



# Article Geometric Morphometric Analysis of Genus *Chaetocnema* (Coleoptera: Chrysomelidae: Alticini) with Insights on Its Subgenera Classification and Morphological Diversity

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**Abstract:** In taxonomy, qualitative methods are commonly used to analyze morphological characters, which can lead to dramatic changes in higher taxa. Geometric morphometrics (GM) has proven to be useful for discriminating species in various taxonomy groups. However, the application of geometric morphometrics in supraspecies classification is relatively scarce. In this study, we tested the controversial two subgenera classification of *Chaetocnema* with geometric morphometrics; a total of 203 *Chaetocnema* species representing 50% of all known species from around the world were selected for the analysis. We analyzed the shape of the pronotum, elytron, head, aedeagus, and spermatheca. The results showed that the two traditional subgenera distinctly differed from each other; therefore, we propose using two subgenera arrangements to facilitate the understanding and taxonomy of the *Chaetocnema* species (especially in the Oriental, Palearctic, and Nearctic regions). Additionally, the morphological diversity of the abovementioned structures of *Chaetocnema* was analyzed, and it was found that the highest morphological diversity was in the spermatheca, which was greater than that in the aedeagus. Our research demonstrated that GM could be useful for detecting morphological delimitation of the supraspecies taxa. It also showed that GM methods are applicable to insects as small as 2 mm in body size.

Keywords: morphological diversity; flea beetles; subgenus; taxonomy; quantitative; morphology

## 1. Introduction

Taxonomy is central to the exploration and understanding of biodiversity [1]. Species identification and population discrimination are essential for the conservation of biodiversity and natural resource management [2]. Traditionally, the diagnostic characters of many groups were evaluated using qualitative methods. However, quantitative methods such as geometric morphometrics may be an efficient addition for the discrimination of supraspecies taxa.

Geometric morphometrics offers a comprehensive and effective approach to the study of shape via the multivariate statistical analysis of anatomical landmarks or outlines of biological homology [3–5]. This tool not only has a certain statistical power but also serves



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**Copyright:** © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). the purpose of visualizing, interpreting, and communicating results, which presents an obvious advantage [6–8]. Geometric morphometrics has been used in taxonomy across different groups (lizards, snakes, beetles, parasitoid wasps, etc.) [7,9–14]. It has been found to be more powerful compared to traditional morphological analyses for discriminating between species and populations of 'morphologically ambiguous' taxa [10]. However, the applicability of geometric morphometrics in higher taxa classification is relatively scarce [2].

*Chaetocnema* Stephens (Chrysomelidae, Galerucinae, Alticini) is a genus including species with extremely tiny body sizes, usually around 2 mm. It is one of the few genera with a cosmopolitan distribution and comprises more than 400 species [15]. There have been exemplary revisional studies in recent years on the Oriental [16], Palearctic [15], Afrotropical [17,18], and Nearctic species [19]. These studies offer not only descriptive but also precise graphic morphological data, which make *Chaetocnema* an excellent group for quantitative morphological research.

The traditional two subgenera of *Chaetocnema* (subgenus *Chaetocnema* s. str. Stephens, 1831 [20] and subgenus *Udorpes* Motschulsky, 1845 [21]) have been recognized by many authors, including Maulik [22], Heikertinger and Csiki (world species) [23], Heikertinger (Palearctic and Oriental species) [24], Gressitt and Kimoto (Chinese and Korean species) [25], Samuelson (Oceania species) [26], Döberl (Palearctic and Oriental species) [27] and Ruan et al. (Oriental species) [16]. However, both White [19] and Konstantinov et al. [15] found that the previous delimitation of the two subgenera was problematic for some species, and Konstantinov et al. [15] suggested not using any subgeneric classification until rigorous phylogenetic analysis was conducted. Ruan et al. [16,28,29] tentatively used two subgenera (subgenus *Chaetocnema* s. str. and subgenus *Udorpes*) for Oriental *Chaetocnema* species based on the traditionally used characters and the shape of the spermatheca. Alternatively, Özdikmen [30] proposed another subgeneric arrangement of the *Chaetocnema* species based on the shape of the spermatheca, comprising as many as 13 subgenera.

