Morphological Adaptation of Cave-Dwelling Ground Beetles in China Revealed by Geometric Morphometry (Coleoptera, Carabidae, Trechini)

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Simple Summary: Cavernicolous ground beetles dwelling in China are one of the most diverse and underground-adapted coleopteran group in the world. The tribe Trechini is, among them, the most representative group constituting over 170 known species with a narrow and elongated body and long appendages or a stout body and short appendages. However, very little information about their morphology has been explored. The aim of this study was to analyze the morphological adaptations of this group using geometric morphological methods. The beetles were divided into four different morphological types, including aphaenopsian, semi-aphaenopsian, anophthalmic, and surface-dwelling, and the analysis is based on the morphology of their head, pronotum, and elytra. Our findings indicate that the overall morphological variation of cave trechine beetles has gradually specialized from an anophthalmic to semi-aphaenopsian to aphaenopsian type. Different types have different directions of variation in the head, pronotum, and elytra, but the pronotum is more differentiated and morphologically diverse than the head and elytra.

Abstract: Cave-dwelling ground beetles in China represent the most impressive specific diversity and morphological adaptations of the cavernicolous ground beetles in the world, but they have not been systematically examined in quantitative terms. The present study focuses on the application of geometric morphological methods to address the morphological adaptations of the tribe Trechini, the most representative group in China. We have employed a geometric morphometry analysis of the head, pronotum, and elytra of 53 genera of Trechini, including 132 hypogean and 8 epigean species. Our results showed that the overall morphological variation of cave carabids has gradually specialized from an anophthalmic to semi-aphaenopsian to aphaenopsian type. There were extremely significant differences ($p < 0.01$) among four different adaptive types including aphaenopsian, semi-aphaenopsian, anophthalmic, and surface-dwelling Trechini when their adaptability to a cave environment was used as the basis for grouping. Furthermore, there were differences in the phenotypic tree of the head, pronotum, and elytra, and an integrated morphology. To the best of our knowledge, this is the first report on the analysis of the head, pronotum, and elytra of four different adaptive types of ground beetles in order to clarify the morphological adaptations of cavernicolous carabids to the cave environment.

Keywords: adaptive type; cave environment; morphological variation; phenotypic development
1. Introduction

China is very rich in cave-dwelling ground beetles. At present, 202 species of cave carabids belonging to 8 tribes and 71 genera have been recorded, among which the Trechini is the most diverse group including 175 species from 63 genera [1,2]. All of them are troglobions and completely lack eyes, except for four troglobiline with more or less degenerated eyes [3]. In addition to the disappearance of their eyes, cavernicolous carabids underwent morphological modifications during long-term adaptation to the subterranean environment. These are manifested in the loss of pigment and metathoracic wings, as well as their more slender body and thin legs [4,5]. According to the adaptability of characteristic appearances and biological information, cave trechine beetles were divided into three morphological types [6–8]: aphaenopsian, semi-aphaenopisan, and anophthalmic (Figure 1). The former means that these carabids have an extreme elongation of their body and appendages, while the latter refers to their stout body and shorter appendages. The semi-aphaenopsian is considered to be a transitional type, with morphological characteristics lying between the above two. The surface-dwelling trechine beetles have darker body color and compound eyes (Figure 1).

![Morphological characteristics of four different adaptive types of ground beetles.](image)

Geometric morphometry is an approach that relies on the quantitative analysis of the geometry of the target structure and the further performance of statistical analyses [9]. Different types of data, such as landmark coordinates, outline curves, and surfaces are used to define the shape [10]. The original morphological information is usually obtained through Cartesian coordinates, which are used to remove the interference of non-morphological variation in the analysis so that the punctuation overprint analysis of all samples can be visualized and displayed [11]. Geometric morphometry began to be used in the 1980s, and in the 21st century it has been widely used in entomology, medicine, archaeology, and other fields [12–14]. Recently, geometric morphometry has developed from two-dimensional to three-dimensional. Three-dimensional scanning, electron microscope scanning, micro-CT scanning, etc., provide advanced technical support for the development of geometric morphometry [15–17].
The application of geometric morphometry to coleopteran insects is very extensive. It is often used to explore morphological differences between species with sexual dimorphism \cite{18,19}, intersubspecies, sister groups \cite{20,21}, and high-level categories \cite{22,23}. It is also possible to infer the ancestral form of an existing taxa in order to study its origin and evolution \cite{24}. Geometric morphometry has gradually been applied to different groups of Coleoptera, including Carabidae \cite{25–27}, Lucanidae, Chrysomelidae \cite{28}, Scarabaeidae \cite{29–31}, and Silphidae \cite{32}. However, studies of the geometric morphometry of cave-dwelling ground beetles have rarely been reported \cite{33}.

Based on a geometric morphometric approach, the present paper provides, for the first time, an analysis of the head, pronotum, and elytra of four different adaptive types of ground beetles in order to clarify the morphological adaptations of cavernicolous carabids to the cave environment. In addition, the phenotypic relationship was obtained with a clustering analysis in the genetic category to explore the morphological evolution of cave-dwelling ground beetles.

2. Materials and Methods

2.1. Studied Materials

For the materials used in this study, we implemented the following principles: (1) Sampling as many genera and species as possible, including type species. (2) We did not deal with subgenus separately; species taxonomic treatment was based on the latest publications. (3) We used bibliographic data to obtain the morphological adaptation types of known taxa, e.g., \cite{34–38}.

A total of 140 species in 53 genera of Trechini (49 genera and 132 species from caves and 4 genera and 8 species of surface-dwelling beings) were examined in this study (Table A1). Among them, 97 specimens were deposited at South China Agricultural University, e.g., \cite{39–41}.

