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Original article (Orijinal araştırma)

Geometric morphometric analysis of pronotum shape in two isolated populations of *Dorcadion anatolicum* Pic, 1900 (Coleoptera: Cerambycidae) in Turkey[1]

Dorcadion anatolicum Pic, 1900 (Coleoptera: Cerambycidae)' un Türkiye'deki izole popülasyonlarında pronotum şekil değişiminin geometrik morfometri analizleri

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Abstract

The genus *Dorcadion* Dalman, 1817 (Coleoptera: Cerambycidae) comprises species living at ground surface and having lost the ability to fly. Hence the populations of the species are easily isolated from each other. These biological features are considered to be important factors for the acceleration of speciation in this genus. The effects of population isolation can be measured through the morphological characters of samples. The morphological characters that enabled the taxonomists to identify this genus are mainly subjective. This situation causes some systematic problems. *Dorcadion anatolicum* Pic, 1900 (Coleoptera: Cerambycidae) is an endemic species in Turkey and the subspecies status of some populations is debated. The aim of this study was to determine pronotum shape variation via geometric morphometrics from two isolated localities and to contribute to knowledge of the taxonomic and evolutionary status of *D. anatolicum*. The samples were collected from two different localities of Turkey (Kahramanmaraş and Konya Provinces) in March-April 2018. Results of morphometric analysis revealed that the pronotum shape variations of the samples allowed morphological discriminations of populations.

Keywords: Coleoptera, *Dorcadion anatolicum*, geometric morphometrics, landmark, pronotum

Öz

Dorcadion Dalman, 1817 (Coleoptera: Cerambycidae) cinsi türleri toprak yüzeyinde yaşayan ve uçma kabiliyetleri olmayan türlerdir. Ancak bu türlerin popülasyonları birbirlerinden kolayca izole olabilirler. Bu tarz biyolojik özellikler türleşme sürecini hızlandıran önemli faktörler olarak düşünülebilir. İzole popülasyon en etkileri örneklerin morfolojik karakterleri üzerinden ölçülebilir. Taksonomistlerin cinsin tanımlamasında kullandığı morfolojik karakterler genel olarak özneldir. Bu durum bazı sistematik problemlere neden olmaktadır. Türkiye'ye endemik olan *Dorcadion anatolicum* Pic, 1900 (Coleoptera: Cerambycidae) türünün bazı popülasyonlarının alttür statüsü tartışmalıdır. Bu çalışmanın amacı, iki izole lokaliteden alınan örneklerin pronotum şekil farklılıklarını geometrik morfometri analizleri ile belirlemek ve *D. anatolicum*'un taksonomik ve evrimsel durumuna katkıda bulunmaktır. Çalışmada kullanılan örnekler Türkiye'nin iki farklı popülasyonundan (Kahramanmaraş ve Konya) 2018 yılı mart ve nisan aylarında toplanmıştır. Morfometrik analiz sonuçları örneklerin pronotum şekil değişikliklerinin, popülasyonların morfolojik anlamda ayrımına izin verdiğini ortaya koymuştur.

Anahtar sözcükler: Coleoptera, *Dorcadion anatolicum*, geometrik morfometri, homolog referans noktası, pronotum

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Introduction

Isolated populations are good models for the study of the process of speciation. Studies can focus on genetic, ecological and behavioral variation, but the morphological approach still remains essential because morphology is used to discriminate species. Geometric morphometrics (Bookstein, 1991; Rohlf, 1993; Rohlf & Marcus, 1993) allows quantification of geometric variation of the anatomical structures and the visualization of morphological variation among samples of organisms. The main advantage of geometric morphometrics is that it captures geometry of analyzed objects by landmark coordinates, and preserves this information throughout the analysis (Bookstein, 1996). Unlike earlier techniques, geometric morphometrics has the ability to show shape changes like deviation of displacement vectors from the mean value or deformation grids in original sample space on each of the landmarks. Visualized shape variations can help to characterize populations within species or sexes.

