

# *Holothuria (Mertensiothuria) viridiaurantia* sp. nov. (Holothuriida, Holothuriidae), a new sea cucumber from the Eastern Pacific Ocean revealed by morphology and DNA barcoding

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## Abstract

*Holothuria (Mertensiothuria) viridiaurantia* sp. nov. is described based on specimens from rocky reefs of northern Chocó in the Colombian Pacific Ocean; however, it also occurs along the Eastern Pacific Ocean from Mexico and Panama. Although specimens from Mexico and Panama were previously identified as *Holothuria (Mertensiothuria) hilla* Lesson, 1830 the new species is easily distinguished morphologically and via mtDNA. In terms of morphology, the species can be identified by its olive-green background and white-orange papillae and tentacles, larger tentacles with deep indentations and also by larger buttons on the dorsal and ventral body wall, papillae and tube feet; large, thick and rough tentacle rods, and the absence of ossicles in the longitudinal muscles. The new species is included in the subgenus *Mertensiothuria* considering molecular evidence.

## Keywords

16S, COI, Chocó, Colombia, Echinodermata, Cabo Marzo, Gulf of Cupica, Gulf of Tribugá, Holothuroidea, mtDNA, morros, riscales, rocky reefs

## Introduction

The family Holothuriidae Ludwig, 1894 currently includes 211 valid species, with the genus *Holothuria* Linnaeus, 1867 being the most diverse, containing 165 formally described species (WoRMS 2019a). Sixteen new *Holothuria* species have been described from different localities around the world since 2000; two of them from the Central and Tropical Eastern Pacific (Laguarda-Figueras and Solís-Marín 2009; Honey-Escandón et al. 2011). The diversity of *Holothuria* will likely continue to grow considering some “cryptic” species currently recognised based on molecular evidence (COI mtDNA) and morphological characteristics, such as colouration, as reported for the *Holothuria* (*Thymiosycia*) *impatiens* complex (Michonneau 2015). In addition, the exploration of poorly known regions could generate new information on the diversity of holothurians and other marine organisms. In particular, an area that warrants further exploration is the north of the Colombian Pacific (Chocó), part of the Tumbes-Magdalena-Chocó biogeographical hotspot that is considered a mega-diverse area (Cortés 1997).

*Holothuria* (*Mertensiothuria*) Deichmann, 1958, one of the 18 *Holothuria* subgenera, was reviewed by Samyn and Massin (2003), who emended its diagnosis by incorporating ossicles from the longitudinal muscles. According to Samyn and Massin (2003) there were six species in the subgenus; four of them were previously recognised: *H. (M.) albofusca* Cherbonnier, 1988, *H. (M.) fusciorubra* Théel, 1886, *H. (M.) leucospilota* (Brandt, 1835), and *H. (M.) papillifera* Heding in Mortensen, 1938; and two were transferred from the subgenus *Thymiosycia* into *Mertensiothuria*: *H. hilla* Lesson, 1830 and *H. aphanes* Lampert, 1885. Samyn and Massin (2003) also removed four species from the subgenus either because of the absence of ossicles in the longitudinal muscles (*Holothuria arenacava* Samyn et al., 2001 and *Holothuria platei* Ludwig, 1898) or because this characteristic was unknown (*Holothuria artensis* Cherbonnier & Féral, 1984 and *Holothuria exilis* Koehler & Vaney, 1908). According to WoRMS (2019b), *Mertensiothuria* currently includes the same six species accepted by Samyn and Massin (2003), although *H. (M.) papillifera* is considered species inquirenda. In addition, the subgenus includes three more species: *H. (M.) isuga* Mitsukuri, 1912, *H. (M.) arenacava* and *H. (M.) artensis*; the last two species excluded in the revision by Samyn and Massin (2003) have been transferred by WoRMS but with no reference.

Among the species in this subgenus *H. (M.) hilla* Lesson, 1830 is the most widespread species, reported from the Red Sea to Madagascar and across the Indian Ocean and the Pacific Ocean to the Central and Tropical Eastern Pacific (Purcell et al. 2012). It is a common species in the Central and Tropical Eastern Pacific occurring in its common colour morph, comprised of a yellow background and white papillae (Samyn and Massin 2003; Purcell et al. 2012). However, specimens with different colours, such as an olive-green background and white-orange papillae, have been reported by several authors (Solís-Marín et al. 2009, Lam. 30A; Sotelo-Casas et al. 2015: fig. 2E; Molina et al. 2015: fig. 3C). Specimens with yellow and green colour patterns were collected in the Colombian Pacific Ocean in 2016, allowing comparison of the morphology and mitochondrial DNA. The purpose of this paper is to describe a new species of *Holothuria* from the Eastern Pacific and to indicate how it differs from *Holothuria* (*Mertensiothuria*) *hilla*.

## Materials and methods

The specimens reviewed were collected as part of the project “Riscales”, developed by the Instituto de Investigaciones Marinas y Costeras – INVEMAR ([www.invemar.org.co](http://www.invemar.org.co)), seeking to characterise the biodiversity of the rocky reefs (called locally “riscales” and “morros”) located in northern Chocó in the Colombian Pacific Ocean. These ecosystems are important for regional fisheries and conservation (Díaz-Fahrenberger et al. 2016). Specimens were collected by hand using SCUBA diving at three rocky reefs between 10 and 15 m depth, during two sampling events in April and October 2016 (Fig. 1A, B). The specimens were placed in plastic bags with seawater, relaxed using magnesium chloride, fixed and preserved in 96% ethanol. They are deposited at the Museo de Historia Natural Marina de Colombia (MHNMC) – Makuriwa of INVEMAR (**INV EQU**).

External and internal morphology were reviewed to record standard data for sea cucumbers. Tissue from papillae, dorsal body wall, tube feet, ventral body wall, tentacles, and internal organs (longitudinal muscles, respiratory trees, tentacle ampullae, cloaca, and intestine) was removed and dissolved in fresh household bleach. Ossicles were observed and photographed using light microscopy and, at least, ten ossicles of each type were measured using the software ImageJ (Schneider et al. 2012). Type of ossicles, shape, and size were compared with those described by Lesson (1830) and Samyn and Massin (2003).

Ethanol-fixed tissues of the sea cucumbers collected during the project were processed to obtain sequences of the mitochondrial cytochrome oxidase I (COI) and 16S (large subunit) genes; in this paper, only the data of the specimens of interest are shown. Genomic DNA was extracted using the QIAGEN extraction kit (DNeasy Blood & Tissue Kit) and COI and 16S were amplified using the primers COIceF (ACTGCCACGCCCTAGTAATGATATTTTTTATGGTNATGCC) and COIceR (TCGTGTGTCTACGTCCATTCTACTGTRAACATRTG) (Hoareau and Boissin 2010) and 16SA (CGCCTGTTTATCAAAAACAT) and 16SB (CTCCGGTTT-GAACTCAGATCA) (Palumbi 1996). PCRs were carried out following the conditions described by Hoareau and Boissin (2010). PCR products were purified and sequenced using the BigDye 3.1 (Applied Biosystems) technology. The obtained nucleotide sequences were edited using Mega 7. We analysed a fragment of 443 bp of 16S genes (including gaps) and 439 bp of COI. Sequences of COI were translated into amino acids to ensure their integrity and accuracy. The sequences obtained in the present study were submitted to GenBank (Table 1). Available sequences of *H. (M.) hilla*, *H. (M.) leucospilota*, *H. (T.) arenicola*, and *H. (T.) impatiens* from GenBank were included in the analysis (Table 1). 16S sequences were aligned using the L-INS-i method implemented in MAFFT 6 (Katoh et al. 2002) and COI with Clustal W (Thompson et al. 1994). Distances using Kimura 2 parameters correction were calculated and neighbour-joining trees were generated using Mega 7 (Kumar et al. 2016). Within-group genetic differences were analysed on the species level also based on Kimura 2-parameter distances. The best substitution model was searched using the Akaike information criterion implemented in jModelTest (Posada 2008). Phylogenetic relationships were inferred using Bayesian Inference (BI) and Maximum Likelihood (ML).

BI was performed with MrBayes v. 3.2.6 (Ronquist and Huelsenbeck 2003) using unlinked GTR+G evolutionary model for each gen; the data set was run twice, using four Markov chains for ten million generations for each analysis to estimate posterior probabilities. ML analysis was performed in Mega 7; support was assessed in this case by 1000 bootstrap pseudoreplicates.

## Results

**Order Holothuriida Miller, Kerr, Paulay, Reich, Wilson, Carvajal & Rouse, 2017**

**Family Holothuriidae Burmeister, 1837**

**Genus *Holothuria* Linnaeus, 1767**

**Subgenus *Mertensiothuria* Deichmann, 1958**

***Holothuria* (*Mertensiothuria*) *viridiaurantia* sp. nov.**

<http://zoobank.org/3CF88C56-4A82-4758-B74A-395DF68C7F68>

Figures 1–7; Tables 1–3

*Holothuria* (*Mertensiothuria*) *hilla* Solís-Marín et al. 2009: 110–111, fig. 30A–G; Santos-Beltrán and Salazar-Silva 2011: fig. 2A; Honey-Escandón et al. 2012; Sotelo-Casas et al. 2015: 3–4, figs 2E, 3(4–8), 4(4–6); Molina et al. 2015: fig. 3C.

**Material examined. Holotype:** INV EQU4309, one specimen, total length 70 mm; collected in La Viuda rocky reef, Gulf of Cupica, northern Chocó, Colombia (6°37.9812'N, 77°29.985'W), by G. Borrero, 24 Oct 2016; at 15 m depth under rocks and attached; GenBank nucleotide sequences COI MK477997 and 16S MK477991 (Fig. 2A–D).

**Paratype:** INV EQU4234, one specimen, total length 35 mm; collected in El Faro rocky reef, Cabo Marzo, northern Chocó, Colombia (6°49.4802'N, 77°41.3976'W), by M.J. Vanegas, 24 April 2016; at 13 m depth under rocks and attached; GenBank nucleotide sequences 16S MK477992 (Fig. 2E); **Paratype:** INV EQU4312, one specimen, total length 25 mm; collected in Morromico rocky reef, Gulf of Tribugá, northern Chocó, Colombia (5°52.3194'N, 77°18.6426'W), by G. Borrero, 20 Oct 2016; at 10 m depth under rocks; GenBank nucleotide sequences COI MK477998 (Fig. 2F).

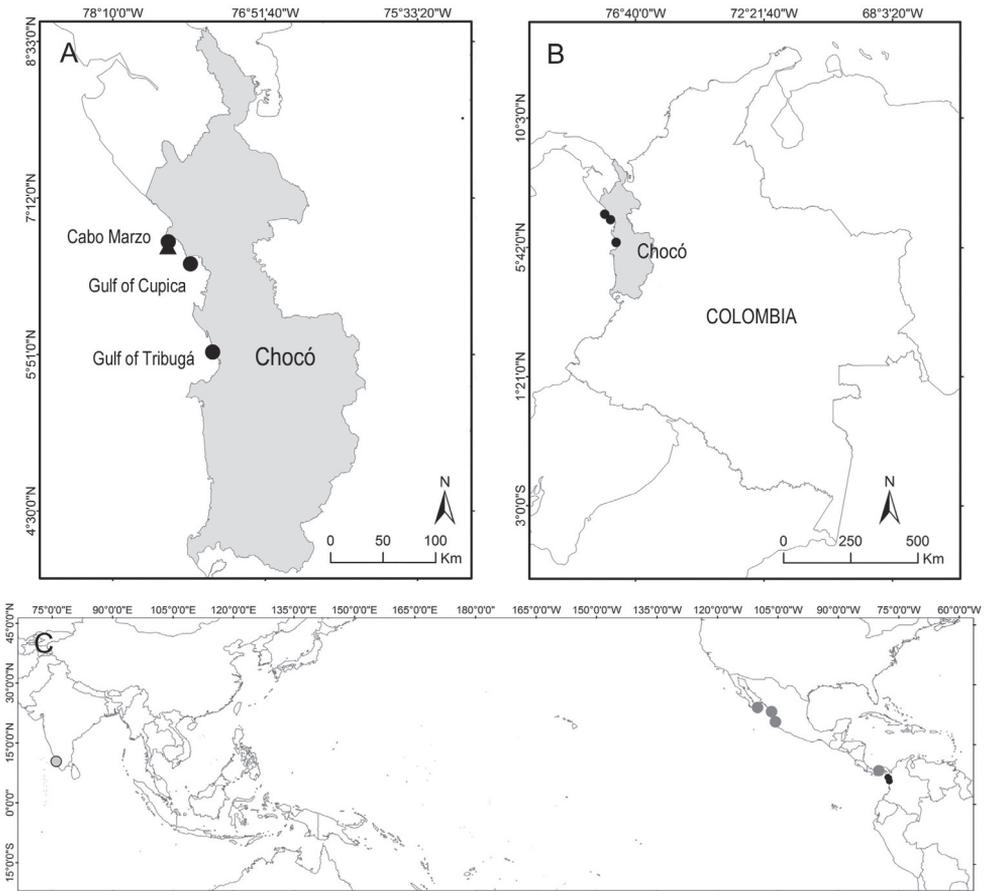
**Comparative material *Holothuria* (*Mertensiothuria*) *hilla*:** INV EQU4245, one specimen, total length 75 mm; collected in Piedra de Rodrigo rocky reef, Cabo Marzo, northern Chocó, Colombia (6°47.0346'N, 77°41.6148'W), by M.J. Vanegas, 25 April 2016; at 19 m depth under rocks; INV EQU4310, four specimens, total length 70–100 mm; by G. Borrero, 26 Oct 2016; same locality, depth, and habitat as previous; GenBank nucleotide sequences 16S MK477993 (Fig. 2G, H); INV EQU4311, one specimen, total length 65 mm; by G. Borrero, 26 Oct 2016; same locality, depth, and habitat as previous; GenBank nucleotide sequences COI MK477996 and 16S MK477994 (Fig. 2I, J).

**Table 1.** Specimens of *Holothuria (Mertensiothuria) viridiaurantia* sp. nov. from Colombia and GenBank sequences analysed for the partial cytochrome oxidase subunit 1 (COI) and/or 16S genes. Sequence-Voucher Location column includes the origin of the sequences according to the GenBank references where they have been generated; sequences from this study include the catalogue number at the MHNMC-INVEMAR (INV EQU). An asterisk (\*) in the species column indicates changes in the GenBank ID, where the sequences were previously identified as *H. (Mertensiothuria) hilla*.

Species	GenBank Accession number		Sequence-Voucher, Location	Reference
	COI	16S		
<i>H. (M.) viridiaurantia</i> sp. nov.	MK477997	MK477991	Colombia (Pacific) Holotype (INV EQU4309)	This study
	MK477998	–	Colombia (Pacific) Paratype (INV EQU4312)	This study
	–	MK477992	Colombia (Pacific) Paratype (INV EQU4234)	This study
<i>H. (M.) viridiaurantia</i> sp. nov.*	JN207616	JN207515	Mexico	Honey-Escandón et al. 2012
	KP780302	–	India	Deepa and Bijukumar, unpub.
<i>H. (M.) hilla</i>	MK477996	MK477994	Colombia (Pacific) (INV EQU4311)	This study
	–	MK477993	Colombia (Pacific) (INV EQU4310)	This study
	KX874337	KX856783	Mariana Islands, Guam	Miller et al. 2017
	–	EU822442	–	Uthicke and Byrne, unpub.
	–	FJ223856	Malaysia	Kamarul et al. 2006
	–	FJ223864	Malaysia	Kamarul et al. 2006
<i>H. (M.) leucospilota</i>	JN207617	JN207541	Marshall Islands (Majuro)	Honey-Escandón et al. 2012
	KC405566	KY986424	Pangkor Island, Malaysia	Kamarudin and Rehan 2015
	KY986417	KY986423	Pangkor Island, Malaysia	Kamarudin and Rehan 2015
	KC405565	KY986422	Pangkor Island, Malaysia	Kamarudin and Rehan 2015
<i>H. (T.) impatiens</i>	MK477999	MK477995	Colombia (Pacific) (INV EQU4236)	This study
	JN207632	JN207526	Mexico (Pacific)	Honey-Escandón et al. 2012
<i>H. (T.) aff. impatiens</i>	–	FJ223857	Malaysia	Kamarudin et al. 2010
<i>H. (T.) arenicola</i>	JN207608	JN207556	Florida (USA)	Honey-Escandón et al. 2012
<i>Isothicopus fuscus</i>	MK477908	MK477869	Panama (Pacific) IFTa210	This study

**Diagnosis.** Olive-green background with white-orange dorsal papillae, tube feet and tentacles; buttons >75 µm in length; large tentacles with deep indentations; tentacle rods thick, rough and with some perforations; longitudinal muscles without ossicles.

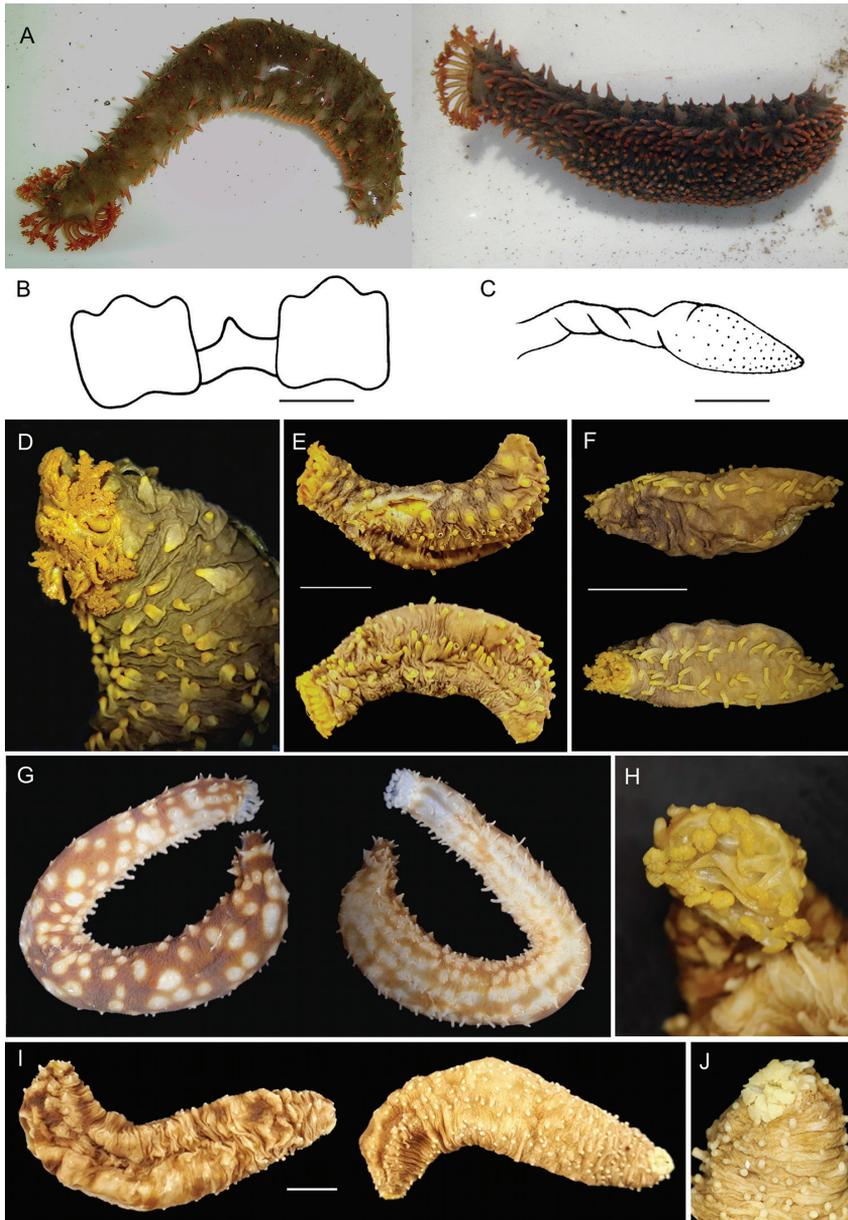
**Description.** External appearance: medium-sized species, holotype preserved specimen 70 mm long and 21 mm wide; body loaf like (length < 4× diameter) length/width ratio 2.3. Body shape of living ex situ specimen cylindrical in cross-section (Fig. 2A), tapering posteriorly and widening anteriorly, ending in a large crown of tentacles. Body wall soft and thin (2–3 mm thick). Anus terminal surrounded by small papillae. Mouth directed ventrally in live and preserved specimens, encircled by large papillae (Fig. 2A, D). Large peltate tentacles 20; ca. 5–6 mm total length, and 4–5 mm width shield; with



**Figure 1.** Maps showing the geographic distribution of *Holothuria (Mertensiothuria) viridiaurantium* sp. nov. **A, B** Detailed distribution in Chocó, Colombia; the triangle indicates the only locality where *H. (Mertensiothuria) hilla* specimens were collected **C** Wider distribution of *H. (M.) viridiaurantium* sp. nov. showing other localities from Panama and Mexico where the species have been identified through photographs, and the locality in India from where the GenBank sequence KP780302 originated. Colombian localities are represented by exact coordinates, and all other localities were derived from specific localities mentioned in the literature.

deep indentions 2–3 mm. Few large, long and slender conical papillae scattered on the dorsal surface, although a vague arrangement into four rows is observed, two of them are lateral, where they are a little larger; smaller papillae scattered among the largest. Ventral tube feet cylindrical, large and thick, densely distributed throughout the ventral surface.

**Colour.** Background of living specimens olive-green; base of the papillae is a light or whitish green that changes to orange from the middle to the ends, however, the tips of the papillae are whitish. Ventral surface similar to dorsum, with orange tube feet and white suckers; tentacles orange, same colour as papillae and tube feet (Fig. 2A). Dark brownish green in preserved specimens with papillae, tube feet, and tentacles a dark yellow (Fig. 2D–F).



**Figure 2.** Type specimens of *Holothuria (Mertensiothuria) viridiaurantia* sp. nov. (**A–F**) and comparative material of *Holothuria (Mertensiothuria) hilla* (**G–J**). **A** Dorsal and ventral view of the alive holotype of *H. (M.) viridiaurantia* sp. nov. from Gulf of Cupica, Northern Chocó, Colombia (INV EQU4309, L = 70 mm) **B** calcareous ring and **C** stone canal and madreporite of the Holotype **D** detail of the preserved holotype tentacles **E** paratype from Cabo Marzo; Northern Chocó, Colombia (INV EQU4234, L = 35 mm) **F** smallest paratype from Gulf of Tribugá, Northern Chocó, Colombia (INV EQU4312, L = 25 mm) **G, H** alive specimen of *H. (M.) hilla* from Cabo Marzo, Northern Chocó, Colombia, and detail of tentacles in the preserved specimen (INV EQU4310, L = 100 mm) **I, J** preserved specimen of *H. (M.) hilla*, same locality as **G, H** and detail of tentacles (INV EQU4311, L = 65 mm). Scale bars: 2 mm (**B, C**); 1 cm (**E, F, I**).

**Internal anatomy.** Square radial plates in the calcareous ring, 3 mm wide and 3 mm high, with three anterior rounded processes, and posterior margin with shallow rounded indentation; interradial plates slender, 1.5 mm high and 2.5 mm wide, pointed anterior margin and rounded posterior margin (Fig. 2B). One free stone canal, 4 mm long, and a helicoidally madreporite, 4 mm long (Fig. 2C). Tubular tentacle ampullae, 3–4 mm long and striped coloured. Tube-like polian vesicle, 17 mm long. Longitudinal muscles pair flat, thinner in the middle of each pair, irregularly wide, 3–4 mm wide each band, or 2–2 mm wide, attached, with narrow free edges. Gonads absent. Cuvierian organ present. Right respiratory tree extending to anterior end; left respiratory tree attached to the intestine until the middle of the body.

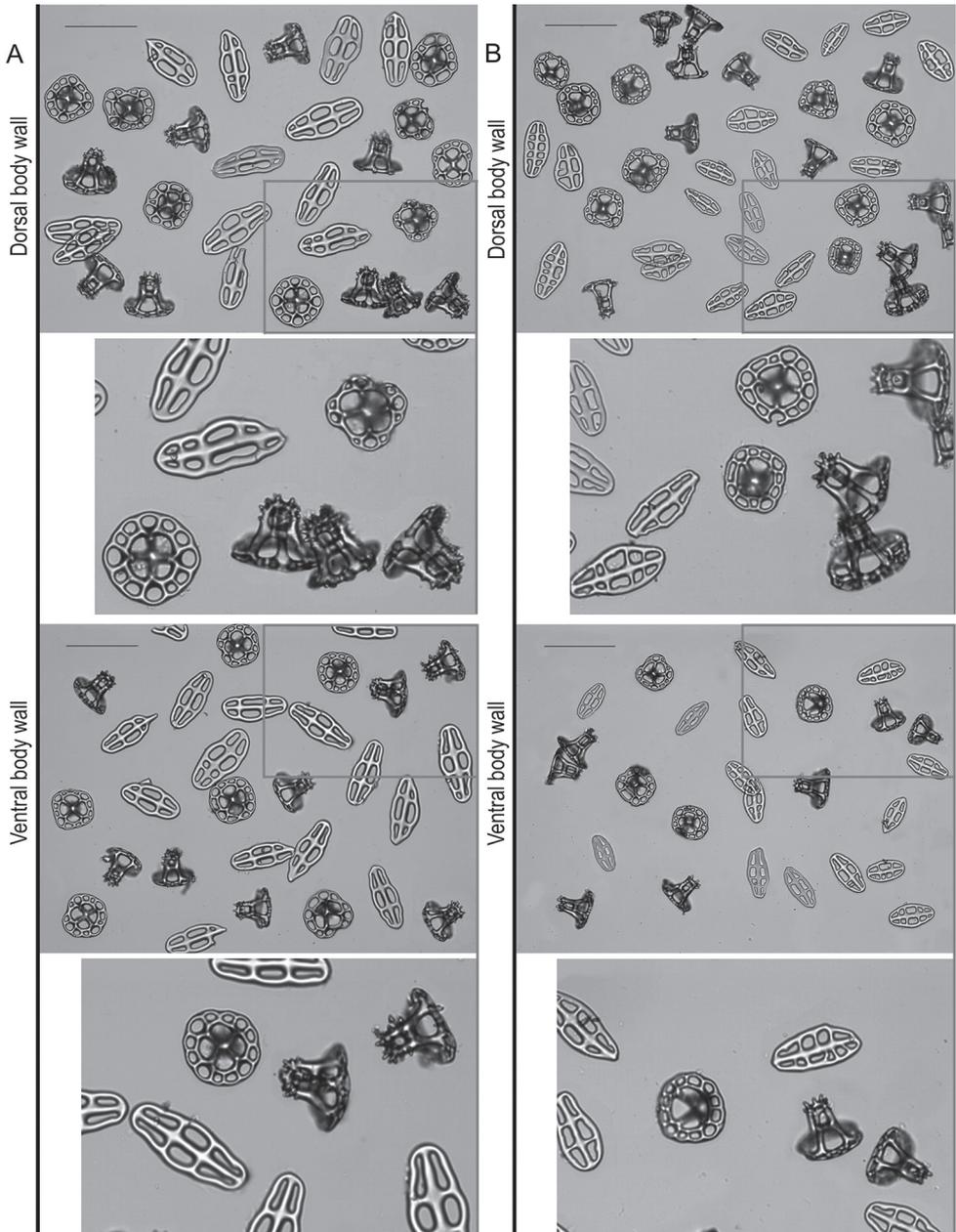
**Ossicles:** Dorsal and ventral body wall include similar tables and buttons, with dorsal tables taller and dorsal buttons larger than ventral (Table 2, Fig. 3A). Tables disc circular to quadrangular in outline; rim of the disc smooth; with four large central perforations and 7–12 smaller peripheral holes, arranged in one ring; spire with four pillars, single crossbeam, spiny crown with a small central hole (Fig. 3A). Dorsal tables 60–81  $\mu\text{m}$  across disc ( $\bar{x}$  = 68  $\mu\text{m}$ ) and 43–54  $\mu\text{m}$  height ( $\bar{x}$  = 49  $\mu\text{m}$ ); ventral tables 57–71  $\mu\text{m}$  across disc ( $\bar{x}$  = 63  $\mu\text{m}$ ) and 40–49  $\mu\text{m}$  height ( $\bar{x}$  = 44  $\mu\text{m}$ ). Buttons with smooth rim but irregular contour, usually with three pairs of holes, sometimes four pairs or three-four unpaired holes (Fig. 3A). Dorsal buttons 79–115  $\mu\text{m}$  long ( $\bar{x}$  = 101  $\mu\text{m}$ ); ventral buttons 82–108 long ( $\bar{x}$  = 94  $\mu\text{m}$ ). Dorsal papillae with tables, buttons, button-like plates, rods and at the very tip one small plate and small rods (Table 2, Fig. 4A). Tables and buttons similar in shape and size to the ones in the body wall, although table's spires are thicker and buttons are larger, up to 130  $\mu\text{m}$ . Rods 167–203  $\mu\text{m}$  long ( $\bar{x}$  = 187  $\mu\text{m}$ ) with distal or median perforations; small plates at the top of the papillae 99–134  $\mu\text{m}$  ( $\bar{x}$  = 187  $\mu\text{m}$ ) and small rods 37–58  $\mu\text{m}$  ( $\bar{x}$  = 48  $\mu\text{m}$ ). Ventral tube feet or pedicels with tables, buttons, plates, and end plates (Table 2, Figs 4A, 5A). Tables and buttons similar in shape and size to the ones in ventral body wall, although buttons are larger, up to 140  $\mu\text{m}$ . Plates 105–133  $\mu\text{m}$  long ( $\bar{x}$  = 117  $\mu\text{m}$ ) and 72–129  $\mu\text{m}$  wide ( $\bar{x}$  = 116  $\mu\text{m}$ ); end plates 578–581  $\mu\text{m}$  wide. Tentacles with large and small rods (Table 2; Fig. 5A); large rods are thick plate-like and very rough, usually with perforations at the extremities or along its length, 113–261  $\mu\text{m}$  long and 33–150  $\mu\text{m}$  width. Small rods thin and few spinose (58–107  $\mu\text{m}$ ). Longitudinal muscles without ossicles (Fig. 5A), as well as in the other internal organs, such as respiratory trees, tentacle ampullae, cloaca, and intestine.

**Paratypes:** Juveniles, 35 and 25 mm long, 12 and 8 mm wide respectively (Fig. 2E, F). External morphology different to the holotype, which is much larger at 70 mm long. Small dorsal papillae in the four main rows, as described for the holotype; and three rows of tube feet, two lateral and one in the middle of the ventral side which includes two irregular lines of pedicels (Fig. 2F). Dorsal and ventral body wall buttons are smaller in the juvenile, although there is not a considerable difference in size; however, in shape they are more rounded at the extremities and frequently present more than three pairs of holes (Table 2, Fig. 6A, B). Tables showed more changes during growth in comparison with buttons: the tables spire are taller and narrower, pointed-like without cross beam clearly noted, with few spines around the top; and the tables disc diameter is larger, with peripheral holes less in number and larger in size in the juvenile (Table 2,

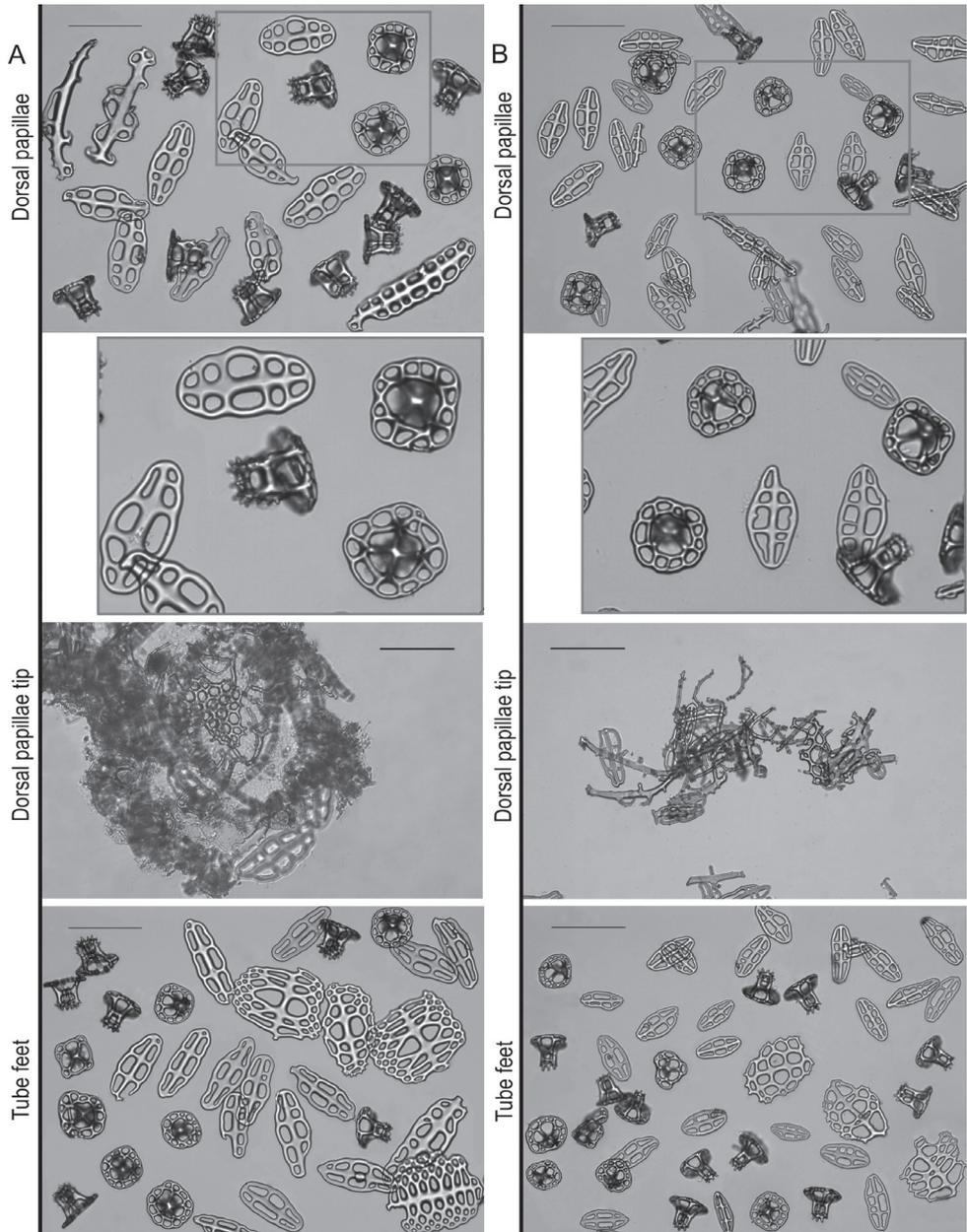
**Table 2.** Comparison of ossicle size in *Holothuria* (*Mertensiothuria*) *viridiaurantia* sp. nov. holotype, juvenile paratype, and *H. (M.) hilla* of similar body size.

Characteristics	<i>H. (M.) viridiaurantia</i> sp. nov. Paratype INV EQU4312		<i>H. (M.) viridiaurantia</i> sp. nov. Holotype INV EQU4309		<i>H. (M.) hilla</i> INV EQU4311	
	L = 25 mm		L = 70 mm		L = 65 mm	
	Range (µm)	Average	Range (µm)	Average	Range (µm)	Average
<b>Dorsal body wall</b>						
Buttons length	88–119	99	79–115	101	47–70	61
Tables disc diameter	62–84	73	60–81	68	51–75	59
Tables height	56–68	62	43–54	49	35–45	41
Width spires	14–15	15	23–31	29	21–26	23
<b>Ventral body wall</b>						
Buttons length	78–104	90	82–108	94	50–73	61
Tables disc diameter	54–80	63	57–71	63	47–61	53
Tables height	45–56	51	40–49	44	34–44	39
Width spires	9–17	14	22–29	25	16–20	18
<b>Dorsal papillae</b>						
Buttons, Buttons-like plates length	80–111	99	106–130	117	64–91	79
Tables disc diameter	51–95	74	60–82	71	48–66	58
Tables height	58–71	64	42–62	52	43–47	45
Width spires	19–28	25	30–42	36	20–25	22
Rods	97–168	143	167–203	187	102–152	129
Plates	–	–	99–134	118	104–107	106
Small Rods	–	–	37–58	48	43–67	57
<b>Ventral tube feet</b>						
Buttons, Buttons-like plates length	93–116	105	91–140	110	52–84	70
Tables disc diameter	54–75	63	53–73	61	41–57	51
Tables height	46–54	50	41–51	45	34–43	38
Width spires	15–23	18	23–31	28	17–24	21
Plates length	66–122	96	105–133	117	76–127	96
Plates width	46–83	65	72–129	116	68–99	84
End Plates	391–401	396	578–581	580	570–584	577
<b>Tentacles</b>						
Large Rods Length	132–259	197	185–261	221	166–267	227
Large Rods Width	21–39	29	33–150	75	10–27	18
Small Rods Length	37–91	56	58–107	74	37–65	47
<b>Longitudinal muscle</b>						
C-O shape ossicles length	NA	NA	NA	NA	13–33	24

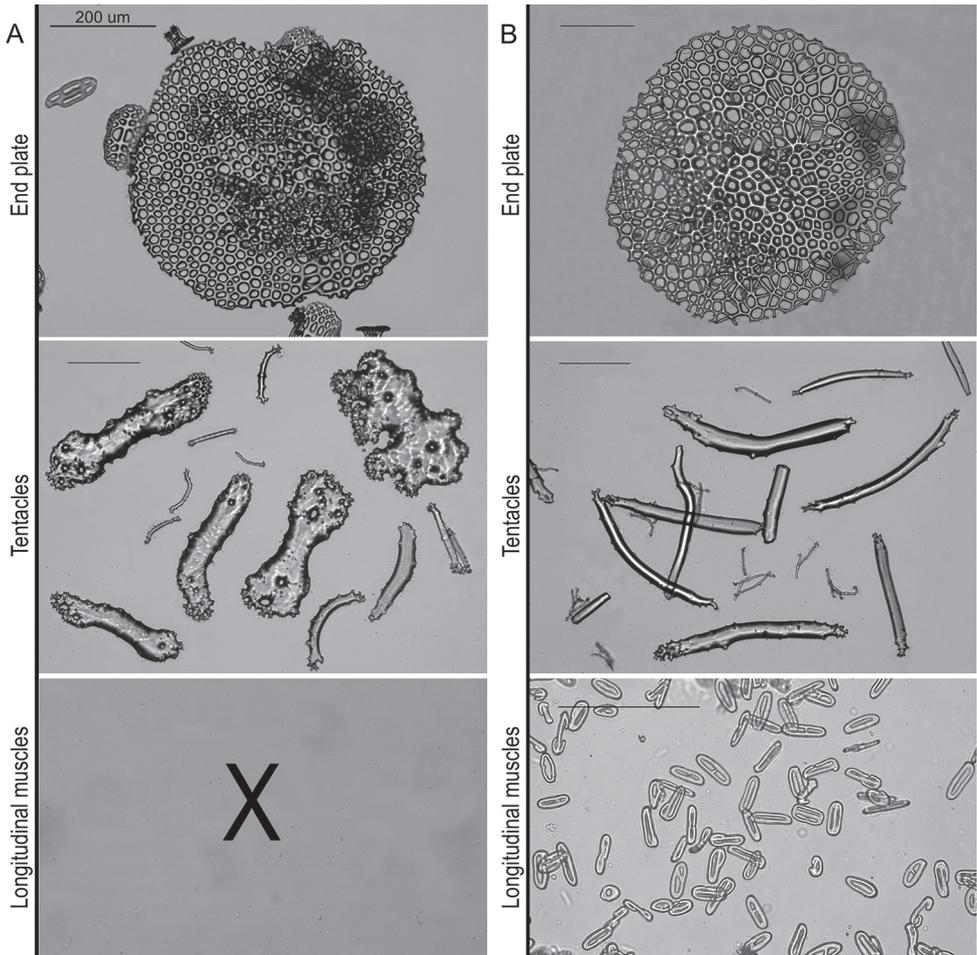
Fig. 6A, B). Dorsal papillae and tube feet present similar pattern of change during growth in buttons and tables when comparing the juvenile with the holotype; however, tables in dorsal papillae and tube feet in the juveniles are less pointed-like and one cross beam is clearly noted in most of the tables in comparison with those from the dorsal and ventral body wall (Fig. 6C, D). In addition, rods in dorsal papillae are smaller in size; it was not possible to observe the small plates and rods at the very top of the papillae. Supporting plates and end plates in the tube feet are also smaller in the juvenile (Table 2, Fig. 6D, E). Tentacle rods are not well developed in the paratype, being almost similar in length but less thick than those of the holotype, however, they are thicker than those in the *H. (M.) hilla* individual of 65 mm in length (Table 2, Figs 5A, B; 6F).



**Figure 3.** Ossicle comparison between *Holothuria (Mertensiothuria) viridiaurantia* sp. nov. and *Holothuria (Mertensiothuria) hilla*. **A** Holotype of *H. (M.) viridiaurantia* sp. nov. (INV EQU4309, L = 70 mm) **B** *H. (M.) hilla* (INV EQU4311, L = 65 mm); showing ossicle set from dorsal body wall (tables, buttons) and ventral body wall (tables, buttons); grey squares indicated in the images are presented enlarged below each image. Scale bar: 100  $\mu$ m.



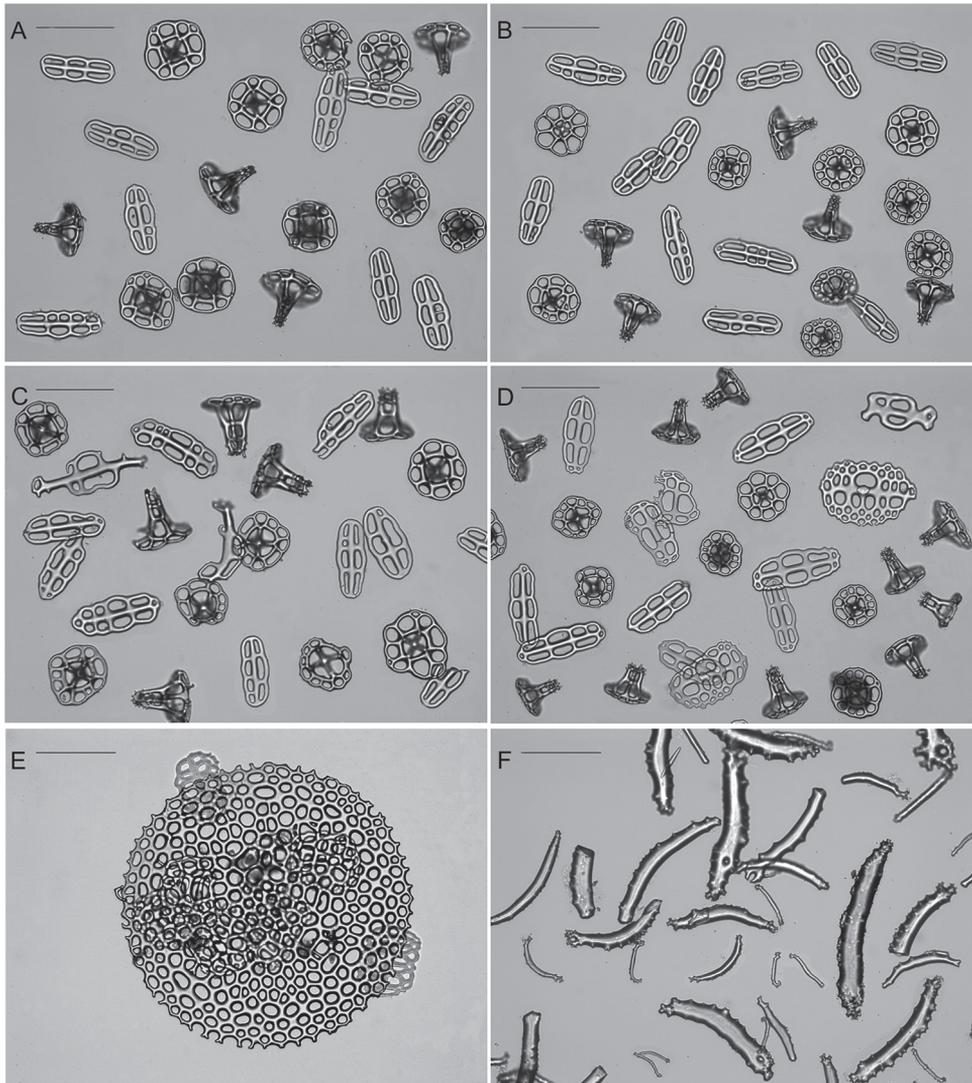
**Figure 4.** Ossicles comparison between *Holothuria (Mertensiothuria) viridiaurantia* sp. nov. and *Holothuria (Mertensiothuria) hilla*. **A** Holotype of *H. (M.) viridiaurantia* sp. nov. (INV EQU4309, L = 70 mm) **B** *H. (M.) hilla* (INV EQU4311, L = 65 mm); showing ossicle set from dorsal papillae (tables, buttons, rods), dorsal papillae tip (showing the plate and small rods at the tip) and tube feet (tables, buttons and supporting plates); grey squares indicated in some images are presented enlarged below each image. Scale bar: 100  $\mu$ m.



**Figure 5.** Ossicles comparison between *Holothuria (Mertensiothuria) viridiaurantia* sp. nov. and *Holothuria (Mertensiothuria) hilla*. **A** Holotype of *H. (M.) viridiaurantia* sp. nov. (INV EQU4309, L = 70 mm) **B** *H. (M.) hilla* (INV EQU4311, L = 65 mm); showing end plates from tube feet and ossicle set from tentacles (large and small rods) and longitudinal muscles (C's and O's ossicles). Scale bars: 100 µm (except **A** upper).

**Etymology.** From the Latin *viridis* (green) and *aurantius* (orange-coloured), referring to the living colour with olive-green background and orange-white papillae, tube feet, and tentacles (feminine).

**Distribution.** *Holothuria (Mertensiothuria) viridiaurantia* sp. nov. is known and confirmed along the Eastern Pacific from Mexico (as *Holothuria (Mertensiothuria) hilla*, Solís-Marín et al. 2009; Santos-Beltrán and Salazar-Silva 2011; Honey-Escandón et al. 2012; Sotelo-Casas et al. 2015), Panama (as *H. (M.) hilla*, Molina et al. 2015) and Colombia (present study) (Fig. 1). However, a GenBank sequence of one specimen from Kerala coast, India (Accession number KP780302.1) suggests that the new species could have a wider geographical distribution across the Indian Ocean and the Pacific Ocean to the Central and Tropical Eastern Pacific, like *H. (M.) hilla* (Fig. 1C).



**Figure 6.** Ossicles of the juvenile paratype of *Holothuria (Mertensiothuria) viridiaurantia* sp. nov. (INV EQU4312, L = 25 mm). **A** Dorsal body wall (tables, buttons) **B** ventral body wall (tables, buttons) **C** dorsal papillae (tables, buttons, rods) **D** tube feet (tables, buttons, supporting plates) **E** tube feet (end plate) **F** tentacles (large and thick rods, small rods). Scale bars: 100  $\mu$ m.

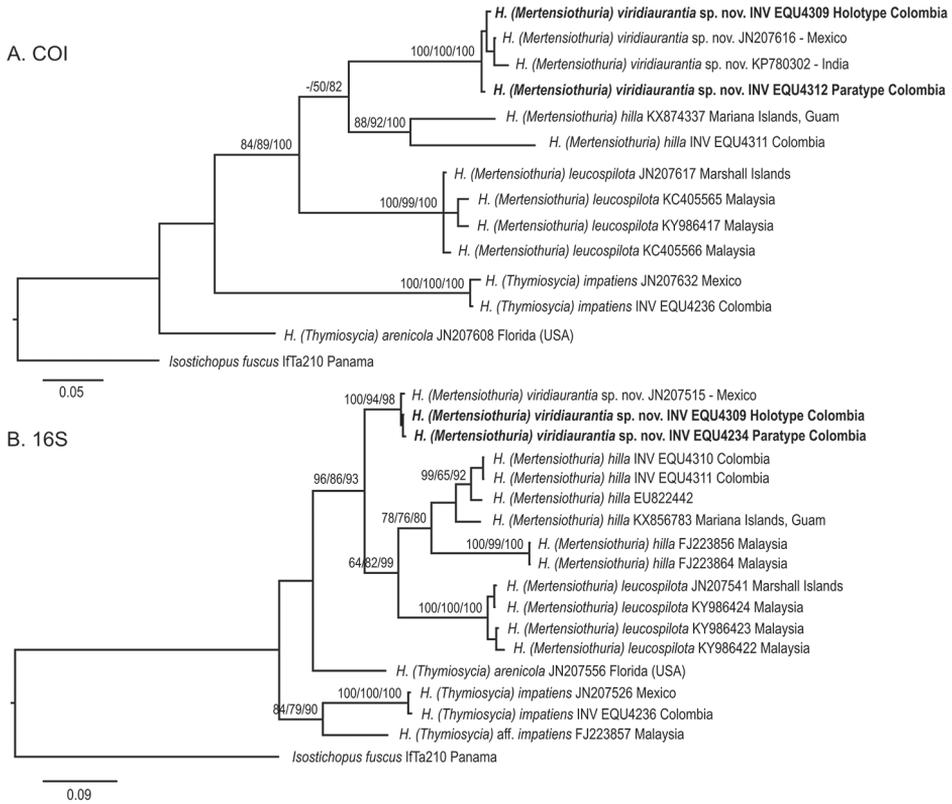
However, it was not possible to review the specimen belonging to the sequence, so colouration and morphological characteristics described in the present paper should be reviewed and confirmed. Notably, images of green-coloured *H. (M.) hilla* from the Philippines are presented by Dolorosa et al. (2017; Fig. 2J).

**Habitat.** *Holothuria (Mertensiothuria) viridiaurantia* sp. nov. is associated with rocky bottoms from the intertidal to 15 m depth (Molina et al. 2015; present study). Specimens collected in Colombia were found attached under medium rocks, differing

from *H. (M.) hilla* which were found under rocks but on a sandy substrate instead of a rocky substrate. *Holothuria (M.) viridiaurantia* sp. nov. was collected in three different rocky reefs in the northern Chocó, whereas *H. (M.) hilla* was found only in one, during both collection trips in 2016. Several specimens of *H. (M.) hilla* were found regenerating new anterior ends, this was not observed in specimens of the new species.

**Conservation status.** As the specimens of *Holothuria (Mertensiothuria) viridiaurantia* sp. nov. were previously assigned to *H. (M.) hilla*, the conservation status of this species must be considered. Currently *H. (M.) hilla* is included in the IUCN Red List in the category of Least Concern, and in addition, it is classified as a low-value species (about USD3 kg<sup>-1</sup> dried in the Philippines) among commercially important sea cucumbers of the world (Purcell et al. 2012). There is no fishery reports of *H. (M.) hilla* in the Eastern Pacific Ocean, however, *H. (M.) hilla* is fished commercially in the Philippines, Indonesia and Madagascar, that could include the new species considering the potential wider distribution of *H. (M.) viridiaurantia* sp. nov.