2.2. Geometric Morphometric Approach

2.2.1. Image Acquisition

Photographs of existing samples were taken with a Keyence VHX-5000 digital microscope (Figure S1). Due to the lack of specimens for 44 species, images were obtained from related original literature, e.g., \cite{42–44} (Figure S1). For Minimaphaenops (Enshiaphaenops) senecali Deuve, 2016, we gathered the data from the specimen we collected as well as the additional figure of the type specimen from the original publication. After editing using Adobe Photoshop CS6, we imported the data into tps-Util 1.78 \cite{45}.

2.2.2. Landmark Data

The shape of the head, pronotum, and elytra and the positions of stable pores on the elytra were chosen as morphological indicators. We selected the configurations of 50 semilandmarks of each object, except for the stable pores on the elytra, which were represented by 6 landmarks (Figure 2). The landmarks and semilandmarks in each image were digitized using the tps-Dig 2.31 software (Landmark Data S1). The Tps-Small 1.34 software \cite{46} was used to detect the data correlation of the tps files after the landmarks to verify whether the data correlation met the requirements.
2.2.3. Statistical Analysis

The morphological data obtained by the standardized processing of different cave environment-adapted types were imported into the MorphoJ 1.07A software. Generalized Procrustes Analysis (GPA) was used to perform Procrustes superimposition on the overall samples to extract shape variables [47,48]. We used calculational processing to ensure that the sum of the squares of the distances between landmarks of the same serial number was minimized. Additionally, we calculated the overall average shape to compare the degree of difference between the individual and overall average shape (measured by Procrustes distance). On this basis, we applied principal component analysis (PCA).

We selected the first two principal components (PC) to construct scatterplots to show the morphological differences of carabids in different cave environment adaptation types. An energy map of the extreme points of the coordinate origin arrangement was obtained from a thin-plate spline (TPS) analysis using the MorphoJ 1.07A software, where differences in the landmarks were displayed in a visual form.

On the basis of PCA, we set the different cave environment adaptation types as the basis for grouping and performed canonical variate analysis (CVA). The results were displayed through the Mahalanobis distance and Procrustes distance.

2.2.4. Clustering Analysis

The original TPS file was split into 53 subfiles according to genera using the tps-Util 1.78 software; then we used the tps-Super 2.05 software to calculate the average form of each genus. Procrustes distances between genera were preformed using the tps-Small 1.34 software; then we used the unweighted group averaging method (UPGMA) in the NTSYSpc 2.10e software [49] to analyze the Procrustes distance matrix.

3. Results

3.1. Internal Correlation of the Original Data

The original data were converted from camber Kendall space to Euclidean tangent space. For the head, pronotum, and elytra, the correlation coefficients of the data before
and after the conversion were 0.999995, 0.999992, and 1.000000, respectively, which met the requirements.

3.2. PCA of the Morphological Variation in Head, Pronotum and Elytra

Principal component analysis was performed on the morphological data of the head, pronotum, and elytra for 141 species of carabid beetles, with 96, 96, and 108 principal components being obtained, respectively. Among them, the first principal component (PC1) accounted for 89.86%, 84.95%, and 39.35% of the overall variance, while the second principal component (PC2) accounted for 3.94%, 7.75%, and 27.20%, respectively. Using PC1 and PC2, which affect the morphological variation, as the abscissa and ordinate, respectively, a scatterplot of the morphological variation was obtained, and a 90% equal frequency ellipse was constructed based on the cave adaptation type of these carabid beetles (Figure 3a,c,e).

From the perspective of the morphological variation of the head (Figure 3a) and pronotum (Figure 3c), it was found that the aphaenopsian had no overlap and could be distinguished well from anophthalmic and surface-dwelling carabid beetles. The semi-aphaenopsian carabid beetle type was between the aphaenopsian and anophthalmic types and was more similar to the anophthalmic type because of its larger overlaps. The latter was closer to the surface-dwelling carabid beetles. Regarding the morphological variation of the elytra (Figure 3e), these four types overlapped more overall. No significant differences were found between elytra of the semi-aphaenopsian, aphaenopsian, and anophthalmic types.

The energy map of the coordinate origin and the extreme points of the PCA scatterplot of carabid beetles’ head, pronotum, and elytra (Figure 3b,d,f) show that the length/width ratio of the head and pronotum has a tendency to decrease significantly, while their lateral edges expand outward in the positive direction of PC1. The widest point of the head moves to the front, but the front edge of the pronotum tends to be wider than the rear edge. Elytra tend to be more slender and the scutellum appears to be narrower. The anterior of the edge side of the elytra has a tendency to undergo adduction, with the shoulders disappearing and the position of the hair pores moving to the distal end of the elytra. In the positive direction of PC2, the posterior edge of the head is sunken inward and the front and caudal
corners of the pronotum tend to become acute from an obtuse angle. The first four elytra hair pores are more scattered and the last three are closer together.

3.3. CVA of the Morphological Variation in Head, Pronotum, and Elytra

According to the results of the PCA, CVA was used to analyze the morphological variation in the distances of the head, pronotum, and elytra among all the genera of carabid beetles. The results showed that the Mahalanobis distance and Procrustes distance between aphaenopsian and surface-dwelling types were largest when adaptability to a cave environment was used as the basis for grouping. For the head, pronotum, and elytra, the maximum Mahalanobis distance (Table 1) was 28.5719, 20.8313, and 20.7926, respectively, while the maximum Procrustes distance (Table 2) was 0.3429, 0.3258, and 0.1032, respectively.

