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Phyllodiaptomus (*Phyllodiaptomus*) *roietensis*, a new diaptomid copepod (Copepoda, Calanoida) from temporary waters in Thailand and Cambodia, with a key to the species

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Abstract

Phyllodiaptomus (*Phyllodiaptomus*) *roietensis* **sp. nov.** was collected from temporary water bodies in Roi Et and Nakhon Ratchasima provinces in northeastern Thailand and Kampong Thom Province in central Cambodia. The new species is closely related to *Phyllodiaptomus* (*P.*) *surinensis* Sanoamuang & Yindee, 2001 in that it shares common morphological characters in the males: urosomites 2–3, P5 intercoxal sclerite, right P5 Exp-2, and left P5 Exp. Minor differences on the right antennule, right caudal ramus, P5 basis and Enp exist. The females differ in their Pdg 5, genital double-somite, and P5. An updated key to the species of the genus *Phyllodiaptomus* Kiefer, 1936 is provided.

Keywords

Diaptomidae, freshwater, rare species, Southeast Asia, taxonomy, temporary water bodies

Introduction

The genus *Phyllodiaptomus* Kiefer, 1936, is among the most common freshwater copepods in Southeast Asia (Sanoamuang 1999). To date, eleven valid species have been recorded in Asia (Walter and Boxshall 2018): *Phyllodiaptomus* (*Phyllodiaptomus*) *blanci* (Guerne & Richard, 1896) from Uzbekistan; *P.* (*Ctenodiaptomus*) *annae* (Apstein, 1907) from Sri Lanka; *P.* (*P.*) *tunguidus* Shen & Tai, 1964 from China; *P.* (*P.*) *longipes* Kiefer, 1965 from Indonesia; *P.* (*C.*) *sasikumari* Ranga Reddy & Venkateswarlu, 1989 and *P.* (*C.*) *wellekensae* Dumont & Ranga Reddy, 1993 from India; *P.* (*C.*) *praedictus* Dumont & Ranga Reddy, 1994, *P.* (*P.*) *christineae* Dumont, Ranga Reddy & Sanoamuang, 1996, *P.* (*P.*) *surinensis* Sanoamuang & Yindee, 2001, and *P.* (*P.*) *thailandicus* Sanoamuang & Teeramaethee, 2006 from Thailand; and *P.* (*P.*) *irakiensis* Khalaf, 2008 from Iraq. In addition, Alekseev et al. (2013) reported *P.* (*C.*) *praedictus sulawesensis* as a subspecies of *P.* (*C*) *praedictus* from Indonesia; this subspecies was later found in the Philippines (Guinto et al. 2018).

During seasonal sampling collections of freshwater copepods from several localities in Thailand and Cambodia, we encountered another hitherto unknown species of *Phyllodiaptomus*. In this paper, we describe *Phyllodiaptomus* (*P.*) *roietensis* sp. nov. from two localities in Roi Et and Nakhon Ratchasima provinces, northeast Thailand, and two localities in Kampong Thom Province in central Cambodia (Fig. 1).

Materials and methods

Samples were collected using a plankton net with a mesh size of 60 μm and preserved immediately in 70% ethanol. Adult copepods were selected under an Olympus SZ51 stereomicroscope at 40-x magnification and placed in a mixture of glycerol and 70% ethanol (ratio ~ 1:10 v/v). After 10 min the animals were transferred to pure glycerol. The animals were dissected and prepared in a glycerin-mounted slide under a stereomicroscope at 40–100-x magnifications. The dissected specimens were mounted in pure glycerin on a glass slide and sealed under a cover glass with transparent nail varnish. All un-dissected specimens were stored in 70% ethanol in 1.5 mL microtubes.

All appendages and body ornamentation were examined at 1000-x magnification under an Olympus CX31 compound microscope. The drawings were made using an Olympus U-DA drawing tube mounted on a compound microscope. The final versions of the drawings were made using the CORELDRAW 12.0 graphic program.

Specimens for scanning electron microscopy (SEM) were dehydrated in an ethanol series (50%, 70%, 80%, 90%, 95%, 100%, and 100%) for 15 min at each concentration. Specimens were dried in a critical-point dryer and were mounted on stubs using adhesive tape under a stereomicroscope. Dried specimens were coated with gold in a sputter-coater. The SEM photographs were taken using a scanning electron microscope (FEI Helios NanoLab G3 CX).

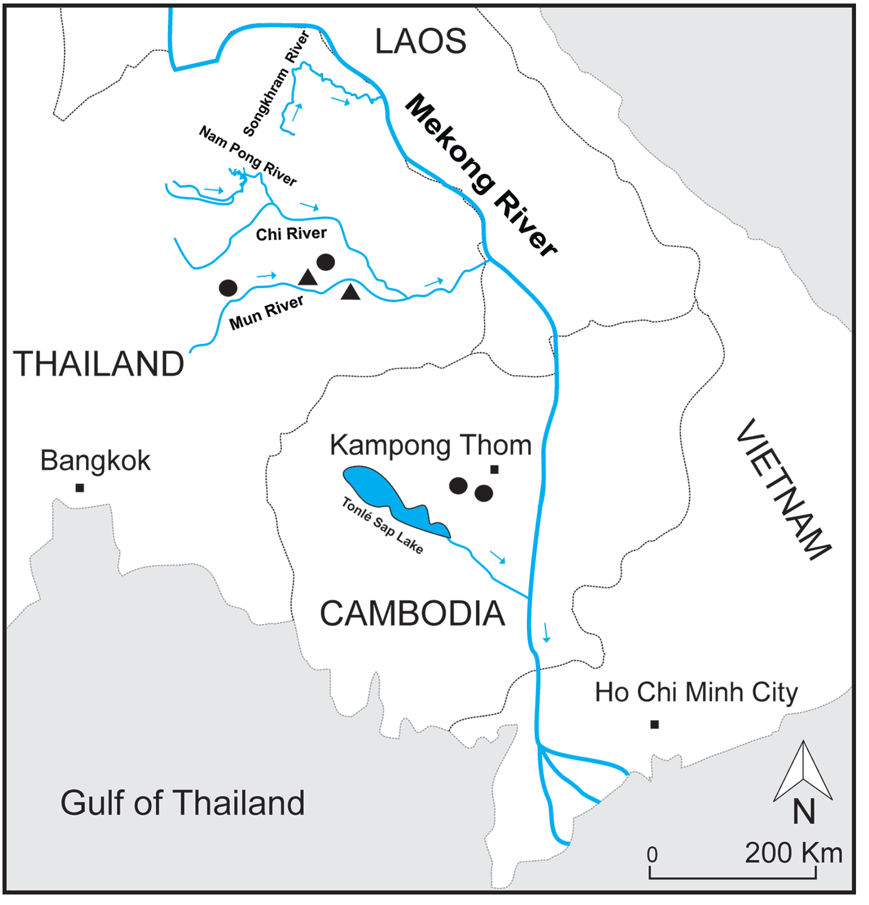

Figure 1. Distribution of *Phyllodiaptomus* (*P.*) *roietensis* sp. nov. and *P.* (*P.*) *surinensis*. Key: black square = city, black circle = *P.* (*P.*) *roietensis* sp. nov., black triangular = *P.* (*P.*) *surinensis*, blue arrows indicate water flow direction.

Specimens were deposited at the Natural History Museum, London, United Kingdom (**NHMUK**) and at the Applied Taxonomic Research Center, Khon Kaen University (Thailand) (**KKU**).

Abbreviations used in this paper are as follows:

ae	aesthetasc;	**Pdg**	pediger;
Enp	endopod;	**Pdg 1–5**	pedigers 1–5;
Exp	exopod;	**P1–P5**	legs 1–5;
Exp/Enp-n	exopodal segment n/endopodal segment n;	**sp**	spine.

The descriptive terminology follows Huys and Boxshall (1991).

Taxonomic section

Order Calanoida Sars, 1903
Family Diaptomidae Baird, 1850
Sub-family Diaptominae Kiefer, 1932
Genus *Phyllodiaptomus* Kiefer, 1936
Subgenus *Phyllodiaptomus* Dumont, Ranga Reddy & Sanoamuang, 1996

***Phyllodiaptomus* (*P.*) *roietensis* sp. nov.**
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Figs 2–8

Type locality. A pool in the rice field at Ban Nakae, Khilek Subdistrict, Pathum Rat District, Roi Et Province, northeastern Thailand; pH of water 8.6, water conductivity 126 µS cm^{-1}.

Type material. ***Holotype***: one adult male completely dissected (NHMUK 2019.7, one slide), Ban Nakae (15°37'37"N, 103°28'06"E), Khilek Subdistrict, Pathum Rat District, Roi Et Province, northeastern Thailand; collected on 12 June 1999 by L. Sanoamuang. ***Allotype*:** one adult female completely dissected (NHMUK 2019.8, one slide); same data as for holotype. ***Paratypes***: two adult females and three adult males undissected preserved in 70% ethanol (NHMUK 2019.9–13), one adult female completely dissected (KKU-COP-2019-S-01); one adult female with eggs and three adult males undissected preserved in 70% ethanol (KKU-COP-2019-T-01); same data as for holotype.

Other localities. (1) a temporary pond, Ban Non Lakki (15°10'55"N, 102°23'46"E), Than Lalot Subdistrict, Phimai District, Nakhon Ratchasima Province, northeastern Thailand; collected on 17 October 2017 by N. Plangklang; (2) a roadside canal, Tropeang Chouk village (no geographical co-ordinates), Baray District, Kampong Thom Province, central Cambodia; collected on 14 June 2007 by R. Chaicharoen; (3) a temporary pond, Kropeu village (no geographical co-ordinates), Steung Sen District, Kampong Thom Province, central Cambodia; collected on 14 June 2007 by R. Chaicharoen.

Description of adult female. Total body length measured from anterior margin of rostrum to posterior margin of caudal rami, 0.9–1.3 mm. Rostrum (Fig. 3G) with bifid process in distal margin, pointed backward; each with short spine at tip. Prosome length: urosome plus caudal rami ratio about 2.6:1, ratio of width to length of prosome = 1:2.4, urosomites 1–3 = 1.3:3.0:1.1, caudal ramus = 1:1.5. Prosome (Figs 2A, 3A) ovoid, cephalosome with transversal groove in anterior part of somite length; Pdg 4 and 5 fused, partly separated laterally, with few tiny hair-like spinules scattered laterally (Fig. 3B, C). Pdg 5 (Figs 2C, E, 3C–F) with asymmetrical postero-lateral wings; right one rounded; left one longer and triangular; each wing with dorsal and posterior spines (former spine slightly larger than later one). Urosome (Figs 2A, 3A) with asymmetrical genital double-somite. Genital double-somite (Figs 2C–E, 3C–F) longer than urosomite 2, anal somite and caudal ramus combined. Left side with obvi-

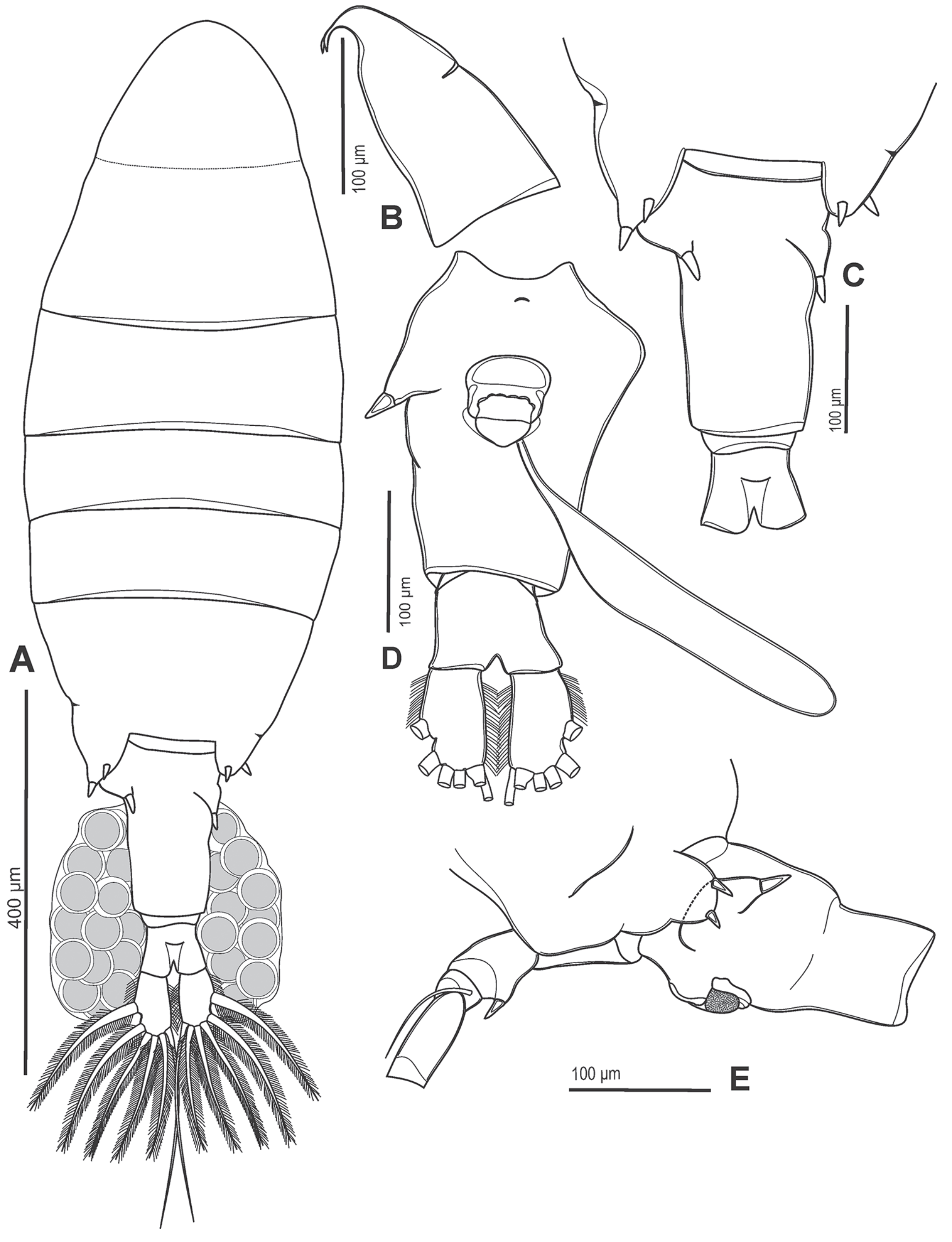

Figure 2. *Phyllodiaptomus* (*P.*) *roietensis* sp. nov., female: **A** habitus, dorsal view **B** cephalosome with rostrum, lateral view **C** lateral wings on Pdg 5 and urosome (without caudal rami), dorsal view **D** urosome, ventral view **E** Pdg 5 with P5 and genital double-somite, lateral view from left side.

ously laterally dilated proximal part of genital-double segment; dilatation dorsally with large and blunt spine distally, tip of spine oriented medially. Right side with slightly dilated proximal part of genital double-somite; elongated into triangular outgrowth

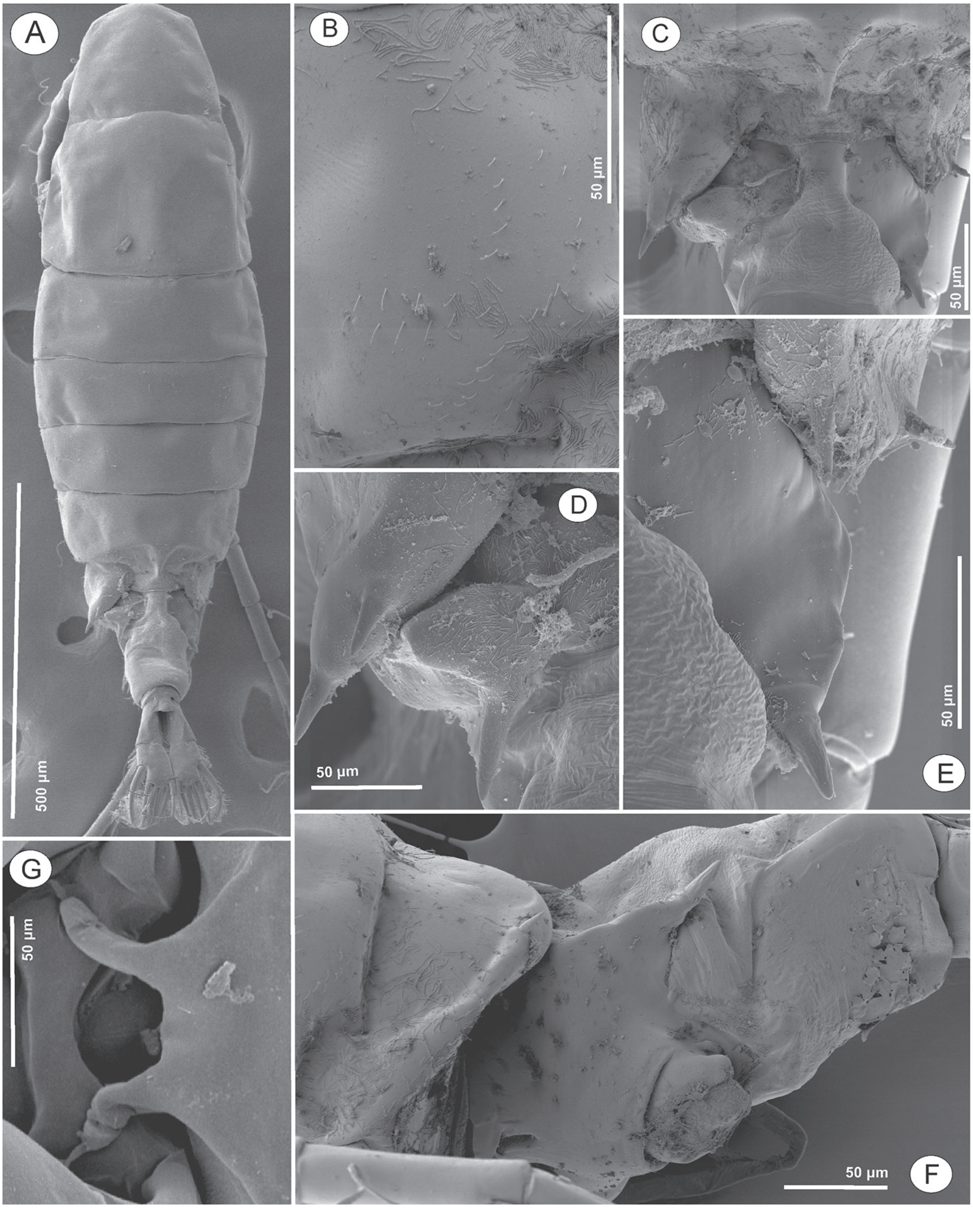

Figure 3. *Phyllodiaptomus* (*P.*) *roietensis* sp. nov., female: **A** habitus, dorsal view **B** Pdg 4, lateral view **C** Pdg 5 and genital double-somite, dorsal view **D** Pdg 5 and genital double-somite spines, lateral view from left side **E** Pdg 5 and genital double-somite spines, lateral view from right side **F** Pdg 5 and genital double-somite, lateral view **G** rostral spines.

with blunt spine at tip; spine orientated ventro-laterally. A pair of gonopores located beneath genital operculum, at about one-half length of genital double-segment. Adult female bears one egg sac with 20–25 eggs (Fig. 2A). Urosomite 2 symmetrical, very short. Anal somite (Figs 2C, D, 3A) as long as wide; anal operculum small, free margin convex. Caudal rami (Fig. 2A, D) symmetrical, with row of setules along inner and outer margins. Ramus with six setae (seta II–VII), subequal in length, all plumose but dorsal (VII); dorsal seta articulated, longest.

Antennule (Fig. 4A) symmetrical, 25-segmented, reaching beyond the end of caudal setae. Setal formula (Roman numerals in parentheses refer to segment number): 1+ae (I), 3+ae (II), 1+ae (III), 1(IV), 1+ae (V), 1 (VI), 1+ae (VII), 1+sp (VIII), 2+ae (IX), 1 (X), 1 (XI), 1+ae+sp (XII), 1 (XIII), 1+ae (XIV), 1 (XV), 1+ae (XVI), 1 (XVII), 1 (XVIII), 1+ae (XIX), 1 (XX), 1 (XXI), 2 (XXII), 2 (XXIII), 2 (XXIV), 4+ae (XXV).

Antenna (Fig. 4B) biramous. Coxa and basis with one and two inner setae on distal corner, respectively. Exp-1–7 with 1, 3, 1, 1, 1, 1, and 1 inner seta, respectively; Exp-7 with three additional apical setae. Enp-1 with two inner median setae. Enp-2 with eight inner and seven apical setae.

Mandible (Fig. 4C) with six strongly chitinized teeth and one dorsal seta on gnathobase. Basis with four inner setae. Enp-1 with four inner distal setae; Enp-2 with nine apical setae plus tiny spinules along posterior surface. Exp-1–4 with 1, 1, 1, 3 setae, respectively.

Maxillule (Fig. 4D) with 13 setae on praecoxal arthrite. Coxal endite and coxal epipodite with four and nine setae, respectively. Proximal and distal endites each with four setae; basal exite with a single seta. Enp with seven apical setae. Exp with six setae.

Maxilla (Fig. 4E) with two praecoxal and two coxal endites; each with three apical setae. Allobasis with three setae. Enp-1 and 2 with three setae each.

Maxilliped (Fig. 4F) with four endites on syncoxa, with 1, 2, 3, 4 apical setae respectively. Basis with three setae along median margin. Enp-1–6 with 2, 3, 2, 2, 2, 4 setae, respectively.

P1–P4 (Fig. 5A–D) with round and bare intercoxal sclerite. Coxa with inner seta. P1 basis with reduced outer seta. Exp longer than Enp, Exp and Enp three-segmented except P1 Enp bi-segmented. Armature formula of P1–P4 as follows (Arabic and Roman numerals indicate number of setae and spines, respectively; outer-inner or outer-apical-inner indicate seta/spine):

	Coxa	Basis	Exp			Enp		
			1	2	3	1	2	3
P1	0-1	0-0	I-1	0-1	I-3-2	0-1	1-2-3	–
P2	0-1	0-0	I-1	I-1	I-3-3	0-1	0-2	2-2-3
P3	0-1	0-0	I-1	I-1	I-3-3	0-1	0-2	2-2-3
P4	0-1	1-0	I-1	I-1	I-3-3	0-1	0-2	2-2-3

P5 (Fig. 5E) asymmetrical. Coxa with blunt, stout spine on distal outer margin. Basis with thin, bare seta on distal outer margin, reaching beyond 3/4 of Exp-1. Exp-1 sub-rectangular, more than twice as long as wide, longer than Enp. Exp-2 triangular, right side stout and shorter than left one; with row of strong spinules along margins and proximolateral spine at basal Exp-3; with two longitudinal grooves on anterior

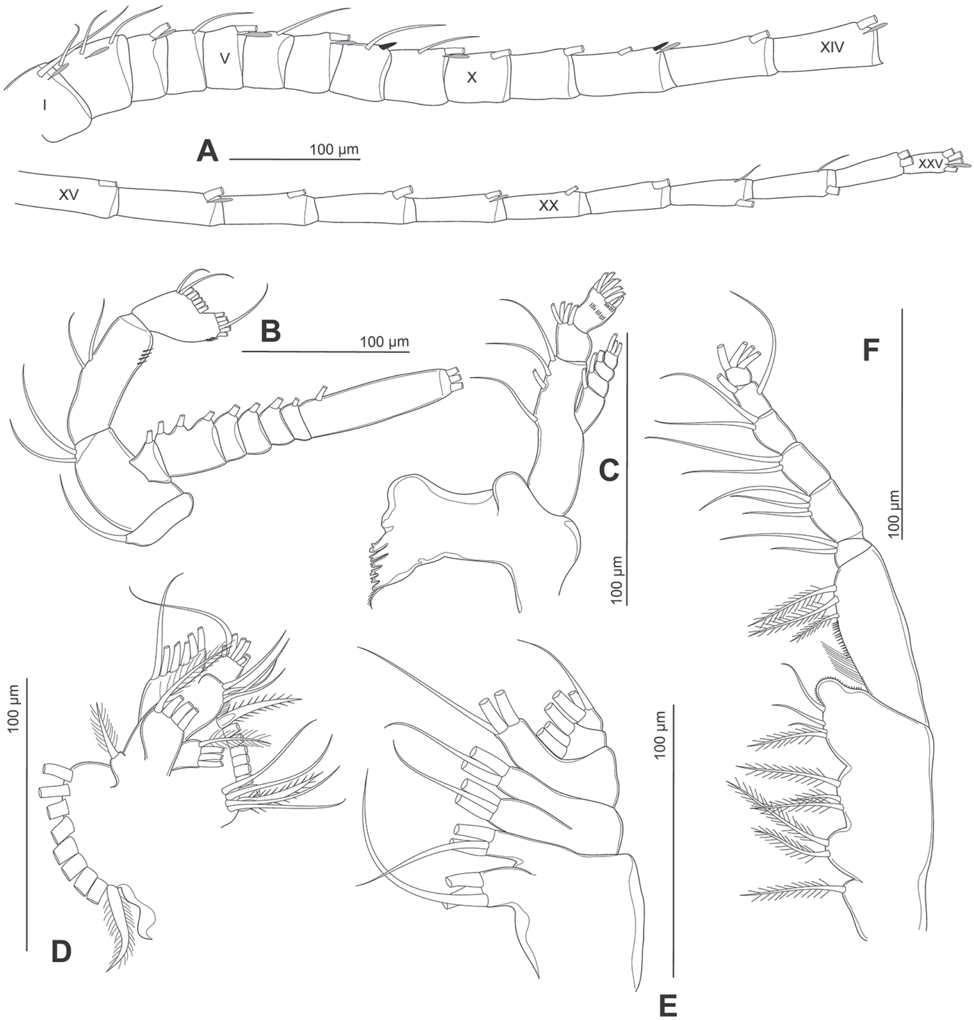

Figure 4. *Phyllodiaptomus* (*P.*) *roietensis* sp. nov., female: **A** antennule **B** antenna **C** mandible **D** maxillule **E** maxilla **F** maxilliped.

view (Fig. 5F, G). Exp-3 represented by small distal prominence produced into short distolateral spine and longer medial spine. Enp subconical, Enp-1 slightly rectangular. Enp-2 tipped with circular row of spinules.

Description of adult male. Body length (Figs 6, 7A) without caudal setae, 0.8–1.1 mm (mean = 1.0 mm, n = 5), smaller than female. Prosome length: urosome plus caudal rami ratio about 2.1:1, ratio of width to length of prosome = 1:2.1, urosomites 1–5 = 2.3:1.0:1.0:1.3:1.0, caudal ramus = 1:1.9. Prosome similar to that of female except lateral wings on Pdg 5. Lateral wings (Figs 6A, 7B–D) asymmetrical, round on right and more triangular on left side; posterior spine on right wing larger compared to left side. Urosome (Figs 6A, B, 7A) 5-segmented, asymmetrical, curved downward to right side.

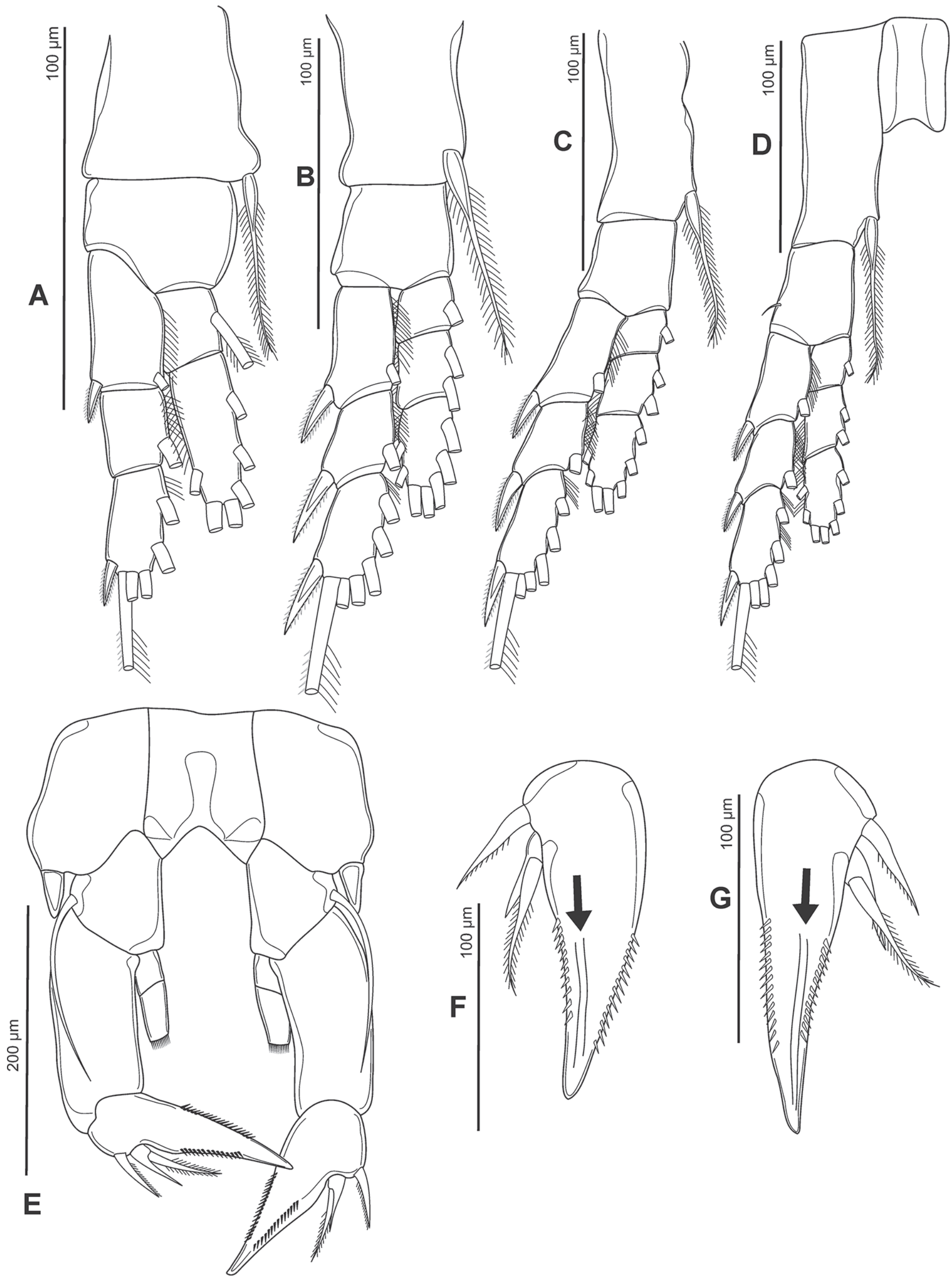

Figure 5. *Phyllodiaptomus* (*P.*) *roietensis* sp. nov., female: **A** P1 **B** P2 **C** P3 **D** P4 **E** P5 **F, G** right and left P5 Exp-2 (black arrows indicate longtitudinal ridges) **A–E** posterior view **F, G** anterior view.

Genital somite (Figs 6, 7B) dilated postero-laterally on right side, with spine at distal outer corner; longer than that on Pdg 5 wings. Genital aperture located on mid-ventral region. Urosomites 2–3 (Figs 6B, 7D) without ornamentation. Urosomite 4 (Fig. 6A, B) with

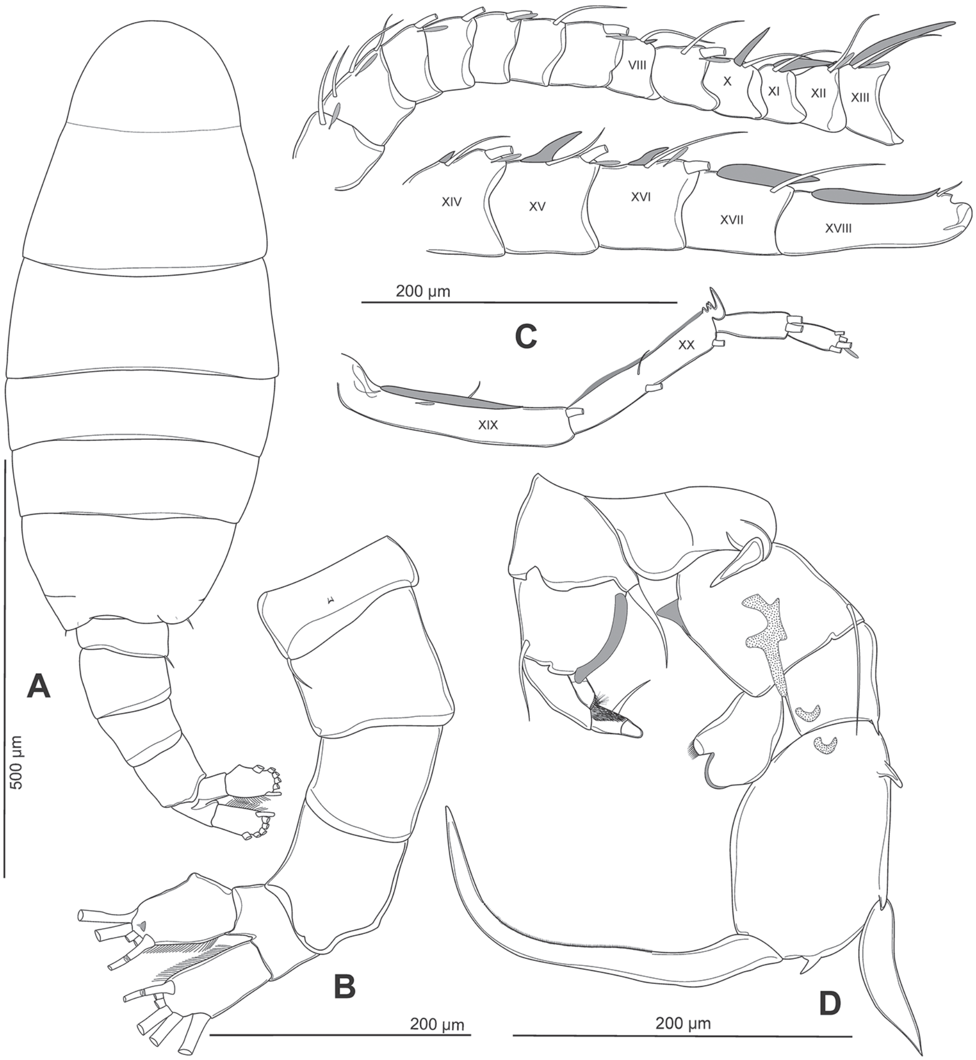

Figure 6. *Phyllodiaptomus* (*P.*) *roietensis* sp. nov., male: **A** habitus, dorsal view **B** urosome, ventral view **C** right antennule, with grey objects indicating antennular spines **D** P5, with grey and dotted objects indicating hyaline lamella and chitinous prominences respectively, posterior view.

irregularly dilated posterior margin. Anal somite (Fig. 6A, B) asymmetrical and twisted to right side. Caudal rami (Figs 6A, B, 7E, F) asymmetrical, right ramus with two triangular prominences: one proximolateral and one distoventral; setation similar to female.

Antennule (Figs 6C, 7G, H) asymmetrical, with geniculated right side. Right antennule 22-segmented, with setal formula as 1+ae (I), 3+ae (II), 1+ae (III), 1 (IV), 1+ae (V), 1 (VI), 1+ae (VII), 1+sp (VIII), 2+ae (IX), 1+sp (X), 1+sp (XI), 1+ae+sp (XII), 1+ae+sp (XIII), 2+ae+sp (XIV), 2+ae+sp (XV), 2+ae+sp (XVI), 1+sp (XVII), 1+sp (XVIII), 2+ae+sp (XIX), 3+sp (XX), 2 (XXI), 4+ae (XXII); geniculated between segments 18 and 19; segment 20 (antepenultimate) with serrated process distally (3–4 teeth), and with longitudinal hyaline membrane along outer margin.

Left antennule, antenna, mouthparts, and P1–P4 as in female.

P5 (Figs 6D, 8A, G) intercoxal sclerite with rounded lobe on free margin. Right P5: coxa with acute, stout spine on posterior lobe. Basis (Fig. 8B, G) with large proximo-medial triangular lamella at one-fourth length of inner margin; with large three-lobed chitinous medial prominence on posterior surface; distal outer margin with long, thin seta, slightly extending beyond Exp-1. Enp (Fig. 8B, H, G) with bi-lobed distal margin, tipped with spinules and hyaline lamella on inner and outer lobes, respectively; reaching downward to approximately one-third of Exp-2. Exp-1 (Fig. 8A, B, H) with semi-circular knob on distomedial margin; distolateral margin with small acute process. Exp-2 (Fig. 8C, H) elliptic, with three accessory lateral spines, one proximal, middle, and distal on lateral margin. Principal lateral spine articulated, located at two-third length of Exp-2, flat, thick, digitiform, with sharp tip; long, with approximately half of segment bearing it; slightly twisted in posterolateral direction. End claw (Figs 6D, 8A) medially sickle-shaped, slender towards tip, more than 1.5 times as long as Exp-2; medial margin serrated with row of tiny spinules.

Left P5 (Figs 6D, 8D): coxa with moderate strong seta inserted on posterior lobe at distal inner corner, slightly shorter than distal margin of basis. Basis with flap of longitudinal hyaline lamella at medial margin; with long, thin seta at posterolateral margin, extending to approximately half of Exp-1. Exp-1 (Fig. 8F) tapering towards posterior margin, medial margin concave, with field of setules and tiny spinules. Exp-2 smaller than Exp-1, conical; with large seta at mid-length of medial margin, as long as Exp-2 and apical process combined; with few setules proximally and widespread with spinules distally along inner margin, thickness of spinules increased from proximal to distal; apical process stout, bare, and blunt-tip. Enp (Fig. 8F, J) bi-segmented, longer than Exp-1, Enp-2 tipped with row of spinules distally.

Differential diagnosis. *Phyllodiaptomus* (*P.*) *roietensis* sp. nov. with the male P5 Exp-2 displays an affinity to the subgenus *Phyllodiaptomus* sensu Dumont et al. (1996): the lateral side of the right Exp-2, medially concave in posterior view, principal lateral spine inserted on distal to mid-outer margin and three accessary spines arranged from proximal, middle, and distal, respectively; the left Exp-2 with patch of strong spinules along medial margin.

The male of the new species has serrated outgrowth on the antepenultimate segment of the right antennule. The right caudal ramus with small chitinous spine near distal margin on ventral side and triangular prominence along proximal one-third length of outer margin. The P5 intercoxal sclerite produced, with convex distal margin. The right P5 with (1) short, strong spine on posterior lobe of coxa, (2) triangular hyaline lamella on proximal inner margin and large chitinous outgrowth on posterior surface of basis, (3) acute distal outer corner of Exp-1 (4) Exp-2 oval and concave, with strong, flat, curved principal spine and three accessary spines, and (5) bi-lobed Enp. The left P5 with long and narrow hyaline lamella along inner margin, Exp-2 with patch of strong spinules along medial margin, and bi-segmented Enp.

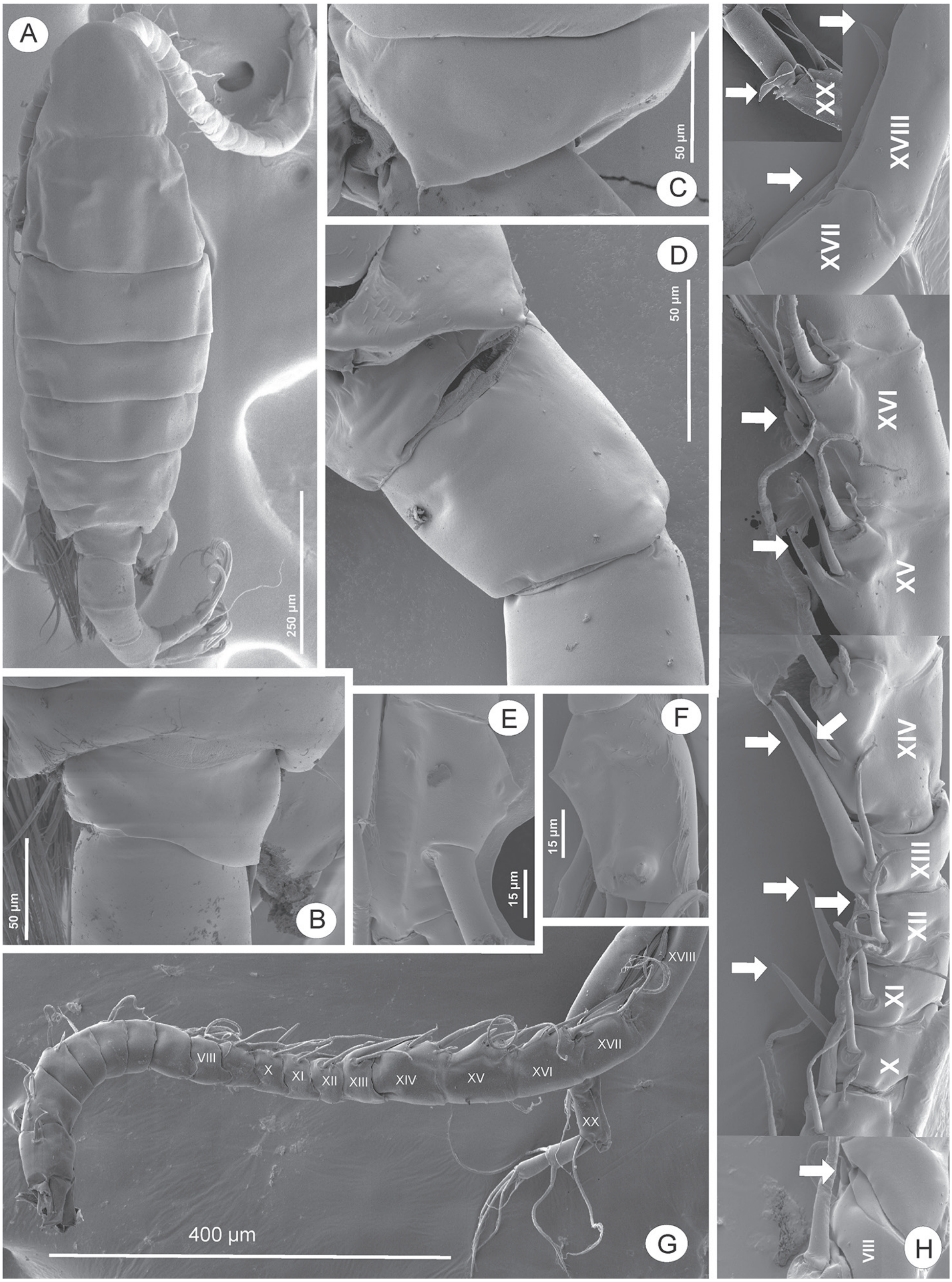

Figure 7. *Phyllodiaptomus* (*P.*) *roietensis* sp. nov., male: **A** habitus, dorsal view **B** Pdg 5 and genital somite, dorsal view **C** Pdg 5 lateral wing, left view **D** Pdg 5, genital somite and urosomites 2 and 3, ventrolateral view **E, F** right caudal ramus in dorsal (**E**) and ventral (**F**) views **G, H** right antennule (white arrows indicate spines on segments 8, 10–18, 20.

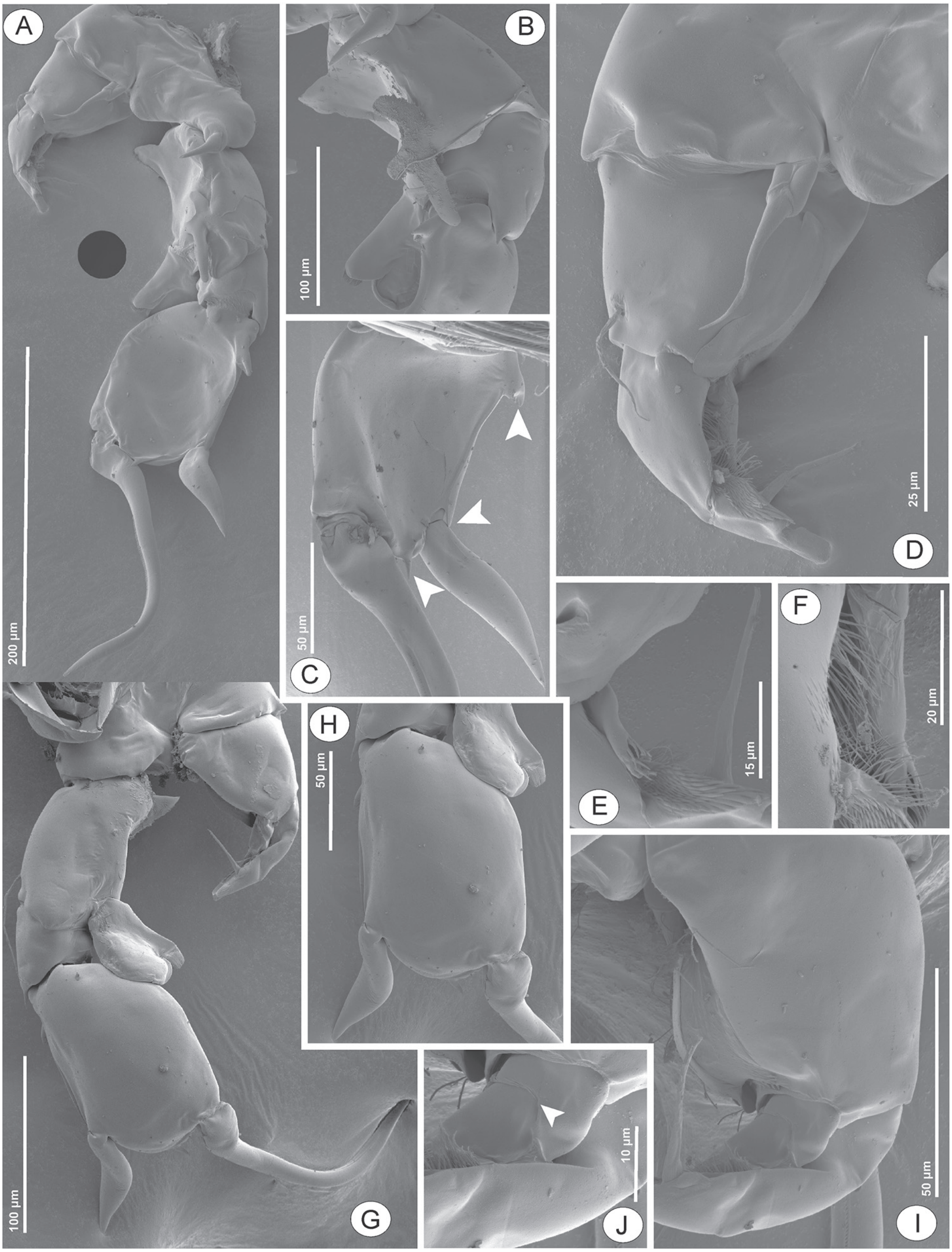

Figure 8. *Phyllodiaptomus* (*P.*) *roietensis* sp. nov., SEM photographs, male: **A** P5 in posterior view **B** right P5 basis, Exp-1 and Enp, posterior view **C** right P5 Exp-2 in posterior view (white arrows indicate accessory spines) **D** left lobe of P5, posterior view **E** left P5 Exp-2 and Enp, posterior view **F** left P5 Enp, posterior view **G** P5, anterior view **H** right P5 Exp-1–2 and Enp, anterior view **I** left P5 basis, Exp and Enp, anterior view **J** left P5 Enp (white arrow indicates Enp segmented point), anterior view.

Female with asymmetrical Pdg 5 wings, left wing more elongated in posterio-lateral direction; posterior and dorsal spines short and strong. Genital double-somite with posterolateral directed process on right side. One pair of genital spines on lateral side slightly symmetrical and strong. P5 Exp-2 with conveyor canal on anterior surface. P5 with bi-segmented Enp.

Etymology. The specific name *roietensis* is taken after the type locality, Roi Et Province. The name with the Latin suffix "*-ensis*" is the adjective for a location.

Distribution. Known only from four temporary water bodies from Roi Et and Nakhon Ratchasima provinces, Thailand and Kampong Thom Province, Cambodia (Fig. 1). Presence of specimens was recorded in early monsoon period. The new species is rare, as it was found in 0.4% of all the localities sampled in Cambodia. The new species was found together with six diaptomids including *Dentodiaptomus javanus* (Grochmalicki, 1915), *Eodiaptomus sanoamuangae* Ranga Reddy & Dumont, 1998, *Mongolodiaptomus calcarus* (Shen & Tai, 1965), *M. malaindosinensis* (Lai & Fernando, 1978), *Neodiaptomus laii* Kiefer, 1974, and *Phyllodiaptomus* (*Phyllodiaptomus*) *christineae* Dumont, Ranga Reddy & Sanoamuang, 1996.

Discussion

To date, the genus *Phyllodiaptomus* has been recorded in Asia, including south China, Turkey, Israel, Uzbekistan, Iran, Iraq, India, Sri Lanka, Nepal, Indonesia, Thailand, Laos, Philippines and Cambodia (Dumont and Ranga Reddy 1993; Ranga Reddy 1994; Dumont et al. 1996; Ranga Reddy et al. 1998; Sanoamuang 1999; Sanoamuang and Yindee 2001; Sanoamuang and Teeramaethee 2006; Khalaf 2008; Alekseev et al. 2013, 2016; Marrone et al. 2014; Bekleyen et al. 2017; Guinto et al. 2018; Sanoamuang and Watiroyram 2018). Most species are considered endemic to specific countries. Three species (*P.* (*C.*) *annae*, *P.* (*C.*) *wellekensae*, and *P.* (*C.*) *sasikumari*) are endemic to India; two species (*P.* (*P.*) *thailandicus* and *P.* (*P.*) *surinensis*) are endemic to Thailand; *P.* (*P.*) *tunguidus*, *P.* (*P.*) *irakiensis*, and *P.* (*P.*) *longipes* are endemic to China, Iraq, and Indonesia, respectively. Only *P.* (*P.*) *blanci* is widely distributed, extending across many countries. Five species have been recorded in Thailand, namely *P.* (*C.*) *praedictus*, *P.* (*P.*) *christineae*, *P.* (*P.*) *thailandicus*, *P.* (*P.*) *surinensis*, and *P.* (*P.*) *roietensis* sp. nov. (Sanoamuang 2002; this study). Among the Thai sister species, *P.* (*P.*) *surinensis* and *P.* (*P.*) *roietensis* sp. nov. are rare. In 3,000 samples collected within Thailand, each has been recorded in only two localities in the northeast. This is in contrast to another endemic Thai species, *P.* (*P.*) *thailandicus*, which is widely distributed in both temporary and permanent water bodies in the east and south of Thailand (Sanoamuang 2002).

