural features, integrating both species.

Fu (1999) in a complete study of bisexual and unisexual *Darevskia*, said that: "The DNA data do not support the species status of *Lacerta dryada* (Darevsky and Tuniyev, 1997)". Three of his five specimens of *D. clarkorum* were "from the geographic range of *L. dryada*" [sic.!, from Tskali Gorge, Georgia, the other two were from Mahden-Maden-, Turkey, attributed to true *D. clarkorum* by him], and morphologically they also resemble [sic.!] the diagnostic characters of *D. dryada*". However, two of the three supposed *D. dryada* (Tskali Gorge remains untraceable and the comment "also resembles the diagnostic characters" would have not been included if they were from the type locality) shared identical ATPase 6 sequence with two specimens of their *D. clarkorum*. The other specimen was one bp different. By all this, Fu considered the species status of *D. dryada* questionable, and also mentioned an unpublished allozyme study that did not detect any fixed alleles between the two proposed species (MacCulloch, pers. comm., in Fu, 1999).

Murphy et al. (2000) classified 15 species of *Darevskia* into 3 main clades using mtDNA and alloenzymes that they called: clade-*caucasica*, clade-*saxicola* and clade-*rudis*, respectively. According to that study, *D. clarkorum* would be included in the clade-*caucasica*, as a sister taxon of *D. mixta* (Mehely, 1909). Kupriyanova & Odierna (2002) also included *D. clarkorum* in the *caucasica*-clade, following Murphy et al (2000).

Panner (2001) conducted a study on the reproductive biology of *D. clarkorum* in a terrarium, giving the first data on its reproduction (clutches from two to five eggs).

Schmidtler et al. (2002) reviewed a good sample of *D. clarkorum* (75 specimens from several Turkish locations and three specimens of *D. dryada* from Georgia) captured between 250 and 1750 m asl, by uni- and multivariate methods. They divided the sample into two groups, a low altitude population (which would coincide with *D. dryada*) and a higher altitude population (assimilable to *D. clarkorum*), while the samples from intermediate elevations would represent the link between both groups, although somewhat more similar to those from higher altitudes. For these authors, the key was in the relationship between climate and elevation, with lower altitude populations having small-sized scales but higher scale counts (as dorsalia). Schmidtler et al. (2002) used a statistical method that was not very appropriate to discriminate samples; PCA (Principal Components Analysis) does not look for differences between samples but takes all together and treats each individual separately, so in practice specimens from two

similar taxa may be closer than one conspecific of a different size or with a more extreme scalation value. The study unit in PCA is the specimen, not the population, and that makes it difficult to draw conclusions about their differentiability.

Ciobanu et al. (2002), studying CLsat Tandem Repeats (and with 145-146 bp), found no differences between their samples of *D. clarkorum* and *D. dryada* (both badly spelled as “driada” and “clarcorum”, but without informing about their localities of origin). Ciobanu et al. (2003 and 2004) specified that their samples of *D. clarkorum* come from “Magden” (sic., Maden, Tk) and *D. dryada* from “Girgami” (Adzharia, Ge), a locality also untraceable. In the first paper, Ciobanu et al. (2003) mentions that *D. dryada* dwells in lowlands (up to 300 m above sea level), and its population is small and depressed (very low numbers), whereas *D. clarkorum* inhabits mountainous regions up to 1700 m asl and is currently undergoing an explosive population growth (sic.); its numbers being approximately 50 times higher than that of *D. dryada*. Both species plus *D. mixta* differ only by between zero and two nucleotides in the CLsatI and CLsatII monomer sequences, with exceptionally high similarity, explained by these authors by their very recent separation [“It would be reasonable to consider these three species (*dryada*, *clarkorum*, and *mixta*) as morphs of a single ancient species, since they in essence do not differ genetically”].

Bischoff & Tarkhnishvili (2002) and Bischoff (2003) treated the Charnaly River lizards as *D. clarkorum* and mentioned a curious biological fact; they do not start their activity until about 11 am in a very hot environment, while other sympatric species are already active two hours before this. They also cited *D. clarkorum* from Mt. Mtirala, about 600 m asl. Bischoff (2003) indicated that the species (*D. clarkorum*) is less scarce than previously thought and is not in danger of extinction. In its type locality, *Darevskia dryada* lives in very humid areas covered with dense forest, with large boulders and tree stumps, always close to water (maximum 50 m away).

Bosch & Bischoff (2004) accepted the proposal of synonymy from Schmidtler et al. (2002) and commented that: “it is astounding that this form, described from the Charnaly river valley just northeast northeast (sic.! south-east) of Batumi on the Black Sea coast, could have been considered to be different as there is absolutely no biological barrier between this area and the terra typica of *D. clarkorum* just 20 km to the south in Turkey (Cankurtaran pass). All the more surprising, since Darevsky was involved in the description of both forms.” However, all these commentaries are opinions and these authors do not provide a specific study in their paper. In addition, the fact that both type localities are very close and there are no barriers between them is a circular argument, since there may be several sympatric *Darevskia* and hence there could be several types described even from the same locality. As mentioned by Tarkhnishvili (2012) “up to four species can coexist at a single rock face, and up to eight species may occur within the area of 10 km<sup>2</sup>”, although strict syntopy usually correspond to species from different groups within *Darevskia*. With a better reformulation of the surprise of Bosch & Bischoff (2004), the interesting question here is to ask why Darevsky saw that there were clearly different types even being so close and similar.

According to Brown’s (2005) study based on 16S rDNA, *D. clarkorum* appeared more closely related to a sample of *D. derjugini* than to *D. mixta*, *D. caucasica* (Mehely, 1909) and *D. daghestanica* (Darevsky, 196), with which it formed a group.

Grechko et al. (2006) also used the same samples as Ciobanu et al. (2003) (see above for doubts about the *D. dryada* origin) and confirmed that *mixta*+*clarkorum*+*dryada* belong to the same group (the *mixta*-clade in their scheme) and shared 3 diagnostic substitutions in CLsatI. Intragroup differentiation in the CLsatII (*mixta*+*dryada*+*clarkorum*) is small (between 0–1%). *Darevskia dryada* and *D. clarkorum* differed in 1 bp in the CLsatII, and about 3% in CLsatIII. But it was also said that they were identical and not distinguishable (its presence in *D. clarkorum* was only corroborated by DNA hybridization but it was not sequenced). In relation to the relative content of every CLsat family, *D. dryada* has less CLsatI than *D. clarkorum*, but strikingly much more CLsatII and CLsatIII.

Ilgaz (2007) included the largest sample of *D. clarkorum* studied to date (103 specimens) with univariate methods, providing new information about its distribution, morphology and taxonomy. Kurnaz & Kutrup (2018) cited the species from a high area (1730 m asl) that is incidentally also the most southern area known. Finally, Altunışık & Eksilmez (2018) studied the demography (age structure) of Turkish *D. dryada* from two nearby locations, one of them the only Turkish population included in the original description of the species (“Subashi-Kaj” = Subaşı vilage), and estimated that these lizards attain sexual maturity at the age of one or two years and the potential reproductive life span for males and females is six and five years, respectively.

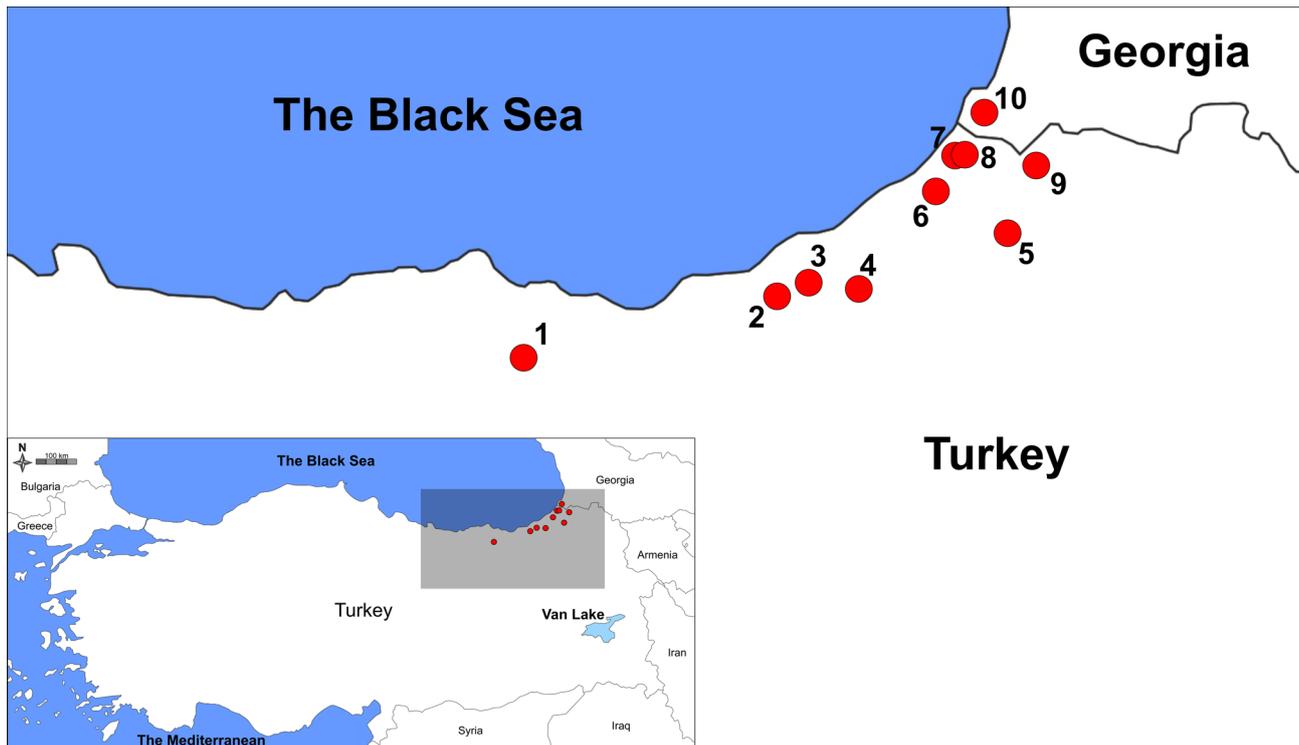
In the present study, a large number of specimens belonging to rock lizards of the *D. clarkorum*/*D. dryada* complex from northeastern Anatolia, Turkey were evaluated using morphological and osteological data and their taxonomic status was assessed.

