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The morphological and molecular identity of *Longidorus piceicola* Lišková, Robbins & Brown, 1997 from Romania (Nematoda, Dorylaimida)

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Abstract

Longidorus piceicola, a new geographical and host record from Romania, was described and illustrated on the basis of two populations originating from a coniferous and a deciduous forest. The main morphological characters of specimens from Romania correspond very well with the type material collected from the soil around *Picea abies* L. (Slovakia) except for the shorter body and tail. The D2-D3 fragment of 28S rDNA from both populations was amplified and sequenced, and the sequences were identical to *L. piceicola* sequence from Slovakia. The partial 18S-ITS1-5.8S-ITS2 rDNA regions from one of the populations were sequenced for the first time. The evolutionary relationships between *L. piceicola* and the closest species *L. intermedius* based on D2-D3 sequence divergence and single-nucleotide polymorphisms are discussed. Although having very low sequence dissimilarity (0.3–0.9 %) both species have distinct morphology and biology. *Longidorus piceicola* differs from *L. intermedius* in having a much longer odontostyle, body, distance anterior end - guide ring, a wider lip region, more ventromedian supplements (11 *vs* 5–7) in the male, and develops through four rather than three juvenile stages. Furthermore, *L. piceicola* occurs more frequently in association with conifers, while *L. intermedius* is found mainly in oak forests.

Keywords
D2–D3 expansion region rDNA, ITS, juvenile stages, new record, phylogeny, SNPs

Introduction

Longidorus piceicola Lišková, Robbins & Brown, 1997 was originally described from Slovakia (Lišková et al. 1997) in association with *Picea abies* L. Subsequently, it was recovered from different localities in Bosnia and Herzegovina, Serbia and Montenegro (Barsi and Lamberti 2001), and Poland (Kornobis and Peneva 2011) in forests dominated by coniferous trees. Here two new findings of this species in Romania are reported. The aims of this paper are to characterize morphologically and molecularly the populations recovered and to discuss the phylogenetic relationships with the most closely related species.

Materials and methods

Sampling and processing

Specimens were collected from the rhizosphere of a *Larix decidua* Mill. forest near to Bran, Braşov County, Romania (45.3050N, 25.2156E), ca 760 m a.s.l. on 15.10.2013, and from the soil around roots of deciduous trees (*Quercus* sp., *Tilia* sp., and *Fraxinus* sp.), Cernica forest, Ilfov County (44.2637N, 26.16514E) and ca 60 m a.s.l. on 4.08.2014. Nematodes were isolated from soil samples by a decanting and sieving technique (Cobb 1918); specimens recovered were heat killed at 55 °C for two minutes, fixed in a 4 % formalin/1 % glycerol mixture, processed to anhydrous glycerol (Seinhorst 1959), and mounted on glass microscope slides. Drawings were prepared using an Olympus BX51 compound microscope with differential interference contrast (DIC). Photographs were taken using an Axio Imager.M2-Carl Zeiss compound microscope equipped with a digital camera (ProgRes C7) and specialised software (CapturePro Software 2.8). Measurements were made using an Olympus BX41 light microscope, a digitising tablet (CalComp Drawing Board III, GTCO CalCom Peripherals, Scottsdale, AZ, USA), and computer Digitrak 1.0f programme, (Philip Smith, Scottish Crop Research Institute, Dundee, UK) and a Leica DMLB microscope with a Leica DFC 295 camera and LAS V 4.2 software.

DNA extraction, amplification and sequencing

The genomic DNA extraction, amplification, and sequencing of single specimens of *L. piceicola* from both populations in Romania were carried out independently in two laboratories: one at the Institute for Sustainable Plant Protection, Bari, Italy and the

other at the Institute of Biodiversity and Ecosystem Research, Sofia, Bulgaria. Both protocols are presented separately below.

Institute for Sustainable Plant Protection (Bari Unit): specimens (Cernica locality) for molecular analysis were kept in DESS solution (Yoder et al. 2006) before extraction. Genomic DNA was extracted from six individual female nematodes as described by De Luca et al. (2004). The crude DNA isolated from each individual nematode was directly amplified. The partial 18S-ITS1-5.8S-ITS2 regions were amplified using the forward primer TW81 (5'-GTTTCCGTAGGTGAACCTGC-3') and the reverse primer AB28 (5'-ATATGCTTAAGTTCAGCGGGT-3') (Subbotin et al. 2001) and the D2-D3 expansion segments of 28S rDNA was amplified using the D2A (5'-ACAAGTACCGT-GAGGGAAAGTTG-3') and D3B (5'-TCGGAAGGAACCAGCTACTA-3') primers (De Ley et al. 1999). PCR cycling conditions used for amplification were: an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 50s, annealing at 55°C for 50s and extension at 72°C for 1 min and a final step at 72°C for 7 min. The size of amplification products was determined by comparison with the molecular weight marker ladder 100 (Fermentas, St. Leon-Rot, Germany) following electrophoresis of 10 ml on a 1 % agarose gel. PCR products of the ITS and D2-D3 regions were purified for cloning and sequencing using the protocol provided by the manufacturer (High Pure PCR elution kit, Roche, Germany). Purified ITS fragments were cloned in TA cloning vector (Invitrogen) and several clones were sequenced using an ABI Prism 377 sequencer (PE Applied Biosystem, Foster City, CA).