Beetle bodies (or a body part such as head, pronotum, femur and elytra) have been the subject of geometric morphometric analysis in the past (Pizzo et al., 2006; Benitez, 2013; Qubaiova et al., 2015; Zuniga-Reinoso & Benitez, 2015). External shape morphology evolution in two polymorphic sister species of the genus *Onthophagus* Latreille*,* 1802 (Coleoptera: Scarabaeidae) were analyzed (Pizzo et al., 2006). Body morphometrics can help to characterize populations within species and sexes, as shown by the analysis of *Ceroglossus* Solier, 1848 (Coleoptera: Carabidae) (Benitez, 2013). Body shape variation has also been used for cryptic species of *Nyctelia* Berthold, 1827 (Coleoptera: Tenebrionidae) to enable identification (Zuniga-Reinoso & Benitez, 2015). There are also two remarkable studies of genus *Oreoderus* Burmeister, 1842 (Coleoptera: Scarabaeidae) (Li et al., 2016) and *Ablattaria* Reitter, 1884 (Coleoptera: Silphidae) (Qubaiova et al., 2015) using geometric morphometrics.

With six genera, and 17 subgenera, Dorcadionini is a tribe belonging to the subfamily Lamiinae and includes a total of 278 species of which 227 are endemic in Turkey (Özdikmen, 2016). The members of this genus generally cannot fly due to the atrophy of the flight wings. The larvae of *Dorcadion* appear at the end of May or June and feed on the grass roots. They became pupae after about 13-14 weeks after wintering as mature larvae. The adults emerge after 2-3 weeks and crawl on meadow vegetation (Baur et al., 2002; Kumral et al., 2012).

Dorcadion anatolicum Pic, 1900 (Coleoptera: Cerambycidae) is endemic to Central and Southeastern Anatolian Regions of Turkey (Özdikmen, 2010). This species has three subspecies: *Dorcadion anatolicum seydisehirense* Breuning*,* 1946; *Dorcadion anatolicum brignolii* Breuning*,* 1946*; Dorcadion anatolicum postapertum* Breuning*,* 1946 *(*Sama, 1982). However, these subspecies have not been used recently. According to Özdikmen (2010), the subspecific structure of *D. anatolicum* needs to be clarified.

Considering the biology of *Dorcadion*, isolated populations of various species belonging to this genus may be the results of anthropogenic and environmental effects. Over time, the reflection of isolated gene pools and different environmental interactions on individuals belonging to these isolated populations can be observed quantitatively and qualitatively. It is also important to understand the process of evolution of this species. Thus, we used landmark-based geometric morphometrics method to analyze pronotum shape morphology in two distant localities of *D. anatolicum*.

Materials and Methods

Samples of the *Dorcadion anatolicum* were collected from two different localities of Turkey (Kahramanmaraş and Konya Provinces) on March-April 2018 (Figure 1).

Sexes of samples were distinguished by the shape and size of the fore tarsus and confirm by using gonads. The study was evaluated on only male individuals to eliminate variations that may arise from sexual dimorphism. A total of 73 specimens (35 from Kahramanmaraş and 38 from Konya) were used in this study. A single image was taken by a camera attached to Leica EZ4HD microscope for each specimen of pronotum.

Figure 1. The localities from where samples were collected. Locality 1: Kahramanmaraş Province (Göksun-Kayseri Road, around Mehmetbey Town, 38º6’36” N, 36º28’17” E); Locality 2: Konya Province (Taşkent District, Avşar Town, Feslekan Plateau, 36º51’9” N, 32º30’44” E) (Anonymous, 2019).

Landmark-based morphometric methods were chosen as they are the most effective technique in learning about the shape information of an organism and eligibility to use powerful statistical methods for testing differences in shape. In this study, 10 landmarks on the pronotum were digitized on photographs using tpsDig 2.17 (Rohlf, 2013). The position of landmarks is given in Figure 2.