## MATERIAL AND METHODS

### Morphology

A total of 180 specimens (177 specimens from Turkey, plus 3 from Georgia) were studied, detailed as follows (locality numbers are the clustered samples represented in Fig. 1):

Turkish specimens were collected from different localities in 2001, 2002 and 2014. Turkish specimens were incorporated into the collection of ZDEU (Zoology Department of Ege University) and are kept in the Zoology Lab of the Department of Biology at Faculty of Science, Buca, İzmir, Turkey. For analyses (only adults, as a few juveniles were excluded to avoid allometry changes), 171 specimens were finally used (92 males and 79 females), the largest sample studied so far [75 specimens in Schmidtler et al. (2002) and 103 in Ilgaz, (2007)].



**FIGURE 1.** Map showing the localities from where specimens have been studied. **1.** Sümela, Maçka, Trabzon, northeastern Anatolia, Turkey, **2.** Çataldere, Kaptanpaşa, Rize, northeastern Anatolia, Turkey, **3.** 11 km southwest of Hemşin, Rize, northeastern Anatolia, Turkey, **4.** Ayder Plateau, Çamlıhemşin, Rize, northeastern Anatolia, Turkey, **5.** Hatila Plateau, Artvin, northeastern Anatolia, Turkey, **6.** 16 km northeast of Ortacalar, Arhavi, Artvin, northeastern Anatolia, Turkey, **7.** Subaşı Village, Hopa, Artvin, northeastern Anatolia, Turkey, **8.** Cankurtaran, Hopa, Artvin, northeastern Anatolia, Turkey, **9.** Karagöl, Borçka, Artvin, northeastern Anatolia, Turkey, **10.** Charnaly, Georgia

### Specimens studied

Repeated numbers (a, b, c) are small samples geographically close that have been grouped for statistical treatment.

**1)** 1 M, 9 F.—Sümela, Maçka, Trabzon, Northeastern Anatolia (Turkey), 05/08/2014, 1300 m, (n° 58/2014), [40°41'08.1", 39°39'30.9"].

**2 a)** 12 M, 8 F.—Çataldere Village, Kaptanpaşa, Rize, Northeastern Anatolia (Turkey), 05/09/2002, 600 m, (n° 139/2002), [40°56'05.5", 40°46'33.3"].

**2 b)** 8 M, 2 F, 1 Juv.—Çataldere Village, Kaptanpaşa, Rize, Northeastern Anatolia (Turkey), 04/06/2019, 600 m, (n° 47/2019), [40°56'05.5", 40°46'33.3"].

**3)** 16 M, 14 F, 2 Juvs.- 11 km southwest of Hemşin, Rize, Northeastern Anatolia (Turkey), 07/09/2002, 500 m (n° 148/2002), [40°57'38.14", 40°51'47.99"].

**4 a)** 6 M, 6 F.—Ayder Plateau, Çamlıhemşin, Rize, Northeastern Anatolia (Turkey), 08/07/2001, 1330 m, (n°

164/2001), [40°57'11.45", 41° 6'1.42"].

**4 b)** 9 M, 6 F.—Ayder Plateau, Çamlıhemşin, Rize, Northeastern Anatolia (Turkey), 04/06/2019, 1330 m, (n° 46/2019), [40°57'11.45", 41° 6'1.42"].

**5)** 7 M, 6 F.—Hatila Plateau, Artvin, Northeastern Anatolia (Turkey), 09/06/2008, 1450 m, (n° 96/2019), [41°09'27.59", 41°46'08.01"].

**6)** 8 M, 13 F, 1 Juv.—16 km northeast of Ortacalar, Artvin, Northeastern Anatolia (Turkey), 13/07/2002, 750 m, (n° 126/2002), [41°16'48.05", 41°26'13.67"].

**7 a)** 2 M, 1 F.—Subaşı Village, Hopa, Artvin, Northeastern Anatolia (Turkey); 02/06/2019, 190 m, (n° 26/2019), [41°23'08.1', 41°30'04.1"].

**7 b)** 6 M, 5 F.—Yoldere Village, Hopa, Artvin, Northeastern Anatolia (Turkey), 02/06/2019, 80 m, (n° 27/2019), [41°23'38.7", 41°28'17.6"].

**8 a)** 4 M, 4 F.—Cankurtaran Pass, between Hopa and Borçka 14. km, Artvin, Northeastern Anatolia (Turkey), 23/07/2015, 685 m, (n° 23/2015), [41°23'31.5", 41°32'14.0"].

**8 b)** 3 M.—Cankurtaran Pass, between Hopa and Borçka 14. km, Artvin, Northeastern Anatolia (Turkey), 02/06/2019, 685 m, (n°62/2019), [41°23'31.5", 41°32'14.0"].

**8 c)** 2 M.—8 km west of Borçka, Artvin, Northeastern Anatolia (Turkey), 12/07/2002, 550 m, (n° 121/2002), [41°22'58.2", 41°34'08.2"].

**9)** 6 M, 4 F, 5 Juvs.—Karagöl, Borçka, Artvin, Northeastern Anatolia (Turkey), 04/09/2002, 850 m, (n° 135/2002), [41°23'13.72", 41°51'13.72"].

**10)** 2 M, 1 F.—Charnaly River Gorge, Chelvachauri District, Adjara (W Georgia), 25/6/1994 (OA94062501-03), [41°34'14.69', 41°37'06.75"].

## Characters studied

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), posttemporal 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 4<sup>th</sup> 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") for the multivariate analyses. Rostral-Internasal (R-I) and Postocular-Parietal (POST-PAR) contacts were also examined and codified as 0 (no contact), 0.5 (contact only at one point or on one side) and 1 (contact).

Also frontonasal scale proportions were measured from a limited series of 26 Turkish specimens (13 Turkish *D. dryada* and 13 *D. clarkorum*) and 3 *D. dryada* from Georgia (from the type locality of *D. dryada*, the Charnaly River Gorge) to check for a concrete diagnostic character mentioned in Darevsky & Tuniyev (1997; Fig 1), the shape of the frontonasal scale.

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 Turkish males and females, and later for the whole sample (including Georgian ones) excluding the sexual dimorphic characters.

## Statistical analyses

### Multivariate techniques

The statistical analysis used in the morphological study was Canonical Discriminant Analysis (CDA) (Sokal & Rohlf 1995; Blackith & Reyment 1971; Legendre & Legendre 1998, and online help in the statistical programs used; see below). In this analysis, all specimens appear graphically displayed, but each population can be represented by a centroid (a hypothetical middle individual) from which UPGMA and Minimum-Length Spanning Tree (MST) computed from the Mahalanobis' distance matrix among these centroids can be calculated. MST (not represented graphically) helps to detect the nearest neighbours based on their position in the multidimensional space and also avoids a distortion of trees by the reciprocal pairwise distance recalculation at every stage during the construction of UPGMA trees. The cophenetic correlation coefficient was calculated to ascertain the goodness of fit of the UPGMA aggrupation-derived matrix of cophenetic (ultrametric) distances to the original distance matrix (Rohlf, 2000).

We tested the significance of the differences among pre-established groups (the different samples) by conducting an Analysis of Similarity (ANOSIM) (Clarke 1988, 1993), which tests whether the assigned groups are meaningful (i.e. whether they are more similar within groups than with samples from different groups); see more details and examples of its use in taxonomy in Arribas (2010) and Arribas et al. (2013, 2014). We checked for significance by running resampling tests (1,000 randomizations) to test whether the given results could occur by chance. If the value of R was significant, this was evidence that the samples within groups were more similar than would be expected by random chance. Furthermore pair-wise tests among populations allowed testing of the significance of the differences among the concerned groups and detection of which ones were really different from the others.

Particular characters, the measurement of the length and width of the frontonasal (or internasal) scale were taken and converted to an index (length/width) to test one of the diagnostic features suggested by Darevsky & Tuniyev (1997; Fig 1). It was measured in 12 Turkish *D. dryada*, three *D. dryada* from the Charnaly River (Georgia, type locality) and 12 specimens of *D. clarkorum* from various locations included in this study (including the type locality of the species). Also, special attention was paid to the presence or absence of a differentiated masseteric plate, an alleged diagnostic character in Darevsky & Tuniyev (1997).

A Factor Analysis (FA) (not represented graphically) was completed to identify what scalation characters varied in parallel, as in Arribas (1999b), and what specific character best reflected the variation in this scalation number. This character was found to be DS, the number of dorsal scales (higher correlation with the first factor, reflecting general body scalation). A matrix of dissimilitude of the variation in this character (DS) among populations was used later to compare with matrices of dissimilitude in some climatic and geographic parameters, in order to determine if there is climatic parallel variation, as suggested by Schmidlter et al. (2002).

Mahalanobis' (squared) distance matrices were compared using a Mantel Test (with 1,000 permutations) (Mantel, 1967; Sokal 1979) with the following matrices constructed using Euclidean (squared) distances: 1. Temperatures during the activity period (April to September); 2. Temperature during lizard egg incubation (July and August); 3. Precipitation during activity period (April to September); 4. Precipitation during incubation period (July and August); 5. Aerial (straight line) geographical distances; 6. Altitude of the sample localities; and 7. Distance to the Black Sea coast. Climatic data (precipitation and temperatures) were extracted from Steinhauser (1970). In some cases, the Smouse et al. (1986) test was used to calculate the correlation among two matrices, excluding the effect of a third one.

Dorsalia average values of each sample were compared with their relative position in geographic longitude and latitude by means of the Spearman Rank Correlation coefficient (Sokal & Rohlf 1995) to detect possible clinal variations.

Multivariate analyses (CDA and ANOSIM) were performed with Community Analysis Package 6.0<sup>®</sup> (Seaby & Henderson, 2019). UPGMA, MST and Mantel tests were calculated with NTSYSpc 2.1<sup>©</sup> (Rohlf, 2000). Descriptive statistics, correlations and the preliminary Factor Analysis (not shown) were processed with NCSS 2001<sup>®</sup> package (Hintze 2007).

