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A new species of *Strongylacron* (Copepoda, Harpacticoida, Cletodidae) from intertidal mudflats in the Korean peninsula

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Abstract

A new species, *Strongylacron glabrum* **sp. n.** is described from intertidal mudflats in the Korean peninsula. The new species is assigned to the monospecific genus *Strongylacron* Gee & Huys, 1996 in accordance with the generic morphological features of the rostrum, antennary exopod, and thoracic legs. However, *Strongylacron glabrum* **sp. n.** is clearly distinguished from the type species, *S. buchholtzi* (Boeck, 1873), by the naked anterior margin of rostrum, the elongate exopod and endopodal lobe of female P5 approximately 3.5 and 2.7 times as long as width, respectively, and the presence of 8–10 rod-like projections on prosomites. The genus *Strongylacron* is first recorded from Korean waters by the present study.

Keywords

Cletodidae, *glabrum*, harpacticoid, Korea, new species, *Strongylacron*, taxonomy

Introduction

The harpacticoid copepod genus *Strongylacron* Gee & Huys, 1996 belonging to the family Cletodidae T. Scott, 1904 was established by Gee and Huys (1996) as a part of an effort to resolve the relationship among members of *Enhydrosoma* Boeck, 1873.

The latter genus was recognized as a heterogeneous group by Fiers (1987) and Mielke (1990). After the studies of Lang (1936, 1948), this genus was distinguished from *Cletodes* Brady, 1972 by subtle morphological characteristics such as the form and setation of the distal segment on legs 3 and 4 (Gee 1994, 2001; Gee and Huys 1996). In the revision of *Enhydrosoma*, Gee (1994) first proposed the *buchholtzi*-species group representing differences from others in the rostrum, antenna, maxillule and male P3 endopod. In addition, he suggested that this group should be removed from *Enhydrosoma*. Thereafter, the *buchholtzi*-species group was revised and the following three genera were established: *Schizacron* Gee & Huys, 1996, *Spinapecruris* Gee, 2001, and *Strongylacron* Gee & Huys, 1996 (Gee 1994, 2001; Gee and Huys 1996). *Strongylacron* is a monospecific genus containing *St. buchholtzi* (Boeck, 1873) (Walter and Boxshall 2016). The type species has been chiefly reported from European waters and occurs usually around estuary sediments containing high organic content (Boeck 1873; Sars 1909; Wells 1963; Gee and Huys 1996).

In South Korea, ten cletodid harpacticoids have been reported from various environments: Lee and Chang (2007) reported three species, *Limnocletodes behningi* Borutzky, 1926, *L. angustodes* Shen & Tai, 1963, and *Kollerua longum* (Shen & Tai, 1979), from salt marshes and estuaries; Kim (2013) recorded *Enhydrosoma curticauda* Boeck, 1873 from coral sands in Udo, Jeju Island; six species, *E. coreana* Kim, Trebukhova, Lee & Karanovic, 2014, *E. apimelon* Karanovic, Kim & Lee, 2015, *E. robustum* Karanovic, Kim & Lee, 2015, *E. kosmetron* Karanovic, Kim & Lee, 2015, *Geehydrosoma intermedia* (Chislenko, 1978), and *Paracrenhydrosoma kiai* Song, Dahms, Lee, Ryu & Khim, 2014, were recently reported from intertidal and subtidal muddy bottoms (Kim et al. 2014; Song et al. 2014; Karanovic et al. 2015).

While studying harpacticoid copepods from Korean waters as a part of the 'Survey of indigenous biological resources of Korea', a new harpacticoid copepod belonging to the genus *Strongylacron* was discovered and reported here as *Strongylacron glabrum* sp. n. along with detailed description and illustrations.

Materials and methods

Sampling was performed with a sieve of 212 µm mesh from intertidal mudflats on the south-western coasts of Korea. In ebb tides, surface sediments (< 5 cm sediments depth) were obtained by using large spoons. Samples remaining on sieve were fixed initially with 5% formaldehyde-seawater solution. Harpacticoid specimens were preserved with 99.9% ethanol after sorting in the laboratory. They were dissected by using tungsten needles under stereo microscope (Discovery, V8; Carl Zeiss, Germany) and then mounted on polyvinyl lactophenol or lactophenol. The observations and drawings were performed by light microscope (ECLIPSE 80*i*; Nikon, Japan) equipped with a drawing tube. Several specimens were examined in a scanning electron microscope (SEM). They were cleaned by an ultrasonic machine, prefixed by 4% glutaraldehyde, postfixed by 2% OsO_4, dehydrated through graded ethanol solutions, air-dried, and coated with gold. The dried materials were observed under SEM (VEGA 3 LM; Tescan, Czech Republic), with an accelerating voltage of 20 kV and working distances between 17.90–20.50 mm. Descriptions and line drawings examined under 400–1,000× magnifications were made based on the paratypes. All materials examined were deposited in Chosun University and the National Institute of Biological Resources (NIBR), Korea.

The terminology of the body and appendage morphology follows Huys and Boxshall (1991). Abbreviations used in the text and figures are:

ae	aesthetasc;
exp	exopod;
enp	endopod;
exp (enp)-1 (2, 3)	to denote the proximal (middle, distal) segment of a three-segmented ramus;
P1-P6	first to sixth thoracic legs.

Systematic accounts

Family Cletodidae T. Scott, 1904
Genus *Strongylacron* Gee and Huys, 1996

***Strongylacron glabrum* sp. n.**
http://zoobank.org/A16A2690-3712-4FDC-A7CD-067CD78D8B25
Figs 2–10

Type locality. South Korea, Jeollanam-do Province: Jindo-gun County, Imhoe-myeon, Namdong-ri, 34°21.666'N, 126°09.449'E, intertidal mudflats (Fig. 1).

Material examined. Holotype ♀ (NIBRIV0000326503) and allotype ♂ (NIBRIV0000326504), both undissected and preserved in 99.9% ethanol. Paratypes: 2♀♀ (NIBRIV0000326505, NIBRIV0000326506) and 2 ♂♂ (NIBRIV0000326507, NIBRIV0000326508) dissected and mounted on each slide, respectively. All materials were collected from mud flats on type locality on 9 April 2013.

Additional material. South Korea, Jeollanam-do Province: 15 ♀♀, Muan-gun County, Haeje-myeon, Songseok-ri, 35°9.477'N, 126°20.864'E, 9 April 2013; 2 ♀♀, Goheung-gun County, Gwayeok-myeon, Noil-ri, 34°41.275'N, 127°19.447'E, 30 April 2013. For the photographs of SEM: 4 ♀♀ and 2 ♂♂, Jeollanam-do Province, Sinan-gun County, Aphae-myeon, Sinyong-ri, 34°52.990'N, 126°17.867'E, 6 April 2012. All materials collected from sediments composed of mud or muddy sand on each locality (Fig. 1).

Diagnosis. Habitus semi-cylindrical, approximately 900 μm; each prosomite with 8 or 10 rod-like projections. Rostrum fused to cephalothorax basally, slightly recurved dorsally; anterior margin rounded and naked. Genital field with vestigial P6 represented

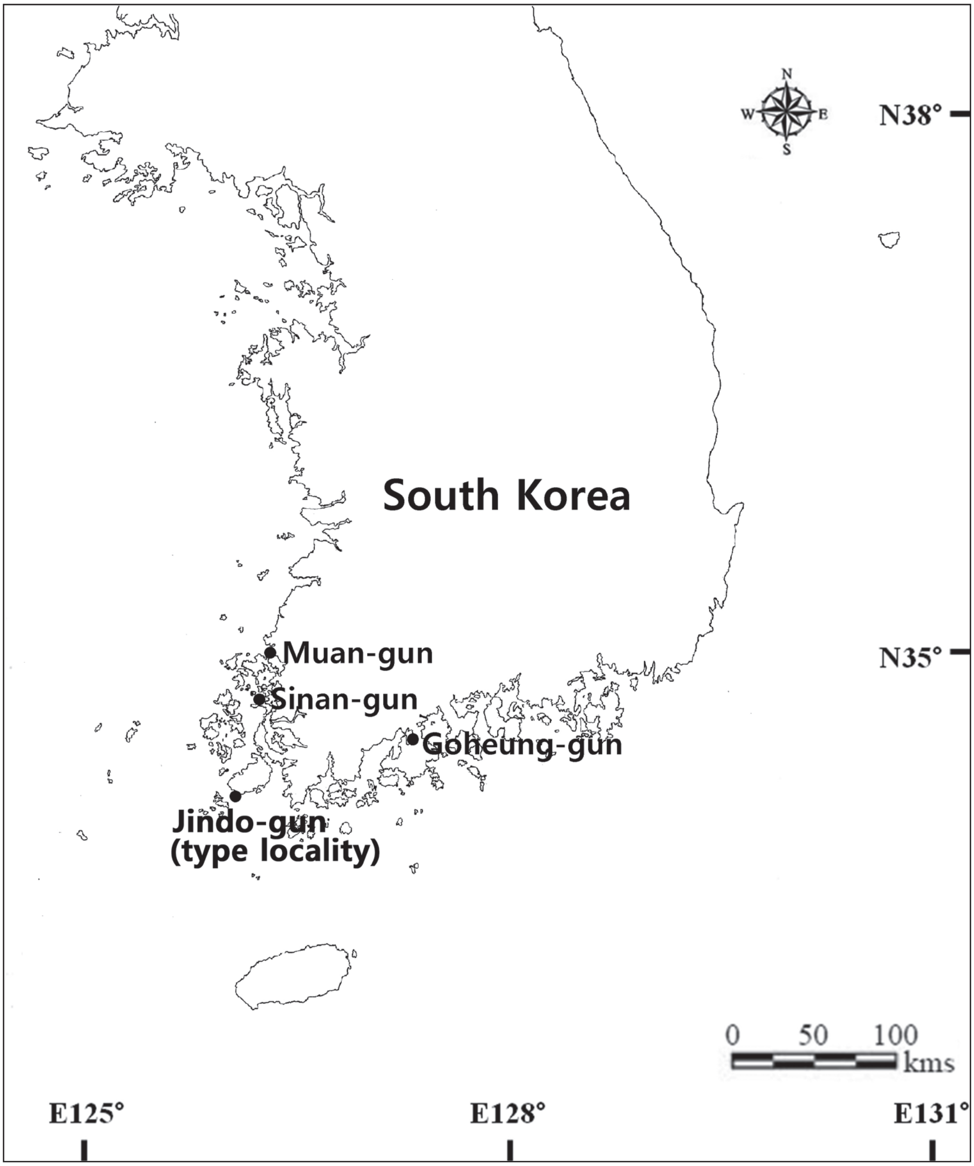

Figure 1. Localities of the sampling stations of the present study in South Korea.

by seta. Caudal rami approximately 2.5 times (female) and 3.6 times (male) as long as width; tube pore on outer margin inserted proximally; caudal seta VI shorter than seta IV. Antennary endopod with stout spine-like seta at distal corner. Mandibular gnathobase with 3 bicuspid teeth, without seta; basis with 2 short and 1 long setae. Female P5 exopod 3.5 times as long as width; innermost seta on exopod shorter than exopod in length; endopodal lobe approximately 2.7 times as long as width. Male P5 endopodal lobe, outer most seta on endopodal lobe reaching half of middle seta on exopod.

Female. Body (Figs 2A, B, 8A) semi-cylindrical, tapering posteriorly, with inconspicuous boundary between prosome and urosome; total length including tip of rostrum and caudal rami from 889.1 to 923.1 µm (mean 905.3 µm, *n* = 3) in lateral view. All somites covered with fine setules on surface; posterior border with row of setules except for anal somite. Rostrum (Figs 3A, 8E) well-developed, fused to cephalothorax basally, slightly recurved dorsally, with pair of subapical sensilla; anterior margin rounded, naked, slightly concave midway; posterior surface with tube pore.

Prosome (Figs 2A, B, 8B–D) 4-segmented, comprising cephalothorax and 3 free pedigerous somites. Cephalothorax slightly shorter than succeeding somites combined, with 8 rod-like projections. Each posterior border of 3 free somites (Fig. 8C) with 8, 10, 8 rod-like projections bearing sensillum, respectively.

Urosome (Figs 2A, B, 4A) 5-segmented, comprising P5-bearing somite, genital double-somite, and 3 postgenital somites. P5-bearing somite with 8 rod-like projections bearing sensillum on posterior border dorsally. Genital double-somite, dorsal and lateral surfaces completely divided by suture, but ventral surface (Fig. 4A) partially fused; each posterior border with 6 and 8 rod-like projections bearing sensillum, respectively. Genital field (Fig. 4D) with vestigial P6 represented by seta. Urosomite 4 with 6 rod-like projections bearing sensillum on posterior margin. Urosomite 5 with pair of lateral protrusions covered with setules. Anal somite (Fig. 6D) with semi-circular operculum bearing pair of setae and 1 row of setules on posterior margin; lateral margin of each side with extra tube pore.

Caudal rami (Figs 6D, 9E, F) cylindrical, tapering posteriorly, as long as anal somite in length, with 2 tube pores and 7 caudal setae: lateral seta I along with seta II inserted in proximal fifth of ramus; seta III half of caudal ramus in length; seta IV small, fused to well-developed seta V at its base; seta V 3.0 times as long as caudal ramus; seta VI shortest, located at inner distal corner; seta VII located in dorsal surface proximally, articulated basally.

Antennule (Figs 3B, 8F) 5-segmented, short, blunt; surface (Fig. 9A) ornamented with small papillae. Segment 1 short, with 3 rows of setules on surface and row of stout spinules on anterior margin. Segment 2 longest. Segments 3 and 5 with aesthetasc fused to seta at its base, respectively. Segment 4 shortest. Setal formula as follows: 1-[1], 2-[8], 3-[7+ae], 4-[1], 5-[10+ae].

Antenna (Figs 3C, 9B). Coxa small. Allobasis long, with 2 abexopodal setae; antennary exopod small, peduncle-like, with long bipinnate seta. Endopod 1-segmented, slightly shorter than allobasis; anterior margin with row of setules, row of subdistal spinules, and 2 spines; distal margin with 5 non-geniculate spines, 1 slender seta, and 1 tube pore; surface with row of spinules distally.

Mandible (Fig. 3D). Coxal gnathobase well-developed, with 3 bicuspid teeth; outer distal corner broad, rounded; surface with group of setules. Palp 1-segmented; basis with 2 short and 1 long plumose setae; exopod and endopod fused to basis, each represented by plumose seta.

Maxillule (Fig. 3E). Praecoxa with patch of setules on surface; arthrite armed with 5 spines on distal margin, 1 pinnate seta on lateral margin and 2 tube setae on anterior

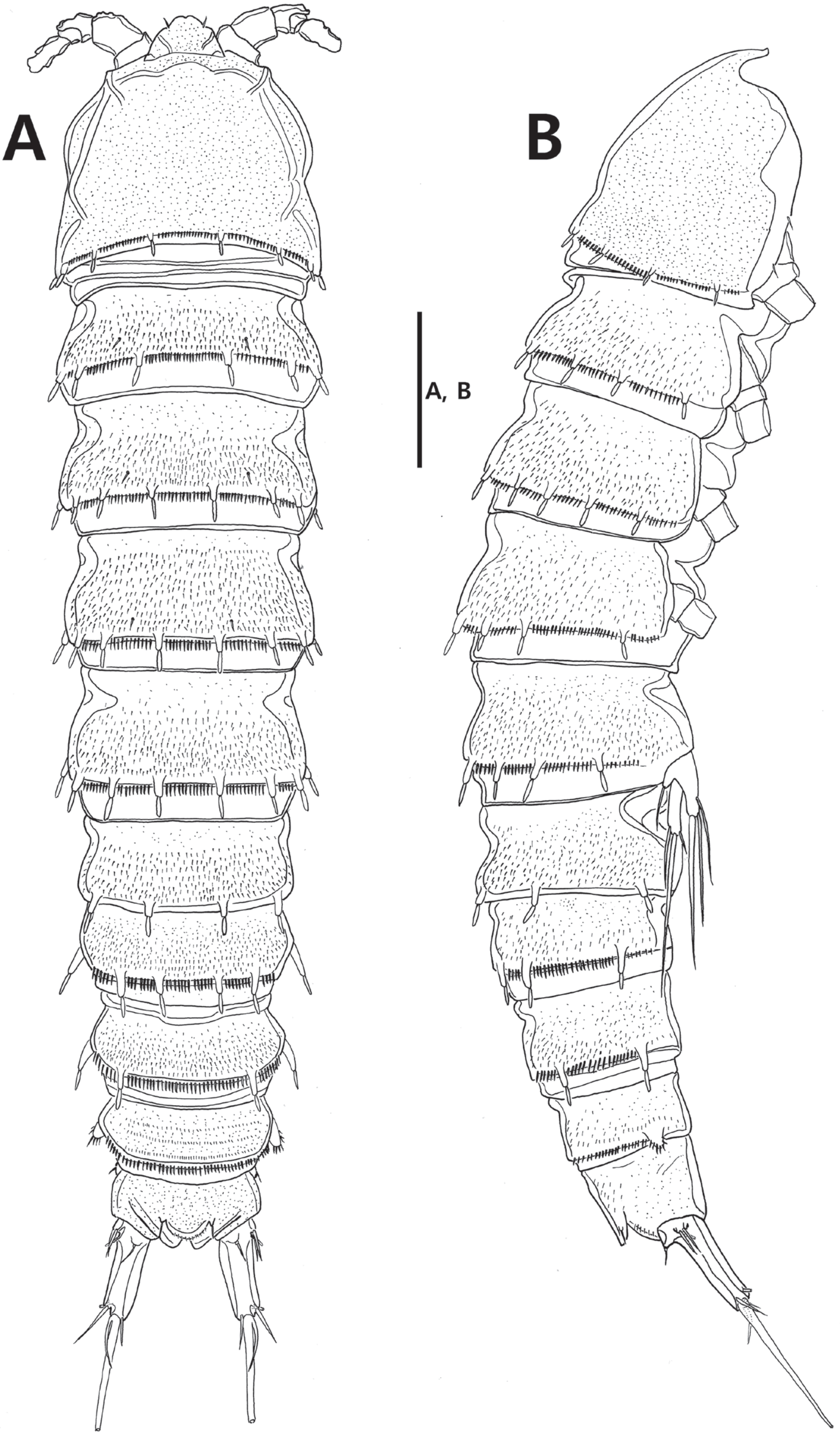

Figure 2. *Strongylacron glabrum* sp. n. female. **A** habitus, dorsal **B** habitus, lateral. Scale bar 100 µm.

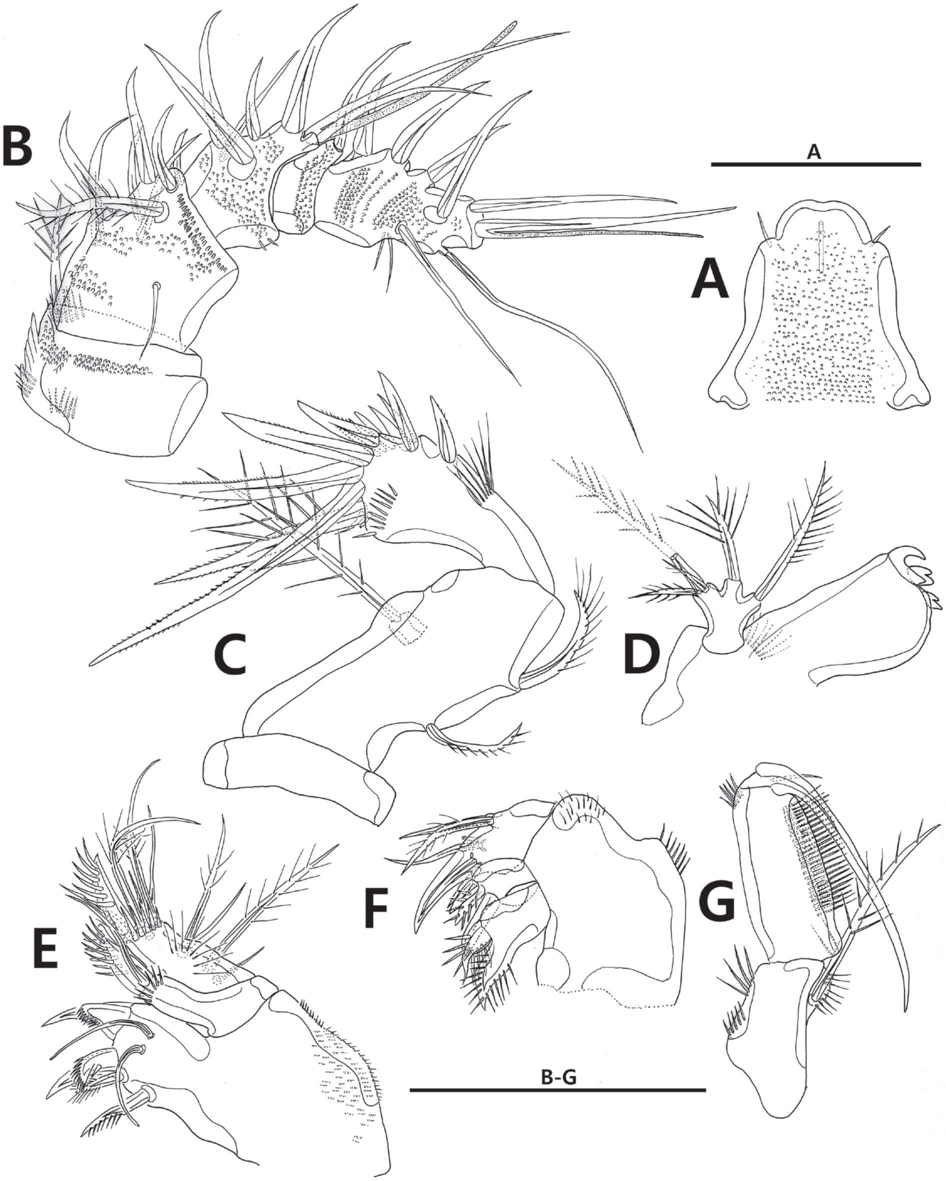

Figure 3. *Strongylacron glabrum* sp. n. female. **A** rostrum **B** antennule **C** antenna **D** mandible **E** maxillule **F** maxilla **G** maxilliped. Scale bars 50 µm.

surface. Coxal endite with 1 stout bipinnate and 1 slender setae; surface with row of spinules. Basal endite with 6 elements, 1 row of spinules and 1 row of setules. Both rami incorporated into basis, each represented by 1 plumose and 1 naked setae.

Maxilla (Fig. 3F). Syncoxa with 1 row of setules and 1 patch of setules along outer margin, bearing 2 endites: proximal endite with 2 stout pinnate (one fused to endite proximally) and 1 bare setae; distal endite with 1 pinnate and 2 bare setae. Allobasal endite forming claw like, with 3 elements and 1 tube pore. Endopod incorporated into allobasis and represented by 2 setae. Exopod absent.

Maxilliped (Fig. 3G). Syncoxa with 3 rows of setules and 1 long bipinnate seta. Basis elongate, with 2 rows of setules along palmar margin; outer distal margin with row of setules distally. Endopod represented by claw, longer than length of basis, with accessory seta.

P1 (Fig. 4B). Coxa with row of spinules on anterior surface. Basis with 2 rows of spinules on anterior surface, 1 outer seta, and 1 pinnate inner spine. Exopod 3-segmented, slightly longer than endopod; each segment ornamented with rows of outer spinules and inner setules; exp-1 and -2 with outer spine, respectively; exp-3 with 2 outer spines, 2 apical setae, and 1 posterior tube pore. Endopod 2-segmented; each segment ornamented with rows of outer spinules and inner setules; enp-2 1.5 times as long as preceding one, and armed with 1 short inner seta, 1 long apical seta, and 1 outer spine.

P2–P4 (Figs 4C, 5A, B). Praecoxa with row of spinules on distal margin. Coxa with row of spinules on anterior surface. Basis with 1 or 2 rows of spinules and 1 tube pore on anterior margin, and 1 outer seta. Exopod 3-segmented; each segment ornamented with rows of outer spinules and inner setules; exp-1 and -2 without inner seta; exp-3 with tube pore on anterior margin. Endopod 2-segmented; endopod of P4 very short and slightly exceeding end of P4 exp-1.

Setal formula of P1–P4 as follows:

	Exopod	**Endopod**
P1	I-0, I-0, II,2,0	0-0, I,1,1
P2	I-0, I-0, II,2,0	0-0, 0,2,0
P3	I-0, I-0, II,2,1	0-0, I,2,0
P4	I-0, I-0, II,2,1	0-0, I,1,1

P5 (Figs 5C, 9C, D) distinctly U-shaped, covered with fine setules. Baseoendopod with anterior tube pore and peduncle bearing outer seta; endopodal lobe reaching to 2/3 of exopod, with 3 pinnate setae, 2 tube pores, and 1 row of spinules. Exopod indistinctly separated from baseoendopod, 3.5 times as long as width, with 1 anterior tube pore and 3 pinnate setae; innermost seta shorter than exopod.

Male. Body (Figs 6A, 10A, B) 700.0–934.1 µm (mean 799.8, *n* = 3) in length, measured from anterior margin of rostrum to end of caudal rami.

Urosomites 2 and 3 (Figs 6B, 10A) not fused. P6 (Fig. 6B) asymmetrical, and with 2 rows of setules on posterior margin and plate on one side of body. Urosomite 3, ventral surface asymmetrical ventrally, with 1 row of setules and 1 row of delicate setules.

Caudal rami (Figs 6A, B, 10C–E) longer than female, 1.9 times longer than anal somite in length.

Antennule (Figs 7A, 10F) 6-segmented, subchirocer. Segment 1 with 3 rows of spinules on surface. Segment 4 swollen; proximal corner with 1 row of spinules and 2 spines; small peduncle on inner surface with aesthetasc and seta. Segment 5 shortest

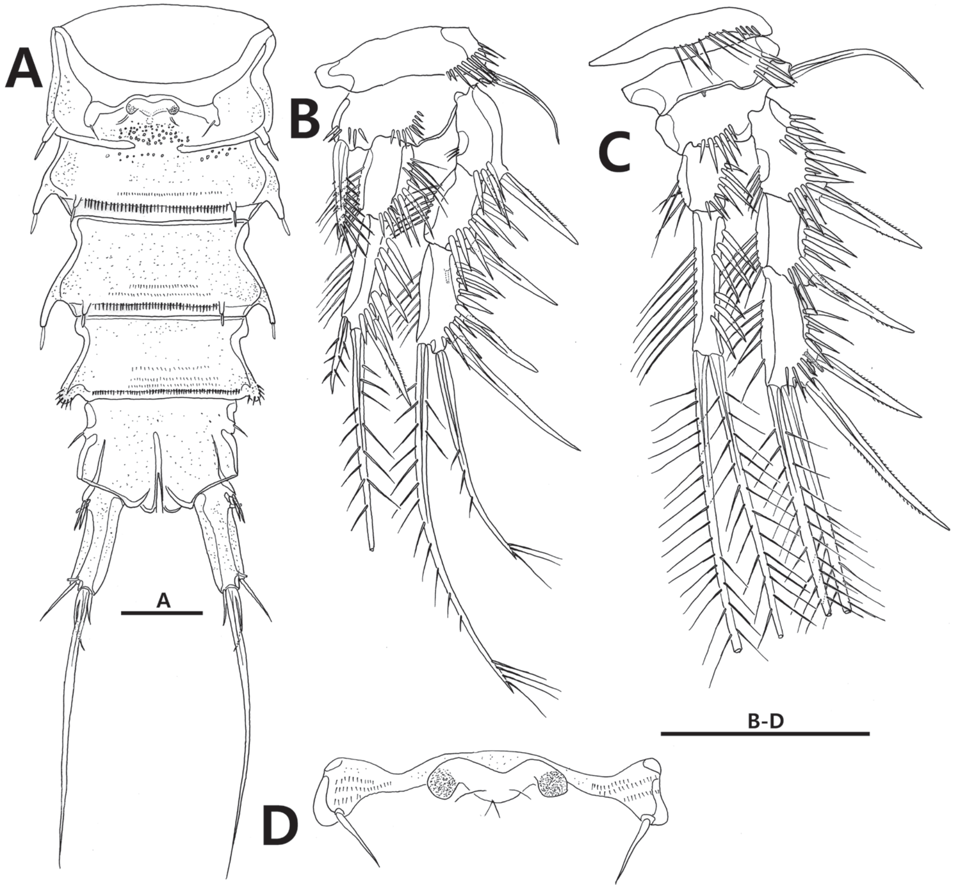

Figure 4. *Strongylacron glabrum* sp. n. female. **A** urosome except P5-bearing somite, ventral **B** genital field **C** P1 **D** P2. Scale bars 50 μm.

with protrusions at distal corner. Each aesthetasc on segments 4 and 6 fused to seta at its base. Setal formula as follows: 1-[1], 2-[8], 3-[10], 4-[12+ae], 5-[0], 6-[9+ae].

P3 (Fig. 7B). Endopod 3-segmented, modified; enp-2 with recurved apophysis at inner distal edge; enp-3 small, with 2 plumose apical setae. Exp-3 with tube pore on anterior surface.

P5 (Fig. 6C). Baseoendopod and exopod confluent. Endopodal lobe very small, with 2 setae, 2 tube pores, and 1 row of spinules; inner seta half of outer one in length. Exopod with 1 tube pore and 3 setae, innermost seta approximately 1/3 of outermost seta in length.

Distribution. The south-western coasts of South Korea.

Etymology. The epithet of the specific name, *glabrum*, is derived from the Latin adjective *glaber*, meaning 'hairless' or 'bare'. This name refers to the naked anterior rostral margin of the new species.

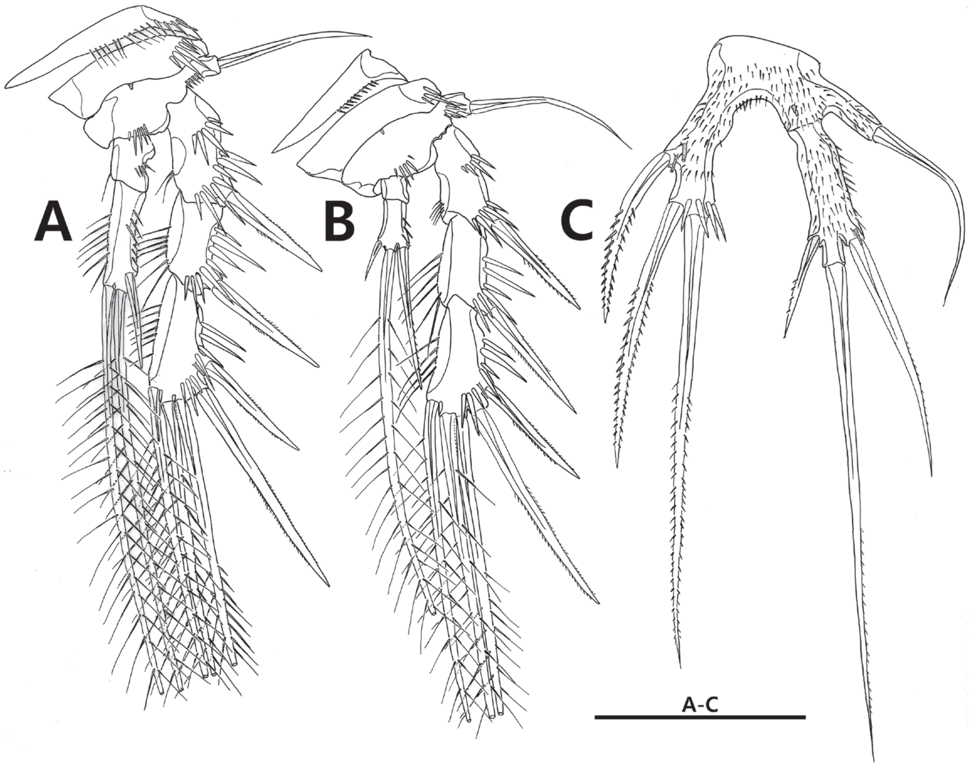

Figure 5. *Strongylacron glabrum* sp. n. female. **A** P3 **B** P4 **C** P5. Scale bar 50 µm.

Remarks. Gee and Huys (1996) redefined the taxonomic status of four *Enhydrosoma* species, *E. buchholtzi* (Boeck, 1873), *E. barnishi* Wells, 1967, *E. bifurcarostratum* Shen & Tai, 1965, and *E. vervoorti* Fiers, 1987, belonging to the *buchholtzi*-species group (see Gee 1994), and they established two genera, *Schizacron* Gee & Huys, 1996 and *Strongylacron* Gee & Huys, 1996. These genera share a distinctive U-shaped female P5, which is known as a unique structure of the family Cletodidae T. Scott, 1904, but they are typically divided in terms of the structure of rostrum (Gee and Huys 1996). *Schizacron* is characterized by having a recurved dorsally and markedly bifid anterior rostral margin, while *Strongylacron*'s rostrum is non-recurved and has a broadly rounded anterior margin (Gee and Huys 1996). Additionally, they recognized the presence of a row of fine setules on the anterior rostral margin as a significant generic characteristic of *Strongylacron*. The genus *Strongylacron* was erected based on only one species, *St. buchholtzi*, with a restricted distribution in the north Atlantic Ocean (northwestern Europe and Canada) (Boeck 1873; Sars 1909; Willey 1929; Wells 1963; Gee and Huys 1996).

In the genus *Strongylacron*, the presence of fine setules on the anterior margin of rostrum seems to be not a generic characteristic but a specific feature to distinguish species due to the discovery of the new species, *St. glabrum* sp. n., having a naked rostrum. In

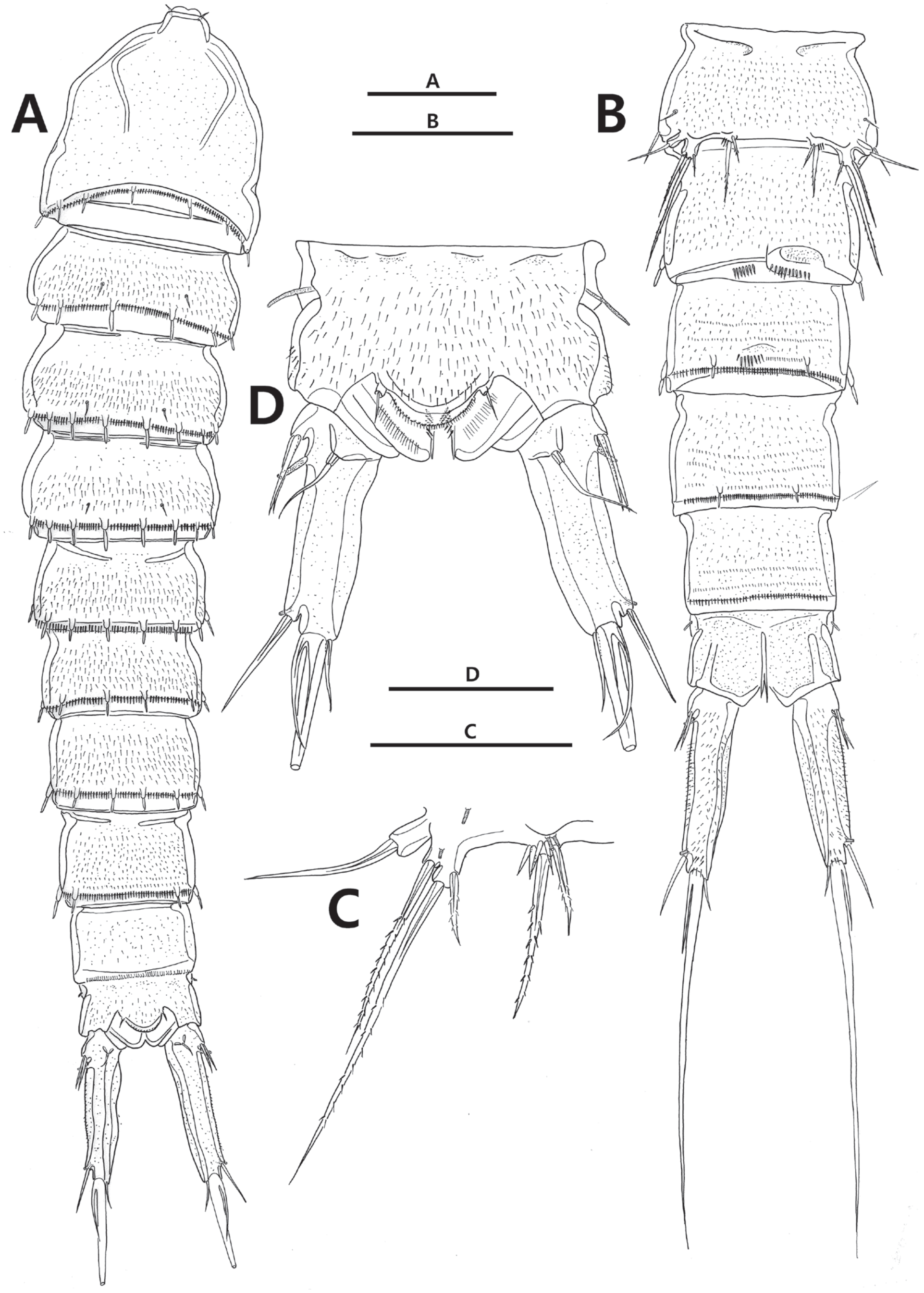

Figure 6. *Strongylacron glabrum* sp. n. **A–C** male: **A** habitus, dorsal **B** urosome, ventral **C** P5. **D** female: **D** anal somite and caudal rami, dorsal. Scale bars 100 µm (**A, B**), 50 µm (**C, D**).

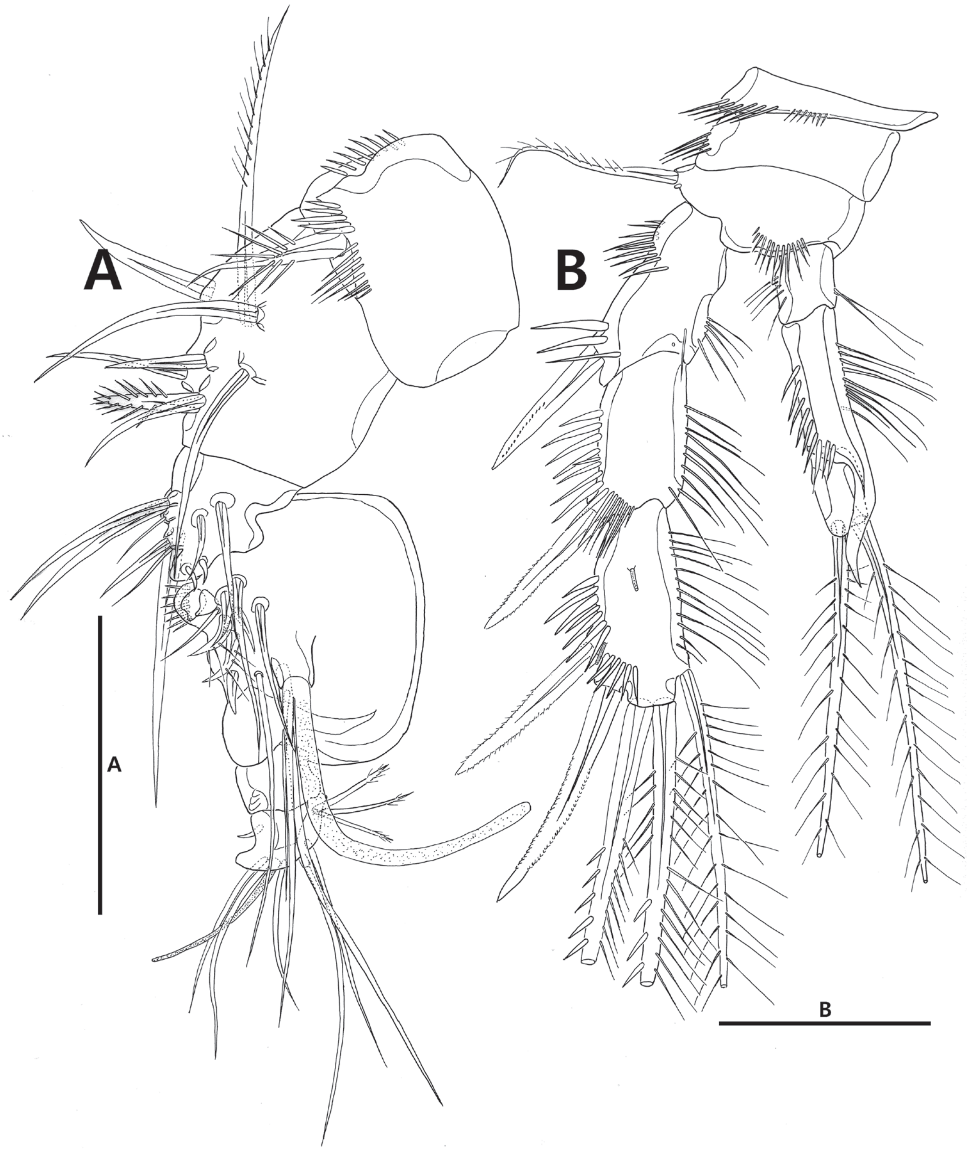

Figure 7. *Strongylacron glabrum* sp. n. male. **A** antennule **B** P3. Scale bars 50 μm.

related genera such as *Cletodes* Brady, 1972, *Enhydrosoma* Boeck, 1873, and *Schizacron* Gee & Huys, 1996, the rostrum of most species are usually naked except for *Cletodes macrura* Fiers, 1991 and *Schizacron barnishi* (Wells, 1967) (Wells 1967; Fiers 1991; Gee 1994; Gee and Huys 1996). There is an additional difference on the position of the tube pore on the caudal rami between *St. glabrum* sp. n. and the generic diagnosis given by Gee and Huys (1996). The tube pore on outer margin is located proximally in *St. glabrum* sp. n., while it is inserted medially in the generic diagnosis (Gee and Huys 1996).

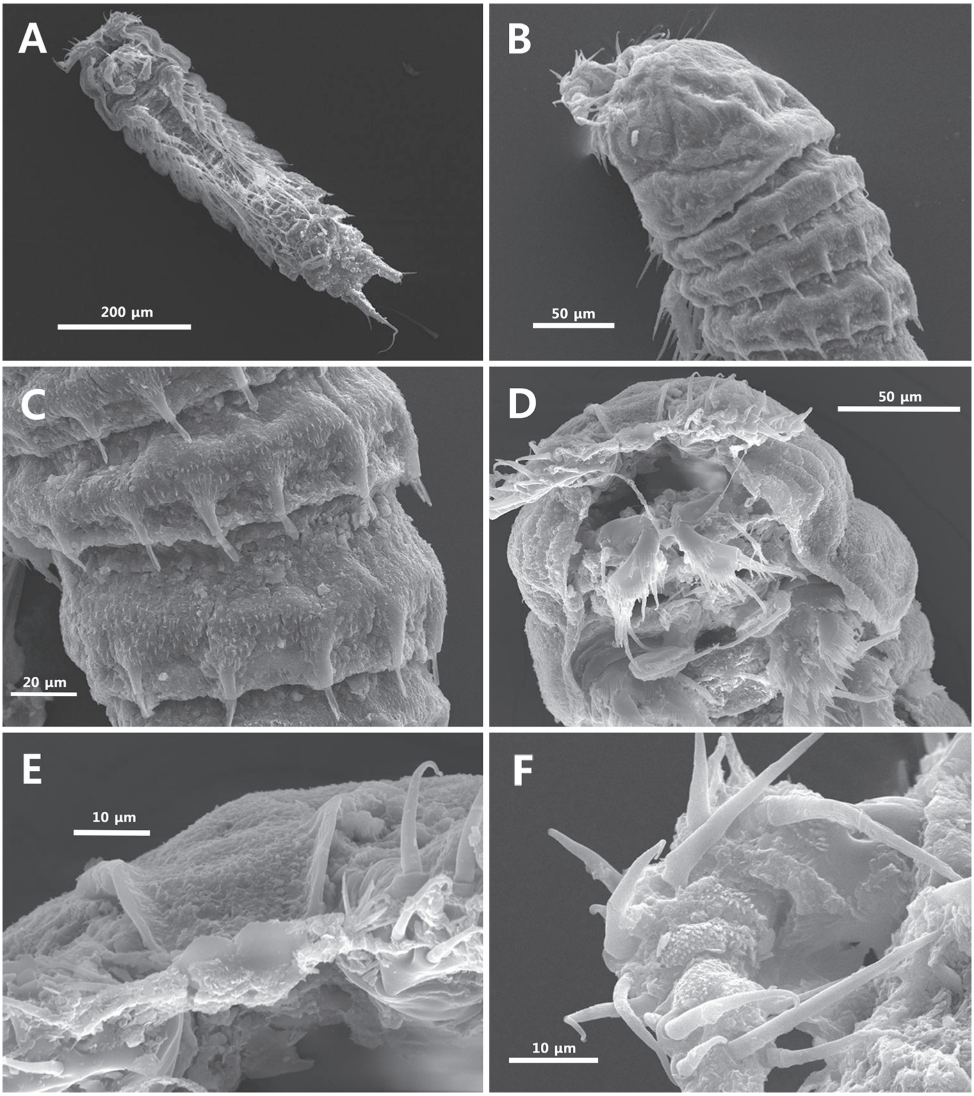

Figure 8. Scanning electron microscope photographs. *Strongylacron glabrum* sp. n. female: **A** habitus, ventral **B** prosome, dorsolateral **C** thoracic somites 2–4, dorsolateral **D** cephalothorax, ventral **E** rostrum, anterior **F** antennules, dorsal.

Nevertheless, the new species can be placed in the genus *Strongylacron* without doubt based on its possession of the following morphological features: (1) each third exopodal segment of P1–P4 with four, four, five, five setae/spines, (2) rostrum with non-recurved anterior margin dorsally, (3) a minute antennary exopod with a pinnate seta, (4) female P5 distinctly U-shaped, (5) a recurved apophysis on the second endopodal segment of male P3, and (6) two apical setae on the third endopodal segments of male P3.

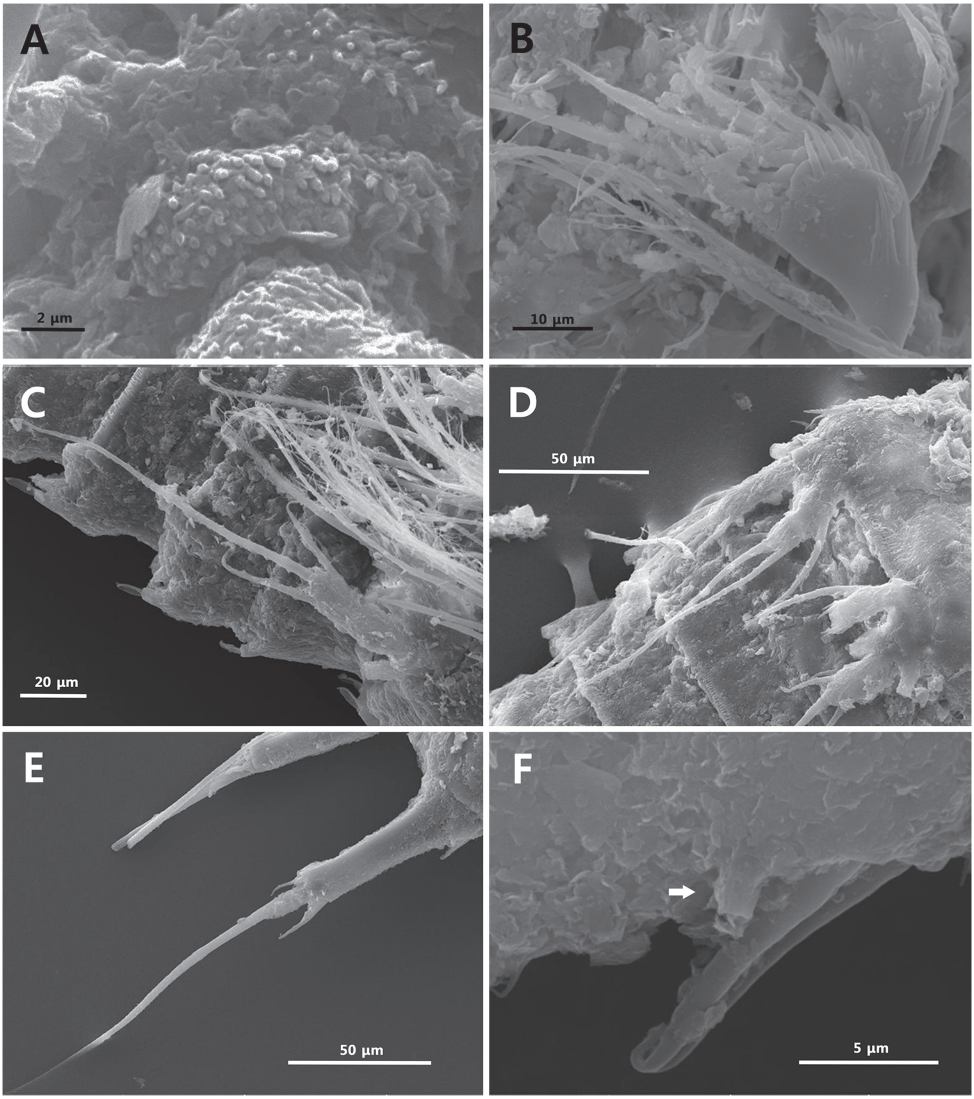

Figure 9. Scanning electron microscope photographs. *Strongylacron glabrum* sp. n. female: **A** surface of antennules, dorsal **B** antennary endopod **C, D** P5 **E** caudal rami **F** caudal setae I, II and tube pore (arrow).

Strongylacron glabrum sp. n. shows many differences from *St. buchholtzi*, including: (1) the anterior margin of the rostrum is slightly concave in the middle and naked between sensilla (vs. having row of fine setules (Sars 1909, pl. CXCVIII, R + a1.; Gee and Huys 1996, Fig. 1B)); (2) the outermost seta on the distal margin of the antennary endopod is stout as neighboring one (vs. slender than the neighboring one (Sars 1909, pl. CXCVIII, a2.; Gee and Huys 1996, Fig. 1C)); (3) the mandibular basis has one long and two short setae (vs. two long and one short setae (Gee and Huys

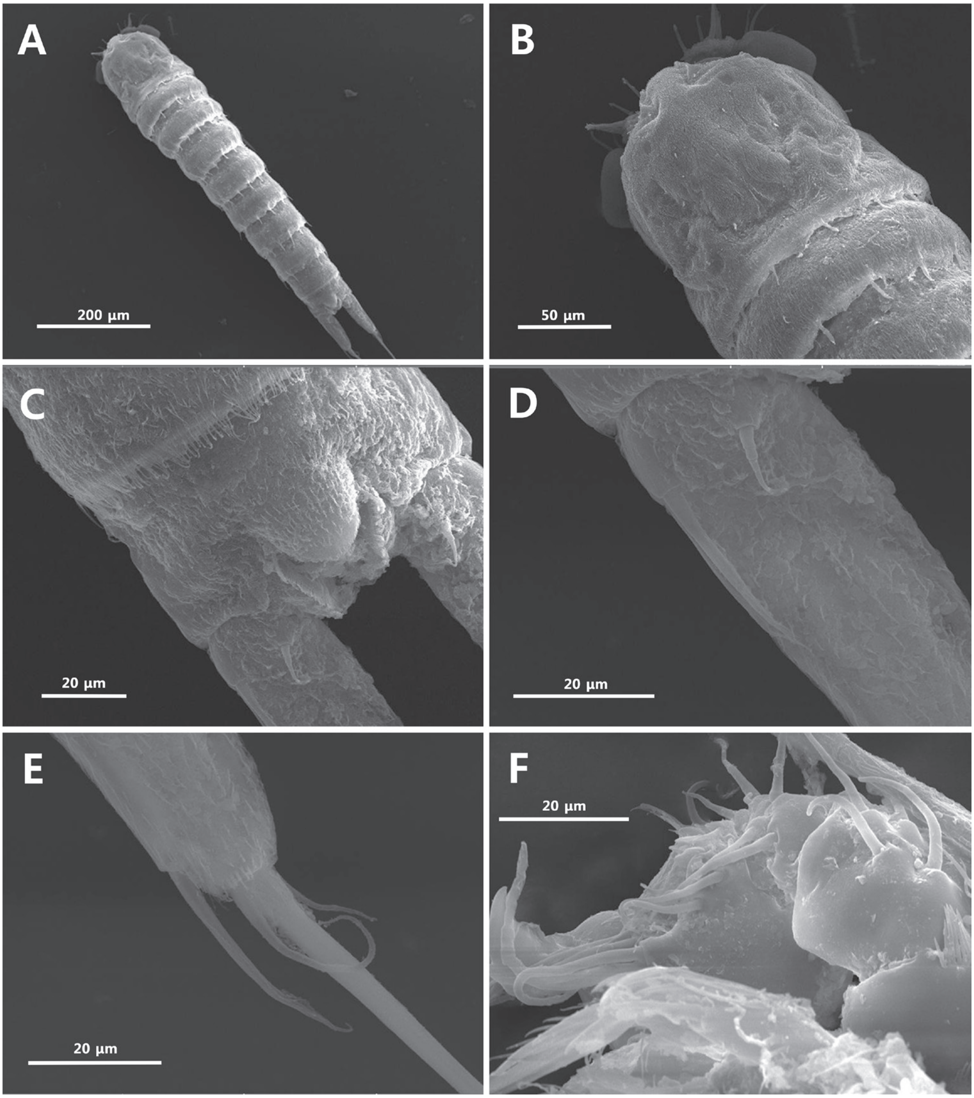

Figure 10. Scanning electron microscope photographs. *Strongylacron glabrum* sp. n. male: **A** habitus, dorsal **B** cephalothorax and thoracic somite 2, dorsal **C** anal somite, dorsal **D** proximal part of caudal ramus **E** distal part of caudal ramus, dorsal **F** antennule, ventral.