| Table 1. Mahalanobis distances from four adaptive types of ground beetles based on the head, pronotum, and elytra, respectively. |
|---|---|---|
| Aphaenopsian | Semi-Aphaenopsian | Anophthalmic |
| Semi-aphaenopsian | 11.1596/8.9433/12.6030 |  |
| Anophthalmic | 13.9436/10.7282/15.3457 | 6.6324/7.6456/7.0232 |
| Surface-dwelling | 28.5719/20.8313/20.7926 | 23.0163/16.3761/18.8595 |

| Table 2. Procrustes distances from four adaptive types of ground beetles based on the head, pronotum, and elytra, respectively. |
|---|---|---|
| Aphaenopsian | Semi-Aphaenopsian | Anophthalmic |
| Semi-aphaenopsian | 0.1319/0.1409/0.0516 |  |
| Anophthalmic | 0.2451/0.2342/0.0854 | 0.1227/0.0995/0.0485 |
| Surface-dwelling | 0.3429/0.3258/0.1032 | 0.2275/0.1981/0.0644 |

The Mahalanobis distance (Table 3) and Procrustes distance (Table 3) of the head, pronotum, and elytra were tested to determine the significance of the differences. It was shown that the four different types (aphaenopsian, semi-aphaenopsian, anophthalmic, and surface-dwelling carabid beetles) had high significant differences from each other (p < 0.01).

| Table 3. P-values of the differences in Mahalanobis and Procrustes distances for the four adaptive types of ground beetles (10,000 permutation test, consistent for the head, pronotum, and elytra). |
|---|---|---|
| Aphaenopsian | Semi-Aphaenopsian | Anophthalmic |
| Semi-aphaenopsian | <0.0001 |  |
| Anophthalmic | <0.0001 | <0.0001 |
| Surface-dwelling | <0.0001 | <0.0001 |

3.4. The Phenotypic Evolutionary Relationship between Cave Trechini Genera

Based on the Procrustes distance matrix of the average morphology among 53 genera of carabid beetles, a cluster analysis was performed to construct a morphological phenotypic tree, including the head, pronotum, elytra, and all three (Figure 4). The results revealed that there were differences between the four phenotypic trees, but the variation trend of the head and the integrated morphological phenotypic tree was relatively close. When the 53 genera branched for the first time, the aphaenopsian and semi-aphaenopsian genera clustered into a clade, while the anophthalmic and surface-dwelling genera of carabid beetles clustered into another clade.
In the head phenotypic tree (Figure 4a), Sidublemus was the first to be differentiated into a single branch. Dongodytes, Sinaphaenops, Giraffaphaenops, and Pilosaphaenops were all found to be closely related. However, Shuangheaphaenops, Uenotrechus, Xuedytes, Yanzaphaenops, and Minimaphaenops were mixed together with mostly semi-aphaenopsian genera carabid beetles. From the integrated phenotypic tree (Figure 4d), it could be seen that Giraffaphaenops and Xuedytes were the earliest to differentiate, and they were located far from other genera. The relationship between Yanzaphaenops of the aphaenopsian and semi-aphaenopsian group was relatively close, while Wanhuaphaenops of the anophthalmic group had a close relationship to those of the aphaenopsian group.

In the pronotum phenotypic tree (Figure 4b), all the aphaenopsian group except for Yanzaphaenops was combined into a clade with Wanhuaphaenops, Shenaphaenops, and Huoyanodytes. The remaining three types of carabids genera were clustered together, while about 1/3 of the anophthalmic type were grouped with surface-dwelling carabid beetles in another clade. The result of the elytra phenotypic tree (Figure 4c) showed that Dianotrechus was the first to be differentiated into a single branch. The aphaenopsian group and a small part of the semi-aphaenopsian group were clustered into a clade. Among them, Dongodytes is closely related to Xuedytes, but the same highly specialized Sinaphaenops was far away from the other genera in the aphaenopsian group. Part of the semi-aphaenopsian and anophthalmic groups were grouped together with surface-dwelling carabids.

Figure 4. Phenotypic tree of ground beetle genera based on the Procrustes distance. (a) Head, (b) pronotum, (c) elytra, and (d) integrated morphology. Aphaenopsian: red letters; semi-aphaenopsian: green letters; anophthalmic: blue letters; surface-dwelling: purple letters.
4. Discussion

4.1. Morphological Variation Direction of Cave-Adapted Trechine Beetles

The highly specialized morphological characteristics of cave-dwelling ground beetles have long attracted the attention of researchers [50]. Most previous studies in this area have focused on changes in the morphology of cave-dwelling ground beetles after their long-term adaptation to cave life [51–53]. The present research is the first to attempt to study the morphological adaptation and variation direction of cave-dwelling ground beetles using geometric morphological analysis.

In the extreme environment of caves, animals often show the adaptive characteristics of convergent evolution due to environmental pressure [54,55]. Luo et al. [56,57] found that the cave-dwelling *S. wangorum* shows a distinct head posterior constriction and elongated pronotum combined with long and slender legs. Our results showed that the more slender their body is the higher the degree to which the ground beetles had adapted to the cave environment. This is mainly manifested in the fact that the widest point of the head gradually moves to the front, while the anterior edge of the pronotum tends to be narrower than the posterior edge in surface-dwelling compared to aphaenopsian carabids. Surprisingly, there is little available information concerning the elytra vitiation of cavernicolous carabids or other beetles [58]. We found that the position of the hair pores gradually moved towards the edge of the elytra, except for the scutellum, with the elytra becoming slenderer in cave carabids. One of the reasons why elytra is slenderer is a consequence of reducing or the disappearance of hind wings (also known as humeral calli) [59], and this situation is more distinct among the cave-dwelling species, especially the highly specialized ones.

In addition, aphaenopsian species mostly wander on stalactite walls or roofs in complete darkness [60], while semi-aphaenopsian species run on low rock walls or along the ground [61]. Anophthalmic species often live under small rocks or under damp dead wood in caves, and their habits are relatively close to those of surface-dwelling species [62–64]. It is speculated that the extension of the head and pronotum of cave-adapted ground beetles effectively increases the flexibility of the head, which may help this species to find prey in caves where food is scarce [65]. In contrast, surface-dwelling carabids may face great survival challenges [66,67] and their strong bodies will help them to fight and escape when faced with threats.

