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A new feather mite species of the genus *Proterothrix* Gaud, 1968 (Acarina, Proctophyllodidae) from the Large Niltava, *Niltava grandis* (Passeriformes, Muscicapidae) – an integrative description

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Abstract

A new species of the feather mite genus *Proterothrix* (Proctophyllodidae: Pterodectinae) is described from the Large Niltava *Niltava grandis* (Blyth) (Passeriformes, Muscicapidae) in northeast India (Meghalaya, Jaintia Hills, Shnongrim village). *Proterothrix chachulae* Constantinescu, **sp. n.** differs from all known species of the genus by having in males the aedeagus with bilobate tip. The morphological description is supplemented with molecular characterisation of a fragment f near the 5` terminus of the mitochondrial COI gene.

Keywords

Taxonomy, feather mite, new species, Pterodectinae, *Proterothrix*

Introduction

The bird genus *Niltava* Hodgson belongs to the family of Old World Flycatchers (Passeriformes: Muscicapidae), currently includes six valid species and is distributed in Indo-Malayan biogeographic region (Clements et al. 2016). Feather mites were previously recorded only in two of these species: *Analges* sp., *Anisodiscus* sp., *Mesalgoides* sp., *Proctophyllodes cotyledon* Trouessart, 1899, *Bicentralges distinctus* Orwig, 1968, *Proterothrix* sp., and *Trouessartia* sp. ex *Niltava grandis* (Blyth); *Analges* sp., *Proctophyllodes elegans* Atyeo and Braasch, 1966, *Proterothrix* sp., *Therisalges* sp., *Trouessartia* sp., and *Xolalges* sp. ex *Niltava sundara* Hodgson (Atyeo 1973; Atyeo and Braasch 1968; Orwig 1968). An unidentified *Proterothrix* species, reported by Atyeo (1973) from *Niltava grandis* in Asia is a potentially a new species, but has never been described. In this paper we describe a new species of *Proterothrix* collected from *N. grandis grandis* in Meghalaya (Northeast India).

The genus *Proterothrix* Gaud, 1968 (Proctophyllodidae: Pterodectinae) includes 28 species known exclusively from the Old World (Ethiopian, Oriental and Australasian regions) (Hernandes and Valim 2014). In the Oriental region, the genus appears to be one of the most widespread proctophyllodid genera, with 13 described species (from Malaysia, Taiwan, China, Vietnam and India) and 56 potentially new species recorded by Atyeo (1973), but never described elsewhere.

Species of the genus *Proterothrix* have been identified to date on birds from the orders Coraciiformes (Alcedinidae) and Passeriformes (Dicruridae, Eurylaimidae, Leiothrichidae, Monarchidae, Muscicapidae, Paradisaeidae, Paradoxornithidae, and Pellorneidae). According to modern concepts, this genus, along with seven more genera belongs to the "*Phroterothrix* generic group" incorporating archaic pterodectines with setae *ps3* anterior to adanal suckers in males (Mironov and Proctor 2009, Hernandes and Valim 2014). Twenty six out of the 28 species were arranged in three species groups: *megacaula* (3 species), *schizothyra* (4 species) and *wolffi* (19 species) (Gaud 1952, 1962, 1968, 1979, Mironov et al. 2008, 2010, 2012, Mironov and Proctor 2009, Mironov and Tolstenkov 2013, Constantinescu et al. 2014). The new species of *Proterothrix* described herein belongs to the *wolffi* species group, in having almost closed coxal fields III in males and parallel-sided terminal cleft in females.

Materials and methods

The material used in the present paper was collected in Shnongrim (Meghalaya, India) in January 2014. The birds were captured using mist–nets, identified and visually checked for the presence and collection of mites and released back to the wild. Mite specimens were collected manually with a needle and placed in tubes with 96%

ethanol. Later, in laboratory conditions, mite specimens were cleared in 90% lactic acid for 24 hours, and mounted on microscope slides in Hoyer's medium. Drawings were made using an Olympus CX21 microscope, with a camera lucida drawing device. The bird specimens were identified according to Rasmussen and Anderton (2012) and Grimmett et al. (2011), and the taxonomy of birds follows Clements et al. (2016). The body setation of mites follows that of Griffiths et al. (1990) with the modifications by Norton (1998) concerning coxal setae, while the setation of legs follows Gaud and Atyeo (1996). The description of the new species of *Proterothrix* is given according to the current format used for species of pterodectine mites (Mironov and Fain 2003, Mironov 2006, Valim and Hernandes 2006, Mironov et al. 2008) and the measuring techniques of particular structures used were described by Mironov and Proctor (2009).

The full set of measurements is given for a holotype (male) and a range of measurements for corresponding paratypes. All measurements are in micrometers (μm). The holotype and paratypes of the new species are deposited in the Acarological Collection of the "Grigore Antipa" National Museum of Natural History, Bucharest, Romania (inventory numbers are given in brackets for all type specimens).

Three paratype specimens of *Proterothrix chachulae* sp. n. (two males ANA747, ANA748 and one female ANA749) were used to isolate DNA using DNAeasy Tissue Kit (Qiagen). All three specimens used for molecular analyses were mounted and kept as reference vouchers for morphological examination. The specimens preserved in ethanol 96% were transferred in 180μl ATL Buffer with 20 μl of Proteinase K and incubated overnight at 56ºC on a shaking thermoblock. After 24h, 5μl of Proteinase K were added and incubation was continued until 72h. For the rest of the protocol we followed the manufacturer specifications and the modification suggested by Dabert et al. (2008).

The 663bp fragment near the 5` terminus of the COI gene was used as DNA barcode region, amplified by PCR with the degenerate primers bcdF05 (5`- TTTTC-TACHAAYCATAAAGATATTGC-3`) and bcdR04 (5`- TATAAACYTCDGGAT-GNCCAAAAAA-3`), according to Dabert et al. (2008). The PCRgenotyping reaction was performed in a 50 μL total volume containing DNA template, 1X Green GoTaq® Flexi Buffer, 2.5 mM MgCl2, each dNTP at 0.1 mM, each primer at 0.5 μM (the primers were M13 tailed) and 1.5 units of GoTaq® DNA polymerase (5U/μl) (Promega, Madison, USA). The PCR products were isolated from samples containing visible bands and sent for sequencing to Macrogen (Seoul, Korea).

Sequence chromatograms were edited and assembled with CodonCode Aligner version 3.7.1. Pairwise distances between sequences were computed with MEGA version 6 (Tamura et al. 2013) using K2P distance model (Kimura 1980). DnaSP v5 was used to obtain data about the genetic polymorphism in the studied specimens (Librado and Rozas 2009).

Results

Family Proctophyllodidae Trouessart et Mégnin, 1884
Subfamily Pterodectinae Park et Atyeo, 1971
Genus *Proterothrix* Gaud, 1968

***Proterothrix chachulae* Constantinescu, sp. n.**
http://zoobank.org/9CC8B15E-BCFB-4288-9EAA-265ED462C931
Figs 1–6

Type material. Male holotype (ANA672), 6 male (ANA671, ANA673, ANA674, ANA675, ANA747(P2♂), ANA748(P1♂)) and 6 female (ANA676, ANA677, ANA678, ANA679, ANA680, ANA749(P1♀) paratypes, 27.01.2014, from the Large Niltava *Niltava grandis grandis* (Blyth) (Passeriformes, Muscicapidae); INDIA**:** Meghalaya, Jaintia Hills, Shnongrim village, (25°21'12.36"N, 92°31'3.06"E); 1151 m; subtropical forest; collector D. K. B. Mukhim.

Description. MALE (Figs 1; 2; 3; holotype, range for 6 paratypes in parentheses): Pseudorutellar lobes with long and acute lateral extensions. Length of idiosoma 284 (284–288), width 104 (104–109), length of hysterosoma 184 (190–192). Prodorsal shield entire, anterolateral extensions short and with acute tips, lateral margins without incisions, posterior margin with wide blunt-angular extension, posterior angles well expressed, length 96 (94–96), width 82 (82–88), surface with ovate lacunae (Fig. 1). Scapular setae *se* separated by 36 (35–42). Scapular shields narrow. Humeral shields narrow, separated from outer sclerotized areas of epimerites III. Setae *cp* situated ventrally, setae *c2* filiform, situated dorsally, both pairs on striated tegument. Subhumeral setae *c3* lanceolate, 18 (18–20) × 6 (6–8). Hysteronotal shield with anterior margin concave, anterior angles rounded, distance from anterior margin to bases of setae *h3* 174 (180–186), greatest width in anterior part 74 (72–80), surface with small circular lacunae. Opisthosomal lobes roughly trapezoidal, short, each with angular median expansion on posterior margin, setae *h3* situated slightly posterior to setae *h2*. Terminal cleft V–shaped, 16 (16–20) in length; margins of terminal cleft without membranes. Supranal concavity clearly outlined, triangular. Setae *f2* slightly posterior to bases of setae *ps2*. Setae *h1* near lateral margins of opisthosoma. Setae *ps1* filiform, length 6 (5–8), situated near lateral margins of opisthosomal lobes, anterior to bases of setae *h3*. Setae *c1* present, setae *h3* flattened and enlarged in basal part, shorter than *h2*. Dorsal measurements: *c2–d2* 68 (64–68), *d2–e2* 70 (72–78), *e2–h3* 42 (40–46), *d1–d2* 33 (31–34), *e1–e2* 24 (20–26), *h1–ps2* 10 (7–11), *h2–h2* 36 (34–36), *h3–h3* 20 (20–22), *ps2–ps2* 44 (46–48).

Epimerites I fused into a V, posterior end connected with epimerites II by transverse sclerotized bands. Epimerites II long, with posterior ends free. Coxal field I closed, coxal field II open, coxal fields III almost closed, coxal fields IV with narrow sclerotized areas at bases of trochanters IV. Epimerites IVa present, well developed, their anterior tips bearing bases of setae *4a* (Fig. 2). Genital arch 12 (8–10) long, 18 (14–18) wide,

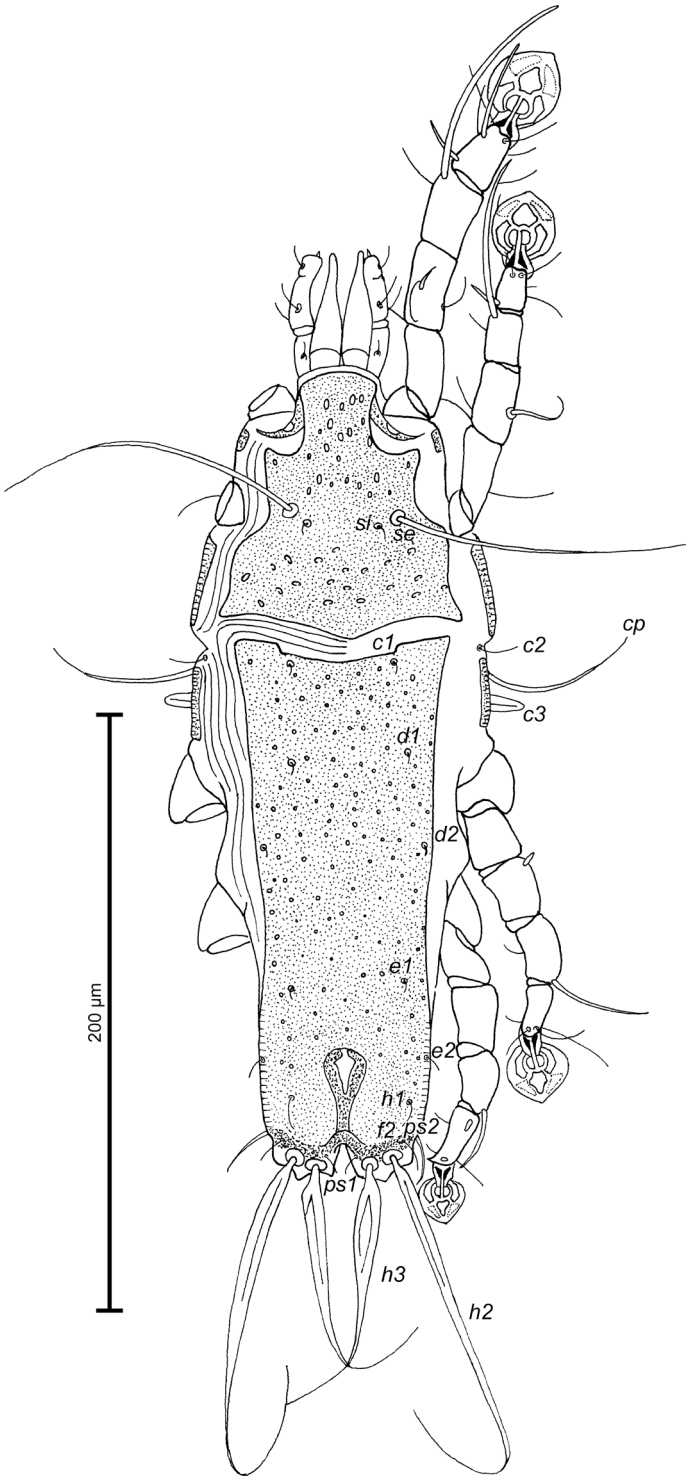

Figure 1. *Proterothrix chachulae* sp. n., male holotype: dorsal view of idiosoma.

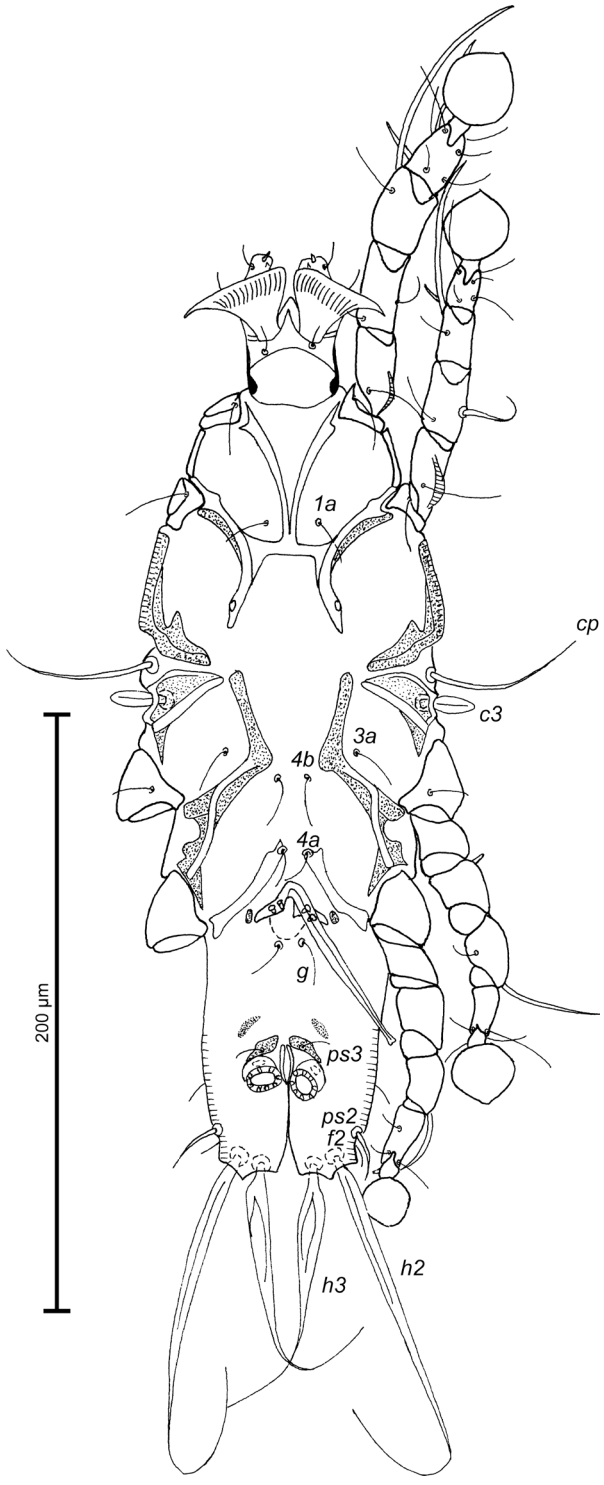

Figure 2. *Proterothrix chachulae* sp. n., male holotype: ventral view of idiosoma.

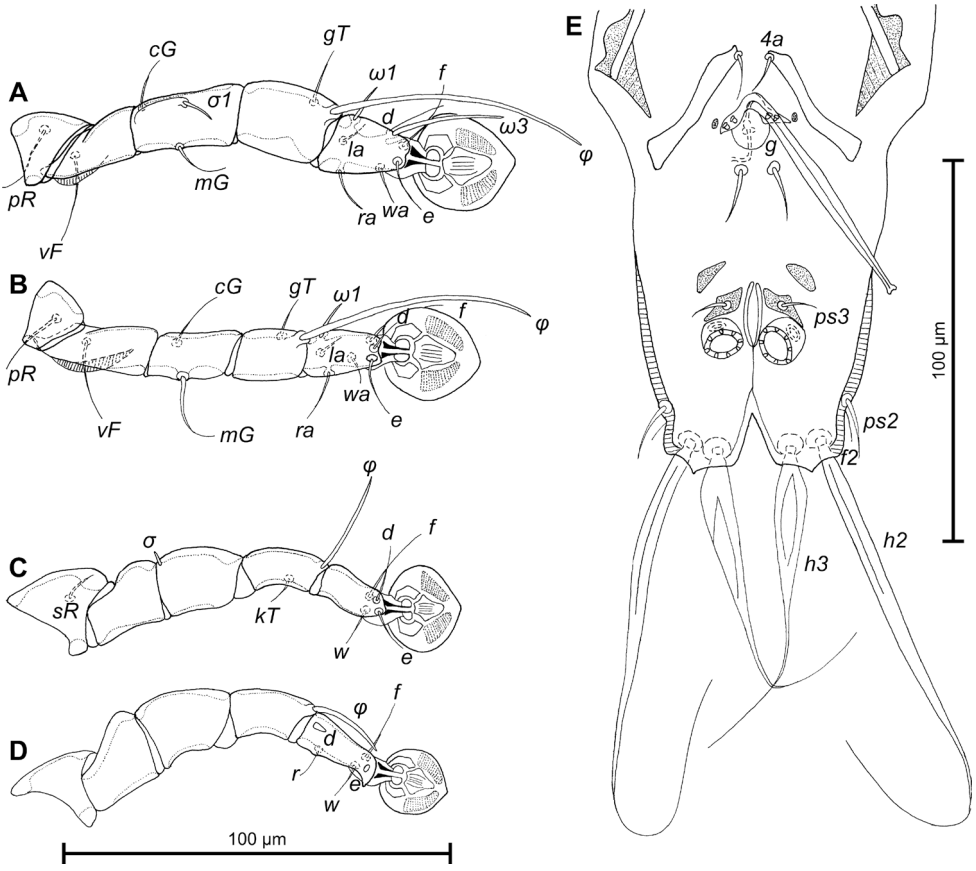

Figure 3. *Proterothrix chachulae* sp. n., **A–D** details of male legs, dorsal view: **A** leg I **B** leg II **C** leg III **D** leg IV **E** opisthosoma of male, ventral view.

basal sclerite of genital apparatus rounded posteriorly; aedeagus with bilobate tip 64 (64–69) long, extending to level of setae *ps3*. Genital papillae situated at level of genital arch. A pair of small ovoid sclerites located near tips of genital arch. Two pairs of adanal shields (median and anterolateral) represented by small triangular plates, setae *ps3* situated on median pairs. Anal suckers 12 (11–12) in diameter, corolla indented, with 8 small teeth. Ventral measurements: *3a–4b* 20 (18–22), *4b–4a* 30 (28–30), *4a–g* 30 (27–32), *g–ps3* 36 (37–40), *ps3–ps3* 17 (16–18), *ps3–h3* 36 (36–40).

Legs I longer than legs II, femora I and II with ventral crest (Fig. 3A, B). Seta *e* of tarsus I filiform. Setae *mG*II thickened basally, with filiform apex, setae *d* of tarsi II, III much shorter than corresponding setae *f*. Tarsus IV 23 (22–24) long, with apical claw-like process, setae *d, e* button-like, seta *d* bigger in diameter than *e*, situated in basal and apical parts of segment, respectively (Fig. 3D). Length of solenidia: *ω1*I 10 (10–14), *ω1*II 9 (8–10), *φ*I 56 (54–60), *φ*II 46 (42–44), *φ*III 25 (22–26), *φ*IV 22 (18–20).

FEMALE (Figs 4; 5; 6A–E; range for 6 paratypes): Pseudorutellar lobes with long and acute lateral extensions as in males. Length of idiosoma 392–396, width 125–132,

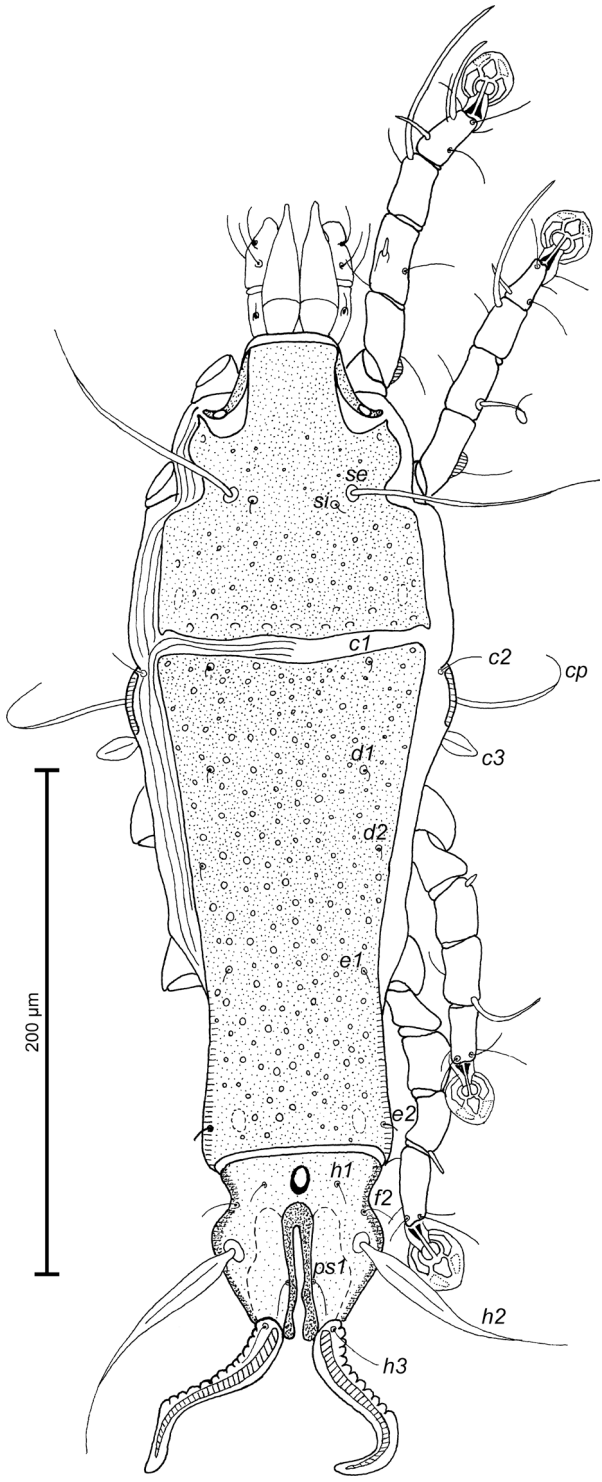

Figure 4. *Proterothrix chachulae* sp. n., female paratype: dorsal view of idiosoma.

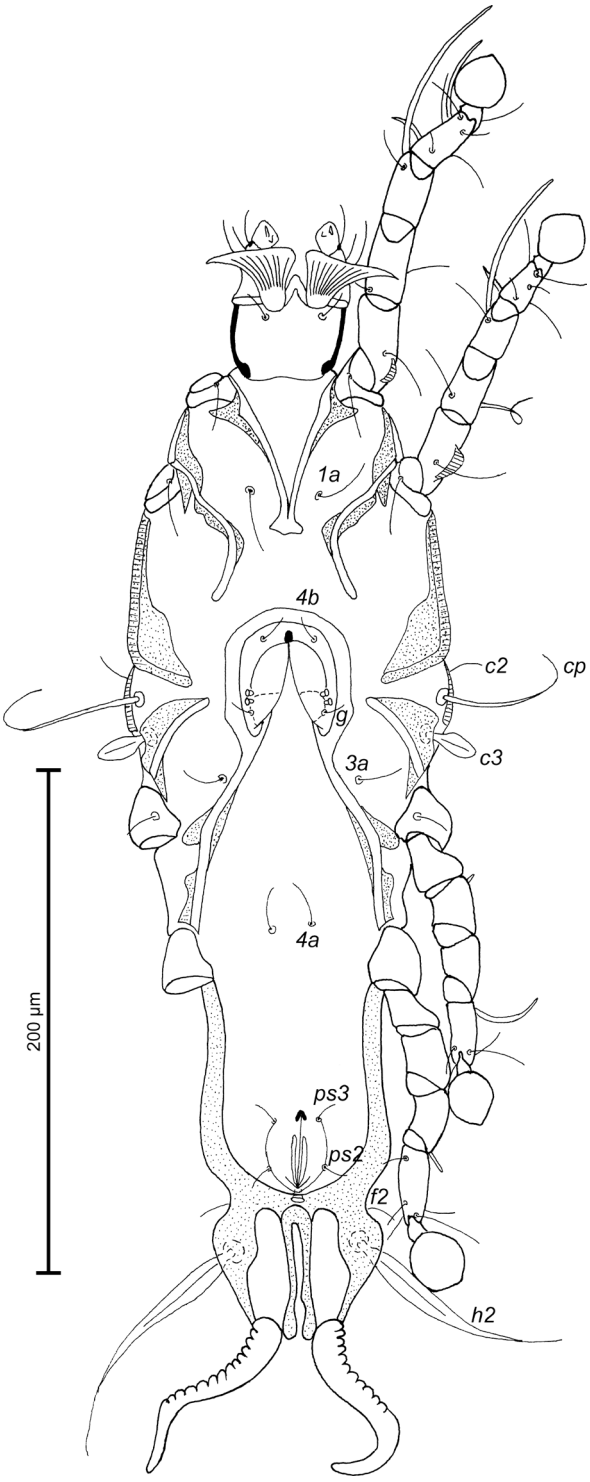

Figure 5. *Proterothrix chachulae* sp. n., female paratype: ventral view of idiosoma.

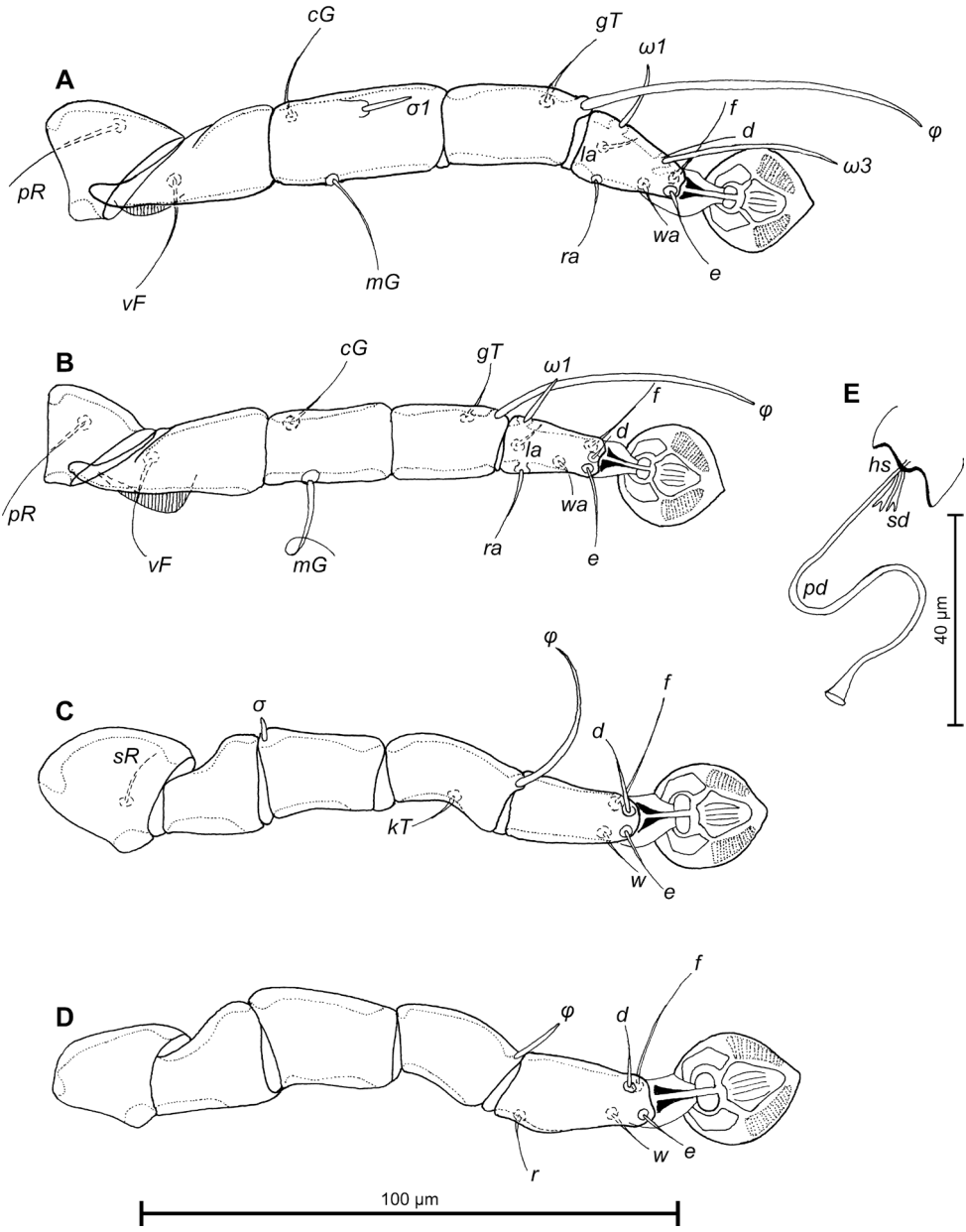

Figure 6. *Proterothrix chachulae* sp. n., **A–D** details of female legs, dorsal view: **A** leg I **B** leg II **C** leg III **D** leg IV **E** spermatheca and spermaducts of female. Abbreviations: hs–head of spermatheca; pd–primary spermaduct; sd–secondary spermaduct.

and length of hysterosoma 270–280. Prodorsal shield entire, anterolateral extensions with rounded tips, lateral margins without deep incisions, posterior margin almost straight, length 117–121, width 107–110, surface with small circular lacunae in an-

terior part and bigger ovate and circular lacunae in posterior part (Fig. 4). Scapular setae *se* separated by 43–46. Scapular shields narrow. Humeral shields narrow, separated from epimerites III. Setae *c2* filiform, situated dorsally on striated tegument. Subhumeral setae *c3* lanceolate, 18–22 × 6–8. Anterior hysteronotal shield roughly rectangular, anterior and posterior margins concave, greatest length 189–190, greatest width in anterior part 99–101, surface with sparsely disposed circular lacunae. Length of lobar region 74–79, width at level of setae *h2* 63– 65. Terminal cleft parallel-sided, narrow, with almost touching margins posterior to level of setae *ps1*, length 45–52. Supranal concavity well developed, ovoid. Setae *h1* on lobar shield, at midlevel of supranal concavity; surface of lobar shield without ornamentation. Setae *h2* spindle-shaped, with terminal filament, 73–81 × 6–8. Setae *ps1* closer to inner margin of opisthosomal lobes, setae *h3* 17–18 long, about 1/3 from the length of terminal appendages. Dorsal measurements: *c2–d2* 83–84, *d2–e2* 99–105, *e2–h2* 44–47, *h2–h3* 32–35, *d1–d2* 34–47, *e1–e2* 40–42, *h1–h2* 28–32, *h2–ps1* 26–30, *h1–h1* 29–31, *h2–h2* 49–51.

Epimerites I fused as a V, with short lateral extensions. Coxal fields I, II with small sclerotized areas, epimerites IVa absent (Fig. 5). Translobar apodemes of opisthosomal lobes present, fused to each other anterior to terminal cleft. Epigynum horseshoe-shaped, greatest width 49–53. Secondary spermaducts short, about 10 long (Fig. 6E). Distance between pseudanal setae: *ps2–ps2* 20–23, *ps3–ps3* 16–18, *ps2–ps3* 16–20.

Legs I slightly longer than legs II; femora I, II with wide ventral crest; setae *mG*II thickened basally, with filiform apex. Length of solenidia: *ω1*I 9–11, *ω1*II 6–9, *φ*I 59–67, *φ*II 46–48, *φ*III 30–35, *φ*IV 5–7 (Fig. 6A–D).

Etymology. This species is named in honor of Oana Mirela Chachula (a biologist, the National Museum of Romanian History, Romania), for her support of our research of ectoparasites of birds from Meghalaya (India).

Remarks. *Proterothrix chachulae* sp. n. belongs to the *wolffi* species group by having almost closed coxal fields III in males and parallel-sided terminal cleft in females. Males of the new species differ from all known males of the genus by having the aedeagus with bilobate tip. Among all species of the genus, *P. chachulae* is closely related to *P. cyornis* Mironov and Tolstenkov, 2013 from *Cyornis tickelliae* Blyth (Passeriformes: Muscicapidae) by having the pseudorutelar lobes with acute lateral extensions in both sexes. Males of both species have the opisthosomal lobes roughly trapezoidal and short, setae *h3* flattened and enlarged in basal part, a similar general proportions of aedeagus and epimerites IVa well developed, their anterior tips bearing bases of setae *4a*. *Proterothrix chachulae* differs from that species by the following features: in males, the prodorsal shield has posterior margin with wide blunt-angular extension, the opisthosomal lobes have angular median expansion on posterior margin, a pair of small ovoid shields is located at the tips of the genital arch, seta *d* is bigger in diameter then *e* on tarsus IV; in females, the prodorsal shield bears in anterior part a few small circular lacunae, the lobar shield is without ornamentation, and the setae *h3* have about 1/3 from the length of terminal appendages. In males of *P. cyornis*, the prodorsal shield has posterior margin slightly convex, the opisthosomal lobes have posterior margin with a small median invagination, small sclerites near the tips of genital arch are absent, seta *d* and *e* on tarsus

IV are subequal in diameter; in females, the prodorsal shield bears big ovate lacunae in anterior part, the lobar shield bears few circular lacunae in anterior part, and the setae *h3* have about 1/5 from the length of terminal appendages.

DNA barcode. *Representative DNA sequences*. GenBank accession numbers for molecular voucher: ANA747 P2 male KY594726; ANA748 P1 male KY594724; ANA749 P1 female KY594725.

We sequenced a 660-pb fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene for two male and one female paratypes. In the resulted alignment we identified 8 variable sites. Two haplotypes were identified: H1 (ANA_748_P1_M and ANA_749_P1_F) and H2 (ANA_747_P2_M).

Intraspecific genetic distance between the analyzed specimens using K2P model is 0.8% (SE 0.3). All observed nucleotide substitutions were synonymous and did not change the amino acid sequence.

This reported genetic distance in the nucleotide sequence of the DNA barcode is comparable with genetic distances found for other Analgoidea species like *Proctophyllodes cetti* (0.87%) (Badek et al. 2008).

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Taxonomic notes on the armored spiders of the families Pacullidae and Tetrablemmidae (Arachnida, Araneae) from Singapore

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Abstract

Eight species of armored spiders belonging to two families, Pacullidae Simon, 1894 and Tetrablemmidae O. Pickard-Cambridge, 1873, are reported from Singapore. Five species are documented as new to science: *Paculla bukittimahensis* Lin & Li, **sp. n.** (male and female), *P. globosa* Lin & Li, **sp. n.** (male and female), *Ablemma malacca* Lin & Li, **sp. n.** (male and female), *Singaporemma lenachanae* Lin & Li, **sp. n.** (male and female), and *Sulaimania brevis* Lin & Li, **sp. n.** (male). The three known species are *Brignoliella besutensis* Lin, Li & Jäger, 2012, *B. michaeli* Lehtinen, 1981, and *Singaporemma singulare* Shear, 1978, of which the female of *B. besutensis* is described for the first time. For comparison, types of *Singaporemma adjacens* Lehtinen, 1981 from Vietnam, *Singaporemma halongense* Lehtinen, 1981 from Vietnam, *Singaporemma singulare* from Singapore and *Sulaimania vigelandi* Lehtinen, 1981 from Malaysia are studied and photographed.

Keywords

Taxonomy, pacullids, tetrablemmids, morphology, southeast Asia

Introduction

Tetrablemmids and pacullids are collectively known as armored spiders because their abdomen is characteristically armor-plated with complicated abdominal scuta. Family placement of these haplogyne spiders has gone through a rather tortuous journey. Tetrablemmidae was first established by O. Pickard-Cambridge (1873) with *Tetrablemma* as its type genus. The family name Pacullidae was first used by Thorell (1898). He had taken the cue from Simon (1889, 1894) who had replaced the preoccupied name *Phaedima* in Phaedimonidae (Thorell 1881) with *Paculla,* and grouped it, along with *Perania* and *Tetrablemma* in Paculleae, but under the family Theridiidae. Roewer (1963) placed *Paculla*, *Tetrablemma*, and a few other tetrablemmids under another family, viz., Hadrotarsidae. The family placements of these armored spiders were negated when Levi and Levi (1962) and Levi (1968) transferred them out of Theridiidae and Hadrotarsidae respectively. Believing that they were monophyletic, Brignoli (1973) subsumed Pacullidae (*sensu* Thorell) under Tetrablemmidae. In an extensive survey of the armored spiders, Shear (1978) pointed out that some of the *Paculla* described by Simon, Roewer and Brignoli were not the *Paculla* (*sensu Phaedima*) as initially conceived by Thorell (1898). More significantly, while agreeing that Pacullidae and Tetrablemmidae were closely related, he argued that more study was needed before the two families were combined. Nevertheless, Lehtinen (1981) decided to incorporate Pacullidae, then with a sole genus *Paculla*, as a sub-family under Tetrablemmidae. More recently, however, based on target-gene analyses from extensive spider taxa, Wheeler et al. (2016) have restored the family status of Pacullidae and circumscribed Tetrablemmidae, with redefined diagnoses and composition. Here, their family placement in reporting the armored spiders from Singapore are adopted.

According to Murphy and Murphy (2000: 548, fig 59.1–3), Singapore was home to four species of armored spiders. They included *Singaporemma singulare* Shear, 1978, which was the type species of the genus named after Singapore. The remaining three armored spiders comprised an unidentified species of *Paculla* collected from Bukit Timah in Singapore, and two species previously described from Malaysia and Vietnam: *Brignoliella michaeli* Lehtinen, 1981 and *Singaporemma halongense* Lehtinen, 1981. Our current study suggests that the *Singaporemma* in Singapore comprise *S. singulare* and a second *Singaporemma* not identical to *S. halongense,* but a new species closely related to it. This study further suggests that the unidentified *Paculla* referred to by Murphy and Murphy (2000: 370, fig 59.1) is probably one of the two new species of *Paculla* described in this paper.

Altogether, this paper records a total of eight species of armored spiders in Singapore. They include two new species of *Paculla* (Pacullidae). Among the Tetrablemmidae described and re-described in this paper are *S. singulare*, along with a new species each of *Ablemma*, *Singaporemma* and *Sulaimania*. The presence of *Brignoliella besutensis* Lin, Li & Jäger, 2012 in Singapore was ascertained for the first time, while the presence of *B. michaeli* was revalidated.

Materials and methods

All specimens were collected from July to August 2015 from various locations in Singapore by sifting leaf litter. Specimens were preserved in 95% ethanol. They were examined and measured under a Leica M205 C stereomicroscope. Further details were studied under an Olympus BX43 compound microscope. Vulvae were removed and treated in lactic acid. To reveal the course of the spermatic duct, the palpal bulbs were treated in lactic acid and mounted in Hoyer's Solution. Photographs were taken with a Canon EOS 60D wide zoom digital camera (8.5 megapixels). The images were combined using Helicon Focus 3.10.3 software (Khmelik et al. 2006).

All measurements are in millimetres. Height of carapace is measured with tubercle. Leg measurements are given in the following sequence: total length (femur, patella, tibia, metatarsus, and tarsus). Abbreviations in figures are as follows: **A** – anal plate; **ALG** – anterolateral groove of preanal plate; **AT** – atrium; **AV** – anterior ventrolateral plate; **EF** – epigynal fold; **EP** – epigynal pit; **ep** – embolic part of apes of palpal organ; **IVP** – inner vulval plate; **L** – lateral plate; **LH** – lateral horn; **MV** – median ventrolateral plate; **MVB** – bridge fragments of MV; **P** – pulmonary plate; **PA** – preanal plate; **PG** – postgenital plate; **PLC** – posterolateral corner of PA; **PMC** – posteromedial corner of PA; **PV** – posterior ventrolateral plate; **sd** – spermatic duct; **sl** – subterminal lamella; **SR** – seminal receptaculum; **VD** – vulval duct; **VS** – vulval stem. Abbreviations in text include: **AER** – anterior eye row; **ALE** – anterior lateral eye; **AME** – anterior median eye; **PLE** – posterior lateral eye. References to figures in the cited papers are listed in lowercase (fig. or figs); figures from this paper are noted with an initial capital (Fig. or Figs).

All types of the new species are deposited in the Lee Kong Chian Natural History Museum, National University of Singapore (**LKCNHM**). Other material used in the current work are deposited in the Natural History Museum of the Sichuan University (**NHMSU**) in Chengdu, China; the Zoological Museum of the University of Turku (**ZMUT**) in Turku, Finland and the American Museum of Natural History in New York, USA (**AMNH**).

Taxonomy

Family Pacullidae Simon, 1894

Genus *Paculla* Simon, 1887

Type species. *Phaedima granulosa* Thorell, 1881 from New Guinea (see Lehtinen 1981).

***Paculla bukittimahensis* Lin & Li, sp. n.**
http://zoobank.org/E95B9FE6-2604-4D4C-BC86-C5E701064012
Figs 1, 2, 3

Type material. Holotype ♂ (LKCNHM), SINGAPORE: Bukit Timah Nature Reserve, Catchment Path, altitude 107 m, 1°21'12.5"N, 103°46'50.6"E, 20 August 2015, S. Li and Y. Tong leg. **Paratypes** 1♂ and 5♀ (LKCNHM), same data as holotype.

Other material examined. 1♂ and 3♀ (NHMSU), SINGAPORE: Bukit Timah Nature Reserve, Catchment Path, altitude 107 m, 1°21'12.5"N, 103°46'50.6"E, 20 August 2015, S. Li and Y. Tong leg.

Etymology. The specific name refers to the type locality; adjective.

Diagnosis. This new species can be distinguished from all congeners with the exception of *P. mului* Bourne, 1981 and *P. wanlessi* Bourne, 1981 by the wide, short embolus (Fig. 2A, C–D), the well-developed postgenital scutum (Fig. 1G) and the nearly rectangular atrium (Fig. 3B). It differs from *P. mului* (see Bourne, 1981: 220, figs 11–17) by the normal male femur I lacking a subdistal-ventral process, the longer, particularly furcated embolus (Fig. 2A, C–D) and the presence of three disjunct bridge fragments of MV (Fig. 3B); and from *P. wanlessi* (see Bourne, 1981: 217, figs 1–10) by the larger bulb (Fig. 2C–D), the more pointed embolus (Fig. 2A–B), the nearly trapeziform preanal scutum (Figs 1G, 3B) and the triangular median ventrolateral plate (Figs 1G, 3A–B). It can be separated from *P. globosa* sp. n. (Figs 5A–D, 6A–B) by the wider, shorter embolus (Fig. 2A–B), the slightly compressed bulb (Fig. 2C–D), and the three shorter, disjunctive bridge fragments of MV (Fig. 3B) and the nearly rectangular atrium (Fig. 3B).

Description. Male (holotype). Coloration: body dark reddish brown; legs reddish-brown.

Measurements: total length 4.05; carapace 1.80 long, 1.35 wide, 1.12 high; abdomen 2.35 long, 1.65 wide, 1.78 high; clypeus 0.45 high; sternum 1.05 long, 0.90 wide. Length of legs: I 7.01 (2.12, 0.53, 2.00, 1.52, 0.84); II 5.85 (1.81, 0.50, 1.54, 1.25, 0.75); III 4.82 (1.45, 0.45, 1.20, 1.10, 0.62); IV 6.64 (2.00, 0.51, 1.83, 1.62, 0.68).

Prosoma (Fig. 1A–B, E): carapace finely granulated, margin rugose, covered with thin setae; eyes white, ALE>AME=PLE; cephalic part moderately raised; cervical groove distinct; clypeus vertical anteriorly; labium triangular, distally obtuse; sternum rough, marginally rugose, posterior corner protruded. Legs: cuticle striated, weakly granular.

Opisthosoma (Fig. 1A–B, E): dorsal scutum long, oval, smooth, modified by tiny pits, covered with thin setae; ventral scutum rugose; lateral scutum I short, perigenital scutum triangular, postepigastral scutum same width as preanal scutum.

Palp (Fig. 2A–D): femoral cuticle slightly striated, approximately 2.5 times as long as patella; patella proximally narrow, distally wide; tibia large, swollen, 1.5 times as wide as femur; cymbium compressed, distally bifurcate; bulb tomato-shaped, surface smooth (Fig. 2C–D); embolus long, proximally sclerotized, distally rugose membranous, starting from subdistal-ventral 1/3 position of bulbous surface, and curved downwards; embolic tip flexuous, and asymmetric split ends (Fig. 2A–B).

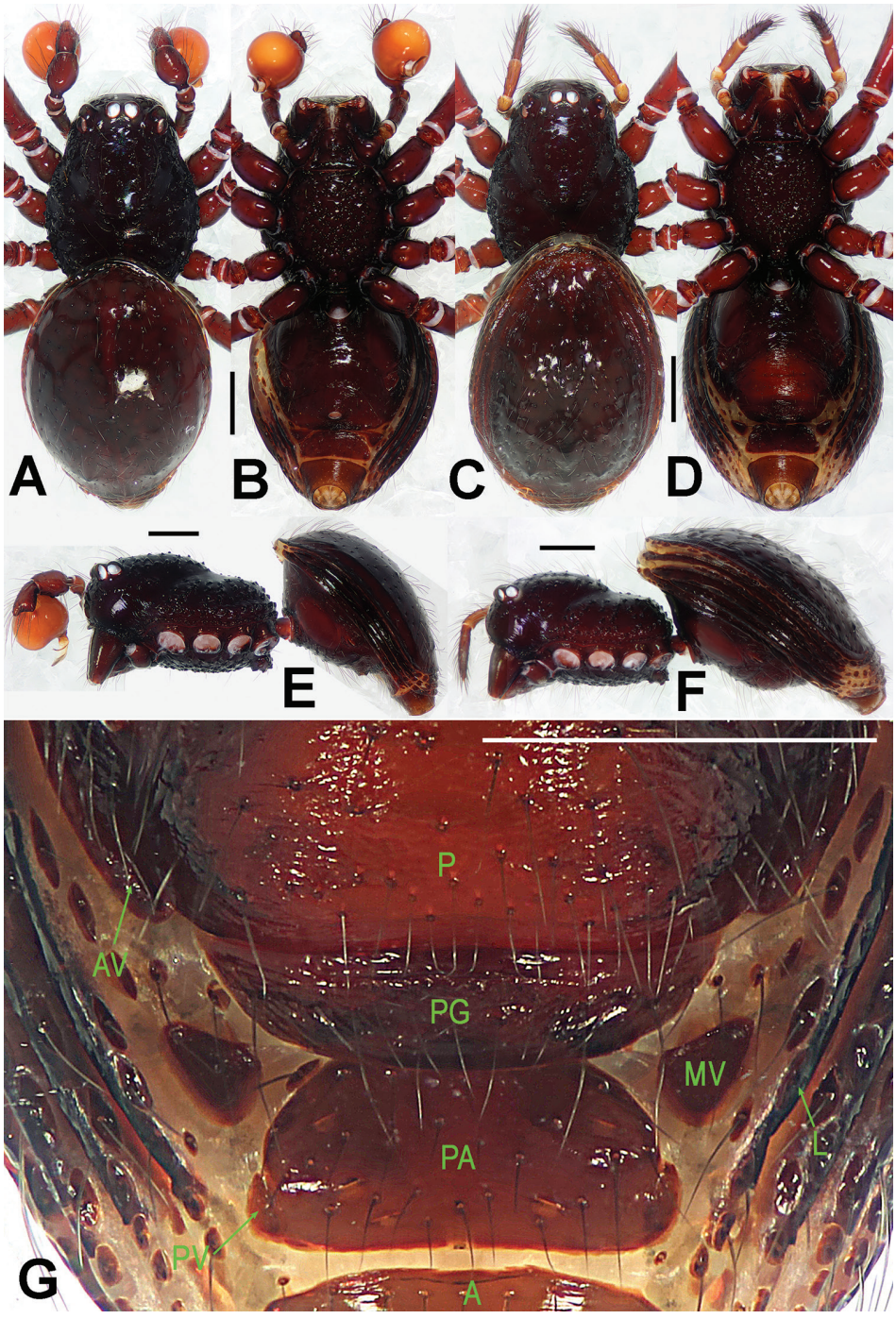

Figure 1. *Paculla bukittimahensis* sp. n., male holotype (**A–B, E**) and female paratype (**C–D, F–G**). **A–F** habitus **G** genital area (untreated). **A, C** dorsal **B, D, G** ventral **E–F** lateral. Abbreviations: A = anal plate; AV = anterior ventrolateral plate; L = lateral plate; P = pulmonary plate; PA = preanal plate; PG = postgenital plate; PV = posterior ventrolateral plate. Scale bars: 0.50 mm.

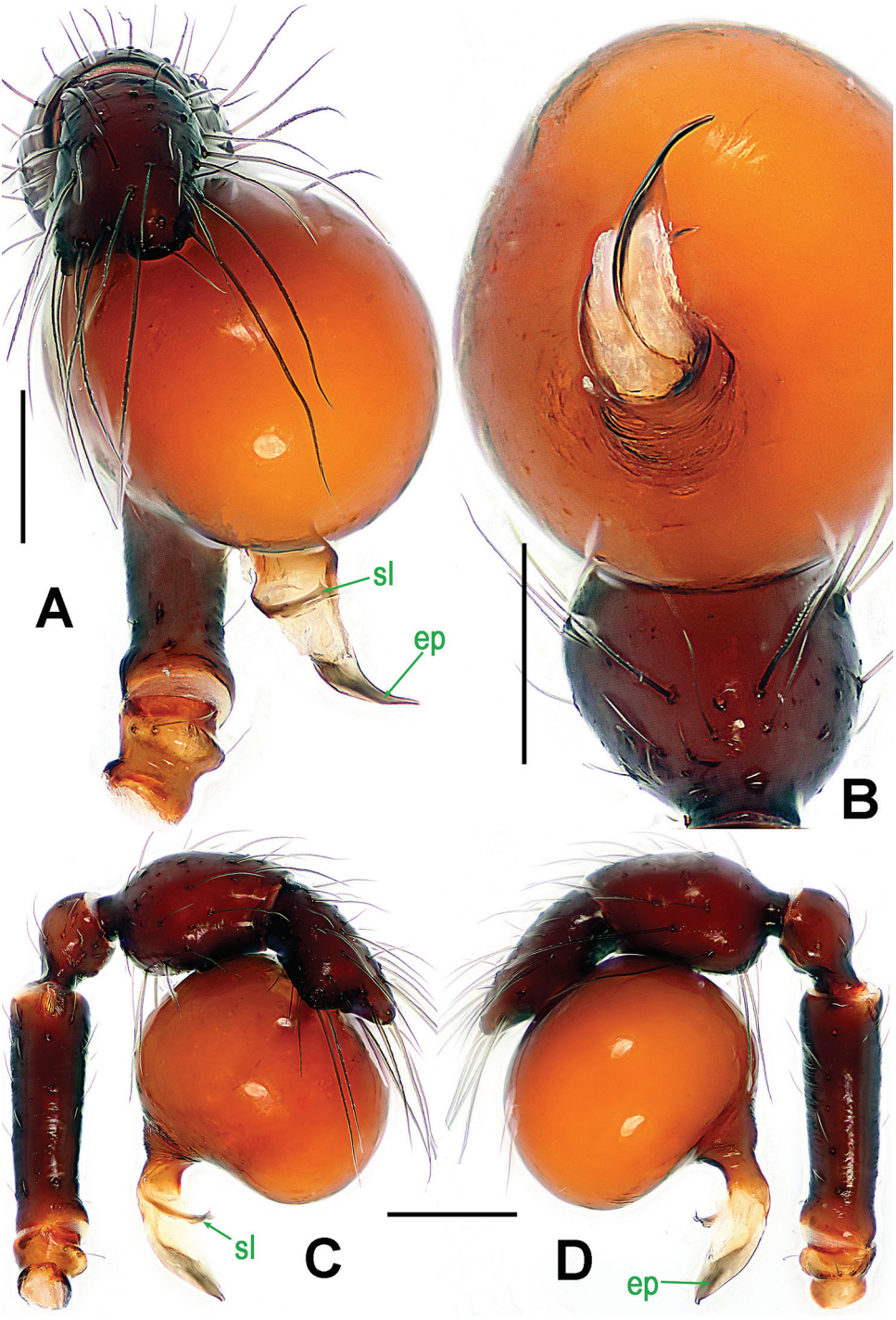

Figure 2. *Paculla bukittimahensis* sp. n., male holotype. **A, C–D** left palp **B** palpal bulb. **A** anterior **B** ventral **C** prolateral **D** retrolateral. Abbreviations: ep = embolic part of apes of palpal organ; sl = subterminal lamella. Scale bars: 0.20 mm.