The right antennule is mainly used as a clasping organ in all males of the family Diaptomidae, and it normally bears spines or spinous processes on segments 8, 10–16, and 20 (Kulkarni et al. 2018). However, *P.* (*P.*) *roietensis* sp. nov., *Mongolodiaptomus loeiensis* Watiroyram & Sanoamuang, 2017, and *Mongolodiaptomus mekongensis* Sanoamuang & Watiroyram, 2018 differ from *P.* (*P.*) *surinensis* and other diaptomids by having additional spines on segments 17–19 (see Figs 5C, 6H; Watiroyram and Sanoamuang 2017: fig. 4F; Sanoamuang and Watiroyram 2018: fig. 6D). The males of these species may manage to mate more easily with females using the unique ornamentation of antennule and caudal ramus. In females, Pdg 5 wings and genital double-somites are probably important for species recognition and mating behavior of their males (Ohtsuka and Huys 2001; Ali et al. 2014). Although the male morphological features of the two parapatric *Phyllodiaptomus* are different, they are able to differentiate their conspecific females during mating, as the females of the new species can be distinguished from its congeners by the presence of posterolateral process on both sides of genital double-somite, which are absent in other congeners except *P.* (*P.*) *thailandicus*. However, the characteristic that differentiates the new diaptomid from *P.* (*P.*) *thailandicus* is the presence of a single process on each side of the genital double-somite; *P.* (*P.*) *thailandicus* has two processes only on the right side (Figs 2C–E, 3C–F; Sanoamuang and Teeramathee 2006: figs 1, 24). In contrast to their males, the new species and *P.* (*P.*) *surinensis* have unique females which can be easily differentiated. The female P5 Exp-2 of the new species is obviously asymmetrical compared with that of *P.* (*P.*) *surinensis* which has a slightly asymmetrical P5 Exp-2. Dumont and Ranga Reddy (1993) observed that the conveyor canal on the P5 Exp-2 in females is species-specific and unique to the genus *Phyllodiaptomus*: the new species has two longitudinal ridges on the anterior surface versus multi-longitudinal ridges in *P.* (*P.*) *surinensis* (Fig. 5F, G; Sanoamuang and Yindee 2001: fig. 39). The clasping site on the genital double-somite of the new species is wider than those in *P.* (*P.*) *surinensis.* The new species has substantial left genital double-somite proximal bulging versus only slight asymmetry in *P.* (*P.*) *surinensis.* The genital double-somite of *P.* (*P.*) *surinensis* has a bi-lobed hyaline outgrowth ventrally, which is absent in the new species. The genital spines in the female of the new species are oriented to a posterolateral direction in dorsal view, whereas they are pointed to the lateral direction in *P.* (*P.*) *surinensis*. The new species has tiny spinules on Pdg 4–5 laterally; these are present dorsally in *P.* (*P.*) *surinensis* (Fig. 3B; Sanoamuang and Yindee 2001: fig. 2).

The male of *P.* (*P.*) *roietensis* sp. nov. has a number of morphological differences from other members of the *blanci*-species group as follows:

a) Antepenultimate segment with a serrated process versus smooth in *P.* (*P.*) *longipes*.
b) Urosomite(s) with a long hair or hair-like setae versus bare in *P.* (*P.*) *thailandicus*, *P.* (*P.*) *christineae*, and *P.* (*P.*) *blanci*.
c) Right caudal ramus with ventral prominences as in *P.* (*P.*) *surinensis* and *P.* (*P.*) *tunguidus*. However, a ventral prominence is also present on the left ramus of *P.* (*P.*) *tunguidus* (but is absent in the left ramus of the new species) and there are only two prominences in the new species but five in *P.* (*P.*) *surinensis*.
d) Intercoxal sclerite is modified distally into single lobe versus two lobes in *P.* (*P.*) *irakiensis* and *P.* (*P.*) *thailandicus*. The new species has a round or semi-circular distal margin versus triangular in *P.* (*P.*) *blanci*, *P.* (*P.*) *christineae*, *P.* (*P.*) *longipes*, and *P.* (*P.*) *tunguidus*.

e) Right P5 coxal spine is strong and acute versus rectangular in *P.* (*P.*) *thailandicus* and slender in *P.* (*P.*) *christineae*.
f) Right P5 basis with a three-lobed chitinous prominence on posterior surface versus bare in *P.* (*P.*) *irakiensis* and *P.* (*P.*) *blanci*. In addition, three species, *P.* (*P.*) *longipes*, *P.* (*P.*) *christineae*, and *P.* (*P.*) *tunguidus*, have a longitudinal ridge on the posterior surface, which is absent in the new species (the first one has two minute prominences on the ridge). The right P5 basis has a triangular hyaline lamella at inner margin versus elongated in *P.* (*P.*) *christineae*, *P.* (*P.*) *longipes*, and *P.* (*P.*) *tunguidus*, and round in *P.* (*P.*) *blanci*. The left P5 basis has inner lamella versus bare in *P.* (*P.*) *irakiensis* and *P.* (*P.*) *longipes*, digitiform in *P.* (*P.*) *tunguidus*, and small in *P.* (*P.*) *blanci*. The new species lacks any ornamentation on the anterior surface but *P.* (*P.*) *surinensis* has two minute lateral spines (see Sanoamuang and Yindee 2001: fig. 54).
g) Right P5 Exp-2 with three accessary lateral spines versus bare in *P.* (*P.*) *tunguidus* and *P.* (*P.*) *blanci*, and one in *P.* (*P.*) *irakiensis*, *P.* (*P.*) *christineae*, and *P.* (*P.*) *longipes*.
h) Right P5 Enp with a bi-lobed shape versus conical in the rest of the species except *P.* (*P.*) *surinensis*.
i) Left P5 with bi-segmented Enp versus one-segmented in *P.* (*P.*) *thailandicus*, *P.* (*P.*) *surinensis*, *P.* (*P.*) *blanci*, *P.* (*P.*) *christineae*, and *P.* (*P.*) *longipes*.

With regard to the comparative morphology above, the male of the new species is most similar to those of *P.* (*P.*) *surinensis*. However, there are three major differences among the males, i.e. the right caudal ramus, left P5 basis, and left P5 Enp as described above. The fine detail on its inner hyaline lamella on the right P5 basis is also different: triangular in the new species versus oval bi-lobed in *P.* (*P.*) *surinensis*.

Ranga Reddy (1994) provided the first key to species and included six species of *Phyllodiaptomus* (*P.* (*P.*) *tunguidus*, *P.* (*P.*) *blanci*, *P.* (*P.*) *longipes*, *P.* (*C.*) *annae*, *P.* (*C.*) *wellekensae*, and *P.* (*C.*) *sasikumari*); he also gave morphological descriptions of these six species. In this study, the key is updated as follows:

Keys to worldwide species of *Phyllodiaptomus* Kiefer, 1936

Males:

1	Left P5 Exp-2 with a serrate hyaline fan on inner margin	**2 (subgenus *Ctenodiaptomus*)**
–	Left P5 Exp-2 with a field of spinules on inner margin	**5 (subgenus *Phyllodiaptomus*)**
2	Inner margin of P5 intercoxal sclerite with conical lobe, blunt tip	***P.* (*C.*) *sasikumari***
–	Inner margin of P5 intercoxal sclerite with triangular lobe, acute tip...........	**3**
3	Right P5 Exp-1 without acute process on distal outer corner	***P.* (*C.*) *wellekensae***
–	Right P5 Exp-1 with acute process on distal outer corner	**4**

4 Right P5 Exp-2 without hyaline lobe on distal outer corner.. ***P.* (*C.*) *praedictus***
– Right P5 Exp-2 with hyaline lobe on distal outer corner.......... ***P.* (*C.*) *annae***
5 Antepenultimate segment with smooth process...................... ***P.* (*P.*) *longipes***
– Antepenultimate segment with serrated process **6**
6 Urosomite 2–3 or only 2 with hair or hair-like setae **7**
– Urosomite 2–3 without hair or hair-like setae **9**
7 Inner margin of P5 intercoxal sclerite with two lobes..... ***P.* (*P.*) *thailandicus***
– Inner margin of P5 intercoxal sclerite with triangular lobe.......................... **8**
8 Right P5 Exp-2 with slender principal spine..................... ***P.* (*P.*) *christineae***
– Right P5 Exp-2 with thick principal spine .... ***P.* (*P.*) *blanci***
9 Inner margin of P5 intercoxal sclerite with two lobes.......... ***P.* (*P.*) *irakiensis***
– Inner margin of P5 intercoxal sclerite with single lobe **10**
10 Right P5 Exp-1 without acute process on distal outer corner .... ***P.* (*P.*) *tunguidus***
– Right P5 Exp-1 with acute process on distal outer corner **11**
11 Right P5 basis with one-lobed hyaline lamella on inner margin .... ***P.* (*P.*) *roietensis* sp. nov.**
– Right P5 basis with two-lobed hyaline lamella on inner margin .... ***P.* (*P.*) *surinensis***

Females:

1 Genital double-somite with postero-laterally oriented outgrowth................ **2**
– Genital double-somite without postero-laterally oriented outgrowth **3**
2 Genital double-somite with two postero-laterally oriented outgrowths on right side.. ***P.* (*P.*) *thailandicus***
– Genital double-somite with single postero-laterally oriented outgrowth on right side.. ***P.* (*P.*) *roietensis* sp. nov.**
3 P5 Enp one-segmented .... ***P.* (*P.*) *longipes***
– P5 Enp two-segmented **4**
4 Pdg 5 left wing bi-lobed... **5**
– Pdg 5 left wing round or triangular.. **6**
5 Pdg 5 wings symmetrical .... ***P.* (*P.*) *tunguidus***
– Pdg 5 wings asymmetrical.. ***P.* (*P.*) *irakiensis***
6 Pdg 5 right wing round or triangular **7**
– Pdg 5 right wing bi-lobed **8**
7 Genital double-somite with ventral hyaline outgrowth........ ***P.* (*P.*) *surinensis***
– Genital double-somite without ventral hyaline outgrowth ***P.* (*P.*) *christineae***
8 Genital double-somite dilated at the proximal left side **9**
– Genital double-somite non-dilated at the proximal left side...................... **10**
9 Genital double-somite dilated at the middle of right side.... ***P.* (*C.*) *praedictus***
– Genital double-somite non-dilated at the middle of right side .... ***P.* (*C.*) *wellekensae***

10	Genital double-somite dilated at the middle of right side	***P.* (*P.*) *blanci***
–	Genital double-somite non-dilated at the middle of right side	**11**
11	P5 basis with short lateral seta, not reaching over Exp-1	***P.* (*C.*) *annae***
–	P5 basis with long lateral seta, reaching over Exp-1	***P.* (*C.*) *sasikumari***

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RESEARCH ARTICLE

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New distribution records of subterranean crustaceans from cenotes in Yucatan (Mexico)

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Abstract

New records of 14 stygobiont crustacean species pertaining to six Malacostraca orders from 32 cenotes are presented, with their associated caves of the state of Yucatan, Mexico, together with an individual account for each species. Species composition of most of the investigated cenotes is examined for the first time. A thermosbaenacean and two amphipod species were not formally recorded to the cenote ecosystems of the state of Yucatan prior to our research. Distribution data of a cirolanid isopod previously known only from its type locality is also provided. Barcodes of mitochondrial cytochrome c oxidase subunit I for the reported peracarid species previously lacking this information have been included in present study as tools for species identification and a baseline of further molecular genetic analyses.

Keywords

anchialine ecosystems, barcode sequences, biodiversity, endemic, Eucarida, Peracarida, stygobiont, Yucatan Peninsula

Introduction

'Cenotes' (the local name for water-filled sinkholes) are typical karst features of the Yucatan Peninsula in Mexico. In many cases, far-reaching networks of submerged subterranean cave passages extend from them (Mercado-Salas et al. 2013). Due to the mixing of fresh and saline water, a distinct stratification can be observed inside these anchialine systems (Bishop et al. 2015). Intrusion of saline water is found deeper as the distance from the coastline increases (Bauer-Gottwein et al. 2011). Therefore, most inland cenotes within the state of Yucatan are exclusively freshwater systems, except for a few rather deep ones with haloclines below 50 m in depth, and those located near the northern coastline of the Peninsula (Álvarez et al. 2005; Angyal et al. 2018).

Anchialine ecosystems in Yucatan contain a crustacean-dominated fauna that is adapted to hypogene conditions, such as the lack of sunlight and the low food resource availability (Mejía-Ortíz et al. 2013). Stygobiont species are restricted to aquatic subterranean habitats (Botosaneanu 1986), and often exhibit conspicuous morphological adaptations to hypogene life, known as troglomorphisms. Such adaptations include structural reductions (e.g., loss of visual organs and pigmentation) or extensions (e.g., lengthening of appendages and complexity of sense organs) (Mejía-Ortíz et al. 2006; González et al. 2018) and physiological modifications (e.g., reduced metabolic rates and starvation resistance) (Hervant et al. 1999, 2001; Bishop and Iliffe 2009). In 2016, prior to our systematic sampling, 47 stygobiotic crustacean species had been reported from anchialine ecosystems of the Mexican federal states of the Yucatan Peninsula, of which 22 were known from cenotes and submerged caves of the state of Yucatan (e.g., Holsinger 1977; Kallmeyer and Carpenter 1996; Álvarez et al. 2005; Suárez-Morales et al. 2006). Fourteen percent of these species belong to the subclass Copepoda (9 spp.), while the remainder belong to the orders Mysida (1 sp.), Stygiomysida (2 spp.), Amphipoda (1 sp.), Isopoda (5 spp.), and Decapoda (4 spp.).

According to the database of the Secretaría de Desarrollo Sustentable (SDS Yucatan), there are more than 3,000 registered cenotes and caves within this state. Current efforts are being directed to complete the descriptions of all registered cenotes, despite that only a small fraction of them have been biologically investigated to date. Ongoing research and explorations are necessary to describe the true biodiversity of these subterranean habitats, their geographical patterns, and changes in time. Thus, our aim was to improve our knowledge on the distribution and ecology of the stygobiotic crustacean fauna of the cenotes and their associated cave passages in the state of Yucatan. We aimed to provide data from cenotes that had never been investigated from a zoological point of view in order to extend the geographical range of crustacean species distribution and contribute to a precise biodiversity mapping of stygofauna in Yucatan. Additionally, we intended to collect samples for molecular and morphological studies so as to gain and make available to the public mitochondrial cytochrome c oxidase subunit I sequences (COI) of species that were lacking barcode information, setting the standard for studies and tools for species identification.

Materials and methods

Sampling sites and sampling

We collected stygobiotic macro-crustaceans from 32 cenotes between May 2016 and January 2018 in cenotes of the state of Yucatan (shorter form: Yucatan) (Figure 1, Table 1). Most of the cenotes studied are several kilometers away from the coast and contain only freshwater. In contrast, some cenotes near the coast have a halocline that divides the cave into freshwater and saline water habitats. Some of the cenotes studied belong to the 'Ring of Cenotes', a fracture zone with high density of sinkholes identified as the outer rim of the crater where the famous asteroid impacted Chicxulub 66 million years ago (González-Herrera et al. 2002; Bauer-Gottwein et al. 2011) (Figure 1). Macro-crustaceans were collected during scientific cave dives using 50 ml sample

Table 1. Location data and identification codes of the studied cenotes.

Cenote nr. (see Figure 1 map)	Cenote name	CenoteAndo cenote code	Municipality	Settlement	Coordinates latitude	Coordinates longitude
1	Ayun-Nah	01980007Y_	Cacalchen	Cacalchen	20°58'49.6"N	89°14'39.4"W
2	Bebelchen	00028064YC	Uman	Sanahcat	20°44'11.4"N	89°43'55.4"W
3	Cervera	00090028YC	Dzilam de Bravo	Yalsihom	21°22'29.5"N	88°50'01.8"W
4	Chihuo Hol	00080001YC	Abala	Mucuyche	20°38'06.1"N	89°36'42.3"W
5	Dzalbay	00585085YC	Temozon	Dzalbay	20°49'53.4"N	88°03'23.0"W
6	Dzonbakal	00125101YC	Uman	San Antonio Mulix	20°40'11.4"N	89°46'43.9"W
7	Dzonotila	00168001YC	Abala	Mucuyche	20°37'44.0"N	89°39'33.0"W
8	Flor de Liz	-	Tixkokob	Tixkokob	21°00'16.0"N	89°23'33.0"W
9	Ixim Ha	00164037YC	Tixkakal	Tixkakal	20°37'49.0"N	89°06'40.0"W
10	Kakuel	00142001YC	Abala	Mucuyche	20°37'40.3"N	89°34'26.8"W
11	Kampepen	00042076YC	Tecoh	Chinquila	20°42'00.8"N	89°22'41.6"W
12	Kankirixche	00002001YC	Abala	Mucuyche	20°38'13.8"N	89°37'58.8"W
13	Kankal	-	Homun	Homun	20°39'38.3"N	89°16'42.5"W
14	Kanun	01730036Y_	Homun	Homun	20°44'44.2"N	89°14'40.7"W
15	Nayah	00009076YC	Tecoh	Pixyah	20°38'47.5"N	89°24'16.9"W
16	Noh'Chunck	00229011YC	Chunchumil	Celestun	20°48'48.5"N	90°11'47.8"W
17	Nohmozon	00010076YC	Tecoh	Pixyah	20°62'32.5"N	89°38'42.0"W
18	Pixton	00064064YC	Huhi	Huhi	20°43'13.3"N	89°10'08.5"W
19	Pol Box	00321023YC	Chochola	Chochola	20°41'24.3"N	89°48'54.5"W
20	Sabtun 1	00230011YC	Chunchumil	Celestun	20°51'00.7"N	90°14'08.1"N
21	San Elias	01171036Y_	Homun	Homun	20°41'21.0"N	89°14'19.0"W
22	San Juan	00063036YC	Homun	Homun	20°44'02.6"N	89°17'18.6"W
23	Santito	00108045YC	Kopoma	Kopoma	20°38'58.1"N	89°53'44.3"W
24	El Virgen	-	Sotuta	Sotuta	20°32'01.9"N	89°02'19.4"W
25	Tres Oches	-	Homun	Homun	20°43'55.7"N	89°16'20.0"W
26	Tza Itza	00050076YC	Tecoh	Tecoh	20°43'49.1"N	89°27'57.9"W
27	Xaan	00423036YC	Homun	Homun	20°43'39.3"N	89°15'24.6"W
28	X'baba	00162023YC	Chochola	Chochola	20°40'42.5"N	89°49'00.7"W
29	X-Batun	00005023YC	Uman	San Antonio Mulix	20°40'23.8"N	89°46'22.8"W
30	X'kokob	00650093YC	Ekmul	Ekmul	20°56'51.0"N	89°20'41.0"W
31	Yaal Utsil	00003001YC	Abala	Mucuyche	20°37'26.0"N	89°36'24.0"W
32	Yax-Kis	00091001YC	Abala	Mucuyche	20°37'33.7"N	89°35'35.7"W

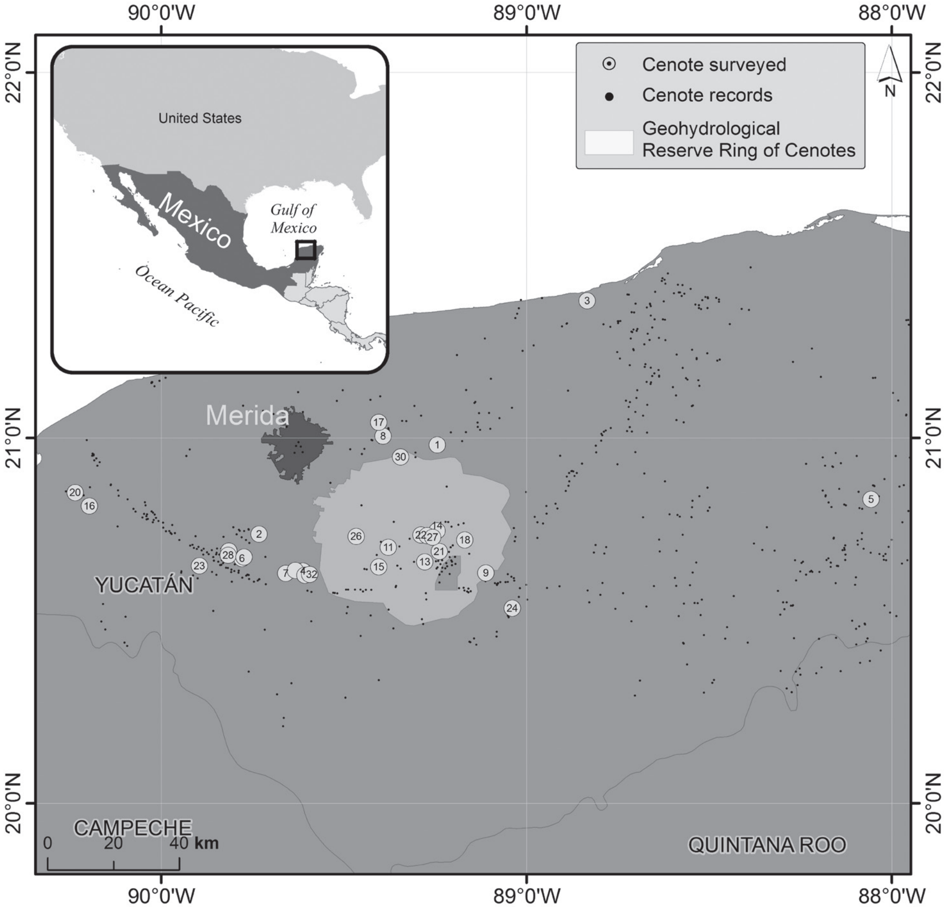

Figure 1. Map of the state of Yucatan and location of the 32 investigated cenotes. Details of the numbered cenotes can be found in Table 1. The light area represents the Geohydrological Reserve in Yucatan, while the dark area depicts the urban extension of the city of Merida.

tubes and 10 cm diameter hand nets. Habitat data (e.g., depth, temperature, collected in cavern or cave, position relative to halocline) at the collection site of each individual was recorded along with photographs and video-recordings of the observed crustaceans and their habitats. All crustaceans were individually placed into 70 or 96% ethanol containing tubes immediately after collection. All specimens were collected under the permits of the Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT/SPGA/DGVS/05263/14; SEMARNAT/SPGA/DGVS/02068/17). The collected material was deposited in the Yucatán Collección de Crustáceos, Unidad Multidisciplinaria de Docencia e Investigación, Universidad Nacional Autónoma de México in Sisal (**UNAM UMDI-Sisal**), the Collección Nacional de Crustáceos, Instituto de Biología, **UNAM** in Mexico City, or in the Collection of Crustaceans of the Hungarian Natural History Museum (**HNHM**), Budapest.

Morphological analysis

Individuals were examined using a stereo-microscope. Specimens of thermosbaenaceans, stygiomysids, mysids, and amphipods were studied as follows: cleared and stained exoskeletons were dissected under a Leica M125 stereo microscope. The dissections were then mounted on slides and examined using a Leica DM 1000 compound light microscope (Fišer et al. 2009; Angyal et al. 2015). For the identification of the collected material the following publications were used: Álvarez et al. 2005; Álvarez and Iliffe 2008; Angyal et al. 2018; Botosaneanu and Iliffe 1999, 2000, 2002, 2006; Bowman 1966, 1977; Bruce 1986; Creaser 1936; Hobbs and Hobbs 1976; Hobbs et al. 1977; Hobbs 1979; Holsinger 1977, 1990; Horwitz et al. 1995; Kallmeyer and Carpenter 1996; Lowry and Myers 2013; Meland et al. 2015; Pérez-Aranda 1983a, 1983b, 1984a, 1984b; Tinnizi and Quddusi 1993; Wagner 1994. Photographs were made using an OMAX 14 OMP digital USB microscope camera, a Nikon D5300, and a Nikon D7000 with 60 mm macro lens.

Molecular studies (COI barcode sequences)

DNA extraction of the peracarids studied was performed using QIAamp DNA Microkit (QIAGEN), following the manufacturer's instructions. A few pereopods of each animal provided the necessary material to extract DNA. For PCR amplification of mitochondrial COI, we used the primer pair LCO 1490 and HCO 2198 (Folmer et al. 1994). PCR reactions (25 µl) contained 13.85 µl mQ water, 2.5 µl 10× PCR buffer, 2.5 µl dNTP mix (2mM), 1.5 µl of each primers (5µM), 0.15 µl Fermentas Dream Taq (5U/µl), and 3 µl DNA extract. PCR temperature conditions were set as follows: initial denaturation for 3 min at 94 °C, denaturation for 45 sec at 94 °C, hybridization for 45 sec at 48 °C, and polymerization for 1 min at 72 °C. After thirty cycles, a final extension for 3 min at 72 °C was performed. PCR products were purified using Exo SAP-IT Express PCR Product Cleanup (Affymetrix) according to the manufacturer's instructions. The fragments were sequenced in both directions using PCR amplification primers with an ABI 3130 sequencer. Contigs were assembled and sequences were edited using BioEdit 7.1.11 sequence alignment editor software (Hall 1999): chromatograms of complement reverse and forward strings were compared, gaps were eliminated, while indels and stop codons were checked. 605-651 bp COI barcode sequences have been uploaded to the NCBI GenBank database. Accession numbers and localities are listed in Table 2.

Results

A total of 14 stygobiont crustacean species, belonging to six Malacostraca orders, was collected (Figures 2, 3). New records of each species at each cenote were assessed after an exhaustive literature investigation (Table 3). This evaluation was based only on the

Table 2. Locality data and GenBank accession number of COI gene fragments of one individual of each newly collected stygobiotic peracarid species.

Taxon	Locality (cenote)	Voucher	GenBank accession nr.	Cited in
Tulumella unidens (Thermosbaenacea)	Sabtun 1	YUC-CC-255-11-004-656	MK900685	present study
Stygiomysis cokei (Stygiomysida)	Dzonotila	YUC-CC-255-11-004-638	MK900690	present study
Stygiomysis cf. *holthuisi* (Stygiomysida)	Kankal	YUC-CC-255-11-004-621	MK900689	present study
Antromysis cenotensis (Mysida)	Pol Box	YUC-CC-255-11-004-694	MK981568	present study
Mayaweckelia troglomorpha (Amphipoda)	Dzonbakal	CNR 34392	MF589977	Angyal et al. 2018
Mayaweckelia cenoticola (Amphipoda)	Ayun-Nah	YUC-CC-255-11-003923	MF589975	Angyal et al. 2018
Tuluweckelia cernua (Amphipoda)	Kankirixche	YUC-CC-255-11-003924	MF589983	Angyal et al. 2018
Creaseriella anops (Isopoda)	Tza Itza	HNHM-YUC_Isopoda-01	MK900687	present study
Yucatalana robustispina (Isopoda)	Kankirixche	YUC-CC-255-11-004-715	MK900686	present study
Cirolana yunca (Isopoda)	Tres Oches	HNHM-YUC-Isopoda-02	MK900688	present study

Table 3. Records of stygobiotic crustacean species collected between May 2016 and January 2018 in 32 cenotes of Yucatan. Bold-faced locality names represent new records for the cenote, while bold-faced locality names with an asterisk (*) represent new records for the state of Yucatan.

Taxon	Cenote
THERMOSBAENACEA	
Tulumella unidens Bowman & Iliffe, 1988	**Cervera***, **Sabtun 1***
STYGIOMYSIDA	
Stygiomysis cokei Kallmeyer & Carpenther, 1996	**Tres Oches**, **San Elias**, Dzonotila, **Yax-Kis**
Stygiomysis cf. *holthuisi* (Gordon, 1958)	**Tres Oches**, **Tza Itza, X-Batun**, **Kanun**, **Kankirixche**, **Kakuel**, **Santito**, **Pol Box**, **Kankal**, **Flor de Liz**, **Bebelchen**, **Chihuo Hol**, **Yax Kis**
MYSIDA	
Antromysis cenotensis Creaser, 1936	**Tza Itza**, **Dzonbakal**, **Nayah**, **Kampepen**, **Kanun**, **Xaan**, **Kakuel**, Kankirixche, **Santito**, **Pol Box**, **Kankal**, **Dzonotila**, **Ixim Ha**, **Noh'Chunck**, **X'kokob**, **Flor de Liz**, **Pixton**, **Bebelchen**, **El Virgen**, **Chihuo Hol**
AMPHIPODA	
Mayaweckelia cenoticola Holsinger, 1977	**Ayun-Nah**, **Dzonotila**, **Ixim Ha**, **Bebelchen**
Mayaweckelia troglomorpha Angyal, 2018	**Dzonbakal***, **Kanun***, **Xaan***, **Kankirixche*, Dzonotila***, **X'kokob***, **Chihuo Hol***, **Yax-Kis***
Tuluweckelia cernua Holsinger, 1990	**San Juan***, **Dzonbakal***, **Tres Oches***, **Xaan***, **Kakuel***, **Kankirixche***, **Santito***, **X'baba***, **Sabtun 1***, **Pixton***, **Yax-Kis***
ISOPODA	
Creaseriella anops (Creaser, 1936)	**San Juan,** Cervera, **Tza Itza**, **Tres Oches**, Kankirixche, **Chihuo Hol**
Yucatalana robustispina Botosaneanu & Iliffe, 1999	**Xaan**, Kakuel, Kankirixche, **Yaal Utsil**, **Tza Itza, Pol Box**, **Dzonotila**, **X'baba**, **El Virgen**, **Chihuo Hol**, **Yax Kis**
Cirolana yunca (Botosaneanu & Iliffe, 2000)	**Tres Oches**, **X'baba**, **Chihuo Hol**
DECAPODA	
Typhlatya dzilamensis Alvarez, Iliffe & Villalobos, 2005	Cervera, **Sabtun 1**
Typhlatya mitchelli Hobbs & Hobbs, 1976	San Juan, Tza Itza, **Dzonbakal**, **Kampepen**, **Ayun-Nah**, **Tres Oches**, Kakuel, Kankirixche, **Sabtun 1**, **Bebelchen**, **El Virgen**, **Chihuo Hol**
Typhlatya pearsei Creaser, 1936	**Tres Oches**, **Xaan**, Kankirixche, Nohmozon
Creaseria morleyi (Creaser, 1936)	Tza Itza, **Kampepen**, **Kakuel**, Kankirixche, **Santito**, **Kankal**, **Bebelchen**, **El Virgen**, **Dzalbay**

collected material that has been deposited in scientific collections. Additional data based on observations, however, are mentioned in the "Remarks" section in each case. An individual account for each species is subsequently discussed. 605-651 base-pair COI barcode sequences of the analyzed species (Table 2) were obtained and uploaded to NCBI GenBank (https://www.ncbi.nlm.nih.gov/genbank/).

Subphylum: Crustacea

Class: Malacostraca
Superorder: Peracarida
Order: Thermosbaenacea
Family: Tulumellidae

***Tulumella unidens* Bowman & Iliffe, 1988**
Figure 2A

Material examined. 4 individuals; **Cenote Cervera**, depth 25.6-26.2 m, cave, in hydrogen sulfide layer, around and below halocline, 26 °C, Yalsihom, Yucatan, Mexico; 8 May 2016; colls. D. Angyal & E. Chávez Solís. 4 individuals; **Cenote Sabtun 1**, depth 24.0-25.0 m, cavern, above and around halocline, 25 °C, Chunchumil, Yucatan, Mexico; 10 December 2017; colls. D. Angyal, E. Chávez Solís, S. Drs, Q. Hernández & S. Reyes.

Previous distribution. Iliffe 1992; Iliffe 1993; Bowman and Iliffe 1988; Rocha et al. 1998; Pohlman et al. 2000; Pesce and Iliffe 2002; Álvarez et al. 2015; Olesen et al 2015; Benítez et al. 2019.

Type locality is Cenote Naharon (Cristal) in Quintana Roo. This species had only been reported from Quintana Roo from cenotes Calavera (Temple of Doom), Mayan Blue, Actun Ha (Carwash), Muknal, Na'ach Wennen Ha, Bang, Odyssey, Tabano, and Quebrada.

Remarks. Our findings extend the distribution area of this thermosbaenacean, previously endemic to Quintana Roo, to the cenotes located in the coastal areas north of Dzilam de Bravo and the east of Celestun. It is most likely that this species has a coastal distribution along the anchialine systems of the Yucatan Peninsula. Previous records were reported from cenotes located 2-10 km from the coastline near Tulum, where they occurred mostly above and at the halocline (Álvarez & Iliffe 2008; Álvarez et al. 2015; Benítez et al. 2019). In Cenote Cervera, 3.6 km inland from the northern coast of the Yucatan Peninsula, we observed individuals both above and below the halocline, as well as in the hydrogen sulfide layer.

Order: Stygiomysida
Family: Stygiomysidae

***Stygiomysis cokei* Kallmeyer & Carpenther, 1996**
Figure 2B

Material examined. 1 individual; **Cenote Tres Oches**, depth 21.6 m, cave, freshwater, 27 °C, Homun, Yucatan, Mexico; 5 June 2016; colls. D. Angyal & E. Chávez Solís. 2 individuals, **Cenote San Elias**, depth 28.2 m and 32.0 m, cavern, freshwater, 26

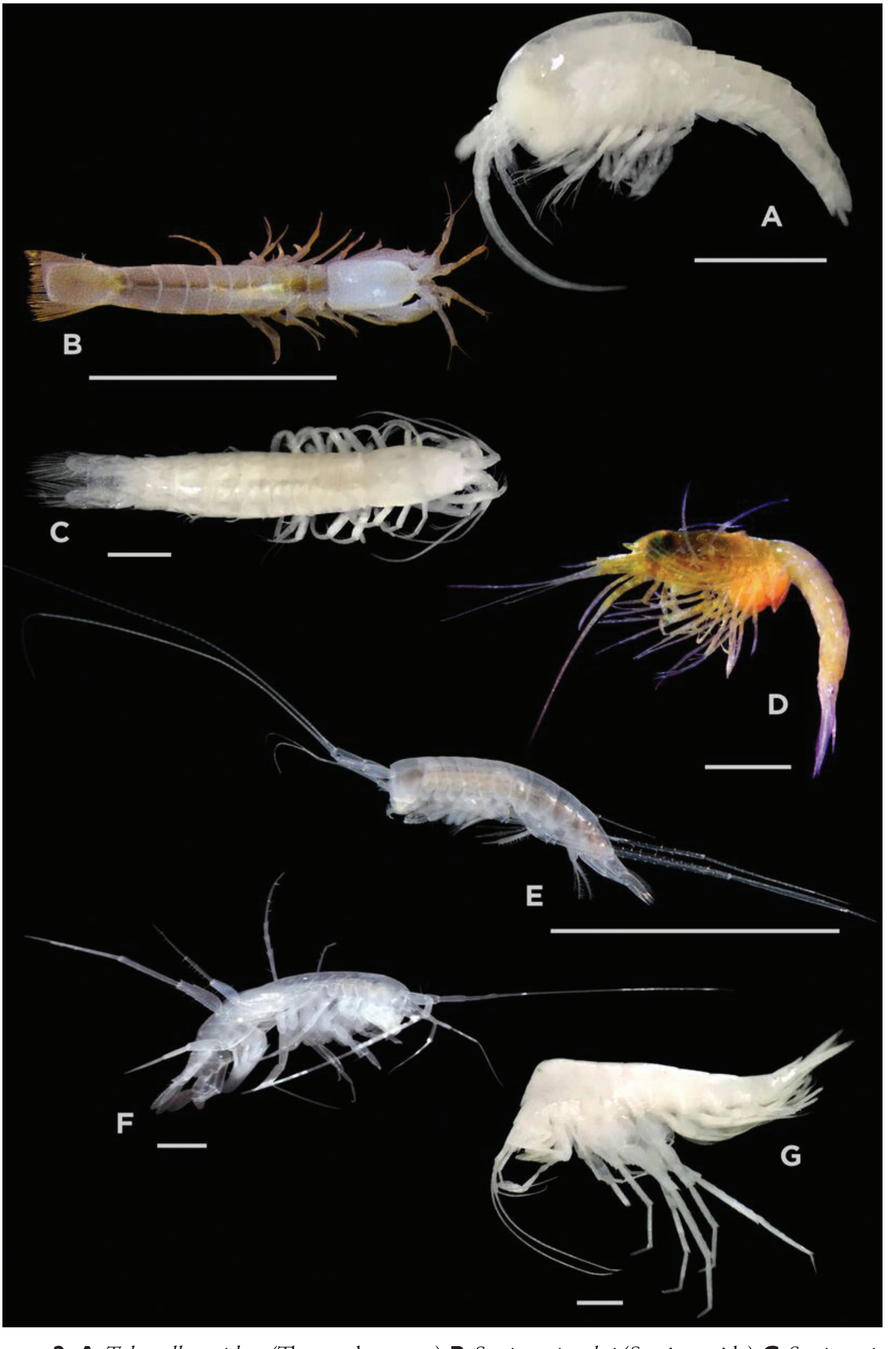

Figure 2. A *Tulumella unidens* (Thermosbaenacea) **B** *Stygiomysis cokei* (Stygiomysida) **C** *Stygiomysis* cf. *holthuisi* (Stygiomysida) **D** *Antromysis cenotensis* (Mysida) **E** *Mayaweckelia troglomorpha* (Amphipoda) **F** *Mayaweckelia cenoticola* (Amphipoda) **G** *Tuluweckelia cernua* (Amphipoda). Scale bars: 1 mm (**A, C, D, F, G**); 10 mm **(B, E)**.

°C, Homun, Yucatan, Mexico; 19 November 2017; colls. D. Angyal, E. Chávez Solís, S. Drs & L. Liévano. 2 individuals; **Dzonotila**, depth 20.8 m and 28.0 m, cavern, freshwater, 27 °C, Mucuyche, Yucatan, Mexico; 20 November 2017; colls. D. Angyal, E. Chávez Solís, S. Drs & B. Magaña. 1 individual; **Yax-Kis**, depth 12.1 m and 27.0 m, cave, freshwater, 27 °C, Mucuyche, Yucatan, Mexico; 27 January 2018; colls. D. Angyal, S. Drs & L. Liévano.

Previous distribution. Kallmeyer and Carpenter 1996; Pesce and Iliffe 2002; Álvarez and Iliffe 2008; Álvarez et al. 2015; Benítez et al. 2019.

Type locality is Cenote Calavera (Temple of Doom) in Quintana Roo. Further known localities in Quintana Roo are cenotes Mayan Blue, Naharon (Cristal), Escondido, Actun Ha (Carwash), Actun Ko, Na'ach Wennen Ha, Muknal and Tabano. From Yucatan the species was known from cenotes Papakal, San Eduardo, Kankirixche, Yaal Utsil and Dzonotila.

Remarks. Our records show that this species is distributed in cenotes of central Yucatan and along the Ring of Cenotes. Among the two *Stygiomysis* species of the region, *S. cokei* proved to be rarer than *Stygiomysis* cf. *holthuisi*. New occurrences were recoded between 12-32 m deep in freshwater. In cenotes San Elias, Dzonotila and Yax-Kis it co-occurred with *S.* cf. *holthuisi*. Previously the species had also been reported in brackish habitats (Álvarez and Iliffe 2008; Álvarez et al. 2015).

***Stygiomysis* cf. *holthuisi* (Gordon, 1958)**

Figure 2C

Material examined. 2 individuals; **Cenote Tres Oches**, depth 21.6 m, cave, freshwater, 27 °C, Homun, Yucatan, Mexico; 5 June 2016; colls. D. Angyal & E. Chávez Solís. 1 individual; **Cenote Tza Itza**, depth 18.9 m, cavern, freshwater, 27 °C, Tecoh, Yucatan, Mexico; 10 May 2016; colls. D. Angyal & E. Chávez Solís. 1 individual; **Cenote X-Batun**, depth 19.3 m, cavern, freshwater, 27 °C, San Antonio Mulix, Yucatan, Mexico; 14 May 2016; colls. R. Acosta, D. Angyal, J. Baduy & S. Reyes. 3 individuals; **Cenote Kanun**, depth 10.9-13.0 m, cave, freshwater, 26 °C, Homun, Yucatan, Mexico; 4 June 2016; colls. R. Acosta, D. Angyal, J. Baduy, B. Magaña & S. Reyes. 1 individual; **Cenote Kakuel**, depth 29.8 m, cave, freshwater, 27 °C, Mucuyche, Yucatan, Mexico; 10 June 2016; colls. D. Angyal & E. Chávez Solís. 1 individual; **Cenote Kankirixche**, depth 3 m, cavern, freshwater, 27 °C, Mucuyche, Yucatan, Mexico; 11 June 2016; colls. D. Angyal & E. Chávez Solís. 1 individual; **Cenote Santito**, depth 5.4 m, cavern, freshwater, 27 °C, Kopoma, Yucatan, Mexico; 10 November 2017; colls. D. Angyal, D. Drs & L. Liévano. 1 individual; **Cenote Pol Box**, depth 3.0 m, cavern, freshwater, 27 °C, Chochola, Yucatan, Mexico; 12 November 2017; colls. D. Angyal, S. Drs, L. Liévano & E. Sosa. 4 individuals; **Cenote Kankal**, depth 6.0-27.0 m, cavern, freshwater, 25 °C, Homun, Yucatan, Mexico; 12 November 2017; colls. D. Angyal, S. Drs, L. Liévano & E. Sosa. 2 individuals; **Cenote Flor de Liz**, depth 3.0 m, cavern, freshwater, 27 °C, Tixkokob, Yucatan, Mexico; 17 December 2017; colls. D. Angyal,

S. Drs, L. Liévano & S. Reyes. 1 individual; **Cenote Bebelchen**, depth 30.0 m, cavern, freshwater, 25 °C, Sanahcat, Yucatan, Mexico; 18 December 2017; colls. D. Angyal, S. Drs, L. Liévano & S. Reyes. 2 individuals; **Cenote Chihuo Hol**, depth 16.0 and 25.0 m, cavern, freshwater, 27 °C, Mucuyche, Yucatan, Mexico; 26 January 2018; colls. D. Angyal, S. Drs, L. Liévano, B. Magaña & N. Simoes. 3 individuals; **Yax Kis**, depth 9.0-25.0 m, cave, freshwater, 27 °C, Mucuyche, Yucatan, Mexico; 27 January 2018; colls. D. Angyal, S. Drs & L. Liévano.

Previous distribution. Gordon 1958; Botosaneanu 1980; Bowman et al. 1984; Pesce and Iliffe 2002; Álvarez and Iliffe 2008, Álvarez et al. 2015, Benítez et al. 2019.

Type locality is Devil's Hole, St. Martin, Lesser Antilles (France). The species is known from the Bahamas, Anguilla, Puerto Rico, and the Yucatan Peninsula. In Quintana Roo *S.* cf. *holthuisi* was recorded from cenotes Mayan Blue, Casa Cenote, Na'ach Wennen Ha, Bang, Odyssey, Muknal, and Tabano. From Yucatan the species was previously known only from a single locality, Cenote Mucuyche.

Remarks. We have also recorded the species from cenotes Yaal Utsil, San Elias, and Dzonotila in freshwater bodies in both cavern and cave sections, between 3 and 30 m deep. Álvarez and Iliffe (2008) and Álvarez et al. (2015) reported observations in both freshwater and around the halocline from cenotes in Quintana Roo.

Order: Mysida
Family: Mysidae

***Antromysis cenotensis* Creaser, 1936**
Figure 2D

Material examined. 21 individuals; **Cenote Tza Itza**, depth 12.7-13.5 m, cavern, freshwater, 27 °C, Tecoh, Yucatan, Mexico; 10 May 2016; colls. D. Angyal & E. Chávez Solís. 1 individual; **Cenote Dzonbakal**, depth 25.3 m, cavern, freshwater, 27 °C, San Antonio Mulix, Yucatan, Mexico; 14 May 2016; colls. R. Acosta, D. Angyal, J. Baduy & S. Reyes. 1 individual; **Cenote Nayah**, depth 27.9 m, entrance of cave part, freshwater, 26 °C, Pixyah, Yucatan, Mexico; 17 May 2016; colls. D. Angyal & B. Magaña. 3 individuals; **Cenote Kampepen**, depth 9.3-12.5 m, cavern, freshwater, 27 °C, Chinquila, Yucatan, Mexico; 17 May 2016; colls. D. Angyal & B. Magaña. 4 individuals; **Cenote Kanun**, depth 0.5 m, cenote entrance, freshwater, 26 °C, Homun, Yucatan, Mexico; 4 June 2016; colls. R. Acosta, D. Angyal, J. Baduy, B. Magaña & S. Reyes. 4 individuals; **Cenote Xaan**, depth 22.2-24.2 m, cavern and cave, freshwater, 27 °C, Homun, Yucatan, Mexico; 9 June 2016; colls. D. Angyal & E. Chávez Solís. 15 individuals; **Cenote Kakuel**, depth 7.2-10.8 m, cavern, freshwater, 27 °C, Homun, Yucatan, Mexico; 10 June 2016; colls. D. Angyal & E. Chávez Solís. 1 individual; **Cenote Kankirixche**, depth 9.0 m, cavern, freshwater, 27 °C, Mucuyche, Yucatan, Mexico; 11 June 2016;

colls. D. Angyal & E. Chávez Solís. 4 individuals; **Cenote Kankirixche**, depth 10.0-25.0 m, cavern, freshwater, 27 °C, Mucuyche, Yucatan, Mexico; 25 January 2018; colls. D. Angyal, S. Drs, B. Magaña & L. Liévano. 18 individuals; **Cenote Santito**, depth 0.2-1.0 m, cavern, freshwater, 27 °C, Kopoma, Yucatan, Mexico; 10 November 2017; colls. D. Angyal, S. Drs & L. Liévano. 17 individuals; **Cenote Pol Box**, depth 5.2-9.3 m, cavern, freshwater, 27 °C, Chochola, Yucatan, Mexico; 12 November 2017; colls. D. Angyal, S. Drs, L. Liévano & E. Sosa. 1 individual; **Cenote Kankal**, depth 24.6 m, cavern, freshwater, 25 °C, Homun, Yucatan, Mexico; 18 November 2017; colls. D. Angyal, E. Chávez Solís, S. Drs & L. Liévano. 21 individuals; **Dzonotila**, depth 3.0-27.0 m, cavern, freshwater, 27 °C, Mucuyche, Yucatan, Mexico; 20 November 2017; colls. D. Angyal, E. Chávez Solís, S. Drs & B. Magaña. 5 individuals; **Cenote Ixim Ha**, depth 10.0 m, cavern, freshwater, 25 °C, Tixkakal, Yucatan, Mexico; 25 November 2017; colls. D. Angyal, E. Chávez Solís, S. Drs, L. Liévano & E. Sosa. 1 individual; **Cenote Noh'Chunck**, depth 12.0 m, cavern, freshwater, 25 °C, Chunchumil, Yucatan, Mexico; 25 November 2017; colls. D. Angyal, E. Chávez Solís, S. Drs, Q. Hernández & S. Reyes. 11 individuals; **Cenote X'kokob**, depth 1.0-4.0 m, cavern, freshwater, 25 °C, Ekmul, Yucatan, Mexico; 17 December 2017; colls. D. Angyal, S. Drs, L. Liévano & S. Reyes. 14 individuals; **Cenote Flor de Liz**, depth 0.3-3.0 m, cavern, freshwater, 27 °C, Tixkokob, Yucatan, Mexico; 17 December 2017; colls. D. Angyal, S. Drs, L. Liévano & S. Reyes. 19 individuals; **Cenote Pixton**, depth 3.0 m, cavern, freshwater, 27 °C, Huhi, Yucatan, Mexico; 18 December 2017; colls. D. Angyal & L. Liévano. 11 individuals; **Cenote Bebelchen**, depth 27.0 m, cavern, freshwater, 25 °C, Sanahcat, Yucatan, Mexico; 18 December 2017; colls. D. Angyal, L. Liévano & S. Reyes. 6 individuals; **Cenote El Virgen**, depth 25.0 m, cavern, freshwater, 26 °C, Sotuta, Yucatan, Mexico; 20 December 2017; colls. L. Liévano & N. Simoes. 3 individuals; **Cenote Chihuo Hol**, depth 11.0 m, cavern, freshwater, 27 °C, Mucuyche, Yucatan, Mexico; 20 December 2017; colls. D. Angyal, S. Drs, B. Magaña, L. Liévano & N. Simoes.

Previous distribution. Creaser 1936, 1938; Nicholas 1962; Bowman 1977; Reddell 1977, 1981; Holsinger 1990; Iliffe 1992, 1993; Fiers et al. 1996; Rocha et al. 1998, 2000; Suárez-Morales and Rivera Arriaga 1998; Pohlman et al. 2000; Pesce and Iliffe 2002; Schmitter-Soto et al. 2002; Álvarez and Iliffe 2008; Álvarez et al. 2015; Benítez et al. 2019.

Type locality is Grutas de Balankanche (Yucatan). Widely distributed in the central and northern parts of the Yucatan Peninsula, known from several wells, cenotes and caves of Quintana Roo and Yucatan.

Remarks. *Antromysis cenotensis* was present in all the cenotes studied, except for Cenote Cervera. Álvarez et al. (2015) mentions that *A. cenotensis* occurs mostly above or occasionally below the halocline up to a depth of 16 m. In the present study, the species was only observed in freshwater habitats, in some cases as deep as the scope of the survey. Our findings prove this species as a common representative of the stygofauna of Yucatan, as it was found in more than 95% of the visited sites. *Antromysis cenotensis* is listed as "threatened" in the Mexican Red List of Threatened Species (NOM-059 SEMARNAT 2010).

Order: Amphipoda
Family: Hadziidae

Mayaweckelia troglomorpha **Angyal, 2018**
Figure 2E

Material examined. 2 individuals; **Dzonbakal**, depth 26.3 and 26.5 m, cave, freshwater, 27 °C, San Antonio Mulix, Yucatan, Mexico; 14 May 2016; colls. R. Acosta, D. Angyal, J. Baduy & S. Reyes. 1 individual; **Cenote Kanun**, depth 24.3 m, cave, freshwater, 26 °C, Homun, Yucatan, Mexico; 4 June 2016; colls. R. Acosta, D. Angyal, J. Baduy, B. Magaña & S. Reyes. 1 individual; **Cenote Xaan**, depth 25.4 m, cave, freshwater, 27 °C, Homun, Yucatan, Mexico; 9 June 2016; colls. D. Angyal & E. Chávez Solís. 2 individuals; **Cenote Kankirixche**, depth 20.4 and 33.3 m, cavern and cave, freshwater, 27 °C, Homun, Yucatan, Mexico; 11 June 2016; colls. D. Angyal & E. Chávez Solís. 5 individuals; **Dzonotila**, depth 11.0-17.7 m, cavern, freshwater, 27 °C, Mucuyche, Yucatan, Mexico; 20 November 2017; colls. D. Angyal, E. Chávez Solís, S. Drs & B. Magaña. 2 individuals; **Cenote X'kokob**, depth 4.0-10.0 m, cavern, freshwater, 26 °C, Ekmul, Yucatan, Mexico; 17 December 2017; colls. D. Angyal, E. Chávez Solís, S. Drs & B. Magaña. 2 individuals; **Cenote Chihuo Hol**, depth 8.0-27.2 m, cavern, freshwater, 27 °C, Mucuyche, Yucatan, Mexico; 26 January 2018; colls. D. Angyal, S. Drs, L. Liévano, B. Magaña & N. Simoes. 1 individual; **Cenote Yax-Kis**, depth 8.0 m, cave, freshwater, 27 °C, Mucuyche, Yucatan, Mexico; 27 January 2018; colls. D. Angyal, S. Drs & L. Liévano.

Previous distribution. Angyal et al. 2018. Type locality is Dzonbakal (Yucatan. Allotype female is from Cenote Kankirixche, paratypes are from Dzonbakal and cenotes Kanun, Xaan and Kankirixche (all in Yucatan).

Remarks. At present, collected material is available from eight localities and a small *M. troglomorpha* population was also observed in Cenote San Elias. All the individuals were found in freshwater habitats, both in cave and cavern sections, where water temperature was between 26 and 27 °C. In cenote Kankirixche, some individuals were observed below 45 meters in depth. As a species recently described by our research group, one of the outcomes of present expeditions. As *M. troglomorpha* was found in approximately 30% of the visited sites, it does not appear to be a rare freshwater stygobiotic element in the Yucatan cenotes.