4.2. Geometric Morphology Analysis to Judge the Phylogeny of Cavernicolous Carabids

The molecular phylogeny of cave Trechini in China was analyzed based on two mitochondrial and two nuclear genes [68]. The preliminary study showed that the Chinese cave Trechini of Carabidae does not form a monophyletic lineage but rather is composed of four main independent evolutionary clades, each of which contains at least one highly convergent troglomorphic species.

In our study, certain differences exist in the morphological phenotypic trees of the head, pronotum, and elytra based on the Procrustes distance of carabids. For example, typical aphaenopsian genera—such as Dongodytes, Giraffaphaenops, Sinaphaenops, and Xuedytes—show extreme morphological specialization, but they are not clustered into same clade phylogenetically (Figure 4) [69]. Moreover, the semi-aphaenopsian genera of Shenaphaenops and Huoyanodytes and the anophthalmic type of Wanhuaphaenops are more closely related to the aphaenopsian type. A similar situation was also found in the Pyrenean subterranean Trechini, where the phylogenetic relationship between species of the same morphological type was not found to be close [70]. It may therefore be inferred that various Trechini lineages were settled multiple times independently in caves and underwent parallel morphological changes.

Furthermore, we did not classify the subgenus as an independent taxon in the present study, but the morphological differences between some subgenera in the same genus are relatively considerable. These differences may have a certain impact on the results of overall average shape.
Moreover, the length between the clade of Giraffaphaenops + Xuedytes and other genera is extensive in the integrated morphological phenotypic tree (Figure 4). This may have been caused by long periods of geographical isolation, or there may still be large gaps between these two genera and others. Future geometric morphometry of research for these groups could focus on adding the missing new genera and combining molecular phylogeny and biogeography for analysis.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/insects12111002/s1, Figure S1: Photographs of samples, Landmark Data S1: Semi-landmarks of samples.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Basal information of the studied specimens used in the geometric morphometric analysis.

| No. | Species完成 | Locality | Adaptive Type |
|-----|-------------|----------|---------------|
| 1   | Dongodytes (Dongodytes) buxian Tian, 2011 | Guangxi, Du’an County | aphaenopsian |
| 2   | D. (D.) elongatus Tian, Yin & Huang, 2014 | Guangxi, Du’an County | aphaenopsian |
| 3   | D. (D.) foetidus Deuve, 1995 | Guangxi, Bama County | aphaenopsian |
| 4   | D. (D.) giraffa Ueno, 2005 | Guangxi, Tian’e County | aphaenopsian |
| 5   | D. (D.) grandis Ueno, 1998 | Guangxi, Fengshan County | aphaenopsian |
| 6   | D. (D.) ianu Tian, Yin & Huang, 2014 | Guangxi, Du’an County | aphaenopsian |
| 7   | D. (D.) tongziti Yang, Huang & Tian, 2018 | Guangxi, Bama County | aphaenopsian |
| 8   | D. (D.) troglodytes Tian, Yin & Huang, 2014 | Guangxi, Du’an County | aphaenopsian |
| 9   | D. (Dongodytodes) brevipenis Tian, Yin & Huang, 2014 | Guangxi, Du’an County | aphaenopsian |
| 10  | D. (D.) delargeni Tian, 2011 | Guangxi, Du’an County | aphaenopsian |
| 11  | D. (D.) inexpectatus Tian, Yin & Huang, 2014 | Guangxi, Du’an County | aphaenopsian |
| 12  | D. (D.) juzhuansi Tian, Yin & Huang, 2014 | Guangxi, Du’an County | aphaenopsian |
| 13  | Giraffaphaenops clarkei Deuve, 2002 | Guangxi, Dahu County | aphaenopsian |
| 14  | G. yangi Tian & Luo, 2015 | Guangxi, Tianlin County | aphaenopsian |
| 15  | M. (Enskiaphaenops) senca Deuve, 2016 | Hubei, Enshi Autonomous Prefecture | aphaenopsian |
| 16  | M. (Minimaplaenops) lipae Deuve, 1999 | Chongqing, Fengjie County | aphaenopsian |
| 17  | Pilosaphaenops pilosulus Deuve & Tian, 2008 | Guangxi, Huanjiang County | aphaenopsian |
| 18  | P. (P. alatini Tian, 2010 | Guangxi, Huanjiang County | aphaenopsian |
| 19  | Huangheaphaenops elegans Tian, 2017 | Guizhou, Suiyang County | aphaenopsian |
| 20  | Sinaphaenops (Dongaphaenops) xiaoxici Deuve & Tian, 2014 | Guizhou, Fan County | aphaenopsian |
| 21  | S. (Sinaphaenops) banchunica Chen & Tang, 2017 | Guizhou, Guiding County | aphaenopsian |
| 22  | S. (S.) gracilior Ueno & Ran, 1998 | Guizhou, Libo County | aphaenopsian |
| 23  | S. (S.) orthogenys Ueno, 2002 | Guizhou, Duyu City | aphaenopsian |
| 24  | S. (S.) troglodytes Deuve & Tian, 2008 | Guizhou, Sandu County | aphaenopsian |
| 25  | S. (S.) micracanthus Ueno & Wang, 1991 | Guizhou, Libo County | aphaenopsian |
| 26  | S. (S.) michungsia Tian & Huang, 2015 | Guizhou, Duyu City | aphaenopsian |
| 27  | S. (S.) orthogenys Ueno, 2002 | Guizhou, Sandu County | aphaenopsian |
Table A1. Cont.

