



Original article (Orijinal araştırma)

Sexual dimorphism in the Anatolian endemic tiger beetle, *Cephalota circumdata* ssp. *cappadocica* Franzen, 1996 (Coleoptera: Carabidae: Cicindelinae): a study showing the effectiveness of geometric morphometrics¹

Anadolu endemik kaplan böceği, *Cephalota circumdata* ssp. *cappadocica* Franzen, 1996 (Coleoptera: Carabidae: Cicindelinae)'de eşeyssel dimorfizm: geometrik morfometrinin etkinliğini gösteren bir çalışma

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Abstract

Sexual dimorphism is an important source of intraspecies variation in tiger beetles. However, little is known about sexual dimorphism in tiger beetles. This article contributes the literature in the field of sexual dimorphism by comparing the morphology of males and females in the context of phenotypic changes in the head and pronotum of endemic tiger beetle *Cephalota circumdata* ssp. *cappadocica* Franzen, 1996 (Coleoptera: Carabidae: Cicindelinae). All the specimens examined in the study were gathered during May and August of 2016 from salty soils around Seyfe Lake located in Kırşehir Province, Turkey. Specifically, the efficacy of geometric morphometrics was assessed in the analysis of sexual dimorphism of tiger beetles. Statistically significant differences were found in the head and pronotum shape variation and regression results indicated that size has little influence on the differentiation of shape among sexes. Moreover, the jackknifed cross-validated correct classification percentages for head and pronotum were 88% and 85%, respectively when using only the shape variables. Consequently, geometric morphometrics is an effective and useful method to determine sexual dimorphism in tiger beetles.

Keywords: *Cephalota circumdata* ssp. *cappadocica*, Cicindelinae, geometric morphometrics, sexual dimorphism

Öz

Eşeyssel dimorfizm, kaplan böceklerinde türler arası varyasyonun önemli bir kaynağıdır. Buna rağmen, kaplan böceklerindeki eşeyssel dimorfizm hakkında az şey bilinmektedir. Bu makale, endemik kaplan böceği *Cephalota circumdata* ssp. *cappadocica* Franzen, 1996 (Coleoptera: Carabidae: Cicindelinae)'nin baş ve pronotumundaki fenotipik değişiklikler bağlamında erkek ve dişilerin morfolojisini karşılaştırarak eşeyssel dimorfizm alanındaki literatüre katkıda bulunmaktadır. Araştırmada incelenen tüm örnekler, 2016 yılında Mayıs-Ağustos ayları arasında Kırşehir (Türkiye) ilinde bulunan Seyfe Gölü çevresindeki tuzlu topraklardan toplanmıştır. Spesifik olarak, kaplan böceklerinin eşeyssel dimorfizminin analizinde geometrik morfometrinin etkinliği değerlendirilmiştir. Baş ve pronotum şekil varyasyonlarında istatistiksel olarak anlamlı farklılıklar bulunmuş ve regresyon sonuçları cinsiyetler arası bu şekil farklılaşmasında büyüklüğün etkisinin çok az olduğunu göstermiştir. Ayrıca sadece şekil değişkenlerinin kullanıldığında, Jack-knife çapraz geçerlenmiş doğru sınıflandırma yüzdesi sırasıyla baş için %88 ve pronotum için %85 olarak bulunmuştur. Sonuç olarak geometrik morfometri, kaplan böceklerinde eşeyssel dimorfizmin belirlenmesinde etkili ve kullanışlı bir yöntemdir.

Anahtar sözcükler: *Cephalota circumdata* ssp. *cappadocica*, Cicindelinae, geometrik morfometri, eşeyssel dimorfizm

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Introduction

Sexual differences are often dramatic and widespread among taxa (Wyman et al., 2013). It is possible to see many striking examples of this among animal species where dramatic changes have been identified in males and females (Teder, 2014). For insects in particular, knowledge of the amount of sexual dimorphism is a critical source of information for exploring and exploiting variation in the life history, morphology, physiology and behavior. Of these, morphology is still used extensively in insect taxonomy. Morphological sexual dimorphism, if not taken into account, could lead to a taxonomic misunderstanding because males and females may be described as separate species. Additionally, a better documenting of subtle differences between the sexes could be of significance in understanding the biology, especially diagnosis and accurate identification of insects (Punzalan & Hosken, 2010; Allen et al., 2011; Virginio et al., 2015; Baig et al., 2016; Moraes et al., 2016).