Institute of Biodiversity and Ecosystem Research: Genomic DNA was extracted from two single female worms *L. piceicola* from Bran locality using a standard nematode digestion protocol (Holterman et al. 2006). The D2–D3 expansion segments of the 28S rRNA gene were amplified using the same primers D2A and D3B (De Ley et al. 1999). Each PCR reaction was performed under the following conditions: initial denaturation 94°C for 5 min; 40 cycles (denaturation 94°C for 30 secs; primer annealing 50°C for 30 secs; extension 72°C for 1 min), and final extension 72°C for 10 min. For further details, see Nedelchev et al. (2014). The amplified products were sequenced by Eurofins MWG Operon, Germany.

Sequence and phylogenetic analysis

The sequences of the *L. piceicola* have been deposited in GenBank with the following accession numbers: KY086070 and LT669801 for D2-D3 expansion domains of 28S rRNA gene; LT669802 and LT669803 for the ITS region. The D2-D3 and ITS sequences were compared with those of other nematode species available at the GenBank sequence database using BLASTN similarity search tool revealing similar results for both regions. The closest D2-D3 sequences to *L. piceicola* were aligned using ClustalX 2.1 (Larkin et al. 2007). The estimates of evolutionary divergence between the sequences of *L. piceicola* and *L. intermedius* Kozlowska & Seinhorst, 1979 (numbers of base differences and p-distances) and Single Nucleotide Polymorphism (SNP) varia-

tions (six transitions and four transversions) were performed with MEGA7 (Kumar et al. 2016). Furthermore, sequences revealing the highest similarity to *L. piceicola* were used for phylogenetic analyses; however only a midpoint rooted tree based on a reduced number of sequences (26) comprising several related species was presented here. The multiple sequence alignments used for phylogeny reconstructions were carried out using GUIDANCE2 Server (http://guidance.tau.ac.il) with the default settings (Sela et al. 2015). Bayesian Inference (BI) algorithm implemented in MrBayes 3.2.5 was used for phylogenetic relationships reconstruction (Huelsenbeck and Ronquist 2001; Ronquist et al. 2012). For further details, see Lazarova et al. (2016).

Results

Longidorus piceicola Lišková, Robbins & Brown, 1997

Material examined. Eleven females and 21 juveniles, two females and one juvenile from Cernica forest, Ilfov County, Romania on slide numbers NE 35–37 stored at the reference collection of the National Phytosanitary Laboratory, Voluntari, Romania, 9 females and 20 juveniles - at the personal collection of the first author; nine females and 30 juveniles from Bran, Braşov County, Romania, stored in the nematode collection of IBER, Bulgaria, slide numbers N2-29/2/1-19.

Description. Figures 1–7.

Measurements See Tables 1–3.

Females (Figs 1A, B1–B4, G2–G4, 5E, 6E, J, O, 7) based on the *Larix* population, Bran, Braşov County.

Habitus spiral shaped, more strongly coiled in posterior part of body. Cuticle 3–4 µm thick at guide ring region, *ca* 3 µm in mid-body, and 5–6 µm on tail posterior to anus. Lip region broadly rounded anteriorly, rounded laterally, almost continuous with rest of body. Amphideal fovea pocket-shaped, varying from not lobed to symmetrically bilobed at base (according to terminology proposed by Decraemer and Coomans 2007) extending to *ca* half the distance anterior end-guide ring. Left and right fovea of about equal size (12.7 (11–14) µm, n = 5), sensillar pouch (fusus) just posterior the guide ring, the distance from the fovea to fusus 24 (23–29 µm). Pharyngeal bulb occupying 25 (22–29) % of total pharynx length; dorsal nucleus located at 29.5 (27–32) % (n = 7) of bulb length; ventro-sublateral nuclei at 54 (48–57) % (n = 8) (left) and 54 (52–56.5) % (n = 8) (right); opening of the dorsal gland at 9 (7.5–11) % and opening of the ventro-sublateral glands at 84 (80.5–90.5) % of the distance from anterior end of pharyngeal bulb, respectively. In one female, a small vestigium (5 µm) observed in wall of slender pharynx. Two nerve rings observed, the first one at 207.2 ± 8.8 (193–218) µm from anterior end, surrounding about mid-odontophore; the second at 329 ± 11.6 (313–344) µm from anterior end, n = 6, (first at 235.7 ± 12.7 (215–255) and second at 329.3 ± 18.6 (290–343) µm from anterior end, n = 7, Cernica forest). Tail bluntly conical, dorsally convex, flat or shallowly concave