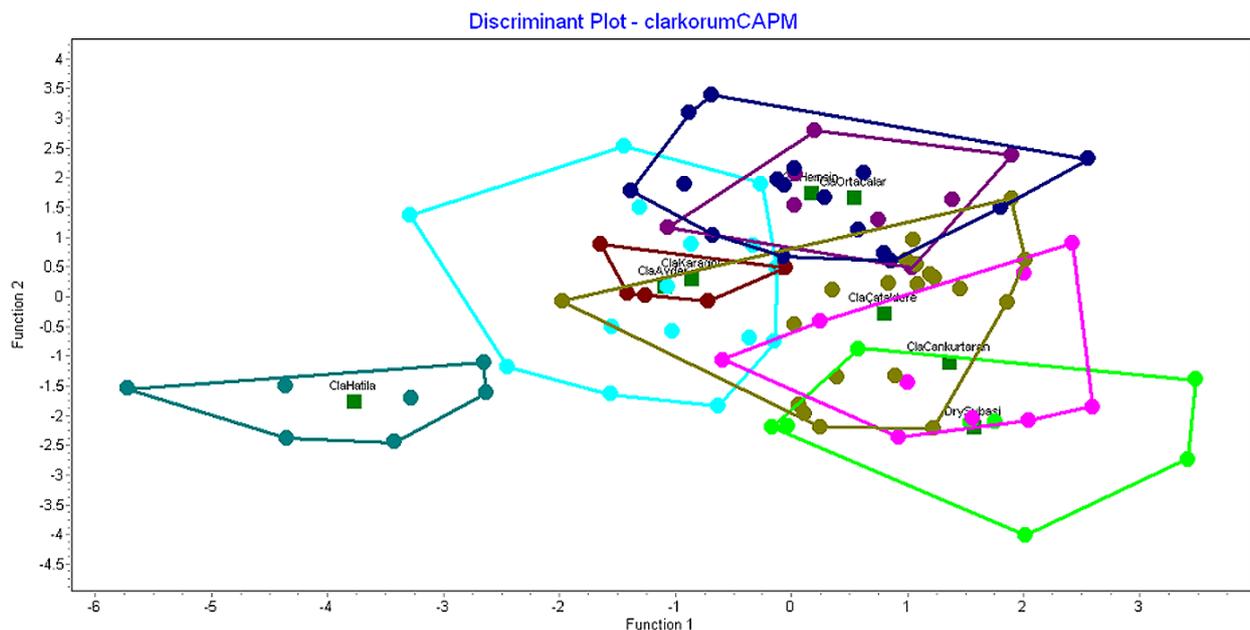
## Osteological study

Alcohol preserved specimens were cleaned with 1% KOH in deionized water and bones stained with alizarin red. They were posteriorly differentiated, and the excess of pigment eliminated with Mall solution (80% of the previous cleaning solution plus 20% glycerol), and preserved permanently in glycerol following the procedures of Taylor (1967) and Durfort (1978). Osteological nomenclature follows Arribas (1998). Information about the studied specimens and their localities is given in Appendix I.

## RESULTS

### Males Analysis

**Canonical Discriminant Analysis (Males from Turkey).** Discriminant Analysis (CDA) maximizes the discrimination of the samples between the first two significant axes (Eigenvalues greater than one; Chi square 310.66 [ $P = 0.00000025$ ] and 228.22 [0.00000018] for the first and second axes, respectively). The bidimensional plot (see Fig. 2) is a plane defined by the first axis (Eigenvalue 2.14; 31.1% of the variability explained) and the second axis (1.79; 25% explained) that separates the samples, but with great overlap between them. Both axes together explain 56% of the total variability. Wilks Lambda is 0.0133 ( $F_{161} = 2.27$ ,  $P = 2.29 \cdot 10^{-11}$ ). As  $P < 0.05$ , this demonstrates the existence of significant differences among the samples analyzed. Bartlett's Sphericity Tests further proved that the variables were uncorrelated, a prerequisite for successful discriminant analysis (Bartlett's Chi-Squared Test Statistic = 312.82, 161 degrees freedom;  $P = 2.10^{-7}$ ).



**FIGURE 2.** Canonical Discriminant Analysis (CDA) plots for males. Specimens, sample centroids and group perimeters are represented. For details, percentage of variance explained, and other data, see text.

As shown in Fig. 2, the first axis (31.1% of variability explained) separates Hatila males in the negative part, characterized by greater values of POST-PAR (-0.593255, in contact in all the specimens) and TS2 (-0.438039) and smaller SVL (0.570458), TS1 (0.383353) and SRL (0.374612). The second axis (25% of variability explained) does not discriminate any population from the rest. Its negative part appears to include the Turkish “*dryada*”, characterized by higher values of TS2 (-0.660798), PI (-0.499682) and MASSET (-0.404006), and smaller values of LS (0.602008).

However, observing these two first axes, except Hatila, the remaining samples overlap widely. Visually, there is no relationship between the positions of the sample centroids and the altitude (Ayder at 1330 m asl appears in

the middle of the plot) or their geographic position (the most different one, Hatila, is close to others which are not differentiated and the geographically more distant Çataldere, Ayder and Hemşin remain in the middle of the undifferentiated ones).

This CDA warrants the discrimination of 80.9% of the specimens. The best discriminated population is Cankurtaran (88.8%), whereas the worst one is Hemşin (68.7%).

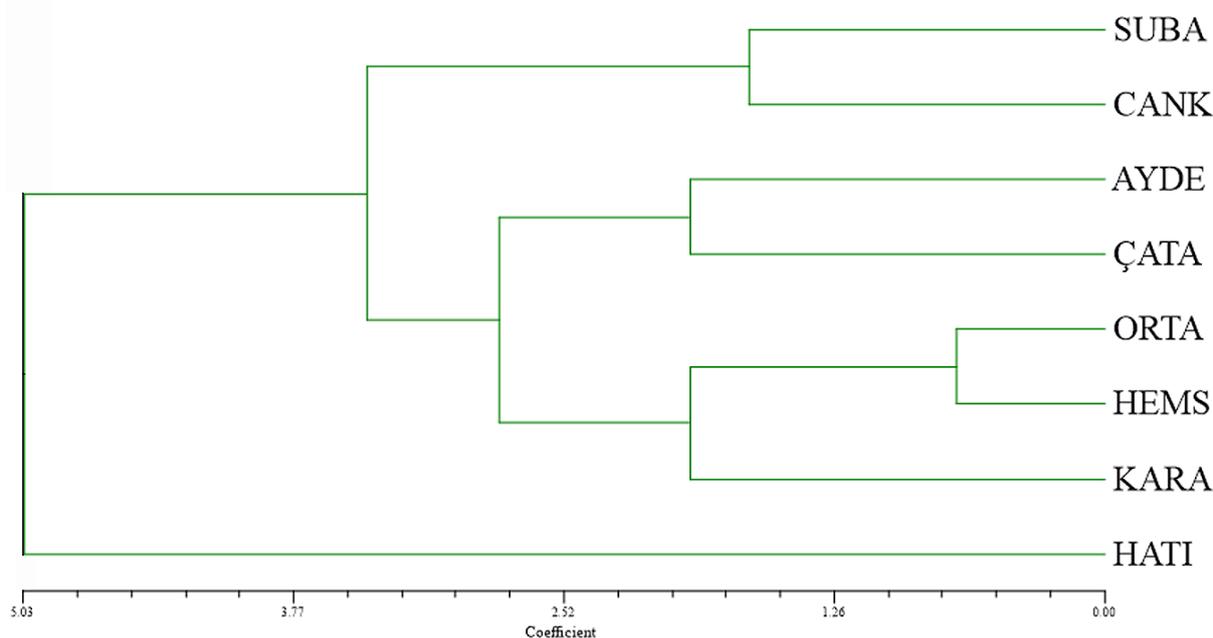
The UPGMA tree derived from Mahalanobis' distances among centroids (Table 1) (Cophenetic Correlation Index  $r = 0.90868$ , reflects the original distances matrix very well, without distortion) (Fig. 3) clusters Subaşı & Yoldere (the Turkish "*D. dryada*" hereafter) with only Cankurtaran Pass specimens (the type locality of *D. clar-korum*). Ayder clusters with its neighboring Çataldere, and Ortacalar clusters with Hemşin (not geographically closer, but morphologically very similar) and both clusters together with Karagöl. Hatila is the most different sample, as reflected by the CDA plot (Fig. 2).

**TABLE 1.** Mahalanobis' distances among samples. AYDE – Ayder, CANK – Cankurtaran, ÇATA – Çataldere, KARA – Karagöl, ORTA – Ortacalar, HEMS – Hemşin, HATI – Hatila, SUME – Sümela, CHAR – Charnaly, SUBA – Subaşı & Yoldere, DRYA – Charnaly.

MALES										
	DRYA	AYDE	CANK	ORTA	KARA	ÇATA	HEMS	HATI		
DRYA	0									
AYDE	3.90	0								
CANK	1.65	4.00	0							
ORTA	4.04	3.35	2.91	0						
KARA	3.43	1.97	2.76	2.05	0					
ÇATA	2.72	1.93	3.17	3.22	2.67	0				
HEMS	4.14	2.80	3.22	0.69	1.80	2.88	0			
HATI	5.77	4.11	5.21	5.61	3.66	5.43	5.40	0		
FEMALES										
	DRYA	AYDE	ORTA	KARA	ÇATA	HEMS	HATI	CANK	SUME	
DRYA	0									
AYDE	4.35	0								
ORTA	4.46	3.50	0							
KARA	6.76	4.16	3.23	0						
ÇATA	4.77	4.15	2.82	4.29	0					
HEMS	6.61	3.57	2.67	2.33	4.32	0				
HATI	5.34	2.39	5.71	6.38	5.34	5.53	0			
CANK	1.32	3.13	4.04	6.11	2.49	5.78	4.03	0		
SUME	4.36	3.5	5.35	7.32	4.94	6.00	2.78	3.33	0	
TOTAL (MALES & FEMALES)										
	CHAR	SUBA	AYDE	CANK	ORTA	KARA	ÇATA	HEMS	HATI	SUME
CHAR	0									
SUBA	4.86	0								
AYDE	7.41	2.48	0							
CANK	5.17	1.01	2.25	0						
ORTA	6.6	3.35	2.4	2.44	0					
KARA	7.56	3.63	1.6	2.73	1.51	0				
ÇATA	7.3	2.73	1.07	2.38	2.49	2.24	0			
HEMS	7.71	4.1	2.24	3.17	1.31	0.72	2.7	0		
HATI	7.91	3.9	3.02	3.57	4.63	3.54	3.98	4.19	0	
SUME	7.54	3	2.27	2.94	4.33	3.43	2.95	4.15	1.55	0

The MST (Minimum Spanning Tree) (not represented graphically) shows that the most similar samples are Hemşin and Ortacalar (0.69), and the most different is Hatila, which clusters with Karagöl (3.66). Turkish *D. dryada* seems to be more related to Çataldere (2.72) and Cankurtaran (1.65). Çataldere relates with Ayder (1.93), Ayder with Karagöl (1.97), and Karagöl with Hemşin (1.80). The most connected sample to the other ones (indicative of “centrality” inside the species) is the Karagöl sample which connects both with the geographically closer Hatila and with more distant ones such as Hemşin and Ayder.