1996, Fig. 3B)); (4) the mandibular gnathobase does not have one plumose seta (vs. having a short and stout pinnate seta (Gee and Huys 1996, Fig. 3B)); (5) caudal seta VI is shorter than seta IV in length (vs. seta VI is longer than seta IV (Sars 1909, pl. CXCVIII, F.; Gee and Huys 1996, Fig. 2A)); (6) the tube pore on outer margin of caudal rami is proximally inserted (vs. medially (Gee and Huys 1996, Fig. 2A)); (7) the length to greatest width ratio of caudal ramus in male is about 3.6:1 (vs. 2.6:1 (Gee and Huys 1996, Fig. 2B)); (8) the length to greatest width ratios of female P5 exopod

Table 1. Morphological differences between *Strongylacron buchholtzi* and *S. glabrum* sp. n.

Character	*S. buchholtzi*	*S. glabrum* sp. n.
Body		
length (μm)♀	500–810	889–923
Prosomite		
number of rod-like projections on posterior border	14–18	8–10
Rostrum		
anterior margin	with row of fine setules	naked
Antenna		
outermost seta on distal margin of endopod	slender	stout
Mandible		
gnathobase	with seta	without seta
basis	with 2 long, 1 short	with 1 long, 2 short
P5		
exopod, length ratio to greatest width♀	at most 3.0:1	approximately 3.5:1
endopodal lobe, length ratio to greatest width♀	at most 1.8:1	approximately 2.7:1
length of outer seta to baseoendopod to middle seta on exopod ♂	slightly short	half
Caudal ramus		
position of tube pore on lateral margin♀	median	proximal
seta III, length ratio to caudal ramus♀	1.1:1	0.4:1
seta VI, length ratio to caudal ramus♀	0.84:1	0.3:1
length ratio to greatest width ♂	2.6:1	3.6:1

and endopodal lobe are approximately 3.5:1 and 2.7:1, respectively (vs. at most 3.0:1 and 1.8:1, respectively (Sars 1909, pl. CXCVIII, p5.; Gee and Huys 1996, Fig. 3D)); (9) the innermost seta on female P5 exopod is shorter than the length of exopod (vs. longer than exopod (Sars 1909, pl. CXCVIII, p5.; Gee and Huys 1996, Fig. 3D)); (10) the outermost seta on the endopodal lobe of male P5 is reaching half of the middle one on exopod (vs. slightly shorter (Gee and Huys 1996, Fig. 4C)); (11) each posterior border of the prosomite has eight or ten rod-like projections (vs. 14–18 (Gee and Huys 1996, Fig. 1C)) (Table 1).

Members of the family Cletodidae are known as mud-burrowers from shallow and sublittoral marine habitats (Por 1986; Boxshall and Halsey 2004; Kim et al. 2014; Song et al. 2014). *Strongylacron* species were also reported from muddy bottoms. *Strongylacron buchholtzi* was known from intertidal and sublittoral (depth of 20 m) habitats on the Atlantic Ocean (northwestern Europe, Canada) (Boeck 1873; Sars 1909; Willey 1929; Wells 1963; Gee and Huys 1996). *Strongylacron glabrum* sp. n. was found from intertidal mudflats on the south-western coasts of South Korea. The genus *Strongylacron* herein is first recorded from the Pacific Ocean.

Key to known species of the genus *Strongylacron*

1 Rostrum furnished with densely fine setules on anterior margin; female P5 exopod and endopodal lobe at most 3.0 and 1.8 times as long as greatest width, respectively; each prosomite in female with 14–18 rod-like projections .... ***S. buchholtzi* (Boeck, 1873)**

– Rostrum naked between sensilla on anterior margin; female P5 exopod and endopodal lobe approximately 3.5 and 2.7 times as long as greatest width, respectively; each prosomite in female with 8–10 rod-like projections .... ***S. glabrum* sp. n.**

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RESEARCH ARTICLE

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A new species of the genus *Petrolisthes* Stimpson (Crustacea, Decapoda, Porcellanidae) from the Central Pacific, with remarks and new records for *P. aegyptiacus* Werding & Hiller

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Abstract

Petrolisthes paulayi **sp. n.** is described from specimens collected in French Polynesia. The new species belongs to an assemblage of morphologically similar Indo-West Pacific (IWP) species, here designated as the "mesobranchial-spine group". All species in the group bear carapace spines, including one or more mesobranchial spines, and transverse, piliferous striations on the dorsal surface of carapace and chelipeds. *Petrolisthes paulayi* sp. n. is distinguishable from all species in the group by its forwardly produced, trilobate front, and a characteristic combination of carapace spines. We also report on the range extension and live coloration of *P. aegyptiacus* Werding & Hiller, another species of the mesobranchial-spine group, so far considered a Red Sea endemic. Specimens from the Mascaréne Islands confirm that the geographic range of the species extends to the southern Indian Ocean. While specimens morphologically similar to *P. aegyptiacus*, and collected in the Line and Society Islands, suggest a large range extension to the Central Pacific, it is probable that these individuals represent an undescribed species closely related to *P. aegyptiacus*.

Keywords

Crustacea, Porcellanidae, *Petrolisthes*, new species, Indo-West Pacific, species complex, range extension

Introduction

The genus *Petrolisthes* Stimpson, 1858, is the most species-rich genus of the family Porcellanidae (Crustacea, Decapoda, Anomura, Galatheoidea), with 109 species worldwide (Dong et al. 2010; Hiller and Werding 2010; Osawa and McLaughlin 2010; Osawa and Maenosono 2011; Osawa and Uyeno 2013; Naderloo and Apel 2014; Werding and Hiller 2015). All species of the genus share a flattened body shape, but they are morphologically highly diverse, and were therefore classified by Haig (1960) into five "natural divisions". The largest and worldwide distributed division is characterized by teeth and spines on carapace and pereopods, and contains Ortmann's (1897) *P. galathinus*-group (surface of carapace and pereopods with marked transverse, pilliferous striations) and *P. lamarckii*-group (surface of carapace and pereopods relatively smooth). Kropp (1984) upgraded this group with newly described species. The two groups are taxonomically difficult because they contain numerous complexes of species with high intraspecific variation in diagnostic characters that sometimes overlap interspecifically. Some of these complexes have been disentangled by examining large series of specimens from different localities (e.g. Kropp 1984; Werding and Hiller 2015; Osawa and Ng 2016), and by comparing DNA sequences from different conspecific and sympatric morphotypes suspected to represent different species (Hiller et al. 2006; Hiller and Werding 2007, 2010).

While all Atlantic and East Pacific (EP) species (with one exception, *Petrolisthes sanfelipensis* Glassell, 1936) of the "*P. galathinus* group" do not bear spines on the mesobranchial margins of the carapace, a group of morphologically similar Indo-West Pacific (IWP) species is characterized by a set of one or more such spines. This group was represented until the end of the 1970s by *P. scabriculus* (Dana, 1852) and *P. militaris* (Heller, 1862) only. In the last three decades, six morphologically similar species were described: *P. celebesensis* Haig, 1981, *P. perdecorus* Haig, 1981, *P. heterochrous* Kropp, 1986, *P. nanshensis* Yang, 1996, *P. aegyptiacus* Werding & Hiller, 2007, and *P. holthuisi* Hiller & Werding, 2010. Only *P. aegyptiacus* is restricted to the Indian Ocean, and has been so far reported as the only endemic porcellanid from the Red Sea (Werding and Hiller 2007). We designate this assemblage of species as the mesobranchial-spine group. Here, we describe a new species of this group, *Petrolisthes paulayi* sp. n., from specimens recently collected in the Central Pacific by the Florida Museum of Natural History. We also provide a comprehensive and summarized table with the combination of morphological characters leading to the identification of all species of the group, with minimal ambiguity. Additionally, we discuss new information on the geographic range and coloration of *P. aegyptiacus*.

Material and methods

Specimens deposited in the collections of the Florida Museum of Natural History, University of Florida (UF), Gainesville, U.S.A., the Muséum National d'Histoire

Naturelle (MNHN), Paris, France, and the Justus-Liebig University (JLU), Giessen, Germany, were examined. Individuals of the new species and of *P. aegyptiacus* were counted and sexed, and carapace length and width of the holotype and largest paratype male and female were measured in mm using a stereoscope with a micrometer. Table 1 lists morphological diagnostic characters useful to identify all nominal species of the IWP mesobranchial-spine group, including *P. paulayi* sp. n.

Results

Systematic account

Family Porcellanidae

Petrolisthes paulayi sp. n.

http://zoobank.org/505F537B-319C-460F-9C53-47BF862FE799
Figs 1–3

Material. Holotype: UF43955, male, Line Islands, Palmyra Atoll, N side of Atoll, outer reef slope, from dead *Pocillopora* cf. *verrucosa* head, 10.6 m.

Paratypes: UF43956, 1 male, same collection data as holotype; UF10692, 1 male (with bopyrid), 1 ovigerous female, Kiribati, Line Islands, Tabuaeran Atoll, SSW side of Atoll, outer reef slope, under rock, 10-23 m; UF10693, 2 females (1 ovigerous), Kiribati, Line Islands, Tabuaeran Atoll, outer reef slope, from *Halimeda* sample, 10-23 m; UF10711, 1 female, Line Islands, Tabuaeran Atoll, W side, S of Main Reef Pass, outer reef slope, from dead *Pocillopora* cf. *verrucosa* head, 10-15 m; UF15894, 1 male, (photographed specimen, Fig. 3), French Polynesia, Society Islands, Moorea, Haapiti, just NW of Matauvau Pass, outer reef slope, 15-23 m.

Other material. UF10588, 12 specimens, Line Islands, Palmyra Atoll, Line, N side of Atoll, outer reef slope, dead *Pocillopora* cf. *verrucosa* head, 10.6 m; UF41926, 1 male, Kiribati, Line Islands, Starbuck, Starbuck Island, dead *Pocillopora*, 12 m; UF41916, 1 female, Kiribati, Line Islands, Starbuck, Starbuck Island, 12 m; UF40590, 2 males, 1 ovigerous female, Kiribati, Line Islands, Starbuck, Starbuck Island, 7 m; UF41980, 4 males, 4 females (3 ovigerous), Kiribati, Line Islands, Vostok, Vostok Island, dead *Pocillopora*, 10 m.

Measurements. Male holotype: carapace length 4.5 mm; carapace width 4.2 mm.

Largest male paratype: carapace length 4.8 mm; carapace width 4.5 mm.

Largest female paratype: carapace length 4.5 mm; carapace width 4.3 mm.

Description. Carapace (Figs 1, 3) slightly longer than broad. Front (Fig. 2a) trilobate, markedly produced beyond eyes, median lobe broadest, rounded, overreaching the slender, forwardly produced lateral lobes; frontal borders finely serrated. Orbits deeply rounded, inner margin armed with a small supraorbital spine, followed mostly by a second tubercle-like elevation that is sometimes armed with a spine; infraorbital

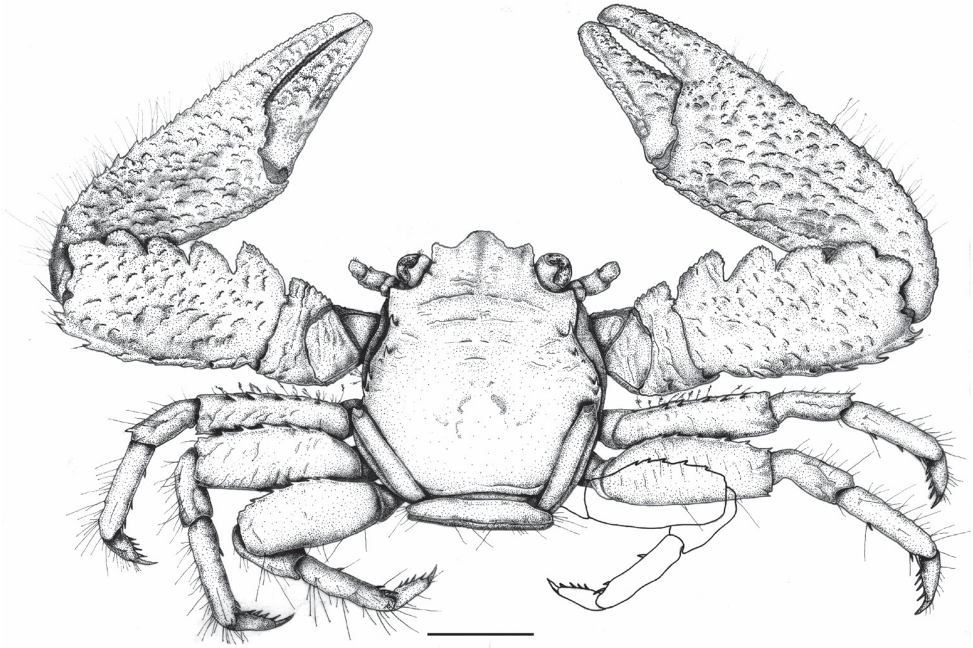

Figure 1. *Petrolisthes paulayi* sp. n. Male, holotype, dorsal view, UF43955, Line Islands, Palmyra Atoll. Right third walking leg supplemented. Scale bar: 2 mm.

angles forming an acute edge. Lateral margins (Fig. 2b) with a prominent epibranchial spine on epibranchial edge, and followed by a second smaller spine; two strong spines on mesobranchial margin. Carapace with few transverse, piliferous striations on protogastric ridge and on gastric region; epibranchial region rugose.

Third thoracic sternite (Fig. 2c) anteriorly trilobate, median lobe broad, lateral lobes slender, exceeding median lobe.

Telson (Fig. 2d) with seven plates.

First movable segment of antenna with an anterodistal slender projection bearing a narrow tooth. Basal segments of antennular peduncle bearing acute, irregular spines on anterior margin.

Chelipeds (Figs 1, 3) subequal, robust, dorsal surface somewhat convex, covered with interrupted scale-like ridges, granulated on ventral side. Merus with a large, serrate-edged tooth on anterior margin; carpus about 2.5 times as long as broad, with 4 rounded, serrated teeth on anterior margin, the proximal ones spine-tipped in some specimens; anterodistal edge with an additional blunt tooth; posterior margin with scale-like granules forming a row of 5 to 6 curved, upright spines, distally increasing in size. Manus moderately broad, posterior margin serrated but without spines, fringed with an irregular set of stiff and feathered setae; gape of fingers without distinct setation.

Walking legs (WL; Figs 1, 3) relatively robust, irregularly covered with scattered, simple and feathered setae of different size; merus of WL1 without spur-like spine on ventral, mid-distal margin; merus of WL1 and 2 with continuous transverse ridges;

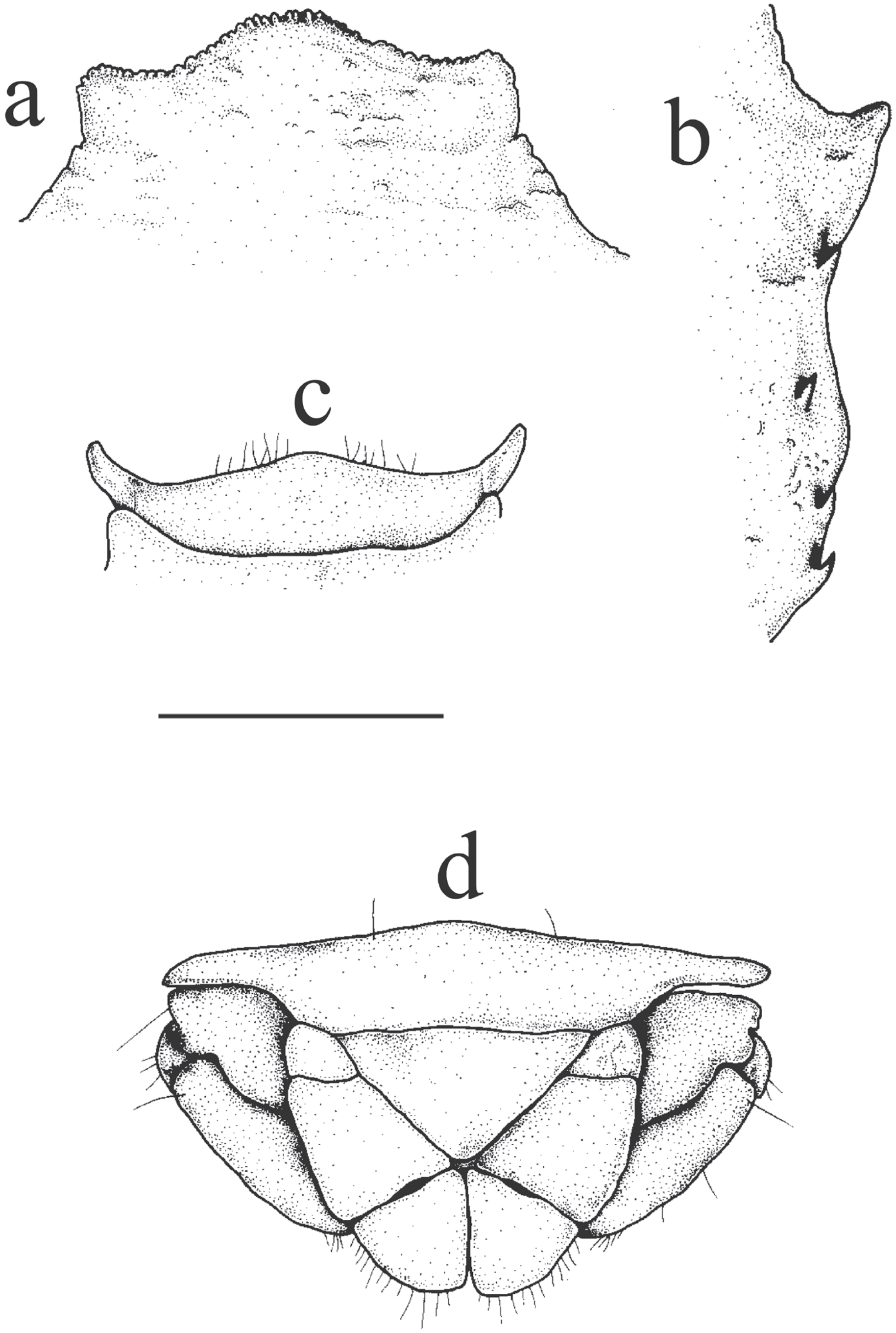

Figure 2. *Petrolisthes paulayi* sp. n. Male, holotype, UF43955, Line Islands, Palmyra Atoll. **a** Carapace front, dorsal view **b** right, lateral margin of carapace showing epibranchial and mesobranchial spines, dorsal view **c** third thoracic sternite, ventral view **d** last abdominal segment, telson and uropods, external view. Scale bar: 1 mm.

Figure 3. *Petrolisthes paulayi* sp. n., UF15894, Society Islands, Moorea. Photographed by the Moorea Biocode Marine Invertebrate Team (catalogue number dMBC08_03896, Florida Museum of Natural History).

merus of WL3 without such ridges; merus of all WL with row of spines on anterior margin, distributed as follows: WL1 and 3: 5, WL2: 4-5. Merus of WL1 and WL2 with posterodistal spine; Dorsodistal edge of carpus in WL1 produced into a sharp spine. Propodus of all WL with 1 or 2 movable spines in addition to the terminal spine-triplet on posterior margin. Dactylus of all WL with 4 movable spines on posterior margin.

Coloration (Fig. 3). The carapace has a yellow-orange background with a white, reticulate pattern in the posterior half. The white markings on the gastric region fuse into an irregular, transversal stripe towards the epibranchial edges. The inverted figure of a butterfly is depicted by a broad, white, curved band connecting the hepatic margins on both sides, two semicircular white spots mesial to the orbits, and another backwardly curved white band behind the front. The reticulate pattern continues to the anterior part of the abdomen and the proximal parts of the walking legs, covering half to most of the merus. This pattern is then replaced by a dark purple band that increases in size, and is followed by a narrower white band bordering the articulation with the purple-colored carpus. Dactylus is also purple. White bands on both ends of the propodus give the walking legs a uniform, ring-like aspect. The chelipeds exhibit a similar ground color that becomes darker distally, with white marks forming irregular rows of round spots on carpus and manus.

Ecology. The specimens examined were collected in depths between 7 and 23 m, on the outer reef slope, from *Halimeda* and dead *Pocillopora*. Further collections will probably confirm that *P. paulayi* sp. n. inhabits other exposed coral environments of the tropical western Pacific.

Distribution. The new species is known only from the Line and Society Islands in the Central Pacific.

Etymology. The new species is named after Gustav Paulay for supporting this and other studies on Porcellanidae, and for entrusting us with the porcellanid collection of the Florida Museum of Natural History.

Remarks. *P. paulayi* sp. n. can be be easily distinguished from other *Petrolisthes* species of the Indo-West Pacific by its unique color pattern, and by the combination of the following characters on the carapace: two mesobranchial spines, two epibranchial spines and a conspicuous trilobate front. The later character is known only in *P. elegans* Haig, 1981, which lacks mesobranchial spines, and only bears one epibranchial spine.

Petrolisthes aegyptiacus Werding & Hiller, 2007
Fig. 4

Petrolisthes aegyptiacus Werding & Hiller, 2007: 5, fig. 4 (type locality: Egypt, Red Sea).

Material. UF12962, 1 ovigerous female, Mascaréne Islands, La Réunion Island, Saint-Leu, Sec Jaune, rocky slope, basalt blocks, fore reef, under rocks, 10-19 m; UF13075, 1 male, La Réunion Island, Boucan Canot, Paine au Sucre, 10-15 m; UF33079, 1 male, 1 ovigerous female, Red Sea, Saudi Arabia, Thuwal, Al-Fahal reef, 1-37 m; UF36734, 1 specimen (identified from photograph), Red Sea, Saudi Arabia, offshore of Farasan Banks, Shib Radib, fore reef wall and barrier reef flat, 7-9 m; UF15472, 1 male, French Polynesia, Society Islands, Moorea Island, mid N coast, off Sheraton Hotel, outer reef slope, from within rubble; UF15474, 1 female (ovigerous), same data as UF15472.

Description. Coloration. Fresh specimens from the Indian Ocean and the Central Pacific are white or beige on the anterior part of carapace and chelipeds (Fig. 4). The chelipeds may be entirely white, but usually show vivid red spots towards the finger-tips. A row of small, purple spots may border the outer edge of carpus and manus. The front of the carapace may be fringed with some irregular, purple-brown spots, while a larger purple spot delimits the infra-ocular edge. The metabranchial regions bear a large, semi-lunar red blotch that extends towards the basal parts of the walking legs. The walking legs show a ring-like pattern. The surface of legs can be dark-purple or red with white marks, one of them on the distal margin of the merus, one at half distance of the propodus, and another near the articulation with the dactylus.

The red marks of the posterior part of the carapace extend to the lateral part of the segments of the abdomen that are visible from dorsal view. The median part of the first two or three segments of the abdomen is beige, interrupted by irregular red spots. The remaining posterior segments of the abdomen are entirely purplish. The whitish

Figure 4. *Petrolisthes aegyptiacus* Werding & Hiller 2007, female, Red Sea, Egypt, Dahab. Scale bar: 3 mm.

and reddish color, typical of *P. aegyptiacus*, suggests a camouflaging strategy, as the color of the substrate inhabited by the species is usually spotted with red Foraminifera (*Homotrema* Hickson, 1911).

Distribution. Previously only known from the Red Sea and the Mascaréne Islands in the southwestern Indian Ocean, and the Society and Line Islands in the Central Pacific Ocean (for the occurrence in the Pacific, see discussion below).

Discussion

Petrolisthes paulayi sp. n. was first found in the Line Islands, and subsequently in the Society Islands. The new species is probably confined to the Central and West Pacific, and seems to have a wide ecological range, as it occurs in shallow and deeper waters (7–23 m), and is adapted to different habitats, including corals and rocks. *P. paulayi* sp. n. is morphologically most similar to *P. heterochrous* because the two species bear an identical combination of carapace spines (Table 1). However, the new species is distinguishable from *P. heterochrous* by its conspicuous, trilobate front. These two species can

Table 1. Diagnostic characters for identification of the *Petrolisthes* species comprising the Indo-West Pacific mesobranchial-spine group. Diagnostic characters for identification of the *Petrolisthes* species comprising the Indo-West Pacific mesobranchial-spine group. SOS = number of supraocular spines; EBS = number of epibranchial spines; MBS = number of mesobranchial spines; CTF = conspicuous trilobate front; SP-WL1 = presence of spur-like spine on walking leg 1; LSC = abundant, long setae on carapace.

Species	SOS	EBS	MBS	CTF	SP-WL1	LSC
P. aegyptiacus	1	1	2	No	No	No
P. celebesensis	0	1	1	No	No	No
P. heterochrous	1	2	1-2	No	No	No
P. holthuisi	1	2	2	No	Yes	No
P. militaris	1	2	>2	No	No	No
P. nanshensis	0	2	1	No	No	No
P. paulayi sp. n.	1-2	2	2	Yes	No	No
P. perdecorus	1	2	2-3	Yes	No	Yes
P. scabriculus	2	2	>2	No	No	No

be distinguished from the other members of the mesobranchial-spine group by their lack of long, conspicuous setae on the carapace, typical of *P. perdecorus*, and by the lack of a spur-like spine on the ventral, mid-distal margin of the merus of the first walking legs, specific of *P. holthuisi* (see fig. 3 in Hiller and Werding 2010).

Petrolisthes aegyptiacus was originally considered a potential Red Sea endemic, based on few specimens collected in Quseir, Egypt, and Sanganeb Atoll, Sudan, and deposited in an old collection of the Museum für Naturkunde, Stuttgart, Germany (Werding and Hiller 2007). The species is here confirmed as a non-endemic of the Red Sea region, as the new material suggests that the species is also distributed in the southern Indian Ocean and the Central Pacific. However, preliminary comparisons of mitochondrial DNA sequences of specimens from the Indian Ocean and the Central Pacific (Hiller, unpublished data) suggest that specimens from the Line and Society Islands probably represent an undescribed species closely related to *P. aegyptiacus*.

According to the present findings and those by Werding and Hiller (2007), no porcellanid species is endemic to the Red Sea. From the 18 species occurring in this sea, 12 extend their ranges into the West Pacific, and the rest into the southern Indian Ocean. These observations confirm those previously made by Lewinsohn (1969), who questioned the presence of endemic anomuran decapods in the Red Sea.

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Revision of the genus *Gryposmylus* Krüger, 1913 (Neuroptera, Osmylidae) with a remarkable example of convergence in wing disruptive patterning

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Abstract

The charismatic lance lacewing genus *Gryposmylus* Krüger, 1913 (Osmylidae: Protosmylinae) from South East Asia is revised with a new species (*G. pennyi* **sp. n.**) described from Malaysia. The genus is diagnosed and both species in the genus redescribed and figured. An extraordinary example of morphological convergence is presented, with disruptive camouflaging wing markings in *G. pennyi* **sp. n.** being remarkably similar to the South American green lacewing *Vieira leschenaulti* Navás (Chrysopidae).

Keywords

Osmylidae, convergence, camouflage, lacewing

Introduction

Lance lacewings (Osmylidae) are a charismatic family of Neuroptera found throughout the world except North America. The family currently contains almost 300 species, both extant and extinct (Oswald 2016). Osmylids are relatively basal representatives of Neuroptera, and are closely related to families such as Nevrorthidae and Sisyridae, as well as Coniopterygidae and Dilaridae (Winterton et al. 2010; Wang et al. 2016). The major works on the family were by Krüger (1912–1915b) early during the previous century, where he erected numerous genera often based on spurious wing venation features; today only approximately 25 genera are considered valid (Kimmins 1940; New 1986; Wang and Liu 2009). The division of the extant representatives of the family into eight subfamilies, including Eidoporisminae, Gumillinae, Kempyninae, Osmylinae, Porisminae, Protosmylinae, Spilosmylinae and Stenosmylinae, is far more stable, with each subfamily relatively easily diagnosable (Krüger 1912–1915b; New 1989). The extinct subfamily (Mesosmylininae) comprising at least seven genera is known from Late Triassic to Mid Cretaceous aged deposits (e.g., Jepson et al. 2009, 2012; Khramov 2014), while another extinct subfamily Cratosmylinae, containing the single species *Cratosmylus* Myskowiak, is known from Brazilian Cretaceous aged deposits (Myskowiak et al. 2015). Finally, the extinct subfamily, Epiosmylinae, is considered a junior synonym of Gumillinae (Lambkin 1988; Wang et al. 2009; Khramov 2014). The putative sister family to Osmylidae is Archaeosmylidae (Late Permian to Early Triassic) and is differentiated from the former based on several wing venation features, such as a non-pectinately branched forewing CuP (see Khramov 2014).

The osmylid subfamily Protosmylinae comprises four extant genera and at least four extinct genera and shares a close relationship with the subfamilies Spilosmylinae (Wang et al. 2010) and Gumillinae. The close relationship between these subfamilies is exhibited by similarities in the wing venation, most notably with the hind wing vein CuP being unbranched, while in other subfamilies the vein is highly pectinately branched along the posterior wing margin. Spilosmylinae and Protosmylinae share a series of features in the male genitalia, including a narrowly arched and apilose gonarcus, parameres present and fused apically into an arch-like shape (subarcus *sensu* Tjeder 1957) in most genera, narrow entoprocesses, abdominal scent glands absent and tergites 8 and 9 separate. In the female genitalia, both subfamilies share the reduction in size of sternite 8 with a concomitant migration of the sclerite posteriorly. During copulation, sternite 8 acts against a depression in the intersegmental membrane immediately posterior to sternite 7. In other subfamilies sternite 8 is regularly shaped and positioned immediately posterior to sternite 7, and is acted upon during copulation by gonopophyses 9.

The Oriental genera *Heterosmylus* Krüger and *Gryposmylus* Krüger are placed in Protosmylinae, along with the monotypic genus *Paryphosmylus* Krüger from Ecuador (Krüger 1912–1915b; Wang et al. 2010). Recent studies using molecular and morphological data also place the Oriental genus *Lysmus* Navás in the subfamily (SLW unpublished data). Fossil protosmylines are known from the Tertiary, namely *Protosmylus* Krüger (Hagen in Berentdi 1856) from Baltic amber and *Osmylidia* Cockerell from Florissant shale deposits (Cockerell 1908; Carpenter 1943). Older fossils are known also from the Mid Jurassic (e.g., *Juraheterosmylus* Wang et al.) (Wang et al. 2010), Late Jurassic (e.g., *Jurosmylus* Makarkin & Archibald) (Khramov 2014b) and Early Cretaceous (e.g., *Protosmylina* Jepson et al.) (Jepson et al. 2009). Clearly, while Protosmylinae are presently distributed only in the Oriental and Neotropical regions, the subfamily has had a much broader distribution previously, with fossils present throughout the Holarctic region.

Herein we revise the genus *Gryposmylus*, including a redescription of the type species (*G. pubicosta* (Walker)) (Fig. 1) and a description a new species (*G. pennyi* sp. n.) (Fig. 2). A key to species is provided and both species are figured. The new species of *Gryposmylus* presents a remarkable example of convergence in disruptive camouflage markings in the wings, exhibiting an amazing similarity with a very distantly related green lacewing (Chrysopidae) from South America.

Materials and methods

Terminology follows Tjeder (1957), Adams (1969) and Wang et al. (2011) with the following modifications based of detailed examinations and a recent assessment of structural homology across all genera of Osmylidae and related families of Neuroptera (SLW, unpublished data). Figure 5 depicts the various male genitalic structures (colour-coded) typically exhibited in Protosmylinae. In the male genitalia the baculum (*sensu* Tjeder 1957) is the anterior arm of the gonarcus (red colour). This structure is present in some subfamilies and may be articulated (e.g., Spilosmylinae) or fused with the rest of the gonarcus (e.g., Kempyninae, Protosmylinae). The parameres (green colour) herein are equivalent to the subarcus of Tjeder (1957). Parameres are present only in Osmylinae (paired structures), Spilosmylinae (fused ventrally and U-shaped) and Protosmylinae (fused dorsally and arch-shaped). The parameres are always closely associated with the mediuncus (pink colour), either immediately flanking the mediuncus (e.g., Osmylinae), cradling from below (e.g., Spilosmylinae) or immediately anterior to it (Protosmylinae). We follow Adams (1969) in the use of mediuncus for the main intromittent organ, which is equivalent to the parameres of some authors (e.g., Tjeder 1957; New 1986), or more recently the gonocoxite 10 complex of Aspöck and Aspöck (2008) and Martins et al. (2016), or gonocoxites by Ardila-Camacho and Noriega (2014). The gonarcus is variable in form depending on the subfamily, being relatively narrow, arching medially and lacking setae in Protosmylinae and Spilosmylinae. In Porisminae, Stenosmylinae, Kempyninae, Eidoporisminae and especially Osmylinae, the gonarcus is large and observable externally, with distinct setal pile evident. Entoprocesses (blue colour) are fused laterally on the gonarcus (*sensu* Tjeder 1957), and are equivalent to gonocoxite 9 of Adams (1969). In Spilosmylinae and Protosmylinae they are narrow and elongate, while in Osmylinae they are more reduced to shorter and broader processes. In Kempyninae and Stenosmylinae they are larger and subtriangular in shape. Aspöck and Aspöck (2008) and Martins et al. (2016) interpreted the gonarcus and entoprocessus as a complex of gonocoxite 9 and gonopophyses 9, respectively. In the female genitalia (Fig. 6), the structure of the female sclerites is also variable among subfamilies. The enlarged gonocoxite 9 (=gonopophyses lateralis) is closely associated anteriorly with gonopophysis 9 (=sternite 9 of Wang et al. (2011)) in Protosmylinae, Spilosmylinae and Osmylinae and two separate sclerites are clearly visible. By contrast, in more derived subfamilies Porisminae, Kempyninae, Eidoporisminae and Stenosmylinae, gonopophyses 9 is not closely associated as a regular sclerite with gonocoxite 9, but instead is more elaborately shaped as a single articulating sclerite which acts upon sternite 8. In all subfamilies there is a terminal stylus (gonostylus 9). Sternite 8 (*sensu* New 1986) has also been referred to as the subgenitale (*sensu* Tjeder 1957), or as a fusion of gonocoxite8+gonopophyses 8 by Aspöck and Aspöck (2008) and Martins et al. (2016). The shape and position of this sclerite is highly variable. In Osmylinae, Porisminae, Kempyninae, Eidoporisminae and Stenosmylinae it is a plate-like sclerite immediately posterior to sternite 7. In Kempyninae and Stenosmylinae it is frequently modified into a bowl-like structure. In Protosmylinae and Spilosmylinae, sternite 8 is much smaller in size and located posteriorly as a small knob-like process.

Wing vein terminology used here follows Makarkin et al. (2011) with regard to the identity of vein MA in both wings. Based on recent unpublished studies on wing venation and tracheation (SLW unpublished data) we disregard the assumption that the MA vein is fused anteriorly with R and that it is represented in both wings as the posterior-most vein of the Rs field (*sensu* Kukalová-Peck and Lawrence 2004) (Fig. 4). Consequently, we do not consider the sigmoid vein as the remnant of vein MA.

Genitalia were macerated in 10% KOH to remove soft tissue, then rinsed in distilled water and dilute glacial acetic acid, dissected in 80% ethanol and subsequently stained with a solution of Chlorazol Black in 40% ethanol. The dissected genitalia were placed in glycerine in a genitalia vial mounted on the pin beneath the specimen.

Taxonomy

Gryposmylus Krüger, 1913: 32.

Figs 1–4

Type species. *Chrysopa pubicosta* Walker 1860: 183, original designation.

Diagnosis. Forewing length 15–18 mm. Antennae much shorter than forewing length; head with posterior genal area relatively wide; prothorax length subequal to width; female forecoxa lacking pedicellate setae or anterior processes; wing ovoid, not falcate along posterior margin; costal area broad basally with basal crossveins arranged radially, costal crossveins simple with occasional forking; interlinking crossveins absent from entire wing margin; wing venation with relatively few crossveins; two gradate series well defined, divergent in orientation; single basal subcostal crossvein present; forewing with seven branches of Rs present, basal branch of Rs diverging close to origin of Rs on R_1; forewing M vein branching in proximal half of wing, basal to origin of basal branch of Rs; hind wing CuP as a single vein branching just before wing margin; male genitalia with gonarcus narrowly arched medially and apilose, anterior arms of gonarcus (=baculum) present, non-articulated; parameres present, ends fused anteriorly and forming an arch-shape; entoprocesses narrow, spatulate distally and curved dorsally; male tergites 8 and 9 as separate sclerites, scent glands absent; female genitalia with sternite 8 positioned posteriorly, small and knob-like (=subgenitale), hollowed depression in the membrane immediately posterior to sternite 7; sternite 7 unmodified; spermatheca as single lobe, folded onto itself; spermathecal duct greatly elongate and coiled around spermatheca.

Included species. *Gryposmylus pubicosta* (Walker); *Gryposmylus pennyi* sp. n.

Comments. *Gryposmylus* is a distinctive Oriental genus that is the putative sister genus of *Lysmus*. Both genera have the basal branch of forewing vein Rs diverging close to the origin of Rs on R. In *Gryposmylus* forewing vein M forks basally, or equal with, the origin of the basal branch of Rs, while in *Lysmus* this fork is distal to the origin of the basal Rs branch. The costal margin of *Gryposmylus* is wider basally than in all other Protosmylinae genera, and the basal 7–8 costal crossveins are arranged in a slight radiating pattern, while in other genera they are usually parallel, or only the basal 2-3 veins are radially oriented. Also in *Gryposmylus* the forewing gradate series is generally divergent in orientation, while in other Protosmylinae genera they are subparallel. There is some variation among individuals in both *Gryposmylus* and *Lysmus* and the distinction of the genera is not consistently defined. At this time we maintain them as separate genera until more species are known and the limits of this variation are known.

Key to species of *Gryposmylus*

1 Head, thorax and abdomen almost entirely black with brown mottled patterning; forewing extensively marked along posterior margin and distally, distinct dark streak apically in both wings, pterostigmal marking relatively large (Fig. 3B) .... ***Gryposmylus pennyi* sp. n.**

– Head, thorax and abdomen mostly yellow with extensive reticulated brown patterning; mesoscutum and parts of pleuron white; extent of forewing markings highly variable, from relatively few markings (Fig. 3A) to more extensive, but rarely concentrated in any region of wing, apical streak lacking, hind wing unmarked except for pterostigma, pterostigmal markings relatively small...... .... ***Gryposmylus pubicosta* (Walker)**

Gryposmylus pubicosta Walker

Figs 1, 3A

Chrysopa pubicosta Walker 1860: 183

Gryposmylus pubicosta (Walker)– Krüger 1913: 32; Carpenter 1943: 755 [text figure 1].

Material examined. Lectotype [sex not determined]. **INDIA**: “Hindostan” (Natural History Museum, London). Herein designated.

Other material examined. INDIA: Himchal Pradesh Prov.: male, Kano, S[h]imla, McLachlan Collection, B.M. 1938- 674 (Natural History Museum, London); Uttarakhand Prov.: female, “Masuri” [Mussoorie], 7000 feet, 18.vi.1868, Lang McLachlan Collection, B.M. 1938- 674 (Natural History Museum, London). **MALAYSIA**: Sabah (Borneo): female, Crocker Range National Park, HQ Station Road, 9.viii.2003, Whiting, Svensen, Bybee (California State Collection of Arthropods); 2 females,

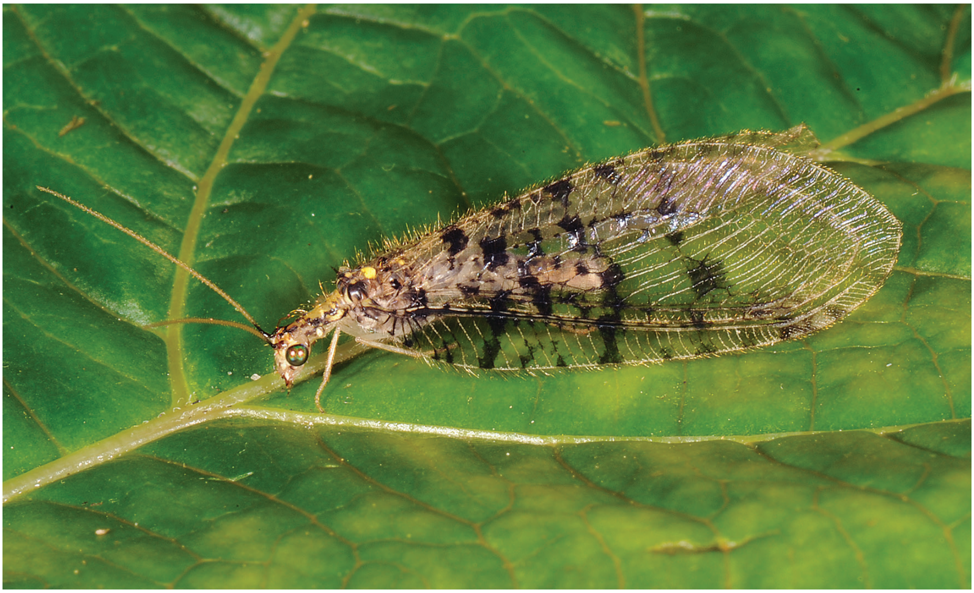

Figure 1. *Gryposmylus pubicosta* (Walker), male (dark form) Sabah, Malaysia (photograph credit: Stephen D. Gaimari).

Penampang Distr., Crocker Range Gunung Alab, 1660 m, 5°48'47"N 116°20'16"E [5°48.78', 116°20.26'] S. Gaimari, M. Hauser, 16–18.x.2011, ex. Mercury vapour light (California State Collection of Arthropods).

Diagnosis. Head and body largely yellow with brown reticulated markings; mesoscutum and parts of pleuron white; forewing markings mottled, highly variable; hind wing unmarked except region immediately around pterostigma.

Redescription. Forewing length: 21–22.0 mm; hind wing length: 16–17.5 mm. *Head*. Dark yellow with brown and white markings; palpi dark yellow; frons with dark, subtriangular marking below antennal socket, clypeus often with smaller marking laterally; dark genal mark small or large; vertex dark yellow with white area laterally, ocelli pale, surrounded with dark marking medially, dark vertex marking extending posteriorly as dark stripe from lateral ocellus; dark marking on gena along posterior margin of eye; scape dark brown, dark yellow on anterior surface; pedicel dark brown-black; flagellum dark yellow except for black basal three flagellomeres. *Thorax*. Prothorax dark yellow with black and white markings along lateral margins, setae relatively elongate, especially along lateral edge; mesothorax dark yellow with extensive dark brown-black markings, anteriorly with a dark spot and laterally with radiating pattern of brown streaks, a tuft of elongate dark setae is present anteriorly on the mesoscutum; mesoscutellum black laterally and anteriorly, vivid yellow-white posteriorly; metathorax dark yellow with dark brown spot medially, metascutellum black laterally, yellow-white posteriorly; pleuron dark yellow with broken white stripes; legs dark yellow, setal pale; claws brown. *Wings*. Rounded, venation brown with elongate setae on all veins on both surfaces of wings; wings hyaline with brown markings; extent of forewing markings highly variable, ranging from few markings to extensive markings in basal half of wing (Fig. 1), consistent markings in forewing of all specimens include: dark markings at base of costal area, at origin of M from R, crossveins 2–3cua-cup, distal crossvein ma-mp and extending along inner gradate series, distal crossveins r-rs and pterostigma; hind wing largely hyaline except for dark markings in pterostigma and distal crossveins r-rs, hind wing venation pale except for wing apex. *Abdomen*. Pale to dark yellow on all segments with dark brown reticulate pattern on tergites 1–8 and sternites 1–5; pale erect setae sparsely distributed on all segments. *Male genitalia*. Tergite 8 and sternite 8 quadrangular, sparsely distributed setae on sclerites and intersegmental membrane; tergite 9 relatively narrow, extending ventrally below level of ectoproct; sternite 9 subtriangular, fused partially to gonarcus laterally; ectoproct rounded with thickened area along posterolateral margin, callus cercus relatively large with *ca*. 45 setae; genitalia typical for subfamily, gonarcus as narrow arch medially, narrow entoprocessus extending posteriorly, curved dorsally and spatulate distally; gonarcus extending anteriorly as non-articulated rod-shaped apodemes (=baculum), gonarcus fused laterally to sternite 9 at junction of entoprocessus and gonarcus anterior apodeme; parameres narrow, arch-shaped with medial thickening; mediuncus curved with paired-flanges, connected membranously to medial arch of gonarcus. *Female genitalia*. Tergite 8 large and subquadrate, sternite 8 as small and knob-like process, directed posteriorly, adjacent to tergite 9; tergite 9 narrow, extending ventrally to articulate with gonopophysis 9 + gonocoxite 9 (=gonapophysis lateralis); gonopophyses 9 and gonocoxite 9 closely associated; gonocoxite 9 elongate with a dark longitudinal band laterally, distally articulated with a relatively long stylus (=gonostylus 9); ectoproct rounded, callus cercus relatively large; spermathecae folded medially, expanded basally and connecting with a very long coiled spermathecal duct.

Comments. The specific type locality for this species is listed as “Hindostan” by Walker (1860), which is a common geographic term for the entire northwestern portion of India. The McLachlan collection, now in the Natural History Museum collection, contains multiple specimens of *G. pubicosta*, presumably identified by Walker. Walker (1860) does not indicate that the description is based on a series of specimens, and the measurements provided in the description suggest that it was based on a single specimen. Moreover, at least one specimen in the McLachlan collection was collected in 1868, years after the original description of the species was published. Consequently, we do not consider these additional specimens as part of the syntype series but herein designate a Lectotype to clarify the status of this species.

This species is variable in the extent of body and wing markings, with some species being very pale with few wing markings (Fig. 3A) to others being very dark with extensive wing markings (Fig. 1). The male and female genitalia are very similar between both species in the genus.

Gryposmylus pennyi sp. n.

http://zoobank.org/4E470ED2-B2DF-4D17-B5B1-F71AAFBDA440
Figs 2, 3B, 4–6

Material examined. Holotype male. **VIETNAM**: Ninh Binh Prov.: Cuc Phueng National Park, 390m, 20°21'03"N. 105°35'36"E [20°21.05', 105°35.6'], S.D. Gaimari, M. Hauser, Pham H.T., 26.iii.2012, ex. Mercury vapour light (California State Collection of Arthropods).

Paratype female. **CHINA**: Yunnan Prov.: Mengla, Wangtianshu, 4.V.2005, Xiaoshuan Bai (China Agricultural University Collection).

Diagnosis. Head and body largely black with dark brown markings; forewing markings with distinct dark pattern, especially basally, and elongate band apically; hind wing with markings along posterior margin and in wing apex.

Figure 2. *Gryposmylus pennyi* sp. n., male, Sabah, Malaysia (photograph credit: Stephen D. Gaimari).

Figure 3. *Gryposmylus* spp.: **A** *G. pubicosta* (Walker) (pale form) (Forewing length 16.5 mm) **B** *G. pennyi* sp. n. (Forewing length 16.0 mm).

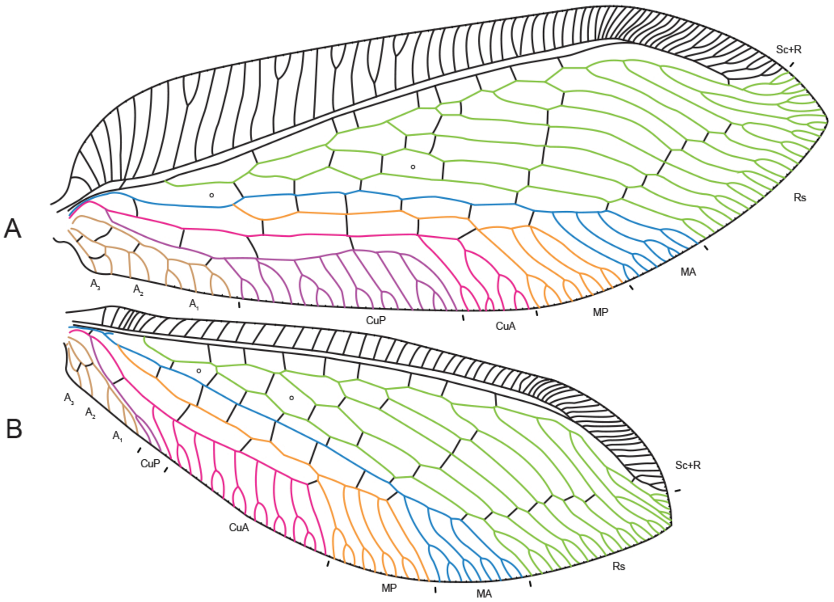

Figure 4. Wing venation of *Gryposmylus pennyi* sp. n.: **A** forewing **B** hind wing. Major wing veins are colour coded.

Description. Forewing length: 16.0–16.5 mm; hind wing length: 13.0–13.5 mm. *Head*. Predominantly black; frons cream-white with black opposing chevrons; clypeus with two black spots; gena with black spot; palpi white with dark bands on each segment; vertex black with lateral eye margin and ocelli white; antennal scape black, white on anterior surface; pedicel black; flagellum cream-white with basal flagellomere black. *Thorax*. Prothorax slightly narrowed anteriorly, predominantly black, white laterally and with three white spots along posterior margin; posterior intersegmental membrane white; prothoracic pile erect and a mixture of black and white setae; mesoscutum and metascutum black; pleuron with white and black longitudinal stripes, legs white, tibiae dark brown basally and setae on tibiae and tarsi yellowish. *Wings* (Figs 3B, 4). Forewing costal area broad with crossveins mostly simple, admixed with some forked veins (variable between wings and individuals); wing venation brown with elongate setae on all veins on both surfaces of wings; wings hyaline with extensive dark brown markings arranged in a broad sigmoid pattern (Fig. 2), extensive markings in posterior region of forewing, along both gradate series and apically along distal Rs veins; pterostigma very dark; hind wing mostly hyaline, venation pale; dark markings and venation at wing base, along posterior margin gradate series and from pterostigma to wing apex. *Abdomen*. Uniformly black, with dark brown markings. *Male genitalia* (Fig. 5). Tergite 8 and sternite 8 quadrangular, sparsely distributed setae on sclerites and intersegmental membrane; tergite 9 relatively narrow, extending ventrally below level of ectoproct; sternite 9 subtriangular, fused partially to gonarcus laterally; ectoproct rounded with thickened area along posterolateral margin, callus cercus relatively

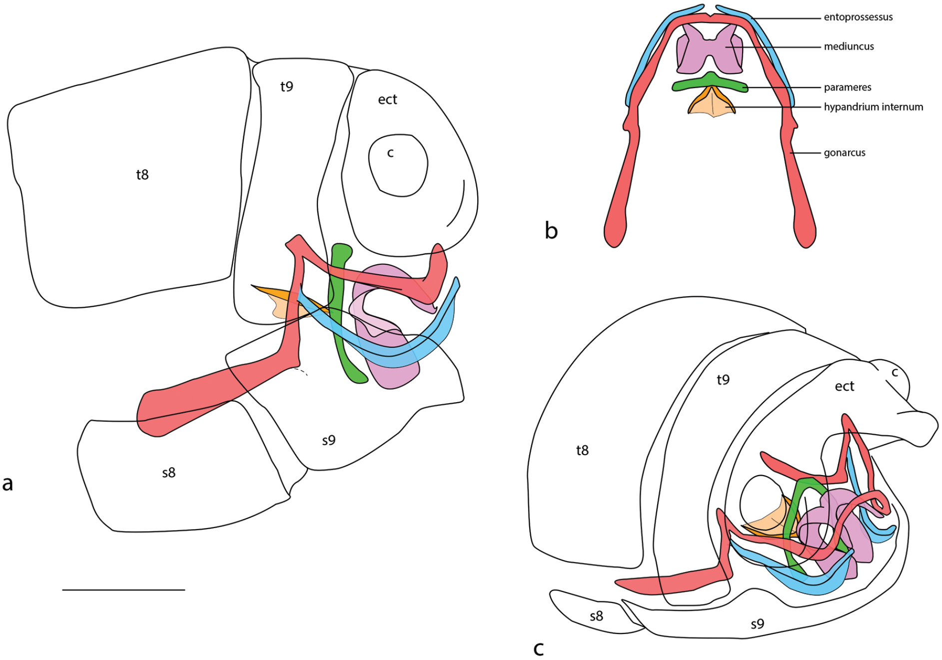

Figure 5. Male genitalia of *Gryposmylus pennyi* sp. n.: **A** lateral view **B** dorsal view **C** oblique view. Colour key: gonarcus (red), ectoprocessus (blue), mediuncus (purple), parameres (green), hypandrium internum (orange). Abbreviations: *t8*, tergite 8; *s8*, sternite 8; *t9*, tergite 9; *s9*, sternite 9; *ect*, ectoproct; *c*, callus cercus. Scale bar: 0.2 mm.

