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Description of larvae of three Opatrini species (Coleoptera: Tenebrionidae: Blaptinae) from China, with molecular species delimitation and diagnoses

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Abstract

Opatrini Brullé, 1832 is the most species-rich tribe in the subfamily Blaptinae Leach, 1815. In this study, a preliminary phylogenetic relationship among 14 species of five genera of Opatrini is hypothesized based on mitochondrial gene (*COI*) fragment. Based on the phylogenetic topology and the results of three molecular species delimitation analyses, three larval specimens are assessed for the adults. Additionally, the larvae of these three species are described and illustrated: *Gonocephalum bilineatum* (Walker, 1858), *Gonocephalum gracile* (Bates, 1879), and *Mesomorphus latiusculus* Chatanay, 1917.

Key words: darkling beetles, immature stage, species delimitation, morphology

Introduction

The tribe Opatrini is robust species richness in Blaptinae with over 2000 species of 117 genera (Lumen *et al.* 2020; Kamiński *et al.* 2021). Currently, only larvae of 73 species of 26 genera have been described: *Melanesthes* Dejean, 1834 (13 species), *Penthicus* Faldermann, 1836 (12 species), *Gonocephalum* Solier, 1834 (11 species), *Opatrum* Fabricius, 1775 (5 species), *Eumylada* Reitter, 1904 (4 species), *Scleropatrum* Reitter, 1887 (4 species), *Blapstinus* Sturm 1826 (2 species), *Eremostibes* Koch, 1963 (2 species), *Parastizopus* Gebien, 1938 (2 species), *Trichoto* Hope, 1841 (2 species), *Adavius* Mulsant & Rey, 1859 (1 species), *Amathobius* Gebien, 1920 (1 species), *Ammobius* Guérin-Méneville, 1844 (1 species), *Anatrum* Reichardt, 1936 (1 species), *Caedius* Blanchard, 1845 (1 species), *Heterotarsus* Latreille, 1829 (1 species), *Jintaium* Ren, 1999 (1 species), *Myladina* Reitter, 1889 (1 species), *Mesomorphus* Miedel, 1880 (1 species), *Nemanes* Fairmaire, 1888 (1 species), *Opatroides* Brullé, 1832 (1 species), *Penthicinus* Reitter 1896 (1 species), *Periloma* Gebien, 1938 (1 species), *Psammogaster* Koch, 1953 (1 species), *Sinorus* Mulsant & Revelière, 1860 (1 species) (Zhang & Yu 2004; Jia *et al.* 2013; Kamiński *et al.* 2019). Larval morphology is important for understanding the systematics of different genera or species of Coleoptera, and it has been used to support the close relationships among genera (Beutel *et al.* 1999; Grebennikov & Scholtz 2004; Lawrence *et al.* 2011). However, the larval morphology is currently known for less than 4% of the adult species in the tribe Opatrini.

The tribe Opatrini, with one87 species of 22 genera from China and the larval morphology of 45 species of 11 genera, are currently known (Ren & Yang 2006; Iwan & Löbl 2020). In previous studies, it was difficult to accurately identify species based on larval morphological characteristics. In this study, we constructed a preliminary molecular phylogeny for the tribe Opatrini and applied molecular species delimitation methods to verify the taxonomic status of larval specimens. In addition, larvae of three known species are described and illustrated: *Gonocephalum bilineatum* (Walker, 1858), *Gonocephalum gracile* (Bates, 1879), and *Mesomorphus latiusculus* Chatanay, 1917.

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Material and methods

Morphological examination

The examined larval specimens of this study were deposited at the Museum of Hebei University, Baoding, China (MHBU).

The photos were taken with the imaging system: (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), 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 v. 22.1.0 to form the final figure plates.

Label data are presented verbatim. A slash (/) separates text on different lines of label.

Taxon sampling, DNA extraction, PCR amplification, and sequencing

Adult and larval specimens were collected in the field from Hainan, Xizang, and Yunnan, China. To correlate the different stages, the molecular data were collected from larval and adult individual.

DNA was extracted from pygopod tissue of the larva using Insect DNA Isolation Kit (BIOMI-GA, Hangzhou, China) following the manufacturer's protocols. The DNA extracted was stored at -20°C. 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, an 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). In total, all sequences were collected from 54 individuals (51 adults and 3 larvae), 45 known sequences were downloaded from NCBI (accession number in Fig. 1). Detail information in this study for the new samples is provided in Table 1. We also used five previously published sequence of three species of the genus *Gnaptorina* as outgroup, which has been considered as close relatives of the tribe Opatrini (Kamiński *et al*. 2021).

Phylogenetic analyses

Phylogenetic analysis was based on mitochondrial gene fragment (Cytochrome C Oxidase I, *COI*) by Maximum Likelihood (ML). 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 conducted using IQ-TREE v1.6.8 (Nguyen *et al.* 2015) that was integrated into PhyloSuite. The ML tree was inferred using an edge-linked partition model for 5000 ultrafast bootstraps (1000 replicates) (Minh *et al.* 2013).

Molecular species delimitation analyses

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). Hence, it has been advocated to use them in a complementary way to better assess species boundaries. Here, we used a combination of three distinct methods to assess the boundaries of species with Opatrini.