Analysis of Similarity (ANOSIM) of the males sample data show poor group assignation; the test value is positive but relatively low (R-statistic = 0.164493,  $P < 0.001$ ; 1000 permutations). As the general test is significant, the different samples differ more between themselves than internally. Turkish *D. dryada* differs from Ortacalar ( $P = 0.001$ ), Karagöl ( $P = 0.004$ ), Hemşin ( $P = 0.005$ ) and Hatila ( $P = 0.001$ ), but does not differ from Ayder ( $P = 0.271$ ), Cankurtaran ( $P = 0.156$ ) and Çataldere ( $P = 0.26$ ). From the other *D. clarkorum* samples, Ayder only differs from Çataldere ( $P = 0.002$ ). Cankurtaran differs from Ortacalar ( $P = 0.011$ ), Karagöl ( $P = 0.022$ ), Hemşin ( $P = 0.03$ ) and especially from Hatila ( $P = 0.001$ ). Ortacalar differs from Hatila ( $P = 0.001$ ), Karagöl from Hatila ( $P = 0.007$ ), Çataldere from Hatila ( $P = 0.001$ ) and Hemşin ( $P = 0.001$ ), and finally Hemşin from Hatila ( $P = 0.022$ ).



**FIGURE 3.** UPGMA trees (males) derived from Mahalanobis’ distances among sample centroids. See text for interpretation.

## Females Analysis

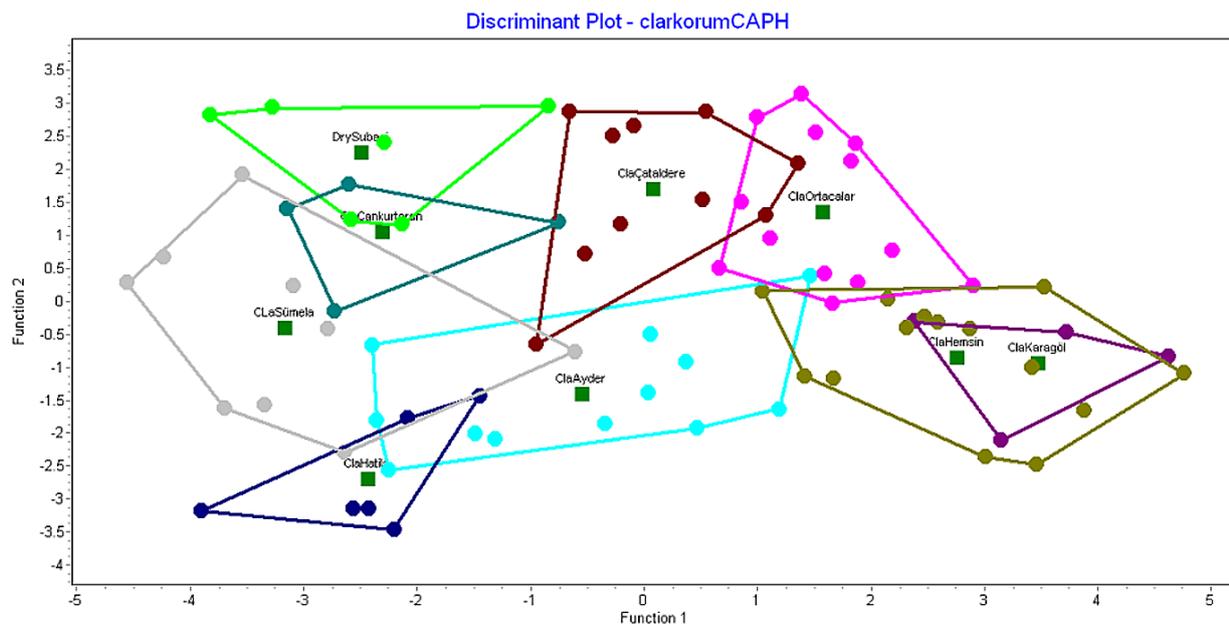
**Canonical Discriminant Analysis (Females from Turkey).** Canonical Discriminant Analysis (CDA) maximizes the discrimination of the samples between the first two significant axes (Eigenvalues greater than one; Chi square 391.3 [ $P = 0.00000026$ ] and 278.79 [0.00000017] for the first and second axes, respectively). The bidimensional plot (see Fig. 4) is a plane defined by the first axis (Eigenvalue 5.42; 42.5% of the variability explained) and the second axis (2.46; 19% explained) that represent the samples with great overlap. Both together explain 61.8% of the total variability, higher than in the male analysis. No population appears discriminated in this analysis. However, no centroid falls within another population’s polygon of dispersion, except for Karagöl and Hemşin which overlap more widely.

Wilks Lambda is 0.00155218 ( $F_{184} = 2.71$ ,  $P = 1.80 \cdot 10^{-16}$ ). 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 a successful discriminant analysis (Bartlett’s Chi-Squared Test Statistic = 394.554, 184 degrees freedom;  $P = 0.000000258$ ).

As shown in Fig. 4, the first axis (42.5% of variability explained) spreads all the populations with considerable overlap among them (but not their centroids, except Karagöl and Hemşin, as mentioned above). The positive part of

the axis shows large overlaps between Karagöl and Hemşin, and also Ortacalar, characterized by higher scores for SCG (0.618406) and COLL (0.525869) and lower values for PI (-0.598382) and DS (-0.541183), separated from Cankurtaran, Sümela, Hatila and the Turkish *D. dryada*, which appear in the extreme negative part of the axis and are characterized by the contrary scores. Ayder and Çataldere remain in intermediate position and character scores.

The second axis (19.3% of variability explained) does not discriminate any population from the rest. Its positive part has Turkish *D. dryada*, Çataldere, Ortacalar and Cankurtaran characterized by the highest scores of DS (0.822319) and SPTMP (0.552382) and the lowest value for SCP (-0.44461), though with very similar scores. The remaining populations appear widespread up to Hatila in the negative part, with the contrary tendency in the above-mentioned characters.



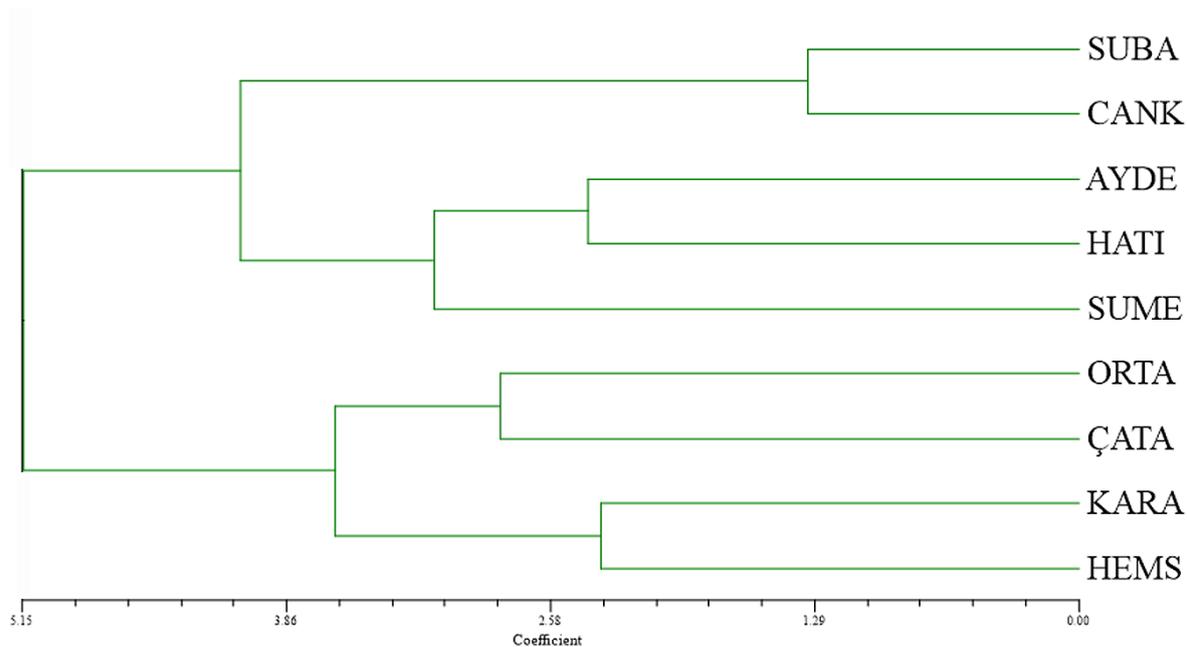
**FIGURE 4.** Canonical Discriminant Analysis (CDA) plots for females. Specimens, sample centroids and group perimeters are represented. For details, percentage of variance explained, and other data, see text.

There is no visual relationship, as can be seen in Fig. 4, between the position in the axes and the longitude, latitude, or the altitude. Populations from the east such as Cankurtaran, Hatila or the Turkish “*dryada*” appear together, while Sümela from the west part of the area of *D. clarkorum*, and Karagöl (from east) and Hemşin (centre) appear to fully overlap. Çataldere and Ayder do not overlap with Hemşin (all belong to the geographic center of the species’ distribution).

This discriminant analysis warrants the discrimination of 93.59% of the specimens (better than male analysis). All the samples have 100% correct classification except for Ayder (91.6%) and Hemşin (92.8%).

The UPGMA tree derived from Mahalanobis’ distances among centroids (Table 1) (Cophenetic Correlation Index  $r = 0.71896$ , reflects the original distances matrix fairly well, without much distortion) (Fig. 5) clusters the Turkish *D. dryada* (Subaşı and Yoldere: SUBA) with Cankurtaran Pass specimens (the type locality of *D. clarkorum*). Ayder clusters with Hatila, and both with Sümela. This later group clusters later with Cankurtaran plus the Turkish *D. dryada*. Another group includes Ortacalar and Çataldere, and Karagöl and Hemşin, all they cluster basally with the remaining ones (see Fig. 5).

MST (Minimum Spanning Tree) (not represented graphically) shows that the most similar samples (as in the male analysis) are just Turkish “*D. dryada*” and Cankurtaran (1.32). This latter connects with Çataldere and Ayder (at 2.49 and 3.13, respectively). Çataldere clusters with Ortacalar (at 2.82), and this later with Hemşin at (2.67). Hemşin connects with Karagöl (2.33), Ayder with Hatila (2.39) and this later with Sümela (2.78). The most “central” (connected with minimum distances to other different populations) is Cankurtaran, which connects with three other samples.