*Tlanoma* Motschulsky, 1845 has been erroneously used as a subgenus name for a very long time. In fact, *Tlanoma* is a subjective junior synonym of *Chaetocnema* in a strict sense, as pointed out and fully discussed by Konstantinov et al. in 2011 [15]. Therefore, we followed Konstantinov et al. [15] and used the subgenus name *Chaetocnema* (s. str.) (type species: *C. concinna*) and *Udorpes* (type species: *C. splendens*).

To test the traditional subgeneric classification of *Chaetocnema*, we tentatively attributed the studied species to either subgenus *Chaetocnema* s. str. or *Udorpes* and analyzed the morphological variations using a geometric morphometric technique. The pronotum, elytron, head, aedeagus, and spermatheca were selected for analysis as they are commonly used morphological characters in flea beetles and are frequently illustrated in taxonomic studies. The research was based on a large dataset consisting of 203 *Chaetocnema species* from the Oriental, Palearctic, and Nearctic regions, representing approximately 50% of all known species from around the world.

# 2. Materials and Methods

## 2.1. Taxa Examined

This study analyzed a total of 203 *Chaetocnema* species, comprising 79 species from the Oriental region, 75 species from the Palearctic region, and 59 species from the Nearctic region. Additionally, two outgroup flea beetle species were included in the analysis, covering 97.16% of all described extant *Chaetocnema* species from the three biogeographic regions and approximately 50% of all described extant species worldwide (Table S1). Species from other biogeographic regions were not included in the analysis due to insufficient published images. The subgeneric arrangement followed the classification proposed by Ruan et al. [16] (Figure 1). Images of the species in the geometric morphometric analyses were collected from previously published studies [15,16,19].



**Figure 1.** The morphological differences between subgenus *Chaetocnema* s.str and *Udorpes*. (**A**) Representatives of *Chaetocnema* species, 1–4; subgenus *Chaetocnema* s. str., 5–8; subgenus *Udorpes*; 1; *C. confinis* Crotch, 2; *C. nigrica* Motschulsky, 3; *C. picipes* Stephens, 4; *C. semicoerulea* (Koch), 5; *C. cylindrica* (Baly), 6; *C. ingenua* (Baly), 7; *C. concinnipennis* Baly, 8; *C. concinnicollis* (Baly). (**B**) A typical image of the head of the subgenus *Chaetocnema* s. str. (*C. constricta* Ruan et al.). (**C**) A typical image of the head of the subgenus *Udorpes* (*C. bella* (Baly)). (**D**) A typical image of the spermatheca of the subgenus *Chaetocnema* s. str. (**E**) A typical image of the subgenus *Udorpes*. (**F**,**G**) Head of the subgenus *Chaetocnema* s.str. and *Udorpes*.

# 2.2. Morphological Delimitation of the Two Subgenera of Chaetocnema

The selected species were assigned to subgenus *Chaetocnema* s. str. or *Udorpes* for analysis based on the following characters. Details of the species information are provided in Supplementary File S1.

**Subgenus** *Chaetocnema* **s. str.:** (1) Frontolateral sulcus of head well developed and represented by a row of deep and large punctures; frontal ridge (interantennal space) convex, impunctate or with extremely minute punctures, usually narrow; frons impunctate or only punctate at sides near frontolateral sulcus. (2) Vertex of head without punctures

