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Morphological variations based on geometric morphometrics between male and female pronota of *Oxythyrea cinctella* (Schaum, 1841) (Coleoptera: Scarabaeidae, Cetoniinae)

***Oxythyrea cinctella*'nın (Schaum, 1841) (Coleoptera: Scarabaeidae, Cetoniinae) erkek ve dişi pronotumu arasındaki geometrik morfometriye dayalı morfolojik varyasyonlar**

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Abstract
In this study, pronotum size and shape were used as an exemplar characteristic to evaluate the utilit of pronotal morphology on the sexual dimorphism determination of the scarab beetle *Oxythyre cinctella* (Schaum, 1841). Using geometric morphometrics, the sexual size and shape dimorphism c pronotum of 85 specimens (45 females, 40 males) collected from Ankara Province (Turkey) wer analyzed. Results of geometric morphometrics revealed that there is statistically significant sexual siz and shape dimorphism in pronotum. Further, the mean centroid sizes of the females was significantl larger than that of the males. Finally, multivariate regression results indicated that size has negligibl influence on the differentiation in pronotum shape between sexes. We hope the results here presente would be helpful for the further understanding of the sexual dimorphism in the beetle genus *Oxythyre*

Özet
Bu çalışmada, pronotal morfolojinin *Oxythyrea cinctella* (Schaum, 1841)'nın eşeysel dimorfizmini tayini için pronotum büyüklüğü ve şekli örnek bir özellik olarak kullanılmıştır. Ankara ilinden (Türkiye toplanan 85 örneğin (45 dişi, 40 erkek) pronotumu geometrik morfometri ile eşeysel boyut ve şek dimorfizmi analiz edildi. Geometrik morfometrinin sonuçları, pronotumda istatistiksel olarak anlam eşeysel boyut ve şekil dimorfizmi olduğunu ortaya koydu. Ayrıca, dişilerin ortalama centroid boyut erkeklerden önemli ölçüde daha büyüktü. Son olarak, çok değişkenli regresyon sonuçları, boyutu eşeyler arasındaki pronotum şeklindeki farklılaşma üzerinde ihmal edilebilir bir etkiye sahip olduğun göstermiştir. Burada sunulan sonuçların *Oxythyrea* cinsi böceklerde eşeysel dimorfizmin daha i anlaşılması için yararlı olacağını umuyoruz.

INTRODUCTION

Oxythyrea cinctella (Schaum, 1841) belongs to the genus *Oxythyrea* Mulsant, 1842 in the subfamily Cetoniinae Leach, 1815 within the family Scarabaeidae Latreille, 1802. Like *O. cinctella*, phytophagous beetles of Scarabaeidae are plant pests of great economic importance in agriculture, forestry, and horticulture (Jackson and Klein 2006). *O. cinctella* is an economically significant pest that has the potential to cause regular damage to agriculture and native ecosystems. This species was included in the lists of harmful fauna as examples reported in apricot orchards (Öztürk et al. 2004, Apak 2021), cherry orchards (Tezcan and Pehlivan 2001), oil-bearing rose production fields (Demirözer and Karaca 2011, Demirözer et al. 2011), temperate region fruit trees (Özbek 2008), ornamental plants and saplings (Kaygin et al. 2008) in Turkey and the neighbouring countries (Modarres Awal 2006, Augul 2016, Bunalski et al. 2016, Yusifov et al. 2016).

Geometric morphometrics has increasingly become more available tool to reveal previously undocumented morphological patterns in entomological research (Tatsuta et al. 2018). Internal or external rigid structures of the many insects such as pronotum, elytra, mandible, genitalia, horn, hind tibia, hind femur, metendosternite have been widely used in the geometric morphometric-based assessment of morphological variations (Pretorius et al. 2000, Garnier et al. 2005, Rodrigues et al. 2005, Polihronakis 2006, Romiti et al. 2017, Da Silva et al. 2018, Juache et al. 2018, Kerman et al. 2018, Zhang et al. 2019). Of these, the pronotum, the hardened dorsal plate of the prothorax, is of great importance not only to bear important muscles and support the locomotion of prothoracic legs (Garnier et al. 2005, Zhang et al. 2019) but also provide some constant characters, present some differences between the species and offer useful characters in classification (Wood 1963, Ali 1978). Besides, previous studies have indicated that the morphological characteristics of the pronotum are used to help determine sexual dimorphism (Assing 2006, Pomfret and Knell 2006, Petrov et al. 2007, Reaney and Knell 2015, Li et al. 2020). Moreover, much of our understanding of the morphological variations between male and female pronota are derived from geometric morphometric researches (Vergara et al. 2014, Eldred et al. 2016, Carillo and Dela Cruz 2018, Vesović et al. 2019, Regueira et al. 2020).