Mayaweckelia cenoticola **Holsinger, 1977**
Figure 2F

Material examined. 1 individual; **Cenote Ayun-Nah**, depth 14.0 m, cave, freshwater, 27 °C, Cacalchen, Yucatan, Mexico; 22 May 2016; colls. D. Angyal, B. Magaña & E. Sosa Rodríguez. 1 individual; **Dzonotila**, depth 18.0 m, cavern, freshwater, 27 °C, Mucuyche, Yucatan, Mexico; 20 November 2017; colls. D. Angyal. E. Chávez Solís, S.

Drs & B. Magaña. 1 individual; **Cenote Ixim Ha**, depth 4.7 m, cavern, freshwater, 25 °C, Tixkakal, Yucatan, Mexico; 25 November 2017; colls. D. Angyal, E. Chávez Solís, S. Drs, L. Liévano & E. Sosa. 3 individuals; **Cenote Bebelchen**, depth 0.5-7.3 m, cavern, freshwater, in water column and in roots at cavern entrance, 25 °C, Sanahcat, Yucatan, Mexico; 18 December 2017; colls. D. Angyal, S. Drs, L. Liévano & S. Reyes.

Previous distribution. Holsinger 1977, 1990; Reddell 1981; Álvarez and Iliffe 2008, Álvarez et al. 2015, Angyal et al. 2018, Benítez et al. 2019.

Type locality is Cenote Xtacabiha (Yucatan). From Yucatan the species was also known from Cueva de Orizaba, Cenote Nohchen, Grutas de Tzab-Nah and Grutas de Santa Maria. From Quintana Roo there were records from Cenote Actun Ha (Carwash), Cenote de las Ruinas, Cenote de San Martin, Cenote de Santo Domingo, Cueva de Tancah, Odyssey, Bang and Tabano. From the state of Campeche, the species was known from the Volcán de los Murciélagos cave.

Remarks. *Mayaweckelia cenoticola* proved to be rarer than *M. troglomorpha*, since it was recorded from only four cenotes. In Cenote Bebelchen we found some individuals in the roots of trees near the surface at the entrance region. Holsinger (1990) found that the species is associated mainly with freshwater habitats, with few populations occurring in weak brackish water. Individuals found in the Ox Bel Ha System (Quintana Roo) by Álvarez et al. (2015) and Benítez et al. (2019) also occurred in freshwater.

Tuluweckelia cernua Holsinger, 1990

Figure 2G

Material examined. 3 individuals; **Cenote San Juan**, depth 27.0-27.1 m, cave, freshwater, 27 °C, Homun, Yucatan, Mexico; 7 May 2016; colls. D. Angyal & E. Chávez Solís. 2 individuals; **Cenote Dzonbakal**, depth 29.0 m, cave, freshwater, 27 °C, San Antonio Mulix, Yucatan, Mexico; 22 May 2016; colls. D. Angyal, J. Baduy & B. Magaña. 10 individuals; **Cenote Tres Oches**, depth 15.8-22.9 m, cave, freshwater, 27 °C, Homun, Yucatan, Mexico; 5 June 2016; colls. D. Angyal & E. Chávez Solís. 3 individuals; **Cenote Xaan**, depth 22.7-26.6 m, cave, freshwater, 27 °C, Homun, Yucatan, Mexico; 9 June 2016; colls. D. Angyal & E. Chávez Solís. 3 individuals; **Cenote Kakuel**, depth 32.2-38 m, cave, freshwater, 27 °C, Mucuyche, Yucatan, Mexico; 10 June 2016; colls. D. Angyal & E. Chávez Solís. 3 individuals; **Cenote Kankirixche**, depth 20.4-49.6 m, cavern and cave, freshwater, 27 °C, Mucuyche, Yucatan, Mexico; 11 June 2016; colls. D. Angyal & E. Chávez Solís. 2 individuals; **Cenote Santito**, depth 5.3-6.0 m, cavern, freshwater, 27 °C, Kopoma, Yucatan, Mexico; 10 November 2017; colls. D. Angyal, S. Drs & L. Liévano. 1 individual; **Cenote X'baba**, depth 26.0 m, cavern, freshwater, 27 °C, Chochola, Yucatan, Mexico; 26 November 2017; colls. S. Drs, L. Liévano & E. Sosa. 1 individual; **Cenote Sabtun 1**, depth 25.0 m, cavern, above the halocline, 25 °C, Chunchumil, Yucatan, Mexico; 10 December 2017; colls. D. Angyal, S. Drs, E. Chávez Solís, Q. Hernández & S. Reyes. 1 individual; **Cenote Pixton**, depth 7.0 m, cavern, freshwater, 26 °C, Huhi, Yucatan, Mexico; 18 December

2017; colls. D. Angyal & L. Liévano. 3 individuals; **Cenote Yax-Kis**, depth 23.4-32.0 m, cave, freshwater, 27 °C, Mucuyche, Yucatan, Mexico; 27 January 2018; colls. D. Angyal, S. Drs & L. Liévano.

Previous distribution. Holsinger 1990; Álvarez and Iliffe 2008; Álvarez et al. 2015; Angyal et al. 2018; Benítez et al. 2019.

Type locality is Cenote Calavera (Temple of Doom) in Quintana Roo. This species was known only from coastal caves of Quintana Roo: Mayan Blue, Actun Ha (Carwash), Mojara, Naharon (Cristal), Na'ach Wennen Ha, Bang, Muknal, Odyssey, and Tabano.

Remarks. *Tuluweckelia cernua* was both the most frequent and abundant stygobiotic amphipod in the present study. Additional observations were from cenotes Yaal Utsil, El Virgen, and Dzalbay. In contrast with previous reports (e.g. Holsinger 1990), *T. cernua* always occurred in freshwater habitats. Individuals were collected between depths of 5-50 m. The species co-occurred with *M. troglomorpha* in five cenotes. These are the first distributional records of *T. cernua* for the state of Yucatan. Known localities of this species have almost tripled, increasing its distribution range into the Yucatan inland area.

Order: Isopoda
Family: Cirolanidae

***Creaseriella anops* (Creaser, 1936)**
Figure 3A

Material examined. 3 individuals; **Cenote San Juan**, depth 20.0-28.0 m, cavern and cave, freshwater, 27 °C, Homun, Yucatan, Mexico; 7 May 2016; colls. D. Angyal & E. Chávez Solís. 1 individual; **Cenote Cervera**, depth 24.0 m, cave, below halocline, 26 °C, Yalsihom, Yucatan, Mexico; 8 May 2016; colls. D. Angyal & E. Chávez Solís. 2 individuals; **Cenote Tza Itza**, depth 12.5-13.5 m, cavern, freshwater, 27 °C, Tecoh, Yucatan, Mexico; 10 May 2016; colls. D. Angyal & E. Chávez Solís. 2 individuals; **Cenote Tres Oches**, depth 18.2-21.7 m, cave, freshwater, 27 °C, Homun, Yucatan, Mexico; 5 June 2016; colls. D. Angyal & E. Chávez Solís. 1 individual; **Cenote Kankirixche**, depth 3.0 m, cavern, freshwater, 27 °C, Mucuyche, Yucatan, Mexico; 11 June 2016; colls. D. Angyal & E. Chávez Solís. 1 individual; **Cenote Chihuo Hol**, depth 15.0 m, cavern, freshwater, 27 °C, Mucuyche, Yucatan, Mexico; 26 January 2018; colls. D. Angyal, S. Drs, L. Liévano, B. Magaña & N. Simoes.

Previous distribution. Creaser 1936, 1938; Nicholas 1962; Reddell 1977, 1981; Holsinger 1990; Iliffe 1992, 1993; Fiers et al. 1996; Rocha et al. 1998; Botosaneanu and Iliffe 1999, 2002; Álvarez et al. 2005; Iliffe and Botosaneanu 2006; Álvarez and Iliffe 2008; Sánchez-Rodríguez 2008; Ruíz-Cancino et al. 2013; Álvarez et al. 2015; Ortiz and Chazaro-Olvera 2015; Benítez et al. 2019.

Type locality is Cenote Sambula (Motul, Yucatan). Known from numerous caves and cenotes in Quintana Roo and Yucatan, and a well in Campeche.

Remarks. The species was also observed in cenotes Yaal Utsil, Pol Box, X'kokob, Bebelchen, Kankal, San Elias, Dzonotila, Yax-Kis, Xaan and X'baba. *Creaseriella anops* was found both in cavern and cave sections, between 3 and 40 m deep. Our observations generally agree with the records of Iliffe and Botosanenau (2006) and Álvarez et al. (2015) as a freshwater species. However, as Benítez et al. (2019) reported, we also observed individuals around or below the halocline. *Creaseriella anops* is listed as "threatened" in the Mexican Red List of Threatened Species (NOM-059-SEMARNAT 2010).

***Yucatalana robustispina* Botosaneanu & Iliffe, 1999**
Figure 3B

Material examined. 1 individual; **Cenote Xaan**, depth 27.6 m, cave, freshwater, 27 °C, Homun, Yucatan, Mexico; 9 June 2016; colls. D. Angyal & E. Chávez Solís. 1 individual; **Cenote Kakuel**, depth 19.9 m, cavern, freshwater, 27 °C, Mucuyche, Yucatan, Mexico; 10 June 2016; colls. D. Angyal & E. Chávez Solís. 5 individuals; **Cenote Kankirixche**, depth 20-49.3 m, cavern and cave, freshwater, 27 °C, Mucuyche, Yucatan, Mexico; 11 June 2016; colls. D. Angyal & E. Chávez Solís. 3 individuals; **Cenote Kankirixche**, depth 10.0-27.0 m, cavern, freshwater, 27 °C, Mucuyche, Yucatan, Mexico; 26 January 2018; colls. D. Angyal, S. Drs, L. Liévano & B. Magaña. 1 individual; **Cenote Yaal Utsil**, depth 35.5 m, cave, freshwater, 27 °C, Mucuyche, Yucatan, Mexico; 3 November 2017; colls. D. Angyal, S. Drs & E. Chávez Solís. 1 individual; **Cenote Tza Itza**, depth 15.0 m, cavern, freshwater, 27 °C, Tecoh, Yucatan, Mexico; 3 November 2017; colls. D. Angyal, S. Drs & L. Liévano. 1 individual; **Cenote Pol Box**, depth 3.0 m, cavern, freshwater, 27 °C, Chochola, Yucatan, Mexico; 12 November 2017; colls. D. Angyal, S. Drs, L. Liévano & E. Sosa. 2 individuals; **Dzonotila**, depth 14.0 and 16.0 m, cavern, freshwater, 27 °C, Mucuyche, Yucatan, Mexico; 12 November 2017; colls. D. Angyal, S. Drs, E. Chávez Solís & B. Magaña. 1 individual; **Cenote X'baba**, depth 12.0 m, cave, freshwater, 25 °C, Chochola, Yucatan, Mexico; 12 November 2017; colls. S. Drs, L. Liévano & E. Sosa. 1 individual; **Cenote El Virgen**, depth 12.6 m, cavern, freshwater, 26 °C, Sotuta, Yucatan, Mexico; 20 December 2017; colls. L. Liévano & N. Simoes. 1 individual; **Cenote Chihuo Hol**, depth 20.6 m, cavern, freshwater, 27 °C, Mucuyche, Yucatan, Mexico; 26 January 2018; colls. D. Angyal, S. Drs, L. Liévano, B Magaña & N. Simoes. 3 individuals; **Cenote Yax Kis**, depth 12.0-33.0 m, cave, freshwater, 27 °C, Mucuyche, Yucatan, Mexico; 27 January 2018; colls. D. Angyal, S. Drs & L. Liévano.

Previous distribution. Botosaneanu and Iliffe 1999, 2002, 2006; Álvarez and Iliffe 2008.

Type locality is Cenote Pabakal (Papakal), Yucatan. It was also found in cenotes Kankirixche, Kakuel, Chuih-Hol Dos, Xacha, and San Geronimo (all in Yucatan).

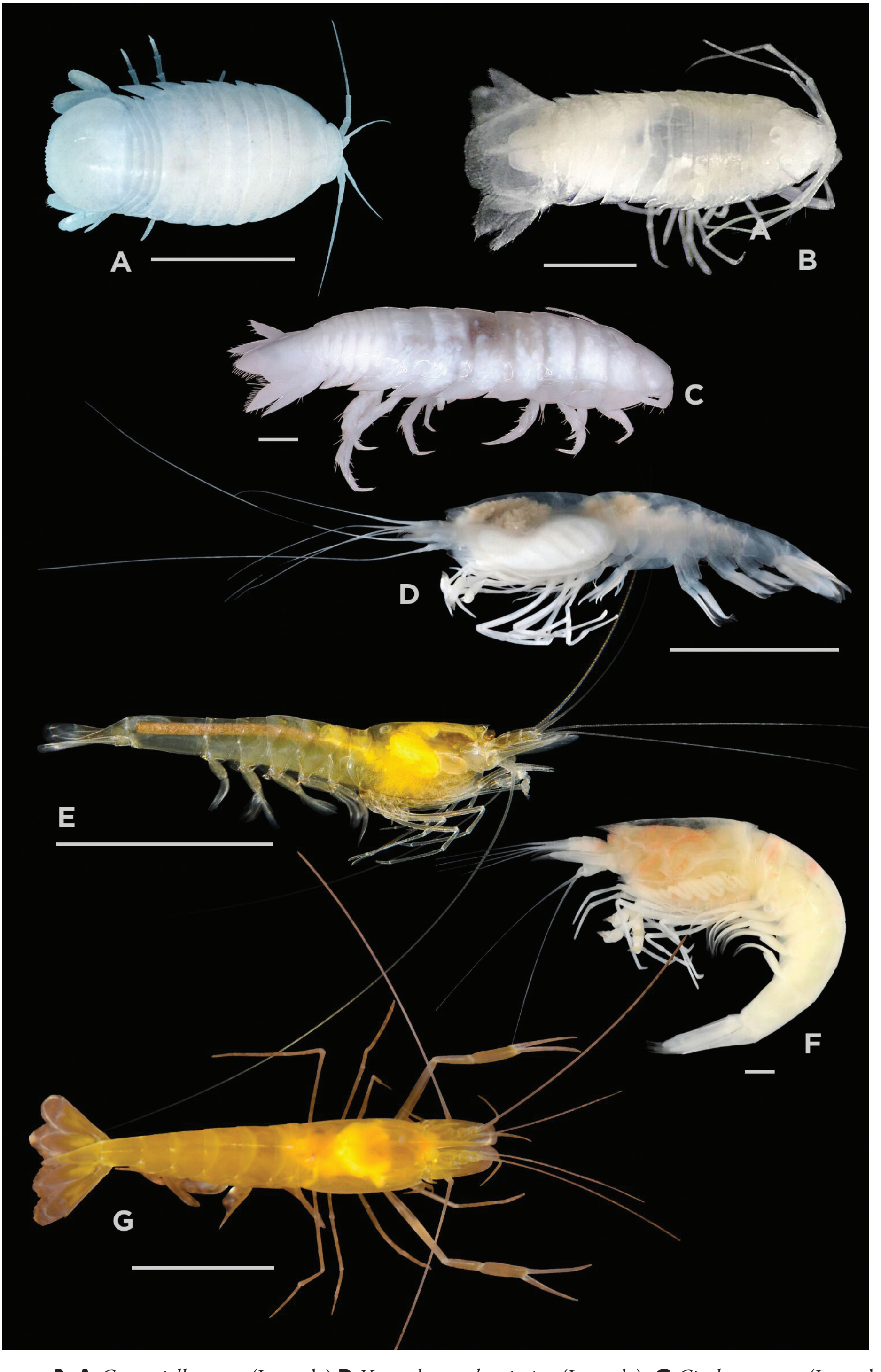

Figure 3. A *Creaseriella anops* (Isopoda) **B** *Yucatalana robustispina* (Isopoda); **C** *Cirolana yunca* (Isopoda) **D** *Typhlatya dzilamensis* (Decapoda) **E** *Typhlatya mitchelli* (Decapoda) **F** *Typhlatya pearsei* (Decapoda) **G** *Creaseria morleyi* (Decapoda). Scale bars: 1 mm (**B, C, F**); 10 mm (**A, D, E, G**).

Remarks. Individuals of *Y. robustispina* were collected in a third of all localities visited, where it occurred in freshwater between 3 and 49 m in depth. In eight cenotes *Y. robustispina* co-occurred with the isopod *C. anops*. Agreeing with our observations, previous records referred specimens caught in freshwater between 5-50 m in depth (Botosaneanu and Iliffe 1999, 2002, 2006). Known localities of this species have been doubled.

Cirolana yunca (Botosaneanu & Iliffe, 2000)

Figure 3C

Material examined. 1 individual; **Cenote Tres Oches**, depth 22.4 m, cave, freshwater, 27 °C, Homun, Yucatan, Mexico; 5 June 2016; colls. D. Angyal & E. Chávez Solís. 1 individual; **Cenote X'baba**, depth 25.0 m, cave, freshwater, 25 °C, Chochola, Yucatan, Mexico; 26 November 2016; colls. S. Drs, L. Liévano & E. Sosa. 1 individual; **Cenote Chihuo Hol**, depth 19.0 m, cavern, freshwater, 27 °C, Mucuyche, Yucatan, Mexico; 26 January 2018; colls. D. Angyal, S. Drs, L. Liévano, B Magaña & N. Simoes. 1 individual.

Previous distribution. Botosaneanu and Iliffe 2000, 2006; Álvarez and Iliffe 2008; Rocha-Ramírez et al. 2009.

Type locality is Cenote Sabak Ha (Yucatan). This species had only been collected from its type locality until our expeditions.

Remarks. We here provide the first records after the original description, which was based on a single specimen collected at 60 m in depth near the halocline at a salinity of 1.4 g/l (Botosaneanu and Iliffe 2000, 2006). The three newly collected individuals were found in freshwater habitats, both in cavern and cave zones below 19 m in depth. The species was found in approximately 10% of the studied cenotes always as solitary individuals. Therefore, *C. yunca* seems to be a rare element of the Yucatan freshwater cenote ecosystems.

Superorder: Eucarida
Order: Decapoda
Family: Atydae

Typhlatya dzilamensis Álvarez, Iliffe & Villalobos, 2005

Figure 3D

Material examined. 1 individual; **Cenote Cervera**, depth 27.4 m, cave, below halocline, 27 °C, Yalsihom, Yucatan, Mexico; 8 May 2016; colls. D. Angyal & E. Chávez Solís. 1 individual; **Cenote Sabtun 1**, depth 28 m, cavern, below halocline, 26 °C, Chunchumil, Yucatan, Mexico; 10 Dec 2017; colls. D. Angyal & E. Chávez.

Previous distribution. Álvarez et al. 2005, 2015; Álvarez and Iliffe 2008; Benítez et al. 2019; Espinasa et al. 2019.

Type locality is Buya Uno, allotype was collected from Cenote Cervera and paratypes from Dzilamway, all cenotes in Dzilam de Bravo region (Yucatan north coast). This species was recently recorded at the Ox Bel Ha system south of Tulum (Benítez et al. 2019) and the Ponderosa system north of Tulum (Espinasa et al. 2019).

Remarks. In accordance with previous records by Álvarez et al. (2005, 2015), our specimens were also collected in fully marine water. Recent observations of this species increase the expected distribution, suggesting an underground coastal and saline habitat that could extend from the south of Quintana Roo (Ox Bel Ha) to the west coast of Yucatan (Sabtun 1).

Typhlatya mitchelli Hobbs & Hobbs, 1976

Figure 3E

Material examined. 3 individuals; **Cenote San Juan**, depth 4.3-9.1 m, cave and cavern, freshwater, 27 °C, Homun, Yucatan, Mexico; 7 May 2016; colls. D. Angyal & E. Chávez Solís. 11 individuals; **Cenote Tza Itza**, depth 4.3-16.5 m, cave, freshwater, 27 °C, Tecoh, Yucatan, Mexico; 10 May 2016; colls. D. Angyal & E. Chávez Solís. 1 individual; **Cenote Dzonbakal**, depth 9.3 m, cavern, freshwater, 27 °C, San Antonio Mulix, Yucatan, Mexico; 14 May 2016; colls. R. Acosta, D. Angyal, J. Baduy & S. Reyes. 1 individual; 1 individual; **Cenote Dzonbakal**, depth 14 m, cavern, freshwater, 27 °C, San Antonio Mulix, Yucatan, Mexico; 29 May 2016; colls. D. Angyal, J. Baduy & B. Magaña. 5 individuals; **Cenote Kampepen**, depth 10.1 m, cavern, freshwater, 27 °C, Chinquila, Yucatan, Mexico; 17 May 2016; colls. D. Angyal & B. Magaña. 2 individuals; **Cenote Ayun-Nah**, depth 9 m, cave, freshwater, 27 °C, Cacalchen, Yucatan, Mexico; 22 May 2016; colls. D. Angyal, B. Magaña & E. Sosa Rodríguez. **Cenote Tres Oches**, depth 8.1-22 m, cave, freshwater, 27 °C, Homun, Yucatan, Mexico; 5 June 2016; colls. D. Angyal & E. Chávez Solís. 7 individuals; **Cenote Kakuel**, depth 5-25.8 m, cave and cavern, freshwater, 27 °C, Mucuyche, Yucatan, Mexico; 10 June 2016; colls. D. Angyal & E. Chávez Solís. 1 individual; **Cenote Kankirixche**, depth 30.2 m, cavern, freshwater, 27 °C, Mucuyche, Yucatan, Mexico; 10 December 2016; colls. D. Angyal & E. Chávez Solís. 2 individuals; **Cenote Sabtun 1**, depth 24.0 and 25.0 m, cavern, above the halocline, 25 °C, Chunchumil, Yucatan, Mexico; 10 December 2017; colls. D. Angyal, E. Chávez Solís, S. Drs, Q. Hernández & S. Reyes. 1 individual; **Cenote Bebelchen**, depth 34.0 m, cavern, freshwater, 25 °C, Sanahcat, Yucatan, Mexico; 18 December 2017; colls. D. Angyal, S. Drs, L. Liévano & S. Reyes. 1 individual; **Cenote El Virgen**, depth 19.9 m, cavern, freshwater, 26 °C, Sotuta, Yucatan, Mexico; 20 December 2017; colls. L. Liévano & N. Simoes. 1 individual; **Cenote Chihuo Hol**, depth 26.0 m, cavern, freshwater, 27 °C, Mucuyche, Yucatan, Mexico; 26 January 2018; colls. D. Angyal, S. Drs, B. Magaña, L. Liévano & N. Simoes.

Previous distribution. Hobbs and Hobbs 1976; Hobbs et al. 1977; Hobbs 1979; Reddell 1977, 1981; Iliffe 1992; Rocha et al. 1998; Webb 2003; Botello and Álvarez 2013; Benítez 2014; Álvarez et al. 2015; Chávez Solís 2015; Benítez et al. 2019.

Type locality is Cenote Kabahchen (Yucatan). The species occurs in numerous caves and cenotes throughout the peninsula in Quintana Roo and Yucatan.

Remarks. Our findings corroborate that *T. mitchelli* is a widespread common crustacean in the freshwater cenotes of Yucatan. This species was caught from the shallow zones to 34 m in depth, indicating a wide vertical range as well as a wide geographical range. The species was also observed (but not collected) in cenotes Yaal Utsil, Santito, Pol Box, Kankal, San Elias, Dzonotila, X'baba, X'kokob, Pixton, Dzalbay, and Yax-Kis. *Typhlatya mitchelli* is listed as "least concern" in the IUCN Red List (De Grave et al. 2013a) and as "threatened" in the Mexican Red List of Threatened Species (NOM-059-SEMARNAT 2010).

Typhlatya pearsei **Creaser, 1936**
Figure 3F

Material examined. 1 individual; **Cenote Tres Oches**, depth 21.6 m, cave, freshwater, 27 °C, Homun, Yucatan, Mexico; 6 June 2016; colls. D. Angyal & E. Chávez Solís. 2 individuals; **Cenote Xaan**, depth 25.8 and 26.1 m, cave, freshwater, 27 °C, Homun, Yucatan, Mexico; 9 June 2016; colls. D. Angyal & E. Chávez Solís. 1 individual; **Cenote Kankirixche**, depth 3 m, cavern, freshwater, 27 °C, Mucuyche, Yucatan, Mexico; 11 June 2016; colls. D. Angyal & E. Chávez Solís. **Cenote Nohmozon**, depth 12.2 m, cavern, freshwater, 25 °C, Pixyah, Tecoh, Yucatan, Mexico; 11 March 2016; colls. E. Chávez Solís.

Previous distribution. Creaser 1936; Nicholas 1962; Hobbs et al. 1977; Holthuis 1977; Hobbs 1979; Reddell 1977, 1981; Pérez-Aranda 1983a; Holsinger 1990; Iliffe 1992; Webb 2003; Hunter et al. 2007; Yager and Madden 2010; Botello and Álvarez 2013; Mejía-Ortíz et al. 2013; Benítez 2014; Pakes et al. 2014; Álvarez et al. 2015; Chávez Solís 2015; Benítez et al. 2019.

Type locality is 'Balam Canche Cave' (Grutas de Balankanche, Yucatan). The species is widely distributed within the northern part of the Yucatan Peninsula; it occurs in Quintana Roo, Yucatan, and Campeche.

Remarks. Despite previous studies stating that *T. pearsei* has the largest of *Typhlatya*'s distribution range in the Yucatan Peninsula (Álvarez et al. 2015), we only collected individuals in a few localities, where it occurred in freshwater, both near the surface in open cenote pools and in deeper cave passages up to 26 m in depth. This species is listed as "least concern" in the IUCN Red List (De Grave et al. 2013b) and as "threatened" in the Mexican Red List of Threatened Species (NOM-059-SEMARNAT 2010).

Family: Palaemonidae

***Creaseria morleyi* (Creaser, 1936)**
Figure 3G

Material examined. 2 individuals; **Cenote Tza Itza**, depth 15.4 m, cavern, freshwater, 27 °C, Tecoh, Yucatan, Mexico; 10 May 2016; colls. D. Angyal & E. Chávez Solís. 2 individuals; **Cenote Kampepen**, depth 6-9.5 m, cavern, freshwater, 27 °C, Chinquila, Yucatan, Mexico; 17 May 2016; colls. D. Angyal & B. Magaña. 2 individuals; **Cenote Kakuel**, depth 3 and 13.9 m, cavern, freshwater, 27 °C, Mucuyche, Yucatan, Mexico; 10 June 2016; colls. D. Angyal & E. Chávez Solís. 1 individual; **Cenote Kankirixche**, depth 3.6 m, cavern, freshwater, 27 °C, Mucuyche, Yucatan, Mexico; 11 June 2016; colls. D. Angyal & E. Chávez Solís. 1 individual; **Cenote Santito**, depth 4.0 m, cavern, freshwater, 27 °C, Kopoma, Yucatan, Mexico; 10 November 2017; colls. D. Angyal, S. Drs & L. Liévano. 1 individual; **Cenote Kankal**, depth 0.3 m, cavern, freshwater, 25 °C, Homun, Yucatan, Mexico; 18 November 2017; colls. D. Angyal, S. Drs, E. Chávez Solís & L. Liévano. 1 individual; **Cenote Bebelchen**, depth 30.0 m, cavern, freshwater, 25 °C, Sanahcat, Yucatan, Mexico; 18 December 2017; colls. D. Angyal, L. Liévano & S. Reyes. 1 individual; **Cenote El Virgen**, depth 25.0 m, cavern, freshwater, 26 °C, Sotuta, Yucatan, Mexico; 20 December 2017; colls. L. Liévano & N. Simoes. 1 individual; **Cenote Dzalbay**, depth 4.3 m, cavern, freshwater, 23 °C, Sotuta, Yucatan, Mexico; 20 December 2017; colls. D. Angyal & L. Liévano.

Previous distribution. Creaser 1938; Hobbs and Hobbs 1976; Holthuis 1977; Hobbs et al. 1977; Reddell 1977, 1981; Hobbs 1979; Pérez-Aranda 1983b; Iliffe 1992; Botello and Álvarez 2006; Botello and Álvarez 2010; Benítez 2014; Álvarez et al. 2015; Chávez Solís 2015; Chávez Solís et al. 2017; Benítez et al. 2019.

Type locality is San Isidro Cave (Yucatan). Widely distributed in cenotes and caves of Yucatan, Campeche, and Quintana Roo.

Remarks. Reddell (1981) mentions the species as an "ever-present element of fauna of pools and lakes in caves in the Yucatan Peninsula". In addition to the above listed localities, we also observed the species in cenotes Yaal Utsil, Pol Box, San Elias, Dzonotila, Flor de Liz, X'baba, Chihuo Hol, and Yax-Kis. Specimens were recorded in both cave and cavern sections, up to 38 m in depth. Benítez et al. (2019) also found individuals around and below the halocline in cenotes belonging to the Ox Bel Ha system. *Creaseria morleyi* is listed as "threatened" in the Mexican Red List of Threatened Species (NOM-059-SEMARNAT 2010) and as "least concern" in the IUCN Red List (De Grave et al. 2013c).

Discussion

While there are more than 3,000 registered cenotes in the state of Yucatan (SDS Yucatan census), less than five percent have been zoologically investigated. Results herein confirm that the region deserves more attention and that the geographical, bathymetric, and fresh/salt water distribution of stygobiotic species is far from being fully understood. In order to contribute to the management of the vulnerable cenote ecosystems and their highly specialized endemic stygofauna, collecting as much information as possible about the biology of Yucatan aquifers would be paramount. This data should include reports on the species' distribution, density and rarity, taxonomy, ecology, as well as characteristics of their habitats related to their biology, such as the amount of epigean originated organic sources or the degree of anthropogenic pollution in cenotes.

Prior to this study, the amphipod *T. cernua* was only known from Quintana Roo, mostly associated with saltwater habitats in anchialine cenotes near the northeastern coastline of the Peninsula (Holsinger 1990; Rocha et al. 1998; Álvarez and Iliffe 2008; Álvarez et al. 2015). Contrary to previous findings, all individuals were found in freshwater habitats during our study (Angyal et al. 2018). Rocha et al. (1998) and Pesce and Iliffe (2002) mentioned observation records of 'thermosbaenaceans' from cenotes Yuncu, Mucuyche, Pabakal (Papakal), and Grutas de Tzab-Nah (all in Yucatan). However, these individuals had never been identified at the species level and it seems no voucher information of the potentially collected specimens is available. The present study confirms first records for *T. cernua* and *T. unidens* in the state of Yucatan. Together with the amphipod *M. troglomorpha*, which was discovered and described within the frame of herein presented expeditions (Angyal et al. 2018) and the new cave isopod *Curassanthura yucatanensis* Álvarez, Benítez, Iliffe & Villalobos, 2019 (Álvarez et al. 2019), the list of stygobiotic crustaceans recorded for the state of Yucatan raised from 22 (in 2016) to 26. In addition, the cirolanid isopod *C. yunca* was only known from its type locality, but we now provide distribution data for this species in three other localities. Our results show that the stygiomysid *S.* cf. *holthuisi* has historically been unrecognized, unsampled or ignored. This specific contribution proves that inland cenotes have been understudied and distribution patterns of stygofauna are still unknown. Due to the previously lacking zoological information for the vast majority of the cenotes investigated in our study, most of the distribution records presented here are new.

A closer morphological and molecular analysis of the *Typhlatya* species in Yucatan is recommended in order to distinguish cryptic species that may be causing confounding biodiversity and ecological patterns in the Yucatan Peninsula.

Among the 14 crustacean species listed, prior to this study, cytochrome c oxidase subunit I sequences were publicly available only for the decapods *T. mitchelli*, *T. pearsei*, *T. dzilamensis*, and *C. morleyi*. The currently published COI barcode gene fragments can aid future molecular research on the peracarid fauna of Yucatan's cenote ecosystems by facilitating their identification, as well as in the recognition of cryptic species.

The mysid *A. cenotensis*, the atyid shrimps *T. mitchelli* and *T. pearsei* and the palaemonid shrimp *C. morleyi* are listed in the Mexican and IUCN red lists of threatened species (SEMARNAT 2010; De Grave et al. 2013a, b, c). These species are present in most cenotes throughout the Yucatan Peninsula and can be considered a selected group of species whose protection will act as an umbrella in protecting other less common ones. On the other hand, there are rare species with an extremely narrow distribution range, which are not yet under legal protection. This makes these species even more vulnerable to urbanization and environment deterioration. Therefore, we suggest the inclusion of narrow endemic species into the national and international protection lists, such as the isopod *C. yunca* or the atyid shrimp *T. dzilamensis*.

The number of new records provided in this work shows a historic lack of biodiversity surveys in underwater caves of inland cenotes of the state of Yucatan. Most of the biodiversity and its distribution patterns are currently biased towards large populations, easily accessible sites, and touristic attractions. Our efforts yield a greater understanding of the distribution patterns of stygofauna in Yucatan cenotes.

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RESEARCH ARTICLE

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Four new species of the primitively segmented spider genus *Qiongthela* from Hainan Island, China (Mesothelae, Liphistiidae)

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Abstract

The primitively segmented spider genus *Qiongthela* Xu & Kuntner, 2015 consists of seven species that are distributed in Hainan Island, China and southern Vietnam. Of the seven species, five are known from Hainan Island. In this study, four more *Qiongthela* species collected from Hainan Island are diagnosed and described as new to science based on morphological characters: *Q. baoting* **sp. nov.** (♂♀), *Q. qiongzhong* **sp. nov.** (♂♀), *Q. sanya* **sp. nov.** (♂♀), *Q. yinggezui* **sp. nov.** (♂♀). To facilitate future identification, the GenBank accession codes of the DNA barcode gene, cytochrome c oxidase subunit I (COI), for all the type specimens are also provided.

Keywords

Abdominal tergites, COI, genital morphology, taxonomy, trapdoor spiders

Introduction

As the sole extant lineage of the suborder Mesothelae, the primitively segmented spider family Liphistiidae is unique in having segmented plates on the abdomen (i.e., abdominal tergites) and in bearing spinnerets centrally on the ventral abdomen (Pocock 1892; Platnick and Gertsch 1976; Coddington and Levi 1991; Haupt 2003; Xu et al. 2015a, b). Its members live in underground burrows with a trapdoor, are long-lived, and have a limited dispersal ability (Bristowe 1976; Coddington and Levi 1991; Haupt 2003; Xu et al. 2015a, b). Liphistiidae is relatively species-poor, currently containing 131 described species in eight genera of two subfamilies, Liphistiinae Thorell, 1869 and Heptathelinae Kishida, 1923. It is constrained to East (China and Japan) and Southeast (Indonesia (Sumatra), Laos, Malaysia, Myanmar, Thailand, and Vietnam) Asia (Xu et al. 2015a, b, 2016; World Spider Catalog 2020). The subfamily Heptathelinae contains seven genera: *Ganthela* Xu & Kuntner, 2015 and *Sinothela* Haupt, 2003 limited to China only, *Heptathela* Kishida, 1923 and *Ryuthela* Haupt, 1982 restricted to Japan only, and the other three genera (*Qiongthela* Xu & Kuntner, 2015, *Songthela* Ono, 2000, and *Vinathela* Ono, 2000) occur in both China and Vietnam (Xu et al. 2015a, b, c, 2016, 2017a, b; World Spider Catalog 2020).

The genus *Qiongthela* was established by Xu and Kuntner in 2015 based on both morphological and molecular characters (Xu et al. 2015a, b). Until now, there are only seven named species, five of which are known from Hainan Island, China: *Q. baishensis* Xu, 2015, *Q. bawang* Xu, Liu, Kuntner & Li, 2017, *Q. jianfeng* Xu, Liu, Kuntner & Li, 2017, *Q. wuzhi* Xu, Liu, Kuntner & Li, 2017, and *Q. yini* Xu, Liu, Kuntner & Li, 2017 (Fig. 1C); the other two of which, *Q. australis* (Ono, 2002) and *Q. nui* (Schwendinger & Ono, 2011), are distributed in southern Vietnam (Fig. 1C) (Ono 2002; Schwendinger and Ono 2011; Xu et al. 2015a, b, 2017b; Word Spider Catalog 2020). In this study, we diagnosed and described four more new *Qiongthela* species collected from Hainan Island based on both male and female genital morphology. In addition, we also provided the COI sequences of the holotypes for facilitating future identification.

Materials and methods

All specimens were collected from Hainan Island, China. All the type and voucher specimens are deposited at the College of Life Sciences, Hunan Normal University (HNU), Changsha, Hunan Province, China. We collected the spiders alive and fixed them in absolute ethanol if they were adults. For juvenile/subadult males, we took them back to the laboratory and reared them until they reached adulthood. We removed the right four legs of adults, preserved them in 100% ethanol and kept at –80°C for molecular work. We preserved the remains in 80% ethanol as vouchers for morphological identification and examination.

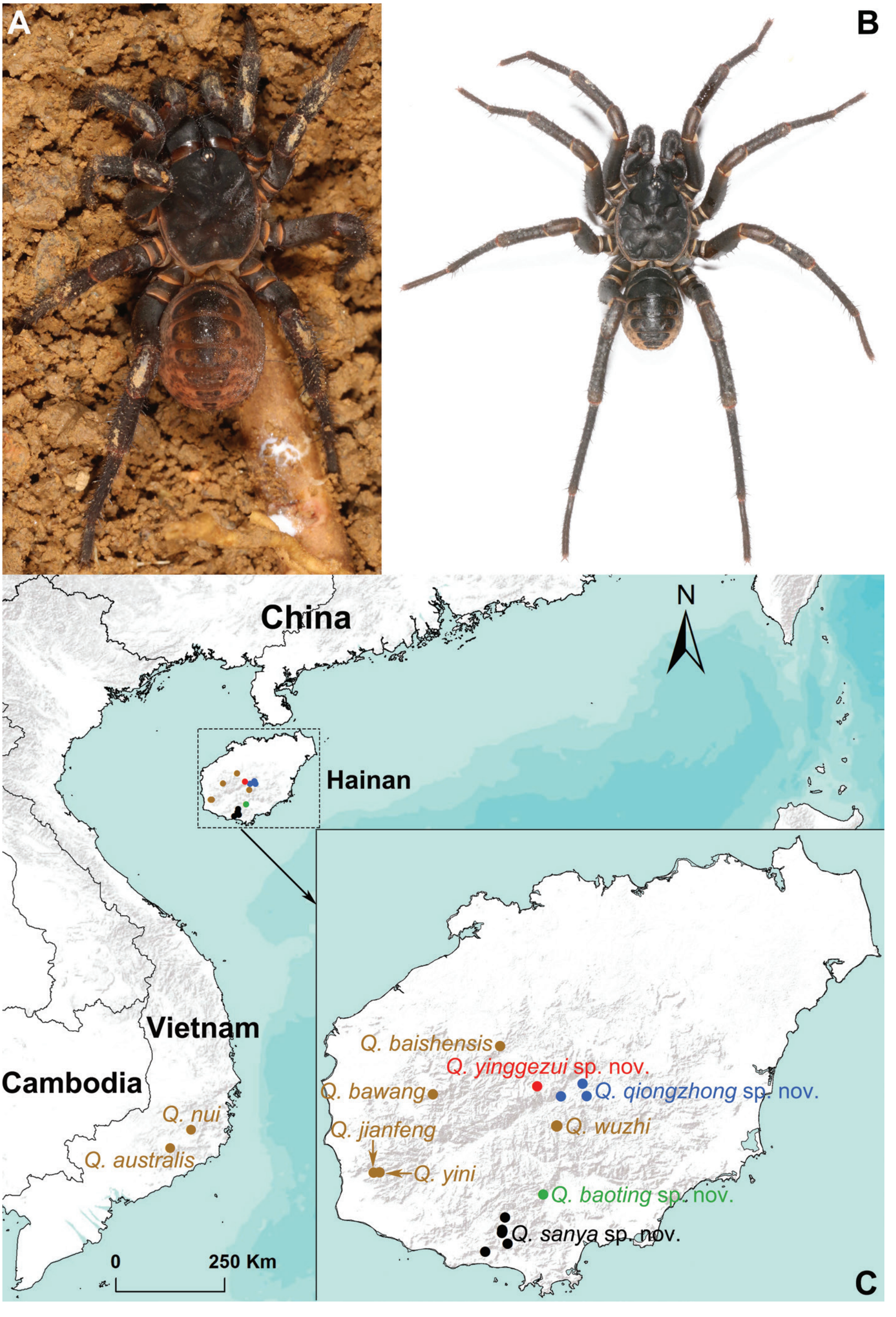

Figure 1. General somatic morphology of *Qiongthela baoting* sp. nov. and a map showing the type localities of seven known *Qiongthela* species and all sites of four new *Qiongthela* species in southern Vietnam and Hainan Island, China. **A** female (XUX–2017–196) **B** male (XUX–2017–195) **C** geographical map. Seven known species are indicated in brown solid circles, and four new species are indicated in red, blue, green, and black solid circles.

We examined and dissected the specimens using an Olympus SZ51 stereomicroscope. We cleaned the female genitalia in 10 mg/ml trypsase (Bomei Biotech Company, Hefei, Anhui, China) for at least 3 hours at the room temperature to dissolve soft tissues. We took the photos under the Olympus BX53 compound microscope using a digital camera CCD, and generated compound focussed images using Helicon Focus v6.7.1. All measurements were carried out under a digital camera MC170HD mounted on stereomicroscope Leica M205C and given in millimeters. Leg and palp measurements are given in the following order: leg total length (femur + patella + tibia + metatarsus + tarsus), palp total length (femur + patella + tibia + tarsus).

Abbreviations used are as follows: ALE = anterior lateral eyes; AME = anterior median eyes; BL = body length; CL = carapace length; Co = conductor; CT = contrategulum; CW = carapace width; E = embolus; OL = opisthosoma length; OW = opisthosoma width; PC = paracymbium; PLE = posterior lateral eyes; PME = posterior median eyes; RC = receptacular cluster; T = tegulum.

Taxonomy

Genus *Qiongthela* Xu & Kuntner, 2015

Type species. *Qiongthela baishensis* Xu, 2015

Diagnosis. *Qiongthela* males can be distinguished from those of all other Heptathelinae genera by the blade-like conductor narrowing towards the tip (Figs 2A–D, 3A–E, 4A–G, 6A–E), and by the tegulum bearing two obvious apophyses (Figs 2A–E, 3A–E, 4A–E, 6A–E). *Qiongthela* females differ from those of all other Heptathelinae genera by two paired receptacular clusters with numerous granula (Fig. 5A–H) (Xu et al. 2017b).

Species composition. *Q. australis* (Ono, 2002), *Q. baishensis* Xu, 2015, *Q. bawang* Xu, Liu, Kuntner & Li, 2017, *Q. jianfeng* Xu, Liu, Kuntner & Li, 2017, *Q. nui* (Schwendinger & Ono, 2011), *Q. wuzhi* Xu, Liu, Kuntner & Li, 2017, *Q. yini* Xu, Liu, Kuntner & Li, 2017.

Distribution. China (Hainan), Vietnam.

Qiongthela baoting sp. nov.

http://zoobank.org/C104261D-DBFB-4A70-84FD-5CF0BD15B82E
Figure 2

Type material. Holotype: CHINA · 1 ♂; Hainan Province, Baoting County, Maogan Town, Zaye Village; 18.60°N, 109.57°E; alt. 410 m; 21 August 2017; D. Li, F.X. Liu and X. Xu leg.; XUX–2017–195 (matured on 25 August 2018 at HNU). **Paratype:** CHINA · 1 ♀; same data as for holotype; XUX–2017–196.

Diagnosis. Male of *Q. baoting* sp. nov. can be distinguished from that of *Q. baishensis*, *Q. jianfeng*, *Q. nui*, *Q. wuzhi*, and the other three new species by the con-

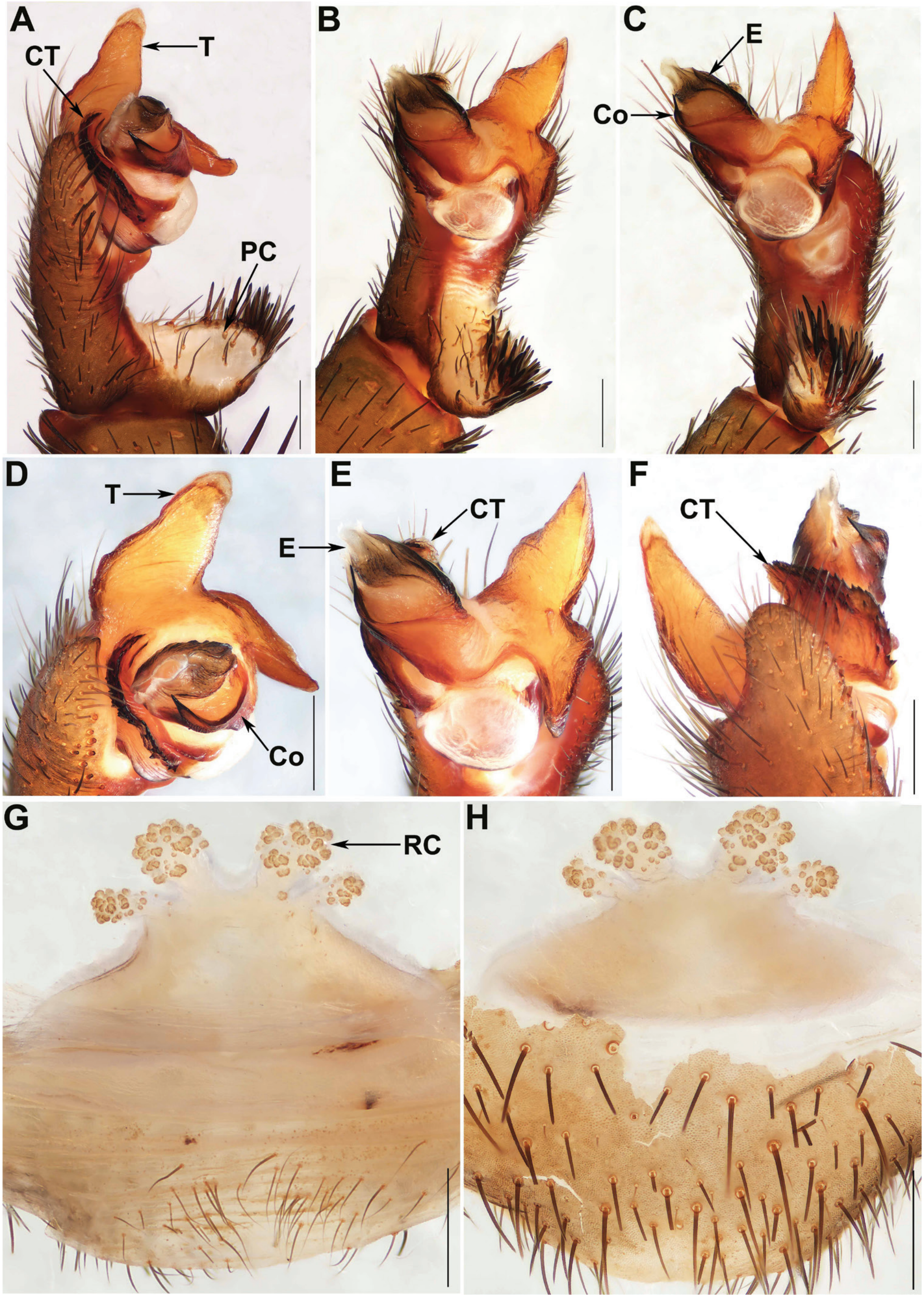

Figure 2. Male and female genital anatomy of *Qiongthela baoting* sp. nov. **A** palp prolateral view **B** palp ventral view **C** palp retrolateral view **D–F** palp distal view **G** vulva dorsal view **H** vulva ventral view. **A–F** XUX–2017–195 (holotype) **G–H** XUX–2017–196. Scale bars: 0.5 mm.

ductor with a pointed apex (Fig. 2A–D); from all the other *Qiongthela* species by the contrategulum with four edges distally (Fig. 2A, D), and by the marginal apophysis of the tegulum with a flake-like, semi-translucent apex (Fig. 2A, D, F). Female of *Q. baoting* sp. nov. differs from that of *Q. baishensis* and *Q. nui* by the base of the lateral receptacular clusters close to the inners, and by the genital stalks of the inners thicker than those of the laterals (Fig. 2G, H); from the other *Qiongthela* species by two paired receptacular clusters all along the anterior margin of the bursa copulatrix, with distinct genital stalks, and the inners larger than the laterals (Fig. 2G, H).

Description. ***Male*** (holotype, Fig. 1B). Carapace dark brown; opisthosoma light brown, with 12 dark brown tergites, close to each other, the first 2–7 larger than others, and the fourth largest; sternum narrow, much longer than wide; a few fine pointed hairs running over the ocular area; chelicerae with promargin of cheliceral groove bearing 9 denticles of variable size; legs with firm hairs and spines; 7 spinnerets. Measurements: BL 12.39, CL 6.17, CW 5.62, OL 6.44, OW 4.52; ALE > PLE > PME > AME; leg I 17.60 (5.15 + 1.65 + 3.82 + 4.28 + 2.69), leg II 16.95 (4.71 + 1.48 + 3.67 + 4.30 + 2.79), leg III 19.26 (4.56 + 1.31 + 3.73 + 6.04 + 3.63), leg IV 25.77 (6.52 + 1.61 + 5.32 + 7.68 + 4.64).

Palp. Cymbium with a short, thick projection dorsally (Fig. 2F); paracymbium unpigmented and unsclerotised prolaterally, with numerous setae at the tip (Fig. 2A, B). Contrategulum with an irregular dentate edge proximally and four edges distally: the inner edge sharp, very short; the middle two edges serrate, one towards the proximal portion of contrategulum, the other ended at the centre of the contrategulum; the outer edge short, smooth, slightly sclerotised (Fig. 2A, D, F). The marginal apophysis of tegulum long, wide basally, with a flake-like and semi-translucent apex distally (Fig. 2A, D), a proximally directed terminal apophysis of tegulum with smooth margin, narrowing to a slightly hooked apex (Fig. 2A–E). Conductor situated ventro-proximally on embolus, basal portion fused with embolus, distal free narrowing to a pointed apex (Fig. 2A–E). Embolus largely sclerotised, with a wide, flat opening of sperm duct distally (Fig. 2A, D, E).

Female (Fig. 1A). Carapace dark brown; opisthosoma reddish brown, with 12 red-brown tergites, close to each other, the first 2–7 larger than the others, and the fourth largest; sternum narrow, nearly twice as long as wide; a few fine pointed hairs running over the ocular area; chelicerae robust with promargin of cheliceral groove containing 10 denticles of variable size; legs with firm hairs and spines; 7 spinnerets. Measurements: BL 16.35, CL 7.30, CW 6.12, OL 7.59, OW 6.25; ALE > PLE > PME > AME; palp 10.09 (3.66 + 1.01 + 2.60 + 2.82), leg I 11.78 (3.69 + 1.16 + 3.02 + 2.35 + 1.57), leg II 12.34 (3.94 + 1.44 + 2.69 + 2.58 + 1.70), leg III 10.99 (3.21 + 1.04 + 2.35 +2.90 + 1.47), leg IV 20.26 (5.85 + 1.93 + 4.17 + 5.45 + 2.86).