at middle; most species with only a few punctures on each side near the eyes. (3) Sides of spermathecal receptacle not sinuated, spermatheca usually pear-shaped, flask-shaped, or cylindrical. Species list: C. aenigmatica, C. alutacea, C. appendiculata, C. arizonica, C. babai, C. baoshanica, C. basalis, C. bicolor, C. bicolorata, C. blatchleyi, C. breviuscula, C. brunnescens, C. californica, C. cheni, C. chlorophana, C. compressa, C. concinna, C. conducta, C. confinis, C. constricta, C. coyei, C. crenulata, C. dapitanica, C. delarouzeei, C. densa, C. depressa, C. deqinensis, C. discreta, C. dispar, C. duvivieri, C. ectypa, C. elongatula, C. excavata, C. extenuata, C. fallaciosa, C. fortecostata, C. fulvida, C. furthi, C. fusiformis, C. gentneri, C. gracilis, C. granulicollis, C. granulosa, C. granulosa, C. hainanensis, C. heptapotamica, C. hongkongensis, C. jinxiuensis, C. kanmiyai, C. kimotoi, C. kingpinensis, C. koreana, C. labiosa, C. livida, C. longipunctata, C. lubischevi, C. magnipunctata, C. major, C. mandschurica, C. melonae, C. montivaga, C. nebulosa, C. nigrica, C. nigrilata, C. obesula, C. opulenta, C. orientalis, C. parafusiformis, C. pelagica, C. philippina, C. picipes, C. pulicaria, C. puncticollis, C. punctifrons, C. purerulea, C. quadricollis, C. repens, C. resplendens, C. rileyi, C. sabahensis, C. salixis, C. scheffleri, C. schlaeflii, C. semicoerulea, C. septentrionalis, C. serpentina, C. shabalini, C. simplicifrons, C. sticta, C. subbasalis, C. subconvexa, C. subviridis, C. sumatrana, C. taiwanensis, C. tibialis, C. tonkinensis, C. transbaicalica, C. trapezoidus, C. tristis, C. vesca, C. wallacei, C. warchalowskii, C. yaosanica, C. yiei, C. yulongensis, and C. yunnanica.

**Subgenus** *Udorpes*: (1) Frontolateral sulcus of head absent or obsolete; frontal ridge (interantennal space) flattend and evenly punctate, usually wide; frons evenly punctate. (2) Vertex entirely covered with numerous large and deep punctures. (3) Sides of spermathecal receptacle sinuated. Species list: C. acuminata, C. acupunctata, C. aequabilis, C. aerosa, C. afghana, C. albiventris, C. alticola, C. angustifrons, C. angustula, C. anisota, C. arenacea, C. arida, C. aridula, C. balanomorpha, C. belka, C. bella, C. belli, C. bergeali, C. borealis, C. bretinghami, C. coacta, C. cognata, C. concinnicollis, C. concinnipennis, C. confusa, C. costata, C. costulata, C. cribrata, C. cribrifrons, C. cylindrica, C. denticulata, C. difficilis, C. eastafghanica, C. floridana, C. franzi, C. fuscata, C. glabra, C. gottwaldi, C. grandis, C. greenica, C. hortensis, C. igori, C. imitatrix, C. ingenua, C. irregularis, C. jelineki, C. kabakovi, C. klapperichi, C. kumaoensis, C. latapronotus, C. leonhardi, C. ljudmilae, C. malayana, C. mannerheimii, C. megachora, C. megasticta, C. merguiensis, C. midimpunctata, C. minitruncata, C. minuta, C. modesta, C. modiglianii, C. montenegrina, C. nocticolor, C. obesa, C. obliterata, C. oblonga, C. opacula, C. ordinata, C. paganettii, C. paragreenica, C. paraumesaoi, C. perturbata, C. pinguis, C. procerula, C. producta, C. prolata, C. protensa, C. psylloides, C. pusaensis, C. reteimpunctata, C. rufofemorata, C. sahlbergii, C. shanxiensis, C. singala, C. sinuata, C. splendens, C. subcoerulea, C. sulcicollis, C. tarsalis, C. tbilisiensis, C. texana, C. truncata, C. umesaoi, C. ussuriensis, C. westwoodi, and C. zangana.

It should be noted that although *C. cylindrica* and *C. concinnicollis* have narrow frontal ridges, they have sinuated spermathecal receptacles, and the vertex is densely covered with large punctures. Therefore, they were assigned to the subgenus *Udorpes*. Although *C. orientalis* and *C. conducta* have relatively wide and flat frons, they could be attributed to the subgenus *Chaetocnema* s. str. because their spermathecal receptacle is not sinuated, and there are very few punctures on the vertex. Although *C. depressa* has large punctures all over the vertex, it could be attributed to the subgenus *Chaetocnema* s. str. because its frontal ridge is narrow and its spermathecal receptacle is not sinuated. Nearly all the species in the subgenus *Chaetocnema* s. str. from the Nearctic region have wide frons, but they were placed in the subgenus *Chaetocnema* s. str. because their frontolateral sulcus is present and there are very few punctures on the vertex and frons.