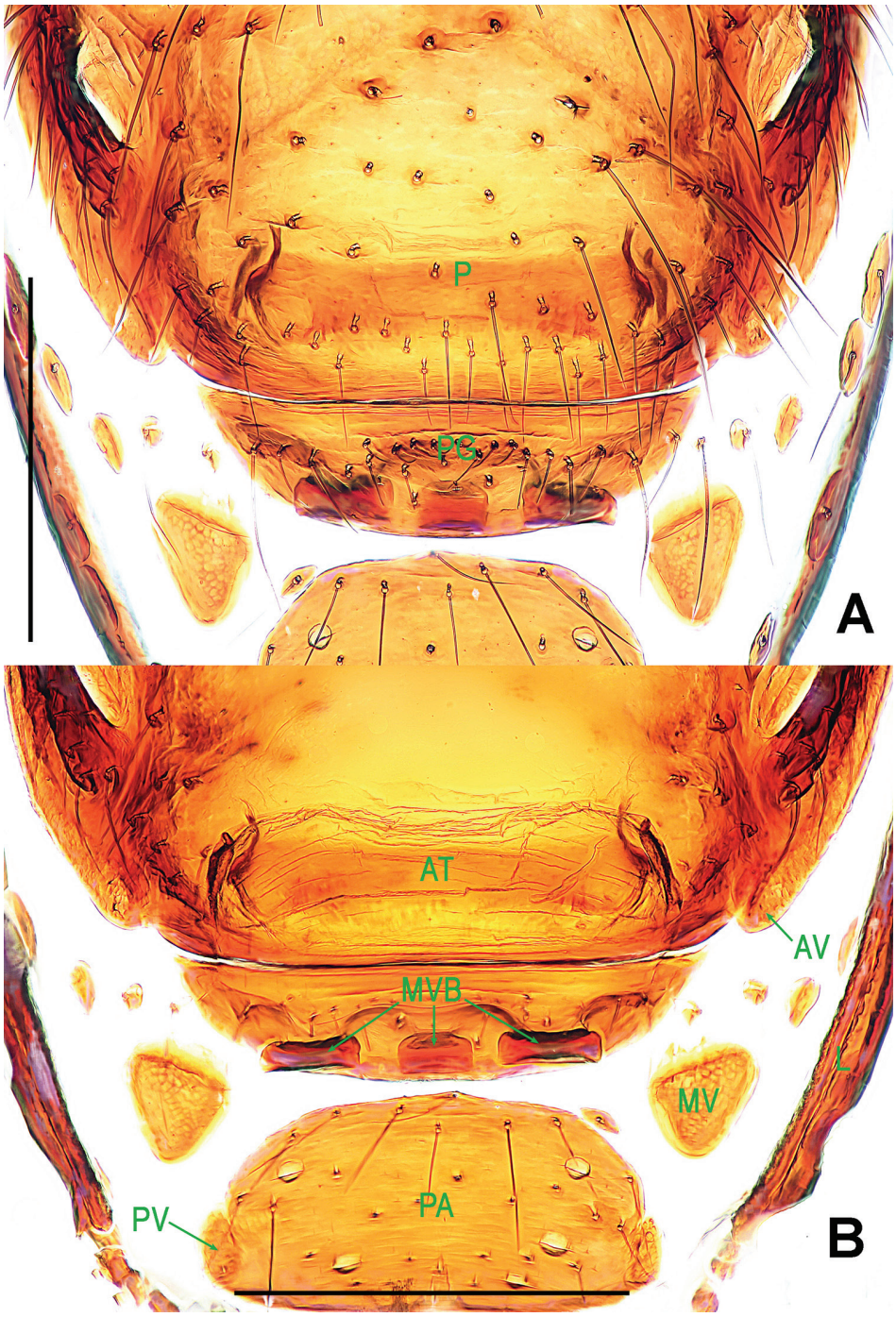

Figure 3. *Paculla bukittimahensis* sp. n., female paratype. **A** genital area (lactic acid-treated), ventral **B** *ditto*, dorsal. Abbreviations: AT = atrium; AV = anterior ventrolateral plate; MV = median ventrolateral plate; MVB = bridge fragments of MV; P = pulmonary plate; PA = preanal plate; PG = postgenital plate; PV = posterior ventrolateral plate. Scale bars: 0.50 mm.

Female (one of paratypes). Coloration as in male.

Measurements: total length 4.42; carapace 1.85 long, 1.40 wide, 1.13 high; abdomen 2.85 long, 1.85 wide, 2.03 high; clypeus 0.43 high; sternum 1.10 long, 0.97 wide. Length of legs: I 7.37 (2.27, 0.55, 2.10, 1.60, 0.85); II 6.11 (1.90, 0.50, 1.60, 1.38, 0.73); III 5.19 (1.55, 0.49, 1.30, 1.20, 0.65); IV 7.19 (2.15, 0.52, 2.00, 1.77, 0.75). Length of palp: 1.62 (0.48, 0.21, 0.35, 0.58).

Carapace, abdomen, and legs as in male (Fig. 1C–D, F); clypeus slightly lower than in male.

Genitalia (Figs 1G; 3A–B): epigynal area strongly sclerotized (Fig. 1G); postgenital scutum wider than preanal scutum; median ventrolateral plate triangular (Fig. 3A). Vulva with a large, nearly rectangular atrium; three bridge fragments of MV disjunctive, the medial shorter than the laterals, below the atrium, and closed to the dorsoposterior margin of postgenital scutum (Fig. 3B).

Distribution. Singapore.

***Paculla globosa* Lin & Li, sp. n.**

http://zoobank.org/CAD62F40-4F41-4D91-8E0E-7E9AAAA1632C

Figs 4, 5, 6

Type material. Holotype ♂ (LKCNHM), SINGAPORE: Bukit Timah Nature Reserve, Jungle Fall Stream, altitude 118 m, 1°21'25.4"N, 103°46'25.3"E, 21 August 2015, S. Li and Y. Tong leg. **Paratype** 1♀ (LKCNHM), SINGAPORE: Bukit Timah Nature Reserve, Bukit Timah Summit, altitude 163 m, 1°21'16.7"N, 103°46'35.0"E, 19 August 2015, S. Li and Y. Tong leg.

Etymology. The specific epithet derives from the Latin word "*globosus*" = globular, drawing attention to the shape of palpal bulb; adjective.

Diagnosis. This new species can be distinguished from all congeners with the exception of *P. sulaimani* Lehtinen, 1981 by the globular bulb (Fig. 5C–D), the long, finger-like embolus (Fig. 5A–D) and the presence of three bridge fragments of MV (Fig. 6B). Its male differs from *P. sulaimani* (see Lehtinen, 1981: 18, fig. 13) by the narrower embolus with weakly lobed end (Fig. 5A, C–D). The female differs from *P. bukittimahensis* sp. n. (Fig. 3B) by the anterior median part of the atrium protruding broadly and the larger, bridge fragments of MV almost touching each other (Fig. 6B).

Description. Male (holotype). Coloration: body dark reddish brown; legs reddish-brown.

Measurements: total length 4.40; carapace 1.95 long, 1.40 wide, 1.20 high; abdomen 2.50 long, 1.90 wide, 2.30 high; clypeus 0.49 high; sternum 1.30 long, 1.00 wide. Length of legs: I 8.28 (2.53, 0.65, 2.35, 1.75, 1.00); II 6.95 (2.10, 0.60, 1.85, 1.50, 0.90); III 5.75 (1.70, 0.54, 1.41, 1.30, 0.80); IV 8.16 (2.50, 0.62, 2.30, 1.91, 0.83).

Prosoma (Fig. 4A–B, E): carapace low, sparsely granulated over whole surface, but surface between the granules smooth; eyes white, ALE=AME=PLE; cephalic part moderately raised; cervical groove distinct; thoracic fovea shallow; clypeus vertical an-

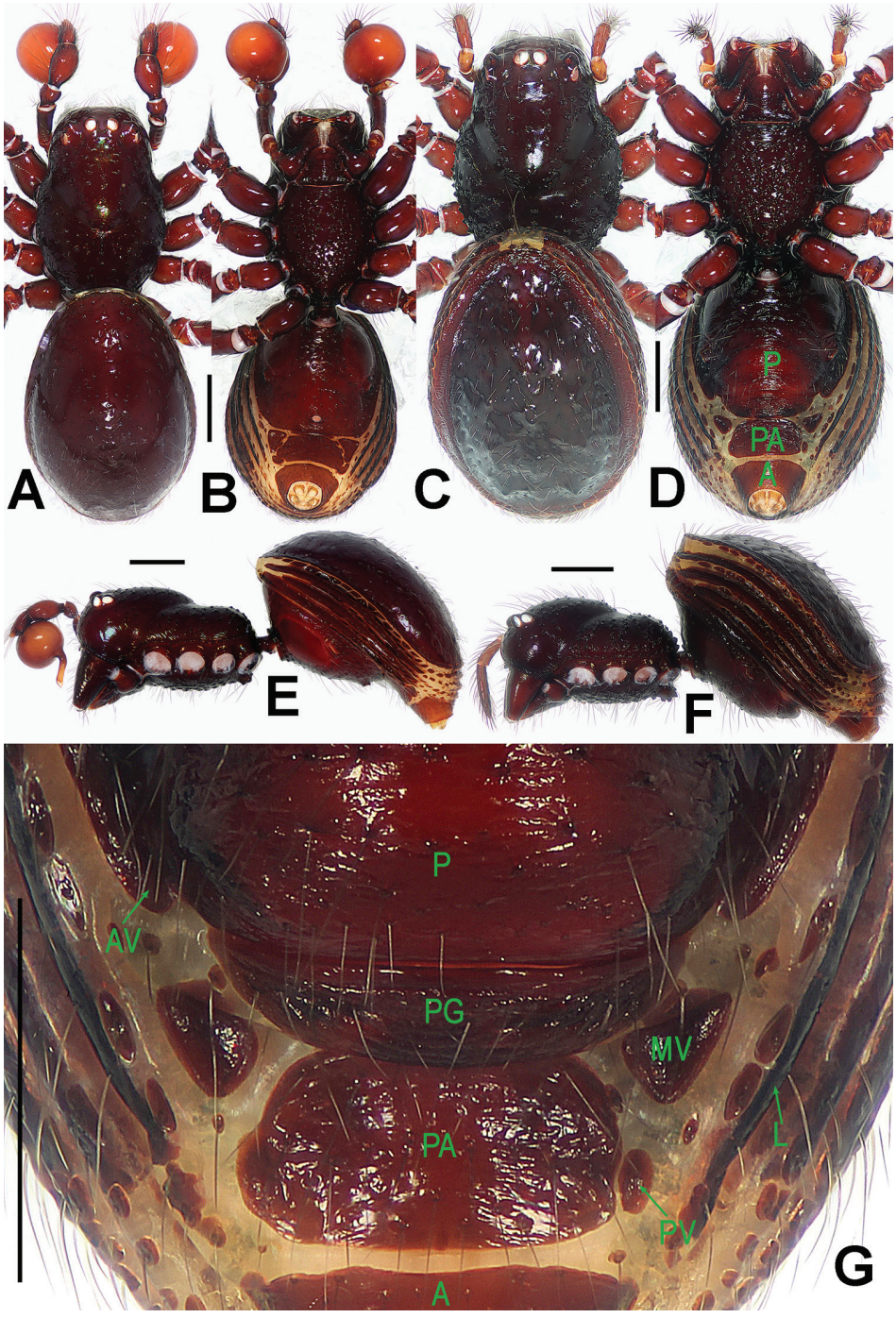

Figure 4. *Paculla globosa* sp. n., male holotype (**A–B, E**) and female paratype (**C–D, F–G**). **A–F** habitus **G** genital area (untreated). **A, C** dorsal **B, D, G** ventral **E–F** lateral. Abbreviations: A = anal plate; AV = anterior ventrolateral plate; L = lateral plate; MV = median ventrolateral plate; P = pulmonary plate; PA = preanal plate; PG = postgenital plate. Scale bars: 0.50 mm.

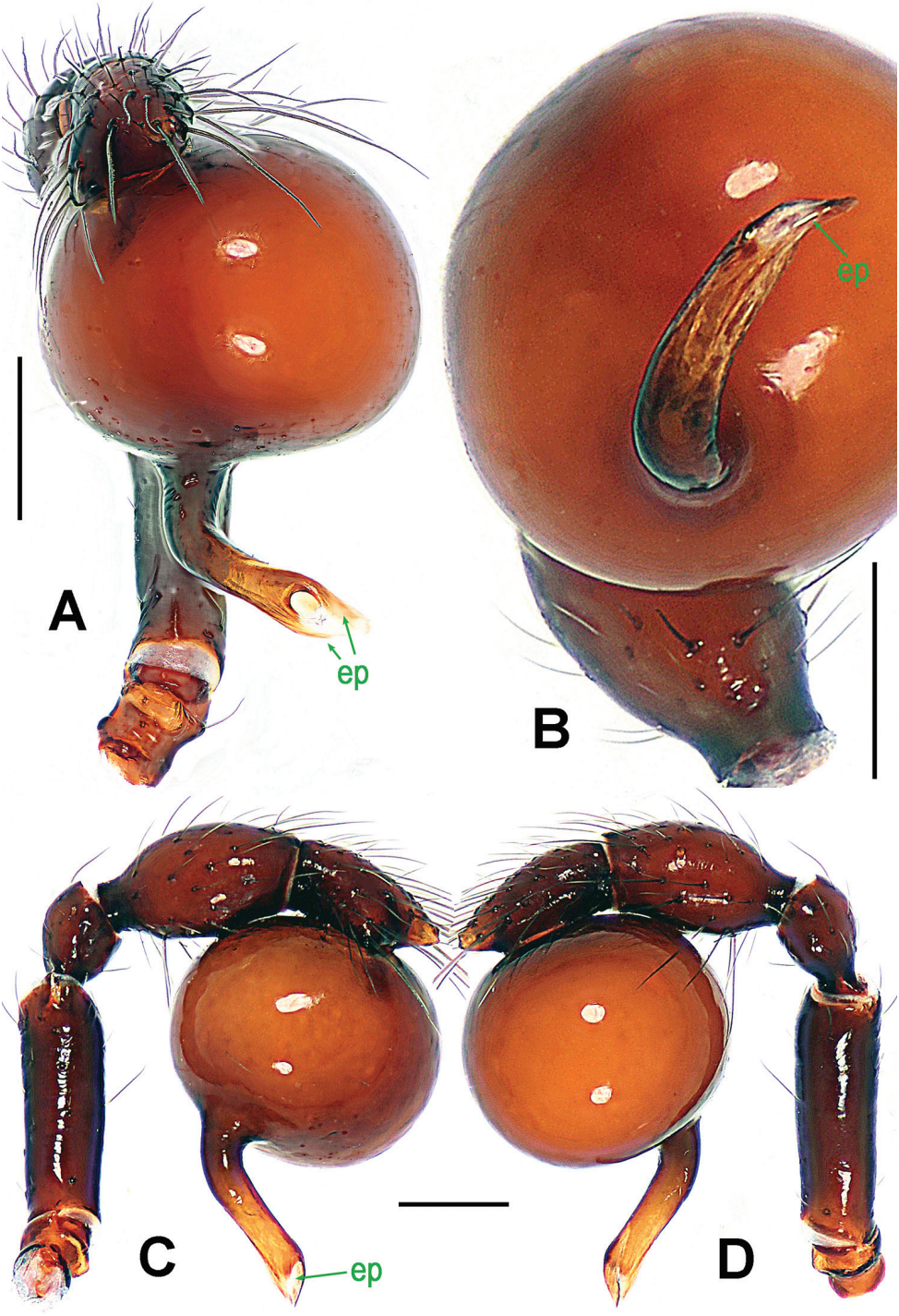

Figure 5. *Paculla globosa* sp. n., male holotype. **A, C–D** left palp **B** palpal bulb. **A** anterior **B** ventral **C** prolateral **D** retrolateral. Abbreviations: ep = embolic part of apes of palpal organ. Scale bars: 0.20 mm.

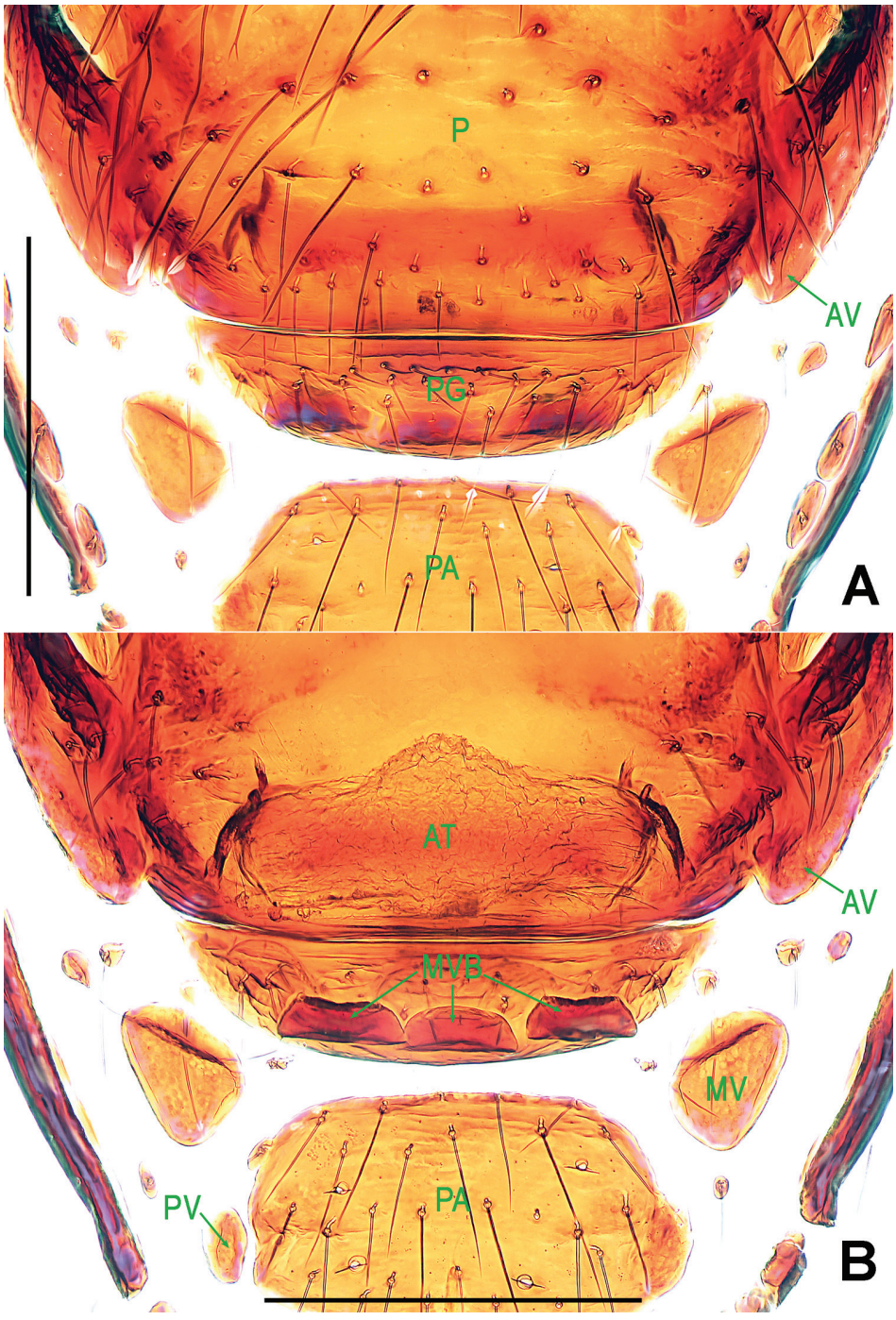

Figure 6. *Paculla globosa* sp. n., female paratype. **A** genital area (lactic acid-treated), ventral **B** *ditto*, dorsal. Abbreviations: AT = atrium; AV = anterior ventrolateral plate; MV = median ventrolateral plate; MVB = bridge fragments of MV; P = pulmonary plate; PA = preanal plate; PG = postgenital plate; PV = posterior ventrolateral plate. Scale bars: 0.50 mm.

teriorly; labium triangular, distally obtuse; sternum rough, covered with thin setae, posterior corner protruded. Legs: cuticle striated, weakly granular.

Opisthosoma (Fig. 4A–B, E): dorsal scutum long, oval, covered with thin setae, margin rugose, center modified by sparse pits; rows of small sclerites between lateral scuta, lateral scutum I short; ventral scutum smooth, margin rugose; perigenital scutum broad, triangular.

Palp (Fig. 5A–D): femoral cuticle smooth, approximately two times as long as patella; patella proximally narrow, distally wide; tibia broad, swollen, 1.3 times as wide as femur; cymbium compressed, slightly shorter than tibia, distally obtuse bifurcate; bulb globular, surface smooth (Fig. 5C–D); embolus long, clavate, starting from subdistal-ventral 1/3 position of bulbous surface (Fig. 5B), proximally sclerotized and bent, distally shallow furcated; embolic tip faint, with split ends (Fig. 5A).

Female (paratype). Coloration as in male.

Measurements: total length 4.50; carapace 1.85 long, 1.45 wide, 1.20 high; abdomen 2.67 long, 1.80 wide, 2.21 high; clypeus 0.43 high; sternum 1.14 long, 0.97 wide. Length of legs: I 7.35 (2.25, 0.55, 2.10, 1.65, 0.80); II 6.23 (1.95, 0.53, 1.65, 1.35, 0.75); III 5.22 (1.55, 0.50, 1.30, 1.22, 0.65); IV 7.20 (2.15, 0.54, 2.00, 1.80, 0.71). Length of palp: 1.58 (0.45, 0.21, 0.34, 0.38).

Carapace, abdomen, and legs as in male (Fig. 4C–D, F); clypeus slightly lower than in male.

Genitalia (Figs 4G; 6A–B): epigynal area strongly sclerotized (Fig. 4G); postgenital scutum rugose, wider than preanal scutum; median ventrolateral plate triangular; preanal scutum rugose, wider than long (Fig. 6A). Vulva with a large, nearly rectangular atrium, laterally rugose, and protruding anteromedially; three adjacent, equal sizes bridge fragments of MV below the atrium, and close to the dorso-posterior margin of postgenital scutum (Fig. 6B).

Distribution. Singapore.

Family Tetrablemmidae O. Pickard-Cambridge, 1873

Genus *Ablemma* Roewer, 1963

Type species. *Ablemma baso* Roewer, 1963 from Sumatra (see Lehtinen 1981).

Ablemma malacca Lin & Li, sp. n.

http://zoobank.org/4404C598-0F48-44E6-A95D-8B8A1D05DFA8
Figs 7, 8, 9

Type material. Holotype ♂ (LKCNHM), SINGAPORE: Central Catchment Nature Reserve, altitude 60 m, 1°21'21.7"N, 103°48'3.8"E, 26 August 2015, S. Li and Y. Tong leg. **Paratypes** 1♂ and 2♀ (LKCNHM), same data as holotype.

Other material examined. 1♂ and 1♀ (NHMSU), SINGAPORE: Central Catchment Nature Reserve, altitude 60 m, 1°21'21.7"N, 103°48'3.8"E, 26 August 2015, S. Li and Y. Tong leg.

Etymology. The specific epithet refers to the Strait of Malacca, which separates Singapore from the Indonesian island of Sumatra; noun.

Diagnosis. This new species can be distinguished from all its congeners with the exception of *A. datahu* Lehtinen, 1981, *A. makiling* Lehtinen, 1981, *A. shimojanai* (Komatsu, 1968), *A. singalang* Lehtinen, 1981, and *A. unicornis* Burger, 2008 by the absence of a long, pointed tooth on male cephalic area posteriorly (Fig. 7E), the elongated palpal bulb (Fig. 8B–C), and by the long, claviform inner vulval plate (Fig. 9C). It differs from *A. datahu* (see Lehtinen, 1981: 48, figs 176, 190, 196) and *A. makiling* (see Lehtinen, 1981: 50, figs 192, 187) by the trifurcated embolic end (Fig. 8C) and the narrower, longer and straighter inner vulval plate (Fig. 9C); from *A. shimojanai* (see Shear, 1978: 32, figs 82–87) by the lack of cheliceral horn and posteriorly cephalic process in male (Fig. 7E, G), the larger embolus (Fig. 8B–C), the inner vulval plate with a bent end and the stouter lateral horns (Fig. 9C); from *A. singalang* (see Lehtinen, 1981: 48, fig. 170) by the shorter, trifurcated embolus (Fig. 8B–C); and from *A. unicornis* (see Burger, 2008b: 253, figs 1, 2, 5, 11, 13) by the higher cephalic process in male ocular area (Fig. 7E), the six eyes in both sexes (Fig. 7A, C), the longer embolus (Fig. 8B–C) and the narrower base of inner vulval plate (Fig. 9C).

Description. Male (holotype). Coloration: body brownish-yellow; legs yellowish-orange.

Measurements: total length 1.08; carapace 0.49 long, 0.44 wide, 0.47 high; abdomen 0.66 long, 0.50 wide, 0.40 high; clypeus 0.24 high; sternum 0.31 long, 0.30 wide. Length of legs: I 1.14 (0.39, 0.13, 0.28, 0.16, 0.18); II 1.01 (0.33, 0.12, 0.23, 0.16, 0.17); III 0.88 (0.28, 0.11, 0.18, 0.17, 0.14); IV 1.19 (0.37, 0.13, 0.30, 0.22, 0.17).

Prosoma (Fig. 7A–B, E, G): carapace finely reticulated, margin rugose; eyes white, ALE>PME>PLE, ocular area protruded (Fig. 7E, G); clypeus very high, sloping forward, marginally rounded; cephalic part raised, flat top; thoracic part radial furrow distinctly smooth; chelicerae robust, with a small basal tuber and an anterodistal tooth (Fig. 7E), cheliceral lamina well developed; labium triangular, distally blunt; sternum finely reticulated, with sparse setae. Legs: cuticle striated; femur I slightly swollen, tibia I with a distal-laterally ventral tuber (Fig. 8A).

Opisthosoma (Fig. 7A–B, G): dorsal scutum oval, dimpled with tiny pits, smooth between the pits, covered with sparse setae; ventral scutum rugose; perigenital scutum absent; postepigastral scutum straight, nearly same width as preanal scutum; preanal scutum rectangular, with thick posterolateral corners.

Palp (Fig. 8B–C): femoral cuticle sculptured and ventrally granulated, approximately 2 times longer than patella; tibia not swollen, with a distally dorsal trichobothrium; cymbium small, cup-shaped; bulb long pear-shaped, its surface smooth; spermatic duct basally broad, distally narrow; embolus short, foot-shaped, distally strongly sclerotized, and forming a trifurcated terminal.

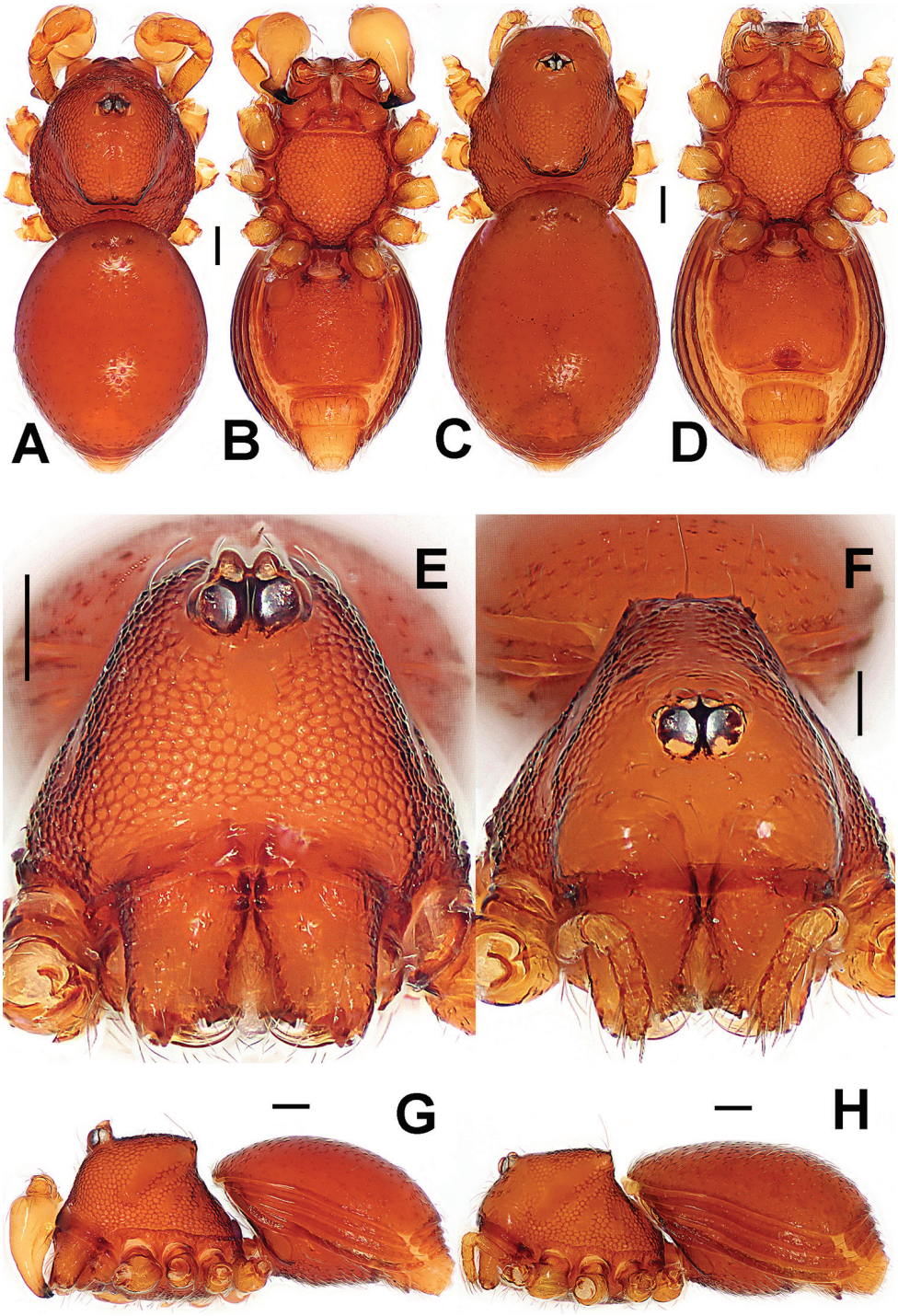

Figure 7. *Ablemma malacca* sp. n., male holotype (**A–B, E, G**) and female paratype (**C–D, F, H**). **A–D, G–H** habitus **E–F** prosoma. **A, C** dorsal **B, D** ventral **E–F** anterior **G–H** lateral. Scale bars: 0.10 mm.

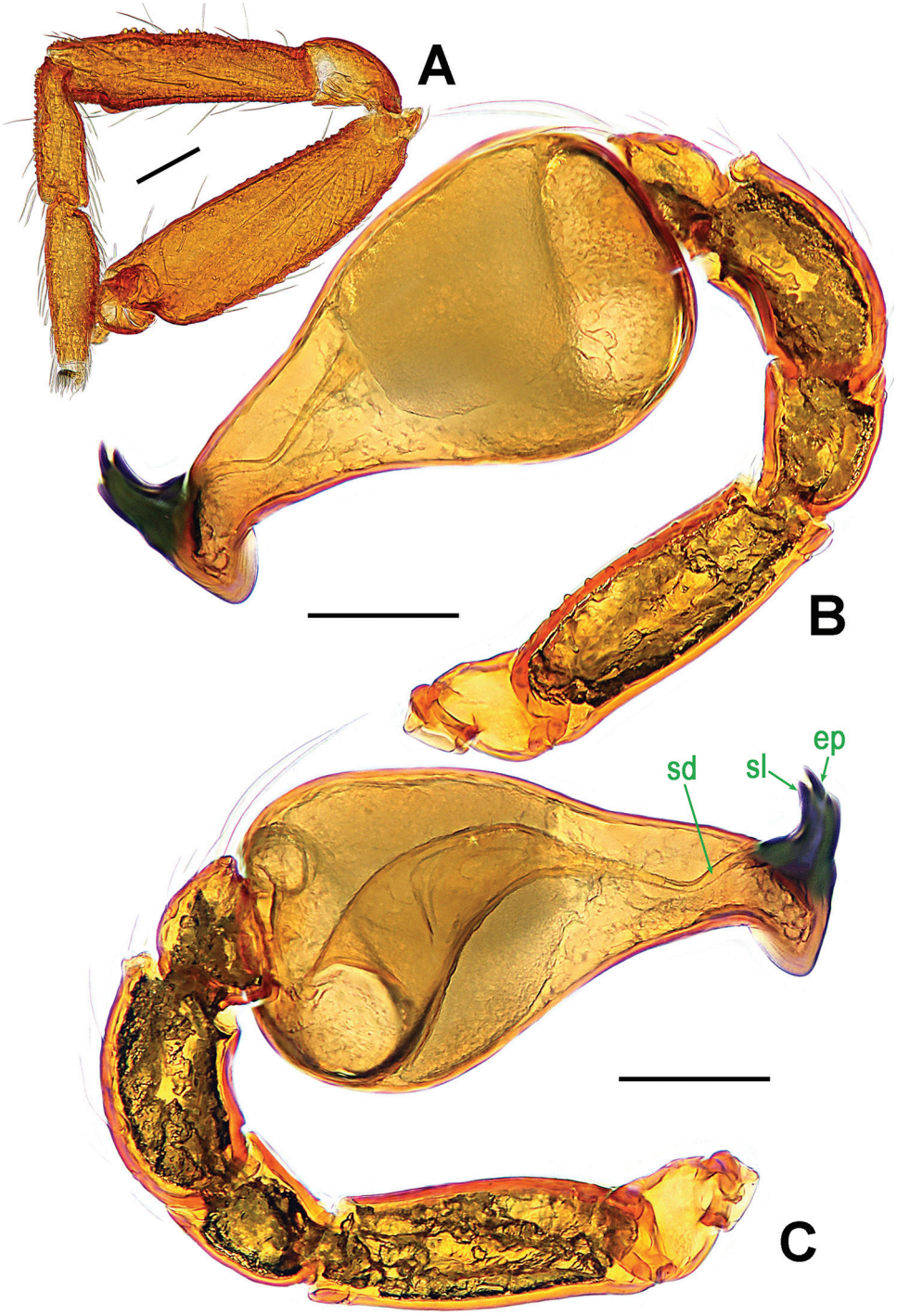

Figure 8. *Ablemma malacca* sp. n., male holotype. **A** left leg I, retrolateral **B** left palp, retrolateral **C** *ditto*, prolateral. Abbreviations: ep = embolic part of apes of palpal organ; sd = spermatic duct; sl = subterminal lamella. Scale bars: 0.10 mm.

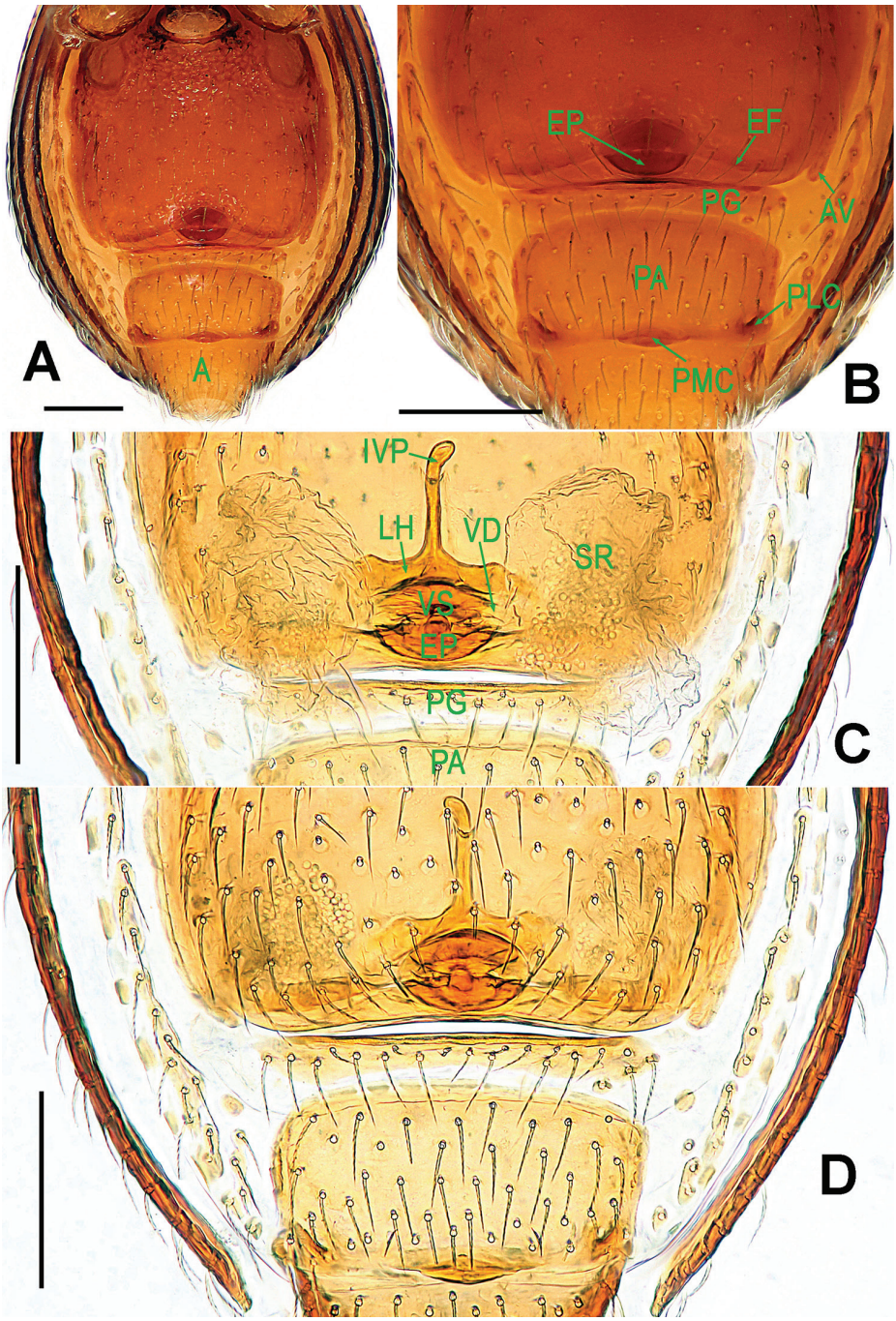

Figure 9. *Ablemma malacca* sp. n., female paratype. **A** opisthosoma **B** genital area (untreated) **C** cleared vulva (lactic acid-treated) **D** *ditto*. **A–B, D** ventral **C** dorsal. Abbreviations: A = anal plate; AV = anterior ventrolateral plate; EF = epigynal fold; EP = epigynal pit; IVP = inner vulval plate; LH = lateral horn; PA = preanal plate; PG = postgenital plate; PLC = posterolateral corner of PA; PMC = posteromedial corner of PA; SR = seminal receptaculum; VD = vulval duct; VS = vulval stem. Scale bars: 0.10 mm.

Female (one of paratypes). Coloration: same as in male.

Measurements: total length 1.10; carapace 0.52 long, 0.42 wide, 0.42 high; abdomen 0.72 long, 0.54 wide, 0.42 high; clypeus 0.14 high; sternum 0.31 long, 0.31 wide. Length of legs: I 1.14 (0.38, 0.14, 0.25, 0.17, 0.20); II 1.00 (0.32, 0.13, 0.22, 0.16, 0.17); III 0.88 (0.26, 0.12, 0.17, 0.18, 0.15); IV 1.16 (0.36, 0.13, 0.29, 0.20, 0.18).

Prosoma (Fig. 7C–D, F, H): clypeus lower than in male, smooth, bears sparse setae; ocular area not protruded; cephalic part lower than in male, palps reduced; other features as in male.

Opisthosoma (Figs 7C–D, H; 9A–B): dorsal and ventral scuta as in male; lateral scutum I long, extending beyond posterior rim of booklung cover; cover centrally smooth, laterally rugose; postgenital scutum narrow, slightly curved; perigenital scutum absent; preanal scutum rectangular, with sparse serrated setae and one posteromedial and two posterolateral corners.

Genitalia (Fig. 9B–D): epigynal pit and vulval stem forming to an oval structure, strongly sclerotized (Fig. 9B, D); vulval duct and lateral horn weakly sclerotized, connected to the translucent, saccular seminal receptaculum (Fig. 9C); inner vulval plate long, distal end slightly bent (Fig. 9C); central process absent.

Distribution. Singapore.

Genus *Brignoliella* Shear, 1978

Type species. *Polyaspis acuminata* Simon, 1889 from New Caledonia (see Burger 2008a; Lehtinen 1981).

Brignoliella besutensis Lin, Li & Jäger, 2012

Figs 10, 11, 12

B. besutensis Lin et al., 2012: 56, figs 1A–F, 2A–C, 3A–C (male) from Malaysia.

Material examined. 1♂ and 8♀ (NHMSU), SINGAPORE: Pulau Ubin, altitude 2 m, 1°25'18.0"N, 103°56'25.4"E, 22 August 2015, S. Li and Y. Tong leg.

Diagnosis. *B. besutensis* is similar to *B. caligiformis* Tong & Li, 2008 from Hainan Island, China (see Tong, 2013: 76, fig. 91A–G), but male can be distinguished by the non-inflated palpal tibia, the pear-shaped bulb, the horn-shaped embolus, and the slightly sinuous course of the spermatic duct (Fig. 11A–B, also see Lin et al., 2012: fig. 2A–C vs. Tong, 2013: fig. 91F–G). Female distinguished by the straight postgenital scutum (Fig. 12A–D vs. Tong, 2013: fig. 91D–E), the larger, adjacent pits of the preanal scutum (Fig. 12B–D vs. Tong, 2013: fig. 91D–E), the narrower lateral horn and the flatter vulval stem (Fig. 12C vs. Tong, 2013: fig. 91E).

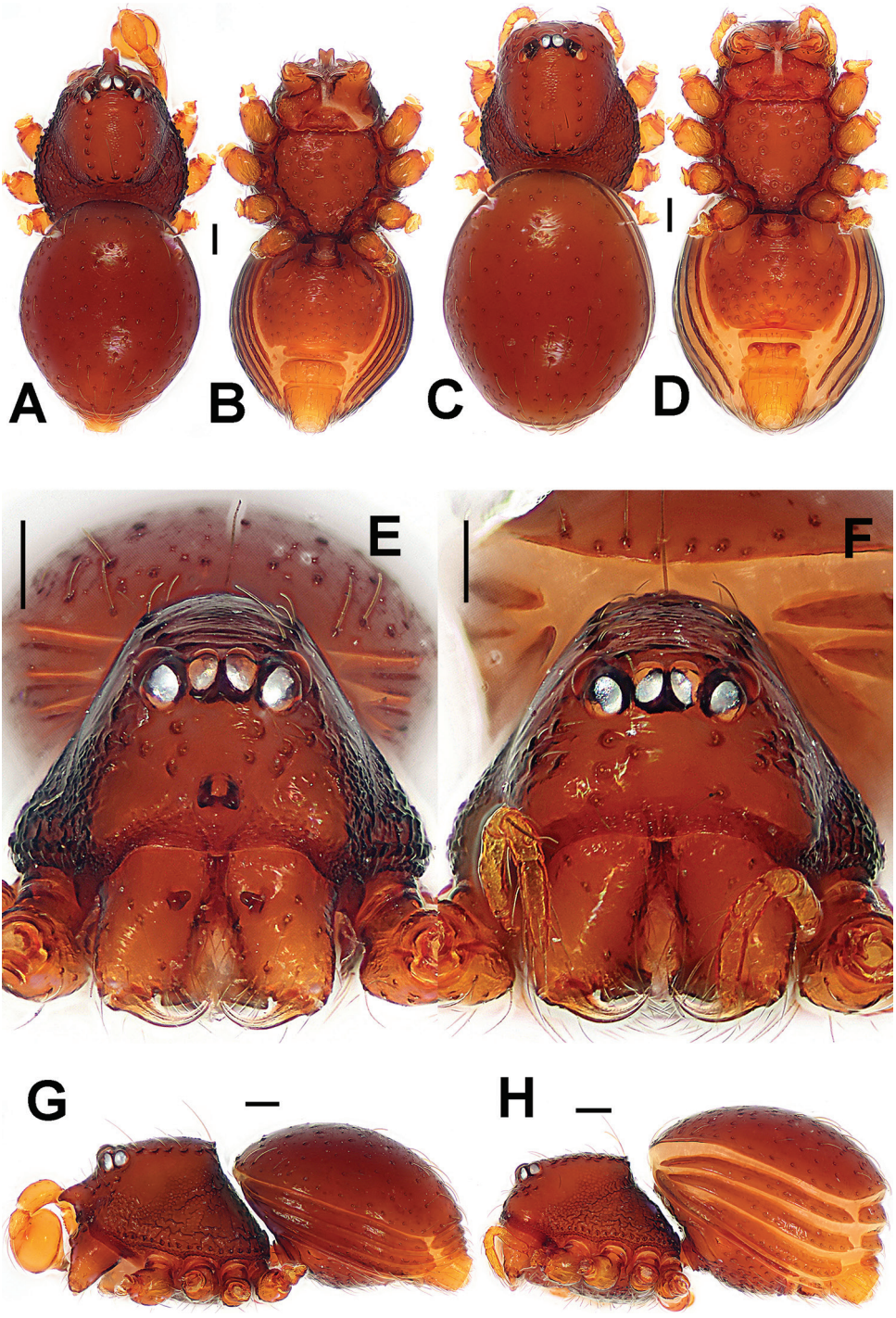

Figure 10. *Brignoliella besutensis* Lin, Li & Jäger, 2012, male (**A–B, E, G**) and female (**C–D, F, H**) from Singapore. **A–D, G–H** habitus **E–F** prosoma. **A, C** dorsal **B, D** ventral **E–F** anterior **G–H** lateral. Scale bars: 0.10 mm.

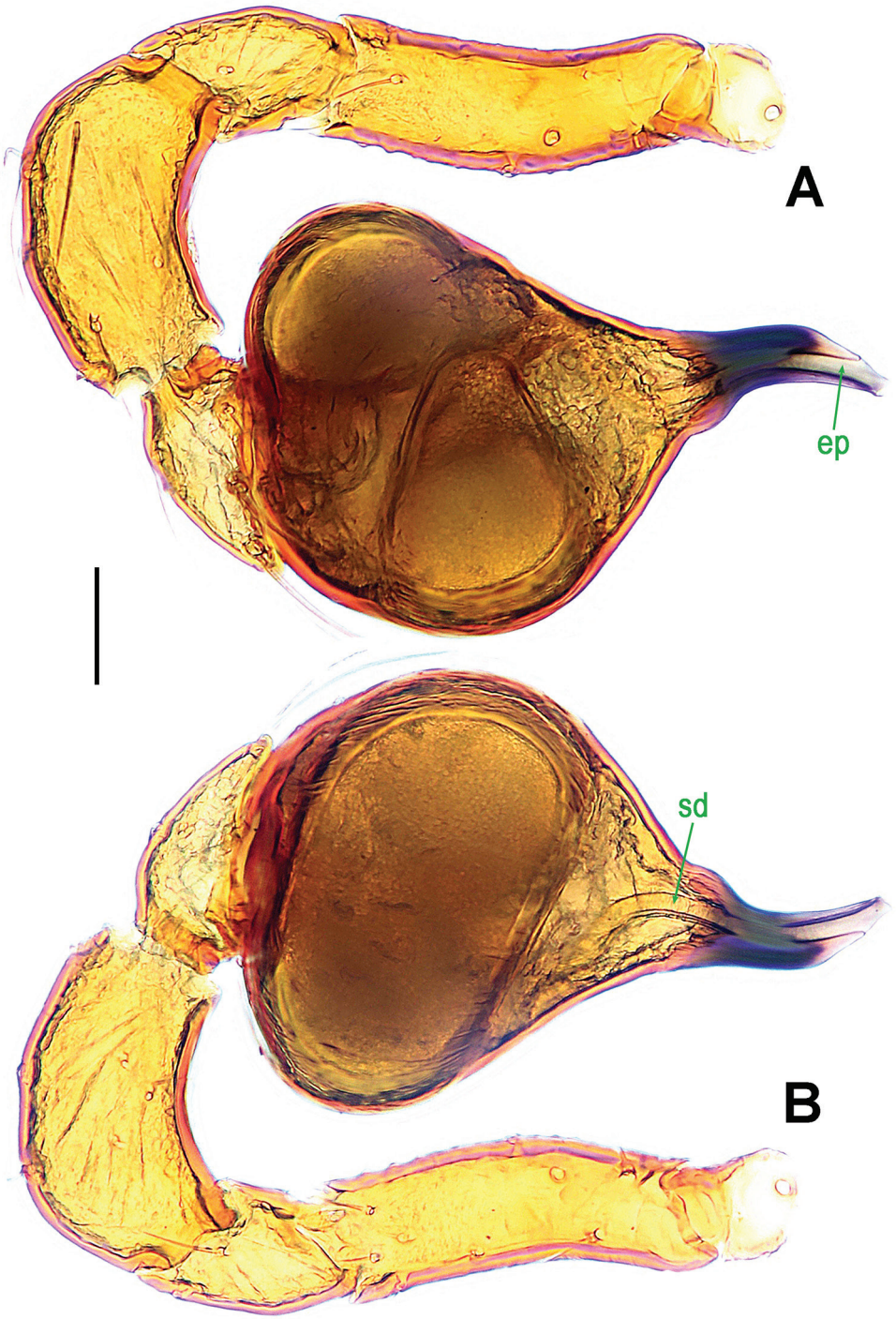

Figure 11. *Brignoliella besutensis* Lin, Li & Jäger, 2012, male from Singapore. **A** left palp, retrolateral **B** *ditto*, prolateral. Abbreviations: ep = embolic part of apes of palpal organ; sd = spermatic duct. Scale bars: 0.10 mm.

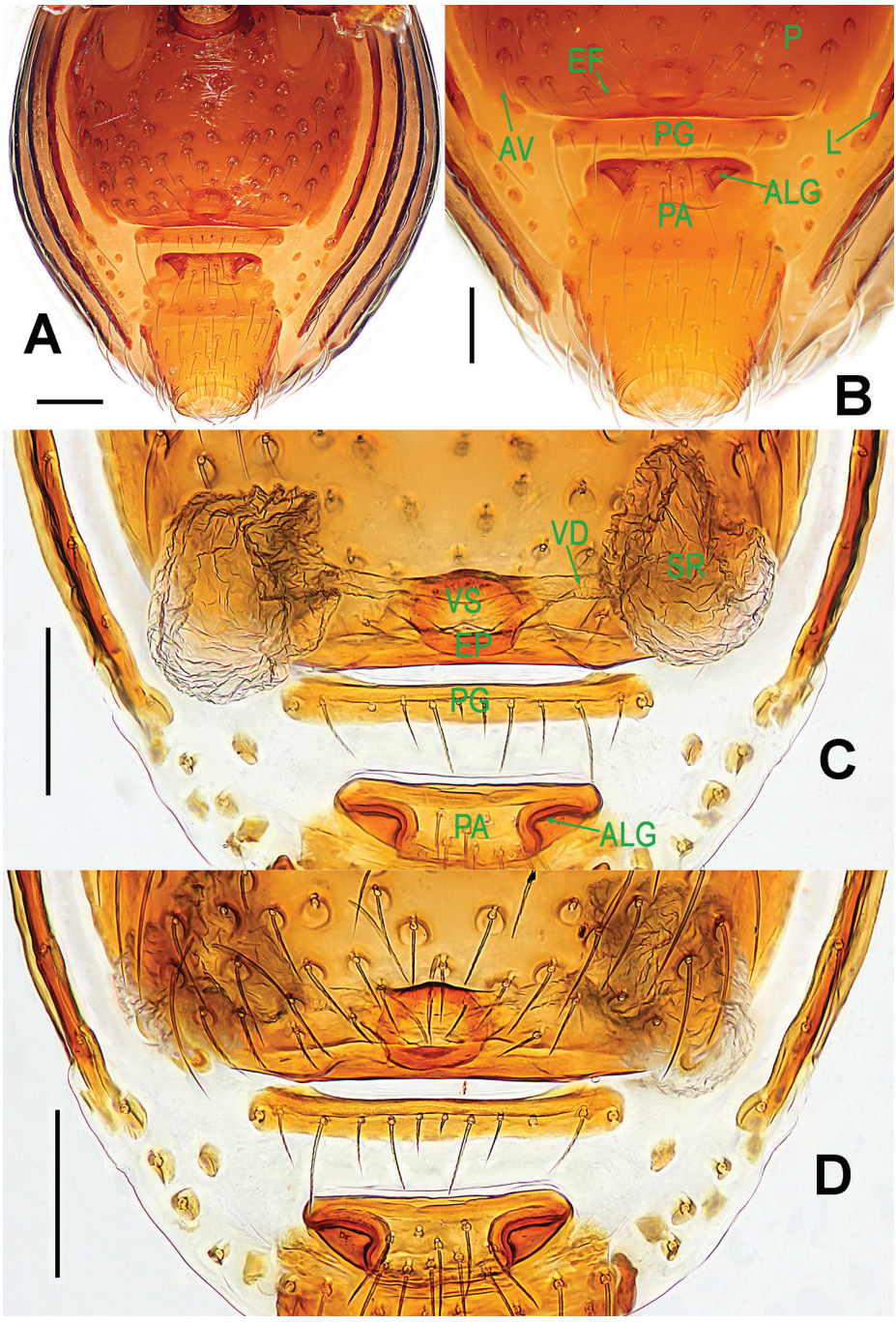

Figure 12. *Brignoliella besutensis* Lin, Li & Jäger, 2012, female from Singapore. **A** opisthosoma **B** genital area (untreated) **C** cleared vulva (lactic acid-treated) **D** *ditto*. **A–B, D** ventral **C** dorsal. Abbreviations: ALG = anterolateral groove of preanal plate; AV = anterior ventrolateral plate; EF = epigynal fold; EP = epigynal pit; L = lateral plate; P = pulmonary plate; PA = preanal plate; PG = postgenital plate; SR = seminal receptaculum; VD = vulval duct; VS = vulval stem. Scale bars: 0.10 mm.

Description. Male. Coloration: body reddish-brown; legs pale reddish-brown to yellowish-brown.

Measurements: total length 1.16; carapace 0.58 long, 0.48 wide, 0.47 high; abdomen 0.72 long, 0.56 wide, 0.48 high; clypeus 0.16 high; sternum 0.32 long, 0.32 wide. Length of legs: I 1.25 (0.40, 0.14, 0.28, 0.21, 0.22); II 1.14 (0.36, 0.13, 0.25, 0.19, 0.21); III 1.01 (0.30, 0.12, 0.21, 0.19, 0.19); IV 1.29 (0.40, 0.13, 0.30, 0.26, 0.20).

