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Identification and morphological description of the larva of *Uloma* (*Uloma*) *excisa excisa* Gebien, 1914 (Coleoptera: Tenebrionidae: Ulomini)

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Abstract

The larva of *Uloma* (*Uloma*) *excisa excisa* Gebien, 1914, from southern China, is associated with the adult by using molecular phylogenetic analyses based on mtDNA *COI* sequences. The morphological characteristics of the larva of this species are described and illustrated in detail for the first time. A key to the larvae of three Chinese *Uloma* species is provided.

Key words: *Uloma* (*Uloma*) *excisa excisa*, immature stages, morphology, *COI*, China

Introduction

The tribe Ulomini Blanchard, 1845 is globally distributed except for Antarctica, belonging to the family Tenebrionidae Latreille, 1802 of Coleoptera. Ulomini currently includes more than 350 described species and subspecies of 26 genera (Merkl & Ando 2018; Bouchard *et al*. 2021). *Uloma* Dejean, 1821 is the most speciose genus in this tribe, with more than 200 described species and subspecies. The genus is especially diverse in tropical regions (Schawaller 2000, 2015; Merkl & Ando 2018). However, the immature stages of *Uloma* are still poorly known. Most research focused on the mature stage. For example, the larvae for only 3% (six species) and the pupae for 2.25% (five species) of the numerous known *Uloma* species have been described (Perris 1877; Korschefsky 1943; Byzova & Keleinikova 1964; Hayashi 1966; Skopin 1978; Fontes 1979; Steiner 1995; Klausnitzer 1996; Cherney 2005, 2006; Liu *et al*. 2022; Niu *et al*. 2023). Even so, some of these descriptions are incomplete for good morphological comparisons, and the figures are more limited.

During the study of insects in the Nanling Mountains region, which is located at the junction of Guangdong Province, Guangxi Zhuang Autonomous Region, Hunan Province, and Jiangxi Province of south China, we found several adults and larvae of *Uloma* in soft decaying tissue on dead standing tree trunks in warm and moist forests of Yangshan County, Guangdong Province (Fig. 1). The adults were identified to belong to one species, *Uloma* (*Uloma*) *excisa excisa* Gebien, 1914, on the basis of morphological characteristics. The morphological structures of these larvae were extremely similar to each other, so the larvae may belong to one morphospecies. However, we were unable to reliably identify them to associate them with adults of *U.* (*U.*) *excisa excisa* by morphological evidence. Therefore, the molecular phylogenetic methods were used to resolve the associated problem, based on the cytochrome oxidase subunit I (*COI*) gene fragment. Finally, the larvae were confirmed to belong to *U.* (*U.*) *excisa excisa*.

In this paper, the larva of *U.* (*U.*) *excisa excisa* is described and illustrated in detail for the first time. *U.* (*U.*) *excisa excisa* is the third *Uloma* species from China whose larvae are described after *U.* (*U.*) *metogana* Ren, 2004 and *U.* (*U.*) *intricornicula* Liu, Ren & Wang, 2007 (Liu *et al*. 2022; Niu *et al*. 2023). A key to these three larvae is provided for promoting the classification and identification of immature stages of Ulomini. In addition, three

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samples of *U.* (*U.*) *intricornicula* are tested for molecular comparisons, *COI* sequences for this species is also provided.

FIGURE 1. Habitats for *Uloma* (*Uloma*) *excisa excisa* Gebien, 1914. Photoed by Xing-Long Bai at Dakeng, Chengjia, Yangshan, Qingyuan, Guangdong, China, on May 14, 2021.

Material and methods

Morphology

The specimens of *Uloma* (*Uloma*) *excisa excisa* Gebien, 1914 were collected by hand primarily beneath the bark of rotten trunks in the wild. They were preserved in 95% ethanol and then transported to the laboratory. The morphological characteristics and molecular data establish the identity of larvae and adults of *U.* (*U.*) *excisa excisa*. All specimens were deposited at the Museum of Hebei University, Baoding, China (MHBU).

