RESEARCH ARTICLE



## Two new species of Acanthobothrium Blanchard, 1848 (Onchobothriidae) in Narcine entemedor Jordan & Starks, 1895 (Narcinidae) from Acapulco, Guerrero, Mexico

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Academic editor: <i>B</i> .	Georgiev	Received 14 August 2018	Accepted 25 April	il 2019	Published 5 June 2019
	http://	zoobank.org/0CAC34BD-1C75	-415F-973D-C37E45	57D06F	

**Citation**: Zaragoza-Tapia F, Pulido-Flores G, Violante-González J, Monks S (2019) Two new species of *Acanthobothrium* Blanchard, 1848 (Onchobothriidae) in *Narcine entemedor* Jordan & Starks, 1895 (Narcinidae) from Acapulco, Guerrero, Mexico. ZooKeys 852: 1–21. https://doi.org/10.3897/zookeys.852.28964

#### Abstract

Two species of *Acanthobothrium* (Onchoproteocephalidea: Onchobothriidae) are described from the spiral intestine of *Narcine entemedor* Jordan & Starks, 1895, in Bahía de Acapulco, Acapulco, Guerrero, Mexico. Based on the four criteria used for the identification of species of *Acanthobothrium*, *A. soniae* **sp. nov.** is a Category 2 species (less than 15 mm in total length with less than 50 proglottids, less than 80 testes, and with the ovary asymmetrical in shape). *Acanthobothrium vidali* **sp. nov.** is a Category 6 species (more than 15 mm in total length with more than 50 proglottids, fewer than 80 testes, and the ovary is asymmetrical). The new species differ from similar species from the Pacific Ocean by total length, the number of proglottids, diameter of the accessory sucker, the length of the cirrus sac, the number of testes per proglottid and the measurements of hooks. With the recognition of *A. soniae* **sp. nov.** and *A. vidali* **sp. nov.**, 42 species of *Acanthobothrium* have been reported from the Pacific coast of the Americas. This is the first report of species of *Acanthobothrium* from a member of *Narcine* from Mexico and it brings the number of species reported from the Pacific Coast of Mexico to 13.

#### Keywords

Acanthobothrium, Elasmobranchii, Helminth, Narcine entemedor, Onchoproteocephalidea, Torpediniformes

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## Introduction

Acanthobothrium Blanchard, 1848 is one of the richest genera within Onchoproteocephalidea (Maleki et al. 2013; Caira et al. 2017), but relatively few occurrences have been documented in Mexico. To date, the best-studied locality is the Gulf of California (Sea of Cortez; Mar de Cortés, Golfo de California) with the descriptions of 10 species (Appy and Dailey 1973; Caira and Burge 2001; Caira and Zahner 2001; Ghoshroy and Caira 2001). The only other species known from the Pacific Coast of Mexico was described from the more southern state of Jalisco (Monks et al. 1996) (see Merlo-Serna and García-Prieto 2016). More recently, A. cartagenensis Brooks & Mayes, 1980 was reported from Quintana Roo, Mexico (Caribbean) (Monks et al. 2015a) and A. marquesi was described from Campeche, Mexico (Gulf of Mexico) by Rodríguez-Ibarra et al. (2018). As part of a collaborative project to extend the knowledge of the helminth fauna of marine fishes in Mexico, rays were collected from the coastal waters off Acapulco, Guerrero, a region with few studies of the parasites of rays. There are six reports of parasites of rays from Acapulco, none for Acanthobothrium (see Merlo-Serna and García-Prieto 2016). In this paper, two new species of Acanthobothrium are described from Narcine entemedor Jordan & Starks, 1895 (Elasmobranchii: Torpediniformes: Narcinidae); one Category 2 species (Ghoshroy and Caira 2001) and one Category 6 species. The new species constitute the first records from the Southern Pacific Coast of Mexico and the first record of species of Acanthobothrium reported in Narcine entemedor from Mexico.

## Materials and methods

Eleven recently killed specimens of Narcine entemedor Jordan & Starks, 1895 (Giant electric ray or Cortez Numbfish) were purchased from local fishermen at Playa Las Hamacas, Bahía de Acapulco, Guerrero (16°51'10.80"N, 99°53'59.02"W) in February, April, May, June 2011 and June 2012. Rays were transported to the laboratory (Universidad Autónoma de Guerrero, Unidad Académica de Ecología Marina) in ice chests containing ice where the spiral intestine was removed and opened by longitudinal incision. Collection, preservation and mounting follow Monks et al. (2015b). Stained specimens were examined using a compound photomicroscope (Leica DM-LB2) equipped with both normal light optics and differential interference contrast (DIC-Nomarski) optics. Figures were drawn with the aid of a drawing tube. Measurements are presented as ranges and number of specimens from which the measurements were taken is given in parentheses. Hook measurements follow Euzet (1959) as modified by Ghoshroy and Caira (2001). Measurements are in micrometers unless otherwise stated. Other hook terminology follows that of Caira (1985). Designation of proglottid apolysis follows Caira et al. (1999) and Franzese and Ivanov (2018). Microtriches on the scolex and peduncle were not examined using SEM, so they are referred to by the general name "microtriches" without qualifying them as proposed by

Chervy (2009). The categorical method suggested by Ghoshroy and Caira (2001) and Fyler and Caira 2006 was used to facilitate comparisons among species of *Acanthobo-thrium* from the Eastern Pacific Ocean and other congeners with similar morphological characters described in the Pacific Ocean. Specimens from several museums were examined, the acronyms are as follows:

CNHE	(Colección Nacional de Helmintos del Instituto de Biología, Universidad
	Nacional Autónoma de México, México);
HWML	(University of Nebraska State Museum, Harold W. Manter Laboratory,
	Division of Parasitology, Lincoln, Nebraska, USA);
CHE	(Colección de Helmintos, Centro de Investigaciones Biológicas, Univer-
	sidad Autónoma del Estado de Hidalgo, Pachuca, México).

Type material was deposited in CNHE (holotype and paratypes), HWML (paratypes), and CHE (paratypes). Application and validity of scientific names, authorities, and common names of fish are in accord with Froese and Pauly (2018) and Last et al. (2016).

## Systematic accounts

Order Onchoproteocephalidea Caira, Jensen, Waeschenbach, Olson & Littlewood, 2014 Family Onchobothriidae Braun, 1900 Genus *Acanthobothrium* Blanchard, 1848 Type species *Acanthobothrium coronatum* (Rudolphi, 1819) Blanchard, 1848

*Acanthobothrium soniae* sp. nov. http://zoobank.org/4E0635D5-3E38-4FEC-8192-4B0AEF53C76C Figures 1A–E; 2A–D

**Type material.** Holotype (CNHE-11136), 3 paratypes (CNHE-11137), 3 paratypes (HWML-139978), and 1 paratype (CHE-P00081).

**Other material examined.** Acanthobothrium bullardi Ghoshroy & Caira, 2001 (CNHE–4046, México) paratype; A. campbelli Marques, Brooks & Monks, 1995 (CNHE–3033, Costa Rica; HWML–38546, Costa Rica) voucher and paratype; A. costarricense Marques, Brooks & Monks, 1995 (CNHE–3034, Costa Rica) 2 vouchers; A. dasi Ghoshroy & Caira, 2001 (CNHE–4044, México; HWML–15549, 15550, 15551, México) 4 paratype; A. franus Marques, Centritto & Stewart, 1997 (CNHE–3140, Costa Rica) paratype; A. inbiorium Marques, Centritto & Stewart, 1997 (CNHE–3138, Costa Rica) paratype; A. puntarenasense Marques, Brooks & Monks, 1995 (CNHE–4076, Costa Rica) paratype; A. rajivi Ghoshroy & Caira, 2001 (CNHE–4039, México) paratype; A. vargasi Marques, Brooks & Monks, 1995 (HWML 38545, Costa Rica).



**Figure I.** Holotype of *Acanthobothrium soniae* sp. nov. (CNHE-11136). **A** Scolex **B** hooks **C** mature proglottid **D** terminal proglottid **E** genitalia. Scale bars: 200 μm (**A**, **D**); 100 μm (**B**, **E**); 150 μm (**C**).

**Type host.** *Narcine entemedor* Jordan & Starks, 1895 (Elasmobranchii: Torpediniformes: Narcinidae).

**Type locality.** Bahía de Acapulco (Playa Las Hamacas: 16°51'11"N, 99°53'59"W), Guerrero, México.

Site of infection. Spiral intestine.

**Quantitative descriptors of parasite populations (Bush et al. 1997).** Prevalence= 9.0% (1 ray of 11 was infected); abundance = 0.73 (8 helminths in 11 rays); mean intensity = 8 (8 helminths in 1 infected ray).

**Etymology.** The species is named in remembrance of Sonia Virginia Flores León, former player of the Pumas Club Women's Basketball Team, UNAM, daughter of Virginia León-Règagnon and Martín Ignacio Flores-Carbajal and dear friend of SM and GP-F; she will not be forgotten.

**Diagnosis.** *Acanthobothrium soniae* sp. nov. is a Category 2 species. It is small, with a range of 10–13 acraspedote proglottids. The testes are wider than long with a range of



**Figure 2.** Light microscope photographs of holotype of *Acanthobothrium soniae* sp. nov. (CNHE-11136). **A** Scolex **B** details of bothridium **C** peduncle cephalic **D** genitalia. Scale bars: 200 μm (**A**); 40 μm (**B**), 100 μm (**C**, **D**). Abbreviations: mt microtriches; vg vagina; cs cirrus sac; gp genital pore.

31–47 testes per proglottid. The arms of the ovary are unequal (asymmetrical). Finally, this species also can be distinguished from similar congeners by total length, number of proglottids, diameter of accessory sucker, the length of the cirrus sac, number of testes per proglottid, and size of the hooks.

**Description.** [Based on 5 complete worms and 3 partial specimens] Worms 2.9– 6.7 mm (n = 5) long, euapolytic; 10–13 (n = 5) proglottids per worm. Scolex 380–420 (n = 6) long by 280–320 (n = 6) wide, with four bothridia. Maximum width of scolex at level of middle loculus (Figs 1A, 2A). Bothridia free posteriorly, tri-locular, 340–380 (n = 6) long by 140–160 (n = 6) wide, with anterior muscular pad (Figs 1A, 2A). Muscular pad 105–130 (n = 6) wide, with apical sucker 45–50 (n = 6) and one pair of bipronged hooks at posterior margin (Figs 1A, 2A). Anterior loculus of bothridia 175–205 (n = 6) long; middle loculus 60–90 (n = 6) long; posterior loculus 80–95 (n = 6) long (Figs 1A, 2A); loculus length ratio (anterior:middle:posterior) 1:0.38:0.46. Velum between medial margins of bothridia in dorsal or ventral pairs not seen (Figs 1A, 2A). Hooks bipronged, hollow, with tubercle on proximal surface of axial prong; internal channels of axial and abaxial prongs continuous, smooth, the base and anterior part of each hook embedded in musculature of scolex, tips of prongs free (Figs 1A, B, 2A). Bases (handles) of medial and lateral hooks articulate to one another (Figs 1B, 2A). Lateral hook measurements (n = 6): A 43–45, B 88–105, C 83–93, D 125–138; Medial hook measurements (n = 6): A' 38-45, B' 83-108, C' 80-98, D' 125-143. Cephalic peduncle 450-630 (n = 6) long by 70-95 (n = 6) wide, not all the cephalic peduncle is covered with prominent microtriches (Figs 1A, 2C). Scolex is covered with microtriches (Fig. 2B). Proglottids acraspedote. Immature proglottids 55-110 (n = 8) long by 60-115 (n = 8) wide, mature proglottids 225-800 (n = 8) long by 125-215 (n = 8) wide (Fig. 1C), terminal proglottids 585–1,425 (n = 7) long by 160–275 (n = 7)7) wide (Fig. 1D). Genital pore marginal, irregularly alternating, 56%-68% (n = 7) from anterior end of proglottid; genital atrium present (Fig. 1E). Testes in single layer, arranged in two irregular columns, one on each side of the uterus (Fig. 1C, D). Testes generally wider than long in mature proglottids, 25-63 (n = 8) long by 13-28 (n = 8) wide (Fig. 1C). Total number of testes 31-47 (n = 8), aporal 16-26 (n = 8), poral 15–21 (n = 8), preporal 11–17 (n = 8), and postporal 3-5 (n = 8); all testes located anterior to ovarian isthmus. Cirrus sac pyriform, extending anteriorly (Figs 1C, D, E, 2D), 55–90 (n = 6) long by 63–96 (n = 6) wide in mature proglottids, 85-140 (n = 6) long by 48-90 (n = 6) wide in termial proglottids. Cirrus armed.

Vagina extending laterally from common genital atrium, following anterior margin of cirrus sac, weakly sinuous posteriorly along medial line of proglottid to oötype (Figs 1C, D, E, 2D); vaginal sphincter absent. Seminal receptacle not seen. Ovary inverted A-shaped in frontal view in mature and terminal proglottids (Fig. 1C, D). Arms of ovary unequal (Fig. 1C, D); aporal arm always longer than poral arm. Aporal arm 78–275 (n = 7) long in mature proglottids, 243–625 (n = 7) long in terminal proglottids; poral arm 68–213 (n = 7) long in mature proglottids, 190–550 (n = 7) long in terminal proglottids and Mehlis' gland posterior to ovarian isthmus. Vitellarium follicular form lateral bands, extending from near anterior margin of proglottid to near posterior margin of proglottid (Fig. 1C, D); follicles 15–23 (n = 6) long by 10–13 (n = 6). Uterus thick-walled, saccate, extending from anterior margin of proglottid to near posterior margin of proglottid. Excretory ducts laterally. Gravid proglottids and eggs not seen.

**Remarks.** There are 42 Category 2 species (sensu Ghoshroy and Caira 2001) of *Acanthobothrium* that have been described worldwide. Of these, 17 species have been found in the Pacific Ocean, 14 of which are amphi-American (Table 1).

Acanthobothrium soniae sp. nov. is a Category 2 species (sensu Ghoshroy and Caira 2001): Category 2 species have a total length  $\leq 15$  mm (the length of *A. soniae* sp. nov. is 2.9–6.7 mm), a strobila made up of  $\leq 50$  proglottids (*A. soniae* sp. nov. has 10–13 proglottids), the number of testes per proglottids  $\leq 80$  (*A. soniae* sp. nov. has 31–47 testes per proglottid), and the arms of the ovary are asymmetrical.

As presented in Table 1, the new species can be distinguished from similar Category 2 species of *Acanthobothrium* that have been described from the Pacific Coast of the Americas (amphi-American species), and from others parts of Pacific Ocean by the measurements given in the Table 1. The total length of the new species (2.9–6.7 mm) is shorter than that of *A. campbelli* (0.99–1.8 mm). The number of proglottids of the new species (10–13) is less than that of *A. annapinkiensis* Carvajal & Goldstein, 1971 (15–26), *A. cimari* Marques, Brooks & Monks, 1995 (14–33), *A. puntarenasense* 

**Table 1.** Comparison of *Acanthobothrium soniae* sp. nov. vs. Category 2 species of the genus from the Pacific Ocean. Abbreviations: No. Number; A Base (handle) length; B Axial prong length; C Abaxial prong length; D Total hook length. Note: the use of "–" without numerical values are measurement ranges that overlap those of *A. soniae* sp. nov.

Species of Acanthobothrium	Total	No. of	Diameter	Length	No. of	Measurements of hook		(µm)	
	length	proglottids	of accesory	of cirrus	testes per	A	В	С	D
	(mm)		sucker (µm)	sac (µm)	proglottid				
Pacific coast of the Americas						_			
A. soniae sp. nov.	2.9-6.7	10-13	45-50	55–90	31-47	43-45	88-105	83–93	125-138
<i>A. annapinkiensis</i> Carvajal & Goldstein, 1971	-	15–26	~ 120	-	-	60–80	180–250	160–240	240-310
A. brachyacanthum Riser, 1955	-	-	-	123–135	-	-	66	57–60	90
<i>A. bullardi</i> Ghoshroy & Caira, 2001	_	_	_	113–175	_	-	-	43–78	_
A. campbelli Marques, Brooks & Monks, 1995	0.99–1.8	3–6	16–38	-	15–23	16-41	-	_	95–120
A. cimari Marques, Brooks & Monks, 1995	_	14–33	_	148–180	_	-	-	57–82	-
<i>A. coquimbensis</i> Carvajal & Jeges, 1980	-	-	16-44	200–400	-	-	-	96–136	-
<i>A. costarricense</i> Marques, Brooks & Monks, 1995	-	-	-	110–236	-	-	-	54–66	-
<i>A. dasi</i> Ghoshroy & Caira, 2001	-	-	-	100–153	-	-	-	50–75	68–125
<i>A. olseni</i> Dailey & Mudry, 1968	-	-	24-34	96–168	-	-	-	-	91–115
<i>A. puntarenasense</i> Marques, Brooks & Monks, 1995	-	23–27	14-15	151–183	-	-	72–82	75–81	107–114
<i>A. rajivi</i> Ghoshroy & Caira, 2001	-	-	-	-	9–13	28–35	63–73	58–68	88–98
A. unilateralis Alexander, 1953	-	-	-	150–160	-	58–64	118	118	173–182
A. vargasi Marques, Brooks & Monks, 1995	-	5–7	22-41	-	22–29	-	-	-	-
Other parts of the Pacific Oc	ean								
A. guanghaiense Yang, Sun, Zhi, Iwaki, Reyda & Yang, 2016	-	13–28	28–32	95–132	-	-	_	94–124	-
<i>A. masnihae</i> Fyler & Caira, 2006	-	23-43	-	-	6–12	-	69–88	-	99–123
A. popi Fyler, Caira & Jensen, 2009	-	14-20	50-88	108–152	-	-	-	-	-
<i>A. tetabuanense</i> Reyda & Caira, 2006	-	25-36	-	-	6-12	-	-	-	-

(23–27), A. guanghaiense Yang, Sun, Zhi, Iwaki, Reyda & Yang, 2016 (13–28), A. masnihae Fyler & Caira, 2006 (23–43), A. popi Fyler, Caira & Jensen, 2009 (14–20), and A. tetabuanense Reyda & Caira, 2006 (25–36), and the number of proglottids of the new species is greater than that of A. campbelli (3–6) and A. vargasi (5–7). The diameter of the accessory sucker of the new species (45–50) is shorter than that A. annapinkiensis (-120) and A. popi (50–88), and the diameter of the accessory sucker of the new species is longer that A. campbelli (16–38), A. coquimbensis Carvajal & Jeges, 1980 (16–44), A. olseni Dailey & Mudry, 1968 (24–34), A. puntarenasense (14–15), A. vargasi (22–41), A. guanghaiense (28–32) and A. popi (50–88). The length of the cirrus sac of the new species (55–90) is shorter than that of *A. brachyacanthum* Risen, 1955 (123–135), *A. bullardi* (113–175), *A. cimari* (148–180), *A. coquimbensis* (200–400), *A. costarricense* (100–236), *A. dasi* (100–153), *A. olseni* (96–168), *A. puntarenasense* (151–183), *A. unilateralis* Alexander, 1953 (150–160), *A. guanghaiense* (95–132), and *A. popi* (108–152). The number of testes per proglottid of the new species (31–47) is less than that of *A. campbelli* (15–23), *A. rajivi* (9–13), *A. vargasi* (6–12), and *A. tetabuanense* (6–12). Finally the measurements of the hooks of the 18 species can be found in Table 1.

#### Acanthobothrium vidali sp. nov.

http://zoobank.org/9D9106EF-7772-4E61-A44F-B2D151782329 Figures 3A–D; 4A–D

**Type material.** Holotype (CNHE-11134), 7 paratypes (CNHE-11135), 3 paratypes (HWML-139979, 139980, 139981), and 7 paratypes (CHE-P00082).

**Other material examined.** Acanthobothrium franus Marques, Centritto & Stewart, 1997 (CNHE–3140, Costa Rica) paratype; A. inbiorium Marques, Centritto & Stewart, 1997 (CNHE–3138, Costa Rica) paratype; A. obuncus Marques, Brooks & Barriga, 1997 (CNHE–3032A, 3167B, Ecuador) holotype; A. soberoni Ghoshroy & Caira, 2001 (CNHE–4042, México).

**Type host.** *Narcine entemedor* Jordan & Starks, 1895 (Elasmobranchii: Torpediniformes: Narcinidae).

**Type locality.** Bahía de Acapulco (Playa Las Hamacas: 16°51'11"N, 99°53'59"W), Guerrero, México.

Site of infection. Spiral intestine.

**Quantitative descriptors of parasite populations (Bush et al. 1997).** Prevalence = 36.36% (4 of 11 rays were infected); abundance = 1.91 (21 helminths in 11 rays); mean intensity = 5.25 (21 helminths in 4 infected rays).

**Etymology.** The species is named in honor of Dr Victor Vidal Martínez (Departamento de Recursos del Mar, CINVESTAV-IPN, Merida, Yucatan, Mexico), for his contribution to our knowledge of helminths of fishes from Mexico.

**Diagnosis.** Acanthobothrium vidali sp. nov. is a Category 6 species. This species is large, with a range of 164–214 craspedote proglottids, with a range of 50–76 testes per proglottid, and the arms of ovary unequal in length (asymmetrical). This new species also can be distinguished from similar congeners by total length, number of proglottids, diameter of accessory sucker, the length of the cirrus sac, number of testes per proglottid, and size of the hooks.

**Description.** [Based on 5 complete worms and 16 partial specimens] Worms 26.5–70.9 mm (n = 5) long, greatest width at level of mature proglottids, euapolytic; 164–214 (n = 5) proglottids per worm. Scolex 880–1,400 (n = 20) long by 680–1,170 (n = 20) wide, with four bothridia; maximum width of scolex at level of posterior margin of anterior loculus (Figs 3A, 4A). Bothridia free posteriorly, tri-locular, 770–1,230

 $(n = 20) \log by 320-570 (n = 20) wide, with anterior muscular pad (Figs 3A, 4A).$ Muscular pad 250-325 (n = 18) wide, with apical sucker 75-150 (n = 19) and one pair of bipronged hooks at posterior margin (Figs 3A, 4A). Anterior loculus of bothridia 400-650 (n = 20) long; middle loculus 170-310 (n = 20) long; posterior loculus 150–340 (n = 20) long (Figs 3A, 4A); loculus length ratio (anterior:middle:posterior) 1:0.48:0.50. Velum between medial margins of bothridia in dorsal or ventral pairs not seen (Figs 3A, 4A). Hooks bipronged, hollow, with tubercle on proximal surface of axial prong; internal channels of axial and abaxial prongs continuous, smooth, base and anterior part of each hook embedded in musculature of scolex, tips of prongs free (Figs 3A, B, 4A). Bases (handles) of medial and lateral hooks articulate with one another (Figs 3B, 4A). Lateral hook measurements (n = 15): A 140-170, B 200-285, C 140-305, D 360-465; Medial hook measurements (n = 15): A' 100-165, B' 225-300, C' 200-270, D' 300-425. Cephalic peduncle 2.38-9.13 mm (n = 15) long by 0.15-0.23 mm (n = 15) wide, microtriches not seen on the scolex or cephalic peduncle (Figs 3A, 4A, B, C). Proglottids craspedote. Immature proglottids 50–230 (n = 17) long by 240–520 (n = 17) wide, mature proglottids 260–700 (n = 10) long by 300–790 (n = 10) wide (Fig. 3C), terminal proglottids  $1,120 (n = 1) \log by 480 (n = 1)$  wide. Genital pore marginal, irregularly alternating, 49%-63% (n = 9) of proglottid length from anterior end in mature proglottids; genital atrium present (Figs 3C, D, 4D). Testes arranged in two to three irregular columns on each side of the uterus, in frontal view testes wider than long in mature proglottids, 50-125 (n = 6) long by 40-50 (n = 6) wide (Fig. 3C). In terminal proglottids, anteriormost testes wider than long and posteriormost testes longer than wide. Total number of testes 50-76 (n = 10), aporal 26-40 (n = 10), poral 23-36 (n = 10), preporal 17-26 (n = 10), postporal 5-11 (n = 10). All testes located anterior to ovarian isthmus. Cirrus sac pyriform, 125-175 (n = 6) long by 30-75 (n = 6) wide in mature proglottids (Figs 3C, D, 4D). Cirrus armed. Vagina anterior to cirrus sac (Figs 3C, D, 4D), walls relatively thick, covered with gland cells. Vagina extending laterally from common genital atrium, following anterior margin of cirrus sac, weakly sinuous posteriorly along medial line of proglottid to oötype (Fig. 3C); vaginal sphincter absent. Seminal receptacle not seen. Ovary in mature proglottids H-shaped in frontal view (Fig. 3C); posterior lobes wider than anterior lobes. Ovarian isthmus approximately 2/3 of the distance from anterior end of ovary. Arms of ovary unequal in length, aporal arm always longer than poral arm (Fig. 3C). Aporal arm 150–260 (n = 8) long, reaching to posterior margin of cirrus sac, poral arm 125–225 (n = 8) long in mature proglottids, not reaching posterior margin of cirrus sac. Mehlis' gland posterior to ovarian isthmus. Vitiellarium follicular, forming lateral bands, extending from near anterior margin of proglottid to near posterior margin of proglottid (Fig. 3C); follicles 15-20 (n = 8) long by 10-15 (n = 8) wide. Uterus thin-walled, saccate, extending from anterior margin of proglottid to near posterior margin of proglottid. Excretory ducts lateral. Gravid proglottids and eggs not seen.

**Remarks.** There are 14 Category 6 species of *Acanthobothrium* that have been described worldwide. Of these, seven species have been found in the Pacific Ocean, four of which are amphi-American (Table 2). *Acanthobothrium vidali* sp. nov. also is a



**Figure 3.** Holotype of *Acanthobothrium vidali* sp. nov. (CNHE-11134). **A** Scolex **B** hooks **C** mature proglottid **D** genitalia. Scale bars: 400 μm (**A**); 200 μm (**B**–**D**).

Category 6 species, bringing the total number to 15. Category 6 species have a total length >15 mm (the length of *A. vidali* sp. nov. is 26.5–70.9 mm), a strobila made up of > 50 proglottids (*A. vidali* sp. nov. has 164–214 proglottids), number of testes per proglottids  $\leq$  80 (*A. vidali* sp. nov. has 50–76 testes per proglottid), and the arms of the ovary are asymmetrical.

As presented in Table 2, the new species can be distinguished from similar Category 6 species of *Acanthobothrium* that have been described from the Pacific Coast of the Americas (amphi-American species), and from others parts of Pacific Ocean by the measurementes given in Table 2. The total length of the new species (26.5–70.9 mm) is longer than that of *A. aetiobatidis* (Shipley, 1900) Southwell, 1925. The number of proglottids of the new species (164–214) is greater than that of *A. gonzalesmugaburoi* Severino & Sarmiento, 1979. The diameter of accessory sucker of the new species (75–150) is larger than that of *A. obuncus* (33–48) and *A. soberoni* (40–65). The length of the cirrus sac of the new species (125–175) is shorter than that of *A. obuncus* (258–322), *A. aetiobatidis* (200–250), and *A. rodmani* Fyler, Caira & Jensen, 2009 (190–234). The number



**Figure 4.** Light microscope photographs of holotype of *Acanthobothrium vidali* sp. nov. (CNHE-11134). **A** Scolex **B** details of bothridium **C** peduncle cephalic **D** genitalia. Scale bars: 400  $\mu$ m (**A**); 40  $\mu$ m (**B**);100  $\mu$ m (**C**, **D**). Abbreviations: vg vagina; cs cirrus sac; gp genital pore.

Table 2. Comparison of Acanthobothrium vidali sp. nov. vs. Category 6 species of the genus from the Pa-
cific Ocean. Abbreviations: No. Number; A Base (handle) length; B Axial prong length; C Abaxial prong
length; D Total hook length. Note: the use of "-" without numerical values are measurement ranges that
overlap those of <i>A. vidali</i> sp. nov.

Species of	Total	No. of	Diameter	Length	No. of	Measurements of hook (µm)			μm)	
Acanthobothrium	length	proglottids	of accesory	of cirrus	testes per	A	В	С	D	
	(mm)		sucker (µm)	sac (µm)	proglottid					
Pacific coast of the Ameri	Pacific coast of the Americas									
A. vidali sp. nov.	26.5-70.9	164–214	75-150	125-175	50–76	140-170	200-285	140-305	360-465	
A. gonzalesmugaburoi Severino & Sarmiento, 1979	-	38–63	-	-	-	50-87	67–123	57–120	146–219	
A. maculatum Riser, 1955	-	-	_	-	-	72–78	72–78	75–78	135-141	
<i>A. obuncus</i> Marques, Brooks & Barriga, 1997	-	-	33–48	258–322	-	66	60–64	63–64	126–130	
<i>A. soberoni</i> Ghoshroy & Caira, 2001	-	-	40-65	-	-	45-88	43–90	65–100	86–158	
Other parts of the Pacific	Ocean									
A. aetiobatidis (Shipley, 1900), Southwell, 1925	15–20	-	-	200–250	23–28	-	120-130	120–130	250–280	
<i>A. arlenae</i> Campbell & Beveridge, 2002	-	-	-	_	17–26	-	160–179	-	289–344	
A. rodmani Fyler, Caira & Jensen, 2009	-	-	_	190–234	17–26	-	_	-	335–357	

Table 3. Comparison of species of Acanthobothrium that have been reported from Narcine entemedor. Ab-
breviations: No. Number; A Base (handle) length; B Axial prong length; C Abaxial prong length; D Tota
hook length. Note: Information taken from the original descriptions and this study.

Species of	Total	No. of	Diameter	Length of	No. of	Me	asurement	s of hook (	μm)
Acanthobothrium	length	proglottids	of accesory	cirrus sac	testes per	A	B	С	D
	(mm)		sucker (µm)	(µm)	proglottid				
Reported from Nar	Reported from Narcine entemedor								
A. franus Marques,	16.0-40.0	68-141	60–159	102-281	24-59	118-175	245-319	223-322	354-465
Centritto &									
Stewart, 1997*									
A. inbiorium	28.0-82.0	156-223	20-75	122-285	44-73	35-50	65–75	50-60	95-120
Marques, Centritto									
& Stewart, 1997									
A. soniae sp. nov.	2.9-6.7	10-13	45-50	55–90	31-47	43-45	88–105	83–93	125-138
A. vidali sp. nov.	26.5-70.9	164-214	75–150	125-175	50-76	140-170	200-285	140-305	360-465

of testes per proglottid of the new species (50-76) is greater than that of A. aetiobatidis (23-28), A. arlenae Campbell & Beverage, 2002 (17-26), and A. rodmani (17-26). Finally, the measurements of the hooks of the eight species can be found in Table 2.

The new species is the fourth species of Acanthobothrium reported from N. entemedor, preceded by A. franus and A. inbiorium (Category 5 species), and A. soniae sp. nov. (Category 2 species) described above. All species have been reported from the Pacific Coast of the Americas. Acanthobothrium vidali sp. nov. can be distinguished from these other species by number of proglottids (164-214) is greater than that of A. franus (68-141) and A. soniae sp. nov. (10-13). The total length of the lateral hook of A. vidali sp. nov. (360-465) is longer than that of A. inbiorium  $(95-120 \mu m)$ . The length of the axial prong of the lateral hook of A. vidali sp. nov. (200-285) is longer than that of *A. inbiorium* (65–75  $\mu$ m) (Table 3).

#### Discussion

To date, 190 valid species of Acanthobothrium have been reported from different regions of the world (Caira et al. 2017; Rodríguez-Ibarra et al. 2018; Franzese and Ivanov 2018). Forty species of Acanthobothrium have been described from the Pacific coast of the Americas (eleven species from USA, eleven from México, eight from Costa Rica, four from Ecuador, four from Peru, and three from Chile). With these descriptions of A. soniae sp. nov. and A. vidali sp. nov., 13 species of Acanthobothrium have been reported from the Pacific Coast of Mexico. A list of amphi-American species of Acanthobothrium from the Pacific coast, their hosts, and localities is given in Table 4.

Host specificity of most species of Acanthobothrium appears to be rather strict (Ivanov, 2005; Vardo-Zalik and Campbell 2011; Franzese and Ivanov 2018). According to the reports of species of the genus (type localities, additional localities, type host, and additional host), 82% of the species of Acanthobothrium show strict host specificity. In contrast, 33 of the 190 valid species of Acanthobothrium have been reported in more than one species of host (see the reports of Rudolphi 1819; Yoshida **Table 4.** Species of *Acanthobothrium* reported from the Pacific Ocean of the Americas (amphi-American species).  $\ddagger$  Category designation obtained from Ghoshroy and Caira (2001). Category designations not included in Ghoshroy and Caira (2001) were calculated for this study using the original descriptions. Sources were as given by that author or the original descriptions used for this study.