**FIGURE 5.** UPGMA trees (females) derived from Mahalanobis' distances among sample centroids. See text for interpretation.

Analysis of Similarity (ANOSIM) of the sample data show poor group assignment; the test value is positive but relatively low (R-statistic = 0.228934,  $P < 0.001$ ; 1000 permutations). As the general test is significant, the different samples differ more between themselves than internally. Turkish *D. dryada* differ significantly (but near the significance threshold) from Çataldere ( $P = 0.036$ ), Hemşin ( $P = 0.01$ ), Hatila ( $P = 0.03$ ) and Sümela ( $P = 0.033$ ), but are statistically not different (but also very near the threshold) from Ayder ( $P = 0.056$ ), Ortacalar ( $P = 0.058$ ), Karagöl ( $P = 0.052$ ), and are largely similar to Cankurtaran ( $P = 0.081$ ).

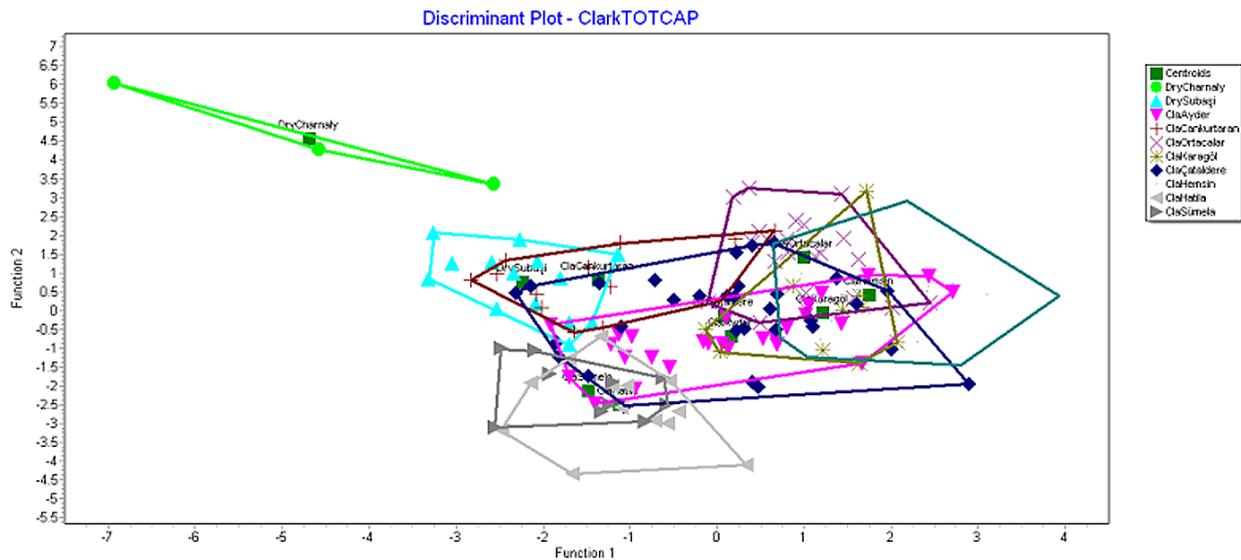
Ayder differs from Ortacalar ( $P = 0.018$ ), Hemşin ( $P = 0.03$ ) and Hatila ( $P = 0.031$ ), but does not reach significance with Karagöl, Çataldere, Sümela, and especially Cankurtaran (all  $p > 0.05$ ). Ortacalar differs from Çataldere ( $P = 0.022$ ), Hatila ( $P = 0.001$ ), Cankurtaran ( $P = 0.016$ ) and Sümela ( $P = 0.002$ ) and does not differ from Karagöl and Hemşin ( $p > 0.05$ ). Karagöl differs from Hatila ( $P = 0.004$ ), Cankurtaran ( $P = 0.01$ ) and Sümela ( $P = 0.001$ ) and does not differ from Çataldere and Hemşin ( $p > 0.05$ ). Çataldere differs from Hemşin ( $P = 0.025$ ), Hatila ( $P = 0.002$ ) and Sümela ( $P = 0.002$ ) and does not differ from Cankurtaran ( $p > 0.05$ ). Hemşin differs from Hatila, Cankurtaran and Sümela (all  $P = 0.01$ ). Hatila differs from Sümela ( $P = 0.036$ ) but is not different from Cankurtaran ( $p > 0.05$ ), and finally, Cankurtaran does not differ from Sümela ( $p > 0.05$ ).

### Conjoint Analysis (Males+Females, Turkey+Georgia)

**Canonical Discriminant Analysis (Both Sexes).** After deleting near invariant and/or dimorphic characters (TVP, LVP and Post-Par), a Discriminant Analysis (CDA) was run for the complete set of adult specimens (*D. dryada* and *D. clarkorum* from Turkey), including three *D. dryada* from the type locality in Georgia (172 specimens in total, the largest sample studied so far).

In the Discriminant Analysis (CDA) the first two significant axes (Eigenvalues greater than one; Chi square 603.22 [ $P = 0.00000033$ ] and 431.87 [0.00000023] for the first and second axes, respectively) are examined. The bidimensional plot (see Fig. 6) is a plane defined by a first axis (Eigenvalue 2.02; 34.2% of the variability explained) and the second axis (1.64; 27.8% explained) that separates the samples in a very similar form in the male and female analyses except for the differentiation of the Georgian *D. dryada*. Both axes together explain 62% of the total variability. Wilks Lambda is 0.0204094 ( $F_{189} = 3.78$ ,  $P = 4.95 \cdot 10^{-45}$ ). As  $p < 0.05$ , this demonstrates the existence of sig-

nificant differences among the samples analysed. Bartlett's Sphericity Tests further proved that the variables were uncorrelated, a prerequisite for a successful discriminant analysis (Bartlett's Chi-Squared Test Statistic = 605.168, 189 degrees freedom;  $P = 0.00000034$ ).



**FIGURE 6.** Canonical Discriminant Analysis (CDA) plots for males and females together, including Georgian specimens. Specimens, sample centroids and group perimeters are represented. For details, percentage of variance explained, and other data, see text.

As shown in Fig. 6, the first axis (34.2% of variability explained) separates the Charnaly River specimens of *D. dryada* in its negative part, with a few overlaps of the unique female specimen (short headed), with the Turkish samples. This negative part (Charnaly specimens) is characterized by higher values of PI (-0.628652), TS2 (-0.550267), MASSET (-0.492901) and DS (-0.334385), and lower scores for LS (0.399345), COLL (0.231288) and PA2 (0.212269).

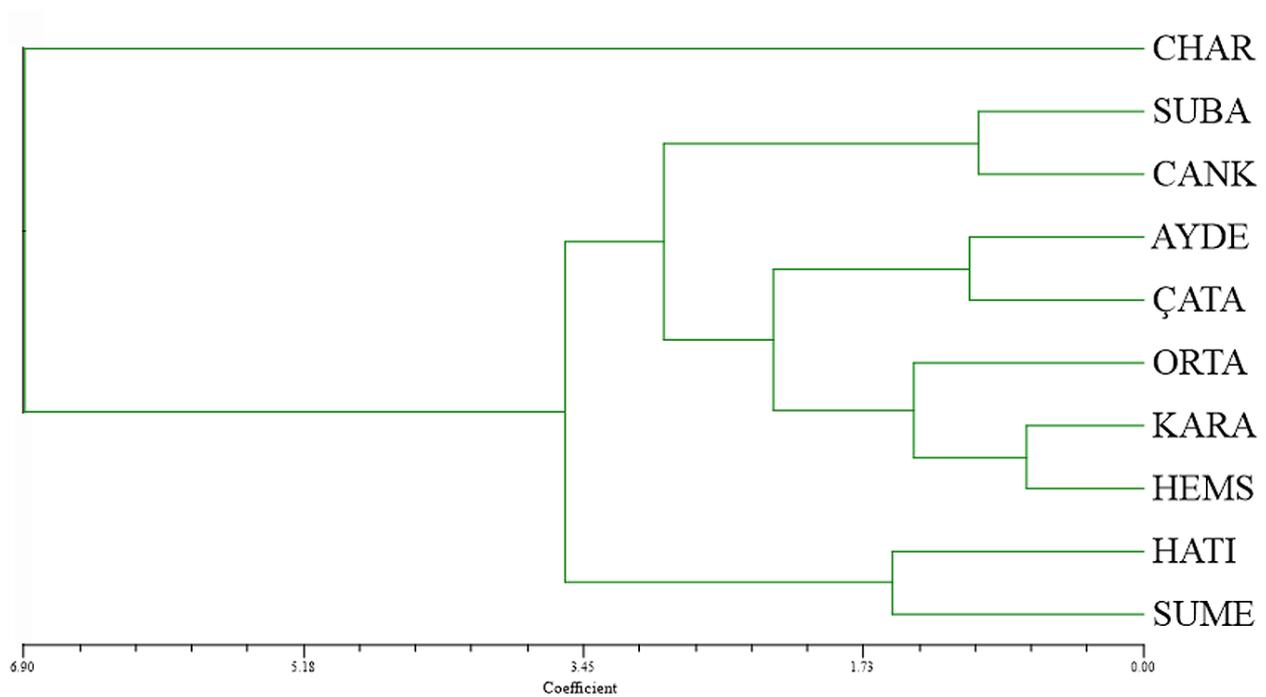
The second axis (27.8% of variability explained) separates the Charnaly specimens of *D. dryada* in its positive part, almost without overlap, characterized by MASSET (0.55051), DS (0.448868), SVL (0.385235), SRL (0.361477) and TS2 (0.334565) with respect to the Turkish samples (both attributed to *D. dryada* and *D. clarkorum*).

As was clear from the male and female analyses, all the Turkish samples more or less overlap, but the Charnaly River specimens from Georgia are clearly different in several morphological characters.

This discriminant warrants the discrimination of 71.5% of the specimens. The best discriminated population is Charnaly (100%) followed by Sümela (90%), with the remaining samples discriminated between 55% (the worst classification, Çataldere) and 80% (Hemşin).

