ZooKeys 1190: 1–37 (2024)
DOI: 10.3897/zookeys.1190.110552

Research Article

# A new ant species of the genus *Carebara* Westwood, 1840 (Hymenoptera, Formicidae, Myrmicinae) with a key to Chinese species

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## Abstract

A new Chinese ant species *Carebara laeviceps* **sp. nov.** is described based on the major and minor workers. This species is most similar to *C. lusciosa* (Wheeler, 1928) due to a spineless propodeum, the absence of horns, and a smooth head capsule. It is distinguished by the following features: (1) antenna 10-segmented; (2) katepisternum rugose-reticulate; (3) in major workers, lateral sides of head in full-face view parallel; (4) metanotal groove distinct, anterodorsal corner forming an acute tooth behind metanotal groove. Moreover, an updated key to Chinese *Carebara* species is presented based on major workers, with a checklist comprising a total of 36 Chinese *Carebara* species and subspecies. Morphological structures and scanning electron micrographs of the newly discovered species' minor and major workers are provided.

**Key words:** *Carebara laeviceps*, China, East Asia, new species, Sichuan Province, taxonomy

OPEN ACCESS

**Academic editors:** Sebastian Salata
**Received:** 4 August 2023
**Accepted:** 5 December 2023
**Published:** 22 January 2024

**ZooBank:** https://zoobank.org/21B7440C-0C41-41FD-A6BF-39A74AD7C619

**Citation:** Liu Z-y, Zhong Y, Huang Y-y, Ran H, Song F (2024) A new ant species of the genus *Carebara* Westwood, 1840 (Hymenoptera, Formicidae, Myrmicinae) with a key to Chinese species. ZooKeys 1190: 1–37. https://doi.org/10.3897/zookeys.1190.110552

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## Introduction

The genus *Carebara* Westwood, 1840 is a large genus of ants that contains 234 valid species (including 9 fossil species) and 22 subspecies (Bolton 2023). It is a worldwide genus mainly recorded in the tropical and subtropical regions (Bharti and Kumar 2013; Azorsa and Fisher 2018). They nest in soil or termite mounds, and some species also inhabit rotten wood (Bharti and Kumar 2013). Most members of *Carebara* are minute in size and subterranean, mainly feeding on dead insects and other invertebrates. Some species are aggressive ground predators with mass raiding habits (Moffett 1988), like those of the former genus *Pheidologeton* Mayr, 1862. Studies regarding the ethology and life cycle of *Carebara* are still limited.

The genus was established based on the type species *C. lignata* Westwood, 1840. It was originally incorporated into the formerly valid subfamily Attidae after its establishment (Smith 1858). Later, the taxonomic status underwent

several changes among the tribes Solenopsidini (Forel 1893; Emery 1895; Wheeler 1910, 1922; Kusnezov 1964) Pheidologetini (Emery 1913, 1914, 1924; Bolton 1994), and the *Pheidologeton* genus group (Ettershank 1966; Bolton 1987) due to the incomplete records for different castes and polymorphism. The genus *Carebara* was considered as a senior synonym of the genera *Aeromyrma* Forel, *Afroxyidris* Belshaw & Bolton, *Amauromyrmex* Wheeler, *Aneleus* Emery, *Crateropsis* Patrizi, *Erebomyrma* Wheeler, *Hendecatella* Wheeler, *Idrisella* Santschi, *Lecanomyrma* Forel, *Neoblepharidatta* Sheela & Narendran, *Nimbamyrma* Bernard, *Oligomyrmex* Mayr, *Paedalgus* Forel, *Parvimyrma* Eguchi & Bui, *Pheidologeton* Mayr, *Solenops* Karavaiev, *Spelaeomyrmex* Wheeler, and *Sporocleptes* Arnold (Fischer et al. 2014), and finally placed in Crematogastrini by Ward et al. (2015) based on a comprehensive phylogenetic analysis of 11 genes.

Taxonomic changes at the genus level have superseded some early regional revisions of *Carebara* species. Moreover, the lack of a comprehensive revision, especially for Old World species, has created difficulties in species identification (Fischer et al. 2015). To address these issues, recent regional studies have been conducted in India (Bharti and Kumar 2013; Bharti et al. 2014; Akbar and Bharti 2017), Saudi Arabia (Sharaf and Aldawood 2013), the Afrotropical region (Fischer et al. 2014, 2015), Brazil (Baccaro et al. 2015), Madagascar (Azorsa and Fisher 2018), and Australia (Heterick 2021). The studies about species groups initially concentrated on New World species, and Fernández (2004, 2010) reviewed American species and proposed five species groups based on the morphology of worker caste. In the Old World, the species groups of *Carebara* were first studied by Bharti and Kumar (2013). They placed 11 Indian species into three groups and recommended the fusion of the *C. concinna* group with the *lignata* group based on the eyeless minor worker of *C. asina* (Forel, 1902). Fischer et al. (2014) established the *C. polita* group, which shares morphological similarities with *Pheidologeton*, and included six Afrotropical species and two Neotropical species. This study also synonymized *Pheidologeton* with *Carebara*. Later, Fischer et al. (2015) established the *phragmotica* group, and the *acutispina* group was proposed by Hosoishi et al. (2022), both including phragmotic species.

In China, *C. castanea* Smith, 1858 was the first Chinese *Carebara* species to be described from Hong Kong. Subsequently, *C. sauteri* (Forel, 1912) and *C. yanoi* (Forel, 1912), followed by the queen caste-established species *C. amia* (Forel, 1913), were all collected in Taiwan. Wheeler (1921) described a species from Zhejiang, namely *C. vespillo* (Wheeler, 1921). Later, Wheeler (1928) described three new species and two new subspecies of China; among them, *Oligomyrmex silvestrii taiponicus* was raised to species status by Bolton (1995) and is now known under the name *C. taiponica* (Wheeler, 1928). Another species *Oligomyrmex silvestrii* was considered a secondary homonym of *Aneleus silvestrii* Santschi, 1914, later renamed *Oligomyrmex wheeleri* Ettershank, 1966, but now known as *C. wheeleri* (Ettershank, 1966). The remaining three taxa are *C. lusciosa*, *C. polyphemus* (Wheeler, 1928), and *C. capreola laeviceps* (Wheeler, 1928).

Later, Wu and Wang (1995) revised some ant genera from the Chinese mainland, including the former valid genera *Pheidologeton* and *Oligomyrmex* (now both in *Carebara*). They also described three new species, namely *C. hunanensis* (Wu & Wang, 1995), *C. jiangxiensis* (Wu & Wang, 1995), and *C. pseudolusciosa* (Wu & Wang, 1995). It is worth mentioning that Wu and Wang (1995) erroneously illus-

trated these three species in relation to eye position, tooth numbers, and cephalic indices (Xu 2003). Zhou and Zheng (1997) as well as Li and Tang (1986) conducted comprehensive studies on *Carebara* from Guangxi Province, and described four new species, *C. nanningensis* (Li & Tang, 1986), *C. latinoda* (Zhou & Zheng, 1997), *C. melasolena* (Zhou & Zheng, 1997), and *C. trechideros* (Zhou & Zheng, 1997).

The very first comprehensive revision of former *Oligomyrmex* species in China was presented by Xu (2003), who studied this genus in more depth, conducting a revision of 26 species in China, and described eight new species, namely *C. altinodus* (Xu, 2003), *C. curvispina* (Xu, 2003), *C. striata* (Xu, 2003), *C. acutispina* (Xu, 2003), *C. obtusidenta* (Xu, 2003), *C. bihornata* (Xu, 2003), *C. rectidorsa* (Xu, 2003) and *C. reticapita* (Xu, 2003) from China. Additionally, Xu excluded *C. cribriceps* (Wheeler, 1927). Despite Zhou's (2001) description of *C. cribriceps* in Guangxi, Zhou's illustration of this species displays a minor concavity in the posterior margin of the head, in contrast to Wheeler's (1927) account, which distinctly portrays a pronounced concavity in the posterior margin. This disparity suggests that the species documented by Zhou may be an undescribed species from China (Xu 2003). Later, a new and different species, *C. zengchengensis* (Zhou et al., 2006) was described from Guangdong.

*Carebara* species from Taiwan were mostly studied by Terayama (2009), who made several revisions and described two new species *C. qianliyan* Terayama, 2009 and *C. sakamotoi* Terayama et al., 2012. Currently, there are a total of 36 valid species and subspecies in China.

As a contribution to the taxonomy of the *Carebara* species of China, we report a new species: *C. laeviceps* sp. nov. High-resolution images and scanning electron micrographs (SEM) of the minor and major workers of the new species are provided. An updated key to Chinese *Carebara* species is also provided based on the major worker.

## Material and methods

All samples were collected from Kaijiang County, Sichuan Province, China by direct sampling on the ground and preserved in 75% EtOH, then deposited in the Forest Insect Herbarium, Ant Specimen Branch of Southwest Forestry University, Kunming, China (**SWFU**). Specimens were observed under a Phenix XSP-02 microscope. Photographs were taken by Samsung SM-N9860, and SEM photographs were taken by a FEI Quanta 450 at 12.50 kV. To observe the microstructure and preserve the specimens, some of the specimens were disassembled before observation under SEM. The specimens were sputter-coated with gold for 30 min. Image stacking using Helicon Focus software. Morphological terminology and standard measurements mostly follow Bolton (1994), all measurements are given in millimeters:

**HL** Head Length. Maximum length from the mid-point of the anterior clypeal margin to the mid-point of the posterior margin measured in full-face view.

**HW** Head Width. Maximum width of the head measured in full-face view.

**EL** Eye Length. Maximum length of the eye measured in lateral view.

**SL** Scape Length. Maximum length of the antennal scape measured in full-face view.

**WL** Weber's Length. Maximum diagonal length from the most anterior point of the pronotal slope to the most posteroventral margin of propodeal lobe measured in lateral view.

**PNW** Pronotum Width. Maximum width of pronotum measured in dorsal view.

**PNH** Pronotum Height. Maximum height of pronotum measured in lateral view from index of procoxa to the highest point of the dorsal pronotum.

**MNH** Promesonotum Height. Maximum height of promesonotum measured in lateral view from the index of mesocoxa to the highest point of the dorsal pronotum.

**PDH** Propodeum Height. Maximum height of propodeum, measured in lateral view from the highest point of the dorsopropodeum perpendicular to a line that marks the lateroventral borders of the katepisternum and the propodeum.

**PTL** Petiolar Length. Maximum length of petiole measured in lateral view from most anteroventral point of the peduncle, at or below the propodeal lobe, to most posterodorsal point at the junction with helcial tergite.

**PTH** Petiolar Height. Maximum height of petiole measured in lateral view from the highest (median) point of the node, orthogonally to the ventral outline of the node.

**PTW** Petiolar Width. Maximum width of petiole measured in dorsal view.

**PPL** Postpetiolar Length. Maximum length of postpetiole measured in dorsal view from the anterior end of the node to the posterior end of the node.

**PPH** Postpetiolar Height. Maximum height of postpetiole measured in lateral view from the highest point of the node to the lowest point of the ventral process, often in an oblique line.

**PPW** Postpetiolar Width. Maximum width of postpetiole measured in dorsal view.

## Ratios

**CI** Cephalic index: HW / HL × 100;

**SI** Scape index: SL / HW × 100;

**EI** Eye index: EL / HW × 100;

**LPpI** Lateral postpetiole index: PPL / PPH × 100;

**DPpI** Dorsal postpetiole index: PPW / PPL × 100;

**PpWI** Postpetiole width index: PPW / PTW × 100;

**PpLI** Postpetiole length index: PPL / PTL ×100;

**PpHI** Postpetiole height index: PPH / PTH × 100;

**PPI** Postpetiole index: PPW / PNW × 100.

## Taxonomy

### Genus *Carebara* Westwood, 1840

*Carebara* Westwood, 1840: 86. Type species: *Carebara lignata* Westwood, 1840: 86, Indonesia (Java). Indomalaya.

= *Pheidologeton* Mayr, 1862: 750. Synonymized by Fischer et al. 2014: 63.

= *Oligomyrmex* Mayr, 1867: 110. Synonymized by Fernández 2004: 194.

= *Aeromyrma* Forel, 1891: 198. Synonymized by Fernández 2004: 194.

= *Aneleus* Emery, 1900: 327. Synonymized by Fernández 2004: 194.
= *Erebomyrma* Wheeler, 1903: 138. Synonymized by Fernández 2004: 194.
= *Paedalgus* Forel, 1911: 217. Synonymized by Fernández 2004: 194.
= *Lecanomyrma* Forel, 1913: 56. Synonymized by Fernández 2004: 194.
= *Spelaeomyrmex* Wheeler, 1922: 9. Synonymized by Fernández 2004: 194.
= *Hendecatella* Wheeler, 1927: 93. Synonymized by Fernández 2004: 194.
= *Amauromyrmex* Wheeler, 1929: 1. Synonymized by Fischer et al. 2014: 63.
= *Solenops* Karavaiev, 1930: 207. Synonymized by Fernández 2004: 194.
= *Idrisella* Santschi, 1937: 372. Synonymized by Fischer et al. 2014: 66.
= *Crateropsis* Patrizi, 1948: 174. Synonymized by Fernández 2004: 194.
= *Sporocleptes* Arnold, 1948: 219. Synonymized by Fernández 2004: 194.
= *Nimbamyrma* Bernard, 1953: 240. Synonymized by Fernández 2004: 194.
= *Afroxyidris* Belshaw & Bolton, 1994: 631. Synonymized by Fernández 2004: 194.
= *Neoblepharidatta* Sheela & Narendran, 1997: 88. Synonymized by Fernández 2004: 194.
= *Parvimyrma* Eguchi & Bui, 2007: 40. Synonymized by Fernández 2010: 195.

**Synopsis of members of Carebara from China.** Currently, there are 36 *Carebara* species and subspecies in China, with the majority in the southern and southwestern regions (Fig. 1). The highest diversity is observed in Guangdong, Guangxi, Yunnan, and Sichuan provinces (Xu 2003; Zhou and Zheng 1997).

Here we provide a brief overview of the provisional definition of Chinese *Carebara* species groups. In addition to the former *Pheidologeton* species, other *Carebara* members in China (except *C. amia*) align with the *concinna-lignata* group proposed by Bharti. It is worth mentioning that the criteria for classification vary among different studies. Bharti and Kumar (2013) suggested merging the *concinna* and *lignata* groups, while Fischer et al. (2015) retained the *lignata* group, defining its features based on Fernández (2004): workers typically small with 9-segmented antenna, mandibles 3- or 4-toothed, absent eyes and propodeal teeth, and a rounded dorsum of the propodeum. The queens are generally much larger than the workers. Hosoishi et al. (2022) established the *acutispina* group; however, it is highly probable that this is an artificial group within the *concinna-lignata* group. Due to the lack of comprehensive revisions of all castes (especially queens and males), the current definition of species groups relies predominantly on the morphology of the worker caste. However, this approach introduces uncertainties due to the potential influence of convergent evolution and a global investigation into *Carebara* species, utilizing molecular data, is imperative for a more accurate understanding of the phylogenetic relationship between groups. To prevent the proposal of multiple species groups, our definition is mainly based on Bharti and Kumar (2013).

There are 11 species and subspecies of China belonging to the previously valid genus *Pheidologeton*: *C. affinis* (Jerdon, 1851), *C. diversa* (Jerdon, 1851), *C. diversa draco* (Santschi, 1920), *C. diversa laotina* (Santschi, 1920), *C. latinoda*, *C. melasolena*, *C. nanningensis*, *C. trechideros*, *C. vespillo*, *C. yanoi*, *C. zengchengensis*. These species are identified by their 11-segmented antennae, distinct polymorphic worker castes, and multifaceted eyes (Fischer et al. 2015). Fischer et al. (2014) indicated that former *Pheidologeton* species would be split into two groups: one with a polymorphic worker caste and the other with a dimorphic worker caste, and the 11 Chinese taxa mentioned above belong to the former group.

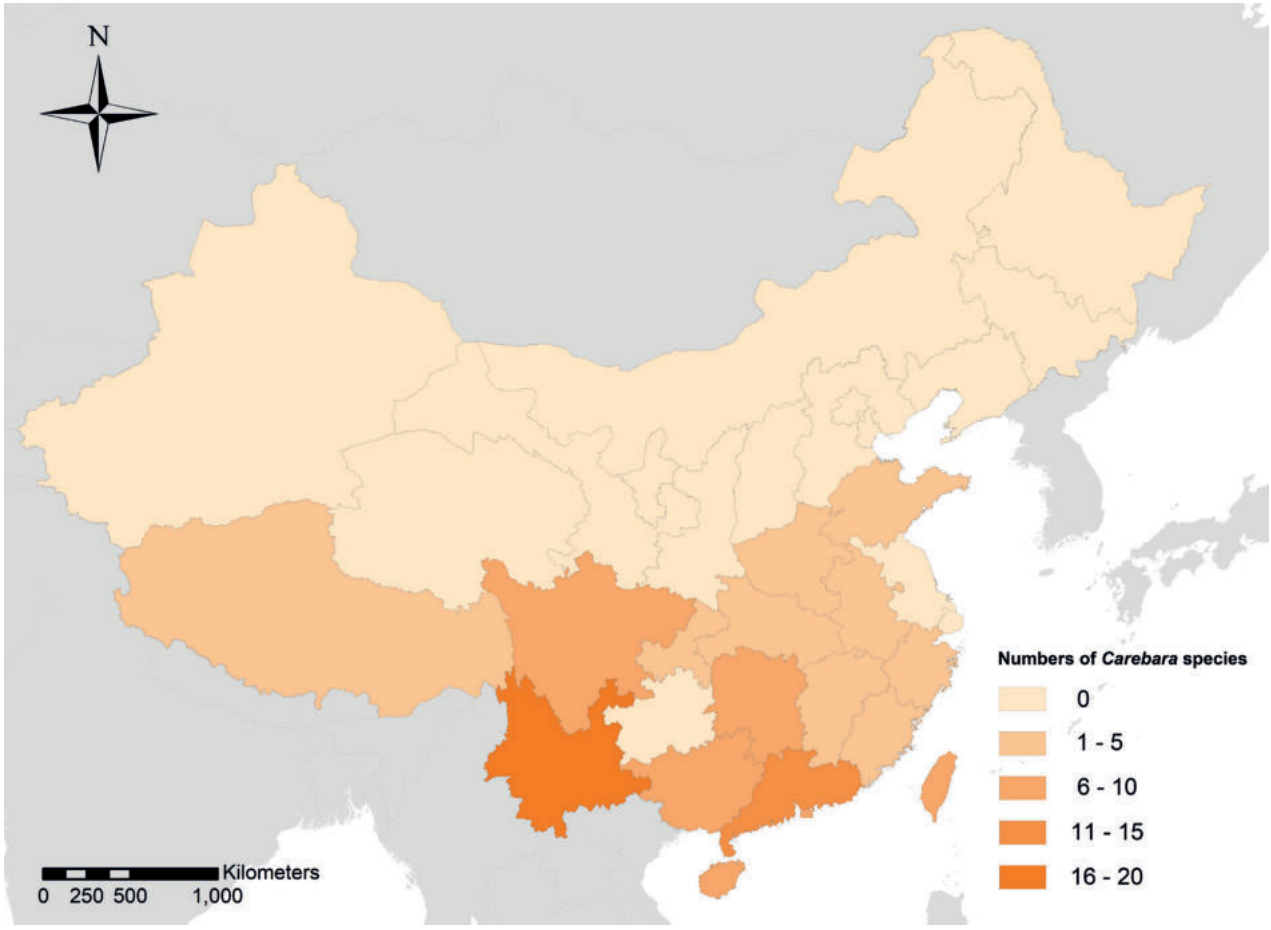


**Figure 1.** Map of the diversity of known Chinese *Carebara* species. Shades from pale to dark indicate species numbers from low to high.

The *lignata* group was originally established by Fernández (2004) to group New World species characterized by minor workers with 9-segmented antenna and the absence of eyes. The minor workers of this group mainly lack propodeal spines. Major workers present or absent. Members of the *concinna* group with dimorphic workers, eyes present in both major and minor workers, propodeum armed or only angulated, antenna 9- to 11- (rarely 8-) segmented. Bharti and Kumar (2013) suggested redefining the *concinna* group including the *lignata* group; this alteration was based on the observation that the major worker of *C. asina* aligned with the *concinna* group, whereas the minor worker lacked eyes and propodeal spines, consistent with the traditional *lignata* group proposed by Fernández (2010).

Certain Chinese *Carebara* species, like *C. bihornata* and *C. sakamotoi*, also form a bridge between the *lignata* and *concinna* groups. Similar to *C. asina*, *C. bihornata* exhibit eyeless minor workers with an unarmed propodeum, while the major workers accord with the features of *concinna* group. Some species provide additional insights into the *concinna-lignata* group, such as *C. capreola* and *C. curvispina*, both characterized by eyeless major and minor workers. This suggests that the features of the *concinna-lignata* group include: (1) workers monomorphic or dimorphic; (2) antenna 9- to 11- (rarely 8-) segmented; (3) propodeal spines present or absent in major and minor workers; (4) eyes present or absent in major and minor workers.

## Key to *Carebara* species of China based on major worker caste

This key is based on Xu (2003) and Zhou et al. (2006), including 34 species and subspecies; some illustrations of the key were drawn from Xu (2003) and Terayama (1996). The following species are excluded from this key because descriptions of the major workers are unavailable: *C. amia*, *C. castanea*, and *C. lignata*. Some distribution data about Chinese *Carebara* species in previous studies are ambiguous. After verification with Xu (pers. comm. July 21, 2023), some records are not accepted in this study and the following species are excluded from the Chinese fauna: *C. asina*, *C. bengalensis* (Forel, 1902), *C. bruni* (Forel, 1913), *C. cribriceps* (Wheeler, 1927), and *C. pumilia* Fischer et al., 2014.

1 Antenna 11-segmented (Fig. 2A) ..... **2**
– Antenna 9- or 10-segmented (Fig. 2B, C) ..... **17**
2 Worker caste polymorphic, with continuous series of intermediates between minor and largest major worker ..... **3**
– Worker caste dimorphic ..... **13**

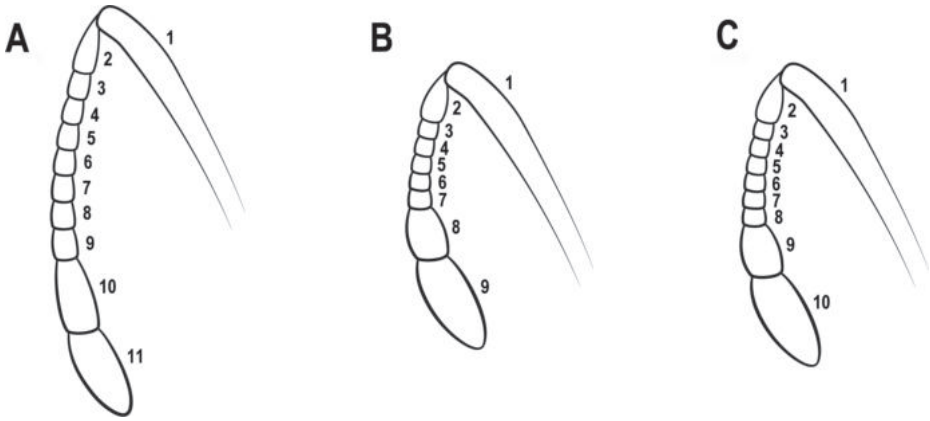


**Figure 2.** Antenna **A** 11-segmented **B** 9-segmented **C** 10-segmented.

3 Propodeal spines long, > 1/2 of the distance between the base of two spines (Fig. 3A) ..... **4**
– Propodeal spines short, < 1/3 of the distance between the base of 2 spines (Fig. 3B) ..... **9**

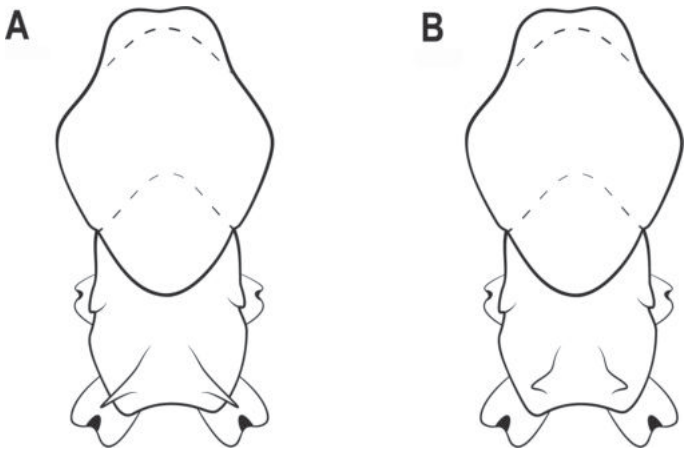


**Figure 3.** Mesosoma **A** longer spine **B** shorter spine.

4 Largest major worker with mesoscutellum strongly convex in lateral view and with single ocellus on the front of head (Fig. 4A, C) ..... **5**

– Largest major worker with mesoscutellum broadly convex in lateral view and with no ocelli on the front of head (Fig. 4B, D) ..... **7**

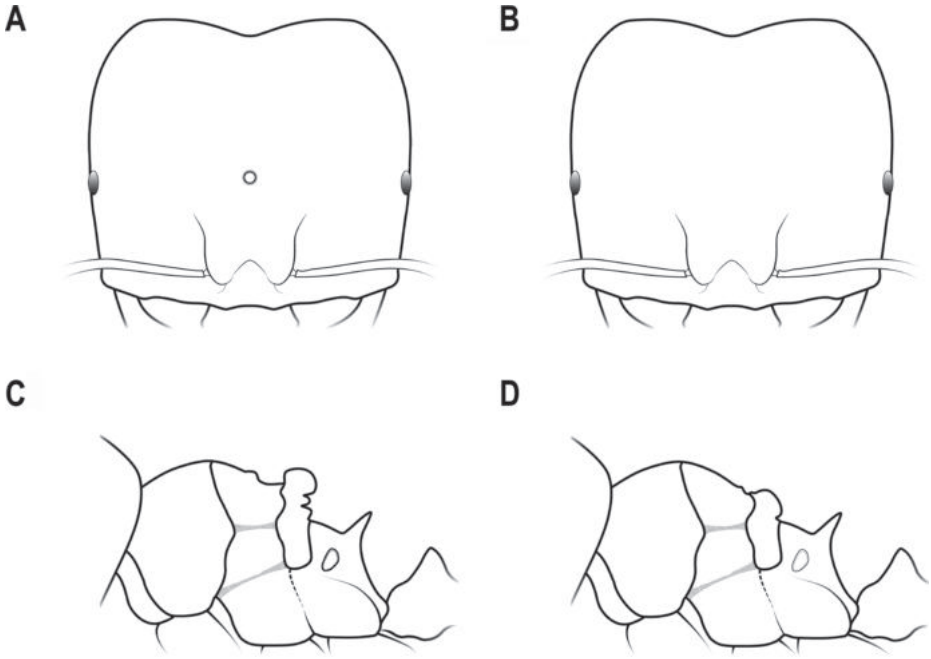


**Figure 4.** Head in full-face view and mesosoma in lateral view (the largest major worker) **A** head with an ocellus **B** head with no ocelli **C** mesoscutellum strongly convex **D** mesoscutellum broadly convex.

5 Largest major worker distinctly smaller with TL 11–12 mm..... ..... ***C. diversa draco* (Santschi, 1920)**

– Largest major worker distinctly larger with TL ~ 16 mm..... **6**

6 The posterior quarter of the head with transverse and very large wrinkles; the smooth frontal space more extensive (Fig. 5A)..... ..... ***C. diversa laotina* (Santschi,1920)**

– The posterior 1/3 of head with diverged wrinkles; smooth frontal space more narrowed (Fig. 5B) ..... ***C. diversa* (Jerdon)**

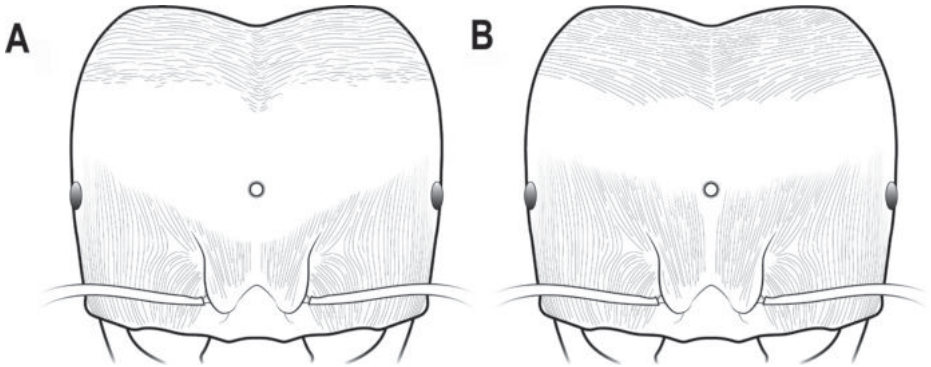


**Figure 5.** Heads of *C. diversa* and *C. diversa laotina* in full-face view (the largest major worker) **A** *C. diversa laotina*, head with broader smooth space, wrinkles mostly transverse **B** *C. diversa*, head with more narrowed smooth space, wrinkles fine and diverged.

7 Propodeal spines curving forward and inclined (Fig. 6A) ..... ***C. yanoi* (Forel)**
– Propodeal spines directed backwards or erect (Fig. 6B)..... **8**

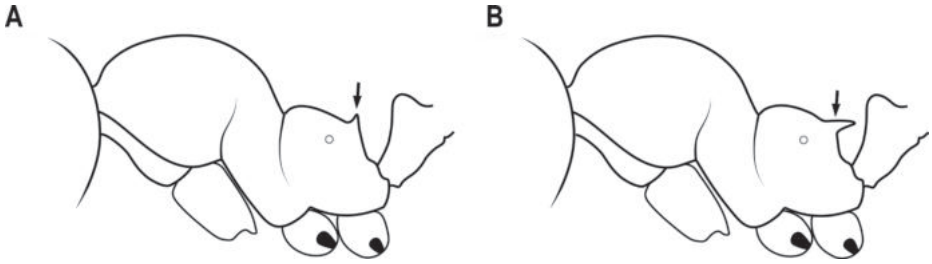


**Figure 6.** Pronotal spines **A** curving forward **B** backwards.

8 Promesonotum slightly convex in lateral view; first tergite of gaster > 2× as wide as second tergite ..... ***nanningensis* (Li & Tang)**
– Promesonotum distinctly convex in lateral view; first gastric tergum almost as wide as second tergum ..... ***C. affinis* (Jerdon)**
9 Head and body mostly smooth and shiny (Fig. 7A) ..... **10**
– Head and body coarsely striate (Fig. 7B)..... **12**

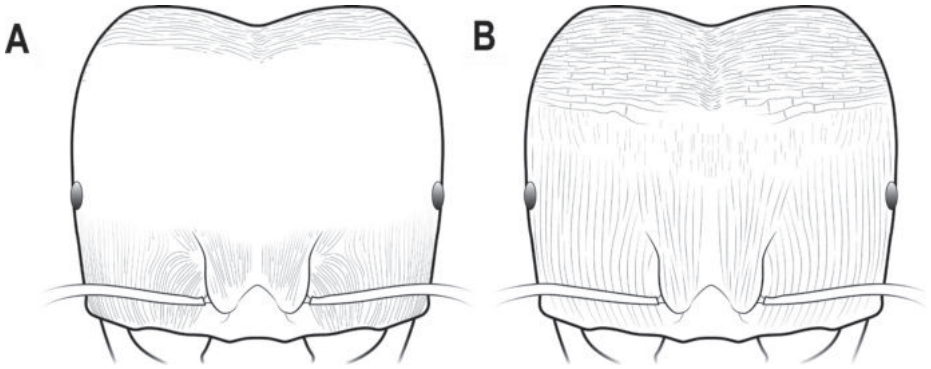


**Figure 7.** Head in full-face view **A** head mostly smooth and shiny **B** head mostly striate.

10 Propodeal spines pointing backwards in the largest major worker; petiolar node broadly rounded above in profile view; head without a coarse black line in median longitudinal groove (Fig. 8A, C) ..... ***C. latinoda* (Zhou & Zheng)**
– Propodeal spines curving forward in the largest major worker; petiolar node narrowed above, triangular in profile view; head with a coarse black line in median longitudinal groove (Fig. 8B, D) ..... **11**

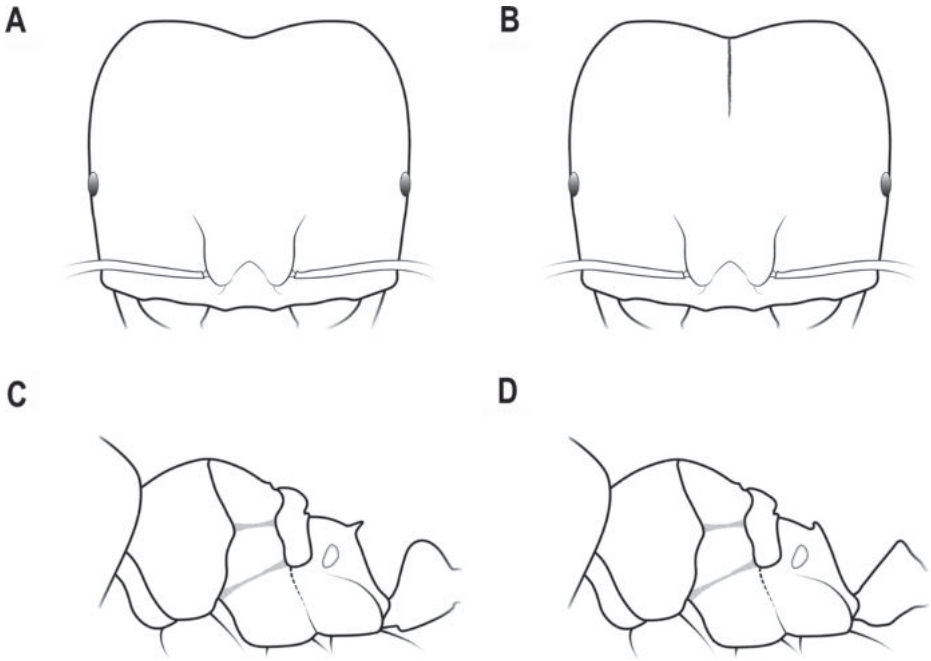


**Figure 8.** Head in full-face view, mesosoma and petiole in lateral view **A** head without a coarse black line **B** head with a coarse black line in median longitudinal groove **C** propodeal spines pointing backward, petiolar node round above **D** propodeal spines curving forward, petiolar node narrowed above.

11 Postpetiole approximately as long as wide; hairs sparse (Fig. 9A, C) ..... ..... ***C. vespillo* (Wheeler)**

– Postpetiole distinctly broader than long; hairs abundant (Fig. 9B, D)..... ..... ***C. melasolena* (Zhou & Zheng)**

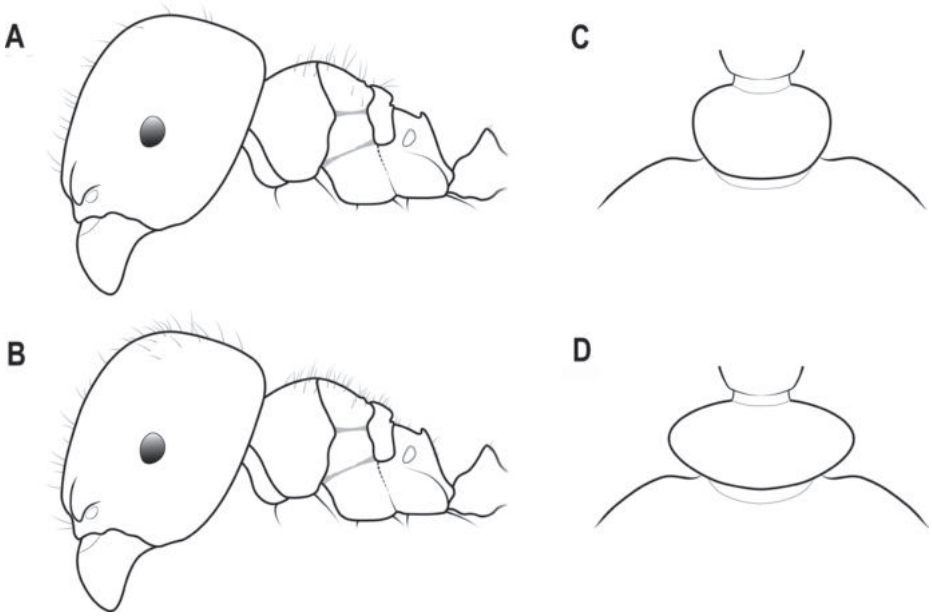


**Figure 9.** Head and mesosoma in lateral view, postpetiolar node in dorsal view **A** *C. vespillo*, hairs sparse **B** *C. melasolena*, hairs abundant **C** *C. vespillo*, postpetiolar node nearly as long as wide **D** *C. melasolena*, postpetiolar node distinctly wider than long.

12 Propodeal spines laterally compressed and curving forward; mandibles with longitudinal striations on the base in full-face view; interspaces on the head between striations punctured (Fig. 10A, C) ..... ***C. trechideros* Zhou & Zheng**

– Propodeal spines thick and straight, not curved; mandibles smooth in full-face view; interspaces between striations smooth (Fig. 10B, D) ..... ***C. zengchengensis* (Zhou et al.)**

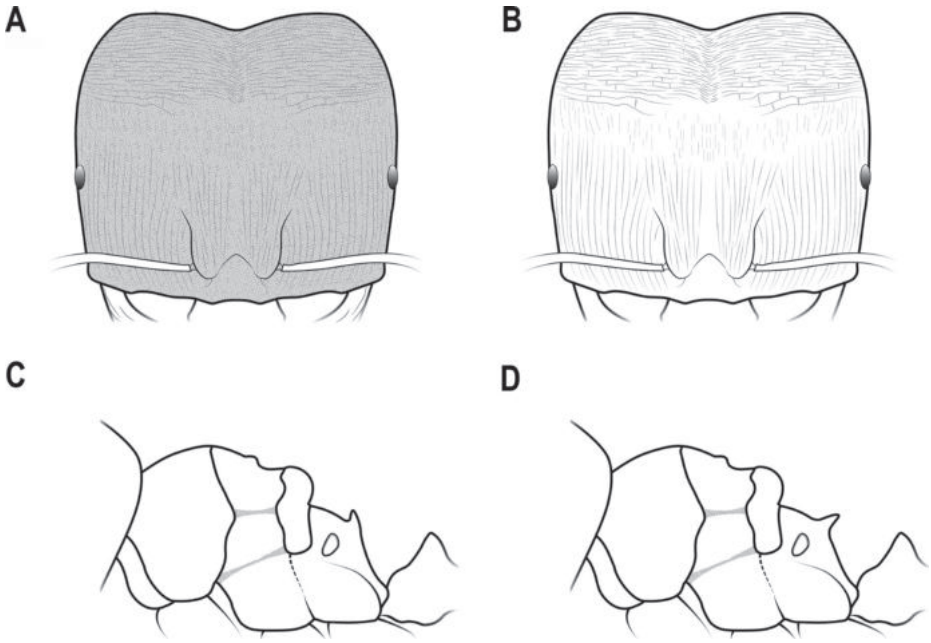


**Figure 10.** Head in full-face view and mesosoma in lateral view (the largest major worker) **A** head of *C. trechideros*, mandibles with longitudinal striations, interspaces between striations punctured **B** head of *C. zengchengensis*, mandibles without striations, interspaces between striations smooth **C** propodeal spines curving forward **D** propodeal spines straight and not curved.

13 Mandible with 5 teeth on masticatory margin (Fig. 11A) ..... **14**

– Mandible with 6 teeth on masticatory margin (Fig. 11B) ..... **16**

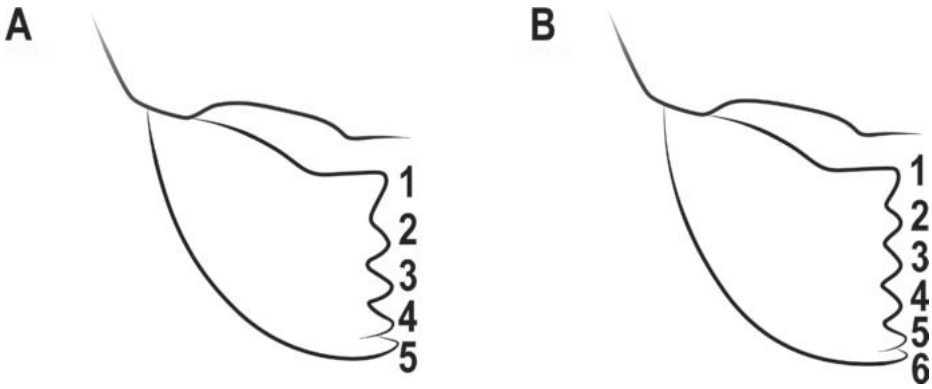


**Figure 11.** Mandibles **A** mandible with 5 teeth **B** mandible with 6 teeth.

14 Posterolateral corners of head with minute tubercles; eyes present; head slightly longer than wide (Fig. 12A).....***C. altinodus* (Xu)**
– Posterolateral corners of head with developed horns; eyes absent; head distinctly longer than wide (Fig. 12B).....**15**

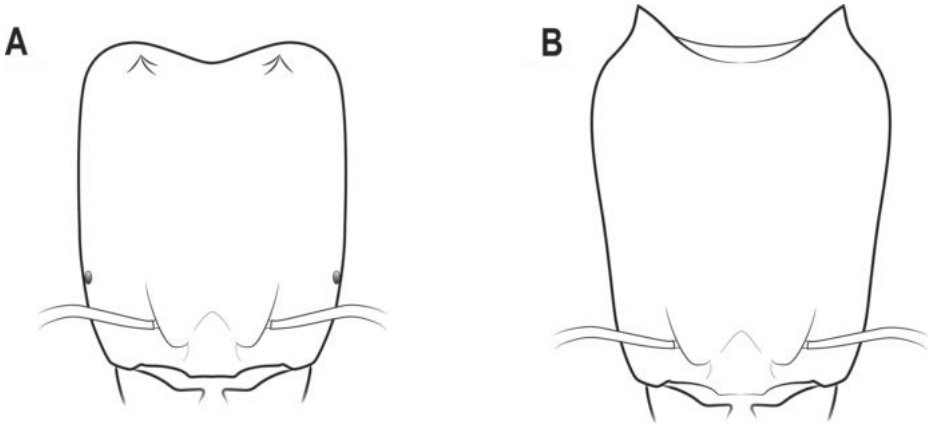


**Figure 12.** Horns **A** posterolateral corners of head with minute tubercles **B** posterolateral corners of head with developed horns.

15 Head broader behind than in front with more punctures; head and thorax with dense hairs ..... ***C. capreola* (Wheeler)**
– The posterior portion of head almost as wide as the anterior portion; head with less punctures, smoother and more shiny; head and thorax with sparser and shorter hairs.....***C. capreola laeviceps* (Wheeler)**
16 Eyes absent; propodeal denticles downwardly inclined; first segment of gaster finely punctuate (Fig. 13A) .....***C. curvispina* (Xu)**
– Eyes present; propodeal denticles dorsoposteriorly pointed; first segment of gaster densely longitudinally striate (Fig. 13B).....***C. striata* (Xu)**

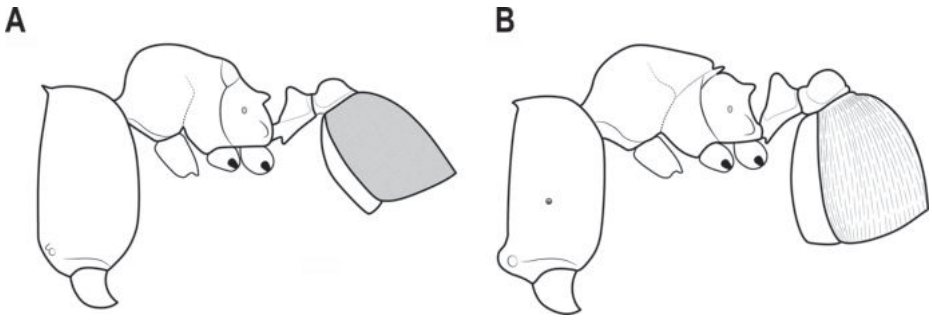


**Figure 13.** Two *Carebara* species in lateral view **A** *C. curvispina*, propodeal denticles down-inclined, first segment of gaster finely punctuate, eyes absent **B** *C. striata*, propodeal denticles dorsoposteriorly pointed, first segment of gaster densely longitudinally striate, eyes present.

17 Mandible with 6 teeth on masticatory margin (Fig. 14C) .....**18**
– Mandible with 4 or 5 teeth on masticatory margin (Fig. 14A, B).....**19**

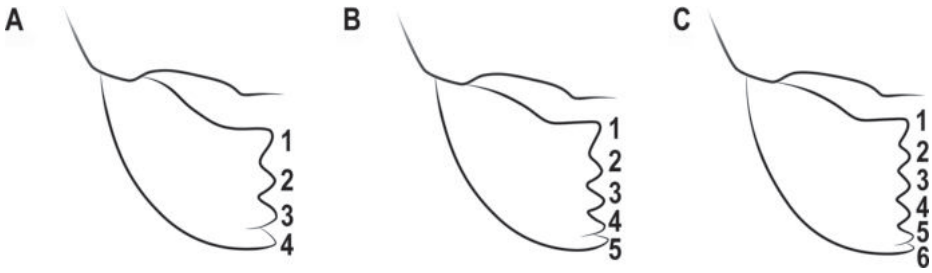


**Figure 14.** The number of teeth **A** 4-teethed **B** 5-teethed **C** 6-teethed.

18 HL < 1 mm; first gastral tergum smooth and shiny.....***C. oni* (Terayama)**
– HL > 1 mm; first gastral tergum punctate and microreticulate with longitudinal striations (Fig. 15A, B).....***C. qianliyan* Terayama**

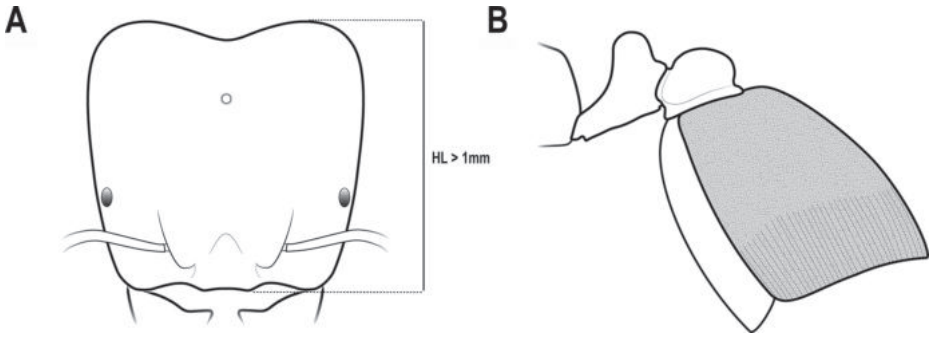


**Figure 15.** Head in full-face view and tergum in lateral view (*C. qianliyan*) **A** HL > 1 mm **B** first gastral tergum punctate with longitudinal striations.

19 Posterolateral corners of head with a pair of distinct horns or small tubercles (Fig. 16A, B).....**20**
– Head with no horns or tubercles (Fig. 16C).....**29**

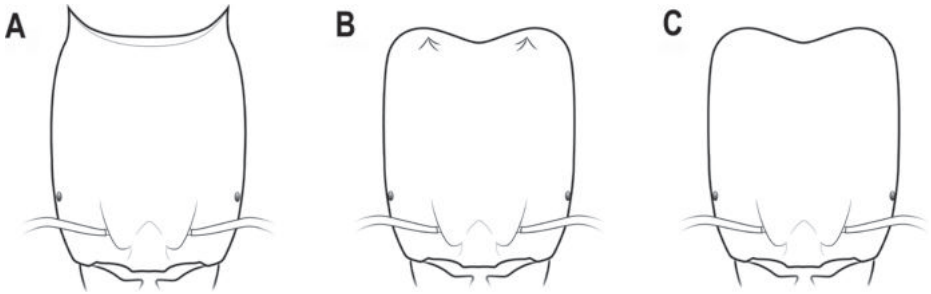


**Figure 16.** Horns or tubercles on the posterior corner of head **A** distinct horns **B** small tubercles **C** no horns or tubercles.

20 Propodeum with a pair of protruding denticles (Fig. 17A) .....**21**
– Propodeum without a pair of protruding denticles; posterodorsal corner of propodeum rounded or forms an obtuse or right angle (Fig. 17B, C).....**23**

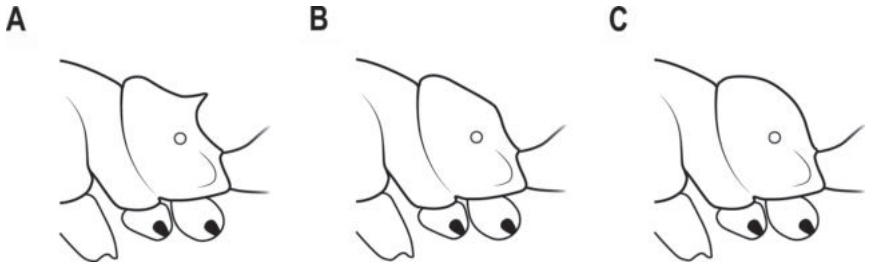


**Figure 17.** Propodeum in lateral view **A** propodeum with protruding denticles **B** propodeum forming an obtuse angle **C** propodeum rounded.

21 Posterior area of head without transverse striations; metanotal groove impressed shallowly; body smaller with TL 1.4 mm (Fig. 18C) ..... ..... ***C. acutispina* (Xu)**

– Posterior area of head with transverse striations; metanotal groove deeply impressed; body larger with TL 2.1–2.6 mm (Fig. 18A, B) .....**22**

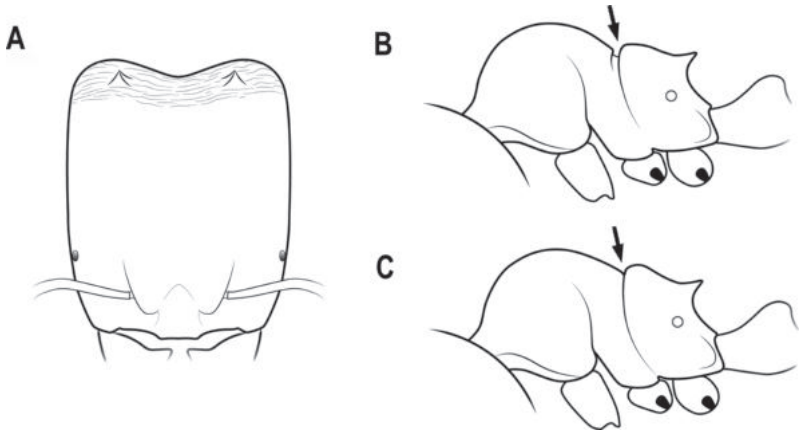


**Figure 18.** Striations and metanotal groove **A** head with transverse striations **B** metanotal groove impressed deeply **C** metanotal groove impressed shallowly.

22 Mandible with 4 teeth on masticatory margin; clypeus with the anterior margin of median portion concave indistinctly.....***wheeleri* (Ettershank)**

– Mandible with 5 teeth on masticatory margin; clypeus with the anterior margin of median portion concave distinctly..... ***C. obtusidenta* (Xu)**

23 Horns connected by a developed transverse ridge (Fig. 19A) .....**24**

– Horns not connected by a developed transverse ridge (Fig. 19B) .....**25**

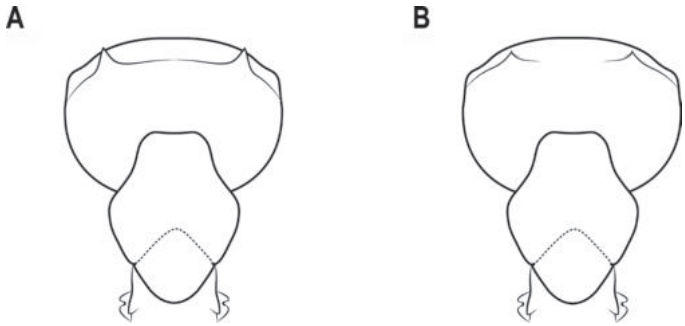


**Figure 19.** Horns in dorsal view **A** horns connected by a transverse ridge **B** horns not connected by a transverse ridge.

24 Head capsule thin with straight anterior margin in lateral view (Fig. 20A) .... .....***C. bihornata* (Xu)**

– Head capsule thick with convex anterior margin in lateral view (Fig. 20B).... .....***C. sakamotoi* Terayama et al.**

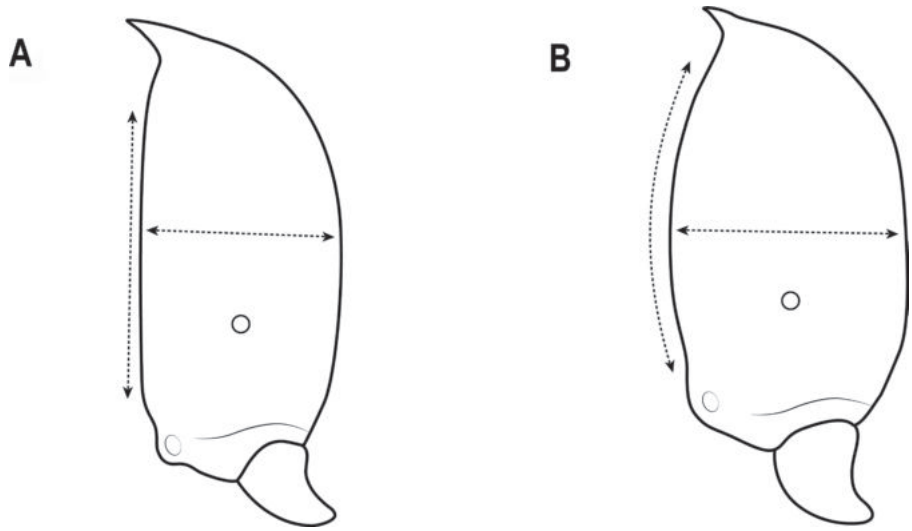


**Figure 20.** Head capsule in lateral view **A** head capsule thin with anterior margin straight **B** head capsule thick with anterior margin convex.

25 Body larger with TL 3.0–3.5 mm ..... ***C. polyphemus* (Wheeler)**

– Body smaller with TL 1.5–2.3 mm .....**26**

26 Mandible with 4 teeth on masticatory margin..... ***C. taiponica* (Wheeler)**

– Mandible with 5 teeth on masticatory margin.....**27**

27 Head coarsely microreticulate; frons and vertex with many striae (Fig. 21A) .....***C. yamatonis* (Terayama)**

– Head largely smooth and shiny (Fig. 21B).....**28**

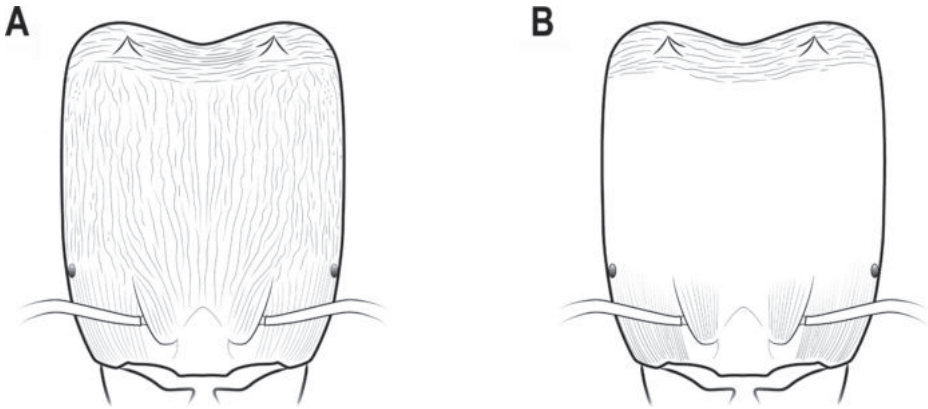


**Figure 21.** Head in full-face view **A** head with dense microreticulation **B** head largely smooth and shiny.

28 Anterodorsal corner of propodeum prominent, forming an acute tooth behind metanotal groove (Fig. 22A).....***C. sauteri* (Forel)**
– Anterodorsal corner of propodeum not forming an acute tooth (Fig. 22B) .... .....***C. rectidorsa* (Xu)**

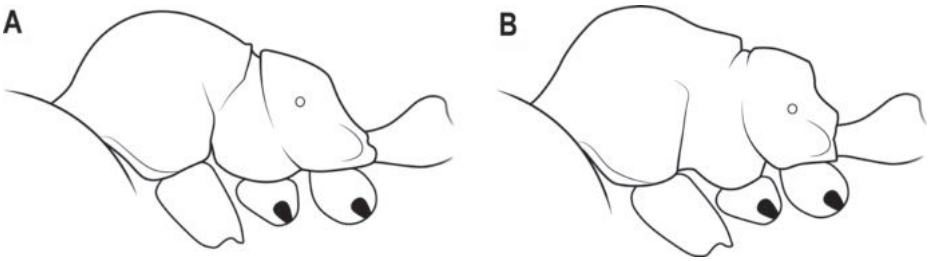


**Figure 22.** Mesosoma in lateral view **A** anterodorsal corner of propodeum forming an acute tooth **B** anterodorsal corner of propodeum not forming an acute tooth.

29 Head nearly square, ~ as long as broad; eyes with 16 facets; head with 3 ocelli; dorsum of mesosoma straight (Fig. 23A, B)..... ***C. hunanensis* (Xu)**
– Head rectangular, longer than broad; eyes with < 10 facets; head without ocelli; dorsum of mesosoma not straight.....**30**

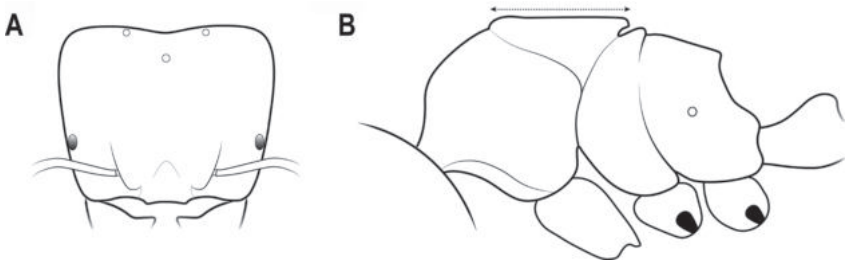


**Figure 23.** *C. hunanensis* **A** head with 3 ocelli in full-face view **B** dorsum of mesosoma straight in lateral view.

30 Propodeum with a pair of acute teeth; head with fine reticulations ..... ..... ***C. reticapita* (Xu)**
– Propodeum forms an obtuse angle; head smooth, at most sparsely punctured .....**31**
31 Posterodorsal corner of propodeum forming a right angle of ~ 90° (Fig. 24A) ..... ***C. pseudolusciosa* (Wu & Wang)**
– Posterodorsal corner of propodeum forming an obtuse angle of more than 90° (Fig. 24B).....**32**

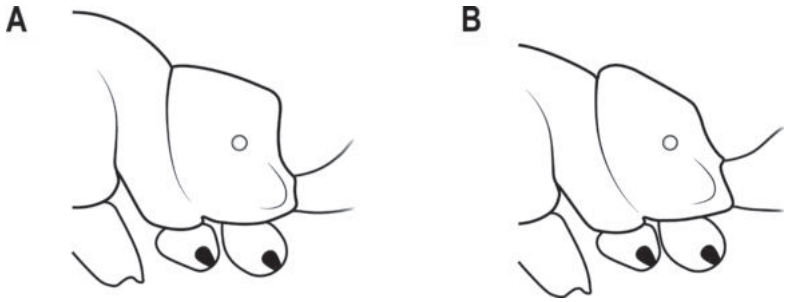


**Figure 24.** Propodeum in lateral view **A** posterodorsal corner forming an right angle **B** posterodorsal corner forming an obtuse angle.

32 Vertex with transverse striations..... ***C. jiangxiensis* (Wu & Wang)**
– Vertex smooth and with no striations.....**33**
33 Antenna 10-segmented; katepisternum rugose-reticulate; body distinctly larger with TL > 2.6 mm (Fig. 25A).....***C. laeviceps* sp. nov.**
– Antenna 9-segmented; katepisternum smooth and shiny; body smaller with TL ~ 2 mm (Fig. 25B) .....***C. lusciosa* (Wheeler)**

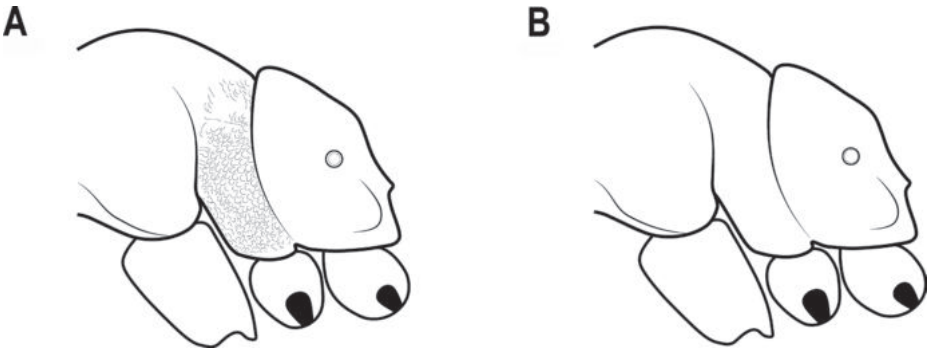


**Figure 25.** Katepisternum in lateral view **A** katepisternum rugose-reticulate **B** katepisternum smooth.

***Carebara laeviceps* Liu & Zhong, sp. nov.**
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Figs 26–31

**Type material.** SWFU; ***Holotype*.** China: 1 major worker, Sichuan Province, Dazhou City, Kaijiang County, 31°12'24"N, 107°55'43"E, alt. 1100 m, 27.VI.2022, Gui-chuan Nie, SWFU A22-955. ***Paratypes*.** China: 3 major workers and 4 minor workers, same data as holotype, SWFU A22-955.

**Description of major worker. *Measurements*.** Holotype major worker: HL 0.84, HW 0.63, EL 0.02, SL 0.38, WL 0.73, PNW 0.42, PNH 0.29, MNH 0.48, PDH 0.32, PTL 0.30, PTH 0.23, PTW 0.22, PPL 0.21, PPH 0.18, PPW 0.25, CI 75, SI 60, EI 3, LPpI 117, DPpI 119, PpWI 114, PpLI 70, PpHI 78, PPI 60. Paratype major workers (*n* = 3): HL 0.88 (0.85–0.90), HW 0.68 (0.66–0.70), EL 0.03 (0.02–0.05), SL 0.36 (0.35–0.36), WL 0.76 (0.75–0.78), PNW 0.43 (0.42–0.44), PNH 0.33 (0.31–0.35), MNH 0.44 (0.43–0.44), PDH 0.33 (0.30–0.35), PTL 0.30 (0.26–0.33), PTH 0.23 (0.22–0.23), PTW 0.20 (0.19–0.20), PPL 0.17 (0.14–0.19), PPH 0.18 (0.15–0.20), PPW 0.26 (0.25–0.27), CI 78 (77–78), SI 52 (51–55), EI 4.38 (3–7.14), LPpI 107 (94–127), DPpI 144 (131–153), PpWI 132 (130–135), PpLI 59 (57–61), PpHI 75 (65–83), PPI 60 (58–61). ***Head*.** Large, subrectangular with lateral margins straight and parallel in full-face view, distinctly longer than broad, ~ 1.3× as long as wide; posterior margin slightly concave medially; posterolateral corner rounded and without horns in lateral view. Mandible triangular with five teeth on the masticatory margin. Clypeus steep and flat in lateral view; anterior margin of clypeus nearly straight with median portion indistinctly concave. Frontal lobes concealing condylar bulb. Ocelli absent. Eyes minute, located a little behind the anterior 1/3 length of head, ~ 0.3 mm from mandibular insertions to eyes. Antenna 10-segmented with a 2-segmented club; scape short, ~ 0.4× as long as HL; apex of scape below mid-length of distance from antennal insertion to vertexal corner when scape is laid back. Dorsum of head

flat in lateral view. ***Mesosoma.*** In lateral view, promesonotum slightly convex with moderately rounded dorsum; the sides of pronotum strongly convex and rounded in dorsal view; promesonotal suture indistinct. Metanotal groove deeply impressed. Anterodorsal corner of propodeum forms an acute tooth behind the metanotal groove in lateral view; propodeum lower than promesonotum with flat dorsum; the declivity and dorsum of propodeum forming an obtuse angle in lateral view; declivitous edge of propodeum with a pair of indistinct carinae; lateral margins of propodeum strongly convex in dorsal view. ***Waist.*** Petiole ~ 0.8× as high as long with a long peduncle; petiolar node wider than long in dorsal view. In lateral view, the peduncle without angled tooth in anteroventral corner and the ventral margin of peduncle slightly convex; dorsum of petiole rounded in lateral view; anterior and posterior surfaces of petiolar node moderately convex. Postpetiolar node slightly lower than petiolar node, roundly convex. In dorsal view, postpetiole wider than petiole (PPW 0.25, PTW 0.22), both petiolar and postpetiolar nodes with convex lateral margins. ***Gaster.*** Long and oval. ***Sculpture and hairs.*** Mandibles, Median portion of clypeus and area from frons to posterior margin of head smooth and shiny, except genae and frontal lobes longitudinally striate. Posterior area of head without striations or carinae. Dorsum and lateral face of pronotum mostly smooth and shiny; anterior face of pronotal disc with fine reticular rugae. Mesonotum smooth; anepisternum and katepisternum strongly rugose-reticulate. In dorsal view, metanotal groove with several longitudinally parallel rugulae; propodeum mostly smooth in dorsal view; lateral face and declivity of propodeum weakly rugose-reticulate and with indistinct transverse rugulae in lateral view. Dorsum of petiolar node smooth; the lateral faces of node and peduncle rugose-reticulate; postpetiole weakly reticulate in dorsal view; ventral area of petiole and postpetiole strongly reticulate in lateral view. Gaster smooth and shiny. Head capsule covered with erect to subdecumbent hairs; while hairs on scapes and mandibles mostly decumbent. Dorsum of pronotum and mesonotum with abundant long erect hairs in lateral view; hairs on lateral face of mesosoma and dorsum of propodeum much sparser. Dorsum of petiole and postpetiole, and gaster with long erect to decumbent hairs; the ventral margin of petiole and postpetiole with no hairs in lateral view. ***Color.*** Head yellowish brown with clypeus and genae slightly darker; masticatory margin of mandible black. Mesosoma and petiole yellowish brown. Color of appendages and gaster paler.

**Description of minor worker.** ***Measurements.*** Paratype minor workers (*n* = 4): HL 0.46 (0.44–0.48), HW 0.44 (0.42–0.46), EL 0.01, SL 0.31 (0.30–0.32), WL 0.52 (0.51–0.52), PNW 0.28 (0.27–0.29), PNH 0.20 (0.20–0.21), MNH 0.29 (0.27–0.31), PDH 0.21 (0.19–0.23), PTL 0.18 (0.17–0.19), PTH 0.15 (0.14–0.15), PTW 0.13 (0.12–0.13), PPL 0.12 (0.11–0.12), PPH 0.11 (0.10–0.11), PPW 0.16, CI 94 (88–98), SI 70 (65–74), EI 2, LPpI 110 (100–120), DPpI 139 (133–145), PpWI 128 (123–133), PpLI 64 (61–71), PpHI 73 (67–79), PPI 57 (55–59). ***Head.*** Much smaller (HL 0.44–0.48, HW 0.42–0.46) than the head of major worker. In full-face view head subquadrate with lateral margins convex, slightly longer than broad and narrowed both anteriorly and posteriorly, ~ 1.1× as long as wide. Posterior margin of head slightly concave medially, posterolateral corners rounded in full-face view. Dorsum of head broadly convex in lateral view. Anterior margin of clypeus almost straight. Mandible triangular with five teeth on masticatory margin. Eyes minute, situated at the anterior 1/2

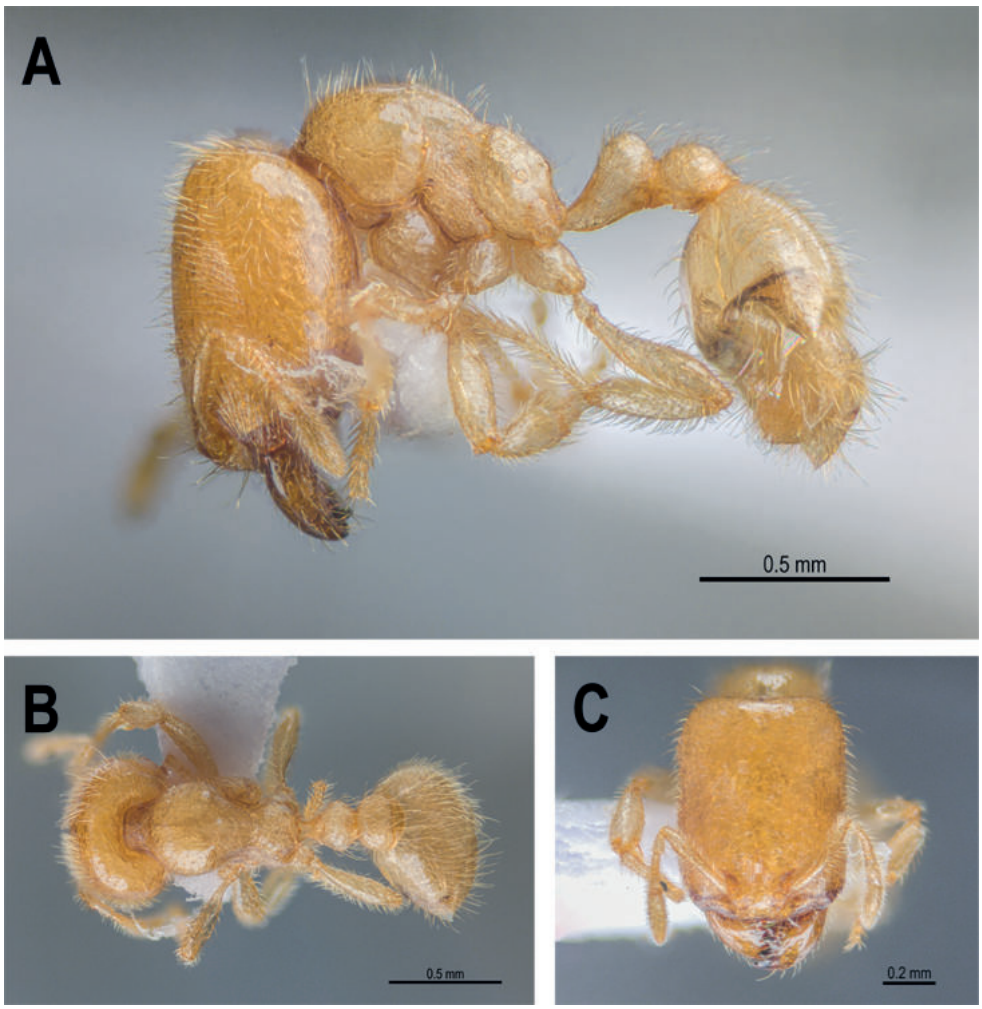


**Figure 26.** *Carebara laeviceps* Liu & Zhong, sp. nov. Holotype (major worker) **A** body in lateral view **B** body in dorsal view **C** head in full-face view.

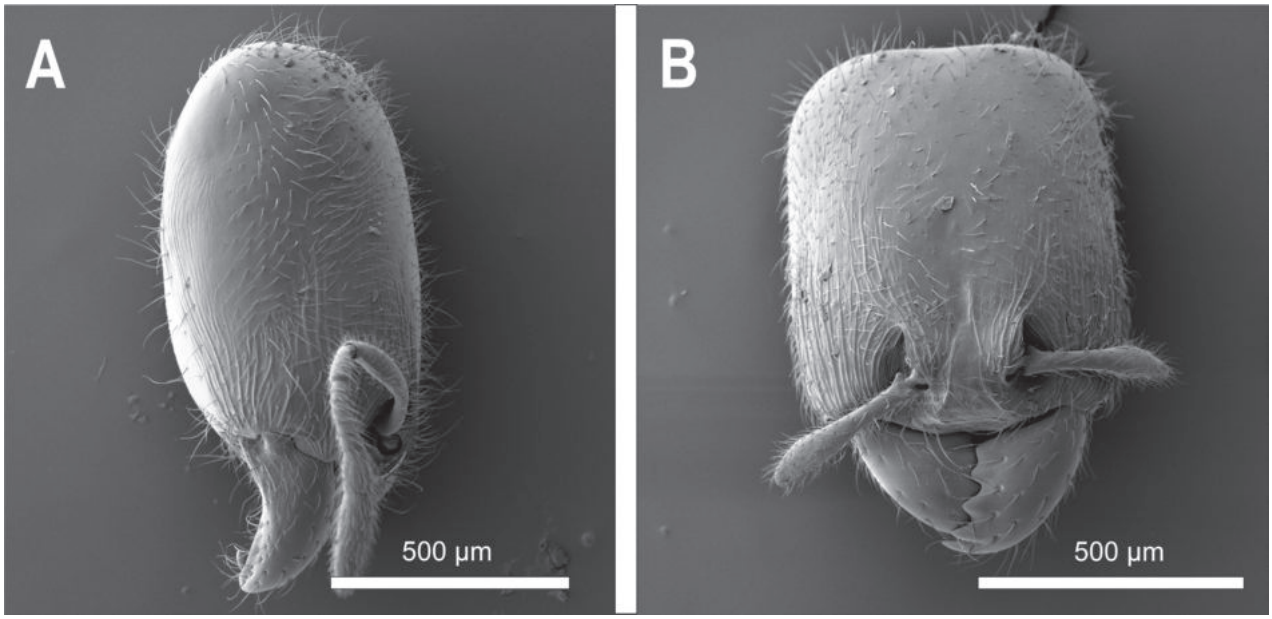


**Figure 27.** *Carebara laeviceps* Liu & Zhong, sp. nov. Major worker under SEM (not holotype) **A** head in lateral view **B** head in full-face view.

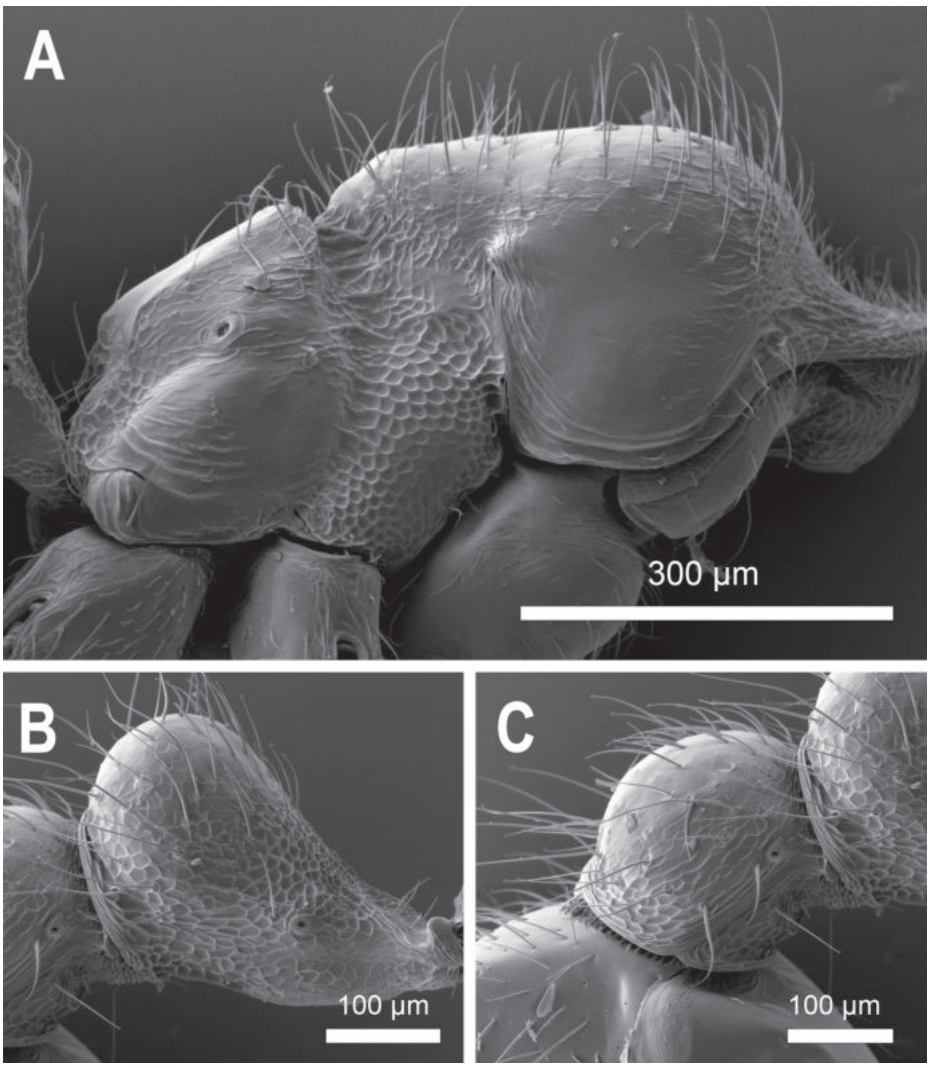


**Figure 28.** *Carebara laeviceps* Liu & Zhong, sp. nov. Major worker under SEM (not holotype) **A** mesosoma in lateral view **B** petiole in lateral view **C** postpetiole in lateral view.

length of head, ~ 0.2 mm from mandibular insertions to eyes. Antenna 10-segmented with a 2-segmented club; scape 0.70× as long as HW; apex of scape reaching 3/5 of the distance from antennal insertion to vertexal corner when scape is laid back. Dorsum of head broadly convex in lateral view. ***Mesosoma*.** Promesonotum with dorsal profile slightly arched in lateral view, nearly flat; suture indistinct. Metanotum absent; metanotal groove distinct and strongly impressed; In lateral view, propodeum spineless; the dorsal face of propodeum straight, forming an obtuse angle with the declivity of propodeum; declivity nearly straight, with median portion slightly concave; anterodorsal corner forming an acute tooth behind metanotal groove in lateral view. ***Waist*.** Petiole longer than high with long peduncle (PTL 0.18, PTH 0.15) in lateral view; ventral margin of petiole slightly convex; petiolar node broader than long with anterodorsal and posterodorsal faces convex in dorsal view. In lateral view, combined profile of anterior face of node and peduncle convex distinctly. Declivity of the posterior face of petiole slightly steeper than anterior face. Postpetiole with lower node than petiole, both dorsa of petiolar and postpetiolar nodes roundly convex. ***Gaster*.** Oval, relatively short. ***Sculpture and hairs*.** In full-face view, head

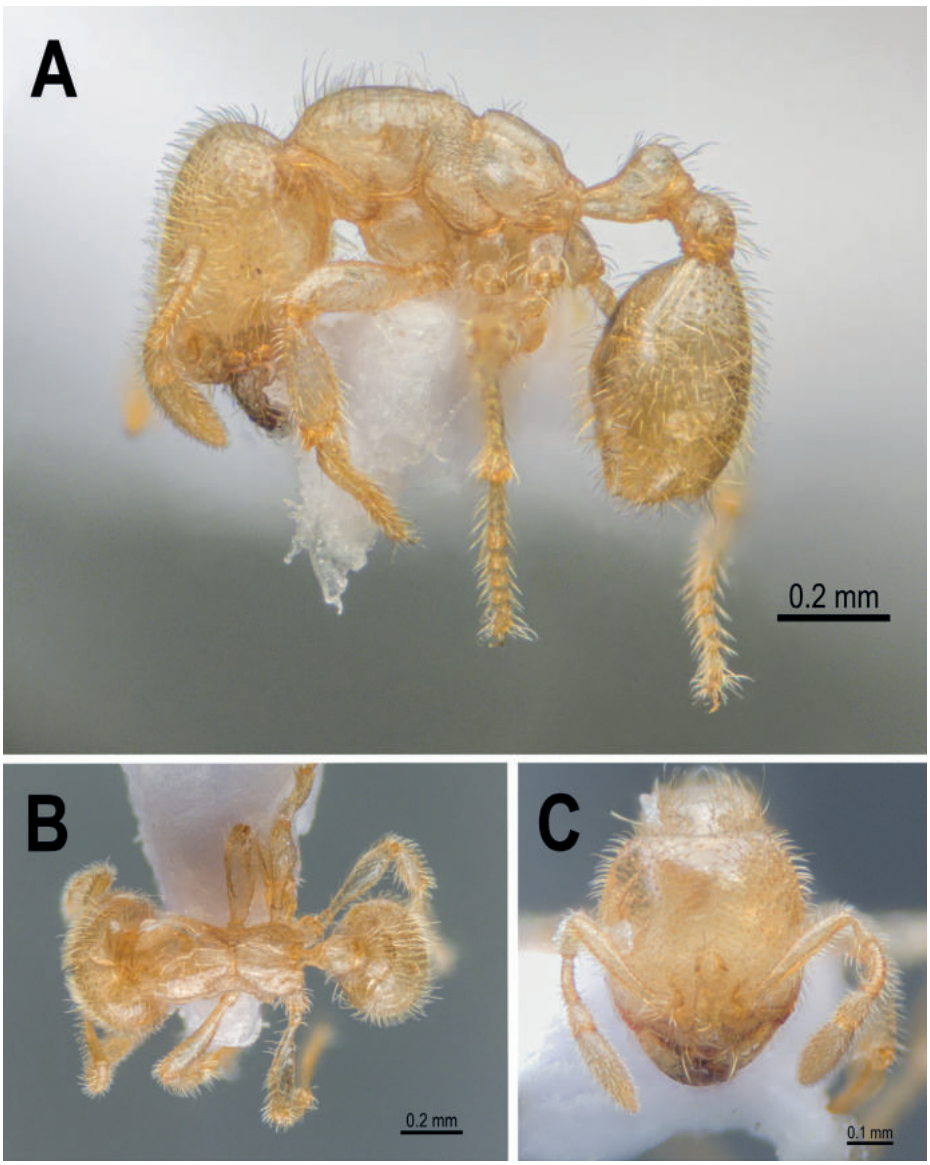


**Figure 29.** *Carebara laeviceps* Liu & Zhong, sp. nov. Paratype minor worker **A** body in lateral view **B** body in dorsal view **C** head in full-face view.

capsule, clypeus, and mandibles mostly smooth; only gena and frontal lobe with indistinct longitudinal rugulae; sculpture on mesosoma same as major workers. Gaster smooth and shiny. Whole head with abundant erect to suberect hairs; hairs on frons slightly sparser; scapes and lateral margin of mandibles with dense decumbent hairs. Dorsal and lateral faces of promesonotum with long erect hairs and short suberect hairs; propodeum with very sparse hairs. Hairs on waist and gaster like major worker. ***Color.*** Whole body yellowish white.

**Etymology.** The specific epithet *laeviceps* refers to the smooth and shiny head of the major workers.

**Biology.** Little known, the type material was collected in the grassland of Hanlin Village, Kaijiang City. The species nests underground and feeds on small invertebrates. Some major workers exhibit a swollen gaster, serving as a storage organ for reserves during foraging.

**Remarks.** *Carebara laeviceps* is most similar to *C. lusciosa*, *C. bouvardi* (Santschi, 1913) and *C. rectangulata* Bharti & Kumar, 2013, but can be easily

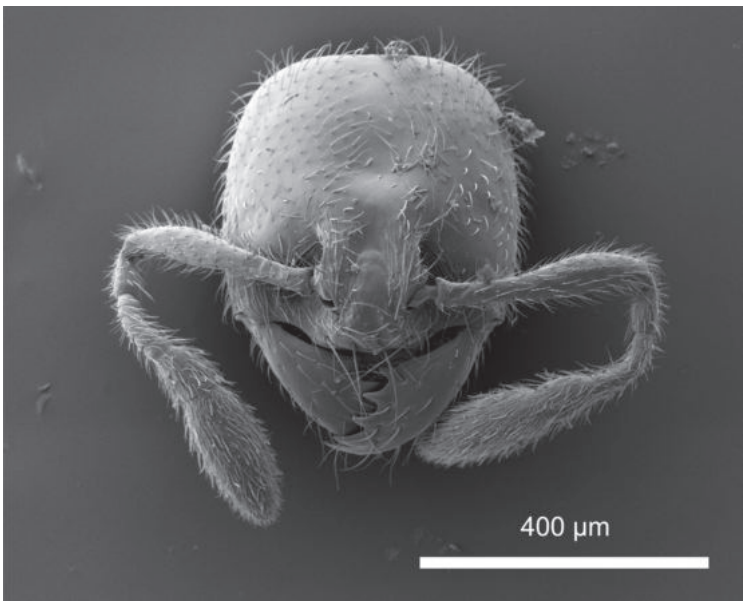


**Figure 30.** *Carebara laeviceps* Liu & Zhong, sp. nov. Minor worker under SEM. head in full-face view.

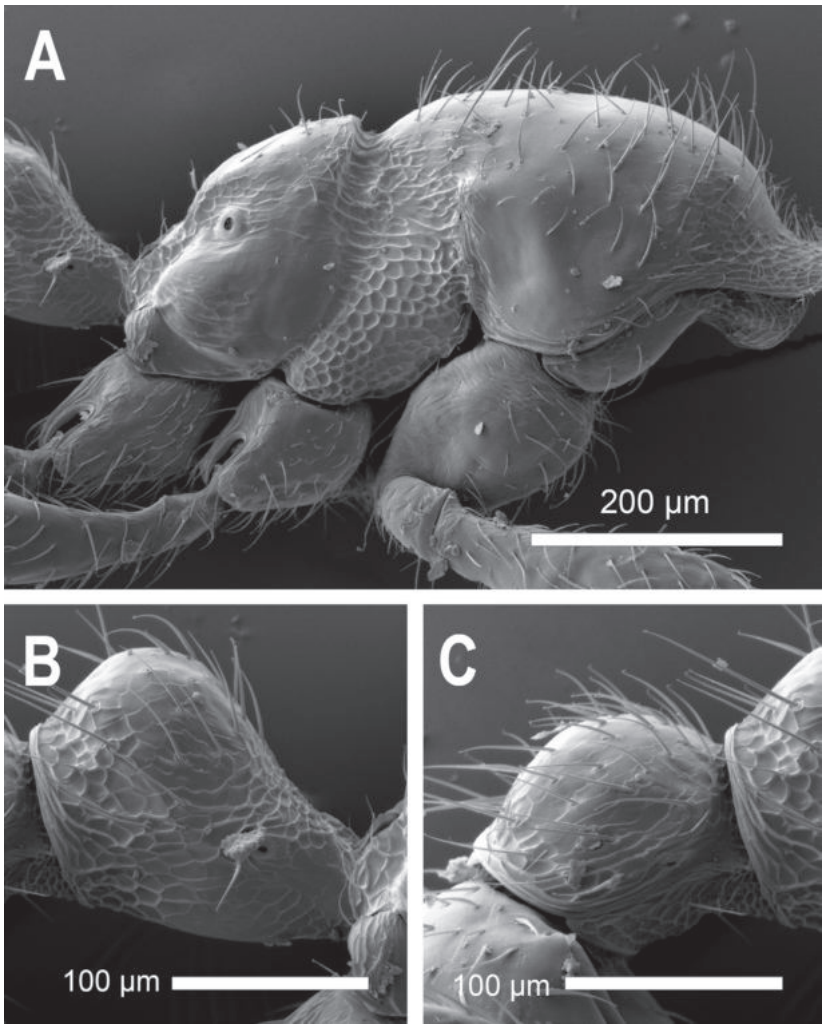


**Figure 31.** *Carebara laeviceps* Liu & Zhong, sp. nov. Minor worker under SEM **A** body in lateral view **B** petiole in lateral view **C** postpetiole in lateral view.

distinguished from these three species by combination of the following features: antenna 10-segemented (9-segmented in *C. lusciosa*, *C. bouvardi*, and *C. rectangulata*); posterior margin of head without a transverse carina in major worker (with a transverse carina in *C. rectangulata*); lateral profile of head in major worker parallel in full face view (subparallel in *C. lusciosa*); katepisternum finely rugose-reticulate in major worker (smooth in *C. lusciosa*, punctured in *C. rectangulata*); ventral face of petiole moderately convex (straight in *C. bouvardi* and *C. rectangulata*); distinctly larger with TL ~ 2.8 mm (*C. lusciosa*: 2 mm, *C. rectangulata*: 2.41 mm, *C. bouvardi*: ~ 2.4 mm).

## Taxonomic checklist of *Carebara* species in China

A checklist of all known *Carebara* species in China is presented here based on Xu (2003), Zhou et al. (2006), and Terayama et al. (2012). The changes in taxonomic status, diagnostic features and distribution data of each species are provided. The checklist is arranged alphabetically.

### *C. acutispina* (Xu, 2003)

*Oligomyrmex acutispinus* Xu, 2003: 315, figs 16–19 (s.w.) China (Yunnan). Indomalaya.
*Carebara acutispina* (Xu, 2003). Combination in *Carebara*: Guénard and Dunn 2012: 41.

**Geographic distribution.** China (type locality. Sichuan, Yunnan).

**References.** Xu (2003); Fontanilla et al. (2019); He et al. (2020); Liu et al. (2020); Hosoishi et al. (2022).

### *C. affinis* (Jerdon, 1851)

*Oecodoma affinis* Jerdon, 1851: 110 (s.w.) India. Indomalaya.
*Pheidole affinis* (Jerdon, 1851). Combination in *Pheidole*: Smith 1858: 174.
*Pheidologeton affnis* (Jerdon, 1851). Combination in *Pheidologeton*: Roger 1863: 30.
*Carebara affinis* (Jerdon, 1851). Combination in *Carebara*: Fischer et al. 2014: 71.

**Geographic distribution.** Widespread in Australasia and Indomalaya region: Bangladesh, Borneo, China (Guangdong, Guangxi, Hainan, Hong Kong, Taiwan, Xizang, Yunnan), India (type locality), Indonesia, Laos, Malaysia, Myanmar, Nicobar Island, Philippines, Sri Lanka, Thailand, Australia, Papua New Guinea.

**References.** Zhou and Zheng (1997); Zhou (2001); Lin and Wu (2003); Zhou et al. (2006); Terayama (2009); Guénard and Dunn (2012); Liu et al. (2020).

### *C. altinodus* (Xu, 2003)

*Oligomyrmex altinodus* Xu, 2003: 312, figs 5–8 (s.w.) China (Yunnan). Indomalaya.
*Carebara altinodus* (Xu, 2003). Combination in *Carebara*: Guénard and Dunn 2012: 41.

**Geographic distribution.** China (type locality. Hainan, Jiangxi, Xizang, Yunnan).

**References.** Xu (2003); Chen et al. (2011); Guénard and Dunn (2012); Liu (2012); Song et al. (2013); Liu et al. (2016); Lu and Chen (2016); Liu et al. (2017); Fontanilla et al. (2019); He et al. (2020); Lee et al. (2020); Zhang et al. (2022).

***C. amia* (Forel, 1913)**

*Solenopsis amia* Forel, 1913: 191 (q.) China (Taiwan). Indomalaya.
*Aneleus amia* (Forel, 1913). Combination in *Aneleus*: Emery 1923: 60.
*Oligomyrmex amia* (Forel, 1913). Combination in *Oligomyrmex*: Ettershank 1966: 123.
*Carebara amia* (Forel, 1913). Combination in *Carebara*: Fernández 2004: 235.

**Geographic distribution.** China (type locality. Taiwan).

**References.** Lin and Wu (2003); Terayama (2009).

**Remarks.** This species only with queen caste described and not similar to any known species.

***C. bihornata* (Xu, 2003)**

*Oligomyrmex bihornatus* Xu, 2003: 317, figs 24–27 (s.w.) China (Yunnan). Indomalaya.
*Carebara bihornata* (Xu, 2003). Combination in *Carebara*: Guénard and Dunn 2012: 41.

**Geographic distribution.** China (type locality. Yunnan).

**References.** Xu (2003); Guénard and Dunn (2012); Liu et al. (2020).

***C. capreola* (Wheeler, 1927)**

*Oligomyrmex* (*Hendecatella*) *capreolus* Wheeler, 1927: 93, fig. 5 (s.w.m.) Vietnam. Indomalaya.
*Carebara capreola* (Wheeler, 1927). Combination in *Carebara*: Fernández 2004: 235.

**Geographic distribution.** China (Guangdong, Macao), Vietnam (type locality).

**References.** Xu (2003); Guénard and Dunn (2012).

***C. capreola laeviceps* (Wheeler, 1928)**

*Oligomyrmex* (*Hendecatella*) *capreolus* subsp. *laeviceps* Wheeler, 1928: 24 (s.) China (Macao).
*Carebara capreola laeviceps* (Wheeler, 1928). Combination in *Carebara*: Guénard and Dunn 2012: 41.

**Geographic distribution.** China (type locality. Guangdong, Macao).

**References.** Wheeler (1930); Guénard and Dunn (2012).

***C. castanea* Smith, 1858**

*Carebara castanea* Smith, 1858: 178 (q.) China (Hong Kong). Indomalaya.

**Geographic distribution.** China (type locality. Hong Kong), Laos, Thailand.
**References.** Xu (1999); Guénard and Dunn (2012).

***C. curvispina* (Xu, 2003)**

*Oligomyrmex curvispinus* Xu, 2003: 313, figs 9–12 (s.w.) China (Yunnan). Indomalaya.
*Carebara curvispina* (Xu, 2003). Combination in *Carebara*: Guénard and Dunn 2012: 41.

**Geographic distribution.** China (type locality. Yunnan).
**References.** Xu (2003); Guénard and Dunn (2012).

***C. diversa* (Jerdon, 1851)**

*Oecodoma diversa* Jerdon, 1851: 109 (s.w.) India (Kerala). Indomalaya.
*Pheidole diversa* (Jerdon, 1851). Combination in *Pheidole*: Smith 1858: 174.
*Pheidologeton diversa* (Jerdon, 1851). Combination in *Pheidologeton*: Roger 1863: 30.
*Carebara diversa* (Jerdon, 1851). Combination in *Carebara*: Fischer et al. 2014: 71.

**Geographic distribution.** Widespread species, mainly in Indomalayan region: Bangladesh, Borneo, Cambodia, China (Fujian, Guangdong, Guangxi, Hainan, Hong Kong, Macao, Taiwan, Yunnan), Guinea, India (type locality), Indonesia, Japan, Laos, Malaysia, Myanmar, Philippines, Singapore, Sri Lanka, Thailand, Vietnam.
**References.** Wu and Wang (1995); Zhou and Zheng (1997); Zhou (2001); Lin and Wu (2003); Zhou et al. (2006); Terayama (2009); Guénard and Dunn (2012).

***C. diversa draco* (Santschi, 1920)**

*Pheidologeton diversus* st. *draco* Santschi, 1920: 163 (s.w.q.) Vietnam. Indomalaya.
*Pheidologeton diversus draco* Santschi, 1920. Subspecies of *Pheidologeton diversus*: Wheeler 1929: 44.
*Carebara diversa draco* (Santschi, 1920). Combination in *Carebara*: Fischer et al. 2014: 71.

**Geographic distribution.** China (Guangdong, Hainan), Vietnam (type locality).
**References.** Wheeler (1930); Zhou et al. (2006); Guénard and Dunn (2012).

***C. diversa laotina* (Santschi, 1920)**

*Pheidologeton diversus* var. *laotina* Santschi, 1920: 162 (s.w.q.) Laos, Vietnam. Indomalaya.
*Pheidologeton diversus laotina* Santschi, 1920. Subspecies of *Pheidologeton diversus*: Wheeler 1930: 68.

*Pheidologeton laotina* (Santschi, 1920). Status as species: Ettershank 1966: 119 (error).
*Carebara diversa laotina* (Santschi, 1920). Combination in *Carebara*: Fischer et al. 2014: 71.

**Geographic distribution.** Cambodia, China (Fujian, Guangdong, Hongkong, Macao), Laos (type locality), Vietnam (type locality).

**References.** Wheeler (1930); Zhou et al. (2006); Huang and Zhou (2007); Guénard and Dunn (2012).

***C. hunanensis* (Wu & Wang, 1995)**

*Oligomyrmex hunanensis* Wu & Wang, 1995: 75, figs 90, 93 (s.w.) China (Hunan). Indomalaya.
*Carebara hunanensis* (Wu & Wang, 1995). Combination in *Carebara*: Guénard and Dunn 2012: 41.

**Geographic distribution.** China (type locality. Hong Kong, Hunan).

**References.** Xu (2003); Guénard and Dunn (2012).

***C. jiangxiensis* (Wu & Wang, 1995)**

*Oligomyrmex jiangxiensis* Wu & Wang, 1995: 75, 194, figs 91, 94 (s.w.) China (Jiangxi). Indomalaya.
*Carebara jiangxiensis* (Wu & Wang, 1995). Combination in *Carebara*: Guénard and Dunn 2012: 41.

**Geographic distribution.** China (type locality. Guangdong, Jiangxi, Sichuan, Yunnan, Zhejiang).

**References.** Xu (2003); Zhao et al. (2009); Guénard and Dunn (2012); Staab et al. (2014); Huang et al. (2019); He et al. (2020).

***C. latinoda* (Zhou & Zheng, 1997)**

*Pheidologeton latinodus* Zhou & Zheng, 1997: 165, figs 4–6 (s.w.) China (Guangxi). Indomalaya.
*Carebara latinoda* (Zhou & Zheng, 1997). Combination in *Carebara*: Fischer et al. 2014: 72.

**Geographic distribution.** China (type locality. Guangdong, Guangxi).

**References.** Zhou (2001); Zhou et al. (2006); Guénard and Dunn (2012).

***C. lignata* Westwood, 1840**

*Carebara lignata* Westwood, 1840: 86, pl. 2, fig. 6 (q.) Indonesia (Java). Indomalaya.

**Geographic distribution.** Widespread in Indomalaya region: Bangladesh, China (Yunnan), India, Indonesia (type locality), Nepal.

**References.** Xu (1999); Guénard and Dunn (2012); Song et al. (2013); Lu et al. (2017).

***C. lusciosa* (Wheeler, 1928)**

*Oligomyrmex lusciosus* Wheeler, 1928: 22 (s.w.) China (Guangdong). Indomalaya.
*Carebara lusciosa* (Wheeler, 1928). Combination in *Carebara*: Fernández 2004: 235.

**Geographic distribution.** China (type locality. Guangdong).
**References.** Xu (2003); Guénard and Dunn (2012).

***C. melasolena* (Zhou & Zheng, 1997)**

*Pheidologeton melasolenus* Zhou & Zheng, 1997: 163, figs 1–3 (s.w.) China (Guangxi). Indomalaya.
*Carebara melasolena* (Zhou & Zheng, 1997). Combination in *Carebara*: Fischer et al. 2014: 72.

**Geographic distribution.** China (type locality. Chongqing, Guangxi, Hainan, Henan, Hong Kong, Hubei, Hunan, Jiangxi, Sichuan, Yunnan, Zhejiang).
**References.** Zhou (2001); Zhang and Zheng (2002); Zhou et al. (2006); Huang and Zhou (2007); Guénard and Dunn (2012); Staab et al. (2014); Liu et al. (2015); Liu et al. (2020).
**Remarks.** The status of this species is somewhat ambiguous, In Zhou and Zheng (1997) and Zhou et al. (2006), this species can be distinguished from *C. vespillo* (Wheeler, 1921) by the following characteristics: the coarse black line present in the median longitudinal groove of the head; postpetiolar node distinctly broader than long; and hairs sparser on the head and body. However, in Chen et al. (2021), *C. vespillo* was recorded with the presence of the black line. Accordingly, some former specimens of *C. vespillo* may have been misidentified as *C. melasolena* due to the presence of the black line. In Zhou and Zheng (1997), the authors pointed the postpetiolar node of *C. melasolena* is 1.5× broader than long, in Zhou et al. (2006) the node is 2× broader than long, maybe this ratio is a also an unstable morphological trait.
Above all, the features and separation of these two species needs further examination of the type specimens, it is possible that *C. melasolena* is a synonym of *C. vespillo*, but here we still list *Carebara melasolena* as a valid species based on former studies.

***C. nanningensis* (Li & Tang, 1986)**

*Pheidologeton nanningensis* Li & Tang, 1986: 162 (s.w.) China (Guangxi). Indomalaya.
*Carebara nanningensis* (Li & Tang, 1986). Combination in *Carebara*: Fischer et al. 2014: 72.

**Geographic distribution.** China (type locality. Guangxi).
**References.** Zhou and Zheng (1997); Zhou et al. (2006); Guénard and Dunn (2012).

***C. obtusidenta* (Xu, 2003)**

*Oligomyrmex obtusidentus* Xu, 2003: 316, figs 20–23 (s.w.) China (Yunnan). Indomalaya.
*Carebara obtusidenta* (Xu, 2003). Combination in *Carebara*: Guénard and Dunn 2012: 41.

**Geographic distribution.** China (type locality. Hunan, Chongqing, Sichuan, Xizang, Yunnan), India.

**References.** Xu (2003); Huang (2005); Chen et al. (2011); Guénard and Dunn (2012); Liu (2012); Song et al. (2013); Fontanilla et al. (2019); Luo et al. (2019).

***C. oni* (Terayama, 1996)**

*Oligomyrmex oni* Terayama, 1996: 20, figs 38–43 (s.w.) Japan. Palearctic.
*Carebara oni* (Terayama, 1996). Combination in *Carebara*: Terayama 2009: 151.

**Geographic distribution.** China (Taiwan), Japan (type locality).

**References.** Lin and Wu (2003); Terayama (2009); Guénard and Dunn (2012); Terayama et al. (2012).

***C. pseudolusciosa* (Wu & Wang, 1995)**

*Oligomyrmex pseudolusciosus* Wu & Wang, 1995: 76, 195, figs 92, 95 (s.w.q.) China (Hubei, Anhui). Indomalaya.
*Carebara pseudolusciosa* (Wu & Wang, 1995). Combination in *Carebara*: Guénard and Dunn 2012: 41.

**Geographic distribution.** China (type locality. Anhui, Guangxi, Henan, Hubei).

**References.** Xu (2003); Guo (2006); Guénard and Dunn (2012); Lu (2013); Guo et al. (2015).

***C. polyphemus* (Wheeler, 1928)**

*Oligomyrmex polyphemus* Wheeler, 1928: 21 (s.) China (Guangdong). Indomalaya.
*Carebara polyphemus* (Wheeler, 1928). Combination in *Carebara*: Fernández 2004: 235.

**Geographic distribution.** China (type locality. Guangdong, Yunnan).

**References.** Xu (2003); Zhao et al. (2009); Guénard and Dunn (2012).

***C. qianliyan*** Terayama, 2009

*Carebara qianliyan* Terayama, 2009: 152, figs 230, 231 (s.w.) China (Taiwan). Indomalaya.

**Geographic distribution.** China (type locality. Taiwan).

**References.** Terayama (2009); Guénard and Dunn (2012); Terayama et al. (2012).

***C. rectidorsa*** (Xu, 2003)

*Oligomyrmex rectidorsus* Xu, 2003: 319, figs 32–35 (s.w.) China (Yunnan). Palearctic.
*Carebara rectidorsa* (Xu, 2003). Combination in *Carebara*: Guénard and Dunn 2012: 41.

**Geographic distribution.** China (type locality. Chongqing, Hainan, Henan, Hubei, Hunan, Sichuan, Xizang, Yunnan), India.

**References.** Xu (2003); Huang (2005); Guo (2006); Guénard and Dunn (2012); Guo et al. (2015); Fontanilla et al. (2019); Huang et al. (2019); Luo et al. (2019); He et al. (2020); Lee et al. (2020).

***C. reticapita*** **(Xu, 2003)**

*Oligomyrmex reticapitus* Xu, 2003: 319, figs 38–41 (s.w.) China (Yunnan). Palearctic.
*Carebara reticapita* (Xu, 2003). Combination in *Carebara*: Guénard and Dunn 2012: 41.

**Geographic distribution.** China (type locality. Guangxi, Hainan, Sichuan, Xizang, Yunnan).

**References.** Xu (2003); Chen et al. (2011); Guénard and Dunn (2012); Liu (2012); Chen et al. (2013); Song et al. (2013); Cheng et al. (2015); Liu et al. (2016); Liu et al. (2017); Fontanilla et al. (2019); He et al. (2020); Lee et al. (2020).

***C. sakamotoi*** **Terayama et al., 2012**

*Carebara sakamotoi* Terayama et al., 2012: 2, figs 4–7 (s.w.) China (Taiwan). Indomalaya.

**Geographic distribution.** China (type locality. Taiwan).

**References.** Terayama et al. (2012).

***C. sauteri*** **(Forel, 1912)**

*Oligomyrmex sauteri* Forel, 1912: 56 (s.) China (Taiwan, Zhejiang). Indomalaya.
*Carebara sauteri* (Forel, 1912). Combination in *Carebara*: Fernández 2004: 235.

**Geographic distribution.** China (type locality. Taiwan, Zhejiang), Japan.

**References.** Lin and Wu (2003); Xu (2003); Terayama (2009); Guénard and Dunn (2012); Terayama et al. (2012).

**Remarks.** In Wu and Wang's (1992) study, *C. hunanensis* was mistakenly identified as *C. sauteri* but was later corrected by Terayama (Wu and Wang 1995).

***C. striata*** **(Xu, 2003)**

*Oligomyrmex striatus* Xu, 2003: 314, figs 13–15 (s.) China (Yunnan). Palearctic.
*Carebara striata* (Xu, 2003). Combination in *Carebara*: Fernández 2010: 202.

**Geographic distribution.** China (type locality. Sichuan, Yunnan).
**References.** Xu (2003); Guénard and Dunn (2012); He et al. (2020).

***C. taiponica* (Wheeler, 1928)**

*Oligomyrmex silvestrii* subsp. *taiponicus* Wheeler, 1928: 24 (s.) China (Hong Kong). Palearctic.
*Oligomyrmex taiponicus* Wheeler, 1928. Status as species: Bolton 1995: 300.
*Carebara taiponica* (Wheeler, 1928). Combination in *Carebara*: Fernández 2004: 235.

**Geographic distribution.** China (type locality. Hong Kong, Yunnan), Laos.
**References.** Xu (2003); Guénard and Dunn (2012).

***C. trechideros* (Zhou & Zheng, 1997)**

*Pheidologeton trechideros* Zhou & Zheng, 1997: 167, figs 7–9 (s.w.) China (Guangxi). Indomalaya.
*Carebara trechideros* (Zhou & Zheng, 1997). Combination in *Carebara*: Fischer et al. 2014: 72.

**Geographic distribution.** China (type locality. Guangdong, Guangxi, Hunan, Jiangxi, Sichuan, Yunnan), Thailand, Vietnam.
**References.** Zhou (2001); Zhou et al. (2006); Huang and Zhou (2007); Li et al. (2009); Zhao et al. 2009); Chen et al. (2012); Guénard and Dunn (2012); Song et al. (2013); Zhang et al. (2014); Fontanilla et al. (2019); Huang et al. (2019); Luo et al (2019).

***C. vespillo* (Wheeler, 1921)**

*Pheidologeton vespillo* Wheeler, 1921: 533 (s.w.) China (Zhejiang). Indomalaya.
*Carebara vespillo* (Wheeler, 1921). Combination in *Carebara*: Fischer et al. 2014: 72.

**Geographic distribution.** China (type locality. Guangxi, Henan, Hong Kong, Hunan, Jiangxi, Shandong, Zhejiang), Vietnam.
**References.** Wu and Wang (1992); Bolton (1995); Wu and Wang (1995); Zhou et al. (2006); Guénard and Dunn (2012); Lu (2013); Zhang et al. (2014); Cheng et al. (2015); Guo et al. (2015).

***C. wheeleri* (Ettershank, 1966)**

*Oligomyrmex wheeleri* Ettershank, 1966: 124. Replacement name for *Oligomyrmex silvestri* Wheeler, 1928: 23 (s.w.) China (Hong Kong). Palearctic.
*Carebara wheeleri* (Ettershank, 1966). Combination in *Carebara*: Fernández 2004: 235.

**Geographic distribution.** China (type locality Hong Kong, Yunnan).
**References.** Xu (2003); Guénard and Dunn (2012); Huang et al. (2019).

***C. yamatonis* (Terayama, 1996)**

*Oligomyrmex yamatonis* Terayama, 1996: 23, figs 48–51 (s.w.) Japan. Palearctic.
*Carebara yamatonis* (Terayama, 1996). Combination in *Carebara*: Terayama 2009: 151.

**Geographic distribution.** China (Hubei, Hunan), Japan (type locality).
**References.** Lin and Wu (2003); Huang (2005); Guénard and Dunn (2012).

***C. yanoi* (Forel, 1912)**

*Pheidologeton yanoi* Forel, 1912: 57 (w.q.) China (Taiwan). Indomalaya.
*Carebara yanoi* (Forel, 1912). Combination in *Carebara*: Fischer et al. 2014: 72.

**Geographic distribution.** China (type locality. Taiwan).
**References.** Wheeler (1929); Lin and Wu (2003); Zhou et al. (2006); Terayama (2009).

***C. zengchengensis* (Zhou et al., 2006)**

*Pheidologeton zengchengensis* Zhou et al., 2006: 871, figs 1, 2 (s.w.) China (Guangdong). Indomalaya.
*Carebara zengchengensis* (Zhou et al., 2006). Combination in *Carebara*: Fischer et al. 2014: 72.

**Geographic distribution.** China (type locality. Fujian, Guangdong, Macao).
**References.** Zhou et al. (2006); Zhang and Hou (2009); Zhao et al. (2009).

## Discussion

In this study, a new *Carebara* species, *C. laeviceps* sp. nov. is described and the key and checklist of Chinese *Carebara* species are updated. Chinese *Carebara* species are predominantly small and subterranean, making the collection and identification quite challenging. Previous studies (Liu et al. 2020; Chen et al. 2021) have recorded several undescribed species. As future research advances, certain widely distributed Chinese species, such as *C. melasolena*, may reveal an extensive presence within the Indomalayan region. Furthermore, the Indo-China Peninsula may share certain widespread species with Yunnan and Guangxi provinces, such as *C. castanea* (Fig. 32).

The definition of the *Carebara* species groups is a complex question that requires large-scale research. Current studies, however, are mostly limited to a regional level. Unraveling the phylogenetic relationships among species groups in different faunas also demands a substantial amount of molecular data. Therefore, there is a need for a more comprehensive survey and taxonomic revision of *Carebara* species of the Old World.

A provisional definition of Chinese *Carebara* species groups is provided in this research, and some features of the *concinna-lignata* group have been updated. It is possible that these species might be divided into several distinct groups in future studies; for example, *C. altinodus*, *C. hunanensis*, *C. oni*, and

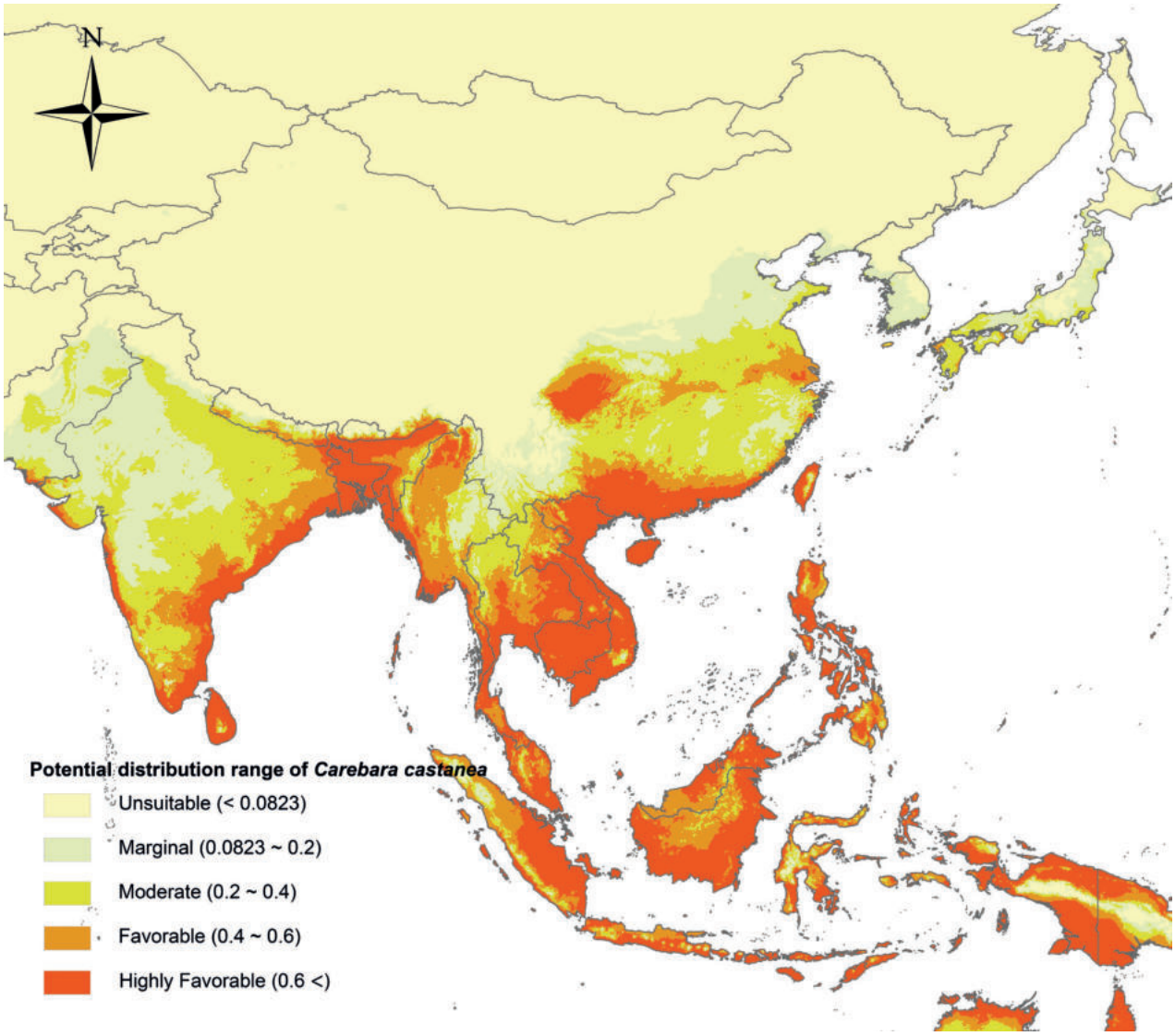


**Figure 32.** Potential distribution range of *C. castanea* under the current climate.

*C. qianliyan* could potentially form a single group due to various shared features, such as a massive mesosoma, the head capsule relatively short (CI > 90), large size (TL > 3.4 mm), and ocelli mostly present. Similarly, there may also be the *acutispina* species group but due to the lack of molecular data and to avoid making polyphyletic groups, we have maintained the classification proposed by Bharti and Kumar (2013).

## Acknowledgments

We would like to thank Prof. Zheng-hui Xu and Shan-yi Zhou for their guidance and help during this study. We express our gratitude to Gui-chuan Nie for his contribution to the specimens.

## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

## Ethical statement

No ethical statement was reported.

## Funding

This work is supported by the Biodiversity Survey and Assessment Project of the Ministry of Ecology and Environment, China (No. 2019HJ2096001006), Expert Workstation in Zhaotong, Yunnan (Nos. 2019ZTYX03, 2021ZTYX05), the Young Elite Scientist Sponsorship Program by CAST(No. YESS20200106) and the 2115 Talent Development Program of the China Agricultural University.

## Author contributions

Zhi-yu Liu: Illustration drawing, SEM photo shooting, drafting of the original manuscript; Ying Zhong: Illustration drawing, photography of holotype and paratype, writing and reviewing; Yu-yuan Huang: Supervision and reviewing; Hao Ran: Writing and reviewing; Fan Song: Supervision and reviewing.

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## Data availability

All of the data that support the findings of this study are available in the main text.

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ZooKeys 1190: 39–73 (2024)
DOI: 10.3897/zookeys.1190.109539

Research Article

# Revision of the macropterous subgenus *Curtonotus* from east China, with the description of a new species (Carabidae, Zabrini, *Amara*)

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## Abstract

Species from east China belonging to the subgenus *Curtonotus* were studied, resulting in the description of a new species, *Amara* (*Curtonotus*) *beijingensis* **sp. nov.** The type locality is Xiaolongmen Forest Park in Beijing. All the known macropterous *Curtonotus* species from eastern China are reviewed and for each species taxonomical notes, illustrations, and new provincial records are noted. An improved key for their identification is provided as well.

**Key words:** Coleoptera, key, secondary sexual characteristics

OPEN ACCESS

Academic editor: Borislav Guéorguiev
Received: 14 July 2023
Accepted: 20 December 2023
Published: 22 January 2024

ZooBank: https://zoobank.org/9CE8F6C6-28B7-4CDA-8140-BA78B59FF68F

Citation: Li Y, Li H, Shi H (2024) Revision of the macropterous subgenus *Curtonotus* from east China, with the description of a new species (Carabidae, Zabrini, *Amara*). ZooKeys 1190: 39–73. https://doi.org/10.3897/zookeys.1190.109539

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## Introduction

Ground beetles belonging to *Amara* Bonelli, 1810 subgenus *Curtonotus* Stephens, 1827, are commonly observed in various provinces of China. They are easily distinguished from representatives of the other subgenera of the genus by their relatively large size and constricted pronotum base. The subgenus has a Holarctic distribution and is especially diverse in China, with a total of 94 valid species known so far (Anichtchenko 2023). Among these, 15 species are found in the Nearctic Realm (seven of them also present in the Palearctic Realm), while 79 species are confined to the Palearctic Realm (Hieke 2017). In total, 41 *Curtonotus* species are recorded from China, with many of them being widely distributed in the northern regions and others restricted to the southwestern mountainous areas.

A recent taxonomic revision of Chinese subgenus *Curtonotus* published by Hieke (2010) defined two main groups and described eight new species. Since then, no further new *Curtonotus* species have been reported from China. Recently, during the examination of a large number of carabid specimens collected from the Xiaolongmen Forest Park in Beijing, an undescribed species of *Curtonotus* was found. This species is most similar to *A.* (*C.*) *macronota* Solsky, 1875, but differs significantly in the shape of the apical lamella of male genitalia. While examining this material, several interesting records of *Curtonotus* from China were also discovered.

In this paper, we present the description of a new species of the subgenus *Curtonotus* found in Beijing. Additionally, we provide detailed notes on all the "macropterous *Curtonotus*" species documented in eastern China. The new species is thoroughly characterized through extensive descriptions and illustrations. For other macropterous *Curtonotus* species recorded in eastern China, we offer identification keys and illustrations, except in cases where materials were unavailable. The primary purpose of the current work is the identification of all macropterous *Curtonotus* species known in eastern China, encompassing all provinces except Xinjiang and Xizang.

It is worth noting that the males of most species from subgenus *Curtonotus* exhibit modified mesotibiae. In light of this, we represent a brief discussion for male mesotibiae projections. This sexually dimorphic trait not only holds taxonomical significance but also potentially plays a role in copulation and might be linked to sexual conflicts between the sexes.

## Material and methods

This work was based primarily on the examination of specimens from China. Institutional and private collections cited in the present paper are indicated by the following abbreviations:

**IZAS** Institute of Zoology, Chinese Academy of Sciences, Beijing, China;
**CBJFU** Forest Entomology Laboratory, Beijing Forestry University, Beijing, China;
**CLHY** collection of Haoyuan Li, Beijing, China;
**CLYH** collection of Yihang Li, Beijing, China;
**CCJH** collection of Jiaheng Chen, Puning, Guangdong, China;
**CUMZ** University Museum of Zoology, Cambridge, United Kingdom;
**CMNC** Canadian Museum of Nature, Ottawa, Canada;
**ITLJ** National Institute of Agro-environmental Sciences, Tsukuba, Ibaraki, Japan;
**MNHN** Muséum National d'Histoire Naturelle, Paris, France;
**MNHU** Museum für Naturkunde der Humboldt-Universität zu Berlin, Berlin, Germany;
**MSNG** Museo Civico di Storia Naturale (Giacomo Doria), Genova, Italy;
**NHML** The Natural History Museum, London, United Kingdom;
**NHRS** Naturhistoriska Rihsmuseet, Stockholm, Sweden;
**NMPC** Narodni Muzeum Prirodovedecke Muzeum, Prague, Czech Republic;
**TMB** Természettudományi Múzeum Allattara, Budapest, Hungary;
**ZRAS** Zoological Institute, Russian Academy of Science, St. Petersburg, Russia;
**ZMUC** Zoological Museum, University of Copenhagen, Copenhagen, Denmark;
**ZMUM** Moscow State University, Moscow, Russia.

Male genitalia were dissected using fine forceps and glued on mounting cards. For each species, illustrations of left-lateral and dorsal views of the median lobe of aedeagus and dorsal view of right paramere are provided for one specimen of each species. The gonocoxites of ovipositors were pulled out using fine forceps but not removed from the apex of the abdomen.

Most morphological terms in the present paper follow their general applications. When referring to the orientation of the median lobe of male genitalia,

"left" or "right" was determined in dorsal view with the apex of the median lobe pointing anteriorly and the base ventrally.

Measurements and abbreviations are as follows: length of body (**BL**), the linear distance from the apex of labrum to elytral sutural apex; head maximum width (**HW)** across the outer margin of eyes; pronotum maximum width (**PW**); pronotum length (**PL**), measured along median line; pronotum anterior width measured along tips of anterior angles (**PAW**); pronotum basal width measured along tips of posterior angles (**PBW**); elytra length (**EL**), the linear distance from apex of scutellum to elytra sutural apex; elytra width (**EW**) maximum width of elytra.

For each taxon, original and important taxonomic references are cited. Genus combination, information on the name-bearing types, newly recorded localities, and other important comments are listed in parentheses after each reference. Newly recorded localities are labeled with an asterisk. Full-body photographs of all species were captured by a Nikon D7200 camera with LAOWA 60 mm F2.8 2:1 Super Macro lens; male genitalia, pronotum, mesotibial projections, and female ovipositors were captured by the same camera with a LAOWA 25 mm F2.8 2.5–5X Ultra Macro lens. For each final image, several photographs were taken at different focal planes, combined with ZereneStacker software to obtain one synthesized photograph, and finally edited by Photoshop Elements 2022 Editor 20.0.

## Taxonomic account

### Subgenus *Curtonotus* Stephens, 1827

**Type species.** *Amara convexiuscula* (Marsham, 1802); type locality: "England".

The subgenus *Curtonotus* is recognizable among genus *Amara* by the combination of the following characteristics: 1) medium to large sized species (7–25 mm); 2) prosternal process not bordered, without setae at apex; 3) inner margin of male mesotibiae with projection in most species (present as one to three distinct denticles); 4) right paramere without a terminal hook; 5) pronotum more or less cordate, constricted to base, distinctly narrower than elytral base; 6) mesofemora mostly with only two posterior setae.

The subgenus *Curtonotus* was regarded as the most basal clade of the tribe Zabrini (Sánchez-Gea et al. 2004), but the intra-group relationships among the species of *Curtonotus* are still unclear. *Curtonotus* is similar to the subgenera *Bradytulus* Tschitscherine and *Tibetamara* Makarov & Sundukov in external appearances (Hieke 2010; Makarov and Sundukov 2021). The subgenus *Bradytulus* is different from *Curtonotus* by the generally much smaller body size, prosternal process with reduced border, and male metatibiae mostly with tufted ensiform setae. *Tibetamara* is different from *Curtonotus* by the male mesotibiae without a projection, right paramere shortened, and a relatively wide pronotum base. Some large species of the subgenus *Bradytus* may also be similar to *Curtonotus* but can be distinguished by prosternal process bordered, grooves of pronotum basal fovea shorter and usually shallower, and the right paramere usually with an apical hook. The subgenera *Amathitis* Zimmermann, *Ammoleirus* Tschitscherine, *Cribramara* Kryzhanovskij, *Harpalodema* Reitter, and *Hyalamara* Tschitscherine also have a constricted pronotum base, but can be readily distinguished from *Curtonotus* by having multiple setae on the mesofemora.

*Curtonotus* species can be found in various open habitats, including grassland, alpine meadow, coastal areas, riparian flood plains, riverbanks, and forest margins. Throughout our observations, we noted that many inhabit similar environments together with other *Amara* and *Harpalus* Latreille species. Both adults and larvae of *Curtonotus* demonstrate omnivorous feeding habits, consuming other insect larvae as well as plant seeds (Sasakawa 2010). Among them, species like *A.* (*C.*) *gigantea* Motschulsky, 1844 are aggressive predators. Macropterous species can be attracted by light sources, and some species climb on stalks or leaves for foraging. Certain widely distributed species, like *A.* (*C.*) *gigantea* and *A.* (*C.*) *macronota*, are commonly found in urban areas and agrarian lands, suggesting potential roles in pest and weed control (Sasakawa 2009). Moreover, other species such as *A.* (*C.*) *brevicollis* Chaudoir, 1850, *A.* (*C.*) *dux* Tschitscherine, 1894, and *A.* (*C.*) *fodinae* Mannerheim, 1825 have also been recorded in proximity to residential areas.

According to Hieke's (2010) work, the Chinese *Curtonotus* species were classified into two groups, mainly based on the shape of metepisternum. Group A includes the macropterous *Curtonotus*, with hind wings usually well developed (rarely shortened), metepisternum long, with length on outer margin nearly twice as long as the anterior width. Most species of this group have widespread distributional ranges, more commonly seen in the northern and eastern provinces of China. The Group B contains brachypterous *Curtonotus*, with hind wings usually rudimentary (rarely shortened); metepisternum short, with length on outer margin subequal to the anterior width. Most species of this group are narrowly distributed, only known from the high mountain areas of west China.

In the present paper, we focus on the macropterous *Curtonotus* from the eastern provinces of China with describing a new species and providing supplementary notes on all other species so far recorded in this area. Based on available materials, four species groups of the macropterous *Curtonotus* were recognized in the eastern China: *gigantea* group, *tumida* group, *brevicollis* group, and *macronota* group, mainly based on the characteristics of head size, number of supraorbital setae, shape of pronotum, male mesotibiae projection, and the apex of male genitalia. Other *Curtonotus* species not recorded from this area may have relationships with some of the above species groups, but they have not been treated in the present paper.

## Key to the macropterous species of the subgenus *Curtonotus* from central and eastern China

1 Larger species, body length > 16 mm; male mesotibiae projection composed of a very large triangular proximal denticle and two small distal denticles.....***A.* (*C.*) *gigantea* (*gigantea* group)**

– Smaller species, body length < 14 mm; male mesotibiae projection not as above, if with triangular proximal denticle, only one distal denticle present.....**2**

2 Dorsum reddish brown, elytra usually with faint bronze luster; pronotum with fine and dense punctures except at middle..... **3 (*brevicollis* group)**

– Dorsum black or dark brown; pronotum mid-anterior area without or with large and sparse punctures, mostly concentrated at middle; pronotum lateral sides impunctate or with punctures confined in lateral grooves .....**4**

3 Pronotum mid-lateral setae present; posterior angles acute and distinctly laterally protruded (Fig. 5B); male mesotibiae gradually swollen near middle, without definite proximal denticle; apical lamella of aedeagus longer, rounded-truncated at apex (Fig. 5F)..... ***A.* (*C.*) *dux***

– Pronotum mid-lateral setae absent; posterior angles usually obtuse or nearly rectangular, much less protruded than previous species (Fig. 6B); male mesotibiae with distinct proximal denticle near middle; apical lamella of aedeagus shorter, rounded-triangular at apex (Fig. 6F) ..... ..... ***A.* (*C.*) *brevicollis***

4 Head with one supraorbital seta (posterior one absent); pronotum mid-lateral setae always absent ..... **5 (*tumida* group)**

– Head with two supraorbital setae; pronotum mid-lateral setae present ..... ..... **9 (*macronota* group)**

5 Pronotum lateral margins almost straight near posterior angles; lateral sides of abdomen sternites without punctures or wrinkles ..... ..... ***A.* (*C.*) *hyperborea***

– Pronotum lateral margins distinctly sinuate near posterior angles; lateral sides of abdomen sternites punctate or wrinkled..... **6**

6 Pronotum lateral grooves a little deeper, distinctly punctate (Fig. 3B)..... ..... ***A.* (*C.*) *gansuensis***

– Pronotum lateral grooves shallower, at most very sparsely punctate..... **7**

7 Pronotum lateral margins moderately sinuate before posterior angles (Fig. 4B); apical lamella of aedeagus nearly straight (Fig. 4F); female gonocoxite 2 (Fig. 4G) shorter and wider, approx. 1.6 times as long as wide..... ..... ***A.* (*C.*) *goniodera***

– Pronotum lateral margin strongly sinuate before posterior angles (Habu 1953: fig. 3); apical lamella of aedeagus more or less deflected to right; female gonocoxite 2 longer, 2.0–2.3× as long as wide ..... **8**

8 Pronotum widest after middle; apical lamella of aedeagus longer ..... ..... ***A.* (*C.*) *tumida***

– Pronotum widest near middle; apical lamella shorter ...***A.* (*C.*) *shinanensis***

9 Elytra without or only with very faint microsculpture after middle; male mesotibiae with three denticles (Fig. 9C) ..... ***A.* (*C.*) *hiogoensis***

– Elytra with distinct isodiametric microsculpture; male mesotibiae at most with two denticles ..... **10**

10 Pronotum strongly constricted to base, widest point near anterior third (Fig. 8B); elytra with very strong isodiametric microsculpture; male mesotibiae projection composed of two distinct denticles, the proximal one very large and near middle of tibia (Fig. 8C)..... ***A.* (*C.*) *banghaasi***

– Pronotum less constricted to base, widest point near middle; elytra with distinct isodiametric microsculpture, but shallower than in previous species; male mesotibiae projection in a different form, if composed of two denticles, the proximal one near apical third of tibia ..... **11**

11 Pronotum lateral margins faintly sinuate before posterior angles; posterior angles slightly laterally protruded (Fig. 1B); apical lamella of aedeagus rounded, slightly declined to right side (Fig. 1F)..... ***A.* (*C.*) *beijingensis* sp. nov.**

– Pronotum lateral margins distinctly sinuate before posterior angles; posterior angles strongly laterally protruded; apical lamella of aedeagus rounded-triangular, not declined to right side (Fig. 11F)..... **12**

12 Pronotum mid-anterior region impunctate (Fig. 7B); male mesotibiae slightly bent at base, gradually swollen near middle, without well-defined denticles (Fig. 7C); apex of gonocoxite 2 attenuate (Fig. 7G) ..... ***A.* (*C.*) *fodinae***

– Pronotum mid-anterior region sparsely punctate; male mesotibiae with two well-defined denticles (Figs 10C, 11C); apex of gonocoxite 2 widely rounded (Figs 10G, 11G) ..... **13**

13 Pronotum more distinctly narrowed to base; outer ridge of pronotal basal fovea strongly convex; pronotum basal region and elytra striae with very coarse punctures (Fig. 11B); gonocoxite 2 of ovipositor with length subequal to greatest width (Fig. 11G) ..... ***A.* (*C.*) *macronota***

– Pronotum less narrowed to base; outer ridge of pronotal basal fovea moderately convex; pronotum basal region and elytra striae with finer punctures (Fig. 10B); gonocoxite 2 of ovipositor much longer than greatest width (Fig. 10G) ..... ***A.* (*C.*) *harpaloides***

## New species descriptions

***Amara* (*Curtonotus*) *beijingensis* sp. nov.**
https://zoobank.org/AAEEA378-8EC4-4967-B5F3-1A71916DBCB9
Fig. 1

**Type material.** ***Holotype*:** male (IZAS), Beijing, Mentougou District, Xiaolongmen Forest Park, 1100 m, 2018.08.16–21 [in Chinese]. ***Paratypes***, a total of 13 males and 29 females (6 males, 15 females in IZAS, 7 males, 14 females in CBJFU), all with the same localities as holotype, but in different collecting date: 2 females, 2014.08.17–22; 5 males, 7 females, 2015.08.16–21; 2 males, 11 females, 2016.08.17–22; 1 male, 1 female, 2017.08.16–21; 1 male, 1 female, 2018.08.17–22; 1 female, 2019.08.16–21; 5 females, 2021.08.16–21; 4 males, 1 female, 2022.08.17–23.

**Chinese common name.** 北京暗步甲.

**Diagnosis.** A species from the *macronota* species group, with black dorsum, relatively wide head, and with two supraorbital setae; pronotum widest a little before middle; lateral margins evenly curved, narrowed and slightly sinuate before posterior angles; posterior angles evidently protruded laterally; mid-anterior area densely punctate; elytra striae distinctly punctate except near to apex; male mesotibial projection distinct, composed of two denticles: the proximal denticle is larger, its basal margin extended to form a wide triangular projection; the distal denticle is much smaller (~ 1/3 length of the proximal one), near midpoint between the proximal denticle and tibial apex; apical lamella of male genitalia large, slightly declined to right, apex rounded.