Family / Host species	Acanthobothrium species	Type locality	Source	Category designation
Heterodontidae				
Heterodontus francisci (Girard, 1855)	A. bajaensis Appy & Dailey, 1973	San Quintin Bay, Baja California, Mexico	Appy and Dailey (1973)	4‡
	A. puertecitense Caira & Zahner, 2001	Puertecitos, Gulf of California, Mexico	Caira and Zahner (2001)	4
<i>H. mexicanus</i> Taylor & Castro-Aguirre, 1972	A. santarosaliense Caira & Zahner, 2001	Santa Rosalia, Gulf of California, Mexico	Caira and Zahner (2001)	3
Rhinobatidae	Į.	L	1	1
Pseudobatos productus (Ayres, 1854)	A. olseni Dailey & Mudry, 1968	Newport Beach, California, USA	Dailey and Mudry (1968)	2‡
	A. rhinobati Alexander, 1953	Santa Monica Harbor, California, USA	Alexander (1953)	9(5)‡
	A. robustum Alexander, 1953	Long Beach Harbor, California, USA	Alexander (1953)	4‡
Platyrhinidae				
<i>Platyrhinoidis triseriata</i> (Jordan & Gilbert, 1880)	A. goldsteini Appy & Dailey, 1973	Seal Beach, California, USA	Appy and Dailey (1973)	5(9)‡
Narcinidae				
<i>Diplobatis ommata</i> (Jordan & Gilbert, 1890)	A. dollyae Caira & Burge, 2001	Bahía de Los Angeles, Gulf of California, Mexico	Caira and Burge (2001)	1
	A. maryanskii Caira & Burge, 2001	Loreto, Gulfo of California, Mexico	Caira and Burge (2001)	5
	A. royi Caira & Burge, 2001	Punta Arena, Gulf of California, Mexico	Caira and Burge (2001)	1
Narcine entemedor Jordan & Starks, 1895	A. franus Marques, Centritto & Stewart, 1997	Cuajiniquil Beach, Gulf of Santa Helena, Guanacaste, Costa Rica	Marques et al. (1997b)	5(8)‡
	<i>A. inbiorium</i> Marques, Centritto & Stewart, 1997	Cuajiniquil Beach, Gulf of Santa Helena, Guanacaste, Costa Rica	Marques et al. (1997b)	5‡
	A. soniae sp. n.	Playa las Hamacas, Bahía de Acapulco, Guerrero, Mexico	This study	2‡
	A. vidali sp. n.	Playa las Hamacas, Bahía de Acapulco, Guerrero, Mexico	This study	6‡
Torpedinidae				
<i>Tetronarce californica</i> (Ayres, 1855)	A. hispidum Riser, 1955	Monterey Bay, California, USA	Riser (1955)	5‡
Rajidae				
<i>Raja stellulata</i> (Gilbert, 1915)	A. brachyacanthum Riser, 1955	Monterey Bay, California, USA	Riser (1955)	2‡
Zearaja chilensis (Guichenot, 1848)	<i>A. annapinkiensis</i> Carvajal & Goldstein, 1971	Anna Pink Hay, Chile	Carvajal-G. and Goldstein (1971)	2‡
Arhynchobatidae				
<i>Psammobatis scobina</i> (Philippi, 1857)	<i>A. psammobati</i> Carvajal & Goldstein, 1969	South Pacific Ocean, between Papudo and Talcahuano, Chile	Carvajal-G. and Goldstein (1971)	5‡
<i>Sympterygia brevicaudata</i> (Cope, 1877)	A. lusarmientoi Severino & Verano, 1980	Callao, Lima, Peru	Severino and Verano (1980)	7
Gymnuridae				
<i>Gymnura afuerae</i> (Hildebrand, 1946)	<i>A. atahualpai</i> Marques, Brooks & Barringa, 1997	Puerto Bolivar, Provincia de El Oro, Ecuador	Marques et al. (1997a)	1‡
Dasyatidae				
<i>Hypanus dipterurus</i> (Jordan & Gilbert, 1880)	<i>A. bullardi</i> Ghoshroy & Caira, 2001	Bahía de Los Angeles, Gulf of California, Mexico	Ghoshroy and Caira (2001)	2‡
	A. dasi Ghoshroy & Caira, 2001	Puertecitos, Gulf of California, Mexico	Ghoshroy and Caira (2001)	2‡
	<i>A. rajivi</i> Ghoshroy & Caira, 2001	Puertecitos, Gulf of California, Mexico	Ghoshroy and Caira (2001)	2‡
	<i>A. soberoni</i> Ghoshroy & Caira, 2001	Puertecitos, Gulf of California, Mexico	Ghoshroy and Caira (2001)	6‡

Family / Host species	Acanthobothrium species	Type locality	Source	Category designation
H. longus (Garman, 1880)	A. cimari Marques, Brooks & Monks, 1995	Punta Morales, Puntarenas Province, Costa Rica	Marques et al. (1995)	2‡
	A. cleofanus Monks, Brooks & Lonce de Leon, 1996	Chamela Bay, Jalisco, Mexico	Monks et al. (1996)	3‡
	A. costarricense Marques, Brooks & Monks, 1995	Punta Morales, Puntarenas Province, Costa Rica	Marques et al. (1995)	2‡
	A. obuncus Marques, Brooks & Barriga, 1997	Puerto Hualtaco, Provincia de El Oro, Ecuador	Marques et al. (1997a)	6‡
	A. puntarenasense Marques, Brooks & Monks, 1995	Punta Morales, Puntarenas Province, Costa Rica	Marques et al. (1995)	2‡
	<i>A. vargasi</i> Marques, Brooks & Monks, 1995	Punta Morales, Puntarenas Province, Costa Rica	Marques et al. (1995)	2‡
Potamotrygonidae			· · · · · · · · · · · · · · · · · · ·	-
Potamotrygon motoro (Müller & Henle, 1841)	A. peruviense Reyda, 2008	Madre de Dios River at Boca Manu, Madre de Dios Department, Peru	Reyda (2008)	1(8)
Urotrygonidae				
Urobatis halleri (Cooper, 1863)	A. parviuncinatum Young, 1954	San Diego Bays, California, USA	Young (1954)	8‡
U. tumbesensis (Chirichigno F. & McEachran, 1979)	A. minusculus Marques, Brooks & Barriga, 1997	Puerto Hualtaco, Provincia de El Oro, Ecuador	Marques et al. (1997a)	1‡
Urotrygon chilensis (Günther, 1872)	A. campbelli Marques, Brooks & Monks, 1995	Costa de Pajaros, Puntarenas, Costa Rica	Marques et al. (1995)	2‡
Myliobatidae				
Myliobatis californicus Grill,	A. holorhini Alexander, 1953	Long Beach Harbor, California, USA	Alexander (1953)	3‡
1865	A. maculatum Riser, 1955	Monterey Bay, California, USA	Rêgo et al. 1968	6(3)‡
	A. microcephalum Alexander, 1953	Long Beach Harbor, California, USA	Alexander (1953)	4‡
	A. unilateralis Alexander, 1953	Long Beach Harbor, California, USA	Alexander (1953)	7(2)‡
M. chilensis Philippi, 1892	<i>A. coquimbensis</i> Carvajal & Jeges, 1980	Antofagasta, Chile	Carvajal-G. and Jeges-G. (1980)	2‡
<i>M. peruvianus</i> Garman, 1913	<i>A. gonzalesmugaburoi</i> Severino & Sarmiento, 1979	Callao, Lima, Peru	Severino and Sarmiento (1979)	7(6)
Aetobatidae				
Aetobatus narinari (Euphrasen, 1790)	A. monksi Marques, Brooks & Barriga, 1997	Puerto Jelí, Provincia de El Oro, Ecuador	Marques et al. (1997a)	1‡
	<i>A. nicoyaense</i> Brooks & McCorquodale, 1995	Punta Morales, Golfo de Nicoya, Costa Rica	Brooks and McCorquodale (1995)	1‡
Scombridae (Perciformes)				
Sarda chiliensis (Cuvier, 1832)	A. chilensis Rego, Vincednte & Herrera, 1968	Paita, Piúra, Peru	Rêgo et al. (1968)	3‡

1917; MacCallum 1921; Léon-Borcéa 1935; Baer 1948; Euzet 1952; Yamaguti 1952; Young 1954; Riser 1955; Rees and Williams 1965; Goldstein 1967; Campbell 1969; Williams 1969; Carvajal-G. and Jeges-G. 1980; Rodriguez and Tantaleán-Vidaurre 1980; Brooks et al. 1981; Mayes and Brooks 1981; Escalante-A. 1986; Tantaleán-Vidaurre 1991; Marques et al. 1997a; Campbell and Beveridge 2002; Friggens and Brown 2005; Lacerda et al. 2008; and, Jannacone et al. 2011).

Prior to de Carvalho and Last (2016), the genus *Narcine* Henle, 1834 was composed of 20 species. To date, those taxa have been divided into two genera; 15 species of *Narcine* (tail length about equal to disc length or width) and 5 species of *Narcinops* de Carvalho & Last, 2016 (tail much longer tan disc length or width), this latter distributed only in

Narcine	Species of	Category	Habitat of host	Type locality	Source
Narcine sp. (Reported as N. braunii, synonym of N. brasiliensis)	<i>A. indicum</i> Subhapradha, 1955	‡ 5	Northern Indian Ocean	Madras Coast, India	Subhapradha (1955)
<i>N. bancroftii</i> (Griffith & Smith, 1834) [reported as <i>N. brasiliensis</i> (Olfers, 1831)]	A. lintoni Goldstein, Henson & Schlicht, 1968	† 1(8,9,5)	North Carolina to northeastern Brazil	Gulf of Mexico, Texas, USA	Goldstein et al. (1969)
N. brasiliensis	A. electricolum Brooks & Mayes, 1978	†9	Brazil to northern Argentina	Caribbean Sea, near Cartagena, Colombia	Brooks and Mayes (1978)
<i>N. entemedor</i> Jordan & Starks, 1895	<i>A. franus</i> Marques, Centritto & Stewart, 1997	† 5(8)	Baja California to northen Peru	Cuajiniquil Beach, Gulf of Santa Helena, Guanacaste, Costa Rica	Marques et al. (1997b)
	A. inbiorium Marques, Centritto & Stewart, 1997	† 5	Baja California to northen Peru	Cuajiniquil Beach, Gulf of Santa Helena, Guanacaste, Costa Rica	Marques et al. (1997b)
	<i>A. soniae</i> sp. nov.	2	Baja California to northen Peru	Playa las Hamacas, Bahía de Acapulco, Guerrero, Mexico	This study
	A. vidali sp. nov.	6	Baja California to northen Peru	Playa las Hamacas, Bahía de Acapulco, Guerrero, Mexico	This study

**Table 5.** Species of *Acanthobothrium* reported in species of *Narcine*. † Data from Ghoshroy and Caira (2001); ‡ Data from Fyler and Caira (2006).

Australia (Last et al. 2016). No helminths have been reported from the former members of *Narcine* that are now assigned to *Narcinops*. Five valid species of *Acanthobothrium* have been reported worldwide from three species of *Narcine* (Table 5) (Subhapradha, 1955; Goldstein et al. 1969; Brooks and Mayes 1978; Marques et al. 1997b), but no species of *Acanthobothrium* in *Narcine* have been reported from Mexico (Merlo-Serna and García-Prieto 2016). In Mexico, only two species of helminth have been reported previously from *Narcine: Anaporrhutum euzeti* Curran, Blend & Overstreet, 2003 and *Nagmia rodmani* Curran, Blend & Overstreet, 2009).

The categorical method suggested by Ghoshroy and Caira (2001) and Fyler and Caira, (2006) was used to facilitate comparisons among the 190 valid species of *Acan-thobothrium*. Ghoshroy and Caira (2001) proposed the categories to facilitate comparisons among taxa from the same geographic region. Because of the large number of species worldwide, it is necessary to focus only on those species from the same region that possessing the same combination of characters as the new species; thus, delimiting the comparison between similar species that could be confused with a new species and not comparing each new species to all of the 190 valid species currently described. We agree that comparisons with each species of this expanding group is unnecessary and, as the number of species increases, an exercise in futility. In agreement with previous authors, this categorical method is useful but does not reflect groupings from a rigorous phylogenetic hypothesis (i.e., is phenetic) (Campbell and Beveridge 2002; Ivanov 2005; Reyda and Caira 2006; Twohig et al. 2008; Fyler and Caira 2010; Yang et al. 2016).

Although not all species of the genus have been examined, Franzese and Ivanov (2018) suggest that the pattern of microthiches is quite uniform among species of *Acanthobothrium*; (i.e., all species have filitriches covering most surfaces of the worms,

interspersed with gladiate spinitriches on proximal bothridial surface, scolex proper and the cephalic peduncle). Because of insufficient material, it was not possible to make a study of this species using the SEM, so we cannot provide detailed information on the microtriches.

## Acknowledgements

The authors thank all those who made possible the collection and examination of specimens. The authors would like to thank the curator Luis García-Prieto of the CNHE and Scott L Gardner and Gabor Racz (curator and collection manager, respectively) of the HWML, for providing access to laboratories and specimens. Students from the Laboratorio de Morfología Animal, Universidad Autónoma del Estado de Hidalgo, and the Unidad Académica de Ecología Marina, Universidad Autónoma de Guerrero, helped with the collection of specimens. This study was supported by funds from the project "Inventario Ambiental y Establecimiento de Indicadores Regionales de la Red Temática: Calidad Ambiental y Desarrollo Sustentable (PROMEP–SEP)". The Consejo Nacional de Ciencia y Tecnología (CONACYT) provided a scholarship (no. 432427) to FZ–T.

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## Indonaia rectangularis (Tapparone-Canefri, 1889), comb. nov., a forgotten freshwater mussel species from Myanmar (Bivalvia, Unionidae)

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Academic editor: Y. Mutafchiev | Received 15 February 2019 | Accepted 25 April 2019 | Published 6 June 2019 http://zoobank.org/48C1EFF9-105F-4D2C-A801-A61D320F3B26

**Citation:** Bolotov IN, Vikhrev IV, Lopes-Lima M, Gofarov MY, Konopleva ES, Lunn Z, Chan N, Bogan AE (2019) *Indonaia rectangularis* (Tapparone-Canefri, 1889) comb. nov., a forgotten freshwater mussel species from Myanmar (Bivalvia, Unionidae). ZooKeys 852: 23–30. https://doi.org/10.3897/zookeys.852.33898

#### Abstract

Unio rectangularis Tapparone-Canefri, 1889 is a little-known nominal species of freshwater mussels described from a tributary of the Ayeyarwady River in Myanmar. This taxon was considered a synonym of *Gibbosula laosensis* (Lea, 1863), a margaritiferid species. However, the range of *Gibbosula laosensis* does not encompass the Ayeyarwady River watershed. Here we re-examine the holotype of *Unio rectangularis* and provide a conchological re-description of this species. Based on conchological features such as the shell shape, elevated umbo, and the structure of lateral and pseudocardinal teeth, we transfer this taxon to the genus *Indonaia* Prashad, 1918 and propose *I. rectangularis* (Tapparone-Canefri, 1889), **comb. nov.** It appears to be a rare freshwater mussel species with a restricted range, because it has not been found since the original description. Two additional species in this genus are known from Myanmar, i.e. *Indonaia andersoniana* (Nevill, 1877) and *I. subclathrata* (Martens, 1899).

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#### **Keywords**

Ayeyarwady River, Indochinellini, Margaritiferidae, Parreysiinae, Southeast Asia, Unionidae

## Introduction

*Gibbosula laosensis* (Lea, 1863) (Bivalvia, Margaritiferidae) is an endangered freshwater mussel species from Southeast Asia (Bolotov et al. 2014), which has recently been transferred from *Margaritifera* Schumacher, 1816 to *Gibbosula* Simpson, 1900 (Lopes-Lima et al. 2018). Several enigmatic nominal taxa and nomina dubia were linked to this species as its putative synonyms or closely related species, i.e. *Unio rectangularis* Tapparone-Canefri, 1889, *U. sula* Simpson, 1900, *U. sella* Haas, 1912, and *Margaritanopsis woodthorpi* Godwin-Austen, 1919 (Simpson 1900; Haas 1912; Godwin-Austen 1919; Prashad 1922). While the complete story of the three latter names remains to be discussed, a taxonomic reassignment of *Unio rectangularis* is presented here.

Leonardo Fea, an adventurous Italian pioneer and explorer, found a single shell of *Unio rectangularis* among large collections of non-marine molluscs from British Burma during his travels in 1885–1887 (Tapparone-Canefri 1889; Prashad 1922). Cesare Maria Tapparone-Canefri, a famous Italian malacologist, studied this sample and published a comprehensive paper (Tapparone-Canefri 1889) with a description of numerous new taxa of terrestrial and freshwater molluscs, including *Unio rectangularis*.

Later, Prashad (1922) revisited Tapparone-Canefri's unionid taxa in his broad-scale review of freshwater mussels described from British Burma. In the account on *Gibbosula laosensis*, Prashad (1922: 93) stated that: "The species described as *Unio rectangularis* by Tapparone-Canefri [...] is based on a single very young shell. It is undoubtedly to be referred to the genus *Margaritanopsis* and probably represents another species of the genus. Owing, however, to a single young shell being available I do not feel disposed to consider it as a distinct species...". After that generic reassignment of *Unio rectangularis*, this species was completely forgotten, lost to malacological taxonomy, and it was not listed in the subsequent taxonomic checklists on freshwater mussels of the Oriental Region (Brandt 1974; Subba-Rao 1989; Bolotov et al. 2017a; Zieritz et al. 2018) and in the most authoritative global revisions (Haas 1969; Graf and Cummings 2007).

We revise the generic placement of *Unio rectangularis* and discuss its prospective taxonomic status based on morphological study of the type specimen.

#### Material and methods

We studied the holotype of *Unio rectangularis* in the Museo Civico di Storia Naturale di Genova (MSNG), Genova, Italy. The images of the specimen were taken with Canon EOS 7D DSLR camera (Canon Inc., Japan). Shell measurements were performed with Adobe Photoshop CS using digital photographs of the holotype. Samples of *Indonaia andersoniana* (Nevill, 1877) (N = 37 specimens) and *I. subclathrata* (Martens, 1899) (N = 7 specimens) were studied in the Russian Museum of Biodiver-

sity Hotspots (RMBH), Federal Center for Integrated Arctic Research of the Russian Academy of Sciences, Arkhangelsk, Russia. Three shell dimensions at each specimen of the studied taxa (length, height, and width of the shell, all taken at the maximum diameter) were measured using calipers ( $\pm 0.1$  mm). The comparative analysis of shell morphology was carried out with regard to the main distinguishing traits, such as shell shape, umbo position, structures of pseudocardinal and lateral teeth, as well as muscle attachment scars (Konopleva et al. 2019).

## Results

Family Unionidae Rafinesque, 1820 Subfamily Parreysiinae Henderson, 1935 Tribe Indochinellini Bolotov, Pfeiffer, Vikhrev & Konopleva, 2018 Genus *Indonaia* Prashad, 1918

*Indonaia rectangularis* (Tapparone-Canefri, 1889), comb. nov. Figure 1A–F

*= Unio rectangularis* Tapparone-Canefri 1889: 354. *=Margaritanopsis rectangularis* (Tapparone-Canefri, 1889). – Prashad 1922: 93, pl. 2, fig. 5.

**Type.** Holotype in MSNG, without ID number (Fig. 1A–C). Original label: "*Unio rectangularis* Tapp. Can. Teinzo, Mti E. di Bhamo (L. Fea)" (Fig. 1D). Secondary labels: "*Margaritanopsis* var.? juv. detto B. Prashad!" (Fig. 1E) and "*Margaritanopsis* var.? Young specimen" (Fig. 1F). The original label does not have a collecting date, but Fea's sample of another freshwater mussel species from Teinzo residing in the MSNG is dated "Marzo 1886" that may also be applicable to the holotype of *U. rectangularis*.

**Type locality.** Teinzo (presently Teinthaw village), 24.3978N, 97.2519E, Moolay River (Mole Chaung in Burmese), hills E of Bhamo (L. Fea), alt. 110 m a.s.l., Ayeyarwady Basin, Myanmar (Tapparone-Canefri 1889).

**Diagnosis.** As Tapparone-Canefri (1889) stated, the shell of *Indonaia rectangularis* is not similar to any other freshwater mussel species known from Myanmar. This species cannot be mistaken with the two congeners, *Indonaia andersoniana* and *I. subclathrata* (Fig. 2), that were described from the Ayeyarwady Basin. Both these species differ from *Indonaia rectangularis* by a more elongated shell and less developed lateral, and pseudocardinal teeth. The pseudocardinal teeth in *I. rectangularis* are lamellar, thick, and very elongated. Additionally, a unique feature of *I. rectangularis* is the presence of regular ridges crossing the growth lines in the posterior-dorsal area forming a clear rectangular pattern that was never seen in other species (Fig. 1C).

**Redescription.** Shell length 34.2 mm, height 20.4 mm, width 16.2 mm. Shell thick, obovate, inequilateral, with broader posterior side. Dorsal margin slightly convex. Ventral margin nearly straight. Anterior margin rounded. Posterior margin slightly

Α В Museo Civico di Storia Naturale di Genova Inio rectangularij Eopp. Com. Veinzo ME 8. Di Bhama (L. Jean) D С Museo Civico di Storia Naturale di Genova Kargaritanopsis Var Margaritanopsis var? Vorug specimen B. Frashad! E

**Figure 1.** Holotype of *Indonaia rectangularis* (Tapparone-Canefri, 1889), comb. nov. [MSNG]. **A**, **B** Shell, lateral view: inner side of the left valve and outer side of the right valve (**A**); vice versa (**B**) **C** shell, dorsal view **D** original label [probably by C.M. Tapparone-Canefri] **E**, **F** secondary labels [probably by B. Prashad]. Scale bar: 5 mm. (Photos: Ilya V. Vikhrev).

elevated posteriorly, with an inconspicuous wing. Umbo prominent, elevated, rounded, slightly eroded. Shell surface mostly smooth. In the posterior-dorsal area, regular ridges cross the growth lines and form a clear rectangular pattern. In the anterior-dorsal area, curved, lamella-like ridges closely spaced along growth lines. Periostracum light olivebrown, with two parallel, slightly curved green bands along posterior-dorsal area; inner band with a broad greenish extension posteriorly. Nacre silver-white. Umbo cavity deep. Anterior adductor scar round, shallow but well marked. Posterior adductor scar oval, very shallow, unclear. Mantle attachment scars absent. Pseudocardinal teeth are thick, lamella-like, very elongated, two teeth in the right valve and one tooth in the left valve. Lateral teeth well developed, thick, elongated and straight, one tooth in the right valve and two teeth in the left valve. Soft body morphology and anatomy unknown.

**Remarks.** Unio rectangularis was originally described based on a single specimen with a shell 34.2 mm long, 20 mm high and 16 mm wide (Tapparone-Canefri 1889). The single



**Figure 2.** Specimens of *Indonaia andersoniana* (Nevill, 1877) and *I. subclathrata* (Martens, 1899) from Myanmar [RMBH biv450\_2 and RMBH biv347\_2, respectively]. **A, B** Shell of *I. andersoniana*, lateral view: inner side of the left valve and outer side of the right valve (**A**); vice versa (**B**) **C** shell of *I. andersoniana*, dorsal view **D, E** shell of *I. subclathrata*, lateral view: inner side of the left valve and outer side of the right valve (**D**); vice versa (**E**) **F** shell of *I. subclathrata*, dorsal view. Scale bar: 5 mm. (Photos: Ekaterina S. Konopleva).

shell labelled as *Unio rectangularis* and deposited in the MSNG studied here corresponds in dimensions to the specimen described by Tapparone-Canefri. The original label (Fig. 1D) also affirms that this shell is the holotype of *Unio rectangularis* designated by monotypy.

**Distribution.** This species is known only from the type locality, a tributary of the Ayeyarwady River (Fig. 3). It appears to be a rather restricted and rare species, because it has not been recorded since the original description, even during a recent broad-scale survey of freshwater mussels in Myanmar (Bolotov et al. 2017a, b, 2018; Konopleva et al. 2019). However, there has been no studies on freshwater mussels from the Mole River published since Tapparone-Canefri (1889).



**Figure 3.** Map of the type locality of *Indonaia rectangularis* (Tapparone-Canefri, 1889), comb. nov. (dark blue circle). The digital elevation model and other layers of the map were added from the Esri Data & Maps 10 dataset.

## Discussion

The results of our study reveal that *Unio rectangularis* is not a margaritiferid because it does not have mantle attachment scars, the most prominent diagnostic feature of the Margaritiferidae (Lopes-Lima et al. 2018). *Unio rectangularis* can be placed within the genus *Indonaia* based on conchological features, i.e. the shell shape, elevated umbo, and the structure of lateral and pseudocardinal teeth (Figs 1, 2). Prashad (1922) considered that the holotype of *Unio rectangularis* is a "very young shell". However, all *Indonaia* species are rather small mussels (Fig. 2), and this holotype shell surely represents an adult specimen.

*Indonaia* represents the most divergent phylogenetic clade among the tribe Indochinellini (Bolotov et al. 2017a, 2018; Pfeiffer et al. 2018; Konopleva et al. 2019). This genus contains three species from India and three species from Myanmar (Konopleva et al. 2019). The taxonomic relationship of *Indonaia* with another Oriental genus, *Radiatula* Simpson, 1900, is still to be resolved, because molecular sequences of *Unio crispisulcatus* Benson, 1862, the type species of *Radiatula*, are still not available.

## Key to species of Indonaia from Myanmar

1	Shell	surface	with	radial	ridges	covering	the	entire	shell
	disc				-	I. subclathr	ata (N	lartens, 1	1899)*
_	Shell s	urface witl	hout rad	ial ridges	s on the sl	nell disc			2
2	Shell s	urface mo	stly smo	oth, whi	le regular	ridges cross	the gi	rowth lin	es and
	form a	clear recta	ngular p	attern in	the poster	rior-dorsal ai	rea, and	d curved,	lamel-
	la-like ridges closely spaced along growth lines in the anterior-dorsal area							a	
		-	I. recta	angulari	s (Tappar	one-Canefri	i, 1889	), comb.	<b>nov.</b> **
_	Shell s	urface smo	oth	_		I. anderson	<i>iana</i> (I	Nevill, 18	877)***

## Acknowledgements

This work was partly funded by grants from the Russian Ministry of Education and Science (Project No. 6.2343.2017/4.6), Federal Agency for Scientific Organizations (Project No. AAAA-A18-118012390161-9), National Geographic Society (Project No. NGS-274R-18), Russian Foundation for Basic Research (Project Nos. 16-34-00638 and 18-34-20033), and Northern Arctic Federal University. Fundação para a Ciência e a Tecnologia – FCT supported MLL under grant No. SFRH/BD/115728/2016. We thank Maria Tavano, curator of the invertebrate collection of MSNG (Genova, Italy), for her great help during work with Tapparone-Canefri's collection. Dirk van Damme and Daniel Graf greatly helped us to improve an earlier version of the paper.

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<sup>\*</sup> Manipur, Chindwin rivers, middle reaches of the Ayeyarwady River; common species (Konopleva et al. 2019)

<sup>\*\*</sup> Mole River, Ayeyarwady Basin; rare species

<sup>\*\*\*</sup> Middle reaches and downstream of the Ayeyarwady River; common species (Bolotov et al. 2017a, 2018; Konopleva et al. 2019)

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RESEARCH ARTICLE



# Morphological and molecular study on Yininemertes pratensis (Nemertea, Pilidiophora, Heteronemertea) from the Han River Estuary, South Korea, and its phylogenetic position within the family Lineidae

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Academic editor: Y. Mutafchiev   Received 21 December 2018   Accepted 5 May 2019	Published 5 June 2019
http://zoobank.org/542BA27C-9EDC-4A15-9C72-5D84DDFEEF6A	

**Citation:** Park T, Lee S-H, Sun S-C, Kajihara H (2019) Morphological and molecular study on *Yininemertes pratensis* (Nemertea, Pilidiophora, Heteronemertea) from the Han River Estuary, South Korea, and its phylogenetic position within the family Lineidae. ZooKeys 852: 31–51. https://doi.org/10.3897/zooKeys.852.32602

## Abstract

Outbreaks of ribbon worms observed in 2013, 2015, and 2017–2019 in the Han River Estuary, South Korea, have caused damage to local glass-eel fisheries. The Han River ribbon worms have been identified as *Yininemertes pratensis* (Sun & Lu, 1998) based on not only morphological characteristics compared with the holotype and paratype specimens, but also DNA sequence comparison with topotypes freshly collected near the Yangtze River mouth, China. Using sequences of six gene markers (18S rRNA, 28S rRNA, histone H3, histone H4, 16S rRNA, and COI), the phylogenetic position of *Y. pratensis* was inferred among other heteronemerteans based on their sequences obtained from public databases. This analysis firmly placed *Y. pratensis* as a close relative to *Apatronemertes albimaculosa* Wilfert & Gibson, 1974, which has been reported from aquarium tanks containing tropical freshwater plants in various parts of the world as well as a wild environment in Panama.

## Keywords

Anguilla japonica, brackish-water invertebrates, freshwater invertebrates, Yellow Sea

## Introduction

An explosive proliferation of unidentified, brackish-water heteronemerteans was observed in the Han River Estuary, South Korea, in the spring of 2013. Our morphological observation of the Han River ribbon worms indicated that they represent Yininemertes pratensis (Sun & Lu, 1998), a brackish-water heteronemertean known only by its original description from the Yangtze (Changjiang) River Estuary, China (for the nomenclature of the genus, see Sun and Lu 2008; Özdikmen 2009; Kajihara 2014). Outbreaks of Y. pratensis in the Han River Estuary were also observed in 2015, 2017, 2018, and 2019. Reportedly, the worms have caused severe damage (Lee 2015; Noh 2019) to local fisheries of glass eels, which are juveniles of Anguilla japonica Temminck & Schlegel, 1847, a valuable fishery resource in East Asian countries showing dramatic declines in recent years (Tzeng 1997; Tatsukawa 2003; Tseng et al. 2003). As causes for the eel declines, overfishing and habitat loss due to human activities (e.g., Chen et al. 2014) and oceanic-atmospheric factors such as changes in ocean circulation (Chang et al. 2018) have been suggested. To what extent the nemerteans have been contributing to the anguillid declines is not known. For glass-eel fisheries, fishermen set long, conical nets on the estuarine bottoms with apertures directing downstream. At the end of each net, ascending catches are to be concentrated mostly during flood tide. In the 2015 bloom, more than 90% of catches were worms, with none to only a few eels that were dead (Lee 2015) probably due to yet-unidentified neurotoxic substances (Kwon et al. 2017) in worm mucus within the concentrated net catches. These neurotoxins might have been discharged from epidermal cells and contained in the secreted mucus (cf. Tanu et al. 2004; Asakawa et al. 2013). To our knowledge, this is the first record of damage to fisheries directly caused by nemertean outbreaks, although a potentially indirect case is known. At certain Alaskan localities in the 1983–1984 and 1984–1985 brooding seasons of the red king crab Paralithodes camtschaticus (Tilesius, 1815), a widespread outbreak of the decapod-egg-predatory nemertean Carcinonemertes regicides Shields et al., 1989, and possibly Ovicides paralithodis Kajihara & Kuris, 2013 as well, caused high egg mortality (Kuris et al. 1991), which could have led to a subsequent decline in the red king crab population (e.g., Loher and Armstrong 2005). In addition, the milky ribbon worm Cerebratulus lacteus (Leidy, 1851) has been identified as an important threat to populations of the softshell clam Mya arenaria Linnaeus, 1758, which is one of the commercial bivalves in Atlantic Canada, although no outbreak has ever been reported for C. lacteus (cf. Bourque et al. 2001, 2002).

Facing a plethora of undescribed species with dwindling number of experts, some nemertean taxonomists agreed that taxonomic descriptions of ribbon worms will have to shift from traditional, internal-anatomy-based style to histology-free one with a combination of high-quality external images and molecular phylogeny (Strand and Sundberg 2011; Strand et al. 2014; Kajihara 2015; Sundberg et al. 2016). On the other hand, in the case of Heteronemertea, only about 10% of ~100 genera (Gibson 1995; Kajihara et al. 2008) have been represented by type species in terms of sequences for

multi-locus analysis (Thollesson and Norenburg 2003; Andrade et al. 2012; Kvist et al. 2014, 2015). Logically, until the rest of ~90 genera are also represented in the same manner, examination of internal morphology will remain indispensable to genus-level identification (e.g., Chernyshev et al. 2018). Moreover, most heteronemertean genera currently diagnosed are non-monophyletic. This has been repeatedly pointed out in previous studies (e.g., Sundberg and Saur 1998; Schwartz 2009; Puerta et al. 2010; Hiebert and Maslakova 2015). Therefore, as many type species of genus-group names (such as *Yininemertes*) as possible should be placed in molecular phylogenetic context for proper application of genus names in many other species of heteronemerteans as long as Linnaean binominal nomenclature is employed.

In this paper, we report the identity of Han River nemerteans based on morphological characteristics in comparison to the type material of *Y. pratensis* as well as DNA barcoding data from the type locality. Also, we infer the phylogenetic position of *Y. pratensis* among Heteronemertea based on a multi-locus molecular analysis.