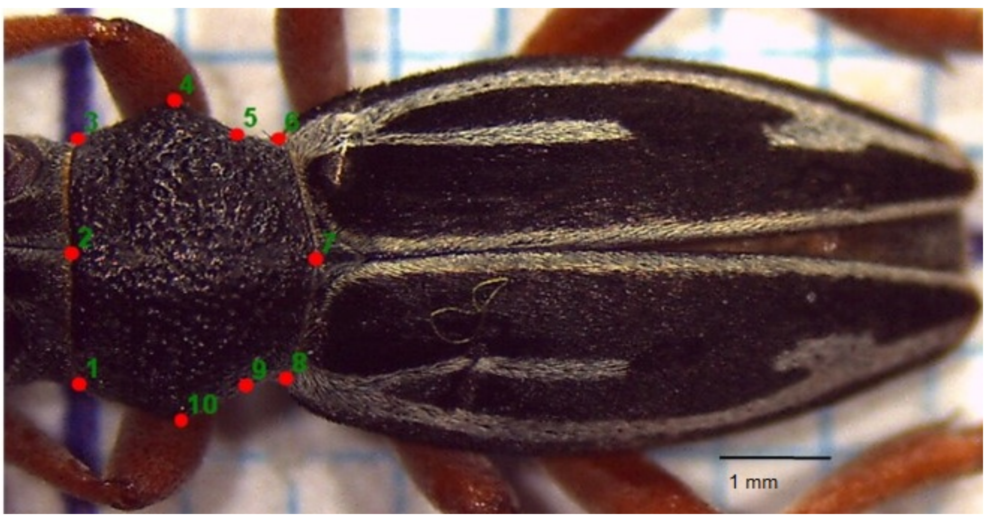

Figure 2. Selected landmarks on *Dorcadion anatolicum* male, representing the dorsal side of the pronotum: 1) Anterior margin left edge; 2) middle of anterior margin; 3) anterior margin right edge; 4) right spine apex; 5) right protuberance posterior limit; 6) posterior right edge; 7) middle of posterior margin; 8) posterior margin left edge; 9) left protuberance posterior limit; and 10) left spine apex.

A generalized Procrustes analysis (GPA) has been developed to superimposition of landmark configurations and to eliminate the effects of translation, rotation and scale (Rohlf, 1999). GPA, multivariate descriptions of the shape variables, relative warp analysis (principal component analysis of the partial warp scores) and visualization of transformation grids allowed us to describe shape variations. Principal components analysis (PCA) performed using the covariance matrix of the Procrustes shape coordinates to summarize multivariate data by building linear combinations of the original variables that are uncorrelated and maximize the sample total amount of variance explained (Viscosi & Cardini, 2011). We used PCA of partial warps using MorphoJ. PC scores were used as dependent shape variables and MANOVA were performed using IBM SPSS 25 to compare the variation of the pronotum shape between the localities.

The discriminant analysis (DA) is probably one of the most widely used statistical method for investigating taxonomic differences and is generally used when only two groups are compared (Viscosi & Cardini, 2011). Discriminant analysis was conducted on the PC scores of pronotum to obtain a classification matrix based on shape variation using IBM SPSS 25. We used the percentages of correct classification to evaluate the discrimination of pronotum shape between populations. To compare overall pronotum size among populations, the centroid size (the square root of the sum of the square distances between each landmark and the centroid) (Bookstein, 1996) was computed for each population and tested by independent samples t-test. Regression analysis were used to explore how shape varies with size. Size correction using log-transformed centroid size effects on shape were tested using MorphoJ (Klingenberg, 2011).

Results and Discussion

PCA of all specimens explained 46.1% of shape variation within samples by the two first PC axes extracted from the variance-covariance matrix (PC1 explains 26.6% and PC2, 19.5%). A total of up to nine axes were required to cover more than 90% of the shape variation. In the PCA plots, individuals of the two populations were mixed and did not form any distinct cluster (Figure 3).

The multivariate analysis of variance (MANOVA) of pronotum shape showed a significant difference between the two populations (Hotelling's Trace = 0.614, F = 4.24, p = 0.000). Discriminant function was performed using the first nine PCs to determine the degree of morphological separation between the two groups. The DA conducted on the PC scores of pronotum evidenced that 94.7% of Konya population and 88.86% of Kahramanmaraş population were correctly classified. The percentage of correct classifications were high for all leave-out-one cross-validated groups (Konya 71.1%; Kahramanmaraş 71.4%) (Figure 4). The DA found significant differences between means in Procrustes distances (P < 0.0001) for the two populations. Pronotum shape variation measurements has allowed to separate the samples from the two different habitats. The pronotum of Konya samples were observed to be shorter than the Kahramanmaraş samples in anterior and posterior directions and less pointed in lateral edges (revealed with landmarks 2 and 7).