Although geometric morphometric studies have currently focused on the determination of intraspecific shape variations (Polihronakis 2006), morphological differentiation patterns in polyphenic sister species (Pizzo et al. 2006), differentiating new species (Li et al. 2016), the characterization of biogeographically isolated populations (Barros et al. 2020) of various scarab beetles, none attempt has been made on *Oxythyrea* beetles so far. Here we thus aimed to assess the degree of sexual dimorphism between the sexes of *O. cinctella* through pronotum morphology by using landmark-based analyses for the first time.

MATERIALS AND METHODS

In this study, specimens of *O. cinctella* were collected from Ankara Province (Ayaş, Kazan, Kızılcahamam) (Figure 1), Turkey on March-April 2020 and preserved in 70% ethanol. All individuals were dissected using fine forceps and insect pins under a stereomicroscope to observe genitalia for sexing them.

The pronotum of the specimens were photographed in a dorsal view using a Leica camera attached to a Leica binocular microscope. TPSdig2 (Rohlf 2017) program was used to digitize and save 13 landmarks for the dorsal view of the pronotum (Figure 2). The landmark coordinates of 85 specimens (45 females, 40 males) were used for the pronotum size and shape analysis.

Figure 1. Adult specimens of *Oxythyrea cinctella* on various plants (© Y. Koçak)

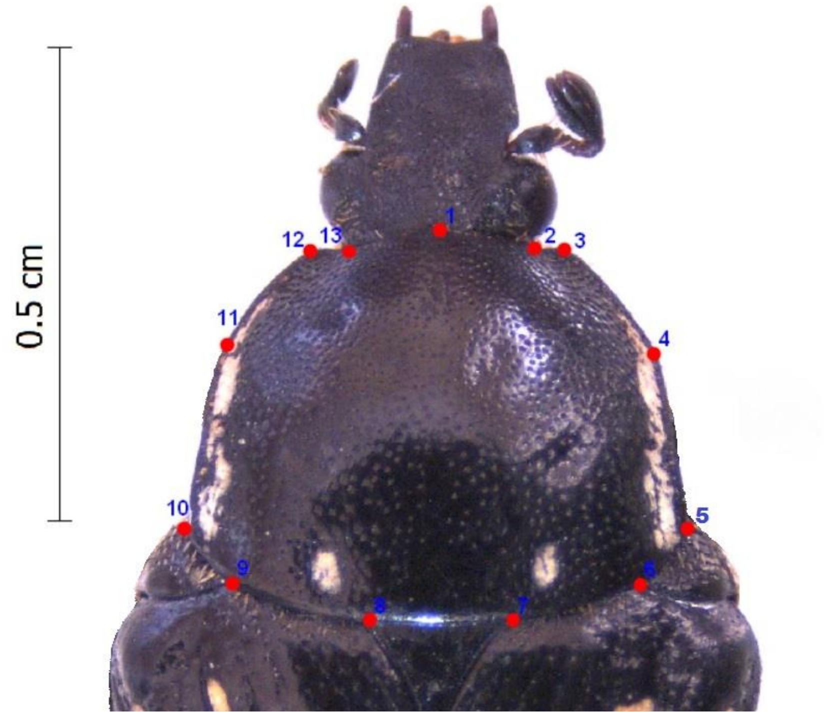

Figure 2. Dorsal view of *Oxythyrea cinctella* showing positions of 13 landmarks used to define the shape of pronotum

Landmark-based morphometric methods were used asses to pronotum shape and size between sexes and MophoJ v1.07a (Klingenberg 2011) was used for all analyses of landmarks configurations. To compare pronotum size between sexes, the centroid size (CS) (square root of the sum of the square distances between each landmark and the centroid) (Bookstein 1986) was computed. The independent samples t-test was performed using the IBM SPSS25 to test pronotum size variation between sexes. 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). Principal component analysis (PCA) was used to reduce the dimensionality of the landmark dataset and so into finding new variables that are linear functions of those in the landmark dataset, that successively maximize variance, and that are uncorrelated with each other. Then multivariate regression analysis was performed to determine the effect of size on shape. Finally, discriminant function analysis (DFA) with cross validation was performed to determine the degree of morphological distinction between sexes.

RESULTS AND DISCUSSION

Size Variation

The independent samples t-test showed that the CS means of males is significantly different from that of females (Table 1). Figure 3 shows a box-plot of CS between sexes. Further, the females appear to be larger than the males for CS of pronotum.