**Comparison.** Among the Chinese species of subgenus *Curtonotus*, *A.* (*C.*) *beijingensis* sp. nov. is most similar to *A.* (*C.*) *macronota* for the following characteristics: two supraorbital setae present, pronotum basal area and elytral striae both distinctly punctate; pronotum anterior angles slightly protruded; male mesotibiae with two distinct denticles on inner margin; gonocoxite 2 of ovipositor very short. However, the new species is different from *A.* (*C.*) *macronota* in the following characteristics: elytral striae, pronotum basal and mid-anterior area with fine and dense punctures; pronotum lateral margins very

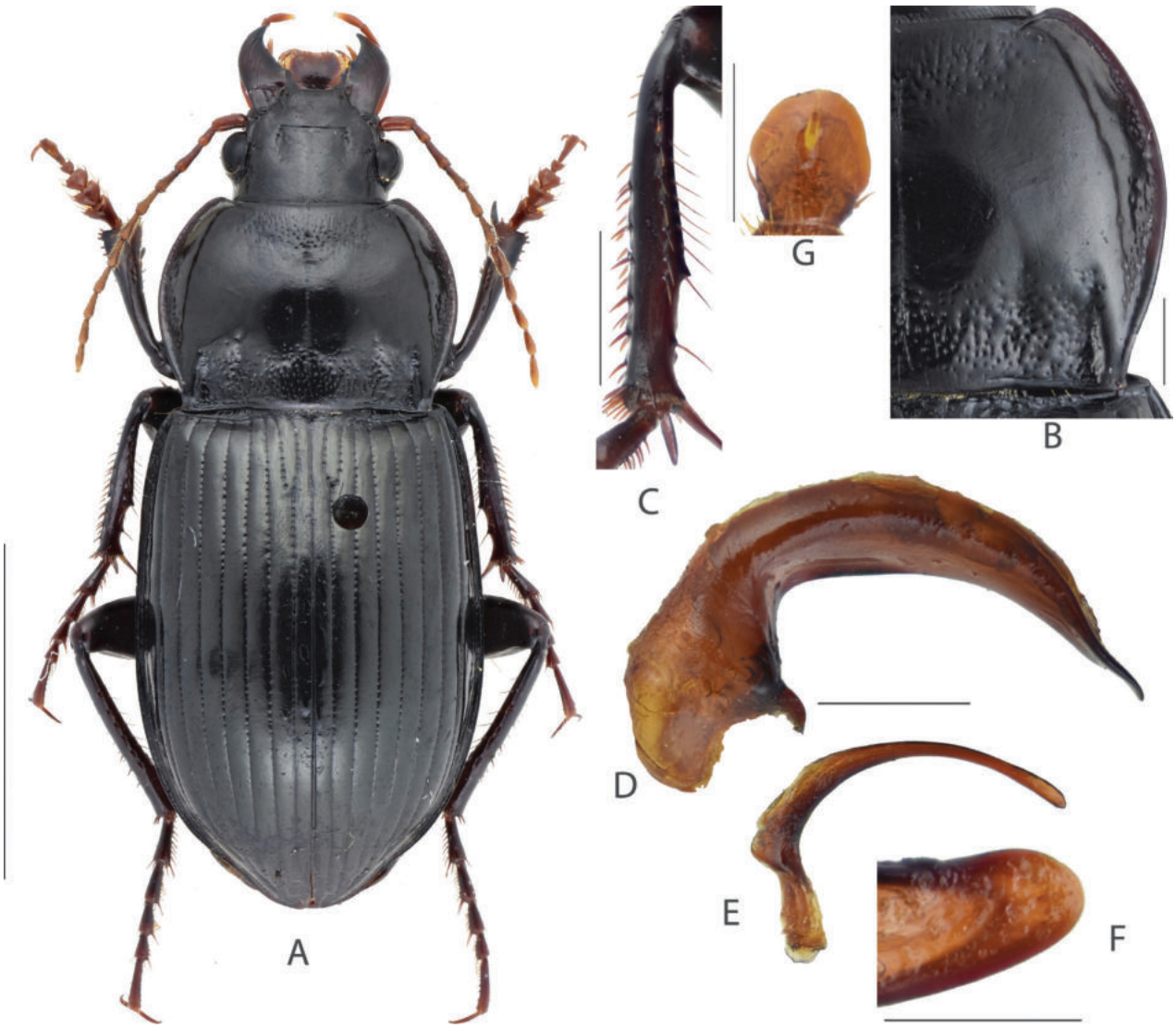


**Figure 1.** *Amara* (*Curtonotus*) *beijingensis* sp. nov. **A** dorsal habitus, male (holotype) **B** pronotum posterior angle **C** male mesotibia **D** lateral view of aedeagus **E** right paramere **F** dorsal view of apical lamella of aedeagus **G** gonocoxite 2 of ovipositor. Scale bars: 5 mm (**A**); 1 mm (**B–E**); 0.5 mm (**F, G**).

slightly sinuate before the posterior angles which are less laterally protruded; elytra basal border curved upward, and apical lamella of median lobe much longer and wider, and more evidently declined to the right side, in dorsal view, apex widely rounded but not attenuate. In contrast, *A.* (*C.*) *macronota* has much coarser and denser punctures on elytral striae, pronotum basal and mid-anterior area; pronotum lateral margins evidently sinuate before the posterior angles which are clearly protruded laterally; elytra basal border nearly straight; and male genitalia with the apical lamella rounded-triangular, evidently attenuate to apex, not declined to the right side.

The new species *A.* (*C.*) *beijingensis* resembles other two species from China, *A.* (*C.*) *hiogoensis* Bates, 1873 and *A.* (*C.*) *harpaloides* Dejean, 1828. Compared with the new species, *A.* (*C.*) *hiogoensis* can be distinguished in the elytra with very indistinct microsculpture; pronotum without punctures on the mid-anterior area; and gonocoxite 2 of ovipositor a little longer. *A.* (*C.*) *harpaloides* can be distinguished from the new species in the finer punctures on pronotum and

elytral striae; apical lamella of male genitalia different in shape; gonocoxite 2 of ovipositor much longer; and female with stronger elytra microsculpture.

There are six other species (*A.* (*C.*) *brevicollis*, *A.* (*C.*) *dux*, *A.* (*C.*) *gansuensis* Jedlicka, 1957, *A.* (*C.*) *banghaasi* Baliani, 1933, *A.* (*C.*) *fodinae*, and *A.* (*C.*) *harpaloides*) of *Curtonotus* in the mountain region situated west of Beijing, which may live in sympatry with *A.* (*C.*) *beijingensis* sp. nov. Compared with the new species, *A.* (*C.*) *brevicollis and A.* (*C.*) *dux* are different in the reddish brown dorsal surface and very fine pronotal punctures; *A.* (*C.*) *gansuensis* is different in the head with only one supraorbital seta; *A.* (*C.*) *banghaasi* is different in the pronotum widest point far before middle and anterior angles not protruded; *A.* (*C.*) *fodinae* is different in narrower pronotum and pronotum lateral margin curved longer before posterior angle.

We also compared the new species with all *Curtonotus* species recorded from nearby countries including Russia, Japan, Mongolia, North Korea and South Korea. *A.* (*C.*) *beijingensis* sp. nov. can be distinguished from most of these species by the combination of following characteristics: head with two supraorbital setae; antennomere 1 in similar color as rest segments; pronotum widest near middle, posterior angles acute and laterally protruded; pronotum disc with distinct punctures confined in the mid-anterior area. Among these species, *A.* (*C.*) *beijingensis* sp. nov. is externally very similar to *A.* (*C.*) *gebleri* Dejean, 1831 Dejean which was recorded from Mongolia and the Russian Far East. *A.* (*C.*) *gebleri* is different from the new species in the head a little more thickened; apical lamella of male genitalia rounded-triangular, gradually attenuate to apex, with length lesser than greatest width; gonocoxite 2 of ovipositor much more elongate. *A.* (*C.*) *beijingensis* sp. nov. is also similar to *A.* (*C.*) *propinqua* Ménétriés, 1832 which was recorded from Mongolia and Middle Asia. *A.* (*C.*) *propinqua* is different from the new species in the pronotum widest point far before middle; pronotum mid-anterior area only with very scarce punctures; right margin of the median lobe of male genitalia strongly swollen at middle.

**Description.** (Habitus in Fig. 1A) Size relatively large in the genus, BL = 13.0–14.6 mm, body form rather robust; dorsum black, elytra black with shinny surface; antennae, mouthparts and tarsomeres dark reddish brown; venter black. ***Head*** relatively thick, distinctly shorter than pronotum (HW/PW = 0.5–0.6); frons sparsely wrinkled, frontal fovea short, reaching middle level of eyes; with two supraorbital setae; eyes large, hemispheric; antennae nearly reaching pronotum base. ***Pronotum*** (Fig. 1B) nearly circular, widest a little before middle; pronotum wider than long (PW/PL = 1.44–1.50); basal margin a little longer than anterior margin (PAW/PBW = 0.74–0.78); anterior angles slightly protruded, rounded at apex; lateral margins evenly curved, slightly constricted before posterior angles, mid-lateral setae before middle; posterior angles small, acuminate, distinctly protruded, forming evident denticle. Basal surface densely punctate, punctures reaching region between basal fovea; inner groove short and faint, a little distant from basal margin; outer groove well incised, with distinct outer margin, reaching basal margin, approximately 1/4 length of pronotum. Disc convex, smooth at middle, slightly transversely wrinkled; mid-anterior region densely punctate; lateral grooves slightly widened, distinctly punctate; median line fine but distinct. ***Elytra*** oblong, EL/EW = 1.40–1.50, widest point near middle, lateral margins subparallel before middle; basal border weakly bent forward, extending toward humeral angles; basal border and

lateral margin forming an obtuse angle, with small and narrow denticle, not protruded. Basal setigerous pores absent; parascutellar striae deep as other striae, its apex joint to stria 1; all striae well incised, distinctly punctate except near to apex; punctures dense and coarse near base, gradually reducing in size to elytral apex; third interval without discal setigerous pore; ninth interval with umbilical series regularly composed of 13 pores. Elytra with isodiametric microsculpture in both sexes, quite evident on disc, very shallow near apex. Hind wings fully developed. ***Ventral side.*** Proepisternum, mesepisternum, and metepisternum heavily punctate, metepisternum lateral side twice length as basal width; abdominal sternites III–IV punctate and wrinkled except in middle, coarse area gradually narrowed from proximal sternites to distal ones; sternite VII with two pairs of marginal setae in females, with one pair in males. ***Legs.*** Male mesotibiae projection composed of two denticles on inner margin (Fig. 1C): proximal denticle larger, near middle of tibiae, with acute apex; distal one much smaller, on the middle point of the proximal denticle and distal end of tibiae. All tarsomeres with setae underside. ***Male genitalia.*** (Fig. 1D). Median lobe of aedeagus bent greater than 90 degrees; apex in lateral view gradually attenuate, a little deflected and then bent downward; dorsal margin gradually narrowed toward apex; ventral margin near straight at middle; in dorsal view, apical lamella (Fig. 1F) with maximum width subequal to length, apex rounded, slightly declined to right; apical orifice small, nearly middle placed; right paramere (Fig. 1E) long, apical half slender and gradually curved, a little thickened to apex, apex rounded without hook. ***Female genitalia.*** (Fig. 1G) Gonocoxite 2 of ovipositor very short, length a little greater than greatest width, inner and outer margin each with one ensiform seta before widest point, apex widely rounded.

**Distribution.** Only known from the type locality in western Beijing, China. Considering it has fully-developed hind wings, this species may also be found in nearby provinces.

**Etymology.** The scientific name of the new species comes from its type locality, Beijing.

**Remarks.** All types of this new species were collected by the undergraduate students of Beijing Forestry University attending Forestry Cognition Field Practice in the same locality (Xiaolongmen Forest Park, Mentougou, Beijing) in late August repeatedly during the past nine years. Many of these specimens were collected by pitfall traps under (or beside) different types of forest and others were randomly hand-collected along trails. For each year, more than 100 students attend this field practical. Thus, very few of these specimens have the collector's name recorded. Moreover, due to the difficulty to identify *Curtonotus* species in the field and mixture of specimen from various habitats, the specific habitat of the new species remains unknown for now. It is inferred that *A.* (*C.*) *beijingensis* sp. nov. inhabits in forest edges, like many other *Curtonotus* species found in the same area such as *A.* (*C.*) *harpaloides* and *A.* (*C.*) *gansuensis*. Although the new species is most similar to *A.* (*C.*) *macronota*, we hypothesize that these two species prefer different habitats and altitudes in the area around Beijing. *Amara* (*C.*) *beijingensis* sp. nov. was only collected in temperate broadleaf forests above 1000 m elevation, while *A.* (*C.*) *macronota* was found in various open habitats in plain areas of Beijing. Besides, *A.* (*C.*) *beijingensis* sp. nov. possibly shares the same habitat with *A.* (*C.*) *harpaloides*, as both species were collected by light trap around Xiaolongmen Forest Park.

## Supplementary notes on recorded species from eastern and central China

### *Amara* (*Curtonotus*) *gigantea* species group

This species group contains only one species which is special among all Chinese *Curtonotus* for its largest body size, strongly thickened head, well-developed projection on male mesotibiae, and large apical lamella of the male genitalia.

### *Amara* (*Curtonotus*) *gigantea* (Motschulsky, 1844)

Fig. 2

*Leirus gigantea* Motschulsky, 1844: 173. (type locality: "O.-Siberia" [= east Siberia]; syntypes in ZMUM); Bates 1873: 290; Lewis 1879: 188; Andrewes 1928: 22; Matsumura 1929: 194; Kryzhanovskij 1975: 94; Sasakawa 2010: 358.

*Amara herculeana* Tschitschérine, 1894: 381 (type locality: "Chingan mer" [in northern China]; syntypes in ZRAS); synonymized by Kryzhanovskij 1975: 94.

**Specimens examined.** 1 male (CLYH), China, Beijing, Changping district, Hedi Road, 40.138031°N, 116.313659°E, 40 m, 2022.06.14, Yihang Li leg.; 1 male (CLYH), China, Beijing, Yuhuangmiao Village, 40.515125°N, 115.895570°E, 556 m, 2022.08.06, Yihang Li leg.; 1 male (CLHY), China, Hebei, Saihanba Forest Park, 1650 m, 2021.06, Sikai Du leg. ; 1 male (CLYH), China, Henan, Baotianman Eco-tourist Area, 2020.07, Haoyi Liu leg.; 4 males, 1 female, China, Inner Mongolia, Tongliao, Horqin Left Rear banner, Jinbaotun Town, 43.372914°N, 123.545808°E, 2022.06, Hongliang Li, leg.; 15 males, 15 females (CLHY), China, Jiangsu, Yangzhou, Jiangdu District, Heping Road, 32.377533°N, 119.574815°E, 119 m, 2023. 04, Wang leg.; 2 males, 1 female, (CBJFU), China, Jilin, Qianjin County, Jiaohe Forest Station, 43.9555°N, 127.6971°E, 397 m, 2018. 08.31–09.01, Hongliang Shi leg.; 1 male (CBJFU), China, Shaanxi, Yangxian, Huayang township, 2017.VIII.4, Weifeng Yan leg.; 1 male (CLHY), China, Zhejiang, Zhoushan, Shengsi county, Caiyuan town, 30.709870°N, 122.462928°E, 52.78 m, 2023.06.17, Haoyuan Li leg.

**Chinese common name.** 巨暗步甲.

**Diagnosis.** The largest Chinese species of the subgenus, BL = 16.0–25.0 mm; dorsum black, legs dark brown to black; head strongly thickened, only a little shorter and narrower than pronotum maximum width; head with one or two supraorbital setae. Pronotum cordate (Fig. 2B), finely and densely punctate through mid-anterior, basal and lateral regions; lateral margins strongly sinuate before posterior angles; posterior angles nearly rectangular, slightly laterally protruded. Elytra elongated; lateral sides of abdominal sternites smooth. Male mesotibiae projection composed of one large denticle and two small denticles (Fig. 2C): proximal denticle widely triangular, with a row of setae, two distal denticles present between the proximal one and tibiae distal apex. Male genitalia with the apical lamella of aedeagus strongly elongate (Fig. 2F), gently declined to right, a little attenuate to tip. Gonocoxite 2 (Fig. 2G) of ovipositor elongated, length ~ 1.5× greatest width, a little widened to apex, apex widely rounded.

**Distribution.** China (Beijing, Hebei, Gansu, Heilongjiang, Liaoning, Jilin, Inner Mongolia, Jiangsu, Sichuan, Shaanxi, Shanxi, Shanghai, Shandong, Henan*,

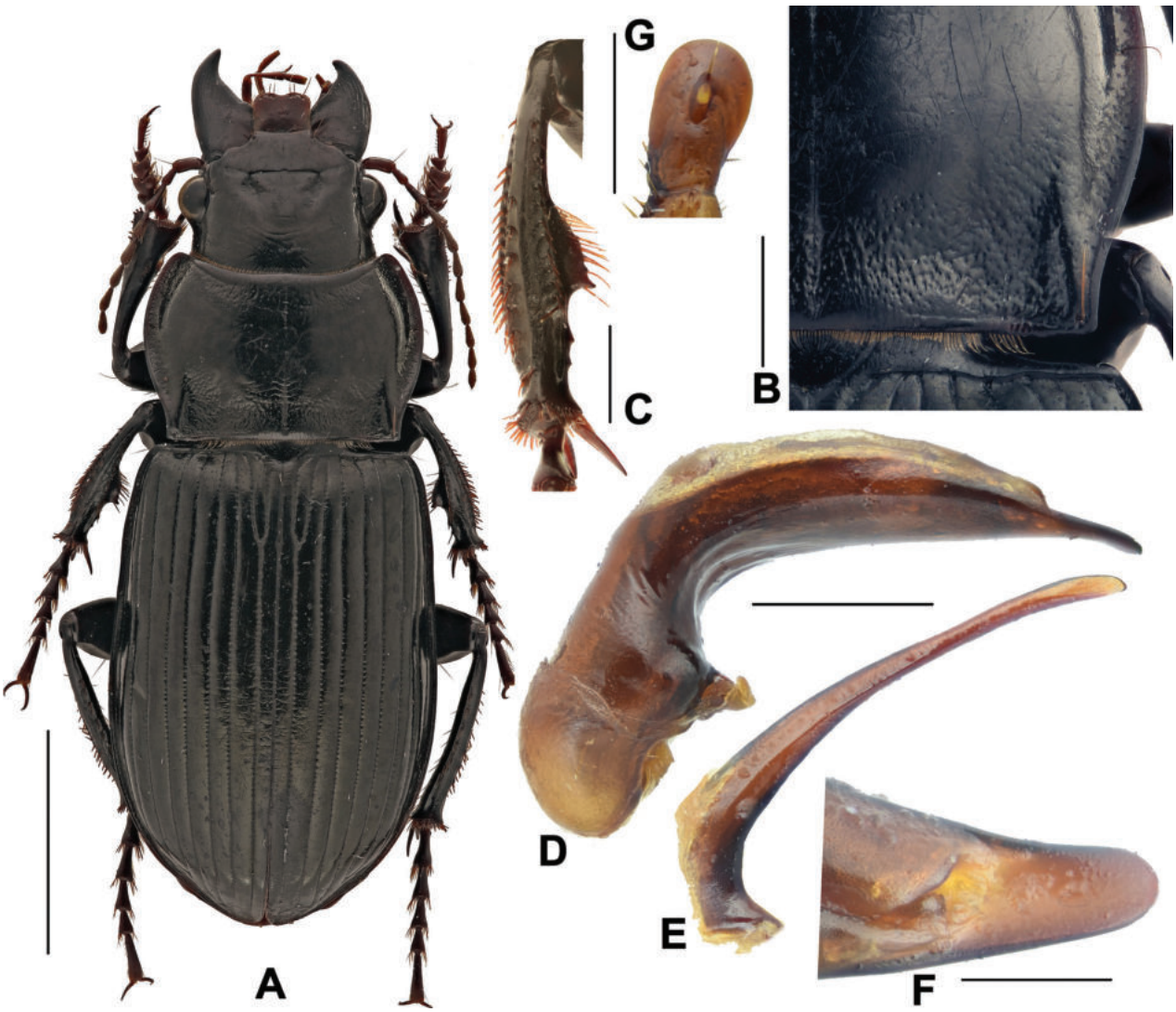


**Figure 2.** *Amara* (*Curtonotus*) *gigantea* **A** dorsal habitus, male (Fengning, Hebei, taken by Xiaoran Yang) **B** pronotum posterior angle (taken by Xiaoran Yang) **C** male mesotibia (taken by Xiaoran Yang) **D** lateral view of aedeagus **E** right paramere **F** apical lamella **G** female gonocoxite (Tongliao, Inner Mongolia). Scale bars: 5 mm (**A**); 1 mm (**B–E**); 0.5 mm (**F, G**).

Zhejiang, Taiwan), Japan, North Korea, South Korea, Russia (Far East, east Siberia), Oriental Region.

### *Amara* (*Curtonotus*) *tumida* species group

Five species distributed in eastern China belong to this species group. They are characterized by the head with only one supraorbital seta each side and the pronotum lateral setae absent. These two distinctive features make them easily differentiated from all other *Curtonotus* species known from eastern China.

### *Amara* (*Curtonotus*) *gansuensis* Jedlička, 1957

Fig. 3

*Amara* (*Curtonotus*) *gansuensis* Jedlička, 1957: 26 (type locality: Gansu, China; holotype in NMPC); Hieke 1993: 98.

*Amara* (*Curtonotus*) *pseudoseishini* Hieke, 1990: 269 (type locality: "Chin Ling Shan" [= Qinling mountains, China], approx. 34°N, 108°O; holotype in ZMUC); synonymized by Hieke 1993: 98.

**Specimens examined.** 8 males, 9 females (CBJFU), China, Beijing, Mentougou District, Xiaolongmen Forest Park, 1100 m, 2014.8~2019.8; 1 female (CBJFU), China, Beijing, Huairou District, Education Center of Beijing University of Agriculture, 2016.07.30, Pingzhou Zhu leg.; 1 male, 3 females (CBJFU), China, Beijing, Songshan National Nature Reserve, 40.50806°N, 115.79111°E, 778 m, 2013.07.22–08.5, Liubo leg.; 1 female (CBJFU), China, Hebei, Chongli, CN2, 40.8814°N, 114.9499°E, 718 m, 2018. 10, Wenhao Hu leg.; 1 female (CBJFU), China, Shanxi, Gujiao, Kangjialiang Village, 37.5316°N, 112.19°E, 1230 m,

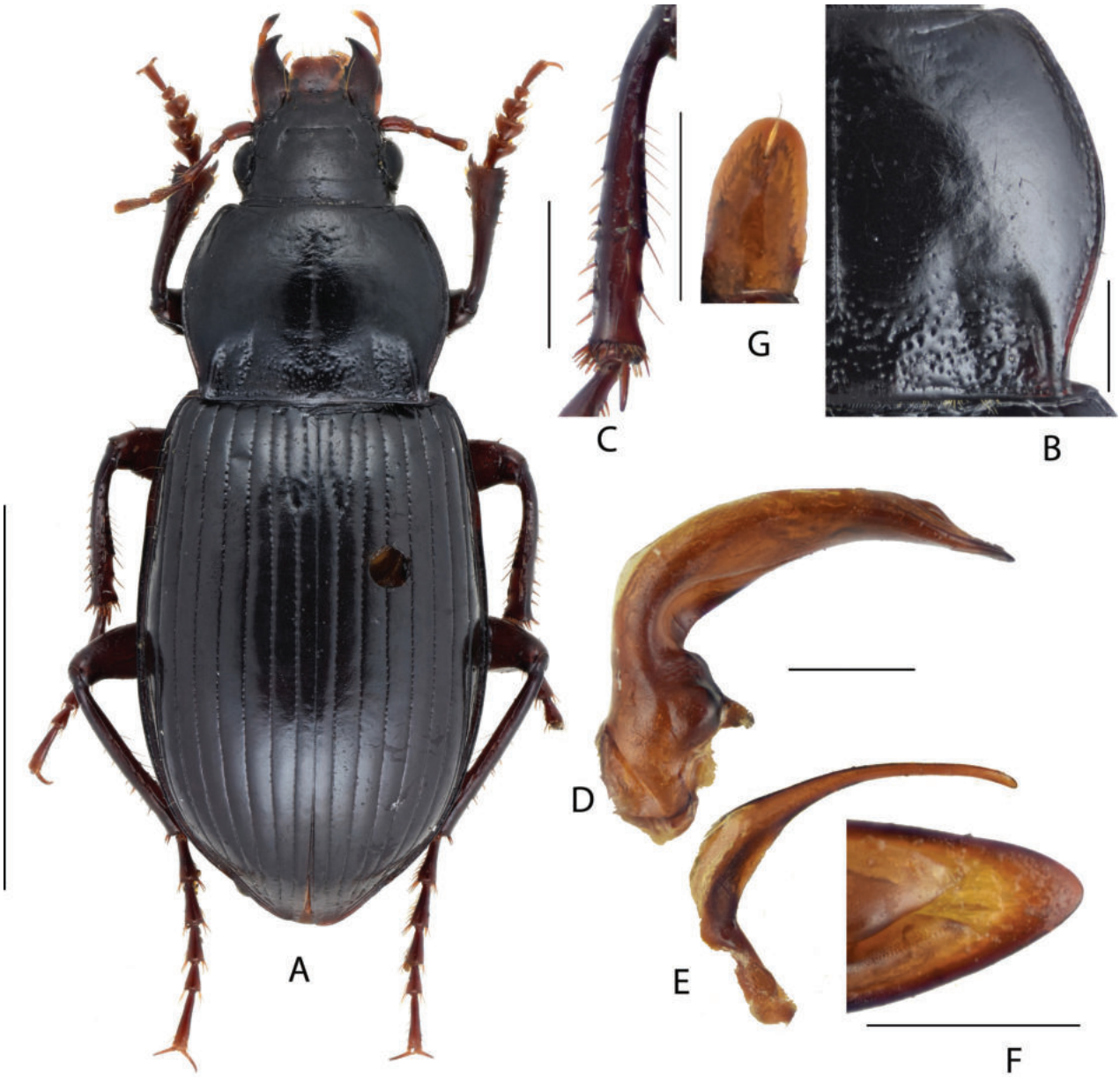


**Figure 3.** *Amara* (*Curtonotus*) *gansuensis* **A** dorsal habitus, male (Mentougou District, Beijing) **B** pronotum posterior angle **C** male mesotibia **D** lateral view of aedeagus **E** right paramere **F** apical lamella **G** female gonocoxite (Chongli, Hebei). Scale bars: 5 mm (**A**); 1 mm (**B–E**); 0.5 mm (**F, G**).

2021.09.10, Xiaojie Sun leg.; 2 females (CLYH), China, Hebei, Zhangjiakou, Hailiutu, Dayuedai, 41.176428°N, 114.512037°E, 1390 m, 2022.09.11, Cong Wang leg.

**Chinese common name.** 甘肃暗步甲.

**Diagnosis.** Medium-sized species, BL = 9.6–12.0 mm; dorsum shining black, legs reddish brown; head small, ~ 1/2 of pronotum maximum width, with one supraorbital seta. Pronotum cordate (Fig. 3B), densely punctate at base, sparsely punctate at mid-anterior region; lateral margins strongly sinuate before posterior angles; lateral grooves deeply incised, a little expanded, and distinctly punctate; posterior angles acute and laterally protruded. Elytra elongated, widest after middle; sides of abdominal sternites densely punctate. Male mesotibiae projection (Fig. 3C) composed of two small denticles; the proximal denticle acutely pointed a little beyond middle; distal denticle on the midpoint between the proximal one and tibiae apex. Male genitalia with apical lamella (Fig. 3F) elongate, subtriangular, almost straight, narrowed to apex; gonocoxite 2 (Fig. 3G) of ovipositor elongated, length ~ 1.6× greatest width, apex a little narrowed.

**Comparison.** This species can be distinguished from the other four Chinese species belonging to this species group by its deeply incised and punctate pronotum lateral grooves.

**Distribution.** China (Beijing, Hebei*, Gansu, Liaoning, Shaanxi, Shanxi*), North Korea, Russia (Far East).

**Remarks.** According to our examined specimens, the pronotum of this species exhibits a variable length of the sinuation before the posterior angles.

### ***Amara* (*Curtonotus*) *goniodera* Tschitschérine, 1895**

Fig. 4

*Amara* (*Curtonotus*) *goniodera* Tschitschérine, 1895: 164 (type locality: Korea; holotype in TMB); Hieke 1990: 256.

**Specimens examined.** 2 males (CBJFU), China, Inner Mongolia, Genhe, Daxinganling Ecological Station, 50.8061°N, 121.5824°E, 726 m, 2018.08.28, Hongliang Shi leg.; 1 male (CBJFU), China, Jilin, Baishan, Fusong County, west of Changsongling tunnel, 41.7789°N, 127.9400°E, 1577 m, 2019.08.09, Hongliang Shi & Yizhou Liu leg.; 1 male (CBJFU), China, Jilin, Antu County, north slope of Changbaishan mt, the waterfall of Tianchi, 42.0373°N, 128.0544°E, 1959 m, 2019.08.06, Hongliang Shi & Yizhou Liu leg.; 1 female (CLYH), China, Jilin, Baishan, Antu County, north slope of Changbaishan mt, the waterfall of Tianchi, 2023.06.14, Taoqi Wang leg.

**Chinese common name.** 宽瓣暗步甲.

**Diagnosis.** Medium-sized species, BL = 10.8–12.2 mm; dorsum black, legs dark drown; head small, its width ~ 1/2 of pronotum maximum width; head with one supraorbital seta each side. Pronotum (Fig. 4B) cordate, densely punctate at base, sparsely punctate at mid-anterior region; lateral margins shallowly sinuate before posterior angles; posterior angles acute or nearly rectangular, slightly laterally protruded. Elytra elongated, widest near middle; sides of abdominal sternites densely punctate. Male mesotibiae projection (Fig. 4C) not so distinct as *A.* (*C.*) *gansuensis*; the proximal denticle blunt, present near apical third of tibia, distal denticles blunt and smaller than the proximal one, present

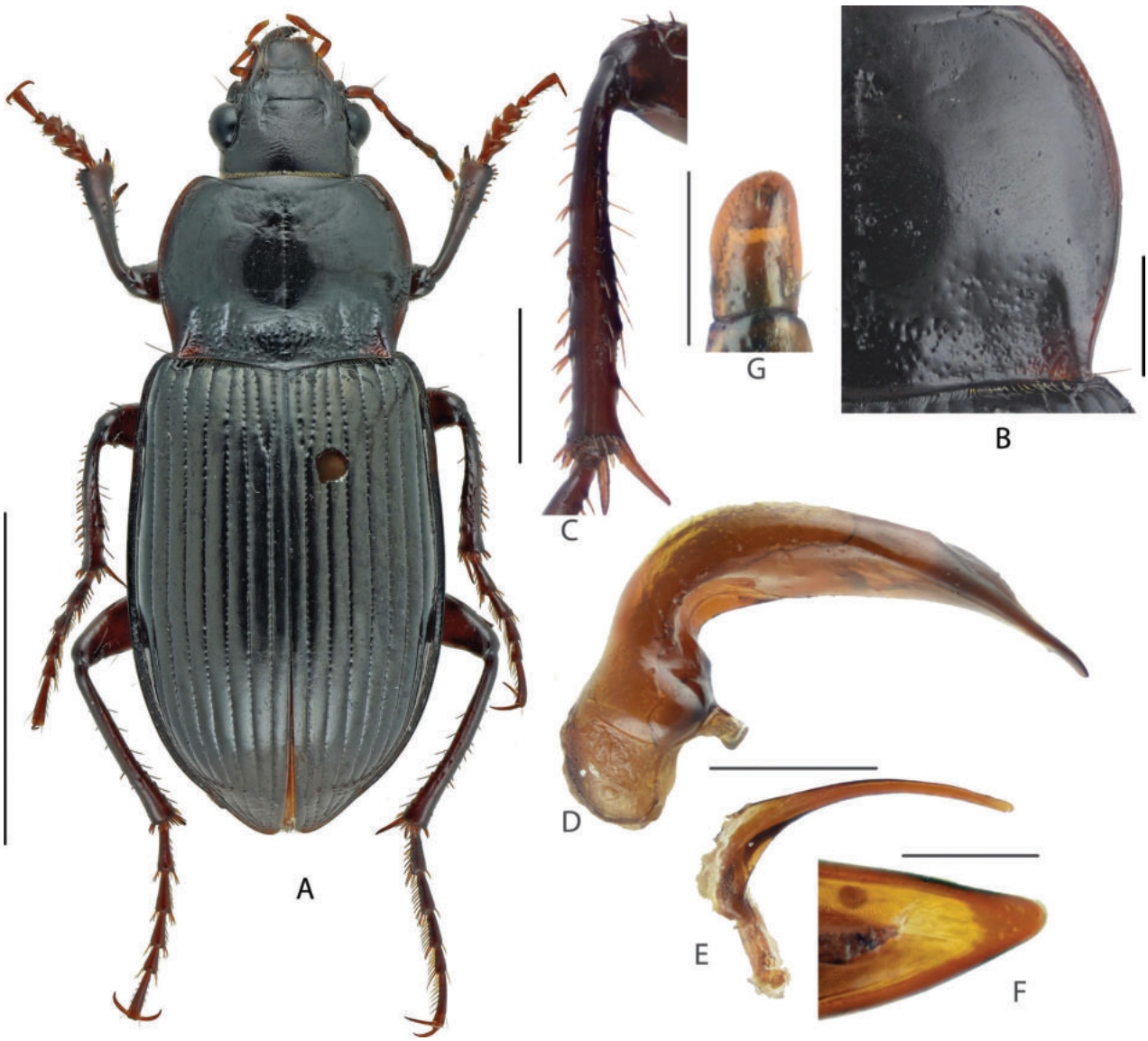


**Figure 4.** *Amara* (*Curtonotus*) *goniodera* **A** dorsal habitus, male (Zhangbei, Hebei) **B** pronotum posterior angle **C** male mesotibia **D** lateral view of aedeagus **E** right paramere **F** apical lamella **G** female gonocoxite (Zhangbei, Hebei). Scale bars: 5 mm (**A**); 1 mm (**B–E**); 0.5 mm (**F, G**).

at the midpoint between the proximal one and tibial distal apex. Male genitalia with apical lamella (Fig. 4F) long and straight, subpointed and narrowed at tip; gonocoxite 2 (Fig. 4G) of ovipositor elongate, broadly expanded, ~ 1.4–1.6× as long as wide, slightly attenuate to apex, apex narrowly rounded.

**Comparison.** This species can be distinguished from the other four Chinese species belonging to this species group by having a much longer and somewhat pointed apical lamella of the median lobe. Compared with *A.* (*C.*) *tumida* and *A.* (*C.*) *shinanensis*, *A.* (*C.*) *goniodera* has wider and stouter gonocoxite; compared with *A.* (*C.*) *gansuensis*, *A.* (*C.*) *goniodera*, it has narrower pronotum with narrower lateral groove and shorter lateral marginal sinuation before posterior angles; compared with *A.* (*C.*) *hyperborea*, *A.* (*C.*) *goniodera*, *A.* (*C.*) *goniodera* has distinctly punctate abdominal sternites.

**Distribution.** China (Gansu, Heilongjiang, Qinghai, Jilin, Shaanxi, Inner Mongolia*, Hebei*), North Korea, South Korea, Mongolia, Russia (Far East, east Siberia).

### ***Amara* (*Curtonotus*) *hyperborea* Dejean, 1831**

*Amara hyperborea* Dejean, 1831: 800 (type locality: "Labrador" [= Newfoundland and Labrador, Canada]; holotype in MNHN); Putzeys 1865: 338; Putzeys 1866: 257; Jakobson 1906: 262; Lindroth 1953: 18; Lindroth 1954: 134, 135; Lindroth 1955: 98; Hieke 1990: 252; Hieke 1993: 147.

*Curtonotus elongatus* LeConte, 1850: 207 (type locality: "Lake Superior" [Probably in USA]; holotype in CUMZ); synonymized by Lindroth 1954: 134.

*Leirus ovipennis* Motschulsky, 1859: 156 (type locality: "Californie" [actually in western Alaska]; syntypes in ZMUM); synonymized by Hieke 1993: 147.

*Leirus longicollis* Motschulsky, 1860: 95 (type locality: "Daourie orientale" [in Kamchatka, Russia]; syntypes in ZMUM); Tschitschérine 1894: 389; synonymized by Putzeys 1865: 338.

*Amara* (*Leirus*) *peregrina* Morawitz, 1862: 258 (type locality: "Kulussutai" [in southeastern Siberia]; syntypes in ZRAS); synonymized by Lindroth 1953: 18.

*Curtonotus canadensis* Putzeys, 1866: 256 (type locality: "Canada boréal." [= northern Canada]; holotype in MNHN); synonymized by Lindroth 1968: 678.

*Curtonotus dejeani* Putzeys, 1866: 258 (type locality: "Kamchatka"; holotype in MNHN); synonymized to *A. longicollis* by Tschitschérine 1894: 389.

*Curtonotus pedestris* Putzeys, 1866: 254 (type locality: "Udskoe Okhotsk" [= Udskoye, Region Chabarowsk, Russia; holotype in MNHN); synonymized to *A.* (*C.*) *hyperborea* Dejean by Hieke 1990: 252.

*Curtonotus tristis* Putzeys, 1866: 255 (type locality: "Oowho-Bay" [= Hudson Bay, Canada]; holotype in MNHN); synonymized to *A.* (*C.*) *hyperborea* by Lindroth 1968: 253.

*Harpalus simulans* Sahlberg, 1880: 44 (type locality: "Tschornaja ostrow" [= Yenisey range, Russia]; syntypes in NHRS); synonymized to *A.* (*C.*) *hyperborea* by Jakobson 1906: 262.

*Curtonotus imperfectus* Brown, 1930: 232 (type locality: "Bradore Bay" [in Quebec, Canada]; holotype in CMNC); synonymized to *A.* (*C.*) *hyperborea* by Lindroth 1954: 135.

*Amara* (*Curtonotus*) *coreana* Baliani, 1937: 182 (type locality: "Ompo" [= Onbo, North Hamgyeong Province, North Korea]; holotype in MSNG); synonymized to *A.* (*C.*) *hyperborea* by Hieke 1990: 255.

**Chinese common name.** 极北暗步甲.

**Diagnosis.** Small- to medium-sized species, BL = 9.0–13.0 mm; dorsum brown, legs reddish brown; head small, ~ 1/2 of the pronotum maximum width; head with one supraorbital seta. Pronotum cordate, punctate at base, sporadically punctate at mid-anterior region; lateral margins nearly straight before posterior angles; posterior angles nearly rectangular, slightly laterally protruded. Elytra long, widest after middle; lateral sides of abdominal sternites smooth. Male mesotibiae projection composed of two denticles; proximal denticle acutely pointed beyond middle; distal denticle smaller, present between the proximal one and tibiae apex. Male genitalia with short apical lamella, slightly declined toward right, narrowed to apex; gonocoxite 2 of ovipositor elongated, length ~ 1.7–2.0× as greatest width, narrowed to apex.

**Comparison.** this species can be distinguished by having relatively elongated body, impunctate sides of abdominal sternites, nearly smooth pronotum anterior portion, and nearly straight pronotum lateral edge before posterior angle.

**Distribution.** China (Heilongjiang, Jilin, Xinjiang), Russia (Far East, east Siberia, west Siberia), Mongolia, North Korea, Europe, North America.

**Remarks.** This species, as well as the following two species (*A.* (*C.*) *tumida*, *A.* (*C.*) *shinanensis*), was recorded from the provinces of northeastern China (Habu 1953; Hieke 1990). However, we did not find any specimens collected from eastern China that accord with these three species although several specimens of *Curtonotus* were examined from northeastern China. Considering many species of this subgenus are superficially similar externally, we suspect that these three species records from China could be based on misidentifications of similar species. We hope future research can corroborate their precise distribution in China.

### *Amara* (*Curtonotus*) *tumida* Morawitz, 1862

*Amara* (*Leirus*) *tumida* Morawitz, 1862: 258 (type locality: "Zagan-olui" [in Zabaykalsky Krai, Russia]; lectotype in ZRAS); Tschitschérine 1894: 390; Hieke 1990: 259 (lectotype designation).

*Leirus tibialis* Motschulsky, 1844: 343 (type locality: "Kamchatka"; holotype in ZMUM); junior secondary homonym of *Amara* (*Amara*) *tibialis* Paykull 1798; synonymized by Tschitschérine 1894: 390.

*Amara* (*Curtonotus*) *tumida tunkunensis* Hieke, 1990: 265 (type locality: "Quellgebiet des fl. Irkut" [= source of Irkut River, Buryatia, Russia]; holotype in MNHU).

**Chinese common name.** 膨胸暗步甲.

**Diagnosis.** Small- to medium-sized species, BL = 9.0–11.0 mm; dorsum black, legs dark brown; head small, ~ 1/2 of pronotum maximum width, with one supraorbital seta. Pronotum cordate, widest near middle, densely punctate at base, sparsely punctate at mid-anterior region; lateral margins sinuate before posterior angles; posterior angles laterally protruded, acute or nearly rectangular. Elytra relatively long, widest near middle; lateral sides of abdominal sternites densely punctate. Male mesotibiae projection composed of two denticles; proximal denticle acutely pointed a little beyond middle; distal denticles smaller, present between the proximal one and tibial distal apex. Male genitalia with apical lamella slightly declined rightward, narrowed to apex; gonocoxite 2 of ovipositor elongated, length ~ 2.3× as greatest width, apex narrow.

**Comparison.** This species is most similar to *A.* (*C.*) *shinanensis*, which can only be distinguished from it by having longer apical lamella, more constricted pronotum base, and relatively longer body. Compared with *A.* (*C.*) *gansuensis*, *A.* (*C.*) *tumida* has more distinct mesotibiae denticles and narrower pronotum lateral groove; compared with *A.* (*C.*) *goniodera*, *A.* (*C.*) *tumida* has more sinuate lateral margins before posterior angles, longer gonocoxite and shorter apical lamella; compared with *A.* (*C.*) *hyperborea*, *A.* (*C.*) *tumida* has punctate abdominal sternites and more constricted pronotum base.

**Distribution.** China (Heilongjiang, Inner Mongolia), Russia (east Siberia).

**Remarks.** According to Hieke (1990), this species has shortened wings, to ~ 1/3 the length of elytra. We could not confirm it based on current material. If this is true, it will be an important feature to distinguish *A.* (*C.*) *tumida* from similar species, like *A.* (*C.*) *shinanensis*.

### *Amara* (*Curtonotus*) *shinanensis* Habu, 1953

*Curtonotus shinanensis* Habu, 1953: 43 (type locality: "Flow of the Tenryu at Iijima-mura" [in Nagano, Japan]; holotype in ITLJ); Hieke 1993: 99.

*Amara* (*Curtonotus*) *seishini* Jedlička, 1957: 25 (type locality: "Seishin, Olto" [= Chongjin, North Korea]; holotype in NMPC); synonymized to *A.* (*C.*) *shinanensis* by Hieke 1993: 99.

**Chinese common name.** 悠游暗步甲.

**Diagnosis.** Small- to medium-sized species, BL = 9.5–11.0 mm; dorsum dark brown or nearly black, legs brown; head small, ~ 1/2 of pronotum maximum width; head with one supraorbital seta. Pronotum cordate, widest near middle, densely punctate at basal and mid-anterior regions; lateral margins sinuate before posterior angles; posterior angles slightly acute or nearly rectangular, laterally protruded. Elytra relatively short, widest near middle; lateral sides of abdominal sternites finely punctate. Male mesotibiae projection composed of two denticles (Habu 1953), which are smaller in some individuals (Hieke 1990); the proximal denticle acutely pointed a little beyond middle, distal denticles smaller, present between the proximal one and tibiae apex. Male genitalia with short apical lamella, slightly declined rightward, narrowed at apex; gonocoxite 2 of ovipositor elongated, length ~ 2× as greatest width, apex narrowed.

**Comparison.** This species is most similar to *A.* (*C.*) *tumida*, which can be distinguished by having shorter apical lamella, relatively short and oval body, and wider pronotum base. To distinguish from the remaining three species, referring to comparison part of *A.* (*C.*) *tumida*.

**Distribution.** China (Heilongjiang, Liaoning, Jilin), Japan, North Korea, Mongolia, Russia (Siberia).

### *Amara* (*Curtonotus*) *brevicollis* species group

This group contains two species distributed in eastern China. They are characterized by a reddish brown dorsum, sometimes with a faint coppery luster, and the pronotum densely and finely punctate at anterior and lateral portions.

### *Amara* (*Curtonotus*) *dux* Tschitschérine, 1894

Fig. 5

*Amara* (*Curtonotus*) *dux* Tschitschérine, 1894: 383 (type locality: "Chingan mer." [= south Chingan Mountains, China]; holotype in ZRAS); Hieke 1993: 100.

*Amara* (*Curtonotus*) *suensoni* Hieke, 1990: 249 (type locality: "Si-wan-tse" [= Xiwanzi Town, Hebei, China]; holotype in MNHU); synonymized by Hieke 1993: 100.

**Specimens examined.** 1 male (CBJFU), China, Beijing, Songshan National Nature Reserve, 40.50806°N, 115.79111°E, 778 m, 2013.08.12, Liubo leg.; 6 males, 6 females (CBJFU), Beijing, Songshan National Nature Reserve, 40.50806°N, 115.79111°E, 778 m, 2013.08.10, Bo Liu leg.; 1 male (CBJFU), Beijing, Xiaolongmen Forest Park, 1100 m, 2014.08.17–22; 1 female (CLYH), Hebei., Zhangjiakou, Zhangbei, Caoyuantianlu West Line, 41.00010926°N, 114.62708997°E, 1523 m, 2022. 09.04, Cong Wang leg.; 1 male (CLYH), Hebei, Zhangjiakou, Hailiutu, Dayuedai, 41.176428°N, 114.512037°E, 1390 m, 2022.09.11, Cong Wang leg.; 1 male (CLYH), Henan, Gongyi, Shihuiwu Village, 34.763760°N, 113.031417°E, 2022.09.07, Zheng Zhi leg.; 2 males, 1 female (CLYH), China, Gansu, Lanzhou, Yuzhong, Lanzhou University, 35.942353°N, 104.158454°E, 2022.09.22, Hanyu Yu leg.

**Chinese common name.** 点胸暗步甲.

**Diagnosis.** Medium- to large-sized species, BL = 13.0–14.4 mm; dorsal surface dark brown, legs yellowish brown; head relatively large, slightly narrower

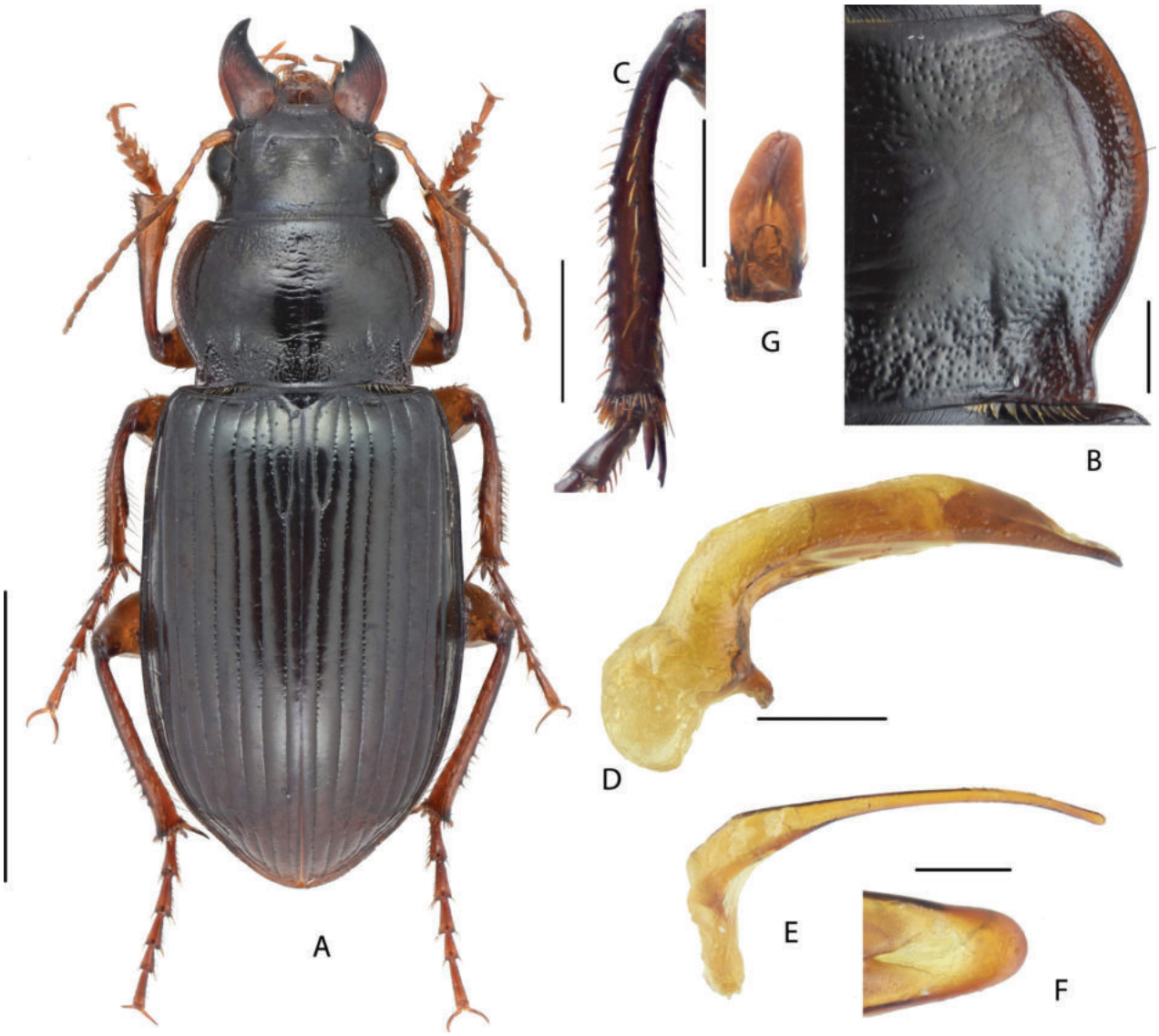


**Figure 5.** *Amara* (*Curtonotus*) *dux* **A** dorsal habitus, male (Gongyi, Henan) **B** pronotum posterior angle **C** male mesotibia **D** lateral view of aedeagus **E** right paramere **F** apical lamella **G** female gonocoxite (Lanzhou, Gansu). Scale bars: 5 mm (**A**); 1 mm (**B–E**); 0.5 mm (**F, G**).

than pronotum maximum width; head with two supraorbital setae. Pronotum (Fig. 5B) transverse, widest near middle; finely and densely punctate through basal, mid-anterior, and lateral regions; lateral margins strongly sinuate before posterior angles; posterior angles strongly laterally protruded, apex acute or near rectangular. Elytra basal border slightly curved; lateral sides of abdominal sternites sparsely wrinkled and punctate. Male mesotibiae (Fig. 5C) without distinct denticles, swollen near middle which different from females. Male genitalia with apical lamella (Fig. 5F) wide, apex rounded-truncated, indistinctly declined to right side; gonocoxite 2 (Fig. 5G) of ovipositor elongate, length ~ 2× greatest width, narrowed to apex.

**Comparison.** This species is very similar to *A.* (*C.*) *brevicollis*, but can be distinguished from the latter species in having pronotum lateral margins always evidently sinuate before the acute posterior angles, pronotum mid-lateral setae present, elytra basal border slightly curved, and longer apical lamella of male genitalia. It has overlapped distribution with *A.* (*C.*) *brevicollis*, but is less common.

**Remarks.** In Hieke's work (1990), *Amara* (*Curtonotus*) *suensoni*, a synonym of this species, was described as having three similarly sized denticles on the male mesotibial projection. We also observed two or three very tiny denticles (much smaller than and alike the denticles in males of several other species) in our examined male specimens of this species. However, considering some female individuals also have similar-sized denticles on their mesotibiae, we do not think these additional small denticles are male-specific character.

**Distribution.** China (Ningxia, Hebei, Beijing, Henan*, Gansu*, Liaoning, Inner Mongolia), Mongolia, North Korea, South Korea, Russia (east Siberia, Far East).

### ***Amara* (*Curtonotus*) *brevicollis* Chaudoir, 1850**

Fig. 6

*Leirus brevicollis* Chaudoir, 1850: 151 (type locality: "O. Siberia" [= east Siberia]; holotype in MNHN); Lindroth 1968: 665; Hieke 1999: 170.

*Curtonotus transversicollis* Putzeys, 1866: 236 (type locality: "Amér. Russe.: Akina" [= Akima, Zabaykalsky Krai, Russia]; syntypes in MNHN); synonymized by Lindroth 1968: 665.

*Amara* (*Curtonotus*) *kuznetzovi* Lutshnik, 1928: 46 (type locality: "See Issyk-kul" [= Issyk-Kul, Kyrgyzstan]; holotype originally in Lutshnik collection, now could be lost); synonymized by Hieke 1999: 170.

**Specimens examined.** 7 males, 5 females (CBJFU), China, Beijing, Songshan National Nature Reserve, 40.50806°N, 115.79111°E, 778 m, 2013.07.21~2013.8.10, Bo Liu leg; 4 males, 4 females (CBJFU), China, Beijing, Chinese Agricultural University, 2003. 09.14, Ye Liu leg.; 2 males, 8 females (CBJFU), China, Beijing, Mentougou, Xiaolongmen Forestry Park, date between 2014.VIII-2019.VIII; 1 male (CBJFU), Beijing, Huairou, Sidaohe, 2017.VI.10–14; 1 female (CBJFU), Beijing, Shunyi, Hanshiqiao, 2016.IX.17, Zhu pingzhou leg.; 1 female (CLYH), China, Beijing, Haidian district, Shucun Park, 40.024101°N, 116.305901°E, 45 m, 2021.05, Yihang Li leg.; 1 female (CBJFU), China, Jilin, Yanbian, Helong City, 42.5506°N, 128.9951°E, 435 m, 2019.08.02, Yizhou Liu leg.; 1 female (CBJFU), China, Heilongjiang, Muleng, Ziping Mt, 2017.07.10, Zhengtong Wang leg.; 2 males, 1 female

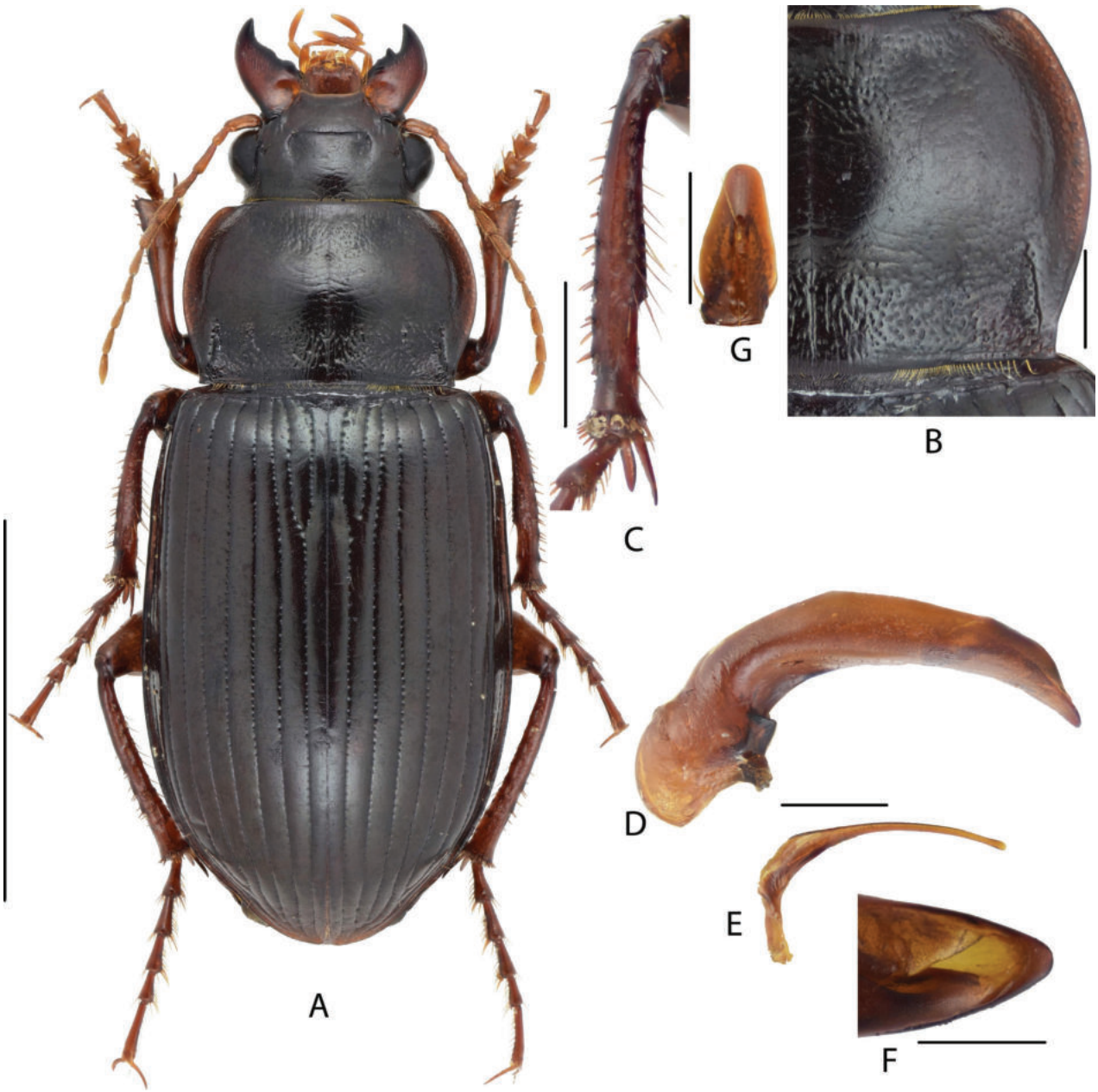


**Figure 6.** *Amara* (*Curtonotus*) *brevicollis* **A** dorsal habitus, male (Qilian, Qinghai) **B** pronotum posterior angle **C** male mesotibia **D** lateral view of aedeagus **E** right paramere **F** apical lamella **G** female gonocoxite (Lanzhou, Gansu). Scale bar: 5 mm (**A**); 1 mm (**B–E**); 0.5 mm (**F, G**).

(CBJFU), China, Qinghai, Qilian County, Babao Township, 38.1804°N, 100.2454°E, 2727 m, 2019.08.16, Weifeng Yan leg., 1 male (CBJFU), China, Qinghai, Menyuan county, Xianmi, Taihua village, 37.2329°N, 102.1135°E, 2784 m, 2017.07.15, Pingzhou Zhu leg.; 2 females (CLYH), China, Gansu, Lanzhou, Yuzhong, Lanzhou University, 35.942353°N, 104.158454°E, 2022.09.22, Hanyu Yu leg.

**Chinese common name.** 短胸暗步甲.

**Diagnosis.** Medium-sized species, BL = 9.5–12.5 mm; dorsal surface dark brown, legs yellowish brown; head relatively large, > 1/2 pronotum maximum width; head with two supraorbital setae. Pronotum (Fig. 6B) transverse, widest near middle; finely and densely punctate through basal and mid-anterior regions; lateral margins usually shallowly sinuate before posterior angles;

posterior angles usually rectangular or obtuse, less protruded than the previous species. Elytra basal border nearly straight; lateral sides of abdominal sternites sparely wrinkled and punctate. Male mesotibiae projection composed of only one large denticle (Fig. 6C): proximal denticle very large, a little beyond midpoint of tibiae, significant dilated; distal denticle absent. Male genitalia with apical lamella (Fig. 6F) shorter than the previous species, a little narrowed to apex, apex rounded-triangular; gonocoxite 2 (Fig. 6G) of ovipositor elongate, length ~ 2× greatest width, apex narrowed.

**Comparison.** This species is most similar to *A.* (*C.*) *dux*, but different by the absence of pronotum mid-lateral setae, presence of a distinct denticle near middle of male mesotibiae, and much shorter and narrower apical lamella of male genitalia. Besides these above, in most specimens of *A.* (*C.*) *brevicollis*, the pronotum posterior angles are obtuse or nearly rectangular, much less protruded than in *A.* (*C.*) *dux*. However, we also examined a few specimens of *A.* (*C.*) *brevicollis* from north China which has the pronotum outline almost identical to *A.* (*C.*) *dux*.

**Distribution.** China (Beijing, Gansu, Guizhou, Hebei, Ningxia, Heilongjiang, Hubei, Jilin, Qinghai, Sichuan, Shaanxi, Xinjiang, Inner Mongolia), Russia (east Siberia, west Siberia, Far East, South European Territory), Mongolia, North Korea, South Korea, Kazakhstan, Kyrgyzstan, Turkmenistan, Europe.

### *Amara* (*Curtonotus*) *macronota* species group

This species group includes six Chinese species. They are characterized by the dorsal surface being black or dark brown; head with two supraorbital setae on each side; pronotum mid-lateral setae present; pronotum impunctate or sparsely punctate on anterior portion; male mesotibiae projection varied.

### *Amara* (*Curtonotus*) *fodinae* Mannerheim, 1825

Fig. 7

*Amara fodinae* Mannerheim, 1825: 20 (type locality: "Barnaul" [in Altai Krai, Ruaasia]; syntypes could be lost); Morawitz 1862: 234; Tschitschérine 1894: 387; Kryzhanovskij 1975: 92.

*Leirus altaicus* Motschulsky, 1844: 174 (type locality: "Altai" [in Altai Krai, Russia]; syntypes in Motschulsky's personal collection); synonymized by Morawitz 1862: 234.

*Amara* (*Curtonotus*) *primitiva* Jedlička, 1957: 28 (type locality: "Quellgebiet des fl. Irkut im Ostsajan-Gebirge" [= Headwaters of the Irkut river in the east Sayan Mountains, Buryatia, Russia]; holotype in NMPC); synonymized by Kryzhanovskij 1975: 92.

*Amara fodinae vicina* Tschitschérine, 1894: *Amara fodinae* var. *vicina* Tschitschérine 1894: 387 (type locality: "Amdo-Plateau: Ankhur-kashan" [in Qinghai, China]; syntypes in ZRAS).

**Specimens examined.** 6 males, 3 females (CBJFU), China, Inner Mongolia, Genhe, Daxinganling Ecological Station, 50.8061°N, 121.5824°E, 726 m, 2018.08.28, Hongliang Shi leg.; 2 males (CLYH), China, Hebei, Zhangjiakou, Zhuolu, Lingshan

Scenic Spots, 40.054300°N, 115.487502°E, 1788 m, 2021.08.02, Yihang Li leg.; 1 male, 1 female (CLYH), China, Hebei, Shijiazhuang, Chang'an District, 2022.08.20–22, Ran Meng leg.; 3 males (CLYH), China, Hebei, Zhangjiakou, Zhangbei, Caoyuantian Road West Line, 41.00010926°N, 114.62708997°E, 1523 m, 2022.09.04, Cong Wang leg.; 4 males, 2 females (CBJFU), China, Hebei, Chongli, DF2, 41.0527°N, 115.3240°E, 1447 m, 2018.09, Wenhao Hu leg.; 1 female (CBJFU), China, Beijing, Mentougou District, eastern slope of Donglingshan Mt, 40.0451°N, 115.4897°E, 1710 m, 2022.07.20, Hongliang Shi & Ganyan Yang leg.; 2 male, 1 female (CBJFU), China, Beijing, Xiaolongmen Forestry park, 2014.VIII.17–22; 1 male, 2 females (CBJFU), Beijing, Songshan, 2005.8.21; 1 male (CLYH), China, Qinghai, Xining, The Party School of Qinghai Provincial committee of CPC, 36.631848°N, 101.778172°E, 2243 m, 2021.09, Bohan Cui leg.; 1 male (CBJFU), China, Qinghai, Menyuan county, Xianmi, Talihua vill. 2784 m, N37.2329 E102.1135, 2017.VII.15, Shi HL et al leg.; 1 male (CBJFU), China, Qinghai, Gangca county, Qonj Xiang, Qinghaihu bank, 3200 m, 37.1973, 99.8039, 2017.VII.19 Shi HL leg.; 1 male (CBJFU), Qinghai, Menyuan county, Meihua vill. 2784 m, N37.2574 E102.0869, 2020.VII.30 Liu YZ, Yin WQ lgt.

**Chinese common name.** 掘暗步甲.

**Diagnosis.** Medium to large-sized species, BL = 11.0–13.0 mm; dorsum black or dark brown, legs usually dark brown; head relatively small, ~ 1/2 of pronotum maximum width, with two supraorbital setae. Pronotum (Fig. 7B) cordate, widest near middle; densely punctate at basal region, impunctate at mid-anterior region; lateral margins with long sinuation before posterior angles; posterior angles nearly rectangular or acute, not or slightly protruded. Elytra elongated, widest near middle, with isodiametric microsculpture; lateral sides of abdominal sternites wrinkled. Male mesotibiae projection (Fig. 7C) without defined denticle, distal half of tibiae prominently dilated. Male genitalia with short triangular apical lamella (Fig. 7F), slightly bent rightward, narrowed to apex; gonocoxite 2 (Fig. 7G) of ovipositor elongate, length ~ 2× as greatest width, distinctly attenuate to apex.

**Comparison.** This species is different from most of Chinese *Curtonotus* by its male mesotibiae lacking denticles. From the shape of pronotum, *A.* (*C.*) *fodinae* is most similar to *A.* (*C.*) *banghaasi*, but these two species are different in many aspects: in *A.* (*C.*) *fodinae* the pronotum is widest near middle, but widest clearly before middle in *A.* (*C.*) *banghaasi*; in *A.* (*C.*) *fodinae*, the male mesotibiae has no distinct denticles but has two distinct denticles in *A.* (*C.*) *banghaasi*; in *A.* (*C.*) *fodinae*, the apex of apical lamella is a little acute and slightly bent rightwards, but more widely rounded and near straight in *A.* (*C.*) *banghaasi*; in *A.* (*C.*) *fodinae*, the gonocoxite 2 is strongly elongate and attenuate to apex, while in *A.* (*C.*) *banghaasi* it is widely rounded at apex. Some small-sized individuals of *A.* (*C.*) *harpaloides* also might be confused with *A.* (*C.*) *fodinae*, but can be differentiated by the pronotum distinctly punctate on mid-anterior region, male mesotibiae with two distinct denticles and females with gonocoxite 2 much stouter.

**Remarks.** Two subspecies have been recognized under this species (Hieke, 1993): *A.* (*C.*) *fodinae vicina* is smaller and less robust and distributed in the western provinces of China, while the nominotypical subspecies is a little larger and more robust.

**Distribution.** China (Gansu, Hebei, Beijing, Heilongjiang, Jilin, Inner Mongolia, Shaanxi, Shanxi, Qinghai, Sichuan, Xinjiang, Mongolia, Tibet), Russia (Far East, east Siberia, west Siberia), Kyrgyzstan, Kazakhstan, Tajikistan, Turkmenistan, Europe.

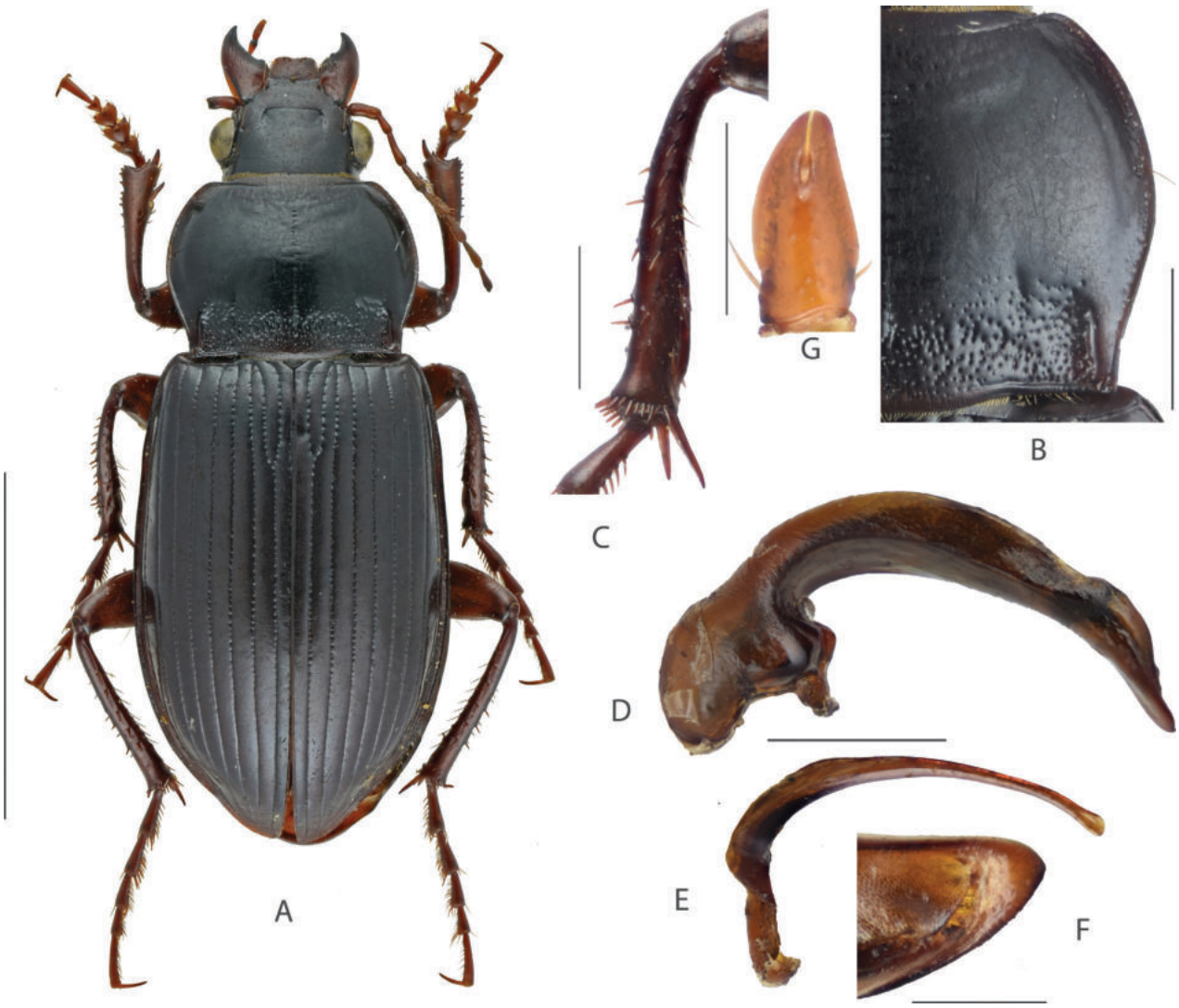


**Figure 7.** *Amara* (*Curtonotus*) *fodinae* **A** dorsal habitus, male (Zhangbei, Hebei) **B** pronotum posterior angle **C** male mesotibia **D** lateral view of aedeagus **E** right paramere **F** apical lamella **G** female gonocoxite (Chongli, Hebei). Scale bar: 5 mm (**A**); 1 mm (**B–E**); 0.5 mm (**F, G**).

### ***Amara* (*Curtonotus*) *banghaasi* Baliani, 1933**

Fig. 8

*Amara* (*Curtonotus*) *banghaasi* Baliani, 1933: 90 (type locality: Pechino [= Peking, Beijing]; holotype in MSNG).

**Specimens examined.** 3 males, 5 females (CBJFU), China, Inner Mongolia, Hexigten Banner, Dalinuoer Lake Nature Reserve, 1200 m, 2006.07.06, Hongliang Shi leg.; 1 female (CBJFU), China, Beijing, Mentougou District, Donglingshan Mt, 40.0252°N, 115.4542°E, 1974 m, 2022. 08.22, Hongliang Shi leg.; 1 female (CLYH), China, Gansu, Zhangye, Gaotai, near Heihe, 2015.08.07–09, Deyao Zhou leg.; 1 male (CLYH), China, Gansu, Zhangye, Gaotai, Hongshahe, 2015.07.30, Deyao Zhou leg.; 1 female (CBJFU), China, Ningxia Hui Autonomous Region, Lingwu, Lingwu Farm, 2017.08.03, Yidan Zhang leg; 1 male, 1 female (CBJFU), China, Qinghai, Hainan, 36.42681°N, 100.9955°E, 3323 m, 2019.08.13, Shihao Wang leg. ; 1 male (CBJFU), China, Qinghai, Gangca, Qonj

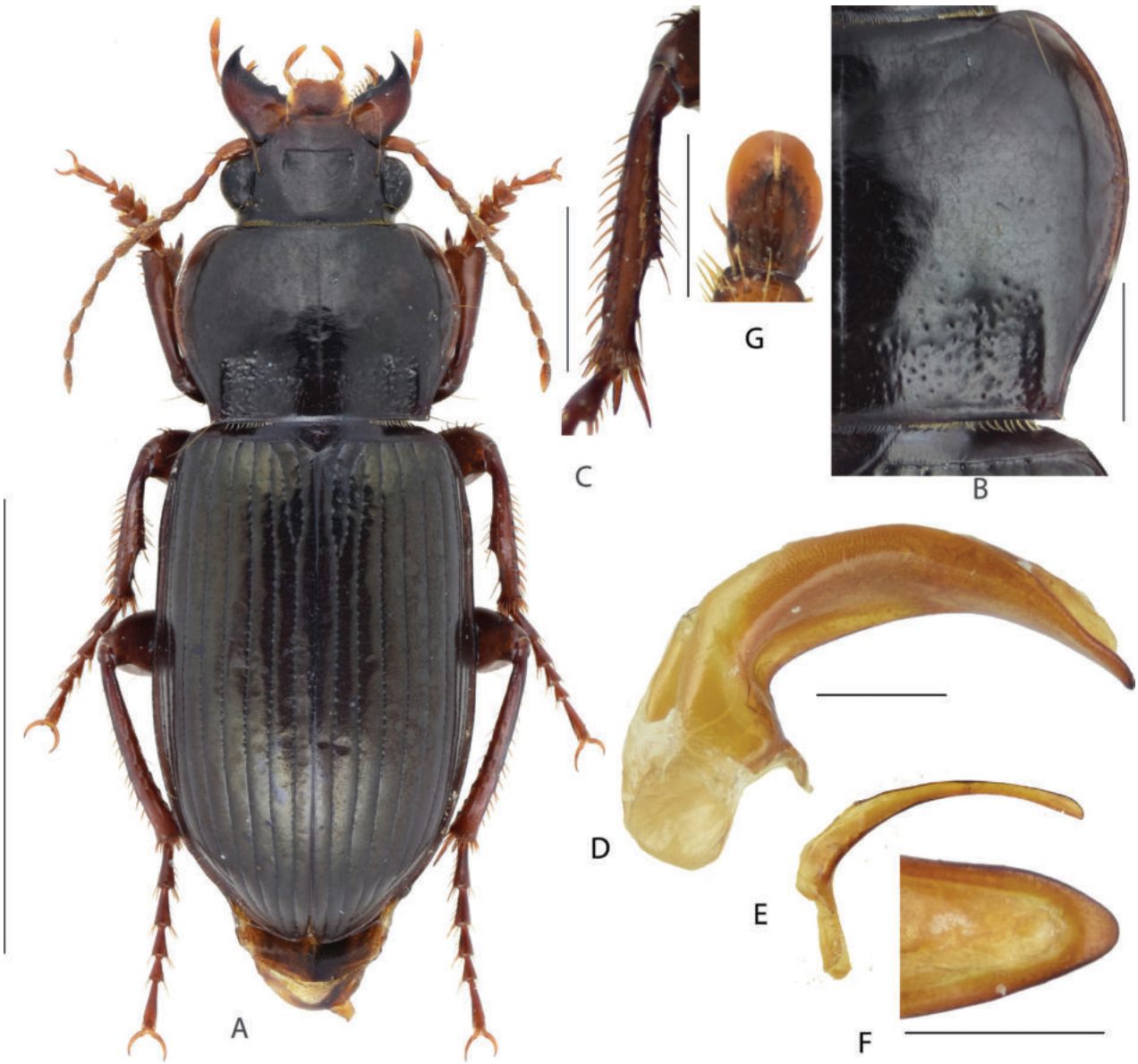


**Figure 8.** *Amara*(*Curtonotus*) *banghaasi* **A** dorsal habitus, male (Gangca, Qinghai) **B** pronotum posterior angle **C** male mesotibia **D** lateral view of aedeagus **E** right paramere **F** apical lamella **G** female gonocoxite (Hainan, Qinghai). Scale bar: 5 mm (**A**); 1 mm (**B–E**); 0.5 mm (**F, G**).

Xiang, Qinghaihu bank, 32.1973°N, 99.8039°E, 3200 m, 2017.07.19, Hongliang Shi leg.

**Chinese common name.** 棒暗步甲.

**Diagnosis.** Medium to large-sized species, BL = 11.5–13.0 mm; dorsum black or dark brown, legs dark brown; head relatively large, more than half length of pronotum maximum width, with two supraorbital setae. Pronotum (Fig. 8B) cordate, widest near anterior third; densely and coarsely punctate at basal region, impunctate or scarcely punctate at mid-anterior region; lateral margins with sinuation long and distinct before posterior angles; posterior angles nearly rectangular or a little acute, apex not protruded. Elytra elongated, widest behind middle, with very strong isodiametric microsculpture; lateral sides of abdominal sternites sparsely wrinkled and punctate. Male mesotibiae projection composed of two denticles (Fig. 8C); proximal denticle very large, acutely pointed,

a little after the middle; distal denticle much smaller, present between the proximal one and tibiae apex. Male genitalia with apical lamella shortly triangular (Fig. 8F), slightly bent leftward, narrowed to tip, apex rounded; gonocoxite 2 (Fig. 8G) of ovipositor stout, length ~ 1.5× greatest width, apex widely rounded.

**Comparison.** This species is most similar to *A.* (*C.*) *fodinae* among the eastern Chinese species of Curtonotus. Comparisons between them are provided under the latter species.

**Distribution.** China (Beijing, Heilongjiang, Hubei, Liaoning, Qinghai, Inner Mongolia*, Gansu*).

**Remarks.** *Amara* (*Curtonotus*) *daurica* Motschulsky, 1844 was recorded from Heilongjiang and Qinghai provinces of China (Hieke, 2017), but there is no reliable record anywhere else in China of this species either from the literature or in our examined specimens. Due to its similar external appearance to *A.* (*C.*) *banghaasi*, we hesitate these records might be based on a misidentification of *A.* (*C.*) *banghaasi* or other similar *Curtonotus* species.

### ***Amara* (*Curtonotus*) *hiogoensis* (Bates, 1873)**

Fig. 9

*Curtonotus hiogoensis* Bates, 1873: 291 (type locality: "Hiogo" [in Japan]; syntypes in MNHN and NHML); Lewis 1879: 190; Tschitschérine 1898: 76; Matsumura 1929: 194; Habu 1953: 41.

**Specimens examined.** 3 females (CBJFU), China, Jilin, Qianjin County, Jiaohe Forest Station, 43.9555°N, 127.6971°E, 2018.09.01, Hongliang Shi leg.; 1 male (CBJFU), China, Jilin, Antu, Erdaobaihe, 733 m, N42.4021 E128.1068, 2018.9.3, Shi Hongliang leg.; 1 female, 1 male (CBJFU), China, Hubei, Xuanen, Changtanhe, Houhe Village, 30.033006°N, 109.724061°E, 1210 m, 2017.V.7–9, Yizhou Liu leg.; 1 female (CBJFU), China, Hubei, Shennongjia, Hongping Town, 1500 m, 2013.08.15, Hao Huang leg.; 1 female (CLYH), China, Hubei, Shiyan, Zhuxi County, Shuangping Village Committee, 1151 m, 2023.06.07, Qianle Lu leg.

**Chinese common name.** 兵库暗步甲.

**Diagnosis.** Large-sized species, BL = 13.5–14.0 mm; body black, legs dark brown to black; head relatively large, greater than half of pronotum maximum width, with two supraorbital setae. Pronotum (Fig. 9B) strongly transverse, slightly cordate, basal region densely punctate, mid-anterior region impunctate, sometimes finely wrinkled; lateral margins slightly sinuate near posterior angles; posterior angles wide, with indistinct denticles, barely protruded laterally; elytra oblong, widest near middle, without or only with very faint isodiametric microsculpture after middle; lateral sides of abdominal sternite punctate. Male mesotibiae projection composed of three small denticles (Fig. 9C), proximal denticle acutely pointed clearly beyond the middle, and two slightly smaller distal denticles between the proximal one and tibiae apex. Male genitalia with relatively long apical lamella (Fig. 9F), slightly bent rightward, apex widely rounded; gonocoxite 2 (Fig. 9G) of ovipositor elongate, length ~ 1.5× greatest width, apex widely rounded.

**Distribution.** China (Anhui, Fujian, Hubei, Sichuan, Shaanxi, Zhejiang, Jilin*), Japan, North Korea, South Korea, Russia (Far East).

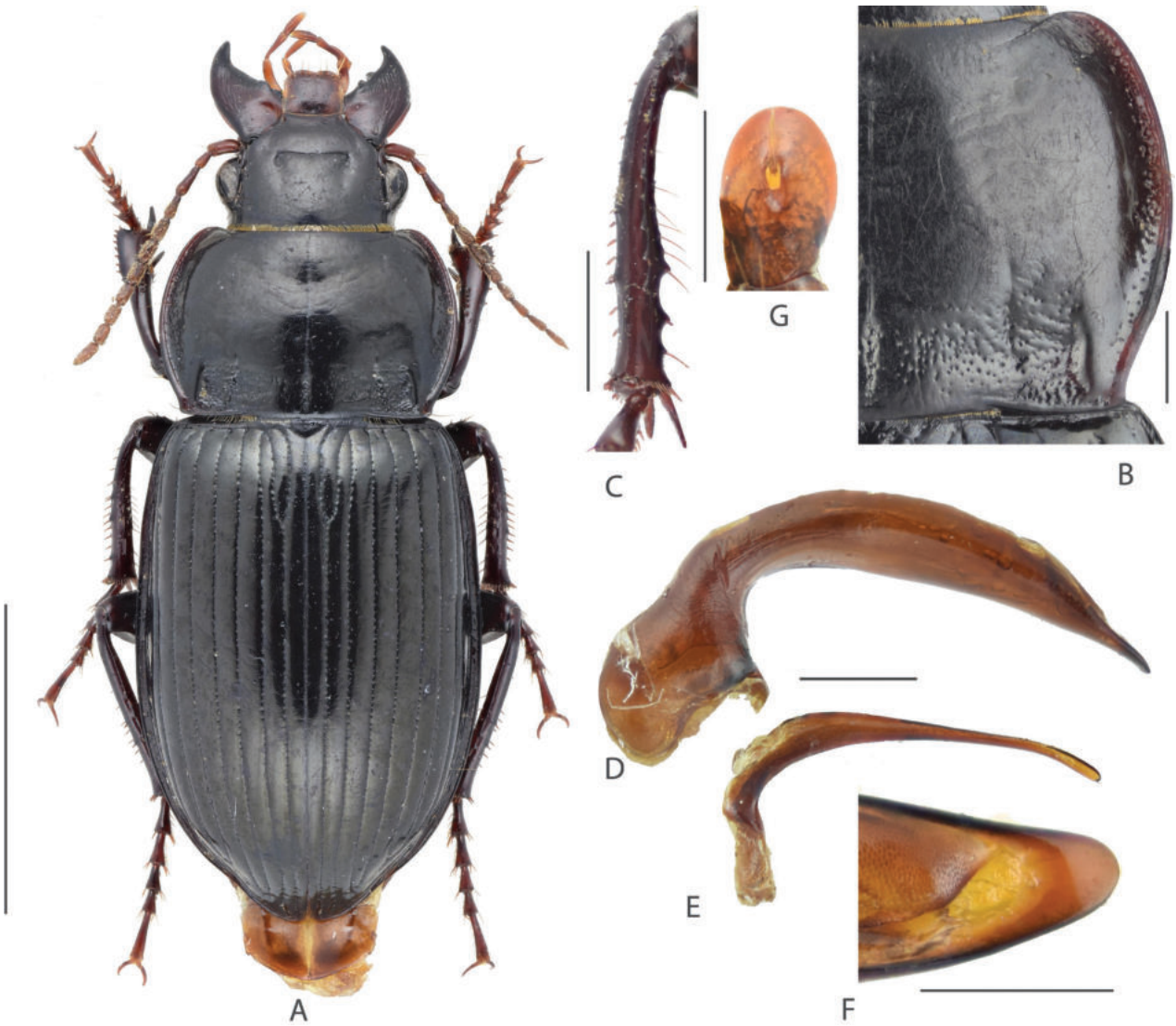


**Figure 9.** *Amara* (*Curtonotus*) *hiogoensis* **A** dorsal habitus, female (Jiaohe, Jilin) **B** pronotum posterior angle (Jiaohe, Jilin) **C** male mesotibia (Jiaohe, Jilin) **D** lateral view of aedeagus (Jiaohe, Jilin) **E** right paramere (Jiaohe, Jilin) **F** apical lamella (Jiaohe, Jilin) **G** female gonocoxite. Scale bars: 5 mm (**A**); 1 mm (**B–E**); 0.5 mm (**F, G**).

### ***Amara* (*Curtonotus*) *harpaloides* Dejean, 1828**

Fig. 10

*Amara harpaloides* Dejean, 1828: 514 (type locality: "Sibirien" [= Barnaul, Altai Krai, Russia]; syntypes in MNHN); Hieke 1999: 162.

*Curtonotus convexicollis* Putzeys, 1866: 232 (type locality: "Siberia"; holotype in MNHN); synonymized by Hieke 1999: 162.

**Specimens examined.** 2 males (CLYH), China, Hebei, Zhangjiakou, Zhuolu, Lingshan Scenic Spots, 40.054300°N, 115.487502°E, 1788 m, 2021.08.02, Yihang Li leg.; 1 female (CLYH), China, Beijing, Songshan National Reserve, 40.534820°N, 115.7541325°E, 1380 m, 2022.08.07, Yihang Li leg.; 1 male (CLHY), China, Beijing, Qingshui Town, Hongkou Village, light trap, 39.99464407°N, 115.48366919°E, 950 m, 2022.07.24, Haoyuan Li leg.; 19 males, 17 females (CBJFU), China, Inner Mongolia, Genhe, Greater Khingan Ecological Station,

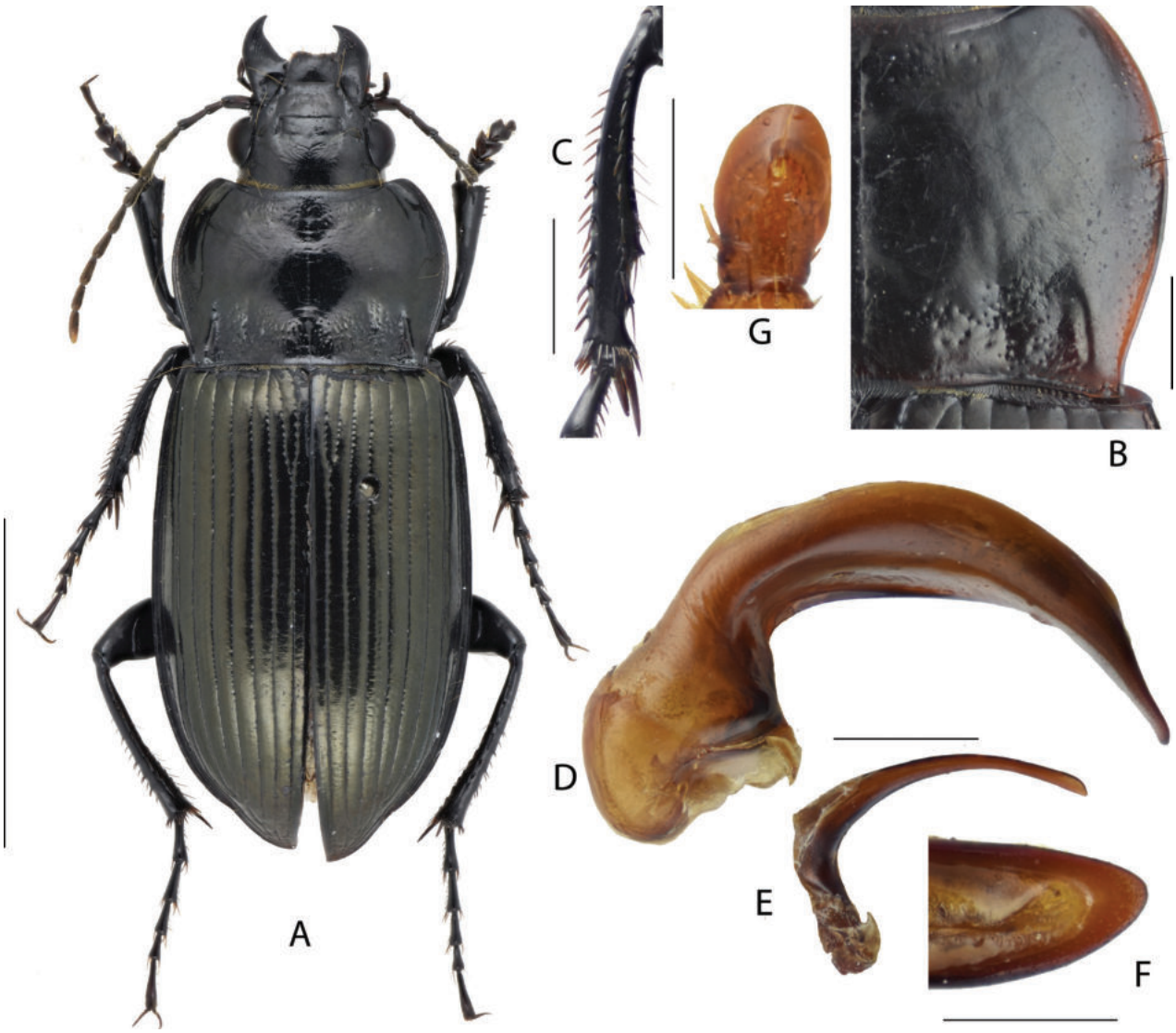


**Figure 10.** *Amara* (*Curtonotus*) *harpaloides* **A** dorsal habitus, male (Zhangbei, Hebei) **B** pronotum posterior angle (Tianjun, Qinghai) **C** male mesotibia **D** lateral view of aedeagus **E** right paramere **F** apical lamella **G** female gonocoxite (Yongdeng, Gansu). Scale bar: 5 mm (**A**); 1 mm (**B–E**); 0.5 mm (**F, G**).

50.8061°N, 121.5824°E, 726 m, 2018.08.28, Hongliang Shi leg.; 1 male (CLYH), China, Hebei, Zhangjiakou, Hailiutu, Dayuedai, 41.176428°N, 114.512037°E, 1390 m, 2022.09.11, Cong Wang leg.; 1 female (CBJFU), China, Gansu, Yongdeng, Liancheng Township, 36.5940°N, 102.8326°E, 1927 m, 2021. 08.04, Youyan Huang & Hanshuo Liu leg.; 1 female (CBJFU), China, Qinghai, Haixi, Tianjun County, Kuaier'ma, 37.4724°N, 98.7772°E, 3765 m, 2022.08.05, Hongliang Shi leg.; 3 males, 11 females (CBJFU), China, Qinghai, Zekong county, Maixiu forestry center, 35.2706°N, 101.9304°E, 2962 m, 2019.08.23, Weifeng Yan leg.

**Chinese common name.** 婪暗步甲.

**Diagnosis.** Medium to large-sized species, BL = 10.0–12.0 mm; body black, legs dark brown to black; head small, ~ 1/2 of pronotum maximum width, with two supraorbital setae. Pronotum (Fig. 10B) subcordate, widest near middle, densely punctate at basal region, sparsely punctate at mid-anterior region; lateral margins weakly sinuate or nearly straight on posterior half; posterior angles more or less protruded laterally, nearly rectangular,

apex without denticulate. Elytra oblong, widest near middle; finely punctate on basal half of striae; humeral tooth strongly and straightly protruded; elytra with isodiametric microsculpture, stronger in females; lateral sides of abdominal sternites wrinkled. Male mesotibiae projection composed of two denticles (Fig. 10C); proximal denticle acutely pointed, near apical third of tibiae; distal denticle slightly smaller and wider than proximal one, between the proximal one and tibiae apex. Male genitalia with slightly long apical lamella (Fig. 10F), straight and widely triangular, gradually narrowed to apex, apex rounded; gonocoxite 2 (Fig. 10G) of ovipositor elongate, length ~ 1.5× greatest width, apex widely rounded.

**Comparison.** Among this species group, *A.* (*C.*) *harpaloides* is most similar to *A.* (*C.*) *macronota* and *A.* (*C.*) *beijingensis*, and can be distinguished from these two by having smaller elytra punctures, stronger elytral microsculpture in females, shallowly sinuate pronotum lateral margins before the posterior angles, and humeral tooth more strongly protruded.

**Distribution.** China (Gansu, Hebei, Beijing, Heilongjiang, Qinghai, Sichuan, Shanxi, Inner Mongolia, Sichuan), Russia (west Siberia, east Siberia, Far East).

### ***Amara* (*Curtonotus*) *macronota* (Solsky, 1875)**

Fig. 11

*Curtonotus macronotus* Solsky, 1875: 265 (type locality: "Nikolskoje" [= Nikolskoye, Kamchatka Krai, Russia]; holotype in ZRAS); Matsumura 1929: 194; Lafer 1989: 180; Hieke 1995: 322; Sasakawa 2009: 107; Hieke et al. 2012: 61.

*Curtonotus nitens* Putzeys, 1866: 234 (type locality: "Chine boréale" [= northern China]; holotype in MNHN); Solsky 1875: 265; Lewis 1879: 189; Bates 1888: 370; Tschitschérine 1894: 385; Habu 1953: 43; Junior secondary homonym of *Amara nitens* Letzner, 1852.

*Amara* (*Curtonotus*) *jureceki* Jedlička, 1957: 29 (type locality: "Wladiwostok" [= Vladivostok, Russia]; holotype in NMPC); synonymized by Lafer 1989: 180.

*Amara* (*Curtonotus*) *ovalipennis* Jedlička, 1957: 30 (type locality: "Kyoto" [in Japan]; holotype in NMPC); synonymized by Hieke 1995: 322.

**Specimens examined.** 1 male (CLYH), China, Beijing, Haidian district, Baiwangshan Forest Park, 40.033893°N, 116.256957°E, 100 m, 2021.03.12, Yihang Li leg.; 2 males (CLYH), China, Beijing, Changping district, Hedi Road, 40.139454°N, 116.305624°E, 40 m, 2022.06.13, Yihang Li leg.; 3 males (CLYH), China, Sichuan, Mianning County, Tuowu Mountain, 2200 m, 2022.04.15, Yuan Li leg.; 1 female (CLHY), China, Beijing, Haidian district, Yuanmingyuan Park, 40 m, 2022.02.06, Haoyuan Li leg.; 3 males, 2 females (CBJFU), China, Beijing, Shunyi District, Hanshiqiao Wetland Reserve, 2016.10.17, Pingzhou Zhu leg.; 2 males (CBJFU), China, Shanxi, Gujiao, Yunding Mountain Preserve, 35.5246°N, 111.3553°E, 1790 m, 2021.09.10, Xiaojie Sun leg.; 1 female (CLYH), China, Hunan, Huaihua, Subaoding Mt, 1800 m, 2022. 07. 14, Yihang Li leg.; 1 male (CLYH), China, Guizhou, Changshun county, Changshun No.1 Primary School, 2020.08.25, local collector leg.; 4 males, 5 females (CCJH), China, Guangxi, Ziyuan, Shilipingtan, Zijinshan Mt, 26.169585°N, 110.499607°E, 1734 m, Jiaheng Chen leg.

**Chinese common name.** 巨胸暗步甲.

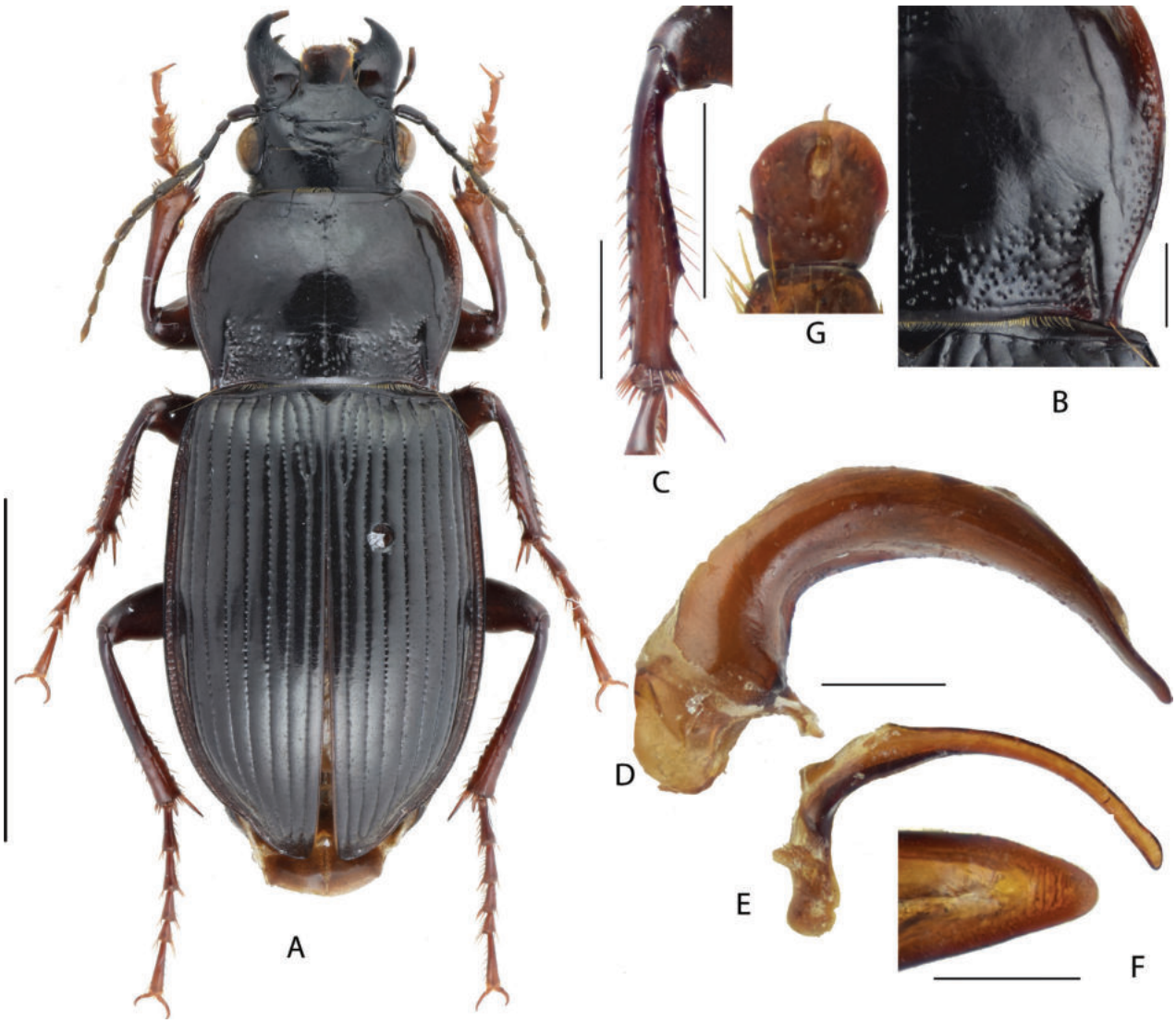


**Figure 11.** *Amara* (*Curtonotus*) *macronota* **A** dorsal habitus, male (Gujiao, Shanxi) **B** pronotum posterior angle **C** male mesotibia **D** lateral view of aedeagus **E** right paramere **F** apical lamella **G** female gonocoxite (Shunyi, Beijing). Scale bars: 5 mm (**A**); 1 mm (**B–E**); 0.5 mm (**F, G**).

**Diagnosis.** Large-sized species, BL = 10.5–13.5 mm; body completely black, legs reddish brown to black; head medium sized, more than half length of pronotum, with two supraorbital setae. Pronotum (Fig. 11B) cordate, widest at middle; densely and very coarsely punctate at basal region, very sparsely punctate at anterior portion; lateral margins strongly sinuate before posterior angles; posterior angle strongly protruded laterally, apex sharp, nearly rectangular. Elytra oblong, widest after middle; coarsely punctate on basal two-thirds of striae; humeral tooth protruded, but smaller than the previously one, apex a little bent backward; microsculpture isodiametric in both sexes; lateral sides of abdominal sternites punctate and wrinkled. Male mesotibiae projection composed of two denticles (Fig. 11C); proximal denticle acutely pointed, near apical third of tibiae; distal denticle slightly smaller, present between the proximal one and tibiae apex. Male genitalia with relatively long apical lamella (Fig. 11F), nearly straight and widely triangular, gradually narrowed to apex, apex rounded; gonocoxite 2 (Fig. 11G) of ovipositor stout, length subequal to greatest width, apex widely rounded.

**Comparison.** This species can be distinguished from related species by having very strongly sinuate lateral margin before posterior angles, nearly straight elytra basal border, heavy punctate on pronotum basal region and elytra striae, and very short gonocoxite.

**Distribution.** Beijing, Fujian, Gansu, Guangdong, Guizhou, Hebei, Heilongjiang, Henan, Hubei, Jiangsu, Jilin, Jiangxi, Liaoning, Inner Mongolia, Sichuan, Shaanxi, Shanxi, Shanghai, Shandong, Tianjin, Yunnan, Zhejiang, Japan, North Korea, South Korea, Russia (Far East), Russia (east Siberia).

## Discussion

In the subgenus *Curtonotus*, the male modified mesotibiae projections have taxonomic importance. In some cases, closely related species can be readily distinguished by the differences on male mesotibiae. According to the examined material of macropterous *Curtonotus* species, the most basic form of the male mesotibiae is composed of an acute proximal denticle beyond the middle of tibiae, and a smaller distal denticle near the midpoint between the proximal denticle and tibial apex (e.g., Fig. 10C). This form is commonly seen in several unrelated species and thought to be plesiomorphic. In other Chinese species, the male mesotibiae are modified in different ways. It is inferred that all these different forms are derived from the basic form of a simple proximal denticle and a distal denticle.

The proximal denticle is always single but different in shape among species. In *A.* (*C.*) *brevicollis* (Fig. 6C), *A.* (*C.*) *banghaasi* (Fig. 8C), and *A.* (*C.*) *macronota* (Fig. 11C), a widened proximal denticle is present, with its basal margin extended to form a wide triangular projection. Among these three species, the proximal denticle is more developed in *A.* (*C.*) *banghaasi* than in other two. The proximal denticle in *A.* (*C.*) *gigantea* (Fig. 2C) is similar to the above three species, but much stronger with a sharply projected apex and serrated basal margin. In *A.* (*C.*) *dux* (Fig. 5C) and *A.* (*C.*) *fodinae* (Fig. 7C), the proximal denticle is absent, with mesotibiae slightly dilated and curved on inner margin.

The distal denticle also varies among different species. Different from the typical form with a single small acute distal denticle, *A.* (*C.*) *gigantea* (Fig. 2C) and *A.* (*C.*) *hiogoensis* (Fig. 9C) both have two equal-sized small distal denticles, whereas in *A.* (*C.*) *dux* (Fig. 5C), *A.* (*C.*) *brevicollis* (Fig. 6C), and *A.* (*C.*) *fodinae* (Fig. 7C), the distal denticle is completely absent.

The specialization of the middle legs is also observed in other carabid clades. *Discoderus* LeConte, a central American genus belonging to the Harpalini, has bowed mesotibiae in males (Shpeley and Ball 1978). Some species of the American harpaline genus *Stenomorphus* Dejean also have arced and hairy mesotibiae in males (Ball et al. 1991). A well-known example is *Agra schwarzeneggeri* Erwin, in which the male of this species "has an enormously enlarged middle femur" (Erwin 2002: 46).

In our study on *Curtonotus*, we put forth the hypothesis that the pattern of mesotibial denticles might be correlated with sexually antagonistic selection within a species. Through our observations of *Curtonotus* copulation images, we observed that certain species (e.g., *Amara* (*C.*) *aulica* Panzer) prefer to mate on stalks or flowers, posing a risk of the male falling off. During this process, the male mesotibiae constantly grip the female's elytra lateral borders or shoul-

ders. This adaptation becomes essential as in most carabid beetles the outer edge of elytra border forms a minute upward reflex, and the large denticles on the male's mesotibiae facilitate securing the female's body by grasping her elytral border reflex, preventing any mishap if the female struggles during mating. Moreover, it may also enable the male to protect the female from disturbances by other males.

Similar functions have been observed in various insect clades, such as the well-known example of the water strider genus *Rheumatobates* Bergroth (Gerridae, Hemiptera), where males possess specialized antennae to grasp and control females during mating (Khila et al. 2012). In the case of the blister beetle *Linsleya convexa* Leconte (Meloidae, Coleoptera), males have spinose tubercles at the base of each foreleg femur, as well as shortened foreleg tibiae and reduced foreleg tibial spurs, all regarded as adaptations for clasping the female and supporting the male's body during mating (Selander and Pinto 1967). Similarly, within the Adephaga, the tiger beetle genus *Manticora* Fabricius exhibits large and asymmetrical mandibles in the males, which are used to guard the female by grasping her thorax (Oberprieler and Arndt 2000).

Among *Curtonotus* species, the one with the largest denticle is *A.* (*C.*) *gigantea*, which also has the largest size and a robust body, indicating more difficulty in controlling the female. The presence of large denticles in males may assist in better control over females. Conversely, the *Curtonotus* species in the *tumida* group, having smaller body sizes within the subgenus, display less specialized mesotibiae. This conclusion might be applicable to other *Amara* species with mesotibiae denticles, such as members of the subgenus *Bradytulus*. However, species like *A.* (*C.*) *dux* and *A.* (*C.*) *fodinae* have medium to large body sizes but lack denticles. We hypothesize that the independent loss of denticles in *A.* (*C.*) *dux* and *A.* (*C.*) *fodinae* may have occurred due to differing behavior strategy, reducing the selective pressure of competition between the opposite sexes. Nevertheless, the specific evolutionary dynamics behind this behavior require further exploration.

It is essential to consider that the specialized male mesotibiae might serve multiple functions during the mating process. For instance, the unique shape of male mesotibiae may serve as a recognition tool for females, preventing copulation between different species. The denticles may also provide a species-specific, localized tactual stimulus for the female, as observed in the blister beetle (Selander and Pinto 1967). Due to limited materials, we have not examined many other species, especially those with distributions outside of China. Additionally, there is scarce documentation of the mating process of *Curtonotus* species. We hope that future research can encompass a broader range of *Curtonotus* species and carefully observe their mating process to further substantiate our hypothesis.

## Acknowledgements

We wish to thank Mr. Tengfei Zhao, Mr. Yulong Shi and Mr. Ge Wang from Beijing Baihuashan National Nature Reserve for their continued help to our field investigation and practice in Xiaolongmen Forestry Park for over ten years. Thanks are also to Dr. Meiying Lin (Institute of Zoology, Chinese Academy Science) for her support when collecting around Beijing. Special gratitude is to Mr. Cong Wang (Beitaoxu Elementary School) for donating multiple specimens

and providing important distributional information for several *Curtonotus* species. We also thank Mr. Ran Meng (Shijiazhuang), Mr. Xiaoran Yang (Northeast Forestry University), Mr. Yuan Li (Deyang), Mr. Sikai Du (Nanjing Nature Watching Science Center), Mr. Bohan Cui (China Pharmaceutical University), Mr. Haoyi Liu (Zhengzhou), Mrs. Hanyu Yu (Lanzhou University), Mr. Deyao Zhou (Shanghai Agricultural Science and Technology), Mr. Taoqi Wang (Northeast Normal University), Mr. Zheng Zhi (Zhengzhou Business University), Mr. Qianle Lu (Shenzhen University), and Mr. Jiaheng Chen (Beijing Forestry University) for providing specimens in the present research. We especially wish to thank Dr. Borislav Gueorguiev (Sofia, Bulgaria) and Dr. Jiri Hejkal (Czech Republic) for their valuable comments on the article.

## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

### Funding

No funding was reported.

### Author contributions

Conceptualization: YL. Investigation: YL. Resources: HS. Visualization: HL. Writing – original draft: YL. Writing – review and editing: HS, HL.

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### Data availability

All of the data that support the findings of this study are available in the main text.

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ZooKeys 1190: 75–89 (2024)
DOI: 10.3897/zookeys.1190.113808

Research Article

# Complete mitochondrial genome of *Guigarra cailaoensis* Wang, Chen & Zheng, 2022 (Cypriniformes, Cyprinidae) and its phylogenetic implications

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## Abstract

*Guigarra cailaoensis* is a member of family Cyprinidae, subfamily Labeoninae (Cypriniformes) which was recently discovered in southwestern China. Following its initial description, additional information on this species has remained notably scarce. In the current study, we assemble the complete mitochondrial genome (mitogenome) of *G. cailaoensis* using the Illumina sequencing platform. The mitogenome is identified as a circular, double-stranded DNA sequence of 16,593 base pairs, encompassing 13 protein-coding genes (PCGs), 22 transfer RNA genes, two ribosomal RNA genes, and a putative control region. Maximum-likelihood and Bayesian-inference approaches were used to construct phylogenetic trees for three datasets: (i) PCG sequences of the complete mitogenome (dataset 1); (ii) PCG sequences of the complete mitogenome combined with nuclear DNA (ncDNA) (*Rag1*) sequence (dataset 2); and (iii) ncDNA (*Rag1*) sequences (dataset 3). Phylogenetic analyses position *G. cailaoensis* as a sister taxon to the lineage consisting of *Paraqianlabeo lineatus* Zhao, Sullivan, Zhang & Peng, 2014 and *Pseudogyrinocheilus prochilus* Fang, 1933 in dataset 1, and to *Pseudogyrinocheilus prochilus* in dataset 2, species lacking an oral disc on the lower lip. However, *G. cailaoensis* showed a close relationship to the lineage consisting of *Discogobio* and *Discocheilus* in dataset 3, species possessing an oral disc on the lower lip. Nonetheless, a variety of species with an oral disc on the lower lip are clustered into different lineages across the three datasets that may indicate that the development of the oral disc is homoplastic within the subfamily Labeoninae. The outcomes of this study have the potential to support conservation efforts for this species and to enrich our understanding of genetic resources in the area.

**Key words:** Illumina, Labeoninae, phylogeny, southwestern China

OPEN ACCESS

Academic editor: Nina Bogutskaya
Received: 7 October 2023
Accepted: 18 December 2023
Published: 22 January 2024

ZooBank: https://zoobank.org/01B2AB92-FB2C-4CAA-BC96-C8E85DB2B528

Citation: Zheng L-P, Geng Y-M (2024) Complete mitochondrial genome of *Guigarra cailaoensis* Wang, Chen & Zheng, 2022 (Cypriniformes, Cyprinidae) and its phylogenetic implications. ZooKeys 1190: 75–89. https://doi.org/10.3897/zookeys.1190.113808

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## Introduction

*Guigarra cailaoensis* Wang, Chen & Zheng, 2022, a recently described genus and species in the subfamily Labeoninae of the family Cyprinidae (Cypriniformes), is a small fish adapted to torrent-water environments. To date, it has only been recorded in a small tributary of the Hongshuihe River in Guangxi Province, China. There it inhabits small streams in the upper reaches of the tributary,

while being notably absent from the lower reaches (Wang et al. 2022). These environments are very fragile, rendering *G. cailaoensis* a potential indicator of local ecological conditions.

The subfamily Labeoninae, recognized by its unique oral morphology (Zhang et al. 2000), comprises more than 40 genera and 500 species. Within this subfamily, eight genera and nearly 200 species are characterized by a structurally varied oral disc on the lower lip (Wang et al. 2022). *Guigarra* Wang, Chen & Zheng, 2022, which also exhibits this feature, is a recently discovered genus found in the karst region in southwestern China, following the discovery of *Lanlabeo* Yao, He & Peng, 2018 in the same area.

Previous research on the subfamily Labeoninae has predominantly focused on taxonomy, particularly the description of new genera and species, as well as molecular phylogenetics. In recent years, various new Labeoninae genera and species from the karst region of southwestern China have been described, including *Sinigarra* Zhang & Zhou, 2012, *Paraqianlabeo* Zhao, Sullivan, Zhang & Peng, 2014, *Prolixicheilus* Zheng, Chen & Yang, 2016, *Zuojiangia* Zheng, He, Yang & Wu, 2018, *Lanlabeo* Yao, He & Peng, 2018, and *Guigarra* Wang, Chen & Zheng, 2022 (Zhang and Zhou 2012; Zhao et al. 2014; Zheng et al. 2016, 2018; Yao et al. 2018; Wang et al. 2022), highlighting the rich species diversity in this subfamily. Furthermore, molecular phylogenetic studies have elucidated the phylogenetic relationships within the subfamily and validated the classification of genera (Yang and Mayden 2010; Zheng et al. 2010, 2012; Yang et al. 2012). Yang et al. (2012) identified four primary clades within the subfamily Labeoninae, with Zheng et al. (2016) defining the karst group as part of the fourth clade. Wang et al. (2022) further established that *G. cailaoensis* belongs to this karst group.

Mitochondrial genomes (mitogenomes) are characterized by a simple molecular structure, strict maternal inheritance, minimal recombination, and a rapid evolutionary rate, making them valuable markers in studies of molecular population genetics and phylogenetics (Xiao and Zhang 2000). As mitogenomic research has advanced, the mitogenomes of fewer than 100 species of Labeoninae have been sequenced and deposited in GenBank. In this study, we successfully sequenced the complete mitogenome of *G. cailaoensis*. Our findings could contribute to the conservation of this species and further enrich genetic resources.

## Materials and methods

### Sample collection, DNA extraction, and quality testing

The sample used in this study was collected from the Cailaohe River, Fengshan, Guangxi, China (24.61°N, 106.97°E). Total genomic DNA was extracted from fin-tissue samples using a DNA isolation kit (Qiagen) with a final elution volume of 50 µl. The quality and purity of the isolated DNA were assessed prior to downstream applications. Agarose gel electrophoresis was used to analyze DNA integrity and assess the presence of contaminants. DNA purity was evaluated using a NanoDrop One spectrophotometer (Thermo Fisher Scientific, USA). Final DNA concentrations were accurately determined using a Qubit 3.0 Fluorometer (Thermo Fisher Scientific, USA).

## Library construction, mitogenome assembly, and annotation

The collected DNA sample was used for paired-end (PE) library construction using standard protocols of the NEBNext Ultra II DNA Library Prep Kit for Illumina (NEB, USA) in accordance with the manufacturer's instructions. It was sequenced using the Illumina NovaSeq 6000 platform (Illumina, USA) with a 350-bp insert size. Adaptor and low-quality reads were filtered using fastp (Chen 2023), resulting in a total of 69.21 Mb of clean reads (150 bp). The mitogenome was *de novo* assembled using MitoZ (Meng et al. 2019). The assembled mitogenome was annotated using the online tool MITOS using the default parameters (Bernt et al. 2013). The protein-coding sequences were checked and confirmed using Geneious R10 (Kearse et al. 2012). Start/stop codons, codon usages, relative synonymous codon usage (RSCU), and nucleotide composition were analyzed using MEGA v. 7 (Kumar et al. 2016) and PhyloSuite (Xiang et al. 2023). Skew compositions were calculated using: AT-skew = (A − T) / (A + T) and GC-skew = (G − C) / (G + C) (Perna and Kocher 1995). TRNAscan-SE v. 2.0 (Lowe and Chan 2016) was used to predict the secondary structures and anticodons of transfer RNAs (tRNAs). The online mitochondrial visualization tool OGDRAW (Greiner et al. 2019) was used to draw a graphical map of the complete mitogenome. The newly generated complete mitogenome sequence and its annotation were submitted to GenBank using BankIt (accession number OR492308).

## Phylogenetic analysis

To determine the phylogenetic position of *G. cailaoensis*, 92 complete mitogenomes and 68 *Rag1* sequences of Labeoninae were downloaded from GenBank, and one species of Torinae, two species of Xenocypridinae, and three species of Opsariichthyinae were used as the outgroups (Mayden et al. 2009). Three datasets were constructed for analyses: (i) protein-coding gene (PCG) sequences of the complete mitogenome (dataset 1); (ii) PCG sequences of the complete mitogenome combined with nuclear DNA (ncDNA) (*Rag1*) sequences (dataset 2); and (iii) ncDNA (*Rag1*) sequences (dataset 3). All sequences were first aligned using MAFFT v. 7.475 (Katoh and Standley 2013), then trimmed using trimAl (Salvador et al. 2009). Maximum-likelihood (ML) and Bayesian-inference (BI) approaches were used to construct phylogenetic trees based on the three datasets. The ML analysis was performed using IQ-TREE v. 2.1.4 (Minh et al. 2020) based on the best-substitution model selected by ModelFinder in the IQ-TREE package (Kalyaanamoorthy et al. 2017). Nodal support was assessed based on 1,000 bootstrap replicates (Felsenstein 1985). The BI analysis was performed using MrBayes v. 3.2.7 (Ronquist et al. 2012), with the best-fit nucleotide substitution model also determined using ModelFinder. Four chains (three hot, one cold) were run for 5 million generations, with tree sampling every 1,000 generations and the first 25% of samples discarded as burn-in. Convergence was confirmed by ascertaining that the average standard deviation of split frequencies was below 0.01. The phylogenetic trees were viewed and edited using FigTree v. 1.4.4 (Rambaut 2014).

## Results

### Mitogenome composition and organization

The mitogenome of *Guigarra cailaoensis* was identified as a circular double-stranded DNA sequence of 16,593 base pairs (bp) in length and included 13 protein-coding genes, 22 tRNA genes, two ribosomal RNA (rRNA) genes, and a putative control region (Table 1, Fig. 1). The base composition of *G. cailaoensis* was A = 32.2%, G = 15.5%, T = 26.4%, and C = 26.0%, with higher AT content (58.6%) than GC content (41.4%) (Table 2).

### PCGs and codon usage

The PCGs had a total length of 11,412 bp, accounting for 68.78% of the total length of the complete mitogenome. The *ND5* coding DNA sequence (CDS) had the highest number of base pairs (1 824 bp), while ATPase8 had the lowest (165 bp).

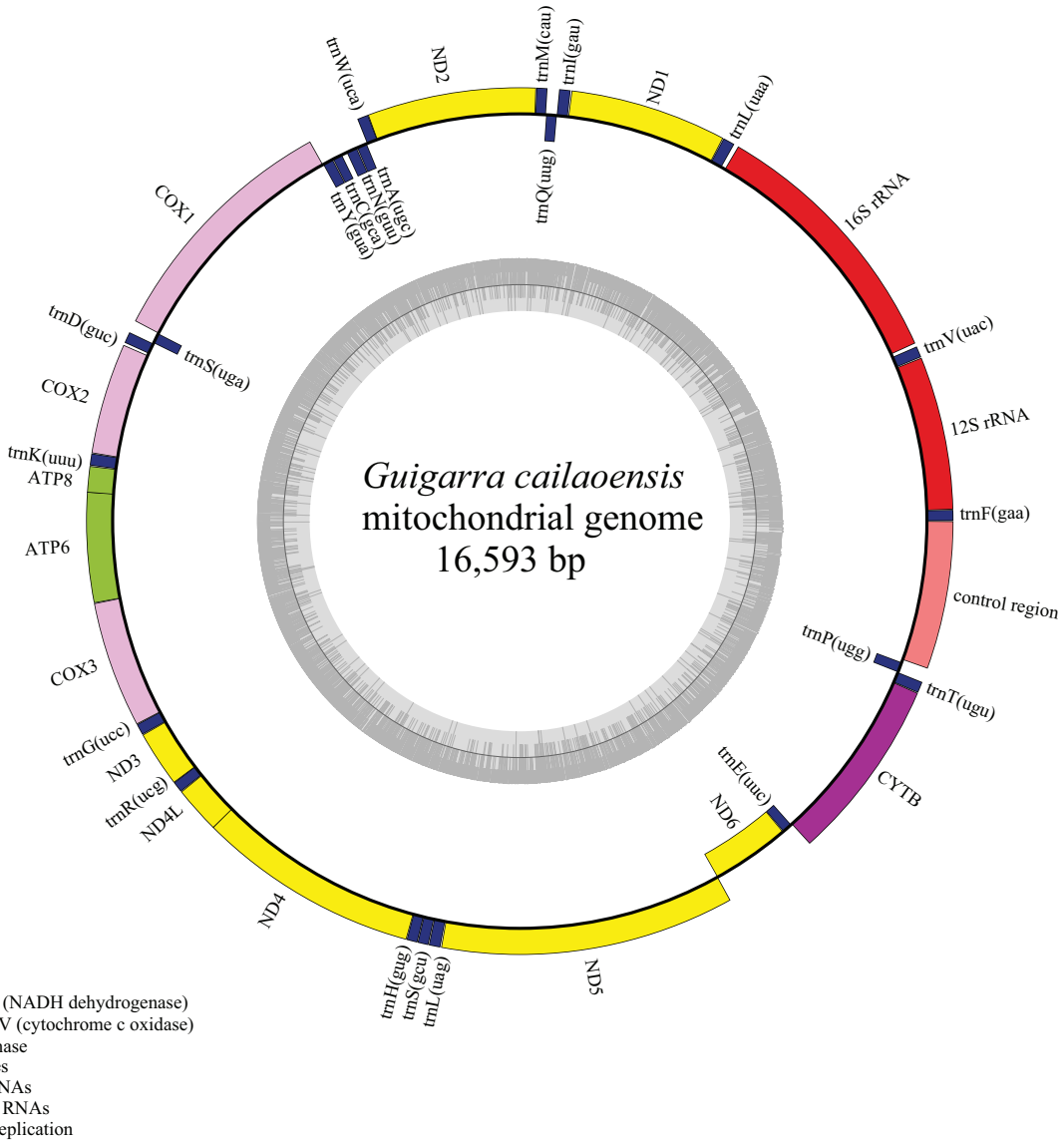


**Figure 1.** Circular map of complete mitogenome of *Guigarra cailaoensis*.

**Table 1.** Summary of genetic components of *Guigarra cailaoensis* mitogenome.

| Gene | Type | Initial bp | Final bp | Length | Direction | Strand | Start codon | Stop codon | Anticodon |
|---|---|---|---|---|---|---|---|---|---|
| *trnF* | tRNA | 1 | 69 | 69 | forward | H | | | GAA |
| *12S rRNA* | rRNA | 70 | 1021 | 952 | forward | H | | | |
| *trnV* | tRNA | 1024 | 1095 | 72 | forward | H | | | TAC |
| *16S rRNA* | rRNA | 1115 | 2755 | 1641 | forward | H | | | |
| *trnL1* | tRNA | 2781 | 2856 | 76 | forward | H | | | TAA |
| *ND1* | CDS | 2858 | 3832 | 975 | forward | H | ATG | TAA | |
| *trnI* | tRNA | 3837 | 3908 | 72 | forward | H | | | GAT |
| *trnQ* | tRNA | 3907 | 3977 | 71 | reverse | L | | | |
| *trnM* | tRNA | 3979 | 4047 | 69 | forward | H | | | CAT |
| *ND2* | CDS | 4048 | 5094 | 1047 | forward | H | ATG | TAG | |
| *trnW* | tRNA | 5093 | 5163 | 71 | forward | H | | | TCA |
| *trnA* | tRNA | 5166 | 5234 | 69 | reverse | L | | | TGC |
| *trnN* | tRNA | 5236 | 5308 | 73 | reverse | L | | | GTT |
| OL | rep_origin | 5311 | 5342 | | | | | | |
| *trnC* | tRNA | 5342 | 5407 | 66 | reverse | L | | | GCA |
| *trnY* | tRNA | 5409 | 5479 | 71 | reverse | L | | | GTA |
| *COX1* | CDS | 5481 | 7031 | 1551 | forward | H | GTG | TAA | |
| *trnS1* | tRNA | 7032 | 7102 | 71 | reverse | L | | | GCT |
| *trnD* | tRNA | 7106 | 7177 | 72 | forward | H | | | GTC |
| *COX2* | CDS | 7191 | 7881 | 691 | forward | H | ATG | T-- | |
| *trnK* | tRNA | 7882 | 7957 | 76 | forward | H | | | TTT |
| *ATP8* | CDS | 7959 | 8123 | 165 | forward | H | ATG | TAG | |
| *ATP6* | CDS | 8117 | 8800 | 684 | forward | H | ATG | TAA | |
| *COX3* | CDS | 8800 | 9585 | 786 | forward | H | ATG | TAA | |
| *trnG* | tRNA | 9585 | 9656 | 72 | forward | H | | | TCC |
| *ND3* | CDS | 9657 | 10007 | 351 | forward | H | ATG | TAG | |
| *trnR* | tRNA | 10006 | 10075 | 70 | forward | H | | | TCG |
| *ND4L* | CDS | 10076 | 10372 | 297 | forward | H | ATG | TAA | |
| *ND4* | CDS | 10366 | 11746 | 1381 | forward | H | ATG | T-- | |
| *trnH* | tRNA | 11747 | 11815 | 69 | forward | H | | | GTG |
| *trnS2* | tRNA | 11816 | 11884 | 69 | forward | H | | | TGA |
| *trnL2* | tRNA | 11886 | 11958 | 73 | forward | H | | | TAA |
| *ND5* | CDS | 11962 | 13785 | 1824 | forward | H | ATG | TAA | |
| *ND6* | CDS | 13782 | 14303 | 522 | reverse | L | ATG | TAA | |
| *trnE* | tRNA | 14304 | 14372 | 69 | reverse | L | | | TTC |
| *CYTB* | CDS | 14377 | 15517 | 1141 | forward | H | ATG | T-- | |
| *trnT* | tRNA | 15518 | 15589 | 72 | forward | H | | | TGT |
| *trnP* | tRNA | 15589 | 15658 | 70 | reverse | L | | | TGG |
| D-loop | D-loop | 15676 | 16593 | 918 | forward | H | | | |

**Table 2.** Base composition and skewness of the mitogenome of *Guigarra cailaoensis*.

| Regions | Size (bp) | T(U) | C | A | G | AT (%) | GC (%) | AT skewness | GC skewness |
|---|---|---|---|---|---|---|---|---|---|
| *ATP6* | 684 | 30.7 | 25.3 | 31.3 | 12.7 | 62.0 | 38.0 | 0.009 | −0.331 |
| *ATP8* | 165 | 27.3 | 26.1 | 35.2 | 11.5 | 62.5 | 37.6 | 0.126 | −0.387 |
| *COX1* | 1551 | 30.0 | 25.4 | 27.6 | 17.0 | 57.6 | 42.4 | −0.043 | −0.199 |
| *COX2* | 691 | 26.9 | 25.9 | 30.7 | 16.5 | 57.6 | 42.4 | 0.065 | −0.222 |
| *COX3* | 786 | 27.2 | 28.0 | 29.0 | 15.8 | 56.2 | 43.8 | 0.032 | −0.279 |
| *CYTB* | 1141 | 28.9 | 26.7 | 30.9 | 13.5 | 59.8 | 40.2 | 0.032 | −0.329 |
| *ND1* | 975 | 27.9 | 26.9 | 31.4 | 13.8 | 59.3 | 40.7 | 0.059 | −0.320 |
| *ND2* | 1047 | 24.4 | 30.2 | 33.2 | 12.2 | 57.6 | 42.4 | 0.154 | −0.423 |
| *ND3* | 351 | 30.2 | 27.9 | 27.6 | 14.2 | 57.8 | 42.1 | −0.044 | −0.324 |
| *ND4* | 1381 | 27.9 | 26.4 | 32.4 | 13.3 | 60.3 | 39.7 | 0.075 | −0.330 |
| *ND4L* | 297 | 29.3 | 27.9 | 27.3 | 15.5 | 56.6 | 43.4 | −0.036 | −0.287 |
| *ND5* | 1824 | 27.0 | 27.7 | 33.1 | 12.1 | 60.1 | 39.8 | 0.101 | −0.392 |
| *ND6* | 522 | 42.7 | 11.7 | 15.3 | 30.3 | 58.0 | 42.0 | −0.472 | 0.443 |
| PCGs | 11412 | 28.6 | 26.3 | 30.3 | 14.7 | 58.9 | 41.1 | 0.028 | −0.282 |
| rRNAs | 2593 | 20.2 | 23.8 | 35.4 | 20.6 | 55.6 | 44.4 | 0.274 | −0.073 |
| tRNAs | 1562 | 27.2 | 20.7 | 29.0 | 23.1 | 56.2 | 43.8 | 0.032 | 0.056 |
| CR | 918 | 34.0 | 18.7 | 33.9 | 13.4 | 67.9 | 32.1 | −0.002 | −0.166 |
| Full | 16593 | 26.4 | 26.0 | 32.2 | 15.5 | 58.6 | 41.5 | 0.100 | −0.254 |

The base percentage composition revealed a lower G + C content (41.1%) compared to the A + T content (58.9%). All PCGs were encoded on the heavy (H) strand, except for the *ND6* gene, which was encoded on the light (L) strand. All PCGs were initiated with the methionine codon ATG, except for *COX1*, which was initiated with GTG, consistent with previous labeonine mitochondrial DNAs (Wang et al. 2019). Two types of stop codon were identified: TAA (*ATP6*, *COX1*, *COX3*, *ND1*, *ND4L*, *ND5*, and *ND6*) and TAG (*ATP8*, *ND2*, and *ND3*). Incomplete stop codons were detected for *COX2*, *CYTB*, and *ND4* (Table 1).

The RSCU results indicated that six codons, CUA (2.35%), CGA (2.35%), CCA (2.33%), GGA (2.20%), UCA (2.19%), and GUA (2.16%), were the most frequently used. Additionally, the amino acids Pro, Thr, Leu1, Arg, Ala, Ser2, Val, and Gly were encoded by four codons, while all the other amino acids were encoded by two codons (Fig. 2).

## Ribosomal and transfer RNA genes

The *12S rRNA* and *16S rRNA* were 952 and 1,641 bp in length, respectively. They were located between *trnF* and *trnL1*, separated by *trnV*. The nucleotide composition of the rRNAs was A = 35.4%, C = 23.8%, G = 20.6%, and T = 20.2%. Thus, *G. cailaoensis* displayed a higher percentage of AT (55.6%) than GC (44.4%) (Table 2).

Twenty-two tRNA genes were identified in *G. cailaoensis* mitogenome, including two for *trnL* and *trnS*, and one for each of the other amino acids (Table 1). Of these, 21 tRNA genes exhibited the typical cloverleaf secondary structure with four domains, while the *trnS1* gene lacked the D domain (D-stem and D-loop) (Fig. 3).

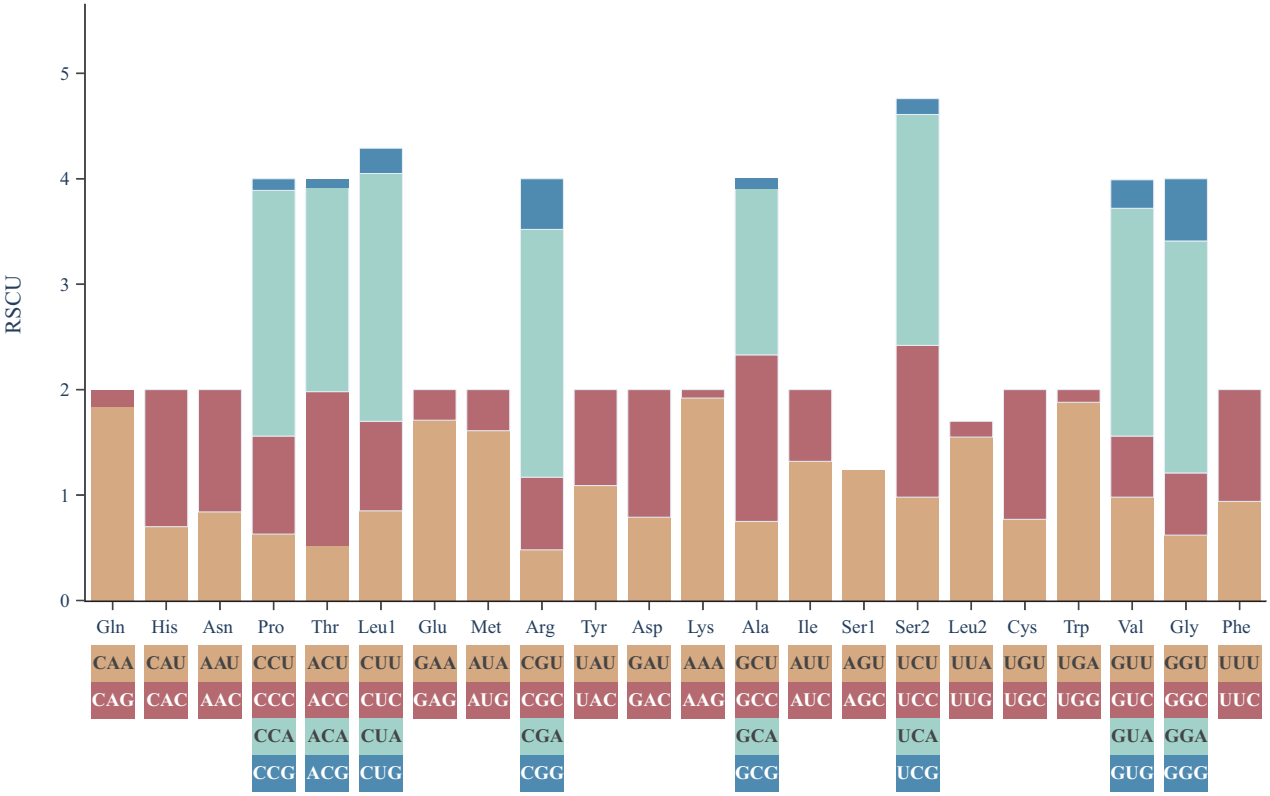


**Figure 2.** Relative synonymous codon usage (RSCU) in mitogenomes of *Guigarra cailaoensis*.

Fourteen tRNAs were encoded on the H-strand, while the remaining tRNAs were encoded on the L-strand (*trnQ*, *trnA*, *trnN*, *trnC*, *trnY*, *trnS1*, *trnE*, and *trnP*; Table 1). The length of these tRNAs varied, ranging from 66 bp (*trnC*) to 76 bp (*trnL1* and *trnK*), with a total length of 1,562 bp and accounting for 9.41% of the total mitogenome. Nucleotide composition of the tRNAs was A = 29.0%, C = 20.7%, G = 23.1%, and T = 27.2%, showing a higher AT content (56.2%) than GC content (43.8%) (Table 2).

## Non-coding region

The non-coding control region in the mitogenome, identified as the D-loop, was located between the *trnP* and *trnF* genes (Fig. 1). Spanning 918 bp in length, the region accounted for 5.53% of the whole mitogenome. The region exhibited a higher AT content (67.9%) than GC content (32.1%), with a nucleotide composition of A = 33.9%, T = 34.0%, C = 18.7%, and G = 13.4% (Table 2).