## Materials and methods

## Specimen collection and processing

Approximately 700 individuals of ribbon worms were collected from local fishermen's glass-eel nets for *Anguilla japonica*, set at about 37°36'08"N, 126°48'23"E, in Goyang, South Korea, approximately 40 km upstream of the mouth of the Han River (Figs 1A, B, 2A) on April 6, 2015 by TP. A total of 12 topotype specimens of *Y. pratensis* were collected at two sites in Shanghai by TP, Kwang-Soo Kim, Seul Yi, SS, and Guang Xi: i) Bailonggang, 31°15'40.0"N, 121°44'11.8"E, on May 13, 2016; and ii) Chongming



**Figure 1.** Maps showing sampling localities indicated by arrow heads. **A** The Han River and Yangtze River Estuaries are about 840 km apart from each other across the Yellow Sea **B** sampling locality in Goyang, Korea **C** two sampling localities, Chongming Island and Bailonggang, China.

Island, 31°34'39.4"N, 121°54'34.9"E, on May 14, 2016 (Figs 1A, C, 2C). Specimens from the Han River were anesthetized with 7% MgCl<sub>2</sub> solution before fixed in either 7% neutral-buffered formalin for morphological observation (~300 individuals) or 100% ethanol for DNA extraction (~300 individuals). Of these 12 specimens collected from Shanghai, nine were fixed in 70% EtOH for DNA extraction while three were used for taking photographs. Anterior portion of one formalin-fixed specimen from the Han River was dehydrated in ethanol series, cleared in xylene, embedded in paraffin (melting point: 56–57 °C), and transversely sectioned at thickness of 8 µm. Serial sections were stained with Mallory's trichrome method (Gibson 1994). Specimens were deposited in National Institute of Biological Resources Invertebrate Collection, Incheon, Korea (NIBR IV) and Invertebrate Collection of the Hokkaido University Museum, Sapporo, Japan (ICHUM) (Table 1). For comparison, the holotype (DH005A) and a paratype (DH005C) of *Y. pratensis* deposited in Ocean University of China, Qingdao, People's Republic of China, were also examined.

#### Molecular phylogeny

Small pieces of tissue taken from 22 specimens collected from the Han River and seven specimens from Yangtze River were used for total genomic DNA extraction using DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Partial sequences of six gene markers (nuclear 18S rRNA, 28S rRNA, histone H3, and histone H4; mitochondrial 16S rRNA, and COI) were used for molecular analyses using the same primers published by Andrade et al. (2012). For PCR amplification, the following mixture was prepared in a total volume of 50  $\mu$ L: 50 ng of template genomic DNA, 2.5 mM dNTPs, 5  $\mu$ L of 10× Ex Taq<sup>™</sup> buffer, 2  $\mu$ L of each 10 pM primer, and 1 U (0.5  $\mu$ L) of TaKaRa Ex Taq<sup>™</sup> polymerase. Thermal cycling condition comprised an initial denaturation at 94 °C for 30 sec followed by 35 cycles of denaturation at 72 °C for 1 min. A final extension step at 72 °C for 10 min was then followed. Amplified PCR products were sequenced using an ABI 3730 sequencer (Applied Biosystems, Foster City, CA, USA) from both directions. All sequences generated de novo in this study were deposited at GenBank (Table 2).

To assess phylogenetic affinity of the Han River nemerteans, maximum likelihood (ML) analysis and Bayesian Inference (BI) were carried out with 31 lineid heteronemertean species for which the aforementioned six gene sequences were available in public databases (Table 3). Outgroups were chosen to include *Baseodiscus mexicanus* (Bürger, 1893) and *B. unicolor* Stiasny-Wijnhoff, 1925 (cf. Andrade et al. 2012; Kvist et al. 2014). Sequence alignment was performed using MAFFT ver. 7 (Katoh and Standley 2013) with *E-INS-i* option for 18S, 28S, and 16S. For the protein-coding H3, H4, and COI, sequences were aligned straightforward without gaps. Sequences were edited and concatenated using MEGA ver. 5.2 (Tamura et al. 2011). Gaps and incompletely determined nucleotides accounted for 24.9% of the entire dataset of these sequences.

**Table 1.** List of specimens identified as *Yininemertes pratensis* (Sun & Lu, 1998) in this study with catalogue numbers at the National Institute of Biological Resources Invertebrate Section, Incheon, Korea (NIBR IV) and the Invertebrate Collection of the Hokkaido University Museum, Sapporo, Japan (ICHUM) as well as their sampling date and locality.

Catalogue number	Sampling date and locality	Remarks		
NIBR IV 0000409587-0000409590	6 April 2015, Govang South Korea	> 300 individuals fixed in 10%		
	o ripin 2019, Goyang, oouur Rorea	formalin		
NIBR IV 0000409591-0000409595	6 April 2015, Goyang, South Korea	> 300 individuals fixed in 100% EtOH		
NURD IV 0000/0050( 0000/00/17	6 April 2015 Covena South Korea	22 voucher specimens used for DNA		
NIBK 1V 0000409990-000040901/	o April 2013, Goyang, South Rolea	extraction		
NIBR IV 0000758851	13 May 2016, Bailonggang, China	1 specimen fixed in 70% EtOH		
NIBR IV 0000758852	13 May 2016, Bailonggang, China	1 specimen fixed in 70% EtOH		
NIBR IV 0000758853	13 May 2016, Bailonggang, China	1 specimen fixed in 70% EtOH		
NIBR IV 0000758854	13 May 2016, Bailonggang, China	1 specimen fixed in 70% EtOH		
NIBR IV 0000758855	13 May 2016, Bailonggang, China	1 specimen fixed in 70% EtOH		
NIBR IV 0000758856	13 May 2016, Bailonggang, China	1 specimen fixed in 70% EtOH		
NIBR IV 0000758857	13 May 2016, Bailonggang, China	1 specimen fixed in 70% EtOH		
NIBR IV 0000758848	14 May 2016, Chongming, China	1 specimen fixed in 70% EtOH		
NIBR IV 0000754958	14 May 2016, Chongming, China	1 specimen fixed in 70% EtOH		
ICHUM 5259	6 April 2015, Goyang, South Korea	8 specimens fixed in 10% formalin		
		Serial transverse sections of the anterior		
ICHUM 5260	6 April 2015, Goyang, South Korea	portion of a specimen, Mallory		
		trichrome, 36 slides.		

**Table 2.** GenBank accession numbers of sequences determined in the present study from voucher specimens of *Yininemertes pratensis* (Sun & Lu, 1998) deposited in the National Institute of Biological Resources Invertebrate Collection, Incheon, Korea (NIBR IV).

NIBR IV	18S rRNA	28S rRNA	Histone H3	Histone H4	16S rRNA	COI
0000409596	KY274047	KY274069	KY274091	KY274113	KY274025	KY274003
0000409597	KY274048	KY274070	KY274092	KY274114	KY274026	KY274004
0000409598	KY274049	KY274071	KY274093	KY274115	KY274027	KY274005
0000409599	KY274050	KY274072	KY274094	KY274116	KY274028	KY274006
0000409600	KY274051	KY274073	KY274095	KY274117	KY274029	KY274007
0000409601	KY274052	KY274074	KY274096	KY274118	KY274030	KY274008
0000409602	KY274053	KY274075	KY274097	KY274119	KY274031	KY274009
0000409603	KY274054	KY274076	KY274098	KY274120	KY274032	KY274010
0000409604	KY274055	KY274077	KY274099	KY274121	KY274033	KY274011
0000409605	KY274056	KY274078	KY274100	KY274122	KY274034	KY274012
0000409606	KY274057	KY274079	KY274101	KY274123	KY274035	KY274013
0000409607	KY274058	KY274080	KY274102	KY274124	KY274036	KY274014
0000409608	KY274059	KY274081	KY274103	KY274125	KY274037	KY274015
0000409609	KY274060	KY274082	KY274104	KY274126	KY274038	KY274016
0000409610	KY274061	KY274083	KY274105	KY274127	KY274039	KY274017
0000409611	KY274062	KY274084	KY274106	KY274128	KY274040	KY274018
0000409612	KY274063	KY274085	KY274107	KY274129	KY274041	KY274019
0000409613	KY274064	KY274086	KY274108	KY274130	KY274042	KY274020
0000409614	KY274065	KY274087	KY274109	KY274131	KY274043	KY274021
0000409615	KY274066	KY274088	KY274110	KY274132	KY274044	KY274022
0000409616	KY274067	KY274089	KY274111	KY274133	KY274045	KY274023
0000409617	KY274068	KY274090	KY274112	KY274134	KY274046	KY274024
0000754958	KY274138	KY274140	KY274144	KY274146	KY274136	KY274142
0000758857	KY274137	KY274139	KY274143	KY274145	KY274135	KY274141

**Table 3.** GenBank accession numbers of sequences used in the present phylogenetic analysis (Histone H4 sequences indicated by asterisks (\*) were kindly provided by Dr Sebastian Kvist).

	18S rRNA	28S rRNA	Histone H3	Histone H4	16S rRNA	COI	Reference
Apatronemertes albimaculosa Wilfert & Gibson, 1974 <sup>a</sup>	JF293030	HQ856860	JF277733	JF277666	JF277587	HQ848584	Andrade et al. (2012)
<i>Cerebratulus lacteus</i> (Leidy, 1851)	JF293044	HQ856857	JF277728	JF277653	JF277575	HQ848576	Andrade et al. (2012)
<i>Cerebratulus</i> <i>marginatus</i> Renier, 1804	JF293042	HQ856858	JF277729	JF277652	JF277576	HQ848575	Andrade et al. (2012)
Gorgonorhynchus albocinctus Kajihara, 2015	LC010650	LC010651	-	-	-	LC010649	Kajihara (2015)
Gorgonorhynchus cf. bermudensis Wheeler, 1940 <sup>b</sup>	KF935300	KF935356	KF935412	*	KF935467	KF935517	Kvist et al. (2014)
<i>Kulikovia</i> <i>alborostrata</i> (Takakura, 1898) <sup>c</sup>	-	AJ436877	-	-	AJ436822	AJ436932	Thollesson and Norenburg (2003)
<i>Kulikovia</i> <i>manchenkoi</i> Chernyshev et al., 2018 <sup>d</sup>	JF293035	HQ856856	JF277730	JF277683	JF277572	HQ848574	Andrade et al. (2012)
<i>Lineus acutifrons</i> Southern, 1913	JF304778	HQ856855	JF277727	JF277681	JF277573	GU590937	Andrade et al. (2012)
<i>Lineus bilineatus</i> (Renier, 1804)	JF293041	HQ856844	JF277731	JF277682	JF277571	-	Andrade et al. (2012)
<i>Lineus lacteus</i> (Rathke, 1843) <sup>e</sup>	JF293065	HQ856850	JF277725	JF277656	JF277584	HQ848583	Andrade et al. (2012)
<i>Lineus longissimus</i> (Gunnerus, 1770)	_	AJ436880	_	_	AJ436825	AJ436935	Thollesson and Norenburg (2003)
<i>Lineus ruber</i> (Müller, 1774) <sup>f</sup>	JF293040	HQ856853	JF277718	JF277655	JF277583	HQ848580	Andrade et al. (2012)
<i>Lineus sanguineus</i> (Rathke, 1799) <sup>g</sup>	KF935301	KF935357	KF935413	*	KF935468	KF935518	Kvist et al. (2014)
<i>Maculaura</i> <i>alaskensis</i> (Coe, 1901a) <sup>h</sup>	_	AJ436882	AJ436981	-	AJ436827	AJ436937	Thollesson and Norenburg (2003)
<i>Micrura</i> <i>chlorapardalis</i> Schwartz & Norenburg, 2005	KF935292	KF935348	KF935404	*	KF935459	KF935512	Kvist et al. (2014)
<i>Micrura dellechiajei</i> (Hubrecht, 1879)	KF935294	KF935350	KF935406	*	KF935461	KF935514	Kvist et al. (2014)
<i>Micrura fasciolata</i> Ehrenberg, 1828	JF293038	HQ856846	JF277721	JF277660	JF277585	HQ848577	Andrade et al. (2012)
<i>Micrura ignea</i> Schwartz & Norenburg, 2005	JF293043	HQ856859	JF277734	JF277664	JF277588	HQ848587	Andrade et al. (2012)
<i>Micrura purpurea</i> (Dalyell, 1853)	JF293036	HQ856845	JF277726	JF277663	JF277577	HQ848586	Andrade et al. (2012)
	18S rRNA	28S rRNA	Histone H3	Histone H4	16S rRNA	COI	Reference
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<i>Micrura verrilli</i> Coe, 1901a	KF935288	KF935344	KF935400	*	KF935455	KF935508	Kvist et al. (2014)
<i>Micrura</i> sp. <sup>i</sup>	KF935293	KF935349	KF935405	*	KF935460	KF935513	Kvist et al. (2014)
<i>Notospermus</i> <i>geniculatus</i> (Delle Chiaje, 1828)	KF935295	KF935351	KF935407	*	KF935462	-	Kvist et al. (2014)
Notospermus sp. 1 (SK76)	KF935296	KF935352	KF935408	*	KF935463	KF935515	Kvist et al. (2014)
Notospermus sp. 2 (SK65)	KF935297	KF935353	KF935409	*	KF935464	-	Kvist et al. (2014)
Notospermus sp. 3 (SK50)	KF935298	KF935354	KF935410	*	KF935465	KF935516	Kvist et al. (2014)
Parborlasia corrugata (McIntosh, 1876)	JF293037	HQ856851	JF277732	JF277662	JF277578	-	Andrade et al. (2012)
Parvicirrus dubius (Verrill, 1879)	-	AJ436885	-	-	AJ436830	AJ436940	Thollesson and Norenburg (2003)
<i>Pseudomicrura</i> <i>afzelii</i> Strand & Sundberg, 2011	GU445924	GU445919	_	-	GU445914	GU392013	Strand and Sundberg (2011)
<i>Riseriullus occultus</i> Rogers et al., 1993	JF293031	HQ856848	JF277724	JF277679	JF277581	HQ848581	Andrade et al. (2012)
<i>Tenuilineus bicolor</i> (Verrill, 1892)	-	AJ436878	AJ436980	-	AJ436823	AJ436933	Thollesson and Norenburg (2003)
Zygeupolia rubens (Coe, 1895)	JF293045	HQ856861	JF277735	JF277661	JF277574	HQ848585	Andrade et al. (2012)
<i>Yininemertes</i> <i>pratensis</i> (Sun & Lu, 1998)	KY274047	KY274069	KY274091	KY274113	KY274025	KY274003	Present study
Outgroup		i					
Baseodiscus mexicanus (Bürger, 1893)	KF935281	KF935337	KF935393	*	KF935449	KF935503	Kvist et al. (2014)
Baseodiscus unicolor Stiasny-Wijnhoff, 1925	KF935284	KF935340	KF935396	*	KF935451	KF935505	Kvist et al. (2014)

<sup>a</sup>Left unidentified in Andrade et al. (2012); the species identification herein follows that of Kajihara et al. (2016) <sup>b</sup>Identified as *Cerebratulus leucopsis* (Coe, 1901b) in Kvist et al. (2014); re-examination of the voucher material at the Museum of Comparative Zoology at Harvard University (MCZ IZ 135331) revealed that it had a branched proboscis (Gonzalo Giribet, pers. comm. to HK)

'Transferred to Kulikovia by Chernyshev et al. (2018)

<sup>d</sup>Identified as *Lineus torquatus* Coe, 1901a in Andrade et al. (2012); the identification herein follows that of Chernyshev et al. (2018)

<sup>c</sup>Identified as *Ramphogordius lacteus* in Andrade et al. (2012); its generic affiliation follows that of Ament-Velásquez et al. (2016)

<sup>f</sup>Identified as *Ramphogordius sanguineus* in Andrade et al. (2012); the identification herein follows that of Kang et al. (2015) <sup>g</sup>Identified as *Lineus* sp. in Kvist et al. (2014); the species identification herein follows that of Kang et al. (2015), and its generic affiliation follows that of Ament-Velásquez et al. (2016)

<sup>h</sup>Transferred to *Maculaura* by Hiebert and Maslakova (2015)

<sup>1</sup>Identified as *Micrura rubramaculosa* Schwartz and Norenburg, 2005 in Kvist et al. (2014); the species identification has been corrected at the database of the Museum of Comparative Zoology at Harvard University for the voucher specimen (MCZ IZ 132531).

PartitionFinder ver. 1.1 (Lanfear et al. 2012) was used to determine the best partition scheme for ML and BI. For BI, the most suitable substitution model for each partition was also selected: GTR+I+G for 16S and 28S; GTR+G for COI (1<sup>st</sup> codon), H3 (1<sup>st</sup> and 3<sup>rd</sup> codons), and H4 (1<sup>st</sup> and 2<sup>nd</sup> codons); K80+I+G for 18S and H4 (3<sup>rd</sup> codon); F81+I+G for COI (2<sup>nd</sup> codon); HKY+I+G for COI (3<sup>rd</sup> codon); and JC for H3 (2<sup>nd</sup> codon). ML analysis was performed using RAxML ver. 8.0.0 (Stamatakis 2014) with a GTR+G model of nucleotide substitution for all partitions consisting of 1000 rapid bootstraps. BI was carried out using MrBayes ver. 3.2.3 (Ronquist and Huelsenbeck 2003; Altekar et al. 2004) with two independent Metropolis-coupled analyses (four Markov chains of 10,000,000 generations for each analysis). Trees were sampled every 100 generations. Values of run convergence indicated that sufficient amounts of trees and parameters were sampled (average standard deviation of split frequencies = 0.006616; minimum estimated sample size of tree lengths = 706.26; potential scale reduction factor of tree lengths = 1.001). Run convergence was also assessed with Tracer ver. 1.6 (Rambaut et al. 2014).

#### **Population genetics**

Using 29 sequences (22 from Korea, seven from China) of 658-bp partial COI gene, haplotype network analyses were performed with Network ver. 5.0.0.1 (available at http://www.fluxus-engineering.com) using median-joining method (Bandelt et al. 1999) and TCS ver. 1.2.1 (Clement et al. 2000) using statistical parsimony (Templeton et al. 1992). Calculation of genetic distances was carried out using MEGA ver. 5.2 (Tamura et al. 2011). Calculations for haplotype diversity, nucleotide diversity, Tajima's *D*, and Fu's *F*s values were done with ARLEQUIN ver. 3.5.2.2 (Excoffier et al. 2005).

## Results

#### Morphology

The external feature of the Han River nemerteans agreed with the original description of *Y. pratensis* in that these worms were variously dark brown, brick red, and tinged with violet sometimes (Fig. 2A, B). Generally, their body color became paler posteriorly. Sun and Lu (1998) have reported that specimens from the Yangtze River Estuary sometimes show light-red, 4–10 transverse rings arranged on the body. Such ring arrangement was also found in specimens from the Han River Estuary (Fig. 2B) as well as topotype specimens (Fig. 2E, G) collected from muddy sediment with or without vegetation (Fig. 2C, D).

In specimens collected from the Han River, the proboscis was not branched, and reddish in color (Fig. 3A). Serially sectioned specimen (ICHUM 5260) showed



**Figure 2.** Photographs of *Yininemertes pratensis* (Sun and Lu, 1998) taken in life. **A** A haul of a glasseel net at the Han River Estuary, South Korea, on 6 April 2015 **B** magnification of a swarm of the same worms as in **A** from the Han River Estuary taken in the laboratory; arrow heads indicating the characteristic transverse narrow rings in the intestinal region **C** a specimen dug from clayey mud sediment with vegetation at Bailonggang in the Yangtze River Estuary, China, May 13, 2016 **D** a specimen dug from non-vegetated clay sediment at Chongming Island in the Yangtze River Estuary, China, 14 May 2016 **E** topotype from the Yangtze River Estuary showing an overview of whole specimen **F** topotype from China showing magnification of head, ventral view **G** topotype from China, magnification of intestinal region, showing the characteristic narrow transverse rings, indicated by arrow heads.



**Figure 3.** *Yininemertes pratensis* (Sun and Lu, 1998), photograph in life (**A**) and photomicrographs of transverse sections (**B**, **D**, **E**, **G**, **H** ICHUM 5260 **C** DH005C, paratype **F** DH005A, holotype). **A** Anesthetized state with proboscis partially protruded, NIBR IV 0000409596 **B**, **C** proboscis; large arrow heads indicating fibers contributing to muscle cross; small arrow heads showing rhabdoids **D** cerebral region showing type-3 neuron **E**, **F** foregut region, arrow heads indicating intra-epithelial somatic muscle fibers **G**, **H** cephalic region showing well-developed cephalic lacuna.

the following internal anatomical features: *i*) the proboscis had two muscle crosses (Fig. 3B), similar to that in the paratype of *Y. pratensis* (Fig. 3C); ii) the rhynchocoel outer circular musculature was not interwoven with the adjacent body-wall longitudinal musculature; iii) the nervous system had type-3 neurons (cf. Beckers 2015) along the inner portion of the brain (Fig. 3D); iv) the foregut wall had intraepithelial somatic muscle fibres that appeared to be circular or diagonal (Fig. 3E), similar to that observed in the holotype (Fig. 3F); *v*) the body-wall dermal glandular layer was not separated from the body-wall outer longitudinal muscle layer by connective tissue layer (Fig. 3E); and *vi*) the blood system comprised spacious cephalic lacuna (Fig. 3G, H), an alimentary vascular plexus (Fig. 3E), and a mid-dorsal blood vessel.

#### Molecular phylogeny

Lengths of the six gene markers determined for Korean and Chinese materials were: 16S, 507–508 bp; 18S, 1000–1003 bp; 28S, 1132 bp; COI, 658 bp; H3, 331 bp; and H4, 160 bp. Resulting ML tree (ln L = -51290.378661) and BI tree (harmonic mean of estimated marginal likelihood for two runs = -52096.68) were topologically more or less the same, with *Y. pratensis* being a sister of *Apatronemertes albimaculosa* Wilfert & Gibson, 1974 in both trees with 100% bootstrap support value and 1.0 posterior probability (Fig. 4). The inter-specific K2P distance between the COI sequences of *Y. pratensis* and *A. albimaculosa* was 0.163–0.196. More basal relations between this clade (= *Y. pratensis* + *A. albimaculosa*) and other heteronemerteans included in this analysis were poorly resolved.

#### **Population genetics**

Median-joining and statistical parsimony networks were identical in shape, comprising eight haplotypes with a maximal difference of five mutations (Fig. 5). From 29 specimens analysed (22 from Korea, seven from China), a total of nine haplotypes were detected, of which two were shared by Korean and Chinese populations. Eleven of 22 sequences from Korea were represented by the same haplotype, which was also the main haplotype among the Chinese population (shared by five of seven Chinese individuals analysed). Eight COI haplotypes from Korea differed by 0.000–0.006 from each other in terms of both uncorrected *p*-distance and K2P. The Korean population showed higher values of nucleotide diversity and haplotype diversity than the Chinese ones (Table 4). Tajima's *D* and Fu's *F*s values were all negative for the Korean population, the Chinese population, and the total population, although not significantly different from zero except for the Fu's *F*s values for the Korean population and total population.



**Figure 4.** Maximum likelihood tree (ln L = -51290.378661) for heteronemerteans based on concatenated 18S rRNA, 28S rRNA, histone H3, histone H4, 16S rRNA, and COI dataset showing phylogenetic position of *Yininemertes pratensis* (Sun and Lu, 1998). Numbers near nodes are bootstrap values for maximum-likelihood analysis and posterior probability for Bayesian inference. Scale bar indicates the number of substitutions per site.



**Figure 5.** Median-joining network for eight haplotypes detected among 29 *Yininemertes pratensis* specimens (22 from Han River, Korea; 7 from Yangtze River, China; statistical-parsimony method yielded the same topology). Numbers in each circle (pie chart) indicate sample size which is proportional to the size of each pie diagram.

**Table 4.** Number of individuals analysed for population genetic analysis, number of haplotypes, nucleotide diversity, haplotype diversity, Tajima's *D*, and Fu's *F*s based on 658-bp partial COI gene sequences from populations of *Yininemertes pratensis* (Sun and Lu, 1998) in the Han River and Yangtze River Estuaries.

Locality	Number of individuals	Number of haplotypes	Nucleotide diversity (S.D.)	Haplotype diversity (S.D.)	Tajima's D	Fu's <i>F</i> s	
Han River	22	0	0.001849	0.7216 (0.0807)	0.80	-3.87*	
Estuary, Korea	22	0	(0.001365)	0./310 (0.089/)	-0.80		
Yangtze River	7	2	0.000868	0 5229 (0 2096)	1.22	0.02	
Estuary, China	/	3	(0.000907)	0.9298 (0.2080)	-1.23	-0.92	
total	20	0	0.001632	0 (8/7 (0 0800)	1 1 0	5 0.0**	
total	29	2	(0.001234)	0.0847 (0.0899)	-1.10	-9.08	

\*P = 0.003; \*\*P = 0.000

## Discussion

Because fundamental biological aspects of *Y. pratensis* such as diet, life duration, breeding season, reproductive strategy (semelparous/iteroparous) and mode (oviparous, viviparous, and ovoviviparous), and larval ecology (if the species produces larvae in the first place) are unknown, the causes for the *Y. pratensis* outbreaks since 2013

in the Han River Estuary, South Korea, are open to speculation. One of the potential factors conceivable to explain the *Yininemertes* outbreaks is that the species might be capable of asexual reproduction. Until recently, asexual reproductive capacity among Heteronemertea had been confirmed only in the lineid Lineus sanguineus (Rathke, 1799) and L. pseudolacteus (Gontcharoff, 1951) (cf. Ament-Velásquez et al. 2016). However, asexual reproduction by fragmentation in the valenciniid Baseodiscus delineatus (Delle Chiaje, 1825) (Ikenaga et al. 2019) and B. hemprichii (Ehrenberg, 1831) (Kajihara and Hookabe 2019), as well as head-regenerative ability in the lineid Cerebratulus lineolatus Coe, 1905 (Zattara et al. 2019), Lineus pictifrons Coe, 1904 (Coe 1932), and L. rubescens Coe, 1904 (Coe 1930), have been documented. Asexual reproductive capacity may have evolved in more lineages than previously thought among heteronemerteans, possibly including Y. pratensis. Another hypothesis is that the Han River ribbon worms might have been introduced from other, unidentified localities. However, this hypothesis sounds rather unlikely, because the haplotype diversity in the Korean population (0.7316), which was greater than the Chinese one (0.5238), suggests that a stable population have existed in the Han River Estuary, probably since long before the first bloom observed in 2013. While Tajima's D and Fu's Fs values were overall negative, we cannot draw any robust conclusion about the population dynamics because most of the values were statistically not significant. Future study is needed to pinpoint possible environmental factors that are responsible for the Y. pratensis outbreaks, as well as to elucidate the species' basic biology for obtaining countermeasures against the economic loss to local glass-eel fisheries caused by such blooms.

The family Lineidae McIntosh, 1874 currently contains about 90 genera and 370 species of heteronemerteans, which are morphologically characterized by having horizontal lateral cephalic slits and three apical organs. Most are marine, but six species (each in a monotypic genus) have been described from freshwater or brackish-water habitat. These are Planolineus exsul Beauchamp, 1928 from Indonesia; Siolineus turbidus Du Bois-Reymond Marcus, 1948 from Amazon; Hinumanemertes kikuchii Iwata, 1970 from Japan; A. albimaculosa from freshwater tanks in Germany (Wilfert and Gibson 1974), Austria (Senz 1993), USA (Smith 2001), and Japan (Kajihara et al. 2016), as well as in submerged logs and rocks in a pond in Panama (Kvist et al. 2018); Amniclineus zhujiangensis Gibson & Qi, 1991 from Zhujiang, China; and Y. pratensis from China and Korea (Sun and Lu 1998; present study). Our phylogenetic tree indicates that A. albimaculosa and Y. pratensis form a highly supported clade, suggesting that the remaining fresh- and brackish-water forms, especially those in Southeast and East Asia, may also belong to the same clade. At this moment, however, neither morphological nor molecular synapomorphy between A. albimaculosa and Y. pratensis can be perceived; for instance, the characteristic outer cephalic vessels in A. albimaculosa are not found in Y. pratensis. Both species are reddish in body color, but this may be due to convergent evolution, as freshwater monostiliferous hoplonemerteans in the genus Prostoma Dugès, 1828 also possess reddish body. Future studies with expanded taxon sampling, along with detailed morphological examination, should clarify the evolution of these freshwater heteronemerteans.

#### Acknowledgements

We are grateful to Dr Sebastian Kvist for sharing his unpublished histone H4 sequences; to Kwang-Soo Kim, Seul Yi, and Guang Xi for their help in collecting specimens from Shanghai; and to Professor Gonzalo Giribet for the information on a specimen deposited in the Museum of Comparative Zoology (MCZ IZ 135331). Dr Malin Strand (as a reviewer), two anonymous reviewers, and Dr Yasen Mutafchiev (as an editor) gave us thoughtful comments that improved earlier version of the manuscript. The present study was partially supported by a grant (NIBR201601111) from the National Institute of Biological Resources (NIBR) funded by the Ministry of Environment (MOE), Republic of Korea for TP; and Japan Society for the Promotion of Science (JSPS) Grant-in-Aid for Scientific Research (grant number 17K07520) for HK.

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RESEARCH ARTICLE



# A new species of the genus Cristimenes Ďuriš & Horká, 2017 (Decapoda, Caridea, Palaemonidae)

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Academic editor: I.	Wehrtmann	Received 30 March 201	9   Accepted 29 April 2019	Published 5 June 2019
	http://zo	oobank.org/B0F71A68-41E9	4F4D-8186-D85513DCDD14	[ 

Citation: Park J-H, De Grave S, Kim W (2019) A new species of the genus *Cristimenes* Ďuriš & Horká, 2017 (Decapoda, Caridea, Palaemonidae). ZooKeys 852: 53–71. https://doi.org/10.3897/zookeys.852.34959

## Abstract

A new species of crinoid-associated shrimp, *Cristimenes brucei* **sp. nov.**, is described based on specimens from Korea, although the species also occurs in Hong Kong and is likely more widespread. The new species is morphologically very similar to *C. commensalis*, but can be distinguished by the reduced supraorbital tooth on the carapace. *Cristimenes brucei* **sp. nov.** is clearly recovered as a monophyletic species through COI barcode and molecular phylogenetic analyses based on four genetic markers (COI, 16S, H3, 18S).

## Keywords

Cristimenes brucei sp. nov., crinoid associate, Indo-West Pacific, Hong Kong, Korea, phylogeny

# Introduction

The genus *Cristimenes* Ďuriš & Horká, 2017 is associated with echinoderms (Ďuriš and Horká 2017) and currently consists of three species: *C. cristimanus* (Bruce, 1965), *C. zanzibaricus* (Bruce, 1967) (both associated with echinoids), and *C. commensalis* (Borradaile, 1915), associated with crinoids. All three species are widespread across the tropical regions of the Indo-West Pacific, and morphologically easily distinguished from related genera by the unique carpo-propodal articulation of the second pereiopod (Borradaile 1915; Barnard 1958; Bruce 1965, 1967, 1982a, 1984, 1989, 1996; Hayashi and Honma 2004; Marin and Savinkin 2007; Ďuriš and Horká 2017).

*Cristimenes commensalis* differs from the other two species by its host affiliation and can also be easily distinguished by the morphology of the ambulatory dactyli (Bruce 1965, 1967, 1980; Ďuriš and Horká 2017). The species has been recorded from various host crinoid species across the Indo-West Pacific, after it was described from Murray Island, Torres Strait, Australia in 1915 (Barnard 1958; Bruce 1982b; Marin and Savinkin 2007). Bruce (1979) already remarked upon variation in the supraorbital tooth and the lateral carina of specimens from Hong Kong in comparison to Indonesian specimens (Bruce 1982a, 1983). Specimens matching this morphology were obtained from Korea, and based on a morphological comparison as well as a molecular analysis are herein reported as a new species.

#### Materials and methods

Fieldwork for this study was carried out and organised in Korea (2012–2018), the Philippines (2014, 2018), and Vietnam (2016–2018) by Seoul National University (**SNU**), Korea Institute of Ocean Science and Technology (**KIOST**), the University of the Philippines Visayas (**UPV**), and the Institute of Tropical Biology (**ITB**). Host crinoids were collected during scuba diving and associated shrimps separated. All shrimps and tissue of host crinoids were preserved in 80% ethanol. The type series is deposited in the Marine Arthropod Depository Bank of Korea, Seoul National University, Seoul, Korea (**MADBK**); National Institute of Biological Resources, Incheon, Korea (**NIBR**) and the Oxford University Museum of Natural History, Oxford, United Kingdom (**OUMNH.ZC**). Postorbital carapace length (pocl, in mm) is used as the standard size measurement.

Molecular phylogenetic analyses were performed to elucidate the phylogenetic position of the new species within Cristimenes. Four species of Cristimenes (C. commensalis, C. cristimanus, C. zanzibaricus, and the new species) and three crinoid-associated shrimps Araiopontonia odontorhyncha Fujino & Miyake, 1970, Laomenes amboinensis (De Man, 1888), and Unguicaris pilipes (Bruce & Zmarzly, 1983) were selected as the ingroup, with Palaemonella pottsi (Borradaile, 1915) as the outgroup. Total genomic DNA was isolated from fifth pleopod tissue or eggs using the QIAamp<sup>®</sup> DNA Micro Kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions. Two mitochondrial DNA fragments (cytochrome c oxidase subunit I (COI) and 16S rRNA) and two nuclear DNA fragments (histone 3 (H3) and 18S rRNA (18S)) were amplified by polymerase chain reaction (PCR) with the primer pairs LCO1490/HCO2198 (Folmer et al. 1994), 16S-ar/16S-1472 (Crandall and Fitzpatrick 1996; Palumbi et al. 2002), H3F/H3R (Colgan et al. 1998), 18Sa2.0/18S9r (Whiting 2002), respectively. PCR protocols followed Horká et al. (2016), with PCR products sent to Macrogen Inc. (Seoul, Korea) for purification and Sanger sequencing. Geneious v11.1.5 (http://www. geneious.com) was used to manipulate and confirm the sequencing data from both DNA strands before data analysis. Newly obtained sequences and additional sequences from GenBank are listed in Table 1.