The UPGMA tree derived from Mahalanobis' distances among centroids (Table 1) (Cophenetic Correlation Index  $r = 0.93867$ , reflects the original distances matrix very well, without distortion) (Fig. 7) joins the Turkish samples two by two: Subaşı (Turkish *D. dryada*) with Cankurtaran, Ayder and Çataldere, Karagöl and Hemşin (the most similar) and unites to a third, Ortacalar. These three subgroups cluster progressively and later with this fourth one: Hatila plus Sümela. The Charnaly sample (Georgian *D. dryada*) is the most different and basal to all these other clusters.

The MST (Minimum Spanning Tree) (not represented graphically) shows that the most different sample is Charnaly which clusters far with Subaşı, the Turkish *D. dryada* at 4.86. The most similar samples are Hemşin and Karagöl (0.72), and the other *D. clarkorum* clusters with them at discrete minimum distances. Again, the Turkish *D. dryada* clusters just with the Cankurtaran samples (type locality of *D. clarkorum*) at 1.01. Ayder clusters with Cankurtaran, Çataldere, Sümela and Karagöl (at 2.25, 1.07, 2.27 and 1.6, respectively). The other clusters are Karagöl and Hemşin (0.72), Hemşin with Ortacalar (1.31), and Sümela with Hatila (1.55). The sample most connected to others is Ayder (connects with the other four samples).



**FIGURE 7.** UPGMA trees derived from Mahalanobis' distances among sample centroids. See text for interpretation (males and females together, Turkey and Georgia).

Analysis of Similarity (ANOSIM) of the sample data showed poor group assignment; the test value is positive but relatively low (R-statistic = 0.188968,  $P < 0.001$ ; 1000 permutations). As the general test is significant, the different samples differ more between themselves than internally. Charnaly (Georgian *D. dryada*) differs from all the Turkish samples; less with Subaşı ( $P = 0.014$ ) and more with Hatila and Ortacalar ( $P = 0.001$  both). Turkish *D. dryada* (Subaşı & Yoldere) differs from Ortacalar ( $P = 0.002$ ), Karagöl ( $P = 0.001$ ), Çataldere ( $P = 0.027$ ), Hemşin ( $P = 0.003$ ), Hatila ( $P = 0.001$ ) and Sümela ( $P = 0.006$ ), but does not differ from Ayder ( $P = 0.06$ ) nor Cankurtaran ( $P = 0.114$ ). Ayder differs from Ortacalar ( $P = 0.01$ ), Çataldere ( $P = 0.005$ ) and Hemşin ( $P = 0.006$ ), but does not differ from Cankurtaran, Karagöl, Hatila and Sümela (all  $p > 0.05$ ). Cankurtaran differs from Ortacalar ( $P = 0.002$ ), Karagöl ( $P = 0.001$ ), Hemşin ( $P = 0.004$ ), Hatila ( $P = 0.001$ ) and Sümela ( $P = 0.007$ ) but does not differ from Çataldere ( $p > 0.05$ ). Ortacalar differs from Çataldere ( $P = 0.008$ ), Hatila and Sümela (both  $P = 0.001$ ) and does not differ from Karagöl ( $p > 0.05$ ). Karagöl differs from Hatila ( $P = 0.003$ ) and Sümela ( $P = 0.001$ ) but does not differ from Çataldere and Hemşin ( $p > 0.05$ ). Çataldere differs from Hemşin ( $P = 0.002$ ), Hatila and Sümela (both  $P = 0.001$ ). Hemşin differs from Hatila and Sümela (both  $P = 0.001$ ). Finally, Hatila does not reach significant differences with Sümela ( $p > 0.05$ ).

### Frontonasal index as discriminant character

The frontonasal index (longitude divided by width) runs from 0.65 to 0.92 (average  $0.82 \pm 0.07$ ). There are no differences between the Georgian *D. dryada* (Charnaly), Turkish *D. dryada* (Subaşı) and *D. clarkorum* ( $F_{25,2} = 0.20$ ,  $P = 0.817446$ ). Average results for Charnaly River (*D. dryada*) are  $0.83 \pm 0.05$  (0.78-0.88), Turkish *D. dryada* mean index is  $0.82 \pm 0.07$  (0.7-0.92) and Turkish *D. clarkorum*  $0.81 \pm 0.06$  (0.65-0.88).

The frontonasal index shows considerable overlap between the samples. In fact, the specimens from the *D. dryada* type locality (Charnaly River) do not even have highest index values, so we deduce that the character is not valid to differentiate *D. dryada* or any *D. clarkorum* population.

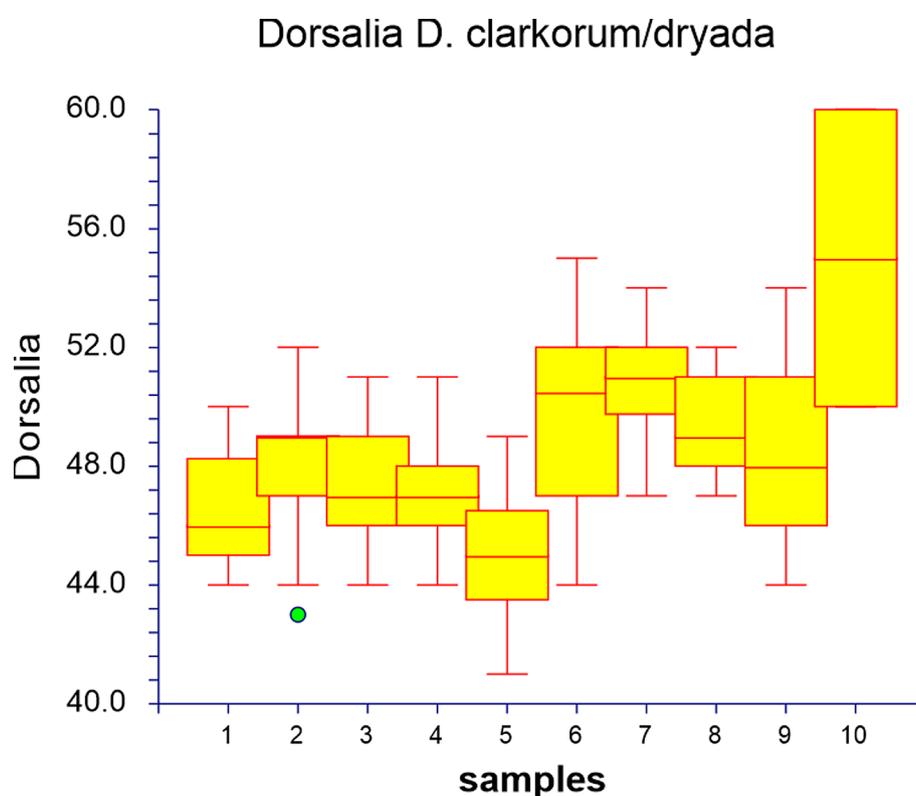
## Masseteric plate as a diagnostic character

In 86% of the Turkish specimens attributed to *D. dryada* (n=14), there was a developed masseteric plate. This percentage is higher but fairly similar to the Turkish *D. clarkorum*, 73.6% (n=163). These percentages do not differ statistically (Mann Whitney U-test:  $Z=1.1$ ,  $p=0.27$ , NS). The total percentage for all the Turkish specimens studied is 74.6%. The three Georgian specimens of *D. dryada* studied had well developed masseteric plates.

As can be seen, the presence/absence of a developed masseteric plate is not a diagnostic character, as pointed out by Schmidtler et al. (2002).

## Differentiation and climatic parameters

Two matrices were compared with several geographic and climatic parameters; one is the general differentiation for the matrix of Mahalanobis' distances among centroids derived from the CDA and the other is a matrix of differences among the mean values for dorsalia (the best descriptor of the overall body covering scalation, as derived from factor analysis –not shown–) (see Fig. 8 for the Box Plot of dorsalia values in different populations-numbers of samples, as in Materials and Methods section). The results of the correlations and Mantel tests are given in Table 2.



**FIGURE 8.** Box Plots of dorsalia values in different populations of the *D. clarkorum/dryada* studied (numbers of samples as in Material and Methods): **1)** Sümela, **2)** Çataldere, **3)** Hemşin, **4)** Ayder, **5)** Hatila Plateau, **6)** Ortacalar **7)** Subaşı & Yoldere (Turkish “*dryada*”), **8)** Cankurtaran Pass, **9)** Karagöl, **10)** Charnaly River Gorge (Georgian *D. dryada*). The center is the median, the length of the rectangle is the interquartile range (from the 25th to the 75th percentile) and represents the central 50% of data. Line is the spread from minimum to maximum values. Green point is a severe outlier.

**TABLE 2.** Comparison (Mantel test) among Mahalanobis distances (all morphological characters used in CVA) and Euclidean distances (dorsalia) matrices, with other matrices inferred using Euclidean distances of several geographic and climatic parameters among these localities. The Smouse et al. (1986) test was used to calculate the correlation among two matrices, excluding the effect of a third one. For interpretation of the results, see text. Significant results are highlighted in bold.

MANTEL TESTS		
Parameter	DORSALIA	GENERAL (all studied characters)
Altitude	<b>r = 0.35</b> <b>t= 2.03</b> <b>P = 0.03 (*)</b>	<b>r = 0.42</b> <b>t= 2.34</b> <b>P = 0.0040 (**)</b>
Distance to the coast	r = 0.21 t= 1.05 P = 0.18 (NS)	r = 0.27 t= 1.27 P = 0.15 (NS)
Geographic Latitude	<b>r = 0.43</b> <b>t= 2.68</b> <b>P = 0.01 (**)</b>	<b>r = 0.43</b> <b>t= 2.59</b> <b>P = 0.01 (**)</b>
Geographic Longitude	r = 0.17 t= 0.75 P = 0.22 (NS)	r = -0.08 t= -0.34 P = 0.36 (NS)
Precipitation Activity (April-September)	r = 0.16 t= 0.42 P = 0.82 (NS)	r = 0.21 t= 1.15 P = 0.18 (NS)
Precipitation Incubation (July-August)	r = 0.36 t= 1.91 P = 0.97 (NS)	<b>r = 0.46</b> <b>t= 2.36</b> <b>P = 0.03 (*)</b>
Temperature Activity (April-September)	r = 0.35 t= 1.55 P = 0.07 (NS)	<b>r = 0.49</b> <b>t= 2.03</b> <b>P = 0.03 (*)</b>
Temperature Incubation (July-August)	r = 0.19 t= 0.80 P = 0.17 (NS)	r = 0.26 t= 1.03 P = 0.16 (NS)
Altitude (extracting Latitude effect)	r = 0.26 t= 1.62 P = 0.06 (NS)	<b>r = 0.34</b> <b>t= 2.03</b> <b>P = 0.03 (*)</b>
Smouse-Long-Sokal 3-way Mantel test		
Latitude (excluding Altitude effect)	<b>r = 0.37</b> <b>t= 2.38</b> <b>P = 0.01 (**)</b>	<b>r = 0.35</b> <b>t= 2.24</b> <b>P = 0.03 (*)</b>
Smouse-Long-Sokal 3-way Mantel test		

Mahalanobis' distances (which include all the scalation traits and a few biometric ones) are significantly correlated with height (altitude) and with latitude, and both correlations are almost identical ( $r=0.42$  and  $0.43$ , respectively;  $P = 0.004$  and  $0.01$ , respectively). These factors could appear to be the same, as the sea level is to the north and the highest localities are in the south. We also applied a Smouse, Long & Sokal test to both correlations, excluding the effect of the third one, and both were still almost equal and significant ( $r=0.34$  with height, and  $0.35$  with latitude,  $P = 0.03$  in both cases) despite extracting the effect of the alternative factor. This suggests that there is another factor underlying height and latitude and that both are not the same. Although less markedly, Mahalanobis' distances are significantly correlated with precipitation during embryonic development ( $r=0.46$ ;  $P = 0.03$ ) (but not during the whole activity period), and with temperature during the activity period (April-September) ( $r = 0.49$ ,  $P = 0.03$ ) (but not only during the incubation one).