## Phylogenetic analysis

The best-fit models for ML and BI analyses were identified, as shown in Table 3. Phylogenetic trees derived from dataset 1 and 2 were remarkably similar, while those from dataset 3 showed slight variations. Within each dataset, the trees generated from ML and BI analyses were consistent across all taxa, differing only slightly in their support values. Consequently, the ML trees were presented here together with the nodal support values generated by ML and BI analyses, respectively. Notably, all taxa within Labeoninae were recovered as a

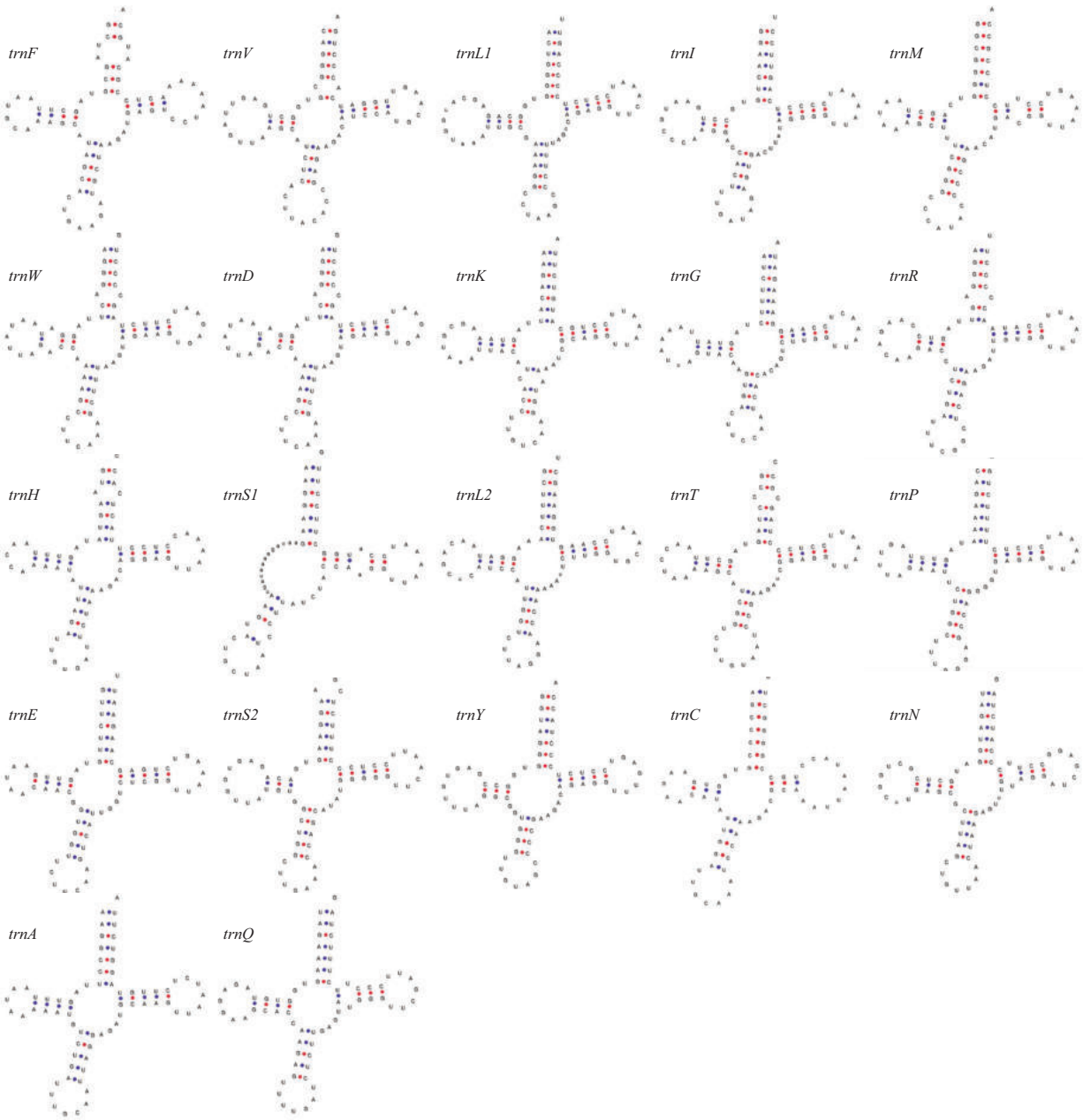


**Figure 3.** Secondary structures of 22 tRNA genes in *Guigarra cailaoensis*.

**Table 3.** The best-fit models selected by ModelFinder for three datasets.

| | ML | BI |
|---|---|---|
| Dataset 1 (Mitogenome) | TIM2 + F + R6 | GTR + F + I + G4 |
| Dataset 2 (Mitogenome+*Rag1*) | GTR + F + R6 | GTR + F + I + G4 |
| Dataset 3 (*Rag1*) | TIM2e + I + G4 | SYM + I + G4 |

monophyletic clade, further subdivided into four major lineages. In dataset 1, *G. cailaoensis* formed a sister taxon to the lineage consisting of *Paraqianlabeo lineatus* and *Pseudogyrinocheilus prochilus*, and in dataset 2 formed a sister taxon to *Pseudogyrinocheilus prochilus*. However, in dataset 3, *G. cailaoensis* formed a sister taxon to the lineage consisting of *Discogobio* and *Discocheilus* (Figs 4–6).

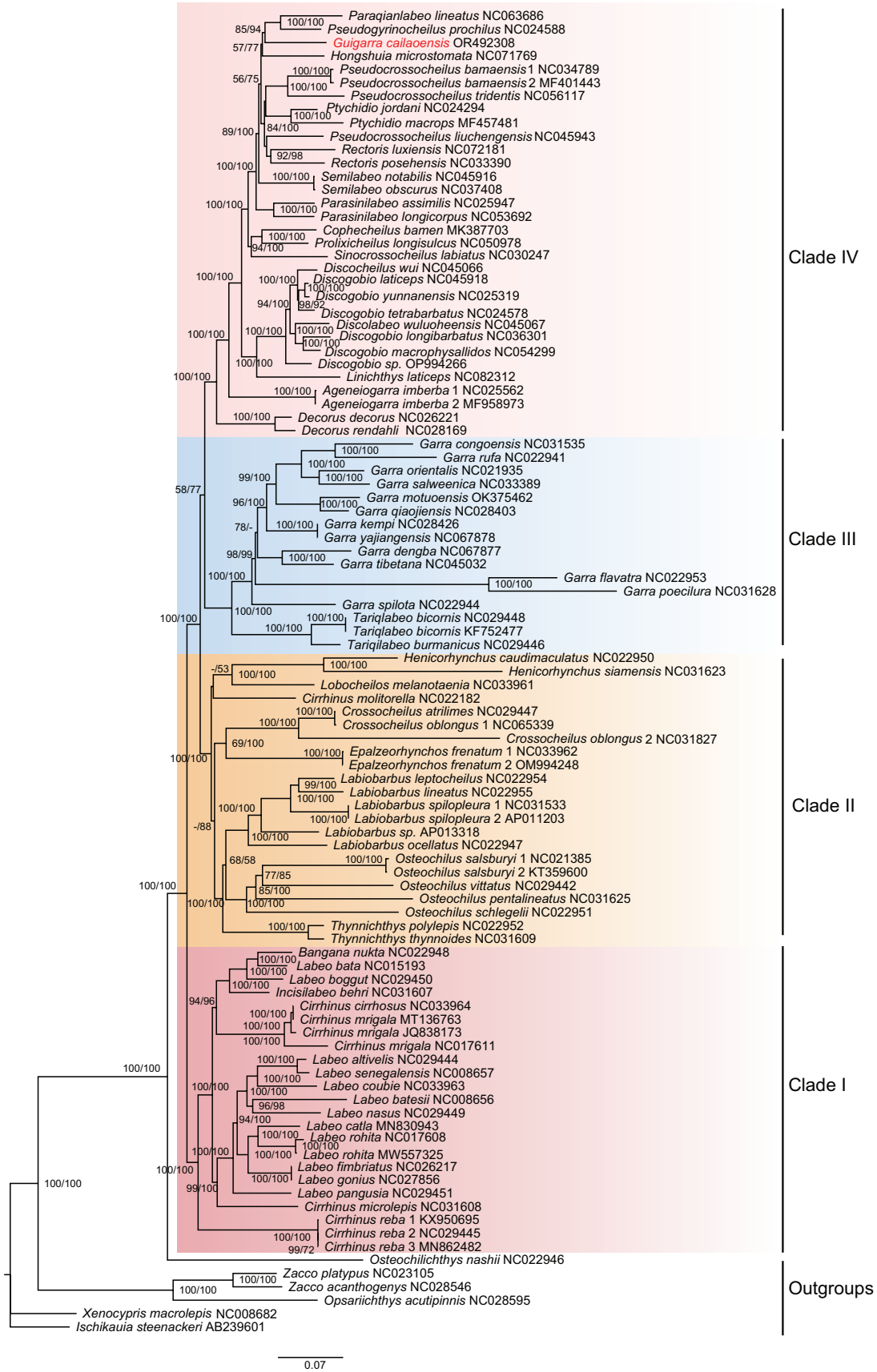


**Figure 4.** Phylogenetic tree of *Guigarra cailaoensis* and 98 species downloaded from GenBank based on PCG sequences of complete mitogenomes (dataset 1). Nodal numbers are ML bootstrap values and BI posterior probability values, respectively. Only values above 50% are given.

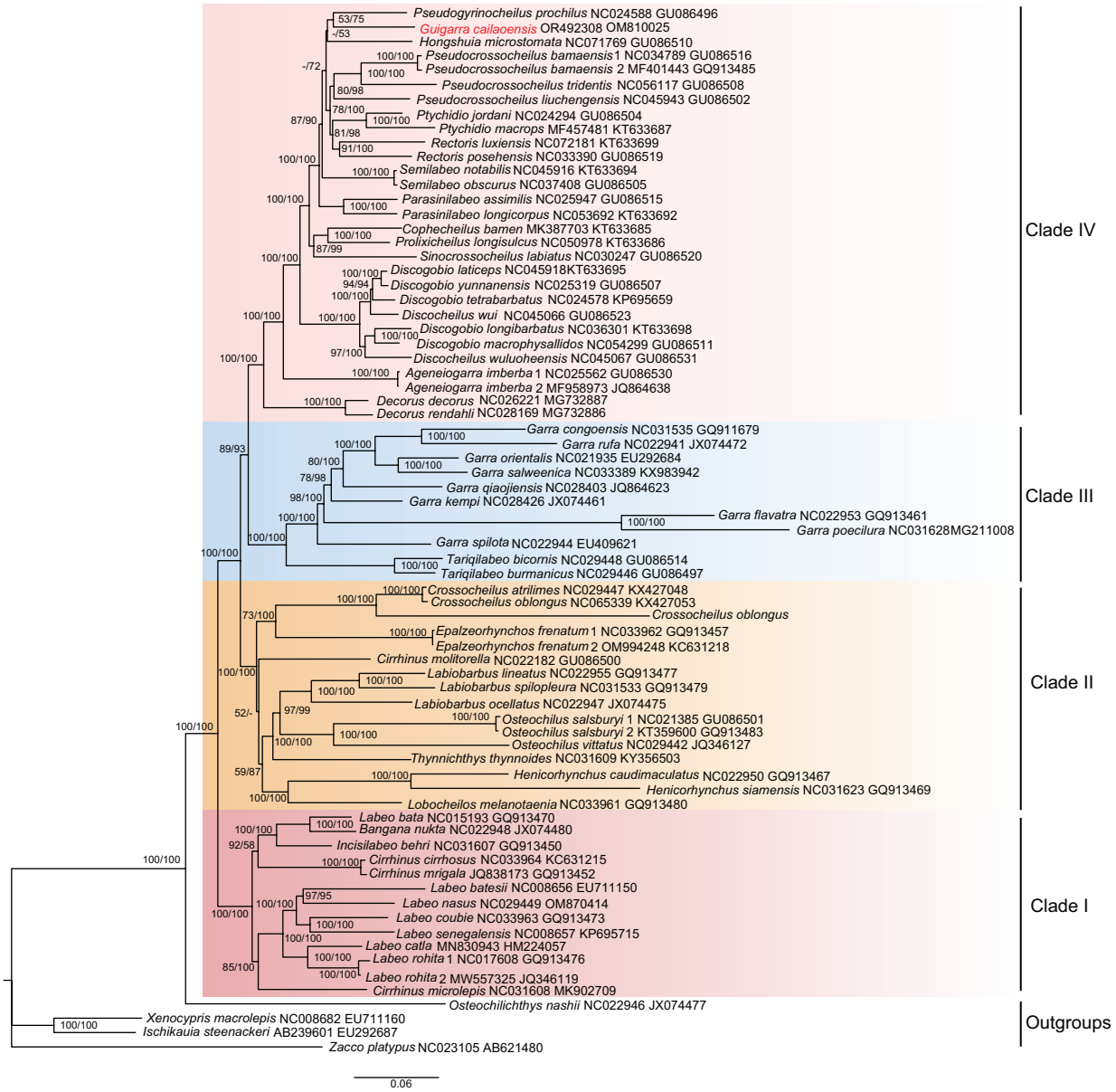


**Figure 5.** Phylogenetic tree of *Guigarra cailaoensis* and 72 species downloaded from GenBank based on PCG sequences of complete mitogenome combined with ncDNA (*Rag1*) sequences (dataset 2). Nodal numbers are ML bootstrap values and BI posterior probability values, respectively. Only values above 50% are given.

## Discussion

The mitochondrial gene structure in *Guigarra cailaoensis* is congruent with that of other vertebrate animals, consisting of double-stranded circular DNA spanning approximately 15–20 kb (Miya et al. 2001). Furthermore, its base composition is also consistent with results observed in other Labeoninae fish species (Zheng and Yang 2017; Zhang et al. 2023), and the features of its tRNA genes are consistent with those observed in metazoan mitochondrial DNA (Watanabe et al. 2014).

Our phylogenetic analyses of the subfamily Labeoninae across three datasets identified four lineages, which is consistent with the results of Yang et al. (2012). When considering the species common to all three datasets, the

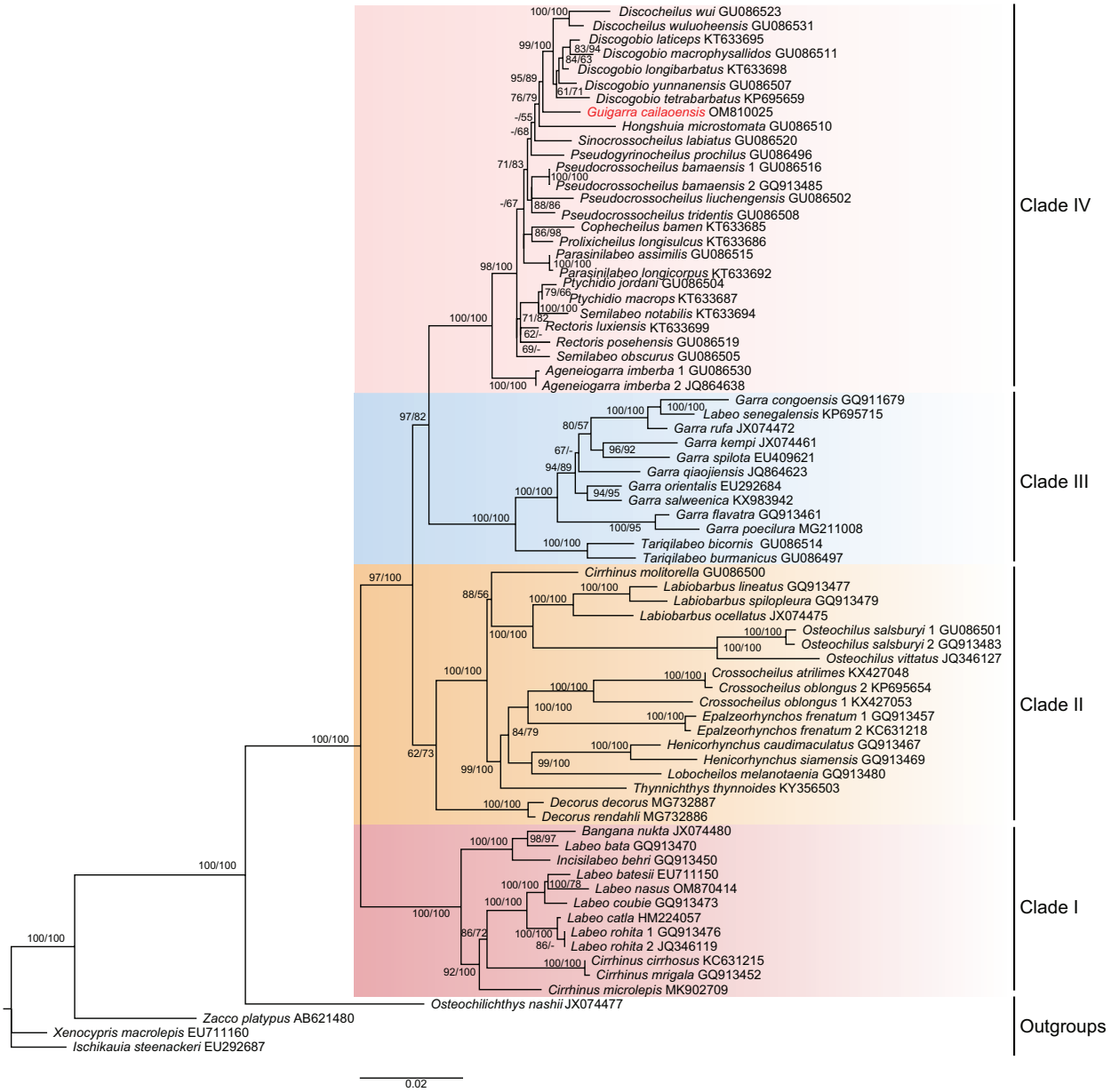


**Figure 6.** Phylogenetic tree of *Guigarra cailaoensis* and 72 species downloaded from GenBank based on ncDNA (*Rag1*) sequences (dataset 3). Nodal numbers are ML bootstrap values and BI posterior probability values, respectively. Only values above 50% are given.

phylogenetic trees derived from datasets 1 and 2 were nearly identical, while those derived from dataset 3 differed slightly. The main divergence was observed in the placement of the *Decorus decorus* and *Decorus rendahli* lineage, located in Clade IV in datasets 1 and 2 but in Clade II in dataset 3. *Guigarra cailaoensis* was positioned in Clade IV, corresponding to the fourth clade described by Yang et al. (2012) and within the karst group defined by Zheng et al. (2016). Currently, approximately eight genera within Labeoninae are characterized by the presence of an oral disc on the lower lip, including *Ageneiogarra*, *Ceratogarra*, *Discocheilus*, *Discogobio*, *Garra*, *Guigarra*, *Sinigarra*, and *Placocheilus* (Wang et al. 2022). Our results revealed that the genera with oral discs are distributed across different lineages in the three datasets. Although *G. cailaoensis* possesses an oral disc on its lower lip, it was not closely related

to other oral disc-bearing species based on datasets 1 and 2. In dataset 1, it is closely related to *Paraqianlabeo lineatus* and *Pseudogyrinocheilus prochilus*, and in dataset 2, it is closely related to *Pseudogyrinocheilus prochilus*, neither of which possess an oral disc on the lower lip. The results derived from dataset 1 and 2 are essentially consistent, because only the complete mitogenome sequences of *Pseudogyrinocheilus prochilus* are available in dataset 1 and 2, and *Paraqianlabeo lineatus* was not included in dataset 2. However, in dataset 3, *G. cailaoensis* is closely related to the lineage consisting of *Discogobio* and *Discocheilus*, both of which possess an oral disc on the lower lip. The phylogenetic position of *G. cailaoensis* and its closely related taxa derived from dataset 3 are consistent with the results of Wang et al. (2022), who reported a close affinity between *G. cailaoensis*, *Discogobio*, and *Discocheilus* based on one nuclear and two mitochondrial genes. We hypothesize that the observed inconsistencies among the results of different datasets likely stem from differences in the phylogenetic signal contribution between mitogenome and nuclear gene loci, as discussed by Zheng et al. (2010). Nonetheless, a variety of species with an oral disc on the lower lip were clustered into different lineages in the results from three datasets that may indicate that the development of the oral disc is homoplastic within the subfamily Labeoninae. In conclusion, the successful assembly of the complete mitogenome of *G. cailaoensis* not only enhances our understanding of its genetic background but may also prove valuable for conservation and resource restoration strategies in the area.

## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

This study does not require ethical approval because no procedures were performed on live animals, and the tissue samples used were collected from the dead specimens.

### Funding

This work was supported by the National Natural Science Foundation of China (31960103) and Yunnan Provincial Basic Research Special Project (202301AT070254).

### Author contributions

Conceptualization: LPZ. Data curation: LPZ. Funding acquisition: LPZ. Methodology: LPZ, YMG. Project administration: LPZ. Software: LPZ, YMG. Supervision: LPZ. Visualization: LPZ. Writing – original draft: LPZ. Writing – review and editing: LPZ, YMG.

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### Data availability

All of the data that support the findings of this study are available in the main text. The genome sequence data are openly available in GenBank of NCBI at (https://www.ncbi.nlm.nih.gov/) under the accession no. OR492308.

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ZooKeys 1190: 91–106 (2024)
DOI: 10.3897/zookeys.1190.113126

Research Article

# The adult, pupa, and larva of a new species of *Gnaptorina* Reitter, 1887 (Coleoptera, Tenebrionidae, Blaptini) from the Tibetan Plateau, with molecular phylogenetic inferences

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## Abstract

The adult, pupa and larva of a new species, *Gnaptorina* (*Gnaptorina*) *lhorongica* Li, **sp. nov.**, from northeastern Xizang, China are described and illustrated. The species was identified using molecular phylogenetic analyses based on three mitochondrial fragments and one nuclear gene fragment (*COI*, *Cytb*, *16S*, and *28S*-D2). The taxonomic status of the new species is confirmed using a combination of molecular and morphological datasets. This study provides valuable molecular and morphological data for phylogenetic studies of the tribe Blaptini.

**Key words:** Beetle, China, DNA sequence, morphological description

OPEN ACCESS

Academic editor: Patrice Bouchard
Received: 22 September 2023
Accepted: 4 January 2024
Published: 23 January 2024

ZooBank: https://zoobank.org/12561B99-6F89-414E-BAB5-AF6D84DD75CD

Citation: Ji B-Y, Ma X-T, Rong J-D, Ren G-D, Pan Z, Li X-M (2024) The adult, pupa, and larva of a new species of *Gnaptorina* Reitter, 1887 (Coleoptera, Tenebrionidae, Blaptini) from the Tibetan Plateau, with molecular phylogenetic inferences. ZooKeys 1190: 91–106. https://doi.org/10.3897/zookeys.1190.113126

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## Introduction

The genus *Gnaptorina* Reitter, 1887 belongs to the subtribe Gnaptorinina Medvedev, 2001 of the tribe Blaptini Leach, 1815 of the subfamily Blaptinae Leach, 1815 (Kamiński et al. 2021). The Gnaptorinina is a species-rich subtribe in Blaptini, consisting of 11 genera. Larval and pupal morphology is important for understanding the systematics of different groups of Coleoptera, and it has been used to support the close relationships of genera or subtribes (Grebennikov and Scholtz 2004; Lawrence et al. 2011; Chigray 2019; Kamiński et al. 2019). To date, the morphology of immature stages of seven genera and 40 species are known within Blaptini: *Blaps* Fabricius, 1775 (larvae of 25 spp. and pupae of 10 spp.), *Prosodes* Eschscholtz, 1829 (larvae of five spp.), *Dila* Fischer von Waldheim, 1844 (larvae of two spp.), *Nalepa* Reitter, 1887 (larvae of two spp.), *Gnaptorina* Reitter, 1887 (larvae of two spp.), *Agnaptoria* Reitter, 1887 (larvae of two spp.) and *Itagonia* Reitter, 1887 (larva of one sp.) (Yu et al. 1993a, 1993b, 1996, 1999a, 1999b, 2000; Ren et al. 2000; Zhang et al. 2000, 2005; Yu and Zhang 2004, 2005; Zhao et al. 2009; Zhu and Ren 2014; Li et al. 2022, 2023; Tang et al. 2023).

* These authors contributed equally to this work and should be considered co-first authors.

*Gnaptorina* is the third largest genus of the subtribe Gnaptorinina with 38 described species (Medvedev 2009; Shi 2013; Ren et al. 2016; Li et al. 2019; Bai et al. 2020). All *Gnaptorina* species have been recorded in China, except for *G. sikkimensis* Kaszab, 1965, which is endemic to northern India, and *G. brucei* Blair, 1923, which occurs in Nepal and northern India. However, the larval morphology of only two species (*G. felicitana* Reitter, 1887 and *G. cylindricollis* Reitter, 1889) have been described (Yu et al. 1996; Zhu and Ren 2014), and pupal morphology is still unknown due to the lack of specimens.

In this study, the adults, pupa and larva of a new *Gnaptorina* species are described based on morphological and molecular evidence. In addition, we construct a molecular phylogeny for the genus and apply it to verify the taxonomic status of the new species.

## Materials and methods

### Morphological examination

In total, 64 adults, two larvae, and one pupa of the new species were examined for this study and deposited at the Museum of Hebei University, Baoding, China (**MHBU**).

The photos 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 v. 22.1.0 to form the final figure plates.

Label data are presented verbatim. A slash (/) separates text on different lines of label. A double slash (//) separates text on separate lines of a label; authors' remarks are enclosed in brackets "[]".

### Taxon sampling, DNA extraction, PCR amplification, and sequencing

Larval specimens were collected in the field together with adults from the Tibetan Plateau, China. To correlate the different stages, the molecular data were collected from six individuals (four adults, one larva, and one pupa).

DNA was extracted from the pygopod tissues of the larva and pupa, and from the leg muscle tissue of the adults using the Insect DNA isolation Kit (BIOMIGA, Dalian, China) following the manufacturer's protocols. The DNA extracted was stored at –20 °C. Fragments of three mitochondrial markers (cytochrome

oxidase subunit I, *COI*; cytochrome b, *Cytb*; *16S* ribosomal DNA, *16S*), and one nuclear marker (*28S* ribosomal DNA domain D2, *28S*-D2) were amplified and sequenced. The primers and the annealing temperatures are shown in Table 1.

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 1 min, annealing for 45 s, 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). Altogether, all molecular data were collected from 82 individuals (80 adults, one larva, and one pupa); 89 new sequences from 26 individuals of nine species were generated, and 211 sequences were previously published (Li et al. 2021). We used previously published sequences of four Platyscelidini Lacordaire, 1859 species as the outgroup, which has been considered a close relative of the tribe Blaptini (Kamiński et al. 2021). Detailed information for all the samples used in this study is provided in Suppl. material 1.

**Table 1.** Primer sequences for PCR.

| Gene | Primer (forward/reverse) | Sequence (forward and reverse) 5'→3' | PCR conditions (annealing) | References |
|---|---|---|---|---|
| *COI* | F 2183 | CAACATTTATTTTGATTTTTTGG | 50 °C | Monteiro and Pierce 2001 |
| | R 3014 | TCCAATGCACTAATCTGCCATATTA | | |
| *Cytb* | F revcb2h | TGAGGACAAATATCATTTTGAGGW | 50 °C | Simmons and Weller 2001 |
| | R rebcbj | TCAGGTCGAGCTCCAATTCATGT | | |
| *16S* | F 13398 | CGCCTGTTTATCAAAAACAT | 50 °C | Simon et al. 1994 |
| | R 12887 | CCGGTCTGAACTCAGATCAT | | |
| *28S-D2* | F 3665 | AGAGAGAGTTCAAGAGTACGTG | 58 °C | Belshaw and Quicke 1997 |
| | R 4068 | TTGGTCCGTGTTTCAAGACGGG | | |

### Phylogenetic analyses

Phylogenetic analyses were based on the concatenated dataset under the maximum likelihood (ML) criterion in IQ-TREE v. 1.6.6 (Nguyen et al. 2015), as implemented in the dedicated IQ-TREE web server (http://iqtree.cibiv.univie.ac.at/, accessed 2023-6-1). The ML tree was inferred under an edge-linked partition model for 5000 ultrafast bootstraps (1000 replicates) (Minh et al. 2013). The consensus phylogenetic tree was visualized in Figtree v. 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree, accessed 2023-6-1).

## Results

### Morphological study and diagnosis

***Gnaptorina* (*Gnaptorina*) *lhorongica* Li, sp. nov.**
https://zoobank.org/0CB92107-F71F-48C0-98FD-9EF1FA9CA7F5
Figs 1, 3, 5–7

**Type locality.** Lajiu Township, Lhorong County, Xizang.

**Type materials (Adults). *Holotype*:** China • ♂//西藏洛隆腊久乡 [Lajiu Township, Lhorong County, Xizang]/ 30°28.714'N, 95°53.593'E/ Alt. 4680 m /labeled

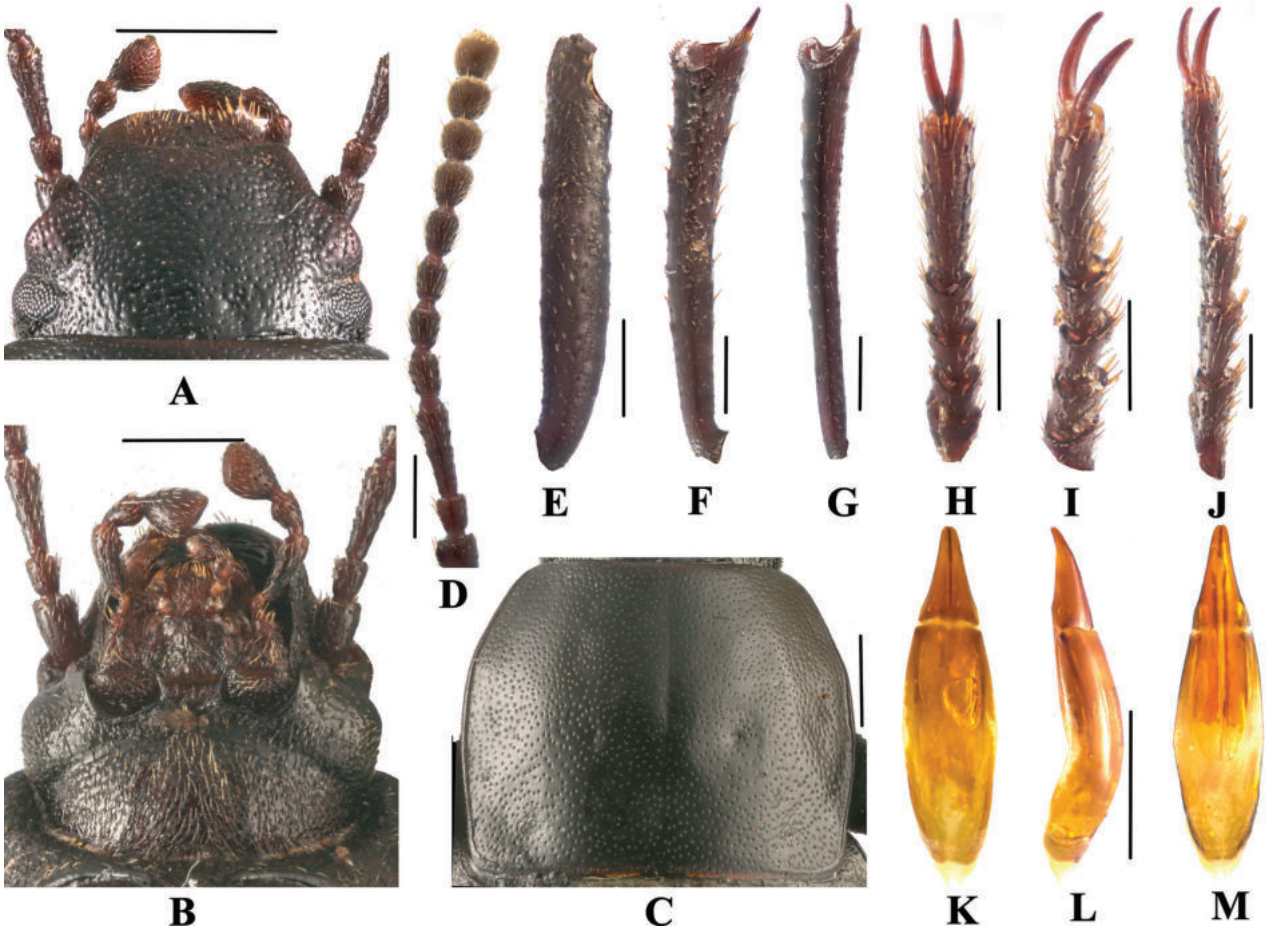


**Figure 1.** *Gnaptorina* (*Gnaptorina*) *lhorongica* Li, sp. nov. Holotype **A** head, dorsal view **B** head, ventral view **C** pronotum **D** antenna **E** protibia **F** mesotibia **G** metatibia **H** protarsus **I** mesotarsus **J** metatarsus **K** aedeagus, dorsal view **L** aedeagus, lateral view **M** aedeagus, ventral view. Scale bars: 1.0 mm (**A–C, K–M**); 0.5 mm (**D–J**).

30.Jul. 2019/ 任国栋， 李亚林， 白兴龙 [Guo-Dong Ren, Ya-lin Li & Xing-Long Bai leg.]. ***Paratypes***: 4♂6♀// 西藏洛隆腊久乡 [Lajiu Township, Lhorong County, Xizang]/ 30°28.714'N, 95°53.593'E/ Alt. 4680 m/ labeled 30. Jul. 2019/ 任国栋，李亚林，白兴龙 [Guo-Dong Ren, Ya-lin Li & Xing-Long Bai leg.]; 6♂4♀// 西藏洛隆腊久乡[Lajiu Township, Lhorong County, Xizang]/ 30°25.203'N, 96°5.950'E/ Alt. 3910 m/ labeled 30.Jul. 2019/ 任国栋, 李亚林， 白兴龙 [Guo-Dong Ren, Ya-lin Li & Xing-Long Bai leg.]; 6♂8♀// same data as holotype; 10♂8♀// 西藏洛隆孜托镇 [Zituo Township, Lhorong County, Xizang]/ 30°32.515'N, 95°46.774'E/ Alt. 4031 m/ labeled 30.Jul. 2019/ 任国栋， 李亚林， 白兴龙 [Guo-Dong Ren, Ya-lin Li & Xing-Long Bai leg.]; 3♂8♀// 西藏洛隆达翁拉山 [Daonla mountain, Lhorong County, Xizang]/ 30°46.204'N, 95°33.758'E/ Alt. 3854 m/ labeled 11.Jul. 2015/ 任国栋， 白兴龙 [Guo-Dong Ren & Xing-Long Bai leg.].

**Other examined materials. Larva.** 2 ex. // 西藏洛隆腊久乡 [Lajiu Township, Lhorong County, Xizang]/ 30°28.714'N, 95°53.593'E/ Alt. 4680 m/ labeled 30.Jul. 2019/ 任国栋， 李亚林， 白兴龙 [Guo-Dong Ren, Ya-lin Li & Xing-Long Bai leg.].

**Pupa.** 1♂// 西藏洛隆腊久乡 [Lajiu Township, Lhorong County, Xizang]/ 30°28.714'N, 95°53.593'E/ Alt. 4680 m/ labeled 30.Jul. 2019/ 任国栋， 李亚林，白兴龙 [Guo-Dong Ren, Ya-lin Li & Xing-Long Bai leg.].

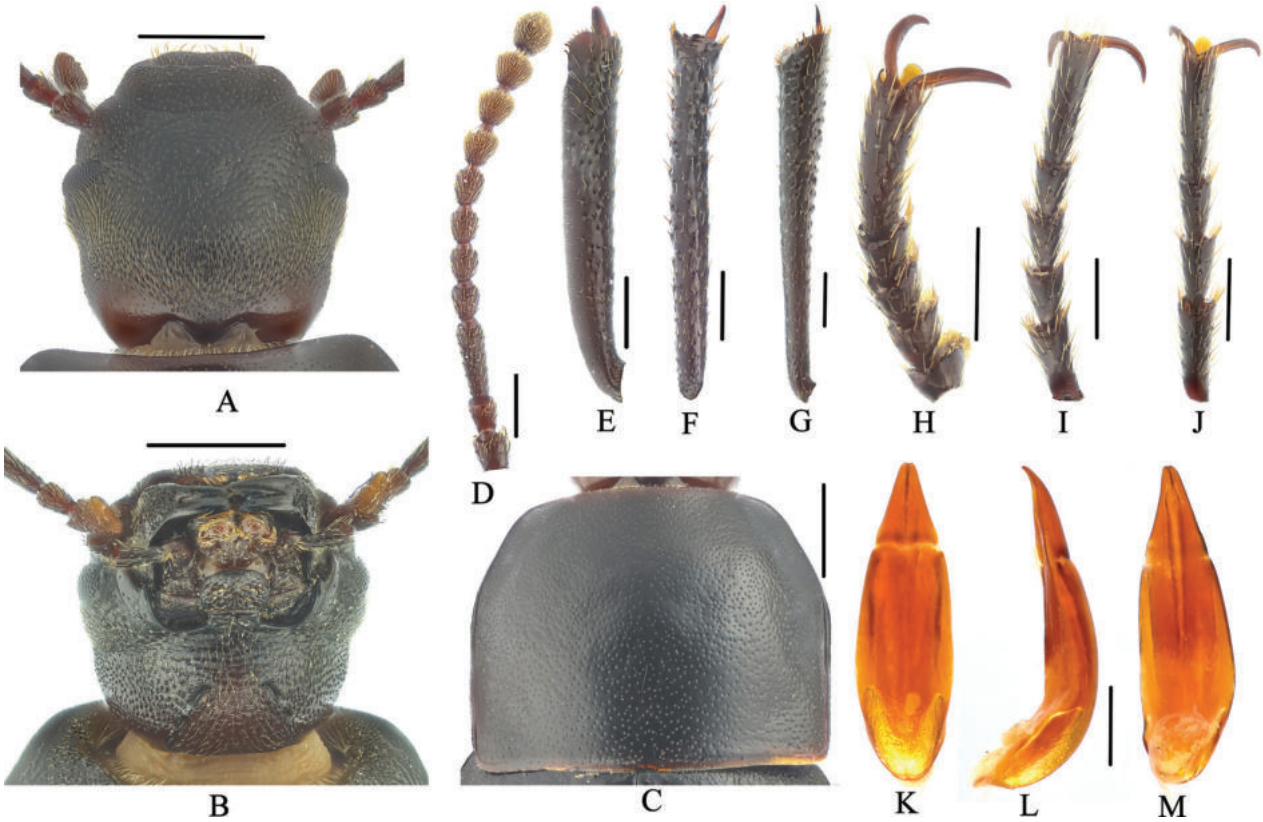


**Figure 2.** *Gnaptorina* (*Gnaptorina*) *dongdashanensis* Shi, 2013 **A** head, dorsal view **B** head, ventral view **C** pronotum **D** antenna **E** protibia **F** mesotibia **G** metatibia **H** protarsus **I** mesotarsus **J** metatarsus **K** aedeagus, dorsal view **L** aedeagus, lateral view **M** aedeagus, ventral view. Scale bars: 1.0 mm (**A–C**); 0.5 mm (**D–M**).

**Description of adult.** Body length 10.5–11.2mm, width 5.8–6.0 mm; body shiny, black; antennae, palpi, and tarsi brown.

**Male** (Figs 1A–L, 2). ***Head*:** (Fig. 1A). Anterior margin of clypeus weakly sinuate. Lateral margin of head with distinct emargination between epistome and genae. Head widest at eye level. Lateral margin of head with pair of projections between antennal base and oculus, brownish red. Genal margin arcuately converging before eyes. Eyes barely protruding beyond contour of head. Vertex flat or slightly convex, with uniform punctures. Antennae (Fig. 1D) slender and long, reaching beyond pronotal base when posteriorly extended, antennomeres VIII–X oval, XI spindle-shaped. Length (width) ratio of antennomeres II–XI as follows: 10.0(8.0): 26.0(8.0): 13.0(8.0): 12.0(8.0): 12.0(8.0): 13.0(9.0): 12.0(10.0): 11.0(10.0): 11.0(11.0): 11.0(11.0).

***Prothorax*.** Pronotum (Fig. 1C) transverse, 1.31–1.33 times as wide as long. Broadest at middle, 1.80–1.85 times as wide as head. Ratio of width at anterior margin to its maximum width and base 23: 37: 34. Anterior margin straight or weakly sinuate, laterally beaded. Lateral margins weakly wider from base to middle and narrowing toward anterior angles arcuately. Anterior angles obtuse, rounded apically; posterior angles weakly obtuse, nearly rectangular. Disc convex, with shallow and circular depressions laterally before base, surface with dense, fine punctures. Prothoracic hypomeron weakly concave, with longitudinal wrinkles and sparse and minute granules. Prosternal process steeply sloping behind procoxae and forming wide and flat prominence at end of declivity.

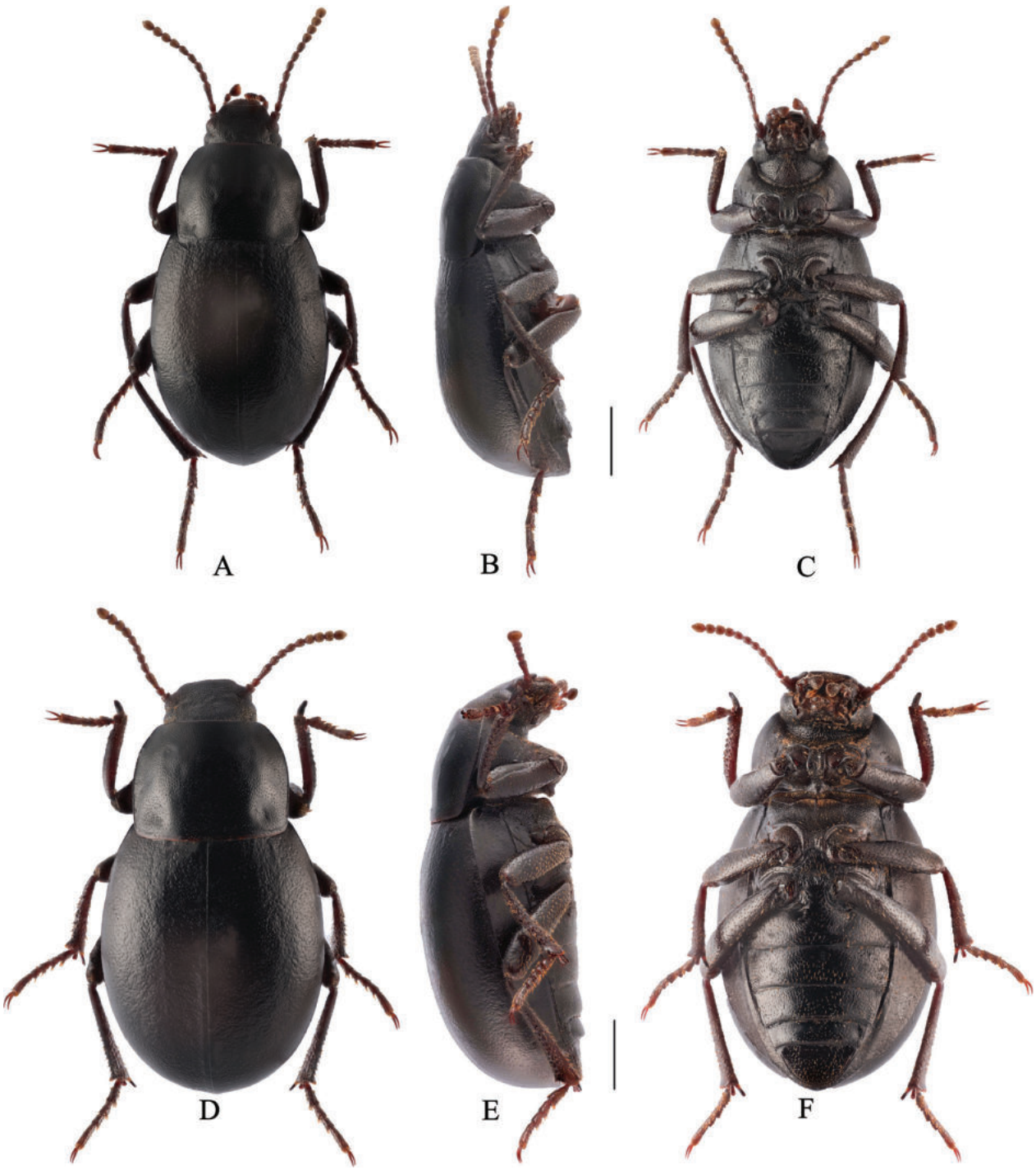


**Figure 3.** *Gnaptorina* (*Gnaptorina*) *lhorongica* Li, sp. nov. **A–C** male, holotype **D–F** female, paratype **A**, **D** dorsal view **B, E** lateral view **C, F** ventral view. Scale bars: 2.0 mm.

***Pterothorax.*** Elytra widely oval and convex, 1.12–1.26 times as long as wide, 1.61–1.77 times as wide as pronotum, widest before middle. Surface with shallow, fine, sparse punctures and irregular, short wrinkles. Lateral margins reaching sutural elytral angle, visible dorsally in anterior third and apex. Surface of epipleura smooth, with shallow wrinkles.

***Legs*** (Fig. 1E–J) slender and long. Profemora with obtuse tooth. Protibiae straight, distal apical spur obviously shorter than protarsomere 1, lower spur shorter; ventral surface of protarsomeres 1 and 2 with hairy brush. Mesotibiae

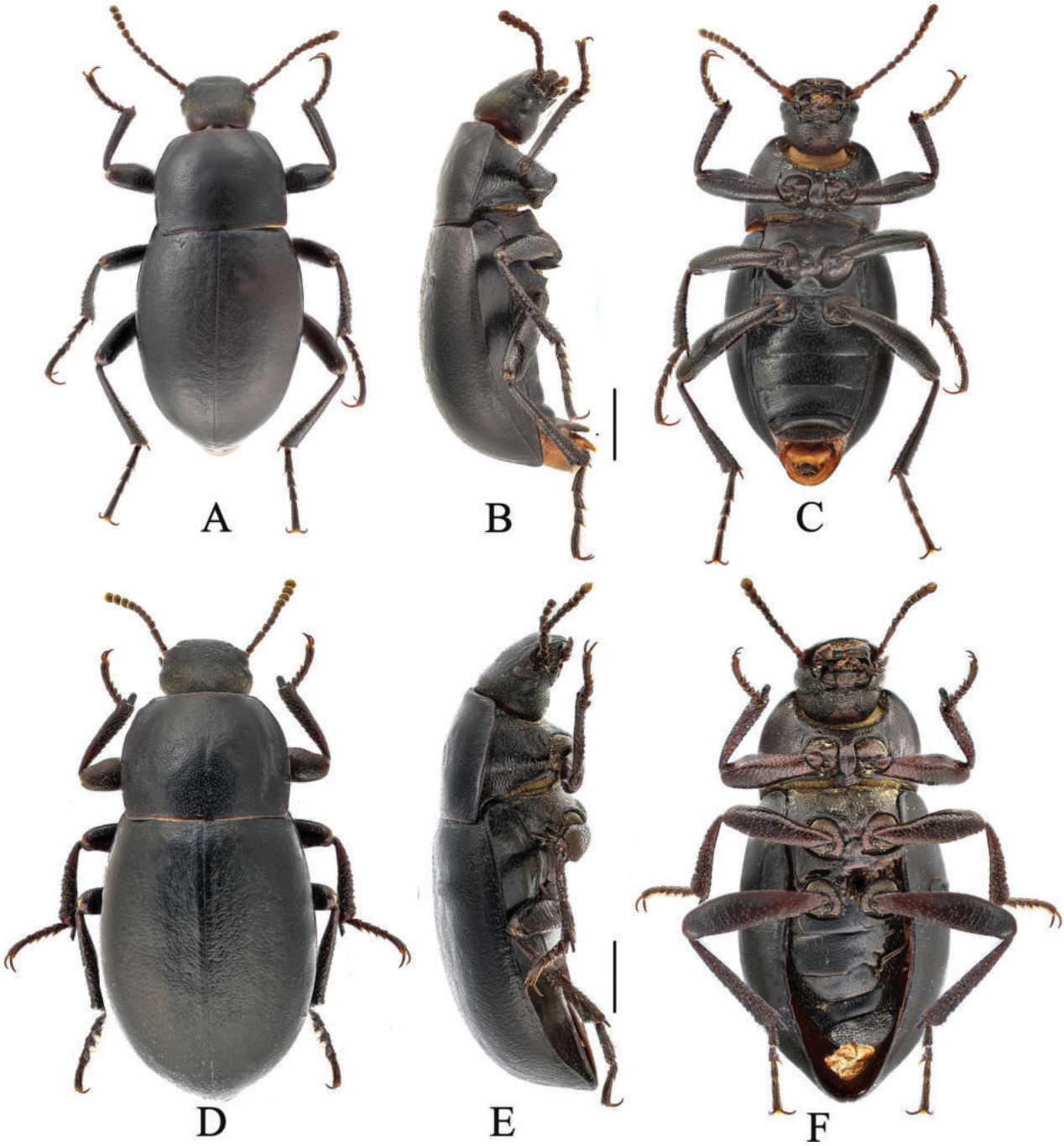


**Figure 4.** *Gnaptorina* (*Gnaptorina*) *dongdashanensis* Shi, 2013 **A–C** male **D–F** female **A**, **D** dorsal view **B, E** lateral view **C, F** ventral view. Scale bars: 2.0 mm.

weakly curved; ventral surface of mesotarsomere 1 with hairy brush at apex. Metatibiae straight, regularly widening apicad. Ratio of length(width) pro-, meso-, and metatibiae: 80.0(15.0): 95.0(17.0): 138.0(20.0), that of metatarsomeres I–IV as follows: 19.0(11.0): 20.0(10.9): 17.0(9.6):37.0(9.8).

***Aedeagus*.** (Fig. 1K–M) 2.20 mm long and 0.58 mm wide. Parameres 0.53 mm long and 0.37 mm wide, conical, widest at base, with outer margins weakly sinuate near middle, and regularly narrowing towards apex laterally in dorsal view; dorsal side nearly straight, slightly curved to ventral side apically in lateral view.

**Figure 5.** Larva of *Gnaptorina* (*Gnaptorina*) *lhorongica* Li, sp. nov. **A–C** habitus **A** dorsal view **B** ventral view **C** lateral view **D** head, dorsal view **E** head, fore foot, and mesoleg, in ventral view **F** pygopods, in dorsal view **G** pygopods, in ventral view. Scale bars: 2 mm (**A–C**); 1 mm (**D–G**).

**Female** (Fig. 3). Body length 11.2–11.5mm, width 5.9–6.2 mm. Body wider than male. Head 1.14 times as wide as interocular distance. Pronotum 1.28–1.30 times as wide as long, widest in middle, lateral margins subparallel from base to middle, then narrowing toward anterior angles arcuated. Elytra more convex, 1.13 times as long as wide. Antennae shorter than in male. Upper spur of protibiae wide and flat; lower spur fine and pointed. Ventral surface of pro and mesotarsomeres I–IV with hairy brush.

**Etymology.** Named after the county of Lhorong, where the type locality is located.

**Distribution.** China: Xizang.

**Diagnosis.** This new species is morphologically similar to *G.* (*G.*) *dongdashanensis* Shi, 2013 but can be distinguished from it by the following male character states: (1) genal margin arcuately converging before eyes (genal margin parallel before eyes in *G. dongdashanensis*); (2) antennomeres IV–VII long and cylindrical (antennomeres IV–VII nearly spherical in *G. dongdashanensis*); (4) elytral widely oval (elytral elongate-oval in *G. dongdashanensis*). (Figs 2, 4)

**Description of larva. *Body*.** (Fig. 5A–C) Mature larvae length 23.0–25.0mm, width 2.5–3.0mm. Body subcylindrical; 9$^{th}$ abdominal tergite conical and urogomphi not sharp; body brownish yellow, shiny; body wall ossified; median line obvious on first four segments; pairs of setae grow on each tergite; terga I–VIII with four pairs of long setae, two pairs anterior and two pairs posterior.

***Head*** (Figs 5D, E, 6A–C). Prognathous slightly narrower than width of prothorax, slightly convex dorsally, and sides rounded (Fig. 5D). Labrum transverse;

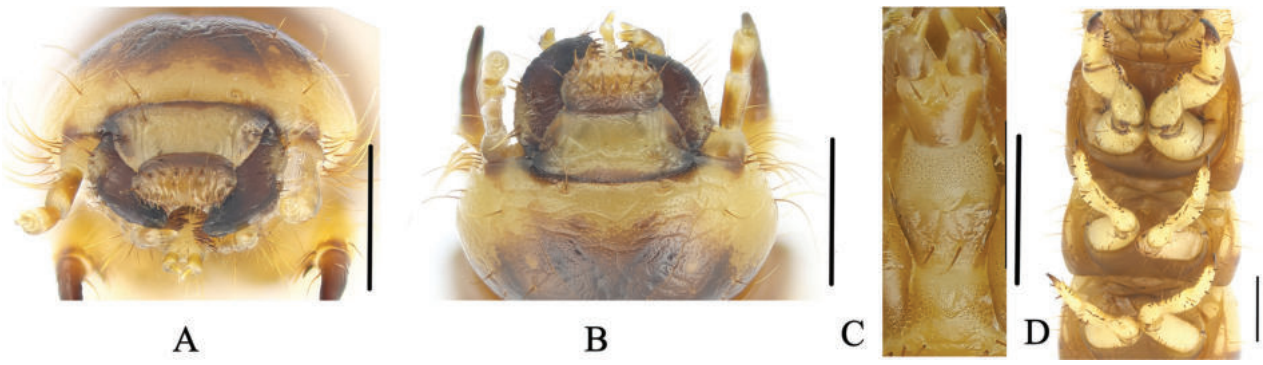


**Figure 6.** Larva of *Gnaptorina* (*Gnaptorina*) *lhorongica* Li, sp. nov. **A** head **B** labrum and clypeus, in dorsal view **C** labium **D** legs. Scale bars: 1 mm (**A, B, D**); 0.5 mm (**C**).

apical part with six setae. Mandibles well developed; left and right symmetrical; each mandible with two pair of setae; clypeus transverse, trapezoidal, left and right marginal sides with two pairs of short setae (Fig. 6A, B). Epicranial stem Y-shaped (Fig. 5E); frons and epicranial plate slightly convex, lateral margin with densely long setae, frons with four pairs of setae. Maxillary palps three-segmented, cylindrical, and conical at apex; I widest, II longest, I as long as III (Fig. 5D, E). Labial palps (Fig. 5D) two-segmented, short; II conical. Mentum convex, U-shaped, base of mentum straight, prementum with two long setae, mentum with two long setae on posterior margin, submentum with eight setae on mid-posterior part (Fig. 6C). Antennae well developed, three-segmented, dome-like at apex; segment I wider and as long as II; segment III shortest and narrowest.

***Thorax*** (Fig. 5A–C). Thoracic segmentation C-shaped in dorsal view, parallel-sided, widest at middle, with transverse plicae. Each thoracic tergum with two pairs of elongate setae on anterior and posterior margins. Anterior and posterior border of prothorax with brown longitudinal stripes, with pair of irregular brown spots on tergite, pair of brown bands on top dorsal margins of mesothorax and metathorax; two pairs of irregular brown spots on tergites IV–XI. Pronotum longest, about twice as long as meso- and metanotum, mesonotum shortest.

***Legs*** (Figs 5E, 6D). Legs well developed. Prothoracic leg noticeably stronger, longer, and thicker than meso- and metathoracic legs; profemur and protibia with a row of spines and denser long setae (Fig. 5E). Protarsungulus strongly sclerotized, sharp, claw-like; one strong, long seta on inner side of the base of the protarsungulus, and one strong, short spine on posterior outer side. Profemora and protibiae gradually narrowing towards apex; inner margin setal formula of foreleg 4–5(3): 6(5): 2(2); outer margin of tibiae with two setae; outer margin of femora with two setae; trochanter with three setae. Mesotarsus with one short, broad spine at base; inner margin setal formula of mesothoracic leg 4(3): 5(3): 2(2); outer margin of tibiae with two short spines; outer margin of femora with two spines; outer margin of trochanters with three setae. Metatarsus with one short, broad spine at base, the inner margin setal formula of metathoracic leg 4(3): 4(3): 2(2), outer margin of tibiae with two short spines, outer margin of femora with two to three spines, outer margin of trochanters with two setae. Profemora about half length of protibia; meso- and metathoracic legs moderately shorter than prothoracic one, tarsungulus highly ossified, hooked, with a row of spines and sparse setae.

***Abdomen*** (Fig. 5A–C). Approximately 3.6 times as long as thorax; segments I–VIII subcylindrical, with transverses plicae, faintly rugose, and with sparse elongate setae ventrally; tergum of IX 0.75 times as long as tergum VIII, distinctly narrower than tergum VIII; with a row of short spines each side (five spines on left, four spines on right); last segment conical in dorsal view, surface of convex disc with sparse long setae in ventral view; urogomphi suddenly upturned to apex in lateral view, apex truncated, with two thorn-like processes.

***Spiracles*** (Fig. 5B). Pair of circular thoracic spiracles, situated ventrolaterally on anterolateral margins of terga I–VIII.

**Diagnosis of larva.** The larva of new species is morphologically very similar to *G.* (*G.*) *cylindricollis* Reitter, 1889, but can be distinguished from it by the following characters: (1) lateral margins of the head with dense long setae (lateral margins of the head with sparse long setae in *G. cylindricollis*); (2) mentum with two long setae on the side of the posterior margin and submentum with eight setae on located in the middle, posteriorly (mentum with four long setae on the side of the posterior margin and submentum with five setae in the posterior part of the center in *G. cylindricollis*).

The larva of new species is morphologically very similar to *G.* (*G.*) *felicitana* but can be distinguished from the latter by the following characters: (1) lateral margins of the head with dense, long setae (*G.* (*G.*) *felicitana* with sparse, long setae); (2) terga I–VIII with four pairs of long setae, two anterior pairs and two posterior pairs (*G.* (*G.*) *felicitana* with six pairs of long setae, three anterior pairs and three posterior pairs); (3) frons with eight setae, two on upper margin, six on apex (*G.* (*G.*) *felicitana* with eight setae, two on upper margin, two at center, and four at posterior margin in); (4) apex of antennomere III without long setae (*G.* (*G.*) *felicitana* with one long seta at the apex); (5) side posterior margin of mentum with two long setae and middle of submentum with eight setae posteriorly (*G.* (*G.*) *felicitana* with mentum and submentum both with 10 setae).

**Description of pupa.** ***Body*** (Fig. 7A–C). Length 13.5 mm, width 3.6 mm. Body moderately elongated, slightly flattened, tapering towards posterior and with pronotum widest, creamy white.

***Head*** (Figs. 7B, C). Invisible in dorsal view. Smooth, with transverse wrinkles. Head bending towards underside of prothorax, slightly elevated at center of head, with sparse, short setae on margins. Labrum and mandible smooth, covered with sparse, short setae; anterior margin of clypeus straight, sides weakly curved. Antennae rod-shaped, gradually thickened. Maxillary and labial palpi visible clearly.

***Thorax*** (Fig. 7A–C). Pronotum semicircular and with posterior margin straight, about 1.6 times as long as wide, widest in middle. Pronotum depressed medially, with transverse plicae, with sparse short setae on top to anterior margin and sparse short setae lateral margin in dorsal view. Elytra narrowed proximally to form alaria, surface smooth but with sparse short setae.

***Legs*** (Fig. 7B, C). Legs similar to adults. Femora and tibiae with minute setae; tarsi glabrous, extended anteriorly. Fore leg shortest; hind leg longest.

***Abdomen*** (Fig. 7A, C, D). Abdomen nine-segmented, with distinct midline. Terga broad, convex, covered with minute, short setae. Terga I–VI flanked by outwardly projecting, plate-like lateral processes; with sparse, short setae. Lateral process of tergum VII triangular; lateral process of tergum VIII less developed. Tergum IX with a pair of elongate urogomphi at apex 1.13 mm long. (Fig. 7E).

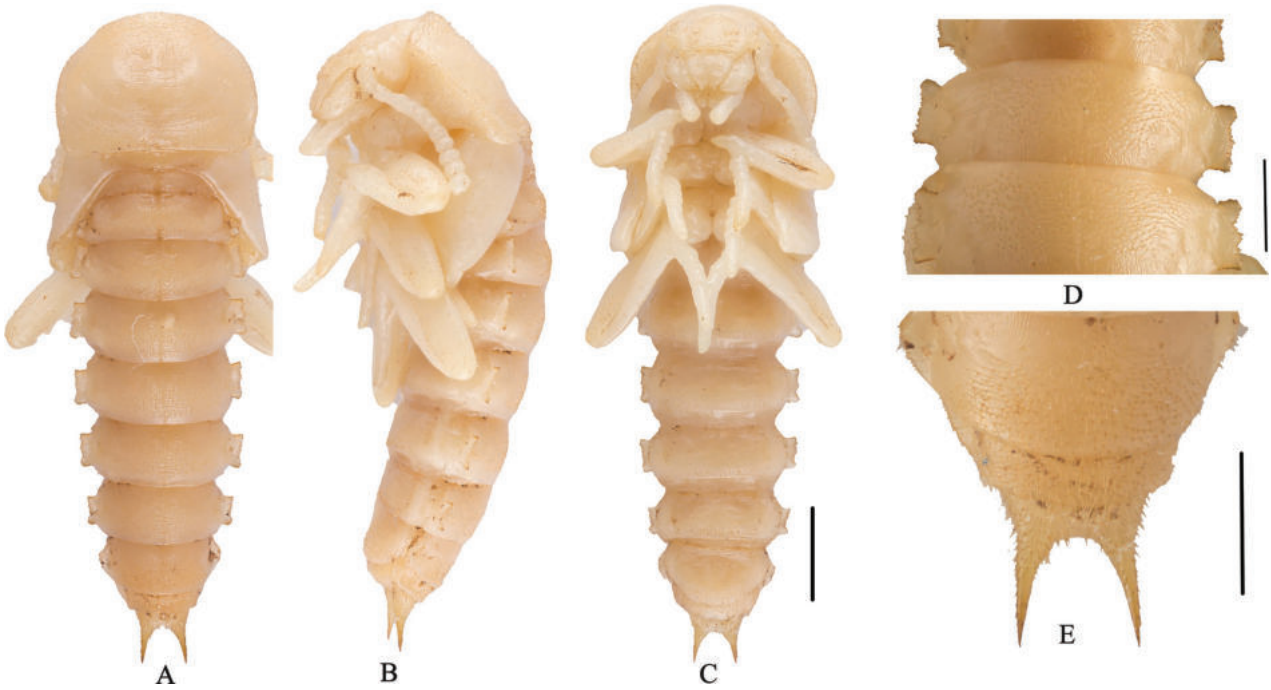


**Figure 7.** Pupa of *Gnaptorina* (*Gnaptorina*) *lhorongica* Li, sp. nov. **A–C** habitus **A** dorsal view **B** ventral view **C** lateral view **D** lateral process of abdominal terga, in dorsal view **E** urogomphy, in dorsal view. Scale bars: 2 mm (**A–C**); 1 mm (**D, E**).

## Phylogenetic relationships

The final, concatenated dataset was 2321-bp long, including 300 sequences from 82 specimens of 32 described species and six specimens of the new species (*COI*, 648 bp; *Cytb*, 579 bp; *16S*, 496 bp; *28S-D2*, 443 bp). IQ-TREE analyses yielded a topology, and the preliminary phylogenetic relationship was hypothesized for the genus *Gnaptorina* (Fig. 8).

The ML tree revealed that there was a reasonable correlation of membership of these major clades. The monophy of the subgenera was well supported overall. The individuals of *Gnaptorina* were grouped into three well-supported clades: clade C1 (*Gnaptorina*, uBV = 96), clade C2 (*Austroptorina*, uBV = 100) and clade C3 (*Hesperoptorina*, uBV = 87). The taxonomic status of the new species is confirmed by phylogenetic relationships and morphological evidence: *G.* (*G.*) *lhorongica* Li, sp. nov. The adult, pupa, and larva cluster into a single well-supported clade (uBV = 100). Based on the above results, the larval and pupal samples are confirmed as adult stages of *G.* (*G.*) *lhorongica* Li, sp. nov. The molecular analyses also indicate that the new species is a closely related and sister to *G.* (*G.*) *dongdashanensis*.

## Discussion

The adults, pupa, and larva of *G. lhorongica* Li, sp. nov. were collected in the field; hence, it was rather difficult to judge the larval developmental stage. The larva used for the description above was inferred to be in its final instar stage based on previous research on the larval biology of the Blaptini.

The genus *Gnaptorina* comprises 38 species (Bai et al. 2020), which are mostly distributed in high elevations on the Tibetan Plateau (Li et al. 2021). It is difficult to obtain larvae and pupae by rearing adults, because habitat

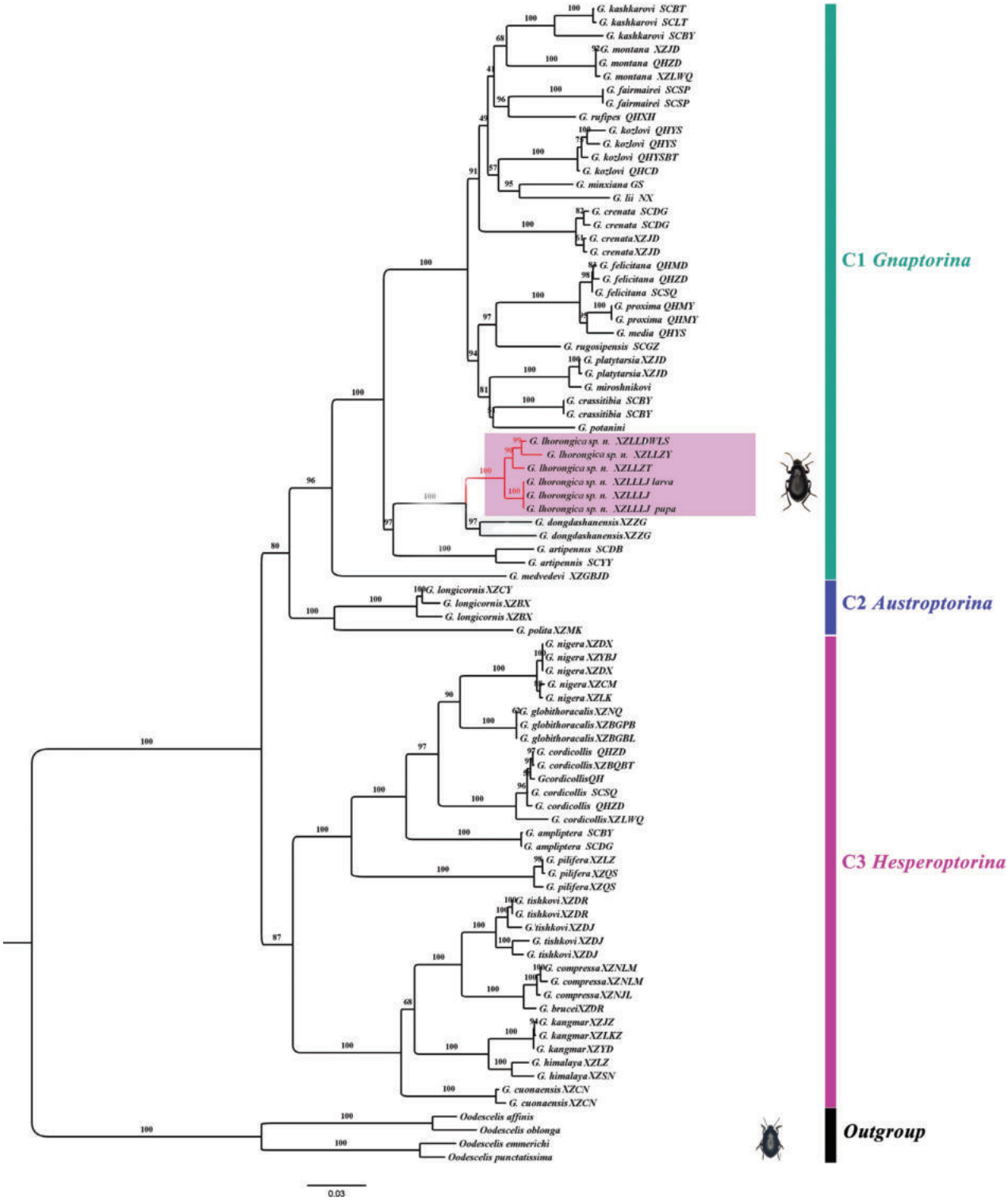


**Figure 8.** Maximum-likelihood phylogenetic tree based on 2321 bp of mitochondrial and nuclear DNA sequences (*COI*, *Cytb*, *16S*, and *28S*-D2) within the genus *Gnaptorina*. Support for each node is represented by ultrafast bootstrap values (uBV).

conditions of *Gnaptorina* in the wild are not possible to replicate. To date, the larval morphology of only *G. felicitana* and *G. cylindricollis* have been described and pupal morphology in *Gnaptorina* has not been updated before the present study (Yu et al. 1996; Zhu and Ren 2014). Our present description of the pupa

for *G.* (*G.*) *lhorongica* Li, sp. nov . is the first for the genus. Thus, it is currently impossible to provide a generic diagnosis for pupae of *Gnaptorina*, and our understanding of the morphological diversity within *Gnaptorina* is clearly in its infancy; it would be premature to attempt a diagnosis for larvae and pupae of the genus. We hope to discover additional larvae and pupae and associate them with their respective adults by rearing or molecular analysis. Only with the discovery of larvae and pupae for additional *Gnaptorina* species may we offer sound generic diagnoses and more robust hypotheses of relationships. Meanwhile, the molecular database of the genus *Gnaptorina* offers strong support for studying unknown species at any stage of development.

## Acknowledgements

We thank Xinglong Bai for his kind help with fieldwork and for providing samples from Xizang. We also thank Junxia Zhang for additional comments and corrections. Finally, we are very grateful by two anonymous reviewers for their constructive suggestions on the manuscript.

## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

### Funding

This research was funded by the National Natural Science Foundation of Hebei (C202101020), the National Natural Science Foundation of China (32170477), and National Innovative Entrepreneurship Training Program for college students (S202310075045).

### Author contributions

Conceptualization: B-YJ, ZP, X-ML. Data curation: X-MLand X-TM. Formal analysis: B-YJ, X-ML. Writing – original draft: B-YJ, X-TM. Visualization: X-TM, J-DR. Funding acquisition: G-DR, X-ML. Writing – review and editing: B-YJ, J-DR, ZP, G-DR.

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### Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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## Supplementary material 1

### List of specimens used in this study with the corresponding accession number

Authors: Xiu-Min Li

Data type: docx

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/zookeys.1190.113126.suppl1

ZooKeys 1190: 107–119 (2024)
DOI: 10.3897/zookeys.1190.115573

Research Article

# A revision of some species of *Souvanna* Breuning, 1963, *Mispila* Pascoe, 1864, and *Athylia* Pascoe, 1864 (Coleoptera, Cerambycidae, Lamiinae)

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## Abstract

*Alidus signatus* Pic, 1926 is transferred from *Mispila* to *Souvanna*, and *Souvanna signata* (Pic, 1926), **comb. nov.** is proposed. The lectotype of *Alidus signatus* is designated. The following synonyms are proposed: *Souvanna signata* = *Athylia* (*s. str.*) *quadristigma* (Gressitt, 1940), **syn. nov.** = *Souvanna phoumai* Breuning, 1963, **syn. nov.** = *Mispila* (*Dryusa*) *coomani* Breuning, 1968, **syn. nov.**, *Mispila* (*s. str.*) *tenuevittata* (Pic, 1930) = *Mispila* (*s. str.*) *assamensis* Breuning, 1938, **syn. nov.** The gender of the holotype of *Alidus multilineatus* Pic, 1925 is determined. New distributional records for *Souvanna signata*, *Mispila curvilinea* Pascoe, 1869, *M. subtonkinea* Breuning, 1968 and *M. tenuevittata* are provided.

**Key words:** Gender definition, lectotype designation, new combination, new faunistic records, synonyms

OPEN ACCESS

**Academic editor:** Francesco Vitali
**Received:** 10 November 2023
**Accepted:** 31 December 2023
**Published:** 24 January 2024

**ZooBank:** https://zoobank.org/6EE1FEFE-0A45-4780-AA44-C2F38FE8B6F5

**Citation:** Huang G-Q, Weigel A, Chang E-M, Zhang G-M (2024) A revision of some species of *Souvanna* Breuning, 1963, *Mispila* Pascoe, 1864, and *Athylia* Pascoe, 1864 (Coleoptera, Cerambycidae, Lamiinae). ZooKeys 1190: 107–119. https://doi.org/10.3897/zookeys.1190.115573

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## Introduction

Breuning (1963a) established the monotypic genus *Souvanna* for *Souvanna phoumai* from Laos (Vientiane). Pascoe (1864) established the genus *Athylia* for *Athylia avara* Pascoe, 1864. Gressitt (1940) described *Enispia quadristigma* from China (Hainan), and Breuning (1960) transferred it to the genus *Athylia* Pascoe, 1864. *Athylia* presently consists of two subgenera: *Athylia* (*s. str.*) with 22 species and one subspecies, and *Pulchrathylia* Breuning, 1964 with two species. All species and subspecies of the above subgenera are distributed in the Oriental Region (Tavakilian and Chevillotte 2023). Breuning (1938, 1968a) described *Mispila assamensis* from India (Assam) and *Mispila* (*Dryusa*) *coomani* from Vietnam (Tonkin), respectively. Yan et al. (2023) and Xie et al. (2023) revised some species of *Mispila* Pascoe, 1864. *Mispila* currently consists of three subgenera: *Mispila* (*s. str.*) with 37 species and one subspecies, *Dryusa* Pascoe, 1864 with six species, and *Trichomispila* Breuning, 1939 with two species. All species and subspecies of the above subgenera are distributed in East, South, and Southeast Asia, as well as in Oceania (Tavakilian and Chevillotte 2023).

During the study of the genera *Souvanna*, *Athylia*, and *Mispila*, we found that the taxonomic status of *Athylia quadristigma*, *Souvanna phoumai*,

*Mispila coomani*, and *Mispila assamensis* were doubtful; additionally, the gender of the holotype of *Alidus multilineatus* Pic, 1925 (currently, a junior synonym of *Mispila curvilinea* Pascoe, 1869) was unknown. Therefore, we studied these issues and report our results here.

## Material and methods

Specimens examined are deposited in following institutions and private collections:

**BMNH** The Natural History Museum, London, United Kingdom;
**BPBM** Bernice Pauahi Bishop Museum, Honolulu, Hawaii, USA;
**CAS** California Academy of Sciences, San Francisco, CA, USA;
**CSG** Collection Andre Skale, Gera, Germany;
**CWW** Collection Andreas Weigel, Wernburg, Germany;
**LPSNU** School of Biological Science and Technology, Liupanshui Normal University, Liupanshui, Guizhou, China;
**MNHN** Muséum national d'Histoire naturelle, Paris, France;
**SYSU** Sun Yat-sen University, Guangzhou, Guangdong, China.

Photographs of Figs 1A–C, M–O, 3F–H were taken by Xavier Gouverneur, Fig. 1D–G by Bing-Lan Zhang, Fig. 1H–K by Rachel Diaz-Bastin, Figs 1L, 5A by Nobuo Ohbyashi, Figs 3D, 6G by Guang-Lin Xie, Figs 2A, 4A, B by En-Ming Chang, and Figs 2B–D, 4C–E, 5B, 6I–K by Andreas Weigel. All photographs were edited with Adobe Photoshop CS 5.

## Taxonomy

### Apomecynini

### *Souvanna* Breuning, 1963

*Souvanna* Breuning, 1963a: 39: Breuning 1964a: 5; Breuning 1964b: 428. Type species: *Souvanna phoumai* Breuning, 1963.

### *Souvanna signata* (Pic, 1926), comb. nov.

Figs 1, 2

*Alidus signatus* Pic, 1926: 13 (type locality "Tonkin, Vietnam").
*Mispila* (*Mispila*) *signata*: Breuning 1961: 281 (catalogue); Breuning 1963b: 473 (key), 478 (redescription).
*Enispia quadristigma* Gressitt, 1940: 156 (type locality "Central Hainan: Sam-ts 'uen-kai-hui, southeast of Lai-mo-leng and Fan-ta, southeast of Nam-fung"), pl. 4, fig. 12; Gressitt 1951: 482 (catalogue); Breuning 1963c: 5; Hua et al. 2009: 76, pl 76, fig. 871 (holotype, ♂). syn. nov.
*Athylia quadristigma*: Breuning 1960: 166 (catalogue); Hua 1982: 69 (catalogue); Hua 2002: 197 (catalogue); Löbl and Smetana 2010: 230 (catalogue); Lin and Tavakilian 2019: 331 (catalogue); Danilevsky 2020: 323 (catalogue).

*Souvanna phoumai* Breuning, 1963a: 39 (type locality "région de Vientiane, Laos"), figs pp. 39 and 40; Breuning 1964b: 429; Rondon and Breuning 1970: 365 (catalogue), fig. 12h. syn. nov.

*Mispila* (*Dryusa*) *coomani* Breuning, 1968a [nec *Mispila coomani* (Pic, 1934)]: 858 (type locality "Hoa Binh, Tonkin, Vietnam"). syn. nov.

**Body length.** 6.5–10.7 mm (♂), 9.6 mm (♀). The gender of the types of *Alidus signatus* (lectotype), *Souvanna phoumai* (syntype), and *Mispila coomani* (holotype) were unknown, so the body length was determined from the types of *Enispia quadristigma* and additional materials examined.

**Type material examined.** ***Alidus signatus***: lectotype (MNHN), Hoa Binh Tonkin (handwritten with black ink on a rectangular white label) / type (handwritten with black ink on a rectangular white label) / *Alidus signatus* n sp (handwritten with black ink on a rectangular white label) / TYPE (printed with black ink on a rectangular red label) / Museum Paris Coll. M. Pic (printed with black ink on a rectangular white label with black borders); examined from three photographs (Fig. 1A–C). ***Enispia quadristigma***: holotype, ♂ (SYSU), Hainan Is., South China. Sam-ts' uen-kai-hui. SE. of Lai-mo-ling (Mt. range). Ting-an Dist. July 4–6. 1935. F. K. To (printed with black ink on a rectangular yellow label) / HOLOTYPE ENISPIA 4-STIGMA J.L. Gressitt ("HOLOTYPE J.L. Gressitt" printed and "ENISPIA 4-STIGMA" handwritten with black ink on a rectangular red label) / 四点凸额天牛*Enispia quadristigma* Gressitt ♂ 鉴定人:华立中 2008 ("四点凸额天牛*Enispia quadristigma* Gressitt ♂ 2008" handwritten and "鉴定人:华立中" printed with black ink on a rectangular white label with black borders) / En-420876 SYS plus a QR-code (printed with black ink on a rectangular white label with black borders); examined from four photographs (Fig. 1D–G); paratype, ♂ (CAS), Fan Ta, Hainan Id VII-17-35 (printed with black ink on a rectangular white label) / L. Gressitt Collector (printed with black ink on a rectangular white label) / L. Gressitt Collection (printed with black ink on a rectangular white label) / PARATYPE ENISPIA 4-STIGMA J.L. Gressitt ("PARATYPE J.L. Gressitt" printed and "ENISPIA 4-STIGMA" handwritten with black ink on a rectangular yellow label) / CASENT 8556282 plus a QR-code (printed with black ink on a rectangular white label with black borders); examined from four photographs (Fig. 1H–K). ***Souvanna phoumai***: syntype, ♂ (BPBM), région de Vientiane, Laos, XI.1962; examined from one photograph (Fig. 1L). ***Mispila* (*Dryusa*) *coomani*:** holotype (MNHN), TYPE (printed with black ink on a rectangular red label) / *Mispila* (*Dryusa*) *coomani* mihi Breuning dét. Typ ["*Mispila* (*Dryusa*) *coomani* mihi typ" handwritten with blue ink and "Breuning dét." printed with black ink on a rectangular white label]/ TONKIN HOA BINH A DE COOMAN (printed with black ink on a rectangular white label with black borders) / MUSÉUM PARIS 1952 COLL R OBERTHUR (printed with black ink on a rectangular white label with black borders); examined from three photographs (Fig. 1M–O).

**Additional material examined.** **China** • 1♂ (LPSNU, fig. 2A), Menglun Reservoir, Menglun Town, Mengla County, Xishuangbanna Dai Autonomous Prefecture, Yunnan Province, 5.XII.2016, leg. Ri-Xin Jiang. **Thailand** • 1♂ (CSG, fig. 2B): Ko Kut Isl., Trat Province, 24.V–8.VI. 2022, leg. A. Skale. **Vietnam** • 1♂ (CWW, fig. 2C), vic. Me Linh (IEBR station) [Institute of Ecology and Biological Resources], vic. Ngoc Thanh, Thái Nguyên Province, 21°23'3"N, 105°42'44"E, Alt. 60–80 m, 12.V.2012, leg. A. Weigel • 1♀ (CWW, fig. 2D): River valley, Son O

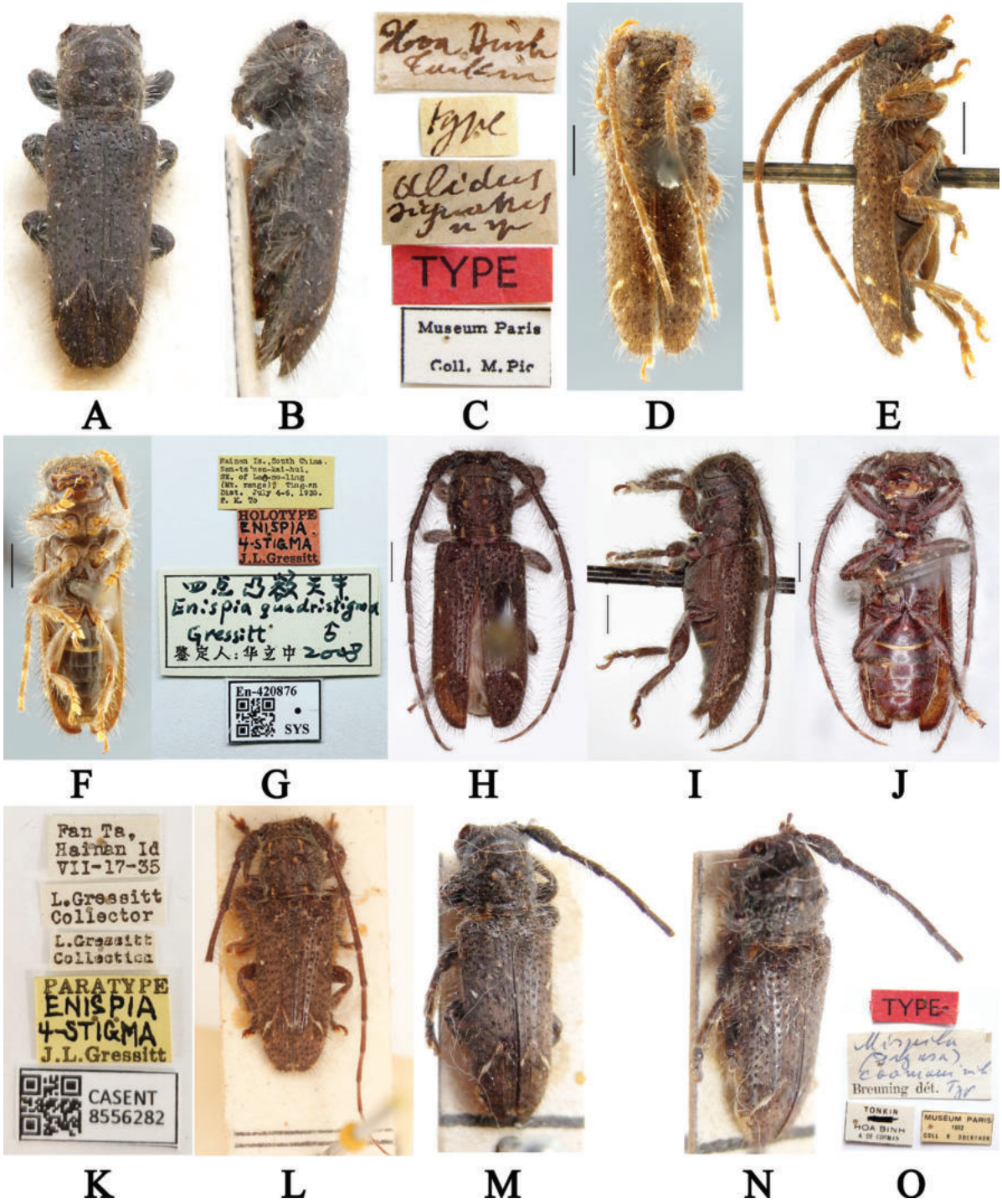


**Figure 1.** **A–C** *Alidus signatus*, lectotype **A** dorsal habitus **B** lateral habitus **C** labels **D–K** *Enispia quadristigma* **D–G** holotype **D** male dorsal habitus **E** male lateral habitus **F** male ventral habitus **G** labels **H–K** paratype **H** male dorsal habitus **I** male lateral habitus **J** male ventral habitus **K** labels **L** *Souvanna phoumai*, syntype, dorsal habitus **M–O** *Mispila coomani*, holotype **M** dorsal view **N** lateral view **O** labels. Scale bars: 1 mm (**D–F, H–J**).

Lau, ca. 30 km NW Hue, Thừa Thiên Huế Province, 16°31'3"N, 107°15'36"E, Alt. 30 m, 11.V.2019, leg. A. Weigel, KL [umbrella].

**Comments.** Having compared the types of *M. signata* (Fig. 1A, B), *A. quadristigma* (Fig. 1D–F, H–J), *S. phoumai* (Fig. 1L[REMOVED HYPERLINK FIELD]) and *M. coomani* (Fig. 1M, N), we found that the above four species are

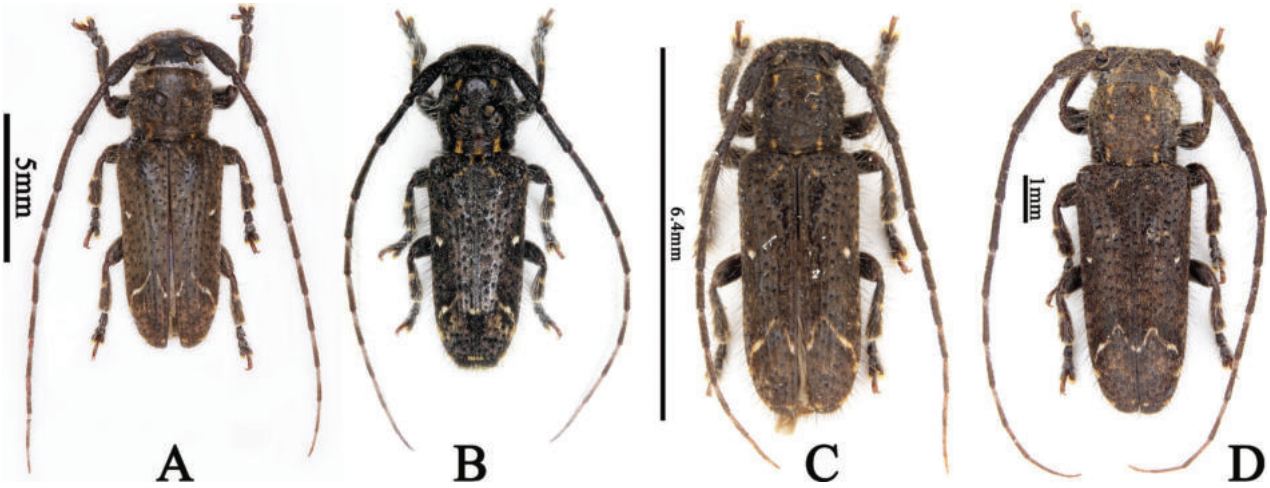


**Figure 2.** *Souvanna signata*, dorsal habitus **A** male from Yunnan, China **B** male from Trat, Thailand **C** male from Thái Nguyên, Vietnam **D** female from Thừa Thiên Huế, Vietnam.

identical; thus, we treat *A. quadristigma*, *S. phoumai* and *M. coomani* as junior synonyms of *M. signata*.

*Athylia* and *Souvanna* belong to the tribe Apomecynini, while *Mispila* belongs to the tribe Pteropliini. According to the key to tribes of Lamiinae and the key to genera of Apomecynini (Rondon and Breuning 1970), the difference between Apomecynini and Pteropliini is that the middle tibiae have an outer groove in front of the apex in Apomecynini and the middle tibiae have no outer groove in front of the apex in Pteropliini. The difference between *Athylia* and *Souvanna* is that the eyes are deeply emarginated in *Souvanna* and subdivided in *Athylia*. Furthermore, according to Breuning (1964b) and our examined materials, the 3$^{rd}$ antennal joint is longer than the 4$^{th}$ in *Athylia*, while the 3$^{rd}$ antennal joint is shorter than the 4$^{th}$ in *Souvanna*. To confirm these differences, we also have examined material of *Athylia avara* Pascoe, 1864 from the Ternate Island (Indonesia), the type species of *Athylia*.

After our examination of these materials, we can confirm that this species belongs to the tribe Apomecynini, and, according to the mentioned features, it belongs to the monotypical genus *Souvanna*.

In the description of *Alidus signatus*, Pic (1926) mentioned "Long. 7–9 mill", which indicates that Pic examined at least two specimens; thus, we designate syntype examined by us (Fig. 1A, B) as the lectotype following the recommendations of ICZN (1999; Art. 74.7).

**Distribution.** China (Hainan, Yunnan), Laos (Vientiane), Thailand (Trat), Vietnam (Hoa-Binh, Thái Nguyên, Thừa Thiên Huế).

### Pteropliini

### *Mispila* Pascoe, 1864

*Mispila* Pascoe, 1864: 90: Gemminger and Harold 1873: 3092; Hüdepohl 1995: 295; Kariyanna et al. 2017: 192. Type species: *Mispila venosa* Pascoe, 1864.
*Mispila* (*Mispila*) Aurivillius 1922: 275; Breuning 1961: 281; Breuning 1963b: 471 (redescription); Rondon and Breuning 1970: 380 (key), 414 (key).
*Diatylus* Lacordaire 1872: 565. Type species: *Diatylus zonarius* Lacordaire, 1872.

### ***Mispila* (*s. str.*) *curvilinea* Pascoe, 1869**
Figs 3, 4

*Mispila curvilinea* Pascoe, 1869: 206 (type locality "India"); Gemminger and Harold 1873: 3092 (catalogue); Hua 1982: 96 (catalogue); Hua et al. 2009: 91, pl. 91, fig. 1051 (♂, ♀); Weigel 2012: 411 (distribution), pl. 32, fig. h; Kariyanna et al. 2017: 193 (catalogue); Barševskis 2018: 291 (distribution).

*Mispila* (*Mispila*) *curvilinea* Aurivillius, 1922: 275 (catalogue): Breuning 1961: 281 (catalogue); Breuning 1963b: 472 (key), 474 (redescription); Rondon and Breuning 1970: 415 (catalogue), fig. 23 i (♀); Hua 1981: 178 (new distribution); Hua 2002: 216 (catalogue); Löbl and Smetana 2010: 315 (catalogue); Lin and Tavakilian 2019: 366 (catalogue); Danilevsky 2020: 451 (catalogue); Xie et al. 2023: 248 (catalogue), fig. 3 (a–d holotype, ♂; e, f ♀; g, h ♂).

*Alidus multilineatus* Pic, 1925: 24 (type locality "Tonkin, Vietnam"): Breuning 1963b: 475 (synonymized).

**Body length.** 15.0–18.0 mm (♂), 12.0–18.2 mm (♀). The body length was determined from the holotypes of *Mispila curvilinea*, *Alidus multilineatus*, and additional materials examined. The body length of the holotype of *A. multilineatus* was mentioned in the original paper (Pic 1925).

**Type material examined. *Mispila curvilinea*:** holotype, ♂ (BMNH), *Mispila curvilinea* (handwritten with black ink on a rectangular white label with a straight-line black border) / *Mispila curvilinea* typ Pasc (handwritten with black ink on a rectangular white label) / India (handwritten with black ink on a fan-shaped green label) / Pascoe Coll. 93–60 (printed with black ink on a square white label) / Type (printed with black ink on a circular white label with circular red borders) / NHMUK 014596491 plus a QR-code (printed with black ink on a rectangular white label); examined from five photographs (Fig. 3A–E). ***Alidus multilineatus*:** holotype, ♂ (MNHN), Pho-vi (Tonkin) 9. 07 (handwritten with black ink on a rectangular white label) / Type (handwritten with black ink on a rectangular yellow label) / ex Buquet (handwritten with black ink on a rectangular white label) / *Alidus multilineatus* Pic (handwritten with black ink on a rectangular white label) / TYPE (printed with black ink on a rectangular red label); examined from three photographs (Fig. 3F–H).

**Additional material examined. China** • 3♂♂, 3♀♀ (LPSNU), Hulukou, Xima Town, Yingjiang County, Dehong Dai and Jingpo Autonomous Prefecture, Yunnan Province, Alt. 1200 m, VI–VII.2018, leg. Wei-Zong Yang • 1♂ (CWW): vic. Guo Men Shan (NNNR), 37 km NW Jinghong City, Xishuangbanna Dai Autonomous Prefecture, Yunnan Province, 22°14'43"N, 100°36'12"E, Alt. 1100 m, 18.VI.2019, leg. A. Weigel LFF [light trap]. **Laos:** 1♂, 2♀♀ (CWW): Phou Pan (Mt.), Ban Saleui, Hua Phan Province, 20°12'N, 104°01'E, Alt. 2060 m, V.2017, leg. local collector. **Malaysia** • 1♀ (CWW, fig. 4E): 35 km SE Ipoh, Tanah Rata, Cameron Highland, Pahang, 4°28'N, 101°23'E, Alt. 1500 m, 19–31.III.2003, leg. M. Nèmec.

**Comments.** In the original description of *A. multilineatus*, Pic (1925) did not mention the gender of the holotype. Breuning (1963b: 475) treated it as a junior synonym of *M. curvilinea* and provided a supplementary description of this species, including the sexual differences: "antennae are more than 0.5 time longer than body in males, antennae are slightly shorter than body in females." According to above characters and the material examined, we could confirm that the holotype of *A. multilineatus* is a male.

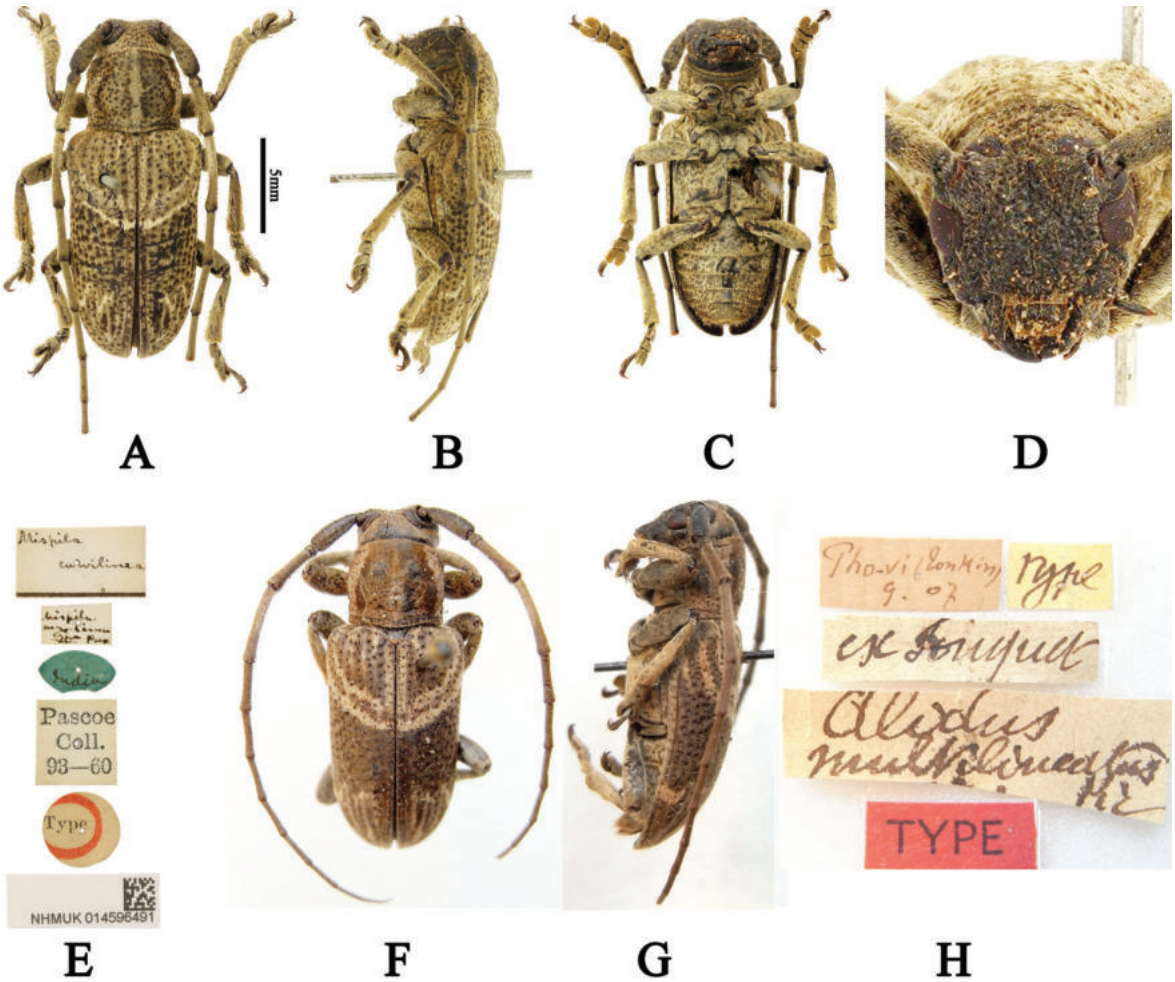


**Figure 3. A–E** *Mispila curvilinea*, holotype (photographs of Fig. 3A–C, E reproduced from Xie et al. 2023) **A** male dorsal habitus **B** male lateral habitus **C** male ventral habitus **D** male frontal view **E** labels **F–H** *Alidus multilineatus*, holotype **F** male dorsal habitus **G** male lateral habitus **H** labels.

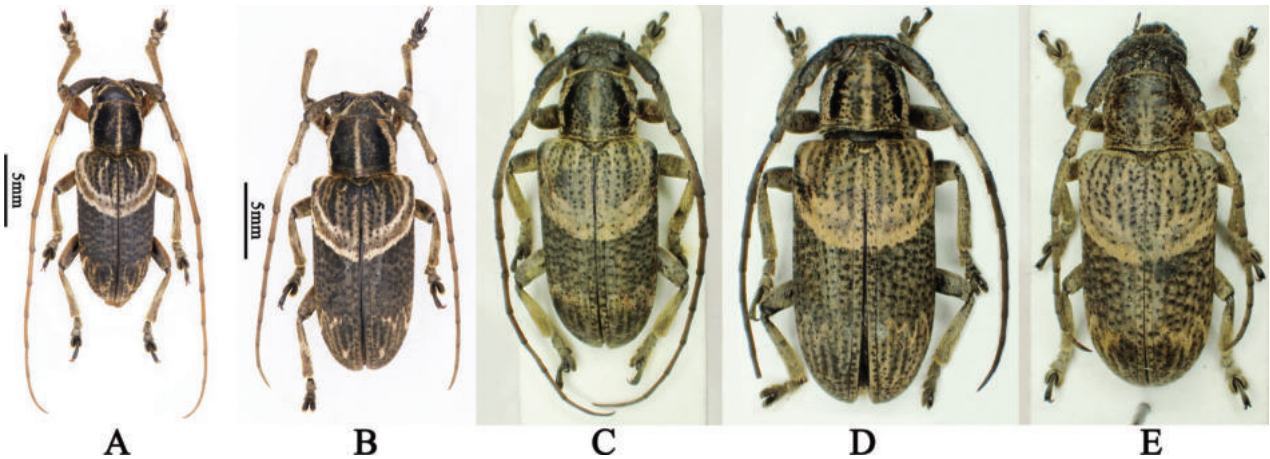


**Figure 4.** *Mispila curvilinea*, dorsal habitus **A** male from Yunnan, China **B** female from Yunnan, China **C** male from Hua Phan, Laos **D** female from Hua Phan, Laos **E** female from Pahang, Malaysia.

**Distribution.** Bengal (Klimpong, Samsingh), Cambodia, China (Guangxi, Yunnan), India (Sikkim), Laos (Bokeo, Hua Phan, Khammouane, Vientiane), Malaysia (Pahang), Vietnam (Ha Giang).

### ***Mispila* (*s. str.*) *subtonkinea* Breuning, 1968**
Fig. 5

*Mispila* (*s. s.*) *subtonkinea* Breuning, 1968b: 21 (type locality "Vientiane, Laos").
*Mispila* (*s. str.*) *subtonkinea* Rondon and Breuning 1970: 415 (catalogue), fig. 24a (holotype, ♂); Xie et al. 2023: 260 (catalogue), fig. 13g–l (♂).

**Body length.** 8.0–9.0 mm (♂). The body length is determined from the holotype of *M. subtonkinea* and additional materials examined. The body length of the holotype of *M. subtonkinea* is referred to in the original paper (Breuning 1968b).

**Type material examined.** ***Holotype***, ♂ (BPBM), Vientiane, Laos, 15 février (= February) 1965; examined from one photograph (Fig. 5A).

**Additional material examined. Vietnam:** 1 ♂ (CWW, fig. 5B): 6 km SW von Thanh Son, Tay Yen Tu Nat. Res., Bắc Giang Province, 21°10.830'N, 106°43.427'E, Alt. 200 m, 18–21.V.2015, leg. A. Weigel, KS [clearing] LFF [light trap].

**Distribution.** Laos (Vientiane), Vietnam (Bắc Giang).

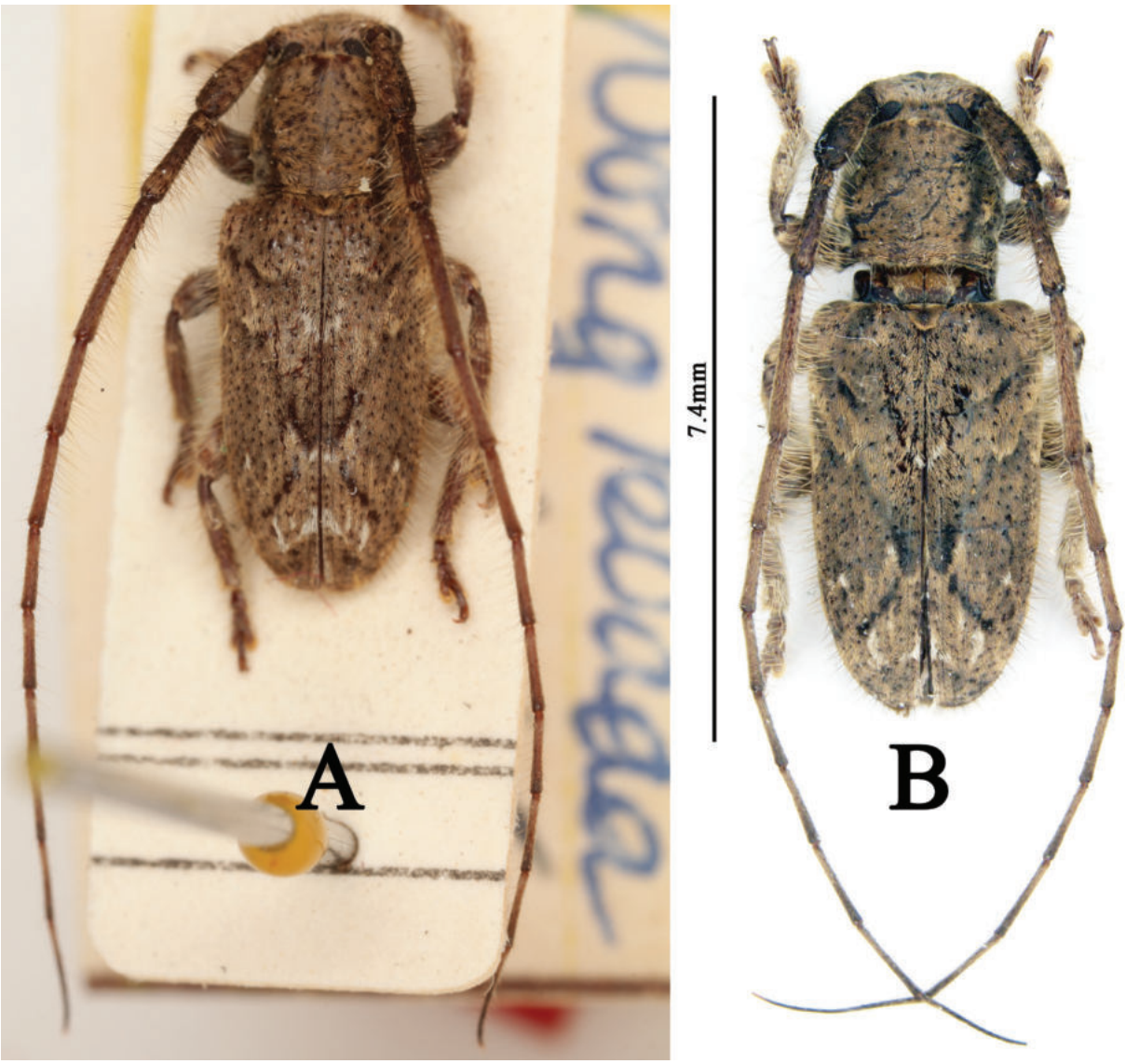


**Figure 5.** *Mispila subtonkinea*, males, dorsal habitus **A** holotype **B** specimen from Vietnam.

***Mispila* (*s. str.*) *tenuevittata* (Pic, 1930)**
Fig. 6

*Sodus tenuevittatus* Pic, 1930: 19 (type locality: "Chapa, Tonkin, Vietnam").
*Mispila* (*Mispila*) *venosa* m. *tenuevittata*: Breuning 1961: 281 (catalogue); Breuning 1963b: 486 (catalogue).
*Mispila* (*s. str.*) *tenuevittata*: Yan et al. 2023: 4 (stat. resurrected, catalogue), figs 2A–H, 3A–D; Xie et al. 2023: 255 (catalogue), fig. 8 (e–g ♂, h–j ♀).
*Mispila* (*Dryusa*) *sonthianae* Breuning, 1963d: 59 (type locality: "Vientiane, Laos"), fig. (holotype); Rondon and Breuning 1970: 415 (catalogue), fig. 24b (holotype, ♂); Löbl and Smetana 2010: 315 (catalogue); Lin and Tavakilian 2019: 366 (catalogue); Danilevsky 2020: 451 (catalogue).
*Mispila sonthianae*: Hua 2002: 216 (catalogue); Hua et al. 2009: 92, pl. 92, fig. 1053 (♂, ♀).
*Mispila assamensis* Breuning, 1938: 381 (type locality: "Mtes. Patkai, Assam, India"); Kariyanna et al. 2017: 193 (catalogue); Mitra et al. 2017: 87 (distribution); Xie et al. 2023: 257 (catalogue), fig. 11 (a–d holotype, ♀; e–h ♂). syn. nov.
*Mispila* (*Mispila*) *assamensis*: Breuning 1961: 281 (catalogue); Breuning 1963b: 472 (key), 487 (redescription).

**Body length.** 7.1–12.0 mm (♂), 7.6–13.7 mm (♀). The body length was determined from Yan et al. (2023), the holotype of *M. assamensis*, and additional materials examined.

**Type material examined. *Sodus tenuevittatus*:** see Yan et al. 2023. ***Mispila assamensis*:** holotype, ♀ (BMNH), *Mispila assamensis* mihi Typ det. Breuning ("*Mispila assamensis* mihi Typ" handwritten and "det. Breuning" printed with black ink on a rectangular white label) / Assam Patkai Mt. (handwritten with black ink on a rectangular white label) / Doherty (handwritten with black ink on a rectangular white label)/ Fry Coll. 1905. 100. (printed with black ink on a rectangular white label) / 61563 (handwritten with black ink on a rectangular white label) / Type (printed with black ink on a circular white label with circular red borders) / NHMUK 014596495 plus a QR-code (printed with black ink on a rectangular white label); examined from five photographs (Fig. 6D–H).

**Additional material examined. Thailand** • 1♂ (CWW): vic. Khao Lak, Takuapa distr., Phang-nga Province, 08°37.623'N, 98°15.091'E, Alt. 50 m, 23.VIII–02.IX.2010, leg. A. Skale • 3♂♂, 3♀♀ (CWW): vic. Khao Lak, Takuapa distr., Phang-nga Province, 08°37'N, 98°15'E, 07.VIII.2012, leg. A. Weigel • 1♀ (CWW, fig. 6J): Yai island, Kam, 14 km W Na Kha, Ranong Province, 09°29.652'N, 98°21.385'E, 09.VIII.2012, leg. A. Weigel, UWP [primary forest] KÜ [coast]. **Vietnam** • 2♂♂ (CWW, fig. 6K): Son Hurang River, Hue, Thừa Thiên Huế Province, 16°27'36"N, 107°34'7"E, 10 m [a. s. l.], 11.V.2019, leg. A. Weigel, LFF [light trap] (Hotel).

**Comments.** After having compared the holotypes of *Mispila tenuevittata* (Fig. 6A, B) and *M. assamensis* (Fig. 6D–G), we found that both holotypes are identical, except for the different genders. Thus, we treat *M. assamensis* as a junior synonym of *M. tenuevittata*. Yan et al. (2023) marked the holotype of *M. tenuevittata* as a female in the legend, while they considered it as a male in the comments.

**Distribution.** China (Guangxi, Hainan, Yunnan), India (Assam), Laos (Bokeo, Mekong, Vientiane), Thailand (Phang-nga, Ranong), Vietnam (Chapa, Thừa Thiên Huế).

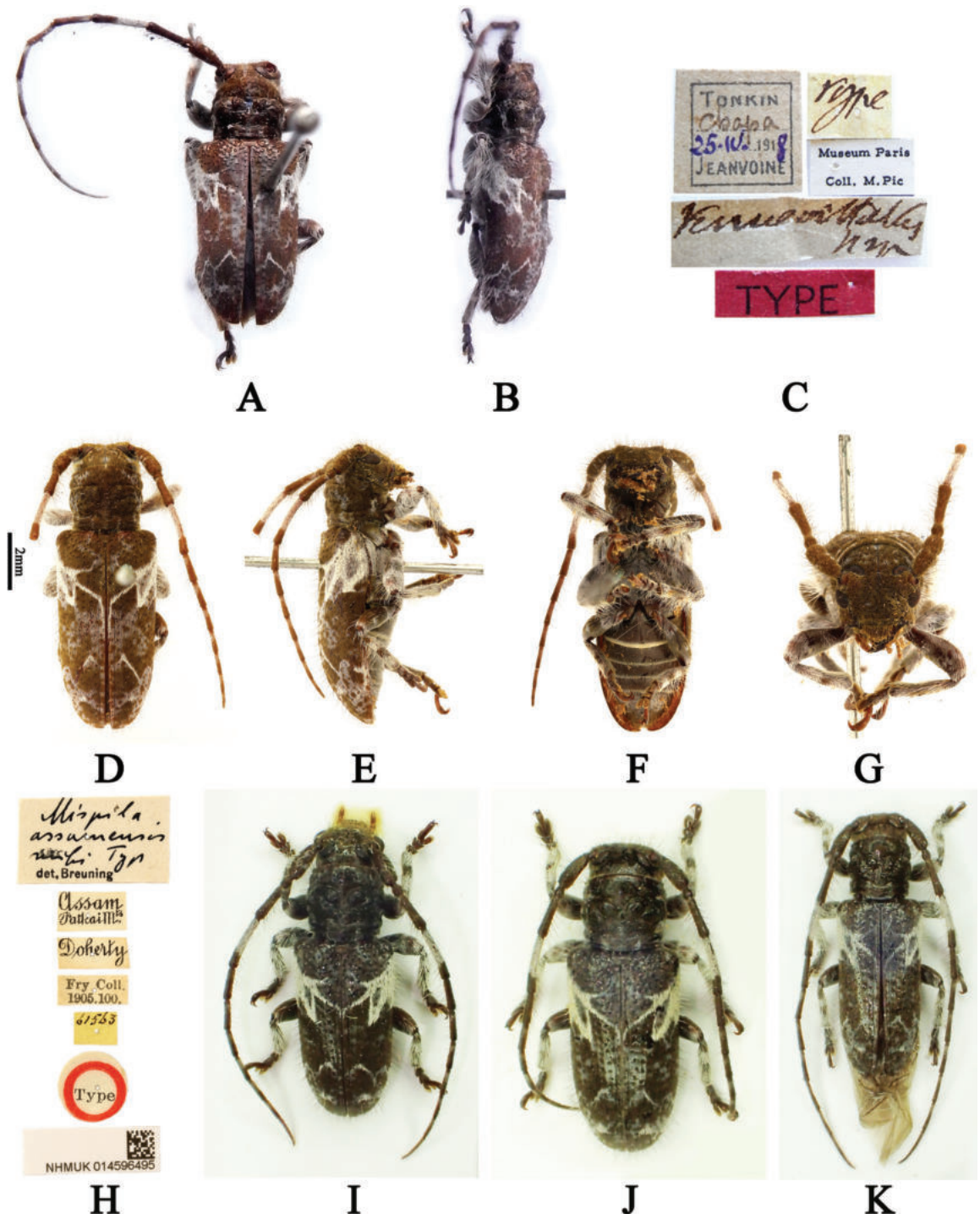


**Figure 6.** *Mispila tenuevittata* **A–C** *Sodus tenuevittatus*, holotype **A** male dorsal habitus **B** male lateral habitus **C** labels (photographs of Fig. 6A–C reproduced from Yan et al. 2023) **D–H** *Mispila assamensis*, holotype (photographs of Fig. 6D–F, H reproduced from Xie et al. 2023) **D** female dorsal habitus **E** female lateral habitus **F** female ventral habitus **G** female frontal view **H** labels **I** male dorsal habitus, from Phang-nga, Thailand **J** female dorsal habitus, from Ranong, Thailand **K** male dorsal habitus, from Thừa Thiên Huế, Vietnam.

## Acknowledgements

We sincerely appreciate Xavier Gouverneur (Rennes, France) for providing the lectotype photographs of *Alidus signatus*, holotype photographs of *Mispila coomani* and *Alidus multilineatus*; Guang-Lin Xie (Yangtze University, Jingzhou,

Hubei, China) for providing the holotype photographs of *Mispila curvilinea* and *Mispila assamensis*; Hong Pang (SYSU) and Bing-Lan Zhang (SYSU) for providing the holotype photographs of *Enispia quadristigma*; Rachel Diaz-Bastin (CAS) for providing the paratype photographs of *Enispia quadristigma*; Nobuo Ohbyashi (Miura City, Japan) gave us the permission to use the syntype photograph of *Souvanna phoumai* and the holotype photograph of *Mispila subtonkinea*. We are very grateful to Fa-Lei Wang (Chongqing, China) for donating material of *Mispila curvilinea*, to Ri-Xin Jiang (Guizhou University, Guizhou, China) for donating material of *Souvanna signata*. We also thank Andre Skale (CSG) for providing data of *Souvanna signata*, Ping Wang (Yangtze University, Jingzhou, Hubei, China), Zhu Li (Southwest University, Chongqing, China), Guang-Lin Xie, Xavier Gouverneur and Si-Yao Huang (Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany) for providing literatures. We express our appreciation to Larry G. Bezark (Sacramento, California, USA) and Francesco Vitali (Academic Editor of ZooKeys for Cerambycidae) for improving our manuscript.

## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

### Funding

This research was supported by the Fund Project of the Education Department of Guizhou Province (黔教技[2022]091号, 黔教技[2022]054号, 黔教技[2022]338号), the Guizhou Provincial Science and Technology Foundation (黔科合基础-ZK[2022]一般527).

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Funding acquisition: GH, GZ. Writing – original draft:GH, EC. Writing – review and editing: GH, AW.

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### Data availability

All of the data that support the findings of this study are available in the main text.

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ZooKeys 1190: 121–130 (2024)
DOI: 10.3897/zookeys.1190.114201

Research Article

# Review of the genus *Laena* Dejean, 1821 (Coleoptera, Tenebrionidae) from Gansu Province, China, with the description of a new species

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## Abstract

A new species of the genus *Laena* from Xiaolongshan in Gansu Province, China is described as *Laena hui* **sp. nov.** All *Laena* species known to occur in Gansu Province are reviewed, and an identification key is provided. The mitochondrial gene COI to confirm the identity of the new species, which is morphologically most similar and phylogenetically close to *L. fengileana*. The new species can be recognized by features of elytra and tibiae.

**Key words:** COI gene, DNA barcoding, identification key, Laenini, Lagriinae

OPEN ACCESS

Academic editor: Aaron Smith
Received: 18 October 2023
Accepted: 8 January 2024
Published: 24 January 2024

ZooBank: https://zoobank.org/2D7C511A-83A4-4379-9ACA-6E9B587AAE95

Citation: Wei Z, Ren G (2024) Review of the genus *Laena* Dejean, 1821 (Coleoptera, Tenebrionidae) from Gansu Province, China, with the description of a new species. ZooKeys 1190: 121–130. https://doi.org/10.3897/zookeys.1190.114201

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## Introduction

The genus *Laena* Dejean, 1821 belongs to the tribe Laenini, subfamily Lagriinae, family Tenebrionidae, which is widely distributed in Asia, southern Europe, and southern Africa (Bouchard et al. 2021). Adults are found under loose bark, in leaf litter, in crevices of wood, or under stones in steppe, the alpine zone, and even in subdeserts, while larvae and pupae are found in rotten wood (Wei and Ren 2019a) and in soil for xerophylic species.

In China, the species diversity of the genus *Laena* is extraordinarily high. In the last two decades, more than 100 *Laena* species have been described from China (Schawaller 2001, 2008, 2021; Zhao and Ren 2011, 2012a, 2012b; Schawaller and Aston 2017; Wei and Ren 2019b, 2019c, 2023; Wei et al. 2020, 2021; Schawaller and Bellersheim 2023; Wei and Ren 2023).

Southern Gansu is an important part of the Qingling Mountains, which is an important zone of species diversity in China, and four *Laena* species have been recorded from Gansu Province to date (Reitter 1889; Schawaller 2001, 2008). During an investigation into insect diversity in Xiaolongshan, Gansu Province, another undescribed *Laena* species was collected in southern Gansu.

In this study, *Laena hui* sp. nov. is described and illustrated. An identification key to the five *Laena* species from Gansu is also provided; it is based on examined specimens. DNA barcoding has been widely used in species delimitation in insects (Hebert et al. 2004; Hajibabaei et al. 2006; Smith et al. 2007; Liu et al. 2018; Han et al. 2022; Li et al. 2022) since it was initially proposed by Hebert et al. (2003). To clarify the taxonomic status of the new species, the mitochondrial gene COI was sequenced, and a maximum-likelihood phylogenetic tree was constructed to explore the position of *L. hui* sp. nov. in the genus *Laena*.

## Materials and methods

The examined *Laena* specimens are deposited in the China West Normal University (**CWNU**), the Museum of Hebei University (**MHBU**), and Institute of Zoology, Chinese Academy of Sciences (**IZAS**). The whole genomic DNA was extracted from leg and thorax muscle tissues of *Laena* specimens using the Ezup Column Animal Genomic DNA Purification Kit (Shanghai, China) following the manufacturer's instructions. The polymerase chain reactions (PCR) were conducted under the conditions as specified by Wei and Ren (2023). The bidirectional sequencing of mitochondrial gene COI was conducted by Sangon Biotech Co. Ltd (Shanghai, China). The new sequences were checked and edited using SeqMan v. 7.1.0 and BioEdit v. 7.1.11. All the sequences were aligned and trimmed using ClustalW and trimAl v. 1.2, respectively. The best-fit model was calculated using Modelfinder based on the Bayesian information criterion. The maximum-likelihood (ML) tree was constructed using IQtree v. 1.6.8 integrated in PhyloSuite v. 1.2.2 (Zhang et al. 2020) and based on default parameters values. The original ML tree was edited and visualized using FigTree v. 1.43 and Photoshop cc 2019. In total, 31 COI sequences of 17 *Laena* species were used for the phylogenetic analyses, including 25 previously known and the six new sequences provided in this study (Table 1). Five species of the genus *Anaedus* Blanchard, 1842, *Hypolaenopsis* Masumoto, 2001, and *Grabulax* Kanda, 2016 were used as outgroups.

## Results

### Phylogenetic analyses

The ML tree was reconstructed based on GTR+I+G4+F model. The phylogenetic tree (Fig. 1) showed that all the *Laena* species form a single clade with low value support (43). The target species, *L. hui* sp. nov., is close to *L. fengileana* in the ML tree with high value support (96).

### Taxonomy

**Tribe Laenini Seidlitz, 1895**
**Genus *Laena* Dejean, 1821**

### A key to five *Laena* species recorded from Gansu Province

1 All femora without teeth on inner sides near apex.....**2**
– All femora with teeth on inner sides near apex.....**4**

2 Pronotal lateral margins beaded ..... **3**
– Pronotal lateral margins not beaded (Fig. 3A) ..... ***L. bifoveolata***
3 Elytral interval with irregular and small punctures ..... ***L. haigouica***
– Elytral interval with a row of small punctures..... ***L. langmusica***
4 Pronotal disc with two impressions in median portion; protibia distinctly broadened at base at inner side; body length 5.7–5.9 mm (Fig. 2H) ..... ..... ***L. fengileana***
– Pronotal disc without impressions in median portion; protibia gradually broadened from base to apex; body length 7.1–7.7 mm (Fig. 2D) ..... ..... ***L. hui* sp. nov.**

**Table 1.** The taxa were used for phylogenetic analysis in this study.

| No. | Taxa | Collection information | GenBank no. | References |
|---|---|---|---|---|
| GS12 | *Laena fengileana* Masumoto, 1996 | 2022-VII-3, China, Gnasu, Li County, Taopingxiang, Nanshan, elev. 2190 m | OR682144 | This study |
| SC21 | *Laena qinlingica* Schawaller, 2001 | 2023-IX-8, China, Sichuan, Wangcang County, Micangshan, Shuiliandong elev. 1650 m | OR682145 | This study |
| GS31 | *Laena bifoveolata* Reitter, 1889 | 2022-VI-23, China, Gansu, Qingshui County, Shanmenzhen, Dajicun, elev. 1784 m | OR682146 | This study |
| GS32 | *Laena bifoveolata* Reitter, 1889 | 2022-VI-23, China, Gansu, Qingshui County, Shanmenzhen, Dajicun, elev. 1784 m | OR682147 | This study |
| GS4 | *Laena hui* sp. nov. | 2021-VIII-29, China, Gansu Province, Tianshui City, Dongchazhen, Dongcha forest farm, elev. 1840 m | OR682148 | This study |
| YN01 | *Laena yulongica* Schawaller, 2001 | 2022-V-7, China, Yunnan, Weixi County, Nilidicun, elev. 2300 m | OR682149 | This study |
| n/a | *Laena haigouica* Schawaller, 2001 | 2022-VII-23, China, Sichuan, Songpan, Huanglong, Dawan, elev. 2920 m | OR721926 | Wei and Ren 2023 |
| n/a | *Laena kangdingica* Schawaller, 2001 | 2022-VIII-5, China, Sichuan, Yajiang, Waduozhen, elev. 2600 m | OR721927 | Wei and Ren 2023 |
| n/a | *Laena bowaica* Schawaller, 2001 | 2022-VIII-1, China, Sichuan, Danba, Bianerxiang, Erwacao, elev. 2470 m | OR721930 | Wei and Ren 2023 |
| n/a | *Laena bowaica* Schawaller, 2001 | 2022.VIII.1, China, Sichuan, Danba, Bianerxiang, Erwacao, elev. 2470 m | OR721931 | Wei and Ren 2023 |
| n/a | *Laena bifoveolata* Reitter, 1889 | 2022.VIII.26, China, Gansu, Longnan, Taopingxiang Taoping Forestry Farm, elev. 2576 m | OR721932 | Wei and Ren 2023 |
| n/a | *Laena bifoveolata* Reitter, 1889 | 2022.VIII.26, China, Gansu, Longnan, Taopingxiang Taoping Forestry Farm, elev. 2576 m | OR721933 | Wei and Ren 2023 |
| n/a | *Laena puetzi* Schawaller, 2001 | 2022.VII.31, China, Sichuan, Jinchuan, Dusongxiang, Dusonggou, elev. 2264 m | OR721934 | Wei and Ren 2023 |
| n/a | *Laena maowenica* Schawaller, 2008 | 2022.VII.20, China, Sichuan, 6 KM Eastern Mao County, elev. 1896 m | OR721935 | Wei and Ren 2023 |
| n/a | *Laena maowenica* Schawaller, 2008 | 2022.VII.20, China, Sichuan, 6 KM Eastern Mao County, elev. 1896 m | OR721936 | Wei and Ren 2023 |
| n/a | *Laena fengileana* Masumoto, 1996 | 2022.VII.22, China, Sichuan, Songpan, Mounigou, Shangzhai, elev. 3070 m | OR721937 | Wei and Ren 2023 |
| n/a | *Laena becvari* Schawaller, 2001 | 2022.VIII.7, China, Sichuan, Litang, Junba, elev. 3050 m | OR721938 | Wei and Ren 2023 |
| n/a | *Laena becvari* Schawaller, 2001 | 2022.VIII.7, China, Sichuan, Litang, Junba, elev. 3050 m | OR721939 | Wei and Ren 2023 |
| n/a | *Laena mounigouica* Wei & Ren, 2023 | 2022.VII.21, Sichuan, Songpan, Mounigou, Tuguanzhai, elev. 2978 m | OR721941 | Wei and Ren 2023 |
| n/a | *Laena mounigouica* Wei & Ren, 2023 | 2022.VII.21, Sichuan, Songpan, Mounigou, Tuguanzhai, elev. 2978 m | OR721942 | Wei and Ren 2023 |
| n/a | *Laena shaluica* Schawaller, 2001 | 2022.VIII.5, China, Sichuan, Yajiang, Waduozhen, Ridui, elev. 3100 m | OR721943 | Wei and Ren 2023 |
| n/a | *Laena shaluica* Schawaller, 2001 | 2022.VIII.5, China, Sichuan, Yajiang, Waduozhen, Ridui, elev. 3100 m | OR721944 | Wei and Ren 2023 |
| n/a | *Laena barkamica* Schawaller, 2008 | 2022.VII.26, China, Sichuan, Heishui, Yangyong, Hade, elev. 2600 m | OR721945 | Wei and Ren 2023 |
| n/a | *Laena barkamica* Schawaller, 2008 | 2022.VII.26, China, Sichuan, Heishui, Yangyong, Hade, elev. 2600 m | OR721946 | Wei and Ren 2023 |
| n/a | *Laena fengileana* Masumoto, 1996 | 2022.VII.23, China, Sichuan, Songpan, Huanglongxiang, Dawan, elev. 2920 m | OR721947 | Wei and Ren 2023 |
| n/a | *Laena fengileana* Masumoto, 1996 | 2022.VII.23, China, Sichuan, Songpan, Huanglongxiang, Dawan, elev. 2920 m | OR721948 | Wei and Ren 2023 |
| n/a | *Laena yajiangica* Schawaller, 2001 | 2022.VIII.4, China, Sichuan, Daofu, Xiatuoxiang, Yiwu, elev. 2780 m | OR721949 | Wei and Ren 2023 |
| n/a | *Laena yajiangica* Schawaller, 2001 | 2022.VIII.4, China, Sichuan, Daofu, Xiatuoxiang, Yiwu, elev. 2780 m | OR721950 | Wei and Ren 2023 |
| n/a | *Laena dentithoraxa* Wei & Ren, 2023 | 2022.VIII.6, China, Sichuan, Yajiang Yizhan, elev. 2800 m | OR721951 | Wei and Ren 2023 |
| n/a | *Laena puetzi* Schawaller, 2001 | 2022.VII.29, China, Sichuan, Barkman, Shaerzong, Dazatou, elev. 2690 m | OR721952 | Wei and Ren 2023 |

| No. | Taxa | Collection information | GenBank no. | References |
|---|---|---|---|---|
| n/a | *Laena puetzi* Schawaller, 2001 | 2022.VII.29, China, Sichuan, Barkman, Shaerzong, Dazatou, elev. 2690 m | OR721953 | Wei and Ren 2023 |
| n/a | *Hypolaenopsis nomurai* (Schawaller, 2001) | 2022.VII.23, China, Sichuan, Songpan, Huanglongxiang, Dawan, elev. 2920 m | OR721929 | Wei and Ren 2023 |
| n/a | *Hypolaenopsis* sp. | 2022.VII.26, China, Sichuan, Heishui, Yangrong, Hade, elev. 2600 m | OR721940 | Wei and Ren 2023 |
| n/a | *Hypolaenopsis hongyuanica* (Schawaller, 2001) | 2022.VII.26, China, Sichuan, Hongyuan, Shuajingsi, elev. 3160 m | OR721928 | Wei and Ren 2023 |
| n/a | *Grabulax darlingtoni* Kanda, 2016 | Colombia, Sierra Nevada de Santa Marta | KU233834 | Kanda et al. 2015 |
| n/a | *Anaedus brunneus* Ziegler, 1844 | n/a | MN448231 | Direct submission |

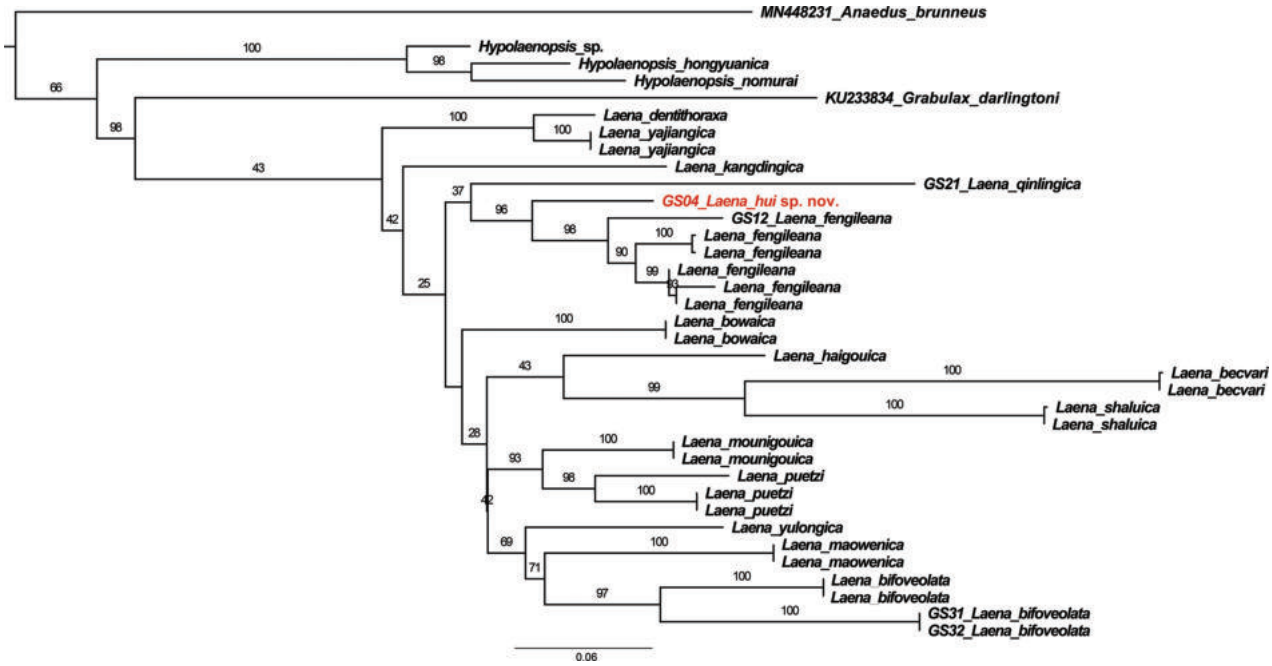


**Figure 1.** The maximum-likelihood tree of *Laena* species based on mitochondrial gene COI. The new species is in red.

***Laena hui* sp. nov.**
https://zoobank.org/DD047226-D95F-4642-BD96-053044595E53
Fig. 2A–G

**Type locality.** China, Gansu Province, Tianshui City, Dongchazhen.

**Type specimens.** ***Holotype*:** China • ♂; Gansu Province, Tianshui City, Dongchazhen, Dongcha forest farm; 34°15'54"N, 106°35'39"E; elev. 1840 m; 2021-8-29; Qi Liu leg.; MHBU. ***Paratype*:** China • 1♀ (in 95% ethanol); the same data as holotype; CWNU.

**Diagnosis.** Based on morphological characteristics (as provided in the identification key) and the phylogenetic position in the ML tree, the new species is most similar and closest to *L. fengileana*. However, *L. hui* sp. nov. can be distinguished from *L. fengileana* by the following characters: body larger, length 7.1–7.7 mm (5.7–5.9 mm in *L. fengileana*); body surface with very short setae, elytral intervals glabrous (elytral intervals each with a row of small punctures in *L. fengileana*); all tibiae distinctly hooked at their inner apex, protibiae gradually broadened from base to apex (abruptly widened at base in *L. fengileana*; Fig. 2H); and apices of parameres rounded and constricted, lateral margins nearly straight (distinctly concave in posterior in *L. fengileana*).

**Description.** Holotype (Fig. 2A–G). Body length 7.7 mm, width 3.0 mm. Body black; antennae, maxillary palpi, and legs blackish brown; dorsal surface shiny, with sparse punctures bearing short setae.