The specimens were observed and described using a Nikon SMZ800. The photographs were taken with three imaging systems: (a) Canon EOS 5D Mark III (Canon Inc., Tokyo, Japan) connected to a Laowa FF 100 mm F2.8 CA-Dreamer Macro 2× or Laowa FF 25 mm F2.8 Ultra Macro 2.5–5× (Anhui Changgeng Optics Technology Co., Hefei, China); (b) a Leica M205A stereomicroscope equipped with a Leica DFC450 camera (Leica Microsystems, Singapore, Singapore), which was controlled using the Leica application suite v. 4.3; (c) JVC KY-F75U (JVC Kenwood, Long Beach, CA, USA) digital camera attached to a Leica Z16 APO dissecting microscope (Leica Microsystems, Buffalo Grove, IL, USA) with an apochromatic zoom objective and motor focus drive, using a Syncroscopy Auto-Montage System (Synoptics, Cambridge, UK) and software. Multiple images were used to construct the final figures. Images were illuminated with either an LED ring light attached to the end of the microscope column, with incidental light filtered to reduce glare, or by a gooseneck illuminator with bifurcating fiberoptics; image stacks were white-balance corrected using the system software (Synoptics, Cambridge, UK). Montaged images were edited using Adobe Photoshop CC 2019 to form the final figure plates.

The morphological terminology of larval structures follows that of Matthews *et al*. (2010) and Niu *et al*. (2023). More than one larva was examined, so the range of values was given.

DNA extraction, PCR amplification, and sequencing

To correlate the different stages, the molecular data were collected from larval and adult individuals. There were 17 samples used for molecular comparisons and analyses. Most of them were preserved in 95 % alcohol.

DNA was extracted from the pygopod tissue of the larva and the leg muscle tissue of the adult using the Insect DNA Isolation Kit (BIOMI-GA, Hangzhou, China) following the manufacturer's protocols. The DNA extracted was stored at -20°C. The fragment of mitochondrial molecular marker (cytochrome oxidase subunit I, *COI*) was amplified with the primers F 2183 and R 3014 (Folmer *et al*. 1994). The profile of the PCR amplification consisted of an initial denaturation step at 94°C for 4 min, 35 cycles of denaturation at 94°C for 45 s, annealing at 47°C for 1.5 min, and extension at 72°C for 1 min, and a final 8 min extension step at 72°C. PCR was performed using TaKaRa Ex Taq (TaKaRa, Dalian, China). PCR products were subsequently checked by 1% agarose gel electrophoresis, and sequencing was performed at General Biol Co. (Anhui, China). We used sequences of three species of the tribe Amarygmini as outgroups.

The newly obtained sequences are deposited in GenBank. Sequences of a few samples were obtained from GenBank. All sample information is listed in Table 1.

TABLE 1. Experimental sample and sequence information.