Table	Specimens u	sed in the phy	logenetic analys	is, with co	llection	location,	GenBan	k accession	num-
bers (C	OI, 16S, H3, a	nd 18S), and	source reference	s. N/A - n	ot availa	ble.			

Taxa	Location	Voucher ID	G	GenBank accession numbers		Source	
			COI	168	H3	185	
Cristimenes brucei sp. nov.	Korea	MADBK 120532_012	MK688394	MK688410	MK688426	MK688442	Present study
	Korea	SNU KR JH537	MK688395	MK688411	MK688427	MK688443	Present study
	Korea	MADBK 120532_015	MK688396	MK688412	MK688428	MK688444	Present study
	Korea	MADBK 120532_015	MK688397	MK688413	MK688429	MK688445	Present study
Cristimenes commensalis	Philippines	SNU PH PI13	N/A	MK688414	MK688430	MK688446	Present study
	Philippines	SNU PH PI36	N/A	MK688415	MK688431	MK688447	Present study
	Philippines	SNU PH PI261	MK688398	N/A	N/A	N/A	Present study
	Philippines	SNU PH PI263	MK688399	N/A	N/A	N/A	Present study
	Philippines	SNU PH PI264	MK688400	MK688416	MK688432	MK688448	Present study
	Taiwan	UO Tw12-48B	KU064993	KU170697	KU065081	KU064912	Horká et al. 2016
Cristimenes cristimanus	Philippines	SNU PH PC49	MK688401	MK688417	MK688433	MK688449	Present study
	Vietnam	SNU VI VI109	MK688402	MK688418	MK688434	MK688450	Present study
	Vietnam	SNU VI VI110	MK688403	MK688419	MK688435	MK688451	Present study
	Vietnam	UO V08-34	KU064994	KU064838	KU065082	KU064913	Horká et al. 2016
Cristimenes zanzibaricus	Taiwan	UO Tw12-86	KU065011	KU170696	KU065096	KU064925	Horká et al. 2016
Laomenes amboinensis	Philippines	SNU PH12	MK688405	MK688420	MK688436	MK688452	Present study
	Philippines	SNU PH PH76	MK688404	MK688421	MK688437	MK688453	Present study
	Taiwan	UO Tw12-49	KU064979	KU064825	KU065063	KU064898	Horká et al. 2016
Unguicaris pilipes	Philippines	SNU PH PI57	MK688406	MK688422	MK688438	MK688454	Present study
	Philippines	SNU PH PI68	MK688407	MK688423	MK688439	MK688455	Present study
Unguicaris sp.	Taiwan	NTOU 6687-09	KU065020	KU064863	KU065108	KU064937	Horká et al. 2016
Palaemonella pottsi	Philippines	SNU PH PI56	MK688408	MK688424	MK688440	MK688456	Present study
	Philippines	SNU PH PI58	MK688409	MK688425	MK688441	MK688457	Present study

COI sequence divergence within and between species were calculated using the Neighbor-Joining method (Saitou and Nei 1987) and the Kimura 2-parameter (K2P) distance method (Kimura 1980) within the MEGA6 (Tamura et al. 2013). Multiple sequence alignment was performed using MAFFT v7 (Katoh and Standley 2013) under the default parameters and then checked by eye; phylogenetic trees for the combined dataset were constructed by maximum likelihood (ML) analysis and Bayesian Inference (BI) approaches. The best-fitting substitution model for COI (HKY+I+G), 16S (HKY+G), H3 (GTR) and 18S (GTR+I+G) was determined by jModelTest v2.1.10 (Darriba et al. 2012) according to the Akaike Information Criterion (AIC; Akaike 1974). The ML analysis was carried out using RAxML v8.2.4 (Stamatakis 2006) using the model GTRGAMMA for each partition with 1,000 bootstrap runs. The BI analysis was carried out using MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003). The combined dataset was run for 10 million generations, sampling every 500 generations, with the first 50% trees discarded as burn-in. Phylogenetic trees were visualised in iTOL v4.0.3 (Letunic and Bork 2016).

#### **Systematics**

## Infraorder Caridea Dana, 1852 Family Palaemonidae Rafinesque, 1815 Genus *Cristimenes* Ďuriš & Horká, 2017

#### Cristimenes brucei sp. nov.

http://zoobank.org/05D90862-52D5-4C2F-A5CF-5E4F3251A40E Figures 1–7, 8A–B, 9A–C, 10A–C

Periclimenes commensalisl: Bruce 1982a: 236-238, fig. 2.

**Material examined.** *Holotype.* KOREA – Jeju Special Self-Governing Province • 1 ov.  $\bigcirc$  (pocl 3.80 mm); Munseom Island; 33°13'37"N, 126°34'8"E; depth 20 m; 16 Oct. 2015; JH Park leg.; on *Anneissia japonica* (Müller, 1841); NIBRIV0000841118; *Paratypes.* KOREA – Jeju Special Self-Governing Province • 3  $\bigcirc \bigcirc$  (pocl 2.2, 1.86, 1.83 mm); Munseom Island; 33°13'37"N, 126°34'8"E; depth 20 m; 16 Oct. 2015; JH Park leg.; on *A. japonica*; MADBK 120532\_006 • 1 ov.  $\bigcirc$  (pocl 3.65 mm); same data; 16 Oct. 2015; JH Park leg.; on *A. japonica*; OUMNH.ZC.2018-03-022 • 2  $\bigcirc \bigcirc$ , 1  $\oslash$  (pocl 3.34, 3.1, 1.6 mm); same data; 17 Oct. 2015; JH Park leg.; on *A. japonica*; MADBK 120532\_007 • 3  $\bigcirc \bigcirc$ , 1  $\oslash$  (pocl 2.85, 2.2, 1.5, 1.98 mm); same data; 08 Jul. 2016; JH Park leg.; on *A. japonica*; NIBRIV0000841119 • 1  $\bigcirc$  (pocl 1.67 mm); same data; 08 Aug.2016; JH Park leg.; on *A. japonica*; OUMNH.ZC.2018-03-023 • 1  $\bigcirc$  (pocl 2.45 mm); same data; 31 Mar. 2018; JH Park leg.; on *A. japonica*; MADBK 120532\_017.

Additional material. KOREA – Dadohaesang National Park • 1  $\bigcirc$  (pocl 2.7) mm); Geomundo Island; 34°3'35"N, 127°16'57"E; depth 20 m; 5 Jul. 2014; JH Park leg.; on A. japonica; MADBK 120532\_002 – Gyeongsangbuk-do • 1  $\mathcal{D}$ , 3  $\mathcal{J}\mathcal{J}$  (pocl 1.95, 2.0, 1.7, 1.6 mm); Pohang-si, Guryongpo; 36°0'25"N, 129°35'10"E; depth 15 m; 22 Sep. 2016; JH Park leg.; on A. japonica; MADBK 120532 015 – Jeju Special Self-Governing Province • 5 ♀♀, 1 ♂ (pocl 2.5, 2.3, 2.2, 1.7, 1.45, 2.0 mm); Beomseom Island; 33°13'7"N, 126°30'50"E; depth 20 m; 28 Feb. 2015; JH Park leg.; on A. solaster (Clark, 1907); MADBK 120532\_003 • 8 ♀♀, 1 ♂ (pocl 2.77, 2.6, 2.6, 2.5, 2.5, 2.4, 2.3, 2.1, 1.9 mm); same data; 16 May 2015; JH Park leg.; on Catoptometra *rubroflava* (Clark, 1908); MADBK 120532\_004 • 1 ov.  $\bigcirc$ ,  $6 \bigcirc \bigcirc$ ,  $3 \circlearrowright \circlearrowright$  (pocl 3.43, 3.48, 3.21, 3.1, 3.08, 2.37, 2.3, 2.89, 2.54, 1.97 mm); same data; 17 May 2015; JH Park leg.; on *A. japonica*; MADBK 120532\_005 • 1 ♀, 1 ♂ (pocl 1.83, 1.83 mm); Saeseom Island; 33°14'2"N, 126°33'49"E; depth 20 m; 30 Jan. 2016; JH Park leg.; on A. japonica; MADBK 120532\_011 • 3 99, 2 33 (pocl 2.45, 2.24, 1.2, 2.0, 1.68) mm); Seopseom Island; 33°13'55"N, 126°35'51"E; depth 15 m; 28 Jan. 2016; JH Park leg.; on *A. japonica*; MADBK 120532\_010 • 1 ♀, 2 ♂♂ (pocl 3.0, 2.16, 1.89) mm); same data; 28 Apr. 2016; JH Park leg.; on A. japonica; MADBK 120532\_013 • 1 3 (pocl 2.27 mm); same data; 28 Apr. 2016; JH Park leg.; on *A. japonica*; OUM-NH.ZC.2018-03-024 • 1 ♀ (pocl 3.5 mm); same data; 28 Apr. 2016; JH Park leg.;



3.0 mm

Figure 1. *Cristimenes brucei* sp. nov., lateral aspect. Ovigerous female holotype pocl 3.65 mm (NI-BRIV0000841118).

on *A. japonica*; OUMNH.ZC.2018-03-025 • 1  $\overset{\circ}{\circ}$  (pocl 1.89 mm); same data; depth 30 m; 16 Jul. 2015; JH Park leg.; on *A. solaster*; MADBK 120532\_008 • 2  $\overset{\circ}{\circ} \overset{\circ}{\circ}$ , 2  $\overset{\circ}{\circ} \overset{\circ}{\circ}$  (pocl 2.74, 1.05, 1.53, 1.47 mm); same data; depth 27 m; 31 Jan. 2016; JH Park leg.; on *A. japonica*; MADBK 120532\_012 • 1  $\overset{\circ}{\circ}$  (pocl 1.9 mm); Jeongbang Waterfall point; 33°14'36"N, 126°34'16"E; depth 15 m; 18 Jul. 2015; JH Park leg.; on A. solaster; MADBK 120532\_009 • 2  $\overset{\circ}{\circ} \overset{\circ}{\circ} \overset{\circ}{\circ}$  (pocl 3.5, 2.76, 2.55, 2.0 mm); Jigwido Island; 33°13'36"N, 126°39'12"E; depth 20 m; 14 Apr. 2013; JH Park leg.; on *A. japonica*; MADBK 120532\_001 • 1  $\overset{\circ}{\circ}$  (pocl 1.77 mm); Unjin Port point; 33°13'2"N, 126°14'42"E, depth 18 m; 20 Oct. 2016; SH Lee leg.; on *A. japonica*; MADBK 120532\_016.

**Diagnosis.** Rostrum well developed, with dorsal and ventral teeth. Carapace smooth, without epigastric tooth; lateral carinae feebly developed; supraorbital tooth reduced, blunt; inferior orbital angle pointed; antennal and hepatic teeth well developed. Fourth thoracic sternite without median process. Abdomen with rounded pleura. Telson with two pairs of small dorsal spiniform setae, and with three pairs of posterior spiniform setae. Eyes with hemispherical cornea. Basal antennular segment with two acute distolateral teeth. Antennal basicerite with sharp distoventral tooth; scaphocerite with large distolateral tooth, not reaching distal end of lamella. Epistome rounded. Mandible without palp; molar process robust; incisor process with four or five terminal teeth. Maxillula with bilobed palp. Maxilla with blunt palp, basal endite well developed, bilobed. First maxilliped with simple palp; basal and coxal endites fused; exopod with developed caridean lobe; epipod bilobed. Second maxil-



**Figure 2.** *Cristimenes brucei* sp. nov., female pocl 2.74 mm (A, B, C, E) (MADBK 120532\_012), ovig. female paratype pocl 3.65 mm (D) (OUMNH.ZC.2018-03-022). **A** carapace, lateral view **B** carapace, eyes, right antennule and antenna, dorsal view **C** anterior carapace, lateral view **D** distal end of telson, dorsal view **E** fifth and sixth abdominal segments, telson, and uropod, lateral view **F** left uropodal exopod, distolateral armature.

liped with subquadrate epipod, without podobranch. Third maxilliped with slender exopod; arthrobranch rudimentary. First pereiopods slender, fingers subspatulate with entire cutting edges. Second pereiopods equal in shape and subequal in size; palm articulated subproximally; cutting edges of fingers feebly dentate proximally, serrated distally. Dactyli of ambulatory pereiopods biunguiculate; corpus with two or three acute dorsodistal spinules, with acute preterminal accessory tooth. Uropodal exopod with distolateral tooth and movable acute spine.

**Description.** Rostrum (Figs 2A, B, 9A, B) well developed, slightly overreaching distal end of antennular peduncle; upper margin slightly convex with 6–8 dorsal teeth, ventral margin convex with 0–3 ventral teeth.

Carapace (Figs 2A, B, 9A, B) smooth without epigastric tooth; lateral carinae feebly developed; supraorbital tooth reduced, blunt (Fig. 9C); inferior orbital angle pointed; antennal and hepatic teeth (Fig. 2C) well developed, antennal tooth long and slender, hepatic tooth larger than antennal tooth; pterygostomial angle rounded.

Thoracic sternite (Fig. 3D) without special features; fourth thoracic sternite without finger-like median process.

Abdomen (Fig. 1) smooth; pleura of first to fifth segments rounded; sixth segment with pointed posterolateral angle, posteroventral angle blunt (Fig. 2E).



**Figure 3.** *Cristimenes brucei* sp. nov., female pocl 2.74 mm (MADBK 120532\_012). **A** eye, interocular region, and epistome, dorsal view **B** antennule, ventral view **C** antenna, ventral view **D** fifth (top) and fourth thoracic sternite.

Telson (Fig. 2D, E) 0.78 of pocl, 3.2 times longer than proximal width; two pairs of small dorsal spiniform setae at 0.53 and 0.82 of telson length, with three pairs of posterior spiniform setae, outer pair short, inner pair long and stout.

Eye (Figs 2B, 3A) with hemispherical cornea, dorsolaterally with nebenauge; eye-stalk 1.2 times as long as wide.

Antennule (Fig. 3B) well developed; basal segment with two acute distolateral teeth, with submarginal medioventral tooth; stylocerite reaching to middle of proximal segment; intermediate and distal segment subequal in length; upper flagellum biramous, proximal four segments fused, shorter free ramus with five segments, 0.3 of longer free ramus.

Antenna (Fig. 3C) well developed; basicerite with sharp distoventral tooth; ischiocerite and merocerite unarmed; carpocerite reaching to 0.4 of scaphocerite; scaphocerite 2.4 times as long as maximal wide, distolateral tooth large, not reaching distal end of lamellae.

Mandible (Fig. 4A) without palp; molar process robust, with four strong teeth and brush-like setae; incisor process with four or five terminal teeth.

Maxillula (Fig. 4B) with bilobed palp; upper lacinia broad, with stout and simple spines, with plumose setae on lower margin; lower lacinia robust with long spines distally, with plumose setae on lower margin.



**Figure 4.** *Cristimenes brucei* sp. nov., female pocl 3.48 mm (MADBK 120532\_005). **A** mandible **B** maxillule **C** maxilla **D** first maxilliped **E** second maxilliped **F** third maxilliped.

Maxilla (Fig. 4C) with blunt palp, with sparsely plumose setae; coxal endite obsolete; basal endite well developed, bilobed, with sparsely plumose setae; scaphognathite 2.9 times as long as wide.

First maxilliped (Fig. 4D) with long simple palp, with sparsely plumose setae along the medial margin of the palp; basal and coxal endites fused, with serrulate setae medially; exopod with developed caridean lobe, flagellum with long simple seta; epipod bilobed.

Second maxilliped (Fig. 4E) with subquadrate epipod, without podobranch; merus and carpus without special features; propodus with slender simple setae; dactylus 2.7 times as long as wide, with dense serrulate setae distally.

Third maxilliped (Fig. 4F) with endopod slightly overreaching distal end of carpocerite; ischiomerus approximately six times longer than wide, medially sparsely setose; penultimate segment 0.56 length of ischiomerus, medially with long serrulate setae; terminal segment tapering, slightly downcurved distally, subequal to penultimate segment, with transverse rows of setae and group of terminal hamate setae; exopod slender with plumose setae distally; coxa with large rounded epipod, arthrobranch rudimentary.

First pereiopod (Fig. 5A, B) overreaching distal end of scaphocerite; ischium 0.56 length of merus, unarmed; merus and carpus subequal in length; carpus 1.36



**Figure 5.** *Cristimenes brucei* sp. nov., female pocl 2.74 mm (MADBK 120532\_012). A first pereiopod, ventrolateral view **B** same, chela, lateral view **C** third pereiopod, lateral view **D** same, dactylus and distal propodus, lateral view **E** fourth pereiopod, lateral view.

times length of chela with row of serrulate setae along distomesial margin; chela 1.9 times longer than deep; palm with transverse row of serrulate setae ventrolaterally; fingers subspatulate, 0.89 times length of palm, cutting edge straight, entire, with groups of setae.

Second pereiopods (Figs 6A, B, 10A) equal in shape and subequal in size; ischium 0.7 length of merus, unarmed; merus 2.0 times as long as carpus, unarmed; carpus short, 1.2 times as long as maximal width, articulated subterminally (Fig. 6C); palm cylindrical, 1.1 times as long as finger, articulated subproximally; fingers stout with curved tip; cutting edges of fingers feebly dentate proximally, serrated distally (Figs 6D, E, 10C, D).

Ambulatory pereiopods (Fig. 5C, E, F) of usual shape for genus, third pereiopod overreaching end of scaphocerite by distal margin of carpus. Third pereiopod (Fig. 5C) with ischium 0.54 length of merus, unarmed; merus 0.85 times length of propodus, unarmed; carpus 0.46 times length of propodus, unarmed; propodus with three distolateral spiniform setae including single distoventral one; dactylus 0.18 times length of propodus, biunguiculate; corpus with three acute dorsodistal spinules, with acute preterminal accessory tooth, ventral margin straight, with simple distal setae laterally; unguis 0.58 times as long as corpus (Fig. 5D).



**Figure 6.** *Cristimenes brucei* sp. nov., female pocl 2.74 mm (MADBK 120532\_012). **A** minor right second pereiopod, ventrolateral view **B** major left second pereiopod, lateral view **C** same, carpo-propodal articulation, dorsal view **D** same, dactylus, lateral view **E** same, distal margin of cutting edge of dactylus.

Fourth pereiopod (Fig. 5E) with ischium 0.57 length of merus, unarmed; merus 0.80 times length of propodus, unarmed; carpus 0.42 times length of propodus, unarmed; propodus with four distolateral spiniform setae including two distoventral ones; dactylus 0.18 times length of propodus, biunguiculate; corpus with two acute dorsodistal spinules, with acute preterminal accessory tooth, ventral margin straight, with simple distal setae laterally; unguis 0.64 times as long as corpus.

Fifth pereiopod (Fig. 5F) with ischium 0.54 length of merus, unarmed; merus 0.74 times length of propodus, unarmed; carpus 0.38 times length of propodus, unarmed; propodus with five mesial spiniform setae, distolateral one absent; dactylus 0.18 times length of propodus, biunguiculate; corpus with two acute dorsodistal spinules, with acute preterminal accessory tooth, ventral margin straight, with simple distal setae laterally; unguis 0.70 times as long as corpus.

Pleopods as usual for genus. First pleopod of male (Fig. 7A) with endopod 2.8 times longer than wide. Second pleopod of male (Fig. 7B) with appendix masculina with stout, long setae; appendix interna slightly longer than appendix masculina. Second pleopod of female (Fig. 7C) as usual for genus.



**Figure 7.** *Cristimenes brucei* sp. nov., male pocl 2.54 mm (**A**, **B**) (MADBK 120532\_005), female pocl 2.74 mm (**C**) (MADBK 120532\_012) **A** endopod and basal half exopod of first pleopod **B**, **C** endopod of second pleopod.

Uropod (Fig. 2E) overreaching distal end of telson; exopod with distolateral tooth and movable acute spine.

**Etymology.** The new species is named in honour of Dr AJ (Sandy) Bruce, in recognition of his considerable contribution to the systematics of Palaemonidae.

**Colour.** Body colour (Fig. 8A, B) orange or reddish-brown adapted to the colour of the host crinoids; creamy white line extending from the tip of the rostrum to the posterior dorsal margin of the carapace; similar, but thinner and lighter line extending from posterior ventral angle of the sixth abdominal segment to the lateral side of the first antennular peduncle.

**Ecology.** The specimens were collected from the crinoids *Anneissia japonica*, *A. solaster* and *Catoptometra rubroflava* at a depth of 15 – 27 m. Bruce (1982a) reported that the Hong Kong specimens were collected from *Tropiometra afra* (Hartlaub, 1890).

**Distribution.** Presently only known from the type locality, Jeju Special Self-Governing Province, Korea as well as Hong Kong (Bruce 1982a).

Remarks. The new species is morphologically very similar to the other crinoid-associated species in the genus, *C. commensalis* (Fig. 8C). Within the genus, both species



**Figure 8.** Colour pattern of three species of *Cristimenes*. **A** *Cristimenes brucei* sp. nov. from Korea (MADBK 120532\_017) **B** same, with host crinoid species **C** *Cristimenes commensalis* (Borradaile, 1915) from Vietnam (SNU VI VI305) **D** *Cristimenes cristimanus* (Bruce, 1965) from Vietnam (SNU VI VI297).

share the following characteristics: subspatulate fingers of the first pereiopods; proximally dentate and distally serrate cutting edges of the fingers of the second pereiopods (Fig. 10C, D); and the presence of accessory spinules on the anterior margin of the dactyli of the ambulatory pereiopods. The new species can, however, be easily distinguished from *C. commensalis* by the reduced, blunt supraorbital tooth (Fig. 9A–C) and reduced rostral carinae (vs. well-developed supraorbital tooth (Fig. 9D) and rostral carinae in *C. commensalis*).

*Cristimenes brucei* sp. nov. can easily be distinguished from the echinoid-associated species *C. cristimanus* (Fig. 8D) and *C. zanzibaricus* by the reduced supraorbital tooth and rostral carinae (vs. extremely developed supraorbital tooth and rostral carinae), the presence of accessory spinules on the anterior margin of the ambulatory dactylus (vs. absent), and a different host affiliation, with the latter two species being associated with echinoids.

The crinoid-associated genera *Araiopontonia* Fujino & Miyake, 1970, *Laomenes* Clark, 1919, and *Unguicaris* Marin & Chan, 2006 are phylogenetically closely related to *Cristimenes*. The new species shares a morphological trait with *Araiopontonia odon-torhyncha* Fujino & Miyake, 1970 in having accessory spinules on the anterior margin of the ambulatory dactylus, but the new species can easily be distinguished from *A. odontorhyncha* by the reduced supraorbital teeth and rostral carinae, the presence of a hepatic tooth on the carapace, and the low and rounded epistome (vs. developed supraorbital tooth and rostral carinae, absence of hepatic tooth, and well developed rounded epistomial horns in *A. odontorhyncha*). All species in the genus *Laomenes* can



**Figure 9.** *Cristimenes brucei* sp. nov., female pocl 2.7 mm (MADBK 120532\_002) (**A**, **C**), male pocl 2.3 mm (MADBK 120532\_005) (**B**), *Cristimenes commensalis* from Vietnam, female pocl 2.1 mm (SNU VI\_VI229) (**D**) **A** carapace, lateral view **B** carapace, dorsal view **C** supraorbital tooth, lateral view **D** carapace, dorsal view.

be distinguished from the new species by having more strongly developed supraorbital teeth and rostral carinae, well developed sharp epistomial horns and simple biunguiculat ambulatory dactylus. The new species is morphologically similar to *U. novaecaledoniae* (Bruce, 1968) among species of the genus *Unguicaris*. The new species shares with *U. novaecaledoniae* similar first chelipeds, proximally dentate but distally serrate cutting edges of the fingers of the second pereiopods, and the presence of well-developed accessory spinules on the anterior margin of the ambulatory dactyli. The new species can, however, be distinguished from *U. novaecaledoniae* by the presence of reduced supraorbital teeth (vs. absent).

## **Phylogenetic analyses**

We obtained fragments of 658, 462, 293, and 655 bp for the COI, 16S, H3, and 18S markers, respectively. Barcode COI regions were calculated for 13 specimens across all four species of *Cristimenes*, with the maximum K2P intraspecific divergence being 0.15%, 1.09% and 1.25% in *Cristimenes* brucei sp. nov., *C. commensalis*, and *C. cristimanus* (Table 2), whilst mean K2P interspecific distances between *C. brucei* sp. nov.



**Figure 10.** *Cristimenes brucei* sp. nov., female pocl 2.7 mm (MADBK 120532\_002) (**A**, **C**, **D**), female pocl 3.34 mm (MADBK 120532\_007) (**E**, **F**), *Cristimenes commensalis* from Vietnam, female pocl 2.0 mm (SNU VI\_VI155) (**B**) **A**, **B** chela and carpus of second pereiopod **C** same, distally serrate margin of fixed finger **D** same, proximally dentate margins of fingers **E** dactylus and distal propodus of third pereiopod **F** same, distal dorsal spinules of dactylar corpus, and unguis.

and *C. commensalis*, *C. cristimanus* and *C. zanzibaricus* being 18.2%, 13.8%, and 15.1%, respectively (Table 2).

Phylogenetic analyses were conducted on 21 specimens of seven species of four genera (Table 1). The combined 2068 bp fragments had 253 parsimony-informative sites for COI, 145 for 16S, 42 for H3, and 102 for 18S. The ML and BI analyses showed the same topology, except for *Laomenes* and *Unguicaris* (Fig. 11). The resulting



**Figure 11.** Phylogenetic tree of *Cristimenes* and related crinoid associated genera resolved by Maximum Likelihood (ML) and Bayesian Inference (BI) analysis based on the combined dataset for four genes (COI, 16S, H3, and 18S). BI posterior probabilities and ML bootstrap support (BI/ML) as shown. **A** The topology based on the BI tree **B** Condensed part of ML tree.

**Table 2.** Kimura 2-Parameter model distances for COI within and among species of *Cristimenes*. N/A - not available.

Species	Maximum distance	Mean distance between species				
	within species	1	2	3	4	
1. Cristimenes brucei sp. nov.	0.15 %					
2. Cristimenes cristimanus	1.25 %	13.78 %				
3. Cristimenes zanzibaricus	N/A	15.10 %	10.95 %			
4. Cristimenes commensalis	1.09 %	18.17 %	17.25 %	14.61 %		

phylogeny clearly indicates the monophyly of *Cristimenes* with high support values, both in ML and BI analyses. *Cristimenes brucei* sp. nov. is clearly recovered as a monophyletic species but as a sister group to the echinoid associated *C. cristimanus*, whilst the crinoid associated *C. commensalis* is a sister group to the clade containing *C. brucei* sp. nov., *C. cristimanus*, and *C. zanzibaricus*.

#### Acknowledgements

We thank Dr Nguyen Van Tu (ITB, Vietnam) for the invitation and management of fieldwork in Vietnam. We are also grateful to Dr Hyi-Seung Lee (KIOST, Korea) and Dr Wilfredo L. Campos (UPV, Philippines) for the invitation to participate in the Philippine fieldwork. Funding for fieldwork in Vietnam and Philippines was provided through the Marine Biotechnology Program (No. 20170488) funded by the Ministry of Ocean and Fisheries (MOF). JHP is also grateful to Dr Chang-Rae Lee (National Park Research Institute, Korea), Dr Tae Seo Park (NIBR, Korea), and Dr Myung-Hwa Shin (National Marine Biodiversity Institute, Korea) for the invitation to participate in the exploration of the Dadohaesang National Park, the Jeju Special Self-Governing Province, and the East Sea of Korea, respectively. This study is also supported by grant from the National Institute of Biological Resources (No. 20170431) funded by the Ministry of Environment and Marine Biotechnology Program (No. 20170431) funded by the MOF of Korean Government. The authors are grateful to the editor and two reviewers for their valuable comments to this study.

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RESEARCH ARTICLE



# Description on two species of genus *Platythomisus* (Araneae, Thomisidae) from China and Singapore

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Academic editor: A. Pérez-González   Received 11 March 2019   Accepted 25 April 2019   Published 5 June 2019
http://zoobank.org/8AFB6728-CAAC-463F-9E8A-A26BB2723B37

**Citation:** Lin Y, Koh JKH, Shao L, Li S (2019) Description on two species of genus *Platythomisus* (Araneae, Thomisidae) from China and Singapore. ZooKeys 852: 73–84. https://doi.org/10.3897/zookeys.852.34436

#### Abstract

Two species of the genus *Platythomisus* Doleschall, 1859 are studied: *P. xiandao* Lin & Li, **sp. nov.** is described based on male and female specimens from Yunnan, China, and *P. octomaculatus* (C. L. Koch, 1845), the type species of the genus, is redescribed based on female specimens from Singapore. Its male, also from Singapore, is described for the first time.

# Keywords

African region, new species, Oriental region, taxonomy, type species

# Introduction

The spider genus *Platythomisus* Doleschall, 1859 includes 13 species, of which nine are known in the African region and four are distributed in the Oriental region, viz., *P. jucundus* Thorell, 1894 ( $\mathcal{J}$ , Indonesia), *P. octomaculatus* (C. L. Koch, 1845) ( $\mathcal{Q}$ , Brunei, India, Indonesia, Malaysia, Singapore, Thailand), *P. quadrimaculatus* Hasselt, 1882 (juvenile, Indonesia), and *P. sudeepi* Biswas, 1977 ( $\mathcal{J}\mathcal{Q}$ , India, Sri Lanka) (WSC 2019; Li and Quan 2017).

We have since found matched pairs of two *Platythomisus* species in collections from Singapore and China. The discovery of one of these species, *Platythomisus xiandao* Lin & Li sp. nov., from Yunnan represents the first record of this genus in China. Furthermore, specimens of *P. octomaculatus* (C. L. Koch, 1845) from Singapore have allowed us to provide the first description of the male of the species.

#### Material and methods

All specimens were preserved in 80% ethanol. Dissected genitalia were cleared in warm 10% potassium hydroxide (KOH) solution before study. Specimens were examined under a LEICA M205C stereomicroscope. Photomicroscopy images were taken with an Olympus C7070 zoom digital camera (7.1 megapixels). Laboratory habitus photographs were taken with a Canon 5D Mark III digital camera equipped with a Canon MP-E 65 mm lens. Photos were stacked with Helicon Focus (version 6.7.1) or Zerene Stacker (version 1.04) and processed in Adobe Photoshop CC2018. Field photographs were taken with a Nikon D800E with a Tamron 90 mm macro lens.

All measurements are in millimeters and were obtained with a LEICA M205C stereomicroscope. Eye sizes are measured as the maximum diameter from either the dorsal or frontal view. Leg measurements are given as follows: total length (femur, patella, tibia, metatarsus, tarsus). The terminology used in the text and figures follows Ono (1988). Distribution maps were generated using ArcMap software (version 10.2).

The types of *Platythomisus xiandao* Lin & Li, sp. nov. are deposited at the Institute of Zoology, Chinese Academy of Sciences in Beijing (**IZCAS**). The voucher specimens of *P. octomaculatus* of this study are kept at the Lee Kong Chian Natural History Museum, National University of Singapore (**LKCNHM**).

To confirm the species delimitation, a fragment of the cytochrome c oxidase subunit I (COI) was amplified and sequenced. Primer sets for the PCR and cycle sequencing reactions in this study are from Folmer et al. (1994). The GenBank accession numbers are provided in Table 1. MEGA7.0.16 (Kumar et al. 2016) was used for subsequent manual adjustment of the sequences and calculation of pairwise comparisons of uncorrected K2P-distances. COI sequences of *Thomisus* Walckenaer, 1805 were also obtained from GenBank to calculate intraspecific genetic distance.

Abbreviations: ALE anterior lateral eyes, AME anterior median eyes, PLE posterior lateral eyes, PME posterior median eyes, E embolus, ITA intermediate tibial apophysis, RTA retrolateral tibial apophysis, VTA ventral tibial apophysis, At atrium, CD copulatory duct, S spermathecae.

Species	Length (bp)	GenBank accession number
Platythomisus octomaculatus	647 bp	MK774520
Platythomisus xiandao sp. nov.	647 bp	MK774521

**Table 1.** The accession numbers for two species in this paper.

# Family Thomisidae Sundevall, 1833 Subfamily Thomisinae Sundevall, 1833

#### Genus Platythomisus Doleschall, 1859

Type species. Thomisus 8-maculatus C.L. Koch, 1845, from Ostindien.