Head hexagonal, surface smooth, with dense, large punctation bearing short setae. Genae distinctly raised, surface without punctures in apical part, and sides with small punctures. Eyes ovate and prominent. Epistome trapezoidal, with anterior margin weakly emarginated; surface slightly convex at middle, with shallow, small punctures, and each lateral side with a longer seta near anterior angle. Fronto-clypeal suture indistinct, not depressed. Frons distinctive longitudinal convex at middle, with large, sparse large punctures; lateral parts depressed, with large, dense punctures. Vertex weakly convex, with large, sparse punctures on middle. Antennae slender, reaching pronotal base when directed backwards; antennomere III approximately 2.1× as long as antennomere II, the relative ratio of the length of antennomeres II–XI as follows: 0.16:0.3 3:0.26:0.24:0.26:0.26:0.26:0.26:0.28:0.42.

Pronotum widest at anterior 1/3, widened anteriorly and significantly convergent from anterior 1/3 to anterior margin; anterior margin slightly emarginated at middle; lateral margins neither marked nor beaded; basal margin neither bent downwards nor beaded; disc strongly convex, surface with large, sparse punctures, and distance between punctures 0.5–3.0× puncture diameter; anterior and posterior angles rounded, not produced. Prothoracic hypomera with punctures as large as those on disc, but with shorter setae. Prosternal process widest at middle and bent downwards behind coxae; surface with dense and large punctures bearing very short setae.

Elytra (Fig. 2C) elongate-oval, widest at middle, approximately 1.6× longer than wide; lateral sides arcuate; humeral angles rounded. Elytral surface smooth, with rows of punctures without striate, bearing very short setae; punctures in rows as large as those on pronotal disc; elytral intervals with few punctures nearly invisible, interval IX with three setigerous pores (one on anterior part, two on posterior part). Elytral apices significantly prolonged and with apex obtuse.

Abdomen ovoid, approximately 1.7× as long as wide. Surface convex, smooth, with punctures gradually became smaller from ventrites I–IV, bearing short setae; posterior part of ventrites IV distinctly convex transversely at posterior part before posterior margin; ventrites V with setae at posterior part longer than those on anterior part.

Legs (Fig. 2D–F) long and slender. Femora with sharp teeth near apex on inner sides; tooth on profemora rounded at apex, and meso- and metafemoral teeth acute and pointed at apex. All tibiae slender and distinctly hooked at inner apex; protibiae gradually becoming broader from base to apex, metatibiae slightly S-shaped on inner sides.

Aedeagus (Fig. 2G) subfusiform, length 2.2 mm, width 1.8 mm. Parameres trapezoidal, widest at base and narrowing to apex, with rounded apex; lateral sides of parameres shortly constricted before apex.

**Sexual dimorphism.** Female. Body length 7.1 mm, width 3.0 mm. Apex of tibiae not hooked at inner sides.

**Distribution.** China: Gansu.

**Etymology.** The name of this species honors the late Prof. Jinchu Hu (China West Normal University, Nanchong City, China) who is a famous expert on the Giant Panda.

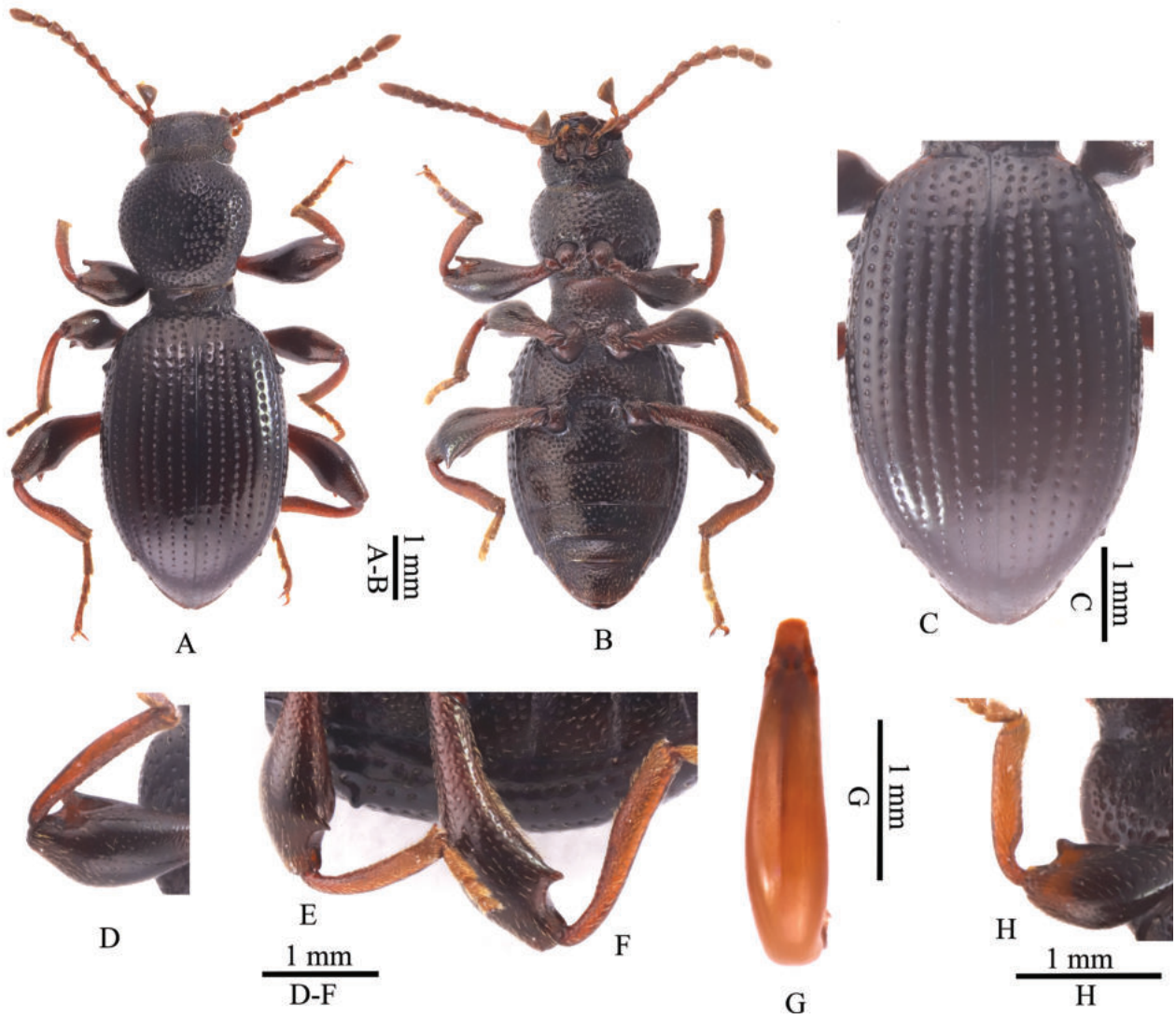


**Figure 2.** *Laena* species **A–G** *Laena hui* sp. nov., holotype **A, B** dorsal and ventral views **C** elytra **D–F** pro- meso- and meta-leg, in ventral view **G** aedeagus **H** proleg of *L. fengileana*.

**Note.** The specimens were collected by sifting leaf litter in a mixed forest. The paratype was preserved in 95% alcohol, and a hind leg was used to extract the whole genome. The mitochondrial gene COI of this new species is provided in Table 1.

### ***Laena bifoveolata*** **Reitter, 1889**

Fig. 3A

*Laena bifoveolata* Reitter, 1889: 709; Schawaller 2001: 7; Schawaller 2008: 404; Wei et al. 2020: 523.

**Examined specimens.** China – Ningxia Hui Autonomous Region • 4♂4♀;·Liupanshan, Longtan forestry station; 35.3898°N, 106.3451°E; elev. 1936 m, 2008-VI-23, Qiaohe Lou leg., IZAS – Gansu Province • 1♂ (in 95% ethanol); Dingxi City, Zhang County, Xinsizhen, Dishuiya; 34.6025°N, 104.5713°E; elev.

1930 m; 2022-VI-30; Qi Liu leg.; CWNU • 2♀ (in 95% ethanol); Hui County, Xiaolongshan, 33.6522°N, 106.2938°E; elev. 1920 m; 2022-VII-9; Qi Liu leg.; CWNU • 2♀ (in 95% ethanol); Qingshui County, Shanmenzhen, Dajicun; 34.2153°N, 106.3372°E; elev. 1784 m; 2022-VI-23; Qi Liu leg.; CWNU • 1♂4♀ (in 95% ethanol); Dingxi City, Zhang County, Malizhen, Huihuiliang; 34.5019°N, 104.7097°E; elev. 2300 m; 2022-VII-7; Qi Liu leg.; CWNU • 1♀ (in 95% ethanol); Qingshui County, Shanmen forest farm; 34.4056°N, 106.2222°E; elev. 1666 m; 2022-VI-21; Qi Liu leg.; CWNU.

**Distribution.** China: Shaanxi, Ningxia, Gansu, Hubei, and Sichuan.

### ***Laena fengileana*** **Masumoto, 1996**

Figs 2H, 3B

*Laena fengileana* Masumoto, 1996: 180; Schawaller 2001: 15; Schawaller 2008: 404; Yuan and Ren 2018: 698; Wei and Ren 2023: 79.

**Examined specimens.** China – Gansu Province • 1♂ (in 95% ethanol); Li County, Shangpingxiang, Changankan; 34.1416°N, 104.8240°E; elev. 2550 m; 2022-VII-2; Qi Liu leg.; CWNU • 3♂ (in 95% ethanol); Li County, Taopingxiang, Nanshan; 34.0745°N, 104.8977°E; elev. 2190 m; 2022-VII-3; Qi Liu leg.; CWNU • 1♂1♀; Woniushan forest park; 34.4832°N, 104.8311°E; elev. 2650 m; 2022-VI-28; Qi Liu leg.; CWNU – Shaanxi Province • 2♂; Qinling Shan Mt. range, W pass on road Xi'an to Shagoujie, 45 km, SW Xi'an; 33°52'N, 108°46'E ; elev. 2800 m; 2001-VII-25; A. Metana leg.; MHBU.

**Distribution.** China: Sichuan, Shaanxi, and Gansu.

### ***Laena haigouica*** **Schawaller, 2001**

Fig. 3C

*Laena haigouica* Schawaller, 2001: 19; Schawaller 2008: 405; Wei et al. 2020: 526; wei and Ren 2023: 79.

**Examined specimens.** China – Sichuan Province • 1♂ (in 95% ethanol); Songpan, Huanglongxiang, Dawancun; elev. 2920 m; 2022-VII-23; Zhonghua Wei leg.; CWNU.

**Distribution.** China: Gansu and Sichuan.

### ***Laena langmusica*** **Schawaller, 2001**

Fig. 3D

*Laena langmusica* Schawaller, 2001: 25; Schawaller 2008: 405; Yuan and Ren 2018: 699; Wei et al. 2020: 526.

**Examined specimens.** China – Sichuan Province • 1♂1♀; West of Zhier (= Zier); elev. 4241 m; 28°20.87'N, 101°28.36'E; 5-VI-2004; R. Sehnai and M. Tryzna leg.; MHBU.

**Distribution.** China: Shaanxi, Gansu, and Sichuan.

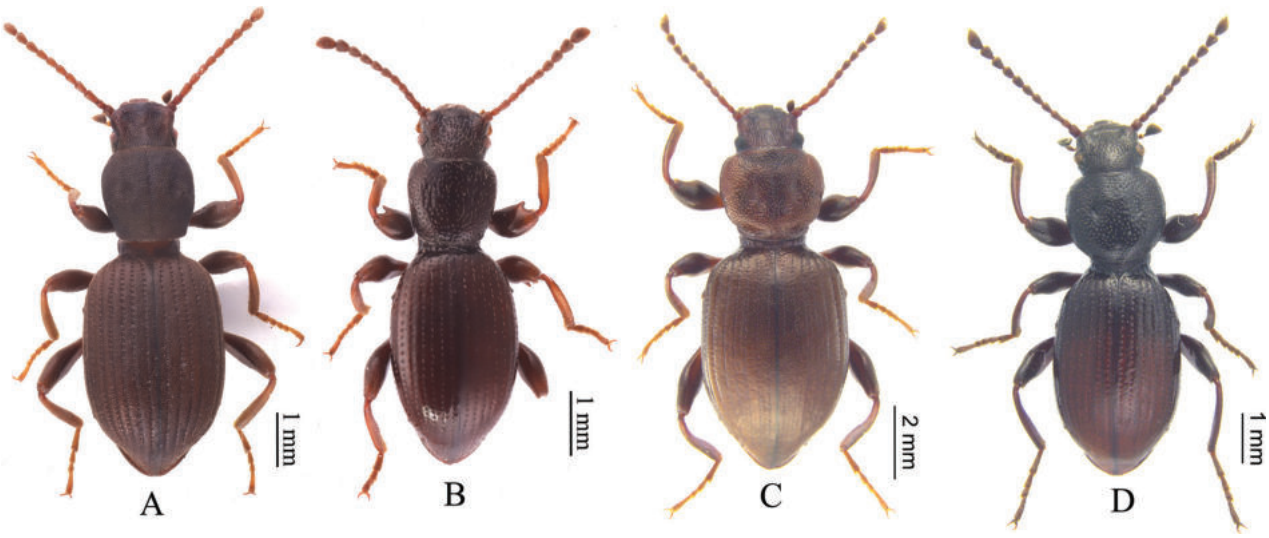


**Figure 3.** The four *Laena* species previously known from Gansu Province **A** *L. bifoveolata* **B** *L. fengileana* **C** *Laena haigouica* **D** *L. langmusica*.

## Acknowledgments

We thank Mr. Qi Liu (Hebei University, Baoding, Hebei, China) for collecting the specimens, and Dr. Menglin Wang (China West Normal University, Nanchong, Sichuan, China) for revising the manuscript. We cordially thank reviewers Dr. Wolfgang Schawaller and Dr. Maxim Nabozhenko for their valuable comments on the manuscript.

## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

### Funding

This study was supported by the Natural Science Foundation of Sichuan Province (2022NSFSC1615) and the Doctoral Scientific Research Foundation of China West Normal University (20E054).

### Author contributions

Conceptualization: ZHW, GDR. Data curation: ZHW. Formal analysis: ZHW. Funding acquisition: MLW. Visualization: ZHW. Writing – original draft: ZHW. Writing – review and editing: GDR, MLW.

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### Data availability

The new sequences of the mitochondrial gene COI are available in NCBI (OR682144–OR682149).

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ZooKeys 1190: 131–152 (2024)
DOI: 10.3897/zookeys.1190.109886

Research Article

# A new species of *Odorrana* Fei, Ye & Huang, 1990 (Amphibia, Anura, Ranidae) from central Guangxi, China with a discussion of the taxonomy of *Odorrana* (*Bamburana*)

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## Abstract

A new species of odorous frog, *Odorrana damingshanensis* **sp. nov.**, was found at the Damingshan National Nature Reserve in Guangxi, China. This species can be distinguished from its congeners by a combination of the following characters: medium body size (SVL 52.3–54.8 mm in males and 74.8–81.2 mm in females), sawtooth spinules on the upper lip, obtusely rounded snout that extends beyond the lower margin, distinct dorsolateral folds, horny tubercles on the rear of the back, presence of outer metatarsal tubercles, dilated nuptial pad with velvety spinules, distinct maxillary gland with tiny spines, and external lateral vocal sac. Through analysis of the 16S mitochondria gene, the new species is closely related to *O. nasica* and *O. yentuensis*, but the genetic divergence between the new species and the latter exceeds 7% (uncorrected *p*-distance). Currently, the new species is only known from its original discovery site. Furthermore, a discussion on the taxonomy of *Odorrana* (*Bamburana*) was conducted, identifying seven species within the subgenus *Odorrana* (*Bamburana*).

**Key words:** Cryptic species, morphology, Odorous frog, phylogeny, taxonomy

OPEN ACCESS

Academic editor: Luis Ceríaco
Received: 22 July 2023
Accepted: 29 December 2023
Published: 25 January 2024

ZooBank: https://zoobank.org/CA8761BF-F1A7-487B-9509-5894630A5664

Citation: Chen W-C, Mo Y-M, Lin L, Qin K (2024) A new species of *Odorrana* Fei, Ye & Huang, 1990 (Amphibia, Anura, Ranidae) from central Guangxi, China with a discussion of the taxonomy of *Odorrana* (*Bamburana*). ZooKeys 1190: 131–152. https://doi.org/10.3897/zookeys.1190.109886

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## Introduction

The genus *Odorrana* Fei, Ye & Huang, 1990 is currently known to consist of 62 species, primarily found in East and Southeast Asia (AmphibiaChina 2023; Frost 2023). Within China, there have been records of 40 species, with 18 species identified in Guangxi (AmphibiaChina 2023). Among these, *Odorrana versabilis* (Liu & Hu, 1962) was previously believed to have a wide distribution across southern and central China, including provinces such as Zhejiang, Guizhou, Anhui, Fujiang, Jiangxi, Hunan, Guangdong, Guangxi, and Hainan (Guo et al. 1966; Hu et al. 1973, 1978; Liu et al. 1973; Ma et al. 1982; Zou 1983; Pan et al. 1985; Zong and Ma 1985; Wu et al. 1986; Huang et al. 1990; Chen 1991; Sichuan Institute of Biology 1974; Fei et al. 2009; Mo et al. 2014; AmphibiaChina 2023).

Li et al. (2001) conducted a comparison of specimens from different geographic populations and observed distinct morphological divergences, indicating the presence of a species complex within *O. versabilis*. Based on both morphological and molecular data, Li et al. (2001) proposed that the *O. versabilis* species complex consisted of three separate species: *O. exiliversabilis* Li, Ye & Fei, 2001, *O. nasuta* Li, Ye & Fei, 2001, and *O. versabilis*. *Odorrana exiliversabilis* is found in Fujiang, Zhejiang, Anhui, and Jiangxi provinces, with Huangkeng County in Fujiang serving as the type locality (Li et al. 2001; AmphibiaChina 2023). *Odorrana nasuta* is restricted to Hainan Island. *Odorrana versabilis*, on the other hand, occurs in Guizhou, Anhui, Jiangxi, Hunan, Guangdong, and Guangxi, with Longsheng and Jinxiu counties in Guangxi as the type locality. Subsequently, Fei et al. (2005) assigned these three species to the subgenus *Bamburana* within the genus *Odorrana*, based on several distinguishing characters. These included the presence of dorsolateral folds in the subgenus *Bamburana* (absent in the subgenus *Odorrana*), the upper lip adorned with sawtooth spinules (absent in the subgenus *Odorrana*), a xiphisternum without a notch (deeply notched in the subgenus *Odorrana*), and a widened posterior sternum (sternum not widened posteriorly in the subgenus *Odorrana*).

In 2010, Fei et al. conducted a revision of the genus *Odorrana*, reorganizing it into four separate and valid genera within the tribe Odorranini: *Bamburana*, *Eburana*, *Matsuirana*, and *Odorrana*. Fei et al. (2010) proposed that the genus *Bamburana* consisted of seven species: *B. exiliversabilis*, *B. montivaga* (Smith, 1921), *B. nasica* (Boulenger, 1903), *B. nasuta*, *B. trankieni* (Orlov, Le & Ho, 2003), *B. tormota* (Wu, 1977), and *B. versabilis*. However, the idea of dividing the tribe Odorranini into four genera did not gain widespread acceptance. In 2012, Fei et al. followed up on their previous classification (Fei et al. 2005), which had divided the genus *Odorrana* into the subgenera *Odorrana* (*Odorrana*) and *Odorrana* (*Bamburana*). Chen et al. (2013) subsequently confirmed the monophyly of the genus *Odorrana* and divided it into seven distinct clades (clades A–G). Clade F included *O. exiliversabilis*, *O. nasica*, *O. nasuta*, *O. tormota*, and *O. versabilis*, and corresponded to the subgenus *Odorrana* (*Bamburana*). However, Chen et al. (2013) did not specifically address the validity of *Odorrana* (*Bamburana*) but only confirmed that this group forms a monophyletic cluster. Furthermore, there was a lack of molecular data available to support the inclusion of *O. trankieni* in the subgenus *Odorrana* (*Bamburana*). To date, no further research has discussed the validity of *Odorrana* (*Bamburana*) or its constituent species.

The distribution of *O. versabilis* in Guangxi has been previously discussed by Fei et al. (2005) and Mo et al. (2014). They argued that it was widely present in counties such as Wuming, Shangsi, Longshen, Jinxiu, and Ziyuan. Previous studies have also indicated the occurrence of *O. nasuta* and *O. nasica* in Guangxi (Zhang and Wen 2000; Fei et al. 2005; Chen 2018; Huang et al. 2020). However, *O. nasuta* was specifically documented in Shiwandashan and Damingshan National Nature Reserves (Chen 2018; Huang et al. 2020), while information on the distribution of *O. nasica* is only available on AmphibiaChina (2023) without specifying its precise location. It is important to note that these findings are solely based on morphological descriptions and lack molecular evidence.

In recent years, we conducted herpetological surveys in various nature reserves in Guangxi (Fig. 1), where we collected a series of specimens resembling

**Figure 1. A** distributions of the subgenus *Odorrana* (*Bamburana*), and **B** habitat of *O.* (*B.*) *damingshanensis* sp. nov. Abbreviations: DMS = Damingshan, DYS = Dayaoshan, SWDS = Shiwandashan, HP = Huaping, DD = Diding.

*O. versabilis*. However, through phylogenetic analyses, we discovered that these specimens did not form a monophyletic group. Instead, they were distributed across five distinct branches, suggesting the presence of cryptic species within the *O. versabilis* species complex. The objective of this study is to investigate the species diversity within the *O. versabilis* species complex, describe the potential new species that have been identified, and assess the validity of the subgenus *Odorrana* (*Bamburana*) and its constituent species.

## Material and methods

Fifty-four specimens from five different species were collected from various national nature reserves in Guangxi, China, including Shiwandashan (**SWDS**), Dayaoshan (**DYS**), Damingshan (**DMS**), Huaping (**HP**), and Diding (**DD**) reserves, between 2013 and 2022 (Fig. 1, Appendix 1). The specimens were euthanized using isoflurane, then fixed in 10% formalin and stored in 75% ethanol. Muscle tissues were obtained from each specimen before formalin fixation and preserved in 100% ethanol for molecular analyses (Luo et al. 2021). Morphological measurements were taken to the nearest 0.1 mm using digital calipers, following the methods described by Fei et al. (2009) and Luo et al. (2021). Abbreviations of characters used in the paper are as follows:

**SVL** snout-vent length, distance from the tip of snout to the posterior margin of vent;
**HDL** head length, distance from the tip of snout to the rear of jaw;
**HDW** maximum head width, the greatest width between the left and right articulations of jaw;
**SNT** snout length, distance from the tip of snout to the anterior corner of eye;
**EN** eye-nostril distance, distance from the anterior of eye to nostril;
**EYE** eye diameter, horizontally from the anterior to posterior corner of eye;
**IN** internarial space, the shortest distance between the inner margins of nostrils;
**IOD** interorbital distance, the shortest distance between the anterior corners of orbits;
**TMP** tympanum diameter;
**TEY** tympanum-eye distance, from the anterior edge of tympanum to the posterior corner of eye;
**TIB** tibia length, distance from knee to tarsus;
**THL** thigh length, distance from vent to knee;
**PL** pes length, distance from the tip of the fourth toe to the base of the inner metatarsal tubercles;
**FLL** forelimb length, distance from elbow to the tip of the third finger;
**ML** manus length, distance from the tip of the third digit to the base of tubercle on prepollex;
**$FD_3$** diameter of the third finger disc;
**$TD_4$** diameter of the fourth toe disc.

Genomic DNA was extracted from muscle tissues using DNeasy tissue extraction kits (Qiagen). Three fragments of mitochondrial DNA (mtDNA) were amplified, targeting segments of the 12S (~ 750 bp) and 16S (~ 1000 bp)

ribosomal RNA genes, as well as the COI (~ 630 bp) gene. The primer sequences and PCR conditions followed the protocols outlined by Chen et al. (2013) for the 12S and 16S regions, and by Che et al. (2012) for the COI region. To confirm successful amplification, the PCR products were directly sequenced using an ABI 3730 automated DNA sequencer. The obtained sequences were then validated for accuracy and specificity through BLAST searches (Altschul et al. 1997) and deposited in GenBank (Table 1).

The DNA sequences obtained were aligned using the ClustalW algorithm implemented in Mega v. 7 (Kumar et al. 2016) with default settings. Homologous DNA sequences from GenBank were downloaded for phylogenetic analyses (Table 1). The uncorrected paired divergence (*p*-distance) was calculated using Mega v. 7. The best-fitting models of DNA substitution for the molecular data were determined using the Akaike Information Criterion (AIC) implemented in

**Table 1.** Localities, voucher information and GenBank accession numbers for all samples used in this study.

| ID | Species | Locality | Voucher | GenBank accession no. | | |
|---|---|---|---|---|---|---|
| | | | | 12S | 16S | COI |
| 1 | *O.* (*B.*) *damingshanensis* sp. nov. | Wuming, Guangxi, China | NNU00689 | ON791444 | ON791419 | ON791392 |
| 2 | *O.* (*B.*) *damingshanensis* sp. nov. | Wuming, Guangxi, China | NNU00690 | ON791445 | ON791420 | ON791393 |
| 3 | *O.* (*B.*) *damingshanensis* sp. nov. | Wuming, Guangxi, China | NNU00691 | ON791446 | ON791421 | ON791394 |
| 4 | *O.* (*B.*) *damingshanensis* sp. nov. | Wuming, Guangxi, China | NNU00692 | ON791447 | ON791422 | ON791395 |
| 5 | *O.* (*B.*) *damingshanensis* sp. nov. | Wuming, Guangxi, China | NNU00693 | ON791448 | ON791423 | ON791396 |
| 6 | *O.* (*B.*) *nasica* | Jingxi, Guangxi, China | NNU00663 | ON791466 | ON791443 | ON791418 |
| 7 | *O.* (*B.*) *trankieni* | Shangsi, Guangxi, China | NHMG1303003 | ON791449 | ON791424 | |
| 8 | *O.* (*B.*) *trankieni* | Shangsi, Guangxi, China | NHMG140108 | ON791451 | ON791426 | |
| 9 | *O.* (*B.*) *trankieni* | Shangsi, Guangxi, China | NHMG141103 | MH665665 | MH665671 | ON791406 |
| 10 | *O.* (*B.*) *trankieni* | Shangsi, Guangxi, China | NHMG141104 | MH665666 | MH665672 | ON791405 |
| 11 | *O.* (*B.*) *trankieni* | Shangsi, Guangxi, China | NHMG141107 | MH665667 | MH665673 | ON791403 |
| 12 | *O.* (*B.*) *trankieni* | Shangsi, Guangxi, China | NHMG141111 | ON791450 | ON791425 | |
| 13 | *O.* (*B.*) *trankieni* | Shangsi, Guangxi, China | NHMG141113 | MH665668 | MH665674 | ON791404 |
| 14 | *O.* (*B.*) *trankieni* | Wuming, Guangxi, China | NNU20042913 | ON791452 | ON791427 | ON791397 |
| 15 | *O.* (*B.*) *trankieni* | Wuming, Guangxi, China | NNU20042914 | ON791453 | ON791428 | ON791398 |
| 16 | *O.* (*B.*) *trankieni* | Wuming, Guangxi, China | NNU20042915 | ON791454 | ON791429 | ON791399 |
| 17 | *O.* (*B.*) *trankieni* | Wuming, Guangxi, China | NNU20210302 | ON791455 | ON791430 | ON791400 |
| 18 | *O.* (*B.*) *trankieni* | Wuming, Guangxi, China | NNU20210303 | ON791456 | ON791431 | ON791401 |
| 19 | *O.* (*B.*) *trankieni* | Wuming, Guangxi, China | NNU20210304 | ON791457 | ON791432 | ON791402 |
| 20 | *O.* (*B.*) *versabilis* | Jinxiu, Guangxi, China | NNU00637 | ON791460 | ON791435 | ON791409 |
| 21 | *O.* (*B.*) *versabilis* | Jinxiu, Guangxi, China | NNU00638 | ON791461 | ON791436 | ON791410 |
| 22 | *O.* (*B.*) *versabilis* | Jinxiu, Guangxi, China | NNU00639 | ON791462 | ON791437 | ON791411 |
| 23 | *O.* (*B.*) *versabilis* | Jinxiu, Guangxi, China | NNU00640 | ON791463 | ON791438 | ON791412 |
| 24 | *O.* (*B.*) *versabilis* | Jinxiu, Guangxi, China | NNU00641 | ON791464 | ON791439 | ON791413 |
| 27 | *O.* (*B.*) *versabilis* | Jinxiu, Guangxi, China | NNU00647 | ON791465 | ON791440 | ON791414 |
| 28 | *O.* (*B.*) *versabilis* | Longsheng, Guangxi, China | NNU201908005 | ON791458 | ON791434 | ON791407 |
| 29 | *O.* (*B.*) *versabilis* | Longsheng, Guangxi, China | NNU201908010 | ON791459 | ON791433 | ON791408 |

| ID | Species | Locality | Voucher | GenBank accession no. | | |
|---|---|---|---|---|---|---|
| | | | | 12S | 16S | COI |
| 30 | *O.* (*B.*) *yentuensis* | Shangsi, Guangxi, China | NHMG1401035 | MH665669 | MH665675 | ON791416 |
| 31 | *O.* (*B.*) *yentuensis* | Shangsi, Guangxi, China | NHMG1401036 | ON791467 | ON791441 | ON791415 |
| 32 | *O.* (*B.*) *yentuensis* | Shangsi, Guangxi, China | NNU00230 | ON791468 | ON791442 | ON791417 |
| 33 | *O. andersonii* | Longchuan, Yunnan, China | HNNU001YN topotype | KF185021 | KF185057 | |
| 34 | *O. anlungensis* | Anlong, Guizhou, China | HNNU1008I109 topotype | KF185013 | KF185049 | |
| 35 | *O. chapaensis* | Lai Chau, Vietnam | Genbank | DQ283372 | DQ283372 | |
| 36 | *O. chloronota* | Ha Giang, Vietnam | Genbank | DQ283394 | DQ283394 | |
| 37 | *O.* (*B.*) *exiliversabilis* | Wuyishan, Fujian, China | HNNU0607032 topotype | KF185020 | KF185056 | |
| 38 | *O.* (*B.*) *exiliversabilis* | Wuyishan, Fujian, China | LSU20200716WY02 topotype | MT934403 | MT934403 | MT934403 |
| 39 | *O. fengkaiensis* | Fengkai C*O.*, Guangdong, China | SYS a002262 Paratype | KT315354 | KT315375 | |
| 40 | *O. grahami* | Kunming, Yunnan, China | HNNU1008II016 topotype | KF185015 | KF185051 | |
| 41 | *O. graminea* | Wuzhishan, Hainan, China | HNNU0606123 topotype | KF185002 | KF185038 | |
| 42 | *O. hainanensis* | Wuzhishan, Hainan, China | HNNU0606105 topotype | KF184996 | KF185032 | |
| 43 | *O. hejiangensis* | Hejiang, Sichuan, China | HNNU1007I202 topotype | KF185016 | KF185052 | |
| 44 | *O. hosii* | Kuala Lumpur, Malaysia | Genbank | AB511284 | AB511284 | |
| 45 | *O. huanggangensis* | Wuyishan, Fujian, China | HNNU0607001 paratype | KF185023 | KF185059 | |
| 46 | *O. ishikawae* | Amami Island, Ryukyu | Genbank | AB511282 | AB511282 | |
| 47 | *O. jingdongensis* | Jingdong, Yunan, China | 20070711017 topotype | KF185014 | KF185050 | |
| 48 | *O. junlianensis* | Junlian, Sichuan, China | HNNU002 JL topotype | KF185022 | KF185058 | |
| 49 | *O. kuangwuensis* | Nanjiang, Sichuan, China | HNNU 0908II185 topotype | KF184998 | KF185034 | |
| 50 | *O. leporipes* | Shaoguan, Guangdong, China | HNNU1008I099 topotype | KF185000 | KF185036 | |
| 51 | *O. liboensis* | Maolan National Nature Reserve, Libo County, Guizhou, China | GZNU20180608007 holotype | MW481339 | MW481350 | |
| 52 | *O. lipuensis* | Lipu, Guangxi, China | NHMG1303018 paratype | MH665670 | MH665676 | |
| 53 | *O. lungshengensis* | Longsheng, Guangxi, China | HNNU70028 topotype | KF185018 | KF185054 | |
| 54 | *O. margaretae* | Dujiangyan City, Sichuan, China | SYS a003214 | KT315370 | KT315391 | |
| 55 | *O. mutschmanni* | Cao Bang Province, Vietnam | IEBR 3723 holotype | KU356761 | KU356765 | |
| 56 | *O. nanjiangensis* | Nanjiang, Sichuan | HNNU1007I291 topotype | KF185006 | KF185042 | |
| 57 | *O. narina* | Okinawa Island, Ryukyu | Genbank | AB511287 | AB511287 | |
| 58 | *O.* (*B.*) *nasica* | Ha Tinh, Vietnam | AMNH A161169 | DQ283345 | DQ283345 | |
| 59 | *O.* (*B.*) *nasica* | Tam Dao, Vinh Phu Prov., Vietnam | ROM 18031 | | DQ204493 | |
| 60 | *O.* (*B.*) *nasica* | Tam Dao, Vinh Phu Prov., Vietnam | ROM 20235 | | DQ204494 | |
| 61 | *O.* (*B.*) *nasuta* | Wuzhishan, Hainan, China | HNNU051119 topotype | KF185017 | KF185053 | |
| 62 | *O.* (*B.*) *nasuta* | Limu shan, Hainan, China | HNNU-A0019L | KX269223 | KX269223 | |
| 63 | *O. schmackeri* | Yichang, Hubei, China | HNNU 0908II349 topotype | KF185011 | KF185047 | |
| 64 | *O. swinhoana* | Taibei, Taiwan, China | HNNUTW1 | KF185009 | KF185045 | |
| 65 | *O. tianmuii* | Linan, Zhejiang, China | HNNU 0707071 paratype | KF185004 | KF185040 | |
| 66 | *O. tiannanensis* | Hekou, Yunnan, China | HNNUHK001 topotype | KF185008 | KF185044 | |
| 67 | *O. tormota* | Huangshan, Anhui, China | AM04005, topotype | DQ835616 | DQ835616 | DQ835616 |
| 68 | *O. tormota* | Huangshan, Anhui, China | SCUM052069 | NC009423 | NC009423 | NC009423 |
| 69 | *O.* (*B.*) *trankieni* | Son La province, Vietnam | VNMN04035 | - | KX893900 | |
| 70 | *O.* (*B.*) *trankieni* | Hoa Binh Province, Vietnam | IEBR A.2015.69 | - | KX893889 | |
| 71 | *O.* (*B.*) *trankieni* | Bac Giang Province, Vietnam | IEBR A.2013.74 | - | KX893890 | |
| 72 | *O.* (*B.*) *versabilis* | Leishan, Guizhou, China | HNNU003 LS | KF185019 | KF185055 | |
| 73 | *O. wuchuanensis* | Wuchuan, Guizhou, China | HNNU019 L topotype | KF185007 | KF185043 | |
| 74 | *O. yizhangensis* | Yizhang, Hunan, China | HNNU1008I075 topotype | KF185012 | KF185048 | |
| 75 | *O.* (*B.*) *yentuensis* | Vietnam | IEBR A.2015.38 | | KX893891 | |
| 76 | *Babina daunchina* | Emeishan, Sichuan, China | HNNU20060103 topotype | KF185029 | KF185065 | |
| 77 | *Rana chensinensis* | Ningshan, Shanxi, China | HNNU 20060359 | KF185025 | KF185061 | |

MrModeltest v. 2.3 (Nylander 2004), resulting in the selection of the GTR + I + G model. Phylogenetic relationships within the genus *Odorrana* were reconstructed using Bayesian inference (BI) with MrBayes v. 3.2 (Ronquist et al. 2012). A majority-rule consensus tree was constructed to calculate the Bayesian posterior probabilities (BPP) for the nodes in the tree. Maximum likelihood (ML) trees were inferred using the CIPRES Science Gateway server (https://www.phylo.org/portal2; Miller et al. 2010) with the estimation of the proportion of invariable sites and 1000 bootstrap pseudo replicates.

## Results

### Molecular analyses

Both BI and ML analyses produced similar results, which align with previous studies conducted by Chen et al. (2013) and Luo et al. (2021). Our specimens were categorized into five distinct lineages based on preliminary phylogenetic analyses (Fig. 2). Firstly, the specimens from DMS were divided into two separate lineages. One lineage, referred to as DMS_I specimens, showed close relation to specimens from SWDS and *O. trankieni* from Vietnam, forming a monophyletic group. The other lineage, referred to as DMS_II specimens, formed a well-supported monophyletic group (BS = 100; PP = 1.00) and exhibited close affinity to *O. nasica* and *O. yentuensis*. The SWDS specimens, DMS_I specimens, and *O. trankieni* clustered together, forming a monophyletic group. The specimens from DYS and HP (type locality: Longsheng and Jinxiu counties, Guangxi, China), with *O. versabilis* from Leishan, Guizhou, formed a monophyletic group. The specimen from DD was found to be nested within *O. nasica* from Vietnam, indicating that they belong to the same species. *Odorrana yentuensis* from SWDS and Vietnam formed a monophyletic group and showed close affinity to *O. nasica*. Lastly, *O. nasuta* from Hainan Island formed a distinct monophyletic group (Fig. 2).

The uncorrected *p*-distances for the 16S fragments within *O. versabilis* species complex are remarkably low, ranging from 1.3% to 3.1%. For instance, the genetic distances between *O. versabilis* and *O. nasuta* range from 1.8% to 2.4%, while those between *O. versabilis* and *O. nasica* range from 0.7% to 2.8% (Suppl. material 1: table S1). However, the DMS_II specimens show significant genetic divergences (> 5.0%) from their congeners. When examining the COI fragments, the smallest genetic distance among them is greater than 4.0% (Suppl. material 1: table S2). Specifically, the genetic distances between the specimens from DYS and SWDS range from 5.1% to 6.3%. On the other hand, the genetic divergences between the specimens from DMS and SWDS are very low, ranging from 0.3% to 0.8%, suggesting that these specimens are congeners. Nevertheless, the proposed new species (DMS_II specimens) exhibits distinct genetic differences from other similar species, with divergence values exceeding 5.0% for 16S and 10.0% for COI (Suppl. material 1: tables S1, S2).

### Morphological analyses

Table 2 presents the morphological differences observed. The DMS_II specimens can be distinguished from other similar species by various morphological

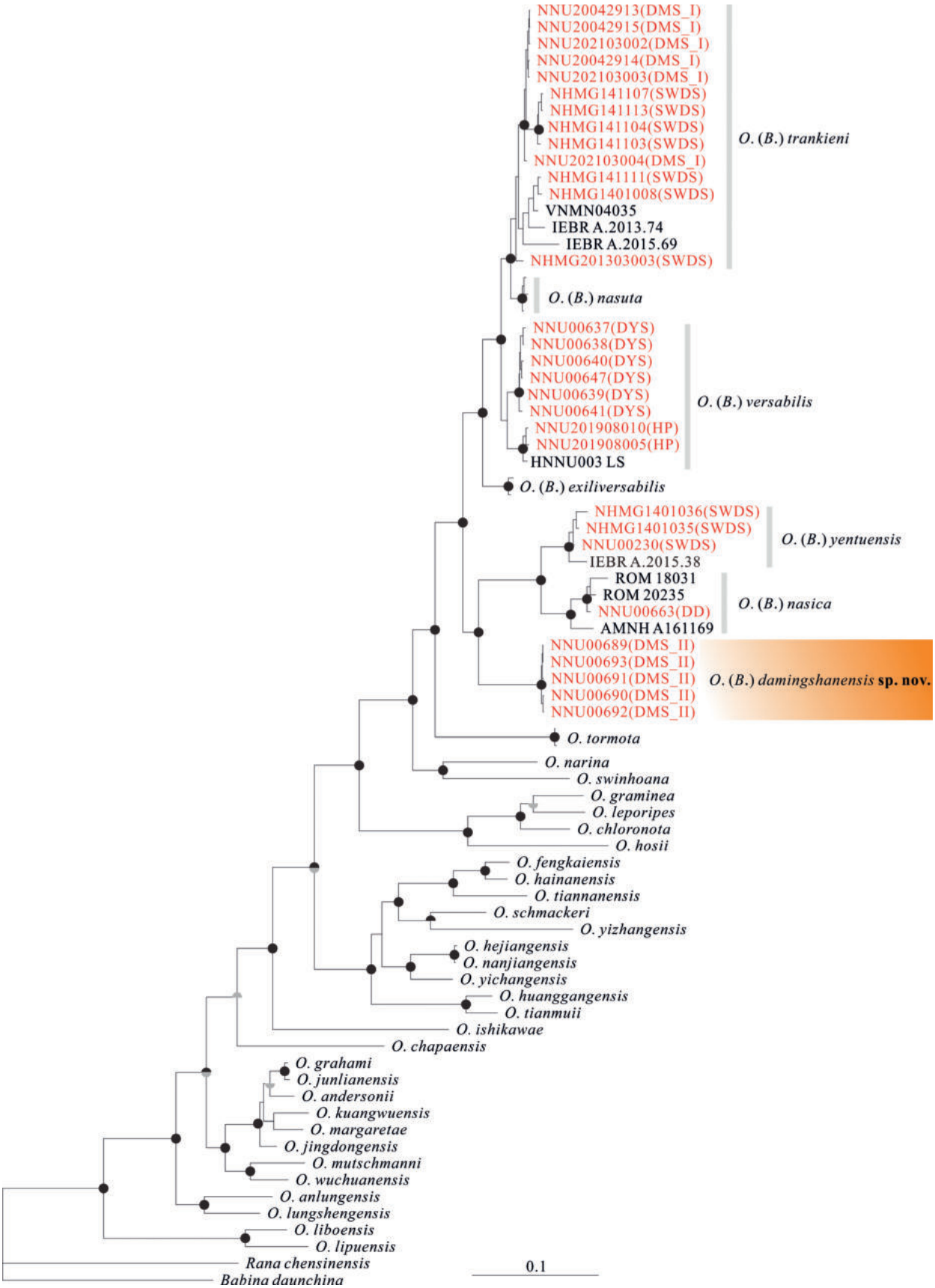


**Figure 2.** Maximum likelihood tree reconstructed based on 12S, 16S, and COI genes sequences. Note supports are shown on branches as bootstrap supports (upper half; > 70% < 90% = grey, > 90% = black) and Bayesian posterior probabilities (lower half; > 0.95 = grey, 1 = black). Red indicates the newly collected specimens in this study.

**Table 2.** Morphological comparisons. Abbreviations: DMS = Damingshan, DYS = Dayaoshan, SWDS = Shiwandashan, SVL = snout-vent length, SD = Standard deviation.

| Character | SVL (mean ± SD, ranges) | | SVL of female / SVL of male | Tip of snout strongly projecting beyond margin of the lower jaw | Vocal sac | Rear of the back with horny tubercles | Pineal ocellus | Sternum widened posteriorly without notch | Upper lip with sawtooth spinules | Relative lengths of fingers | References |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Male | Female | | | | | | | | | |
| DMS_II *O.* (*B.*) *damingshanensis* sp. nov. | 53.3 ± 1.3 (52.3–54.8) *n* = 3 | 78.0 ± 4.5 (74.8–81.2) *n* = 2 | 1.46 | No | External | Yes | Yes | Yes | Yes | II < IV < I < III | This study |
| DYS *O.* (*B.*) *versabilis* | 76.4 ± 4.1 (71.7–79.4) *n* = 3 | 78.0 ± 1.9 (77.0–82.4) *n* = 7 | 1.02 | Yes | Internal | No | Yes | Yes | Yes | II < IV < I < III | This study |
| DMS_I *O.* (*B.*) *trankieni* | 72.5 ± 2.9 (70.2–76.2) *n* = 4 | 75.4 ± 5.9 (69.0–80.6) *n* = 3 | 1.04 | Yes | External | No | Yes | Yes | Yes | IV < II < I < III | This study |
| SWDS *O.* (*B.*) *trankieni* | 71.7 ± 1.3 (70.0–73.1) *n* = 5 | 80.4 ± 3.5 (76.2–83.9) *n* = 4 | 1.12 | Yes | External | No | Yes | Yes | Yes | IV < II < I < III | This study |
| SWDS *O.* (*B.*) *yentuensis* | 43.7 ± 1.3 (40.1–46.9) *n* = 13 | 59.9 ± 1.3 (54.1–65.3) *n* = 6 | 1.37 | No | External | Yes | Yes | Yes | Yes | II < I < IV < III | This study |
| *O.* (*B.*) *exiliversabilis* | 48.7 (42.7–52.4) *n* = 20 | 58.1 (51.8–61.8) *n* = 24 | 1.19 | No | Internal | No | Yes | Yes | Yes | II < IV < I < III | Fei et al. 2001, 2012; Li et al. 2001; AmphibiaChina 2023 |
| *O.* (*B.*) *nasica* | 41.0–46.0; *n* = 4 | 60.0–70.7; *n* = 2 | / | Yes | External | No | No | Yes | Yes | II < IV < I < III | Boulenger 1903; Zhang and Wen 2000; Stuart and Chan-ard 2005; Tran et al. 2008; Yang 1991 |
| *O.* (*B.*) *nasuta* | 59.2 (57.1–63.2) *n* = 10 | 73.4 (73.1–73.6) *n* = 2 | 1.24 | Yes | External | No | Yes | Yes | Yes | II < IV < I < III | Fei et al. 2001, 2012; Li et al. 2001; Chen 2018; Huang et al. 2020; AmphibiaChina 2023 |
| *O. tormota* | 33.8 (32.0–36.3) | 59.5 (59.0–60.0) | 1.76 | No | External | No | – | No | No | I ≈ II < IV < III | Wu 1977; Fei et al. 2012; AmphibiaChina 2023 |
| *O.* (*B.*) *trankieni* | 75.2–84.1, *n* = 7 | 86.2–95.8, *n* = 5 | / | Yes | External | No | – | Yes | Yes | IV < II < I < III | Orlov et al. 2003; Pham et al. 2020 |
| *O.* (*B.*) *versabilis* | 72.4 (69.3–77.8) *n* = 5 | 77.0 (70.0–81.4) *n* = 6 | 1.06 | Yes | Internal | No | Yes | Yes | Yes | II < IV < I < III | Liu and Hu 1962; Fei et al. 2001, 2012; Li et al. 2001; AmphibiaChina 2023 |
| *O.* (*B.*) *yentuensis* | 44.5 (44.3–45.5) *n* = 4 | 60.9 (59.3–61.9) *n* = 6 | 1.37 | No | External | Yes | Yes | Yes | Yes | II < I < IV < III | Tran et al. 2008; Lu et al. 2016 |

characteristics listed in Table 2, such as SVL, vocal sac, horny tubercles on the rear of the back, and sawtooth spinules on the upper lip. For further information, please refer to the Taxonomic account section below.

## Taxonomic results

Based on the analysis of morphological characters and molecular data, it has been determined that the specimens from SWDS, DMS_I, and those previously identified as *O. trankieni* from Vietnam, belong to the same species, namely *O. trankieni*. The specimens from HP and DYS have been identified as *O. versabilis*. The DD specimen has been classified as *O. nasica*. Furthermore, the DMS_II specimens have been found to represent an undescribed species of *Odorrana*, which will be described below.

***Odorrana damingshanensis* sp. nov.**
https://zoobank.org/448AD82C-76AE-4D51-88DF-D7790C2FC408
Figs 3, 4

**Type material examined. *Holotype*.** NNU 00690, adult male, from the Damingshan National Nature Reserve, Wuming District, Nanning City, Guangxi, China (23.4637°N, 108.4869°E; elevation 1159 m), collected by Weicai Chen on 25 April 2022. ***Paratypes*.** NNU 00689 and NNU 00691, adult males; NNU 00692 and NNU 00693, adult females, collected at the same site and time as the holotype.

**Diagnosis.** Based on both molecular analyses and specific morphological traits, these specimens were assigned to the genus *Odorrana*. The distinguishing morphological characteristics of these species include dilated and tapering tips of the digits, disks with circummarginal grooves and a longer vertical diameter than horizontal diameter, fully webbed toes, the absence of a tarsal fold, a thick first finger with a distinct nuptial pad, sawtooth spinules on the upper lip, and well-defined dorsolateral folds (Fei et al. 2001, 2005).

*Odorrana damingshanensis* sp. nov. can be distinguished from other species in its genus by the following combination of characters: (1) medium body size (SVL 52.3–54.8 mm in males and 74.8–81.2 mm in females); (2) SVL of female/SVL of male = 1.46; (3) sawtooth spinules on the upper lip; (4) snout obtusely rounded without significantly extending beyond the lower jaw; (5) well-defined dorsolateral folds; (6) horny tubercles on the rear of the back; (7) presence of outer metatarsal tubercles; (8) absence of a supratympanic fold; (9) highly dilated nuptial pad with velvety spinules on the dorsolateral surface of Finger I; (10) distinct expansion of the tips of the second, third, and fourth fingers, with the first slightly enlarged; expanded tips of the toes with distinct circummarginal grooves; (11) distinct maxillary gland with tiny spines; and (12) external lateral vocal sac (Fig. 3A–I).

**Description of holotype.** Head longer than wide (HDL/HDW = 1.23); snout obtusely rounded in dorsal view, but not strongly projecting beyond margin of lower jaw (Fig. 3G); canthus rostral distinct, loreal region concave; nostrils oval, oblique, and closer to tip of snout than eye; upper lip with sawtooth spinules (Fig. 3G); eye diameter less than snout length (EYE/SNT = 0.88); interorbital region flat with a pineal; interorbital distance less than eye diameter (IOD/EYE

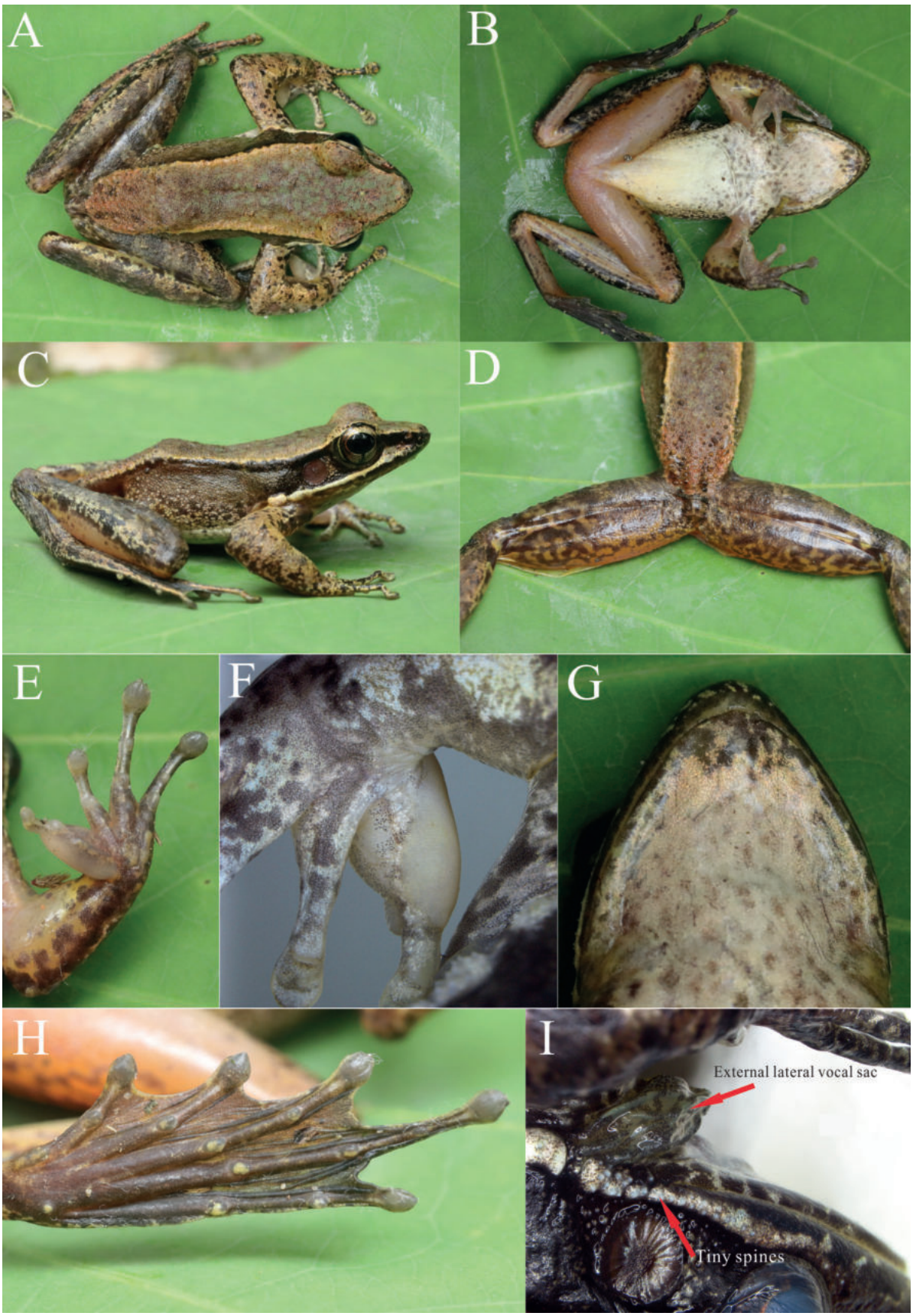


**Figure 3.** The holotype of *O.* (*B.*) *damingshanensis* sp. nov. (NNU 00690) **A** dorsal view **B** ventral view **C** dorsolateral view **D** rear of the back with horny tubercles and dorsal view of thighs **E** ventral view of hand **F** nuptial pad with velvety spinules **G** ventral view of snout **H** ventral view of foot, and **I** external lateral vocal sac and tiny spines on maxillary glands.

= 0.75); internostril distance less than eye diameter (IN/EYE = 0.88); tympanum distinct, rounded, 57% eye diameter, slightly concave relative to skin of temporal region; supratympanic fold absent; vomerine teeth on two oblique ridges, closed each other than choana; tongue elongated, deeply notched posteriorly; pupil horizontally oval; and external lateral vocal sac (Fig. 3I).

Forelimbs stout, relative length of fingers II < IV < I < III; tips of the second, third and fourth distinctly expanded, but the first slightly enlarged; tips of all fingers with circummarginal grooves; Finger III disk width less than tympanum diameter ($FD_3$/TMP = 0.62); finger webbing absent; subarticular tubercles prominent, rounded, formula 1, 1, 2, 2; inner and outer palmar tubercles distinct; nuptial pad on lateral surface of Finger I strongly dilated with velvety spinules, extending from hand base to level of subarticular tubercle (Fig. 3E, F). Tips of toes expanded, with distinct circummarginal grooves; relative length of toes I < II < III ≈ V < IV; toes entirely webbed; subarticular tubercles distinct, rounded, formula 1, 1, 2, 3, 2; inner metatarsal tubercle elongated; outer metatarsal tubercle conical (Fig. 3H).

Body surface shagreened; rear of the back with horny tubercles; ventral surface of venter, forelimbs, and thighs smooth; flanks shagreened; dorsal of forelimbs and hindlimbs shagreened, and hindlimbs with sparse tubercles; two distinct maxillary glands with tiny spinules (Fig. 3I).

**Coloration in life.** Dorsum grey-beige, with irregular grass-green blotches; a discrete darker brown stripe from tip of snout, across canthus rostral, along the inferior dorsolateral fold, finally ending at the anterior of groin; pineal gland grass-green; cream white stripe from anterior of upper lip to maxillary glands; tympanic region brown, some creamy white tubercles around the tympanum; the upper part of belly, chest, and throat with irregular grey cloud, but the lower part of belly creamy white without spots; ventral surface of thighs and forelimbs incarnadine without spots; forelimbs and hindlimbs with pale brown crossbars, three on lower arm, four on thigh and four on tibia; pupil black with orange border; iris creamy yellow, but the posterior iris pale jacinth; and velvety nuptial pad creamy white (Fig. 3A–I).

**Coloration in preservative.** Dorsum brown; bars on forelimbs and hindlimbs darker brown; horny tubercles on the rear of the back turned into creamy white; creamy white nuptial pad turned into grey; the upper part of belly, throat, and chest with brown cloud; the lower part of belly immaculate creamy white; the ventral surface of the hindlimbs creamy yellow; external lateral vocal sac pale green and projecting distinctly (Fig. 3I).

**Etymology.** The specific name of this species, *damingshanensis*, is derived from its discovery locality, Damingshan National Nature Reserve. In English, it is suggested to be called the Damingshan Bamboo-leaf Frog. In Chinese, it is known as大明山竹叶蛙(Dà Míng Shān Zhú Yè Wā).

**Distribution and ecology.** *Odorrana damingshanensis* sp. nov. is a newly discovered species found in the Damingshan National Nature Reserve, located in Wuming District, Nanning City, Guangxi, China. This species was observed near slow-flowing rocky streams, which were ~ 2–3 m wide and 10–30 cm deep (Fig. 1B). The frogs were often seen sitting on rocks, and interestingly, one specimen (NNU 00691) was found on a leaf near a stream, while another specimen (NNU 00693) was perched on a dry branch above a stream. The surrounding vegetation in the area consists of evergreen forest, and the elevation

is ~ 1200 m with an ambient temperature of 20 °C in April. During the survey, no advertisement calls were heard; however, both female specimens were gravid, carrying creamy yellow eggs without black poles (Fig. 4A). Additionally, amplexus behavior was observed when males encountered females indoors (Fig. 4B). The breeding season of *O. damingshanensis* sp. nov. is speculated to occur between April and May. Other sympatric species in the area include *Gracixalus jinxiuensis* and *Quasipaa shini*.

**Sexual dimorphism and variation.** The measurements of *O. damingshanensis* sp. nov. are provided in Table 3. Females of this species were observed to be significantly larger than males in terms of SVL, with a ratio of 1.46. The specimens show variation in dorsal colors: NNU 00691 displays a grass green coloration with brown blotches (Fig. 4C), while NNU 00689 exhibits a pale beige coloration with grass green blotches (Fig. 4D). Males have a higher density of horny tubercles on the rear of their backs compared to females (Fig. 4C–F).

**Comparisons.** *Odorrana damingshanensis* sp. nov. shares morphological similarities with closely phylogenetically related species, including *O. exiliversabilis*, *O. nasica*, *O. nasuta*, *O. tormota*, *O. trankieni*, *O. versabilis,* and *O. yentuensis* (Fig. 4G–J, Table 2). These similarities can be observed in its protruding snout, slender limbs, sawtooth spinules on the upper lip, and distinct dorsolateral folds. However, *O. damingshanensis* sp. nov. can be distinguished from *O. exiliversabilis* by the noticeably larger body size in females (SVL 74.8–81.2 mm vs SVL 51.8–61.8 mm); presence of horny tubercles on the rear of the back (vs absence); and presence of an external lateral vocal sac (vs an internal subgular vocal sac) (Fei et al. 2001, 2012; Li et al. 2001). *Odorrana damingshanensis* sp.

**Table 3.** Measurements of *O.* (*B.*) *damingshanensis* sp. nov. (in mm). Abbreviations are defined in the text.

| Characters | NNU 00689 | NNU 00690 | NNU 00691 | NNU 00692 | NNU 00693 |
|---|---|---|---|---|---|
| Sex | Male | Male | Male | Female | Female |
| SVL | 52.3 | 54.8 | 52.8 | 74.8 | 81.2 |
| HDL | 19.2 | 20.4 | 18.0 | 25.5 | 27.4 |
| HDW | 16.0 | 16.6 | 16.6 | 23.1 | 25.9 |
| SNT | 7.7 | 7.8 | 7.7 | 11.1 | 11.6 |
| EN | 4.0 | 4.3 | 4.2 | 5.2 | 5.7 |
| EYE | 6.4 | 6.9 | 6.6 | 8.8 | 8.3 |
| IN | 5.7 | 6.1 | 6.2 | 8.4 | 8.1 |
| IOD | 4.9 | 5.2 | 5.2 | 7.5 | 7.4 |
| TMP | 3.7 | 3.9 | 3.8 | 5.0 | 4.7 |
| TEY | 1.9 | 2.1 | 1.7 | 2.8 | 3.0 |
| TIB | 30.3 | 32.1 | 32.5 | 44.2 | 48.9 |
| THL | 27.0 | 29.3 | 28.2 | 39.4 | 44.0 |
| PL | 29.5 | 29.3 | 28.7 | 41.4 | 43.7 |
| FLL | 23.0 | 25.4 | 25.2 | 35.7 | 38.6 |
| ML | 13.6 | 14.6 | 14.0 | 21.1 | 23.5 |
| $FD_3$ | 2.1 | 2.4 | 2.2 | 3.1 | 3.4 |
| $TD_4$ | 1.8 | 2.0 | 1.8 | 2.6 | 2.8 |

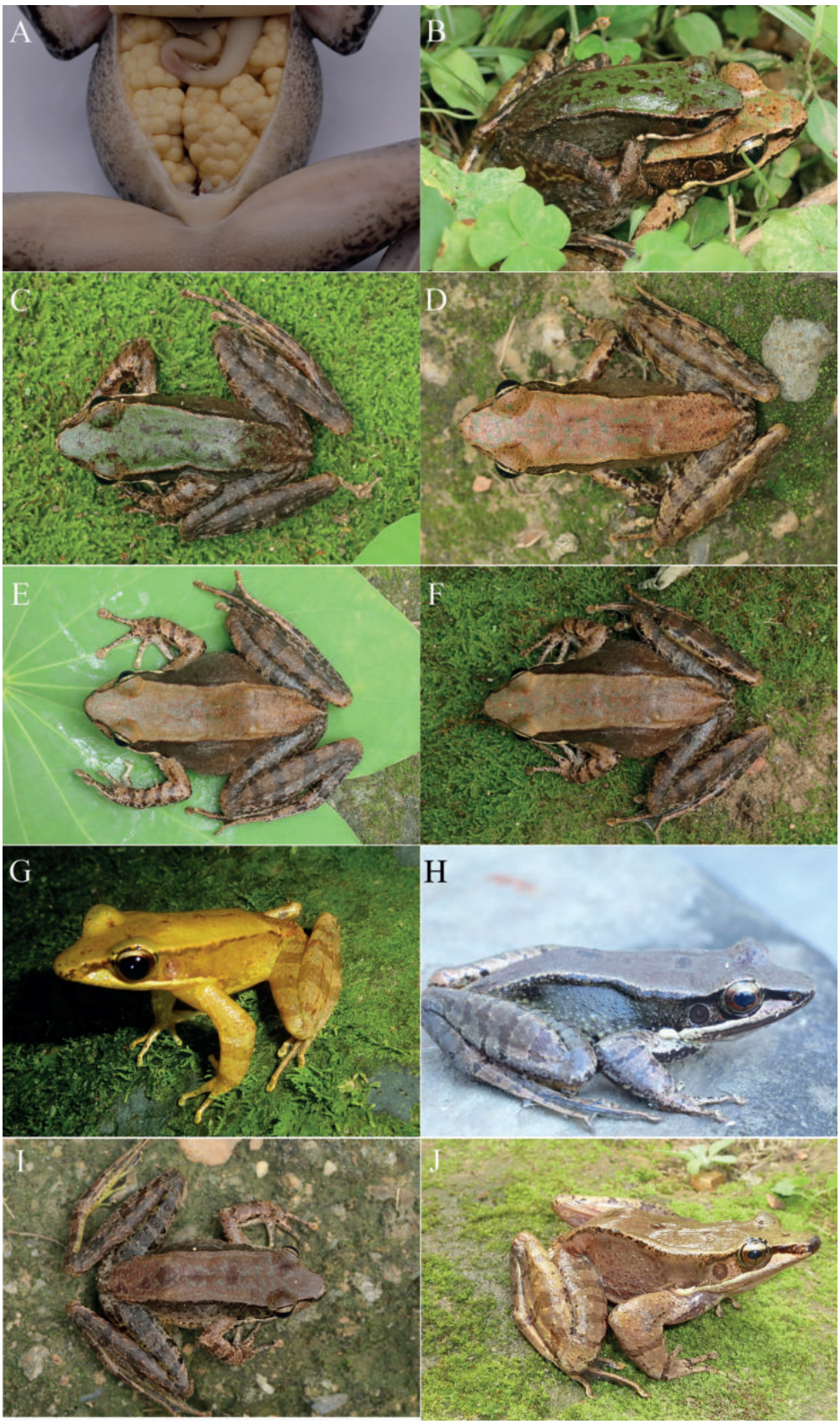


**Figure 4. A** female with creamy yellow eggs without pigmented poles **B** amplexus **C** dorsal view of NNU 00691 **D** dorsal view of NNU 00689 **E** dorsal view of NNU 00692 **F** dorsal view of NNU 00693 **G** dorsolateral view of *O.* (*B.*) *yentuensis* (NHMG1401036, adult male) **H** dorsolateral view of *O.* (*B.*) *versabilis* (NNU00638, adult male) **I** dorsal view of *O.* (*B.*) *nasica* (NNU00663, adult female) **J** dorsolateral view of *O.* (*B.*) *trankieni* (NHMG141107, adult male).

nov. differs from *O. nasica* in having a relatively larger body size in males (SVL 52.3–54.8 mm vs SVL 41.0–46.0 mm); absence of strongly protruding snout (vs presence); a distinct pineal body (vs invisible); an elongated inner metatarsal tubercle (vs an oval inner metatarsal tubercle); absence of whitish spinules scattered ventrally near the groin (vs presence of such spinules) (Yang 1991; Zhang and Wen 2000; Stuart and Chan-ard 2005; Tran et al. 2008). *Odorrana damingshanensis* sp. nov. differs from *O. nasuta* in having a relatively smaller body size in males (SVL 52.3–54.8 mm vs SVL 57.1–63.2 mm); SVL of female/SVL of male = 1.46 (vs the ratio of 1.24), distinct maxillary gland with tiny spines (vs absent tiny spines on maxillary gland); presence of horny tubercles on the rear of the back (vs smooth); absence of strongly protruding snout (vs presence) (Fei et al. 2001, 2012; Li et al. 2001). *Odorrana damingshanensis* sp. nov. differs from *O. tormota* by the absence of a deeply sunk tympanum forming an external auditory canal (vs presence of a deeply sunk tympanum); conspicuously larger body size (SVL 52.3–54.8 mm in males and 74.8–81.2 mm in females vs SVL 32.0–36.3 mm in males and 59.30–60.0 mm in females); presence of sawtooth spinules on upper lip (vs absence) (Wu 1977; Fei et al. 2012; AmphibiaChina 2023). *Odorrana damingshanensis* sp. nov. differs from *O. trankieni* in having a conspicuously smaller body size (SVL 52.3–54.8 mm in males and 74.8–81.2 mm in females vs SVL 75.2–84.1 mm in males and 86.8–95.9 mm in females); SVL of female/SVL of male = 1.46 (vs similar body sizes for males and females); absence of strongly protruding snout (vs presence); presence of horny tubercles on the rear of the back (vs smooth); relative lengths of fingers II < IV < I < III (vs IV < II < I < III); distinct maxillary gland with tiny spines (vs absent tiny spines)(Orlov et al. 2003; Pham et al. 2020). *Odorrana damingshanensis* sp. nov. differs from *O. versabilis* in having a conspicuously smaller body size in males (SVL 52.3–54.8 mm vs SVL 70.4–77.2 mm); presence of horny tubercles on the rear of the back (vs smooth); distinct maxillary gland with tiny spines (vs absent tiny spines); external lateral vocal sac (vs internal subgular vocal sac) (Liu and Hu 1962; Fei et al. 2001, 2012; Li et al. 2001). *Odorrana damingshanensis* sp. nov. differs from *O. yentuensis* in having a notably larger body size (SVL 52.3–54.8 mm in males and 74.8–81.2 mm in females vs SVL 41.7–46.2 mm in males and 59.3–65.7 mm in females); shagreened body surface (vs smooth); distinct maxillary gland with tiny spines (vs absent tiny spines on maxillary gland); irregular grey cloud on the upper part of belly, chest, and throat, but creamy white the lower part of belly without spots (vs yellowish white ventral side of body without spots)(Tran et al. 2008; Lu et al. 2016).

Finally, *O. damingshanensis* sp. nov. can be distinguished from other *Odorrana* species by the presence of sawtooth spinules on the upper lip (vs absent sawtooth spinules on the upper lip, *O. absita*, *O. amamiensis*, *O. andersonii*, *O. anlungensis*, *O. arunachalensis*, *O. aureola*, *O. bacboensis*, *O. banaorum*, *O. bolavensis*, *O. cangyuanensis*, *O. chapaensis*, *O. chloronota*, *O. concelata*, *O. dulongensis*, *O. fengkaiensis*, *O. geminata*, *O. gigatympana*, *O. grahami*, *O. graminea*, *O. hainanensis*, *O. heatwolei*, *O. hejiangensis*, *O. hosii*, *O. huanggangensis*, *O. ichangensis*, *O. indeprensa*, *O. ishikawae*, *O. jingdongensis*, *O. junlianensis*, *O. khalam*, *O. kuangwuensis*, *O. kweichowensis*, *O. leporipes*, *O. liboensis*, *O. lipuensis*, *O. livida*, *O. lungshengensis*, *O. macrotympana*, *O. margaretae*, *O. mawphlangensis*, *O. monjerai*, *O. morafkai*, *O. mutschmanni*, *O. nanjiangensis*, *O. narina*, *O. orba*, *O. sangzhiensis*, *O. schmackeri*, *O. sinica*, *O. splendida*, *O. supranarina*, *O. swinhoana*,

*O. tianmuii*, *O. tiannanensis*, *O. tormota*, *O. utsunomiyaorum*, *O. wuchuanensis,* and *O. yizhangensis*); the presence of external lateral vocal sac (vs absent vocal sac, *O. arunachalensis*, *O. concelata*, *O. heatwolei*, *O. hosii*, *O. ichangensis*, *O. kuangwuensis*, *O. leporipes*, *O. liboensis*, *O. lipuensis*, *O. livida*, *O. margaretae*, *O. mawphlangensis*, *O. monjerai*, *O. mutschmanni*, *O. narina*, *O. sangzhiensis*, *O. schmackeri*, *O. splendida*, *O. supranarina*, *O. wuchuanensis*); well-defined dorsolateral folds (vs absent dorsolateral folds, *O. andersonii*, *O. anlungensis*, *O. arunachalensis*, *O. aureola*, *O. bacboensis*, *O. cangyuanensis*, *O. chapaensis*, *O. chloronota*, *O. concelata*, *O. dulongensis*, *O. fengkaiensis*, *O. geminata*, *O. grahami*, *O. hainanensis*, *O. heatwolei*, *O. hejiangensis*, *O. huanggangensis*, *O. ichangensis*, *O. ishikawae*, *O. jingdongensis*, *O. junlianensis*, *O. kuangwuensis*, *O. kweichowensis*, *O. liboensis*, *O. lipuensis*, *O. lungshengensis*, *O. macrotympana*, *O. margaretae*, *O. mawphlangensis*, *O. morafkai*, *O. mutschmanni*, *O. nanjiangensis*, *O. sangzhiensis*, *O. schmackeri*, *O. sinica*, *O. splendida*, *O. swinhoana*, *O. tianmuii*, *O. tiannanensis*, *O. wuchuanensis,* and *O. yizhangensis*); horny tubercles on the rear of the back (vs absent horny tubercles on the rear of the back, *O. absita*, *O. amamiensis*, *O. andersonii*, *O. anlungensis*, *O. arunachalensis*, *O. aureola*, *O. bacboensis*, *O. banaorum*, *O. bolavensis*, *O. cangyuanensis*, *O. chapaensis*, *O. chloronota*, *O. concelata*, *O. dulongensis*, *O. fengkaiensis*, *O. geminata*, *O. gigatympana*, *O. grahami*, *O. graminea*, *O. hainanensis*, *O. heatwolei*, *O. hejiangensis*, *O. hosii*, *O. huanggangensis*, *O. ichangensis*, *O. indeprensa*, *O. ishikawae*, *O. jingdongensis*, *O. junlianensis*, *O. khalam*, *O. kuangwuensis*, *O. kweichowensis*, *O. leporipes*, *O. liboensis*, *O. lipuensis*, *O. livida*, *O. lungshengensis*, *O. macrotympana*, *O. margaretae*, *O. mawphlangensis*, *O. monjerai*, *O. morafkai*, *O. mutschmanni*, *O. nanjiangensis*, *O. narina*, *O. orba*, *O. sangzhiensis*, *O. schmackeri*, *O. sinica*, *O. splendida*, *O. supranarina*, *O. swinhoana*, *O. tianmuii*, *O. tiannanensis*, *O. utsunomiyaorum*, *O. wuchuanensis,* and *O. yizhangensis*).

## Discussion

The specimens in our study were classified into five species: *O. damingshanensis* sp. nov., *O. nasica*, *O. trankieni*, *O. versabilis*, and *O. yentuensis*, indicating a significant diversity of species in Guangxi, China. Our findings challenge previous research (Zhang and Wen 2000; Fei et al. 2005; Chen 2018; Huang et al. 2020) that identified SWDS and DMS_I specimens as *O. trankieni* instead of *O. nasuta* or *O. nasica*, based on morphology and phylogeny (Fig. 2). Chen (2018) and Huang et al. (2020) suggested the presence of *O. nasuta* in SWDS and DMS but lacked molecular data to support their claims. By combining phylogenetic and morphological data, we confirm that Chen (2018) and Huang et al. (2020) misidentified their specimens (Voucher nos.: NHMG 1303003, NHMG 141103–04 for Chen; Huang 201808296–98 for Huang et al.). Zhang and Wen (2000) proposed the occurrence of *O. nasica* in Debao County and DMS (voucher no. 830354, female, SVL 60.0 mm, collected in Debao County, Guangxi, China). However, they did not provide any supporting evidence for their findings. Upon examining the description provided by Zhang and Wen (2000), we observed that our DMS_I specimens exhibited similar body size, color pattern, and other diagnostic features to their specimen (Table 2; Zhang and Wen 2000). Considering the geographical proximity of Debao County to DD, we conclude that the Debao specimen and DD specimen represent the same species, *O. nasica*.

Upon examination of the series of specimens collected across Guangxi, we discovered that *O. damingshanensis* sp. nov., *O. nasica*, *O. nasuta*, *O. trankieni*, and *O. versabilis* exhibit similar body sizes and morphological characteristics in females, but not in males (Table 2). These factors have often led to misidentification of these species. Fig. 1 indicates that *O. exiliversabilis* and *O. versabilis* are present in the northern region of the Xi River, while *O. damingshanensis* sp. nov., *O. nasica*, *O. nasuta*, and *O. trankieni* are found in the southern region of the Xi River. However, further research is required to determine the extent to which the Xi River plays a significant role in the separation of these species. Currently, we know that *O. exiliversabilis* occurs in Southeastern China (Fujian, Zhejiang, Anhui, and Jiangxi provinces), *O. nasica* is found in the Sino-Vietnamese region (Yunnan, Guangxi, and Northern Vietnam), *O. nasuta* is limited to Hannan Island, *O. trankieni* is distributed in Northern Vietnam (Son La, Hoa Binh, and Bac Giang provinces) and Guangxi, China (this study), representing a new country record for China. *O. versabilis* inhabits Southern China (Guizhou, Guangxi, Guangdong, Hunan, and Jiangxi provinces), and *O. yentuensis* occurs in the Sino-Vietnamese region (AmphibiaChina 2023; Frost 2023). *Odorrana damingshanensis* sp. nov. is only known from DMS. It should be noted that *O. damingshanensis* sp. nov. and *O. trankieni* are sympatric species. However, *O. trankieni* is typically found at lower altitudes ranging from 200 to 900 m, while *O. damingshanensis* sp. nov. is found at higher altitudes exceeding 1000 m. *Odorrana trankieni* is commonly encountered in small cascade streams or wide streams with a slow current, whereas *O. damingshanensis* sp. nov. was specifically found in small, slow-flowing rocky streams (Fig. 1B).

Fei et al. (2005) initially proposed dividing the genus *Odorrana* into two subgenera, *Odorrana* (*Bamburana*) and *Odorrana* (*Odorrana*), based on several distinguishing characters. These characters included the presence of dorsolateral folds (absent in the latter), upper lip with sawtooth spinules (absent in the latter), xiphisternum without a notch (deeply notched in the latter), and posterior widening of the sternum (not widened in the latter). In a subsequent revision by Fei et al. (2010), the genus *Odorrana* was elevated to the generic level and divided into four genera within the tribe Odorranini: *Bamburana*, *Eburana*, *Matsuirana*, and *Odorrana*. Fei et al. (2010) suggested that the genus *Bamburana* comprised seven species: *B. exiliversabilis*, *B. montivaga*, *B. nasica*, *B. nasuta*, *B. tormota*, *B. trankieni*, and *B. versabilis*. However, this revision has not been widely accepted (AmphibiaChina 2023; Frost 2023). In a subsequent study by Fei et al. (2012), they did not adopt this revision and instead followed their original proposal of *Odorrana* (*Bamburana*) and *Odorrana* (*Odorrana*) (Fei et al. 2005). Phylogenetically, Chen et al. (2013) confirmed the monophyly of the genus *Odorrana* and divided it into seven major branches (clades A–G). They did not support the genera *Bamburana*, *Eburana*, *Matsuirana*, and *Odorrana* proposed by Fei et al. (2010): *Bamburana*, *Eburana*, and *Matsuirana* formed monophyletic groups, while *Odorrana* was paraphyletic. Furthermore, except for *Bamburana*, no diagnostic characters corresponded to the four genera proposed by Fei et al. (2010). According to Fei et al. (2005), the genus *Odorrana* can be divided into two subgenera: *Odorrana* (*Bamburana*) and *Odorrana* (*Odorrana*). By following this classification, differentiating *Odorrana* (*Bamburana*) from *Odorrana* (*Odorrana*) becomes easier based on four distinct characteristics: distinct dorsolateral folds, sawtooth spinules on the upper lip, absence of

a notch in the xiphisternum, and posterior widening of the sternum. Our phylogenetic trees also provide support for the monophyly of *Odorrana* (*Bamburana*) species (BS = 100; PP = 1.00), which include eight species: *O.* (*B.*) *damingshanensis* sp. nov., *O.* (*B.*) *exiliversabilis*, *O.* (*B.*) *nasica*, *O.* (*B.*) *nasuta*, *O.* (*B.*) *tormota*, *O.* (*B.*) *trankieni*, *O.* (*B.*) *versabilis*, and *O.* (*B.*) *yentuensis*. However, it is important to note that *O.* (*B.*) *tormota* has a deeply sunk tympanum forming an external auditory canal (Wu 1977). Furthermore, *O.* (*B.*) *tormota* lacks sawtooth spinules on the upper lip and has a deep notch on the xiphisternum. Therefore, we suggest excluding *O.* (*B.*) *tormota* from the subgenus *Odorrana* (*Bamburana*). Further investigation is necessary to determine if the subgenus *Odorrana* (*Odorrana*) can be further subdivided.

In this study, we identified five of seven species of the subgenus *Odorrana* (*Bamburana*) that occur in Guangxi: *O.* (*B.*) *damingshanensis* sp. nov., *O.* (*B.*) *nasica*, *O.* (*B.*) *trankieni*, *O.* (*B.*) *versabilis*, and *O.* (*B.*) *yentuensis*. This finding highlights the significant species diversity of *Odorrana* (*Bamburana*) in Guangxi. It is worth noting that the presence of *O.* (*B.*) *trankieni* is a new record for China.

## Acknowledgments

We express our gratitude to the staff of Shiwandashan, Dayaoshan, Damingshan, Huaping, and Diding National Nature Reserves for their valuable assistance during the fieldwork. Additionally, we would like to acknowledge Tao Thien Nguyen for enhancing the manuscript.

## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

### Funding

This work was supported by the National Natural Science Foundation of China (Nos. 32060116 and 32360128) and Guangxi Natural Science Foundation, China (No. 2020GXNSFDA238022).

### Author contributions

CWC and MYM conceived and designed the study and prepared the manuscript. CWC measured the specimens, performed the molecular experiments, and analyzed the data. CWC, LL, and QK conducted field surveys. All authors read and approved the final version of the manuscript.

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### Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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## Appendix 1

**Table A1.** Specimens examined. Abbreviations: NNU = Nanning Normal University; NHMG = Natural History Museum of Guangxi.

| ID | Species | Vouchers | Sex | Locality |
|---|---|---|---|---|
| 1 | *O.* (*B.*) *damingshanensis* sp. nov. | NNU00689 | Adult, male | Wuming, Guangxi, China |
| 2 | *O.* (*B.*) *damingshanensis* sp. nov. | NNU00690 | Adult, male | Wuming, Guangxi, China |
| 3 | *O.* (*B.*) *damingshanensis* sp. nov. | NNU00691 | Adult, male | Wuming, Guangxi, China |
| 4 | *O. (B.) damingshanensis* sp. nov. | NNU00692 | Adult, female | Wuming, Guangxi, China |
| 5 | *O. (B.) damingshanensis* sp. nov. | NNU00693 | Adult, female | Wuming, Guangxi, China |
| 6 | *O. (B.) damingshanensis* sp. nov. | NNU00201 | Sub-adult | Wuming, Guangxi, China |
| 7 | *O. (B.) nasica* | NNU00663 | Adult, female | Jingxi, Guangxi, China |
| 8 | *O. (B.) trankieni* | NHMG1303003 | Adult, male | Shangsi, Guangxi, China |
| 9 | *O. (B.) trankieni* | NHMG140108 | Adult, female | Shangsi, Guangxi, China |
| 10 | *O. (B.) trankieni* | NHMG141103 | Adult, female | Shangsi, Guangxi, China |
| 11 | *O. (B.) trankieni* | NHMG141104 | Adult, female | Shangsi, Guangxi, China |
| 12 | *O. (B.) trankieni* | NHMG141107 | Adult, male | Shangsi, Guangxi, China |
| 13 | *O. (B.) trankieni* | NHMG141111 | Adult, female | Shangsi, Guangxi, China |
| 14 | *O. (B.) trankieni* | NHMG141112 | Adult, male | Shangsi, Guangxi, China |
| 15 | *O. (B.) trankieni* | NHMG141113 | Adult, male | Shangsi, Guangxi, China |
| 16 | *O. (B.) trankieni* | NHMG141116 | Adult, male | Shangsi, Guangxi, China |
| 17 | *O. (B.) trankieni* | NNU20042908 | Adult, male | Wuming, Guangxi, China |
| 18 | *O. (B.) trankieni* | NNU20042909 | Adult, male | Wuming, Guangxi, China |
| 19 | *O. (B.) trankieni* | NNU20042910 | Adult, male | Wuming, Guangxi, China |
| 20 | *O. (B.) trankieni* | NNU20210304 | Adult, male | Wuming, Guangxi, China |
| 21 | *O. (B.) trankieni* | NNU202103001 | Adult, female | Wuming, Guangxi, China |
| 22 | *O. (B.) trankieni* | NNU202103002 | Adult, female | Wuming, Guangxi, China |
| 23 | *O. (B.) trankieni* | NNU202103003 | Adult, female | Wuming, Guangxi, China |
| 24 | *O. (B.) versabilis* | NNU00637 | Adult, female | Jinxiu, Guangxi, China |
| 25 | *O. (B.) versabilis* | NNU00638 | Adult, male | Jinxiu, Guangxi, China |
| 26 | *O. (B.) versabilis* | NNU00639 | Adult, female | Jinxiu, Guangxi, China |
| 27 | *O. (B.) versabilis* | NNU00640 | Adult, female | Jinxiu, Guangxi, China |
| 28 | *O. (B.) versabilis* | NNU00641 | Adult, male | Jinxiu, Guangxi, China |

| ID | Species | Vouchers | Sex | Locality |
|---|---|---|---|---|
| 29 | *O. (B.) versabilis* | NNU00642 | Adult, female | Jinxiu, Guangxi, China |
| 30 | *O. (B.) versabilis* | NNU00643 | Adult, female | Jinxiu, Guangxi, China |
| 31 | *O. (B.) versabilis* | NNU00644 | Adult, female | Jinxiu, Guangxi, China |
| 32 | *O. (B.) versabilis* | NNU00645 | Adult, male | Jinxiu, Guangxi, China |
| 33 | *O. (B.) versabilis* | NNU00647 | Adult, female | Jinxiu, Guangxi, China |
| 34 | *O. (B.) versabilis* | NNU201908005 | Sub-adult | Longsheng, Guangxi, China |
| 35 | *O. (B.) versabilis* | NNU201908010 | Adult, female | Longsheng, Guangxi, China |
| 36 | *O. (B.) yentuensis* | NHMG1401035 | Adult, male | Shangsi, Guangxi, China |
| 37 | *O. (B.) yentuensis* | NHMG1401036 | Adult, male | Shangsi, Guangxi, China |
| 38 | *O. (B.) yentuensis* | NNU00230 | Adult, male | Shangsi, Guangxi, China |
| 39 | *O. (B.) yentuensis* | NHMG1505001 | Adult, male | Shangsi, Guangxi, China |
| 40 | *O. (B.) yentuensis* | NHMG1505002 | Adult, male | Shangsi, Guangxi, China |
| 41 | *O. (B.) yentuensis* | NHMG1505003 | Adult, male | Shangsi, Guangxi, China |
| 42 | *O. (B.) yentuensis* | NHMG1505004 | Adult, male | Shangsi, Guangxi, China |
| 43 | *O. (B.) yentuensis* | NHMG1505005 | Adult, male | Shangsi, Guangxi, China |
| 44 | *O. (B.) yentuensis* | NHMG1505006 | Adult, male | Shangsi, Guangxi, China |
| 45 | *O. (B.) yentuensis* | NHMG1505007 | Adult, male | Shangsi, Guangxi, China |
| 46 | *O. (B.) yentuensis* | NHMG1505008 | Adult, male | Shangsi, Guangxi, China |
| 47 | *O. (B.) yentuensis* | NHMG1505009 | Adult, male | Shangsi, Guangxi, China |
| 48 | *O. (B.) yentuensis* | NHMG1505010 | Adult, male | Shangsi, Guangxi, China |
| 49 | *O. (B.) yentuensis* | NHMG1505011 | Adult, female | Shangsi, Guangxi, China |
| 50 | *O. (B.) yentuensis* | NHMG1505012 | Adult, female | Shangsi, Guangxi, China |
| 51 | *O. (B.) yentuensis* | NHMG1505013 | Adult, female | Shangsi, Guangxi, China |
| 52 | *O. (B.) yentuensis* | NHMG1505014 | Adult, female | Shangsi, Guangxi, China |
| 53 | *O. (B.) yentuensis* | NHMG1505015 | Adult, female | Shangsi, Guangxi, China |
| 54 | *O. (B.) yentuensis* | NHMG1505016 | Adult, female | Shangsi, Guangxi, China |

## Supplementary material 1

### Supplementary information

Authors: Wei-Cai Chen, Yun-Ming Mo, Li Lin, Kun Qin

Data type: xls

Explanation note: **table S1.** Uncorrected p-distance based on 16S gene fragment. **table S2.** Uncorrected *p*-distance based on COI gene fragment.

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Link: https://doi.org/10.3897/zookeys.1190.109886.suppl1

ZooKeys 1190: 153–162 (2024)
DOI: 10.3897/zookeys.1190.115839

Research Article

# Two new species of the genus *Psilalcis* Warren, 1893 (Geometridae, Ennominae, Boarmiini) from Hainan, China

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## Abstract

Two new species, *Psilalcis subalbibasis* Liu, **sp. nov.** and *Psilalcis subconceptaria* Liu, **sp. nov.**, are described from Hainan Island, China. Adult males and females of both species, including their genitalia, are figured and compared to closely related species.

**Key words:** Geometridae, new species, *Psilalcis*, *P. subalbibasis*, *P. subconceptaria*, taxonomy

OPEN ACCESS

Academic editor: Axel Hausmann
Received: 16 November 2023
Accepted: 4 January 2024
Published: 25 January 2024

ZooBank: https://zoobank.org/557E09C6-7C85-41DF-AD32-0A944FE69923

Citation: Liu B (2024) Two new species of the genus *Psilalcis* Warren, 1893 (Geometridae, Ennominae, Boarmiini) from Hainan, China. ZooKeys 1190: 153–162. https://doi.org/10.3897/zookeys.1190.115839

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## Introduction

The genus *Psilalcis*, belonging to the tribe Boarmiini in the subfamily Ennominae of the Geometridae, was established by Warren (1893) with *Tephrosia inceptaria* Walker, 1866 from Flores, Indonesia as its type species. Two other new species, *Psilalcis atrifasciata* Warren and *Psilalcis dentilinea* Warren, both from Sikkim, were also described as members of this new genus in Warren's article; the former was treated as a synonym of *Parapholodes fuliginea* (Hampson) by Sato (2000); the latter was transferred to *Prochasma* by Prout (1926), primarily based on the presence of the metallic mesothoracic crest. Only a few species and subspecies were included in *Psilalcis* over the following one hundred years (Warren 1899; Inoue 1956, 1964; Sato 1993a, 1993b). Subsequently, Holloway [1994] placed *Paralcis* Warren, 1894 (type species: *Menophra conspicuata* Moore, 1888) as a synonym of *Psilalcis* because of the similar genitalic characters and proposed a broad sense of *Psilalcis* that included the genera *Heterarmia* Warren, 1895, *Polymixinia* Wehrli, 1943, and *Protoboarmia* McDunnough, 1920. He also thought that the genus *Phanerothyris* Warren, 1895 might be referable to *Psilalcis* but had a more distinctive valve structure. In addition, he summarized the characters for the whole group, primarily based on the features of the male and female genitalia, and treated four Bornean species as members of *Psilalcis* with two new species. In the following nearly 30 years, a number of new members, including many newly described species, were added to *Psilalcis* (Sato 1995, 1996, 1998, 1999, 2002, 2008a, 2008b, 2013, 2020, 2023; Inoue 1998; Beljaev and Stüning 2000; Orhant 2001; Sato and Wang 2006, 2016; Stüning 2018). The present *Psilalcis* is a complex with large numbers of

species belonging to several different groups on the basis of external characters, and there is evidently much more revisional work to be done.

Recently, two new species, *Psilalcis subalbibasis* sp. nov. and *Psilalcis subconceptaria* sp. nov., were collected from Hainan Island, China; the former is similar in external appearance and genitalia to its close relatives *P. albibasis* (Hampson, 1895), *P. benefica* (Sato, 1993) and *P. sumatrana* Sato, 2013; the latter, together with its close relatives *P. conceptaria* Holloway, 1994, *P. paraceptaria* Sato, 1996 and *P. vietnamensis* Sato, 1996, share unique features of a trifid valve structure and a setose ampulla at the base of a central laminate lobe on the male genitalia, which perhaps can be treated as a separate group. In the present paper, these two new species are described, and their definitive diagnoses are given with respect to closely related species.

## Materials and methods

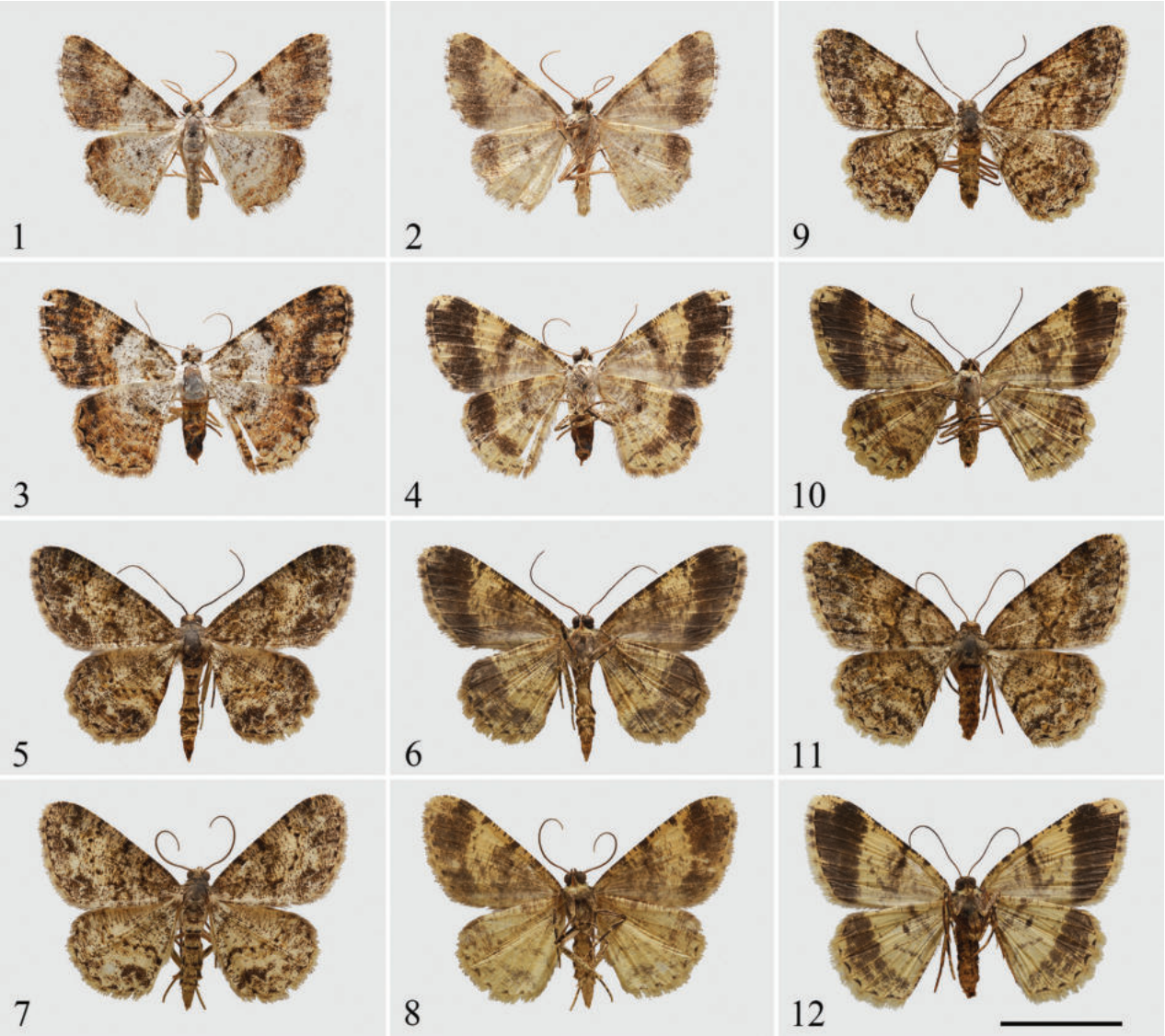
All specimens of *Psilalcis* treated herein were collected by light traps on Hainan Island, China and currently are deposited in Coconut Research Institute, Chinese Academy of Tropical Agricultural Sciences, Wengchang, China (**CRICATAS**). For long-term preservation, most of the type specimens of the two new species, including the holotypes, will be transferred to the Institute of Zoology, Chinese Academy of Sciences, Beijing, China (**IZCAS**) and some of the paratypes will be transferred to the Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany (**ZFMK**). Terminology for wing venation followed the Comstock-Needham System (Comstock 1918) as adopted for Geometridae by Scoble (1992) and Hausmann (2001), and that of the genitalia was based on Klots (1970) and Skou and Sihvonen (2015). Abdomens were removed and placed in 10% NaOH solution for examination of the genitalia. Genitalia were dissected in 10% alcohol solution and stained with Chlorazol Black E. Photographs of adults were taken with a Nikon D750 camera using a Nikon AF-S Micro 60 mm f/2.8G ED lens. Photos of genitalia were taken with a KUY NICE E31SPM digital camera attached to a Nikon SMZ745T microscope.

## Taxonomic account

***Psilalcis subalbibasis* sp. nov.**
https://zoobank.org/8E0EAB41-CFAB-4E7F-BA60-D865FDD6F543
Figs 1–4, 13, 15, 18

**Type-material.** ***Holotype*:** ♂, China, Hainan Province, Lingshui, Diaoluoshan, 922 m, 19.VI.2023, Bo Liu leg., gen. prep. no. CRICATAS00112 (CRICATAS, will be transferred to IZCAS in the future). ***Paratype*:** 1 ♀, China, Hainan Province, Lingshui, Diaoluoshan, 922 m, 19.VI.2023, Bo Liu leg., gen. prep. no. CRICATAS00113 (CRICATAS, will be transferred to IZCAS in the future).

**Diagnosis.** *Psilalcis subalbibasis* is very similar in appearance to its close relatives *P. albibasis* (Sato 1999: 37, figs 19, 40; Sato 2020, pl. 25: 18), *P. benefica* (Sato 1993a: 18, pl. 36: 21, fig. 153; Sato 1999: 37; Sato 2013, figs 28, 36; Sato 2020, pl. 25: 17) and *P. sumatrana* (Sato 2013: 250, 251, figs 16–18, 27, 35), all of which have a similar wing pattern of white basal half and reddish-brown terminal half together with a broad dark band. It can be distinguished from




**Figures 1–12.** Adults of *Psilalcis* spp. **1–4** *Psilalcis subalbibasis* sp. nov. **1** male, holotype, upperside **2** male, holotype, underside **3** female, paratype, upperside **4** female, paratype, underside **5–12** *Psilalcis subconceptaria* sp. nov. **5** male, holotype, upperside **6** male, holotype, underside **7** male, paratype, upperside **8** male, paratype, underside **9** female, paratype, upperside **10** female, paratype, underside **11** female, paratype, upperside **12** female, paratype, underside. Scale bar: 1 cm.

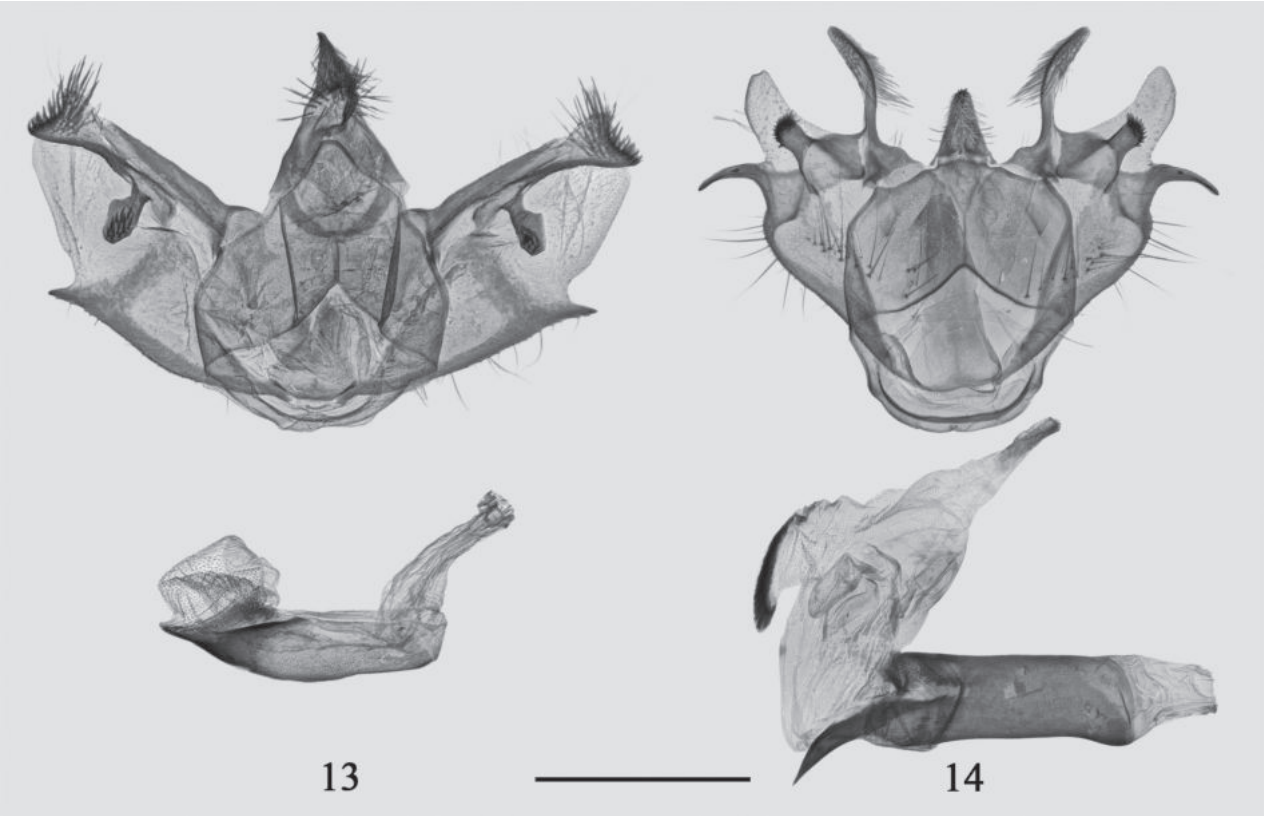
its relatives by the following genitalia characters: 1) cucullus crescent-shaped, strongly concave apically in *P. subalbibasis*, triangular and not concave apically in the other three closely related species; 2) setose ampulla narrow at base and broad at apex; and 3) female genitalia with a rather large, sclerotized, uniquely constructed lamella postvaginalis.

**Description.** Forewing length: male 12.3 mm; female 13.5 mm. Faces more vivid and contrasty in female. ***Head.*** Antennae fasciculate, with moderately long ciliate ventrally in male; filiform in female. Frons not protruding, covered with short scales. Labial palpus curved upwards beyond frons, covered with long, intermingled, dark and fawn scales, third segment not extended in female. Vertex with lamellar, fawn scales, posterior scales erect. Chaetosemata present, small, near eye-margin. ***Thorax.*** Patagia and tegulae with lamellar, white, slightly fawn-colored scales, with longer, pale fawn hair-scales on tegulae only. Prothorax ventrally covered with lamellar, white scales. Legs slender, fawn,

chequered black, hind tibia dilated, with a fawn scent brush in male, index of spurs 0-2-4. Forewings with apex angled, termen minutely concave between vein-ends. Fovea present in male, with posterior flexure of the anal vein to accommodate it. Hindwing with apex rounded, termen moderately concave between vein-ends. Wings dark deer-red, mottled dark, with a large white patch at base half, hindwing patch much larger, extending close to submarginal line in male. Antemedial and medial lines dark fawn, faintly visible on forewing, hardly visible on hindwing. Postmedial line fine, dark, slightly sinuous. Discal dot oval, dark, clearly visible, fused with costal patch forming a barred patch on forewing. Submarginal line rather fine, zigzag-shaped, white, faintly visible. Outside of postmedial line bearing a large dark band. Area of apex and between $M_3$ and $CuA_1$ on forewing without dark colouration. Distal band present only on upper half of hindwing. Marginal line black, inwardly concave. Fringes identical with the ground colour, interspersed with some dark. Underside brownish-yellow, covered with dark streaks. Distal band similar to upperside, but broader and more prominent. Discal dot clearly visible. Medial line more conspicuous in female. ***Venation.*** Forewing: $R_1$ and $R_2$ coincident; $R_1$+$R_2$ arising from upper vein of cell, then running almost parallel to the stem of $R_{3-4}$ and $R_{3-5}$; stem of $R_{3-5}$ arising shortly before anterior angle of cell; $M_2$ from nearly the middle of the discocellular vein; $CuA_1$ from before posterior angle of cell; the base of the anal vein concave downwards. Hindwing: Sc+$R_1$ running closely parallel but not anastomosing with upper vein of cell at base; Rs from before anterior angle of cell; $CuA_1$ from before posterior angle of cell; 3A present. ***Pregenital abdomen.*** Dorsally scaled white and fawn, scattered with some black scales. Ventrally with pale fawn scales. Setal comb (straight field) of minute setae present on abdominal sternite 3. Tympanal organs moderately sized, without lacinia. A pair of long sterno-tympanal processes present laterally on sternite 1+2, with moderately long free end, reaching the tympanal cavity. Tergite and sternite of segment 7 short, length about 1/2 of width. Tergite and sternite of segment 8 slightly elongate, length approximately equal to width in male.

***Male genitalia.*** Uncus hood-like, base broad, triangular, apex short, strongly sclerotized, curved ventrad at 90 degrees, with strong setae dorsally. Gnathos vestigial, socii not visible. Juxta short, basally broad, apically slightly bifurcated. Saccus rounded, slightly extended. Valvae parallelogram, costa rod-shaped. Cucullus dilated, crescent-shaped, strongly concave apically. Setose digitate ampulla located at the ventral edge of the valve costa, narrow at base, dilated at apex. Valve lamina membranous, central laminate lobe weakly sclerotized. Sacculus sclerotized, distally with a short, slightly inwardly curved spine-like process. Aedeagus short, apex tapering, with a minute spine near the tip. Vesica without cornuti.

***Female genitalia.*** Ovipositor slightly elongated, papillae anales narrow, covered with short setae. Anterior apophyses short, about 2/5 length of posterior apophyses. A thin needle-like sclerite present between the bases of posterior apophyses. Lamella antevaginalis narrow, ribbon-shaped. Lamella postvaginalis rather large, strongly sclerotized; centrally squared, distally concave in the middle; lateral processes expanded, bent dorsad, centrally concave inwards. Posterior part of bursa rather short, with an irregular week of narrow sclerotized band. Anterior part of bursa slightly broader than posterior part, but no clear demarcation visible. Signum absent.




**Figures 13, 14.** Male genitalia of *Psilalcis* spp. **13** *Psilalcis subalbibasis* sp. nov. paratype, gen. prep. no. CRICATAS00112 **14** *Psilalcis subconceptaria* sp. nov. paratype, gen. prep. no. CRICATAS00143. Scale bar: 1 mm.

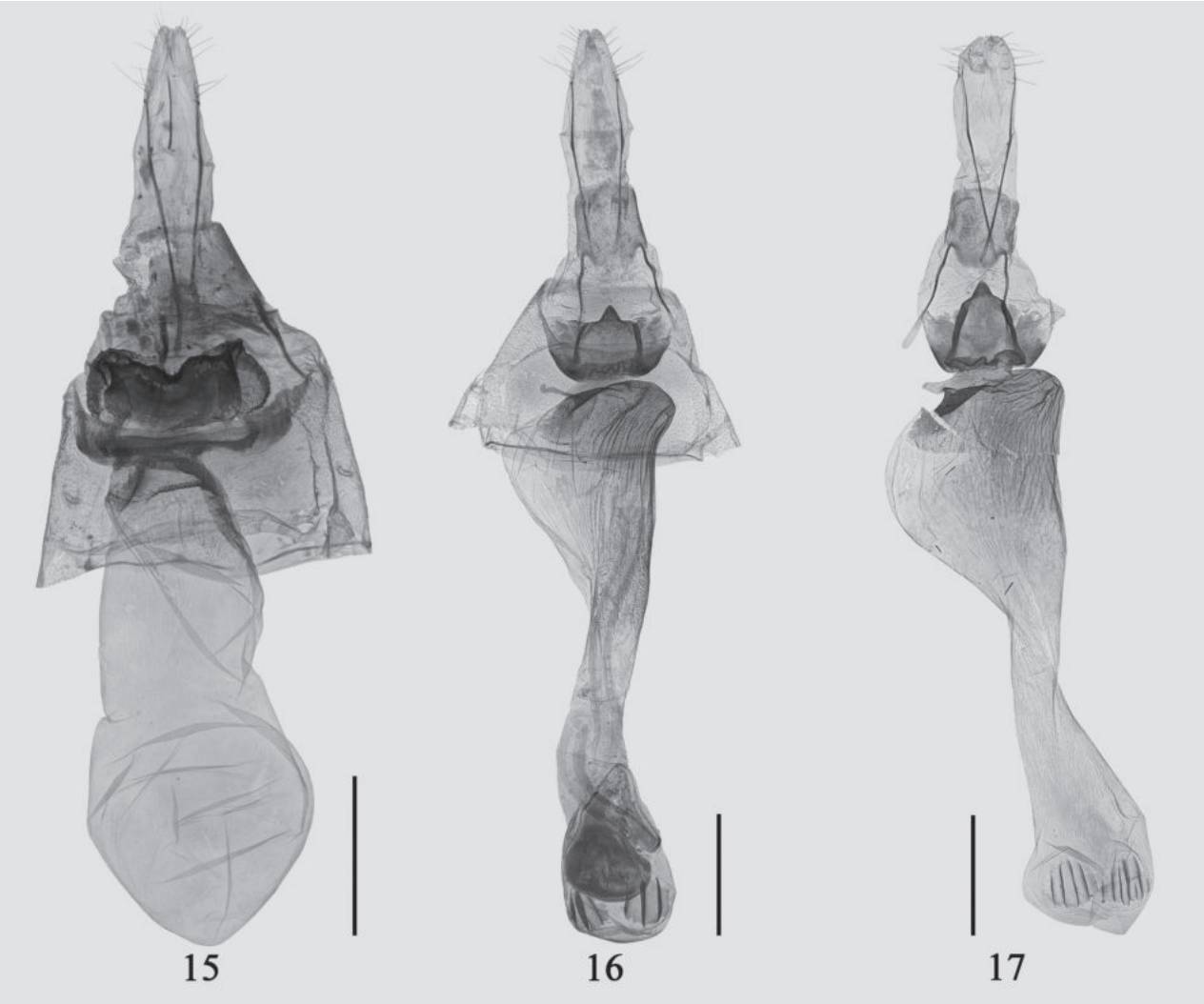
**Etymology.** This new species, *Psilalcis subalbibasis*, is highly similar to *P. albibasis* (Hampson) in wing pattern and male genitalia.

**Distribution.** China (Hainan).

***Psilalcis subconceptaria* sp. nov.**
https://zoobank.org/DDCF3EEE-FE44-41C3-99E2-B1E0A18A68E8
Figs 5–12, 14, 16, 17, 19–21

**Type material.** ***Holotype*:** ♂, China, Hainan Province, Lingshui Li Autonomous County, Diaoluoshan, 922 m, 19.VI.2023, Bo Liu leg. (CRICATAS, will be transferred to IZCAS). ***Paratypes*:** 1 ♂, Hainan Province, Qiongzhong Li and Miao Autonomous County, Yinggeling, 496m, 3.III.2023, Bo Liu leg.; 3 ♂ 2 ♀, China, Hainan Province, Wuzhishan City, Wuzhishan, 756 m, 25.III.2023, Bo Liu leg.; 4 ♂ 10 ♀, China, Hainan Province, Lingshui Li Autonomous County, Diaoluoshan, 922 m, 20.IV.2023, Bo Liu leg. gen. prep. no. CRICATAS00147; 5 ♂ 4 ♀, China, Hainan Province, Lingshui Li Autonomous County, Diaoluoshan, 922 m, 10.V.2023, Bo Liu leg. including gen. prep. nos. CRICATAS00143, CRICATAS00146; 2 ♂ 5 ♀, China, Hainan Province, Lingshui Li Autonomous County, Diaoluoshan, 922 m, 19.VI.2023, Bo Liu leg. (CRICATAS, will be transferred to IZCAS and ZFMK)

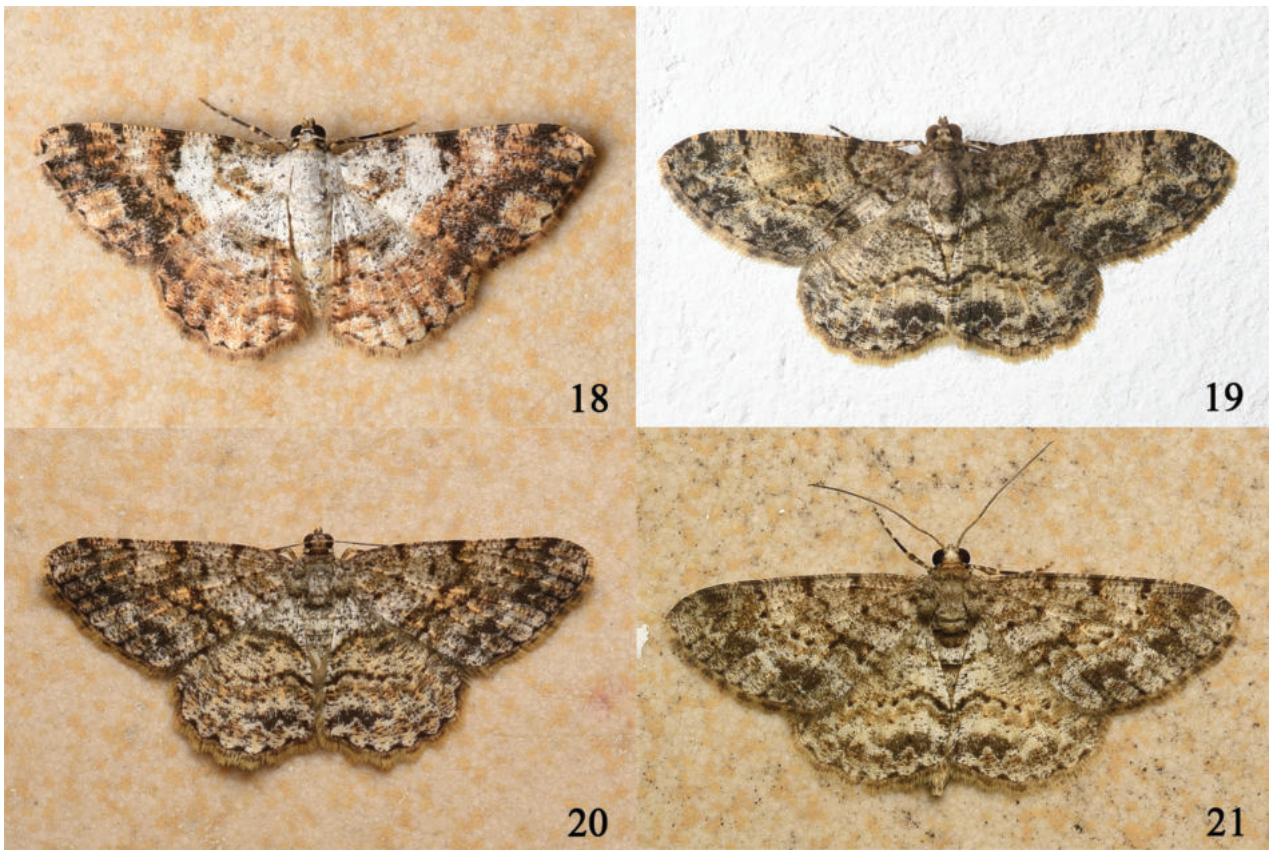
**Diagnosis.** *Psilalcis subconceptaria* shares a very similar wing pattern and similar trifid valve structure of the male genitalia with *P. conceptaria* (Holloway [1994: 235], pl. 15: 13, figs 497, 499; Sato 1996, figs 71, 79), *P. paraceptaria* (Sato 1996: 66, 67, figs 47–50, 72, 80), and *P. vietnamensis* (Sato 1996: 66,




**Figures 15–17.** Female genitalia of *Psilalcis* spp. **15** *Psilalcis subalbibasis* sp. nov. paratype, gen. prep. no. CRICATAS00113 **16** *Psilalcis subconceptaria* sp. nov. paratype, gen. prep. no. CRICATAS00146 **17** *Psilalcis subconceptaria* sp. nov. paratype, gen. prep. no. CRICATAS00147. Scale bars: 1 mm.

figs 41–44, 73, 78; Sato 2020, pl. 25: 6, 7). It can be easily distinguished from the other three related congeners by the following genitalia characters: 1) signum located at the anterior of corpus bursae, smaller; 2) costal process elongated, longer than that of the other three relatives; and 3) apex of valve lamina slender, narrower than that of the other three relatives.

**Description.** Forewing length: male 12.1–14.5 mm; female 13.3–15.0 mm. Wing pattern variable among individuals, usually more vibrant in females. ***Head*.** Antennae fasciculate, with moderately long ciliate ventrally in males; filiform in females. Frons not protruding, covered with short scales, upper half dark, lower half pale brown. Labial palpus curved upwards beyond frons, covered with long, intermingled, dark and brownish scales, third segment not extended. Vertex with lamellar, brownish scales, posterior scales erect. Chaetosemata present, small, near eye-margin. ***Thorax*.** Patagia and tegulae with lamellar, brownish and dark scales, with longer, dark brownish hair-scales on tegulae only. Prothorax ventrally covered with lamellar, brownish scales. Legs slender, yellow, chequered black, hind tibia dilated, with a yellow scent brush in males,




**Figures 18–21.** Living specimens of *Psilalcis* spp. **18** *Psilalcis subalbibasis* sp. nov. female **19** *Psilalcis subconceptaria* sp. nov. male **20** *Psilalcis subconceptaria* sp. nov. female **21** *Psilalcis subconceptaria* sp. nov. female.

index of spurs 0-2-4. Forewings with apex angled, termen minutely concave between vein-ends. Fovea present in males, with posterior flexure of the anal vein to accommodate it. Hindwing with apex rounded, termen moderately concave between vein-ends. Wings brownish, dotted with white and black scales. Postmedial lines of both wings punctuated, sometimes joined in lines, sinuous, black. Medial and postmedial lines of forewing converge below $CuA_2$, then separate. Submarginal line very fine, zigzag-shaped, white, faintly visible. Marginal line black, inwardly concave. Distal band serrated, narrow, evident on forewing, only visible near tornus on hindwing. Discal dot small. Fringes colored brownish, interspersed with some black. Underside brownish-yellow, covered with dark streaks. Distal band sometimes absent or not conspicuous on hindwings. ***Venation.*** Forewing: $R_1$ and $R_2$ coincident; $R_1$+$R_2$ arising from upper vein of cell, then running close to the stem of $R_{3\text{-}4}$ and $R_{3\text{-}5}$; stem of $R_{3\text{-}5}$ arising from anterior angle of cell; $M_2$ from 1/4 of the discocellular vein close to $M_1$ at base; $CuA_1$ from before posterior angle of cell; the base of the anal vein concave downwards. Hindwing: Sc+$R_1$ running closely parallel but not anastomosing with upper vein of cell at base; Rs from before anterior angle of cell; $CuA_1$ from before posterior angle of cell; 3A present. ***Pregenital abdomen.*** Abdomen scaled pale brown, scattered with some black scales. Setal comb (straight field) of minute setae present on the third sternite. Tympanal organs moderately sized, without lacinia. A pair of long sterno-tympanal processes present laterally on sternite 1+2, with moderately long free end, reaching the tympanal cavity. Tergite and sternite of segment 8 strongly elongate in males, length nearly twice the width.

***Male genitalia*.** Uncus hood-like, short, weakly curved ventrally, dorsally with short setae. Gnathos and socii absent. Juxta tongue-like, broad at base, slightly pointed at tip. Saccus rounded, slightly extended. Valvae trifid, costal process elongate, cucullus vestigial. Setose ampulla located at the centre of valve laminate lobe. Apex of sacculus bearing a long, strongly curved spine. Valve lamina membranous, distally elongated, central laminate lobe sclerotized. Aedeagus stout, apex with a curved, slender spine, vesica with a cluster of needle-like cornuti on a lateral lobe.

***Female genitalia*.** Ovipositor slightly elongated, papillae anales narrow, covered with short setae. Anterior apophyses short, about 3/5 length of posterior apophyses. The needle-like sclerite between the bases of posterior apophyses absent. Lamella antevaginalis narrow, ribbon-shaped. Lamella postvaginalis very large, centrally triangular, distally triangularly convex at the centre, lateral processes extended, slightly curved dorsad. Posterior part of bursa much narrower than anterior part, rather short, membranous. Anterior part of bursa elongated, posteriorly projected at both sides, with sclerotized corrugations, centrally with a constriction, anteriorly bearing a pair of small, circular, opposed sclerotized patches, with three to five longitudinal ridges.

**Etymology.** The specific name, *subconceptaria*, is derived from its closely related species, *P. conceptaria*.

**Distribution.** China (Hainan).

## Acknowledgements

I am indebted to Rikio Sato, Niigata, Japan, for confirming the identifications of the two new species and sending me his articles, essential for the present paper, and to Dieter Stüning, Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany, for his suggestions to improve the manuscript. I would also like to thank Jiexiong Fu, Hainan Tropical Rainforest National Park, Lingshui, China, and Wei Yan, Coconut Research Institute, Chinese Academy of Tropical Agricultural Sciences, Wenchang, China, for their assistance in collecting specimens.

## Additional information

### Conflict of interest

The author has declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

### Funding

This work was supported by the Key Research and Development Programs of Hainan Province (No. ZDYF2022XDNY214 and ZDYF2018136) and the Hainan Provincial Natural Science Foundation of China (No. 321QN344).

### Author contributions

The author solely contributed to this work.

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## Data availability

All of the data that support the findings of this study are available in the main text.

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ZooKeys 1190: 163–193 (2024)
DOI: 10.3897/zookeys.1190.113898

Research Article

# Molecular phylogeny and taxonomic position of *Macrobrachium lanchesteri* (De Man, 1911), with descriptions of two new species from Thailand (Decapoda, Caridea, Palaemonidae)

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## Abstract

*Macrobrachium lanchesteri* (De Man, 1911), a translucent freshwater prawn has a wide distribution range throughout mainland Southeast Asia. A high morphological variation and genetic divergence between different geographical *M. lanchesteri* populations in Thailand have peculiarly extended the uncertainty of species boundaries and blended confusingly with several *Macrobrachium* species. To clarify these circumstances, broad sample examinations of the morphological variation, including topotype specimens, and phylogenetic reconstruction based on the concatenated mitochondrial dataset (16s rRNA and COI genes) were performed. Broad morphological examination of *M. lanchesteri* has shown congruency with phylogenetic analyses by revealing prominent lineages of *M. lanchesteri* sensu stricto and two new sibling lineages with interspecific variation between 6.48–8.76% for COI and 3.06–4.23% for 16S. Descriptions of two new species, named herein as *M. panhai* Chaowvieng & Siriwut, **sp. nov.** and *M. rostrolevatus* Chaowvieng & Siriwut, **sp. nov.** are provided. Morphological investigation of rostral form suggested plasticity in *M. rostrolevatus* populations showing the morphological trait associated with their habitat preferences. Furthermore, phylogenetic positions of the three taxa affirmed the hidden diversity of Thai freshwater *Macrobrachium* fauna correlated with the river network in the Mekong and Chao Phraya basins, Thailand. The genetic data and distribution records obtained in this study may also assist future river conservation plans as well as the sustainable management of freshwater prawn diversity.

**Key words:** Edible prawns, Lower Mekong Basin, morphological plasticity, new species

OPEN ACCESS

Academic editor: Célio Magalhães
Received: 9 October 2023
Accepted: 4 January 2024
Published: 29 January 2024

ZooBank: https://zoobank.org/B41FADCF-F7F0-4195-83C5-A203D0290B04

Citation: Chaowvieng A, Sutcharit C, Chanabun R, Srisonchai R, Jeratthitikul E, Siriwut W (2024) Molecular phylogeny and taxonomic position of *Macrobrachium lanchesteri* (De Man, 1911), with descriptions of two new species from Thailand (Decapoda, Caridea, Palaemonidae). ZooKeys 1190: 163–193. https://doi.org/10.3897/zookeys.1190.113898

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## Introduction

Palaemonid freshwater prawns of genus *Macrobrachium* Spence Bate, 1868 have shown high species richness comprising 271 species worldwide (WoRMS 2023). This genus has a broad geographical distribution and is commonly found in the Oriental Region of Asia (De Grave et al. 2008). Several *Macrobrachium*

species demonstrate economic impacts, serving as protein resources and for utilisation in ornamental fish aquaculture (Cai et al. 2004; Wowor et al. 2004). According to its remarkable species richness and diversifications of aquatic and terrestrial invertebrate faunas in Indochina, the intensive fauna exploration and historical biogeography using both morphology and genetics were reinvestigated systematically in several taxa such as river prawns, bivalves, land snails and millipedes (De Bruyn et al. 2014; Pholyotha et al. 2021; Jeratthitikul et al. 2022; Likhitrakarn et al. 2023). Indochinese *Macrobrachium* prawns have gained attention recently, especially in the context of taxonomy and systematics (Cai et al. 2004; Wowor et al. 2004; Hanamura et al. 2011). Several molecular taxonomic studies have been verified nominal species and consequently supplemented the taxonomic account of some cryptic *Macrobrachium* prawns (Mar et al. 2018; Siriwut et al. 2020; Jurniati et al. 2021; Saengphan et al. 2021). Additionally, the DNA barcoding and molecular delimitation methods were implemented to clarify the taxonomic boundaries of several *Macrobrachium* species. Moreover, the phylogenetic positions of several species have been addressed some morphological complexity groups based on barcode gap distance threshold (Siriwut et al. 2021).

Currently, 34 species have been documented in Thailand (Cai et al. 2004; Cai and Vidthayanon 2016; Saengphan et al. 2018, 2019, 2020, 2021; Siriwut et al. 2020, 2021). Two major river basins, the Chao Phraya and the Greater Mekong, have been discussed as being significant hotspots for native *Macrobrachium* faunal diversity (Cai and Ng 2002; Hanamura et al. 2011). Some Thai *Macrobrachium* species have been reported to show narrow distribution within these basins, such as *M. chainatense* Saengphan, Panijpan, Senapin, Laosinchai, Ruenwongsa, Suksomnit & Phiwsaiya, 2019 which was only found in Central Thailand, and *M. spelaeus* Cai & Vidthayanon, 2016 that live in stygobiotic habitats. Contrastingly, some widespread species have also been documented about their distribution occupancy crossed inland basins and some insular territory of Southeast Asia, such as *M. sintangense* (De Man, 1898), and *M. dienbienphuense* Dang & Nguyen, 1972 (Cai et al. 2004; Wowor et al. 2004; Hanamura et al. 2011). For this reason, freshwater faunas in Thailand and neighbouring countries are capable linkage in terms of species composition, reaching an occurrence data of coexistence and cryptic species according to the connection of the river network (Hanamura et al. 2011; Siriwut et al. 2020).

A small translucent and common *M. lanchesteri* (De Man, 1911) dominantly occupies all river basins throughout mainland Southeast Asia with scattered distribution records from Malaysia, Singapore, Indonesia; it has even expanded northward to South China (Wowor and Choy 2001; Cai and Ng 2002; Cai et al. 2004). This species was originally found in southern Thailand and was diagnosed as having a straight and short rostrum not exceeding the scaphocerite and slender, thin second pereiopods (Kemp 1918; Holthuis 1950). The lectotype designation and morphological study of *M. lanchesteri* by Chong and Khoo (1988) advocated diagnostic character variation, particularly on rostral structure and body size variation in male regarding sexual dimorphism. Additionally, *M. lanchesteri* was mentioned with an argument on taxonomic boundary with some other congeners such as *M. peguense* (Tiwari, 1952), *M. kistnense* (Tiwari, 1952), and *M. tiwarii* Jalihal, Shenoy & Sankolli, 1988.

Moreover, *M. lanchesteri* also blended confusingly with the juveniles of several species such as *M. idae* (Heller, 1862) and *M. lar* (Fabricius, 1798) (Lanchester 1902; Kamita 1966).

Previous phylogenetic and population genetic studies of *M. lanchesteri* in Thailand have also detected high genetic diversity, both between and within populations (Reingchai et al. 2009; Khanarnpai et al. 2019; Siriwut et al. 2021). Moreover, the possible existence of cryptic species within several *Macrobrachium* species in Thailand under traditional morphological discrimination criteria was reported, including *M. lanchesteri*, based on DNA barcode delimitation thresholds (Siriwut et al. 2021). The lack of intensive collection from different river basins impeded comprehensive genetic and morphological information that would contribute to taxonomic boundary clarification and phylogenetic relationships of *M. lanchesteri* and other native species in this area. To elucidate the taxonomic confusion of several coexistent translucent *Macrobrachium* prawns, the integration of traditional morphological identification and molecular analysis could be investigated concurrently. Therefore, this study aimed to clarify the taxonomic boundaries of *M. lanchesteri* in Thailand by broad-scale sampling and reconstruct the phylogenetic relationships with various related translucent species based on COI gene and 16S rRNA markers, which have been used extensively to investigate the phylogenetic relationships between crustaceans (Costa et al. 2007; Pileggi and Mantelatto 2010; Castelin et al. 2017; Jamaluddin et al. 2019; Rossi et al. 2020). This study will contribute to elucidate the taxonomic status of *M. lanchesteri* s. str.and its closely related species as well as assist economical freshwater prawn management in the future.

## Materials and methods

### Sample collection and preparation

Prawn specimens were collected from various freshwater basins in Thailand. Live specimens were photographed to document body coloration using a Nikon D5300 camera with a micro-Nikkor 105 mm f/2.8 IF-ED Macro Lens. Prawns were gradually euthanised following the protocols approved by the Mahidol University-Institute Animal Care and Use Committee (MU-IACUC) under approval number MUSC66-026-656. Specimens were preserved in 95% ethanol and stored into a container for further morphological examination and molecular analysis. Voucher specimens were deposited at the Chulalongkorn University Museum of Zoology, Bangkok, Thailand (**CUMZ**) and Mahidol University Museum of Natural History, Department of Biology, Faculty of Science, Mahidol University, Thailand (**MUMNH**). Traditional identifications were carried out based on previous taxonomic studies of *Macrobrachium* species: Lanchester (1902), Holthuis (1950), Chong and Khoo (1988), Cai and Ng (2002), Cai et al. (2004), Wowor et al. (2004), and Hanamura et al. (2011). The morphological variation of prawn specimens was observed and illustrated under a stereomicroscope. A list of abbreviations used in the descriptions is given as follows: **Fin** (finger), **Pal** (palm), **Car** (carpus), **Mer** (merus), **Che** (chela), **Dac** (dactylus), **Pro** (propodus), **cl** (carapace length), **rl** (rostrum length). All morphological characters were measured using Dinocapture software v. 2.0 and reported in millimetres.

## DNA extraction and PCR protocol

All prawn specimens used for molecular analysis in this study are listed in Table 1. Genomic DNA was extracted from pleonal muscle tissue by using DNA extraction kits (NucleoSpin Tissue kit: MACHEREY-NAGEL). Genomic DNA quality was evaluated and visualised by gel electrophoresis and a UV illuminator. Two mitochondrial genes, 16S rRNA and cytochrome c oxidase subunit I (COI), were amplified. Three sets of primer such as 16Sa-L (5' CGC CTG TTT ATC AAA AAC AT 3') and 16Sbr-H2 (5' CTC CGG TTT GAA CTC AGA TCA 3') following Palumbi (1996) for 16S gene, LCO1490 (5'GGT CAA CAA ATC ATA AAG ATA TTG G 3'; Folmer et al. (1994), MacroNancy (5' GCG GGT AGR ATT AAR ATR TAT ACT TC 3'; Siriwut et al. (2020), HCOoutout (5' GTA AAT ATA TGR TGD GCTC 3'; Schulmeister et al. (2002) for COI were used in this study. PCR was performed using T100™ thermal cycler (BIO-RAD) with a gradient temperature function. The PCR profile consisted of the following steps: 94 °C for 5 min as an initial step followed by 34 cycles 94 °C for 30 sec for denaturing, 45–49 °C for 40 sec, 72 °C for 15 sec for extension, and final extension at 72 °C for 10 min. PCR products were run by 1% agarose gel electrophoresis stained with SYBR Safe illuminant (Invitrogen, USA). The purified products were sent for sequencing by a commercial company (Macrogen and Bioneer, Korea) using an Applied Biosystems automatic sequencer.

## Phylogenetic analyses

Sequences were aligned and corrected using the ClustalW algorithm in MEGA 11 (Tamura et al. 2021). All sequences have been registered and deposited in GenBank database under accession numbers OR575072–OR575118 for COI and OR578642–OR578698 for 16S (Table 1). The voucher specimen locality of each species used in molecular analysis is illustrated in Fig. 1. The DNA dataset for phylogenetic analyses was assembled including ten deposited COI sequences of *Macrobrachium* species in GenBank database. To depict the clade of *M. lanchesteri* sensu De Man (1911), topotype sequences were selected as representative indicators. *Macrobrachium villosimanus* (Tiwari, 1949) was used as the rooting outgroup.

Phylogenetic trees were constructed using maximum likelihood (ML) and Bayesian inference (BI) methods throughout the online CIPRES Science Gateway server (Miller et al. 2010). The concatenated dataset of two markers with the partitioned file for nucleotide substitution model fit was prepared in Kakusan 4 (Tanabe 2007). ML tree was visualised in RAxML v. 8.2.12. (Stamatakis 2014). The GTR+G model was set as the model for all gene partitions with 1,000 bootstrap replicates performed to verify tree topology and clade support. BI tree was estimated using MrBayes v. 3.2.7 (Ronquist et al. 2012). Markov chain Monte Carlo (MCMC) was configured as 10,000,000 generations of the sampling process; the first 25% of obtained trees were discarded as burn-in. Finalised trees were estimated for the consensus tree topology. The annotation and illustration of clade and branch length were performed in Figtree (Rambaut 2010). Node posterior probabilities of 0.95 were considered statistically significant for BI, and bootstrap support values greater than 70 were considered highly supported for ML (Huelsenbeck and Hillis 1993; Larget and Simon 1999). Pairwise genetic distance of intra and interspecific of each gene dataset was calculated using the p-distance method in MEGA 11 (Tamura et al. 2021).