Prosoma (Fig. 10A–B, E, G): carapace strongly sclerotized; cephalic part smooth, slightly raised, with two rows of wart-like knots behind ocular area; thoracic part irregularly reticulated, margin rugose and denticulate; ocular area situated anteriorly, with six eyes in three diads, PME absent, ALE>AME=PLE in eye size. Clypeus with few wart-like knots, clypeal horn short, distally bifid; chelicerae surface smooth, with a fronto-mesial, short cheliceral apophysis, cheliceral lamina developed, translucent. Endites basally wide, distally narrow; labium distally blunt, subtriangular. Sternum long same as wide, with relatively large wart-like knots, marginally crinkled. Legs with lateral veins.

Opisthosoma (Fig. 10A–B, G): dorsal scutum oval, with sparse small pits, setae inserted in pits; ventral scutum anteriorly slightly crinkled, posteriorly with pits, booklung cover smooth; lateral scutum I long; postgenital scutum present, preanal scutum rectangular and smooth.

Palp (Fig. 11A–B): femur ventrally granulated, with three long setae; patella almost half as long as tibia, without modification; tibia slightly bent, with a distal-dorsal trichobothrium; cymbium subtriangular in lateral view; bulb pear-shaped; spermatic duct course simple; embolus short, sclerotized, distinctly narrowed and slightly bent distally, horn-shaped.

Female (First description). Coloration: same as in male.

Measurements: total length 1.18; carapace 0.56 long, 0.48 wide, 0.46 high; abdomen 0.84 long, 0.64 wide, 0.52 high; clypeus 0.12 high; sternum 0.33 long, 0.34 wide. Length of legs: I 1.22 (0.40, 0.14, 0.26, 0.20, 0.22); II 1.14 (0.36, 0.13, 0.24, 0.20, 0.21); III 0.98 (0.30, 0.12, 0.19, 0.20, 0.17); IV 1.26 (0.39, 0.13, 0.28, 0.24, 0.22).

Prosoma (Fig. 10C–D, F, H) as in male, but lacking clypeal horn and cheliceral apophysis, and clypeus lower than in male. Legs also as in male.

Opisthosoma (Figs 10C–D, H; 12A–B): dorsal and ventral scuta as in male; lateral scutum I short, and not exceeding anterior margin of preanal scutum; postgenital scutum straight, slightly wider than preanal scutum; preanal scutum trapezoidal, with two large grooves at anterolateral corners (Fig. 12A–B).

Genitalia (Fig. 12C–D): epigynal fold distinct; epigynal pit and vulval stem forming into a sclerotized ring; central process and inner vulval plate absent; lateral horn and vulval duct weak, connected to the translucent, saccular seminal receptaculum (Fig. 12C).

Distribution. Malaysia, Singapore.

Brignoliella michaeli **Lehtinen, 1981**
Figs 13, 14, 15

B. michaeli Lehtinen, 1981: 41, figs 103–106, 108, 111 (male and female) from Penang, Malaysia.

Material examined. 19♂ and 29♀ (NHMSU), SINGAPORE: Central Catchment Nature Reserve, near Mandai Agrotechnology Park, altitude 46 m, 1°24'53.7"N, 103°47'56.2"E, 1 September 2015, S. Li and Y. Tong leg.

Diagnosis. *B. michaeli* can be distinguished from all congeners with the exception of *B. besutensis*, *B. caligiformis*, *B. maoganensis* Tong & Li, 2008, *B. maros* Lehtinen, 1981, *B. martensi* (Brignoli, 1972), and *B. massai* Lehtinen, 1981 by the long, pear-shaped bulb or by the long, furcated clypeal horn. It differs from *B. besutensis* (Figs 10A–B, E; 11A–B, 12B–C), *B. caligiformis* (see Tong, 2013: 76, fig. 91A, E, F–G) and *B. maoganensis* (see Tong, 2013: 77, fig. 92A, E, F–G) by the longer clypeal horn in male, the more pointed embolus, the larger pits of preanal scutum, and the narrower lateral horn (Figs 13A–B, E; 14A–B, 15B–C); from *B. maros* (see Lehtinen, 1981: 39, figs 97, 116, 125) and *B. massai* (see Lehtinen, 1981: 40, figs 93, 95, 115, 124) by the longer clypeal horn and larger cheliceral horn in male (Fig. 13A–B, E), the longer pear-shaped bulb, the curving embolus (Fig. 14A–B) and the wider vulval stem (Fig. 15C); and from *B. martensi* (see Lehtinen, 1981: 38, figs 98, 112, 117) by the narrower, longer clypeal horn in male (Fig. 13A), the longer bulb and the curving, tapering embolus (Fig. 14A–B), and the nearly quadrate preanal scutum and the distinct epigynal fold (Fig. 15A–B, D).

Description. Male. Coloration: body brownish-yellow; legs yellowish-orange.

Measurements: total length 1.38; carapace 0.74 long, 0.56 wide, 0.60 high; abdomen 0.82 long, 0.65 wide, 0.56 high; clypeus 0.20 high; sternum 0.37 long, 0.38 wide. Length of legs: I 1.45 (0.46, 0.16, 0.34, 0.25, 0.24); II 1.34 (0.42, 0.15, 0.30, 0.24, 0.23); III 1.21 (0.36, 0.14, 0.26, 0.23, 0.22); IV 1.53 (0.48, 0.15, 0.36, 0.30, 0.24).

Prosoma (Fig. 13A–B, E, G): carapace strongly sclerotized; cephalic part smooth, distinctly raised, with two rows of wart-like knots behind ocular area; thoracic cuticle irregularly rugose, marginally denticulate; ocular area situated anteriorly, with six eyes in three diads, PME absent, ALE>AME=PLE in eye size, ALE and PLE adjacent. Clypeus with wart-like knots, anteromargin rugose, clypeal horn long, distally bifid; chelicerae surface smooth, with a fronto-subbasal, short cheliceral apophysis, cheliceral lamina developed, translucent. Endites basally wide, distally narrow; labium subtriangular. Sternum long same as wide, with finely large ring-like pits, marginally crinkled. Legs with shallow annular grains.

Opisthosoma (Fig. 13A–B, G): dorsal scutum short oval, with sparse small pits, setae inserted in pits, smooth between the pits (Fig. 13A, E); ventral scutum modified by ring-like pits, booklung cover smooth; lateral scutum I long, and just over anteromargin of preanal scutum; postgenital scutum present, same wide as preanal scutum; preanal scutum rectangular, with an anterior long fold and two anterolaterally shallow grooves (Fig. 13B).

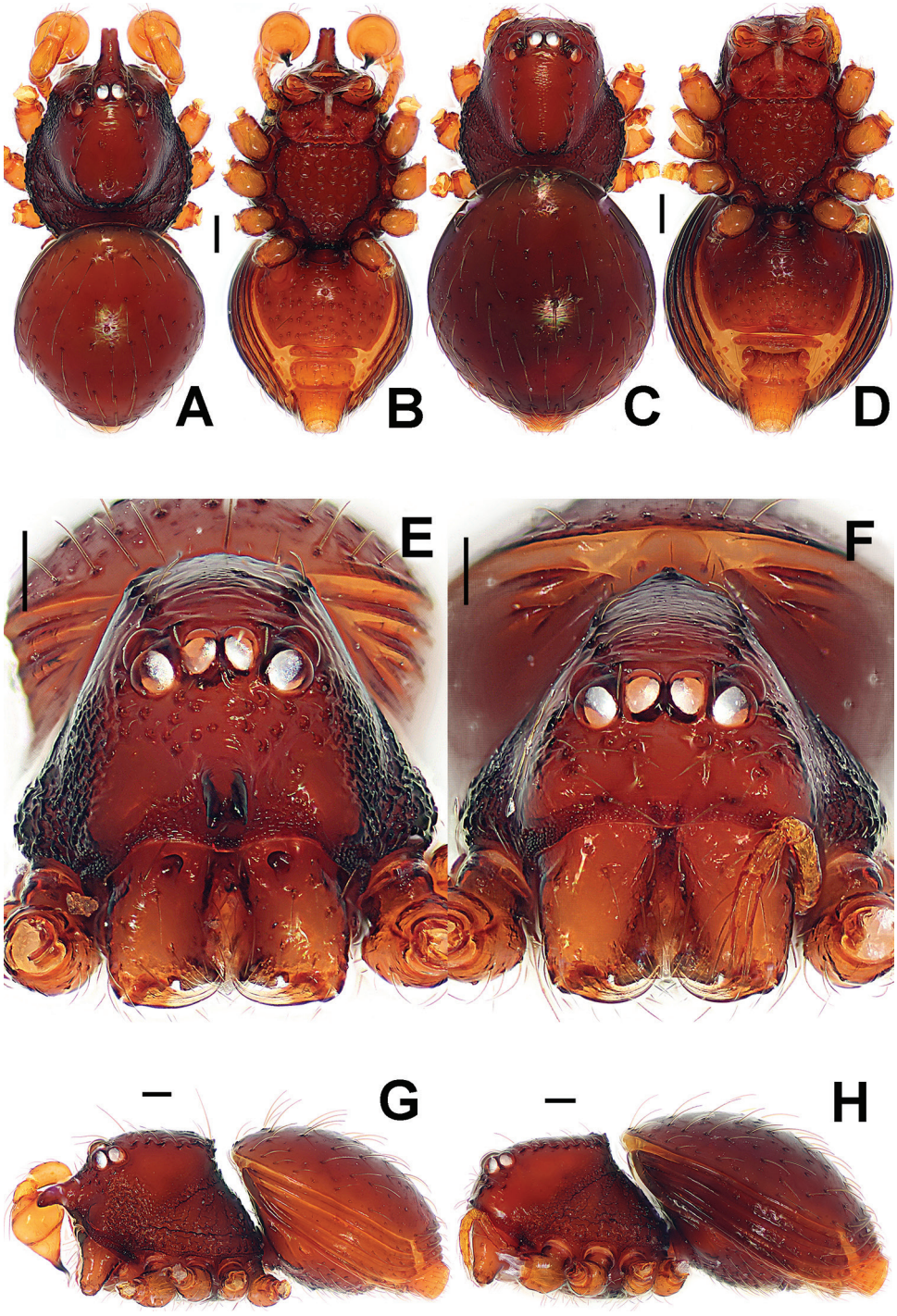

Figure 13. *Brignoliella michaeli* Lehtinen, 1981, male (**A–B, E, G**) and female (**C–D, F, H**) from Singapore. **A–D, G–H** habitus **E–F** prosoma. **A, C** dorsal **B, D** ventral **E–F** anterior **G–H** lateral. Scale bars: 0.10 mm.

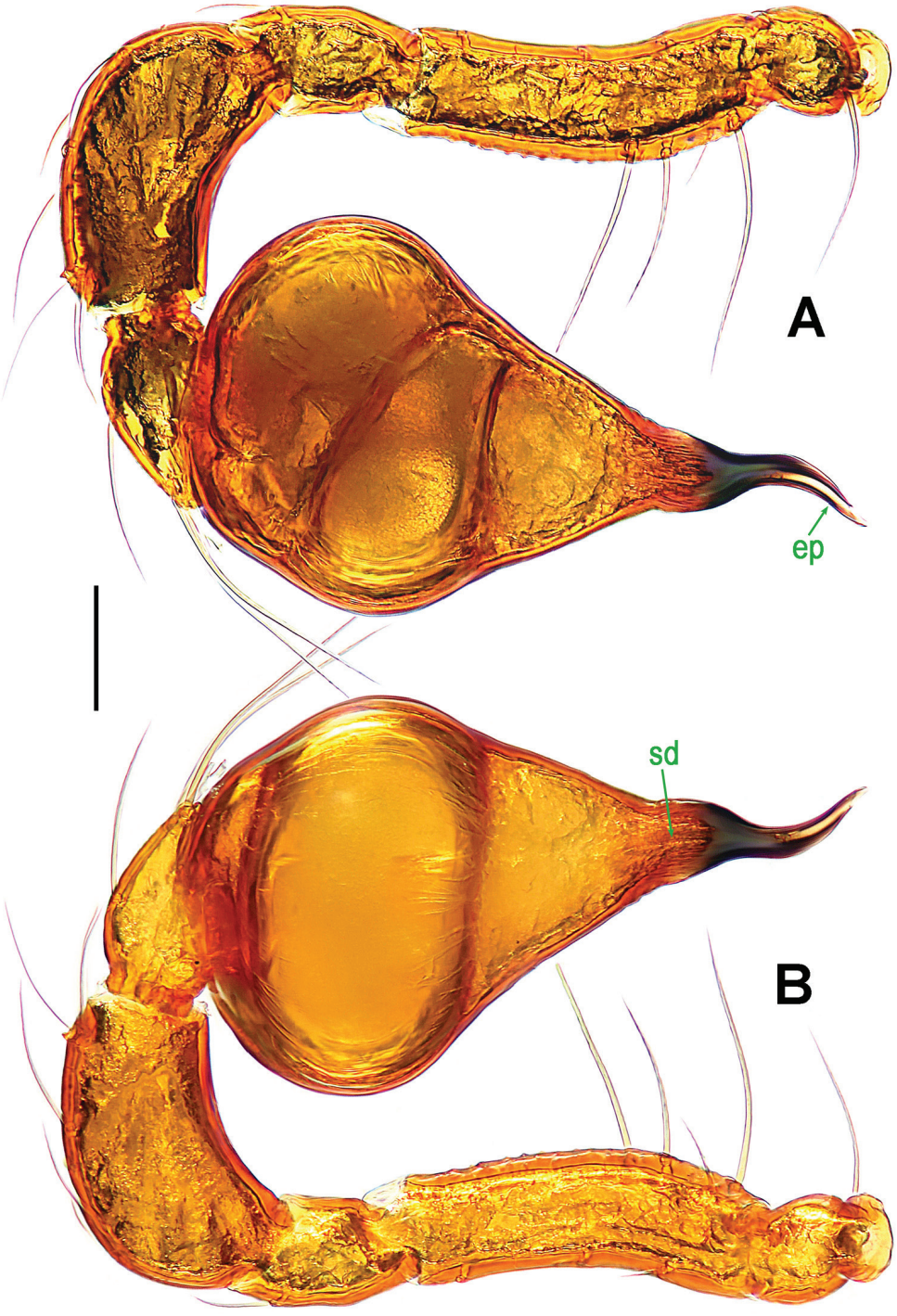

Figure 14. *Brignoliella michaeli* Lehtinen, 1981, male from Singapore. **A** left palp, retrolateral **B** *ditto*, prolateral. Abbreviations: ep = embolic part of apes of palpal organ; sd = spermatic duct. Scale bars: 0.10 mm.

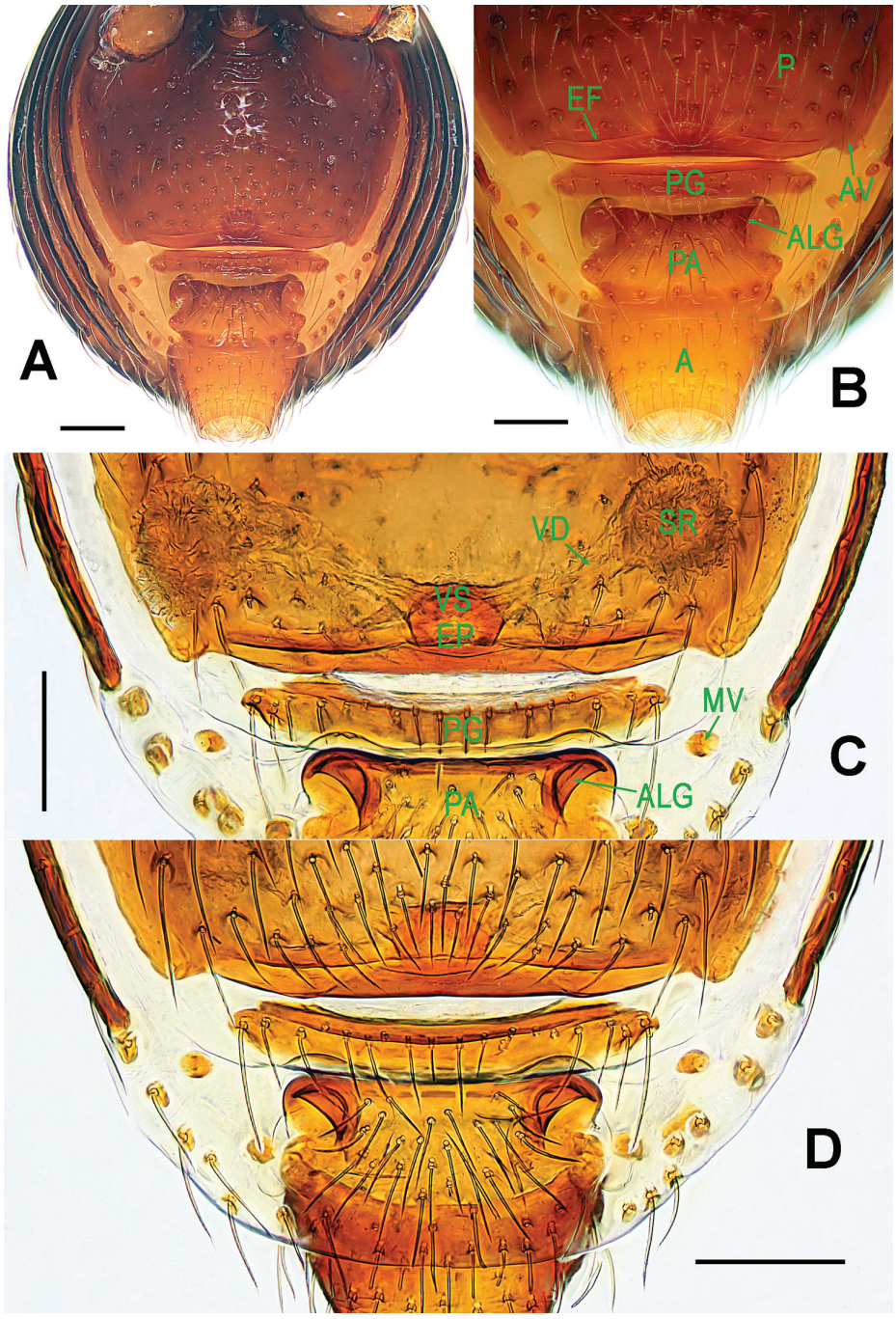

Figure 15. *Brignoliella michaeli* Lehtinen, 1981, female from Singapore. **A** opisthosoma **B** genital area (untreated) **C** cleared vulva (lactic acid-treated) **D** *ditto*. **A–B, D** ventral **C** dorsal. Abbreviations: A = anal plate; ALG = anterolateral groove of preanal plate; AV = anterior ventrolateral plate; EF = epigynal fold; EP = epigynal pit; MV = median ventrolateral plate; P = pulmonary plate; PA = preanal plate; PG = postgenital plate; SR = seminal receptaculum; VD = vulval duct; VS = vulval stem. Scale bars: 0.10 mm.

Palp (Fig. 14A–B): femur slightly bent, ventral cuticle granulated, approx. 2.2 times patella in length; tibia slightly swollen, approx. 2/3 times femur in long, with a distal-dorsal trichobothrium; cymbium short as patella; bulb pear-shaped, its surface with filamentous veins (Fig. 14A); spermatic duct base wide, and tapering to the apex of bulb after coiled a loop; embolus strongly sclerotized, mesially bent and distally sharp.

Female. Coloration: same as in male, but deeper in opisthosoma.

Measurements: total length 1.34; carapace 0.66 long, 0.56 wide, 0.56 high; abdomen 0.90 long, 0.72 wide, 0.66 high; clypeus 0.14 high; sternum 0.36 long, 0.38 wide. Length of legs: I 1.55 (0.50, 0.17, 0.35, 0.27, 0.26); II 1.44 (0.46, 0.16, 0.32, 0.26, 0.24); III 1.29 (0.39, 0.15, 0.28, 0.25, 0.22); IV 1.63 (0.51, 0.16, 0.38, 0.32, 0.26).

Prosoma (Fig. 13C–D, F, H) as in male; but lack of clypeal horn and cheliceral apophysis, and clypeus lower than in male. Legs also as in male.

Opisthosoma (Figs 13C–D, H; 15A–B): dorsal and ventral scuta as in male; lateral scutum I short, not extending beyond anteromargin of preanal scutum; postgenital scutum straight, wider than preanal scutum and in male, with an anterior fold; preanal scutum sub-rectangular, with two large, inverted, pocket-shaped grooves at anterolateral corners (Fig. 15A–B, D).

Genitalia (Fig. 15C–D): epigynal fold long; epigynal pit large, and vulval stem short; central process and inner vulval plate absent; lateral horn and vulval duct narrow, translucent, connected to the rugose, saccular seminal receptaculum (Fig. 15C).

Distribution. Malaysia, Singapore.

Genus *Singaporemma* Shear, 1978

Type species. *Singaporemma singulare* Shear, 1978 from Singapore (see Shear 1978).

Singaporemma lenachanae Lin & Li, sp. n.

http://zoobank.org/0A6AB9AF-31B6-4153-A353-5AA16C45E72D
Figs 16, 17, 18, 19B–I, 20

Material. Holotype ♂ (LKCNHM), SINGAPORE: Bukit Timah Nature Reserve, Seraya Loop, altitude 118 m, 1°21'25.4"N, 103°46'25.3"E, 17 August 2015, S. Li and Y. Tong leg. **Paratypes** 4♂ and 2♀ (LKCNHM), same data as holotype.

Other material examined. 3♂ and 2♀ (NHMSU), SINGAPORE: Bukit Timah Nature Reserve, Seraya Loop, altitude 118 m, N1°21'25.4", E103°46'25.3", 17 August 2015, S. Li and Y. Tong leg.

Other species studied for comparison. *Singaporemma halongense* Lehtinen, 1981 (Figs 19A, 21A–D; Lehtinen, 1981: 31, figs 43, 49, 54, 58, 62). Paratype 1♂ (ZMUT), VIETNAM: Quang Ninh, Ha Long, in litter of brook valley, altitude 30 m, 11 October 1978, P.T. Lehtinen leg.

Etymology. Patronymic in honour of Dr Lena Chan from the National Biodiversity Centre, Singapore in recognition of her support of this study; noun (name) in genitive case.

Diagnosis. This new species can be distinguished from all other congeners with the exception of *S. banxiaoensis* Lin & Li, 2014, *S. halongense* and *S. singulare* by the slender, straight embolus without any furcated end (Fig. 17B–E), the lack of central process, and the "T"-shaped inner vulval plate (Fig. 20C). It differs from *S. banxiaoensis* (see Lin and Li, 2014: 42, fig. 5A–D) by the lower initial position of embolus (Fig. 17A vs. fig. 5A), the wider embolic end (Fig. 17D, 19B–C vs. Fig. 5D), the wider, shorter preanal scutum (Fig. 20B vs. Fig. 6B), the narrower lateral horns, and the "T"-shaped inner vulval plate (Fig. 20C vs. Fig. 6C). Differs from *S. halongense* (see Figs 19A, 21A–D, and Lehtinen, 1981: 31, fig. 58) by the sharper, narrower embolic end (Fig. 19B–I vs. Fig. 19A), the wider preanal scutum and the "T"-shaped inner vulval plate (Fig. 20B–C vs. fig. 58). From *S. singulare* (see Figs 23A–E, 25A–E) by the shorter ovate bulb (Fig. 18A–F vs. Fig. 23A–C, 25D–E), the bent embolic end (Fig. 17D, 19B–I vs. Fig. 23D, 25C), the more swollen palpal tibia (Fig. 18A–F vs. Fig. 23A–B, 25D–E), the narrower postgenital scutum and preanal scutum (Fig. 20B vs. Fig. 23H, 26B), and the larger inner vulval plate (Fig. 20C vs. Fig. 26C).

Description. Male (holotype). Coloration: body reddish-brown; legs yellowish-brown.

Measurements: total length 0.86; carapace 0.42 long, 0.38 wide, 0.40 high; abdomen 0.58 long, 0.49 wide, 0.39 high; clypeus 0.17 high; sternum 0.24 long, 0.27 wide. Length of legs: I 0.95 (0.31, 0.11, 0.22, 0.15, 0.16); II 0.87 (0.27, 0.10, 0.20, 0.15, 0.15); III 0.77 (0.24, 0.10, 0.16, 0.14, 0.13); IV 0.99 (0.33, 0.11, 0.23, 0.17, 0.15).

Prosoma (Fig. 16A–B, E, G): carapace finely reticulated; eyes white, AME=ALE>PLE in size, ARE procured; clypeus high, sloping forward, marginally rounded (Fig. 16C, 16G); cephalic part raised, quadrate (Fig. 16A); thoracic part smooth in radial grooves; Chelicerae without any horn or process, cheliceral lamina developed; endites basally wide, distally narrow; labium triangular, distally blunt; sternum finely reticulated, scutellate, posterior corner blunt, margin rugose, with sparse setae. Legs cuticle striated.

Opisthosoma (Fig. 16A–B, G): covered with serrated setae; dorsal scutum oval, but anteriorly truncated, finely reticulated; ventral scutum reticulated; lateral scutum long, and exceeding beyond the posterior margin of preanal scutum; perigenital scutum present; postgenital scutum straight, narrow, same wide as preanal scutum; preanal scutum shallow rectangular.

Palp (Figs 17A–E, 18A–F, 19B–I): femoral cuticle sculptured and granulated, approximately 2.5 times patella in length; patella short, and small; tibia remarkably swollen, about 2.3 times femur in width (Fig. 17B–C, E); cymbium relatively small, bearing long setae; bulb egg-shaped, its surface with irregular lines (Fig. 17A–B); embolus long, straight, starting at the subapical 1/3 position of bulb prolaterally (Fig. 17B–C, 18A–F), embolic end slightly bent, sharp and flexible (Figs 17D, 19B–I).

Female (one of paratypes). Coloration: same as in male.

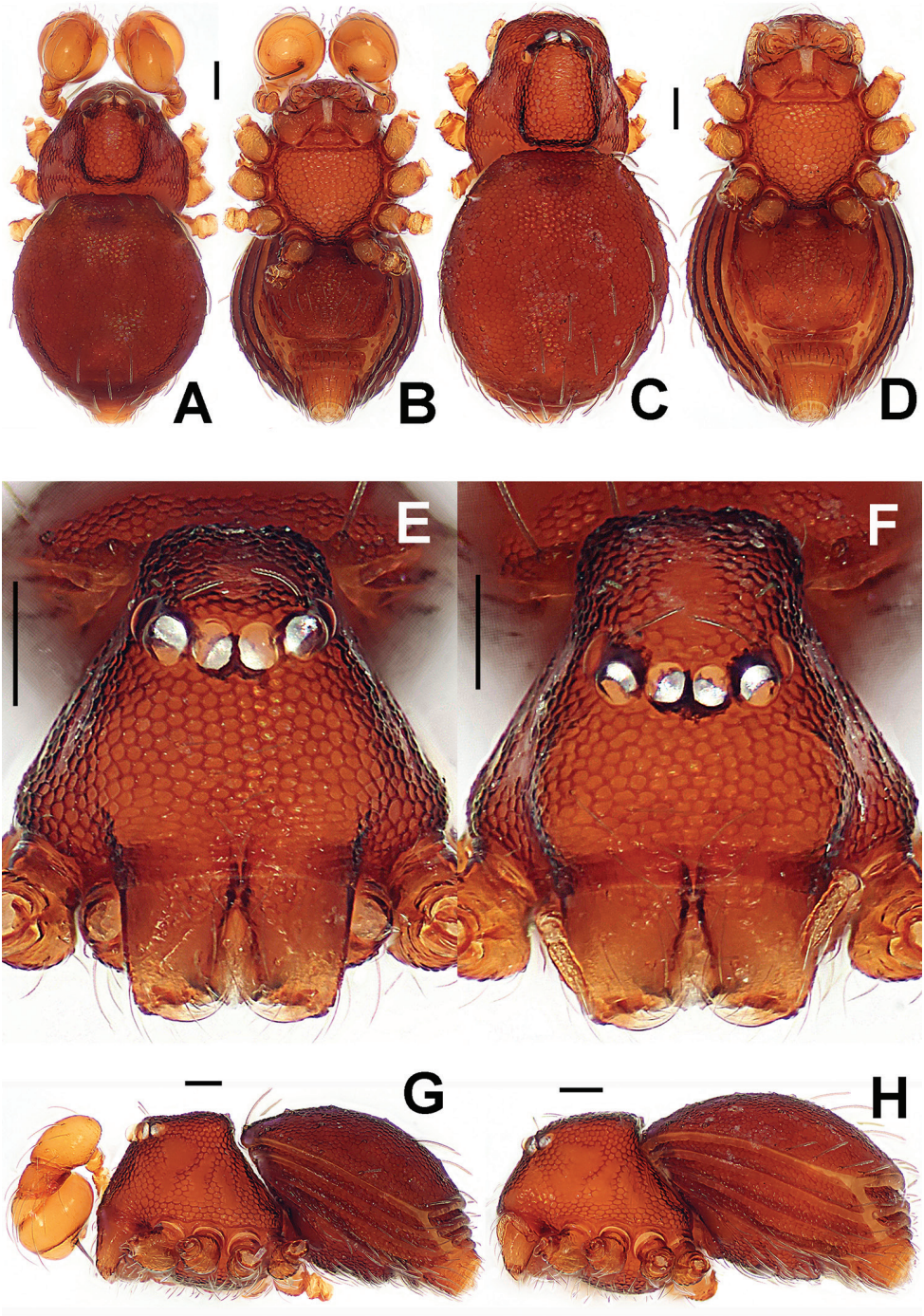

Figure 16. *Singaporemma lenachanae* sp. n., male holotype (**A–B, E, G**) and female paratype (**C–D, F, H**). **A–D, G–H** habitus **E–F** prosoma. **A, C** dorsal **B, D** ventral **E–F** anterior **G–H** lateral. Scale bars: 0.10 mm.

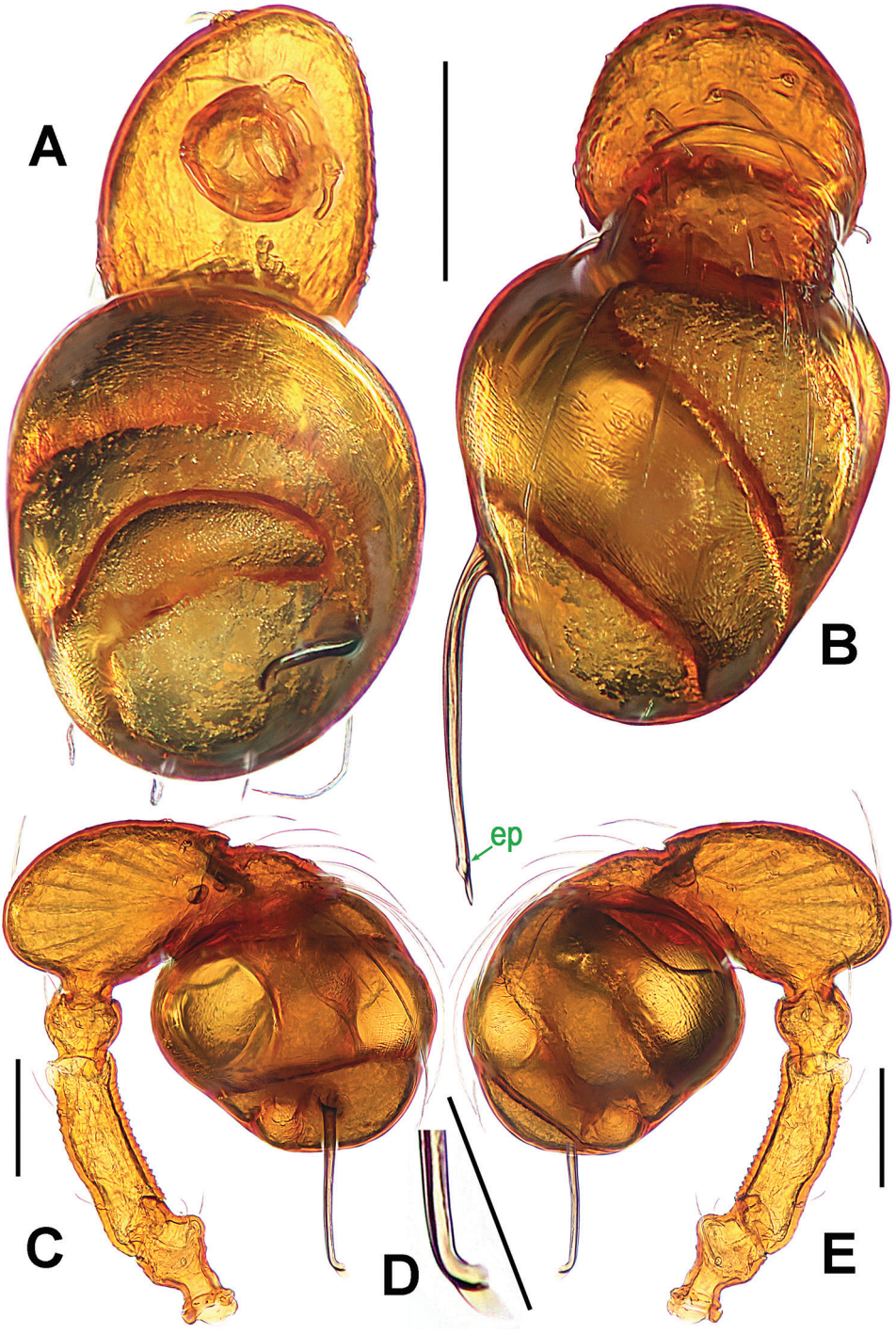

Figure 17. *Singaporemma lenachanae* sp. n., male holotype. **A** palpal bulb, ventral **B** left palp, anterior **C** *ditto*, prolateral **D** embolic end, prolateral **E** left palp, retrolateral. Abbreviation: ep = embolic part of apes of palpal organ. Scale bars: 0.10 mm.

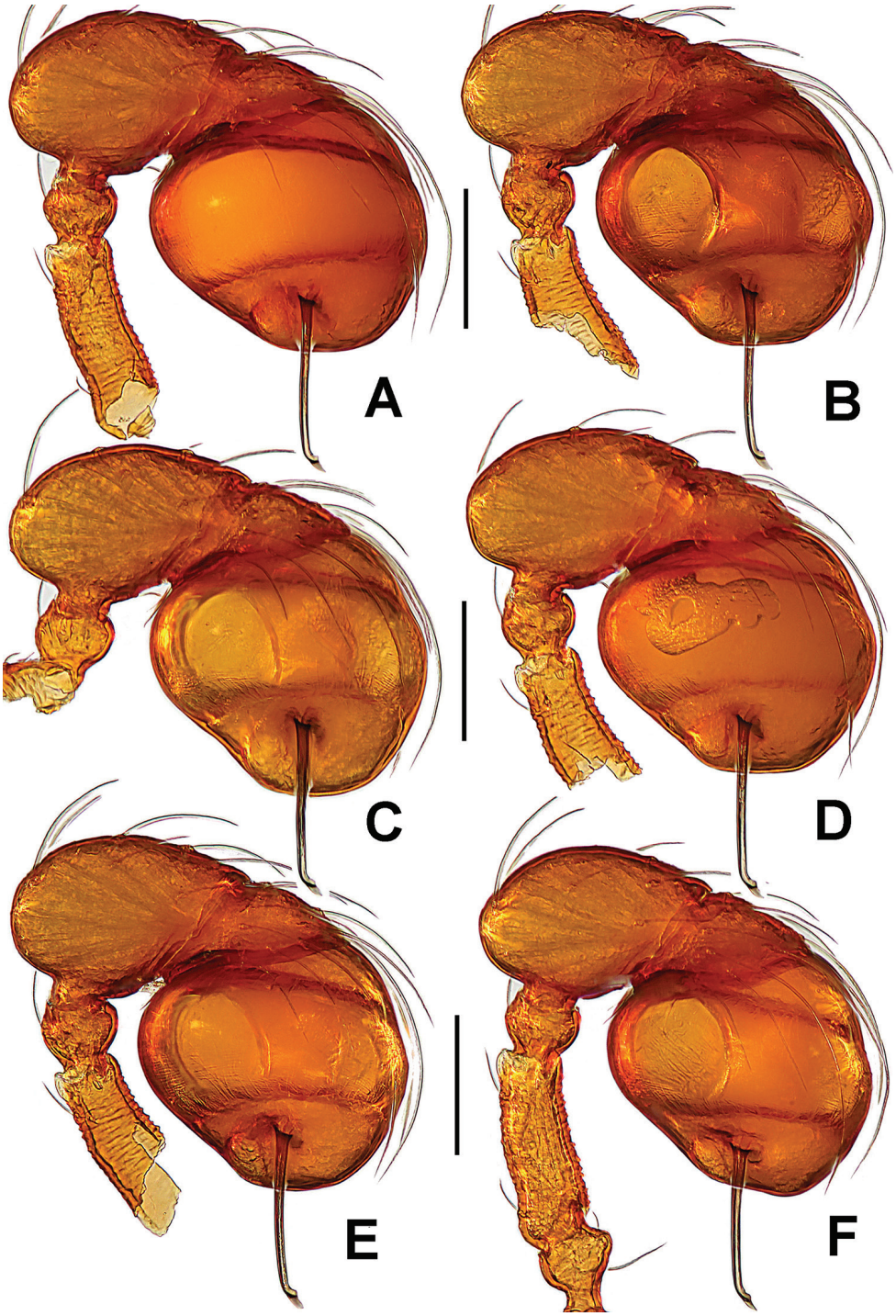

Figure 18. *Singaporemma lenachanae* sp. n., male paratypes. **A–F** left palps, prolateral. Scale bars: 0.10 mm.

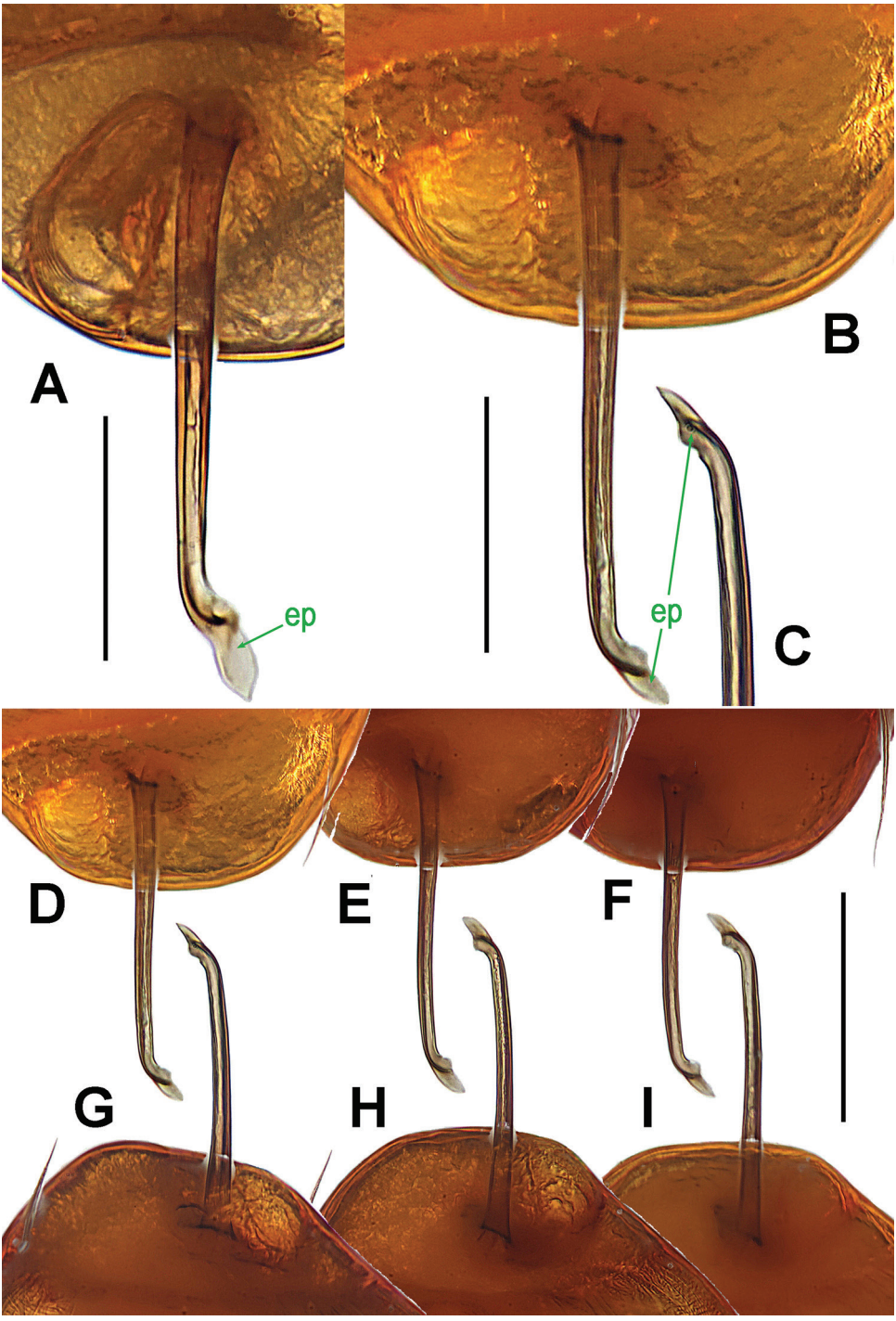

Figure 19. *Singaporemma halongense* Lehtinen, 1981, male paratype (**A**), and *S. lenachanae* sp. n., male paratypes (**B–I**). **A** embolic end, prolateral **B–I** *ditto***,** prolateral. Abbreviations: ep = embolic part of apes of palpal organ. Scale bars: 0.10 mm.

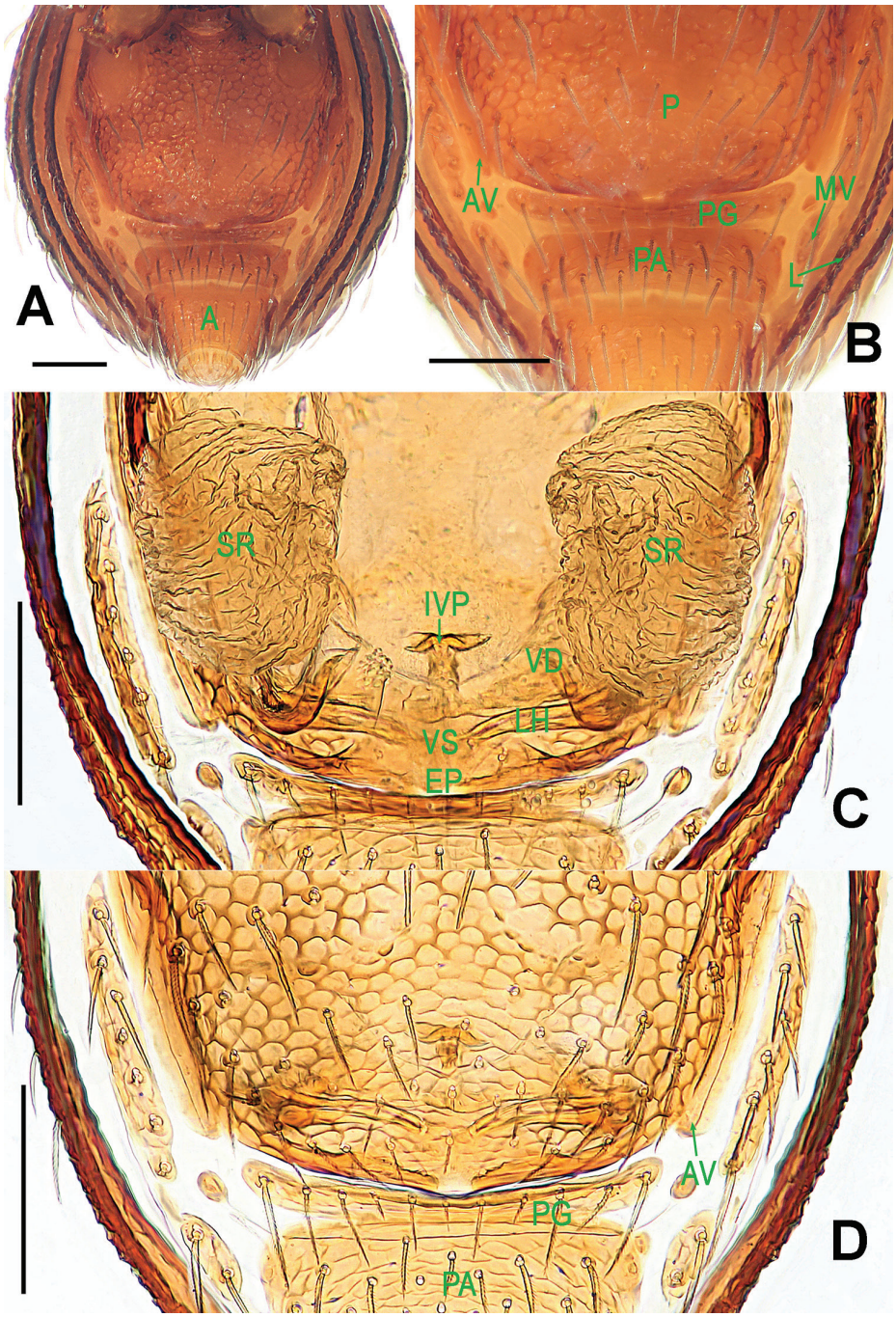

Figure 20. *Singaporemma lenachanae* sp. n., female paratype. **A** opisthosoma **B** genital area (untreated) **C** cleared vulva (lactic acid-treated) **D** *ditto*. **A–B, D** ventral **C** dorsal. Abbreviations: A = anal plate; AV = anterior ventrolateral plate; EP = epigynal pit; IVP = inner vulval plate; L = lateral plate; LH = lateral horn; MV = median ventrolateral plate; PA = preanal plate; PG = postgenital plate; SR = seminal receptaculum; VD = vulval duct; VS = vulval stem. Scale bars: 0.10 mm.

Measurements: total length 0.96; carapace 0.44 long, 0.38 wide, 0.41 high; abdomen 0.64 long, 0.52 wide, 0.40 high; clypeus 0.15 high; sternum 0.26 long, 0.27 wide. Length of legs: I 0.91 (0.28, 0.12, 0.20, 0.15, 0.16); II 0.83 (0.26, 0.11, 0.18, 0.14, 0.14); III 0.76 (0.24, 0.10, 0.16, 0.13, 0.13); IV 0.98 (0.32, 0.11, 0.22, 0.17, 0.16).

Prosoma (Fig. 16C–D, F, H) as in male, but clypeus slightly lower than in male. Palps distinctly reduced. Legs also as in male.

Opisthosoma (Figs 16C–D, H; 20A): dorsal and ventral scuta as in male; lateral scutum I long, extending beyond posterior margin of preanal scutum; perigenital scuta small, oval; postgenital scutum slightly curved, wider than preanal scutum; preanal scutum sculptured, shallow rectangular, with sparse serrated setae.

Genitalia (Fig. 20B–D): epigynal pit distinct, oval (Fig. 20B, D); vulval posterior margin weakly sclerotized (Fig. 20C); vulval stem triangular; central process absent; inner vulval plate "T"-shaped (Fig. 20C); lateral horn narrow, and weakly sclerotized; vulval ducts relatively wide, membranous and translucent, connected to the rugose, large, saccular seminal receptaculum (Fig. 20C).

Distribution. Singapore.

Singaporemma singulare Shear, 1978

Figs 22, 23A–E, G–H, 24, 25, 26

S. singularis Shear, 1978: 36, figs 108–111; Lehtinen, 1981: 31.

Examined materiral. Holotype ♂ and **paratype** 1♀ (AMNH), SINGAPORE: near MacRitchie Reservoir, 25 October 1950, M.W.F. Tweedie leg.

Other material. 10♂ and 6♀ (LKCNHM), SINGAPORE: Central Catchment Nature Reserve, treetop walk, 1°21'13.3"N, 103°48'29.4"E, 28 August 2015, S. Li and Y. Tong leg; 5♂ and 4♀ (NHMSU), SINGAPORE: Central Catchment Nature Reserve, Treetop Walk, 1°21'13.3"N, 103°48'29.4"E , 28 August 2015, S. Li and Y. Tong leg.

Other species studied for comparison. *Singaporemma adjacens* Lehtinen, 1981 (Fig. 23F; Lehtinen, 1981: 31, figs 47, 51, 64). Holotype ♂ (ZMUT), VIETNAM: Quang Ninh, Ha Long, in litter of dense jungle close to seashore, altitude 10 m, 12 October 1978, P.T. Lehtinen leg.

Diagnosis. *S. singulare* is distinguished from *S. bifurcata* (see Lin and Li, 2010: 26, figs 35–37) and *S. wulongensis* (see Lin and Li, 2014: 46, fig. 8A–F) by the embolus without any furcate end; from *S. adjacens* (see Fig. 23F and Lehtinen, 1981: 31: fig. 64a–b) by the narrower embolus. It is similar to *S. banxiaoensis* (see Lin and Li 2014: 42, fig. 5A–D), *S. halongense* (see Figs 19A, 21C–D and Lehtinen, 1981: 31, fig. 62a–b), and *S. lenachanae* sp. n. (Figs 17A–E, 18A–F, 19B–I) in having a straight embolus with modified end, but the male can be distinguished by the initial position of embolus (Figs 23A, 25D) and the knife-shaped embolic end (Figs 23D, 25C). Female distinguished by the absence of central process (Fig. 26C) and the punctured rather than reticulated clypeal area (Fig. 24F).

Description. Male. Coloration: body reddish-brown; legs yellowish-brown.

Measurements: total length 1.14; carapace 0.50 long, 0.45 wide, 0.44 high; abdomen 0.73 long, 0.60 wide, 0.52 high; clypeus 0.19 high; sternum 0.30 long, 0.33 wide. Length of legs: I 1.24 (0.40, 0.13, 0.30, 0.19, 0.22); II 1.10 (0.34, 0.12, 0.26, 0.18, 0.20); III 0.99 (0.30, 0.12, 0.22, 0.17, 0.18); IV 1.36 (0.43, 0.14, 0.34, 0.23, 0.22).

Prosoma (Fig. 24A–B, E, G): carapace finely reticulated, except for the radial grooves in thoracic area, marginally denticulate (Fig. 24A, E); eyes with black base, ALE>AME>PLE, ALE and PLE adjacent, ARE straight; cephalic part raised, top flat, covered with long setae (Fig. 24G); clypeus high, sharply sloping forward, bears densely short setae, anterior margin rugose (Fig. 24E); Cheliceral frontal surface sculptured, but lack of process, cheliceral lamina developed; sternum finely reticulated, marginally rugose, and posteriorly truncated. Legs striated, cuticle scalelike.

Opisthosoma (Figs 24A–B, G; 26A): covered with serrated setae; dorsal scutum oval, reticulated, bears sparse nodules and setae; ventral scutum reticulated, margin rugose; booklung cover rounded, smooth; lateral scutum I long, exceeding by far the posterior margin of preanal scutum; perigenital scutum large; postgenital scutum wide, its posterior margin overlaps joint of anterior margin of preanal scutum; preanal scutum rectangular, with blunt corners.

Palp (Figs 23A–E; 25A–E): femoral cuticle granular, striated, approximately 2.6 times as long as patella; patella short; tibia swollen, 1.8 times as wide as femur; cymbium wide and compressed; bulb long egg-shaped, surface smooth; spermatic duct basally wide, and tapering to the base of embolus after coiling a loop; embolus thin and long, weakly sclerotized, starting from the prolateral pericenter of bulbous surface, and almost straight downwards (Figs 23A, 25D); embolic end flexuous, knife-shaped (Figs 23D, 25C).

Female. Coloration: body slightly lighter than in male; legs yellowish-brown.

Measurement: total length 1.18; carapace 0.50 long, 0.44 wide, 0.44 high; abdomen 0.74 long, 0.63 wide, 0.56 high; clypeus 0.17 high; sternum 0.29 long, 0.32 wide. Length of legs: I 1.24 (0.40, 0.14, 0.28, 0.20, 0.22); II 1.14 (0.37, 0.13, 0.25, 0.19, 0.20); III 1.04 (0.32, 0.12, 0.22, 0.18, 0.20); IV 1.39 (0.44, 0.13, 0.35, 0.24, 0.23).

Prosoma (Fig. 24C–D, F, H) as in male, except for clypeal area no reticulated, but covered with short setae. Palp reduced. Legs also as in male.

Opisthosoma (Figs 24C–D, H; 26A): dorsal and ventral scuta as in male; lateral scutum I long, exceeding by far posterior margin of preanal scutum; perigenital scutum large, long oval; postgenital scutum slightly curved, faintly wider than preanal scutum, overlapped joint the anterior margin of preanal scutum; preanal scutum smooth, nearly rectangular, with sparse serrated setae.

Genitalia (Figs 23H, 26B–D): epigynal pit small, indistinct (Fig. 26B, D), closed to vulval posterior margin (Fig. 21C); vulval stem wide, connected with lateral horns; central process absent; inner vulval plate "T"-shaped, basally sclerotized (Fig. 26C); lateral horn narrow, and straight; vulval ducts relatively wide, upward curved, translucent, connected to the saccular seminal receptaculum (Fig. 26C).

Distribution. Singapore.