The insect order Coleoptera possess a large number and great diversity of sexually dimorphic species. Sexual differences are generally unnoticeable in most beetle species (at least visually). The male and female can be distinguished only by minor (often microscopic) morphological differences (Kawano, 2006; Maeno et al., 2012; Benitez et al., 2013; Hsiao et al., 2015). Hence, the challenge is finding appropriate groups of species on which to test models of sexual dimorphism. One group of beetles that may be ideal for these purposes is the family Carabidae (tiger beetles), which have sclerotized body parts suitable for morphometric descriptions (Pearson, 1988; Pearson & Vogler, 2001). Some previous studies also lend support to this notion. Kritsky & Simon (1995) examined the mandibles of five tiger beetle species to determine sexual dimorphism in their size and tooth arrangement. Satoh et al. (2003) measured the mandible length of nine tiger beetle species to show sexual dimorphism. Satoh & Hori (2004) reported that *Lophyridia angulata* (Fabricius, 1798) (Coleoptera: Carabidae: Cicindelinae) has sexual dimorphism in mandible length and has large interpopulation differences in mandible size. Franzen & Heinz (2005) evaluated sexual dimorphism in the enigmatic tiger beetle, *Mantica horni* Kolbe, 1896 (Coleoptera: Carabidae: Cicindelinae) based on morphometric values of elytral length. Jaskula (2005) detected sexual dimorphism in Polish population of the tiger beetle *Cicindela hybrida* ssp. *hybrida* Linnaeus, 1758 (Coleoptera: Carabidae: Cicindelinae) via biometric studies measured some parameters of the mandibles, elytra and pronota. Cassola & Bouyer (2007) mentioned the strongly sexually dimorphic labrum for the African tiger beetle genus *Neochila* Basilewsky, 1953 (Coleoptera: Carabidae: Cicindelinae). Franzen (2007) investigated several populations of tiger beetles of the *Cicindela campestris* Linnaeus, 1758 (Coleoptera: Carabidae: Cicindelinae) group from southern Turkey and Lebanon with respect to 10 morphometric ratios of head, pronotum, elytra, aedeagus and antenna considering possible sex dependent variations. Ball et al. (2011) stated that the genus *Manticora* Fabricius, 1792 (Coleoptera: Carabidae: Cicindelinae) has the pronouncedly sexually dimorphic mandibles. Young (2015) obtained various body measurements including length of the mandibles, elytra and mesothoracic legs to show possible size differences between the sexes. Jaskula et al. (2016) tested whether variation of morphometric traits between males and females in *Calomera littoralis* (Fabricius, 1787) (Coleoptera: Carabidae: Cicindelinae) that included head, pronotum, elytra and mandible measurements.

In the studies of sexual dimorphism in tiger beetles or other beetles, standard morphometrics has tended to focus on measuring linear distances such as length, width and height, until geometric morphometrics was created and has gained prominence over time. Among the phenotypic tools, this technique has recently been increasingly used in insect taxonomy and systematics to resolve complex taxonomic issues at the species level (Baracchi et al., 2011; Gómez et al., 2013; Liu et al., 2016). Besides, equally importantly, geometric morphometrics can be a valuable means to reveal, quantify and analyze subtle variations in the case of males and females presenting quite similar external morphology (Camargo et al., 2015). Exact geometric descriptions of morphological differences between the same structures in both sexes are produced via geometric morphometric analysis (Pretorius & Scholtz, 2001). Geometric

morphometrics, thus, has become an indispensable tool for sexual dimorphism studies in beetles (Benitez et al., 2013; Jun-Yan et al., 2015; Eldred et al., 2016; Vesović et al., 2019). Of these, less familiar is the use of geometric morphometrics to detect the degree of sexual dimorphism in species of tiger beetles except for recent work on the four tiger beetle species. Jones & Conner (2018) quantified shape differences in the mandibles of these species via geometric morphometric technique to look at both intraspecific sexual dimorphism as well as interspecific differences.