Female genitalia. Two pairs of receptacular clusters along the anterior margin of the bursa copulatrix, close to each other, the inner ones distinctly larger than the laterals, with genital stalks thicker than those of the laterals (Fig. 2G, H).

Etymology. The species epithet, a noun in apposition, refers to the type locality.

Distribution. Hainan (Baoting), China.

GenBank accession number. Holotype (XUX–2017–195): MN911989.

Qiongthela qiongzhong **sp. nov.**
http://zoobank.org/09106528-8A15-461F-9042-3026C7C9E099
Figure 3

Type material. Holotype: CHINA · 1 ♂; Hainan Province, Qiongzhong County, Yinggen Town, Chaocan Village; 19.08°N, 109.74°E; alt. 440 m; 15 August 2017; D. Li, F.X. Liu and X. Xu leg.; XUX–2017–156 (matured on 6 November 2017 at HNU). **Paratypes:** CHINA · 2 ♂♂, 2 ♀♀; same data as for holotype; XUX–2017–159, 161 (♂ matured on 6 November 2017 at HNU), XUX–2017–163 (♀ matured on 3 June 2018 at HNU), XUX–2017–158 · 6 ♂♂; Hainan Province, Qiongzhong County, Yinggen Town, Nabai Village; 19.03°N, 109.76°E; alt. 320 m; 14 August 2017; D. Li, F.X. Liu and X. Xu leg.; XUX–2017–148, 151, 154 (matured on 6 November 2017 at HNU), XUX–2017–149, 155 (matured on 10 November 2017 at HNU), XUX–2017–150 (matured on 14 January 2018 at HNU) · 1 ♀; Hainan Province, Qiongzhong County, Hongmao Town, Caohui Village; 19.03°N, 109.65°E; alt. 345 m; 14 August 2017; D. Li, F.X. Liu and X. Xu leg.; XUX–2017–144 · 2 ♂♂, 2 ♀♀; same locality as for holotype; 19.08°N, 109.74°E; alt. 420 m; 17 August 2019; D. Li, F.X. Liu, X. Xu and L. Yu leg.; XUX–2019–111 (♂, matured on 16 October 2019 at HNU), XUX–2019–112 (♂, matured on 23 October 2019 at HNU), XUX–2019–108, 109.

Diagnosis. Males of *Q. qiongzhong* sp. nov. resemble those of *Q. yinggezui* sp. nov., but can be distinguished from those of the latter by the marginal apophysis of the tegulum with a blunt apex (Fig. 3A, D); from *Q. baoting* sp. nov. by the tegulum marginal apophysis with a non-translucent apex (Fig. 3A, D), by the contrategulum with two edges distally (Fig. 3A, D), and by the cymbial projection long and thin (Fig. 3G); from *Q. australis* by the conductor with a slightly bent apex (Fig. 3C, E, G), and by the contrategulum lacking beak-like extension (Fig. 3F); from *Q. jianfeng* by the terminal apophysis of the tegulum abruptly narrowed distally (Fig. 3A–C); from *Q. nui*, *Q. sanya* sp. nov., and *Q. wuzhi* by the marginal apophysis of the tegulum with a blunt apex (Fig. 3A, D). Females of *Q. qiongzhong* sp. nov. can be distinguished from those of *Q. bawang* and *Q. jianfeng* by the receptacular clusters with indistinct genital stalks (Fig. 3H, J); from those of *Q. baishensis*, *Q. baoting* sp. nov., *Q. nui*, *Q. yini*, and *Q. wuzhi* by the similar-sized receptacular clusters or the laterals slightly larger than the inners (Fig. 3H–M).

Description. ***Male*** (holotype). In alcohol carapace light reddish brown; opisthosoma light brown, with brown 12 tergites, close to each other, the first 2–7 larger than others, and the fourth largest; sternum narrow, nearly twice as long as wide; a few fine pointed hairs running over the ocular area; chelicerae with promargin of cheliceral groove containing 10 denticles of variable size; legs with firm hairs and spines; 7 spinnerets. Measurements: BL 13.34, CL 6.13, CW 5.61, OL 7.17, OW 5.50; ALE > PLE > PME > AME; leg I 16.64 (4.88 + 1.54 + 4.04 + 4.03 + 2.15), leg II 16.25 (4.62 + 1.32 + 3.60 + 4.51 + 2.20), leg III 17.39 (4.57 + 1.34 + 3.48 + 5.32 + 2.68), leg IV 22.50 (6.06 + 1.49 + 5.11 + 6.81 + 3.04).

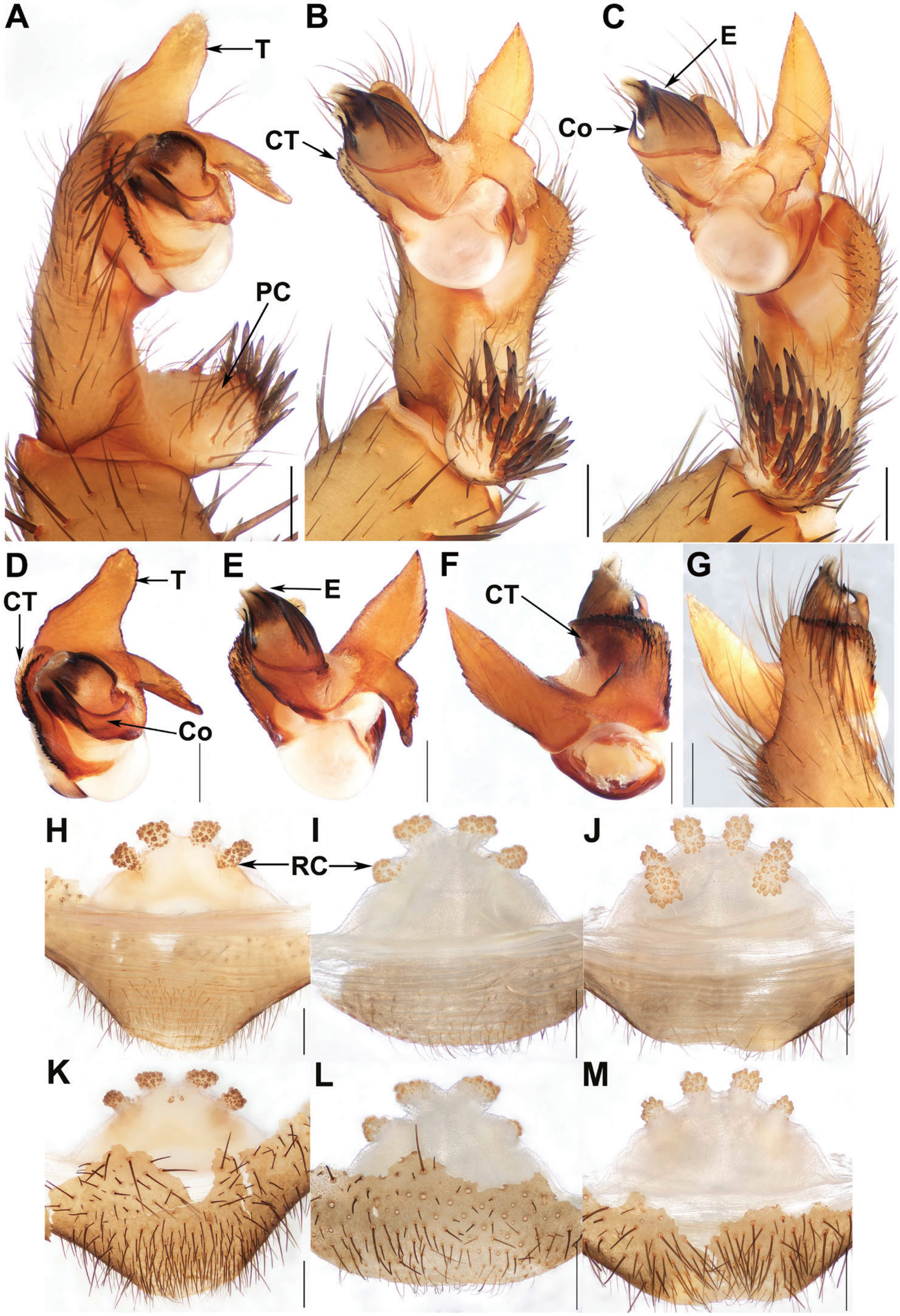

Figure 3. Male and female genital anatomy of *Qiongthela qiongzhong* sp. nov. **A** palp prolateral view **B** palp ventral view **C** palp retrolateral view **D–G** palp distal view **H–J** vulva dorsal view **K–M** vulva ventral view. **A–C, G** XUX–2017–156 (holotype) **D–F** XUX–2017–159 **H, K** XUX–2017–158 **I, L** XUX–2017–144 **J, M** XUX–2017–163. Scale bars: 0.5 mm.

Palp. Cymbium with a long, thin projection dorsally (Fig. 3G); paracymbium unpigmented and unsclerotised prolaterally, with numerous setae at the tip (Fig. 3A, B). Contrategulum with a proximally irregular dentate edge and two distal edges: the inner one irregularly dentate, and the outer one sharp, semi-translucent (Fig. 3A, D–F). The marginal apophysis of tegulum with a blunt, slightly dentate apex distally, a proximally directed terminal apophysis of tegulum with several denticles and an abruptly narrowed and slightly hooked apex (Fig. 3A–E). Conductor situated ventro-proximally on embolus, the basal portion fused with embolus, distal free, narrowing to a slightly bent apex (Fig. 3A–C, E). Embolus largely sclerotised, retrolaterally with numerous longitudinal ribs, and with a wide, flat sperm duct opening distally (Fig. 3A, D, F).

Female (XUX–2017–158). In alcohol carapace reddish brown; opisthosoma brown; opisthosoma with 12 dark brown tergites, separated from each other, the first 2–7 larger than others, and the fourth largest; sternum narrow, nearly twice as long as wide; a few fine pointed hairs running over the ocular area; chelicerae with promargin of cheliceral groove containing 10 strong denticles of variable size; legs with firm hairs and spines; 7 spinnerets. Measurements: BL 16.59, CL 6.93, CW 6.18, OL 9.48, OW 7.83; ALE > PLE > PME > AME; palp 11.02 (3.80 + 1.20 + 2.86 + 3.16), leg I 13.69 (4.53 + 1.52 + 2.99 + 2.87 + 1.77), leg II 12.61 (3.80 + 1.39 + 2.77 + 2.78 + 1.87), leg III 12.06 (3.71 + 1.04 + 2.46 + 3.27 + 1.58), leg IV 20.31 (6.08 + 1.67 + 4.24 + 5.55 + 2.77).

Female genitalia. Two pairs of receptacular clusters along the anterior margin of the bursa copulatrix, receptacular clusters similar size or the inner ones slightly smaller than the lateral ones, with indistinct genital stalks (Fig. 3H–M).

Variation. Males and females vary in body size. The range of measurements in males (N = 11): BL 12.43–17.24, CL 5.99–7.80, CW 5.61–7.12, OL 6.52–9.52, OW 4.67–7.02; females (N = 5): BL 9.93–16.59, CL 4.91–7.38, CW 4.25–6.51, OL 4.93–9.48, OW 3.48–7.83. In addition, female genitalia show considerable intraspecific variation: the receptacular clusters vary in shape: triangular (Fig. 3H, J), or oval (Fig. 3I, L); the ventral side of the bursa copulatrix with two small granula (Fig. 3K); the posterior part of genital area arched (Fig. 3I, L), or with a slightly notch in the middle (Fig. 3J, M).

Etymology. The species epithet, a noun in apposition, refers to the type locality.

Distribution. Hainan (Qiongzhong), China.

GenBank accession number. Holotype (XUX–2017–156): MN911987.

Qiongthela sanya **sp. nov.**
http://zoobank.org/F46F043A-D2BD-4BE0-B24D-771C53F26BDB
Figures 4, 5

Type material. Holotype: CHINA · 1 ♂; Hainan Province, Sanya City, Tianya District, Zhaka Village; 18.50°N, 109.41°E; alt. 240 m; 22 August 2017; D. Li, F.X. Liu and X. Xu leg.; XUX–2017–219. **Paratypes:** CHINA · 1 ♀; same data as for holotype; XUX–2017–218 · 1 ♀; Hainan Province, Sanya City, Heshangling; 18.35°N, 109.32°E; alt. 130 m; 1 August 2017; D. Li, F.X. Liu, Z.T. Zhang and X. Xu leg.;

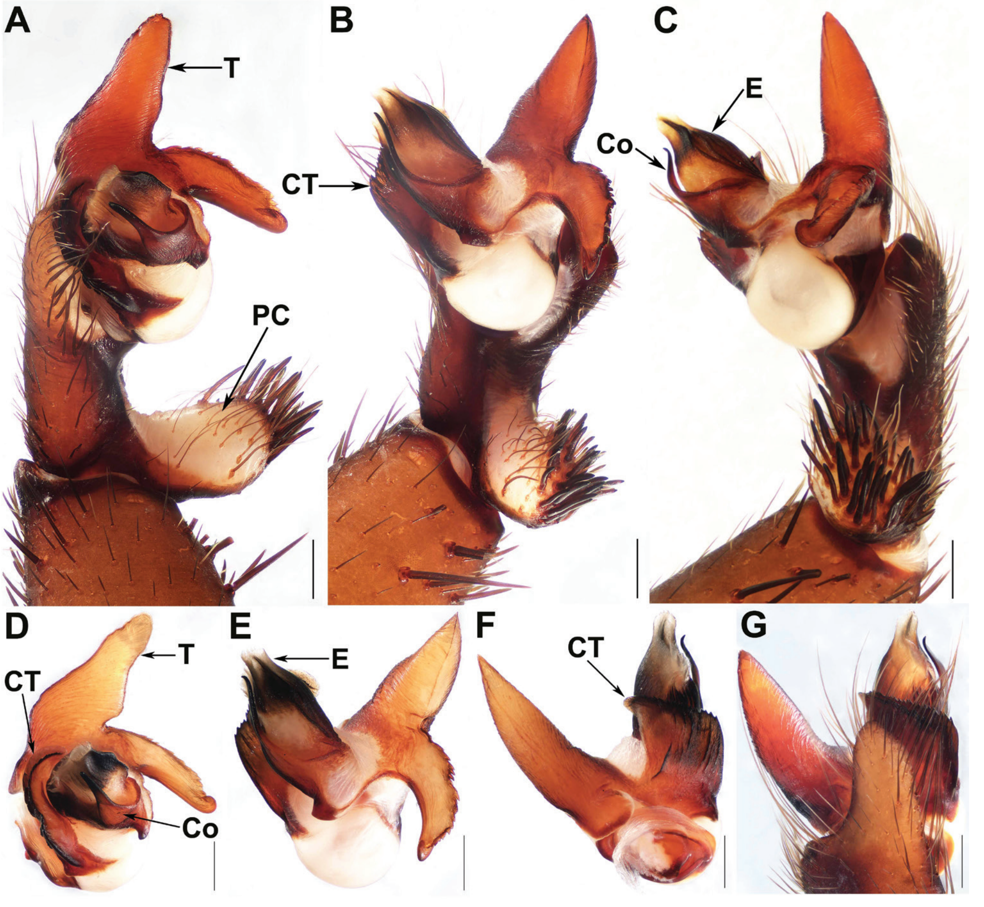

Figure 4. Male genital anatomy of *Qiongthela sanya* sp. nov. **A** palp prolateral view **B** palp ventral view **C** palp retrolateral view **D–G** palp distal view. **A–C, G** XUX–2017–219 (holotype) **D–F** XUX–2019–134. Scale bars: 0.5 mm.

XUX–2017–025 · 1 ♂, 2 ♀♀; Hainan Province, Sanya City, Tianya District, Baoqian Village; 18.39°N, 109.42°E; alt. 195 m; 22 August 2017; D. Li, F.X. Liu and X. Xu leg.; XUX–2017–205 (♂ matured on 29 October 2017 at HNU), XUX–2017–202, 209 · 1 ♂, 10 ♀♀; Hainan Province, Sanya City, Tianya District, Nandao Farm, Sanmudong; 18.44°N, 109.40°E; alt. 200 m; 21 August 2019; D. Li, F.X. Liu, X. Xu and L. Yu leg.; XUX–2019–134 (♂ matured on 2 October 2019 at HNU), XUX–2019–136 to 137H · 9 ♀♀; Hainan Province, Sanya City, Tianya District, Nandao Farm, Haiyan Group; 18.45°N, 109.40°E; alt. 215 m; 22 August 2017; D. Li, F.X. Liu and X. Xu leg.; XUX–2017–214 to 217, XUX–2017–221, 222, XUX–2017–225 to 227 · 1 ♀; Hainan Province, Sanya City, Tianya District, between Hongxing Farm and Zhaka Village; 18.50°N, 109.41°E; alt. 235 m; 22 August 2017; D. Li, F.X. Liu and X. Xu leg.; XUX–2017–220 · 1 ♀; Hainan Province, Sanya City, Tianya District, Nandao Farm, Haiying Group; 18.43°N, 109.39°E; alt. 200 m; 21 August 2019; D. Li, F.X. Liu, X. Xu and L. Yu leg.; XUX–2019–131.

Diagnosis. Males of *Q. sanya* sp. nov. can be distinguished from those of *Q. baoting* sp. nov. by the longer tegulum marginal apophysis with a non-translucent apex (Fig. 4A, D), and by the conductor with a bent apex (Fig. 4C, E, F, G); from those of the other *Qiongthela* species by the conductor base with a triangular apophysis ventrally (Fig. 4A–E). Females of *Q. sanya* sp. nov. can be distinguished from *Q. australis*, *Q. yini* and *Q. yinggezui* sp. nov. by the inner receptacular clusters smaller than the lateral ones (Fig. 5A–H); from those of the other *Qiongthela* species by the inner receptacular clusters along the anterior margin of the bursa copulatrix, the laterals located slightly on the dorsal wall of the bursa copulatrix, and by the trapezoidal bursa copulatrix (Fig. 5A–H).

Description. ***Male*** (holotype). In alcohol carapace reddish dark; opisthosoma brown, with 12 reddish dark tergites, close to each other, the first 2–7 larger than others, and the fourth largest; sternum narrow, nearly twice as long as wide; a few fine pointed hairs running over the ocular area; chelicerae with promargin of cheliceral groove containing 9 denticles of variable size; legs with firm hairs and spines; 7 spinnerets. Measurements: BL 13.40, CL 6.47, CW 5.87, OL 6.80, OW 5.20; ALE > PLE > PME > AME; leg I 22.06 (6.30 + 1.62 + 5.26 + 5.97 + 2.90), leg II 20.17 (5.16 + 1.50 + 4.81 + 5.77 + 2.94), leg III 22.02 (5.65 + 1.62 + 4.41 + 6.83 + 3.52), leg IV 28.13 (7.15 + 1.87 + 6.00 + 8.93 + 4.17).

Palp. Cymbium with a short projection dorsally (Fig. 4G); prolateral side of paracymbium unpigmented and unsclerotised, with numerous setae at the tip (Fig. 4A–C). Contrategulum with two distal edges: the inner one strongly dentate, and the outer one smooth, sharp, semi-translucent (Fig. 4A, D, F). Tegulum with a long, pointed, distally directed marginal apophysis, the proximally directed terminal apophysis with a dentate margin and continuously narrowing to a rounded, hooked apex (Fig. 4A–E). Conductor situated ventro-proximally on embolus, fused with embolus at the basal portion, distal free narrowing to a bent apex (Fig. 4B, C, E–G); conductor base with a triangular apophysis ventrally (Fig. 4A–E). Embolus largely sclerotised, with a wide, flat sperm duct opening distally, retrolaterally with numerous longitudinal ribs (Fig. 4B, C, E).

Female (XUX–2017–215). In alcohol carapace reddish dark; opisthosoma dark brown, with 12 reddish dark tergites, close to each other, the first 2–7 larger than others, and the fourth largest; sternum narrow, much longer than wide; a few fine pointed hairs running over the ocular area; chelicerae with promargin of cheliceral groove containing 10 strong denticles of variable size; legs with firm hairs and spines; 7 spinnerets. Measurements: BL 25.50, CL 11.95, CW 10.97, OL 13.00, OW 10.75; ALE > PLE > PME > AME; palp 18.86 (6.35 + 2.20 + 4.73 + 5.58), leg I 23.55 (8.14 + 3.11 + 5.51 + 4.44 + 2.35), leg II 21.33 (7.25 + 2.68 + 4.87 + 4.25 + 2.28), leg III 22.43 (7.19 + 2.50 + 4.99 + 4.98 + 2.78), leg IV 34.17 (10.27 + 3.17 + 7.11 + 9.07 + 4.55).

Female genitalia. The inner receptacular clusters along the anterior margin of the bursa copulatrix, the lateral ones located slightly on the dorsal wall of the bursa copulatrix; the inner ones smaller than the lateral ones, with short or long genital stalks. The bursa copulatrix trapezoidal (Fig. 5A–H).

Variation. Males and females vary in body size. The range of measurements in males (N = 3): BL 13.40–15.01, CL 6.47–7.21, CW 5.87–6.53, OL 6.16–7.53, OW

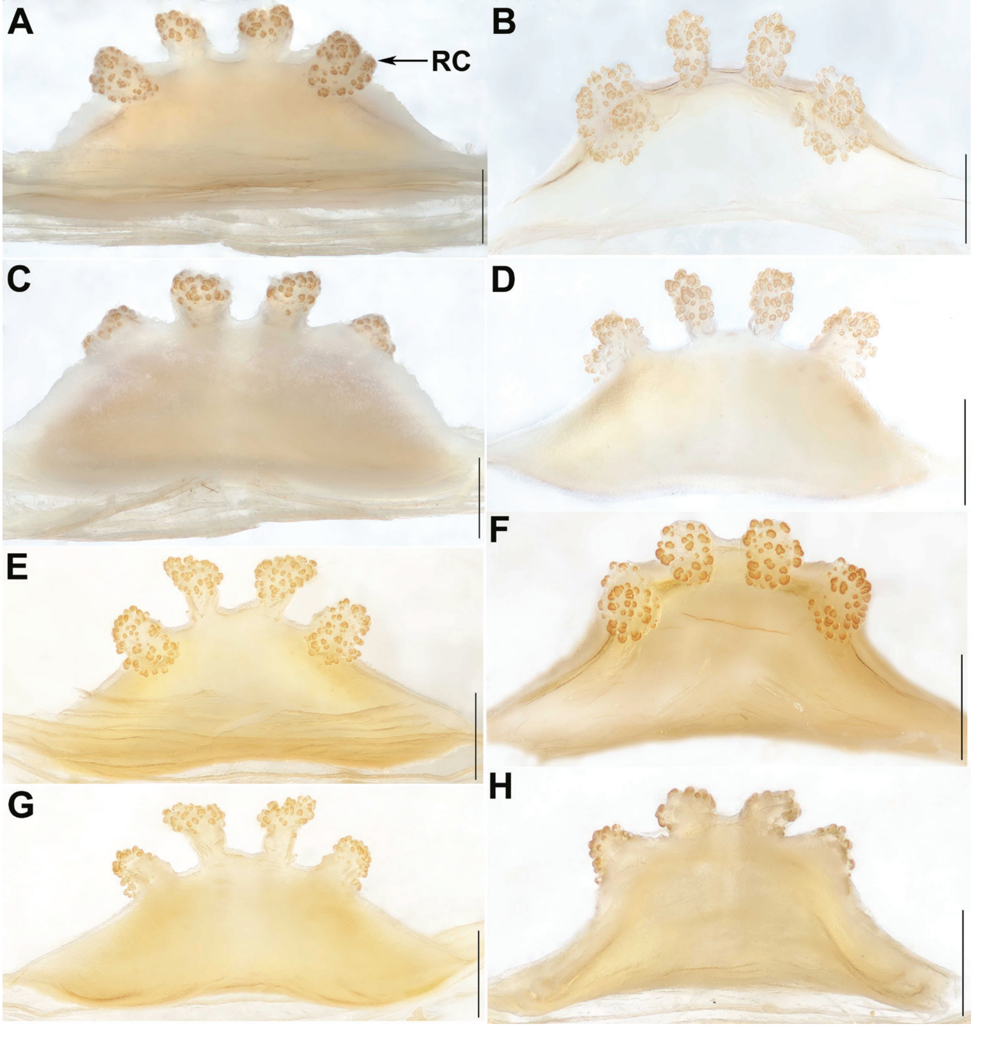

Figure 5. Female genital anatomy of *Qiongthela sanya* sp. nov. **A, B, E, F** vulva dorsal view **C, D, G, H** vulva ventral view. **A, C** XUX–2017–215 **B, D** XUX–2017–025 **E, G** XUX–2017–226 **F, H** XUX–2017–227. Scale bars: 0.5 mm.

4.47–5.20; females (*N* = 25): BL 15.41–27.74, CL 7.32–14.14, CW 6.23–11.59, OL 7.33–13.49, OW 5.70–11.84. In addition, female genitalia show intraspecific variation: the inner pair of the receptacular clusters along the anterior margin of the bursa copulatrix upward, with short or long genital stalks (Fig. 5A–E, G), or clusters toward the dorsal margin (Fig. 5F, H).

Etymology. The species epithet, a noun in apposition, refers to the type locality.

Distribution. Hainan (Sanya), China.

GenBank accession number. Holotype (XUX–2017–219): MN911990.

***Qiongthela yinggezui* sp. nov.**
http://zoobank.org/72CEC4E7-BE97-4E42-8F90-559DAA2AC067
Figure 6

Type material. Holotype: CHINA· 1 ♂; Hainan Province, Qiongzhong County, 3.7 Km to Yinggezui; 19.07°N, 109.55°E; alt. 710 m; 11 August 2017; D. Li, F.X. Liu, Z.T. Zhang and X. Xu leg.; XUX–2017–114 (matured on 29 September 2017 at HNU). **Paratypes:** CHINA · 3 ♀♀; same data as for holotype; XUX–2017–115, 116, 121.

Diagnosis. Male of *Q. yinggezui* sp. nov. differs from that of *Q. australis* by the conductor base wide and with a bent apex (Fig. 6A–F), and by the shorter paracymbium (Fig. 6A); from *Q. nui* by the embolus with a smooth surface retrolaterally (Fig. 6B, C, E); from *Q. baoting* sp. nov. by the cymbium with an elongated projection (Fig. 6F), and by the conductor with a bent apex (Fig. 6B–E); from *Q. jianfeng*, *Q. qiongzhong* sp. nov. and *Q. sanya* sp. nov. by the scutiform marginal apophysis of the tegulum thick basally and pointed distally (Fig. 6A–F), and by the embolus with a smooth surface retrolaterally (Fig. 6B, C, E). Females of *Q. yinggezui* sp. nov. can be distinguished from those of *Q. australis* by the similar-sized receptacular clusters, and the lateral ones slightly located on the dorsal wall of the bursa copulatrix (Fig. 6G); from *Q. yini* by the receptacular clusters with more granula (Fig. 6G, H); from *Q. sanya* sp. nov. by the lack of genital stalks (Fig. 6G, H); from those of the other *Qiongthela* species by the inner receptacular clusters situated at the anterior margin of bursa copulatrix, the lateral pair located on the dorsal wall of the bursa copulatrix (Fig. 6G, H).

Description. Male (holotype). In alcohol carapace light reddish brown; opisthosoma light brown, with 12 brown tergites, separated from each other, the first 2–7 larger than others, and the fourth largest; sternum narrow, nearly twice as long as wide; a few fine pointed hairs running over the ocular area; chelicerae with promargin of cheliceral groove containing 9 denticles of variable size; legs with firm hairs and spines; 7 spinnerets. Measurements: BL 13.60, CL 5.99, CW 6.30, OL 7.29, OW 5.48; ALE > PLE > PME > AME; leg I 17.44 (4.82 + 1.55 + 4.10 + 4.51 + 2.46), leg II 17.50 (4.73 + 1.43 + 3.97 + 4.80 + 2.57), leg III 16.73 (4.68 + 1.36 + 2.40 + 5.59 + 2.70), leg IV 25.19 (6.52 + 1.62 + 5.28 + 8.00 + 3.77).

Palp. Cymbium with an elongated projection dorsally (Fig. 6F); prolateral side of paracymbium unpigmented and unsclerotised, with numerous setae at the tip (Fig. 6A, B). Contrategulum with a proximally irregular dentate edge and two distal edges: the inner one dentate, the outer one smooth, sharp, semi-translucent, fused with the inner one at the middle portion of contrategulum (Fig. 6A, D–F). The marginal apophysis of tegulum long, pointed with a sharp apex, a proximally directed terminal apophysis with finely dentate margin and continuously narrowing to a rounded, hooked apex (Fig. 6A–E). Conductor situated ventro-proximally on embolus, fused with embolus at the basal portion, distal free narrowing to a bent apex (Fig. 6A–C, E). Embolus largely sclerotised, with a wide, flat sperm duct opening, and with a smooth surface retrolaterally (Fig. 6A–E).

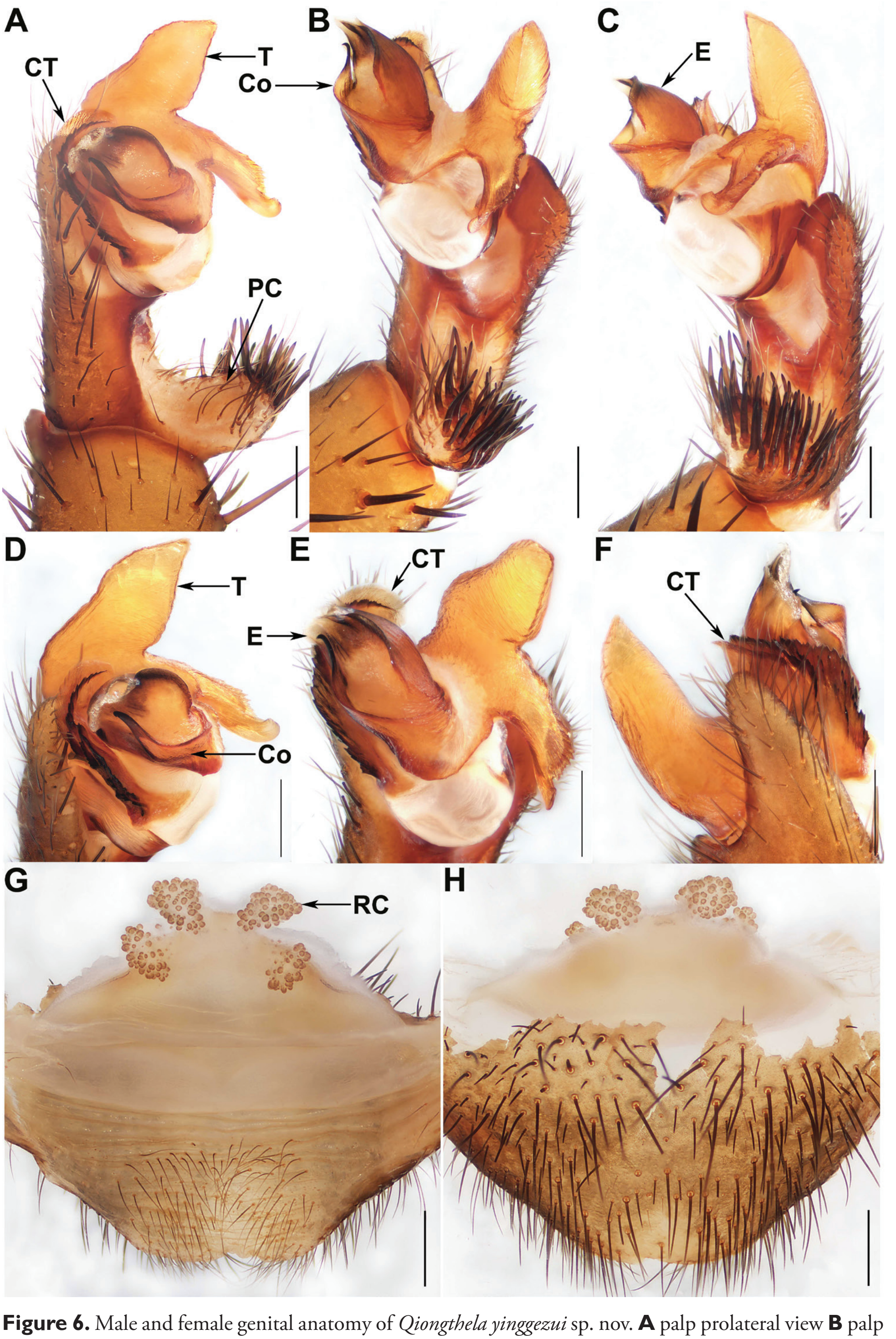

Figure 6. Male and female genital anatomy of *Qiongthela yinggezui* sp. nov. **A** palp prolateral view **B** palp ventral view **C** palp retrolateral view **D–F** palp distal view **G** vulva dorsal view **H** vulva ventral view. **A–F** XUX–2017–114 (holotype) **G–H** XUX–2017–121. Scale bars: 0.5 mm.

Female (XUX–2017–121). In alcohol carapace reddish brown; opisthosoma brown; opisthosoma with 12 tergites, closed to each other, the first 2–7 larger than others, and the fourth largest; sternum narrow, more than twice the width; a few fine pointed hairs running over the ocular area; chelicerae with promargin of cheliceral groove containing 10 denticles of variable size; legs with firm hairs and spines; 7 spinnerets. Measurements: BL 14.76, CL 7.03, CW 6.39, OL 7.82, OW 6.03; ALE > PLE > PME > AME; palp 13.30 (5.30 + 1.23 + 2.85 + 3.91), leg I 14.35 (4.84 + 1.54 + 3.21 + 2.95 + 1.82), leg II 12.72 (3.54 + 1.24 + 2.97 + 2.99 + 1.98), leg III 13.78 (4.20 + 1.28 + 2.60 + 3.71 + 1.99), leg IV 20.21 (5.29 + 1.38 + 4.52 + 5.78 + 3.24).

Female genitalia. Two paired of the similar-sized receptacular clusters, the inner ones along the anterior margin of the bursa copulatrix, and the lateral ones located slightly on the dorsal wall of the bursa copulatrix, without genital stalks (Fig. 6G, H).

Variation. Females vary in body size. The range of measurements in females (N = 3): BL 11.51–14.76, CL 4.68–7.03, CW 4.54–6.39, OL 5.54–7.82, OW 4.32–6.03.

Etymology. The species epithet, a noun in apposition, refers to the type locality.

Distribution. Hainan (Yinggezui), China.

GenBank accession number. Holotype (XUX–2017–114): MN911988.

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RESEARCH ARTICLE

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Asianopis gen. nov., a new genus of the spider family Deinopidae from Asia

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Abstract

A new genus of the spider family Deinopidae C.L. Koch, 1850 is described from Asia: *Asianopis* Lin & Li **gen. nov.**, with *A. zhuanghaoyuni* Lin & Li **sp. nov.** as the type species. The new genus is divided into two species groups, of which the *liukuensis*-group includes two species: *A. dumogae* (Merian, 1911) **sp. reval. comb. nov.** (♀) and *A. liukuensis* (Yin, Griswold & Yan, 2002) **comb. nov.** (♂♀); and the *zhuanghaoyuni*-group comprises five species: *A. celebensis* (Merian, 1911) **comb. nov.** (♂), *A. konplong* (Logunov, 2018) **comb. nov.** (♂), *A. wangi* Lin & Li **sp. nov.** (♂♀), *A. wuchaoi* Lin & Li **sp. nov.** (♂♀), and *A. zhuanghaoyuni* Lin & Li **sp. nov.** All previously described species are transferred from *Deinopis* MacLeay, 1839. *Deinopis scrubjunglei* Caleb & Mathai, 2014 is treated as a **junior synonym** of *Asianopis liukuensis* **comb. nov.**

Keywords

New combination, new species, species groups, systematics, taxonomy

Introduction

The spider family Deinopidae C.L. Koch, 1850 (Araneae, Deinopoidea), known as net-casting or ogre-faced spiders, is a small family that consisted of two genera and 64 species prior to the current study (World Spider Catalog 2019). The genus *Deinopis* was established by MacLeay (1839) based on *Deinopis lamia* MacLeay, 1839 (♂♀) from Cuba. The other genus, *Menneus*, was established by Simon (1876) based on *Menneus tetragnathoides* Simon, 1876 (♂) from Angola.

Ten species of Deinopidae were known from Asia: *Deinopis aruensis* Roewer, 1938 (♀) and *D. celebensis* Merian, 1911 from Indonesia; *D. fasciculigera* Simon, 1909 (♀) and *D. konplong* Logunov, 2018 (♂) from Vietnam; *D. scrubjunglei* Caleb & Mathai, 2014 (♂♀) from India; *D. gubatmakiling* Barrion-Dupo & Barrion, 2018 (juvenile), *D. labangan* Barrion-Dupo & Barrion, 2018 (♀), and *D. luzonensis* Barrion-Dupo & Barrion, 2018 (♀) from the Philippines; *D. kollari* Doleschall, 1859 (♂) from Myanmar and Malaysia; *D. liukuensis* Yin, Griswold & Yan, 2002 (♂♀) from China. Here, we describe a new genus and three new species, and present a molecular phylogenetic analysis of these spiders.

Material and methods

All specimens were preserved in 80% ethanol. Metatarsi and tarsi were removed for preservation in 100% ethanol for subsequent molecular work. Epigynes were cleared in proteinase K at 56 °C to dissolve non-chitinous tissues for three hours. Specimens were examined under a LEICA M205C stereomicroscope. Photomicroscope images were taken with an Olympus C7070 zoom digital camera (7.1 megapixels). Laboratory habitus photographs were taken with a Canon 5D Mark III digital camera equipped with a Canon MP-E 65 mm lens. Photos were stacked with Helicon Focus (version 6.7.1) or Zerene Stacker (version 1.04) and processed in Adobe Photoshop CC 2018. Photographs of *Asianopis celebensis* comb. nov. were taken by a KEYENCE. Photographs of *Asianopis liukuensis* comb. nov. from India (i.e., the type materials of *D. scrubjunglei*) were taken using a Leica DFC500 HD camera mounted on a Leica M205A stereomicroscope.

All measurements are in millimetres. Eye sizes are measured as the maximum diameter from either the dorsal or frontal view. Leg measurements are given as follows: total length (femur, patella+tibia, metatarsus, tarsus). Copulatory duct turns are defined by the number of apparent loops on the lateral margin of the copulatory/fertilization duct complex in dorsal view. The length of the embolic tip fold is measured as from the beginning of the fold to the embolic tip (Fig. 22D, E). The terminology used in the text and figures follows Coddington et al. (2012). Distribution maps were generated using ArcMap software (version 10.2).

A total of 31 specimens of Deinopidae were collected for phylogenetic analysis (Suppl. material 1: Table S1). Sequences of seven specimens were from the National Center for Biotechnology Information (NCBI) public data, and the other 24 were from recent field collections. Whole genomic DNA was extracted from 2–4 legs using a TIANamp Genomic DNA kit (TIANGEN Inc., Beijing, China) following the manufacturer's protocol. Seven gene fragments were amplified in 20-μL reactions: COI (~640 bp), 12S (~330 bp), 16S (~470 bp), 18S (~1700 bp), 28S (~1200 bp), H3 (~310 bp) and wnt (~330 bp). Primers and PCR conditions for each locus are listed in Suppl. material 1: Table S2. Sequence chromatograms were proofed and edited using Sequencher version 4.2 Demo (Gene Codes Corporation, Ann Arbor, MI USA). The COI, H3 and wnt fragments were translated in MEGA version 7 (Kumar et al. 2016) to check for the presence of stop codons. A representative of the family Uloboridae was used as the outgroup, with the corresponding sequences downloaded from NCBI. The complete list of 32 taxa and GenBank accession numbers are provided in Suppl. material 1: Table S1.

Multiple sequence alignments were carried out with MAFFT version 7.243 (Katoh and Standley 2013). Alignments of the protein-coding COI, H3 and wnt genes were produced using the L-INS-i method. As for the highly variable ribosomal genes, the E-INS-i method was used to generate alignments of 12S, 16S, 18S, and 28S. To exclude the ambiguously aligned regions, alignments of the ribosomal genes were processed with the program trimAl version 1.3 (Capella-Gutiérrez et al. 2009). The alignments are shown in the supplementary data.

The concatenated gene matrix was partitioned by gene using PartitionFinder version 1.1.1 (Lanfear et al. 2012). The best partitioning scheme was selected based on the Akaike information criterion (AIC) (Suppl. material 1: Table S3). Maximum likelihood (ML) analysis was performed using RAxML version 8.2.9 with a GTR + Γ + I model applied to each partition (Stamatakis 2014). One thousand non-parametric bootstrap replicates were conducted to obtain the best-scoring ML tree.

Bayesian analysis was performed using MrBayes version 3.2.6 (Ronquist et al. 2012). Two independent runs, each with four independent chains, were carried out for 20,000,000 generations and were sampled every 1,000 generations with a burn-in of 25%. Partitions and models followed the result of PartitionFinder. Convergence of the runs was determined with the standard deviation of split frequencies (<0.01). Effective sampling sizes (>200) of all parameters were checked in Tracer version 1.6 (Rambaut et al. 2014). A 50% majority-rule consensus tree was then constructed from the post-burnin sampled trees to estimate posterior probabilities (PP).

Abbreviations

ALE	anterior lateral eye	**ETA**	embolic terminal apophysis
AME	anterior median eye	**FD**	fertilization duct
CD	copulatory duct	**MA**	median apophysis
CO	copulatory opening	**MABL**	median apophysis–basal lobe
E	embolus	**MADL**	median apophysis–distal lobe
EMA	embolic middle apophysis	**MP**	median plate
EO	embolic opening	**PLE**	posterior lateral eye

PME posterior median eye
S spermatheca
SD sperm duct
SpD spermathecal duct
T tegulum.

Museum abbreviations

HNU Hunan Normal University, Changsha, China
IZCAS Institute of Zoology, Chinese Academy of Sciences, Beijing, China
MMUE Manchester Museum of the University of Manchester, UK
NMB Naturhistorisches Museum Basel, Basel, Switzerland
SRC-ZSI Southern Regional Centre, Zoological Survey of India, Kolkata, India

Taxonomy

Family Deinopidae C.L. Koch, 1850

Genus *Asianopis* Lin & Li, gen. nov.

http://zoobank.org/C8CA3BB7-776C-4BB9-9E19-F819587E87AB

Type species. *Asianopis zhuanghaoyuni* Lin & Li, sp. nov.

Etymology. The generic name is a combination of the word "*Asia*", referring to the distribution of the genus, and the generic name *Deinopis*. The gender is feminine.

Diagnosis. *Asianopis* gen. nov. can be easily distinguished from *Deinopis* by the following characters: a prominent setal fringe can be found above the posterior median eyes in both sexes of *Asianopis* species (Fig. 4A, B), which is absent in *Deinopis* (Coddington et al. 2012: fig. 3a); the embolic tip of male *Asianopis* has an embolic middle apophysis (*liukuensis*-group, Fig. 21A), an embolic terminal apophysis or is weakly folded apically (*zhuanghaoyuni*-group, Fig. 21B–E), whereas none of these characters is present in *Deinopis* (Coddington et al. 2012: fig. 11m); the MADL in *Asianopis* is small and has a basal lobe, while in *Deinopis*, the median apophysis is larger than the MABL and covers the entire base (Coddington et al. 2012: fig. 11m); female chelicerae with many denticles between the promarginal and retromarginal teeth (Fig. 2F) or female chelicerae without denticles (Fig. 2H), in contrast, denticles are only at the center of any two adjoining retromarginal teeth in *Deinopis* (Coddington et al. 2012: fig. 5c); femora I enlarged proximally in *Asianopis* gen. nov. (*liukuensis* group, Fig. 2I) or not enlarged (*zhuanghaoyuni*-group, Fig. 2J), but they are enlarged distally in *Deinopis* (Coddington et al. 2012: fig. 3b); epigynal median plate lateral margins anchor-shaped in *Asianopis* gen. nov. (Figs 3A, 6A), but ellipsoid in *Deinopis* (Coddington et al. 2012: fig. 9b); SpD is consistently narrow in *Asianopis* gen. nov. (Figs 3B, 6B) but tapering in *Deinopis* (Coddington et al. 2012: fig. 9d).

Description. Male. Total length 12.14–16.10 (*n* = 8), carapace pear-shaped, yellow-brown (*liukuensis*-group) or brown (*zhuanghaoyuni*-group) with white edge, white line extending from cephalic area to posterior margin and small spines sparsely distributed; fovea longitudinal, indistinct. Chelicerae with a promarginal tooth and one or two retromarginal teeth (*liukuensis*-group) or with four promarginal teeth and 2–6 retromarginal teeth (*zhuanghaoyuni*-group), no denticles. Endites and labium brown, distally white; sternum diamond-shaped, brown with median light band and few small spines. Legs brown, ventrally with black pattern and short spines, leg formula 1243. Opisthosoma cylindrical, brown or dark-brown with small black spots and irregular pattern. Cribellum entire, spinnerets brown (Figs 4, 10, 13, 16).

Female. Total length 14–24 (n = 13). Chelicerae with four promarginal teeth and seven retromarginal teeth, many denticles in between the promarginal and retromarginal teeth (*liukuensis*-group) or four promarginal teeth and 8–13 retromarginal teeth, without denticles (*zhuanghaoyuni*-group). Appearance of carapace, opisthosoma and legs as in male but femora of legs I enlarged basally (*liukuensis*-group) (Fig. 2I).

Male palpal tibia longer than cymbium; cymbium almost round; tegulum distinctly wider than the diameter of embolic coil (*liukuensis*-group) or tegulum obscured by embolic coil (*zhuanghaoyuni*-group) (Figs 17, 18); embolus long and strongly coiled around MA, embolic base beginning at 7–8 o'clock position, coiled 1200° (*liukuensis*-group) or more than 1500° (*zhuanghaoyuni*-group), embolic tip straight (*liukuensis*-group) or widened subapically, folded and without apophysis (*zhuanghaoyuni*-group); MA small, directed at 7–8 o'clock position, with two lobes, a small lobe at the base, and a narrow distal lobe with two apophyses (*liukuensis*-group) or large, with two lobes, a large lobe at the base and a kidney-shaped distal lobe (*zhuanghaoyuni*-group).

Epigyne with anchor-shaped median plate, CO distinct, CD with three turns, S oval, SpD consistently wide (*liukuensis*-group) or with a well-developed MP, obscuring CO, CD with 7–8 turns, S oval, SpD consistently thin (*zhuanghaoyuni*-group).

Molecular phylogeny. The molecular phylogenetic analysis indicates with strong support that all the species in this study do not belong to *Deinopis*. Based on the 4893 bp-aligned sequences of seven gene fragments, the ML and Bayesian analyses produced the same topology, showing a split of a Southwest China clade from other clades and is strongly supported (Bootstrap value: 88; PP: 0.98) (Fig. 1). Our results are consistent with the results of Chamberland et al. (2018) who conducted a global phylogenetic analysis of *Deinopis*. Therefore, the Southwest China clade can be classified as a new genus with strong support (Bootstrap value: 100; PP: 1). Although intraspecific support values are low in both ML and Bayesian analyses results, basal nodes are strongly supported, including the sister relationship of *A. wangi* Lin & Li, sp. nov. & *A. zhuanghaoyuni* Lin & Li, sp. nov. (Bootstrap value: 95; PP: 1).

Natural habitat. All the species of *Asianopis* gen. nov. were collected from bushes in low-elevation forests.

Composition. This new genus comprises two species groups: the *liukuensis*-group with two species: *A. dumogae* (Merian, 1911) sp. reval. comb. nov. and *A. liukuensis* (Yin, Griswold & Yan, 2002) comb. nov. and the *zhuanghaoyuni*-group with five species: *A. celebensis* (Merian, 1911) comb. nov., *A. konplong* (Logunov, 2018) comb. nov., *A. wangi* sp. nov., *A. wuchaoi* sp. nov., and *A. zhuanghaoyuni* sp. nov.

Distribution. China (Fujian, Yunnan, Hong Kong, Guangxi, Hainan), India, Indonesia, and Vietnam.

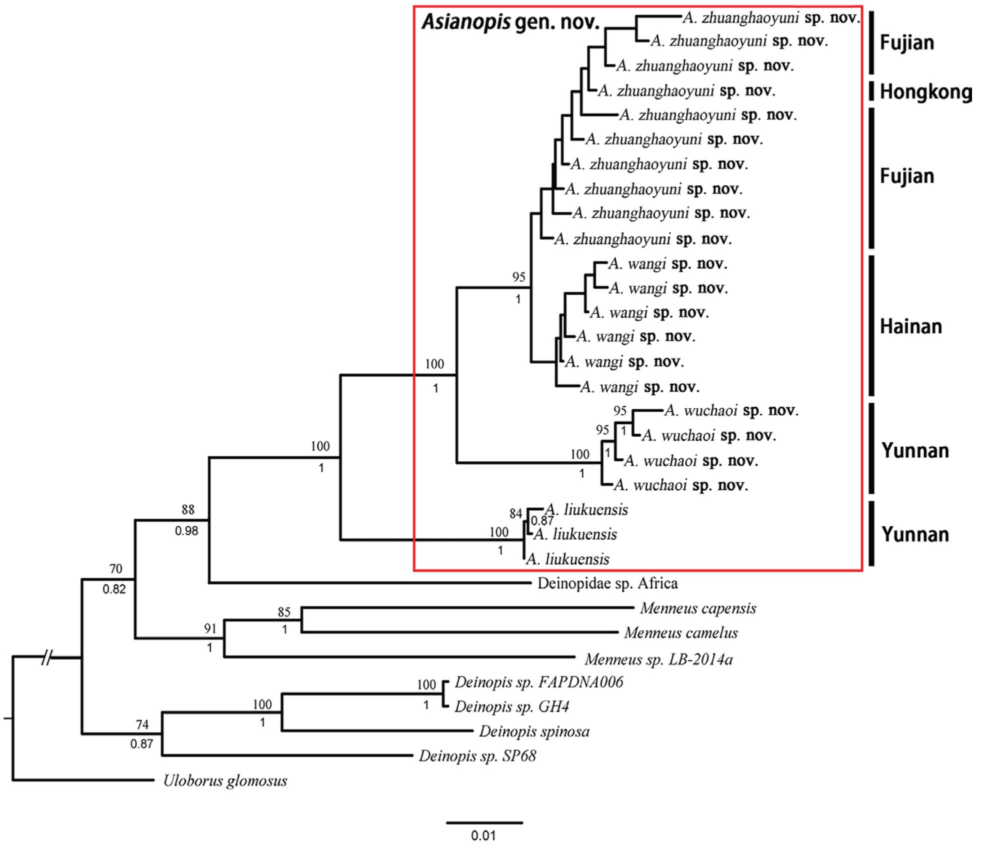

Figure 1. Phylogenetic tree of Deinopidae spiders based on 31 specimens. Numbers on nodes indicate Maximum Likelihood bootstrap values and Bayesian posterior probabilities.

The *liukuensis*-group

Asianopis dumogae (Merian, 1911), sp. reval. comb. nov.
Fig. 3

Dinopis dumogae Merian, 1911: 171 (♀ only, ♂ mismatched).

Type material examined. 1♀ (NMB-ARAN-00514a), "Wald bei Duluduo", Sulawesi Utara, forest near Duluduo, 00°31'33"N, 123°57'10"E, Sulawesi, Indonesia.

Diagnosis. This species can be distinguished from *A. liukuensis* comb. nov. by the MP nearly covering the CO, S round, and the overall equal thickness of the CD (Figs 4, 6).