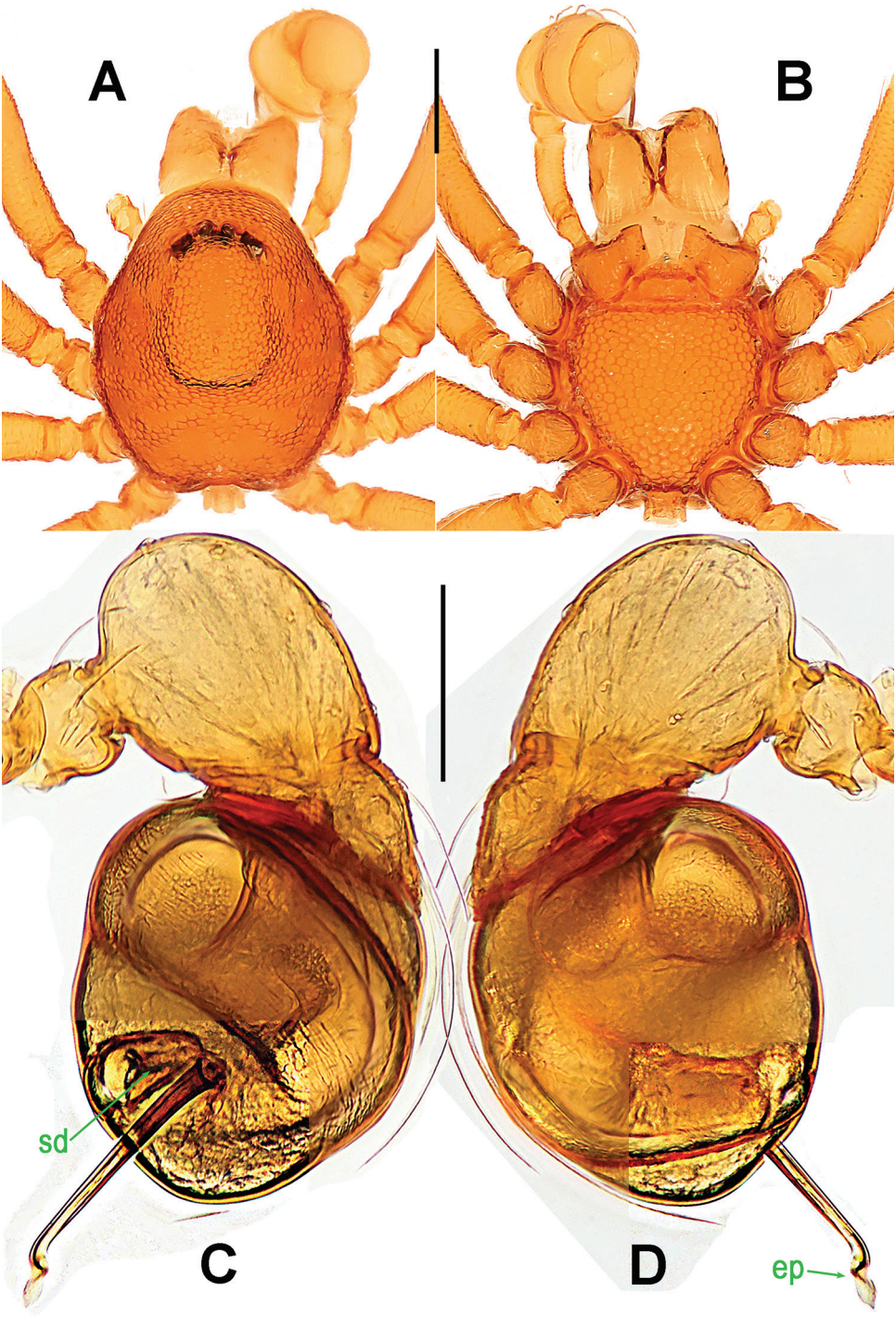

Figure 21. *Singaporemma halongense* Lehtinen, 1981, male paratype. **A** prosoma, dorsal **B** *ditto*, ventral **C** left palp, prolateral **D** *ditto*, retrolateral. Abbreviations: ep = embolic part of apes of palpal organ; sd = spermatic duct. Scale bars: 0.10 mm.

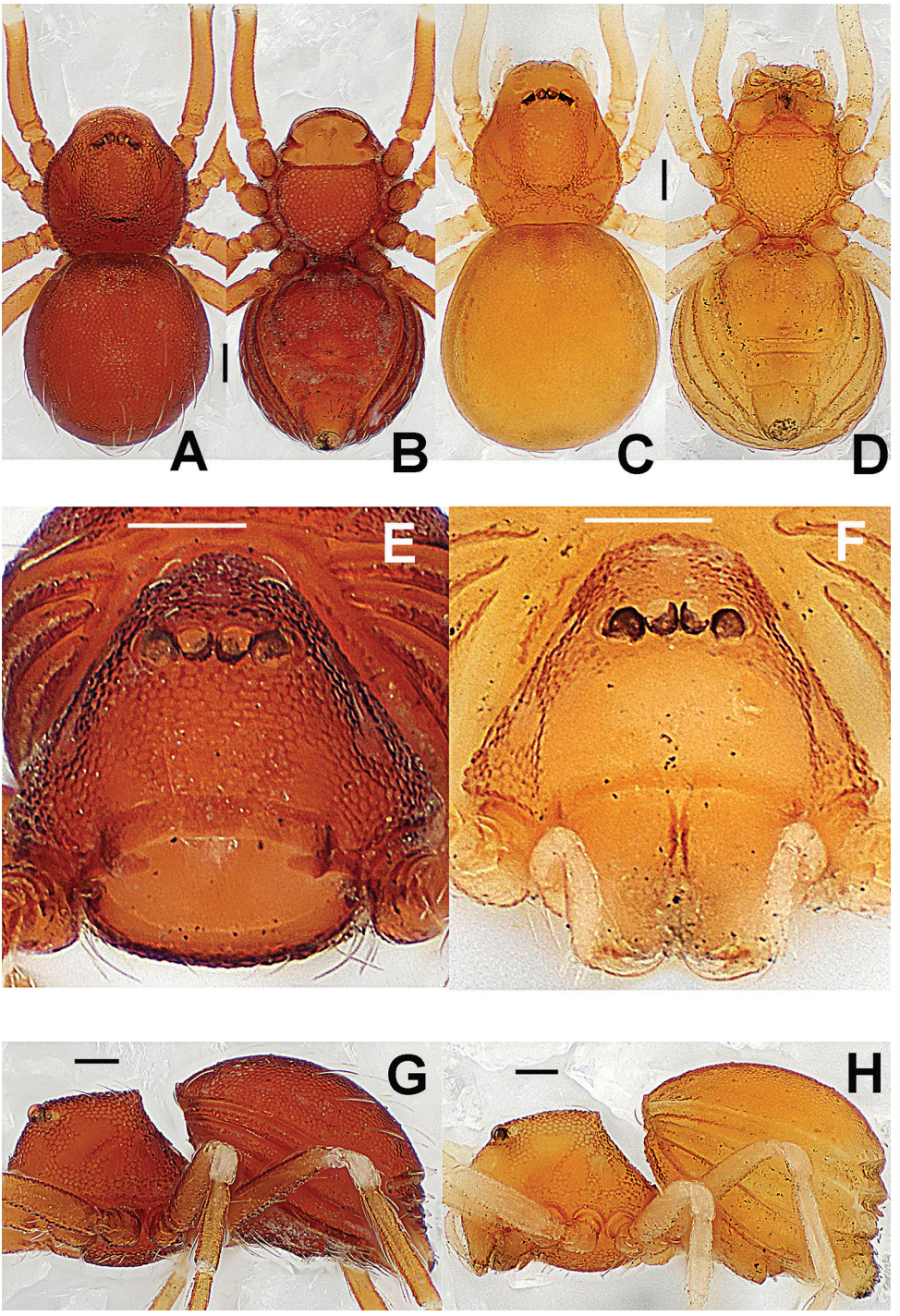

Figure 22. *Singaporemma singulare* Shear, 1978, male holotype (**A–B, E, G**) and female paratype (**C–D, F, H**). **A–D, G–H** habitus **E–F** prosoma. **A, C** dorsal **B, D** ventral **E–F** anterior **G–H** lateral. Scale bars: 0.10 mm.

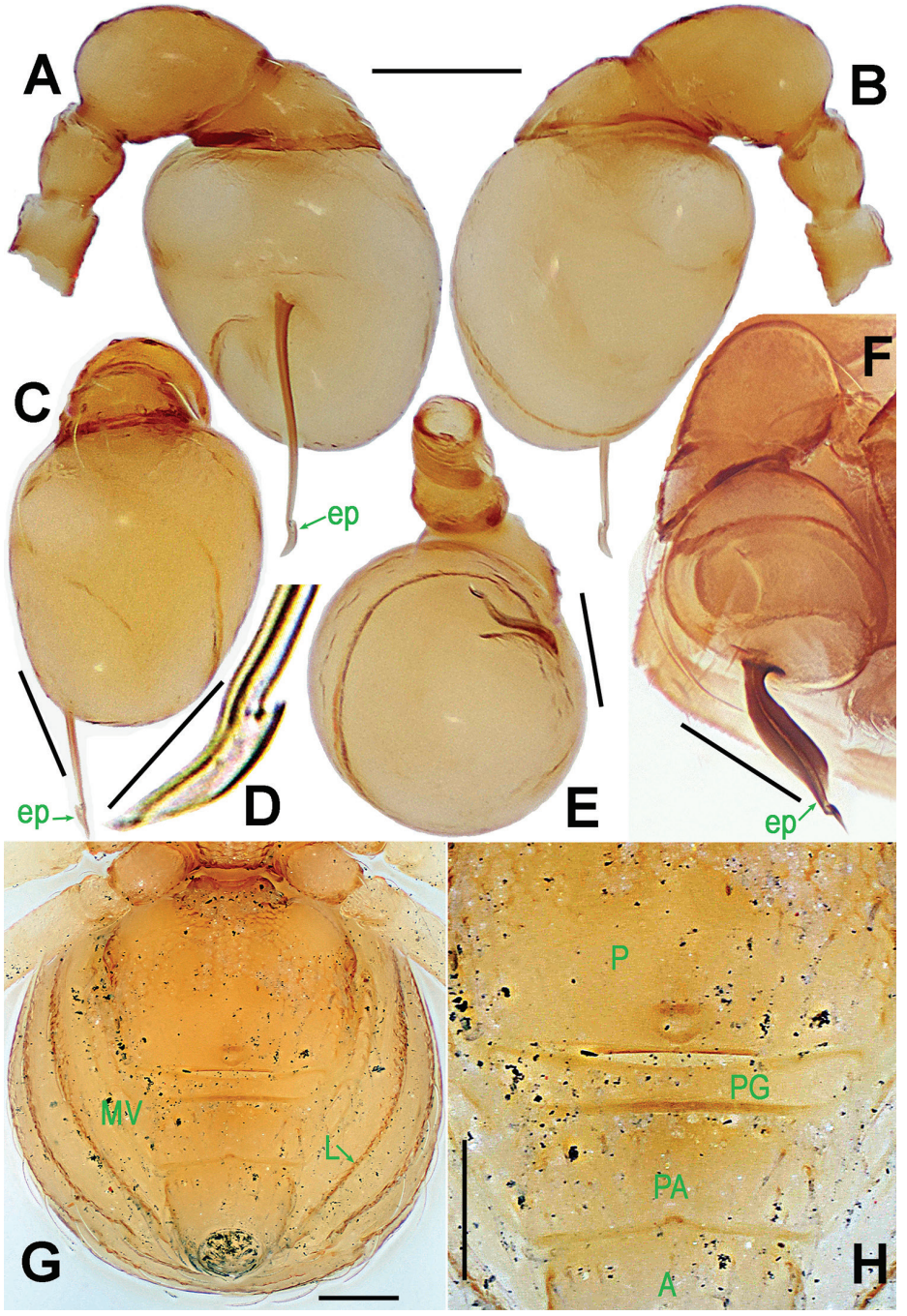

Figure 23. *Singaporemma singulare* Shear, 1978, male holotype (**A–E)** and female paratype **(G–H**), and *S. adjacens* Lehtinen, 1981, male holotype (**F**). **A–C, E–F** left palp **D** embolic end **G** opisthosoma **H** Genital area (untreated). **A, D** prolateral **B, F** retrolateral **C** anterior **E, G–H** ventral. Abbreviations: A = anal plate; ep = embolic part of apes of palpal organ; L = lateral plate; MV = median ventrolateral plate; P = pulmonary plate; PA = preanal plate; PG = postgenital plate. Scale bars: 0.10 mm, **D** 0.05 mm .

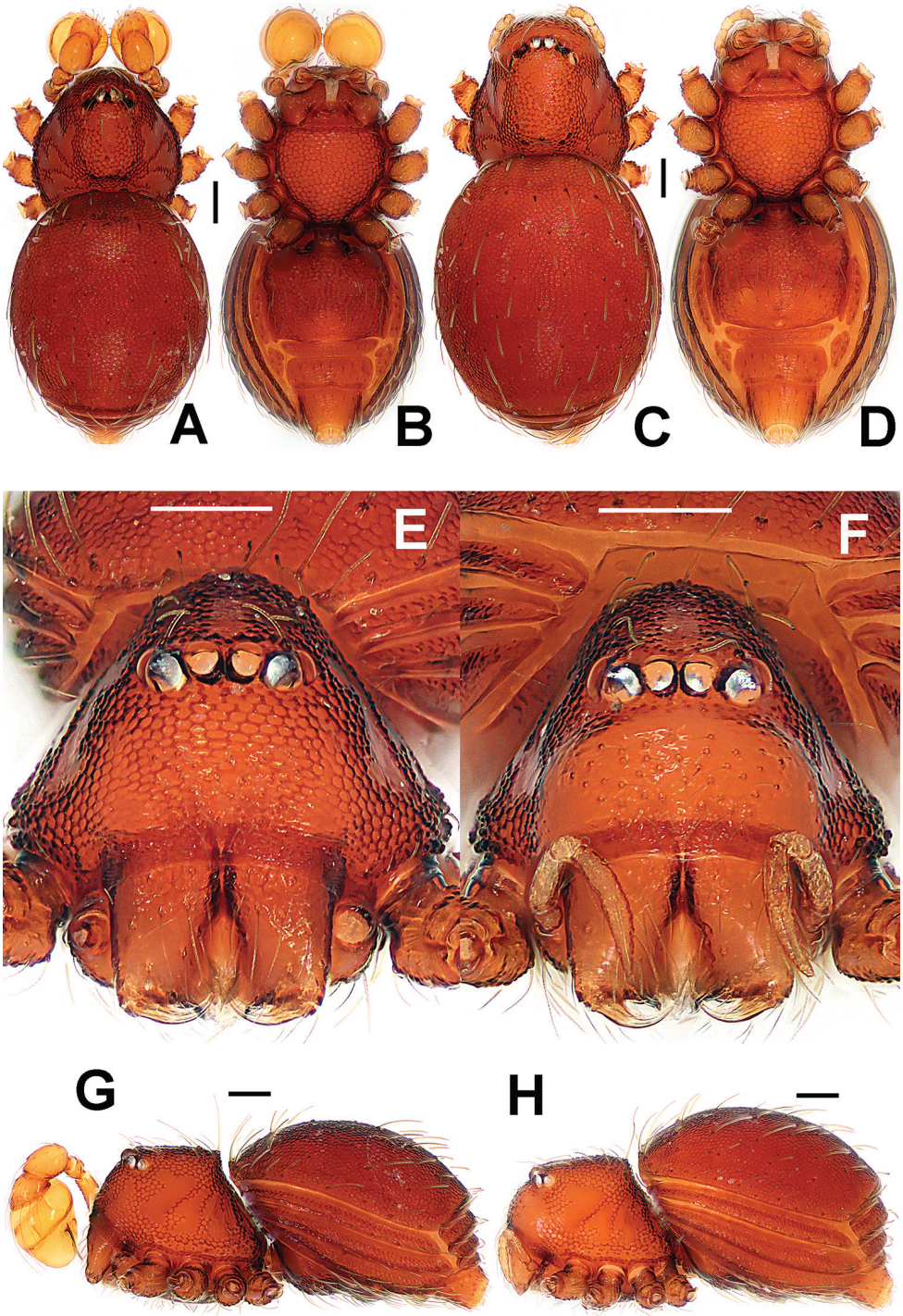

Figure 24. *Singaporemma singulare* Shear, 1978, male and female specimens from Singapore. **A–D, G–H** habitus **E–F** prosoma. **A, C** dorsal **B, D** ventral **E–F** anterior **G–H** lateral. Scale bars: 0.10 mm.

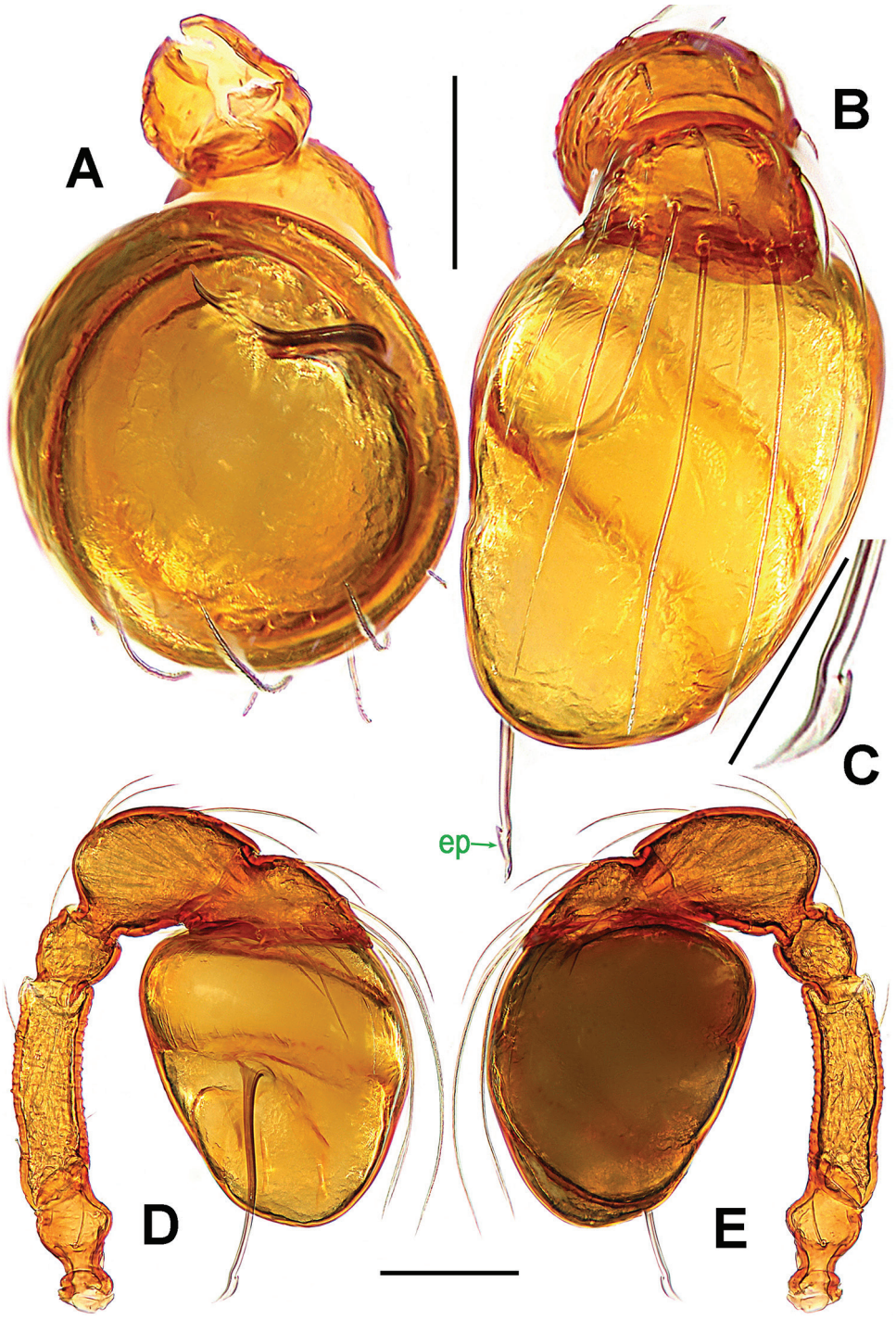

Figure 25. *Singaporemma singulare* Shear, 1978, male specimen from Singapore. **A** palpal bulb, ventral **B** left palp, anterior **C** embolic end, prolateral **D** left palp, prolateral **E** *ditto*, retrolateral. Abbreviations: ep = embolic part of apes of palpal organ. Scale bars: 0.10 mm.

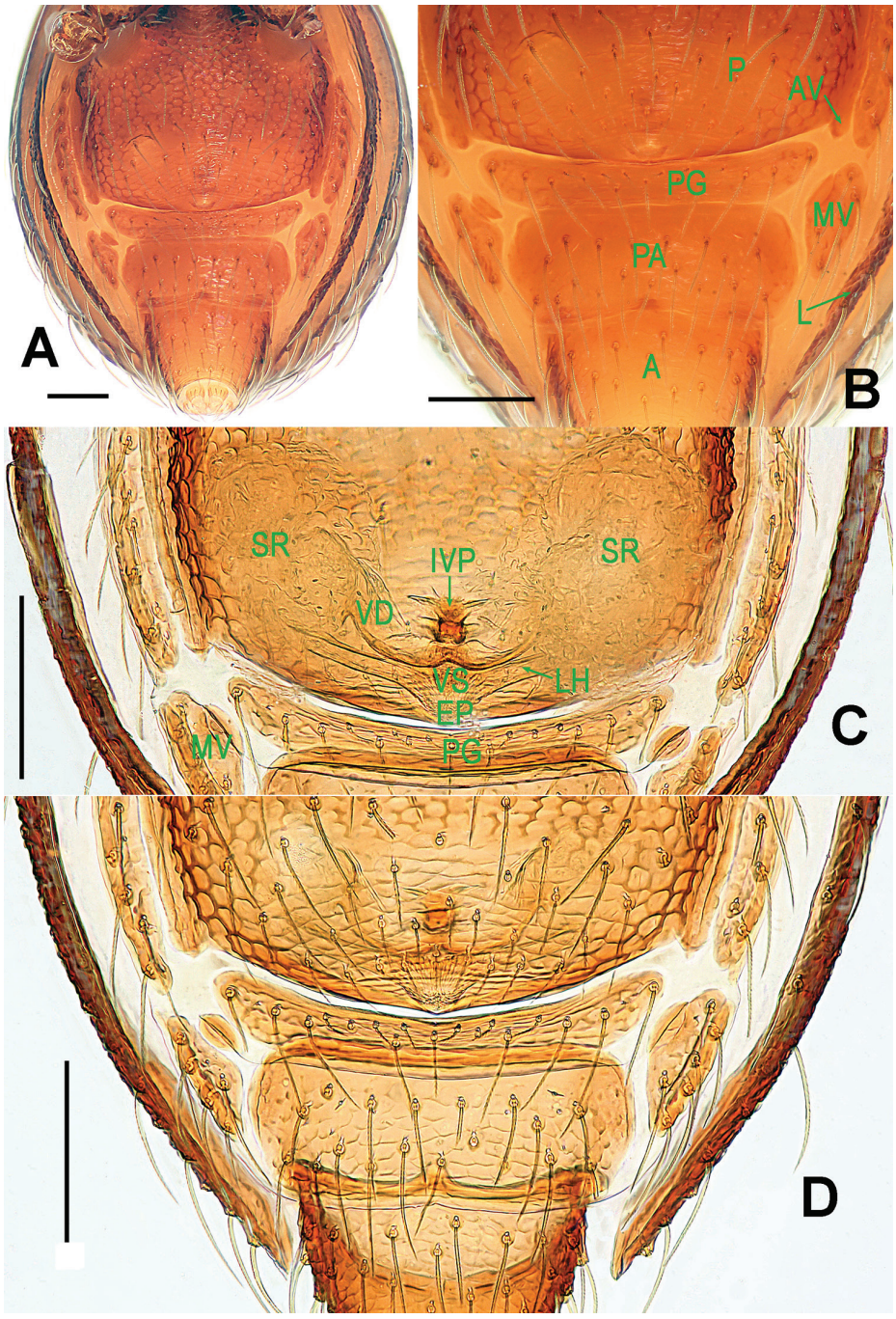

Figure 26. *Singaporemma singulare* Shear, 1978 , female specimen from Singapore. **A** opisthosoma **B** genital area (untreated) **C** cleared vulva (lactic acid-treated) **D** *ditto*. **A–B, D** ventral **C** dorsal. Abbreviations: A = anal plate; AV = anterior ventrolateral plate; EP = epigynal pit; IVP = inner vulval plate; L = lateral plate; LH = lateral horn; MV = median ventrolateral plate; P = pulmonary plate; PA = preanal plate; PG = postgenital plate; SR = seminal receptaculum; VD = vulval duct; VS = vulval stem. Scale bars: 0.10 mm.

Remarks. This species is originally described from Singapore and was designated as the type species of the genus *Singaporemma* by Shear (1978). Based on the only male specimen available at that time, he had illustrated an embolus of the right palp that was said to be "curving sharply posteriorly" (Shear, 1978: 36, figs 109–110), i.e. bent at right angles at about mid-length. Schwendinger and Košulič (2015) suggested that Shear's description of *S. singulare* was based on an atypical specimen. They noted that five other *Singaporemma* male specimens from Singapore, one of them from the type locality itself, all had "essentially straight emboli (only slightly bent ventrad) on both palps". We have since reexamined the holotype of the species and photographed its left palp (Fig. 23A–E). We can now confirm that Shear (1978) had indeed described a deformed embolus.

Genus *Sulaimania* Lehtinen, 1981

Type species. *Sulaimania vigelandi* Lehtinen, 1981 from Malaysia (see Lehtinen 1981).

Sulaimania brevis Lin & Li, sp. n.

http://zoobank.org/B62F8B41-FE14-49A7-B5E3-DC75AD24A40C
Fig. 27

Material. Holotype ♂ (LKCNHM), SINGAPORE: Bukit Timah Nature Reserve, Jungle Fall Stream, altitude 118 m, 1°21'25.4"N, 103°46'25.3"E, 21 August 2015, S. Li and Y. Tong leg. **Paratype** 1♂ (LKCNHM), same data as holotype.

Other material examined. 2♂ (NHMSU), SINGAPORE: Bukit Timah Nature Reserve, Jungle Fall Stream, altitude 118 m, N1°21'25.4", E103°46'25.3", 21 August 2015, S. Li and Y. Tong leg.

Other species studied for comparison. *Sulaimania vigelandi* (Fig. 28A–E; Lehtinen, 1981: 51, figs 126–130, 133). Paratype 1♂ (ZMUT), MALAYSIA: Johor, Kota Tinggi District, Jalan Lombong, Biological Field Station, in litter of rain forest, 31 October to 4 November 1976, P.T. Lehtinen leg.

Etymology. The specific epithet derives from the Latin word "*brevis*" = short, and refers to the short embolus; adjective.

Diagnosis. This new species shares certain characteristics of the genus *Sulaimania,* including eye arrangement, habitus, and the general configuration of the male palp similar to that of the type species, *S. vigelandi* (see Fig. 28D–E and Lehtinen 1981: 51, figs 126–130, 133). However, it may be distinguished from *S. vigelandi* by the shorter embolus without a bent embolic end (Fig. 27F–H vs. Fig. 28D–E and Lehtinen 1981: fig. 129), the larger body size, the oblong carapace (Fig. 22A vs. Fig. 28A and Lehtinen 1981: fig. 127), and the sternum with reticular margins (Fig. 22B vs. Fig. 28B).

Description. Male (holotype). Coloration: body and legs light brownish yellow.

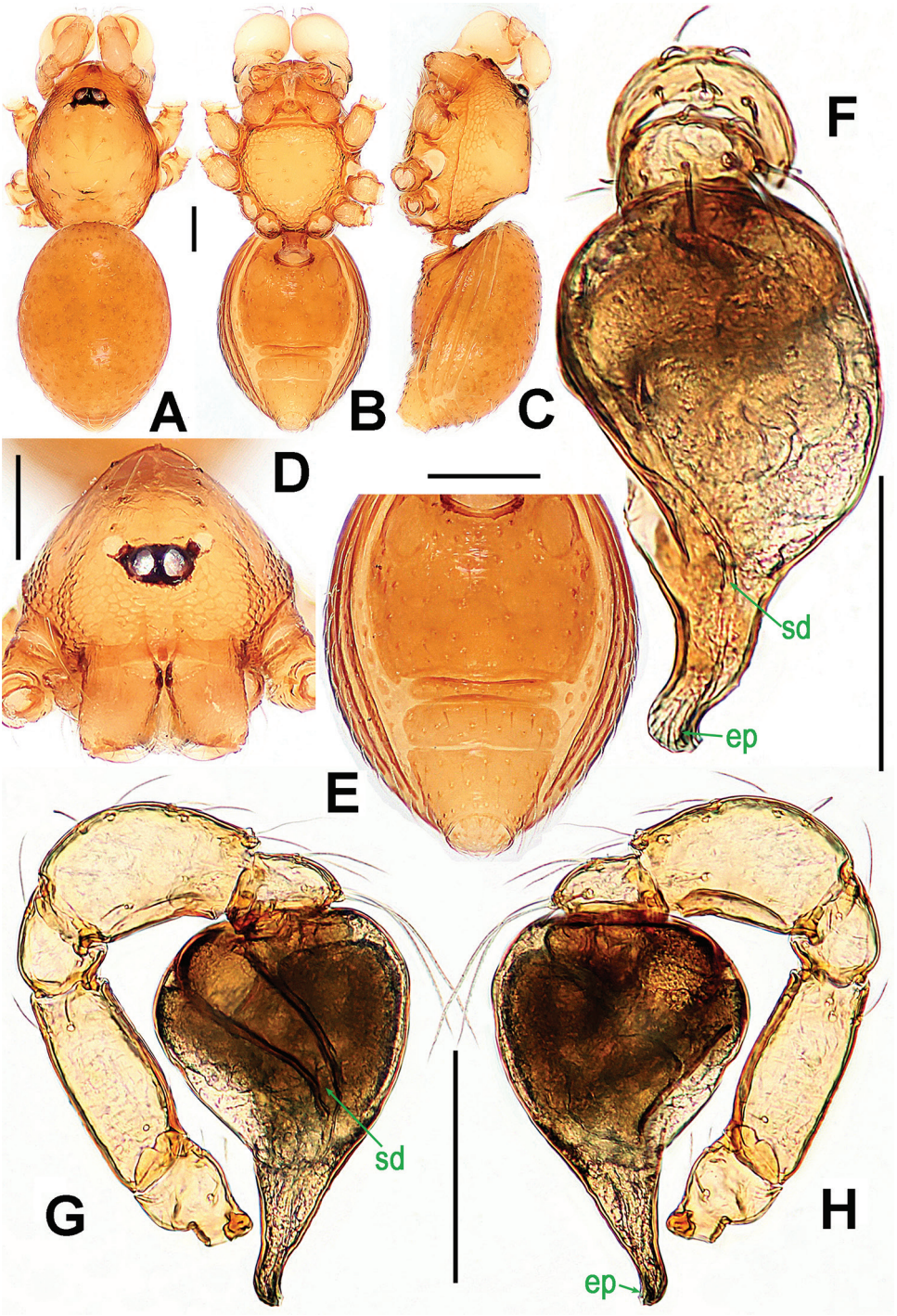

Figure 27. *Sulaimania brevis* sp. n., male holotype. **A–C** habitus **D** prosoma **E** opisthosoma **F–H** left palp. **A** dorsal **B, E** ventral **C** lateral **D, F** anterior **G** prolateral **H** retrolateral. Abbreviations: ep = embolic part of apes of palpal organ; sd = spermatic duct. Scale bars: 0.10 mm.

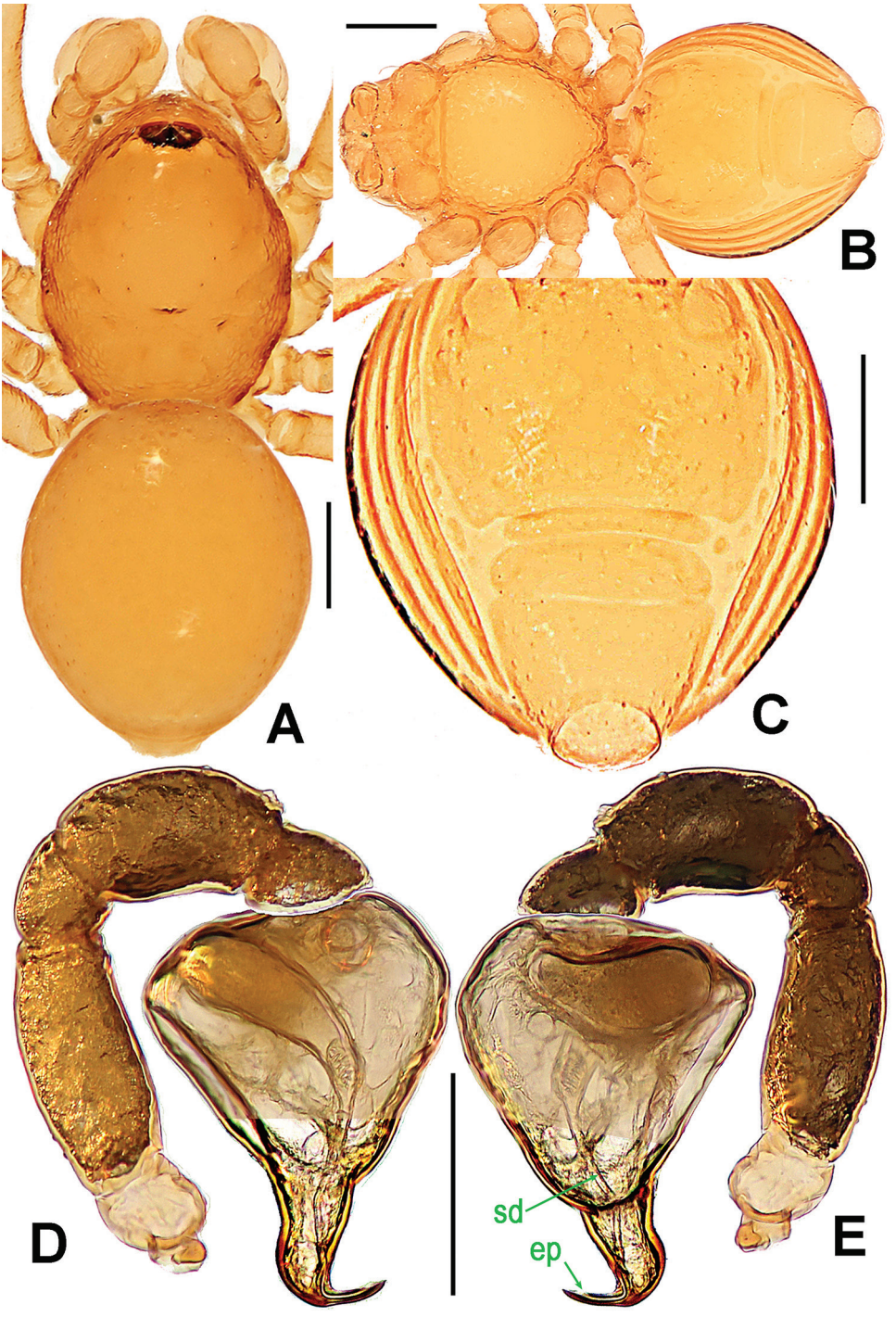

Figure 28. *Sulaimania vigelandi* Lehtinen, 1981, male paratype. **A–B** habitus **C** opisthosoma **D–E** left palp. **A do**rsal **B–C** ventral **D** prolateral **E** retrolateral. Abbreviations: ep = embolic part of apes of palpal organ; sd = spermatic duct. Scale bars: 0.10 mm.

Measurements: total length 0.78; carapace 0.46 long, 0.31 wide, 0.27 high; abdomen 0.48 long, 0.34 wide, 0.28 high; clypeus 0.10 high; sternum 0.23 long, 0.22 wide. Length of legs: I 0.78 (0.25, 0.10, 0.18, 0.12, 0.13); II 0.71 (0.22, 0.09, 0.15, 0.12, 0.13); III 0.61 (0.18, 0.08, 0.12, 0.11, 0.12); IV 0.80 (0.25, 0.08, 0.19, 0.14, 0.14). Leg formula: IV-I-II-III.

Prosoma (Fig. 27A–D): finely reticulated at clypeus and thoracic margin; cephalic area flat and smooth, bearing several long setae; AME with black base, larger than PLE in size; clypeus sharply sloping anteriorly; sternum reticulated, marginally rugose, centrally smooth, covered with sparse long setae. Legs: cuticle striated; all tibiae with 3 trichobothria, but only one at each metatarsus.

Opisthosoma (Fig. 27A–C, E): dorsal scutum long, oval, bearing short setae, anterior edge and center granulated; ventral scutum sparsely granulated, margin rugose; lateral scutum I short, perigenital scutum broad.

Palp (Fig. 27F–H): femoral cuticle faintly striated, approximately 2.5 times as long as patella; patella short, length equal to width; tibia swollen, 2 times as long as patella, 1.5 times as wide as femur, with a dorsal-distal trichobothrium; bulb inverted pyriform, surface smooth; spermatic duct looming and spiral, basally wide, and gradually tapering to embolic tip (Fig. 22F–G); embolus short, weakly sclerotized, starting from bulbous apex; embolic tip truncated, slight curving (Fig. 22F–H).

Female. Unknown.

Distribution. Singapore.

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Two psammophilic noctuids newly associated with beach plum, *Prunus maritima* (Rosaceae): The Dune Noctuid (*Sympistis riparia*) and Coastal Heathland Cutworm (*Abagrotis benjamini*) in Northeastern North America (Lepidoptera, Noctuidae)

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Abstract

Beach plum, *Prunus maritima* Marshall, 1785 not Wangenh., 1787 (Rosaceae), currently under development as a potential crop, represents an under-acknowledged host plant for several Lepidoptera that have undergone declines in the northeastern USA. The Coastal Heathland Cutworm, *Abagrotis nefascia* (Smith, 1908), and the Dune Noctuid, *Sympistis riparia* (Morrison, 1875), are unrelated species of psammophilic noctuines (Lepidoptera: Noctuidae) regularly encountered on a localized basis in coastal southern New England and New York, and whose precise life history requirements are undocumented. We inferred and, based on field observation and rearing, corroborated beach plum as a larval host for these species in Massachusetts; the plant's role in sustaining other moths with limited or contracting regional distributions is discussed. *Sympistis riparia*, belonging to a widely distributed complex of closely related species, has been associated specifically with both maritime and freshwater dunes. The eastern populations of *Abagrotis nefascia* represent a conspicuous range disjunction, separated from the nearest western populations by more than 2000 miles, and originally described by Franclemont as race *benjamini* of *A. crumbi*, both

later synonymized with *A. nefascia*. Following examination of types and other material, an evaluation of putatively diagnostic features from both the original description and our own observations, genitalic characters, and the results of provisional barcode analyses, *Abagrotis benjamini* Franclemont, **stat. rev.**, is elevated to the rank of a valid species rather than representing eastern populations of *Abagrotis nefascia* (=*crumbi*) to which it originally referred.

Keywords
Abagrotis, *Sympistis*, beach plum, *Prunus maritima,* Noctuidae, psammophile

Introduction

Distributions of herbivorous insects are often constrained by ecological or edaphic factors, in addition to the presence of their host plants. In northeastern North America, some of the most threatened terrestrial plant communities, including pitch pine-scrub oak barrens, maritime heathlands, shrublands, and grasslands, are limited to sandy, well-drained soils geologically derived from glacial outwash or ancient lake beds, sometimes collectively referred to as sandplains. Lepidoptera associated with such soils are often localized and hence prominent among those considered regionally endemic or locally threatened, and in the Northeast are heavily concentrated in coastal areas. Habitat associations and life history requirements of many sandplain insects remain undocumented, potentially due to a combination of regional variation in host specificity and the fact that observations of adult moths may belie the precise location of their larval hosts. Dunes and related habitats, such as barrier beaches, are obvious features of coastal areas, often adjacent to strictly terrestrial sandplain habitats, but are commonly overlooked as potential resources for invertebrates and may further confound our inferences of host associations. In several cases, moths' habitat associations, if not host plants themselves, have been inferred or asserted in the absence of corroborating life history information, even to the point of being reflected in the animals' common names.

The Dune Noctuid, *Sympistis riparia* (Morrison), and the Coastal Heathland Cutworm, *Abagrotis nefascia* (Smith), are cases in point. Both represent large noctuid genera with numerous cryptic species (Lafontaine 1998; Troubridge 2008) variously associated with Ericaceae, Rosaceae, and other "low plants" (cf. Crumb 1956). The highly speciose genus *Sympistis* is composed of several distinct groups of variously cryptic and localized species. The smaller genus *Abagrotis* includes a range of benign and pest species, many of them widespread, particularly in western North America. Both *S. riparia* and *A. nefascia* are considered widespread in North America but in the Northeast are heavily concentrated in or confined to coastal areas, where they are observed in association with a narrow range of plant communities primarily on sandy outwash soils. Finally, the habitat associations of these two species were thought to be well-enough understood to have yielded common names referencing dunes and maritime heathlands, respectively.

The range of *S. riparia* extends westward to California and British Columbia, and although the eastern portion includes sporadic inland occurrences and coastal areas

from New Brunswick to the mid-Atlantic, records are most heavily concentrated along the southern New England coast and the southern shores of the Great Lakes. The distribution of *Abagrotis nefascia*, in contrast, is more conspicuously centered in western North America, where it is considered a major pest of grapes (Lowery and Mostafa 2010), and the species' disjunct occurrence in the Northeast is confined almost entirely to coastal areas from New Brunswick to southern New England and New York, with the nearest conspecific populations roughly 2000 miles away. The northeastern populations were described under the trinomen *Abagrotis crumbi* race *benjamini* Franclemont 1955, and later synonymized with *nefascia* by Lafontaine (1998: 221) along with nominate *crumbi*. Because of their ecologically and geographically narrow distributions in the Northeast, both *S. riparia* and *A. nefascia* are listed as species of conservation concern in one or more New England states, including Massachusetts, where they represent two of three state-listed noctuids whose host plant associations have not been documented (the other being *Apamea inebriata* Ferguson, a possible associate of *Andropogon glomeratus* (Walt.) Britton, Sterns & Poggenb.).

This project began with the modest goal of elucidating the life history and host plant of *S. riparia*, which we all-too-slowly grew to suspect included one of the most common dune shrubs, beach plum (*Prunus maritima*). Well-known regionally for its fruit, this shrub has a checkered history of cultivation (Uva 2003), but has recently returned to the fore under development as a potential crop (Uva and Whitlow 2007). Although beach plum is recorded as a host for several oligophagous insects (bees as well as moths), herbivores of *Prunus* are in general not known for strictly monophagous feeding habits. Having guessed that any putative botanical specialization on the part of *S. riparia* was as much a function of substrate as plant chemistry, our efforts were focused accordingly on areas in the vicinity of dune systems already familiar to us for their unique occurrences of insects. These efforts led in turn to our examination of *Abagrotis* in association with beach plum. Our purpose here is thus to document our observations of host use for both species and clarify the taxonomic status of eastern *Abagrotis nefascia* based on type material as well as reared specimens and DNA barcode data.

Materials and methods

Larval and adult surveys

Despite its well-known association with both maritime and inland dunes, the host(s) of *S. riparia* have eluded lepidopterists for decades. Among the candidate host plants suggested on the basis of their prevalence in dunes was American beachgrass (*Ammophila breviligulata* Fern.), which would account for a high concentration of records along the Atlantic Coast as well as the shores of the Great Lakes. Beachgrass represents not only an important structural and stabilizing component of dunes but an important host for herbivorous insect biodiversity, including other noctuid dune associates such as *Apamea lintneri* (Grote, 1873) (Quinter 2009: 103). With over 190 species

in North America, *Sympistis* represents the most speciose noctuid genus on the continent, but relatively few species have documented larval life histories or host associations. Those which are known involve broad-leaved plants including Scrophulariaceae, Ericaceae, Caprifoliaceae and most particularly Rosaceae. Given this background, an association with a grass seemed highly unlikely: graminivory in Lepidoptera tends to be either highly conserved or associated with polyphagous feeding habits not known from any species of *Sympistis.* Feeding on grasses requires a complex of behaviors and morphological features necessary to consume and digest siliceous plant material, a suite of attributes unlikely to evolve frivolously. To our knowledge, no isolated species of graminivores are known to be nested within groups of folivores of broad-leaved plants, and an isolated *Ammophila*-feeder within a lineage dominated by Rosaceae feeders would be unique for Noctuidae, if not Lepidoptera.

With this in mind, we began our survey of potential host plants with what we surmised to be more likely candidates, the shadbushes or serviceberries *Amelanchier* Medikus. *Amelanchier* was also among the hosts recorded by Crumb (1956: 121) for *A. nefascia* (as *Abagrotis* n. sp.) (Lafontaine 1998). Although we recognized that *S. riparia* is unlikely to be strictly monophagous, it seemed plausible that the local distribution of *Amelanchier* in a range of coastal habitats, and the wider occurrence of various *Amelanchier* species would account for both the regional distribution of *S. riparia* and the more diffuse local distribution of adult moths. Based on our understanding of other *Sympistis*, we expected *riparia* to overwinter in the egg stage, and given that adults had been recorded as early as mid-June, we expected that a narrow larval development period was accommodated by feeding, at least initially, on floral tissues available in early spring. In this region, *Amelanchier* is the first flowering rosaceous shrub of the season, referred to as "shadbush" for the coincidence of its flowering with the return of shad to their freshwater spawning grounds.

As a state-listed species, observations of *S. riparia* are tracked by the Massachusetts Natural Heritage Program, and upon examining these data we initiated our survey at a series of sites near Moshup Trail in Aquinnah, Massachusetts (Figs 1–2), which at the time supported the highest known concentration of recent observations (Fig. 3a, b). This geologically unique area comprises a structurally complex mosaic of morainal heathlands, shrublands, and perched fens enclosing migrating dunes interspersed with cranberry (*Vaccinium macrocarpon* Aiton), sundew (*Drosera intermedia* Hayne) and bushy bluestem (*Andropogon glomeratus*). This unusual admixture of plant communities supports a unique assemblage of Lepidoptera (Goldstein, in prep.), including a number of regionally localized or unique occurrences. Among those species that had been recorded in significant numbers was *A. nefascia*, but given its wider distribution both in Massachusetts and on Martha's Vineyard (Fig. 4a, b), we did not initially suspect it shared a primary host plant with *S. riparia.*

A casual survey for *Sympistis* larvae on *Amelanchier* in April 2011 was timed to coincide with the plant's flowering season, but was unsuccessful. Later the same season, however, light trapping efforts at a site approximately three kilometers distant yielded adult moths in high numbers which were unprecedented in our experience. Although

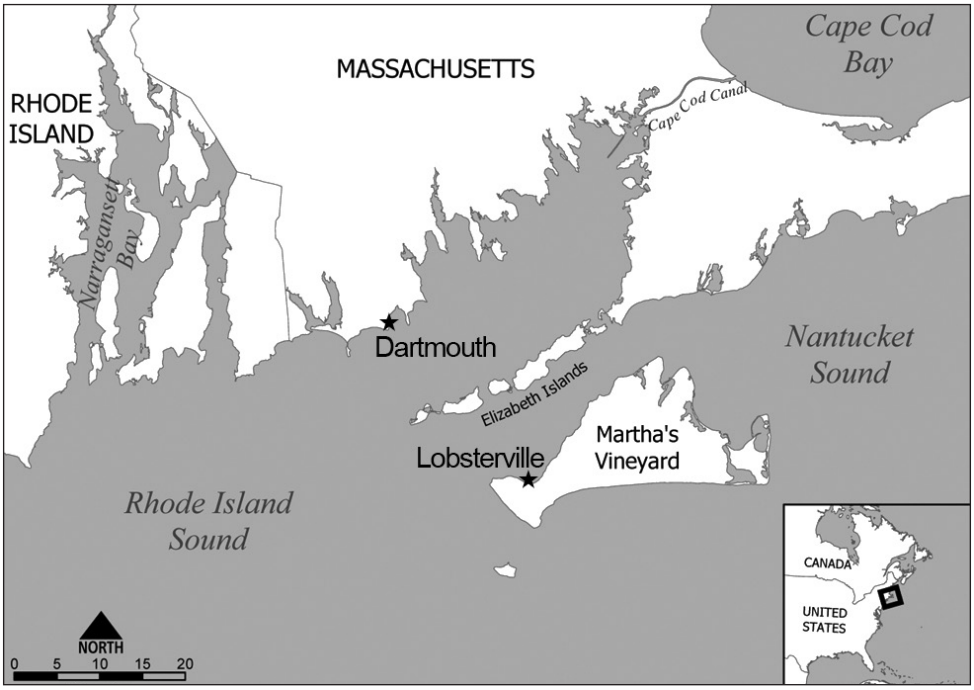


Figure 1. Section of coastal southern New England including Cape Cod and the Massachusetts offshore islands. Study sites in Aquinnah and Dartmouth marked with a ★.

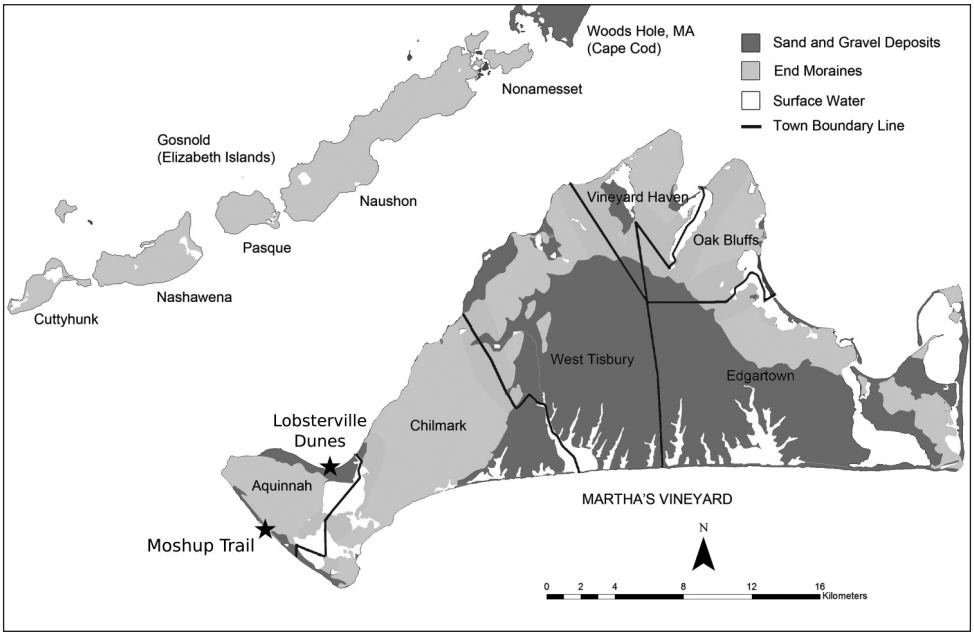


Figure 2. Martha's Vineyard and neighboring islands comprising Dukes County with indication of town boundary lines and relevant geologic formations and substrates overlain. Moshup Trail and Lobsterville Dunes sites marked with a ★.

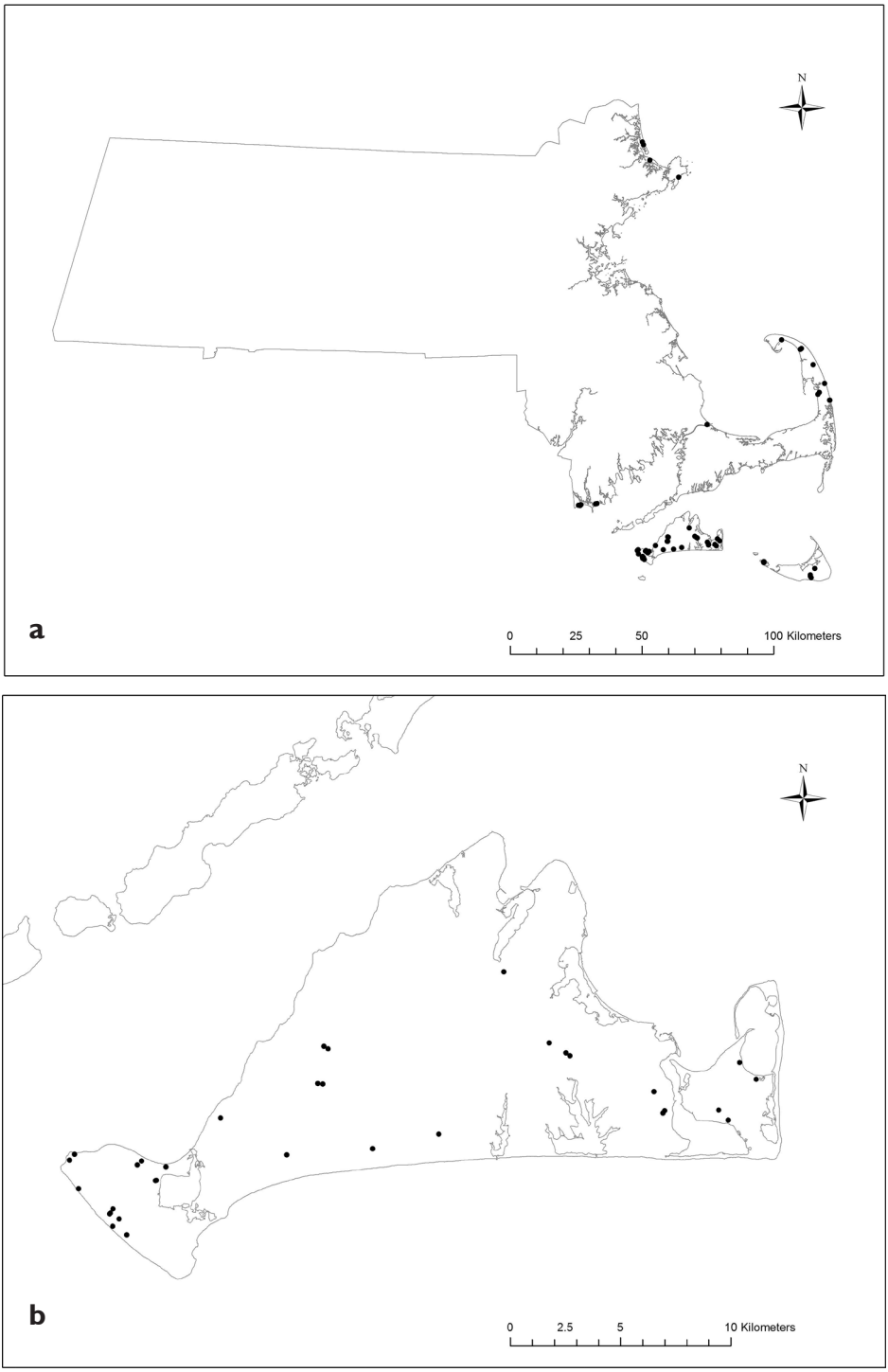


Figure 3. a Distribution of *Sympistis riparia* adults recorded from Massachusetts as of 2016 (MNHESP), **b** Distribution of *Sympistis riparia* adults recorded from Martha's Vineyard as of 2016 (MNHESP).