## 2.3. Data Analysis

Seven characters of the five structures were examined and analyzed. The pronotum, elytron, aedeagus in lateral view, aedeagus in ventral view, spermathecal receptacle, and spermathecal pump were represented by one curve on the outline, which was resampled into 50, 50, 50, 50, 35, and 30 equally spaced semi-landmarks (SLM), respectively (Figure 2, Supplementary File S2). The head was represented by two curves in the frontal view: one started on the upper end of the superorbital sulcus, which almost touched the margin of



the eye, and ended at the lower part of the frontal lateral sulcus, which is situated at the same horizontal level as the lower part of the antennal socket. Each of these curves was resampled into 10 equally spaced semi-landmarks (Figure 2, Supplementary File S2).

**Figure 2.** The curves used in the geometric morphometric analysis. (**A**) Pronotum: one curve resampled into 50 semi-landmarks; (**B**) elytron: one curve resampled into 50 semi-landmarks; (**C**) head (represented by the grooves on the head): two curves resampled into 20 semi-landmarks; (**D**) aedeagus in lateral view: one curve resampled into 50 semi-landmarks; (**E**) aedeagus in ventral view: one curve resampled into 50 semi-landmarks; (**F**) spermathecal receptacle: one curve resampled into 35 semi-landmarks; (**G**) spermathecal pump: one curve resampled into 30 semi-landmarks.

The curves were digitized using tpsDig 2.05 software [31]. The data files used for the morphological analysis were prepared by converting semi-landmarks into landmarks in text files [32–34]. The landmark configurations were scaled, translated, and rotated against the consensus configuration using the Procrustes superimposition method [3]. The principal component analysis (PCA) and the canonical variate analysis (CVA) were performed using MorphoJ 1.06a software [35] (Figures 3–8). The variability in the shape space was assessed using PCA. The morphological diversity was quantified as the Procrustes variance in PCA (Figures 5 and 6, Tables S1 and S2), which measures the dispersion of all observations around the mean shape of the respective taxa [6,36]. The statistical significance of pairwise differences was determined using permutation tests (10,000 replications) with the Procrustes and Mahalanobis distances in CVA (Tables 1, 2 and S3); both tests were used to assess significance because *p*-values can differ due to the anisotropy (directional dependency) of the shape variation [37]. The Mahalanobis distance indicates how different an individual is from the others in the sample, thus reflecting how well the groups are separated from each other. The Procrustes distance is a measure of the absolute magnitude of the shape deviation, indicating the significance of the shape differences between the average group shapes. In this paper, there would be a significant difference if the obtained *p*-values for the Mahalanobis distance and the Procrustes distance among groups were less than 0.05. The mean configuration (average shapes) of the analyzed structures for each subgenus were



presented to visualize the shape variation (Figure 3H), and lollipop graphs of deformation were used to portray the resulting shape variations along the first two PC axes (Figure 4H).

**Figure 3.** Canonical variate analysis (CVA) for different characters of the two subgenera. Species of subgenus *Chaetocnema* s. str., *Udorpes*, and outgroups are marked with red, green, and blue, respectively. The 90% equal frequency ellipses containing 90% of the data points are shown. (**A**) Pronotum; (**B**) elytron; (**C**) head; (**D**) aedeagus in lateral view; (**E**) aedeagus in ventral view; (**F**) spermathecal receptacle; (**G**) spermathecal pump; (**H**) average shapes of the two subgenera. **1**(**B**) Pronotum; **2**(**B**) elytron; **3**(**D**) head; **4**(**1**) aedeagus in lateral view; **5**(**1**) aedeagus in ventral view; **6**(**1**) spermathecal receptacle; **7**(**1**) spermathecal pump.



**Figure 4.** Principal component analysis (PCA) of different characters of *Chaetocnema* species. Species of subgenus *Chaetocnema* s. str., *Udorpes*, and outgroups are marked with red, green, and blue, respectively. The 90% equal frequency ellipses containing 90% of the data points are shown. (**A**) Pronotum; (**B**) elytron; (**C**) head; (**D**) aedeagus in lateral view; (**E**) aedeagus in ventral view; (**F**) spermathecal receptacle; (**G**) spermathecal pump; (**H**) deformations from the origin along with the first two PC axes are shown as shape models with a scale factor of +0.1. 10® Pronotum; 2® elytron; 3® head; (**①**1) aedeagus in lateral view; (**⑤**12) aedeagus in ventral view; (**⑥**13) spermathecal receptacle; (**⑦**14) spermathecal pump.