Description. See Merian (1911). Photos of the epigyne of the syntype are given in Figure 6.

Distribution. Indonesia (North Sulawesi).

Comments. Merian (1911) reported *D. celebensis* based on three specimens from different localities in Sulawesi, Indonesia. One male (NMB-ARAN-00514b, "Zen-

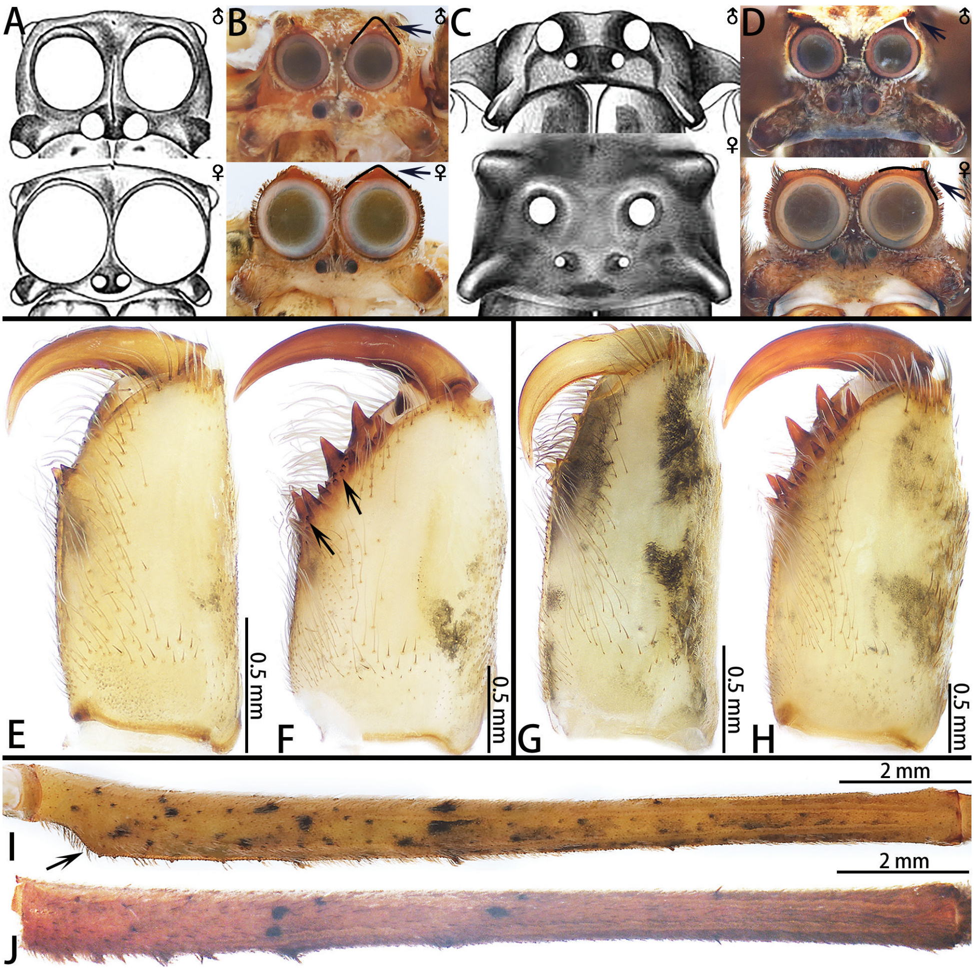

Figure 2. Prosoma (frontal view, upper ♂, lower ♀) (**A–D**), chelicerae (**E–H**) and leg I (**I–J**). Figures **A** and **C** modified from Coddington et al. (2012). **A** *Deinopis spinosa* **B** *Asianopis liukuensis* comb. nov. **C** *Menneus dromedarius* **D** *Asianopis zhuanghaoyuni* sp. nov. **E** Chelicerae of male *A. liukuensis* comb. nov. **F** Chelicerae of female *A. liukuensis* comb. nov. (Arrows indicate the denticles) **G** Chelicerae of male *A. zhuanghaoyuni* sp. nov. **H** Chelicerae of female *A. zhuanghaoyuni* sp. nov. **I** Left leg I of female *A. liukuensis* comb. nov. Arrow shows enlarged femur **J** Left leg I of female *A. zhuanghaoyuni* sp. nov.

tral-Celebes, nördlich vom Golf von Bone", South Sulawesi, north of the Gulf of Boni (precise locality not known), one female from North Sulawesi (NMB-ARAN-00514a, "Wald bei Duluduo", Sulawesi Utara, forest near Duluduo, 00°31'33"N, 123°57'10"E and one female from Central Sulawesi (NMB-ARAN-00514c, Larga, südlich vom Posso-See, unterhalb Patiro Rano, bei 900 m, Central Sulawesi, south of Lake Poso at an elevation of 900 m (the localities "Larga" and "Patiro Rano" could not be located on maps; the epigyne of this specimen is missing, but the specimen is clearly larger than the others).

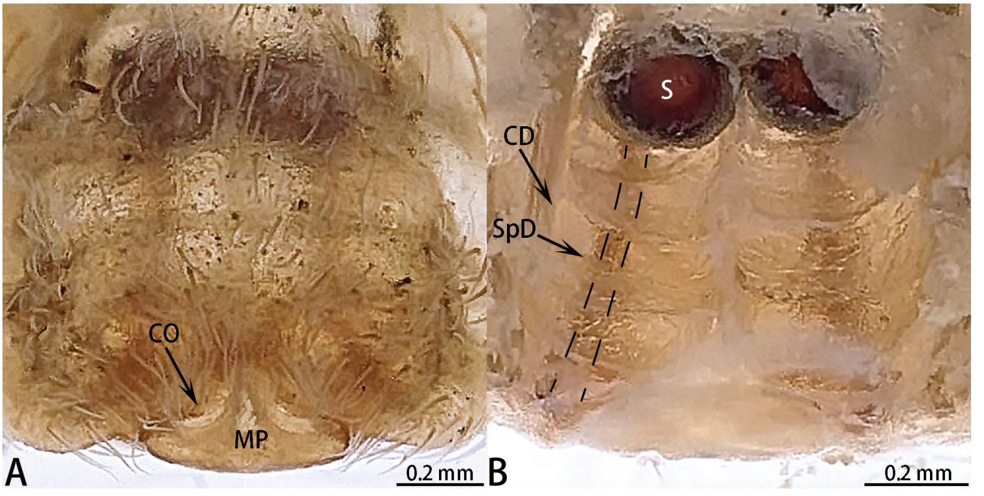

Figure 3. *Asianopis dumogae* sp. reval. comb. nov., female type. **A** Epigyne **B** Vulva, dorsal view.

Merian (1911) stated that the male and the females may not represent the same species and suggested the name *D. celebensis* for the male, and *D. dumogae* for the female. According to the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature 1999: Article 11.5.1), such conditionally proposed species names are potentially available as valid names if published before 1961. The species has not been listed in any of the catalogues. We examined the types and concluded the male and the two females are indeed three different species. The palp of the male *D. celebensis* exhibits features of the *zhuanghaoyuni* group: the tegulum is obscured by the embolic coil, and the embolus is long and strongly coiled around the MA. The female from North Sulawesi (Doloduo) has features of the *liukuensis* group: an anchor-shaped median plate, CO distinct, CD with three turns. Thus, we revalidated the female *D. dumogae* as *Asianopis dumogae* (Merian, 1911), sp. reval. comb. nov.

***Asianopis liukuensis* (Yin, Griswold & Yan, 2002), comb. nov.**
Figs 2B, E, F, I, 4–8, 19, 21A, 22A, G, 23

Deinopis liukuensis Yin et al., 2002: 610, figs 1–7 (♂♀)
Deinopis liukuensis Zhang & Wang, 2017: 238 (♂♀)
Deinopis scrubjunglei Caleb & Mathai, 2014: 2, figs 1–20 (♂♀) syn. nov.

Type. *Holotype.* ♂ (HNU, no. 00-LK-1, lost), China, Yunnan Province, Liuku, Mt Gaoligong, 25°30'48"N, 98°30'36"E, elevation ca 800 m, 26.VI.2000, Heng-Mei Yan leg.

Type materials of *Deinopis scrubjunglei* examined. ♂ (SRC-ZSI I/SP 19), Madras Christian College, Chennai, Tamil Nadu, 12°55'12.7"N, 80°07'24.6"E, elevation ca 32 m, 5.XII.2013, John Caleb T.D. leg.; ♀ (SRC-ZSI I/SP 20), 22.IV.2014, same location, John Caleb T.D and Karthy leg.

Figure 4. *Asianopis liukuensis* comb. nov., male from Xishuangbanna and female from Jianfengling. **A** Male prosoma, frontal view **B** Female prosoma, frontal view **C** Male habitus, dorsal view **D** Male habitus, ventral view **E** Female habitus, dorsal view **F** Female habitus, ventral view.

Figure 5. *Asianopis liukuensis* comb. nov., left palp, male from Xishuangbanna. **A** Prolateral view **B** Retrolateral view.

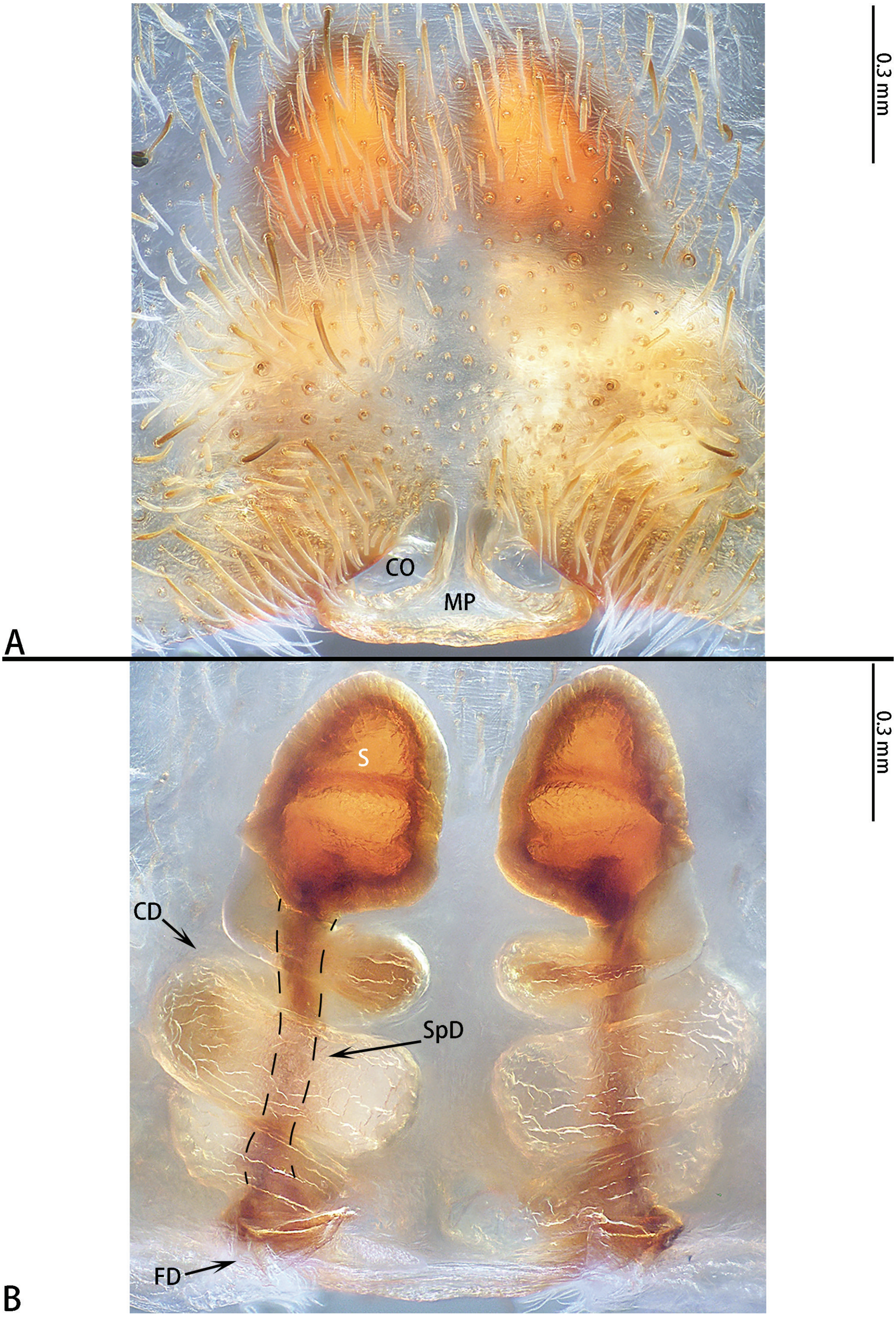

Figure 6. *Asianopis liukuensis* comb. nov., female from Jianfengling. **A** Epigyne **B** Vulva, dorsal view.

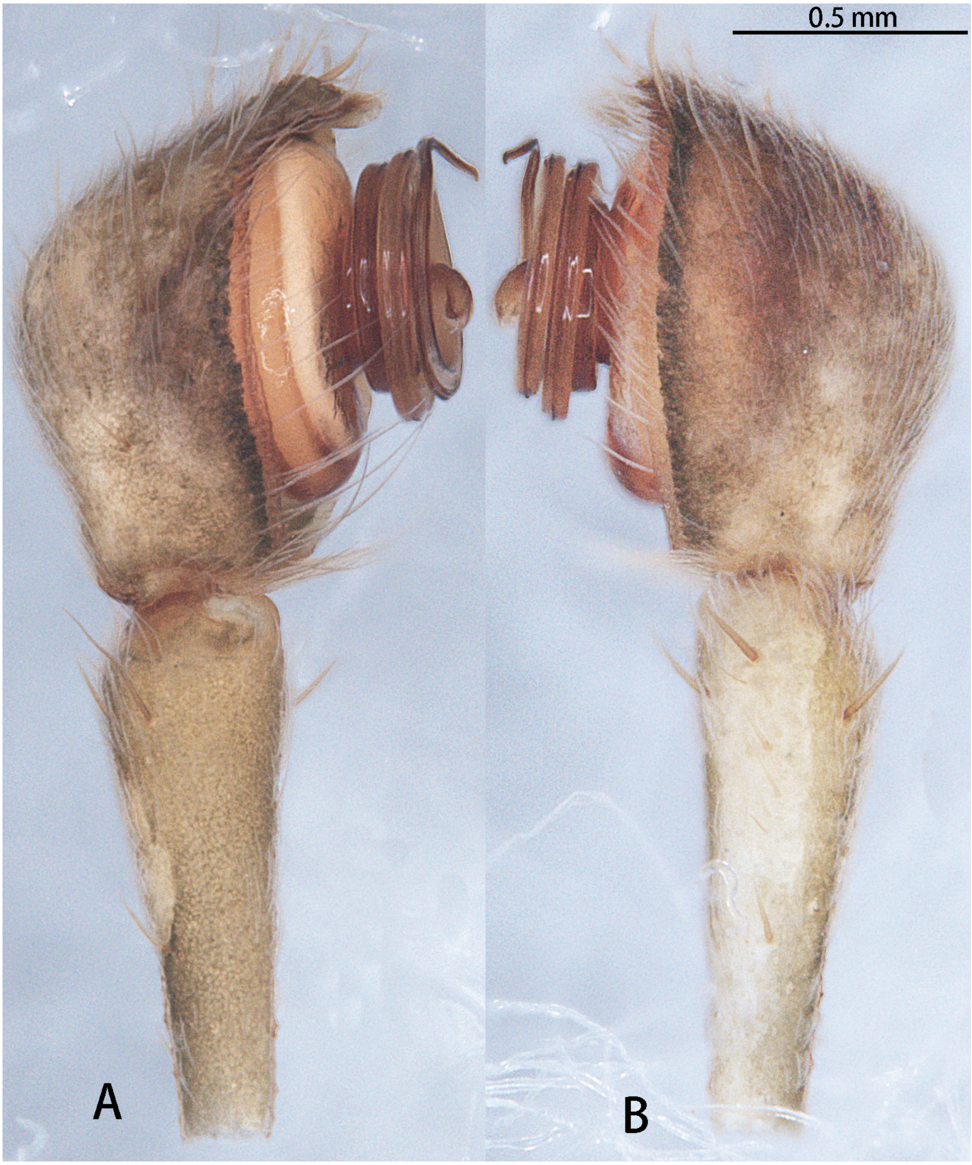

Figure 7. *Asianopis liukuensis* comb. nov., left palp, holotype male of *Deinopis scrubjunglei* syn. nov. **A** Prolateral view **B** Retrolateral view.

Other material examined. 2♂, China, Yunnan Province, Xishuangbanna Dai Autonomous Region, rubber tree plantation near Jinghong City, 28.IV.2016, Chaotai Wei leg.; 1♀, China, Hainan Island, Ledong County, Jianfengling National Park, 13.VII.2019, Zixuan Lin leg.

Diagnosis. This species can be distinguished from other congeners by the distinct female copulatory opening, oval S, and CD tapering from the copulatory opening to spermatheca (Figs 6, 8).

Description. See Yin et al. (2002) and Caleb and Mathai (2014).

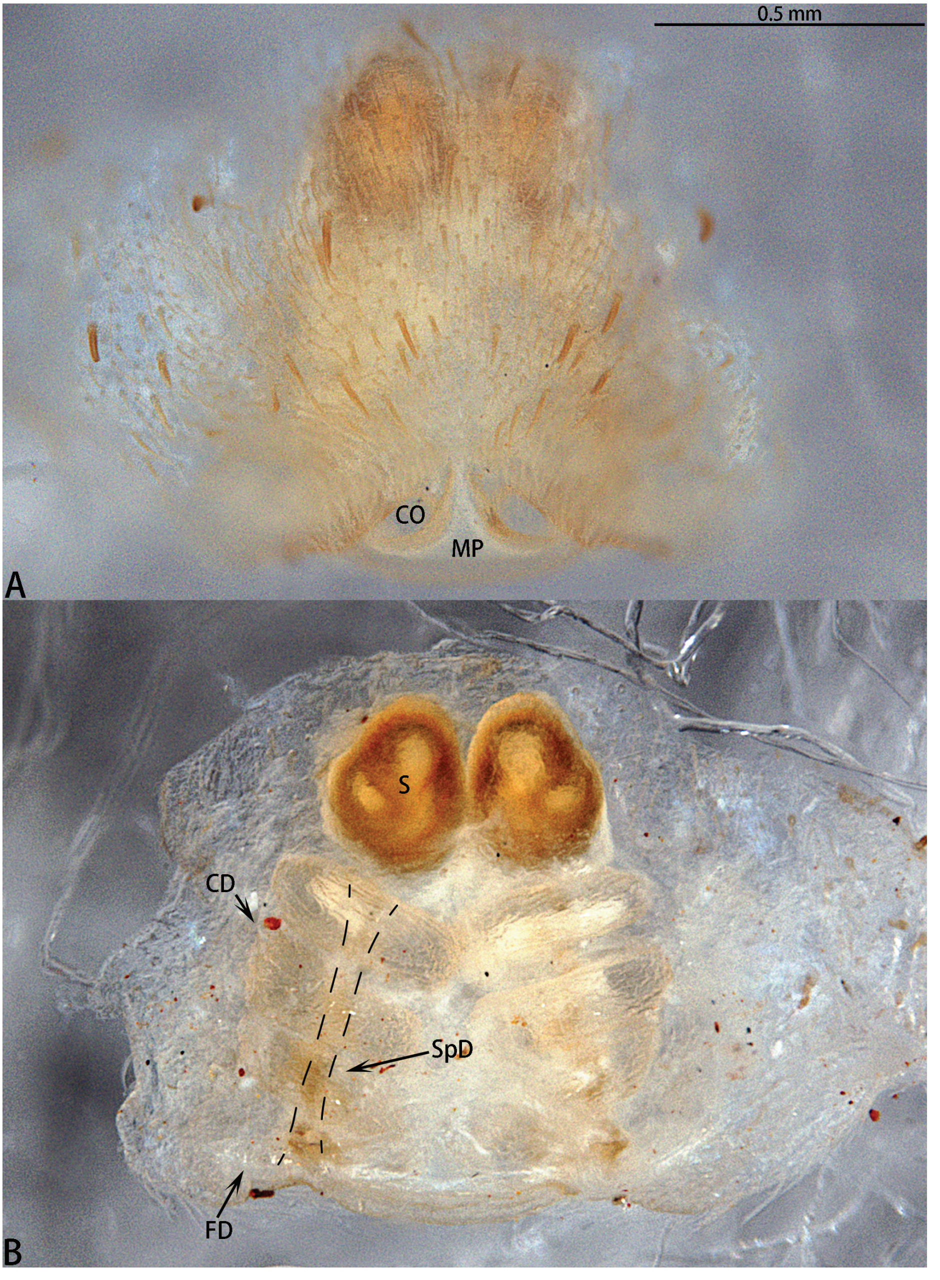

Figure 8. *Asianopis liukuensis* comb. nov., paratype female of *Deinopis scrubjunglei* syn. nov. **A** Epigyne **B** Vulva, dorsal view.

Distribution. China (Yunnan, Guangxi, Hainan), India.

Comments. Type materials of *D. scrubjunglei* syn. nov. were examined and no differences between *A. liukuensis* and *D. scrubjunglei* were observed. Thus, we consider *D. scrubjunglei* to be a synonym of *A. liukuensis*, and the figures of *D. scrubjunglei* are given for comparison (Figs 7, 8, 19C).

The *zhuanghaoyuni*-group

Asianopis celebensis **(Merian, 1911), comb. nov.**
Fig. 9A–F

Dinopis celebensis Merian, 1911: 167, figs A, B (♂only, ♀ mismatched).

Type material examined. ♂ (NMB), NMB-ARAN-00514b, "Zentral-Celebes, nördlich vom Golf von Bone", South Sulawesi, north of the Gulf of Boni (precise locality not known).

Diagnosis. The male can be distinguished from other congeners by having the distal lobe of the MA distinctly smaller than the basal lobe; in other *Asianopis* spp., the distal lobe is slightly smaller than the basal lobe (Fig. 9A, C).

Description. See Merian (1911). Photos of holotype male habitus and palps are shown in Fig. 9A–F.

Distribution. Indonesia (Sulawesi).

Comments. One male and two females were types for *Asianopis celebensis* (Merian, 1911) comb. nov. after Merian (1911). Based on the current study, one type female from North Sulawesi is *Asianopis dumogae* (Merian, 1911) sp. reval. comb. nov., and the other type female from South Sulawesi is a member of the *zhuanghaoyuni*-group, but its status at the species level is uncertain because of the missing epigyne.

Asianopis konplong **(Logunov, 2018), comb. nov.**

Deinopis konplong Logunov, 2018: 141, figs 1–7 (♂).

Type. Holotype ♂ (MMUE, G7579.37) from Vietnam, Kon Tum Province, Kon Plong District, 14 km north of Kon Plong, 14°43'20"N, 108°18'59"E, elevation ca 1030 m, 3–12.VI.2016, A.A. Abramov leg. Not examined.

Diagnosis. This species can be distinguished from other *Asianopis* species by the short palp (ratio of the length of the palpal tarsus to the length of the cymbium: 1:1) and upturned embolic tip (Logunov 2018: fig. 4).

Description. See Logunov (2018).

Distribution. Vietnam (Kon Tum).

Asianopis wangi **Lin & Li, sp. nov.**
http://zoobank.org/64A4C3D1-03A5-4D7A-B2E6-E30EA28DC41C
Figs 10–12, 20B, 21C, 22C, D, H, 23

Type. ***Holotype.*** ♂ (IZCAS-Ar39681), China, Hainan Province, Wuzhishan City, Wuzhishan Nature Reserve, Diewupo, 17.V.2019, Dongdong Wang leg.

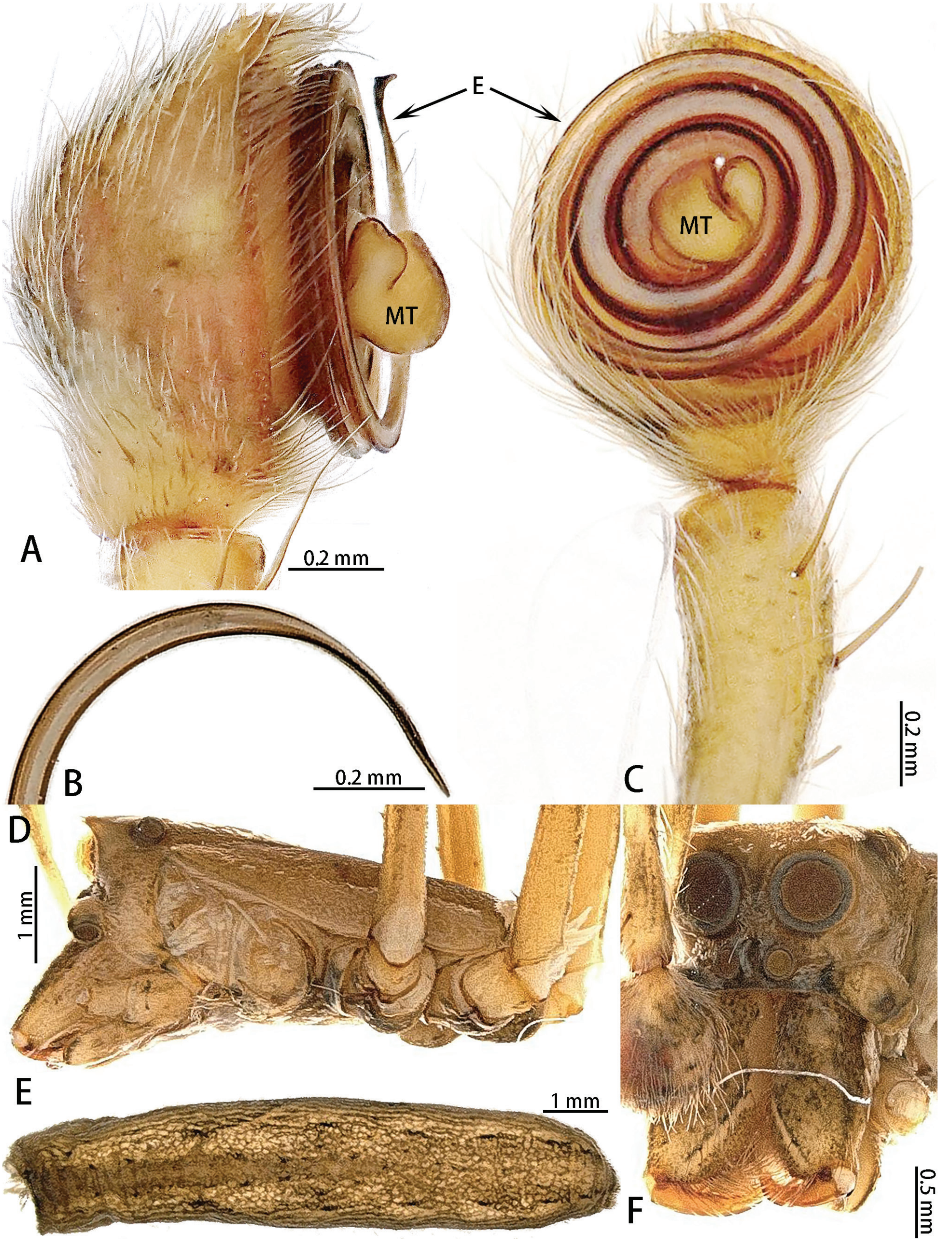

Figure 9. *Asianopis celebensis* comb. nov., male type. **A** Male right palp, prolateral view **B** Embolic tip **C** male left palp (embolic tip detached), retrolateral view **D** Male prosoma, lateral view **E** Male opisthosoma, dorsal view **F** Male prosoma, frontal view.

Paratypes. 1♂1♀ (IZCAS-Ar39682-Ar39683), same data as holotype; 1♂2♀ (IZCAS-Ar39684-Ar39686) China, Hainan Province, Wuzhishan City, Nansheng Town, Maoxiang Village, 18.V.2019, Dongdong Wang leg.

Etymology. The species is named after Mr Dongdong Wang, the collector of the holotype; noun (name) in genitive case.

Diagnosis. The males resemble *A. zhuanghaoyuni* sp. nov. but can be distinguished from other species by the ratio of the length of the embolic opening to the length of the embolic tip fold, which is 1:6 in *A. wangi* sp. nov. and 1:8 in *A. zhuanghaoyuni* sp. nov. The fold is more developed in *A. wangi* sp. nov. (Fig. 21C, D). The median plate is triangular in *A. wangi* sp. nov. and subtriangular in *A. zhuanghaoyuni* sp. nov. (Figs 12, 19).

Description. Male holotype (Figs 10A, C, D, 11, 20B, 21D, 22C). Total length 15.31, carapace 6.22 long, 4.60 wide, opisthosoma 9.32 long, 2.10 wide. Eye sizes and interdistances: AME 0.30, ALE 0.38, PME 0.65, PLE 0.34, AME–AME 0.30, AME–ALE 0.97, PME–PME 0.23, PME–PLE 0.69, AME–PME 0.24, ALE–PLE 1.82. Clypeus height 0.10. Chelicerae with four promarginal and 10–13 retromarginal teeth. Leg measurements: leg I: 84.08 (21.13 + 26.50 + 29.53 + 6.92), leg II: 59.70 (18.39 + 19.55 + 15.80 + 5.96), leg III: 36.14 (12.05 + 11.79 + 10.26 + 2.04), leg IV: 37.23 (11.92 + 12.37 + 11.28 + 1.66). Leg formula: 1243.

Male palp (Figs 11, 20B, 21D). Cymbium hemispherical; tegulum flat, obscured by embolic coils; embolus long and strongly coiled, originating at 10 o'clock and coiling 1500° around MA; embolic tip widened subapically, strongly folded and without apophysis. MA large, with two lobes.

Female paratype (Figs 10B, E, F, 12, 22D). Total length 24.04, carapace 7.56 long, 5.32 wide, opisthosoma 16.28 long, 6.86 wide. Eye sizes and interdistances: AME 0.28, ALE 0.38, PME 1.34, PLE 0.42, AME–AME 0.13, AME–ALE 1.03, PME–PME 0.39, PME–PLE 1.30, AME–PME 0.22, ALE–PLE 1.92. Clypeus height 0.34 (n = 1). Chelicerae with four promarginal and 8–13 retromarginal teeth (8(n = 1), 13(n = 1)). Leg measurements: Leg I: 54.24 (16.22 + 16.83 + 17.63 + 3.56), leg II: 50.59 (15.90 + 16.41 + 15.00 + 3.28), leg III: 30.84 (10.96 + 10.38 + 7.88 + 1.62), leg IV: 30.28 (10.13 + 10.58 + 8.27 + 1.30). Leg formula: 1234.

Epigyne (Fig. 12) with a median plate, CD with 7 or 8 turns, S oval, SpD consistently narrow.

Distribution. China (Hainan).

Asianopis wuchaoi **Lin & Li, sp. nov.**

http://zoobank.org/F05E46B7-98E7-4DA1-B7DF-AD440C2E05B6

Figs 13–15, 21B, 22B, 23

Type. ***Holotype.*** ♂ (IZCAS-Ar39687), China, Yunnan Province, Jinghong City, Mount Jinuo, 10.V.2019, Chao Wu leg.

Paratypes. 2♀ (IZCAS-Ar39688-Ar39689), China, Yunnan Province, Jinghong City, Mengla County, Mengxing Village, 16.VI.2019, Yi Li leg.; 1♀ (IZCAS-

Figure 10. *Asianopis wangi* sp. nov., male holotype and female paratype. **A** Male prosoma, frontal view **B** Female prosoma, frontal view **C** Male habitus, dorsal view **D** Male habitus, ventral view **E** Female habitus, dorsal view **F** Female habitus, ventral view.

Figure 11. *Asianopis wangi* sp. nov., left palp, male holotype. **A** Prolateral view **B** Retrolateral view.

Ar39690), China, Yunnan Province, Jinghong City, Situlaozhai Village, 20.V.2019, Chaotai Wei leg.

Etymology. The species is named after Mr Chao Wu, the collector of the holotype male; noun (name) in genitive case.

Diagnosis. The males can be easily distinguished by the length of the palpal tibia which is almost equal to the length of the cymbium; simple embolic tip with ETA (Fig. 21B); embolus coiling almost 3300° around MA. Epigyne with a well-developed, subtriangular median plate, obscureing CO, and CD with 9 turns (Fig. 14).

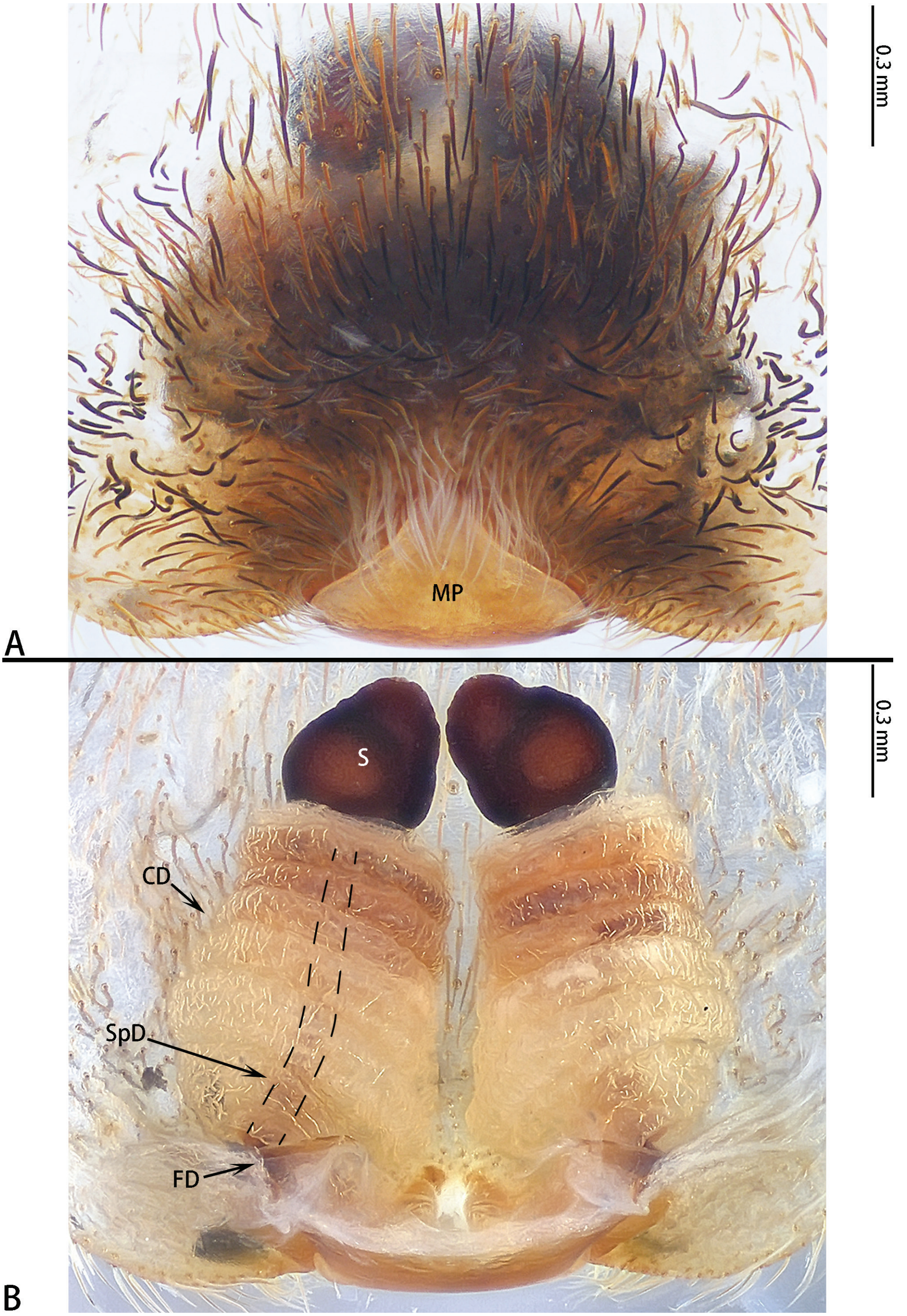

Figure 12. *Asianopis wangi* sp. nov., female paratype. **A** Epigyne **B** Vulva, dorsal view.

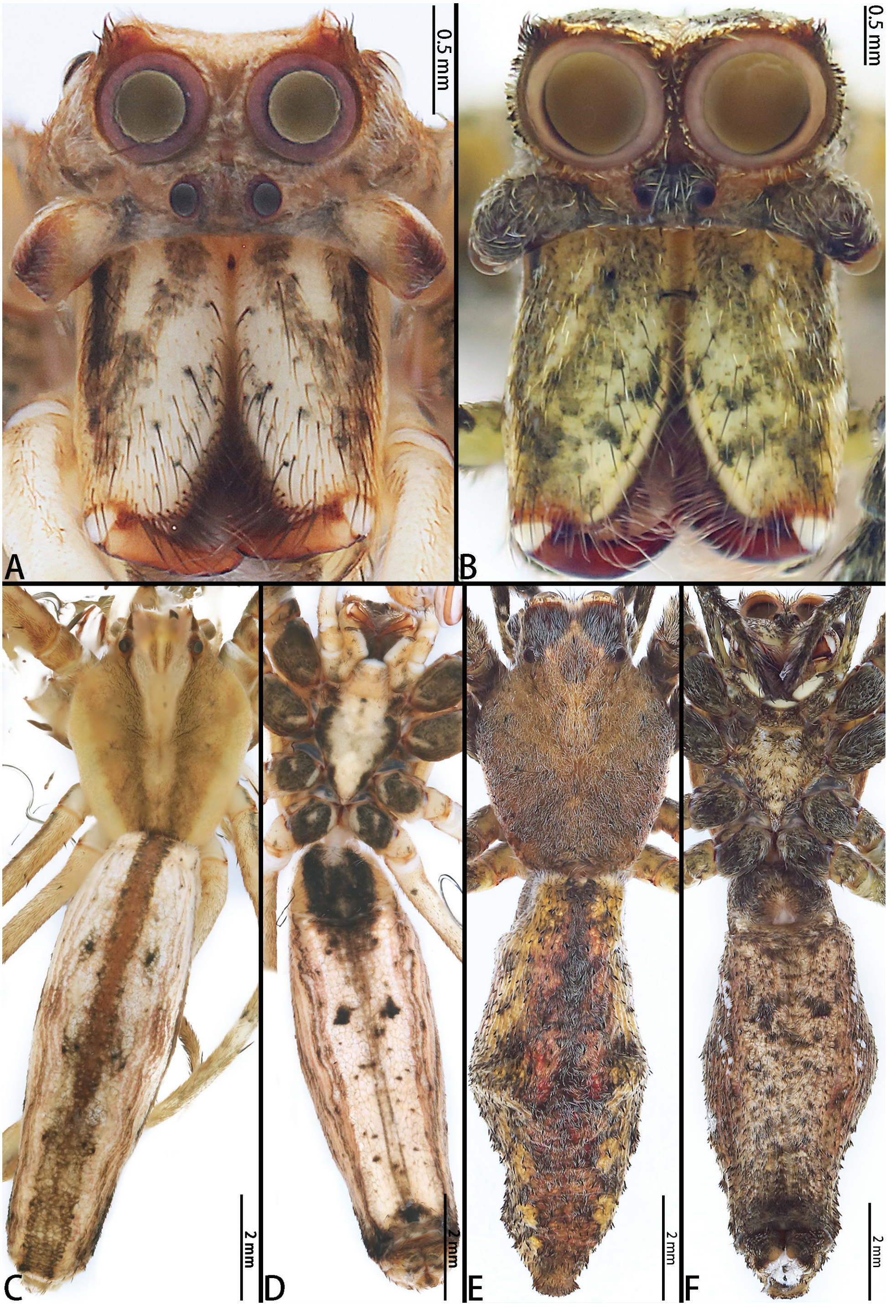

Figure 13. *Asianopis wuchaoi* sp. nov., male holotype and female paratype. **A** Male prosoma, frontal view **B** Female prosoma, frontal view **C** Male habitus, dorsal view **D** Male habitus, ventral view **E** Female habitus, dorsal view **F** Female habitus, ventral view.

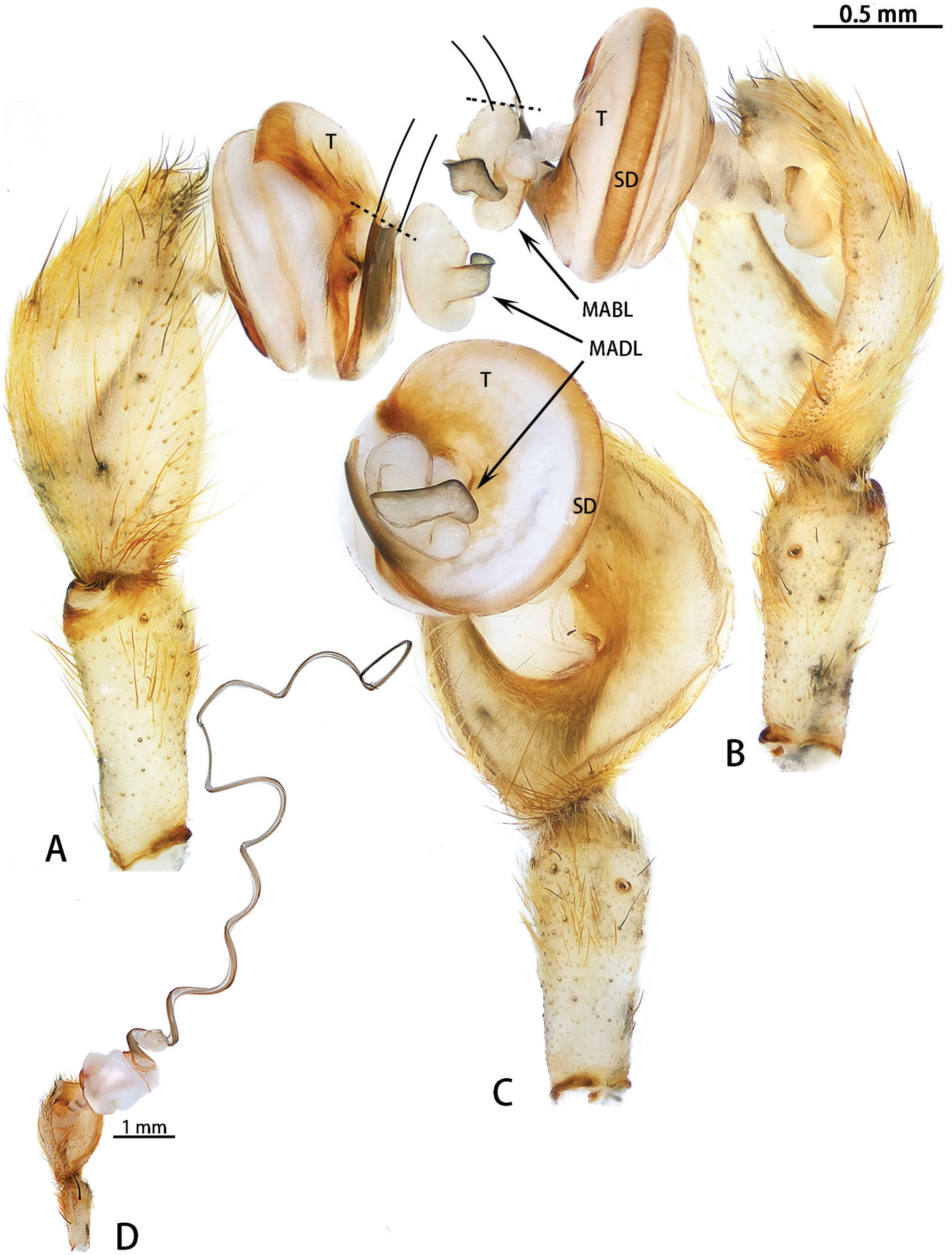

Figure 14. *Asianopis wuchaoi* sp. nov., male holotype. **A** Right palp (flipped horizontally), prolateral view **B** Right palp (flipped horizontally), retrolateral view **C** Left palp, prolateral view **D** Left palp, prolateral view.

Description. Male holotype (Figs 13A, C, D, 14, 21A). Total length 12.14, carapace 4.00 long, 3.40 wide, opisthosoma 8.14 long, 2.4 wide. Eye sizes and interdistances: AME 0.15, ALE 0.26, PME 0.52, PLE 0.29, AME–AME 0.17, AME–ALE

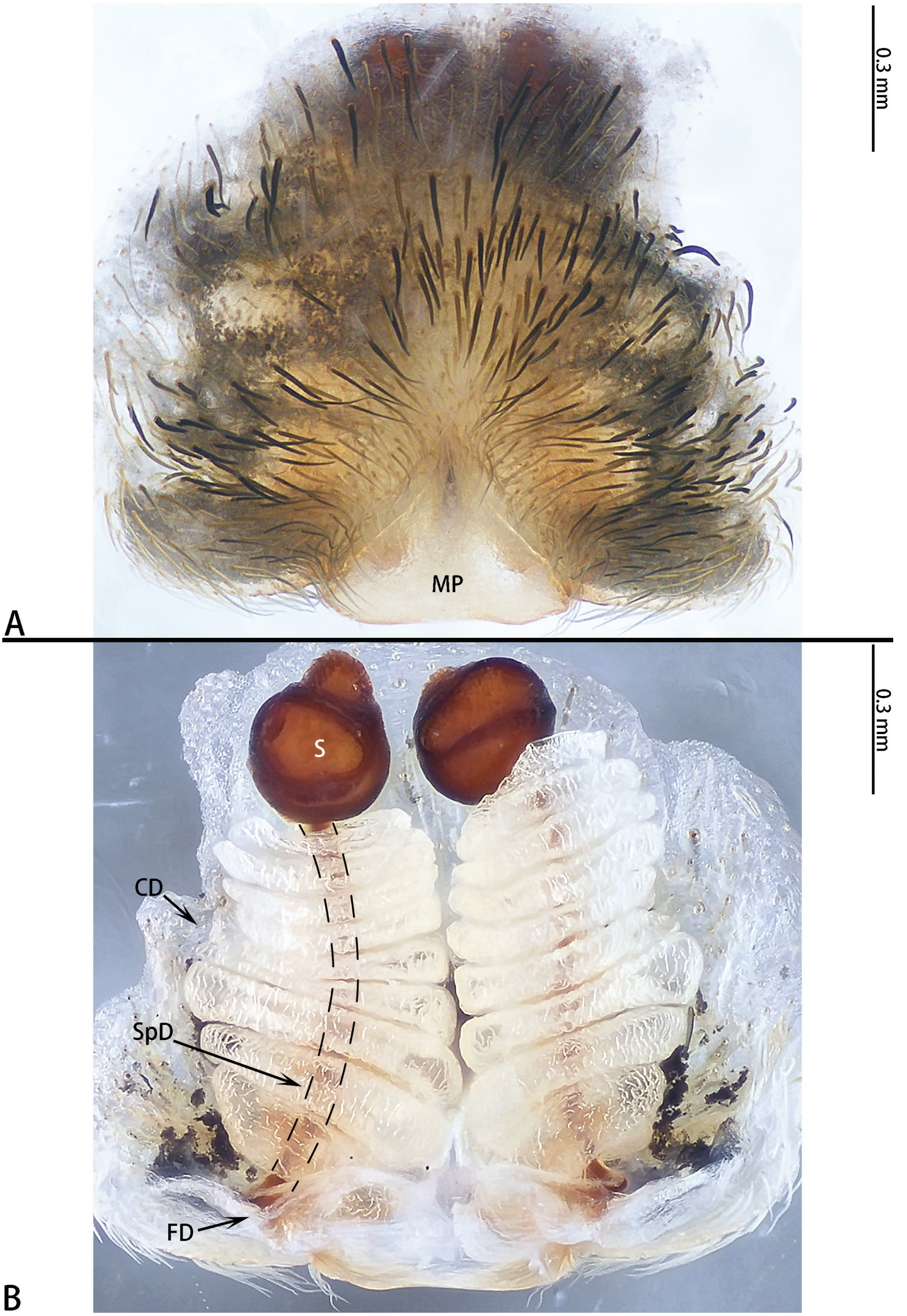

Figure 15. *Asianopis wuchaoi* sp. nov., female paratype. **A** Epigyne **B** Vulva, dorsal view.

0.70, PME–PME 0.16, PME–PLE 0.61, AME–PME 0.11, ALE–PLE 0.95. Clypeus height 0.05. Chelicerae with four promarginal and six retromarginal teeth. Leg measurements: leg I: damaged, leg II: damaged, leg III: (6.92 + 6.86 + ? + 1.44), leg IV: 21.82 (6.91 + 7.18 + 6.35 + 1.38).

Male palp (Figs 14, 21A). Cymbium hemispherical; tegulum flat, obscured by embolic coils; embolus long and strongly coiled, originating at five o'clock and coiling 3300° around MA. MA large, with two lobes.

Female paratype (Figs 13B, E, F, 15). Total length 14.60, carapace 6.28 long, 4.10 wide, opisthosoma 9.29 long, 3.72 wide. Eye sizes and interdistances: AME 0.11, ALE 0.34, PME 0.94, PLE 0.29, AME–AME 0.30, AME–ALE 1.03, PME–PME 0.06, PME–PLE 0.64, AME–PME 0.14, ALE–PLE 1.33. Clypeus height 0.13 (n = 1). Chelicerae with four promarginal and 8–13 retromarginal teeth (8(n = 1), 10(n = 1), 13(n = 1)). Leg measurements: Leg I: 39.82 (12.11 + 11.67 + 13.01 + 3.03), leg II: 36.81 (11.47 + 11.79 + 10.83 + 2.72), leg III: 23.53 (9.47 + 6.79 + 5.83 + 1.44), leg IV: 21.71 (7.18 + 7.76 + 5.70 + 1.07). Leg formula: 1234.

Epigyne (Fig. 15) with a median plate, obscuring CO, CD with 9 turns, S oval, SpD is consistently thin.

Distribution. China (Yunnan).

Note. The male died during ecdysis so some legs are damaged or curled, and the palps are expanded.

Asianopis zhuanghaoyuni **Lin & Li, sp. nov.**

http://zoobank.org/21A5E514-F8EE-4479-9338-51D419AA6E4A

Figs 2D, G, H, J, 16–18, 20, 21D, 22E, F, H, 23

Type. ***Holotype.*** ♂ (IZCAS-Ar39691), China, Fujian Province, Fuzhou City, Minhou County, Xiyuan Reservoir, 26°03'15.5"N, 119°06'05.4"E, elevation ca 102 m, 25.VI.2018, Haoyun Zhuang and Zhuoheng Jiang leg.

Paratypes. 1♀ (IZCAS-Ar39692), same data as holotype, Haoyun Zhuang leg.; 1♂1♀ (IZCAS-Ar39693-Ar39694), same locality data as holotype, but 15.V.2018, Haoyun Zhuang leg.; 1♂4♀ (IZCAS-Ar39695-Ar39699), same locality data as holotype, but 19.VI.2019, Haoyun Zhuang leg.; 1♂1♀ (IZCAS-Ar39700-Ar39701), same locality data as holotype, but 26.V.2019, Haoyun Zhuang leg.

Etymology. The species is named after Mr Haoyun Zhuang, the collector of the type specimens; noun (name) in genitive case.