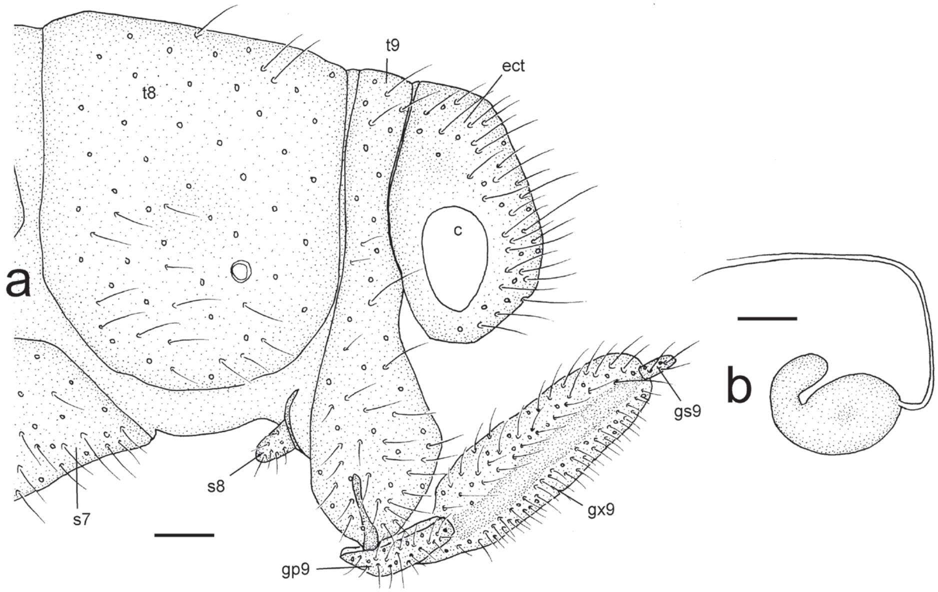

Figure 6. Female genitalia of *Gryposmylus pennyi* sp. n.: Additional abbreviations: *gx9*, gonocoxite 9; *gp9*, gonopophysis 9; *gs9*, gonostylus 9. Scale bars: 0.2 mm.

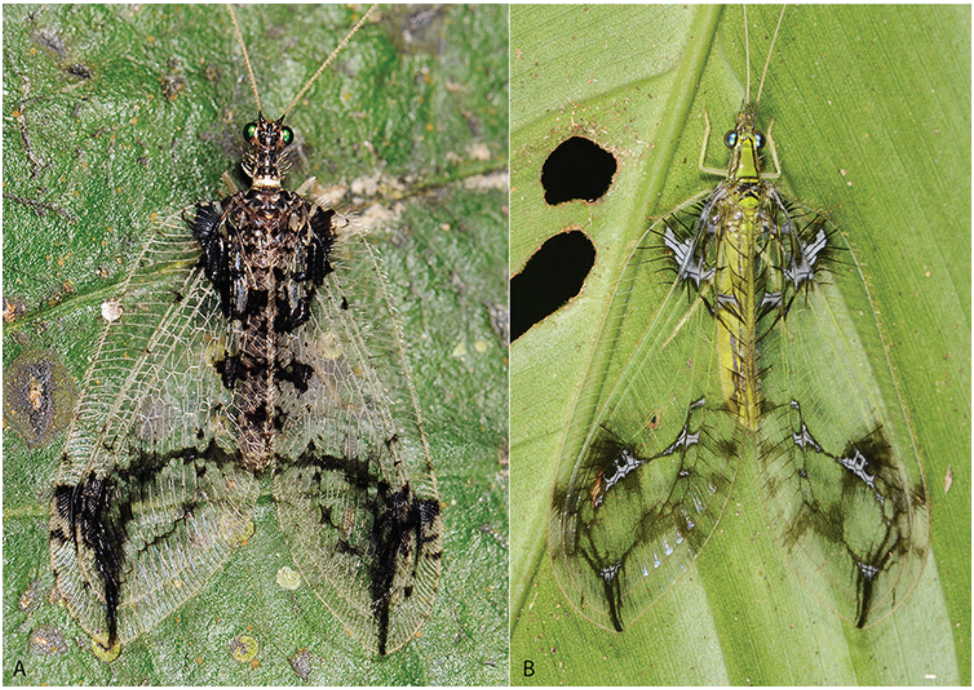

Figure 7. Comparison of *Gryposmylus pennyi* sp. n. (**A**) (Oriental) and *Vieira leschenaulti* Navás (**B**) (Chrysopidae) (Neotropical) (photograph credits: **A** Stephen D. Gaimari **B** Arthur Anker).

large with *ca*. 45 setae; gonarcus as narrow arch medially, narrow entoprocessus extending posteriorly, reflexed dorsally and spatulate distally; gonarcus extending anteriorly as non-articulated rod-shaped apodemes (=baculum), gonarcus fused laterally to sternite 9 at junction of entoprocessus and gonarcus anterior apodeme; parameres narrow, arch-shaped with medial thickening dorsally; mediuncus curved with paired-flanges, connected membranously to medial arch of gonarcus. *Female genitalia* (Fig. 6). Tergite 8 large and subquadrate, sternite 8 as small and knob-like process, directed posteriorly, adjacent to tergite 9; tergite 9 narrow, extending ventrally to articulate with gonopophysis 9 + gonocoxite 9 (=gonapophysis lateralis); gonopophyses 9 and gonocoxite 9 closely associated; gonocoxite 9 elongate with a dark longitudinal band laterally, distally articulated with a relatively long stylus (=gonostylus 9); ectoproct rounded, callus cercus relatively large; spermathecae folded medially, expanded basally and connecting with a very long coiled spermathecal duct.

Comments. *Gryposmylus pennyi* sp. n. is distributed in northern Vietnam and adjoining southern China. A specimen was also recently photographed from Sabah, Malaysia, with the image posted on social media website 'Facebook'; the specimen was identified but it was not collected. *Gryposmylus pennyi* sp. n. has distinctive wing markings (Fig. 7A), which show a peculiar similarity to those wing markings of an unrelated chrysopid, *Vieira leschenaulti* from the Amazon region of South America (Fig. 7B). This is a dramatic example of convergent wing patterning in distantly related lacewings, presumably associated with disruptive camouflage patterning to break up the outline of the individual as it sits on the underside of leaves in dense forested habitats.

Etymology. We have the great honour of naming this species after the Late Norman Penny (1946–2016). Norm was a wonderful colleague and excellent researcher of Neuroptera, with numerous publications on various lacewing families, especially on New World Chrysopidae.

Acknowledgements

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RESEARCH ARTICLE

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Parvitermes (Isoptera, Termitidae, Nasutitermitinae) in Central America: Two new termite species and reassignment of *Nasutitermes mexicanus*

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Abstract

The termite genus *Parvitermes* is now recognized on the Central American mainland to include *P. mexicanus*, **new combination** (previously in *Nasutitermes*) and two new species, *P. mesoamericanus* **sp. n.** and *P. yucatanus* **sp. n.**, herein described from soldiers and workers. These three species, nine West Indian *Parvitermes*, and *Antillitermes subtilis* all share characteristic enteric valve spines that orientate against intestinal flow. All species are subterranean nesters and cellulose feeders. Evidence is mounting that generic-level endemicity may be completely absent among the West Indian nasutitermitine fauna and that its origins stem from Central America.

Keywords

Neotropics, soldier key, enteric valve armature, new combination, taxonomy

Introduction

Emerson (1949) erected the genus *Parvitermes* to accommodate six small nasutiform termites: *Nasutitermes brooksi* Snyder, 1925 from Cuba, *Constrictotermes discolor* Banks, 1919 and *Nasutitermes wolcotti* Snyder, 1924a from Puerto Rico, *Constrictotermes flaveolus* Banks, 1919 and *C. pallidiceps* Banks, 1919 from Hispaniola, and *Nasutitermes laticephalus* Snyder, 1926 from Bolivia. Three additional *Parvitermes* species from Hispaniola were later added (*P. subtilis* Scheffrahn & Krecek, 1993, *P. collinsae* Scheffrahn & Roisin, 1995, and *P. dominicanae* Scheffrahn et al., 1998). The distribution of *P. brooksi* and *P. wolcotti* was expanded to include the central Bahamas (Scheffrahn et al. 2006) and the British and U.S. Virgin Islands (Scheffrahn et al. 2003), respectively.

Roisin et al. (1996) revised the small nasutes of the West Indies based mainly on worker morphology. The following taxa were reassigned to *Parvitermes*: *Constrictotermes toussainti* Banks, 1919, *Nasutitermes aequalis* Snyder, 1924b, and *Eutermes antillarum* Holmgren, 1910. Furthermore, *P. discolor* was placed in a new genus, *Caribitermes* Roisin, 1996, and *P. subtilis* was placed in another new genus, *Antillitermes* Roisin, 1996. The removal of *Parvitermes* as a Neotropical mainland genus was completed by Roisin et al. (1996), who transferred *P. laticephalus* to *Velocitermes*, and by Cuezzo and Cancello (2009), who showed that the Brazilian *P. bacchanalis* Mathews, 1977 should also be excluded from *Parvitermes*.

In the present paper, *Parvitermes* is shown to be a widespread endemic genus of the Central American mainland as *Parvitermes mexicanus* (Light, 1933), comb. n. and as two new Central American species, *P. mesoamericanus* and *P. yucatanus*. All three species are described mainly by the shape of soldier nasus and their enteric valve armature.

Materials and methods

All material is from the University of Florida Termite Collection (UF) at the author's address. Photographs (Figs 1A, 3–5) were taken as multi-layer montages using a Leica M205C stereomicroscope controlled by Leica Application Suite version 3 software. Preserved specimens were taken from 85% ethanol and suspended in a pool of Purell® Hand Sanitizer to position the specimens over a transparent Petri dish background. Microphotographs (Figs 1B, 1C, 2, 6) were taken from slide mounts in PVA medium (BioQuip, Rancho Dominquez, CA) using a Leica CTR 5500 compound microscope with bright field lighting. The distribution map (Fig. 7) was created using ArcGIS Desktop ver. 10.3 (ESRI, Redlands, CA).

Taxonomy

Key to soldiers of Central American species of *Parvitermes*

1 Head capsule widest near posterior third (Fig. 3A), nasus angled slightly above plane of vertex (Fig. 3B) .... ***P. mexicanus***

– Head capsule widest near middle (Fig. 3C, E), nasus angled in line or below plane of vertex (Fig. 3D, F) **2**

2(1) Nasus, in lateral view, nearly cylindrical in apical 2/3 (Fig. 3D) .... ***P. mesoamericanus* sp. n.**

– Nasus, in lateral view, conical (Fig. 3F) .... ***P. yucatanus* sp. n.**

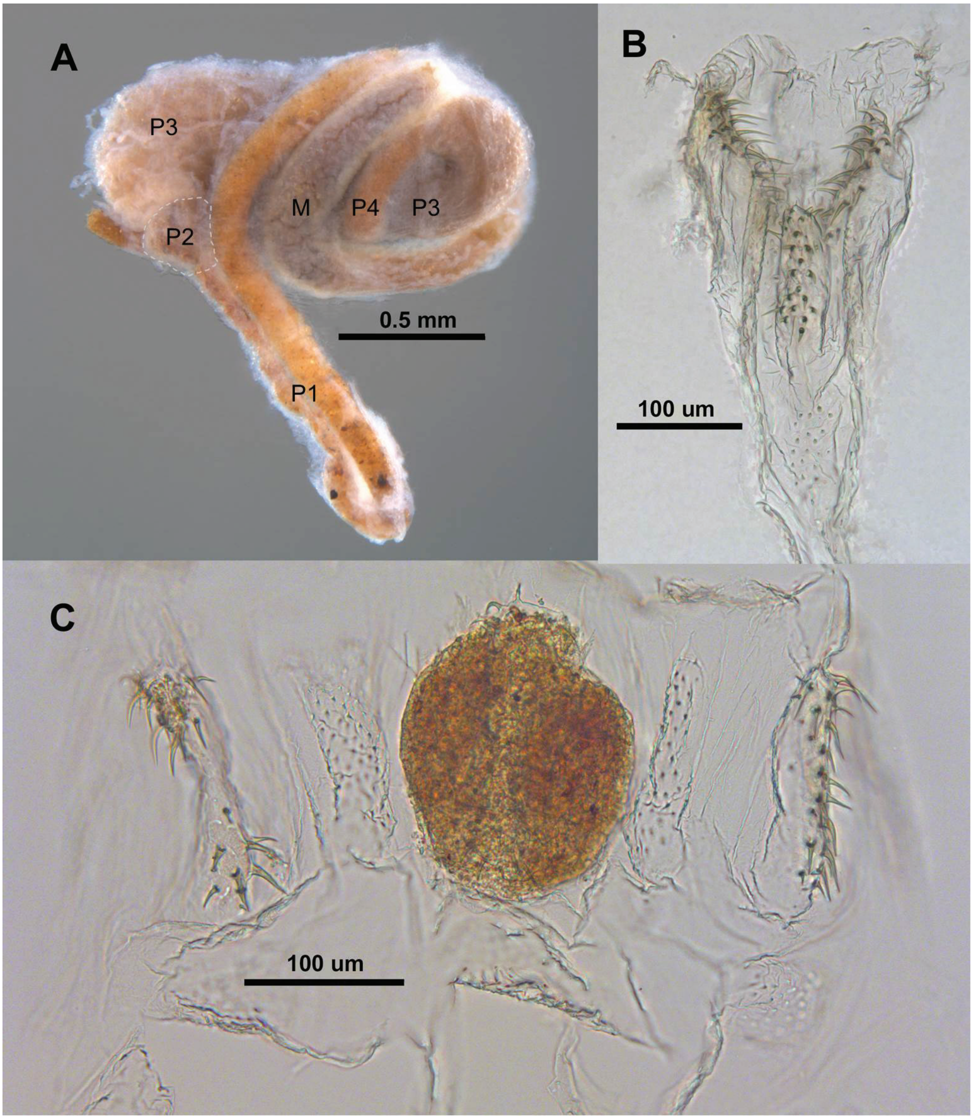

Figure 1. *Parvitermes brooksi*. **A** Dorsal left view of gut: M = mesenteron, P1-P4 = proctodeal segments 1-4 (limits of P2 highlighted) **B** Whole mount of P2 with musculature removed. Posterior (end attached to P3) at top of image **C** P2 splayed open; bacterial pellet attached to spines of the central pad.

Genus *Parvitermes* Emerson, 1949

Type species. *Nasutitermes brooksi* Snyder, 1925. Type: soldier; Cuba, Cienfuegos, Soledad.

Remarks. The nomenclatural summary for *Parvitermes* is provided by Krishna et al. (2013). The generic redescription of *Parvitermes* by Roisin et al. (1996) is relevant

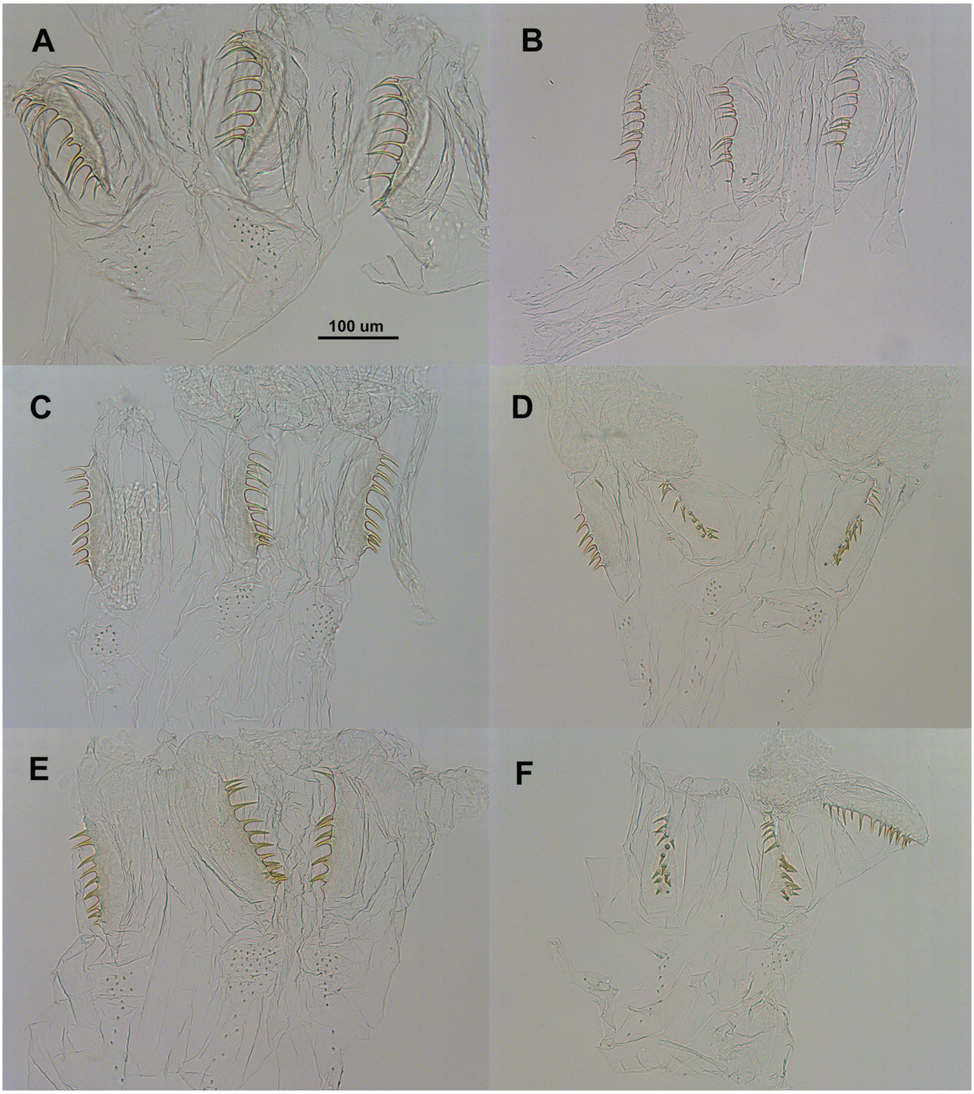

Figure 2. Enteric valve armatures. *Parvitermes mexicanus* comb. n., **A** worker **B** soldier. *Parvitermes mesoamericanus* sp. n. **C** worker **D** soldier. *Parvitermes yucatanus* sp. n. **E** worker **F** soldier. Posterior (end attached to P3) at top of each image.

to all three *Parvitermes* species described herein with the exception that *P. mexicanus* comb. n. has a shorter first proctodeal segment (P1) compared to all others.

Diagnosis. The spine arrangement and counter-current orientation of the *Parvitermes* enteric valve armature (EVA), with the exception of *Antillitermes*, is unique among all termite genera. In addition to *Parvitermes*, only three other nasutitermitine genera are found from Mexico to Nicaragua, including *Nasutitermes*, *Subulitermes*, and *Tenuirostritermes* (*Atlantitermes* from Nicaragua in Scheffrahn et al. (2005) is an error). Compared to mainland *Parvitermes*, head capsules of *Nasutitermes* soldiers are larger and darker (with the exception of *N. glabritergus* Snyder & Emerson, 1949 from Honduras, unpubl. record), those of *Subulitermes* are much smaller with much narrower cylindrical nasi, and the head capsules of *Tenuirostritermes* are very constricted near their middle.

Workers and soldiers. The EVA arises within the second proctodeal segment (P2) which forms a swelling at the terminus of a very long (shorter and thicker in *P. mexicanus*), U-shaped P1. The P2 constricts somewhat at its attachment near the dorsal surface of the third proctodeal segment (P3 or paunch) to form a pear-shaped segment (Fig. 1A). The posterior EV ring (*sensu* Noirot 2001) of both workers and soldiers is uniquely composed of three keel-shaped pads covered with about 7-15 long spines directed into the P2 lumen (Fig. 1B). The spines are curved or angled counter to the direction of the food flow. The spiny pads are separated with or without additional patches of tiny conical teeth (Figs 1C, 2). In preserved specimens, the *Parvitermes* spines of each pad are imbedded into a congealed pellet of presumed bacterial cells (Fig. 1C).

Parvitermes mexicanus (Light, 1933), comb. n.

Nasutitermes mexicanus Nickle & Collins, 1988 (soldier). Type localities: MEXICO: Colima: Colima, Jala, and Madrid.

Material examined. MEXICO, 76 km S. Oaxaca, 16.49, -96.74, 11 Jan 1997, T.G. Myles & D.A. Muruvanda, UF no. MX23; Chamela, 19.5314, -105.0832, 1 Apr 1996, G. Thompson, MX99; Aguaje de la Anona, 15.7731, -96.2168 , 27 May 2006, T. Atkinson, MX572. Soldiers of these three colony samples were identified based on the following: congruence with both dorsal and lateral head capsule photographs from the original description (Figs L, O, Light 1933), Light's 1933 rostrum (nasus) diagnosis stating that it is "slightly uplifted distally", proximity of the Chamela sample to the type localities, and Light's 1933 measurements. Furthermore, scanning electron micrographs of a *P. mexicanus* soldier from Chamela (figs 15D, H, Nickle and Collins 1988) agree perfectly with the examined soldiers.

Comparisons. See below under *P. mesoamericanus* sp. n.

Alate. Unknown.

Soldier (Table 1, Fig. 2B, 3A, B). Monomorphic; however some rare divergent size morphs reported by Light 1933. Head capsule and pronotum light orange-brown; nasus darker. In dorsal view, cephalic gland duct partially or completely visible from nasus to reservoir. Many small and a few longer setae scattered on head; setae on nasus very small and numerous.

In dorsal view, head capsule outline, without nasus, subtrapazoidal; nasus about 2/3 as long as rest of head capsule; head capsule slightly constricted behind antennal sockets; widest at posterior 1/3. In lateral view, vertex with slight concavities near

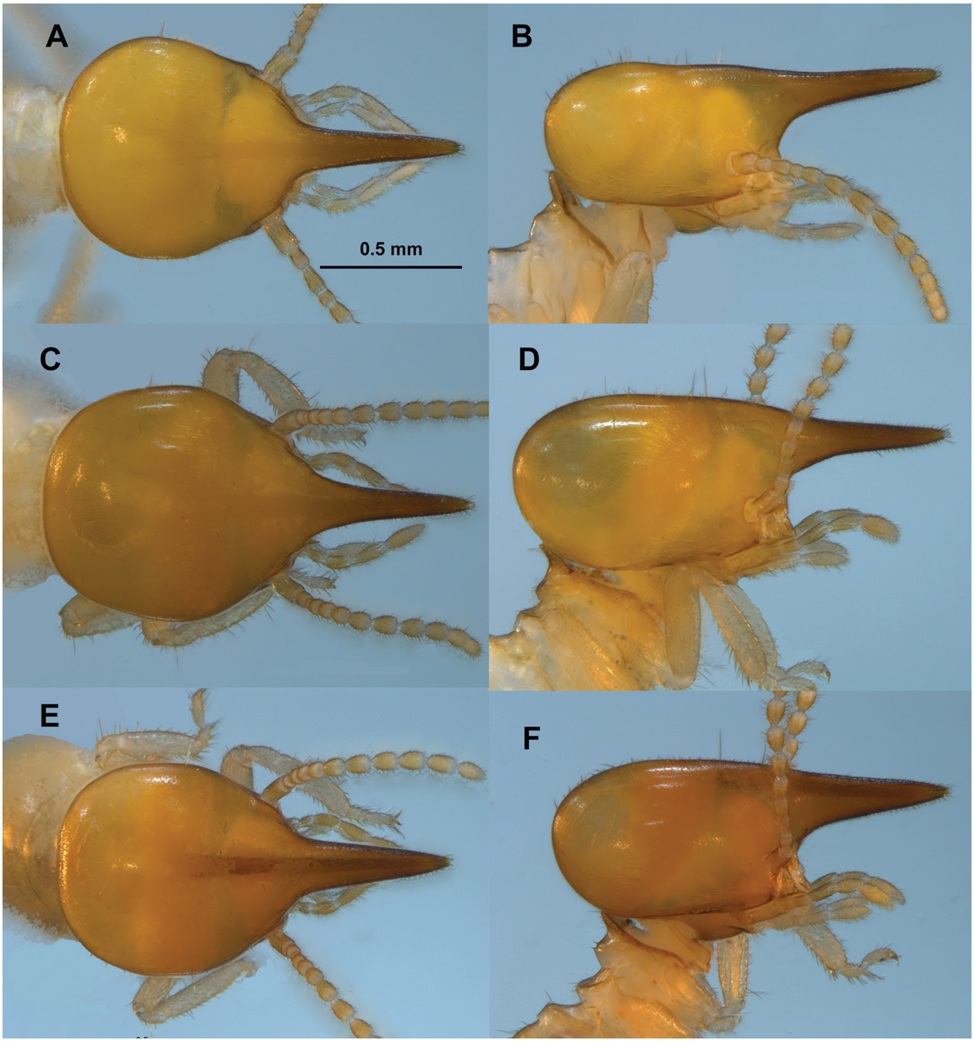

Figure 3. *Parvitermes* soldier head capsule. *Parvitermes mexicanus* comb. n., **A** dorsal **B** lateral. *Parvitermes mesoamericanus* sp. n. **C** dorsal **D** lateral. *Parvitermes yucatanus* sp. n. **E** dorsal **F** lateral.

midpoint; second slight concavity at base of nasus; plane of vertex parallel with ventral margin of head capsule. In dorsal view nasus is narrowly conical, about twice its width at base compared to midpoint. In lateral view nasus narrowly conical; angled ca. 5°above plane of vertex. Mandibles without points. Antennal with 13 articles (1>2<3>4). Hind tibia longer than head width. Pronotum with scattered microscopic setae (0.03 mm); anterior lobe evenly convex and ca. 90° from plane of posterior lobe, posterior lobe more blunt. Each tergite with 3-4 long (0.1 mm) setae and dozens of microscopic (0.03 mm) setae. EVA consists of three irregular rows of sharp, narrow, and down-curved spines; a few small scale-like spines in the anterior ring.

Table 1. Measurements (mm) of *Parvitermes mexicanus* comb. n. soldiers.

Colony	Head length to end of nasus	Head width (max.)	Pronotal width	Hind tibia length
MX23 (n=3)	1.44–1.52	0.80–0.82	0.42–0.46	0.94–0.96
MX99 (n=2)	1.50–1.52	0.88–0.92	0.44–0.45	0.92–0.93
MX572 (n=8)	1.34–1.46	0.72–0.80	0.44–0.46	0.89–0.96
Range	1.34–1.52	0.72–0.92	0.42–0.46	0.89–0.96
Mean	1.43	0.80	0.45	0.92

Table 2. Measurements (mm) of *Parvitermes mexicanus* comb. n. workers.

Colony	Head length to end of postclypeus	Postclypeal length	Head width	Pronotal width	Hind tibia length
MX23 (n=3)	0.84–1.06	0.21–0.22	0.88–1.06	0.44–0.48	0.72–0.84
MX99 (n=1)	0.92	0.23	0.96	0.42	0.74
MX572 (n=11)	0.80–1.01	0.21–0.26	0.90–1.04	0.40–0.55	0.74–0.94
Range	0.80–1.06	0.21–0.26	0.88–1.06	0.40–0.55	0.72–0.94
Mean	0.93	0.23	0.96	0.44	0.78

Worker (Table 2, Figs 2A, 4A, 5A, 6A). Monomorphic. Head capsule very pale yellow with two slightly darker yellowish-orange dorso-lateral patches; pronotum very pale yellow; body, antennae, and legs hyaline. Antennal with 12 articles. Postclypeus considerably inflated in lateral view; scattered short and medium setae on head capsule. Abdomen with many short and a few scattered longer setae. Mandibles with about eight ridges on molar plate, molar plate with distinct dorsal notch; apical and first marginal teeth of similar shape and projection; third marginal smaller, separated from first by slightly concave cutting edge. Gut with P1 U-shaped turn near midpoint, bottom of turn extending only to dorso-lateral aspect of abdomen. EVA consists of three irregular rows of about 10-12 sharp, narrow, and down-curved spines; anterior ring with three patches of small scale-like spines.

Distribution. Tropical Pacific slope of Mexico (Fig. 7).

***Parvitermes mesoamericanus* sp. n.**

http://zoobank.org/C83A167F-3E4F-43DE-9329-9232DD812A92

Type-locality. Honduras, S. Pinalillo, 15.0860, -88.2160, 144 m elev.

Holotype. Soldier, 2 Jun 2007, Scheffrahn et al. cols., UF no. HN822 (in microvial).

Paratypes. GUATEMALA: Salama, 15.1055, -90.3261 , 28 May 2006, Scheffrahn et al., GUA16; Road to Rabinal, 15.1045, -90.3722, 28 May 2006, Scheffrahn et al., GUA33; HONDURAS: Coyolito, 13.3149, -87.6227, 31 May 2007, Scheffrahn et al., HN431; NICARAGUA: Los Cardones, 12.8851, -86.0534, 30 May 2004, Scheffrahn et al., NI114.

Table 3. Measurements (mm) of *Parvitermes mesoamericanus* sp. n. soldiers.

Colony	Head length to end of nasus	Head width (max.)	Pronotal width	Hind tibia length
GUA16 (n=12)	1.38–1.50	0.76–0.84	0.36–0.41	0.66–0.78
GUA33 (n=12)	1.48–1.63	0.83–0.91	0.42–0.48	0.76–0.84
HN431 (n=12)	1.37–1.49	0.76–0.82	0.41–0.43	0.68–0.76
HN822 (n=10)	1.37–1.46	0.71–0.80	0.36–0.39	0.64–0.75
NI114 (n=12)	1.30–1.43	0.69–0.75	0.40–0.42	0.64–0.75
Range	1.30–1.63	0.69–0.91	0.36–0.48	0.64–0.84
Mean	1.44	0.78	0.40	0.72

Imago. Unknown.

Soldier (Table 3, Figs 2D, 3CD, 8). Monomorphic. Head capsule and pronotum light orange-brown; nasus darker. In dorsal view, cephalic gland duct partially or completely visible from nasus to reservoir. Many small, some medium, and a few longer setae scattered on head; setae on nasus small and numerous. In dorsal view, head capsule outline, without nasus, ovoid; nasus about 2/3 as long as rest of head capsule; head capsule barely constricted behind antennal sockets; widest in middle. In lateral view, vertex nearly in a flat plane; vertex and ventral margin of head capsule converge to front. In dorsal view nasus is narrowly conical, about thrice its width at base compared to midpoint; In lateral view nasus conical; projecting directly anterior below plane of vertex. Mandibles with short, very narrow, points. Antennal with 12 articles (1>2<3=4). Hind tibia about as long as or shorter than maximum head width. Pronotum with scattered microscopic setae (0.05 mm); anterior lobe evenly convex and ca. 90° from plane of posterior lobe, posterior lobe more blunt. Each tergite with 3-4 long (0.13 mm) setae and dozens of microscopic (0.05 mm) setae. EVA consists of three irregular rows of about 8-12 long subtriangulate, and very slightly down-curved spines; a few small scale-like spines in the anterior ring.

Worker (Table 4, Figs 2C, 4B, 5B, 6B, 8). Monomorphic. Head capsule very pale yellow with two slightly darker yellowish-orange dorso-lateral patches; pronotum very pale yellow; body, antennae, and legs hyaline. Antennal with 12-13 articles. Postclypeus considerably inflated in lateral view; scattered short, medium, and a few longer setae on head capsule. Abdomen with many short and longer setae. Mandibles with about eight ridges on molar plate, molar plate with slight dorsal notch; apical and first marginal teeth of similar shape and projection; third marginal smaller, separated from first by slightly emarginate cutting edge. Gut with very long P1; U-shaped turn near midpoint, bottom of turn extending to ventro-lateral aspect of abdomen. EVA consists of three irregular rows of about 8-12 long subtriangulate, and very slightly down-curved spines; three patches with small scale-like spines in the anterior ring.

Etymology and distribution. Named for Middle America which encompasses Guatemala, Honduras, and Nicaragua; the known range of this termite. The distribution habitat of *P. mesoamericanus* (Fig. 7) is more xeric than adjacent regions lacking this termite.

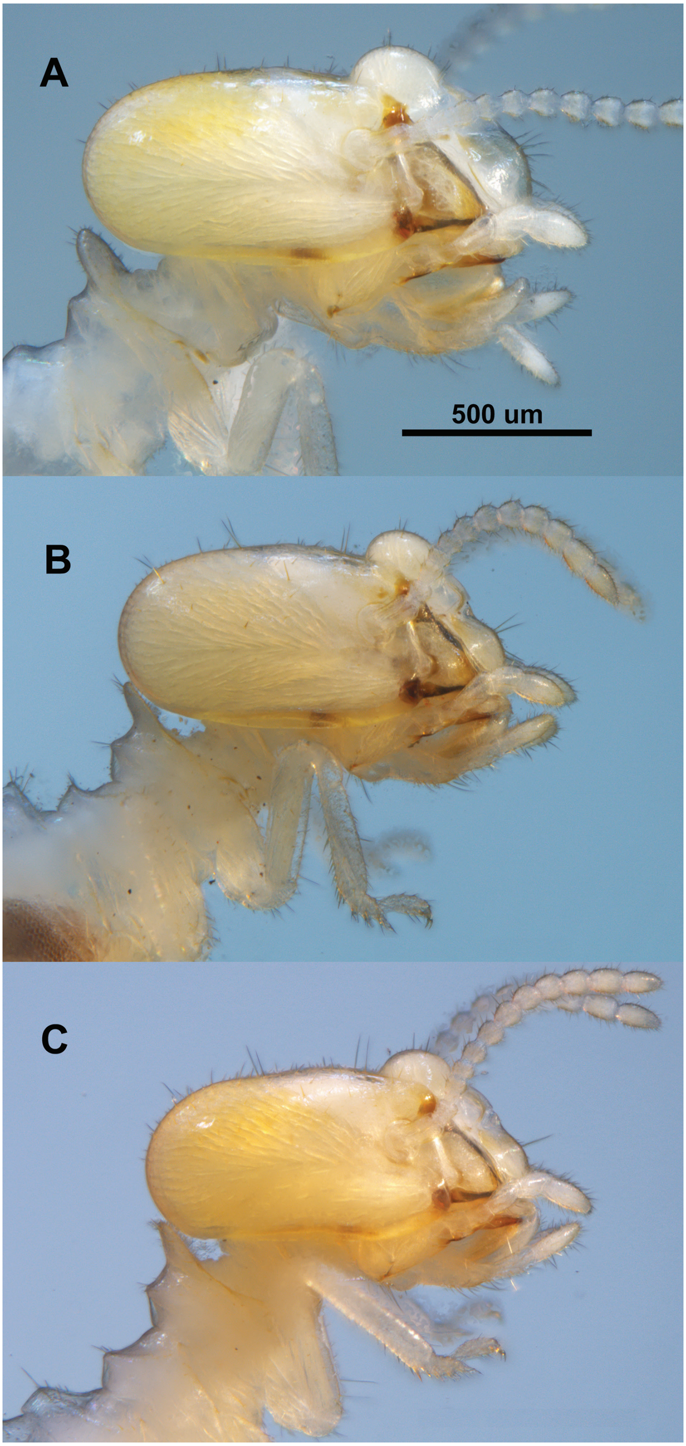

Figure 4. *Parvitermes* worker head and thorax, lateral views. **A** *P. mexicanus* comb. n. **B** *P. mesoamericanus* sp. n. **C** *P. yucatanus* sp. n.

Table 4. Measurements (mm) of *Parvitermes mesoamericanus* sp. n. workers.

Colony	Head length to end of postclypeus	Postclypeal length	Head width	Pronotal width	Hind tibia length
GUA16 (n=12)	0.74–0.92	0.17–0.23	0.80–0.92	0.39–0.52	0.54–0.75
GUA33 (n=12)	0.85–0.98	0.20–0.23	0.87–0.92	0.44–0.54	0.63–0.80
HN431 (n=12)	0.70–0.88	0.17–0.20	0.77–0.90	0.37–0.48	0.53–0.70
HN822 (n=10)	0.76–0.86	0.17–0.20	0.76–0.86	0.36–0.46	0.58–0.74
NI114 (n=12)	0.76–0.85	0.18–0.20	0.77–0.86	0.40–0.46	0.60–0.75
Range	0.70–0.98	0.17–0.23	0.76–0.92	0.36–0.54	0.53–0.80
Mean	0.83	0.20	0.84	0.45	0.66

Comparisons. The soldier of *P. mesoamericanus* has the nasus directed forward, the head capsule widest in the middle, a few scattered long setae on the vertex, and points on the mandibular stubs while in *P. mexicanus*, the nasus is slightly upturned, the head is widest in the posterior third, the vertex lacks scattered long setae, and the mandibular stubs have points. The worker of *P. mesoamericanus* has a much longer and more ventrally positioned P1, stouter and less curved EVA spines, and longer setae on the vertex, while in *P. mexicanus* the P1 is shorter and more dorsal, the EVA spines are thinner and more curved, and the setae on the vertex are shorter. The *P. mesoamericanus* worker is proportionally smaller to its soldier as compared *P. mexicanus*. Both castes of *P. mexicanus* have longer hind tibia than *P. mesoamericanus*.

Parvitermes yucatanus sp. n.

http://zoobank.org/0A5B0FFA-48A8-4DF0-84EA-0051AD9CA0E5

Type-locality. Mexico, 0.9 km N. gate of Punta Sam, 21.2423, -86.8056, 2 m elev.

Holotype. Soldier. 9 Dec 1997, J. Chase, J. Mangold cols., UF col. no. MX161 (in microvial).

Paratypes. GUATEMALA: P. N. Tikal, 17.1371, -89.6803, 30 May 2006, Scheffrahn et al., GUA222; MEXICO: Hwy 307, 1 km S Marine, 20.5803, -87.1424, 8 Dec 1997, J. Chase, J. Mangold, MX148; same data, MX152; Chicana Ecovillage, 18.5178, -89.4846, 21 Jan 2001, MX281; 10.5 km W Coba toward Chemax, 20.5514, -87.8049, 22 Jan 2003, J. Chase, J. Mangold, MX492.

Alate. Unknown.

Soldier. (Table 5, Figs 2F, 3E, F). In all respects, similar to *P. mesoamericanus* except for the following: In dorsal view nasus conical, about 1.6x its width at base compared to midpoint; in lateral view nasus broadly conical. Mandibles with short, very narrow, points. Antennal with 11-12 articles (1>2<3=4). Hind tibia usually shorter than maximum head width. Pronotum with a few longer setae (0.10 mm) along margin of anterior lobe.

Worker. (Table 6, Figs 2E, 4C, 5C, 6C). In all respects, similar to *P. mesoamericanus* except for the following: Mandibles with about seven ridges on molar plate, mo-

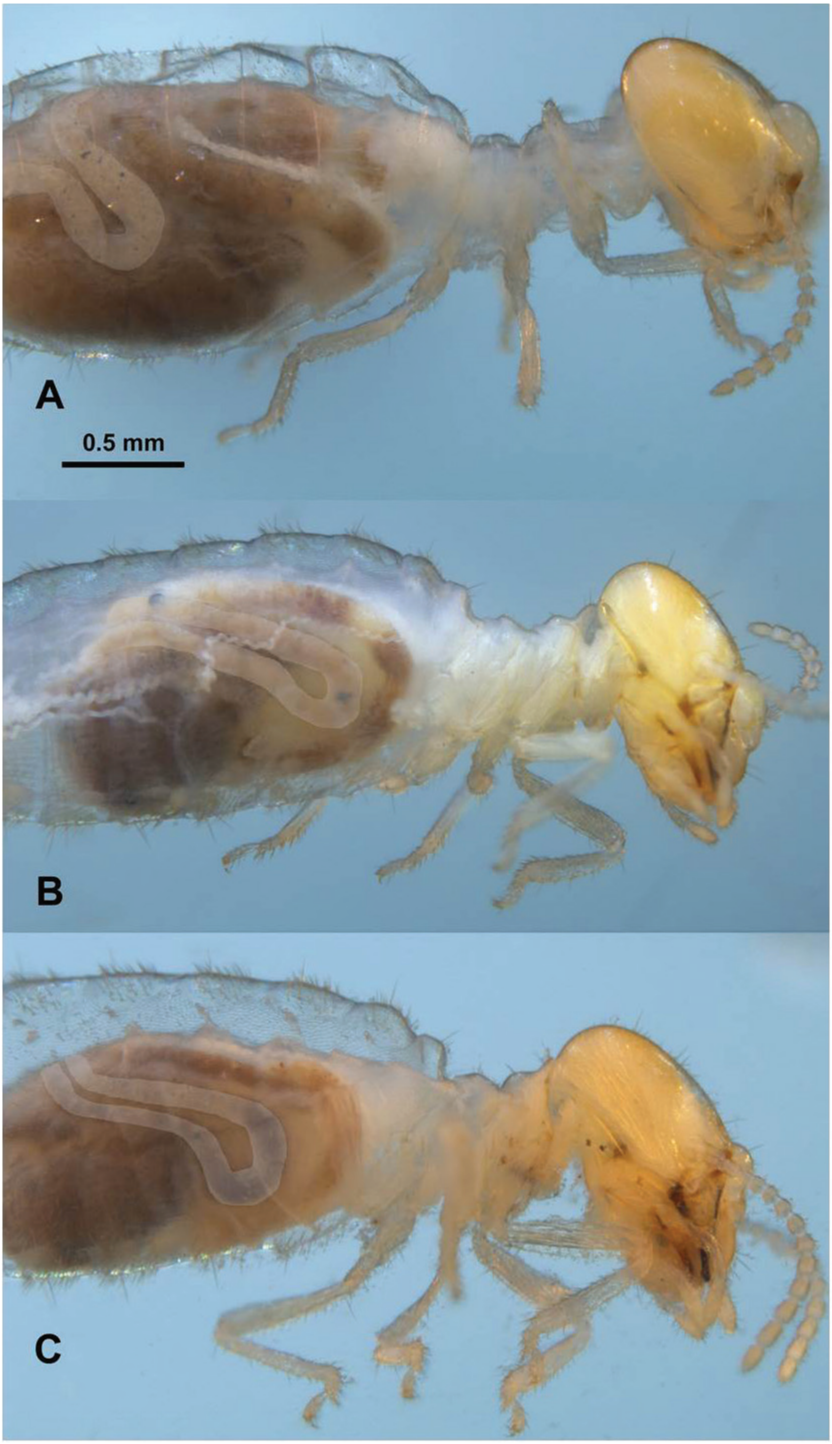

Figure 5. *Parvitermes* worker bodies with P1 highlighted. **A** *P. mexicanus* comb. n., dorsolateral view **B** *P. mesoamericanus* sp. n., ventrolateral view **C** *P. yucatanus* sp. n., ventrolateral view.

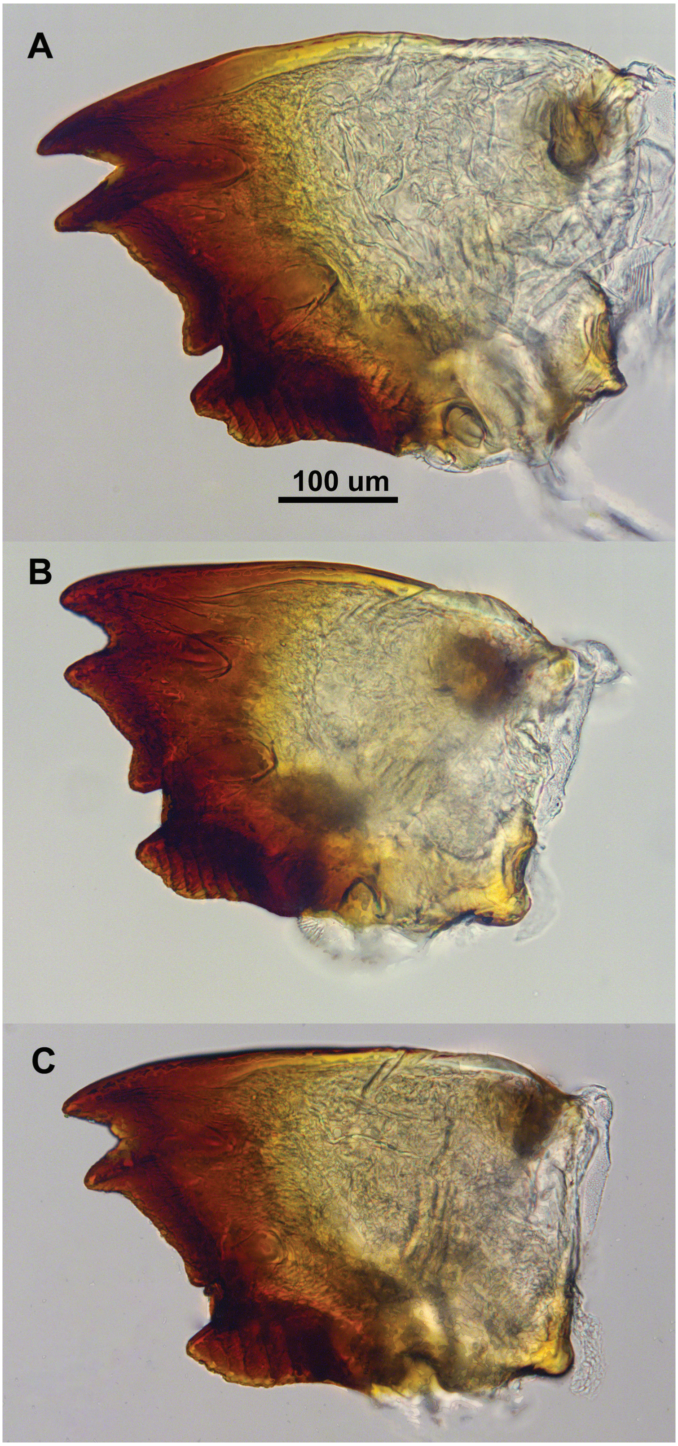

Figure 6. *Parvitermes* ventral views of left worker mandibles. **A** *P. mexicanus* comb. n. **B** *P. mesoamericanus* sp. n. **C** *P. yucatanus* sp. n.

Table 5. Measurements (mm) of *Parvitermes yucatanus* sp. n. soldiers.

Colony	Head length to end of nasus	Head width (max.)	Pronotal width	Hind tibia length
GUA222 (n=12)	1.36–1.45	0.70–0.78	0.36–0.40	0.65–0.70
MX148 (n=2)	1.28–1.29	0.66	0.36	0.58
MX152 (n=12)	1.34–1.42	0.68–0.74	0.34–0.40	0.64–0.70
MX161 (n=12)	1.36–1.46	0.74–0.78	0.38–0.44	0.66–0.74
MX281 (n=12)	1.38–1.45	0.72–0.78	0.34–0.39	0.64–0.76
MX492 (n=12)	1.32–1.42	0.72–0.78	0.34–0.38	0.64–0.70
Range	1.28–1.46	0.66–0.78	0.34–0.44	0.58–0.76
Mean	1.38	0.72	0.37	0.67

Table 6. Measurements (mm) of *Parvitermes yucatanus* sp. n. workers.

Colony	Head length to end of postclypeus	Postclypeal length	Head width	Pronotal width	Hind tibia length
GUA222 (n=12)	0.73–0.85	0.17–0.20	0.76–0.86	0.36–0.46	0.54–0.67
MX148 (n=4)	0.73–0.80	0.17–0.19	0.73–0.80	0.36–0.39	0.51–0.58
MX152 (n=12)	0.68–0.84	0.18–0..23	0.71–0.85	0.32–0.44	0.54–0.70
MX161 (n=12)	0.78–0.87	0.17–0.19	0.77–0.84	0.40–0.53	0.56–0.74
MX281 (n=12)	0.76–0.84	0.17–0.19	0.78–0.84	0.36–0.44	0.60–0.74
MX492 (n=12)	0.75–0.85	0.17–0.19	0.74–0.82	0.36–0.40	0.54–0.67
Range	0.68–0.87	0.17–0.23	0.71–0.86	0.32–0.53	0.51–0.74
Mean	0.78	0.18	0.79	0.38	0.61

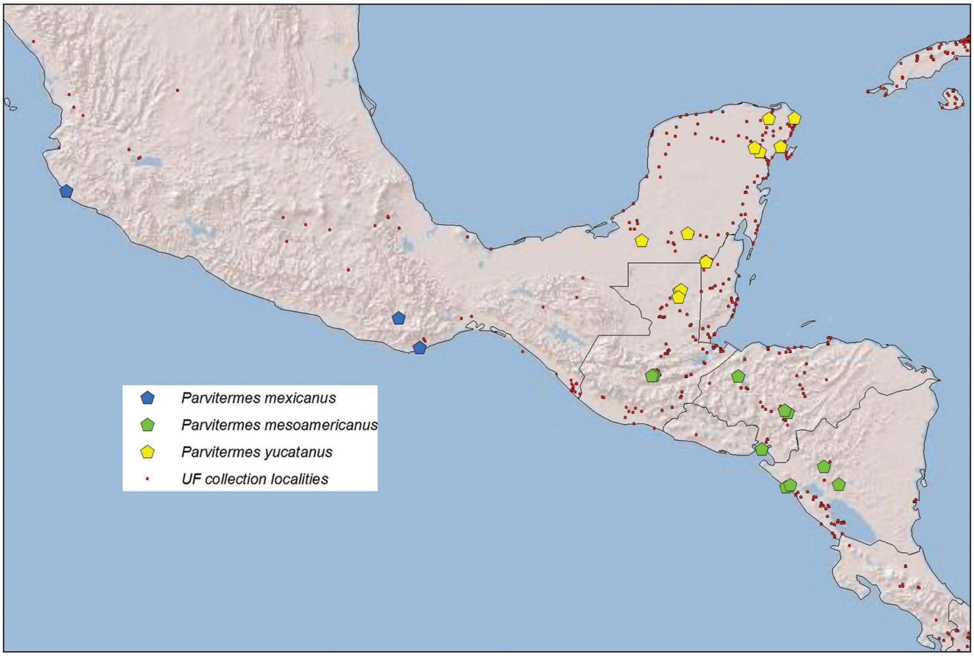

Figure 7. Collection localities of three *Parvitermes* species in the UF Termite collection. The far western sample of *P. mexicanus* comb. n. was taken near the type locality.

Figure 8. Field photograph of *P. mesoamericanus* sp. n. foragers feeding within a crevice of damp wood (Coyolito, Honduras; paratype locality, HN431).

lar plate without dorsal notch; apical and first marginal teeth of similar shape and projection; third marginal smaller, separated from first by slightly concave cutting edge. EVA consists of three irregular rows of about 7–12 long, narrow, subtriangulate, and slightly down-curved spines.

Etymology and distribution. Named for the Yucatan Peninsula which encompasses Belize, Mexico, and Guatemala; the known range of *P. yucatanus* (Fig. 7). This region has a pronounced dry winter season.

Comparisons. The soldiers of *P. yucatanus* and *P. mesoamericanus* are very similar with the following exception: the nasus of *P. yucatanus*, in lateral view, is more conical and broader at the base than that of *P. mesoamericanus*. The workers of *P. yucatanus* and *P. mesoamericanus* are indistinguishable. The distributions of *P. yucatanus* and *P. mesoamericanus* appear to be allopatric (Fig. 7) with the latter species occupying a more arid zone.

Biology. The Central American *Parvitermes* are wood-surface feeders. They typically attack wood in contact with the ground where they encase their surroundings with dark carton material (Fig. 8) reminiscent of *Amitermes* and build narrow foraging galleries to above-ground feeding sites (Light 1933, Weesner 1970 for *P. mexicanus*). Colonies nest in the soil underneath rocks and logs where brood and larvae have been found in weak cells of thin dark carton. In the West Indies, *Parvitermes* are often collected in hollowed-out stems of woody herbaceous plants (*P. brooksi* and *P. wolcotti*). In the arid lands of the Dominican Republic, *P. flaveolus* attacks wooden fence posts, and after rains, will feed on dried grass bunches that they cover with a thin arcade of soil.

Discussion

The current study reveals that *Parvitermes* is no longer a genus exclusive to the West Indies (Roisin et al. 1996) but has a widespread mainland complement of three species. This leaves only the monospecific genera *Antillitermes* and *Caribitermes* as the remaining endemics of all termite genera in the West Indies (excluding the continental islands of Trinidad and Tobago). The gestalt of the *Antillitermes subtilis* EVA closely resembles that of *Parvitermes* s. str. (Roisin et al. 1996) and suggests that the EVA is a mainland synapomorphy of both genera. *Antillitermes subtilis* may very well be a species of *Parvitermes*. *Caribitermes discolor* may also have a mainland lineage as it resembles an undescribed species from Panama (PN1315, Scheffrahn unpubl.). Therefore, it is quite possible, with the exception of the relict *Constrictotermes guantanamensis* from Cuba (Krěcěk et al. 1996), that all West Indian termites share congeneric species on the Central American mainland and that the West Indian fauna arose from Pleistocene/Miocene (Krishna and Grimaldi 2009) overwater dispersal events from Central America (Darlington 1938, Hedges 1996) or the more recent late Pleistocene land connections (Scheffrahn et al. 2006).