**Diagnosis.** Sexual dimorphism is distinct in *Platythomisus*. Females can be easily distinguished from most other thomisids by their extraordinary large size [up to 20 mm in length in some specimens of the type species *P. octomaculatus* (C. L. Koch, 1845)] with strikingly contrasting color patterns on the carapace and opisthosoma. Typically, the epigyne has sclerotized margins and a conspicuous epigynal atrium; spermathecae longer than wide, well-sclerotized, and not divided into compartments. The male is much smaller than female (1:3 or more). Palp with VTA, ITA and RTA; tegulum flat, disk-shaped; tegular ridge present; embolus slender.

Distribution. Oriental and African zoogeographic regions.

#### Platythomisus octomaculatus (C. L. Koch, 1845)

Figs 1A-C, 2A, C, 3A, B, 4A-D, 5A, B, 6

*Thomisus 8-maculatus* C.L. Koch 1845: 55, fig. 990 (♀). *Platythomisus phryniformis* Doleschall 1859: 60 pl. 3, fig. 10 (♀). *Platythomisus octomaculatus* van Hasselt 1882: 42, pl. 3, fig. 4 (♀); Simon 1895: 1017, fig. 1076 (♀).

**Type material.** The holotype of *P. octomaculatus* was not examined as it could not be located in any of the databases of all the major museums in Europe.

**Specimens examined.**  $\bigcirc$  (LKCNHM), Singapore, Pasir Ris Road, Pasir Ris Nature Park, mangrove foliage, 09.X.2018, J. Koh leg.;  $\bigcirc$  (LKCNHM), Singapore, Neo Tiew Crescent, Sungei Buloh Wetland Reserve, mangrove foliage, 01°26'49"N, 103°43'45"E, 20.IV.2016, M. Tan leg.  $\bigcirc$  (LKCNHM), Singapore, Neo Tiew Crescent, Sungei Buloh Wetland Reserve, mangrove foliage, 01°26'53"N, 103°43'42"E, 13.I.2013, J. Koh leg.

Diagnosis. See diagnosis of the species *Platythomisus xiandao* sp. nov.

**Description. Male** (Figs 1A–C, 2A, C, 4C, D, 5B): total length 3.72, carapace 1.62 long, 1.5 wide, opisthosoma 2.18 long, 1.52 wide. Carapace reddish brown. Eye region orange, AER and PER recurved. Eye sizes and interdistances: AME 0.06, ALE 0.09, PME 0.04, PLE 0.08, AME–AME 0.26, AME–ALE 0.20, PME–PME 0.37, PME–PLE 0.28, AME–PME 0.17, ALE–PLE 0.19. Clypeus 0.13 high, red. Chelicerae red, with ridge, without any teeth. Endites and labium red anteriorly and black posteriorly. Sternum black, with sparse hairs. Legs white, coxae faint black,



**Figure 1.** Male palps of *Platythomisus octomaculatus* (C. L. Koch, 1845) (**A**, **B**, **C**) and *Platythomisus xiandao* sp. nov. (**D**, **E**, **F**). **A** Left, prolateral view **B** Same, ventral view **C** Same, retrolateral view **D** Right male palp (Horizontal flip), prolateral view **E** Same, ventral view **F** Same, retrolateral view.



**Figure 2.** Expanded male palps (treated with 10% KOH) of *Platythomisus octomaculatus* (C. L. Koch, 1845) (**A**, **C**) and *Platythomisus xiandao* sp. nov. (**B**, **D**). **A**, **B** Right, ventral view **C**, **D** Same, tarsus apophysis retrolateral view.

tarsi, metatarsi and tibiae have a longitudinal black line dorsally each. Legs translucent when alive. Leg I: 6.45 (2.05 + 2.26 + 1.23 + 0.91), leg II: 6.60 (2.18 + 2.35 + 1.19 + 0.88), leg III: 3.84 (1.34 + 1.44 + 0.54 + 0.52), leg IV: 3.56 (1.31 + 1.30 + 0.46 + 0.49). Leg formula: 2134. Opisthosoma broadly pentagonal, dorsum yellowcinnamon, ventrum reddish-brown. Dorsum with three distinct black spots and four smaller faint brown spots, with red folds laterally, ventrum with a large shield-shaped black patch. Spinnerets black.

Male palp (Figs 1A–C, 2A, C). Femur white. Tibia black, VTA club-shaped, with a thick bristle near the apex. ITA boot-shaped, terminally flat with a laterally pointing extension. RTA long, terminal slightly bent. Cymbium black. Tegulum flat, diskshaped, with a tegular ridge. Embolus spiral, thin, the base of embolus arising from a 1:30-o'clock-position, the length of embolus to the length of embolus base 5:1 (Fig. 2A).

Female (Figs 3A, B, 4A, B, 5A) markedly larger and different from male. Total length 17.05, carapace 8.21 long, 7.63 wide, opisthosoma 10.00 long, 7.31 wide. Carapace yellow with three pairs of black spots. The anterior pair cover the ALE and PLE, adjacent to AME and PME; middle spots extremely small. AER and PER slightly recurved. Eye sizes and interdistances: AME 0.14, ALE 0.19, PME 0.10, PLE 0.16, AME-AME 1.14, AME-ALE 0.93, PME-PME 1.61, PME-PLE 1.01, AME-PME 0.54, ALE-PLE 0.42. Clypeus height 0.68, orange. Chelicerae orange, with ridge, margin has a thin brown ring pattern, without any teeth. Endites and labium orange. Sternum orange, with sparsely set hairs. Legs two-colored, with coxa, trochanter, femur and patella in orange, and metatarsus and tarsus black. Coloration of the tibiae varies: black in tibiae I and II, while orange in tibiae III and IV. Leg I: 26.84 (8.14 + 9.62 + 6.02 + 3.06), leg II: 27.24 (8.21 + 9.94 + 6.09 + 3.00), leg III: 15.64 (5.58 + 5.90 + 2.50 + 1.66), leg IV: 15.81 (5.32 + 6.47 + 2.47 + 1.55). Leg formula: 2143. Opisthosoma sub-rectangular with a bluntly pointed posterior end, yellow, laterally pleated. Dorsum with seven blue-grey spots, ventrum with an oblong black patch. Spinnerets black, surrounded by a narrow black ring.

Epigyne (Figs 3A, B) with atrium shaped like a funnel with a broad neck, the length of atrium to the middle width of the atrium 1:1.5, the length of anterior margin to the length of posterior margin 2:1. CD short, approximately half the length of the spermathecae. Spermathecae stout, with almost parallel sides anteriorly.

**Intraspecific variations:** While the holotype has eight spots on the dorsum, as illustrated by Koch (1845), mature specimens seen or photographed in Singapore, Brunei, Thailand, and East and West Malaysia possess only seven dorsal spots but with the eighth spot on the ventrum. In some juvenile females, there are only five dorsal spots on the opisthosoma. The number of spots on the carapace varies between two and four pairs in mature females. The general coloration of live male specimens varies from yellow to red.

**Distribution.** The type locality "Ostindien", meaning "East Indies", refers to Indonesia today. Within Indonesia, the species was recorded in Java as *Platythomisus phryniformis* by Doleschall (1859) and in Padang, Sumatra by Hasselt (1882). However, we failed to find any specimens of the species during field trips in the country. This species has been recorded in Singapore, Brunei, Thailand, and East and West Malaysia (Koh and Leong 2014). A report of *P. octomaculatus* from Assam, India (Yadav et al. 2017) is clearly misidentified. We suggest it may be *P. xiandao* sp. nov.

**Natural history.** Individuals live among low-lying tree foliage in or around degraded forests and mangrove swamps. Eggs are laid between leaves and sealed with thick silk. The egg sacs are guarded by the mother until the spiderlings hatch in about 2 weeks.

#### Platythomisus xiandao sp. nov.

http://zoobank.org/E45077FE-3209-4887-AD5A-A7DA989FC2FF Figs 1B–D, 2B, D, 3C, D, 4E–H, 5C, D, 6

**Holotype.**  $\bigcirc$  (IZCAS), China, Yunnan Prov., Jinghong City, Mount Jinuo, hatched from paratype egg sac. Hatched 12.X.2017, matured 10.IV.2018, C.T. Wei leg. **Paratype:**  $\bigcirc$  (IZCAS), same locality data as holotype, collected 06.X.2017, C.T. Wei leg.

**Etymology.** The specific name is derived from the Chinese word "xiandao" (noun), the name of the Strategic Priority Research Program of the Chinese Academy of Sciences (CAS). The program has made it possible for the biodiversity research team in the CAS to remain as an integral cluster to fulfil all its ambitious goals.

**Diagnosis.** Male of *P. xiandao* sp. nov. can be easily distinguished from that of *P. octomaculatus* by the length of embolus to the length of embolus base is 3:1 (Fig. 2B), but 5:1 in *P. octomaculatus* (Fig. 2A), the base of embolus arising from a 3:30-o'clock-position in *P. xiandao* sp. nov. (Fig. 1E), and 1:30-o'clock-position in *P. octomaculatus* (Fig. 1B); the atrium of *P. xiandao* sp. nov. is bell-shaped (Fig. 3C) while that of *P. octomaculatus* is funnel-shaped (Fig. 3A); the spermathecae of *P. xiandao* sp. nov. (Fig. 3D) are more slender than those in *P. octomaculatus* (Fig. 3B).

The two species can also be diagnosed by their somatic differences. The males of *P. xiandao* sp. nov. have seven black spots on the opisthosoma (Fig. 4G) and a reddish sternum (Fig. 4H); the males of *P. octomaculatus* have only three distinct black spots on the opisthosoma (Fig. 4C) and a black sternum (Fig. 4D). The females of *P. xiandao* sp. nov. have larger black patches on the carapace (Fig. 4A) and a black patch at the center of ventrum extending posteriorly to connect with the black area surrounding the spinnerets through a narrow neck (Fig. 4F), while the black patch at the ventrum of *P. octomaculatus* females is broadly oblong and well-separated from the spinnerets (Fig. 4B).

**Description. Male** (Figs 1D–F, 2B, D, 4G, H, 5D): total length 3.92, carapace 1.55 long, 1.38 wide, opisthosoma 2.40 long, 1.70 wide. Carapace red. Eye region orange, AER and PER recurved. Eye sizes and interdistances: AME 0.05, ALE 0.09, PME 0.03, PLE 0.06, AME–AME 0.24, AME–ALE 0.17, PME–PME 0.32, PME–PLE 0.28, AME–PME 0.13, ALE–PLE 0.19. Clypeus 0.13 high, red. Chelicerae red, with ridge, without any teeth. Endites and labium red. Sternum red, sparsely set hairs. Legs white, coxa black, tarsus, metatarsus, tibia, patella and femur of leg I and II have two black lines. Legs transparent when alive. Leg I: 5.73 (1.86 + 2.00 + 1.08 + 0.79), leg II: 6.04 (2.02 + 2.10 + 1.10 + 0.82), leg III: 3.20 (1.00 + 1.20 + 0.51 + 0.49), leg



Figure 3. Epigyna and vulvae of *Platythomisus octomaculatus* (C. L. Koch, 1845) (**A**, **B**) and *Platythom-isus xiandao* sp. nov. (**C**, **D**). **A**, **C** Epigyne, ventral view **B**, **D** Vulva, dorsal view.

IV: 3.27 (1.21 + 1.11 + 0.46 + 0.49). Leg formula: 2143. Opisthosoma oval, white, dorsum cinnamon, ventrum reddish-brown. Opisthosoma dorsum with seven black spots, first one largest, lateral sides with red folds, ventrum reddish-brown, with a large shield-shaped black pattern. Spinnerets black.

Male palp (Figs 1D–F, 2B, D). Femur white. Tibia black, VTA club-shaped, slightly bent, with a bristle near the apex. ITA boot-shaped, terminally flat with lateral exten-



**Figure 4.** Habitus of *Platythomisus octomaculatus* (C. L. Koch, 1845) (**A–D**) and *Platythomisus xiandao* sp. nov. (**E–H**). **A, E** Female habitus, dorsal view **B, F** Female habitus, ventral view **C, G** Male habitus, dorsal view; **D, H** Male habitus, ventral view.

sion. RTA long and thin, terminal slightly bent. Cymbium black. Tegulum flat, diskshaped, with tegular ridge. Embolus slender and spiral, the base of embolus arising from a 3:30-o'clock-position, the length of embolus to the length of embolus base 3:1 (Fig. 2B).

**Female** (Figs 3C, D, 4E, F, 5C) distinctly different from male. Total length 13.01, carapace 5.20 long, 5.05 wide, opisthosoma 8.78 long, 6.35 wide. Carapace yellow with three pairs of big black subcircular patches. The anterior pair extend to the protruding ends on both lateral sides of the ocular tubercles, covering the ALE, PME and PLE, and adjoining the AME. The median pair of black disks smaller than the



Figure 5. Photos of alive *Platythomisus octomaculatus* (C. L. Koch, 1845) (**A**, **B**) and *Platythomisus xi-andao* sp. nov. (**C**, **D**) **A**, **C** Female **B**, **D** Male.

anterior and posterior pairs. AER and PER slightly recurved. Eye sizes and interdistances: AME 0.11, ALE 0.12, PME 0.05, PLE 0.09, AME–AME 0.81, AME–ALE 0.54, PME–PME 1.02, PME–PLE 0.71, AME–PME 0.37, ALE–PLE 0.36. Clypeus height 0.56, orange. Chelicerae orange, with ridge, margin has a black ring pattern, without any teeth. Endites and labium orange. Sternum orange, sparsely set hairs. Legs bicolored, coxa, trochanter, the femur of leg I and II black, the patella of leg I and II and the femur of leg III and IV orange with a black inverted triangle spot. Other legs orange. Leg I: 18.09 (5.70 + 6.41 + 3.96 + 2.02), leg II: 18.54 (5.96 + 6.54 + 4.00 + 2.04), leg III: 10.82 (3.64 + 4.04 + 1.88 + 1.26), leg IV: 11.16 (3.92 + 4.35 + 1.66 + 1.23). Leg formula: 2143. Opisthosoma pentagonal, yellow. Opisthosoma dorsum with seven black spots, with the largest in the anteriormost spot, lateral folded, ventrum yellow, with a shield-shaped black pattern coalescing with the black ring surrounding the black spinnerets.

Epigyne (Fig. 3C, D) with bell-shaped atrium, the length of atrium to the middle width of the atrium 1:1.4, the length of anterior margin to the length of posterior margin 1:1. CD short, about one-fourth the length of the spermathecae. Spermathecae comparatively slender, shaped like a sea cucumber or hot-dog sausage.

Distribution. China (Yunnan).



Figure 6. Known distribution of *Platythomisus* species in Oriental Region.

Natural history. Individuals of this species hide under the leaves.

**Remarks.** Based on the 647 bp-aligned sequences, the COI uncorrected K2P-distance between *P. octomaculatus* and *P. xiandao* sp. nov. is 0.073. The result far exceeded the maximum value of intraspecific genetic distance for Thomisidae.

# Acknowledgements

The manuscript benefitted greatly from comments by Abel Pérez-González, Hirotsugu Ono, Yuri Marusik, André Wanderley do Prado, and Suresh Benjamin. Jishen Wang and Hotaru Amamiya suggested the name of the new species. Paul Y.C. Ng contributed the image of a live male *P. octomaculatus*, Guiqiang Huang and Xuankong Jiang helped with the images of live *P. xiandao* sp. nov. Zhengzhong Huang provided the habitus images of preserved female specimens of both species. This study was supported by the National Natural Science Foundation of China (NSFC-31530067) to Shuqiang Li.

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RESEARCH ARTICLE



# A new genus and a new species in the subfamily Polyzosteriinae (Blattodea, Blattidae) from China

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Academic editor: Pavel	Stoev	Received 24 Janu	ary 2019	Accepte	d 16 April	2019	Published 5	5 June 2019
	http://	zoobank.org/BD58F	C49-AC47-	-489D-A00	9-3E8C02C3	SD8E6		

**Citation:** Liao S, Wang Z, Che Y (2019) A new genus and a new species in the subfamily Polyzosteriinae (Blattodea, Blattidae) from China. ZooKeys 852: 85–100. https://doi.org/10.3897/zookeys.852.33325

#### Abstract

*Laevifacies quadrialata* gen. et sp. nov. is described from Hainan Province, China based on morphological data. COI data (DNA barcodes) is utilized to confirm the sexual dimorphism occurring in *Laevifacies quadrialata* gen. et sp. nov. *Melanozosteria nitida* Brunner von Wattenwyl, 1865, is reported from Guangxi Province, China. A key to the Chinese Polyzosteriinae is provided.

# Keywords

Blattaria, cockroaches, Laevifacies, Melanozosteria, molecular identification, morphology

# Introduction

Polyzosteriinae is a relatively species-abundant subfamily in the Blattidae. The subfamily is flightless (except Methanini), having lobiform vestigial tegmina or being totally apterous. Some species have pits or tubercles scattered on the pronotum (Rentz 2014), short tarsi and large pulvilli and arolia (Mackerras 1968b). Members of Polyzosteriinae were firstly mentioned by Burmeister (1838), with the establishment of genus *Polyzosteria* Burmeister, 1838. Tepper (1893) erected the subfamily Polyzosteriinae with two genera, *Polyzosteria* and *Platyzosteria* Brunner von Wattenwyl, 1865. The revisionary works of Mackerras (1965a, 1965b, 1965c, 1966a, 1966b, 1967a, 1967b, 1968a,

1968b) included 16 genera, e.g. *Polyzosteria* Burmeister, 1838; *Platyzosteria* Brunner von Wattenwyl, 1865; *Cosmozosteria, Melanozosteria, Zonioploca* Stål, 1874; *Methana* Stål, 1877; *Anamesia, Drymaplaneta, Leptozosteria, Pseudolampra* and *Temnelytra* Tepper, 1893; *Desmozosteria, Euzosteria* and *Scabina* Shelford, 1909; *Eppertia* Shaw, 1925 and *Megazosteria* Mackerras, 1966a. In the catalogue, Princis (1966) recorded *Eurycotis* Stål, 1874. McKittrick (1964) provided a detailed description of the genitalia and proventriculus of the Polyzosteriinae female and male for the first time. Mackerras (1965a) divided the Australian members into two tribes: Polyzosteriini and Methanini, compiling the most complete account of Polyzosteriinae to date. Grandcolas (1997) described 5 genera from New Caledonia. Up to now, Polyzosteriinae contains 22 genera and 305 species (Beccaloni 2014), most of which are distributed in Australia, Southeast Asia, America, and the Pacific Islands.

The genus Melanozosteria was established with Polyzosteria nitida Brunner von Wattenwyl, 1865 as type species (Stål 1874). After that, Mackerras (1967b) did not agree with Melanozosteria as a synonym of Platyzosteria owing to the misidentification of one species of Platyzosteria by Shelford (1909), and placed Melanozosteria and Leptozosteria in Platyzosteria as subgenera. Roth (2003) re-established the taxonomic status of Melanozosteria as a genus. Currently 44 species are known of Melanozosteria, which are mainly distributed in Australia (Beccaloni 2014). Two Melanozosteria species are currently recorded in China (Melanozosteria nitida Brunner von Wattenwyl, 1865 and Melanozosteria soror Brunner von Wattenwyl, 1865). Melanozosteria nitida from Taiwan was originally determined as Periplaneta polita Walker, 1868. Then Shelford (1909) proposed that *Periplaneta polita* is a synonym of *Cutilia nitida* Brunner von Wattenwyl, 1865. Until now, they have both been considered synonyms of Melanozosteria nitida. In the catalogue, Princis (1966) recorded Melanozosteria nitida from Taiwan, China, but he guestioned its distribution on Mainland China. The other species, Melanozosteria soror, is mainly distributed in Australia and the Pacific Islands. Walker (1868) firstly recorded this species from Taiwan, China (it was originally described as Periplaneta philpotti, but later synonymized under Melanozosteria soror in Princis (1957)). Then Shiraki (1931) recorded this species from Hainan, but no further information was provided.

DNA barcodes have been proven to be a helpful method to identify species and to successfully match male and female. Barcoding has been applied to resolve the problems of sexual dimorphism and even to identify nymphs in cockroaches (Evangelista et al. 2013; Qiu et al. 2017; Che et al. 2017; Wang et al. 2018). To date, members of the Polyzosteriinae have been identified primarily on the basis of morphological characters (Mackerras 1965a, 1965b, 1965c, 1966a, 1966b, 1967a, 1967b, 1968a, 1968b; Rentz 2014) and DNA Barcoding has not been employed to investigate the diversity of Polyzosteriinae. In this paper, *Laevifacies quadrialata* gen. et sp. nov. is described from China and the sexual dimorphism is revealed via DNA barcoding. We also record a specimen from Guangxi, thus proving that *Melanozosteria nitida* is also distributed in Mainland China. A key to the known Polyzosteriinae species from China is provided.

# Materials and methods

#### Morphological study

Morphological terminology used in this paper mainly follows McKittrick (1964), Mackerras (1965a) and Roth (2003). Measurements are based on specimens examined. Genital segments of the examined specimens were macerated in 10% NaOH for 20 minutes and rinsed with distilled water, observed in glycerin jelly using a MOTIC K400 stereomicroscope. Photographs of the specimens were taken using a Canon<sup>®</sup> 50D plus a Canon<sup>®</sup> EF 100mm f/2.8L IS USM Macro lens combined with Helicon Focus<sup>®</sup> software. Photos of other characters were taken using a Leica<sup>®</sup> M205A stereomicroscope. All photographs mentioned above were modified in Adobe Photoshop<sup>®</sup> CS6. The type materials are deposited in the Institute of Entomology, College of Plant Protection, Southwest University, Chongqing, China.

#### DNA extraction, PCR, and sequencing

We used two cockroach specimens for COI sequencing in this study in order to resolve the sexual dimorphism. Both sequences are deposited in GenBank with the accession numbers: MK798103, MK798104 (Table 1). The extraction procedure was according to the Hipure Tissue DNA Mini Kit (Magen Biotech, Guangzhou). Fragments of COI were amplified using PCR. Primers for the amplifications are LCO1490 (5'-GGTCAACAAATCATAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAAATCA-3') (Folmer 1994). The amplification conditions were: initial denaturation at 94 °C for 3 min, followed by 35 cycles for 30 s at 94 °C, 30 s at 49 °C, and 1 min at 72 °C, with a final extension of 10 min at 72 °C.

# Sequence processing and phylogenetic analyses

A total of ten COI sequences were analyzed (two sequences of *Laevifacies* species from our study, six sequences of Blattidae, and two sequences of a mantid outgroup downloaded from GenBank) (Table 1). All COI sequences were aligned using MEGA 7.0 and adjusted visually after translation into amino acid sequences. Finally, for the phylogenetic analysis we acquired COI sequences whose lengths were 658 bp, except for *Angustonicus lifou* whose sequence was only 650 bp. The genetic divergence value was quantified based on the Kimura 2-parameter (K2P) distance model (Kimura 1980), using MEGA 7 (Kumar et al. 2016) with 1000 bootstrap replicates. Maximum Likelihood (ML) analysis was implemented in RAxML 7.3.0 (Stamatakis et al. 2008) using GTRGAMMA model with 1000 bootstrap replicates.

#### Results

#### Phylogenetic analysis based on COI

In this study, we acquired two COI sequences, whose length, excluding primers, was 658 bp each. The genetic divergence value between male and female of *Laevifacies quadrialata* sp. nov. is 0.9%; however, the interspecific K2P genetic divergence among *Laevifacies quadrialata* sp. nov. and other species ranged from 10.4 to 13.1%.

The ML phylogenetic tree (Figure 1) revealed that male and female of *Laevifacies quadrialata* sp. nov. grouped together with a high support value (MLB = 100).

#### Taxonomy

#### Subfamily Polyzosteriinae Tepper, 1893

#### Key to Species of Polyzosteriinae in China

1	Sexual dimorphism present. Body small; tegmina and hind wings vestigial in
	male; tegmina vestigial and hind wings absent in female
_	Sexes similar. Body large; tegmina vestigial and hind wings absent2
2	Terga and abdomen uniformly dark reddish brown to black
_	Margin of terga with continuous and broad yellow stripes, the middle black;
	sometimes abdomen with continuous or discontinuous yellow stripes
	Melanozosteria soror

#### Laevifacies gen. nov.

http://zoobank.org/EC93B8A9-1413-4EB1-B139-63AF641FD6E3

#### **Type species.** *Laevifacies quadrialata* sp. nov. here designated.

Generic diagnosis. Body small to medium, thinner in male, thorax slightly broader than abdomen. Surface smooth and shining. Pronotum slightly semicircular, vertex barely exposed. Male with vestigial tegmina and hind wings on mesonotum and metanotum respectively, both nearly triangular; female only with vestigial tegmina, its shape similar to that of male, without hind wings. Legs strong but short, coxae with

Polyzosteriinae Tepper, 1893: 32; Princis 1950: 170; Princis 1960: 447; Princis 1966: 561; McKittrick 1964: 66; Mackerras 1965a: 841; Rentz 2014: 121.

Tab	le	<ol> <li>Species</li> </ol>	used	in t	his	stud	y
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Fam	nily	Species	Accession number	Reference
Outgroups	Mantidae	Mantis religiosa	KM529415	Hebert et al. 2015 (Unpublished)
		Mantis religiosa	KR148854	Hebert et al. 2016
Ingroups	Blattidae	Laevifacies quadrialata sp. nov.	MK798103	
		Laevifacies quadrialata sp. nov.	MK798104	
		Periplaneta australiasiae	KX640825	Ma et al. 2017
		Shelfordella lateralis	KU684413	Cheng et al. 2016
		Neostylopyga rhombifolia	KP986425	Legendre et al. 2015
		Hebardina concinna	KF640073	Yue et al. 2014
		Methana parva	KP986422	Legendre et al. 2015
		Angustonicus lifou	KP986393	Legendre et al. 2015



**Figure 1.** Maximum likelihood (ML) tree derived from COI gene analysis with 1000 bootstrap replicates. Number above branch indicates MLB.

punctation, front femora Type  $A_2$ . Mid and hind metatarsus with strong spines, claws symmetrical. Cerci strong, short and symmetrical. Styli long and symmetrical. Supraanal plate in male short, triangular; subgenital plate broad and short, slightly quadrilateral and symmetrical. L1 divided into two parts, L3 bifurcated, one branch short, the other one long, R1 nearly claw-like and R2 large, hooked.

**Etymology.** The name *Laevifacies* is derived from two Latin words *laevis* and *facies*, referring to the smooth and shining surface of terga. The gender of *Laevifacies* is feminine.

Remarks. Based on former studies (Gutiérrez 2013, 2014; Mackerras 1965a, 1965b, 1965c, 1966a, 1966b, 1967a, 1967b, 1968a, 1968b; Rehn and Hebard 1927), the Polyzosteriinae is characterized as follows: species having semicircular pronotum, lobiform vestigial tegmina, angles of T2-T7 produced, tarsi usually short, bare or with hind and sometimes mid metatarsi spiny (Laevifacies with mid and hind metatarsi spiny, while in Mackerras (1968b), Australian species of Blattinae and Polyzosteriinae from other Blattidae with all metatarsi spiny), large pulvilli and arolia, cerci strong, short and symmetrical, L1 with hollow finger-like projection and sclerotized projection and R1 claw-like and margin with projection; thus, *Laevifacies* is placed in the subfamily Polyzosteriinae. Laevifacies has common features with Melanozosteria, Eurycotis, Leptozosteria, and Platyzosteria, such as body small to large, and shining, usually with vestigial tegmina, angles of T5-T6 acute, T6-T7 with punctation and hind metatarsus usually spiny (Gutiérrez 2013, 2014; Mackerras 1965c, 1968b). Laevifacies is similar to the Melanozosteria and Eurycotis in general appearance, but it can be distinguished from *Melanozosteria* by the following characters: 1) body thin and small in male (Figure 2A, B), while in Melanozosteria, it is broad and large (Figure 4A, B, F, G); 2) the surface of terga smooth (Figure 2A), vs. surface with punctation in *Melanozosteria* (Figure 4A, F); 3) male with vestigial tegmina and hind wings (Figure 2A), but in Melanozosteria only with vestigial tegmina or apterous (Figure 4A, F); 4) the margin of L2d smooth and posterior of L2d finger-like with more small spines (Figure 3A), while in Melanozosteria the margin strongly denticulate and posterior of L2d with acute angle (Figure 4J, M); 5) L3 bifurcated, one short and the other long (Figure 3A), however, L3 unbifurcated or bifurcated with branches of equal length in Melanozosteria (Figure 4J, M); 6) R1 fist-shaped (Figure 3C), while in *Melanozosteria* foot-shaped or finger-shaped (Figure 4K, M); and 7) R2 only with one large and long uniform structure (Figure 3C), while in Melanozosteria, two unequal forked structures present (Figure 4K, M); and it can be distinguished from *Eurycotis* by the following characters: 1) tibiae not specialized, while in *Eury*cotis, one group of which species have smooth surface, uniform black body and lateral tegmina, with highly specialized caudal tibiae; 2) R2 is hook-like, while in Eurycotis R2 is pincer-like. In addition, Eurycotis is restricted to South and North America and Cuba, while Laevifacies gen. nov. is found in East Asia. Laevifacies is similar to the Methana in the following genitalia characteristics, the margin of L2d smooth, R1 as a strongly claw-like sclerotized process, both of L1 have two structures, L1 of Methana has strong finger-like sclerotization and a membranous lobe, while *Laevifacies* has a finger-like membrane and a strongly sclerotized lobe (Figure 3A, B).

Geographical distribution. China (Hainan).



**Figure 2. A–K** *Laevifacies quadrialata* sp. nov. **A–F**, **I–K** male holotype **A** in dorsal view **B** in ventral view **C** pronotum, in dorsal view **D** head, in ventral view **E** femur, in ventral view **F** tibia, in ventral view **I** fore tarsus, in ventral view **J** mid tarsus, in ventral view **K** hind tarsus, in ventral view. **G–H** female paratype **G** in dorsal view **H** in ventral view. Scale bars: 5 mm (**A–B, G–H**); 1 mm (**C–F, I–K**).

#### Laevifacies quadrialata sp. nov.

http://zoobank.org/CB699FB7-9F08-4830-A85D-948A8A48E629

**Diagnosis.** Sexual dimorphism. Body small and black. Surface smooth and shining except last two terga with punctation. Tegmina and hind wings vestigial in male, tegmina vestigial and hind wings absent in female. Angles of T2–T7 sharp and protruded. Legs strong. Supra-anal plate short and triangular. Styli long and symmetrical.

**Description. Measurements.** Male, pronotum: length × width  $5.5-6.2 \times 7.9-8.0$  mm, overall length: 15.6-17.7 mm. Female, pronotum: length × width  $7.0-7.1 \times 10.5-10.7$  mm, overall length: 17.0-21.0 mm.

Body black, smooth, shining. Vertex and frons black. Clypeus to part of labrum brown to dark brown, maxillary palpi and labial palpi dark brown to black. Eyes black when the specimens are fresh, fading after a long time (Figure 2D). Antennae dark brown with near middle segments and tip segments milky white (Figure 2A, B). Pronotum black, surface smooth and shining (Figure 2C). Tegmina and hind wings black, terga smooth except last two terga with punctation (Figure 2A, G). Sterna and legs dark brown to black. Cerci dark brown to black, apex yellowish brown (Figure 2A, B, G, H).

Size small to medium, female larger than male. Body oval, vertex nearly unexposed (Figure 2A, B, G, H). Ocelli present, small and round (Figure 2D). Pronotum nearly semicircular, anterior margin arc-shaped, posterior margin nearly straight, posterior



**Figure 3. A–G** male genitalia features from holotype **A** left phallomere, in dorsal view **B** L1 of left phallomere, in dorsal view **C** right phallomere, in dorsal view **D** subgenital plate, in ventral view **E** supraanal plate, in dorsal view **F–G** female genitalia features from paratype **F** subgenital plate, in ventral view **G** supra-anal plate and genitalia, in dorsal view. Abbreviations: **a.a.**, anterior arch; **acc.pr.**, accessory process; **bsv.**, basivalvula; **c.a.**, central apodeme; **i.p.p.**, inner posterior process; **lat.st.IX–X**, laterosternal of the ninth-tenth segment; **L.ph.**, left phallomere; **L1–L3**, parts of left phallomere; **o.p.p.**, outer posterior process; **R.ph.**, right phallomere; **R1–R3**, parts of right phallomere; **v.I–III**, first-third valve; **v.ph.**, ventral phallomere. Scale bars: 1 mm (**A, C–G**); 0.5 mm (**B**).

angles blunt (Figure 2C). Small, vestigial tegmina and hind wings present in male, both extending to notal hind margin, only vestigial tegmina in female (Figure 2A, G); angles of T2–T7 sharp and protruded, sterna smooth and shining (Figure 2A, B, G, H). Legs strong, fore coxae with punctation; front femora Type A<sub>2</sub> (Figure 2E); mid and hind metatarsus with a row of spines; hind metatarsus fairly long with pulvillus which occupies nearly one-quarter of its length, remainder of surface with hair, claws moderately symmetrical and unspecialized (Figure 2E, F, I, K). Male: supra-anal plate short, triangular, divided into two round lobes (Figure 3E); subgenital plate broad and

short, posterior margin round; styli long and symmetrical (Figure 3D). Cerci symmetrical and strong, with indistinct segmentation, ends sharp (Figure 3E). Female: supra-anal plate with higher sclerotization (Figure 3G).