**Figure 5.** Morphological diversity of the seven characters of genus *Chaetocnema* and two subgenera. Aedeagus (L): aedeagus in lateral view; aedeagus (V): aedeagus in ventral view.



**Figure 6.** Morphological diversity of the seven characters of Oriental, Palearctic, and Nearctic *Chaetocnema*. Aedeagus (L): aedeagus in lateral view; aedeagus (V): aedeagus in ventral view; OR: Oriental region; PA: Palearctic region; NE: Nearctic region.



**Figure 7.** Principal component analysis (PCA) of the different characters of the three faunas. Oriental, Palearctic, Nearctic *Chaetocnema*, and outgroups are marked with green, blue, red, and purple, respectively. The 90% equal frequency ellipses containing 90% of the data points are shown. (**A**) Pronotum; (**B**) elytron; (**C**) head; (**D**) aedeagus in lateral view; (**E**) aedeagus in ventral view; (**F**) spermathecal receptacle; (**G**) spermathecal pump.

**Table 1.** The *p*-values for the Mahalanobis distances and the Procrustes distances of the seven characters between subgenus *Chaetocnema* s. str. and *Udorpes*. MD: Mahalanobis distances; PD: Procrustes distances.

	Pronotum	Elytron	Head	Aedeagus in Lateral View	Aedeagus in Ventral View	Spermathecal Receptacle	Spermathecal Pump
<i>p</i> -values for MD <i>p</i> -values for PD	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	<0.0001	0.3499	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001



**Figure 8.** Canonical variate analysis (CVA) of the different characters of the three faunas. Oriental, Palearctic, Nearctic *Chaetocnema*, and outgroups are marked with green, blue, red, and purple, respectively. The 90% equal frequency ellipses containing 90% of the data points are shown. (**A**) Pronotum; (**B**) elytron; (**C**) head; (**D**) aedeagus in lateral view; (**E**) aedeagus in ventral view; (**F**) spermathecal receptacle; (**G**) spermathecal pump.

**Table 2.** The *p*-values for the Mahalanobis distances and the Procrustes distances of the seven characters among Oriental (OR), Palearctic (PA), and Nearctic (NE) *Chaetocnema*. MD: Mahalanobis distances; PD: Procrustes distances.

		Pronotum	Elytron	Head	Aedeagus in Lateral View	Aedeagus in Ventral View	Spermathecal Receptacle	Spermathecal Pump
OR vs. PA	<i>p</i> -values for MD	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	<i>p</i> -values for PD	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0002	0.6871	0.0030
OR vs. NE	<i>p</i> -values for MD	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	-	-
	<i>p</i> -values for PD	< 0.0001	< 0.0001	0.0003	0.0797	0.0773	-	-
PA vs. NE	<i>p</i> -values for MD	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	-	-
	<i>p</i> -values for PD	< 0.0001	0.3282	0.0015	0.0001	0.0003	-	-

# 3. Results

# 3.1. Morphological Differences between Subgenera Chaetocnema s. str. and Udorpes

The two subgenera showed significant differences in pronotum, head, aedeagus, and spermatheca shapes, with *p*-values for both Mahalanobis and Procrustes distances of less than 0.05 (Table 1). The average shapes of the elytron between the two subgenera were not significantly different, with a *p*-value of 0.3499 for the Procrustes distance. However, the

morphological boundaries of elytra of the two subgenera were distinctly separated from each other, according to the *p*-value for the Mahalanobis distance.

Based on the average shapes of the two subgenera (Figure 3H), the pronotum of subgenus *Chaetocnema* s. str. was wider than *Udorpes*. The frons of subgenus *Udorpes* were noticeably wider than those of *Chaetocnema* s. str. The aedeagus in the lateral view of the subgenus *Udorpes* exhibited a more curved shape compared to *Chaetocnema* s. str., and the shape of the aedeagus in the ventral view of the subgenus *Udorpes* appeared wider than *Chaetocnema* s. str. The spermathecal receptacle of the subgenus *Chaetocnema* was pearshaped and not sinuated, whereas in *Udorpes* it was sinuated. The apex of the spermathecal pump of subgenus *Udorpes* was more flattened than that of *Chaetocnema* s. str.