Table 1. Independent t-test results

						Levene's Test for Equality of Variances		t-test for Equality of Means		
	Sex	N	Mean	Std. Deviation	Std.Error Mean	F	Sig.	t	df	Sig.
CS	Female	45	1092.86	90.25	13.45	1.354	0.248	2.594	83	0.011
	Male	40	1045.28	77.29	12.22					

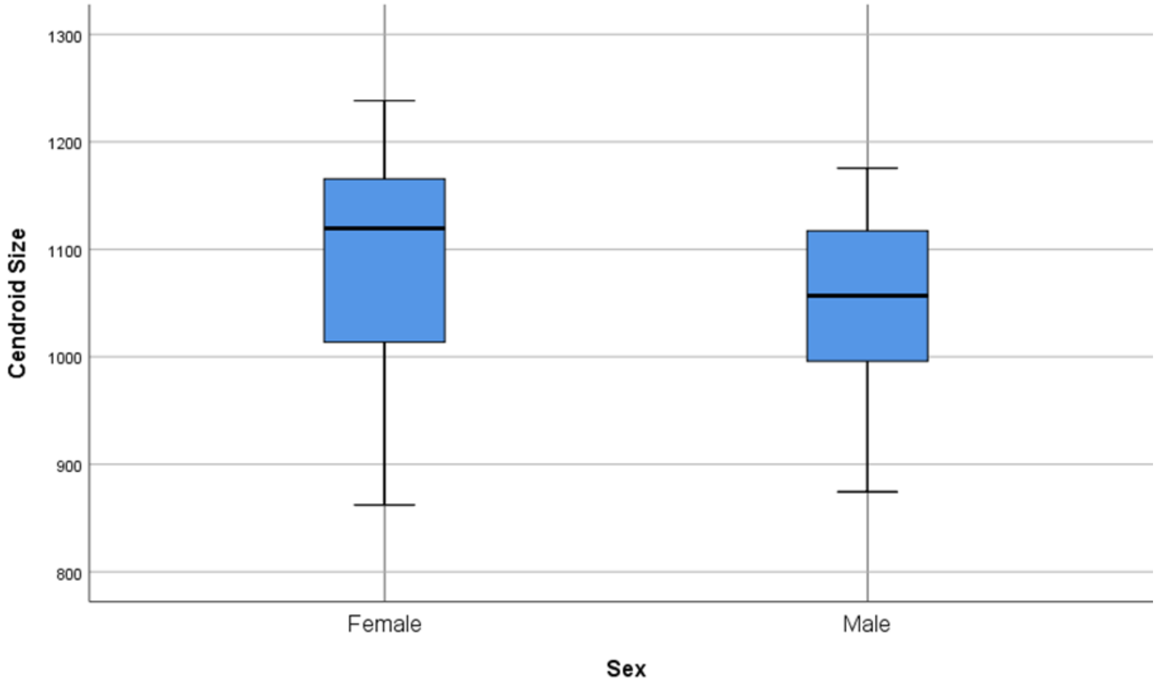

Figure 3. Box-plot of centroid size for pronotum for males and females of *Oxythyrea cinctella*

Shape Variation

Principal component analysis (PCA) showed that 41.3% of the total variation of pronotum shape was explained by the first two principal components (PC1 explains 26.7% and PC2, 14.6%) (Table 2). Twenty-two main components were required to explain the total shape variation of pronotum.

Table 2. Principal component analysis results

	Eigenvalues	Variance%	Cumulative%
1	0.00049861	26.669	26.669
2	0.00027354	14.631	41.299
3	0.00021696	11.604	52.903
4	0.00015034	8.041	60.944
5	0.00011493	6.147	67.091
6	0.00010848	5.802	72.894
7	0.00008214	4.393	77.287
8	0.00006132	3.280	80.567
9	0.00005999	3.209	83.775
10	0.00005784	3.094	86.869
11	0.00005080	2.717	89.586
12	0.00003809	2.037	91.623
13	0.00002949	1.577	93.200
14	0.00002504	1.340	94.540
15	0.00002427	1.298	95.838
16	0.00001916	1.025	96.863
17	0.00001607	0.860	97.723
18	0.00001302	0.696	98.419
19	0.00001018	0,545	98.963
20	0.00000759	0.406	99.369
21	0.00000603	0.323	99.692
22	0.00000576	0.308	100.000

Although multivariate regression of shape on centroid size was statistically significant that explained only 4.64 percent of the total variation in the shape of pronotum (4.64%, $p = 0.0003$). On the other words, pronotum shape differences have not related to the size of the pronotum.

Discriminant function analysis (DFA) was performed to determine the degree of morphological separation between the sexes. Cross-validated correct classification percentage was 82.36% between sexes (86.7% for females and 77.5% for males) (Figure 4). DFA showed that there is a statistically significant difference between means of procrustes distance of sexes ($p < 0.0001$).