**Remarks.** The new species was previously assigned to *Holothuria (Mertensiothuria) hilla* (Solís-Marín et al. 2009; Santos-Beltrán and Salazar-Silva 2011; Honey-Escandón et al. 2012; Molina et al. 2015; Sotelo-Casas et al. 2015), however there is no mention of the distinct and striking colouration of the specimens reported in those papers in comparison with *H. (M.) hilla*. Perhaps the identification of this species was based on the similar external appearance (shape of the body and papillae) and apparent similar ossicles at first sight; without regard to the colouration, which has been traditionally considered to be intra-specific variability in echinoderms. However, recent research demonstrates that it can be a diagnostic characteristic, for example in the species complex *H. (T.) impatiens* (Michonneau 2015); this subject requires careful and exhaustive study, especially the purpose of colouration in sea cucumbers (Clark 1922; Michonneau 2015). In this study, a detailed revision of specimens from the new species and *H. (M.) hilla*, showed not only the colouration as a diagnostic feature, but also the size and shape of the tentacles, which are larger and with deeper indentations in the new species (Fig. 2). In reference to the ossicles, although similar in shape at first sight, a detailed revision showed several diagnostic characteristics: 1) differences in the size of the complete ossicle sets from the dorsal and ventral body wall, dorsal papillae and tube feet; specifically, the tables are taller and thicker with wider discs and the buttons are larger in the new species, in both juvenile and large specimens (Table 2; Figs 3–6); size of the buttons is the most diagnostic trait for the species; 2) the size and shape of the tentacle rods, being wide (plate-like), thick and very rough, and with some perforations in the new species compared to slender rods in *H. (M.) hilla* (Table 2; Figs 5A, B, 6F); 3) longitudinal muscle ossicles are absent in the new species, contrary to *H. (M.) hilla* (Table 2; Fig. 5A, B). In general, the morphological structures of the new species are thicker and stronger than those of *H. (M.) hilla*, which is a more delicate species. Among the morphological characteristics of the new species, the absence of ossicles in the longitudinal muscles, larger size of the perforated plates of the tube feet, and size and shape of the tentacle ossicles, match those considered by Samyn and Massin (2003) for excluding *Holothuria arenacava* and *Holothuria platei* from *Mertensiothuria*. However, the decision for including the new species in this subgenus was made based on the mtDNA evidence.



**Figure 7.** Bayesian inference tree of mitochondrial genes of the specimens analysed for the present study. **A** COI **B** 16S. The numbers on the nodes indicate Neighbour Joining (bootstrap %) / Maximum Likelihood (bootstrap %) / Bayesian posterior probability. Hyphen (-) indicates nodes not supported in some trees. Sequences from Colombia obtained in this study include the catalogue number at the MHNMC - INVEMAR (INV EQU); GenBank ascension number is included for the other sequences (see Table 1).

**Molecular characteristics.** We obtained COI and 16S sequence data from three specimens of *Holothuria (Mertensiothuria) viridiaurantia* sp. nov. and two of *Holothuria (Mertensiothuria) hilla* from the rocky reef in northern Chocó, Colombia. Specimens of *H. (M.) viridiaurantia* sp. nov. from Colombia (type specimens) were recovered in a well-supported clade, separated from *H. (M.) hilla* for both, COI and 16S genes (Fig. 7). Two sequences, derived from one specimen from Mexico (GenBank Accession No. JN207616–COI and JN207515–16S) and one from India (KP780302–COI), were recovered in the same clade as type specimens from Colombia. However, different tree topologies for COI and 16S sequence data were recovered. For COI *H. (M.) viridiaurantia* sp. nov. appears sister to *H. (M.) hilla*, with *H. (M.) leucospilota* positioned sister to them (Fig. 7A). However, for 16S *H. (M.) hilla* and *H. (M.) leucospilota* appeared as sister species with *H. (M.) viridiaurantia* sp. nov. as sister clade (Fig. 7B). Species from *Thymiosycia* subgenus appear separated from *Mertensiothuria* subgenus for both genes and all tree reconstruction methods (Fig. 7A, B). Evidence for species status

**Table 3.** Kimura 2 parameter distances (%) within specimens of *Holothuria* (*Mertensiothuria*) *viridiaurantia* sp. nov. and between the *Holothuria* species included in the analysis. COI distances are below diagonal and 16S distances above. The numbers in bold lettering along the diagonal represent average within species distances for COI and 16S (COI / 16S).

	Species	1	2	3	4	5	6
1	<i>H. (M.) viridiaurantia</i> sp.nov.	<b>1.3 / 0.5</b>	12.5	11.8	15.7	13.5	32.6
2	<i>H. (M.) hilla</i>	16.7	<b>13.8 / 7.9</b>	13.9	19.4	16.3	33.9
3	<i>H. (M.) leucospilota</i>	15.6	17.7	<b>1.9 / 1.6</b>	19.4	17.2	34.8
4	<i>H. (T.) impatiens</i>	21.3	21.5	21.8	<b>0.9 / 8.8</b>	15.5	33.2
5	<i>H. (T.) arenicola</i>	18.0	19.9	17.6	19.4	<b>nc / nc</b>	34.3
6	<i>Isostichopus fuscus</i>	24.4	22.5	24.0	24.5	20.7	<b>nc / nc</b>

of *H. (M.) viridiaurantia* sp. nov. comes from the COI and 16S genetic distances. Inter-specific distances between the two previously recognised *Mertensiothuria* species included in the analysis is 17.7% for COI and 13.9% for 16S; and distances between the new species and them are 16.7 and 15.6% for COI and 12.5 and 11.8% for 16S; inter-specific distances among species of *Mertensiothuria* and *Thymiosycia* showed larger values (Table 3). In addition, intra-specific distances for *H. (M.) viridiaurantia* sp. nov. were 1.31% for COI and 0.5% for 16S, the lowest values in all the species analysed. Intra-specific distances for *H. (M.) hilla* (13.8% for COI and 7.9% for 16S) could be showing a species complex, similar to what was described by Michonneau (2015) for *H. (T.) impatiens*, which is also recovered here with 8.8% for 16S, including one specimen identified as *H. (T.)* aff. *impatiens* (Table 3). Lower intraspecific distance for COI (0.9%) for *H. (T.) impatiens* is explained because the sequence for COI was not available for this specimen. There is, therefore, strong molecular evidence that *H. (M.) viridiaurantia* sp. nov. is an undescribed species different from *H. (M.) hilla*, a finding also supported by the morphological characteristics described previously.

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# Four new Caribbean *Sigambra* species (Annelida, Pilargidae), and clarifications of three other *Sigambra* species

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## Abstract

*Sigambra grubii* Müller, 1858 has been reported from many different coastal environments in Brazil and the Grand Caribbean. However, more than one species was thought to be included under this species group name. After the study of several subtle and consistent differences in specimens fitting the description *S. grubii*, a new Grand Caribbean species is herein recognized and described as *S. hernandezi* **sp. nov.** Further, the study of other *Sigambra* specimens prompted the examination of type specimens of *S. bassi* (Hartman, 1947), and of *S. wassi* Pettibone, 1966 to clarify some morphological features, and three other new species are recognized and newly described: *S. diazi* **sp. nov.** and *S. ligneroi* **sp. nov.** from the southeastern Caribbean (Venezuela), and *S. olivai* **sp. nov.** from the northwestern Caribbean (México). Morphological features are also clarified for *S. grubii* by comparison with specimens from the type locality, Florianópolis, Brazil, and with type specimens of *S. bassi* from Florida (U.S.A.), and non-type specimens of *S. wassi* from Virginia (USA). A key to identify all species of *Sigambra* is also included.

## Keywords

dorsal hooks, Fritz Müller, key to species, morphology, polychaetes, taxonomy

## Introduction

Fritz Müller (1822–1897) was regarded by Darwin as the ‘Prince of Observers’ after his careful studies of many different plant and animal groups in Santa Catharina, Brazil (Hartfelder 2019). Müller’s research interests resulted in 263 published articles, which are certainly remarkable even after current standards. Müller wrote two papers dealing with polychaetes (Hartman 1951): one was part of his series of arguments and examples supporting Darwin, where he referred to an amphinomid living on goose barnacles; the other one was a report about the polychaetes found from Santa Catharina Island, Florianópolis, Brazil (Müller 1858). A panoramic description of the main benthic and pelagic organisms was part of a letter dated November 1856 and sent to his brother Hermann (Möller 1921: 9). Müller (1858) proposed eight new genera and described nine new species from Santa Catharina Island. Six of his genus-group names are listed as valid in WoRMS: *Cherusca*, *Glycinde*, *Hermundura*, *Isolda*, *Magelona*, and *Sigambra*. *Sigambra grubii*, the type species for the genus, was briefly described based on a living specimen in 13 text lines and three figures of the anterior end, parapodium, and dorsal hook.

Hartman (1947: 483) hesitated about recognizing *Sigambra*; she thought it could be the same as *Ancistrosyllis* McIntosh, 1879, and indicated that its status should be solved by the study of topotypes (non-type specimens from the type locality). She later regarded *Sigambra* as questionably the same as *Ancistrosyllis* (Hartman 1959: 195). Pettibone (1966: 156, 157, 179 ff) reinstated *Sigambra* and since then, it has been recorded in many localities along Brazilian coasts. Currently, *S. grubii* is regarded among the most abundant marine benthic polychaete species along south and southeastern Brazil, in sediments of up to 150 m depth (Rohr and Almeida 2006). However, it has been recorded in over 110 studies along Brazilian coasts (Amaral et al. 2013) from estuarine areas to marine deep-water sediments (1000–3000 m), including the northern Amazonian mangrove region (Cutrim et al. 2018, Ribeiro et al. 2018). Given such a wide bathymetric range, more than one species might be included under the same name. Further, because of the lack of a clarification of the morphology of *S. grubii*, some other unlikely species records have been reported in literature from the region. For example, de Almeida et al. (2012) recorded *S. pettiboneae* Hartmann-Schröder, 1979, a species originally described from Australia, from Santa Catharina State, Brazil.

The present study was prompted by the finding of *Sigambra* specimens in brackish waters of Tamiahua Lagoon in the Mexican coastal states of Veracruz and Quintana Roo, and the need to clarify the details of some widely distributed *Sigambra* species including the amphiamerican *S. bassi* (Hartman, 1947). Type material of *S. bassi*, *S. wassi* Pettibone, 1966, and topotype specimens of *S. grubii* were studied and compared to specimens from the Grand Caribbean and southern Brazil. We found some subtle and consistent differences in the Grand Caribbean specimens, and they are herein described as new. We also provide additional observations for the topotypes to clarify their differences. A key to identify all species in the genus is also included.

## Materials and methods

Tamiahua Lagoon is on the Mexican Gulf of México coast, in the northern part of Veracruz. After a heavy rain season in 1999 a mass benthic mortality or defaunation occurred, prompting a study on the recovery of polychaete benthic communities. Six systematic samplings were carried out at the site, through a network of four transects each with four stations, during November 1999, March, August, and November 2000, July 2001, and February of 2002 (Sánchez-Hernández 2009). Sediments were taken with an Eckman dredge and washed through a 0.5 mm mesh screen; polychaetes were fixed in a 10% formalin solution and preserved in 70% ethanol.

Material from the southern Brazilian coast was collected from a non-vegetated tidal flat adjacent to salt marshes and mangroves in Pontal da Daniela (27°27'11"S, 48°31'47"W), Santa Catharina Island, Florianópolis. Sediment samples were taken during low tide with a manual PVC corer (10 cm diameter x 15 cm depth), in summer and winter of 2018. Sediments were washed through a 0.5 mm mesh. Specimens were removed and then fixed in a 6% formalin solution in sea water, with Rose Bengal. Specimens were later sorted and transferred to 70% ethanol.

Specimens were often twisted and measuring their length or counting their chaetigers became problematic. For the length, the specimen was carefully set along a ruler and the width measured at approximately chaetiger 10 including dorsal cirri. For twisted specimens, the number of chaetigers is given as an estimate (ca.) when they exceeded approximately 90. Start of dorsal hooks was determined from specimens mounted in a 1:1 solution of glycerin and 70% ethanol and scanned with a compound microscope. For indicating the relative size of median to lateral antennae, specimens were observed in profile, because tips of the median antenna were often damaged. If possible, these appendages were measured directly with a mini scale such as BioQuip 4828M. The first presence of dorsal hooks depended on the visibility of the hook, or at least on their broken handles, when the specimens were viewed along their dorsal parapodial surfaces.

Specimens were often temporally stained with Methyl Green or Shirlastain-A; the latter was especially useful for detecting papillae in the basal pharyngeal ring. Digital photographs were made in both stereo- and compound microscope, and for some species, SEM micrographs were made in the El Colegio de la Frontera Sur (ECOSUR) facility. Photo series were compressed by using HeliconFocus software. The sequence of species described in Systematics is alphabetical.

Specimens are deposited in the following collections:

- ECOSUR** El Colegio de la Frontera Sur, Chetumal, México.
- LACM** Allan Hancock Polychaete Collection, Los Angeles County Museum of Natural History, Los Angeles, U.S.A.
- UANL** Polychaete Collection, Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, Monterrey, México.
- USNM** National Museum of Natural History, Smithsonian Institution, Washington, USA

## Systematics

Order Phyllodocida Dales, 1962

Suborder Nereidiformia Glasby, 1993

Family Pilargidae de Saint-Joseph, 1899

Subfamily Pilarginae de Saint-Joseph, 1899

*Sigambra* Müller, 1858

*Sigambra* Müller, 1858: 214; Pettibone 1966: 179 (reinstated); Licher and Westheide 1997: 2 (key to species); Nishi et al. 2007: 65 (table with characters of all species).

**Type species.** *Sigambra grubii* Müller, 1858, by monotypy.

**Diagnosis.** Pilarginae with body depressed, usually obconic. Prostomium with three antennae, longer than palps; palps biarticulate. Tentacular cirri as long as half width of tentacular segment. Parapodia biramous. Dorsal and ventral cirri foliose to tapered, dorsal ones usually longer than ventral ones. Notopodia include dorsal hooks along many segments, sometimes with accessory capillaries. Neuropodia with shorter pectinates, medium-sized denticulates, and longer finely denticulate capillaries, often twisted distally.

**Remarks.** *Sigambra* species were reviewed by Licher and Westheide (1997), and they modified the orthography for the type species, using *grubei* instead of *grubii*, as originally introduced, and included a key to species. However, *Sigambra grubii* does not need an orthographic modification. As was customary in the times, Müller (1858) did not include etymologies for his new taxa. Licher and Westheide (1997: 4) referred to article 31a of the code (ICZN 1985: 61, 1999: 37) in an aim to change the orthography for the specific epithet to *grubei*. This was incorrect because of three reasons: First, they apparently misunderstood the corresponding examples for the same section in the code, especially the last one (reiterated in the most recent edition): ‘Cuvier, if Latinized to Cuvierius, gives *cuvierii*.’ Second, the original epithet was not modified by either De Quatrefages (1866: 89), nor Pettibone (1966: 182), both had a good knowledge of Latin, and Licher and Westheide (1997: 3) referred to these publications. And third, Licher and Westheide overlooked the proposals of two other species using the same epithet (*Onuphis grubii* von Marenzeller, 1866, and *Arenicola grubii* Claparède, 1869), which would underline the fact that the original orthography was correctly formed once the last German name was Latinized. Consequently, the original orthography must be retained.

*Sigambra* Müller, 1858 resembles *Ancistrosyllis* McIntosh, 1879 by having dorsal hooks above the dorsal cirri (Salazar-Vallejo and Rizzo 2009: 431). They differ by the relative size of the antennae, tentacular and dorsal cirri, and body papillation. In *Sigambra* these appendages are long, foliose to tapered, usually antennae are longer than palps, and the integument is mostly smooth, whereas in *Ancistrosyllis* appendages are short, usually digitate, palps are longer than antennae, and integument is mostly papillate.

Diagnostic features for all the then known species were tabulated by Nishi et al. (2007). Specific diagnostic features are included below in the key to species. Anterior end features include the relative length of median antennae, the length of tentacular

segment and presence of modifications along its anterior margin, the presence of ventral cirri on chaetiger 2, and of a constriction on anterior chaetigers. Parapodial features include the relative size of dorsal and ventral cirri, the start of dorsal hooks and their presence along body, and the type of neurochaetae. For the pharynx, the number of marginal papillae is especially useful. There are two patterns regarding the start of dorsal hooks. In the first, their start tends to be more or less stable, with a very small variation (2–4 chaetigers) disregarding variations in total size or number of chaetigers. In the other pattern, notohooks start at an earlier chaetiger in smaller specimens, and they are apparently displaced posteriorly during ontogeny, such that larger specimens will have dorsal hooks from a more posterior chaetiger. Further, notohooks along a few anterior chaetigers are often embedded in the notopodial bases, such that it is necessary to observe the specimen under a compound microscope to precise on which chaetiger notohooks arise. This implies that a series of specimens of different size, collected from the same date, and from similar depths, and sediment types, should be analyzed before deciding which alternative to follow in the key below. If available, size ranges were included in parenthesis to help guide decisions in the key, following Nishi et al. (2007).

There are four other potentially useful characters. First, the prostomial dorsal surface between the palps (interpalpal region) can be characterized by its anterior margin as blunt or depressed, and by the lateral depressions being widened posteriorly, or rectangular if the lateral depressions are more or less parallel. Second, in some species there is a deep antennal furrow for each lateral antenna; they can be easily noted if distinct, or as indistinct if they are difficult to see; further, antennal furrows are often narrower medially, and then they can diverge slightly, being almost parallel, or markedly divergent. Third, in some species, the ventral cirri can be short, not reaching neuropodial lobes tips, whereas in other species, ventral cirri can reach and even surpass neurochaetal lobes in medial or posterior parapodia. Fourth, the number of posterior chaetigers without hooks: in posterior chaetigers the dorsal hooks tend to be more exposed and are usually larger than those present in medial chaetigers or larger than parapodial lobes.

***Sigambra bassi* (Hartman, 1947)**

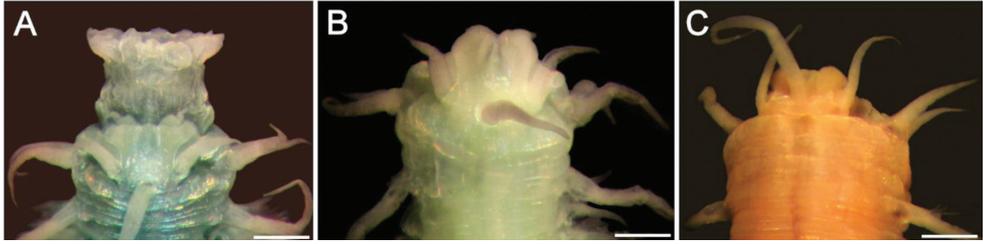
Fig. 1

*Ancistrosyllis bassi* Hartman, 1945: 6 (contents), 9 (substrate), 15 (non-diagnostic characters), nomen nudum.

*Ancistrosyllis bassi* Hartman, 1947: 501–504, pl. 61, figs 1–7; Hartman 1951: 36–38, pl. 11, figs 1–6 (figures rearranged from Hartman 1947).

*Sigambra bassi*: Pettibone 1966: 186, fig. 16, comb. nov.

**Type material.** *Holotype* (LACM 142), Gulf of México, southwestern Florida, Lemon Bay, Chadwick Beach (26°55'25"N, 82°21'40"W), sandy shore, low tide, 17 Jan. 1938, O. Hartman, coll. *Paratype* (LACM 1549), northwestern Atlantic, Beaufort, Bogue Sound, North Carolina, near US Fisheries Laboratory on Pivers Island (34°43'11"N, 76°40'19"W), sandy shore, 6 Jun. 1940, O. Hartman, coll.



**Figure 1.** *Sigambra bassi* (Hartman, 1947) **A** holotype (LACM 142), anterior end, dorsal view **B** paratype (LACM 1549), anterior end, dorsal view **C** non-type specimen (LACM 6614), anterior end, dorsal view. Scale bars: 0.3 mm (**A**, **C**), 0.2 mm (**B**).

**Additional material. Northeastern Pacific, Los Angeles.** Non-type specimen (LACM 6614), opposite Berth 79, at old Municipal Fish Market, in mid-channel, Sta. LA-26 (33°33'17"N, 118°16'34"W), 12 m, black mud, 14–15 Jun. 1954, D.J. Reish et al. coll.

**Clarification.** The holotype (Fig. 1A) was found in the Gulf of México side of Florida, whereas the paratype (Fig. 1B) was found in North Carolina. Both specimens are colorless, without any trace of pigmented glands. An additional specimen (Fig. 1C) was included in the same species, but it was collected in Los Angeles Harbor, and it has some brownish glands in both dorsal tentaculophores and dorsal cirri of chaetiger 1. There are some other differences between these specimens. For example, in the holotype the interpalpal area is slightly projected anteriorly and its margins are slightly divergent posteriorly, the lateral antennae are three times longer than wide, and the lateral antennal depressions are markedly divergent and expanded, such that the anterior region is wider than posterior one. In the paratype the interpalpal area is tapered, reduced anteriorly, with its margins expanded posteriorly, the lateral antennae are 4 times longer than wide, and the lateral antennal depressions diverge laterally such that the anterior region is shorter than the posterior one. The non-type specimen has a truncate interpalpal region, with its anterior margin slightly bilobed, and margins more or less parallel, the lateral antenna are more than 12 times longer than wide, and the lateral antennal depressions are barely distinct, visible only along the inner side of each antenna.

**Remarks.** If these specimens are conspecific, the species would have a Gulf of México-Atlantic and Eastern Pacific distribution, which is untenable, for the following reasons. A strong genetic discontinuity between Gulf and Atlantic populations has been noted for specimens living in Florida (Soltis et al. 2006, Bijak et al. 2018), and ampho-American species have been progressively recognized as including more than one distinct species upon morphological features and genetic markers combined (Carrera-Parra and Salazar-Vallejo 2011), or even after the analysis of morphological differences (Conde-Vela and Salazar-Vallejo 2015). Consequently, *S. bassi* should be regarded as a northwestern Atlantic species restricted to the Gulf of México; new names and descriptions will be provided for the specimens from Beaufort, NC, and Los Angeles, California in a subsequent publication. Chances are, however, that the same species might extend from the Gulf of México to Beaufort, NC. On the other hand, it should be noted that specimens from San Francisco have been regarded as belonging to a different species since 2013 (Norris 2013).

***Sigambra diazi* sp. nov.**

<http://zoobank.org/B8CC2C1F-6108-47CC-8427-08AF56D99593>

Fig. 2

*Sigambra tentaculata*: Liñero-Arana and Díaz-Díaz 2005: 68–69, fig. 2 (**non** Treadwell 1941).

**Type material.** *Holotype* (ECOSUR 214) and *paratype* (ECOSUR 215), southern Caribbean, Venezuela, Laguna de Chacopata (10°39'50"N, 63°48'30"W), 1.5 m, sediments, 15 May 2000, O. Díaz-Díaz, coll.

**Diagnosis.** *Sigambra* with median antenna reaching chaetiger 5–7, twice as long as laterals; tentacular segment four times wider than long, anterior margin smooth; dorsal cirri larger than ventral ones; chaetiger 2 without ventral cirri; notopodia with dorsal hooks and capillaries from chaetiger 4–5; median and posterior chaetigers with ventral cirri reaching neuropodial lobes tips; pharynx with 13–16 marginal papillae.

**Description.** Holotype (ECOSUR 214) an anterior fragment, 7.5 mm long, 1.5 mm wide, 41 chaetigers, right parapodia of chaetigers 16 and 40 removed for observing parapodial features. Body obconic, cylindrical along chaetigers 1–22, depressed thereafter. Dorsal integument rugose, weakly areolate, especially along chaetigers 4–16 (Fig. 2A).

Prostomium blunt, three times wider than long. Palps with palpophores massive, directed ventrally, palpostyles digitate, with a basal oblique mark; interpalpal area distinct, right longitudinal depression better defined than left one, expanded posteriorly. Antennae tapered, median antenna twice as long as laterals, laterals barely surpassing palp tips, median antenna reaching chaetiger 2–3. Lateral antennal depressions distinct, more or less parallel to anterior margin of tentacular segment.

Pharynx barely exposed (Fig. 2B). Basal ring not exposed. Distal margin with 14 papillae, four centrolateral ones twice larger than others, each papilla prismatic, tips globular, variably developed.

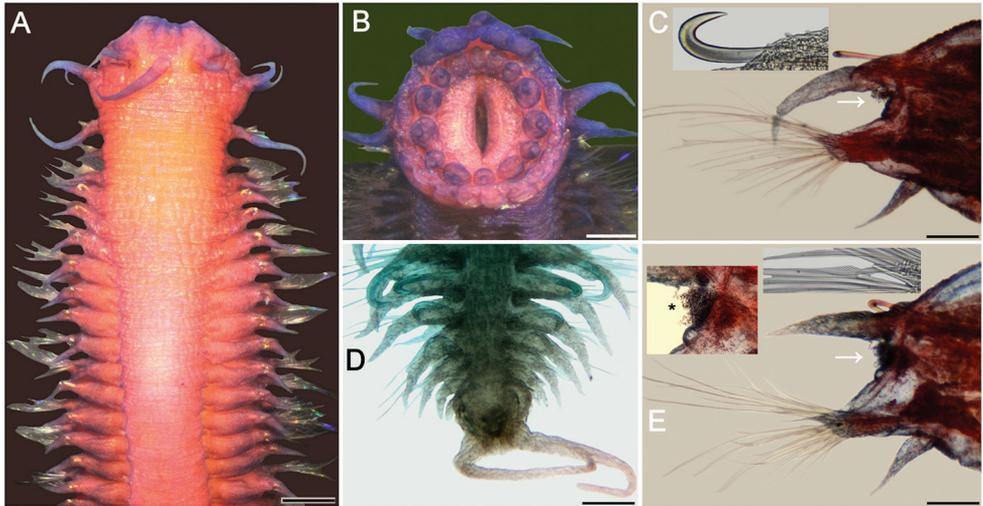
Tentacular segment three times wider than long; dorsal tentacular cirri slightly longer than ventral ones, approximately half as long as dorsal cirri of chaetiger 1.

Parapodial cirri tapered throughout body. Dorsal cirri tapered, not basally expanded, longer than ventral ones (Fig. 2C, D). Ventral cirri shorter than neurochaetal lobes in anterior chaetigers, as long as neurochaetal lobes in median and posterior chaetigers, missing on chaetiger 2. Prechaetal lobe truncate, not projected along its upper margin, postchaetal lobes long, acute. Anterior and median parapodia with hypertrophied gonopores, some showing abundant sperm (Fig. 2D, inset).

Notochaetae include dorsal hooks from chaetiger 4, heads of hooks fully exposed initially, progressively larger and exposing handles; one accessory capillary present from chaetiger 13. Neurochaetae include three or four short wide pectinates, approximately ten large thin pectinates, and many long finely denticulate capillaries.

Posterior region missing. In paratype tapered to a blunt cone (Fig. 2E). Pygidium laterally expanded, anus terminal, anal cirri ventrolateral, as long as last five or six chaetigers.

Oocytes not seen in parapodial spaces.



**Figure 2.** *Sigambra diazi* sp. nov. **A** holotype (ECOSUR 214), stained with Shirlastain-A, anterior region, dorsal view **B** same, frontal view of pharynx **C** Chaetiger 16, right parapodium, anterior view (arrow points to gonopore; inset: notohook) **D** Paratype (ECOSUR 215), stained with methyl green, posterior end, dorsal view **E** Chaetiger 40, right parapodium, anterior view (arrow points to gonopore; inset: hypertrophied gonopore with sperm, indicated with an asterisk, and central pectinate neurochaetae). Scale bars: 0.3 mm (**A**, **B**), 150  $\mu$ m (**C**), 0.1 mm (**D**), 90  $\mu$ m (**E**).

**Etymology.** This species is named after Oscar Díaz-Díaz, Venezuelan polychaete specialist, working now in Chile, as a modest homage to his many publications on polychaetes, and especially because he sampled and processed the specimens including this newly described species. The specific epithet is a noun in the genitive case (ICZN 1999, Art. 31.1.2).

**Variations.** The paratype was complete. It is 9.5 mm long with 70 chaetigers, but last three chaetigers are hookless. The median antenna is twice as long as the laterals, and the tentacular cirri reach chaetiger 2 or 3 as in the holotype. In the paratype the dorsal hooks start on chaetiger 4, as in the holotype, but although in the holotype the additional capillary is seen from chaetiger 13, in the paratype they are visible from chaetiger 24.

**Remarks.** *Sigambra diazi* sp. nov. resembles *S. tentaculata* (Treadwell, 1941) and it has been identified as such in previous studies, but the redescription by Moreira and Parapar (2002) of *S. tentaculata* helps for clarifying their differences. They differ in the relative shape of dorsal cirri, and in the size of ventral cirri in comparison to neurochaetal lobe. In *S. diazi* dorsal cirri are tapered, without basal enlargement, and ventral cirri in median and posterior chaetigers are as long as neurochaetal lobes, whereas in *S. tentaculata*, dorsal cirri are basally widened, and ventral cirri are shorter than neurochaetal lobes along body.

**Distribution.** Only known from the southern Caribbean coast of Venezuela, in shallow muddy bottoms.

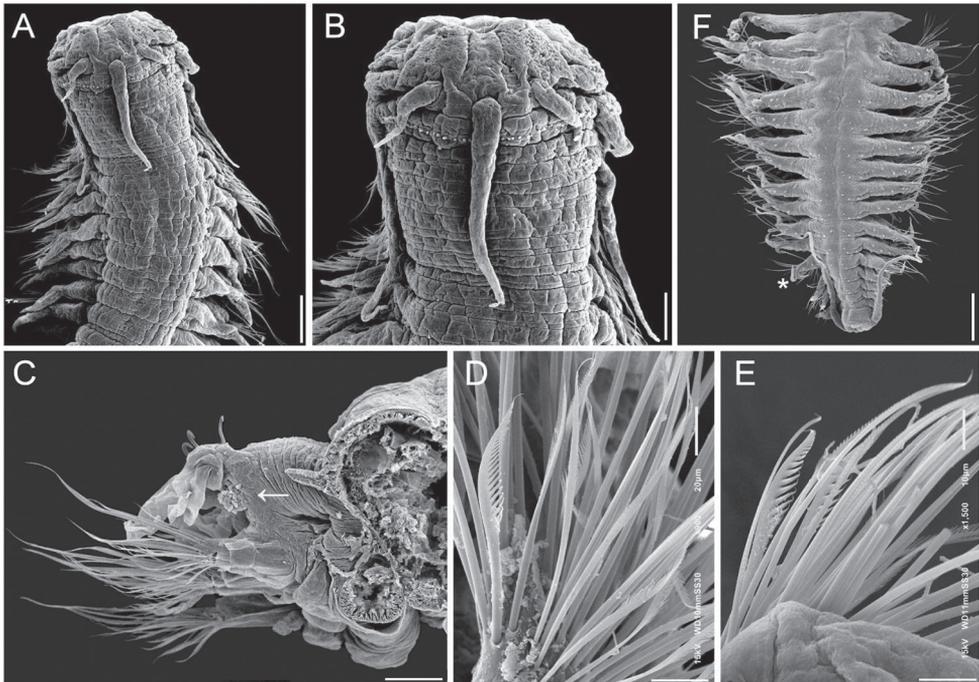
***Sigambra grubii* Müller, 1858**

Figs 3, 4

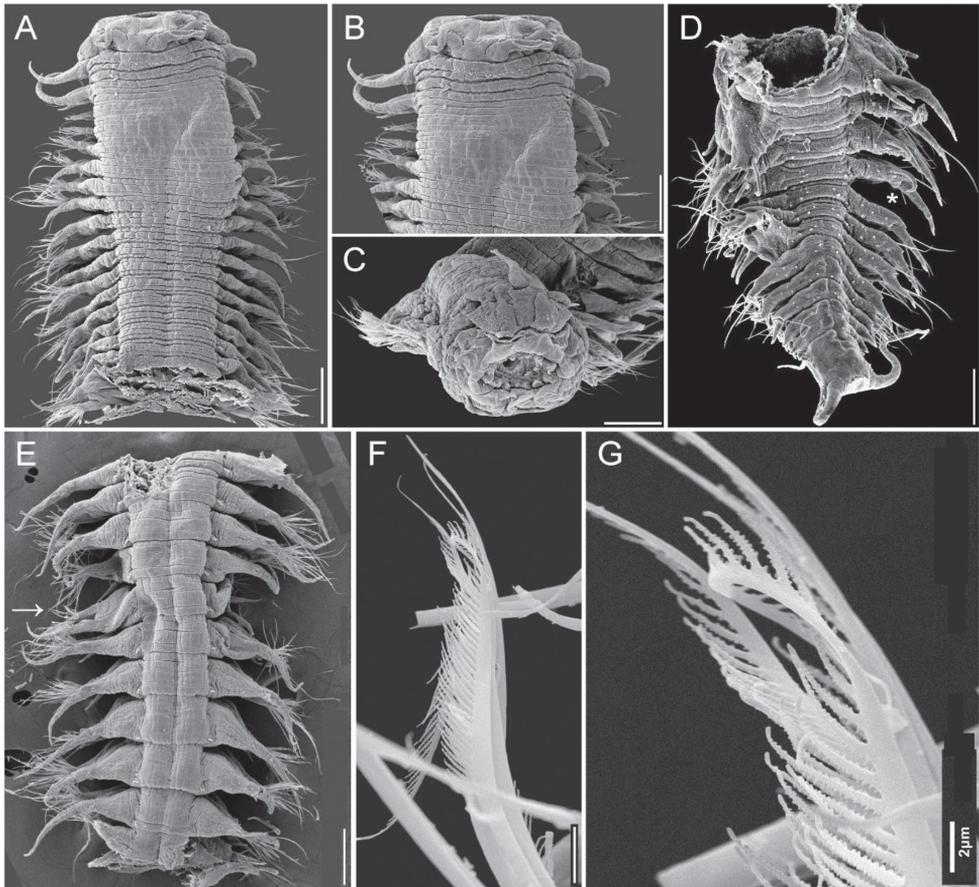
*Sigambra grubii* Müller, 1858: 214–215, pl. 6, figs 7–9; Pettibone 1966: 182, fig. 13a–c (reinstatement); Salazar-Vallejo 1990: 508–511, figs 1, 2, 4A–C, table 1 (redescription, key to species); Hartwich 1993: 104 (1 syntype).

*Sigambra grubei*: Licher and Westheide 1997: 3 (new orthography).

**Material examined. Brazil.** Pontal da Daniela (27°27'11"S, 48°31'47"W), in the Santa Catharina Island, Florianópolis. One specimen, 13RC1, July 2018 [mature female, anterior fragment (6.5 mm long, 0.9 mm wide, 54 chaetigers, first dorsal hooks from chaetiger 17)]. One specimen, S1R2, Feb. 2018 [anterior fragment (3.5 mm long, 0.5 mm wide, 27 chaetigers; first dorsal hooks from chaetiger 14)]. One specimen, S2R2C1, Feb. 2018 [anterior fragment (3.7 mm long, 0.8 mm wide, 26 chaetigers; first dorsal hooks from chaetiger 21)]. One specimen, S3R1C1, Feb. 2018 [postlarva (1 mm long, 0.2 mm wide, 13 chaetigers; first dorsal hooks from chaetiger 9)]. Four specimens for SEM (ECOSUR).



**Figure 3.** *Sigambra grubii* topotype, small specimen (ECOSUR SEM P1) **A** anterior region, dorsal view **B** same, close-up of anterior end **C** right parapodium, median chaetiger, of larger specimen (6.5 mm long), anterior view (arrow points to gonopore) **D** same, pectinate chaetae **E** same, another parapodium, pectinate chaetae **F** same, posterior region, dorsal view (asterisk indicates last chaetiger with notohooks). Scale bars: 200 µm (**A**, **F**), 110 µm (**B**), 100 µm (**C**), 22 µm (**D**), 12 µm (**E**).



**Figure 4.** *Sigambra grubii* topotypes, large specimen (ECOSUR SEM P1) **A** anterior region, dorsal view **B** same, close-up of anterior end **C** another specimen, anterior end, frontal view **D** same, posterior region (asterisk indicates last chaetiger with notohooks) **E** specimen in A, median chaetigers (arrow points enlarged chaetae) **F** pectinate chaetae **G** same, detail showing bipinnate denticulation. Scale bars: : 280  $\mu\text{m}$  (**A**), 250  $\mu\text{m}$  (**B**), 170  $\mu\text{m}$  (**C**), 110  $\mu\text{m}$  (**D**), 230  $\mu\text{m}$  (**E**), 8  $\mu\text{m}$  (**F**), 2  $\mu\text{m}$  (**G**).

**Description.** Prostomium blunt, two or three times wider than long (Figs 3A, B, 4A, B). Palpophores massive, palpostyles barely projected (Fig. 4C); interpalpal area distinct, anteriorly depressed, slightly expanded posteriorly. Antennae tapered, median one two or three times longer than laterals, laterals surpassing tips of palps, median antenna reaching chaetiger 6 or 7. Lateral antennal depressions indistinct.

Pharynx not exposed (Fig. 4C), with 14 thick, prismatic papillae, four lateral ones larger; tips indistinct. Basal pharynx apparently smooth.

Tentacular segment 4–5 times wider than long, with a single transverse row of globular tubercles, with two or three additional short middorsal series; dorsal tentacular cirri slightly longer than ventral ones, as long (Fig. 4A) or half as long (Fig. 3A, B) as dorsal cirri of chaetiger 1.

Parapodial cirri tapered throughout body. Dorsal cirri slightly expanded basally, longer than ventral ones. Ventral cirri as long as neurochaetal lobes in anterior and median chaetigers, longer in posterior ones, missing on chaetiger 2. Prechaetal lobes truncate, projected along its upper margin (Fig. 3C), postchaetal lobes long, acute. Anterior and median parapodia with hypertrophied gonopores, margin granulose (Fig. 3C, arrow).

Notochaetae include dorsal hooks from chaetiger 9–21 (size related), barely exposed initially, handles progressively exposed, without accessory capillaries. Neurochaetae include two or three supracicular shorter wider pectinates (Fig. 4F, G), sometimes with tiny paired denticles along each main tooth, approximately five infra-acicular narrower pectinates (Fig. 3D, E), and abundant finely denticulate capillaries (Fig. 3E).

Posterior region tapered into a truncate cone (Figs 3F, 4D); last 4–7 chaetigers without hooks. Pygidium with two ventrolateral anal cirri, as long as last eight chaetigers.

**Remarks.** As indicated in the key below, *Sigambra grubii* Müller, 1858 resembles *S. hernandezi* sp. nov. by having dorsal hooks without accessory capillaries. Their main differences are in the size of median antenna, and in the number of posterior chaetigers without hooks. In *S. grubii* the median antenna is medium-sized, reaching chaetiger 3 or 4, and there are 4–6 posterior chaetigers without hooks, whereas in *S. hernandezi* the median antenna is shorter, reaching chaetiger 2 or 3, and there are only two posterior hookless chaetigers. Another subtle difference is the relative size of papillae along body: which are larger in *S. grubii* compared to *S. hernandezi*.

**Distribution.** The species was described from Florianópolis, southern Brazil, and although it has been reported from a wide bathymetric range along the Brazilian coast, further investigations of this material might show the species to be restricted to shallow water sediments. An interesting fact about Ilha do Desterro (type locality), former name for Florianópolis is the origin of the name. Florianópolis was meant to be a tribute to Marshal Floriano Peixoto, the second President (1891–1894) of the Republic of the United States of Brazil, by combining the first name with the Greek term *polis*, meaning city. Up to 1893, the city was called Nossa Senhora do Desterro (Our Lady of Banishment) or simply Desterro.

***Sigambra hernandezi* sp. nov.**

<http://zoobank.org/366BBD1A-5C74-4AAC-B2E2-C01DAF53053C>

Figs 5–7

*Sigambra grubii*: Liñero-Arana and Díaz-Díaz 2005: 68, fig. 1 (**non** Müller 1858).

**Type material.** *Holotype* (ECOSUR 216), and *paratypes* (8) (ECOSUR 217), Northwestern Caribbean, Chetumal Bay, Quintana Roo, México, Cayo Venado (18°45'04.51"N, 88°06'58.81"W), sandy substrate, 1.5 m, 5 Aug. 2004, J.A. Hoil-Baeza, coll.

**Additional material.** **Gulf of México.** Tamiahua Lagoon, Veracruz, México, collected by J. A. de León González and M. E. García Garza. One specimen (UANL

4048), collapsed, 4 Nov. 1999 (6.5 mm long, 0.5 mm wide, 86 chaetigers, first dorsal hooks from chaetiger 24). Two specimens for SEM. One mature (UANL 4047), breaking into two parts, Sta. 1-09, 5 Nov. 1999 (11.5 mm long, 0.8 mm wide, 104 chaetigers, first dorsal hooks from chaetiger 14). Another one (UANL 5799), 23 Aug. 2002 (13 mm long, 0.9 mm wide, 103 chaetigers; first dorsal hooks from chaetiger 26). 131 specimens: One, Sta. T-02, 21°42'01"N, 97°35'54"W, 2.2 m. One, Sta. T-09, 21°36'10"N, 97°37'39"W, 2.5 m, 4 Nov. 1999. Three, Sta. T-02, 21°42'01"N, 97°35'54"W, 2.8 m, 7 Mar. 2000; Two, Sta. T-01, 21°42'01"N, 97°39'00"W, 2.8 m; Three, Sta. T-02, 21°42'01"N, 97°35'54"W, 2.8 m; three, Sta. T-04, 21°42'01"N, 97°32'52"W, 2.8 m; One, Sta. T-09, 21°36'10"N, 97°37'39"W, 3 m; one, Sta. T-10, 21°36'10"N, 97°34'45"W; one, Sta. T-11, 21°36'10"N, 97°32'09"W, 4 m; one, Sta. T-12, 4 m, 16 Aug. 2000; One, Sta. T-01, 21°42'01"N, 97°39'00"W, 3 m; three, Sta. T-02, 21°42'01"N, 97°35'54"W, 2.5 m; four, Sta. T-04, 21°42'01"N, 97°32'52"W, 1.5 m; three, Sta. T-05, 21°38'47"N, 97°39'13"W, 2 m; one, Sta. T-07, 21°38'47"N, 97°34'01"W, 3.5 m; two, Sta. T-08, 21°38'47"N, 97°31'14"W, 2.8 m; one, Sta. T-09, 21°36'10"N, 97°37'39"W, 2.5 m; one, Sta. T-10, 21°36'10"N, 97°34'45"W, 3.5 m; five, Sta. T-12, 21°36'10"N, 97°28'59"W, 3.8 m; four, Sta. T-13, 21°32'09"N, 97°36'04"W, 2 m; one, Sta. T-15, 21°32'09"N, 97°30'47"W, 2.5 m; one, Sta. T-16, 21°32'09"N, 97°27'19"W, 2.5 m, 25 Nov. 2000; four, Sta. T-01, 21°42'01"N, 97°39'00"W, 3.5 m; six, Sta. T-02, 21°42'01"N, 97°35'54"W, 2.5 m; one, Sta. T-05, 21°38'47"N, 97°39'13"W, 2.5 m; one, Sta. T-06, 21°38'47"N, 97°36'46"W, 3.8 m; six, Sta. T-07, 21°38'47"N, 97°34'01"W, 4 m; one, Sta. T-08, 21°38'47"N, 97°31'14"W, 2.5 m; five, Sta. T-09, 21°36'10"N, 97°37'39"W, 2 m; two, Sta. T-10, 21°36'10"N, 97°34'45"W, 3 m; five, Sta. T-11, 21°36'10"N, 97°32'09"W, 3.5 m; five, Sta. T-12, 21°36'10"N, 97°28'59"W, 3.5 m; eight, Sta. T-13, 21°32'09"N, 97°36'04"W, 2 m; four, Sta. T-14, 21°32'09"N, 97°32'48"W, 2.5 m; one, Sta. T-15, 21°32'09"N, 97°30'47"W, 2 m; one, Sta. T-16, 21°32'09"N, 97°27'19"W, 3 m, 12 Jul. 2001; three, Sta. T-01, 21°42'01"N, 97°39'00"W, 2.5 m; four, Sta. T-02, 21°42'01"N, 97°35'54"W, 2 m; two, Sta. T-03, 21°42'01"N, 97°34'19"W, 2.5 m; one, Sta. T-04, 21°42'01"N, 97°32'52"W, 1.8 m; two, Sta. T-05, 21°38'47"N, 97°39'13"W, 2 m; two, Sta. T-06, 21°38'47"N, 97°36'46"W, 2.2 m; three, Sta. T-07, 21°38'47"N, 97°34'01"W, 2.6 m; one, Sta. T-09, 21°36'10"N, 97°37'39"W, 2 m; one, Sta. T-10, 21°36'10"N, 97°34'45"W, 2.8 m; five, Sta. T-12, 21°36'10"N, 97°28'59"W, 3 m; one, Sta. T-13, 21°32'09"N, 97°36'04"W, 1 m; two, Sta. T-14, 21°32'09"N, 97°32'48"W, 2.5 m; one, Sta. T-15, 21°32'09"N, 97°30'47"W, 2 m; three, Sta. T-16, 21°32'09"N, 97°27'19"W, 2 m, 23 Feb. 2002. **Chetumal Bay, Q. Roo, México.** Two specimens (ECOSUR), N off Isla Tamalcab (18°38'30.45"N, 88°11'12.84"W), 1 m, sand, 1 Oct. 1996, P. Salazar-Silva, coll. (both complete, 9–10 mm long, 1.1–1.4 mm wide, 69–77 chaetigers; first dorsal hooks from chaetiger 22–28; two last chaetigers hookless). Three specimens (ECOSUR), Río Hondo (18°29'21.99"N, 88°18'32.97"W), sandy mud, 1.5 m, 3 Aug. 2004, J.A. Hoil-Baeza, coll. (anterior fragments, 3.0–3.5 mm long, 0.3–0.5 mm wide, 7–26 chaetigers; dorsal hooks from chaetiger 13–15). Seven specimens (ECOSUR), Punta Amainada (18°42'21.64"N, 88°09'12.36"W), sandy substrate,

1.5 m depth, 4 Aug. 2004, J.A. Hoil-Baeza, coll. (1.7–7.0 mm long, 0.2–0.4 mm wide, 29–68 chaetigers; dorsal hooks from chaetiger 10–32). Twelve specimens (ECOSUR), Cayo Venado (18°45'04.51"N, 88°06'58.81"W), sandy substrate, 1.5 m, 5 Aug. 2004, J.A. Hoil-Baeza, coll. (1.8–11.3 mm long, 0.2–0.8 mm wide, 25–63 chaetigers; dorsal hooks from chaetiger 11–28). Five specimens for SEM (ECOSUR), Cayo Venado, sandy substrate, 1.5 m, 5 Aug. 2004, J.A. Hoil-Baeza, coll. (1.7–6.5 mm long, 0.2–0.4 mm wide, 25–70 chaetigers; dorsal hooks from chaetiger 11–23). **Southwestern Caribbean, Venezuela.** Chacopata or El Maguey lagoons, seven specimens (ECOSUR), 2 m, 15 Feb. 2000, M. Liñero & O Díaz-Díaz, coll. (damaged, some without posterior region, antennae or tentacular cirri broken; smallest specimen with subdermal eyespots in bases of lateral antennae; 4.5–13.0 mm long, 1–2 mm wide, 56–102 chaetigers, first dorsal hooks from chaetiger 13–24).

**Diagnosis.** *Sigambra* with median antenna reaching chaetiger 2–3, twice as long as laterals; tentacular segment 4 times wider than long, anterior margin smooth; dorsal cirri larger than ventral ones; chaetiger 2 without ventral cirri; notopodia with dorsal hooks from chaetiger 4–5, without capillaries; posterior region with two hookless chaetigers; pharynx with 13–16 marginal papillae.

**Description.** Holotype (ECOSUR 216) slightly twisted along posterior region. Body contracted, cylindrical anteriorly, depressed medially and posteriorly, 9 mm long (paratypes 2.7–9.0 mm long), 2 mm wide (paratypes 0.6–2.0 mm wide), 76 chaetigers (paratypes with 33–82 chaetigers); right parapodia of chaetigers 19, 36, 37, and 62 removed for observing parapodial features. Dorsal integument smooth along chaetigers 1–9 (Figs 5A, 6A), areolate along chaetigers 10–22, smooth, annulated medially and posteriorly.

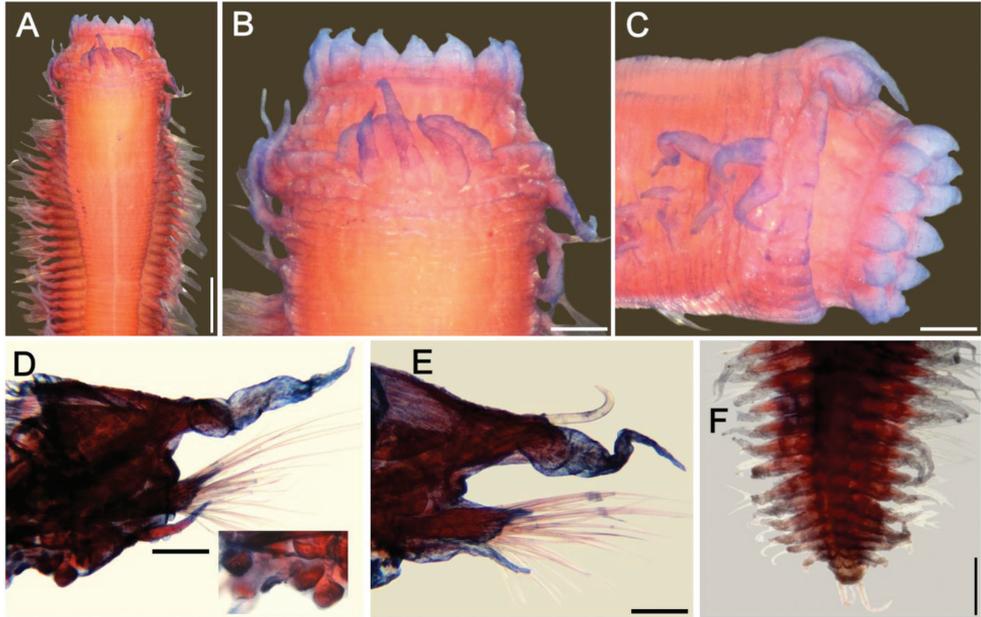
Prostomium blunt, 2.5 times wider than long (Fig. 5B). Palps with palpophores massive, as long as wide, palpostyles minute, short, barely exposed; interpalpal area indistinct. Antennae tapered, median antenna twice as long as laterals (smaller in smallest specimen, Fig. 6B, larger in largest specimen, Fig. 6F), laterals surpassing palps anterior margin, median antenna reaching chaetiger 2 or 3. Lateral antennal depressions indistinct.

Pharynx fully exposed (Figs 5B, C, 6D, J), with 14 thick, prismatic papillae of similar size, with a short, globular tip. Basal pharynx ring rugose, without distinct papillae.

Tentacular segment six times wider than long, with a single series of transverse globular papillae, barely duplicate or with a few middorsal papillae (Fig. 6G); dorsal tentacular cirri slightly longer than ventral ones, approx. half as long as dorsal cirri of chaetiger 1.

Parapodial cirri tapered throughout body. Dorsal cirri slightly expanded basally, longer than ventral ones. Ventral cirri as long as neurochaetal lobes in anterior and median chaetigers, longer in posterior ones (Fig. 5D, E), missing on chaetiger 2. Prechaetal lobes truncate, not projected along its upper margin, postchaetal lobes long, acute. Anterior and median parapodia with hypertrophied gonopores, margin smooth (Fig. 6F, H, I).

Some smaller features are worth mentioning after their observation with SEM. In anterior chaetigers (Fig. 7A) there are some small round papillae or tubercles in the upper, posterior surface of parapodia (Fig. 7B, C), but their tips have 2–5 long cilia, and they probably are sensory structures. The hypertrophied gonopore has a cover of globular smooth, external protuberances (Fig. 7B, D).