Within the tiger beetle genus *Cephalota* Dokhtourov, 1883 (Coleoptera: Carabidae: Cicindelinae), the endemic tiger beetle *Cephalota circumdata* ssp. *cappadocica* Franzen, 1996 (Coleoptera: Carabidae: Cicindelinae) distributes along banks of the salt lakes in the central Anatolia (Franzen, 1996; Cassola, 1999; Gebert, 1999; Azadbakhsh & Nozari, 2015; Matalin & Chikatunov, 2016). The degree of morphological sexual dimorphism in *C. c.* ssp. *cappadocica* is slight and the differences between males and females are not quite as marked. Furthermore, there are no known studies on the description of sexual dimorphism on *C. c.* ssp. *cappadocica*. These factors, therefore, stimulated our interest in this beetle in which we specifically opted and compared head and pronotum.

The principal goal of this study is to evaluate the performance of geometric morphometrics to elucidate sexual dimorphism in the head and pronotum of tiger beetle species, using *C. c.* ssp. *cappadocica* as a model species. Results of this research could provide the groundwork for follow up studies.

Materials and Methods

Data collection and landmark digitizing

All the specimens employed in the study were gathered during May and August of 2016 with entomological hand net from salty soils around Seyfe Lake in Kırşehir Province, Turkey (Figure 1) and preserved in 70% ethanol. Morphological identification was performed by second author (Yavuz Koçak). Specimens of the two sexes were recognized by dissecting the genitalia since it was not possible to visually assess the sex of adult beetles.



Figure 1. Seyfe Lake in Kırşehir Province, Turkey (satellite image from Anonymous, 2020).

Each specimen was photographed with a camera attached to the Leica microscope separately for head and pronotum. Twelve landmarks on the head and 10 landmarks on the pronotum were digitized once for each image using TPSdig2 (Rohlf, 2017) (Figure 2a, b) (Table 1). The landmark coordinates of 60 specimens (30 females and 30 males) were used for the head and pronotum shape analyses. MorphoJ v1.03a (Klingenberg, 2011) was used for analyses configurations of landmarks.

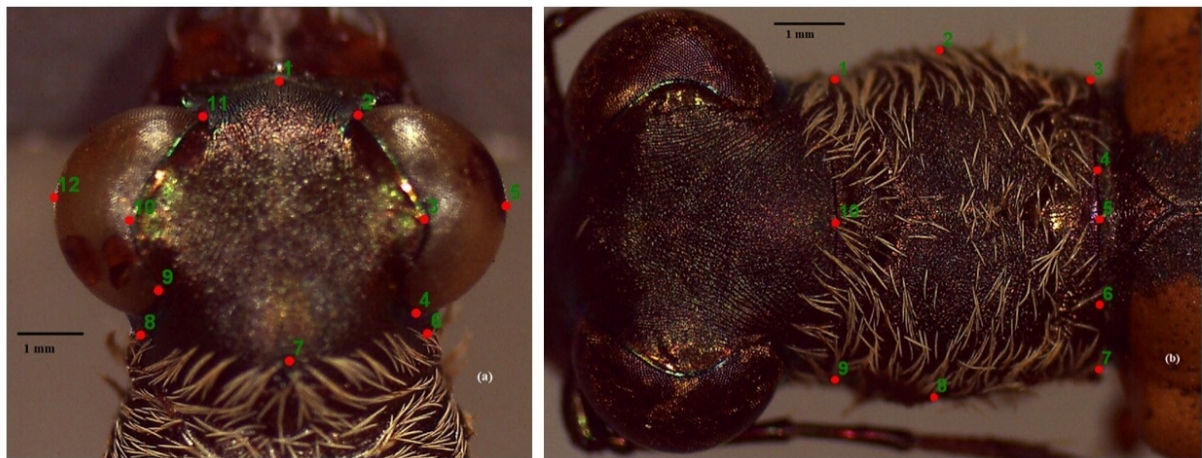


Figure 2. Dorsal views of the landmarks used to define the shape of a) head and b) pronotum of *Cephalota circumdata* ssp. *cappadocica* specimens (see description of landmarks in Table 1).