These results strongly support the two subgenera arrangement of *Chaetocnema* in our analysis.

#### 3.2. Morphological Diversity of Different Characters in Chaetocnema

The first two principal components of the seven characters (pronotum, elytron, head, aedeagus in lateral view, aedeagus in ventral view, spermathecal receptacle, and spermathecal pump) from all analyzed *Chaetocnema* species accounted for 87.55%, 94.37%, 84.58%, 73.85%, 79.33%, 84.35%, and 71.95%, respectively, of the variation among the species. This indicated that the first two components could effectively represent the main shape variation for all of the characters analyzed. The first two principal components were plotted to illustrate the variation along the two axes, with 90% equal-frequency ellipses containing approximately 90% of the specimens in each group (Figures 4 and 7).

Based on the lollipop graphs of deformation, the results indicated that the main shape change of the pronotum was observed in the posterior margin and posterolateral angles, whereas a secondary shape change was observed in the length/width ratio and anterior margin. The elytron showed the main shape change in the length/width ratio, with a secondary shape change observed in the humeral and apical parts. For the head, the main shape change was observed in the supraorbital sulci, with a secondary shape change in the width of the frontal ridge (interantennal space). In the aedeagus in lateral view, the main shape change was observed in the length/width ratio, and a secondary shape change could be observed in the length/width ratio. The main shape change of the aedeagus in the ventral view was observed in the length/width ratio, and a secondary shape change in the length/width ratio, with a secondary shape change in the length/width ratio, with a secondary shape change in the ventral view was observed in the length/width ratio, and a secondary shape change in the length/width ratio, with a secondary shape change in the length/width ratio, with a secondary shape change in the length/width ratio, with a secondary shape change in the length/width ratio, with a secondary shape change in the length/width ratio, with a secondary shape change in the lateral curvature. Finally, the main shape change in the spermathecal pump was observed at the apex (Figure 4H).

Based on the PCA results, the morphological diversity of these seven characters was not equal in *Chaetocnema* (Figure 5, Table S2). The highest morphological diversity and most apparent differentiation among species were found in the spermatheca (spermathecal receptacle and spermathecal pump). The head was the third most diverse character, followed by the pronotum, aedeagus in lateral view, elytron, and aedeagus in ventral view. There was a minor difference in the morphological diversity of the pronotum and aedeagus between the two subgenera (Figure 5, Table S2). Subgenus *Chaetocnema* s. str. exhibited much greater morphological diversity than *Udorpes* in the head, elytron, and spermathecal pump, but much smaller morphological diversity than that of *Udorpes* in the spermathecal receptacle. The aedeagus in the ventral view showed the lowest morphological diversity in both subgenera was found in the spermatheca, specifically in the spermathecal pump among the subgenus *Chaetocnema* s. str. Species and the spermathecal receptacle among the subgenus *Udorpes* species.

## 3.3. Morphological Differences of Oriental, Palearctic, and Nearctic Chaetocnema Species

This study observed the greatest morphological diversity in Oriental species in the spermathecal pump, whereas the aedeagus in the ventral view showed the least diversity. In

Palearctic species, the greatest morphological diversity was observed in the spermathecal receptacle, whereas the aedeagus in the ventral view showed the least diversity. The greatest morphological diversity in Nearctic species was observed in the head, whereas the aedeagus in the ventral view showed the least diversity. The spermatheca (including the spermathecal receptacle and the spermathecal pump) demonstrated obviously greater morphological diversity compared to other characters in Oriental and Palearctic species. Additionally, the head exhibited significant morphological variations in *Chaetocnema* for all three regions. The morphological diversity of the aedeagus in the lateral view was greater than that in the ventral view for all three regions (Figure 6, Table S3).

This study found that for most morphological features, the morphological variations in the Palearctic species were greater than those of the Oriental and Nearctic species. This result was consistent with the size of 90% equal-frequency ellipses, where the ellipse of the Palearctic species was always the largest (Figure 7).