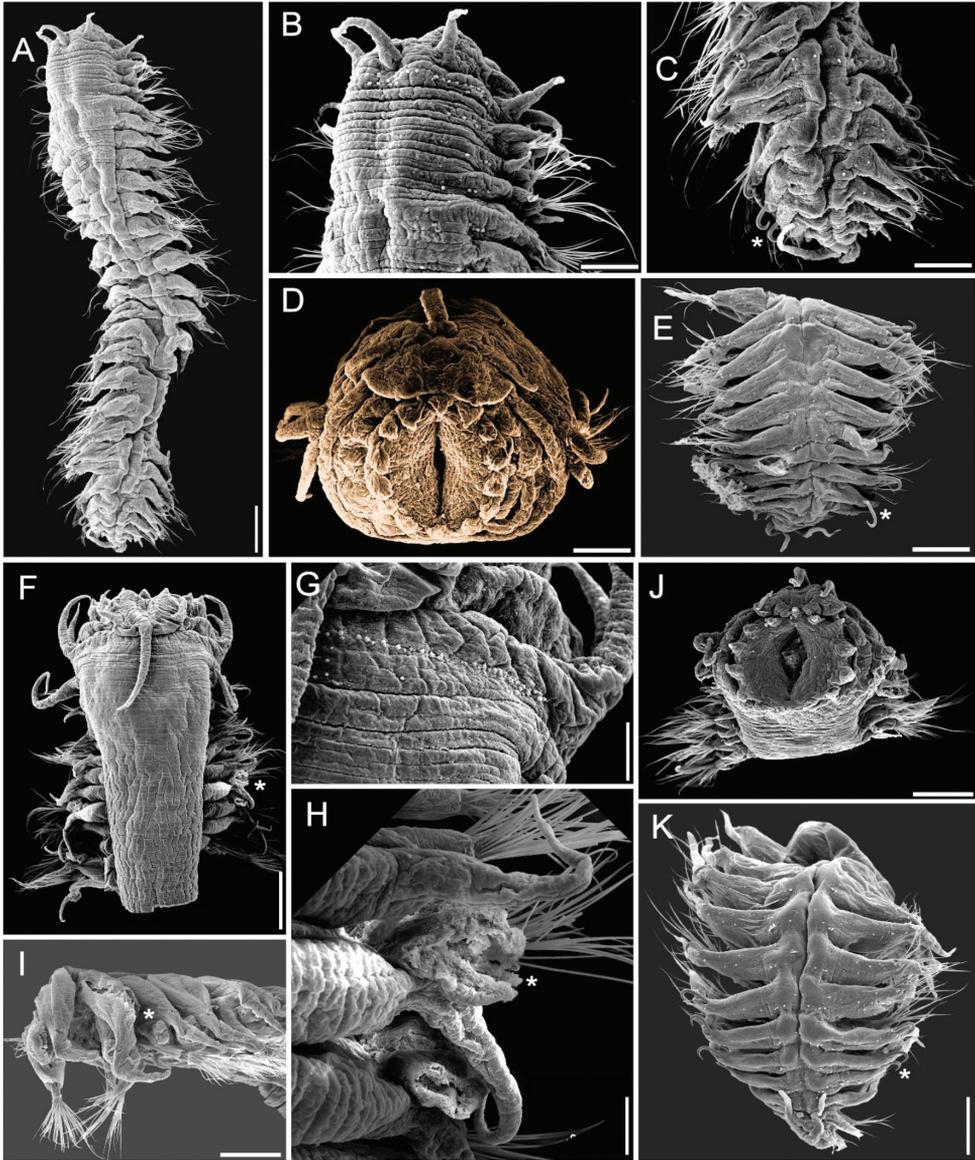
**Figure 5.** *Sigambra bernandezii* sp. nov., holotype (ECOSUR 216), stained with Shirlastain-A **A** anterior region, dorsal view **B** anterior end, dorsal view **C** same, right lateral view **D** chaetiger 19, right parapodium, posterior view (inset: close-up of oocytes exposed after body wall fracture) **E** chaetiger 62, right parapodium, posterior view **F** posterior region, dorsal view. Scale bars: 0.6 mm (**A**), 0.2 mm (**B**, **C**), 140  $\mu$ m (**D**), 80  $\mu$ m (**E**), 0.3 mm (**F**).

Notochaetae include dorsal hooks from chaetiger 28 (in paratypes from chaetigers 11–28), barely exposed initially, handles progressively exposed, without accessory capillaries. Neurochaetae include two or three supracicular, short wide pectinates, 4–6 infra-acicular narrow pectinates, and abundant, long, finely denticulate capillaries.

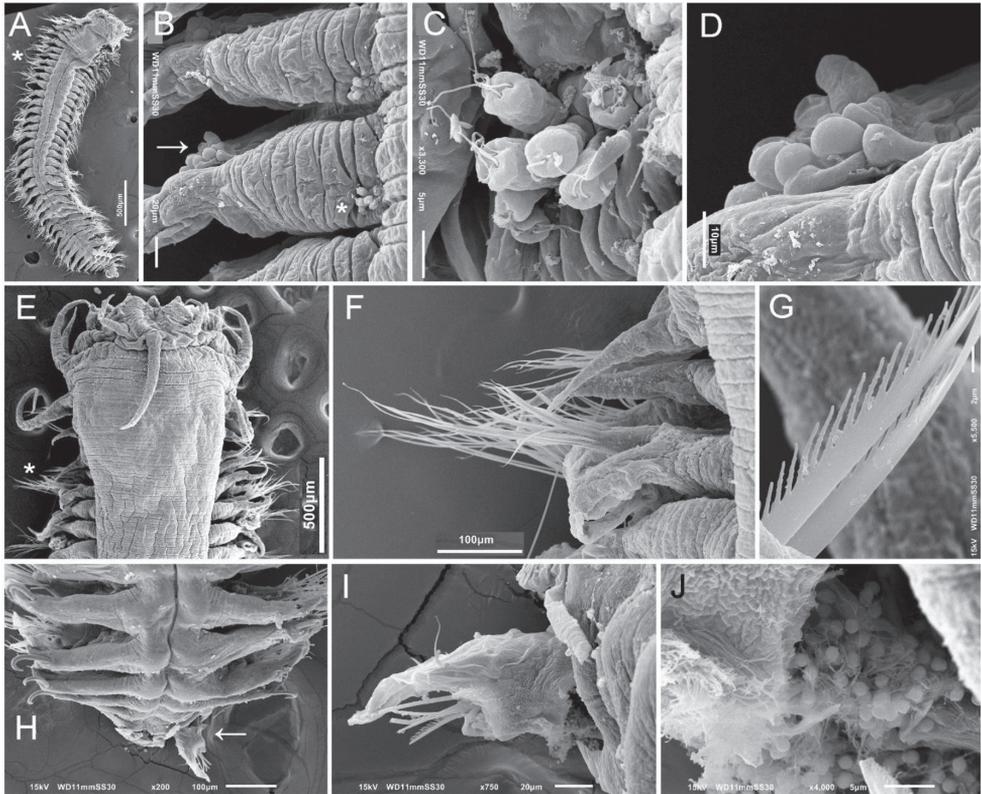
Posterior region (Figs 5F, 6C, E, K) tapered into a small blunt cone; last two chaetigers hookless. Pygidium with two ventrolateral anal cirri, as long as last 2–4 chaetigers.

Oocytes inside parapodial spaces (Fig. 5D, inset), ca. 100  $\mu$ m in diameter.

**Variation.** Specimens were 1.7–13.0 mm long, 0.2–2.0 mm wide, 25–104 chaetigers, with dorsal hooks starting on chaetiger 10–28 with a posterior displacement in larger specimens. The specimens from Tamiahua were slightly larger than those in Chetumal (up to 13 mm long, 104 chaetigers) but because the dorsal hooks start from chaetiger 24–26 whereas they start from chaetiger 14 in the smallest specimen (6.5 mm long), they are regarded as belonging to the same species as the one from Chetumal (up to 11.3 mm long, 76 chaetigers, dorsal hooks from chaetiger 26–28; specimens 6–7 mm long had dorsal hooks on chaetiger 19–26). There were no accessory capillaries with notohooks. The supracicular, wider pectinates (Fig. 7E–G) have small lateral, paired denticles, when compared to those present in *S. grubii*. Oocytes and sperm are present along coelom, but in the posterior region, there are mostly spermatids (Fig. 7H–J), each ca. 1.3  $\mu$ m in diameter.



**Figure 6.** *Sigambra hernandezi* sp. nov. **A** small specimen, dorsal view, body dehydrated **B** same, anterior end, oblique dorsal view **C** same, posterior region, dorsal view (asterisk indicates last chaetiger with hooks) **D** another specimen, anterior end, frontal view **E** another specimen, posterior region, dorsal view (asterisk indicates last chaetiger with hooks). largest specimen (UANL 5799) **F** Anterior region, dorsal view (asterisk indicates globular structures enlarged in **C**) **G** same, close-up of papillae series in tentacular segment **H** same, close-up of interramal globular structures, outer wall broken **I** left parapodia, chaetigers 11–16, oblique frontal view (asterisk indicates gonopore) **J** another specimen (UANL 4047), anterior end, oblique frontal view **K** same, posterior region, dorsal view (asterisk indicates last chaetiger with hooks). Scale bars: 150  $\mu\text{m}$  (**A**), 80  $\mu\text{m}$  (**B**, **C**), 140  $\mu\text{m}$  (**D**), 170  $\mu\text{m}$  (**E**), 330  $\mu\text{m}$  (**F**), 90  $\mu\text{m}$  (**G**), 60  $\mu\text{m}$  (**H**), 200  $\mu\text{m}$  (**I**), 220  $\mu\text{m}$  (**J**), 140  $\mu\text{m}$  (**K**).



**Figure 7.** *Sigambra hernandezii* sp. nov. **A** larger specimen, Chetumal, dorsal view (asterisk indicates left chaetigers 8–9) **B** same, chaetigers 8–9, left parapodia, partial dorsal view (asterisk indicates cilia, arrow points to gonopore) **C** chaetiger 9, left parapodium, axillary ciliated papillae, seen from above **D** same, gonopore papillae, seen from above **E** large specimen, Tamiahua, anterior region, dorsal view, asterisk indicates left parapodia of chaetigers 3–4 **F** same, close-up of chaetae, seen from above **G** same, rotated, details of denticles in pectinate chaeta **H** another specimen, posterior region, dorsal view (arrow points to broken posterior chaetiger **I** same, rotated about 90 degrees, showing inner contents **J** same, spermatozoa. Scale bars inserted in micrographs.

**Etymology.** This species is named after Dr. Héctor A. Hernández-Arana, quantitative benthic ecologist in ECOSUR-Chetumal, in recognition of his many studies on brackish water environments in southeastern México, and especially because he led the research study where Chetumal Bay specimens were collected. The specific epithet is a noun in the genitive case (ICZN 1999, Art. 31.1.2).

**Remarks.** *Sigambra hernandezii* sp. nov. resembles *S. grubii* Müller, 1858 because they have dorsal hooks without capillaries. They differ especially in the relative size of median antenna, and in the number of posterior chaetigers without hooks. In *S. hernandezii* the median antenna is short, reaching chaetiger 2–3, and there are two hookless chaetigers in the end of body, whereas in *S. grubii* the median antenna is medium sized, reaching chaetiger 3–4, and there are six hookless posterior chaetigers. Another subtle difference is the relative size of papillae along body, which are smaller in *S. hernandezii* and larger in *S. grubii*.

In Tamiahua Lagoon, *S. hernandesi* was an important member of the colonizing benthic polychaetes, being rare after defaunation and progressively becoming one of the more abundant species (Sánchez-Hernández 2009), along a wide range of salinity (5–30‰). In Chetumal Bay, *S. hernandesi* was not among the most abundant species (Hoil-Baeza 2009), and it was common in brackish water seagrasses (*Halodule* sp.). The specimens included in this study were collected along the western shore of Chetumal Bay, which has the lowest salinity values (Carrillo et al. 2009). The specimens from Venezuela are included here with hesitation because they are in suboptimal conditions, but they have a similar pattern in parapodial development and start of the dorsal hooks.

**Distribution.** Grand Caribbean region, from Tamiahua Lagoon in northern Veracruz, México, to Chetumal Bay, Quintana Roo, in sediments in shallow brackish water; probably reaching the southeastern Caribbean in similar environments.

***Sigambra ligneroi* sp. nov.**

<http://zoobank.org/8AE4464D-22F6-45E8-AD25-3337E85BC40F>

Fig. 8

*Sigambra wassi*: Liñero-Arana and Díaz-Díaz 2005: 69–70, fig. 3 (**non** Pettibone 1966).

**Type material. Holotype** (ECOSUR 218), southern Caribbean, Venezuela. 15 km west off Barcelona (10°06'50"N, 64°51'20"W), dredge, 22 m, 30 May 2000, I. Liñero-Arana & O. Díaz-Díaz, coll.

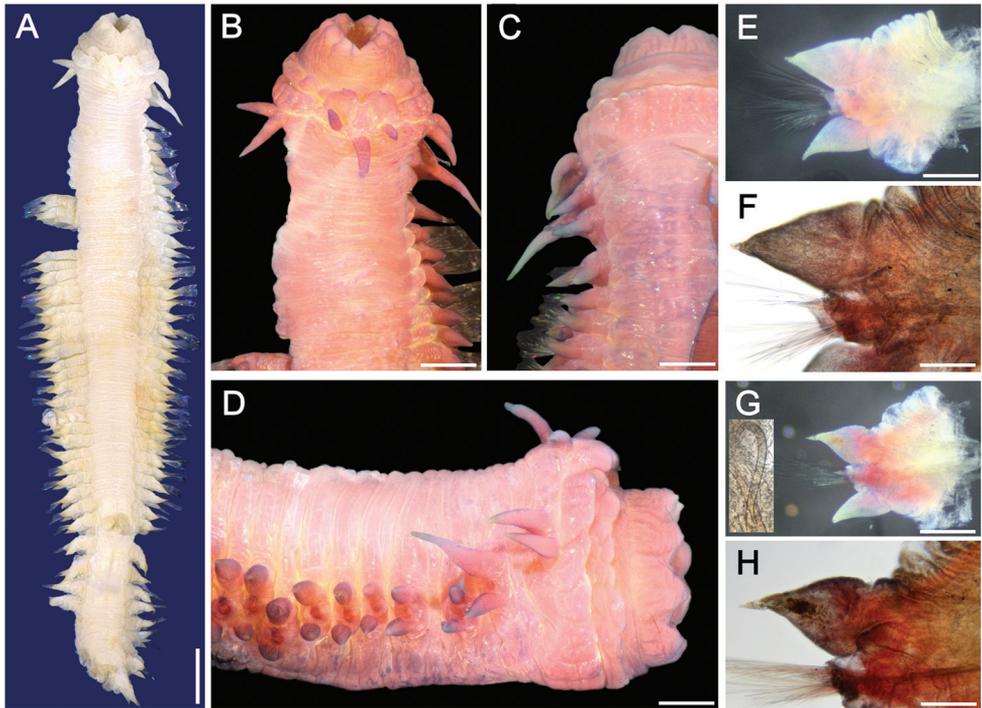
**Diagnosis.** *Sigambra* with median antenna twice as long as laterals, reaching chaetiger 1–2; dorsal cirri larger than ventral cirri; chaetiger 2 with ventral cirri; dorsal hooks from chaetiger 26–28, without capillaries; pharynx with 8 marginal papillae.

**Description.** Holotype (ECOSUR 218), anterior fragment, bent dorsally, slightly damaged (Fig. 8A). Body contracted, cylindrical anteriorly, depressed medially and posteriorly, 17 mm long, 4 mm wide (excluding chaetae), 49 chaetigers. Dorsal integument rugose, segment margins better defined along first 8 chaetigers, then crenulated along medial and posterior segments. Left parapodia of chaetigers 1–9, 13, 14, and right parapodia of chaetigers 45–49 previously removed. Right parapodia of chaetigers 16 and 36 removed for observing parapodial features.

Prostomium blunt, three times wider than long (Fig. 8B). Palps with palpophores massive, as long as wide, palpostyles tiny, directed laterally; interpalpal area distinct, truncate anteriorly, expanded posteriorly. Antennae digitate, median antenna twice as long as laterals, laterals not reaching tips of palps, median antenna reaching chaetiger 1–2. Lateral antennal depressions indistinct.

Pharynx exposed with two rings (Fig. 8D). Basal ring rugose, with ca. 22 globular projections, better defined dorsally and laterally, basally shorter. Distal ring with 8 short, wide, conical papillae, arranged as 4 per side.

Tentacular segment five or six times wider than long; dorsal tentacular cirri slightly longer than ventral ones, approx. half as long as dorsal cirri of chaetiger 1.



**Figure 8.** *Sigambra ligneroi* sp. nov., holotype (ECOSUR 218) **A** dorsal view **B** anterior region, dorsal view, after Shirlastain-A staining **C** same, ventral view **D** same, right lateral view **E** chaetiger 16, right parapodium, anterior view **F** same, dorsal cirrus and chaetal lobes **G** chaetiger 36, right parapodium, anterior view (inset: notohook) **H** same, dorsal cirrus and chaetal lobes. Scale bars: 1.4 mm (**A**), 1 mm (**B**), 1.7 mm (**C**), 1.6 mm (**D**), 0.3 mm (**E**, **G**), 180  $\mu$ m (**F**), 250  $\mu$ m (**H**).

Parapodial cirri tapered along chaetigers 1–4 (5), foliose, longer than wide thereafter; dorsal cirri longer than ventral ones, triangular along anterior chaetigers (Fig. 8E, F), basally wider in posterior ones (Fig. 8G, H), tips long, with globular brownish glands concentrated subdistally, especially along posterior chaetigers. Ventral cirri longer than neurochaetal lobes. Chaetiger 1 with dorsal cirri at least twice as long as ventral ones (Fig. 8C, D). Chaetiger 2 with dorsal cirri slightly longer than ventral ones. Prechaetal lobes rounded, more projected along its upper margin; postchaetal lobes long, acute.

Notochaetae include dorsal hooks from chaetiger 27, barely exposed, without accessory capillaries. Neurochaetae of two types: smaller and medium-sized fine denticulates and long smooth capillaries. No pectinate chaetae present.

Posterior region features unknown. Oocytes not seen.

**Variation.** A larger specimen was recorded by Liñero-Arana and Díaz-Díaz (2005), and they also provided several illustrations, probably combining some features of both specimens. Their largest specimen was also an anterior fragment but twice as large and with more chaetigers (33 mm long, 5 mm wide, ca. 90 chaetigers). All body features match and they also included an illustration of the far posterior chaetigers; parapodial

cirri are tapered, about twice as long as wide, dorsal hooks become more exposed and there is an additional chaeta, thick, slightly falcate, which might correspond to an acicula, instead of a capillary.

**Etymology.** The species name is after Dr. Ildefonso (Mikel) Liñero-Arana, polychaete specialist from the Universidad de Oriente, Instituto Oceanográfico de Venezuela, Cumaná, in recognition of his many publications about Venezuelan polychaetes. The specific epithet is a noun in the genitive case (ICZN 1999, Art. 31.1.2).

**Remarks.** *Sigambra ligneroi* sp. nov. resembles *S. wassi* Pettibone, 1966 in having similar body, parapodia and prostomial shapes, such that the holotype plus another specimen were identified as the latter species. They differ, however, in several features, the most important ones being the relative size of antennae, the length of parapodial cirri on chaetiger 1, and the number of marginal pharyngeal papillae, or at least their shape. In *S. ligneroi* the median antenna is twice as long as laterals, dorsal cirri are three times longer than ventral one on chaetiger 1, and there are eight regular papillae on the pharynx. By comparison, in *S. wassi* antennae are subequal, dorsal cirri are twice as long as the ventral ones (cf. Wolf 1984: 29.7, fig. 29.4c), and there are ca. eight irregularly-defined papillae on the pharynx.

As indicated in the key below, *S. ligneroi* also resembles *S. healyae* Gagaev, 2008 because both have ventral cirri on chaetiger 2, and their pharynx has 8 papillae. They differ by the relative size of antennae, and in the start of dorsal hooks; in *S. ligneroi* median antenna is twice as long as laterals, and dorsal hooks start on chaetiger 26–28, whereas in *S. healyae* antennae are subequal and dorsal hooks start on chaetiger 4.

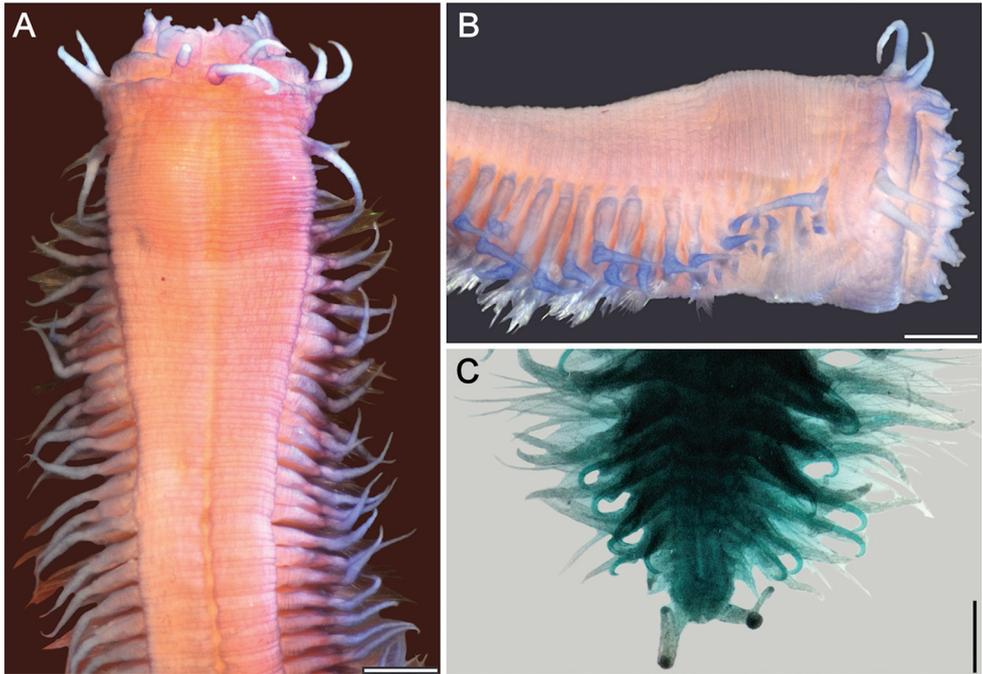
**Distribution.** Only known from the type locality, off Barcelona, Venezuela, in sediments of water depths of 22 m.

***Sigambra olivai* sp. nov.**

<http://zoobank.org/EF7981AA-9A3A-4BF2-98D1-B324ECE2C478>

Figs 9, 10

**Type material.** **Holotype** (ECOSUR 219), Northwestern Caribbean, México, Nichupté Lagoon, NW sector, *Halodule*, Sta. 1 (21°08'55.60"N, 86°47'51.29"W), 1.5 m, 30 Oct 1987, M.S. Jiménez & J.J. Oliva, coll. **Paratypes (9)**: One (ECOSUR 220), Nichupté Lagoon, NW sector, *Halodule*, Sta. 1 (21°08'55.60"N, 86°47'51.29"W), 1.5 m, 30 Oct 1987, M.S. Jiménez & J.J. Oliva, coll. One (ECOSUR 221), Nichupté Lagoon, NW sector, *Halodule*, Sta. 1 (21°08'55.60"N, 86°47'51.29"W), 1.5 m, 22 Apr. 1988, M.S. Jiménez & J.J. Oliva, coll. One (ECOSUR 222), Nichupté Lagoon, NE sector, Bojórquez Lagoon, *Halodule*, Sta. 2 (21°07'58.38"N, 86°45'10.39"W), 1.5 m, 27 Oct. 1987, M.S. Jiménez & J.J. Oliva, coll. One (ECOSUR 223), Nichupté Lagoon, NE sector, Bojórquez Lagoon, *Halodule*, Sta. 2 (21°07'58.38"N, 86°45'10.39"W), 1.5 m, 1 Feb. 1988, M.S. Jiménez & J.J. Oliva, coll. Two (ECOSUR 224), Nichupté Lagoon, NE sector, Bojórquez Lagoon, *Halodule*, Sta. 2 (21°07'58.38"N, 86°45'10.39"W), 1.5 m, 5 Jul. 1988, M.S. Jiménez & J.J. Oliva, coll. One (ECOSUR 225), Nichupté Lagoon, NE



**Figure 9.** *Sigambra olivai* sp. nov., holotype (ECOSUR 219), stained with Shirlastain-A **A** anterior region, dorsal view **B** anterior region, lateral view **C** posterior end, dorsal view, stained with Methyl green. Scale bars: 1.0 mm (**A**), 0.4 mm (**B**), 0.2 mm (**C**).

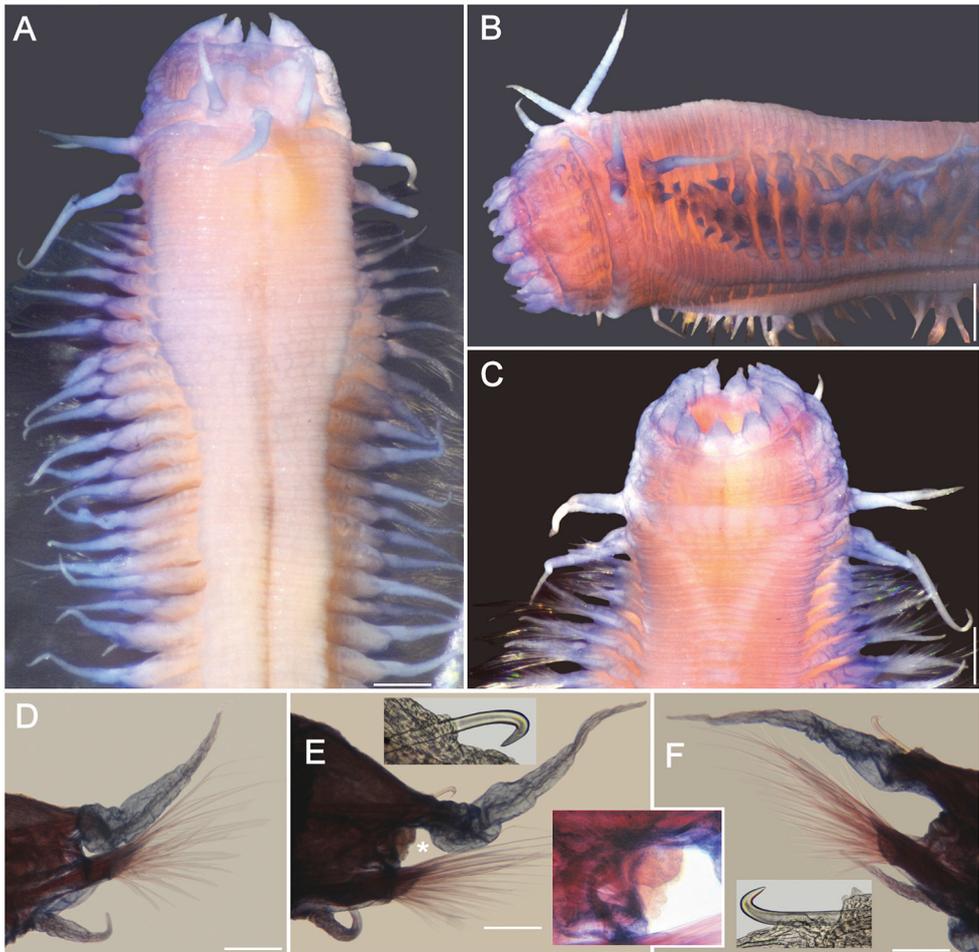
sector, Bojórquez Lagoon, *Halodule*, Sta. 2 (21°07'58.38"N, 86°45'10.39"W), 1.5 m, 5 Jul. 1988, M.S. Jiménez & J.J. Oliva, coll. Two (ECOSUR 226), Nichupté Lagoon, NE sector, Bojórquez Lagoon, *Thalassia*, Sta. 3 (21°07'01.24"N, 86°45'41.01"W), 1.5 m, 20 Abr. 1988, M.S. Jiménez & J.J. Oliva, coll.

**Additional material. Northwestern Caribbean, México.** One specimen (ECOSUR), Nichupté Lagoon, NE sector, Bojórquez Lagoon, *Thalassia*, Sta. 3 (21°07'01.24"N, 86°45'41.01"W), 1.5 m, 29 Oct. 1987, M.S. Jiménez & J.J. Oliva, coll.

**Diagnosis.** *Sigambra* with median antenna twice as long as laterals; chaetiger 2 without ventral cirri; dorsal cirri larger than ventral ones; dorsal hooks from chaetiger 30–39, without capillaries; posterior chaetigers without capillary notochaetae; pharynx with 13–16 marginal papillae.

**Description.** Holotype (ECOSUR 219) twisted, broken into two pieces, larger fragment plus posterior end, some median parapodia with hypertrophied gonopores. Body contracted, cylindrical anteriorly, depressed medially and posteriorly, 20 mm long (anterior fragment 14 mm long, posterior one 6 mm long), 2.8 mm wide, 152 chaetigers (90 + 62). Dorsal integument rugose, areolate, especially after chaetigers 6–7 (Figs 9A, 10A). Parapodia removed from paratype.

Prostomium blunt, four times wider than long. Palps with palpophores massive, as long as wide, palpostyles blunt, short, with an oblique basal mark; interpalpal area dis-



**Figure 10.** *Sigambra olivai* sp. nov., paratype (ECOSUR 221), stained with Shirlastain-A **A** anterior region, dorsal view **B** same, left, slightly oblique lateral view **C** same, anterior end, ventral view **D** another paratype (ECOSUR 226, complete), chaetiger 30, right parapodium, posterior view **E** same, chaetiger 35, right parapodium, posterior view; asterisk indicates the gonopore (insets: notohook and close-up of interramal gonopore) **F** same, chaetiger 90, right parapodium, anterior view (inset: notohook). Scale bars: 0.3 mm (**A–C**), 0.2 mm (**D**), 150  $\mu$ m (**E, F**).

tinct, blunt anteriorly, expanded posteriorly. Antennae tapered, median antenna twice as long as laterals (left lateral antenna broken), laterals surpassing tips of palps, median antenna reaching chaetiger 2 or 3. Lateral antennal depressions indistinct.

Pharynx with distal ring exposed (Fig. 9A, B), with 15 papillae of similar size, each conical with a globular mucron (rarely duplicated). Basal pharynx ring exposed in one paratype (Fig. 10A–C), with 3–5 series of short, globular papillae, better visible laterally.

Tentacular segment eight or nine times wider than long; dorsal tentacular cirri slightly longer than ventral ones (Figs 9B, 10B, C), about half as long as dorsal cirri of chaetiger 1.

Parapodial cirri tapered throughout body. Dorsal cirri basally expanded, longer than ventral ones. Ventral cirri as long as neurochaetal lobes in anterior and median chaetigers, longer in posterior ones (Fig. 10D–F), missing on chaetiger 2. Prechaetal lobes truncate, slightly projected along its upper margin, postchaetal lobes long, acute. Median parapodia with hypertrophied gonopores, showing distinct globular, papillate or smooth surfaces (Fig. 10E, inset).

Notochaetae only dorsal hooks from chaetiger 38, barely exposed initially, more projected posteriorly, without accessory capillaries. Neurochaetae include two or three supracicular shorter wide pectinates, 4–5 infra-acicular narrow pectinates, especially along anterior and median segments, and abundant, long finely denticulate capillaries.

Posterior region tapered into a small blunt cone; last two chaetigers hookless. Pygidium with two ventrolateral anal cirri (Fig. 9C).

Oocytes inside parapodial spaces, ca. 100  $\mu\text{m}$  in diameter.

**Variation.** Complete specimens 15–24 mm long, 128–150 chaetigers. Antennae are easily eroded during sieving, but in undamaged specimens the median one is twice as long as lateral ones. Dorsal hooks start in chaetigers 30–39, apparently a size-dependent variation. There are no capillary chaetae with dorsal hooks in posterior chaetigers; last two have no hooks.

**Etymology.** This species is named after José Juan Oliva-Rivera, amphipod taxonomist in ECOSUR, in recognition of his efforts sampling and processing benthic invertebrates from Nichupté Lagoon, Cancún, México. The specific epithet is a noun in the genitive case (ICZN 1999, Art. 31.1.2).

**Remarks.** *Sigambra olivai* sp. nov. resembles *S. constricta* (Southern, 1921) by having median antenna twice as long as the laterals, and dorsal hooks from chaetigers 30–40. They especially differ in the presence of a constriction on chaetiger 4, and of capillary notochoetae in posterior chaetigers. In *S. olivai* there is no constriction on chaetiger 4, and there are no capillaries in posterior chaetigers, whereas in *S. constricta* the body has a constriction on chaetiger 4, and there is a single capillary notochoetae in posterior chaetigers.

**Distribution.** Only known from Nichupté Lagoon, Cancún, México, in seagrasses, mostly *Halodule* sp., in sediments of about 1 m depth.

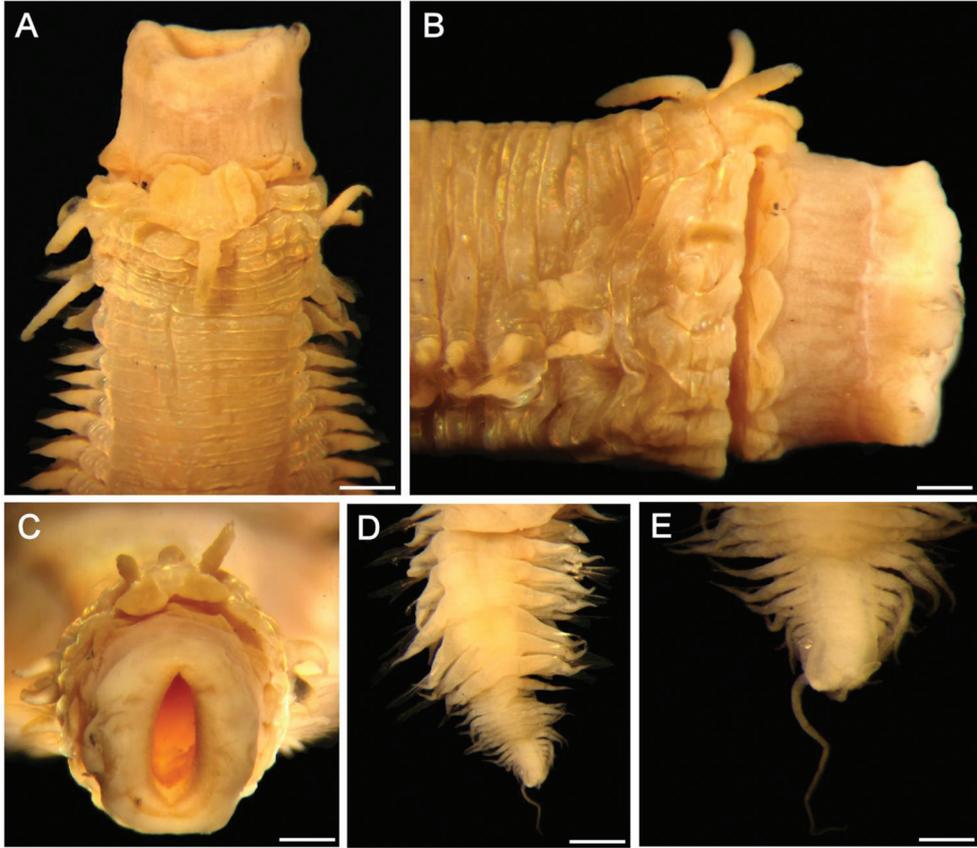
### *Sigambra wassi* Pettibone, 1966

Fig. 11

*Sigambra wassi* Pettibone, 1966: 186–190, figs 17,18; Wolf 1984: 29-8, fig. 29-4a–j.

**Type material.** *Holotype* (USNM 30988), NW Atlantic, Chesapeake Bay, off Rappahanock River (37°37.3'N, 75°59'W), 11 m, sand, Jun. 1962, M. Wass, coll., incomplete posteriorly. *Paratype* (USNM 30987), off Rappahanock River (37°37'N, 76°11'W), 13 m, mud, Jul. 1961, M. Wass, coll., broken and in three pieces.

**Clarification.** The original illustrations and descriptions deserve some clarifications. Antennae are certainly of about the same length, but they are not tapered but digitate, and surpass the tips of palps, at least the right one (Fig. 11A). The pharynx is



**Figure 11.** *Sigambra wassi* Pettibone, 1966 **A** holotype (USNM 30988), anterior region, dorsal view **B** same, right lateral view **C** same, frontal view **D** paratype (USNM 30987), posterior region, dorsal view **E** same, posterior end. Scale bars: 0.6 mm (**A, C**), 0.9 mm (**B**), 0.5 mm (**D**), 0.2 mm (**E**).

fully exposed, and the basal ring has about 20 irregular hemispherical lobes, but the anterior margin is eroded and damaged (Fig. 11B), such that its marginal papillae are difficult to detect; there are vague indications of the presence of approx. eight large papillae (Fig. 11C). They would include some round projections which might represent the eroded base of papillae, or that they were collapsed when the specimen was compressed into the container.

The posterior end is twisted in the paratype. It had to be pressed by a glass slide in order to have a better observation of the pygidium and anal cirri (Fig. 11D). Anal cirri are delicate, three times longer than pygidial width and tapered (Fig. 11E), not subcylindrical as originally illustrated.

**Remarks.** After the study of type material, the number of marginal papillae is not defined for *S. wassi* Pettibone, 1966. Their number might be the same as in *S. ligneroi* sp. nov. described above, but better specimens are needed to clarify this. On the other hand, most diagnostic features for the species were confirmed by Wolf (1984), the fragility of anal cirri might explain why they were not observed by him.

**Key to species of *Sigambra* Müller, 1858\***

(modified after Salazar-Vallejo 1990 and Licher and Westheide 1997)

- 1 Dorsal cirri larger than ventral cirri ..... **2**  
 – Dorsal and ventral cirri subequal..... **20**
- 2 Chaetiger 2 without ventral cirri ..... **3**  
 – Chaetiger 2 with ventral cirri ..... **21**
- 3 Pharynx with 8 marginal papillae ..... **4**  
 – Pharynx with 13–16 marginal papillae..... **7**
- 4 Dorsal hooks from chaetigers 3–8 ..... **5**  
 – Dorsal hooks from chaetigers 15–17 (5–18 mm long; up to chaetiger 30?); median and posterior notopodia with 1 capillary; median antenna long, reaching chaetiger 7 ..... ***S. vargasi* Dean, 1999 (Pacific Costa Rica)**
- 5 Median and posterior notopodia with capillaries..... **6**  
 – Notopodia without capillaries; all neurochaetae with tips entire; median antennae reaching chaetiger 5 (17–23 mm long).....  
 ..... ***S. papagayu* Bamber in Muir and Bamber 2008 (Hong Kong)**
- 6 Some neurochaetae with bifid tips; median antenna barely longer than laterals, reaching chaetiger 2; median and posterior notopodia with 2 capillaries.....  
 ..... ***S. bidentata* Britayev & Saphronova, 1981 (Sea of Japan)**
- All neurochaetae with tips entire; median antenna markedly longer than laterals, reaching chaetiger 3; median and posterior notopodia with 1 capillary.....  
 ..... ***S. qingdaoensis* Licher & Westheide, 1997 (Yellow Sea)**
- 7 Dorsal hooks from anterior chaetigers (4–18) ..... **8**  
 – Dorsal hooks from median chaetigers (30–40); median antenna twice as long as lateral ones, or longer ..... **19**  
 – Dorsal hooks from posterior chaetigers (42–66), or beyond that (14 mm long); median antenna as long as lateral ones, barely reaching chaetiger 1 .....  
 ..... ***S. rugosa* Fauchald, 1972 (Western México)**
- 8 Tentacular segment about twice wider than long..... **9**  
 – Tentacular segment 4 times wider than long ..... **10**
- 9 Tentacular segment with anterior margin with rounded projected lobes, external to lateral antennae..... ***Sigambra* sp. indet. (Brazil (AER))**  
 – Tentacular segment with anterior margin smooth, without projected lateral lobes; dorsal hooks from chaetiger 3–4 (14 mm long).....  
 ..... ***S. setosa* Fauchald, 1972 (Western México)**

\* The key includes two undescribed species being recorded with other names, or not completely identified, but in both cases, they are distinct and deserve to be described.

- 10 Tentacular segment with rounded projected lobes in anterior margin; median antenna slightly longer than lateral ones, reaching chaetiger 3–4; dorsal hooks from chaetiger 4–5 (6–12 mm long).....***S. parva* (Day, 1963) (South Africa)\*\***  
 – Tentacular segment with anterior margin smooth, without rounded projected lobes ..... **11**
- 11 Median and posterior notopodia with capillaries..... **12**  
 – Notopodia without capillaries ..... **18**
- 12 Median antenna short, reaching up to chaetigers 3–4..... **13**  
 – Median antenna medium-sized, reaching chaetigers 5–7 ..... **14**  
 – Median antenna long, reaching chaetigers 9–12; dorsal hooks from chaetigers 11–15 (40 mm long); lateral antennae without lateral depressions .....  
 ..... ***S. bassi* sensu Blake, 1994 (California)**
- 13 Dorsal hooks from chaetiger 4 (15 mm long); median antenna slightly longer than laterals; first dorsal cirri slightly longer than dorsal tentacular ones.....  
 ..... ***S. tentaculata* sensu Blake, 1994 (NE Pacific)**  
 – Dorsal hooks from chaetiger 12–18 (16 mm long); median antenna twice as long as laterals; first dorsal cirri markedly longer than dorsal tentacular ones.....  
 ..... ***S. elegans* Britayev & Saphronova, 1981 (Sea of Japan)**
- 14 Median antenna slightly longer than lateral ones, reaching chaetiger 4–6; dorsal hooks from chaetiger 7–10 (5.5 mm long) .....  
 ..... ***S. pettiboneae* Hartmann-Schröder, 1979 (NW Australia)**  
 – Median antenna twice as long as lateral ones ..... **15**
- 15 Median antenna reaching chaetiger 7–8..... **16**  
 – Median antenna reaching up to chaetiger 5–6; dorsal hooks from chaetiger 4–5.  
 ..... **17**
- 16 Dorsal hooks from chaetiger 3–9 (5–20 mm long) .....  
 .... ***S. hanaokai* (Kitamori, 1960) (Seto Island, Japan), Reinst. Nishi et al. 2007**  
 – Dorsal hooks from chaetiger 12–18 (24 mm long) .....  
 ..... ***S. bassi* (Hartman, 1947) (Florida\*\*\*)**
- 17 All parapodia with ventral cirri shorter than neuropodial lobes; dorsal cirri basally wider.....  
 ..... ***S. tentaculata* (Treadwell, 1941) (NW Atlantic), Moreira and Parapar 2002**  
 – Median and posterior parapodia with ventral cirri long, reaching tip of neuropodial lobes; dorsal cirri tapered, not wider basally.....***S. diazi* sp. nov. (southern Caribbean, Venezuela\*\*\*\*)**

\*\* Redescr. Moreira and Parapar 2002 (including *S. cf. tentaculata* Katzmann et al., 1974 *vide* Moreira and Parapar 2002)

\*\*\* N.C. specimens, after Gardiner 1976: 122, fig. 9d, have shorter median antenna

\*\*\*\* cf. *S. bassi* sensu Licher and Westheide 1997 Washington

- 18 Median antenna medium-sized, reaching chaetigers 3–4; posterior region with 4–6 hookless chaetigers; body papillae large .....  
 ..... *S. grubii* Müller, 1858 (southern Brazil)
- Median antenna short, reaching chaetiger 2–3; posterior region with 2 hookless chaetigers; body papillae small .....  
 ..... *S. hernandezi* sp. nov. (NW Atlantic, brackish water)
- 19 Median antenna slightly longer than laterals; body without a constriction on chaetiger 4; dorsal hooks from chaetiger 30–39 (15–24 mm long); posterior chaetigers without capillary notochaetae .....  
 ..... *S. olivai* sp. nov. (Northwestern Caribbean, México)
- Median antenna twice as long as laterals; body with a constriction on chaetiger 4; dorsal hooks from chaetiger 30–40 (16–24 mm long); posterior chaetigers with a single capillary notochaetae .....  
 ..... *S. constricta* (Southern, 1921) (Northeastern India, brackish water)
- 20 Dorsal hooks from chaetiger 6 (1.5 mm long) .....  
 ..... *S. ocellata* (Hartmann-Schröder, 1959) (El Salvador, brackish water \*\*\*\*)
- Dorsal hooks from chaetiger 3 (3.7 mm long) .....  
 ..... *S. magnuncus* Paterson & Glover, 2000 (NE Atlantic, abyssal)
- 21 Pharynx with 8 marginal papillae ..... 22
- Pharynx with 14–16 marginal papillae ..... 24
- 22 Median antenna slightly longer than laterals ..... 23
- Median antenna twice as long as laterals; dorsal hooks from chaetiger 26–28 .....  
 ..... *S. ligneroi* sp. nov. (southern Caribbean, Venezuela)
- 23 Dorsal hooks from chaetiger 4 (0.7 mm wide), tentacular segment as long as wide .....  
 ..... *S. healyae* Gagaev, 2008 (Arctic Ocean)
- Dorsal hooks from chaetigers 23–30 (45–70 mm long); tentacular segment 4–5 times wider than long .....  
 ..... *S. wassi* Pettibone, 1966 (Northwestern Atlantic)
- 24 Dorsal hooks from chaetiger 7, with accessory capillaries; dorsal cirri progressively longer from chaetiger 2 .....  
 ..... *Sigambra* sp. Imajima, 2001 (Eastern Japan)
- Dorsal hooks from chaetigers 43–70, without capillary chaetae; dorsal cirri of chaetiger 2 smaller than those in following chaetigers .....  
 ..... *S. robusta* (Ehlers, 1908) (Southwestern Africa)

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\*\*\*\* Licher and Westheide (1997:10) indicated that it lacks ventral cirri on chaetiger, 2 but they did not examine type material.

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# Jawless land snail *Sinorachis*, a new bradybaenine genus from China (Eupulmonata, Camaenidae)

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## Abstract

A new land snail species that represents a new genus is reported from Hubei, China. The snail has a conical shell with pits and/or granules on embryonic whorls and a smooth teleoconch with straight peristome. The head of the animal has a developed wart. The mantle lobe is only developed on the left side. At the front of the buccal mass there is no chitinous jaw that is without exception seen in Chinese camaenids. Its radular teeth are usually slender and tongue-shaped, not typical in bradybaenine snails. The genital system is typical of Bradybaeninae and is characterized by the absence of a membranous sac surrounding the terminal genitalia, penial caecum or flagellum; a well-developed penis sheath; a symmetrical dart sac apparatus; and one distally branched mucous gland. The new species *Sinorachis baihu* Wu & Chen, **gen. and sp. nov.**, is assigned to the type species of the new genus, in which all the known Chinese *Rachis* species are included. Thereby, the new genus is composed of three species, namely *Sinorachis onychinus* (Heude), **comb. nov.**, *Sinorachis aureus* (Heude), **comb. nov.** and the new species.

## Chinese abstract

从湖北报道了一个陆生贝类新物种并指定其为新属华霜螺属 *Sinorachis* Wu & Chen, **gen. nov.** 的模式种。该属因生殖系统具有巴蜗牛亚科（坚螺科）的典型矢囊结构而被列入巴蜗牛亚科。模式种白虎华霜螺 *Sinorachis baihu* Wu & Chen, **gen. and sp. nov.** 口球前端的颚片阙如，系已知中国的坚螺科中首个口球缺乏颚片的物种。此外，白虎华霜螺齿舌的齿多呈细舌状且排列紧密，与常见的巴蜗牛亚科的齿舌不同。华霜螺属的鉴别特征为：贝壳锥形；胚螺层具微小凹坑和/或颗粒；成螺光滑；壳口缘不反折。头痛发达；外套膜左缘具小叶。颚片阙如。

生殖系统基部膜囊缺乏；具交接器鞘；鞭状体无；矢囊器官对称；粘液腺一根、分支。几何形态学分析亦表明本属具有与霜纳螺 *Rachis* Albers、中国艾纳螺各属及巴蜗牛亚科的拟锥螺 *Pseudobuliminus* Gredler 相异的贝壳特征。由之将中国原艾纳螺总科霜纳螺属的所有物种移入本新属。华霜螺属由爪华霜螺 *Sinorachis onychinus* (Heude), **comb. nov.**、金华霜螺 *Sinorachis aureus* (Heude), **comb. nov.** 与本新种组成。

## Keywords

Bradybaeninae, Enoidea, Hubei, taxonomy

## Introduction

*Rachis* Albers, 1850 (type species *Bulimus punctatus* Anton, 1838; SD Martens in Albers 1860) is an enoid genus comprised of many known species from Africa and India (Schileyko 1998b). Two Chinese land snail species with conical shells were assigned to this genus based on shell morphology (Gredler 1887; Yen 1939; Wu 2018). Although Möllendorff (1901) placed them in *Buliminus* and thought they appeared to be related to the group, including *Bulimus cantori* Philippi, 1844 (= *Mirus cantori*), he wondered about the absence of an angular nodule that is ubiquitously observed in the Chinese enids. The Chinese *Rachis* species differed from the true *Rachis* species by the presence of embryonic shell sculpture that is unseen in *Rachis* (Schileyko 1998b). However, this sculpture may have been overlooked by early researchers.

The shells of Chinese *Rachis* species are, in many respects, untypical of the bradybaenine genera. In our recent work on the land snails from Hubei Province, comparison of the shell morphology revealed an arboreal snail that was firmly believed to fall into the same category of Chinese *Rachis*. The genital pattern of this species, however, totally differs from those of these enoids; rather, the developed dart sac apparatus suggests it belongs to the camaenid subfamily Bradybaeninae.

## Materials and methods

Living specimens were relaxed by drowning in fresh water before being transferred to 70% ethanol for fixation, which was replaced with ethanol of the same concentration after three days. Just after the specimens were relaxed, a piece of foot was cut and preserved in 99.7% ethanol for future molecular analysis. The shell and genitalia were measured with digital vernier calipers and from photographs to the nearest 0.1 mm. Whorl number was recorded as described by Kerney and Cameron (1979), with 0.125 (=  $\frac{1}{8}$ ) whorl accuracy. Soft parts were measured after the specimens were sufficiently fixed in 70% ethanol. Directions used in descriptions: proximal = towards the genital atrium; distal = away from the genital atrium.

The buccal mass was removed and treated in 10% sodium hydroxide solution under 60 degrees Celsius for up to 10 min before extracting the radula, which was cleaned by water using an ultrasonic cleaner. Then the radula was transferred into 75% ethanol. Radulae and shell were examined under a scanning electron microscope (Sigma 500).

Geometric morphometric methods were used to explore the conchological relationship among the new species described herein, the enoids and the high-shelled bradybaenine species distributed in mainland China. Shell morphological variation analyses were performed in the tps series software including tpsUtil32 (Rohlf 2004) and tpsDig32 (Rohlf 2005), using the geometric morphometric methods based on the landmarks and semi-landmarks on the profile of the aperture-viewed shell as per Schilthuizen et al. (2012). The designs of the landmarks and semi-landmarks are as follows (Fig. 9B):

- LM1** the crossing of peristome and left profile of body whorl;
- LM2** the columella insertion;
- LM3** the right insertion of peristome onto body whorl;
- LM4 and LM10** the right and left terminal points on last suture, respectively;
- LM5 and LM9** the right and left terminal points on penultimate suture, respectively;
- LM6 and LM8** the right and left terminal points on suture above the penultimate one, respectively;
- LM7** apex of shell;
- LM11–18** eight semi-landmarks on the left profile between LM10 and LM1 by length;
- LM19–36** eighteen semi-landmarks on the peristome between LM1 and LM3 by length.

The usually used landmark point crossed by the right profile and the last part of suture (arrowed on Fig. 9B) was not chosen in this study because the point is not present on all the aperture-view images of the specimens observed herein. The landmarks and the semi-landmarks were treated indiscriminately. The geometric morphometric analysis employed aperture-viewed images of a total of 232 shells including most of Chinese enids (112 specimens of 112 species, including one from Wu and Xu 2011, 111 from Wu 2018; see Appendix 1), some Chinese *Pseudobuliminus* Gredler, 1886 and one *Stenogyropsis* Möllendorff, 1899 species (102 specimens of 20 species: SMF and HBUMM specimens; see Appendix 1), *Rachis* Albers, 1850 (five specimens of two species: SMF specimens and Raheem et al. 2014: fig. 39a; see Appendix 1) and *Rhachistia* Connolly, 1925 (seven specimens of four species in Raheem et al. 2014: figs 39B–40B), and *Sinorachis* gen. nov. (five specimens of three species: SMF and HBUMM specimens; one image of *Buliminus aureus* Heude, 1890 from Heude 1890: Pl. 35, fig. 21). The detailed information of the specimens used in this work is listed in the Appendix 1. Full Procrustes fitting, covariance matrix generation, and subsequent

canonical variate analysis (CVA) were performed using MorphoJ (version 1.05f; Klingenberg 2011).