**Table 1.** Locality and GenBank accession numbers of specimens used in phylogenetic analyses.

| Taxa | Voucher IDs | Localities | Coordinates | GenBank accession no. | | References |
|---|---|---|---|---|---|---|
| | | | | COI | 16S | |
| *M. lanchesteri* (De Man, 1911) | MUMNH_MP00221.1- M421 | Yom, Pong, Phayao | 19°06'24.3"N, 100°15'58.5"E | OR575100 | OR578680 | This study |
| | MUMNH_MP00230.1-M420 | Pai, Mueang Mae Hong Son, Mae Hong Son | 19°19'36.89"N, 97°56'34.78"E | OR575099 | OR578679 | This study |
| | MUMNH_MP00350-M400 | Nam Lao, Mae Taeng, Chiang Mai | 19°12'16.4"N, 98°40'51.3"E | OR575091 | OR578671 | This study |
| | MUMNH_ MP00222.1-M379 | Mae Mang, Bo Kluea, Nan | 19°08'12.6"N, 101°09'03.1"E | OR575081 | OR578661 | This study |
| | MUMNH_MP00245.1-M437 | Khlong Khlung, Wang Sai, Kamphaeng Phet | 16°11'51.7"N, 99°36'53.0"E | OR575108 | OR578688 | This study |
| | MUMNH_MP00259.1-M443 | Klong Un, Phu Phan, Sakon Nakhon | 17°00'22.1"N, 103°54'50.5"E | OR575112 | OR578692 | This study |
| | MUMNH_MP00259.1-M433 | Klong Un, Khok Phu, Phu Phan, Sakon Nakhon | 17°00'22.1"N, 103°54'50.5"E | OR575106 | OR578686 | This study |
| | MUMNH_MP00262.1-M442 | Huai Sathot, Huai Phueng, Kalasin | 16°41'32.6"N, 103°51'20.1"E | OR575111 | OR578691 | This study |
| | MUMNH_MP00264.1-M399 | Chi River, Mueang Maha Sarakham, Maha Sarakham | 16°13'01.1"N, 103°16'44.1"E | OR575090 | OR578670 | This study |
| | MUMNH_MP00274.1-M435 | Lam Takhong, Pak Chong, Nakhon Ratchasima | 14°33'00.7"N, 101°27'34.1"E | OR575107 | OR578687 | This study |
| | MUMNH_MP00278.1-M452 | Nong Prue Mai Kaeo, Plaeng Yao, Chachoengsao | 13°33'31.5"N, 101°17'44.0"E | OR575118 | OR578698 | This study |
| | MUMNH_MP00293.1-M412 | Yang Chum, Tha Yang, Phetchaburi | 12°46'55.7"N, 99°40'43.65"E | OR575098 | OR578678 | This study |
| | MUMNH_MP00300.1-M398 | Pak Nam, Mueang Krabi, Krabi | 8°04'50.7"N, 98°55'07.3"E | OR575089 | OR578669 | This study |
| | MUMNH_MP00301.1-M422 | Klong Na Thap, Chana, Songkhla | 7°01'20.1"N, 100°43'51.5"E | OR575101 | OR578681 | This study |
| | CUMZ_MP00089-M078 | Sathing Phra, Songkhla | 7°25'01.4"N, 100°25'04.0"E | MW845498 | OR578643 | Siriwut et al. 2021 |
| | CUMZ_MP00090-M079 | Sathing Phra, Songkhla | 7°25'01.4"N, 100°25'04.0"E | MW845497 | OR578644 | Siriwut et al. 2021 |
| | CUMZ_MP00093-M082 | La-un, Ranong | 10°06'37.1"N, 98°45'32.5"E | MW845495 | OR578645 | Siriwut et al. 2021 |
| *M. panhai* sp. nov. | MUMNH_MP00307.1-M441 | Klong Tron, Thong Sang Khan, Uttaradit | 17°35'39.1"N, 100°29'10.3"E | OR575110 | OR578690 | This study |
| | MUMNH_MP00351-M447 | Klong Hi, Seka, Bueng Kan | 17°54'17.9"N, 103°57'12.0"E | OR575115 | OR578695 | This study |
| | MUMNH_MP00309.1-M404 | Nam Suai, Mueang Nong Khai, Nong Khai | 17°45'01.1"N, 102°51'00.5"E | OR575092 | OR578672 | This study |
| | MUMNH_MP00310.1-M425 | Mekong River, Khong Chiam, Ubon Ratchathani | 15°19'10.3"N, 105°29'53.2"E | OR575103 | OR578683 | This study |
| | MUMNH_MP00313.1-M439 | Ang Kep Nam Nam Khuen Nam Lang, Wang Pong, Phetchabun | 16°25'19.8"N, 100°48'48.2"E | OR575109 | OR578689 | This study |
| | CUMZ_MP00302-M382 | Sri Nakarin Dam, Si Sawat, Kanchanaburi | 14°24'12.1"N, 99°07'24.7"E | OR575082 | OR578662 | This study |
| | MUMNH_MP00320.1-M405 | Huai Raeng, Bo Rai, Trat | 12°23'48.4"N, 102°39'15.1"E | OR575093 | OR578673 | This study |
| | CUMZ_MP00020-M003 | Nam Pat, Uttaradit | 17°43'47.0"N, 100°41'24.3"E | MW845582 | OR578642 | Siriwut et al. 2021 |
| | CUMZ_MP00146-M147 | Klaeng, Rayong | 12°47'05.7"N, 101°40'59.6"E | MW845580 | OR578651 | Siriwut et al. 2021 |
| *M. rostrolevatus* sp. nov. | CUMZ_MP00323-M368 | Bueng Khong Long, Bueng Kan | 17°59'59.1"N, 104°01'06.9"E | OR575076 | OR578656 | This study |
| | MUMNH_MP00324.1-M387 | Bueng Khong Long, Bueng Kan | 17°59'59.1"N, 104°01'06.9"E | OR575083 | OR578663 | This study |
| | MUMNH_MP00324.2-M408 | Bueng Khong Long, Bueng Kan | 17°59'59.1"N, 104°01'06.9"E | OR575095 | OR578675 | This study |
| | MUMNH_MP00325.1-M392 | Nam Suai, Mueang Nong Khai, Nong Khai | 17°45'01.1"N, 102°51'00.5"E | OR575085 | OR578665 | This study |
| | MUMNH_MP00326.1-M388 | Si Charoen, Phen, Udon Thani | 17°42'47.7"N, 102°50'57.4"E | OR575084 | OR578664 | This study |
| | MUMNH_MP00352-M407 | Tha Rae, Mueang Sakon Nakhon, Sakon Nakhon | 17°15'08.5"N, 104°09'32.0"E | OR575094 | OR578674 | This study |
| | MUMNH_MP00330.1-M424 | Nam Chan, Akat Amnuai, Sakon Nakhon | 17°35'46.1"N, 104°00'21.6"E | OR575102 | OR578682 | This study |
| | MUMNH_MP00334.1-M367 | Bueng Aram, Yang Talat, Kalasin | 16°24'21.8"N, 103°20'26.4"E | OR575075 | OR578655 | This study |

| Taxa | Voucher IDs | Localities | Coordinates | GenBank accession no. | | References |
|---|---|---|---|---|---|---|
| | | | | COI | 16S | |
| *M. rostrolevatus* sp. nov. | MUMNH_MP00334.2-M409 | Bueng Aram, Yang Talat, Kalasin | 16°24'21.8"N, 103°20'26.4"E | OR575096 | OR578676 | This study |
| | MUMNH_MP00334.3-M432 | Bueng Aram, Yang Talat, Kalasin | 16°24'21.8"N, 103°20'26.4"E | OR575105 | OR578685 | This study |
| | MUMNH_MP00353-M364 | Suk San, Khun Han, Si Sa Ket | 14°35'27.7"N, 104°29'29.3"E | OR575072 | OR578652 | This study |
| | MUMNH_MP00340.1-M444 | Huai Khayung, Kantharalak, Si Sa Ket | 14°34'42.1"N, 104°38'48.1"E | OR575113 | OR578693 | This study |
| | MUMNH_MP00343.1-M371 | Mun River, Tha Tum, Surin | 15°17'38.5"N, 103°30'42.4"E | OR575077 | OR578657 | This study |
| | MUMNH_MP00348.1-M445 | Sawai Riang, Non Sung, Nakhon Ratchasima | 15°16'13.3"N, 102°22'37.0"E | OR575114 | OR578694 | This study |
| | MUMNH_MP00341.1-M448 | Klong Thap Than, Rattanaburi, Surin | 15°16'55.4"N, 103°58'38.1"E | OR575116 | OR578696 | This study |
| | MUMNH_MP00354-M450 | Tha Yang, Phu Kradueng, Loei | 16°53'38.3"N, 101°52'53.1"E | OR575117 | OR578697 | This study |
| | MUMNH_ MP00346.1-M411 | Sathaet, Mueang Yang, Nakhon Ratchasima | 15°27'35.2"N, 102°59'46.6"E | OR575097 | OR578677 | This study |
| | CUMZ_MP00096-M085 | Mueang, Mahasarakam | 16°11'01.4"N, 103°27'24.4"E | MW845577 | OR578646 | Siriwut et al. 2021 |
| | CUMZ_MP00097-M086 | Udonthani | 17°19'02.5"N, 102°35'53.0"E | MW845578 | OR578647 | Siriwut et al. 2021 |
| *M. rosenbergii* (De Man, 1879) | MUMNH_MP00355-M377 | Pak Chan, Kra Buri, Ranong | 10°31'38.0"N, 98°50'01.8"E | OR575080 | OR578660 | This study |
| | MUMNH_MP00356-M394 | Pak Chan, Kra Buri, Ranong | 10°31'38.0"N, 98°50'01.8"E | OR575087 | OR578667 | This study |
| | CUMZ_MP00100-M094 | Mueang, Maha Sarakham | 16°11'01.4"N, 103°27'24.4"E | MW845593 | OR578648 | Siriwut et al. 2021 |
| | CUMZ_MP00118-M115 | Mueang, Ranong | 9°53'13.5"N, 98°38'01.2"E | MW845595 | OR578650 | Siriwut et al. 2021 |
| *M. sintangense* (De Man, 1898) | MUMNH_MP00357-M374 | Huai Yang, Ta Phraya, Sa Kaeo | 14°00'44.4"N, 102°38'39.9"E | OR575078 | OR578658 | This study |
| | MUMNH_MP00358-M393 | Huai Yang, Ta Phraya, Sa Kaeo | 14°00'44.4"N, 102°38'39.9"E | OR575086 | OR578666 | This study |
| | MUMNH_MP00359-M366 | Khwae Noi, Mueang Kanchanaburi, Kanchanaburi | 13°58'18.4"N, 99°18'25.9"E | OR575074 | OR578654 | This study |
| | MUMNH_MP00360-M430 | Kui Buri, Prachuap Khiri Khan | 12°05'29.7"N, 99°48'18.1"E | OR575104 | OR578684 | This study |
| | MUMNH_MP00361-M365 | Tha Di, Nakhon Si Thammarat | 8°23'28.5"N, 99°52'27.4"E | OR575073 | OR578653 | This study |
| *M. villosimanus* (Tiwari, 1949) | MUMNH_MP00362-M376 | Chan Thi, Mueang Trat, Trat | 12°15'55.0"N, 102°36'08.8"E | OR575079 | OR578659 | This study |
| | MUMNH_MP00363-M396 | Trang, Kantang, Trang | 7°26'02.9"N, 99°30'54.9"E | OR575088 | OR578668 | This study |
| | CUMZ_MP00116-M113 | La-ngu, Satun | 6°54'22.3"N, 99°48'42.2"E | MW845638 | OR578649 | Siriwut et al. 2021 |

# Results

## Molecular phylogeny and genetic divergence

Forty-seven sequences of partial COI and 57 sequences of partial 16S genes were successfully amplified and obtained (Table 1). COI sequence contained 627 bp with 417 bp of conserved sites, 210 bp of variable sites and 202 of parsimony informative sites. 16S sequence contained 554 bp with 373 bp of conserved sites, 181 bp of variable sites and 154 bp of parsimony informative sites. The proportional range of genetic variations in *M. lanchesteri* species complex and other *Macrobrachium* species were revealed by p-distance. Inter and intraspecific variations ranged from 15.12–20.68% for COI, 8.6–16.18% for 16S and 0.9–5.79% for COI and 1.08–3.19% for 16S, respectively.

Both ML and BI trees based on 1,181 bp concatenated dataset of the COI and 16S gene fragments revealed the six *Macrobrachium* species as monophyletic groups with strong statistical support values (Fig. 2). Clade C comprised all *M. sintangense* sequences. Phylogenetic tree also showed that *M. rosenbergii* (De Man, 1879) is closely related to *M. lanchesteri* species complex clade, forming clade D. The genetic distance between *M. rosenbergii* and *M. lanchesteri* species complex clade was 15.12% for COI and 8.6% for 16S. In

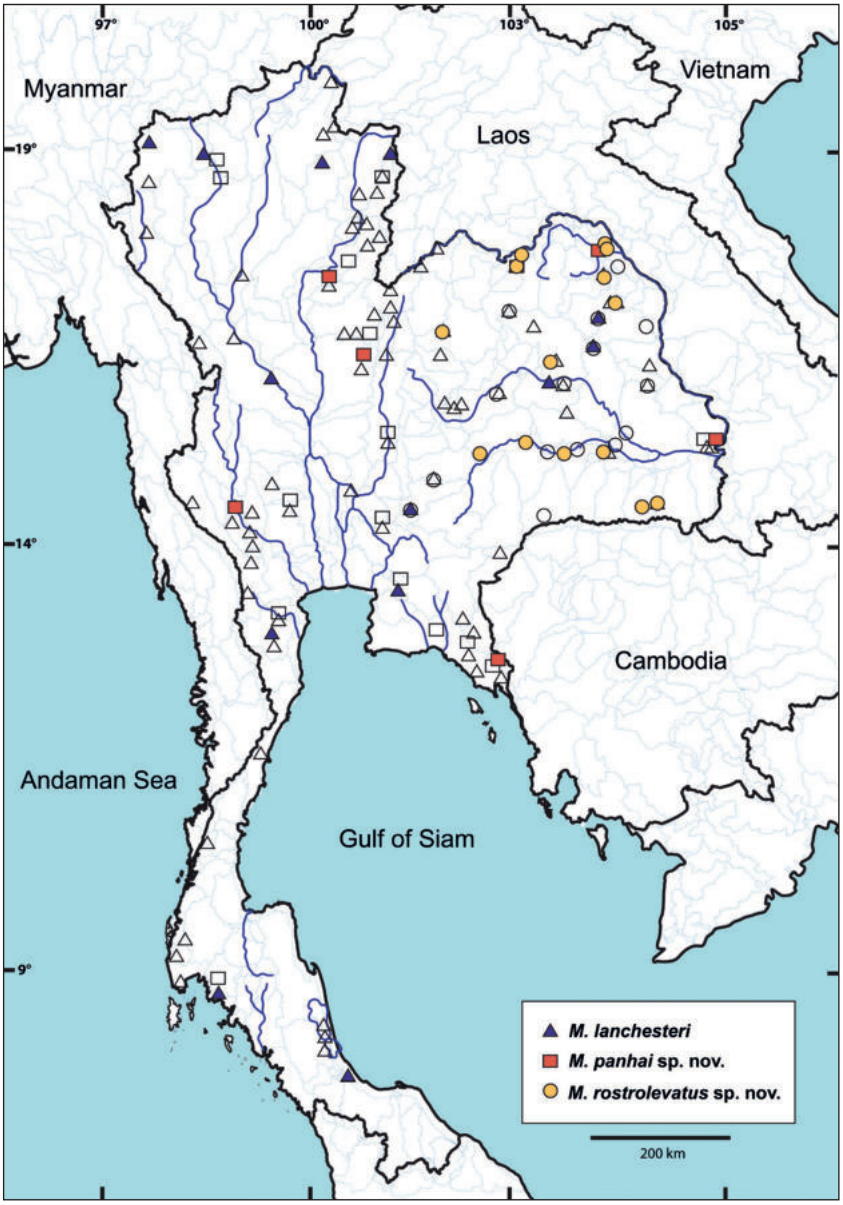


**Figure 1.** Distribution map of three *Macrobrachium* species in Thailand. A colour symbol indicates the locality of specimen used in phylogenetic analyses. A transparent symbol indicates the locality of specimen examined based on morphology. Equivalent symbols, whether coloured or not, indicate the same species.

the clade E, *Macrobrachium lanchesteri* species complex was divided into three monophyletic groups with high statistical supports for both ML (100) and BI (1). The interspecific variation ranged from 6.48–8.76% for COI and 3.06–4.23% for 16S. The intraspecific variation also ranged from 0.92–2.27% for COI and 0.7–2.23% for 16S. In the results of this study, clade H was shown as *M. lanchesteri* based on the topotype sequences assembled. The monophyletic group of *M. lanchesteri* s. str. herein represents two subclades, lower Isthmus of Kra (Clade I) and upper Isthmus of Kra populations. *Macrobrachium panhai* sp. nov. (Clade J) was nested as a sister clade of *M. lanchesteri* s. str. with sufficient support in ML (74), but partial support in BI (86). *Macrobrachium rostrolevatus* sp. nov. (Clade F) was separated from congeneric members of *M. lanchesteri* species complex and all samples in this clade were strictly distributed inside freshwater basins on the Khorat Plateau, i.e. the Mun, Chi and Songkhram Rivers.

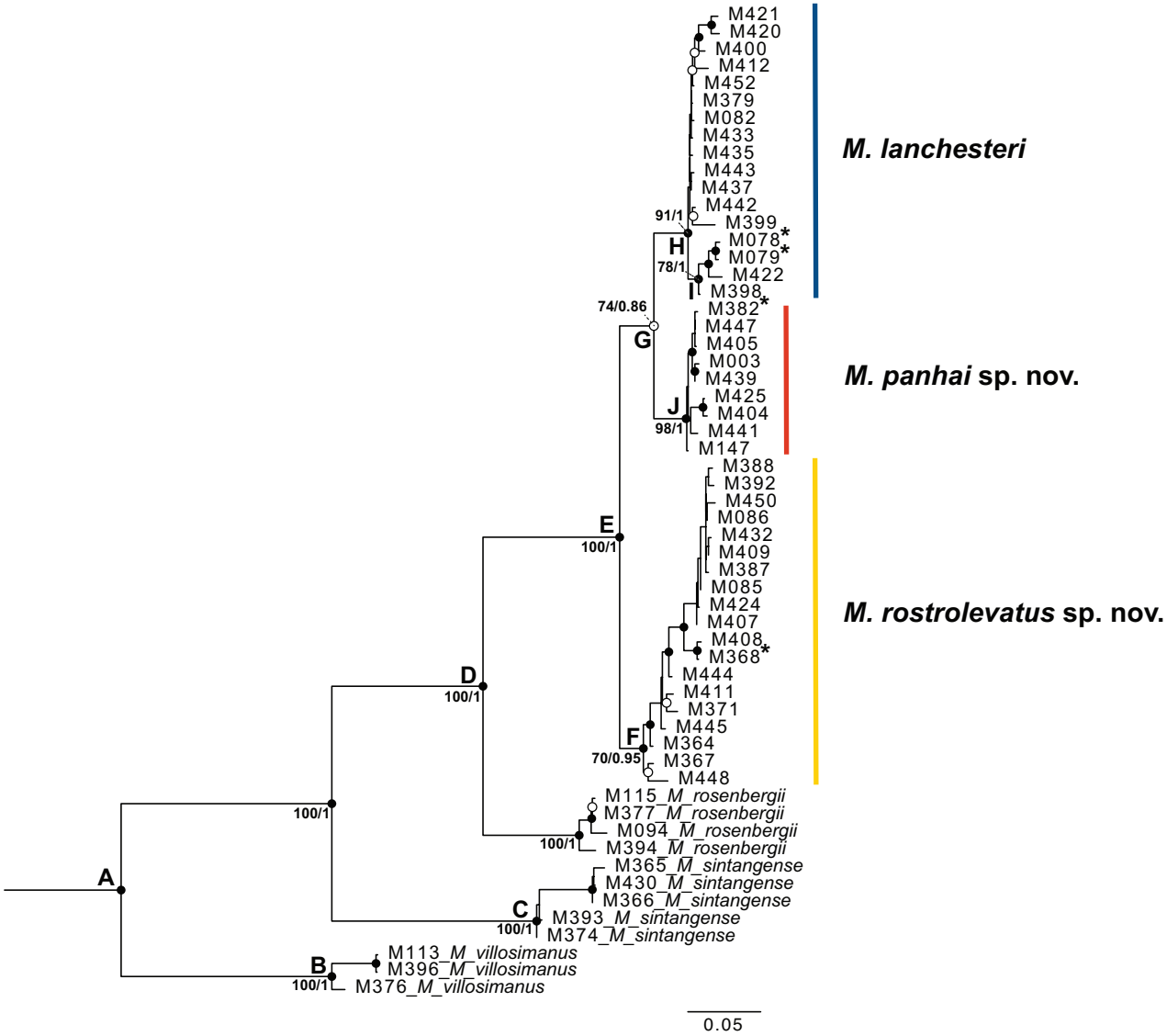


**Figure 2.** Phylogenetic tree based on a concatenation of COI and 16S genes. Nodes of a phylogenetic tree marked with a black circle indicate statistical support from both ML and BI (≥ 70 bootstrap values and ≥ 0.95 posterior probability scores). A white circle indicates statistical support for either ML or BI. An asterisk indicates the topotype in *M. lanchesteri* and holotype in the new species.

## Taxonomic account

***Macrobrachium lanchesteri* (De Man, 1911)**
Figs 3A, B, 4

*Palaemon paucidens* Lanchester, 1902: 568, pl. 33, fig. 4. Type locality: Singora [Songkhla Province, Thailand]. [Not De Haan (1844) and Hilgendorf (1893)].
*Palaemon* (*Eupalaemon*) *lanchesteri* De Man, 1911: 264. Replacement Name.
*Palaemon lanchesteri*: Kemp 1918: 257.
*Macrobrachium lanchesteri*: Suvatti 1937: 49; Suvatti 1967; 139; Holthuis 1950: 139; Holthuis 1980: 96; Johnson 1961: 56; Naiyanetr 1980: 17; Naiyanetr 1992: 17; Naiyanetr 1998: 32; Chong and Khoo 1988: 196; Cai and Ng. 2002: 77; Cai et al. 2004: 586; Wowor et al. 2004: 349; Hanamura et al. 2011: 13.
*Macrobrachium lar*: Kamita 1966: 138.

*Cryphiops* (*Macrobrachium*) *lanchesteri*: Johnson 1968: 233.
*Macrobrachium* cf. *lanchesteri*: Ng 1994: 75, fig. 2.

**Material examined. *Topotypes*: Songkhla** • 11 ♀♀, 3 ♂♂, Wat Pho Klang, Khu Khut, Sathing Phra; 7°25'26.2"N, 100°25'05.8"E; MUMNH MP00216. • 25 ♀♀, 3 ♂♂, Laem Wang, Khu Khut, Sathing Phra; 7°27'12.3"N, 100°25'12.9"E; MUMNH MP00217. • 5 ♀♀, 3 ♂♂, Bang Khiat, Singhanakhon; 7°20'19.2"N, 100°25'36.8"E; MUMNH MP00218.

**Additional material. Chiang Rai** • 12 ♀♀, 16 ♂♂, Chiang Khong Street Market, Wiang, Chiang Khong; 20°15'57.7"N, 100°24'21.7"E; MUMNH MP00219. **Chiang Mai** • 1 ♀, Nam Lao, Pa Pae, Mae Taeng; 19°12'16.4"N, 98°40'51.3"E; MUMNH MP00350. **Phayao** • 5 ♀♀, 1 ovigerous, 4 ♂♂, Wean, Ban Pa Suk, Chiang Kham; 19°30'04.7"N, 100°16'32.9"E; MUMNH MP00220. • 17 ♀♀, 14 ♂♂, Yom, Ban Duea, Pong; 19°06'24.3"N, 100°15'58.5"E; MUMNH MP00221. **Nan** • 4 ♀♀, Mae Mang, Bo Kluea Tai, Bo Kluea; 19°08'12.6"N, 101°09'03.1"E; MUMNH MP00222. • 10 ♀♀, 1 ovigerous, 8 ♂♂, Ban Hua Fai, Sisaket, Na Noi; 18°19'33.4"N, 100°43'25.6"E; MUMNH MP00223. • 1 ovigerous, Huai Pa Sak, Sisaket, Na Noi; 18°19'33.4"N, 100°43'25.6"E; MUMNH MP00224. • 1 ♀, 1 ♂, Ban Huai Lao, Chiang Khong, Na Noi; 18°18'42.3"N, 100°54'18.2"E; MUMNH MP00225. • 2 ♀♀, 1 ovigerous, 4 ♂♂, Huai Hin, Sathan, Na Noi; 18°14'21.8"N, 100°41'57.7"E; MUMNH MP00226. • 2 ♀♀, Ban Na Bua, Nong Daeng, Mae Charim; 18°45'12.8"N, 101°00'39.7"E; MUMNH MP00227. • 3 ♀♀, 1 ♂, Nan River, Tan Chum, Wiang Sa; 19°00'59.5"N, 100°46'47.3"E; MUMNH MP00228. **Lampang** • 1 ♀, 2 ♂♂, Ban Mae Pa, Thoen; 17°38'17.9"N, 99°15'39.6"E; MUMNH MP00229. **Mae Hong Son** • 3 ♀♀, 8 ♂♂, Pai, Pang Mu, Mueang Mae Hong Son; 19°19'36.9"N, 97°56'34.8"E; MUMNH MP00230. • 2 ♀♀, 2 ♂♂, Yuam, Mae Sariang; 18°09'26.0"N, 97°55'37.8"E; MUMNH MP00231. • 8 ♀♀, 4 ♂♂, Nong Pong Sila, Khun Yuam; 18°50'26.4"N, 97°56'21.3"E; MUMNH MP00232. **Uttaradit** • 3 ♀♀, 5 ♂♂, Huai Nam Muet, Phak Khuang, Thong Sang Khan; 17°28'21.5"N, 100°22'25.6"E; MUMNH MP00233. • 4 ♀♀, 1 ovigerous, 3 ♂♂, Klong Tron, Ban Bueng Pra Kot, Thong Sang Khan; 17°35'39.1"N, 100°29'10.3"E; MUMNH MP00234. • 4 ♀♀, 15 ♂♂, Nam Pat, Fak Tha; 18°00'04.4"N, 100°52'42.7"E; MUMNH MP00235. • 2 ♀♀, Ban Rai Phana Wan, Muang Chet Ton, Ban Khok; 18°08'48.7"N, 101°02'11.0"E; MUMNH MP00236. **Tak** • 2 ♀♀, 2 ♂♂, Ban Klong Haui Sai, Nong Bua Tai, Mueang Tak; 16°46'24.2"N, 99°06'45.0"E; MUMNH MP00237. • 1 ♀, Klong Mae Sot, Phra That Pha Daeng, Mae Sot; 16°42'24.7"N, 98°36'52.1"E; MUMNH MP00238. **Phitsanulok** • 13 ♀♀, 7 ♂♂, Khek, Kaeng Sopha, Wang Thong; 16°53'15.4"N, 100°39'13.3"E; MUMNH MP00239. • 7 ♀♀, 3 ♂♂, Ban Bo, Wang Nok Aen, Wang Thong; 16°51'13.6"N, 100°36'43.2"E; MUMNH MP00240. **Phetchabun** • 16 ♀♀, 5 ♂♂, Ban Pho Ngam, Tha Phon, Mueang Phetchabun; 16°35'29.5"N, 101°07'41.6"E; MUMNH MP00241. • 4 ♀♀, 2 ♂♂, Si Thep Historical Park, Si Thep;15°28'19.7"N, 101°08'48.2"E; MUMNH MP00242. • 1 ovigerous, 1 ♂, Nam Khuen Nam Lang, Wang Hin, Wang Pong; 16°24'20.6"N, 100°47'56.5"E; MUMNH MP00243. • 5 ♀♀, 1 ♂, Klong Nam Phung, Hin Hao, Lom Kao; 16°58'47.8"N, 101°12'51.5"E; MUMNH MP00244. **Kamphaeng Phet** • 4 ♀♀, 2♂♂, Khlong Khlung, Wang Sai; 16°11'51.7"N, 99°36'53.0"E; MUMNH MP00245. **Nong Khai** • 4 ♀♀, 4 ovigerous, 2 ♂♂, Nam Suai, Song Hong, Mueang Nong Khai; 17°45'01.1"N, 102°51'00.5"E; MUMNH MP00246. **Loei** • 5 ovigerous, 1 ♂, Mekong River, Hat Bia, Pak Chom; 18°03'39.4"N, 101°47'51.8"E; MUMNH MP00247. • 3 ♀♀, 1 ovigerous, 8 ♂♂, Hueang, Na Chan, Chaing Khan; 17°47'13.7"N, 101°34'31.1"E; MUMNH

MP00248. • 2 ♀♀, 1 ovigerous, 4 ♂♂, Klong Nam Man, Na Ho, Dan Sai; 17°19'35.6"N, 101°08'54.5"E; MUMNH MP00249. • 4 ♀♀, Klong Khok Khamin, Khok Khamin, Wang Saphung; 17°10'24.7"N, 101°50'52.1"E; MUMNH MP00250. • 4 ♀♀, 3 ovigerous, 1 ♂, Ban Wang Kum, Dan Sai; 17°07'27.6"N, 101°10'40.7"E; MUMNH MP00251. • 4 ♀♀, 2 ♂♂, Ban Tha Yang, Phu Kradueng; 16°53'47.6"N, 101°53'18.3"E; MUMNH MP00252. **Udon Thani** • 1 ♀, Klong Nam Khong, Thap Kung, Nong Saeng; 17°10'01.5"N, 102°46'03.2"E; MUMNH MP00253. • 1 ♀, 2 ovigerous, 2 ♂♂, Huai Yang, Nong Ya Sai, Wang Sam Mo; 16°59'19.2"N, 103°21'50.0"E; MUMNH MP00254. • 2 ♀♀, 2 ovigerous, Huai Yang, Nong Ya Sai, Wang Sam Mo; 16°57'22.9"N, 103°22'19.9"E; MUMNH MP00255. **Sakon Nakhon** • 2 ♀♀, 3 ♂♂, Nam Chan, Akat, Akat Amnuai; 17°35'46.1"N, 104°00'21.6"E; MUMNH MP00256. • 13 ♀♀, 4 ♂♂, Klong Lak, Chiang Khruea, Mueang Sakon Nakhon; 17°15'33.7"N, 104°07'00.1"E; MUMNH MP00257. • 2 ♀♀, 1 ♂, Nong Han, Tha Rae, Mueang Sakon Nakhon; 17°15'08.5"N, 104°09'32.0"E; MUMNH MP00258. • 3 ♀♀, 2 ♂♂, Klong Un, Khok Phu, Phu Phan; 17°00'22.1"N, 103°54'50.5"E; MUMNH MP00259. **Mukdahan** • 3 ♀♀, Klong Bang I, Nong Weang, Nikhom Kham Soi; 16°23'43.8"N, 104°34'33.7"E; MUMNH MP00260. **Kalasin** • 1 ovigerous, 4 ♂♂, Bueng Aram, Khlong Kham, Yang Talat; 16°24'21.8"N, 103°20'26.4"E; MUMNH MP00261. • 2 ♀♀, 3 ♂♂, Huai Sathot, Kham Bong, Huai Phueng; 16°41'32.6"N, 103°51'20.1"E; MUMNH MP00262. **Maha Sarakham** • 6 ♀♀, 1 ovigerous, 2 ♂♂, Chi River, Tha Tum, Mueang Maha Sarakham; 16°10'58.2"N, 103°27'19.9"E; MUMNH MP00263. • 1 ♀, Chi River, Koeng, Mueang Maha Sarakham; 16°13'01.1"N, 103°16'44.1"E; MUMNH MP00264. **Chaiyaphum** • 6 ♀♀, Huai I Muet, Khon San; 16°31'34.6"N, 101°39'28.0"E; MUMNH MP00265. • 3 ♀♀, 1 ovigerous, 2 ♂♂, Pha Iang Waterfall, Huai Ton, Mueang Chaiyaphum; 15°57'42.2"N, 101°54'17.8"E; MUMNH MP00266. • 1 ♀, Na Siao, Mueang Chaiyaphum; 15°55'05.1"N, 102°06'13.8"E; MUMNH MP00267. • 2 ♀♀, 2 ovigerous, Tat Ton, Na Siao, Mueang Chaiyaphum; MUMNH MP00268. **Roi Et** • 5 ♀♀, 4 ♂♂, Ban Nong Phue, Chaturaphak Phiman; 15°49'07.7"N, 103°30'36.9"E; MUMNH MP00269. **Ubon Ratchathani** • 2 ♀♀, 2 ovigerous, 2 ♂♂, Mekong River, Khong Chiam, Khong Chiam; 15°19'10.3"N, 105°29'53.2"E; MUMNH MP00270. • 4 ♀♀, Tung Lung, Nong Saeng Yai, Khong Chiam; 15°20'12.2"N, 105°24'02.6"E; MUMNH MP00271. **Si Sa Ket** • 1 ♀, Bueng Bun Local Market, Bueng Bun; 15°19'18.6"N, 104°03'01.2"E; MUMNH MP00272. • 2 ♀♀, 1 ovigerous, 2 ♂♂, Khayung, Thung Yai, Kantharalak 14°34'42.8"N, 104°38'47.5"E; MUMNH MP00273. **Nakhon Ratchasima** • 9 ♀♀, 1 ovigerous, 3 ♂♂, Lam Takhong, Mu Si, Pak Chong; 14°33'00.7"N, 101°27'34.1"E; MUMNH MP00274. • 2 ♀♀, 1 ♂, Sema, Sung Noen; 14°55'11.0"N, 101°47'53.5"E; MUMNH MP00275. **Lopburi** • 3 ♀♀, 1 ovigerous, 6 ♂♂, Lop Buri Local Market, Tha Sala, Mueang Lop Buri; 14°47'25.2"N, 100°40'27.7"E; MUMNH MP00276. **Nakhon Nayok** • 1 ♀, Khao Perm, Ban Na; 14°21'14.5"N, 101°05'06.9"E; MUMNH MP00277. **Chachoengsao** • 1 ♂, Nong Prue Mai Kaeo, Wang Yen, Plaeng Yao; 13°33'31.5"N, 101°17'44.0"E; MUMNH MP00278. **Sa Kaeo** • 3 ♀♀, 1 ovigerous, 3 ♂♂, Huai Yang, Thap Rat, Ta Phraya; 14°07'24.9"N, 102°40'03.6"E; MUMNH MP00279. **Chanthaburi** • 3 ♀♀, 5 ♂♂, Wang Kra Prae, Soi Dao; 12°58'18.9"N, 102°16'12.5"E; MUMNH MP00280. • 6 ♀♀, 5 ovigerous, 6 ♂♂, Koh Phasuk, Makham; 12°40'48.9"N, 102°12'08.6"E; MUMNH MP00281. • 10 ♀♀, 11 ♂♂, Phra Sathueng, Thap Chang, Soi Dao; 13°12'29.1"N, 102°10'07.0"E; MUMNH MP00282. • 2 ♀♀, 3 ♂♂, Klong I Ngaeo, Sung, Khlung; 12°27'13.8"N, 102°16'56.1"E; MUMNH MP00283. **Trat** • 1 ♀, Tha Sen, Khlong Yai; 12°06'53.3"N, 102°43'16.8"E; MUMNH MP00284. **Suphan Buri** • 6 ♀♀, 4 ovigerous, 12 ♂♂, Ban Chara Mai, Ban Kong, U

Thong; 14°34'51.0"N, 99°52'04.7"E; MUMNH MP00285. • 1 ovigerous, 1 ♂, Kra Siao, Dan Chang; 14°50'01.9"N, 99°40'38.4"E; MUMNH MP00286. **Kanchanaburi** • 1 ♀, 1 ovigerous, 3 ♂♂, Huai Khayeng, Khayeng, Thong Pha Phum; 14°38'59.0"N, 98°34'31.3"E; MUMNH MP00287. • 1 ♀, Huai I Su, Nong Ri, Bo Phloi; 14°32'33.3"N, 99°22'41.4"E; MUMNH MP00288. • 3 ♀♀, 5 ♂♂, Taphoen, Lat Ya, Mueang Kanchanaburi; 14°08'14.0"N, 99°22'57.8"E; MUMNH MP00289. • 23 ♀♀, 3 ovigerous, 15 ♂♂, Huai Mae Pla Soi, Na Suan, Si Sawat; 14°34'03.4"N, 99°12'13.6"E; MUMNH MP00290. • 2 ♀♀, 5 ♂♂, Klong Phachi, Klon Do, Dan Makham Tia; 13°55'05.2"N, 99°22'59.3"E; MUMNH MP00291. **Ratchaburi** • 3 ♀♀, 1 ovigerous, 2 ♂♂, Khok Mu, Ta Nao Si, Suan Phueng; 13°28'29.5"N, 99°15'48.8"E; MUMNH MP00292. **Phetchaburi** • 2 ♀♀, Yang Chum, Klat Luang, Tha Yang; 12°46'55.7"N, 99°40'43.7"E; MUMNH MP00293. • 4 ♀♀, 2 ♂♂, Ban Yang Nam Klat Tai, Nong Ya Plong; 13°06'33.3"N, 99°43'22.8"E; MUMNH MP00294. **Prachuap Khiri Khan** • 2 ♂♂, Phongprasan, Bang Saphan; 11°12'53.1"N, 99°30'09.6"E; MUMNH MP00295. **Ranong** • 1 ovigerous, Bang Khun Paeng, Bang Phra Nuea, La-un; 10°04'06.0"N, 98°43'04.1"E; MUMNH MP00296. **Phang Nga** • 1 ♂, Phu Ta Jor, Le, Kapong; 8°46'04.7"N, 98°27'19.8"E; MUMNH MP00297. • 6 ♀♀, 1 ovigerous, 1 ♂, Thung Maphrao, Thai Mueang; 8°32'56.4"N, 98°19'23.4"E; MUMNH MP00298. **Phuket** • 1 ♂, Bang Pae, Pa Klok, Thalang; 8°02'17.8"N, 98°24'12.4"E; MUMNH MP00299. **Krabi** • 9 ovigerous, 1 ♂, Pak Nam, Mueang Krabi; 8°04'50.7"N, 98°55'07.3"E; MUMNH MP00300. **Songkhla** • 2 ♀♀, 1 ♂, Klong Na Thap, Chana; 7°01'20.1"N, 100°43'51.5"E; MUMNH MP00301.

**Diagnosis.** Rostrum straight or proximal convex and distal margin gently upward. Rostrum length reaches beyond end of antennular peduncle and as long as scaphocerite. Rostral formula: 6–10/1–6 teeth including 1–3 distal teeth with small gap separate from rest. Carapace smooth. Epistome bilobed. First pereiopods reaching end of scaphocerite. Second pereiopods long and slender, similar in form and length, exceeding scaphocerite. Fingers covered with scattered setae, slightly shorter than palm. Translucent razor on cutting edge present anteriorly. Proximal quarter of cutting edges with one or two tiny teeth. Carpus cylindrical shape and articulation margin expanded. Carpus 1.5× longer than chela. Merus subcylindrical. Carpus 1.5× longer than merus. Third pereiopods long and slender, reaching end of scaphocerite. Dactylus curved distally with short setae. Propodus 2× longer than dactylus. Propodus with 4–8 pairs of spines distributed along its length and fine setae at articulation. Propodus 2× longer than carpus. Sixth and seventh thoracic sternites usually smooth. Eighth thoracic sternite with acute median process. First and second pleonal sternites with small median process. Third and fourth pleonal sternites smooth. Fifth pleonal sternite with triangular ridge. Uropodal diaeresis with inner movable spine shorter than outer angle.

**Composite description.** ***Rostrum*** (Fig. 4B). Straight or slightly convex proximally and upward distally. Rostrum length exceeding end of antennular peduncle and slightly shorter than scaphocerite. Dorsal margin with 6–10 teeth including 1–3 teeth distally with small gap from rest. Postorbital margin with one or two teeth, reaching to one-fourth of carapace length. Ventral margin with 1–6 teeth, starting from middle to distal margins. Short setae present between rostral teeth.

***Cephalon*** (Fig. 4B). Well-developed eye. Ocular beak without laterally expanded tip. Cornea longer and broader than stalk. Postantennular carapace margin rounded. Cornea osculum longer than stalk. Antennular peduncle longer than wide with fine setae, basal segment short, second segment shorter than third

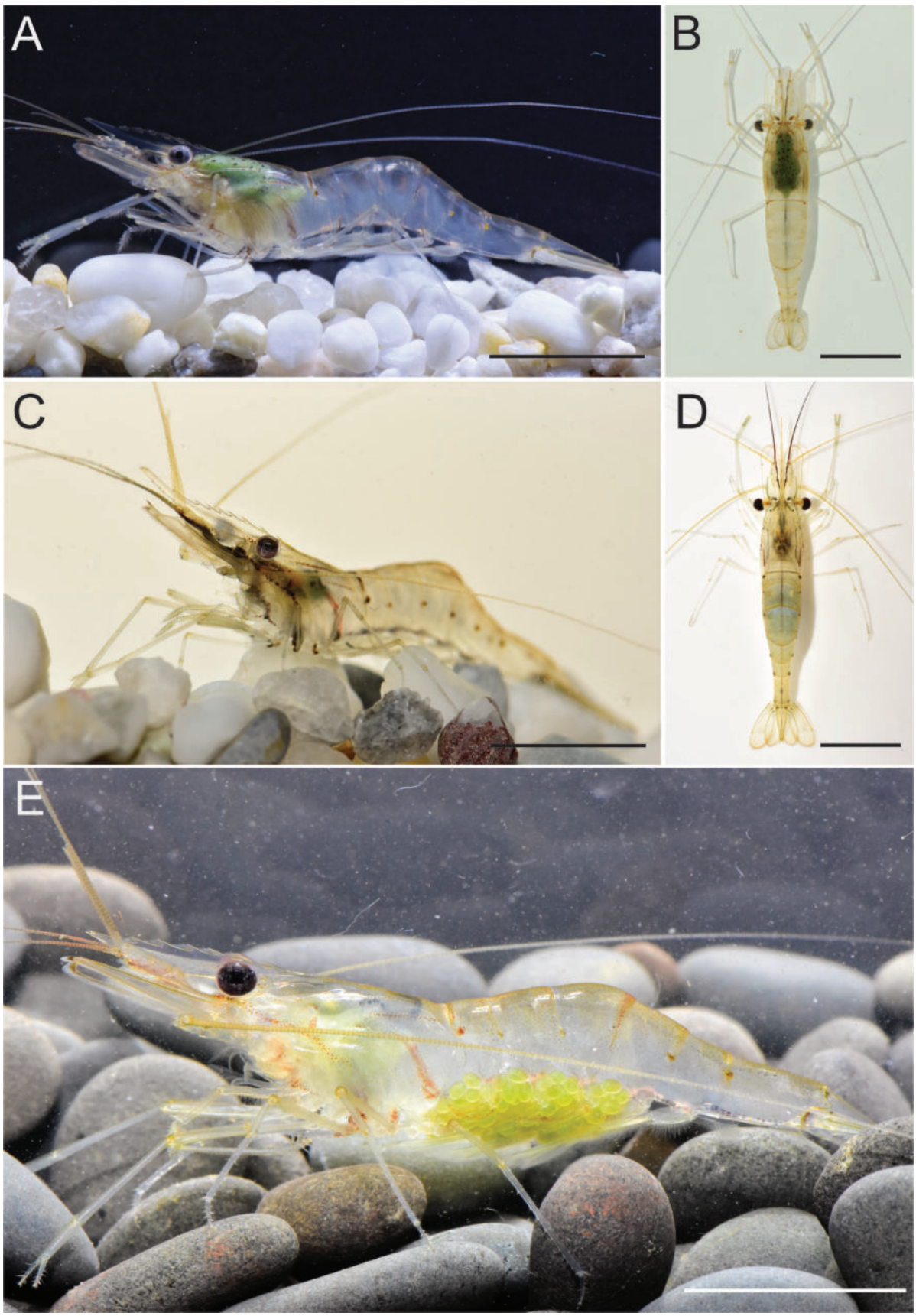


**Figure 3.** Living habit of specimens of three *Macrobrachium* species **A, B** *M. lanchesteri* from Bang Khiat, Singhanakhon, Songkhla, Thailand **C, D** *M. rostrolevatus* sp. nov. from Bueng Khong Long, Bueng Kan, Thailand **E** *M. panhai* sp. nov. from Sri Nakarin Dam, Tha Kradan, Si Sawat, Kanchanaburi, Thailand. Scale bars: 1 cm.

segment. Stylocerite projection sharp, reaching beyond basal segment. Antennal spine sharp situated below orbital margin. Hepatic spine slightly larger than antennal spine, positioned posteriorly and lower than antennal spine. Scaphocerite with straight margin, distolateral tooth sharp and not reaching end of lamella. Epistome bilobed (Fig. 4C). Branchiostegal suture starting from carapace margin to behind hepatic spine. Carapace surface smooth.

***First pereiopods.*** Long and slender, reaching end of scaphocerite. Fingers as long as palm, tips with fine setae. Series of setae present at anterior inner part of palm. Carpus slightly longer than merus. Distal articulation of carpus with series of fine setae. Ischium shorter than merus. Scattered setae present on all segments.

***Second pereiopods*** (Fig. 4D). Long and slender, similar in form and exceeding scaphocerite. Fingers subcylindrical covered with scattered setae. Palm 1.1–1.4× longer than fingers. Fingers with translucent razor edge present anteriorly and one or two tiny teeth on proximal quarter of cutting edges. Tip of fingers crossed and covered by fine setae (Fig. 4E). Carpus cylindrical shape and articulation margin expanded. Carpus 1.3–1.5× longer than chela. Merus subcylindrical. Carpus 1.1–2× longer than merus. Scattered short setae present on all segments.

***Third pereiopods*** (Fig. 4F). Long and slender, reaching end of scaphocerite. Dactylus short and curved distally. Propodus with 4–8 pairs of spines along inferior-lateral margin and fine setae at distal articulation, 2× longer than dactylus. Propodus 2× longer than carpus. Short setae present on all segments.

***Fourth and fifth pereiopods.*** Long and slender, exceeding scaphocerite. Propodus of fourth pereiopods with 5–10 pairs of spines distributed along its length, 2× longer than dactylus. Propodus slightly longer than merus. Ischium shorter than merus. Propodus with fine setae at distal articulation. Scattered short setae present on all segments. Propodus of fifth pereiopods with 7–13 pairs of spines distributed along its length and fine setae at distal articulation. Propodus 2× longer than carpus. Propodus as long as merus. Scattered short setae present on all segments.

***Thoracic sternum.*** Fourth and fifth thoracic sternites with transverse plate. Sixth and seventh thoracic sternites smooth. Eighth thoracic sternite with or without acute median process.

***Pleon.*** Smooth. All pleonal sternites with transverse ridge. First and second pleonal sternites usually with small median process. Third and fourth pleonal sternites smooth. Fifth pleonal sternite with triangular ridge. Preanal carina present, obtuse ridge developed without spine or setae. Ventral margin of pleural tergum with small setae.

***Telson*** (Fig. 4G). Tapered posteriorly, protruding point on middle margin with lateral spines and few fine setae. Inner spines longer than outer spines. Dorsal surface with two pairs of small spines similar in size.

***Uropods*** (Fig. 4G). Uropodal diaeresis with inner movable spine, usually shorter than outer angle. Exopods longer than endopods.

**Remarks.** The specimen collected in this study generally agrees with the original description in Lanchester (1902), and a subsequent description of the lectotype provided by Chong and Khoo (1988). Previous studies reported that male specimens tended to display the sexual dimorphism with a large body size, tomentose fingers, and minute spinules on all segments (except fingers) of second pereiopods. In this study, only one large male specimen, collected from Loei Province, Thailand, exhibits this characteristic. Typically, both male and

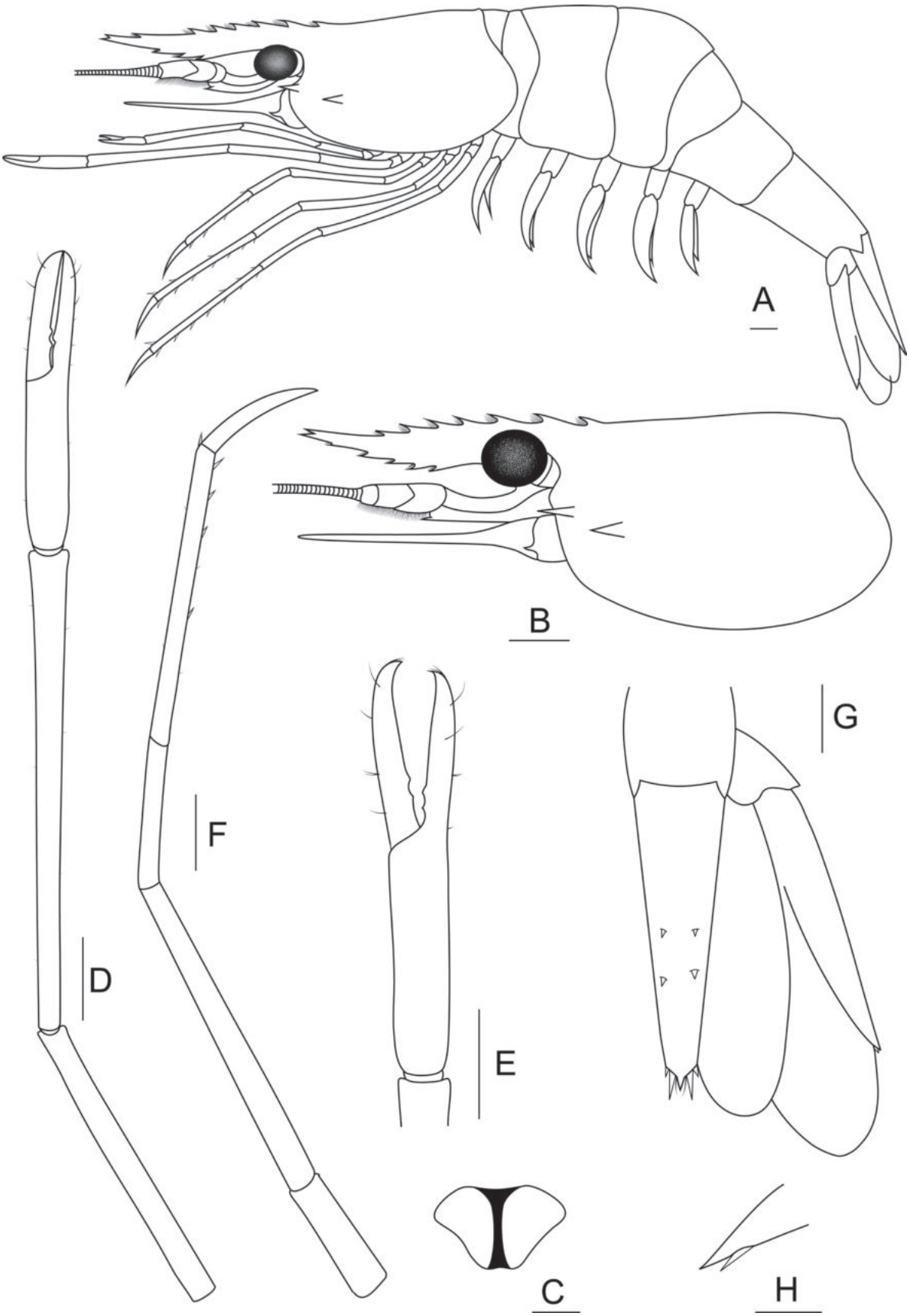


**Figure 4.** Morphological characteristics of *Macrobrachium lanchesteri* (**A** female topotype MUMNH MP00218 **B–H** female topotype MUMNH MP00216.1) **A** lateral view **B** carapace **C** epistome **D** second pereiopod **E** teeth between fingers **F** third pereiopod **G** uropod and **H** movable spine at uropodal diaeresis. Scale bars: 1 mm.

female specimens possess fine setae on fingers and scattered setae on surface of second pereiopods. Furthermore, this study also observed two variable characters occurring on the second pereiopods. Firstly, the proportional length and form of second pereiopods were found to be variable in specimens from Krabi population. Their second pereiopods are shown to be prominently long and robust, similar to those of *M. sintangense* (a common riverine species). The palm margin is laterally inflated and slightly shorter than fingers, and the chela slightly longer than the carpus. Additionally, Chong and Khoo (1988) reported the presence of two tiny teeth on the basal portion of cutting edges of fingers in *M. lanchesteri* as a diagnostic character. In this study, one or two tiny teeth were present on the cutting edges of fingers and vary among *M. lanchesteri* populations. Historically, *M. lanchesteri* was noted to resemble several other species including *M. idae*, *M. peguense* (see under remarks of *M. panhai* sp. nov.), *M. sankollii* Jalihal, Shenoy & Sankolli, 1988, *M. unikarnatakae* Jalihal, Shenoy & Sankolli, 1988, and *M. sintangense*. Further phylogenetic relationships and phylogenetic placement of aforementioned taxa should be tested to elucidate and verify their taxonomic identities.

*Macrobrachium lanchesteri* has a wide distribution across mainland Southeast Asia and southern China. This species can live in various freshwater ecosystems by inhabiting aquatic vegetation in stagnant freshwater habitats such as ponds, lakes, and paddy fields.

***Macrobrachium panhai* Chaowvieng & Siriwut, sp. nov.**
https://zoobank.org/42A0A555-C9CF-4DBF-B707-ED49A971F523
Figs 3E, 5

**Material examined. *Holotype*: Kanchanaburi** • Ovigerous ♀ from Sri Nakarin Dam, Tha Kradan, Si Sawat; 14°24'12.1"N, 99°07'24.7"E; CUMZ MP00302. ***Paratypes*:** 7 ♀♀, 8 ovigerous, 6 ♂♂ from the same locality of holotype; MUMNH MP00303.

**Additional material. Chiang Mai** • 6 ♀♀, 6 ovigerous, Nong Han, San Sai; 18°53'44.9"N, 99°01'05.7"E; MUMNH MP00304. • 1 ♀, Ping, Ki Lek, Mae Taeng; 19°04'37.0"N, 98°56'59.8"E; MUMNH MP00305. **Nan** • 1 ♀, Ban Na Bua, Nong Daeng, Mae Charim; 18°45'12.8"N, 101°00'39.7"E; MUMNH MP00306. **Uttaradit** • 1 ♂, Klong Tron, Ban Bueng Pra Kot, Thong Sang Khan; 17°35'39.1"N, 100°29'10.3"E; MUMNH MP00307. • 3 ♀♀, Klong Tron, Nam Khai, Nam Pat; 17°36'15.3"N, 100°32'15.3"E; MUMNH MP00308. **Buengkan** • 1 ♀, Klong Hi, Sang, Seka; 17°54'17.9"N, 103°57'12.0"E; MUMNH MP00351. **Nong Khai** • 2 ♀♀, Nam Suai, Song Hong, Mueang Nong Khai; 17°45'01.1"N, 102°51'00.5"E; MUMNH MP00309. **Ubon Ratchathani** • 1 ♀, 1 ovigerous, 2 ♂♂, Mekong River, Khong Chiam; 15°19'10.3"N, 105°29'53.2"E; MUMNH MP00310. • 1 ♀, 1 ovigerous, Sae Hua Maew Waterfall, Nong Saeng Yai, Khong Chiam; 15°20'12.2"N, 105°24'02.6"E; MUMNH MP00311. **Phitsanulok** • 6 ♀♀, 1 ♂, Ban Bo, Wang Nok Aen, Wang Thong; 16°51'13.6"N, 100°36'43.2"E; MUMNH MP00312. **Phetchabun** • 1 ♀, Ang Kep Nam Nam Khuen Nam Lang, Wang Hin, Wang Pong; 16°25'19.8"N, 100°48'48.2"E; MUMNH MP00313. **Nakhon Nayok** • 2 ovigerous, Ban Na, Pa Kha, Ban Na; 14°17'11.2"N, 101°04'13.7"E; MUMNH MP00314. **Suphan Buri** • 10 ♀♀, 8 ovigerous, 4 ♂♂, Ban Chara Mai, Ban Kong, U Thong; 14°34'51.0"N, 99°52'04.7"E; MUMNH MP00315. **Sa Kaeo** • 1 ovigerous, Huai Yang, Ta Phraya; 14°00'46.5"N, 102°38'37.0"E; MUMNH MP00316. **Chachoengsao** • 2 ♀♀, 3 ovigerous, Nong Prue Mai Kaeo,

Wang Yen, Plaeng Yao; 13°33'31.5"N, 101°17'44.0"E; MUMNH MP00317. **Rayong** • 12 ovigerous, Khao Chuk, Kong Din, Klaeng; 12°51'32.0"N, 101°46'12.0"E; MUMNH MP00318. • 7 ♀♀, 11 ovigerous, 12 ♂♂, Koh Phasuk, Makham, Makham, Chanthaburi; MUMNH MP00319. **Trat** • 1 ♀, 2 ovigerous, Huai Raeng, Dan Chumphon, Bo Rai;12°23'48.4"N, 102°39'15.1"E; MUMNH MP00320. • 1 ♀, 2 ovigerous, Ang Kep Nam Dan Chumphon, Dan Chumphon, Bo Rai; 12°27'45.4"N, 102°38'24.6"E; MUMNH MP00321. **Phetburi** • 1 ♂, Klong Prachan, Yang Nam Klat Tai, Nong Ya Plong; 13°06'33.27"N, 99°43'22.75"E; MUMNH MP00322.

**Diagnosis.** Rostrum straight proximally and slightly upward distally. Rostrum length reaching beyond end of antennular peduncle and exceeding the scaphocerite. Rostral formula: 8–12/3–6 teeth including two or three distal teeth with small gap separate from rest. Carapace smooth. Epistome bilobed. First pereiopods reaching end of scaphocerite. Second pereiopods thin and long, similar in form and equals in length, exceeding scaphocerite. Fingers covered with scattered setae, slightly shorter than palm. Translucent razor edge present anteriorly between fingers and no teeth on inner side of cutting edges. Carpus cylindrical shape and articulation margin expanded. Carpus 1.5× longer than chela. Merus subcylindrical. Carpus 1.5× longer than merus. Third pereiopods thin and long, reaching end of scaphocerite. Dactylus curved distally with short setae. Propodus 2× longer than dactylus. Propodus with three or four pairs of spines and fine setae present scarcely on articulation margin. Propodus 2× longer than carpus. Sixth to eighth thoracic sternites smooth. First and second pleonal sternites with small median process or smooth. Third and fourth pleonal sternites smooth. Fifth pleonal sternite with triangular ridge. Uropodal diaeresis with inner movable spine slightly longer than outer angle.

**Composite description (holotype in parentheses).** ***Rostrum*** (Fig. 5B). Straight or proximal convex and slightly distal upward. Rostrum length exceeding end of antennular peduncle and slightly exceeding scaphocerite (rl 7.32 mm). Dorsal margin with 8–12 (10) teeth including two or three (3) teeth distally with small gap from rest. Postorbital margin with one or two (1) teeth, reaching one-third of carapace length. First dorsal tooth positioned slightly behind hepatic spine. Ventral margin with 3–6 (4) teeth, starting from middle to distal margin. Short setae present between rostral teeth.

***Cephalon*** (Fig. 5B). Eye well developed. Ocular beak without laterally expanded tip. Cornea longer and broader than stalk. Postantennular carapace margin rounded. Cornea osculum longer than stalk. Antennular peduncle longer than wide, with fine setae. Basal segment short, second segment shorter than third segment. Stylocerite projection sharp, reaching beyond basal segment. Antennal spine sharp, situated below orbital margin. Hepatic spine slightly larger than antennal spine, positioned posteriorly and lower than antennal spine. Scaphocerite with straight margin, distolateral tooth sharp and not reaching end of lamella. Epistome bilobed (Fig. 5C). Branchiostegal suture beginning at carapace margin to behind hepatic spine. Carapace surface smooth (cl 5.76 mm).

***First pereiopods.*** Thin and long, reaching end of scaphocerite. Fingers as long as palm, tips with fine setae. Series of setae present on anterior inner part of palm. Carpus slightly longer than merus. Distal articulation of carpus with series of fine setae. Ischium shorter than merus. Scattered setae present on all segments.

***Second pereiopods*** (Fig. 5D). Thin and long, similar in form and exceeding scaphocerite. Fingers subcylindrical covered with scattered setae. Palm 1.1–1.5×

longer than fingers (Fin 1.06: Pal 1.39 mm). Fingers with translucent razor edges present anteriorly and cutting edge between fingers smooth. Tip of fingers crossed and covered by fine setae (Fig. 5E). Carpus cylindrical shape and articulation margin expanded. Carpus 1.2–2× longer than chela (Che 2.45: Car 4.22 mm). Merus subcylindrical. Carpus 1.2–1.7× longer than merus (Mer 2.85: Car 4.22 mm). Ischium as long as merus. Scattered short setae present on all segments.

***Third pereiopods*** (Fig. 5F). Thin and slender, reaching end of scaphocerite. Dactylus short and curved distally. Propodus 2× longer than dactylus. Propodus with three or four pairs of spines along inferior-lateral margin and fine setae at distal articulation, 2× longer than carpus. Ischium shorter than carpus. Scattered short setae present on all segments.

***Fourth and fifth pereiopods.*** Long and slender, exceeding scaphocerite. Propodus of fourth pereiopods with 3–6 (4) pairs of spines distributed along its length, 2.5× longer than dactylus. Propodus as long as merus. Ischium shorter than merus. Propodus with fine setae at distal articulation. Scattered short setae present on all segments. Propodus of fifth pereiopods with 4–8 pairs of spines distributed along its length and fine setae at distal articulation. Propodus 2.5× longer than carpus. Propodus as long as merus. Scattered short setae present on all segments.

***Thoracic sternum.*** Fourth and fifth thoracic sternites with moderately transverse plate without median process, and seventh thoracic sternite smooth. Eighth thoracic sternite usually smooth.

***Pleon.*** Smooth. All pleonal sternites with transverse ridges. First and second pleonal sternites with or without small median processes. Third and fourth pleonal sternites smooth. Fifth pleonal sternite with triangular ridge. Preanal carina present, obtuse ridge developed without spine or setae. Ventral margin of pleural tergum with small setae.

***Telson*** (Fig. 5G). Tapered posteriorly, protruding point on middle margin with lateral spines and few fine setae. Inner spines longer than outer spines. Dorsal surface with two pairs of small spines, similar in size.

***Uropods*** (Fig. 5G). Uropodal diaeresis with inner movable spine, as long as or slightly longer than outer angle. Exopods longer than endopods.

**Etymology.** The specific name *panhai* is dedicated to Prof. Dr. Somsak Panha, a taxonomist from Faculty of Science, Chulalongkorn University, Thailand well known for his remarkable contributions and endorsement to the study of invertebrate fauna in Thailand.

**Distribution.** This species is distributed in the Chao Phraya and Mekong River Basins, Thailand.

**Remarks.** *Macrobrachium panhai* sp. nov. differs from *M. lanchesteri* s. str. due to having the rostral formula with 8–12/3–6 teeth (vs 6–10/1–6 teeth in *M. lanchesteri*). Movable spine at uropodal diaeresis is slightly longer than outer angle (vs movable spine is shorter in *M. lanchesteri*). Third propodus has 3–6 pairs of spines (vs 4–8 pairs of spines in *M. lanchesteri*). The teeth between fingers of second pereiopods are absent (vs 1 or 2 teeth on fixed and movable finger in *M. lanchesteri*). The ratio between rostrum and carapace length is 0.89–1.53 (vs 0.78–1.14 in *M. lanchesteri*) and the ratio between chela and carpus is 1.2–2.08 (vs 1.15–1.72 in *M. lanchesteri*). *M. panhai* sp. nov. occasionally co-exists with *M. lanchesteri* in the Chao Phraya and Mekong River Basins.

This new species also differs from *M. peguense* sensu Tiwari (1952) by processes of rostral formula 8–12/3–6 teeth (vs 6–9/2–4 teeth in *M. peguense*).

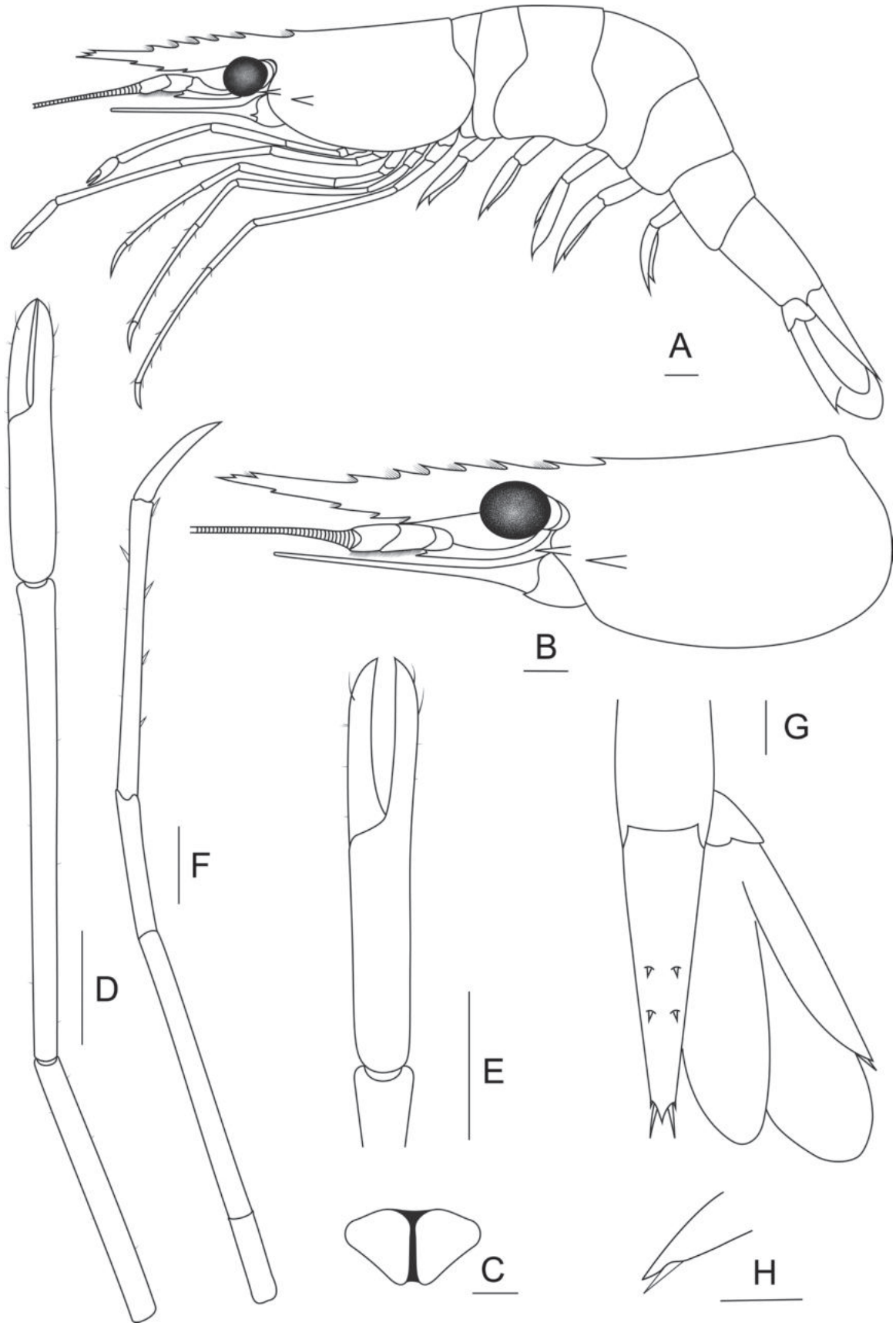


**Figure 5.** Morphological characteristics of *Macrobrachium panhai* sp. nov. (**A, F** ovigerous female paratype MUMNH MP00303 **B–E, G–H** ovigerous female holotype CUMZ MP00302) **A** lateral view **B** carapace **C** epistome **D** second pereiopod **E** teeth between fingers **F** third pereiopod **G** uropod and **H** movable spine at uropodal diaeresis. Scale bars: 1 mm.

Second pereiopods had palms shorter than half of carpus (vs palm slightly more than half of carpus in *M. peguense*). Propodus of third pereiopods are 2× longer than dactylus (vs 3 in *M. peguense*). Dorsal surface of telson is without depression (vs longitudinal depression in *M. peguense*). Movable spine at uropodal diaeresis is slightly longer than outer angle (vs movable spine is shorter in *M. peguense*). Cai and Ng (2002) also mentioned that the egg size can be used to distinguish *M. peguense* and *M. lanchesteri* group (1.15–1.5 × 1.6–2.1 mm and 0.6–0.7 × 0.8–1 mm, respectively). Currently, the distribution range of *M. peguense* was found only from Myanmar.

***Macrobrachium rostrolevatus*** **Chaowvieng & Siriwut, sp. nov.**
https://zoobank.org/58FE013B-93A1-43A0-9A96-7F45FBD759F9
Figs 3C, D, 6

**Material examined.** ***Holotype*****: Bueng Kan** • Ovigerous ♀ from Bueng Khong Long; 17°59'59.1"N, 104°01'06.9"E; CUMZ MP00323. ***Paratypes*****:** 10 ♀♀, 13 ovigerous, 7 ♂♂ from the same locality of holotype; MUMNH MP00324.

**Additional material. Nong Khai** • 3 ♀♀, 2 ♂♂, Nam Suai, Song Hong, Mueang Nong Khai; 17°45'01.1"N, 102°51'00.5"E; MUMNH MP00325. **Udon Thani** • 10 ♀♀, 4 ovigerous, 2 ♂♂, Si Charoen, Ban That, Phen; 17°42'47.7"N, 102°50'57.4"E; MUMNH MP00326. • 3 ♀♀, 1 ovigerous, Nam Khong, Thap Kung, Nong Saeng; 17°10'01.5"N, 102°46'03.2"E; MUMNH MP00327. **Loei** • 1 ♀, Tha Yang, Phu Kradueng; 16°53'38.3"N, 101°52'53.1"E; MUMNH MP00354. **Nakhon Phanom** • 2 ♀♀, 3 ♂♂, Klong Kam, Na Khu, Na Kae; 16°57'42.3"N, 104°31'33.2"E; MUMNH MP00328. • 1 ♀, Huai Saab, Sam Phong, Si Songkhram; 17°43'59.2"N, 104°09'19.9"E; MUMNH MP00329. **Sakon Nakhon** • 1 ♀, Nam Chan, Akat, Akat Amnuai; 17°35'46.1"N, 104°00'21.6"E; MUMNH MP00330. • 1 ♂, Klong Lak, Chiang Khruea, Mueang Sakon Nakhon; 17°15'33.7"N, 104°07'00.1"E; MUMNH MP00331. • 1 ♂, Klong Un, Khok Phu, Phu Phan; 17°00'22.1"N, 103°54'50.5"E; MUMNH MP00332. • 1 ♀, Tha Rae, Mueang Sakon Nakhon; 17°15'08.5"N, 104°09'32.0"E; MUMNH MP00352. **Kalasin** • 1 ♀, 2 ♂♂, Huai Sathot, Kham Bong, Huai Phueng; 16°41'32.6"N, 103°51'20.1"E; MUMNH MP00333. • 18 ♀♀, 2 ovigerous, 10 ♂♂, Bueng Aram, Khlong Kham, Yang Talat; 16°24'21.8"N, 103°20'26.4"E; MUMNH MP00334. **Khon Kaen** • 10 ♀♀, 2 ♂♂, Kong Kaeo Reservoir, Si Bun Rueang, Chonnabot; 16°05'47.2"N, 102°37'05.4"E; MUMNH MP00335. **Maha Sarakham** • 3 ♂♂, Ban Tha Tum, Mueang Maha Sarakham; 16°10'55.8"N, 103°27'10.6"E; MUMNH MP00336. **Yasothon** • 8 ♀♀, 11 ♂♂, Se Bai, Sawat, Loeng Nok Tha; 16°10'13.5"N, 104°32'21.1"E; MUMNH MP00337. • 11 ♀♀, 7 ovigerous, 12 ♂♂, Klong Wai, Fa Yat, Maha Chana Chai; 15°30'59.5"N, 104°15'12.3"E; MUMNH MP00338. **Si Sa Ket** • 13 ♀♀, 16 ovigerous, 2 ♂♂, Bueng Bun Local Market, Bueng Bun; 15°19'18.6"N, 104°03'01.2"E; MUMNH MP00339. • 2 ♀♀, Huai Khayung, Thung Yai, Kantharalak; 14°34'42.1"N, 104°38'48.1"E; MUMNH MP00340. • 1 ovigerous, Suk San, Phran, Khun Han; 14°35'27.7"N, 104°29'29.3"E; MUMNH MP00353. **Surin** • 3 ♀♀, Klong Thap Than, Yang Sawang, Rattanaburi; 15°16'55.4"N, 103°58'38.1"E; MUMNH MP00341. • 28 ♀♀, 9 ♂♂, Mun River, Tha Tum; 15°19'53.3"N, 103°38'34.9"E; MUMNH MP00342. • 12 ♀♀, 13 ♂♂, Mun River, Krapho, Tha Tum; 15°17'38.5"N, 103°30'42.4"E; MUMNH MP00343. • 3 ♀♀, Ban Kut Chum Saeng, Yawuek, Chumphon Buri; 15°18'57.6"N,

103°15'24.2"E; MUMNH MP00344. **Buri Ram** • 2 ovigerous, 1 ♂, Lam Chi, Non Charoen, Ban Kruat; 14°26'43.9"N, 103°12'55.8"E; MUMNH MP00345. **Nakhon Ratchasima** • 1 ♀, 7 ♂♂, Sathaet, Krabueang Nok, Mueang Yang; 15°27'35.2"N, 102°59'46.6"E; MUMNH MP00346. • 4 ♀♀, 3 ♂♂, Sema, Sung Noen; 14°55'11.0"N, 101°47'53.5"E; MUMNH MP00347. • 3 ♀♀, 2 ♂♂, Sawai Riang, Than Prasat, Non Sung; 15°16'13.3"N, 102°22'37.0"E; MUMNH MP00348. • 1 ♂, Lam Takhong, Mu Si, Pak Chong; 14°33'00.7"N, 101°27'34.1"E; MUMNH MP00349.

**Diagnosis.** Rostrum long and thin, proximal half straight and uplifted distal half. Rostrum length reaching beyond end of antennular peduncle and prominently exceeding scaphocerite. Rostral formula: 6–11/4–9 teeth including 2–4 teeth distally with large gap from rest. Apical teeth usually present with trifid. Carapace smooth. Epistome bilobed. First pereiopods reaching end scaphocerite. Second pereiopods thin and long, similar in form and length, exceeding end of scaphocerite. Fingers covered with scattered setae with translucent razor edge present anteriorly between fingers and one tooth on proximal quarter of cutting edges. Palm 1.25× longer than fingers. Carpus cylindrical shape and articulation margin expanded. Carpus 1.5–2× longer than chela. Merus subcylindrical. Carpus 1.5× longer than merus. Third pereiopods thin and long, slightly exceeding scaphocerite. Dactylus curved distally with short setae. Propodus 2× longer than dactylus. Propodus with 3–6 pairs of spines distributed along its length and fine setae at its articulation. Propodus 2× longer than carpus. Sixth to eighth thoracic sternites smooth. First and second pleonal sternites with small median process. Third and fourth pleonal sternites smooth. Fifth pleonal sternite with triangular ridge. Uropodal diaeresis with inner movable spine slightly longer than outer angle.

**Composite description (holotype in parenthesis).** ***Rostrum*** (Fig. 6B). Tapered and long, proximal half of rostrum straight and uplifted distally. Rostrum length exceeding end of antennular peduncle and distinctly exceeding scaphocerite (rl 10.34 mm). Dorsal margin with 6–11 (9) teeth including 2–4 (4) teeth distally separated from rest. Apical teeth usually present with trifid. Postorbital margin with 1 or 2 (1) teeth reaching one-third of carapace length. First dorsal tooth positioned slightly behind hepatic spine. Ventral margin with 4–9 (7) teeth, starting from middle to distal margin. Short setae present between rostral teeth.

***Cephalon*** (Fig. 6B). Eye well developed; ocular beak without laterally expanded tip. Cornea longer and broader than stalk. Postantennular carapace margin rounded. Cornea osculum longer than stalk. Antennular peduncle longer than wide, with fine setae. Basal segment short, second segment being shorter than third segment. Stylocerite projection sharp, reaching beyond basal segment. Antennal spine sharp, situated below orbital margin. Hepatic spine slightly larger than antennal spine, positioned posteriorly and lower than antennal spine. Scaphocerite with straight margin, distolateral tooth sharp and not reaching end of lamella. Epistome bilobed (Fig. 6D). Branchiostegal suture starting from carapace margin to behind hepatic spine. Carapace surface smooth (cl 7.14 mm).

***First pereiopods.*** Long and slender, reaching end of scaphocerite. Fingers as long as palm, tips with fine setae. Series of setae present at anterior inner part of palm. Carpus slightly longer than merus. Distal articulation of carpus with series of fine setae. Ischium shorter than merus. Scattered setae present on all segments.

***Second pereiopods*** (Fig. 6E). Long and slender, similar in form and distinctly exceeding the scaphocerite. Fingers subcylindrical covered with scattered

setae. Palm 1.2–1.7× longer than fingers (Fin 1.47: Pal 1.84 mm). Fingers with translucent razor edge present anteriorly and one tooth on cutting edges. Tip of fingers crossed and covered by fine setae (Fig. 6F). Carpus cylindrical shape and articulation margin expanded. Carpus 1.5–2.0× longer than chela (Che 3.31: Car 6.19 mm). Merus subcylindrical. Carpus 1.3–1.6× longer than merus (Mer 3.99: Car 6.19 mm). Ischium as long as merus. Scattered short setae present on all segments.

***Third pereiopods*** (Fig. 6G). Long and slender, slightly exceeding scaphocerite. Dactylus curved distally with short setae. Propodus 2× longer than dactylus (Dac 1.19: Pro 3.94 mm). Propodus with 3–6 (4) pairs of spines along inferior-lateral margin and fine setae at distal articulation, 2× longer than carpus (Car 1.83: Pro 3.94 mm). Ischium shorter than carpus. Scattered short setae present on all segments.

***Fourth and fifth pereiopods.*** Long and slender, exceeding scaphocerite. Propodus of fourth pereiopods with 4–7 (5) pairs of spines distributed along its length, 2× longer than dactylus. Propodus slightly shorter than merus. Ischium shorter than merus. Propodus with fine setae at distal articulation. Scattered short setae present on all segments. Propodus of fifth pereiopods with 4–10 pairs of spines (holotype damaged) distributed along its length and fine setae at distal articulation. Propodus 2.5× longer than carpus. Propodus as long as merus. Scattered short setae present on all segments.

***Thoracic sternum.*** Fourth and fifth thoracic sternites with moderately transverse plate. Sixth to eighth thoracic sternites usually smooth.

***Pleon.*** Smooth. All pleonal sternites with transverse ridge. First and second pleonal sternites with or without median process. Third and fourth pleonal sternites smooth. Fifth sternite with triangular ridge. Preanal carina present, obtuse ridge developed without spine or setae. Ventral margin of pleural tergum with small setae.

***Telson*** (Fig. 6H). Tapered posteriorly, protruding point on middle margin with lateral spines and few fine setae. Inner spines longer than outer spines. Dorsal surface with two pair of small spines, similar in size.

***Uropods*** (Fig. 6H). Uropodal diaeresis with inner movable spine, as long as or slightly longer than outer angle. Exopods longer than endopods.

**Etymology.** The specific epithet *rostrolevatus* is from the Latin compound words *rostro*, for rostrum, and *levatus*, referring to lifted.

**Distribution.** This species is distributed in freshwater basins of Khorat Plateau, Northeast Thailand.

**Remarks.** *Macrobrachium rostrolevatus* sp. nov. differs from *M. lanchesteri* s. str. based on the presence of single tooth on movable and fixed fingers of second pereiopods (vs 1 or 2 teeth on movable and fixed fingers in *M. lanchesteri*), movable spine at uropodal diaeresis slightly longer than the outer angle (vs shorter than outer angle in *M. lanchesteri*), and the presence of 3–6 pairs of spines on propodus of third pereiopods (vs 4–8 pairs of spines in *M. lanchesteri*). This new species also differs from *M. villosimanus* sensu Tiwari (1949) and *M. rosenbergii* sensu De Man (1879) by having 6–11/4–9 rostral teeth (vs 12–14/7–10 rostral teeth in *M. villosimanus*; 9–13/10–15 rostral teeth in *M. rosenbergii*). The second pereiopods are smooth and covered with fine setae (vs spinules in entire cheliped, movable finger densely pubescent and fixed finger sparsely pubescent in *M. villosimanus*; coarse velvet hairs on movable

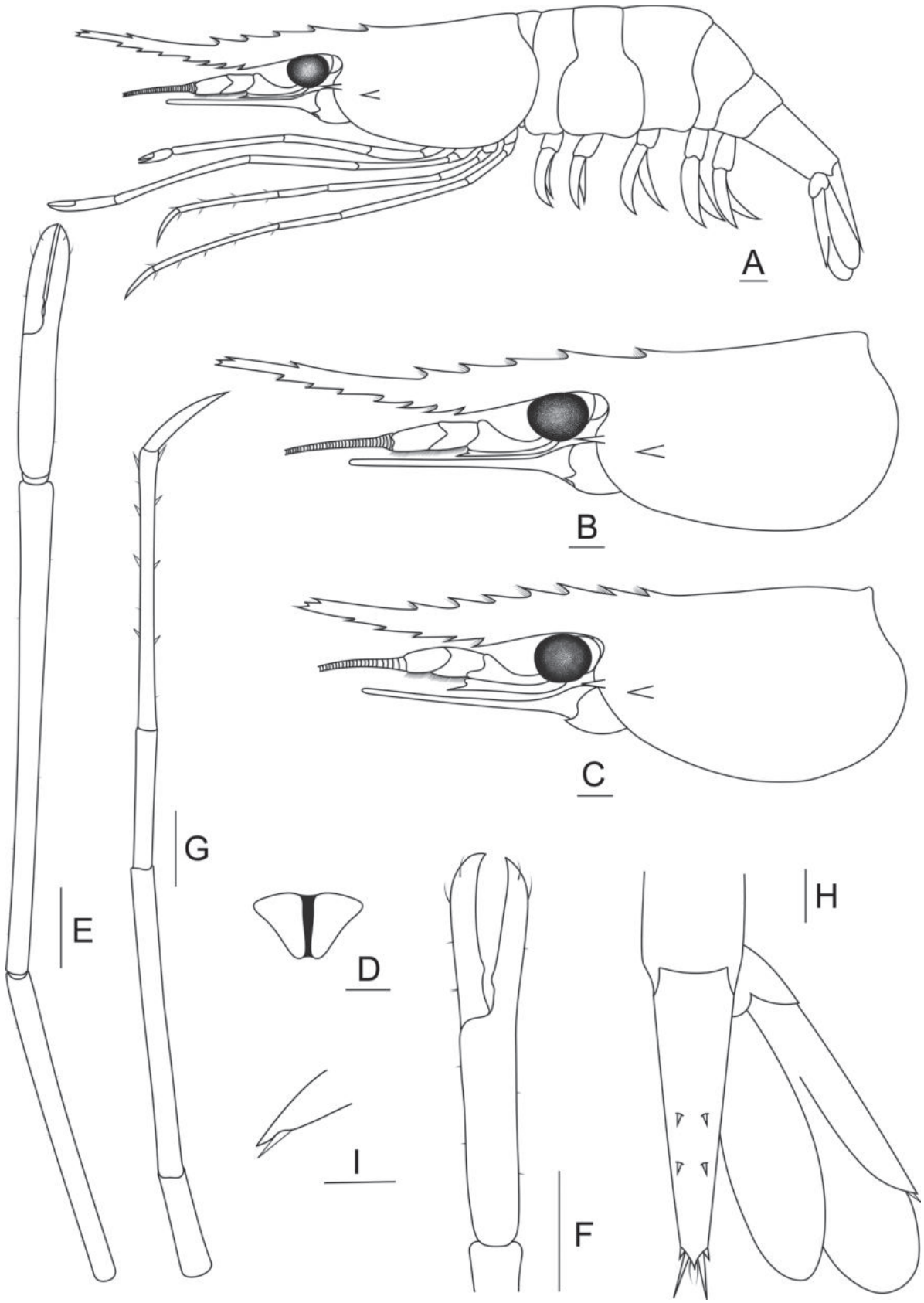


**Figure 6.** Morphological characteristics of *Macrobrachium rostrolevatus* sp. nov. (**A, B, D–I** ovigerous female holotype CUMZ MP00323 **C** ovigerous female specimen MUMNH MP00338.1) **A** lateral view **B** carapace **C** rostral variation **D** epistome **E** second pereiopod **F** teeth between fingers **G** third pereiopod **H** uropod and **I** movable spine at uropodal diaeresis. Scale bars: 1 mm.

finger except its tip, and fixed finger covered with numerous short spines in *M. rosenbergii*). Moreover, this new species differs from *M. lamarrei* sensu H. Milne Edwards (1837) by processes 6–11/4–9 rostral teeth (vs 6–11/5–9 rostral teeth in *M. lamarrei*). The movable spine at uropodal diaeresis is slightly longer than outer angle (vs without movable spine in *M. lamarrei*). Further description of *M. lamarrei* was provided by Cai and Ng (2002). In addition, *M. rostrolevatus* sp. nov. is present only in freshwater basins on the Khorat Plateau, and lives in various habitats such as lakes, ponds, and river whereas *M. villosimanus*, *M. rosenbergii* and *M. lamarrei* typically inhabit brackish water territory.

*Macrobrachium rostrolevatus* sp. nov. exhibits phenotypic plasticity in rostral shape. The population in a lentic habitat such as a pond, paddy field and lake have an upcurved on distal half of rostrum. On the other hand, some populations have slightly convex at basal and upturned distally with a smaller gap between distal and proximal teeth of rostrum (Fig. 6C). The taxonomic discrimination based on rostrum form in genus *Macrobrachium* is cautioned due to controversial situation found in this study. Additionally, the uncertain identity found from Thai *Macrobrachium* specimens was also mentioned in previous records. For example, Naiyanetr (1998) reported *M. palaemonoides* Holthuis, 1950 [= *Tenuipedium palaemonoides* in Wowor and Ng (2010)] from Surin Province. Re-examination of the collection by Cai et al. (2004) placed those specimens back under a typical variation of *M. lanchesteri* and mentioned that *T. palaemonoides* s. str. was known only from the original type locality in the west coast of Sumatra. The unique characteristics of *T. palaemonoides* are shown to be distinct from *Macrobrachium* such as its long branchiostegal groove, the second pereiopod is as wide as first pereiopod and the fourth and fifth pereiopods are longer than the second pereiopod.

## Key to species of *M. lanchesteri* and closely related species in Thailand

1 Rostrum upturned distally and exceeding the scaphocerite by one-third its length ..... **2**
– Rostrum straight and as long as scaphocerite ..... **4**
2 Fingers of second pereiopods without pubescence, fifth pereiopods with spiniform setae..... ***M. rostrolevatus* sp. nov.**
– Finger of second pereiopods with pubescence, fifth pereiopods with extremely spiniform setae ..... **3**
3 Rostrum with 7–10 ventral teeth, carpus subequal to chela, densely pubescent on movable finger and sparsely pubescent for fixed finger ..... ..... ***M. villosimanus***
– Rostrum with 10–14 ventral teeth, carpus longer than palm, coarse velvet hairs on movable finger except the tip..... ***M. rosenbergii***
4 Second pereiopods more robust and longer than body length, rostrum with 10–14 dorsal teeth..... ***M. sintangense* group**
– Second pereiopods slenderly without spine and shorter than body length, rostrum with 6–12 dorsal teeth..... **5**
5 Fingers with two tiny teeth on cutting edges, movable spine at uropodal diaeresis shorter than outer angle ..... ***M. lanchesteri* s. str.**
– Fingers without teeth on cutting edges, movable spine at uropodal diaeresis longer than outer angle ..... ***M. panhai* sp. nov.**

## Discussion

Morphological and genetic analyses revealed three distinct lineages (prior assumption as geographical variation of *M. lanchesteri*), which are recognised herein as *M. lanchesteri* s. str., *M. panhai* sp. nov., and *M. rostrolevatus* sp. nov. Previously, the taxonomic identity of *M. lanchesteri* s. l. was investigated based on the morphological examination of and reinvestigation of type specimens (Lanchester 1902; Chong and Khoo 1988). In this study, the clarification of species boundaries and phylogenetic positions were supplemented by molecular analyses. The phylogenetic position of *M. lanchesteri* is closely related to *M. rosenbergii*, although some morphological characteristics might appear similar to the *M. sintangense* species group. Current observation noted that a juvenile of *M. sintangense* and *M. lanchesteri* were morphologically overlapping. Ecologically, they commonly co-exist in several habitats such as riverbanks and lentic reservoirs in mainland Southeast Asia. Their life histories were supposedly influenced by a convergent evolutionary mechanism (Wowor et al. 2009), the same example as noted in other species with abbreviated larval development (ALD) such as *Macrobrachium* species: *M. platycheles* Ou & Yeo, 1995, *M. sundaicum* (Heller, 1862), and *M. malayanum* (Roux, 1935) (Murphy and Austin 2005). The independent lineages of ALD species were hypothesised as evidence of multiple invasions of marine ancestors (Liu et al. 2007; Murphy and Austin 2005; Wowor et al. 2009). To elucidate the effect of environmental conditions and feeding preferences altering morphological characteristics among coexisting species, comprehensive materials along an environmental gradient could be investigated. Additionally, *M. rosenbergii* showed distinctiveness in both morphological characters and a reproductive strategy different from *M. lanchesteri*. The life cycle of *M. lanchesteri* is completed typically in freshwater as opposed to *M. rosenbergii*, which had larval development and egg hatching occurring in brackish water. The close phylogenetic relationship between *M. lanchesteri* and *M. rosenbergii* seem to potentially derive from a common ancestor through evolutionary divergence processes.

The evidence of genetic divergence and composition differences in Thai invertebrate population are often documented between the lower and upper Isthmus of Kra regions. This evidence was sparsely seen in *M. lanchesteri* s. str. The same patterns of genetic divergence correlated to subregional populations were also detected in the widespread *M. spinipes* (Schenkel, 1902). This species shows a wide distribution range in the Indo-Australasian region due to a historical event during the last glacial maximum (De Bruyn and Mather 2007; Ng and Wowor 2011). Currently, the geographical distribution of *M. lanchesteri* in Southeast Asia seems to possibly include the introduction by human activities, particularly from local fishery-related activities such as in Sabah and Brunei Darussalam (Ng 1994; Wowor and Choy 2001). Thai *M. lanchesteri* s. str. failed to show a strong subregional pattern despite widespread distribution records, and a similar pattern was also observed in some freshwater gastropods collected from different parts of Thailand (Saijuntha et al. 2021). This might be the consequence of the commercial trade of aquatic plants in Thailand that accidentally introduced freshwater gastropods throughout the area. Contrastingly, *M. rostrolevatus* sp. nov. has a narrow distribution range and a dense population specifically found in the sub-basins of the Songkhram, Chi, and Mun rivers on the Khorat Plateau.

However, a comprehensive survey of the adjacent sub-basins along the Lower Mekong River Basin should be implemented to affirm its geographic range.

*Macrobrachium* prawns exhibit a vast variation of morphological characters, with several species demonstrating sexual dimorphism and morphological plasticity (Holthuis 1950; Dimmock et al. 2004; Short 2004). These phenomena increased the uncertainty of species boundaries and the complication of taxonomic discrimination criteria for various *Macrobrachium* species groups. Recent studies have employed tools, including molecular identification using mitochondrial gene datasets, to clarify and resolve taxonomically ambiguous situations (Liu et al. 2007; Carvalho et al. 2013; Castelin et al. 2017; Rossi et al. 2020; Saengphan et al. 2021). In this study, the mitochondrial genes 16S and COI showed potential to be useful for taxonomic clarification between closely related taxa and revealed the existence of cryptic species, as in the cases of *M. panhai* sp. nov. and *M. lanchesteri* s. str. Although *M. panhai* sp. nov. shares morphological characteristics with *M. lanchesteri*, genetic differentiation falls within the delimitation gap suggested by Siriwut et al. (2021). For this reason, the delimitation threshold based on inter- and intraspecific variations of *Macrobrachium* species would be considered an additional tool for cryptic fauna exploration and delineation of morphologically ambiguous groups of *Macrobrachium* prawns.

*Macrobrachium rostrolevatus* sp. nov. has different forms of rostrum that appear to be associated with habitat preference. The long and upcurved rostrum is prevalent in lentic habitats i.e., ponds and lakes, whereas the shorter and straight rostrum is dominant in lotic habitats like river tributaries. This rostral shape variability may indicate phenotypic plasticity, similar to observations in *M. australe* (Guérin-Méneville, 1838) and members of the genus *Caridina* H. Milne Edwards, 1837, where rostral shape is influenced by water current speed. In an area with fast-flowing current, the long rostrum can be more fragile and impede movement whereas the shorter, more robust, and straight rostrum might better resist the strong water current (Zimmermann et al. 2011; Mazancourt et al. 2017). Moreover, the variation in morphological traits influenced by environment was also found in *M. australiensis* Holthuis, 1950, an endemic Australian freshwater prawn and *M. nipponense* (De Haan, 1849), a widespread species in Taiwan (Dimmock et al. 2004; Chen et al. 2015). This study provided additional evidence that the diagnostic characters of *Macrobrachium* can be influenced by the environment. Therefore, morphological identification alone should be implemented carefully, especially for species with high morphological variability (Liu et al. 2007; Siriwut et al. 2020). The integration of other molecular markers such as nuclear markers and morphometric analysis could be used to further enhance the accuracy of taxonomic identification and phylogenetic relationships of *Macrobrachium* in the future.

## Acknowledgement

The authors are grateful to Y. Ponrujee, S. Pichsinee, K. Macharoenboon, and S. Klomthong for kind support during the field collections and data analysis. This research has been supported by Center of Excellence on Biodiversity (BDC), Office of Higher Education Commission (BDC-PG1-166011). We also sincerely thank the Department of Biology, Faculty of Science, Mahidol University for assistance and facilities. The authors are grateful to Daisy Wowor, Célio Magalhães, and anonymous reviewer for constructive comments and suggestions on the manuscript.

# Additional information

## Conflict of interest

The authors have declared that no competing interests exist.

## Ethical statement

This research has been conducted using animal care protocols approved by the Mahidol University-Institute Animal Care and Use Committee (MU-IACUC) under approval number MUSC66-026-656.

## Funding

This research has been supported by Center of Excellence on Biodiversity (BDC), Office of Higher Education Commission (BDC-PG1-166011).

## Author contributions

Conceptualization: WS. Funding acquisition: WS, CS. Investigation: AC, WS, EJ, CS. Resources: EJ, RC, RS. Supervision: WS. Writing – original draft: AC. Writing – review and editing: CS, EJ, RS, WS.

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## Data availability

All of the data that support the findings of this study are available in the main text.

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ZooKeys 1190: 195–212 (2024)
DOI: 10.3897/zookeys.1190.111583

Research Article

# Notes on species of *Talaus* Simon, 1886 (Araneae, Thomisidae) from China, with descriptions of two new species

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## Abstract

Taxonomic notes on the *Talaus* species from China are provided. Two new species, *T. yuyang* Yao & Liu, **sp. nov.** and *T. zhangjiangkou* Yao & Liu, **sp. nov.** are described and illustrated, and a further three species are redescribed based on their genitalic characters: *T. dulongjiang* Tang, Yin, Ubick & Peng, 2008, *T. niger* Tang, Yin, Ubick & Peng, 2008, and *T. sulcus* Tang & Li, 2010. The species *T. xiphosus* Zhu & Ono, 2007 is considered a junior synonym of *T. triangulifer* Simon, 1886 based on an examination of many recently collected female and male specimens from Guangxi Province, China. Diagnoses, detailed illustrations and a map of distributional records of the six treated species of *Talaus* in China are provided.

**Key words:** Crab spiders, new synonym, Southeast Asia, taxonomy

OPEN ACCESS

**Academic editor:** Ingi Agnarsson
**Received:** 24 August 2023
**Accepted:** 15 January 2024
**Published:** 29 January 2024

**ZooBank:** https://zoobank.org/793327D7-F62D-4343-AD8F-CC1F39AAA82B

**Citation:** Li C-z, Yao Y-b, Xiao Y-h, Xu X, Liu K-k (2024) Notes on species of *Talaus* Simon, 1886 (Araneae, Thomisidae) from China, with descriptions of two new species. ZooKeys 1190: 195–212. https://doi.org/10.3897/zookeys.1190.111583

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## Introduction

The crab spider genus *Talaus* Simon, 1886 comprises 13 species, of which 12 are endemic in Southeast Asia (WSC 2023); the validity of the other species, *T. limbatus* Simon, 1895 recorded from South Africa based on a juvenile specimen (Simon 1895), was doubted by Benjamin (2020). The genus has been neglected for the past ten years and only four species are known from Yunnan, Guangxi and Hainan in southwest and south China (Li and Lin 2016).

*Talaus* is currently non-monotypic with its type species, *Talaus triangulifer* Simon, 1886, described from Sumatra, Indonesia based on a female specimen (Benjamin et al. 2008; WSC 2023). Based on the combination of morphological characters of *T. beccarii* Benjamin, 2020, *T. nanus* Thorell, 1890, *T. oblitus* O. Pickard-Cambridge, 1899, *T. opportunus* (O. Pickard-Cambridge, 1873) and *T. xiphosus* Zhu & Ono, 2007, Benjamin (2020) re-defined the genus with the following features: the male palp with a retrolateral tibial apophysis and a ventral tibial apophysis, with a dorsal tibial apophysis in some species, and a short or long spiniform embolus with a broad base; the epigyne lacks a median septum, has a short copulatory duct, and the spermathecae are rounded to irregular in shape. Furthermore, Benjamin (2020) highlighted questions about the species to be included in this genus, and suggested that *T. limbatus* from South Africa

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was probably misplaced, that *T. xiphosus* might be a synonym of the type species *T. triangulifer*; and that *T. elegans* Thorell, 1890 might also be a junior synonym of *T. triangulifer*. When these problems are resolved, this genus will be unambiguous in the spider family Thomisidae Sundevall, 1833.

The present paper describes six species based on ample material (including some paratypes) from China which have been collected by spider enthusiasts, our colleagues, and the authors. Two new *Talaus* species are identified as undescribed and examination of these specimens reveals that *T. xiphosus* is a junior synonym of *T. triangulifer*.

## Material and methods

The new materials were collected in the bush with the malaise trap, fogging and beating method. Specimens were examined using a SZ6100 stereomicroscope. Both male and female copulatory organs were dissected and examined in 80% ethanol using an Olympus CX43 compound microscope with a KUY NICE CCD camera (Beijing Tiannuoxiang Scientific Instrument Co., Ltd, China). Epigynes were cleared with pancreatin solution (Álvarez-Padilla and Hormiga 2007).

The measurements were taken using a stereomicroscope (AxioVision SE64 Rel. 4.8.3) and are given in millimeters. The body lengths of all specimens exclude the chelicerae and spinnerets. Terminology of the male and female copulatory organs follows Benjamin (2020). Leg measurements are given as total length (femur, patella, tibia, metatarsus, tarsus). The abbreviations used in the figures are as follows:

| | |
|---|---|
| **ALE** | anterior lateral eye; |
| **AME** | anterior median eye; |
| **CD** | copulatory duct; |
| **CO** | copulatory opening; |
| **d** | dorsal; |
| **Em** | embolus; |
| **EH** | epigynal hood; |
| **FD** | fertilization duct; |
| **MOA** | median ocular area; |
| **p** | prolateral; |
| **PLE** | posterior lateral eye; |
| **PME** | posterior median eye; |
| **r** | retrolateral; |
| **RTA** | retrolateral tibial apophysis; |
| **Spe** | spermatheca; |
| **TR** | tegular ridge; |
| **v** | ventral; |
| **VTA** | ventral tibial apophysis. |

Depositories of all specimens examined are abbreviated as:

| | |
|---|---|
| **ASM-JGSU** | Animal Specimen Museum, College of Life Science, Jinggangshan University, Ji'an, China; |
| **CAS** | California Academy of Sciences, San Francisco, USA; |
| **HNU** | Hunan Normal University, Changsha, China. |

## Taxonomy

### Family Thomisidae Sundevall, 1833

### Genus *Talaus* Simon, 1886

**Type species.** *Talaus triangulifer* Simon, 1886

**Notes.** Before this study, the genus included 13 species (WSC 2023). One-third of these species are recorded from southern China; the validity of one species, *T. xiphosus* was doubted by Benjamin (2020) and is confirmed as a synonym in this study; therefore, only 12 *Talaus* species are restricted to Southeast Asia and the Indomalayan Realm (Bhutan, China, India, Indonesia, Myanmar, Sri Lanka, Vietnam; WSC 2023). The 13$^{th}$ species, *T. limbatus* from South Africa, is probably misplaced.

### *Talaus dulongjiang* Tang, Yin, Ubick & Peng, 2008

Figs 1, 2

*Talaus dulongjiang* Tang et al., 2008: 63, figs 1–12.

**Type material examined.** ***Paratypes*.** 2 ♂ (DHK-2004-068): China, Yunnan. Province, Gongshan County, Dulongjiang Township, 2.3–3.3 km south of Longyuan Village along Dulongjiang, 28.00532°N, 98.32145°E, 1685 m, 2 November 2004, David Kavanaugh leg. (Tho-159, paratypes examined, HNU); 4 ♀ (Tang-04-08), Mokewang Bridge, 27.83827°N, 98.32103°E, 1455 m, 6–7 November 2004, Guo Tang leg., other data same as previous (Tho-159). Holotype not examined.

**Diagnosis.** The male of this species is similar to *T. niger* Tang, Yin, Ubick & Peng, 2008 (Tang et al. 2008: 65, figs 16–18) in having the same position of the tegular ridge and the filiform embolus, but can be separated from it by the retrolateral tibial apophysis strongly bending forward (vs slightly) and the longer ventral tibial apophysis (vs relatively short) (Fig. 1C–F). The females can be easily separated from *T. niger* (Tang et al. 2008: 65, fig. 20) by the helical copulatory duct (vs S-shaped) and the slightly separated swollen spermathecae (vs clearly separated) (Fig. 2D).

**Description.** Male habitus as in Fig. 1A, B, palp in Fig. 1C–F. ***Palp*:** tibia with two apophyses, the ventral one slightly shorter than retrolateral one, but longer than tibia, with a strongly curved apex directed prolaterally; retrolateral one relatively thin, basally slightly curved antero-retrolaterally in ventral view, longer than tibia; tegular ridge long, basally arising from ~ 12 o'clock position of the tegulum; embolus filiform, spiraling nearly 3/4 coil, arising from 3 o'clock and ending at ~ 1 o'clock on tegulum. Female habitus as in Fig. 2A, B, epigyne in Fig. 2C, D. ***Epigyne*:** copulatory openings relatively large, sub-antero-laterally located; copulatory ducts broad and long, looping 1.75 coils; spermathecae swollen, slightly separated by ~ 1/7 of their width; fertilization ducts located posteriorly, directed anterolaterally.

**Distribution.** Known from Yunnan Province, China (Fig. 12).

**Remarks.** According to Tang et al. (2008) the original material consisted of three males and seven females; two males and four females were deposited in HNU and one male and three females in CAS. However, only two males and

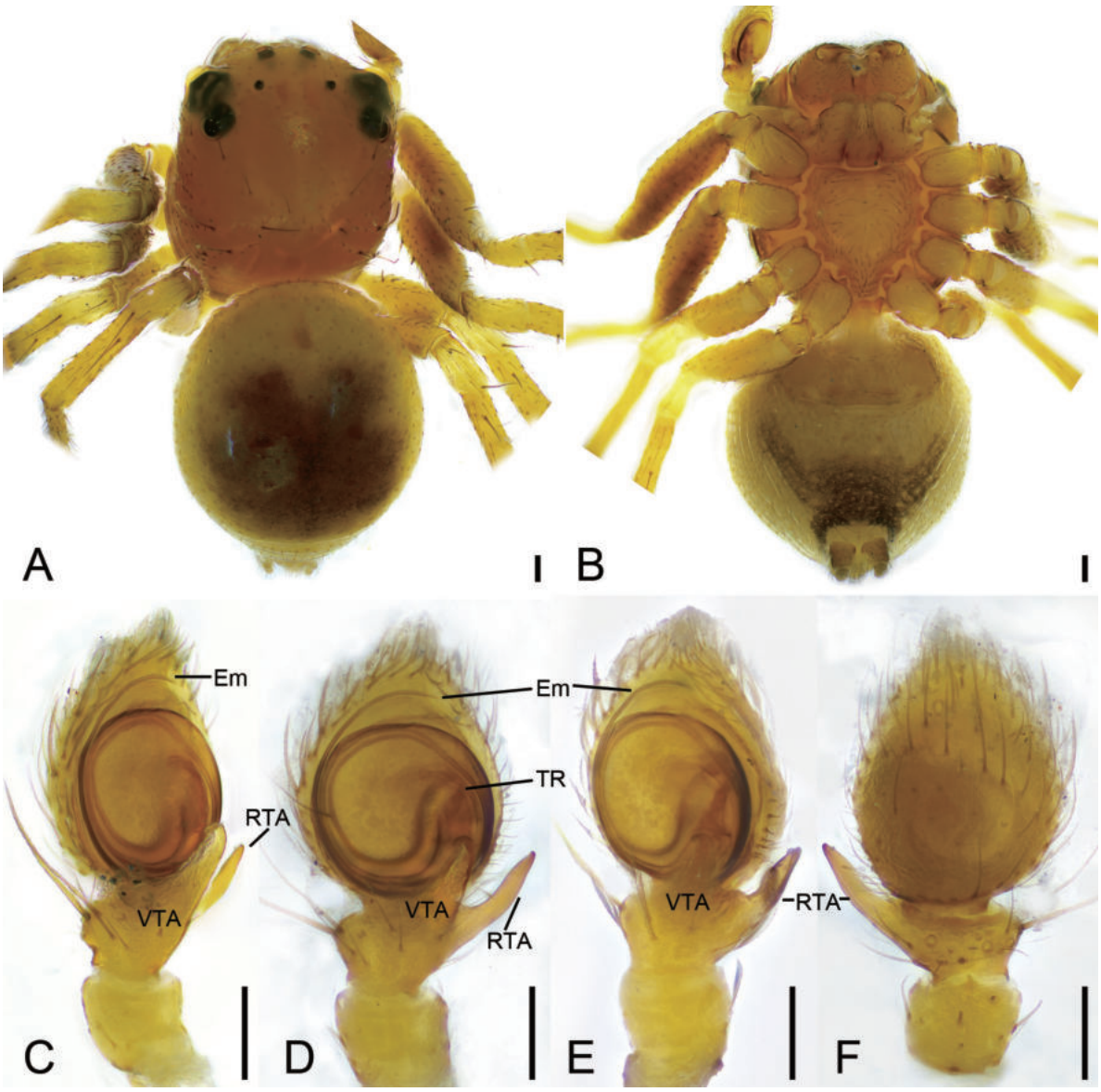


**Figure 1.** *Talaus dulongjiang* Tang, Yin, Ubick & Peng, 2008, male **A** habitus, dorsal view **B** habitus, ventral view **C** palp, prolatero-ventral view **D** palp, ventral view **E** palp, ventro-retrolateral view **F** palp, dorsal view. Abbreviations: Em – embolus, RTA – retrolateral tibial apophysis, TR – tegular ridge, VTA – ventral tibial apophysis. Scale bars: 0.1 mm.

four females (but not the holotype) were found in HNU and there are no striking markings on them: the label data match the localities of the paratypes recorded by Tang et al. (2008: 63).

***Talaus niger* Tang, Yin, Ubick & Peng, 2008**
Fig. 3

*Talaus niger* Tang et al., 2008c: 65, figs 13–23.

**Type material examined. *Paratype*.** 2 ♀ (GKJ020): CHINA, Yunnan Province, Tengchong County, Wuhe Township, Tongjiazhuang Village, Longchuanjiang

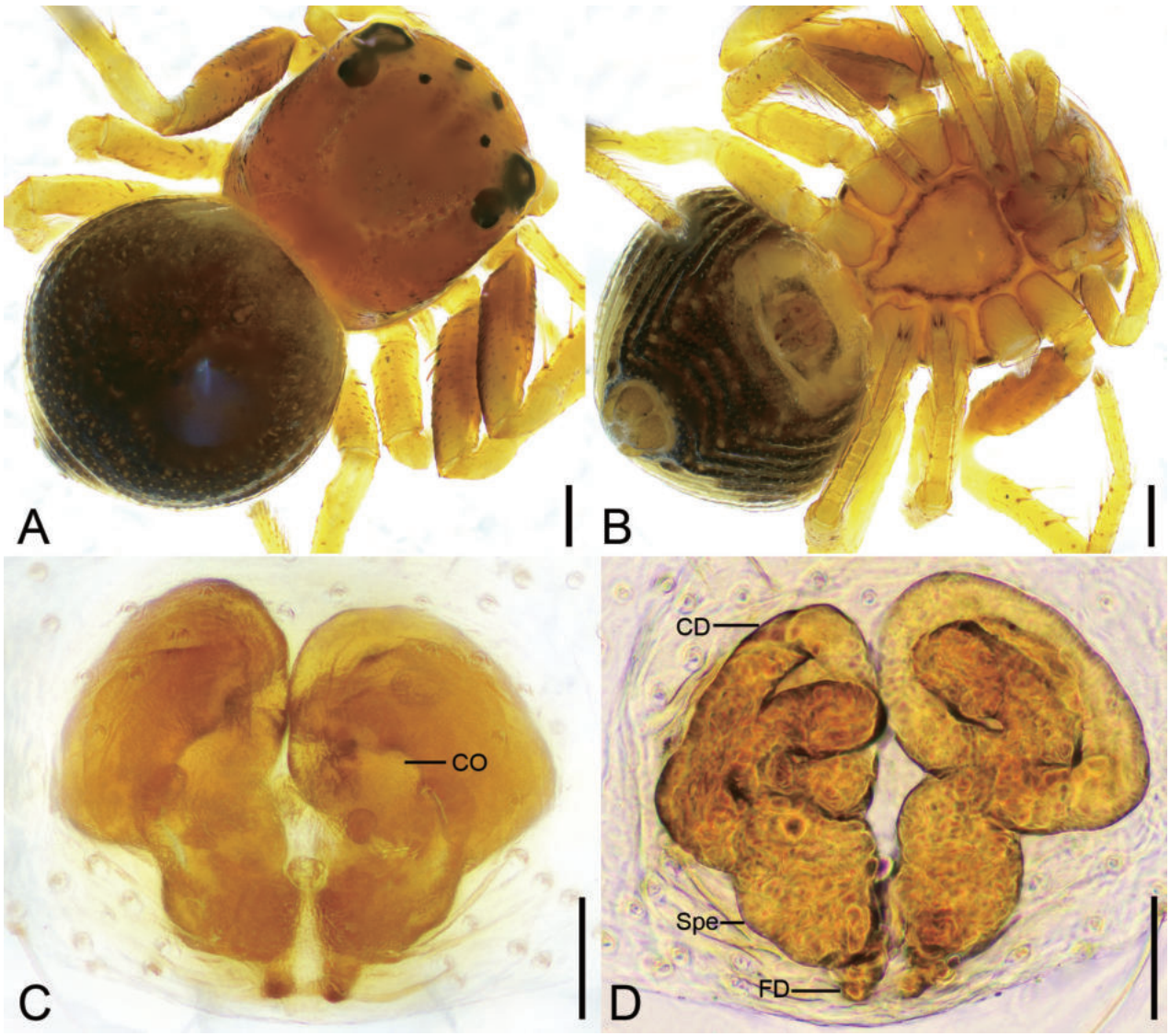


**Figure 2.** *Talaus dulongjiang* Tang, Yin, Ubick & Peng, 2008, female **A** habitus, dorsal view **B** habitus, ventral view **C** epigyne, ventral view **D** epigyne, dorsal view. Abbreviations: CD – copulatory duct, CO – copulatory opening, FD – fertilization duct, Spe – spermatheca. Scale bars: 0.2 mm (**A, B**); 0.05 mm (**C, D**).

River (Longjiang Bridge), along river, 24.89284°N, 98.67439°E, 1210 m, 24 May 2005, Heng-mei Yan & Ke-ji Guo leg. (Tho-202). Holotype not examined.

**Diagnosis.** The female can easily be recognized by the S-shaped copulatory ducts with a swollen median part (Fig. 3D).

**Description.** Female habitus as in Fig. 3A, B, epigyne in Fig. 3C, D. ***Epigyne*:** copulatory openings relatively large, antero-medially located, with sclerotized and round margins; epigynal hood posteriorly located, thumb-like; copulatory ducts S-shaped, anterior part relative narrow, L-shaped, medial and posterior part swollen, extending transversally; spermathecae triangular, widely separated by approximately half of their width; fertilization ducts located posteriorly, directed anteriorly.

**Distribution.** Known only from the type locality in Yunnan Province, China (Fig. 12).

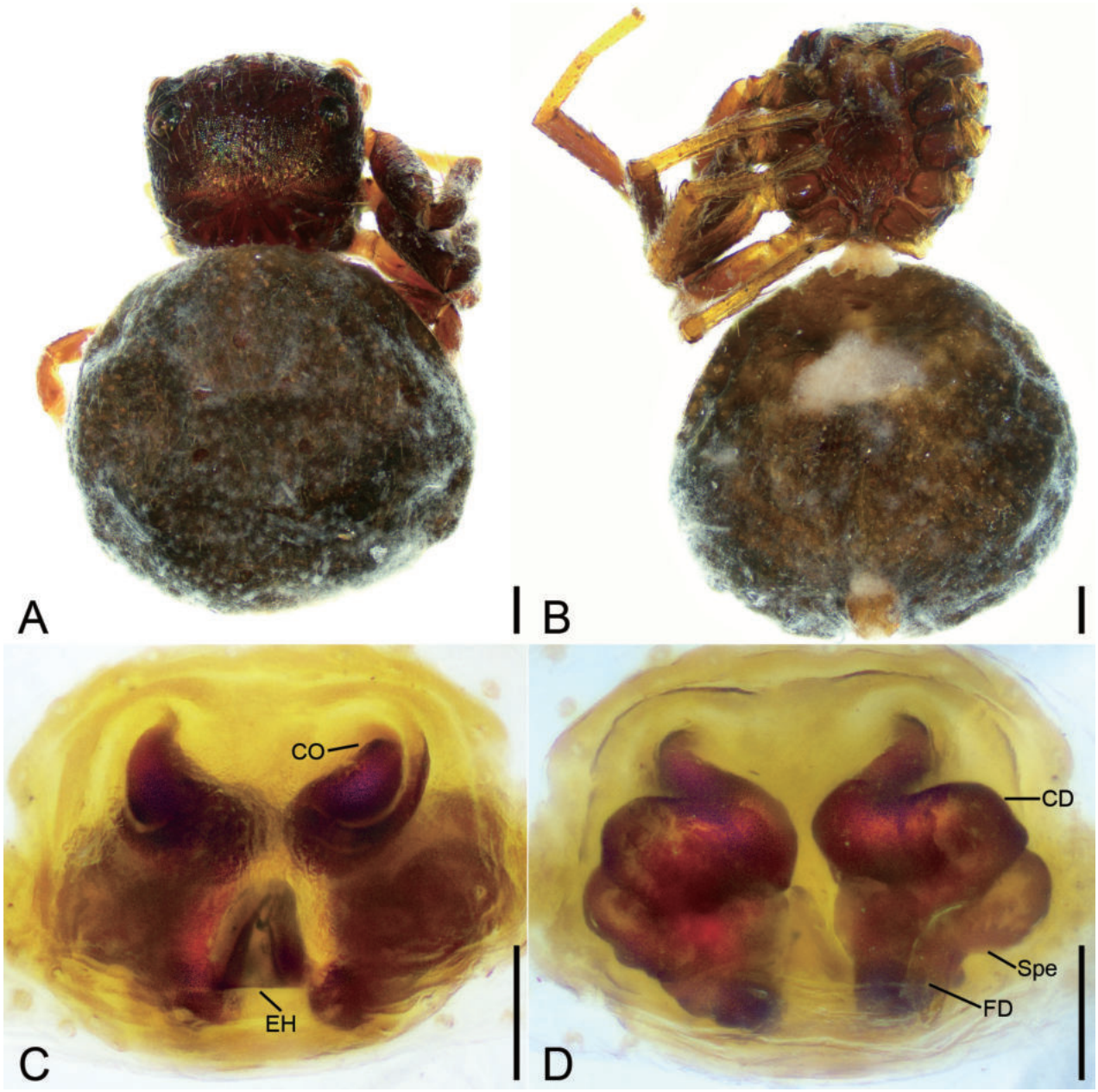


**Figure 3.** *Talaus niger* Tang, Yin, Ubick & Peng, 2008, female **A** habitus, dorsal view **B** habitus, ventral view **C** epigyne, ventral view **D** epigyne, dorsal view. Abbreviations: CD – copulatory duct, CO – copulatory opening, EH – epigynal hood, FD – fertilization duct, Spe – spermatheca. Scale bars: 0.2 mm (**A, B**); 0.05 mm (**C, D**).

**Remarks.** According to Tang et al. (2008: 65), the original materials consisted of a male and two females, one female deposited in HNU and one female in CAS. However, two females and no male holotype were found in HNU and there are no labels designating the paratypes. Based on the locality information and the original illustration given in their paper, these specimens can be confirmed as the paratypes of *T. niger* and have been labelled as such. They are somewhat shriveled after alcohol evaporated during storage, but their epigynes still can be clearly recognized after pancreatin digestion and are now placed on labelled slides.

***Talaus sulcus*** **Tang & Li, 2010**
Figs 4, 5

*Talaus sulcus* Tang & Li, 2010b: 93, f. 71A–D, 72A–F, 73A–D.

**Other material examined.** 2 ♀: China, Guangxi Zhuang Autonomous Region, Chongzuo City, Jiangzhou District, Zuozhou Town, Nongxue Village, 22°36.024'N, 107°24.93'E, 252 m, 5 September 2015, Bing Zhou, Wang Liu, Ji-he Liu, Qu Cai, Xian-feng Huang & Da Li leg. (Tho-334, HNU); 1 ♀, Guanghe Village, Pairutun, Nongzui, 22°32.556'N, 107°26.970'E, 311 m, 11 September 2015, other data same as previous (Tho-334, HNU); 1 ♀, Quxi Village, Nongqiong, 22°34.208'N, 107°25.003'E, 276 m, 31 August 2015, other data same as previous (Tho-334, HNU); 1 ♀, Longzhou County, Nonggang National Nature Reserve, Longjiang Station, Checkpoints, 22.4777°N, 106.9092°E, 204 m, 28 October 2017, Ai-lan He, Ke-ke Liu, Qu Cai, Ji-he Liu, Jin-xin Liu & Zong-guang Huang leg. (Tho-335, HNU).

**Diagnosis.** Female resembles *Talaus triangulifer* (Fig. 7C, D) in having a bean-shaped spermathecae, but can be easily recognized by the copulatory

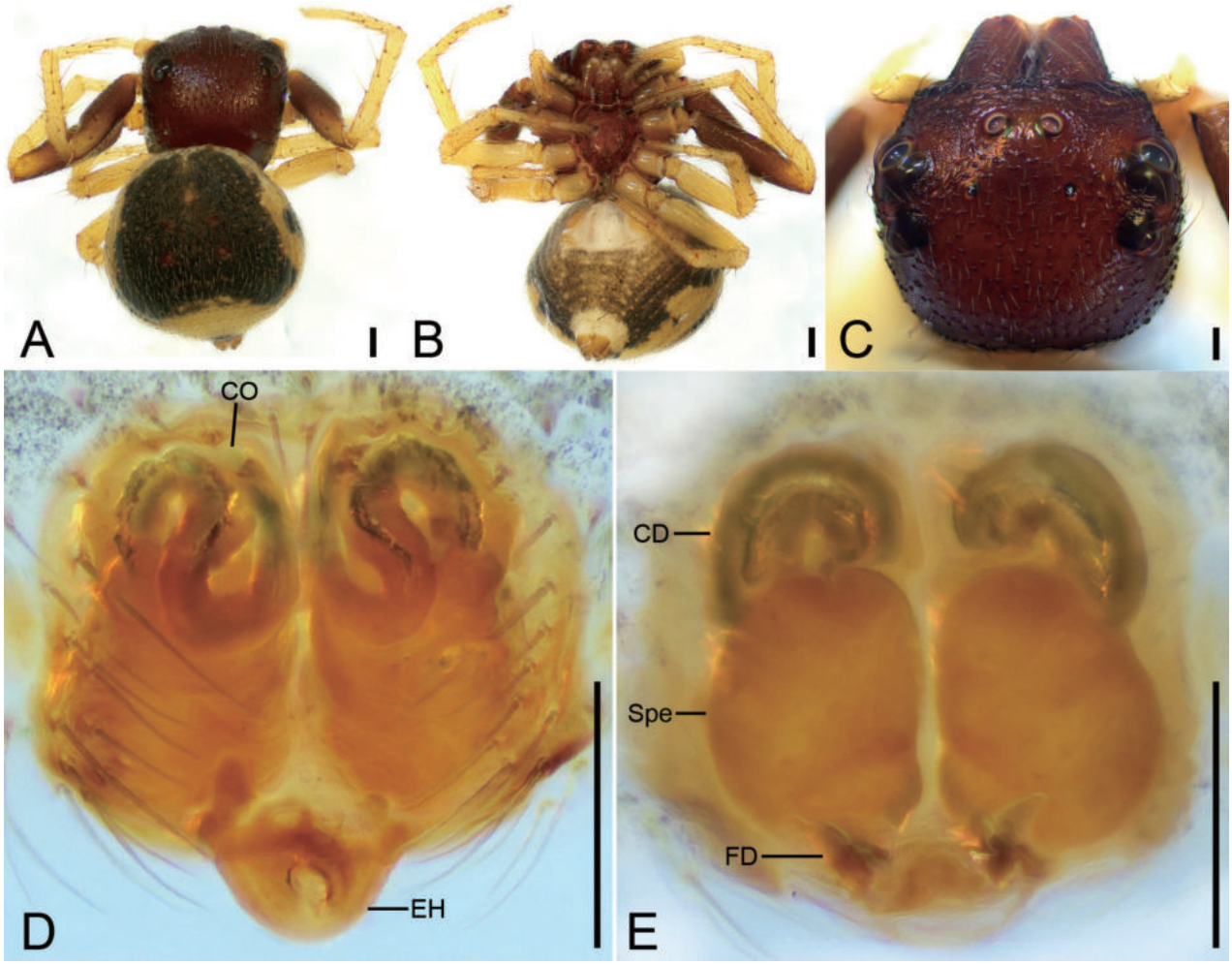


**Figure 4.** *Talaus sulcus* Tang & Li, 2010, female **A** habitus, dorsal view **B** habitus, ventral view **C** prosoma, dorso-frontal view **D** epigyne, ventral view **E** epigyne, dorsal view. Abbreviations: CD – copulatory duct, CO – copulatory opening, EH – epigynal hood, FD – fertilization duct, Spe – spermatheca. Scale bars: 0.2 mm (**A, B**); 0.1 mm (**C–E**).

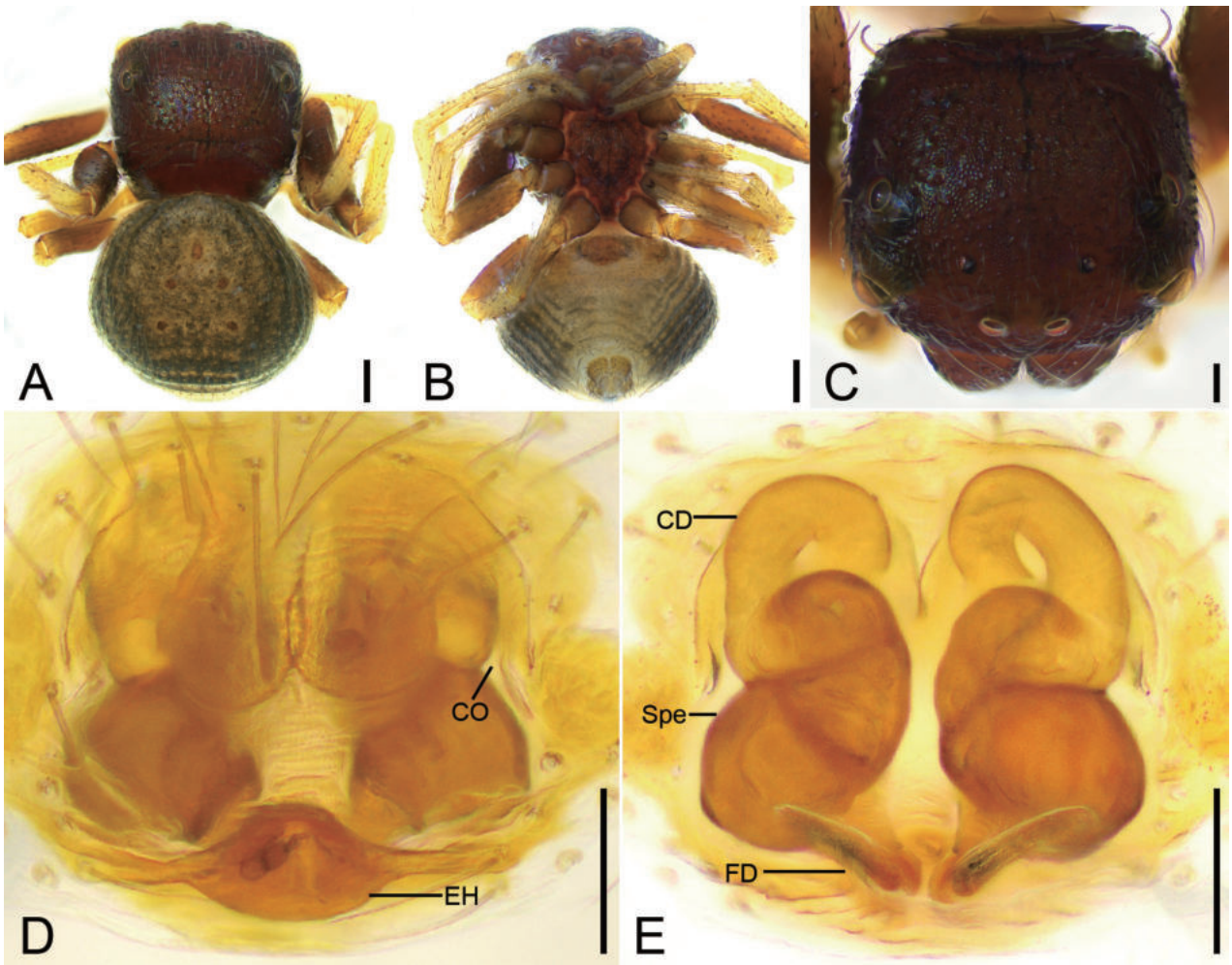


**Figure 5.** *Talaus sulcus* Tang & Li, 2010, female **A** habitus, dorsal view **B** habitus, ventral view **C** prosoma, dorsal view **D** epigyne, ventral view **E** epigyne, dorsal view. Abbreviations: CD – copulatory duct, CO – copulatory opening, EH – epigynal hood, FD – fertilization duct, Spe – spermatheca. Scale bars: 0.2 mm (**A, B**); 0.1 mm (**C**); 0.05 mm (**D, E**).

openings located antero-medially and directed posteriorly (vs located antero-medially and directed anteriorly, or located latero-medially) and the S-shaped or spiral copulatory ducts (vs C-shaped) (Figs 4D, E, 5D, E).

**Description.** Female habitus as in Figs 4A, B, 5A, B, eyes as in Figs 4C, 5C, epigyne as in Figs 4D, E, 5D, E. ***Epigyne*:** copulatory openings small, antero-medially located, separated by half of width of epigynal hood; epigynal hood postero-medially located, looks like a semi-circular extrusion; copulatory ducts long, S-shaped in ventral view, C-shaped in dorsal view; spermathecae ovoid, swollen, slightly separated from each other; fertilization ducts postero-medially located, directed anterolaterally. The epigynes also include the other variations: copulatory openings small, slit-like, latero-medially located; epigynal hood postero-medially located, triangular, curved forward; copulatory ducts long, like an oval ring in dorsal view; spermathecae sac-shaped, with obvious constrictions, nearly sac-shaped, anterior slightly separated from each other anteriorly; fertilization ducts postero-medially located, touching their bases, directed anterolaterally.

**Distribution.** Known from Yunnan (Tang and Li 2010) and Guangxi Province (present records), China (Fig. 12).

**Remarks.** The detailed study of newly collected specimens from Guangxi revealed that they have two variants of the epigyne conformation as shown for paratype specimens from Yunnan by Tang and Li (2010). Specimens from Jiangzhou District have S-shaped (in ventral view) and C-shaped (in dorsal view) copulatory ducts and ovoid spermathecae as illustrated in Tang and Li (2010: fig. 72C, D); the specimens from Longzhou County have loop-like copulatory ducts and nearly C-shaped spermathecae similar to the illustration in Tang and Li (2010: fig. 72E, F). However, our new female specimens do not have a yellow abdomen dorsally bearing a large subtriangular spot subposteriorly, which is an acceptable color-variation proposed by Tang and Li (2010: 93).

***Talaus triangulifer* Simon, 1886**
Figs 6, 7

*Talaus triangulifer* Simon, 1886: 172); Benjamin 2020: 414, figs 2C, H–J, 4E, 7A–B.
*Talaus xiphosus* Zhu & Ono, 2007: 81, figs 1–5; Benjamin 2020: 415 ("probably a synonym of *T. triangulifer*"). syn. nov.

**Other material examined.** 3 ♂: China, Guangxi Zhuang Autonomous Region, Chongzuo City, Longzhou County, Nonggang National Nature Reserve, Sanlian Station, Longdan, 22.53470°N, 106.83697°E, 307 m, 31 October 2017, Ai-lan He, Ke-ke Liu, Qu Cai, Ji-he Liu, Jin-xin Liu & Zong-guang Huang leg. (Tho-029, HNU); 1 ♀, 30 October 2017, other data as previous; 14 ♂ 11 ♀, Nonggang Station, Boarded-up Houses, 22.46444°N, 106.92359°E, 188 m, 28 October 2017, other data as previous; 5 ♂ 11 ♀, Core Area, 22.46415°N, 106.93238°E, 228 m, 26 October 2017, other data as previous; 4 ♂ 3 ♀, 27 October 2017, other data as previous; 6 ♂ 6 ♀, Longjiang Ligatures, 22.4770°N, 106.90921°E 204 m, 28 October 2017, other data as previous; 12 ♂ 1 ♀, 27 October 2017, other data as previous; 12 ♂ 8 ♀, Longhengtun, 22.47450°N, 106.98307°E, 270 m, 29 October 2017, other data as previous; 1 ♂ 3 ♀, 22.47166°N, 106.97051°E, 163 m, other data as previous; 1 ♂ 1 ♀, Nanning City, Wuming County, Damingshan National Nature Reserve, Sanbao Station, Chaoyang, 23°31'13.679"N, 108°23'4.560"E, 3 November 2018, 593 m, Ai-lan He, Ke-ke Liu, Hui-juan Sheng, Ji-he Liu, Jin-xin Liu & Zong-guang Huang leg.; 9 ♂ 1 ♀, Ganlan Station, 23°34'15.380"N, 108°25'16.284"E, 7 November 2018, 485 m, other data as previous; 1 ♂ 2 ♀, Shanglin County, Zhaojiang Station, 23°27'1.8"N, 108°23'32.639"E, 6 November 2018, 263 m, other data as previous; 1 ♂ 1 ♀, Jilong Station 23°26'5.279"N, 108°26'32.639"E, 591 m, 5 November 2018, other data as previous; 17 ♂ 28 ♀, Chongzuo City, Jiangzhou District, Zuozhou Town, Guanghe Village, Hecuntun, Nongyao, 22°36.318'N, 107°25.677'E, 224 m, 9 September 2015, Bing Zhou, Wang Liu, Ji-he Liu, Qu Cai, Xian-feng Huang & Da Li leg.; 8 ♂ 7 ♀, Longmitun, Nongxing, 22°34.190'N, 107°26.283'E, 272 m, 7 September 2015, other data as previous; 11 ♀, Hecuntun, Nongteng, 22°35.074'N, 107°25.430'E, 235 m, 4 September 2015, other data as previous; 1 ♂ 11 ♀, Nongdan, 22°34.054'N, 107°24.295'E, 296 m, 30 August 2015, other data as previous; 2 ♂, 12 ♀, Pairutun, 22°34.911'N, 107°25.684'E, 226 m, 3 September 2015, other data as previous; 7 ♂, 26 ♀, Nongheng, along the mountain road, 22°34.740'N, 107°24.915'E, 271 m, 29 August 2015, other

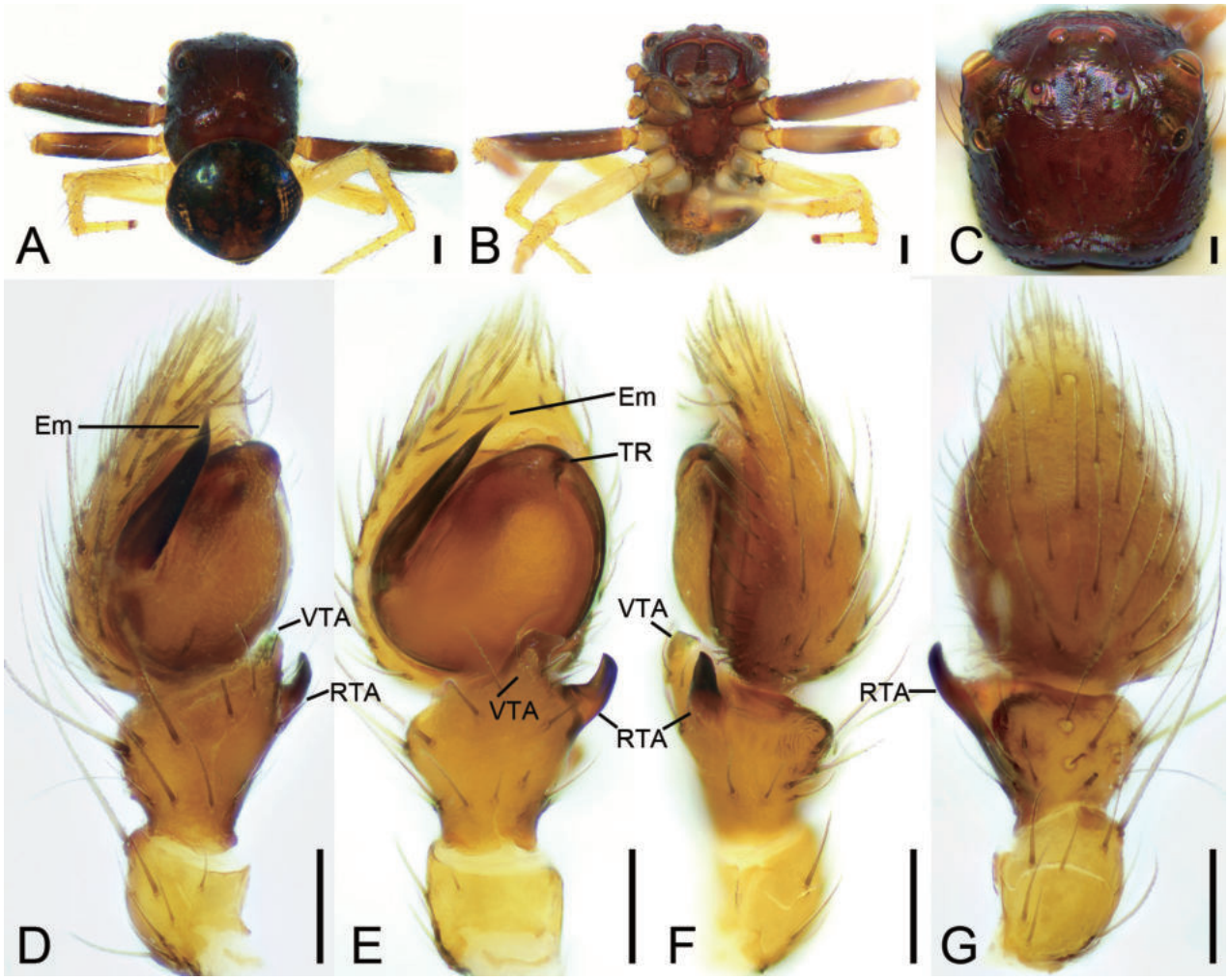


**Figure 6.** *Talaus triangulifer* Simon, 1886, male **A** habitus, dorsal view **B** habitus, ventral view **C** prosoma, dorsal view **D** palp, prolatero-ventral view **E** palp, ventral view **F** palp, retrolateral view **G** palp, dorsal view. Abbreviations: Em – embolus, RTA – retrolateral tibial apophysis, TR – tegular ridge, VTA – ventral tibial apophysis. Scale bars: 0.2 mm (**A, B**); 0.1 mm (**C–G**).

data as previous; 2 ♂ 4 ♀, Duolu Town, Duobai Village, Longquantun, Nongquan, 22°32.392'N, 107°27.221'E, 145 m, 10 September 2015, other data as previous.