**Male genitalia.** Left phallomere consisting of three parts: L1, L2, and L3. L1 with two parts L1a and L1b, L1a with membranous finger-like projection; L1b with sclerotized projection. L2 consisting of L2d and L2v, L2d strongly sclerotized in anterior part, the posterior part with finger-like and with more small spines; L2va simple and broad, L2vb sclerotized and the posterior with a spinous projection. L3 with a simple hook, elongate to the right and bifurcated (Figure 3A, B). Right phallomere consisting of R1, R2, and R3. R1 large, claw-like, right margin with a prominent spine; R2 large, curved hook-like, the base strong and gradually becoming thinner, bent to the right; R3 large and cucullate, highly sclerotized (Figure 3C).

**Female genitalia.** The first valve (v.I) long, slightly broad and crescent-shaped, terminal membranous; the second valve (v.II) small, flaky and obscured by the v.I; the third valve (v.III) broader than v.I, terminal membranous; paraprocts (pp.) symmetrical and the middle concave; the middle of anterior arch (a.a.) concave; basivalvula (bsv.) trapezoidal (Figure 3G); inner posterior process of the laterosternal shelf (i.p.p.) divided in two parts, which are connected by hairy membrane; outer posterior process of the laterosternal shelf (o.p.p.) symmetrical, terminal with hairs (Figure 3F).

Material examined. HOLOTYPE: male, CHINA, Hainan Prov., Baisha, Yinggeling Nature Reserve, 20-VIII-2010, Guo Zheng leg. PARATYPES: 1 male, same data as holotype; 1 male, Hainan Prov., Mt. Wuzhishan, 18-21-V-2014, Shunhua Gui, Xinran Li & Jianyue Qiu leg.; 1 male, Hainan Prov., Diaoluoshan, 18-IV-2015, Lu Qiu & Qikun Bai leg. (GenBank accession number: MK798103); 2 females, Hainan Prov., Lingshui, Mt. Diaoluoshan, 22-V-2014, Jianyue Qiu, Xinran Li & Shunhua Gui leg. (GenBank accession number: MK798104).

**Etymology.** The species epithet comes from the Latin word *quadrialata* in reference to the male having four triangular vestigial wings.

**Remarks.** In our study the interspecific K2P genetic divergence among *L. quadrialata* sp. nov. and other cockroach species ranged from 10.4 to 13.1%. But the genetic divergence value between male and female of *L. quadrialata* sp. nov. is only 0.9%, so we pair them based on their similar morphology combined with this COI data. Sexual dimorphism occurs in *L. quadrialata* sp. nov.: 1) females without hind wings, but males with vestigial hind wings (Figure 2A, 2G); 2) male with narrower body, while female with broader body (Figure 2A, B, G, H).

Geographical distribution. China (Hainan)

#### Melanozosteria Stål, 1874

*Melanozosteria* Stål, 1874: 13; Kirby 1904: 129; Shelford 1909: 265; Shelford 1910: 5 (as synonym of *Polyzosteria*); Princis 1966: 569; Mackerras 1968a: 237 (as subgenus); Roth 2003: 167; Rentz 2014: 151.

- *Cutilia* Stål, 1877: 36; Kirby 1904: 134; Shelford 1909: 289; Shelford 1910: 7; Hanitsch 1915: 99; Princis 1949: 10.
- Symtomaptera Tepper, 1893: 106 (as a subgenus of Periplaneta); Kirby 1904: 129; Shelford 1909: 265 (as a synonym of Polyzosteria); Princis 1949: 10 (as a synonym of Melanozosteria).

#### Melanozosteria nitida Brunner von Wattenwyl, 1865

**Diagnosis.** Body broad oval and reddish brown to black. Pronotum slightly arched, surface with punctation. Vestigial tegmina sectorial with punctation, separated from mesonotum for nearly whole length, hind wings absent. Surface with punctation. Angles of T2–T7 protruded and sharp. The medial aspects to the styli with stubby and sharp spines.

**Redescription.** Measurements. Male, pronotum: length  $\times$  width 7.4  $\times$  12.5 mm, overall length: 26.1 mm.

Body uniformly deep reddish brown to black (Figure 4A, B, F, G). Eyes and ocelli yellowish white. Margin of clypeus and labrum dark brown. Vertex and frons black. Antennae brown or black, middle joints creamy-white (Figure 4D). Pronotum, tegmina, abdomen, legs and cerci all uniformly deep reddish brown to black (Figure 4C).

Body large, broad oval and convex, surface shining. Pronotum slightly arched, surface with punctation. Anterior margin of pronotum roundly protruded, and posterior margin straight (Figure 4C). Tegmina vestigial, sectorial, and separated from mesonotum, surface with punctation. Angles of metanotum protruded. Hind wings absent. Surface of all terga shining and with punctation; angles of T2–T7 protruded and sharp, T9 not protruded (Figure 4A, F). Legs short and thick. Fore coxae with slightly punctation; front femora Type  $A_2$  (anterior with two long spines, posterior with many small and slightly equal spines). Tibiae hair-brushes; hind tibiae with a row of spines, hind metatarsus with pulvillus occupying one-quarter to one-third of its length, remainder of ventral surface with spines (Figure 4E). All pulvilli large, claws symmetrical (Figure 4E). Supra-anal plate long, symmetrical and quadrilateral, side edge at gradient, angles of posterior round, the middle of posterior margin concave and with hair. Cerci thick, with blurry segmentation and the terminal segment spinous distally (Figure 4F, G, L). Subgenital plate nearly quadrilateral, short. The medial aspects to the styli with stubby and sharp spines (Figure 4I).

**Male genitalia.** Left phallomere includes L1, L2, and L3. L1 with three parts (a, b, c). L1a slightly sclerotized, posterior not sclerotized, membranous and blunt. L1b more sclerotized and posterior sharp. L1c anterior slightly sclerotized and posterior blunt membrane. L2 includes L2d and L2v. L2d with a well-sclerotized, strongly denticulate in anterior margin, while the posterior of the sclerite becomes more delicate and ends in a sharp point; L2v usually single, L3 is a simple hook, but the posterior divides into two small forks which resemble an elephant's nose (Figure 4J, 4M). Right phallomere includes R1, R2, and R3. R1 large, elongate, foot-like with broad down-



Figure 4. A–E, I–L *Melanozosteria nitida* from Guangxi, male A in dorsal view B in ventral view C pronotum, in dorsal view D head, in ventral view E tarsus, in ventral view F–H Lectotype of *Melanozosteria nitida*, male F in dorsal view G in ventral view H labels I subgenital plate, in ventral view J left phallomere, in dorsal view K right phallomere, in dorsal view L supra-anal plate, in dorsal view M genitalia of *Melanozosteria nitida* in Mackerras (1968a) F–H provided by H. Bruckner, Natural History Museum Vienna, NOaS Image Collection. Scale bars: 10 mm (A–B, F–G); 1 mm (C–E, I–M).

turned "thumb" and 5–6 strongly denticulate on medial edge, R2a long, fairly broad, tapering slightly towards medial corner; R2b shorter, more strongly sclerotized and tapering to long narrow elongation. R3 with structure of folded sclerite (Figure 4K, 4M).

Materials examined. 1 male, CHINA, Guangxi Prov., Shangsi, Nadang, 15-XI-1958, Dexiang Gu & Jinting Liang leg.

**Type specimen examined.** Lectotype of *Polyzosteria nitida*, male, Ternate (Natural History Museum Vienna), "Ternate Jeynalle CoII. Br. V. W.", "LECTOTYPE", "LECTOTYPE of *Polyzosteria nitida* Brunn. Selected by KHL Key, 1963."; holotype of *Periplaneta polita*, male, Taiwan (Natural History Museum), "Holotype", "*Periplaneta polita* Walker", "BMNH (E) #878036", presented by Beccaloni (2014).

**Remarks.** We compared the lectotype of *M. nitida* (from Ternate, Indonesia) with the specimen from Guangxi and found there are minor differences between them: the styli are straight in the Guangxi individual (Figure 4I), but in the lectotype of *M. nitida*, slightly bent (Figure 4F, G). We also compared the genitalia between the Guangxi individual and the illustration in Mackerras (1968a); they share the typical characters of L1b spinous projection and serration along the margin of L2d, but they are also different in the following characteristics: 1) the terminal of L3 divided into two small forks, which resemble an elephant's nose in the Guangxi individual (Figure 4]), while in the Mackerras (1968a) individual, L3 has one blunt hook (Figure 4M); 2) L2v broad and sclerotized, and posterior of L3 membranous in the Guangxi individual (Figure 4]), while L2v thin, long and with sharp sclerotized terminus in the Mackerras (1968a) individual (Figure 4M). And the variation of supra-anal plate between samples from Queensland and New Guinea were treated as intraspecific differences in different locations (Mackerras 1968a). Considering Mackerras (1968a) also recorded that the *M. nitida* is a widely distributed tropical species from Taiwan, Malaya, Moluccas, and Philippines, and due to our specimens being inadequate, the minor difference in the Guangxi individual and the lectotype of *M. nitida* are temporarily considered as the intraspecific differences of different populations.

**Geographical distribution.** Australia, Philippines, Malaysia, New Guinea, New Caledonia, New Zealand, China, Thailand.

# Discussion

Almost all members in the Polyzosteriinae are brachypterous or apterous (excepting the tribe Methanini), and display high developmental stochasticity (Rentz 2014). The Australian Polyzosteriinae exhibit the best examples of aposematic coloration. They are often being metallically colored, or spotted and barred with bright orange, red, or yellow markings (Rentz 1996; Roach and Rentz 1998). When disturbed, they may first display a warning signal before resorting to defensive measures (Bell et al. 2007). However, *Laevifacies quadrialata* sp. nov. did not attract our attention due to their bland appearance and life in a hidden habitat (usually hidden in bushes, Lu Qiu, pers. obs.), even with sexual dimorphism. Sexual dimorphism is very common in cockroaches, some of which beinng

so extreme that it is a challenge for taxonomists to match the two sexes (Roth 1992). In this study, sexual dimorphism is revealed for the first time in Polyzosteriinae on the basis of COI data, and exhibits mainly in the body size and the vestigial hind wings.

#### Acknowledgements

We thank Dr Guo Zheng (Shenyang Normal University, China), Dr Shunhua Gui, Dr Xinran Li, Dr Lu Qiu, Dr Jianyue Qiu, and Mr Qikun Bai (all SWU) for collecting the valuable specimens. We also thank Harald Bruckner (Natural History Museum Vienna) for providing us with lectotype photos of *M. nitida*. And we are grateful to Prof John Richard Schrock (Department of Biological Sciences, Emporia State University) for revising the manuscript before submission. Sonia Lopes (Federal University of Rio de Janeiro, Brazil) and one anonymous reviewer are acknowledged for their valuable comments and suggestions to the manuscript. This study is supported by the National Natural Sciences Foundation of China (No. 31772506), the Program of the Ministry of Science and Technology of the People's Republic of China (2015FY210300) and the Fundamental Research Funds for the Central Universities (XDJK2017B032).

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RESEARCH ARTICLE



# Description of larva and pupa of *Laena haigouica* (Tenebrionidae, Laenini) from China based on morphology and four DNA makers

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Academic editor: W. Schawaller   Received 2 December 2018   Accepted 11 April 2019   Published 5 June 2019
http://zoobank.org/DAD8CDBE-BDBD-4BE4-B6FA-23F0D7E028B4

**Citation:** Wei Z, Ren G (2019) Description of larva and pupa of *Laena haigouica* (Tenebrionidae, Laenini) from China based on morphology and four DNA makers. ZooKeys 852: 101–109. https://doi.org/10.3897/zookeys.852.32095

#### Abstract

The larvae, pupae, and adults of *Laena haigouica* Schawaller, 2001 were collected during recent fieldwork in the Sichuan Province (China). Since the pupal morphology of *Laena* has never been investigated this created an opportunity to provide the first description. Moreover, prior to this study larval characters of only one species, *Laena starcki* Reitter, 1887, were known. Therefore, description of the larva of *L. haigouica* enabled the first verification of the intrageneric stability of larval characters revealed for other *Laena* species. Association of the studied immature stages with the adults was confirmed by analysing COI sequences. Additionally, three other loci (16S, Cytb, 28S) were sequenced for *L. haigouica* during this study.

#### **Keywords**

DNA markers, Laena, larva, pupa, darkling beetle, systematics

# Introduction

The genus *Laena* contains approximately 261 species in Palaearctic region (Schawaller 2008). The larva of *Laena starcki* Reitter, 1887 was first described by Byzova (1958). Watt (1974) stated that he examined the larvae of *Laena viennensis* Sturm, 1807 from

Slovenia, but he did not provide a detailed description. In that paper, Watt also corrected the mistaken description of Byzova, which was that the antennae of Laena larvae have two segments not three segments. However, the authors of this study demonstrated that antennae of Laena larvae with three segments (Fig. 2D). Laena larvae have a body shape of the tenebrionid type, like wireworms. Knowledge of their morphology, development and habits is very scarce. Doyen (1988) estimated that there are approximately 240 genera and 300 species of tenebrionid larvae described. Recently, many pupae of tenebrionid beetles were described (Steiner 1995; Bouchard and Steiner 2004; Iwan and Schimrosczyk 2008; Purchart and Nabozhenko 2012; Wagner and Gosik 2016; Kamiński et al. 2018). Kamiński et al. (2018) summarized the known data on pupae of the 'Opatrinoid' clade and provided a checklist. However, few pupa of Lagriinae have been described, including Lagria villosa Fabricius, 1781 described by Spilman (1978) and pupa of Centorus procerus moldaviensis Reitter, 1920 described by Cherney (2005). Of the Laena species, only one species has larval stages described, and no pupal stages described. The morphology of larvae of the genus Laena has been barely dealt with, and only two species have been utilized for phylogenetic studies (Aalbu et al. 2017).

Recently, the larvae, pupae, and adults of *Laena haigouica* Schawaller, 2001 were collected during fieldwork in damp deadwood in Sichuan Province of China. Therefore we speculated that the larvae of *L. haigouica* were feeding on deadwood or fungi. The larva and pupa are described, photographed, and figured for the first time in this paper.

# Materials and methods

Larvae, pupae and adults of *Laena haigouica* were collected on 27 July 2016 from Zhongcha rangeland, alt. 2870 m, Jiuzhaigou County (Sichuan Province, China) by Xiumin Li, Xinglong Bai, Xianlei Shao and Runyang Zhang. All examined specimens were preserved in 70% alcohol and deposited in the Museum of Hebei University, Baoding, China.

Larvae were observed and described using Nikon SMZ800. Photographs of larvae and pupae were taken with a desktop SEM Hitachi TM3000 and Leica M205A stereomicroscope equipped with a drawing tube, and a Leica DFC450 camera.

Total genomic DNA was extracted from larval, pupal, and adult tissue using EZNA Insect DNA Kit (Omega Bio-tek, USA), following manufacturer's protocols. One fragment of the mitochondrial protein-coding gene (COI) was amplified respectively from larva, pupa, and an adult; one fragment of the mitochondrial protein-coding gene (Cytb) was amplified from an adult; one fragment of the mitochondrial ribosomal RNA gene (16S) was amplified from an adult; and one fragment of nuclear rRNA gene (28S) was amplified from an adult. The detailed methods of the molecular studies are the same as those used in Li et al. (2018). Sequences were aligned using the ClustalW algorithm (Thompson et al. 1994) as implemented in BioEdit 7.0.9.0. (Hall 1999).

#### Taxonomy

#### Laena haigouica Schawaller, 2001

Laena haigouica Schawaller, 2001: 19–20, figs 41–44. Type locality: China, Sichuan, Jiuzhaigou.

**Specimens examined.** Larvae (11 ex), pupae (2 ex), adults  $(437^{\circ})$ , Sichuan, Jiuzhaigou, Zhongcha rangeland, 2870 m, 27.VII.2016, Xiumin Li, Xinglong Bai, Xianlei Shao & Runyang Zhang leg., HBUM.

Larva. *Diagnosis.* The larva of *L. haigouica* Schawaller, 2001 can be separated from *L. starcki* Reitter, 1887 by following characters: clypeus transverse, 3.3 times wider than long, surface with four long erect setae; abdominal spiracles on lateral margins of tergites III–VIII in middle or just before middle.

**Description.** The description is based on what is probably a later instar larva. Body length 15–17 mm. Body (Fig. 1A–C) elongate, parallel-sided, subcylindrical; setose; integument soft; white to light brown in colour; thoracic and abdominal segments subcylindrical. Abdomen without defensive glands.

Head. (Fig. 2A-B). Light brown. Weakly declined; width slightly narrower than prothorax; distinctly constricted before occipital foramen; sides rounded; punctation minute, dense, separated by 1-3 puncture diameters. Epicranial suture stem length approximately one-half head capsule length; frontal arms Y-shaped. Frons smooth. Epicranial plates light brown, smooth, with sparse short and few long erect setae; lateral portion with both short and long erect, setation denser than dorsal part; ventral portion of each plate with three long erect setae and sparse short setae. One small stemmata present on each epicranial plate, before Y-shaped frontal arms. Labrum transverse, widest in middle, surface convex, with six to seven long erect setae; anterior margin weakly protruding; lateral margins curved, contracted at apical part. Clypeus transverse, surface convex, with four long erect setae. Mandible well developed, with two small teeth on anterior inner sides. Maxillary palpi cylindrical, apex (Fig. 2E) with nine sensilla knobble. Ligula (Fig. 2C) apex with two long erect setae. Mentum longer than wide, widest in middle. Anterior margin of gula distinctly narrower than posterior margin. Antenna (Fig. 2D) short than head, tri-segmented, clavate; second segment longer than first, third segment small and rounded, prominent, surrounded by seven long erect setae.

**Thorax.** Thoracic tergites light yellow. Prothoracic tergum subquadrate, longer than wide, 1.4–1.5 times as long as meso- or metatergites; surface with short and long erect setae. Meso- and metatergites wider than long; surface with short and long erect setae. Spiracles oval; prothoracic and metathoracic without spiracle; mesothoracic spiracles on anterolateral part, largest, approximately twice size of abdominal spiracles. Metathoracic tergum distinctly wider than pro- and mesothoracic tergum. Coxal cavity distinctly separate.

Abdomen. Abdominal tergites light brown, slightly darker than sternites; surface smooth, with short and long erect setae. Abdominal tergites I-VIII wider than long,



Figure 1. Larva of Laena haigouica Schawaller, 2001 A-C habitus, in dorsal, ventral, and lateral views.

widest in middle; lateral margins of abdominal tergites curved. Abdominal tergite IX distinctly narrower than tergite VIII; anterior margin distinctly wider than posterior margin; tergite IX posteriorly round, armed with pair of acute urogomphi (Fig. 2F, J, K). Abdominal spiracles on lateral margins of tergites III–VIII in middle or just before middle.

*Legs.* Surface with long erect setae; proleg (Fig. 2G) distinctly longer, slightly thicker than meso- and metalegs (Fig. 2H–I); tarsungulus sickle-shaped, prothoracic tarsungulus more sclerotized than meso- and metathoracic tarsungulus; prothoracic trochanter shortest and thickest; posterior trochanter short and thick; prothoracic tibia curved, slightly longer and slender than meso- and metathoracic tibia.

**Pupa**. *Diagnosis*. Compared to the known pupa of *Lagria villosa* described by Spilman (1978) and pupa of *Centorus procerus moldaviensis* described by Cherney (2005) in subfamily Lagriinae, the pupa of *Laena* can be easily separated by light color, sparse setae, urogomphi 0.7 (n=2) times length of tergite IX, lateral margins of abdominal segments I– VII each with tubercle bearing long erect setae, abdominal tergites I–VIII with tubercles.

**Description.** Body length 10.5–11.2 mm, body width 2.5–2.9 mm; body white to light brown (Fig. 3A–C), with darker apices of spine on urogomphi, with black eyes, and light brown mandible apices; body with sparse long erect setae, setae yellow; abdominal tergites with developed lateral processes bearing two to three long erect setae; abdominal tergum IX with paired upturned urogomphi.



**Figure 2.** Larval morphology of *Laena haigouica* Schawaller, 2001 **A–B** head, in dorsal and ventral views **C** mouth and appendages, in ventral view **D** antenna, in lateral view **E** apex of maxillary palpus **F** segments IX, in dorsal view **G–I** pro-, meso- and metalegs, in ventral view **J–K** urogomphi in ventral and lateral views.

*Head.* Dorsal surface smooth, concealed (invisible in dorsal view). Anterior margin of labrum rounded, with sparse short setae. Clypeus with two long erect setae on each anterolateral side. Frons and vertex with sparse granules bearing long erect setae.



**Figure 3.** Pupal morphology of *Laena haigouica* Schawaller, 2001 **A–C** habitus, in dorsal, ventral and lateral views **D** tubercles of lateral margins of abdominal segments **E** setae of abdominal tergites.

Eye ovate, black. Mandible apices brown. Antenna long and thick; antennomeres IV–XI with small brown spots on apex in dorsal view, without setae.

**Thorax.** Pronotum transverse. Anterior margin straight, with eight granules bearing long erect setae; anterior angles subrectangular, posterior angles rounded; lateral margins curved, each side with seven granules bearing long erect setae. Disc surface convex, with sparse granules bearing long erect setae. Mesonotum and metanotum distinctly narrower than pronotum, each with four setae posteriorly. Mesoventrite, metaventrite and elytra glabrous.

*Abdomen.* Abdominal spiracles ovate. Tergites I–VIII each with four pairs of setae (Fig. 3E). Lateral margins of abdominal segments I–VII each with tubercle (Fig. 3D) bearing two to three long erect setae, VIII and IX each with three small tubercles each bearing long erect seta. Tergites I–VII transversely convex in middle. Tergite IX poste-

riorly rounded, with pair of apically sclerotized urogomphi bearing one to two setae at base, ventrite with setae denser than tergite. Ventrites V–VI with four long erect setae. Ventrites VII–IX with setae denser than ventrites I–VI.

*Legs.* Prolegs distinctly longer than meso- and metalegs. Femora covered with sparse erect setae. Tibiae and tarsi glabrous, without setae.

*Comment.* These two specimens probably represent early stage pupa, which have the body colour light and antenna and tarsi near translucent.

**DNA markers.** The mtDNA COI sequences respectively from the larva, pupa and adult were identical after sequence alignment. The gene fragments of 16S, 28S, COI, and Cytb are deposited in GenBank with the accession numbers MK227697, MK227698, MK227699 (pupa), MK227700 (larva), MK227701, and MK227702.

# Discussion

The morphological characteristics of larvae of subfamily Lagriinae were summarized by Hayashi (1964) and Matthews et al. (2010). Larvae of *Laena* species resemble those of *Centorus* species in subfamily Lagriinae. These larvae can be distinguished from other larvae of the subfamily Lagriinae by having three-segmented antennae (Byzova 1958; Cherney 2005). The former can be easily differentiated from the latter by following characters: (1) *Laena* species with Y-shaped frontal arms (*Centorus* species with U-shaped frontal arms); (2) *Laena* species with antennomere II distinctly longer than antennomere I, antennomere III very small and rounded (*Centorus* species with antennomere II nearly equal to antennomere I, antennomere III short and columned); (3) *Laena* species with stemmata before Y-shaped frontal arms (*Centorus* species with stemmata behind U-shaped arms); (4) abdominal tergites with tubercles each bearing a long erect seta.

The description provided above for *L. haigouica* is the first contribution to the knowledge on the pupal stages of Laenini. Pupa possess lateral processes which were considered to be plesiomorphic among the whole Tenebrionidae (Steiner 1995). The pupae of *Laena* species can be easily separated from known pupae of *Lagria* species (Spilman 1978) and *Centorus* species (Cherney 2005) in Lagriinae by having a body with sparse long setae and abdominal tergites with tubercles each bearing a long erect seta.

# Acknowledgements

We are grateful to Xiumin Li and Xinglong Bai for their collaboration in field work in Sichuan. We also thank Dr. Marcin Kamiński and anonymous reviewer for their valuable suggestions. This work was supported by the National Natural Science Foundation of China under Grant No. 31572309, the Ministry of Science and Technology of the People's Republic of China under Grant No. 2015FY210300.

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RESEARCH ARTICLE



# The genus Sternocampsus Fleutiaux, 1927 (Coleoptera, Elateridae, Oxynopterinae), with description of a new species from South China

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Academic editor: Hume Douglas   Received 13 November 2018   Accepted 24 April 2019   Pu	ıblished 5 June 2019

**Citation:** Liu Z, Jiang S-h (2019) The genus *Sternocampsus* Fleutiaux, 1927 (Coleoptera, Elateridae, Oxynopterinae), with description of a new species from South China. ZooKeys 852:111–124. https://doi.org/10.3897/zookeys.852.31611

## Abstract

*Sternocampsus coriaceus*, **sp. nov.** is described and illustrated from China. A new combination, *Campsosternus castaneus* (Jiang & Wang, 1999) is proposed. A key and a checklist of the known species, together with a distribution map of Chinese *Sternocampsus* species, are provided.

## **Keywords**

China, distribution, Elateroidea, key, new taxon, new combination, taxonomy

## Introduction

The Oriental genus *Sternocampsus* Fleutiaux, 1927 (Coleoptera: Elateridae) was established for a single species, viz. *S. villosus* Fleutiaux, 1927, from Pahang, Malaysia. Although these *Sternocampsus* are large-bodied beetles, specimens are rarely collected. After 72 years, a second species, *S. castaneus* Jiang, 1999, was discovered in Yunnan, China (Jiang and Wang 1999). However, we propose to transfer it to *Campsosternus* for reasons below.

Sternocampsus belongs to the subfamily Oxynopterinae Candèze, 1857 and morphologically resembles Oxynopterus Hope, 1842, the monotypic genus Sinuaria Jordans, 1894 and Campsosternus Latreille, 1834. Sinuaria can be easily distinguished from *Sternocampsus* by the strongly sinuate pronotal edges, strongly retracted head and non-serrate antenna. Flabellate antennae are diagnostic for *Oxynopterus*, however antennae are serrate in *Sternocampsus* and *Campsosternus*. *Sternocampsus* is also like *Campsosternus* in having a prominent mesoventrite and the male aedeagus has parameres with hook-like apices. But *Sternocampsus* differs from *Campsosternus* by the following (*Campsosternus* characteristics in parentheses): having a smaller and a somewhat retracted head (larger and less retracted); the pronotum is flat from lateral view and wider across the hind angles than the median length (often evenly convex and usually as wide as long); hind angles long and strongly divergent (short and less divergent); the prosternum is concave sublaterally (often flat or slightly concave); elytra without striae (often with striae); and the body dark without a metallic sheen (often with metallic sheen) (Latreille 1834; Fleutiaux 1927; Kishii 1987).

Here, we describe a new species of *Sternocampsus* from South China and propose a new combination (i.e., *Campsosternus castaneus* (Jiang & Wang, 1999), **comb. nov**.). The new species is illustrated along with a key to the two known species of *Sternocampsus*.

## Material and methods

Studied specimens belong to the following collections:

BMNH,	British Museum of Natural History, London, UK
MHBU,	the Museum of Hebei University, Baoding, China
MNHN,	Muséum National d'Histoire Naturelle, Paris, France
SNUC,	Insect Collection of the Shanghai Normal University, Shanghai, China
SWU,	Institute of Entomology, Southwest University, Chongqing, China
SZPT,	School of Applied Chemistry and Biological Technology, Shenzhen Polytech-
	nic, Shenzhen, Guangdong Province, China.

The terminology used mainly follows Costa et al. (2010) and Douglas (2011). The classification follows Cate et al. (2007). Observations and measurements were made under a stereomicroscope Motic SMZ-168. Photographs were made using a digital microscope (LY-WN-YH 3D system), Canon EOS-1 camera with Canon EF 100 mm, 65 mm and 55–250 mm lens.

Measurements: body length was measured along the midline from the anterior edge of the head capsule to the apex of elytra; body width was measured across the broadest part (usually across the elytra). Pronotal length was measured along the midline; the pronotal width was measured across the broadest part (usually across the hind angles).

Specimens were mounted on paper cards. The genitalia were removed, cleaned and fixed under the body of the specimen in glycerol mounts following Prosvirov and Savitsky (2011).

## Taxonomy

## Campsosternus castaneus (Jiang & Wang, 1999), comb.nov.

Figs 1a-c, 2a-h, 3a, d

Sternocampsus castaneus: Jiang and Wang 1999: 34; Jiang 1993: 136 [nomen nudum].

**Material examined.** Holotype of *C. castaneus*, China (SZPT). ♂, "Mengla (650m), Yunnan, 1982. IV.12, Jin Gentao, No. 0545".

Syntype (images) of *Campsosternus argentipilis* (Candéze, 1874) (BMNH), label 1 "type", label 2 "Siam", label 3 "*Campsosternus argentipilis* Type Cdze".

Syntype (images) of *C. saundersi* (Candéze, 1874) (BMNH), label 1 "type", label 2 "Siam Laos", label 3 "Laos Mouhot", label 4 "*Campsosternus saundersii* Cdze Type ex coll. Saunders", label 5 "*Campsosternus saundersii* type", label 6 "Jancon coll. 1903-130.", label 7 "BMNH (E) #1024842".

**Diagnostic note.** Based on the original description, this species has: body length 30.5–32.0 mm, elongate, chestnut brown integument, covered with silvery white pubescence (Fig. 1a–c). Antenna (Fig. 2c) reaching beyond apex of hind angle of pronotum by length of four apical antennomeres. Pronotum 1.2 times wider than long, slightly convex, with punctures medially, spaces between punctures 5 puncture diameters wide, nearly impunctate laterally; hind angles long, acute, divergent, with a distinct carina. Scutellar shield (Fig. 2e) 1.1 times wider than long, with punctures, spaces between punctures 5 to 8 puncture diameters wide. Elytra wider than prothorax, 3.5 times longer than prothorax; convex with micro-striae, interstrial punctures sparse and smallest of all punctures on body; interstriae flat with very shallow punctures, irregularly distributed, faintly transversally rugose. Penis (Fig. 2f) narrowed to acute with sides convex near base, straight near apex, parameres with longitudinal carina, hook-like at apex.

**Notes.** Based on examination of the types this species should be transferred from genus *Sternocampsus* to *Campsosternus* because of its convex and nearly square-shaped pronotum (anterior quarter 2/3 width of hind angles), the larger protruding head (less than half of eyes hidden by thorax in dorsal view) and the striate elytra. This species is similar to several dark colored *Campsosternus* species (cf. Fig. 3b, e: *C. argentipilis* (Candéze, 1874) and Fig. 3c, f: *C. saundersi* (Candéze, 1874)). They share several characters: prominent, large head with only slightly protruding frons, long pubescence, striate elytra. Furthermore, the green metallic sheen is somewhat present on the scutellum and medio-longitudinal area of the ventral part of *C. castaneus* (Figs 1b, 2e), shared with most *Campsosternus* spp. *Campsosternus castaneus* also differs from *C. argentipilis* by the density of the pubescence, punctures on pronotum and elytra, and body size, and is distinguished from *C. saundersi* by the shape of the pronotum, body size and ratios.

Distribution. China (Yunnan).



Figure 1. Habitus of *Campsosternus castaneus* (Jiang & Wang, 1999). comb. nov., holotype, male: **a** dorsal view **b** ventral view **c** lateral view.



**Figure 2.** *Campsosternus castaneus* (Jiang & Wang, 1999). comb. nov., holotype, male: **a** head, dorsal view **b** prothorax, ventral view **c** antennomeres 1–10, dorsal view **d** middle leg, ventral view **e** scutellar shield, dorsal view **f** aedeagus **g** abdomen, ventral view **h** elytra, dorsal view.



Figure 3. Habitus of *Campsosternus* spp. (all BMNH, photos by Dr. Yong-ying Ruan): a-c Dorsal view of *Campsosternus* spp. a *C. castaneus* (Jiang & Wang, 1999). comb. nov. b *C. argentipilis* (Candéze, 1874)
c *C. saundersi* (Candéze, 1874) d-e Lateral view of *Campsosternus* spp. d *C. castaneus* (Jiang & Wang, 1999). comb. nov. e *C. argentipilis* (Candéze, 1874) f *C. saundersi* (Candéze, 1874).

#### Sternocampsus Fleutiaux, 1927

*Sternocampsus* Fleutiaux, 1927: 104; Jiang 1993: 136; Jiang and Wang 1999: 34; Cate et al. 2007: 94.

## Type species. Sternocampsus villosus Fleutiaux, 1927, by monotypy.

**Diagnosis.** Head narrow compared to pronotum (ratio of head width between eyes to pronotum width across hind angles varied from 1/5 to 1/6). Frons flattened medially to level of labrum, frontal carina incomplete medially; mandibles arched, protruding. Antenna of both sexes, exceeding hind angles of pronotum, compressed, and serrate from 3<sup>rd</sup> to 10<sup>th</sup> antennomeres. Pronotum narrowed anteriorly, both sides distinctly sinuate, strongly flanged laterally, weakly convex medio-longitudinally; hind angles divergent, acute, apex recurved. Suture between meso- and metaventrite weak. Elytra attenuate, apically with a spine, surface almost smooth, with slightly irregular furrows posteriorly. Penis narrowed to apex, parameres with hook-like angles apically.