TABLE 1. List of specimens used in this study with the corresponding accession number.

Species	Developmental stage	Sampling locality	Elevation (m)	Date of collection	Collectors	Accession numbers
Gonocephalum bilineatum	Adult	Wulie Town, Changjiang County, Hainan, China (HNCJ)		5.II.2023	G.-D. Ren *et al*.	PQ360862
Gonocephalum bilineatum	Adult	Yulong County, Yunnan, China (YNYL)	1784	6.VIII.2015	G.-D. Ren *et al*.	PQ360864
Gonocephalum bilineatum	Larva	Wulie Town, Changjiang County, Hainan, China (HNCJ)		16.XII.2022	G.-D. Ren *et al*.	PQ360863
Gonocephalum gracile	Adult	Cawarong, Zayü County, Xizang, China (XZCY)	1906	3.V.2023	X.-L. Bai *et al*.	PQ360866
Gonocephalum gracile	Adult	Cawarong, Zayü County, Xizang, China (XZCY)	1833	3.V.2023	X.-L. Bai *et al*.	PQ360867
Gonocephalum gracile	Larva	Cawarong, Zayü County, Xizang, China (XZCY)	1906	3.V.2023	X.-L. Bai *et al*.	PQ360865
Mesomorphus latiusculus	Adult	Cangshan, Yangbi Yi Autonomous County, Yunnan, China (YNYB)	1658	1.V.2023	X.-L. Bai *et al*.	PQ360859
Mesomorphus latiusculus	Adult	Cangshan, Yangbi Yi Autonomous County, Yunnan, China (YNYB)	1658	1.V.2023	X.-L. Bai *et al*.	PQ360861
Mesomorphus latiusculus	Larva	Cangshan, Yangbi Yi Autonomous County, Yunnan, China (YNYB)	1658	1.V.2023	X.-L. Bai *et al*.	PQ360860

