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Geometric morphometric study of two species of the psammophilous genus *Erodiontes* (Coleoptera: Tenebrionidae) from the Lute desert, Central Iran

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A geometric morphometric study of the genus *Erodiontes* was carried out using the Elliptic Fourier method to demonstrate the effect of vicariance on populations of these psammophilous species. Four populations of *Erodiontes*, comprising *E. aelleni* and *E. pfaundleri*, were sampled from Chartaghi, Aboozeidabad, Meser and Hojatabad. Differences between the shapes of the conspecific populations are minute, suggesting that the separation of conspecific populations is recent. In contrast, populations of *E. pfaundleri* showed significant differences in pronotum size whereas it was similar in size in populations of *E. aelleni*. This difference is believed to be due to environmental factors such as temperature and nutrition.

Key words: geometric morphometric, Erodiontes, Lute desert, Central Iran

INTRODUCTION

Deserts form 25-30% of the earth's land surface, and approximately 20% of world deserts are covered with sand. Sandy substrates cover 64% of the Sahara, 41% of the Libyan, 52% of the Arabian (Fet et al., 1998), and 34% of the Iranian deserts (Ahmadi, 1998). Sandy deserts support many animal species adapted to thrive in sand. Like other stenotopes, it is believed that psammophilous species are prone to greater diversification than eurytopes. It has been observed that strictly thinophilous species are unable to migrate to adjacent non-sandy areas or to sandy areas with different composition, compactness, and granularity. Non-sandy substrata alter the behavior of these animals and interrupt their reproduction (Prendini, 2001). Consequently, any separation of sand dunes could result in reproductive isolation of their psammophilous inhabitants.

Several large ergs are distributed in southeast and central Iran, of which the erg of Lute desert the largest, with an area greater than 10,000 Km² (Mahmoodi, 2002).

Erodiontes (Coleoptera: Tenebrionidae) are psammophilous beetles and are endemic to the central dunes of Iran. They live on sandy substrates and, like some other psammophilous darkling beetles (Henwood, 1975), exhibit bimodal daily activity, being active during the morning and late afternoon and remaining beneath the surface during the heat of the day and at night. In the morning they emerge from the sand and before the sand gets too hot at noon, individuals burrow into the sand and remain there to escape the heat. After several hours when the temperature of surface decreases, they come to surface again and remain there until dusk. The known distribution of *Erodiontes* spp. is presented in Table 1.

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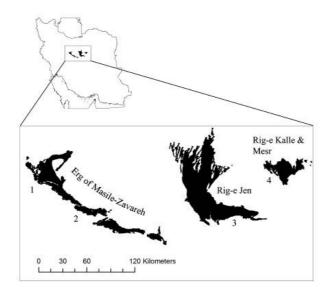


FIGURE 1.- The shape and location of the ergs (Mahmoodi, 2002) in central Iran and species collected: 1) Chartaghi: E. aelleni 2) Aboozeidabad: E. aelleni 3) Hojatabad: E. pfaundleri 4) Mesr: E. pfaundleri

TABLE 1. - The distribution of <i>Erodiontes</i> spp. in the Lute desert(Kaszab, 1979).
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Species	Localities			
E. aelleni	Chartaghi (between Kashan & Maranjab)			
E. pfaundleri	Rig-Jen desert, between Tabas & Anarak, Yazd			

TABLE 2.- Details of samples and origins of *Eriodontes* spp. From the Lute desert.

Locality	District	Species	n	Male	Female	Unknown	Sex Ratio F/M
Chartaghi	Kashan	E. aelleni	20	12	8	0	1.5
Aboozeidabad	S. Kashan	E. aelleni	39	23	15	1	1.53
Mesr	Jandagh	E. pfaundleri	52	35	14	3	2.5
Hojatabad	Jandagh	E. pfaundleri	56	32	23	1	1.39

Source software or format	Destination software or format		
.tps (outline)	.dat (EFA)		
.tps (outline)	PAST		
.tps (outline)	Morpheus		
.tps (outline)	CSV (comma separated value)		
NTS	CSV		
EFA (.out)	PAST		

TABLE 3.- Geometric Morphometric Tools Package data format conversions.

The objective of the study was to determine the different species of genus *Erodiontes* and evaluate the effect of vicariance on the morphology of the pronotum of *E. aelleni* and *E. pfaundleri* using the Elliptic Fourier method. A software application for facilitating data conversion and size calculation was developed.

MATERIAL AND METHODS

Sampling

Specimens of *Erodiontes* were collected, during their active phase, from the surface of the central dunes of Iran at 4 locations (Table 2). All specimens were killed, mounted, labeled, and numbered for photography and subsequent digitizing. Photographs were taken by Canon Power shot SX100 IS using a stand and lighting equipment. The software Remote Capture was used for controlling the camera by computer, which allows a preview of the picture before shooting. A millimeter scale was used for scaling the specimens.

Digitizing

TpsDig v2.12 was used for digitizing points around the pronotum using the Background Curve tool. The outline of the pronotum of all specimens was digitized at 150 points (Figure 2) and scaled for calculating the size variables.