Table 1. Morphological landmarks used in this study

Landmark	Description	Landmark	Description
1	The center of the anterior margin of the clypeus	1	The top of the right fore pronotal angle
2	Anteriormost of the right eye	2	Point at half the length of the right lateral pronotal margin
3	Leftmost at maximum width of the right eye	3	The top of the right hind pronotal angle
4	Posteriormost of the right eye	4	The right pronotal base emargination
5	Rightmost at maximum width of the right eye	5	The center of the pronotal base
Head	6 The intersection of the pronotum with the right posterior of the head	Pronotum	6 The left pronotal base emargination
7	Center of the posterior part of the head	7	The top of the left hind pronotal angle
8	The intersection of the pronotum with the left posterior of the head	8	Point at half the length of the left lateral pronotal margin
9	Posteriormost of the left eye	9	The top of the left fore pronotal angle
10	Rightmost at maximum width of the left eye	10	The center of the anterior pronotal margin
11	Anteriormost of the left eye		
12	Leftmost at maximum width of the left eye		

Geometric morphometric analyses

Landmark-based morphometric methods were used as these are efficient in capturing information about the biological shape and lead to potent statistical approaches for analyze the difference of shape. In addition, these methods allow quantitatively accurate and clear viewing of shape changes (Rohlf & Marcus,

1993). Generalized procrustes analysis (GPA) superimposed the specimens into a common coordinate system and mathematically eliminated the effects of digitizing position, orientation and scale (Rohlf & Slice, 1990). The software package MorphoJ was used to perform the GPA. To compare overall head and pronotum size between sexes, the centroid size (square root of the sum of the square distances between each landmark and the centroid) (Bookstein, 1986) was computed. Centroid size (CS) was used for it is a measure of size that is independent of shape in absence of allometry (Bookstein, 1991).

Centroid size and shape variables were used in following statistical analyses. First, the mean CS of sexes were compared for each structure by using independent groups t-test and visualized using a boxplot. Second, a principle component analysis using the covariance matrix of the procrustes shape coordinates was conducted to degrade dimensionality of the data to ensure the necessity of the parametric test. Covariance matrix of the shape coordinates and PC scores was generated in MorphoJ. A multivariate analysis of variance (MANOVA) was performed using the PC scores as shape variables to test whether sex have significant effects on the average shape of head and pronotum. Then multivariate regression of shape onto size was used to explore how shape varies with size by using PAST 3 (Hammer et al., 2001). Finally, discriminant function analyses (DFA), using jackknifed cross validation, was performed for head and pronotum structures, separately, using only the shape variables and then using shape and CS by using PAST 3. The independent samples t-test and MANOVA were performed using the IBM SPSS 25.

Results and Discussion

For head and pronotum, Kolmogorov-Smirnov test revealed a normal distribution of each sex group ($p > 0.05$) and Levene's test showed that variance of centroid size is equal across sex groups for head ($F = 3.25$, $p = 0.076$) but not equal for pronotum ($F = 14.4$, $p = 0.000$). The independent groups t-test showed that the CS mean of males is significantly different from that of the females, for both structures. (for head $t = 6.68$, $df = 58$, $p = 0.000$, and for pronotum $t = 2.54$, $df = 40$, $p = 0.015$). Figure 3 shows box plots of CS for head and pronotum. Further, distributions of females appear to be more variable with respect to CS than males and females are larger than males for both head and pronotum.

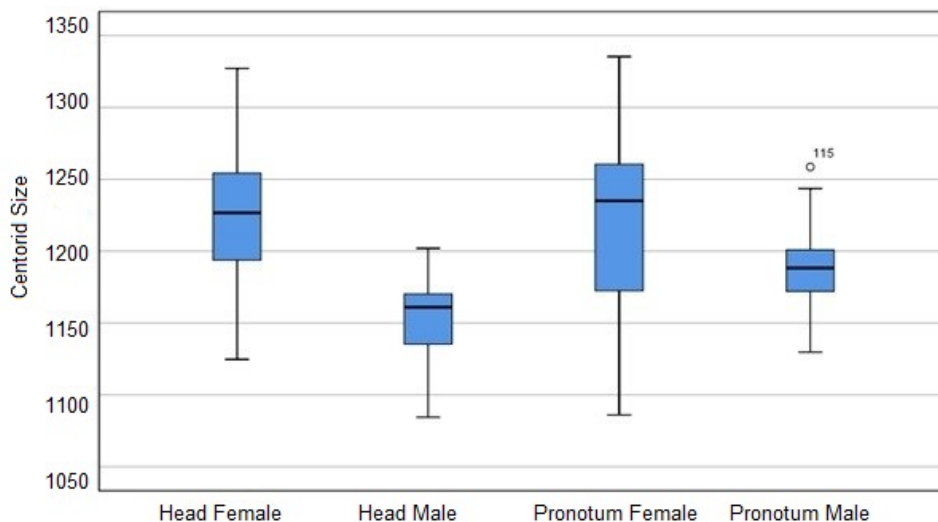


Figure 3. Boxplots of centroid size for head and pronotum for males and females of *Cephalota circumdata* ssp. *cappadocica*.