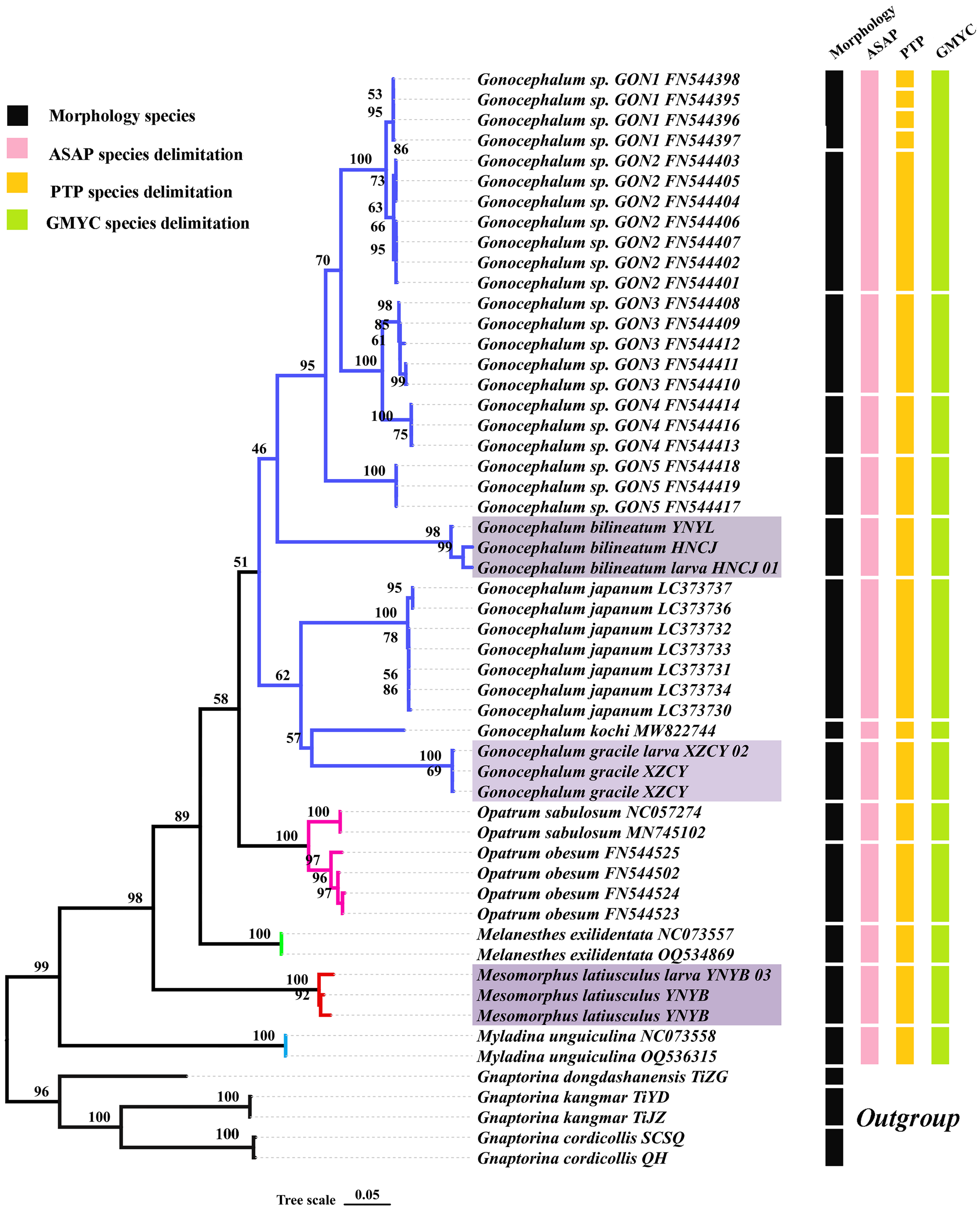

FIGURE 1. Maximum-likelihood phylogenetic tree based on *COI* sequences data. Support for each node is represented by ultrafast bootstrap values (uBV, %). Vertical colored bars delineate extant morphospecies (black), and the results of three separate molecular analyses delimiting species (pink, yellow and light green).

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* gene fragment, outgroups were also 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. The analysis was conducted in R using the package GMYC with default settings (100 trees randomly selected, 250 million generations with a burn-in of 25 million and a thinning parameter of 100). PTP analysis relied on the best-score ML tree from the IQ-TREE analysis, and was conducted on the web server of the Exelixis Lab (http://species.h-its.org/ptp/) using default settings.

Results

Phylogenetic relationships and species delimitation

The final IQ-TREE analysis produced a preliminary hypothesis based on *COI* sequence data, involving 54 individuals from 17 species (Fig. 1). The ML tree revealed that Clade 1 had low branch support (C1=*Gonocephalum*, uBV=51), while the monophyly of the other four clades was well supported: C2 (*Opatrum*, uBV=100), C3 (*Melanesthes*, uBV=100), C4 (*Mesomorphus*, uBV=100), and C5 (*Myladina*, uBV=100).

The ML tree and three molecular species delimitation methods consistently associate the larvae and adults of different species with congruent results. Larvae and three known species cluster into a single well-supported clade respectively (uBV ≥ 98). Three molecular species delimitation results showed that the samples HNCJ01, XZCY02, and YNYB03 consistently grouped individuals from three known species. Therefore, we conclude that the above assumption is correct: the sample HNCJ01 is the larva of *G. bilineatum*, the sample XZCY02 is the larva of *G. gracile*, and the sample YNYB03 is the larva of *M. latiusculus*.

Larval diagnoses of the tribe Opatrini Brullé, 1832

The larvae of the tribe Opatrini are elongated, cylindrical, and highly ossified, ranging in color from light yellow to grey-black. The middle of the labrum and clypeus has two setae or two rod-shaped spines; there are eight (3+2+3) setae on the anterolateral margin of the labrum. Ocelli 3–5 on the antennae. The last segment is conical or capsule-shaped, with more than eight spines on the lateral posterior margin (Dai & Yu 2000).

Descriptions of larval morphology

***Gonocephalum bilineatum* (Walker, 1858)**
Chinese common name: 二纹土甲
(Fig. 2)

Opatrum bilineatum Walker, 1858: 284.
Gonocephalum kamischaticum Motschulsky, 1860: 139.
Blapstinus latifrons Leconte, 1874: 57.
Opatrum orarium Lewis, 1894: 380.
Gonocephalum seriatum Fauvel, 1904: 164.
Gonocephalum bilineatum: Chatanay 1917: 238; Gebien 1910: 322; Reichardt 1936: 109; Kaszab 1952: 641; Boddy 1965: 171; Kwon & Choi 1986: 106; Schawaller 1997: 3; Hua 2002: 138; Ren & Yang 2006: 142.

Examined larval materials. 1 ex. (MHBU): Wulie Town, Changjiang County, Hainan/ 2022-XII-16/ Guo-Dong Ren leg.

Description. Body (Fig. 2A–C): Larval body subcylindrical; last segment capsule; body yellowish brown, shiny; body wall ossified; median line obvious on thorax and abdominal segment I.

Head (Fig. 2D–G): Width of Head slightly narrower than prothorax; labrum transverse, apex with two setae; mandible left-right unsymmetrical; membranous elevation with four setae; clypeus transverse, trapezoidal, margin dark brown, with two setae at middle and margin respectively. Epicranial stem Y-shaped; anterior margin of frons covered with dark brown, with sparse long setae on lateral margins. Ocelli evident. Maxillary palpi three-segmented, cylindrical, and conical at apex; I widest, II longest. Labial palps two-segmented, II conical; mentum convex, U-shaped, base of mentum straight; prementum with two setae; mentum wide and short, with four long setae; submentum with four setae. Antennae three-segmented, cylindrical at apex; II longest and widest; III shortest and narrowest.

Thorax (Fig. 2A): Thoracic segment parallel-sided, widest in middle, with transverse plicae. Pronotum longest, 2.92 times as long as mesonotum, 2.00 times as long as metanotum, mesonotum shortest.