Geometric Morphometric Tools Package

The Geometric Morphometric Tools Package (GMTP1) was recommended by the Rodents Research Department of Ferdowsi University of Mashhad and programmed by the senior author (Siavash Taravati) to facilitate the process of data conversion and size calculations. This software is able to perform conversions of several data formats non-programmed in TPS software (Table 3). In addition, the program GMTP was used to calculate centroid; centroid size, perimeter, and the cumulative area within the outline of all specimens of each .tps file and export the results in Comma Separated Values (CSV) format.

Elliptic Fourier analysis

The programs EFA and EFAWIN were used to conduct an elliptic Fourier analysis. Data were converted to EFA format (.dta) by GMTP and loaded into the EFAWIN software. The 15th harmonics were used, and results were chosen to be invariant to size, location, and rotation. The output file (.out) was converted to PAST format by GMTP, and a Canonical Variate Analysis (CVA) was performed to analyze the data.

Size

Three variables were used for analyzing size. Centroid size, area, and perimeter were calculated to determine significant differences among groups. For variables with normal distribution and similar variances, one-way ANOVA was employed, and for others, the Kruskal-Wallis test was used in the



FIGURE 2.- Pronotum of E. aelleni from Chartaghi showing 150 points around the outline.

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FIGURE 3.- Geometric Morphometric Tools Package (GMTP) screenshot

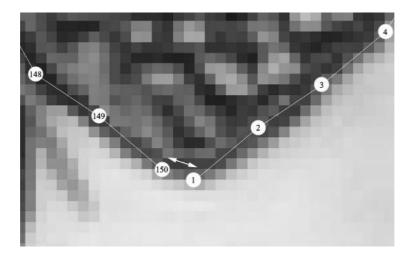


FIGURE 4.- Cropped photograph of the pronotum showing the anterior left portion of pronotum and the gap between the start and end point.

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Choose the type of calculation:	Open curve
Continue	Closed curve

FIGURE 5.- A window in GMTP asking the user to calculate the closed or open contour.

TABLE 4.- Tukey's pairwise comparisons for centroid Size (over diagonal) and perimeter (under diagonal) showing the *P* (same) value. Asterisks (*) indicate significant differences.

Population	Chartaghi	Aboozeidabad	Mesr	Hojatabad
Chartaghi		0.476	*7.721E-06	*8.125E-06
Aboozeidabad	0.5964		*7.721E-06	*0.0005177
Mesr	*7.72E-06	*7.72E-06		*1.239E-05
Hojatabad	*7.91E-06	*1.32E-04	*4.12E-05	

TABLE 5.- Mann-Whitney pairwise comparisons (Bonferroni corrected) of pronotum area. Asterisks

 (*) indicate significant differences.

Population	Chartaghi	Aboozeidabad	Mesr	Hojatabad
Chartaghi				
Aboozeidabad	0.5656			
Mesr	*9.76E-10	*8.28E-13		
Hojatabad	*1.31E-05	*6.75E-05	*1.4E-06	

PAST program to compare means and medians. For visualizing size differences among groups, a 95% confidence interval graph was plotted using SPSS 16.

All variables were calculated using GMTP. Data were imported to Excel and, subsequently, to PAST. The pronotum length and width were measured by tpsDig 2.

As the software EFA only accepts open outline coordinates (open polygons), the first and last point's coordinates will not coincide, and the program assumes that it must complete the contour itself. Therefore, the gap between the first and last point (Figure 4) will lead to incorrect area and perimeter calculation. GMTP solves this by automatically closing the contour for area and asking the user, in the case of perimeter calculation, to stipulate open or closed contour (Figure 5). When the Closed option is chosen, the program adds the gap between first and last point to the total length of the curve.

Allometry

For assessing the relation between size and shape, the correlation test was used between CS and PCA scores using the Pearson product-moment correlation coefficient and the PCA with highest correlation was plotted against CS.

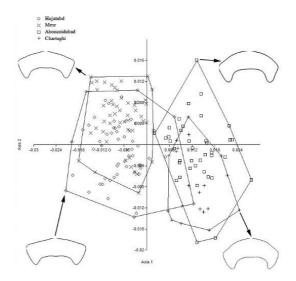


FIGURE 6.- Canonical variate analysis scatter plot of four samples of *Erodiontes* spp. Species are shown with different shape.

RESULTS

The CVA scatter plot of Elliptic Fourier coefficients showed that populations of each species occupy different areas of the graph but have some overlap (Fig.6). Conspecific populations have much more overlap than heterospecific populations.

ANOVA assumes data normality and homogeneity of variances. The CS data and perimeter were checked for normality (p>0.05) for each population, and the homogeneity of variances were checked by Levene's test (p>0.05). The *P* value for ANOVA of CS was 1.586E-21 (< 0.05) and 4.75E-21 for perimeter; therefore we rejected the null hypothesis and concluded that the means of both pronotum CS and perimeter for at least two populations are significantly different from each other. Tukey's test was used to determine which populations were different from one another. The results of Tukey's pairwise comparisons for both CS and perimeter variables are given in Table 4.