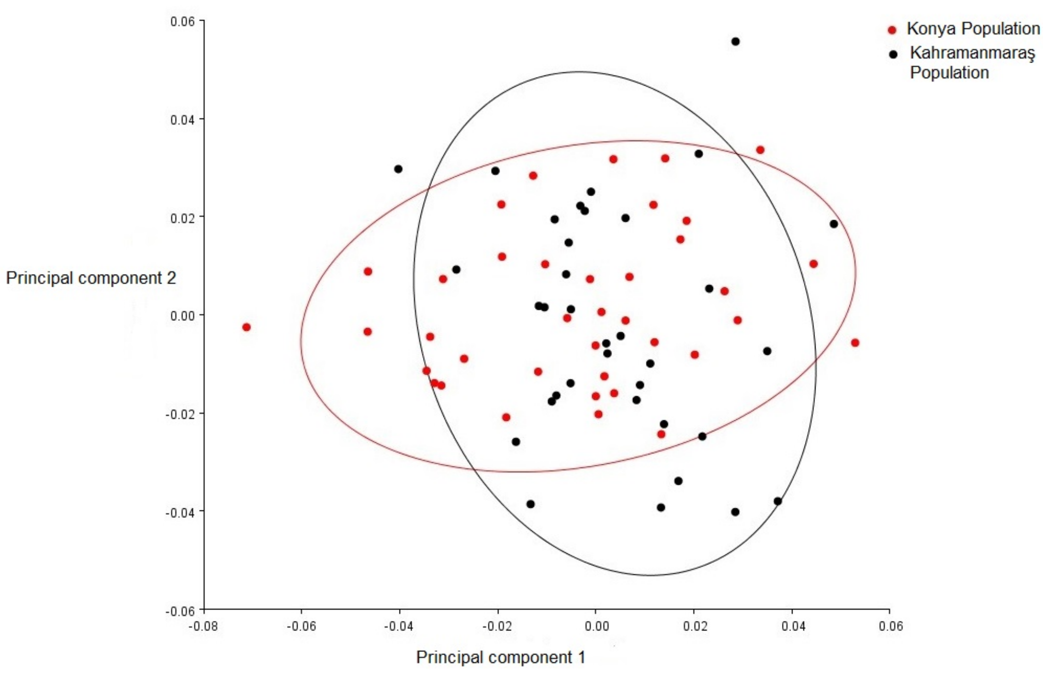

Figure 3. Shape differences between populations, Konya (red) and Kahramanmaraş (black).

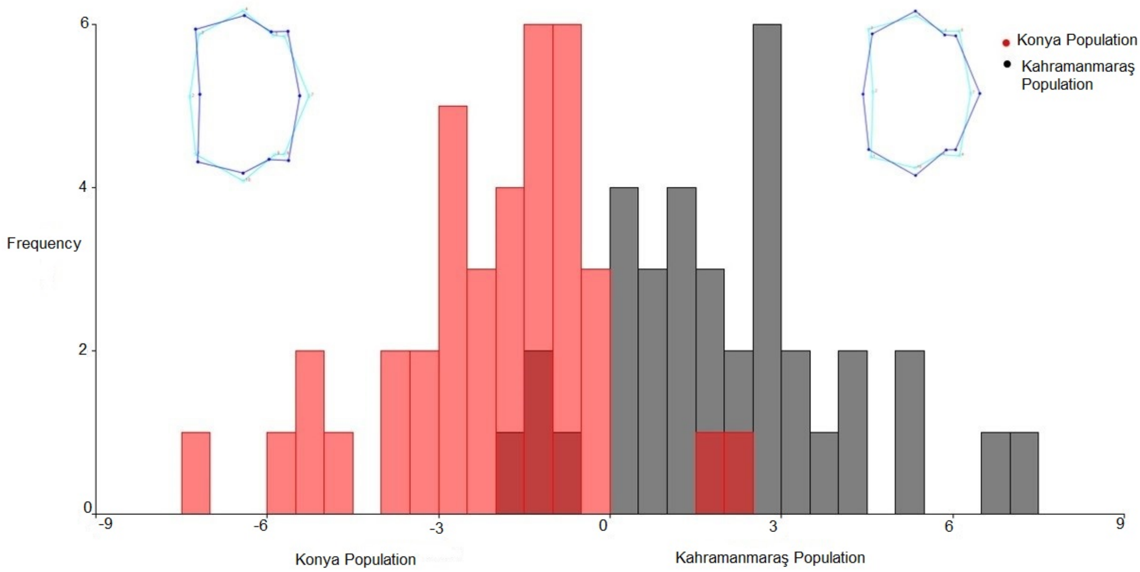

Figure 4. Cross validation scores of shape variables of Konya and Kahramanmaraş populations of the different groups. The violet lines show the extreme shape change in positive and negative direction. Light blue lines are the mean shape and violet lines show the shape change of the pronotum (scales are -5.0 and +5.0, respectively).