Legs (Fig. 2H, I): Prothoracic leg noticeably stronger, longer, and thicker than meso- and metathoracic legs. Protarsungulus strongly sclerotized, sharp, claw-like; tarsungulus with one long spine on inner side, and one short spine on outer side at base. Profemora and protibiae gradually narrowing towards apex; tibiae with two long spines on inner side, femora with three short rod-shaped spines on inner side, trochanter with two short rod-shaped spines on inner side. Mesotarsus with one sharp spine on inner side, and 1 strong, short spine on outer side; tibiae with two spines on inner side, and with one spine on outer side; femora with two spines on inner and outer side, trochanters with two spines on inner side, two minute setae on outer side. Metatarsus with one spine on inner and outer side at base, tibiae and femora with two spines on inner and outer side, trochanters with two spines on inner side.

Abdomen (Fig. 2A, C): Approximately 3.46 times as long as thorax; abdominal segments I–VIII subcylindrical, with transverses plicae; posterior margin of abdominal segment VIII and base of abdominal segment IX with thick setae. Last segment IX capsule, 0.74 times as long as abdominal segment VIII, distinctly narrower than VIII; surface of convex disc with sparse long setae in ventral view, with row of short spines on each side (seven spines on left, five spines on right); urogomphi upturned and apex truncated, with 6 short spines (Fig. 2J–L).

Spiraculae (Fig. 2C): Mesothoracic spiracle almost twice as large as abdominal segment I spiracle; lateral margin of abdominal segments I–VIII and mesothorax each with pair of oval spiraculae, abdominal segment I spiracle largest, tapers downwards.

Distribution. China: Fujian, Guangdong, Hainan, Hong Kong, Guangxi, Sichuan, Yunnan, Xizang.

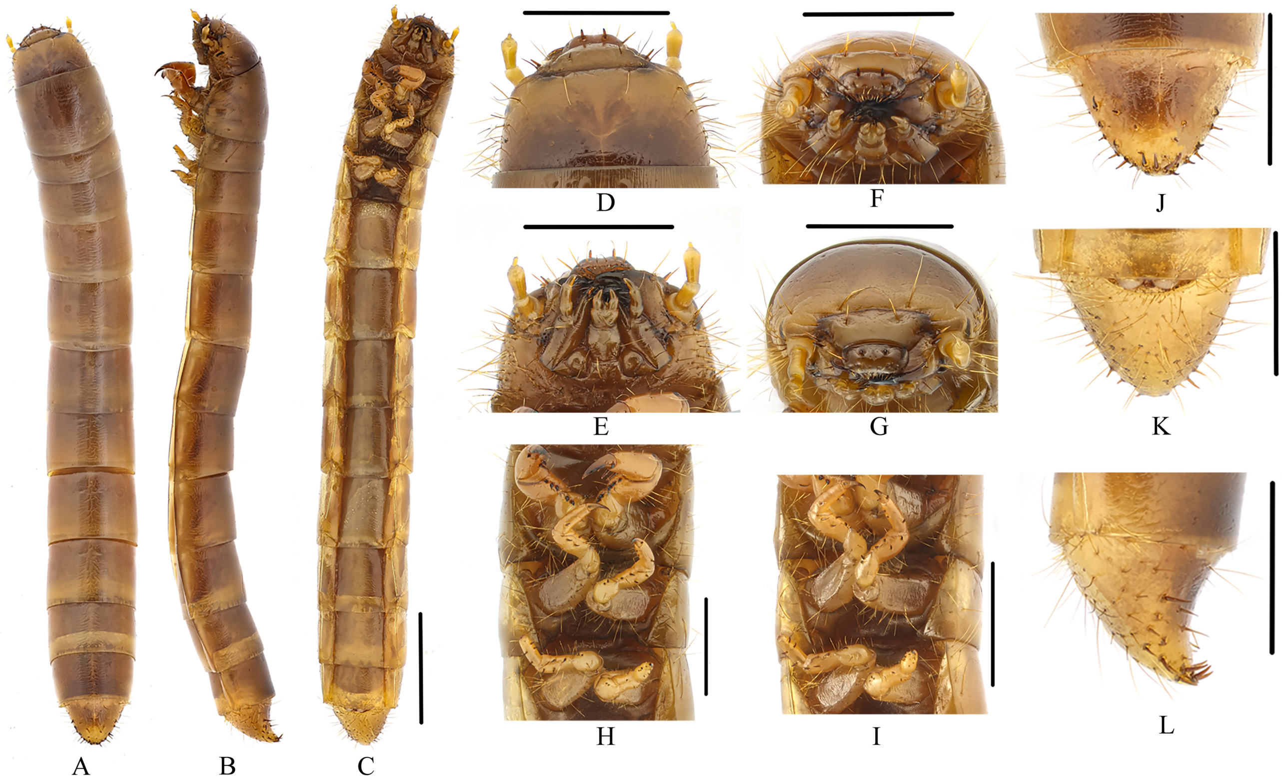

FIGURE 2. Larva of *Gonocephalum bilineatum* (Walker, 1858) **A.** Habitus, dorsal view. **B.** Habitus, lateral view. **C.** Habitus, ventral view. **D.** Head, dorsal view. **E.** Head, ventral view. **F, G.** Head, vertex view. **H.** Legs. **I.** metathoracic leg lateral view. **J.** Pygopods, dorsal view. **K.** Pygopods, ventral view. **L.** Pygopods, lateral view. Scale bars: 2 mm (**A**–**C**), 1 mm (**D**–**L**).