**Diagnosis.** The species can be easily differentiated from other *Talaus* species by the long straight xiphoid embolus [vs curved in *T. beccarii* (Benjamin, 2020: 406, fig. 1A); short in *T. opportunus* (Benjamin 2020: 411, fig. 6A, B); flagelliform and curved in other species]. Females resemble those of *T. opportunus* (Benjamin 2020: 411, fig. 6C, D) in having a pair of question-mark-like copulatory ducts, but can be easily distinguished from it by the oval spermathecae (vs irregular in *T. opportunus*). The females are similar to those of *T. sulcus* (Tang and Li 2010: 93, fig. 72C–F) in having a semi-circular epigynal scape and the swollen spermathecae, but can be separated from it by the question-mark-like copulatory ducts (vs S-shaped or spiral in *T. sulcus*).

**Description. Male** habitus as in Fig. 6A, B, eyes as in Fig. 6C, palp as in Fig. 6D–G. ***Palp*:** tibia with two apophyses: the ventral one bird-head-like in ventral view, with a sharp, narrowed apex directed retrolaterally; retrolateral one horn-like, well sclerotized, longer than ventral one; tegular ridge arising from ~ 1 o'clock position; embolus (Em) xiphoid, arising from 8 o'clock and ending at

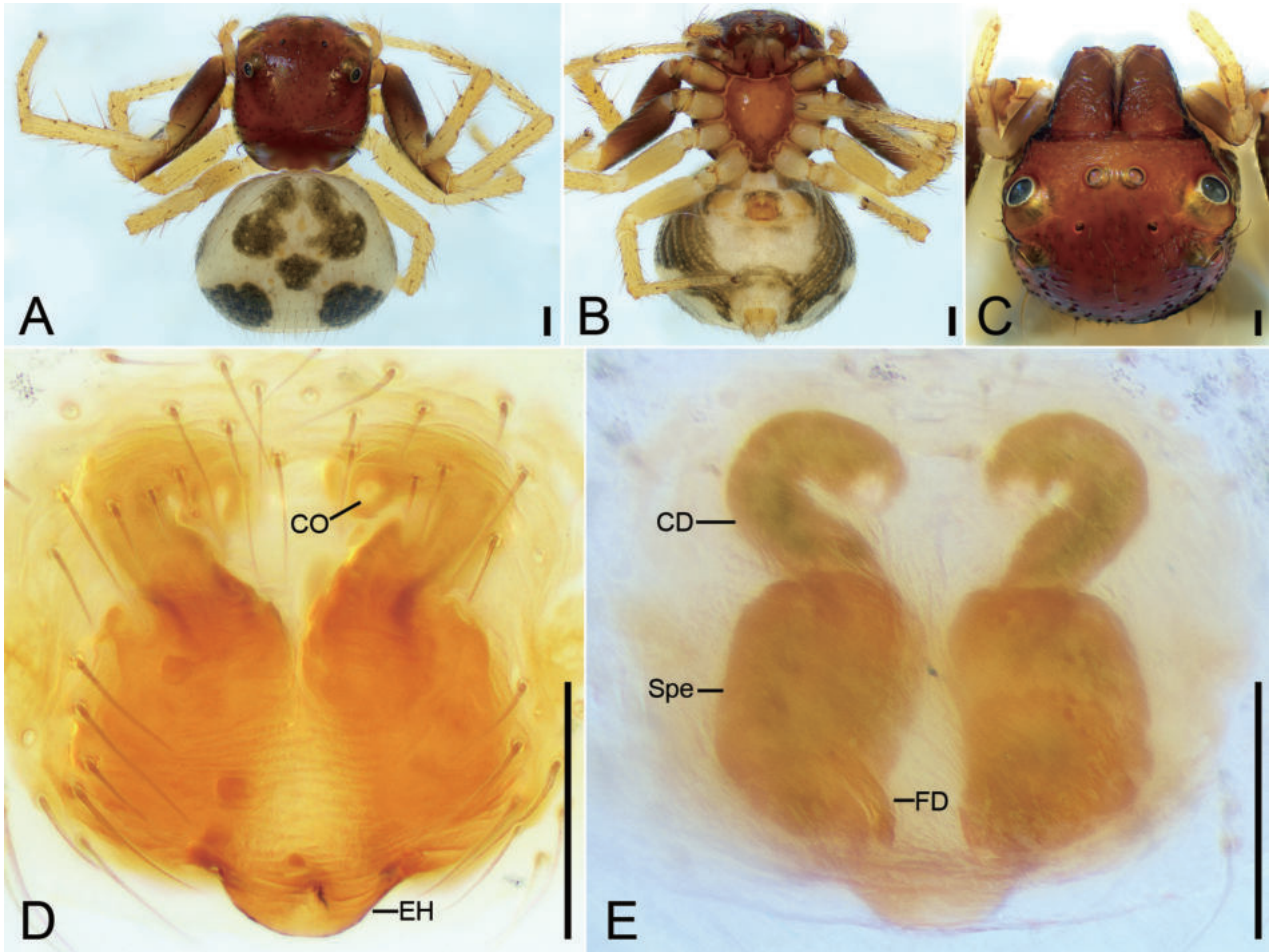


**Figure 7.** *Talaus triangulifer* Simon, 1886, female **A** habitus, dorsal view **B** habitus, ventral view **C** prosoma, dorso-frontal view **D** epigyne, ventral view **E** epigyne, dorsal view. Abbreviations: CD – copulatory duct, CO – copulatory opening, EH – epigynal hood, FD – fertilization duct, Spe – spermatheca. Scale bars: 0.2 mm (**A, B**); 0.1 mm (**C–E**).

~ 12 o'clock. Female habitus as in Fig. 7A, B, epigyne as in Fig. 7D, E. ***Epigyne*:** copulatory openings small, directed backwards, separated by half width of spermathecae; epigynal hood located posteriorly, semi-circular; copulatory ducts question-mark-like; spermathecae nearly oval, swollen, anterior part nearly touching, posterior part separated by less than half of spermathecal width.

**Distribution.** Known from Indonesia (Borneo, Sumatra; WSC 2023), Malaysia (Sabah; Benjamin 2020), and China (new records, Yunnan and Guangxi provinces; Fig. 12).

**Remarks.** According to Zhu and Ono (2007), the original materials of *Talaus xiphosus* were collected from Longzhou County, Chongzuo City in Guangxi Zhuang Autonomous Region. Of these specimens, some were collected from Chongzuo City, the others from Nanning City. Although there is 100 km from the locality of the holotype (Ningming County in Chongzuo City) to the nearest point of the locality of our specimens, and there is intraspecific variation in the abdomen color in the specimens from Zhu and Ono (2007), we did not find any noticeable differences in the male palps or in the female epigynes and therefore confirm Benjamin's (2020) proposal that *T. xiphosus* is a junior synonym of *T. triangulifer*. The results presented here suggest that this species has a wide distribution in Southeast Asia.

***Talaus yuyang*** **Yao & Liu, sp. nov.**
https://zoobank.org/0B1D782D-8049-473F-9399-E05EE564F269
Figs 8, 9, 11

**Type material.** ***Holotype*** ♂, China: Chongqing Municipality: Chengkou County, Hongjun Park, 31°56'56.89"N, 108°40'15.84"E, late October 2022, Y.Y. Zhou leg. (Tho-322, ASM-JGSU). ***Paratypes*****:** 1 ♂ 7 ♀, same data as holotype; 1 ♂, 1 ♀; Ledong County, Jianfengling National Natural Reserve, Mingfenggu scenic spot, 18°44'25.87"N, 108°50'47.83"E, 1–31 May 2021, Yun-hu Mo leg. (Tho-322, ASM-JGSU).

**Etymology.** The specific name is taken from the first name of Mr Yuyang Zhou, who collected the specimens at Hongjun Park; noun in apposition.

**Diagnosis.** The male is similar to that of *Talaus sulcus* (Tang and Li 2010: 93, fig. 71B–D) in having the horn-like retrolateral tibial apophysis and the filariform embolus, but can be easily distinguished from it by the tegular ridge arising from ~ 8 o'clock position (vs 9 o'clock) and the very long finger-like ventral tibial apophysis (vs short, hump-like). The females of the new species can be easily

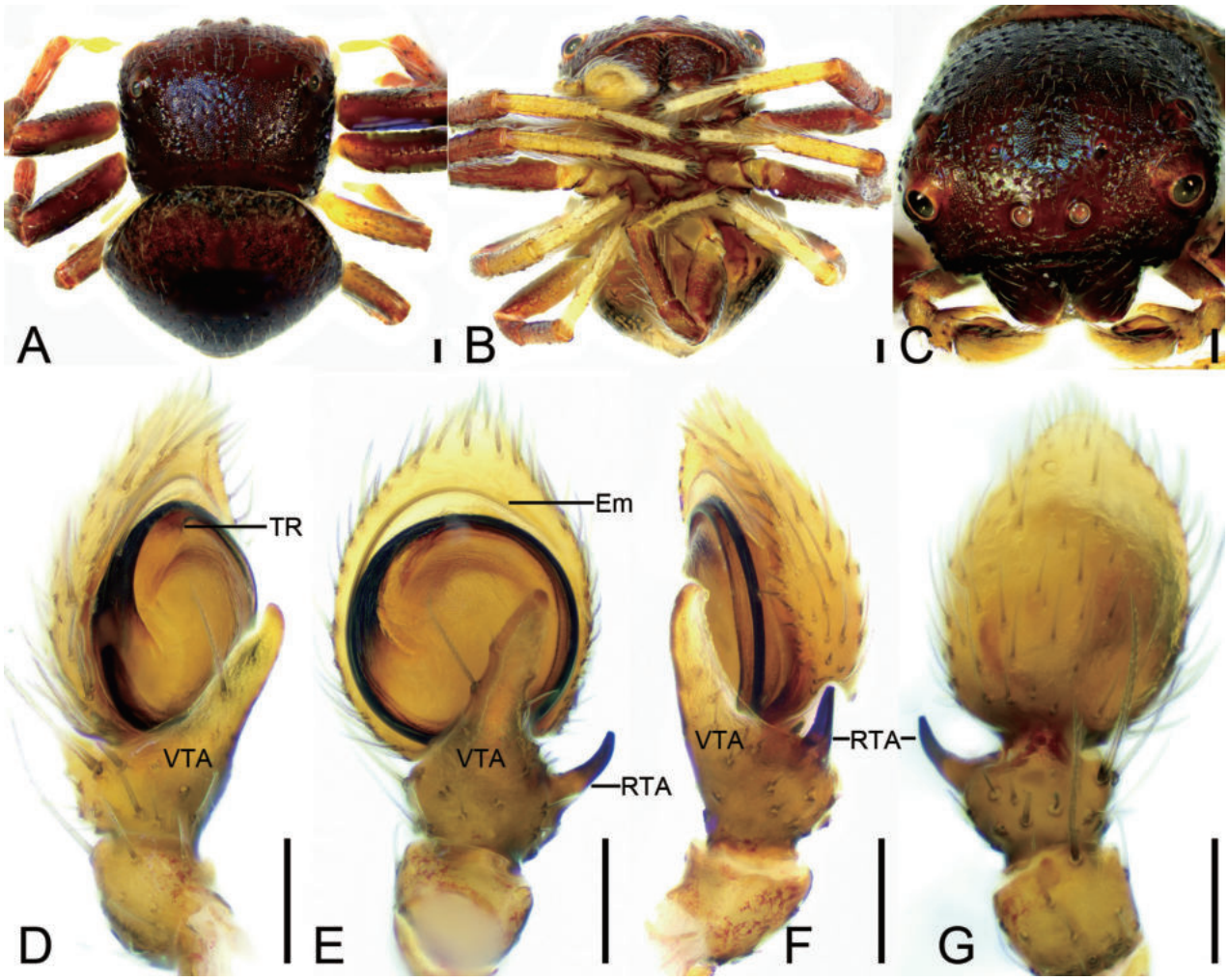


**Figure 8.** *Talaus yuyang* sp. nov., male holotype **A** habitus, dorsal view **B** habitus, ventral view **C** prosoma, dorso-frontal view **D** palp, prolatero-ventral view **E** palp, ventral view **F** palp, retrolateral view **G** palp, dorsal view. Abbreviations: Em – embolus, RTA – retrolateral tibial apophysis, TR – tegular ridge, VTA – ventral tibial apophysis. Scale bars: 0.1 mm.

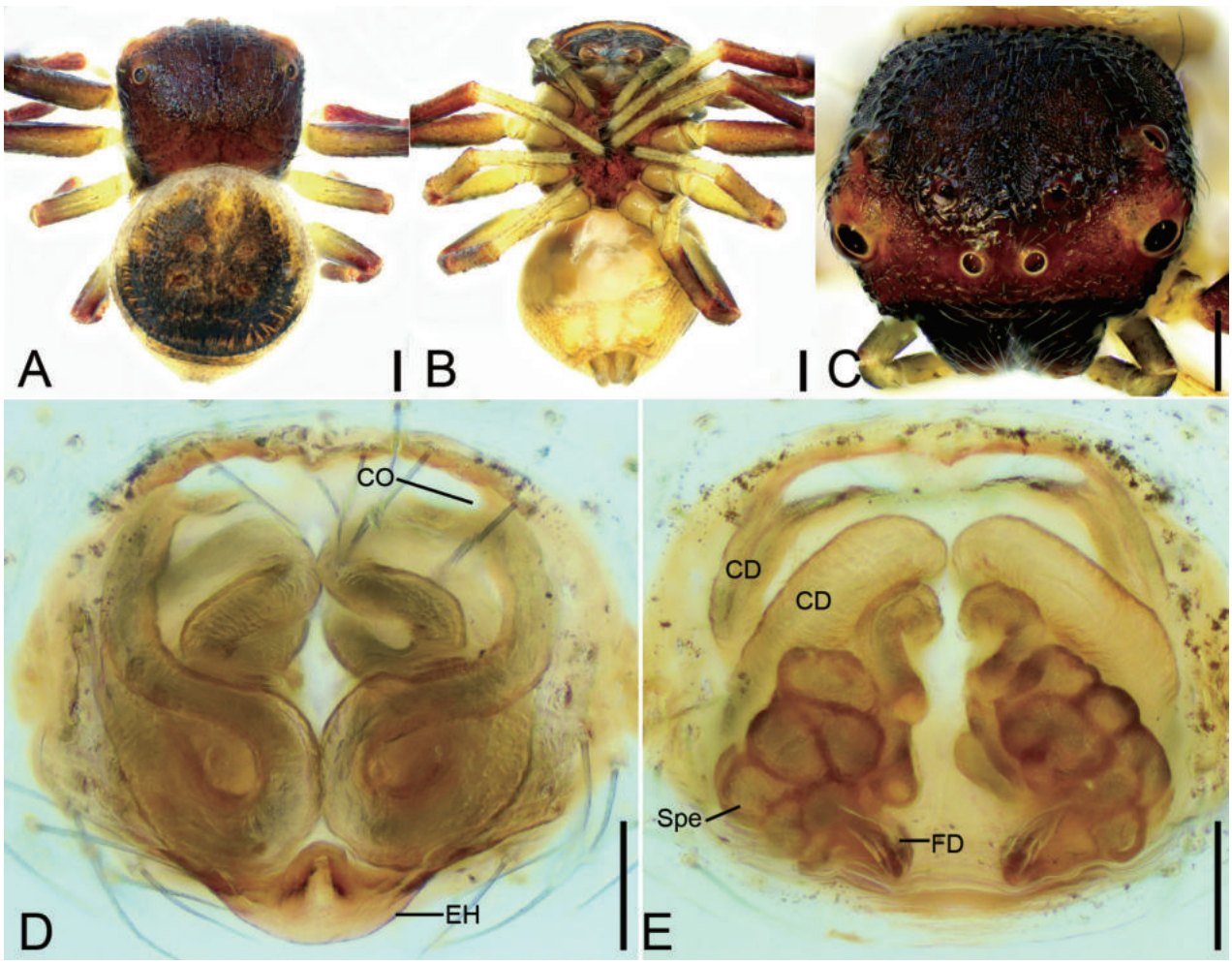


**Figure 9.** *Talaus yuyang* sp. nov., female paratype **A** habitus, dorsal view **B** same, ventral view **C** prosoma, dorso-frontal view **D** epigyne, ventral view **E** same, dorsal view. Abbreviations: CD – copulatory duct, CO – copulatory opening, EH – epigynal hood, FD – fertilization duct, Spe – spermatheca. Scale bars: 0.2 mm (**A–C**); 0.05 mm (**D, E**).

recognized by the tunnel-like copulatory openings located anterolaterally, the very long copulatory ducts, and the spermathecae with many constrictions.

**Description. Male** (holotype). ***Habitus*** (Figs 8A, B, 11). Total length 1.68. ***Carapace*** (Fig. 8A) red brown, length 0.71, width 1.0, with densely short setae. ***Eyes*** (Fig. 8C) diameters and interdistances: AME 0.05, ALE 0.11, PME 0.05, PLE 0.07; AME–AME 0.13, ALE–AME 0.26, PME–PME 0.27, PLE–PME 0.26, AME–PME 0.14, AME–PLE 0.42, ALE–ALE 0.76, PLE–PLE 0.79, ALE–PLE 0.18. MOA 0.24 long, front width 0.24, back width 0.38. Chelicerae red brown, straight, robust, without retromarginal or promarginal teeth. Endites yellow brown, longer than wide. Labium yellow brown, longer than wide. Sternum red brown, longer than wide. Legs red brown except yellow metatarsi and tarsi (Fig. 8A, B); measurements: I 2.33 (0.71, 0.29, 0.51, 0.5, 0.32); II 2.41 (0.71, 0.29, 0.54, 0.53, 0.34); III 1.63 (0.48, 0.23, 0.35, 0.31, 0.26); IV 1.62 (0.49, 0.24, 0.38, 0.29, 0.22); spination: I Pa: d1; Ti: d2, p2, r2, v2; Mt: d4, p2, r2, v4; II Pa: d1; Ti: d2, p2, r2, v2; Mt: d4, p1, r2, v4; III Pa: d1; Ti: d2, p1; Mt: d2; IV: Pa: d1; Ti: r1; Mt: d1, p1, r1. ***Abdomen*** (Fig. 8A, B) 0.98 long, 1.12 wide, ovoid, black brown; venter yellow.

***Palp*** (Fig. 8D–G). Ventral tibial apophysis digitiform, longer than tibia, apex slightly curved retrolaterally. Retrolateral tibial apophysis slightly shorter than

tibia, horn-like, slightly curved in ventral view. Tegular ridge located at ~ 10 o'clock. Embolus filiform, arising from 9 o'clock and ending at ~ 3 o'clock, spiraling 1.5 coils.

**Female** (paratype). ***Habitus*** (Fig. 9A, B). As in male except as follows. Total length 1.84. ***Carapace*** (Fig. 9A) broadly square, length 0.83, width 0.95, with densely short setae. ***Eye*** (Fig. 9C) diameters and interdistances: AME 0.05, ALE 0.09, PME 0.05, PLE 0.07; AME–AME 0.11, ALE–AME 0.26, PME–PME 0.24, PLE–PME 0.26, AME–PME 0.16, AME–PLE 0.41, ALE–ALE 0.72, PLE–PLE 0.71, ALE–PLE 0.19. MOA 0.24 long, front width 0.22, back width 0.33. Chelicerae yellow, with abundant thick setae on frontal surface. ***Legs measurements*:** I 1.98 (0.65, 0.27, 0.41, 0.36, 0.29); II 2.09 (0.71, 0.31, 0.45, 0.34, 0.28); III 1.32 (0.33, 0.24, 0.28, 0.23, 0.24); IV 1.51 (0.48, 0.22, 0.35, 0.27, 0.19); spination: I Fe: d2, p2; Ti: p2, v2; Mt: d4, p2, v2; II Ti: p2; Mt: d4, p2, v2; III Fe: d1; Pa: d1; Mt: d2, p2; IV: Fe: d1; Pa: d1; Mt: d2, p1. ***Abdomen*** (Fig. 9A, B) ovoid, 1.01 long, 1.05 wide, yellow to black brown, with yellow margin dorsally; venter yellow.

***Epigyne*** (Fig. 9D, E). Copulatory openings located at antero-lateral part of atrium, transversal, tunnel-shaped. Copulatory ducts very long, convoluted, double S-shaped in ventral view, splay in dorsal view. Spermathecae sac-shaped, with many constrictions its surface. Fertilization ducts blade-like, directed anterolaterally.

**Distribution.** Known only from the Chongqing Municipality and Hainan Province of China (Fig. 12).

***Talaus zhangjiangkou*** **Yao & Liu, sp. nov.**
https://zoobank.org/6945DACB-01F3-472D-9459-95C013CD83D1
Fig. 10

**Type material.** ***Holotype*** ♂, China: Fujian Province: Zhangzhou City, Yunxiao County, Dongxia Town, Fujian Zhangjiangkou National Mangrove Nature Reserve, 23°55'38.08"N, 117°24'52.91"E, 4 March 2023, H.T. Song, Z.H. Qi, R.X. Su, and B. Ding leg. (Tho-347, ASM-JGSU).

**Etymology.** The specific name is taken from the type locality; noun in apposition.

**Diagnosis.** This new species is similar to that of *Talaus triangulifer* (Benjamin 2020: 414, figs 2I, J, 7A, B) in having the longer, broad-based embolus and the configuration of the tibial apophyses, but can be easily distinguished from it by the retrolateral tibial apophysis with a distinctly curved tip directed mostly dorsally as seen in retrolateral view (vs directed mostly ventrally) and the embolus with a furcate tip (vs pointed) in ventral view (Fig. 10E–G).

**Description.** ***Habitus*** (Fig. 10A, B). Total length 2.24. ***Carapace*** (Fig. 10A) red brown, length 1.07, width 1.14, with densely short setae. ***Eyes*** (Fig. 10C) diameters and interdistances: AME 0.07, ALE 0.12, PME 0.05, PLE 0.08; AME–AME 0.12, ALE–AME 0.31, PME–PME 0.32, PLE–PME 0.3, AME–PME 0.17, AME–PLE 0.5, ALE–ALE 0.88, PLE–PLE 0.88, ALE–PLE 0.21. MOA 0.29 long, front width 0.25, back width 0.41. Chelicerae red brown, straight, robust, without retromarginal or promarginal teeth. Endites yellow brown, longer than wide. Labium yellow brown, longer than wide. Sternum red brown, longer than wide, with long dense setae. Legs red brown except yellow metatarsi and tarsi (Fig. 10A, B); measurements: I 3.24 (1, 0.42, 0.77, 0.61, 0.44); II 3.49 (1.04, 0.48, 0.81, 0.7,

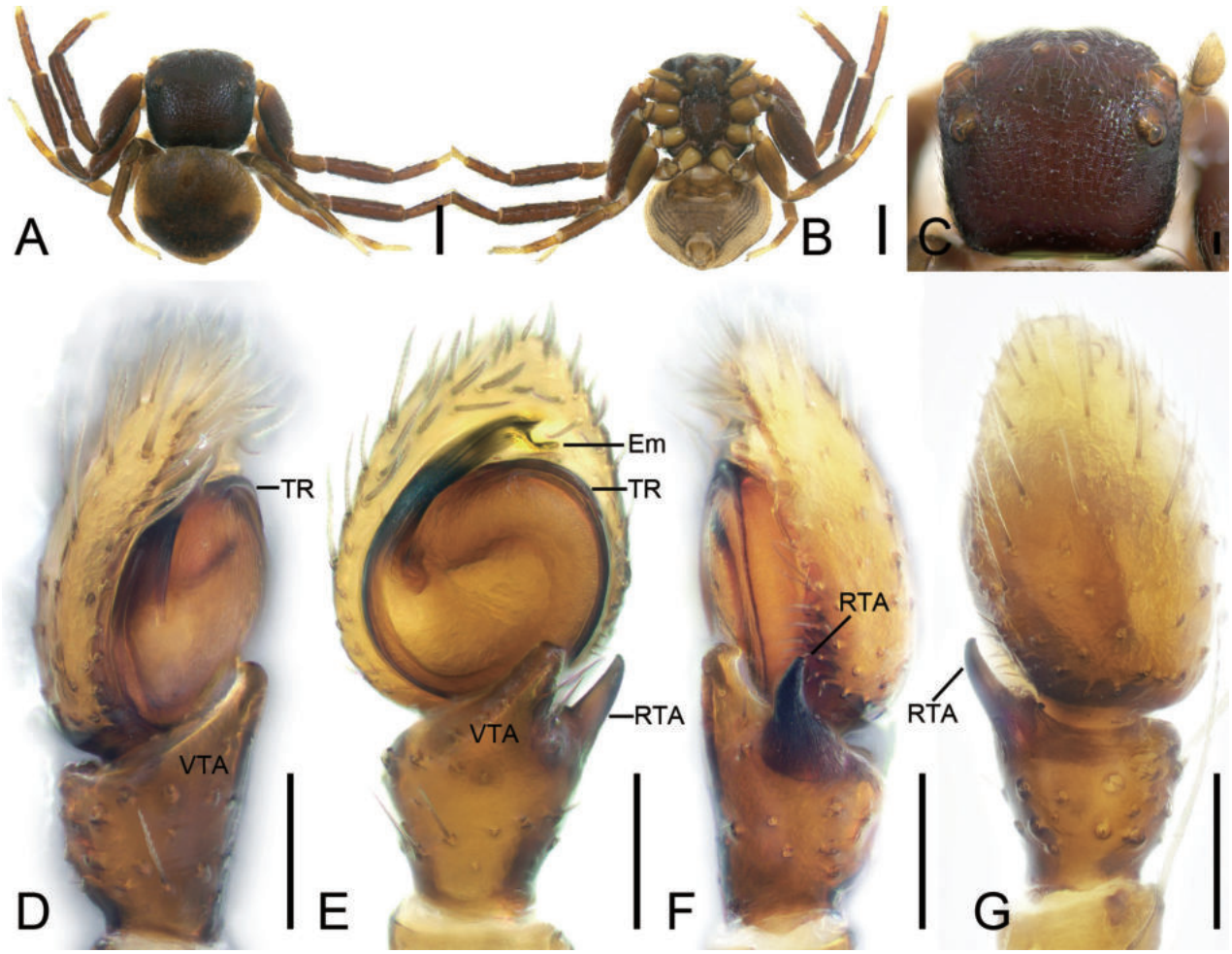


**Figure 10.** *Talaus zhangjiangkou* sp. nov., male holotype **A** habitus, dorsal view **B** habitus, ventral view **C** prosoma, dorsal view **D** palp, prolatero-ventral view **E** palp, ventral view **F** palp, retrolateral view **G** palp, dorsal view. Abbreviations: Em – embolus, RTA – retrolateral tibial apophysis, TR – tegular ridge, VTA – ventral tibial apophysis. Scale bars: 0.1 mm (**A, B**); 0.1 mm (**C–G**).

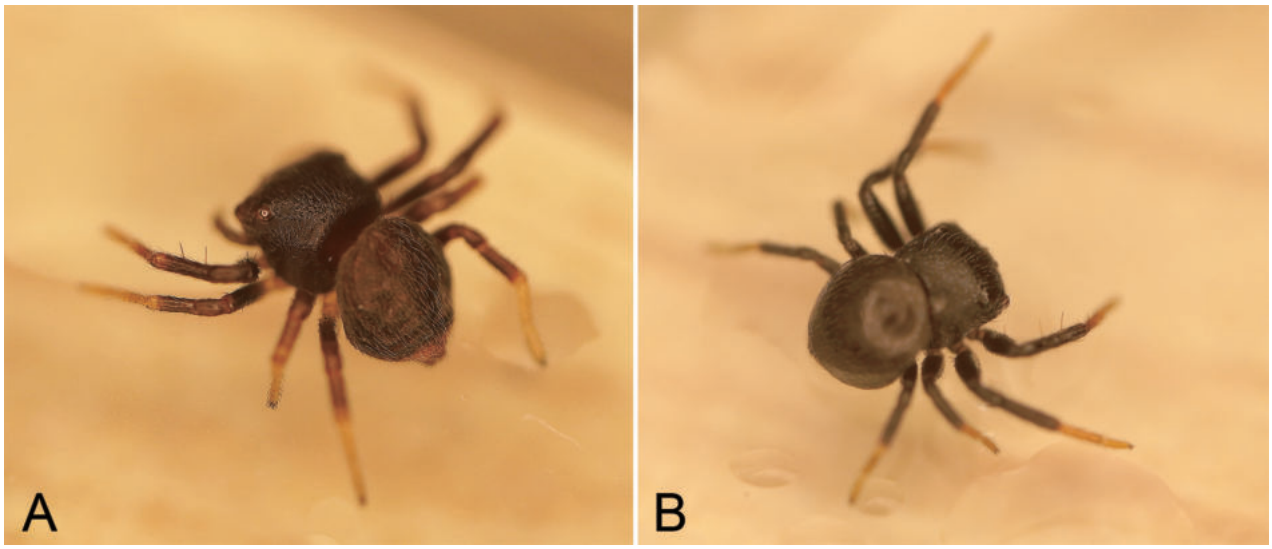


**Figure 11.** Photographs of live specimen from China **A, B** *Talaus yuyang* sp. nov., male.

**Figure 12.** Distributional records of *Talaus dulongjiang* Tang, Yin, Ubick & Peng, 2008 (yellow circles), *T. niger* Tang, Yin, Ubick & Peng, 2008 (black circle), *T. sulcus* Tang & Li, 2010 (red triangles), *T. triangulifer* Simon, 1886 (green circles), *T. yuyang* sp. nov. (blue circles) and *T. zhangjiangkou* sp. nov. (red square) from China.

0.46); III 2.23 (0.68, 0.32, 0.54, 0.38, 0.31); IV 2.08 (0.67, 0.3, 0.48, 0.37, 0.26); spination: I Pa: d1, p1; Ti: d3, p2, r3, v2; Mt: d3, p1, r1, v4; II Ti: d3, p3, r3, v2; Mt: d3, p2, r1, v3; III Pa: d1; Ti: d1, r1, v1; Mt: d2, p1, r1, v1; IV: Ti: d1, r1; Mt: d1, p1, r1, v1. ***Abdomen*** (Fig. 10A, B) 1.17 long, 1.37 wide, ovoid, with a round and a fan-shaped black-brown spots dorsally; venter yellow, with black sloping stripes.

***Palp*** (Fig. 10D–G). Ventral tibial apophysis digitiform, shorter than tibia, apex slightly curved retrolaterally. Retrolateral tibial apophysis nearly as long as tibia, horn-like, slightly curved in retrolateral view, directed mostly dorsally. Tegular ridge arising from ~ 12 o'clock. Embolus stout, with broad base, apically furcate, arising from 9 o'clock and ending at ~ 1 o'clock.

**Female.** Unknown.

**Distribution.** Known only from the Fujian Province of China (Fig. 12).

## Acknowledgements

We are grateful to Guo Tang, David Kavanaugh, Heng-mei Yan, Ke-ji Guo, Bing Zhou, Wang Liu, Qu Cai, Xian-feng Huang, Da Li, Ji-he Liu, Ai-lan He, Jin-xin Liu,

Zong-guang Huang, Hui-juan Sheng, Yu-yang Zhou, Hai-tian Song, Zhi-hao Qi, Rong-xiang Su, Bi Ding and Yun-hu Mo for collecting the specimens. We also thank Dr Nathalie Yonow for improving the English of the manuscript and the three reviewers Dr Alexander A. Fomichev, Dr Suresh Benjamin, and Dr Ansie Dippenaar, as well as the subject editor Dr Ingi Agnarsson for providing detailed corrections and suggestions.

## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

### Funding

This study was supported by the Natural Science Foundation of China (32000301/32160243/32070429), the Fujian Forestry Science and Technology Project (2023FKJ19) and the Science and Technology Project of Fujian Province (2023R1051).

### Author contributions

Yong-hong Xiao, Xiang Xu and Ke-ke Liu desiged the MS. Cong-zheng Li and Yan-bin Yao provided the data. Ke-ke Liu drafted the early MS.

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### Data availability

All of the data that support the findings of this study are available in the main text.

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ZooKeys 1190: 213–257 (2024)
DOI: 10.3897/zookeys.1190.109586

Research Article

# Philippine herpetology (Amphibia, Reptilia), 20 years on: two decades of progress towards an increasingly collaborative, equitable, and inclusive approach to the study of the archipelago's amphibians and reptiles

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## Abstract

A first review of the history, status, and prospects for Philippine herpetology conducted more than two decades ago (2002) summarized the diverse topics studied and highlighted the development and achievements in research up to the year 2000. This study revisits and re-assesses what Philippine herpetology has accomplished, both as a discipline and a community, during the last two decades (2002–2022). A total of 423 herpetological publications was collated, revealing a substantial increase in annual publications, rising from approximately four per year during 2002–2008 to around 28 per year in 2009–2022. Half of the published studies focused on squamate reptiles (lizards 30.5%, snakes 21%) and 28.4% on amphibians, 5.9% on turtles, and 2.6% on crocodiles. The remaining 11.6% of studies focused simultaneously on multiple taxa (i.e., faunal inventories). Diversity and distribution (35.2%) and ecological (26.5%) studies remained popular, while studies on taxonomy (14.9%), phylogenetics and biogeography (11.8%), and conservation (11.6%) all increased. However, geographical gaps persist urging immediate surveys in many understudied regions of the country. Finally, we found a balanced representation between Filipino and foreign first authors (1.0:1.1), yet a substantial gender gap exists between male and female first authors (7.1:1.0). Nonetheless, the steep increase in publications and the diversity of people engaged in Philippine herpetology is a remarkable positive finding compared to the 20 years preceding the last review (1980–2000). Our hope is that the next decades will bring increasingly equitable, internationally collaborative, and broadly inclusive engagement in the study of amphibians and reptiles in the Philippines.

**Key words:** Biodiversity, conservation, distribution, Southeast Asia, systematics, taxonomy

OPEN ACCESS

**Academic editor:** Fedor Konstantinov
**Received:** 15 July 2023
**Accepted:** 4 January 2024
**Published:** 30 January 2024

**ZooBank:** https://zoobank.org/3C99730E-4FF6-4189-9BCD-C435E31A36DD

**Citation:** Meneses CG, Pitogo KME, Supsup CE, Brown RM (2024) Philippine herpetology (Amphibia, Reptilia), 20 years on: two decades of progress towards an increasingly collaborative, equitable, and inclusive approach to the study of the archipelago's amphibians and reptiles. ZooKeys 1190: 213–257. https://doi.org/10.3897/zookeys.1190.109586

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## Introduction

Sustained, regionally focused, field-based research programs focusing on the ecology and evolution of amphibians and reptiles provide insight into many conceptually intriguing, unique, and fundamental questions relating to the

* These authors contributed equally to this work.

origin, accumulation, and continued survival of Earth's biodiversity (Brown and Alcala 1970a; Brown et al. 2013a; Gainsbury and Meiri 2017; Greenberg et al. 2018; Meiri et al. 2020; Sheu et al. 2020; Zimin et al. 2022; Camaiti et al. 2023). Regionally oriented research by herpetological systematists, biogeographers, and macroecologists have elevated our understanding of the global phylogenetic and evolutionary underpinnings of amphibian and reptile biodiversity by relating the distribution of this diversity to the geographical template itself (Esselstyn et al. 2010; Böhm et al. 2013; Barley et al. 2014, 2015; Roll et al. 2017; Vidan et al. 2019; Gumbs et al. 2020; Bernstein et al. 2021a). Similarly, regional studies on topics ranging from natural histories of species from particular areas to global analyses of clade-wide trait variation (Vidan et al. 2019; Camaiti et al. 2022) and organismal genomic variation (Formenti et al. 2022; Chan et al. 2022; Hutter et al. 2022) now contribute significantly to our collective understanding of conservation challenges facing highly imperiled amphibian and reptile populations (Böhm et al. 2013; González-del-Pliego et al. 2019; Tingley et al. 2019; Gumbs et al. 2020; Chapple et al. 2021; Cox et al. 2022; McDonald et al. 2022; Womack et al. 2022; Slavenko et al. 2023).

The Philippine Archipelago, which has been the focus of sustained herpetological inquiry for more than a century (Taylor 1921, 1922a, b, 1928; Leviton 1963a; Brown and Alcala 1970a, 1978, 1980; Brown et al. 2002a, 2012b, 2013a; Diesmos and Brown 2011; Diesmos et al. 2014, 2015; Leviton et al. 2014, 2018), is situated adjacent to the Southeast Asian mainland, between the Western Philippine Sea and the Western Pacific Ocean (Fig. 1). This unique archipelago is home to numerous remarkable evolutionary radiations of amphibians and reptiles (Brown and Guttman 2002; Brown et al. 2002a, 2009a, b, 2013a, 2015a; Evans et al. 2003; Brown and Diesmos 2009; Siler and Brown 2010, 2011; Siler et al. 2010a, b, c, d, 2011a, b, c, 2013, 2014a, b, c; Welton et al. 2013a; 2014a, b; Weinell and Brown 2017; Weinell et al. 2020a, b, c Chan et al. 2021, 2022; Flury et al. 2021), many of which have only recently been characterized, and some of which remain to be studied in depth (e.g., Brown et al. 2010a, 2011a, b, 2012a; Linkem et al. 2010a, b, c; Linkem and Brown 2013; Wynn et al. 2016; Oliver et al. 2018; Davis et al. 2020; Bernstein et al. 2021b; Eliades et al. 2021). Currently, there are approximately 475 recognized species of amphibians and reptiles in the Philippines, 76.2% of which are endemic, and most of which can be characterized as (1) geographically circumscribed species, whose distributions coincide with one of the archipelago's 5–7 major faunal regions (Brown and Alcala 1970a, 1978, 1980; Brown et al. 2017); (2) range-restricted species from isolated islands (e.g., Brown and Alcala 1974; Brown et al. 1997; Ferner et al. 2001; Linkem et al. 2010a; Oliveros et al. 2011; Brown et al. 2018; Meneses et al. 2022), or (3) species limited to geologically isolated and/or upland habitats (e.g., Brown and Alcala 1961, 1970b, 1982a; Ferner et al. 1997; Brown et al. 1999a, b, 2020; Linkem et al. 2010b; Siler at al. 2010c). However, this estimate is expected to change over time with ongoing biodiversity inventories, taxonomic revisionary studies, critical reappraisals of earlier works, and the novel application of technologies (e.g., genomic data, ecological niche modeling) still emerging today (Brown et al. 2002a; Brown 2006; Brown and Siler 2013; Diesmos et al. 2015).

Just more than two decades ago, Brown et al. (2002a) conducted a comprehensive review of Philippine herpetological studies. The authors summarized the

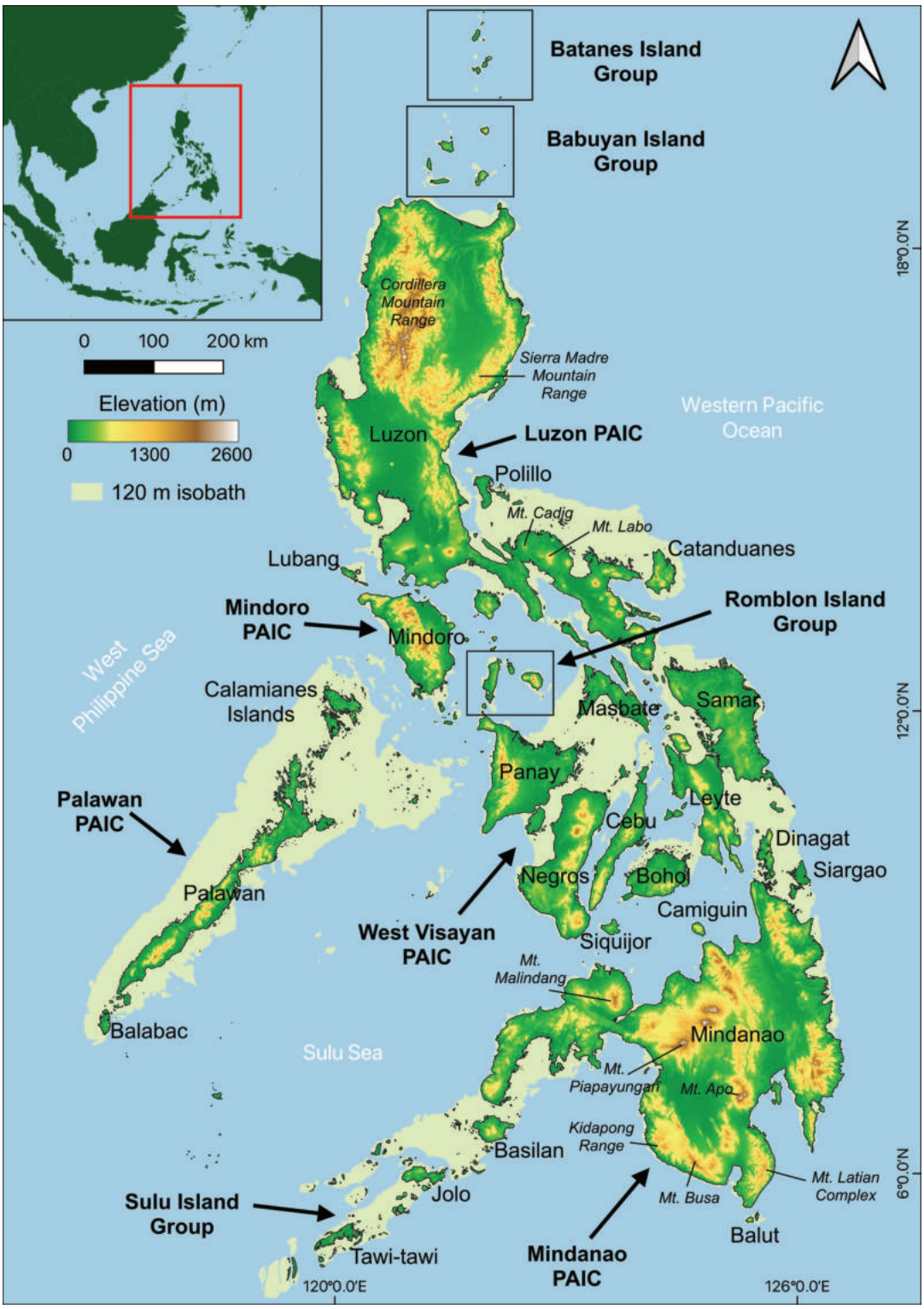


**Figure 1.** Map of the Philippine archipelago, situated in Southeast Asia (inset map), showing the recognized Pleistocene Aggregate Island Complexes (PAICs) and small island groups.

history of amphibian and reptile studies and included the distinct periods which characterized the development of herpetological research in the country, highlighting the important contributions of biologists during the last decades leading up to the turn of the century. That review centered on five topical themes or areas of research focus: (1) diversity and distribution, (2) taxonomy, (3) ecology,

(4) phylogenetic systematics and biogeography, and (5) conservation. As a result of that exercise, it became abundantly clear that most of the archipelago's earlier herpetological studies (prior to the 2000s) were predominantly focused on species diversity, taxonomy, and biogeography (and, to a lesser extent, ecology, and conservation). Despite the many papers focused on single species descriptions, but also including synthetic reviews (e.g., Brown and Alcala 1978, 1980), an immense amount of taxonomic work was still needed by the early 2000s. This is because the majority of studies to that date were descriptive, based solely on traditional morphological characters (i.e., measurements, meristic data like scale counts, and comparisons of discrete character states), and only selected clades had been comprehensively reviewed with the goal of synthetic considerations of those particular faunal groups (Taylor 1921, 1922a, b; Inger 1954; Leviton and Brown 1958; Leviton 1962, 1963a, b, 1964a, b, c, d, 1965a, b, 1967, 1968, 1979, 1983; Brown and Alcala 1974, 1980, 1994). As discussed by Brown (2006, 2007) and Brown and Stuart (2012), the use of multiple data streams and integrative approaches (including phylogeny) for more robust, pluralistic, and quantitative approaches to species recognition was just in their infancy (Brown and Diesmos 2002; Brown and Guttman 2002; Diesmos et al. 2002; Brown et al. 2003).

Brown et al. (2002a) also highlighted some of the gaps in other research areas. For instance, on the subject of biogeography and conservation, they emphasized how understanding patterns of Philippine amphibian and reptile distributions would be essential to formulating effective conservation and management strategies (Brown and Alcala 1961, 1986; Alcala and Custodio 1995; Diesmos et al. 2002). However, at the time of their review, Brown et al. (2002a) emphasized that the full informative potential of a comprehensive understanding of verified species distributions had not been fully realized due to limited information from many unexplored islands groups (i.e., the Batanes, Bubuyans, Lubang, Leyte, Masbate, Mindoro, the Romblon Island Group, Siquijor, the Sulu Archipelago, and Samar), as well as numerous high-elevation mountain ranges of the archipelago's largest islands, Luzon and Mindanao. At that time (Brown et al. 2002a), documentation of patterns of species occurrences and community structure along elevational gradients was just beginning to take shape (Brown and Alcala 1961; Custodio 1986; Alcala and Custodio 1995; Alcala et al. 1995; Brown et al. 1996, 2000a; Heaney et al. 2000). Numerous other topics were highlighted, including formative areas of research that had become a focus by mid-century, but for which no follow-up investigations had been forthcoming during the last 20–40 years (Brown et al. 2002a). These included studies of reproductive biology (Alcala and Brown 1956, 1982; Alcala 1962; Brown and Alcala 1982b), physiology (Alcala and Brown 1966), development (Alcala 1962; Alcala and Brown 1982), and basic population biology and demography (Alcala 1967, 1970; Alcala and Brown 1967).

In general, Brown et al. (2002a) emphasized that these conspicuous gaps in Philippine herpetological research would most likely be addressed most effectively through collaborative efforts of teams of institutions (universities, local government units, non-governmental organizations, stakeholder communities) and the participation of diverse groups of foreign and local researchers, students, and local community representatives. Ultimately, the results accessible via open-access peer-reviewed publications–such studies reinforced by integrative analyses of multiple data types that are freely accessible through

web-based platforms (e.g., HerpNet, VertNet, etc.) and specimen-associated data provided by natural history museums–could be harnessed in such a way that a more inclusive, transparent, broadly participatory future of Philippine herpetology could be realized (e.g., Brown et al. 2012b, 2013b). With the turn of the millennium and given the inevitable shift in herpetological research that was coming, Brown et al. (2002a) reviewed and took stock of the accomplishments, discoveries, strengths, and shortcomings of what they envisioned as a global community of herpetologists interested in Philippine biodiversity. Today, we revisit that same general topic, but we do so with the sense that another, pronounced, and unique period of the historical development of herpetology in the Philippines is coming in the years ahead.

In this paper, we revisit studies conducted from 2002–2022 to re-assess the state of Philippine herpetological research, 20 years after the review of Brown et al. (2002a). We summarized the last two decades of published studies in five general areas or topical themes, as discussed in the previous review. One of our goals was to explore whether these studies addressed gaps previously identified (Brown et al. 2002a). Trends during the last two decades of taxonomic studies were examined, including attention to data types (e.g., morphology versus molecular data), modern quantitative methodology (e.g., phylogenetics, advanced statistical procedures, quantitative biogeographical inference), and how these were integrated or used in statistical species delimitation (e.g., Barley et al. 2013; Welton et al. 2013a). We also sought to obtain an explicitly geographical or spatial overview of the last twenty years of research and identify priority areas that receive little attention and now represent challenges for the future. Finally, to determine who is primarily involved in Philippine herpetological research–and to critically and transparently address the question of diversity, equity, and inclusion in our field (Have we broadened engagement? Is our community more diverse now than it was a quarter century ago?)–we classified published studies by the lead author's nationality and gender and explored the diversity of people who study Philippine herpetology.

## Methods

### Literature review

We employed a systematic literature review of Philippine herpetology published from 2002–2022, following the guidelines from the updated Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Page et al. 2021). We compiled a dataset of peer-reviewed journal articles by searching through Google Scholar and Web of Science. The following keywords were used: "Philippine amphibians," "Philippine reptiles," "Philippine lizards," "Philippine snakes," and "Philippine anurans/frogs." The list of papers compiled was manually supplemented to include articles not captured in the preliminary search. This includes articles addressing other areas or topics, but which included substantial Philippine specimens (or data derived from Philippine studies, and now available in the public domain) to inform their results, and other articles published in locally refereed scientific journals. We made every effort to be as comprehensive as possible, but it is conceivable that a few published papers meeting our criteria for inclusion may have been missed by our search process. Nevertheless,

our objective was primarily focused on capturing and characterizing the general trends and overall patterns, which may be inferred from appropriately, ethically, and transparently communicated (i.e., subject to peer-review, properly documented, accessibly archived, and demonstrably repeatable) scientific research in topics relating to the biology of Philippine amphibians and reptiles.

For each article, we extracted the following information to determine trends and patterns in Philippine herpetological research: (1) year of publication, (2) major focal taxon (amphibians [frogs/toads or caecilians], lizards, snakes, crocodiles, turtles, and a combined category "multiple taxa" for studies involving combinations of each major group), and each paper's (3) research theme as discussed above (see Table 1). Then, we performed a Poisson regression and local polynomial regression to test for a trend in the number of papers published during the last two decades. Because we wanted to address the technological advances of the last quarter century, we characterized the variable categories of evidence, employed by papers that included new species descriptions (see also Brown and Stuart 2012). We achieved this by classifying every taxonomic publication according to the type of data that was used (e.g., morphological, bioacoustic, molecular, and/or combinations of these data).

**Table 1.** The categories used to identify types of peer-reviewed papers involving Philippine herpetology published from 2002–2022 (based on the five topical themes of Brown et al. 2002a).

| Category | Description |
|---|---|
| Diversity and distribution | surveys, checklists, distributions (range extensions, new island records), and measures of diversity |
| Taxonomy | species descriptions and taxonomic revisions |
| Ecology | natural history, community ecology, and population biology |
| Phylogenetics and biogeography | phylogenetics (excluding new species description), evolutionary biology, and biogeography |
| Conservation | conservation, outreach, threats, and methods |

## Geographical patterns

Available geographic coordinates were extracted from articles focusing on new species descriptions, species distribution records, natural history notes, and targeted herpetological inventories. To assess country-wide geographical patterns of published herpetological studies from the literature, we projected occurrence records on a map of the archipelago and georeferenced all points by referring to museum records and biodiversity information resources (below), if necessary. For articles that did not report geographic coordinates but which did include specific locality information (e.g., island, province, municipality, barangay, or other unique identifiers), we georeferenced occurrence data in Quantum GIS v. 3.22 using the Philippine gazetteer available from DIV-GIS Database (https://www.diva-gis.org/). We included regions, areas, or single sites, which have been recently surveyed, and for which all specimen-associated data have been properly curated (e.g., in museum databases linked to accessible voucher specimens, ensuring repeatability and transparency; and from the community of the biodiversity repository institutions which provide unrestricted access to specimen-associated data) and published in accessible, publicly available databases such as the Global Biodiversity Information Facility (GBIF; https://www.gbif.org/), VertNet (http://vertnet.org/), and iDigBio (https://www.idigbio.org).

Our presentation of geographical patterns made use of the last half-century's prevailing context for biogeographical studies in the archipelago, namely the Pleistocene Aggregate Island Complexes (PAIC) model (Brown and Diesmos 2002; Brown and Guttman 2002; Brown et al. 2013a). This model attempts to capture the biogeographic terrestrial subregions of the archipelago based on earlier studies that traced underwater bathymetric contours around the archipelago's major landmasses (Inger 1954; Leviton 1963a; Brown and Alcala 1970a), so as to reflect patterns of island amalgamations, terrestrial connections involving major landmasses and adjacent island banks, and other exposure of land caused by oscillating Pleistocene sea levels (review: Brown et al. 2013a). Together, the frequently discussed "PAIC Paradigm" illustrates the locations of simplified, reasonably accurate, inferred land connections that may have partitioned and isolated terrestrial vertebrates into the variable and distinct faunal compositions we find today, on the islands which together make up each unique faunal subregion: The Luzon PAIC, and those of Mindanao, Mindoro, West Visayan (also referred to as Negros-Panay), Palawan, and the smaller Romblon Island Group (RIG), the Sulu Island Group (SIG) (Fig. 1), and a few small islands which are associated with larger landmasses but which were never fully connected to them. This latter group includes Siquijor Island, adjacent to the West Visayan PAIC; Camiguin Sur Island, adjacent to the Mindanao PAIC); and finally, isolated island groups that form minor but unique subcenters of biodiversity, but which are not part of or strongly associated with, the major PAICs (e.g., Babuyan and Batanes Island Groups, north of Luzon).

### First authorship nationality and gender gaps

Finally, as a simplified but important general first step towards exploring disparity and gender gaps in Philippine herpetology, we also determined the nationality (Filipino vs all non-Filipino nationalities/"foreign") and traditional biological gender (male vs female) of the first author of each article. Although we feel this somewhat crude, excessively binary view will not capture nuances needed to truly assess equity gaps in Philippine herpetology, we consider it a first step and a point from which we hope future discussions and steps towards broadening engagement can begin (see Ramírez-Castañeda et al. 2022). In particular, we emphasize that our use of the term "gender" and its binary assignment in this preliminary analysis is used solely for the purposes of assessing gender gaps in Philippine herpetological research and does not imply a binary nature of the term (see Rock et al. 2021). All analyses and visualizations were performed in R Studio v. 4.2.2 (R Core Team 2022). R code and documentation (R markdown HTML, Suppl. materials 1, 2) are available on GitHub (https://github.com/csupsup/PhilHerpsRev).

## Results

We compiled a total of 423 peer-reviewed scientific articles on Philippine herpetology, published from 2002–2022 (see Suppl. material 1). There was a significant increase in publications during the years based on our Poisson regression analysis ($X^2_{(1,\ n\ =\ 21)} = 131.9$, $p < 0.001$). Approximately four publications per year, for the majority of the first decade (2002–2008), was a general trend that

increased sharply to approximately 28 publications per year across the second decade (2009–2022; Fig. 2). The years with the highest number of publications were all during the past 11 years: 2020 (*n* = 47), 2021 (*n* = 42), 2014 (*n* = 32), 2011 (*n* = 31), and 2022 (*n* = 31). Notably, this dramatic seven-fold increase in publication rates during the last 11 years, comprises a remarkable 83.4% of all publications in Philippine herpetology since 2002.

## Research themes

Of the 423 articles we reviewed, half the papers were conducted primarily on reptiles, with lizards and snakes comprising nearly equivalent proportions of the total, or 30.5% (*n* = 129), and 21% (*n* = 89), respectively (Fig. 3A). Amphibians comprised 28.4% (*n* = 120), turtles 5.9% (*n* = 25), and crocodiles 2.6% (*n* = 11). The remaining papers involved multiple taxa (11.6%, *n* = 49), with the predominant combinations of taxa most often employed by regional faunal studies: including all amphibian and reptile species recorded for a given island, region, local area, or specific site (Fig. 3A). Of these same 423 papers, 35.2% fell under the category Diversity and Distribution (*n* = 149), 26.5% were focused on Ecology (*n* = 112), 14.9% constituted Taxonomy (*n* = 63), 11.8% were classified as Phylogenetics and Biogeography (*n* = 50), and 11.6% were in the Conservation (*n* = 49) category (Fig. 3B).

A quarter of the Diversity and Distribution studies were targeted toward herpetological inventories and surveys (multiple taxa, 25.8%), while the remaining constituted brief distribution reports on amphibians (30.2%) and reptiles (45%), most of which consisted of single occurrence records, provided for a single species, not previously reported for that island, region, or province. Studies in Ecology, including short natural history notes, were almost equally represented among amphibians (31.2%), lizards (20.4%), and snakes (26.8%) but with few papers on turtles (7.1%) and crocodiles (4.5%). Notably, most publications in taxonomy during the last two decades focused on lizards (63.5%), with amphibians and snakes comprising 20.6% and 15.9%, respectively. Similarly, Phylogenetics and Biogeography publications primarily were conducted on lizards (44%), followed by amphibians (34%), and snakes (12%). All taxa were represented well in Conservation studies (10–20%).

## New species descriptions

A total of 95 herpetological species (72 lizards, 14 amphibians, and 9 snakes) was reported as new to science and formally described during a prolific period of taxonomic activity spanning the last two decades (Fig. 4A). New species descriptions started to increase in 2009 with peaks in 2010 (18 new species), 2014 (10 new species), and 2020 (11 new species). Species description papers employing only morphological data were published more frequently between 2000 and 2012 but continue to decrease until the present time. Although the first use of molecular data (primarily mitochondrial DNA sequences) in systematic and biogeographical studies of Philippine herpetofauna occurred earlier, the use of molecular data as a form of evidence to justify new species recognition in taxonomic publications first began as a

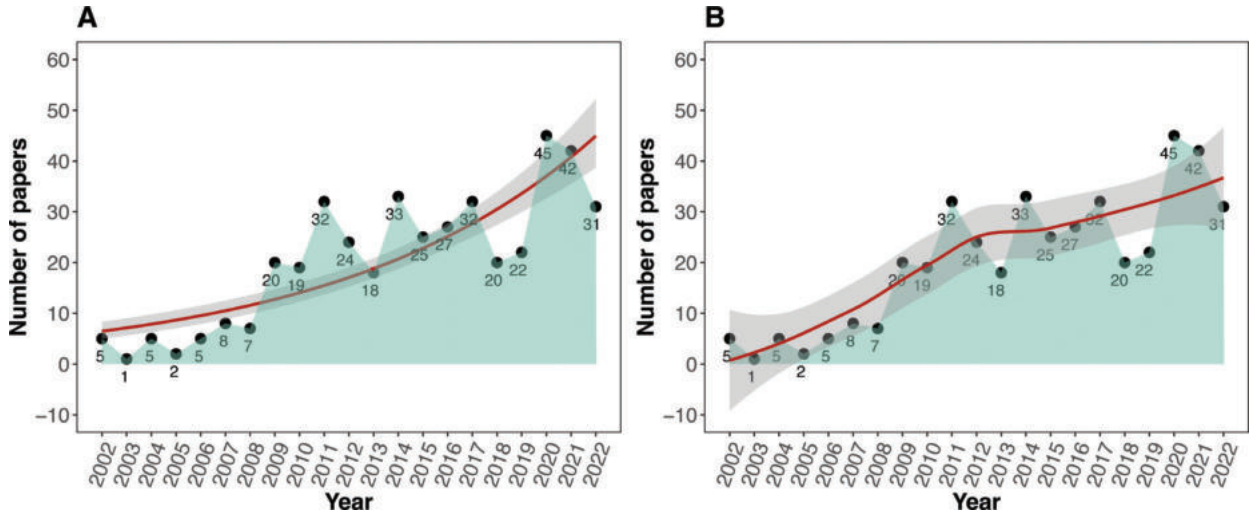


**Figure 2.** Philippine herpetological research papers published (2002–2022) **A** with Poisson regression line and **B** local polynomial regression. The gray shading represents the 95% confidence interval of the regression models, and the light green shade indicates the trend in terms of the number of papers published per year.

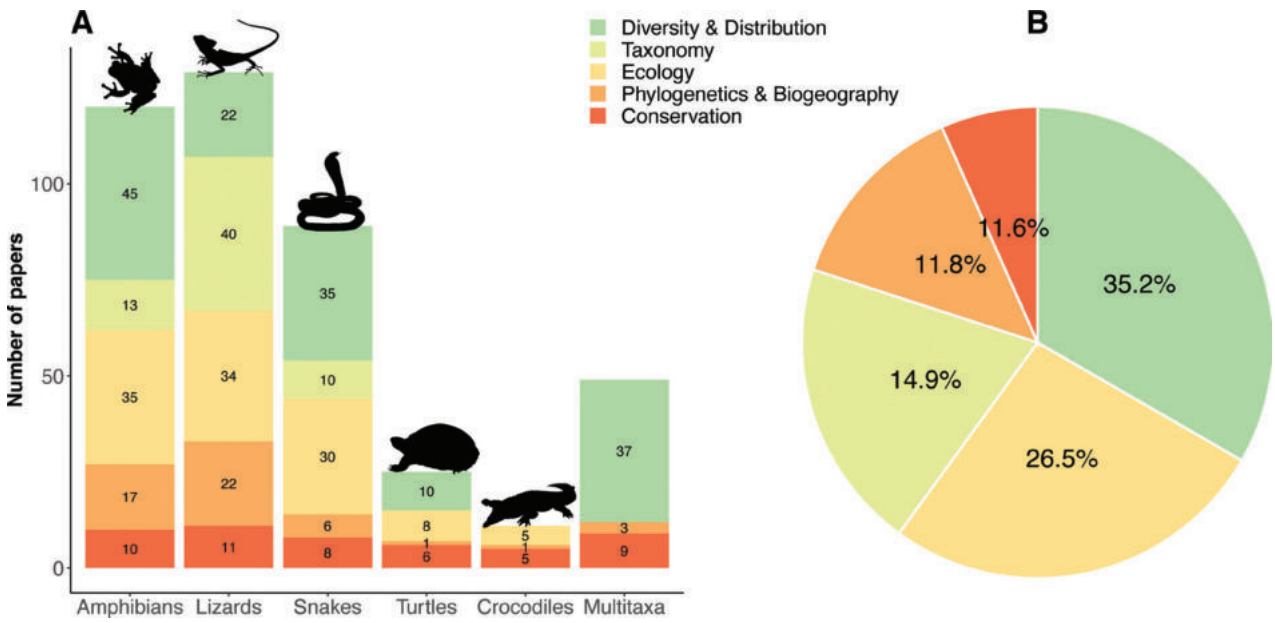


**Figure 3.** Total of published papers compiled (2002–2022) **A** category per taxon and **B** category proportions in percentages.

practice in 2009 and steadily increased, eventually becoming nearly (but not quite) routine by 2022 (Fig. 4B). When we mapped the geographical position of origin for holotype specimens of these new species (see Suppl. material 2), we found that most of the holotypes originated on Luzon PAIC islands (43.2%) or were from the Mindanao PAIC landmasses (22.1%), whereas few came from the Palawan PAIC (9.5%), the West Visayan PAIC (8.4%), Mindoro Island/PAIC (7.4%), small islands of Romblon Province (RIG; 3.2%), the Sulu Archipelago (SIG; 2.1%), the Babuyan and/or Batanes groups of islands (2.1%) and the isolated island of Camiguin Sur (2.1%) (Fig. 5A; total number of points = 95).

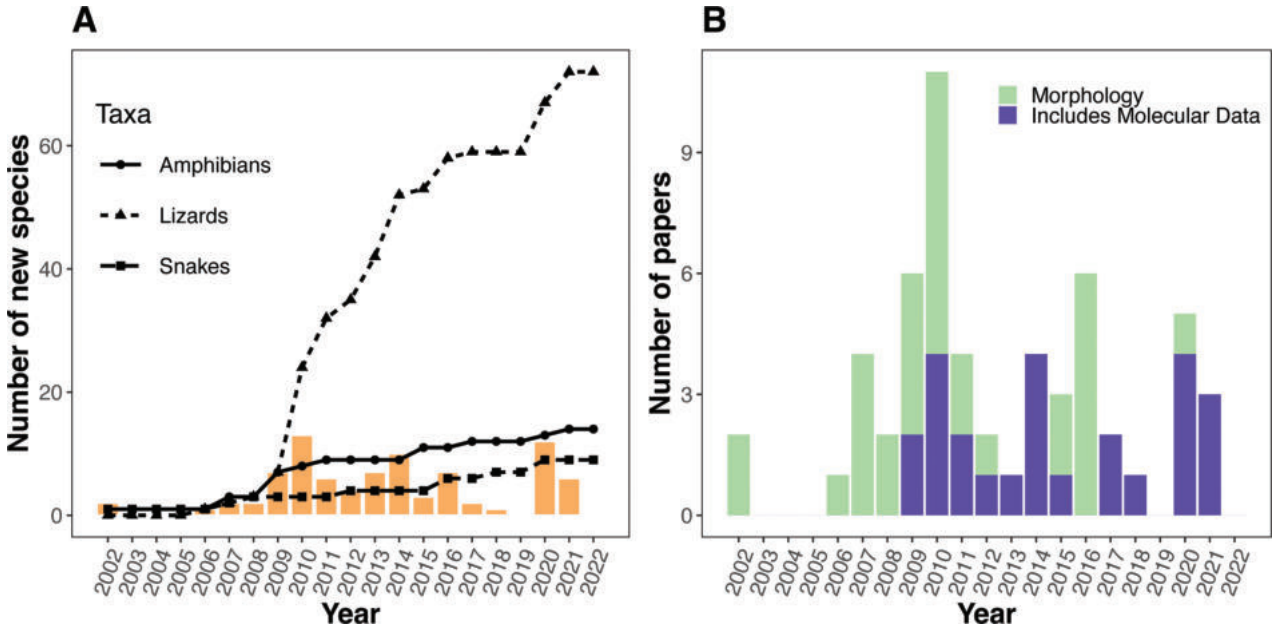


**Figure 4. A** Accumulation curves depicting the relationships between the cumulative total number of species per taxon (key) and the year of description. The orange bars indicate the number of new species described annually **B** number of papers published under the Taxonomy category, grouped by data type.

## Geographical patterns

Brief distribution records, focused on single occurrences for single species from areas where they had not previously been reported were published primarily in one journal (Herpetological Review), on the basis of records from the PAICs of Mindanao (31.5%), Luzon (27%), and the West Visayas (16.9%) (Fig. 5B; total number of points = 89; see Suppl. material 2). The majority of new distribution records from the Mindanao PAIC were from the central and southern regions of this large island, with few notes reporting occurrences of particular species from the eastern and western regions of Mindanao or from the northern Mindanao PAIC islands of Samar, Leyte, and Bohol. In contrast, single species distribution records from the Luzon PAIC were not grouped geographically and were more randomly distributed (Fig. 5B). On the West Visayan PAIC, the islands of Cebu, Negros, and Masbate were the focus of most new geographical distribution records. Records from Palawan (11.2%) primarily were from the northernmost portion of this island, as well as the Calamian group of islands, to its north. The remaining distribution records originated in the RIG (6.7%), SIG (2.2%), Mindoro Island/PAIC (2.2%), the Batanes Island Group (1.1%), and Siquijor (1.1%). Occurrence points from natural history notes (Fig. 5B; total number of points = 83) were biased towards the two largest PAICs, Luzon (45.7%) and Mindanao (37.3%).

Targeted herpetological surveys or sustained site-based studies attempting to characterize comprehensively, whole island communities, or regional faunas were concentrated during the last 20 years most heavily on the Luzon (35.6%) and Mindanao (33.1%) PAICs (Fig. 5C; total number of points = 247). Within the Luzon PAIC, targeted fieldwork was conducted mostly in several more northern regions of this large island, but a few sites were sampled well, along southern Luzon's Bicol Peninsula. Within the Mindanao PAIC, surveys were conducted primarily in the northern and northeastern regions (including several large

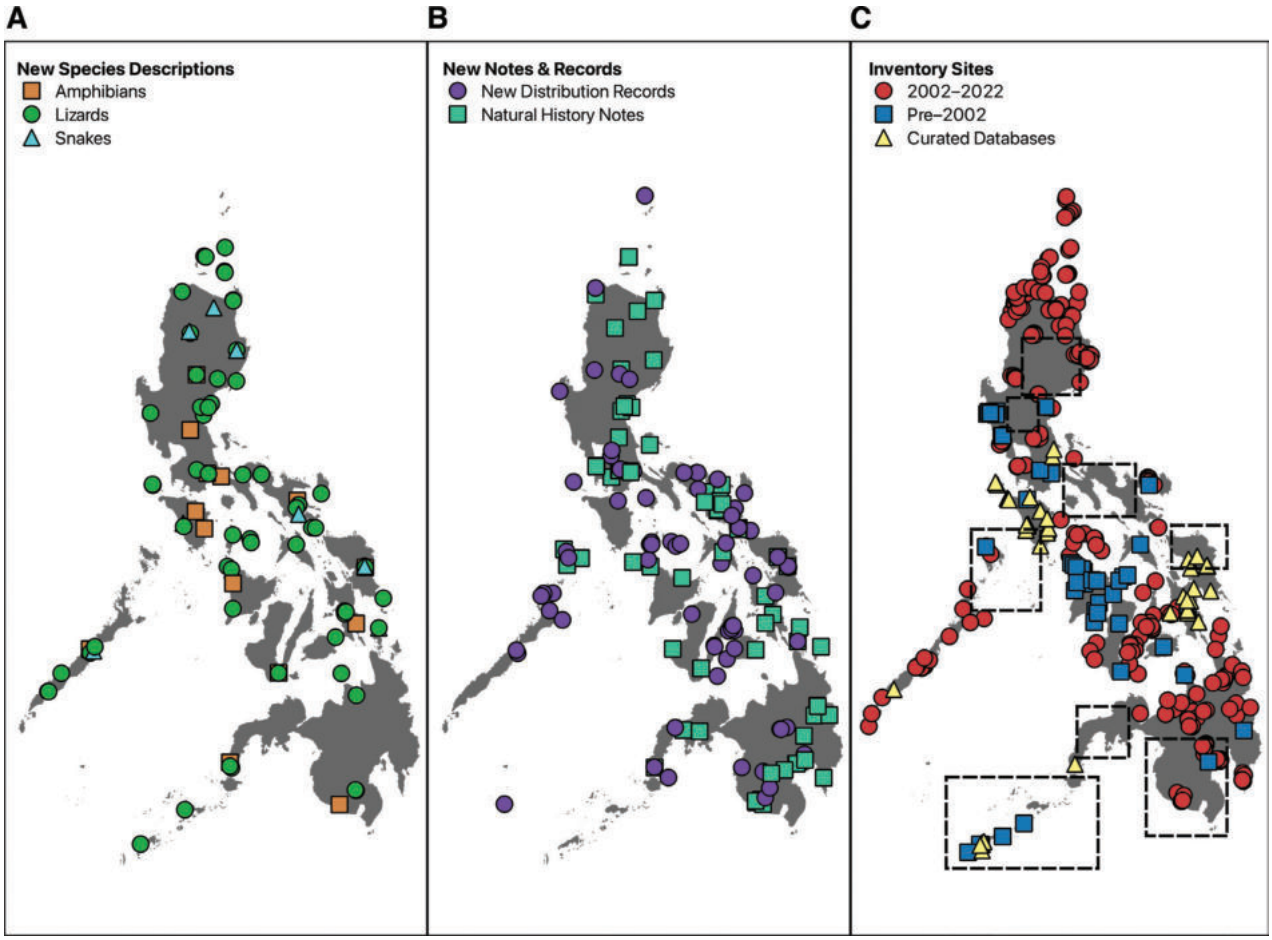


**Figure 5.** Maps showing the type localities of **A** new species described in the past two decades **B** localities of new distribution records and natural history notes, and **C** the inventory sites available from publications and curated databases. Dashed squares indicate understudied regions (**C**) where comprehensive surveys have not yet been published, but natural history notes and new geographical records have begun to fill spatial gaps.

mountains on northern Mindanao Island itself, but also on the East Visayan islands of Samar and Leyte). Only a relatively few sites have been sampled reasonably well in the southern portion of Mindanao Island during the last two decades. The West Visayan PAIC received attention in the form of a few surveys (10.1%) in recent years, most notably on Cebu, Panay, and Negros Islands. Approximately equal numbers of survey points were collated from the RIG (5.6%), Palawan (4.8%), and the island groups of Babuyan (5.3%) and Batanes (3.2%). There were also survey efforts in Camiguin Sur (1.6%) and Siquijor (0.4%).

## First authorship nationality and gender gaps

We found approximately equal cases of Filipino- versus foreign-first authorships (1.0:1.1), with the number of Filipino first authors steadily increasing during the period of 2002–2022 (Fig. 6A). In the last decade (2013–2022), 63.7% of papers were first-authored by Filipinos which is a three-fold increase from the 2002–2012 estimate (21.1%). We identified a wide gender gap in Philippine herpetology, with males outnumbering females when quantified as a function of first authorship (7.1:1.0) during the past 20 years; only approximately 12.3% of all papers were first-authored by females (Fig. 6B).

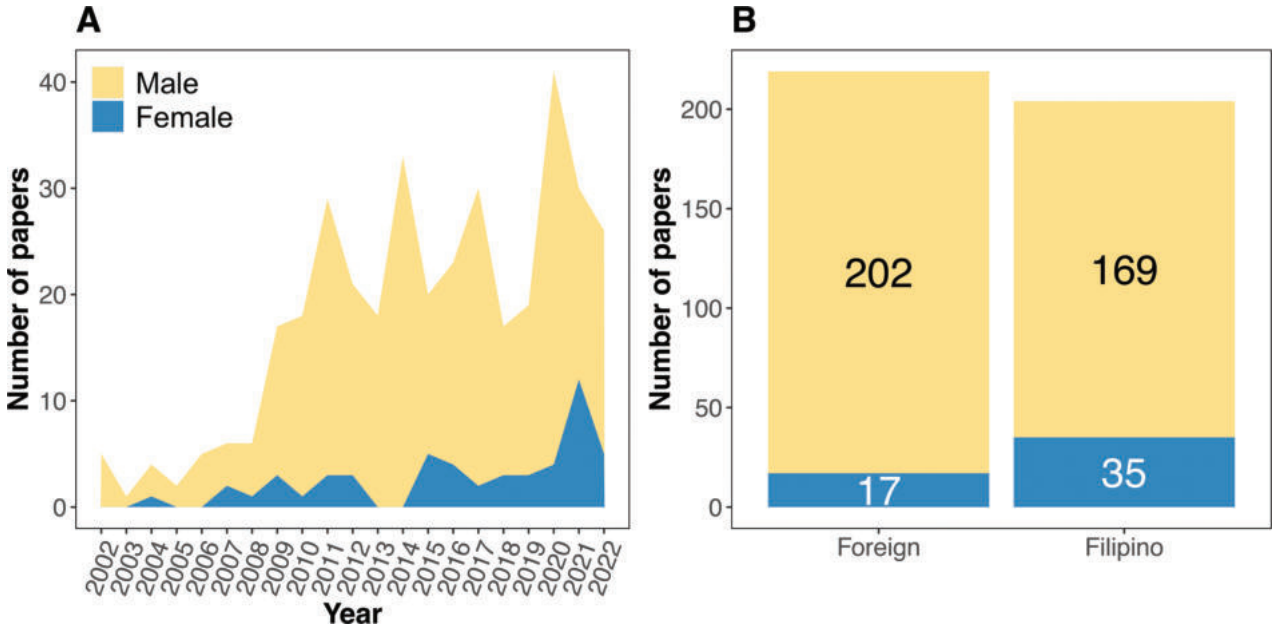


Figure 6. The total number of papers grouped by the first author's **A** gender (7.13 male: 1.0 female) and **B** nationality (1.07 foreign: 1.0 Filipino).

## Discussion

We are greatly encouraged by the increasingly collaborative and much more equitable and inclusive nature of Philippine herpetology. Great strides have been made during the past two decades in terms of our collective knowledge of herpetological biodiversity in the archipelago, public awareness and interest in amphibians and reptiles, and an increasingly vocal environmentalist movement–combined with the establishment of new protected areas and new laws for protecting historically underappreciated groups such as amphibians and reptiles (Diesmos and Brown 2011; Brown et al. 2012a; Diesmos et al. 2014; Gonzalez et al. 2018). The past 20 years provide us with a clearer picture of the progress made, gaps filled and unfilled, and collaborations formed, which now serve as a springboard for or guide to additional progress in the coming decade for our community.

### Herpetology studies during the last two decades

Our appreciation of the diversity and distribution of amphibians and reptiles in the Philippines has increased during the last 20 years, with the continued efforts towards conducting targeted faunal inventories and resurveys, supplemented by short distribution notes reporting single species occurrences (Brown et al. 2012a, 2013a). Faunal inventories have dominated herpetological studies during the last two decades, which is beneficial for informing habitat conservation measures (Margules and Pressey 2000) and these works positively impact species conservation assessments (see Gonzalez et al. 2018; IUCN 2022). Importantly, data from these studies help address knowledge gaps in species distributions, broaden stakeholder inclusion and participation in Philippine herpetology, and improve our collective knowledge and understanding of biodiversity (Hortal et al. 2015).

Region-wide herpetological inventories in recent years covered a wide range of habitats and utilized multiple datasets like field records and museum specimens (Oliveros et al. 2011; Siler et al. 2011d, 2012b; Brown et al. 2012a, 2013b; Devan-Song and Brown 2012; Mcleod et al. 2012; Sanguila et al. 2016; Supsup et al. 2016). Complementing these regional inventories were site-based studies along different environmental gradients in inland montane habitats (Alcala et al. 2004; Plaza and Sanguila 2015; Supsup et al. 2017, 2020; Gojo-Cruz et al. 2018; Pitogo et al. 2021a; Sanguila et al. 2021) and island ecosystems (Bucol et al. 2011; Siler et al. 2012a; Venturina et al. 2020; Clores et al. 2021; Meneses et al. 2022). Species accounts resulting from these targeted surveys provide a much clearer picture of herpetological communities occurring in several critically threatened habitats, which serve as important baseline studies to track long-term changes in amphibian and reptile communities over time (e.g., Brown et al. 2000a, vs Siler et al. 2011d; Siler et al. 2012b, vs Meneses et al. 2022; Brown and Alcala 1986, vs Supsup et al. 2016; Brown 2015, vs Pitogo et al. 2021a). Continued conducting of targeted surveys and resurveys are still urgently needed to gather empirical field data and species occurrences throughout the Philippines, especially in understudied regions, small islands (see discussion on geographical patterns), and other threatened habitats (e.g., karst habitats, lowland forest fragments, and understudied protected areas). Repeated resurveys spanning time, seasonal variation, space, and disturbance (both anthropogenic [deforestation, mining, road-building] and natural [typhoons, earthquakes, tsunamis]; Peterson et al. 2017) in key areas and habitat types, whenever resources are available, are strongly recommended (Brown et al. 2012a; Sanguila et al. 2016).

Many of the ecology studies of Philippine herpetofauna are short observations on species' reproductive biology, natural history, habitat use, diet, and behavior (see Suppl. material 1); all of these biodiversity information products supplement our limited understanding of the natural history of many endemic and native species (i.e., Meneses 2020; Brown et al. 2021a, b, c; Maglangit et al. 2021; Pitogo 2021). However, more in-depth ecological and herpetological community composition studies have bolstered patterns that were apparent two decades ago (Brown et al. 2002a) and revealed new trends. Elevational studies have repeatedly characterized a positive relationship between elevation and proportional endemism (the portion of species at a given site that is endemic to the Philippines), but an inversely proportional relationship between elevation and species diversity (Brown and Alcala 1961; Brown et al. 2000a; Ferner et al. 2001; Diesmos et al. 2004a; Siler et al. 2011d; Brown et al. 2013a; Pitogo et al. 2021b; Brown et al. 2022; Meneses et al. 2022), with a potential mid-elevation peak in species diversity was detected in some areas (Gojo-Cruz et al. 2019; Supsup et al. 2022). There is evidence that elevation strongly shapes broad-scale distribution patterns of Philippine herpetological communities, but other habitat characteristics were also found to influence the fine-scale distribution of species (Siler et al. 2011c; Pitogo et al. 2021b; Seidl et al. 2019; Supsup et al. 2020, 2022). Functional traits potentially drive distribution patterns (Pitogo et al. 2021b) and may influence species' responses to environmental changes (Scheffers et al. 2013). Resource partitioning has also been recently investigated in amphibians (Shooman-Goodier et al. 2019; Plaza et al. 2021) and reptiles (Garcia et al. 2014; see also Auffenberg and Auffenberg 1988), improving our

knowledge of trophic interactions among co-occurring species. These hypothesis-driven studies are welcomed developments in Philippine herpetology and should pave the way for a greater understanding of herpetological communities, limiting resources, and habitats critical for individual species' persistence and community resilience. Despite advances in research focusing on community ecology, there has been limited progress in studies involving reproductive biology, development (see Flores et al. 2023), physiology, and behavior (Lama and Senarillos 2023) during the past 20 years–as was also the case during the first review (Brown et al. 2002a).

Complementing field-based studies are works utilizing genetic data to understand the evolutionary history and phylogenetic relationships of species. The subdisciplines of model-based statistical phylogenetic systematics (Hillis et al. 1996) and quantitative biogeographical inference (Ronquist 1997) were just emerging at the time of the first review (Brown et al. 2002a). During the last two decades, numerous studies involving robust genetic datasets from Philippine species (and/or whole clades), from multilocus Sanger sequence datasets to genome-scale datasets, orders of magnitude more expansive, have provided inference into biogeographic patterns and a diversity of underlying processes. These studies have contributed greatly to our appreciation of true species diversity, species' distributions, routes of colonization, and the dynamic geographical template's role in contributing to evolutionary diversification within the archipelago (Brown and Diesmos 2009; Blackburn et al. 2010, 2013; Siler et al. 2012a, 2014a, b, c; Brown et al. 2013a, 2015a, 2016; Linkem et al. 2013a; Oaks et al. 2013, 2019, 2022; Brown 2016; Chan and Brown 2017). These studies also provide insights into surprising and intriguing evolutionary relationships not observed in other vertebrate groups (Weinell and Brown 2017; Meneses et al. 2020a; Weinell et al. 2020b), and patterns of cryptic speciation (Sanguila et al. 2011; Barley et al. 2013, 2021; Linkem and Brown 2013; Welton et al. 2013a, 2017; Weinell et al. 2020a, c; Abraham et al. 2021; Chan et al. 2021), many of which have informed taxonomic developments, fueling the description of nearly 100 new species during the past twenty years (see Taxonomic Progress, below). We expect an increase in this type of work in coming years, especially with the widespread availability and declining cost of genomic data (Carter et al. 2023), which increases accuracy in phylogenetic inference, while greatly clarifying previously obfuscated species boundaries by allowing nuanced insight into related phenomena, such as gene flow, admixture, hybridization, lineage sorting, and retention of ancestral polymorphism (Alexander et al. 2016; Wood et al. 2020; Chan et al. 2021).

## Conservation of Philippine herpetofauna

The last two decades involved increasing numbers of conservation studies, particularly focused on large and charismatic species like crocodiles (van de Ven et al. 2009; van der Ploeg et al. 2011; Brown et al. 2021a), turtles (Abreo et al. 2016; Sy et al. 2020), monitor lizards (Welton et al. 2013b, 2020; Abaño-Sarigumba et al. 2018), and sailfin lizards (Siler et al. 2014d; Heinrich et al. 2021). These species are most often the highly traded and threatened species in the Philippines (Cruz and Lagunzad 2021). Although amphibians receive less attention in the conservation literature, several efforts were made to highlight their high

vulnerability to environmental perturbations and climatic changes (Alcala et al. 2004, 2012a, b; Brown et al. 2012a; Diesmos 2012; Diesmos et al. 2014). Recent amphibian rediscoveries of "lost" species have captured public attention (e.g., Bittel 2015; Pitogo and Saavedra 2021), which bolsters public interest and stimulates conservation research and action in overlooked taxa (e.g., Brown and Alcala 2000; Pitogo and Saavedra 2023). Overall, peer-reviewed literature on the conservation of Philippine herpetofauna has increased substantially from 20 years ago (Brown et al. 2002a). We are encouraged by these developments and hope to maintain this momentum, build further on these gains (including the development of more outreach materials in support of conservation objectives), and strive to improve the conservation attention to Philippine amphibians and reptiles (Brown et al. 2012a; Gonzalez et al. 2018).

Based on the most recent and updated IUCN assessments on Philippine herpetofauna (ca. 475 species), approximately 13.2% (63 species) and 13% (62 species) are threatened and data deficient, respectively (IUCN 2023). Upwards of 5.9% (28 species) are still unassessed. Notably, 131 of these 153 species are Philippine endemics, many of which have not been observed since their original descriptions. Many additional unrecognized and unprotected species await taxonomic descriptions and, as such, are at increased risk of extinction (McDonald et al. 2022). Continued lowland habitat loss, brought about by forest conversion into less complex habitats that are not suitable to support high levels of biodiversity is likely the most substantial threat to these taxa (Heaney and Regalado 1998; Brown et al. 2002a; Diesmos et al. 2004a; Suarez and Sajise 2010; BMB-DENR 2016; Gojo-Cruz et al. 2019; Decena et al. 2020; Supsup et al. 2020). Although there has been an increase in the establishment of protected areas to avert the threat of habitat loss, many of these protected areas (PAs; i.e., national parks, natural parks, biotic areas, protected landscapes) do not overlap with key biodiversity areas (Mallari et al. 2015; Supsup et al. 2023), have poor to fair management effectiveness (Madarang et al. 2017), have never been properly inventoried for terrestrial biodiversity despite their establishment decades ago (Brown et al. 2002b), and can only marginally reduce forest cover loss (Apan et al. 2017). Despite these challenges, we acknowledge that habitat protection through the establishment of PAs and other effective area-based conservation measures are the first steps and are still the most effective measures for long-term species conservation and survival/persistence. Additionally, public education and societal awareness concerning threats posed by emerging infectious diseases (Swei et al. 2011a, b; Diesmos et al. 2012; O'Hanlon et al. 2018; Byrne et al. 2019) and pet-trade facilitated introductions of highly competitive alien and invasive species (Diesmos et al. 2006; Pili et al. 2019, 2021) are all on the rise.

## Taxonomic progress

At the time that the state of Philippine herpetology was last reviewed, Brown et al. (2002a) recognized a total of 101 species of Philippine amphibians (77% endemic) and approximately 258 species of Philippine reptiles (65% endemic). During the last 20 years, during which nearly 100 new species were added to the archipelago's fauna, we recorded differing, taxonomically dependent proportional increases in the number of additionally recognized reptiles (258 vs 361, 28%)

versus amphibians (101 vs 114, an 11% increase) between 2002 and 2022 (Fig. 3A), indicating that the archipelago's native species diversity is far from comprehensively understood. This taxonomic shortfall warrants continued surveys and resurveys, plus targeted taxonomic revisionary attention to particular clades before we might conclude that the fauna is reasonably well characterized. As such, we can anticipate additional increases in cumulative total species diversity estimates in the coming decades, but the question remains, by how much?

Based on the numbers of suspected undescribed species known at that time, Brown et al. (2002a) suggested that herpetological species diversity might increase from 101 to possibly as many as 125–130 (~ 22%) amphibians and from 258 to approximately 275–280 (~ 8%) reptiles. Explanations for why amphibian diversity proportionally increased by less (11%) than the 22% estimated by Brown et al. (2002a) or why reptiles species diversity increased by proportionally far more (28%) than the 8% estimated by Brown et al. (2002a) appears to be a simple function of the fact that researchers (who were actively completing taxonomic studies) showed more interest in systematic and biogeographic questions for which Philippine reptiles represented preferable focal study subjects (e.g., Siler et al. 2009a, 2010a, b, c, d, 2011a, b, c, 2012a, 2014a, b, c, e; Welton et al. 2009, 2010a, b; Brown et al. 2010a, b, c; Linkem et al. 2010a, b, c, 2013; Barley et al. 2013, 2020, 2021; Weinell et al. 2020a, b, c; Eliades et al. 2021) than was the case for research topics involving amphibian study systems (Brown et al. 2009, 2015a, b; Siler et al. 2009b, c, 2010e; Fuiten et al. 2011; Brown 2015; Diesmos et al. 2020; Herr et al. 2021).

Recent efforts to conduct comprehensive herpetological surveys and resurveys have provided a near-complete estimation of the amphibian and reptile diversity and endemism of several islands (e.g., Siler et al. 2012b vs Meneses et al. 2022), mountain ranges (Brown et al. 2000a vs Siler et al. 2011d), or other conspicuous geographical subcenters of diversity in the archipelago through the years (Plaza and Sanguila 2015; Sanguila et al. 2016, 2021). The notable progress of an increased number of described species has been species descriptions (Brown et al. 2010a, c, 2011a, b; Brown 2015; Barley et al. 2021), resurrections of species (e.g., Brown et al. 2000b; Siler et al. 2020), redescriptions of poorly understood taxa (Davis et al. 2014, 2015; Wynn et al. 2016), and revisionary considerations of species boundaries within entire clades (e.g., Linkem and Brown 2013; Brown et al. 2015a, b; Barley et al. 2020). The vast majority of these studies involved the integration of traditional morphological characters (typical of the early 2000s; Brown and Stuart 2012) with molecular data, bioacoustic analyses, ecological information, or other independent data streams (Fig. 4B).

The majority of the 95 species newly described or recognized during the last twenty years are lizards (Fig. 3A); these are dominated by new species of the genera *Brachymeles* (*n* = 12) and *Pseudogekko* (*n* = 5; see Siler at al. 2020; Brown et al. 2020). The genus *Parvoscincus* was expanded by 13 species (Brown et al. 2010c; Linkem and Brown 2013; Siler et al. 2014e) and the genus *Eutropis* by nine new taxa (Barley et al. 2020, 2021). Other new lizard species were described from the genera *Lepidodactylus*, *Gekko*, *Luperosaurus*, *Varanus*, *Cyrtodactylus*, and *Lygosoma*; in total, more than 72 previously unrecognized lizard species have been identified in the past two decades. In many ways, the most spectacular lizard discovery of the past two decades involved the

description of a third species of frugivorous monitor lizard, *Varanus bitatawa* from the southern Sierra Madre Mountain Range of Luzon Island (Welton et al. 2010c, 2012), which has since been confirmed from northern Luzon, including the northernmost reaches of the Cordillera Mountain Range or western Luzon (Abaño-Sarigumba et al. 2018; Meneses et al. 2020b). Five other species of Philippine-endemic monitor lizards in the genus *Varanus* were described during the last twenty years as well (Koch et al. 2010; Welton et al. 2014b).

In amphibians, the greatest taxonomic activities were associated with seven descriptions of new Ceratobatrachid frogs in the genus *Platymantis* (Siler et al. 2007, 2009a, 2010e; Brown et al. 2015a, b; Diesmos et al. 2020). Additional discoveries and descriptions of new species were assigned to the genera *Limnonectes*, *Sanguirana*, *Pulchrana*, *Leptobrachium*, and *Kaloula* (Brown et al. 2009a, 2016, 2017; Siler et al. 2009b; Fuiten et al. 2011; Abraham et al. 2021; Herr et al. 2021). These recorded a total increase of 14 amphibian species in two decades, and we suspect that many more await discovery, because many amphibian clades are in urgent need of taxonomic resolution (e.g., *Platymantis*, *Philautus*, and *Occidozyga*; Brown et al. 2015a, Chan et al. 2021, 2022; Flury et al. 2021), which is currently challenged by limited numbers of recordings (insufficient sample sizes necessary to permit quantitative analyses and statistical tests for species delimitation) or a complete lack of acoustic data for particular species or species groups (Brown and Alcala 1994; Alcala and Brown 1999; Hertwig et al. 2011; Herr et al. in press; Khalighifar et al. 2021).

One notable and striking recent discovery was the characterization of an ancient, archipelago-wide clade–a nearly 40 million-year-old endemic Philippine evolutionary radiation, now recognized as the archipelago's only endemic reptile family: snakes of the clade Cyclocoridae (Weinell and Brown 2017). Two surprising elements of this discovery were apparent; first, the unpredicted finding that snakes of the genera *Cyclocorus*, *Hologerrhum*, *Oxyrhabdium*, and *Myersophis* were a monophyletic group (they had previously and variably been treated as members of separate families or left incertae sedis, of unknown taxonomic affinities; McDiarmid et al. 1999). Second, it was clear from multilocus phylogenetic analyses that an unnamed, genus-level lineage had been overlooked (Weinell and Brown 2017; Weinell et al. 2020a). Description of the new miniaturized genus and species of snake of the family Cyclocoridae, *Levitonius mirus*, was based on three specimens of this secretive, fossorial snake from Samar and Leyte Islands. *Levitonius* exhibits highly distinctive morphology associated with its miniaturized body form, fossorial habitat, and unique diet, consisting solely of soil invertebrates (Weinell et al. 2020a). This discovery also used CT-scan, a novel method to characterize deep internal anatomy, together with molecular data, traditional morphological characters, diet, and ecological niche data (Weinell et al. 2020a), a novel degree of data-type integration to be associated with a taxonomic description for a Philippine species. Subsequent phylogenomic analyses (Das et al. 2023) confirmed the early-branching phylogenetic placement of Cyclocoridae, closely related to the globally distributed snake clade Elapoidea, which includes coral snakes and cobras. Other, highly unique, or unpredicted snake species discovered included a new species of blind snake phenotypically similar to *Acutotyphlops* (Wallach et al. 2007) from northern Luzon and the highly distinctive, krait-like *Calliophis salitan* (Brown et al. 2018, 2021b). The former is a genus otherwise restricted to the Solomon

Islands, which creates a conspicuously unusual and disjunct distribution (the single Philippine species has yet to be included in a phylogenetic analysis, which would be necessary to evaluate this disjunct and somewhat suspect taxonomic/geographic placement). In contrast, *Calliophis salitan* is related to the giant, long-glanded tropical coral snakes of the *C. bivirgata* group and most likely constitutes a separate, unique invasion of the archipelago, apart from other Philippine elapid snakes. In summary, nine species of snakes were described or newly recognized, in the genera *Acutotyphlops*, *Calamaria*, *Calliophis*, *Dendrelaphis*, *Hemibungarus*, *Levitonius*, *Malayotyphlops*, and *Lycodon* (Gaulke 2002, 2011; Wallach et al. 2007; Siler et al. 2013; Wynn et al. 2016; Weinell and Brown 2017; Leviton et al. 2018; Weinell et al. 2019, 2020a, b, c).

## Geographical patterns of herpetological surveys

Our synthesis of available geographic data suggests that the last two decades were characterized by a significant surge of herpetological research across the archipelago. Herpetological surveys conducted on most major islands, particularly those that were not visited before (e.g., island groups of Babuyans, Batanes, and Romblon Province) have led to a stunning number of new species discoveries, elevating sharply the herpetological diversity of the country. Rediscoveries of poorly known species have also provided new insights about their population status and ignited the needed hope for conservation (e.g., Diesmos et al. 2004b; Siler et al. 2011b, c; Bittel 2015; Oliver et al. 2020; Supsup and Carestia 2020; Brown et al. 2021b; Pitogo and Saavedra 2021, 2023; Meneses et al. 2022). However, despite the highly celebrated discoveries, much work is still needed because many small islands and isolated habitats remain unexplored or have not been surveyed thoroughly (see Fig. 6). The islands of Jolo and Basilan in the Sulu Archipelago are the notably less explored areas of the country despite their zoogeographic importance (Seale 1917; Taylor 1918; Gaulke 1993, 1994, 1995). Few biologists have visited these islands during the last century due to logistical and security constraints. The only attempt at a comprehensive study of this archipelago (with specimens collected and still available for reconsideration) was the work of Taylor (1918); unfortunately, many of his specimens from the region, including holotypes of several of the Sulu Archipelago's endemic species (e.g., *Luperosaurus joloensis*, *Brachymeles vermis*, *B. suluensis*) were lost during World War II (Brown and Rabor 1967; Brown and Alcala 1974; Gaulke 1993, 1994, 1995; Uetz et al. 2023). Because of the lack of studies on these islands, many wildlife biologists and biogeographers are still puzzled by uncertain taxonomic affinities and conservation status of the endemic biodiversity of the Sulu Archipelago (Siler et al. 2012c; Spinks et al. 2012; Brown and Siler 2013; Chan et al. 2021).

The apparent absence of peer-reviewed herpetological studies on the islands of Polillo (east of Luzon) and Siargao Island (northeast of Mindanao) is somewhat artefactual, and due to the fact that the majority of fieldwork conducted on these two small islands is only available as unpublished reports (but see Ross and Lazel 1991; Nuñeza and Galorio 2015; Sanguila et al. 2016; Quibod et al. 2021); nevertheless, some collection information (specimens deposited in accessible biodiversity repositories such as Smithsonian National Museum Natural History [USNM], University of Kansas Natural History Museum

[KUNHM], Philippine National Museum of Natural History [PNMNH], and Father Saturnino Urios University [FSUU]) are readily accessible via online biodiversity repositories (e.g., GBIF, iDigBio). In addition to herpetologically unexplored regions of the country, the intact forest habitats of the central Sierra Madre Mountain Range of Luzon (in particular, higher elevations), including the relatively large but fragmented forests to the west have not been thoroughly explored (but see Brown et al. 2000a, 2007, 2010c, 2013b; Siler et al. 2011c; Gojo-Cruz et al. 2018, 2019). Similarly, isolated high-elevation forest habitats in southern Luzon along the borders of Quezon and Bicol Provinces and in Mindoro have not been explored well. Except for the relatively well-explored Caraga region in northeastern Mindanao (Sanguila et al. 2016), many of Mindanao's forests remain herpetologically underexplored, a condition which has persisted during the last two decades (and last century); this is particularly true of western, central, and southern Mindanao, from Mt. Piapayungan southward to the Mt. Latian complex (Taylor 1921, 1922a, b; Sanguila et al. 2016; Pitogo et al. 2021a; Maglangit et al. 2022). As in the Sulu Archipelago, the limited availability of published results from field studies on Mindanao is due, in part, to logistical and security challenges, as well as a lack of local regional expertise, training, and experience with field-based herpetological inventories (Sanguila et al. 2016; Pitogo et al. 2021a; Pitogo and Saavedra 2021).

Despite these gaps, we should note that during the last two decades, there have been significant field-based efforts focused on survey-resurvey studies at important, formerly incompletely understood areas. Several key studies have revisited areas that were targeted in periods before 2002, with the general goal of reassessing, completing, and/or providing a time series (before and after comparison) to enable a temporal perspective on faunal investigations conducted previously, and in light of deforestation, land use change, and global climate change: Zambales Mountains (Brown et al. 1996; Devan-Song and Brown 2012), the central Sierra Madre Mountains of eastern Luzon (Brown et al. 2000a; Siler et al. 2011d), the northern portions of the Sierra Madre Mountains (Brown et al. 2013b), the northern Cordillera Mountain Range of western Luzon (Diesmos et al. 2004a; Brown et al. 2012b), Panay Island (Ferner et al. 2001; Gaulke 2011), Cebu Island (Brown and Alcala 1986; Supsup et al. 2016), and Negros Island (Brown and Alcala 1955, 1961, 1963, 1986; Alcala 1958; Bucol et al. 2019), as well as recent resurveys focused on reassessments of the faunas of northeastern, central eastern, and southern Mindanao (Sanguila et al. 2016, 2021; Pitogo et al. 2021a; Plaza et al. 2021).

Many additional, earlier faunal studies (published before 2001; other areas that have been surveyed, but as of yet, have not been published) are now urgent priorities for survey-resurvey studies, hopefully in the near future (Fig. 5C). We recommend undertaking this work as soon as possible because such areas may contain critically important populations of endemic species, 'lost' species (i.e., species not encountered since their original descriptions and for which holotype specimens were destroyed in World War II), exceedingly rare species, and species awaiting rediscovery and discovery (currently unknown to science), all of which may already be facing cryptic extinction risk brought by habitat degradation and destruction (McDonald et al. 2022). We strongly encourage researchers to consider understudied areas as top priorities for field-based biodiversity research in the coming years (Fig. 5C). Also, for areas that

have not been explored due to logistical obstacles and/or security challenges (e.g., the Sulu Archipelago, southern and southwestern Mindanao Island, etc.), we strongly encourage capacity-building activities for local institutions, universities, and other community stakeholders (e.g., training of residents, students, and other community members) to conduct field-based biodiversity research involving amphibians and reptiles. Such an approach is most likely the safest, most cost-effective, and most feasible strategy for moving forward to address geographical hiatuses and knowledge gaps represented by unexplored, politically charged, or otherwise sensitive areas (see Ramírez-Castañeda et al. 2022). Training residents to effectively survey their local biodiversity reduces reliance on foreign institutions (or groups that are not residents of an area), builds the research capacity of stakeholders who may not have had the opportunity to engage in science, maximizes scholarly equity, and increases the potential for local governance and conservation action.

## Diversity, equity, and inclusion in herpetology research

The rise of Filipino-first authorship in Philippine herpetological studies during the last two decades (Fig. 6B) is an encouraging development since the first review (Brown et al. 2002a). This substantial increase indicates an increased interest among early-career Filipino researchers in the discipline, which formerly was limited to a few Filipinos and their foreign collaborators. It is apparent that a large proportion of the last two decades of Filipino-led studies were field surveys and descriptive studies, whereas the majority of sophisticated studies utilizing genetic data relying on large molecular datasets or genomic analyses were led by non-Filipinos. This disparity reflects the relatively limited capacity for genomics in the Philippines, highlighting the importance of equitable collaborations to ensure skill and technology transfers between local and foreign researchers. A collaborative approach provides opportunities for capacity development for less experienced researchers (e.g., mentorship, writing, decision-making) and may further improve local interest in scientific research (Ramírez-Castañeda et al. 2022). We have seen many multi-national collaborations during the past two decades, which are consequential and contribute to our present understanding and appreciation of Philippine herpetofauna. However, we also acknowledge that access to these opportunities, including advancement in scientific careers, is limited by many socioeconomic factors.

Women are historically underrepresented in herpetology but there has been an increase in female authorship in research on amphibians and reptiles, potentially narrowing the gap if this positive trajectory continues (Rock et al. 2021). Nevertheless, the gender gap in Philippine herpetology (7.13 male per 1.0 female, as first authors) is far from the global average (1.95 male per 1.0 female first authors); ameliorating this disparity will require more representation from women in scientific publications. Despite the substantial gender gap, we are inspired by the continued emergence of next-generation Filipinas and greatly value their contributions to the advancement of the field during the past 20 years. We hope that many additional early-career women researchers, along with other historically underrepresented Filipino groups, will be encouraged to participate in the study of amphibians and reptiles toward a more inclusive and equitable scientific community in the Philippines. Additionally, finding ways to

communicate the results of our studies to the public while narrowing the gap between the scientific community and the lay-/citizen science community will be an important step for rendering our science more accessible to the public and policymakers, while bridging the gaps between science and policy (Young et al. 2014; Ramírez-Castañeda et al. 2022).

### Future directions

Clearly at a crossroads of topical shifts in research themes, increased engagement, equity, inclusion, and representation of diversity in collaborations, Philippine herpetology has undergone a demonstrable maturation since the first review (Brown et al. 2002a). This work sets the stage for what we hope and anticipate will be an increasingly collaborative and inclusive engagement by diverse kinds of herpetologists during the coming several decades, in continuation of the rich history and development of our collective understanding of the amphibians and reptiles in the archipelago. Much progress has been made in some areas (increased general public education and lay-public interest, increased numbers of publications by a broad array of early-career herpetologists, narrowing of the first authorship gap between Filipinos versus foreigners), whereas other equity gaps (e.g., first authorship gender) still remain, requiring increased attention towards fostering diversity and encouraging the engagement of people from a broader array of backgrounds. It is our hope, in compiling this synthesis, that we can challenge the community of individuals, groups, and institutions interested in amphibians and reptiles of the Philippines to pursue some of the conspicuous gaps in research themes identified here and encourage early-career herpetologists to pursue research topics that have advanced in surrounding countries during the last quarter century, but which have not received comparable interest or attention in the Philippines (e.g., amphibian larval biology, developmental studies, etc.). Similarly, historically understudied geographical gaps identified in this review should be viewed as opportunities for increased attention and enhanced collaboration, both among Philippine institutions and between Filipino and foreign herpetologists. The challenge issued by Walter C. Brown a few years before the last review (e.g., 'The State of Philippine Herpetology;' Brown et al. 2002a) still stands: "Rather than view Philippine herpetology as something you might be tempted to divide up, why not just see how much you can accomplish, together, in collaboration?" (W. C. Brown to RMB and A. C. Alcala, personal communication 1998). In accordance with this perspective, and in light of the progress made during the last two decades, we are quite sure that the future of Philippine herpetology will profit most from increased engagement, involving a diversity of people, and embracing increasingly broad thematic research questions in collaboration, all with the common goal of understanding, appreciating, and conserving the archipelago's spectacularly unique amphibian and reptile fauna.

## Acknowledgements

Dedicated to the memory of Angel C. Alcala (1929–2023) and Walter C. Brown (1913–2002) in recognition of their numerous fundamental contributions to the study of the amphibians and reptiles of the Philippines; with particular recogni-

tion for the research synergy they accomplished during the most trying of political times and with respect for the breakthrough international collaboration they forged–transcending historical, racial, societal, and political stereotypes–and all in the name of the passion they shared for the archipelago's herpetological fauna. We thank Justin Bernstein, Marites Sanguila, an anonymous reviewer, and ZooKeys editors for constructive comments on earlier drafts of this manuscript.

## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

### Funding

During the preparation of this manuscript, we were supported by a grant from the US National Science Foundation (DEB 1654388 + Pandemic Supplement to RMB), a KU College of Liberal Arts and Sciences Graduate Assistantship in Support of Faculty Research award, a KU Biodiversity Institute Research and Exploration Fund grant, and funding from the South, Southeast, and East Asia Fund of the KU Office of International Affairs, University of Kansas.

### Author contributions

All authors participated in the design and conduct of the study. The literature search, data analysis, and preparation of tables and figures were all performed by CGM, KMEP, and CES. All authors wrote and reviewed the early drafts and approved the final version of the manuscript.

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### Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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## Supplementary material 1

### Scientific articles on Philippine herpetology, published from 2002–2022

Authors: Camila G. Meneses, Kier Mitchel E. Pitogo, Christian E. Supsup, Rafe M. Brown

Data type: xlsx

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## Supplementary material 2

### Brief distribution records

Authors: Camila G. Meneses, Kier Mitchel E. Pitogo, Christian E. Supsup, Rafe M. Brown

Data type: xlsx

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Link: https://doi.org/10.3897/zookeys.1190.109586.suppl2

ZooKeys 1190: 259–280 (2024)
DOI: 10.3897/zookeys.1190.114958

Research Article

# The millipede family Polydesmidae Leach, 1816 (Diplopoda, Polydesmida) from Vietnam, with a description of a new cavernicolous species

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## Abstract

The millipede family Polydesmidae Leach, 1816 is reviewed in the scope of the Vietnamese fauna. The distribution of the species, *Polydesmus vietnamicus* Nguyen, 2009 is extended northward to Ha Giang Province. A new cavernicolous polydesmid, *Pacidesmus tuachua* **sp. nov.**, is described from two caves in northwestern Vietnam, representing the first record of the genus from Vietnam. Extensive illustrations and DNA barcodes are provided for both species, a revised key is presented to all 12 species of *Pacidesmus* Golovatch, 1991, as well as a key to all eight genera of Asian Polydesmidae.

**Key words:** Asia, cave fauna, COI sequence, diversity, new species, taxonomy

OPEN ACCESS

Academic editor: Dragan Antić
Received: 31 October 2023
Accepted: 11 January 2024
Published: 30 January 2024

ZooBank: https://zoobank.org/A5D5D928-CE84-4E13-BC31-ED2393B3403D

Citation: Nguyen AD, Vu TTT, Eguchi K (2024) The millipede family Polydesmidae Leach, 1816 (Diplopoda, Polydesmida) from Vietnam, with a description of a new cavernicolous species. ZooKeys 1190: 259–280. https://doi.org/10.3897/zookeys.1190.114958

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## Introduction

The millipede family Polydesmidae Leach, 1816 is almost strictly Holarctic, consisting of more than 60 nominal genera or subgenera and nearly 400 species and subspecies (Hoffman 1980; Golovatch 1991). The family is mostly distributed in the Mediterranean area, whereas Central and East Asia, as well as the entire Nearctic Region, show lower generic and, to a lesser degree, species diversity (Golovatch 1991; Djursvoll et al. 2001). Only a few macropolydesmid genera are found in Asia and Indochina including *Epanerchodus* Attems, 1901, *Pacidesmus* Golovatch, 1991, *Polydesmus* Latreille, 1802–1803, *Nipponesmus* Chamberlin & Wang, 1953, *Gleninea* Turk, 1945, *Jaxartes* Verhoeff, 1930, *Schizoturanius* Verhoeff, 1931, and *Uniramidesmus* Golovatch & Mikhaljova, 1979 (Golovatch 1991; Geoffroy and Golovatch 2004; Mikhaljova 2004; Golovatch and Geoffroy 2006, 2014; Golovatch et al. 2007; Nguyen 2009; Antić et al. 2019; Liu and Golovatch 2020). In addition, a fossil species of the rather small western to central European genus *Propolydesmus* Verhoeff, 1895 has recently been described from the mid-Cretaceous amber of Myanmar (burmite, 99–100 Mya) (Su et al. 2023).

Vietnam has been known to harbour a rich fauna of millipedes with about 250 recorded species (Enghoff et al. 2004 and updated). Many new species have been discovered recently, including cave millipedes, e.g. *Hyleoglomeris alba* Nguyen et al., 2022 (Kuroda et al. 2022), *Tylopus nguyeni* Golovatch, 2019, and *Hylomus srisonchai* Golovatch, 2019 (Golovatch 2019), but only one polydesmid has hitherto been revealed: *Polydesmus vietnamicus* Nguyen, 2009 from Tam Dao National Park, Vinh Phuc Province (Nguyen 2009).

The present paper updates the knowledge of the family Polydesmidae in Vietnam, with the description of a new cavernicolous species found in northwestern Vietnam. An updated key to all 12 *Pacidesmus* species is also presented, as well as a key to all eight genera of Polydesmidae reported so far from Asia.

## Materials and methods

Millipede specimens were hand-collected from forests and caves in northern Vietnam and preserved in 85–90% ethanol. Morphological characters were investigated with an Olympus SZX16 stereomicroscope. Gonopods were dissected for morphological examination and photographed. Colour images were taken at various focal planes using a Nikon imaging system (Nikon-Br) coupled with a SMZ800N Nikon stereomicroscope. UV images were taken using a Sony a6000 digital camera attached to the aforementioned SMZ800N Nikon stereomicroscope under the UV flashlight Nichia Convoy. Images were stacked using Helicon Focus version 7.0 and assembled in Adobe Photoshop CS6. Scanning electron microscope (**SEM**) images were taken using the system Prisma E (ThermoFisher Scientific) in the Institute of Ecology and Biological Resources.

Total DNA was extracted using Qiagen DNeasy Blood and Tissue Kits. A 680-bp fragment of the mitochondrial gene, cytochrome c oxidase subunit I (**COI**), was amplified and sequenced using a pair of universal primers, LCO1490 and HCO2198 (Folmer et al. 1994). Polymerase chain reaction (**PCR**) conditions for amplification of the COI gene follow those of (Nguyen et al. 2017). The successfully amplified PCR products were sent to the FirstBase Company (Malaysia) for purification and sequencing. COI sequences were checked and confirmed using BLASTN 2.6.0+ search (Zhang et al. 2000) and registered for GenBank accession numbers.

Morphological terminology follows Liu and Golovatch (2020). All specimens reported here, including types, are deposited in the Institute of Ecology and Biological Resources (**IEBR**), Vietnam Academy of Science and Technology, Hanoi, Vietnam.

### Abbreviation

**IEBR-Myr** Institute of Ecology and Biological Resources, Myriapod collection.

## Results

### Taxonomy

**Order Polydesmida Pocock, 1887**
**Family Polydesmidae Leach, 1816**

### Genus *Polydesmus* Latreille, 1802–1803

**Type species.** *Julus complanatus* Linnaeus, 1761, by monotypy.

**Remarks.** *Polydesmus* is certainly the largest genus within the family Polydesmidae, with over 200 species and subspecies which mainly occur in Europe and the Mediterranean, west of the central Caucasus (Hoffman 1980; Djursvoll et al. 2001). A few species have been found in the Oriental Region, these being *Polydesmus japonicus* Miyosi, 1956, *P. miyosii* Murakami, 1966, *P. tanakai* Murakami, 1970, and *P. tangonis* Murakami, 1973 from Japan; *P. moorei* Pocock, 1895 and *P. paludicola* Pocock, 1895 from eastern China; *P. liber* Golovatch, 1991 from Hong Kong, southern China (Golovatch 1991); and *P. vietnamicus* Nguyen, 2009, the only species of the genus from northern Vietnam (Nguyen 2009). The two old species of Pocock (1895) from mainland China are only provisionally to be assigned to *Polydesmus*, as both require revision. The distribution of *Polydesmus* seems to be amphi-Palaearctic (Golovatch 1991).

### *Polydesmus vietnamicus* Nguyen, 2009

Figs 1, 2–8

**Materials examined.** Vietnam – Vinh Phuc Province • 1 ♂, 1 ♀; Vinh Phuc Province, Tam Dao National Park, near town; 1,000 m a.s.l.; 1 March 2005; Anh D. Nguyen leg.; natural secondary forest • 1 ♂; Vinh Phuc Province, Tam Dao National Park, on the way to Thac Bac waterfall; 1,000 m a.s.l.; 22 March 2005; Anh D. Nguyen leg.; bamboo forest, near stream • 1 ♂, 3 ♀s, 1 juvenile; Vinh Phuc Province, Tam Dao National Park, around the town; 900–1,000 m a.s.l.; 15–18 October 2010; Anh D. Nguyen leg.; mixed forest; IEBR-Myr 967 • 3 ♀♀; Tam Dao National Park, on way to Tam Dao 2; 1,100 m a.s.l.; 25 February 2017; Anh D. Nguyen leg.; natural forest; IEBR-Myr 604 – Ha Giang Province • 2 ♂♂, 1 ♀; Bac Me Natural Reserve, Lac Nong commune, Ban Khen; 22°45'30.8"N, 105°14'04.5"E; 11 December 2019; Anh D. Nguyen leg.; regenerated forest; IEBR-Myr 808.

**Diagnosis.** A typical polydesmid with 20 body rings and three transverse rows of bosses with setae on metaterga. Gonopodal solenomere rather well developed, conspicuously shaped. Endomere elongate and strongly falcate, directed caudally, starting laterally and basally of recurvature point of seminal groove, set off from femorite by a sulcus, with a pair of strong teeth at about midway (**mt**). Seminal groove largely mesal, crossing the femorite diagonally, terminal lateral loop relatively short and turning around a distofemoral process (**ap**). Solenomere (**sl**) short, but evident and bifid.

The species differs from the morphologically particularly similar *Polydesmus liber* Golovatch, 1991 in being larger (33.0–38.4 mm vs 21.0–23.0 mm in length) and in the gonopod endomere (with a pair of teeth at about its midlength vs with two pairs of moderate teeth at 1/3 and 2/3 of its length).

It is particularly noteworthy that all East and Southeast Asian species undoubtedly belonging to *Polydesmus*, however few, share the symplesiomorphy of densely setose gonopod coxites, which contrasts with very poorly setose ones observed in the much more numerous western Palaearctic counterparts (Golovatch 1991).

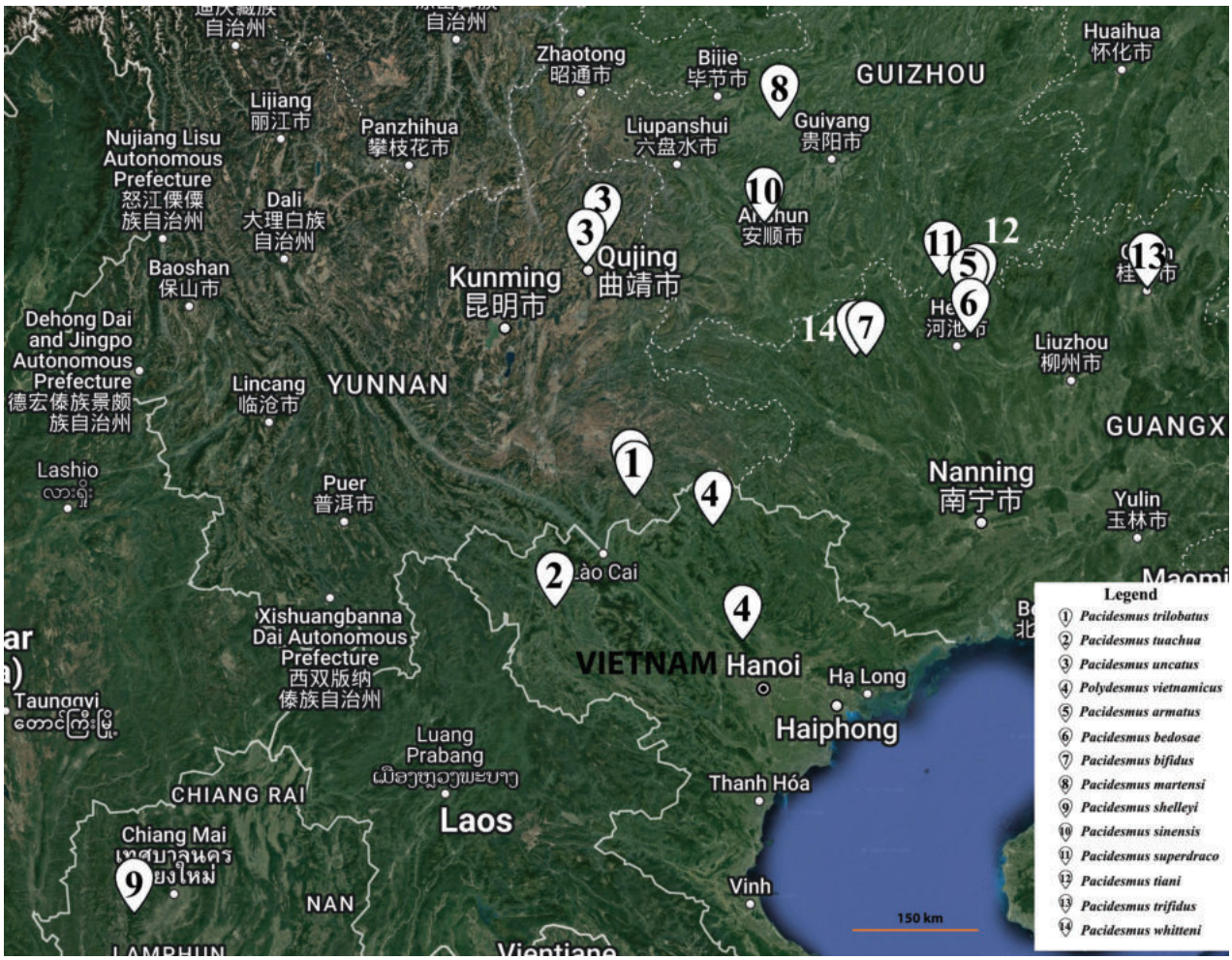


**Figure 1.** Records of polydesmid species in Vietnam and of all known *Pacidesmus* species.

**DNA barcode.** The COI fragment (660 bp) was uploaded to GenBank with accession numbers PP118038 and PP118039. *Polydesmus vietnamicus* has a close COI identity to *Pseudopolydesmus pinetorum* (Bollman, 1888) (MT739870) and *Pseudopolydesmus serratus* (Say, 1821) (MT739862), with 89.8% (query coverage 83%) and 88.71% (query coverage 83%), respectively.

**Remarks.** This species was previously known from only its type locality, Tam Dao National Park (Nguyen 2009). Currently, its distribution is extended northward to Ha Giang Province. There are no significant morphological variations between the type specimens and those samples collected in Ha Giang.

### Genus *Pacidesmus* Golovatch, 1991

**Type species.** *Pacidesmus shelleyi* Golovatch, 1991, by original designation.

**Remarks.** *Pacidesmus* contains 12 species found in southern China and northern Thailand (listed below). While the type species, *P. shelleyi* Golovatch, 1991, is known from forest litter at 2,200 m a.s.l. on Mount Doi Inthanon in northern Thailand (Golovatch 1991), the remaining species seem to be troglobionts restricted to caves in southern China, especially Guangxi and Guizhou provinces (Golovatch et al. 2010; Golovatch and Geoffroy 2014; Liu and Golovatch 2020).

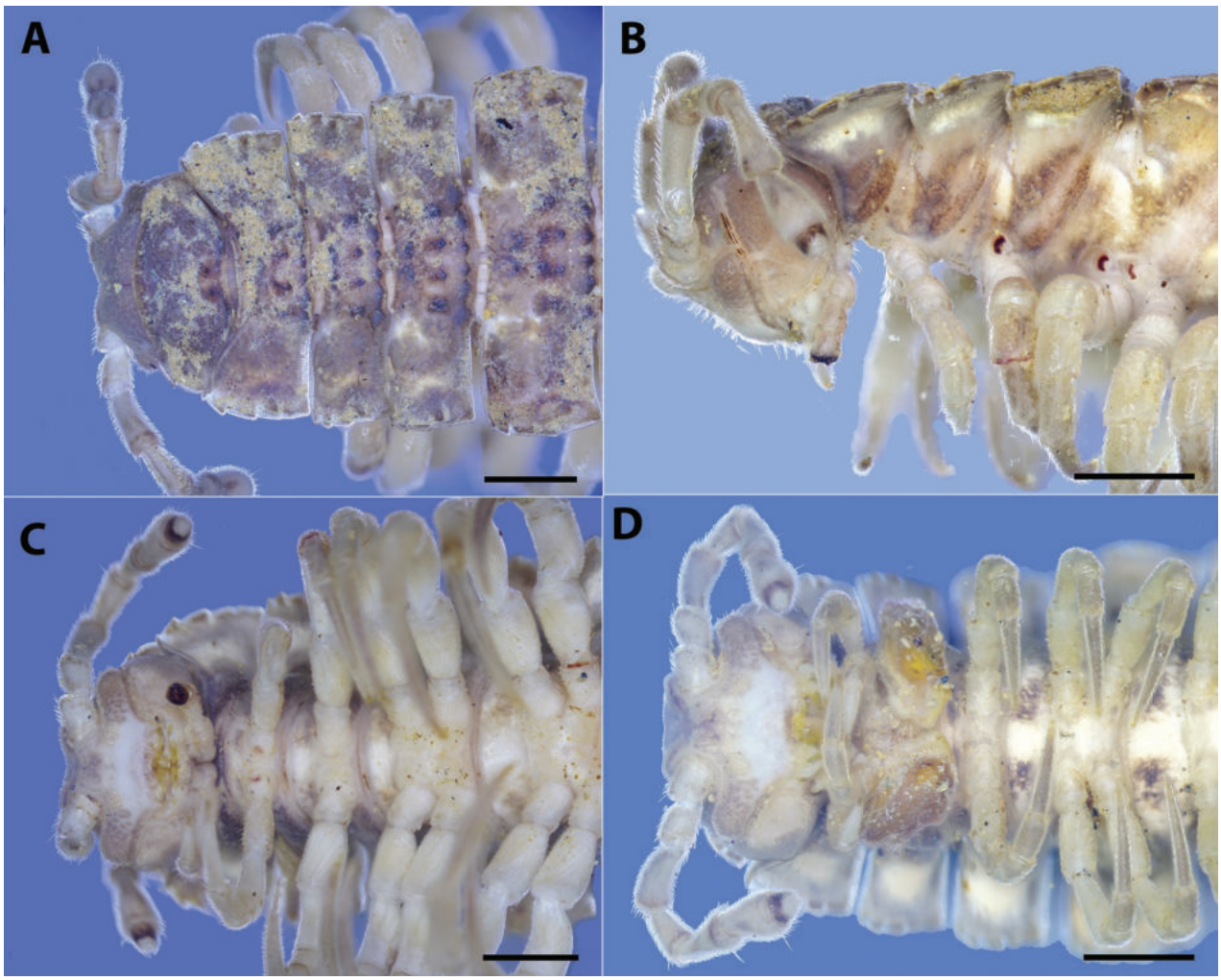


**Figure 2.** *Polydesmus vietnamicus* Nguyen, 2009 from Ha Giang Province (IEBR-Myr 808) **A–C** ♂. Anterior part of body **A** dorsal view **B** lateral view **C** ventral view **D** ♀, anterior part of body, ventral view. Scale bars: 1 mm.

***Pacidesmus tuachua* sp. nov.**
https://zoobank.org/CB8E9D35-3365-4416-81CA-589284A6D42F
Figs 1, 9–14

**Materials examined.** ***Holotype*.** Vietnam • ♂; Dien Bien Province, Tua Chua District, Xa Nhe commune, Xa Nhe cave; 600 m a.s.l.; 21°52'37"N, 103°24'48"E; 12 April 2022; Anh D. Nguyen leg.; **IEBR-Myr 951H.**

***Paratypes*.** Vietnam – Dien Bien Province • 5 ♂♂, 10 ♀♀; Tua Chua District, Xa Nhe commune, Kho Chua La cave; 600 m a.s.l.; 21°52'36.9"N, 103°24'47.9"E; 12 January 2021; Anh D. Nguyen leg.; IEBR-Myr 899 • 3 ♂♂, 4 ♀♀; Tua Chua District, Xa Nhe commune, Kho Chua La cave; 600 m a.s.l.; 21°52'36.9"N, 103°24'47.9"E; 12 January 2021; Anh D. Nguyen leg.; IEBR-Myr 900 • 4 ♂♂, 5 ♀♀; Tua Chua District, Xa Nhe commune, Kho Chua La cave; 600 m a.s.l.; 21°52'36.9"N, 103°24'47.9"E; 12 April 2022; Anh D. Nguyen leg.; IEBR-Myr 951P • 4 ♂♂, 5 ♀♀; Tua Chua District, Xa Nhe commune, Xa Nhe cave; 600 m a.s.l.; 21°52'37"N, 103°24'48"E; 12 April 2022; Anh D. Nguyen leg.; IEBR-Myr 952 • 2 ♂♂, 4 ♀♀; Tua Chua District, Xa Nhe commune, Kho Chua La cave; 600 m a.s.l.; 21°52'36.9"N, 103°24'47.9"E; 12 April 2022; Anh D. Nguyen leg.; IEBR-Myr 953.

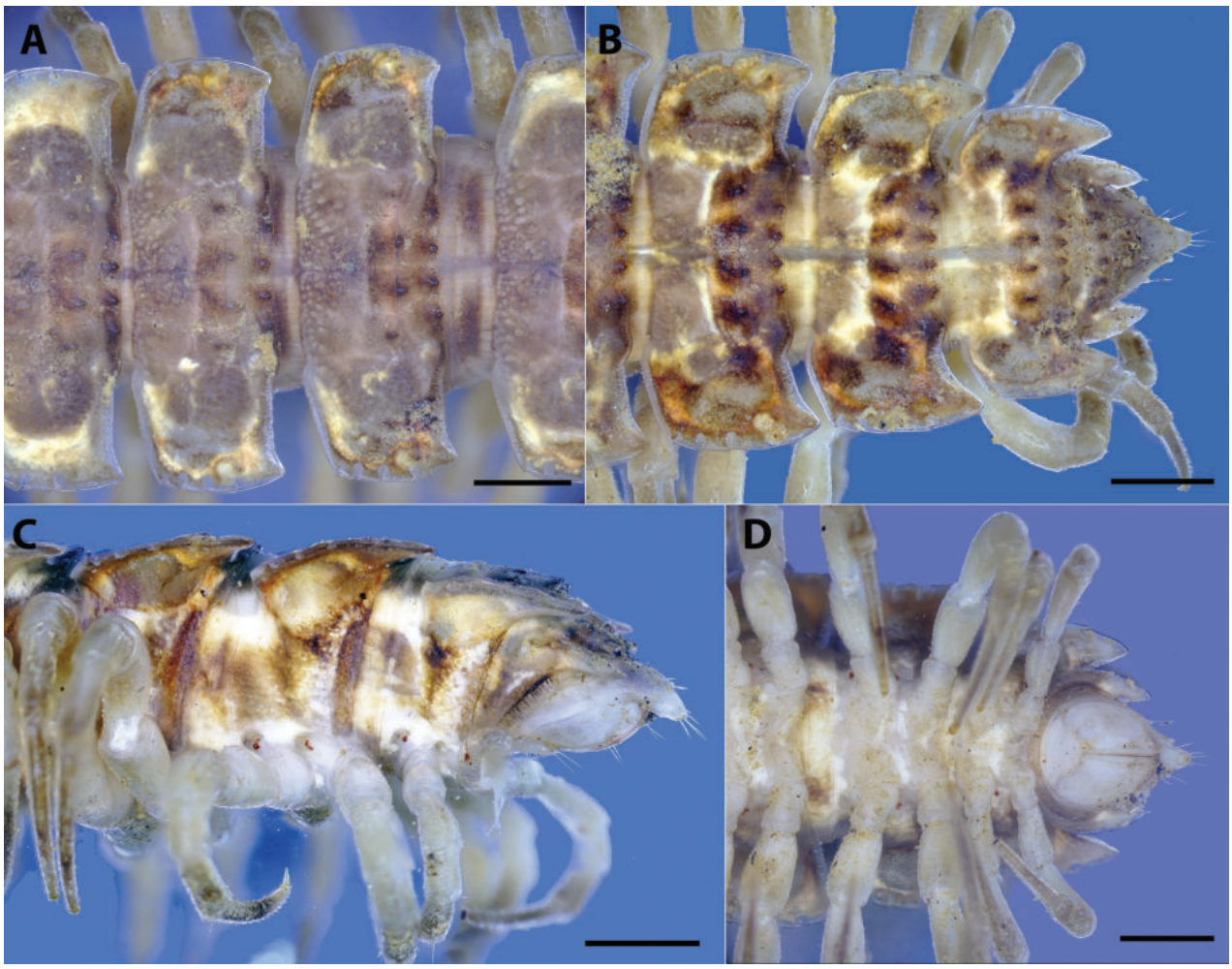


**Figure 3.** *Polydesmus vietnamicus* Nguyen, 2009 from Ha Giang Province (IEBR-Myr 808) ♂ **A** midbody segment 8–11, dorsal view **B** posterior part of body, dorsal view **C** posterior part of body, lateral view **D** posterior part of body, ventral view. Scale bars: 1 mm.

**Diagnosis.** The new species can be distinguished from its congeners by a combination of the following features: unpigmented colouration, small size (midbody width <4.0 mm), head narrower than collum, absence of sphaerotrichomes, lateral budges on male prefemora, subfalcate gonopod telopodite, absence of exomere, endomere with an acute triangular process distally and a broad triangular process medially, and endomere tip slightly and unequally bifid.

The species is truly cavernicolous, characterized by white or unpigmented colour and living within a cave. As a troglobiont species, it groups with all 12 troglobiont or troglophile congeners from China (Table 1). However, this species differs from all of these, except *P. bifidus* from the Hengli Xin Don Cave, Guangxi Province in the absence of an exomere and the gonopod telopodite showing no additional processes; the endomere also has two additional processes, and the tip of the endomere bears two tiny teeth. The new species is similar to *P. bifidus* in having a troglomorphic appearance, the absence of an exomere, and a bifid tip of the endomere, but it differs in having two tiny teeth at the tip of the endomere tip (vs two long processes in *P. bifidus*).