All of the *p*-values for both the Mahalanobis distances and the Procrustes distances between the three geological groups were less than 0.05 for the pronotum and the head (Table 2), indicating significant differences in the pronotum and the head between the three geological groups. It can also be seen from the *p*-values that there were significant differences in aedeagus (both in the lateral and ventral view) between Oriental and Palearctic, and Nearctic and Palearctic *Chaetocnema*. There were significant differences in the elytron between Palearctic and Oriental, and Nearctic and Oriental *Chaetocnema*. Additionally, there were significant differences in the spermathecal pump between Oriental and Palearctic *Chaetocnema*.

According to the Procrustes distances, the average shapes of Nearctic and Palearctic *Chaetocnema* had the greatest difference compared to the other region pairs in the pronotum (Table S4). However, the average shapes of this pair had the smallest difference in the head. The average shape of the aedeagus in the lateral view of the Oriental *Chaetocnema* was more similar to that of the Nearctic species than to the Palearctic species. The Mahalanobis distances for the pronotum, head, and aedeagus in the lateral view between Palearctic and Nearctic *Chaetocnema* were the largest among all region pairs, consistent with the smallest overlaps of these three characters between Palearctic and Nearctic *Chaetocnema* compared to the other region pairs (Table S4, Figure 8).

# 4. Discussion

## 4.1. The Subgenera Arrangement and Morphological Diversity of Chaetocnema

In this study, we evaluated the validity of the two subgenera of *Chaetocnema* using geometric morphometrics with five external and internal structures (pronotum, elytron, head, spermatheca, and aedeagus). Additionally, we revealed the morphological diversity of *Chaetocnema* in the Oriental, Palearctic, and Nearctic regions. According to the CVA analyses of the two subgenera, the studied specimens were clustered into two groups for all seven shape variables.

Significant differences were observed between the subgenus *Chaetocnema* s. str. And *Udorpes* in the pronotum, head, aedeagus, and spermatheca. Surprisingly, the shapes of the pronotum were also significantly different between these two subgenera, although it was hard to attribute the species to either subgenus by measuring the width of the pronotum. The average shape of the pronotum of the subgenus *Chaetocnema* s. str. Was wider than that of the subgenus *Udorpes*. These results strongly support the distinct differences between the two traditional subgenera. Özdikmen [30] pointed out that the shapes of the spermatheca could be classified into as many as 13 groups, based on which he proposed 13 subgenera for the genus *Chaetocnema*. However, this subgeneric classification was based merely on the shape of the spermatheca. For a more comprehensive subgeneric classification, more characters should be taken into consideration, especially those on the head, such as the width of the frontal ridge, the presence or absence of the frontolateral sulcus, and the abundance or deficiency of punctures on the vertex and frons. These characters exhibit significant morphological diversity and can be helpful in distinguishing subgeneric groups.

The morphological diversity of the seven characters examined was not equivalent. The highest morphological diversity and most evident differentiation among species was found in the spermatheca, followed by the head. The morphological diversity of spermatheca was even higher than that of aedeagus among the *Chaetocnema* species. This indicates that female genitalia, like spermatheca, are also very useful and have excellent diagnostic value in taxonomy. Similar scenarios have been observed in other insect groups. For instance, in Cydnidae (Hemiptera), the spermathecal characters have great taxonomic value at specific, generic, and supra-generic levels [38]; in dung beetles (Scarabaeidae: Scarabaeinae), the spermathecal structures play an important role in separating and diagnosing the tribes [39]; in Carabidae, Curculionidae, and Elateridae, the spermatheca contributes to the recognition of species and genera [40–42]. In various taxa of Chrysomeloidea, the spermatheca provides characters for diagnosis at various ranks (including subfamilies, genera, and species) [43–49].

The male genitalia have been well-studied and treated as one of the most important structures providing diagnoses in many insect groups because of their rapid divergence due to sexual selection [50–54]. However, our analysis showed that the aedeagus of *Chaetocnema* has rather low diversity among the seven characters. This may be explained as follows: in the ventral view, the basal half of the aedeagus of *Chaetocnema* usually has a similar shape, cylindrical in general, and the variation usually only occurs in the apical part; in the lateral view, the aedeagus usually has similar ventrally curved outlines.

The grooves on the head also have great morphological diversity, which provides important information regarding subgenera delimitation. The main shape variations were in the angle between the supraorbital sulci and the width of the frontal ridge, as shown by the PCA results.