Dorsalia differences (that reflect the overall body-covering scalation) are significantly correlated with differences in altitude ( $r = 0.35$ ,  $P = 0.03$ ) and latitude ( $r = 0.43$ ,  $P = 0.01$ ), as with the overall Mahalanobis distances (see above), that include also other scalation and biometric characters. Correlation with latitude continues if the influence of altitude is extracted ( $r = 0.37$ ,  $P = 0.01$ ), but the correlation with altitude disappears when the influence of latitude is extracted ( $r = 0.26$ ,  $P = 0.06$ ) although not far from significance. Also, contrasting with global differences, dorsalia does not show significant correlation with the temperature during activity period nor the precipitation during the incubation period (see Table 2)

As can be seen, there are some kinds of relationship between the scalation with the height (altitude) and latitude. Both seem related, as the altitude increases in general from north to south, but if their mutual influence is reciprocally eliminated, the relationship continues significantly or very near the significance threshold (as in dorsalia, representing pure body-covering scalation) or fully significantly with overall differentiation (Mahalanobis' distances). As dorsalia is not correlated with the temperature or precipitation, this suggests that there is another subjacent factor (solar radiation?, evapotranspiration?) not tested here, but that could be responsible for the correlations.

Concerning the overall differentiation (Mahalanobis distances that include other independent characteristics of scalation, not strictly the body recovering ones, and biometry of the head proportions), there is a correlation with the temperatures in the activity period, and interestingly, precipitation during the egg-incubation period.

### Clinal variation

Dorsalia averages of each sample were compared with their geographic longitude and latitude. There is no significant correlation between dorsalia and longitude (Spearman Rank correlation coefficient:  $\rho = 0.32$ ;  $P = 0.36$ , NS) nor latitude ( $\rho = 0.55$ ;  $P = 0.09$ , NS), so we can affirm that there is no clinal variation in the overall scalation.

### Osteological Study

The osteology of *D. clarkorum* is very similar to other *Darevskia* species but has a tendency to have increased presacral vertebral number (as occurs in the *D. raddei* complex). As a whole, *D. clarkorum* specimens have between 7 and 9 premaxillary teeth (usually 7, more rarely 9 or 8). This character has few variables in *Darevskia*, which almost invariably have 7, but *D. chlorogaster* (Boulenger, 1908) regularly has 9. This presence of 8 or 9 teeth together with the usual number of seven can be interpreted as a sign of primitivism inside the group, as in *D. chlorogaster*. The presence of primitive species in the Colchian and Hyrcanian refuges of the Caucasian area was recalled on several occasions, as in Tuniyev (1990) and Darevsky & Tuniyev (1997).

*Darevskia clarkorum* has between 12 and 19 (average 17) maxillary teeth and between 17 and 22 (average 20) dentary teeth. Males usually have 28 presacral vertebrae, more rarely 27. Females, correspondently, have 28 or 29 presacral vertebrae in similar proportion. As a curiosity, a male specimen from Cankurtaran Pass had asymmetry, with 27 presacral (five short ribbed ones) on one side and 28 (with 6 short ribbed ones) on the other side. These numbers and the anecdotal case of asymmetry show us a tendency to increase the usual number in *Darevskia* (27 and 28 in males and females, respectively), with 6 (rarely five or seven) short ones in the lumbar area. There is no trace of short vertebrae associated with the third presacral vertebrae. Tail has preautotomic vertebrae type A, very rarely B (Arnold 1973; Arnold et al. 2007). The clavicles studied are usually opened (marginated), and very rarely closed (emarginated). Interclavicle is typical cruciform and the sternal fontanelle is rounded, oval, or irregular, frequently fairly small. Costal formula is (3+2) with, or rarely without, one inscriptional rib. The postorbital bone is much shorter and smaller than the postfrontal one. The anteromedial process of postocular and anterodistal at the postfrontal are both present. Squamosal and postocular bones overlap along less than a quarter of the longitude of the latter. Turkish specimens attributed to *D. dryada* (from Subaşı and Yoldere villages) are totally similar to the other *D. clarkorum*.

Two true *D. dryada* from Charnaly River (Georgia) have 7 premaxillary teeth, a slightly higher teeth count for maxillary (15–20, average 17.25) and specially high dentary teeth count (22–26, average 24) which does not even overlap with *D. clarkorum* scores, but clearly corresponds with the fairly bigger head of the true *D. dryada* males. Presacral vertebrae are 27 in the two males studied. Clavicles are open or closed (one case of each), whereas the closed ones are fairly rare in *D. clarkorum*. From one third to one sixth overlap between the squamosal and postocular bones is present. All the other characters are similar to *D. clarkorum* (presence of the studied processes in postocular and postfrontal bones, absence of ribs associated to the third presacral vertebrae, sternal xiphisternal formula (3+2), one inscriptional rib in both specimens, postocular very small and shorter compared to postfrontal, interclavicle cruciform and sternal fontanelle oval well developed).

Differences in the true *D. dryada* (type locality) should be approached with caution due to the small sample, but are consistent with bigger male heads and the fixation of otherwise "odd" characters that appear usually as individual variations in small and inbred populations (Arribas & Odierna, 2004).

## DISCUSSION and CONCLUSIONS

As can be seen in our CVA analyses, all the Turkish samples, both males and females, are very closely related and there is considerable overlap among them. There is no visual relationship among the population centroids in the CVA and their geographic or altitudinal position. Both in terms of UPGMA and MST, the Turkish populations assimilated to *D. dryada* (Subaşı+Yoldere) are closer to the type locality of *D. clarkorum* (Cankurtaran Pass) and are no more different than other *D. clarkorum* samples. Hence, the Turkish *D. dryada* are *D. clarkorum*, and this later has no relevant geographic variability that could be separated taxonomically.

However, as can be seen in our conjoint analysis, the Georgian specimens of *D. dryada* (from Charnaly River, the type locality) are different from the Turkish specimens of *D. clarkorum* and also to those specimens erroneously attributed to *D. dryada* (indistinguishable from Turkish *D. clarkorum*, as said above). Our two Georgian male specimens of true *D. dryada* are clearly bigger than any of the *D. clarkorum* studied here, including the Turkish specimens so far erroneously attributed to *D. dryada* (SVL 71.67 and 71.44 mm, vs.  $59.59 \pm 0.47$ , range from 44.4 to 69.01, in 90 males). The female specimen is also in the upper range of *D. clarkorum* (68.05 mm vs  $59.31 \pm 0.52$ , range from 47 to 71.41 mm, in 78 females). This size difference is clearly visible in Darevsky & Tuniyev (1997; Fig. 2 c & d). Turkish specimens previously attributed to *D. dryada* (even with a paratype in the original description) are clearly smaller than Georgian *D. dryada*, and of similar size to the other Turkish *D. clarkorum*.

Although the presumed diagnostic characteristics of *D. dryada* are not clearly specified in the original description (Darevsky & Tuniyev, 1997), apart from the larger size, the relative presence of developed masseteric and the shape of internasal scale, Schmidtler et al. (2002) say that both taxa differ in the original description in dorsalia, dorsalia/10 ventral scales, femoral pores, and longitudinal and transverse gularia and tibialia according to original tables. In our CDA the Charnaly specimens had greater PI (Pileus comparatively longer), TS2 (longitudinal rows of temporal scales between tympanic and parietal plates), MASSET (greater frequency –the three specimens studied- of masseteric plate developed), DS (dorsalia), SVL (greater size) and SLR (number of supralabial scales); and contrarily, comparatively smaller values of LS (longitudinal rows of scales on ventral surface of thigh between the femoral pores and the outer row of enlarged scales), COLL (collaria), and PA2 (number of circumanalia scales, surrounding the anal plate). The differences in femoral pores and gularia from Darevsky & Tuniyev (1997) tables should be investigated with more data from the Georgian samples. It is a highly threatened population, so studies should be carried out in vivo or reducing the population impact.

The frontonasal index (longitude divided by width), proposed to be a diagnostic character for *D. dryada* (Darevsky & Tuniyev, 1997), is not valid as no differences exist between the Georgian *D. dryada* (Charnaly) studied by us, the Turkish *D. dryada* (Subaşı and Yoldere), and *D. clarkorum* values. The results of the index are 0.83, 0.82 and 0.81 respectively for Charnaly River *D. dryada*, Turkish specimens attributed to *D. dryada* and Turkish *D. clarkorum*, with considerable overlap between them. In fact, the specimens of the *D. dryada* type locality (Charnaly River) do not even have the highest index values, so we deduce that the character is not valid for separating *D. dryada* or any *D. clarkorum* population.