Specimens code	Species	Stage	Locality	Latitude and longitude	GenBank ID *COI*	Source of data
GDQY001	*Uloma* (*Uloma*) *excisa excisa*	Larva	Dakeng, Chengjia, Yangshan, Qingyuan, Guangdong, China	24°47'N, 112°50'E	PP977069	This study
GDQY002		Larva	Dakeng, Chengjia, Yangshan, Qingyuan, Guangdong, China	24°47'N, 112°50'E	PP977070	This study
GDQY003		Larva	Dakeng, Chengjia, Yangshan, Qingyuan, Guangdong, China	24°47'N, 112°50'E	PP977071	This study
GDQY004		Adult (male)	Dakeng, Chengjia, Yangshan, Qingyuan, Guangdong, China	24°47'N, 112°50'E	PP977072	This study
GDQY005		Adult (female)	Dakeng, Chengjia, Yangshan, Qingyuan, Guangdong, China	24°47'N, 112°50'E	PP977073	This study
GDQY006		Adult (male)	Dakeng, Chengjia, Yangshan, Qingyuan, Guangdong, China	24°47'N, 112°50'E	PP977075	This study
GXGL001		Adult (male)	Huaping, Guilin, Guangxi, China	25°63'N, 109°91'E	PP977074	This study
HNLD003	*Uloma* (*Uloma*) *intricornicula*	Larva	Jianfengling, Ledong Li, Hainan, China	18°44'N, 108°51'E	PP977078	This study
HNLD002		Adult (female)	Jianfengling, Ledong Li, Hainan, China	18°44'N, 108°51'E	PP977077	This study
HNLD001		Adult (male)	Jianfengling, Ledong Li, Hainan, China	18°44'N, 108°51'E	PP977076	This study
XZLZ001	*Uloma* (*Uloma*) *metogana*	Larva	Duoka, Yi'ong, Nyingchi, Xizang, China	30°07'N, 95°01'E	OL828345	Liu *et al*., 2022
XZLZ002		Pupa	Duoka, Yi'ong, Nyingchi, Xizang, China	30°07'N, 95°01'E	OL828344	Liu *et al*., 2022
XZLZ003		Adult (female)	Duoka, Yi'ong, Nyingchi, Xizang, China	30°07'N, 95°01'E	OL828343	Liu *et al*., 2022
-	*Uloma* sp.	Larva	-	-	KT876915	Linard *et al*., 2016
Outgroup1	*Plesiophthalmus colossus*	Adult	Huangniushi, Jiulianshan, Longnan, Jiangxi,China	24°33′N, 114°25′E	PP977079	This study
Outgroup2	*Plesiophthalmus davidis*	Adult	Mihouyuan, Yuanqu, Shanxi,China	35°24'N, 112°1'E	PP977080	This study
Outgroup3	*Plesiophthalmus longipes*	Adult	Guangtang, Anhe, Quanzhou, Guangxi, China	25°44′N 110°31′E	PP977081	This study

Phylogenetic analyses and Molecular species delimitation

Phylogenetic analysis was based on the *COI* sequences by Maximum Likelihood (ML) method. A best-fit model was tested according to the corrected Akaike's Information Criterion (AICc) using ModelFinder (included in IQ-TREE) with the software PhyloSuite v1.2.2 (Zhang *et al*. 2020). The ML tree search was performed in IQ-TREE v1.6.8 (Nguyen *et al*. 2015), which was also plugged into PhyloSuite. The ML tree was inferred using an edge-linked partition model for 5000 ultrafast bootstraps (1000 replicates) (Minh *et al*. 2013). Support for each node is represented by ultrafast bootstrap values (uBV).

We used a combination of three distinct methods (ASAP, GMYC, and PTP) to assess the boundaries of species within the genus *Uloma*. We relied on the Assemble Species by Automatic Partitioning (ASAP) approach as implemented on the online web application (https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html) (Puillandre *et al*. 2021). ASAP analysis was carried out based on *COI*, and outgroups were excluded. In addition to the distance-based ASAP method, we also performed tree-based analyses using two distinct methods: the General Mixed Yule Coalescent (GMYC) model and Poisson-tree-processes (PTP) (Pons *et al*. 2006; Zhang *et al*. 2013). Accordingly, GMYC analysis was conducted on an ultrametric tree from the BEAST analysis, with all outgroups removed (Bouckaert *et al*. 2014). The analysis was on the online web application (https://species.h-its.org/gmyc/). PTP analysis relied on the best-score ML tree from the IQ-TREE analysis and was carried out on the web server of the Exelixis Lab (http://species.h-its.org/ptp/) using default settings.