Abbreviations used in the text and figures are as follows:

<b>A</b>	anus;	<b>HG</b>	hindgut;
<b>AS</b>	accessory sac, a sac both inserted by mucous glands and opening into the chamber containing the love dart or opening into the dart sac chamber;	<b>K</b>	kidney;
<b>At</b>	atrium;	<b>MC</b>	mantle collar;
<b>AU</b>	auricle;	<b>MG</b>	mucous glands;
<b>BC</b>	bursa copulatrix;	<b>P</b>	penis;
<b>BCD</b>	bursa copulatrix duct;	<b>PC</b>	pericardium;
<b>DS</b>	dart sac;	<b>PG</b>	pallial gland;
<b>DtC</b>	love dart chamber, the chamber secreting and containing the love dart;	<b>PR</b>	penial retractor muscle;
<b>Ep</b>	epiphallus;	<b>PS</b>	penis sheath;
<b>FO</b>	free oviduct;	<b>PV</b>	principal pulmonary vein;
<b>HBUMM</b>	mollusk collection of the Museum of Hebei University, Baoding, China;	<b>SMF</b>	Forschungsinstitut und Naturmuseum Senckenberg, Frankfurt;
		<b>U</b>	ureter;
		<b>UO</b>	ureteric orifice;
		<b>V</b>	ventricle;
		<b>Va</b>	vagina;
		<b>VD</b>	vas deferens.

## Systematics

### Helicoidea Rafinesque, 1815

### Camaenidae Pilsbry, 1895

### Bradybaeninae Pilsbry, 1898

### *Sinorachis* Wu & Chen, gen. nov.

<http://zoobank.org/98AD47D3-B40F-4EE7-9678-5F9657D0FCD4>

**Type species.** *Sinorachis baihu* Wu & Chen, gen. and sp. nov.

**Diagnosis.** Shell conical. Embryonic shell with pits and/or granules. Adult shell smooth. Peristome not reflexed. Head wart developed. Mantle lobe only present on left side. Jaw absent. Membranous sac surrounding terminal genitalia absent. Penis sheath present. Penial caecum absent. Flagellum absent. Dart sac apparatus symmetrical. Mucous glands one; branched.

**Description.** *Shell* conical. Whorls slightly convex. Suture impressed. Protoconch brownish purple; shiny; with tiny pits and/or granules. Adult shell smooth, not hairy

or scaly. Body whorl large. Peristome not reflexed. Aperture not expanded. Umbilicus a slit. Shell glossy; banded or not.

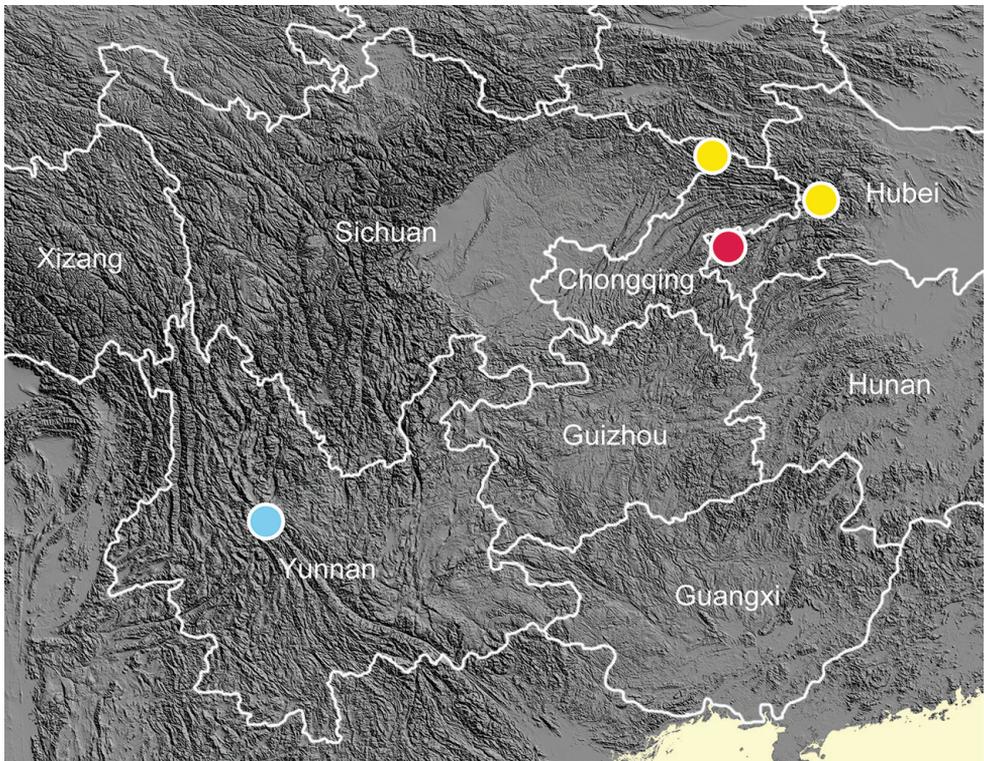
**General anatomy.** Eversible head wart between ommatophore insertions developed. Lobe on mantle collar present on left but absent on right. Jaw absent. Crop thin, indistinguishable from the remaining alimentary tract.

**Pallial complex.** Ureter closed. Kidney triangular, not bilobed.

**Genitalia.** Penis sheath present. Penis externally simple; internally with several pilasters. Flagellum absent. Epiphallus and vas deferens distinctly demarcated. Membranous sac surrounding terminal genitalia absent. Dart sac apparatus symmetrical. Accessory sac present. Poly-layered structure developed in dart sac. Mucous gland branched; inserting into dart sac through one peduncle.

**Etymology.** This new genus is named after *sino* (China) and *rachis*, an enoid genus in which the old species of the new genus were placed.

**Distribution.** Hubei (Badong, Lichuan), Chongqing (Chengkou), Yunnan (Dali) (Fig. 1).



**Figure 1.** Distribution map of *Sinorachis* Wu & Chen, gen. nov. Blue dot, *Sinorachis aureus* (Heude, 1890) comb. nov.; yellow dots, *Sinorachis onychinus* (Heude, 1885) comb. nov.; red dot, *Sinorachis baihu* Wu & Chen, gen. and sp. nov.

**Remarks.** *Sinorachis baihu* Wu & Chen, gen. and sp. nov. shares most conchological features with the other two known species, namely *Buliminus onychinus* Heude, 1885 and *Buliminus aureus* Heude, 1890, which were treated by some authors as species of the enoid genus *Rachis* (Gredler 1887). But based on pallial complex and genital system, the new species described herein should not be assigned to the genus *Rachis* Albers, 1850 (Einoidea) that belongs to Orthurethra and there is no dart apparatus in genitalia. The new genus is entirely in character for the subfamily Bradybaeninae in possessing the typical dart sac apparatus that does not differ from that commonly seen in all the Chinese bradybaenine genera. In our opinion, based on shell morphology, *B. onychinus* and *B. aureus* should also belong to the genus *Sinorachis* gen. nov.

The new genus is the only one that lacks a jaw in the subfamily Bradybaeninae. Like *Bradybaena* Beck, 1837 and some other bradybaenine genera (Wu et al. 2019), the genus shows a leaf-shaped appendage on the left mantle collar. The shells of the new genus are quite different from those of the high-shelled genus *Pseudobuliminus* Gredler, 1886 in having a sculptured embryonic shell and a distinctly large body whorl. In aspects of general shell morphology, the shell of the new genus, the genus *Rachis* Albers, 1850 distributed in Africa and India, the genus *Rhachistia* Connolly, 1925 distributed in eastern Africa and Asia, Chinese enid genus and Chinese *Pseudobuliminus* spp. can be discriminated with the aid of the geometric morphometric methods (Fig. 10) based on the landmarking scheme employed herein (Fig. 9B).

In comparison with Chinese species of another bradybaenine genus, *Pseudobuliminus*, that also has a high spired adult shell and embryonic sculpture, the new genus has a poly-layered structure, an accessory sac, and a single branch of mucous gland in the dart sac apparatus, but has no membranous sac surrounding terminal genitalia. If only focusing on the characteristic spectrum of genitalia (table 1, in Wu 2019), the genus is closest to *Ponsadenia* Schileyko, 1978 but these two genera can be distinguished by presence/absence of the poly-layered shell structure and the structure of accessory sac which looks like a bridge in the latter genus.

***Sinorachis baihu* Wu & Chen, sp. nov.**

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Figs 1–10

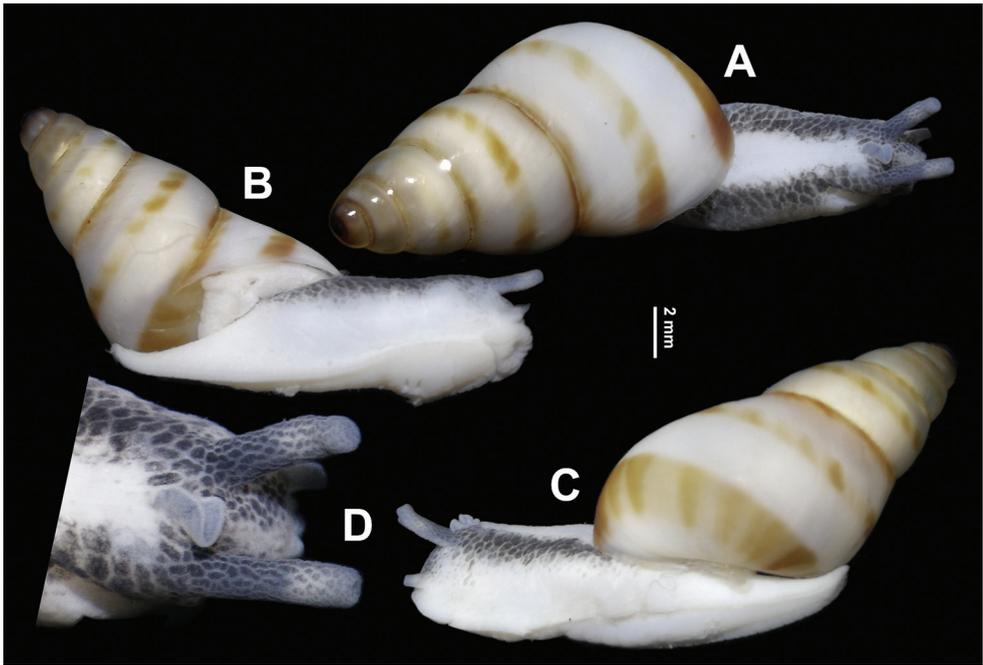
**Type material.** *Holotype*: CHINA • fully mature animal; Hubei, Lichuan, Liangwuxiang, Shanchacun; 108.837°E, 30.274°N; 1.XI.2018; Liwan Zhang leg.; HBUMM08296-specimen 1. *Paratype*: one juvenile animal; same data as for preceding; HBUMM08296-specimen 2. Partial foot was cut off in both specimens and preserved in 99.7% ethanol at -20 °C; HBUMM08296a-specimens 1, 2.

**Diagnosis.** Embryonic shell with pits, each having a central hump. Shell with three bands.



**Figure 2.** A–D *Sinorachis onychinus* (Heude, 1885) comb. nov., shells A SMF42825 B SMF42826 C SMF42827, SW Hupei D SMF104593 E *Sinorachis baihu* Wu & Chen, gen. and sp. nov., HBUMM08296-specimen 1, holotype.

**Description.** *Shell* (Figs 2, 6D, E). Conical; thin but solid; dextral. Whorls slightly convex. Suture impressed. Umbilicus a slit. Columella almost vertical. Protoconch densely and evenly covered with fine centrally-uplifted pits (Fig. 6D, E). Teleoconch without spiral furrows. Aperture oblique; not sinuate at peristome. Body whorl not descending behind aperture. Shell surface without ribs. Growth lines fine. Adult shell not hairy or scaly. Adult body whorl rounded at periphery; with bottom convex. Ring-like thickening within aperture absent. Peristome thin; not reflexed. Callus thin and



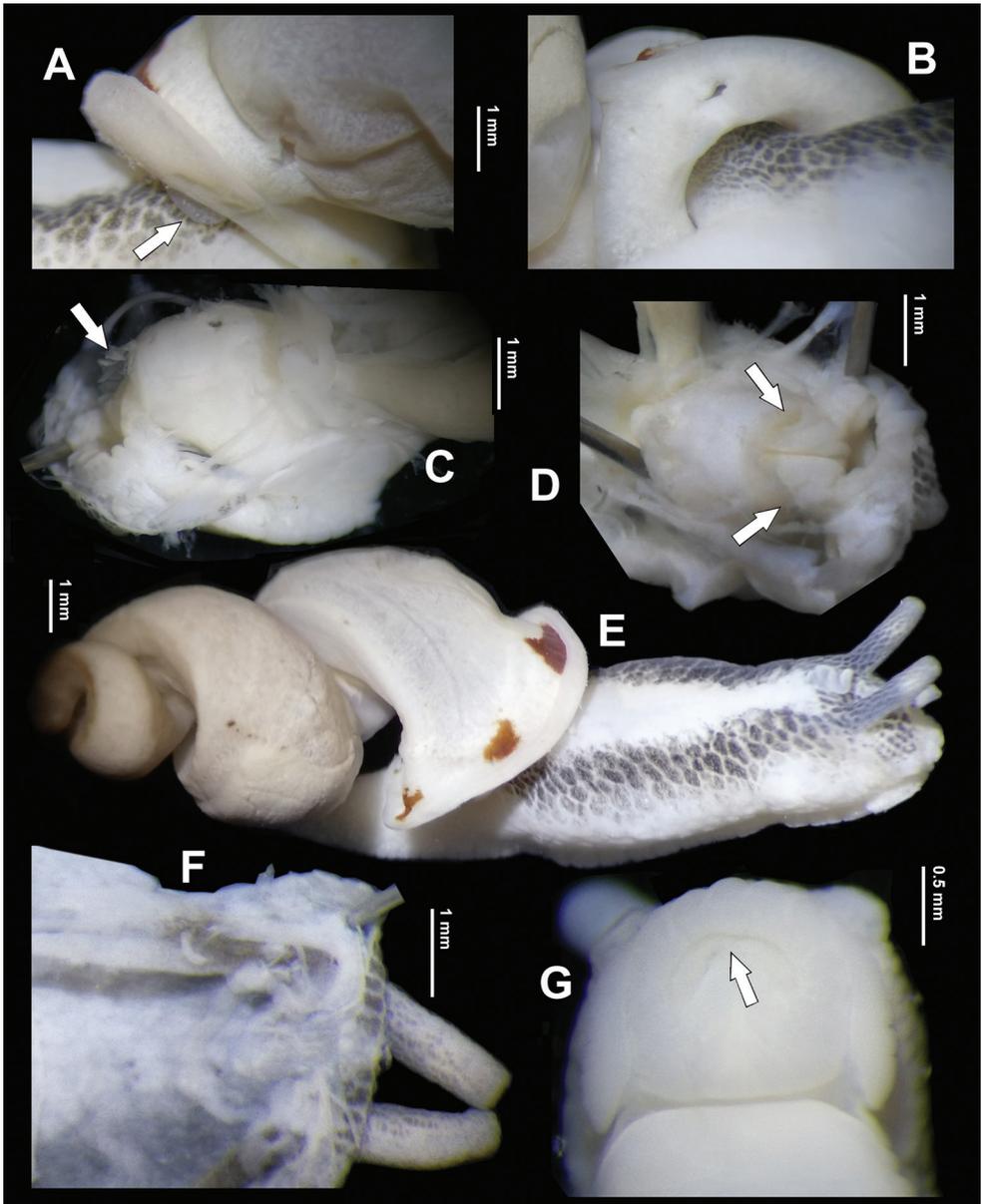
**Figure 3.** *Simorachis baihu* Wu & Chen, gen. and sp. nov., HBUMM08296-specimen 1, holotype **A–C** living animal **D** magnified view of head.

transparent. Shell glossy; white. A suture band, a supra-peripheral band and a subperipheral band more or less broken. Measurements (holotype): shell height 14.7 mm, shell breadth 8.7 mm, aperture height 5.3 mm, aperture width 3.5 mm, embryonic shell whorls 1.500, whorls 5.125, shell height/ breadth ratio 1.70.

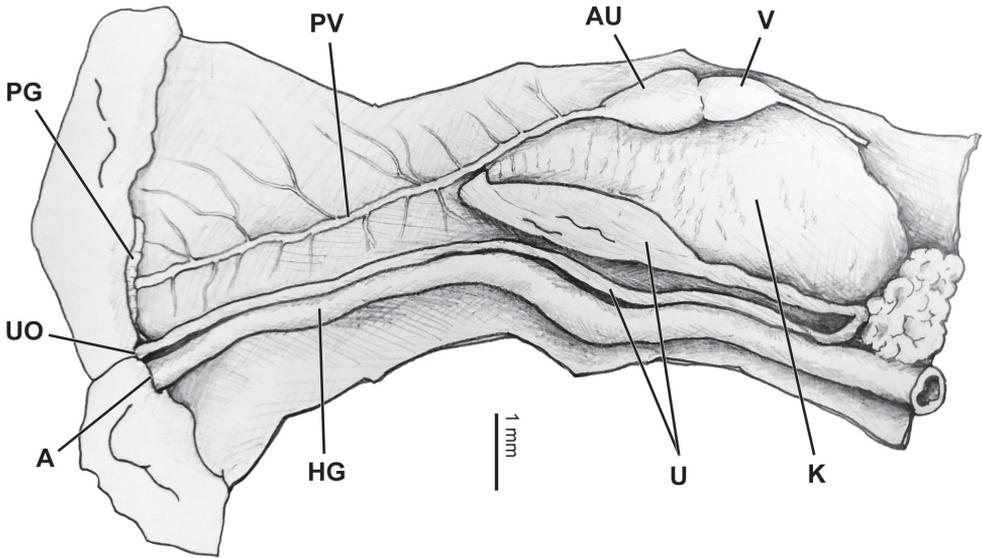
**General anatomy** (Figs 3, 4). A crest-like head wart between and slightly behind ommatophore insertions present. On left edge of mantle collar a leaf-shaped appendage present. Body dorsally white; symmetrically with two lateral black pigmented stripes that become lighter near sole. Sole creamy white. Jaw absent (Fig. 4C, D, G).

**Pallial complex** (Fig. 5). Pallial roof not pigmented. Pallial gland thin, parallel to mantle collar. Hindgut running parallel to parietal-palatal margin for length of pallial chamber. Ureter slender, typical sigmurethrous, about 1/5 breadth of hindgut, adhering to hindgut for all its length. Secondary ureter developed. Kidney triangular, not bilobed, about as long as 1/2 of pallial chamber. Heart as long as 1/3 – 1/2 of kidney. Main pulmonary vein running along contour and apex of kidney, then diffusing into thinner veins mostly concentrated on anterior half.

**Radula** (Fig. 6A–C): Teeth arranged in transversal rows, each row containing about 151 (75–1–75) closely arranged teeth. Central tooth tricuspid, narrowly tongue-shaped (Fig. 6A). Lateral teeth slightly thickened at inner edge; bicuspid (L1–2 or



**Figure 4.** **A–F** *Sinorachis baihu* Wu & Chen, gen. and sp. nov., HBUMM08296-specimen 1, holotype. **A** left margin of mantle collar with the lobe arrowed **B** right margin of mantle collar **C** lateral dorsal view of buccal mass with an oral curtain (a sheet of curtain-like tissue on the most anterior of the buccal mass) arrowed **D** dorsal view of buccal mass with an oral curtain arrowed **E** shell-removed animal, showing three pigmentation patches near mantle margin **F** internal body wall of head, showing no obvious head gland between the ommatophore tentacles **G** HBUMM08296-specimen 2, paratype, mouth of ventral view with an oral curtain arrowed.



**Figure 5.** Pallial complex of *Sinorachis baihu* Wu & Chen, gen. and sp. nov., HBUMM08296-specimen 2, paratype.

L1–3) (Fig. 6A), tricuspid (from L2 or L3 on) (Fig. 6A, B) and gradually transformed to marginals with one endocone and three or four ectocones (Fig. 6C).

**Genital system** (Figs 7, 8). Penis sheath long, covering approximately 3/4 of penis. Penis thin; externally simple; internally with three pilasters. Epiphallus subequal to penis in length; without epiphallic papilla. Flagellum absent. Vas deferens ca. 1/2 length of epiphallus; of even thickness. Epiphallus and vas deferens sharply demarcated (Fig. 7). Dart sac apparatus large in size; distal 1/3 with a distinct accessory sac ventrally that is internally solid. Love dart very short, approximate 0.7 mm long; sharply tapering from distal end; transparent. Mucous gland with one common peduncle; simply branched. Vagina as long as penis. Bursa copulatrix small, ball-shaped.

**Measurements of holotype.** DS–5.9 mm long, 1.5 mm broad; DtC–0.7 mm; MG–2.7 mm; P–7.3 mm; Ep–7.5 mm; VD–3.8 mm; PR–5.5 mm; Va–5.7 mm; FO–3.1 mm; BC plus BCD–5.7 mm.

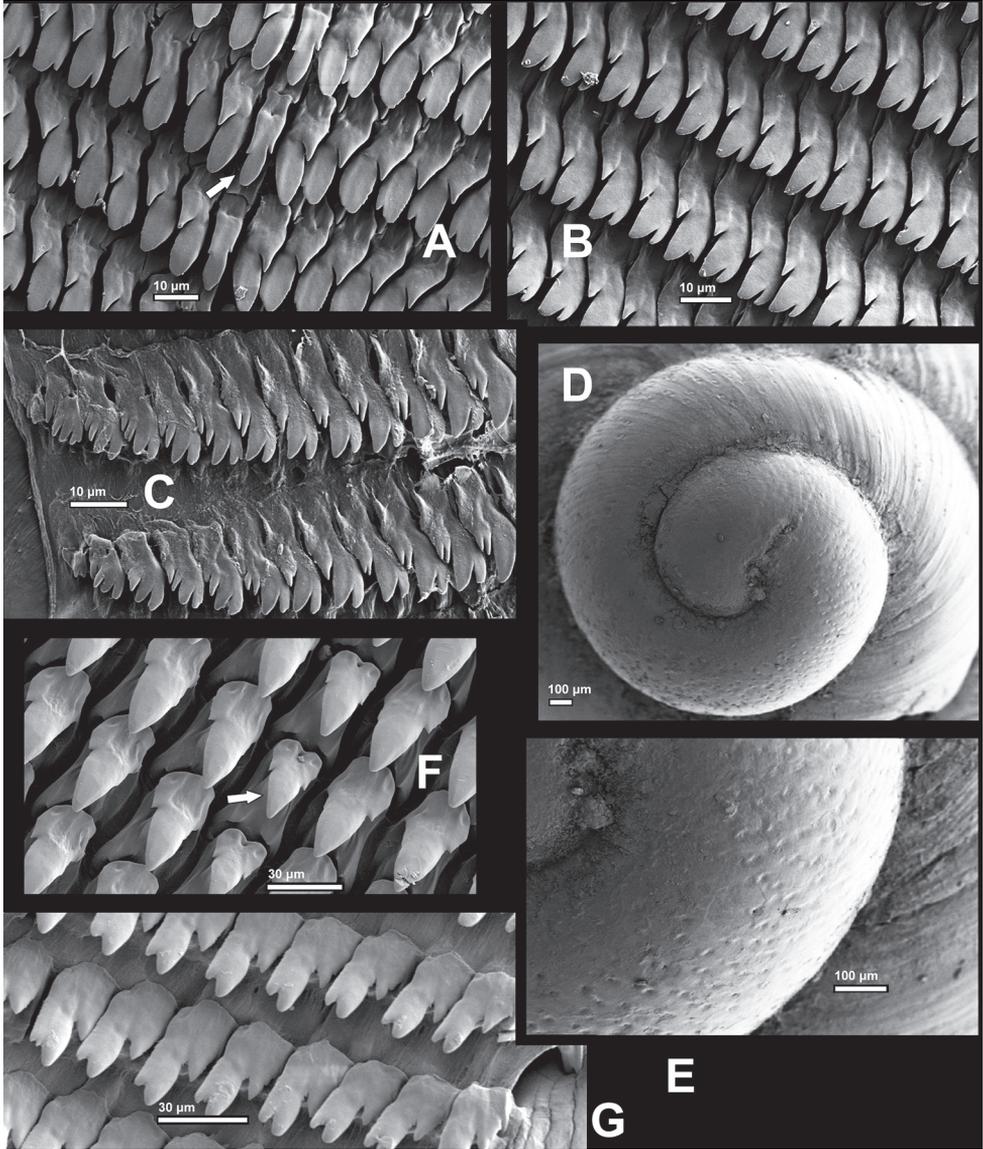
**Etymology.** This species is named after *baihu* (=白虎 in Chinese, means white tiger) which is the totem of the local Tujia people.

**Type locality.** Lichuan, only known from the type locality (Fig. 1).

**Distribution.** Hubei.

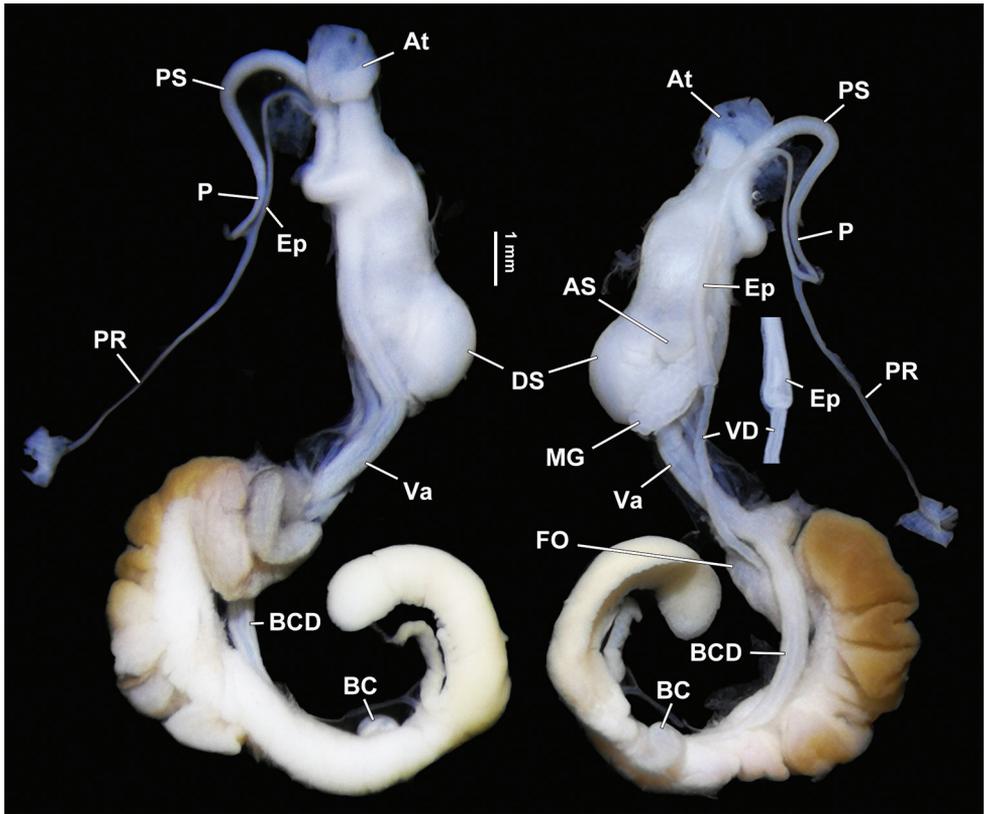
**Ecology.** This species was only found on the trunk of a tree (Fig. 9A).

**Taxonomic remarks.** The new species and the two species that were once placed in the genus *Rachis* share many conchological features. However, typical sigmurethrous pallial complex (Solem 1985) is observed in the new species. The new species is slightly smaller than and obviously thinner than *Sinorachis onychinus* (height 16 mm, diam.



**Figure 6.** SEM images **A–E** *Sinorachis bairu* Wu & Chen, gen. and sp. nov. HBUMM08296-specimen 2, paratype: **A** radula, showing central tooth (arrowed) and several lateral teeth **B** radula, showing tricuspid lateral teeth **C** radula, showing most lateral part **D** embryonic shell **E** magnified embryonic shell, showing some pits **F–G** radula of *Laeocathaica prionotropis* Möllendorff, 1899, HBUMM08299-spec.1: **F** showing central tooth (arrowed) and nearby lateral teeth **G** marginal part of radula.

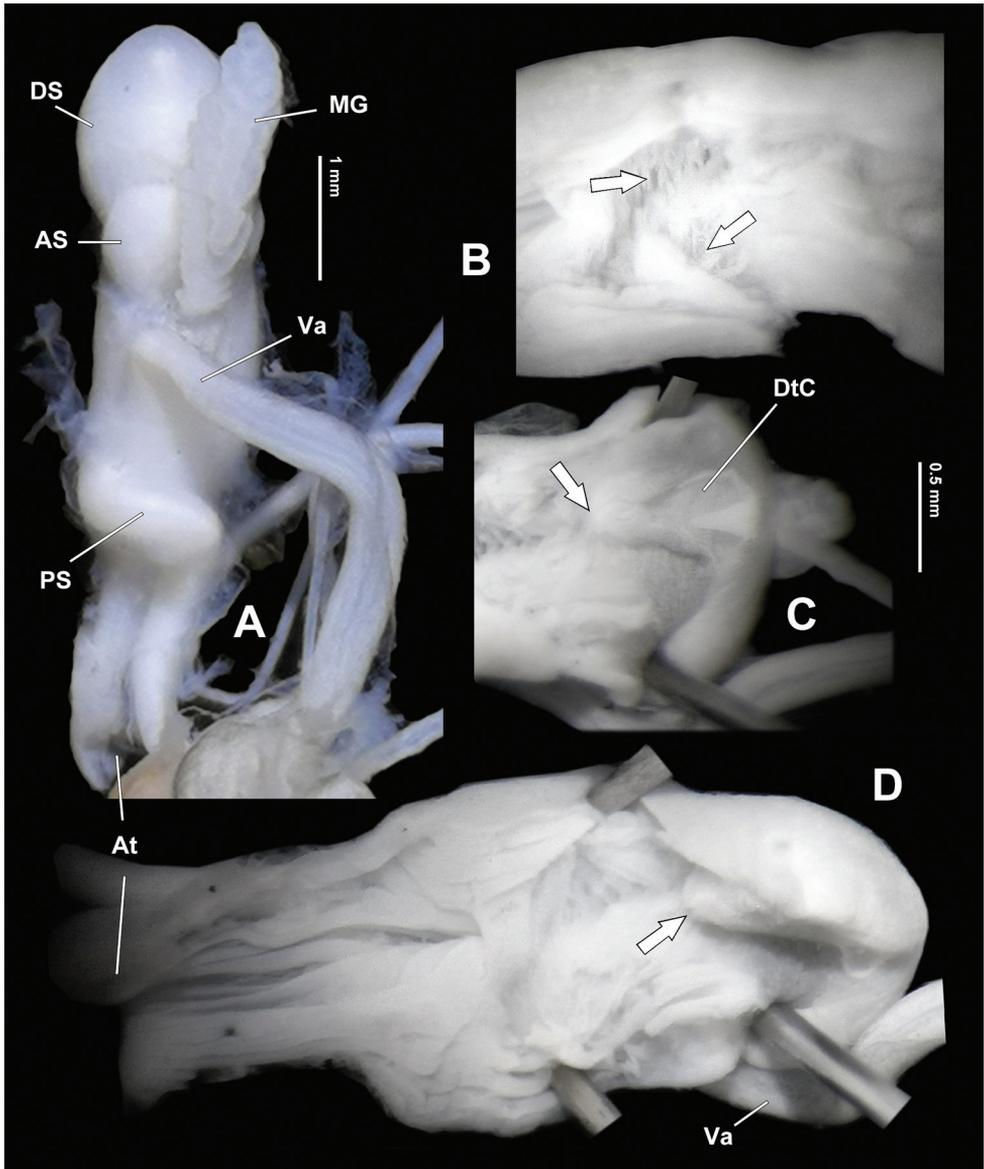
maj. 11 mm: Heude 1885: 114, pl. 30, fig. 5). The new species can be distinguished from *S. onychinus* by having evenly distributed pits each centrally with a hump on the embryonic shell. In *S. onychinus*, the embryonic shell is smooth on the first 0.5 whorl,



**Figure 7.** *Sinorachis baihu* Wu & Chen, gen. and sp. nov., HBUMM08296-specimen 1, holotype. Both sides of genitalia. The portion with the demarcation between epiphallus and vas deferens is magnified.

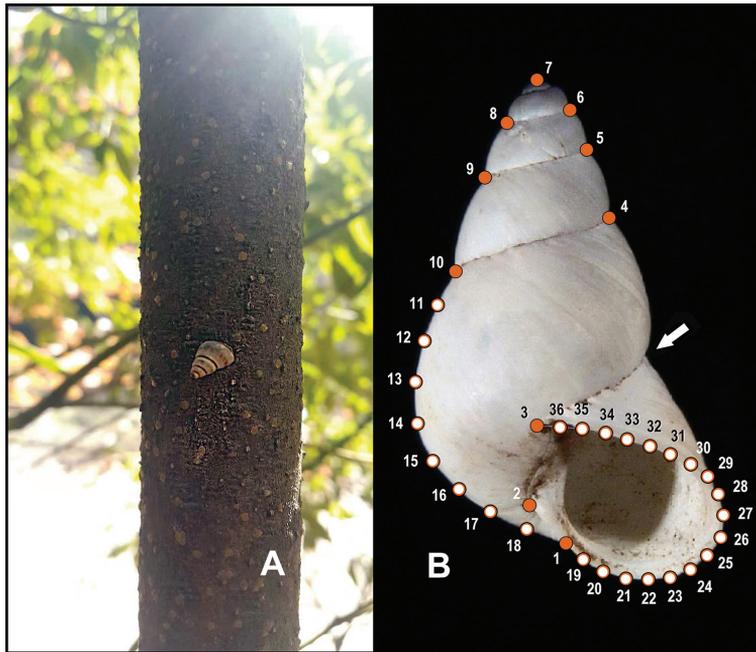
and is axially wrinkled on the subsequent protoconch whorls (0.5–1.25 whorl). On the remaining embryonic whorls, the sculpture is shown as evenly distributed tiny pits [examined material: SMF42825, SMF42826: Patung, Hupei, Mlldff. G., Slg. Kobelt u. Bttgr. SMF42827: *Sinorachis onychinus* (not paratypes of *Rhachis chalcidonicus* as mentioned in Yen 1939: 91, pl. 8, fig. 47), SW Hubei, Gredler G., Slg. Mlldff. SMF1045593] that become weak or disappear altogether.

In pulmonates the presence of a jaw is a ubiquitous characteristic related to herbivorous/ detritivorous/ fungivorous foraging strategies, while the absence of a jaw is correlated with predation/carnivorous foraging strategies (Mordan and Wade 2008). In addition, the absence of a jaw also occurs in some non-carnivorous groups, such as Achatinellidae, which are fungivorous and have the jaw weakly developed or absent (Schileyko 1998a). The comparison between the new species and *Laeocathaica prionotropis* Möllendorff, 1899 (Bradybaeninae) (Fig. 6F, G. HBUMM08299-spec. 1, Bikou, Wenxian, Gansu. Coll. Li, Q., April 2019) indicates they are two different types of radula. The latter species, a typical ground-dweller, is herbivorous snail, which has the robust cone-shaped and sparsely arranged radular teeth that seem to be typical

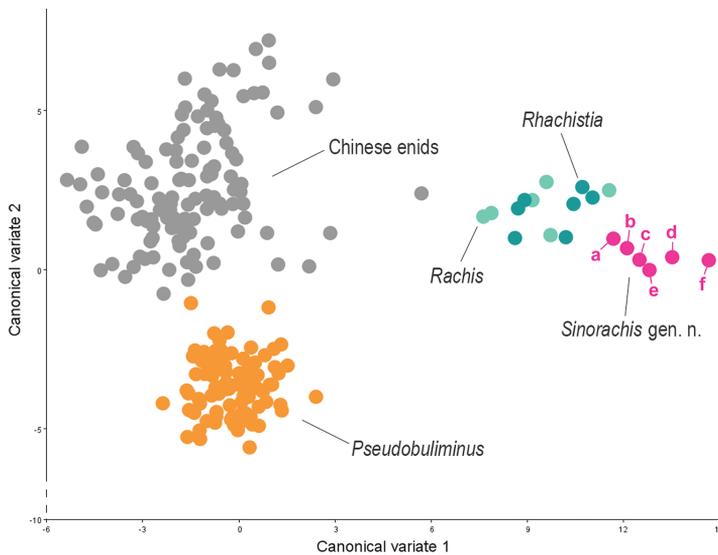


**Figure 8.** *Sinorachis baihu* Wu & Chen, gen. and sp. nov., HBUMM08296-specimen 1, holotype **A** bottom view of dart sac apparatus **B** partial dorsally exposed dart sac apparatus, showing arrowed poly-layered structure **C** distal part of dorsally exposed dart sac apparatus, showing opened love dart chamber, with opening of the love dart chamber arrowed **D** dorsally exposed dart sac apparatus with opening of love dart chamber arrowed.

in bradybaenine snails (e.g., compare it with fig. 4 in Páll-Gergely and Hunyadi 2016), while the new species has more slender and densely arranged radular teeth, which suggest the diet range of this species might not cover large plants and animals.



**Figure 9.** **A** habitat of *Sinorachis baihu* Wu & Chen, gen. and sp. nov., HBUMM08296-specimen 2, paratype **B** a diagram showing design of landmarks (solid orange dots) and semi-landmarks (empty orange dots). Arrow indicates the crossing point made by the right profile and the last part of the suture.



**Figure 10.** Scatter plot of canonical variate 1 against canonical variate 2 (Canonical Variate Analysis), showing the shell morphological relationship among *Rachis* Albers, 1850 (bright blue dots), *Rhachistia* Connolly, 1925 (grey-blue dots), Chinese *Pseudobuliminus* spp. (orange dots), Chinese enid species (grey dots) and *Sinorachis* gen. nov. (pink dots). a, *Sinorachis onychinus* (Heude), SMF42826; b, *S. onychinus*, SMF104593; c, *S. baihu* Wu & Chen, gen. and sp. nov., holotype; d, *S. onychinus*, SMF42825; e, *S. aureus* (Heude), based on an image from Heude (1890); f, *S. onychinus*, SMF42827.

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## Appendix I

Brief information of specimens and photographs used in the geometric morphometric analysis.

### ***Pseudobuliminus* (s. l.) Gredler, 1886 + *Stenogyropsis* Möllendorff, 1899**

*Pseudobuliminus achatinus* (Möllendorff, 1899)

SMF9026 (1, number of specimen), SMF9027 (2), HBUMM00523 (2): China (label lost), HBUMM04429 (2): (Gansu), HBUMM05421 (1): Gansu.

*Pseudobuliminus buliminoides* (Heude, 1882)

SMF24668 (2).

*Pseudobuliminus buliminus strigatus* (Möllendorff, 1899)

SMF9011 (1), HBUMM01187 (2): Sichuan, HBUMM04458 (2): Gansu, HBUMM04613 (2): Gansu, HBUMM05506 (1): Gansu, HBUMM05558 (1): Gansu, HBUMM05562 (2): Gansu, HBUMM05773 (1): Gansu.

*Pseudobuliminus cerasinus* (Gredler, 1892)

SMF95044 (2).

*Pseudobuliminus certus* (Zilch, 1938)

SMF24673 (1), SMF24751 (4).

*Pseudobuliminus chineensis* (Bavay & Dautzenberg, 1908)

SMF186520 (2).

*Pseudobuliminus cristatellus* (Möllendorff, 1902)

HBUMM06746B (2): Sichuan, HBUMM00503 (2): Gansu.

*Pseudobuliminus gracilispirus* (Möllendorff, 1899)

SMF9028 (1), SMF9029 (2), HUMM04456 (2): Gansu.

*Pseudobuliminus hirsutus* (Möllendorff, 1899)  
SMF9022 (1), SMF9023 (3), HBUMM05535 (1): Gansu, HBUMM05582 (1): Gansu, HBUMM06565 (2): Sichuan, HBUMM06677B (1): Gansu.

*Pseudobuliminus incertus* (Schmacker & Böttger, 1891)  
SMF24675 (1), SMF36015 (1), SMF36951 (1), SMF96649 (1).

*Pseudobuliminus meiacoshimensis* (Adams & Reeve, 1850)  
SMF294303 (2).

*Pseudobuliminus paradoliolus* Zilch, 1951  
SMF36081 (2), SMF50090 (1).

*Pseudobuliminus piligerus* (Möllendorff, 1899)  
SMF9024 (1), SMF9025 (1), HBUMM04428 (2): Gansu, HBUMM05428 (2): Gansu, HBUMM05556 (2): Gansu, HBUMM06745B (1): Gansu, HBUMM06902B (2): Sichuan, HBUMM04432 (2): Gansu, HBUMM04448 (2): Gansu.

*Pseudobuliminus subcylindricus* (Möllendorff, 1899)  
SMF9018 (3), SMF9019 (1), SMF9021 (1), HBUMM04449 (2): Gansu.

*Pseudobuliminus subdoliolus* (Haas, 1935)  
SMF42549 (1), SMF9323 (1).

*Pseudobuliminus superbus* (Möllendorff, 1888)  
SMF9147 (1),

*Pseudobuliminus turritus* (Gude, 1900)  
SMF96652 (3), SMF24682 (2)

*Stenogyropsis potanini* (Möllendorff, 1899)  
SMF9032, SMF9034 (2), HBUMM05404 (1): Gansu, HBUMM05596 (2): Gansu, HBUMM05643 (2): Gansu, HBUMM05698 (2): Gansu, HBUMM05701 (2): Gansu, HBUMM05722 (2): Gansu

### ***Rachis* Albers, 1850**

*Rachis zonulata* (L. Pfeiffer, 1846)  
SMF74313 (3).

*Rachis* sp.  
SMF426914 (2): original label "*Rachis succincta*".

*Rachis punctatus* (Anton, 1838)  
After fig. 39A (Raheem et al. 2014).

### ***Rhachistia* Connolly, 1925**

*Rhachistia bengalensis* (Lamarck, 1822)  
After fig. 39B (Raheem et al. 2014).

*Rhachistia praetermissus* (W.T. & H.F. Blanford, 1861)  
After fig. 39C–E (Raheem et al. 2014).

*Rhachistia pulcher* (Gray, 1825)  
After figs 39F, 40A (Raheem et al. 2014).

*Rhachistia trutta* (Blanford, 1866)  
After fig. 40B (Raheem et al. 2014).

### **Chinese enid species**

After Wu (2018) except *Funiculus songi* Wu & Xu, 2011.



# A new species in the genus *Acartia* Dana, 1846 (Crustacea, Copepoda, Calanoida, Acartiidae) from the South Pacific coastal waters of Nadi Bay, Fiji

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## Abstract

A new species in the genus *Acartia*, *Acartia nadiensis* **sp. nov.**, is described from Fijian coastal waters. This species belongs to the subgenus *Odontacartia* based on the following morphological features: presence of a rostral filaments, a pointed process on the last prosomite, a serrated terminal spine on female P5, and the absence of a protrusion on the basis of the male right P5. This new species can be differentiated from its congeners by the combination of the absence of a spine on the first segment of the antennules, the short outer seta of female P5, and a medial spine on the exp-2 of the left male P5. Phylogenetic analyses using mitochondrial COI partial sequences show that the new species is distinct from its congeners.

## Keywords

*Odontacartia*, planktonic copepod, mtCOI, taxonomy, South Pacific

## Introduction

The genus *Acartia* Dana, 1846 is one of the most dominant groups of planktonic copepods and has a worldwide distribution in estuarine, coastal, and even oceanic waters (Bradford 1976; Walter and Boxshall 2019). Sixty-five species in this genus have been

reported from various locations ranging from tropical to polar regions (Soh et al. 2013; Razouls et al. 2019; Srinui et al. 2019), and these species have been allocated to six subgenera: *Acartiura* Steuer, 1915, *Euacartia* Steuer, 1915, *Hypoacartia* Steuer, 1915, *Acanthacartia* Steuer, 1915, *Odontacartia* Steuer, 1915, and *Acartia* (= *Plankacartia*) Dana, 1846 (Steuer 1915, 1923). Among these subgenera, the subgenus *Odontacartia* contains 13 species: *Acartia amboinensis* Carl, 1907; *A. australis* Farran, 1936; *A. bispinosa* Carl, 1907; *A. bowmani* Abraham, 1976; *A. centura* Giesbrecht, 1889; *A. edentata* Srinui, Ohtsuka & Metillo, 2019; *A. erythraea* Giesbrecht, 1889; *A. japonicus* Mori, 1940; *A. lilljeborgi* Giesbrecht, 1889; *A. mertoni* Steuer, 1917; *A. ohtsukai* Ueda & Bucklin, 2006; *A. pacifica* Steuer, 1915; and *A. spinicauda* Giesbrecht, 1889.

During a survey of the diversity of planktonic copepods in Fijian waters, we collected an undescribed species of the genus *Acartia* that clearly belongs to the subgenus *Odontacartia*. In this study, we describe the morphological characters of the new species from Nadi Bay, Fiji. Partial mtCOI sequences were also obtained and compared with related species to determine if this new species is also genetically distinct from its congeners.

## Materials and methods

### Sample collection and identification

Specimens were collected from Nadi Bay, Fiji, using a 100 µm mesh plankton net having a 30 cm diameter mouth, and then preserved in 99% ethanol. Specimens were dissected in lactic acid, and mounted on slides with lactophenol. Preparations were sealed with transparent nail varnish. All drawings were prepared using a drawing tube attached to an Olympus BX51 differential interference contrast microscope. For scanning electron microscope (SEM) preparation, specimens were dehydrated in a series of graded ethanol solutions, then placed in isoamyl acetate, critical point dried, mounted on stubs, coated in platinum, and observed under a Hitachi S4700 field-emission electron microscope at Eulji University, Seoul, Korea. Descriptive terminology was adopted from Huys and Boxshall (1991).

### DNA extraction and amplification

For DNA extraction, ethanol was removed from fixed specimens (99% EtOH) by washing with distilled water, and DNA was extracted using a tissue DNA purification kit (COSMO GENETECH, Co. Ltd, Korea). DNA was extracted from individual specimens. mtCOI DNA was amplified in 20 µl reaction volumes containing extracted tissue DNA and primers LCO-1490 (5'-GGT CAA CAA ATC ATA AAG ATA AAG ATA TTG G-3') and HCO-2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') (Folmer et al. 1994). PCR conditions comprised initial denaturation at 94 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 46 °C for 2 min, and extension at 72 °C for 3 min. This was followed by a final extension

step at 72 °C for 10 min. PCR products were evaluated by electrophoresing amplification products on 1% agarose gel containing ethidium bromide. Purification of amplified products was performed using a PCR purification kit (COSMO GENETECH Co. Ltd, Korea), and both strands were sequenced using an ABI 3730XL sequencer (COSMO GENETECH Co. Ltd, Korea).

### Phylogenetic analysis

Sequences were aligned and edited using CLUSTAL W (Thompson et al. 1994) within MEGA6 (Tamura et al. 2013). For the phylogenetic analysis, three *Acartia* species (*A. erythraea*, *A. japonica*, and *A. ohtsukai*) belonging to the subgenus *Odontacartia* were collected from South Korea and Japan for this study (Table 1). Sequences of *A. pacifica* and *A. spinicauda* were obtained from the NCBI database for comparison. Phylogenetic analysis and pairwise distance analysis were conducted using MEGA6 software using neighbor-joining and minimum-evolution algorithms, respectively, and the Tamura-Nei model of sequence evolution was applied (Tamura and Nei 1993). Codon positions were set as follows: 1<sup>st</sup> + 2<sup>nd</sup> + Noncoding. All positions containing gaps and missing data were eliminated. *Acartia* (*Acartiura*) *omorii* Bradford, 1976 was used as outgroup.

### Systematics

**Order Calanoida G. O. Sars, 1903**

**Family Acartiidae G. O. Sars, 1900**

**Genus *Acartia* Dana, 1846**

**Subgenus *Odontacartia* Steurer, 1915**

***Acartia nadiensis* sp. nov.**

<http://zoobank.org/DD2852BB-7AAE-4B65-85F1-4A741FD85F7F>

Figures 1–8

**Type locality.** Coastal water (17°45.848'S, 177°22.348'E), Nadi Bay, Fiji.

**Materials examined.** All specimens have been deposited in the Marine Biodiversity Institute of Korea (MABIK). Holotype 1 ♀ (MABIK CR00246502) and Allotype 1 ♂ (MABIK CR00246503) undissected and preserved in 70% ethanol. Paratype: 2 ♀♀ (MABIK CR00246504–CR00246505) dissected on 13 and 10 slides, respectively; 2 ♂♂ (MABIK CR00246506–CR00246507) dissected on 14 and 8 slides, respectively; 10 ♀♀ (MABIK CR00246508–CR00246517) and 4 ♂♂ (MABIK CR00246518–CR00246521) undissected and preserved in 70% ethanol. 4 ♀♀ and 4 ♂♂ dried, mounted on stub, and coated with platinum for SEM. All specimens are from the type locality and were collected by S. Lee on 10 October 2013. The illustrations are based on the paratypes (♀, MABIK CR00246504; ♂, MABIK CR00246506).

**Table 1.** List of species analyzed for molecular comparison.

Species	Locality	GenBank no.	References
<i>A. (Odontacartia) erythraea</i>	Mokpo, Korea	MN603769–MN603773	Present study
<i>A. (Odontacartia) japonica</i>	Okinawa, Japan	MN603774	Present study
<i>A. (Odontacartia) nadiensis</i>	Nadi Bay, Fiji	MN603766–MN603768	Present study
<i>A. (Odontacartia) obtusukai</i>	Busan, Korea	MN603775–MN603777	Present study
<i>A. (Odontacartia) pacifica</i>	Nakajima Island, Japan	KC287267	Bucklin and Blanco-Bercial 2014
	Nakajima Island, Japan	DQ071177	Ueda and Bucklin 2006
<i>A. (Odontacartia) spinicauda</i>	Xiamen waters, China	DQ665253–DQ665254	Liu et al. 2006
<i>A. (Acartiura) omorii</i>	Gwangyang Bay, Korea	MN603778	Present study

**Etymology.** The specific name refers to the type locality of Nadi bay, Fiji.

**Description of female.** Total body length 975–1050  $\mu\text{m}$  (mean  $\pm$  SD = 1018  $\pm$  26  $\mu\text{m}$ ,  $n = 10$ , holotype 1015  $\mu\text{m}$ ) as measured from anterior margin of cephalosome to posterior margin of the caudal rami. Body surface armed with some sensillae (Fig. 1A). Prosome:urosome length ratio = 3.52:1.