| No. | Species | Locality | Adaptive Type |
|-----|---------|----------|---------------|
| 28  | S. (S.) wangerum Ueno & Ran, 1998 | Guizhou, Libo County | aphaenopsian |
| 29  | S. (S.) yaoensis Tian, Chen & Yang, 2017 | Guizhou, Duyun City | aphaenopsian |
| 30  | S. (Thaumastaphaenops) bairdaciensis Ueno, 2002 | Guizhou, Ziyun County | aphaenopsian |
| 31  | S. (T.) pulcherrimus Magrini, Vanni & Zanone, 1997 | Guizhou, Ziyun County | aphaenopsian |
| 32  | Uenotrechus deunei Tian & Chen, 2017 | Guangxi, Du’an County | aphaenopsian |
| 33  | U. gejianhuangui Tian & Wei, 2017 | Guangxi, Huangjiang County | aphaenopsian |
| 34  | U. libensis Deuve & Tian, 1999 | Guizhou, Maolan County | aphaenopsian |
| 35  | U. nandianensis Deuve & Tian, 2010 | Guizhou, Nandan County | aphaenopsian |
| 36  | Xuedytes bellus Tian & Huang, 2017 | Guangxi, Du’an County | aphaenopsian |
| 37  | Yanaphaenops hirtipennis Tian & Huang, 2005 | Hubei, Shennongjia | aphaenopsian |
| 38  | Aspidaphaenops dufou Tian & Huang, 2018 | Guizhou, Xingyi County | semi-aphaenopsian |
| 39  | A. masaki Ueno, 2006 | Guizhou, Xingyi County | semi-aphaenopsian |
| 40  | A. reflexus Ueno, 2006 | Guizhou, Xingyi County | semi-aphaenopsian |
| 41  | A. volatilis Ueno, 2006 | Guizhou, Anlong County | semi-aphaenopsian |
| 42  | A. xiongda Tian & Huang, 2018 | Hubei, Shennongjia | semi-aphaenopsian |
| 43  | Boraphaenops angustus Ueno, 2002 | Guangxi, Liping County | semi-aphaenopsian |
| 44  | Guanaphaenops deunei Tian, Feng & Wei, 2016 | Guangxi, Liping County | semi-aphaenopsian |
| 45  | G. lingnanensis Deuve, 2000 | Guizhou, Lipanshui City | semi-aphaenopsian |
| 46  | Guizhaphaenops (Guizhaphaenops) giganterus Ueno, 2000 | Yunnan, Zhenxiong County | semi-aphaenopsian |
| 47  | G. (G.) lipsorum Deuve, 1999 | Guizhou, Pan County | semi-aphaenopsian |
| 48  | G. (G.) pouilliei Deuve & Quenneveu, 2014 | Guizhou, Lipanshui City | semi-aphaenopsian |
| 49  | G. (G.) stratis Ueno, 2000 | Guizhou, Zijin County | semi-aphaenopsian |
| 50  | G. (G.) zhijingensis Ueno & Ran, 2004 | Yunnan, Zhenxiong County | semi-aphaenopsian |
| 51  | G. (S. semiaphaenops) lipsorum zhijingensis Deuve & Tian, 2018 | Yunnan, Zhenxiong County | semi-aphaenopsian |
| 52  | G. (S.) martini Deuve, 2001 | semi-aphaenopsian |
| 53  | G. (S.) yundongensis Deuve & Tian, 2016 | semi-aphaenopsian |
| 54  | Huangaphaenops taiawalensis Tian & Huang, 2016 | semi-aphaenopsian |
| 55  | Jiangaphaenops longiceps Ueno & Clarke, 2007 | semi-aphaenopsian |
| 56  | Luxiophaenops deunei Tian & Yin, 2013 | semi-aphaenopsian |
| 57  | L. yun Tian & Huang, 2015 | semi-aphaenopsian |
| 58  | Plesioaphaenops annae Deuve & Tian, 2011 | semi-aphaenopsian |
| 59  | Shenaphaenops humenensis Ueno, 1999 | semi-aphaenopsian |
| 60  | Shijianaphaenops majusculus Ueno, 1999 | semi-aphaenopsian |
| 61  | Toshiaphaenops globiceps Ueno, 1999 | semi-aphaenopsian |
| 62  | T. tibialis Ueno, 1994 | semi-aphaenopsian |
| 63  | Zhiaphaenops grovialis Ueno & Ran, 2002 | semi-aphaenopsian |
| 64  | Z. haozicus Deuve & Tian, 2018 | semi-aphaenopsian |
| 65  | Z. xiaolong Deuve & Tian, 2015 | semi-aphaenopsian |
| 66  | Z. lii Ueno & Ran, 2002 | semi-aphaenopsian |
| 67  | Z. lii Deuve & Tian, 2015 | semi-aphaenopsian |
| 68  | Z. multisetifer Deuve & Tian, 2018 | semi-aphaenopsian |
| 69  | Z. pubescens Ueno & Ran, 2002 | semi-aphaenopsian |
| 70  | Z. wenganicus Deuve & Tian, 2018 | semi-aphaenopsian |
| 71  | Bathytrechus ruairi Ueno, 2005 | semi-aphaenopsian |
| 72  | Cathaphaenops (Ameggialolophus) angulipennis Ueno, 2000 | semi-aphaenopsian |
| 73  | C. (A.) chuanlongzhensis Deuve, 1999 | semi-aphaenopsian |
| 74  | C. (A.) cyclorhodies Deuve & Tian, 2016 | semi-aphaenopsian |
| 75  | C. (A.) dracorius Deuve, 1999 | semi-aphaenopsian |
| 76  | C. (A.) eriensis Deuve & Tian, 2016 | semi-aphaenopsian |
| 77  | C. (A.) lagrodactylus Deuve, 2016 | semi-aphaenopsian |
| 78  | C. (A.) lynchi Deouve & Tian, 2008 | semi-aphaenopsian |
| 79  | C. (A.) signigalilantii Deuve, 1999 | semi-aphaenopsian |
| 80  | C. (C.) inachus Deuve & Tian, 2015 | semi-aphaenopsian |
| 81  | C. (C.) mirabilis Ueno, 2002 | semi-aphaenopsian |
| 82  | C. (Cathaphaenops) delphini Deuve, 1999 | semi-aphaenopsian |
| 83  | Cimmeritodes (Cimmeritodes) huangii Deuve, 1996 | semi-aphaenopsian |
| 84  | C. (Dianocimmerites) crassifemoralis Deuve & Tian, 2016 | semi-aphaenopsian |
| 85  | C. (Shimenites) shimenensis Deuve & Tian, 2017 | semi-aphaenopsian |
| 86  | C. (Xiangcimmerites) zhongfanjiangensis Deuve & Tian, 2016 | semi-aphaenopsian |
| 87  | C. (Z.) (Z.) zhejiangensis Deuve & Tian, 2015 | semi-aphaenopsian |
| 88  | Devereaphaenops (Devereaphaenops) genicinctus Tian & Huang, 2017 | semi-aphaenopsian |
| 89  | D. (Paraglossus) gelatinosus Tian & Huang, 2017 | semi-aphaenopsian |
| 90  | Dianotrophus guorongxi Deuve, 2016 | semi-aphaenopsian |
| 91  | Dangoblemus kemadongicus Deuve & Tian, 2016 | semi-aphaenopsian |
| 92  | Dianocimmerites lipsorum Deuve & Tian, 2016 | semi-aphaenopsian |
| 93  | Gracilibrum lipingensis Deuve & Tian, 2016 | semi-aphaenopsian |
| 94  | Guiaphaenops yudongensis Deuve, 1997 | semi-aphaenopsian |
| 95  | Guanaphaenops tunsipidiceras Ueno, 1997 | semi-aphaenopsian |
| 96  | Libotrichus duanensis Lin & Tian, 2014 | semi-aphaenopsian |
| 97  | L. nishiharae Ueno, 1998 | semi-aphaenopsian |
| 98  | Microblemus rieui Ueno, 2007 | semi-aphaenopsian |
| 99  | Oodinotrechus (Oodinotrechus) kishimototai Ueno, 1998 | semi-aphaenopsian |
Table A1. Cont.