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Taxonomic revision of the New World genus *Callotillus* Wolcott (Cleridae, Tillinae), with the description of the new genus *Neocallotillus*, and an illustrated key of identification to species

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Abstract

The New World checkered beetle genus *Callotillus* Wolcott, 1911 is revised and the new genus *Neocallotillus* established. The subspecies *Callotillus elegans vafer* Wolcott is synonymized with the nominal subspecies, *C. elegans elegans* (Erichson), which is transferred to, and designated as the type species of *Neocallotillus* **gen. n.** as *Neocallotillus elegans* (Erichson, 1847), **comb. n.** Two additional species are transferred from *Callotillus* to the new genus: *Neocallotillus intricatus* (Wolcott & Dybas, 1947), **comb. n.** and *N. crusoe* (Wolcott, 1923), **comb. n.**, the latter tentatively and based on Wolcott's original description. *Callotillus* is now composed of two species: *C. eburneocinctus* Wolcott, 1911 and *C. bahamensis* Vaurie, 1952. All abovementioned species except *N. crusoe* are diagnosed and redescribed. In the absence of reference material of *Neocallotillus crusoe*, Wolcott's original description is transcribed. An illustrated key to species is provided. Characters of taxonomic relevance are illustrated and discussed. Updated distribution maps and locality data for all specimens examined are presented.

Keywords

West Indies, North America, Central America, geographic range, taxonomy

Introduction

Callotillus Wolcott is a genus of checkered beetles restricted to the Americas. The group has a wide distribution in the New World, extending throughout much of North and Central America and the West Indies (Fig. 8). Wolcott (1911) erected *Callotillus* for *Tillus elegans* Erichson and *Callotillus eburneocinctus* Wolcott, designating the latter as the type species. In later works, *C. vafer* Wolcott, *C. crusoe* Wolcott, and *C. intricatus* Wolcott & Dybas were described from North America, Puerto Rico and Costa Rica, respectively (Wolcott 1921, 1923; Wolcott and Dybas 1947). Barr (1950) recognized *C. vafer* as a subspecies of *C. elegans* on the basis of apparent discontinuity in the geographic distribution of these species and subtle morphological variations observable throughout their geographic range. Finally, an expeditionary work in the West Indies by Vaurie (1952) retrieved a new species, *Callotillus bahamensis*. The genus *Callotillus* thus presently includes five species and two subspecies: *Callotillus bahamensis* Vaurie, *C. crusoe* Wolcott, *C. eburneocinctus* Wolcott, *C. elegans* Wolcott (including the subspecies *C. e. elegans* (Erichson) and *C. e. vafer* Wolcott), and *C. intricatus* Wolcott. In this paper we investigate the species of *Callotillus* within the context of a generic revision. All species comprising the two genera are diagnosed and redescribed, except *N. crusoe*, where the original description given by Wolcott (1923) is transcribed. An illustrated key and updated distribution maps are given for all the species treated here.

Material and methods

The taxonomic sampling consisted of approximately 300 specimens collected in North and Central America, and the West Indies. Male genitalia were dissected if more than one male per species was available. Genitalia extraction and dissection procedures are similar to those outlined by Ekis (1977). Most morphological terminology follows the work of Ekis (1977), Rifkind (1993), and Opitz (2010). Specimens were examined with a Leica MZ 7.5 stereomicroscope. Images were taken and measured using a Leica DFC 500 digital camera, and stacked using the software Zerene Stacker V. 1.04.

Information reflected in the distribution maps was obtained from the locality data of the material examined in this study, the original descriptions of all the species, and the works of Wolcott (1947), Corporaal (1950), Papp (1960), Barr (1975), Rifkind (1996), Leavengood (2008), and Burke et al. (2015).

Material used in this work was borrowed from the following collections:

AMNH	American Museum of Natural History, New York, NY, USA
BMNH	British Museum of Natural History Collection, London, UK
CASC	California Academy of Sciences Collection, San Francisco, California, USA
CIUM	Colección de Insectos de la Universidad Autónoma del Estado de Morelos, Centro de Investigación en Biodiversidad y Conservación, UAEM, Mexico
CNHM	Cincinnati Museum of Natural History, Cincinnati, Ohio, USA

CNIN	Colección Nacional de Insectos, Instituto de Biología, UNAM, DF, Mexico
EMEC	Essig Museum of Entomology, University of California, Berkeley, USA
FMNH	Field Museum of Natural History, Chicago, Illinois, USA
FSCA	Florida State Collection of Arthropods, Gainesville, FL
EWC	James E. Wappes Collection, San Antonio, TX, USA
JNRC	Jacques Rifkind Collection, Valley Village, CA, USA
KSUC	Kansas State University Museum of Entomological and Prairie Arthropod Research Collection, Kansas State University, Manhattan, KS, USA
MSUC	Michigan State University Insect Collection, East Lansing, Michigan, USA
NMNH	Smithsonian National Museum of Natural History, Washington, D.C., USA
RHTC	Robert H. Turnbow Jr. Collection, Enterprise, AL, USA
SEMC	Snow Museum Entomological Collection, The University of Kansas, Lawrence, Kansas, USA
TAMU	Texas A&M Insect Collection, Texas A&M University, College Station, TX, USA

Taxonomy

Key to species of *Callotillus* and *Neocallotillus*

1 Antennomeres 4-9 of males pectinate (Fig. 3A–C), antennomeres 4-9 of females robust, serrate, (Fig. 3D–F); terminal maxillary palpomeres conical; slender to moderately robust species (Figs 1A–F, 4A) .... ***Neocallotillus*** (2)

– Antennomeres 4-9 of males strongly serrate (Fig. 3G–H), antennomeres 4-9 of females moderately serrate (Fig. 3I–J); terminal segments of maxillary palps cylindrical; robust species (Figs 1G–H, 4B) .... ***Callotillus*** (4)

2 Elytral disc finely punctate, punctations irregularly arranged; median region of each elytron adorned with a transverse, light testaceous to almost whitish, slightly protruding fascia, and one protruding macula on the anterior half of the elytral disc, this macula can be absent in some specimens (Fig. 1A–D) ... . ***Neocallotillus elegans***

– Elytral disc devoid of punctations; fasciae pattern not as above, elytral disc variously adorned, larger individuals **3**

3 Each elytron with a testaceous, broad and procurved fascia that initiates at the elytral suture and extends from the median region of the elytral disc to the elytral apex, and small, narrow, moderately oblique marking at the median region of the elytral disc; last third of elytral disc with a semicircular macula (Fig. 1F) .... ***Neocallotillus crusoe***

– Each elytron adorned with a pair of elaborate, pale fasciae, and one macula arranged as follows: macula located on the anterior fourth, posterior to the humeral angle; one fascia located on anterior half of elytral disc, and strongly procurved, initiating on elytral suture and ending just before epipleural fold;

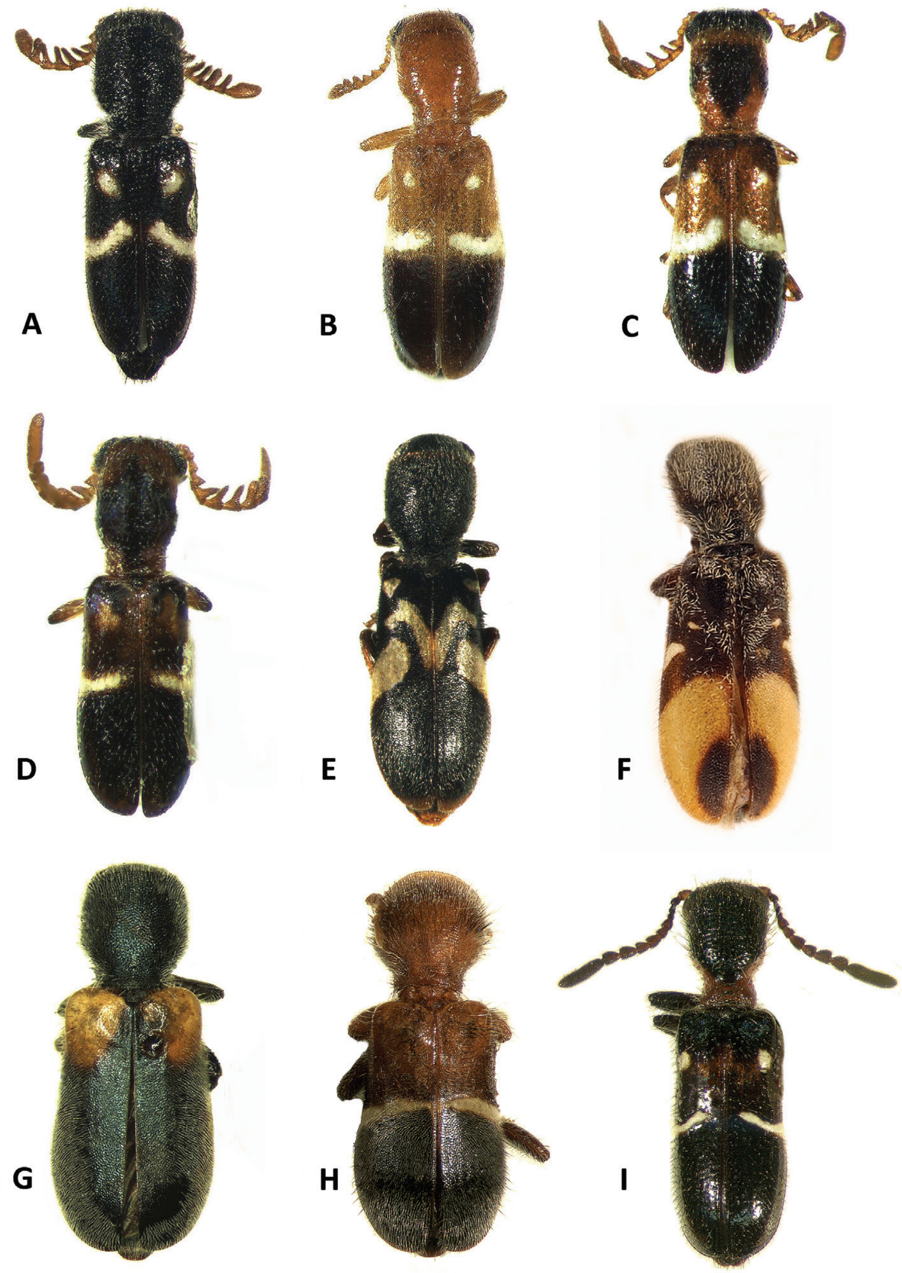

Figure 1. Habitus of: **A** *Neocallotillus elegans* (*Callotillus elegans elegans*) male **B** *N. elegans* (*C. e. vafer*) female **C** *N. elegans* (*C. e. vafer*) male **D** *N. elegans* (*C. e. elegans*) male **E** *N. intricatus* (*C. intricatus*) male **F** *N. crusoe* (*C. crusoe*) holotype male, (image courtesy of The American Museum of Natural History, New York) **G** *Callotillus bahamensis* male **H** *C. eburneocinctus* male **I** *Barrotillus kropotkini* male.

second fascia moderately oblique, located immediately posterior to the other fascia, initiating at the epipleural fold and not reaching the elytral suture (Fig. 1E) .... ***Neocallotillus intricatus***

4 Pronotal disc dark testaceous to rufous; anterior half of elytral disc same color as pronotum, posterior half piceous, color transition interrupted by a median, transverse, pale fascia that extends from epipleural fold to elytral suture; median region of elytral disc depressed in lateral view (Fig. 1H) ....
.... ***Callotillus eburneocinctus***

– Pronotal integument piceous; elytral disc same color as pronotum, except humeral area testaceous to almost ferrugineous; elytral disc lacking any fascia or maculation; median region of elytral disc feebly depressed in lateral view (Fig. 1–G).. ***Callotillus bahamensis***

Neocallotillus **Burke, gen. n.**

http://zoobank.org/08D462B4-C0D4-4BE0-82BC-A604244AAAE6

Type species. *Neocallotillus elegans* (Erichson, 1847), here designated.

Differential diagnosis. *Neocallotillus* is most closely related to *Callotillus*. The new genus can be differentiated from *Callotillus* based on the following combination of characters: in *Neocallotillus* species the male has antennomeres 1-2 filiform; the third antennomere is moderately serrate; antennomeres 4-9 are strongly pectinate; and the tenth antennomere is ovoid in shape and laterally depressed (Fig. 3A–C); the length of the tenth antennomere may vary by species. Females have antennomeres 1-3 filiform; the fourth antennomere is moderately serrate; and antennomeres 4-9 are robust, moderately, and gradually increase is size toward the distal end (Fig. 3D–F); the tenth antennomere of females is similar to that of the males. Males of *Callotillus* have antennomeres 1-2 filiform; the third antennomere is moderately serrate; antennomeres 4-9 are strongly serrate and approximately equal in length; and the tenth antennomere is broadly ovoid and about the same length as antennomeres 8-9 combined (Fig. 3G, H). The antennal structure of females is similar to that of males, except antennomeres 4-9 are moderately serrate and the tenth antennomere is cylindrical to moderately ovoid (Fig. 3I, J). Additionally, *Neocallotillus* species are relatively slender and elongate (Figs 1A–E, 4A), while *Callotillus* species are conspicuously more robust (Figs 1F–G, 4B). *Neocallotillus* species lack an elytral swelling present on the anterior third of the elytral disc of *Callotillus* (Fig. 4A–B). *Neocallotillus* also resembles *Barrotillus* Rifkind (Fig. 1I), however, the antenna of *Neocallotillus* is composed of 10 antennomeres (Fig. 3A–F), while the antenna of *Barrotillus* has 11 antennomeres (Fig. 3K). The restricted distribution of *Barrotillus*, recorded only from a confined locality in Honduras, will also serve to separate it from the widely distributed *Neocallotillus*.

Description. Size: 3–7 mm. Color: Light testaceous to dark brown (Fig. 1A–F); costae on elytral disc variously adorned, ranging from light testaceous to brown. Form: small to medium sized individuals; body elongate; elytra subparallel to moderately expanded posteriorly.

Head: Eyes medium sized, moderately taller than wide, conspicuously bulging laterally, strongly emarginate at antennal insertion; diameter of ommatidia small (Fig. 4A); clypeus approximately 3× the width of eye emargination and moderately emarginate medially. Antennae composed of 10 antennomeres; sexual dimorphism observable in antennal shape, where the antennae are moderately pectinate and strongly depressed dorsoventrally in males (Fig. 3A–C), but serrate and somewhat depressed dorsoventrally in females (Fig. 3D–F); tenth antennomere ovoid in both sexes. Labrum elongate, subquadrate; terminal maxillary palp conical, acuminate posteriorly; terminal labial palp securiform.

Thorax: Shape of pronotum scutiform, rounded laterally, narrower than anterior margin of elytra; disc feebly to moderately convex; inconspicuously broader at middle, feebly sinuate, conspicuously constricted on last fourth; anterior depression and antescutellar impression absent. Tibial spur formula 2-2-2, pulvillar formula 4-4-4. Prosternum: Smooth to feebly puncticulate; conspicuously wider than long. Mesoventrite: smooth, puncticulate. Metepisternum visible throughout its length in lateral view, not concealed by elytron. Metaventrite: moderately to strongly convex; variously punctate.

Elytra: Slender to feebly expanded posteriorly, elongate; median region of elytral disc feebly depressed in lateral view; sides subparallel to moderately expanded posteriorly in dorsal view; elytral declivity feebly to moderately gradual; elytral markings always present in various shapes, may be protruding or not.

Legs: Femora smooth, variably vested. Tibiae feebly to moderately rugulose, weakly expanded posteriorly, variously vested. Two tarsal denticles conspicuously separated from each other, inner tarsal denticles trigonal, outer tarsal denticles digitiform.

Abdomen: Smooth to glossy, moderately vested, feebly to moderately convex, with six visible ventrites; lateral margins of ventrites 1-5 parallel, posterior margins truncate; sixth ventrite triangular to subquadrate in shape; male pygidia moderately differentiated from female pygidia (Fig. 5A–F).

Aedeagus: Moderately robust; phallobasic apodeme short, slender distally; endophallic struts elongate, slender throughout their length.

Etymology: This generic name, which is preceded by the Latin prefix *neo* (new), refers to the superficial similarity to the genus *Callotillus*.

Remarks. Expressing tentative assignment of some of his species to *Callotillus*, Wolcott (1923) wrote: "*Callotillus crusoe*, as well as *C. elegans* and *C. vafer*, are placed in *Callotillus* provisionally only, as it differs from the other members of the genus in several important characters. No doubt, eventually, the creation of a new genus will be necessary for the reception of this new species and *C. elegans* and *C. vafer*. In *C. eburneocinctus*, the terminal segment of the maxillary palps is sub-cylindrical, the eyes are emarginate internally and the abdomen has but five segments. In *C. elegans*, *C. vafer*, and *C. crusoe* the maxillary palpi have the terminal segment conical, the eyes are deeply emarginate anteriorly, and the abdomen has six distinct well developed segments". The morphological differences listed by Wolcott, together with the presence of pectinate antennae on males of *Neocallotillus* versus serrate antennae on males of *Callotillus* (Fig. 3A–C, G–H), and an elytral swelling present in *Callotillus* but absent in *Neocallotillus* (Fig. 4 A–B), support the recognition of two genera within the group. The monotypic *Barrotillus* was also examined in this study in order to assess possible congenericity with *Neocallotillus*. The structure of the antennae and number of antennomeres serve as evidence to conclude that these closely related genera should be considered as separate taxa (Fig. 3A–K).

Neocallotillus elegans (Erichson, 1847), comb. n.

Tillus elegans Erichson 1847: 85.
Callotillus occidentallis Gorham 1882: 129.
Callotillus vafer Wolcott 1921: 270 **syn. n.**

Holotype depository. Zoologisches Museum Berlin, Germany (ZMB). Holotype locality: "Republica Peruana".

Distribution. USA: AZ, CA, LA, NM, NV, TX, UT; Mexico: Baja California, Baja California Sur, Chiapas, Chihuahua, Guerrero, Jalisco, Morelos, Nayarit, Oaxaca, Sonora, Tamaulipas, Yucatan; Central America: Costa Rica, Guatemala, Honduras, Nicaragua (Fig. 8A).

Differential diagnosis. *N. elegans* can be differentiated from similar species based on the integument color, fascia pattern, and wide geographic distribution. The species is most similar to *N. intricatus* but can be easily differentiated from the latter based on the fasciae pattern on the elytral disc. *Neocallotillus elegans* has the elytra adorned with a light testaceous to almost whitish median, longitudinal, slightly protruding fascia, and a pair of protruding maculae on the anterior half near the humeral angles (Fig. 1A–C), these maculae may be absent in some individuals (Fig. 1D). *Neocallotillus intricatus* has the elytral disc decorated with an intricate design of light testaceous fasciae and a pair maculae arranged in the following manner: each elytron with one macula situated posterior to the humeral angle; one strongly procurved fasciae located on the anterior half of the elytral disc, this fascia initiates on the elytral suture and do not reach the epipleural fold; and a second fascia situated immediately posterior to the first, this band is strongly oblique, initiating on the epipleural fold and not reaching the elytral suture (Fig. 1E). The geographic distribution of these species can also serve to separate them. *Neocallotillus elegans* is found from the United States to Costa Rica (Fig. 8A), while *N. intricatus* is restricted to Costa Rica and Panama (Fig. 8B).

Redescription. Form: Small individuals, feebly to moderately slender (Fig. 1A–D). Body: elongate, slender. Color: body integument variously colored, from piceous to ferruginous, with tones ranging from fuscous to testaceous; each elytron with one macula and one fascia, both markings ranging from almost albus to testaceous; thefascia is located on the median region of the elytral disc and can range from conspicuously wide to almost imperceptible; the macula is located on the median region of the first third of the elytral disc, initiating on the epipleural fold and almost reaching the elytral suture; these markings can be medially interconnected or not. The maculae may be absent in some specimens (Fig. 1D).

Head: Including eyes wider than pronotum; eyes conspicuously bulging laterally, taller than wide, large, finely faceted, very strongly emarginate; emargination subtriangular, extending 3/4 the eye width; integument moderately to strongly punctate; antennal notch located in front of antennal emargination; frons feebly to moderately bi-impressed. Antennae consisting of 10 antennomeres; antennomeres 2-3 small, bead-like; fourth antennomere strongly serrate, robust; antennomeres 4-9 pectinate, gradually increasing in size toward distal end; last antennomere enlarged, ovoid in shape, laterally compressed (Fig. 3A, B). Anterior portion of clypeus wide, approximately 3× the length of eye emargination (Fig. 6A).

Thorax: Pronotum longer than broad, moderately to strongly punctate, punctations ranging from coarse and deep to moderately shallow and fine; sides subparallel in dorsal view, then abruptly constricted on posterior fourth; disc feebly convex. Prosternum smooth to feebly punctate; punctations coarse, finely to moderately vested with pale, recumbent setae. Mesoventrite smooth, feebly punctate; finely vested with some pale, semi-recumbent to recumbent setae. Metaventrite moderately punctate; strongly convex; surface smooth, vested with fine, recumbent and semi-recumbent setae; longitudinal depression present; metaventral process absent.

Elytra: Humeri indicated, slender, elongate; lateral margins subparallel, slightly to moderately broader on last third, then moderately to strongly depressed on second third, and conspicuously convex again on last third; sculpture consisting on shallow, irregularly arranged punctations; elytral apices subtriangular to almost rounded, feebly dehiscent; interstices at elytral base about 3× the width of punctuation; scutellum subquadrate, not depressed; epipleural fold complete, narrowing toward apex.

Legs: Femora swollen on posterior half; shiny; very feebly rugulose; weakly clothed with some semi-recumbent setae. Tibiae more profusely vested than femora.

Abdomen: Six ventrites; ventrites 1-5 shiny, smooth, subquadrate, not depressed laterally. Fifth ventrite subquadrate; lateral margins subparallel; posterior margin broadly, shallowly emarginate. Sixth ventrite small, conspicuously excavated, moderately, coarsely punctate, conspicuously broader than long; lateral margins strongly oblique, procurved; posterior margin broadly, moderately deeply, U-shaped emarginate; posterolateral angles broadly rounded (Fig. 5A–B). Fifth tergite subquadrate, moderately, coarsely punctate; posterior margin broadly, shallowly emarginate. Sixth tergite concave, wider than long; surface smooth; lateral margins moderately oblique; posterior margin broadly, moderately deeply, U-shaped emarginate. Posterolateral angles broadly rounded, fully covering sixth ventrite from dorsal view.

Aedeagus: Phallobasic apodeme present; phallus with copulatory piece moderately swollen at apex; phallic plate devoid of denticles; intraspicular plate present, elongate; phallobasic apodeme short, expanded distally; phallobase subparallel; phallobasic lobes free; tegmen complete, fully covering phallus; phallobasic lobes pointed anteriorly; endophallic struts long, extending beyond the length of tegmen; endophallic struts slender throughout their length, weakly robust distally (Fig. 7A–B).

Female variation: Females can be distinguished from males based on the antennal structure and the shape of the last abdominal segment. The antennal shape of females is moderately to strongly serrate; antennomeres 2-3 are slender, filiform; antennomeres 4-9 are serrate, the serrations gradually increase in size toward distal end (Fig. 3D, E). The posterior margin of the sixth ventrite of females is strongly procurved, producing a semicircular pygidium (Fig. 5C–D).

Remarks. The species *Callotillus occidentalis*, described by Gorham (1882) for individuals collected in Guatemala and Nicaragua, was later synonymized with *C. elegans* by Schenkling (1903). We examined one female paratype of *C. occidentallis* (Fig. 2A) and agree with Schenkling's synonomy. Barr (1950) proposed that *Callotillus vafer* be reclassified as a subspecies of *C. elegans* on the basis of integument color, geographic distribution discontinuity, and differences in the structure of the elytral punctation. Individuals inhabiting Arizona, California, Nevada, New Mexico, western Texas, Utah, and the Baja California peninsula were classified by Barr as *C. e. vafer* while specimens from western Louisiana, eastern Texas, Mexico and Central America were recognized as *C. e. elegans*. Barr also indicated the existence of intermediate forms of these subspecies in the United States and Baja California; however, we have found intermediate forms exist throughout much of the geographic range of the species. It is possible to find both color morphotypes (Fig. 1A–B), including intermediate forms (Fig. 1C–D), as well as conspicuously similar antennal forms (Fig. 3A, B), throughout North and Central America. Aside from integument variation, no other morphological evidence was found to differentiate these subspecies as separate taxa. As more material from these subspecies has been accumulated, we consider *N. elegans* a species with a wide spectrum of color variation throughout an extensive geographic distribution (Fig. 8A).

Material examined. PARATYPE: 1 female: [*Callotillus occidentalis* Gorham], Pantaleon, 1700 ft., Champion, paratype depository: BMNH. (Fig. 2A).

Additional material examined (N= 241). USA: 2 males: SW Hidalgo Co., TX, 17-III-1946, George B. Vogt, beating flowers and foliage, on *Prosonis juliflora* DeCandolie; 1 male, 3 females: Riverside Co., CA, Chuckawalla Mts., Corn Spg. Campground, 25-IV-1987, A. J. Mayor; 1 female: Imperial Co., CA, 9 mi N Winterhaven, 2-IV-1997, F. G. Andrews and A. J. Gilbert, sweeping *Prosopis*; 1 female: TX, 12 mi W Guthrie, 14-VII-1969, K. Polk; 1 male, 1 female: Hidalgo Co., TX, Sta. Ana Natl. Refuge, vic. Willow Lake, T. C. McRea; 1 male, 1 female: Val Verde Co., TX., Pecos River, 29-VIII-1970, no collector data; 1 male: Rio Grande City, Starr Co., TX, on *Prosopis*; 1 male: NM, 12 mi W Carlsbad, 25-IV-1971, on mesquite, C. R. Ward; 1 male: San Diego Co., CA, Borrego State Park, 17-20-IV-1969, no collector data; 1 female: Painted Canyon, Riverside Co., Calif., 25-III-1962, F. G. Andrews; 1 male: N. M., Hidalgo Co., Coronado Natl. Forest, 26-V-1976, W. Iselin; 3 males, 2 females: AZ, Sta. Catalina Mts., Pima Canyon, 7-IX-1970, K. Stephan; 1 female: TX, 5 mi NW of Alpine, 17-VI-1965, on *Sapindus drummondii*, G. H. Nelson; 2 males: Starr Co., TX, 2 mi W of Sullivan City, reared from *Pithecelobium flexicaule*, G. H. Nelson; 1 female: Socorro Co., NM, Bosque de Apache Nat. Wildlife Ref., 2-VII-2000, F. W. Skillman Jr.; 2 females: AZ, Sta. Catalina Mts., Pima Canyon, Bred ex Palo Verde, K. Stephan; 1 female AZ, Sta. Catalina Mts., Sabino Canyon, 11-VIII-1961, G. H. Nelson;; 1 female: CA, Imperial Co., 7 mi N of Glamis, 29-VIII-1987, on *Cercidium*

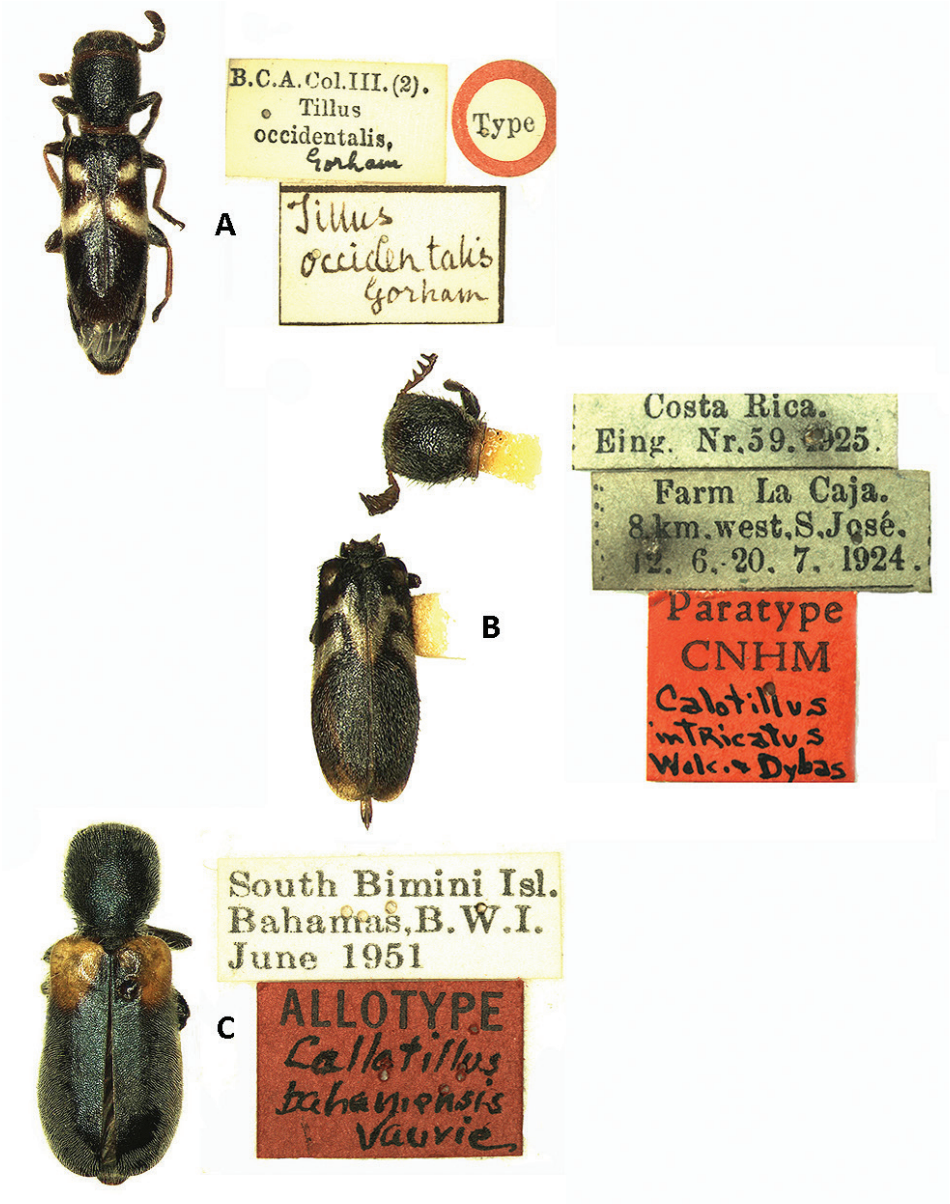

Figure 2. Types and corresponding labels of: **A** *Tillus occidentalis* (Gorham) [*Neocallotillus elegans*], female **B** *Callotillus intricatus* (Wolcott and Dybas) [*Neocallotillus intricatus*], male **C** *Callotillus bahamensis* Vaurie, female.

floridum, Wood; 2 males, 1 female: Imperial Co., CA, Frink Spr., 7-VII-1993, on *Olneya tesota,* G. H. Nelson; 1 female: San Diego Co., CA, 3 mi E of Jacumba, reared *Acacia greggii*, 22-V-1987, G. H. Nelson; 1 male, 3 females: AZ, Pima Co., Green

Valley, 15-VII-976, no collector data; 1 male, 1 female: Eddy Co., NM, 26 mi E of Carlsbad, 2-VI-1977, no collector data; 1 female: Dona Ana Co., NM, 9 miles west of Santa Teresa, 8-V-1999, J. C. Schaffner; 1 female: Bastrop Co., TX, Bastrop, 3-VI-1997, S. G. Wellso; 1 female: Cochise Co., AZ, 12 mi N of Douglas, 24-VII-1982, J. E. Wappes; 1 male: TX, 3 mi southeast Presidio, 12-IV-1968, J. G. Hafernik; 1 male, 1 female: Pima Co., AZ, Mt. Lemon, V-17-1976, R. Lenczy; 1 male: Cochise Co., AZ, Wilcox Dry Lake, 6-VI-1970, A. R. Hardy; 1 male: TX, Brownsville, VII-1937, H. S. Barber; 2 males: AZ, Tucson, VIII-193[ ], Bryant; 1 female: Riverside Co., CA, 25-III-1962, F. G. Andrews; 10 males, 5 females: CA, Mecca, 20-V-1924, B. Warwick; 1 female: CA, Calipatria, 6-V-1924, B. Warwick; 1 female: CA, Calipatria, 1-V-1924, B. Warwick; 1 male: CA, Calipatria, 10-V-1924, B. Warwick; 10 females: Hidalgo Co., TX, J. N. Knull, 28-III-1954; 3 males, 4 females: TX, Brownsville, , 25-V-1934, J. N. Knull; 2 females: Cameron Co., TX, 25-III-1952, J. N. Knull; 5 males, 3 females: Hidalgo Co., TX, 26-III-1957, J. N. Knull; 6 males, 6 females: Hidalgo Co., TX, 20-III-1952, D. J. and J. N Knull; 1 male: AZ, Huachuca Mt., 5-VI D. J. and J. N. Knull; 2 females: Starr Co., TX, D. J. and J. N. Knull, 28-III-1950; 1 male: Uvalde Co., TX, 20-V, D. J. Knull; 1 male, 2 females: Hidalgo Co., TX, 29-III-1968, D. J. and J. N Knull; 2 females: Hidalgo Co., TX, 26-III-1953, D. J. and J. N. Knull; 1 male, 1 female: Hidalgo Co., TX, 24-III-1954, D. J. and J. N. Knull; 1 male: Hidalgo Co., TX, 28-III-1954, D. J. and J. N. Knull; 1 male, 4 females: CA, Santa Rosa L., VIII, J. L. Knull; 3 males, 1 female: AZ, Wilcox, 4-VII-1951, D. J. and J. N. Knull; 2 males: AZ, Wilcox, 6-VI-1954, D. J. and J. N. Knull; 1 female: AZ, Patagonia Mts., 2-VII-1953, D. J. and J. N. Knull; 1 male: Culberson Co., TX, 9-VII-1953, D. J. and J. N. Knull; 2 male, 2 females: Pima Co., AZ, 9-VII-1975, N. M. Downy; 1 male: Bell Co., TX, Holland, 12-VII-1988, S. G. Wellso; 2 males: Hidalgo Co. TX, Sta. Ana Natl. Refugee, VIII-1977, J. E. Wappes; 1 male, 1 female: Calipatria Co., CA, 15-VII-1925, B. Warwick; 1 female: Uvalde Co., TX, VII-27, J. N. Knull; 1 female: CA, Mecca, 12-V-1924, B. Warwick; 4 males, 1 female: CA, Calipatria, 1-6-V-1924, B. Warwick; 15 males, 9 females: AZ, Chiricahua Mts., 1-3-VI, J. N. Knull; 1 male, 2 females: AZ, Tucson, VIII-19, J. N. Knull; 2 females: Hidalgo Co., NM, 24-III-1954; D. J. and J. N. Knull; AZ, Wilcox, 11-VI-1954, D. J. and J. N. Knull; 2 males: Imperial Co., CA, 15 mi W of Calexico, 5-6-VI-1961, light trap, H. F. Howden; 1 female: CA, Palm Springs, 15-VI-1948, D. J. and J. N. Knull; 1 female: TX, Davis Mts., 24-VI-1957, D. J. and J. N. Knull; 4 males, 7 females: TX, Chisos Mts., V-25, J. N. Knull; 2 males: Jeff Davis Co., TX, 20-VI-1957, D. J. and J. N. Knull; 1 male: TX, on live oak, 17-V-1965, J. L. Bottmer; 2 males: AZ, Mt. Huachuca, 5-8-VI, D. J. and J. N. Knull; 3 males: Hidalgo Co., TX, 20-IV-1968, D. J. and J. N Knull; 4 males, 6 females: Jim Wells Co., TX, 8 mi S of Alice, 6-8-April-1984, S. G. Wellso; 2 females: Jim Wells Co., TX, Alice, 15-IV-1986, S. G. Wellso; 1 male: Brewster Co., TX, Castolon, 14-IV-1983, S. G. Wellso; 1 female: Brewster Co., TX, Big Bend Natl. Park, 16-IV-1983, S. G. Wellso; MEXICO: 1 male, 1 female: Chiapas, Mex., 4 mi NW of Pueblo Nuevo River Bajada, 15-VII-1965, G. H. Nelson; 1 male: Baja Calif. S., Mex., 4 mi S La Paz, 14-IX-1978, B. K. Dozier; 1 male: Baja Calif., Mex., Catavina, riparian palm oasis, on

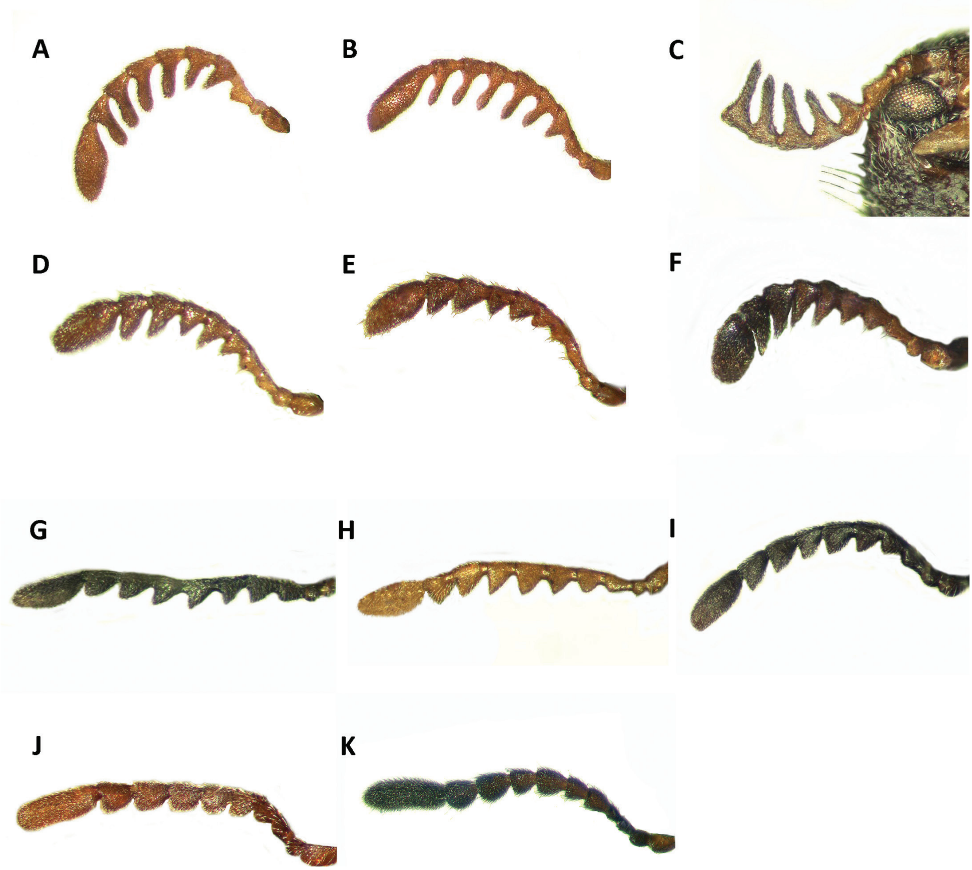

Figure 3. Antennae of: **A** *Neocallotillus elegans* (*Callotillus elegans elegans*) male **B** *N. elegans* (*C. e. vafer*) male **C** *N. intricatus* (*C. intricatus*) male holotype **D** *Neocallotillus elegans* (*C. e. elegans*) female **E** *N. elegans* (*C. e. vafer*) female **F** *N. intricatus* (*C. intricatus*) female **G** *Callotillus bahamensis* male **H** *C. eburneocinctus* male **I** *C. bahamensis* female **J** *C. eburneocinctus* female **K** *Barrotillus kropotkini* male.

Acacia greggii, G. H. Nelson; 1 male, 2 females: Baja Calif. S., Mex., La Paz, 29-VI-1973, B. F. Chamberlain; 2 females: Baja Calif. S., Mex., 1-3 mi E Cabo San Lucas, G. Riley; 1 female: Baja Calif. Sur, Mex, 9 mi N San Jose del Cabo, G. Riley; 2 males: Baja Calif. S., Mex., 66 km NE Insurgentes nr. Ultima Agua, on *Prosopis articulata*, 13-IV-1994, D. Yanega; 2 males: Morelos, Mex., Tlaquiltenango, Huaxtla, 18.37598 N, 99.04804 W, 1053 m, 13-XII-2009, V. H. Toledo; 1 male: Sonora, Mex., 29 km SE Tecoripa y 3 km S Rancho Las Peñitas, 733 m, on *Acacia* sp., 22-IV-2004, V. H. Toledo; 2 males, 1 female: Baja Calif. S., Mex., Las Barrancas, 27-V-1984, P. DeBach, Malaise trap; 1 female: Yucatan, Mex., Tekom, 04-VIII-1940, I. Sanderson; 1 male: Baja Calif., Mex., Santa Rosa, 08-10-I-1914, G. Beyer; 1 male, 2 females: Chiapas, Mex., 4 mi NW of Pueblo Nuevo, 15-VII-1968, G. H. Nelson; 1 male: Tamaulipas, Mex., El Encino, 15-IV-1984, S. G. Wellso. 8 males, 3 females: Baja Calif. S., Mex. 6 km E of San Antonio, 350 m, on *Prosopis articulata*, 11-IV-1994, no collector data;

CENTRAL AMERICA: 2 males: Guanacaste, [Costa Rica], Cerro El Hacha, 800m, 12 km SE La Cruz, 320000, 364000, 1998; 1 male: Heredia Province, Costa Rica, Sarapiqui, Chilamate, La Marita Farm, 26-II-1992, R. L. Johnson and R. Ochoa; 1 female: Rivas, Nicaragua, San Juan del Sur, 11' 15° N, 82' 52° W, 10-III-1998, L. J. Clark; 1 female: Granada, Nicaragua, Volcan Mombacho, Finca San Joaquin, 15-V-1998, malaise trap, in organic coffee, J. M. Maes.

Neocallotillus crusoe **(Wolcott, 1921), comb. n.**

Holotype depository. (AMNH). Holotype locality: Camuy, Puerto Rico.

Distribution. Puerto Rico (Fig. 8C).

Differential diagnosis. *N. crusoe* is similar to *N. elegans* but differs from the latter species by the absence of seriate elytral punctures, its larger size, its broader form, and the impunctate metaventrite and abdomen. The differently formed and arranged raised fasciae or maculae are also distinguishing characters. The head and pronotum in *N. crusoe* are densely pubescent, sparsely so in the *N. elegans* species; the antennae are differently formed, having a greater number of triangular segments; the color pattern is unique; the arrangement of the pubescence in basal half of elytra is distinctive; and the densely pilose elytral tubercles are present only in *C. eburneocinctus*.

The following is Wolcott's original description. N=1. Form: Moderately slender. Color: Black. Dorsal surface rather feebly shining; ventral surface very shining; front of head narrowly rugulose; antenna (apical two segments black) and labrum at sides testaceous; elytra black with the apical half in large part pale yellow; a large, ovate, ante-apical, black maculation; sides at middle with an oblique, elevated, white maculation; a similar minute, slightly transverse maculation at basal fourth at middle of width of each elytron.

Head: Including the not prominent eyes, equal in width to pronotum at apex; surface rather coarsely rugoso-punctate; pubescence dense, semi-recumbent, grayish white. Antennae slightly longer than head and prothorax, ten-segmented; basal segment short, very stout; second small, subtriangular; third to ninth triangular, their apices acute; ninth and tenth forming an elongate ovate mass; tenth narrower than eighth, nearly as long as seventh and eighth together; color testaceous; ninth and tenth segments black, the former narrowly testaceous at base.

Pronotum: Slightly longer than wide; apical margin truncate; sides parallel to slightly behind the middle, then rather strongly arcuately narrowing to about basal fourth, thence subparallel to base; base truncate, the extreme edge with a fine elevated margin; subapical constriction wanting; subapical transverse impression nearly obsolete, only faintly indicated in certain lights; surface with sculpture same as that of head; pubescence same as that of head but with long, sparse, erect, black hairs intermixed.

Elytra: Base nearly twice as wide as pronotum at base; length 1/3× times width at base; humeri obtusely rounded; sides from humeri to middle straight, nearly parallel, behind the middle gradually broadening to apical fourth, thence arcuately narrowing

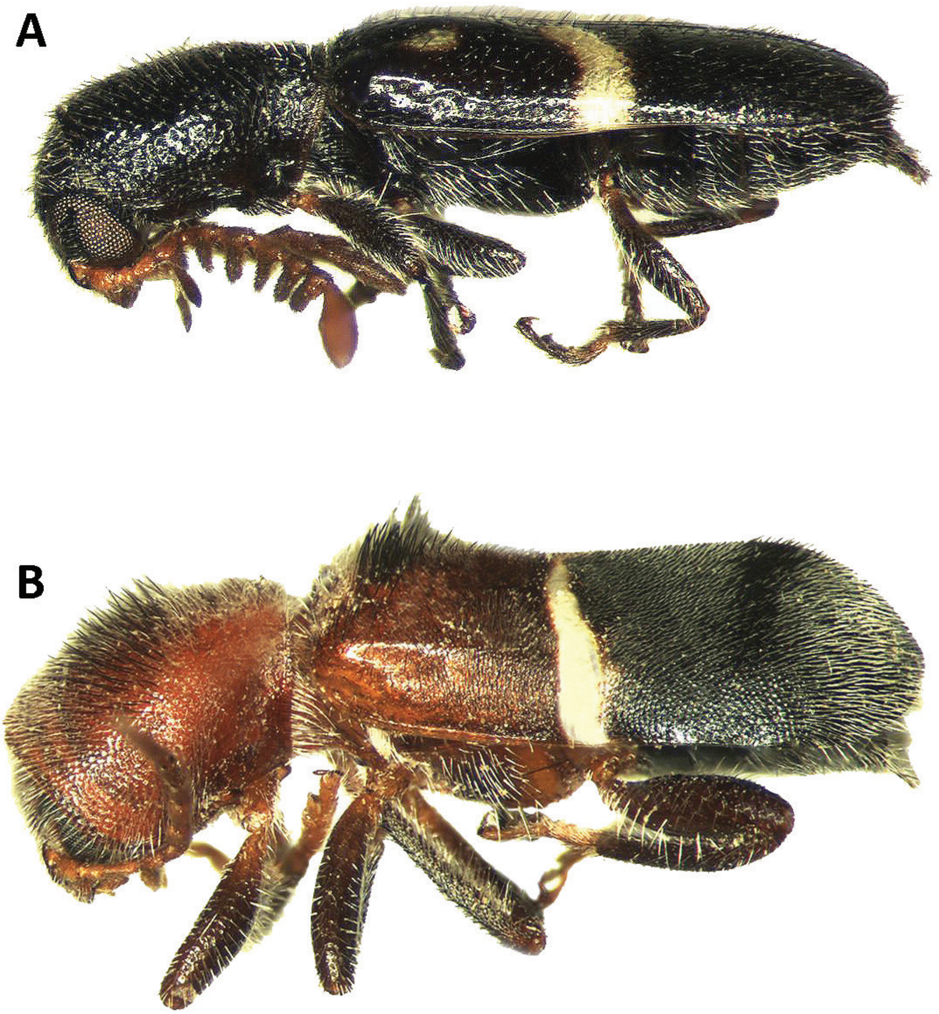

Figure 4. Habitus in lateral view of: **A** *Neocallotillus elegans*, male **B** *Callotillus eburneocinctus*, male.

to the conjointly rounded apices; color black, apical half pale yellow, anterior margin of yellow portion convex; in apical third a large, elongate ovate, common, sutural maculation, extending very nearly to apical margin, black; sides slightly anterior to middle with a feebly arcuate, linear, elevated, white maculation, this extending obliquely and attenuate forward from lateral margin halfway to suture; at basal fourth a minute, slightly transverse, elevated, white maculation midway between the lateral margin and the suture; base with a broad triangular area, having one angle on suture, and an oblique fascia each side, extending from immediately behind the humeri to the suture at a point slightly before the middle, composed of dense, coarse, grayish-white pubescence; a large, feebly elevated, subbasal tubercle, midway between lateral margin and suture, densely clothed with a tuft of long, black hairs; black portions densely clothed with short, semi-recumbent, black pubescence, longer and erect in humeral region; the yellow portion densely clothed with pale yellowish pubescence, a few nearly erect, long, black hairs intermixed; surface finely and sparsely punctate at extreme base, becoming closer at about basal fourth, and a little coarser toward the apex; punctuation irregular throughout, showing no tendency to become seriate.

Abdomen: Impunctate; very sparsely clothed with long, black hairs. Meso[ventrite] sternum smooth; moderately clothed with semi-recumbent, grayish-white pubescence. Legs rather short and stout; moderately clothed with rather long, white hairs. Length, 4.2 mm.

Remarks. Wolcott (1923) described *Callotillus crusoe* from a single male specimen collected near Camuy, Puerto Rico. Wolcott concluded that this species was allied to *C. elegans* and *C. vafer* but could be differentiated from the latter two based on the absence of elytral punctations, a relatively larger size and broader body shape, and the absences of punctations on the metaventrite and abdomen. Based on Wolcott's illustration and his descriptive work, the shape of the antennae appear to be serrate, and the species seems to be comparatively larger and broader than remaining *Neocallotillus* species. These characteristics may suggest a relationship to *Callotillus*. Due to the absence of material of *N. crusoe*, a redescription of this species is not presented in this study; however, in order to complement the revision of the newly erected genus *Neocallotillus*, the descriptive work given by Wolcott (1923) is transcribed above. Based on Wolcott's assessment, we tentatively place *C. crusoe* within *Neocallotillus*. Further examination of material from this species will serve to corroborate the relatedness of this species with those species composing *Neocallotillus*, or conversely, its reassignment to *Callotillus*.

***Neocallotillus intricatus* (Wolcott & Dybas, 1947), comb. n.**

Holotype depository. Naturalis Biodiversity Center, Leiden, The Netherlands (RMNH). Holotype locality: Farm La Caja, 8 km. west of San Jose, Costa Rica.

Distribution. Costa Rica, Panama (Fig. 8B).

Differential diagnosis. *N. intricatus* is most similar to *N. elegans*. The two species can be differentiated based on the fasciae pattern on the elytra disc. *Neocallotillus intricatus* has the elytral disc decorated with an intricate design of light testaceous fasciae and a pair of maculae arranged in the following manner: Each elytron with one macula situated posterior to the humeral angle; one strongly procurved fasciae located on the anterior half of the elytral disc, this fascia initiates on the elytral suture and do not reach the epipleural fold; and a second fascia situated immediately posterior to the first, this band is strongly oblique, initiating on the epipleural fold and not reaching the elytral suture (Fig. 1E). *Neocallotillus elegans* has the elytra adorned with a light testaceous to almost whitish median, longitudinal, slightly protruding fascia, and a pair of protruding maculae on the anterior half near the humeral angles (Fig. 1A–C), these maculae may be absent in some

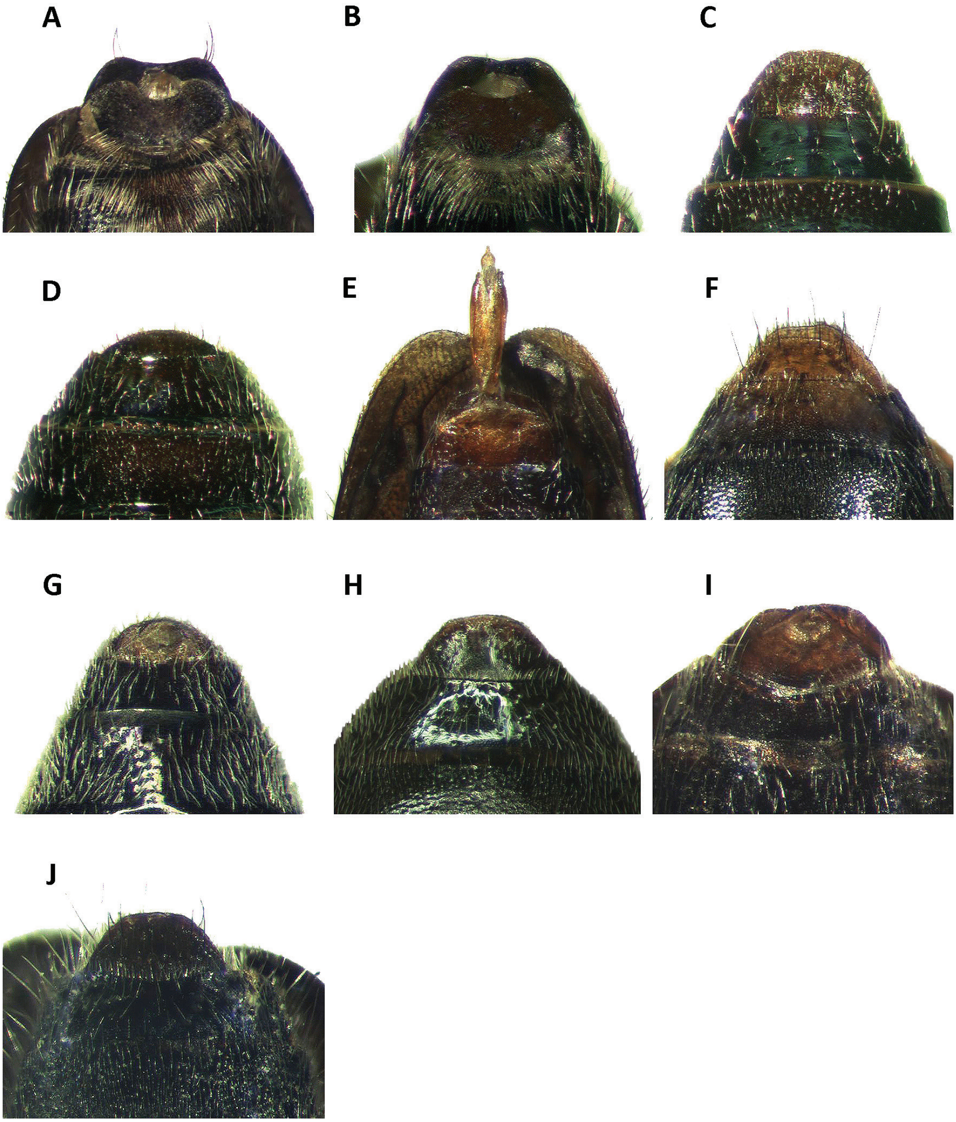

Figure 5. Pygidium of: **A** *Neocallotillus elegans* (*Callotillus elegans elegans*) male **B** *N. elegans* (*C. e. vafer*) male **C** *N. elegans* (*C. e. elegans*) female **D** *N. elegans* (*C. e. vafer*) female **E** *N. intricatus* (*C. intricatus*) male **F** *N. intricatus* (*C. intricatus*) female **G** *Callotillus bahamensis* male **H** *C. bahamensis* female **I** *C. eburneocinctus* male **J** *C. eburneocinctus* female.

individuals (Fig. 1D). The geographic distribution of these species can also serve to separate them. *Neocallotillus intricatus* is limited to Costa Rica and Panama (Fig. 8B) while *Neocallotillus elegans* is found from the United States to Costa Rica (Fig. 8A).