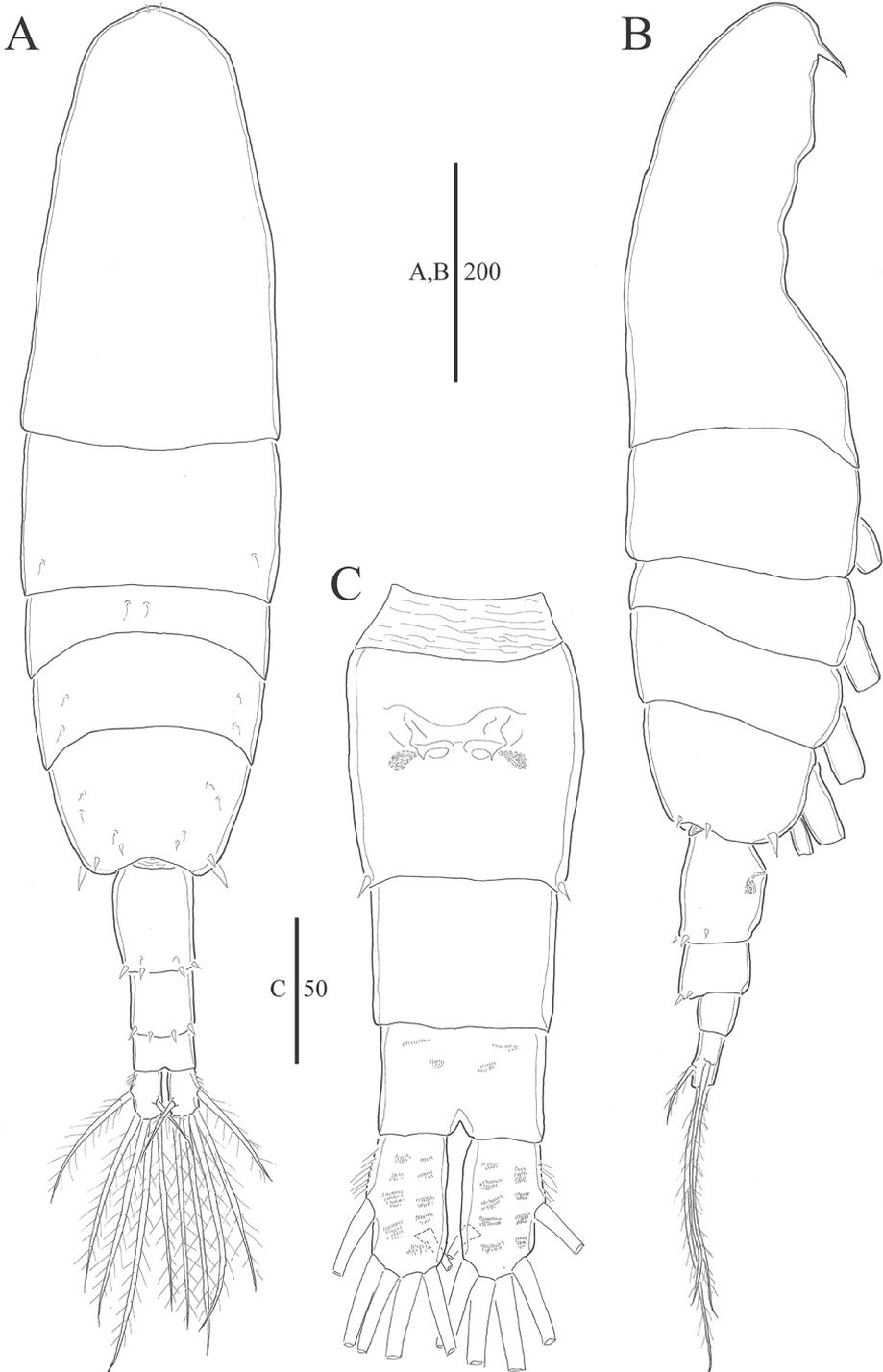
Prosome 5-segmented (Fig. 1A, B), cephalosome and first pedigerous somite completely separate; fourth and fifth pedigerous somite fused. Posterior corners of fifth pedigerous somite rounded, each with three spines. Rostral filaments thick and short (Figs 2A, 7A).

Urosome 3-segmented (Figs 1A–C, 7D–H, 8A), genital double somite slightly swollen anterolaterally, with paired gonopores ventromedially, each gonopore covered with pointed operculum; first and second urosomites each with four spines on postero-dorsal margin. Caudal rami bearing short hairs on lateral margin. Proportional lengths of urosomites and caudal rami as 38:23:17:22 = 100.

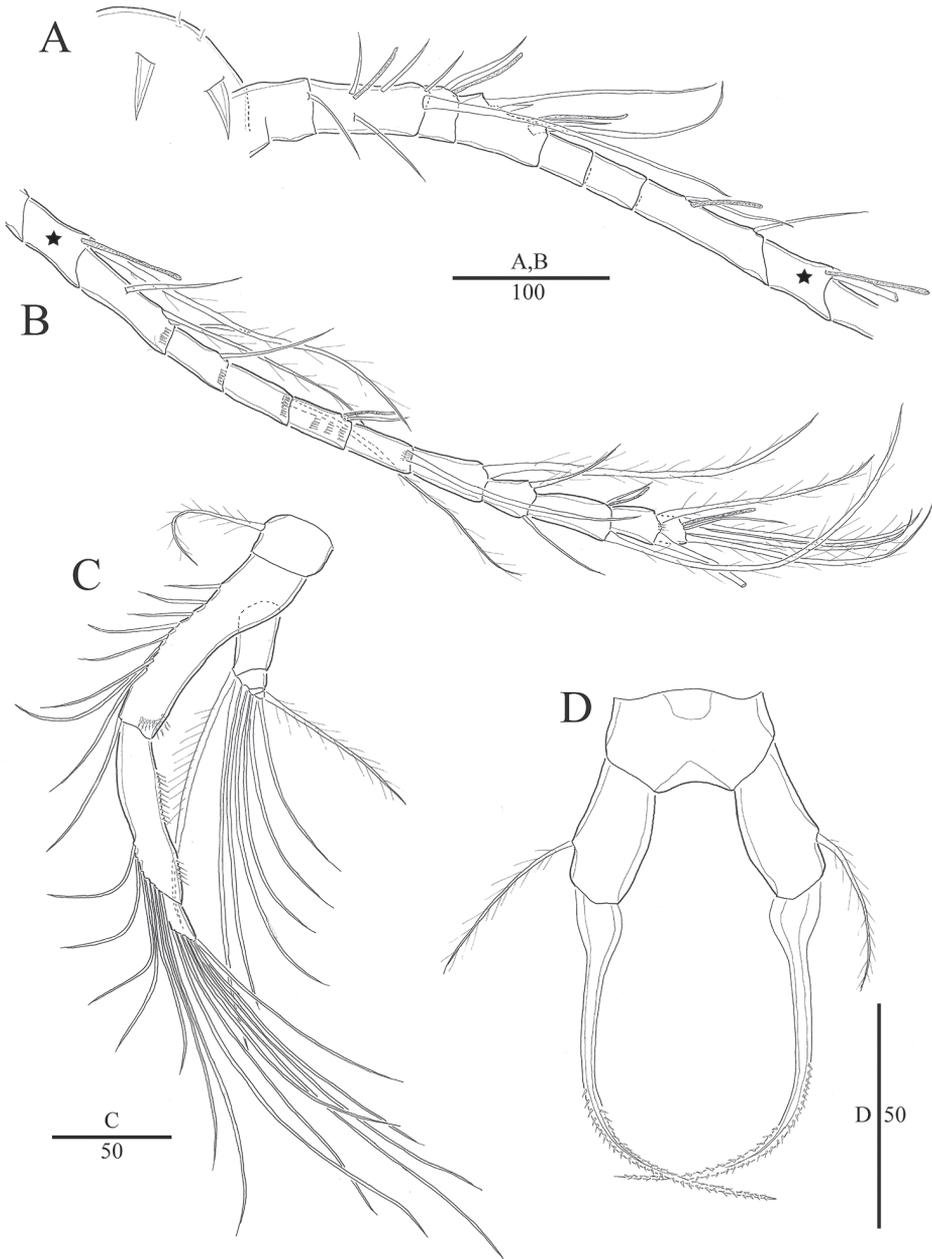
Antennule incompletely 18-segmented (Fig. 2A, B), fourth to seventh segments partly fused on dorsal surface; ninth to eleventh segment each with one row of setules, twelfth segment with three rows of setules, thirteenth and seventeenth segment each with one row of setules; segmentation and setation patterns as follows: (1) I-[1], (2) II-VI-[5+ae], (3) VII-[1+ae], (4) VIII-XI-[4(1spiniform)+ae], (5) XII-[0], (6) XIII-[0], (7) XIV-XV-[2+ae], (8) XVI-[1+ae], (9) XVII-XVIII-[2+ae], (10) XIX-[1], (11) XX-[1], (12) XXI-[1+ae], (13) XXII-[1], (14) XXIII-[1], (15) XXIV-[2(1+1)], (16) XXV-[2(1+1)+ae], (17) XXVI-[2(1+1)], (18) XXVII-XXVIII-[4+ae].

Antenna (Fig. 2C): coxa with seta; basis and first endopodal segment fused to form elongated allobasis bearing eight setae medially and one seta terminally along inner margin, and spinular row on distal area; second endopodal segment elongated, with seven setae, rows of spinules on lateral margin; third exopodal segment short, with seven setae. Exopod 4-segmented; setation formula 1, 2, 2, 3.

Mandible: (Fig. 3A) coxa with well developed gnathobase bearing eleven teeth; basis with seta and row of setules on lateral and posterior margins; endopod 2-segmented, first endopodal segment with two setae, second segment with seven setae; exopod 5-segmented, setation formula as 1, 1, 1, 1, 2.

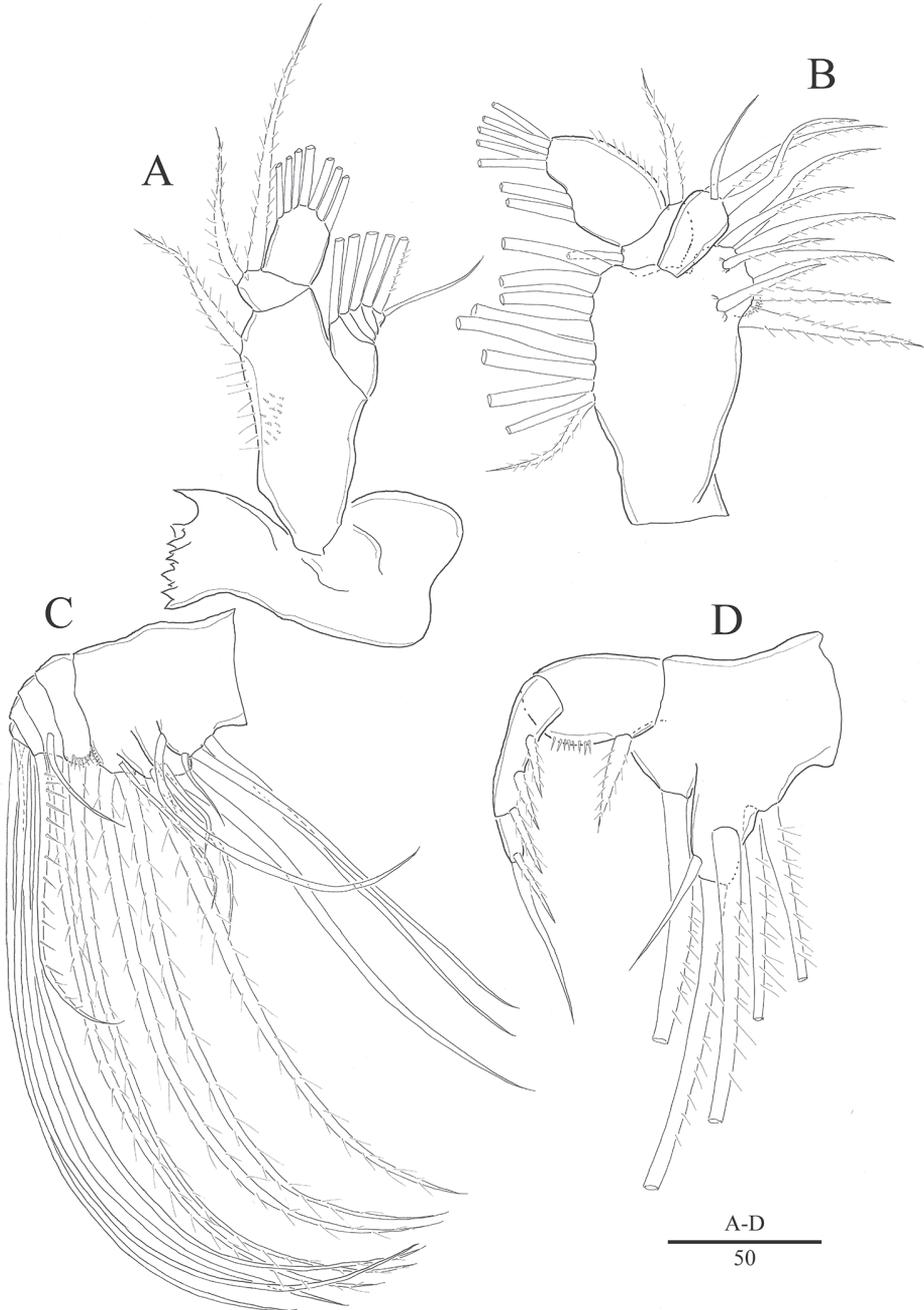


**Figure 1.** *Acartia nadiensis* sp. nov. female. **A** Habitus, dorsal **B** habitus, lateral **C** urosome, ventral. Scale bars: in  $\mu\text{m}$ .



**Figure 2.** *Acartia nadiensis* sp. nov. female. **A** Rostrum and antennule (part, 1<sup>st</sup> to 8<sup>th</sup> segment) **B** antennule (part, 8<sup>th</sup> to 18<sup>th</sup> segment) **C** antenna **D** P5. Scale bars: in  $\mu\text{m}$ .

Maxillule: (Fig. 3B) precoxa and coxa incompletely fused, praecoxal arthrite with eight setae; coxal endite with three setae; one short seta and eight long setae on coxal epipodite; basal endite with one seta; basal exite with one seta; 1-segment exopod with two setae laterally and five setae terminally; endopod absent.



**Figure 3.** *Acartia nadiensis* sp. nov. female. **A** Mandible **B** maxillule **C** maxilla **D** maxilliped. Scale bars: in  $\mu\text{m}$ .

Maxilla: (Fig. 3C) pre-coxa and coxa incompletely fused, setation formula of endites 4, 2, 2, 3; basal endite with a seta and row of spinules on distal margin; endopod 3-segmented, with setation formula 2, 2, 3.

Maxilliped (Fig. 3D) comprising syncoxa with six setae; basis with spiniform seta; endopod 2-segmented, first segment with three setae, second segment with two setae.

Legs 1–4 (Fig. 4A–D) biramous, each with 3-segmented exopod and 2-segmented endopod, and spinules along inner and outer margins as illustrated. Intercoxal sclerites well developed. Spine and setal formulae as follows:

	Coxa	Basis	Exopod	Endopod
P1	0-0	0-0	I-1; I-1; II, 1, 4	0-1; 1, 2, 3
P2	0-0	0-0	0-1; 0-1; 0, I, 5	0-2; 1, 2, 4
P3	0-0	0-0	0-1; 0-1; 0, I, 5	0-2; 1, 2, 4
P4	0-0	1-0	0-1; 0-1; 0, I, 5	0-3; 1, 2, 3

P5 (Figs 2D, 7B, C) symmetrical, 3-segmented; basis ovate, with outer seta; exopod tapering, thick, bent at midlength, distal portion serrated, base slightly swollen.

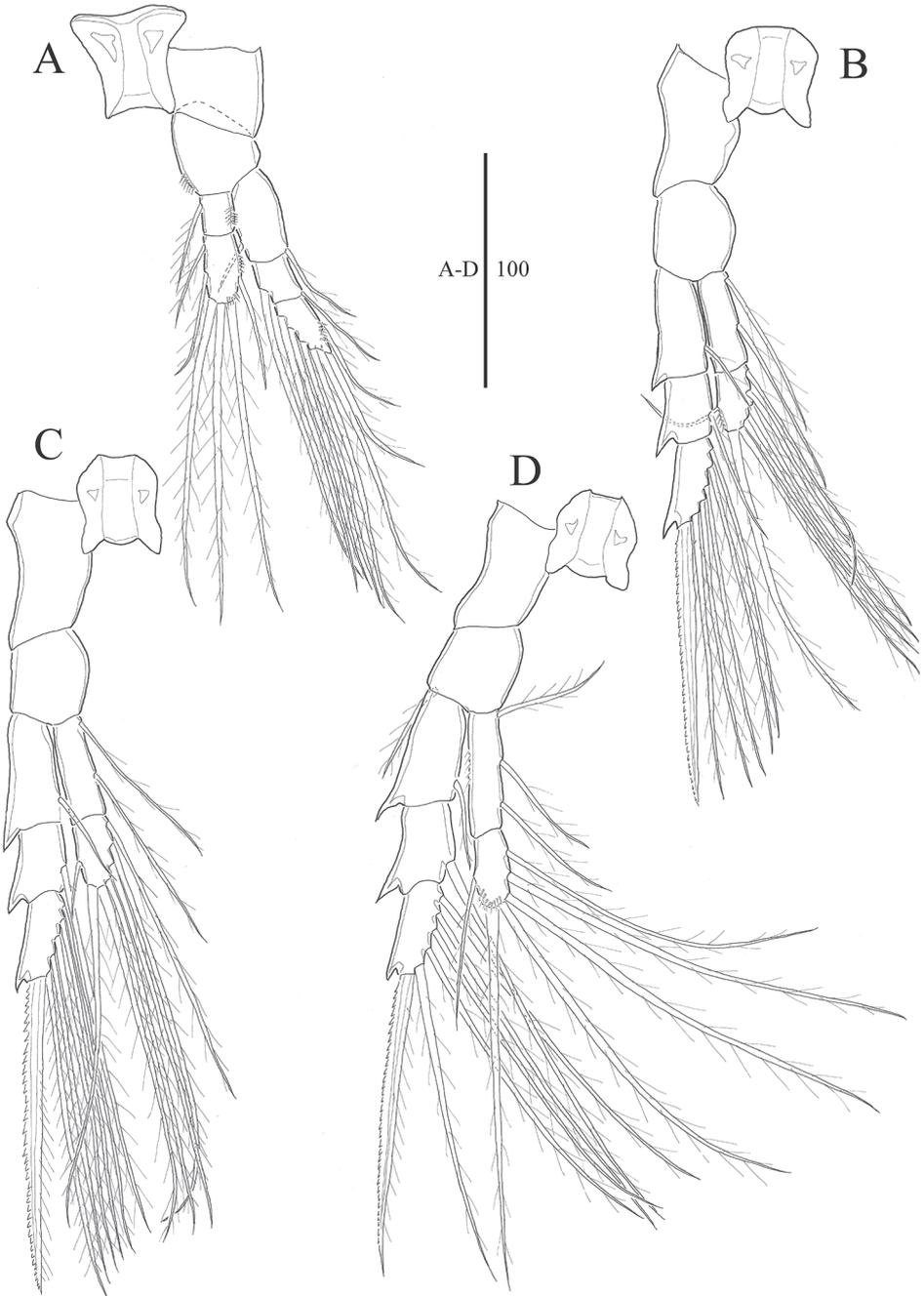
**Description of male.** Total body length 910–952  $\mu\text{m}$  (mean  $\pm$  SD = 931  $\pm$  16  $\mu\text{m}$ ,  $n = 5$ , allotype 930  $\mu\text{m}$ ) measured from anterior margin of cephalosome to posterior margin of caudal rami. Body surface armed with some sensilla (Fig. 5A, B). Prosome:urosome length ratio = 3.12:1.

Prosome (Fig. 5A, B) 5-segmented. Rostral filaments thin (Figs 5A, B, 8B). Fifth prosomite with six spines on posterior margin.

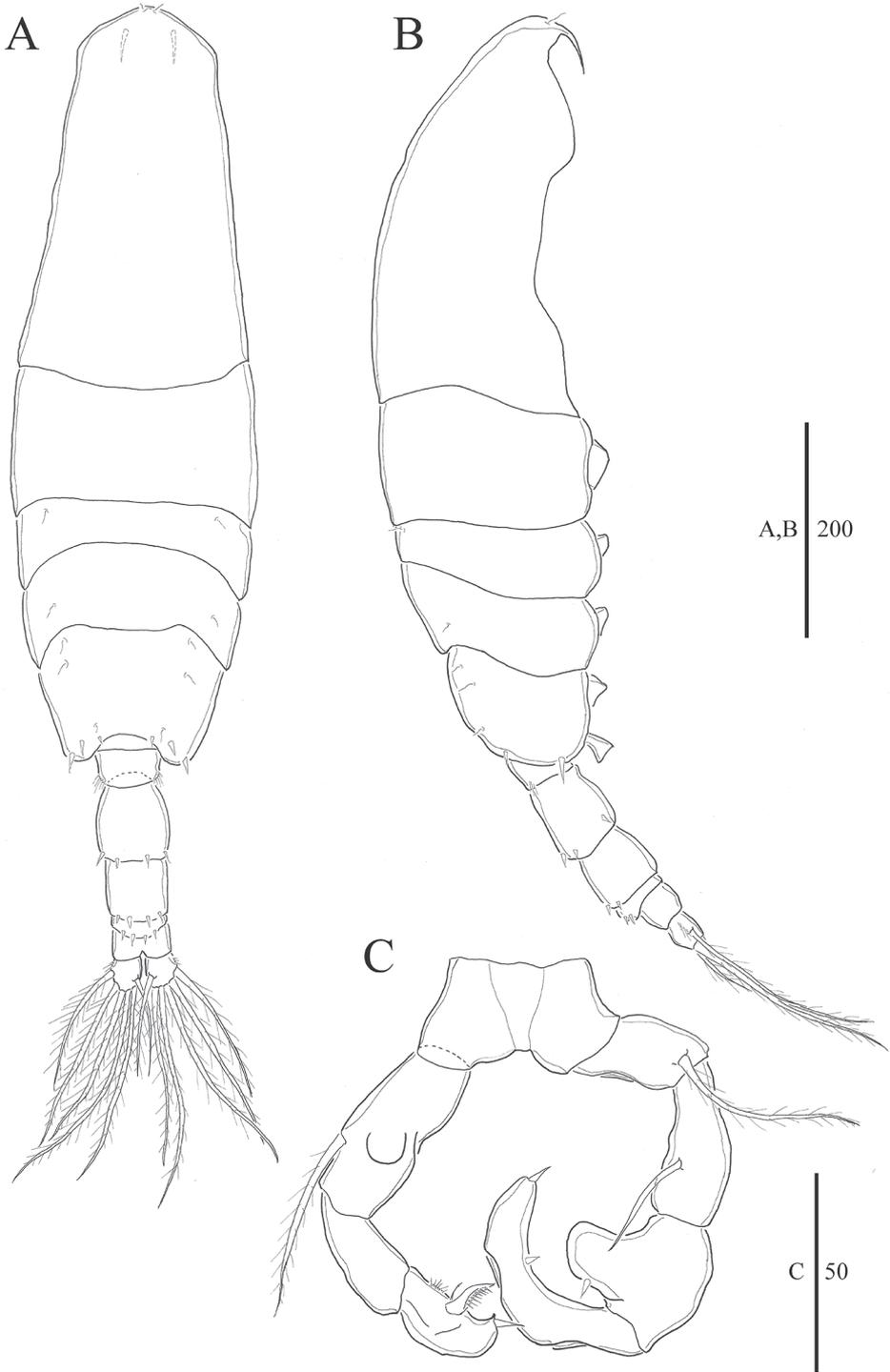
Urosome (Figs 6C, D, 8C–H) 5-segmented. Second urosomite with four spines on posterodorsal margin and two spines on posteroventral margin; pair of sensillae on dorsal surface. Third and fourth urosomites each with four spines on posterodorsal margin. Caudal rami bearing short hairs on lateral margin. Length proportions of urosomites to caudal rami as 16:31:21:7:12:14 = 100.

Left antennule 22-segmented (Fig. 6A). Segmentation and setation pattern as follows: (1) I-[1], (2) II-VII-[3+ae], (3) VIII-[2], (4) IX-[1+ae], (5) X-[2(1spiniform)], (6) XI-[2+ae], (7) XII-[0], (8) XIII-[0], (9) XIV-[2(1spiniform)+ae], (10) XV-[1], (11) XVI-[1+ae], (12) XVII-[1], (13) XVIII-[1+ae], (14) XIX-[1], (15) XX-[1], (16) XXI-[1+ae], (17) XXII-[1], (18) XXIII-[1], (19) XXIV-[2(1+1)], (20) XXV-[2(1+1)+ae], (21) XXVI-[2(1+1)], (22) XXVII-XXVIII-[4+ae]. Right antennule 18-segmented (Fig. 6B), with geniculation with fourteenth and fifteenth segments. Segmentation and setation pattern as follows: (1) I-[1], (2) II-VII-[3+ae], (3) VIII-[2], (4) IX -[1+ae], (5) X-XI-[3(1spiniform)+ae], (6) XII-[0], (7) XIII-[0], (8) XIV-[2(1spiniform)+ae], (9) XV-[1], (10) XVI-[1+ae], (11) XVII-[1], (12) XVIII-[1+ae], (13) XIX-[1], (14) XX-[1], (15) XXI-XXIII-[3+ae], (16) XXIV-XXV-[4(2+2)+ae], (17) XXVI-[2(1+1)], (18) XXVII-XXVIII-[4+ae].

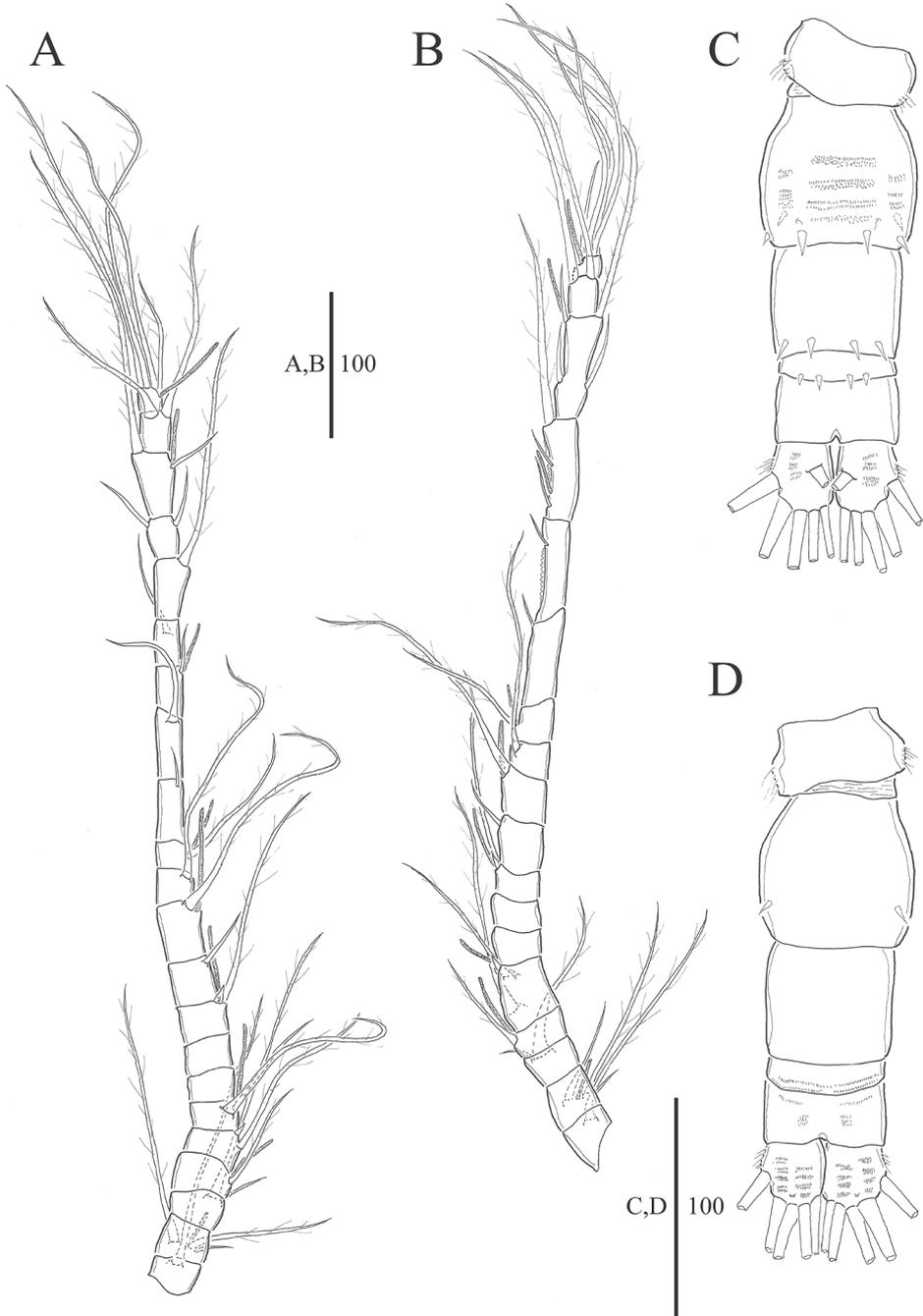
Other mouthparts and P1–P4 as in female. P5 asymmetrical (Fig. 5C); intercoxal sclerite distinct. Left leg 4-segmented; basis armed with posterolateral seta and rounded lobe on posterior surface; exopod 2-segmented, exp-1 unarmed; exp-2 with hairs, and one spine with teeth on medial margin and one small spine distally. Right leg 5-segmented, basis armed with posterolateral seta. Exopod 3-segmented, exp-1 with long slender seta; exp-2 with oblong inner lobe bearing one spine on distal margin; exp-3 with one spine on medial margin and one spine distally.



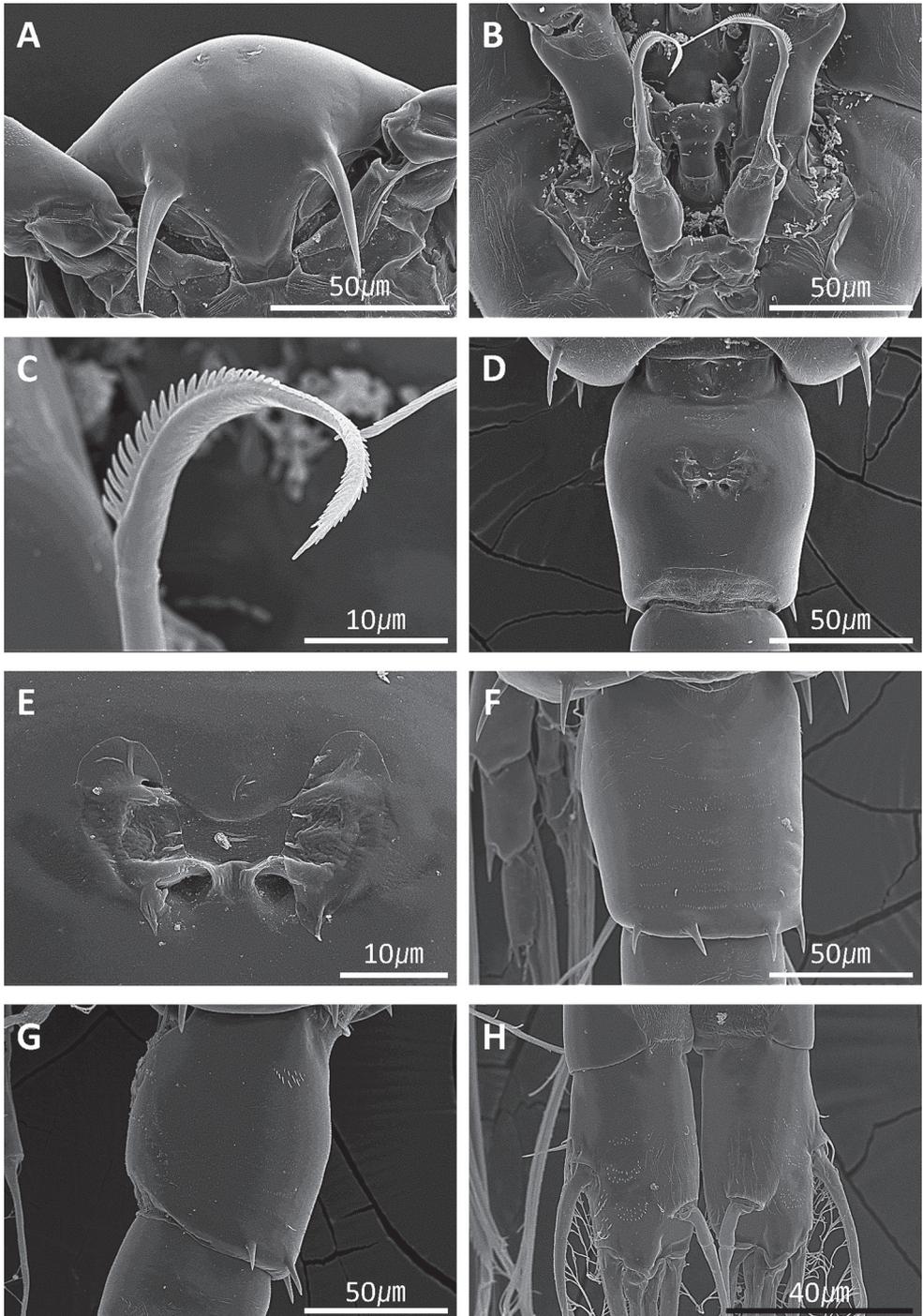
**Figure 4.** *Acartia nadiensis* sp. nov. female. **A** P1 **B** P2 **C** P3 **D** P4. Scale bars: in µm.



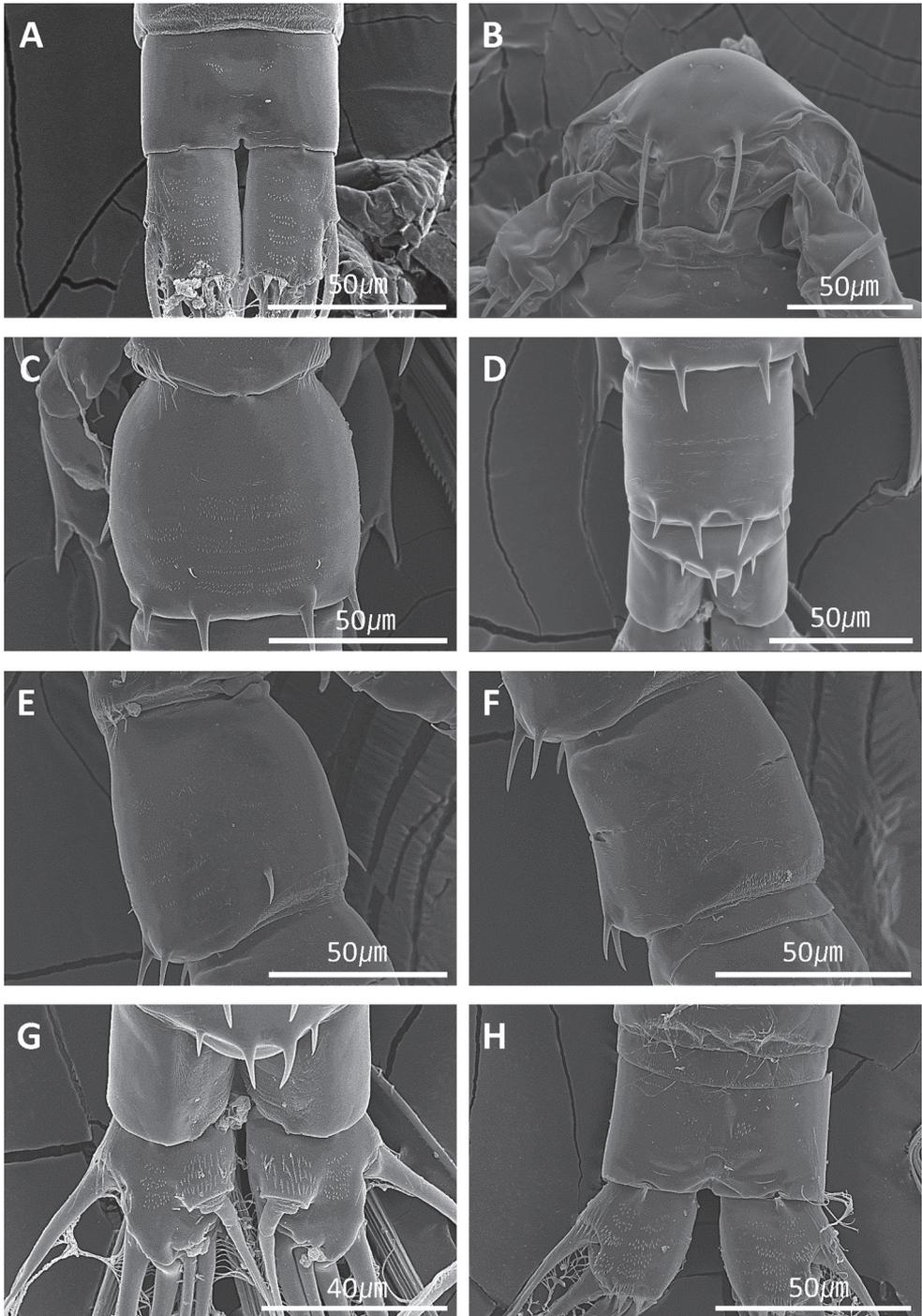
**Figure 5.** *Acartia nadiensis* sp. nov. male. **A** Habitus, dorsal **B** habitus, lateral **C** P5. Scale bars: in μm.



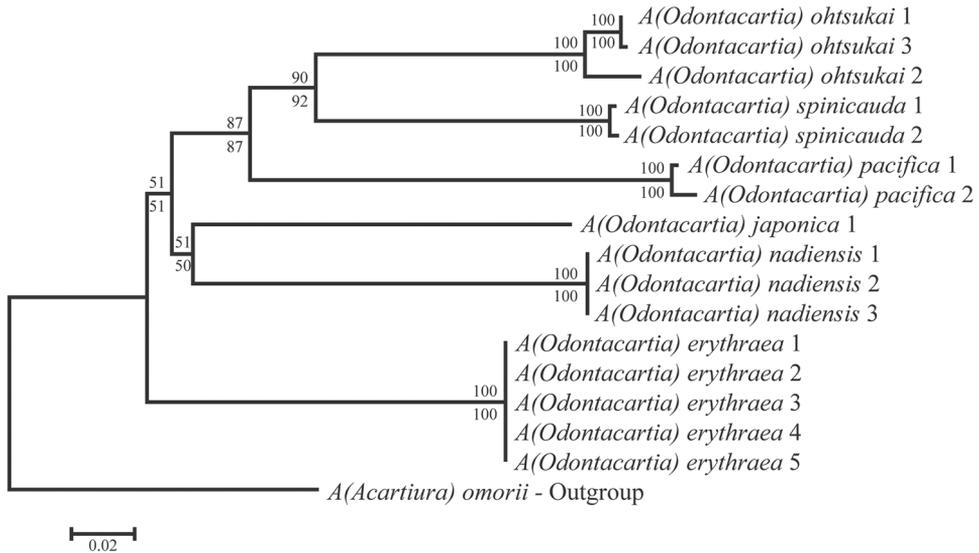
**Figure 6.** *Acartia nadiensis* sp. nov. male. **A** Antennule (left) **B** antennule (right) **C** urosome, dorsal **D** urosome, ventral. Scale bars: in  $\mu\text{m}$ .



**Figure 7.** *Acartia nadiensis* sp. nov. Scanning electron micrographs. **A** Female, rostrum **B** female, P5 **C** female, P5, terminal spine **D** female, genital double-somite **E** female, genital field **F** female, 1<sup>st</sup> urosomite, dorsal view **G** female, 2<sup>nd</sup> urosomite, lateral view **H** female, caudal rami, dorsal view. Scale bars: in  $\mu\text{m}$ .



**Figure 8.** *Acartia nadiensis* sp. nov. Scanning electron micrographs. **A** Female, urosome and caudal rami, ventral view **B** male, rostrum **C** male, 1<sup>st</sup> urosomite **D** male, 2<sup>nd</sup>–4<sup>th</sup> urosomite, dorsal view **E** male, 1<sup>st</sup> urosomite, lateral view **F** male, 2<sup>nd</sup> urosomite, lateral view **G** male, 5<sup>th</sup> urosomite and caudal rami, dorsal view **H** male, 4<sup>th</sup> urosomite and caudal rami, ventral view. Scale bars: in μm.



**Figure 9.** Phylogenetic tree based on mtCOI sequences (581 bp) of *Odontacartia* species including *A. (Acartiura) omorii* as outgroup. One-thousand bootstrap replicates were performed by MEGA6 using neighbor joining and minimum evolution methods. Neighbor joining bootstrap values shown above branches; minimum evolution bootstrap values are below branches.

## Molecular analysis

A 581 bp partial region of mtCOI was sequenced from five species: *A. nadiensis* sp. nov., *A. erythraea*, *A. japonica*, *A. ohtsukai*, and *A. omorii*. Sequences of two species (*A. pacifica* and *A. spinicauda*) were obtained from NCBI and also included in the analysis. All species belong to the subgenus *Odontacartia* except *A. omorii*, which belongs to the subgenus *Acartiura* and was used as the outgroup. The mtCOI sequences of *A. nadiensis* differed in a 24.1% from *A. japonica*, and in up to 29.0% from *A. pacifica* (Table 2). Neighbor joining and minimum evolution phylogenetic analyses using the Tamura-Nei model showed that *A. nadiensis* was clearly distinct from its congeneric species (Fig. 9).

## Discussion

The new species, *Acartia nadiensis* sp. nov., clearly belongs to the subgenus *Odontacartia*. This subgenus displays the following diagnostic characters compared to the other five subgenera of *Acartia*: presence of rostral filaments, symmetrical pointed process on the posterior corner of the last prosomite, minutely serrated terminal spine on female P5, and absence of protrusion on the basis of male right P5 (Steuer 1915; Ueda and Bucklin 2006; Soh 2010). The new species can be distinguished from other *Odontacartia* species by several distinctive characters (Table 3). First, *Odontacartia* species, including the new



**Table 3.** Morphological differences among species within the subgenus *Odontacartia* (Calanodia: Acartiidae: *Acartia*).

	<i>A. nadiensis</i> sp. nov.	<i>A. amboinensis</i>	<i>A. australis</i>	<i>A. bispinosa</i>	<i>A. boumani</i>	<i>A. centura</i>	<i>A. adenata</i>	<i>A. erythraea</i>	<i>A. japonica</i>	<i>A. liljeborgi</i>	<i>A. mertoni</i>	<i>A. obisakai</i>	<i>A. pacifica</i>	<i>A. spinicauda</i>
<b>Female</b>														
Body length	975–1050	1340–1510	1290–1400	1320–1530	1200–1300	1350–1400	1190–1230	1400	1350–1410	1330–1400	ND	1190–1230	1190–1210	1250
Spine on 1 <sup>st</sup> seg of antennules	absent	present	present	present	absent	absent	absent	present	present	present	absent	absent	absent	absent
<b>P5</b>														
Basis length/width ratio	2	4	2.5	2.4	1.5	1.6	1.4	2.3	2.1	1.4	2	1.8	1.4	1.4
Length ratio of P5 outer seta/terminal spine	0.4	1.8	1.2	1.4	0.9	1.6	1.3	1.6	0.7	1.5	1	1	1.8	1.2
<b>Urosome</b>														
Dorsal spines on 1 <sup>st</sup> urosomite	4	2	2	2	0	2	0	2	2	(small spinules)	2	2	2	2
Dorsal spines on 2 <sup>nd</sup> urosomite	4	4	0	0	2	2	2	2	(small spinules)	(small spinules)	2	2	2	2
Caudal rami length/width ratio	1.8	1.3	1.1	1.8	2	1.7	3	1.4	1.2	1.5	2	3	2.5	3
<b>Male</b>														
Body length	910–952	ND	1170–1230	1070–1160	1100	1250–1280	1080–1150	ND	1190–1240	1100	ND	1030–1050	1030–1160	ND
<b>Left P5</b>														
Length ratio of medial process/segment on 2 <sup>nd</sup> exopodite	0.5	ND	0.7	0.4	0.9	0.8	2	0.4	1	0.5	3.5	1.4	1.6	0.9
Type of medial process on 2 <sup>nd</sup> exopodite	Spine with teeth	ND	Spine	Spine with fine setae	Spine	Spine	Long seta	Spine	Spine with teeth	Spine	Long seta	Long seta	Long seta	Spine
<b>References</b>	This study	Tanaka 1965	Ueda 1986	Nishida 1985; El-Sherbiny and Al-Aidaros 2014	Abraham 1976	Abraham 1976	Srimui et al. 2019	Mori 1964	Ueda 1986	Giesbrecht 1892	Steuer 1923; Ueda and Bucklin 2006	Ueda and Bucklin 2006	Ueda and Bucklin 2006	Giesbrecht 1982; Mori 1964

species, can be divided into two groups based on the presence of a spine on the first segment of antennules (Steuer 1923; Srinui et al. 2019). Species with this spine include *A. amboinensis*, *A. australis*, *A. bispinosa*, *A. erythraea*, *A. japonica*, and *A. lilljeborgi*. Species lacking spine include *A. bowmani*, *A. centura*, *A. mertoni*, *A. ohtsukai*, *A. pacifica*, and *A. spinicauda*. *Acartia nadiensis* sp. nov. also lacks a spine on the first segment of antennules. Second, the outer seta of the female P5 of *A. nadiensis* sp. nov. is much shorter than the terminal spine, and the length ratio of the outer seta/terminal spine is 0.4. Most species of *Odontacartia* have an outer seta that is longer than the terminal spine in female P5. There are two species (*A. bowmani* and *A. japonica*) that have a short terminal seta on female P5, and the length ratio of outer seta/terminal seta are 0.9 and 0.7, respectively. Third, the male P5 of *A. nadiensis* is clearly distinguishable from the rest of species based on its length and the type of medial process on the exp-2 of the left leg. Furthermore, the new species shows other minor differences compared to the other 13 *Odontacartia* species, such as the number of dorsal spines on the urosomite, the length/width ratio of the female P5 basis, and the length/width ratio of caudal rami.

To supplement the morphological evidences, we conducted molecular phylogenetic analyses using partial mtCOI sequences of six *Odontacartia* species, including the new species. The mtCOI gene is widely used to identify sibling species due to its higher evolutionary rate than 16s and 18s rDNA (Knowlton and Weight 1998; Hebert et al. 2003; Schindel and Miller 2005; Karanovic et al. 2018). In previous studies of calanoid copepods, mtCOI sequence divergence between species have been shown to range from 13.0–22.0% (Bucklin et al. 1999), 17.6–26.7% (Eyun et al. 2007), and 21.0–23.0% (Soh et al. 2013). The mtCOI partial sequence of *A. nadiensis* sp. nov. differed by 24.1–29.0% from the sequences of congeneric species, which is greater than the range of interspecific differences reported in previous studies.

The length ratio of the outer seta/terminal spine of the female P5 is the most diagnostic morphological feature in *Odontacartia* species. However, this character is also used to determine the subgenus *Euacartia* (Soh et al. 2013). This confusion between subgenus systems has been documented previously (Madhupratap and Haridas 1994). Barthélémy (1999) compared female genital structure of 25 species of Acartiidae using light and scanning electron microscopy and concluded that there is no support for the current subdivision of *Acartia* into subgenera. Although the new species *A. nadiensis* belongs to the subgenus *Odontacartia* based on the current identification system, the validity subgeneric taxa, as proposed by Steuer (1915, 1923), within *Acartia* should be reevaluated.

### Key to species of the subgenus *Odontacartia* Steuer, 1915

- |   |   |                       |
|---|---|-----------------------|
| 1 | Presence of spine on 1 <sup>st</sup> to 2 <sup>nd</sup> segments of female antennule..... | 2                     |
| – | Absence of spine on 1 <sup>st</sup> to 2 <sup>nd</sup> segments of female antennule.....  | 5                     |
| 2 | Small spinule row present on dorsal surface of female 1 <sup>st</sup> urosomite .....     |                       |
|   | .....   | <i>A. lilljeborgi</i> |
| – | Strong spines present on dorsal surface of female 1 <sup>st</sup> urosomite .....         | 3                     |

3	Absence of processes (spines and spinules) on dorsal surface of female 2 <sup>nd</sup> urosomite.....	4
–	Small spinule row present on dorsal surface of female 2 <sup>nd</sup> urosomite .....	<i>A. japonica</i>
–	2 strong spines present on dorsal surface of female 2 <sup>nd</sup> urosomite.....	<i>A. erythraea</i>
–	4 strong spines present on dorsal surface of female 2 <sup>nd</sup> urosomite.....	<i>A. amboinensis</i>
4	Length-width of female caudal rami are almost similar; medial process on 2 <sup>nd</sup> exopodite of male left P5 as spine .....	<i>A. australis</i>
–	Female caudal rami almost twice longer than wide; medial process on 2 <sup>nd</sup> exopodite of male left P5 as spine with fine seta.....	<i>A. bispinosa</i>
5	Dorsal surface of female 1 <sup>st</sup> urosomite devoid of processes (spines and spinules).....	6
–	Spine present on dorsal surface of female 1 <sup>st</sup> urosomite .....	7
6	Female caudal rami twice longer than wide; medial process and 2 <sup>nd</sup> exopodite segment of male left P5 almost similar in length .....	<i>A. boumani</i>
–	Female caudal rami three times longer than wide; medial process of male left P5 twice longer than 2 <sup>nd</sup> exopodite segment.....	<i>A. edentata</i>
7	Dorsal surface of female 1 <sup>st</sup> and 2 <sup>nd</sup> urosomite with two strong spines.....	8
–	Four strong spines on dorsal surface of female 1 <sup>st</sup> and 2 <sup>nd</sup> urosomite .....	<i>A. nadiensis</i> sp. nov.
8	Length of female P5 outer seta and terminal spine similar .....	9
–	Female P5 outer seta is longer than terminal spine.....	10
9	Female caudal rami is twice as long as wide.....	<i>A. mertoni</i>
–	Female caudal rami three times longer than wide.....	<i>A. obtusukai</i>
10	Length-width ratio of female caudal rami as 1.7; medial process on 2 <sup>nd</sup> exopodite male left P5 as spine.....	<i>A. centura</i>
–	Length-width ratio of female caudal rami as 2.5; medial process on 2 <sup>nd</sup> exopodite of male left P5 as long seta .....	<i>A. pacifica</i>
–	Length-width ratio of female caudal rami as 3; medial process on the 2 <sup>nd</sup> exopodite of male left P5 as spine.....	<i>A. spinicauda</i>

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# Two new species of the genus *Caligus* (Crustacea, Copepoda, Siphonostomatoida) from the Sea of Japan, with a note on the establishment of a new species group

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## Abstract

Two new species of *Caligus* are described from the Japanese coast of the Sea of Japan. *Caligus chinglonglini* **sp. nov.** is based on a male specimen found in a plankton sample, whereas *C. kajii* **sp. nov.** was collected from the body surface of the host flathead *Platycephalus* sp. These two new species can be assigned to a distinct species group, the *pseudorhombi* group newly named and defined by the morphology of the genital complex in both sexes, and by the structure and armature of legs 2 and 4. The species group so far accommodates 19 species including these two new species. The morphology, host specificity and zoogeography of the species group are discussed herein and keys to species groups of *Caligus* and to species of the *C. pseudorhombi* species group are provided.

## Keywords

caligid copepods, plankton, *Platycephalus*, sea lice, taxonomy

## Introduction

Members of the genus *Caligus* Müller, 1785 are known as sea lice and several species are known to cause serious economic losses in marine fish farming facilities (Ho and Lin 2004b; Johnson et al. 2004, Costello 2009; Shinn et al. 2015a, b). The life cycle of sea lice and their host specificity have been the subject of intensive studies designed to develop methods of controlling these pests. The life cycles of caligid copepods have been shown to be more diverse than expected. The general pattern is for a species to utilize a single host for all post-copepodid stages, after infection of the host fish (Ho and Lin 2004b; Dojiri and Ho 2013). A less common type of life cycle is found in the so-called “planktonic caligids” such as *Caligus undulatus* Shen & Li, 1959, *C. ogawai* Venmathi Maran, Ohtsuka & Shang, 2012 and *C. ilhoikimi* Suárez-Morales & Gasca, 2016, which exhibit a dual mode of life with adults found both on the host fish and free in the water column (Shen and Li 1959; Ho and Lin 2004a; Venmathi Maran and Ohtsuka 2008; Venmathi Maran et al. 2012a, b, 2016; Suárez-Morales et al. 2012a, b; Suárez-Morales and Gasca 2016; Kim et al. 2019). A third life cycle pattern is found in species which conduct host switching after the final molt of the chalimus phase and require both an intermediate and a final host (Hayward et al. 2009; Ohtsuka et al. 2018). The third type is rare and has so far been recorded only in three species infecting farmed fish (Hayward et al. 2008, Ohtsuka et al. 2018), although Cressey and Cressey (1980) suspected that the adults of *Caligus biserioidentatus* Shen, 1957 occurred on different host species from the immature stages.

An undescribed species of *Caligus* was found in a plankton sample collected at Ashibe Port, Iki Island, Nagasaki Prefecture, Japan on May 24, 2014. Another undescribed species was found infecting the flathead *Platycephalus* sp. caught off Shimonoseki City, Yamaguchi Prefecture, Japan in the Sea of Japan in 2016. Since these two undescribed species belong to a distinct species group of *Caligus*, they are described together in the present paper, together with remarks on taxonomy, host specificity and distribution of members of the species group.