Results

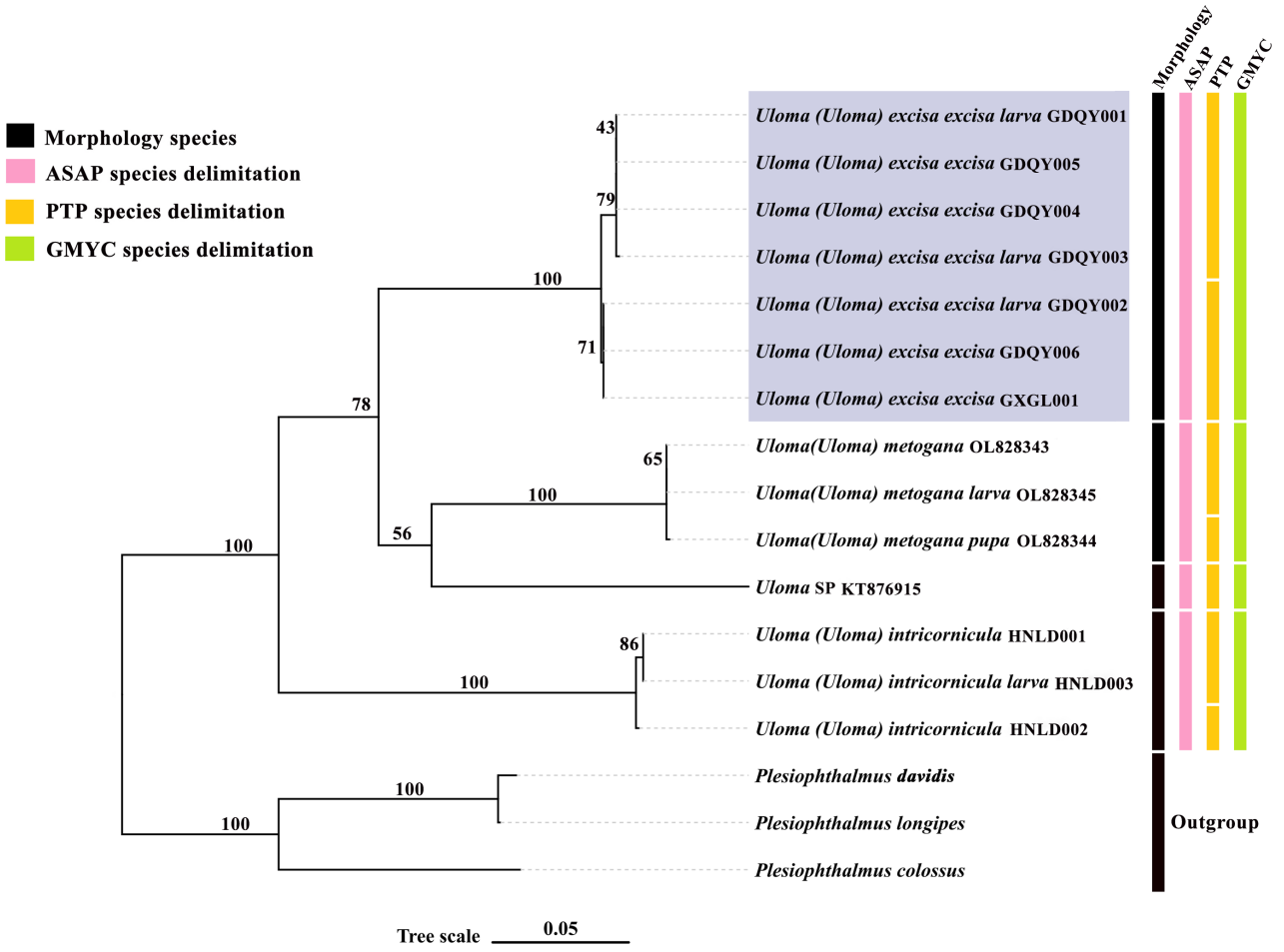


FIGURE 2. Maximum-likelihood phylogenetic tree based on 758 bp of the *COI* gene fragments within *Uloma*. Vertical colored bars delineate extant morphospecies (black), and the results of three separate molecular analyses delimiting species (pink, yellow and light green).