Redescription. Form: Body elongate; head, pronotum and anterior half of elytra slender, feebly expanded behind second half of elytral margins. Color: Head, pronotum, thorax, abdominal segments 1-4 and femora griscent to fuscous; anterior margin of pronotum, antennae, mouthparts, tibiae, abdominal segments 5-6 and elytral apex light-ferruginous. Elytra adorned with an intricate array of two pale-testaceous fasciae and a pair of maculae of the same color, the position of these elytral markings is as follows: the first fascia is located on the anterior half of the elytral disc, this band is strongly procurved, initiating on the elytral suture and not reaching the epipleural fold; the second fascia is located immediately posterior to the first band and is moderately oblique, initiating on the epipleural fold and not reaching the elytral suture; the two macuale are located posterior to the humeral angles. Elytral pattern not elevated from elytral disc (Fig. 1E).

Head: Including eyes not wider than pronotum; eyes strongly emarginate, taller than wide, feebly bulging laterally, rather small, finely faceted; emargination subtriangular; integument moderately punctate; antennal notch anterior to antennal emargination; frons moderately bi-impressed. Antennae of male consisting of 10 antennomeres; antennomeres 2-3 small, beadlike; fourth antennomere serrate; antennomeres 4-9 pectinate, gradually increasing in size toward distal portion of antenna; last antennomer enlarged, as long as ninth antennomere, ovoid in shape, laterally compressed.

Thorax: Pronotum longer than broad; surface rugulose and strongly, finely punctate; punctations numerous, shallow; sides subparallel in dorsal view, then abruptly constricted on posterior fourth; disc convex. Prosternum feebly convex; surface smooth; conspicuously punctate, punctations shallow. Mesoventrite smooth; surface feebly punctate; finely vested. Metaventrite globate; surface smooth, strongly convex and finely punctate; longitudinal depression and metaventral process absent; metepisternum exposed but profusely covered with short, fine, pale setae observable in lateral view. Scutellum ovoid in shape.

Elytra: Humeri indicated; slender on anterior half and then gradually expanding behind middle; surface convex on first third, then strongly depressed on second third, and then conspicuously convex on last third; elytral sinuosity observable in lateral view; sculpture on elytral disc consisting on abundant, very shallow, irregularly arranged punctations almost imperceptible in some individuals; elytral apices rounded, moderately dehiscent; interstices on elytral base about 2× the width of punctuation; epipleural fold complete, narrowing toward apex.

Legs: Femora swollen; surface shiny, smooth; vestiture consisting of some semi-recumbent setae, then abruptly vested with numerous pale, semi-recumbent, rather stout setae on distal face. Tibiae more profusely vested than femora; vestiture consisting on stout, pale, short, recumbent setae interspaced with some semierect setae.

Abdomen: Six ventrites; ventrites 1-4 broadly convex, smooth, rugulose, subquadrate, not depressed laterally; posterior margins truncate. Fifth ventrite shiny; lateral margins moderately obtuse; posterior margin broadly, shallowly emarginate. Sixth ventrite small; surface moderately excavated, shiny, feebly punctate, conspicuously broader than long; lateral and posterior margins strongly oblique, nearly semicircularly rounded (Fig. 5E). Fifth tergite sub-quadrate; surface moderately, coarsely punctate; posterior margin broadly, shallowly emarginate. Sixth tergite feebly concave, wider than long; surface smooth; lateral margins moderately oblique; posterior margin trun-

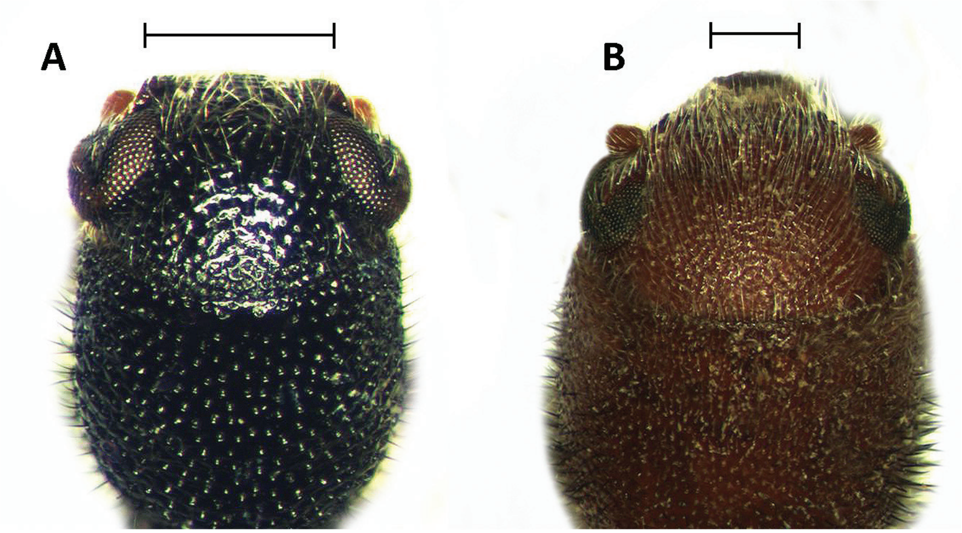

Figure 6. Head of: **A** *Neocallotillus elegans* (*Callotillus elegans elegans*) **B** *Callotillus eburneocinctus*. Scale bars indicate width of frons.

cate; posterolateral angles subquadrate. Sixth tergite extending beyond apical margin of sixth ventrite, fully covering sixth ventrite from dorsal view.

Aedeagus: Moderately robust; distal portion of phallus petiolate; phallobasic apodeme present; phallus with copulatory piece moderately swollen distally; intraspicular plate present, elongate; phallobasic lobes moderately procurved; tegmen complete, fully covering phallus; phallobasic lobes acuminate distally (Fig. 7C).

Female variation: The antennal shape of females is strongly serrate (Fig. 3F), rather than pectinate, as observed in males (Fig. 3C). Females have antennomeres 1-3 slender, antennomeres 4-5 are moderately serrate, and antennomeres 6-9 are strongly serrate, serrations gradually increase in size toward the distal end. Females also have the lateral and posterior margins of the sixth ventrite subquadrate (Fig. 5F), producing a somewhat semicircular pygidium. All females in the material examined were moderately larger than males.

Remarks. Wolcott and Dybas (1947) described *Callotillus intricatus* based on two specimens, one male and one female (Fig. 2B), collected from a single locality 8 km west of San Jose, Costa Rica. This species is here transferred to *Neocallotillus* based on the pectinate antennae of male individuals (Fig. 3C) and the feebly pectinate to almost serrate antennae in females (Fig. 3F), these antennal shapes are very similar to those observed on *N. elegans* (Fig. 3A–B, D–E). Other characters that are similar in these species are general body shape (Fig. 1A–E), elytral sculpturing, and the conical shape of the terminal segment of the maxillary palps.

Material examined. PARATYPE: 1 male: Farm La Caja, 8 km W San José, Costa Rica, Eing, 12-VI to 20-VII-1924, hand written red label paratype depository: CNHM.

Additional material examined (N=9). 2 females: Costa Rica, Guanacaste, 3 km SW of R. Naranjo, 11-18-III-1992, F. D. Parker; 1 male, 3 females: Costa Rica, Guanacaste, 14 km S Cañas, 2-III-1990, F. D. Parker; 1 male, 2 females: Panama, Coclé province, El Valle [Anton], 19-II-1999, W. E. Wappes.

Callotillus Wolcott, 1911

Type species. *Callotillus eburneocinctus* Wolcott, 1911 (original designation).

Differential diagnosis. *Callotillus* is most similar to the new genus *Neocallotillus*. A list of useful characters to properly differentiate these genera is provided above in the differential diagnosis of *Neocallotillus*.

Redescription. Size: 6–10 mm. Color: Testaceous and ferrugineous to almost black; fasciae on elytral disc may be present or not, if present, ranging from light testaceous to brown. Body moderately robust, expanded posteriorly.

Head: Rather small, longer than wide; eyes inconspicuously bulging laterally (Fig. 6B), strongly emarginate at antennal insertion; clypeus feebly emarginate medially; antennae composed of 10 antennomeres; sexual dimorphism observable in antennal composition, with antennomeres 2-9 in males conspicuously serrate (Fig. 3G, H) but moderately serrate in females (Fig. 3I, J), last antennomere compressed laterally in both sexes; labrum moderately constricted laterally, subquadrate; terminal maxillary palps sub-cylindrical; terminal labial palps securiform.

Thorax: Pronotum moderately to conspicuously globose, narrower than anterior margin of elytra; disc moderately to strongly convex, inconspicuously broader at middle, feebly sinuate behind middle, then feebly to moderately constricted on last fourth; anterior depression and antescutellar impression absent. Prosternum: smooth to feebly puncticulate; conspicuously wider than long. Mesoventrite smooth, feebly to moderately puncticulate; metepisternum partially visible in lateral view, not fully concealed by elytron. Metaventrite moderately to strongly convex; surface variously punctate.

Elytra: Moderately robust, elongate, expanded posteriorly; median region of elytral disc feebly to moderately depressed in lateral view; subbasal elytral swellings present; elytral apex declivity feebly to moderately steep; transverse fasciae may be present on elytral disc or not, if present elevated from elytral disc.

Legs: Femora wide; surface rugulose to smooth. Tibiae rugulose, moderately expanded posteriorly; tibial spur formula 2-2-2. Two tarsal denticles, inner tarsal denticles trigonal, outer tarsal denticles digitiform.

Abdomen: Six ventrites; surface smooth, moderately convex; ventrites 1-5 with lateral margins parallel and posterior margins truncate; male pygidium moderately differentiated form that of females.

Aedeagus: Slender and moderately sclerotized; phallobasic apodeme robust distally; endophallic struts short, robust throughout their length.

Remarks. In accordance to the taxonomic changes previously discussed, in this revision we treat *Callotillus* as a genus containing two species: *C. bahamensis* Vaurie

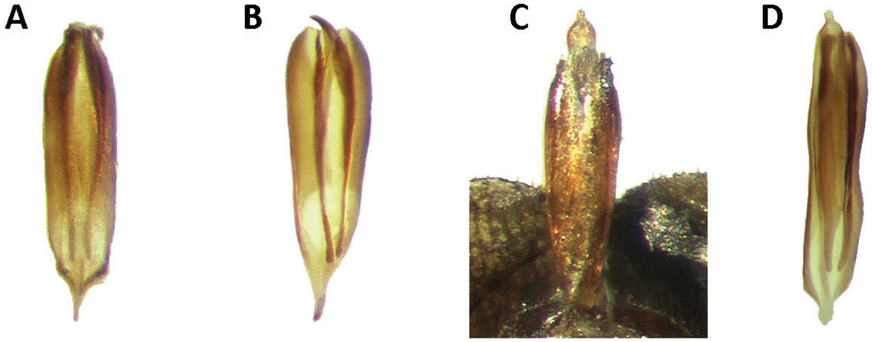

Figure 7. Aedeagus of: **A** *Neocallotillus elegans* (*C. e. elegans*) **B** *N. elegans* (*C. e. vafer*) **C** *N. intricatus* (*C. intricatus*) **D** *Callotillus eburneocinctus.*

(Figs1G, 2C), a species currently recorded from the Bahamas and the Cayman Islands (Fig. 8D), and *C. eburneocinctus* Wolcott (Figs 1H, 4B), a species restricted to the southernmost tip of the Florida peninsula, including the Florida Keys (Fig. 8E).

Callotillus bahamensis Vaurie, 1952

Holotype depository. American Museum of Natural History (AMNH). Holotype locality: South Bimini Island, Bahamas, British West Indies.

Distribution. The Bahamas, Cayman Islands (Fig. 8D).

Differential diagnosis. *Callotillus bahamensis* is most similar to *C. eburneocinctus*. The two species can be differentiated with ease based on the color pattern on the elytral disc. *Callotillus bahamensis* has the elytral disc predominantly piceous, except a light testaceous area surrounding the humeral angles, this testaceous area extends from the anterior fourth of the epipleural fold and may reach the scutellum or not (Fig. 1G). *Callotillus eburneocinctus* has the anterior half of the elytral disc rufous and the posterior portion fuscous, this coloration shift is interrupted by a transverse, moderately elevated, pale band which runs from the elytral suture to the epipleural fold (Fig. 1H).

Redescription. Form: Moderately robust; elytra gradually expanded toward apex, then abruptly narrowing behind distal fourth. Color: Anterior portion of femora, trochanters, coxae, and anterior fourth of the elytral disc light testaceous, this testaceous pattern on the elytral disc reaches the humeral region laterally and the scutellum internally; remaining body uniformly fuscous; the elytral disc is devoid of any bands or fasciae (Fig. 1G).

Head: Including eyes not wider than pronotum; eyes taller than wide, not bulging laterally, rather small, finely faceted, strongly sub-triangularly emarginate; integument rugose, feebly punctate, punctuation rather small; antennal notch located in front of emargination; frons not bi-impressed. Antennae of males composed of 10 antennomeres; second antennomere short, robust, beadlike in shape; third antennomere about 2× the length of previous antennomere, moderately serrate; fourth antennomeres slightly longer than third antennomere; antennomeres 4-9 about the same length, strongly serrate; last antennomere elongate, about 2.5× the length of ninth antennomere, slightly ovoid in shape, laterally compressed (Fig. 3G).

Thorax: Pronotum globose, slightly broader than long; surface shiny, finely, deeply punctate; sides subparallel, then abruptly constricted on posterior fourth; disc strongly convex; anterior transverse depression and subbasal tumescence absent. Prosternum wider than long; surface smooth. Mesoventrite rugulose; surface finely punctate, feebly vested with fine, pale, semierect setae. Metepisternum partially visible in lateral view; conspicuously clothed with recumbent, pale setae. Metaventrite globose; strongly convex; surface shiny; longitudinal depression present; metaventral process absent.

Elytra: Convex, robust; humeri indicated, gradually expanding toward elytral apex, then abruptly narrowing behind elytral fourth; conspicuously vested with fine, pale, recumbent and semi-recumbent setae, vestiture density is reduced on anterior fourth where elytral disc acquires a testaceous tone; elytral disc rugulose throughout the surface; elytral apices rounded, moderately dehiscent; epipleural fold complete, narrowing toward apex.

Legs: Femora swollen anteriorly; surface shiny, smooth, very finely rugulose. Tibiae longitudinally rugulose; two tarsal denticles, outer denticle digitiform, interior denticle triangular in shape.

Abdomen: Ventrites 1-4 broadly convex, smooth, subquadrate, feebly punctate, not depressed laterally. Fifth visible ventrite convex, shiny and moderately depressed medially; lateral margins subparallel; posterior margin broadly truncate. Sixth ventrite triangular in shape; small; moderately excavated; shiny; feebly punctate; conspicuously broader than long; lateral margins strongly oblique, feebly arcuate; posterior margin small, broadly, deeply emarginate; posterolateral angles broadly rounded (Fig. 5G). Fifth tergite subquadrate; strongly convex; rugulose; feebly punctate; posterior margin truncate. Sixth tergite subquadrate; rugulose; wider than long; surface moderately convex; coarsely punctate; lateral margins oblique, posterior margin truncate; posterolateral angles rounded. Sixth tergite extending beyond apical margin of sixth ventrite, fully covering sixth ventrite in dorsal view.

Aedeagus: Not available.

Female variation: Females of *C. bahamensis* can be distinguished from male specimens based on the antennal structure and the shape of the last abdominal segment. The females have antennomeres 4-9 moderately serrate (Fig. 3I), rather than strongly serrate, as observed in males (Fig. 3G). Additionally, the last ventrite and the last tergite are subquadrate in shape (Fig. 5H), and not emarginate, as in males (Fig. 5G).

Material examined. ALLOTYPE: 1 female: South Bimini Island, Bahamas, B. W. I., VI-1951, M. Cazier and C and P Vaurie, handwritten red label, allotype depository: SMNH.

Additional material examined (N=2). 1 male, 1 female: Cayman, Little Cayman, 3 km SE of Spot Bay, 27-V-2009, R. Turnbow.

Remarks. In her original description, Vaurie (1952) indicated that *C. bahamensis* is most closely related to *C. crusoe*. After examination of specimens of *C. bahamensis*,

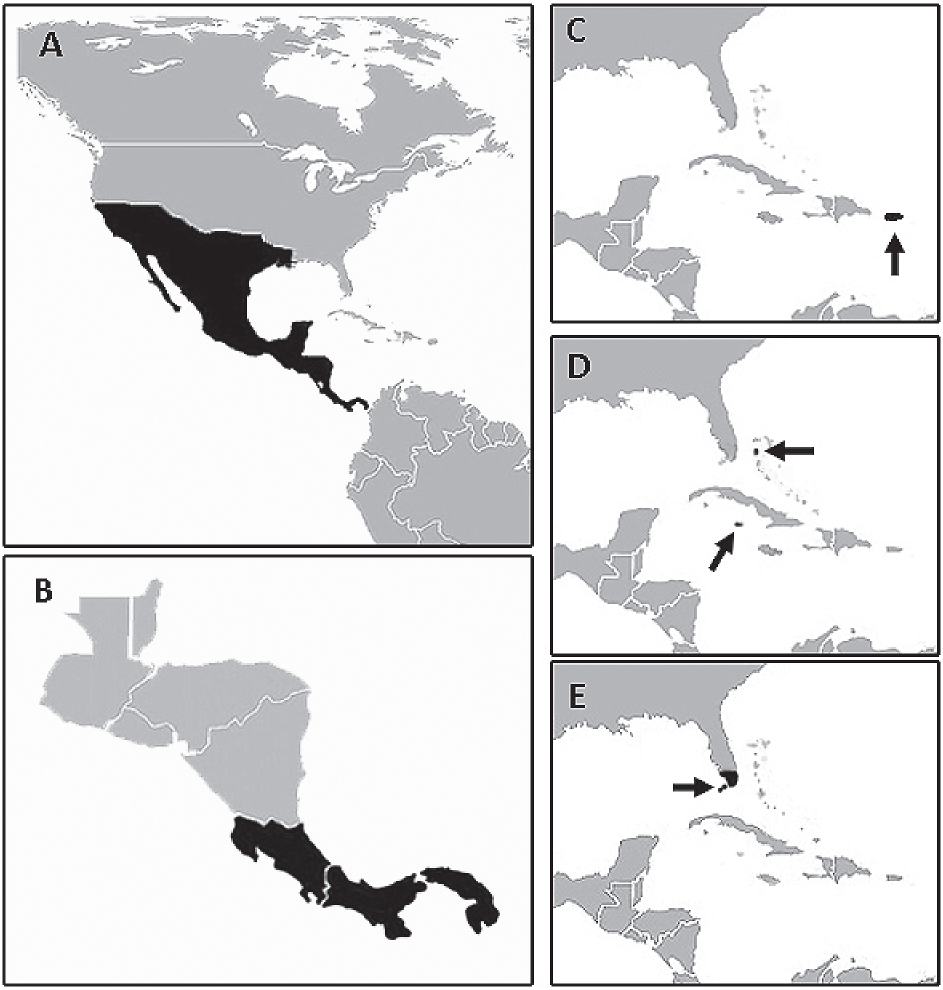

Figure 8. Distribution map of: **A** *Neocallotillus elegans* **B** *N. intricatus* **C** *N. crusoe* **D** *Callotillus bahamensis* **E** *C. eburneocinctus.*

this species is most similar to *C. eburneocinctus*. Based on Wolcott's description (1921), we place *C. crusoe* within *Neocallotillus*. Examination of material of *C. crusoe* will be essential to clarify the status of this rare species.

Callotillus eburneocinctus Wolcott, 1911

Holotype depository. United States National Museum of Natural History (USNM). Holotype locality: Key West, Monroe Co., Florida.

Distribution. USA: FL (Fig. 8E).

Differential diagnosis. *Callotillus eburneocinctus* is most similar to *C. bahamensis*. Characters to distinguish these species appear in the diagnosis section of *C. bahamensis.*

Redescription. Form: Body moderately elongate, robust; head and pronotum somewhat slender; elytra gradually expanded toward apex, then abruptly narrowing behind distal fourth. Color: head, antennae, mouthparts, pronotum, and anterior half of elytral disc testaceous to rufous; legs brunneous; distal end of mandibles, abdomen and posterior half of elytral disc fuscous; thorax bicolored, metaventrite anteriorly and internally ferrugineous, posteriorly and distally fuscous; each elytron with a transverse, median, pale fascia that runs from the epipleural fold to the elytral suture, this band may be protruded in most individuals (Fig. 1H).

Head: Including eyes not wider than pronotum; eyes small, taller than wide, not bulging laterally, finely faceted, strongly, sub-triangularly emarginate; surface integument corrugate; antennal notch located in front of antennal emargination; frons not bi-impressed. Antennae of males consisting of 10 antennomeres; second antennomere short, robust; third antennomere about 2× the length of previous antennomere, moderately serrate; fourth antennomeres slightly longer than third antennomere; antennomeres 4-9 about the same length, strongly serrate; last antennomere elongate, about 2× the length of ninth antennomere, slightly ovoid in shape, laterally compressed (Fig. 3H). Anterior portion of clypeus narrow, approximately 2× the length of eye emargination (Fig. 6B).

Thorax: Pronotum globose, as broad as long; surface rugulose, profusely, finely punctate; punctations narrow, shallow; sides subparallel in dorsal view, then abruptly constricted on posterior fourth; disc strongly convex; anterior transverse depression and subbasal tumescence absent. Prosternum wider than long; surface smooth, rugulose, glabrous. Mesoventrite smooth, shiny, glabrous; surface very finely punctate. Metaventrite strongly convex; surface smooth, feebly, finely punctate; longitudinal depression present; metaventral process absent.

Elytra: Robust; humeri indicated, gradually expanding toward elytral apex; surface convex on first third, then moderately depressed on second third, and conspicuously convex again on last third; a pair of long, stiff, erect tuft of dark setae located on the anterior fourth each elytron; surface of elytral disc rugulose; sculpturing absent; elytral apices rounded, moderately dehiscent; epipleural fold complete, narrowing toward apex.

Legs: Femora moderately swollen; surface shiny, smooth. Tibiae longitudinally rugulose; two tarsal denticles, outer denticle digitiform in shape, interior denticle triangular.

Abdomen: Ventrites 1-3 broadly convex, smooth, shiny, subquadrate, feebly punctate, not depressed laterally. Fourth ventrite moderately punctate, medially depressed. Fifth ventrite shiny, strongly excavated; lateral margins subparallel; posterior margin broadly, shallowly emarginate. Sixth ventrite triangular in shape; small; moderately excavated; feebly punctate; conspicuously broader than long; lateral margins strongly oblique, moderately arcuate; posterior margin shallowly, narrowly emarginate; posterolateral angles rounded (Fig. 5I). Fifth tergite subquadrate; moderately, coarsely punctate; posterior margin truncate. Sixth tergite subtriangular in shape; feebly convex; wider than long; surface moderately punctate; lateral margins strongly oblique, moderately arcuate; posterior margin very shallowly, narrowly emarginate; posterolateral angles broadly rounded. Sixth tergite extending beyond apical margin of sixth ventrite, fully covering sixth ventrite in dorsal view.

Aedeagus: Phallobasic apodeme present; phallus with copulatory piece feebly swollen at apex, petiolate; phallobase subparallel; phallic plate armed with one irregular row of denticles; intraspicular plate present, elongate; phallobasic lobes free; tegmen complete, fully covering phallus; phallobasic lobes rounded distally; phallobasic apodeme moderately short, expanded distally; endophallic struts short, robust distally (Fig. 7D).

Female variation: The structure of the antennae will help to differentiate females of *C. eburneocinctus* from males. Females have the third antennomere feebly serrate and the antennomeres 4-9 are moderately serrate (Fig. 3J), males have the third antennomere moderately serrate and antennomeres 4-9 are strongly serrate (Fig. 3H). Females also have the sixth ventrite subquadrate in shape and the posterior margin is broadly truncate (Fig. 5J), males have this segment subtriangular in shape with the posterior margin narrow and feebly emarginate (Fig. 5I).

Material examined (N=35). 2 males, 3 females: Monroe Co., FL, Big Pine Key, 17-IV-1978, E. Giesbert; 1 male, 1 female: Monroe Co., FL, Everglades Nat. Park, Flamingo, 16-V-1991, R. Morris; 1 male, 2 females: FL, Miami, 13-VI-1963, B. K. Dozier; 1 female: FL, Tavernier, on Key Largo, 19-VI-1970, beating *Laguncularis racemosa* (L.), G. H. Nelson; 2 males, 3 females: FL, Miami, Virginia Key, 23-VI-1970, beating *Conocarpus erecta* L., G. H. Nelson. 1 male: Miami-Dade Co., FL, Miami, 25-VI-1965, B.K. Dozier; 4 specimens: FL, No Name Key, 29-V-1997, R. Turnbow, ex. *Metopium toxiferum* L., emerged 31-III-1979, R. Turnbow; 1 specimen: FL, Sugarloaf Key, 2-V-2000, 30-V-1997, R. Turnbow; 4 males, 9 females: Monroe Co., FL, Big Pine Key, reared from wood, E. Giesbert.

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Five new species of *Trigonopeltastes* Burmeister and Schaum from Central America with new country records for other New World Trichiini (Coleoptera, Scarabaeidae, Cetoniinae)

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Abstract

Five new species of *Trigonopeltastes* Burmeister and Schaum, 1840 are described: *Trigonopeltastes arborfloricola* **sp. n.** from Nicaragua, *T. formidulosus* **sp. n.** from Costa Rica, *T. henryi* **sp. n.** from Costa Rica, *T. mombachoensis* **sp. n.** from Nicaragua, and *T. warneri* **sp. n.** from Belize and Guatemala. An updated key to species of *Trigonopeltastes* is presented. *Trigonopeltastes nigrinus* Bates, 1889 and *Trigonopeltastes carus* Bates, 1889 are placed in synonymy with *Trigonopeltastes geometricus* Schaum, 1841, **syn. n.**. The males of *Trigonopeltastes aurovelutinus* Curoe, 2011 and *Trigonopeltastes simplex* Bates, 1889 are described for the first time.

New country records are given for the following: *Giesbertiolus ornatus* Howden, 1988: Costa Rica; *Paragnorimus sambucus* Howden, 1970: Guatemala; *Trichiotinus bibens* (Fabricius, 1775): Canada; *Trigonopeltastes archimedes* Schaum, 1841: Guatemala and Costa Rica; *Trigonopeltastes frontalis* Bates, 1889: Belize, Guatemala, and Honduras; *Trigonopeltastes glabellus* Howden, 1988: Guatemala; *Trigonopeltastes geometricus* Schaum, 1841: Honduras; *Trigonopeltastes sallaei sallaei* Bates, 1889: Guatemala and Honduras; *Trigonopeltastes simplex* Bates, 1889: Mexico; *Trigonopeltastes variabilis* Howden, 1968: Honduras.

Keywords

Taxonomy, Central America, Mexico, Scarabaeoidea, Trichiina

Introduction

The genus *Trigonopeltastes* Burmeister and Schaum, 1840 (Coleoptera: Scarabaeidae: Cetoniinae: Trichiini: Trichiina) is distributed from the southern United States of America to northern Argentina, with most species occurring in Mexico and Central America. Specimens are most often collected in flowers of various shrubs and trees and also turn up in flight intercept traps and by beating vegetation. The rarity of many species in natural history collections emphasizes the need for targeted collecting efforts using specific methods in order to survey New World Trichiini fauna.

The taxonomy of the genus was modernized by Howden (1968), which allowed additional species to be discovered and described by Howden (1988), Howden and Ratcliffe (1990), Howden and Joly (1998), Ricchiardi (2003), and Curoe (2011). While curating and identifying specimens in natural history collections, I discovered five new species of *Trigonopeltastes* from Belize, Guatemala, Nicaragua, and Costa Rica. In addition, the examination of more specimens and longer series added several significant new distributional records and helped to clarify the taxonomic status of *Trigonopeltastes nigrinus* Bates, 1889.

The purpose of this paper is to describe five new species of *Trigonopeltastes*, update the identification key for *Trigonopeltastes*, synonymize *Trigonopeltastes nigrinus* with *Trigonopeltastes geometricus* Schaum, 1841, report new country records in the New World for a number of Trichiini species, and describe the previously unknown males of *Trigonopeltastes aurovelutinus* Curoe, 2011 and *Trigonopeltastes simplex* Bates, 1889. The genus *Trigonopeltastes* now contains 26 species.

Materials and methods

Specimens

More than 500 specimens were examined to form the basis of this review. The following institution and private collections (curators in brackets) are cited in the text as repositories for specimens. The term allotype follows the definition of Santiago-Blay et al. (2008).

CMNC	Canadian Museum of Nature, Ottawa, Ontario, Canada (François Génier, Robert Anderson)
DCCC	David C. Carlson Collection, Fair Oaks, California, United States of America
DEBU	Insect Collection, University of Guelph, Guelph, Ontario, Canada (Steve Paiero, Steve Marshall)
EMEC	Essig Museum of Entomology, University of California, Berkeley, California, United States of America (Cheryl Barr)
FSCA	Florida State Collection of Arthropods, Gainesville, Florida, United States of America (Paul Skelley)

MNCR	Museo Nacional de Costa Rica, San José, Costa Rica (formerly at Instituto Nacional de Biodiversidad (INBio), Santo Domingo de Heredia, Costa Rica) (Angel Solís)
RACC	Rich A. Cunningham Collection, Chino, California, United States of America
SEMC	Snow Entomological Museum, University of Kansas, Lawrence, Kansas, United States of America (Zack Falin)
UNSM	University of Nebraska State Museum, Lincoln, Nebraska, United States of America (Brett Ratcliffe, M.J. Paulsen)
USNM	United States National Museum, Washington, District of Columbia, United States of America (currently housed at UNSM) (Brett Ratcliffe, M.J. Paulsen)

Label data, specimen images, and maps

The verbatim label data is given for specimens in quotation marks with slashes to indicate a new line of text on the label. The specimen images were taken at the CMNC using Leica imaging equipment and the Leica Application Suite software. The maps were created using the SimpleMappr website (http://www.simplemappr.net/). The specimen images and maps were modified and plates constructed in Adobe Photoshop.

Taxonomy

Trigonopeltastes arborfloricola sp. n.

http://zoobank.org/111C866F-E384-4251-BCF7-18D3B9EA932E

Type locality. Reserva Privada Silvestre Domitila, Granada, Nicaragua.

Type series. Holotype male at USNM labeled “NICARAGUA: Granada Prov. / Reserva Privada Silvestre / Domitila, 70 meters / 11°42.51'N, 85°57.16'W / 10 June 2007 N.E. Woodley / S.W. Lingafelter - tree flowers” (typeset). The holotype bears my red holotype label.

Description of holotype (Figs 1–6). Male. Length 9.0 mm, width 4.0 mm. Color: dorsal surface black with orange markings on elytra and light scales on pronotum, pygidium (Figs 1–2, 5–6). Mesofemur and metafemur tan along outside half, black along inside half; profemur, tarsi black. *Head:* Surface with short, dense setae medially on disc (sparsely setose to glabrous on apex of clypeus, base of frons) (Fig. 5), clypeus without longitudinally elongate punctures (punctures on head dense, round). Clypeus slightly wider than long with midline not elevated, apex emarginate. Antenna with 10 antennomeres, club length slightly shorter than length of antennomeres 2–7. Maxilla with long, thin brush protruding beyond clypeus in dorsal view. Mentum densely setose, obscuring surface. *Pronotum:* Surface of disc dull-black with shiny mi-

Figures 1–6. *Trigonopeltastes arborfloricola* sp. n., male holotype. **1** Dorsal habitus **2** Oblique habitus **3** Lateral genitalia **4** Parameres **5** Head and pronotum **6** Pygidium.

cropunctures. Marginal bead complete (obscured mediobasally), with complete ring of short, dense, yellow setae inside marginal bead. Pronotal disc with more-or-less complete inverted triangle grooved into the surface; groove with short, dense, yellow setae (Fig. 5). *Scutellum:* Surface with short, dense, white setae. *Elytra:* Surface glabrous, matt with shiny micropunctures, without cretaceous bands. Elytral striae 1–3 distinctly impressed, especially towards apex; remaining striae weakly defined with rows of punctures not impressed into surface. Orange markings consisting of a large mediobasal patch and smaller medioapical patch on each elytron (Figs 1–2). *Pygidium:* Surface without cretaceous makings, with complete ring of short, dense, yellow setae (Fig. 6). Disc with distinct, transverse microridges; surface evenly convex with apex slightly deflexed. *Venter:* Sternum and abdominal sternites without cretaceous markings, covered with dense, short, yellow setae. *Legs:* Protibia with 2 teeth near apex (Fig. 5). Mesotibia robust with edges weakly bowed outward medially. Tibial spurs acute, unmodified. Holotype with part of right protarsus missing. *Parameres:* Robust with lateral notches towards apex (Figs 3–4).

Etymology. The name *arborfloricola* approximates "tree flower dweller" in Latin. The name is a noun in apposition. As indicated on the label, the holotype was collected in tree flowers.

Distribution (Fig. 56). NICARAGUA - Granada (1): Reserva Privada Silvestre Domitila.

Temporal data. June (1).

Remarks. This species is fairly distinct in the complete lack of cretaceous markings and orange color pattern on the elytra. Hopefully more specimens will turn up in the lowlands of southern Nicaragua and northern Costa Rica so the intraspecific variation can be described.

Trigonopeltastes formidulosus sp. n.

http://zoobank.org/7A845763-31AC-4A47-A969-C016851917C2

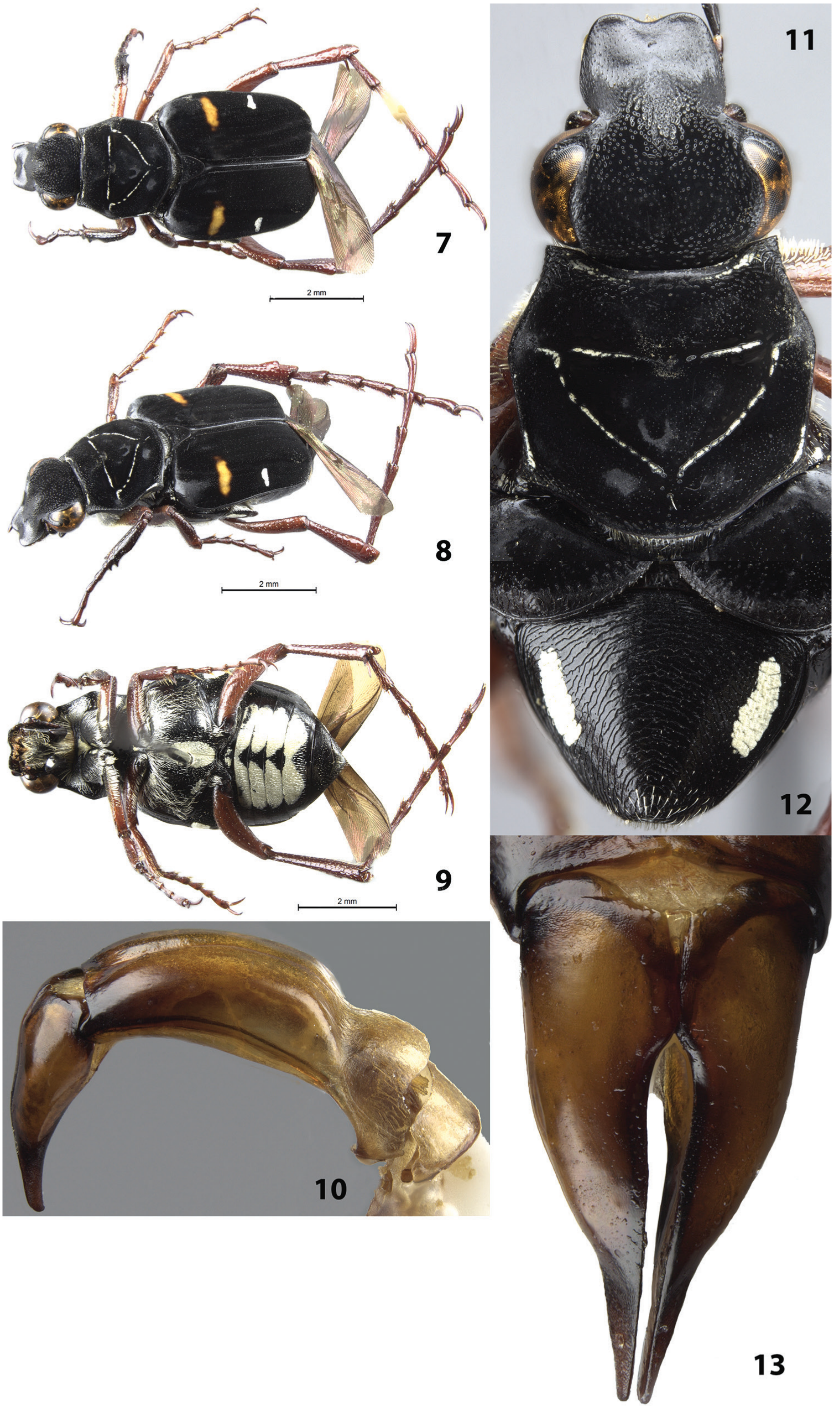
Type locality. Monteverde, 1500 m, Puntarenas, Costa Rica.

Type series. Holotype male, allotype female, 9 male paratypes, and 11 female paratypes. Holotype male, allotype female, and one male paratype at CMNC and one male paratype and one female paratype at UNSM labeled "COSTA RICA, Puntarenas / Monteverde, 1500m / May 11-13, 1996 / E. Giesbert, coll." (typeset). One male and one female paratype at CMNC labeled "COSTA RICA, Puntarenas / San Luis (Monteverde) / 3900' May 12-13, 1996 / E. Giesbert, coll." (typeset). One male paratype (database # INBIOCRI000567265) at MNCR labeled "Est. G. Brenes, 1300m, / Res. Biol. Monteverde, / Prov. Punt. COSTA RICA / E. Bello, Jun 1991, / L-N-249750,450075" (typeset) and "Trigonopeltastes femoratus? / DET. / H. F. HOWDEN 94" (handwritten and typeset). One female paratype (database # INBIOCRI000601568) at MNCR labeled "Est. G. Brenes, 1300m, / Res. Biol. Monteverde, / Prov. Punt. COSTA RICA / E. Bello, Jun 1991, / L-N-249750,450075" (typeset) and "ADN Barcodeado / 2011 / Elena Ulate A." (typeset). One male paratype (database # INBIOCRI001857744) at MNCR labeled "San Luis, Monteverde, R. B. Monteverde, / A. C. Arenal, Prov. Punta, COSTA RICA. / 1000-1350 m. Ene 1994, Z. Fuentes, L N / 449250_250850 # 2609" (typeset) One female paratype (database # INBIOCRI001894631) at MNCR labeled "Buen Amigo, San Luis Monteverde, A. C. / Arenal, Prov. Punta, COSTA RICA. 1000- / 1350 m. May 1994, Z. Fuentes, L N / 250850_449250 # 2926" (typeset). One male paratype (database # INBIOCRI001895044) at MNCR labeled "Buen Amigo, San Luis Monteverde, A. C. / Arenal, Prov. Punta, COSTA RICA. 1000- / 1350 m. May 1994, Z. Fuentes, L N / 250850_449250 # 2926" (typeset) and "ADN Barcodeado / 2011 / Elena Ulate A." (typeset). One female paratype (database # INBIOCRI001895048) at MNCR labeled "Buen Amigo, San Luis Monteverde, A. C. / Arenal, Prov. Punta, COSTA RICA. 1000- / 1350 m. May 1994, Z. Fuentes, L N / 250850_449250 # 2926" (typeset) and "ADN Barcodeado / 2011 / Elena Ulate A." (typeset). One male paratype (database # INBIOCRI001895061) at MNCR labeled "Buen Amigo, San Luis Monteverde, A. C. / Arenal, Prov. Punta, COSTA RICA. 1000- / 1350 m. May 1994, Z. Fuentes, L N / 250850_449250 # 2926" (typeset). One female paratype (database # INBIOCRI001923144) at MNCR labeled "San Luis, Monteverde, R. B. Monteverde / A. C. Arenal, Prov. Punta, COSTA RICA. / 1000-1350 m, 20-27 Jun 1994, Z. / Fuentes, L N 250850_449250 # 3029" (typeset). One female paratype (database # INBIOCRI001923145) at MNCR labeled "San Luis, Monteverde, R. B. Monteverde / A. C. Arenal, Prov. Punta, COSTA RICA. / 1000-1350 m, 20-27 Jun 1994, Z. / Fuentes, L N 250850_449250 # 3029" (typeset). One female paratype (database # INBIOCRI001923255) at MNCR labeled "Buen Amigo, San Luis Monteverde, A. C. / Arenal, Prov. Punta, COSTA RICA. 1000- / 1350 m. 8-12 Jun 1994, K. Matínez, L N / 250850_479250 # 3078" (typeset). One male paratype (database # INBIOCRI001992059) at MNCR labeled "Buen Amigo, San Luis Monteverde, Prov. / Punta, COSTA RICA. 1000 - 1350 m. Ago / 1994, Z. Fuentes, L N 250850_449250 # / 3168" (typeset), "Trigonopeltastes simplex Bates? / DET. / H. F. HOWDEN 01" (handwritten and typeset), and "ADN Barcodeado / 2011 / Elena Ulate A." (typeset). One female paratype (database # INBIOCRI001992060) at MNCR labeled "Buen Amigo, San Luis Monteverde, Prov. / Punta, COSTA RICA. 1000 - 1350 m. Ago / 1994, Z. Fuentes, L N 250850_449250 # / 3168" (typeset) and "ADN Barcodeado / 2011 / Elena Ulate A." (typeset). One female paratype (database # INBIOCRI000457825) at MNCR labeled "Fca. Cafrosa, 1300m. Est / Las Mellizas, P. Internac. / La Amistad, Prov. Punt. / COSTA RICA. M. / Ramirez, Jun 1991, / L-S-316100, 596100 ♀" (typeset and handwritten), "Trigonopeltastes / femoratus? / DET. / H.F. HOWDEN 94" (handwritten and typeset), and "ADN Barcodeado / 2011 / Elena Ulate A." (typeset). One male paratype at UNSM labeled "COSTA RICA, PUNTARENAS / MONTEVERDE / APR 19-26 1988 / E. GIESBERT, COLL." (handwritten) and "Tr. / nigrina / DET. group. / H.F. HOWDEN 91" (handwritten and typeset). One female paratype at UNSM labeled "COSTA RICA, PUNTARENAS / MONTEVERDE / APR 19-26 1988 / E. GIESBERT, COLL."

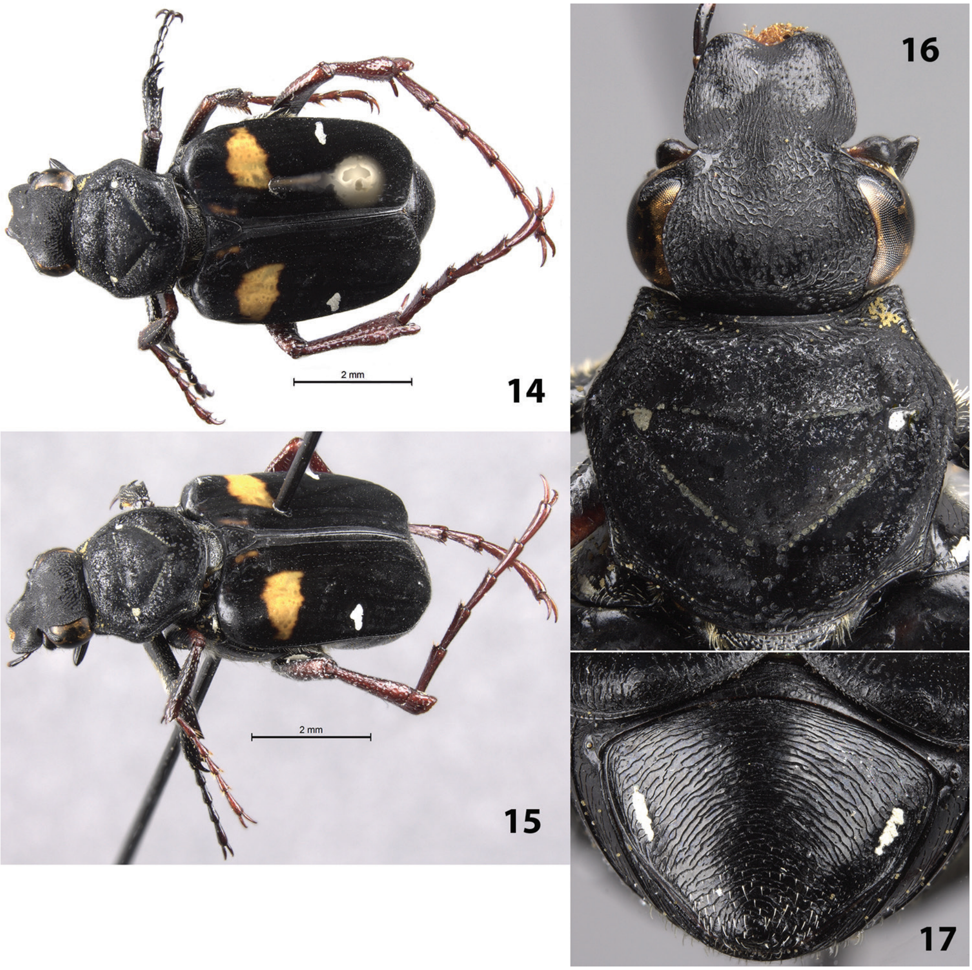
(handwritten). The types listed above bear my red holotype or allotype label or yellow paratype label.

Description of holotype (Figs 7–11, 13). Male. Length 9.0 mm, width 3.5 mm. Color: dorsal surface black with orange band and white, cretaceous band on each elytron (Figs 7–8). Legs tan except protibia and protarsus dark brown. *Head:* Surface setose with short setae medially on disc, without cretaceous markings (Fig. 11), clypeus with longitudinally elongate punctures. Clypeus about as long as wide with midline weakly elevated, apex emarginate. Antenna with 10 antennomeres, club longer than length of antennomeres 2–7. Maxilla with long, thin brush slightly protruding beyond clypeus in dorsal view. Mentum densely setose, obscuring surface. *Pronotum:* Surface of disc dull-black with shiny micropunctures (Fig. 11). Marginal bead complete, without complete ring of setose cretaceous markings inside marginal bead (only a few small patches of cretaceous markings in marginal bead). Pronotal disc with more-or-less complete inverted triangle indented into the surface with thin, cretaceous markings; cretaceous markings of triangle without setae (Fig. 11). *Scutellum:* Surface without cretaceous markings. *Elytra:* Surface glabrous, matt. Transverse cretaceous bands (1 on each side) adjacent to lateral edge approximately halfway between base and apex, length approximately ¼ width of single elytron (Figs 7–8). Elytral striae weakly defined with rows of small punctures, striae not indented into surface. Orange markings consisting of a transverse band adjacent to lateral edge approximately halfway between base and apex, width slightly less that half the width of single elytron (Figs 7–8). *Pygidium:* Surface with large, basiolateral, cretaceous makings; disc with distinct ridges of concentric circles (as in Fig. 12). Disc strongly, evenly convex with apex deflexed, flat. *Venter:* Sternum with large, central, cretaceous marking and smaller transverse, cretaceous markings apicolaterally and basiolaterally; remainder of surface setose (Fig. 9). Visible abdominal sternites 1–4 medially covered with cretaceous markings (except for small, central triangles); with short, white setae scattered throughout (Fig. 9). *Legs:* Protibia with 2 distinct teeth near apex (Fig. 8). Mesotibia robust with edges weakly bowed outward medially. Metatibia clavate. Tibial spurs acute, unmodified. *Parameres:* Robust at base tapering to an acuminate tip apically (Figs 10, 13).

Variation. Female allotype (Figs 14–17): length 9.5 mm, width 4.0 mm. The female allotype differs in the following characters. Color: orange band on elytra twice as thick as on holotype (Figs 14–15). Legs dark brown except mesotarsus, metatibia, and metatarsus tan. *Pygidium:* Surface with smaller basiolateral cretaceous makings. Disc weakly convex with apex not deflexed (Fig. 17). *Venter:* Sternum without cretaceous markings. Visible abdominal sternite 1 with small, lateral, cretaceous spot; sternites 2–4 without cretaceous markings. *Legs:* Protibia with 3 distinct teeth, 2 near apex and 1 medial. Paratypes: length 8.5–10.0 mm. Orange band on elytra either thick or thin, regardless of sex; 3 females had greatly expanded, orange coloration covering much of basal half of elytra. Legs vary from dark tan to dark brown with females generally having darker legs than males. *Pygidium:* Basiolateral cretaceous makings never significantly larger than seen in Figs 12, 17. *Venter:* Sternum and abdominal sternites of



Figures 7–13. *Trigonopeltastes formidulosus* sp. n., male holotype. **7** Dorsal view of habitus **8** Oblique view of habitus **9** Ventral view of habitus **10** Lateral view of genitalia **11** Head and pronotum **12** Pygidium (male paratype used for this photograph) **13** Parameres.



Figures 14–17. *Trigonopeltastes formidulosus* sp. n., female allotype. **14** Dorsal view of habitus **15** Oblique view of habitus **16** Head and pronotum **17** Pygidium.

males with variable-sized cretaceous markings but markings always present in males and never present in females (except on sternite 1).

Etymology. This species is named for the black-and-orange, Halloween-themed coloration of the dorsal surface. The word *formidulosus* is a Latin for "scary". This name is an adjective in the nominative singular.

Distribution (Fig. 56). COSTA RICA - Puntarenas (18): Buen Amigo (San Luis, Monteverde), Estación G. Brenes (Reserva Biológica Monteverde), Finca Cafrosa (Estación Las Mellizas, La Amistad), Monteverde, San Luis (Monteverde).

Temporal data. January (1), May (9), June (6), August (2).

Remarks. This species has many similarities to *T. geometricus* but has consistently different dorsal color pattern and pygidial structure and cretaceous markings. *Trigonopeltastes geometricus* is variable across it's distribution and within populations but typically has more extensive orange color patterns on the elytra and always has thick lateral bands of cretaceous markings on the pygidium often leaving only a medial strip exposed. *Trigonopeltastes formidulosus* consistently has a single orange spot on each elytron without further orange lines and markings (except for the occasional thin basal line adjacent to scutellum and three females have more extensive orange coloring covering much of the basal half of the elytra). The apex of the pygidium in males is deflexed at a 90° angle in *T. formidulosus* while only slightly convex in male *T. geometricus*.