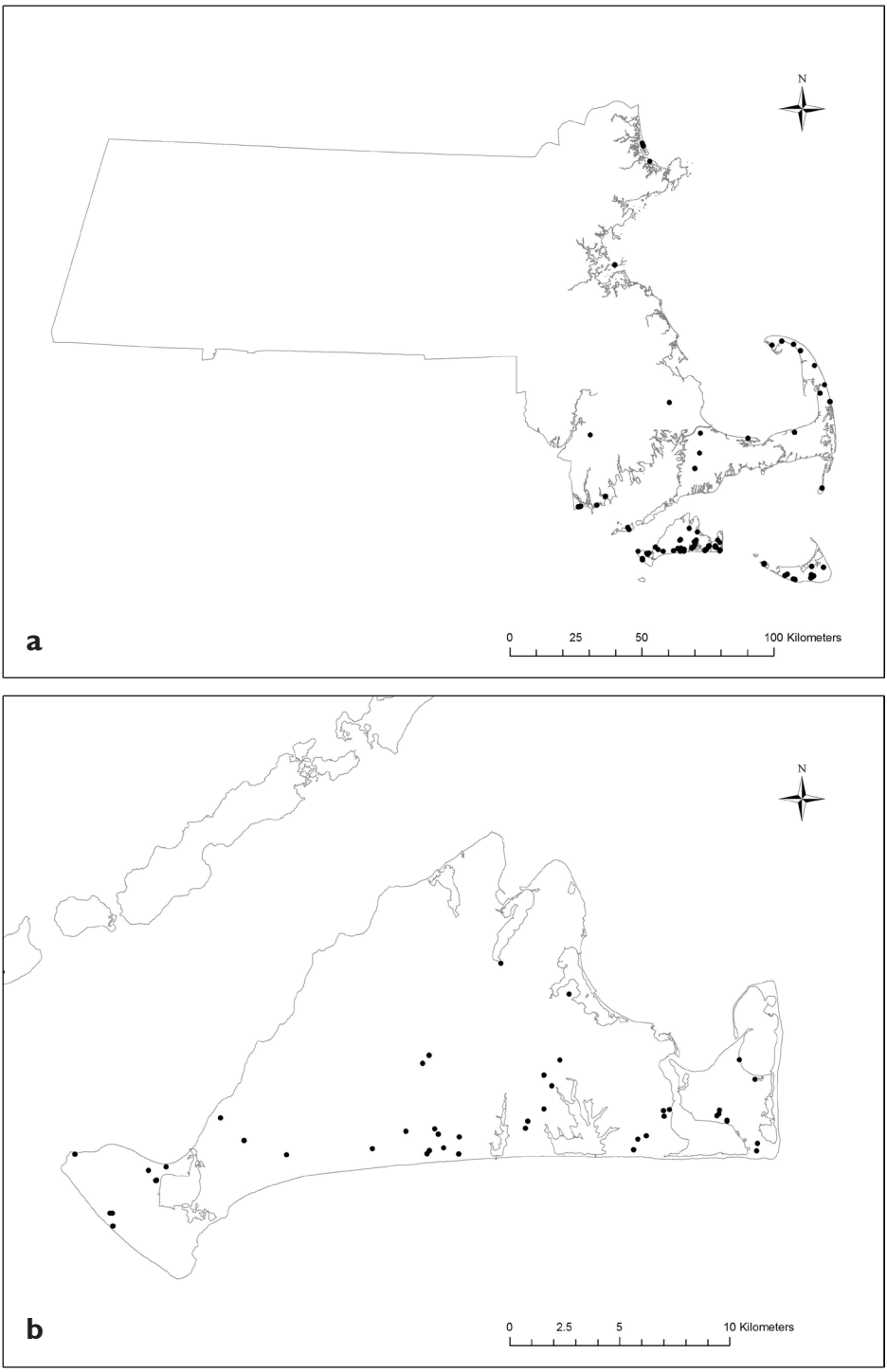


Figure 4. a Distribution of *Abagrotis benjamini* adults recorded from Massachusetts as of 2016 (MNHESP), **b** Distribution of *Abagrotis benjamini* adults recorded from Martha's Vineyard as of 2016 (MNHESP).

radically different from our initial target site in terms of its immediate plant community composition, which includes a mixture of mesic forest and morainal scrub, the trap site overlooks the expansive and under-studied peninsular dune system, Lobsterville Dunes. This site supports a structurally and floristically diverse vegetative cover lichenized and dominated by beach plum *Prunus maritima*, scrub oak *Quercus ilicifolia* Wangenh., and beach heather, *Hudsonia tomentosa* Nutt. (Fig. 5a–c), and pocketed with numerous graminoid and shrub-dominated wetlands and interdunal swales (Fig. 6a, b). The observation of high numbers of *S. riparia* in view of large, highly visible stands of beach plum led to the sudden, strong, and overdue suspicion that one of the most obvious native rosaceous dune plants had been overlooked as a potential host. It also led to our initiating survey efforts targeting beach plum caterpillars beginning the following spring (2012), at which time regular light trapping was also begun within the Lobsterville dune system itself, in parallel with ongoing light trapping at Moshup Trail. This was intended to evaluate whether *S. riparia* did indeed occur in consistently higher numbers within the strict confines of the dune system than had been observed previously. Over 100 light traps were deployed in Aquinnah, including 25 at Lobsterville Dunes between 2012 and 2016. Many specimens were prepared for study, and others frozen in liquid nitrogen for future molecular work.

Morphological examination of *Abagrotis*

Pinned specimens were examined with an incandescent light source. Genitalic preparations varied, the more recent ones following parts of Clarke (1941) and Lafontaine (2004), but using chlorazol black and mounted in euparol, the vesicae everted in water and fixed in ethanol. The most recent dissections were made following an overnight room-temperature soak in supersaturated sodium hydroxide, and examined prior to mounting. Slide preparations were examined with dissecting and compound microscopes. Photographs were made using the Microptics and Visionary Digital imaging systems and images manipulated with Adobe Photoshop® (Adobe Systems, Mountain View, CA). Measurements were made with the aid of an ocular micrometer. Forewing length was measured from the center of the axillary area up to the apex of the forewing (FW). Terminology follows Lafontaine (1998, 2004).

Systematics

Status of *Abagrotis nefascia* in the Northeast

Even a cursory examination of the known distribution of *Abagrotis nefascia* reveals an obvious disjunction between the range occupied by *nefascia* west of the Rocky Mountains and the populations restricted to a narrow band along the northeastern coast of North America. Recognizing this, Franclemont (1955) attributed the north-

Figures 5. Examples of habitats represented at the Lobsterville Dunes. **a** Immediate backdune, dominated by beach plum, also in foreground **b** Floristic diversity on a secondary dune, the vegetation including beach heather (*Hudsonia tomentosa*) and heavy lichenization **c** A section of dune supporting a mixture of salt- and wind-dwarfed oak, including scrub oak (*Quercus ilicifolia*) in the foreground.

Figures 6. Examples of wetlands interspersed among the Lobsterville Dunes. **a** Graminoid wetland edged in part with Atlantic white cedar (*Chamaecyparis thyoides* (L.) Britton, Sterns & Poggenb.) **b** Backdune wetland supporting a colony of black chokeberry (*Aronia melanocarpa* (Michx.) Elliott).

eastern populations as an infraspecific race (*benjamini*) of *Abagrotis crumbi*, which he described based on material reared by S.E. Crumb. Buckett retained both names and their ranks in his (1969c) revision of *Abagrotis* [part], but designated a specimen of *A. crumbi* as the lectotype of *A. nefascia* (Figs 13–18). Lafontaine synonymized both *A. crumbi* and *A. crumbi benjamini* with *A. nefascia,* having traced considerable confusion surrounding the identity of *Abagrotis nefascia* involving no fewer than four specific epithets: *A. nefascia* (Smith, 1908), *A. forbesi* (Benjamin, 1921), *A. crumbi* Franclem-

ont, 1955, and *A. reedi* Buckett, 1969. As Lafontaine (1998: 221) recounts, *A. nefascia* had been predominantly associated with specimens referred to *A. reedi* by Buckett (1968b), who synonymized *A. forbesi* with *A. nefascia*. Although Buckett (1968b: 19) designated a lectotype of *A. nefascia*, the specimen is conspecific with *Abagrotis crumbi* and not with *A. forbesi* as he had supposed. Further confusing matters, the lectotype genitalia that was figured by Buckett (1969: fig. 88) included the aedeagus of the *A. nefascia* lectotype, but the mislabeled valvae belonging to a specimen of *A. forbesi*. In disentangling these threads, Lafontaine reversed Buckett's synonymy, revived *A. forbesi* as a valid species and synonymized both *A. crumbi* and *A. crumbi* race *benjamini* with *A. nefascia*. The history of nomenclatural confusion, including the misspelling of *A. nefascia* as *A. negascia* in the original description, is summarized by Lafontaine (1998: 221): "*Nefascia* was treated as a senior synonym of *forbesi* by Buckett (1968c [cited herein as 1968b]) but the lectotype designated by Buckett (1968c[b]: 19) is clearly referable to the species previously known as *crumbi*. The valve shape and presence of a secondary diverticulum in the subbasal diverticulum of the lectotype are diagnostic for *nefascia* (=*crumbi*)."

Those populations formerly known as *A. crumbi* race *benjamini* have thus not been validly recognized as taxonomically distinct since 1998. Despite the formal synonymy, *benjamini* has been retained as a subspecies of *A. nefascia* outside the taxonomic literature, most prominently in the roster of protected species and subspecies in New York (New York Natural Heritage Program 2016), and Connecticut (Connecticut Dept. of Energy & Environmental Protection 2016) as *Abagrotis nefascia benjamini,* as well as at iNaturalist.org. This usage appears traceable to entries in NatureServe (http://explorer.natureserve.org/), a portal for disseminating information on taxa of conservation concern. Below we revisit the grounds for retaining or elevating the status of *A. benjamini* based on available character information, and revise its status accordingly, providing a diagnosis and re-description of the genitalia. A more detailed treatment of the larvae and life history of these animals will occupy a separate work.

Franclemont wrote in his description of race *benjamini* (1955: 46): "Similar to the typical race, but the markings are a little less distinct, and the color is generally duller. All the specimens are a rather uniform tannish brown with a contrasting purplish gray terminal area." He described the male genitalia as "somewhat larger than the typical race" and the female as having the "ductus bursae more heavily sclerotized; general characteristics similar to those of *forbesi*." The paratypes of *benjamini* comprise specimens from East New York [Brooklyn], NY; Connecticut; and Martha's Vineyard, MA. Upon close examination of these and other specimens, including a series of Massachusetts specimens and the holotypes of *A. crumbi*, *A. nefascia*, *A. forbesi*, and *A. reedi*, we noticed consistent, if subtle, differences between eastern specimens and typical *A. nefascia*. In addition to those differences in coloration noted in Franclemont's (1955) description, we focus on the configuration of spines on the vesica as well as characters adduced by Franclemont (wing pattern and sclerotization of the female ductus), Buckett (number of signa on the bursa copulatrix), and Lafontaine (secondary diverticulum in the male vesica).

Buckett (1968c) characterized *A. crumbi* in a different species group than *A. nefascia* [sensu (Buckett 1968b), not *A. nefascia* (Smith, 1908)]. Although he accurately described the terminal area of the *benjamini* forewing as fading inwardly, he characterized both *A. nefascia* and *A. crumbi* as lacking heavily sclerotized ductus bursae and, more importantly, described the bursae of *A. crumbi* and *benjamini* as having two signa, as in *Abagrotis alternata* (Grote, 1864) (Buckett 1968c: 4) versus a single signum on the bursa of *A. nefascia* (sensu Buckett 1968b: 19). We failed to observe more than one signum on any Massachusetts specimen; most of those we examined from the type series of *A. crumbi*. All the more recently collected western *A. nefascia* possessed two.

As Lafontaine suggested, subtle features of wing pattern may be unreliable indicators of phylogenetic affinity in *Abagrotis*. That said, both Franclemont and Lafontaine acknowledged trends in coloration and shading among eastern *A. nefascia* that may serve to differentiate most specimens from their western counterparts. Eastern specimens range in forewing ground color from a gray brown to brick red; we have not seen any with a dark-maroon forewing ground color.

In addition to exhibiting the secondary diverticulum putatively diagnostic for *A. nefascia* (Lafontaine, 1998: 221), the scobinate plate on the vesicae of eastern specimens tends to be less evenly denticled. The basal sclerotized patch consists of a more heteroideous series of teeth, with two or three very prominent and 5–8 much smaller (Figs 19–23). Specimens with the most conspicuous teeth tend to have them arranged in a nearly co-planar fashion, almost giving them the appearance of a circular saw blade. Western specimens typically have more than 12 teeth comprising a more graduate series of size, and always arranged in multiple rows.

Finally, we observe that the presence of signa on the female bursa has been interpreted inconsistently in this group. Buckett (1969b: 19) specifically recorded *A. nefascia* as "possessing a single signa [sic]" and *A. crumbi* (as a member of the *A. alternata* species group; Buckett 1969c: 4) as "possessing two signae [sic]." Lafontaine (1998: 205) described *Abagrotis* females as having the "bursa lacking signa...or with two signa." In the material we examined, we noted the presence of two signa, although one highly reduced, in both *A. nefascia* and *A. crumbi*, *contra* Buckett (1969c) (Fig. 24). However, we failed to observe a second signum, reduced or otherwise, on the bursae of ten female *A. benjamini* examined.

Barcode data

Interspecific variation in COI sequences in *Abagrotis* is atypically low for a noctuid genus. In the analyses of Zahiri et al. (2014), 29 of 248 genera examined account for 101 identified examples of interspecifically shared haplotypes, with *Abagrotis* accounting for more such incidents (eight) than any other genus examined except *Euxoa* (17). Therefore intraspecific divergences of less than 1% are typically considered more significant within species of *Abagrotis*. Our Massachusetts specimens, however, shared sequences with specimens from New Brunswick, and these collectively differed from a

cluster of reliably determined specimens comprising several species with shared haplotypes, including *A. nefascia*.

We typically eschew the use of distance-based analyses of character data as rigorous arbiters of either phylogenetic relationship or species diagnosis (Goldstein and DeSalle 2011). Such interpretations emulsify potential diagnostic data by masking the visualization of character state distributions on trees, and contradict principles through which statements of monophyly can be legitimately tested, especially for data as minimal as the short sequence conventionally used in DNA barcoding efforts. Instead, we view distance analyses of DNA barcodes as provisional estimates of potential species boundaries, and employ a more transparent cladistic analysis for the limited purpose of summarizing and visualizing character state distributions. In this case, the clustering of eastern "*nefascia*" from Massachusetts and New Brunswick is corroborated by a unique combination of thirteen character state changes, greater than the combined interspecific variation among specimens of seven species in the adjacent cluster. We note an analogous cluster of specimens from British Columbia determined as *A. nefascia.*

Observations of adults and larvae

In Aquinnah, high numbers of adult *S. riparia* were observed, routinely exceeding 200 specimens in a single light trap, the highest numbers appearing between 21 June and 5 July. Based on our capture dates, the flight season of *S. riparia* is somewhat protracted, extending from mid-June through early August. Females retained for obtaining eggs oviposited with varying success, most readily when presented with honey water and in the presence of beach plum foliage. Eggs proved difficult to overwinter without succumbing to desiccation or mold but efforts involving chilled hydration and aureomycin-laden artificial diet are ongoing.

Other species appearing in unusually high numbers included *A. benjamini*, the erebids *Argyrostrotis anilis* (Drury, 1773), *Catocala herodias* Strecker, 1876, and *Drasteria graphica* Hübner, 1818; and the noctuids *Schinia spinosae* (Guenée, 1852) and *Eucoptocnemis fimbriaris* (Guenée, 1852). Species apparently associated with beach plum included regular occurrences of Wild Cherry Sphinx (*Sphinx drupiferarum* J.E. Smith, 1797), which has declined in the Northeast (Wagner, 2012), and now appears confined to coastal areas (Goldstein et al. 2015). During the course of our work we observed other widespread but often scarce *Prunus*-feeding moths that have declined. In particular, both adult *Hyalophora cecropia* (Linnaeus, 1758) and cocoons on beach plum were observed frequently. Of equal ecological interest were the high numbers of moths associated with host plants other than *Prunus* or other signature dune species, and in particular species associated with scrub oak such as *Catocala herodias* and *Cicinnus melsheimeri* (Harris, 1841) (Mimallonidae).

Finally, we collected at least four species of *Abagrotis* in addition to *A. benjamini*, namely *A. alternata*, *A. brunneipennis* (Grote, 1875), *A. cupida* (Grote, 1865), and *A. magnicupida* Lafontaine, 1998, some of which have since been reared as wild-caught

larvae from beach plum. *Abagrotis benjamini* adults first appeared in late June, apparently aestivating and appearing in highest numbers later in the season, primarily in August but extending through September.

Initial efforts to locate larvae on beach plum at Aquinnah in 2012 and 2013 were less immediately successful due to a combination of seasonal conditions and timing. Larvae of *Sympistis riparia* were collected from beach plum and reared to adults at a mainland site in Dartmouth, MA on 16 and 22 May 2013 (Fig. 7a–c) and at Aquinnah on 25 May 2015 and 18–26 May 2016 (Fig. 8a–d). At both sites, *S. riparia* larvae were found alongside those of what turned out to be *Abagrotis benjamini* (Fig. 9a–d). These observations suggest a potential association of both species with beach plum, consistent with known rosaceous hosts recorded from species in both genera. Larvae were not at first found in numbers, when the beach plum was in full but early flower, suggesting a very early hatching (*S. riparia*) or emergence (*A. benjamini*). A more intensive survey over seven nights in May 2016, timed to coincide with the first flowering of beach plum, documented over thirty late-instar larvae of each species. Most larvae were observed feeding on flowers and always on low growth (<1m) after 2100h. In addition to rearing larvae of *S. riparia* and *A. benjamini* to adults (Figs 10, 11), we documented larvae of several other noctuines actively feeding on beach plum, including *Abagrotis alternata* and *Abagrotis magnicupida*.

Larvae of both *Abagrotis benjamini* and *Sympistis riparia* are subterranean by day, and we were impressed in particular by the ease with which *S. riparia* larvae move through sand. As has been observed of other *Sympistis*, larvae of *S. riparia* construct an underground cell within which to pupate. With *S. riparia,* the cell is approximately 3 cm below the surface of the sand, and lined with a thin layer of silk. For this reason, the precise time of pupation was difficult to pinpoint. But, based on our extraction of some larvae and, in the case of *Abagrotis*, their successful pupation in paper towels, the pupal period for *S. riparia* was under 20 days; that of *A. benjamini* was somewhat longer, between 25 and 33 days.

The maturity of the *S. riparia* caterpillars at the time of their observation in the field was surprising given the brief time their host had been in flower (no more than 10 days). Some *Sympistis* (e.g., *S. forbesi* Zacharczenko & Wagner, 2014, *S. piffardi* (Walker, 1862)) are known to over-winter as eggs (McCabe 1985; Zacharczenko et al. 2014), others (e.g. *S. perscripta* (Guenée, 1852)) as pupae (Wagner et al. 2011), but none as larvae. We suspect that larvae of *S. riparia* emerge prior to the expansion and initially feed on buds, the most available meristematic tissues.

Our observations of mature *Abagrotis* larvae, on the other hand, are consistent with those of Rings (1971, 1972a,b), Crumb (1956), and Lafontaine (1998: 206) to the effect that "most, if not all, species [of *Abagrotis*] pass the winter as partially grown larvae." There had been some confusion concerning the diapause of *Abagrotis* in that Buckett (1968a) characterized its members as overwintering as eggs. What makes this life history especially striking in the case of Atlantic Coast dune-dwelling caterpillars, and those at this study site in particular, is the highly disturbance-prone nature of the habitat and the intensity with which it has been visited by recent storms (Fig. 12).

Material examined.

Repository abbreviations.

The following abbreviations refer to collections from which specimen material forms the basis of this work:

AMNH	American Museum of Natural History, New York, USA
CUIC	Cornell University Insect Collection, Ithaca, New York, USA
UCDC	Bohart Museum, University of California, Davis, California, USA
USNM	National Museum of Natural History [formerly, United States National Museum], Washington, District of Columbia, USA

Beyond the history of nomenclatural confusion surrounding *Abagrotis nefascia* and its synonyms, complicating matters further is the difficulty in locating type material, some of which is incompletely labeled or lost, including the genitalic preparation of the *benjamini* holotype. Notwithstanding the apparently mixed type series of *A. nefascia* (Lafontaine 1998: 234), both the holotypes of *A. crumbi* and *A. crumbi benjamini* are unaccompanied by USNM slide numbers, and since the holotype slide of *A. crumbi* was not so labeled, it is unlikely that the *A. benjamini* slide (wherever it resides) was either. The dissection described for the holotype in Franclemont's original description (JGF 1203) appears lost, although a slide bearing the same data with an adjacent number (JGF 1202) was located.

Meanwhile, Benjamin's dissection (FHB 850), which represents the holotype slide of *A. crumbi* as per Franclemont's (1955) description, was originally labeled "Paratype" at the time of its preparation in 1934, more than twenty years prior, while another of Franclemont's paratypes (FHB 843) was labeled "Holotype" by Benjamin; the name *crumbi* having apparently originated with him, based on the type material originally reared by S.E. Crumb in 1933. Two more recent preparations made by Franclemont of male *A. crumbi*, dated 14 May 1990 (JGF 7627 and 7628), are unaccompanied by specimens either at USNM or CUIC.

However, most of Franclemont's paratype material for *A. crumbi* (24 of 27 specimens) and *A. benjamini* (5 of 8 specimens) is intact, as are series of adults and accompanying dissections of Massachusetts *Abagrotis* "*nefascia*" collected by Jones and Kimball on Martha's Vineyard and Nantucket, the dissections made subsequently by A.E. Brower.

Type material.

Abagrotis nefascia. (Figs 13a, b, 18). Lectotype (♂, AMNH): **New Mexico:** Ft Wingate NM VII.21; J.B. Smith Collection Rutgers; *Rhynchagrotis nefascia* ♂ type Sm.; Lectotype *Rhynchagrotis nefascia* Sm. By J.S. Buckett; ♂ Genitalia mounted on slide, F. H. R. no. 12,106; Slide labels: ♂ Genitalia *Rhynchagrotis nefascia* J. B. Smith Ft. Wingate, N.M. VII.27; No. 12,106 LECTOTYPE BALSAM Mounted XI.18.1963 Fred H. Rindge

Abagrotis crumbi. (Figs 15a, b, 20). Holotype (♂, USNM): **Washington:** White Swan VI-5-33; White Swan No. 3; Collector S.E. Crumb; 53; 8; ♂ gen. 850 7 Feb. 34 FHB; HOLOTYPE *Abagrotis crumbi* J.G. Franclemont. Paratypes (12 of 14 ♂, 12 of 13 ♀ designated by Franclemont): **Washington:** White Swan (5♂, 6♀); Ellensburg (6♂, 3♀); Yakima (1♂, 1♀); Tieton (1♀); Cashmere (1♀)

Abagrotis reedi. Holotype (♂, UCDC): Holotype ♂ **California:** *Abagrotis reedi* J.S. Buckett; Tecate Pk Cal San Diego Co VII-21-1963; Bill Reed Collector; 226

Abagrotis forbesi. Holotype (♂, USNM) **Utah:** *Lampra forbesi* Benj. Holotype ♂; T Spalding IX-19-7 Stockton, Ut; Barnes Collection; Slide No. 20640.

Other material examined.

Abagrotis nefascia. USA: **COLORADO** (2♀): Oak Creek Cany Col VII.12; Col. Jacob Doll.; USNM Dissection 148058♀; Glenwood Spgs Colo.; July 24–30; Barnes Collection; USNM Dissection #148049♀. **WASHINGTON** (2♂) (Figs 14a, b, 19a, b): Yakima Co. 4 mi SW Tampico 2 July 2007 BL trap, P.J. Landoldt; Barcode of Life DNA voucher specimen SampleID CCDB-28975-B03 BOLD Proc ID LNAUU1440-15; USNM Dissection 148023; USNMENT01203911; Barcode of Life DNA voucher specimen SampleID CCDB-28975-E03 BOLD Proc ID LNAUU1476-15; USNM Dissection 148024; USNMENT01203941; **WYOMING** (1♀): Converse Co. 0.3 mi S of Glenrock Mormon Canyon Rd. 7 Aug. 2002 42 51.43'N, 106 51.75'W MG Pogue at UV trap; Barcode of Life DNA voucher specimen SampleID CCDB-28975-E02 BOLD Proc ID LNAUU1475-15; USNM Dissection 148025♀; USNMENT01203940. **UTAH** (2♀): Daggett Co. Brown's Park on Green River 1 August 2005 J.D. Hooper Coll.; UT: Daggett Co. elev. 5490' 40.54' 04.2"N, 109.08' 40.3"W; USNMENT01279055; Uintah Co. 11mi NNE of Vernal 1 Sept. 1997 @ light J.D. Hooper Coll.; Unitah Co. elev. 5850' 40.35' 38.8"N, 109.25' 37.1"W; USNMENT01279080.

Abagrotis magnicupida. USA: **MASSACHUSETTS** (2♂, 2♀): West Tisbury, Manuel F. Correllus State Forest, Willow Tree Bottom, 20 July 2004; P.Z. Goldstein leg. (1♂, 3♀); USNM Dissection #148007♀; 148008♂.

Abagrotis brunneipennis. USA: **MASSACHUSETTS:** Edgartown, Manuel F. Correllus State Forest, bathtup deerstand 41°23.770'N, 70°35.550'W, 30 June 2011 @ UV trap P.Z. Goldstein (1♂).

Abagrotis alternata. USA: **MASSSACHUSETTS** (1♂, 2♀): West Tisbury, Manuel F. Correllus State Forest, Willow Tree Bottom 22 August 2000 (1♂, 1♀); USNM Dissection #148009♂; 148010♀; 20 VII 2004; USNM Dissection #148011♀.

Abagrotis benjamini Franclemont, stat. rev.

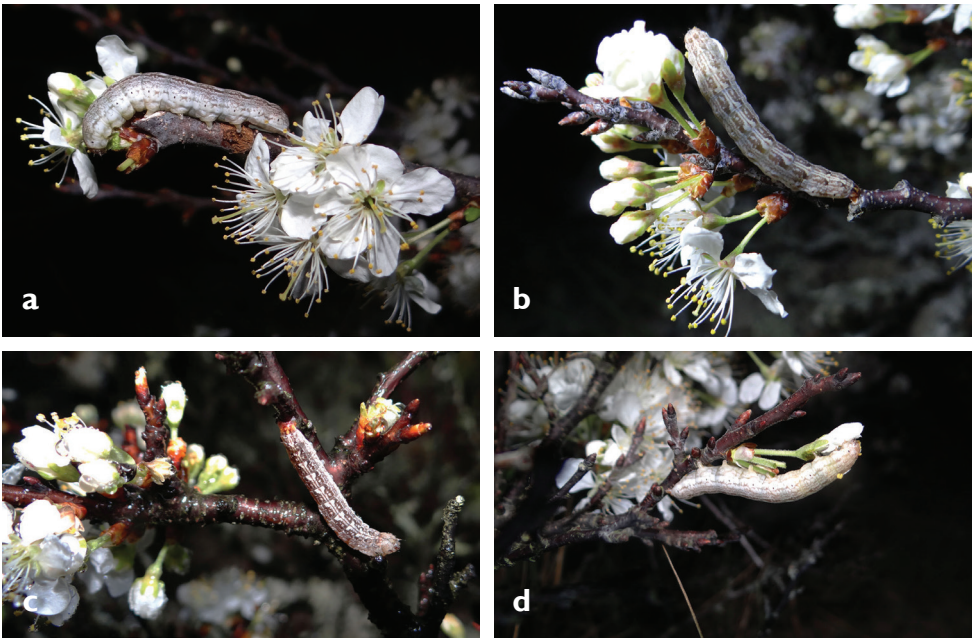
Material examined.

Type material (*Abagrotis crumbi benjamini* Franclemont, 1955)

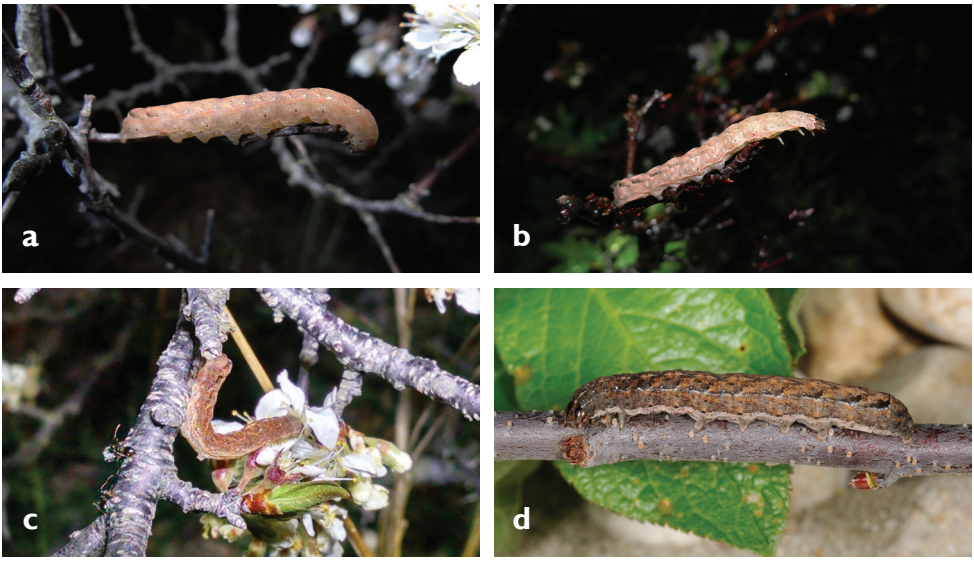
Figures 7. Larvae of *Sympistis riparia*. Dartmouth, MA, 2013 (M. Nelson).

Holotype. (Fig. 16a, b). **New York:** E.N.Y. 7.17.00; Collection BrklynMus; Acc. 17,151; ♂ Gen. #1203 F.H.B. 17 July 1935; HOLOTYPE *Abagrotis crumbi benjamini* J.G. Franclemont.

Slide labels [Paratypes]: (Fig. 22a, b) USNM 36838 ♂; ♂ Gen. #1202 *Lampra crumbi* n. sp. var. E.N.Y. A.C. Weeks 7.17.00 Coll. Brklyn Mus. F.H.B. 17 July 1935. Paratypes (2 of 5♂, 3 of 3♀ designated by Franclemont): **New York:** East New York (2♂, 1♀). **Connecticut:** East River (1♀). **Location uncertain:** "Collection J.B. Smith" (1♀).



Figures 8. a–d Larvae of *Sympistis riparia* photographed *in situ*, Aquinnah, MA, 2016 (P. Goldstein).



Figures 9. a–d Larvae of *Abagrotis benjamini* **a–c** *in situ,* Aquinnah, MA, 2016 (P. Goldstein) **d** Dartmouth, MA, 2012. (M. Nelson).

Figure 10. *Sympistis riparia* Morrison, Dartmouth, MA, *ex larva*. M.W. Nelson.

Figure 11. *Abagrotis benjamini* Franclemont, Dartmouth, MA, *ex larva.* M.W. Nelson.

Other material examined. (not including ~13 with DNA barcodes pending)
MASSACHUSETTS (28♂, 18♀): **Edgartown** (1♂, 2♀): Katama Plain 41°21.472'N, 70°31.256'W, 19 June 2010 (1♂); Wintucket Cove frost bottom 24 July 1991 (1♀); Felix Neck Wildlife Sanctuary 7 VII 1989 P.Z. Goldstein, leg. (1♀). **Aquinnah** (22♂, 15♀): East Pasture Road, 41°20.494'N, 70°47.248'W, @ UV Trap: 30 June 2013 (1♂, 1♀); 1 July 2011 (1♂); 15 July 2011 (1♂); off Moshup Trail/E. of Moshup Trail, 41.324894, -70.810698 / 41°19'29.6'N, 70°48'38.5'W (7♂, 7♀): 17 vii 2003 (3♂, 1♀) [USNM Dissection #s 148015♂; 148029♂ (Fig. 23); 148031♂]; 17 July 2015 (1♀) Barcode of Life DNA voucher specimen SampleID CCDB-28975-E01 BOLD Proc ID LNAUU 1474-15; USNM Dissection #148022♀; 31 July 2014 (1♀);

Figure 12. Damage from storm surge at Lobsterville Dunes following Hurricane Sandy, October 31, 2012 (A.O. Fisher).

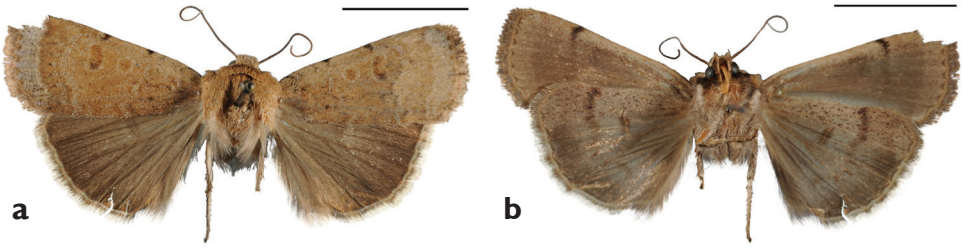


Figure 13. Lectotype of *Rhynchagrotis nefascia* Smith. **a** Dorsal **b** Ventral. Scale bars: 1 cm (Photos C. Richenbacher).

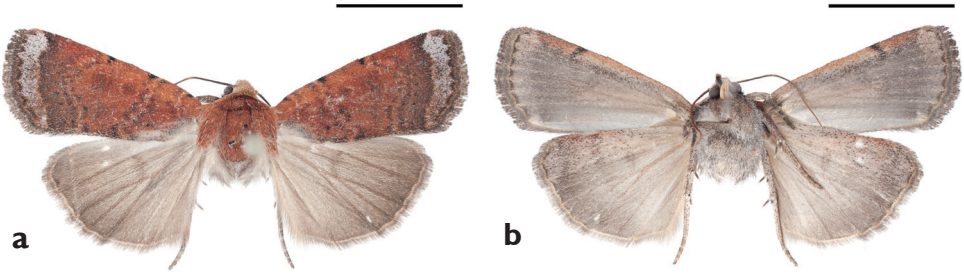


Figure 14. Holotype of *Abagrotis crumbi* Franclemont (=*nefascia*). **a** Dorsal **b** Ventral Scale bars: 1 cm (Photos B. Proshek).

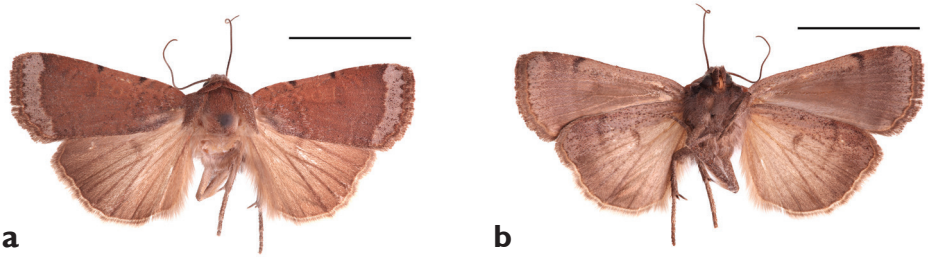


Figure 15. *Abagrotis nefascia* (Smith), Yakima, WA. P. Landoldt. **a** Dorsal **b** Ventral. Scale bars: 1 cm (Photos B. Proshek).

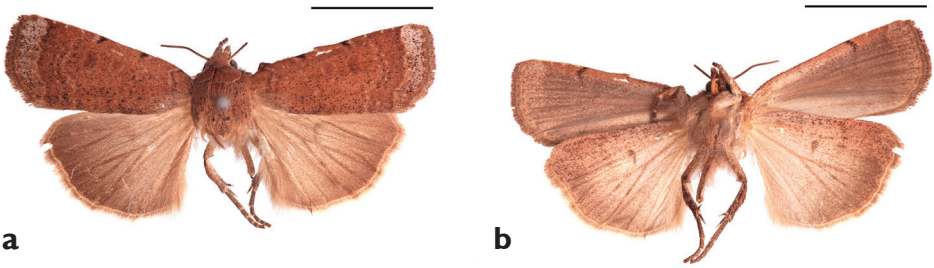


Figure 16. Holotype of *Abagrotis crumbi* race *benjamini* Franclemont. **a** Dorsal **b** Ventral. Scale bars: 1 cm (Photos B. Proshek).

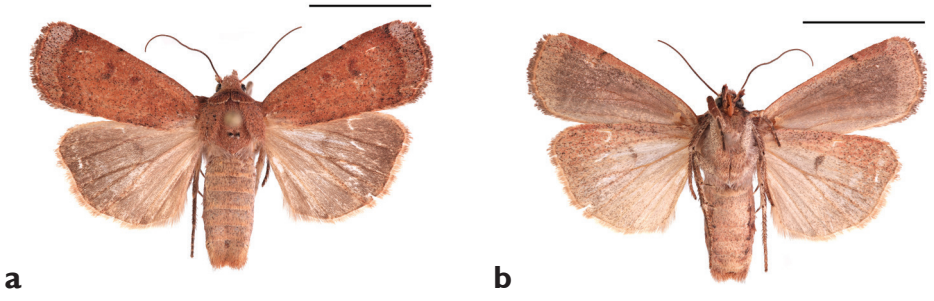


Figure 17. *Abagrotis benjamini* Franclemont, Aquinnah, MA. P.Z. Goldstein. **a** Dorsal **b** Ventral. Scale bars: 1 cm (Photos B. Proshek).

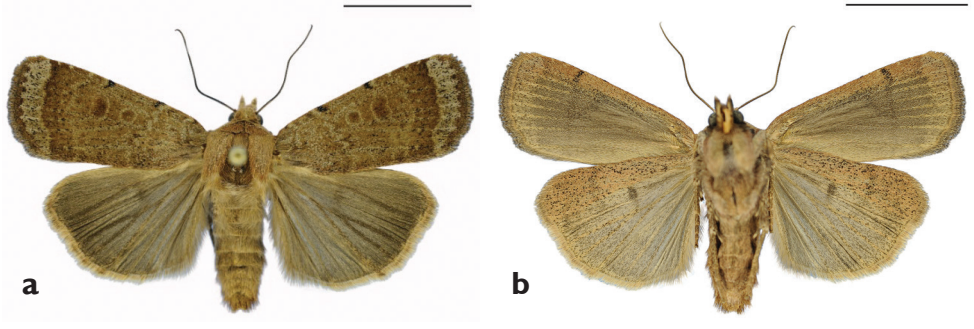


Figure 18. *Abagrotis benjamini* Franclemont, Dartmouth, MA. M.W. Nelson. **a** Dorsal **b** Ventral. Scale bars: 1 cm (Photos B. Proshek).

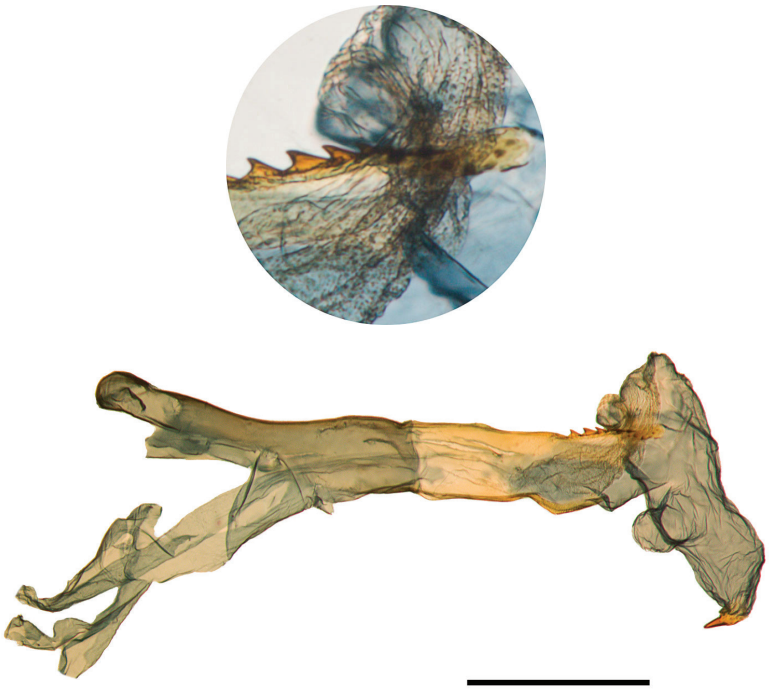


Figure 19. Everted vesica of *A. nefascia*, Holotype. Scale bar: 1 mm.

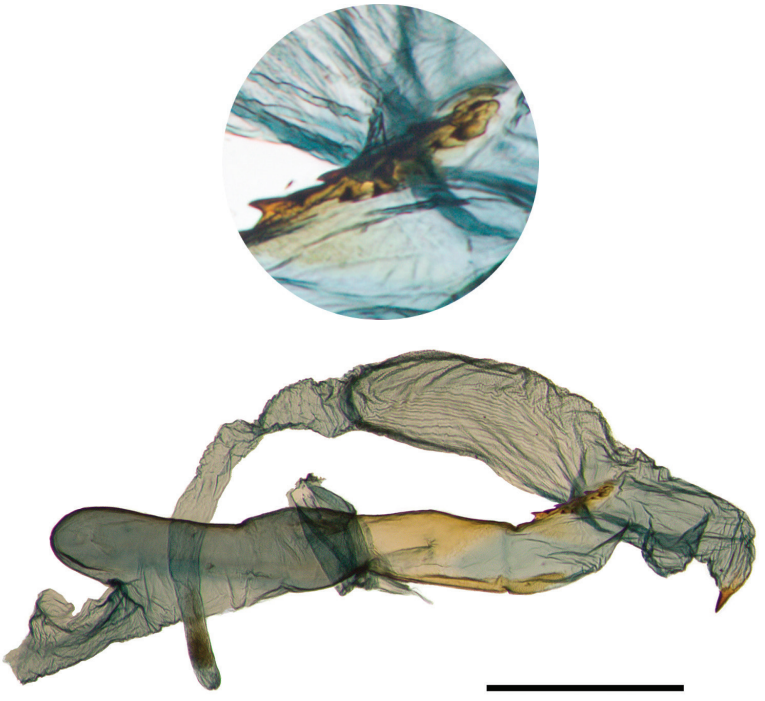


Figure 20. Everted vesica of *A. nefascia*, Yakima, WA. USNM dissection #s 148023, 148024. Scale bar: 1 mm.

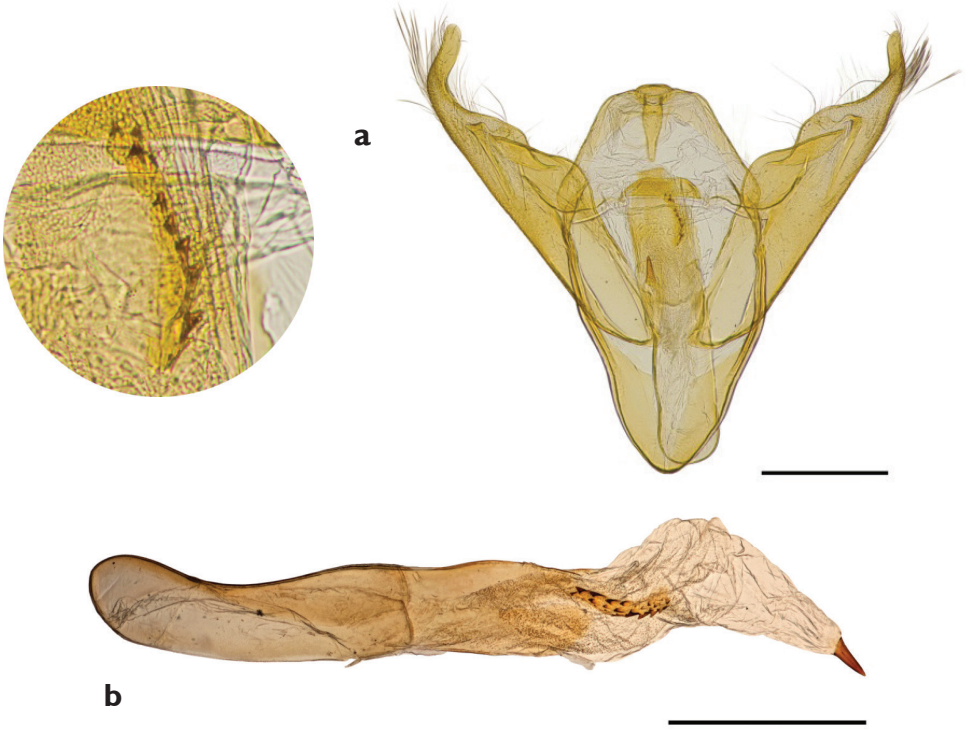


Figure 21. Genitalia of *Abagrotis crumbi* (=*nefascia*). **a** Holotype, ♂. USNM dissection #36819 **b** Paratype, ♂. USNM dissection #36821. Scale bars: 1 mm.

17 July 2003 (3♂, 4♀); 41°19.382'N, 70°48.66'W, 14 August 2002 (1♂); off Moshup Trail, above Zack's Cliffs, 41°19.384'N, 70°48.655'W, (5♂, 1♀): 10 Sept 2002 (3♂); 8 sept 2011 [USNM Dissection 148028♂] (1♂); 12 Sept 2010 (1♂, 1♀); Lobsterville, 41°20'49.7'N, 70°46'54.5'W, (7♂, 6♀): 22 Sept 2012 148026♀ (1♀); 23 August 2012 (2♀); 5 July 2015 148021♂ USNMENT01203938 (1♂); 30 Aug 2014 148019♀ USNMENT01203918 (1♀) (Fig. 17a, b); 3 October 2014 148020♀USNMENT01203909 (1♀); 19 July 2012 (3♂, 1♀); 22 Sept 2012 (3♂). **West Tisbury** (5♂, 1♀): Long Point Wildlife Refuge, maritime heathland E. of Long Cove Pond 41°21.232'N, 70°37.864'W, (2♂, 1♀) 29 June 2010 @ UV trap (USNM Dissection #s 148030♂; 148056♀; 148016♂); 41°21.571'N, 70°36.031'W, 14 June 2010 @ UV trap (1♂); 41°21.572'N, 70°36.032'W, 29 August 2010 @ UV trap (1♂); Maritime heath W. of Long Cove Pond 41°21.165'N, 70°38.328'W, 10 September 2010 @ UV trap (1♂).

Diagnosis. Forewing ground color ranging from pale gray brown to, more commonly, a rusty brown, occasionally reddish brown (paprika colored), never dark; both FW and soma more heavily peppered with black scales than in *A. nefascia*; both fasciae and subterminal line (as dashes on underside, scalloping on upperside) less conspicuous than in *A. nefascia*. Scobinate patch on vesicae of male genitalia with 12 or fewer denticles, at least two of them markedly larger than others. Female bursa with a single signum.

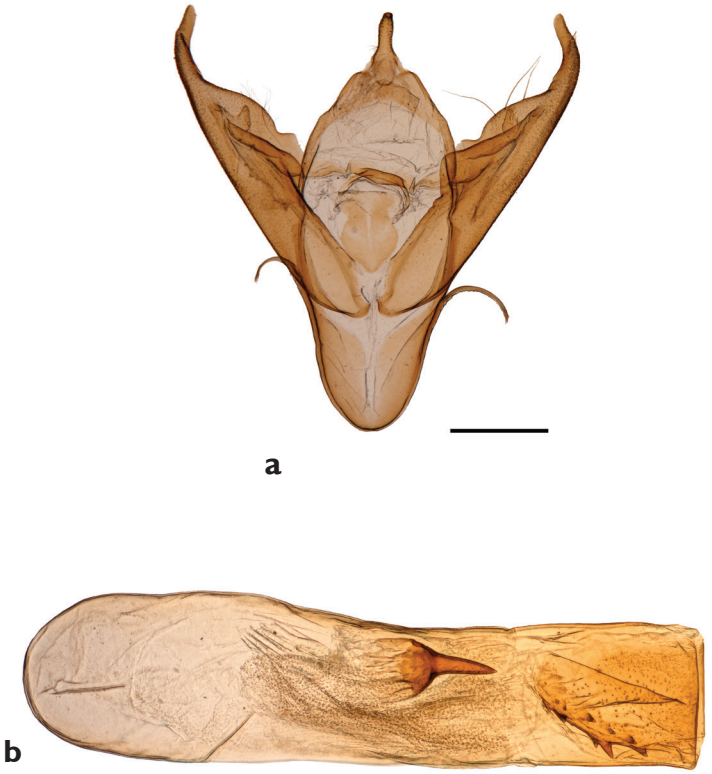


Figure 22. Genitalia of *Abagrotis crumbi benjamini* (Paratype). USNM dissection #36838. **a** Clasper **b** Phallus.

Discussion

This work began as a seemingly challenging attempt to sleuth out what turned out to be an obvious host plant, but led us to understand better the life histories and in one case the taxonomy of two apparently psammophilic species, and to explore an interesting dune fauna that complements conventional understanding of sandplain moths. *Abagrotis benjamini* is endemic to a narrow coastal area of northeastern North America. Long assumed to be an oligophagous species associated with a range of rosaceous and ericaceous plants, *A. benjamini* is here associated specifically with beach plum *Prunus maritima* for the first time based on reared wild caught larvae, observed feeding primarily on flowers. Although the distribution of beach plum in combination with known records of *A. benjamini* and documented high abundance of the moth in dunes dominated by *P. maritima* strongly suggests its role as an important host, co-occurring rosaceous plants, in particular *Amelanchier* cannot be ruled out as alternative hosts. The reinstatement of *A. benjamini* follows a mildly tortuous history of nomenclatural confusion disentangled by Lafontaine (1998) but still awaiting further exploration. Of equal, if not greater interest are the specimens from British Columbia determined as *A. nefascia.* Given the

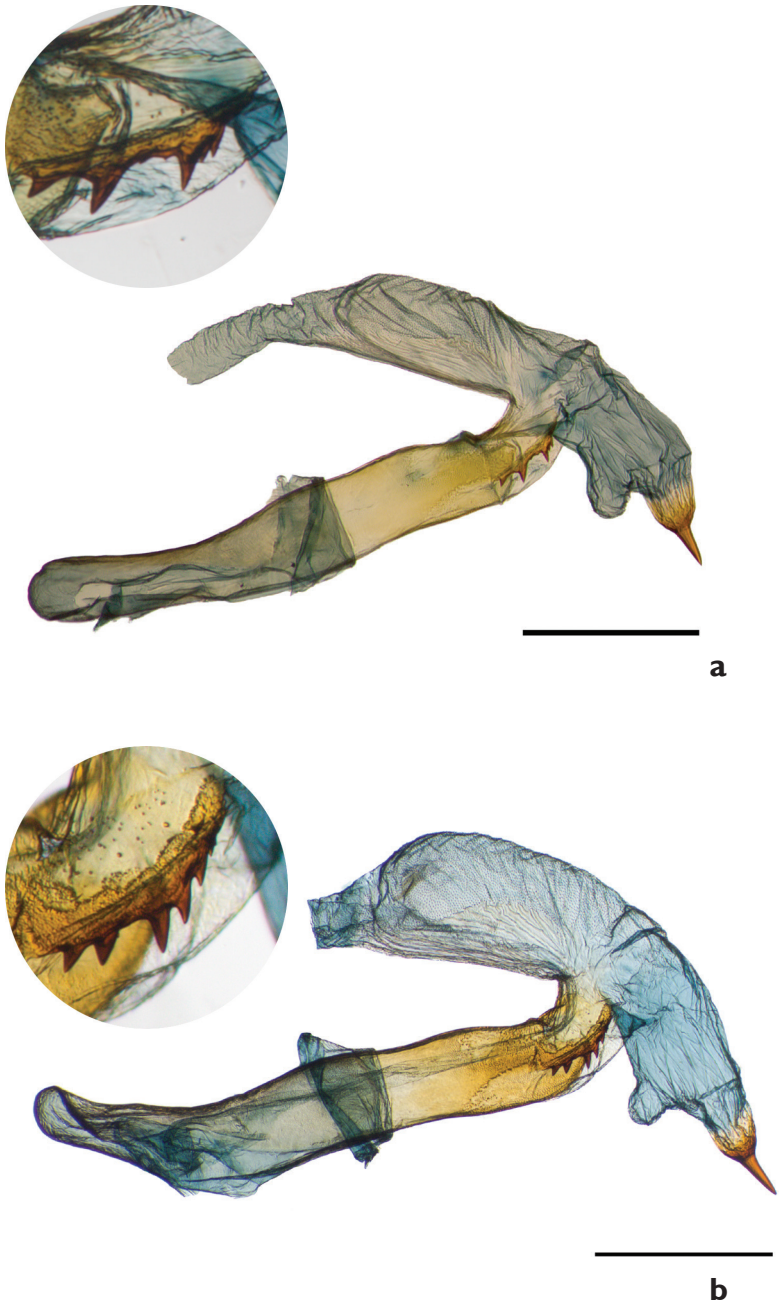


Figure 23. Everted vesica of *Abagrotis benjamini*, Aquinnah, MA. **a** USNM dissection #148029 **b** Everted vesica of *Abagrotis benjamini*, Dartmouth, MA. USNM dissection #148067. Cf. Figs 9d (larva), 11(adult), and 18a, b (adult).