Phylogenetic relationships and species delimitation

The IQ-TREE analysis yielded a topology based on the *COI* gene fragment (758 bp), including 17 sequences from 17 individuals (Fig. 2). The monophyly of each clade was well supported overall.

The ML tree and three molecular species delimitation methods associate the larvae and adults of different species with consistent results. Larvae and adults of known species cluster into a single well-supported clade respectively: *U.* (*U.*) *excisa excisa* (uBV=100), *U.* (*U.*) *metogana* (uBV=100), *U.* (*U.*) *intricornicula* (uBV=100). Therefore, we conclude that the above assumption is correct that the sample is the larva of *U.* (*U.*) *excisa excisa.*

Morphological description

Uloma (*Uloma*) *excisa excisa* Gebien, 1914

Chinese common name: 四突齿甲指名亚种
(Figs 3–12)

Uloma excisa Gebien, 1914: 24 (type locality: China, Taiwan).
Uloma excisa var. *tschungseni* Kaszab 1954: 254.
Uloma excisa excisa: Masumoto & Nishikawa 1986: 26.
Uloma (*Uloma*) *excisa excisa*: Löbl *et al*. 2008: 302.

Larval description. Oligopod. ***Body.*** Length 11.0–14.0 mm, width 1.5–1.7 mm. Body (Figs 3–5) subcylindrical, flat ventrally and with sharp tail-end; evenly sclerotized dorsally and ventrally, more strongly sclerotized at posterior part (Figs 3–4); yellowish brown, darker at posterior part. Vestiture smooth, suffused with large and round punctures dorsally and laterally (Fig. 3, 5).

Head. Head (Figs 6–8) slightly narrower than prothorax, nearly semicircular, convex dorsally; with four long setae near anterior margin, middle two of them longer, and six subsequently, then six near posterior margin in dorsal view (Fig. 7). Frontoclypeal suture (Fig. 7) obvious, nearly straight in middle. Frons (Fig. 7) feebly convex, frontal sutures V-shaped, slightly incurved near middle; median suture barely visible. Clypeus (Fig. 8g) slightly flat, with no puncture on anterior half and dense large punctures on posterior half; anterior margin straight. Labrum (Fig. 8h) transverse, semi-elliptic, with two long erect median setae; anterior margin slightly wavy with several short setae. Ocelli (Fig. 8i) black, divided into two parts of a small upper part and a big lower part. Mandibles (Fig. 6c) well developed, black and dentate at distal part, extended anteriorly; right mandible with three teeth, median tooth obviously larger than dorsal and ventral one; left mandible with two teeth, dorsal tooth obviously larger than ventral one. Maxillae subparallel laterally, with dense thick setae on apical inner margin; maxillary palpi (Fig. 6b) distolateral, subconical, slightly pointed at apices. Labial palpi (Fig. 6d) 2-segmented, short; I nearly equivalent to II, but wider than the latter. Ligula obviously protruding at anterior margin with two short setae. Prementum (Fig. 6e) short, about 1/4 as long as mentum, with two long erect setae. Mentum (Fig. 6f) subhexagonal, widest at basal 3/4, anterior margin wider than posterior margin; smooth, but rough with dense and small punctures at anterior quarter; with two long erect setae on both sides and posterior part respectively, latter much thicker and longer; anterior margin weakly emarginate. Antennae (Fig. 6a) about 1/3 as long as head; antennomere I short; II obviously cylindrical, longest, more than twice as long as I and equivalent to I in width; III thinnest and shortest, about 1/6 as long as II, and with one long erect median seta and three short setae around base of long one at apex of III.