Diagnosis. The males resemble *A. konplong* (Logunov, 2018) comb. nov. but can be distinguished by the embolus originating at five o'clock in *A. zhuanghaoyuni* sp. nov. (9 o'clock in *A. konplong* (Logunov, 2018) comb. nov.); the ratio of the length of the palpal tarsus to the length of the cymbium is 11:9 in *A. zhuanghaoyuni* sp. nov., while in *A. konplong* (Logunov, 2018), comb. nov. it is 1:1 (Figs 18, 22A; Logunov 2018, figs 4–6).

Figure 16. *Asianopis zhuanghaoyuni* sp. nov., male holotype and female paratype. **A** Male prosoma, frontal view **B** Female prosoma, frontal view **C** Male habitus, dorsal view **D** Male habitus, ventral view **E** Female habitus, dorsal view **F** Female habitus, ventral view.

Figure 17. *Asianopis zhuanghaoyuni* sp. nov., male holotype, left palp. **A** Prolateral view **B** Retrolateral view.

Description. Male holotype (Figs 2G, 16A, C, D, 17, 20A, 21E, 22E). Total length 16.54, carapace 5.58 long, 3.84 wide, opisthosoma 11.40 long, 1.90 wide. Eye sizes and interdistances: AME 0.25, ALE 0.30, PME 0.59, PLE 0.30, AME–AME 0.25, AME–ALE 0.85, PME–PME 0.23, PME–PLE 0.59, AME–PME 0.19, ALE–PLE 1.28. Clypeus height 0.20. Chelicerae with four promarginal teeth and a retromarginal tooth. Leg measurements: leg I: 66.35 (18.50 + 22.55 + 18.95 + 6.35), leg II: 52.87 (16.54 + 17.65 + 13.10 + 5.58), leg III: 30.39 (10.78 + 10.83 + 7.18 + 1.60), leg IV: 30.06 (10.42 + 11.12 + 7.18 + 1.34). Leg formula: 1234.

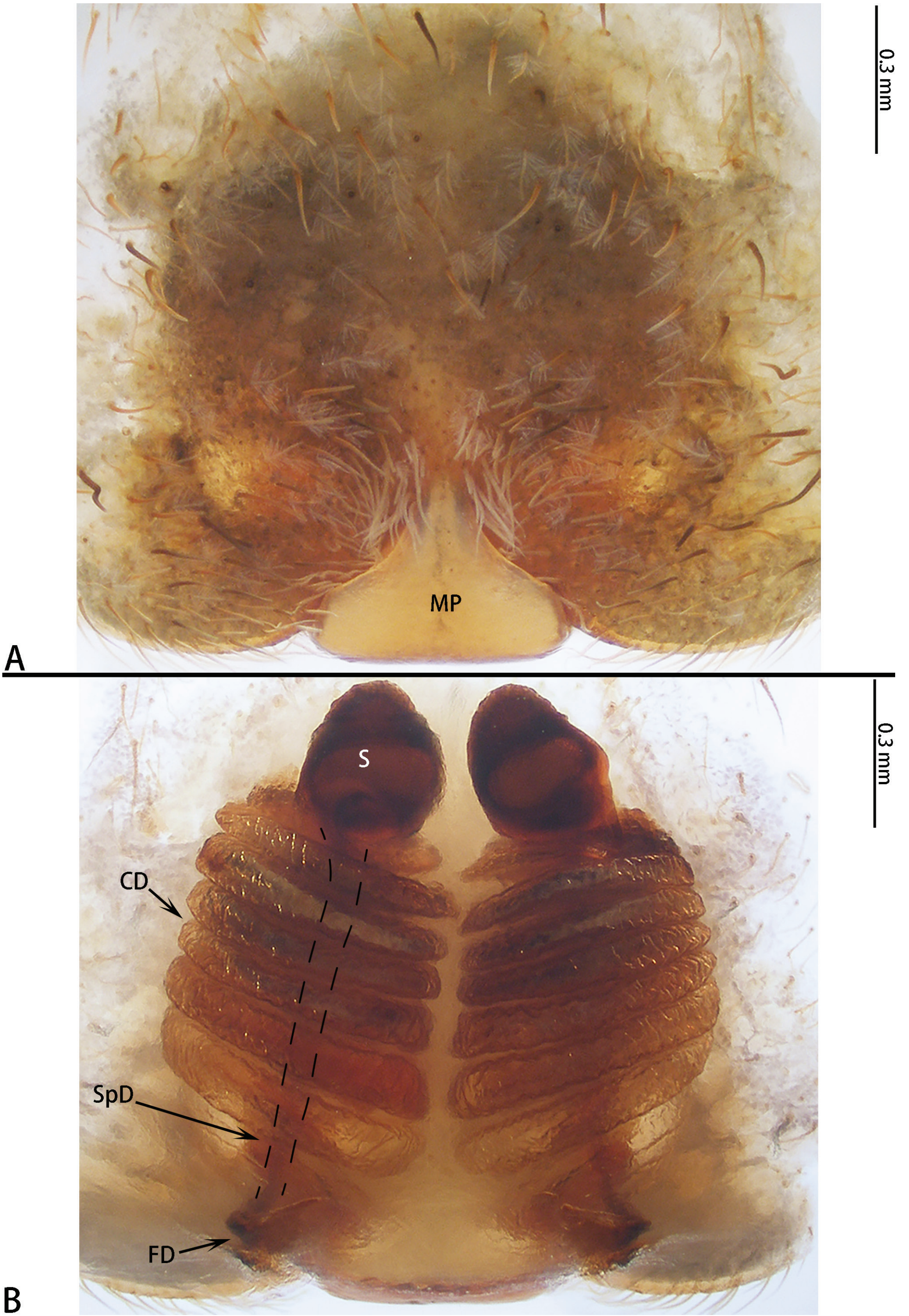

Figure 18. *Asianopis zhuanghaoyuni* sp. nov., female paratype. **A** Epigyne **B** Vulva, dorsal view.

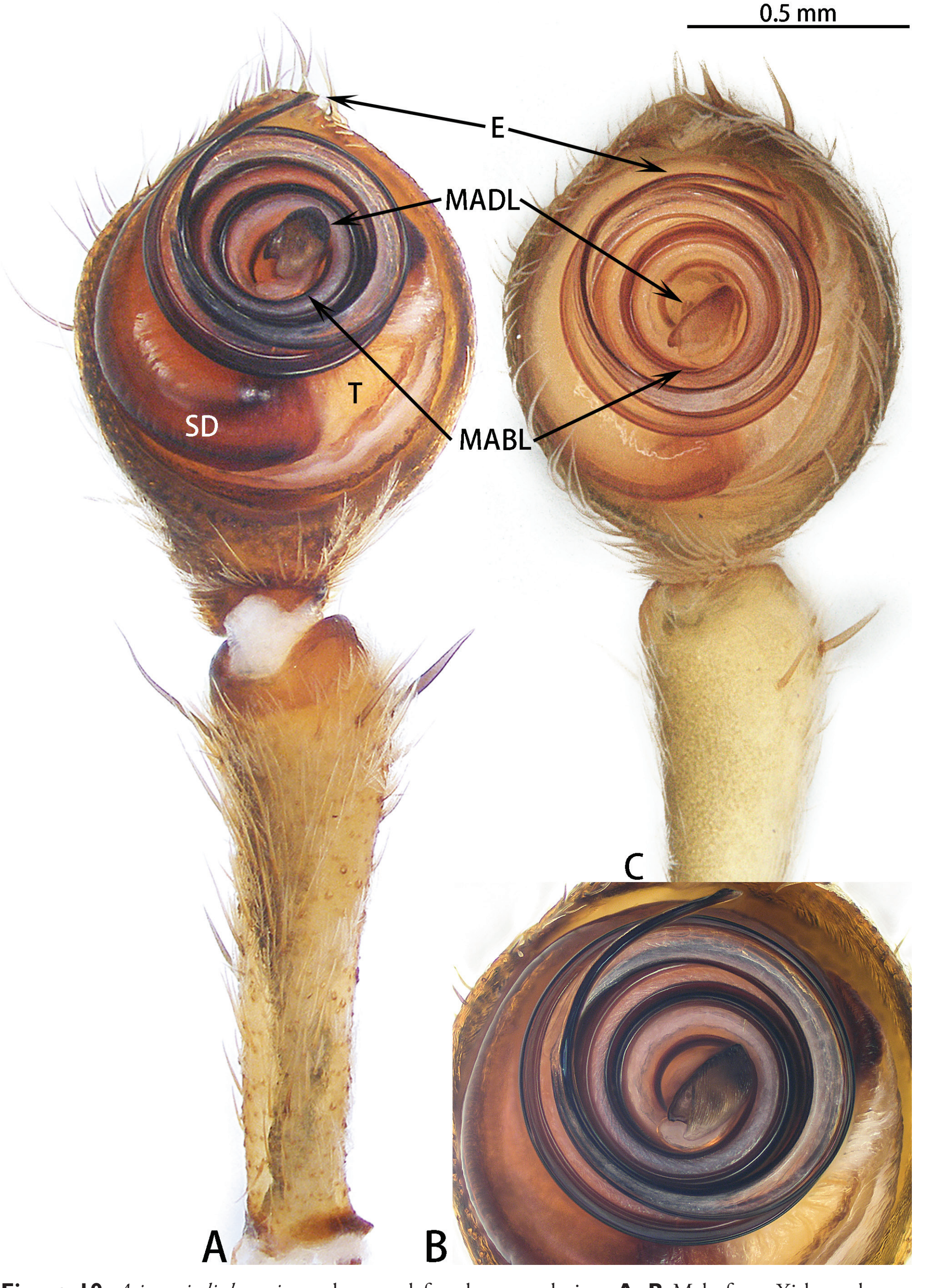

Figure 19. *Asianopis liukuensis* comb. nov., left palp, ventral view. **A, B** Male from Xishuangbanna **C** Male from India, type of *Deinopis scrubjunglei* syn. nov.

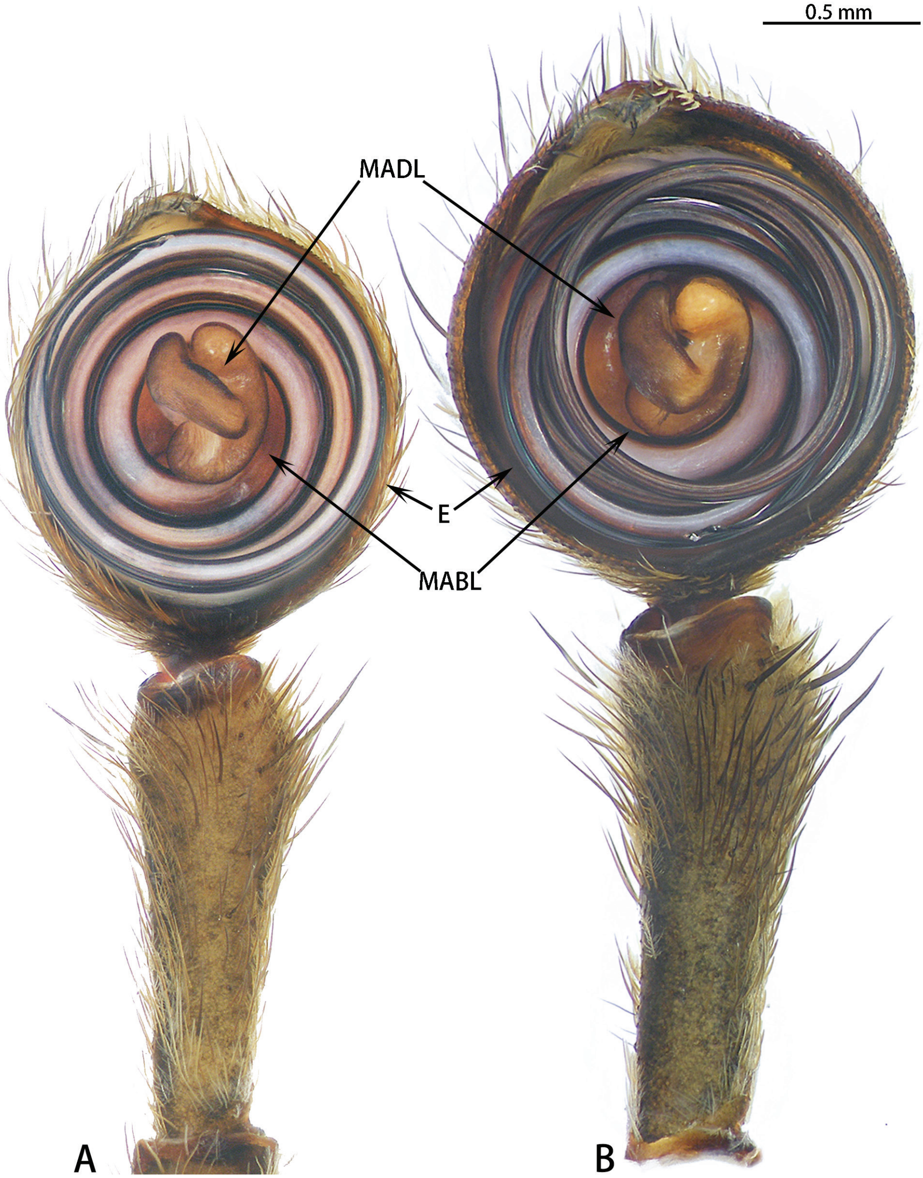

Figure 20. Ventral view of left palp, holotype males. **A** *A. zhuanghaoyuni* sp. nov. **B** *A. wangi* sp. nov.

Male palp (Figs 18, 22A). Cymbium hemispherical; tegulum flat, obscured by embolus coils; originating at five o'clock, coiling 1500° around MA, embolic tip widened subapically, folded and without apophysis. MA large, with two lobes.

Female paratype (Figs 2H, J, 16B, E, F, 18, 22F, H). Total length 22.60, carapace 5.90 long, 4.55 wide, opisthosoma 15.40 long, 5.90 wide. Eye sizes and interd-

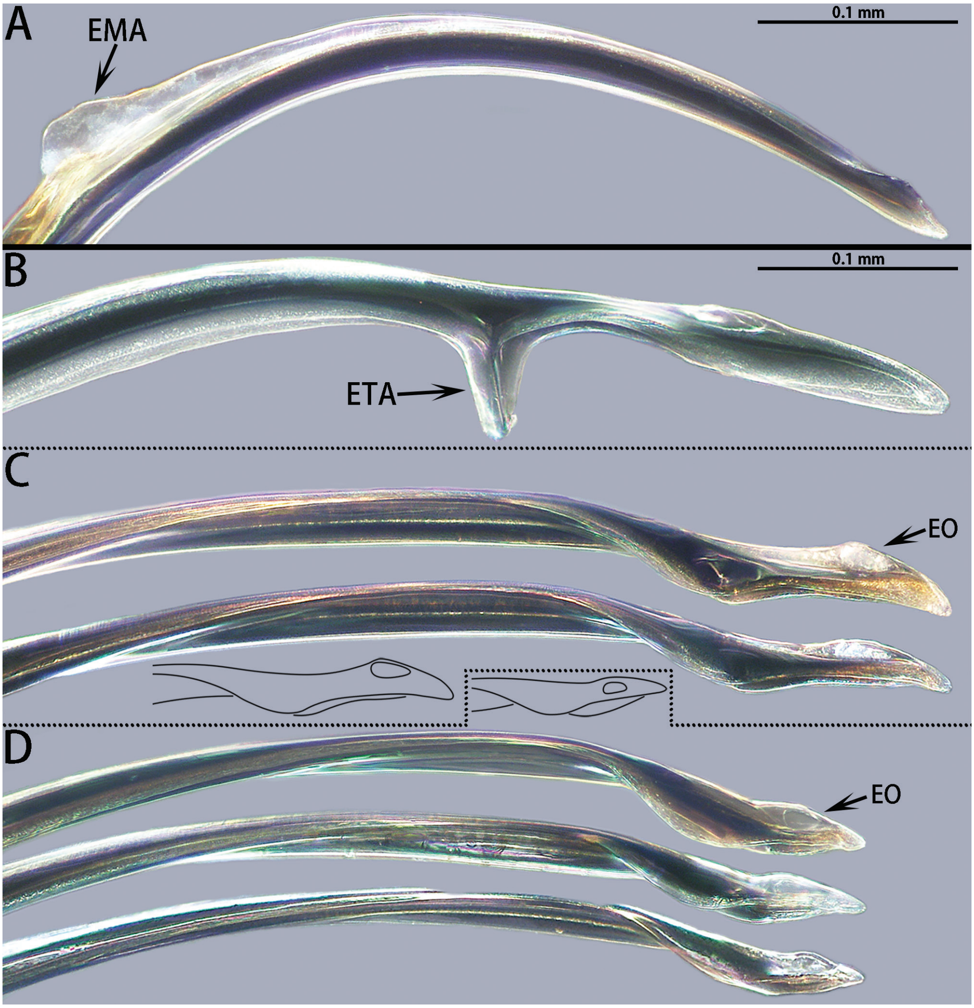

Figure 21. Embolic tips of four species of *Asianopis* gen. nov. **A** *A. liukuensis* (Yin, Griswold & Yan, 2002) comb. nov. **B** *A. wuchaoi* sp. nov. **C** *A. zhuanghaoyuni* sp. nov. **D** *A. wangi* sp. nov.

istances: AME 0.22, ALE 0.35, PME 1.08, PLE 0.33, AME–AME 0.37, AME–ALE 1.22, PME–PME 0.16, PME–PLE 0.98, AME–PME 0.081, ALE–PLE 1.61. Clypeus height 0.59. (*n* = 1). Chelicerae with four promarginal and 10 or 11 (10 (*n* = 2), 11 (*n* = 1)) retromarginal teeth. Leg measurements: Leg I: 49.68 (14.80 + 15.83 + 16.02 + 3.03), leg II: 46.08 (14.71 + 15.20 + 13.33 + 2.84), leg III: 27.79 (9.73 + 9.41 + 7.18 + 1.47), leg IV: 26.78 (9.02 + 9.61 + 6.86 + 1.29). Leg formula: 1234.

Epigyne (Fig. 18) with a median plate, CD with 7–8 turns, S oval, SpD consistently thin.

Distribution. China (Fujian).

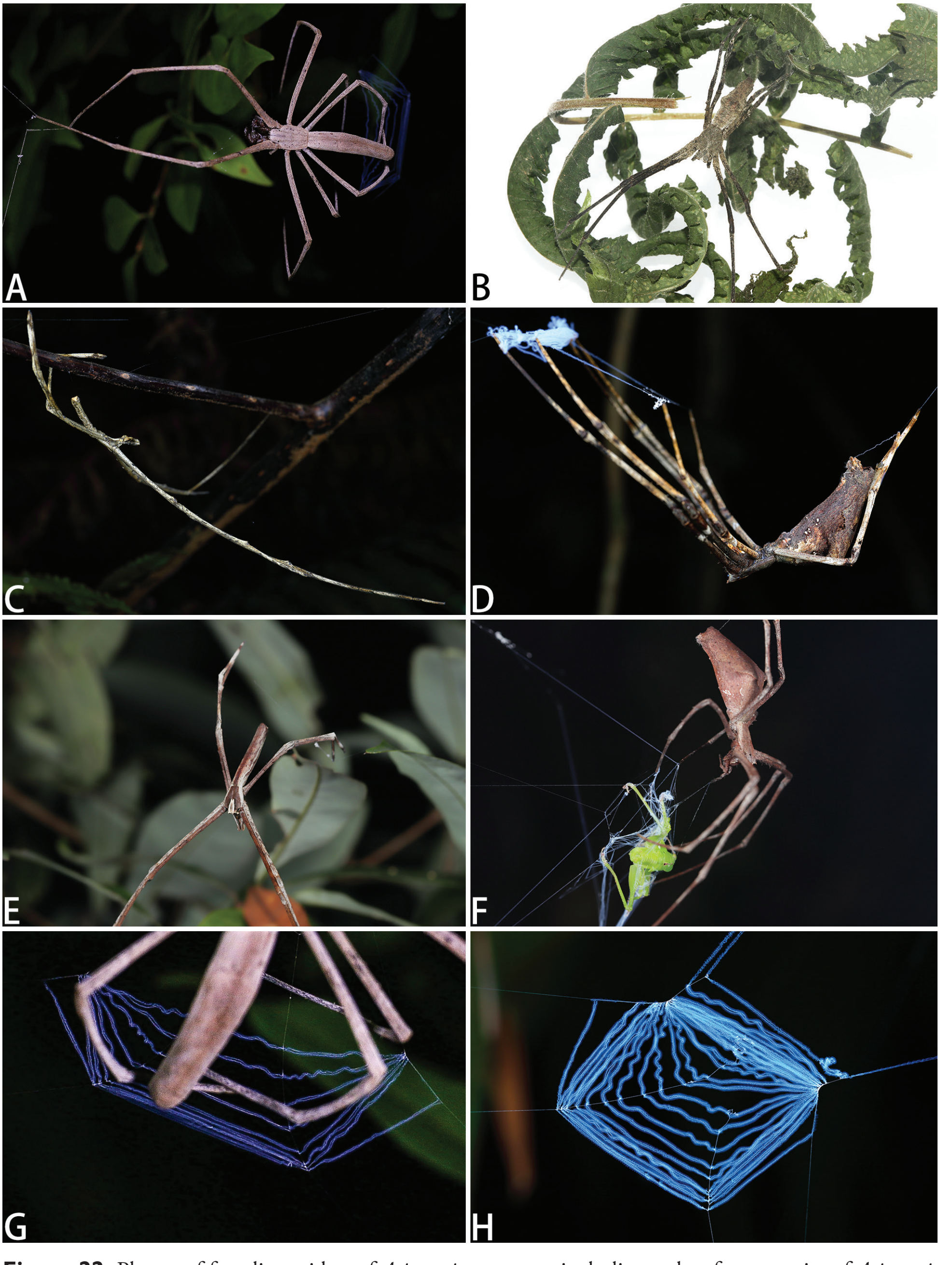

Figure 22. Photos of four live spiders of *Asianopis* gen. nov., including webs of two species of *Asianopis* gen. nov. **A** *A. liukuensis* comb. nov., female **B** *A. wuchaoi* sp. nov., female **C** *A. wangi* sp. nov., male **D** *A. wangi* sp. nov., female **E** *A. zhuanghaoyuni* sp. nov., male **F** *A. zhuanghaoyuni* sp. nov., female **G** Web of *A. liukuensis* comb. nov. **H** Web of *A. wangi* sp. nov.

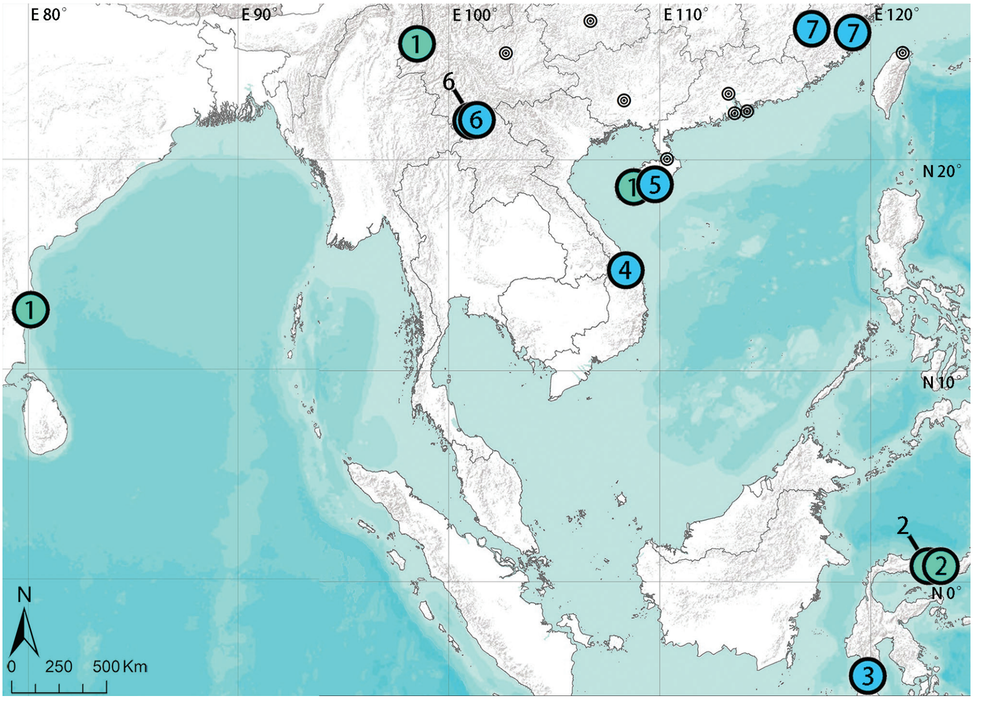

Figure 23. Distribution records of seven species of *Asianopis* gen. nov. in Asia. **1** *A. liukuensis* comb. nov. **2** *A. dumogae* sp. reval. comb. nov. **3** *A. celebensis* comb. nov. **4** *A. konplong* comb. nov. **5** *A. wangi* sp. nov. **6** *A. wuchaoi* sp. nov. **7** *A. zhuanghaoyuni* sp. nov.

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Supplementary material 1

***Asianopis* gen. nov., a new genus of the spider family Deinopidae from Asia**

Authors: Yejie Lin, Lili Shao, Ambros Hänggi, John T.D. Caleb, Joseph K.H. Koh, Peter Jäger, Shuqiang Li

Data type: specimen/primer/DNA sequence

Explanation note: **Table S1.** List of voucher information and GenBank accession numbers. **Table S2.** Primers and PCR conditions for the genetic markers used in this study (modified after Zhao and Li unpublished). **Table S3.** Sequence characteristics and models of DNA evolution selected for the seven sequence regions analyzed.References cited in supplementary tables.

Link: https://doi.org/10.3897/zookeys.911.38761.suppl1

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doi: 10.3897/zookeys.911.38845
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RESEARCH ARTICLE

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A new species of *Astreptolabis* in mid-Cretaceous amber from northern Myanmar, with the discovery of the first male of Astreptolabidinae (Dermaptera)

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Abstract

A new species of one of the basal families among extant Dermaptera, Pygidicranidae, is described from mid-Cretaceous amber of Myanmar based on two females and a male. *Astreptolabis laevis* **sp. nov.**, belongs to the extinct subfamily Astreptolabidinae, sharing the diagnostic combination of features typical of this group, such as the well-developed compound eyes, large pronotum, and straight and tubular cerci. The discovery of a male with its genitalia partly exerted permits characterization of traits for the subfamily and provides further information on the uniqueness and affinities of the subfamily. In addition, the extended hind wing allows for a comparison between the folding mechanism between these fossils and their modern counterparts, demonstrating considerable conservatism in hind wing evolution among Dermaptera.

Keywords

Cenomanian, earwigs, male genitalia, Neodermaptera, Pygidicranidae, taxonomy

Introduction

Earwigs (order Dermaptera) are one of the smaller orders of insects and consist of approximately 2000 modern species segregated into 12 families (Engel and Haas 2007; Engel et al. 2016). These are characteristic insects, with their generally flattened appearances, often leathery integument, tegminized forewings, broad fan-shaped hind wings, and, most distinctive of all, the terminal forceps formed of their modified cerci (Grimaldi and Engel 2005).

Within the Dermaptera, the modern fauna falls entirely within the suborder Neodermaptera (Engel 2003; Grimaldi and Engel 2005; Engel and Haas 2007), with the most basal families falling into the infraorder Protodermaptera. Protodermaptera include the families Diplatyidae, Haplodiplatyidae, Karschiellidae, and Pygidicranidae, all of which plesiomorphically possess equal-sized ventral cervical sclerites, often carinate femora, and in the most basal members a segmented pygidium (Popham 1985; Engel 2003), among other features of the male genitalia. Presently, the earliest definitive Neodermaptera are found in the Lower Cretaceous (Engel et al. 2002, 2011; Engel and Chatzimanolis 2005; Engel and Haas 2007), and there have been 22 taxa described from Cretaceous amber. Of these 22 taxa, six are classified in the family Pygidicranidae, including four adults and two nymphs. Although the record of earwigs preserved in Cretaceous and Cenozoic amber has grown rapidly (e.g., Engel 2009, 2011, 2016, 2017; Perrichot et al. 2011; Ross and Engel 2013; Engel and Perrichot 2014; Engel and Grimaldi 2014; Engel et al. 2011, 2015, 2017; Ren et al. 2017, 2018), well-preserved specimens of adult earwigs are still rather uncommon and it remains a difficulty to associate nymphs with adults when not found as syninclusions. Moreover, the precise phylogenetic placement of many fossil earwigs continues to be poorly understood.

Herein, based on three new specimens from the Upper Cretaceous amber of northern Myanmar, a new species of the extinct pygidicranid subfamily Astreptolabidinae is described and figured. As one of the specimens is a male with its genitalia partly exerted, this discovery also permits an account of the male for the subfamily, providing new characters which emphasize the distinctiveness of this lineage. Based on the new species, the diagnosis of the subfamily is slightly emended to accommodate variations previously unknown.

Materials and methods

The three amber specimens discussed in this study were collected from mines in the Hukawng Valley of Kachin in northern Myanmar. The amber mines are located at the north end of Noije Bum that is at approximately 26.150N, 96.340E, 18 km southwest of Tanai (Shi et al. 2012). The age of Burmese amber is documented as 98.79 ± 0.62 Ma (Shi et al. 2012), which places it precisely at the mid-Cretaceous, in the lowermost Cenomanian near the Albian boundary (Shi et al. 2012; Grimaldi and Ross 2017). The type specimens are housed in the fossil insect collection of the Key Lab of Insect Evolution and Environmental Changes, College of Life Sciences, Capital Normal University, Beijing, China.

The new specimens were examined and photographed using a Leica M205C dissecting microscope with a Leica DFC450 digital camera system. The detailed and enlarged photos were taken by using a Nikon SMZ 25 microscope with a Nikon DS-Ri 2 digital camera system. Line drawings were prepared by using Adobe Illustrator CC and Adobe Photoshop CS5 graphics software. Morphological terminology and the higher classification follow those of Engel and Haas (2007), Giles (1963), and Hincks (1956). The description is based on that of the holotype female and paratype male, with differences in the paratype female discussed separately.

Taxonomy

Family Pygidicranidae Verhoeff, 1902
Subfamily Astreptolabidinae Engel, 2011

Emended diagnosis (modified from Engel (2011)). Tiny earwigs (ca. 3.50–5.30 mm in length); somewhat dorsoventrally compressed, setation variable (either sparsely setose or minutely hirsute); integument somewhat matt. Head prognathous, broad, slightly broader than anterior border of pronotum, apparently mildly tumid, posterolateral corners gently curved, posterior border not concave; compound eyes well developed, prominent; ocelli absent; antenna with at least 14 antennomeres (as noted by Engel (2011)), scape stout, pedicel longer than wide, flagellomeres longer than wide, progressively more elongate from flagellomere II–X, with X–IV subequal in size. Pronotum large, anterior and posterior borders gently convex, lateral borders slightly divergent posteriorly and rounded, anteriorly slightly narrower than head, posteriorly broader than head, all borders not carinate. Tegmina present, without venation, symmetrical, elongate, outer margins convex, apex gently curved and tapering to midline (not truncate), covering first four abdominal segments; hind wings present, with squama slightly exposed from under tegmina. Femora apparently not carinate; tarsi trimerous, second tarsomere shortest or as long as third tarsomere, not extending beneath base of third tarsomere; pretarsal ungues simple; arolium vestigial. Abdomen slender, elongate (eight visible segments for females), most segments only slightly wider than long, apicalmost segment with straight apical margin, without tubercles. Cerci symmetrical, straight, tubular, gently tapering to acute apex, without tubercles, dentition, or serrations; pygidium not evident. Female valvulae scarcely evident apically, largely hidden; male with stout parameres, apically pointed, without accessory teeth or incisions; two virgae present, both directed apically; each distal lobe with a ventral sclerotized accessory structure bearing a comb of prominent teeth below each virga.

Genus *Astreptolabis* Engel, 2011

Diagnosis. Refer to that of subfamily (*vide supra*).

Comments. Given that the subfamily contains a single genus, *Astreptolabis* Engel, 2011, the diagnosis of the subfamily and genus are identical.

The genus presently includes only two species: the type species, *Astreptolabis ethirosomatia* Engel, 2011, and *Astreptolabis laevis* sp. nov.

Astreptolabis laevis sp. nov.

http://zoobank.org/CFBEA9C6-7BF4-49A8-82AD-7D671B82634F
Figs 1– 4

Diagnosis. The new species can be distinguished from *A. ethirosomatia* on the basis of the more sparse setation, particularly on the head, pronotum, and tegmina (distinctly and minutely hirsute in *A. ethirosomatia*); the larger compound eyes, which encompass the entire lateral surface of the head from the antennal articulations to the posterior border (in *A. ethirosomatia* the compound eyes are smaller, distinctly separated anteriorly from the antennal base and posteriorly from the temple margin); distance between compound eyes subequal to compound eye length (distance between compound eyes in *A. ethirosomatia* distinctly greater than compound eye length); absence of ocular setae (present in *A. ethirosomatia*). On the surface there would appear to be further proportional differences between the new species and the type species, but the holotype of *A. ethirosomatia* is poorly preserved and largely compressed with considerable taphonomic distortion (Engel 2011).

Description. ***Female:*** Total length as preserved (including cerci) ca. 3.61 mm (Fig. 1); sparsely setose; head medial length from clypeal apex to posterior border 0.47 mm, maximum width (across level of compound eyes) 0.59 mm; compound eye length 0.25 mm, separated from posterior border of head by minute distance. Pronotum medial length 0.51 mm, anterior width 0.37 mm, posterior width 0.60 mm (Fig. 2A); tegmen length 1.18 mm, maximum width 0.46 mm. Abdominal length as preserved (excluding cerci) 1.43 mm, maximum width 0.54 mm; second tarsomere shortest but almost as long as third tarsomere; arolium vestigial; cercal forceps length 0.61 mm, basal width 0.07 mm, separation between bases 0.05 mm. Integument as preserved dark brown, punctate, somewhat smooth throughout. Legs without spines or bristle-like setae (Fig. 2C). Valvulae extending slightly beyond apex of subgenital plate (Fig. 2D).

Hind wings well developed (Fig. 1); area of hindwing 0.5 mm^2 folded, 2.9 mm^2 unfolded; squama sclerotized, extending a little beyond apex of tegmina; ulnary area distad squama; eight radiating veins and eight intercalary veins in anal area, with concave and convex folding lines between them; ring fold running through anal fan, intersecting with radiating and intercalary veins in broadened areas (Figs 1, 2B).

Male: Total length as preserved (including cerci) ca. 5.30 mm (Fig. 4A, B); sparsely setose; head medial length from clypeal apex to posterior border 0.47 mm, maximum

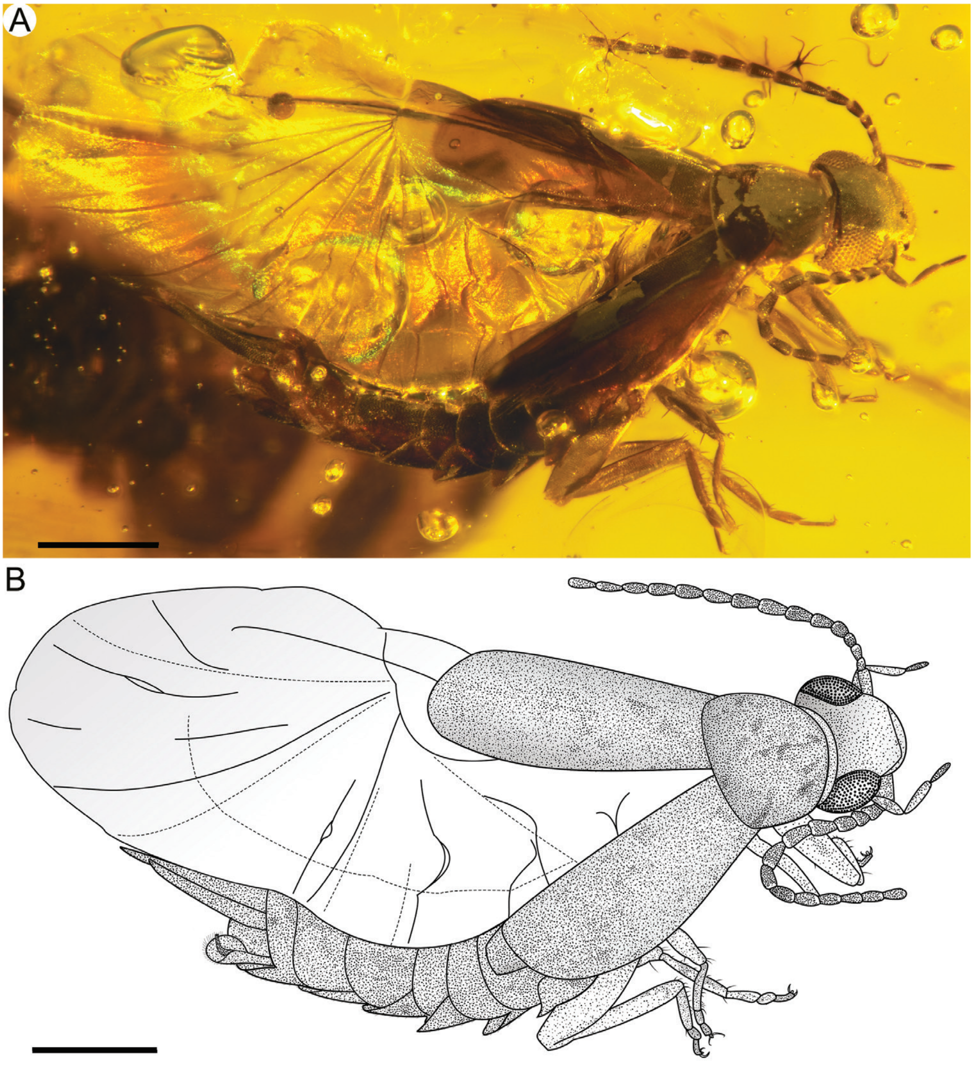

Figure 1. *Astreptolabis laevis* sp. nov., holotype, CNU-DER-MA2018001 **A** photo **B** line drawing. Scale bars: 0.5 mm.

width (across level of compound eyes) 0.71 mm. Pronotum medial length 0.61 mm, anterior width 0.46 mm, posterior width 0.72 mm; tegmen length 1.53 mm, maximum width 0.64 mm. Abdominal length as preserved (excluding cerci) 1.96 mm; second tarsomere shortest but almost as long as the third; arolium vestigial (Fig. 4C); cercal forceps length 0.74 mm. Integument as preserved brown, somewhat smooth throughout. Legs without spines or bristle-like setae. Parameres broad, tapering to acute apex, without incisions or teeth, with a series of sensory setae along inner margin; two virgae extended, apically, with comb-like accessory sclerites positioned ventrally on distal lobes (Fig. 4D, E).

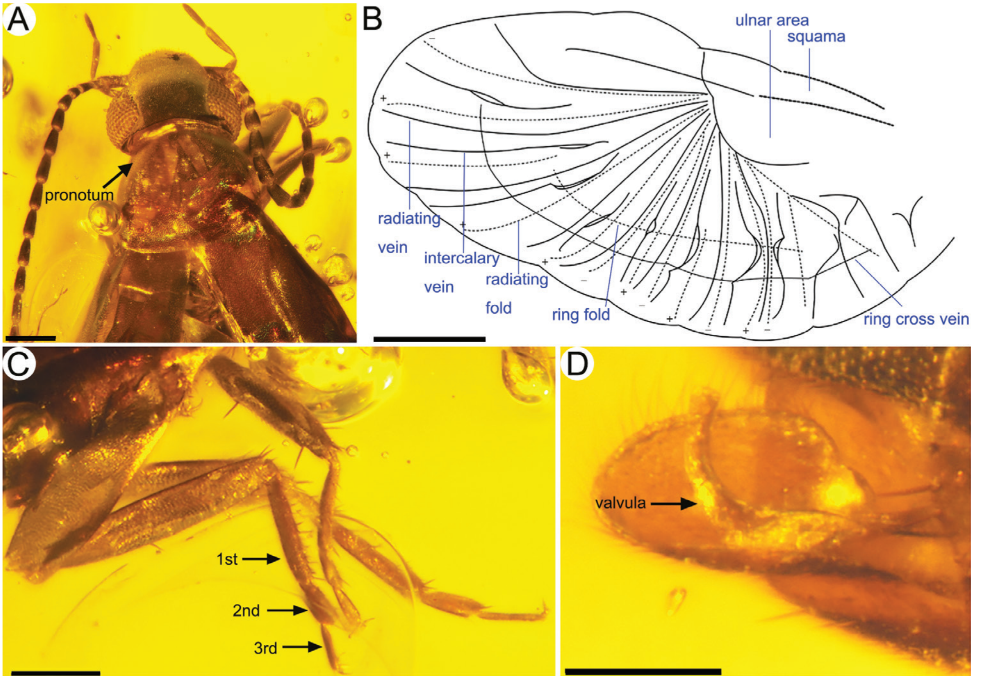

Figure 2. *Astreptolabis laevis* sp. nov., holotype, CNU-DER-MA2018001 **A** photo of pronotum **B** line drawing of hind wing **C** photo of legs **D** photo of vavula. Scale bars: 0.2 mm (**A, C**), 0.5 mm (**B**), 0.1 mm (**D**).

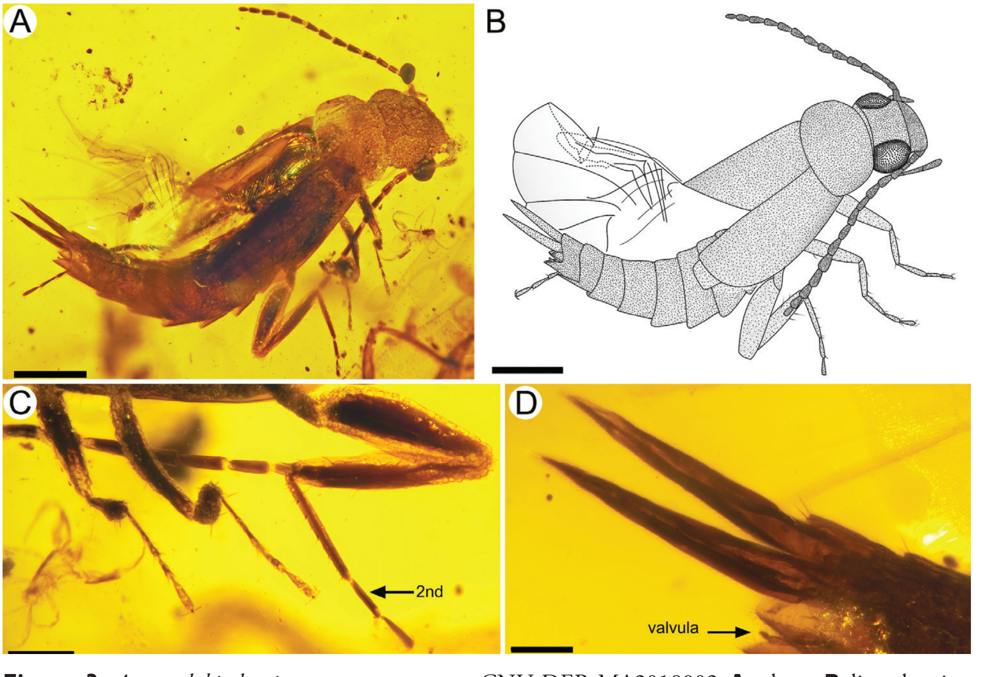

Figure 3. *Astreptolabis laevis* sp. nov., paratype, CNU-DER-MA2018002 **A** photo **B** line drawing **C** photo of legs **D** photo of cerci. Scale bars: 0.5 mm (**A, B**), 0.2 mm (**C**), 0.1 mm (**D**).

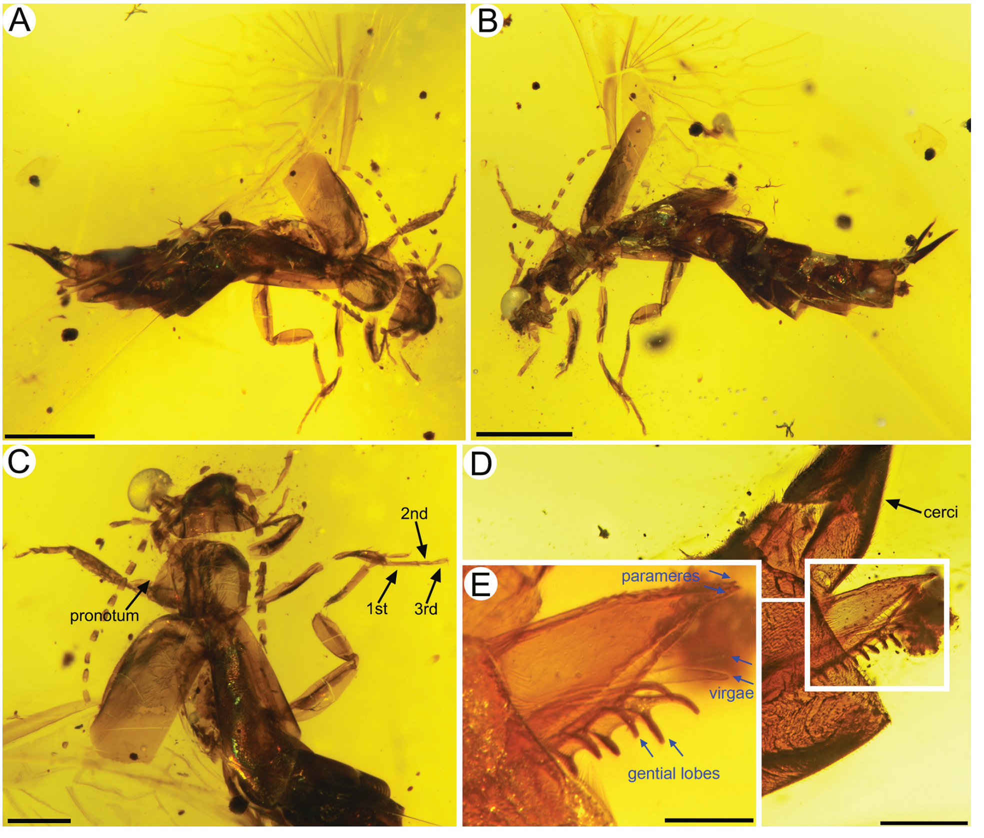

Figure 4. *Astreptolabis laevis* sp. nov., paratype, CNU-DER-MA2018003 **A** dorsal view **B** ventral view **C** photo of pronotum and legs **D** photo of male genitalia **E** enlarged view of male genitalia. Scale bars: 1 mm (**A, B**), 0.5 mm (**C**), 0.2 mm (**D**), 0.1 mm (**E**).

Hind wing well developed, congruent with the description above, and unfolded.

Remarks. The hind wing of the holotype of *A. laevis* is well preserved, and one is unfolded and extended. The base of the hind wing is obscured because of the position of the specimen, but most of the preserved structures are similar to those of extant earwigs. The female paratype CNU-DER-MA2018002 shares the same characters with the holotype, but the integument of this paratype is somewhat roughened and the pronotum seems broader than the holotype; however, these differences may be the result of taphonomy. The male paratype CNU-DER-MA2018003 shares the same characters with the holotype except for a larger body size, which seems to be a sexual difference. Otherwise, differences in body size are mainly reflected in tegmen length and abdominal length which are longer than the female, but otherwise proportional.

Type material. Holotype, ♀, CNU-DER-MA2018001, dorsal view, a well-preserved complete female. Paratype ♀ CNU-DER-MA2018002, dorsal view, a well-preserved complete female. Paratype ♂, CNU-DER-MA2018003. All type material deposited in College of Life Sciences, Capital Normal University, Beijing, China.

Locality and horizon. Hukawng Valley, Kachin State, northern Myanmar; lowermost Cenomanian, mid-Cretaceous.

Etymology. The specific epithet is the Latin word *laevis*, meaning, "polished" or "smooth", in reference the integumental surface of the species.

Discussion

Up to now, many than 1000 species of insects have been reported from Burmese amber (Grimaldi et al. 2002; Ross 2019), including termites (Engel et al. 2016; Zhao et al. 2019), stick insects (Chen et al. 2018, 2019), scorpionflies (Lin et al. 2019), lots of wasps (Zhang et al. 2018), beetles (Cai et al. 2018), lacewings (Liu et al. 2018), etc.; however, earwigs are still quite rare compared to most of the other groups. *Astreptolabis* are the smallest earwigs within Pygidicranidae so far, females being as small as about 3.5 mm in length including cerci. Antenna has at least 14 antennomeres, which is an unusually small number for basal Neodermaptera and likely autapomorphic for the subfamily. Although the known representatives are quite peculiar among living and fossil earwigs, particularly in the straight, tapering, tubular cerci which clearly would have had little force as a grasping structure (contrary to virtually all other Neodermaptera), it has the usual traits typical of the suborder such as the absence of ocelli, the trimerous tarsi, unsegmented cerci, and absence of venation in the tegmina (Engel 2011). The straight and tubular cerci which likely did not function for grasping could imply that astreptolabidines used other strategies for predation, or were scavengers or detritivores and therefore did not hunt. The new species is typical in nearly all traits with the type species, aside from minor differences in largely setation, and proportions of structures. Nonetheless, the new species helps to refine our understanding of the circumscription for the genus and subfamily, and gives us some initial knowledge as to variations that may occur within the lineage. Unfortunately, while it would be revealing to learn more about the structure of the ventral cervical sclerites and thoracic sterna, these cannot be discerned in any of the new specimens and await future discoveries to provide such insights. Nonetheless, the discovery of the male for the group is important, and the uniqueness of the Astreptolabidinae is reinforced by the peculiar features of the male genitalia. Like other Pygidicranidae, the male has two virgae, rather than four terminal virgal sections present in Diplatyidae. However, unlike most other Pygidicranidae the parameres are broader and lack terminal teeth or incisions, and are instead comparatively simple, tapering to an acute apex. These are either specializations apomorphic for the subfamily, or could also suggest that including Astreptolabidinae within Pygidicranidae renders the family paraphyletic. If the latter, then there may be need to elevate the subfamily to family rank (as Astreptolabididae Engel, *nomen translatum*), but such a formal decision must await further character data such as the form of the thoracic sterna which could provide evidence of affinity to one or more subfamilies within Pygidicranidae as currently defined.

However, the female valvulae slightly extending beyond the subgenital plate is a trait known only among the Pygidicranidae (Engel and Grimaldi 2004), and this feature in *A. laevis* tends to corroborate its inclusion among pygidicranids. For the time being, the male of *A. laevis* emphasizes the distinctiveness of the lineage and highlights the need to obtain further character data for this group of peculiar, ancient Neodermaptera. If the subfamily were to fall outside of Pygidicranidae it remains uncertain whether it would be basal to the family or more closely related to Epidermaptera, and perhaps information on sternal forms would aid such a determination. The presence of complex, heavily sclerotized accessory structures ventral to the paired distal lobes is at least reminiscent of the accessory structures sometimes found among diplatyids, although the size and form of those in *Astreptolabis* are drastically different. If these were homologous, then it might suggest that Astreptolabidinae are intermediate in phylogenetic position between the basal families Diplatyidae, Haplodiplatyidae, and Karschiellidae relative to Pygidicranidae.

To date, there are only four Cretaceous amber species of adult pygidicranids published: *Burmapygia resinata* Engel & Grimaldi, 2004, *A. ethirosomatia* Engel, 2011, *Stonychopygia leptocerca* Engel et al., 2017, and *Gracilipygia canaliculata* Ren et al., 2017 (Engel and Grimaldi 2004; Engel et al. 2017; Ren et al. 2017). As noted by the features of the subfamily (*vide supra*), *Astreptolabis* differs greatly from all of these groups.