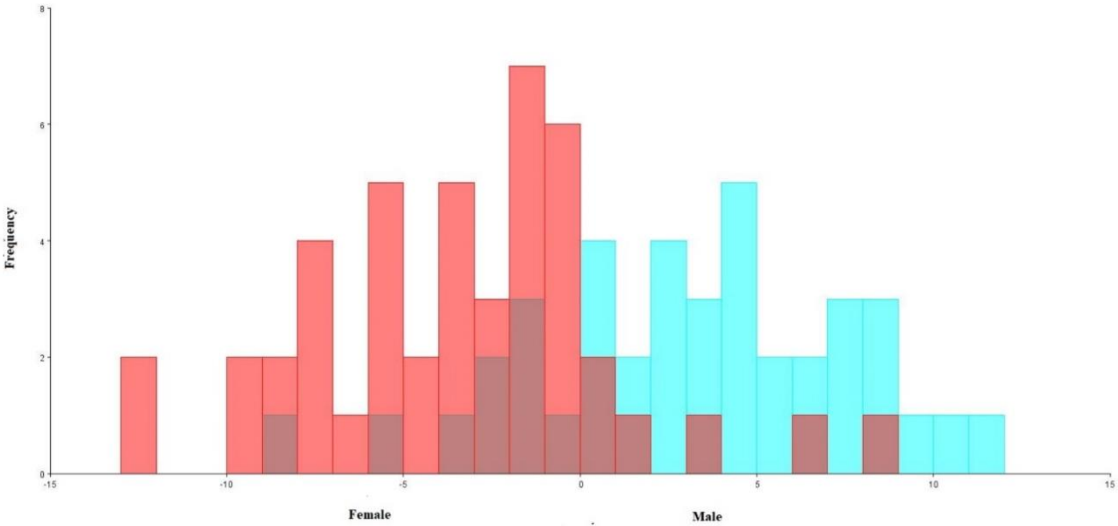

Figure 4. Cross-validation scores of shape variables of pronotum

Also, the results of DFA show that all the landmarks with the greatest variation indicating that females have a wider and shorter pronotum than males. This is also related to elongation and sharpened from both anterior and posterior parts of the pronotum shape in males (Figure 5). According to shape variation in landmarks 3, 4, 5, 6, 8, 9, and 10 females' pronota were wider and shorter than those of the males.

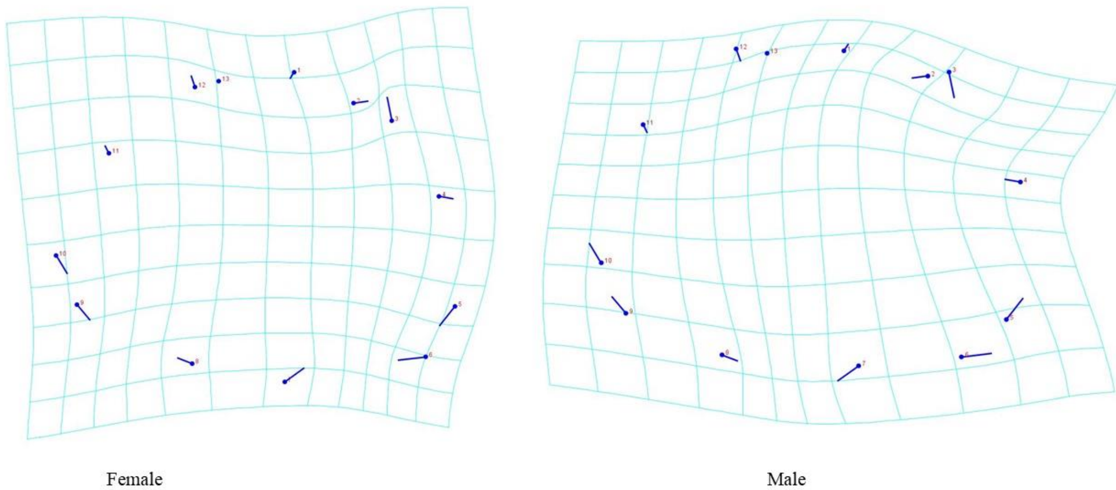

Figure 5. The dots show the average of the female and male specimens (The length of the lines shows the amount of change between the sexes and in which direction this change is directed)

The main goal of the present study is to apply geometric morphometrics for the first time to pronotum of the scarab beetle species, *Oxythyrea cinctella* in order to assess the presence of sexual dimorphism in size and shape. Significant sexually dimorphic differences have been found in the present study confirming earlier pronotal studies in beetles (Eldred et al. 2016, Li et al. 2016, Ober and Connolly 2015, Pizzo et al. 2006). As a conclusion, it can be stated that sexual dimorphism in the pronotum size and shape of *O. cinctella* can be used to discriminate between sexes. Moreover, it shows that the pronotum could be the preferred structure for morphometric analyses since it is particularly suitable for landmark based geometric morphometrics. Finally, additional studies are needed to explore reasons (ecological niche, feeding and reproductive behaviors etc.) behind sexual differences in the pronotum size and shape. Further studies including different populations of this species plus different body parts (head, elytra etc.) should also be performed to understand the process in which sexual dimorphism is affected.

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