Thorax. Thorax 3-segmented (Fig. 10). Dorsomeson (Fig. 3) obvious. Each thoracic tergum nearly rectangular in dorsal view. Prothoracic tergum (Fig. 10) longest, about twice as long as meso- or metathoracic tergum, ratio of length of each thoracic tergum as follows: 1.1: 0.6: 0.8. Prothoracic tergum (Fig. 10) a little longer than width; anterior margin weakly protruding, with a brown broad stripe and eight long setae, lateral two next to each other; posterior margin also weakly protruding, with a brown narrower stripe and six long setae. Mesothoracic tergum (Fig. 10) transverse; anterior margin with a brown narrow stripe, rather dense and large punctures, and two long setae laterally; posterior margin with a brown broader stripe and four long setae. Metathoracic tergum (Fig. 10) transverse; anterior margin with a brown narrow stripe, rather dense and large punctures, and two long setae laterally; posterior margin with a brown broader stripe and six long setae. Mesothoracic spiracles (Fig. 9b) slenderly oval, larger than abdominal spiracles; spiracles visible in ventral view, lying on anterolateral margins of mesothoracic tergum, and near coxal cavity.

FIGURES 3–5. Habitus of the larva of *Uloma* (*Uloma*) *excisa excisa* Gebien, 1914. **3.** Dorsal view. **4.** Ventral view. **5.** Lateral view. Scale bars: 1 mm.

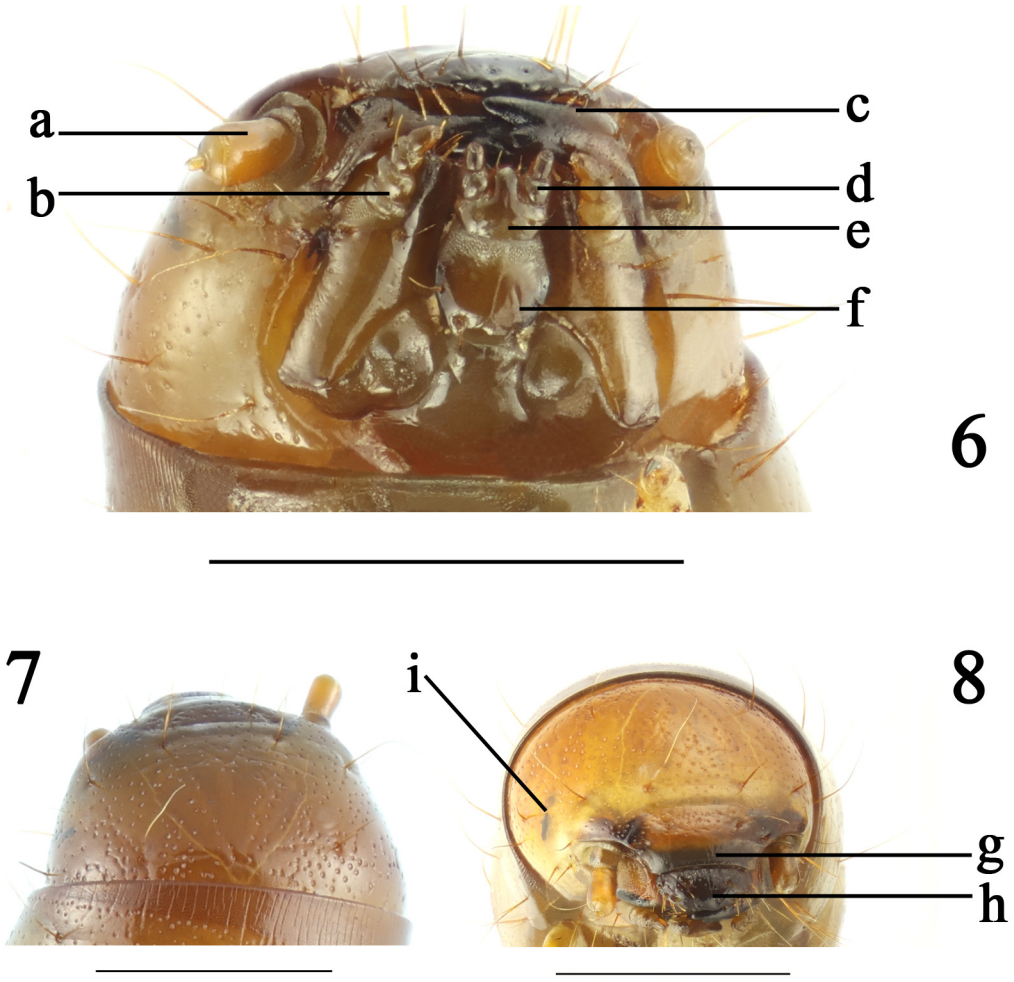
Legs. Legs short, subequal in length, and similar in shape (Figs 9–10). Coxa of prothoracic leg thicker and longer than other segments, with several spines and long setae on inner and outer margins. Prothoracic leg (Fig. 9a, 10f) with inner margin setal formula as follows: 4–5 (1–2): 4 (3): 2 (2). Trochanter subtriangular, with one spine on outer margin; femur subtrapeziform, with three spines on outer margin; tibia subcylindrical, slightly thinner towards to the apex, and with two or three spines on outer margin. Protarsungulus strongly sclerotized, falciform, with two thinner short setae under it. Coxa of mesothoracic leg with dense spines and long setae on inner and outer margin. Mesothoracic leg (Fig. 9c, 10g) with inner margin setal formula as follows: 3–4 (1–2): 4–5 (3): 2 (2). Outer margin of trochanter with one spine, femur with three spines and tibia with two spines. Coxa of metathoracic leg with dense spines and long setae on inner and outer margins. Metathoracic leg (Fig. 9d, 10h) with inner margin setal formula as follows: 2–4 (1–2): 4 (3): 2 (2). Outer margin of trochanter with one spine, femur with three spines and tibia with two spines.