Interestingly, the wing morphology of the hind wings of *A. laevis* is quite similar to extant earwigs. Though the broad attachment and the base of the hind wing is covered by the tegmina, the anal area is relatively clear, and the same areas of folding can be discerned as is found across all Neodermaptera, emphasizing the consistency of this specialization within the order. In addition, the shortened tegmina is known to allow for flexibility in the abdomen and its role in folding the hind wings when not in use and this behavioral repertoire is likely also conserved.

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RESEARCH ARTICLE

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Aurivillius's "Neue oder wenig bekannte Coleoptera Longicornia" (1886–1927), the correct years and page numbers

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Abstract
Aurivillius's work entitled "Neue oder wenig bekannte Coleoptera Longicornia" was published in parts over a period of over four decades. There were two page numbers on most pages of these publications, one ordered by Aurivillius, the other by the journal. Historically, different authors have used different page numbers, and sometimes different years for these publications, which has caused chaos in the citations. Herein, accurate dates of publications for this work, and correct page numbers that should be used are provided and discussed.

Keywords
Correction, misunderstanding, publication date, reference, years of submission, Zoological Record

Introduction

Christopher Aurivillius (1853–1928) was a very important Swedish entomologist, who published 67 references regarding Cerambycidae from 1886 to 1929 (Tavakilian and Chevillotte 2019). Among them, 20 parts were titled as "Neue oder wenig bekannte Coleoptera Longicornia" and numbered from 4 to 23. Most of them (except the 8th part) have two page numbers printed on each page, both of which have been cited by many different authors. In order to determine the correct page numbers and the accurate dates of publication for this significant work, we analyzed all the Cerambycidae literature of Aurivillius.

Materials and methods

Methods of literature collecting

We accessed literature in three ways for this study: a) downloaded pdf files from the Biodiversity Heritage Library: http://www.biodiversitylibrary.org/; b) copied the original pages directly from library holdings (the first author visited the libraries of the Institute of Zoology, Chinese Academy of Sciences, Beijing, China; National Science Library, Chinese Academy of Science, Beijing, China; Muséum national d'Histoire naturelle, Paris, France; Division of Plant Industry, Florida State Collection of Arthropods, Gainesville, Florida, USA; and the National Museum of Natural History (Smithsonian Institution), Washington DC, USA, etc.); c) solicited help from colleagues (especially G. Tavakilian and S. Lingafelter).

Dating the publications

In researching the dates of publication for this work, we consulted five points of reference: a) date printed on first and last pages; b) date shown by the Zoological Record; c) date used by Aurivillius's catalogues; d) date used by literature citing related references; e) date printed on original wrapper.

Results

Historically different ways of citing page numbers

We examined most of the literature citing Aurivillius's "Neue oder wenig bekannte Coleoptera Longicornia" published in the journal "Arkiv för zoologi", and gathered the results herein.

a) Citation using the journal's page numbers: Aurivillius 1912, 1922, 1923, 1928; Breuning 1939, 1940, 1944, 1950, 1956, 1958–1969; Lane 1950; Quentin 1956; Podaný 1968, 1971; Gressitt and Rondon 1970; Rondon and Breuning 1970; Hüdepohl 1985, 1988, 1989, 1990; Lee 1987; Niisato 1989, 2007; Nakamura et al. 1992; Napp 1993; Nýlander 1998; Makihara 1999; Heffern 2002; Makihara and Woro 2002; Morati and Huet 2004; Ohbayashi and Niisato 2007; Bousquet et al. 2009; Morati and Bentanachs 2009; Bentanachs et al. 2010; Sudre et al. 2010;

Juhel 2011; Jiroux 2011; Vitali 2011; Weigel and Skale 2011; Vitali and Vitali 2011; Wallin et al. 2014; Lin 2015; Vitali et al. 2017.

b) Citation using both Aurivillius's and the journal's page numbers, but considering journal numbers as more important: Löbl and Smetana 2010; Heffern 2011; Viktora 2013, 2015a, 2019; Viktora and Tichý 2017; Lin and Yang 2019.

c) Citations using both Aurivillius's and the journal's page numbers, but considering Aurivillius's numbers as more important: Hüdepohl 1992; Juhel and Bentanachs 2010.

d) Citations using Aurivillius's page numbers: Tavakilian 1991; Martins and Galileo 1992; Martins 1997, 1998, 1999, 2002, 2005, 2007, 2009, 2011, 2014; Adlbauer 1998; 2002a, 2002b, 2013; Napp and Mermudes 1999; Vives and Heffern 2001; Vives and Abang 2003; Hüdepohl and Heffern 2004; Heffern 2005; Monné 2005a, 2005b, 2012, 2019a, 2019b, 2019c, 2019d; Monné and Napp 2005; McCarty 2006; Napp 2007; Yokio and Niisato 2009; Juhel and Bentanachs 2009a, 2011, 2012; Vives 2009, 2012, 2015a, 2015b, 2015c, 2015d, 2017; Martins and Santos-Silva 2010; Lin and Yang 2011; Juhel 2012, 2014a, 2015; Monné et al. 2012, 2016; Huang et al. 2014; Jiroux et al. 2014; Lingafelter et al. 2014; Miroshnikov 2014; Gouverneur 2015; Monné and Monné 2015, 2017; Viktora 2015b; Rousset et al. 2016; Santos-Silva and Galileo 2016; Sudre et al. 2016; Viktora and Tichý 2016; Vitali 2016, 2018; Yan and Chen 2016; Yokio and Heffern 2016; Bentanachs and Jiroux 2017; Santos-Silva and Botero 2017. Note that some of these citations could have followed the Titan database (Tavakilian and Chevillotte (2019) since they also cited that database (e.g., Huang et al. 2014; Gouverneur 2015).

e) Random citation method: Sometimes using the journal's page numbers and sometimes using Aurivillius's page numbers in the same paper: Gressitt 1940, 1951; Lingafelter and Hoebeke 2002; Juhel and Bentanachs 2009b; Ślipiński and Escalona 2013; Juhel 2014b, 2016; Nakamura et al. 2014; Lin 2017; Bezark 2019; Lazarev and Murzin 2019.

Common errors encountered when citing this series of papers

The errors occurred in the date (see Table 1), page numbers (see Table 1), information regarding the figures and plates, part numbers, first and last page numbers, journal volume numbers, and so on.

a) Errors regarding the separate plates. There were two kinds of figures in this work, text-figures were inside the content and provided with continuous numbers (see Table 1), while end-plates were printed as separate plates, normally numbered from one. The former can be ignored in the reference, while the latter should be added. For example, Hüdepohl and Heffern (2004) wrote the reference as "Aurivillius, C. 1907. Neue oder wenig bekannte Coleoptera Longicornia. 9. Arkiv för zoologi. 3(18): 93–131. 9 fig.", the "9 fig." would be better stated as "pl. 1: fig. 1–9" or "1 pl.", since there were seven text-figures (figs 35–41) inside the content too, which

Table 1. Bibliographic details of the series "Neue oder wenig bekannte Coleoptera Longicornia" by Aurivillius (1886–1927). AFZ: Arkiv för zoologi; ET: Entomologisk Tidskrift; ICZN: International Code for Zoological Nomenclature.

Publishing year	Title	Journal name	Volume: page numbers by the journal	Page numbers arranged by Aurivillius	Species numbers	Text-figure numbers (inside content)	End-plates (separated from content)	Date of submission (on first page)	Released / printed date (on last page)	Came out date (for the volume normally)
1886	Nya Coleoptera Longicornia	ET	7(2): 89–94.	none (1–6 concluded herein)	1–5	none	none			12 July 1886
1887	Nya Coleoptera Longicornia. II.	ET	8(4): 191–197.	none (7–14 concluded herein)	6–10	figs 1–3.	none			31 Dec. 1887
1891	Neue Coleoptera Longicornia. III	ET	12 (2): 97–106.	15–24	11–20	figs 1–6.	none			18 June 1891
Aurivillius began to arrange continuous page numbers for this series as a whole from the third part in 1891. There were 14 pages for the previous two parts titled "Nya Coleoptera Longicornia", so the third part started on page 15. Though the third part did not use the exact same title, the meaning of the titles were the same.										
1893	Neue oder wenig bekannte Coleoptera Longicornia. 4.	ET	14(3): 177–186.	25–34	21–30	figs 1–12.	none			25 May 1893
Aurivillius fixed the title as "Neue oder wenig bekannte Coleoptera Longicornia" from part 4 in 1893, then kept it to the end. In this table, parts 5 to 23 did not repeat the title but only marked the part numbers. Species number 21 was related to one new genus whose type species was not described by Aurivillius. Later, genera were not numbered again.										
1897	5.	ET	18(4): 241–248.	35–42	31–41	none	Pl. 3: figs 1–8.			19 Jan. 1898
According to Derksen and Scheiding (1963), the date of publication is 1897. All references we searched and the Titan database (Tavakilian and Chevillotte 2019) used 1897, also the Zoological Record indicated 1897. However, the website https://www.biodiversitylibrary.org/item/89782#page/8/mode/1up indicated that the publication date 19 Jan. 1898. "Utgifvet den 19 januari 1898" (= issued/published on January 19th 1898) (printed low on the back side of the original wrapper) should be the official publication date (personal communication with Mikael Sörensson on 10 December 2019).										
1899	6.	ET	20(4): 259–265.	51–57	42–52	figs 13–17.	none			23 Jan. 1900
For Aurivillius's own page numbers, the fifth part ended with page 42, while the sixth part began with page 51. That was because Aurivillius gave one page for each figure, therefore eight figures took the pages 43 to 50. The figures inside the sixth part continued the numbers from the fourth part, while the figures in fifth part arranged as a separate plate were not numbered continuously. "Utgifvet den 23 januari 1900" (= issued/published on January 23rd 1900) (printed low on the back side of the original wrapper) should be the official publication date (personal communication with Mikael Sörensson on 10 December 2019). Also the website https://www.biodiversitylibrary.org/item/43633#page/584/mode/1up indicated the publication date as 23 Jan. 1900. We chose 1899 based on the Zoological Record and Derksen and Scheiding (1963).										
1902	7.	ET	23: 207–224.	59–76 (printed as:1, 2, 61–76)	53–75	figs 18–26.	none			2 Sept. 1902
Aurivillius's page number did not have page 58. That was because every new part began with odd numbers. Therefore, when the previous part ended with odd numbers (parts 6, 9, 12, 13, 15, 16, 17, 19, 20, 22), one even number would be taken by an empty page (page numbers 58, 228, 264, 334, 404, 480, 502) or by a separated plate (page numbers 132, 360, 548).										
1903	8.	AFZ	1: 313–328.	Not printed (but should be 77–92 in conclusion)	76–96	figs 27–34.	none	14 Oct. 1903	27 Nov. 1903	21 Jan. 1904

Publishing year	Title	Journal name	Volume: page numbers by the journal	Page numbers arranged by Aurivillius	Species numbers	Text-figure numbers (inside content)	End-plates (separated from content)	Date of submission (on first page)	Released / printed date (on last page)	Came out date (for the volume normally)
Aurivillius chose the journal AFZ since the eighth part and did not change it again. In this first printing in the AFZ journal, the journal did not include Aurivillius's own page numbers. But from the ninth part, the AFZ printed both the journal's page number on top and Aurivillius' own page number at bottom. The date of publication of the eighth part is confusing. The printing date on the last page is "Tryckt den 27 november 1903", so it should be 1903. However, Aurivillius (1912; 1922) cited this part as 1904, and Zoological Record indicated 1904, which making this a confusing situation. We believe that 1903 is the correct publication year, because Aurivillius corrected it to 1903 in his 1923's catalogue. Most authors cite the eighth part as year 1903 (Heffern 2005, but erroneously missed the first page 313; Makihara 1999; Heffern 2011 (corrected and added page 313)).										
1907	9.	AFZ	3(18): 1–39.	93–131	97–155	figs 35–41.	pl. 1: figs 1–9.	12 Sept. 1906	7 Feb. 1907	24 Sept. 1907
The correct publication date of the ninth part is surely 1907, and most of authors cited it correctly (Aurivillius 1912, 1922, 1923; Breuning 1944; Gressitt 1951; Podaný 1971; Nakamura et al. 1992; Hüdepohl and Heffern 2004; Heffern 2005, 2011; Makihara and Woro 2002; Morati and Bentanachs 2009; Juhel and Bentanachs 2011, 2012; Nakamura et al. 2014; Yokio and Heffern 2016; Bentanachs and Jiroux 2017; Bezark 2019; Lin and Yang 2019). However, some authors used the submission date of 1906 (e.g., Adlbauer 2002b); while some authors used the year 1908 for unknown reason (e.g. Makihara 1999).										
1908	10.	AFZ	4(17): 1–9.	133–141	156–167	figs 42–47	none	11 Mar. 1908	1 May 1908	30 Sept. 1908
1910	11.	AFZ	7(3): 1–44.	143–186	168–223	fig. 48	none	1 June 1910	24 Sept. 1910	25 Jan. 1911
The publication date of the 11th part is confusing. The correct year should be 1910, since on the last page it was printed "Tryckt den 24 september 1910", Aurivillius himself cited it as 1910 (Aurivillius 1912, 1922, 1923), and the Zoological Record also listed it as 1910. Most authors cited it correctly (Quentin 1956; Podaný 1968; Hüdepohl 1992; Martins 1997, 1998, 2002; Vives and Heffern 2001; Heffern, 2002; 2005; Vives and Abang 2003; Monné 2005a, 2012, 2019a, d; Bousquet et al. 2009; Bentanachs et al. 2010; Gouverneur 2015; Monné and Monné 2015; Viktora 2015b; Bezark 2019; Lin and Yang 2019). However, some authors used 1911 because the whole seventh volume was published on January 25, 1911 (Napp 1993; Morati and Huet 2004; Löbl and Smetana 2010; Heffern 2011; Viktora and Tichý 2017).										
1911	12.	AFZ	7(19): 1–41.	187–227	224–291	figs 49–57	none	7 June 1911	8 Dec. 1911	23 Dec. 1911
1913	13.	AFZ	8(22): 1–35.	229–263	292–351	figs 58–68	none	4 June 1913	17 Oct. 1913	31 Dec. 1913
1914a	14.	AFZ	8(29): 1–54.	265–318	352–453	none	pl. 1: figs 1–9	25 Feb. 1914	9 May 1914	22 May 1914
Aurivillius's own page numbers did not state numbers for separate plates, though when the text ended with odd numbers the even numbers could be considered as numbers for the separate plates. There was not page number for the separate plate of the 14th part, since the text ended with an even number 54 (=318) and the 15th part began with 1 (=319).										
1914b	15.	AFZ	9(8): 1–15.	319–333	454–483	none	none	9 Sept. 1914	4 Nov. 1914	15 May 1915
The publication date of the 15th part is confusing. According to ICZN 21.2 and 21.8, the correct date should be 1914, since on the last page it was printed "Tryckt den 4 november 1914", Aurivillius himself cited it as 1914 (Aurivillius 1922, 1923), and the Zoological Record also included it in 1914. Most authors cited it correctly (Adlbauer 2002; Juhel and Bentanachs 2011; Juhel 2014a).										
1916	16.	AFZ	10(19): 1–25.	335–359	484–523	figs 69–72	pl. 1: figs 1–9	7 June 1916	12 Aug. 1916	28 Nov. 1916
The page 26 (=360) is unnecessary to be included, though it doesn't matter so much if it is included. On the page 26 (=360), there was no content written by Aurivillius, but only "Tryckt den 12 augusti 1916. / Uppsala 1916. Almqvist & Wiksells Boktryckeri-A.-B. / -360-" Also, there was an ending mark on the page 25 (=359). In a similar situation that happened to the 12th part, 42 (= 228) normally was not included.										
1920	17.	AFZ	13(9): 1–43.	361–403	523–595	figs 73–81	none	10 Mar. 1920	6 Sept. 1920	11 Oct. 1920
The species number 523 was used twice. It was used for "523. *Hilarolea humeralis*" in the 16th part, and again as "523. *Ophistomis splendida*" in the 17th part. The publication date of this part is surely 1920 and few errors were made. Martins (2005) carelessly used 1922 but he corrected it to 1920 later (Martins 2011, 2014).										
1922	18.	AFZ	14(18): 1–32.	405–436	596–639	figs 82–112	none	7 Dec. 1921	4 April 1922	26 July 1922
The publishing year of the 18th part is surely 1922. However, some authors used the submission date 1921 (e.g., Makihara, 1999), or even 1920 for unknown reasons (e.g. Makihara and Woro 2002).										

Publishing year	Title	Journal name	Volume: page numbers by the journal	Page numbers arranged by Aurivillius	Species numbers	Text-figure numbers (inside content)	End-plates (separated from content)	Date of submission (on first page)	Released / printed date (on last page)	Came out date (for the volume normally)
1923	19.	AFZ	15(25): 1–43.	437–479	640–711	figs 113–133	none	6 June 1923	31 Dec. 1923	Jan. 1924
The date of publication of the 19th part is one of the most confusing cases, since different people used different years and for different reasons. The correct year should be 1923. Many authors used 1923 because of the "Tryckt den 31 december 1923"on the last page (Aurivillius 1928; Breuning 1939, 1944, 1950; Lane 1950; Gressitt and Rondon 1970; Hüdepohl 1985, 1988, 1992; Makihara 1999; Martins 1999, 2009, 2014; Lingafelter and Hoebeke 2002; Hüdepohl and Heffern 2004; Heffern 2005; Makihara and Woro 2002; Monné 2005a, b, 2012; 2019a, b, d; Monné and Napp 2005; Yokio and Niisato 2009; Vives 2011; Monné et al. 2016; Monné and Monné 2017; Bezark 2019). However, since Zoological Record cited 1924 for this work, so do the Titan database (Tavakilian and Chevillotte 2019). Less than half of authors used 1924 as the publication date (Lee 1987; Hüdepohl 1990; Heffern 2011; Lingafelter et al. 2014; Nakamura et al. 2014; Viktora and Tichý 2016; Vitali et al. 2017; Lin and Yang 2019). The first author of this paper Mei-Ying Lin was confused in this case (chose 1924 in the catalogue by Lin and Yang (2019) and an earlier version of this paper, but finally decided to choose the earlier date of "Tryckt den 31 december 1923"). For unknown reasons, 1925 was used by Löbl and Smetana (2010) in the Palaearctic catalogue, and this was followed by Viktora (2013), Viktora and Tichý (2017) and Lazarev and Murzin (2019). Makihara and Woro (2002) erroneously used 1922.										
1925a	20.	AFZ	17A(12): 1–21.	481–501	712–753	figs 134–140	none	4 June 1924	17 Feb. 1925	
The publication date of the 20th part is 1925, however, some authors wrongly used the submission year 1924 from the first page (e.g., Makihara 1999; Makihara and Woro 2002).										
1925b	21.	AFZ	18A(9): 1–22.	503–524	754–795	figs 141–163	none	3 June 1925	17 Nov. 1925	1926
The publication date of the 21st part is also one of the most confusing cases. We consider 1925 as the correct year for two reasons: a) "Tryckt den 17 november 1925" was printed on the last page, which is the correct year according to ICZN items 21.2 and 21.8; b) Titan database and the following literature used 1925 (Makihara 1999; Martins 1999; Heffern 2005; Monné 2005a, b, 2012, 2019a, b, d; Lin and Yang 2011; Lingafelter et al. 2014; Santos-Silva and Galileo 2016; Sudre, et al. 2016; Bezark 2019; Lin and Yang 2019). However, both Zoological Record and the Palaearctic catalogue (Löbl and Smetana 2010) used 1926, as did the following (Gressitt 1940, 1951; Bousquet et al. 2009; Heffern 2011; Ślipiński and Escalona 2013; Lin 2017).										
1927a	22	AFZ	19A(17): 1–23.	525–547	796–844	figs 164–177	pl. 1: figs 1–6	1 June 1927	20 Sept. 1927	3 Nov. 1927
1927b	23	AFZ	19A(23): 1–41.	549–589	845–919	figs 178–202		14 Sept. 1927	21 Dec. 1927	25 Jan. 1928
The publication date of the 23rd part is also one of the most confusing cases. We consider 1927 as the correct year for two reasons: a) "Tryckt den 21 december 1927" was printed on the last page, which is the correct date according to ICZN items 21.2 and 21.8; b) Titan database also used 1927. The following literature used 1927 (Breuning 1944, 1950, 1956; Hüdepohl 1988; Heffern 2005; Weigel and Skale 2011; Vives 2012, 2015b, 2017; Lingafelter et al. 2014; Nakamura et al. 2014; Wallin et al. 2014; Rousset et al. 2016; Vitali 2016; Lin and Yang 2019). However, both Zoological Record and the Palaearctic catalogue (Löbl and Smetana 2010) used 1928, as did the following (Heffern 2011; Lazarev and Murzin 2019). "utkom 25. jan. 1928" was printed on the wrapper. We chose the earlier date based on ICZN item 21.8.										

might cause misunderstanding. Sometimes the separate end-plate was missing in the reference (Monné 2005a, 2005b; Vives 2009; Lingafelter et al. 2014; Monné 2019a, 2019b, 2019d; Monné et al. 2016, missing the plate when citing part 16; Monné 2019a, 2019b, 2019d missing the plate when citing part 22).

b) Errors regarding part numbers. Sometimes authors cited the title without the part numbers (Hüdepohl 1985; Martins 1998; Nýlander 1998; Jiroux 2011; Juhel and Bentanachs 2011; Vives 2011; Monné et al. 2012; Juhel 2014a; Wallin et al. 2014; Ślipiński and Escalona 2016; Bezark 2019 for the second and third part), which is an incomplete citation. Sometimes authors made mistakes on the part numbers (for example, Hüdepohl (1992) wrote part 11 incorrectly as 2; Makihara (1999) wrote part 21 while the journal's information indicates part 22; Vives (2009) wrote part 21–22 while the journal's information indicate part 22, and he used the figure numbers as page numbers). Some authors mixed different parts together, such as Martins (2011) mixing part 13 and 15 together as "13. Arkiv Zool. 9(8): 229–263", while the journal's volume 9(8) belongs to part 15, not part 13.

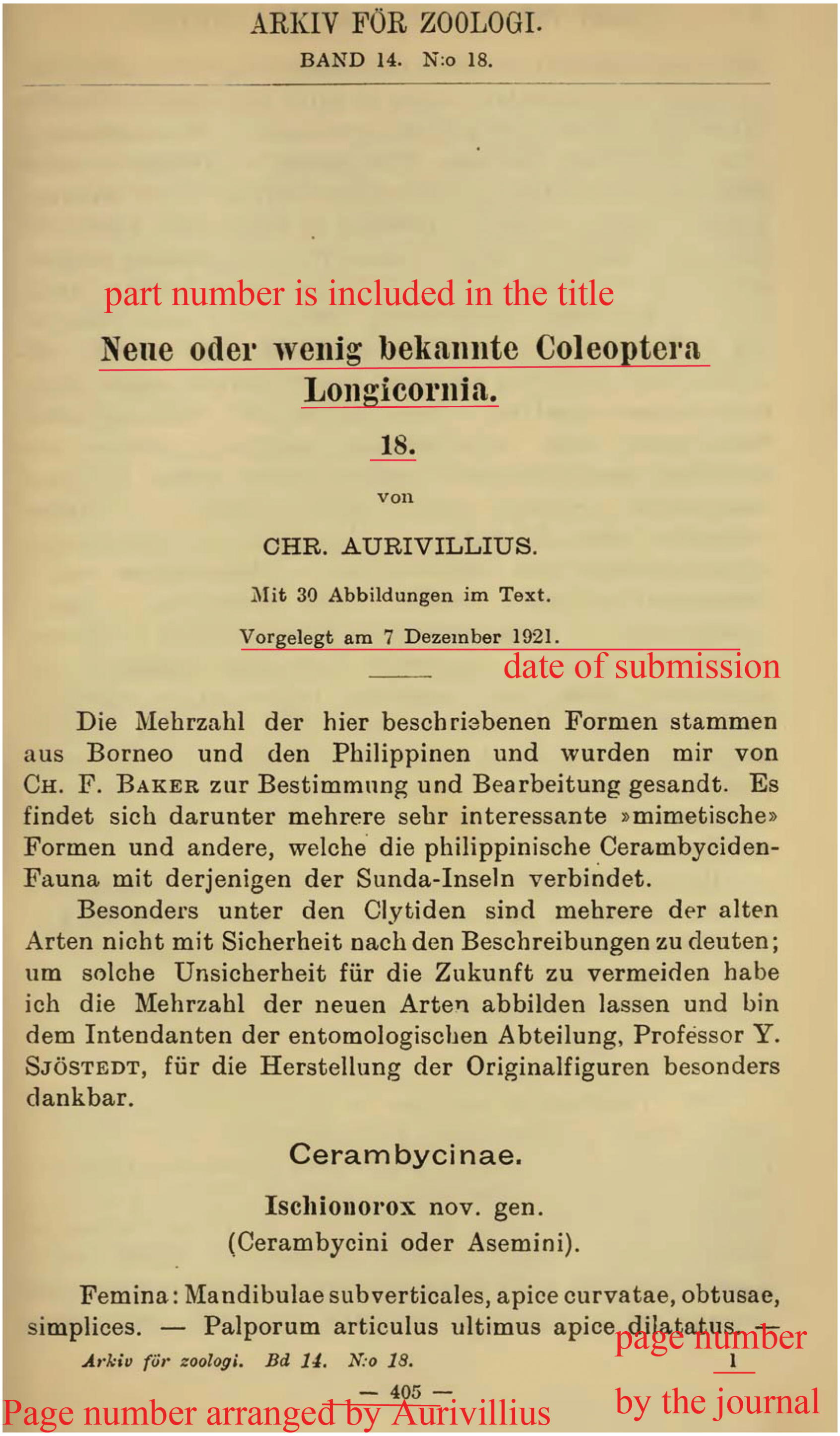
c) Errors regarding first and last page numbers. Sometimes the first page number was missing (Heffern 2005, missing page 313 of part 8), or last page number was missing (Lingafelter and Hoebeke 2002, missing page 224 of part 7, and missing part 7 from the title too; Sudre et al. 2010, missing last 23 pages of part 12; Juhel and Bentanachs 2011, 2012, missing page 39 =131 of part 9; Juhel 2016, missing page 54 = 318 of part 14), or adding one more page (for example, Martins 1997, 1998, 2002, 2005; Monné 2005a, b, 2012; Monné and Monné 2015 and Bezark 2019, added 187 to part 11; Vives 2015d, Yan and Chen 2016 and Lazarev and Murzin 2019, added 228 to part 12). Adding 187 to part 11 is an error that should be corrected, because 187 is the first page number of part 12.

d) Errors regarding about journal volume numbers. Sometimes the volume numbers of the journal were wrongly cited. For example, Podaný (1971) wrote 3 (10) for part 9, while the correct number should be 3 (18); Bentanachs et al. (2010) wrote 7(2) for part 11, while the correct number should be 7 (3); Ohbayashi and Niisato (2007) wrote volume 21 for part 7, while the correct volume number should be 23.

e) Other errors. Some authors cited the figure numbers as page numbers (Vives 2009, 2011), or cited the part number as page number (Löbl and Smetana 2010; Lin 2015, 2017; see fig. 4), or cited page numbers erroneously for unknown reasons (Vives and Abang 2003).

Examples of other types of errors

Nakamura et al. (2014) and Lin (2017) cited part 23 twice in the same paper or book, they used the journal's page numbers and Aurivillius's own numbers in different places in the same publication, and used different years for the same part 23, which made part 23 look like two different articles. Hence, they made mistakes for citations of related taxa. Nakamura et al. (2014) used 1928 for the genus *Mimectatina* in the title, while the origi-



ARKIV FÖR ZOOLOGI.
BAND 14. N:o 18.

Neue oder wenig bekannte Coleoptera Longicornia.

18.

von

CHR. AURIVILLIUS.

Mit 30 Abbildungen im Text.

Vorgelegt am 7 Dezember 1921.

Die Mehrzahl der hier beschriebenen Formen stammen aus Borneo und den Philippinen und wurden mir von Ch. F. Baker zur Bestimmung und Bearbeitung gesandt. Es findet sich darunter mehrere sehr interessante »mimetische» Formen und andere, welche die philippinische Cerambyciden-Fauna mit derjenigen der Sunda-Inseln verbindet.

Besonders unter den Clytiden sind mehrere der alten Arten nicht mit Sicherheit nach den Beschreibungen zu deuten; um solche Unsicherheit für die Zukunft zu vermeiden habe ich die Mehrzahl der neuen Arten abbilden lassen und bin dem Intendanten der entomologischen Abteilung, Professor Y. Sjöstedt, für die Herstellung der Originalfiguren besonders dankbar.

Cerambycinae.

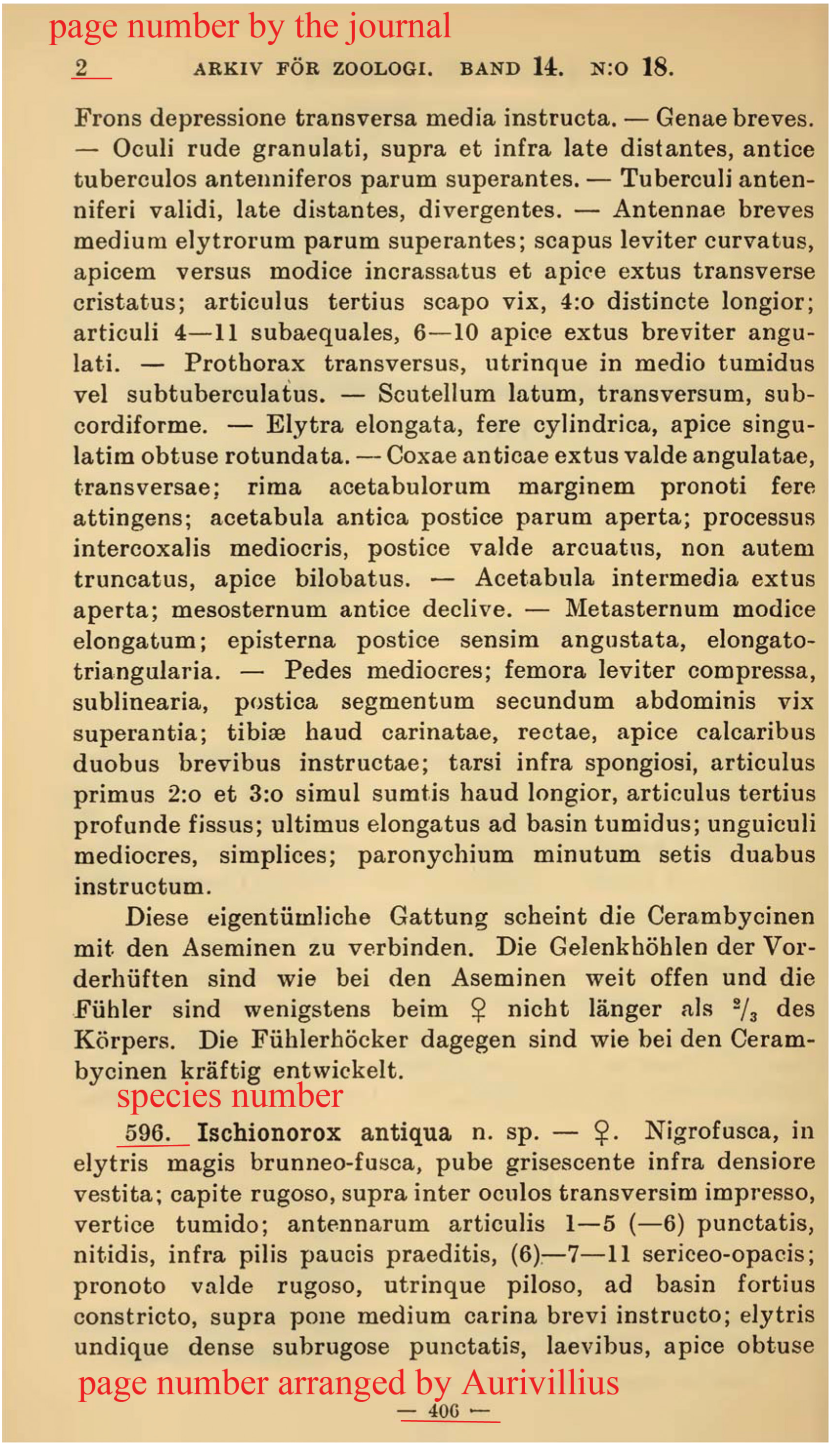
Ischionorox nov. gen.
(Cerambycini oder Asemini).

Femina: Mandibulae subverticales, apice curvatae, obtusae, simplices. — Palporum articulus ultimus apice dilatatus. —

Arkiv för zoologi. Bd 14. N:o 18. 1

— 405 —

Figure 1. First page of part 18, showing the title, date of submission, journal's page number and Aurivillius's page number.



2 ARKIV FÖR ZOOLOGI. BAND 14. N:O 18.

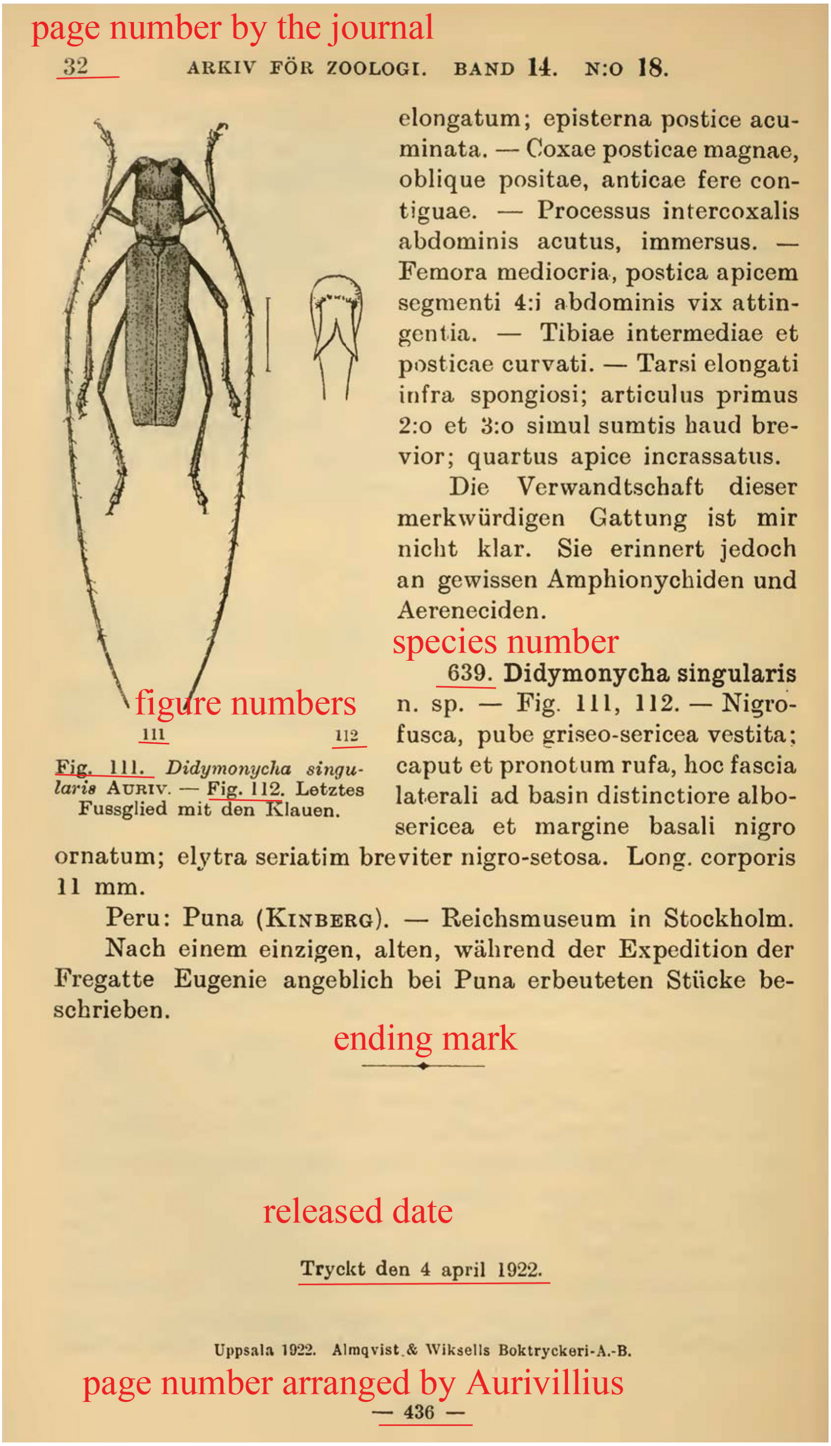
Frons depressione transversa media instructa. — Genae breves. — Oculi rude granulati, supra et infra late distantes, antice tuberculos antenniferos parum superantes. — Tuberculi antenniferi validi, late distantes, divergentes. — Antennae breves medium elytrorum parum superantes; scapus leviter curvatus, apicem versus modice incrassatus et apice extus transverse cristatus; articulus tertius scapo vix, 4:o distincte longior; articuli 4—11 subaequales, 6—10 apice extus breviter angulati. — Prothorax transversus, utrinque in medio tumidus vel subtuberculatus. — Scutellum latum, transversum, subcordiforme. — Elytra elongata, fere cylindrica, apice singulatim obtuse rotundata. — Coxae anticae extus valde angulatae, transversae; rima acetabulorum marginem pronoti fere attingens; acetabula antica postice parum aperta; processus intercoxalis mediocris, postice valde arcuatus, non autem truncatus, apice bilobatus. — Acetabula intermedia extus aperta; mesosternum antice declive. — Metasternum modice elongatum; episterna postice sensim angustata, elongato-triangularia. — Pedes mediocres; femora leviter compressa, sublinearia, postica segmentum secundum abdominis vix superantia; tibiæ haud carinatae, rectae, apice calcaribus duobus brevibus instructae; tarsi infra spongiosi, articulus primus 2:o et 3:o simul sumtis haud longior, articulus tertius profunde fissus; ultimus elongatus ad basin tumidus; unguiculi mediocres, simplices; paronychium minutum setis duabus instructum.

Diese eigentümliche Gattung scheint die Cerambycinen mit den Aseminen zu verbinden. Die Gelenkhöhlen der Vorderhüften sind wie bei den Aseminen weit offen und die Fühler sind wenigstens beim ♀ nicht länger als $^2/_3$ des Körpers. Die Fühlerhöcker dagegen sind wie bei den Cerambycinen kräftig entwickelt.

596. **Ischionorox antiqua** n. sp. — ♀. Nigrofusca, in elytris magis brunneo-fusca, pube grisescente infra densiore vestita; capite rugoso, supra inter oculos transversim impresso, vertice tumido; antennarum articulis 1—5 (—6) punctatis, nitidis, infra pilis paucis praeditis, (6)—7—11 sericeo-opacis; pronoto valde rugoso, utrinque piloso, ad basin fortius constricto, supra pone medium carina brevi instructo; elytris undique dense subrugose punctatis, laevibus, apice obtuse

— 406 —

Figure 2. Second page of part 18, showing the species number, journal's page number and Aurivillius's page number.



32 ARKIV FÖR ZOOLOGI. BAND 14. N:O 18.

elongatum; episterna postice acuminata. — Coxae posticae magnae, oblique positae, anticae fere contiguae. — Processus intercoxalis abdominis acutus, immersus. — Femora mediocria, postica apicem segmenti 4:i abdominis vix attingentia. — Tibiae intermediae et posticae curvati. — Tarsi elongati infra spongiosi; articulus primus 2:o et 3:o simul sumtis haud brevior; quartus apice incrassatus.

Die Verwandtschaft dieser merkwürdigen Gattung ist mir nicht klar. Sie erinnert jedoch an gewissen Amphionychiden und Aereneciden.

111 112

Fig. 111. *Didymonycha singularis* AURIV. — Fig. 112. Letztes Fussglied mit den Klauen.

639. **Didymonycha singularis** n. sp. — Fig. 111, 112. — Nigrofusca, pube griseo-sericea vestita; caput et pronotum rufa, hoc fascia laterali ad basin distinctiore albo-sericea et margine basali nigro ornatum; elytra seriatim breviter nigro-setosa. Long. corporis 11 mm.

Peru: Puna (KINBERG). — Reichsmuseum in Stockholm.

Nach einem einzigen, alten, während der Expedition der Fregatte Eugenie angeblich bei Puna erbeuteten Stücke beschrieben.

Tryckt den 4 april 1922.

Uppsala 1922. Almqvist & Wiksells Boktryckeri-A.-B.

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Figure 3. Last page of part 18, showing the species number, figure numbers, ending mark, release date, journal's page number and Aurivillius's page number.

nal article they used Aurivillius 1927: 27, then type species was written as *Mimectatina singularis* Aurivillius, 1928. Nakamura et al. (2014) variably used 1928 and 1927 in their authorship date for *Mimectatina*, causing confusion. And Nakamura et al. (2014) used the journal page number for the detailed taxon citation "*Cataphrodisium*: Aurivillius: 8", while in the reference they used Aurivillius's own page numbers "93–131", which were incorrect. Nakamura et al. (1992) used 1927 for the type species *Mimectatina singularis* and 1928 for the genus *Mimectatina*. Lin (2017) used 1927 and Aurivillius's page numbers for the genus *Mimectatina* (writing "*Mimectatina* Aurivillius, 1927: 575", which should be corrected to "*Mimectatina* Aurivillius, 1927: 27 (= 575)"), then used 1928 and the journal's page numbers for the genus *Parenes* (Fig. 4, writing "*Parenes* Aurivillius, 1928c: 23", which was copied from Löbl and Smetana (2010) and should be corrected to "*Parenes* Aurivillius, 1927: 29 (= 577)"), wrongly treating the same paper as two separate articles.

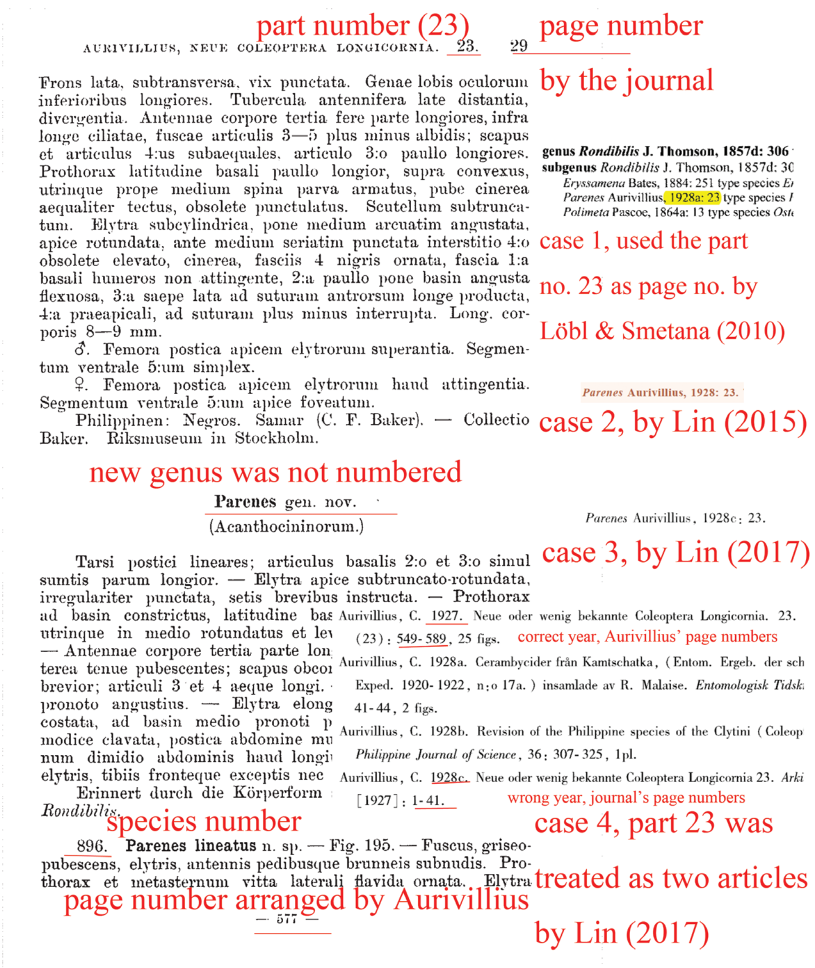

Figure 4. page 29 of part 23, showing the part number, species number, journal's page number and Aurivillius's page number, and four cases of wrong citation.

Discussion

Date of publication we chose

The dates of publication of this series of work contain several confusing cases; the detailed information is shown in Table 1. For parts 5 and 6, we chose the earlier date indicated by the Zoological Record and Derksen and Scheiding (1963), instead of the later date printed low on the back side of the original wrapper, based on IZCN 21.8.1. For parts 11 and 15, we chose the earlier date printed on the last page and indicated by Zoological Record, instead of the later date indicated by the journal, also based on IZCN 21.8.1. For parts 19, 21 and 23, we chose the earlier date printed on the last page, instead of the later date indicated by Zoological Record and the journal, also based on IZCN 21.8.1, Before 2000, an author who distributed separates in advance of the specified date of publication of the work in which the material was published thereby advanced the date of publication.

When we talk about "distribute reprints in advance" in Aurivillius's cases, the authors mean distribute the reprints after the printing date ("tryckt den XX YY 19ZZ") but before the distribute date of the publisher (either printed on the wrapper, normally for the whole volume, or date applied subsequently by the Zoological Record).

Why the journal page numbers should be used

For parts 8 to 23 of Aurivillius's works, the reasons that the journal page numbers should be used include: 1) the works were first officially published in the journal; 2) the large book titled "Neue oder wenig bekannte Coleoptera Longicornia" does not exist; 3) Aurivillius himself used the journal page numbers instead of his own page numbers (Aurivillius 1912, 1922, 1923, 1928); 4) if Aurivillius's own page numbers were chosen, the results are chaotic since the numbers continued between different journals, different years, and additionally, some parts were missing (Table 1: pages 1–14 and 77–92 were not printed); 5) if Aurivillius's own page numbers were chosen, logically there should be pages preceeding them in the same volume. For example, considering "Arkiv för zoologi 13(9): 361–403" instead of "Arkiv för zoologi 13(9): 1–43", logically there should exist "Arkiv för zoologi 13(9): 1–360" (or "Arkiv för zoologi 13: 1–360"), but this is not the case.

How to identify which page number was the journal's page number

1) the page number was printed on the upper left corner (of even pages) or the upper right corner (of odd pages), which was the style of the journal "Arkiv för zoologi" (Aurivillius 1917, 1919, 1925a, 1925b, 1926), except the first page normally appeared on the lower right corner; 2) each part of each volume was numbered from one, which was also the style of journal "Arkiv för zoologi" at that time (Aurivillius 1917, 1919, 1925a, 1925b, 1926).

Aurivillius's own numbers might be chosen for the following reasons

1) it was the choice of the Titan database (Tavakilian and Chevillotte 2019), which is the most exhaustive Cerambycidae database; 2) larger sized numbers appear more important (for some reasons), for parts 3 to 7, which also had two page numbers printed, all were cited with the correct journal's page numbers, because they are larger than Aurivillius's own page numbers (such as Wappes et al. 2011; Ślipiński and Escalona 2016; Souza 2016; Tavakilian and Chevillotte 2019); 3) page numbers on the mid-bottom are more noticeable than page numbers on upper left corner (of even pages) or upper right corner (of odd pages); 4) works were reprinted with the smaller page numbers even though they were originally from a book or journal with the larger page numbers; realizing this subsequent workers may have chosen the larger numbers; 5) to follow author's citing Aurivillius's own page numbers.

The trend

From Fig. 5 we can see that more than half of authors used Aurivillius's page numbers instead of the journal's page numbers. However, from Fig. 6 we can see that more authors used the journal's page numbers than Aurivillius's page numbers before the year 2000, while most authors used Aurivillius's page numbers after the year 2000. Analyzing the references in more detail (Fig. 7), we can see that all authors before 1990 used the journal's page numbers, while more and more authors used Aurivillius's page numbers after 1991. The reasons for this trend might include: a) young authors did not know the history and might choose the bottom page numbers by the first glance; b) many current authors use the Titan database and copy the information from the website.

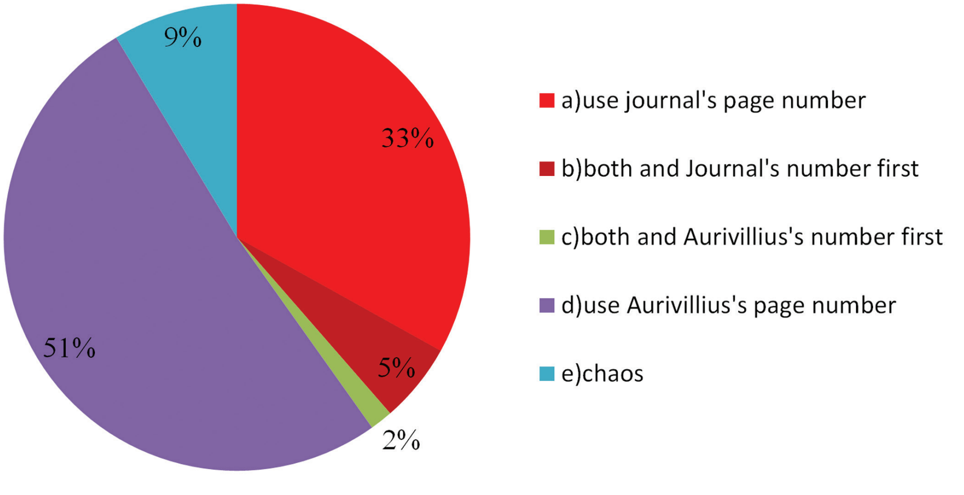

Figure 5. Ratios of different ways to cite Aurivillius's series of works from 1912 to 2019.

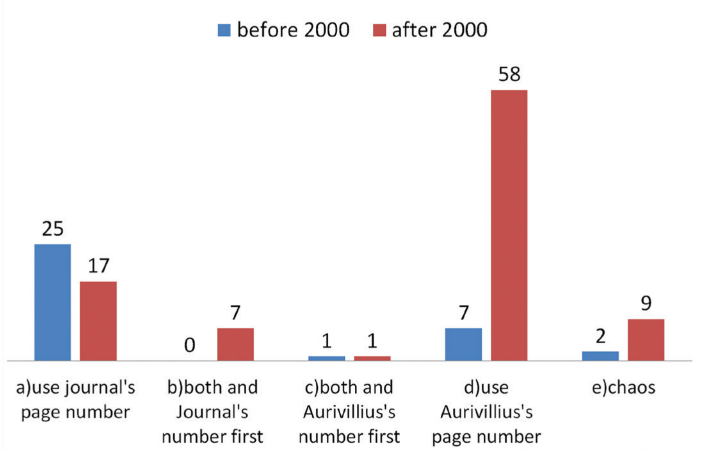

Figure 6. Ratios of different ways to cite Aurivillius's series of works before and after the year 2000.