Gonocephalum gracile **(Bates, 1879)**
Chinese common name: 瘦土甲
(Fig. 3)

Loboderus gracile Bates, 1879: 482; Bates 1890: 75.
Gonocephalum ruficorne: Gravely 1915: 250.
Gonocephalum curvicdle Gridelli, 1934: 66.
Lobodera gracile: Reichardt 1936: 169.
Gonocephalum gracile: Gebien 1939: 447; Kaszab 1952: 546; Schawaller 1997: 6; Hua 2002: 138; Ren & Yang 2006: 147.

Examined larval materials. 2 exx. (MHBU): Cawarong Township, Zayü County, Xizang/ 28.467374°N, 98.469360°E/ Alt. 1906 m/ 2023-V-3/ Xing-Long Bai, Quan-Yu Ji, Kai-Xuan Liu, Wei Zhao leg.; 2 exx. (MHBU): Tunggar Township, Nang County, Xizang/ 2014-VIII-11/ Guo-Dong Ren, Xing-Long Bai, Jun-Sheng Shan leg.

Description. Body (Fig. 3A–C): Larval body subcylindrical; last segment conical; body yellowish brown, shiny; body wall ossified; median line obvious on thorax and abdominal segment I. Abdominal segments II–VIII with transversally arranged brown dots at near base.

Head (Fig. 3D–F): Width of head slightly narrower than prothorax; labrum transverse, apex with two setae; mandible left-right unsymmetrical; membranous elevations with two setae each side; clypeus transverse, subtrapezoidal, with two setae at middle and margin respectively. Epicranial stem Y-shaped; large dark brown color on frons, with sparse long setae on lateral margins. Ocelli evident, 4 in a horizontal row. Maxillary palpi three-segmented, cylindrical, and conical at apex; I widest, II longest. Labial palps two-segmented, II conical; mentum convex, U-shaped, base of mentum straight; prementum with two setae; mentum wider and shorter, with five long setae; two long setae on posterior margin of submentum. Antennae three-segmented, cylindrical at apex; I widest; II longest; III shortest and narrowest.

Thorax (Fig. 3A): Thoracic segment parallel-sided, with transverse plicae. Each thoracic tergum with sparse, slender setae on anterior and posterior margin. Anterior and posterior border of prothorax with brown longitudinal stripes, posterior border of mesothorax and metathorax with brown longitudinal stripe. Pronotum longest, 2.30 times as long as mesonotum, 1.48 times as long as metanotum, mesonotum shortest.