Abdomen. Abdomen 9-segmented (Fig. 5), gradually and slightly darker toward apex, and successively and slightly thicker backwards, except for last segment. Segments I–IX each with much denser punctures near dorsal anterior margin comparing with other parts of abdominal segments, and with a brown broad stripe on posterior margin (Fig. 3). Tergites I–VII (Fig. 3) wider than length, nearly rectangular in dorsal view, with four long erect setae on sides of posterior margins, and only tergite I with two long erect setae on sides of anterior margin. Sternites I–VII (Fig. 4) nearly rectangular, longer than width, and with one long erect seta near four corners respectively, and sternites I with other six long erect setae near anterior margin. Segment VIII (Fig. 12) without pleural sutures; quadrate in dorsal view, with two long erect setae respectively on posterolateral margins; with two erect setae near

anterior margin and six near middle of posterior margin in ventral view. Segment IX (Fig. 11) with dense even large punctures, without non-puncture area near ventral anterior margin; ventral surface with four rows of long erect setae, respectively six, six, two, and two from base to apex; anterior margin almost straight in ventral view (Fig. 12). Segment IX (Figs 11–12) parabolic form, slightly longer than its width, subcircular in cross-section; tip-end slightly pointed with a small papilla but without urogomphi. Anal concealed in posterior part of abdominal tergite VIII, without anal tubes. Abdominal spiracles (Fig. 9e) subcircular, of same size, lying on anterolateral margins of segments I–VIII.

Larval diagnosis. *U.* (*U.*) *excisa excisa* is distinguishable from other *Uloma* species by the following characters: anterior margin of clypeus straight; anterior margin of labrum slightly wavy; mentum subhexagonal, widest at basal 3/4, with dense and small punctures at anterior quarter, anterior margin weakly emarginate; antennomere III about 1/6 as long as II; prothoracic tergum with anterior and posterior margins weakly protruding; protibia with one or two spines on inner margin; mesotibia with one or two spines on inner margin; abdominal segment IX without non-puncture area anteriorly, anterior margin almost straight on ventral surface.