An independent samples t-test of centroid sizes of pronotum did not show statistically significant differences between populations (t = 1.75, p = 0.085) (Figure 5).

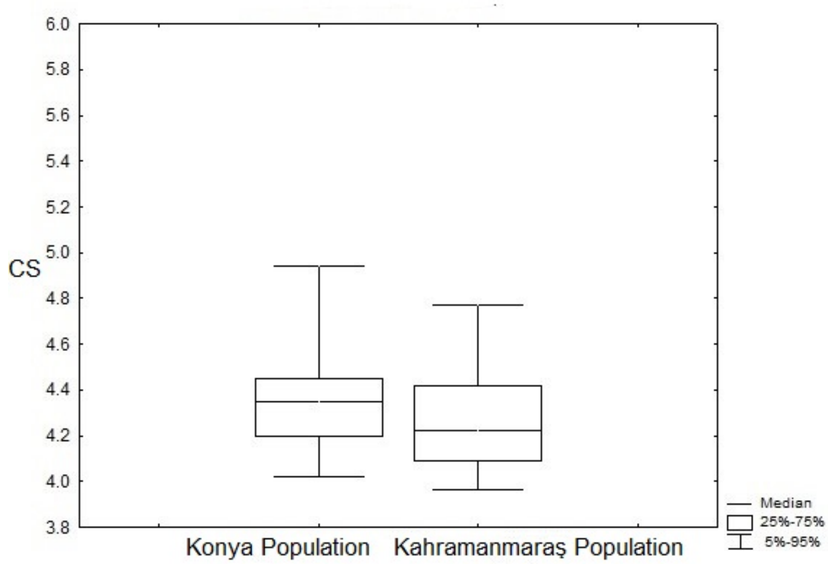

Figure 5. Boxplot of centroid sizes of Konya and Kahramanmaraş populations.

Multivariate regression of the shape variables versus log-transformed centroid sizes were statistically significant with permutation test (P = 0.017), but only 3.45% of variance was explained. This test as well as the large overlap between populations in the scatterplot of regression scores versus size (Figure 6) suggests that the effect of size on shape, although weak, is very similar in the two populations.

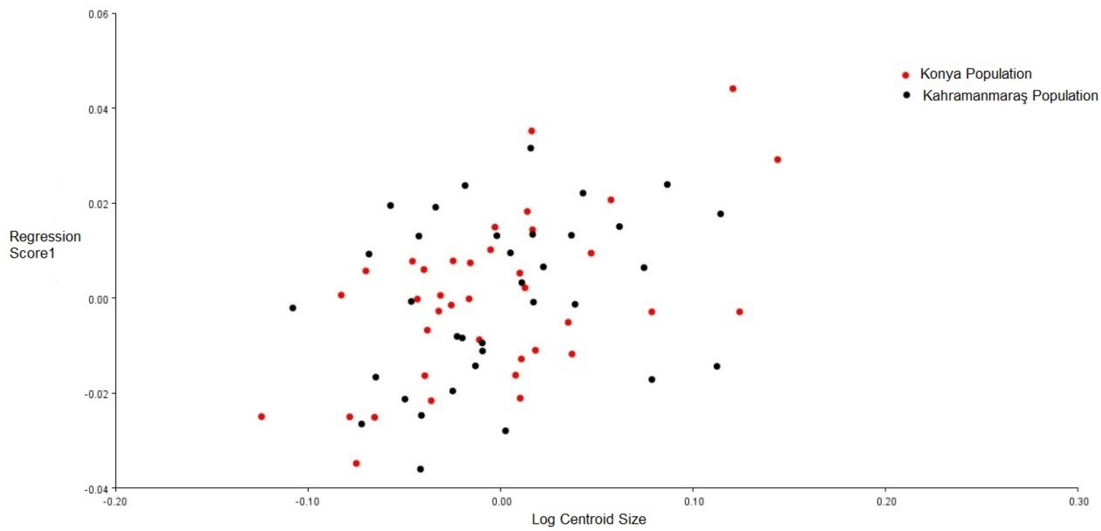

Figure 6. Regression of shape onto log-centroid size pooling within the two analyzed populations.