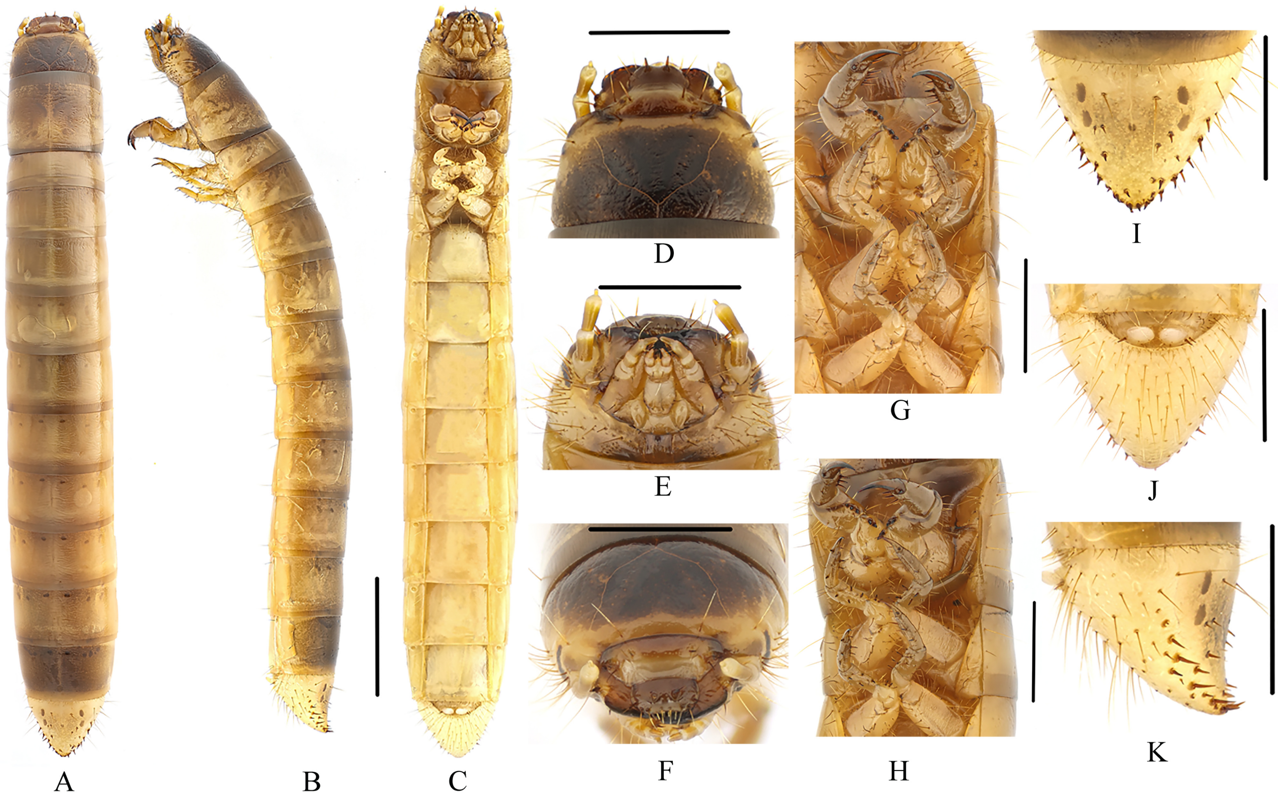

FIGURE 3. Larva of *Gonocephalum gracile* (Bates, 1879). **A.** Habitus, dorsal view. **B.** Habitus, lateral view. **C.** Habitus, ventral view. **D.** Head, dorsal view. **E.** Head, ventral view. **F.** Head, vertex view. **G.** Legs. **H.** Legs, lateral view. **I.** Pygopods, dorsal view. **J.** Pygopods, ventral view. **K.** Pygopods, lateral view. Scale bars: 2 mm (**A**–**C**), 1 mm (**D**–**K**).

Legs (Fig. 3G, H): Prothoracic leg noticeably stronger, longer, and thicker than meso- and metathoracic legs. Protarsungulus strongly sclerotized, sharp, claw-like; tarsungulus with one long spine on inner side, and one short spine on outer side. Profemora and protibiae gradually narrowing towards apex; tibiae with three long spines on inner side, femora with two–3 short rod-shaped spines on inner side, trochanter with two short rod-shaped spines on inner side. Mesotarsus with one sharp spine on inner side, and one strong, short spine on outer side; tibiae with two spines on inner side, one spine on outer side; femora with two spines on inner and outer side, trochanters with two spines on inner side, two minute setae on outer side. Metatarsus with one spine on inner and outer side at base, tibiae and femora with two spines on inner and outer side, trochanters with two spines on inner side, two minute setae on outer side.

Abdomen (Fig. 3A, C): Approximately 3.05 times as long as thorax; abdominal segments I–VIII subcylindrical, with transverses plicae. Basal part of abdominal segments II–VIII with transverse row of elliptic floral spots. Abdominal segment IX as long as abdominal segment VIII and significantly narrower in width than VIII. Last segment conical, surface of convex disc with sparse long setae in ventral view, with row of short spines on each side (10 spines on left, nine spines on right); segment IX dorsally flattened, two pairs of light brown irregular patches, with one0 short setae; urogomphi with two short spines; spurious leg with one to two spines on each side (Fig. 3I–K).

Spiraculae (Fig. 3C): Lateral margins of abdominal segments I–VIII and mesothorax each with one pair of oval spiraculae, mesothoracic spiraculae biggest, abdominal segments I–VIII spiracle gradually shrinking.

Distribution. China: Xinjiang, Xizang.

Mesomorphus latiusculus **Chatanay, 1917**

Chinese common name: 宽褐毛土甲

(Fig. 4)

Mesomorphus latiusculus Chatanay, 1917: 234; Ren & Yang 2006: 47.

Examined larval materials. 1 ex. (MHBU): Cangshan West Town, Yangbi Yi Autonomous County, Yunnan/ 28.467374°N, 98.469360°E/ Alt. 1906 m/ 2023-V-1/ Xing-Long Bai, Quan-Yu Ji, Kai-Xuan Liu & Wei Zhao leg.

Description. Body (Fig. 4A–C): Larval body subcylindrical; last segment conical; body greyish black, shiny; body wall ossified; median line obvious on thorax and abdominal segment I.

Head (Fig. 4D–G): Width of head slightly narrower than prothorax; labrum with two setae mediodorsally; labrum transverse; mandible left-right unsymmetrical; membranous elevations with two setae per side; clypeus subtrapezoidal, with two setae mediodorsally. Epicranial stem Y-shaped; frons convex, with sparse long setae on lateral margins. Ocelli evident, 3 in a horizontal row. Maxillary palpi three-segmented; conical at apex; I thickest, II longest. Labial palps two-segmented, II conical; mentum convex, U-shaped; prementum with two setae at middle; mentum with four long setae; two long setae on lateral margin of submentum. Antennae three-segmented; I widest; II longest; III shortest and narrowest.

Thorax (Fig. 4A): Thoracic segment parallel-sided, widest at middle, with transverse plicae. Pronotum longest, 1.22 times as long as mesonotum, 1.11 times as long as metanotum, mesonotum shortest.

Legs (Fig. 4H–J): Protarsungulus with one spine on inner and outer side at base; femora and tibiae gradually narrowing towards apex; tibia with three long spines on inner side, femora with two spines on inner side, trochanter with two spines on inner side. Mesotarsus with one spine on inner and outer side at base; tibiae with three spines on inner side, and with two spines on outer side; femora with two spines on inner and outer side, trochanters with two spines on inner side. Metatarsus with one spine on inner and outer side at base; tibia with two to three spines on inner side, one to two spines on outer side; femora with two spines on inner and outer side, trochanters with two spines on inner side.