The larvae of the genus *Uloma* can be easily distinguished from other known tenebrionid larvae by a reduced anal region, the absent anal tubes, the absent cerci, the absent pleurosternal sutures on abdominal segment VIII, a paraboloid abdominal segment IX with an apical point, the subcircular cross-section and an elongate anterior extension on the hypopharygeal sclerome (Hayashi 1966; Watt 1974; Liu *et al*. 2022). However, the *Uloma* larvae are very similar to one another in morphological characteristics. Even so, we found the differential characteristics among the known larvae of the genus *Uloma* from China by examining and comparing the specimens directly. The main differences among these species are shown in the key below.



FIGURES 6–8. Larva of *Uloma* (*Uloma*) *excisa excisa* Gebien, 1914. **6.** Head, ventral view. **7.** Head, dorsal view. **8.** Head, anterior view. Scale bars: 1 mm. a. antenna; b. maxillary palpus; c. mandible; d. labial palpus; e. prementum; f. mentum; g. clypeus; h. labrum; i. ocellus.

FIGURES 9–12. Larva of *Uloma* (*Uloma*) *excisa excisa* Gebien, 1914. **9.** Thorax and abdominal segment I, ventral view. **10.** Head and thorax, lateral view. **11.** Abdominal segments VII–IX, lateral view. **12.** Abdominal segments VII–IX, ventral view. Scale bars: 1 mm. a, f. prothoracic leg; b. mesothoracic spiracle; c, g. mesothoracic leg; d, h. metathoracic leg; e. abdominal spiracle.

Examined materials. 7 larvae (MHBU), China, Guangdong Province, Qingyuan City, Yangshan County, Chengjia Township, Dakeng Village, elev. 120 m, 24°47'N, 112°50'E, 2021. V. 14, Xing-Long Bai & Hao-Lin Liu leg.

Distribution. China: Guangdong, Guangxi, Fujian, Taiwan; Korea, Japan, Vietnam.

Key to the known larvae of Chinese *Uloma* (*Uloma*) species

1. Anterior margin of abdominal segment IX emarginate on ventral surface . . . . . ***U.* (*U.*) *metogana* Ren, 2004**
- Anterior margin of abdominal segment IX almost straight on ventral surface 2
2. Mentum relatively slender, widest in middle; protibia with two spines on inner margin . . . . . ***U.* (*U.*) *intricornicula* Liu, Ren & Wang, 2007**
- Mentum widest at basal 3/4, more anteriorly; protibia with one or two spines on inner margin . . . . . ***U.* (*U.*) *excisa excisa* Gebien, 1914**

Discussion

The larvae and adults of *Uloma* (*Uloma*) *excisa excisa* were collected in the wild, so we could not determine the exact developmental stage of the larvae. The larval description above is mainly based on a specimen with more sclerotic and larger body size, which could be a later instar larva.

Furthermore, recent studies have shown that some molecular species definition methods may underestimate or overestimate the number of species (Dellicour & Flot 2018; Luo *et al*. 2018). PTP overestimates the number of species, which is also consistent with the results of previous studies. Therefore, we combined the results of the three molecular species delimitations and concluded that the larval sample was the species of *U.* (*U.*) *excisa excisa* in this study. It provided a good identification method for those indistinguishable larvae. Meanwhile, we hope that more DNA sequences of the genus *Uloma* will be published, which will contribute to the molecular systematics of the tribe Ulomini.

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四突齿甲指名亚种*Uloma* (*Uloma*) *excisa excisa*幼虫的鉴定与形态描述（鞘翅目：拟步甲科：齿甲族）

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摘要：应用基于线粒体*COI*序列的分子系统发育分析方法，对分布于中国南方地区的四突齿甲指名亚种*Uloma* (*Uloma*) *excisa excisa*成、幼虫进行了匹配；首次详细描述了该种幼虫的形态特征，并提供了整体照和特征图；同时提供了中国齿甲属已知幼虫的检索表。

关键词：四突齿甲指名亚种；未成熟阶段；形态学；*COI*；中国