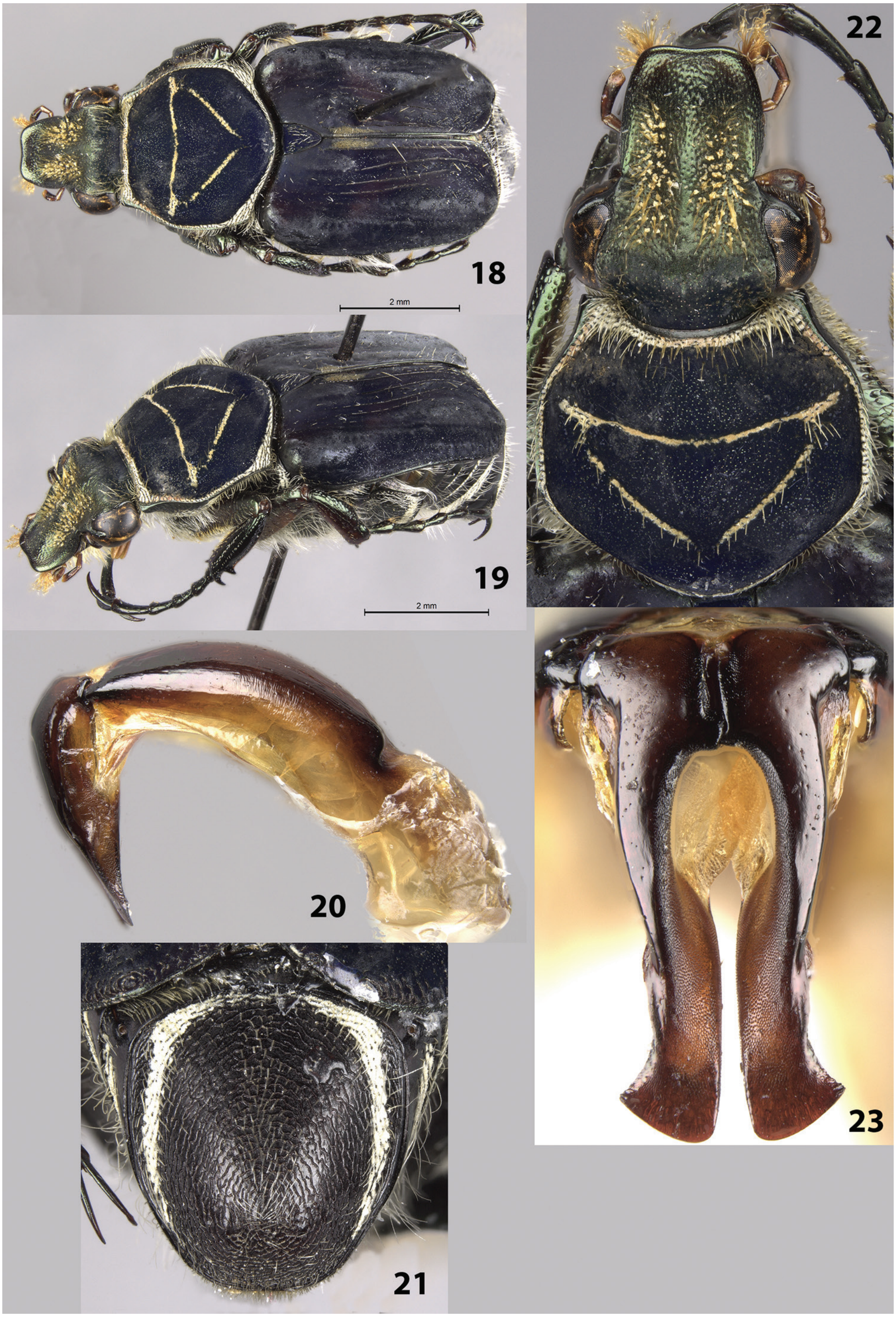
***Trigonopeltastes henryi* sp. n.**

http://zoobank.org/2E3117D5-BB7E-4986-A520-D3F4E42735BC

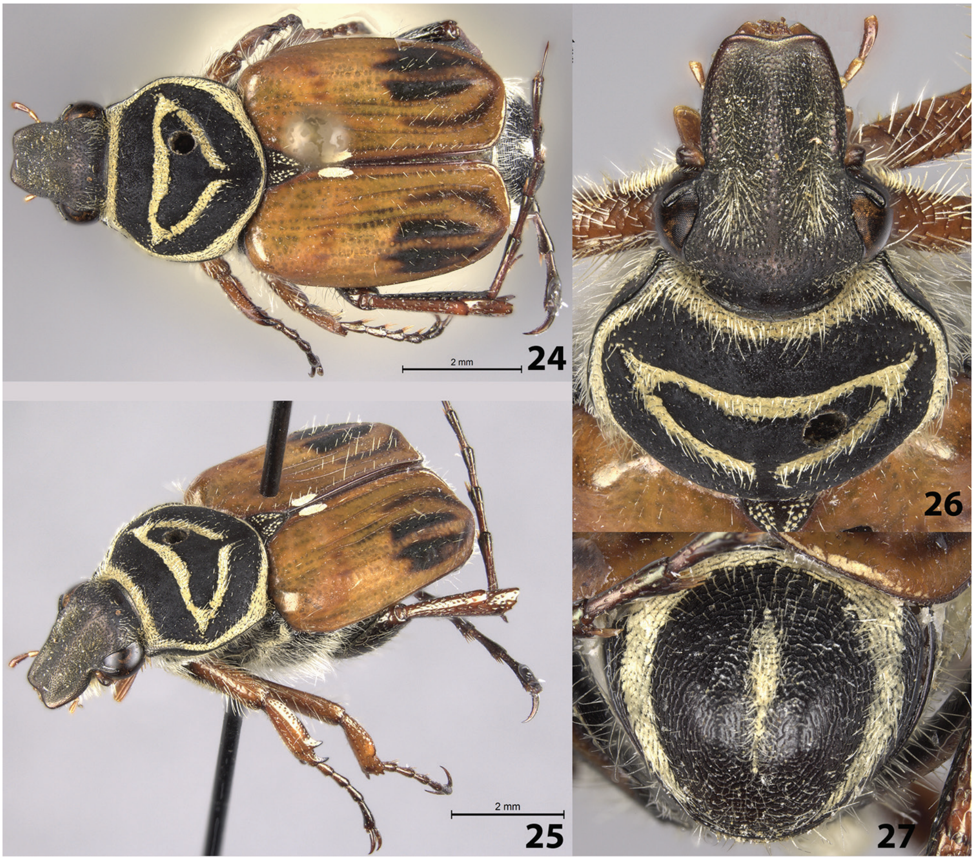
Type locality. San Luis (south of Monteverde), Puntarenas, Costa Rica.

Type series. Holotype male and allotype female. Holotype male at UNSM labeled a) "COSTA RICA, Puntarenas / San Luis (Monteverde) / 3900' May 12-13, 1996 / E. Giesbert, coll." (typeset). Allotype female at CMNC labeled a) "Prov. SAN JOSE / San Ant. / Desamp. 1 Mayo 1976 / Col. U. Ureña." (typeset and handwritten), b) "H. & A. HOWDEN / COLLECTION / Ottawa, Canada" (typeset). Both types bear my red holotype or allotype label.

Description of holotype (Figs 18–23). Male. Length 12.5 mm, width 4.5 mm. Color: head and legs shiny dark metallic green; pronotum and elytra with dull, dark blue, velvety appearance (Figs 18–19, 22); ventral surface shiny black. *Head:* Surface densely setose medially on disc (except along midline); apex, base, and midline of head moderately to sparsely setose (Fig. 22); clypeus with some longitudinally elongate punctures. Clypeus slightly longer than wide with margins and midline distinctly elevated, apex weakly emarginate. Head without cretaceous markings (Fig. 22). Antenna with 10 antennomeres, club length approximately equal to length of antennomeres 2–7. Maxilla with long, thin brush protruding beyond clypeus in dorsal view. Mentum densely setose, obscuring surface. *Pronotum:* Surface of disc dull-blue matt with shiny micropunctures (Fig. 22). Marginal bead complete, with complete ring of setose cretaceous markings inside marginal bead. Pronotal disc with more-or-less complete inverted triangle indented into the surface with cretaceous markings, diagonal lines of triangle with row of setae (Fig. 22). *Scutellum:* Surface setose, without cretaceous markings. *Elytra:* Surface sparsely setose, matt. Transverse cretaceous band (1 on each side) short, located in basal half of elytral suture (Figs 18–19, near pin but obscured by grease); weak cretaceous markings at apex of suture adjacent to pygidium. Elytral striae well defined with weak indentations between humeral angle and elytral suture. *Pygidium:* Surface rugose in a circular, fingerprint pattern; densely setose (especially around margins and midline); with thin, inverted U-shaped cretaceous band along basal and lateral surfaces of disc (Fig. 21). Disc strongly, evenly convex. *Venter:* Sternum with numerous cretaceous markings, densely setose with long setae somewhat obscuring surface. Visible abdominal sternites 2–5 with transverse cretaceous bands thick medially, thin laterally; cretaceous bands setose. *Legs:* Protibia with 2 teeth near apex (Fig. 19). Mesotibia robust with outer edge bowed outward me-



Figures 18–23. *Trigonopeltastes henryi* sp. n., male holotype. **18** Dorsal view of habitus **19** Oblique view of habitus **20** Lateral view of genitalia **21** Pygidium **22** Head and pronotum **23** Parameres.



Figures 24–27. *Trigonopeltastes henryi* sp. n., female allotype. **24** Dorsal view of habitus **25** Oblique view of habitus **26** Head and pronotum **27** Pygidium.

dially. Tibial spurs acute, unmodified. Tarsi with apicoventral tufts of setae. Holotype with metatarsal legs missing except for 1 metafemur. *Parameres:* Apically enlarged with a triangular lateral projection (Figs 20, 23).

Variation. Female allotype (Figs 24–27): length 12.0 mm, width 5.0 mm. The female allotype differs in the following characters. Color: dorsal surface of head bronze with weak green reflections; pronotum and scutellum matt black; elytra dull orange with dull black pattern on apical half (Figs 24–26). Legs tan, metafemur and parts of metatibia shiny black. *Pronotum:* Marginal bead and impressed triangle on disc with thicker cretaceous markings (Fig. 26). *Scutellum:* Surface with small cretaceous spots. *Pygidium:* Disc with longitudinally concave midline, cretaceous markings along midline (Fig. 27). *Venter:* Visible abdominal sternites 2–5 with transverse cretaceous bands thinner, of even thickness, interrupted medially.

Etymology. This species is named for the late Henry Howden (Ottawa, Canada) as thanks for bringing the female allotype to my attention and to honor his great contributions to our knowledge of *Trigonopeltastes* and other Trichiini.

Distribution (Fig. 56). COSTA RICA (2) - Puntarenas (1): San Luis (Monteverde); San José (1): San Antonio de Desamparados.

Temporal data. May (2).

Remarks. The male holotype and female allotype are the only known specimens of this new species. The very different dorsal coloration for these specimens is unusual but not unheard of within this genus. I do have some misgivings about placing these two specimens together as one species but decided to take this conservative approach because the structural characters are similar, the size and shape of the two specimens match well, the general cretaceous patterns are congruent, and the two specimens were collected within close proximity to one another. More specimens will need to be examined to understand the color variation within this species and to test my hypothesis that these male and female specimens belong to the same species.

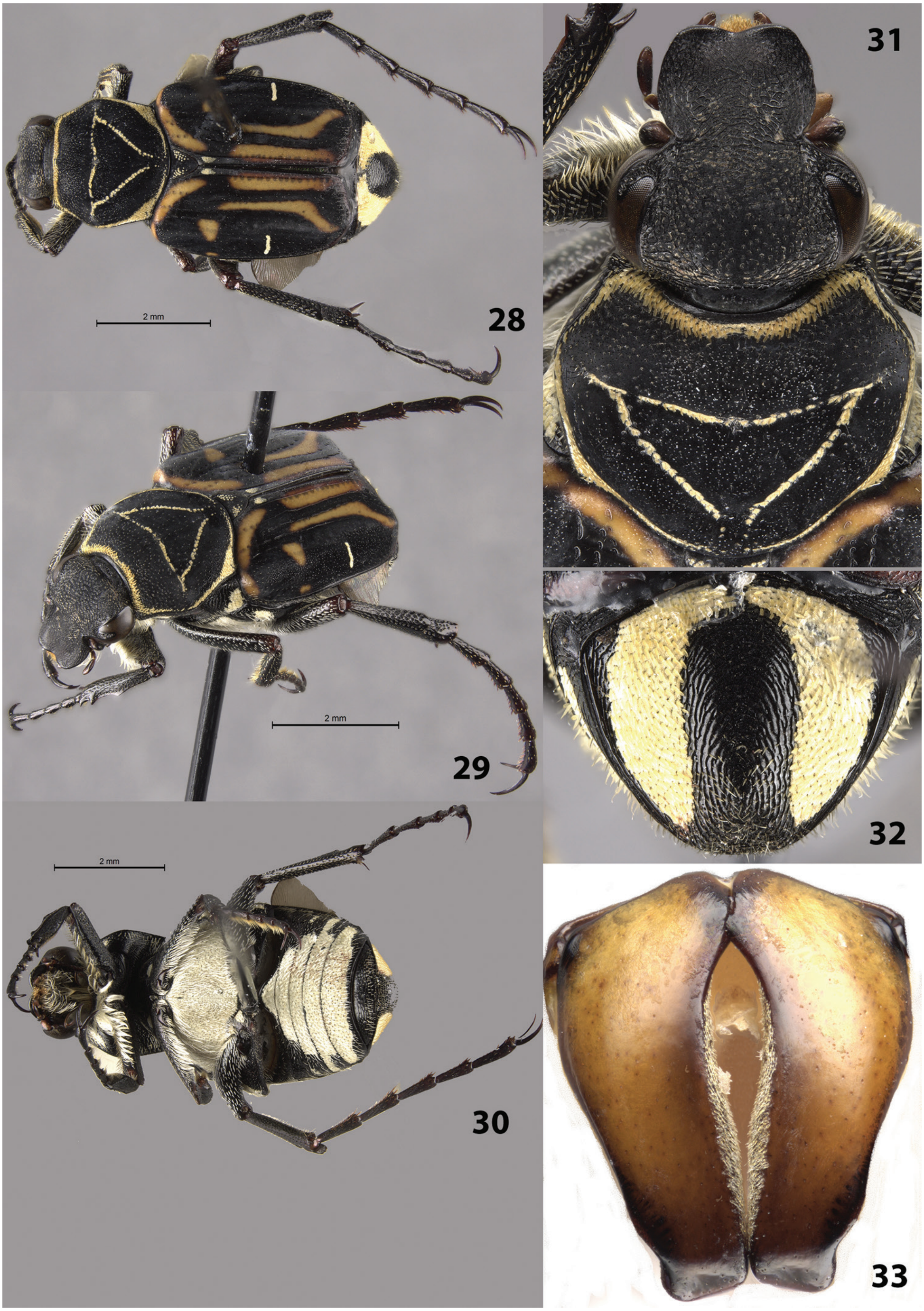
Trigonopeltastes mombachoensis sp. n.

http://zoobank.org/EA928CEE-BC17-4504-8584-6930FCB94F32

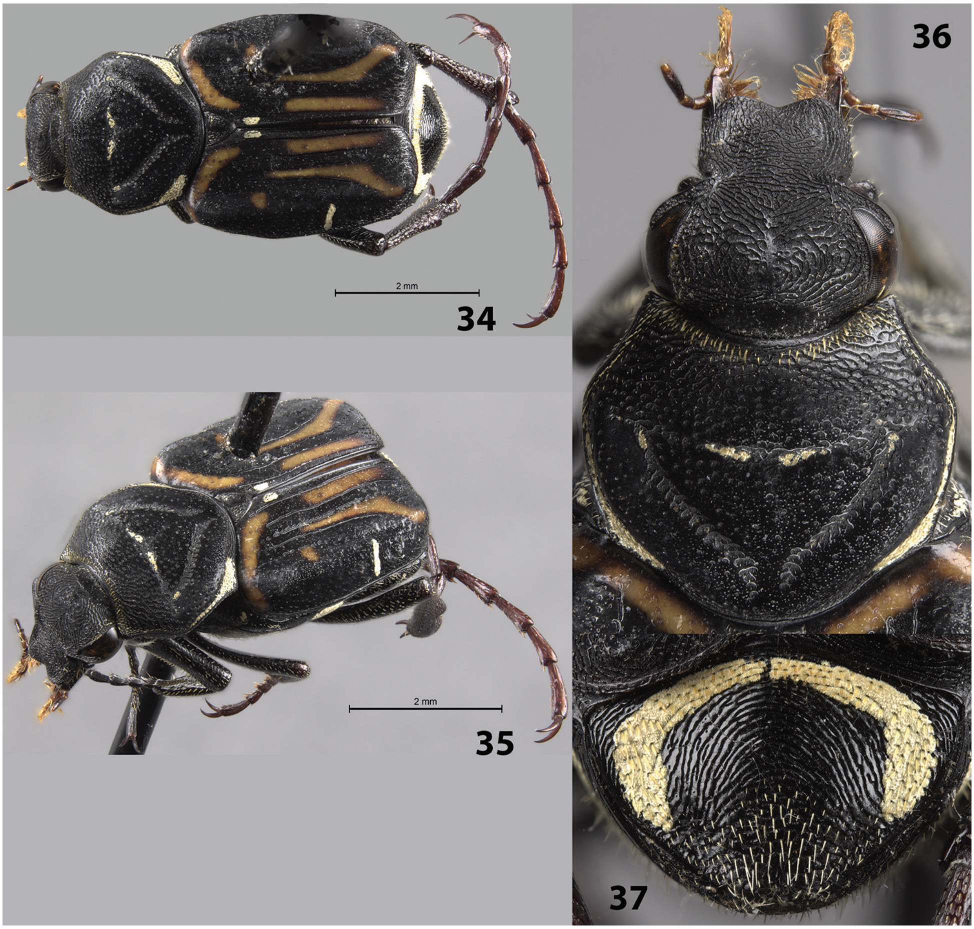
Type locality. Reserva Nacional Volcán Mombacho, Granada, Nicaragua.

Type series. Holotype male, allotype female, and 2 female paratypes. Holotype male at SEMC labeled a) "NICARAGUA: Granada Dept. / Res. Nat. Volcan Mombacho / 1150m 11°50.05'N 85°58.83'W / 3-VI-2002, R. Brooks, Z. Falin, / S. Chatzimanolis ex. misc. / collecting, NIC1BFC02 165" (typeset), b) "SMO533385 / KUNHM-ENT" (typeset beneath barcode). Allotype female at CMNC labeled "NICARAGUA: Grenada Dept. / Volcan Mombacho Res. Nat. / N11°50.0' W85°58.8', 1150 m / elfin cloud forest, beating, / 2-5.VI.2002, R.Anderson / RSA2002-034" (typeset). One female paratype at CMNC labeled "NICARAGUA: Grenada / Dept. Volcan Mombacho Res. / Nat. 1150 m, 11°50.0'N / 85°58.8'W 2-5.VI.2002, R. / Anderson, ex. elfin cloud forest / beating. RSA2002-034X" (typeset). One female paratype at CMNC labeled "NICARAGUA: Granada / Volcan Mombacho / Bosque nuboso #1 / 30-V-1998, malaise trap / J.M. Maes" (typeset). The types listed above bear my red holotype or allotype label or yellow paratype label.

Description of holotype (Figs 28–33). Male. Length 9.0 mm, width 4.0 mm. Color: dorsal surface black with orange markings on elytra and light scales on pronotum, elytra, pygidium (Figs 28–29). Legs black. *Head:* Surface glabrous, clypeus without longitudinally elongate punctures (punctures on head dense, round) (Fig. 31). Clypeus slightly wider than long with midline not elevated, apex emarginate. Antenna with 10 antennomeres, club length approximately equal to length of antennomeres 2–7. Maxilla with long, thin brush protruding beyond clypeus in dorsal view. Mentum densely setose, obscuring surface. *Pronotum:* Surface of disc dull-black with shiny micropunctures (Fig. 31). Marginal bead complete (obscured mediobasally); with complete ring of short, dense, yellow setae and cretaceous markings inside marginal bead. Pronotal disc with more-or-less complete inverted triangle indented into surface; indentation with light cretaceous markings (Fig. 31). *Scutellum:* Surface with



Figures 28–33. *Trigonopeltastes mombachoensis* sp. n., male holotype. **28** Dorsal view of habitus **29** Oblique view of habitus **30** Ventral view of habitus **31** Head and pronotum **32** Pygidium **33** Parameres.



Figures 34–37. *Trigonopeltastes mombachoensis* sp. n., female allotype. **34** Dorsal view of habitus **35** Oblique view of habitus **36** Head and pronotum **37** Pygidium.

oblique, cretaceous markings on each side. *Elytra:* Surface glabrous, matt with shiny micropunctures. Transverse cretaceous band (1 on each side) short, located on basal half of elytral suture. Cretaceous bands (1 on each side) adjacent to lateral edge approximately halfway between base and apex, length approximately ¼ width of elytron (Figs 28–29). Elytral striae 1–4 weakly impressed; remaining striae weakly defined with rows of punctures, not impressed into surface. Orange markings consisting of transverse, basal band; sub-basal, medial spot; 2 longitudinal, medioapical lines (Figs 28–29). *Pygidium:* Surface with thick, lateral bands of cretaceous makings that are narrowly joined basally; non-cretaceous area of disc setose, especially near apex (Fig. 32). Disc with distinct, concentric microridges; surface evenly convex with apex slightly deflexed. *Venter:* Metasternum entirely covered with cretaceous markings and light setae, abdominal sternites with broad, medial cretaceous markings and light setae (Fig. 30). *Legs:* Protibia with 2 teeth near apex, 1 broad medial tooth (Fig. 29). Mesotibia robust with edges weakly bowed outward medially. Tibial spurs acute, unmodified. Holotype with right mesotarsomeres 2–5 missing. *Parameres:* Robust with weak lateral notches towards apex (Fig. 33).

Variation. Female allotype (Figs 34–37): length 8.5 mm, width 3.0 mm. The female allotype differs in the following characters: *Pronotum:* Pronotal indented triangle with minimal cretaceous markings (perhaps due to abrasion, 2 female paratypes have more prominent cretaceous markings in pronotal triangle) (Figs 34–36). *Pygidium:* Surface with much thinner, rounder lateral bands of cretaceous makings along basal and lateral margins (Fig. 37). Disc flat with apex not deflexed. *Venter:* Metasternum with greatly reduced, lateral cretaceous markings, abdominal sternites with reduced, lateral cretaceous markings. *Legs:* Protibia with 3rd medial tooth larger and more distinct. Paratypes: length 9.0–10.0 mm. All characters are similar to those in the female allotype.

Etymology. This species is named for Volcán Mombacho, where all known specimens were collected.

Distribution (Fig. 56). NICARAGUA - Granada (4): Reserva Nacional Volcán Mombacho.

Temporal data. May (1), June (3).

Remarks. This species is similar to *T. intermedius* but can be distinguished by the orange color pattern on the elytra and geographic distribution. *Trigonopeltastes intermedius* has a solid, transverse, sub-basal, orange line while *T. mombachoensis* has a sub-basal, orange spot (Figs 28, 34). *Trigonopeltastes intermedius* is known from Mexico and Guatemala and *T. mombachoensis* is only known from Volcán Mombacho, Nicaragua. Any specimens found of either species in Honduras or northern Nicaragua should be studied to see if these character states are transitional.

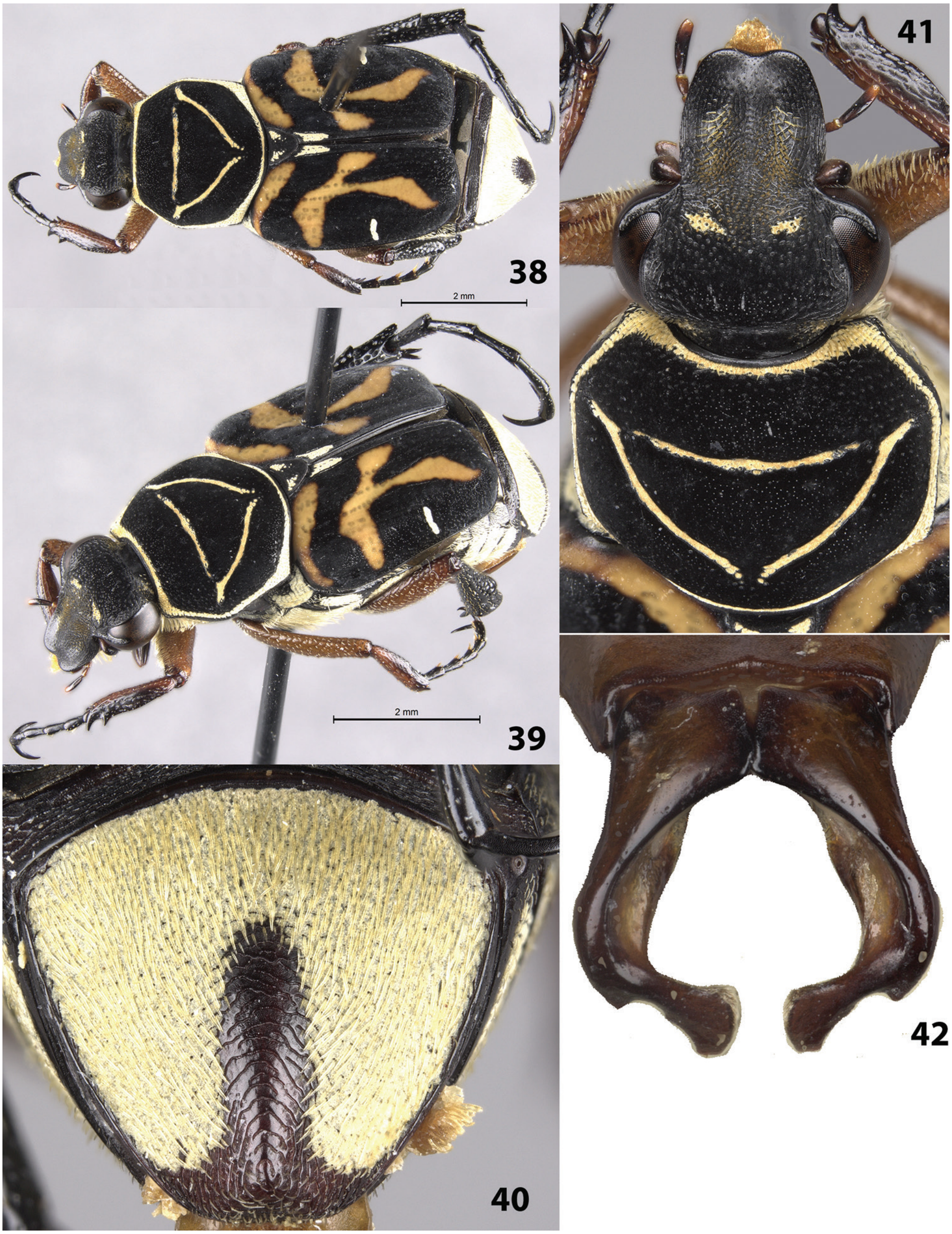
Trigonopeltastes warneri sp. n.

http://zoobank.org/EF644BD9-86EB-44FB-B09C-DC1B9373D89F

Type locality. Las Cuevas Research Station, Chiquibul National Forest, Cayo District, Belize.

Type series. Holotype male, allotype female, and 1 male paratype. Holotype male and allotype female at FSCA labeled "BELIZE: Cayo District / Chiquibul N. F. / Las Cuevas Research Sta. / 16° 43'59"N, 88°59'11"W / 29.V.2003; J.A. Shuey" (typeset). One male paratype at CMNC labeled "GUATEMALA: Petén / Cerro Cahuí / 16.99876 -89.71038 ±206m / 150m, 24.V.2009 / LLAMA #Go-B-05-3-01 / tropical moist forest, beating veg" (typeset). The types listed above bear my red holotype or allotype label or yellow paratype label.

Description of holotype (Figs 38–42). Male. Length 9.5 mm, width 3.5 mm. Color: dorsal surface black with orange markings on elytra and yellow cretaceous markings on head, pronotum, scutellum, elytra, and pygidium (Figs 38–39, 40). Femora tan, protibia and mesotibia half tan and half black longitudinally, metatibia and tarsi black. *Head:* Surface densely setose with short setae medially on disc (ex-



Figures 38–42. *Trigonopeltastes warneri* sp. n., male holotype. **38** Dorsal view of habitus **39** Oblique view of habitus **40** Pygidium **41** Head and pronotum **42** Parameres.

cept along midline), frons with paired cretaceous markings between eyes (Fig. 41), clypeus with longitudinally elongate punctures. Clypeus about as long as wide with midline distinctly elevated, apex emarginate. Antenna with 10 antennomeres, club length approximately equal to length of antennomeres 2–7. Maxilla with long, thin brush protruding beyond clypeus in dorsal view. Mentum densely setose, setae obscuring surface. *Pronotum:* Surface of disc dull-black with shiny micropunctures (Fig. 41). Marginal bead complete; with complete ring of setose, cretaceous markings inside marginal bead. Pronotal disc with more-or-less complete inverted triangle indented into the surface with cretaceous markings, cretaceous markings of triangle without setae (Fig. 41). *Scutellum:* Surface with cretaceous patches in basal corners. *Elytra:* Surface glabrous, matt. Transverse cretaceous band (1 on each side) short, located on basal half of elytral suture. Cretaceous bands (1 on each side) adjacent to lateral edge approximately halfway between base and apex, length approximately ¼ width of elytron (Figs 38–39). Elytral striae weakly defined with rows of punctures not indented into surface. Orange markings consisting of transverse, basal band and continuous, oblique T-shaped pattern on each elytron (Figs 38–39). *Pygidium:* Surface mostly covered with cretaceous makings except for apex and apical 2/3 of medial line (Fig. 40). Disc strongly, evenly convex with apex deflexed, flat. *Venter:* Sternum mostly covered with cretaceous markings and setae. Visible abdominal sternites 1–5 almost entirely covered with cretaceous markings, with erect setae scattered throughout. *Legs:* Protibia with 2 teeth near apex (Fig. 39). Mesotibia robust with outer edge weakly bowed outward medially. Tibial spurs acute, unmodified. Holotype with 1 protarsus and 1 metatarsus missing. *Parameres:* Strongly curved, roughly forming a circle; apex strongly curved inward, enlarged, dorsoventrally flattened (Fig. 42).

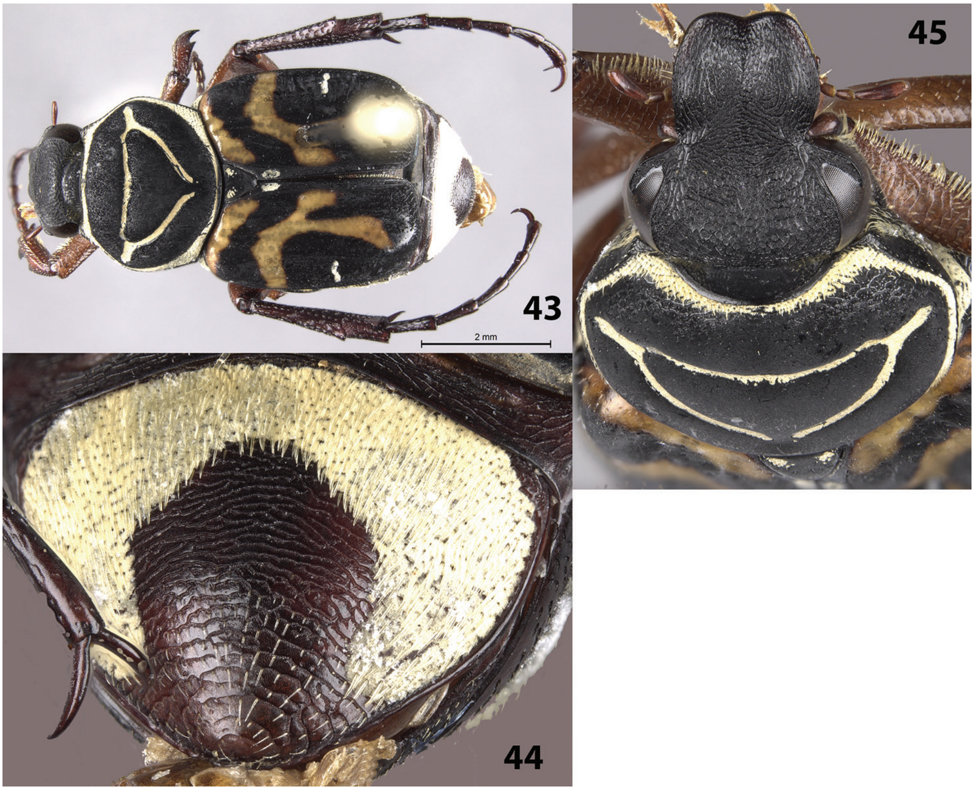
Variation. Female allotype (Figs 43–45): length 9.0 mm, width 4.0 mm. The female allotype differs in the following characters: Color: Legs tan to brown. *Head:* Surface moderately setose with short, obscure setae, head without cretaceous markings (Fig. 45). *Pygidium:* Surface with thick, inverted U-shaped, cretaceous markings along lateral margins and base; apically and medially without cretaceous markings (Fig. 44). Disc evenly convex without deflexed apex. *Venter:* Sternum only partially covered with cretaceous markings. Visible abdominal sternites 1–5 without cretaceous markings medially. *Legs:* Protibia with 3 teeth, 2 near apex and 1 medial. Mesotibia without outer edge bowed outward. Male paratype: length 9.0 mm. Femora and mesotibia orange, protibia half orange and half black longitudinally, metatibia and tarsi black with some dark red. *Head:* Frons with single cretaceous marking extending between eyes. Clypeus with midline setose, not distinctly elevated. The male paratype is similar in all characters to the holotype.

Etymology. This species is named after Bill Warner (Chandler, Arizona) as thanks for bringing the holotype and allotype specimens to my attention.

Distribution (Fig. 57). BELIZE - Cayo (2): Las Cuevas Research Station, Chiquibul National Forest. GUATEMALA – Petén (1): Cerro Cahuí (16.99876°N, 89.71038°W).

Temporal data. May (3).

Remark. This species is similar externally to *T. sallaei sallaei* and *T. intermedius* but has differences in the elytral coloration pattern. *Trigonopeltastes warneri* has distinct male parameres (Fig. 42) and is found in the lowlands of eastern Guatemala and Belize, while *T. sallaei sallaei* and *T. intermedius* are typically found at mid to high elevation localities.



Figures 43–45. *Trigonopeltastes warneri* sp. n., female allotype. **43** Dorsal view of habitus **44** Pygidium **45** Head and pronotum.

Notable new distributional records, male descriptions, and new synonymies for New World Trichiini

Giesbertiolus ornatus Howden, 1988

Distribution. This species was previously recorded from Panama (Howden 1988). The specimens detailed below represent a new country record for Costa Rica.

“Buen Amigo, San Luis Monteverde, A. C. / Arenal, Prov. Punta, COSTA RICA, 1000- / 1350 m. May 1994, Z. Fuentes, L N / 250850_449250 # 2926” (1 male - MNCR).

“COSTA RICA, Prov. Alajuela, San / Cristobal. 600-620m. 18 MAY 1998. / F. A. Quesada. En Flores. / L_N_318056_383200 #50698” (1 male, 1 female - MNCR).

“COSTA RICA. Prov. Guanacaste, / Rincón de la Vieja, Upala, Dos Ríos / San Cristobal, 600-620m, 17 MAY / 1998, F. A. Quesada, En Flor. / I_N_318056_383200 #63528” (1 female - MNCR).

“Est. Cacao, 1000-1400m, / Lado SO Vol. Cacao, / P. N. G., Prov. Guan. / COSTA RICA, C. / Chaves, Jun 1991. / L-N-323300,375700” (1 female - MNCR).

"Est. Cacao, 1000-1400m, / Lado SO Vol. Cacao, P. N. / Guan., Prov. Guanacaste, / Costa Rica, Z. Fuentes, / 21 a 29 may 1992 / L-N 323300,375700" (1 female - MNCR).

"COSTA RICA, Puntarenas, Fca. / Buen Amigo Monteverde, 4Km S. / de la Reserva 1000-1350m. MAY / 1997, Z. Fuentes, Red Mariposa. / L_N_250850_449250 #46800" (1 female - MNCR).

"COSTA RICA, Prov. Alajuela, San / Cristobal. 600-620m. 18 MAY 1998. / F. A. Quesada. En Flores / L_N_318056_383200 #50698" (1 male - CMNC)

"COSTA RICA. Prov. Guanacaste / Rincón de la Vieja, Upala, Dos Ríos, / San Cristobal, 600 – 620m, 17 MAY / 1998. F. A. Quesada, En Flor, / L_N_318056_383200 #63528" (1 female - CMNC)

Paragnorimus sambucus Howden, 1970

Distribution. This species was previously recorded from Mexico (Howden 1970). The specimens detailed below represent a new country record for Guatemala.

"GUATEMALA, Huehuetenango / Nentón, Gracias a Dios, El / Quetzal 1600 m. 20-vi-2006 / J. Monzón y F. Camposeco / COLECCION J. MONZON" (1 male - CMNC).

"GUATEMALA. Huehuetenan- / go. Aguacatán. Río Sn. Juan / 2,212m 6 JUNIO 2009 / 15.368600 – 91.288930 / Col. José Monzón Sierra" (1 male - CMNC).

Trichiotinus bibens (Fabricius, 1775)

Distribution. This species was previously recorded from the United States of America (Howden 1968). The specimens detailed below represent a new country record for Canada. These specimens were likely collected about 100–150 years ago. More collecting is needed around London, Ontario and southwestern Ontario to further verify this record. William Saunders (1835–1914) lived in London from 1847–1886 (Bethune 1914) and was a founding member of the Entomological Society of Canada in 1863 and frequent contributor to the early issues of the *Canadian Entomologist*.

"London / W. Saunders" Canadian scarab database numbers CSD013086–CSD013089 (3 males, 1 female – DEBU)

Trigonopeltastes archimedes Schaum, 1841

Distribution. This species was previously recorded from Mexico and El Salvador (Howden 1968). The specimens detailed below represent new country records for Guatemala and Costa Rica.

"GUAT.: BAJA VERAPAZ / 54.4km S. Purulha, / 850m, 1.VII.1993, / F. Génier, hand coll." (1 male - CMNC).

"GUATML. Zacapa 12-14 / km S Sn Lorenzo 1-2000' / June 3-6 1989 / J. E. Wappes" (2 males - CMNC).

"GUAT., Zacapa Sn / Lorenzo Rd 1500- / 1800'1-10 June / 1991 JE Wappes" (1 male - CMNC).

"GUAT. Zacapa / 12km S. San Lorenzo / 510m 16.VI.1993 / H. & A. Howden" (1 male, 1 female - CMNC).

"COSTA RICA: Guanacaste / Prov., Comelco (50m) / 8 km NW Bagaces / VI-5-1973, P.A. Opler / on: Croton sp." (1 male - CMNC).

"COSTA RICA: Guanacaste, / Parque Nacional Santa Rosa, / Estación Santa Rosa, 295 m. / N10°50'21.4", W 35°37'05.8" / 16-VII-2004 / Barney D. Streit, collector" (2 males, 1 female – CMNC, RACC)

"COSTA RICA: Guanacaste, / Parque Nacional Santa Rosa, / Estación Santa Rosa, / 17-VII-2004 / Barney D. Streit, collector" (2 females – CMNC, RACC)

"COSTA RICA: Guanacaste, / Parque Nacional Santa Rosa, / Estación Santa Rosa, 295 m. / N 10°50'21.4", W 35°37'05.8" / 4-VII-2005 / Barney D. Streit, collector" (2 males – CMNC, RACC)

Trigonopeltastes aurovelutinus Curoe, 2011

Remarks. The male of this species was previously unknown so a description of the key characters is given below. One male specimen was examined labeled "Mex: Guerrero / Acuhuezotla / IX-29-94 Chemsak" (EMEC).

Description of male (Figs 46–51). Color: similar to the description of the females (Curoe 2011; Figs 46–47); dorsal surface black except elytra uniformly orange with darker sutural line. Legs orange with black tarsi and metatibia (Fig. 47). *Head:* Surface glabrous except for light, setose patches at base of clypeus and apex of frons, without cretaceous markings (Fig. 50). *Pronotum:* Surface of disc dull-black with shiny micropunctures (Fig. 50); setae scattered across disc but not obscuring surface as seen in the Curoe (2011) figure of the female (perhaps due to abrasion). Marginal bead complete; with thick, scale-like setae. Pronotal disc with more-or-less complete inverted triangle indented into the surface with thick scale-like setae (Fig. 50). *Elytra:* Orange, glabrous, without cretaceous markings (Figs 46–47). *Pygidium:* Surface with white, scale-like setae covering disc (but not obscuring surface, perhaps due to abrasion), without cretaceous makings (Fig. 49). *Venter:* Surface covered with white, scale-like setae. Visible abdominal sternites with erect, white setae scattered medially. *Legs:* Protibia with 2 teeth near apex (Fig. 47) (female with third medial tooth; Curoe 2011). Metatibia with a distinct, medial protuberance along inner surface (Fig. 46), surface distad to this protuberance smooth with fine striations that may be for stridulation (a key diagnostic character not found in male *T. simplex* and not apparent in the description and figure of the female *T. aurovelutinus*). *Parameres:* Robust with lateral notches towards apex (Figs 48, 51).

Figures 46–51. *Trigonopeltastes aurovelutinus* Curoe, 2011, male from EMEC (label data: "MEX: Guerrero / Acahuezotla / IX-29-94 Chemsak") **46** Dorsal view of habitus **47** Oblique view of habitus **48** Lateral view of genitalia **49** Pygidium **50** Head and pronotum **51** Parameres.

Trigonopeltastes frontalis Bates, 1889

Distribution. This species was previously recorded from Mexico (Howden 1968) and El Salvador (Cave 1983). The specimens detailed below represent new country records for Belize, Guatemala, and Honduras.

"Augustine / Br. Honduras / July 3 1969 / F. D. Bennett" (1 male - CMNC). Augustine is in the Cayo District of Belize.

"GUAT., BAJA VERAPAZ / 5 KM S SAN JERONIMO / 4500', MAY 24-30, 1989 / E. GIESBERT, COLL." (1 male - CMNC)

"HONDURAS Olancho / Dept., P.N. La Muralla / ± 1200m, flowers / 1 July 1995 / DC Carlson/FT Hovore" (1 male - DCCC).

Trigonopeltastes geometricus Schaum, 1841

Trigonopeltastes nigrinus Bates, 1889: 379. **Syn. n.**
Trigonopeltastes carus Bates, 1889, 381. **Syn n.**

Remarks. Bates (1889) originally described *Trigonopeltastes nigrinus* as a variety of *Trigonopeltastes geometricus*, and Howden (1968) later gave *T. nigrinus* full species status. Howden (1968) and Howden and Joly (1998) gave diagnostic characters for each species involving the size, indentation of pronotal triangle, elytral coloration, and pygidial markings but commented that these were based on the examination of "very few specimens" and that further study was needed. In examining longer series of *Trigonopeltastes geometricus*, I have observed that the diagnostic characters used by Howden (1968) to separate *T. geometricus* and *T. nigrinus* break down with a number of individuals exhibiting a blend of the supposed diagnostic characters used. Bates (1889) originally described *Trigonopeltastes carus* as a distinct species, which Howden (1968) synonymized with *T. nigrinus*. Specimens that fall under any of the three preceding names all share the diagnostic characters used in the key and have significant lateral portions of the pygidium covered with yellow, cretaceous markings. Since I could find no justification for maintaining *T. nigrinus* as a separate species, I am here synonymizing this name and *T. carus* under *T. geometricus*. Further study involving molecular data is desirable to test the hypothesis that *T. geometricus* is a single, variable species that is distributed from Mexico to Bolivia.

Distribution. *Trigonopeltastes geometricus* and its synonyms were previously recorded from Mexico, Belize, Guatemala, El Salvador, Nicaragua, Costa Rica, Panama, Venezuela, Colombia, Ecuador, and Bolivia (Howden 1968, Cave 1983, Maes et al. 1997, Howden and Joly 1998). The specimens detailed below represent a new country record for Honduras.

"HOND. Olancho / LaMuralla Pq Nac / 24-27 May 1995 / JE Wappes" (1 female - CMNC).

"HONDURAS: Yoro / PN Pico Pijol, 1300 / N15°09.4' W87°37.6' / 11.V.02, beating / H.Douglas" (1 female - CMNC).

"HONDURAS Atlantida / Dept., ex. log / Lancetilla Bot. Garden / 4 July 1995 / DC Carlson/FT Hovore" (1 female - DCCC).

Trigonopeltastes glabellus Howden, 1988

Distribution. This species was previously recorded from Mexico (Howden 1988). The specimens detailed below represent a new country record for Guatemala.

"Guat. Huehue Finca / Zapote, Rio Lagartero / 5-VI-1991 / Edmund F. Giesbert" (12 males – CMNC, EMEC).

Trigonopeltastes sallaei sallaei Bates, 1889

Distribution. This species was previously recorded from Mexico, El Salvador, Nicaragua, and Costa Rica (Howden 1968). The specimens detailed below represent new country records for Guatemala and Honduras.

"GUAT., Zacapa Sn / Lorenzo Rd 1500- / 1800'1-10 June / 1991 JE Wappes" (1 male - CMNC).

"GUAT.: BAJA VERAPAZ / 54.4km S. Purulha, / 850m, 1.VII.1993, / F. Génier, hand coll." (2 males - CMNC).

"HONDURAS Olancho / Dept., P.N. La Muralla / ± 1200m, flowers / 1 July 1995 / DC Carlson/FT Hovore" (1 male - DCCC).

Trigonopeltastes simplex Bates, 1889

Remarks. The male of this species has never been formally described, and so I have included the description of key characters below with sexually dimorphic characters indicated.

Description of male (Figs 52–55). Color: elytra and legs highly variable (see Figs 52–54); dorsal surface black with elytra all black, all dark orange, or with varying degrees of dark orange basally and black apically; each elytron lacking cretaceous markings or with short, transverse, cretaceous band on basal half of elytra suture and/or short cretaceous band adjacent to lateral edge approximately halfway between base and apex. Legs dark orange, black, or a combination of these colors. *Head:* Surface glabrous, without cretaceous markings. *Pronotum:* Surface of disc dull-black with shiny micropunctures (surface shiny in females). Marginal bead obsolete laterally, without cretaceous markings inside marginal bead (marginal bead complete and with inside cretaceous markings in females). Pronotal disc with more-or-less complete inverted triangle indented into surface and without cretaceous markings (female has cretaceous markings). *Elytra:* See above and Figs 52–54 for color variation. *Pygidium:* Surface

Figures 52–55. *Trigonopeltastes simplex* Bates, 1889, males from CMNC (label data: **52** "Guatemala, Zacapa / rd. to San Lorenzo, 4200' / June 10-15, 1991 / E. Giesbert, coll." **53** "GUAT. Baja Verapaz / 14.5km N.Salamá on / Pantín Rd. 1620 m / 23.V.1991 / H & A Howden" **54** "MEXICO, CHIAPAS / SUMIDERO CYN. 4000' / JUNE 14 1987 / E. GIESBERT, COLL.") **52–54** Dorsal habitus showing three variations of elytral color form **55** Parameres.

without cretaceous makings (present laterally in females), disc with distinct ridges of concentric circles and some setae laterally and apically. *Venter:* Visible abdominal sternites 1–5 almost covered medially with cretaceous markings (except for small, central

Figure 56. Distribution map in Nicaragua and Costa Rica for *Trigonopeltastes arborfloricola* sp. n. (★), *T. formidulosus* sp. n. (●), *T. henryi* sp. n. (▲), and *T. mombachoensis* sp. n. (▼).

triangles); with short, white setae scattered throughout (or long setae just concentrated on sternite 5). *Legs:* Protibia with 2 teeth near apex (female with third medial tooth). *Parameres:* Robust with lateral notches towards apex (Fig. 55).

Distribution. This species was previously recorded from Guatemala (Howden 1968). The specimens detailed below represent a new country record for Mexico. The Mexican specimens have some variation in the metathoracic leg characters and setal characters that bear further investigation.

“MEXICO, CHIAPAS / SUMIDERO CYN, 4000’ / JUNE 14 1987 / E. GIESBERT, COLL.” (1 male - CMNC).

“MEXICO. Chiapas. / Pq. Nac. Sumidero. / 1000m. 25.V.1990 / H.&A. Howden” (1 male - CMNC).

Figure 57. Distribution map in Guatemala and Belize for *Trigonopeltastes warneri* sp. n. (★).

Trigonopeltastes variabilis Howden, 1968

Distribution. This species was previously recorded from Mexico (Chiapas, San Luis Potosi, and Veracruz), Guatemala, and El Salvador (Howden 1968). The specimens detailed below represent a significant range extension north to the Mexican state of Tamaulipas and a new country record for Honduras.

"MEX Tamaulipas / Bocotoma Area 7 km / SSE Gomez Farias / June 1-4 1982 / J. E. Wappes" (1 male - CMNC).

"5 miles sse. of / Gomes Farias, / Tamaulipas, Mexico / July 19-20, 1970 / Murray, Phelps, / Hart, Schaffner" (1 male - CMNC).

"HONDURAS Olancho / Dept., P.N. La Muralla / ± 1200m, flowers / 1 July 1995 / DC Carlson/FT Hovore" (1 male - DCCC).

Updated key to species of *Trigonopeltastes*

Modified from Howden (1968) and Howden and Joly (1998), and best used with the illustrations in those publications as a reference.

The male of *Trigonopeltastes femoratus* Howden is unknown and not included in the key.

The females of *Trigonopeltastes arborfloricola* sp. n., *Trigonopeltastes kerleyi* Ricchiardi, 2003, and *Trigonopeltastes thomasi* Howden & Ratcliffe, 1990 are unknown and not included in the key.