Different morphological characters usually experience unequal selective pressures [34], which may partly explain the observed variation in morphological diversity among the seven characters. Concerning the morphological diversity of *Chaetocnema* from the three biogeographic regions, the morphological diversity of the Palearctic species was greater than that of the Oriental and Nearctic species for most characters, which is consistent with the species richness. This difference in diversity may be due to the larger geological range, more diverse environment, and climate of the Palearctic Region.

## 4.2. Geometric Morphometrics in the Classification of Higher Taxa

Discrete characters are commonly used in traditional taxonomy. However, when dealing with difficult taxonomic problems and a lack of discrete characters, geometric morphometrics can be used to gain valuable insight. In the field of botany and biology, the application of geometric morphometrics contributes to increasing scientific rigor in describing the important aspects of the phenotypic dimension of biodiversity [55]. For example, Viscosi and Cardini [55] applied geometric morphometrics to leaf morphology to explore its potential use in botany and facilitate its use in taxonomy. Ibañez et al. [2] evaluated the applicability of GM methods in the taxonomy of mullets at the genera, species, geographic variant, and stock levels. Karanovic et al. [56] investigated the taxonomic problem of a subterranean copepod species complex using both the barcoding mitochondrial COI gene and geometric morphometrics. Li et al. [57] found that geometric morphometric analyses supported the valid status of the new species of soldier beetles (Coleoptera, Cantharidae). White et al. [58] evaluated the intraspecific and interspecific morphological variations in hominins using three-dimensional geometric morphometric methods. In our study, we used geometric morphometrics to distinguish substantial deformations in specific parts of the five structures (pronotum, elytron, head, aedeagus, and spermatheca). These shapebased morphological measurements played an important role in evaluating morphological diversity and resolving taxonomic problems.

Higher taxa are biological entities that are relatively easy to identify, and the origin of a new higher taxon has a long-term phylogenetic trend, involving the evolutionary changes in a large number of features [59,60]. Taxonomy is crucial for exploring and

understanding biodiversity [1] and the significance of higher taxa in studying biodiversity patterns has been confirmed [61]. The higher-level elements effectively reflect the natural evolutionary process [34,62–65]. In this study, the geometric morphometric analysis of the five structures provides valuable information regarding the subgenera taxonomy of *Chaetocnema*, demonstrating the potential of geometric morphometrics in supraspecies taxa taxonomy.

## 5. Conclusions

In this study, we conducted a geometric morphometric analysis of the five frequently used structures for representative species of *Chaetocnema*, and the results strongly supported the traditional arrangement of the two subgenera in Chaetocnema. Based on our findings, we propose using the two subgenera arrangement to facilitate the taxonomic work of the genus, especially in the Oriental, Palearctic, and Nearctic regions. Additionally, we found the highest morphological diversity in the spermatheca, which was greater than that of the aedeagus. This indicates that female genitalia, like spermatheca, are also very useful in species descriptions and have excellent diagnostic value in taxonomy. For most features, the morphological variations in the Palearctic species were greater than those of the Oriental and Nearctic species, probably due to the larger geological range, and more diverse environment and climate. Despite Chaetocnema species being usually tiny in body size (sometimes as small as 1.3 mm), our study indicated that geometric morphometrics could be applied to insects with tiny body sizes. Although the results are based only on the subgenera level and are limited to the morphological characters of the five structures, our study shows that geometric morphometrics could be useful in supraspecies taxa classification as well as species discrimination.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www. mdpi.com/article/10.3390/d15080918/s1. Supplementary File S1: Consists of four tables. Table S1. List of species in the geometric morphometric analysis. Table S2. Morphological diversity (MD) of the seven characters of the genus *Chaetocnema* and two subgenera. Table S3. Morphological diversity (MD) of the seven characters of Oriental, Palearctic, and Nearctic *Chaetocnema*. Table S4. The Mahalanobis distances and Procrustes distances for the seven characters among Oriental, Palearctic, and Nearctic *Chaetocnema*. Supplementary File S2: Shapes in the GM analysis: (1) outlines of the spermatheca of the subgenus *Chaetocnema* s. str. And *Udorpes*; (2) curves on the head of the subgenus *Chaetocnema* s. str. And *Udorpes*.

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