## Materials and methods

A single male specimen of *Caligus* was found in a plankton sample collected by towing a small plankton net around an underwater fishing light (KU-5MB, Koto Electric Co., Ltd.) at Ashibe Port, Iki Island, Nagasaki Prefecture, Japan (33°48.54'N, 129°45.231'E) during the night-time of May 24, 2014. This becomes the holotype of a new species. A second undescribed species was found infecting the body surface of the flathead *Platycephalus* sp. (total length 58 cm) caught by fishing off Shimonoseki City, Yamaguchi Prefecture, Japan (34°00.686'N, 130°53.756'E) in the morning of August 24, 2016. The copepods were fixed in 70% ethanol immediately after capture. After immersing the copepod specimens in lactophenol, these were examined using Humes and Gooding's (1964) slides on a differential interference microscope (BX-53, Olympus Co., Ltd.) equipped with a drawing tube. Body lengths were measured from

the frontal margin of the cephalothorax to the posterior margin of the caudal ramus excluding the caudal setae. Protists epibiontic on the undescribed caligid collected from Iki Island were photographed with a digital camera (DP21, Olympus Co., Ltd.) attached to the microscope. Terminology essentially follows Ho and Lin (2004b).

Type specimens are deposited at the National Museum of Natural History and Science, Tsukuba, Japan (NSMT-Cr).

## Taxonomy

### Order Shiphonostomatoida Thorell, 1859

### Family Caligidae Burmeister, 1835

### Genus *Caligus* Müller, 1785

#### *Caligus chinglonglini* sp. nov.

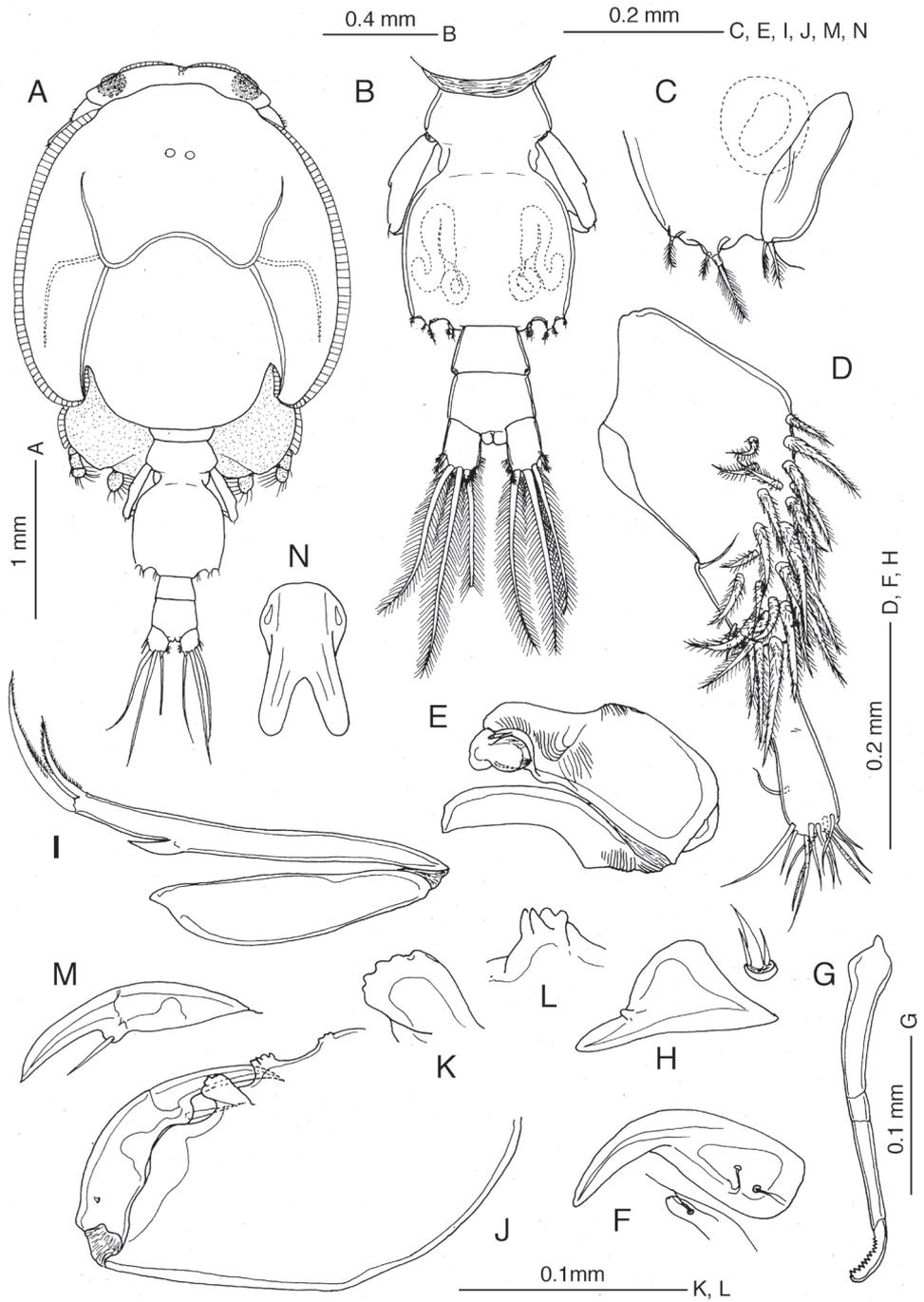
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Figs 1–3, Table 1

**Material examined and type. *Holotype.*** JAPAN • adult ♂; Ashibe Port, Iki Island, Nagasaki Prefecture (33°48.544'N, 129°45.231'E); night, May 24, 2014; partly dissected and mounted on 1 slide, body in vial (NSMT-Cr 26753); S. Ohtsuka leg.

**Description. Male. *Body*** 4.02 mm long. Cephalothorax (Fig. 1A) slightly longer than wide. Pediger 4 (Fig. 1A, B) incompletely fused to genital complex. Genital complex (Fig. 1A, B) about 1.1 times wider than long, about 1.2 times longer than abdominal somites combined, produced posterolaterally into 2 knobs representing leg 5 (Fig. 1C), armed with 1 and 2 setae. Paired genital opercula (Fig. 1C) representing leg 6, each bearing 2 minute unequal setae terminally. Abdomen 2-segmented, second somite ca. 1.7 times longer than first. Caudal ramus (Fig. 1B) furnished with row of fine setules along inner margin; setae II and III located close together subterminally; setae IV–VI well developed; seta VII minute, located at inner distal corner.

Antennule (Fig. 1D) 2-segmented; proximal segment with 26 setae, distal segment with 11 setae and 2 aesthetascs. Antenna (Fig. 1E) 3-segmented; proximal segment unarmed, with adhesion pad distally; middle segment massive, unarmed, with 2 adhesion pads at mid-length and 1 pad terminally; distal segment small, multi-layered flap with 2 small setae proximally. Postantennal process (Fig. 1F) moderately curved, with 2 bisensillate papillae proximally plus bisensillate papilla on adjacent ventral cephalothoracic surface. Mandible (Fig. 1G) with 11 teeth on margin subterminally. Maxillule (Fig. 1H) represented by anterior papilla with 1 thick and 2 fine setae and posterior dentiform process with rounded prominence subterminally. Maxilla (Fig. 1I) 2-segmented, lacertus (syncoxa) unarmed, brachium (basis) slender, with large hyaline membrane at terminal third, plus long calamus and short cana apically. Maxilliped (Figs 1J–M, 3B) heavily chitinized, 2-segmented; corpus (protopod) massive, with 3 unequal processes along myxal margin, proximal process low, middle process with multiple tips, distal process largest,



**Figure 1.** *Caligus chinglonglini* sp. nov., adult male, holotype **A** habitus, dorsal view **B** postcephalothoracic trunk, dorsal view **C** leg 5 and gonopore on right side, ventral view **D** antennule, ventral view **E** antenna **F** postantennal process **G** mandible **H** maxillule **I** maxilla **J** maxilliped **K** middle process on myxal margin of maxilliped **L** distal process on myxal margin of maxilliped **M** subchela of maxilliped **N** sternal furca.

with irregular, undulating distal margin; shaft (endopod) as long as and incompletely fused to claw to form subchela; barbel located on rounded inner basal process of claw. Sternal furca (Fig. 1N) with divergent tines originating close together, rounded at tip.

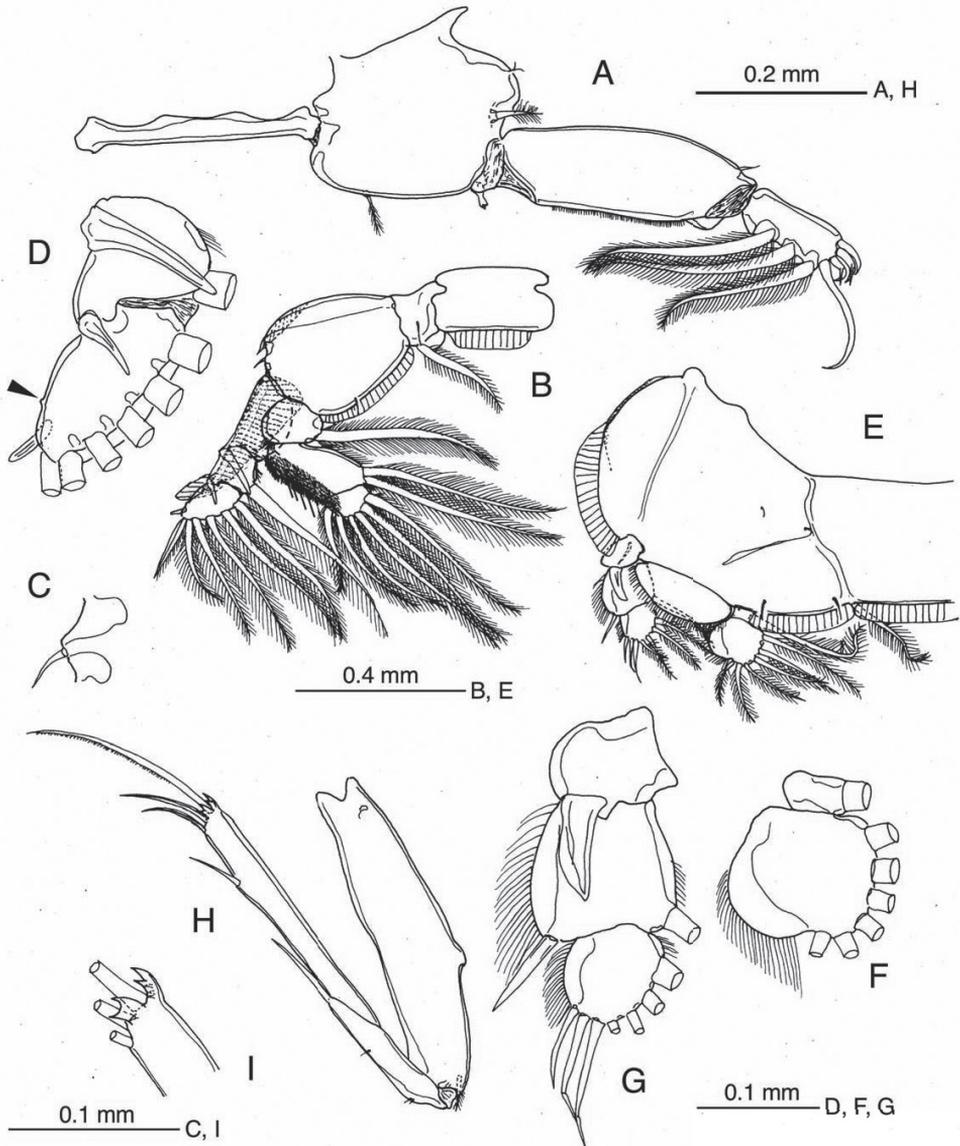
Armature and elements of legs 1–4 as in Table 1. **Leg 1** (Fig. 2A) with massive protopod bearing 1 inner and 1 outer small plumose setae plus bifid setule on outer margin; intercoxal sclerite slender, unornamented; endopod reduced to club-shaped process located near base of exopod; exopod 2-segmented, first segment with row of fine setules along inner margin and 1 naked seta at outer distal corner, second segment with 3 large plumose setae along inner (posterior) margin and 4 elements terminally, middle two of which each bearing accessory process. **Leg 2** (Fig. 2B–D) with intercoxal sclerite ornamented with trapezoidal marginal membrane along posterior margin; coxa with large plumose seta at posterior corner and minute setule on anterior surface; basis ornamented with marginal membrane on both inner and outer edges, bearing 1 minute seta on knob at outer distal corner (Fig. 2C) plus setule near midpoint of inner margin; endopod 3-segmented, outer margins of second and third segments with dense patches of minute setules; exopod 3-segmented, first segment with long outer spine directed obliquely across surface of second segment, second segment with relatively short outer spine, third segment with small outer knob (arrowed in Fig. 2D), 1 reduced outer spine, 1 short terminal spine and 5 plumose setae increasing in size from apical to innermost. **Leg 3** (Fig. 2E–G) apron (protopod) without surface processes, bearing well developed inner seta and 1 minute outer seta, plus 2 relatively long setules along posterior margin; outer basal margin of apron undulating; endopod 2-segmented, proximal segment small, with 1 long plumose seta; velum developed, hirsute along free posterior margin; second segment expanded along outer margin (Fig. 2F); exopod 3-segmented (Fig. 2G), proximal segment small, with slightly curved outer spine not reaching distal border of next segment, middle segment with 1 inner plumose and 1 outer naked seta, third segment with 3 spiniform setae increasing in size distally plus 4 inner setae.

**Leg 4** (Figs 2H, I, 3A) with protopod bearing low outer prominence at mid-length and minute plumose seta at outer distal corner; exopod distinctly 2-segmented, first exopodal segment with long outer spine almost fused basally to segment and reaching more than half distance to origin of proximalmost outer spine on compound second segment; second segment with 1 terminal and 2 slender spines on distal margin plus lateral spine, plus 2 bifurcate processes terminally; each process complex, with 1 or 3 minute prominences basally (Fig. 2I).

**Leg 5** (Fig. 1C) represented by 2 small knobs, outer knob bearing protopodal seta, inner knob representing exopod, bearing 2 plumose setae terminally. Leg 6 (Fig. 1C) consisting of genital operculum, bearing 2 terminal minute setae.

**Table 1.** Armature and elements of legs 1 to 4 of *Caligus chinglonglini* sp. nov.

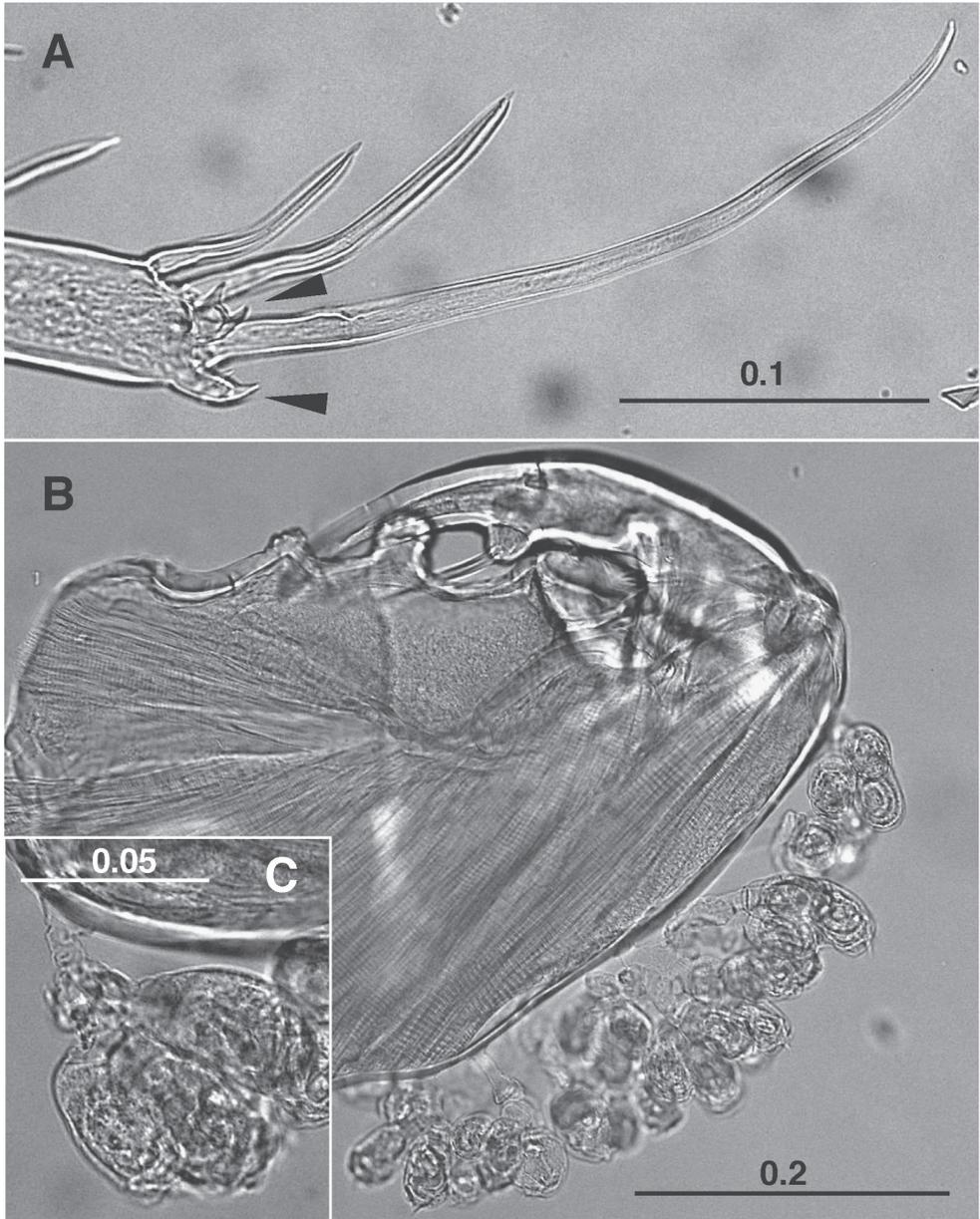
	Protopod (coxa; basis)	Endopod	Exopod
Leg 1	1-1	(vestigial)	1-0; III, 1, 3
Leg 2	0-1; 1-0	0-1; 0-2; 6	I-1; I-1; I, I, 5
Leg 3	1-1	0-1; 6	I-0; I-1; 3, 4
Leg 4	1-0	(absent)	1-0; I, III



**Figure 2.** *Caligus chinglonglini* sp. nov., adult male, holotype **A** leg 1 **B** leg 2 **C** outer seta on basis of leg 2 **D** terminal part of exopod of leg 2, outer knob on segment 3 arrowed **E** leg 3 **F** endopod of leg 3 **G** exopod of leg 3 **H** leg 4 **I** terminal processes of leg 4.

**Female.** Unknown.

**Remarks.** The new species is most closely related to *C. acanthopagri* Ho, Lin & Chen, 1994, *C. dieuzeidei* sensu Shiino (1954b), and *C. latigenitalis* Shiino, 1954 in general appearance and in the structure of the appendages and sternal furca. As Izawa and Choi (2000) and Ho and Lin (2004b) suggested, the minor but most distinct dif-



**Figure 3.** *Caligus chinglonglini* sp. nov., adult male, holotype **A** terminal part of leg 4, bifurcate processes arrowed **B** colony of peritrich ciliates attached along posterior margin of maxilliped corpus **C** peritrich ciliates attached on posterior margin of maxilliped corpus. Scale bars: in mm.

ference can be found in the structure of the pectens of the second exopod segment of leg 4 among these three species. Those of *C. chinglonglini* sp. nov., *C. dieuzeidei* sensu Shiino (1954b) (see Discussion), and *C. latigenitalis* are sharply indented, whereas that

of *C. acanthopagri* is composed of a hyaline membrane. The former three species can be distinguished by the number and shape of dentate processes (divided into 3 or 5 prominences but not hand-like in *C. chinglonglini*; 3 or 4 and hand-like in *C. dieuzeidei* sensu Shiino; 4 or 5 and hand-like in *C. latigenitalis*). In addition, the shape and numbers of processes of the maxillipedal myxal area differ among the males of these three species. In *C. chinglonglini* sp. nov. and *C. latigenitalis*, there are 3 processes arrayed along the myxal margin, but the middle process is furnished with serrated tips in the former but is rounded in the latter. In *C. dieuzeidei* sensu Shiino (1954b), there are only two processes, one quadrangular and the other low triangular, present along the margin.

Although the present new species is described on the basis of a single male, no other species belonging to the newly proposed *pseudorhombi* species group (see Discussion) has so far been recorded from Japanese waters except for *C. latigenitalis* (Nagasawa et al. 2010) in which only females were originally described by Shiino (1954a) and subsequently both sexes were redescribed in detail by Izawa and Choi (2000). These two species are distinguishable as mentioned above. In addition, *C. bifurcus* Shen, 1958, assigned to the same species group was described from Chinese waters based only on two females, but the non-sexually dimorphic characteristics such as sternal furca and legs differ distinctly from those of *C. chinglonglini* sp. nov. Therefore, the establishment of the present new species is justified.

The new species is the fourth species of *Caligus* found exclusively from plankton samples in Japan (see Venmathi Maran et al. 2016, table 2).

Peritrich ciliates were attached along the posterior margin of both maxillipeds (Fig. 3B, C) and on the ventral side of the cephalothorax. Epibiont suctorian and peritrich ciliates have already been recorded from species of *Caligus* and *Lepeophtheirus* von Nordmann, 1832 (Stone and Bruno 1989; Gresty and Warren 1993; Fernandez-Leborans et al. 2005). In *L. salmonis* (Krøyer, 1837) collected from Hokkaido, Japan, the peritrich *Epistylis* sp. attached mainly to the antennae and legs 2 and 3. This is the first record of the occurrence of epibiont peritrich ciliates on “pelagic caligids” (Ho and Lin 2004a; Venmathi Maran and Ohtsuka 2008; Venmathi Maran et al. 2012a, b, 2016).

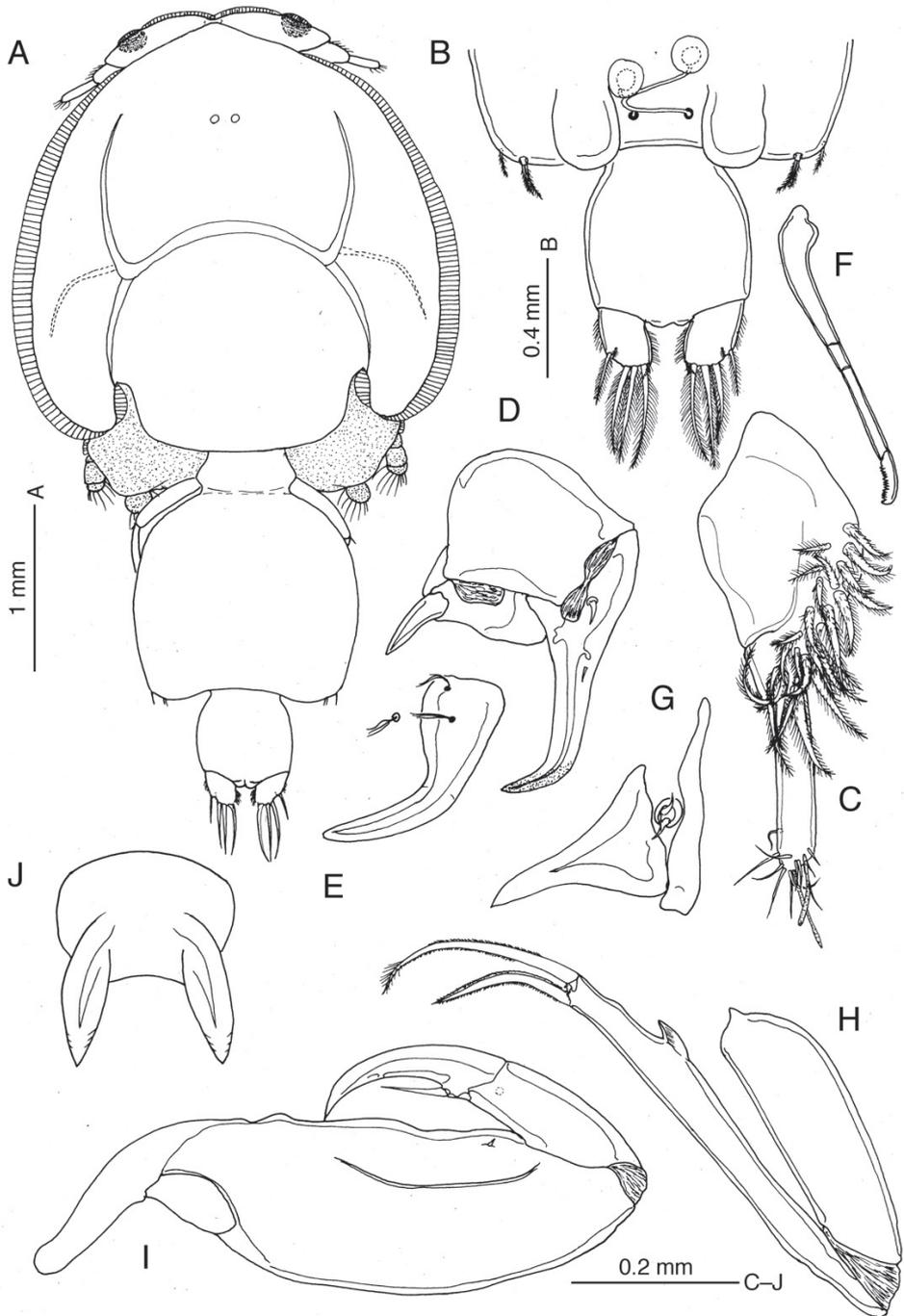
**Etymology.** The new species is named in honor of the late Dr Ching-long Lin who made a great contribution to the taxonomy of parasitic copepods together with Prof. Ju-shey Ho.

***Caligus kajii* sp. nov.**

<http://zoobank.org/9A3A7B46-6140-4865-AAFA-99E718FBA8D6>

Figs 4–6, Table 2

**Material examined.** JAPAN • 38 adult ♀♀ and 14 adult ♂♂; parasitic on body surface of *Platycephalus* sp. (total length 58 cm) collected from a depth of 15 m off Shimomoseki, Yamaguchi Prefecture (34°00.686'N, 130°53.756'E); morning of August 24, 2016; S. Ohtsuka leg.



**Figure 4.** *Caligus kajii* sp. nov., adult female, paratype **A** habitus, dorsal view **B** posterior end of genital complex plus abdomen, ventral view **C** antennule **D** antenna **E** postantennal process **F** mandible **G** maxillule **H** maxilla **I** maxilliped **J** sternal furca.

**Types. Holotype.** JAPAN • 1 ovigerous adult ♀; parasitic on body surface of *Platycephalus* sp. (total length 58 cm) collected from a depth of 15 m off Shimonoseki, Yamaguchi Prefecture (34°00.686'N, 130°53.756'E); morning of August 24, 2016; whole specimen (NSMT-Cr 26754); S. Ohtsuka leg. **Allotype.** JAPAN • 1 adult ♂, same data as in holotype; partly dissected on 1 slide, body in vial (NSMT-Cr 26755); S. Ohtsuka leg. **Paratypes.** JAPAN • 1 ♀, same data as in holotype; partly dissected and bodies in vials (NSMT-Cr 26756); 36 ♀♀ and 13 ♂♂, same data as in holotype; whole specimens (NSMT-Cr 26757); S. Ohtsuka leg.

**Description. Female.** Body length of holotype 6.16 mm, 4.86–6.16 mm in holotype and female paratypes (mean ± standard deviation = 5.49 ± 0.32 mm,  $N = 38$ ). Dorsal cephalothoracic shield subcircular, almost as long as wide (Fig. 4A). Lunules (Fig. 4A) relatively small. Pediger 4 almost completely fused to genital complex. Genital complex (Fig. 4A) subquadrate, about 1.14 times longer than wide, produced posteroventrally into pair of rounded processes between which paired copulatory pores located (Fig. 4B). Spermatophores (Fig. 4B) attached to copulatory pores via fine tubules; spermatophore proper globular, ca. 0.12 mm in diameter. Paired egg strings of holotype female containing 22 and 24 eggs. Abdomen (Fig. 4A, B) 1-segmented, about as long as wide. Caudal ramus (Fig. 4A, B) furnished with rows of setules along inner and outer margins; seta II minute, located near base of seta III on subterminal ventral surface, seta III subterminal, setae IV–VI terminal, well developed, seta VII minute, located at inner distal corner.

Antennule (Fig. 4C) 2-segmented; proximal segment bearing 26 setae on anteroventral surface; distal segment with 1 subterminal and 11 terminal setae and 2 short aesthetascs. Antenna (Fig. 4D) 3-segmented, heavily sclerotized; proximal segment with acutely pointed posterior process; middle segment subrectangular, unarmed; distal segment abruptly curved inward at distal quarter, armed with proximal seta and minute middle seta. Postantennal process (Fig. 4E) as long as distal segment of antenna, smoothly curved inward, with 2 multisensillate papillae basally; similar multisensillate papilla located adjacent to base of process. Mandible (Fig. 4F) with distal part bearing 12 teeth. Maxillule (Fig. 4G) consisting of anterior papilla bearing 3 setae of unequal length and triangular, plate-like posterior process. Maxilla (Fig. 4H) 2-segmented, lacertus (syncoxa) unarmed; brachium (basis) ca. 1.5 times longer than lacertus, with flabellum at about anterior one-third of length; calamus about 1.5 times longer than canna. Maxilliped (Fig. 4I) heavily sclerotized; corpus (protopod) elongate, about 1.7 times as long as subchela, with low proximal lobe located at about 30% along myxal margin, plus minute setule in distal quarter of corpus; shaft (endopod) and claw incompletely fused; claw with antero-proximal barb reaching beyond mid-length. Sternal furca (Fig. 4J) with tines widely separated at base and bluntly pointed.

Armature and elements of legs 1–4 as in Table 2. **Leg 1** (Fig. 5A–C) with protopod bearing 1 marginal bifurcate setule and 2 surface setae; endopod reduced to small knob with vestigial element at tip (Fig. 5B); exopod 2-segmented, proximal segment with row of setules along inner margin and outer distal seta, distal segment with 3 plumose setae along inner margin and 1 naked outer spine, 2 terminal spines each with accessory process (Fig. 5C), plus long spinulose seta (seta 4) terminally.

**Table 2.** Armature and elements of legs 1 to 4 of *Caligus kajii* sp. nov.

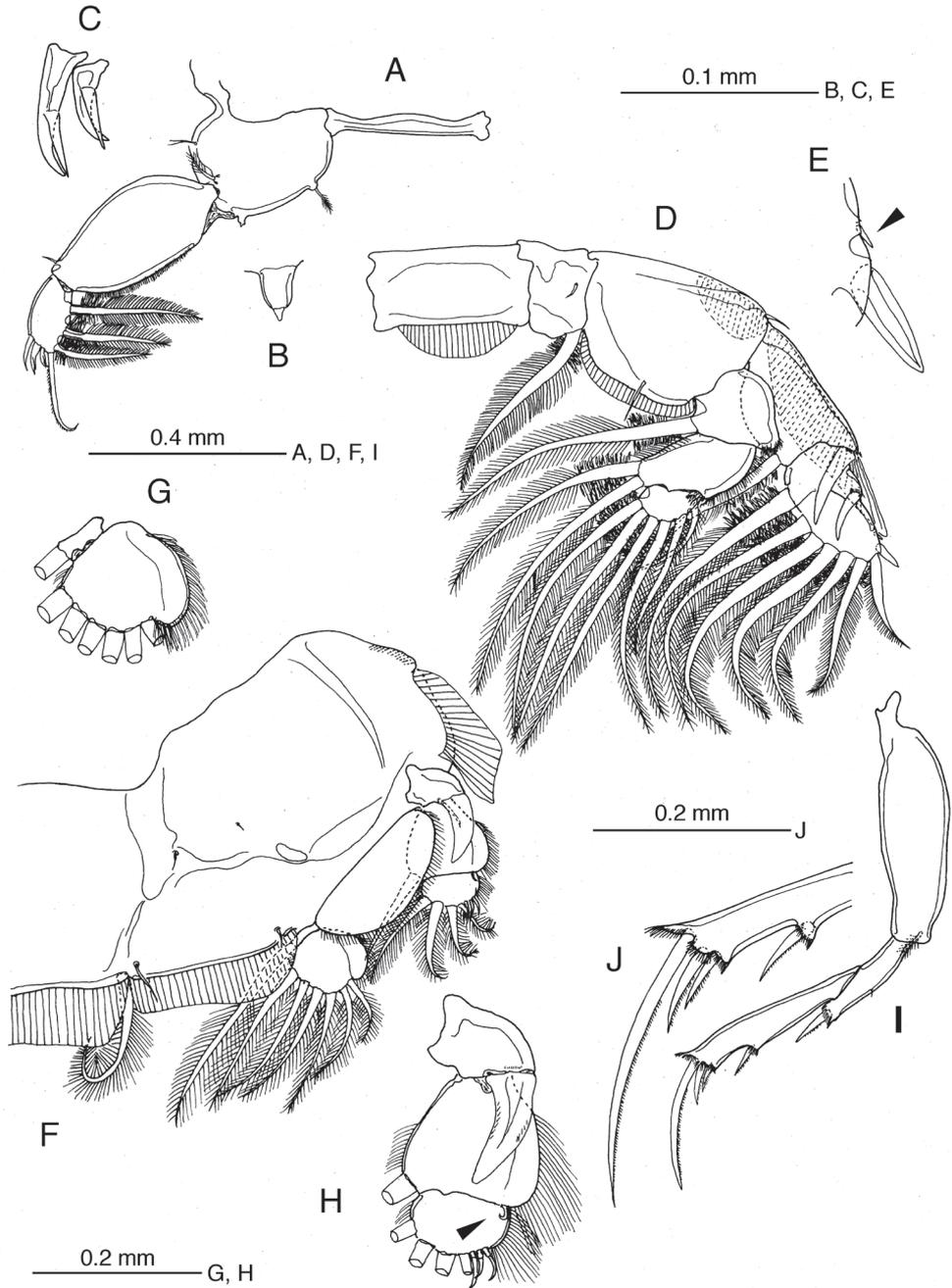
	Protopod (coxa; basis)	Endopod	Exopod
Leg 1	1-1	(vestigial)	1-0; III, 1, 3
Leg 2	0-1; 1-0	0-1; 0-2; 6	I-1; I-1; II, I, 5
Leg 3	1-1	0-1; 6	I-0; 1(vestigial)-1; III, 4
Leg 4	1-0	(absent)	1-0; I, III

**Leg 2** (Fig. 5D, E) with intercoxal sclerite bearing semi-circular marginal membrane along posterior margin; coxa with large plumose seta at posterior corner and setule on anterior surface; basis ornamented with marginal membrane along both inner and outer edges and long setule near midpoint of inner margin; armed with minute seta at distal outer corner; rami 3-segmented; first endopod segment with notch bearing tuft of setules, second segment furnished with row of setules along outer margin, third segment with tuft of setules near base of proximal outer seta; first exopod segment with anterior marginal membrane reflexed dorsally over segment surface and long, stout outer spine directed obliquely across surface of second segment; second segment with smaller outer spine than in first segment; third segment armed with 1 reduced spine (arrowed in Fig. 5E) and 1 moderate-size outer spine. **Leg 3** (Fig. 5F–H) apron (protopod) with no distinct ornamentation on surface, armed with 1 long inner seta and 1 small outer seta terminally; endopod 2-segmented, proximal segment reduced, velum developed, decorated entirely with row of setules along free posterior margin; distal segment with outer margin expanded and hirsute Fig. 5G; exopod 3-segmented, first segment with strong, slightly curved, inward-directed outer spine not reaching distal margin of second segment, second segment with expanded, hirsute outer margin and 1 minute outer seta (arrowed in Fig. 5H), third segment with 3 small spines plus 4 inner setae.

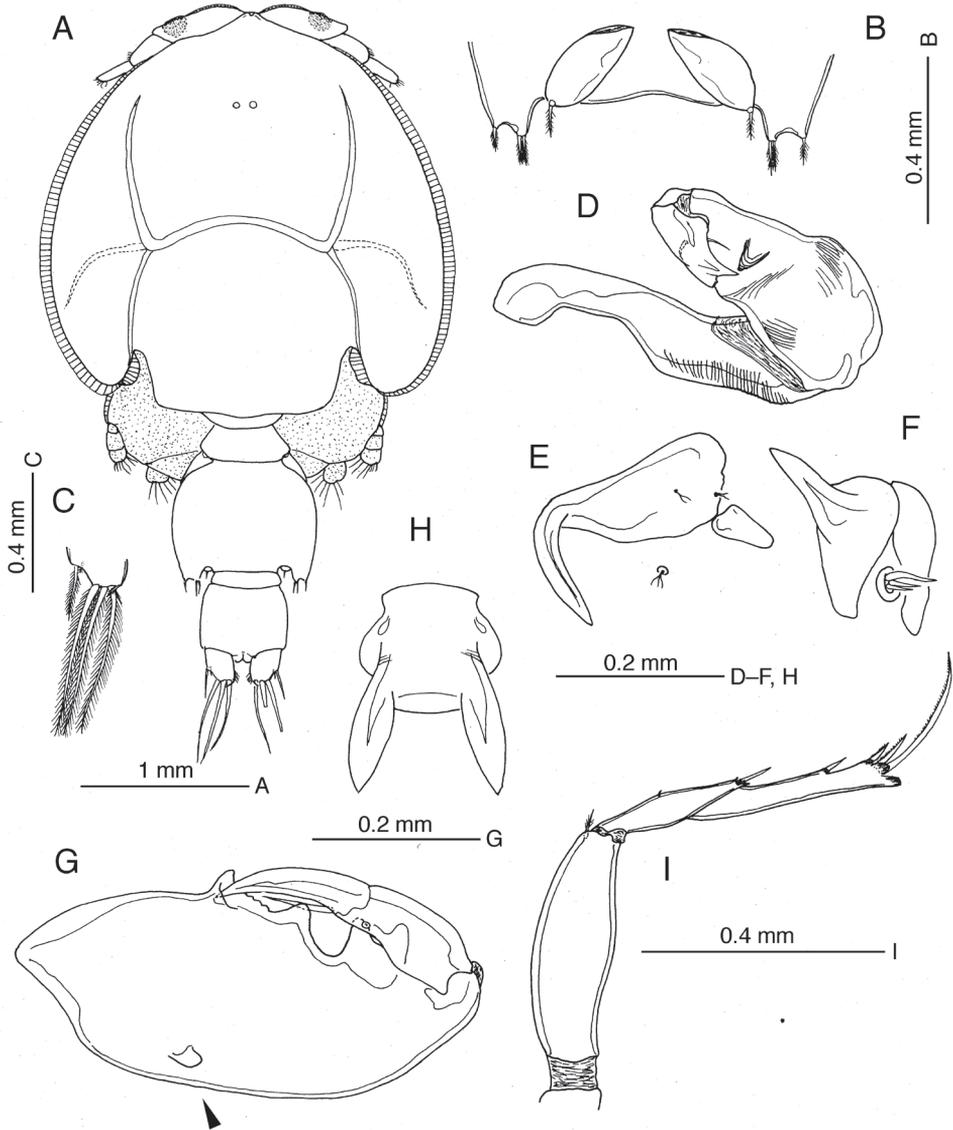
**Leg 4** (Fig. 5I, J) protopod slightly shorter than exopod, bearing minute plumose seta at outer distal corner; exopod 2-segmented, with pecten at base of each exopodal spine; first exopodal segment bearing spinulose spine reaching nearly half of distance to proximalmost outer spine on second segment; innermost terminal spine more than 3 times longer than middle spine. Leg 5 (Fig. 4B) represented by small outer knob bearing protopodal seta and inner (exopodal) knob bearing 2 setae.

**Male.** Body length of allotype 4.36 mm, range 4.09–5.73 mm long in allotype plus all male paratypes ( $4.69 \pm 0.51$  mm long,  $N = 14$ ). Cephalothorax (Fig. 6A) as in female. Pediger 4 (Fig. 6A) separate from genital complex, wider than long. Genital complex (Fig. 6A, B) about 1.4 times wider than long and about 1.2 times longer than abdominal somites combined, expanded posterolaterally into 2 knobs (leg 5), outer knob bearing 1 seta, inner (exopodal) knob with 2 setae; paired genital opercula representing leg 6 (Fig. 6B), each bearing 1 small terminal seta. Abdomen (Fig. 6A) 2-segmented, first segment small, second segment ca. 4.8 times longer than first. Caudal ramus (Fig. 6C) as in female.

Antennule, mandible, maxillule (Fig. 6F) and maxilla as in female. Antenna (Fig. 6D) 3-segmented; proximal segment long, unarmed, with corrugated pad distally;



**Figure 5.** *Caligus kajii* sp. nov., adult female, paratype **A** leg 1 **B** endopod of leg 1 **C** two terminal spines of second exopod segment of leg 1 **D** leg 2 **E** outer spines of terminal exopod segment of leg 2, reduced spine arrowed **F** leg 3 **G** endopod of leg 3 **H** exopod of leg 3 **I** leg 4 **J** terminal part of leg 4.



**Figure 6.** *Caligus kajii* sp. nov., adult male, allotype **A** habitus, dorsal view **B** gonopores, ventral view **C** tip of caudal ramus, dorsal view **D** antenna **E** postantennal process **F** maxillule **G** maxilliped, rounded knob on posterior surface of corpus arrowed **H** sternal furca **I** leg 4.

middle segment massive, with 1 proximal, 2 middle and 2 subterminal corrugated pads; distal segment short, with 2 fine setae at mid-length, 1 bluntly pointed process terminally and 1 membranous truncate projection subterminally. Postantennal process (Fig. 6E) similar to that of female, but more abruptly curved inward than in female. Maxilliped (Fig. 6G) stouter than in female; corpus with rounded knob proximally on posterior surface (arrowhead in Fig. 6G) and 3 distinct processes in myxal area,

proximalmost process socket-like, to receive distal tip of subchela; shaft and claw partly fused, ca. 0.67 times as long as corpus; barb about half length of claw.

**Remarks.** The female of the new species most closely resembles *Caligus bifurcus*, *C. musaicus* Cavaleiro, Santos & Ho, 2010, *C. pectinatus* Shiino, 1965, *C. pseudorhombi* Boxshall, 2018, *C. pterois* Kurian, 1949 and *C. xystercus* Cressey, 1991. All these species share a 2-segmented exopod on leg 4 armed with 4 spines on the distal exopodal segment, the female genital complex is nearly as long as wide and about twice as long as the abdomen, and the abdomen is about as long as wide. However, the present new species is distinguished from these species by the combination of the following characteristics: (1) the genital complex is as long as wide (cf. wider than long in *C. bifurcus*, *C. musaicus* and *C. pterois*; slightly longer than wide in *C. xystercus*); (2) the genital complex is about 2.1 times longer than the abdomen (cf. 1.2 times longer in *C. bifurcus*; 2.2 in *C. musaicus*; 2.1 in *C. pseudorhombi*; 1.6 in *C. pterois*; 3.6 in *C. xystercus*); (3) the corpus of the maxilliped lacks processes (cf. ridge-like process present in *C. pseudorhombi* and *C. pterois*); (4) the tines of the sternal furca taper distally (cf. uniform in width and with a truncate tip in *C. pectinatus*); (5) the terminal exopod segment of leg 1 is furnished with 3 large spines terminally (2 in *C. pseudorhombi*); and (6) the maxillipedal subchela is more than half the length of the corpus (cf. much shorter in *C. musaicus*).

In males, the new species is most similar to *C. musaicus*, *C. nuenonae* Andrews, Bott, Battaglene & Nowak, 2009, *C. pterois*, and *C. priacanthi* Pillai, 1962. These five species share the following characteristics: (1) the genital complex is laterally expanded and produced into 2 posterolateral protuberances representing leg 5, armed with 1 (outer lobe) and 2 setae (inner lobe); (2) the abdomen is completely or incompletely 2-segmented and shorter than the genital complex; (3) the maxillipedal corpus is well developed and carries anteriorly-produced processes on the myxal surface. However, the new species is easily distinguishable from these congeners by the combination of the following features: (1) the maxillipedal corpus has a rounded process on the posterior surface (absent in the other species); (2) the sternal furca has pointed tines which are widely separated (tines that are close at base and with rounded tips in *C. priacanthi*; rounded in *C. nuenonae*); (3) the mxyl surface of the maxilliped carries 3 large, rounded processes along the margin, (cf. the processes are different in shape and number in the other species); (4) the posterior dentiform process of the maxillule lacks a surface ornamentation of minute prominences (present in *C. nuenonae*).

**Etymology.** The new species is named in honor of the late, supremely talented carcinologist Tomonari Kaji who passed away in May 2019.

### Key to species groups currently recognized within the genus *Caligus*

- 1 Leg 1 with 3 inner setae on distal exopod segment lost or highly reduced .....  
..... *C. productus* group
- Leg 1 with 3 inner setae on distal exopod segment well developed ..... 2

- 2 Large denticles present along outer margin of second endopod segment of leg 2....  
..... ***C. bonito* group**
- Large denticles absent along outer margin of second endopod segment of leg 2....3
- 3 Leg 4 is 4-segmented (3-segmented exopod) ..... 4
- Leg 4 is 3-segmented (2-segmented exopod) ..... 5
- 4 Long-bodies; apron of leg 3 with raised bifid cuticular rib and rosette-like array of  
denticles..... ***C. confusus* group**
- Compact bodies; apron of leg 3 without such ornamentation.... ***C. diaphanus* group**
- 5 Distal exopod segment of leg 4 with 1 outer and 3 terminal spines .....  
..... ***C. pseudorhombi* group**
- Distal exopod segment of leg 4 with 3 terminal spines ..... ***C. macarovi* group**

### Key to species within the *C. pseudorhombi* group

#### Female

- 1 Tines of sternal furca widely separated ..... 2
- Tines of sternal furca originating close together..... 9
- 2 Outermost spine of terminal exopod segment of leg 1 reduced or absent ..... 3
- Outermost spine of terminal exopod segment of leg 1 developed ..... 4
- 3 Outermost spine of terminal exopod segment of leg 1 reduced.... ***C. pseudorhombi***
- Outermost spine of terminal exopod segment of leg 1 absent..... ***C. xystercus***
- 4 Maxillipedal corpus with triangular process at mid-length..... ***C. pterois***
- Maxillipedal corpus without distinct process ..... 5
- 5 Tines of sternal furca with truncate tips ..... 6
- Tines of sternal furca with pointed tips ..... 7
- 6 First segment of antenna with sharply pointed posterior process..... ***C. pectinatus***
- First segment of antenna with bluntly pointed posterior process ..... ***C. similis***
- First segment of antenna with spatulate posterior process..... ***C. nuenonnae***
- 7 Tines of sternal furca swollen at mid-length ..... ***C. kajii* sp. nov.**
- Tines of sternal furca tapering distally ..... 8
- 8 Gap between tines narrower than length of tine ..... ***C. bifurcus***
- Gap between tines wider than length of tine ..... ***C. musaicus***
- 9 Maxillipedal corpus with conical process midway..... 10
- Maxillipedal corpus without distinct process ..... 11
- 10 Genital complex as long as wide..... ***C. dieuzeidei***
- Genital complex wider than long ..... ***C. priacanthi***
- 11 Postantennal process with accessory process basally ..... ***C. hobsoni***
- Postantennal process without accessory process ..... 12
- 12 Genital complex more than twice as long as abdomen ..... 13
- Genital complex about 1.4 times longer than abdomen ..... ***C. ligatus***
- Genital complex about 1.7 times longer than abdomen ..... ***C. longirostris***

- 13 First segment of antenna with spatulate posterior process (spine) ..... *C. buechlerae*  
 ..... 14  
 – First segment of antenna with pointed posterior process (spine) ..... 14  
 14 Tines of sternal furca short, thick, about 1.6 times as long as wide .....  
 ..... *C. latigenitalis*  
 – Tines of sternal furca long, slender, about 2.7 times as long as wide ..... *C. olsoni*

## Male

- 1 Both anterior and posterior knobs representing leg 5 produced posteriorly; leg 6 (genital operculum) with 1 (or rarely 2) small setae terminally ..... 2  
 – Only posterior knob representing leg 5 distally produced; leg 6 with 2 (or rarely 3) setae terminally ..... 7  
 2 Tines of sternal furca widely separated ..... 3  
 – Tines of sternal furca close together ..... *C. priacanthi*  
 3 Posterior dentiform process of maxillule covered with minute prominences on medial and apical surfaces ..... *C. nuenonnae*  
 – Posterior dentiform process of maxillule lacking such prominences ..... 4  
 4 Maxilliped with rounded process proximally on posterior surface of corpus .....  
 ..... *C. kajii* sp. nov.  
 – Maxilliped lacking such process on posterior surface of corpus ..... 5  
 5 Third segment of antenna with long hook-like process terminally ..... *C. pterois*  
 – Third segment of antenna with short claw ..... 6  
 6 Maxillipedal corpus with 1 small dentiform and 1 bipartite process in myxal area ..... *C. musaicus*  
 – Maxillipedal corpus with 2 spinous processes proximally and 1 rounded process distally along myxal margin ..... *C. pseudorhombi*  
 7 Genital complex almost as long as abdomen ..... 8  
 – Genital complex longer than abdomen ..... 12  
 8 Tines of sternal furca slender with rounded tip ..... 10  
 – Tines of sternal furca thick with truncate or spatulate tip ..... 11  
 10 Maxillipedal corpus with distinct process at mid-level ..... *C. ligatus*  
 – Maxillipedal corpus without process ..... *C. longirostris*  
 11 Tines of sternal furca with truncate tip ..... *C. similis*  
 – Tines of sternal furca with spatulate tip ..... *C. hobsoni*  
 12 Caudal ramus about 2 times longer than wide ..... *C. dieuzeidei*  
 – Caudal ramus almost as long as wide ..... 13  
 13 Maxillipedal corpus with inner process at mid-length ..... *C. olsoni*  
 – Maxillipedal corpus with 3 inner processes along myxal margin ..... 14  
 14 Terminal exopod segment of leg 4 with dentate processes at base of each terminal spine ..... 15  
 – Terminal exopod segment of leg 4 with hyaline membrane at base of each terminal spine ..... *C. acanthopagri*

- 15 Four or five tips on dentate processes at base of terminal spines of terminal exopod segment of leg 4..... *C. latigenitalis*  
 – Two tips on dentate processes at base of terminal spines of terminal exopod segment of leg 4..... *C. chinglonglini* sp. nov.

## Discussion

Five named species groups were recognized within the genus *Caligus* by Boxshall (2018) but as part of the justification for establishing *C. pseudorhombi* Boxshall, 2018 as a new species, he informally recognized an additional distinct species group. This unnamed species group was diagnosed on the basis of female morphology. The following features are shared by species within the group: (1) the exopod of leg 4 is 2-segmented and the compound distal segment carries 4 spines; (2) the genital complex of the female is as long as wide, without posterolateral lobes, and about twice as long as the abdomen; and (3) the abdomen is about as long as wide (see Boxshall 2018). An additional characteristic which we identify here relates to leg 2: the proximal spine on the outer margin of the third exopodal segment is markedly reduced and the adjacent distal spine is also relatively small in almost all members of the species group for which information on leg 2 is available. The males of the species group are defined for the first time as follows: (1) leg 4 is as in the female; (2) the genital complex is subquadrate with legs 5 and 6 located close together at the posterolateral corner; (3) the abdomen is 1- or, typically, 2-segmented; and (4) the myxal surface of the maxilliped has 1 to 3 pointed or rounded processes (except for *C. longirostris* Hewitt, 1964).

We recognize that the following 19 species can be included in this species group: *C. acanthopagri* (♀♂ known), *C. bifurcus* (♀), *C. buechlerae* Hewitt, 1964 (♀♂), *C. chinglonglini* sp. nov. (♂), *C. dieuzeidei* Brian, 1932 (♀♂), *C. hobsoni* Cressey, 1969, (♀♂), *C. kajii* sp. nov. (♀♂), *C. latigenitalis* (♀♂), *C. ligatus* (♀♂), *C. longirostris* (♀♂), *C. musaicus* (♀♂), *C. nuenonnae* (♀♂), *C. olsoni* Pearse, 1953 (♀♂), *C. pectinatus* (♀), *C. pseudorhombi* (♀♂), *C. priacanthi* (♀♂), *C. pterois* (♀♂), *C. similis* Ho, Kim & Nagasawa, 2005 (♀♂), and *C. xystercus* (♀). Unfortunately for *C. olsoni*, no information is available on leg 2. This species group is newly named as the *pseudorhombi* group, partly because it was first pointed out when *C. pseudorhombi* was originally described by Boxshall (2018), and partly because both sexes of the species were described in detail by Boxshall (2018).