1 Abdominal sternites 1–5 concave or flat; pygidium length approximately equal or greater than width. Males **2**

– Abdominal sternites 1–5 convex; pygidium width greater than length. Females **27**

2 Head color mainly metallic green, pronotum green or blue matt (e.g., Fig. 22) **3**

– Head and pronotum not metallic, mainly black, tan, and/or brown (e.g., Figs 5, 11, 31, 41, 50) **4**

3 Pronotum, scutellum, and elytra blue matt (Fig. 19) .... ***Trigonopeltastes henryi* Smith**

– Pronotum and scutellum green matt, elytra tan .... ***Trigonopeltastes thomasi* Howden & Ratcliffe**

4 Pygidium without cretaceous markings, disc with scales and setae (e.g., Figs 6, 49) **5**

– Pygidium with cretaceous markings (at least along lateral edges), disc with scales and/or setae (e.g., Figs 12, 21, 32, 40) **9**

5 Pronotal disc evenly covered with dense scales. Mexico .... ***Trigonopeltastes discrepans* Howden**

– Pronotal disc with or without scales along margins and within impressed lines forming triangle, but scales not entirely covering pronotal surface **6**

6 Pronotum with distinct, impressed, longitudinal midline (sometimes most easily seen running through triangle impression). Mexico to Guatemala .... ***Trigonopeltastes glabellus* Howden**

– Pronotum without distinctly impressed longitudinal midline. Mexico to Nicaragua **7**

7 Impressed margins of triangle on pronotum glabrous (Figs 52–54) (except small patch of setae sometime present at lateral corners). Mexico to Guatemala .... ***Trigonopeltastes simplex* Bates**

– Impressed margins of triangle on pronotum with thick, scale-like setae (Figs 5, 50). Mexico and Nicaragua **8**

8 Legs bicolored with orange basally and black apically (Fig. 47). Elytra completely orange. Mexico .... ***Trigonopeltastes aurovelutinus* Curoe**

– Legs unicolored, black (Fig. 2). Elytra black with orange markings. Nicaragua .... ***Trigonopeltastes arborfloricola* Smith**

9 Clypeus with apical angles sharp, acute, distinctly reflexed. Durango, Mexico .... ***Trigonopeltastes truncatus* Howden**

– Clypeus with apical angles not sharply angulate or distinctly reflexed **10**

10 Elytral intervals 2–3 medially with transverse cretaceous markings forming (in conjunction with cretaceous marking of the elytral suture) an inverted "T" or "+" shape **11**

– Elytral intervals 2–3 without transverse cretaceous markings forming a medial "T" or "+" shape **12**

11 Mesofemoral and metafemoral surfaces with uniform covering of short, scale-like setae. Mexico to Costa Rica........ ***Trigonopeltastes archimedes* Schaum**

– Mesofemoral and metafemoral surfaces with thin, hair-like setae; without scales. Florida, United States of America........ ***Trigonopeltastes floridanus* (Casey)**

12 Clypeus largely reddish brown. Eastern United States of America........ ***Trigonopeltastes delta* (Forster)**

– Clypeus largely black. Mexico to South America........ **13**

13 Metatibia with inner surface distinctly modified with a basal swelling ending apically with a robust tooth. Mexico.... ***Trigonopeltastes deltoides* (Newman)**

– Metatibia without modifications or teeth along the inner surface........ **14**

14 Species occurring in South America........ **15**

– Species occurring in Mexico and Central America **18**

15 Pronotal triangle lacking scales; penultimate abdominal sternites with conspicuous medial tuft of long, erect setae; metatibia on apical half of inner surface with dense, elongate brush of yellow setae (height of setae approximately equal to width of metatibia). Venezuela........ ***Trigonopeltastes barbatus* Howden & Joly**

– Pronotal triangle with yellow scales (sometimes abraded); penultimate abdominal sternites without distinct medial tuft of setae; metatibia without dense elongate brush of setae on apical half (line of short setae sometime present but height of setae much less than width of metatibia) **16**

16 Pygidium with cretaceous markings filling basolateral corners of disc. Venezuela, Colombia, Ecuador, Bolivia (also in Mexico and Central America) .... ***Trigonopeltastes geometricus* Schaum** (in part)

– Pygidium with cretaceous markings not extending to basolateral corners of disc **17**

17 Elytra with second and fourth intervals weakly elevated, shiny. Bahia, Brazil........ ***Trigonopeltastes kerleyi* Ricchiardi**

– Elytra with second and fourth intervals flat, matt. Brazil, Paraguay, Argentina .... ***Trigonopeltastes triangulus* (Kirby)**

18 Pronotal triangle with apical, transverse line indicated only at midline, obsolete for approximately half of length; pygidial cretaceous markings interrupted basomedially. Mexico to El Salvador .... ***Trigonopeltastes variabilis* Howden**

– Pronotal triangle with apical, transverse line complete or nearly complete; pygidial cretaceous markings usually not interrupted basomedially........ **19**

19 Clypeus and vertex each with wide cretaceous markings on either side of midline; elytra without transverse cretaceous markings along lateral edge .... ***Trigonopeltastes wappesi*** **Howden**

– Clypeus without cretaceous markings, vertex rarely with cretaceous markings; elytra usually with transverse cretaceous markings along lateral edge.......... **20**

20 Abdominal sternites 2–5 without long, erect setae standing out from appressed scales or fine recumbent setae on surface....................................**21**

– Abdominal sternites 2–5 (sometimes only sternite 5) with long, erect setae standing out from appressed scales and recumbent setae on surface **22**

21 Pygidium cretaceous except for thin, longitudinal strip along midline, apex slightly convex but not deflexed. Mexico to Panama (also in South America)... .... ***Trigonopeltastes geometricus*** **Schaum** (in part)

– Pygidium with small, lateral cretaceous spots (Fig. 12); apex with small area of tip deflexed on a roughly 90° angle compared to rest of pygidial surface. Costa Rica .... ***Trigonopeltastes formidulosus*** **Smith**

22 Abdominal sternite 5 only with long, erect setae; all other sternites without long, erect setae. Costa Rica and Panama .... ***Trigonopeltastes pontilis*** **Howden**

– Abdominal sternites 2–5 all with long, erect setae....................................**23**

23 Clypeus basally with prominent patch of short, semierect, tan setae; vertex sometimes with cretaceous markings (Fig. 41); protibia with 2 prominent teeth along outer edge..**24**

– Clypeus without prominent patch of setae (Fig. 31); protibia with 3 prominent teeth along outer edge...**25**

24 Protibia and mesotibia completely tan; elytra more tan than black. Mexico to Honduras .... ***Trigonopeltastes frontalis*** **Bates**

– Protibia and mesotibia tan with prominent, black markings along external edge (Fig. 39); elytra more black than tan. Belize to Guatemala................... ....***Trigonopeltastes warneri*** **Smith**

25 Metafemur with short, appressed scales or setae on much of exposed ventral surface (some long setae sometimes at basal posterior edge); pygidium with basal portion of cretaceous markings usually thick and rarely broken medially; elytra with transverse black mark across interval 2 and 3 adjacent to cretaceous marking on midline or intervals 2 and 3 completely orange adjacent to cretaceous marking on midline; elytra along lateral edges with 2 transverse cretaceous lines (basal line sometimes absent especially in individuals with mainly orange coloration on elytra). Mexico to Costa Rica ....***Trigonopeltastes sallaei*** **Bates**

– Metafemur with long, semierect setae (or very elongate flattened scales) on most of exposed ventral surface; pygidium with basal portion of cretaceous markings reduced and sometimes broken medially; elytra with interval 2 orange and interval 3 with black spot or line adjacent to cretaceous marking on midline; elytra along lateral edges with 1 transverse cretaceous line (sometimes absent) (Fig. 28)....... **26**

26 Basal half of elytra with 1 transverse, solid orange line along base and medial orange spot near humerus (Fig. 28). Nicaragua.. .... ***Trigonopeltastes mombachoensis* Smith**

– Basal half of elytra with 2 transverse, solid orange lines, one along base and one sub-basally. Mexico to Guatemala..........***Trigonopeltastes intermedius* Bates**

27 Pygidium without cretaceous markings, disc with scales and setae **28**

– Pygidium with cretaceous markings (at least along lateral edges), disc with scales and/or setae (e.g., Figs 17, 27, 37, 44) (*Trigonopeltastes formidulosus* sometimes without cretaceous markings but pygidial disc appears glabrous) **29**

28 Head, pronotum, and scutellum densely covered with scales. Mexico ....***Trigonopeltastes discrepans* Howden**

– Head, pronotum, and scutellum not densely covered with scales (mainly glabrous sometimes with patches of scales). Mexico to Guatemala .... ***Trigonopeltastes glabellus* Howden**

29 Species occurring in South America..**30**

– Species occurring in the United States of America, Mexico, and Central America **32**

30 Pygidium not evenly convex, medially often somewhat flattened transversely, apical third swollen. Brazil, Paraguay, Argentina... ....***Trigonopeltastes triangulus* (Kirby)**

– Pygidium evenly convex..**31**

31 Pygidium with cretaceous markings across base continuous or narrowly divided; apex of pygidium lacking small, shiny, triangular tubercle. Venezuela, Colombia, Ecuador, Bolivia (also in Mexico and Central America).............. ....***Trigonopeltastes geometricus* Schaum** (in part)

– Pygidium with cretaceous markings distinctly separated basally; apex of pygidium with small, shiny, triangular tubercle. Venezuela .... ***Trigonopeltastes barbatus* Howden & Joly**

32 Pygidium bilobed either side of depressed midline, disc not evenly convex (Fig. 27). Costa Rica.................................. ***Trigonopeltastes henryi* Smith**

– Pygidium not bilobed and without depressed midline, evenly convex (e.g., Figs 17, 37, 44) **33**

33 Clypeus with apical angles sharp, acute, distinctly reflexed. Durango, Mexico ... .***Trigonopeltastes truncatus* Howden**

– Clypeus with apical angles not sharply angulate or distinctly reflexed **34**

34 Elytral intervals 2–3 medially with transverse, cretaceous markings forming (in conjunction with cretaceous marking of elytral suture) an inverted “T” or “+” shape **35**

– Elytral intervals 2–3 without transverse, cretaceous markings forming a medial “T” or “+” shape... **36**

35 Mesofemoral and metafemoral surfaces with some short, scale-like setae; elytron with prominent cretaceous markings along apex. Mexico to Costa Rica .... ***Trigonopeltastes archimedes*** **Schaum**

– Mesofemoral and metafemoral surfaces with thin, hair-like setae; without scales; elytron without prominent cretaceous markings along apex. Florida, United States of America .... ***Trigonopeltastes floridanus*** **(Casey)**

36 Clypeus largely reddish brown. Eastern United States of America .... ***Trigonopeltastes delta*** **(Forster)**

– Clypeus largely black. Mexico to Panama **37**

37 Metafemur slender, no wider than apex of metatibia; pygidium with ovoid, basal, cretaceous markings distinctly separated from lateral margins. Costa Rica .... ***Trigonopeltastes femoratus*** **Howden**

– Metafemur stocky, wider than apex of metatibia; pygidium with cretaceous markings extending to lateral margins **38**

38 Length 10.5 mm or more. Mexico .... ***Trigonopeltastes deltoides*** **(Newman)**

– Length 10.0 mm or less (with head in vertical position) **39**

39 Pronotal triangle with apical, transverse line indicated only at midline, obsolete for approximately half of length; disc within triangle with fine to moderately-sized punctures. Mexico to El Salvador .... ***Trigonopeltastes variabilis*** **Howden**

– Pronotal triangle with apical, transverse line complete or nearly complete; if incomplete then disc within triangle with large punctures **40**

40 Clypeus and vertex each with wide, cretaceous markings either side of midline; elytra without transverse, cretaceous markings along lateral edge .... ***Trigonopeltastes wappesi*** **Howden**

– Clypeus without cretaceous markings, vertex rarely with cretaceous markings; elytra usually with transverse cretaceous markings along lateral edge **41**

41 Specimens with all of the following: pygidium with cretaceous markings well separated mediobasally; elytra with humeral area orange or reddish brown; pronotal surface shiny, not matt; clypeus with medial punctures or rugae not running longitudinally. Mexico to Guatemala .... ***Trigonopeltastes simplex*** **Bates**

– Specimens without the above combination of characters **42**

42 Clypeal length approximately equal to width, medial portion of disc with punctures or rugae running longitudinally (e.g., Fig. 16) **43**

– Clypeus wider than long, medial portion of disc with punctures or rugae running transversely or randomly (e.g., Figs 36, 45) **45**

43 Pygidium with small, lateral cretaceous spots (Fig. 17) (sometimes absent) .... ***Trigonopeltastes formidulosus*** **Smith**

– Pygidium with more extensive cretaceous markings covering either lateral and dorsal portions of pygidium or most of pygidium except midline (e.g., Figs 37, 44) **44**

44 Pygidium with cretaceous markings rounded and even in thickness, cretaceous markings not reaching basolateral corners of pygidium. Costa Rica and Panama.. ***Trigonopeltastes pontilis*** **Howden**

– Pygidium with cretaceous markings much thinner in some parts compared to others, cretaceous markings filling basolateral corners of pygidium. Mexico to Panama (also in South America) ....***Trigonopeltastes geometricus*** **Schaum** (in part)

45 Femora tan to reddish brown..**46**

– Femora black to dark brown (if tan, from Nicaragua)...............................**47**

46 Clypeus with a group of short, semierect setae on either side near lateral margins; elytral interval 2 with orange coloration extending apically past lateral cretaceous marking. Mexico to Honduras... ***Trigonopeltastes frontalis*** **Bates**

– Clypeus with inconspicuous, fine setae (Fig. 45); elytral interval 2 with orange coloration not extending apically beyond level of lateral cretaceous marking (Fig. 43). Belize to Guatemala ....***Trigonopeltastes warneri*** **Smith**

47 Elytra with transverse, black mark across interval 2 and 3 adjacent to cretaceous marking on midline, or intervals 2 and 3 completely orange adjacent to cretaceous marking on midline; elytra along lateral edges with 2 transverse, cretaceous lines (basal line sometimes absent especially in individuals with mainly orange coloration on elytra); elytral humeral swelling usually either completely black or orange, without continuous orange line across base. Mexico to Costa Rica.................................... ***Trigonopeltastes sallaei*** **Bates**

– Elytra with interval 2 orange and interval 3 with black spot or line adjacent to cretaceous marking on midline; elytra along lateral edges with 1 transverse, cretaceous line (sometimes absent); elytral humeral swelling with continuous orange line across the base (e.g., Figs 34–35) **48**

48 Basal half of elytra with 1 lateral, solid orange line along base and medial orange spot sub-basally (Figs 34–35). Nicaragua..
.... ***Trigonopeltastes mombachoensis*** **Smith**

– Basal half of elytra with 2 lateral, solid orange lines, one along base and one sub-basally. Mexico to Guatemala........ ***Trigonopeltastes intermedius*** **Bates**

Checklist of the New World Trichiini

Apeltastes Howden, 1968

Apeltastes chiapasensis Howden, 1994 – Mexico
Apeltastes elongatus Howden, 1968 – Mexico

Dialithus Parry, 1849

Dialithus magnificus (Parry, 1849) – Mexico, Belize, Guatemala, Honduras, Nicaragua, Costa Rica
    Dialithus castaneipennis Kraatz, 1897 (synonym)
Dialithus scintillans Howden, 1972 – Panama

Giesbertiolus Howden, 1988

Giesbertiolus curoei Ramírez-Ponce, 2014 – Panama
Giesbertiolus festivus (Howden, 1972) – Mexico
Giesbertiolus linnaei Krikken, 2008 – Costa Rica
Giesbertiolus ornatus Howden, 1988 – Costa Rica, Panama

Gnorimella Casey, 1915

Gnorimella maculosa (Knoch, 1801) – Canada, United States of America
    Trichius bigsbii Kirby, 1827 (synonym)
    Gnorimus dissimilis Gory & Percheron, 1833 (synonym)

Iridisoma Delgado-Castillo & Morón, 1991

Iridisoma acahuizotlensis Delgado-Castillo & Morón, 1991 – Mexico

Paleotrichius Poinar, 2011

Paleotrichius dominicanus Poinar, 2011 – Dominican Republic (fossil)

Paragnorimus Becker, 1910
    Peltotrichius Howden, 1968 (synonym)

Paragnorimus aenescens (Bates, 1889) – Mexico
Paragnorimus atratus Smith, 2010 – Guatemala
Paragnorimus glaseri (Howden, 1971) – Guatemala
Paragnorimus guatemalensis Howden, 1970 – Guatemala
Paragnorimus hondurensis Smith, 2010 – Honduras, Nicaragua
Paragnorimus howdeni Smith, 2010 – Guatemala
Paragnorimus linea (Burmeister, 1841) – Mexico
    Trigonopeltastes quadrisignatus Schaum, 1841 (synonym)
Paragnorimus sambucus Howden, 1970 – Mexico, Guatemala
Paragnorimus velutinus Becker, 1910 – Mexico
    Paragnorimus flohri Becker, 1910 (synonym)

Trichiotinus Casey, 1915
  Trichinus Kirby, 1827 (synonym)

Trichiotinus affinis (Gory & Percheron, 1833) – Canada, United States of America
  Trichius variabilis Burmeister & Schaum, 1841 (synonym)
  Trichius mutabilis Schaum, 1844 (synonym)
  Trichiotinus venticosus Casey, 1915 (synonym)
  Trichiotinus parvulus Casey, 1915 (synonym)
Trichiotinus assimilis (Kirby, 1837) – Canada, United States of America
  Trichius bistriga Newman, 1838 (synonym)
  Trichius variabilis Burmeister & Schaum, 1841 (synonym)
Trichiotinus bibens (Fabricius, 1775) – Canada, United States of America
Trichiotinus lunulatus (Fabricius, 1775) – United States of America
  Trichius viridulus Fabricius, 1775 (synonym)
  Trichius virens Gmelin, 1790 (synonym)
  Trichius mutabilis Schaum, 1844 (synonym)
  Trichius semiviridis Casey, 1914 (synonym)
  Trichius carolinensis Casey, 1914 (synonym)
  Trichius rasilicaudus Casey, 1915 (synonym)
  Trichius rufiventris Casey, 1915 (synonym)
Trichiotinus piger (Fabricius, 1775) – Canada, United States of America
  Trichius drummond Gory & Percheron, 1833 (synonym)
  Trichius rotundicollis Kirby, 1837 (synonym)
  Trichiotinus reductus Casey, 1915 (synonym)
Trichiotinus rufobrunneus (Casey, 1914) – United States of America
  Trichius obesulus Casey, 1914 (synonym)
Trichiotinus texanus (Horn, 1876) – United States of America
  Trichiotinus monticola Casey, 1915 (synonym)
  Trichiotinus intermedius Casey, 1915 (synonym)
Trichiotinus viridans (Kirby, 1837) – Canada, United States of America
  Trichius variabilis Burmeister & Schaum, 1841 (synonym)

Trigonopeltastes Burmeister & Schaum, 1840
  Archimedius Kirby, 1827 (synonym)
  Euclidius Kirby, 1827 (synonym)
  Roplisa Casey, 1909 (synonym)

Trigonopeltastes arborfloricola Smith, 2016 – Nicaragua
Trigonopeltastes archimedes Schaum, 1841 – Mexico, Guatemala, El Salvador, Costa Rica
Trigonopeltastes aurovelutinus Curoe, 2011 – Mexico
Trigonopeltastes barbatus Howden & Joly, 1998 – Venezuela
Trigonopeltastes delta (Forster, 1771) – United States of America

Trigonopeltastes deltoides (Newman, 1838) – Mexico
Trigonopeltastes discrepans Howden, 1968 – Mexico
Trigonopeltastes femoratus Howden, 1968 – Costa Rica
Trigonopeltastes floridanus (Casey, 1909) – United States of America
Trigonopeltastes formidulosus Smith, 2016 – Costa Rica
Trigonopeltastes frontalis Bates, 1889 – Mexico, Belize, Guatemala, El Salvador, Honduras
Trigonopeltastes geometricus Schaum, 1841 – Mexico, Belize, Guatemala, El Salvador, Honduras, Nicaragua, Costa Rica, Panama, Venezuela, Colombia, Ecuador, Bolivia
  Trigonopeltastes nigrinus Bates, 1889 (synonym)
  Trigonopeltastes carus Bates, 1889 (synonym)
Trigonopeltastes glabellus Howden, 1988 – Mexico, Guatemala
Trigonopeltastes henryi Smith, 2016 – Costa Rica
Trigonopeltastes intermedius Bates, 1889 – Mexico, Guatemala
Trigonopeltastes kerleyi Ricchiardi, 2003 – Brazil
Trigonopeltastes mombachoensis Smith, 2016 – Nicaragua
Trigonopeltastes pontilis Howden, 1988 – Costa Rica, Panama
Trigonopeltastes sallaei sallaei Bates, 1889 – Mexico (eastern Mexico), Guatemala, El Salvador, Honduras, Nicaragua, Costa Rica
Trigonopeltastes sallaei sinaloensis Howden, 1968 – Mexico (northwestern Mexico from Sonora to Nayarit)
Trigonopeltastes simplex Bates, 1889 – Mexico, Guatemala
Trigonopeltastes thomasi Howden & Ratcliffe, 1990 – Mexico
Trigonopeltastes triangulus (Kirby, 1819) – Brazil, Paraguay, Argentina
  Trigonopeltastes nigra Burmeister, 1846 (synonym)
Trigonopeltastes truncatus Howden, 1968 – Mexico
Trigonopeltastes variabilis Howden, 1968 – Mexico, Guatemala, El Salvador, Honduras
Trigonopeltastes warneri Smith, 2016 – Belize, Guatemala
Trigonopeltastes wappesi Howden, 1988 – Panama

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RESEARCH ARTICLE

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Rediscovery and identity of *Pumilomyia protrahenda* De Stefani (Diptera, Cecidomyiidae) in Sicily with redescription and reassessment of its taxonomic position

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Abstract

A population of the gall midge *Pumilomyia protrahenda* De Stefani, 1919 causing galls on *Artemisia arborescens* (Asteraceae) was discovered near Palermo (Sicily) in 2008. This species had not been found since 1918. Detailed study of morphological characters of adults, larvae and pupae revealed that *P. protrahenda* belongs to the genus *Rhopalomyia* Rübsaamen, 1892, tribe Rhopalomyiini. The monotypic genus *Pumilomyia* De Stefani, 1919 is therefore synonymized under *Rhopalomyia* Rübsaamen, 1892. *Rhopalomyia protrahenda* **comb. n.** is redescribed, with important morphological characters illustrated. Adults have one-segmented palpi, antennae with 12–13 short flagellomeres and long legs with simple tarsal claws. A neotype is designated in the present paper because the type of this species is lost. The host plant has a circum-Mediterranean distribution but the gall midge is currently known only from Sicily, where it completes several generations between January and May.

Keywords

Rhopalomyia protrahenda, gall midge, *Artemisia arborescens*, Italy

Introduction

De Stefani (1919) found galls on *Artemisia arborescens* L., reared adults, established a new genus *Pumilomyia*, and described male, female, pupa, larva and egg as a new gall midge species, *Pumilomyia protrahenda*, together with a photograph of its gall. He included this new genus in the group Asphondylariae (now: Asphondyliini). In his publication he expressed his doubt that this genus really belonged to this group because it differed from other representatives in some morphological characters. The genus *Pumilomyia* (Cecidomyiidae) with its type species *P. protrahenda* is included in the family Cecidomyiidae (Skuhravá 1986, 2006) and in the list of gall midges of Italy (Skuhravá and Skuhravý 1994) and Sicily (Skuhravá et al. 2007).

Remarkably, this species was known only from De Stefani's original description in 1919 more than 90 years ago. In recent years we have searched for the galls of this species at its type locality in the Botanical Garden in Palermo, where De Stefani found galls and also in its surroundings but without success. In March 2008 we discovered galls of *P. protrahenda* on leaves of *Artemisia arborescens* at Mt. Raffo Rosso (Sicily), west of Palermo.

This recent find of larvae, pupae and adults is important because the De Stefani's collection, including many type specimens of species described by him, is considered to be lost (V. Caleca, pers. comm.). We take this opportunity to redescribe the species and designate a neotype.

Materials and methods

Material for this study was collected at three localities in the area of Palermo (Sicily) in 2008, 2012, 2015 and 2016. Branches of *Artemisia arborescens* with galls attributed to *P. protrahenda* were transferred to the laboratory at the Palermo University and placed in rearing cages where they were maintained at ambient temperature to obtain adults. In the field we bagged branches of *Artemisia arborescens* including galls with fine mesh tulle. Adults which emerged from galls and immature stages that were dissected from galls were preserved in 70% ethanol for subsequent morphological studies. Specimens were examined through a stereomicroscope Wild-Heerbrugg M8. A series of images was taken using a Leica DM2500 compound microscope and a Leica DFC450C mounted camera with Leica Application Suite software. Some galls were photographed with a Canon 7D digital camera provided with a Canon MP-E 65 mm macrolens and photos were integrated using the freeware CombineZP (Hadley 2011). Some adults, larvae and pupae were mounted on microscope slides in Canada balsam and some in Hoyer´s medium. Part of the material is preserved in the collections of B. Massa and G. Cerasa, University of Palermo, Department of Agriculture and Forest Sciences, Palermo, Sicily (BMUP), and microscope slides of gall midges as well as several adults preserved in 70% ethanol are in the collection of M. Skuhravá, National Museum, Praha, Czech Republic (NMPC).

Taxonomy

***Rhopalomyia protrahenda* (De Stefani, 1919), comb. n.**

Pumilomyia protrahenda De Stefani 1919: 72.
Pulmilomyia artemisiae De Stefani 1929: 262.
Pumilomyia artemisiae De Stefani 1929: 263, 264.

Redescription. Female (Figs 1–4, 7–9, 12, 14–17). Body size: 1.8–2.2 mm; wing length 1.6–1.8 mm; wing width 0.68–0.70 mm. Freshly emerged females have head and thorax brown and abdomen orange–reddish. Head with large holoptic eyes, eye bridge about 5 ommatidia long at vertex. Ommatidia relatively small and do not touch. Mouthparts extremely reduced. Frontoclypeus with 18–20 setae. Palpus consists of one short, elliptical segment covered with microtrichia. Antennae: scape obconical, pedicel globular, usually 12 flagellomeres, 1st and 2nd connate, ovoid, without necks, densely covered with microtrichia, each with several long setae; of seven females, five had 12 and two 13 flagellomeres, in accordance with information in De Stefani (1919), who reported that about one third of the females had 13 flagellomeres. Flagellomeres gradually decrease in size to end of antenna; circumfila poorly visible, comprising two transverse and one longitudinal band.

Thorax. Wing with R_5 joining costa at wing apex, well visible only in basal two thirds; Cu barely visible; anterior wing margin with shorter setae, posterior wing margin with long setae; wing surface covered with short setae. Legs long, covered with short hairs. All legs with simple claws, empodia as long as claws.

Abdomen: Second to sixth segments broad, seventh segment narrow; 8th tergite entire. Ovipositor protrusible, cerci fused, ovoid, setose and setulose; hypoproct small, rounded, setulose, visible only in dorsoventral view.

Male (Figs 5, 6, 11, 13, 18–19). Body size: 1.7–1.9 mm; wing length 1.7–1.9 mm; wing width 0.75–0.80 mm.

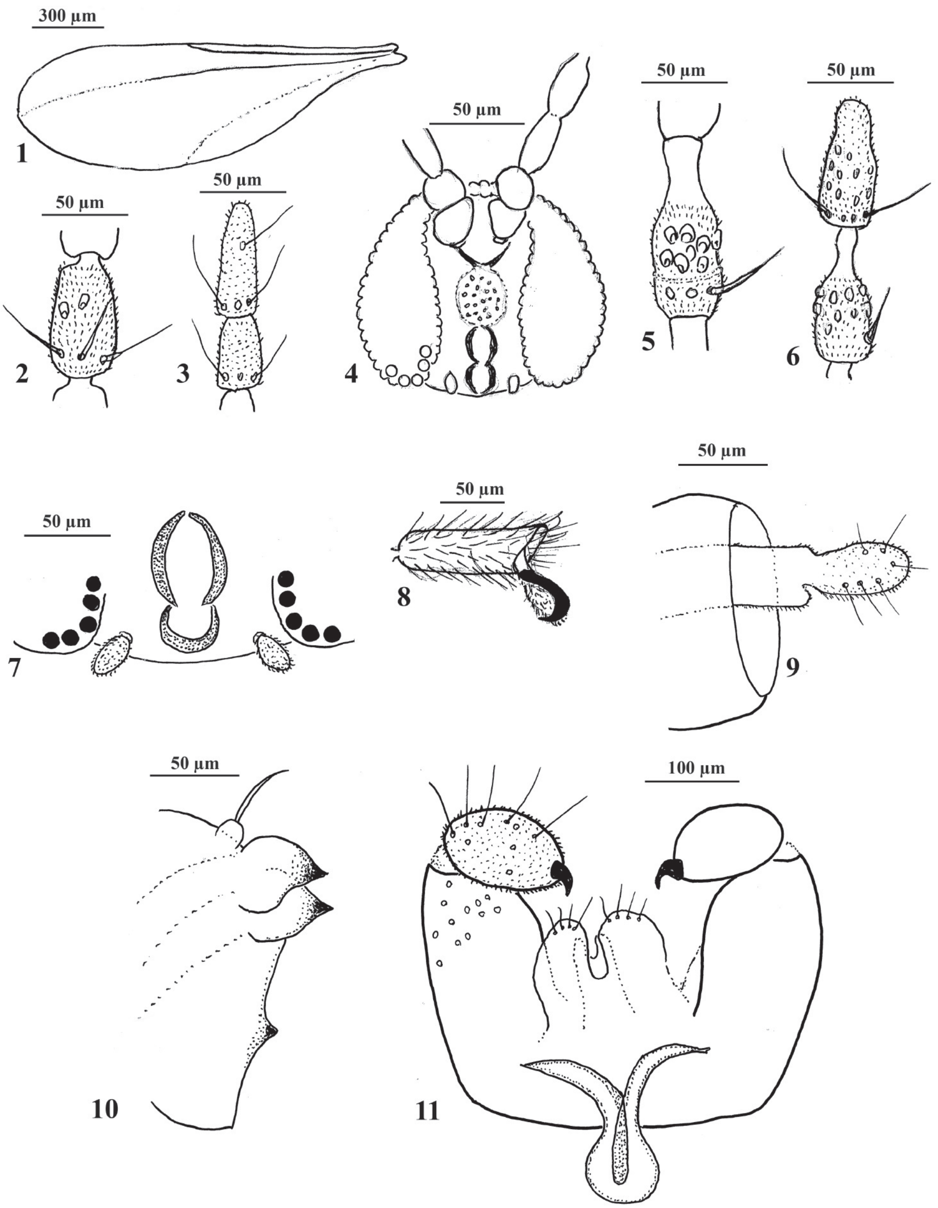
Antennae: scape obconical, pedicel globular, usually 13 flagellomeres, 1st and 2nd connate; each flagellomere composed of basal node and distal neck; neck about one half of node. Of three males, one male had 12 and two males had 13 flagellomeres.

Terminalia: gonocoxites thick, completely setulose, with several long setae; gonostyli ovoid, short and thick, completely setulose, covered with setae, at the tip with large, brown, beak–formed curved tooth; cerci broad, lobes rounded, deeply separated, setulose; hypoproct slender, incised; mediobasal lobes setulose, apically with several long setae; aedeagus rounded at tip, as long as cerci.

Other morphological characters as in female.

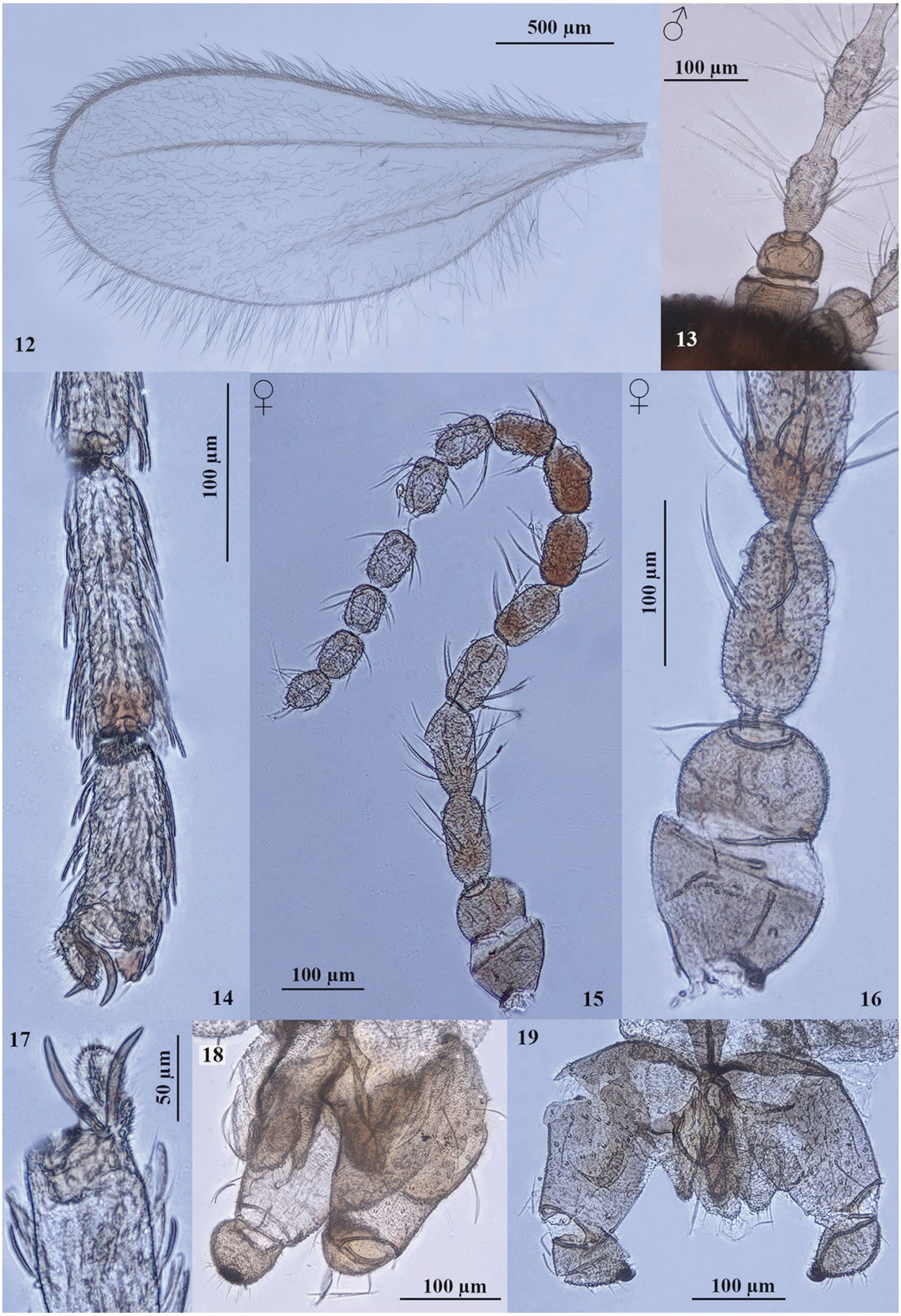
Larva. Body size: 1.2–1.8 mm long, 0.39–0.55 mm broad, cream colored; integument covered densely with very small spiculae; without spatula sternalis, without apparent papillae on ninth abdominal segment.

Pupa (Figs 10, 27, 28). Body size: 1.5–2.2 mm long, 0.6–0.9 mm broad; head and thorax brown, abdomen orange-red, integument with very small spiculae. An-



Figures 1–11. *Rhopalomyia protrahenda* comb. n. **1** Females wing **2** female, fifth flagellomere **3** female, two apical flagellomeres **4** female, head **5** male, fifth flagellomere **6** male, two apical flagellomeres,**7** female, mouthparts, **8** fifth tarsomere with simple claw and empodium **9** terminal part of ovipositor **10** pupal head, lateral view **11** male terminalia.

tennal horns small, dark brown; cephalic papillae bulbous with short setae, face with one small horn; prothoracic spiracle small, in the form of a small bulb. Pupal exuviae hyaline.



Figures 12–19. *Rhopalomyia protrahenda* comb. n. **12** Female, wing **13** male, first flagellomeres **14** female, fifth tarsomeres **15** female, antenna **16** female, first flagellomeres **17** female, claws and empodium **18** male terminalia, lateral view **19** frontal view.

Biology (Figs 20–26, 29). Larvae cause small galls on leaves, leaf stalks and stems of *Artemisia arborescens*. The galls are small hypertrophies of various forms. Only one larva develops in each gall. If galls are abundant, they may cause considerable damage (stunting) of the whole host plant. Larvae pupate in the galls. Several overlapping generations develop per year. New galls may be found on new buds from January onwards, with the emergence of adults until late April and early May. De Stefani (1919) stated that pupae of the last generation use their antennal horns to break out of the gall and drop to the soil, where they remain until the next winter, when the adults emerge. However, his observation is improbable and it is possible that this species diapauses in the larval stage, similar to other *Rhopalomyia* spp. De Stefani (1919) also stated that females of this species are characterized by high fertility and are able to lay a very high number of eggs on leaves of the host plant; he recorded a mean of 127 eggs per female (78–177) (see also Fig. 21).

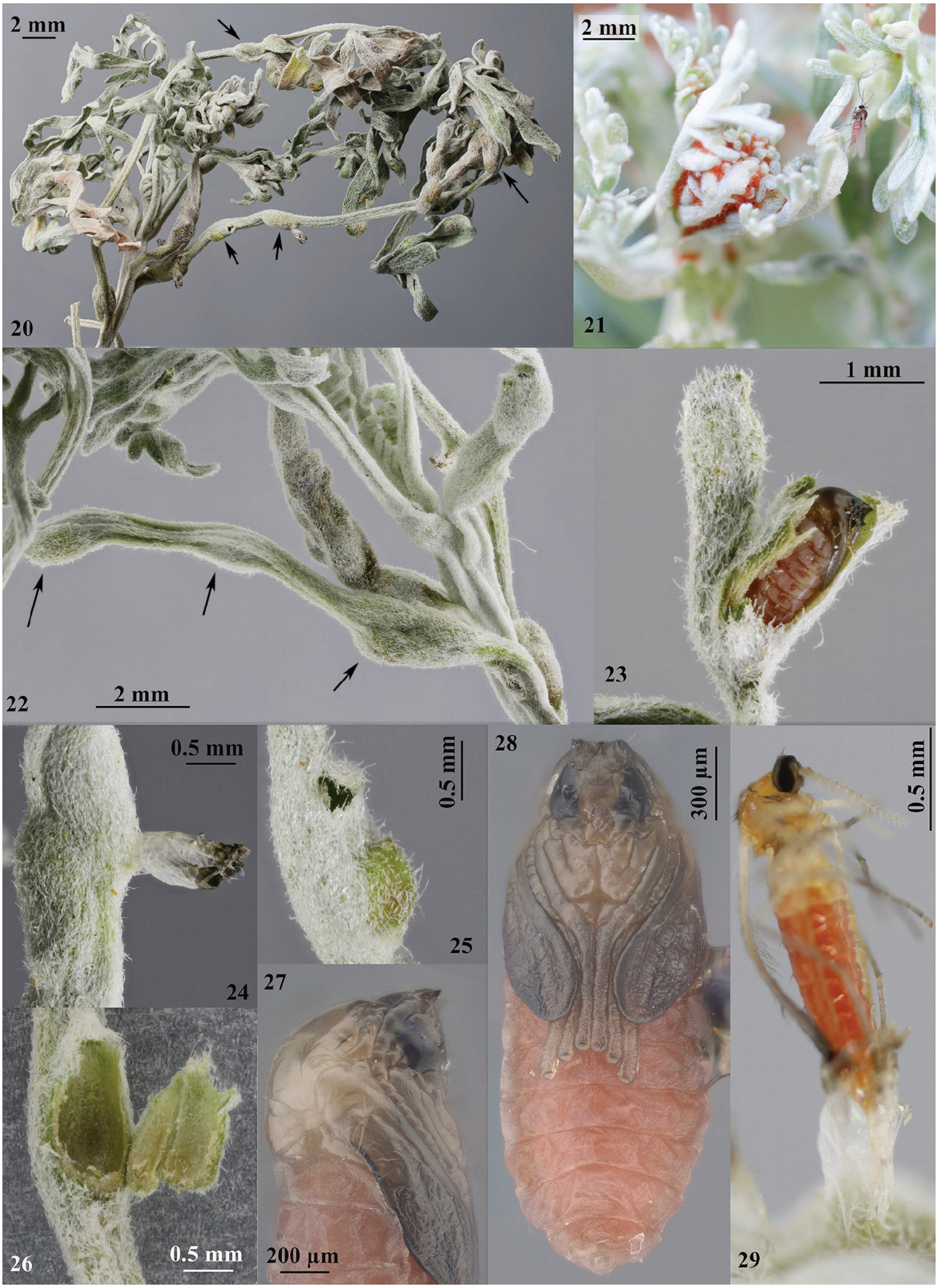
Material examined and neotype designation. The neotype of *Rhopalomyia protrahenda* is designated here: female, Italy, Sicily, Raffo Rosso, reared from small leaf gall on *Artemisia arborescens*, 18.III.2008, leg. G. Cerasa, microscope slide number 9571, in the collection of National Museum, Prague, Czech Republic (coll. Marcela Skuhravá).

Other material. 5♀, 3♂, Italy, Sicily, Palermo, locality Raffo Rosso, galls on *Artemisia arborescens* 3.III.2008, emerged 10–25.III.2008; 3♀, 2♂ same data, emerged 13.II.2012, leg. G. Cerasa; 4♀, 3♂, Italy, Sicily, Cinisi, locality Costa del Furi (Palermo), galls on *Artemisia arborescens* 11.III.2015, emerged 14–16.III.2015, leg. G. Cerasa; 5♀, 3♂, Italy, Sicily, Palermo, locality Raffo Rosso, galls on *Artemisia arborescens* 15.II.2016, emerged 16–25.II.2016, leg. G. Cerasa (in ethanol, BMUP); 2♂, 2♀ (neotype included) mounted (on microscope slides in Canada Balsam, coll. Skuhravà, NMPC); 4♂, 13♀, 6 larvae, 10 pupal exuviae (in Hoyer´s medium, NMPC).

Distribution. The host plant *Artemisia arborescens* is native to the Mediterranean. It is an erect evergreen perennial, with masses of finely–divided aromatic, silvery–white leaves and single–sided sprays of yellow flowers. The plant occurs from the Iberian Peninsula to Israel, the Italian Peninsula, Sicily, the Balkan Peninsula, Turkey and North Africa, and is naturalized in France. *Rhopalomyia protrahenda* has been found only in Sicily.

Taxonomic position. Detailed study of morphological characters of larvae, pupae and adults obtained from galls on *Artemisia arborescens*, found not far from the type locality in Palermo where De Stefani collected galls in the past, revealed that the causer of these galls, described as *Pumilomyia protrahenda* by De Stefani (1919), belongs in the genus *Rhopalomyia* Rübsaamen 1892, and according to the key to the genera of Cecidomyiidae (Skuhravá 1997) it belongs to the tribe Rhopalomyiini and not the Asphondyliini. The genus *Pumilomyia* is therefore synonymized here under *Rhopalomyia* because none of its morphological characters differentiates it from *Rhopalomyia*. De Stefani (1919) incorrectly assessed morphological characters of adult gall midges, mainly the number of antennal flagellomeres (12), shape of female flagellomeres and of ovipositor – and placed his new genus *Pumilomyia* in the Asphondylariae.

The female of *R. protrahenda* has mostly 12 antennal flagellomeres as is typical for all species of *Asphondylia* but the female flagellomeres of *R. protrahenda* are oval and



Figures 20–29. 20 *Artemisia arborescens* covered with galls **21** female of *Rhopalomyia protrahenda* next to apical leaves of the host plant covered with reddish mass of eggs **22** galls, detail **23** dissected gall showing a pupa **24** exuviae protruding from a gall **25** adult emergence hole **26** detail of a leaf gall showing the chamber and thin wall **27** pupa, lateral view **28** pupa frontal view **29** female emerging from gall.

not cylindrical as in females of *Asphondylia*. The female of *R. protrahenda* has the terminal part of ovipositor slender ending with soft, fused cerci, not aciculate and strongly sclerotized as in females of *Asphondylia*.

The genus *Rhopalomyia* Rübsaamen, 1892 is characterized by the combination of the following characters: reduced mouthparts with 1 or 2 segmented palpi, rarely 3; 12-25 flagellomeres; male flagellomeres with long necks, female flagellomeres with short necks; wing with R_5 reaching Costa nearly at wing apex; simple or toothed tarsal claws; male genitalia with stout gonocoxites and short, completely setulose gonostyli, with large claw; mediobasal lobes sheath aedeagus, cerci broad, hypoproct slender; female 8th tergite entire; ovipositor long, soft, not distinctly sclerotized; cerci fused. Larvae of most species do not have a spatula sternalis and their papillae are barely visible; pupae usually have strongly developed antennal horns.

The genus *Rhopalomyia* includes 267 species worldwide (Gagné and Jaschhof 2014). Larvae induce galls on stems, buds and leaves of host plants almost exclusively of the family Asteraceae. Galls of the same species often occur on several organs of the same host plant species. Alternation of plant organs is connected with the development of several generations per year (e. g., *Rhopalomyia tanaceticola* (Karsch 1879) on *Tanacetum vulgare* and *R. millefolii* (Loew 1850) on *Achillea millefolium*). Pupation takes place inside the galls. Species included in the genus are relatively uniform morphologically and show great reduction in taxonomically useful characters. Dorchin et al. (2009) considered that the best means to distinguish among species remains the morphology and structure of their galls together with the identity of the host plant.

Rhopalomyia is relatively species–rich in central, southeast and western Europe, where it contains 38 species. The number of species decreases markedly towards the south. No species were found in the islands of the Mediterranean Sea (other than Sicily) in the past. It is interesting that *Rhopalomyia protrahenda* is the only species of the genus *Rhopalomyia* occurring on a Mediterranean island (Sicily). Many *Rhopalomyia* species have been found to occur in Central Asia, mainly in Kazakhstan (Fedotova 2000, Skuhravá and Skuhravý 2010). The tribe Rhopalomyiini, as defined recently by Gagné in Gagné and Jaschhof (2014), includes two genera: the large genus *Rhopalomyia* with 267 species distributed in all zoogeographic regions and associated mainly with various host plants of the family Asteraceae, and the smaller genus *Psectrosema* with 26 species associated with host plants of the genus *Tamarix* (Tamaricaceae), occurring only in the Palaearctic Region. Only two species belonging to this tribe occur in Sicily: *Rhopalomyia protrahenda* (De Stefani 1919) and *Psectrosema tamaricis* (De Stefani 1902), both found in the Botanical Garden of Palermo by the Sicilian entomologist Teodosio De Stefani.

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RESEARCH ARTICLE

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How reliably can northeast Atlantic sand lances of the genera *Ammodytes* and *Hyperoplus* be distinguished? A comparative application of morphological and molecular methods

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Abstract

Accurate stock assessments for each of the dominant species of sand lances in the northeast Atlantic Ocean and adjacent areas are not available due to the lack of a reliable identification procedure; therefore, appropriate measures of fisheries management or conservation of sand lances cannot be implemented. In this study, detailed morphological and molecular features are assessed to discriminate between four species of sand lances belonging to the genera *Ammodytes* and *Hyperoplus*.

Morphological characters described by earlier authors as useful for identification of the genera are confirmed, and two additional distinguishing characters are added. A combination of the following morphological characters is recommended to distinguish between the genera *Hyperoplus* and *Ammodytes*: the protrusibility of the premaxillae, the presence of hooked ends of the prevomer, the number of dermal plicae, and the pectoral-fin length as a percentage of the standard length. The discriminant function analysis revealed that morphometric data are not very useful to distinguish the species of each of the two genera. The following meristic characters improve the separation of *H. lanceolatus* from *H. immaculatus*: the number of lower arch gill rakers, total number of gill rakers, numbers of caudal vertebrae and total vertebrae, and numbers of dorsal-fin and anal-fin rays. It is confirmed that *A. tobianus* differs from *A. marinus* by its belly scales that are organised in tight chevrons, scales which are present over the musculature at the base of the caudal fin, as well as by the lower numbers of dermal plicae, dorsal-fin rays, and total vertebrae.

In contrast to the morphological data, mitochondrial *COI* sequences (DNA barcodes) failed to separate unambiguously the four investigated species. *Ammodytes tobianus* and *H. lanceolatus* showed an overlap between intraspecific and interspecific K2P genetic distances and cannot be reliably distinguished using the common DNA barcoding approach. *Ammodytes marinus* and *H. immaculatus* exhibited gaps between intraspecific and interspecific K2P distances of 2.73 and 3.34% respectively, indicating that their DNA barcodes can be used for species identification. As an alternative, short nuclear Rhodopsin sequences were analysed and one diagnostic character was found for each of the species *A. marinus*, *H. lanceolatus,* and *H. immaculatus*. *Ammodytes tobianus* can be characterised by the lack of species-specific mutations when compared to the other three species. In contrast to *COI*, the short nuclear sequences represent a useful alternative for rapid species identification whenever an examination of morphological characters is not available.

Keywords

Ammodytes, *COI*, DNA barcoding, *Hyperoplus*, meristic characters, mitochondrial DNA, morphology, morphometrics, northeast Atlantic, nuclear gene, Rhodopsin, Sand lances, species identification

Introduction

Sand lances of the family Ammodytidae are small fishes that live primarily in marine and adjacent brackish waters with sandy substrates of the northern hemisphere, where they are able to quickly dive into the substrate to escape predation (Randall and Ida 2014, Orr et al. 2015). These fishes are characterised by elongated and subcylindrical bodies and possess relatively low elongated dorsal and anal fins without spines, which are separated from the forked caudal fin (e.g. Reay 1986). The number of principal caudal rays is reduced and there is no pelvic fin in most species (e.g. Ida et al. 1994). Sand lances have an increased number of vertebrae in which the number of pre-caudal vertebrae is higher than the number of caudal vertebrae. The lower jaws project beyond the upper jaws. Small and unobtrusive scales are present (e.g. Reay 1986) and the body is often covered in oblique skinfolds (so-called plicae).

The family Ammodytidae comprises 31 species in seven genera (e.g. Randall and Ida 2014, Orr et al. 2015) of which the two genera *Ammodytes* and *Hyperoplus* are distributed circumboreally (Ida et al. 1994). Five species of sand lances belonging to three genera occur in northeast Atlantic waters (Sparholt 2015). This includes the Common sand eel *Ammodytes tobianus* Linnaeus, 1758 and the Lesser sand eel *A. marinus* Raitt, 1934, currently recognised together with four further species in the genus *Ammodytes* (Orr et al. 2015). Additionally, both species of the genus *Hyperoplus*, Corbin´s sand eel *Hyperoplus immaculatus* (Corbin, 1950) and the Greater sand eel *H. lanceolatus* (Le Sauvage, 1824), can be found in the eastern north Atlantic area (Reay 1986), as well as *Gymnammodytes semisquamatus* (Jourdain, 1879). The latter can morphologically be distinguished from the species mentioned above by having a branched lateral line, a body not covered in oblique plicae (Cameron 1959), and scales that are loosely scattered and restricted to the posterior third of the body (Reay 1986), whereas the genera *Hyperoplus* and *Ammodytes* exhibit plicae along the body and an unbranched lateral line.

In identification keys these two genera are often distinguished by showing clear protrusible premaxillae and no vomerine teeth (*Ammodytes*) or no clear protrusible premaxillae and a pair of vomerine teeth (*Hyperoplus*, e.g. Reay 1986). *Hyperoplus lanceolatus* can be separated from *H. immaculatus* by the occurrence of a conspicuous dark spot on either side of the snout below the anterior nostril. This spot is lacking in *H. immaculatus*. *Ammodytes tobianus* is generally distinguished from *A. marinus* by its characteristic belly scales that are organised in tight chevrons and having scales present over the musculature at the base of the caudal fin, whereas these features are not present in *A. marinus* (Reay 1986).

However, the distinguishing features mentioned above are not easy to observe for the untrained eye when comparative material of different species is not available. Furthermore, an accurate species identification, especially of juvenile individuals, is difficult and even sub-adult and adult sand lances are difficult to identify (Sparholt 2015), if identification procedure is restricted to the few morphological characters mentioned above. In this context, Naevdal and Thorkildsen (2002) mentioned the difficulties regarding morphological separation of some of the five species of sand lances found in the northeast Atlantic and suggested a method for successful species identification on the basis of allozyme variation. DNA restriction fragment patterns have also been proposed to distinguish between some of the Atlantic sand eel species (Mitchell et al. 1998) as an alternative to morphological characters.

The difficult identification of sand lance species contributed to the current situation that accurate stock assessments are not available separately for each of the species in the North Sea and adjacent areas (see Sparholt 2015). However, sand lances here are subject to large-scale, industrial exploitation for fish meal and oil production and are also a major prey for many predators such as piscivorous fish, birds, and mammals (e.g. Reay 1986). It is known that exploitation of sand lances affects the food availability for these predators and that the abundance of sand lances is sensitive to recruitment variation (Sparholt 2015). Sand lances are divided into seven stock components for stock assessments in the North Sea based on the most abundant species *A. marinus*. With this approach, the stock situation of the single species cannot be evaluated, as it does not consider that sand lances represent a mix of different species. Clearly, another drawback is that an evaluation of the conservation status of the single species of sand lances is not possible (Thiel et al. 2013).

Molecular-based identification methods of fish species have been developed over the last decades (for an overview see Teletchea 2009). In this context, DNA barcoding constitutes the most popular and effective technique by using partial cytochrome *c* oxidase subunit I (*COI*) sequences for a standardised and routine identification of specimens to species level (Hebert et al. 2003). For a successful application of DNA barcoding as a tool for specimen identification, reliable sequence reference libraries such as the Barcode of Life Database (BOLD, Ratnasingham and Hebert 2007) were developed. Newly generated DNA barcodes can be uploaded and analysed together with data already available on BOLD in order to provide taxonomic identification. Additionally, barcode sequences were automatically analysed on BOLD and a Barcode Index Number (BIN) is assigned according to the calculated sequence clusters (Ratnasingham and Hebert 2013). Taxonomic conflicts apparently occur if sequences assigned to the same species name can be found within different BIN clusters.

For fish, the species discrimination success of DNA barcoding was demonstrated in many studies including freshwater as well as marine faunas from many regions all over the world (e.g. Ward et al. 2005, Hubert et al. 2008, Ward et al. 2008a, Steinke et al. 2009, April et al. 2011, Mabragaña et al. 2011, Costa et al. 2012, Zhang and Hanner 2012, Keskin and Atar 2013, McCusker et al. 2013, Geiger et al. 2014, Knebelsberger et al. 2014, Knebelsberger and Thiel 2014, Knebelsberger et al. 2015). DNA barcodes have also been successfully used to identify fish larvae (Pegg et al. 2006, Victor et al. 2009, Hubert et al. 2010, Kim et al. 2010), and fins (Holmes et al. 2009), and can provide evidence for cryptic diversity (Hubert et al. 2010, Ward et al. 2008b, Zemlak et al. 2009, Puckridge et al. 2013, Geiger et al. 2014, Knebelsberger et al. 2015).

For the North Sea and adjacent areas, two DNA barcoding studies revealed successful differentiation of all investigated species (Knebelsberger et al. 2014, Knebelsberger and Thiel 2014). Altogether, 105 species belonging to 88 genera were analysed. Most of the genera were represented by only one species. As an exception, the genus *Pomatoschistus* was represented by five closely related species.

One of these studies already provided DNA barcodes for the two sand lance species *A. marinus* and *H. immaculatus* and demonstrated a clear separation of these two species (Knebelsberger et al. 2014). A former study from continental Portugal Atlantic waters included DNA barcodes for *H. lanceolatus* but other species of sand lances were missing (Costa et al. 2012). Studies including congeneric species of the genus *Ammodytes* revealed inconsistencies between morphological and DNA barcode-based identification: for two species from the northwest Atlantic Ocean, namely *A. americanus* and *A. dubius*, barcoding fails to separate these species, which may be caused by inadequate taxonomy (McCusker et al. 2013). Inadequate taxonomy may also concern the two species *A. personatus* and *A. hexapterus* from the north Pacific (Turanov and Kartavtsev 2014). In both cases the taxonomic status of the species is questionable and may require comprehensive taxonomic revision. In order to examine the application of DNA barcoding for the identification of sand lances from the North Sea area, all closely related species from this region must be included. This concerns *A. marinus* and *H. immaculatus* as well as *A. tobianus* and *H. lanceolatus*. For the latter two species reliable *COI* data from the North Sea are still missing.

This paper presents the first comprehensive study combining morphological and molecular methods for the discrimination of four species of sand lances belonging to the genera *Ammodytes* and *Hyperoplus* occurring in the northeast Atlantic Ocean and adjacent waters. The suitability of two morphological types of parameters (meristic characters and morphometric measurements) and two genetic approaches (mitochondrial *COI* (DNA barcoding region) and partial nuclear Rhodopsin DNA sequences) for accurate species identification is examined. A detailed and accurate species identification matrix is presented, based on the integration of morphological and molecular traits.