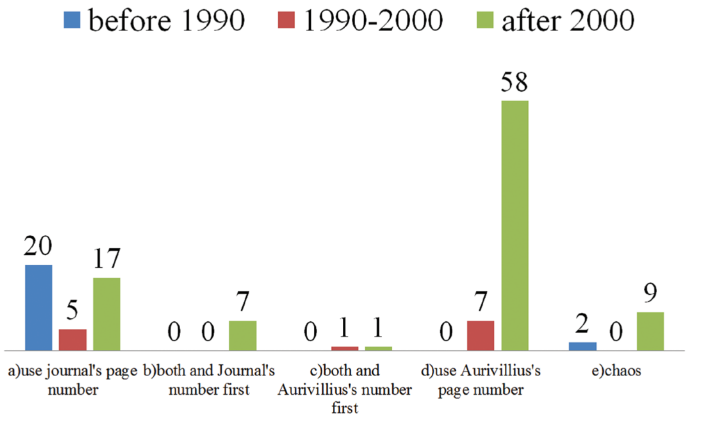

Figure 7. Ratios of different ways to cite Aurivillius's series of works before the year 1990, between 1990–2000, and after the year 2000.

We hope that the Titan database will correct the information and use the journal's page numbers after reading this paper, and authors in the future will cite the related references in correct way.

Correct citation of Aurivillius's works

Based on the above analyses, we suggest that in the future authors cite the work of Aurivillius as follows: the journal page number must be included, Aurivillius's page numbers might be included inside square brackets [] or not included, the internal figure numbers (text-figures) can be included or not, while the supplemental information for the end-plates must be included.

Aurivillius, C. (1886) Nya Coleoptera Longicornia. Entomologisk Tidskrift 7(2): 89–94.
Aurivillius, C. (1887) Nya Coleoptera Longicornia. II. Entomologisk Tidskrift 8(4): 191–197. figs 1–3.
Aurivillius, C. (1891) Neue Coleoptera Longicornia. III. Entomologisk Tidskrift 12(2): 97–106 [=pp. 15–24], figs 1–6.
Aurivillius, C. (1893) Neue oder wenig bekannte Coleoptera Longicornia. 4. Entomologisk Tidskrift 14 (3): 177–186 [=pp. 25–34], figs 1–12.
Aurivillius, C. (1897) Neue oder wenig bekannte Coleoptera Longicornia. 5. Entomologisk Tidskrift 18 (4): 241–248 [=pp. 35–42], pl. 3: figs 1–8.
Aurivillius, C. (1899) Neue oder wenig bekannte Coleoptera Longicornia. 6. Entomologisk Tidskrift 20 (4): 259–265 [=pp. 51–57], figs 13–17.
Aurivillius, C. (1902) Neue oder wenig bekannte Coleoptera Longicornia. 7. Entomologisk Tidskrift 23: 207–224 [=pp. 59–76], figs 18–26.
Aurivillius, C. (1903) Neue oder wenig bekannte Coleoptera Longicornia. 8. Arkiv för zoologi 1: 313–328, figs 27–34.
Aurivillius, C. (1907) Neue oder wenig bekannte Coleoptera Longicornia. 9. Arkiv för zoologi 3(18): 1–39 [=pp. 93–131], pl. 1: figs 1–9; figs 35–41.
Aurivillius, C. (1908) Neue oder wenig bekannte Coleoptera Longicornia. 10. Arkiv för zoologi 4(17): 1–9 [=pp. 133–141], figs 42–47.
Aurivillius, C. (1910) Neue oder wenig bekannte Coleoptera Longicornia. 11. Arkiv för zoologi 7(3): 1–44 [=pp. 143–186], fig. 48.
Aurivillius, C. (1911) Neue oder wenig bekannte Coleoptera Longicornia. 12. Arkiv för zoologi 7(19): 1–41 [=pp. 187–227], figs 49–57.
Aurivillius, C. (1913) Neue oder wenig bekannte Coleoptera Longicornia. 13. Arkiv för zoologi 8(22): 1–35 [=pp 229–263], figs 58–68.
Aurivillius, C. (1914a) Neue oder wening bekannte Coleoptera Longicornia. 14. Arkiv för zoologi. Uppsala 8(29): 1–54 [=pp. 265–318], pl. 1: figs 1–9.
Aurivillius, C. (1914b) Neue oder wenig bekannte Coleoptera Longicornia. 15. Arkiv för zoologi 9(8): 1–15 [=pp. 319–333].
Aurivillius, C. (1916) Neue oder wenig bekannte Coleoptera Longicornia. 16. Arkiv för zoologi 10(19): 1–25 [=pp. 335–359], pl. 1: figs 1–9; figs 69–72.
Aurivillius, C. (1920) Neue oder wenig bekannte Coleoptera Longicornia. 17. Arkiv för zoologi 13(9): 1–43 [=pp. 361–403], figs 73–81.
Aurivillius, C. (1922) Neue oder wenig bekannte Coleoptera Longicornia. 18. Arkiv för zoologi 14(18): 1–32 [=pp. 405–436], figs 82–112.
Aurivillius, C. (1923) Neue oder wenig bekannte Coleoptera Longicornia. 19. Arkiv för zoologi 15(25): 1–43 [=pp. 437–479], figs 113–133.
Aurivillius, C. (1925a) Neue oder wenig bekannte Coleoptera Longicornia. 20. Arkiv för zoologi 17A(12):1–21 [=pp. 481–501], figs 134–140.
Aurivillius, C. (1925b) Neue oder wenig bekannte Coleoptera Longicornia. 21. Arkiv för zoologi 18A(9):1–22 [=pp. 503–524], figs 141–163.
Aurivillius, C. (1927a) Neue oder wenig bekannte Coleoptera Longicornia. 22. Arkiv för zoologi 19A(17): 1–23 [=pp. 525–547], pl. 1: figs 1–6; figs 164–177.

Aurivillius, C (1927b) Neue oder wenig bekannte Coleoptera Longicornia. 23. Arkiv för zoologi 19A(23): 1–41 [=pp. 549–589], figs 178–202.

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Supplementary material 1

Discussions and evidences

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Genetic diversity and population structure of *Terapon jarbua* (Forskål, 1775) (Teleostei, Terapontidae) in Malaysian waters

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Abstract

A background study is important for the conservation and stock management of a species. *Terapon jarbua* is a coastal Indo-Pacific species, sourced for human consumption. This study examined 134 samples from the central west and east coasts of Peninsular (West) Malaysia and East Malaysia. A 1446-bp concatenated dataset of mtDNA COI and Cyt *b* sequences was used in this study and 83 haplotypes were identified, of which 79 are unique haplotypes and four are shared haplotypes. Populations of *T. jarbua* in Malaysia are genetically heterogenous as shown by the high level of haplotype diversity ranging from 0.9167–0.9952, low nucleotide diversity ranging from 0.0288–0.3434, and high F_{ST} values (within population genetic variation). Population genetic structuring is not distinct as shown by the shared haplotypes between geographic populations and mixtures of haplotypes from different populations within the same genetic cluster. The gene flow patterns and population structuring observed among these regions are likely attributed to geographical distance, past historical events, allopatric speciation, dispersal ability and water currents. For instance, the mixture of haplotypes revealed an extraordinary migration ability of *T. jarbua* (>1200 km) via ancient river connectivity. The negative overall value of the neutrality test and a non-significant mismatch distribution are consistent with demographic expansion(s) in the past. The median-joining network concurred with the maximum likelihood haplotype tree with three major clades resolved. The scarcity of information on this species is an obstacle for future management and conservation purposes. Hence, this study aims to contribute information on the population structure, genetic diversity, and historical demography of *T. jarbua* in Malaysia.

Keywords
COI, crescent perch, Cyt *b*, historical demography, ikan mengkerong, Pleistocene

Introduction

A population's genetic structure describes the total genetic diversity in the population, which is shaped by several factors, including the life history, geographical barriers, gene flow, selection and bottlenecks (Wright 1931; Slatkin 1987; Charlesworth 2009; Liu et al. 2019). The patterns of genetic diversity and population structure provide information on the life histories, demography, reproduction and ecology of a species. This information is important for a population's sustainability by implementation of appropriate conservation and management strategies (Okumuş and Çiftci 2003; Zaya et al. 2017).

Terapon jarbua (Forskål, 1775) is a medium-sized fish commonly known as crescent perch, and it is locally known as "ikan mengkerong" in Malaysia (Department of Fisheries Malaysia 2009). This species is classified under the class Actinopterygii, order Perciformes and family Terapontidae (Froese and Pauly 2018). Although it is primarily a marine species, it has also been found in coastal areas, estuaries, freshwaters and in some coastal lagoons (Rao et al. 2000). It is categorized as a catadromous fish, in which the adults spawn in deeper saltwater while the juveniles move to the shallow sandy bottom area near the river mouths. According to Lavergne et al. (2012), the pelagic larval phase of this species is about 25 days. *Terapon jarbua* is classified as least concern (LC) under the IUCN Red List due to its widespread distribution with no known threats (Dahanukar et al. 2017). The native distributional ranges of the crescent grunters include Australia, Bangladesh, Cambodia, China, India, Indonesia, Japan, Malaysia (Du et al. 2019; Shyama et al. 2020), Mediterranean (Golani and Appelbaum-Golani 2010), Myanmar, Philippines, Red Sea, Sri Lanka and Taiwan (Froese and Pauly 2018).

Existing reports on *T. jarbua* are generally limited to their morphometry (e.g., length-weight relationship) and reproductive biology (Miu et al. 1990; Nandikeswari et al. 2014; Musarrat and Masood 2015) while the information on their genetic diversity and population structures is relatively little. Lavergne et al. (2012) and Liu et al. (2015) reported on the population genetic diversity of *T. jarbua* in the Gulf of Aden, Yemen and Taiwanese waters, respectively, using cytochrome *c* oxidase subunit I (COI), cytochrome *b* (Cyt *b*) and microsatellites molecular markers. Also, a phylogeographic survey of *T. jarbua* along with other reef fauna of the western Indian Ocean was reported by Borsa et al. (2016) using COI gene sequences. Mitochondrial DNA (mtDNA) has been widely utilized as the marker of choice to examine the genetic diversity and population structure of marine fishes due to its strict maternal inheritance, rapid mutation rates and the absence of recombination in most species (Whitehead et al. 2003; Dowling et al. 2008; Song et al. 2013).

The main focus of the current study is on the Malaysian populations: Peninsular (West) Malaysia and East Malaysia (Sabah and Sarawak) which are located in the tropical Indo-west Pacific region (Fig. 1). These two land masses are about 1200 km apart, separated by the south-western portion of the South China Sea. In this study COI and Cyt *b* were used as molecular markers to examine the level of gene flow, population genetic differentiation and the historical demography of *T. jarbua* populations in Malaysia. To the best of our knowledge, there is no documented report on the population genetics of *T. jarbua* in Malaysia to date. Hence, this study aims to provide a documented background report as well as to fill the information gap for *T. jarbua* in this region. Homologous COI+ Cyt *b* sequences of four regional representatives of this species from India, Taiwan, Hainan and the Philippines were included in the analysis to provide a wider coverage of the species' natural distribution.

Materials and methods

Sampling

Sampling around major landing sites and local markets was conducted in both East and Peninsular Malaysia where 134 samples of various sizes were collected randomly from five wild populations of *T. jarbua*. Populations were provisionally divided into five groups according to region: 1) Kuala Selangor (KS, $N = 31$) of west Peninsular which is surrounded by the Straits of Malacca; 2) Kuantan, Pahang (KN, $N = 30$) of east Peninsular which is adjacent to the South China Sea; 3) Mukah, Sarawak (MH, $N = 21$) of East Malaysia which is surrounded by the South China Sea; 4) Sandakan (SN, $N = 28$) and 5) Tawau (TW, $N = 24$) of East Malaysia which are surrounded by the Sulu Sea and the Celebes Sea, respectively (Fig. 1). Samples were collected within the period of April 2015 to August 2018. Approximately 20 mg of muscle tissue from each fish sample was removed and immediately preserved in 95% ethanol and stored at -20°C until genetic analysis was performed.

DNA extraction, PCR amplification and DNA sequencing

Genomic DNA was extracted using 10% Chelex Resin following the protocol of Hyde et al. (2005). Approximately 680 bp of the COI-5' gene was amplified using the FishF1 or FishF2 forward primers and FishR1 or FishR2 reverse primer pairs (Ward et al. 2005).

Polymerase Chain Reaction (PCR) amplification of approximately 1000 bp from the 5'-end of the Cyt *b* gene was performed using the primer pairs Glu31 and Thr33 (Liu et al. 2015) and internal primers: Glu231 (5'-CTT ACA GGC CTC TTT CTG GCC AT- 3') and Thr233 (5'- TTT GAG CTA CTA ATG CAG TAT- 3') were designed for this study.

PCR was performed using a Mastercycler epgradient S thermalcycler (Eppendorf, Hamburg, Germany) and 25µl reaction mixtures consisting of 12.5µl exTEN 2X PCR master mix (1st BASE, Selangor, Malaysia), 9.5 µl of sterile distilled water, 1µl each of forward and reverse primers, and 1µl of DNA template. PCR cycling conditions were as follow: initial denaturation for 1 min at 96°C, 36 cycles of denaturation at 95°C for 30 s, annealing for 30 s at 44°C (CO1) or 48°C (Cyt *b*), elongation for 1 min at 72°C, and final elongation for 10 min at 72°C. The amplicons were checked for correct length via electrophoresis on a 1% agarose gel (90V for 25 min). PCR products were sent to Apical Scientific Sdn. Bhd. (Selangor, Malaysia) for purification and DNA sequencing.

Sequence analysis

Multiple sequence alignment was first performed separately for each gene region using the CLUSTAL X (Thompson et al. 1994) program implemented in BIOEDIT ver. 7.0.5 (Hall et al. 2011). The sequences were subsequently trimmed and aligned manually prior to concatenation of COI and Cyt *b* sequences. Analyses performed in this study were based on the final truncated length of 1446-bp concatenated sequences. All haplotype sequences were deposited in Genbank under the accession numbers MN529663–MN52993.

Unique haplotypes were quantified and the genetic diversity, nucleotide diversity, and pairwise distance were calculated using DNASP v. 4.0 (Rozas et al. 2003). The level of gene flow among populations (Nm) based on Hudson et al. (1992) was also calculated in DNASP v. 4.0. Analysis of molecular variance (AMOVA) was performed using ARLEQUIN v.3.5 (Excoffier and Lischer 2010) for the four, hypothetical, region-based groupings (Selangor, Pahang, Sabah and Sarawak) to investigate the partition of genetic variation among regions (F_{CT}), among populations within regions (F_{SC}), and within populations (F_{ST}). The significance of the F-statistics for population comparisons was assessed using 1000 permutations. The Tamura Nei plus gamma rate model (TN93+G) was selected by MEGA v. 7.0 (Kumar et al. 2016) as the best-fitting substitution model based on the Bayesian information criterion. A Maximum Likelihood (ML) tree was reconstructed in MEGA 7.0 to show the level of divergence and relationships among haplotypes of *T. jarbua*. The confidence level at each node was assessed by 1000 bootstrap replications. This tree was compared against the median-joining network generated using the program's default settings of NETWORK 4.5.0.2 (Bandelt et al. 1999).

In addition, a neutrality test of the pairwise differences among all populations was performed to infer historical demographic and deviation of sequence variation from evolutionary neutrality. Deviations from neutrality were evaluated using Fu's Fs (Fu 1997) and Tajima's *D* (Tajima 1989) via DNASP. Statistical tests and confidence intervals for *D* and F's were based on a coalescent simulation algorithm. A large negative value of Fu's Fs or the Tajima's *D* rejecting the null hypothesis of neutrality indicates population expansion(s). The demographic changes were also examined using the mismatch distribution analysis (Rogers and Harpending 1992) in ARLEQUIN with 1000 permutations. The Harpending's raggedness index (Harpending et al. 1993) and the sum of squared deviations (SSD) between observed and expected mismatch for each of the populations under the model of constant population size were analyzed according to Schneider and Excoffier (1999). This method quantifies the smoothness of the observed mismatch distribution and a non-significant result indicates an expanding population. The spatial expansion hypothesis (both raggedness index and SSD) was tested using a parametric bootstrap approach with 1000 replicates.

Results

Genetic diversity

The 1446 bp concatenated COI (631 bp) and Cyt *b* (815 bp) sequences were analyzed for 134 individuals obtained in five different locations (Fig. 1) from East Malaysia and Peninsular Malaysia. The nucleotide composition was 23.0% adenine,

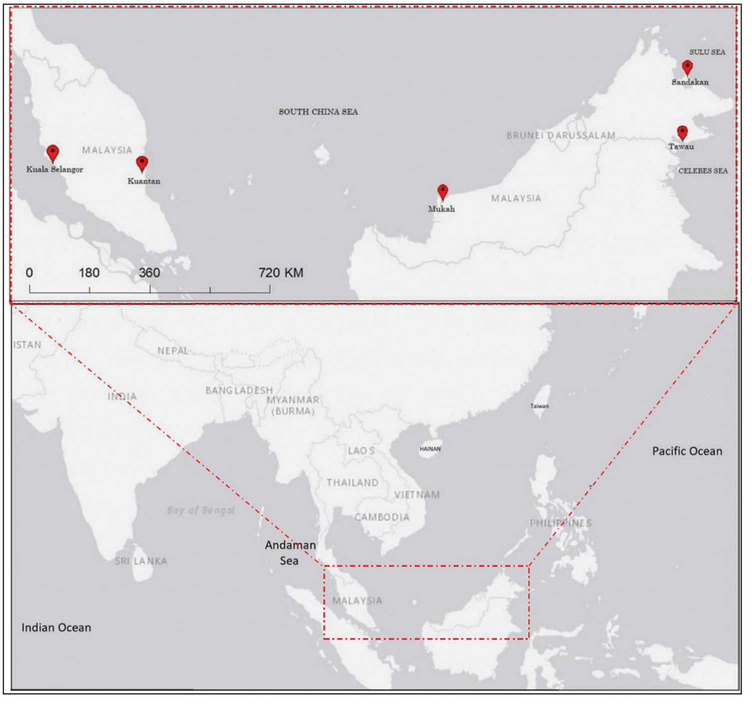

Figure 1. Sampling localities from East (Sandakan and Tawau, Sabah & Mukah, Sarawak) and West (Peninsula) Malaysia (Kuala Selangor, Selangor and Kuantan, Pahang).

28.9% thymine, 32.0% cytosine and 16.1% guanine. The higher A+T content (51.9%) compared to G+C content (48.1%), is common among fishes (Appendix 1). There were 202 polymorphic sites of which 31 (15.4%) were singleton variable sites, and 171 (84.6%) were parsimony informative (Appendix 2). A total of 149 mutations with 66 transitions, 8 transversions and 75 substitutions were found within the dataset (data not shown).

A total of 83 putative haplotypes were derived from the 134 individuals sequenced with 79 of them being unique haplotypes (95.18%) and four were shared haplotypes (4.82%). The dominant haplotype of Malaysian populations is Hap5 (KS, KN, TW, SN, MH, TAI) while other shared haplotypes are Hap3 (KS, KN, TW, MH, IND), Hap8 (KS and KN) and Hap45 (TW and SN). The population from KS recorded the highest total number of haplotypes (22) of which 19 were unique haplotypes, while Tawau recorded the lowest number of haplotypes (15) with 12 unique haplotypes. The nucleotide diversity (π) of *T. jarbua* populations in this study ranged from 0.0288 ±0.0158 (mean ±SD) to 0.3434 ±0.1722 while haplotype diversity (h) ranged from 0.9167 ±0.0482 to 0.9952 ±0.0165 (Table 1). The MH population recorded the highest π and h. A high h and low π indicate that the populations studied were moderate in genetic diversity.

Genetic structure

A ML tree was reconstructed based on the 83 haplotypes of this study and four COI + Cyt *b* sequences from Hainan, Taiwan, India and Philippines which were downloaded from National Center for Biotechnology Information (NCBI) (Appendix 3). The mtDNA concatenated dataset defined the haplotypes into three major clades with no significant clusters corresponding to sampling localities (Fig. 2). Apart from Clade I which consists of eight haplotypes solely from Sarawak, the other four clades include mixtures of haplotypes from various localities without any obvious geographical structuring among them. Clade II consists of haplotypes from MH and KS populations while Clade III consists of the haplotype from the Philippines and haplotypes from Malaysia except MH. Clade III is the most geographically inclusive with haplotypes from India, Hainan, Taiwan, Philippines and all five Malaysian populations.

The general topology of the median-joining network (Fig. 3) corresponded with the ML tree (Fig. 2) with three major clusters identified. In the network presented, shared haplotypes occupy the central area, while the unique haplotypes branched out from the center. This formation provides a star-like profile, which indicates population expansions. Hap3 and Hap5 are the dominant haplotypes in cluster III. The distribution frequency of all the 83 haplotypes in *T. jarbua* populations is presented in Appendix 4. Hap5 recorded the highest distribution frequency with 21 individuals. It is the only common haplotype shared between all five populations from Malaysia and the representative from Taiwan. Hap3 with 16 individuals was found in India and in all locations sampled in Malaysia except for Sandakan.

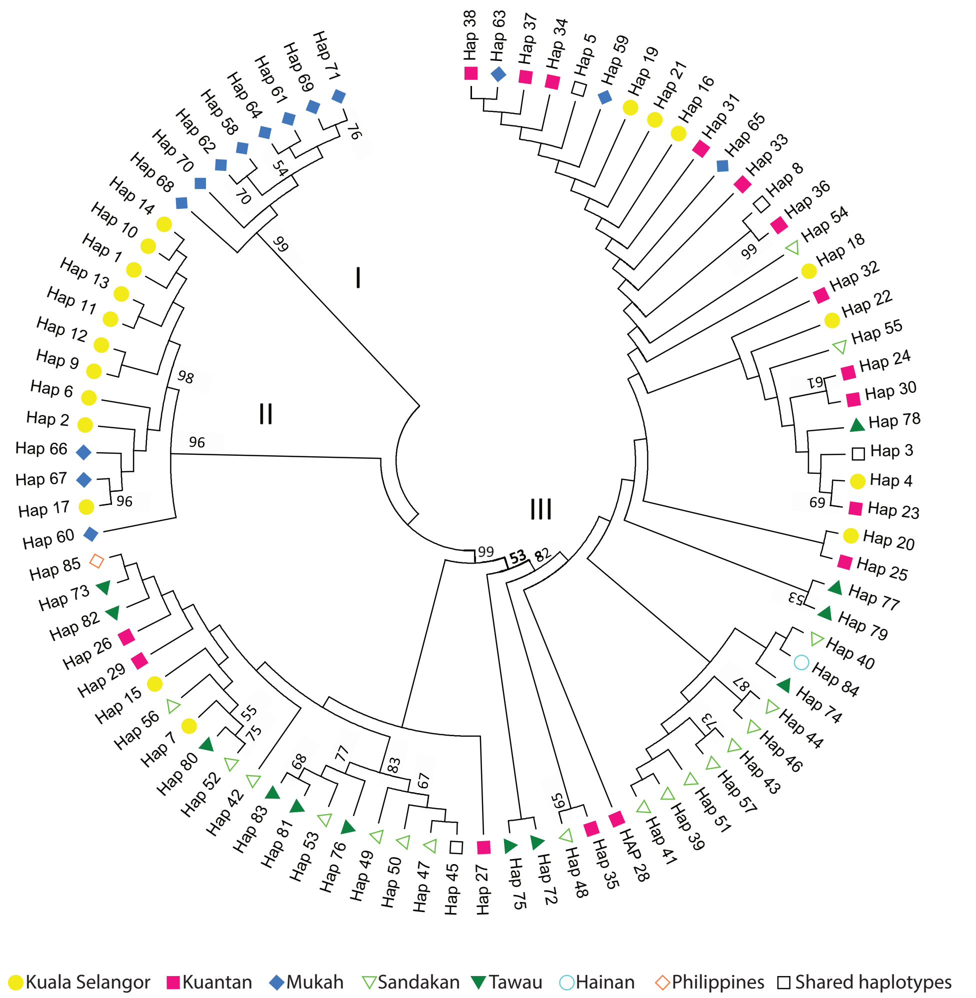

Figure 2. Maximum likelihood haplotype tree reconstructed based on the concatenated mtDNA dataset. The bootstrap values higher than 50% are shown near the nodes.

Table 1. Information and molecular indices of *T. jarbua*. N, number of samples; NH, number of haplotypes; NUH, number of unique haplotypes; *h*, haplotype diversity; *π*, nucleotide diversity; *k*, average number of pairwise differences.

ID	Populations	N	NH	NUH	*h*	*π*	*k*
KS	Kuala Selangor, Selangor	31	22	19	0.9828 ±0.0135	0.1817 ±0.0904	36.5161 ±16.3285
KN	Kuantan, Pahang	30	19	16	0.9678 ±0.0208	0.0288 ±0.0158	5.7885 ±2.8485
MH	Mukah, Sarawak	21	16	14	0.9952 ±0.0165	0.3434 ±0.1722	69.0238 ±31.0005
SN	Sandakan, Sabah	28	20	18	0.9577 ±0.0262	0.0487 ±0.0256	9.7810 ±4.6212
TW	Tawau, Sabah	24	15	12	0.9167 ±0.0482	0.0514 ±0.0271	10.3333 ±4.8857
Total		134	83	79	0.9820 ±0.0050	0.0248 ±0.0031	35.8653 ±12.2638

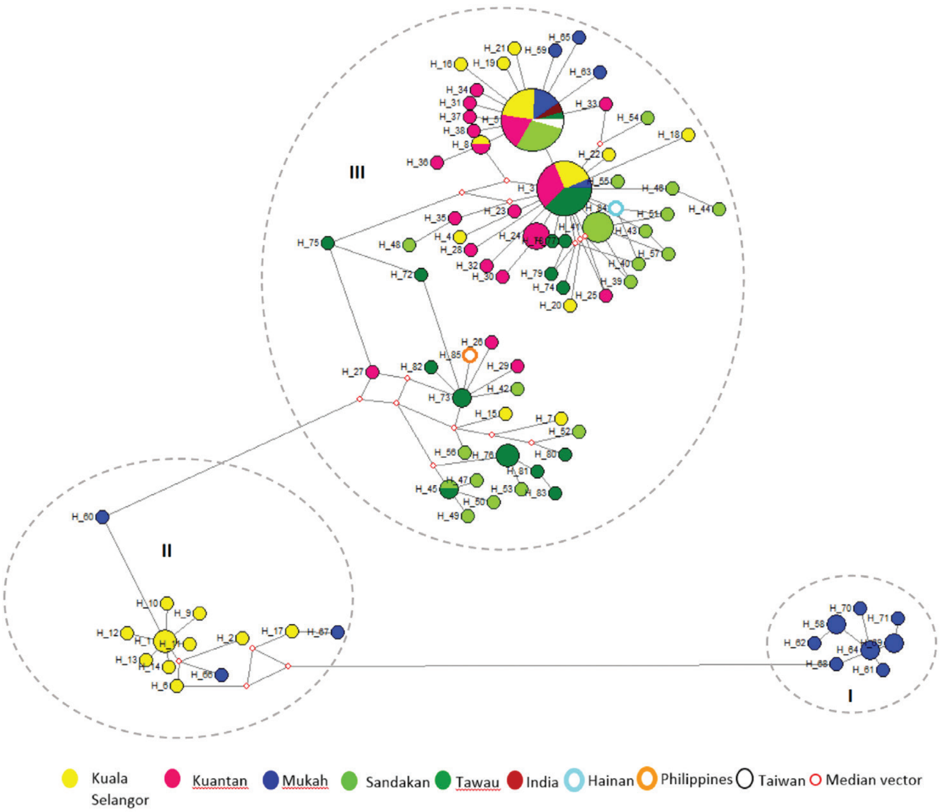

Figure 3. Haplotypes median-joining network corresponding to the ML tree with three observed clusters. The star-like profile observed in cluster III indicates the presence of sudden expansion.

Pairwise F_{ST} comparisons between populations in Malaysia were significant at the 95% confidence level except for the comparison between TW and SN (Table 2). Populations of MH and KN showed the greatest pairwise differentiation (F_{ST} = 0.5353; p < 0.05) while SN and TW showed the least differentiation (F_{ST} = 0.0452; p > 0.05). The pairwise nucleotide divergence among populations (Table 3) showed the same trend as the F_{ST} values and was not correlated with geographical distance. The overall gene flow (Nm) estimated among populations was low at 0.82. The sequence divergence was calculated using the Kimura two parameter (K2P) distance model for both genes (Table 3). The greatest genetic differences (COI: 0.019 and Cyt *b*: 0.029) were observed between MH-KN, MH-SN and MH-TW. The *T. jarbua* populations displayed a low level of conspecific divergence within 2% (COI).

The genetic structure of the *T. jarbua* populations analysed by AMOVA showed little (39.52%) genetic differentiation among regions but high (62.13%) variation within populations (Table 4). This indicates that the populations were not genetically differentiated among regions and the genetic variation was mainly from within the population level. There is essentially no genetic structuring (-0.14% variation) among populations within region.

Table 2. Pairwise F_{ST} (below diagonal) and exact *P*-values (above diagonal) among five populations of *T. jarbua* based on 1000 permutations of the sequence data set. Numbers in bold represent the highest and lowest value. *Significant at $p < 0.05$ by the permutation test. Overall gene flow (N_m) is 0.82.

Populations	KS	KN	MH	SN	TW
KS	-	0.0000*	0.0000*	0.0000*	0.0000*
KN	0.2965	-	0.0000*	0.0270*	0.0090*
MH	0.3310	**0.5353**	-	0.0000*	0.0000*
SN	0.2681	0.0702	0.5038	-	0.0541
TW	0.2633	0.1773	0.4844	**0.0452**	-

Table 3. Net between-group mean distances using Kimura-2-parameter (K2P) model.

	Populations	KS	KN	SN	MH	TW
	KS	-				
	KN	0.003	-			
COI	SN	0.003	0.000	-		
	MH	0.015	0.019	0.019	-	
	TW	0.004	0.001	0.000	0.019	-
	KS	-				
	KN	0.008	-			
Cyt *b*	SN	0.008	0.001	-		
	MH	0.018	0.029	0.029	-	
	TW	0.008	0.001	0.000	0.029	-

Table 4. AMOVA of *T. jarbua* samples based on mtDNA sequences.

Source of variation	Sum of squares	Percentage of variation	F statistic	*P*
Among region (F_{CT})	800.958	39.52	0.3952	0.1896 ± 0.0134
Among populations within region (F_{SC})	11.1610	-0.14	-0.0033	0.0831 ± 0.0082
Within populations (F_{ST})	1476.92	62.13	0.3952	0.0000 ± 0.0000

Historical demography

The overall Tajima's *D* value was negative with an insignificant *p*-value, indicating deviation from evolutionary neutrality. Similarly, the Fu's Fs test which is based on the distribution of haplotypes, revealed negative but significant *p*-values for all five populations studied, indicating an excess of rare haplotypes or rare mutations in the population compared to what is expected under a neutral model of evolution. Following the results of Fu's Fs test, the hypothesis of neutral evolution was rejected.

In the present study, all populations demonstrated bimodal and ragged shaped patterns which points to the population having remained largely constant in size and that the lineage was widespread (Rogers and Harpending 1992). The scatter-plot of the bimodal illustrations is shown in Figure 4. The results of mismatch distribution are contradictory to the results of the neutrality analysis. Hence, to further test the validity of the neutrality test results, we calculated the raggedness index and SSD under the demographic expansion model as shown in Table 5. *P*-

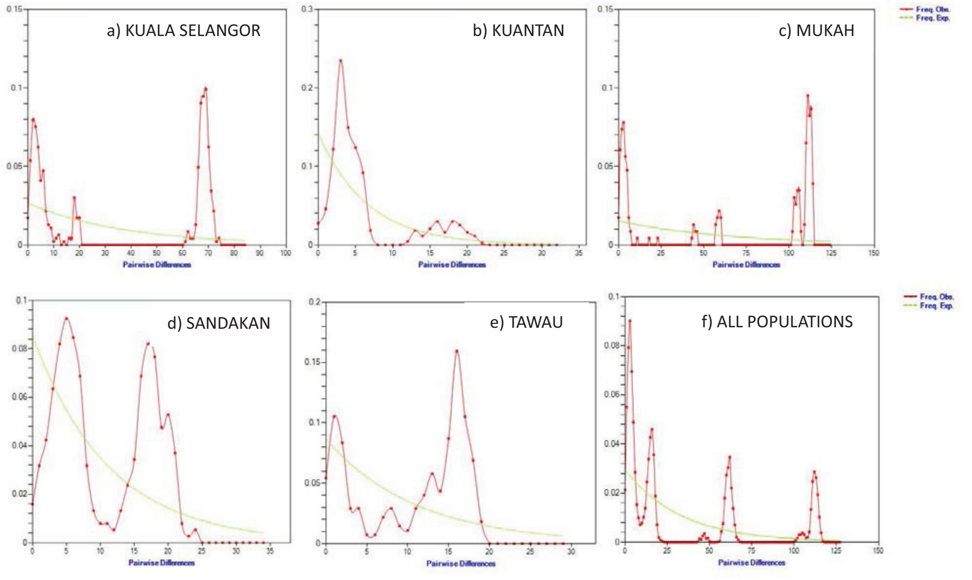

Figure 4. Pairwise number of difference (mismatch distribution) analysis was conducted using the constant population size model to observe the population size changes. The observed frequencies were represented by red dotted line. The frequency expected under the hypothesis of population expansion model was depicted by continuous green line. **a** Kuala Selangor **b** Kuantan **c** Mukah **d** Sandakan **e** Tawau **f** all populations.

Table 5. Parameter estimates of neutrality tests (Tajima's *D* statistic and Fu's Fs) and mismatch distribution (sum of squares deviation (SSD) and r = raggedness index) for each population. Significance (**p* < 0.10) was determined using coalescent simulations.

	Neutrality test		Mismatch distribution		
	Tajima's *D*	**Fu's F_S**	**SSD**	**r**	**Curve**
KS	1.4817	-1.252	0.0296	0.0106	Bimodal
KN	-1.2595	-11.560	0.0113	0.0333	Bimodal
SN	-0.4265	-6.153	0.0301	0.0160	Bimodal
TW	1.0793	-2.075	0.0236	0.0266	Bimodal
MH	2.1863*	-1.093*	0.0288	0.0142	Bimodal
Total	-0.3132	-24.885*	0.0247	0.0201	Bimodal

values of SSD between the observed and expected mismatch distributions were all statistically insignificant ($p > 0.10$), indicating the presence of non-equilibrium and a population expansion event in *T. jarbua*. Besides that, the studied populations showed non-significant raggedness index ($p > 0.10$) indicating the data has relatively good fit to a model of population expansion (Harpending 1994). A ragged distribution suggests that the lineage was widespread (Excoffier et al. 1992; Rogers and Harpending 1992; Rogers 1995).

Discussion

Genetic diversity

Species identification was confirmed by morphological observation and DNA sequence data in which the intraspecific COI divergence was within the 2% threshold value (Hebert et al. 2003; Ward 2009; Srivathsan and Meier 2012). In general, a relatively high haplotype diversity (0.92–0.99) and low nucleotide diversity (0.03–0.34) were observed for the populations in this study. The combination of high haplotype diversity and low nucleotide diversity is common in pelagic marine fishes (Liu et al. 2015). This is likely due to rapid demographic expansion from a small effective population size, assuming there is sufficient time for the number of haplotypes to increase through mutation but insufficient for accumulation of large sequence differences (Grant and Bowen 1998; Avise 2000; Lowe et al. 2004). The inference of population expansion is further supported by the star-like patterns in Figure 3. Our results agree with an earlier study of *T. jarbua* populations from the Taiwanese waters which exhibited similarly high population haplotype diversity ranging from 0.86 to 1.00 as inferred from COI and Cyt *b* sequence data (Liu et al. 2015). In a study on *T. jarbua* populations from the Gulf of Aden, Lavergne et al. (2014) reported similarly high genetic diversities ranging from 0.216 to 0.698. The high number of haplotypes (83 haplotypes) in the present study is likely due to the high mutation rate of the mtDNA genes. Most of the haplotypes are unique to its region which may indicate the presence of different founding populations in the studied localities (Teixeira et al. 2011). The haplotype diversity of a relatively rapid evolving genome within a population often approaches 1.0 as many individuals will tend to have unique haplotypes (Freeland et al. 2011).

Genetic structure

A population's genetic structure is affected by genetic drift, local adaptation, and gene flow. In a marine environment, the development of population structure is greatly influenced by factors that affect dispersal, such as ocean currents, historical variance, and geographic distance coupled with differences in dispersal ability and habitat discontinuity (Saarman et al. 2010). Population structure inferred from mtDNA markers displays less genetic divergence in the pelagic and moderately pelagic species due to their potential to undertake long-distance migrations in oceanic waters (Jaafar 2014).

The haplotype tree (Fig. 2) revealed three major lineages but geographic structuring among the five populations is not distinct. In general, haplotypes specific to certain geographic regions did not form monophyletic groups, but appeared to be randomly distributed across the haplotype tree. Hap5 and Hap3 which recorded the highest distribution frequency are likely the ancestral haplotypes among the populations sampled.

Recent haplotypes were evolved directly or indirectly from the ancestral haplotypes. The existence of two ancestral points indicates that *T. jarbua* in Malaysia probably exist from two different sources. According to the coalescent theory, common haplotypes at the center of a network are inferred to be ancestral, while tip haplotypes at the periphery are derived or descendant from ancestral haplotypes (Akib et al. 2015). The occurrence of star-like patterns radiating from these major haplotypes suggests that *T. jarbua* populations have undergone significant population size expansions in the relatively recent past (Forster et al. 2001; Akib et al. 2015).

F_{ST} values are often used to infer gene flow, in which a lower F_{ST} value indicates low genetic divergence and higher gene flow. F_{ST} values below 0.05, as observed between SN and TW populations, indicate negligible genetic divergence, probably due to active exchange of genetic material between populations through breeding. Furthermore, the pairwise divergence between these populations is not statistically significant. According to Wright (1965), F_{ST} of 0–0.05 is described as little differentiation, 0.05–0.15 as moderate differentiation, 0.15–0.25 as great differentiation and values greater than 0.25 as very great differentiation. All populations studied showed moderate to very great pairwise differentiation except for TW-SN. The overall gene flow recorded was rather low (Nm = 0.82) which suggests limited genetic connectivity among the five populations.

Populations from the same region, i.e., TW and SN of Sabah, were the least genetically variable (Tables 2 and 3), which is likely due to the close geographical distance between these populations. The theory of fish migration across adjacent drainage systems due to flooding, which follows the one-dimensional stepping stone model that allows migration to adjacent population (Song et al. 2013), may apply in the case of *T. jarbua*. The significantly higher genetic differentiation between populations of KS-SN (pairwise F_{ST} = 0.2681) compared to KN-SN (pairwise F_{ST} = 0.0702) may be attributed to distance and physical barrier. Some genetic exchange can be expected since the Straits of Malacca connects the Andaman Sea and South China Sea via the narrow Tebrau strait. It is likely that mixing between the two bodies of water is very limited which supports the F_{ST} value obtained. However, the higher genetic variation between populations of MH-SN (pairwise F_{ST} = 0.5038) as compared to MH-KS (pairwise F_{ST} = 0.3310) implies that geographical distance is not the only driving factor of genetic variation among populations of *T. jarbua* in the Malaysian waters, similar to the results observed in the wider Gulf of Aden (Lavergne et al. 2014) where populations of adjacent locations showed low genetic connectivity despite the absence of a geographic barrier. Populations bordering a common origin such as the South China Sea (KN, MH, TW, and SN) may have evolved independently of each other over time, but there might have been insufficient time for genetic divergence to accumulate in these populations.

Another interesting finding of this study is the occurrence of shared haplotype between the populations from Peninsular and East Malaysia, India, Hainan, Philippines and Taiwan. Common haplotypes between localities and mixed haplotypes of different lineages in some populations in the current study can be explained by the biogeographical history of Southeast Asia (historically known as the Sundaland). Southeast

Asia is believed to have experienced simultaneous glaciation and consequent deglaciation along with its associated decrease and increase of seawater levels during the Pleistocene period, which greatly influenced continental and oceanic configuration (Voris 2000). The shared haplotypes between Malaysian populations and those from as far as India suggests that the range of population expansion after glacial retreat was not restricted to the South China Sea but also extended into the Indian Ocean (Liu et al. 2015). Lavergne et al. (2014) also reported high connectivity between populations in the Gulf of Aden and South China Sea due to the unique sharing of COI haplotypes between both regions. The haplotype sharing and their consequent gene flow may also be attributed to breeding migration, mutation, pelagic larvae, and sharing of common ancestors (Frankham 1996).

The MH population is the most genetically distinct with the highest between-group mean distances, haplotype and nucleotide diversity among the five populations. Geographical isolation of allopatric populations restricts gene flow between two populations, which in turn allows the evolution of a genome adapted to local condition (Hall 1993). Cluster I (MH) is estimated to form after the separation of Peninsular Malaysia from the Borneo Island due to the rise in the depth of the Sunda River between 40 to 100 m. This gradual separation was suspected to have caused accumulative genetic drift. According to Halliday (1993), genetic drift is likely to occur, particularly in small populations that are isolated from the main population and it may become the major source of genetic variation between some populations.

Among the four populations, MH is genetically closest to KS. Geological evidence suggests that the river systems of Sarawak were historically interconnected with most major river systems of Peninsular Malaysia via the Sunda River during Pleistocene glaciation (about 10000 years ago), thus allowing gene flow among these drainages (Kamarudin and Esa 2009). Gene flow from populations in the Straits of Malacca to those in Sarawak has been reported in several studies including Ryan and Esa (2006), Azhar and Hassan (2015), Samani et al. (2016) and Lau et al. (2018). Meanwhile, populations of SN-KN, which are separated by the South China Sea, showed high genetic connectivity (pairwise F_{ST} = 0.0702). This could probably be explained by the high migration ability of *T. jarbua* (>1000 km, Liu et al. 2015), human-mediated transfer through ballast waters (Liu et al. 2019) or past glaciation events. Furthermore, the high similarity in the sequence data (Table 3) perhaps indicates remnants of identical haplotypes from both populations, and that they were essentially similar at one time before the separation (Inger and Chin 2002). Eventually, sea level rise during the last Pleistocene period caused Borneo to be separated from mainland Asia (Peninsular Malaysia), which we suggest, resulted in shelf submergence and subsequent genetic differentiation between grunters from KN and SN. Pleistocene sea level fluctuations could also explain the incomplete divergence of grunters between East and Peninsular Malaysia. Similar evidence of a close genetic relationship between fishes of Borneo and mainland Asia in relation to their biogeographical history was discussed by several other authors (Pin et al. 2001; Nadiatul et al. 2011; Tan et al. 2012; Song et al. 2013).

Historical demography

Historical demographic expansions were determined by analysing the frequency distributions of pairwise differences between sequences (Rogers and Harpending 1992; Ray et al. 2003; Excoffier 2004). Neutrality tests with Tajima's *D* and Fu's Fs statistics estimate the deviation from neutrality, which is based on the expectation of a constant population size at mutation-drift equilibrium. Here, a negative Tajima's *D* signifies an excess of low frequency polymorphisms relative to expectation, indicating population size expansion or positive selection (Tajima 1983). The negative and significant Fu's Fs statistical value provides strong evidence for past population expansion, and rule out the possibility of genetic hitching or background selection, and evolutionary forces that produce a pattern similar to population expansion (Fu and Li 1993; Fu 1997; Okello et al. 2005). The *T. jarbua* populations displayed a genetic pattern typical of a population that has undergone a recent population expansion due to its two common haplotypes (Hap3 and Hap5) present across the range while the rest of the haplotypes are unique. The range expansion was a recent phenomenon and may not have achieved the migration-drift equilibrium, as shown by the lack of phylogeographical structure. Neutrality test statistics were in overall negatively significant and not consistent with a population at drift-mutation equilibrium.

The mismatch distribution is generally displayed as a multimodal pattern for populations showing demographic equilibrium. In contrast, a unimodal pattern depicts populations which have experienced recent demographic expansion (Rogers and Harpending 1992). In the results, all localities presented a multimodal pattern proving recent expansion. The hypothesis that the observed data fit the sudden expansion model was tested using the SSD and the raggedness index. Here, non-significant values for SSD signifies that the observed data do not deviate from that expected under the model of expansion. Non-significant raggedness index also indicates population expansion. Our observations of non-significant values in goodness-of-fit distribution for all populations suggest that population expansion occurred recently (Rogers and Harpending 1992).

Conclusion

To summarize, we found 1) high haplotype diversity but low nucleotide diversity among *T. jarbua* populations in Malaysia; 2) significant results suggesting population expansion of *T. jarbua* in this region; 3) despite the three genetic clusters observed in the haplotype tree and median-joining network, no obvious population structuring was detected among geographically distinct populations. Common haplotypes among populations and haplotypes from several populations in each genetic cluster indicate high genetic connectivity among the populations. This study assesses the genetic diversity and population structure of *T. jarbua* in Malaysia for appropriate conservation and management strategies. Conservation of crescent grunter at its natural variation level is required as it forms a diverse group of taxa with 83 haplotypes distributed across Malaysia. The haplotype composition surveyed in the present study may provide a baseline for future comparisons to monitor the temporal variability of haplotype frequency and population structure. This study also has indirectly revealed the dispersal power of *T. jarbua* through its high mobility and rapid adaptability to a newly colonized area. Further studies can be conducted using larger sample size and temporal replicates, samples collected from other areas of geographical distributions, and sequence data from other mtDNA genes or information based on nuclear DNA. This research contributed useful data for future large scale biogeographical and taxonomic studies of this species.

Animal ethics

The fish species that was employed in this study is not categorized as endangered species under the IUCN list and all the samples were collected from fish markets and landing sites.

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Appendix 1

Percentage of nucleotide composition based on populations.

ID	Nucleotide composition (%)																	
	COI						Cyt b						Combine					
	A	T	C	G	A + T	C + G	A	T	C	G	A + T	C + G	A	T	C	G	A+T	C+G
KS	22.4	28.8	30.9	17.9	51.2	48.8	23.5	28.7	32.9	14.9	52.2	47.8	22.9	28.9	32.0	16.2	51.8	48.2
KN	22.6	28.9	30.7	17.8	51.5	48.5	23.5	28.7	32.8	14.9	52.2	47.7	23.0	29.0	31.9	16.1	52.0	48.0
MH	22.5	28.4	31.4	17.8	50.9	49.2	23.7	28.4	32.9	15.0	52.1	47.9	23.0	28.6	32.2	16.2	51.6	48.4
SN	22.6	28.9	30.7	17.8	51.5	48.5	23.5	28.7	32.8	14.9	52.2	47.7	23.0	29.0	31.9	16.1	52.0	48.0
TW	22.6	28.9	30.8	17.8	51.5	48.6	23.5	28.7	32.9	14.9	52.2	47.8	23.0	28.9	32.0	16.1	51.9	48.1
Total	22.2	29.2	30.9	17.7	51.4	48.6	23.6	28.7	32.9	14.9	52.3	47.8	23.0	28.9	32.0	16.1	51.9	48.1
HAI	22.5	28.9	30.7	17.8	51.4	48.5	23.4	28.8	32.9	14.8	52.2	47.7	23.1	28.9	32.0	16.1	52.0	48.1
IND	22.3	29.5	30.5	17.7	51.8	48.2	23.6	28.8	32.8	14.8	52.4	47.6	22.9	29.7	31.6	15.8	52.6	47.4
PHI	22.9	28.9	30.7	17.5	51.8	48.2	23.4	28.6	32.9	15.1	52.0	48.0	23.2	28.7	32.0	16.1	51.9	48.1
TAI	22.5	28.9	30.7	17.8	51.4	48.5	23.6	28.7	32.9	14.8	52.3	47.7	23.1	28.8	32.0	16.1	51.9	48.1

Appendix 2

Polymorphic site analysis based on COI, Cyt *b* and combined gene. C, conserved site; V, variable site; Pi, parsimony informative sites; S, singleton sites.

ID	COI (631 bp)				Cyt *b* (815 bp)				Combine (1446 bp)			
	C	V	Pi	S	C	V	Pi	S	C	V	Pi	S
KS	602	29	20	9	742	73	53	20	1344	102	73	29
KN	618	13	6	7	793	22	14	8	1411	35	20	15
MH	575	56	54	2	716	99	95	4	1291	155	149	6
SN	617	14	7	7	787	28	20	8	1404	42	27	15
TW	619	12	9	3	798	17	14	3	1417	29	23	6
Total	557	74	60	14	687	128	111	17	1244	202	171	31

Appendix 3

Sequence data used in this study. All data were downloaded from NCBI.

Location	Accession number		Publication
	COI	Cyt *b*	
Hainan	NC027281	NC027281	Wu et al. 2016
Taiwan	KP204162	KP152133	Liu et al. 2015
Kochi, India	KC774674	KC774717	Lenka et al. 2014 (Unpublished)
Philippines	KF999840	KF999856	Canoy and Quilang 2015 (Unpublished)
Malaysia	MN529663–MN529796	MN529797–MN529930	This study

Appendix 4

Frequency distribution of haplotypes according to localities. Highlighted columns indicate shared haplotypes.

Haplotype	Total	KS	KN	TW	SN	MH	PHI	TAI	HAI	IND
Hap_1	3	3								
Hap_2	1	1								
Hap_3	17	4	4	7		1				1
Hap_4	1	1								
Hap_5	21	5	6	1	5	3		1		
Hap_6	1	1								
Hap_7	1	1								
Hap_8	2	1	1							
Hap_9	1	1								
Hap_10	1	1								
Hap_11	1	1								
Hap_12	1	1								
Hap_13	1	1								
Hap_14	1	1								
Hap_15	1	1								
Hap_16	1	1								
Hap_17	1	1								
Hap_18	1	1								
Hap_19	1	1								
Hap_20	1	1								
Hap_21	1	1								
Hap_22	1	1								
Hap_23	1		1							
Hap_24	4		4							

Haplotype	Total	KS	KN	TW	SN	MH	PHI	TAI	HAI	IND
Hap_25	1		1							
Hap_26	1		1							
Hap_27	1		1							
Hap_28	1		1							
Hap_29	1		1							
Hap_30	1		1							
Hap_31	1		1							
Hap_32	1		1							
Hap_33	1		1							
Hap_34	1		1							
Hap_35	1		1							
Hap_36	1		1							
Hap_37	1		1							
Hap_38	1		1							
Hap_39	1				1					
Hap_40	1				1					
Hap_41	5				5					
Hap_42	1				1					
Hap_43	1				1					
Hap_44	1				1					
Hap_45	2			1	1					
Hap_46	1				1					
Hap_47	1				1					
Hap_48	1				1					
Hap_49	1				1					
Hap_50	1				1					
Hap_51	1				1					
Hap_52	1				1					
Hap_53	1				1					
Hap_54	1				1					
Hap_55	1				1					
Hap_56	1				1					
Hap_57	1				1					
Hap_58	2					2				
Hap_59	1					1				
Hap_60	1					1				
Hap_61	1					1				
Hap_62	1					1				
Hap_63	1					1				
Hap_64	2					2				
Hap_65	1					1				
Hap_66	1					1				
Hap_67	1					1				
Hap_68	1					1				
Hap_69	2					2				
Hap_70	1					1				
Hap_71	1					1				
Hap_72	1			1						
Hap_73	2			2						
Hap_74	1			1						
Hap_75	1			1						
Hap_76	3			3						
Hap_77	1			1						
Hap_78	1			1						
Hap_79	1			1						
Hap_80	1			1						
Hap_81	1			1						
Hap_82	1			1						
Hap_83	1			1						
Hap_84	1								1	
Hap_85	1						1			