Dorcadion anatolicum is an endemic species in Turkey and its subspecific structure is doubtful (Özdikmen, 2010). The taxonomic studies of the genus *Dorcadion* are generally based on external morphological characters. Identification of species and subspecies are based on similarities and differences in these characters. These characters generally do not have a quantitative basis; therefore, the studies are sometimes based on ambiguous definitions. Compared to other external morphological characters, pronotum shape variations are important taxonomic characters more commonly and safely used in the *Dorcadion* classification (Önalp, 1990).

These two isolated habitats are located on the Taurus Mountain range and have similar geographic characteristics due to the influence of the Mediterranean climate (Figure 1). This situation may be insufficient to observe the selective pressure caused by geographical differences. However, it may be sufficient to evaluate the genetic isolation of these populations (e.g., genetic drift and founder effect). Generally, separated populations of a species can show variation in different taxonomic characters over time. When these differences are found to be sufficient by taxonomists, the populations may be considered as different subspecies. Even if this does not lead to any systematic category for distinction, it is expected that separate populations contain small or large variations to observe the evolutionary dynamic of the species (Rieseberg et al., 2004; Butlin et al., 2008).

Considering the distance between the two localities and the biological characteristics such as mobility and phenology of *Dorcadion* species, we thought that the effects of isolation between habitats could be quantified by measuring the specimens from the two populations. Therefore, geometric morphometrics was applied and the variation of pronotum shape in *Dorcadion* populations was clearly showed by this technique. Despite the fact that centroid size of the two populations were not significantly different ($t = 1.75$, $p = 0.085$) (Figure 5) the information provided by the analysis of shape variables was significantly different between Konya and Kahramanmaraş populations (Hotelling's Trace = 0.614, $F = 4.24$, $p = 0.000$). As suggested by discriminant function analysis 94.7% of Konya population and 88.9% of Kahramanmaraş population were correctly classified. Geometric variation between populations located in landmarks are 2, 4, 7 and 10 respectively. The Kahramanmaraş population showed a different direction of shape variation by forming a prominent pointed structure at the lateral edges. Differences in landmarks 4 and 10 of Kahramanmaraş samples lead to the presence of rose thorn shaped structure on the both lateral edges. Compared to Konya samples, pronotum variation in landmarks 2 and 7 of the Kahramanmaraş samples gave a clearer ledge in the anterior and posterior media. Therefore, the pronotum produces a triangular recess towards sides of elytra and head. Evaluation of all the pronotum variation, indicated that the pronotum of Konya samples had smoother median points in four planes compared to Kahramanmaraş samples (Figure 4).

Although there many studies have found significant differences in pronotum shape in Coleoptera (Pizzo et al., 2006; Ober & Connolly, 2015; Eldred et al., 2016; Li et al., 2016), geometric morphometrics was applied here to *Dorcadion* for the first time. Combining pronotal shape morphology with phylogenetic analysis Ober & Connolly (2015) showed that the pronotum shape generally reflects phylogenetic relationships, and may be the most important morphological trait for recognizing distinct populations of *Scaphinotus petersi* Roeschke, 1907 (Coleoptera: Carabidae) in the Arizona Sky Islands. Dascălu & Fusu (2012) applied ordinary morphometry analysis to two subspecies of *Dorcadion axillare* Küster, 1847 (Coleoptera: Cerambycidae). Their study showed that all the univariate measures (pronotal length, pronotal width measured at base, maximum elytral length, maximum elytral width and total body length) for the different populations are largely overlapping, even though the differences between the mean values were statistically significant. Based on the results presented here, pronotum shape is an important morphological trait for recognizing distinct populations of *Dorcadion*. It could be said that we observed measurable shape variations of the same magnitude.

Our results also show that geometric morphometric analyses are useful to determine of variations of these habitats at species level. These different shape variations of pronotum can be interpreted as the first observable effects of isolation. While a variation in shape reflects the genetic constitution, the diversity in size of morphological characters between populations usually depends on environmental conditions (Alibert et al., 2001). We also consider that the variation of pronotum shape between these isolated populations occurs as differences in their genetic pools rather than selective pressure. Considering epigenetic effects of the emergence of phenotypes, these quantitative variations can be useful for taxonomical and evolutionary studies. The evaluation of other morphological characters via similar methods and the molecular analysis of genetic structure of these populations may lead to significant outcomes.

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