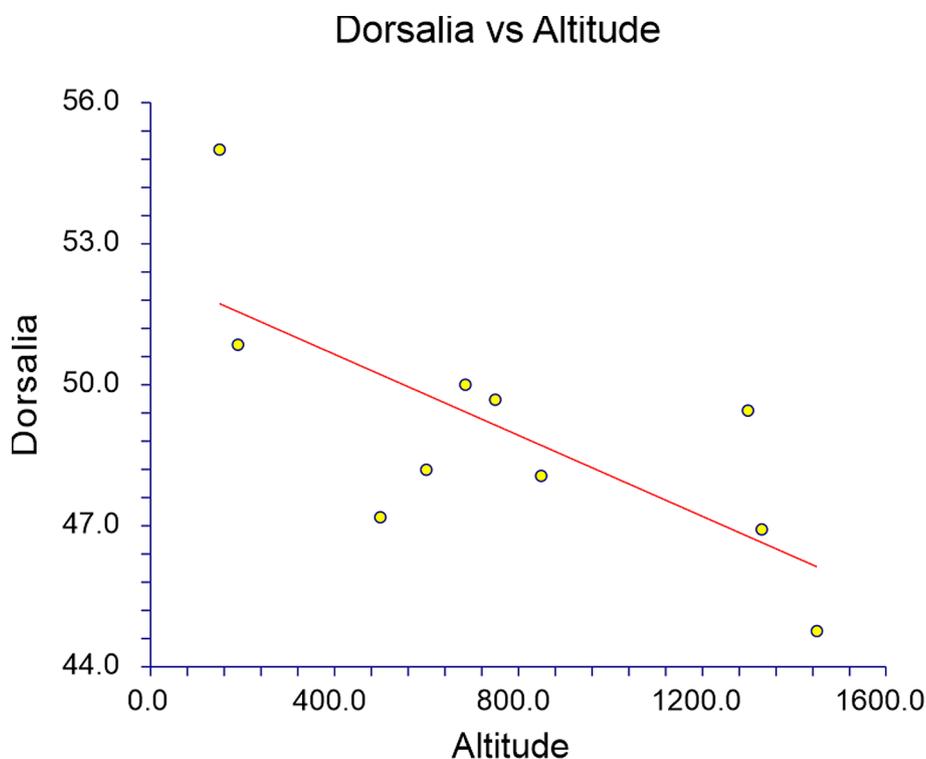
The presence of a masseteric plate was also proposed as a diagnostic character for *D. dryada* (Darevsky & Tuniyev, 1997), who said that was “small, if at all” in *D. clarkorum*, and “large and well expressed (rarely absent)” in *D. dryada*. The three true (Georgian) *D. dryada* studied by us had well developed Masseteric plates, as well as 86% of the Turkish “*D. dryada*”, but this character also appears (three out of each four specimens) in Turkish *D. clarkorum* (73.6%; n=163), without statistical differences among these samples. This lack of diagnostic value was pointed out by Schmidtler et al. (2002).

Two genetic studies (not published, but mentioned in Fu’s thesis; Fu, 1999) compared specimens of *D. clarkorum* and *D. dryada*. Fu (1999) using a mitochondrial marker (ATPase 6) and did not find many differences (the sequences studied were identical or with a single nucleotide difference). He also cited McCulloch data (pers. comm. to Fu) from an unpublished allozyme study that did not detect any fixed alleles between the two proposed species. In any case, the specimens of *D. clarkorum* from Mahden (=Maden) (Turkey) were clearly identified, but the Georgian locality (Adzgarua, Tskali Gorge) is untraceable, so it is not possible to know if they were *D. clarkorum* (also present in Georgia) or *D. dryada*. On the other hand, the comment that their Georgian *D. clarkorum* came from the area of *dryada* (but not specifically from the type locality, uniquely published for *D. dryada* in Georgia), and that its characteristics were consistent with those diagnostic of *D. dryada* (but the most commonly used feature, the developed masseteric, also appears in three out of four *D. clarkorum*, see above), suggests that it may originate

from another place and perhaps represent true *D. clarkorum*. McCulloch provides no information about the origin of their specimens. At that time, it was certainly easier to get specimens of *D. dryada* from the alleged Turkish locality (“Subashi-Kej” (= Subaşı) in Darevsky & Tuniyev, 1997) than from the type locality in Georgia (Charnaly River Gorge), but the former are also *D. clarkorum*, as we demonstrate in this work.

Confusion in photographs (whether due to the assimilation of *D. dryada* to *D. clarkorum* before being described formally, and after being synonymized or due to errors in the photographs), do not help clarify the issue of the distinction between the two taxa. Before the description of *D. dryada*, a specimen was identified as *D. clarkorum* in Szczerbak (2003), and after its “de facto” synonymization, photos of *D. dryada* appear as *D. clarkorum* in Schmidler et al. (2002), Bischoff & Tarknishvili (2002) and Bischoff (2003). There were also curious mistakes, as in Ananjeva et al. (2004), where a *D. rudis* is depicted in the photo corresponding to *D. dryada*, or on the Turkish website (<http://www.turkherptil.org/>) where the *D. dryada* specimens pictured are also *D. rudis*.

Concerning the relationship between morphological variation and climatic parameters, Schmidler et al. (2002) said: “Populations from warmer lowland localities tend to a finer pholidosis with relatively higher scale counts than populations from the cooler high elevation localities. This kind of variation reminds of the principle of “numerical variation of pholidosis correlated with climate” described by Schmidler (1986) from similar data-sets in green lizard species of the genus *Lacerta* (*sensu stricto*)”. This inverse relationship between height and dorsalia values is true in *D. clarkorum*/*D. dryada* (Spearman Rank Correlation coefficient:  $\rho = -0.72$ ;  $P = 0.01$ ) (Fig. 9).



**FIGURE 9.** Plot showing the inverse relationship between altitude and dorsalia mean values in the studied *D. clarkorum*/*D. dryada* populations (Spearman Rank Correlation coefficient:  $\rho = -0.72$ ;  $P=0.01$ ).

According in part to the Schmidler et al. (2002) hypothesis of the “numerical variation of pholidosis correlated with climate”, but checking it with numerical data using the Mantel Test, both the general differentiation between populations and this scalation (dorsalia) appear statistically correlated with altitude and also with latitude. Although it seems that both parameters are the same (because in this region of the Black Sea the lowland localities are to the north and the mountains are to the south), both correlations are preserved by mutually extracting their effects (Smouse-Long-Sokal test), so the doubt remains about whether the underlying factor is the same. The correlation seems to be stronger with morphology in general (multiple scalation characters and head biometry) than only with dorsalia (near the limit of significance with altitude when extracting the effect of latitude). In the case of the general differentiation among samples, it is also significantly correlated with temperatures during the activity period (April-September) and with precipitation during incubation (July-August). If climatic parameters such as temperature and precipitation are not directly correlated with the variation in dorsalia, the relation with altitude (and perhaps

latitude) must be linked with some other climatic parameter not studied here. Our favorite candidates to influence dorsalia and also linked with altitude are solar radiation, and especially evapotranspiration. Size and shape of scales, and their efficiency in the coverage of the skin, can be dramatically influenced by both parameters and this is an interesting topic for future studies of these or other lizards. Also, there is no clinal variation in scalation (tested with dorsalia) in relation with the geographic position (latitude or longitude).

Our true *D. dryada* sample studied, although scarce, are osteologically different from *D. clarkorum* in the number of teeth, especially the dentary ones, without overlap among both forms. This is due to the clearly bigger heads of the *D. dryada* males (the different shape between male heads of both taxa is well depicted in Darevsky & Tuniyev, 1997; see Fig. 1). Also, the vertebral number is primitive in *Darevskia* (27), which also appears and tends to increase to 28 in *D. clarkorum* (even coexisting bilaterally in the same specimen). The clavicles are also open or closed (one case of each), whereas the closed ones are fairly rare in *D. clarkorum*. The Turkish specimens previously considered *D. dryada*, are also osteologically indistinguishable (i.e. in the number of teeth from the dentary) from the other specimens of *D. clarkorum*.

It is normal that species of the same genus appear very similar and almost indistinguishable osteologically. Also there is a certain individual variability, and hence there are individuals with rare characteristics, which in very small and presumably endogamous populations, remain fixed under certain circumstances and distinguish these populations/taxa from other congeneric ones. These circumstances, and the larger size, would be the cause of the characteristics of *D. dryada* with respect to *D. clarkorum*, which nevertheless presents some characteristics derived from the common pattern in the genus *Darevskia*.

With everything explained above, we can say that *D. dryada* cannot be considered a mere synonym of *D. clarkorum*. Its true status (most probably as a ssp. of *D. clarkorum*) can only be established through genetic studies ensuring the origin of the samples from the type locality, the only one where the presence of the nominal *D. dryada* taxon can be warranted. Today, *D. clarkorum* is known from the Pontic Alps (*Doğu Karadeniz Dağları*), from Yavuzkema (Giresun Vilayet) in the west, towards the east entering SW Georgia. The species also crosses the right side of the Çoruh River (Karagöl in Turkey, Mt. Mtirala in Georgia, probably reaching the Kintrishi Gorge to the north –Tuniyev pers. com.-). The true *dryada* are restricted, so far, only to its type locality, the Charnaly River Gorge in Georgia.

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## Appendix I

### Specimens used for osteological study

*Darevskia clarkorum* (Turkish “*dryada*”): Subaşı Village, Hopa, Artvin, Northeastern Anatolia (02/06/2019), 190 m asl (1 male & 1 female).

*Darevskia clarkorum* (Turkish “*dryada*”): Yoldere Village, Hopa, Artvin, Northeastern Anatolia (02/06/2019), 80 m asl (2 males & 2 females).

*Darevskia clarkorum*: Ayder Plateau, Çamlıhemşin, Rize, Northeastern Anatolia (08/07/2001), 1330 m asl (1 male).

*Darevskia clarkorum*: 16 km northeast of Ortacalar, Artvin, Northeastern Anatolia (13/07/2002), 750 m asl (1 male & 1 female).

*Darevskia clarkorum*: Çataldere Village, Kaptanpaşa, Rize, Northeastern Anatolia (04/06/2019), 600 m asl (1 male & 1 female).

*Darevskia clarkorum*: Hatila Plateau, Artvin, Northeastern Anatolia (09/06/2008), 1450 m asl (1 male & 1 female).

*Darevskia clarkorum*: Cankurtaran Pass, between Hopa and Borçka 14. km, Artvin, Northeastern Anatolia (23/07/2015), 685 m asl (1 male & 1 female), (30/09/87) 690 m asl (1 male & 1 female).

*Darevskia clarkorum*: Sümela, Maçka, Trabzon, Northeastern Anatolia (05/08/2014), 1300 m asl (1 male & 1 female).

*Darevskia clarkorum*: Mountain Trial, vicinity of Borçka, Artvin, Northeastern Anatolia (05/1995), (1 male & 1 female),

*Darevskia dryada*: Charnaly River Gorge, Chelvachauri district, Adjara (W Georgia), (25/06/1994), (2 males).