For head, first two principle components explain 60.8% of total variance (PC1 and PC2 explains 40.1% and 20.7%, respectively). Eight components were necessary to explain more than 90% of the head shape variation. For pronotum, first two principle components explain 41.2% of total variance (PC1 and PC2 explains 21.9% and 19.3%, respectively). Nine components were necessary to explain more than 90%

of the pronotum shape variation. MANOVA (PCs as shape variables) procedure detected significant difference between shape of females and males both head and pronotum (for head; Pillai's Trace = 0.613, $p = 0.000$, for pronotum; Pillai's Trace = 0.727, $p = 0.000$). Multivariate regression of shape onto size results indicated that size has only 9.23% influence on the differentiation in head shape among sexes. Similarly, size has only 5.24% influence on the differentiation in pronotum shape among sexes.

DFA for head and pronotum were first run on only the shape variables and then run on both shape variables and CS. DFA, MANOVA and multivariate regression were performed using the first eight principal components for head. The jackknifed cross-validated correct classification percentage for head is 88% (90% for females and 87% for males) when using only the shape variables (Table 2). In addition, when CS is added to DFA, the jackknife cross-validated correct classification percentage increased to 93% for females, but stayed the same for males (87%).

Table 2. Jackknifed correct classification summary of head shape

Head	Shape Variables		Shape Variables and Centroid Size		
	Classification of females (<i>n</i>)	Classification of males (<i>n</i>)	Classification of females (<i>n</i>)	Classification of males (<i>n</i>)	
Female	(27/30) 90.0%	(3/30) 10.0%	(28/30) 93.3%	(2/30) 06.7%	
Male	(4/30) 13.3%	(26/30) 86.7%	(4/30) 13.3%	(26/30) 86.7%	
Total	(31/60) 51.7%	(29/60) 48.3%	(32/60) 53.3%	(28/60) 46.7%	
		88% of cross-validated grouped cases correctly classified		90% of cross-validated grouped cases correctly classified	

DFA, MANOVA and multivariate regression were performed using the first nine principal components for pronotum. The jackknifed cross-validated correct classification percentage for pronotum is 85% (87% for females and 83% for males) when using only the shape variables (Table 3). In addition, when CS is added in the DFA, the jackknifed cross-validated correct classification percentage was same for females but increased to 90% for males. Notably, cross validation results in a higher classification for females than males only except when using both shape variables and centroid size for pronotum.

Table 3. Jackknifed correct classification summary of pronotum shape

Pronotum	Shape Variables		Shape Variables and Centroid Size		
	Classification of females (<i>n</i>)	Classification of males (<i>n</i>)	Classification of females (<i>n</i>)	Classification of males (<i>n</i>)	
Female	(26/30) 86.7%	(4/30) 13.3%	(26/30) 86.7%	(4/30) 13.3%	
Male	(5/30) 16.7%	(25/30) 83.3%	(3/30) 10.0%	(27/30) 90.0%	
Total	(31/60) 51.7%	(29/60) 48.3%	(29/60) 48.3%	(31/60) 51.7%	
		85% of cross-validated grouped cases correctly classified		88% of cross-validated grouped cases correctly classified	

Also, the results of DFA show that the landmarks with the greatest variation were numbers 1, 2, 3, 6, 7, 8, 11 and 12 indicating that females have a wider and shorter head with relatively larger eyes than males. This is also related to elongated and sharpened from both anterior and posterior parts of the head shape in male (Figure 4a). According to shape variation in landmarks 1, 2, 5, 8, 9 and 10 females pronotum was wider and shorter than males. However, the posterior parts of pronotum was slightly narrower in females (Figure 4b).