Abdomen (Fig. 4A, C): Approximately 3.42 times as long as thorax; abdominal segments I–VIII subcylindrical, with transverses plicae; ventral view of abdominal segment I with four pairs of setae on lateral margins, two setae on middle side; abdominal segments II–VIII with two pairs of setae. Last segment conical, 0.54 times as long as abdominal segment VIII, distinctly narrower than VIII; surface of convex disc with sparse long setae in ventral view, with row of short spines on each side (four spines each on left and right); last segment dorsally flattened; urogomphi with two shorter spines (Fig. 4J–L).

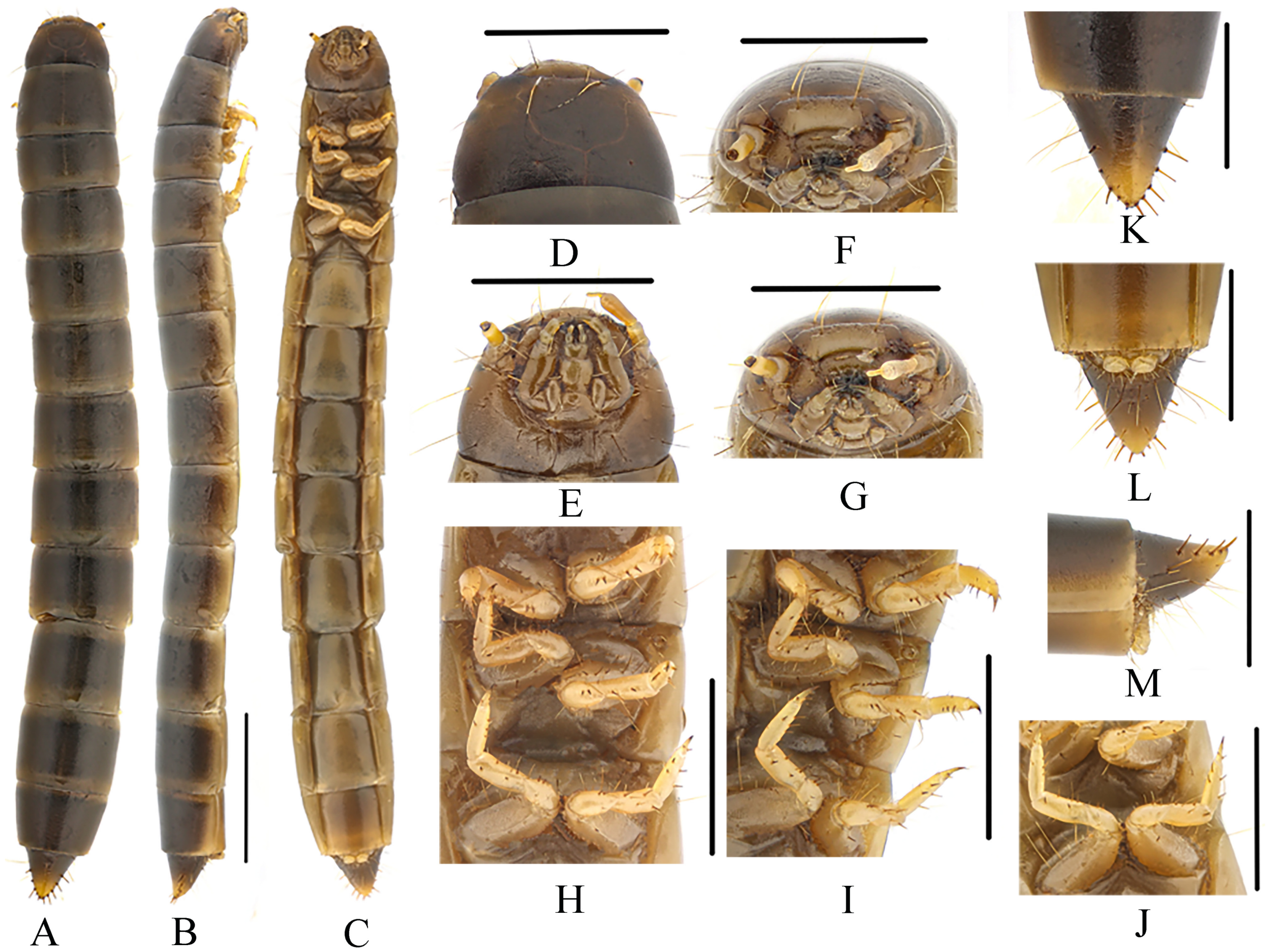

FIGURE 4. Larva of *Mesomorphus latiusculus* Chatanay, 1917. **A.** Habitus, dorsal view. **B.** Habitus, lateral view. **C.** Habitus, ventral view. **D.** Head, dorsal view. **E.** Head, ventral view. **F, G.** Head, vertex view. **H.** Legs. **I.** Legs, lateral view. **J.** Metathoracic leg. **K.** Pygopods, dorsal view. **L.** Pygopods, ventral view. **M.** Pygopods, lateral view. Scale bars: 2 mm (**A**–**C**), 1 mm (**D**–**M**).