**Distribution.** Malaysia (Pahang), China (Guangdong, Guangxi, Hunan, Fujian) (Fig. 9).

#### Sternocampsus coriaceus Liu & Jiang, sp. nov.

http://zoobank.org/E08008CE-DB51-49F6-85C5-5929891CD826 Figs 4a–c, 5a–g, 6a–f, 7a–d, 8

**Material examined.** Holotype.  $\mathcal{E}$ , Guangdong Prov., Nanling Natural Reserve, 12.V.2001, Ming-yi Tian, No. 20180380 (SZPT). Paratypes: 1<sup>Q</sup>, Guangdong Prov., Nanling Natural Reserve, VI–VII.2001, Lei Gao, No. 20180381 (SZPT);  $2 \bigcirc 27 \textcircled{3} \textcircled{3}$ , Guangdong prov., Nanling Natural Reserve, VII-VIII,2011, Bei-kun Chen, Nos. 20180384, 20180385, 20180386, 20180387, 20180388, 20180389, 20180390, 20180391, 20180392 (SZPT); 6승승, Guangdong prov., Nanling Natural Reserve, V.2001, Ming-yi Tian, Nos. 20180393, 20180394, 20180395, 20180396, 20180397, 20180398 (SZPT); 1Å, Guangdong Prov. (light trap), Nanling Natural Reserve, 11.V. 2009, Ding Chen, No. 20180399 (SZPT); 1<sup>(2)</sup>, Guangdong prov. (light trap), Nanling Natural Reserve (1000m), 3.V.2004, Jin-cheng Zeng, No. 20180400 (SZPT); 1Å, Guangdong Prov., Nanling Natural Reserve, VI–VII.2008, Lei Gao No. 20180401 (SZPT);  $1^{\circ}_{\circ}$ , Guangdong Prov. (light trap), Nanling Natural Reserve, VII.2008, Kaixuan Chen, No. 20180402 (SZPT); 1<sup>Q</sup>, Guangdong Prov., Nanling Natural Reserve (Nanling protection station), V.2010, Chen-Hui Zhan, No. 20180405 (SWU); 3 ごう, Guangdong Prov., border between Nanling N.R. and Mangshan N.R., 5.V.2017, Jin-Kun Zhang, Nos. 20180406, 20180407, 20180408 (1 in SNUC, ex SWU; 2 in SWU);  $1 \bigcirc 1 \bigcirc 3$ , Guangxi Prov., Maoer Mts., 3.VII.2003, Min Wang, Nos. 20180382, 20180383 (SZPT); 1Å, Fujian prov., Wuyi Natural Reserve, 5–20.VII.2003, Ming Bai et Guo-dong Ren, No. 20180403 (MHBU); 13, Hunan Prov. (light trap, 1430m),



Figure 4. Habitus of *Sternocampsus coriaceus* sp. nov., holotype, male: **a** dorsal view **b** ventral view **c** lateral view.

Yizhang County, Mangshan Natural Reserve (Xiangsikeng), 2.VII.2017, Ren-Zhi Zhang (SNUC, ex SWU), No. 20180404.

**Diagnosis.** Body not-metallic. Pronotum nearly twice wider (across hind angles) than its median length, flat in dorsal view, with four shallow depressions between longitudinal and transverse middle line. Elytra widest at apical third, shiny, smooth, coriaceous-rugulose sculpture hardly visible, without striae or linear punctures, covered with short pubescence, 1/5 length of diameter antennomere 2. Penis width measured before apical attenuation 3.3 times wider than minimum width of paramere, and penis not reaching beyond parameres.

**Description. Male** (holotype). Body length 47.5 mm, width 14.5 mm. Body dark red-brown to black (Fig. 4a), nearly impunctate. Pronotum, head, ventral parts of body, antenna and legs dark brown to black, elytra dark red-brown, dark laterally, strongly shiny; dorsal pubescence orange, recumbent, and extremely short, denser on pronotum, ventral pubescence longer and denser.

*Head.* Head semi-retracted (concealing most of eyes in dorsal view in Fig. 5c), frons broadly depressed, smooth and impunctate medially, uneven with weak rugose punctures laterally, surface with sparse, short pale orange pubescence, frontal carina incomplete, only present above antennae, moderately protruding above rest of frons in lateral view. Eyes bulbous (Figs 5a, 6a). Mandible strongly incurved, nearly right angled, simple. Labrum semicircular, with dense rugose punctures laterally and long (length 1.8 times longer than diameter of antennomere 2) orange pubescence medi-



**Figure 5.** *Sternocampsus coriaceus* sp. nov., holotype, male: **a** head, dorsal view **b** head, frontal view **c** pronotum, dorsal view **d** antenna, dorsal view **e** surface of pronotum, dorsal view **f** scutellar shield, dorsal view **g** surface of elytra, dorsal view.

ally (Fig. 5a). Last segment of maxillary palpus truncate apically, triangular and 1.7 times longer than wide. Antenna (Fig. 5d) reaching beyond hind angles of pronotum, with long (1/3 length of diameter antennomere 2) orange pubescence, serrate from antennomeres 3 to 10, and gradually narrowing from antennomere 4<sup>th</sup> onward; antennomere 1 clavate, elliptically concave dorsal-subapically, and 1.7 times longer than wide; antennomere 2 shortest, rounded, slightly longer than wide; antennomere 3 2.3



**Figure 6.** *Sternocampsus coriaceus* sp. nov., holotype, male: **a** prothorax, lateral view **b** thorax, ventral view **c** abdomen, ventral view **d** prosternal process, lateral view **e** middle leg, lateral view **f** sternite VII, ventral view.



**Figure 7.** *Sternocampsus coriaceus* sp. nov., **a–c** dorsal, ventral and lateral view of aedeagus **d** bursa copulatrix and thorny plates.

times longer than antennomere 2 and 0.6 times antennomere 4; antennomeres 4 to 10 elongate triangular, attached latero-apically to preceding antennomere; antennomere 11 4.7 times longer than its maximum width, and 1.4 times longer than antennomere 1, with a constriction at apical fourth.

*Thorax.* Pronotum nearly twice wider (across hind angles) than its length, impressed on either side of midline near anterior and posterior thirds (Fig. 5c), bordered by carina at sides and posterad. Disc polished, with short orange pubescence, longer and denser along hind margin; anterior angles broadly protruding anterad near head, pronotal setae long anteriorly to half covering eyes in Fig. 5a; hind angles long, and divergent, upheaved and acute, apices curved downwards, without dorsal carina. Anterior lobe of prosternum rugose-punctate (Fig. 6b). Prosternal sutures sinuate and not bordered by raised carina on hypomeraon; prosternum shiny with small punctures, spaces between punctures 2 to 3 puncture diameters wide and sparse pubescence, length 1/2 of diameter antennomere 2, surface sulcate laterally (Figs 4b, 6a, b); prosternal process straight in lateral view, acuminate in dorsal view, area between the dorsal and ventral apices (*sensu* Douglas 2011) concave, ventral surface shorter than dorsal surface (Fig. 6b, d). Meso- and metaventrite smooth with regular small punctures, covered with thick and orange pubescence, half length of diameter antennomere 2, and suture between ventrites shallow; metaventrite narrowly furrowed medially along entire length (Fig. 6b).

*Scutellar shield.* (Fig. 5f) Flat, widest in anterior third, straight anteriorly with rounded lateral corners and posterior end, broadly concave medially in dorsal view, nearly straight on posterior sides, slightly wider than long, punctate, pubescent.

*Elytra.* 4 times longer than and slightly wider than pronotum (measured across hind angles), elongate (Figure 4a), anterior two-thirds nearly parallel-sided narrowed at posterior third, each apex with spine; shiny, smooth, with fine coriaceous-rugulose sculpture, without striae or linear punctures, covered with pubescence, which 1/5 length of diameter antennomere 2 (Fig. 5g).

*Legs* (Fig. 6e) Covered with extremely dense and regular pubescence. Tarsi with yellow-brown bristle pad underneath, tarsomeres 1 to 4 becoming sequentially shorter, tarsomeres1 nearly equal to tarsomere 5, tarsomere 5 longest, tarsomere 4 shortest. Metacoxal plate (Fig. 6b) with mesal third nearly parallel-sided, then abruptly and strongly narrowed into a narrow strip laterally.

*Abdomen.* General surface like that of metaventrite. Sternite III–VII each with paired round red-brown tubercles laterally, sternite VII emarginate basal-medially, abruptly narrowed posterad, triangular in ventral view, sinuate laterally, with weak longitudinal snowflake-like rugosity near sides anterad (Fig. 6f).

*Genitalia.* Penis width measured before apical attenuation 3.3 times wider than minimum width of paramere, and penis slightly shorter than parameres, apex abruptly and strongly narrowed, with low thorny tubercles; parameres with incision near each base in ventral view, sides nearly straight, and then strongly concave towards apex, with pre-apical acute hook-like expansion (Fig. 7a–c).

**Female.** Like male, except longer (17.0–17.5 mm) and with shorter antennae. Bursa copulatrix with four symmetrical thorny plates inside (Fig. 7d).

**Variations.** Body length: 45.5–51.0 mm (male) or 52.0–53.5 mm (female); body width: 13.5–17.0 mm (male) or 17.0–17.5 mm (female).

Larva. Unknown.

**Etymology.** The specific name "*coriaceus*" (Latin for "leather-like") refers to the coriaceous sculpture of the elytra.

**Distribution.** China: Guangdong (Nanling Natural Reserve), Guangxi (Maoer Mts.), Fujian (Wuyi Mts.), Hunan (Mangshan Natural Reserve) (Fig. 8).

**Biology.** Unknown, but collected at light traps at night. Some specimens collected at the elevation of 1430 m in subtropical forest.

**Remarks.** This species is the second species of the genus *Sternocampsus* Fleutiaux. It differs from the congener *S. villosus* Fleutiaux, 1927 by the following: smaller body (45.5–53.5 mm; 55 mm in *S. villosus*); antennomere 3 shorter than antennomere 1 (antennomere 3 longer than 1 in *S. villosus*); and pubescence of hypomeron sparser and shorter (thicker and longer in *S. villosus*). The shape and arrangement of thorny plates in female bursa copulatrix (Fig. 7d) of *S. coriaceus* differs from *Campsosternus* spp. (Hsieh et al. 2014)), which also supports diagnosis of genus *Sternocampsus* Fleutiaux.

#### Sternocampsus villosus Fleutiaux, 1927

Fig. 9a-h

Sternocampsus villosus Fleutiaux, 1927: 104.

Material examined. Syntype (images) of *C. villosus*, ∂, (MNHN), label 1 "Malay Penins:/ Pahang F.M.S./Cameron Highland/ Tanah Rata/ Febr. 12<sup>th</sup> 1926", label 2 "Musee Kua Lumpur:/Penang/Collection FLEUTIAUX", label 3 "*Sternocampsus villosus* ∂/ Fleut. Type/ Collection FLEUTIAUX", label 4 "Collection E. Fleutiaux", label 5 "TYPE", label 6 "SYNTYPE", label 7 "SYNTYPE *sternocampsus villosus* Fleutiaux, 1927", label 8 "MNHN EC9700". Syntype (images) of *C. villosus* (MNHN), label 1 "Pahang F.M.S./ "Cameron's Highlands"/?/ 4800 ft. Mars. ?. 1924/ H.M.Pendlebury.", label 2 "Musee Kuala Lumpur:/ Penang/ Collection FLEUTIAUX", label 3 "*Sternocampsus villosus*/ Fleut. cotype/ Collection FLEU-TIAUX", label 4 "Collection E. Fleutiaux", label 5 "SYNTYPE", label 6 "SYNTYPE *sternocampsus villosus* Fleutiaux, 1927", label 7 "MNHN EC9701".

**Diagnosis.** According to the original description, this species is characterized by: body length 55 mm, large and robust; elytra not metallic, red-brown; pubescence yellow and thick. Frons depressed medially. Antenna black; similar in both sexes, but somewhat longer in male than in female; antennomere 1 longer than remaining antennomeres, widened apically; antennomere 2 very small; antennomere 3 longer than 1 and shorter than 4. Pronotum smooth. Elytra more or less dark brown, almost smooth, sculpture hardly discernable. Black ventrally, pubescence of hypomeron very thick. Legs black.

**Notes.** We have checked the images of types from the MNHN taken by Dr. Antoine Mantilleri. Elytra were red-brown from the images (Fig. 9a, f), not dark



Figure 8. Distribution map of genus Sternocampsus in China. ■ S. coriaceus sp. nov.

brown as in the original description. From the image (Fig. 9d), the characters of its aedeagus (penis extending slightly beyond parameres, weakly sinuate before acute apex; the outer margin of parameres abruptly incurved before apical hook-like pre-apical expansions) differ from *S. coriaceus*.

Distribution. Malaysia (Pahang).

#### Key to species of genus Sternocampsus Fleutiaux worldwide

## Checklist of Sternocampsus species

Sternocampsus coriaceus Liu & Jiang, sp. nov. [China (Guangdong, Guangxi, Hunan, Fujian)]

Sternocampsus villosus Fleutiaux, 1927 [Malaysia (Pahang)]



**Figure 9.** *Sternocampsus villosus* Fleutiaux, 1927, **a–e** syntype, male: **a** dorsal view **b** ventral view **c** lateral view **d** aedeagus, dorsal view **e** labels of type **f–h** syntype, female: **f** dorsal view **g** ventral view **c** labels of syntype. (Copyright © Antoine Mantilleri. All rights reserved MNHN/A. Mantilleri).

## Acknowledgements

We thank Prof. Kees van Achterberg (Leiden, the Netherlands) and Dr. Hume Douglas (Agriculture and Agri-Food Canada, Canada) for their extensive comments on the manuscript. We are grateful to the collectors for their collecting efforts in the field, and to Prof. Guo-dong Ren (MHBU), Prof. Ming Bai (IOZ, CAS) and Mr. Lu Qiu (SWU) for providing specimens and Dr. Yong-ying Ruan (SZPT) and Dr. Antoine Mantilleri (MNHN) who offered images of type specimens for this study. We also thank the reviewers who critically reviewed the manuscript. Funding for this study was provided by the National Natural Science Foundation of China (#31372231, 31772511) and scientific research project of SZPT (6018K27015).

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DATA PAPER



# Dataset of occurrence and incidence of pine processionary moth in Andalusia, south Spain

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Academic editor: Christian Schmidt   Received 9 August 2018   Accepted 25 February 2019   Published 5 June 201			
http://zoobank.org/BD58FC49-AC47-489D-A009-3E8C02C3D8E6			

**Citation:** Ros-Candeira A, Pérez-Luque AJ, Suárez-Muñoz M, Bonet-García FJ, Hódar JA, Giménez de Azcárate F, Ortega-Díaz O (2019) Dataset of occurrence and incidence of pine processionary moth in Andalusia, south Spain ZooKeys 852: 125–136. https://doi.org/10.3897/zookeys.852.28567

**Resource citation:** Ros Candeira A, Pérez-Luque A J, Suárez Muñoz M, Bonet García F J, Hódar Correa J A (2018). Dataset of occurrence and incidence of pine processionary moth in Andalusia (South Spain). Version 2.4. Sierra Nevada Global Change Observatory. Andalusian Environmental Center, University of Granada, Regional Government of Andalusia. Sampling event dataset https://doi.org/10.15470/s1mxjb accessed via GBIE.org on 2019-05-30

#### Abstract

This dataset provides information about infestation caused by the pine processionary moth (*Thaumetopoea pityocampa* ([Denis & Schiffermüller], 1775)) in pure or mixed pine woodlands and plantations in Andalusia. It represents a long-term series (1993–2015) containing 81,908 records that describe the occurrence and incidence of this species. Data were collected within a monitoring programme known as COPLAS, developed by the Regional Ministry of Environment and Territorial Planning of the Andalusian Regional Government within the frame of the Plan de Lucha Integrada contra la Procesionaria del Pino (Plan for Integrated Control Against the Pine Processionary Moth).

In particular, this dataset includes 4,386 monitoring stands which, together with the campaign year, define the dataset events in Darwin Core Archive. Events are related with occurrence data which show if the species is present or absent. In turn, the event data have a measurement associated: degree of infestation.

#### **Keywords**

Degree of defoliation, forest pest, monitoring, pine plantations, pine woodlands, sampling event, southern Iberian Peninsula, *Thaumetopoea pityocampa* 

## Rationale

Monitoring programmes are conducted in numerous countries and regions affected by this forest defoliator. The detection of this species is simple, since larvae nests are easily visible on affected trees and defoliation becomes obvious at a certain level of infestation. Thus, monitoring often consists on the assignment of infestation indexes to plots based on visual observation and following a discrete scale (see "Methods").

Unfortunately, those existing time series are rarely available for the scientific community. In the case of GBIF, the volume of data regarding this species is scarce (Fig. 1). In the current context of climate change, information about forest pests becomes important since pests can play a fundamental role affecting the physiology of forest ecosystems (Gracia et al. 2005). This data paper, therefore, constitutes an initial step towards the sharing of such time series, aiming to encourage studies using them, especially in relation to management decisions regarding forests phytosanitary status and ecological studies about population dynamics, as well as other research areas.

## Taxonomic coverage and ecological importance

The whole dataset includes 81,908 records that describe the occurrence of a single species: *Thaumetopoea pityocampa* ([Denis & Schiffermüller], 1775).

The pine processionary moth is a well-known species, receiving a great attention by the scientific community, from medicine to ecology. In October 2017, Web of Science referred 400 publications mentioning this species, showing an increasing tendency in the last decades (Fig. 2; see Roques (2016) for a recent review on the taxonomy and biology of the genus). Due to its defoliating activity and to the allergic reactions it causes on animals and humans, scientists have studied this species in detail for a long time. Substantial efforts have been made to understand the life cycle, population dynamics,



**Figure 1.** Distribution of *Thaumetopoea pityocampa* records from GBIF in Spain and records provided in this dataset. Records from GBIF were downloaded on 2018-03-16 using the R package "rgbif" (Chamberlain et al. 2016).

and main factors affecting this species, as well as the mechanisms involved in the urticating reaction caused by larval hairs. Moreover, control measures and management of affected forests are also important research lines.

In Andalusia, Thaumetopoea pityocampa is one of the species that causes the most extensive impact to the pine forests, either natural or planted. The typical distribution area of this species is conditioned by climate and associated with Mediterranean and circum-Mediterranean regions (Battisti et al. 2015), mainly feeding on the genus Pinus. This distribution is explained to a big extent by the minimum winter temperatures as the larval stage takes place during winter (Buffo et al. 2007; Démolin 1969; Hoch et al. 2009). Therefore, increasing winter temperatures favour this species and climate change is thus expected to increase the potential distribution of the species. Because of this, it has become a paradigmatic case study regarding the response of forest pests to climatic change (Netherer and Schopf 2010). Indeed, reports already show presence, outbreaks and potential shifts of the species at higher altitudes and latitudes than before (Battisti et al. 2005; Pimentel et al. 2011). It should be noted that Thaumetopoea pityocampa is not an alien or invasive species in Andalusia, being both the moth and the host pine species autochthonous from the region. However, the combination of climate warming and man-made pine spreading has resulted in a very favourable situation for its expansion in its full distribution area, thus causing its usual consideration as a pest (e.g., EPPO 2004).



**Figure 2.** Number of publications per year about *Thaumetopoea pityocampa* in Web of Science (search date 2017-10-05) since the first publication registered.

#### **Taxonomic ranks**

Kingdom: Animalia Phylum: Arthropoda Class: Insecta Order: Lepidoptera Family: Notodontidae Genus: Thaumetopoea Species: *Thaumetopoea pityocampa* ([Denis & Schiffermüller], 1775) Common Name: pine processionary moth

#### Spatial coverage

## General spatial coverage

Andalusia is located in Southern Spain and covers around  $87,597 \text{ km}^2$ . This is a region characterised by great climate variability. Though the majority of the surface is

classified as Mediterranean climate type (Csa, according to Köppen's classification) (AEMET 2011), there are other bioclimatic zones: subtropical (Mediterranean coast), oceanic (Atlantic coast), mountain (medium and high mountain areas in mountain ranges which reach 2000 m a.s.l.), subcontinental (Guadalquivir Valley and part of oriental Andalusia) and sub-desert (Southeast zone with coastal influence) (Junta de Andalucía 2014). The altitude ranges from sea level to Sierra Nevada summits, where the highest peak reaches 3481 m a.s.l.

The forest area, and specifically coniferous formations which encompass pine forests, has increased intensely during the second half of the 20<sup>th</sup> century. Due to past reforestation projects (Gutiérrez-Hernández et al. 2016), its area has doubled in 51 years (1956–2007) (Muñoz-Rojas et al. 2011). The reasoning in these reforestation was their commercial value and the general economic interest underlying the National Afforestation Plan of the 40s (Junta de Andalucía 2011) and, in eastern Andalusia, the need to control soil erosion. This means that a high percentage of the pine woodlands in Andalusia are or were originally plantations. Muñoz-Rojas et al. (2011) in their research on land cover changes in Andalusia, highlight afforestation as the second major change because of the extension of the transformed area. The evolution of coniferous formations through time in thousands hectares has been as follows: in 1956 they covered 374.6, while in 1989 it was a surface of 764.1, in 1999 around 684.5 and in 2003 a total of 683.7 (Junta de Andalucía 2010).

In this scenario, *Thaumetopoea pityocampa* has found a large surface for its activity producing an impact on forests because of defoliation.

## Coordinates

36°2'35.53"N, 38°37'12.03"N Latitude; 7°26'8.72"W, 1°52'27.71"W Longitude

Temporal coverage 1993–2015 Project details Project title

Plan de Lucha Integrada contra la Procesionaria del Pino (Plan for Integrated Control Against the Pine Processionary Moth)

## Study area description

The target ecosystem of the project is the great majority of Andalusian pure or mixed pine woodlands and plantations (Junta de Andalucía 2013). As noted above, as a result of intense reforestations in past decades the pine forest presence in Andalusia is exten-

sive. The most common species are *Pinus pinea* L., *P. nigra* J.F. Arnold, *P. pinaster* Ait., *P. halepensis* Mill., *P. sylvestris* L., and occasionally *P. sylvestris* subsp. *nevadensis* Christ (Junta de Andalucía 2010). In particular, the surface area covered by the monitoring stands included in this dataset is 7,717.6 km<sup>2</sup>. It should be noted, however, that the present data only include forested areas. Pine processionary moth is also present in non-forested areas with occasional presence of isolated pines (gardens, roadsides, roundabouts), but for reasons of its scant surface they are not included in the monitoring programme.

#### **Design description**

Following European and national regulations regarding forest management and use of phytosanitary products, the Regional Ministry of Environment and Territorial Planning of Andalusian Regional Government implemented the Plan for Integrated Control Against the Pine Processionary Moth (hereafter referred as Plan), which began in 1991. This Plan came into place aiming to assess the evolution of this pest and defining preventive and control measures. As part of that, COPLAS monitoring programme was developed. It consisted of assessing annual defoliation caused by this species on pines and counting of nests through human observation. A survey system was designed to store the generated information, which is collected in a form for each monitoring stand. Within the plan, an important step after assessing the level of damage consists of issuing a proposal for actions or treatments and execute those control measures. According to the incidence of this species, the Plan considers different treatments to maintain the pine processionary moth population below a certain threshold, for example, from manual treatment of the nests or pheromone traps to spray treatments (Junta de Andalucía 2013).

The Plan was designed mainly from a preventive point of view, with the aim of controlling the population of the pest, but contemplating its dynamic character, incorporating large surfaces and new techniques over time. For instance, aerial spraying, a common procedure initiated a few years ago to reduce defoliation impact, is now almost completely forbidden according to EU guidelines (Directive 2019/128/EU). Treatments are, at the present moment, restricted to specific areas in which the pine processionary moth may have a direct impact on human or livestock.

## **Methods**

## Sampling description

For the monitoring programme COPLAS, pine forests were divided into monitoring stands according to administrative and environmental criteria defined in the Plan. Every year, these stands were visited at the end of the defoliating season (from end of winter to beginning of spring) and a defoliation degree was assigned to the plot based on observation of the stand as a whole. The result was the production of a scale ranging from 0 to 5 which represents the degree of infestation by the pine processionary moth:

- Degree 0: None or some very scattered nests are observed through the stand.
- Degree 1: Some nests are observed at the stand edges, in clear areas as well as isolated trees.
- Degree 2: Numerous nests at the edges of the stand, in clear areas and some in the middle of the stand.
- Degree 3: Partial defoliation at the stand edges and isolated trees. Abundant nests in the middle of the stand.
- Degree 4: Very strong defoliations oliations at the stand edges as well as isolated trees and partial defoliations in the rest of the stand.
- Degree 5: Very strong defoliations throughout the stand.

Since this defoliation assessment was used to define further management measures, this initial assessment could be checked and further adjusted by a technician when plots were assigned a degree equal or higher than 3. Plots assigned with a degree of 2 were also checked if they were next to plots assigned with a degree of 3.

Every year, the Plan increased the area covered by the monitoring stands (Junta de Andalucía 2010), which are distributed throughout all the provinces of Andalusia.

## Step description

All data were stored in a normalised database (PostgreSQL) and incorporated into the Information System of Sierra Nevada Global-Change Observatory (Pérez-Pérez et al. 2012). Taxonomic and spatial validations were made on this database (see Quality control description). A custom-made SQL view of the database was performed to gather occurrence data associated to sampling event and other variables associated with occurrence data, specifically, degree of infestation.

The sampling event data, occurrence, and measurement data were accommodated to fulfil the Darwin Core Standard (Wieczorek et al. 2009; 2012). We used Darwin Core Archive Validator tool (http://tools.gbif.org/dwca-validator/) to check whether the dataset meets Darwin Core specifications. The Integrated Publishing Toolkit (IPT v2.0.5) (Robertson et al. 2014) of the Spanish node of the Global Biodiversity Information Facility (GBIF) (http://ipt.gbif.es) was used both to upload the Darwin Core Archive and to fill out the metadata.

The Darwin Core elements for the sampling event data included in the dataset are: eventID, modified, language, institutionCode, collectionCode, continent, country, countryCode, stateProvince, county, eventDate, habitat, minimumElevationIn-Meters, maximumElevationInMeters, decimalLatitude, decimalLongitude, geodetic-Datum, coordinateUncertaintyInMeters, samplingProtocol, sampleSizeValue, sample-SizeUnit, footprintWKT. For the occurrence data the elements are: occurrenceID,



**Figure 3.** Number of monitoring stands per year according to defoliation degree. Gray area represents the total number of monitored stands per year.

catalogNumber, eventID, eventDate, basisOfRecord, scientificName, kingdom, phylum, class, order, family, genus, specificEpithet, scientificNameAuthorship, associatedTaxa, recordedBy, occurrenceStatus. For the measurement data, the Darwin Core elements included were: measurementID, eventID, measurementType, measurement-Value, measurementUnit, measurementDeterminedBy, measurementDetermined-Date, measurementMethod.

#### Quality control description

The scientific names were checked with databases of Catalogue of Life/Species 2000 (Roskov et al. 2018) and a recent review on the phylogeny of the genus *Thaumetopoea* (Basso et al. 2017). We also performed validation procedures (Chapman 2005a; 2005b) (geographic coordinate format, coordinates within provincial/county boundaries, absence of ASCII anomalous characters in the dataset) with Darwin Test (3.3) software (Pando et al. 2017).

## Dataset description

Object name: Darwin Core Archive COPLAS: Dataset of occurrence and incidence of pine processionary moth in Andalusia (South Spain) Character encoding: UTF-8 Format name: Darwin Core Archive Format (Wieczorek et al. 2009) Format version: 1.0 Distribution: http://ipt.gbif.es/resource?r=coplas Publication date of data: 2018-04-20 Language: English Licenses of use: this dataset is licensed under the Creative Commons Attribution 4.0 International License (CC BY 4.0) https://creativecommons.org/licenses/by/4.0/ Metadata language: English Date of metadata creation: 2018-04-20 Hierarchy level: Dataset

## Acknowledgments

We are especially grateful to all the forest rangers that annually collect the raw data for the monitoring programme. Many thanks to the managers involved in COPLAS as Sixto Rodríguez Reviriego and Ángel Carrasco Gotarredona. We also thank Katia Cezón (Spanish GBIF node-CSIC) for technical support and the reviewer Alberto Zilli for his insightful comments. This work has been carried out under the conceptual framework and cooperative spirit of the Sierra Nevada Global Change Observatory and it was supported by the H2020 project "ECOPOTENTIAL: Improving future ecosystem benefits through earth observations" (http://www.ecopotential-project.eu/), which has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 641762. Thanks are due to the projects that fund the research with the following contracts: A. J. Pérez-Luque has a contract within the project LIFE-ADAPTAMED (LIFE14 CCA/ES/000612): "Protection of key ecosystem services by adaptive management of Climate Change endangered Mediterranean socioecosystems" and A. Ros Candeira has a contract within the National Youth Guarantee System and the operational programme "Youth Employment" financed by the European Social Fund.

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RESEARCH ARTICLE



# A new giant Pristimantis (Anura, Craugastoridae) from the paramos of the Podocarpus National Park, southern Ecuador

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Academic editor: A. Crottini | Received 19 February 2018 | Accepted 9 April 2019 | Published 5 June 2019 http://zoobank.org/B2327E50-35B8-4663-A5ED-07B07740BEB5

**Citation:** Yánez-Muñoz MH, Veintimilla-Yánez D, Batallas D, Cisneros-Heredia DF (2019) A new giant *Pristimantis* (Anura, Craugastoridae) from the paramos of the Podocarpus National Park, southern Ecuador. ZooKeys 852: 137–156. https://doi.org/10.3897/zooKeys.852.24557

## Abstract

A new species of frog of the genus *Pristimantis* is described from the paramos of the Nudo de Cajanuma, Podocarpus National Park, on the border between the provinces of Loja and Zamora-Chinchipe, Ecuador. The new species is readily distinguished from all other species of *Pristimantis* by its large body size (snoutvent length: 50.0–50.5 mm in adult females, 34.7–42.5 mm in adult males), thick glandular skin, large warts on flanks, prominent glandular patches on head and legs, and dark brown dorsum. This new species is among the largest and stoutest *Pristimantis* frogs of the high Andes. It is only known from its type locality, where it occurs in paramo bambusoid meadows at elevations between 3300 and 3400 m. It is morphologically similar to *Pristimantis erythros, P. farisorum, P. obmutescens, P. orcesi, P. racemus, P. simoterus, P. simoteriscus*, and *P. thymelensis*. Notorious morphological characters present in this new species are thick glandular patches covering dorsum and limbs and porous skin texture, which are shared with *P. erythros*.

#### Resumen

Describimos una nueva especie de rana del género *Pristimantis* de los páramos del Nudo de Cajanuma, Parque Nacional Podocarpus, en el límite entre las provincias de Loja y Zamora-Chinchipe. La nueva especie se diferencia de otras especies de *Pristimantis* por su gran tamaño corporal (longitud rostro-cloacal: 50,0–50,5 mm en hembras adultas, 34,7–42,5 mm en machos adultos), piel glandular y gruesa, verrugas grandes en los flancos del cuerpo, prominentes parches glandulares en la cabeza y patas, y dorso café oscuro. Esta nueva especie está entre las ranas *Pristimantis* más grandes y fornidas de los altos Andes. Solo se conoce de su localidad tipo, donde habita en herbazales bambusoides de páramo a elevaciones entre 3300 y 3400 m. Es morfológicamente similar a *Pristimantis erythros, P. farisorum, P. obmutescens, P. orcesi, P. racemus, P. simoterus, P. simoteriscus, y P. thymelensis.* Características morfológicas notorias en esta nueva especie son los parches glandulares gruesos que cubren el dorso y las patas y la textura de la piel porosa, las cuales son compartidas con *P. erythros.* 

#### **Keywords**

Amphibia, Andes, Cajanuma, Craugastoridae, Loja, new species, paramo, *Pristimantis*, taxonomy, Zamora-Chinchipe

#### Palabras clave

Amphibia, Andes, Cajanuma, Craugastoridae, Loja, nueva especie, páramo, *Pristimantis*, taxonomía, Zamora-Chinchipe

## Introduction

Pristimantis (Jiménez de la Espada 1870) is the most diverse amphibian genus, representing nearly 8% (532 spp.) of all named anuran species worldwide (Frost 2019). Endemic to tropical America, Pristimantis is distributed from Honduras to Argentina, and is the most-species rich genus in anuran communities of the tropical Andes, from Colombia to Bolivia. Frogs of the genus Pristimantis show vast morphological and ecological diversity, which is associated with a complex and challenging taxonomy (Duellman and Lehr 2009, Pinto-Sánchez et al. 2012, Padial et al. 2014). Ecuador holds 39% of all Pristimantis species (210 spp., Ron et al. 2019), but its diversity is still underestimated, and 61 species have been discovered and scientifically described in the country during the last decade (Ron et al. 2019). Pristimantis from the Andes of southern Ecuador are little known; with new species frequently discovered, new distributional records revealed, collections of putatively new species deposited in museum collections, and several areas unexplored (Bustamante and Mendelson III 2008, Cisneros-Heredia et al. 2009, Reyes-Puig et al. 2010, 2010, 2014, 2015, Yánez-Muñoz et al. 2010, 2010, 2010, 2012, 2016, 2016, 2016, Camacho-Badani et al. 2012, Reyes-Puig and Yánez-Muñoz 2012, Brito and Pozo-Zamora 2013, Urgilés et al. 2014, 2014, 2014, Brito et al. 2017, Urgiles et al. 2017, Sánchez-Nivicela et al. 2018, Reves-Puig et al. 2019).