Similarly, the ANOVA assumptions were tested for pronotum area, and showed that the normality assumption must be rejected. Consequently, the non-parametric Kruskal–Wallis test was conducted, and the result (p=1.466E-18 < 0.05) showed that the null hypothesis of similarity of medians must be rejected, and there is a significant difference between the medians of at least two populations. Table 5 shows the Bonferroni corrected Mann–Whitney pairwise comparison for all populations.

Confidence interval is another way of visualizing the difference between means of two or more populations. Means and 95% confidence intervals for CS and pronotum length are presented in Figure 7. For assessing the power of multivariate analysis, the length/width ratio was calculated for each specimen, and its confidence interval was obtained for comparing the results of multi-width univariate analysis.

For assessing correlation between size and shape, the Pearson product-moment correlation was used to find the highest correlation between the first three PC axes scores and CS. The scores of PC2 had the highest correlation (r = -0.56), which were used to plot against CS (Fig.9).

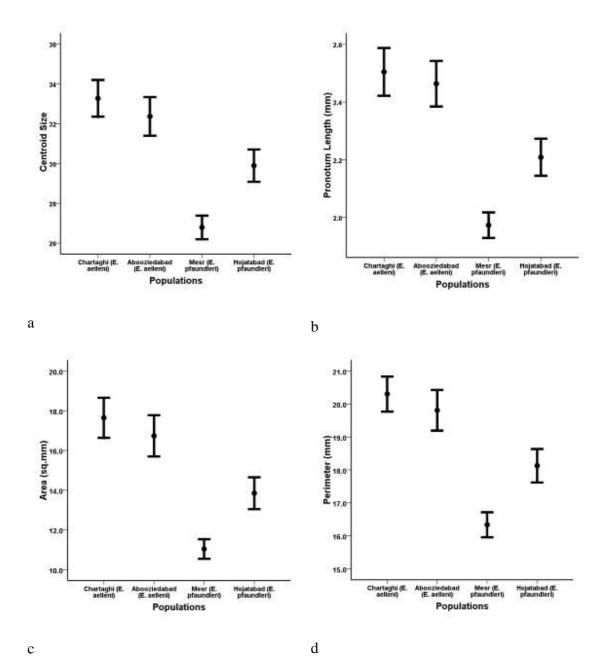


FIGURE 7.- Means and 95% confidence intervals for four size variables: a (centroid size), b (pronotum length) c (area), d (perimeter).

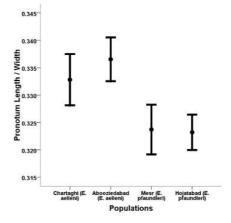


FIGURE 8.- The 95% confidence intervals of pronotum length/width ratio of four *Eriodontes* spp. populations.

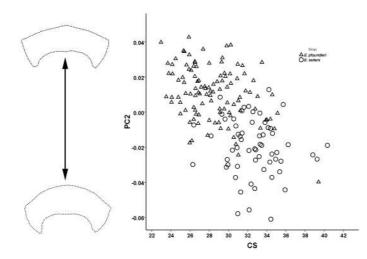


FIGURE 9.- PC2 scores plotted against CS. Species are identified by symbol shape

DISCUSSION

The results of ANOVA for CS and pronotum length showed a significant difference among all populations except those between samples from Chartaghi and Aboozeidabad. The homogeneity of populations of *E. aelleni* was also visible in the confidence interval graphs which show high overlap among these populations, whereas the two populations of *E. pfaundleri* not only did not overlap with those of *E. aelleni* but also did not overlap with each other (Figure 7).

The CVA showed that the shape of the pronotum of the two species differs with respect to curvature. Nevertheless, populations of each species greatly overlap. As a result, a new variable (pronotum length/width) was defined, measured, and analyzed to verify this difference (Figure 8). Similar to the multivariate results, this variable showed significant difference between heterospecific populations. In general, individuals of *E. aelleni* have a larger and more curved pronotum (Fig.9), and the curvature of the pronotum increases with beetle size.

The geographic distance between populations of *E. aelleni* (Chartaghi to Aboozeidabad-Hosseinabad village) is ~52 km and the separation distance of populations of *E. pfaundleri* (Hojatabad to Mesr) is ~65 km. Although these distances are similar, there is a great difference in the continuity of the sand systems between the populations of *E. pfaundleri*. The map of distribution of sandy soils (Fig. 1Error! Reference source not found.) shows the intermediate area between populations of *E. aelleni* to be almost continuously covered with sand and the erg of Maranjab is nearly a unified sand system extending to the habitat of *E. aelleni* at Hosseinabad. In contrast, the sand systems which are inhabited by *E. pfaundleri* are separated by a minimum distance of 40 km. As mentioned, the dependency of psammophilous animals on their sandy substrate prevents them from crossing non-sandy areas. Therefore, the homogeneity of size in *E. aelleni* populations and the significant differences in size in the populations of *E. pfaundleri* could be a result of the discontinuity of the sandy substrate, which itself is highly dependent on climatic conditions.

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