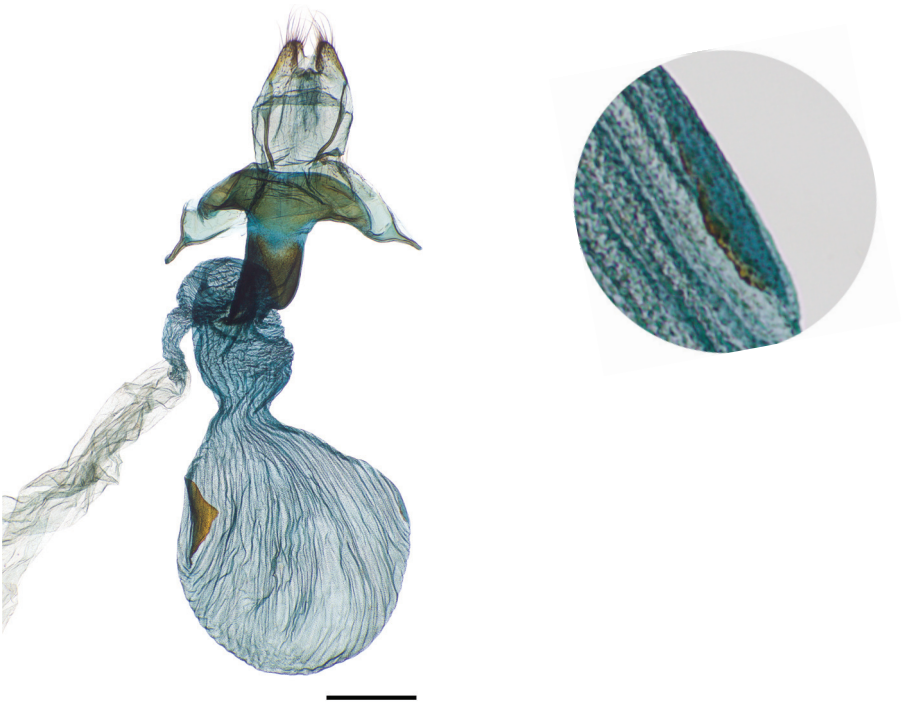


Figure 24. Female genitalia, *A. nefascia* USNM dissection #148066. Note presence of two signa, one reduced.

prominence of western *A. nefascia* among the *Abagrotis* species impacting grapes (Lowery and Mostafa 2010), further work on this complex is warranted.

The association of *Sympistis riparia* with dunes was well documented and accurate; that of *A. nefascia* with coastal heathlands less so. Our elucidation of beach plum as a host was hampered by the fact that each of these moths had been recorded from sites apparently lacking this plant, and possibly by the facultative use of other hosts, especially in the case of *A. benjamini.* We were also misled by the fact that our initial target sites at Moshup Trail, while adjacent to considerable dune habitat, were more conspicuously unique for other reasons. The possibility that the unique habitat in the immediate vicinity of our trapping records had little to do with the high numbers of *Sympistis riparia* did not at first occur to us, particularly since beach plum was not especially dominant; in fact, the heathlands at the site were consistent with what we thought we knew about *Abagrotis benjamini*.

In light of a growing body of literature devoted to desert biodiversity, including that of moths (e.g., Metzler 2014), dunes may represent an underappreciated habitat for insects and biodiversity in general. Perhaps because the habitat associations of moths are not typically highly constrained, coupled with the vagility of the adult stage,

dunes may be overlooked as primary natural areas for Lepidoptera, just as common plants may be under-appreciated as locally important hosts for narrowly distributed organisms. The ecological overlap with and geographic proximity of dunes to other threatened natural communities enhances their services as refugia, and we have noted concentrations of species nevertheless thriving in them. Additionally, the high abundance of species with host plants that are infrequent in dune habitat implies an importance of soils and vegetative structure that may transcend floristic composition, and challenge our assessments of habitat suitability.

Acknowledgments

We are especially grateful to Albert O. Fisher and Brendan O'Neill for access to sites at Moshup Trail, and to Bret Stearns and the Wampanoag Tribe of Aquinnah (Gay Head) for permission to work at Lobsterville. Clara Athearn, Sharon Briton, Kendra Buresch, Oona Carroll, Luanne Johnson, Noah Manning, John Patrick, Matt Pelikan, Estelle Perera, and Tim Simmons assisted in collecting larvae. Ben Proshek (SEL) prepared dissections and generated images; Lucrecia Rodriguez (SEL) generated images. Gary Ouellette (USDA-ARS) generated templates for several maps, and Mark Metz helped construct the final versions.. The staff at Massachusetts Natural Heritage & Endangered Species Program (MNHESP) gave permission to use distribution maps of *S. riparia* and *A. benjamini*. David Grimaldi and Courtney Richenbacher (AMNH) provided access to specimens and generated images of the Lectotype of *Lampra nefascia*. Lynn Kimsey and Steven Heydon (University of California, Davis) kindly loaned type material of *Abagrotis reedi*. Jeremy deWaard and Connor Warne (Centre for Biodiversity Genomics, Biodiversity Institute of Ontario) generously accepted DNA barcode requests and provided initial analyses for *Abagrotis*. Don Lafontaine (CNC) called our attention to the atypical DNA barcode variability in *Abagrotis*. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA; USDA is an equal opportunity provider and employer.

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Molecular phylogeny of *Atractus* (Serpentes, Dipsadidae), with emphasis on Ecuadorian species and the description of three new taxa

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Abstract

We present a molecular phylogeny of snake genus *Atractus*, with an improved taxon sampling that includes 30 of the 140 species currently recognized. The phylogenetic tree supports the existence of at least three new species in the Pacific lowlands and adjacent Andean slopes of the Ecuadorian Andes, which we describe here. A unique combination of molecular, meristic and color pattern characters support the validity of the new species. With the newly acquired data, we propose and define the *A. iridescens* species

group, as well as redefine the *A. roulei* species group. The species *A. iridescens* is reported for the first time in Ecuador, whereas *A. bocourti* and *A. medusa* are removed from the herpetofauna of this country. We provide the first photographic vouchers of live specimens for *A. multicinctus*, *A. paucidens* and *A. touzeti*, along with photographs of 19 other Ecuadorian *Atractus* species. The current status of *A. occidentalis* and *A. paucidens* is maintained based on the discovery of new material referable to these species. With these changes, the species number reported in Ecuador increases to 27, a number that is likely to increase as material not examined in this work becomes available and included in systematic studies.

Keywords
Pacific lowlands, biodiversity, Ecuador, groundsnakes, *Atractus*, phylogeny, new species

Introduction

With 140 species, *Atractus* is the most speciose snake genus in the world, with 33 new species described only during the last ten years (Uetz et al. 2016). Most of these new species have been described using a combination of meristic and morphometric characters (Passos et al. 2009a, 2016, Passos and Lynch 2010, Schargel et al. 2013, Salazar-Valenzuela et al. 2014). However, with the exception of the preliminary phylogeny presented in De Oliveira and Hernández-Ruz (2016), no studies have involved a phylogenetic approach to test species arrangements and boundaries.

One recent work by Passos et al. (2009a) evaluated the taxonomic status of *Atractus* species from the Pacific lowland of Colombia and Ecuador, using a combination of meristic, morphometric, color pattern, and hemipenial characters. These authors described three new species and provided a comprehensive review of all *Atractus* known to occur in the region. However, when referring to this work to compare previously unexamined material from Ecuador, it became clear to us that several Ecuadorian specimens of Pacific lowland *Atractus* could not be assigned to any taxa currently recognized to occur in the country. Some specimens identified as *A. medusa* (Passos et al. 2009a) matched the coloration of the first specimen reported in Ecuador by Cisneros-Heredia and Romero (2015), but they did not match the coloration of the holotype (Passos et al. 2009a). Other specimens were closer in coloration and lepidosis to *A. iridescens* (Peracca, 1860) from Colombia, and others resembled both *A. microrhynchus* (Cope, 1868) and *A. occidentalis* (Savage, 1955). To further complicate matters, the taxonomic validity of *A. occidentalis* and *A. paucidens* (Despax, 1910) was not recognized in Arteaga et al. (2013), owing to their close morphological resemblance to *A. dunni* (Savage, 1955) and *A. modestus* (Boulenger, 1894), respectively.

To resolve these pending issues and to shed light on potentially unclear species boundaries, we report on new material of *Atractus* from Ecuador, review current knowledge on the species occurring in the Pacific lowlands and adjacent Andean slopes, present a new molecular phylogeny, including most Ecuadorian species, and describe three new species of *Atractus*.

Materials and methods

Ethics statement

This study was carried out in strict accordance with the guidelines for use of live amphibians and reptiles in field research compiled by the American Society of Ichthyologists and Herpetologists (ASIH), The Herpetologists' League (HL) and the Society for the Study of Amphibians and Reptiles (SSAR). All procedures with animals (see below) were approved by the Centro de Investigación de la Biodiversidad y Cambio Climático (BioCamb) of the Universidad Tecnológica Indoamérica. They also were reviewed by the Ministerio de Ambiente del Ecuador (MAE) and specifically approved as part of obtaining the following field permits for research and collection: MAE-DNB-CM-2015-0017, granted to Universidad Tecnológica Indoamérica; and permit N°012-IC-FAN-DPEO-MAE, granted to the Museo Ecuatoriano de Ciencias Naturales. Specimens were euthanized with 20% benzocaine, fixed in 10% formalin or 70% ethanol, and stored in 70% ethanol. Museum vouchers were deposited at the Museo de Zoología of the Universidad Tecnológica Indoamérica (MZUTI).

Sampling

Tissue samples from 39 individuals representing 22 species (including three new species described here) were obtained throughout Ecuador. The majority of individuals were located by space-constrained visual examination of ground-level substrates (Campbell and Christman 1982). The remaining individuals were detected by turning over logs, rocks and other surface objects. All specimens included in the genetic analyses were morphologically identified according to Savage (1955, 1960), Cisneros-Heredia (2005), Passos et al. (2009a), Arteaga et al. (2013), Schargel et al. (2013) and Salazar-Valenzuela et al. (2014). We generated sequence data for samples marked with an asterisk under Appendix I, which includes museum vouchers at the Museo de Zoología de la Universidad Tecnológica Indoamérica (MZUTI), the División de Herpetología del Museo Ecuatoriano de Ciencias Naturales (DHMECN) and the Fundación Herpetológica Gustavo Orcés (FHGO).

Laboratory techniques

Genomic DNA was extracted from 96% ethanol-preserved tissue samples (liver, muscle tissue or scales) using a modified salt precipitation method based on the Puregene DNA purification kit (Gentra Systems). We amplified the 16S gene using the primers 16Sar-L and 16Sbr-H-R from Palumbi et al. (1991). Additionally, the Cytb gene was obtained with the primers L14910 and H16064 developed by Burbrink et al. (2000), whereas the gene coding for the subunit 4 of the NADH dehydrogenase was ampli-

fied with the primers ND4 and Leu developed by Arévalo et al. (1994). PCR reactions contained 2 mM (Cytb and ND4) or 3 mM (16S) $MgCl_2$, 200 μM dNTP mix, 0.2 μM (16S and Cytb) or 0.8 μM (ND4) of each primer and 1.25 U (16S and Cytb) or 0.625 U (ND4) Taq DNA Polymerase Recombinant (Thermo Fisher Scientific) in a 25 μL total volume. The nucleotide sequences of the primers and the PCR conditions applied to each primer pair are detailed in Appendix II. PCR products were cleaned with Exonuclase I and Alkaline Phosphatase (Illustra ExoProStar by GE Healthcare) before they were sent to Macrogen Inc (Korea) for sequencing. All PCR products were sequenced in both forward and reverse directions with the same primers that were used for amplification. The edited sequences were deposited in GenBank (Appendix I).

DNA sequence analyses

A total of 126 mtDNA sequences were used to build a mitochondrial phylogenetic tree of the genus *Atractus*. 69 were generated during this work and 57 (all available sequences for the sampled gene fragments) were downloaded from GenBank. A mitochondrial marker dataset, though less powerful to study higher-level phylogenetic relationships, was chosen because it is the most effective to successfully resolve species-level phylogenies (Patwardhan 2014). Recently published works looking to resolve intrageneric relationships within Neotropical dipsadines have done so using phylogenies that are largely based on mitochondrial data (Krysko et al. 2015, Pyron et al. 2016). Specifically, we use the gene Cytochrome-b because it is reported as the most powerful in recovering phylogenetic relationships among closely related taxa (Patwardhan 2014), which is the case for the species of *Atractus* studied here. The mitochondrial genes 16S and ND4 were used to be able to compare with *Atractus* sequences available in GenBank. Novel sequences were edited and assembled using the program Geneious ProTM 5.4.7 (Drummond et al. 2010) and aligned with those downloaded from Genbank (Appendix I) using MAFFT v.7 (Katoh and Standley 2013) under the default parameters in Geneious ProTM 5.4.7. Genes were combined into a single matrix with seven partitions, one per non-coding gene and three per protein coding gene corresponding to each codon position. The best partition strategies along with the best-fit models of evolution were obtained in PartitionFinder 1.1.1 (Lanfear et al. 2012) and jModeltest (Darriba et al. 2012) under the Bayesian information criterion. Phylogenetic relationships were assessed under a Bayesian approach in MrBayes 3.2.0 (Ronquist and Huelsenbeck 2013). Four independent analyses were performed to reduce the chance of converging on a local optimum. Each analysis consisted of 6.7 million generations and four Markov chains with default heating settings. GenBank accession numbers are listed in Appendix I. Trees were sampled every 1,000 generations, resulting in 5,000 saved trees per analysis after 25% of those were arbitrarily discarded as "burn-in." Stationarity was confirmed by plotting the–ln L per generation in the program Tracer 1.2 (Rambaut and Drummond 2003). Genetic distances between *A. esepe* and its closest morphological relatives were calculated using the uncorrected distance matrix in PAUP 4.0 (Swofford 2002).

Morphological data

Our terminology for *Atractus* cephalic shields follows Savage (1960), diagnoses and descriptions generally follow Passos et al. (2009a), and ventral and subcaudal counts follow Dowling (1951). We examined comparative alcohol-preserved specimens from the herpetology collections at the MZUTI, DHMECN, Fundación Herpetológica Gustavo Orcés (FHGO), Museum d'Histoire Naturelle de la Ville de Genève (MHNG), Museo de Historia Natural de la Escuela Politécnica Nacional (EPN), Museo de Zoología de la Pontificia Universidad Católica del Ecuador (QCAZ), National Museum of Natural History (USNM), Muséum National d'Histoire Naturelle (MNHN) and Museo de Zoología de la Universidad San Francisco de Quito (ZSFQ). (Table 1). Morphological measurements were taken with measuring tapes to the nearest 1 mm. When providing the standard deviation, we use the ± symbol. Sex was determined by noting the presence or absence of hemipenes through a subcaudal incision at the base of the tail.

Nomenclatural acts

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature, and hence the new names contained herein are available under that Code from the electronic edition of this article. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix "http://zoobank.org/". The LSID for this publication is: urn:lsid:zoobank.org:pub:7CBF7FB1-EFEA-4DC1-8F64-5BF862694AA0. The electronic edition of this work was published in a journal with an ISSN, and has been archived and is available from the following digital repositories: PubMed Central, LOCKSS.

Results

Molecular phylogeny

The overall topology and support (Fig. 1) is similar to that of Pyron et al. (2015). We consider strong support to be posterior probability values >95%, following Felsenstein (2004). Overall, there is low support for many backbone nodes. Strong support was found for the clade colored in yellow under Fig. 1.

The resulting hypotheses of species relationships for our mitochondrial phylogenetic tree supports Savage's (1960) assumption suggesting independent evolution of the 15 dorsal scale row lineage within *Atractus*, since species with this number of dorsal scale rows, like *A. elaps*, *A. roulei* and *A. duboisi*, belong to different lineages. However, the tree does show that *A. carrioni* (Parker 1930), *A. lehmanni* (Boettger 1898),

Table 1. Locality data for specimens examined in this study. Coordinates represent georeferencing attempts from gazetteers under standard guidelines, though some variation from the exact collecting locality will be present. Similarly, elevations are taken from Google Earth, and may not exactly match the elevations as originally reported.

Species	Voucher	Locality	Latitude	Longitude	Elev.
A. carrioni	DHMECN 4697	Loja, Utuana	-4.36642	-79.72483	2517
A. carrioni	DHMECN 76	Esmeraldas, Copa Quininde (in error)	0.06181	-78.72641	1688
A. carrioni	DHMECN 7668	Loja, Utuana	-4.36642	-79.72483	2517
A. carrioni	MZUTI 4194	Loja, Utuana	-4.36642	-79.72483	2517
A. carrioni	MZUTI 4195	Loja, Utuana	-4.36642	-79.72483	2517
A. duboisi	MHNG 2457.093	Napo, Chiriboga (in error)	-	-	-
A. duboisi	MNHN 0.6147	Ecuador	-	-	-
A. duboisi	MZUTI 3640	Napo, Yanayacu	-0.60071	-77.88927	1924
A. duboisi	MZUTI 62	Napo, Yanayacu	-0.59939	-77.89050	2064
A. dunni	DHMECN 12769	Carchi, Gualpi	0.86439	-78.22435	2104
A. dunni	DHMECN 2215	Pichincha, Río Cambugán	0.17697	-78.50779	1828
A. dunni	DHMECN 3527	Imbabura, Junín	0.27009	-78.64975	1688
A. dunni	DHMECN 3900	Pichincha, Tambo Quinde	0.00967	-78.66906	1870
A. dunni	DHMECN 4159	Pichincha, Pahuma	0.02757	-78.63208	1914
A. dunni	EPN 3127	Santo Domingo, Chiriboga	-0.22841	-78.76725	1813
A. dunni	EPN 3128	Santo Domingo, Chiriboga	-0.22841	-78.76725	1813
A. dunni	FHGO 375	Santo Domingo, La Favorita	-0.22833	-78.76503	1810
A. dunni	FHGO 376	Santo Domingo, La Favorita	-0.22833	-78.76503	1810
A. dunni	FHGO 379	Santo Domingo, La Favorita	-0.22833	-78.76503	1810
A. dunni	FHGO 91	Santo Domingo, La Favorita	-0.22833	-78.76503	1810
A. dunni	MHNG 2441.043	Cotopaxi, Cutzualo	-0.54497	-78.91891	1952
A. dunni	MHNG 2457.091	Santo Domingo, La Favorita	-0.22841	-78.76725	1813
A. dunni	MHNG 2464.03	Cotopaxi, Otonga	-0.41549	-79.00480	2095
A. dunni	MZUTI 2189	Pichincha, Tandayapa–Bellavista	-0.00843	-78.67619	1919
A. dunni	MZUTI 3031	Pichincha, Tandayapa Lodge	0.00268	-78.68131	1757
A. dunni	MZUTI 4097	Imbabura, Santa Rosa de Intag	0.37616	-78.46054	2077
A. dunni	MZUTI 4098	Imbabura, Santa Rosa de Intag	0.37616	-78.46054	2077
A. dunni	MZUTI 4099	Imbabura, Santa Rosa de Intag	0.37616	-78.46054	2077
A. dunni	MZUTI 4100	Imbabura, Below of Siempre Verde	0.37782	-78.46901	1974
A. dunni	MZUTI 4318	Imbabura, Toisán	0.53297	-78.52924	2286
A. dunni	MZUTI 4319	Imbabura, Toisán	0.53297	-78.52924	2286
A. dunni	ZSFQ 1513	Santo Domingo, Guajalito	-0.22875	-78.82248	1801
A. ecuadorensis	DHMECN 5101	Tungurahua, Río Verde	-1.40344	-78.30099	1507
A. elaps	DHMECN 10179	Morona Santiago, Tundayme	-3.57244	-78.46982	790
A. gaigeae	MHNG 2397.044	Morona Santiago, Macas	-2.31670	-78.11670	972
A. gigas	MHNG 2250.035	Santo Domingo, Chiriboga	-0.22841	-78.76725	1813
A. gigas	MHNG 2441.02	Cotopaxi, Otonga	-0.41549	-79.00480	2095
A. gigas	MZUTI 3286	Pichincha, Las Gralarias	-0.00807	-78.73238	1985
A. iridescens	DHMECN 2932	Esmeraldas, Canande	0.52993	-79.03541	594
A. iridescens	DHMECN 5663	Esmeraldas, Tundaloma	1.18236	-78.75250	74
A. iridescens	DHMECN 9633	Esmeraldas, Canande	0.52993	-79.03541	594
A. iridescens	EPN 13920	Carchi, Río Blanco	1.18993	-78.50413	223

Species	Voucher	Locality	Latitude	Longitude	Elev.
A. iridescens	FHGO 10443	Esmeraldas, Tsejpi	0.79930	-78.84527	152
A. iridescens	MZUTI 3548	Esmeraldas, Tundaloma	1.18166	-78.74945	74
A. iridescens	MZUTI 3680	Esmeraldas, Tundaloma	1.18166	-78.74945	74
A. iridescens	MZUTI 4178	Pichincha, Puerto Quito	0.11667	-79.26661	143
A. iridescens	MZUTI 4697	Esmeraldas, Canande	0.52993	-79.03541	594
A. iridescens	ZSFQ 191.101109	Esmeraldas, Tundaloma	1.18166	-78.74945	74
A. lehmanni	DHMECN 7644	Azuay, Reserva Yunguilla	-3.22684	-79.27520	1748
A. lehmanni	DHMECN 7645	Azuay, Reserva Yunguilla	-3.22684	-79.27520	1748
A. major	ANF 1545	Orellana, Estación Científica Yasuní	-0.67781	-76.39819	246
A. major	DHMECN 8343	Sucumbíos, Bloque 27	0.32273	-76.19369	272
A. major	MNHN 0.6149	Ecuador	-	-	-
A. major	MZUTI 4973	Zamora Chinchipe, Maycu	-4.38030	-78.74584	981
A. microrhynchus	DHMECN 2536	El Oro, Buenaventura	-3.65467	-79.76794	524
A. microrhynchus	DHMECN 2586	El Oro, Buenaventura	-3.65467	-79.76794	524
A. microrhynchus	FHGO 897	El Oro, Zambo Tambo	-3.67861	-79.68001	1014
A. microrhynchus	MHNG 2307.017	El Oro, El Progreso	-3.26998	-79.73452	176
A. microrhynchus	MHNG 2397.019	El Oro, El Progreso	-3.26998	-79.73452	176
A. microrhynchus	MHNG 2397.02	El Oro, El Progreso	-3.26998	-79.73452	176
A. microrhynchus	MHNG 2397.021	El Oro, El Progreso	-3.26998	-79.73452	176
A. microrhynchus	MHNG 2459.052	El Oro, El Progreso	-3.26998	-79.73452	176
A. microrhynchus	MZUTI 4122	Manabí, Jama Coaque	-0.11556	-80.12472	299
A. microrhynchus	MZUTI 5109	Los Ríos, Río Palenque	-0.59273	-79.36369	163
A. microrhynchus	QCAZ 1219	Loja, Olmedo	-3.94994	-79.66667	1545
A. microrhynchus	USNM 285473	Los Ríos, Rio Palenque	-0.58333	-79.36667	173
A. microrhynchus	USNM 285474	Los Ríos, Rio Palenque	-0.58333	-79.36667	173
A. modestus	DHMECN 3859	El Oro, Piñas	-3.68041	-79.68253	1019
A. modestus	EPN 13916	Carchi, Chical	0.90327	-78.16201	1437
A. modestus	FHGO 2936	Pichincha, Maquipucuna	0.11757	-78.67446	1490
A. modestus	FHGO 44	Pichincha, Maquipucuna	0.11757	-78.67446	1490
A. modestus	MHNG 2397.041	Cotopaxi, Las Pampas	-0.44036	-78.96663	1590
A. modestus	MZUTI 4760	Pichincha, Gualea	0.08536	-78.74092	1557
A. multicinctus	MZUTI 5106	Esmeraldas, Canandé	0.52581	-79.2088	310
A. occidentalis	EPN 13077	Pichincha, Mindo	-0.04872	-78.77520	1277
A. occidentalis	FHGO 385	Santo Domingo, La Favorita	-0.22833	-78.76503	1810
A. occidentalis	MHNG 2252.079	Cotopaxi, Las Pampas	-0.44036	-78.96663	1590
A. occidentalis	MHNG 2307.068	Pichincha, Tandapi	-0.41522	-78.79728	1455
A. occidentalis	MHNG 2397.028	Cotopaxi, Las Pampas	-0.44036	-78.96663	1590
A. occidentalis	MHNG 2411.085	Pichincha, Tandapi	-0.41522	-78.79728	1455
A. occidentalis	MHNG 2411.086	Pichincha, Tandapi	-0.41522	-78.79728	1455
A. occidentalis	MHNG 2441.044	Pichincha, Nanegalito	0.06181	-78.72641	1688
A. occidentalis	MZUTI 1385	Pichincha, Yellow House	-0.04492	-78.75843	1504
A. occidentalis	MZUTI 2649	Pichincha, Yellow House	-0.05199	-78.76923	1325
A. occidentalis	MZUTI 2650	Pichincha, Yellow House	-0.04371	-78.75351	1520
A. occidentalis	MZUTI 3323	Pichincha,Las Gralarias	-0.00615	-78.73381	1985
A. paucidens	DHMECN 11980	Pichincha, Pedro Vicente Maldonado	0.05361	-78.92109	938
A. paucidens	DHMECN 3975	Santa Elena, Comuna Loma Alta	-1.83442	-80.70291	72

Species	Voucher	Locality	Latitude	Longitude	Elev.
A. paucidens	EPN 8729	Santo Domingo, Finca La Esperanza	-0.27160	-79.10568	616
A. paucidens	EPN 8730	Santo Domingo, Finca La Esperanza	-0.27160	-79.10568	616
A. paucidens	EPN 8731	Santo Domingo, Finca La Esperanza	-0.27160	-79.10568	616
A. paucidens	EPN 8732	Santo Domingo, Finca La Esperanza	-0.27160	-79.10568	616
A. paucidens	MHNG 2309.065	Pichincha, Puerto Quito	0.11667	-79.26661	143
A. paucidens	MNHN 1906.245	Santo Domingo, Santo Domingo	-0.25351	-79.17297	554
A. paucidens	MZUTI 5102	Pichincha, Río Cinto	-0.09070	-78.80299	1409
A. paucidens	MZUTI 5104	El Oro, Buenaventura	-3.65467	-79.76794	524
A. paucidens	MZUTI 5105	Pichincha, Río Cinto	-0.09070	-78.80299	1409
A. resplendens	MZUTI 3996	Tungurahua, Puntzan	-1.41359	-78.40951	1962
A. roulei	MZUTI 4503	Chimborazo, Vicinity of Tixán	-2.16174	-78.81227	2892
A. roulei	MZUTI 4544	Chimborazo, Vicinity of Tixán	-2.16174	-78.81227	2892
A. roulei	QCAZ 6256	Azuay, Hierba Mala	-2.76439	-79.43816	3029
A. roulei	QCAZ 7887	El Oro, Guanazán	-3.44139	-79.49417	2596
A. roulei	QCAZ 7902	El Oro, Guanazán	-3.44139	-79.49417	2596
A. roulei	QCAZ 9643	El Oro, Guanazán	-3.44139	-79.49417	2596
A. roulei	QCAZ 9652	El Oro, Guanazán	-3.44139	-79.49417	2596
A. savagei	DHMECN 3800	Carchi, Río la Plata	0.82381	-78.04584	2256
A. savagei	MZUTI 4916	Carchi, Chilma Bajo	0.86495	-78.04978	2058
A. snethlageae	MNHN 1906.244	Morona Santiago, Gualaquiza	-3.39914	-78.57859	835
A. snethlageae	MNHN 1994.1171	Morona Santiago, Gualaquiza	-3.39914	-78.57859	835
A. touzeti	ANF 2390	Pastaza, Tzarentza	-1.35696	-78.05814	1355
A. trilineatus	MNHN 1898.313	Imbabura, Paramba (in error)	0.81671	-78.35002	698
A. trilineatus	MNHN 1898.314	Imbabura, Paramba (in error)	0.81671	-78.35002	698
A. typhon	DHMECN 9632	Esmeraldas, Canandé	0.52993	-79.03541	594
A. typhon	FHGO 10438	Esmeraldas, Gualpi	0.78173	-79.15993	63
A. typhon	FHGO 10439	Esmeraldas, Gualpi	0.78173	-79.15993	63
A. typhon	MZUTI 3284	Esmeraldas, Itapoa	0.51307	-79.13400	321

A. roulei (Despax, 1910) and *A. pyroni* sp. n., species with 15 scale rows, form a monophyletic group that includes two more species than was suggested by Passos et al. (2013) when naming the *A. roulei* species group (Fig. 1).

Atractus gigas (Myers and Schargel, 2006), *A. modestus*, *A. paucidens*, *A. savagei* (Salazar-Valenzuela et al. 2014), *A. typhon* (Passos et al., 2009a) and *A. zidoki* (Gasc and Rodrigues, 1979) form a poorly supported clade that does not include *A. microrhynchus* and *A. iridescens*, as was suggested by Passos et al. (2009a) when naming the *A. paucidens* species group (Fig. 1). Six species, *Atractus cerberus* sp. n., *A. dunni*, *A. esepe* sp. n., *A. iridescens A. microrhynchus*, and *A. occidentalis*, form a strongly supported clade sister to the *A. paucidens* species group. Here, we name this lineage as the *A. iridescens* species group (Fig. 1).

Atractus occidentalis forms a strongly supported distinct lineage, sister to *A. microrhynchus*. Together, these two species are sister to *A. dunni*. *Atractus typhon* is shown to be the strongly supported sister lineage of *A. gigas*, as is the case for a relationship between *A. roulei* and *A. pyroni* sp. n.

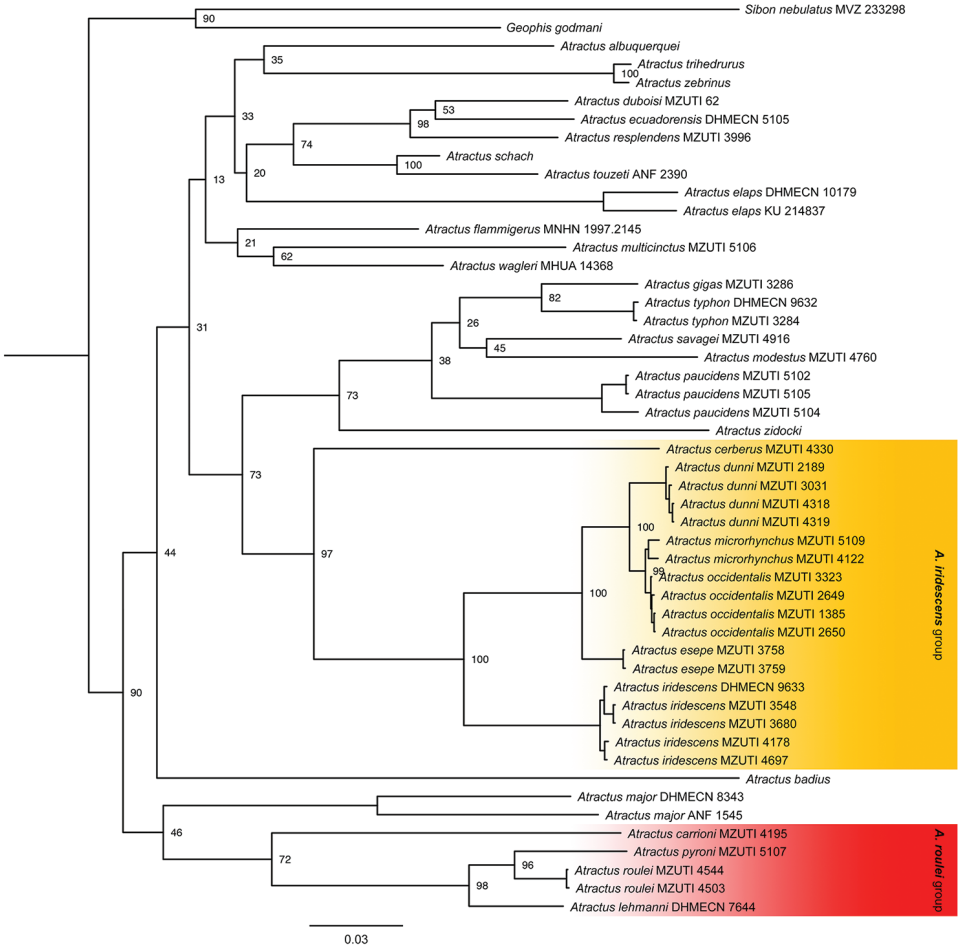

Figure 1. Bayesian consensus phylogeny depicting relationships within colubrid snakes of the genus *Atractus*, summarized from 5 million post-burnin generations in MrBayes 3.2.0. The topology was derived from analysis of 2,564 bp of mitochondrial DNA (gene fragments 16S, Cytb and ND4). Numbers next to branches correspond to posterior probability values. PP values on intraspecific branches are not shown for clarity. Voucher numbers for sequences are indicated for each terminal when available.

New taxa and systematic arrangements derived from the analyses

We seek here to only name or redelimit *Atractus* species groups that are supported in our molecular phylogeny and share features of their coloration pattern and lepidosis. The first such groups is the clade comprising *A. cerberus* sp. n., *A. dunni*, *A. esepe* sp. n., *A. iridescens*, *A. microrhynchus* and *A. occidentalis*. The other is the one comprising *A. carrioni*, *A. lehmanni*, *A. pyroni* sp. n. and *A. roulei*.

Atractus iridescens species group

Diagnosis. 200–360 mm SVL *Atractus* with brown dorsal ground color bearing a pattern of dots or stripes (Fig. 2), generally 17/17/17 smooth dorsals, and 125–163 ventrals (Table 2).

Content. *Atractus cerberus* sp. n., *A. dunni*, *A. echidna*, *A. esepe* sp. n., *A. iridescens*, *A. microrhynchus* and *A. occidentalis*.

Distribution. Pacific lowlands and western Andean slopes in Ecuador and Colombia (Fig. 3).

Comment. Passos et al. (2009a) included *Atractus echidna*, *A. iridescens* and *A. microrhynchus* in the phenetic *A. paucidens* species group. Later, Passos et al. (2012) placed *A. microrhynchus* in the *A. multicinctus* group based on hemipenial characters. Unlike *A. paucidens* or *A. multicinctus* (Jan, 1865), however, the former three species have a brownish color pattern (Fig. 2) and also a lower number of ventral scales (Appendix III). These differences, together with the phylogenetic placement of *A. iridescens* and *A. microrhynchus* support the allocation of these species in the newly formed *A. iridescens* group.

Atractus roulei species group

Diagnosis. 300–450 mm SVL *Atractus* with olive to grayish brown dorsal ground color lacking dots and stripes, 15/15/15 smooth dorsals (occasionally 17/17/17), generally 6 supralabials (sometimes 5), and 135–161 ventrals (Table 3).

Content. *Atractus carrioni*, *A. lehmanni*, *A. pyroni* sp. n. and *A. roulei* (Fig. 1).

Distribution. Western slopes of the Andes and inter-Andean valleys in central and southern Ecuador (Fig. 4).

Comment. Passos et al. (2013) created the *Atractus roulei* species group to accommodate *A. roulei* and its closest morphological relative *A. carrioni*, based mainly on

Table 2. Morphometric data for members of the *Atractus iridescens* species group. Codes are: V=ventrals; SC=subcaudals; D=dorsal scale rows at midbody; PO=postoculars; SL=supralabials; IL=infralabials; MT=maxillary teeth. Data is derived from Appendix III and from the literature.

Species	V		SC		D	PO	SL	IL	MT
	Males	Females	Males	Females					
A. cerberus	152–157	–	25–26	–	17	2	7	7	7
A. dunni	125–136	138–150	26–39	19–26	17	2	6–7	6–8	5–7
A. echidna	127	–	36	–	15	2	7	7	6
A. esepe	149	156	41	30	17	2	7	7	5
A. iridescens	127–150	135–144	33–42	25–37	17	2	6–7	6–7	5–6
A. microrhynchus	133–150	144–163	32–40	24–29	17	1–2	7	6–7	5–7
A. occidentalis	129–141	128–149	33–39	20–37	17	2	6–7	6–7	5–7

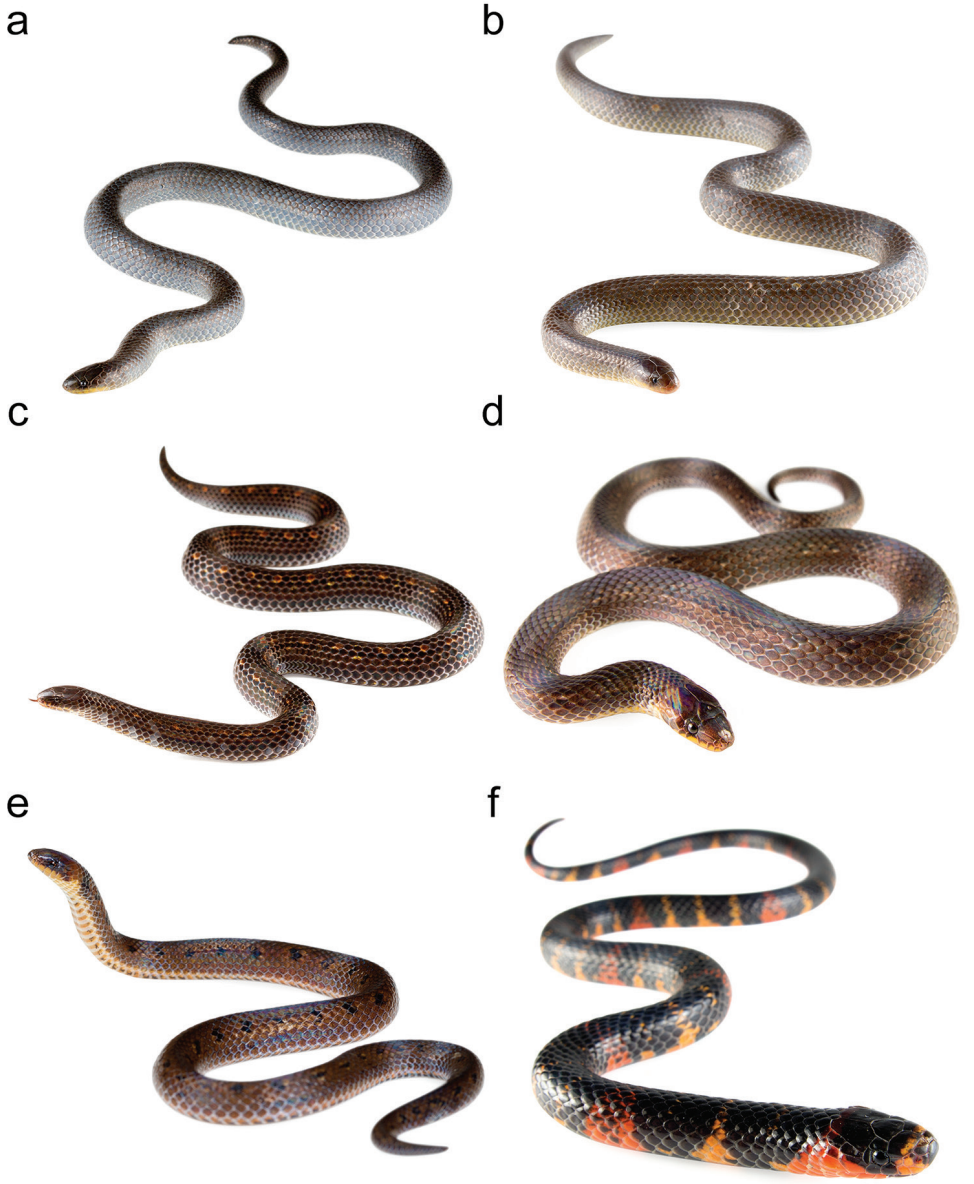

Figure 2. Photographs of some Ecuadorian species of *Atractus* in life: *A. carrioni* MZUTI 4194 (**a**), MZUTI 4195 (**b**), *A. duboisi* MZUTI 3640 (**c**), *A. dunni* MZUTI 4318 (**d**), *A. dunni* MZUTI 2189 (**e**), *A. elaps* AMARU SN (**f**), *A. gigas* MZUTI 3286 (**g**), *A. iridescens* MZUTI 3680 (**h**), *A. iridescens* QCAZ 8072 (**i**), *A. iridescens* MZUTI 4697 (**j**), *A. iridescens* MZUTI 3548 (**k**), *A. major* MZUTI 4973 (**l**), *A. microrhynchus* MZUTI 5109 (**m**), *A. modestus* (**n**), *A. multicinctus* MZUTI 5106 (**o**), *A. occidentalis* MZUTI 1385 (**p**), *A. occidentalis* MZUTI 3323 (**q**), *A. paucidens* MZUTI 5102 (**r**), *A. resplendens* MZUTI 3996 (**s**), *A. roulei* MZUTI 4503 (**t**), *A. savagei* MZUTI 4916 (**u**), *A. snethlageae* (**v**), *A. touzeti* ANF 2390 (**w**), and *A. typhon* MZUTI 5110.

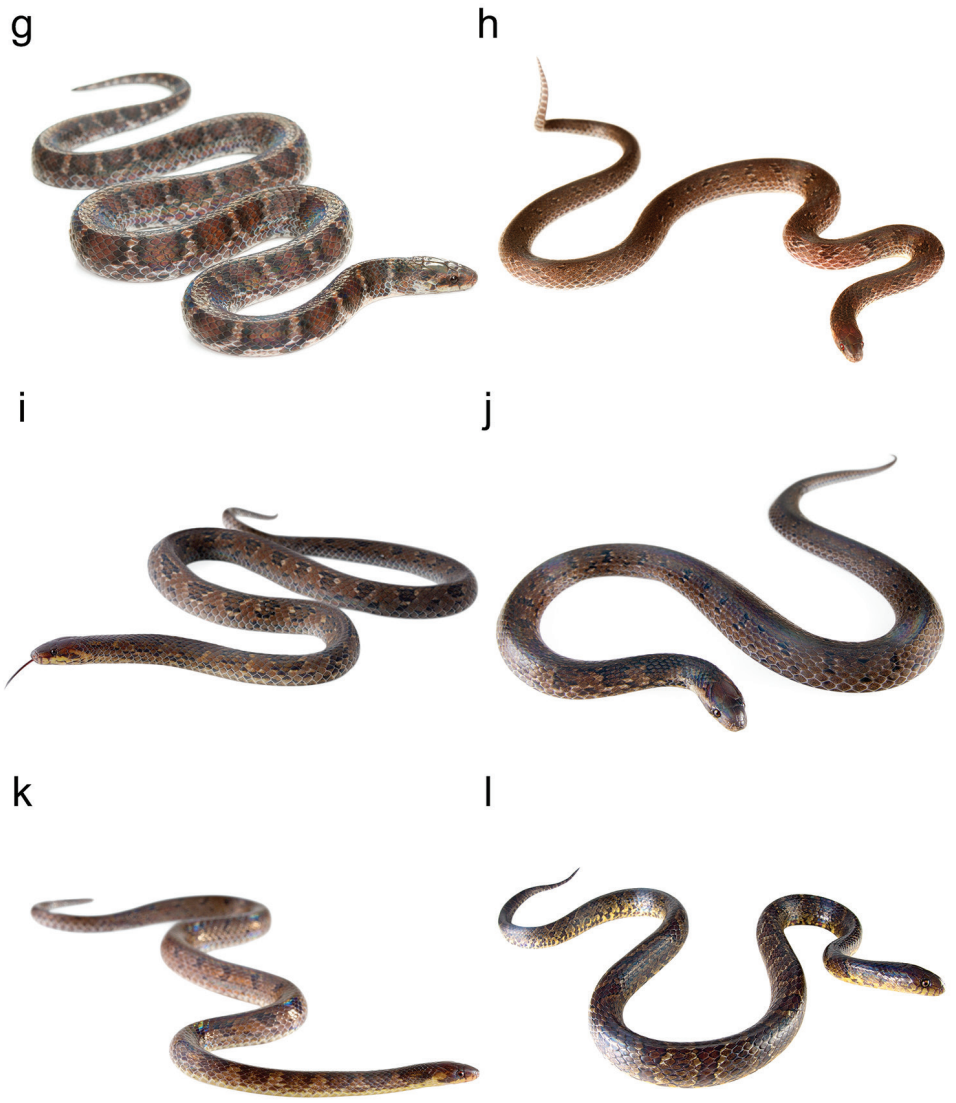

Figure 2. Continued.

Table 3. Morphometric data for members of the *Atractus roulei* species group. Codes are: V=ventrals; SC=subcaudals; D=dorsal scale rows at midbody; PO=postoculars; SL=supralabials; IL=infralabials; MT=maxillary teeth. Data is derived from Appendix III and from the literature.

Species	V		SC		D	PO	SL	IL	MT
	Males	Females	Males	Females					
A. carrioni	136–151	143–161	25–34	18–32	15	1	6	6	7–10
A. lehmanni	141–144	148–153	25–29	20–21	15–17	1	5	6	8–11
A. pyroni	–	143	–	16	15	1	6	5	8
A. roulei	135–146	143–156	20–27	14–23	15	1	5–6	6–7	9–13

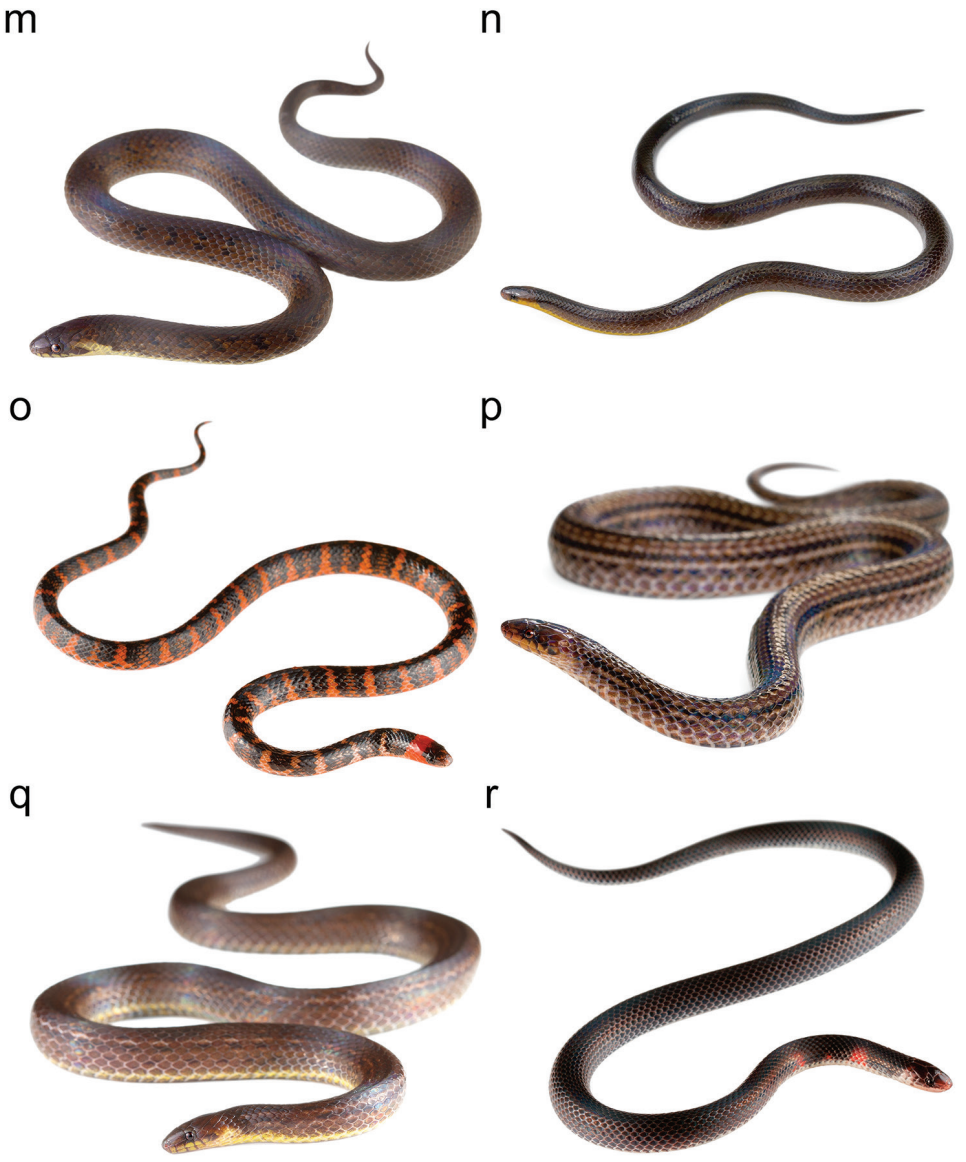

Figure 2. Continued.

their unusual combination of 15/15/15 dorsals and 6 supralabials. Our examination of new material belonging to these two species, and material belonging to *A. pyroni* and *A. roulei* (Appendix III), shows that although the majority of specimens have indeed 6 supralabials, some specimens may have 5, compared with most Ecuadorian *Atractus* which have 7 (Appendix III). One specimen of *A. roulei* from the type locality (MZUTI 4544; Table 1) lacks a loreal scale, which was long thought (Savage 1960;

Figure 2. Continued.

Passos et al. 2013) to be the main feature separating this species from *A. carrioni*. The syntype of *A. lehmanni* (MC 33513) revised by Savage (1960) has 17/17/17 dorsal scale rows. Specimens assignable to *A. lehmanni* have been found only in the vicinity of the type locality (hoya de Cuenca; see Table 1).

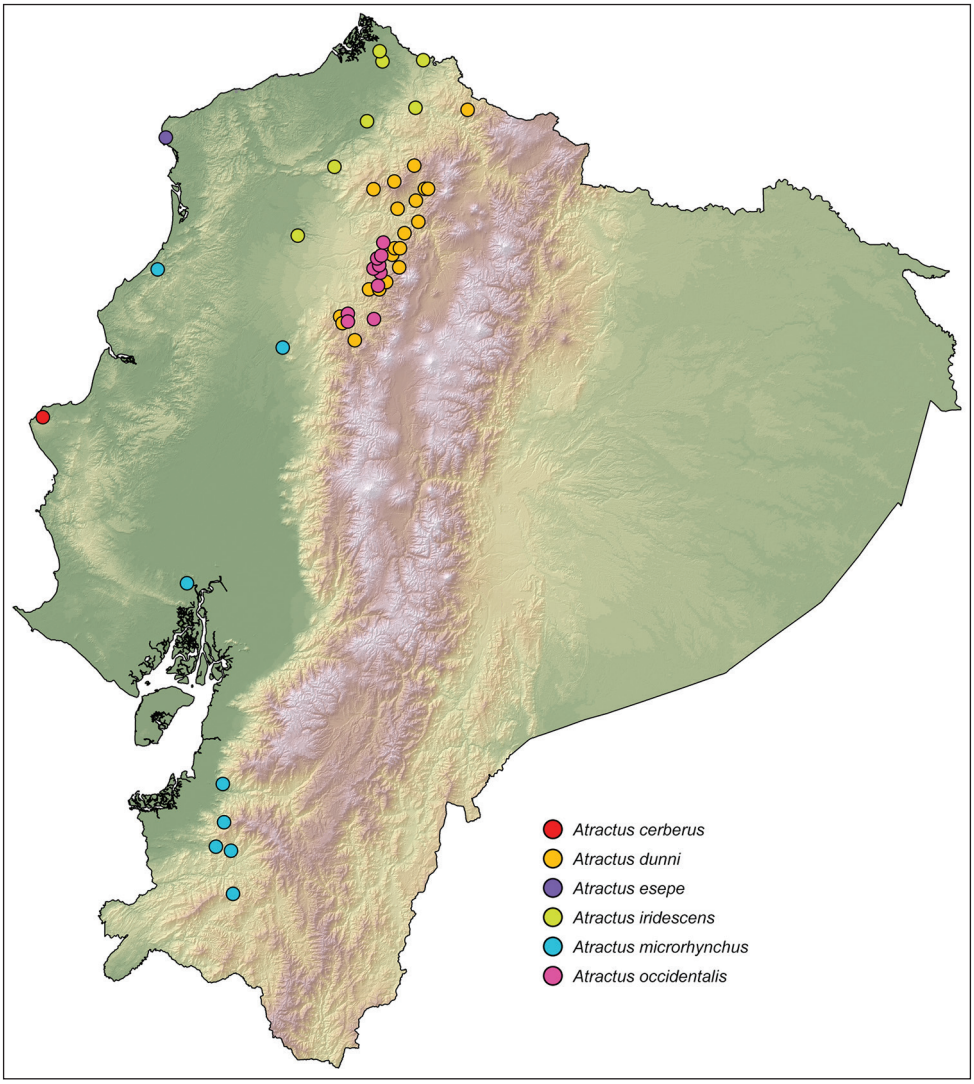

Figure 3. Distribution of Ecuadorian snakes of the *Atractus iridescens* species group. Dots represent known localities.

***Atractus cerberus* sp. n.**
http://zoobank.org/B93B0063-06B6-462F-8C4B-7559D9459714

Proposed standard English name. Cerberus Groundsnake

Proposed standard Spanish name. Tierrera cancerbera

Holotype. MZUTI 4330 (Fig. 5a), adult male collected by José L. Vieira-Fernandes and Alejandro Arteaga on November 06, 2015 at Pacoche, province of Manabí, Ecuador (S1.06664, W80.88123; 280 m).

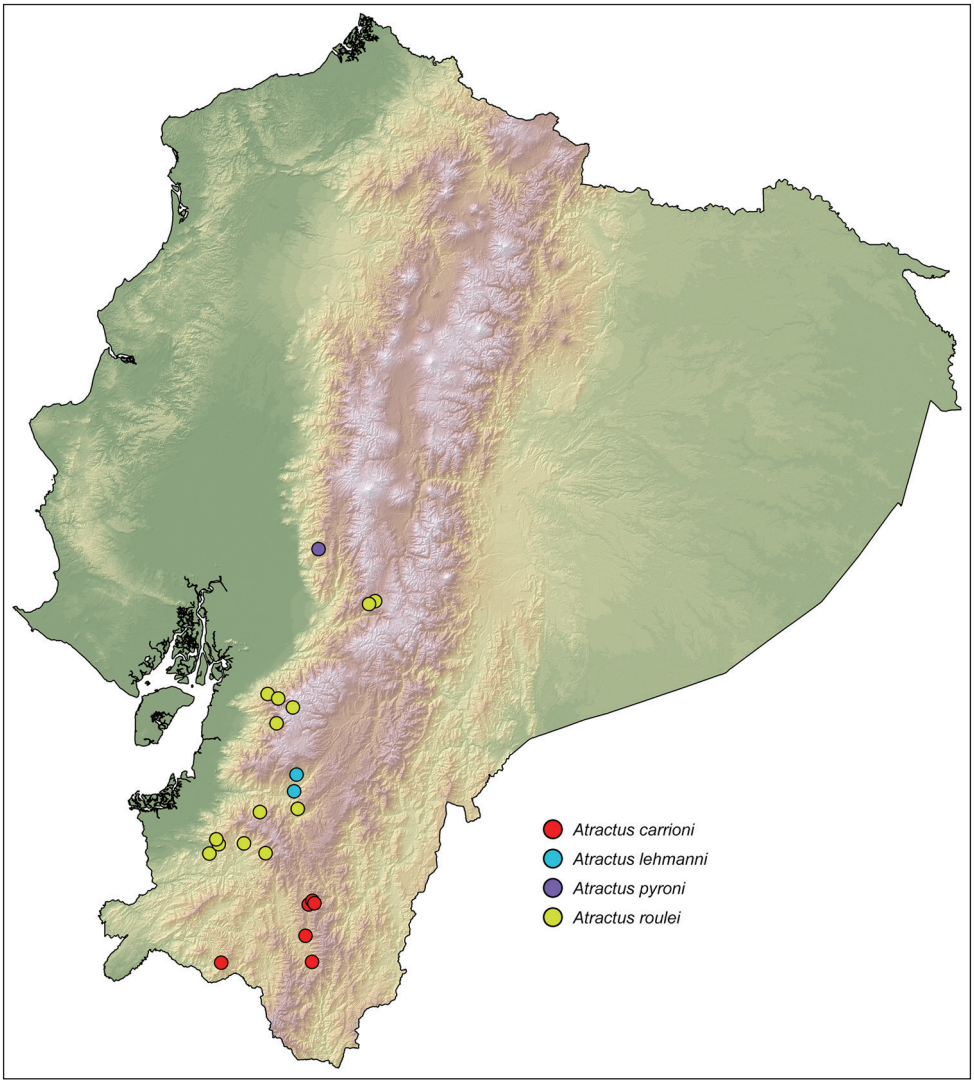

Figure 4. Distribution of Ecuadorian snakes of the *Atractus roulei* species group. Dots represent known localities.