The Podocarpus National Park is located on the southernmost portion of the Cordillera Oriental of the Andes, in the provinces of Loja and Zamora-Chinchipe, southern Ecuador. It protects about 1450 km<sup>2</sup> from 900 to 3600 m elevation, including foothill, low montane, cloud, high montane forests and paramos (MAE 2017). Little

information exists about the herpetofauna of the highlands of Podocarpus National Park. Between 2009 and 2010, herpetological surveys were conducted on the paramos of Cajanuma, western side of the Podocarpus National Park, from 3320 to 3365 m elevation, as part of a project to evaluate the impacts of climate change on the biodiversity of this ecosystem (Salinas Salinas and Veintimilla-Yánez 2010, L Aguirre Mendoza et al. 2015). During these surveys, three putatively new species of *Pristimantis* were collected. Herein, we are pleased to describe and name one of these species.

## Materials and methods

Field work was carried out between December 2009 and April 2010 in the paramos of the Nudo de Cajanuma (nudo is the local name for transverse mountain ranges), Podocarpus National Park, on the border between the provinces of Loja and Zamora-Chinchipe, Ecuador. Paramos are highland Neotropical ecosystems dominated by grasses and forbs and located between the forest upper limit and the permanent snow line in the Andes from Venezuela to northern Peru (Acosta-Solís 1984, Luteyn 1999). While most paramos occur above 3400 m elevation, in southern Ecuador paramos are found from 2080 m elevation due to local climate and geology (Neill 1999, León-Yánez 2011). The physiography of the paramo of Cajanuma is characterised by series of hills with steep slopes, connected by ridges, and dissected by small streams. While paramos in the Podocarpus National Park typically receive an average annual precipitation < 5000 mm, the paramo of Cajanuma is wetter and may receive up to 6000 mm. During most of the year, local weather is characterized by persistent cloud cover, fierce easterly winds, and low temperatures (maximum daily temperature  $\approx 10^{\circ}$  C, minimum typically between 0–3 °C), although a short dry season may occur during November and December (Keating 1999, 2008, Lozano et al. 2009, Aguirre et al. 2015). Vegetation is characterised by a diverse physiognomy of grasses, forbs, shrubs, and treelets. Descriptions of the flora of the paramo of Cajanuma were provided by Keating (1999, 2008) and Eguiguren et al. (2015).

Herpetological surveys were conducted at the paramo of Cajanuma across an area located at the following coordinates: 79.16219444°–79.16111111°W, 4.10861111°– 4.09466667°S, at 3320–3365 m elevation (Salinas Salinas and Veintimilla-Yánez 2010). Coordinates were obtained by means of a Garmin Handheld Navigator GPS (WGS84). Two survey techniques were used: visual encounters during evenings (19:00–22:00) and rake and hoe removal of plant rosettes during mornings (09h00–12h00) (Mueses-Cisneros and Yánez-Muñoz 2009, Heyer et al. 2014). Specimens were photographed alive, euthanised with benzocaine, fixed in 10% formalin, and preserved in 70% ethanol.

Description format, definitions and terminology follows standards proposed by Lynch and Duellman (1997) and Duellman and Lehr (2009). For skin texture, we include a new descriptor: porous skin, which is defined by showing small pores evenly distributed across the skin. Areolate, pustulate and shagreen skin textures differ from porous skin by having dermal modifications (granules, protuberances) raised from the background plane of the skin, while the pores of porous skin are below the background



**Figure 1.** Porous texture of the skin on the middorsum of *Pristimantis andinogigas* sp. nov. (DHMECN 11013, adult male, paratype).

plane (Fig. 1; compare with Duellman and Lehr 2009: fig. 39). Sex and age were determined by direct inspection of gonads. The following measurements were taken with digital calipers to the nearest 0.01 mm and rounded to the nearest 0.1 mm by a single person (David Veintimilla-Yánez): snout-vent length (SVL), straight distance from tip of snout to vent; head width, at angle of jaws; head length, from angle of jaw to tip of snout; eye diameter, horizontally from anterior to posterior corner of eye; interorbital distance, shortest distance between orbits; internarial distance, shortest distance between nostrils; eye-nostril distance, straight distance between anterior corner of eye and posterior margin of nostril; tympanum diameter, greatest horizontal width of tympanum; tibia length, distance from outer border of flexed knee to heel inflection; hand length, distance from base of tenar tubercle to tip of Finger III; and foot length, distance from base of inner metatarsal tubercle to tip of Toe IV. Fingers and toes are numbered preaxially to postaxially from I to IV and I to V, respectively. Comparative lengths of Toes III and V were determined by adpressing both against Toe IV; lengths of Fingers I and II were compared when adpressed against each other. Photographs and field notes were used for descriptions of colouration in life.

Examined specimens are deposited at: División de Herpetología, Instituto Nacional de Biodiversidad, Quito (**DHMECN**); Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá (**ICN**); Laboratorio de Herpetología, Universidad del Valle, Cali (**UVC**), and Museo de Zoología, Universidad San Francisco de Quito (**ZSFQ**). Information on species for comparative diagnoses was obtained from examined specimens and literature, including original species descriptions. The following specimens were examined for comparisons:

ECUADOR: Pristimantis erythros: Provincia del Azuay: Chanlud, 3449 m, DHMECN 12103, holotype, DHMECN 12102, paratype, ZSFQ 034–036. Pristimantis loujosti: Provincia de Tungurahua: Reserva Biológica La Candelaria, 2800 m, DHMECN 4843. Pristimantis orcesi: Provincia de Napo: Cuyuja, 3591 m, DHMECN 2903. Pristimantis thymelensis: Provincia de Carchi: Reserva Ecológica El Ángel, 3900 m, DHMECN 1867-1890; Lagunas del Voladero, 3420 m, DHMECN 2415-2419; Provincia de Napo: Papallacta, 3910 m, DHMECN 1856. COLOMBIA: Pristimantis obmutescens: Departamento del Cauca: Páramo de Puracé, alrededores de la Laguna San Rafael, ICN 2087, holotype. Pristimantis racemus: Departamento del Valle del Cauca, Tenerife, UVC 8131, 8193. Pristimantis simoteriscus: Departamento del Tolima: Municipio de Cajamarca, Páramo de los Valles, SW of Anaime, Anaime-Santa Helena road, ICN 22835, holotype. Pristimantis simoterus: Departamento del Tolima: Páramo de Letras, vereda Albania, Municipio de Herveo, ICN 759, holotype.

Vocalizations were recorded with an Olympus WS-750 digital recorder and a Senheiser K6-C unidirectional microphone. During the recordings, air temperature and relative humidity were measured with a Springfield environmental thermometer. Acoustic analyses were done with Adobe Audition 3.0 software package (Adobe Systems Inc., San Jose, California, USA), at a sampling frequency of 44.1 kHz and 16-bit resolution. Waveform and spectrogram were made using Raven Pro 1.4 software package (Cornell Lab of Ornithology, Ithaca, NY) and analysed with a Fast Fourier Transformation of 512 points. Terminology and definitions follow proposals by Duellman and Pyles (1983), Cocroft and Ryan (1995), Díaz and Cádiz (2006), and the following variables were measured: (1) dominant frequency of the call, (2) dominant frequency of the first note, (3) dominant frequency of the second note, (4) calls per minute, (5) notes per call, (6) call duration, (7) note duration, (8) interval between calls, and (9) intervals between notes within a call.

## Results

## Pristimantis andinogigas sp. nov.

http://zoobank.org/3BF7D08B-5586-4314-AC76-B40D724C1F97 Figures 1–5

*Pristimantis* grp. *orcesi*: L Aguirre Mendoza et al. 2015: 173, 180; Z Aguirre Mendoza et al. 2017: 534–535.

 Common names. English: Giant paramo rainfrog. Spanish: Cutín Gigante de Páramo. Holotype. Adult female; ECUADOR; provincia de Loja, Parque Nacional Podocarpus, Cajanuma; 4.108346°S, 79.162046°W, 3313 m alt.; 27 January 2010; David Veintimilla-Yánez and Karen Salinas leg.; DHMECN 10984 (field number DVY 057).

Paratypes. Same collection data as for holotype; DHMECN 10985-6, adult males, 09 December 2009; DHMECN 10996, adult female, and DHMECN 10991-2, adult males, 10 December 2009; DHMECN 10993-4, adult males, 06 January 2010; DHMECN 10998-9, adult males, 06 January 2010; DHMECN 11000, adult male, 07 January 2010; DHMECN 11005, adult male, 13 January 2010; DHMECN 11008, adult male, 14 January 2010; DHMECN 11010-1, adult males, 27 January 2010; DHMECN 11012-13, 11115, adult males, 31 March 2010; DHMECN 11016, adult male, 06 April 2010; subadult males: DHMECN 10997, 06 January 2010; DHMECN 11001, 07 January 2010; subadult females: DHMECN 10995, 06 January 2010; DHMECN 11002, 07 January 2010; DHMECN 11006, 13 January 2010; DHMECN 11007, 14 January 2010; DHMECN 11018, 12 April 2010; DH-MECN 11021, 22 April 2010; juveniles: DHMECN 10987-8, 10990, 09 December 2009; DHMECN 10989, 10 December 2009; DHMECN 11003-4, 13 January 2010; DHMECN 11009, 26 January 2010; DHMECN 11014, 31 March 2010; DHMECN 11017, 07 April 2010; DHMECN 11019, 14 April 2010; DHMECN 11020, 19 April 2010.

Diagnosis. A new species of Pristimantis diagnosed by the following combination of characters: (1) Skin on dorsum porous, thick and glandular, with large, flat, glandular warts on flanks; dorsolateral folds absent; thick glandular patch on supra/postympanic region, and on dorsal surfaces of humeral, femoral, tibial and tarsal regions; glandular folds in wrists; skin on venter areolate; discoidal fold weakly defined; (2) tympanic membrane and tympanic annulus prominent; tympanic annulus rounded, 36% of eye length, with posterior margin in contact with supratympatic glandular patch; (3) snout rounded in dorsal view; rounded to slightly protruding in lateral view; (4) upper eyelid without tubercles, IOD wider than upper eyelid; cranial crests absent; (5) dentigerous processes of vomers present, oblique, moderately separated, posteromedial to choanae, with 4 to 5 teeth; (6) males with cream-coloured nuptial pads on dorsum of Finger I and vocal slits; (7) Finger I shorter than Finger II; emarginated discs of fingers broadly expanded and elliptical; (8) fingers without lateral fringes; (9) ulnar tubercle present but low or poorly differentiated; (10) heels without tubercles, inner tarsal wart low and poorly differentiated; (11) inner metatarsal tubercle ovoid, about 5–6x the size of subconical, rounded outer metatarsal tubercle; supernumerary plantar tubercles present; (12) toes with narrow lateral fringes; basal toe webbing between toes II-V; Toe V longer than Toe III (disc of Toe III does not reach distal subarticular tubercle on Toe IV, disc on Toe V reaches middle of distal subarticular tubercle on Toe IV); toe discs elliptical, slightly narrower than those on fingers; (13) in life, dorsal surfaces dark brown, chocolate brown, or orange-brown, with or without dark irregular botches, distinctive head markings absent, ventral surfaces brown with irregular pale flecks and blotches, iris bronze with dense black reticulations; in preservative, brown surfaces turn grey; (14) SVL 50.0–50.5 mm in adult females (n = 2), 34.7–42.5 (38.5  $\pm$  2.1 SD, *n* = 10) mm in adult males (Table 1).

**Comparisons.** *Pristimantis andinogigas* sp. nov. is readily distinguished from all other species of *Pristimantis* by its large body size, thick and glandular skin, large warts

Characters	Females $(n = 2)$	Males ( <i>n</i> = 17)
Snout-vent length	50.0-50.5	34.7-42.5 (38.5 ± 2.1)
Head width	19.3–20.1	13.3–15. 9 (14.6 ± 0.8)
Head length	16.0–17.4	11.2–13.9 (12.7 ± 0.72)
Eye diameter	5.6–6.0	4.9–5.6 (5.3 ± 0.2)
Interorbital distance	8.2-8.8	5.5–7.9 (6.1 ± 0.6)
Internarial distance	4.4-4.6	$3.5-4.6~(4.0\pm0.3)$
Eye-nostril distance	4.9–5.3	$3.8-4.9$ ( $4.1 \pm 0.3$ )
Tympanum diameter	2.1–2.7	$1.5-2.1~(1.8\pm0.2)$
Tibia length	22.1–23.2	16.5–19.0 (17.9 ± 0.7)
Hand length	15.7–15.8	11.0–13.3 (12.0 $\pm$ 0.7)
Foot length	23.7–24.3	$16.3-20.4 (18.2 \pm 1.0)$

**Table 1.** Measurements (in mm) of type series of *Pristimantis andinogigas* sp. nov. from Nudo de Cajanuma, Podocarpus National Park, Andes of southern Ecuador. For males, range is followed by means and one standard deviation in parentheses.

on flanks, prominent macroglandular patches on head and legs, and dark brown dorsum. The only species showing a similar combination of characters is *Pristimantis erythros* Sánchez-Nivicela, Celi-Piedra, Posse-Sarmiento, Urgiles, Yánez-Muñoz & Cisneros-Heredía, 2019, which is readily differentiated from *P. andinogigas* sp. nov. by being smaller (38.8-42.6 mm in adult females), having a conspicuous red coloration, and lacking dentigerous processes of vomers. In addition, P. andinogigas sp. nov. resembles the following species by bearing large, flat, glandular warts on flanks, and expanded discs on fingers and toes: Pristimantis farisorum Mueses-Cisneros, Perdomo-Castillo, & Cepeda-Quilindo, 2013, P. obmutescens (Lynch, 1980), P. orcesi (Lynch, 1972), P. racemus (Lynch, 1980), P. simoterus (Lynch, 1980), P. simoteriscus (Lynch), and P. thymelensis (Lynch, 1972). Pristimantis andinogigas sp. nov. is larger than any of these seven species, and furthermore, they differ from P. andinogigas as follows (characters of P. andinogigas sp. nov. in parentheses): areolate or shagreen dorsal skin (porous), thin supratympanic folds (prominent supra/post-tympanic glandular patch), thin glandular patches on legs (thick), and smaller body size, with adult females having 38.4-42.3 mm SVL in P. farisorum, 28.5-38.4 mm SVL in P. obmutescens, 35.2-36.1 mm SVL in P. orcesi, 29.9-37.9 mm SVL in P. racemus, 32.4-37.1 mm SVL in P. simoterus, 25.7-31.4 mm SVL in P. simoteriscus, and 28.0-33.5 mm SVL in P. thymelensis (versus 50.0-50.5 mm SVL in adult females of *P. andinogigas*). In addition, *P. farisorum* has snout subacuminate in dorsal view (rounded), fingers with narrow lateral fringes (absent), dorsum dark brown to black with irregular and elongated orange marking (brown with or without lighter irregular blotches), and inhabits upper montane forests on the Nudo de Pasto, Andes of southern Colombia (Mueses-Cisneros et al. 2013). Pristimantis obmutescens has tympanum concealed beneath skin (visible), fingers with lateral fringes present (absent), small, non-conical tubercles on heel and outer edge of tarsus present (absent), lacks vocal sac and vocal slits in males (present), and inhabits on the Páramo de Puracé, Cordillera Central of the Andes in southern Colombia (Lynch 1980, Lynch et al. 1996). Pristimantis orcesi has skin on head smooth (porous), fingers bearing lateral fringes



**Figure 2.** Holotype of *Pristimantis andinogigas* sp. nov. (DHMECN 10984, adult female, 50.0 mm snout-vent length) in dorsal (**A**) ventral (**B**) and lateral (**C**) views of preserved specimen.

(absent), lacks dentigerous processes of vomers (present), and inhabits paramos on the Andes of north-central Ecuador (Lynch 1972, 1981). Pristimantis racemus has fingers with lateral fringes (absent), dorsum reddish-brown with darker marking (brown with or without darker irregular blotches), lacks vocal sac and vocal slits in males (present), and inhabits paramos on the Cordillera Central of the Andes, central Colombia (Lynch 1980, Lynch et al. 1996). Pristimantis simoterus has fingers with lateral fringes (absent) and inhabits upper montane forests and paramos on the Cordillera Central of the Andes, central Colombia (Lynch 1980, Lynch et al. 1996). Pristimantis simoteriscus has subacuminate snout in dorsal view, fingers with lateral fringes (absent), dorsum grey with dark markings (brown with or without darker irregular blotches), lacks vocal slits in males, and inhabits paramos on the Cordillera Central of the Andes, central Colombia (Lynch et al. 1996). Pristimantis thymelensis has tympanum concealed beneath skin (visible), paraventral folds present (absent), finger bearing lateral fringes (absent), grey to brown dorsum speckled to varying degrees with creamy grey, tan, or black (brown with or without darker irregular blotches), and inhabits paramos on Andes of southern Colombia and northern Ecuador (Lynch 1972, 1981).


**Figure 3.** Dorsal (**A**) and lateral (**B**) views of head, and ventral views of hand (**C**) and foot (**D**) of holotype of *Pristimantis andinogigas* sp. nov. (DHMECN 10984, adult female, head length 16.0 mm, head width 20.1 mm, hand length 15.8 mm, foot length 24.3 mm).

*Pristimantis loujosti* and *P. pycnodermis* also stand out from other species of the genus by their stout body and thick glandular skin on dorsal surfaces of body and limbs, but they differ from *P. andinogigas* sp. nov. as follows (characters of *P. andinogigas* sp. nov. in parentheses): *Pristimantis loujosti* Yánez-Muñoz, Cisneros-Heredia & Reyes-Puig, 2010 has smooth skin on head and granular skin on dorsum and flanks (porous, with large warts on flanks), thick supratympanic fold (prominent glandular supra/ post-tympanic patch), thin glandular patches on legs (thick), subacuminate snout in dorsal view (rounded in dorsal view), fingers bear lateral fringes (absent), black spots on hidden surfaces of limbs (uniformly coloured), light iris with dark reticulation (bronze with dense black reticulations), and it inhabits on cloud forests on the Upper River Pastaza, Cordillera Oriental of the Andes of Ecuador (Yánez-Muñoz et al. 2010).



**Figure 4.** Dorsolateral view of head of holotype of *Pristimantis andinogigas* sp. nov. (DHMECN 10984, adult female) showing supratympanic (**A**) and humeral (**B**) glandular patches, and glandular fold of wrist (**C**). Note porous dorsal skin texture.

*Pristimantis pycnodermis* (Lynch, 1979) differs by having low cranial crests (absent), snout subacuminate in dorsal view and truncate in lateral view (snout rounded in dorsal view; rounded to slightly protruding in lateral view), skin of flanks smooth (with large warts), fingers bear lateral fringes (absent), dark canthal and tympanic marks (head marks absent), large black spots on the flanks (brown with or without dark irregular blotches), 32.5–44.4 mm SVL in adult females (50.0–50.5 mm), and inhabits paramos on the Andes of central-southern Ecuador (Lynch 1979).

**Description of holotype.** Adult female (50.0 mm SVL, Fig. 2); head narrower than body, wider than long (head width 40% of SVL, head length 32% of SVL, head length 80% of head width); snout short (eye nostril 11% of SVL, eye nostril 87% of eye diameter), rounded in dorsal and lateral views; canthus rostralis rounded and weakly concave; loreal area concave; lips flared; eye large (eye diameter 1.14 times eye-nostril distance, eye diameter 38% of head length); nostrils slightly protuberant laterally (Fig. 3). Cranial crest absent; upper eyelids without tubercles; tympanic membrane differentiated, tympanic annulus visible (tympanum diameter 35% of eye diameter), upper and posterior borders of tympanic annulus in contact with prominent, thick glandular patch that covers all dorsal fascia of m. *depressor mandibulae*; large, glandular postrictal tubercles. Choanas small and widely separated from each other, not concealed by palatal shelf of maxilla; dentigerous processes of vomer present, oblique, moderately separated, posterior half not adherent to floor of mouth.



**Figure 5.** Dorsolateral view of three live male paratypes (**A–B, D**) and live female holotype (**C**) of *Pristimantis andinogigas* sp. nov.: DHMECN 10985 (**A**) DHMECN 10993 (**B**) DHMECN 10984 (**C**) DHMECN 11000 (**D**). Photographs by David Veintimilla-Yánez.

Skin on dorsum thick and glandular, surface texture porous (Figs 1, 4), with large, flat, glandular warts on flanks; dorsolateral folds absent; thick glandular patch on dorsal surfaces of humeral, femoral, tibial and tarsal regions; glandular folds in wrists (Fig. 4); skin on venter areolate; discoidal fold weakly defined; skin on ventral surfaces of legs granular; cloaca not protuberant, cloacal region with large warts. Ulnar tubercle present but low; palmar tubercle flat and bifurcate; thenar tubercle elongate, about half the size of palmar tubercle; subarticular tubercles prominent, rounded in ventral and lateral views; supernumerary palmar tubercles rounded, smaller than subarticular tubercles; fingers without lateral fringes; Finger I shorter than Finger II; discs on fingers expanded and elliptical, most prominent on fingers II–IV, while disc on Finger I slightly expanded; all discs bearing ventral pads well defined by circumferential grooves (Fig. 3).

Hind limbs robust (tibia length 46% of SVL; foot length 49% of SVL); heel without tubercles; inner edge of tarsus with one wart low and poorly differentiated; inner metatarsal tubercle ovoid, about 5x round outer metatarsal tubercle; subarticular tubercles rounded; plantar supernumerary tubercles low and inconspicuous, smaller than subarticular tubercles; toes with narrow lateral fringes; basal toe webbing between toes II–V; discs of toes expanded, elliptical, slightly narrower than those on fingers, most prominent on fingers II–V, while disc on Finger I slightly expanded; toes with ventral pads well-defined by circumferential grooves; toe lengths, when adpressed, IV > V > III > II > I; Toe V longer than Toe III; disc of Toe III not reaching distal subarticular tubercle on Toe IV, disc on Toe V reaches middle of distal subarticular tubercle on Toe IV (Fig. 3).

**Measurements (in mm) of holotype.** Snout-vent length 50.0; head width 20.1; head length 16.0; eye-nostril distance 5.3; internarial distance 4.6; interorbital distance 8.8; tympanum diameter 2.1; eye diameter 6.0; tibia length 23.2; hand length 15.8; foot length 24.3.

**Colouration of holotype in life.** Dorsum dark brown; ventral surfaces dark brown with irregular light-yellow flecks and blotches on throat, hands, feet, armpits, and lower venter; iris golden-bronze with dense black reticulations (Fig. 5).

**Colouration of holotype in preservative.** Same pattern as in life, but brown surfaces turned dark grey (Fig. 2).

**Variation.** Males are smaller than females, measurements of the type series are provided in Table 1. Dorsal colouration of body and legs varies from dark brown, chocolate brown, or orange-brown (Fig 5.). Females are darker and have a homogeneous coloration pattern, while males are paler and usually with dark irregular blotches. Some individuals have pale pink flanks and dorsal surfaces of legs (Fig. 5D). Venter colouration varies from completely dark brown to dark brown with irregular light-yellow flecks and blotches. Background dorsal colouration of juveniles is paler, and dorsal dark blotches are darker.

**Etymology.** The specific epithet is coined from the New Latin adjective *andinus* (pertaining to the Andes) and the Latin noun *gigas* (giant). The name alludes to the large and stout body of this new species in comparison with other species of *Pristimantis* from the high Andes.

**Vocalizations.** Males call from grasses at night, in heterogeneous chorus with extensive call superposition. Paratype DHMECN 11016 was calling from bamboos *Neurolepis* sp. ( $T_{air} = 7^{\circ}$  C, relative humidity = 96%). The advertisement call (Fig. 6) has dominant frequencies of 1.63–1.98 kHz (1.80 ± 0.14 SD kHz). Calls were 124–428 ms (231.37 ± 142.76 ms) in duration, with intervals of 2138–5239 ms (3393 ± 1107), and emitted 10.80–24.64 calls per minute (16.61 ± 5.35). Calls were formed by one or two notes, each with 117–148 ms (130 ± 10) in duration, at intervals of 90–157 ms (123 ± 34 ms). In calls with two notes, first note had a dominant frequency (1.65 kHz) lower than the second note (1.89 kHz).

**Distribution, natural history, conservation status, and extinction risk.** *Pristimantis andinogigas* sp. nov. is only known from its type locality, the paramos of the Nudo de Cajanuma, at elevations between 3300 and 3400 m, on the Cordillera Oriental of the Andes of southern Ecuador (Fig. 7). Surveys in other localities of the Nudo de Cajanuma, and on the nearby Nudo de Sabanilla, have not recorded the species (Almendáriz and Orcés 2004, Ron et al. 2019). However, most surveys were conducted at lower elevations, and most paramos in the region lack amphibian inventories. It is possible that *P. andinogigas* inhabits a larger area at the Cajanuma-Sabanilla mountain ridges; but it is unlikely that it occurs farther north on the Cordillera Oriental (e.g., Nudo de Guagrahuma), because of separation by the valley of the River Zamora, reaching elevations as low as 2800 m that may limit species' dispersal.



Figure 6. Advertisement call of Pristimantis and inogigas sp. nov. (paratype, DHMECN 11016).

The ecosystem at the type locality is Paramo Bambusoid Meadow (MAE et al. 2013). The most representative plant genera were *Bomarea*, *Miconia*, *Blechnum*, *Disterigna*, *Epidendrum*, *Gaultheria* and *Puya*; and the most abundant plant species were *Escallonia myrtilloides*, *Puya nitida*, *Hypericum lancioides*, *Tillandsia aequatorialis*, *Neurolepis nana*, *Cortaderia bifida*, *C. jubata*, *Chusquea neurophylla*, *Calamagrostris macrophylla*, *Themistoclesia epiphytica*, *Senecio tephrosioides*, *Disterigma pentandrum*, and *D. empetrifolium*, *Rubus laegaardii* (Eguiguren et al. 2015).

*Pristimantis andinogigas* was found active at night (19h00–22h00) at 6–10° C air temperature and 85–98% relative humidity. All males and some subadults and juveniles were observed active on bamboos (*Neurolepis* spp.); while both adult females were found active on the floor. During the day, individuals were found hidden inside rosettes (*Senecio* spp. and *Puya* spp.) or at the base of bamboos. *Pristimantis andinogigas* was the most abundant species during surveys at the type locality, representing 47 out of 108 anuran records. It was found in sympatry with *Pristimantis percultus*, *Pristimantis* sp. cf. *colodactylus*, *Pristimantis* sp. cf. *colodactylus*, *Pristimantis* sp.

The type locality of *P. andinogigas* is officially protected as part of the Podocarpus National Park, a national protected area created in 1982. The area has little anthropogenic impact, and in general, paramos of the Nudo de Cajanuma and the nearby Nudo de Sabanilla are reported to have a relatively good conservation status (Hofstede et al. 2002). Road infrastructure projects have been proposed in the past, but their development was cancelled (Cisneros et al. 2004, Bernardi de León 2009). However, three expeditions over the last five years have recorded very low numbers of *P. andinogigas*. Although more data are needed, it may be possible that the population of *P. andinogigas* has declined. In the absence of further information about the extinction risk on this newly discovered species, we suggest that *P. andinogigas* should be classified in the IUCN Red List category of Data Deficient (IUCN 2012).



**Figure 7.** Map of southern Ecuador indicating the type locality of *Pristimantis andinogigas* sp. nov. at the Nudo de Cajanuma, Cordillera Oriental, Andes of southern Ecuador.

## Discussion

*Pristimantis andinogigas* sp. nov. is morphologically similar to several species formerly associated under the *P. orcesi* species-group (i.e., *Pristimantis erythros, P. farisorum, P. obmutescens, P. orcesi, P. racemus, P. simoterus, P. simoteriscus,* and *P. thymelensis*). However, we refrain from assigning it to any species-group in the absence of data to conduct an integrative phylogenetic analysis. Morphological characters in *Pristimantis* are by themselves unreliable to assess phylogenetic affinities, and most of the species-groups within *Pristimantis* that were solely defined on morphology have resulted non-monophyletic (Pinto-Sánchez et al. 2012, Padial et al. 2014). The *Pristimantis orcesi* species-group proposed by Lynch (1981) was found to be non-monophyletic by Pinto-Sánchez et al. (2012) and Padial et al. (2014); although the relationships of most species remain unknown since both studies included only two species assigned to the *P. orcesi* species-group (*P. orcesi* and *P. thymelensis*).

*Pristimantis andinogigas* shows two notorious morphological characters that are not extended in the genus: glandular patches covering dorsal surfaces body and limbs, and porous dorsal skin texture. Similar glandular patches were first reported in *P. pycnodermis* by Lynch (1979), subsequently in *P. loujosti* by Yánez-Muñoz et al. (2010) and in *P. erythros* by Sánchez-Nivicela et al. (2018), and we have observed them in *P. orcesi* and an undescribed species of *Pristimantis* from the paramos of southern Ecuador. Porous skin texture has not been reported in any other species of *Pristimantis*, although it could have been confused with shagreen texture when not examined in detail or in preserved specimens. Further analyses are needed to understand the morphology of these characters and their phylogenetic significance.

Over the last decades, field studies in the Podocarpus National Park and nearby areas have revealed extraordinary flora and fauna diversity on the southernmost portion of the Cordillera Oriental of the Andes in Ecuador (Borchsenius 1997, Stattersfield et al. 1998, Brehm et al. 2005, Keating 2008, Rex et al. 2008, Richter et al. 2009). Although information on amphibians and reptiles has not been fully systematised and several areas remain unexplored, available data shows high levels of species richness and endemicity of amphibians and reptiles in the region (Lynch 1979, Cisneros-Heredia and McDiarmid 2006, Yánez-Muñoz et al. 2013, Torres-Carvajal et al. 2017). Further new species of amphibians and reptiles from the Podocarpus National Park, and the nearby Yacuri National Park, will be described in the near future, and discovery of additional new species from unexplored areas is expected.

## Acknowledgments

This work is part of Programa de Investigación Red Terrarana del Ecuador of Instituto Nacional de Biodiversidad INABIO, developed with the support of Universidad Nacional de Loja, Laboratorio de Zoología Terrestre and Museo de Zoología of Universidad San Francisco de Quito USFQ, and Museo de Zoología QCAZ. We thank Karen Salinas who was part of the team that surveyed the herpetological diversity at Cajanuma, to José Villa Esparza and Enrique Armijos, park rangers of the Podocarpus National Park, for their field support, and to Rudolf von May and Alessandro Catenazzi for their comments as reviewers. Research was conducted under authorization by Ministerio del Ambiente del Ecuador. For access to collection specimens and working space, we thank Juan C Sánchez-Nivicela (Universidad del Azuay), Carolina Reyes-Puig (Museo de Zoología, Universidad San Francisco de Quito), Santiago R Ron (Museo de Zoología, Pontificia Universidad Católica del Ecuador PUCE), John D Lynch (Instituto de Ciencias Naturales, Universidad de Colombia), and Raúl Sedano (Colección Herpetológica, Universidad del Valle del Cauca). Revision of type specimens of Colombian Pristimantis was possible thanks to the support of Heinz Schneider of Basel Botanical Garden, Lou Jost, Javier Robayo and Juan P Reyes-Puig of Ecominga Foundation. Work by David Veintimilla-Yánez was supported by Universidad

Nacional de Loja, Centro de Estudios y Desarrollo de la Amazonía CEDAMAZ, and Ministerio del Ambiente de Loja. Work by Diego F Cisneros-Heredia was supported by Universidad San Francisco de Quito USFQ (projects HUBI ID 48 "Taxonomía, Biogeografía y Conservación de Anfibios y Reptiles", ID 1057 "Impact of habitat changes on the biological diversity of the northern tropical Andes", ID 7703 "Estrés fisiológico y molecular en anfibios de los altos Andes tropicales", funded by a grant of the Colegio de Ciencias Biológicas y Ambientales COCIBA-USFQ, ID 12268 "Taxonomía y Conservación del género Pristimantis en los páramos, estribaciones y tierras bajas del Ecuador", ID 13524 "Desarrollo de una plataforma de bioinspiración médica a partir de la biodiversidad", funded by a grant of the Escuela de Medicina, Colegio de Ciencias de la Salud. COCSA-USFQ) and by Programa "Becas de Excelencia", Secretaría de Educación Superior, Ciencia, Tecnología e Innovación (SENESCYT), Ecuador. For their constant encouragement, Mario Yánez-Muñoz expresses his deepest gratitude to Mauro Yánez, Alejandra Figueroa, Joaquín Yánez, and Julieta Yánez; David Veintimilla-Yánez to Carlos Veintimilla, Lucía Yánez, Nikolay Aguirre Mendoza, Max González, Walter Apolo, Katiusca Valarezo, Johana Muñoz, Ivonne González, Tatiana Ojeda Christian Aguirre, and Andreas Fries; and Diego F Cisneros-Heredia to María Elena Heredia, Laura Heredia, and Jonathan Guillemot.

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