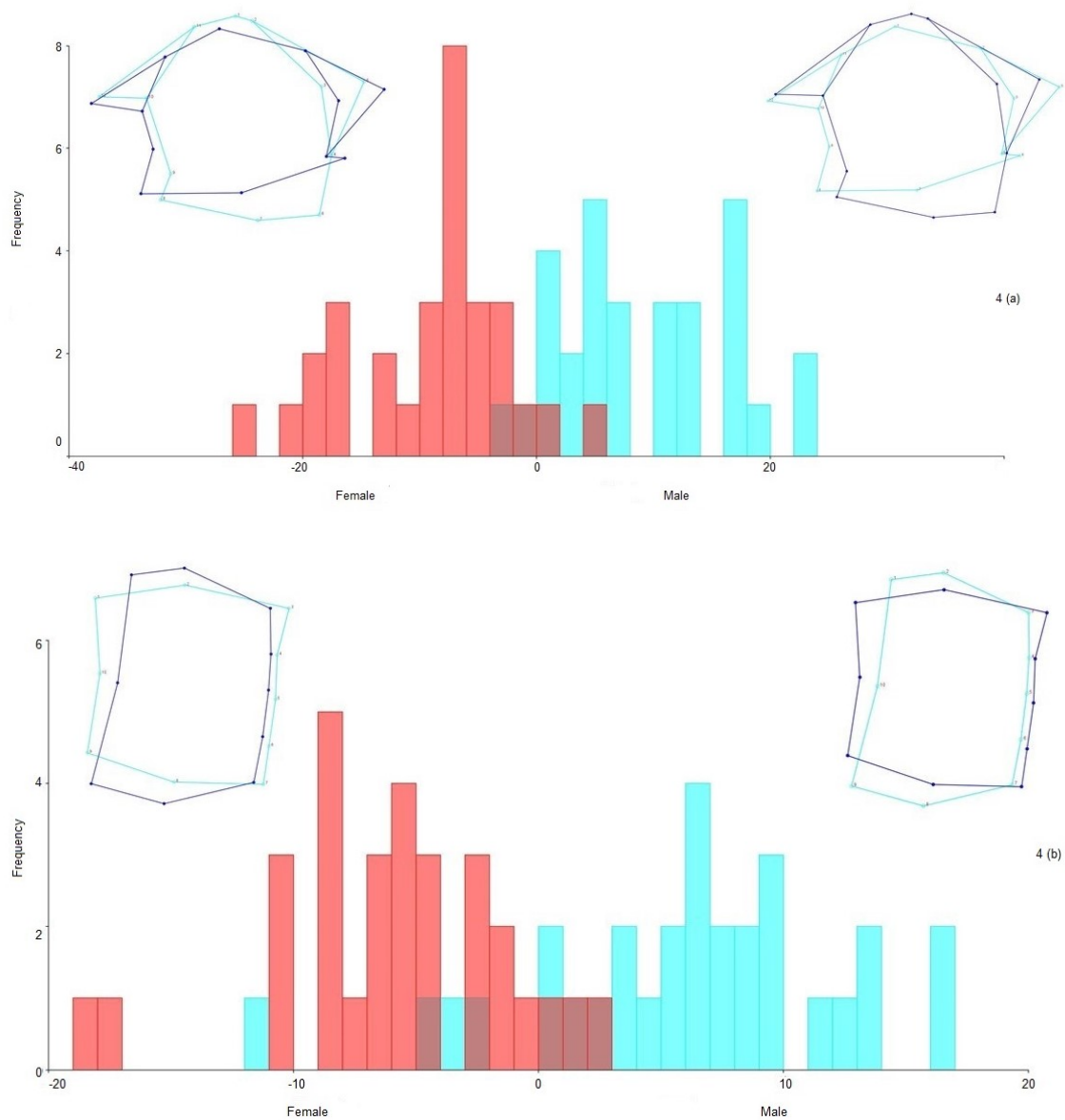


Figure 4. Cross validation scores of shape variables of a) head and b) pronotum. Wire-frame graphs were shown for female and male respectively at the top of left and right of each figure. The extreme changes of shape in positive and negative direction was shown by the violet lines and mean shape of head and pronotum was shown by blue lines. The scale for each figure is; head DA (-20 to 20), pronotum DA (-10 to 10).