Paratopotype. MZUTI 5108 (Fig. 5b), adult male collected by Alejandro Arteaga on September 04, 2016.

Diagnosis. *Atractus cerberus* is placed in the genus *Atractus* as diagnosed by Savage (1960), based on phylogenetic evidence (Fig. 1). It is included in the *A. iridescens* group due to its brown dorsal ground color (Fig. 5) and its phylogenetic position (Fig. 1). The species is diagnosed based on the following combination of characters: (1) 17/17/17 smooth dorsals; (2) two postoculars; (3) loreal moderate; (4) temporals 1+2; (5) seven supralabials, third and fourth contacting orbit; (6) seven infralabials, first four contacting chinshields (7) seven maxillary teeth; (8) three gular scale rows; (9)

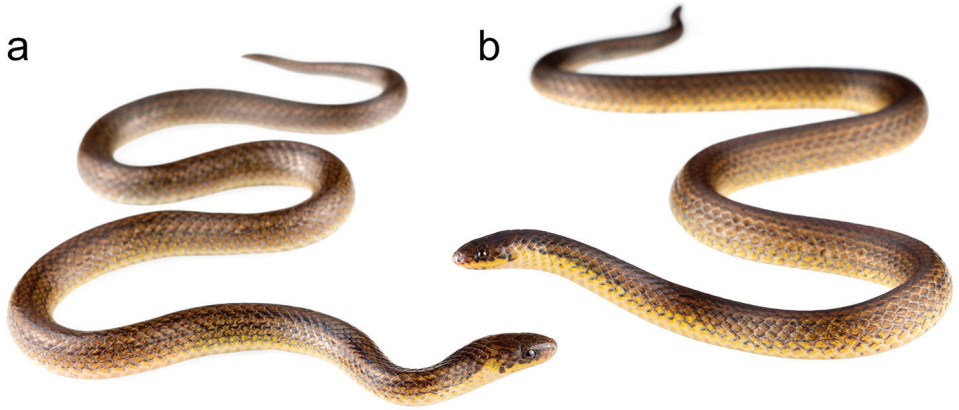

Figure 5. Adult male holotype MZUTI 4330 (**a**) and adult male paratopotype (**b**) of *Atractus cerberus* MZUTI 5108.

three preventrals; (10) 152–157 ventrals; (11) 25–26 subcaudals; (12) dorsal ground color brown with faint black longitudinal bands (Fig. 5); (13) venter light yellow faintly speckled with brownish pigment; (14) 212–309 mm SVL; (15) 23–36 mm TL.

Comparisons. *Atractus cerberus* is included in the *A. iridescens* species group and compared to other Pacific lowland congeners that have a brownish ground color (Fig. 2): *A. boulengerii*, *A. dunni*, *A. echidna*, *A. esepe* sp. n., *A. iridescens*, *A. medusa*, *A. microrhynchus*, and *A. occidentalis*. From *A. boulengerii* and *A. medusa*, it differs in having a striped pattern as opposed to bold black blotches (Fig. 5). From all others, it differs in having yellow ventral surfaces (as opposed to cream or dingy white) and having more than 150 ventrals in males. Finally, the dorsal pattern of *A. cerberus* is less clearly marked than in the majority of the known specimens of the species included in the *A. iridescens* group. Instead of having conspicuous spots, blotches or lines, *A. cerberus* has a series of feebly visible dashes made of pigment slightly darker than the surrounding ground color.

Color pattern. The dorsal ground color is brown with five feebly visible dark-brown to black longitudinal lines that are not continuous throughout the length of the body but broken into spots along some sections (Fig. 5). Between the dark longitudinal lines on each side of the body, there are fields of lighter pigment that on some sections of the body correspond to lines. The head is darker than the rest of the dorsal surfaces and is marked by a dark, irregular postocular stripe that reaches the corner of the mouth (Fig. 5). The top of the supralabials is tinged with black. The ventral surfaces are yellowish cream with scattered brownish speckling that becomes more concentrated towards the tail, which is almost completely brown. The iris is carmine and the pupil is black.

Description of holotype. Adult male, SVL 212 mm, tail length 23 mm (10.8% SVL); body diameter 6.5 mm; head length 7.9 mm (3.7% SVL); head width 4.8 mm (2.3% SVL); interocular distance 3.1 mm; head slightly distinct from body; snout–or-

bit distance 2.8 mm; rostral 1.6 mm wide, about one time broader than high; internasals 1.0 mm wide; internasal suture sinistral relative to prefrontal suture; prefrontals 1.7 mm wide; frontal 2.3 mm wide, with a curvilinear triangle shape in dorsal view; parietals 2.1 mm wide, about twice as long as wide; nasal divided; loreal 1.5 mm long, about 2 times longer than high; eye diameter 1.4 mm; pupil round; supraoculars 1.4 mm wide; two postoculars; temporals 1+2, upper posterior temporal elongate, about four times longer than high, and three times as long as first temporal; seven supralabials, 3rd–4th contacting orbit; symphisial 1.0 mm wide, about twice as broad as long, separated from chin shields by first pair of infralabials; seven infralabials, 1st–4th contacting chin shields; anterior chin shields about three times as long as broad, posterior chin shields absent; three series of gular scales; dorsal scales 17/17/17 rows, smooth without apical pits; preventrals 3; ventrals 157; anal plate single; paired subcaudals 26.

Natural history. The two known specimens of *Atractus cerberus* were found in an isolated patch of deciduous lowland forest surrounded by dry lowland shrubland. MZUTI 4330 was found active on leaf litter at 19h29, in 80% closed canopy secondary forest far from streams. The night was warm and there was drizzle the night before. MZUTI 5108 was found crossing a forest trail close to an open area at 10h00 during a sunny morning after a rainy night.

Distribution. Known only from the type locality, Pacoche, in the Ecuadorian province of Manabí at 280–324 m (Fig. 3). This locality is 3 km airline distance from the shoreline.

Etymology. The specific epithet "*cerberus*" is derived from the name of the Greek monster Kérberos. In Greek mythology, Kérberos is a monstrous multi-headed dog that guards the gates of the underworld, preventing the dead from leaving. Here, we use this word in allusion to the type locality, at the gates of the newly formed "Refinería del Pacífico", a massive industrial oil-processing plant that can easily be likened to the underworld.

Conservation status. Although *Atractus cerberus* belongs to a poorly studied genus of snakes and is known only from two specimens collected recently in a single locality, we consider this species to be Critically Endagered following B1a,b(iii) IUCN criteria because: i) its extent of occurrence is estimated to be less than 50 km^2 (i.e. total area of continous semideciduous forest in the Refugio de Vida Silvestre Pacoche); ii) it has not been detected in any other locality in the province of Manabí despite numerous surveys (Almendáriz and Carr 2007, Cisneros-Heredia 2004, MECN et al. 2013); and iii) and its habitat is severely fragmented, isolated from other such habitats and declining in extent and quality due to deforestation.

***Atractus esepe* sp. n.**
http://zoobank.org/F58E89A5-D398-4703-8098-7474CD6B3E6D

Proposed standard English name. Indistinct Groundsnake

Proposed standard Spanish name. Tierrera indistinta

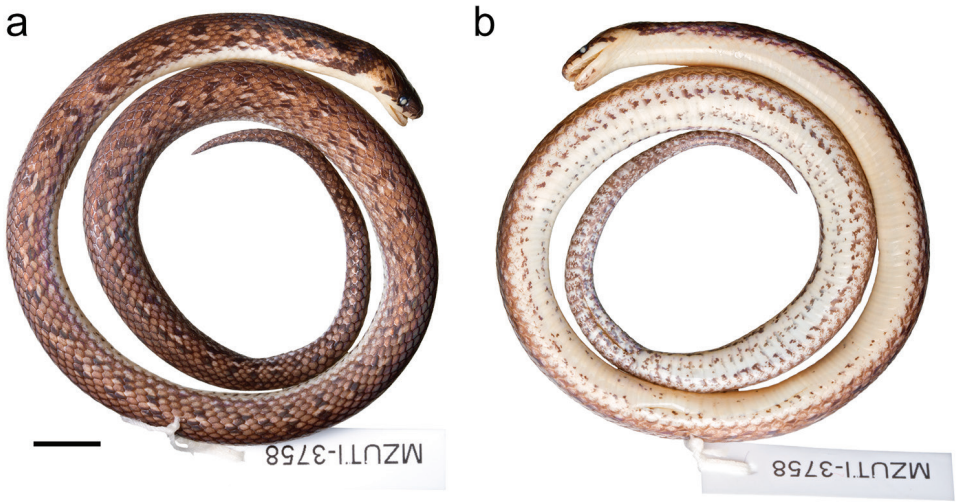

Figure 6. Adult male holotype of *Atractus esepe* MZUTI 3758 in dorsal (**a**) and ventral (**b**) view. Scale = 1 cm.

Holotype. MZUTI 3758 (Fig. 6), adult male collected by Alejandro Arteaga on September 12, 2014 at Caimito, Esmeraldas Province, Ecuador (N0.69620, W80.090472; 102 m).

Paratopotype. MZUTI 3759, adult female collected by Jaime Culebras.

Diagnosis. *Atractus esepe* is placed in the genus *Atractus* as diagnosed by Savage (1960), based on phylogenetic evidence (Fig. 1). It is included in the *A. iridescens* group due to its brown dorsal ground color and its phylogenetic position (Figs 1, 6). The species is diagnosed based on the following combination of characters: (1) 17/17/17 smooth dorsals; (2) two postoculars; (3) loreal long; (4) temporals 1+2; (5) seven supralabials, third and fourth contacting orbit; (6) seven infralabials, first four contacting chinshields (7) seven maxillary teeth; (8) 2–3 gular scale rows; (9) 2–3 preventrals; (10) 149 ventrals in the male holotype, 156 in the female paratype; (11) 41 subcaudals in the male holotype, 30 in the female paratype; (12) dorsal ground color brown with a pattern of complete (MZUTI 3759) or broken (MZUTI 3758) (Fig. 6a) dark lines running parallel along each side of the body and separated from each other by a cream line, but rendering the appearance of a row of dorso-lateral blotches in the broken pattern (MZUTI 3758); (13) venter cream faintly speckled with brownish pigment (Fig. 6b); (14) 232–241 mm SVL; (15) 34–53 mm TL.

Comparisons. *Atractus esepe* is included in the *A. iridescens* species group and compared to other Pacific lowland congeners who have a brownish ground color (Figs 2, 5): *A. boulengerii*, *A. cerberus*, *A. dunni*, *A. echidna*, *A. iridescens*, *A. medusa*, *A. microrhynchus*, and *A. occidentalis*. From these, *A. microrhynchus* and *A. occidentalis* have striped pattern and cream ventral surfaces similar to that of *A. esepe*, but they occur parapatrically (Fig. 3) and can be distinguished from *A. esepe* by a genetic divergence of 5.3–5.7% in a 506 bp

long fragment of the mitochondrial Cytb gene and by having a greater number of subcaudal scales in males (Table 2). Furthermore, adult specimens of *A. microrhynchus* have light brown dorsal surfaces instead of dark brown, and their pattern can be better described as a series of blotches rather than broken longitudinal lines. Specimens of both *A. esepe* and *A. occidentalis* have a pattern of longitudinal lines, but *A. esepe* has a greater number of ventral plus caudal scales than *A. occidentalis* (more than 180 in *A. esepe*) (Table 2).

Color pattern. The dorsal ground color is dark brown with either six longitudinal black lines separated by lighter areas or a pattern of dark brown longitudinally arranged spots that correspond to the longitudinal lines. On each side, the line or series of dark spots along the 2nd and 3rd dorsal scale row is feebly visible, but the other lines or spots are conspicuous. The dorsal surface of the head is dark brown and there is a clearly marked dark postocular stripe running from behind the eye to the edge of the mouth (Fig. 6). The ventral surfaces are dingy white, finely speckled with brown pigment that becomes more concentrated towards the tail. The iris is carmine and the pupil is black.

Description of holotype. Adult male, SVL 232 mm, tail length 53 mm (22.8% SVL); body diameter 7.0 mm; head length 7.9 mm (3.4% SVL); head width 4.8 mm (2.2% SVL); interocular distance 3.4 mm; head slightly distinct from body; snout–orbit distance 3.3 mm; rostral 1.8 mm wide, about one time broader than high; internasals 0.9 mm wide; internasal suture sinistral relative to prefrontal suture; prefrontals 1.9 mm wide; frontal 2.2 mm wide, with a curvilinear triangle shape in dorsal view; parietals 2.1 mm wide, about twice as long as wide; nasal divided; loreal 2.5 mm long, about 3 times longer than high; eye diameter 1.5 mm; pupil round; supraoculars 1.2 mm wide; two postoculars; temporals 1+2, upper posterior temporal elongate, about four times longer than high, and three times as long as first temporal; seven supralabials, 3rd–4th contacting orbit; symphisial 0.8 mm wide, separated from chin shields by first pair of infralabials; seven infralabials, 1st–4th contacting chin shields; anterior chin shields about three times as long as broad, posterior chin shields absent; three series of gular scales; dorsal scales 17/17/17 rows, smooth without apical pits; preventrals 3; ventrals 149; anal plate single; paired subcaudals 41.

Natural history. The two known specimens of *Atractus esepe* were found actively foraging among soil and roots in secondary evergreen lowland forest at least 400 m from the nearest natural body of water. They were found by night at 20h00 after a warm, sunny day.

Distribution. Known only from the type locality, Caimito, in the Ecuadorian province of Esmeraldas at 102 m (Fig. 3). This locality is 1.3 km airline distance from the shoreline.

Etymology. The specific epithet *esepe* is derived from the Spanish pronunciation of "sp.", which is the abbreviation for the Latin word *species*. Here, we use this word in allusion to how the majority of Ecuadorian researchers refer to *Atractus* specimens found in the field.

Conservation status. We consider *Atractus esepe* to be Data Deficient following IUCN criteria because it is known only from its type locality but its occurrence in the biogeographic Choco suggests that it might as well be present in other localities. The

Chocoan forests of Caimito do not appear to be isolated from other similar habitat by geographical or ecological barriers. Therefore, we consider there is inadequate information to make a direct, or indirect, assessment of its extinction risk based on its scarce distribution data.

***Atractus pyroni* sp. n.**
http://zoobank.org/36145E29-02B6-4C66-A097-44EFC1BC3A92

Proposed standard English name. Pyron's Groundsnake

Proposed standard Spanish name. Tierrera de Pyron

Holotype. MZUTI 5107 (Fig. 7), adult male collected by José L. Vieira-Fernandes and Carlos Durán on May 23, 2016 between Balzapamba and Bilován, province of Bolívar, Ecuador (S1.83601, W79.13322; 2026 m).

Diagnosis. *Atractus pyroni* is placed in the genus *Atractus* as diagnosed by Savage (1960), based on phylogenetic (Fig. 1) and morphological (Table 3) evidence. It is included in the *A. roulei* group due to its 15/15/15 dorsal scale rows and its phylogenetic position (Fig. 1). The species is diagnosed based on the following combination of characters: (1) 15/15/15 smooth dorsals; (2) one postocular; (3) loreal long; (4) temporals 1+2; (5) six supralabials, third and fourth contacting orbit; (6) five infralabials, first four contacting chinshields (7) eight maxillary teeth; (8) 3 gular scale rows; (9) 2 preventrals; (10) 143 ventrals; (11) 16 subcaudals; (12) dorsal ground color dark brown with a series of light golden brown paravertebral scales running along the entire dorsum (Fig. 7); (13) venter dark brown with scattered scales of a lighter color; (14) 443 mm SVL; (15) 34 mm TL.

Comparisons. *Atractus pyroni* is compared to members of the *A. roulei* species group: *A. carrioni*, *A. lehmanni*, and *A. roulei* (Fig. 2). From *A. carrioni*, it differs by having a loreal. From *A. lehmanni* and *A. roulei*, it differs in size and color pattern. *Atractus pyroni* is 443 mm in SVL; whereas *A. lehmanni* is 262–321 in SVL, and *A. roulei* is 230–396. Both *A. lehmanni* and *A. roulei* have uniform dorsal ground color, whereas *A. pyroni* has a distinct dorsal bicolored pattern (Fig. 7). Finally, in life, *A. pyroni* is darker than the remaining members of the *A. roulei* species group and has a ventral pattern that, instead of having fine speckles, has conspicuous scattered blotches of a contrasting color.

Color pattern. The dorsal ground color is blackish with a dark vertebral (mid-dorsal) scale row flanked by a dark yellow scale row on either side (the 7th dorsal scale row), irregularly adjoined by one to few additional yellow scales on the 6th dorsal scale row, rendering an appearance of an irregularly edged mid-dorsal striped pattern (Fig. 7). The dorsal and lateral surfaces of the head are dark grayish brown and the labials are dark mustard yellow. All ventral surfaces are glossy grayish black except for the throat and some scattered blotches, which are dark mustard yellow.

Description of holotype. Adult female, SVL 443 mm, tail length 34 mm (7.7% SVL); body diameter 11.6 mm; head length 14.4 mm (3.3% SVL); head width 9.8 mm

Figure 7. Adult female holotype of *Atractus pyroni*. MZUTI 5107. Scale = 1 cm.

(2.2% SVL); interocular distance 5.1 mm; head slightly distinct from body; snout–orbit distance 5.7 mm; rostral 2.8 mm wide, about two times broader than high; internasals 1.5 mm wide; internasal suture sinistral relative to prefrontal suture; prefrontals 2.8 mm wide; frontal 3.5 mm wide, with a curvilinear triangle shape in dorsal view; parietals 4.0 mm wide, about twice as long as wide; nasal divided; loreal 3.7 mm long, about 3 times longer than high; eye diameter 1.8 mm; pupil round; supraoculars 2.1 mm wide; one postocular; temporals 1+2, upper posterior temporal elongate, about five times longer than high, and twice as long as first temporal; six supralabials, 3rd–4th contacting orbit; symphisial 2.4 mm wide, separated from chin shields by first pair of infralabials; five infralabials, 1st–4th contacting chin shields; anterior chin shields about three times as long as broad, posterior chin shields absent; three series of gular scales; dorsal scales 15/15/15 rows, smooth without apical pits; preventrals 2; ventrals 143; anal plate single; paired subcaudals 16.

Natural history. The only known specimen of *Atractus pyroni* was found dead on a dirt road surrounded by silvopastures and remnants of native montane cloudforest.

Distribution. Known only from the type locality, between Balzapamba and Bilován, in the Ecuadorian province of Bolívar at 2026 m (Fig. 7).

Etymology. Named after R. Alexander Pyron, one of the most prolific contemporary herpetologists, in recognition of his invaluable contribution to systematics and evolution of the world's reptiles.

Conservation status. We consider *Atractus pyroni* to be to be Data Deficient following IUCN because there is inadequate information to make a direct, or indirect, assessment of its extinction risk based on its scarce distribution data.

Discussion

Species relationships and taxonomy in the colubrid snake genus *Atractus* are still far from being resolved, and many infrageneric groups are either non-monophyletic, or poorly supported and weakly placed, which may reflect inadequate sampling of taxa (only 30 out of 140 species are included) or characters (only 1 locus is used). No monophyly was found for the groups defined by Savage (1960), which, until further phylogenetic evidence is accumulated or unambiguous diagnostic characters are defined, should not be used.

From the five members of the *A. paucidens* species groups of Passos et al. (2009a) that were sampled in our phylogeny, only *A. paucidens*, *A. savagei*, and *A. typhon* cluster together. *Atractus microrhynchus* and *A. iridescens* belong to another lineage, which is here named the *A. iridescens* species group. This group includes the aforementioned two species plus *A. cerberus*, *A. dunni*, *A. echidna*, *A. esepe*, and *A. occidentalis*. From the species included in this group, we expand the known distribution of all their members (Fig. 3). However, we do not include the specimens ANSP 18114 nor ANSP 26316, from the vicinity of Huigra and identified as *A. occidentalis* by Savage (1960), because their description disagrees with the observed morphological variation reported for *A. occidentalis* in this work. Upon a visit to Huigra, a dry valley dominated by xeric vegetation and rocky outcrops, it became clear to us that it is unlikely for a species like *A. occidentalis*, which is found in evergreen lower-montane forests (Arteaga et al. 2013), to occur in an isolated dry habitat type ca. 250 km airline distance south of the type locality.

We also re-delimit the *A. roulei* species group of Passos et al. (2013) to include *A. carrioni*, *A. lehmanni*, *A. roulei* and *A. pyroni*. We expand the known distribution of *A. roulei* (Fig. 4), but do not include specimen AMNH 17492 from San José de Chimbo (Savage 1960) in the map because this specimen might actually be *A. pyroni* given the morphological similarities between the two species and the geographical proximity to the type locality of *A. pyroni*. Reports of *A. lehmanni* from Colombia (Passos et al. 2009b) are likely misidentifications since *A. lehmanni* has not been registered in Ecuador outside the type locality.

To further clarify the landscape of *Atractus* taxonomy in Ecuador, we analyze the presence of *A. medusa*, *A. melas*, *A. typhon*, *A. badius*, and *A. bocourti* in the country. Cisneros-Heredia and Romero (2015) presented the first country record of *A. medusa* in Ecuador (specimen DFCH-USFQ 191.101109 at Universidad San Francisco de Quito), based on similarities in scalation and coloration between that specimen and the holotype of *A. medusa*, form Gorgona island, Colombia. Certainly, the characters of scalation of the Ecuadorian specimen fit the diagnosis of *A. medusa*. However, they fit just as well the diagnosis of *A. iridescens* provided by Passos et al. (2009a), with the difference that the dorsal pattern of the Ecuadorian specimen resembles more the *A. iridescens* specimen, ICN 10902, pictured in Passos et al. (2009a). The dark brown ground color (as opposed to light cream), the light bordered brown blotches (as opposed to

solid black blotches), and the absence of a black nape band are all characteristics shared by DFCH-USFQ 191.101109 and the other nine specimens of *A. iridescens* presented in Appendix III, with ICN 10902 of Passos et al. (2009a). Therefore, we consider that DFCH-USFQ 191.101109 actually represents the first country record of *A. iridescens* for Ecuador. Based on this new information and re-examination of museum material, we report on 9 additional specimens (Table 1) that expand the current known distribution of this species. Cisneros-Heredia and Romero (2015) suggest that a photographic record of *Atractus* cf. *melas* from the Bilsa Biological Station, province of Esmeraldas, northwestern Ecuador (Ortega-Andrade et al. 2010) corresponds to *A. multicinctus*. The specimen differs from other material assigned to *A. multicinctus* in having whitish rings as opposed to red rings throughout the body (Fig. 2). Although photographic vouchers of *A. typhon* have been presented in MECN et al. (2013), we report on the first museum vouchers of the species in Ecuador (Table 1).

Finally, although Hoogmoed (1980) restricted the type locality of *A. badius* and pointed out that the upper Amazon basin specimens were misidentifications, the species has remained in Ecuadorian faunal lists (Torres-Carvajal et al. 2016), even after Schargel et al. (2013) made compelling cases to exclude this species from the upper Amazon Basin. Other snake, *A. bocourti* was included in the herpetofauna of Ecuador by Pérez-Santos and Moreno (1991) without pointing out to any museum voucher. These authors stated that although they have no information about the distribution of the species in Ecuador, its distribution in Colombia would suggest that it also occurs in Ecuador. Since there is no evidence that neither *A. badius* nor *A. bocourti* occur in Ecuador, we remove them from this country's herpetofauna.

Our analysis of new *Atractus* material supports the evolutionary phylogenetic distinctiveness of at least 22 of the total taxa currently recognized to occur in Ecuador. To include the remaining taxa in future phylogenetic analyses will certainly help resolve species relationships and taxonomic arrangements of cis-Andean Ecuadorian *Atractus*, since the five species that were not included in the phylogeny occur in the Amazonian slopes of the Andes. However, besides including more taxa in future phylogenetic analyses, we feel that a more adequate sampling of molecular markers is needed to overcome the difficulties that mitochondrial-based phylogenies have to capture higher-lever evolutionary relationships. Certainly, future studies can benefit from a phylogeny based on both a nuclear and a mitochondrial dataset.

With these changes, the species number reported in Ecuador increases to 27: *A. carrioni* (Parker, 1930), *A. cerberus*, *A. collaris* (Peracca, 1897), *A. duboisi* (Boulenger, 1880), *A. dunni* (Savage, 1955), *A. ecuadorensis* (Savage, 1955), *A. elaps* (Günther, 1858), *A. esepe*, *A. gaigeae* (Savage, 1955), *A. gigas* (Myers and Schargel, 2006), *A. iridescens* (Peracca, 1860), *A. lehmanni* (Boettger, 1898), *A. major* (Boulenger, 1894), *A. microrhynchus* (Cope, 1868), *A. modestus* (Boulenger, 1894), *A. multicinctus* (Jan, 1865), *A. occidentalis* (Savage, 1955), *A. occipitoalbus* (Jan, 1862), *A. orcesi* (Savage, 1955), *A. paucidens* (Despax, 1910), *A. pyroni*, *A. resplendens* (Werner, 1901), *A. roulei* (Despax, 1910), *A. savage* (Salazar-Valenzuela et al., 2014), *A. snethlageae* (da Cunha & do Nascimento, 1983), *A. touzeti* (Schargel et al., 2013) and *A. typhon* (Passos et al., 2009).

We hope that the novel genetic and morphological data provided herein will promote future researchers to examine species boundaries in *Atractus*, as additional work clearly is waiting.

Author contributions

Conceived and designed the work: AA. Performed the analyses: AA NP. Gathered morphological data: KB JHV DFCH CRP JLVF AA. Analyzed the data: AA KM DFCH JMG. Contributed reagents/materials/analysis tools: JMG NP. Wrote the paper: AA KM JHV DFCH NP CRP JLVF JMG.

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Appendix I

GenBank accession numbers for loci and terminals of taxa and outgroups sampled in this study. Novel sequence data produced in this study are marked with an asterisk (*).

Species	Voucher	16S	CYTB	ND4
A. albuquerquei	–	GQ457726	JQ598918	–
A. badius	–	AF158485	–	–
A. carrioni	MZUTI 4195	KY610046*	–	KY610094*
A. cerberus	MZUTI 4330	KY610047*	KY610073*	KY610095*
A. duboisi	MZUTI 62	KT944041	–	KT944059
A. dunni	MZUTI 2189	KY610048*	–	KY610096*
A. dunni	MZUTI 3031	KY610049*	–	KY610097*
A. dunni	MZUTI 4318	KY610050*	KY610074*	KY610098*
A. dunni	MZUTI 4319	KY610051*	KY610075*	KY610099*
A. ecuadorensis	DHMECN 5105	–	–	KY610100*
A. elaps	DHMECN 10179	KY610052*	KY610076*	KY610101*
A. elaps	KU 214837	–	EF078536	EF078584
A. esepe	MZUTI 3758	KY610053*	KT944052	KY610102*
A. esepe	MZUTI 3759	KT944039	KT944051	KT944058
A. flammigerus	MNHN 1997.2145	AF158471	–	–
A. gigas	MZUTI 3286	KT944043	KT944053	KT944061
A. iridescens	DHMECN 9633	KY610054*	KY610077*	–
A. iridescens	MZUTI 3548	KY610055*	KY610078*	–
A. iridescens	MZUTI 3680	KY610056*	KY610079*	–
A. iridescens	MZUTI 4178	KT944040	KY610080*	–
A. iridescens	MZUTI 4697	KY610057*	KY610081*	–
A. lehmanni	DHMECN 7644	KY610058*	KY610082*	KY610103*
A. major	ANF 1545	KT944045	–	KY610104*
A. major	DHMECN 8343	KY610059*	–	KY610105*
A. microrhynchus	MZUTI 5109	KY610060*	KY610083*	KY610106*
A. microrhynchus	MZUTI 4122	KT944037	KT944049	KT944056
A. modestus	MZUTI 4760	KY610061*	KY610084*	KY610107*
A. multicinctus	MZUTI 5106	KY610062*	KY610085*	KY610108*

Species	Voucher	16S	CYTB	ND4
A. occidentalis	MZUTI 1385	KY610063*	KY610086*	KY610109*
A. occidentalis	MZUTI 2649	KY610064*	KY610087*	KY610110*
A. occidentalis	MZUTI 2650	KT944038	KT944050	KT944057
A. occidentalis	MZUTI 3323	KY610065*	KY610088*	KY610111*
A. paucidens	MZUTI 5102	KY610066*	–	KY610112*
A. paucidens	MZUTI 5104	–	–	KY610113*
A. paucidens	MZUTI 5105	KY610067*	–	KY610114*
A. pyroni	MZUTI 5107	KY610068*	KY610089*	KY610115*
A. resplendens	MZUTI 3996	KT944042	KT944055	KT944060
A. roulei	MZUTI 4503	–	KY610090*	KY610116*
A. roulei	MZUTI 4544	KY610069*	KY610091*	KY610117*
A. savagei	MZUTI 4916	KY610070*	KY610092*	KY610118*
A. schach	–	AF158486	–	–
A. touzeti	ANF 2390	KY610071*	KY610093*	KY610119*
A. trihedrurus	–	GQ457727	JQ598919	–
A. typhon	DHMECN 9632	KY610072*	–	KY610120*
A. typhon	MZUTI 3284	KT944044	KT944054	KT944062
A. wagleri	MHUA 14368	–	GQ334480	GQ334581
A. zebrinus	–	JQ598861	–	–
A. zidocki	MNHN 1997.2046	AF158487	–	–
Outgroups				
Geophis godmani	–	JQ598877	JQ598932	–
Sibon nebulatus	MVZ 233298	EU728583	EU728583	EU728583

Appendix II

List of PCR and sequencing primers and their respective PCR conditions (denaturation, annealing, extension and number of corresponding cycles) used in this study. All PCR protocols included an initial 3-min step at 94 °C and a final extension of 10 min at 72 °C.

Locus	Primer name	Sequence (5'-3')	Reference	PCR profile:
16S	16Sar-L	CGCCTGTTTATCAAAAACAT	Palumbi et al. (1991)	94 °C (45 sec), 53 or 56 °C (45 sec), 72 °C (1 min) [x25-30]
	16Sbr-H-R	CCGGTCTGAACTCAGATCACGT		
Cytb	L14910	GACCTGTGATMTGAAAACCAYCGTTGT	Burbrink et al. (2000)	94 °C (1 min), 58 °C (1 min), 72 °C (2 min) [x30-36]
	H16064	CTTTGGTTTACAAGAACAATGCTTTA		
ND4	ND4	CACCTATGACTACCAAAAGCTCATGTAGAAGC	Arévalo et al. (1994)	94 °C (25 sec), 58 or 60 °C (1 min), 72 °C (2 min) [x25-30]
	Leu	CATTACTTTTACTTGGATTTGCACCA		

Appendix III

Morphometric data and sex for specimens of *Atractus* species examined. Codes are: V=ventrals; SC=subcaudals; D1–3=dorsal scale rows at neck, midbody, and vent; PO=postoculars; SL=supralabials; IL=infralabials; MT=maxillary teeth; SVL=snout-vent length (mm); TL=tail length (mm); M=Male, F=Female.

Species	Voucher	V	SC	D1	D2	D3	PO	SL	IL	MT	SVL	TL	Sex
A. carrioni	DHMECN 4697	144	32	15	15	15	1	6	6	7	361	59	F
A. carrioni	DHMECN 76	157	23	15	15	15	1	6	6	8	333	39	F
A. carrioni	DHMECN 7668	149	28	15	15	15	1	6	6	7	354	58	M
A. carrioni	MZUTI 4195	144	31	15	15	15	1	6	6	8	371	53	M
A. cerberus	MZUTI 5108	152	25	17	17	17	2	7	7	7	309	36	M
A. cerberus	MZUTI 4330	157	26	17	17	17	2	7	7	7	212	23	M
A. duboisi	MHNG 2457.093	166	22	15	15	15	2	7	6	6	455	34	F
A. duboisi	MNHN 0.6147	164	17	15	15	15	2	8	7	–	131	11	F
A. dunni	DHMECN 12769	141	36	17	17	17	2	6	7	7	279	39	–
A. dunni	DHMECN 2215	144	24	17	17	17	2	7	7	6	278	35	F
A. dunni	DHMECN 3527	141	24	17	17	17	2	6	6	6	352	48	F
A. dunni	DHMECN 3900	143	21	17	17	17	2	6	6	–	101	19	–
A. dunni	DHMECN 4159	129	35	17	17	17	2	5	6	6	266	65	–
A. dunni	EPN 3127	–	–	–	–	–	–	–	–	–	355	46	F
A. dunni	EPN 3128	–	–	–	–	–	–	–	–	–	295	63	M
A. dunni	FHGO 375	128	36	17	17	17	2	7	7	6	219	48	M
A. dunni	FHGO 376	143	26	17	17	17	2	7	7	5	278	33	F
A. dunni	FHGO 379	132	35	17	17	17	2	7	7	6	297	61	M
A. dunni	FHGO 91	125	35	17	17	17	2	7	7	6	231	52	M
A. dunni	MHNG 2441.043	145	20	17	17	17	2	7	7	6	205	22	F
A. dunni	MHNG 2457.091	129	34	17	17	17	2	7	6	5	197	39	M
A. dunni	MHNG 2464.03	136	39	16	17	17	2	7	6	5	114	22	M
A. dunni	MZUTI 2189	134	29	17	17	17	2	7	7	6	189	28	M
A. dunni	MZUTI 3031	139	24	17	17	17	2	7	7	5	329	36	F
A. dunni	MZUTI 4097	149	21	17	17	17	2	7	7	6	152	17	–
A. dunni	MZUTI 4098	130	37	17	17	17	2	7	7	6	126	19	–
A. dunni	MZUTI 4099	140	25	17	17	17	2	7	7	–	118	15	F
A. dunni	MZUTI 4100	138	24	17	17	17	2	7	7	–	335	36	F
A. dunni	MZUTI 4318	136	34	17	18	17	2	7	7	6	242	53	M
A. dunni	MZUTI 4319	129	35	15	17	17	2	7	7	5	242	53	M
A. esepe	MZUTI 3758	149	41	17	17	17	2	7	7	5	232	53	M
A. esepe	MZUTI 3759	156	30	17	17	17	2	7	7	5	241	34	F
A. gaigeae	MHNG 2397.044	136	34	17	17	17	2	7	7	5	129	23	M
A. gigas	MHNG 2250.035	168	34	19	17	17	2	6	6	3	272	40	F
A. gigas	MHNG 2441.02	177	31	17	17	17	2	6	6	5	1060	116	F
A. iridescens	DHMECN 2932	138	28	17	17	17	2	6	7	6	252	36	F
A. iridescens	DHMECN 5663	141	32	17	17	17	2	6	6	6	272	46	F
A. iridescens	DHMECN 9633	129	42	16	17	17	2	6	6	6	219	62	M
A. iridescens	FHGO 10443	139	32	17	17	17	2	7	7	5	204	32	F
A. iridescens	MZUTI 3548	131	34	17	17	17	2	7	7	6	200	44	M

Species	Voucher	V	SC	D1	D2	D3	PO	SL	IL	MT	SVL	TL	Sex
A. iridescens	MZUTI 3680	140	40	17	17	17	2	7	7	6	210	46	M
A. iridescens	MZUTI 4178	148		17	17	17	2	7		5	211	37	M
A. iridescens	MZUTI 4697	127	38	17	17	17	2	7	7	5	209	46	M
A. lehmanni	DHMECN 7644	144	29	15	15	15	1	5	6	11	300	35	M
A. lehmanni	DHMECN 7645	144	23	15	15	15	1	5	7	10	321	42	–
A. major	MNHN 0.6149	174	35	17	17	17	2	7	7	6	586	86	F
A. microrhynchus	DHMECN 2586	144	39	17	17	17	1	7	6	6	239	45	M
A. microrhynchus	FHGO 897	149	37	17	17	17	2	7	7	7	239	51	M
A. microrhynchus	MHNG 2307.017	133	34	18	17	17	2	7	6	5	269	55	M
A. microrhynchus	MHNG 2397.019	144	25	17	17	17	2	7	7	6	300	–	F
A. microrhynchus	MHNG 2397.02	147	26	17	17	17	2	7	6	5	225	28	F
A. microrhynchus	MHNG 2397.021	144	24	17	17	17	2	7	6	5	217	28	F
A. microrhynchus	MHNG 2459.052	137	36	17	17	17	2	7	6	5	239	53	M
A. microrhynchus	MZUTI 4122	163	29	17	17	17	2	7	7	7	222	27	F
A. microrhynchus	QCAZ 1219	147	40	17	17	17	2	7	7	7	178	37	M
A. microrhynchus	USNM 285473	152	26	17	17	17	2	7	7	–	335	45	F
A. microrhynchus	USNM 285474	163	28	17	17	17	2	7	7	–	212	21	F
A. modestus	DHMECN 3859		45	17	17	17	2	7	6	–	344	41	–
A. modestus	FHGO 2936	165	41	17	17	17	2	7	7	5	110	20	M
A. modestus	FHGO 44	186	27	17	17	17	2	7	7	6	294	38	F
A. modestus	MHNG 2397.041	146	21	15	15	15	2	7	6	6	200	23	M
A. modestus	MZUTI 4760	147	42	17	17	17	2	6	7	5	273	59	M
A. occidentalis	FHGO 385	128	37	17	17	17	2	7	7	7	188	40	F
A. occidentalis	MHNG 2252.079	145	20	17	17	17	2	6	7	5	262	25	F
A. occidentalis	MHNG 2307.068	141	35	17	17	17	2	6	7	5	272	55	M
A. occidentalis	MHNG 2397.028	137	38	17	17	17	2	6	7	5	117	21	M
A. occidentalis	MHNG 2411.085	138	35	17	17	17	2	7	7	5	253	55	M
A. occidentalis	MHNG 2411.086	129	33	17	17	17	2	7	6	5	122	23	M
A. occidentalis	MHNG 2441.044	134	37	17	17	17	2	7	7	–	274	68	M
A. occidentalis	MZUTI 2649	134	36	17	17	16	2	7	7	6	223	35	F
A. occidentalis	MZUTI 2650	149	24	17	17	17	2	7	7	–	191	21	F
A. occidentalis	MZUTI 3323	134	39	17	17	17	2	7	7	7	332	67	M
A. paucidens	DHMECN 11980	171	43	17	17	17	2	7	7	7	290	50	M
A. paucidens	DHMECN 3975	163	43	17	17	17	2	–	7	7	249	50	M
A. paucidens	EPN 8730	–	–	–	–	–	–	–	–	–	246	53	M
A. paucidens	EPN 8731	–	–	–	–	–	–	–	–	–	237	51	M
A. paucidens	MHNG 2309.065	156	46	15	15	15	2	7	6	6	196	45	M
A. paucidens	MNHN 1906.245	186	40	17	17	17	2	7	7	–	262	42	M
A. pyroni	MZUTI 5107	143	16	15	15	15	1	6	5	8	443	34	F
A. roulei	QCAZ 6256	135	27	15	15	15	1	6	6	9	337	48	M
A. roulei	QCAZ 7887	146	25	15	15	15	1	5	6	9	309	39	M
A. roulei	QCAZ 7902	156	19	15	15	15	1	6	7	11	392	37	F
A. roulei	QCAZ 9643	149	17	15	15	15	1	6	6	11	139	13	F
A. roulei	QCAZ 9652	143	19	15	15	15	1	6	6	13	230	21	F
A. savagei	DHMECN 3800	166	25	17	17	17	2	6	7	7	214	23	F
A. snethlageae	MNHN 1906.244	151	29	17	17	17	2	7	7	7	283	35	F

Species	Voucher	V	SC	D1	D2	D3	PO	SL	IL	MT	SVL	TL	Sex
A. snethlageae	MNHN 1994.1171	160	27	17	17	17	2	7	7	8	315	35	F
A. touzeti	ANF 2390	176	31	17	17	17	2	7	7	7	652	71	F
A. trilineatus	MNHN 1898.313	141	19	15	15	15	2	7	7	8	179	19	M
A. trilineatus	MNHN 1898.314	132	21	15	15	15	2	7	7	7	182	20	M
A. typhon	DHMECN 9632	153	47	15	15	15	2	7	6	7	187	31	M
A. typhon	FHGO 10438	166	41	15	15	15	2	7	7	6	370	68	M
A. typhon	FHGO 10439	158	48	16	16	16	2	7	7	7	349	87	F

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RESEARCH ARTICLE

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Elthusa winstoni sp. n. (Isopoda, Cymothoidae), a new fish parasitic isopod from Hawaii

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Abstract

The new cymothoid species, *Elthusa winstoni* **sp. n.**, a branchial parasite of fishes from the family Acanthuridae Bonaparte, 1835 in Hawaii, is described and figured. The female adults can be distinguished by the strongly vaulted body and compacted body shape; rostrum with a small median point; short antennae which are close together (only 6 articles in both antennula and antenna); short and wide uropods extending to half the length of the pleotelson; short dactyli on pereopod 7; and large recurved robust setae on the maxilla. This is the first record of an *Elthusa* Schioedte & Meinert, 1884 species from the Hawaiian Islands and only the fifth cymothoid described from this region.

Keywords

Marine fish parasite, branchial cavity, Pacific Ocean, *Ctenochaetus strigosus*, *Acanthurus nigroris*

Introduction

The cymothoid genus *Elthusa* Schioedte & Meinert, 1884 was first established in 1884, with *E. emarginata* (Bleeker, 1857) the only known species at the time. Over a century later, Bruce (1990) revised the genus, reclassifying 21 species from *Livoneca* Leach, 1818 into *Elthusa*, and adding two new species. Since then, another four species have been described from New Caledonia and Mexico (Trilles and Justine 2004, 2006, 2010, Rocha-Ramírez et al. 2005). In their review of cymothoids, Smit et al. (2014) stated that there were 28 known *Elthusa* species. Since then, an *Elthusa* homonym has been corrected (Hadfield et al. 2016a), making a current total of 29 recognised species within the genus.

Elthusa has a global distribution (Rocha-Ramírez et al. 2005), being absent only from polar waters. While moderately well-known from the Caribbean, Australia and central Indo-Pacific, the vast central Pacific region has been scarcely studied, with recent records only from New Caledonia (Trilles and Justine 2004, 2006, 2010).

In Hawaii, Bunkley-Williams and Williams Jr. (1996) listed the isopods known from Hawaiian fish hosts, recording only four cymothoid species, namely: *Anilocra gigantea* (Herklots, 1870); *Creniola breviceps* (Schioedte & Meinert, 1881); *Cymothoa recta* Dana, 1853; and *Ichthyoxenus puhi* (Bowman, 1960). This study is the first record of a gill-attaching *Elthusa* species in Hawaii, with both the male and female being described for the new species.

Methods

During a research visit to the Smithsonian National Museum of Natural History in Washington DC (USA) in November 2014, an unnamed specimen originally collected in 1959 from *Acanthurus nigroris* Valenciennes, 1835, from Oahu, Hawaii, was observed. Recently, new material of the same species was collected from the gill rakers of a kole tang, *Ctenochaetus strigosus* (Bennett, 1828), speared by a SCUBA diver at approximately 20 m depth on 28 September 2015 in marine waters adjacent to the island of Niihau, Hawaii (22.00296, -160.11894). The host fish and parasites were stored frozen at-sea and initially examined approximately one month later at the Hawaii Institute of Marine Biology, Kaneohe, Hawaii. Isopods were subsequently removed from the gill chambers of the host fish and preserved in 70% ethanol. All specimens were processed following the techniques recorded in Hadfield et al. (2010, 2013). The female designated as the holotype (and male paratype) were minimally dissected in order to conserve the specimens. The species descriptions were prepared in DELTA (Descriptive Language for Taxonomy) using a general Cymothoidae character set (see Hadfield et al. 2016b). Isopod classification follows Brandt and Poore (2003) and host nomenclature follows that of FishBase (Froese and Pauly 2016) and *Catalog of Fishes* (Eschmeyer 2016).

Abbreviations. AMNH – American Museum of Natural History, New York, USA; **USNM** – National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA; **TL** – total length; **W** – width.

Taxonomy

Suborder Cymothoida Wägele, 1989
Superfamily Cymothooidea Leach, 1814
Family Cymothoidae Leach, 1814

Genus *Elthusa* Schioedte & Meinert, 1884

Elthusa Schioedte & Meinert, 1884: 337.—Bruce 1990: 254.—Trilles 1994: 164.—Trilles and Randall 2011: 453–454.

Type species. *Livoneca emarginata* Bleeker, 1857, by monotypy (Schioedte and Meinert 1884).

Remarks. Diagnostic characters for *Elthusa* include a weakly vaulted body; pleonite 1 as wide as or slightly narrower than pleonite 2; posterior margin of cephalon not trilobed; wide pleon; antennula shorter than antenna, bases not in contact (varying from close together to wide apart); and pleopods all simple, lamellar. A revised diagnosis of the genus was provided by Bruce (1990) as well as Trilles and Randall (2011).

Elthusa can be regarded as one of the most morphologically varied cymothoid genera, with many *Elthusa* species still requiring detailed study and redescription. The antennula position (being close together or far apart), shape of the cephalon anterior margin, as well as the width of the pleon and pleonite 5 are some of the varying characters noted within species of this genus (Bruce 1990). This variation can cause confusion in genus identifications and may lead to incorrect species placement if not taken into account with all of the other genus characters.

Elthusa winstoni sp. n.

http://zoobank.org/FDE6479B-D082-4699-9719-5BAB5181914E
Figures 1–4

Material examined. *Holotype*. Female (17.5 mm TL; 11.5 mm W), partially dissected, from the gill rakers of kole tang, *Ctenochaetus strigosus* (Bennett, 1828), 18–21m deep, Niihau, Hawaii, 27.10.2015, col: Erik Franklin and Morgan Winston (AMNH_IZC 250217).

Paratype. Dissected male (8 mm TL; 3.5 mm W), male (8.5 mm TL; 4 mm W), same data as holotype (AMNH_IZC 250218).

Other material. Ovigerous female (18 mm TL; 14 mm W), male (8 mm; 4 mm W), from the left gill cavity of the bluelined surgeonfish, *Acanthurus nigroris* Valenciennes, 1835, between Diamond Head and Koko Head, Oahu, Hawaii, 10.10.1959 (USNM 1256197).

Description. *Holotype female.* Length 17.5 mm, width 11.5 mm.

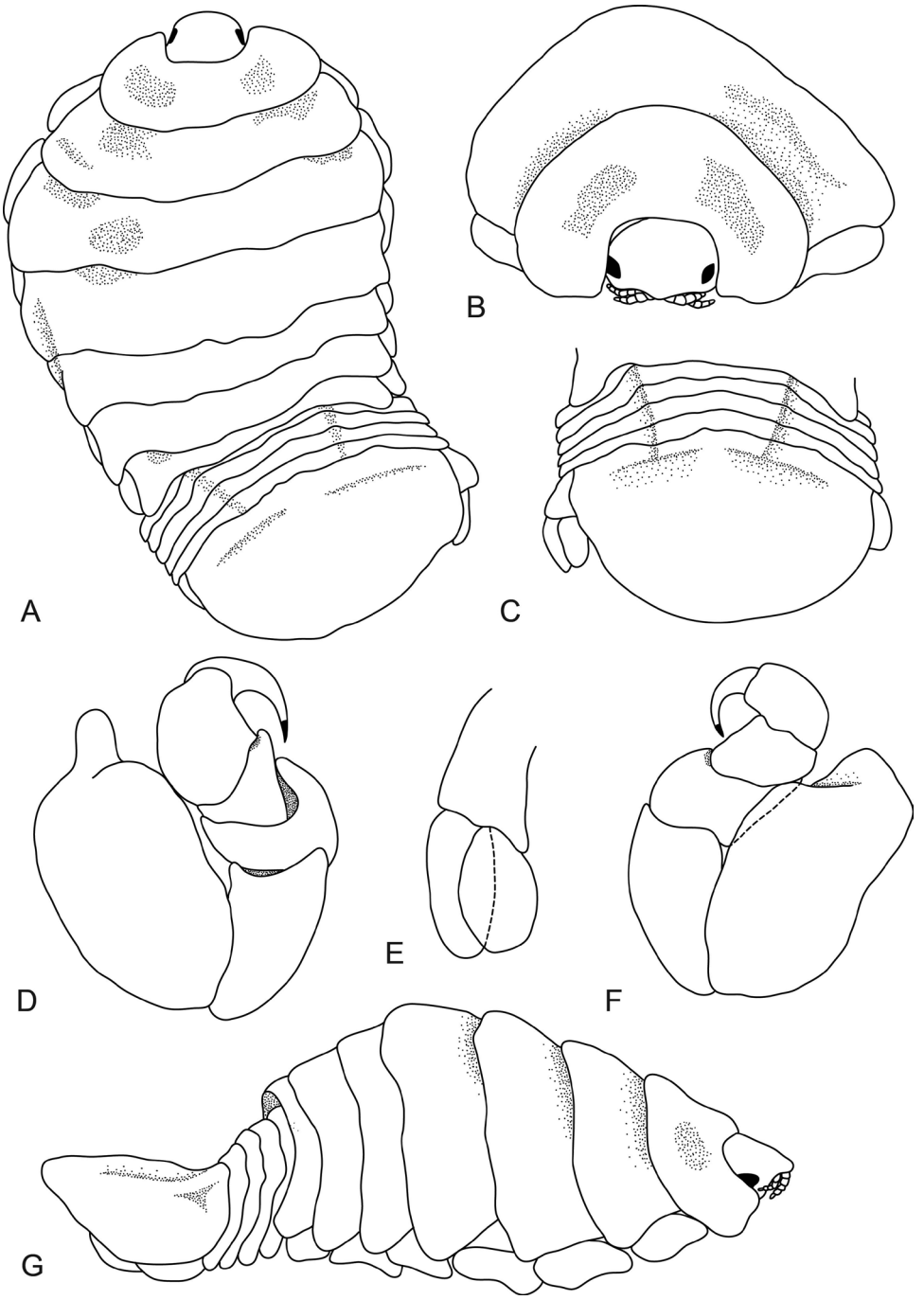

Figure 1. *Elthusa winstoni* sp. n., female holotype (17.5 mm) (AMNH_IZC 250217). **A** dorsal view **B** anterior view of pereonite 1, 2 and cephalon **C** dorsal view of pleotelson **D** pereopod 1 **E** uropod **F** pereopod 7 **G** lateral view.

Body compact, weakly twisted, 1.7 times as long as greatest width, wider anteriorly, dorsal surfaces rugose and strongly arched longitudinally, widest at pereonite 3, most narrow at pereonite 1, lateral margins slightly convex. *Cephalon* 0.7 times longer than wide, visible from dorsal view, square and deeply immersed in pereonite 1. *Frontal margin* forming rounded rostrum with small median point. *Eyes* oval with distinct margins, one eye 0.1 times width of cephalon; 0.25 times length of cephalon. *Pereonite 1* with slight indentations, anterior border straight, anterolateral angle with large broad projections, extend to anterior margin of eyes. Posterior margins of pereonites not smooth, with irregular nodules in certain areas. Coxae 2–3 wide, with posteroventral angles rounded; 4–7 rounded, not extending past pereonite margin. Pereonites 1–3 increasing in length and width; 4–7 decreasing in length and width; becoming more progressively rounded posteriorly. *Pleon* with pleonite 1 largely concealed by pereonite 7 and same width as other pleonites, partially visible in dorsal view; pleonites posterior margin not smooth. Distal ends of pleonite 2 partially overlapped by pereonite 7; posterolateral angles of pleonite 2 narrowly rounded. Pleonites 3–5 similar in form to pleonite 2. *Pleotelson* 0.6 times as long as anterior width, dorsal surface slightly depressed, lateral margins convex, posterior margin rounded.

Antennula approximately the same length as antenna, bases narrowly separated, consisting of 6 articles, extending to anterior margin of eye. *Antenna* consisting of 6 articles, extending to middle of the eye. *Mandibular molar process* ending in an acute incisor, with a single simple seta. *Maxillula* simple with 4 terminal robust setae. *Maxilla* mesial lobe partially fused to lateral lobe; lateral lobe with 3 recurved robust setae; mesial lobe with 1 large recurved robust seta. *Maxilliped* weakly segmented, with lamellar oostegite lobe, article 3 with 3 recurved robust setae.

Pereopod 1 basis 1.5 times as long as greatest width; ischium 0.7 times as long as basis; merus proximal margin with bulbous protrusion; carpus with straight proximal margin; propodus as long as wide; dactylus slender, 0.9 times as long as propodus, 1.8 times as long as basal width. *Pereopod 7* same length as other pereopods, basis 1.8 times as long as greatest width; ischium 0.7 times as long as basis, without protrusions; merus proximal margin with slight bulbous protrusion, distal margin produced, 0.7 times as long as wide, 0.4 times as long as ischium; carpus 0.9 times as long as wide, 0.3 times as long as ischium, without bulbous protrusion; propodus 1.7 times as long as wide, 0.5 times as long as ischium; dactylus slender, 0.7 times as long as propodus, 2.4 times as long as basal width.