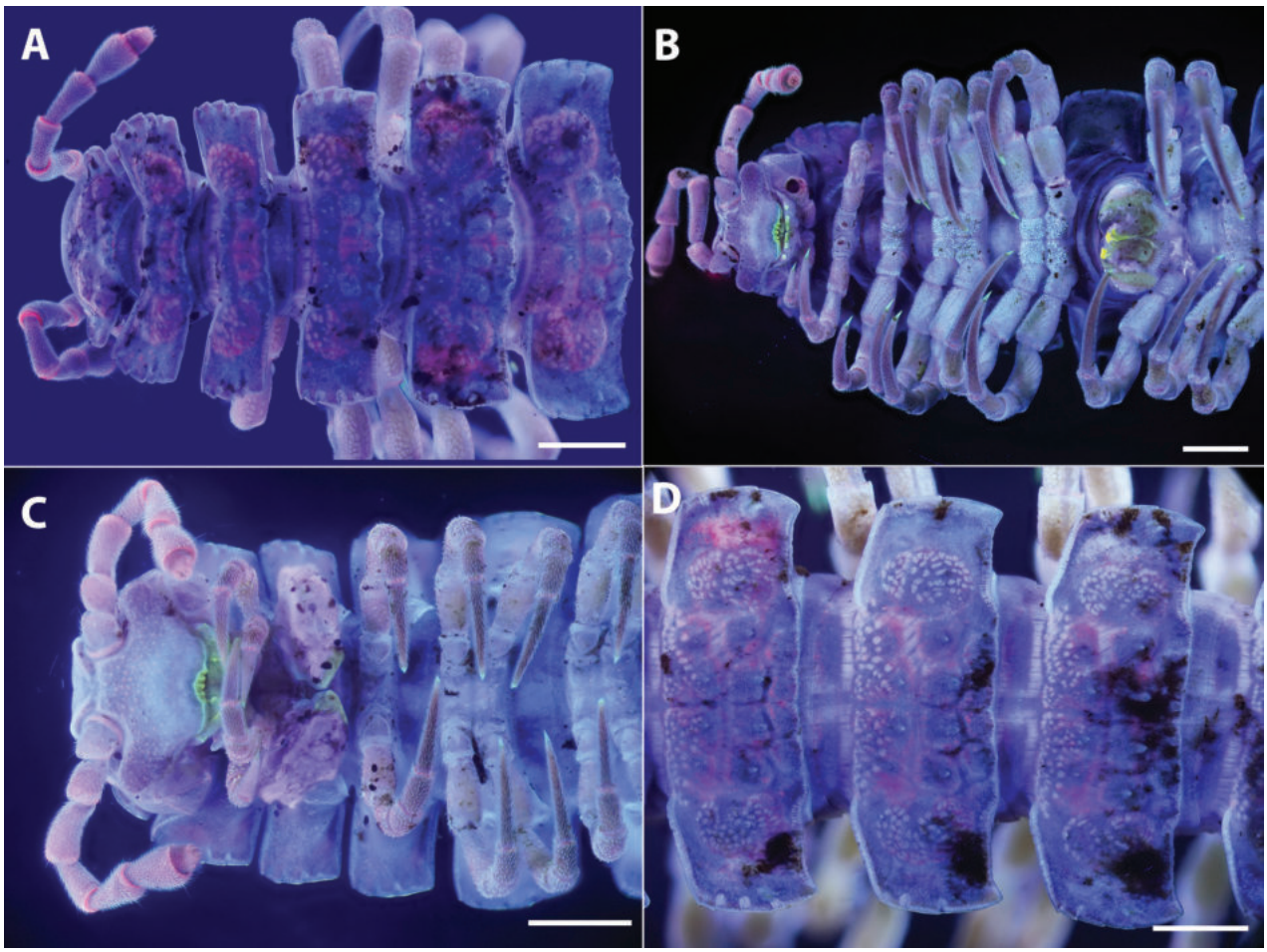


**Figure 4.** *Polydesmus vietnamicus* Nguyen, 2009 from Ha Giang Province (IEBR-Myr 808) ♂ and ♀ under the UV light **A** ♂, anterior part of body, dorsal view **B** ♂, anterior part of body, ventral view **C** ♀, anterior part of body, ventral view **D** ♂, midbody segment 8-10, dorsal view. Scale bars: 1 mm.

**Table 1.** List of all known species of the genus *Pacidesmus* Golovatch, 1991.

| Species | Localities |
|---|---|
| *Pacidesmus armatus* Golovatch, Geoffroy & Mauriès, 2010 | China, Guangxi Prov., Huanjiang, Cave Xiao Lan Dong (Golovatch et al. 2010) |
| *Pacidesmus bedosae* Golovatch, Geoffroy & Mauriès, 2010 | China, Guangxi Prov., Huanjiang, Cave Dong Tu Dong (Golovatch et al. 2010) |
| *Pacidesmus bifidus* Golovatch & Geoffroy, 2014 | China, Guangxi Prov., Cave Hengli Xin Dong near Fengshan (Fengshan Xian) (Golovatch and Geoffroy 2014) |
| *Pacidesmus martensi* Golovatch & Geoffroy, 2006 | China, Guizhou Prov., Qianxi County, Hong Lin Town, Ishui Luo Dong Cave China, Guizhou Prov., Dafang County, Yangzhamba Village, Hei Dong Cave China, Guizhou Province, Qianxi County, Honglin Town, Jisha Village, I Dong Cave (Golovatch et al. 2007; Golovatch and Geoffroy 2006) |
| *Pacidesmus shelleyi* Goolovatch, 1991 | Thailand, Chieng Mai Province, Doi Inthanon National Park (Golovatch 1991) |
| *Pacidesmus sinensis* (Golovatch & Hoffman, 1989) | China, Guizhou Province, Ziyun County, Getuhe National Geopark, Suidao Dong Cave (Liu and Golovatch 2020) A cave in Guizhou Province and Cave Kaikou Dong, Zhenning County, Guizhou Province, China (Loksa 1960; Golovatch and Hoffman 1989; Chen and Meng 1990) |
| *Pacidesmus superdraco* Golovatch, Geoffroy & Mauriès, 2006 | Cave Laitai Dong, Libo County, Guizhou Province (Golovatch et al. 2007) |
| *Pacidesmus tiani* Golovatch, Geoffroy & Mauriès, 2010 | China, Guangxi Prov., Huanjiang, Cave Gang Lai Dong (Golovatch et al. 2010) |
| *Pacidesmus trifidus* Golovatch & Geoffroy, 2014 | China, Guangxi Prov., Guilin County, Grotte des Squelettes (Golovatch and Geoffroy 2014). |
| *Pacidesmus trilobatus* Liu & Golovatch, 2020 | China, Yunnan Province, Maguan County, Pojiao Town, Dayan Dong Cave China, Yunnan Province, Wenshan County, Liujing Town, Laozhai Village, I Dong Cave (Liu and Golovatch 2020). |
| *Pacidesmus uncatus* Liu & Golovatch, 2020 | China, Yunnan Province, Qujing City, Zhanyi County, Tianshengqiao Dong Cave (Liu and Golovatch 2020) |
| *Pacidesmus whitteni* Liu & Golovatch, 2020 | China, Guangxi Zhuang Autonomous Region, Fengshan County, Jinya Town, Hangdong Village, I Dong Cave (Liu and Golovatch 2020) |

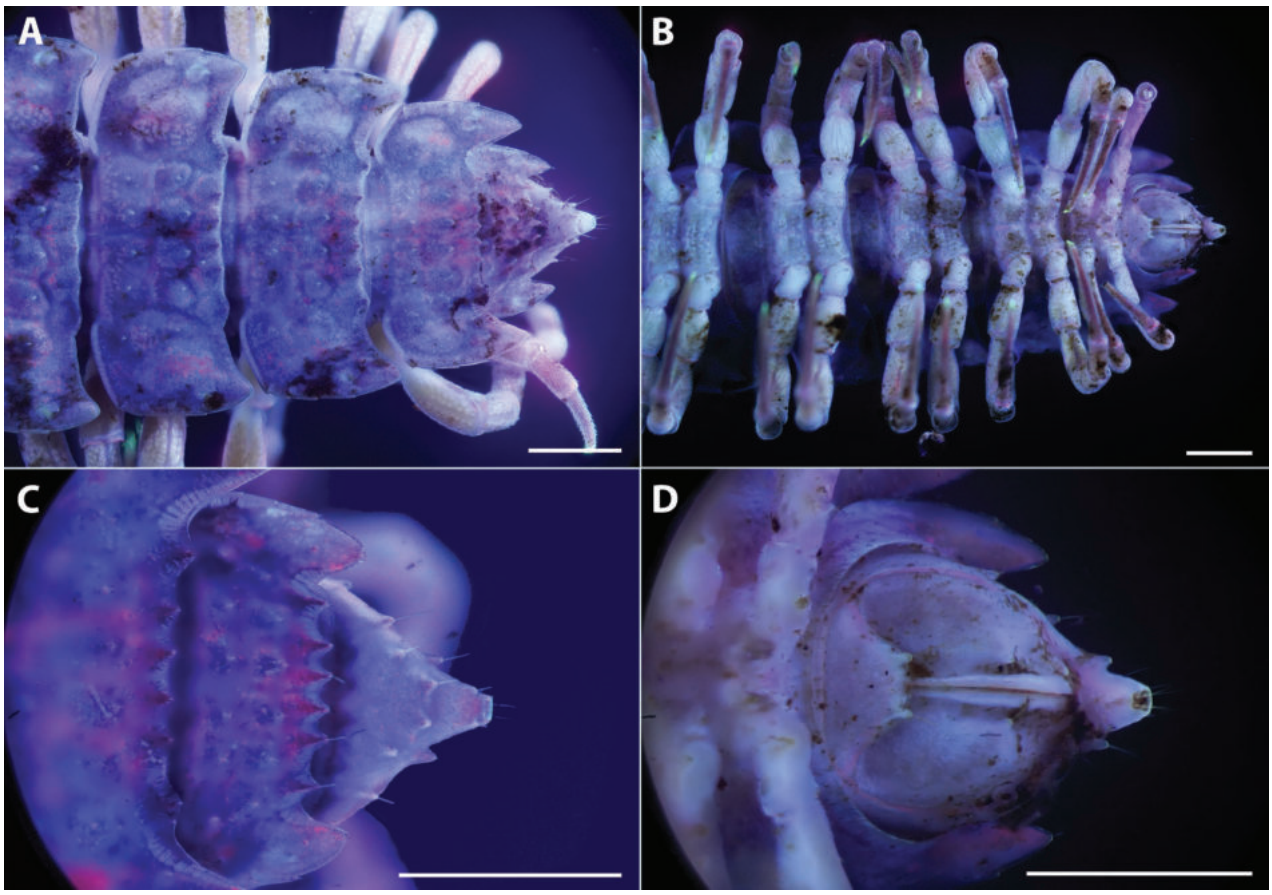


**Figure 5.** *Polydesmus vietnamicus* Nguyen, 2009 from Ha Giang Province (IEBR-Myr 808) ♂, under the UV light **A** posterior part of body, dorsal view **B** posterior part of body, ventral view **C** telson, dorsal view **D** telson, ventral view. Scale bars: 1 mm.

The new species is assigned to *Pacidesmus* because of the following characters: the seminal groove starts mesally, then recurves laterad at the base of a particularly prominent endomere branch to enter an accessory seminal chamber that opens on a setose pulvillus; endomere bears additional processes.

**Etymology.** The specific epithet is treated as a noun in apposition and is based on the "Tua Chua" district where the two caves are located.

**Description.** Holotype length ca 16.3 mm, width of midbody pro- and metazonae 1.0 mm and 1.5 mm, respectively. In width, head < collum < segment 3 = 4 < 2 < 5 = 15, thereafter body gradually tapering towards telson (Figs 9, 10, 12). Colouration in alcohol rather uniformly white (Figs 8, 9). Body with 20 segments. Antennae long and only slightly clavate, possibly reaching past segment 3 if stretching laterally; antennomere 3 longest, approximately 1.3× longer than subequal antennomeres 4–6; antennomeres 5 and 6 each with a small, compact, distodorsal group of bacilliform sensilla; antennomere 7 with a minute dorsoparabasal cone and a distodorsal group of microscopic sensilla (Figs 9A–C, 11C).

Paraterga (Figs 9, 10, 12A–C) strongly developed, set high, starting with collum, dorsum faintly convex; paraterga mostly weakly upturned above dorsum. Caudolateral corner of paraterga acute, postcollum ones extending increasingly past rear tergal margin, especially so in segments 16–18. All poreless segments with three incisions, all pore-bearing ones with four minute incisions at lateral margin. Front margins of metaterga narrowly bordered and forming distinct shoulders.

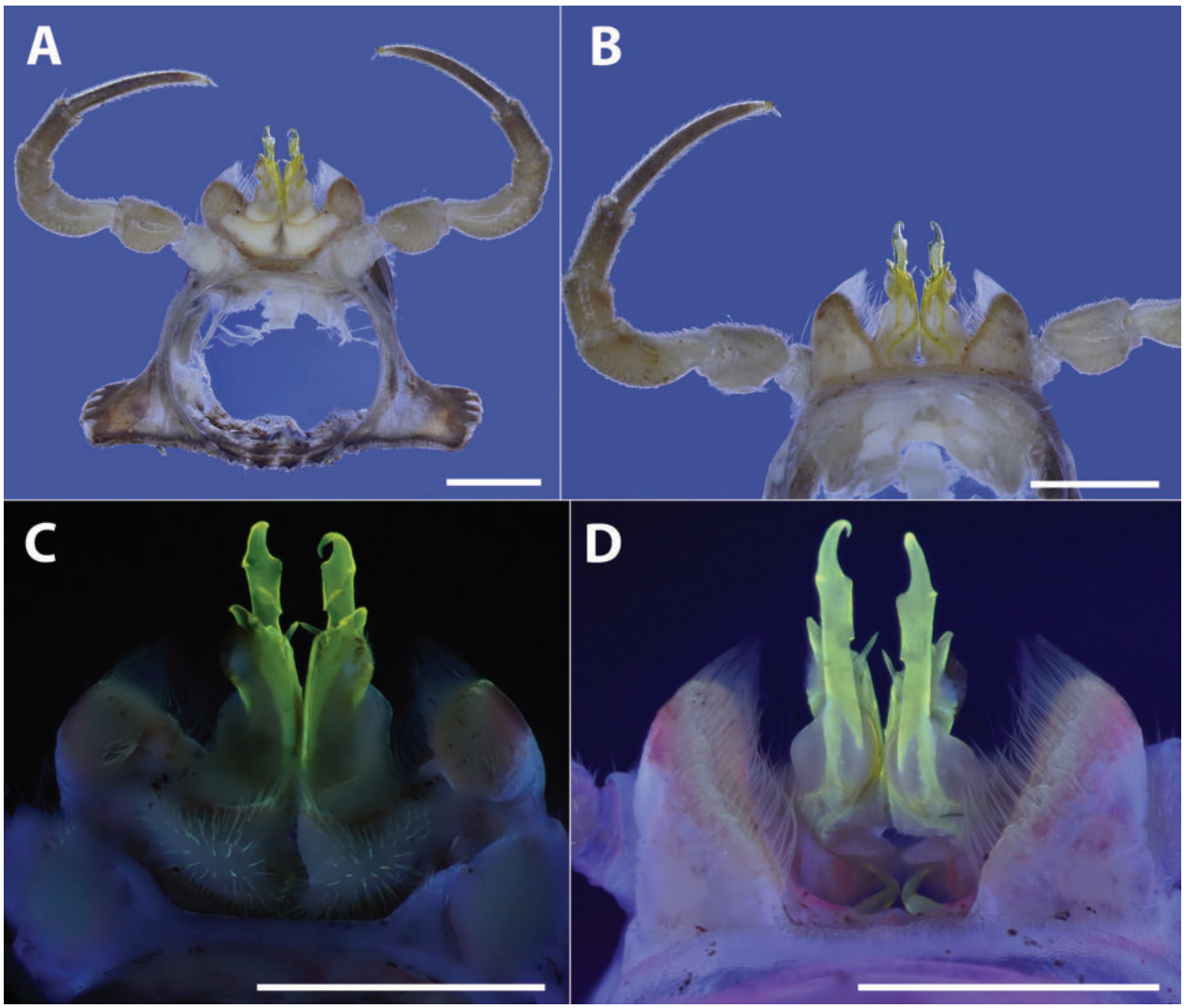


**Figure 6.** *Polydesmus vietnamicus* Nguyen, 2009 from Ha Giang Province (IEBR-Myr 808) ♂, segment 7 with gonopods under the normal (**A, B**) and UV (**C, D**) light **A, C** posterior views **B, D** anterior views. Scale bars: 1 mm.

Ozopores evident, dorsal, located in front of posteriormost marginal incision of paraterga 5, 7, 9, 10, 12–13, 15–19.

Metatergal sculpture typical, poorly developed, obliterate, with three transverse rows of typical (= polydesmid), setigerous, polygonal bosses. Tergal setae short, slightly longer only on collum, simple, often obliterate. Stricture between pro- and metazona wide, shallow and nearly smooth. Limbus exceedingly thin, microdenticulate (Fig. 12A–C). Pleurosternal carinae absent.

Epiproct (Figs 11A, B, 12C, D) short, conical, pre-apical lateral papillae evident. Hypoproct (Figs 11A, 12D) subtriangular; distolateral setiferous knobs small, but distinct and well separated.

Sterna without modifications, but setose (Fig. 10A, C).

Legs generally long and slender, apparently slightly incrassate, approximately 1.7–1.8× as long as midbody height, densely setose, almost all setae simple, poorly branching setae with minute, distal, side branchlets only on slender prefemora, latter devoid of lateral bulges.

Gonopods (Figs 11D, 12, 13) characteristically subfalcate (vs suberect in all other congeners). with large, rectangular coxites (**co**), with a few long setae

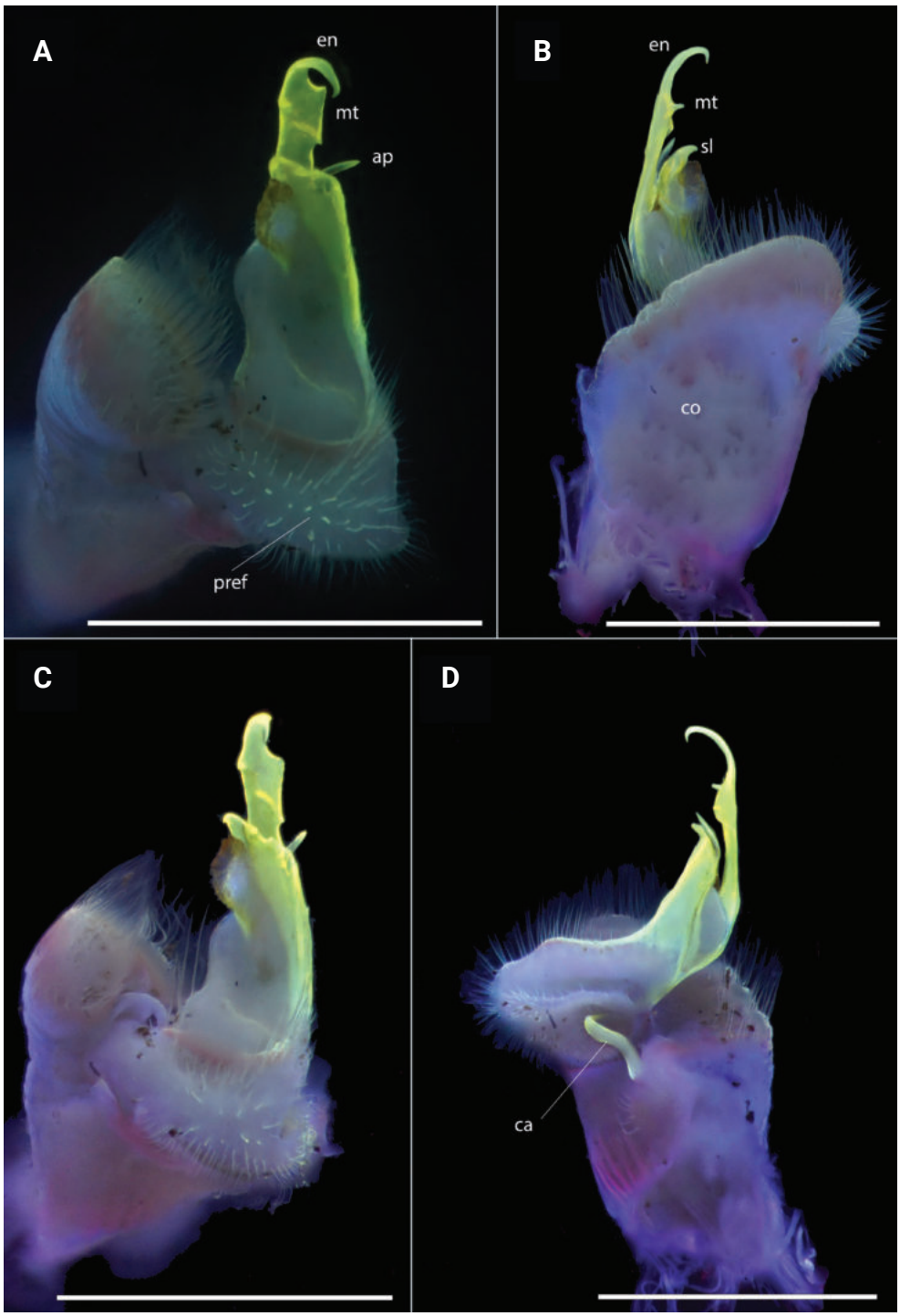


**Figure 7.** *Polydesmus vietnamicus* Nguyen, 2009 from Ha Giang Province (IEBR-Myr 808) ♂, right gonopod under the UV light **A** ventral view **B** lateral view **C** dorsal view **D** mesal view. Scale bars: 1 mm.

ventrally; a long, simple, flagelliform cannula (**ca**) as usual. Telopodite elongate, stout, strongly falcate or C-shaped; prefemorite (**pref**) densely setose; seminal groove starting mesally, then recurving laterad to run to the opening on a hairy pulvillus. Endomere (**en**) with two additional processes, a shorter, larger, broader triangular process at midlength (**p1**), and a longer, acuter, triangular process at ¾ length (**p2**). Tip of endomere unequally bifid, a longer and a shorter branch. Neither an exomere nor a clivus.

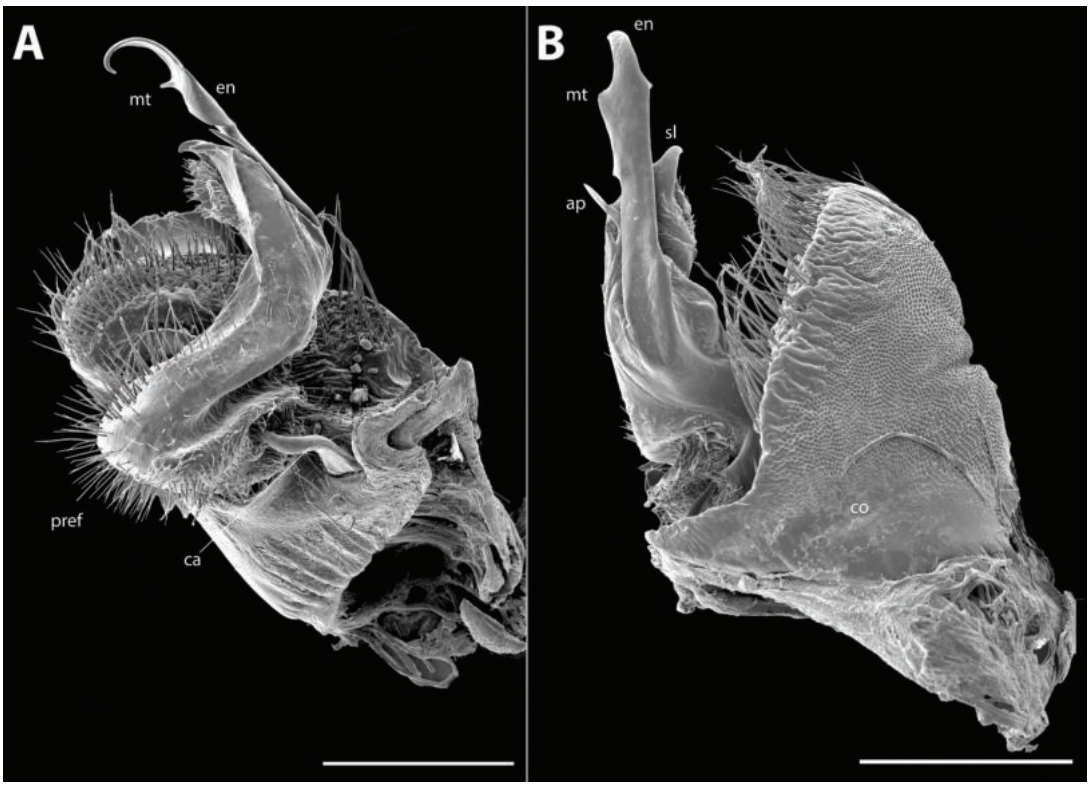


**Figure 8.** *Polydesmus vietnamicus* Nguyen, 2009 from Ha Giang Province (IEBR-Myr 808) ♂, right gonopod **A** mesal view **B** lateral view. Abbreviations: *co* = coxite; *pref* = prefemorite; *ca* = cannula; *en* = endomere; *sl* = solenomere; *mt* = midway teeth; *ap* = additional process. Scale bars: 0.5 mm.

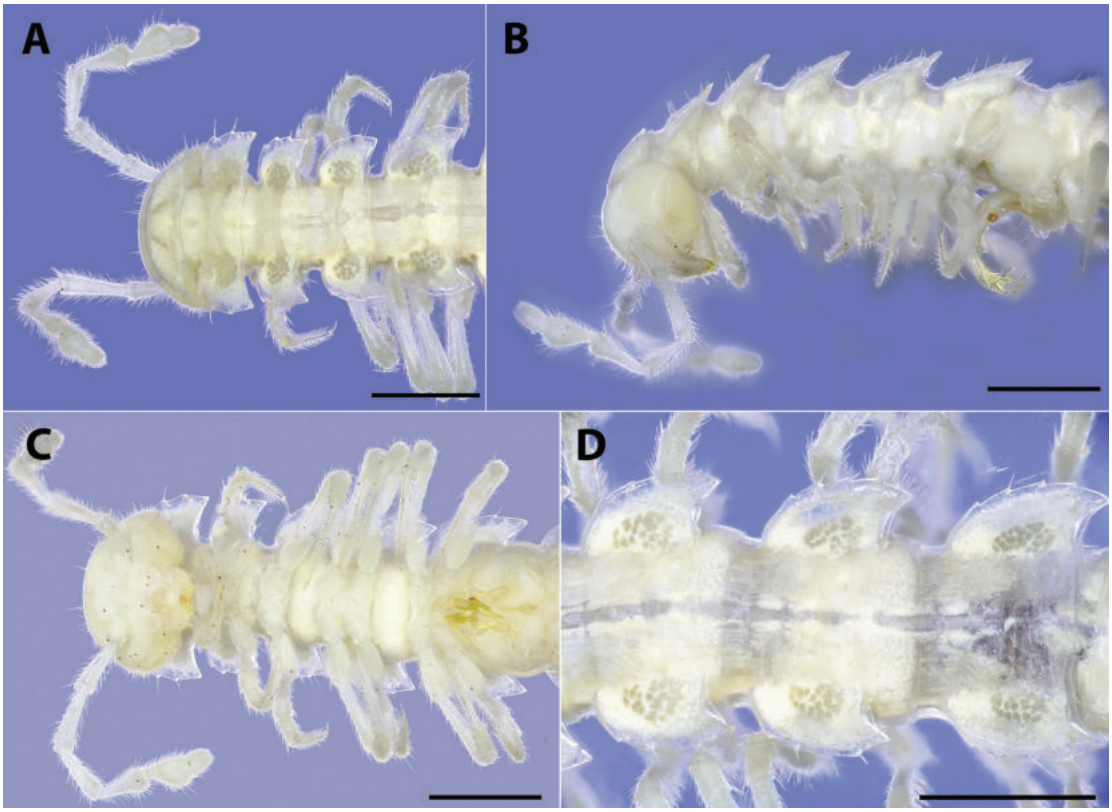


**Figure 9.** *Pacidesmus tuachua* sp. nov., holotype ♂ (IEBR-Myr 951) **A** anterior part of body, dorsal view **B** anterior part of body, lateral view **C** anterior part of body, ventral view **D** segments 8–10, dorsal view. Scale bars: 1 mm.

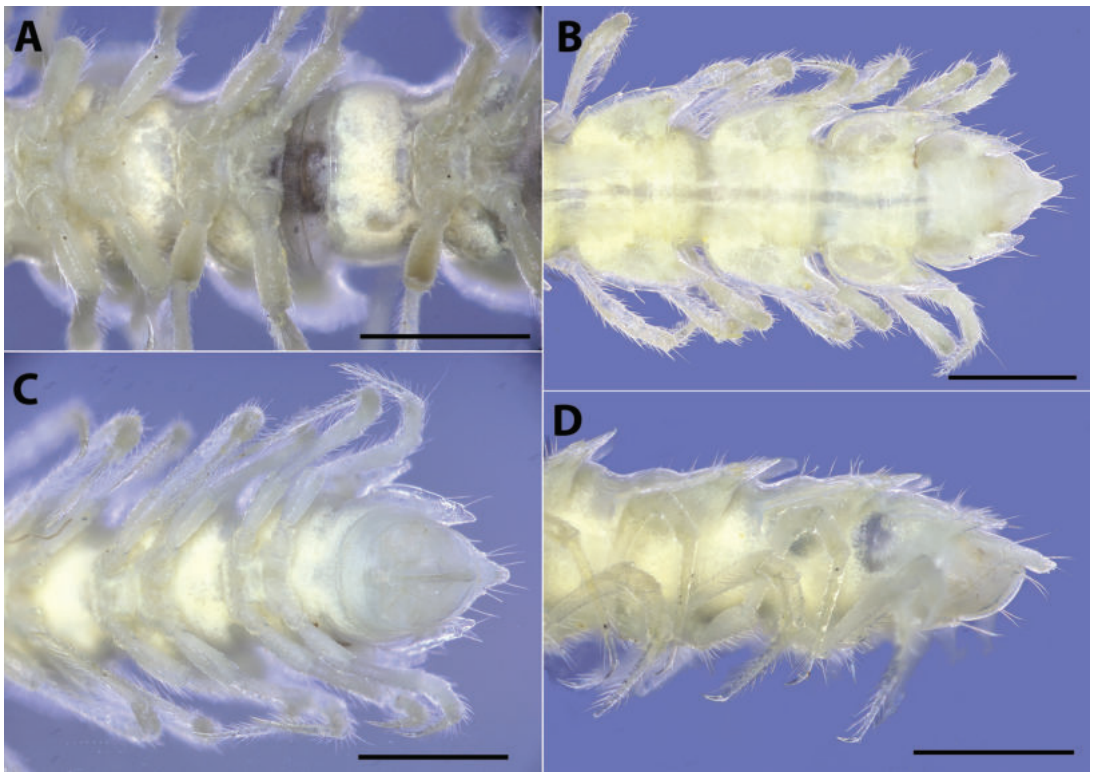


**Figure 10.** *Pacidesmus tuachua* sp. nov., holotype ♂ (IEBR-Myr 951) **A** segments 8–10, ventral view **B–D** posterior part of body, dorsal, vental and lateral views, respectively. Scale bars: 1 mm.

**Female.** Slightly larger than male, length ca 16.8 mm, width of pro- and metazona about 1.1 mm and 1.7 mm, respectively. Paraterga slightly less strongly developed. Legs unmodified, somewhat shorter and more slender. Vulvae highly elevated. Epigynal ridge low.

**DNA barcode.** Two COI sequences (661 bp) were uploaded to the GenBank with the accession numbers PP118040 and PP118041. The new species has a close COI identity to *Epanerchodus koreanus* (NC051495) at 88.72% (query coverage 97%).

**Habitat.** This species is to be considered a true troglobiont because it shows the typical morphological features of a cave-dweller. It was collected exclusively in the dark zone of the caves as described below. Kho Chua La and Xa Nhe caves are both located close together, approximately 500 m in distance. These caves are at the centre of the Xa Nhe commune, Dien Bien Province, northwestern Vietnam. The two caves are tunnel-like: they are high (15–20 m), wide (15–20 m), and long (1,000–1,500 m). The floor is mainly wet, with clay, and some small pools. Several other millipede species have been found in these caves, including *Glyphiulus* sp. (Spirostreptida, Glyphiulidae) and *Eutrichodesmus* sp. (Polydesmida,: Haplodesmidae). The new species was found >1000 m from the entrance.

Kho Chua La and Xa Nhe caves are located on the Tua Chua karst plateau in northeastern Dien Bien Province, northwestern Vietnam. The natural area is about 68,414 ha, and 70% of this area is composed of limestone mountains, which are known for their layers of majestic rugged rock and unique natural landscape. The karst region contains many stunning and well-known caves, such as Kho Chua La, Tham Khem, Hau Chua, Xa Nhe, and Pe Rang Ki (Nguyen et al. 2022). Furthermore, the Tua Chua karst plateau of northwestern Vietnam is close to the

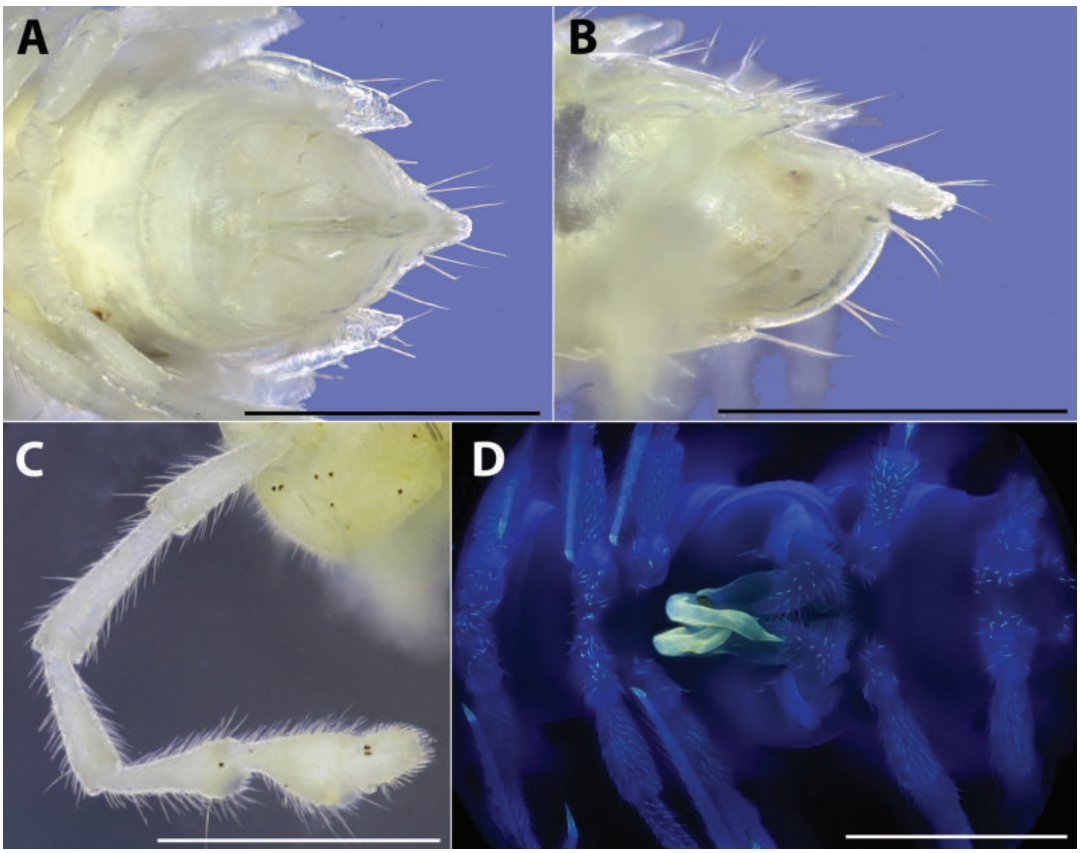


**Figure 11.** *Pacidesmus tuachua* sp. nov., holotype ♂ (IEBR-Myr 951) **A** telson, ventral view **B** telson, lateral view **C** right antenna, anterior view **D** gonopods in situ under UV light, ventral view. Scale bars: 1 mm.

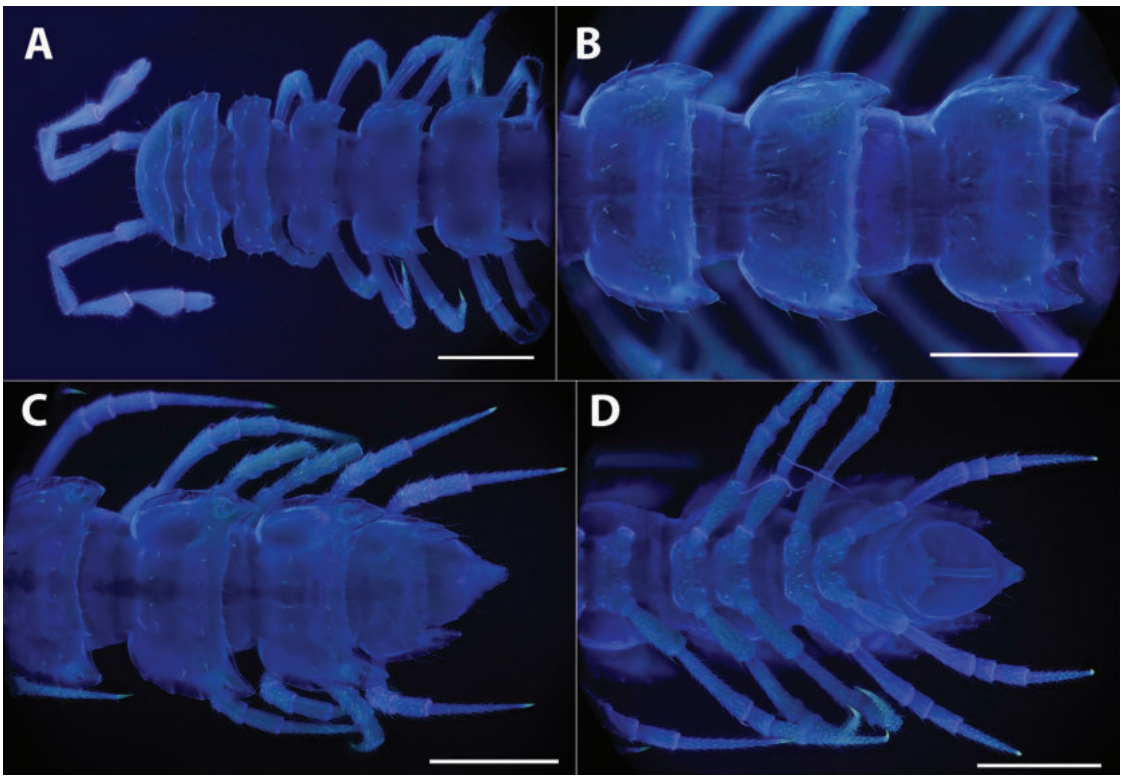


**Figure 12.** *Pacidesmus tuachua* sp. nov., holotype ♂ (IEBR-Myr 951) **A** anterior part of body, under the UV light, dorsal view **B** segments 8–10, dorsal view **C** posterior part of body, dorsal view **D** posterior part of body, ventral view. Scale bars: 1 mm.

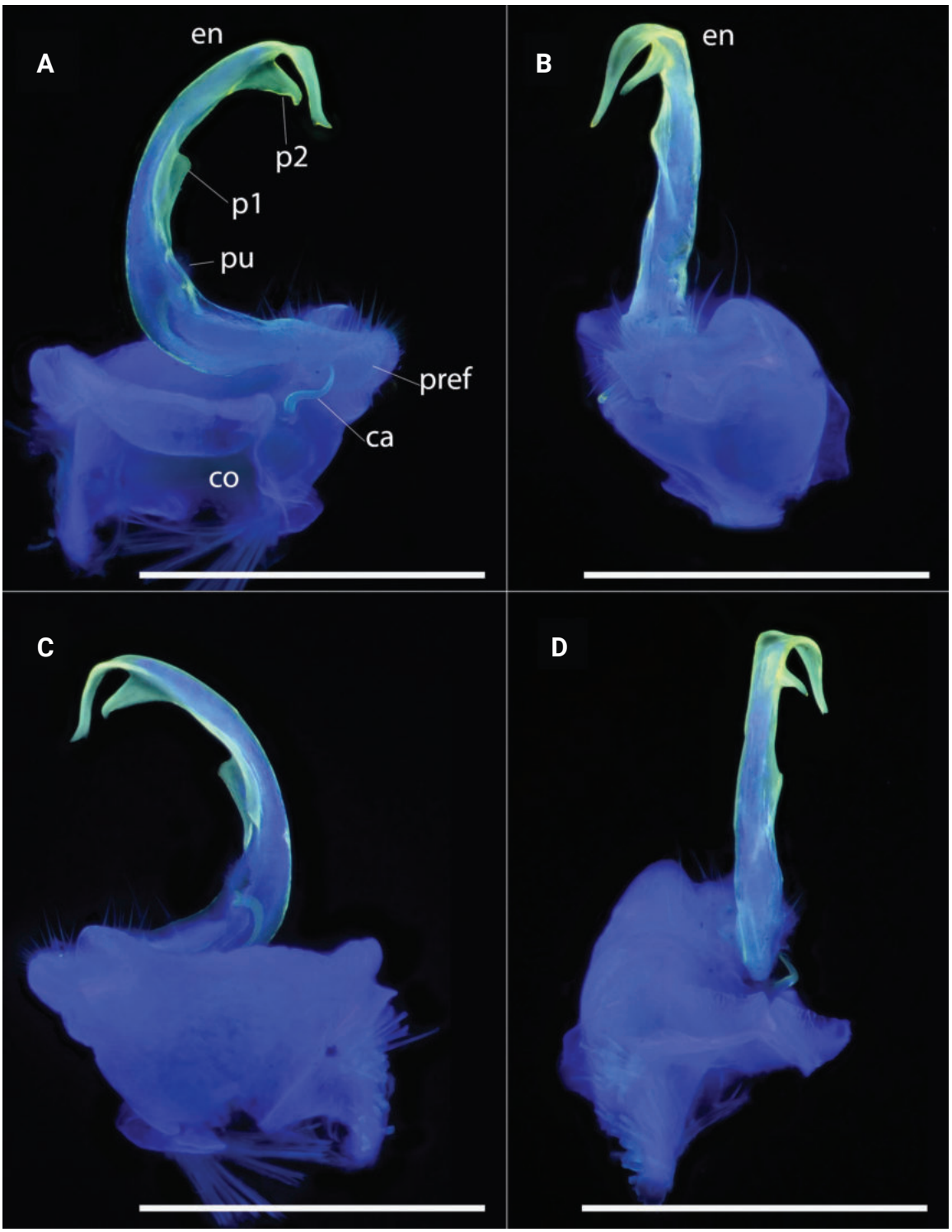


**Figure 13.** *Pacidesmus tuachua* sp. nov., holotype ♂ (IEBR-Myr 951) right gonopod, under UV light **A** mesal view **B** ventral view **C** lateral view **D** dorsal view. Scale bars: 1 mm.

Yunnan and Sichuan karst regions of southwestern China. Given this, it is not surprising to discover the genus *Pacidesmus* in northwestern Vietnam. The distance between *Pacidesmus tuachua* sp. nov. and *Pacidesmus trilobatus* Liu & Golovatch, 2020 from Guangxi Province, China, is about 150 km northeast–southwest (Fig. 1).

**Remarks.** While there remains a noticeable geographical gap between the mountainous northern Thailand species and the troglobionts of southern

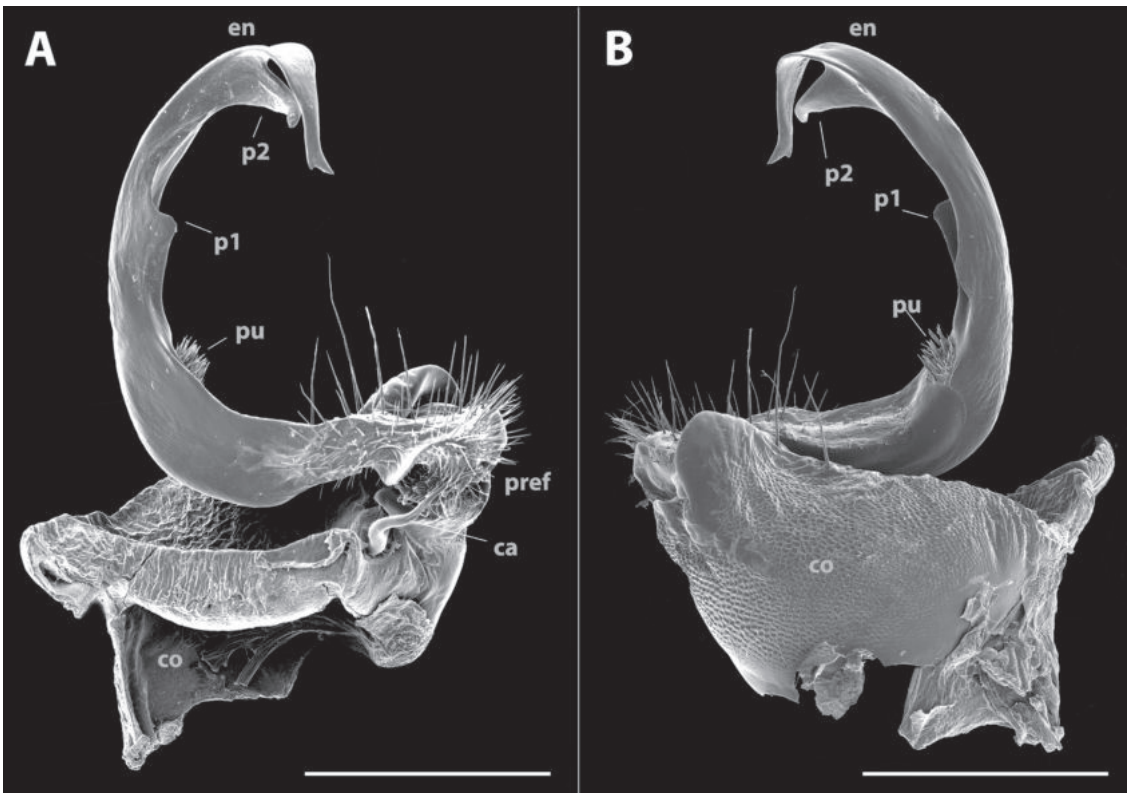


**Figure 14.** *Pacidesmus tuachua* sp. nov., holotype ♂ (IEBR-Myr 951) right gonopod **A** mesal view **B** lateral view. Abbreviations: *co* = coxite; *pref* = prefemorite; *ca* = cannula; *en* = endomere; *pu* = puvillus; *p1* = first process; *p2* = second process. Scale bars: 0.5 mm.

China, the discovery of a new species in northern Vietnam partially fills this gap. Like the more eastern species, the new species is also troglobiotic, and whether these species should be classified in a distinct genus related to a more restricted *Pacidesmus* (including only the type species) needs exploration.

The discovery of a new species marks the first record of the genus *Pacidesmus* in Vietnam.

## Identification key to species of the genus *Pacidesmus* Golovatch, 1991

Modified and updated from Golovatch et al. (2010).

1 Sternal cones between ♂ legs 6 and 7 for accommodation of distal parts of gonopods present. Epigean and high-montane from northern Thailand ..... ***P. shelleyi***

– No such sternal modifications. Cavernicoles from southern China and northern Vietnam..... **2**

2 Gonopod exomere absent ..... **3**

– Gonopod exomere present ..... **4**

3 Gonopod telopodite suberect. Endomere tip clearly, deeply and narrowly bifid; endomere rather stout, not carrying any processes. Guangxi..... ***P. bifidus***

– Gonopod telopodite subfalcate. Endomere tip slightly bifid; endomere slender, carrying two additional processes, a shorter, larger and broader triangular one at midlength, and a longer, acuter triangular one at ¾ length. Northern Vietnam..... ***P. tuachua* sp. nov.**

4 Gonopod exomere without process at base. Endomere tip bifid ..... **5**
– Gonopod exomere with a process at base. Endomere tip either trifid or unifid..... **6**
5 Gonopod endomere less stout. Body length 23–24 mm, width of midbody pro- and metazona 1.5–1.6 and 2.5–2.7 mm, respectively. Paraterga upturned above dorsum only on rings 1–5. Guangxi ..... ***P. bedosae***
– Gonopod endomere stouter. Body length 28–30 mm, width of midbody pro- and metazona 1.4–1.7 and 2.8–3.2 mm, respectively. Paraterga upturned above dorsum until ring 17 (♂) or 14 (♀). Guizhou .... ***P. superdraco***
6 Gonopod endomere rather stout, tip either unifid or trifid. Exomere small..... **7**
– Gonopod endomere slender, tip unifid. Exomere large ..... **8**
7 Only paraterga 1–4 evidently upturned above dorsum. Gonopod endomere slender, subfalcate, carrying a small tooth distally on median surface; tip unifid..... ***P. tiani***
– Paraterga upturned above dorsum starting with paraterga 2. Gonopod endomere stouter, suberect, not carrying any processes. Tip trifid ..... ***P. trifidus***
8 Endomere with two teeth ..... **9**
– Endomere with either one tooth or three teeth..... **10**
9 Caudolateral corners of paraterga strongly triangular. Gonopod exomere larger, finger-shaped..... ***P. whitteni***
– Caudolateral corners of paraterga narrowly rounded to pointed. Gonopod exomere smaller and unciform ..... ***P. armatus***
10 Endomere with one tooth..... **11**
– Endomere with three teeth..... **12**
11 All paraterga clearly upturned above dorsum. Endomere rather long and strongly twisted ..... ***P. uncatus***
– Only anterior paraterga upturned above dorsum. Endomere much shorter and subfalcate ..... ***P. sinensis***
12 Endomere slender and flagelliform, carrying a small denticle frontally at base. Exomere with a large membranous process at base..... ***P. martensi***
– Endomere long and slender, carrying three lobes. Exomere with a short spiniform process at base ..... ***P. trilobatus***

## Discussion

Beyond southern China and Southeast Asia, there are eight macropolydesmid genera occurring in Asia, mainly in Central Asia. The main differences between those genera are presented in Table 2 below.

*Pacidesmus tuachua* sp. nov. is distinguished from members of *Nipponesmus* by having a gonopod endomere; the gonopod telopodite has neither a comb of setae nor slender teeth (vs without endomere, with a conspicuous comb of setae or slender teeth) (Golovatch et al. 2011). The new species differs from members of *Gleninea* in the absence of a gonopod exomere, the gonopod endomere is with only two additional processes, the absence of an accessory seminal chamber (vs the presence of a gonopod exomere, the distal part of the endomere carrying numerous or several spine-like hairs or strong, sometime curved spines, and the presence of an accessory seminal chamber) (Golovatch and Geoffroy 2014).

The new species is also distinguished from *Schizoturanius* and *Uniramidesmus* species by its larger size (16.3 mm vs less than 10.0 mm). The new

**Table 2.** Morphological diagnoses and distribution of all eight macropolydesmid genera in Asia.

| No. | Genus | Diagnosis | Distribution |
|---|---|---|---|
| 1 | *Schizoturanius* | Body small, strongylosomoid (= without prominent paraterga), moniliform; paraterga narrow, only seldom incised laterally; gonopods falcate and bifid distally; an accessory seminal chamber present; gonopod femorite carrying a characteristic process (Mikhaljova 2004) | Ten species in Central Asia and Ukraine, Asian part of Russia, Kazakhstan, northwestern China (Mikhaljova 2004; Nefediev 2023) |
| 2 | *Uniramidesmus* | Body small (usually ca 10 mm long); head covered with dense minute hairs; antennomeres 5–7 each with a small field of tiny bacilli dorsally; metaterga rather convex; paraterga small or medium-sized and set below dorsum, with marginal incisions; metatergal polygonal sculpture ranging from well-developed to poorly-developed; metatergal setae pointed; sphaerotrichs present or absent; gonopods slender, strongly falcate to coiled caudally, relatively simple, in situ crossing each other; seminal groove with a loop parabasally; accessory seminal chamber absent; opening of seminal groove subterminally to terminally on a bare to more or less pubescent pulvillus (Mikhaljova 2004, 2017) | Ten species in central Asia and Asian part of Russia (Mikhaljova 2004, 2017) |
| 3 | *Jaxartes* | Body small (usually ca 10 mm long); metaterga with bosses/tubercles with bacilliform or trichoid setae; paraterga clearly incised laterally; four distal ♂ podomeres with ventral sphaerotrichomes; gonopod coxite without outgrowths other than cannula; the gonotelopodite particularly slender, suberect, with the endomere being considerably longer than a ventrally fringed process (if present at all), also bearing a parabasal tooth and a subtruncate apex, basally with a very evident hairy pulvillus, but no distinct accessory seminal chamber (Antić et al. 2019) | Twelve species in Central Asia (Antić et al. 2019) |
| 4 | *Epanerchodus* | Gonopod endomere mostly absent, rarely present as only a more or less rudimentary structure, while the seminal groove after the recurvature point still makes a long way basad to debauch into a prominent, simple-haired, accessory seminal chamber placed at the bottom of a profound parabasal cavity in the telopodite (Liu and Golovatch 2018; Golovatch 2021). | 120+ species, largely Palaearctic in distribution, mainly from Japan (East Asia) to the western part of China, from Mongolia (Central Asia) in the north to southern China and the Himalaya of Nepal in the south (Liu and Golovatch 2018; Golovatch 2021) |
| 5 | *Pacidesmus* | Body large, up to 30 mm long. Paraterga broad, slightly incised laterally. Metaterga with bosses with setae. Gonopod endomere variable, from relatively short, stout and bifid to long, slender and rather simple; exomere absent or supplied with an outgrowth (Golovatch et al. 2010; Liu and Golovatch 2020) | Twelve species in southern China and northern part of Southeast Asia (Golovatch et al. 2010; Liu and Golovatch 2020) |
| 6 | *Nipponesmus* | Body size large (up to 20 mm in length). Paraterga broad. Gonopod endomere with conspicuous comb of setae or slender teeth. The seminal groove running mostly mesally to recurve neatly between exomere and endomere, then to debauch somewhat basally into a prominent hairy pulvillus which also beset with the same peculiar trichome, and is devoid of an accessory seminal chamber (Golovatch et al. 2011) | Three species in Japan and Taiwan. (Golovatch et al. 2011) |
| 7 | *Gleninea* | Body small to large size (up to 16 mm in length). The third pair of ♂ legs only slightly thickened. Antenomere 5-6, each with a s mall, compact, distodorsal group of bacilliform sensilla. Lateral side of paraterga strongly serrated, 5-6 small, sharp teeth. Gonopod exomere simple, subfalcate; endomere with distal part carrying numerous or several spine-like hairs or strong, sometime curved spines. Accessory seminal chamber present (Golovatch and Geoffroy 2014) | Seven species in the Himalaya of India, Nepal, Bhutan, and China (Golovatch and Geoffroy 2014) |
| 8 | *Polydesmus* | Body size medium to large. Paraterga usually wide. Gonopod solenomere absent to rather well developed, sometimes conspicuously shaped. Exomere from short and slightly curved to very long and strongly falcate, mostly uniramous, directed caudally, starting laterally or apically and basally of recurvature point of seminal groove. Seminal groove, largely mesal; terminal laterad loop relatively short and turning around a distofemoral process (Djursvoll et al. 2001) | About 200 species distributed mainly in the Mediterranean; a few species in East Asia and northern Vietnam (Djursvoll et al. 2001) |

species could possibly be assigned to the genus *Uniramidesmus* based on the simple, slender, falcate gonopod; however, *Uniramidesmus* species are all much smaller (<10.0 mm in length), the gonopods are strongly falcate to coiled caudally and cross each other when in situ; the opening of the seminal groove is subterminal to terminal on a bare to more or less pubescent pulvillus (Mikhaljova 2004). On the contrary, the new species is far larger in size (ca 16.3 mm in length), the gonopods in situ are well separated from each other, and the opening of the seminal groove is on a typical hairy pulvillus.

Compared to *Schizoturanius* (Mikhaljova 2004), *Pacidesmus tuachua* sp. nov. differs well-developed paraterga (vs a strongylosomoid, moniliform body with narrow to almost missing paraterga, which are mostly smooth and only seldom laterally incised); also, the gonopod is without an accessory seminal chamber (vs with an accessory seminal chamber) and there is no gonopod femoral process (vs with a characteristic femoral process). Finally, the new species can hardly be assigned to the genus *Jaxartes*, which is confined to Central Asia, due to its

larger body size (16 mm long), the slender, strongly falcate gonopods without an accessory seminal chamber. On the contrary, the genus *Jaxartes* is diagnosed by its small body (usually ca 1 cm long); the metaterga show bosses or tubercles with bacilliform or trichoid setae, the paraterga are clearly laterally incised, there are four distal male podomeres with ventral sphaerotrichomes, the gonopod coxite is without outgrowths apart from the typical cannula, and the gonotelopodite is particularly slender, suberect, and with the endomere being considerably longer than a ventrally fringed process (if present at all); also, the endomere bears a parabasal tooth and has a subtruncate apex, basally with a very evident hairy pulvillus, but there is no distinct accessory seminal chamber (Antić et al. 2019).

*Pacidesmus tuachua* sp. nov. can hardly be placed in *Epanerchodus* or *Polydesmus* because its paraterga are relatively narrow, the seminal groove starts mesally, as usual, then is recurved laterad at the base of a particularly prominent endomere branch to enter an accessory seminal chamber that opens on a setose pulvillus, and the endomere bears additional processes.

The strongly sigmoid gonopodal telopodite in *P. tuachua* sp. nov is somewhat unusual in comparison to that in other *Pacidesmus* species. This difference may suggest a new genus; however, it currently seems best assigned to *Pacidesmus* based on the above discussion. It is noteworthy that most *Schizoturanius* or *Polydesmus* spp. likewise show only slightly curved gonopod telopodites, but relatively few species in these genera are so strongly sigmoid.

To support further study of Polydesmidae in Vietnam and Southeast Asia, an identification key to macropolydesmid genera occurring in Asia is provided:

## An identification key to macropolydesmid genera in Asia

Based on Turk (1945), Golovatch (1991), Djursvoll et al. (2001), Mikhaljova (2004), Golovatch et al. (2011), Golovatch and Geoffroy (2014), Antić et al. (2019).

1 Gonopods without an accessory seminal chamber (*Uniramidesmus*, *Nipponesmus*, *Jaxartes*) ..... **2**
– Gonopods with an accessory seminal chamber (*Schizoturanius*, *Pacidesmus*, *Gleninea*, *Polydesmus*, *Epanerchodus*) ..... **4**
2 Body moniliform, paraterga narrower. Seminal groove running mostly mesally to recurve neatly between exomere and endomere. Gonopod endomere distally with abundant bacilliform filaments..... ***Nipponesmus***
– Body not moniliform, paraterga broader. Seminal groove running entirely mesally. Gonopod endomere distally without abundant bacilliform filaments ... **3**
3 Seminal groove opening subterminally to terminally on a bare to more or less pubescent pulvillus..... ***Uniramidesmus***
– Seminal groove opening on a distinct, ventral, hairy pulvillus ..... ***Jaxartes***
4 Body moniliform, paraterga narrow. Loop of seminal groove distal ..... ..... ***Schizoturanius***
– Body not moniliform, paraterga broad. Loop of seminal groove not distal .... **5**
5 Paraterga strongly serrated or incised laterally. Gonopod endomere in distal part carrying numerous or several spine-like hairs or strong, sometime curved spines..... ***Gleninea***
– Paraterga not strongly serrated/incised laterally. Gonopod endomere without numerous, mostly strong, sometimes curved spines or bacilli or setae..... **6**

6 Paraterga narrower. Seminal groove starting mesally, then recurving laterad at the base of a particularly prominent endomere branch to enter an accessory seminal chamber that opens on a setose pulvillus; endomere carrying additional processes ..... ***Pacidesmus***

– Paraterga wider. Seminal groove largely running mesally. Gonopod endomere absent.....7

7 An endomere absent. Exomere from short and slightly curved to very long and strongly falcate, mostly uniramous, directed caudally, starting laterally or apically and basally of recurvature point of seminal groove ..... ***Polydesmus***

– An endomere mostly absent, but rather rarely present as only a more or less rudimentary structure, while the seminal groove after the recurvature point still makes a long way basad to debauch into a prominent, simple-haired, accessory seminal chamber placed at the bottom of a profound parabasal cavity in the telopodite..... ***Epanerchodus***

## Conclusion

Two polydesmid genera and species are presently known to occur in Vietnam: *Polydesmus vietnamicus* Nguyen, 2009 and *Pacidesmus tuachua* sp. nov. *Polydesmus vietnamicus* is an epigean, forest-dwelling species, while *Pacidesmus tuachua* is troglobiotic. The diversity of polydesmids in Vietnam is potentially greater given the number of species in other southern Asian regions. The paucity of species is either due to some yet-unknown historical, evolutionary phenomenon or, more likely, reflects insufficient sampling. More extensive surveys are needed to more fully clarify the diversity and biogeography of polydesmids in Southeast Asia.

## Acknowledgements

We thank Dang Van Dong and Nguyen Duc Hiep, from IEBR, for their help in fieldwork. Dr Michael S. Engel (American Museum of Natural History, New York, USA), Dr Sergei I. Golovatch (Russian Academy of Sciences, Moscow, Russia) and Dr Natdanai Likhitrakarn (Faculty of Agricultural Production, Maejo University, Chiang Mai, Thailand) are acknowledged for kindly checking, revising, and correcting the submission. The work is supported by the Vietnam Academy of Science and Technology under the project NCXS01.04/23-25.

## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

### Funding

The work is funded by the Vietnam Academy of Science and Technology under the project NCXS01.04/23-25 “Developing the first-class research team on the discovery of diversity and application potential of hymenopterans, myriapods and soil nematodes in

the limestone mountains of northeastern Vietnam". This study has also been supported by the following funds: the Fund for the Promotion of Joint International Research (Fostering Joint International Research (B)) (JSPS KAKENHI, no. 22KK0087, Leader: K. Eguchi, FY2022–2025), Grant-in-Aid for Scientific Research (C) (JSPS KAKENHI, no 23K05299, Leader: E Oguri, FY2023-2026), the Tokyo Metropolitan University Fund for TMU Strategic Research (leader: Prof. Noriaki Murakami, FY2020–FY2022), and the Asahi Glass Foundation (leader: K. Eguchi, FY2017–FY2022).

## Author contributions

Conceptualization: ADN. Data curation: ADN, KE. Formal analysis: ADN. Funding acquisition: KE, TTTV. Resources: TTTV. Visualization: TTTV. Writing - original draft: ADDN. Writing - review and editing: KE, ADN.

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## Data availability

All of the data that support the findings of this study are available in the main text.

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ZooKeys 1190: 281–302 (2024)
DOI: 10.3897/zookeys.1190.113418

Research Article

# Two new species of Hypodontolaiminae (Nematoda, Chromadorida, Chromadoridae) from the Yellow Sea with a phylogenetic analysis in the subfamily

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## Abstract

Two new species of Hypodontolaiminae, *Dichromadora media* **sp. nov.** and *Neochromadora parabilineata* **sp. nov.**, were isolated and described from the Yellow Sea, China. *Dichromadora media* **sp. nov.** is characterized by four long cephalic setae, the amphidial fovea transverse oval in the male and slit-shaped in the female, the pharynx with a single posterior bulb, spicules curved and distally bifurcated, gubernaculum jointed, four (1+3) precloacal supplements papilliform, and the tail conical elongated with a short spinneret. *Neochromadora parabilineata* **sp. nov.** is characterized by the buccal cavity with one large hollow dorsal tooth and two small subventral teeth, the pharynx with an obvious posterior bulb, spicules L-shaped and widened medially, gubernaculum boat-shaped, seven cup-shaped and equidistant precloacal supplements, and a long and gradually tapering tail. The phylogenetic analysis of maximum likelihood and Bayesian inference based on rDNA sequences confirmed the taxonomic positions of *Neochromadora parabilineata* **sp. nov.** and *Dichromadora media* **sp. nov.** within Hypodontolaiminae. Tree topology in Hypodontolaiminae shows the genera *Neochromadora*, *Dichromadora*, *Ptycholaimellus*, and *Spilophorella* as polyphyletic groups, and the genus *Chromadorita* as a paraphyletic group.

**Key words:** China, *Dichromadora*, marine nematode, *Neochromadora*

OPEN ACCESS

Academic editor: Nic Smol
Received: 28 September 2023
Accepted: 8 January 2024
Published: 30 January 2024

ZooBank: https://zoobank.org/EDBA3DBA-08BD-49AC-9ED5-ABFBDCF40207

Citation: Liang H, Guo W, Wang C (2024) Two new species of Hypodontolaiminae (Nematoda, Chromadorida, Chromadoridae) from the Yellow Sea with a phylogenetic analysis in the subfamily. ZooKeys 1190: 281–302. https://doi.org/10.3897/zookeys.1190.113418

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## Introduction

Nematodes are the most widely distributed and diverse metazoans on the planet, and a large number of nematode species still remain unidentified (Hodda 2022).Chromadoridae Filipjev, 1917 is one of the largest families of nematodes and shown as a monophyletic group with synapomorphies of male monorchid with an anterior testis; precloacal supplements cup-shaped or absent but never tubular; females with two reflexed ovaries, anterior to the right of the intestine, posterior to the left of the intestine (Tchesunov 2014). Chromadoridae has been reviewed systematically by Wieser (1954a), Gerlach and Riemann (1973/1974), Lorenzen (1981, 1994), Tchesunov (2014), and Venekey et al. (2019), and its phylogenetic relationships have been analyzed based on rDNA sequences by Holterman et al. (2008), Leduc et al. (2017), Venekey et al. (2019), and Guo

et al. (2023). The number of rDNA sequences in Chromadoridae have rapidly increased in GenBank: up to now, 250 sequences from 20 genera of Small Subunit (SSU) and 145 sequences from 11 genera of D2–D3 fragment of Large Subunit (LSU) have been deposited in GenBank. In our study of marine nematode taxonomy from the Rizhao coast, at the Yellow China Sea, two new species from the subfamily Hypodontolaiminae are described, *Dichromadora media* sp. nov. and *Neochromadora parabilineata* sp. nov. Ribosome DNA sequences from these two new species and three other species, *Dichromadora sinica* Huang & Zhang, 2010, *Dichromadora major* Huang & Zhang, 2010, and *Dichromadora multisetosa* Huang & Zhang, 2010, are acquired for phylogenetic analysis.

## Materials and methods

### Sample collection

In July 2022, undisturbed samples were collected from intertidal sediments in the Rizhao coast, the Yellow China Sea. Sediments samples were vertically collected with a syringe (2.6 cm internal diameter) to a depth of 8 cm and subdivided into 0–2 and 2–8 cm depth parts. Sediments used for morphological analysis were fixed in a 10% formalin solution in seawater and for molecular analysis were preserved in 95% ethanol. Formalin-fixed samples were stained with 0.1% of Rose Bengal for more than 24 hours. Meiofauna were extracted from the sediment through Ludox centrifugation (Higgins and Thiel 1988), washed through two sieves with mesh sizes of 500 μm and 45 μm with tap water to separate meiofauna from macrofauna (larger than 500 μm), transferred to a grid-lined Petri dish, and sorted under a stereoscopic microscope. Nematodes were transferred into a mixture of ethanol (50%) and glycerin in the ratio 1:9 by volume with the ethanol slowly evaporated away (McIntyre and Warwick 1984). Nematodes were mounted in glycerin on permanent slides. Descriptions were made using an Axiscope–5 differential interference contrast microscope (Zeiss, Germany). Line drawings were made with the aid of iPad (Apple, USA), and photographs were taken with the aid of ZEN software (Zeiss). Type specimens were deposited in the Institute of Oceanology, Chinese Academy of Sciences, Qingdao.

Sediments used for molecular analysis were washed and separated as with formalin-fixed samples except without Rose Bengal dying. Seven male specimens of *D. media* sp. nov., two male specimens of *N. parabilineata* sp. nov., five male specimens of *D. sinica*, two male specimens of *D. major*, and two male specimens of *D. multisetosa* were separated and confirmed on the temporary slides.

### DNA extraction, PCR amplification, and phylogenetic analysis

Genomic DNA was extracted with DNeasy Blood & Tissue kit (Qiagen, Germany) and used as amplification templates for nearly full length SSU rDNA gene, with primers of G18S4F (5' – GCT TGT CTC AAA GAT TAA GCC – 3') / 18PR (5' – TGA TCC WMC RGC AGG TTC AC – 3') (Blaxter et al. 1998), and D2–D3 fragment of LSU rDNA gene with primers of D2A (5' – ACA AGT ACC GTG AGG GAA AGT TG – 3') / D3B (5' – TCG GAA GGA ACC AGC TAC TA – 3') (Nunn 1992). PCR was conducted as described by Zhao et al. (2015). The PCR product was sequenced by Genewiz (China). The sequences were assembled in Genious v.

6.1.2. The newly obtained SSU rDNA sequences have the accession numbers as follows: *D. media* sp. nov. OR479913, *N. parabilineata* sp. nov. OR126985, *D. sinica* OR479916, *D. major* OR479911, and *D. multisetosa* OR479915; the D2–D3 fragment of LSU rDNA sequence accession numbers are *D. media* sp. nov. OR479918, *N. parabilineata* sp. nov. OR135360, *D. sinica* OR479914, *D. major* OR479912, and *D. multisetosa* OR479917. All have been deposited in GenBank.

Sequences of subfamily Hypodontolaiminae in GenBank were used for phylogenetic analysis. Forty-nine SSU rDNA sequences from seven genera (Table 1) longer than 600 bp were selected and aligned with the Muscle algorithm. Substitution models of (GTR (general time-reversible) + G (gamma distribution) + I (proportion of invariable sites)) were selected as the best-fit model and the analysis was rooted with *Latronema whataitai* Leduc & Zhao, 2015 (accession number KR048680). Sixteen D2–D3 fragment of LSU rDNA sequences from five genera (Table 2) were selected and aligned with the Muscle algorithm. Substitution models of (GTR (general time-reversible) + G (gamma distribution)) were selected as the best-fit model, and the analysis was rooted with *Latronema whataitai* (accession number KR04868).

The ML analyses were performed with Mega X with 1000 bootstrap replicates. The BI analyses were constructed with CIPRES (http://www.phylo.org/) and MrBayes on XSEDE v. 3.2.7a were used; the trees were run with chain length of 10,000,000, burn-in frac = 0.25. The topology of the resulting trees was visualized using FigTree v. 1.4.3 and refined with PowerPoint.

**Table 1.** SSU information of samples used for phylogenetic analysis.

| Species | GenBank number | Reference | Locality |
|---|---|---|---|
| *Chromadorita* aff. *leuckarti* | MF409784.1 | Schenk et al. 2018 | Germany |
| *Chromadorita* cf. *leuckarti* | FJ040473.1 | Holterman et al. 2008 | – |
| *Chromadorita humila* | OQ396742.1 | Sun et al. 2023 | China |
| *Chromadorita leuckarti* | FJ969119.1 | van Megen et al. 2009 | – |
| *Chromadorita leuckarti* | KJ636254.1 | Bert et al. 2014 | – |
| *Chromadorita spinicauda* | OK317201.1 | Leduc and Zhao 2023 | New Zealand |
| *Dichromadora major* | OR479911.1 | Wang et al. 2023 | China |
| *Dichromadora media* sp. nov. | OR479913.1 | Wang et al. 2023 | China |
| *Dichromadora multisetosa* | OR479915.1 | Wang et al. 2023 | China |
| *Dichromadora simplex* | MG669747.1 | Macheriotou et al. 2018 | Vietnam |
| *Dichromadora sinica* | OR479916.1 | Wang et al. 2023 | China |
| *Dichromadora* sp. | MN250081.1 | Pereira et al. 2019 | Beaufort Sea (USA) |
| *Dichromadora* sp. | FJ040506.1 | Holterman et al. 2008 | – |
| *Dichromadora* sp. | MN250085.1 | Pereira et al. 2019 | Beaufort Sea (USA) |
| *Dichromadora* sp. | MG669748.1 | Macheriotou et al. 2018 | Netherlands |
| *Dichromadora* sp. | MN250044.1 | Pereira et al. 2019 | Beaufort Sea (USA) |
| *Dichromadora* sp. | MK626828.1 | Tytgat et al. 2019 | Vietnam |
| *Dichromadora* sp. | MG669752.1 | Macheriotou et al. 2018 | Vietnam |
| *Dichromadora* sp. | MG669751.1 | Macheriotou et al. 2018 | Vietnam |
| cf. *Dichromadora* sp. | KJ636253.1 | Bert et al. 2014 | – |
| *Hypodontolaimus inaequalis* | MG669813.1 | Macheriotou 2018 | Netherlands |
| *Hypodontolaimus inaequalis* | MG669812.1 | Macheriotou et al. 2018 | Netherlands |

| Species | GenBank number | Reference | Locality |
|---|---|---|---|
| *Innocuonema tentabundum* | AY854208.1 | Meldal 2005 | Southampton (United Kingdom) |
| *Innocuonema tentabundum* | JN968213.1 | Fonseca et al. 2012 | – |
| *Neochromadora bilineata* | OQ396744.1 | Sun et al. 2023 | China |
| *Neochromadora parabilineata* sp. nov. | OR126985.1 | Wang et al. 2023 | China |
| *Neochromadora poecilosomoides* | OQ396720.1 | Chu et al. 2023 | China |
| *Neochromadora* sp. | AY854210.1 | Meldal 2005 | Southampton (United Kingdom) |
| *Neochromadora* sp. | MG669893.1 | Macheriotou et al. 2018 | Netherlands |
| *Neochromadora* sp. | KX944147.1 | Avo et al. 2017 | Mira estuary (Portugal) |
| *Neochromadora* sp. | MN250121.1 | Pereira et al. 2019 | Beaufort Sea (USA) |
| *Neochromadora* sp. | JN968279.1 | Fonseca et al. 2012 | – |
| *Ptycholaimellus areniculus* | MG669987.1 | Macheriotou et al. 2018 | Vietnam |
| *Ptycholaimellus areniculus* | MG669988.1 | Macheriotou et al. 2018 | Vietnam |
| *Ptycholaimellus brevisetosus* | MK626833.1 | Tytgat et al. 2019 | Vietnam |
| *Ptycholaimellus brevisetosus* | MK626834.1 | Tytgat et al. 2019 | Vietnam |
| *Ptycholaimellus brevisetosus* | MK626809.1 | Tytgat et al. 2019 | Vietnam |
| *Ptycholaimellus brevisetosus* | MG669989.1 | Macheriotou et al. 2018 | Vietnam |
| *Ptycholaimellus ocellatus* | OQ538290.1 | Sun et al. 2023 | China |
| *Ptycholaimellus spiculuncus* | OK317202.1 | Leduc and Zhao 2023 | New Zealand |
| *Ptycholaimellus* sp. | FJ040472.1 | Holterman et al. 2008 | – |
| *Ptycholaimellus* sp. | KX944158.1 | Avo et al. 2017 | Mira estuary (Portugal) |
| *Ptycholaimellus* sp. | JN968285.1 | Fonseca et al. 2012 | – |
| *Ptycholaimellus* sp. | MG669992.1 | Macheriotou et al. 2018 | Vietnam |
| *Ptycholaimellus* sp. | JN968257.1 | Fonseca et al. 2012 | – |
| *Spilophorella aberrans* | MG670031.1 | Macheriotou et al. 2018 | Vietnam |
| *Spilophorella paradoxa* | AY854211.1 | Meldal 2005 | Southampton (United Kingdom) |
| *Spilophorella paradoxa* | JN968274.1 | Fonseca 2012 | – |
| *Spilophorella* sp. | MG670032.1 | Macheriotou et al. 2018 | Vietnam |
| *Latronema whataitai* | KR048680.1 | Leduc and Zhao 2016 | – |

**Table 2.** D2–D3 fragment of LSU information of samples used for phylogenetic analysis.

| Species | GenBank number | Reference | Locality |
|---|---|---|---|
| *Chromadorita humila* | OQ396736.1 | Sun et al. 2023 | China |
| *Chromadorita spinicauda* | OK317226.1 | Leduc and Zhao 2023 | New Zealand |
| *Dichromadora cucullata* | GU003894.1 | Rodrigues et al. 2010 | USA |
| *Dichromadora major* | OR479912.1 | Wang et al. 2023 | China |
| *Dichromadora media* sp. nov. | OR479918.1 | Wang et al. 2023 | China |
| *Dichromadora multisetosa* | OR479917.1 | Wang et al. 2023 | China |
| *Dichromadora sinica* | OR479914.1 | Wang et al. 2023 | China |
| *Dichromadora* sp. | KC755220.1 | Vogt et al. 2014 | Wilhelmshaven (Germany) |
| *Neochromadora* aff. *poecilosoma* | KC755218.1 | Vogt et al. 2014 | Jadebusen (Germany) |
| *Neochromadora parabilineata* sp. nov. | OR135360.1 | Wang et al. 2023 | China |
| *Neochromadora poecilosomoides* | OQ417520.1 | Sun et al. 2023 | China |
| *Neochromadora* sp. | KC755219.1 | Vogt et al. 2014 | Wilhelmshaven (Germany) |
| *Ptycholaimellus ocellatus* | OQ466609.1 | Sun et al. 2023 | China |
| *Ptycholaimellus spiculuncus* | OK317227.1 | Leduc and Zhao 2023 | New Zealand |
| *Spilophorella* sp. | DQ077766.1 | De Ley et al. 2009 | Mexico |
| *Spilophorella* sp. | GU003892.1 | Rodrigues et al. 2010 | USA |
| *Latronema whataitai* | KR048681.1 | Leduc and Zhao 2016 | New Zealand |

## Results

### Taxonomic account

**Order Chromadorida Chitwood, 1933**
**Family Chromadoridae Filipjev, 1917**
**Subfamily Hypodontolaiminae De Coninck, 1965**

**Genus *Dichromadora* Kreis, 1929**

**Diagnosis (based on Venekey et al. 2019).** Cuticle with homogeneous ornamentation and a pronounced lateral differentiation of two longitudinal rows of enlarged dots. Six outer labial papillae and four cephalic setae in separate circles. Amphideal fovea transverse slit-like and loop shaped. Buccal cavity with a triangular hollow dorsal tooth or a large dorsal tooth and two additional ventrosublateral ones; denticles can be present. Peribuccal pharyngeal tissue not swollen anteriorly or with an asymmetrical dorsal swelling; a distinct posterior pharyngeal bulb. Precloacal supplements present or absent.

**Remarks.** The genus *Dichromadora* was erected by Kreis in 1929 with the type species *Dichromadora microdonta* Kreis, 1929 with the genus characters the cuticle with two longitudinal rows of dots, small tooth, pharynx bulb big and round, ovaries paired symmetrical and reflexed, and males with or without precloacal supplements. Six species from the genus *Chromadora* (*C. cephalata* Steiner, 1916, *C. cricophana* Filipjev, 1922, *C. geophila* de Man, 1876, *C. parapoecilosoma* Micoletzky, 1922, *C. sabulicola* Filipjev, 1918, *C. setosa* Bütschli, 1874) were transferred to *Dichromadora* by Kreis (1929). Later, *D. hyalocheile* De Coninck & Schuurmans Stekhoven, 1933, *D. tobaensis* Schneider, 1937, *D. strandi* Allgén, 1940, *D. punctata* Schuurmans Stekhoven, 1950, *D. tenuicauda* Schuurmans Stekhoven, 1950, and *D. abnormis* Gerlach, 1953a were described. Wieser (1954a) described *D. dissipata* Wieser, 1954a, revised the genus characters based mainly on tooth shape and provided new combinations. After Wieser (1954a), *D. apapillata* Timm, 1961, *D. arcospiculum* Timm, 1961, *D. simplex* Timm, 1961, *D. islandica* Kreis, 1963, *D. scandula* Lorenzen, 1966, and *D. cucullata* Lorenzen, 1973 were described and Gerlach and Riemann (1973) presented a list of sixteen species. Later, 12 species (*D. amphidiscoides* Kito, 1981, *D. abyssalis* Bussau, 1993, *D. gathuai* Muthumbi & Vincx, 1998, *D. loiseae* Muthumbi & Vincx, 1998, *D. longicaudata* Muthumbi & Vincx, 1998, *D. quadripapillata* Muthumbi & Vincx, 1998, *D. parasimplex* Dashchenko, 2002, *D. parva* Vermeeren, Vanreusel & Vanhove, 2004, *D. polaris* Vermeeren, Vanreusel & Vanhove, 2004, *D. polarsternis* Vermeeren, Vanreusel & Vanhove, 2004, *D. southernis* Vermeeren, Vanreusel & Vanhove, 2004 and *D. weddellensis* Vermeeren, Vanreusel & Vanhove, 2004) were described. Huang and Zhang (2010) described three species from the China Sea, *D. major*, *D. multisetosa*, and *D. sinica*, and provided a short review of *Dichromadora*. *Dichromadora abyssalis* was considered as valid by Holovachov (2020) based on the high quality descriptions and illustrations despite not following the International Code of Zoological Nomenclature (Venekey et al. 2019). With the addition of *D. rigida* Thanh, Tu & Gagarin, 2016 and *D. agilis* Long, Gagarin & Tu, 2022, 35 species are currently considered as valid (based on Venekey et al. 2019):

1. *Dichromadora abnormis* Gerlach, 1953 (Italy, San Rossore and Tirrenia beaches)
2. *Dichromadora abyssalis* Bussau, 1993 (SE Pacific, Peru Basin)
3. *Dichromadora agilis* Long, Gagarin & Tu, 2022 (Vietnam, Quảng Ninh)
4. *Dichromadora amphidiscoides* Kito, 1981 (Japan, Oshoro Bay)
5. *Dichromadora antarctica* (Cobb, 1914) (Antarctica, Cape Royd; = *Spilophora antarctica* Cobb, 1914)
6. *Dichromadora apapillata* Timm, 1961 (Indian Ocean, Bay of Bengal)
7. *Dichromadora arcospiculum* Timm, 1961 (Indian Ocean, Bay of Bengal)
8. *Dichromadora cephalata* (Steiner, 1916) (Arctic Ocean, Barents Sea; = *Chromadora cephalata* Steiner, 1916, *Chromadora cricophana* Filipjev, 1922)
9. *Dichromadora cucullata* Lorenzen, 1973 (North Sea, Baltic Sea, Helgoland)
10. *Dichromadora dissipata* Wieser, 1954 (Chile, Seno de Reloncaví)
11. *Dichromadora gathuai* Muthumbi & Vincx, 1998 (Indian Ocean, Kenyan coast)
12. *Dichromadora geophila* (de Man, 1876) (North Sea, Netherlands; = *Chromadora canadensis* (Cobb, 1914), *Chromadora geophila* (de Man, 1876), *Hypodontolaimus geophilus* (de Man, 1876), *Spiliphera geophila* de Man, 1876, *Spiliphera spectabilis* Allgén, 1929)
13. *Dichromadora gracilis* (Kreis, 1929) (France, Trebeurden; = *Spilophorella gracilis* Kreis, 1929)
14. *Dichromadora hyalocheile* De Coninck & Schuurmans Stekhoven, 1933 (Belgium, Oostende)
15. *Dichromadora islandica* Kreis, 1963 (Iceland, Eyjafjörður)
16. *Dichromadora loiseae* Muthumbi & Vincx, 1998 (Indian Ocean, Kenyan coast)
17. *Dichromadora longicaudata* Muthumbi & Vincx, 1998 (Indian Ocean, Kenyan coast)
18. *Dichromadora major* Huang & Zhang, 2010 (China, Yellow Sea)
19. *Dichromadora media* sp. nov. (China, Yellow Sea)
20. *Dichromadora microdonta* Kreis, 1929 (France, English Channel)
21. *Dichromadora multisetosa* Huang & Zhang, 2010 (China, Yellow Sea)
22. *Dichromadora parasimplex* Dashchenko, 2002 (New Guinea, Astrolabe Bay)
23. *Dichromadora parva* Vermeeren, Vanreusel & Vanhove, 2004 (Antarctic Sea, Halley Bay)
24. *Dichromadora polaris* Vermeeren, Vanreusel & Vanhove, 2004 (Antarctic Sea, Halley Bay)
25. *Dichromadora polarsternis* Vermeeren, Vanreusel & Vanhove, 2004 (Antarctic Sea, Halley Bay)
26. *Dichromadora punctata* Schuurmans Stekhoven, 1950 (Mediterranean, Villefranche Bay)
27. *Dichromadora quadripapillata* Muthumbi & Vincx, 1998 (Indian Ocean, Kenyan coast)
28. *Dichromadora rigida* Thanh, Tu & Gagarin, 2016 (Vietnam, Yen River Estuary)
29. *Dichromadora scandula* Lorenzen, 1966 (North Sea, Schleswig-Holstein)
30. *Dichromadora simplex* Timm, 1961 (Indian Ocean, Bay of Bengal)
31. *Dichromadora sinica* Huang & Zhang, 2010 (China, Yellow Sea)
32. *Dichromadora southernis* Vermeeren, Vanreusel & Vanhove, 2004 (Antarctic Sea, Halley Bay)
33. *Dichromadora strandi* Allgén, 1940 (Norway, Knivskjaerodden)
34. *Dichromadora tobaensis* Schneider, 1937 (Indonesia, Sumatra)

35. *Dichromadora weddellensis* Vermeeren, Vanreusel & Vanhove, 2004 (Antarctic Sea, Halley Bay)

***Dichromadora media* sp. nov.**
https://zoobank.org/4F7D70E0-54B3-422C-9A87-F496EA14C572
Figs 1, 2, Table 3

**Diagnosis.** Medium body size, cuticle with transverse rows of dots and a lateral differentiation of two longitudinal larger dots, four long cephalic setae, buccal cavity large with one large, hollow and straight dorsal tooth and two small ventrosublateral teeth, amphidial fovea transverse oval in male and slit-shaped in female, pharynx with single posterior bulb, spicules curved and distally bifurcated, gubernaculum jointed, four (1+3) papilliform precloacal supplements, tail conical elongated with short spinneret.

**Material examined.** Four males and three females were measured and studied. ***Holotype*:** ♂ 1 on slide 22ZJT8–1–2; ***Paratypes*:** ♂ 2 on 22ZJT8–1–2, ♂ 3 on 22ZJT8–1–2, ♂ 4 on 22ZJT8–2–5, ♀ 1 on 22ZJT8–2–7, ♀ 2 on 22ZJT8–2–7, and ♀ 3 on 22ZJT8–2–6.

**Type locality and habitat.** Rizhao coast, Shandong Province, China, 35°27'N, 119°35'E, 0–2 cm sediment depth, sandy sediment.

**Measurements.** All measurement data are given in Table 3.

**Description. Males.** Body cylindrical and medium sized (881–925 μm in length). Cuticle with transverse rows of dots and a differentiation consisting of two longitudinal rows of distinct larger dots starting posterior the amphidial fovea and extending to the tail tip (2 μm in width). Transverse bars connecting the two larger dots beginning from the middle of the pharynx to the middle of tail. Somatic setae present sparsely along the lateral differentiation in two longitudinal rows, short in the head and tail (9 μm in length), long in the middle of the body (12 μm in length). Inner and outer labial sensilla papilliform. Four cephalic sensilla setiform at the level of amphidial fovea (1.20–1.36 head diameter in length). Head blunt. Amphidial fovea oval (4 μm in width and 3 μm in length), small (26.7%–28.6% corresponding body diameter) and situated 0.47–0.57 head diameter from the anterior end. Buccal cavity cuticularized with a large, hollow and straight dorsal tooth and two small ventrosublateral teeth. Cheilostoma short with longitudinal cuticularized ribs. Pharynx cylindrical, anterior region surrounding buccal cavity slightly swollen, posterior region swollen into an elongated single bulb with plasmatic interruptions resembling a double bulb (18.8–20.0% of pharynx length). Nerve ring slightly posterior to middle pharynx region (53.6–60.9% of pharynx length). Renette cell of secretory-excretory system situated posterior to pharynx bulb, excretory pore located at anterior buccal cavity (6–8 μm from anterior end). Cardia not observed.

The reproductive system monorchid, with extended testis located to the right of intestine. Spicules equal and slightly curved, 23–25 μm (1.0–1.1 cloacal body diameter) along arc, proximal end slightly widened and distal end bifurcated. Gubernaculum jointed without apophysis. Four (1 + 3) precloacal supplements papilliform, anterior three supplements closely distanced and posteriormost supplement distant from the three anterior ones, distance between the supplements 9–12 μm, 8–9 μm, 16–19 μm, respectively, and posteriormost

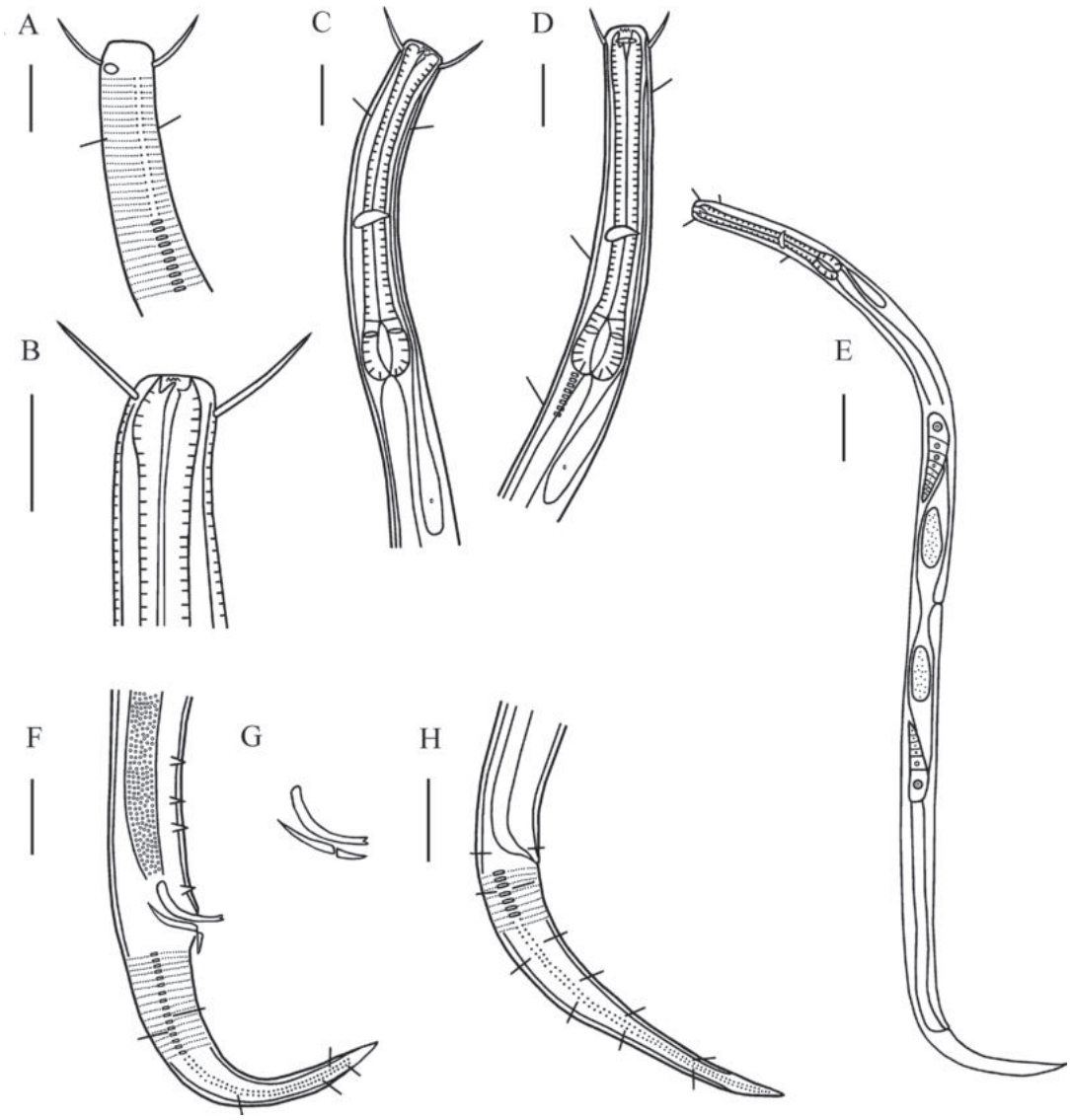


**Figure 1.** *Dichromadora media* sp. nov. **A** lateral view of male anterior region showing cuticle and amphidial fovea (holotype) **B** lateral view of male buccal cavity (holotype); **C** lateral view of male anterior region showing pharyngeal region (holotype) **D** lateral view of female anterior region showing buccal cavity, amphidial fovea and pharyngeal region (22ZJT8-2-7) **E** lateral view of female whole body (22ZJT8-2-7) **F** lateral view of male posterior body, showing precloacal supplements and tail (holotype); **G** lateral view of spicules and gubernaculum (22ZJT8-2-5) **H** lateral view of female posterior body showing tail (22ZJT8-2-7). Scale bars: 20 μm (**A–D, F, H**); 50 μm (**E**).

supplement 7–8 μm from cloaca. Tail elongated conical, gradually tapering, 4.5–5.0 cloacal body diameters. Spinneret very short, 1–2 μm in length.

**Females.** Similar to males in most characteristics. Amphidial fovea slit-like (50.0% corresponding body diameter). Cephalic setae short (10–11 μm in length). Reproductive system didelphic, with opposed and reflexed ovaries. Anterior ovary to right of intestine and posterior ovary to left of intestine. Eggs oval shaped, 8–10 × 10–11 μm. Vulva at the middle of the total body. Vagina short.

**Etymology.** Species epithet *media* refers to the medium body size.

**Remarks.** *Dichromadora media* sp. nov. differs from all other species of the genus *Dichromadora* by the amphidial fovea shape and jointed gubernaculum and it is similar to *D. dissipata*, *D. quadripapillata*, and *D. sinica* in body length and precloacal supplements number. However, it differs from *D. dissipata* in

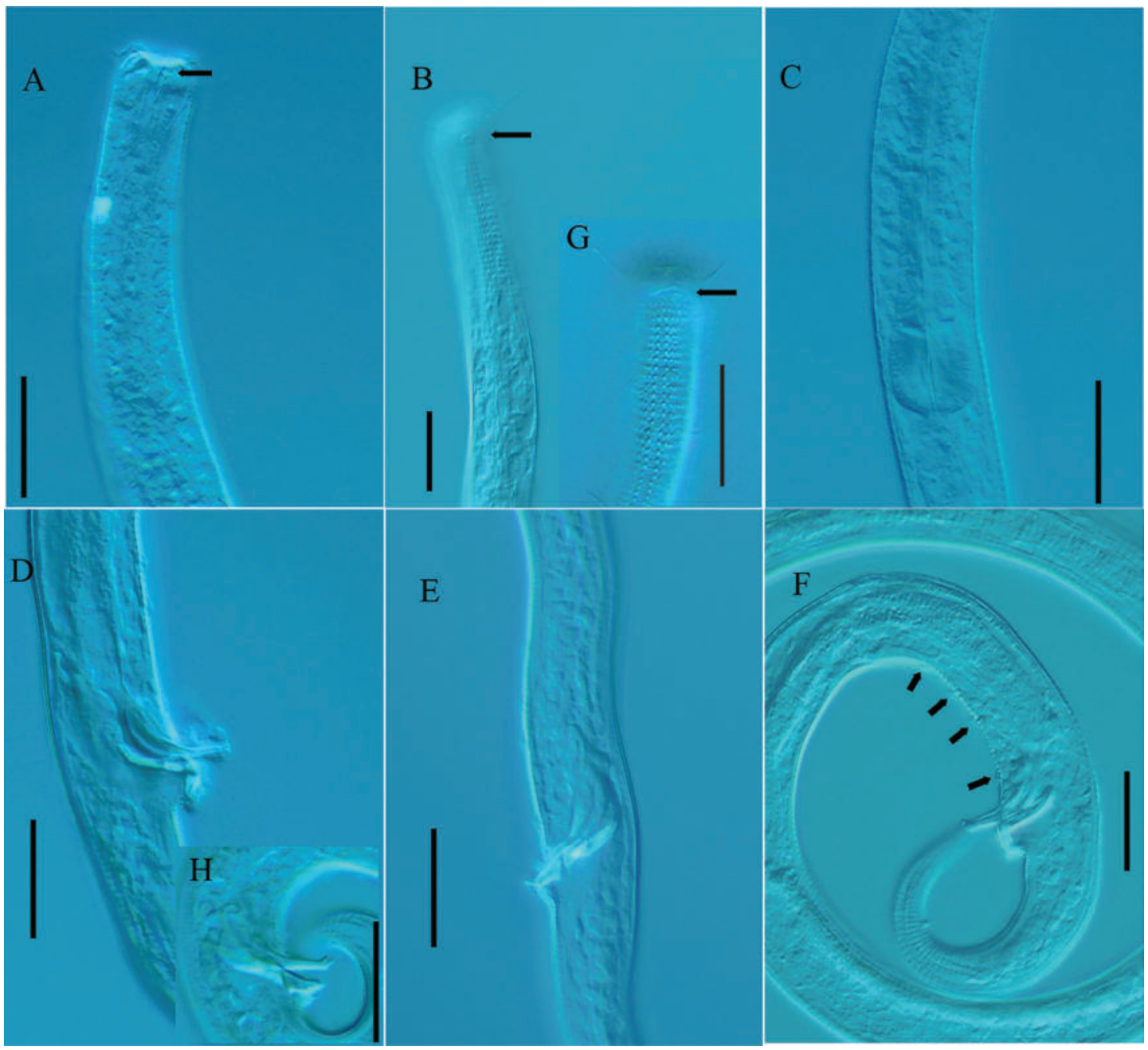


**Figure 2.** *Dichromadora media* sp. nov. **A** lateral view of male anterior region showing tooth (arrow) (holotype) **B** lateral view of male anterior region showing cuticle, amphidial fovea (arrow) (holotype) **C** lateral view of male anterior region showing pharyngeal region (holotype) **D** lateral view of male posterior body, showing spicules (holotype) **E** lateral view of male posterior body, showing gubernaculum (22ZJT8-1-2) **F** lateral view of male posterior body, showing precloacal supplements (arrows) (holotype) **G** lateral view of female anterior region showing amphidial fovea (arrow) (22ZJT8-1-2) **H** lateral view of distal end of spicules (22ZJT8-2-5). Scale bars: 20 μm.

cephalic setae length (10–19 μm vs 9–9.5 μm), spicules length (23–25 μm vs 39 μm), gubernaculum shape (double-jointed without apophysis vs not jointed with dorsal apophysis) and precloacal supplements (1+3 vs 5); differs from *D. quadripapillata* in cephalic setae length (10–19 μm vs 4–5 μm), spicules shape (slightly curved and distally bifurcated vs curved with pointed distal end) and precloacal supplements shape (papilliform vs cup-shaped); differs from *D. sinica* in cuticle differentiation (lateral differentiation with transverse bars vs lateral differentiation without transverse bars), pharynx bulb shape (single bulb with plastic interruptions vs double bulb), spicules shape (slightly curved and distally bifurcated vs distal end with a hook), gubernaculum shape (jointed vs not jointed), and precloacal supplements arrangement (1+3 vs 3+1).

*Dichromadora media* sp. nov. shows a close relationship with *D. sinica* in the phylogenetic trees (Figs 5, 6) based on rDNA sequences and it differs by 2% (39 in 1656 bp, including two gaps) in SSU and 5% (38 in 770 bp, including four

**Table 3.** Measurements of *Dichromadora media* sp. nov. (in µm except for ratios).

| Characters | Holotype | Paratypes | Paratypes |
|---|---|---|---|
| | male | males (*n* = 3) | females (*n* = 3) |
| Total body length | 925 | 895±14.5(881–910) | 812±44.6(761–844) |
| Maximum body diameter | 23 | 22.3±0.6(22–23) | 27.7±0.6(27–28) |
| Head diameter | 15 | 14.3±0.6(14–15) | 16±0(16–16) |
| Length of cephalic setae | 18 | 18.3±0.6(18–19) | 10.7±0.6(10–11) |
| Amphidial fovea width | 4 | 4±0(4–4) | 8±0(8–8) |
| Amphidial fovea from anterior end | 7 | 7.3±0.6(7–8) | 4.7±0.6(4–5) |
| Body diameter at amphidial fovea | 15 | 14.3±0.6(14–15) | 16±0(16–16) |
| Nerve ring from anterior end | 64 | 64.3±5.1(60–70) | 64±4.6(59–68) |
| Body diameter at nerve ring | 18 | 18±0(18–18) | 20.3±1.2(19–21) |
| Pharynx length | 116 | 114.7±2.5(112–117) | 119±4.4(116–124) |
| Pharynx bulb length | 22 | 22.3±0.6(22–23) | 25.3±1.5(24–27) |
| Body diameter at base of pharynx | 19 | 19±0(19–19) | 22.3±1.2(21–23) |
| Cloacal/anal body diameter | 23 | 22.3±0.6(22–23) | 15.7±0.6(15–16) |
| Spicules length along arc | 23 | 24.3±0.6(24–25) | – |
| Gubernaculum length | 23 | 22±1(21–23) | – |
| Vulva from anterior end | – | – | 399±16.1(381–412) |
| Body diameter at vulva | – | – | 27.3±0.6(27–28) |
| V% | – | – | 49.2±0.8(48.6–50.1) |
| Precloacal supplements | 1+3 | 1+3 | – |
| Tail length | 103 | 107±3.5(105–111) | 106.7±6.8(99–112) |
| a | 40.2 | 40.1±1.2(38.9–41.4) | 29.4±1.9(27.2–30.8) |
| b | 8.0 | 7.8±0.1(7.8–7.9) | 6.8±0.3(6.6–7.2) |
| c | 9.0 | 8.4±0.4(7.9–8.7) | 7.6±0.2(7.4–7.7) |
| c’ | 4.5 | 4.8±0.2(4.6–5.0) | 6.8±0.2(6.6–7) |

gaps) in LSU D2–D3 fragment, but they can be morphologically differentiated based on pharynx bulb shape, spicule shape, gubernaculum shape and precloacal supplements.

### Genus *Neochromadora* Micoletzky, 1924

**Diagnosis (based on Venekey et al. 2019).** Cuticle ornamentation heterogeneous and complex, with lateral differentiation visible as two or three longitudinal rows of large dots. Six small outer labial setae or papillae and four cephalic setae in separate circles. Inner labial sensilla may be conspicuous in one species (*N. munita*). Presence of somatic setae in some species. Amphidial fovea transverse slit-like and loop shaped. Buccal cavity with a dorsal tooth and two ventrosublateral teeth, in some species the dorsal one being larger than the others. Denticles can be present. Pharynx anteriorly not swollen or swollen next to the dorsal tooth. Pharynx with a single well-developed posterior bulb. Male usually with numerous precloacal supplements.

**Remarks.** The genus *Neochromadora* was erected by Micoletzky (1924) with the type species *Neochromadora poecilosoma* (de Man, 1893). And six species, *Neochromadora aberrans* (Cobb, 1930), *Neochromadora craspedota* (Steiner, 1916), *Neochromadora edentata* (Cobb, 1914), *Neochromadora izhorica* (Filipjev, 1929), *Neochromadora poecilosomoides* (Filipjev, 1918), *Neochromadora sabuli-*

*cola* (Filipjev, 1918) have been added to *Neochromadora*. Gerlach (1951) described *N. tecta* Gerlach, 1951, redescribed *N. poecilosoma*, *N. izhorica* (Filipjev, 1929), and transferred *Spiliphera trichophora* (Steiner, 1921) to *Neochromadora*. Later Gerlach (1952, 1953b) described *N. attenuate* Gerlach, 1952 and *N. complexa* Gerlach, 1953b. Wieser (1954a) divided *Neochromadora* into two subgenera *Neochromadorina* (Wieser, 1954a) and *Trichodorina* (Wieser, 1954a) based on tooth structure, cervical and somatic setal length, and pharyngeal bulb and described three new species: *N. lateralis* Wieser, 1954a, *N. calathifera* Wieser, 1954a, and *N. torquata* Wieser, 1954a. Later, Wieser (1954b) described another two species *N. amembranata* Wieser, 1954b and *N. brevisetosa* Wieser, 1954b. Afterwards, six species, *N. bonita* Gerlach, 1956, *N. coudenhovei* Wieser, 1956, *N. notocraspedota* Allgén, 1958, *N. appiana* Wieser, 1959, *N. pugilator* Wieser, 1959 and *N. bicoronata* (Wieser, 1959) (synonym *Endeolophos spinosus* (Gerlach, 1957) were described. Wieser (1959) withdrew the subgenus *Trichodorina* with a redescription of *N. poecilosoma* found in Puget Sound. Later, 13 species (*N. alatocorpa* Hopper, 1961, *N. nitida* Timm, 1961, *N. munita* Lorenzen, 1971, *N. paratecta* Blome, 1974, *N. paramunita* Boucher, 1976, *N. angelica* Riemann, 1976, *N. bilineata* Kito, 1978, *N. oshoroana* Kito, 1981, *N. orientalis* Lemzina, 1982, *N. papillosa* Pastor de Ward, 1985, *N. lineata* Pastor de Ward, 1985, *N. nicolae* Vincx, 1986 and *N. alejandroi* Lo Russo & Pastor de Ward, 2012) were described. Hopper (1963) considered *Neochromadora trilineata* Schneider, 1943 as incertae sedis due to the unavailability of specimens. Vincx (1986) considered *N. paramunita* as a synonym of *N. munita*. Up to now, 33 species are currently considered as valid (based on Venekey et al. 2019):

1. *Neochromadora aberrans* (Cobb, 1930) (Antarctic, Commonwealth Bay; = *Spiliphera aberrans* Cobb, 1930)
2. *Neochromadora alatocorpa* Hopper, 1961 (USA, Alabama)
3. *Neochromadora alejandroi* Lo Russo & Pastor de Ward, 2012 (Argentina, San Matías gulf)
4. *Neochromadora amembranata* Wieser, 1954 (Italy, Sampieri)
5. *Neochromadora angelica* Riemann, 1976b (Germany, Helgoland)
6. *Neochromadora appiana* Wieser, 1959 (USA, Washington)
7. *Neochromadora bilineata* Kito, 1978 (Japan, Hokkaido)
8. *Neochromadora bonita* Gerlach, 1956 (Brazil, Cananeia)
9. *Neochromadora brevisetosa* Wieser, 1954 (Italy, Sampieri)
10. *Neochromadora calathifera* Wieser, 1954b (Chile, Seno Reloncavi)
11. *Neochromadora complexa* Gerlach, 1953a (Chile, Seno Ultima Esperanza)
12. *Neochromadora coudenhove*i Wieser, 1956b (Greece, Piraeus)
13. *Neochromadora craspedota* (Steiner, 1916) (Arctic Ocean, Barents Sea; = *Chromadora craspedota* Steiner, 1916)
14. *Neochromadora edentata* (Cobb, 1914) (Antarctic, Cape Royds; = *Nygmatonchus edentata* (Cobb, 1914) Wieser, 1954, *Spiliphera edentata* Cobb, 1914)
15. *Neochromadora izhorica* (Filipjev, 1929) (Baltic Sea, Neva Bay; = *Chromadorella izhorica* Filipjev, 1929)
16. *Neochromadora lateralis* Wieser, 1954 (Chile, Seno Reloncavi)
17. *Neochromadora lineata* Pastor de Ward, 1985a (Argentina, Deseado river)
18. *Neochromadora munita* Lorenzen, 1972 (Germany, Helgoland; = *Neochromadora paramunita* Boucher, 1976)
19. *Neochromadora nicolae* Vincx, 1986 (North Sea, Southern Bight)

20. *Neochromadora nitida* Timm, 1961 (Indian Ocean, Bengal Bay)
21. *Neochromadora notocraspedota* Allgén, 1958 (Uruguay, Uruguay coast)
22. *Neochromadora orientalis* Lemzina, 1982 (Kyrgyzstan, Lake Issyk-Kul)
23. *Neochromadora oshoroana* Kito, 1981 (Japan, Oshoro Bay)
24. *Neochromadora papillosa* Pastor de Ward, 1985 (Argentina, Deseado River)
25. *Neochromadora parabilineata* sp. nov. (China, Yellow Sea)
26. *Neochromadora paratecta* Blome, 1974 (Germany, Sylt)
27. *Neochromadora poecilosoma* (de Man, 1893) (North Sea, English Channel; = *Chromadora poecilosoma* de Man, 1893)
28. *Neochromadora poecilosomoides* (Filipjev, 1918) (Black Sea, Kruglaya Bay and Georgievskii Monastery Bay; = *Chromadora poecilosomoides* Filipjev, 1918)
29. *Neochromadora pugilator* Wieser, 1959 (USA, Washington)
30. *Neochromadora sabulicola* (Filipjev, 1918) (Black Sea, Kruglaya Bay and Georgievskii Monastery Bay; = *Chromadora sabulicola* Filipjev, 1918)
31. *Neochromadora tecta* Gerlach, 1951 (Germany, Amrum Island)
32. *Neochromadora torquata* Wieser, 1954 (Chile, Seno Reloncavi)
33. *Neochromadora trichophora* (Steiner, 1921a) (Spain, Canary Islands; = *Spiliphera trichophora* Steiner, 1921, *Neochromadora longisetosa* Schuurmans-Stekhoven, 1935)

***Neochromadora parabilineata* sp. nov.**
https://zoobank.org/27F8BC81-DA38-470D-AD2B-F4B20F302588
Figs 3, 4, Table 4

**Diagnosis.** Medium body size, buccal cavity with one large hollow dorsal tooth and two small subventral teeth, spicules curved and L-shaped, gubernaculum boat-shaped, seven precloacal supplements cup-shaped, tail conical and gradually tapering.

**Material examined.** Four males and three females were measured and studied. ***Holotype*:** ♂ 1 on slide 22HSB11–2–20; ***paratypes*:** ♂ 2 on 22HSB11–1–21, ♂ 3 on 22HSB11–2–18, ♂ 4 on 22HSB11–2–20, ♀ 1 on 22HSB11–2–18, ♀ 2 on 22HSB11–1–18, and ♀ 3 on 22HSB11–2–18.

**Type locality and habitat.** Rizhao coast, Shandong Province, China, 35°5'N, 119°20'E, 0–2 cm sediment depth, sandy sediment.

**Measurements.** All measurement data are given in Table 4.

**Description. Males.** Body medium sized (864–943 µm), anterior end truncated and posterior end tapered. Cuticle heterogeneous and complex, five transverse rows of small dots present just posterior to cephalic setae, two or three longitudinal rows of larger dots posterior to the cephalic setae to middle part of body, larger dots changing to rectangular markings from middle body to posterior part of cloaca and rectangular markings changing back to larger dots until tail end. Six inner and six outer labial sensilla papilliform, four setiform cephalic sensilla (0.5–0.7 head diameter in length). Somatic setae present in pharynx and tail region (8 µm in length). Amphidial fovea situated at level of cephalic setae, transverse oval, 6–7 µm in width and 2 µm in length (50–58% corresponding body diameter). Buccal cavity shallow, 10–14 µm in depth. Cheilostoma short with cuticularized longitudinal folds. Pharyngostoma with one large hollow dorsal tooth and two small subventral teeth. Pharynx cylindrical, pos-

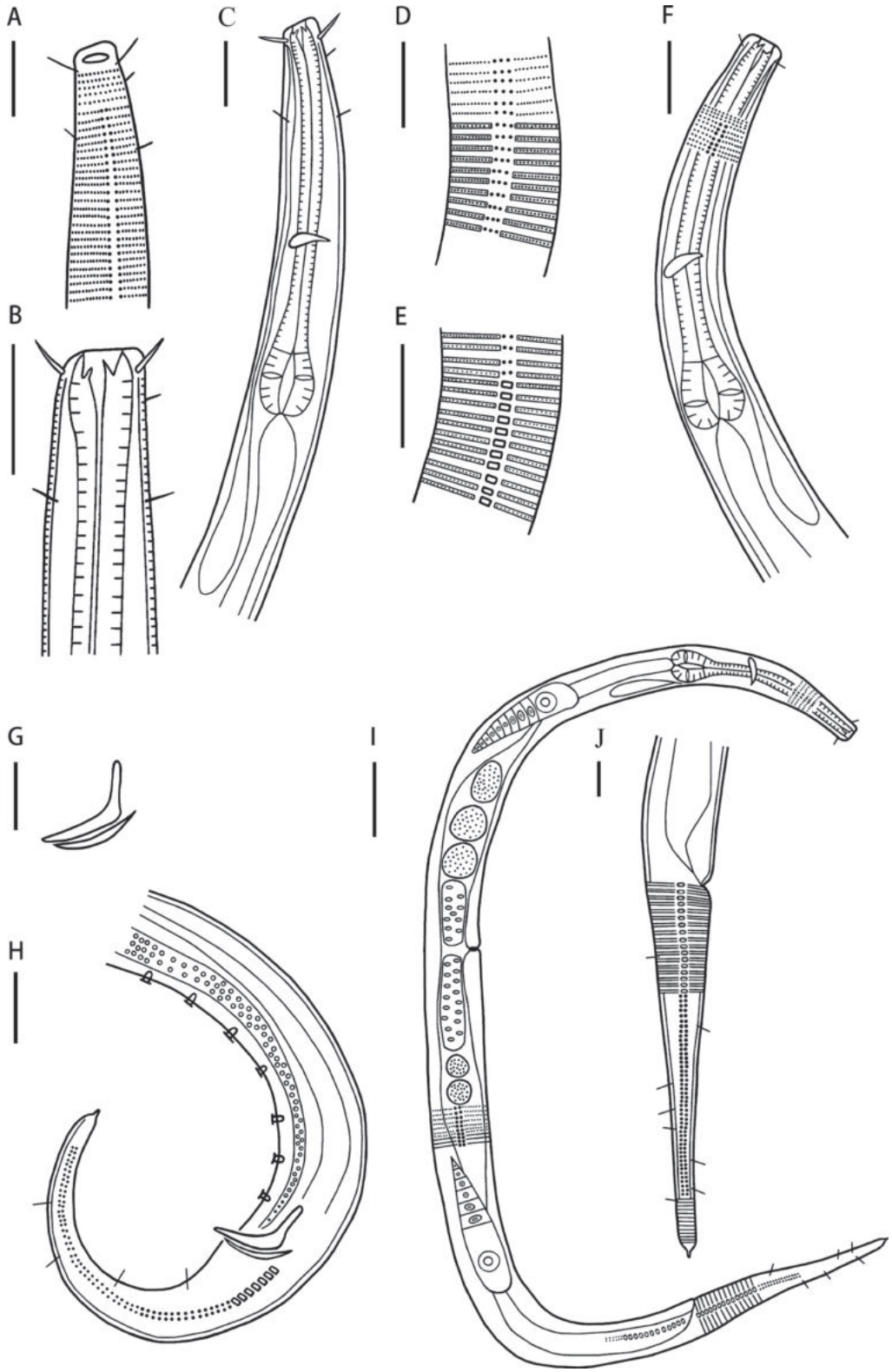


**Figure 3.** *Neochromadora parabilineata* sp. nov. **A** lateral view of male anterior region showing cuticle and amphidial fovea (holotype) **B** lateral view of male buccal cavity (holotype) **C** lateral view of male anterior region showing pharyngeal region (holotype) **D** lateral view of male cuticle at pharynx region (holotype) **E** lateral view of male cuticle at middle body (holotype) **F** lateral view of female anterior region showing buccal cavity and pharyngeal region (22HSB11-1-18) **G** lateral view of spicules and gubernaculum (22HSB11-2-20) **H** lateral view of male posterior body, showing precloacal supplements and tail (holotype) **I** lateral view of female whole body (22HSB11-2-18) **J** lateral view of female posterior body showing tail (22HSB11-2-18). Scale bars: 20 μm (**A–H, J**); 50 μm (**I**).

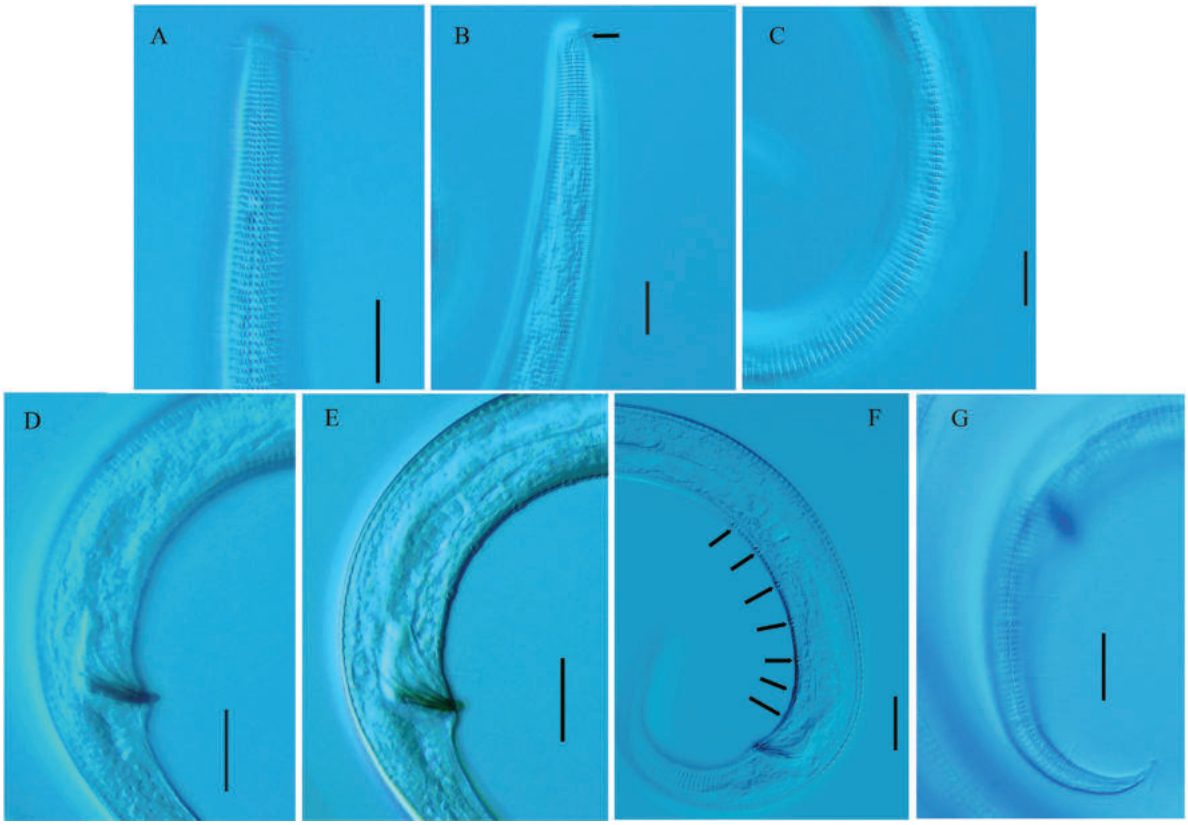


**Figure 4.** *Neochromadora parabilineata* sp. nov. **A** lateral view of male anterior region showing cuticle (holotype) **B** lateral view of female anterior region showing amphidial fovea (arrow) (22HSB11-2-18) **C** lateral view of cuticle at middle body (holotype) **D** lateral view of male posterior body, showing spicules (22HSB11-2-20) **E** lateral view of male posterior body, showing gubernaculum (22HSB11-2-20; **F** lateral view of male posterior body, showing precloacal supplements (arrows) (holotype) **G** lateral view of male posterior body, showing tail and cuticle (22HSB11-2-20). Scale bars: 20 μm.

terior region swollen into an oval bulb (17.5–17.7% of pharynx length). Nerve ring slightly posterior to middle pharynx region (64.2–66.9% of pharynx length). Secretory-excretory system present; renette cell situated posterior to pharynx bulb, excretory pore at level with cephalic setae. Cardia not observed.

Reproductive system with a single, outstretched testis. Spicules curved and L-shaped, widened at the middle part, 28–31 μm (0.81–0.86 cloacal body diameters) along arc. Gubernaculum short and boat-shaped, distal end tapered. Seven precloacal supplements cup-shaped, distance between the anteriormost and cloaca, the posteriormost and cloaca, 100 μm and 18 μm respectively, distance between supplements almost equal-distanced. Tail conical and gradually tapering, 4.4–4.8 cloacal body diameter in length. Spinneret short, 5 μm in length.

**Females.** Similar to males in most characteristics. Tail slightly longer than in males (5.8–6.8 anal body diameters in length). Reproductive system didelphic, with opposed and reflexed ovaries. Anterior ovary to left of intestine and posterior ovary to right of intestine. Spermatheca present. Vulva situated anterior to middle of body. Vagina short and muscularized.

**Etymology.** Species epithet *parabilineata* refers to the new species being similar to *Neochromadora bilineata*.

**Differential diagnosis.** *Neochromadora parabilineata* sp. nov. is similar to *N. bilineata*, *N. izhorica*, *N. complexa*, and *N. poecilosoma* in precloacal supplements number (7–9). But it differs from *N. bilineat*a in body length (864–943 μm

**Table 4.** Measurements of *Neochromadora parabilineata* sp. nov. (in μm except for ratios).

| Characters | Holotype | Paratypes | Paratypes |
|---|---|---|---|
| | male | males (*n* = 3) | females (*n* = 3) |
| Total body length | 878 | 905.3±39.6(864–943) | 913±27.8(881–931) |
| Maximum body diameter | 25 | 25.7±1.5(24–27) | 33.7±5.8(27–37) |
| Head diameter | 12 | 11.7±1.5(10–13) | 11.7±0.6(11–12) |
| Length of cephalic setae | 8 | 7±0(7–7) | 7.3±0.6(7–8) |
| Buccal cavity depth | 10 | 10.7±3.1(8–14) | 7±1(6–8) |
| Amphidial fovea width | 6 | 6.7±0.6(6–7) | 6±0(6–6) |
| Amphidial fovea from anterior end | 3 | 3±0(3–3) | 3±0(3–3) |
| Body diameter at amphidial fovea | 12 | 11.7±0.6(11–12) | 12±0(12–12) |
| Nerve ring from anterior end | 86 | 80.7±3.8(78–85) | 84±6.1(80–91) |
| Body diameter at nerve ring | 23 | 21.3±0.6(21–22) | 23.3±0.6(23–24) |
| Pharynx length | 131 | 124.7±2.1(123–127) | 128±2.6(125–130) |
| Pharynx bulb length | 23 | 22.7±0.6(22–23) | 26±3.6(23–30) |
| Body diameter at the base of pharynx | 24 | 22.3±1.2(21–23) | 26.3±2.9(23–28) |
| Cloacal/anal body diameter | 25 | 24±0(24–24) | 19.3±0.6(19–20) |
| Spicules length along arc | 31 | 29±1(28–30) | – |
| Gubernaculum length | 21 | 20.3±1.5(19–22) | – |
| Vulva from anterior end | – | – | 380.3±34.1(341–401) |
| Body diameter at vulva | – | – | 32±4.6(27–36) |
| Precloacal supplements | 7 | 7 | – |
| V% | – | – | 41.6±2.5(38.7–43.1) |
| Tail length | 113 | 111.3±5.5(105–115) | 121.7±12.6(110–135) |
| a | 35.1 | 35.4±3(32–37.9) | 27.6±4.3(25.1–32.6) |
| b | 6.7 | 7.3±0.3(7–7.6) | 7.1±0.1(7–7.2) |
| c | 7.8 | 8.2±0.7(7.6–9) | 7.6±0.8(6.9–8.5) |
| c’ | 4.5 | 4.6±0.2(4.4–4.8) | 6.3±0.5(5.8–6.8) |

vs 567–852 μm), cephalic setae length (7–8 μm vs 4–6 μm), amphidial fovea width (50–58% vs 45% corresponding body diameter), spicules shape and length (L-shaped and widened in the middle portion, 28–31 μm vs arcuate and gradually narrowing, 23–26 μm), and gubernaculum length (19–22 μm vs 15–18 μm); differs from *N. izhorica* in cephalic seta length (7–8 μm vs 14 μm), pharynx shape (posterior bulb obvious vs posterior bulb weak), spicules length (28–31 μm vs 31.5–34.5 μm), gubernaculum shape (distal end tapered vs distal end with anterior-laterally curved tip) (Riemann 1966); differs from *N. complexa* in body length (864–943 μm vs 642 μm), spicules shape (curved and L-shaped with middle portion widened vs L-shaped even in width), gubernaculum shape (boat-shaped vs dorsal part slenderly extended), distance between precloacal supplements (10–15 μm vs 2–5 μm) (calculation based on Gerlach 1953b: fig. 11); differs from *N. poecilosoma* in body length (864–943 μm vs 1900–2000 μm), cephalic setae length (7–8 μm vs 10–14 μm), spicule length (28–31 μm vs 60–65 μm), gubernaculum shape (boat-shaped vs distal tip with small tooth) (de Man 1893).

## Molecular phylogenetic analysis

The ML topology trees which are obtained based on the rDNA gene sequences are mostly in accordance with the BI topology trees, and only the BI trees are shown in Figs 5, 6.

Sequences of seven genera of the subfamily Hypodontolaiminae, *Chromadorita* Filipjev, 1922, *Dichromadora*, *Hypodontolaimus* de Man, 1886, *Innocuonema* Inglis, 1969, *Neochromadora*, *Ptycholaimellus* Cobb, 1920 and *Spilophorella* Filipjev, 1917 are included in the SSU and LSU rDNA analyses. At genus level, only species of *Chromadorita* cluster in one clade (posterior probability 70 in SSU and 99 in LSU, bootstrap value 99 in LSU) but *Chromadorita* is shown as paraphyletic. *Dichromadora multisetosa* (OR479915), *D. major* (OR479911), *N. poecilosomoides* (OQ396720), and *Innocuonema tentabunda* (JN968213, as *Chromadorita tentabunda*) clustered with the *Chromadorita* clade in the SSU

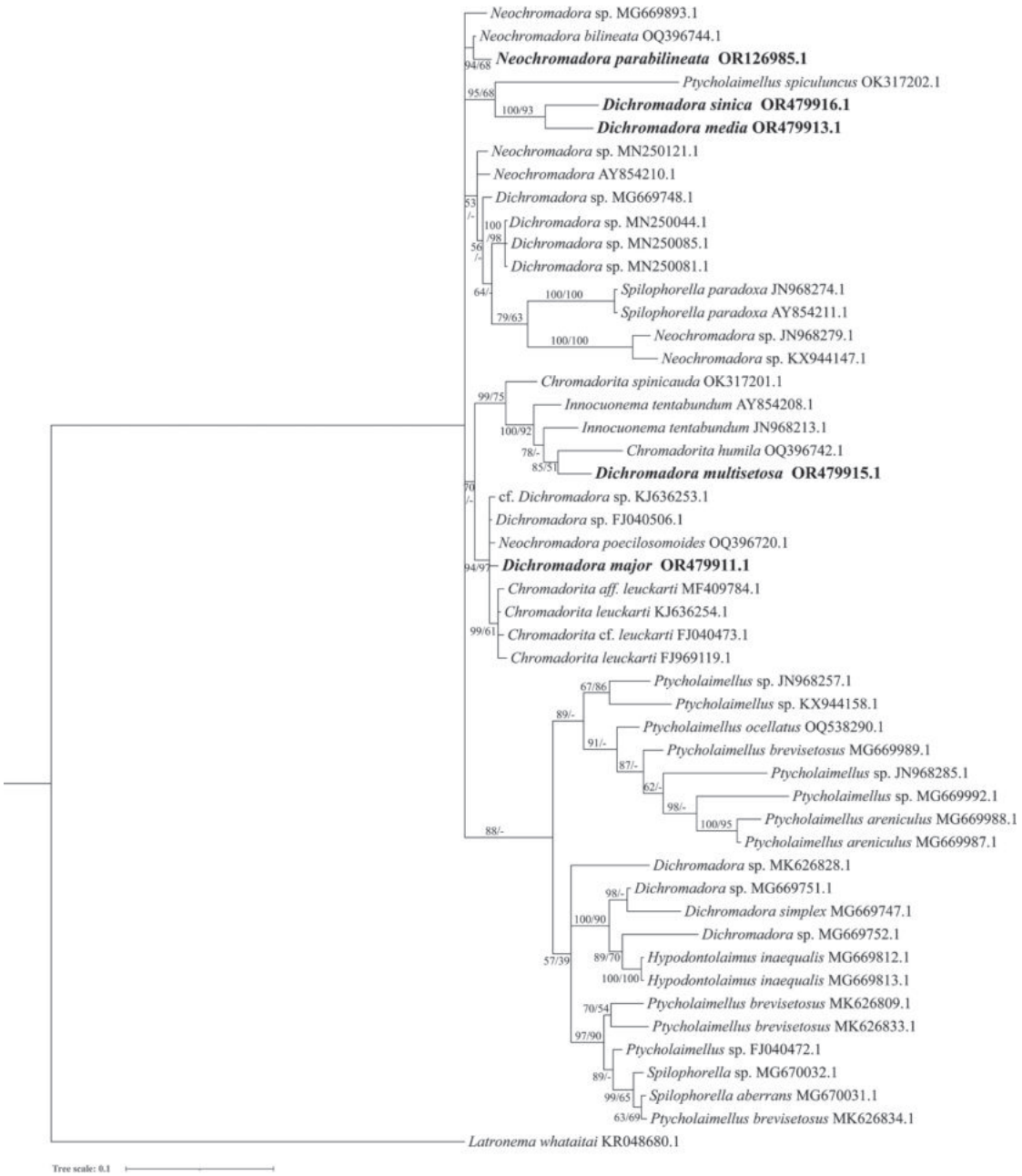


**Figure 5.** Bayesian inference tree of the subfamily Hypodontolaiminae inferred from Small Subunit (SSU) sequences under the general time-reversible (GTR) + gamma distribution (G) + proportion of invariable sites (I) model. Posterior probability (left) and bootstrap values (right) are given on corresponding clades. The sequences obtained in this study are shown in bold. The scales indicate substitutions per site.

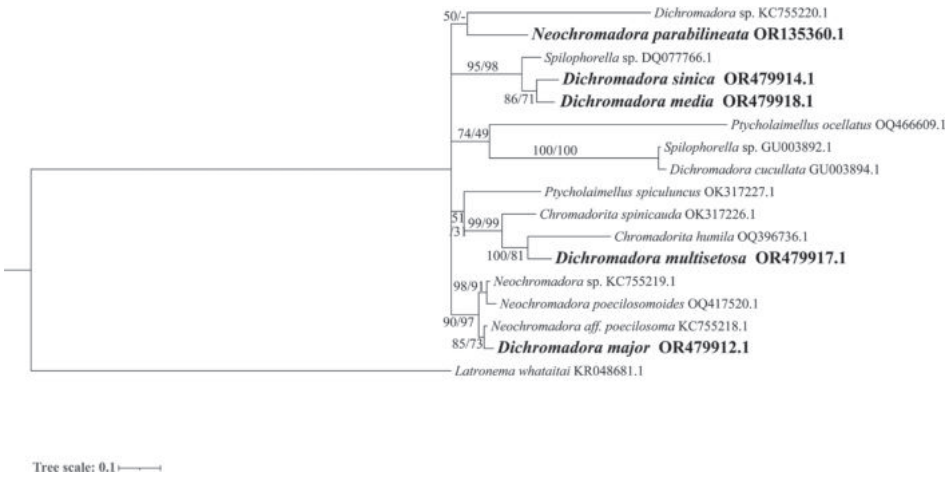


**Figure 6.** Bayesian inference tree of the subfamily Hypodontolaiminae inferred from the D2-D3 fragment of Large Subunit (LSU) sequences under the general time-reversible (GTR) + gamma distribution (G) model. Posterior probability (left) and bootstrap values (right) are given on corresponding clades. The sequences obtained in this study are shown in bold. The scales indicate substitutions per site.

analysis. These species share a common character of peribuccal cavity tissue with an asymmetrical dorsal swelling and only one posterior pharynx bulb. However, *Chromadorita* can be morphologically differentiated from them by having the cuticle homogeneous without any lateral differentiation.

Six sequences of genus *Dichromadora* have been identified to species level, but they are in four different clades in both SSU and LSU analyses and therefore paraphyletic. Among the species of *Dichromadora*, *Dichromadora multisetosa* is the only species with the gubernaculum not being boat-shaped but with dorsal caudal apophysis, and clustered with *Chromadorita humila* (gubernaculum possessing arched dorsal-caudal apophysis) highly supported by the LSU topology tree (posterior probability 85, bootstrap value 51 in SSU; posterior probability 100, bootstrap value 81 in LSU). *Dichromadora sinica* and *D. media* sp. nov. are highly clustered based on rDNA sequences (posterior probability 100, bootstrap value 93 in SSU; posterior probability 86, bootstrap value 71 in LSU), and can be distinguished based on the pharyngeal bulb, precloacal supplements, and gubernaculum shape.

Species of genus *Neochromadora* differ from other genera of Hypodontolaiminae mainly based on the cuticle ornamentation being heterogeneous and complex. However, the cuticle structure is sometimes seen as a variable character (e.g., Leduc et al. 2017), and sequences of *Neochromadora* present as polyphyletic clades in SSU and LSU topology trees. *Neochromadora bilineata* (OQ396744) and *N. parabilineata* sp. nov. have a close relationship in the SSU analysis (posterior probability 94, bootstrap value 68), but the LSU sequence of *N. bilineata* was missing. Relationships between these two species should be further discussed when more molecular data is available, in combination with morphological characters.

The genera *Ptycholaimellus* and *Spilophorella* are paraphyletic clades in both LSU and SSU analyses. *Ptycholaimellus*, *Hypodontolaimus*, and *Dichromadora* all show morphological similarities with each other and clades are weakly supported in the SSU analysis (posterior probability 88 in SSU). Differences between these three genera are slight, and they are clustered within one morphological group based on buccal cavity, peribuccal pharyngeal tissue, and supplements by Venekey et al. (2019).

## Acknowledgements

We are grateful to Dr. Kenji Kito for his kind advice in the *Neochromadora parabilineata* sp. nov. identification, Dr. Huang Yong for sample collecting, and Dr. Wang Yanting for improving the manuscript. We are sincerely grateful to two anonymous referees and the Subject Editor Nic Smol for providing valuable improvements to the manuscript.

## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

### Funding

This work was supported by A Project of Shandong Province Higher Educational Science and Technology Program (No. J18KA152) and Open Project of Liaocheng University Animal Husbandry Discipline (319312101).

### Author contributions

Liang Huixin: phylogenetica ananlysis and description of *N. parabilineata*; Guo Wen: description of *D. media* and sample identification; Wang Chunming: Ms draft

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### Data availability

All of the data that support the findings of this study are available in the main text.