Both sexes of *C. dieuzeidei* were described by Brian (1932) based on material collected in the Mediterranean, but this species has not been found since the original description. This species has been recorded by Shiino (1954b, 1959, 1960). According to Izawa and Choi's (2000) direct observations of Shiino's material: his "*C. dieuzeidei*" from *Acanthopagrus schlegeli* (Shiino 1954b) was identical with *Caligus latigenitalis*, as already pointed out by Lin et al. (1994), and his material of "*C. dieuzeidei*" from *Siganus fuscescens* (Houttuyn, 1782) (Shiino 1959) was *Caligus oviceps* Shiino, 1952 (Izawa and Choi 2000). Shiino's (1960) specimens of "*C. dieuzeidei*" collected from two elasmobranchs were identified as an unknown congener, although the evidence

was not presented. Excluding Shiino's (1960) *C. dieuzeidei*, a total of 19 species, including the two new species described herein can be assigned to this species group. Although *C. dieuzeidei* Brian, 1932 and *C. latigenitalis* were not listed as members of the species group by Boxshall (2018), it is clear that leg 4 and the genital complex and abdomen of the female could fall within the diagnosis (see Brian 1932, Shiino 1954a, b, Izawa and Choi 2000). Unfortunately, leg 2 was neither figured nor mentioned in the text by Brian (1932) in his original description of *C. dieuzeidei*, so the configuration of the spines on leg 2 exopod cannot be confirmed.

The hosts and geographical distributions of the members of the newly recognised species group are summarized in Table 3. The host fish for the species group vary widely and include both pelagic and benthic taxa. The host specificity seems to be relatively low in *C. acanthopagri*, *C. hobsoni*, *C. ligatus*, and *C. xystercus*, but may be higher in other species. Four members of the species group most frequently utilize the family Sparidae as hosts: *C. acanthopagri*, *C. dieuzeidei*, *C. latigenitalis* and *C. xystercus*. Two species are associated with each of the following host families: Atherinidae (*C. ligatus*, *C. olsoni*), Aulostomidae (*C. ligatus*, *C. xystercus*), Pomacanthidae (*C. hobsoni*, *C. xystercus*), and Priacanthidae (*C. priacanthi*, *C. xystercus*). This is the first record of the occurrence of a species (*C. chinglonglini* sp. nov.) belonging to the species group in plankton samples. According to Venmathi Maran et al. (2016), 11 species of *Caligus* were found exclusively from plankton samples. Of these pelagic caligids, only *C. adunctus* is assigned to a species group, the *C. macarovi* group, while the remaining species have not as yet been classified into the five groups defined by Boxshall (2018).

Males of 16 species belonging to the *C. pseudorhombi* group are known, including the present two new species. With the exception of *C. buechlerae*, these males can be divided into two sub-groups on the basis of the morphology of the genital complex: in one sub-group, both of the anterior and posterior knobs representing leg 5 are produced posteriorly, and leg 6 (genital operculum) is armed with 1 (or rarely 2) small setae terminally; whereas in the other sub-group only the posterior (exopodal) knob is distally produced, and leg 6 has 2 (or rarely 3) setae terminally. The first sub-group consists of *C. kajii* sp. nov., *C. musaicus*, *C. nuenonnae*, *C. pseudorhombi*, *C. priacanthi*, and *C. pterois*. The second sub-group comprises *C. acanthopagri*, *C. chinglonglini* sp. nov., *C. dieuzeidei*, *C. hobsoni*, *C. latigenitalis*, *C. ligatus*, *C. longirostris*, *C. olsoni* and *C. similis*. Members of the first sub-group are widely distributed in the Indo-Pacific and the Atlantic, whereas the second sub-group is restricted to the Pacific (Kurian 1949; Pearse 1953; Shen 1958, Heegaard 1962; Hewitt 1964; Lewis 1967; Shiino 1965; Cressey 1969; Pillai 1985; Izawa and Choi 2000; Ho and Lin 2004b; Ho et al. 2005; Andrews et al. 2009; Cavaleiro et al. 2010; Boxshall 2018; present study) (see Table 3).

Five distinct species groups within the genus *Caligus* were defined by Boxshall and El-Rashidy (2009) and Boxshall (2018), namely: *C. bonito* Wilson, 1905 (12 spp. based on Boxshall 2018), *C. confusus* Pillai, 1961 (15 spp.), *C. diaphanus* von Nordmann, 1832 (15 spp.), *C. macarovi* Gusev, 1951 (42 spp.), and *C. productus* Dana, 1852 (14 spp.). In addition, as Boxshall (2018) has already pointed out, a sixth species group, the *C. pseudorhombi* group (19 spp.) is proposed in this study. See Boxshall (2018) for the detailed definition of each species group before using the key.

**Table 3.** Body size, host and locality of species of the *Caligus pseudorhombi* species group.

Species	Body length (mm)	Host	Locality	References
<i>C. acanthopagri</i>	♀ 3.79–5.35 ♂ 3.79–5.35	<i>Acanthopagrus schlegelii</i> (Bleeker, 1854) <i>A. berda</i> (Forsskål, 1775), <i>Rhabdosargus holubi</i> (Steindachner, 1881) <i>Scatophagus argus</i> (Linnaeus, 1766), <i>Thryssa hamiltonii</i> Gray 1835.	Taiwan, south Africa	Ho and Lin 2004b
<i>C. bifurcus</i>	♀ 5.4	<i>Lateolabrax japonicus</i> (Cuvier, 1828)	China	Shen 1958
<i>C. buechleriae</i>	♀ 4.77–5.40 ♂ 3.58–3.85	<i>Tripterygion</i> sp.	New Zealand	Hewitt 1964
<i>C. chinglonglini</i> sp. nov.	♂ 4.02	–	Japan	Present study,
<i>C. dieuzeidei</i>	♀ 5.8 ♂ 6.5	<i>Diplodus sargus</i> (Linnaeus, 1758)	Mediterranean	Brian 1932, Shiino 1954b, Izawa and Choi 2000
<i>C. hobsoni</i>	♀ 2.78–3.45 ♂ 3.9	<i>Chromis punctipennis</i> (Cooper, 1863), <i>Hypsipops rubicundus</i> (Giard, 1854), <i>Rhacochilus toxotes</i> Agassiz, 1854, <i>Medialina californiensis</i> (Steindachner, 1876)	California	Cressey 1969
<i>C. kajii</i> sp. nov.	♀ 4.86–6.16 ♂ 4.09–5.73	<i>Platycephalus</i> sp.	Japan	Present study
<i>C. latigentialis</i>	♀ 3.24–4.33 ♂ 4.1–6.9	<i>Acanthopagrus schlegelii</i>	Japan	Izawa and Choi 2000
<i>C. ligatus</i>	♀ 3.20–3.35 ♂ 2.25–2.65	<i>Aulostomus chinensis</i> (Linnaeus, 1766), <i>Sargocentrus xantherphrum</i> (Jordan & Evermann, 1903), <i>Atherinomorus insularum</i> (Jordan & Evermann, 1903)?, <i>Dacyllus albisella</i> Gill, 1862, <i>Acanthirus dussumieri</i> Valenciennes, 1835, <i>Naso hexacanthus</i> (Bleeker, 1855)	Hawaii	Lewis 1967
<i>C. longirostris</i>	♀ 5 ♂ 6	<i>Pseudophycis barbatus</i> Gunther, 1863, <i>Platycephalus bassensis</i> Cuvier, 1829	Tasmania	Heegaard 1962
<i>C. musaicus</i>	♀ 3.75–5.07 ♂ 3.25–3.64	<i>Platichthys flesus</i> (Linnaeus, 1758)	Portugal	Cavaleiro et al. 2010
<i>C. nuenonnae</i>	♀ 4.27–4.82 ♂ 3.99–5.2	<i>Latris lineata</i> (Foster, 1801)	Tasmania	Andrews et al. 2009
<i>C. olsoni</i>	♀ 3.8 ♂ 3.8	<i>Leuresthes tenuis</i> (Ayres, 1860)	California	Pearse 1953
<i>C. pectinatus</i>	♀ 3.43	<i>Eopsetta jordani</i> (Lockington, 1879)	California	Shiino 1965
<i>C. pseudorhombi</i>	♀ 4.42 ♂ 3.96	<i>Pseudorhombus arsius</i> (Hamilton, 1822)	Australia	Boxshall 2018
<i>C. priacanthi</i>	♀ 2.9 ♂ 1.9	<i>Priacanthus hamrur</i> (Forsskål, 1775)	India	Pillai 1985
<i>C. pierois</i>	♀ 5.8 ♂ 4.4	<i>Pterois russelli</i> Bennett, 1831, <i>P. miles</i> (Bennett, 1828)	India	Pillai 1985
<i>C. similis</i>	♀ 4.95 ♂ 4.72	<i>Neophrynychthys latus</i> (Hutton, 1875)	New Zealand	Ho et al. 2005
<i>C. xystercus</i>	♀ 2.3	<i>Anisotremus virgineus</i> (Linnaeus, 1758), <i>Aulostomus maculatus</i> Valenciennes, 1841, <i>Calamus calamus</i> (Valenciennes, 1830), <i>C. pennatula</i> Guichenot, 1868, <i>Lutjanus apodus</i> (Walbaum, 1792), <i>Pomacanthus arcuatus</i> (Linnaeus, 1758), <i>Heteropriacanthus cruetatus</i> (Lacépède, 1801)	Belize	Cressey 1991

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# A new sawfly of Megalodontesidae (Insecta, Hymenoptera, Pamphilioidea) with pectinate antennae from the Early Cretaceous of China

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## Abstract

A new sawfly of Megalodontesidae, *Jibaissodes peichenae* **sp. nov.**, is described from the Lower Cretaceous Yixian Formation of Northeastern China. It is established mainly based on the pectinate antenna comprising 42 flagellomeres and the proximal 28 bearing apical rami, which gradually shorten in length toward the apex of the flagellum. The pterostigma of the forewing is infuscated apically and on the hind wing, vein 1-Rs is nearly equal to 1r-m and slightly shorter than 1-M. The first tergum is widely excised posteriorly and roundly protruding laterally alike in *Megalodontes*. This find supports that pectinate antennae in extant sawflies of Megalodontesidae originated at least during or before the Early Cretaceous.

## Keywords

ramified antennae, Symphyta, taxonomy, Yixian Formation

## Introduction

Megalodontesidae is a small extant family with only one extant genus comprising 42 species and 12 fossil genera with 23 species (Taeger et al. 2018). Megalodontesidae comprises four subfamilies: three extinct subfamilies, Archoxyelydinae Wang, Rasnitsyn & Ren, 2013, Decorisiricinae Wang, Rasnitsyn, Shih, Sharkey & Ren, 2015, and Praesiricinae Rasnitsyn, 1968 and one extant subfamily, Megalodontesinae Konow, 1897. The sole extant genus, *Megalodontes* Latreille, 1803, which is distributed in temperate regions of the Palearctic (Benson 1968; Goulet 1993; Blank et al. 2001; Taeger et al. 2010), and an extinct genus, *Jibaissodes* Ren, Lu, Guo & Ji, 1995, are characterized by having saw-like or comb-like ramified antennae.

Among extant symphytan insects, ramified antennae are present in various taxa, i.e., in Diprionidae, Pergidae, Tenthredinidae, and Megalodontesidae (Gao et al. 2016). Fossil records of ‘Symphyta’ with ramified antennae are very rare. Up to date, only two species with ramified antennae have been reported: *Jibaissodes bellus* Gao, Shih, Labandeira, Santiago-Blay, Yao & Ren, 2016 with simply ramified antennae from the Lower Cretaceous Yixian Formation, and *Atefia rasnitsyni* Krogmann, Engel, Bechly & Nel, 2012 with the biflabellate antennae from the Lower Cretaceous Crato Formation, assigned to the superfamily Tenthredinoidea s. str. Latreille, 1802. The occurrence of biflabellate antennae in *A. rasnitsyni* has been suggested to indicate the antiquity of insect usage of long-range female attractants (Krogmann et al. 2013).

In this paper, we describe *Jibaissodes peichenae* sp. nov. based on a well-preserved specimen from the mid-Lower Cretaceous Yixian Formation of northeastern China. The new species with distinctly pectinate antennae contributes additional important morphological characters of the family Megalodontesidae.

## Material and methods

For the paper we examined the holotypes of *Jibaissodes peichenae* sp. nov. (specimen no. CNU-HYM-LB2018033, part and counterpart) and *Jibaissodes bellus* Gao, Shih, Labandeira, Santiago-Blay, Yao & Ren, 2016 (specimen no. CNU-HYM-LB2011009, part and counterpart), which are housed in the Key Laboratory of Insect Evolution and Environmental Changes at the Capital Normal University, Beijing, China (CNUB; Dong Ren, curator). Both were collected from the mid-Lower Cretaceous Yixian Formation of Liaoning Province of China. The holotype of *Jibaissodes giganteus* Ren, Lu, Guo & Ji, 1995 (specimen no. BL92105, part, housed in the Geological Museum of China, Beijing, China, Jun Yao, curator) was also examined and redrawn for this paper. It was collected from the Lower Cretaceous Lushangfen Formation of Beijing of China.

The specimens were examined and photographed, either dry or wetted with 95% ethanol, by using a Nikon SMZ25 with an attached camera system. Line drawings were prepared using Adobe Illustrator CC and Adobe Photoshop CC graphics software. The wing venation nomenclature used in the paper was modified after Rasnitsyn (1969, 1980).

## Taxonomy

### Genus *Jibaissodes* Ren, Lu, Guo & Ji, 1995

**Emended diagnosis.** Mesonotum large, notauli strongly impressed, tapering to acutely rounded base. Forewing veins 1-Rs and 1-M nearly in straight line; 1r-rs present, shorter than 2r-rs; 1r-rs reclival and 2r-rs proclival; 1m-cu near base of cell 2rm; 1cu-a at base of cell 1m-cu, connecting to juncture of M+Cu; cell 1m-cu small, nearly rectangular; cell 2rm longer than cell 3rm. Hind wing with 1-Cu nearly perpendicular to cu-a; 1A and 2A strongly curved.

**Type species.** *Jibaissodes giganteus* Ren, Lu, Guo & Ji, 1995

**Emended description.** Fossil incomplete: head appendages, pronotum, legs, abdomen and supposedly posterior parts of wings missing. Head moderately large, near oval; compound eyes large; ocelli not visible. Mesonotum broader than long; mesoscutum small, with distinct medial line and impressed notauli; mesoscutellum indistinct; metanotum narrow and long; metascutum with cenchri present but indistinct. Forewing (Fig. 1E) with Sc absent; pterostigma long, completely infuscated; 1-Rs reclival, slightly shorter than 1-M; 1-M long and straight; Rs-M juncture nearly straight; Rs+M straight, forming a nearly straight line with 2-M, nearly perpendicular to 1-M; 1r-rs slightly shorter than 2r-rs; 2r-rs distinctly proclival, positioned near distal 0.75 of pterostigma; 1cu-a straight, distinctly bent towards wing tip; M+Cu straight; 1-Cu and 2-Cu curved, 1-Cu nearly equal to 2-Cu; 2r-m straight and reclival; 3r-m straight towards wing tip; 1A slightly curved. Costal cell slightly widened before point of origin of Rs; cell 2rm distinctly longer than cell 3rm, cell 3rm trapezoid; cell 1m-cu small, nearly rectangular; cell 2m-cu large, pentagonal. Hind wing (Fig. 1F) with 1-Rs very short, 1r-m straight; 1r-m and 1-M forming a straight line. M+Cu slightly bent; 1-Cu slightly curved, nearly perpendicular to cu-a; cu-a nearly straight; vein 1A strongly arched.

**Other species included.** *Jibaissodes bellus* Gao, Shih, Labandeira, Santiago-Blay, Yao & Ren, 2016; *Jibaissodes peichenae* sp. nov.

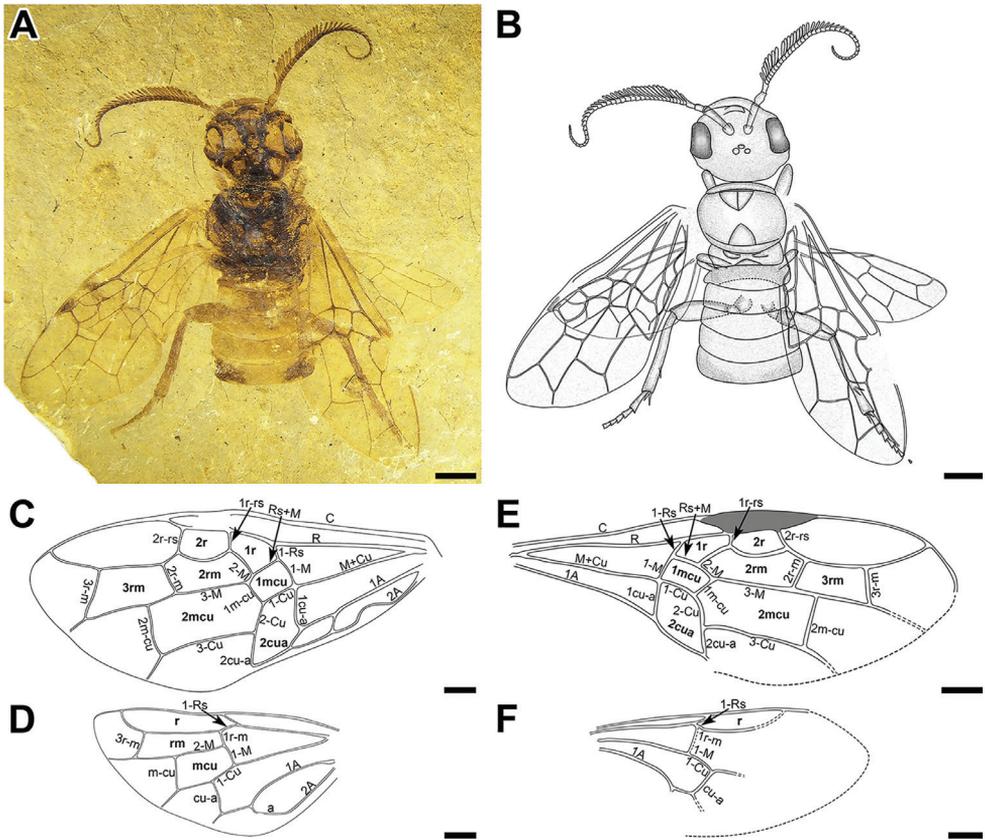
### *Jibaissodes peichenae* Wang, Shih & Gao, sp. nov.

<http://zoobank.org/D752BA0D-21EF-44AB-BBC7-B9E4279A6883>

Figs 1–3

**Type material.** Holotype, specimen no. CNU-HYM-LB2018033p/c, part and counterpart.

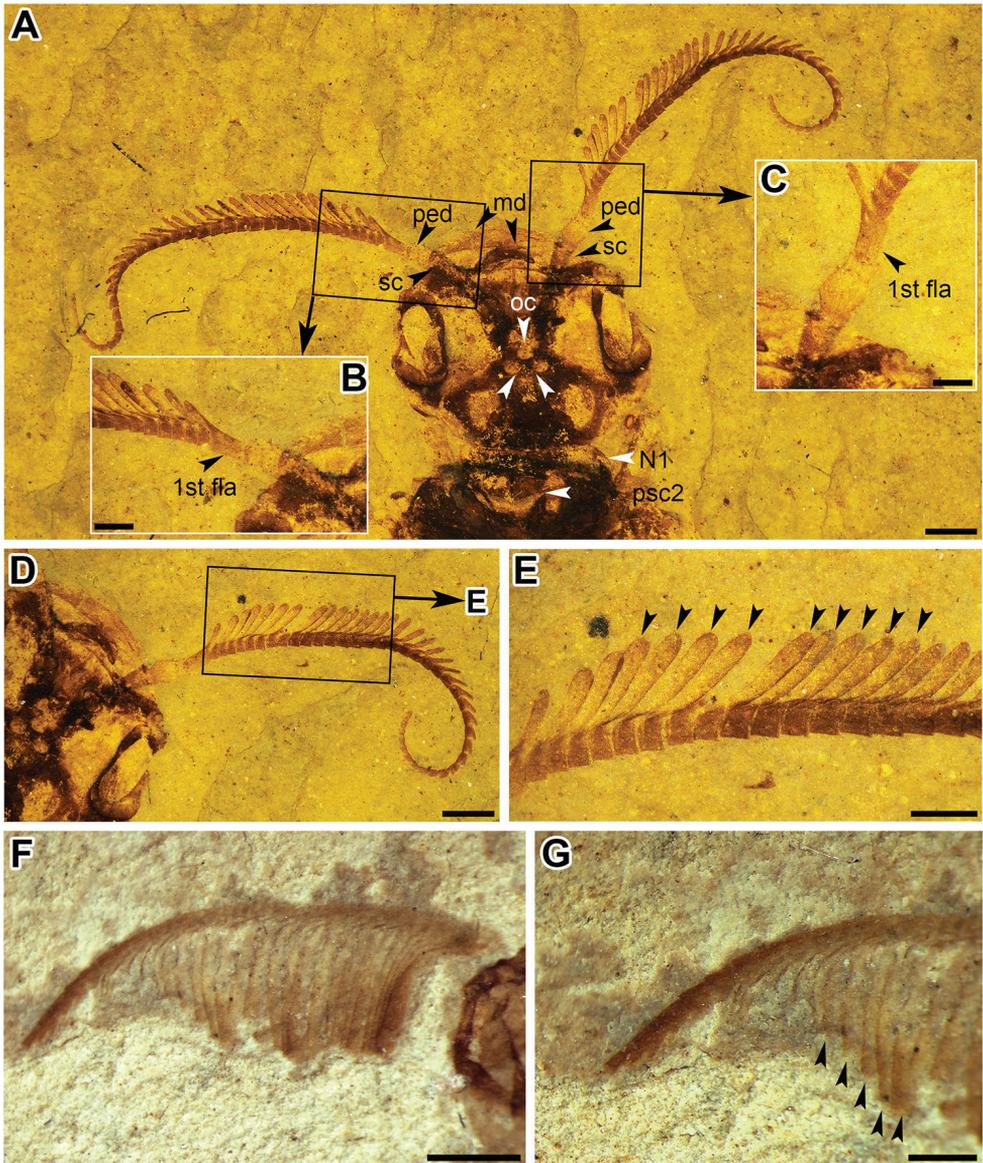
**Diagnosis.** Antenna pectinate, with 42 flagellomeres, flagellum longer than head width, proximal 28 flagellomeres with apical rami, rami gradually shortening in length toward apex of flagellum; scape almost 3 times as long as first flagellomere. Anterior margin of pronotum round, with weakly concave posterodorsal margin. Forewing with pterostigma infuscated apically; vein 1cu-a strongly curved. Hind wing vein 1-Rs nearly equal to 1r-m, slightly shorter than 1-M.



**Figure 1.** **A–D** *Jibaissodes peichenae* sp. nov., holotype, part: **A** photograph of complete specimen **B** line drawing of complete specimen **C** line drawing of forewing **D** line drawing of hind wing **E, F** *Jibaissodes giganteus*, holotype: **E** line drawing of forewing **F** line drawing of hind wing. Scale bars: 2 mm (**A, B**), 1 mm (**C–F**).

**Description. Body** (Figs 1A, B, 3A). Fossil incomplete: distal abdominal segments missing, part of the legs invisible. Middle and surrounding regions of compound eyes and part of posterior head pale, remainder of head dark. Thorax and legs entirely or predominantly dark. First tergum except hind margin and fifth tergum laterally dark. Body about 14.9 mm (but distal abdominal segments missing), antenna 11.4 mm long; forewing about 13.6 mm in length, maximum width 6.0 mm; hind wing about 8.5 mm in length, maximum width 3.8 mm.

**Head.** Large, about as wide as thorax. Head (Fig. 2A) 5.3 mm wide and 4.6 mm long, almost circular. Compound eyes 1.8 mm long, 1.1 mm wide; right mandible large, bent and sickle-shaped; antenna (Fig. 2D, E) with 42 flagellomeres, basal 28 flagellomeres with rami extending from apicolateral angle, apical 14 flagellomeres without distinct rami, longest ramus about 0.8 mm in length, longest ramus slightly longer than total length of following three flagellomeres; rami gradually shortening in length toward apex of flagellum, first flagellomere much shorter than scape, 0.3 mm wide, 0.5 mm long; scape 1.4 mm long, maximum 0.4 mm in width; pedicel 0.8 mm long, maximum 0.5 mm wide.



**Figure 2.** **A–E** *Jibaissodes peichenae* sp. nov., holotype, part: **A** head **B** basis of left antenna **C** basis of right antenna **D** right antenna **E** basal to middle section of right antenna **F, G** *Jibaissodes bellus*, holotype: **F** right antenna **G** apical portion of right antenna. Abbreviations: md = mandible, sc = scape, ped = pedicel, oc = ocelli, 1st fla = 1st flagellomere, N1 = pronotum, psc2 = mesoscutum. Scale bars: 1 mm (**A, D, F, G**); 0.5 mm (**B, C, E**).

**Thorax.** Maximum width 4.9 mm; pronotum short, apex round, with weakly concave posterodorsal margin. Mesoscutum large, with medial line and notauli strongly impressed, tapering to acutely rounded base; mesoscutum without line to mesoscutellum; mesoscutellum tapering to acutely sharp apex; metascutum with

cenchri present and small. Metatibia (Fig. 3B, C) with two preapical (near distal 0.7 of length) and two apical spurs; metabasitarsus long but shorter than remaining tarsomeres combined; metafemur, metatibia, and metabasitarsus lengths 3.4 mm, 4.6 mm, and 1.1 mm, respectively.

**Abdomen.** Five visible segments preserved; maximum width about 5.3 mm; first tergum medially undivided, posteriorly widely excised, laterally roundly protruding, medio-anteriorly dark; fifth tergum laterally dark.

**Forewing.** (Fig. 1B, C) Wing membrane mostly hyaline with weakly infuscated bands covering base and middle regions of forewing, surrounding veins R, M+Cu, 1r-rs, 2-M, m-cu, and 2-Cu; R slightly thickened before pterostigma; pterostigma infuscated apically; 1-Rs (0.5 mm long) short and reclival; 1-M (1.0 mm long) long and slightly curved, about twice as long as 1-Rs; Rs+M straight; 1r-rs length 0.7 mm, slightly shorter than 2r-rs, 0.8 mm long; 2r-rs slightly proclival, positioned near 3/4 pterostigma; 1cu-a distinctly bent towards wing base, 1.4 mm in length; M+Cu nearly straight; 1m-cu 0.8 mm long, 0.7 times as long as 1-Cu (1.1 mm long); 1-Cu nearly equal to 2-Cu (1 mm long) in length; 2r-m slightly curved and reclival; 3r-m straight towards wing tip; 2A with shallow arch midway before 1a-2a cross-vein. Costal cell widened at point of origin of 1-Rs; cell 1r nearly equal in size to cell 2r; cell 2rm longer than cell 3rm, cell 3rm trapezoid; cell 1mcu small, nearly rectangular; cell 2mcu large, pentagonal.

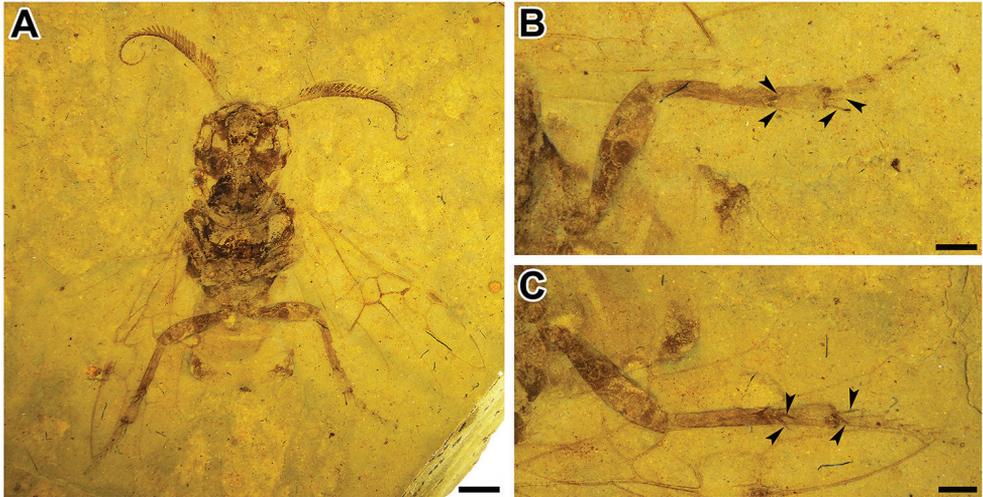
**Hind wing.** (Fig. 1B, D) 1-Rs (0.5 mm long) nearly equal to 1r-m (0.5 mm long), shorter than 1-M (0.7 mm long), 1r-m straight; 1r-m and 1-M forming a nearly straight line; 3r-m present, 0.9 mm in length, strongly bent towards wing tip; 1m-cu (1.0 mm long) and cu-a (1.1 mm long) present, longer than 3r-m, cu-a distinctly bent; M+Cu nearly straight; vein 1A strongly arched.

**Gender.** Unknown.

**Remarks.** The species can be assigned to *Jibaisodes* according to the following diagnostic characters: forewing vein 1r-rs present, shorter than 2r-rs, cell 2rm longer than 3rm and cell 1mcu small. *Jibaisodes* was described from a poorly preserved fossil specimen with a left and a right forewing, a right hind wing, and parts of head and thorax (Ren et al. 1995). *Jibaisodes peichenae* sp. nov. is distinctly differentiated from the type species of *J. giganteus* by the following characters: forewing with pterostigma not completely infuscated; forewing vein 3r-m distinctly longer than 2r-m (about 1.7 times as long as 2r-m); 1cu-a distinctly curved; and hind wing vein 1-Rs nearly equal to 1r-m. Furthermore, *J. peichenae* is distinguished from *J. bellus* by the following characters: antenna pectinate, rami short (vs rami long in *J. bellus*); forewing with pterostigma infuscated apically (vs infuscated medially and apically in *J. bellus*).

**Distribution.** Huangbanjigou, near Chaomidian Village, in Shangyuan County, adjacent to Beipiao City, in Liaoning Province of China. Collected from the mid-Lower Cretaceous Yixian Formation, dated as latest Barremian to earliest Aptian, 125 Ma (Ren et al. 1995; Wang et al. 2015).

**Etymology.** The species epithet is dedicated to Miss Peichen Yao, the daughter of Dr Jun Yao, the specimen donator.



**Figure 3.** *Jibaissoodes peichenae* sp. nov., holotype, counterpart **A** complete specimen **B** right hind leg **C** left hind leg. Arrows indicate spurs. Scale bars: 2 mm (**A**); 1 mm (**B**, **C**).

## Discussion

*Jibaissoodes peichenae* sp. nov. is assigned to Megalodontesidae by two features typical for Megalodontesidae: undivided first tergum and absent Sc (Benson 1968; Goulet 1993; Wang et al. 2016). In addition, *J. peichenae* can be attributed to Megalodontesinae, by the pectinate antennae as a derived character state. Other fossil Megalodontesidae, i.e., Decorisiricinae, Archexyelinae, and Praesiricinae, have a synantennomere 3 (Blank et al. 2013) like the ancestral Xyelidae and a number of fossil taxa, which represents an ancestral character of Hymenoptera (e.g., Rasnitsyn 1996). The semicircular distal excision and the lateral round protuberances of the first abdominal tergum is a putative apomorphy of *Jibaissoodes* + *Megalodontes*, since protrusions are obviously absent in Decorisiricinae, Archexyelinae, and Praesiricinae. On the forewing, M+Cu is straight, while it is curved in Archexyelinae and Praesiricinae.

All fossil megalodontesids share as a plesiomorphy of a curved vein 2A on the forewing (Wang et al. 2015, 2016). The almost straight 2A of the extant *Megalodontes* is an apomorphy of this taxon (Taeger 2002; Taeger et al. 2014). Therefore, we treat *Jibaissoodes* and *Megalodontes* as separate groups. Although *J. peichenae* sp. nov. shares two features with *Megalodontes* (pectinate antennae and laterally protruding, medio-anteriorly dark first tergum; Taeger 2002), it is impossible to evaluate the coloration of head, pronotum, and mesonotum of *J. peichenae* as preserved on fossil with certainty. We need more fossil specimens to address whether *Jibaissoodes* has close affinities with *Megalodontes*.

Since weak sexual dimorphism has been observed within several groups of *Megalodontes* having antennae with relatively long rami in males and slightly short rami in females (Taeger 2002), and thereby, we cannot rule out the possibility that these two species, *J. peichenae* with long ramified antennae and *J. bellus* with short ramified an-

tennae, might be different sexes of the same species. However, *J. peichenae* differs from *J. bellus* also in the following characters: pterostigma of forewing infuscated apically and, particularly, the excised posterior edge of first tergum, which is more similar to *Megalodontes* (Taeger 2002; Taeger et al. 2014). Given that the holotype of *J. peichenae* lacks the tip of the abdomen, it is impossible to properly ascertain whether the two should be considered as the same species. Therefore, we treat them as separate for now, pending future finds of additional fossil specimens to confirm the relationship between these two species.

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# A new petrophilous tiger beetle from the Trans-Pecos region of Texas and revised key to the genus *Amblycheila* (Coleoptera, Carabidae, Cicindelinae)

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## Abstract

A new rock-dwelling (petrophilous) tiger beetle, *Amblycheila katzi* Duran & Roman, **sp. nov.** is described from calcareous canyons and steep hillsides in the Trans-Pecos region of western Texas. It is distinguished from all other *Amblycheila* based on multiple morphological characters, biogeography, and ecology. A revised key to the genus *Amblycheila* is provided.

## Keywords

Caraboidea, Chihuahuan Desert, Nearctic, new species, Omini, petrophile, taxonomy

## Introduction

The New World tiger beetle genus *Amblycheila* Say, 1829 includes seven currently recognized species (Wiesner 1992; Erwin and Pearson 2008; Pearson et al. 2015) exclusively within the Nearctic Realm, and is distributed from central and southwestern US to central Mexico. All members of the genus are nocturnal predators of invertebrates.

They are found in desert or grassland ecosystems, with habitats including rolling hillsides in dry grasslands, rocky washes in deserts, and semi-open brush. Herein we describe *A. katzi* sp. nov., an inhabitant of steep rock canyons in the Trans-Pecos region of western Texas, part of the Chihuahuan Desert.

## Materials and methods

Specimens of an unidentified *Amblycheila* species from Val Verde County were collected by David Katz in 2010 and later by Daniel Sundberg in 2013; these were made available to the authors for study. Additional searches were performed by the authors and colleagues from 2014–2018, and in total 36 wild caught specimens were obtained. The second author collected live adults, 2<sup>nd</sup> and 3<sup>rd</sup> instar larvae from the type locality in 2015, and 59 additional adult beetles were reared through captive breeding. These specimens are indicated in the type series below by “*ex ovum*”.

Type material is deposited in the following institutional and private collections (acronyms used in the text are in parentheses): National Museum of Natural History, Smithsonian Institution, Washington, DC, USA (NMNH), Texas A&M University Insect Collection (TAMUIC), American Museum of Natural History Insect Collection (AMNH), Carnegie Museum of Natural History (CMNH), Florida State Collection of Arthropods (FSCA), Collection of David W. Brzoska (DWBC), Collection of Daniel P. Duran (DPDC), Collection of Stephen J. Roman (SJRC), Collection of David E. Katz (DEKC), Collection of Daniel Sundberg (DSC). Specimens of the new species were compared to material of all congeners, including *A. hoversoni* Gage, 1990, *A. picolominii* Reiche, 1840, *A. nyx* Sumlin, 1991, *A. halffieri* Mateu, 1974, *A. cylindriciformis* (Say, 1823), *A. baroni* Rivers, 1890 and *A. schwarzi* Horn, 1904.

Body measurements are defined as follows. The total body length excludes the labrum and is measured as the distance from the anterior margin of the clypeus to the elytral apex, including the sutural spine, when present. The width of the pronotum is measured to include the lateral margins of the proepisterna. Pronotal width was measured at the widest point of the apex, as well at the narrowest point near the base.

## Taxonomy

### *Amblycheila katzi* Roman & Duran, sp. nov.

<http://zoobank.org/F2A07537-21DD-4371-A52F-2BAB80E8EF84>

Figs 1–3

**Material examined. Holotype:** 1 ♂, USA: Texas / Val Verde Co / 5mi E Langtry / 15-VI-2017 / leg. D. Katz (USNM). **Paratypes:** 2 ♂♂, 2 ♀♀, same label data as holotype. 1 ♀, USA: Texas, 5mi W Langtry / 12-VII-2010 D. Katz (DEKC). 1 ♂, USA: Texas / Val Verde Co / Langtry / 18-VI-2013 / leg. D. Sundberg (DSC). 1 ♂, USA: Texas, /

Val Verde Co / Langtry / 14-VI-2014 / leg. D. Sundberg (DSC). 1 ♂, **USA:** Texas, / Val Verde Co / Langtry / 28-XI-2014 / leg. D. Sundberg (DSC). 1 ♂, **USA:** Texas, / Val Verde Co / Langtry / 06-VII-2013 / leg. D. Sundberg (DSC). 1 ♂, **USA:** Texas, / Val Verde Co / Langtry / \*-VII-2014 / leg. D. Sundberg (DSC). 1 ♂, **USA:** Texas, / Val Verde Co / 3.2mi W. Langtry / 21-VI-2014 / leg. D. Duran (DPDC). 5 ♂♂, 3 ♀♀, **USA:** Texas, / Val Verde Co / 4mi W Langtry / 18-VI-2015 / leg. S. Roman (SJRC). 1 ♀, **USA:** Texas, / Terrell Co / 13mi W Langtry / 18-VI-2015 / leg. S. Roman (SJRC). 3 ♂♂, 4 ♀♀, **USA:** Texas, / Val Verde Co / 3.5mi W Langtry / 01-VI-2017 / leg. S. Roman (SJRC). 2 ♂♂, **USA:** Texas, / Val Verde Co / 3.5mi W Langtry / 01-VI-2017 / leg. S. Roman (SJRC). 2 ♂♂, 2 ♀♀, **USA:** Texas, / Val Verde Co / 3.5mi W Langtry / 01-VII-2019 / leg. S. Roman (SJRC). 1 ♂, 2 ♀♀, **USA:** Texas, / Val Verde Co / 3.5mi W Langtry / 01-VII-2019 / leg. D. Katz (DKC). 1 ♂, **USA:** Texas, / Val Verde Co / 3.5mi W Langtry / 01-VII-2019 / leg. D. Katz (DKC). 4♂, 5 ♀♀, **USA:** Texas, / Val Verde Co / 3.5mi NW Langtry / 23-V-2016 / leg. D. Brzoska (DWBC). 1 ♂, **USA:** Texas, / Val Verde Co / 12.5mi NW Langtry / 23-V-2016 / leg. Brzoska (DWBC). 1 ♂ **USA:** Texas / Brewster Co / Heath Canyon / 22/IX/2019 / leg. J. Chong/S. Roman (SJRC). 6 ♂♂, 5 ♀♀, *ex ovum*, **USA:** Texas, / Terrell Co / 13mi W Langtry / 18-VI-15 / leg. S. Roman (SJRC). 24 ♂♂, 24 ♀♀, *ex ovum*, **USA:** Texas, / Val Verde Co / 13mi W Langtry / 18-VI-15 / leg. S. Roman (SJRC): 1 ♀ (USNM), 1 ♂, 1 ♀ (TAMUIC), 1 ♂, 1 ♀ (AMNH), 1 ♂, 1 ♀ (CMNH), 1 ♂, 1 ♀ (FSCA).

**Diagnosis.** This species can be distinguished from all other *Amblycheila* by the combination of trapezoidal pronotum, almond-shaped elytra with sharply defined dorsal carinae, smooth and polished elytra with sparse irregular rows of setigerous punctures, the lack of a spine-line projection on the male hind trochanter, and small size for the genus (total body length 23–28 mm). No other geographically proximate congeners can be confused with this species. *Amblycheila schwarzi* is most similar in body shape and size, but its dorsal elytral texture is not smooth or polished, it possesses a greater number of shallow setigerous punctures that are arranged more regularly into rows between the dorsal carinae, and the two species are separated by 1300 km, with congeners occurring in the intervening areas.

**Description.** Small-sized *Amblycheila*. Total body length 23.35–27.75 mm, mean ♀ 26.0 mm, mean ♂ 26.0 mm. **Head** (Fig. 2): slightly narrower than anterior margin of pronotum, black throughout, with dark reddish reflections under strong light, moderately polished, 2–8 supraorbital setae next to each eye. Eyes round, proportionately small, not protruding beyond the genae when viewed from above. Frons clearly delimited from clypeus by a visible suture, gradually blending into vertex. Frons surface mostly smooth, lacking striae, but broadly wrinkled with shallow depressions. Vertex smooth, flat, glabrous. Genae smooth, with 0–6 setae present on each side. Clypeus slightly convex, with 2 setae. Labrum black, convex, smooth with 6–12 setae (typically 8–10) set in deep punctae, length 3.0–3.8 mm, width 1.1–1.8 mm, bidentate. Mandibles black, medium-sized, each with 2–12 setae along outside margin. Maxillary palpi dark reddish brown to black, color consistent in each segment. Labial palpi as above. Antennae long, reaching apical third of elytron, slightly longer in male than female;



**Figure 1.** *Amblycheila katzi* Duran & Roman, sp. nov. dorsal habitus (female).

scape dark testaceous to black, with 2 or more subapical setae, sometimes with additional non-apical setae; pedicel dark reddish brown to black, with 2 or more subapical setae; flagellum dark reddish brown, with ring of long apical setae, covered with fine pubescence throughout.

**Thorax:** Pronotum 5.60–6.85 mm in width (mean = 6.30), black, slightly polished, nearly smooth, with fine shallow rugosity, especially near margins; trapezoidal in shape with nearly straight sides, widest near apex, 5.80–7.60 mm (mean = 6.95); base narrow, 4.00–5.30 (mean = 4.70), setae sparse (1–7 per side) and present



**Figure 2.** *Amblycheila katzi* Duran & Roman, sp. nov. frontal habitus (female).

along lateral third of dorsal surface; disc with thin weakly impressed median line and deeply impressed transverse line; notopleural sutures clearly defined, not visible from dorsal view; proepisternum black, dull, glabrous or with few sparse long setae mostly concentrated near the coxae; meso- and metasternum glabrous or with a few setigerous punctures, especially near coxae. Elytra amygdaloid, 13.60–16.40 mm length (mean = 15.15), shape similar in both sexes; sutural spine absent, microserrations absent; elytral dorsal surface smooth and slightly polished, with sparse and irregularly placed setigerous punctae present between dorsal carinae; additional small shallow setigerous punctures are present in lateral areas, especially between dorsal carinae and epipleura.



**Figure 3.** Habitat for *Amblycheila katzi* sp. nov. **A** steep-sided limestone road cut **B** rock ledges with grooves and cracks **C** rock ledge with calcareous soil and third instar *A. katzi* larval burrow **D** closeup of third instar *A. katzi* larval burrow opening.

**Legs:** Pro-, meso- and metacoxae dark reddish brown to black, with sparse setae; trochanters dark reddish brown to black, pro- and mesotrochanter with a single long seta, sometimes with additional shorter setae; metatrochanter glabrous, apex blunt, lacking a produced apical tip; femora black, surface with multiple rows of erect brown setae; tibiae dark reddish brown to black, surface with erect brown setae similar to femora, with additional dense brown setae present at the apices near the tibial spines; tarsi dark reddish brown to black, bearing fine erect setae on lateral and ventral areas, tarsal claw long, simple.

**Abdomen:** Ventrites black, erect setae present and mostly restricted to a distinct row running parallel to the suture between ventrites, abdominal setae on ventrites 1–4 sparser in female than male; terminal abdominal ventrite with scattered setae near apical half in males, no setae present in females except for a dense fringe of setae emerging from the tip of the apex.

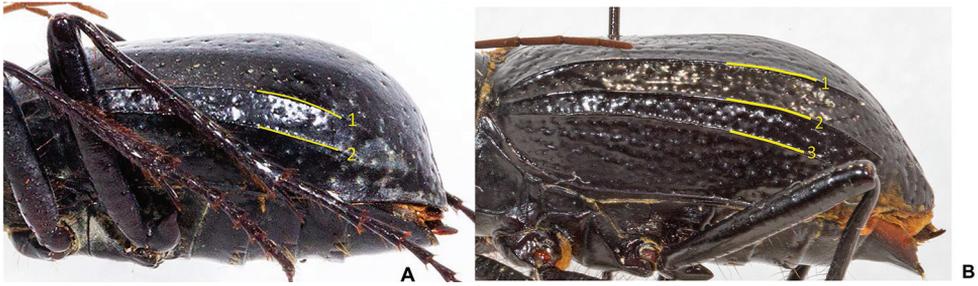
**Etymology.** This new *Amblycheila* is named after David Katz, the discoverer of this remarkable petrophilous insect. We propose the common name of Trans-Pecos Giant Tiger Beetle.

**Distribution and habitat.** *Amblycheila katzi* is currently known only from western Texas in the Trans-Pecos region of the Chihuahuan Desert. All known occurrences are from steep-sided canyons in Val Verde and Terrell Counties, where natural or man-made forces have exposed bedrock, primarily Cretaceous limestone (Freeman 1968)

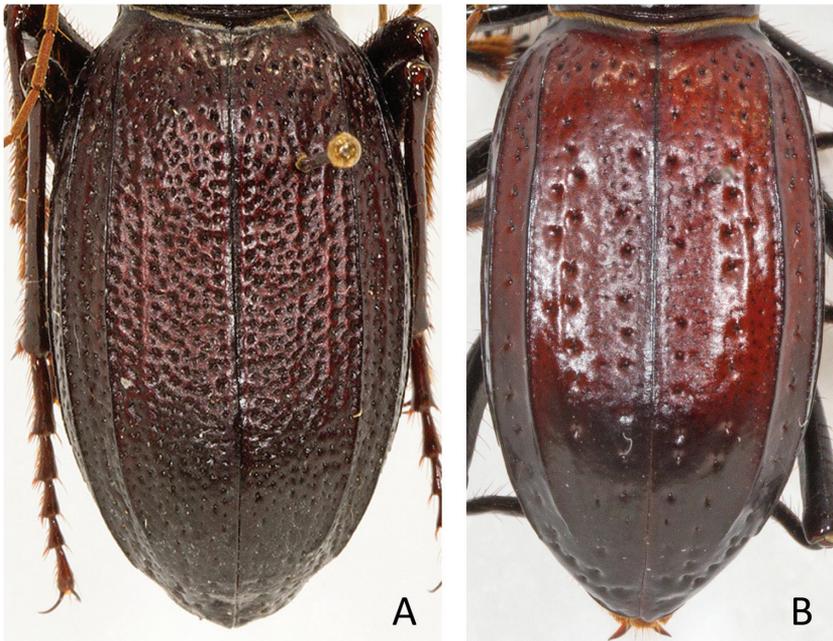
(Fig. 3A). This species appears to prefer vertical surfaces; the authors did not find any specimens on the ground, or in pitfall traps placed at the base of rock walls. All individuals observed by the authors were present 1–5 m above the ground in rock crevices, grooves and ledges (Fig. 3B). Adult beetles were found in the same areas where *Latrodectus* spiders were abundant. The second author discovered 2<sup>nd</sup> and 3<sup>rd</sup> instar larvae in fine calcareous clays within grooves and crevices in vertical limestone walls (Fig. 3C, D).

### Key to the genus *Amblycheila*

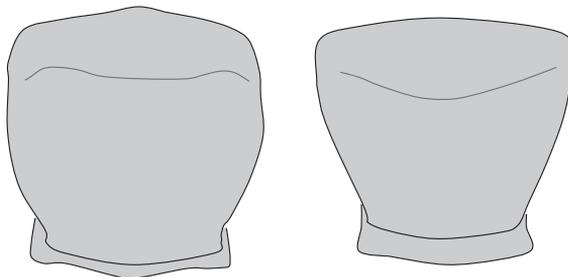
- 1 One or two distinct carinae (pleats) running the length of each elytron (Fig. 4A).... **2**
- Three distinct carinae (pleats) running the length of each elytron (Fig. 4B)..... **3**
- 2 Elytral texture dull; indistinct shallow punctae present. Elytra tapering towards apex. Length 19–26 mm. Southeastern Arizona, northern Mexico ..... ***A. baroni***
- Elytral texture polished; indistinct shallow punctae present along with many small pinpoint punctures. Elytral apex bulbous. Length 26–31 mm. Northern Mexico, possibly southwestern Texas..... ***A. nyx***
- 3 Elytra densely covered with pinpoint punctures. Without rows of larger punctae between dorsal carinae (Fig. 5A)..... **4**
- Punctae between dorsal carinae loosely arranged into rows (Fig. 5B), not densely covering surface. May also possess smaller pinpoint punctures throughout..... **5**
- 4 Elytra uniformly and densely punctate, surface texture dull to matte. Elytra dark red to dark brown. Length 29–40 mm. Great Plains region from South Dakota to Texas..... ***A. cylindriformis***
- Elytral surface polished, bearing small shallow punctures with smooth edges. Elytra black. Length 29–33 mm. Central Mexico ..... ***A. halfferi***
- 5 Pronotum rounded (Fig. 6A). Elytra reddish brown to black. Hind trochanter in male with elongated apex, spine-line. Length 25–37 mm..... **6**
- Pronotum trapezoidal, with nearly straight sides (Fig. 6B). Hind trochanter in male similar to pro- and mesotrochanter, blunt. Elytra black. Length 21–28 mm..... **7**
- 6 Dorsum of each elytron with one well-defined longitudinal row of punctae, with a smaller number of additional irregular punctae; many small shallow pinpoint punctures present. Length 25–31 mm. Arizona to west Texas ..... ***A. picolominii***
- Dorsum of each elytron with deeply impressed punctae, arranged in multiple longitudinal rows or irregularly; shallow pinpoint punctures visible. Length 30–37 mm. South Texas ..... ***A. hoversoni***
- 7 Elytral texture smooth and polished between sparse irregular setigerous punctae. Elytra nearly flat in lateral view, not bulging (Fig. 7A). Length 22–28 mm. Trans-Pecos region of West Texas ..... ***A. katzi***
- Elytral texture dull, not shining; with one or more longitudinal rows of shallow setigerous punctae. Elytra with bulge near apex, especially in male (Fig. 7B). Length 21–27 mm. Mojave Desert region of southeast California to southwest Utah and northwest Arizona ..... ***A. schwarzi***



**Figure 4.** Carinae (pleats) present on *Amblycheila* elytra. **A** two distinct carinae (*A. nyx*) **B** three distinct carinae (*A. halfferi*).



**Figure 5.** Elytral texture. **A** Dense pinpoint punctures present, without rows of larger punctae (*A. cylindriciformis*) **B** with larger punctae loosely arranged into rows (*A. hoversoni*).



**Figure 6.** Pronotal shape **A** rounded **B** trapezoidal.



**Figure 7.** Lateral habitus. **A** *Amblycheila katzi*, sp. nov. male with elytra gradually tapering towards apex **B** *A. schwarzi*. male with elytral bulge and steep decline towards apex.

## Discussion

This species is likely more widespread than presently known. *Amblycheila katzi* can be at low densities, and is present in habitat that would not generally be checked by tiger beetle collectors. No other North American tiger beetle is apparently associated with vertical rock walls, and this unusual behavioral characteristic may further contribute to the lack of known specimens.

Given the fact that all four localities are very close to the US/Mexican border, it is a near certainty that this species occurs in northern Mexico as well. Only two of the eight *Amblycheila* species are known from Mexico, but this is likely due to collecting bias.