Pleopods without setae, simple. *Pleopod 1* exopod 1.3 times as long as wide, lateral margin strongly convex, distally broadly rounded, mesial margin straight; endopod 1.3 times as long as wide, lateral margin convex, distally broadly rounded, mesial margin straight; peduncle 2.8 times as wide as long, without retinaculae. Peduncle lobes absent. Pleopods 2–5 similar to pleopod 1.

Uropod half the length of pleotelson, peduncle 0.8 times longer than rami, peduncle lateral margin without setae. *Endopod* wide, apically rounded, 1.6 times as long as greatest width, lateral margin weakly convex, mesial margin weakly convex, terminating without setae. *Exopod* extending to end of endopod, 2.2 times as long as greatest

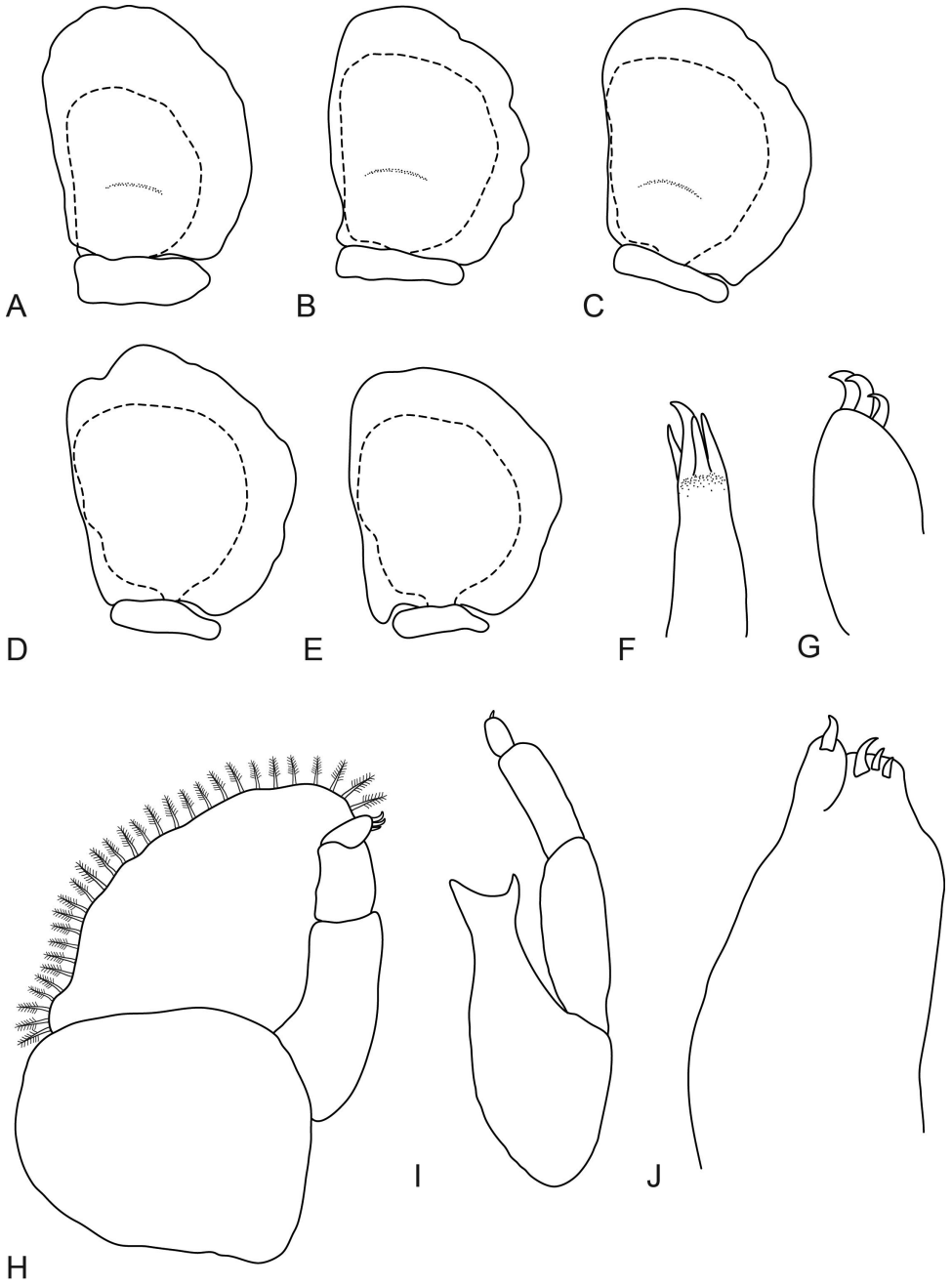

Figure 2. *Elthusa winstoni* sp. n., female holotype (17.5 mm) (AMNH_IZC 250217). **A–E** pleopods 1 to 5 respectively **F** tip of maxillule **G** tip of maxilliped article 3 **H** maxilliped with oostegite **I** mandible **J** maxilla.

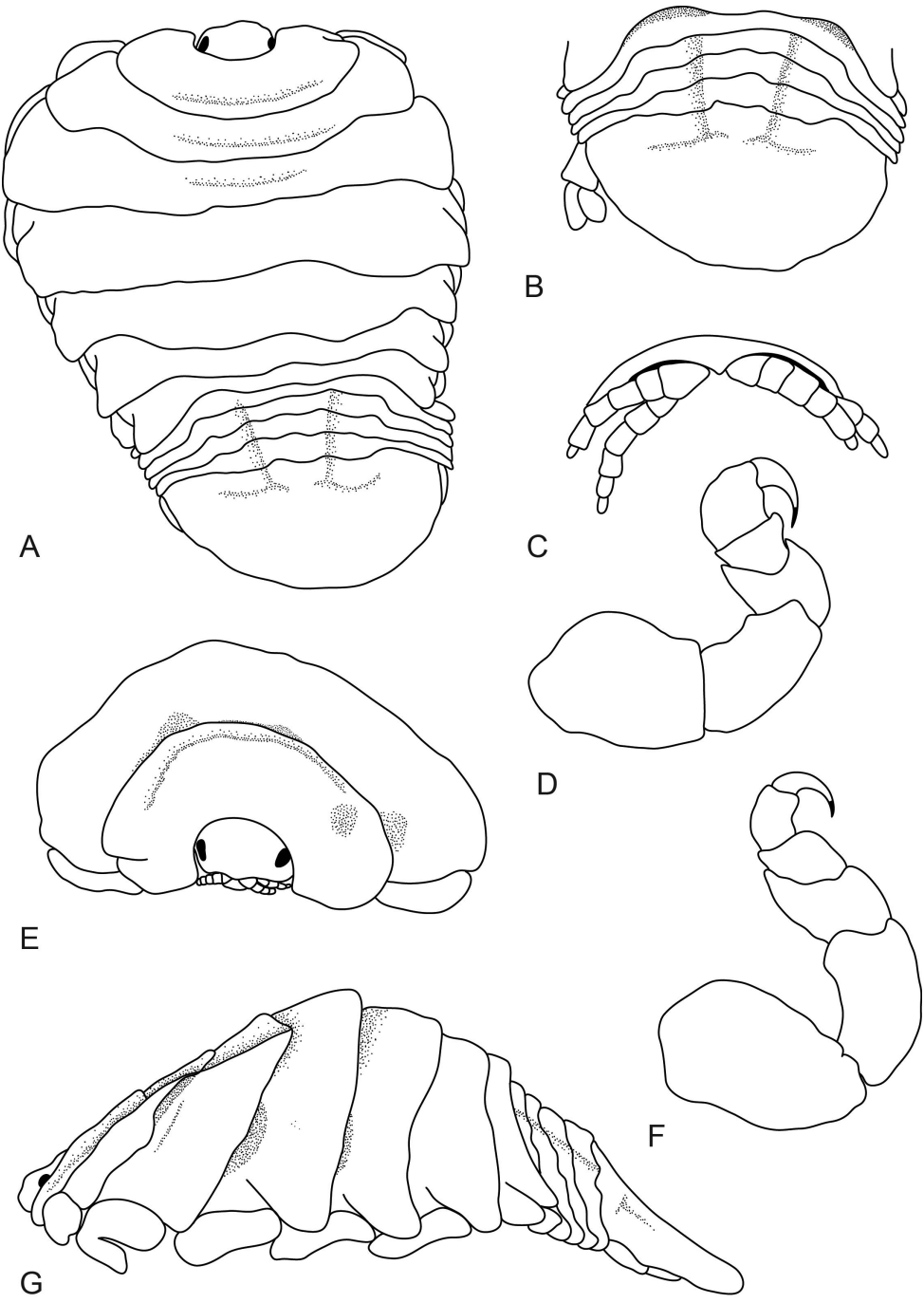

Figure 3. *Elthusa winstoni* sp. n., female (18 mm) (USNM 1256197). **A** dorsal view **B** dorsal view of pleotelson **C** ventral view of cephalon **D** pereopod 1 **E** anterior view of pereonite 1, 2 and cephalon **F** pereopod 7 **G** lateral view.

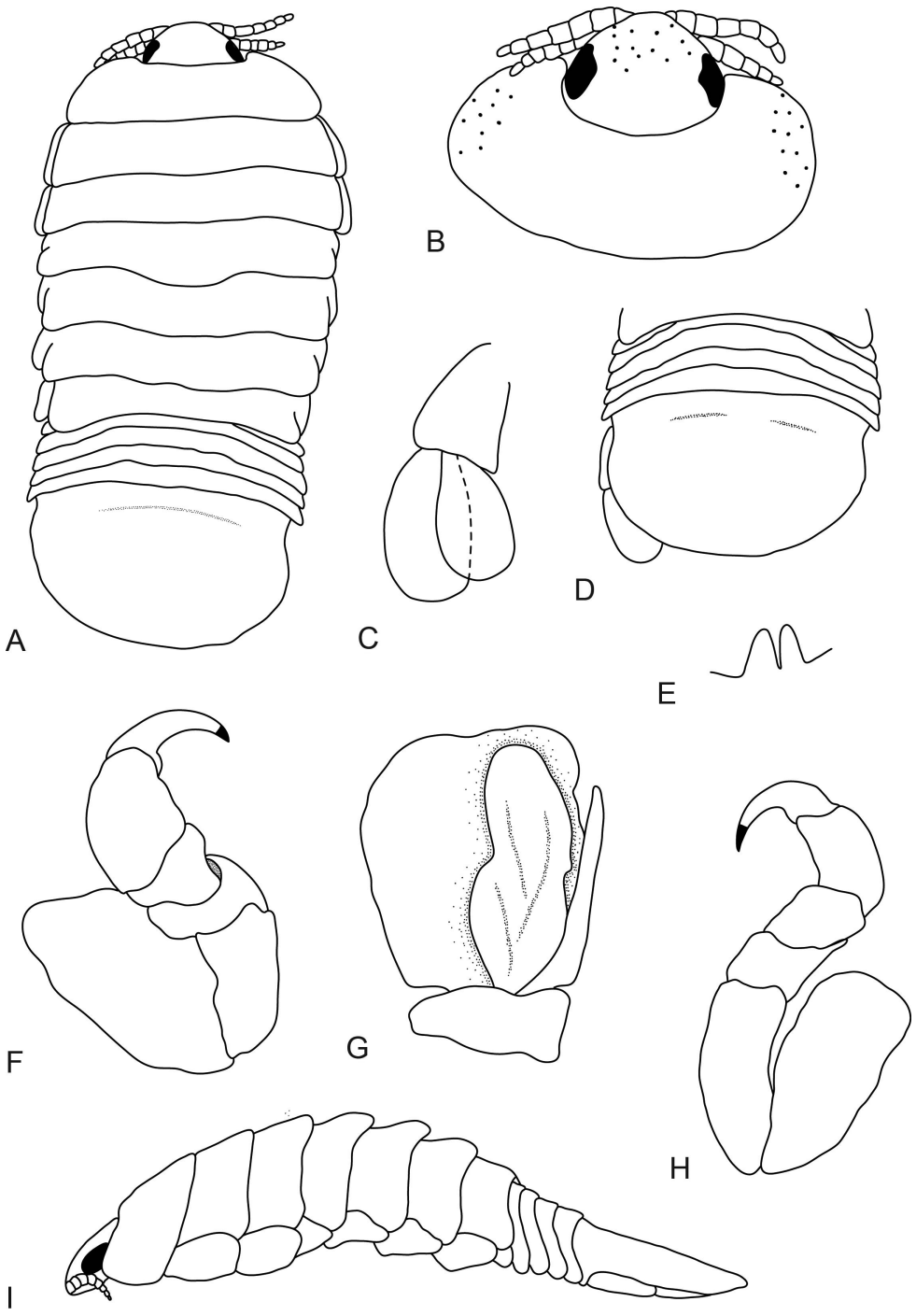

Figure 4. *Elthusa winstoni* sp. n., male paratype (8 mm) (AMNH_IZC 250218). **A** dorsal view **B** dorsal view of pereonite 1 and cephalon **C** uropod **D** dorsal view of pleotelson **E** penes **F** pereopod 1 **G** pleopod 2 with appendix masculina **H** pereopod 7 **I** lateral view.

width, apically rounded, lateral margin straight, mesial margin straight, terminating without setae.

Male. Length 8 mm, width 3.5 mm.

Male similar to female but much smaller. Body rectangular, body 2.1 times as long as wide. *Cephalon* with scattered chromatophores. *Pereonite 1* anterolateral margin broad with scattered chromatophores. *Antennula* bases separated, consisting of 8 articles, extending to posterior margin of eye. *Antenna* consisting of 8 articles, extending to posterior margin of cephalon. *Mandibular molar process* ending in an acute incisor. *Maxillula* simple with 4 terminal robust setae. *Maxilla* with 2 recurved robust setae. *Maxilliped* weakly segmented, with lamellar oostegite lobe, article 3 with 3 recurved robust setae. *Penes* opening flush with surface of sternite 7, tubercules separate, penial process 0.75 times as long as basal width. *Pleopod 2* appendix masculina with parallel margins, 0.8 times as long as endopod, distally narrowly rounded. *Uropods* same length as pleotelson.

Etymology. Named after one of the collectors of the type specimen, Morgan Winston, who collected the type specimens while diving adjacent to the island of Niihau, Hawaii.

Distribution. Known only from Hawaii.

Hosts. Known only from the kole tang, *Ctenochaetus strigosus*, and the bluelined surgeonfish, *Acanthurus nigroris*, both from the family Acanthuridae Bonaparte, 1835.

Remarks. *Elthusa winstoni* sp. n. can be distinguished from all congeners by the irregular, compact body shape; short antennae; short and wide uropods only extending to middle of the pleotelson; and a strongly vaulted body.

This species conforms with many of the *Elthusa* characters in having a wide pleon with pleonite 1 as wide a pleonite 2; the cephalon posterior margin is straight; the antennula bases are not in contact; and all of the pleopods are simple and lamellar. *Elthusa winstoni* sp. n. differs from other *Elthusa* species in the asymmetrical, strongly vaulted body and short antennae not extending past the cephalon (only 6 articles for both antennula and antenna in female, 8 in male). Furthermore, the mandibular palp is slender with only one seta, large recurved robust setae on maxilla, short dactyli on pereopod 7, and a very compact body (1.2 – 1.6 times as long as wide). There are no similar cymothoid species from Hawaii.

Many of the *Elthusa* hosts remain unknown; however, both *Acanthurus nigroris* and *Ctenochaetus strigosus* appear to be new hosts for *Elthusa* isopods. *Creniola breviceps* has previously been located on *Ctenochaetus strigosus* in Hawaii, but *Acanthurus nigroris* is a new host record for cymothoid isopods.

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RESEARCH ARTICLE

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Helobdella blinni sp. n. (Hirudinida, Glossiphoniidae) a new species inhabiting Montezuma Well, Arizona, USA

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Abstract

A new leech species *Helobdella blinni* **sp. n.**, is described from Montezuma Well, an isolated travertine spring mound located in central Arizona, USA. In its native habitat, *H. blinni* had been previously identified as *Helobdella stagnalis* (Linnaeus, 1758), which was later reclassified to *Helobdella modesta* (Verrill, 1872). Similar to the European *H. stagnalis* and North American *H. modesta*, *H. blinni* has six pairs of testisacs, five pairs of smooth crop caecae, one lobed pair of posteriorly-directed crop caecae, one pair of eyes, a nuchal scute, and diffuse salivary glands. However, the pigmentation of this new species ranges from light to dark brown, unlike *H. modesta* which tends to be light grey in color. Also, *H. modesta* produces a clutch of 12–35 pink eggs, whereas *H. blinni* produces smaller clutches of white eggs (7–14, 0.5 ± 0.15 mm, N = 7) and consequently broods fewer young (1–14, 7 ± 3.3 mm, N = 97). *Helobdella blinni* are also able to breed year-round due to the constant warm water conditions in Montezuma Well. Their breeding season is not restricted by seasonal temperatures. These species are morphologically similar, however, comparing the COI mtDNA sequences of *H. blinni* with sequences from nearby populations of *H. modesta* and other *Helobdella* species from GenBank indicate that *H. blinni* is genetically distinct from these other *Helobdella* populations.

Keywords

Leech, Glossiphoniidae, *Helobdella blinni* sp. n., new species, Montezuma Well

Introduction

Montezuma Well is a collapsed travertine spring mound located 72 km south of Flagstaff in the Verde Valley of Northern Arizona (34.6491°N,111.7522°W (DD)) (Fig. 1A). The age of Montezuma Well is estimated to be ~11,000 years (Wagner and Blinn 1987). This location is thermally constant year-round (19–24°C) and is continuously replenished by two vents located at the well bottom. Montezuma Well is 0.76 ha in area and approximately 20 m deep. Most of the shoreline drops off immediately into open water, except at the northeast corner where water drains through a shallow region called the "swallet" and empties into Wet Beaver Creek which is located east of Montezuma Well (Fig. 1A–B). The water within Montezuma Well has unique water chemistry, containing high levels of arsenic (>100μg/L) and dissolved CO_2 (>300mg/L) (Cole and Barry 1973).

Four leech species are known to inhabit Montezuma Well, including an endemic pelagic predator (Govedich et al. 1998), the erpobdellid *Motobdella montezuma* (Davies et al. 1985), and three other glossiphoniid species currently identified as *Helobdella papillata* (Moore, 1952), *H. elongata* (Castle, 1900), and a species currently thought to be *H. stagnalis* (Linnaeus, 1758), all of which inhabit the swallet (Fig. 1B). These Montezuma Well leech populations are thought to have been isolated from other leech populations for as long as 11,000 years (Wagner and Blinn 1987).

In support of this hypothesis, Beresic-Perrins's (2010) description of brood size, parental behavior, and life history of the Montezuma Well population of *H. stagnalis* suggests that this leech is distinct from other known populations of *H. stagnalis*, a species originally described from Europe and which had until very recently been considered to be a widespread cosmopolitan leech species, inhabiting both Europe and North America. Siddall et al. (2005) addressed this problem by resurrecting the original species description for the North American leech, *Helobdella modesta* (Verrill, 1872) which had long been considered to be a synonym of the European *H. stagnalis* (Moore 1898). The molecular analysis by Moser et al. (2011) provided confirmation for the resurrection of *H. modesta* by Siddall et al. (2005). Even though the two species are morphologically indistinguishable (Verrill 1872, Moore 1898, Moore 1952), they differ genetically. Henceforth, we will refer to the North American *H. stagnalis* as *H. modesta*.

Here, we compare key traits, both morphological and molecular, among members of the Montezuma Well *Helobdella* sp. population, several other nearby populations of *H. modesta*, and several other *Helobdella* species. Our molecular analysis includes the cytochrome *c* oxidase subunit I (COI) mitochondrial gene region to test the hypothesis that the Montezuma Well population of *H. modesta* is a distinct species and warrants a new species description. This region is known to be sufficiently variable to reveal interspecific differences and unlikely to suggest differences due to elevated mutation rates (Apakupakul et al. 1999).

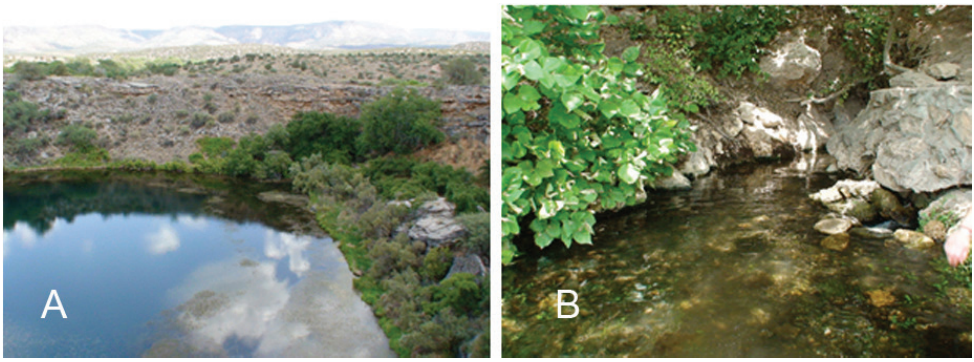

Figure 1. Location of *Helobdella blinni* sp. n. **A** The northeast side of Montezuma Well; and **B** The swallet where the leeches were collected.

Materials and methods

Sampling

A total of 34 individuals of *Helobdella* sp. inhabiting Montezuma Well were collected from the underside of rocks in the swallet: five specimens were collected in June 2011 for molecular analysis and 29 were collected in June 2012 to assess morphological characteristics. For the molecular analysis, the leeches were preserved in 95% ethanol and others, for museum collections, were fixed with buffered formalin overnight and preserved in 70% ethanol. Additionally, a total of 10 specimens of *H.* c.f. *modesta* from Rio de Flag ponds near the Rio de Flag Waste Water Facility outflow in Flagstaff, Arizona (35.18418°N, 111.63294°W (DD)) and Oak Creek, AZ near the Cave Springs campground (34.9961°N, 111.7394°W (DD)) were collected for molecular analyses. These specimens were also fixed in 95% ethanol.

Morphological examination

We documented number of eyes and their placement, color pattern, presence of papillae, number of and structure of gastric caecae, body size, presence of nuchal scute, gonopore placement, egg size and number, and number of offspring using a Nikon binocular dissecting microscope. We then deposited the examined materials in the Invertebrate Zoology collection at the Smithsonian Institution, National Museum of Natural History (USNM).

Molecular analysis

Whole DNA was extracted from the caudal suckers of the individual leeches using a Qiagen DNeasy Blood & Tissue Kit (Cat. No. 69504), with each sample incubated

Table 1. *Helobdella* and outgroup taxa used for our molecular analysis.

Taxon	Locality	Reference
Cystobranchus salmositicus	Outgroup	Williams and Burreson 2006
Ozobranchus margoi	Outgroup	Siddall and Burreson 1998
Gonimosobdella klemmi	Outgroup	Williams and Burreson 2005
Myzobdella lugubris	Outgroup	Siddall and Burreson 1998
Helobdella atli	French Guiana	Oceguera-Figueroa et al. 2010
Helobdella atli	Uruguay	Oceguera-Figueroa et al. 2010
Helobdella atli	Mexico	Oceguera-Figueroa et al. 2010
Helobdella blinni sp. n.	Montezuma Well, AZ, USA	This study
Helobdella blinni sp. n.	Montezuma Well, AZ, USA	This study
Helobdella blinni sp. n.	Montezuma Well, AZ, USA	This study
Helobdella bolivianita	Bolivia	Siddall and Borda 2002
Helobdella bowermani	Oregon, USA	Moser et al. 2013
Helobdella bowermani	Oregon, USA	Moser et al. 2013
Helobdella bowermani	Oregon, USA	Moser et al. 2013
Helobdella californica	California, USA	Kutschera 2011
Helobdella "elongata"	Mexico	Oceguera-Figueroa et al. 2010
Helobdella europaea	Taiwan	Lai et al. 2009
Helobdella europaea	Taiwan	Lai et al. 2009
Helobdella europaea	Taiwan	Lai et al. 2009
Helobdella europaea	Taiwan	Lai et al. 2009
Helobdella europaea	South Africa	Siddall and Budinoff 2005
Helobdella lineata	Michigan, USA	Siddall and Borda 2002
Helobdella fusca	Michigan, USA	Siddall and Borda 2002
Helobdella melananus	Taiwan	Lai et al. 2009
Helobdella melananus	Taiwan	Lai et al. 2009
Helobdella melananus	Taiwan	Lai et al. 2009
Helobdella michaelseni	Chile	Siddall and Borda 2002
Helobdella modesta	Columbus, Ohio, USA	Siddall and Borda 2002
Helobdella modesta	Washington, USA	Oceguera-Figueroa et al. 2010
Helobdella modesta	Washington, USA	Oceguera-Figueroa et al. 2010
Helobdella c.f. *modesta*	Rio de Flag, Flagstaff, AZ, USA	This study
Helobdella c.f. *modesta*	Rio de Flag, Flagstaff, AZ, USA	This study
Helobdella c.f. *modesta*	Oak Creek, AZ, USA	This study
Helobdella c.f. *modesta*	Oak Creek, AZ, USA	This study
Helobdella nununununojensis	Bolivia	Siddall and Borda 2002
Helobdella nununununojensis	Bolivia	Siddall and Borda 2002
Helobdella octatestisaca	Taiwan	Lai et al. 2009
Helobdella octatestisaca	Taiwan	Lai et al. 2009
Helobdella octatestisaca	Taiwan	Lai et al. 2009
Helobdella octatestisaca	Taiwan	Lai et al. 2009
Helobdella octatestisaca	Taiwan	Lai et al. 2009
Helobdella octatestisaca	Taiwan	Lai et al. 2009
Helobdella octatestisaca	Taiwan	Lai et al. 2009
Helobdella octatestisaca	South Africa	Oceguera-Figueroa et al. 2010

Taxon	Locality	Reference
Helobdella octatestisaca	Mexico	Oceguera-Figueroa et al. 2010
Helobdella octatestisaca	Mexico	Oceguera-Figueroa et al. 2010
Helobdella octatestisaca	Mexico	Oceguera-Figueroa et al. 2010
Helobdella octatestisaca	Mexico	Oceguera-Figueroa et al. 2010
Helobdella octatestisaca	Mexico	Oceguera-Figueroa et al. 2010
Helobdella papillata	Michigan, USA	Siddall and Borda 2002
Helobdella papillata	Virginia, USA	Siddall and Borda 2002
Helobdella papillornata	Australia	Siddall and Borda 2002
Helobdella paranensis	Uruguay	Siddall and Borda 2002
Helobdella pichipanan	Bolivia	Siddall et al. 2005
Helobdella "robusta" TXAU1	Texas, USA	Bely and Weisblat 2006
Helobdella "robusta"	California, USA	Bely and Weisblat 2006
Helobdella "robusta" CASA 1	California, USA	Bely and Weisblat 2006
Helobdella "robusta" NYTA	New York, USA	Bely and Weisblat 2006
Helobdella simplex	Argentina	Moser et al. 2006
Helobdella simplex	Argentina	Moser et al. 2006
Helobdella simplex	Argentina	Moser et al. 2006
Helobdella socimulcensis	Mexico	Oceguera-Figueroa et al. 2010
Helobdella socimulcensis	Mexico	Oceguera-Figueroa et al. 2010
Helobdella socimulcensis	Mexico	Oceguera-Figueroa et al. 2010
Helobdella socimulcensis	Mexico	Oceguera-Figueroa et al. 2010
Helobdella socimulcensis	Mexico	Oceguera-Figueroa et al. 2010
Helobdella socimulcensis	Mexico	Oceguera-Figueroa et al. 2010
Helobdella socimulcensis	Mexico	Oceguera-Figueroa et al. 2010
Helobdella sp. Xochimilco	Mexico	Oceguera-Figueroa et al. 2010
Helobdella sorojchi	Bolivia	Siddall and Borda 2002
Helobdella sorojchi	Bolivia	Siddall and Borda 2002
Helobdella stagnalis	United Kingdom	Siddall and Borda 2002
Helobdella "stagnalis"	Mexico	Oceguera-Figueroa et al. 2010
Helobdella "stagnalis"	Mexico	Oceguera-Figueroa et al. 2010
Helobdella transversa	Michigan, USA	Siddall and Borda 2002
Helobdella triserialis	Bolivia	Siddall and Borda 2002
Helobdella triserialis	California, USA	Bely and Weisblat 2006
Helobdella virginiae	Mexico	Oceguera-Figueroa et al. 2010

overnight in a water bath set at 54°C. Using Siddall and Borda's (2002) PCR method, the mitochondrial gene region, cytochrome *c* oxidase subunit I (COI) was amplified. The primers were LCO1490 5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO2198 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (Folmer et al. 1994). The PCR product was purified through the use of the QIAquick PCR Purification Protocol (Cat. No. 28104), checked for PCR product using gel electrophoresis, and sequenced with an ABI Prism 3730 sequencer (Applied Biosystems). We imported the seven "cleanest" sequences and 71 comparative sequences (Table 1) from previous studies (Siddall and Burreson 1998, Siddall and Borda 2002, Siddall and Budinoff

2005, Siddall et al. 2005, Williams and Burreson 2005, Bely and Weisblat 2006, Williams and Burreson 2006, Lai et al. 2009, Oceguera-Figueroa et al. 2010, Kutschera 2011, Moser et al. 2013) from GenBank (http://www.ncbi.nlm.nih.gov/genbank/) into MEGA7.0.18 (Kumar et al. 2016). We aligned the sequences automatically using MUSCLE (Edgar 2004) and then corrected the alignments by hand. We partitioned the data and performed the substitution model test by codon in Partitionfinder (Lanfear et al. 2012). The best substitution model test was General Time Reversal (GTR) +gamma which we used in our maximum-likelihood (ML) analysis (Lanave et al. 1984, Tavare 1986, Rodriguez et al. 1990). For ML analysis, we used RAxML v. 8 (Stamatakis 2014) and included 1,000 nonparametric bootstrap replicates. We used MrBayes for Bayesian inference analysis with ten million generations with a 25% burn-in and our support was assessed based on clade posterior probabilities (Ronquist and Huelsenbeck 2003). These analyses were conducted through CIPRES (Miller et al. 2010). We used PAUP* 4.0 (Swofford 2003) to construct parsimony phylogenies with 100 random additions. We performed the parsimony analysis twice, treating the deletions in the sequences as a 5th state and then as missing data. We performed an uncorrected p-distance analysis to examine nucleotide differences between sequences with 1,000 replicates in MEGA7.0.18 (Kumar et al. 2016).

Results

Family Glossiphoniidae Vaillant, 1890
Genus *Helobdella* Blanchard, 1896

***Helobdella blinni* sp. n.**
http://zoobank.org/B1B3D234-BC3F-4126-BF25-52DA00BA7EB9
Figs 2, 3, 4

Type materials. Holotype. USNM 1186106 (Table 2).

Additional materials. Paratypes. (14 specimens) (USNM 1186107, 1186108, 1186109, 1186110, 1186111, 1186112, 1186113, 1186114, 1186115, 1186116, 1186117, 1186118, 1186119, 1186120) (Table 2)

Type locality. USA, Arizona: Yavapai County, Montezuma Well (34.6491°N, 111.7522°W (DD)), aquatic system, under rocks, 10 June 2012, R.K. Beresic-Perrins.

Etymology. We have named this new species, *Helobdella blinni* in honor of Dr. Dean W. Blinn for his dedication to natural history research at Montezuma Well. For over 20 years at Northern Arizona University, Dr. Blinn studied a wide range of organisms and their interactions at Montezuma Well including predator-prey interactions between *Motobdella montezuma* and the endemic amphipod, *Hyalella montezuma* Cole & Watkins, 1977.

Description. External morphology. Length of specimens 11 to 22 mm (mean + SE 16.6 + 3.2 N=24) and width 3 to 8 mm (5.7 + 1.1 N=28) (Table 3, Figs 2, 3).

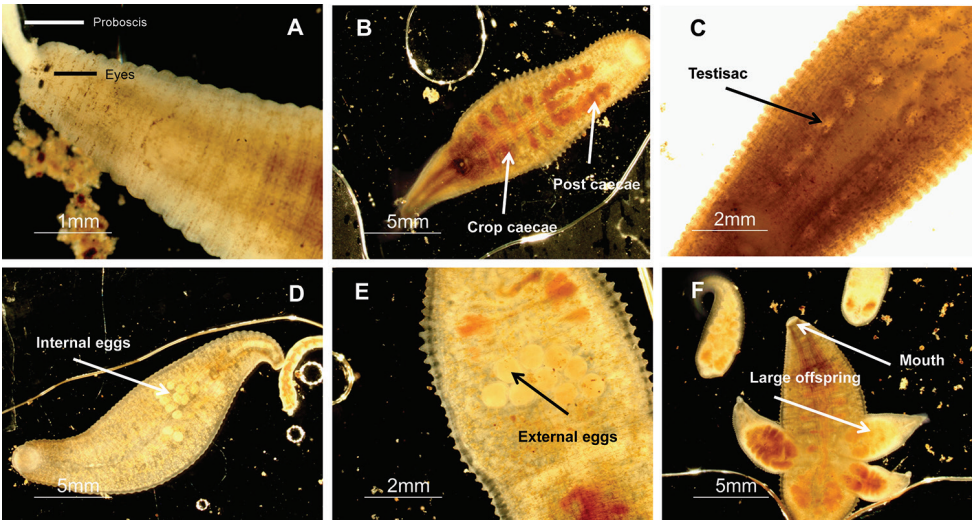

Figure 2. Internal and external morphology of *Helobdella blinni* sp. n. **A** dorsal view of the eyes and extended proboscis **B** crop and post caecae **C** testisacs **D** ventral view of internal eggs which have not been oviposited yet **E** ventral view of white eggs that have been oviposited **F** ventral view of attached and detached offspring.

Table 2. Holotype and paratype collection data and voucher numbers.

Family	Species	Collection data	Voucher #
Glossiphoniidae	*Helobdella blinni* sp. n.	USA: AZ: Yavapai Co., Montezuma Well 34.6491°N, 111.7522°W (DD), 10.VI.2010, aquatic system, under rocks, RK Beresic-Perrins, Holotype (USNM)	1186106
Glossiphoniidae	*Helobdella blinni* sp. n.	(14 specimens) USA: AZ Yavapai Co., Montezuma Well 34.6491°N, 111.7522°W (DD), 10.VI.2010, aquatic system, under rocks, RK Beresic-Perrins, Paratypes (USNM)	1186107
			1186108
			1186109
			1186110
			1186111
			1186112
			1186113
			1186114
			1186115
			1186116
			1186117
			1186118
			1186119
			1186120

Individual color ranges from translucent with brown spots to dark brown (Fig. 4). No dorsal papillae; one pair of eyes located at somite II (0.07 + 0.02 mm diameter, N = 11), distance between eyes 0.1 to 0.03 mm apart (N = 13). A scallop-shaped

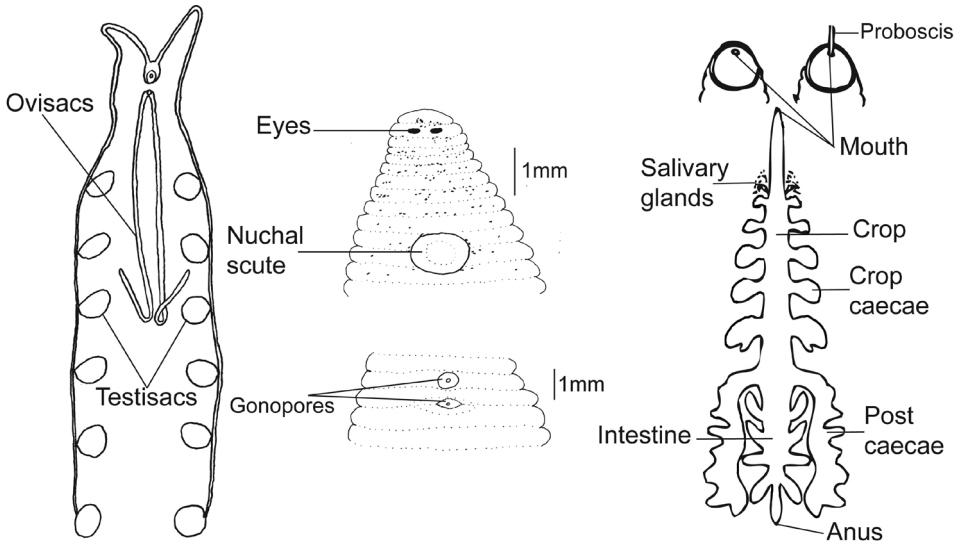

Figure 3. Diagram of the external and internal morphology of *Helobdella blinni* sp. n. (drawn by Rebecca Beresic-Perrins and Fredric Govedich).

Table 3. Morphological measurements of *Helobdella blinni* sp. n.

Trait	Ave	SE	Min	Max	N
body length relaxed (mm)	16.6	3.18	11.3	22.5	24
body width relaxed (mm)	5.7	1.15	3.1	8.0	28
caudal diameter (mm)	1.7	0.3	1.0	2.3	27
egg diameter (mm)	0.5	0.15	0.2	0.7	28
gonopore separation (mm)	0.1	0.08	0.1	0.3	13
nuchal scute length (mm)	0.335	0.05	0.284	0.432	9
nuchal scute width (mm)	0.32	0.04	0.27	0.386	9
proboscis length (mm)	3.5	1.10	2.0	6.2	17
oral sucker diameter (mm)	0.7	0.19	0.4	1.0	15
progeny length (mm)	3.6	1.68	1.6	6.6	18
progeny width (mm)	1.5	0.8	0.7	2.9	18
# eggs	10.0	2.73	7.0	16.0	7
# progeny	7.2	3.35	1.0	14.0	97
eye diameter (mm)	0.1	0.02	0.0	0.1	11
eye distance (mm)	0.1	0.04	0.0	0.2	13

nuchal scute is present on the dorsal side, length 0.293 to 0.432 mm (0.335 + 0.05 N=9) and width 0.27 to 0.386 mm (0.32 + 0.04 N=9). One annulus separates the female and male gonopores. The caudal sucker diameter averages 1.6 + 0.3 mm (N = 27). The eggs (diameter 0.5 + 0.15 mm, N = 28) are laid on the ventral side of the parent in soft-walled transparent cocoons (7–11 eggs per cocoon, N = 7). The mouth is located subterminally in the oral sucker (Figs 2, 3).

Figure 4. Typical pigmentation of *Helobdella blinni* sp. n.

Internal morphology. Average oral sucker diameter is 0.7 + 0.19 mm (N = 15), proboscis length is 3.5 + 1.1 mm (N = 17) (Table 3). Diffuse salivary glands are located near the anterior of the first pair of crop caecae. There are five pairs of smooth crop caecae and one lobed pair of posteriorly directed post caecae. Six pairs of compact testisacs are located in between each of the crop caecae. The intestine contains four pairs of caecae, with the first two pairs anteriorly directed and the other two pairs posteriorly directed. The intestine leads into an unraised anus located two annuli from the caudal sucker (Figure 3).

Development and growth. This species breeds year-round with peaks in spring and fall. Our specimens had an average of 7 to 11 white eggs (diameter 0.5 + 0.15 mm, N = 7) fixed to their ventral surface. Laboratory collections (2007–10) of *H. blinni* documented the eggs hatching 1 to 2 weeks after ovipositing (Beresic-Perrins 2010). Once hatched, the young attach to the ventral surface of the parent, allowing the parent to hunt for food and feed the young, occasionally feeding along with them. Prey consists of oligochaetes and other invertebrates. The average number of young per

adult is 7 + 3.3 (N = 97) ranging from 1 to 14 offspring. The young remain attached to the parent for an additional four to five weeks after hatching. Once the juveniles leave the parent, they tend to aggregate together on rocks (Beresic-Perrins 2010).

Molecular analysis

A Bayesian inference phylogenetic tree of the COI sequence data is presented in Figure 5. We include the posterior probabilities and maximum-likelihood branch supports >50. The Arizona populations of *Helobdella* c.f. *modesta* formed a sister clade to *Helobdella blinni* sp. n., supported by both the Bayesian and parsimony analyses. The results of the uncorrected p-distance analysis revealed a difference of 13.3% (233 nucleotides included) between the two groups (Table 6). The two groups form a larger clade with *H. modesta* (Ohio), *H. stagnalis* (UK), and *H. modesta* (Washington) which is supported by both Bayesian inference and maximum-likelihood. *H. blinni* differed from *H. modesta* (Ohio) by 13.7%, *H. stagnalis* (UK) by 16.3%, and *H. modesta* (Washington) by 16.3% (Table 6).

When we aligned all 78 sequences, there were four, ten-codon deletions within all of the Arizona sequences and *H. atli* (Oceguera-Figueroa and León-Regagnon 2005, Oceguera-Figueroa et al. 2010). When we performed the parsimony analysis, we included deletions as a 5th state in our first analysis and in our second, we treated the deletions as missing data. In the resulting 5th state tree, the two Arizona species remained sister taxa (100% support), but included in the clade was *H. atli* (100% and 58% support). The missing data tree placed *H. blinni* ancestral to *H. modesta* (Washington), *H. modesta* (Ohio), *H. stagnalis* (UK), and *H.* c.f. *modesta* with 100% branch support (Fig. 5).

Discussion

Helobdella blinni sp. n. has morphological and life-history traits similar to other *Helobdella* species, including possession of a nuchal scute, diffuse salivary glands, six pairs of testisacs, and extended parental care for the young (6–7 weeks; Tables 4, 5). *Helobdella blinni*, *H. bowermani* (Moser et al. 2013), *H. octatestisaca* (Lai et al. 2009), and *H.* c.f. *modesta* each have five pairs of smooth crop caecae as opposed to six pairs of lobed crop caecae in *H. californica* (Kutschera 2011) and *H. papillornata* (Govedich and Davies 1998). *Helobdella blinni* and *H. temiscoensis* (Salas-Montiel et al. 2014) share pigmentation characteristics, but they differ internally. *Helobdella temiscoensis* has only four pairs of crop caecae and one descending post caecae as opposed to five pairs and one descending post caecae in *H. blinni*. *Helobdella modesta*, *H. californica, H. atli, H. bowermani,* and *H. octatestisaca* do not resemble the pigmentation of *H. blinni*, running the spectrum from grey to pink. Additionally, they have a descending pair of post caecae, whereas *H. atli, H. californica,* and *H. papillornata* do not. *Helobdella blinni,*

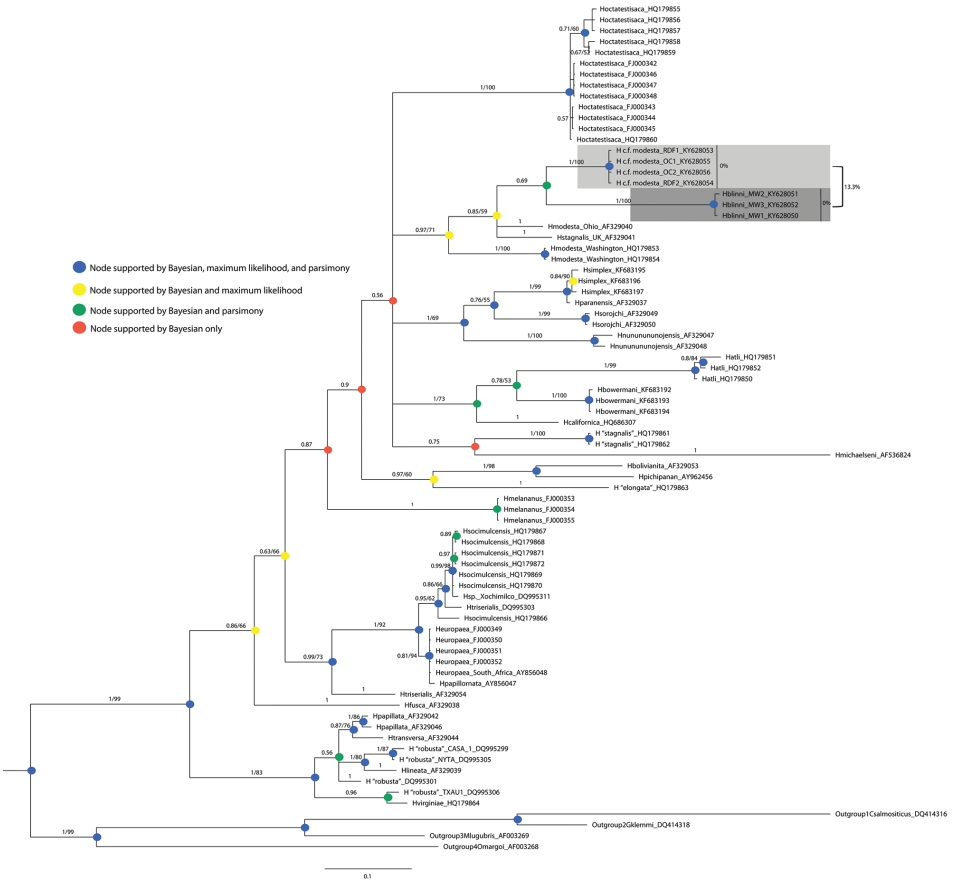

Figure 5. Bayesian Inference phylogenetic tree with 25% burn-in and support was assessed based on clade posterior probabilities tree. We included COI sequences from 31 species of *Helobdella* (family Glossiphoniidae). The Arizona populations are from Oak Creek (OC), Rio de Flag (RDF), and Montezuma Well (MW). Our outgroup included *Cystobranchus salmositicus* (Meyer, 1946), *Gonimosobdella klemmi* (Williams & Burreson, 2005), *Myzobdella lugubris* (Leidy, 1851), and *Ozobranchus margoi* (Davies, 1978). The shaded branches are the Arizona sample sequences. Branch labels include the Bayesian / ML probability. The blue nodes are supported by Bayesian Inference, Maximum-Likelihood, and parsimony analyses. The yellow nodes are supported by Bayesian Inference and Maximum-Likelihood analyses. The green nodes are supported by Bayesian Inference and parsimony analyses. The red nodes are supported by Bayesian Inference analysis only.

H. c.f. *modesta, H. californica, H. temiscoensis, H. atli, and H. bowermani* possess six pairs of testisacs, whereas *H. papillornata* has five pairs and *H. octatestisaca* has four pairs. *Helobdella blinni* also has a larger proboscis than the other *Helobdella* species (mean + SE, *H. blinni* 3.5 mm + 1.1, N=17, *H. californica* mean = 0.7 mm, *H. papillornata* mean= 2 mm). Furthermore, breeding periods also differ between *H. blinni* and the other *Helobdella* species (Tables 4, 5).

Table 4. Morphological comparison of *Helobdella* species

Traits	*H. blinni* sp. n.	*H. modesta*	*H. californica*	*H. papillornata*	*H. temiscoensis*	*H. atli*	*H. bowermani*	*H. octatestisaca*
	(current paper)	(Kutschera 1988: Sawyer 1986)	(Kutschera 1988; 2011)	(Govedich and Davies 1998)	(Salas-Montiel et al. 2014)	(Oceguera-Figueroa and León-Regagnon 2005)	(Moser et al. 2013)	(Lai et al. 2009)
crop caecae	5 pairs, smooth	5 pairs, smooth	6 pairs, lobed	5–6 pairs, lobed	4 pairs	6 pairs	5 pairs, smooth	5 pairs
post caecae	1 pair	1 pair	none	none	1 pair	none	1 pair	1 pair
eyes	1 pair	1 pair	1 pair	1 pair)	1 pair	1 pair	1 pair	1 pair
distance between eyes	0.1 mm	?	?	0.06 mm	?	?	?	?
nuchal scute	yes	yes	yes	no	yes	yes	yes	yes
pairs of testisacs	6 pairs	6 pairs	6 pairs	5 pairs	6 pairs	6 pairs	6 pairs	4 pair
salivary glands	diffuse	diffuse	?	diffuse	diffuse	?	diffuse	diffuse
proboscis length	3.5mm	?	0.7mm	2mm	?	?	?	?
color	transparent with spots to dark brown	transparent to light grey	dark grey	transparent with stripes and papillae	pale brown, blackish - on posterior and mid-body	white-yellowish	pale yellow/ buff, papillae present	brown, pale, gray, and pink
body length	11–22 mm	8–12 mm	10–18 mm	15–40 mm	7.9–13.6 mm	7.5 mm	5.2–9.7 mm	9–14 mm
feeding	small invertebrates	small invertebrates	small invertebrates	small invertebrates	?	?	?	small invertebrates
brooding period	6–7 weeks	6–7 weeks	3–4 weeks	4–6 weeks	?	?	?	?
egg color	white	pink	pink	pink	?	?	?	?
egg diameter	0.5 mm	?	0.5 mm	0.2 mm	?	?	?	?
# eggs	7–14	12–35	8–56	20–50	?	?	?	?

Table 5. Differences in brooding season and size between *H. blinni* sp. n., *H. stagnalis,* and *H.* c.f. *modesta.*

Location	Brooding Season	Average # of offspring	Author
H. blinni sp. n. Montezuma Well, AZ	Year-round	1–14	Beresic-Perrins (2010)
H. modesta Utah Lake, UT	Late spring through summer	12.6–17.4	Tillman and Barnes (1973)
H. modesta Lake Washington, WA	Spring and Summer	14.5	Thut (1969)
H. modesta Marion Lake, BC, CA	Spring and Summer	17.2–19.7	Davies and Reynoldson (1976)
H. modesta Newsome Pond, AB, CA	Late spring through summer	21.3	
H. modesta Cambridge, MA	Spring	31	Castle (1900)
H. modesta Michigan	Late spring through summer	35.3	Sawyer (1972)
H. stagnalis Iceland	Late spring through summer	No data	Bruun (1938)
H. stagnalis River Ely, South Wales	Late spring through summer	No data	Murphy and Learner (1982)
H. stagnalis Whiteknights Lake, UK	Late spring through summer	13–17	Mann (1957)
H. stagnalis Eglwys Nunydd, UK	Late spring through summer	14	Learner and Potter (1974)
H. stagnalis Denmark	Late spring through summer	20	Bennike (1943)

Helobdella blinni, unlike the other *Helobdella* species discussed here, breeds year-round, living in the thermally stable environment of Montezuma Well, with constant (19–24˚C) year-round temperatures (Table 5). Monthly samples have individuals carrying cocoons every month of the year, with peak seasons in the spring and fall, a situation quite different than that for other *Helobdella* species, which have seasonally-constrained reproductive cycles, with egg-laying and brooding beginning in the spring and ending in the fall every year (Table 5). In addition to breeding year-round, *H. blinni* produces smaller broods (7–14 young) when compared to *H. modesta* and *H. stagnalis* (12–35 young) (Tables 4, 5) and has white eggs, unlike the characteristically pink eggs of *H. modesta, H. californica,* and *H. papillornata* (Table 4). The external pigmentation of *H. blinni* also tends to be dark brown, whereas most other *Helobdella* species are grey/brown in color (Fig. 4). *Helobdella blinni* are slightly longer (body length 11–22 mm, 16.6 + 3.2, N=24) than *H.* c.f. *modesta* (8–12 mm) and *H. californica* (10–18 mm), but slightly shorter in length than *H. papillornata* (15–40 mm) (Table 4).

The results from our molecular analysis show *H. blinni* to be genetically distinct from other *Helobdella* species, both from the same region (Rio de Flag and Oak Creek,

Table 6. Uncorrected p-distance pairwise analysis.

Species	Distance - *H. blinni*	Distance - *H.* c.f. *modesta*
H. atli	14.1–15%	16.7%
H. bolivianita	18.5%	19.3%
H. bowermani	15.9%	15.5%
H. blinni	0.0%	13.3%
H. californica	16.7%	17.6%
H. c.f. *modesta*	13.3%	0.0%
H. elongata	18.0%	19.3%
H. europaea	15.5%	16.3%
H. fusca	19.3%	20.6%
H. lineata	16.3%	14.6%
H. melananus	16.3%	17.2%
H. michaelseni	23.2%	20.6%
H. modesta OH	13.7%	8.6%
H. modesta WA	16.3%	14.6%
H. nunununununojensis	17.5–19%	17.5–19.7%
H. octatestisaca	19.7%	16.3%
H. papillata	17.6%	15.8–16.3%
H. papillornata	15.9%	16.7%
H. paranensis	16.3%	13.7%
H. pichipanan	17.2%	19.3%
H. robusta	17.6%	14.6%
H. aff *robusta* CASA	18.9%	17.2%
H. aff *robusta* NYTA	17.6%	15.9%
H. aff *robusta* TXAU1	17.2%	18.0%
H. simplex	16.3%	13.7–14.2%
H. socimulcensis	15.9%	16.7–17.2%
H. sorojchi	18–18.5%	17.6%
H. sp. Xochimilco	15.5%	17.2%
H. stagnalis	20.6%	17.2%
H. stagnalis UK	16.3%	11.6%
H. transversa	16.3%	15.0%
H. triserialis	15.9%	16.7–18.5%
H. virginiae	16.3%	16.7%
Outgroup1 *C. salmositicus*	24.9%	23.2%
Outgroup2 *G. klemmi*	21.5%	21.5%
Outgroup3 *M. lugubris*	18.0%	19.3%
Outgroup4 *O. margoi*	23.2%	20.2%

Arizona populations) and from Europe (UK sample). The sequences yielded a 13.3%–17.4% genetic difference between *H. blinni* and both the Arizona *H.* c.f. *modesta, H. modesta* (Ohio and Washington), and the United Kingdom *H. stagnalis* populations (Table 6). The three Arizona populations belong to their own separate clade, but are closely related to *H. stagnalis* (UK) and the *H. modesta. Helobdella atli, H. bowermani,*

and *H. californica* are located on separate branch tips, but they comprise what Oceguera-Figueroa et al. (2010) designated as the "*stagnalis"* series (Fig. 5).

Based on morphological, life-history, and molecular differences, we propose the *Helobdella* sp. leeches found at Montezuma Well should be considered a new species, likely the result of allopatric isolation. This concept supports our hypothesis that the leech species inhabiting Montezuma Well may have become isolated from other populations as far back as 11,000 years ago (Wagner and Blinn 1987). *Helobdella blinni* sp. n. can be considered a distinct species found in Montezuma Well *and* may also turn out to be endemic to the area. Further sampling and analyses are needed in order to verify endemism.

Although currently classified as *Helobdella* c.f. *modesta*, the Arizona populations from the Rio de Flag and Oak Creek may be an additional undescribed species based on our molecular analysis. Our next step is to investigate these populations more closely, comparing them to other local populations, including White Horse Lake and J.D. Dam Lake, AZ which also contain *H. modesta.*

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