Methods for the selection and use of body parts, and for the evaluation of sexes, are of central importance in sexual dimorphism. Geometric morphometrics is not only a novel tool for detecting morphological variations (Mitteroecker & Gunz, 2009; Breno et al., 2011; Kaliontzopoulou, 2011; Benítez et al., 2013; Meng et al., 2018), but also is the best clue to determine sexual dimorphism between and/or among organisms (Hood, 2000; Kaliontzopoulou et al., 2007; Moneva et al., 2012; Alencar et al., 2014; Jun-Yan et al., 2015; Solis et al., 2015; Minoli et al., 2016; Benítez & Vargas, 2017; Tamagnini et al., 2018). For insects in general, and beetles in particular, past works on sexual dimorphism have concentrated on sclerotized body parts (Pretorius & Scholtz, 2001), such as head and pronotum (Torres et al., 2010; Cruz et al., 2011; Acevedo, 2015; Ober & Connolly, 2015; Eldred et al., 2016; Sukhodolskaya & Saveliev, 2017;

Vesović et al., 2019). Since, an external morphological trait could allow for comparison of intraspecific variation in sexual versus nonsexual characters (Polihronakis, 2006). Of these, the adult head morphology of Coleoptera is interesting in its own right and provide phylogenetically informative characters (Antunes-Carvalho et al., 2016). Also, easily perceived differences in size, structure and function in the prothorax (pronotum constricted behind the head and is the dorsal plate of the prothorax) are usually viewed as taxonomic evidence (Hlavac, 1972). The present study, thus, intended to show that geometric morphometrics can confirm a clear sexual difference in both head and pronotum of *C. c. ssp. cappadocica*.

Of the very large number of geometric morphometrics studies on insect species (Tatsuta et al., 2018), relatively few have examined tiger beetles (Jones & Conner, 2018). As far as is known, no Anatolian tiger beetle has been analyzed via geometrics morphometrics in terms of sexually dimorphic features. For the first time, we aimed at answering the question of whether the sexes of endemic tiger beetle *C. c. ssp. cappadocica* can be morphologically differentiated. Actually, this can be achieved using suitable body structures. As also emphasized-above, it is concluded that forebody parts (head and pronotum) should be regarded as one of the most important external adult features for tiger beetles. Since, the head and the pronotum of a tiger beetle are obvious characters and likely to provide direct support for inferring their taxonomic knowledge, viz., make it a separate coleopteran family in suborder Adephaga. Tiger beetles are easily distinguished from other adephagan beetles by the general structures of the head and the pronotum as followings: head with eyes wider than pronotum and the hind margin of the pronotum is narrower than the base of the elytra (Pearson, 1988; Pearson & Vogler, 2001; Uniyal & Bhargav, 2007; Assmann et al., 2018).

Our results support the view that males of *C. c. ssp. cappadocica* are less variable with respect to CS when compared with the females and females are larger than males in both head and pronotum (Figure 3). Geometric morphometric analysis of shape variation in the *C. c. ssp. cappadocica* revealed statistically significant differences in both the head and pronotum. Also, in this study multivariate regression of shape onto size results indicated that size has little influence on the differentiation in both head and pronotum shape among sexes.

Our attempt allowed us to characterize morphological comparisons between the sexes. Statistically significant shape differences were identified in both the head and pronotum of the *C. c. ssp. cappadocica* using discriminant function analysis. According to results of head, while only shape variables were included in the discriminant function, 88.3% of cross-validated grouped cases correctly classified, this rate increased by only 1.7% when the CS was added (Table 2). Similarly, according to results of pronotum, while only shape variables were included in the discriminant function, 85% of cross-validated grouped cases correctly classified, this rate increased by only 3.3% when the CS was added (Table 3). Thus, discrimination results appear to support multivariate regression of shape against size results; size has little influence on the differentiation in both head and pronotum shape among sexes.

Focusing the head shape *C. c. ssp. cappadocica*, females have a wider and shorter head with relatively larger eyes than males. This is also related to elongated and sharpened from both anterior and posterior parts of the head shape in male (Figure 4a). Our results also show that the female pronotum is wider and shorter than that of males. However, the posterior parts of pronotum was slightly narrower in females (Figure 4b).

In conclusion, our results highlight the importance of considering sexual dimorphism in terms of shape and centroid size of endemic tiger beetle *C. c. ssp. cappadocica*. Here, the evidence of statistically significant shape differences was found among sexes of the *C. c. ssp. cappadocica*. Therefore, geometric morphometrics may be an effective and useful tool especially when the size-independent shape differentiation is too small to be detected by the human eye. In conclusion, this study of the geometric morphometrics of Anatolian tiger beetles revealed unexpected data and enhanced the importance of such analysis in taxonomy and systematic.

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