Materials and methods

Material

In this study 85 specimens representing two species of genus *Ammodytes* and two species of genus *Hyperoplus* were sampled from the North and the Baltic Seas (Suppl. material 1 and 2, Figure 1). For the molecular analysis 70 samples were collected from the North Sea during several cruises conducted by the Thünen Institute of Sea Fisheries (Hamburg, Germany) and the research vessel of the Senckenberg Institute (Wilhelmshaven, Germany). Tissue samples were taken from each of the 70 specimens and preserved in 96% ethanol for molecular analysis at Senckenberg's German Center for Marine Biodiversity Research (DZMB, Wilhelmshaven, Germany). Specimens were preserved in 70% ethanol. The remaining 15 individuals belonging to the species *A. tobianus* were used for morphological analyses only and collected from the Baltic Sea during three different cruises conducted by the German Oceangraphic Museum (Stralsund, Germany). Immediately after catch, specimens were preserved in 4% formaldehyde solution. All

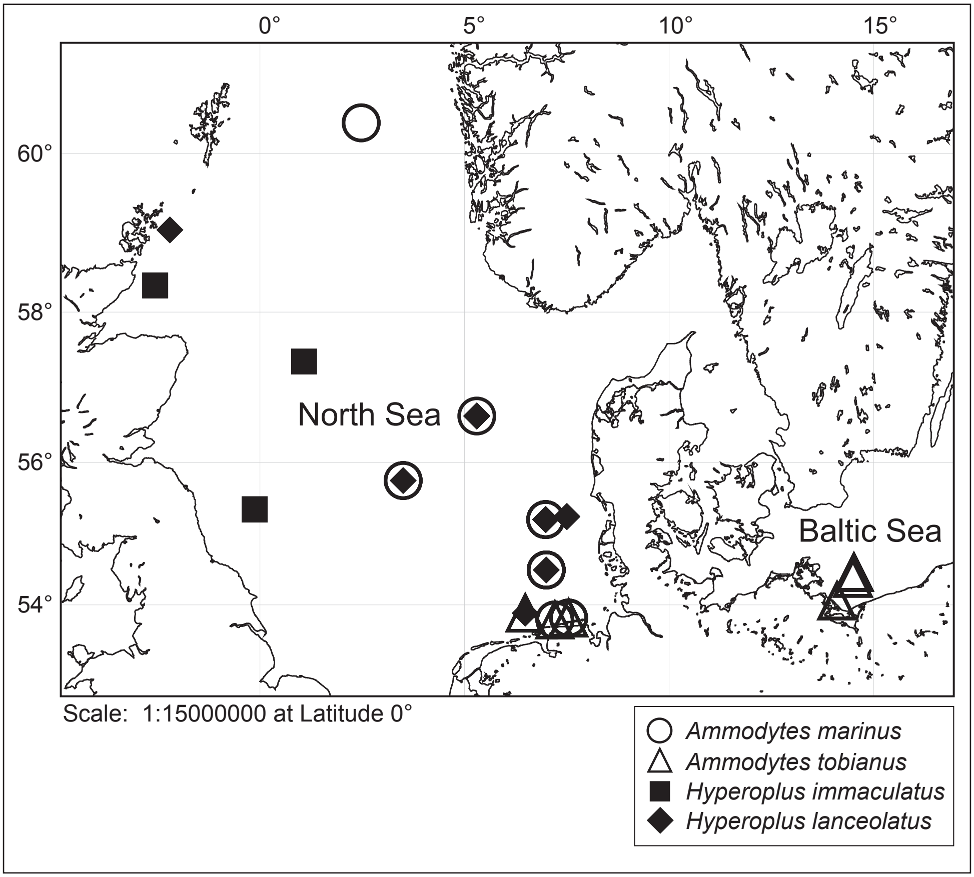

Figure 1. Sampling sites of sand lance species of the genera *Ammodytes* and *Hyperoplus*.

85 voucher specimens were databased and morphologically investigated at the Zoological Museum of the Center of Natural History of the University of Hamburg (ZMH, Hamburg, Germany). Finally, the material was stored for future reference in the ZMH fish collection. All *COI* sequences and related metadata belonging to the 70 voucher specimens from the North Sea are available on the Barcode of Life Data System (www.barcodinglife.org; Ratnasingham and Hebert 2007). DNA barcodes of eight specimens of *H. immaculatus* and 22 specimens of *A. marinus* were obtained from the BOLD project "Barcoding North Sea Fish I" (BNSFI) (Knebelsberger et al. 2014). Newly generated barcodes belonging to five specimens of *A. marinus*, six of *A. tobianus*, and 29 of *H. lanceolatus* were uploaded to the BOLD project "Barcoding North Sea Sand eels" (BNSSE). In addition to *COI*, nuclear Rhodopsin DNA sequences were generated from all 70 North Sea specimens (Suppl. material 1). For comparison, published Rhodopsin data was downloaded from GenBank (*A. tobianus*: AY141306; *H. lanceolatus*: EU492010 and EU492011).

Morphological analyses

Meristic parameters (Table 1) were analysed at the left-hand side of the specimens and supplemented with right-side counts when the left side was damaged. Counts of dorsal, ventral and principal caudal-fin rays as well as of vertebrae were taken from radiographs (Figure 2) made by an X-ray imaging system (Faxitron LX-60). The first caudal vertebra was defined as the first centrum with a long haemal spine, and the centrum fused to the hypural plate was counted as the last vertebra. Counts of dorsal-fin rays were made using the method of Nizinski et al. (1990). Counting dorsal-fin rays began with the first visible ray and excluded the one or two anterior rayless pterygiophores. However, these counts included the last two rays that were each supported by a pterygiophore. Counts of anal-fin rays included all rays visible from the outside. Gill rakers were counted on the lower and upper arch separately. Gill rakers of the lower arch included the raker at the junction between upper and lower parts of the arch. Dermal plicae included those anterior and posterior to the lateral-line pores.

Morphometric measurements (Table 2) were taken by vernier calipers to one tenth of a millimetre. Measurements were done following Hubbs and Lagler´s (1958) method, with the following changes: standard length (SL) was measured from the front of the upper lip in the median plane to the midbase of the caudal fin (end of hypural plate). The front of the upper lip was used as the anterior point of all other horizontal measurements. Head length (HL) was measured from the front of the upper lip to the posterior end of the opercular membrane. Body depth was measured twice, as the depth at the beginning of the base of the dorsal fin (BDD) and as the depth at the beginning of the base of the anal fin (BDA). Body width was measured as the maximum width at the beginning of the base of the dorsal fin (BWD). Orbit diameter (OD) is the maximum fleshy diameter. Interorbital width (IW) is the least fleshy width. Caudal-peduncle depth (CPD) is the smallest depth, and caudal peduncle length (CPL) the horizontal

Table 1. Data of estimated morphological characters of four species of sand lances of the genera *Ammodytes* and *Hyperoplus*. If possible, each meristic character and morphometric measurement is presented with its range (before the semicolon), mean value with standard deviation and the number of specimens analysed (in brackets). Morphometric measurements are given as proportion of SL.

Species	***Ammodytes marinus***	***Ammodytes tobianus***	***Hyperoplus immaculatus***	***Hyperoplus lanceolatus***
Meristic characters				
Dermal plicae (DP)	140–150; 143.1 ± 2.6 (24)	123–135; 128.5 ± 3.0 (21)	179–196; 187.8 ± 5.4 (8)	169–194; 182.6 ± 6.4 (29)
Dorsal-fin rays (DR)	56–62; 59.1 ± 1.4 (27)	51–55; 52.8 ± 1.1 (21)	59–61; 60.1 ± 0.6 (8)	54–57; 55.9 ± 0.8 (29)
Anal-fin rays (AR)	28–31; 29.5 ± 0.8 (27)	25–30; 27.8 ± 1.3 (21)	31–32; 31.8 ± 0.5 (8)	28–30; 29.1 ± 0.8 (29)
Pectoral-fin rays (PR)	12–15; 13.6 ± 0.6 (26)	12–13; 13.0 ± 0.2 (21)	13–15; 14.1 ± 0.6 (8)	13–14; 13.5 ± 0.5 (29)
Principal caudal-fin rays (CR)	15 ± 0 (27)	15 ± 0 (21)	15 ± 0 (8)	15 ± 0 (29)
Upper arch gill rakers (UR)	5–6; 5.0 ± 0.2 (24)	5 ± 0 (21)	5 ± 0 (8)	5 ± 0 (29)
Lower arch gill rakers (LR)	18–20; 18.6 ± 0.8 (24)	20 ± 0 (21)	23–25; 24.3 ± 0.7 (8)	20–22; 20.7 ± 0.8 (29)
Total gill rakers (GR)	23–26; 23.6 ± 0.9 (24)	25 ± 0 (21)	28–30; 29.1 ± 0.6 (8)	25–27; 25.7 ± 0.8 (29)
Precaudal vertebrae (PV)	42–44; 42.9 ± 0.7 (27)	36–41; 38.1 ± 1.4 (21)	42–43; 42.9 ± 0.4 (8)	38–42; 40.3 ± 0.8 (29)
Caudal vertebrae (CV)	26–28; 26.7 ± 0.5 (27)	24–26; 25.1 ± 0.6 (21)	29–30; 29.5 ± 0.5 (8)	25–28; 26.5 ± 0.7 (29)
Total vertebrae (TV)	68–71; 69.6 ± 0.9 (27)	60–66; 63.2 ± 1.4 (21)	72–73; 72.4 ± 0.5 (8)	65–68; 66.8 ± 0.8 (29)
Margins of dorsal and anal fins straight with rays of equal length (MDAS)	yes (27)	yes (21)	yes (8)	yes (29)
Body covered in oblique plicae bearing the scales (BCOP)	yes (27)	yes (21)	yes (8)	yes (29)
Premaxillae clearly protrusible (PCP)	yes (27)	yes (21)	no (8)	no (29)
Two vomerine teeth present (VTP)	no (27)	no (21)	yes (8)	yes (29)
Conspicuous dark spot on either side of snout (DSSS)	no (27)	no (21)	no (8)	yes (29)
Belly scales in tight chevrons (BSTC)	no (27)	yes (21)	yes (8)	not clearly detectable (29)
Scales over musculature at base of caudal fin (SBCF)	no (27)	yes (21)	yes (8)	yes (29)
Scales present in the midline anterior to dorsal fin (SADF)	no (27)	yes (21)	yes (8)	yes (29)

Species	*Ammodytes marinus*	*Ammodytes tobianus*	*Hyperoplus immaculatus*	*Hyperoplus lanceolatus*
Morphometric measurements				
Standard length (SL,mm)	61.2–193; 136.7 ± 29.7 (27)	121.5–146.4; 134.1 ± 7.7 (21)	220.4–270.1; 251.4 ± 15.0 (8)	165.0–291.0; 219.3 ± 32.7 (29)
Percentage standard length				
Body depth at dorsal-fin origin (BDD)	5.9–11.1; 9.1 ± 1.3 (26)	8.3–11.9; 9.5 ± 0.8 (21)	5.8–7.9; 6.8 ± 0.9 (8)	5.6–8.8; 7.0 ± 0.9 (29)
Body depth at anal-fin origin (BDA)	4.9–10.1; 8.1 ± 1.2 (27)	7.3–10.4; 9.4 ± 0.8 (20)	6.6–8.2; 7.5 ± 0.5 (8)	5.2–8.0; 6.7 ± 0.6 (28)
Body with at dorsal-fin origin (BWD)	4.1–6.4; 5.3 ± 0.7 (26)	4.0–7.6; 6.0 ± 0.9 (21)	5.6–8.2; 6.5 ± 0.8 (8)	4.3–7.2; 5.4 ± 0.8 (29)
Head length (HL)	17.6–22.6; 20.0 ± 1.1 (26)	18.4–21.4; 19.6 ± 0.8 (21)	18.3–20.2; 19.4 ± 0.7 (8)	20.2–23.0; 21.7 ± 0.6 (29)
Snout length (SNL)	5.1–6.1; 5.7 ± 0.3 (26)	5.1–6.0; 5.4 ± 0.3 (21)	6.0–6.3; 6.1 ± 0.1 (8)	5.0–8.1; 7.2 ± 0.5 (29)
Orbit diameter (OD)	2.7–4.4; 3.2 ± 0.5 (26)	2.6–3.5; 3.0 ± 0.3 (21)	1.9–2.5; 2.3 ± 0.2 (8)	2.0–4.5; 2.5 ± 0.5 (29)
Interorbital width (IW)	2.0–3.5; 2.4 ± 0.3 (27)	2.1–2.9; 2.4 ± 0.2 (21)	2.8–3.6; 3.2 ± 0.3 (8)	2.7–3.7; 3.3 ± 0.3 (29)
Upper jaw length (UJL)	4.6–10.3; 6.7 ± 1.0 (26)	5.4–7.0; 6.4 ± 0.4 (21)	4.7–6.0; 5.3 ± 0.4 (8)	6.5–8.1; 7.1 ± 0.3 (29)
Caudal peduncle depth (CPD)	2.2 -2.9; 2.6 ± 0.2 (26)	2.8–3.2; 3.0 ± 0.1 (21)	2.5–2.9; 2.7 ± 0.1 (8)	2.3–2.8; 2.5 ± 0.1 (29)
Caudal peduncle length (CPL)	3.2–5.6; 4.1 ± 0.5 (27)	3.5–5.4; 4.3 ± 0.5 (21)	4.4–6.4; 4.8 ± 0.7 (8)	3.9–5.7; 4.9 ± 0.4 (29)
Prepectoral length (PPL)	16.6–21.7; 18.6 ± 1.0 (25)	14.8–20.4; 18.6 ± 1.1 (21)	17.9–18.7; 18.4 ± 0.3 (8)	19.5–22.1; 20.8 ± 0.7 (29)
Predorsal length (PDL)	23.5–28.1; 25.3 ± 1.0 (26)	24.0–27.3; 25.4 ± 1.0 (21)	26.1–27.6; 26.6 ± 0.5 (8)	25.7–30.4; 27.9 ± 1.0 (29)
Preanal length (PAL)	63.4–66.8; 64.9 ± 1.1 (27)	60.3–67.0; 63.7 ± 1.9 (21)	60.2–64.1; 62.8 ± 1.4 (8)	45.4–70.6; 64.9 ± 4.1 (29)
Pectoral-fin length (PFL)	8.6–11.9; 9.9 ± 0.7 (25)	9.4–11.4; 10.4 ± 0.5 (21)	7.2–8.2; 7.7 ± 0.4 (8)	6.7–8.7; 7.7 ± 0.6 (28)
Dorsal-fin base length (DFBL)	66.6–72.0; 69.8 ± 1.4 (27)	66.9–70.8; 69.2 ± 1.1 (21)	68.1–69.8; 69.1 ± 0.7 (8)	65.5–69.2; 67.4 ± 1.1 (29)
Anal-fin base length (AFBL)	28.9–32.4; 30.5 ± 1.0 (27)	26.7–33.4; 31.6 ± 1.9 (21)	30.5–33.8; 32.4 ± 1.2 (8)	28.4–32.1; 29.8 ± 1.0 (29)
Caudal-fin length (CFL)	8.3–11.5; 10.1 ± 0.7 (26)	9.0–12.0; 10.3 ± 0.7 (21)	8.5–9.7; 8.9 ± 0.4 (8)	7.6–10.3; 8.9 ± 0.7 (28)
Dorsal-fin height (DFH)	3.6–6.0; 4.7 ± 0.7 (27)	3.9–6.1; 4.8 ± 0.6 (21)	3.4–4.6; 4.1 ± 0.4 (8)	2.7–5.7; 3.9 ± 0.6 (29)
Anal-fin height (AFH)	2.6–6.2; 4.7 ± 0.8 (27)	3.4–5.8; 4.8 ± 0.5 (21)	2.5–4.6; 3.9 ± 0.7 (8)	2.7–5.6; 4.1 ± 0.6 (29)

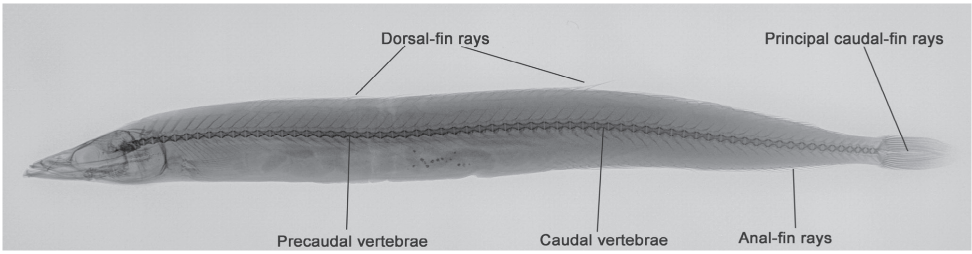

Figure 2. Radiograph of Common sand eel *Ammodytes tobianus* (Linnaeus, 1758) indicating the meristic characters evaluated from X-ray pictures. Depicted specimen: ZMH 26098-3, standard length 128.1 mm.

Table 2. Standardised coefficients of the first three discriminant functions (DF1, DF2, DF3) separating the four species of *Ammodytes* and *Hyperoplus* based on meristic characters. In bold, characters with the greatest weight in DF1 and DF2.

Meristic characters	DF1	DF2	DF3
DP	**0.884**	-0.320	-0.444
DR	0.056	**0.664**	-0.096
AR	0.112	-0.155	0.159
PR	0.015	0.079	-0.172
UR	-0.428	-0.135	-0.289
LR	**0.432**	-0.140	0.891
PV	-0.017	**0.523**	-0.061
CV	0.426	0.516	0.471
Percentage of explained variance	71.438	20.317	8.245
Eigenvalue	40.392	11.488	4.662
Cumulative variance in %	71.438	91.755	100.00

distance between verticals at the rear base of the anal fin and the caudal-fin base. Height of dorsal-fin rays (DFH) and anal-fin rays (AFH) were measured from their tips to the body contour. Caudal-fin length (CFL) was taken horizontally from the caudal-fin base to a vertical point at the tip of the longest ray. The values obtained were standardised by multiplying them by 100 and dividing them by the standard length.

Statistical treatment of morphological data

All morphological data were statistically processed, involving ranges, means, and standard deviations. Morphological data of all specimens that had a complete suite of meristic and morphometric character data were used to conduct two multiple discriminant function analyses (DFA) to determine if the four species of sand lances could be differentiated based on meristic and/or morphometric parameters using XLSTAT (version 2013.0.04, Addinsoft), a statistical analysis add-in for Microsoft Excel®. DFA was used to demonstrate the degree of separation in multivariate space defined by the main patterns of morphological variation among species which is described via the discriminant functions. It also shows which character contributes more to the differentiation. The standardised discriminant function coefficients represent the contributions of every variable to the discriminatory power of the function. Hence, the larger the standardised coefficient, the larger the weight of the variable in the function. Both discriminant analyses were conducted for 76 individuals (22 *A. marinus*, 20 *A. tobianus*, 8 *H. immaculatus*, 26 *H. lanceolatus*). Morphological variables without any variation (e.g. principal caudal-fin rays (CR)), variables, where other variables are included (e.g. total vertebrae (TV)) and qualitative variables (e.g. premaxillae clearly protrusible (PCP)) were excluded from the DFA procedures. The first DFA was performed for the following eight quantitative meristic characters: dermal plicae, dorsal-fin rays, anal-fin rays, pectoral-fin rays, upper arch gill rakers, lower arch gill rakers, precaudal vertebrae, and caudal vertebrae. The second DFA was conducted for the following 19 morphometric parameters: body depth at dorsal-fin origin, body depth at anal-fin origin, body width at dorsal-fin origin, head length, snout length, orbit diameter, interorbital width, upper jaw length, caudal peduncle depth, caudal peduncle length, prepectoral length, predorsal length, preanal length, pectoral-fin length, dorsal-fin base length, anal-fin base length, caudal-fin length, dorsal-fin height, and anal-fin height.

DNA isolation, PCR amplification, and sequencing

Genomic DNA was extracted at the DZMB using the Qiagen DNeasy Blood and Tissue Kit for single columns as described by Knebelsberger and Stöger (2012). A 652-bp fragment of the mitochondrial (mt) *COI* gene was amplified for 38 samples using a M13 tailed primer cocktail (C_FishF1t1-C_FishR1t1) including FishF2_t1 (5'-TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATCGGCAC), FishR2_t1 (5'-CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA), VF2_t1 (5'-TGTAAAACGACGGCCAGTCAACCAACCACAAAGACATTGGCAC) and FR1d_t1 (5'-CAGGAAACAGCTATGACACCTCAGGGTGTCCGAARAAYCARAA) (Ivanova et al. 2007). As a second marker, a 464 bp fragment of the nuclear (nc) Rhodpsin gene was amplified for all 70 samples using the forward primer Rh545 (5'-GCAAGCCCATCAGCAACTTCCG) and the reverse primer Rh1039r (5'-TGCTTGTTCATGCAGATGTAGA) developed by Chen et al. (2003). Each PCR reaction mixture contained 1 µl DNA template, 2.25 µl of 10X reaction buffer (including $MgCl_2$), 0.5 µl dNTPs (2mM each), 0.5 µl of each primer (10 pmol/µl), 0.5 µl Taq polymerase (5 U/µl; Qiagen) and molecular grade water for a total volume of 25µl. Thermal cycling was performed with an initial denaturation for 2 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at the annealing temperature of 50°C, 60 s at 72°C with a final extension of 10 min at 72°C. All PCR products were checked by a 1% agarose gel. Amplicons were purified by incubating 10 µl of PCR products with 0.5 µl of Exonuclease I (20 U/µl) and 2 µl of Alkaline Phosphatase (1 U/µl) for 15 min at 37°C followed by 20 min at 75°C. Purified amplicons were sequenced by Macrogen Europe (Amsterdam, Netherlands).

Sequence alignment and data analyses

Forward and reverse sequences of *COI* and Rhodpsin were assembled and edited using Geneious (version 7.1.9. http://www.geneious.com). Consensus sequences were submitted to GenBank (for accession numbers see Suppl. material 1). Variance in sequence length, base composition, number of invariable sites and the presence of stop codons were analysed using Geneious. The nc and mt sequences were aligned independently using MUSCLE (Edgar 2004) with default settings as implemented in MEGA version 6.06 (Tamura et al. 2013). Primer sequences were cut from the alignment. Rhodopsin gene sequence alignment was checked by eye for species specific diagnostic characters. For *COI*, Kimura-2-parameter (K2P) distances were calculated in MEGA, as K2P is used as standard model for barcoding analyses and enables direct comparison with other studies. Neighbour-Joining (NJ) topology (Saitou and Nei 1987) was built in MEGA using the "pairwise deletion" option for the treatment of gaps and missing data, in order to retain all sites initially, excluding them as necessary. Node support for the NJ topology was evaluated by a non-parametric bootstrap analysis (Felsenstein 1985) with 10,000 replicates. In order to quantify the distinctness between species at the barcode locus, genetic distances were used to calculate the difference between the maximum intraspecific genetic distance and the minimum distance to the nearest neighbor (barcode gap). For the calculation of genetic distances at genus and family level, we used BOLDs "Distance Summary" tool by choosing K2P distance model and MUSCLE (Edgar 2004) alignment algorithm.

On BOLD, DNA barcodes were automatically assigned to operational taxonomic units (OTUs), generated through Refined Single Linkage (RESL) analyses (Ratnasingham and Hebert 2013). Finally, a unique alphanumeric code is assigned to each of the OTUs, constituting the so called barcode index number (BIN). It has been shown that BINs are highly congruent with existing species assignments (Ratnasingham and Hebert 2013). Here, the 'BIN Discordance Report' analysis tool was applied to analyse our dataset together with public sequences on BOLD, and to get hints on cryptic diversity (species) or to identify cases of haplotype sharing between species.

Furthermore, BOLD's "Diagnostic Characters" sequence analysis tool was applied to the COI dataset choosing MUSCLE (Edgar 2004) alignment algorithm. Sequences were grouped by species names in order to categorise consensus bases by their diagnostic potential.

Results

General results of morphological analysis

Meristic characters and morphometric measurements of the four examined species of sand lances are given in Table 1. The number of individuals per analysed character ranged from 24 to 27 for *A. marinus*, from 20 to 21 for *A. tobianus*, from 28 to 29 for *H. lanceolatus*, and was eight individuals for *H. immaculatus* (Table 1).

The data of the present study confirmed that the two genera of *Ammodytes* and *Hyperoplus* can be distinguished by qualitative meristic characters, i.e. by having a clear protrusible premaxillae (PCP) and no vomerine teeth (VTP) (*Ammodytes*) or no clear protrusible premaxillae and two vomerine teeth (*Hyperoplus*) (Table 1). *Hyperoplus* can also be separated from *Ammodytes* by its significantly higher number of dermal plicae (DP). It is also possible to distinguish *Hyperoplus* from *Ammodytes* by its obviously lower pectoral-fin length (PFL), and to a somewhat lesser significance, also by its greater mean snout length (SNL), since no sexual dimorphism has been reported for the last two characters in both genera.

Hyperoplus lanceolatus can be separated from *H. immaculatus* by the presence of a conspicuous dark spot on either side of snout (DSSS) which is lacking in *H. immaculatus* (Table 1). Furthermore, *H. lanceolatus* differs from *H. immaculatus* by its lower numbers of total and lower arch gill rakers (GR, LR), total and caudal vertebrae (TV, CV), as well as dorsal and anal-fin rays (DR, AR).

Ammodytes tobianus can be distinguished from *A. marinus* by having belly scales that are organised in tight chevrons (BSTC) and having scales present over the musculature at the base of the caudal fin (SBCF) and in the midline anterior to dorsal fin (SADF), whereas these characters are not present in *A. marinus* (Table 1). *Ammodytes tobianus* differs from *A. marinus* also by its lower numbers of dermal plicae (DP), dorsal-fin rays (DR), and precaudal and total vertebrae numbers (PV, TV).

Discriminant Function Analysis with meristic characters

DFA based on meristic characters provided three significant functions (Box-Test with $\chi^2=790.916$ and $p<0.0001$; Wilks′ lambda= 0.0003 and $p<0.0001$). These three functions explain 100% of the total variation in the data. The first two functions explain 91,755% of the total variation in the data (Table 2), which is sufficient for the further detailed analysis. The third discriminant function explains 8.245% of total variation.

Individual specimens are projected onto the first two discriminant functions in Figure 3. Because all four species were clearly separated in the discriminant space defined by the first two functions, the third function was not used. The first discriminant function explains 71.438% of total variation (Table 2). It mainly separates *A. tobianus* and *H. immaculatus* and to a lesser extent the species pairs of *A. tobianus* and *H. lanceolatus*, *A. marinus* and *H. immaculatus*, *A. marinus* and *H. lanceolatus* as well as *H. lanceolatus* and *H. immaculatus* (Figure 3). *Ammodytes tobianus* and *A. marinus* cannot be so clearly separated by the first discriminant function.

From the standardised coefficients (Table 2), the two characters that have the greatest influence on the first discriminant function (characters most discriminatory) are the dermal plicae (DP) and lower arch gill rakers (LR) (Table 2). In general *H. immaculatus* and *H. lanceolatus* have a much higher number of dermal plicae than *A. tobianus* and *A. marinus* (Table 1). The numbers of DP of both species of *Hyperoplus* are overlapping, whereas the *Ammodytes* species have different numbers of DP. The

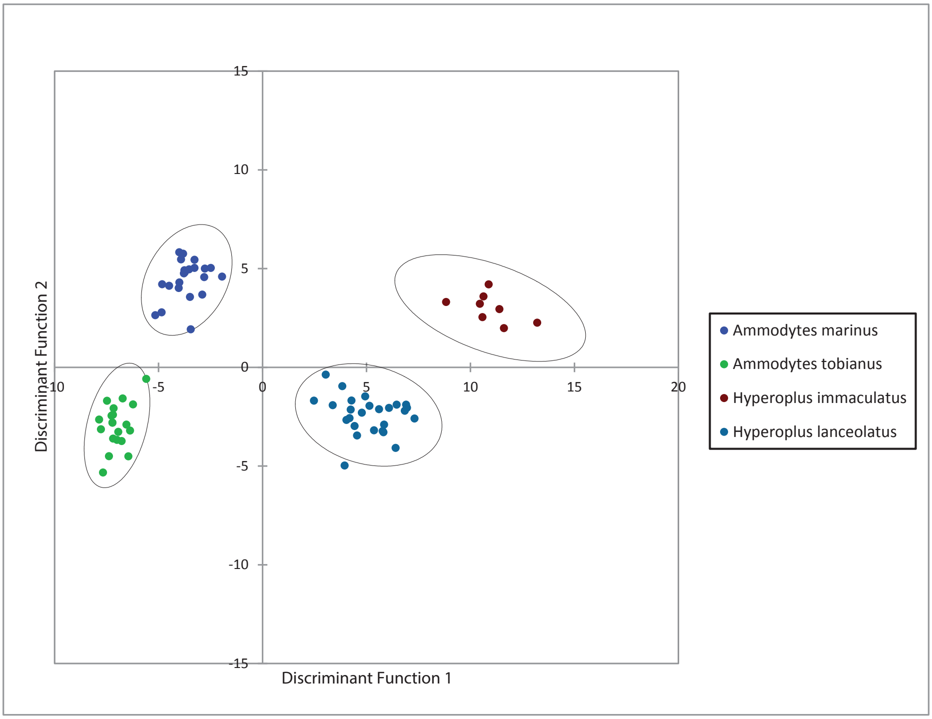

Figure 3. Plot of all analysed *Ammodytes* and *Hyperoplus* specimens onto the first and second discriminant functions based on a set of eight meristic characters. Circles include 95% of specimens in each species.

number of lower arch gill rakers (LR) is higher in *H. immaculatus* in comparison with the other three species, which have overlapping numbers of LR.

The second discriminant function accounts for 20.317% of total variation. *Ammodytes tobianus* and *A. marinus* are clearly and the species pairs of *A. tobianus* and *H. immaculatus*, *A. marinus* and *H. lanceolatus* as well as *H. immaculatus* and *H. lanceolatus* are to a lesser extent discriminated by this function. *Ammodytes tobianus* and *H. lanceolatus* and *A. marinus* and *H. immaculatus* cannot be clearly separated by the second discriminant function. The contrasts between the numbers of dorsal-fin rays (DR) and the numbers of precaudal vertebrae (PV) of the species are mainly responsible for this discrimination. DR is lowest in *A. tobianus* and highest in *H. immaculatus* (Table 1). PV is lowest in *A. tobianus* and highest in *A. marinus* and *H. immaculatus*.

Discriminant Function Analysis with morphometric measurements

Three significant DFA functions were estimated based on morphometric measurements (Box-Test with $\chi^2 = 944.979$ and $p < 0.0001$; Wilks´ lambda = 0.003 and $p < 0.0001$). Together these functions explain 100% of the total variation in the data. The first two functions explain 93.144% of the total variation in the data (Table 3), which

Table 3. Standardised coefficients of the first three discriminant functions (DF1, DF2, DF3) separating the four species of *Ammodytes* and *Hyperoplus* based on morphometric measurements. In bold, characters with the greatest weight in DF1 and DF2.

Morphometric measurements	**DF1**	**DF2**	**DF3**
BDD	-0.135	-0.184	0.085
BDA	-0.178	-0.038	-0.208
BWD	-0.054	0.317	0.222
HL	0.290	-0.084	-0.603
SNL	**0.380**	-0.040	-0.045
OD	-0.231	0.078	0.497
IW	0.198	0.164	0.237
UJL	-0.034	**-0.868**	-0.260
CPD	-0.228	**0.542**	-0.473
CPL	0.112	0.418	-0.147
PPL	0.204	0.101	-0.076
PDL	0.140	0.251	0.143
PAL	-0.020	0.008	0.103
PFL	**-0.612**	-0.280	-0.528
DFBL	-0.060	0.104	0.551
AFBL	0.098	0.429	-0.208
CFL	-0.148	0.520	0.390
DFH	-0.078	-0.125	0.223
AFH	0.136	-0.333	-0.259
Percentage of explained variance	78.576	14.568	6.856
Eigenvalue	20.555	3.811	1.794
Cumulative variance in %	78.576	93.144	100.00

is sufficient for the further detailed analysis. The third discriminant function explains 6.856% of total variation.

Figure 4 presents the individual specimens projected onto the first two discriminant functions. Because all four species were clearly separated in the discriminant space defined by the first two functions, the third function was not used. The first discriminant function explains 78.576% of total variation (Table 3). It mainly separates *A. tobianus* and *H. lanceolatus* and to a lesser extent the species pairs of *A. tobianus* and *H. immaculatus*, *A. marinus* and *H. lanceolatus* as well as *A. marinus* and *H. immaculatus* (Figure 4). The species pairs of *A. marinus* and *A. tobianus* as well as of *H. immaculatus* and *H. lanceolatus* cannot be separated by the first discriminant function.

The two measurement characters that have the greatest weight on the first discriminant function are pectoral-fin length (PFL) and the snout length (SNL) (Table 3). Both species of the genus *Ammodytes* have a greater PFL than both species of the genus *Hyperoplus* (Table 1). In contrast, both *Hyperoplus* species have a greater SNL than both *Ammodytes* species. PFL and SNL are relatively similar for the species of the same genera.

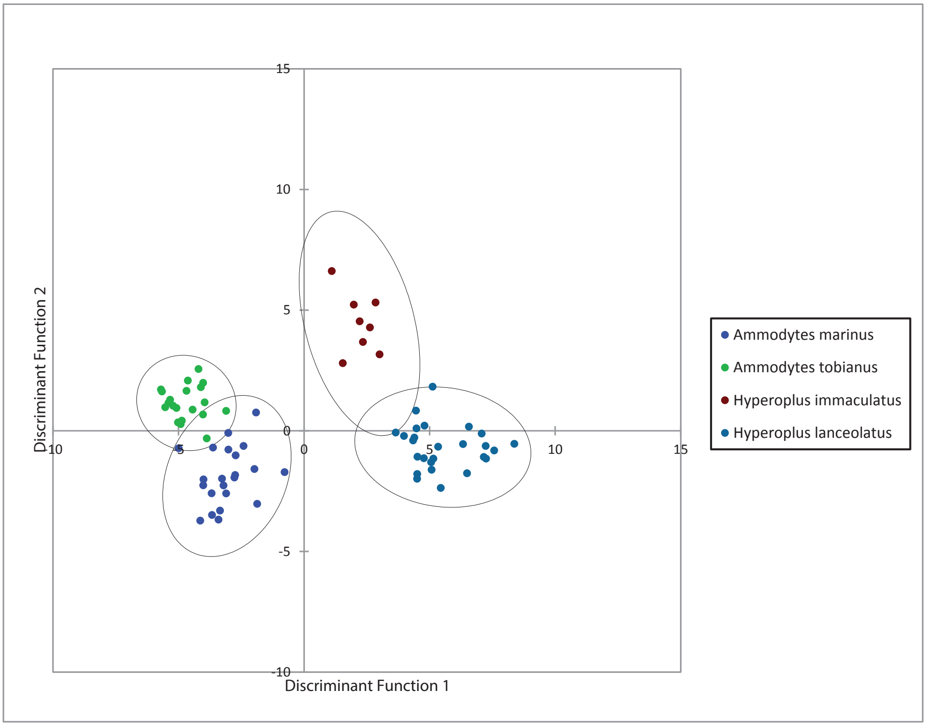

Figure 4. Plot of all analysed *Ammodytes* and *Hyperoplus* specimens onto the first and second discriminant functions based on a set of 19 morphometric characters. Circles include 95% of specimens in each species.

The second discriminant function accounts for 14.568% of total variation. Especially the species within the genera *Ammodytes* and *Hyperoplus*, namely *A. tobianus* and *A. marinus* as well as *H. immaculatus and H. lanceolatus* are separated by this function (Figure 4).

Upper jaw length (UJL) and caudal peduncle depth (CPD) are the two measurements, for which no sexual dimorphism is known, and that have the greatest weight on the second discriminant function (Table 3).

Mt DNA barcoding

Mitochondrial DNA barcodes were obtained for 70 specimens belonging to four species of the family Ammodytidae investigated in this study (Suppl. material 1). The DNA sequences did not show any ambiguous base calls (Ns) or stop codons, and no insertions or deletions were found within the sequence alignment. Sequence length ranged from 619 to 652 bp (mean and standard deviation: 650.5 ± 5.7 bp). The average base composition was 22.8% adenine (A), 29.7% cytosine (C), 18.3% guanine (G) and 29.3% thymine (T); GC content was 48%. The sequence alignment showed 588 identical sites.

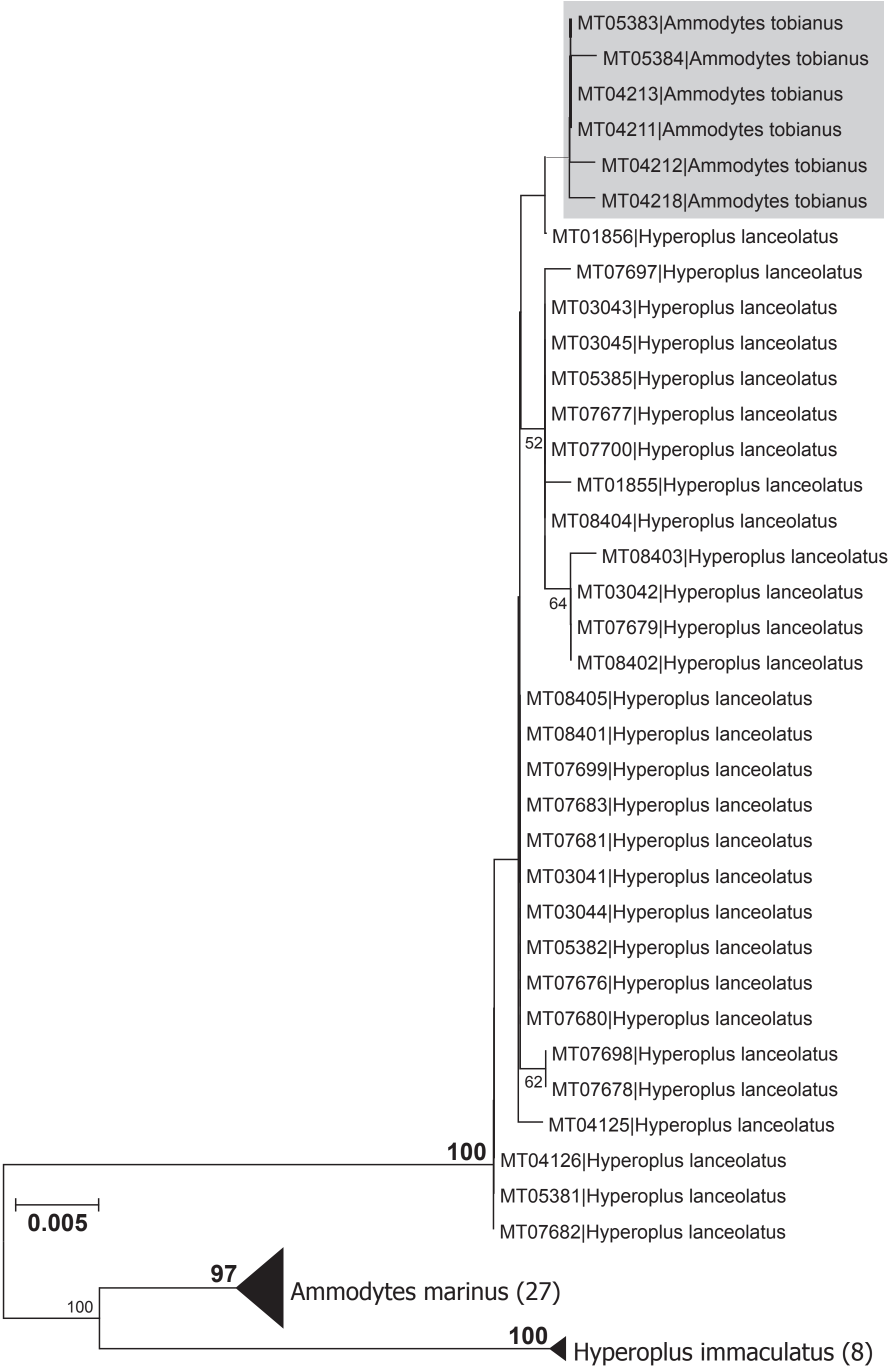

Figure 5. NJ dendrogram based on K2P pairwise genetic distances. Values at nodes indicate the result of the bootstrap test (10.000 pseudo replicates). Only values ≥ 50 are shown. For *Ammodytes tobianus* (grey box) and *Hyperoplus lanceolatus* all analysed individuals are shown. In case of *Ammodytes marinus* and *Hyperoplus immaculatus* the number of specimens is given in brackets.

Table 4. Minimum and maximum intraspecific genetic K2P distances (%) for each species including mean and standard deviation. The barcoding gap indicates the difference between the maximum intraspecific and the minimum interspecific (nearest neighbour) genetic distance. Additionally K2P genetic distances are given in brackets, if they differ from p-distances.

Species	Specimens	Mean Distance	SD*	Minimum Distance	Maximum Distance	Nearest Neighbor	Distance to Nearest Neighbor	Barcoding Gap
A. marinus	27	0.24	0.19	0.00	0.77	*H. immaculatus*	3.50	2.73
A.tobianus	6	0.15	0.01	0.00	0.15	*H. lanceolatus*	0.15	no gap
H. lanceolatus	29	0.22	0.15	0.00	0.62	*A. tobianus*	0.15	no gap
H. immaculatus	8	0.07	0.08	0.00	0.16	*A. marinus*	3.50	3.34

The NJ analysis of the K2P distances revealed well supported monophyletic clusters for the species *A. marinus* and *H. immaculatus* with bootstrap values of 97 and 100, respectively (Figure 5). In contrast, *A. tobianus* and *H. lanceolatus* sequences were grouped together in one monophyletic cluster with a bootstrap support of 100. Within this cluster the sequences of *A. tobianus* were grouped together without bootstrap support indicating that there is no sharing of haplotypes between these two species. The analysis of the K2P genetic distances revealed an overlap between intraspecific (range: 0.0-0.77%; mean and standard deviation: 0.22 ± 0.17%) and interspecific distances (0.15-7.27%; 4.73 ± 1.7%). The overlap was caused by the two species *A. tobianus* and *H. lanceolatus*: in *A. tobianus*, the minimum distance to the nearest neighbour species was even lower than the maximum intraspecific distance, whereas both values were equal in *H. lanceolatus* (Table 4). In contrast, the species *A. marinus* and *H. immaculatus* exhibited barcode gaps of 2.73% and 3.34% respectively, which indicates an undoubtedly separation from the other species. At genus and family level, the genetic distances between species of the same genus varied between 4.46-7.09% and the distances between species belonging to different ranged from 0.15-7.27%.

BIN report

The BIN discordance report tool on BOLD assigned three different BIN numbers to the 70 *COI* haplotypes. The BIN BOLD:ACF3320 was found to be "concordant" and exclusively comprised 32 specimens of the species *Ammodytes marinus*, of which five individuals were not provided by this study. The "discordant" BIN BOLD:AAC5676 comprised 57 specimens, 14 identified as *Ammodytes tobianus* and 43 as *Hyperoplus lanceolatus*. From the former species eight specimens and from the latter 14 specimens were not provided by our study but also support the findings of this study. The third BIN BOLD:AAJ2299 was also specified as discordant and comprised ten specimens, eight (in our study) identified as *Hyperoplus immaculatus* and two identified as *Ammodytes marinus*. The two *A. marinus* entries may represent cases of misidentification as 32 *A. marinus* individuals were grouped together in BIN BOLD:ACF3320.

Table 5. Variable sites identified for the nc Rhodopsin gene fragment sequence alignment. Bases in bold indicate species specific diagnostic characters. The three underlined bases are distinctive for *A. tobianus*.

		Nucleotide position		
Species	**Specimens**	**82**	**433**	**460**
A. marinus	27	C	G	**C**
A.tobianus	7*	<u>C</u>	<u>G</u>	<u>A</u>
H. lanceolatus	30**	**T**	G	A
H. immaculatus	8	C	**A**	A

*one /**two sequences downloaded from GenBank.

Diagnostic characters

The analysis revealed four diagnostic characters for the species *A. marinus* and 16 for *H. immaculatus* (results not shown). The two species *A. tobianus* and *H. lanceolatus* did not show any diagnostic characters on species level. Consequently, only two of the four investigated species can be identified using diagnostic characters on the basis of COI barcode sequences.

Nc DNA analysis

The nc Rhodopsin sequence alignment showed a length of 464 bp after primer trimming. The complete fragment could be amplified and sequenced for all 70 specimens used for the mt DNA barcode analysis. The number of variable sites was very low and the alignment could be easily evaluated by eye. One diagnostic character was found for each of the species *A. marinus* (Table 5; site 460: C instead of A), *H. lanceolatus* (site 82: T instead of C), and *H. immaculatus* (site 433: A instead of G). *Ammodytes tobianus* showed no species specific mutation but could be characterised by a combination of all tree variable sites (Table 5, underlined bases). The Rhodopsin sequences from GenBank were compared with our sequences; the two *H. lanceolatus* sequences (GenBank accessions: EU492010, EU492011) showed concordant results. In the case of the *A. tobianus* sequence (GenBank accession: AY141306) no data was available for site 460 but the two other sites were in agreement with our results.

Discussion

Identification of genera and species using morphological characters

The primary objective of this study was to contribute to robust genera- and species-level identifications, combining morphological and molecular methods, of four closely related species of sand lances of the genera *Ammodytes* and *Hyperoplus* occurring in the northeast Atlantic Ocean and adjacent waters.

The detailed morphological analyses confirmed findings described by other authors (e.g. Duncker and Mohr 1939, Reay 1986): the genus *Ammodytes* can be distinguished by two morphological characters from the genus *Hyperoplus*. *Ammodytes* has clear protrusible premaxillae and no vomerine teeth. In contrast, *Hyperoplus* has no clear protrusible premaxillae and a pair of vomerine teeth. It should be noted here that Kayser (1961) found out that *Hyperoplus* has no real vomerine teeth, but anterior hooked ends of the prevomer instead.

Subsequently, Ida et al. (1994) pointed out that the tip of the prevomer in *Ammodytes* is straight, not protruded from the roof of the mouth, whereas in *Hyperoplus* the tip of the prevomer curved downwards, protruding from the roof of the mouth. According to Wiecaszek et al. (2007), the genus *Ammodytes* also has a longer lower jaw when compared to the length of pectoral-fin, while this relationship is reversed in *Hyperoplus*.

This study adds three more characters helpful in distinguishing between both genera of sand lances based on the four species considered. Firstly, the number of dermal plicae is significantly higher in *Hyperoplus* compared to *Ammodytes*. Secondly, *Hyperoplus* has a lower pectoral-fin length in relation to standard length (SL) than *Ammodytes*. Goltberg (1910) also reported a lower value of pectoral-fin length expressed as a proportion of head length for *H. lanceolatus* than for *A. tobianus*. Thirdly, *Hyperoplus* has a larger mean snout length in % SL than *Ammodytes*. However, the last mentioned character is less recommended for practical taxonomical assignments, since its ranges overlap between the genera to a relatively large extent. Therefore, a combination of the following four characters remains, which seems to be useful to distinguish between the genera *Hyperoplus* and *Ammodytes*: protrusibility of premaxillae, presence of the hooked ends of prevomer, number of dermal plicae, and pectoral-fin length in % SL.

As indicated by the results of discriminant function analysis, morphometric measurements seem not to be characters of the first choice to distinguish the two species of each of the two genera, since they could not be discriminated by the first discriminant function.

According to the results presented here, six meristic characters (the number of lower arch gill rakers, the total number of gill rakers, the number of caudal vertebrae, the number of total vertebrae, and the number of dorsal-fin and anal-fin rays) are more useful than morphometric measurements to distinguish between *H. immaculatus* and *H. lanceolatus*. The use of these additional characters would support and refine the current methods to separate *H. lanceolatus* from *H. immaculatus*. Searching only for the occurrence of a conspicuous dark spot on either side of snout below anterior nostril could be unsuccessful in the case of preserved specimens.

In the case of *A. tobianus* and *A. marinus*, these results support the information on useful distinguishing characters between both species reported for instance by Reay (1986): *A. tobianus* differs from *A. marinus* by its belly scales that are organised in tight chevrons, scales which are present over musculature at base of caudal fin, as well as by lower numbers of dermal plicae, dorsal-fin rays and vertebrae. It should be mentioned that our analyses included also *A. tobianus* from the Baltic Sea, for which no meristic or morphometric data had been published, except for the number of vertebrae and pectoral-fin length (Wiecaszek et al. 2007).

Discrimination of genera and species based on molecular data

The successful discrimination of the two sand lance species *A. marinus* and *H. immaculatus* by DNA barcoding was already demonstrated by Knebelsberger et al. (2014) and could be confirmed by the present study. An additional three specimens of *A. marinus* were added to the dataset and the NJ analysis revealed well-supported monophyletic species clusters for *A. marinus* and *H. immaculatus*, indicating an unambiguous separation of these two species. Successful species discrimination can also be demonstrated by the presence of gaps between intra- and interspecific genetic distances (Meyer and Paulay 2005), which were in case of *A. marinus* and *H. immaculatus* 2.73 and 3.34 respectively. The BIN analysis performed on BOLD revealed two separate species BINs: one concordant BIN exclusively contained sequences which were taxonomically annotated as *A. marinus*, and a second discordant BIN contained all specimens of *H. lanceolatus* and two further entries referring to as *A. marinus*. These two individuals were provided by other sources and may represent cases of misidentification, as all other *A. marinus* entries appeared in the concordant BIN.

Surprisingly, the two species *A. tobianus* and *H. lanceolatus* belonging to different genera cannot be clearly separated on the basis of genetic distances, as the lowest distance (K2P) between these two species was only 0.15% and within species variation was found to be 0.15 and 0.62% respectively. In the NJ dendrogram both species appeared together in a well supported clade and were also found within the same BIN cluster when analysed together with data on BOLD. However, *A. tobianus* and *H. lanceolatus* do not show haplotype sharing, as *A. tobianus* sequences appeared together in a separate cluster. The two species may therefore be separated by applying tree-based approaches like GMYC or model-based ones like ABGD.

In contrast to the barcoding results, both genera of *Ammodytes* and *Hyperoplus* can undoubtedly be separated by morphological character traits as discussed above. DNA barcoding failure between closely related congeneric species is usually more common than between species belonging to different genera (e.g. McCusker et al. 2013, Knebelsberger et al. 2015). For congeneric species of the genus *Ammodytes* inconsistencies between morphological data and DNA barcodes have already been demonstrated. For instance *A. americanus* DeKay, 1842 and *A. dubius* Reinhardt, 1837 from the northwest Atlantic Ocean could not be separated by DNA barcoding, possibly caused by inadequate taxonomy (McCusker et. al. 2013), which may also concern the two species *A. personatus* Girard, 1856 and *A. hexapterus* Pallas, 1814 from the north Pacific (Turanov and Kartavtsev 2014).

In the present work, inadequate taxonomy, erroneous species designation or identification error can be excluded as possible explanation for DNA barcoding failure in unambiguously separating *A. tobianus* from *H. lanceolatus*. In addition, true biological phenomena such as the occurrence of hybridisation or incomplete lineage sorting seem to be unlikely, as no interspecific haplotype sharing was found. In cases where mitochondrial COI sequences fail to distinguish between species, the application of nuclear DNA markers may be tested alternatively. In fish, the nuclear Rhodopsin gene has already been proposed as supplementary marker in order to identify species (Sevilla et al. 2007). However, most studies demonstrated reduced species discrimination success using nuclear Rhodopsin sequences compared to *COI* barcodes (Hanner et al. 2011, Collins et al. 2012, Behrens-Chapuis et al. 2015). In our study, the analysis of a short nuclear Rhodpsin gene fragment revealed diagnostic nucleotides for the species *Ammodytes marinus*, *H. lanceolatus* and *H. immaculatus*. The species *Ammodytes tobianus* can be characterised by the lack of species specific mutations compared to the other three species. Consequently, all four species of sand lances can be identified using the diagnostic character approach in combination with nuclear Rhodopsin sequences. In contrast to that, *COI* provided diagnostic characters only for the two species *A. marinus* and *H. immaculatus*. *Ammodytes tobianus* and *H. lanceolatus* cannot be characterised by this approach.

Our study clearly demonstrated that nuclear Rhodopsin constitutes a preferable alternative marker to discriminate successfully between the four investigated species of sand lances.

Finally, it should be pointed out that the present results are not meant to provide a phylogenetic reconstruction with regard to the genera *Ammodytes* and *Hyperoplus*, since the latter requires a more detailed study of more species of both genera, as well as other members of the group. However, accurate identification of these sand lance species is the basis to assess the status of their stocks and to implement appropriate measures of fisheries management or conservation, and as such, the aim of successfully identifying the NE Atlantic species has been accomplished.

Conclusion

With this study a robust genus- and species-level discrimination of the four most abundant and closely related species of sand lances of the genera *Ammodytes* and *Hyperoplus* in the NE Atlantic Ocean and adjacent waters has been provided. It is expected that these results will facilitate the accurate identification of *A. marinus, A. tobianus*, *H. immaculatus*, and *H. lanceolatus* combining morphological and molecular methods.

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Supplementary material 1

Table S1

Authors: Ralf Thiel, Thomas Knebelsberger

Data type: specimen data

Explanation note: Supplementary metadata for specimens used for both morphological and genetic analyses; Museum and Sample IDs are specimen identifiers, BOLD Process IDs are unique codes automatically generated for each record on BOLD, GenBank Accession NOs represent sequence identifiers.

Supplementary material 2

Table S2

Authors: Ralf Thiel, Thomas Knebelsberger

Data type: specimen data

Explanation note: Museum IDs and collection data for specimens of *Ammodytes tobianus* used for morphological analyses only.