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ZooKeys 1190: 303–317 (2024)
DOI: 10.3897/zookeys.1190.112468

Research Article

# Review of the genus *Prochasma* Warren (Geometridae, Ennominae, Boarmiini), with description of a new species from Hainan, South China

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## Abstract

The few already published generic features of the genus *Prochasma* Warren, 1897 are reviewed and new-found characters are added to make the generic description more comprehensive. A new species, *Prochasma diaoluoensis* Liu & Stüning, **sp. nov.** is described from Hainan Province, China. It is the only *Prochasma* species found on this island and exceptional for its conspicuous pattern, vivid coloration and some morphological characters not observed in other species before. Descriptions and illustrations of adults, their venation, and male and female genitalia are presented. An identification key and an annotated checklist of all presently known species of *Prochasma* are provided. In addition, a DNA barcode sequence is given for the new species, and preliminary phylogenetic estimations of the genus *Prochasma* are discussed.

**Key words:** Checklist, COI, key, male genitalia, morphology, *P. diaoluoensis* sp. nov., taxonomic history, taxonomy

OPEN ACCESS

**Academic editor:** Gunnar Brehm
**Received:** 9 September 2023
**Accepted:** 3 January 2024
**Published:** 31 January 2024

**ZooBank:** https://zoobank.org/EAE92102-F624-4612-A1B8-F89C3C1553FF

**Citation:** Liu B, Stüning D (2024) Review of the genus *Prochasma* Warren (Geometridae, Ennominae, Boarmiini), with description of a new species from Hainan, South China. ZooKeys 1190: 303–317. https://doi.org/10.3897/zookeys.1190.112468

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## Introduction

The genus *Prochasma*, now belonging to the tribe Boarmiini in the subfamily Ennominae, was erected by Warren (1897) with *P. mimica* Warren as its type species and Khasi Hills, India as its type-locality. As a comment following the description, Warren admitted that his "*dentilinea*, wrongly referred to *Psilalcis*" (Warren 1893: 431) is extremely similar to *Prochasma*, but also mentioned differences in neuration and wing pattern, so he did not formally transfer *dentilinea* to *Prochasma*. Hampson (1895) added *Psilalcis dentilinea* to his large concept of *Boarmia* (*Psilalcis* was, like many other genera of Boarmiini, synonymized with *Boarmia* before). Later Hampson (1898: 724) erroneously proposed to add "var. *pulverosa* Warren", which was described as "*Ectropis dentilineata* Moore ab. *pulverosa* nov." by Warren (1896: 403), to *dentilinea* Warren. On the same page he provided the (unnecessary) replacement name *Boarmia flavisecta* Hampson, 1898, nomen novum, for *Prochasma mimica*, which he wrongly cited as *P.* "*minima*" and had found to be preoccupied in his genus *Boarmia*. Almost 30 years later, *Psilalcis dentilinea* Warren was transferred to

*Prochasma* by Prout (1926) mainly based on the presence of the metallic mesothoracic crest, and he also described two more species: *P. scissivestis* Prout, 1926 from Sarawak, Borneo, and *P. albimonilis* Prout, 1927 from Htawgaw, NE Burma. In the latter paper, Prout also questioned the nomenclatoric treatment of Hampson (1898) (see above), but did not correct it. Parsons et al. (1999) listed the four abovementioned species as members of the genus *Prochasma*, but added a further name, *P. squalida* Wileman, 1915, described as "*Boarmia*" from Taiwan, as a synonym of *P. dentilinea*. Sato (2019) revised *Prochasma* and described two new species, *P. kishidana* from Peninsular Malaysia, Sumatra and Borneo, and *P. sasakiana* from Borneo only, as well as restoring *P. squalida* as a distinct species, and transferring *P. scissivestis* Prout, clearly misplaced in *Prochasma*, correctly to the genus *Alcis* Curtis, 1826, as a member of the "*pammicra*-complex" (Sato 2005). Rajaei et al. (2022) listed six species-names in *Prochasma*, with *P. dentilinea* incorrectly as a junior synonym of *P. squalida*. A further new species was later described as *P. parasqualida* by Sato (2023), based on specimens from Vietnam, Laos and Thailand. These specimens were earlier treated as conspecific with *P. squalida* from Taiwan, because of their distinctive similarity of pattern and genitalia. Up to now, there are seven species recorded in the genus *Prochasma*.

Recently, many specimens of *Prochasma* have been collected on Hainan Island, China, which could be confirmed as new to science and will be described here.

## Materials and methods

### Materials

All specimens of the new species were collected by light traps on Hainan Island, S. China and currently are deposited in Coconut Research Institute, Chinese Academy of Tropical Agricultural Sciences, Wengchang, China (**CRICATAS**). For long-term preservation, most of the type specimens of the new species, including the holotype, will be transferred to the Institute of Zoology, Chinese Academy of Sciences, Beijing, China (**IZCAS**) and some of the paratypes will be transferred to the Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany (**ZFMK**).

### Morphology

Terminology for wing venation followed the Comstock-Needham System (Comstock 1918) as adopted for Geometridae by Scoble (1992) and Hausmann (2001), and that of the genitalia was based on Klots (1970) and Skou and Sihvonen (2015). For genitalia examination, abdomens were removed and placed in 10% NaOH solution. Genitalia were dissected in purified water and stained with Chlorazol Black E. Photographs of adults were taken with a Nikon camera (model: D750) equipped with a Nikon lens (AF-S Micro 60 mm f/2.8G ED). Photos of genitalia were taken with a digital camera (KUY NICE E31SPM) attached to a Nikon microscope (model: SMZ745T). Focus stacking images (20 to 30 stacks in 0.25 mm increments were used for each adult image) were generated using Helicon Focus (version: 8.2.2 pro) software.

## DNA barcoding

Genomic DNA was extracted from the legs of dried adult specimens and the barcode fragments were amplified using primers pairs: LCO-1490 and HCO-2198 (Folmer et al. 1994). The PCR products were recovered and cloned and the positive plasmids were sequenced by Sangon Biotech Co., Ltd (Shanghai, China). The obtained sequence information was deposited in the Barcode of Life Data Systems (BOLD: Ratnasingham and Hebert 2007). All sequences utilized in this study, with the exception of the newly described species, were obtained from BOLD Systems. Sequence divergence within and between species was calculated using the Kimura 2-parameter model (Kimura 1980) and the neighbour-joining algorithm (Saitou and Nei 1987), as implemented in BOLD Systems. Genetic distances within and between species are reported as uncorrected pairwise distances (p-distance). Phylogenetic tree construction and species divergence calculations were performed using MEGA 11 (Tamura et al. 2021).

## Taxonomic account

### *Prochasma* Warren, 1897

*Prochasma* Warren, 1897, Novit. zool. 4: 81. Type species: *Prochasma mimica* Warren, 1897.

**Diagnosis.** The genus *Prochasma* Warren currently comprises a total of eight species, including the newly described species presented in this study. These species are united by an apomorphic character, a tuft of well-developed, basally narrow, distally broad and curved, upright scales with metallic gloss on the posterior part of mesothorax in both sexes. This character is unique and distinguishes *Prochasma* from other genera of the tribe Boarmiini, though curved, light-reflecting scales also occur in a number of other geometrid groups; however, these scales are not arranged as an upright brush and other characters such as antennae, transverse lines, fovea (present), venation, male and female genitalia etc. are quite different. There is no genus comparable in size and pattern known to us, with which *Prochasma* could be confused.

**Generic description.** A generic description was provided, besides the original description by Warren (1897), only by Holloway [1994] and by Sato (2019); the latter partly repeated the characters mentioned by Holloway, but added some new features. Herein, we summarize these already published characters of *Prochasma*, with a few corrections, and add new-found, unpublished features.

**General appearance.** Tiny ennomine moths, wingspan 18–25 mm, forewing length: male 10–15 mm, female 11–16 mm, with colourful, yellow, white and black pattern, medial zone of forewings dark in most species. ***Head*.** Male antennae bipectinate, rami arising from basal end of each segment, dorsally unscaled, densely ciliate ventrally, apical one-third of flagellum non-pectinate; female antennae filiform (not "fasciculate", as mentioned by Sato 2019: 138). Frons narrow, rather flat, smooth-scaled, palps curved upwards before frons. Proboscis short, but functional. Chaetosemata present, small, near eye-margin. ***Thorax*.** Patagia and tegulae with large, lamellar, partly elongated scales, teg-

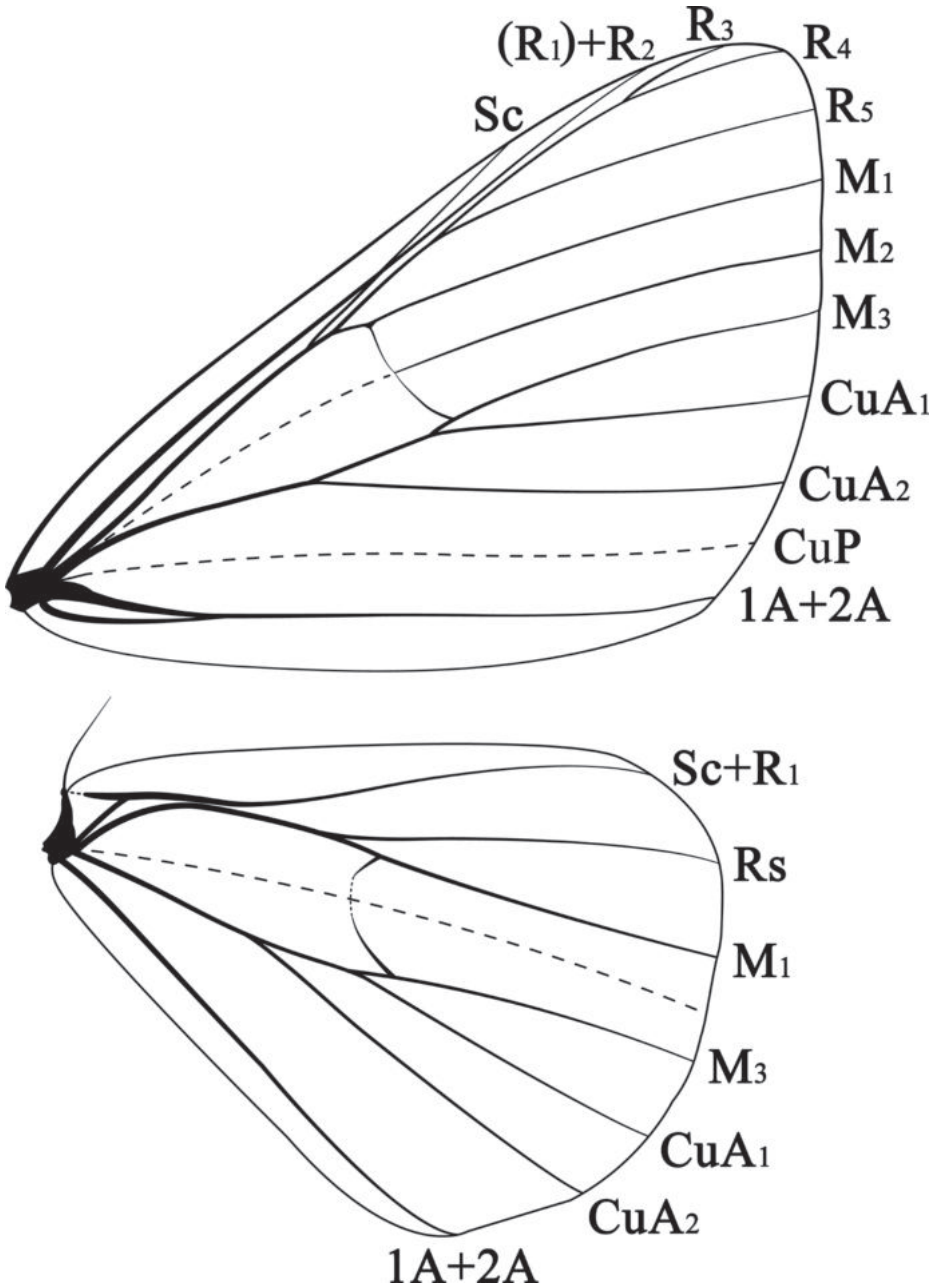



**Figure 1.** Wing venation of *Prochasma diaoluoensis* sp. nov.

ulae in addition with long hair-scales. Mesothorax posteriorly (on mesoscutellum) with a tuft of large, distally curved, metallic scales in both sexes (see Sato (2019, fig. 25); also mentioned by Warren (1897) and Holloway [1994]). Forewing pale yellow or grey (*P. albimonilis*), with distinct dark markings, without fovea in males. Antemedial and postmedial lines thin, black, deeply incurved and outwardly dentate, in some species, reduced to short streaks or dots, bounded distally by a narrow or broader band of the pale ground colour (on proximal side in antemedial lines). Submarginal lines narrow, white, zigzag-shaped where visible. In forewings, the dark band on the outside of the postmedial line broad, variable individually and in different species. The dark band on the inner side of the submarginal line on hindwing also variable in breadth, sometimes narrower,

sometimes extending to the costa, angled outside or reduced to a spot. Discal dot distinct or small, black, visible on both wings, but larger on forewings. Underside similar to upperside, but more blurry and paler. Legs slender, light grey, chequered dark grey or black. Index of spurs 0-2-4, hind tibia hardly swollen, with two pairs of long spurs and with a whitish scent brush in males. ***Venation*** (Fig. 1). $R_1$ and $R_2$ coincident (distal branch of $R_1$ reduced, only $R_2$ reaching costa), the base of the combined veins running closely parallel to vein Sc or anastomosing with it for a short distance. Other veins inconspicuous, vein 3A in hindwing absent. Folds through cells of both wings and those replacing CuP in forewings and $M_2$ in hindwings very vague. ***Pregenital abdomen.*** Tergites and sternites not conspicuously modified. T1 and T2 sclerotized, T1 narrow, T2 of double breadth. Seventh segment distinctly narrow, eighth segment elongate. Coremata absent. Tympanal organs of moderate size, without lacinia. Setal comb present, but modified to a multi-row setal patch, with numerous small, easily detachable setae; when central setae are lost, it may look like "a pair of setal scars" (compare Holloway [1994: 269] and Sato (2019, fig. 24)). Sterno-tympanal process present, but weak, free distal portion short, not reaching the posterior margin of tympanal bulla.

***Male genitalia.*** Uncus triangular, base broad, lateral sides almost straight or slightly rounded, with short setae dorsally, apex short and pointed or more or less narrowly elongated and pointed. Gnathos with strong lateral arms, central part strong, elongate rectangular, with rounded tip. Juxta broad, plate-like, sclerotized, with distal incision in some species or rectangular, with apex slightly narrowed. Saccus strong, triangularly more or less extended, tip rounded. Valvae elongated, parallelogram-shaped in some of the species, sclerotized costa not reaching the weak, narrowed distal part of valvae, which is covered with a moderate to weak cucullus, which is reaching widely basad. Tip of valvae rounded, rarely dorsal margin deeply excavated more basally and carrying a tuft of long, modified setae (so far only found in the new species described below). Ventral margin of valvae at 2/3 to 3/5 length with a short, tooth-like process (i.e., distal process of sacculus). The latter is built as a narrow, sclerotized band along ventral margin of valva and may sometimes be weak or even not visible; distal tooth-like process is variable in size and may rarely be short or almost absent. Basal part of valve lamina less setose and more or less membranous, bordered distally by an oblique, sclerotized ridge. Aedeagus short and stout, vesica containing a single massive cornutus, with significant variations of size and shape between different species, an important specific character.

***Female genitalia.*** Ovipositor short, papillae anales narrow, tapering, covered with short setae. A needle-like sclerite, found between the bases of posterior apophyses in two species so far, may also turn out to be a generic feature. Anterior apophyses distinctly shorter than posterior apophyses, the latter almost double in length. Introitus bursae funnel-shaped, often large, slightly sclerotized. Colliculum absent (Sato (2019: 141) mentions it as "developed", but we could not confirm the presence of a typical colliculum). Posterior part of bursa copulatrix elongated, largely membranous, posteriorly with various types of specifically different sclerotizations. Anterior part of bursa membranous, slightly broader than posterior part, but no clear demarcation visible. Signum absent.

***Prochasma diaoluoensis* sp. nov.**
https://zoobank.org/920827A8-1F55-4604-99B7-6B89898E0366
Figs 2–14

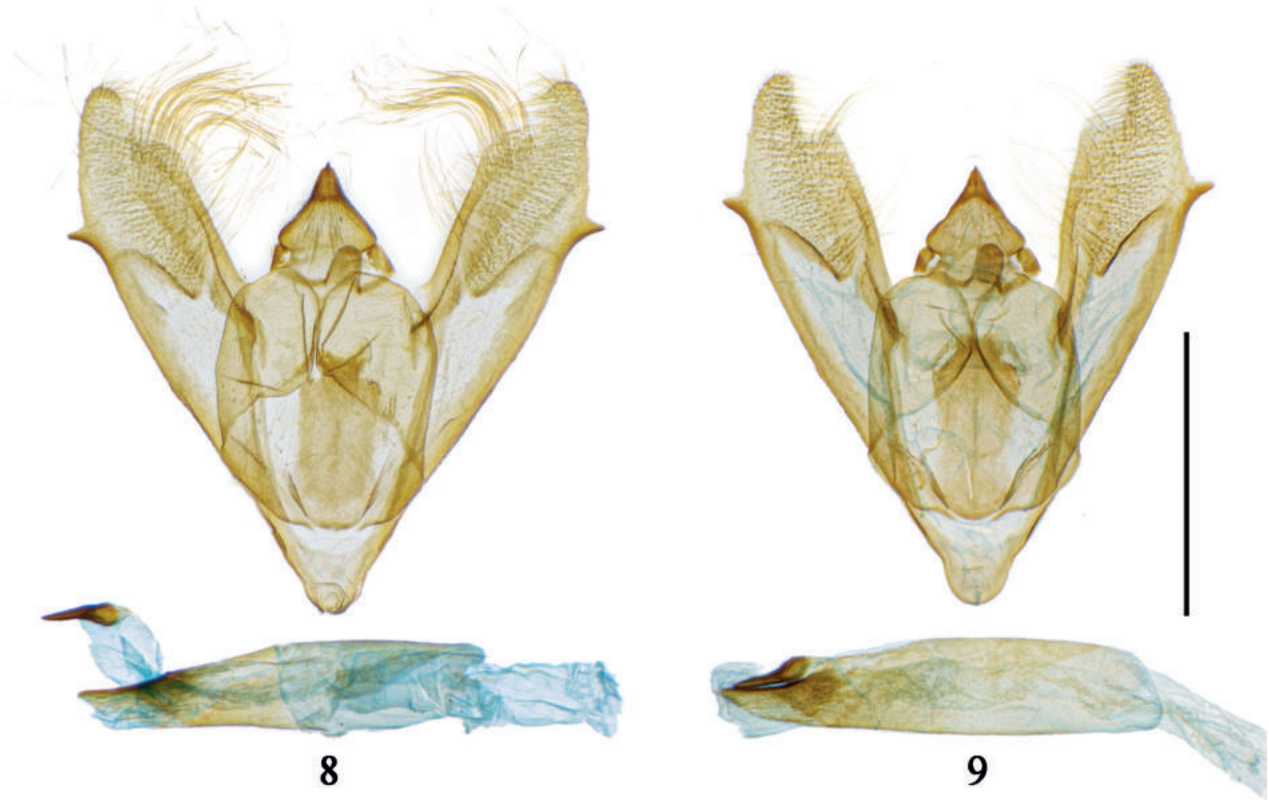
**Type-material. *Holotype*:** male, China, Hainan Province, Lingshui, Diaoluoshan, 922 m, 20.IV.2023, Bo Liu leg. DNA barcode CRICATAS00001 (CRICATAS/ IZCAS). ***Paratypes*** (67 males, 7 females): 13 males 3 females, same locality and collector as holotype, 20.IV.2023, gen. prep. no. CRICATAS00064; 39 males 4 females, same locality and collector as holotype, 10.V.2023, gen. prep. no. CRICATAS00063, gen. prep. no. CRICATAS00071; 6 males, same locality and collector as holotype, 19.VI.2023; 9 males, same locality and collector as holotype, 19.VIII.2023. (CRICATAS/ IZCAS/ ZFMK).

**Diagnosis.** *Prochasma diaoluoensis* is distinguished from its congeners by the following characteristics: 1) Valvae with a deep excavation on dorsal side near apex, basally adjacent a brush of modified setae present, absent in other species (Sato 2023, in litt.); 2) Apex of uncus very short, not narrowly elongated; 3) female genitalia with an elongate, funnel-shaped, sclerotized structure on posterior part of bursa copulatrix and a spoon-shaped lamella postvaginalis; and 4) Ante- and postmedial lines reduced to small denticles, bordered by broad, white lines, more conspicuous than in the congeners. The latter two features are found, but less expressed, also in some other species. The same can be stated about the horizontal, yellow band, traversing both forewings, which is most conspicuous in *P. mimica*, less conspicuous in *diaoluoensis*, but often present, at least in traces, also in the other congeners. Generally, the new species is, though more vividly coloured and with more strongly contrasting pattern, rather similar to its congeners, with exception of *P. albimonilis* which lacks the yellowish ground colour and has homogenous, dark grey pattern elements, almost not separated into basal, medial and postmedial areas. The female genitalia of *albimonilis* are similar to *P. diaoluoensis* in the posterior part of bursa, which is also roundly extended on right side, but the sclerotized part is not funnel-shaped but rather broadly tube-like (Sato 2019, fig. 40). It may even be a functional colliculum. In male genitalia, a narrow dorsal incision is present near apex of valvae in *albimonilis*, but the valvae are broader, especially the sclerotized costal side, and more densely setose.

**Description. *Forewing length*:** male 12.2–13.2 mm; female 12.9–13.6 mm. ***Head*.** Antennae bipectinate on basal two-thirds in males, rami long, length of longest rami about 9 times the diameter of the flagellum segments, filiform in females. Frons not protruding, covered with short scales, upper half pale, lower half dark. Labial palpus curved upwards beyond frons, covered with intermingled, dark and pale scales and longer hair-scales. Vertex with pale scales, a few dark scales near antennae. ***Thorax*.** Patagia and tegulae with lamellar, dark and pale scales, with longer, dark hair-scales on tegulae only, ventrally thorax covered with pale yellow hair-scales. Legs slender, pale, chequered black, hind tibia slightly dilated, with a pale scent brush in males. Forewings with apex angled, termen smoothly curved, without fovea in males. Hindwing with apex rounded. Wings yellow, covered with extensive black scales. Fringes with alternating yellow and smaller black parts. Forewing yellow, with distinct dark markings. Antemedial and postmedial lines both appear as consisting of a few black denticles or dots between $M_1$ and $CuA_2$, bordered by a broad, white band. In females, the denticles are more tooth-like. Submarginal line white, very fine, zigzag-shaped. Area be-

**Figures 2–7.** Adults of *Prochasma diaoluoensis* sp. nov. **2** male, holotype, upperside **3** male, holotype, underside **4** female, paratype, upperside **5** female, paratype, underside **6** male, paratype, living specimen **7** female, paratype, living specimen. Scale bar: 1 cm.

tween $M_3$ and $CuA_1$ appears as a yellow, horizontal band, with or without a few small black spots. Discal dot oval, black, faintly visible. Dark band on inner side of postmedial line of hindwing narrow, reaching from discal dot to inner margin. Dark band on outside broader, the width variable between individuals, slightly broader in females. Submarginal line visible, intermittent, weaker in hindwings. Underside similar to upperside, but more blurry and paler. ***Venation*** (Fig. 1). Forewing: $R_1$ and $R_2$ coincident; $R_1$+$R_2$ arising from upper vein of cell, then shortly anastomosing with Sc, and running almost parallel to the long stem of $R_{3\text{-}4}$; stem of $R_{3\text{-}5}$ arising shortly before anterior angle of cell; $M_2$ from the middle of the discocellulars; $CuA_1$ from before posterior angle of cell. Hindwing: Sc+$R_1$ running

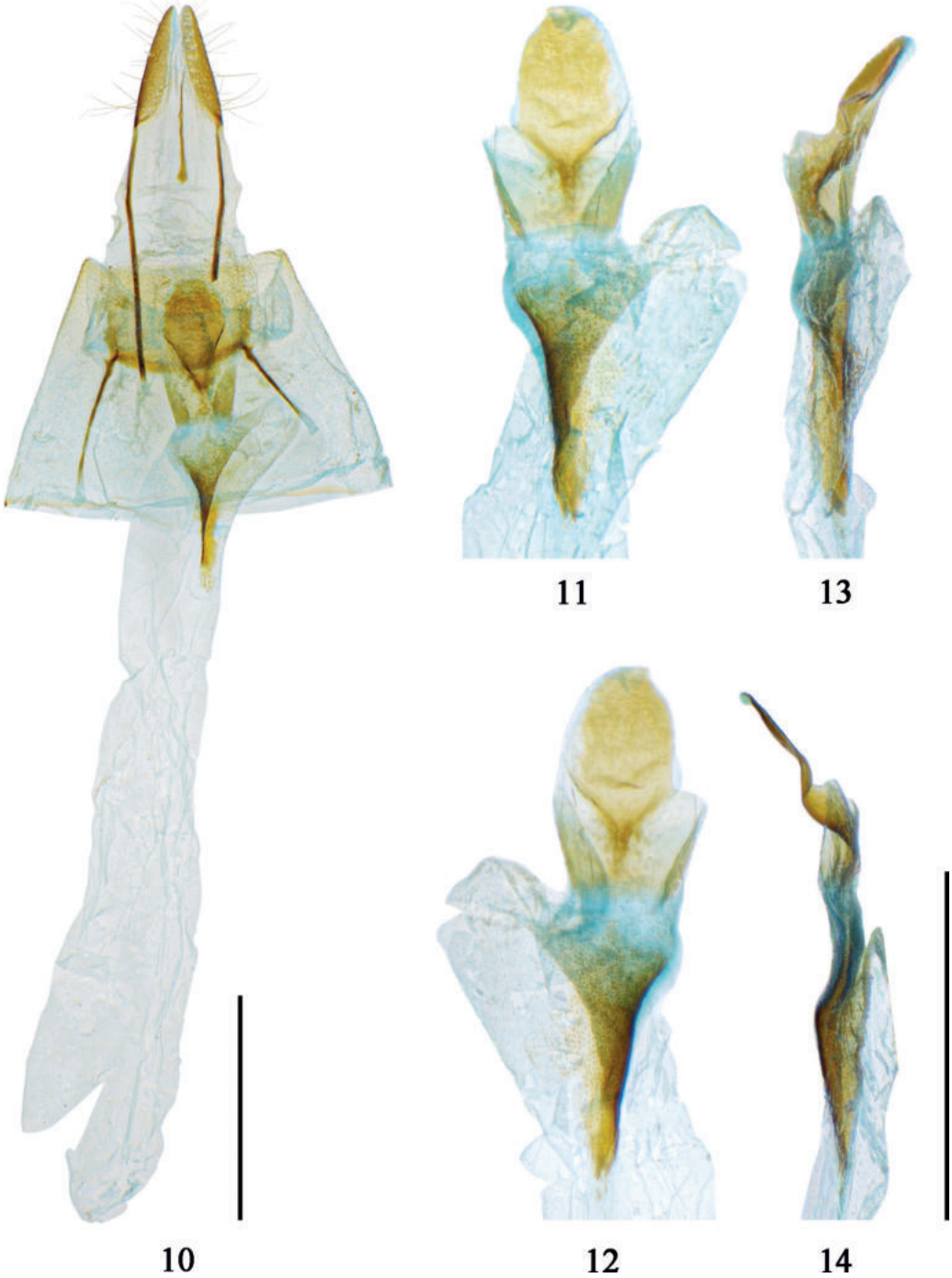



**Figures 8, 9.** Male genitalia of *Prochasma diaoluoensis* sp. nov. **8** paratype (vesica partly everted), gen. prep. no. CRICATAS00063 **9** paratype (brushes of modified setae removed, vesica not everted), gen. prep. no. CRICATAS00064. Scale bar: 1 mm.

closely parallel but not anastomosing with upper vein of cell at base; Rs from before anterior angle of cell; $CuA_1$ from before posterior angle of cell; 3A absent. ***Pregenital abdomen.*** Dorsally scaled pale yellow, with a large black spot on each tergite. Ventrally with pale yellow hair-scales. Tympanal organs and a modified setal comb present, the latter as described in the generic description. Tergite and sternite of segment 7 short, length about 2/5 of width. Tergite and sternite of segment 8 elongate, length slightly greater than width in males, broader in females.

***Male genitalia.*** Uncus triangular, base broad, with short setae dorsally, apex very short, not narrowly elongated. Gnathos with strong lateral arms, central part strong, rectangular, with rounded tip. Juxta rectangular, sclerotized, apex slightly narrowed. Saccus V-shaped, slightly extended. Valvae elongated, apically narrowed ventrally, tip rounded, with a deep excavation on dorsal side. Valve lamina proximally membranous, distally densely covered with setae, without a typical cucullus, with an oblique, sclerotized ridge between both parts. A tuft of long, curved, modified setae, tubular at base, distally flattened, present dorsally near the apex of each valva. Costa straight, sclerotized, basally slightly broadened, distally not reaching tip of valva, ending at excavation. Sacculus sclerotized, distally with a short, tooth-like process, protruding from ventral margin of valva at ¾ of its length. Aedeagus cylindrical, apically broadly elongated and sclerotized on one side. Cornutus short, not stick-like, apex tapering, with bulbous base.

***Female genitalia.*** Ovipositor short, papillae anales narrow, tapering towards apex, covered with short setae. Anterior apophyses short, about 2/3 length of posterior apophyses. A thin needle-like sclerite, roundly enlarged anterior-




**Figures 10–14.** Female genitalia of *Prochasma diaoluoensis* sp. nov. **10** paratype, gen. prep. no. CRICATAS00071 **11–14** Close-ups of lamella postvaginalis, introitus bursae and posterior part of bursa **11** ventral view **12** dorsal view **13** lateral view from right side **14** lateral view from left side. Scale bars: 1 mm.

ly, present between the bases of posterior apophyses. Lamella postvaginalis large, spoon-shaped. Introitus bursae funnel-shaped, slightly sclerotized. Posterior part of bursa elongated, membranous, distally roundly extended on right side; outside with a posteriorly funnel-shaped sclerotized structure formed by a broad sclerite which consists of lamellar plates folded three times, with unknown function (see Figs 11–14). Anterior part of bursa slightly broader than posterior part, but no clear demarcation visible. Signum absent.

**Etymology.** The specific name is derived from the type-locality, Diaoluoshan, Hainan Island, China.

**Distribution.** China (Hainan).

## Preliminary phylogenetic estimations

A barcode sequence based on the COI (658 bp) was obtained from the holotype of *P. diaoluoensis* and submitted to BOLD Systems (BIN: BOLD: AFJ0024, Sample ID: CRICATAS00001, Process ID: CCLEP001-23). There are currently 14 (including the one for *P. diaoluoensis*) *Prochasma*-associated DNA barcoding records on BOLD Systems. Four of them are private and restricted to use only within BOLD Systems, and the remaining ten published records are available but contain nonidentifications and misidentifications. On the basis of the images of the specimens provided on BOLD, the locality information attached to the records and the provided barcode data, most specimens could be identified to species level. However, three species (*P. mimica*, *P. dentilinea*, *P. parasqualida*) are still not represented on BOLD, so a full phylogenetic analysis of the genus is not yet possible. However, based on the data currently available, the following preliminary conclusions can also be drawn: The neighbour-joining tree of *Prochasma* (Fig. 15) clearly shows that *P. diaoluoensis* is a distinct species and most closely related to *P. albimonilis*, with a mean genetic distance of 7.05% (p-dist) (Table 1). Interspecific genetic distances range from 4.9% to 8.7%, intraspecific values range from 0.3% to 2.0%. Furthermore, a phylogenetic tree, offered and constructed by BOLD Systems and

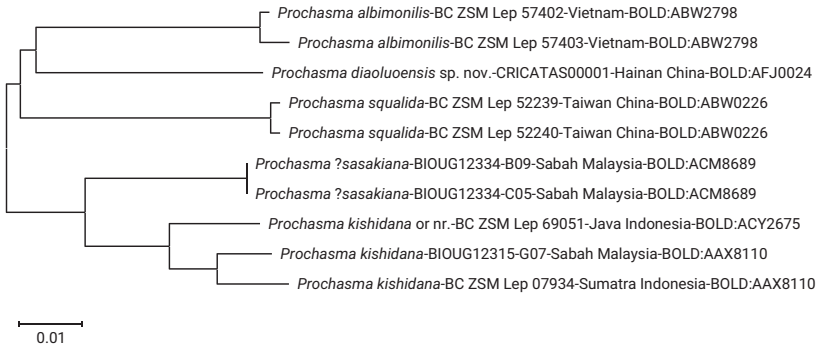



**Figure 15.** Neighbour-joining tree of *Prochasma* taxa on the basis of DNA barcoding.

**Table 1.** Genetic distances (p-distance) within and between species of the genus *Prochasma*.

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *P. diaoluoensis*-CRICATAS00001 | | | | | | | | | | |
| 2 | *P. albimonilis*-BC ZSM Lep 57402 | 6.8% | | | | | | | | | |
| 3 | *P. albimonilis*-BC ZSM Lep 57403 | 7.3% | 0.6% | | | | | | | | |
| 4 | *P. squalida*-BC ZSM Lep 52239 | 7.4% | 7.8% | 7.8% | | | | | | | |
| 5 | *P. squalida*-BC ZSM Lep 52240 | 7.8% | 7.8% | 7.4% | 0.3% | | | | | | |
| 6 | *P. ?sasakiana*-BIOUG12334-B09 | 7.3% | 7.8% | 8.2% | 7.6% | 7.6% | | | | | |
| 7 | *P. ?sasakiana*-BIOUG12334-C05 | 7.3% | 7.8% | 8.2% | 7.6% | 7.6% | 0.0% | | | | |
| 8 | *P. kishidana* or nr.-BC ZSM Lep 69051 | 7.8% | 8.1% | 8.2% | 7.8% | 7.8% | 4.9% | 4.9% | | | |
| 9 | *P. kishidana*-BIOUG12315-G07 | 7.6% | 7.3% | 7.5% | 8.2% | 8.2% | 5.6% | 5.6% | 3.3% | | |
| 10 | *P. kishidana*-BC ZSM Lep 07934 | 8.1% | 7.9% | 8.4% | 8.7% | 8.7% | 5.6% | 5.6% | 2.9% | 2.0% | |

based on the sequences of the "100 nearest neighbours", i.e., the species most closely related to *P. diaoluoensis*, showed that all sequenced *Prochasma* species clustered into a single clade of the phylogenetic tree. This is consistent with the results of our morphology-based study (see generic description and diagnosis). The three not yet sequenced species (i.e., *P. mimica*, *P. parasqualida*, *P. dentilinea*) largely agree with the morphological characters of the others and will not change the homogenous character of the cluster, then representing the genus *Prochasma*.

## Key to *Prochasma* species, based on characters of male genitalia

1 Apical region of valva with excavation or incision on dorsal side.....**2**
– Apical region of valva straight on dorsal side .....**3**
2 Valva narrow, apex with a deep excavation dorsally, a brush of elongated, modified setae present near excavation; cucullus indistinct ..... ..... ***P. diaoluoensis* sp. nov. (Hainan)**
– Valva and costa broad, dorsally near apex with deep incision between both ..... ***P. albimonilis* (Myanmar, Laos, Vietnam)**
3 Cornutus on vesica narrow, stick-like.....**4**
– Cornutus on vesica not stick-like .....**5**
4 Cornutus long, about half the length of aedeagus; dentate process on ventral margin of valva not prominent ..... ***P. mimica* (India, Assam)**
– Cornutus shorter than one-third of aedeagus in length; dentate process on ventral margin of valva prominent..... .....***P. kishidana* (Peninsular Malaysia, Borneo, Sumatra)**
5 Apex of uncus stout and short; dentate process on ventral margin of valva rather short .....**6**
– Apex of uncus slightly elongated; dentate process on ventral margin of valva slightly longer.....**7**
6 Valva narrow; tapering part of cornutus long; dentate process on ventral margin of valva conspicuous..... ***P. squalida* (Taiwan)**
– Valva broad; tapering part of cornutus shorter; dentate process on ventral margin of valva hardly visible.....***P. parasqualida* (Vietnam, Laos, Thailand)**
7 Cornutus large, long, base not bulbous, tapering part less than one-third the length of cornutus, with short, acute tip..... ..... ***P. dentilinea* (India, Nepal, Myanmar, Thailand, Laos, Vietnam)**
– Cornutus smaller, base bulbous, tapering part nearly half the length of cornutus ..... ***P. sasakiana* (Borneo)**

## Checklist of the *Prochasma* species

### Genus *Prochasma* Warren

*Prochasma* Warren, 1897, Novit. zool. 4: 81. Type species: *Prochasma mimica* Warren, 1897.

### *Prochasma mimica* Warren

*Prochasma mimica* Warren, 1897, Novit. zool. 4: 81. Type-locality: Khasi Hills, India.

*Boarmia flavisecta* Hampson, 1898, unnecessary replacement name for *Prochasma* "*minima*" Hampson, nec Warren.

**Distribution.** India.

**Remarks.** Only three specimens are known so far (collection of Natural History Museum, London). Sato (2019) figures a male and a female syntype (figs 1, 2) and male and female genitalia of syntypes (figs 27, 28).

### ***Prochasma albimonilis*** **Prout**

*Prochasma albimonilis* Prout, 1927, J. Bombay nat. Hist. Soc. 31 (4): 943. Type-locality: Htawgaw, Burma; Sato 2019, Tinea 25 (Suppl. 1): 147; Sato 2020, Tinea 25 (Suppl. 2): 84, pl. 29, figs 25, 26.

**Distribution.** Myanmar, Laos, Vietnam.

**Remarks.** Sato (2019) figures the male holotype from NE Myanmar (fig. 5), a male and a female from Vietnam (figs 22, 23) and their genitalia (figs 36, 40).

### ***Prochasma dentilinea*** **(Warren)**

*Psilalcis dentilinea* Warren, 1893, Proc. zool. Soc. Lond. (2): 431. Type-locality: Naga Hills, Sikkim, India.
*Boarmia dentilinea*: Hampson 1895, Fauna Br. India (Moths), 3: 277.
*Prochasma dentilinea*: Prout 1926, Sarawak Mus. J. 3 (2): 207; Prout 1932, J. fed. Malay. St. Mus. 17: 106; Sato 2019, Tinea 25 (Suppl. 1): 139.

**Distribution.** India, Nepal, Myanmar, Thailand, Laos, Vietnam, SW. China (Han H. Beijing, pers. comm.).

**Remarks.** Sato (2019) figures a male syntype from Naga Hills, E. India (fig. 3) and three males and one female from Nepal, Vietnam and Myanmar (figs 7–10), genitalia of male syntype (fig. 29), male and female genitalia from Myanmar (figs 30, 37).

### ***Prochasma kishidana*** **Sato**

*Prochasma kishidana* Sato, 2019, Tinea 25 (Suppl. 1): 138–149, figs 11–13 (adults of holotype and paratypes, males and female), 32, 38 (genitalia of male and female). Type-locality: Holzweg, Prapat, Sumatera Utara, N Sumatra, Indonesia.

**Distribution.** Peninsular Malaysia, Borneo (Brunei, Sarawak), Sumatra.

**Remarks.** Specimens from Borneo have earlier been treated as *P. dentilinea* Warren (Holloway [1994], fig. 574, male genitalia, pl. 17, fig. 36, male adult). Both clearly belong to *P. kishidana*. The female genitalia (fig. 578) is different to those figured by Sato (2019, figs 38, 39) for *kishidana* and *sasakiana*, and may belong to a third, still unknown Bornean species, or the difference may be due to geographical variation, as Sato's figure represents a female from Sumatra.

### ***Prochasma parasqualida* Sato**

*Prochasma parasqualida* Sato, 2023, Tinea 26 (4): 379–385, figs 9–12 (adults of male holotype and female paratype), 16, 19 (male and female genitalia of syntypes). Type-locality: Ban Kalo, Phou Khoun, Luang Prabang, Laos.
*Prochasma squalida*: Sato 2019, Tinea 25 (Suppl. 1): 138–149, figs 19–21, 35, 42; Sato 2020, Tinea 25 (Suppl. 2): 84, pl. 29, fig. 24.

**Distribution.** Vietnam, Laos, Thailand.
**Remarks.** Considered as conspecific with *P. squalida* in Sato (2019, 2020).

### ***Prochasma sasakiana* Sato**

*Prochasma sasakiana* Sato, 2019, Tinea 25 (Suppl. 1): 138–149. figs 14–16 (adults of male holotype and female paratypes), 33, 39 (genitalia of male and female). Type-locality: Trus Madi Mt, Sabah, Borneo.

**Distribution.** Borneo (Sabah).
**Remarks.** Occurring together with *P. kishidana* on Borneo.

### ***Prochasma squalida* (Wileman)**

*Boarmia squalida* Wileman, 1915, Entomologist 48: 282. Type-locality: "Arizan, Formosa" (Alishan, Taiwan, China).
*Prochasma dentilinea*: Prout 1927, J. Bombay nat. Hist. Soc. 31 (4): 943; Inoue 1965, Spec. Bull. Lep. Soc. Japan 1: 34; Parsons et al. 1999, Geometrid Moths of the World, 782.
*Prochasma squalida* Sato, 2019, Tinea 25 (Suppl. 1): 138–149, (stat. rev.), figs 4 (male, holotype), 17, 18 (male, female, Taiwan), 34, 41 (male and female genitalia).

**Distribution.** China (Taiwan).
**Remarks.** This species had been sunk as a synonym of *Prochasma dentilinea* by Prout (1927), but was restored to a valid species by Sato (2019). Specimens from Vietnam, Laos and Thailand identified as conspecific with *P. squalida* in Sato (2019, 2020) were separated and treated as a new species, *P. parasqualida* in Sato (2023).

## Acknowledgements

We would like to express our sincere gratitude to Rikio Sato, Niigata, Japan, for providing us with two of his recent articles, essential for the present paper. We also thank him for re-checking some species of *Prochasma*, not available to us, for uncertain morphological characters. The senior author would like to thank Jiexiong Fu, Hainan Tropical Rainforest National Park, Lingshui, China, and Wei Yan, Coconut Research Institute, Chinese Academy of Tropical Agricultural Sciences, Wenchang, China, for their assistance in collecting specimens.

## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

### Funding

This work was supported by the Hainan Provincial Natural Science Foundation of China (No. 321QN344) and the Key Research and Development Programs of Hainan Province (No. ZDYF2022XDNY214 and ZDYF2018136).

### Author contributions

Conceptualization: BL. Resources: BL, DS. Writing – Original draft: BL. Writing – Review and Editing: BL, DS.

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### Data availability

All of the data that support the findings of this study are available in the main text.

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ZooKeys 1190: 319–345 (2024)
DOI: 10.3897/zookeys.1190.109222

Research Article

# The genus *Neurigona* Rondani, 1856 (Diptera, Dolichopodidae) from Yunnan, China, with descriptions of seven new species and a key to the species of China

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## Abstract

Previously, only three species of the genus *Neurigona* Rondani of the subfamily Neurigoninae were known from Yunnan Province. Here, we reviewed the species of *Neurigona* from Yunnan and added the following seven new species: *N. apicilata* **sp. nov.**, *N. basicurva* **sp. nov.**, *N. brevidigitata* **sp. nov.**, *N. convexa* **sp. nov.**, *N. huanglianshana* **sp. nov.**, *N. quadrimaculata* **sp. nov.**, and *N. ventriprocessa* **sp. nov.** All seven new species are sympatric and were collected from below a reservoir in the Huanglianshan Nature Reserve in Yunnan using three Malaise traps in 2019. This suggests a very high species richness in the Yunnan fauna. A key to the species of *Neurigona* from Chinese mainland is provided.

**Key words:** Identification key, long-legged fly, morphology, Neurigoninae, new taxon, Oriental Realm, sympatric species richness

OPEN ACCESS

**Academic editor:** Marija Ivković
**Received:** 8 July 2023
**Accepted:** 30 December 2023
**Published:** 31 January 2024

**ZooBank:** https://zoobank.org/FFA3D398-7BEA-4665-83A0-E832F702C2FB

**Citation:** Lin C, Wang M, Yang D (2024) The genus *Neurigona* Rondani, 1856 (Diptera, Dolichopodidae) from Yunnan, China, with descriptions of seven new species and a key to the species of China. ZooKeys 1190: 319–345. https://doi.org/10.3897/zookeys.1190.109222

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## Introduction

The genus *Neurigona* Rondani, 1856 belongs to the subfamily Neurigoninae within Dolichopodidae. They usually have non-metallic yellow coloration on much of the thorax, legs, and abdomen, and can be separated from other Dolichopodidae genera by the following features: proboscis with a pair of long and pale ventral hairs; posterior mesonotum distinctly flattened; legs slender and long, and anterior preapical setae on mid and hind femora absent; sternite 5 sometimes with a ventral projection in males; fore tarsomere 1 usually elongated; vein M gently or strongly bent apically and convergent with $R_{4+5}$; surstylus divided into dorsal and ventral lobes; cercus with digitiform internal median projection (Naglis 2003; Kazerani et al. 2022). Adults of most species are regularly observed on tree trunks where the males also exhibit their courtship behavior, sometimes in large numbers (Parent 1938).

*Neurigona* is the most speciose genus of the Neurigoninae with 166 known species from the world, of which 44 species are from the Palaearctic, 44 from the Neotropical, 39 from the Nearctic, 32 from the Oriental, four from the Australian, and three from the Afrotropical realms (De Meijere 1916; Becker 1922;

Parent 1935, 1944; Dyte 1975; Negrobov 1987, 1991; Negrobov and Fursov 1988; Pârvu 1996; Yang 1999; Yang and Saigusa 2001a, b, 2005; Naglis 2003; Zhang et al. 2003; Wang et al. 2006, 2007, 2010, 2020; Yang et al. 2006, 2011; Grichanov 2010; Capellari 2013; Grootaert and Foo 2019; Da Silva et al. 2022; Kazerani et al. 2022). Thirty-one species were known to occur in China (Wang et al. 2010; Yang et al. 2006, 2011, 2018).

Yunnan Province, located in southwestern China, boasts a rich and diverse ecological environment, such as tropical rainforests, subtropical forests, alpine meadows and wetlands. Its eastern part is the Yunnan-Guizhou Plateau, which is one of the four major plateaus in China, and its northwest part is the Hengduan Mountains, which is the longest and widest north-south mountain range in China (Feng et al. 2023). The presence of multiple habitat types within a relatively small geographical area enhances species diversity. With its breathtaking landscapes and abundant biodiversity, it is considered one of the most ecologically significant regions in China. Thus far, only three species, *N. centralis* Yang & Saigusa, *N. ventralis* Yang & Saigusa, and *N. yunnana* Wang, Yang & Grootaert, were recorded from Yunnan (Yang et al. 2006, 2018). In this paper, seven new species of *Neurigona* are added to the fauna of Yunnan, and a key to the males of species of *Neurigona* in Chinese mainland is provided.

## Material and methods

The specimens on which this study is based were collected from below the reservoir in the Huanglianshan National Nature Reserve in Yunnan using three Malaise traps in 2019. All specimens are deposited in the Entomological Museum of China Agricultural University (**CAU**), Beijing. The morphological terminology for adult structures, including the structures of the male genitalia, follow Cumming and Wood (2017). All the figures were taken and drawn by Chen Lin. The following abbreviations are used:

| | |
|---|---|
| **acr** | acrostichal bristle (s); |
| **ad** | anterodorsal bristle (s); |
| **av** | anteroventral bristle (s); |
| **dc** | dorsocentral bristle (s); |
| **pd** | posterodorsal bristle (s); |
| **pv** | posteroventral bristle (s); |
| **v** | ventral bristle(s); |
| **CuAx ratio** | length of dm-cu / length of distal portion of CuA; |
| **LI** | fore leg; |
| **LII** | mid leg; |
| **LIII** | hind leg. |

## Taxonomy

### Key to species (males) of *Neurigona* from Chinese mainland (modified from Yang et al. 2011 and Wang et al. 2010)

1 Abdominal sternite 5 not projected.....**2**
– Abdominal sternite 5 projected, subtriangular or subquadrate.....**17**

2 Fore coxa with wholly black bristles on antero-apical portion ..... **3**
– Fore coxa partly or entirely with yellow apical bristles on antero-apical portion..... **8**
3 Fore tarsomere 1 shorter than fore tibia..... **4**
– Fore tarsomere 1 longer than fore tibia ..... **7**
4 More than 10 paired acr bristles..... **5**
– Four irregularly paired acr bristles..... ..... ***N. yunnana* Wang, Yang & Grootaert, 2007**
5 17–18 paired acr bristles; fore tibia with 1 ad bristle..... ..... ***N. concaviuscula* Yang, 1999**
– 11–12 paired acr bristles; fore tibia without ad bristles ..... **6**
6 Fore tarsomere 4 with basal 1/2 swollen and apical 1/2 concave ventrally, fore tarsomere 5 with basal 1/4 swollen and middle portion concave ventrally (Wang et al. 2010: fig. 6)..... ***N. sichuana* Wang, Chen & Yang, 2010**
– Fore tarsomeres 4 and 5 normal, without swollen or concave portion ..... ..... ***N. hainana* Wang, Chen & Yang, 2010**
7 Fore femur without row of av bristles; mid and hind tibiae wholly yellow; epandrium with two long and wide lateral processes; ventral lobe of surstylus straight and broad, apically with 4 strong hairs (Yang et al. 2011: fig. 999c)..... ..... ***N. guangdongensis* Wang, Yang & Grootaert, 2007**
– Fore femur with row of short av bristles; mid and hind tibiae each with black apex; epandrium with two slender lateral processes; ventral lobe of surstylus curved and slender, apically with 1 thick hair (Yang et al. 2011: fig. 991b) ..... ***N. bimaculata* Yang & Saigusa, 2005**
8 Fore femur with row of yellow av bristles ..... ***N. shennongjiana* Yang, 1999**
– Fore femur without row of yellow av bristles..... **9**
9 Mesonotum with black mid-posterior or lateral spot(s) ..... **10**
– Mesonotum entirely yellow..... **13**
10 Fore tarsomere 5 slightly thickened with long and strong bristles ..... **11**
– Fore tarsomere 5 not thickened, without long and strong bristles ..... **12**
11 Pleuron chiefly yellow; fore claw distinctly elongated, spine-like..... ..... ***N. micropyga* Negrobov, 1987**
– Pleuron chiefly black; fore claw normal..... ***N. yaoi* Wang, Chen & Yang, 2010**
12 Postnotum wholly blackish; mid tibia and tarsus without row of short dense crochet-like av hairs, with only mid tarsomere 1 with row of erect av hairs... ..... ***N. centralis* Yang & Saigusa, 2001**
– Postnotum yellow with a nearly W-shaped dark brown spot; mid tibia and tarsus with row of short dense crochet-like av hairs ..... ..... ***N. brevidigitata* sp. nov.**
13 Fore tibia without ad or pd bristle; hind tarsomere 1 without ventral bristle at base; wing wholly hyaline ..... **14**
– Fore tibia with one ad bristle and one pd bristle; hind tarsomere 1 with ~ 15 short erect spine-like black ventral bristles at base; wing with anterior apical corner brownish..... **15**
14 First flagellomere brown (Fig. 8A); fore tarsus not distinctly shortened ..... ..... ***N. apicilata* sp. nov.**
– First flagellomere brownish with yellow base; fore tarsomeres 3–4 shortened, each 0.4× and 0.2× as long as tarsomere 2 respectively..... ..... ***N. ventralis* Yang & Saigusa, 2005**

15 Fore tarsomere 1 not shorter fore tibia; mid tarsomere 1 not shorter than mid tibia .....**16**
– Fore tarsomere 1 shorter than fore tibia, 0.8× as long as fore tibia; mid tarsomere 1 shorter than mid tibia, 0.9× as long as mid tibia ..... .....***N. xiaolongmensis* Wang, Yang & Grootaert, 2007**
16 Fore tarsomere 1 as long as fore tibia; mid tarsomere 1 longer than mid tibia, 1.4× as long as mid tibia; ventral surstylus rather wide (Yang et al. 2011: fig. 1005c) .....***N. qingchengshana* Yang & Saigusa, 2001**
– Fore tarsomere 1 longer than fore tibia, 1.1× as long as fore tibia; mid tarsomere 1 as long as mid tibia; ventral surstylus relatively narrow (Fig. 6B) ..... .....***N. quadrimaculata* sp. nov.**
17 Abdomen metallic green with basal part yellow .....**18**
– Abdomen mainly yellow with black spot(s) .....**19**
18 Abdomen metallic green, tergum 1 with yellow basal margin; all legs yellow .....***N. grisea* Parent, 1944**
– Abdominal segments 1–3 yellow; legs chiefly yellow except brownish mid coxa and brown to black from hind femur to hind tarsus ..... .....***N. xui* Zhang, Yang & Grootaert, 2003**
19 Mesonotum entirely yellow .....**20**
– Mesonotum with small or large mid-posterior area brown or black .....**27**
20 Fore tarsomere 4 with basal half swollen .....**21**
– Fore tarsomere 4 with no swelling .....**22**
21 Arista dorsal (Yang et al. 2011: fig. 1010a); five dc bristles; 11–12 paired acr bristles; dorsal lobe of surstylus with apical incision (Yang et al. 2011: fig. 1010c) .....***N. xiangshana* Yang, 1999**
– Arista apical (Yang et al. 2011: fig. 1001b); eight dc bristles; 17–18 paired acr bristles; dorsal lobe of surstylus without apical incision (Yang et al. 2011: fig. 1010c).....***N. guizhouensis* Wang, Yang & Grootaert, 2007**
22 Fore tarsomere 1 shorter than or as long as fore tibia .....**23**
– Fore tarsomere 1 distinctly longer than fore tibia, 1.1× as long as fore tibia...**26**
23 Scutellum with small brownish basal spot; fore tarsomere 1 as long as fore tibia..... ***N. basalis* Yang & Saigusa, 2005**
– Scutellum without basal spot; fore tarsomere 1 shorter than fore tibia, between 0.7 to 0.9× respectively .....**24**
24 Fore coxa with six to seven black anterior apical bristles; dorsal lobe of surstylus with short and obtuse process at dorsal corner (Yang et al. 2011: fig. 1006b) ..... ***N. shaanxiensis* Yang & Saigusa, 2005**
– Fore coxa with four to six yellow apical bristles; surstylus not as above ..**25**
25 Mid coxa with black hairs and bristles; dorsal lobe of surstylus with long and thin process at dorsal corner (Yang et al. 2011: fig. 1002c) ..... .....***N. henana* Wang, Yang & Grootaert, 2007**
– Mid coxa with yellow hairs and bristles; dorsal lobe of surstylus without process at dorsal corner (Yang et al. 2011: fig. 1009c)..... ..... ***N. wui* Wang, Yang & Grootaert, 2007**
26 Six dc bristles; 18–19 paired acr bristles; dorsal lobe of surstylus short and wide (Yang et al. 2011: fig. 1011b)..... ***N. xizangensis* Yang, 1999**
– Three dc bristles; acr bristles absent; dorsal lobe of surstylus with wide base and long obtuse apex (Yang et al. 2011: fig. 1003c)..... .....***N. jiangsuensis* Wang, Yang & Grootaert, 2007**

27 Abdominal sternite 5 subtriangular; fore tibia not thickened and bent at base ..... **28**
– Abdominal sternite 5 quadrate (Fig. 9A); fore tibia weakly thickened and bent at base ..... ***N. basicurva* sp. nov.**
28 Wing normal at anterior margin; first flagellomere not triangular ..... **29**
– Wing subapically convex at anterior margin (Fig. 4B); first flagellomere somewhat triangular (Fig. 8D) ..... ***N. convexa* sp. nov.**
29 10–15 paired acr bristles ..... **30**
– 18–19 paired acr bristles ..... **31**
30 Frons and face yellow; first flagellomere quadrate (Fig. 8G); mesonotum brownish to brown, but notopleuron somewhat pale; fore tarsomere 3 shortened and tarsomere 4 ventrally weakly concave with mammillary ventral process at extreme base; M strongly bent, strongly geniculate, nearly in a right angle; abdominal sternite 5 weakly projected, short subtriangular (Fig. 9D) ..... ***N. ventriprocessa* sp. nov.**
– Frons and face wholly metallic green; first flagellomere somewhat oval (Fig. 8E); mesonotum with brown subtriangular spot at middle posterior region; fore tarsus not modified; M distinctly bent, somewhat geniculate, in an obtuse angle; abdominal sternite 5 distinctly projected, long subtriangular (Fig. 9C) ..... ***N. huanglianshana* sp. nov.**
31 Mesonotum with narrow brown mid-posterior spot; mid tibia and tarsus with row of short erect pale av bristles; cercus with long apical process (Yang et al. 2011: fig. 1016b) ..... ***N. zhejiangensis* Yang, 1999**
– Mesonotum with wide black mid-posterior area; mid tibia and tarsus without row of av bristle; cercus without apical process (Yang et al. 2011: fig. 1000b) ..... ***N. guangxiensis* Yang, 1999**

***Neurigona apicilata* sp. nov.**
https://zoobank.org/DDF79F46-4E9A-48E7-B6C7-1EF3B7893122
Figs 1, 8

**Type material.** ***Holotype*:** ♂, **China:** Yunnan, Lvchun, Yakou, Shuikuxiafang, (23°3'25.2"N, 102°46'37.71"E), 1780 m, collected by Malaise trap, 2019. IV.19-V.19, Liang Wang and Xin Li (CAU). ***Paratypes*:** 19 ♂♂, the same data as holotype (CAU).

**Diagnosis.** Male eyes contiguous on face. Mesonotum wholly dark yellow; scutellum dark yellow; postnotum dark brown posteriorly. Laterotergite with two minute dark brown or black spots at anterior margin and one large dark brown spot posteriorly. Hind tarsomere 1 with cluster of short thick posterior bristles at extreme base, several short ventral bristles and four short apical bristles (MSSC). Ventral surstylus rather wide, widened apically.

**Description. Male** (Fig. 1A). Body length 5.0–5.7 mm, wing length 4.1–4.2 mm.

***Head*** metallic green with pale gray pollinosity; face very narrow, eyes contiguous on face. Hairs and bristles on head black, but postocular bristles (except uppermost two bristles) and postero-ventral hairs pale yellow. Antenna (Fig. 8A) yellow except first flagellomere brown; first flagellomere somewhat oval, approximately as long as wide, obtuse apically, with pale white pubescence; arista

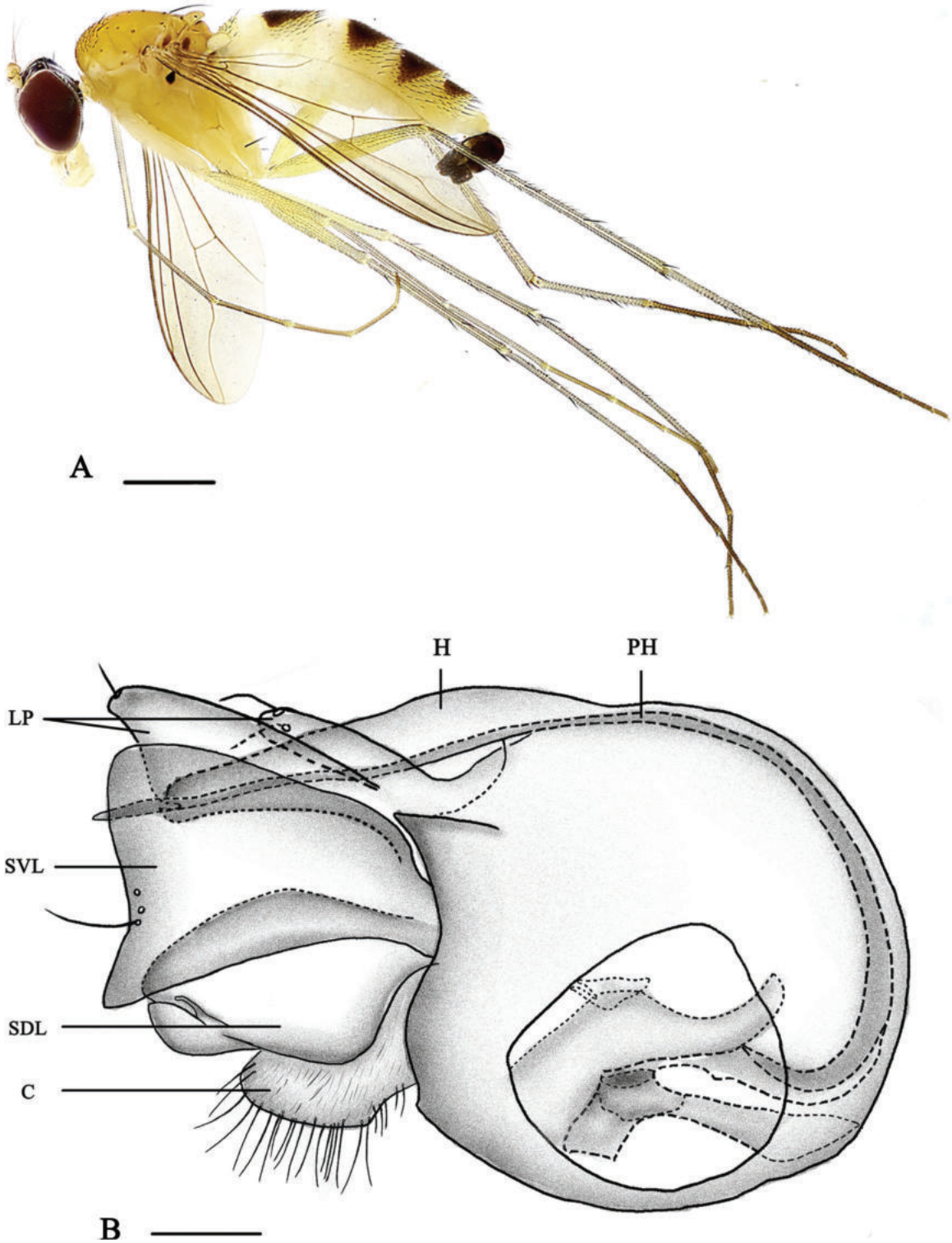


**Figure 1.** *Neurigona apicilata* sp. nov. **A** male habitus, lateral view **B** genitalia, lateral view. Abbreviations: C – cercus; H – hypandrium; LP – lateral process; PH – phallus; SDL – surstylus dorsal lobe; SVL – surstylus ventral lobe. Scale bars: 1 mm (**A**); 0.1 mm (**B**).

subapical, dark brown. Proboscis pale yellow with pale hairs; palpus pale yellow with pale yellow hairs and two to three short black apical bristles.

***Thorax*** dark yellow with fine pale yellow pollinosity; mesonotum dark yellow; postalar callus with a small dark brown spot; postnotum dark brown posteriorly.

Pteropleuron with a small black subtriangular spot. Laterotergite with two-minute dark brown or black spots at anterior margin and one large dark brown spot posteriorly. Hairs and bristles on thorax black, six strong dc, 18–20 irregularly paired acr short hair-like; scutellum with two pairs of bristles, lateral pair long and strong, median pair short and hair-like. Propleuron with one brown bristle on lower portion.

***Legs*** mainly yellow, but fore tarsomeres 2–5 brown and mid and hind tarsomeres 2–5 dark brown. Hairs and bristles on legs black. Fore coxa with three or four thick bristles on antero-apical portion mostly or wholly dark yellow; mid coxa with three anterior and apical bristles; hind coxa with one strong outer bristle at basal 1/3. Mid and hind trochanters each with one outer bristle at middle. Fore tibia devoid of bristles; mid tibia with two ad, two pd and two apical bristles; hind tibia with three ad, four pd, three apical bristles and one row of brownish yellow comb-like bristles. Fore tarsomere 1 with two short thin apical bristles. Mid tarsomere 1 with five short or long apical bristles. Hind tarsomere 1 with cluster of short thick posterior bristles at extreme base, several short ventral bristles and four short apical bristles. Relative lengths of tibiae and five tarsomeres of legs –LI 5.3: 6.2: 3.2: 2.1: 1.9: 0.9; LII 5.9: 9.1: 2.9: 2.1: 1.2: 0.7; LIII 9.8: 4.8: 4.1: 2.2: 1.2: 0.8.

***Wing*** nearly hyaline, tinged brown; veins brown, $M_{1+2}$ gently bent apically and convergent with $R_{4+5}$; CuAx ratio 0.34. Squama yellow with pale yellow hairs. Halter yellow.

***Abdomen*** yellow with yellow pollen, terga 2–5 dark yellow; terga 2–5 each with large blackish spot somewhat narrowed posteriorly. Hairs and bristles on abdomen black except those on venter more or less yellow.

***Male genitalia*** (Fig. 1B) mainly shiny black. Epandrium longer than wide, with two lateral processes (one short and thin, with two apical hairs; the other long and wide, subtriangular). Ventral surstylus rather wide, widened apically; dorsal surstylus rather wide, slightly narrower than ventral surstylus, with very narrow medial incision at tip. Cercus somewhat round, white, bearing short white hairs. Hypandrium long and somewhat thick. Phallus thin, hidden within hypandrium.

**Female.** Unknown.

**Distribution.** China (Yunnan).

**Etymology.** The specific name refers to the ventral surstylus widened apically.

**Remarks.** The species is very similar to *N. qingchengshana* Yang & Saigusa, 2021 from Sichuan, but may be separated from the latter by the fore tarsomere 1 longer than fore tibia and ventral surstylus much widened at extreme tip. In *N. qingchengshana*, the fore tarsomere 1 is as long as the fore tibia, and the ventral surstylus is weakly widened at extreme tip (Yang and Saigusa 2001a; Yang et al. 2011).

***Neurigona basicurva* sp. nov.**

https://zoobank.org/7B2FCEBC-C157-41BE-A196-16FD73B9979A

Figs 2, 8

**Type material.** ***Holotype*:** ♂, **China:** Yunnan, Lvchun, Yakou, Shuikuxiafang, (23°3'25.2"N, 102°46'37.71"E), 1780 m, collected by Malaise trap, 2019. IV.19-V.19, Liang Wang and Xin Li (CAU). ***Paratypes*:** 2 ♂♂, the same data as holotype (CAU).

**Diagnosis.** Mesonotum with three brown longitudinal stripes at middle posterior region. Abdominal sternite 5 quadrate. Fore tibia basally weakly thickened and bent. Ventral surstylus wide, distinctly bent with distinctly bent and narrowed tip; dorsal surstylus short and broad, folded inward, apically furcated.

**Description. Male** (Fig. 2A). Body length 3.8 mm, wing length 3.2 mm.

***Head*** metallic green with pale yellow pollinosity; eyes narrowly separated on middle portion of face. Hairs and bristles on head black, but postocular bristles and postero-ventral hairs pale yellow. Antennal scape and pedicel (Fig. 8B) yellow; first flagellomere dark yellow, almost as long as wide, round apically, with pale brown pubescence; arista subapical, brown, basal segment 0.1× longer than apical segment. Proboscis yellow with pale hairs; palpus pale yellow with two black apical bristles.

***Thorax*** yellow with fine pale yellow pollinosity; mesonotum dark yellow with three brown longitudinal stripes at middle posterior region; scutellum brownish; postnotum brownish with dark yellow basal margin; laterotergite with a small black inner spot at anterior margin. Pteropleuron below wing base with a small black spot. Hairs and bristles on thorax black, six strong dc, 11–12 irregularly paired acr short hair-like; scutellum with two pairs of bristles, lateral pair long and strong, median pair short and hair-like.

***Legs*** mainly yellow, but fore tarsus dark brown, mid and hind tarsi pale brown. Hairs and bristles on legs black. Fore coxa with six mostly yellow bristles on antero-apical portion; mid coxa with three anterior and apical bristles; hind coxa with one strong outer bristle at basal 1/3. Mid trochanter with two bristles, hind trochanter with one bristle. Fore femur with two rows of short dense av. Fore tibia modified, basally weakly thickened and bent, with two rows of long dense black anterior bristles at basal 1/3 and two rows of short dense brownish yellow anterior hairs at remaining 2/3. Mid tibia with one outer bristle and one pd, apically with one bristle. Hind tibia with one pd, apically with three bristles and one row of pale brown comb-like bristles apically. Fore tarsomere 1 modified, weakly bent, with short dense and erect pv; tarsomeres 2–4 with fine but dense ventral hairs. Mid tarsomere 1 with one pv at extreme base. Hind tarsomere 1 with cluster of short erect bristles basally. Relative lengths of tibiae and five tarsomeres of legs – LI 6.7: 5.9: 2.8: 1.8: 1.2: 1.0; LII 7.5: 8.8: 3.0: 2.0: 1.1: 0.8; LIII 12.1: 4.0: 4.0: 2.1: 1.3: 0.7.

***Wing*** nearly hyaline, tinged brown; veins brown, $M_{1+2}$ gently bent apically and convergent with $R_{4+5}$; CuAx ratio 0.45. Squama yellow, but brown at margin, with yellow hairs. Halter dark pale yellow.

***Abdomen*** yellow with pale yellow pollen; terga 1–4 each with large dark brown spot; sternum 5 projected, quadrate (Fig. 9A). Hairs and bristles on abdomen black except those on venter more or less yellow.

***Male genitalia*** (Fig. 2B): epandrium longer than wide, with two lateral processes (one very long and narrow; the other thick, with finger-like outer process near base, apically somewhat bent, and with one yellow bristle at apical 1/4). Ventral surstylus wide, distinctly bent with distinctly bent and narrowed tip; dorsal surstylus short and broad, folded inward, apically furcated. Cercus relatively small, trapezoid, white, with short pale yellow hairs. Hypandrium long and somewhat thick. Phallus long and thin, subapically with a denticle.

**Female.** Unknown.

**Distribution.** China (Yunnan).

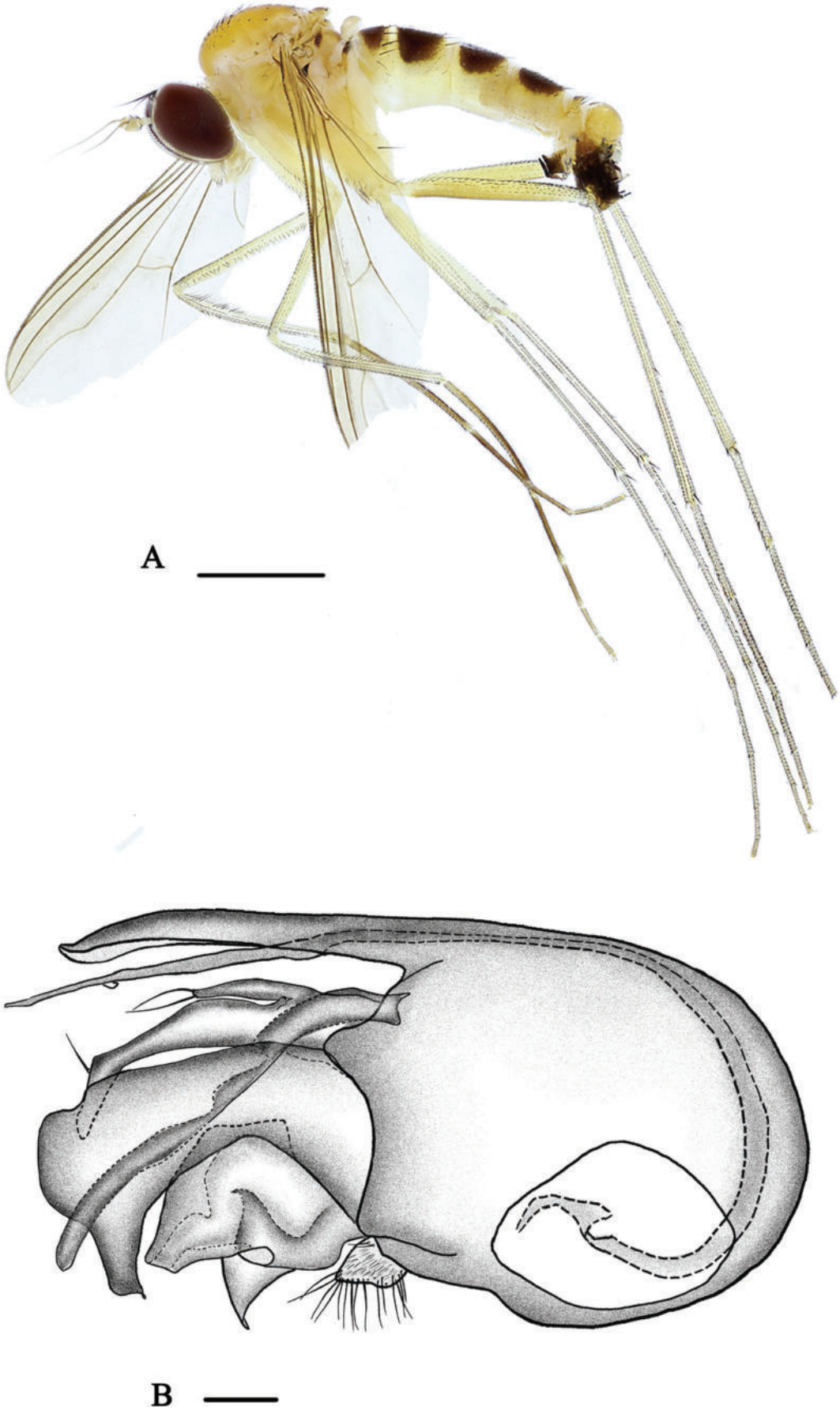


**Figure 2.** *Neurigona basicurva* sp. nov. **A** male habitus, lateral view **B** genitalia, lateral view. Scale bars: 1 mm (**A**); 0.1 mm (**B**).

**Etymology.** The specific name refers to the fore tibia basally weakly thickened and bent.

**Remarks.** The species is peculiar and can be easily separated from other known species of the genus by the quadrate 5th abdominal sternite and fore tibia basally weakly thickened and bent.

***Neurigona brevidigitata* sp. nov.**
https://zoobank.org/E0876F26-7306-4292-9373-9109AC48E7EA
Figs 3, 8

**Type material.** ***Holotype*:** ♂, **China:** Yunnan, Lvchun, Yakou, Shuikuxiafang, (23°3'25.2"N, 102°46'37.71"E), 1780 m, collected by Malaise trap, 2019. IV.19-V.19, Liang Wang and Xin Li (CAU). ***Paratypes*:** 69 ♂♂, 19 ♀♀, the same data as holotype (CAU).

**Diagnosis.** Eyes almost contiguous on middle portion of face. Mesonotum with a brown mid-posterior stripe. Postnotum with a nearly W-shaped dark brown spot. Mid tibia and tarsus with row of short dense crochet-like av hairs. Hind tibia with short blackish comb-like apical bristles on very short and plate-like process. Ventral surstylus rather large and broad, ~ 1.5× wider than dorsal surstylus, apically with four long hairs.

**Description. Male** (Fig. 3A). Body length 5.4 mm, wing length 4.2 mm.

***Head*** metallic green with pale yellow pollinosity; eyes almost contiguous on middle portion of face. Hairs and bristles on head black but postocular bristles and postero-ventral hairs pale yellow. Antenna (Fig. 8C) yellow; first flagellomere somewhat oval, ~ 1.1× as long as wide, round apically, with brown pubescence; arista subapical, brownish. Proboscis yellow with pale hairs, with two long pale hairs, longer than proboscis; palpus pale yellow, but brownish at basal 1/3, with yellow hairs.

***Thorax*** yellow with fine pale yellow pollinosity; mesonotum with one brown longitudinal stripe at middle posterior region; scutellum brown at margin, with minute brown middle spot at basal margin; postnotum with a nearly W-shaped dark brown spot. Pteropleuron below wing base with a small black spot. Laterotergite with one blackish stripe and one minute brown spot at anterior margin. Hairs and bristles on thorax black; six strong dc, 12 irregularly paired acr short and hair-like; scutellum with two pairs of bristles, lateral pair long and strong, median pair short and hair-like. Propleuron with one yellow bristle on lower portion.

***Legs*** mainly yellow; fore tarsomeres 3–5 and mid and hind tarsomeres 2–5 dark brown. Hairs and bristles on legs black except hairs on fore coxa yellow. Fore coxa with four thick dark brownish yellow bristles on antero-apical portion; mid coxa with three or four partly brownish yellow or black anterior and apical bristles; hind coxa with one strong black outer bristle at basal 1/3. Mid and hind trochanters each with one outer bristle at middle. Fore tibia devoid of bristles. Mid tibia with two ad, two pd, row of short dense crochet-like av hairs, and two apical bristles. Hind tibia much elongated, 1.7× longer than fore tibia, with three ad, three pd, seven short thin pv, two thick apical bristles, and short blackish comb-like apical bristles on very short plate-like process. Fore tarsomeres 4 and 5 with slightly long hairs. Mid tarsus with row of short dense crochet-like

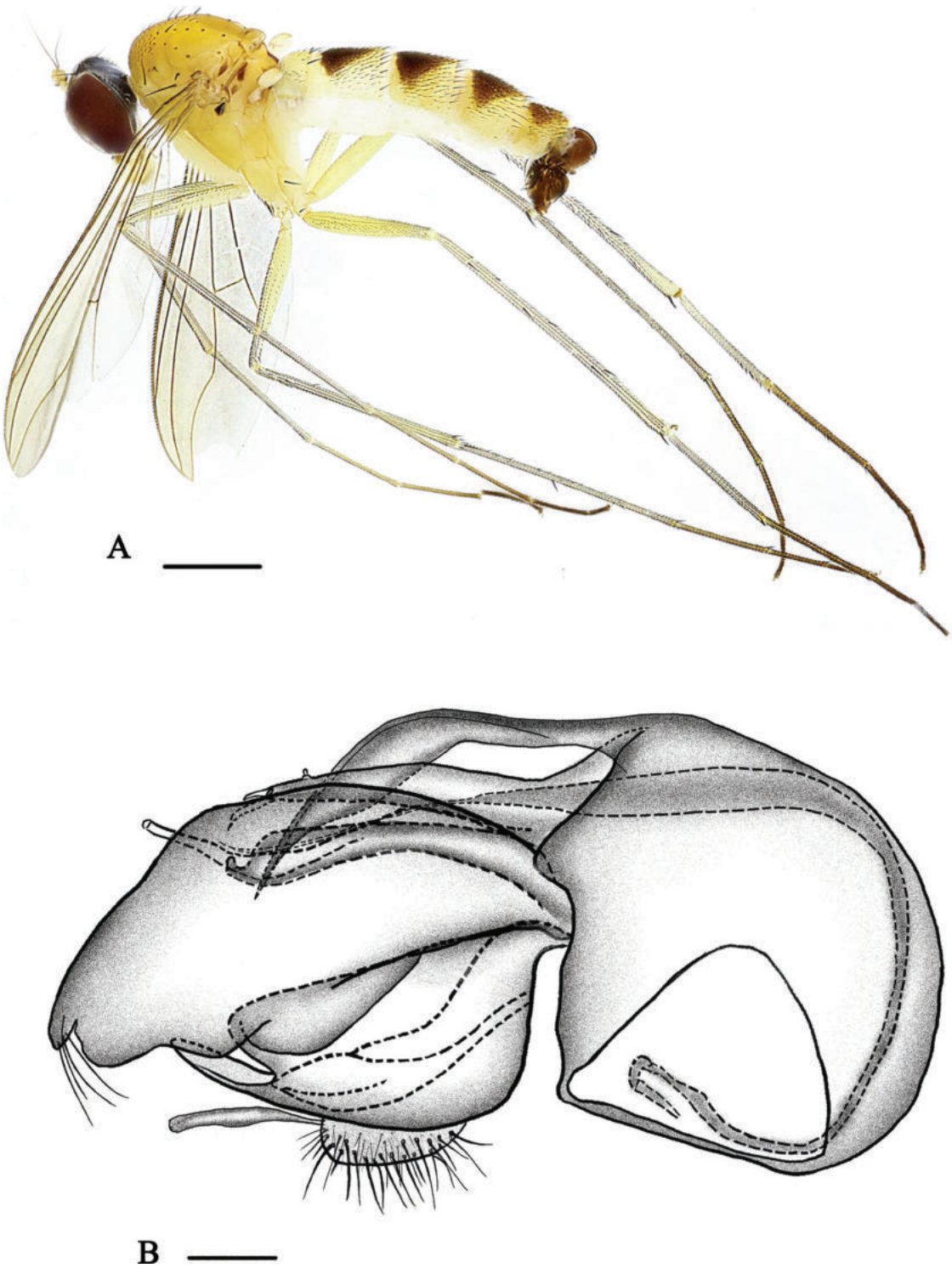


**Figure 3.** *Neurigona brevidigitata* sp. nov. **A** male habitus, lateral view **B** genitalia, lateral view. Scale bars: 1 mm (**A**); 0.1 mm (**B**).

av hairs; tarsomere 1 with two short ad, one long pd at extreme base, five av, and three apical bristles. Hind tarsomere 1 with pale-colored semi-fan-shaped ventral ridge bearing three short and three long hairs at extreme base, three av, and four apical bristles. Relative lengths of tibiae and five tarsomeres of legs – LI 6.8: 4.9: 3.2: 2.9: 2.1: 0.8; LII 8.2: 8.2: 3.5: 2.2:1.2: 0.7; LIII 11.5: 4.7: 4.1: 2.4: 1.3: 0.9.

***Wing*** nearly hyaline, tinged brown; veins brown, $M_{1+2}$ gently bent apically and convergent with $R_{4+5}$; CuAx ratio 0.16. Squama yellow but brown at margin, with yellow hairs. Halter dark yellow, with cluster of hairs at base of knob.

***Abdomen*** yellow with pale yellow pollen; terga 2–5 each with dark brown antero-laterally; hypopygium shiny dark brown. Hairs and bristles on abdomen black except those on venter more or less yellow.

***Male genitalia*** (Fig. 3B): epandrium nearly as long as wide, with two lateral processes (one long, somewhat thick, apically with two short or long and thin processes, and one denticle at apical 1/6; the other very long and thin. Ventral surstylus rather large and broad, ~ 1.5× wider than dorsal surstylus, apically with four long hairs; dorsal surstylus short and broad, apically folded with an oblique finger-like process. Cercus basally somewhat round, white, bearing short white hairs, apically long finger-like. Hypandrium quite long and thin, apically acute. Phallus rather long and thin, apically acute, hidden within hypandrium.

**Female.** Body length 4–5.2 mm, wing length 3.8–4.2 mm. Similar to male, except the following features: eyes distinctly separated on face, narrowest distance in middle of face equal to distance between ocellar bristles; postnotum with a nearly U-shaped dark brown spot. Relative lengths of tibiae and five tarsomeres of legs – LI 4.2: 3.3: 2.1: 1.7: 0.9: 0.6; LII 5.2: 2.8: 2.0: 1.2: 0.7: 0.5; LIII 8.0: 3.1: 2.6: 1.5: 1.0: 0.6.

**Distribution.** China (Yunnan).

**Etymology.** The specific name refers to the dorsal surstylus with a short finger-like process apically.

**Remarks.** The species is very similar to *N. centralis* Yang & Saigusa from Yunnan, but may be separated from the latter by the postnotum yellow with a nearly W-shaped dark brown spot and the mid tibia and tarsus with row of short dense crochet-like av hairs. In *N. centralis*, the postnotum is wholly blackish, and the mid tibia and tarsus do not have the crochet-like av hairs except the mid tarsomere 1 with row of erect av hairs (Yang and Saigusa 2001b; Yang et al. 2011).

### ***Neurigona centralis*** **Yang & Saigusa, 2001**

*Neurigona centralis* Yang & Saigusa, 2001: 173. Type locality: China: Yunnan, Lijiang, Yulongxueshan.

**Diagnosis.** Mesonotum with a black mid-posterior stripe. Postnotum wholly blackish. Mid tibia and tarsus without row of short dense crochet-like av hairs, but mid tarsomere 1 with row of erect av hairs. Epandrium short and wide. Dorsal surstylus short and wide with bifurcated apical process; ventral surstylus long, rather narrow.

**Distribution.** China (Yunnan).

### ***Neurigona convexa*** **sp. nov.**

https://zoobank.org/5591CEE2-411E-4518-9D3A-50885588E162
Figs 4, 8

**Type material.** ***Holotype*****:** ♂, **China:** Yunnan, Lvchun, Yakou, Shuikuxiafang, (23°3'25.2"N, 102°46'37.71"E), 1780 m, collected by Malaise trap, 2019.

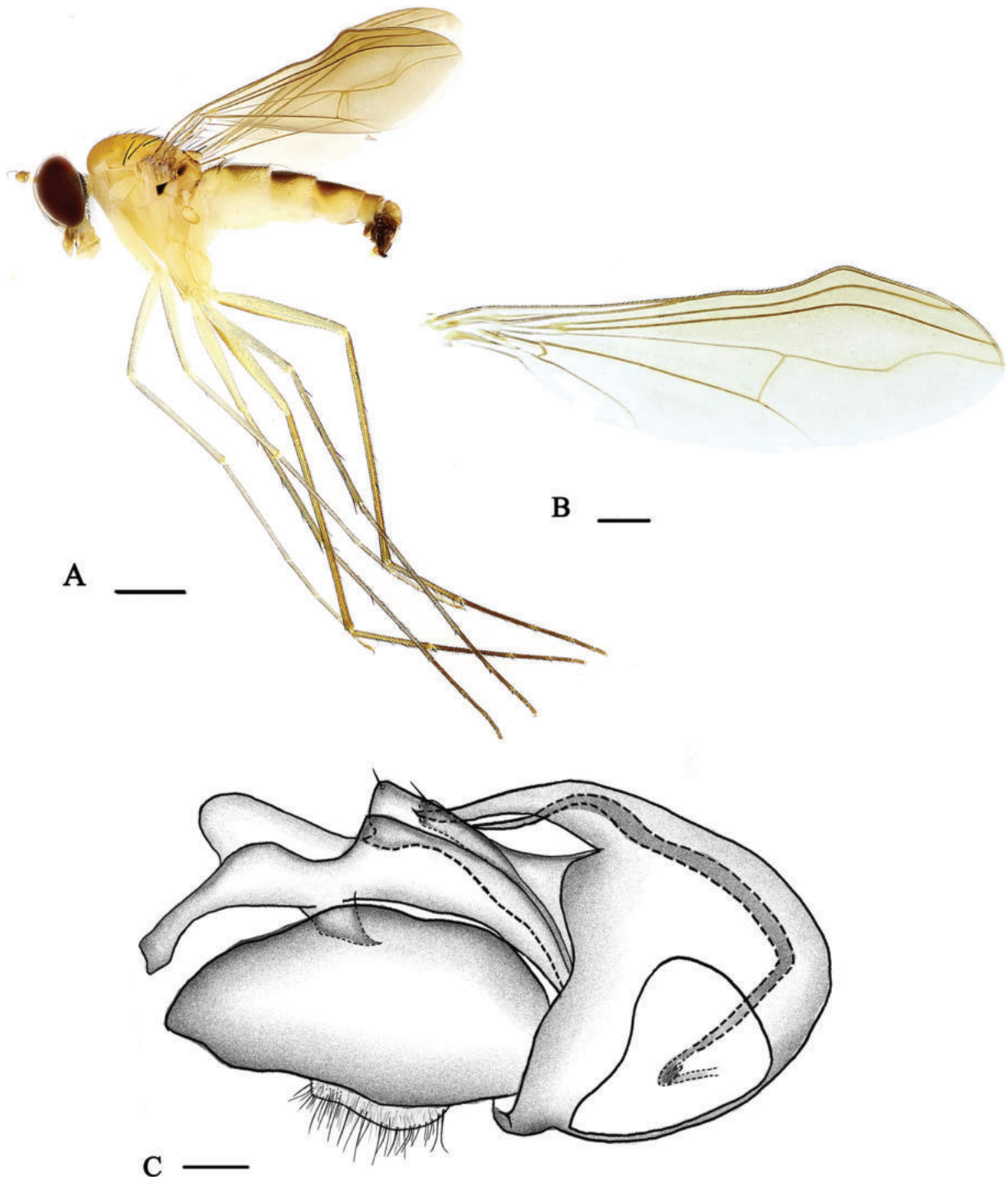


**Figure 4.** *Neurigona convexa* sp. nov. **A** male habitus, lateral view **B** wing **C** genitalia, lateral view. Scale bars: 1 mm (**A**); 0.05 mm (**B**); 0.1 mm (**C**).

IV.19-V.19, Liang Wang and Xin Li (CAU). ***Paratypes*:** 3 ♂♂, the same data as holotype (CAU).

**Diagnosis.** First flagellomere somewhat triangular. Wing subapically convex at anterior margin. Fore tarsomere 4 ventrally weakly swollen at extreme base and then weakly concave for a short distance. Ventral surstylus distinctly widened subapically, apically nearly finger-like; dorsal surstylus nearly as long as ventral surstylus, rather wide, apically somewhat narrow.

**Description. Male** (Fig. 4A). Body length 5.1–5.9 mm, wing length 5.0–5.7 mm.

***Head*** metallic green with pale gray pollinosity; eyes very narrowly separated on middle portion of face. Hairs and bristles on head black, but postocular bristles and postero-ventral hairs pale yellow. Antenna (Fig. 8D) pale yellow, pedicel yellow; first flagellomere dark brown, nearly triangular, almost as long as wide; arista subapical, dark brown, with short brown pubescence. Proboscis yellow with pale hairs; palpus yellow, but brown basally, with blackish setulae and two blackish setae.

***Thorax*** yellow with fine pale yellow pollinosity; mesonotum dark brownish yellow, but brown on flat mid-posterior portion; scutellum dark brownish yellow; postnotum dark brown at basal margin; laterotergite with a small black stripe at anterior margin. Pteropleuron with a very small black subtriangular spot just below wing base. Hairs and bristles on thorax black, six strong dc, 10–11 irregularly paired acr, short, hair-like; scutellum with two pairs of bristles, lateral pair long and strong, median pair short and hair-like. Propleuron with one brown bristle on lower portion.

***Legs*** mainly yellow, but mid and hind tibiae and tarsi brown to dark brown. Hairs and bristles on legs mostly black except hairs on fore coxa pale yellow. Fore coxa with five brownish bristles on antero-apical portion; mid coxa with three brownish anterior and apical bristles; hind coxa with one strong black outer bristle at basal 1/3. Fore tibia with one short ad. Mid tibia with three ad, two pd, and two black strong apical bristles. Hind tibia with three ad, three pd, three short apical bristles, and one row of brownish yellow comb-like bristles. Fore tarsus with very short hairs. Fore tarsomere 4 ventrally weakly swollen at extreme base and weakly incised outward, and with short and nearly erect ventral hairs; tarsomere 5 ventrally with short and nearly erect ventral hairs. Mid tarsomere 1 with two short ad, three short av, and four short thick apical bristles; tarsomeres 2–4 with several short spine-like ventral bristles. Hind tarsomere 1 with thick ventral bristles and three short thick apical bristles. Relative lengths of tibiae and five tarsomeres of legs LI – 7.1: 5.9: 2.2: 1.4:1.1: 0.9; LII 8.1: 8.2: 2.2: 1.3: 0.9: 0.8; LIII 11.2: 3.8: 3.6: 1.5: 1.2: 0.7.

***Wing*** (Fig. 4B) subapically convex forward at anterior margin; nearly hyaline, tinged brown; veins brown; $R_{2+3}$ and $R_{4+5}$ convex forward at apical 1/3 and somewhat parallel; $M_{1+2}$ gently bent apically and convergent with $R_{4+5}$; CuAx ratio 0.75. Squama brown with pale yellow hairs. Halter dark yellow with brown knob.

***Abdomen*** yellow with pale yellow pollen; terga 2–5 each with large dark brown spot at middle; hypopygium shiny dark brown. Abdominal sternite 5 distinctly projected, large subtriangular (Fig. 9B). Hairs and bristles on abdomen blackish except those on venter more or less yellow.

***Male genitalia*** (Fig. 4C): epandrium wider than long, with two lateral processes (one long finger-like; the other long and somewhat thick, apically slightly dilated with very short and acute apico-dorsal process). Ventral surstylus distinctly widened subapically, apically nearly finger-like; dorsal surstylus nearly as long as ventral surstylus, rather wide, apically somewhat narrow. Cercus somewhat round, white, bearing short pale yellow hairs. Hypandrium short and acute. Phallus somewhat short and thin, hidden with hypandrium.

**Female.** Unknown.

**Distribution.** China (Yunnan).

**Etymology.** The specific name refers to the wing subapically convex at anterior margin.

**Remarks.** The new species is peculiar and can be easily separated from other known species of the genus by the wing subapically convex at anterior margin and arista shorter than head width.

***Neurigona huanglianshana* sp. nov.**
https://zoobank.org/1E16CFD0-258E-4930-A199-99719DED5F27
Figs 5, 8

**Type material.** ***Holotype*:** ♂, **China:** Yunnan, Lvchun, Yakou, Shuikuxiafang, (23°3'25.2"N, 102°46'37.71"E), 1780 m, collected by Malaise trap, 2019. IV.19-V.19, Liang Wang and Xin Li (CAU).

**Diagnosis.** Mesonotum with brown subtriangular spot at middle posterior region; scutellum dark brownish yellow with dark yellow posterior margin; postnotum dark brown with dark yellow anterior margin. First flagellomere somewhat oval. Hind tarsomere 1 with cluster of short, erect, and fine ventral bristles basally, three pv on apical 1/3, and two apical bristles. Ventral surstylus apically strongly bent, with bifurcated tip; dorsal surstylus wider than ventral surstylus, nearly quadrate, apically with one knife-like dorsal process.

**Description. Male** (Fig. 5A). Body length 3.0 mm, wing length 3.5 mm.

***Head*** metallic green with gray pollinosity; eyes very narrowly separated on middle portion of face. Hairs and bristles on head black, but postocular bristles and postero-ventral hairs pale yellow. Antenna (Fig. 8E) yellow except first flagellomere dark yellow; first flagellomere somewhat oval, almost as long as wide, somewhat round apically, with dense brownish pubescence; arista subapical, dark brown with dark brownish yellow base. Proboscis yellow with pale bristles and hairs; palpus yellow with two strong black apical bristles.

***Thorax*** yellow with fine pale yellow pollinosity; mesonotum with brown subtriangular spot at middle posterior region; scutellum dark brownish yellow with dark yellow posterior margin; postnotum dark brown with dark yellow anterior margin; laterotergite with blackish stripe at anterior margin and dark brown inner portion. Pteropleuron below wing base with a small black spot. Hairs and bristles on thorax black, six or seven strong dc, 10–12 irregularly paired acr short hair-like; scutellum with two pairs of bristles, lateral pair long and strong, median pair short and hair-like. Propleuron with one yellow bristle on lower portion.

***Legs*** yellow except fore tarsus dark yellow with tarsomere 5 brown and mid and hind tarsi brownish yellow. Hairs and bristles on legs mostly black except hairs on fore coxa brownish yellow to brown. Fore coxa with four long thick brownish yellow bristles on antero-apical portion; mid coxa with three brownish yellow anterior and apical bristles; hind coxa with one strong black outer bristle at basal 1/3. Mid tibia with one ad, one pd, and two short or long apical bristles. Hind tibia with three ad, two pd, two apical ventral bristles, and one row of brownish yellow comb-like bristles. Mid tarsomere 1 with one short and one long dorsal bristles at base. Hind tarsomere 1 with cluster of short erect and fine ventral bristles basally, three pv on apical 1/3, and two apical bristles. Relative lengths of tibiae and five tarsomeres of legs – LI 11.3: 8.0: 5.0: 3.3:1.9: 1.0; LII 13.4: 14.1: 3.7: 2.9:1.6: 2.4; LIII 20.6: 5.9: 6.3: 3.8: 2.7: 1.2.

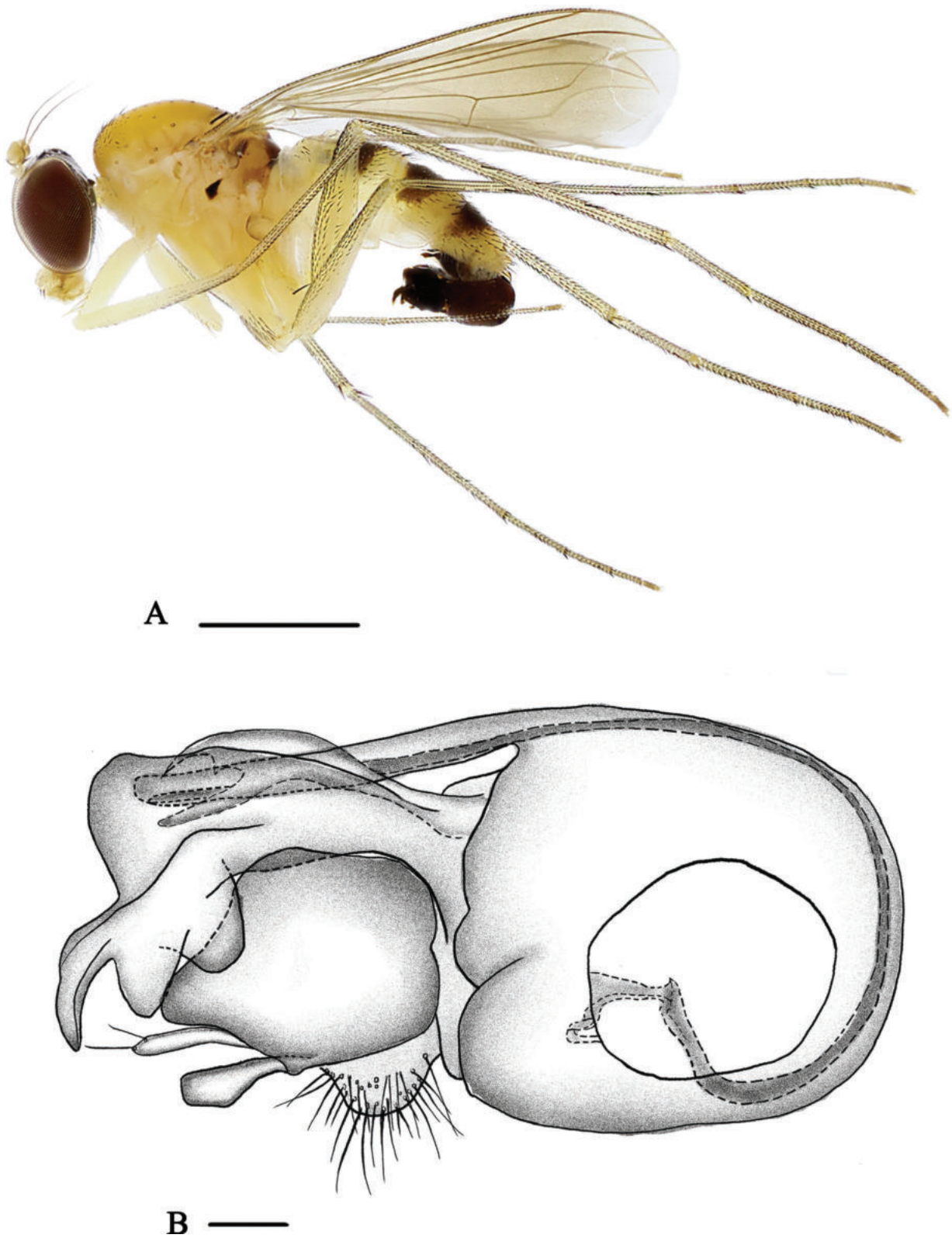


**Figure 5.** *Neurigona huanglianshana* sp. nov. **A** male habitus, lateral view **B** genitalia, lateral view. Scale bars: 1 mm (**A**); 0.1 mm (**B**).

***Wing*** nearly hyaline, tinged brown; veins brown, $M_{1+2}$ distinctly bent, somewhat geniculate, greater than 90 degrees; CuAx ratio 0.49. Squama yellow with yellow hairs. Halter yellow, but base of knob brown with cluster of black hairs.

***Abdomen*** yellow with pale yellow pollen; terga 2–4 each with dark brown basal spot; abdominal sternum 5 distinctly projected, long and subtriangular (Fig. 9C). Hairs and bristles on abdomen black except those on venter more or less yellow.

***Male genitalia*** (Fig. 5B): epandrium almost as long as wide, with two lateral processes (one extremely long and thin; the other long, somewhat wide, apically furcated). Ventral surstylus apically strongly bent, with bifurcated tip; dorsal surstylus wider than ventral surstylus, nearly quadrate, apically with one knife-like dorsal process. Cercus round with long dense yellow hairs, with long finger-like apical process. Hypandrium long and narrow. Phallus long and thin.

**Female.** Unknown.

**Distribution.** China (Yunnan).

**Etymology.** The species is named after the type locality Huanglianshan.

**Remarks.** This species is somewhat similar to *N. henana* Wang, Yang & Grootaert, 2007 from Henan of China, but may be separated from the latter by the arista subapical and mesonotum with one brown subtriangular spot at middle posterior region. In *N. henana*, the arista is dorsal, and the mesonotum is wholly yellow (Wang et al. 2007; Yang et al. 2011).

***Neurigona quadrimaculata* sp. nov.**

https://zoobank.org/65153364-6CA4-4CD4-B4A5-B7B4CFD5CBA7

Figs 6, 8

**Type material.** ***Holotype*:** ♂, **China:** Yunnan, Lvchun, Yakou, Shuikuxiafang, (23°3'25.2"N, 102°46'37.71"E), 1780 m, collected by Malaise trap, 2019.IV.19-V.19, Liang Wang and Xin Li (CAU). ***Paratypes*:** 21 ♂♂, the same data as holotype (CAU).

**Diagnosis.** Eyes contiguous on face. Postnotum and laterotergite dark brown. Thoracic pleuron with four dark spots. Mid tarsus with row of crochet-like av hairs. Ventral surstylus longer than dorsal surstylus, rather narrow, but wide at apex; dorsal surstylus very wide, ~ 3× wider than ventral surstylus, with a long acute apico-dorsal process.

**Description. Male** (Fig. 6A). Body length 5.2–6.1 mm, wing length 4.6–4.9 mm.

***Head*** metallic green with pale gray pollinosity; face very narrow, eyes contiguous on face. Hairs and bristles on head black, but postocular bristles (except uppermost two) and postero-ventral hairs yellow. Antenna (Fig. 8F) yellow except first flagellomere brownish; first flagellomere basally wide, apically narrowed and obtuse, approximately as long as wide, with brown pubescence; arista subapical, dark brown. Proboscis pale yellow with dark yellow hairs; palpus pale yellow with blackish hairs and two short black apical bristles.

***Thorax*** dark yellow with fine pale yellow pollinosity; mesonotum wholly dark yellow, postalar callus with a small dark brown spot; scutellum yellow; postnotum and laterotergite dark brown; mesopleuron, sternopleuron (except posterior portion), and hypopleuron (except postero-dorsal corner) dark brown, pteropleuron brown with a small black spot. Hairs and bristles on thorax black, six strong dc gradually becoming longer backward, 11–12 irregularly paired acr short and hair-like; scutellum with two pairs of sc, lateral pair long and strong, median pair very short and weak. Propleuron with one brown bristle on lower portion.

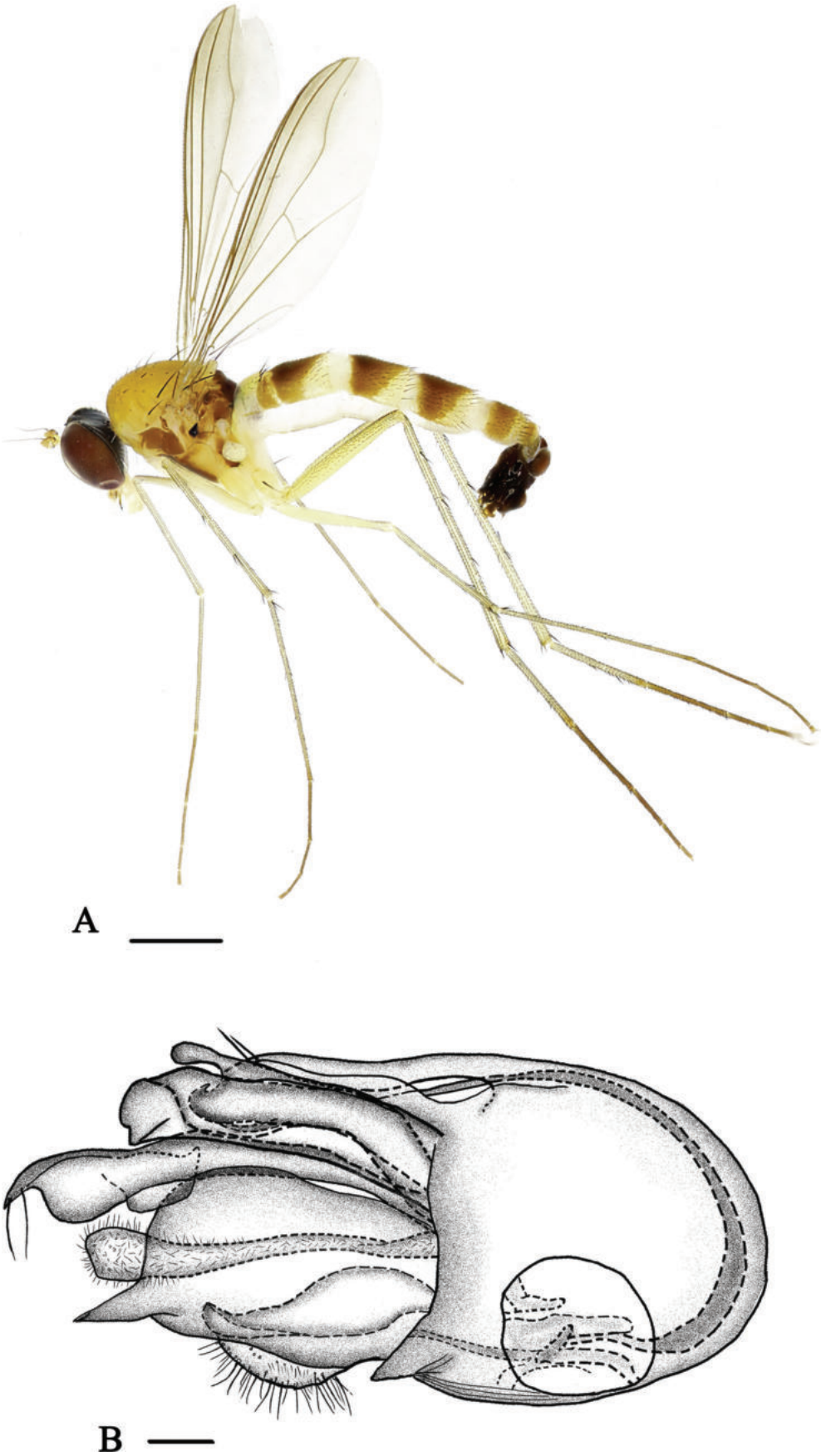


**Figure 6.** *Neurigona quadrimaculata* sp. nov. **A** male habitus, lateral view **B** genitalia, lateral view. Scale bars: 1 mm (**A**); 0.1 mm (**B**).

***Legs*** mainly yellow, but brown or dark brown from tip of tarsomere 1 onward. Hairs and bristles on legs black except hairs on fore coxa yellow. Fore coxa with four thick yellow bristles on antero-apical portion; mid coxa with three mostly dark yellow anterior and apical bristles; hind coxa with one strong black outer bristle at basal 1/3. Mid and hind trochanters each with one spine-like outer bristle at middle. Fore tibia devoid of bristles. Mid tibia with three ad, two pd, and two strong apical bristles. Hind tibia with three ad, three pd, three strong apical bristles, and one row of yellow comb-like bristles. Mid tarsus with row of crochet-like av hairs; tarsomere 1 with one short ad at middle and one long pd at extreme tip. Relative lengths of tibiae and five tarsomeres of legs – LI 5.5: 5.9: 3.2: 2.3: 1.2: 0.8; LII 7.6: 7.9: 3.3: 1.8: 1.1: 0.8; LIII 11.2: 4.2: 4.0: 1.9: 1.2: 0.8.

***Wing*** nearly hyaline, tinged brown; veins brown, $M_{1+2}$ gently bent apically and convergent with $R_{4+5}$; CuAx ratio 0.29. Squama yellow with pale yellow hairs. Halter pale yellow.

***Abdomen*** yellow with yellow pollen; terga 2–5 each with large blackish basal spot. Hairs and bristles on abdomen chiefly black except those on venter more or less yellow.

***Male genitalia*** (Fig. 6B) mainly shiny black. Epandrium longer than wide, with short acute apico-dorsal corner, with three lateral processes, ventral one long and finger-like, middle one long and wide, apically with a nearly U-shaped incision; dorsal one long and wide, curly at tip. Ventral surstylus longer than dorsal surstylus, rather narrow, but wide at apex; dorsal surstylus very wide, ~ 3.0× wider than ventral surstylus, with a long acute apico-dorsal process. Postgonite long and thick, covered with fine hairs. Cercus somewhat round, white, bearing short white hairs. Hypandrium long and wide. Phallus thin, hidden within hypandrium.

**Female.** Unknown.

**Distribution.** China (Yunnan).

**Etymology.** The specific name refers to the thoracic pleuron with four dark spots.

**Remarks.** The species is similar to *N. qingchengshana* Yang & Saigusa, 2021 from Sichuan, but may be separated from the latter by the fore tarsomere 1 longer than fore tibia, 1.1× as long as fore tibia; and narrow ventral surstylus. In *N. qingchengshana*, the fore tarsomere 1 is as long as the fore tibia, and the ventral surstylus is rather wide (Yang and Saigusa 2001a; Yang et al. 2011).

### ***Neurigona ventralis*** **Yang & Saigusa, 2005**

*Neurigona ventralis* Yang & Saigusa, 2005: 759. Type locality: China: Shaanxi, Fuping.

**Diagnosis.** Postnotum dark brown. First flagellomere brownish with yellow base. Fore tarsomeres 3 and 4 shortened, each 0.4× and 0.2× as long as tarsomere 2. respectively; tarsomeres 2 and 3 with long dorsal bristles, tarsomeres 3–5 with two rows of spine-like ventral bristles. Wing wholly hyaline. Dorsal surstylus short and wide with bifurcated apical processes; ventral surstylus long, rather narrow.

**Distribution.** China (Yunnan, Shaanxi).

***Neurigona ventriprocessa* sp. nov.**
https://zoobank.org/8CC18FA7-1D35-48D6-9CB9-E548BE07A403
Figs 7, 8

**Type material.** ***Holotype*:** ♂, **China:** Yunnan, Lvchun, Yakou, Shuikuxiafang, (23°3'25.2"N, 102°46'37.71"E), 1780 m, collected by Malaise trap, 2019.IV.19-V.19, Liang Wang and Xin Li (CAU). ***Paratypes*:** 3 ♂♂, the same data as holotype (CAU).

**Diagnosis.** Frons and face yellow. First flagellomere somewhat quadrate. Mesonotum brownish to brown, but notopleuron somewhat pale; scutellum brown with dark yellow apical margin; postnotum brown with blackish middle line. Fore tarsomere 3 shortened and tarsomere 4 ventrally weakly concave with mammillary ventral process at extreme base. Ventral surstylus distinctly longer than dorsal surstylus, distinctly widened at middle, apically narrowed with short finger-like ventral process; dorsal surstylus short, much widened, ~ 1.5× wider than ventral surstylus.

**Description. Male** (Fig. 7A). Body length 6.9 mm, wing length 5.2 mm.

***Head*** metallic green with gray pollinosity, but yellow ventrally; eyes very narrowly separated on middle portion of face. Hairs and bristles on head black, but postocular bristles and postero-ventral hairs pale yellow. Antenna (Fig. 8G) pale yellow except first flagellomere brown; first flagellomere somewhat quadrate, 1.1× longer than wide, with slightly long, dense brownish pubescence; arista dorsal, dark brown. Proboscis mostly dark yellow, partly brown, with dark yellow and brown hairs; palpus dark yellow, but brown at base, with dark yellow hairs and two short brown bristles.

***Thorax*** mostly dark yellow to brownish yellow with fine pale yellow pollinosity; mesonotum brownish to brown, but notopleuron somewhat pale; scutellum brown with dark yellow apical margin; postnotum brown with blackish middle line. Laterotergite with blackish stripe at anterior margin. Pteropleuron with a small black subtriangular spot. Hairs and bristles on thorax black, five strong dc, 14–15 irregularly acr short hair-like; scutellum with two pairs of bristles, lateral pair long and strong, median pair short and hair-like. Propleuron with one brown bristle on lower portion.

***Legs*** mainly yellow, but hind femur ventrally dark brown at basal 1/3; mid and hind tarsomeres 2–5 brown or dark brown. Hairs and bristles on legs mostly black except hairs on all coxae dark yellow. Fore coxa with dark yellow hairs and six mostly brownish yellow bristles on antero-apical portion; mid coxa with two short black bristles and eight dense dark yellow anterior and apical bristles; hind coxa with dark yellow hairs and one strong outer black bristle at basal 1/3. Mid and hind trochanters each with one outer bristle at middle. All femora distinctly thickened basally. Fore tibia with one short ad and two short pd. Mid tibia with two ad, three pd, one av, two pv, and three apical bristles. Hind tibia with three ad, three pd, two short or long apical bristles, and one row of yellow comb-like bristles. Fore tarsomere 3 shortened with short dense ventral hairs; tarsomere 4 somewhat whitened, ventrally weakly concave, somewhat bare at middle, extreme base distinctly dilated with mammillary ventral process, extreme tip weakly dilated with several short curly brown ventral hairs; tarsomere 5 with quite dense, curly and dark brown ventral hairs. Mid tarsomere 1 with several short or long bristles. Relative lengths of tibiae and five tarsomeres of legs LI – 6.8: 6.7: 2.9: 0.8:1.1: 1.0; LII 8.8: 9.1: 3.7: 2.4:1.3: 0.8; LIII 13.9: 4.9: 4.8: 2.6: 1.8: 0.9.

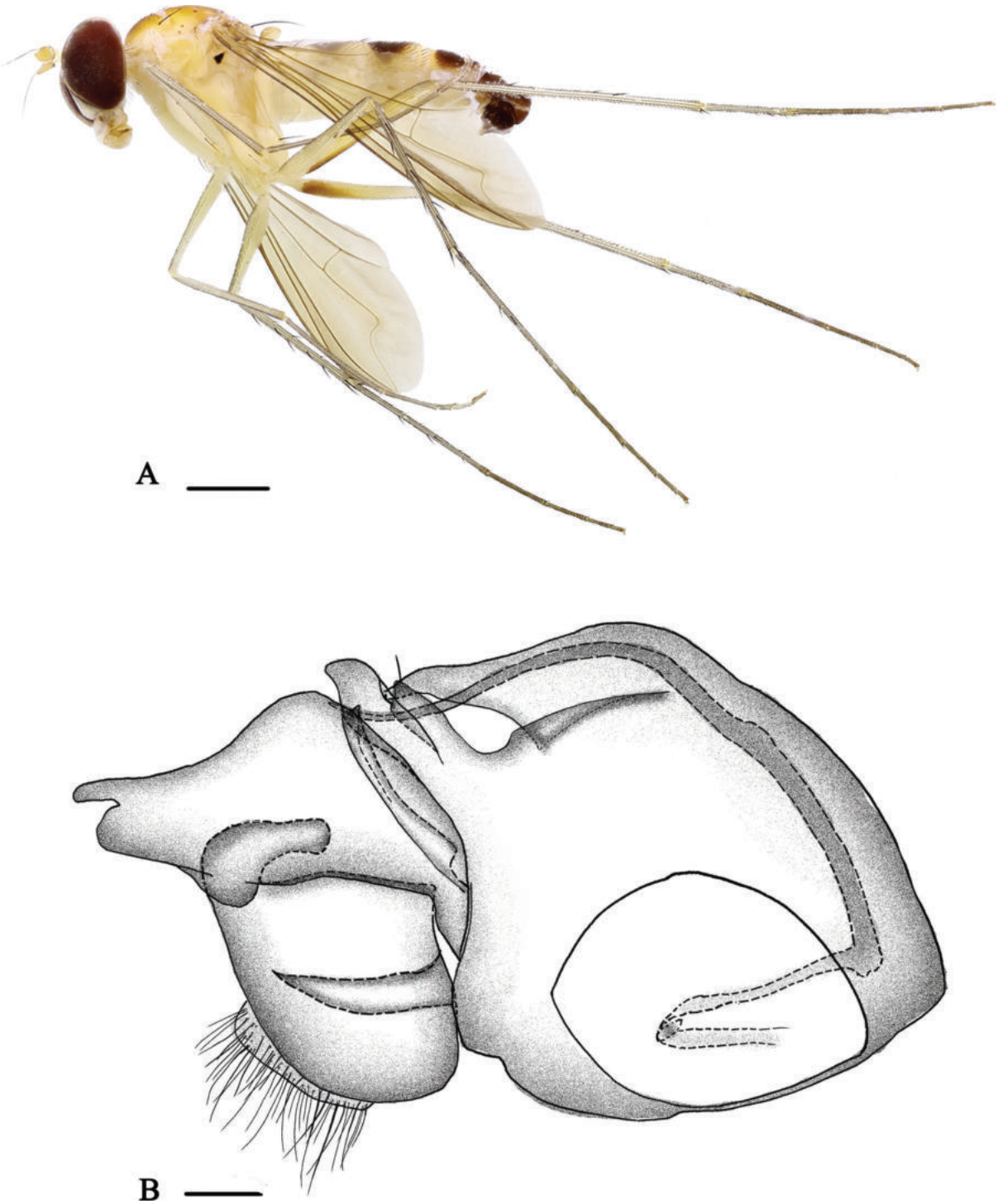


**Figure 7.** *Neurigona ventriprocessa* sp. nov. **A** male habitus, lateral view **B** genitalia, lateral view. Scale bars: 1 mm (**A**); 0.1 mm (**B**).

***Wing*** nearly hyaline, tinged brown; veins dark brown, $M_{1+2}$ strongly bent, geniculate nearly in a right angle; CuAx ratio 0.54. Squama dark yellow with brown margin bearing yellow hairs. Halter dark yellow with dark brown knob.

***Abdomen*** yellow with pale yellow pollen; terga 1–4 each with large dark brown sport, tergum 5 wholly brown; sternum 5 weakly projected, small and subtriangular (Fig. 9D). Hairs and bristles on abdomen black except those on venter more or less yellow.

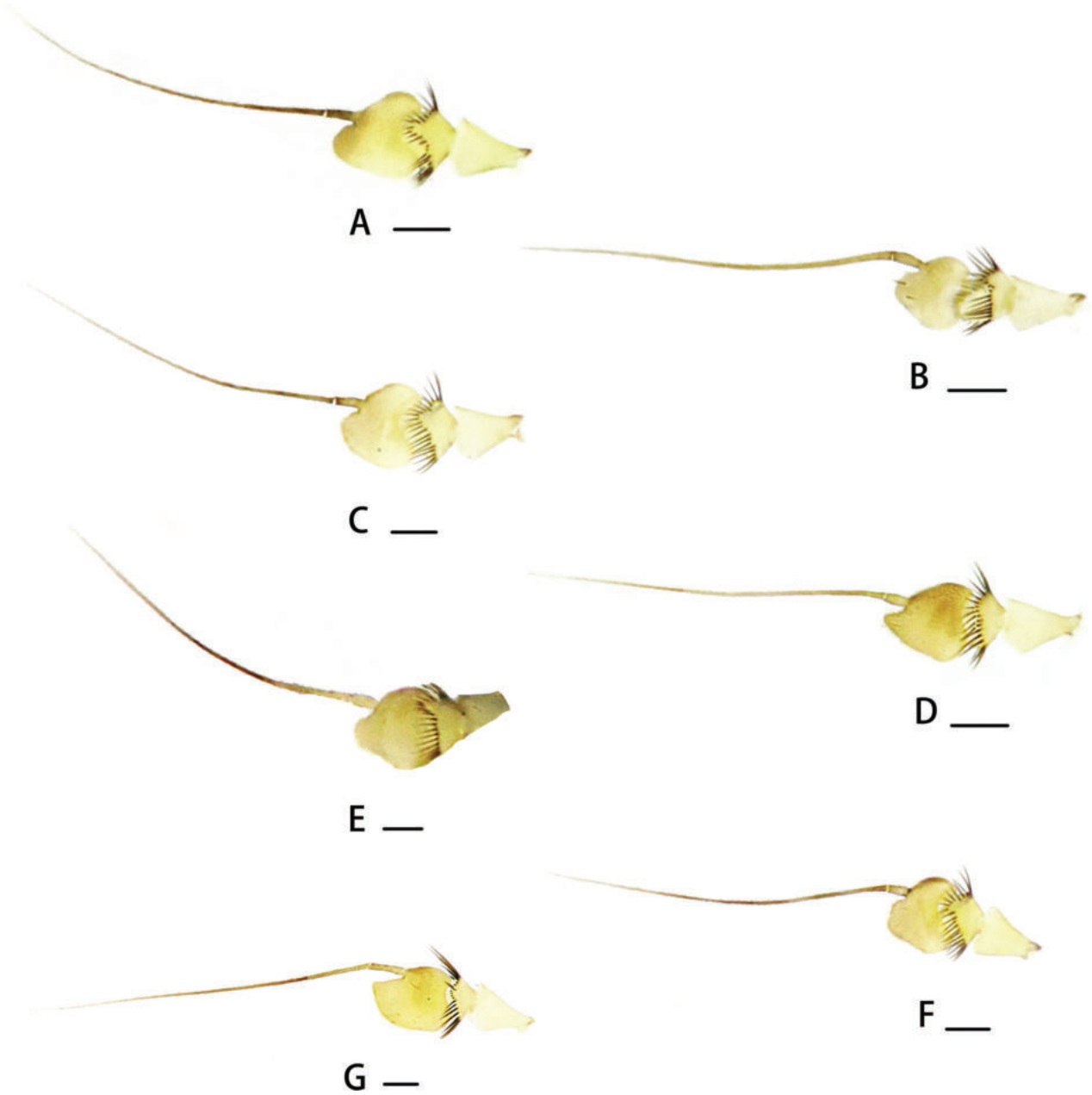


**Figure 8.** Antennae **A** *Neurigona apicilata* sp. nov. **B** *Neurigona basicurva* sp. nov. **C** *Neurigona brevidigitata* sp. nov. **D** *Neurigona convexa* sp. nov. **E** *Neurigona huanglianshana* sp. nov. **F** *Neurigona quadrimaculata* sp. nov. **G** *Neurigona ventriprocessa* sp. nov. Scale bars: 0.1 mm.

***Male genitalia*** (Fig. 7B): epandrium nearly as long as wide, with two lateral processes (one short finger-like; the other slightly long and thick, finger-like). Ventral surstylus distinctly longer than dorsal surstylus, distinctly widened at middle, apically narrowed with short finger-like ventral process; dorsal surstylus short, much widened, ~ 1.5× wider than ventral surstylus. Cercus long, with long dense yellow hairs. Hypandrium basally rather thick, apically narrowed. Phallus long and thin.

**Female.** Unknown.

**Distribution.** China (Yunnan).

**Etymology.** The specific name refers to the fore tarsomere 4 with mammillary ventral process at extreme base.

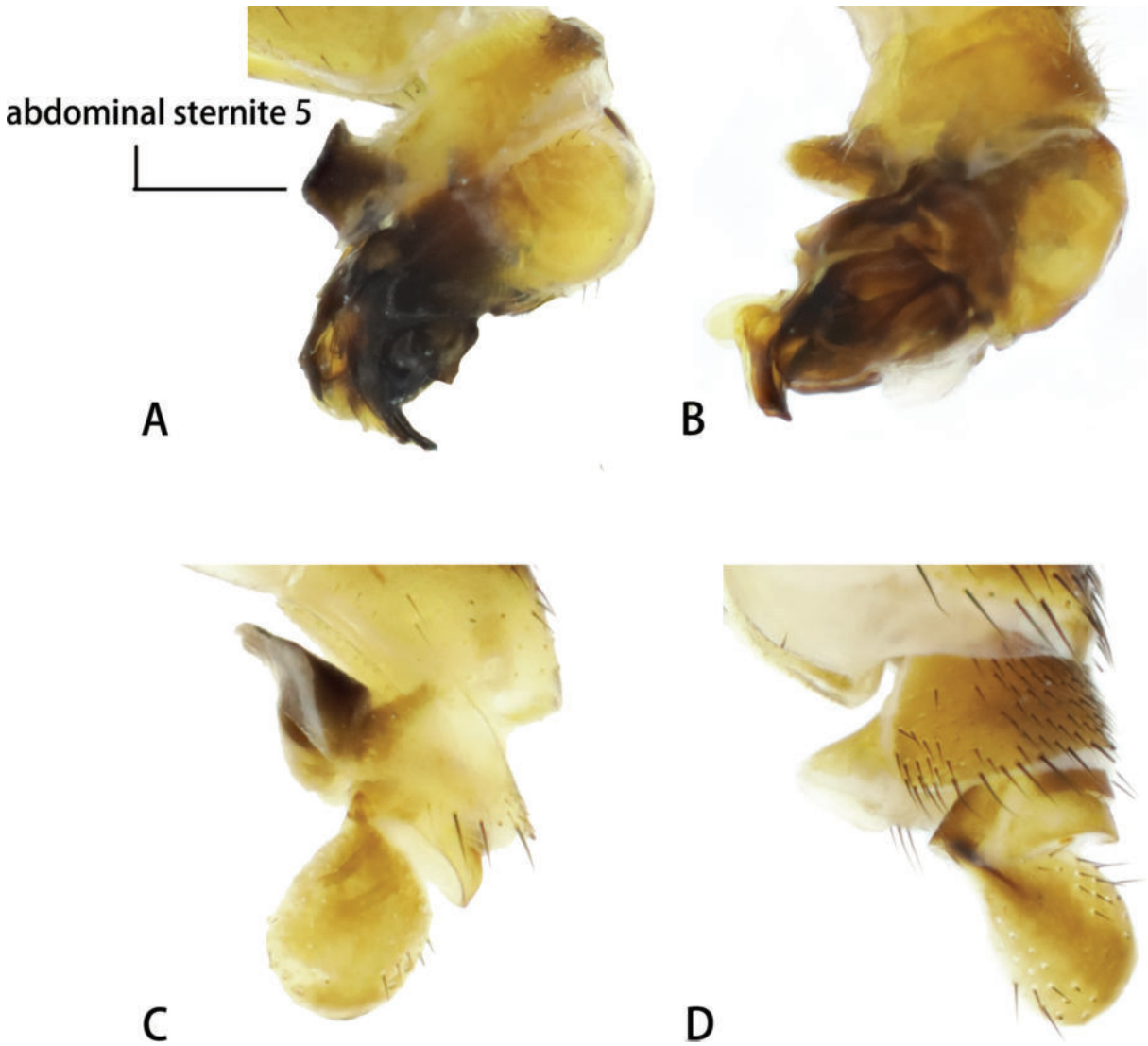


**Figure 9.** Abdominal sternite 5 (male) **A** *Neurigona basicurva* sp. nov. **B** *Neurigona convexa* sp. nov. **C** *Neurigona huanglianshana* sp. nov. **D** *Neurigona ventriprocessa* sp. nov.

**Remarks.** The species is somewhat similar to *N. guangxiensis* Yang, 1999 and *N. zhejiangensis* Yang, 1999 from Oriental China in having the nearly quadrate first flagellomere, but may be separated from them by the mesonotum brownish to brown, fore tarsomere 3 shortened and tarsomere 4 ventrally weakly concave with mammillary ventral process at extreme base. In *N. guangxiensis* and *N. zhejiangensis*, the mesonotum is yellow with markings on the middle posterior portion, and the fore tarsus is not modified (Yang et al. 2011).

### *Neurigona yunnana* Wang, Yang & Grootaert, 2007

*Neurigona yunnana* Wang, Yang & Grootaert, 2007: 38. Type locality: China: Yunnan, Mengla.

**Diagnosis.** Antenna brownish. Four strong dc; quadriseriate acr. Fore coxa with 4–6 black bristles on antero-apical portion. Apex of fore tarsomere 4 and entire

fore tarsomere 5 white with white hairs. Hypopygium yellow. Dorsal surstylus wide with nearly acute tip; ventral surstylus slightly longer than dorsal surstylus, apically distinctly widened.

**Distribution.** China (Yunnan).

## Discussion

The fauna of China has been divided into seven ecoregions: Northeast China, North China, Mongolia-Xinjiang Region, Qinghai-Tibet Regionthat belong to the Palaearctic Realm, and Southwest China, Central China, and South China Region that belong to the Oriental Realm (Zhang 1998). Based on the zoogeographical regions of China, eleven species (*N. centralis*, *N. qingchengshana*, *N. yunnana*, *N. sichuana*, *N. apicilata* sp. nov., *N. basicurva* sp. nov., *N. brevidigitata* sp. nov., *N. convexa* sp. nov., *N. huanglianshana* sp. nov., *N. quadrimaculata* sp. nov., *N. ventriprocessa* sp. nov.) are thus far only recorded from the Southwest China region, nine species (*N. composita*, *N. denudata*, *N. exemta*, *N. gemina*, *N. guangdongensis*, *N. guangxiensis*, *N. pectinata*, *N. xui*, *N. hainana*) are exclusively reported from the South China Region, eight species (*N. basalis*, *N. bimaculata*, *N. henana*, *N. micropyga*, *N. shaanxiensis*, *N. xiangshana*, *N. xiaolongmensis*) are only found in the North China Region, six species (*N. chetitarsa*, *N. guizhouensis*, *N. jiangsuensis*, *N. shennongjiana*, *N. wui*, *N. zhejiangensis*) are discovered in the Central China Region, two species (*N. grisea*, *N. yaoi*) are recorded from the Mongolia-Xinjiang Region, and one species (*N. xizangensis*) is exclusively found in the Qinghai-Tibet Region. Only two species, *N. concaviuscula* and *N. ventralis*, are common in the Central and Southwest China regions, and North and Southwest China regions respectively. Up to now, of the 38 species described from China, 30 species (79%) are recorded from Oriental China, while only eight species (21%) were recorded from Palaearctic China. Within the global zoogeographical realms, the distribution proportions of the *Neurigona* in the Palaearctic and Oriental realms are relatively equal, accounting for 27% and 23%, respectively. This forms a sharp contrast to its proportion in Oriental and Palaearctic China, indirectly suggesting that the current species richness of *Neurigona* in China is still underestimated. Further collections and investigations of *Neurigona* from more areas of China are needed to provide additional data on the distribution of this genus.

The seven new species described in this study were collected by Malaise traps from the same locality, indicating a high level of local sympatry. There are many references indicating that the subfamily Medeterinae also exhibits rich local sympatry. For example, six species of *Systenus* were found from the same locality near Manaus, Brazil (Naglis 2000), eight species of *Systenus* were known only from Malaise traps at a single locality in Guanacaste Province, Costa Rica (Bickel 2015), and six species of *Systenus* were collected at the same collecting site in Yunnan, China (Lin et al. 2023). Furthermore, although *Neurigona* species can also be found in garden or park landscapes, they show a clear preference for wooded sites. The collection site of the material in this study precisely corresponds to such a habitat, which is located underneath an isolated area separated by a dam, with a stream running through the middle and a mixed environment of dense shrubland and broad-leaved forest on both sides (Fig. 10).

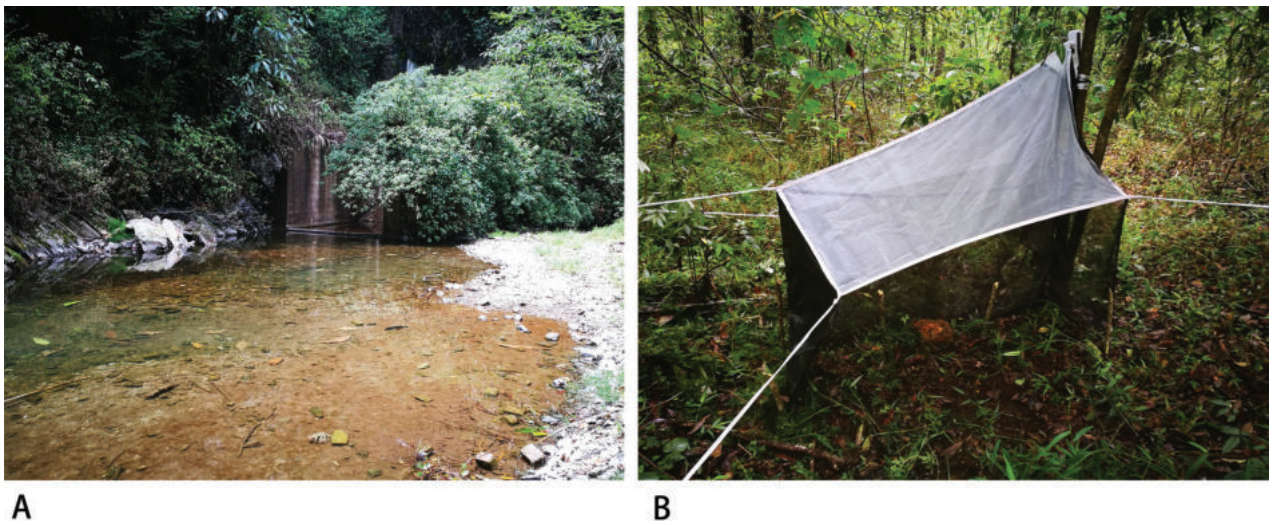


**Figure 10. A** forest habitat in Yunnan Province, Lvchun, Yakou, Shuikuxiafang, 1779 m **B** malaise trap set nearby.

## Acknowledgements

We are very grateful to Dr. Liang Wang and Dr. Xin Li (Beijing) for collecting specimens and providing the habitat photographs and descriptions. We thank Dr. Scott Williams and Dr. Yan Yan (Boston) checking this manuscript. Meanwhile, we would like to extend our gratitude to the subject editor Marija Ivković and the reviewers Dan Bickel, Patrick Grootaert, and Marc Pollet for their invaluable assistance.

## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

### Funding

This research was funded by National Natural Science Foundation of China (No. 32260121), Natural Science Foundation of Inner Mongolia (No. 2022MS03003) and Higher Education Science and Technology Research Project of Inner Mongolia [grant number NJZY22575].

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### Data availability

All of the data that support the findings of this study are available in the main text.

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