| No. | Species | Locality | Adaptive Type |
|-----|---------|----------|---------------|
| 100 | O. (O.) nipponensis | Guangxi, Huanjiang County | anophthalmic |
| 101 | O. (P.) nipponensis | Guanxi, Ping County | anophthalmic |
| 102 | Qianphagnos (Qianphagnos) emersoni | Guizhou, Yanhe County | anophthalmic |
| 103 | Q. (Q.) longicornis | Guizhou, Pengyang County | anophthalmic |
| 104 | Q. (Q.) pilosus | Guizhou, Jiangkou County | anophthalmic |
| 105 | Q. (Q.) rotundicollis | Guizhou, Sinan County | anophthalmic |
| 106 | Q. (Q.) ternius | Guizhou, Fenggang County | anophthalmic |
| 107 | Q. (Q.) phagnos (Qianphagnos) variabilis Tian, Huang & Wang 2015 | Guizhou, Song County | anophthalmic |
| 108 | Q. (Q.) phagnos (Qianphagnos) variabilis Tian, Huang & Wang 2015 | Shaxi, Hanzhong City | anophthalmic |
| 109 | Qianotrechus (Quinotrechus) jani Ueno, 2003 | Sichuan, Gulin District | anophthalmic |
| 110 | Q. (Q.) lacus | Guizhou, Zenghuan County | anophthalmic |
| 111 | Q. (Q.) magnicollis | Guizhou, Suiyang County | anophthalmic |
| 112 | Q. (Q.) tenuicollis | Guizhou, Suiyang County | anophthalmic |
| 113 | Q. (Sanwangius) rosselli, Tian & Chen, 2019 | Chongqing, Wulong District | anophthalmic |
| 114 | Satotrechus longitenuis Deuve & Tian, 2011 | Guangxi, Longlin County | anophthalmic |
| 115 | S. rieae | Guizhou, Anlong County | anophthalmic |
| 116 | Shenolemus minuculius Tian & Fang, 2020 | Anhui, Huangshan City | anophthalmic |
| 117 | Sinotrechus fusiformis Ueno, 2003 | Yunnan, Shilin County | anophthalmic |
| 118 | S. sinatrius Huang & Tian, 2015 | Yunnan, Yiliang County | anophthalmic |
| 119 | Shuaphagnos parvicollis Ueno, 1999 | Chongqing, Jincheng District | anophthalmic |
| 120 | Sichuanotrechus albadracinos Ueno, 2006 | Sichuan, Jiangyou City | anophthalmic |
| 121 | S. dukangensis Huang & Tian, 2015 | Sichuan, Jiangyou City | anophthalmic |
| 122 | Suidublemus solidus Tian & Yin, 2013 | Hunan, Guidong County | anophthalmic |
| 123 | Sinotrechus bedouei Deuve, 1996 | Hunan, Longshan County | anophthalmic |
| 124 | S. dypoligus Ueno, 2009 | Hunan, Sangzhi County | anophthalmic |
| 125 | S. yamangwu Huang, Tian & Faille, 2020 | Hubei, Yangcheng County | anophthalmic |
| 126 | Superbotrechus bennetti Deuve & Tian, 2009 | Hubei, Yichang County | anophthalmic |
| 127 | Tianestrechus tristosus Tian & Tang, 2016 | Guangxi, Tian’e County | anophthalmic |
| 128 | Tianhuaphagnos pinnaniclus Zhao & Tian, 2016 | Guizhou, Tianhu County | anophthalmic |
| 129 | Wanhuaphagnos zhangi Tian & Wang, 2016 | Hunan, Chenzhou City | anophthalmic |
| 130 | Wanotrechus uyi Tian & Fang, 2016 | Anhui, Xuancheng City | anophthalmic |
| 131 | Wulungorbolema taubengoides Ueno, 2007 | Zhejiang, Jiangyao County | anophthalmic |
| 132 | Yunotrechus diumansuis Tian & Huang, 2014 | Yunnan, Maguan County | anophthalmic |
| 133 | Agonotrechus spinangulus Belousoy, Kabak & Liang, 2019 | Sichuan, Muli County | surface-dwelling |
| 134 | Potrechiana crassipes Ueno, 1997 | Sichuan, Meigu County | surface-dwelling |
| 135 | Sinotrechus yunnanus Belousoy, Kabak & Liang, 2019 | Yunnan, Daxaoy County | surface-dwelling |
| 136 | Trechus aghzaius Belousoy & Kabak, 2019 | Xinjiang, Zhaosu County | surface-dwelling |
| 137 | T. cratoecephalus Belousoy & Kabak, 2019 | Xinjiang, Zhaosu County | surface-dwelling |
| 138 | T. salubi Belousoy & Kabak, 2019 | Xinjiang, Xinyuan County | surface-dwelling |
| 139 | T. torayu Belousoy & Kabak, 2019 | Xinjiang, Heiling County | surface-dwelling |
| 140 | T. tsanmensis Belousoy & Kabak, 2019 | Xinjiang, Xinyuan County | surface-dwelling |