Spiraculae (Fig. 4C): Lateral margins of abdominal segments I–VIII and mesothorax each with pair of oval spiraculae, mesothoracic spiraculae much larger than abdominal spiraculae, abdominal segment I spiracle largest, tapers downwards.

Distribution. China: Guangxi, Yunnan.

Discussion

Molecular phylogenetics

The subfamily Blaptinae was resurrected based on molecular phylogenetic analyses and contains eight tribes: Amphidorini LeConte, 1862, Blaptini Leach, 1815, Dendarini Mulsant *et* Rey, 1854, Platyscelidini Lacordaire, 1859, Pedinini Eschscholtz, 1829, Platynotini Mulsant *et* Rey, 1853, Dissonomini Medvedev, 1968, and Opatrini Brullé, 1832, which includes 40 representatives of Opatrini (Kaminski *et al.* 2021; Kaminski *et al.* 2024). In addition, nearly all previous studies on the Opatrini tribe have relied on morphological characteristics, with the exception of phylogenetic analyses of the subtribe Blapstinina based on five molecular markers (Lumen *et al.* 2020). In this study, the preliminary hypothesis reveals weak branch support for the genus *Gonocephalum* (uBV=51). However, the present work is a very preliminary and incomplete molecular phylogenetic analysis of the tribe Opatrini. The resolution to this problem will only be possible with the molecular data analysis of far more genera and species.

Taxonomic remarks

The genus *Gonocephalum* was established by Solier in 1834 and is the most species-rich in the tribe Opatrini, with four17 species. However, the larvae of only 11 known species have been described: *Gonocephalum coriaceum* Motschulsky, 1858, *Gonocephalum japanum* Motschulsky, 1861, *Gonocephalum pubiferum* Reitter, 1904, *Gonocephalum granulatum pusillum* (Fabricius, 1792), *Gonocephalum pygmaeum* (Steven, 1829), *Gonocephalum recticolle* Motschulsky, 1866, *Gonocephalum reticulatum* Motschulsky, 1854, *Gonocephalum rusticum* (Olivier, 1811), *Gonocephalum simplex* (Fabricius, 1801), *Gonocephalum subrugulasum* Reitter, 1887, and *Gonocephalum turchestanicum* Gridelli, 1948 (Iwan *et al.* 2011; Kamiński *et al.* 2019). The genus *Mesomorphus* was established by Miedel in 1880, with 55 species. To date, only the larva of *Mesomorphus villiger* Blanchard, 1853 has been described (Hayashi 1968; Ren & Yang 2006).

Larval morphology has been found to provide numerous characteristics for insect taxonomy and to support the close relationships among genera (Grebennikov & Scholtz 2004; Lawrence *et al.* 2011; Soldati *et al.* 2017; Chigray 2019). Although the taxonomy of Opatrini is well developed, the larval morphology is currently known for less than 4% of adult species. The immature stages of more genera and species must be properly documented to develop an applicable system for larval, pupal, and adult taxonomy in the tribe Opatrini. In this study, the morphological descriptions of the two newly described larvae of *Gonocephalum* are consistent with the characteristics of the genus *Gonocephalum* summarized by Li *et al*. (2013). The species can be distinguished between species by the number of ocelli, the number and arrangement of mesopod tibial spines, and the number of spines on the abdominal segment IX (Li *et al.* 2013). However, there is only one larva of species described in the genus *Mesomorphus* in addition to the new larva in this study. Therefore, it is unclear how to distinguish these larvae based on morphological characteristics. Moreover, there are two larvae reported in the genus *Mesomorphus,* which is not enough to support the classification of genus-level characters.

In this study, most of the larval specimens were collected directly from the field; their taxonomic status is challenging due to the lack of larval information on the known species. In addition, the morphological characters of the larvae are so similar that it is often difficult to distinguish between species within the genus. Our results clearly provide a tool to help associate the larva with known or unknown adult specimens and successfully resolve the problem of larval taxonomic status. Molecular species identification analysis has become an important approach in insect taxonomy (Tautz *et al.* 2002; Hebert *et al.* 2003; Meier *et al.* 2006; Rodriguez *et al.* 2022). These approaches are able to establish correlations between larval and adult stages through DNA sequences, providing valuable reference information for larval taxonomy (Li *et al.* 2022, 2023; Ji *et al.* 2024). Therefore, we hope to continue collecting more larvae and correlating them with their respective adults through rearing or molecular data.

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中国土甲族 Opatrini三种幼虫描述及分子物种界定（鞘翅目：拟步甲科：琵甲亚科）

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摘要：土甲族Opatrini是琵甲亚科Blaptinae中物种最丰富的一个类群。本文基于线粒体基因片段（*COI*）初步推断了Opatrini族5属14种的系统发育关系。基于系统发育树拓扑结构和3种分子物种界定结果将3种幼虫样本与成虫进行匹配。描述了此3个已知种的幼虫，并附插图，即二纹土甲*Gonocephalum bilineatum*、瘦土甲*Gonocephalum gracile*和宽褐毛土甲*Mesomorphus latiusculus*。

关键词：拟步甲；未成熟阶段；物种界定；形态学