When constructing a dichotomous key to the genus *Amblycheila*, we reviewed the literature for potentially informative characters that could diagnose species (Horn 1910; Vaurie 1955; Mateu 1974; Gage 1990; Rumpp unpublished). Not all previously identified characters held up to scrutiny. We found that the placement and number of setae (chaetotaxy) were too variable within species to be of diagnostic value, a view shared by Vaurie (1955). Gage's (1990) description of *A. hoversoni* indicates that it can be distinguished from all other *Amblycheila* based on the presence of a single row of punctae between the dorsal and lateral carinae. We examined long series of *A. hoversoni*, and this character was not always consistent, therefore we did not include it in our key.

## Acknowledgements

The authors thank David Katz, Dan Sundberg, Aaron Chambers, Steve Spomer, and David Brzoska for providing specimens for this study. We thank David Herrmann and Barry Knisley for comments on the dichotomous key. Finally, we thank Jürgen Wiesner and David Pearson for providing comments on the manuscript. The authors have no funding to report. The authors have declared that no competing interests exist.

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# A new species of *Nilobezzia* Kieffer (Diptera, Ceratopogonidae) from the mangrove forest of Hainan Island, China

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<http://zoobank.org/578D1183-362C-4FE0-AE07-53BF7B39680A>

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## Abstract

A new species of *Nilobezzia* Kieffer, *Nilobezzia bamenwana* Li & Li, **sp. nov.**, collected from Bamenwan mangrove forest of Hainan Island, China, is described and illustrated based on female adults. The genus was previously known to have a single species occurring on the island.

## Keywords

biting midges, China, *Nilobezzia bamenwana*, predaceous midges

## Introduction

*Nilobezzia* Kieffer, 1921 is a genus of predaceous ceratopogonid midges in the tribe Johannsenomyiini of the subfamily Ceratopogoninae. It is striking that adult females prey on males while mating (Downes 1969). There are 74 species worldwide (Borkent 2016), of which ten species are distributed in mainland China and Taiwan (Yu et al. 2005). Only one species, *Nilobezzia duodenalis* Liu, Yan & Liu, has been reported from Hainan Island, which was collected from Limu Mountain in the central area of the

island (Liu et al. 1996; Wang et al. 2011). The aim of this contribution is to describe a new species of *Nilobezzia* which was discovered as part of an ongoing investigation of the Ceratopogonidae of Hainan Island.

## Materials and methods

Specimens were collected with a light trap from Bamenwan mangrove forest near Wenchang, Hainan Province, China. The holotype and two paratypes were processed for DNA barcoding of the mitochondrial 5' cytochrome oxidase I gene region, and subsequently mounted onto microscope slides following non-destructive tissue digestion as described by Bellis et al. (2013). DNA barcode sequences compliant with quality assurance criteria of the Barcode of Life Data Systems (BOLD) database (Ratnasingham and Hebert 2007) were submitted with associated specimen details as a dataset (<http://doi.org/10.5883/DS-NILO>). DNA barcode sequences were submitted to GenBank (accession numbers MN135243–MN135245).

Details of colour were taken from specimens kept in ethanol. Measurements of the holotype are provided with the range of variation of the paratypes presented in parentheses. The terminology follows Debenham (1974) and Wirth and Ratanaworabhan (1981). All type specimens are deposited in the Institute of Tropical Agriculture and Forestry, Hainan University, China.

## Taxonomy

### *Nilobezzia* Kieffer, 1921

*Nilobezzia* Kieffer, 1921: 24. Type species: *Nilobezzia armata* Kieffer, 1921 by monotypy.

*Parrotia* Kieffer, 1923: 140. Type species: *Parrotia flaviventris* Kieffer, by original designation. Synonymised by Wirth et al. 1974: 603.

*Crespinia* Kieffer, 1923: 141. Type species: *Crespinia brevipalpis* Kieffer by monotypy. Synonymised by Wirth et al. 1974: 603.

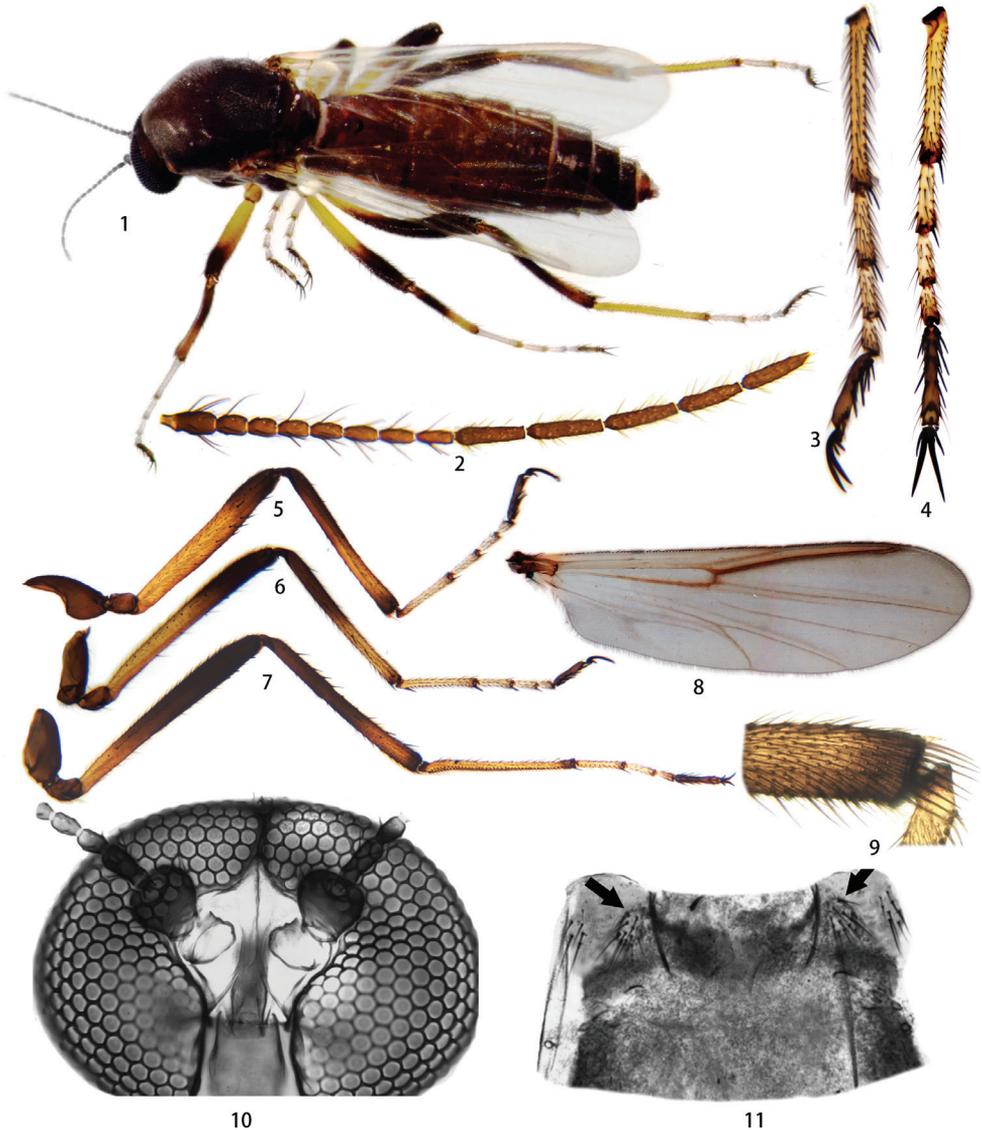
*Sphaerobezzia* Zilahi-Sebess, 1940: 108 (as subgenus of *Bezzia*). Type species: *Bezzia paradoxa* Zilahi-Sebess, by monotypy. Synonymised by Wirth et al. 1974: 603.

### *Nilobezzia bamenwana* Li & Li, sp. nov.

<http://zoobank.org/8E69FC30-F2CC-428E-A9D5-AB6E401833AE>

Figures 1–16

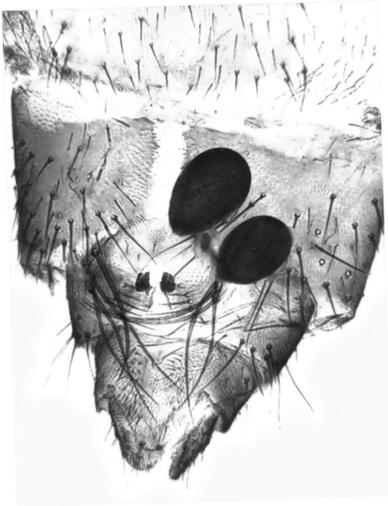
**Type material. Holotype.** CHINA • ♀, slide, Hainan Province, Wenchang City, Bamenwan mangrove forest; 19°37'38"N, 110°47'10"E, 18 Jun 2018; Chunqiao Li leg., light trap; cer250-1, GenBank MN135245.



**Figures 1–11.** Female of *Nilobezzia bamenwana* sp. nov. **1** habitus in dorsal view **2** antenna **3** midtarsus, anterior view **4** foretarsus, ventral view **5** foreleg **6** midleg **7** hind leg **8** wing **9** hind tibial comb **10** head, dorsal view **11** abdominal tergite I, dorsal view.

**Paratypes.** CHINA • 9♀, same data as holotype, six paratypes (cer250-2–cer250-7) mounted on slides, three kept in ethanol (cer250-8–cer250-10). GenBank MN135243 and MN135244.

**Diagnosis.** The only species of *Nilobezzia* with the following combination of characters: body longer than 3.5 mm; femora and tibiae with spines scattered along their length; femora distinctly yellow basally and dark brown distally, tibiae dark brown with



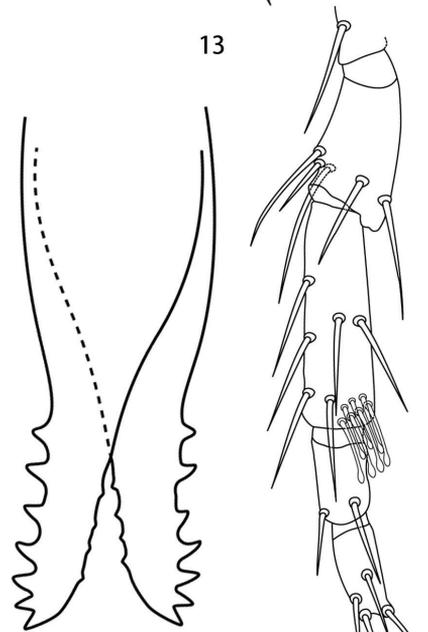
12



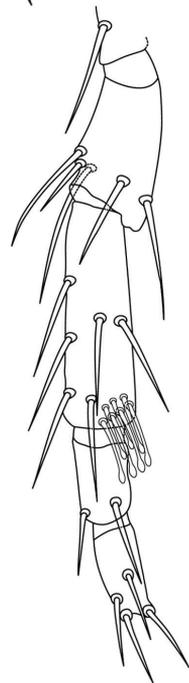
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16

**Figures 12–16.** Female of *Nilobezzia bamenwana* sp. nov. **12** terminal four segments of abdomen, ventral view **13** spermathecae and hair tufts, ventral view **14** proboscis and palpi, anterior view **15** mandibular teeth, anterior view **16** maxillary palpus, anterior view.

subapical pale bands; wing with a single radial cell and spermathecae unequal in size and without necks.

**Description. Female. Body** (Fig. 1) 4.25 (3.85–4.28) mm in length. Wing 2.69 (2.69–2.95) mm in length.

**Head dark brown.** Eyes contiguous, bare (Fig. 10). Antenna dark brown with slightly paler pedicel, basal flagellomeres short and stout, distal 5 flagellomeres each much longer than basal 8 flagellomeres, lengths in ratio of 96:47:47:46:51:50:58:64:129:123:127:111:140; AR 1.37 (1.17–1.37,  $N = 5$ ) (Fig. 2). Maxillary palpi brown, 5-segmented, third segment long, not distinctly swollen, a few scattered hyaline sensillae preapically, lengths in ratio of 17:33:86:43:42, PR 3.07 (2.99–3.44) (Figs 14, 16). Mandible with seven coarse teeth (Figs 14, 15).

**Thorax.** Scutum dark brown, some specimens with humeral area slightly paler, with fine microsetae and several bristles near base of wings. Scutellum and postscutellum concolourous with scutum. Coxae dark brown, trochanters light brown; forefemur, midfemur with basal 2/3 yellow and distal 1/3 dark brown, basal 1/3 of hind femur yellow, distal 2/3 dark brown; basal 1/2 of fore tibia and mid tibia dark brown, distal 1/2 yellow, except narrow dark apex; 2/3 of hind tibia dark brown, apical 1/3 slightly paler, dark apex (Figs 1, 5–7). All femora and tibiae with many scattered black spines over their entire length, variable in number; hind tibial comb (Fig. 9) with nine spines. Tarsomeres I–IV yellow except brown apices; tarsomere V entirely dark brown; foretarsomere and hindtarsomere I–II and midtarsomere III each with single apical spine, midtarsomere I–II with two apical spines, midtarsomere I also with a basal spine, foretarsomere and hind tarsomere III and tarsomere IV of all legs without apical spine, tarsomere V (Fig. 4) with 14 ventral batonnets; claws equal, 0.8 times the length of tarsomere V and bearing two strong basal teeth on the outer surface ca. 0.3 times of length of claw (Figs 3, 4). RL-L 1013:948:377:196:142:80:245 and TR 1.92 (1.78–2.08,  $N = 5$ ) in fore leg, RL-L 1460:1080:533:231:173:91:225 and TR 2.30 (2.15–2.56,  $N = 5$ ) in mid leg, RL-L 1508:1269:1000:320:236:133:240 and TR 3.10 (2.90–3.10,  $N = 5$ ) in hind leg. Wing membrane pale grey, CR 0.82 (0.81–0.82,  $N = 5$ ), a single radial cell (Fig. 8). Haltere white.

Abdominal tergites brown, tergite I (Fig. 11) with lateral tufts of 10–12 short setae arranged in oval area, with anteromesal dark, triangular spot; abdominal segment VIII with a pair of subquadrate genital sclerotations near semi-circular gonopore, ventral hair tufts dark and conspicuous, each comprising a row of four or five long black bristles and a row of four short bristles on each side (Figs 12, 13); cerci brown; two dark brown spermathecae, large, oval, unequal, 129×83 (109–129×67–83)  $\mu\text{m}$  and 94×66 (90–110×57–66)  $\mu\text{m}$ , neck absent, and a third vestigial spermatheca present (Figs 12, 13).

**Male.** Unknown.

**Etymology.** The name *bamenwana* refers to the collecting location of the species.

**Distribution.** Known only from the Bamenwan mangrove forest of Hainan Prov., China.

**Discussion.** Female specimens of *Nilobezzia bamenwana* run to genus *Nilobezzia* in the key of Wirth et al. (1974) and conforms to the diagnosis of *Nilobezzia* provided by Debenham (1974) and Wirth and Ratanaworabhan (1981). The only other species of *Nilobezzia* recorded from mangrove forest is *N. virago* Debenham which was recorded from many different habitats including a single female specimen collected from mangroves in Australia (Debenham 1974).

*Nilobezzia bamenwana* runs to *N. acanthopus* (de Meijere) in the key to Southeast Asian species by Wirth and Ratanaworabhan (1981) but in the latter species the forefemur and midfemur are entirely yellowish and the spermathecae are equal in size. India has 18 described species of *Nilobezzia* (Mazumdar et al. 2009), some of which possess similar leg colour patterns and other characteristics to *N. bamenwana*, but with wings shorter than 2.5 mm and significantly smaller than *N. bamenwana* and none appear to be associated with mangroves. *Nilobezzia bamenwana* runs to *N. opaca* Das Gupta in the key by Mazumdar et al. (2009), but that species can be distinguished by the obvious neck of spermathecae and legs excepting the midfemora, entirely brown. It runs to *N. formosana* (Kieffer) in the key to Chinese species of *Nilobezzia* by Yu et al. (2005), but that species is much smaller in size with a wing length of only 2.11 mm. *Nilobezzia bamenwana* is allied to *N. japana* Tokunaga in general colour, but the latter species is smaller (body length 3.2 mm), has more extensive dark markings on the forefemur and midfemur, and the foretibia lacks spines.

## Acknowledgements

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# A new subspecies of African fire salamander *Salamandra algira* (Urodela, Salamandridae) from the Middle Atlas Mountains, Morocco

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## Abstract

A new subspecies within the *Salamandra algira* species complex from north-western Africa is described. Previous molecular analysis showed that the populations of *S. algira splendens* from north-western Morocco consisted of two well supported clades: clade 1 distributed in the Rif Mountains, from Chefchaouen (type locality) to Al Hoceima, and clade 2, located southern from clade 1 being isolated in the northern and central Middle Atlas Mountains. Clade 2 is herein described as a distinct subspecies: *Salamandra algira atlantica* **ssp. nov.** based on morphological data, allopatric range and molecular divergence. This new subspecies shows an uncorrected pairwise distance of 0.0265 from clade 1 based on cytochrome b DNA sequences. *Salamandra algira atlantica* **ssp. nov.** is a slender and large sized salamander with a highly variable colouration pattern. It can be distinguished from *S. algira splendens* by the greater proportion of coral red in the background colouration, being the only known subspecies of *S. algira* in which coral red can exceed the proportion of black. Variable number (0–5) of yellow to golden yellow dorsal blotches, but usually in lower numbers than the nominotypical subspecies. *Salamandra algira atlantica* **ssp. nov.** inhabits subhumid to humid forests and karstic systems at mid to high elevations. We briefly discuss the phylogenetic and taxonomic issues among the genus *Salamandra* which encompasses more valid species than currently recognised.

## Keywords

Amphibia, mitochondrial DNA, *Salamandra algira atlantica* ssp. nov., taxonomy, threatened species

## Introduction

The batrachofauna of North Africa was considered species-poor and mostly composed of species closely related to the European counterparts. However, several recent studies revealed a high degree of endemism and genotypic divergence (Recuero et al. 2007; Escoriza and Ben Hassine 2019). The genus *Salamandra* Garsault 1764 is a group of terrestrial Urodeles widely distributed across the western Palaearctic, comprising six species: *S. algira* Bedriaga, 1883, *S. atra* Laurenti, 1768, *S. corsica* Savi, 1838, *S. infraimmaculata* Martens, 1885, *S. lanzai* Nascetti, Andreone, Capula & Bullini, 1988 and *S. salamandra* (Linnaeus, 1758) (Dubois and Raffaëlli 2009). The genus is particularly well diversified through the Iberian Peninsula, possibly caused by several events of allopatric speciation during the Plio-Pleistocene climatic cycles (Steinfartz et al. 2000). In North Africa, *S. algira* is the sole representative of the genus (Escoriza and Ben Hassine 2019). The ancestor of *S. algira* possibly colonised the African continent during the Miocene from the Iberian Peninsula (Escoriza et al. 2006; Beukema et al. 2010). Later climatic fluctuations during the Pliocene and Pleistocene have driven allopatric genetic divergence in *S. algira* populations across the mountainous systems of northern Morocco and Algeria (Beukema et al. 2010; Ben Hassine et al. 2016).

*Salamandra algira* shows a fragmented distribution, being mostly confined to humid habitats in mountain ranges between elevations of 30–2455 m above sea level (a.s.l.) (Escoriza and Ben Hassine 2015). Four subspecies are currently recognised (Donaire-Barroso and Bogaerts 2003; Escoriza and Comas 2007; Beukema et al. 2013): *S. algira tingitana* in the north-western Rif, ranging from Ceuta to Moulay Abdessalam; *S. algira splendens*, in the central-eastern Rif, from Chefchaouen to Al Hoceima, including isolated populations in the Middle Atlas Mountains; *S. algira spelaea* restricted to the Beni Snassen massif; and the nominal *S. algira algira* in Algeria, located across the pericoastal mountains of Annaba, Collo, Kabylia, and Blida Atlas (Beukema et al. 2010; Escoriza and Ben Hassine 2014; Hernandez and Escoriza 2017). However, recent phylogenetic analysis suggested that the taxonomy of *S. algira* could require further re-assessments (Ben Hassine et al. 2016; Dinis et al. 2019). *Salamandra algira splendens* includes two distinct allopatric clades separated by 50 km of semi-arid plains. Here, we described one of these clades as a distinct subspecies, based on genetic and morphological evidences.

## Materials and methods

### Field sampling

Voucher specimens (i.e., the three specimens that constituted the type series) were anaesthetised and euthanised in a closed vessel with a piece of cotton wool containing ethyl acetate (Simmons 2002), fixed in 95% ethanol for five hours, and subsequently transferred to 70% ethanol for permanent storage. Type specimens are held at the col-

lections of vertebrates of the Museum of Natural Sciences of Barcelona (MZB) and Madrid (MNCN), Spain. Other specimens (19 adults and one larva) used in this study were measured, photographed and released in situ.

### Morphological examination

All specimens were sexed based on the cloaca morphology, because this is a constant sexual dimorphic character for species of the genus *Salamandra* (Raffaëlli 2013). The morphological comparison between the new taxon and other subspecies were based on voucher specimens, specimens captured in situ (Middle Atlas) and the original descriptions: Donaire-Barroso and Bogaerts (2003), Escoriza and Comas (2007) and Beukema et al. (2013).

We measured 14 morphological characters for adult and larval specimens, using a digital calliper (accuracy 0.01 mm) following Escoriza and Ben Hassine (2019). For adults, the abbreviations are:

<b>TL</b>	total length;	<b>END</b>	eye nostril distance;
<b>SVL</b>	snout-cloaca length;	<b>LHU</b>	humerus length;
<b>HL</b>	head length;	<b>FAL</b>	forearm length;
<b>PAL</b>	parotoid length;	<b>HAL</b>	hand length;
<b>HW</b>	head width;	<b>THL</b>	thigh length;
<b>IOR</b>	interorbital distance;	<b>TIL</b>	tibia length;
<b>ED</b>	eye diameter;	<b>FL</b>	foot length.

For larva the following abbreviations are used:

<b>SVL</b>	snout-cloaca length;	<b>HW</b>	head width;
<b>TAL</b>	tail length;	<b>TL</b>	total length;
<b>HL</b>	head length;	<b>MTH</b>	maximum tail height.

TL has been compared between the sexes of the new subspecies using a Mann-Whitney U test, conducted with Statistica 7.0 (StatSoft, Tulsa, OK).

### Molecular analysis

We inferred a molecular phylogeny based on sequence data from GenBank. Sequences have been obtained for much of the geographical range occupied by *S. algira*, including all major clades (Ben Hassine et al. 2016; Dinis et al. 2019). We aligned the downloaded sequences using ClustalW2 (Thompson et al. 1994). The final alignment included 309 base pairs of cytochrome b DNA partial genes of 106 specimens. The matrix of pairwise uncorrected p-distances was constructed using MEGA-X 10.0.5, estimating

the variation based on 1000 bootstraps (Kumar et al. 2018). The phylogenetic analyses were performed under the Bayesian context using MrBayes v.3.2.5 (Huelsenbeck and Ronquist 2001). The best model of DNA substitution was selected using ModelTest 3.7 (Posada and Crandall 1998). We used the general-time reversible + invariant + gamma (GTR+I+G) substitution model, with 20 million generations of Monte Carlo Markov chains (MCMC), sampling every 10000 and discarding the first 25% of the trees (Huelsenbeck and Ronquist 2001). The convergence of MCMC simulations was determined checking the values of the effective sample sizes for the posterior probability, which was higher than 1000 for all continuous parameters (Ronquist et al. 2012). The phylogenetic tree was generated by iTOL 4.4.2 (Letunic and Bork 2006).

## Results

### Molecular analyses

Mean uncorrected p-distance between *S. algira* from the Atlas Mountains and *S. algira splendens* is  $0.0265 \pm 0.0082$ , similar to the divergence between the eastern subspecies of *S. algira* (*S. algira algira* and *S. algira spelaea*) (Table 1). The phylogenetic tree showed two well supported clades (Bayesian posterior probability, PP = 1.00) (Fig. 1) within the formerly taxonomic unit *S. algira splendens*: one belonging to the northern clade of the Rif Mountains, *S. algira splendens* sensu stricto, and the other one, distributed in the Middle Atlas Mountains, which is herein described as a new subspecies. Therefore, the western Moroccan group of *S. algira* is composed by three main morphologically diagnosable mitochondrial clades having subspecific status: *S. algira tingitana*, *S. algira splendens* and *S. algira atlantica* ssp. nov.

### Taxonomy

#### *Salamandra algira atlantica* ssp. nov.

<http://zoobank.org/22C2B256-0F9B-4063-93B4-7C3DB84AA9E4>

Figs 2–4

Atlas fire salamander

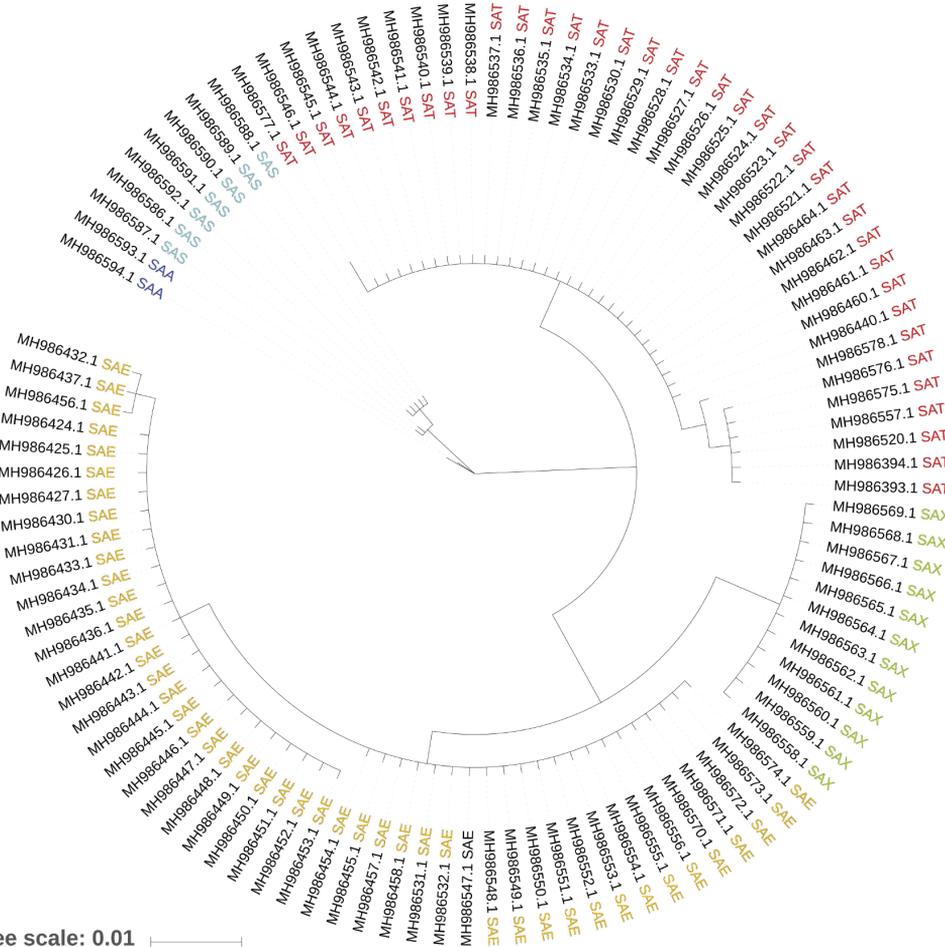
**Holotype.** MNCN 50499 (Fig. 2), an adult male preserved in 70% ethyl alcohol from Jbel Tazekka, Taza Province, Middle Atlas Mountains, northern Morocco (34.15N, 4.00W) at 810 m a.s.l collected on 25 March 2013 by Daniel Escoriza.

**Paratypes.** MZB 2010-0128 and MZB 2010-0129, two adult males preserved in 70% ethyl alcohol collected in the Jbel Tazekka, Taza Province, Middle Atlas Mountains, northern Morocco in December 2009 by Daniel Escoriza and Félix Amat.

**Etymology.** The new taxon is named after the word ‘Atlas’ from Greek Ἀτλας in reference to the Atlas Mountains (Morocco), where this subspecies is found.

**Table I.** Mean uncorrected *p*-distances based on isolate cytochrome b DNA sequences.

	<i>S. algira algira</i>	<i>S. algira spelaea</i>	<i>S. algira tingitana</i>	<i>S. algira splendens</i>
<i>S. algira algira</i>				
<i>S. algira spelaea</i>	0.0233			
<i>S. algira tingitana</i>	0.0573	0.0704		
<i>S. algira splendens</i>	0.0772	0.0756	0.0440	
<i>S. algira atlantica</i>	0.0777	0.0735	0.0518	0.0265



**Figure I.** Phylogenetic relationships among *Salamandra algira* subspecies, inferred from cytochrome b sequences. Abbreviations: SAA = *S. algira algira* (dark blue); SAS = *S. algira spelaea* (sky blue); SAX = *S. algira atlantica* ssp. nov. (lime green); SAE = *S. algira splendens* (orange); SAT = *S. algira tingitana* (red).

**English name.** Atlas fire salamander.

**Diagnosis.** A large subspecies of *S. algira* with a maximum total length of 246.21 mm. Distinguished from the other subspecies by the following combination of characters (Table 2):

**Table 2.** Identification key to *Salamandra algira* subspecies.

	<i>Salamandra algira algira</i>	<i>Salamandra algira spelaea</i>	<i>Salamandra algira tingitana</i>	<i>Salamandra algira splendens</i>	<i>Salamandra algira atlantica</i> ssp. nov.
Total length (in mm)	131–192 ♂ 129–255 ♀	177–194 ♂ 206–236 ♀	151–170 ♂ 140–192 ♀	140–178 ♂ 161–261 ♀	125–246 ♂ 133–205 ♀
Background colouration	Grey-black	Grey-black	Grey-black	Grey-black or coral red	Grey-black or coral red
Number of dorsal yellow blotches	3–10	3–10	0–7	2–7	0–7
Red spots	Usually small	Small	Absent	Small to large	Small to full
White spots on flanks	Present	Present	Absent	Absent	Absent

Background dorsal pattern varies from full grey-black to full coral red, being the only subspecies of *S. algira* in which the coral red can exceed the proportion of grey-black. *Salamandra algira algira* and *S. algira spelaea* usually show only few and little red colouration, mostly around yellow blotches on the dorsum of head, limbs and tail and gular region. *Salamandra algira tingitana* typically lacks red colouration. *Salamandra algira splendens* never shows full red background colouration, being mainly limited to the edges of the yellow blotches, on the dorsum of the head, limbs, tail and gular region.

Variable number (0–7, usually 2–4) of yellow to golden yellow dorsal blotches, but in lower numbers than the eastern subspecies (*S. algira algira* and *S. algira spelaea*, 3–10). *Salamandra algira tingitana* can be completely black or with numerous fragmented little yellow spots or with few large blotches in an arrangement similar to *S. algira atlantica* ssp. nov. *Salamandra algira splendens* has a similar number of yellow blotches than *S. algira atlantica* ssp. nov., but these can be more irregular in their shape in the former. Moreover in *S. algira atlantica* ssp. nov. dorsal yellow blotches can fade progressively into white, composing a pattern that does not appear in *S. algira splendens* (Fig. 3D).

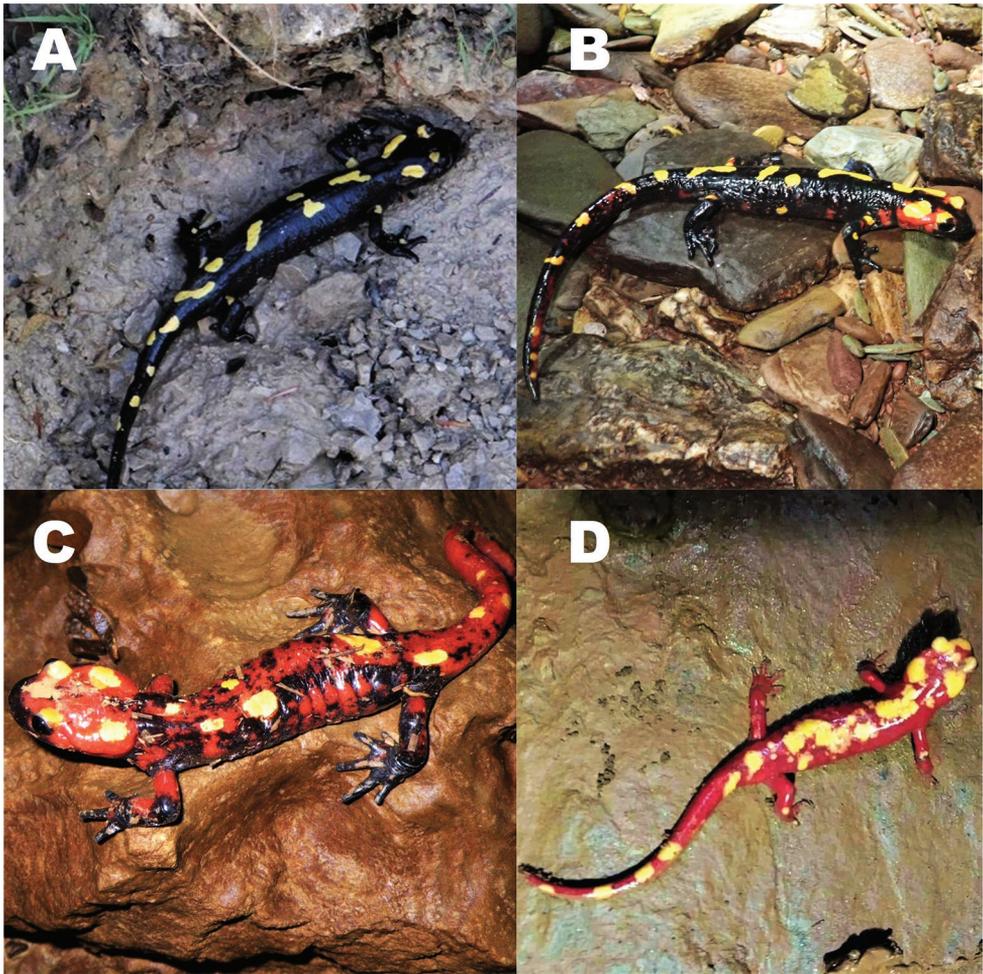
Absence of white spots in the flanks of the body, which appear in the nominotypic subspecies and *S. algira spelaea*.

**Description of holotype.** Snout rounded in a dorsal aspect and in lateral profile. Head large and well differentiated from the body. Nostrils oriented dorsolaterally, situated close to the snout tip. Large-sized parotoids, elongatedly ovoid, slightly divergent anteriorly, showing contrasted glandular pores. Prominent eyes situated laterally, with barely visible vertical oval pupils. Gular fold present. Costal grooves 10. Fingers short and slightly rounded, with a relative length I < IV < II < III. Toes slightly rounded, with a relative length I < V < II < III < IV. Cloaca ovoid. Subcylindrical tail, ended in a blunt tip. Dorsal skin weakly granular. Two pairs of glandular pores disposed in parallel, along the vertebral axis.

**Colour of holotype.** In life the background dorsal colouration is black with four elongated golden yellow spots. In the head appear two golden yellow spots on the orbits and two on the parotoids. Four yellow spots at the base of the limbs and five on the dorsum of the tail. Two yellow spots in lateral parts of the body, close to the insertion of the hindlimbs. One small yellow spot showing red colouration in lateral part of the head, located posterior and ventral to the orbit. Uniform greyish black ventral colouration. Eyes dark brown, almost black.



**Figure 2.** Holotype of *Salamandra algira atlantica* ssp. nov. (MNCN 50499) in life. Photograph by Daniel Escoriza.



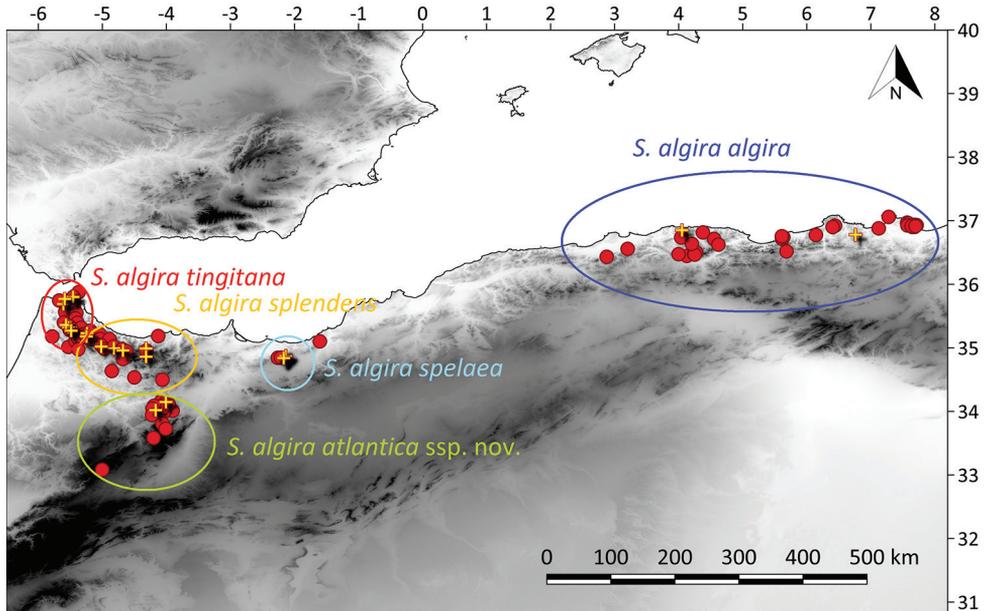
**Figure 3.** Variation in the colouration pattern of *Salamandra algira atlantica* ssp. nov.: **A** adult male from Jbel Sidi Ali, Midelt Province **B** adult female from Grotte de Chaâra, Taza Province **C, D** adult males from Grotte de Chaâra. Photographs by Axel Hernandez.

**Measurements of holotype (in mm).** TL 175.19 mm, SVL 99.16 mm, HL 20.82 mm, PAL 9.89 mm, HW 14.57 mm, IOR 6.17 mm, ED 5.05, END 4.81 mm, LHU 8.01 mm, FAL 11.52 mm, HAL 13.07 mm, THL 6.53 mm, TIL 11.93 mm, FL 14.8 mm.

**Variation.** There are intraspecific population variations regarding colouration pattern in adult specimens (Fig. 3). Within the same population, yellow spots on the dorsum of the head can be divided into four semisymmetric spots on the parotoids and the eyes (21.05% specimens) or they can be merged unilaterally (42.11% specimens) or bilaterally (36.84% specimens). The coral red colour can be limited to a thin colouration on the edge of the yellow spots or be the dominant background colour (Fig. 3). The specimens can show a completely black ventral region, or show some reddish colouration in the throat and / or the cloaca region. More rarely, they can show

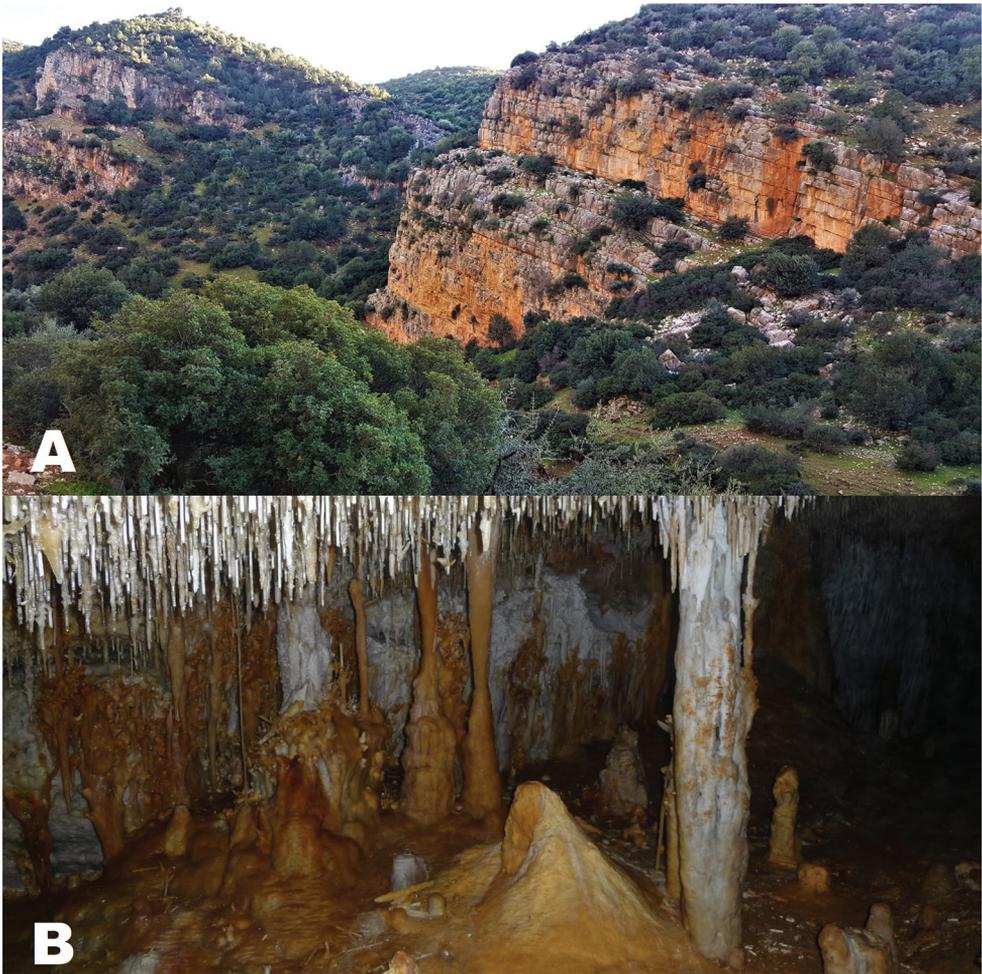


**Figure 4.** Larva of *Salamandra algira atlantica* ssp. nov. Photograph by Daniel Escoriza.



**Figure 5.** Map showing the distribution of the subspecies of *Salamandra algira* (red circles) and the localities included in the phylogenetic analyses (yellow crosses) in northern Morocco and Algeria.

small white spots in the gular region (10.53% specimens). The only known specimen from the southernmost population (Sidi Ali) showed a predominantly black dorsal coloration, with five small yellow patches in the dorsum and seven on the tail (Fig. 3A). The size (TL) of adults ranges from 125.93–246.21 mm in males ( $N = 15$ , mean = 190.65 mm  $\pm$  7.74 standard error, SE) and 133.43–205.81 mm in females ( $N = 4$ , mean = 171.54 mm  $\pm$  16.67 SE). The differences in TL between both sexes were not significant (Mann-Whitney U test:  $U = 23$ ,  $P = 0.484$ ). Males mean SVL 93.71 mm  $\pm$  2.82 SE, HL 21.57 mm  $\pm$  0.76, PAL 12.81 mm  $\pm$  0.53, HW 15.87 mm  $\pm$  0.45, IOR 6.73 mm  $\pm$  0.25, ED 4.04 mm  $\pm$  0.12, END 4.69 mm  $\pm$  0.14, LHU 7.81 mm  $\pm$  0.32, FAL 9.57 mm  $\pm$  0.36, HAL 12.18 mm  $\pm$  0.56, THL 7.49 mm  $\pm$  0.31, TIL 10.26 mm  $\pm$  0.38, FL 15.34 mm  $\pm$  0.59. Females mean SVL 90.72 mm  $\pm$  8.33 SE, HL 20.18 mm  $\pm$  1.90, PAL 12.82 mm  $\pm$  1.11, HW 16.28 mm  $\pm$  1.33, IOR 7.33 mm  $\pm$  0.57, ED 3.71  $\pm$  0.18, END 3.95 mm  $\pm$  0.39, LHU 7.28 mm  $\pm$  0.67, FAL 8.40 mm  $\pm$  0.86, HAL 11.73 mm  $\pm$  1.27, THL 7.36 mm  $\pm$  1.06, TIL 9.64 mm  $\pm$  1.10, FL 15.15 mm  $\pm$  2.69. Males have an ovoid-shaped cloaca during the breeding season and females a flat cloaca. Larvae are characterised by having the following morphological characters: Head depressed, with relatively large eyes situated laterally (Fig. 4). Snout rounded and semi-circular. Gills with three short rami and numerous fimbriae. Four fingers and five toes, narrow and pointed. Ten or eleven costal grooves. Tail equal in length to SVL or slightly smaller. Tail fin short, originating anterior to the pelvic girdle, ended in a bluntly pointed tip. Colour uniformly dark brown with a distinct pale spot at the base of the four limbs. The colouration varies according to the development and



**Figure 6.** **A** Habitat of *Salamandra algira atlantica* ssp. nov. at the type locality, Taza Province, Middle Atlas Mountains, northern Morocco **B** Cave habitat at Grotte de Chaâra. Photographs by Axel Hernandez.

the terminal phases show diffuse yellow spots on the head and dorsum. SVL 29.0 mm, TAL 23.9 mm, HL 10.8, HW 6.2, TL 52.9 mm, MTH 5.6 mm (Taza, Morocco).

**Distribution.** *Salamandra algira atlantica* ssp. nov. is endemic to the northern and central Middle Atlas Mountains, northern Morocco: Tazekka National Park, Bou Iblane Massif and Jbel Sidi Ali (Fig. 5).

**Natural history.** The new subspecies is found from 600–2455 m a.s.l. near springs and streams in humid mesothermal forests of conifers (*Abies pinsapo*, *Cedrus atlantica*, *Pinus halepensis*) and oaks (*Quercus ilex*, *Q. canariensis*, *Q. suber*; Fig. 6 A). There are also troglophile populations at Grotte de Chaâra, Grotte d’Izora. and Gouffre du Friouato which reproduce inside the caves at 400 m from the entrance (Fig. 6B). It is a crepuscular and nocturnal species having a surface activity from autumn to spring.

## Discussion

*Salamandra algira* was briefly described as *Salamandra maculosa* var. *algira* by Bedriaga (1883). As the holotype is not known to exist, a neotype (NHMW 9251) was later designated from Jebel Edough near Bône (Annaba), Algeria by Eiselt (1958). *Salamandra algira* had been considered as a subspecies of *S. salamandra*, until its specific and species status was confirmed by molecular analysis (Veith 1994; Steinfartz et al. 2000). Donaire-Barroso and Bogaerts (2003) described *S. algira tingitana* from Jbel Musa (= Jabal Muse; Tingitana Peninsula, northwestern Morocco), and subsequently *S. algira* was divided into two subspecies by separation of *S. algira tingitana* from the nomotypic *S. algira algira* from Annaba, Algeria. Escoriza et al. (2006) rediscovered a small and isolated population of *S. algira* in the Beni Snassen Massif (north-eastern Morocco). This population was shortly after described as a distinct and micro-endemic subspecies: *S. algira spelaea* (Escoriza and Comas 2007). In 2013, a fourth subspecies, *S. algira splendens* was described from the Rif and Middle Atlas Mountains. The type locality of *S. algira splendens* is Aïn Tissimilan, Jebel el Kelaâ, Chefchaouen, western Rif Mountains (Beukema et al. 2013).

As previously found (Escoriza et al. 2006; Dinis et al. 2019), our phylogenetic analysis recovered two main lineages within *S. algira*, one located west of the Moulouya valley, including the western subspecies (*S. algira tingitana* and *S. algira splendens*) and a second lineage formed by the eastern subspecies (*S. algira algira* and *S. algira spelaea*). Our results confirm that Algerian populations of *S. algira* are genetically distinct from the Moroccan ones (Ben Hassine et al. 2016). Additionally, our phylogenetic analysis corroborates the recent recognition of two main independent lineages in *S. algira tingitana* (Beukema et al. 2010, 2013). These different clades are mostly separated across the boundaries of river Martil, differing at the morphological level and by having two different reproduction modes, viviparous and larviparous (Beukema et al. 2010). The viviparous form of *S. algira tingitana*, which is only distributed in the extreme northern parts of the Tingitana Peninsula, was previously considered as a full valid species by Dubois and Raffaëlli (2009). This consideration is still under debate, despite unanimous recognition of two very divergent phenotypes and genotypes (Beukema et al. 2010, 2013). Consequently, *S. algira tingitana* may warrant taxonomic revision including new phylogenetic and morphological studies.

The separation among allopatric populations over North African mountainous systems can be attributed to the Late Neogene aridification (Griffin 2002; Escoriza and Ben Hassine 2015). The split between both subspecies of the western group, *S. algira tingitana* and *S. algira splendens*, was estimated during the Pliocene, approximately 1.6–3.8 million years ago (Mya) while between the eastern subspecies, *S. algira algira* and *S. algira spelaea*, was estimated during the Plio-Pleistocene boundary, approximately 2.5 Mya (Beukema et al. 2010; Ben Hassine et al. 2016). *Salamandra algira atlantica* ssp. nov. is completely isolated from other populations of *S. algira* by the arid Moulouya and the Saïss plains. These contemporaneous arid barriers precluded the gene flow between Rif and Middle Atlas populations and the Beni Snassen (Escoriza and Ben Hassine 2015).

Despite several attempts, the genus *Salamandra* still represents an outstanding and challenging complex due to conservative morphology, with a high degree of geographical, intraspecific, and genetic variation (Eiselt 1958; Steinfartz et al. 2000; García-París

et al. 2003). The highest diversity is mainly found through the Iberian Peninsula, where nine subspecies are currently recognised (Joger and Steinfartz 1994; Veith 1994, 1996; Steinfartz et al. 2000). The recent classification and systematic arrangement are controversial and under debate (Thiesmeier 2004; Thiesmeier and Grossenbacher 2004; Dubois and Raffaëlli 2009; Raffaëlli 2013). Due to several discordance between nuclear and mtDNA gene trees usually found in previous studies, as a consequence of the retention of ancestral states, insufficient lineage sorting in the diverging populations or also resulting from gene introgression, their classification is difficult to assess (García-París et al. 2003), although *S. salamandra* sensu lato comprises several lineages representing three to four cryptic species (Raffaëlli 2013). In this sense, unique morphological traits, ecological differences and allopatric status, are crucial to evaluate properly new taxa among the genus *Salamandra* following recent descriptions (Malkmus 1983; Joger and Steinfartz 1994; Donaire-Barroso and Bogaerts 2003; Bonato and Steinfartz 2005; Köhler and Steinfartz 2006; Escoriza and Comas 2007; Beukema et al. 2013).

Our results indicated morphological divergence between *S. algira splendens* and *S. algira atlantica* ssp. nov. However, some specimens of this new subspecies are similar to *S. algira splendens*, thus their differentiation has to be supported by the geographical range. The presence of coral red colouration is evident in specimens of both subspecies, but is more extensive in some adult individuals of *S. algira atlantica* ssp. nov. In this sense, differences in the extension or presence of the red colouration was also considered as a diagnostical criterion for some Iberian subspecies: *S. salamandra bejarae*, *S. salamandra crespoides*, *S. salamandra gallaica* and *S. salamandra morenica* (Joger and Steinfartz 1994; Thiesmeier 2004). Reddish colouration is very scarce or absent in some species of the genus (e.g., *S. atra*, *S. corsica*) and several subspecies of *S. salamandra* (Thiesmeier 2004; Raffaëlli 2013; Sparreboom 2014).

In terms of conservation, *S. algira atlantica* ssp. nov. should be regarded as vulnerable. This endemic subspecies is distributed in a poorly prospected area of approximately 1600 km<sup>2</sup>, where it is almost exclusively confined to mid-high elevations. However, the distribution of the subspecies is still not completely understood: e.g., the southernmost population is only known from a single specimen and the hypogeal populations were also been discovered very recently (Hernandez 2018a, b, c). The alteration and destruction of natural habitats are the main threats found in the Middle Atlas Mountains.

## Conclusions

This study increases the current known number of subspecies of *S. algira* found in northern Morocco, from three (*S. algira tingitana*, *S. algira spelaea*, *S. algira splendens*) to four subspecies in describing *S. algira atlantica* ssp. nov. It also supports this region as having the highest intraspecific diversity of *S. algira*. The taxonomic separation of a single widespread species into multiple small-ranged taxa in turn have important implications for the conservation status of the original species. We therefore recommend a re-assessment of the outdated Vulnerable status (VU) of *S. algira* (IUCN 2009) to reflect the current taxonomic revisions and the increasing threats from the international pet trade and habitat loss which have taken place over the last decade.

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