References

1. Fang, J.; Li, W.B.; Wang, X.H.; Tian, M.Y. New cavernicolous ground beetles from Anhui Province, China (Coleoptera, Carabidae, Trechinae, Platynini). Zootaxa 2020, 2923, 33–50. [CrossRef] [PubMed]
2. Tian, M.Y.; Huang, X.L.; Li, C.L. Contribution to the knowledge of subterranean ground beetles from eastern Wuling Mountains, China (Coleoptera: Carabidae: Trechinae). Zootaxa 2021, 4926, 521–534. [CrossRef]
3. Deuve, T.; Tian, M.Y.; Ran, J.C. Trois caraboides remarquables de la réserve nationale de maolan, dans le guizhou meridional, chine (coleoptera, carabidae et trechidae). Revue Française d’Entomologie (N.S.) 1999, 21, 131–138.
4. Barr, T.C.; Holsinger, J.R. Speciation in cave faunas. Annu. Rev. Ecol. Syst. 1985, 16, 313–337. [CrossRef]
5. Faille, A.; Casale, A.; Ribera, I. Phylogenetic relationships of west Mediterranean troglobitic Trechini ground beetle species (Coleoptera: Carabidae). Zool. Scr. 2011, 40, 282–295. [CrossRef]
6. Jeannel, R. Faune de France. In Coleoptères Carabiques; Paul Lechevalier et Fils Publ: Paris, France, 1941; Volume 39, p. 571.
7. Casale, A.; Vigna, T.A.; Juberthie, C. Coleoptera: Carabidae. In Encyclopaedia Biospeologica, 3nd ed.; Juberthie, C., Decu, V., Eds.; Marinela Nazareanu et Violeta Berlescu: Bucharest, Romania, 1998; pp. 1047–1081.
8. Moldovan, O.T. Beetles. In Encyclopedia of Caves, 2nd ed.; White, W.B., Culver, D.C., Eds.; Elsevier: Amsterdam, The Nederland, 2012; pp. 54–62.
9. Adams, D.C.; Rohlf, F.J.; Slice, D.E. Geometric morphometrics: Ten years of progress following the `revolution’. Ital. J. Zool. 2004, 71, 5–16. [CrossRef]
10. Adams, D.C.; Rohlf, F.J.; Slice, D.E. A field comes of age: Geometric morphometrics in the 21st century. Hyrix 2013, 24, 7–14.
11. Bookstein, F.L. Biometrics, biomathematics and the morphometric synthesis. Bull. Math. Biol. 1996, 58, 313–365. [CrossRef]
12. Corti, M. Geometric Morphometrics: An extension of the revolution. Trends Ecol. Evol. 1993, 8, 302–303. [CrossRef]
13. Bai, M.; Yang, X.K. Application of geometric morphometrics in biological researches. Chin. Bull. Entomol. 2007, 44, 143–147.
14. Chen, J.Y.; Schopf, J.W.; Bottjer, D.J.; Zhang, C.Y.; Kudryavtsev, A.B.; Tripathi, A.B.; Wang, X.Q.; Yang, Y.H.; Gao, X.; Yang, Y. Raman spectra of a Lower Cambrian cnenphorid embryo from southwestern Shaanxi, China. Proc. Natl. Acad. Sci. USA 2007, 104, 6289–6292. [CrossRef] [PubMed]
15. Yang, H.D.; Bai, M.; Li, S.; Lu, Y.Y.; Ma, D.Y. A study of the three-dimensional morphological complexity of insect hindwing articulation based on four scarab species (Coleoptera: Scarabaeoidea). Acta Entomol. Sin. 2015, 58, 1322–1330.
45. Rohlf, F.J. tpsUtil, file Utility Program, version 1.64; Department of Ecology and Evolution, State University of New York at Stony Brook: Stony Brook, NY, USA, 2013. Available online: http://smbmorphometrics.org/ (accessed on 12 September 2019).
46. Rohlf, F.J. The Tps Series of Software. Hystrix Ital. J. Mamm. 2015, 26, 9–12.
47. Bookstein, F.L. Thin-plate splines and the atlas problem for biomedical images. In Proceedings of the 12th International Conference on Information Processing in Medical Imaging, Berlin/Heidelberg, Germany, 7–12 July 1991; Colchester, A.C.F., Hawkes, D.J., Eds.; Springer: Berlin, Germany, 1991; pp. 326–342.
48. Rohlf, F.J.; Bookstein, F.L. Computing the uniform component of shape variation. Syst. Biol. 2003, 52, 66–69. [CrossRef] [PubMed]
49. Rohlf, F.J. On the use of shape spaces to model morphometric methods. Hystrix Ital. J. Mamm. 2000, 11, 9–25.
50. Deuve, T.; He, L.; Tian, M.Y. Descriptions of the first semi-aphenopsian troglobitic Patrobini and of a new anophthalmic Trechidae. Bull. Soc. Entomol. France. 2002, 107, 515–523.
51. Howarth, F.G. Ecology of cave arthropods. Annu. Rev. Entomol. 1983, 28, 365–389. [CrossRef]
52. Moldovan, O.T.; Jalžić, B.; Erichsen, E. Adaptation of the mouthparts in some subterranean Cholevinae (Coleoptera, Leiodidae). Natura Croatica 2004, 13, 1–18.
53. White, W.; Culver, D.C. Encyclopedia of Caves; Academic Press: Amsterdam, The Nederland, 2012; p. 945.
54. Forsythe, T.G. The relationship between body form and habit in some Carabidae (Coleoptera). J. Zool. 1987, 211, 643–666. [CrossRef]
55. Pipan, T.; Culver, D.C. Shallow subterranean habitats. In Encyclopedia of Caves, 2nd ed.; White, W., Culver, D.C., Eds.; Academic Press: Amsterdam, The Nederland, 2012; pp. 683–690.
56. Liu, W.X.; Golovatch, S.I.; Wesner, T.; Tian, M.Y. Convergent Evolution of Unique Morphological Adaptations to a Subterranean Environment in Capillipedes (Diplopoda). PLoS ONE 2017, 12, e0170717. [CrossRef] [PubMed]
57. Luo, X.Z.; Wipfler, B.; Ribera, I.; Liang, H.B.; Tian, M.Y.; Ge, S.Q.; Beutel, R.G. The cephalic morphology of free-living and cave-dwelling species of trechine ground beetles from China (Coleoptera, Carabidae). Org. Divers. Evol. 2018, 18, 125–142. [CrossRef]
58. Luo, X.Z.; Wipfler, B.; Ribera, I.; Liang, H.B.; Tian, M.Y.; Ge, S.Q.; Beutel, R.G. The thoracic morphology of cave-dwelling and free-living ground beetles from China (Coleoptera, Carabidae, Trechinae). Arthropod Struct. Dev. 2019, 53, 109900. [CrossRef] [PubMed]
59. Tian, M.Y. New records and new species of cave-dwelling trechine beetles from Mulun Nature Reserve, northern Guangxi, China (Insecta: Coleoptera: Carabidae: Trechinae). Subterr. Biol. 2019, 7, 69–73.
60. Tian, M.Y.; Huang, S.B. Contribution to the knowledge of the cavernicolous beetle genus Aspidaphaenops Uéno from Guizhou (Coleoptera: Carabidae: Trechinae). Zootaxa 2018, 4422, 244–258. [CrossRef]
61. Tian, M.Y.; Huang, S.B. New records and a new species of the cavernicolous genus Güiodytes Tian, 2013 from Guangxi, China (Coleoptera: Carabidae: Scaritinae). Zootaxa 2014, 3861, 355–362. [CrossRef] [PubMed]
62. Lin, W.; Tian, M.Y. Supplemental notes on the genus Libotrechus Uéno, with description of a new species from Guangxi, southern China (Coleoptera: Carabidae: Trechinae). Coleopts Bull. 2014, 68, 429–433. [CrossRef]
63. Deuve, T.; He, L.; Tian, M.Y. Descriptions of the first semi-aphenopsian troglobiotic Patrobini and of a new anophthalmic cave-dwelling Trechini from central Sichuan, China (Coleoptera: Caraboidae). Ann. Soc. Entomol. France 2020, 37, 1–13. [CrossRef]
64. Pang, J.M.; Tian, M.Y. A remarkably modified species of the tribe Platynini (Coleoptera, Carabidae) from a limestone cave in Jiangxi Province, eastern China. ZooKeys 2014, 382, 1–12.
65. Wisen, G.; Gasith, A. An unprecedented role reversal: Ground beetle larvae (Coleoptera: Carabidae) lure amphibians and prey upon them. PLoS ONE 2011, 6, e25161. [CrossRef]
66. Sugiura, S.; Sato, T. Successful escape of bombardier beetles from predator digestive systems. Biol. Lett. 2018, 14, 20170647. [CrossRef]
67. Huang, S.B. Molecular Phylogeny of the Cavernicolous Trechine Beetles in China (Coleoptera: Carabidae). Master’s Thesis, South China Agricultural University, Guangzhou, China, 2016.
68. Huang, S.B.; Tian, M.Y.; Faille, A. A preliminary phylogeny of cave trechine beetles from China (Coleoptera: Carabidae: Trechina). ARPHA Conf. Abstr. 2020, 3, e51897. [CrossRef]
69. Faille, A.; Ribera, I.; Deharveng, L.; Bourdeau, C.; Garnery, L.; Deuve, T. A molecular phylogeny shows the single origin of the Pyrenean subterranean Trechini ground beetles (Coleoptera: Carabidae). Mol. Phylogen. Evol. 2010, 54, 97–106. [CrossRef] [PubMed]