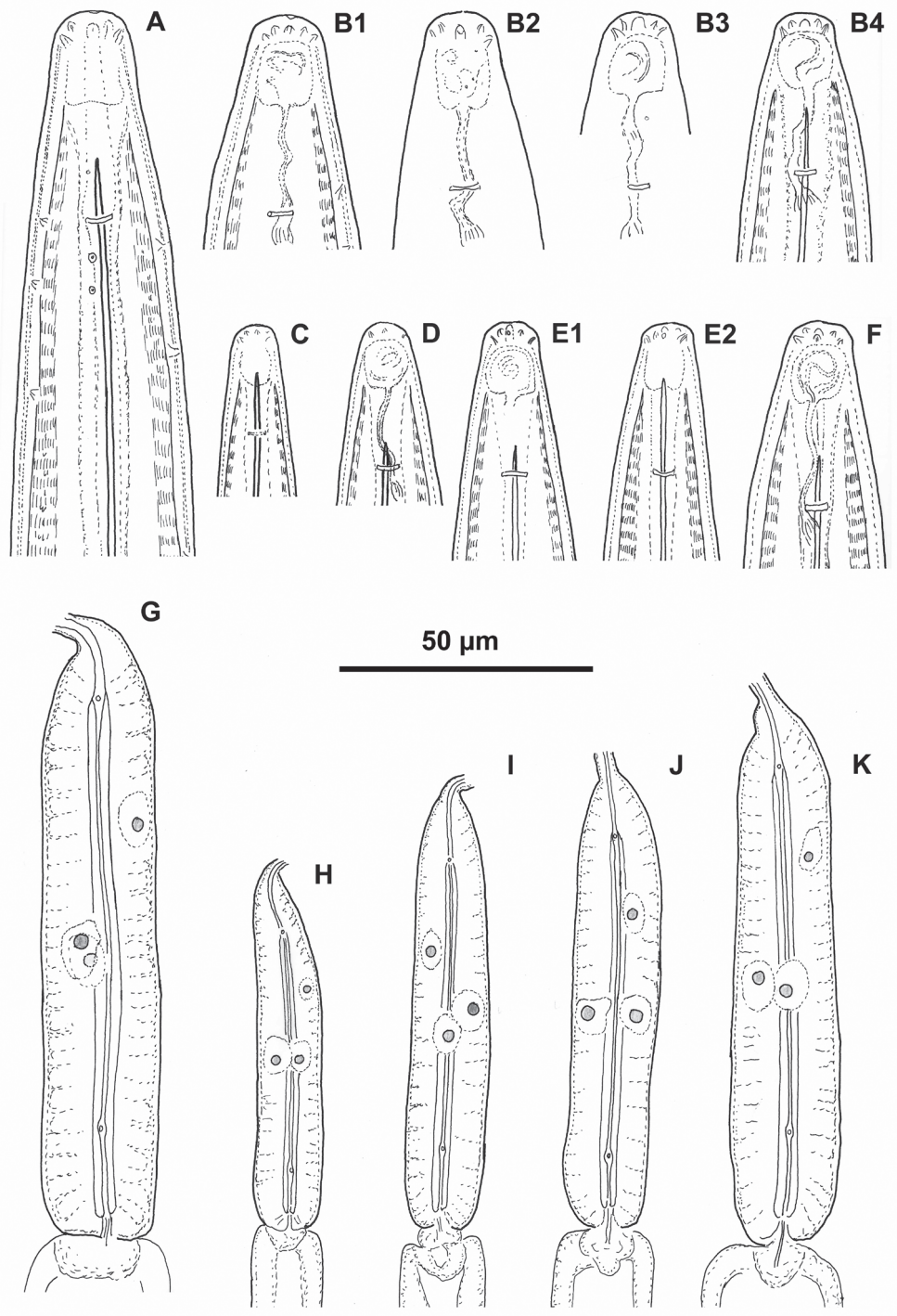


Figure 1. *Longidorus piceicola* Female and juveniles: **A** Neck region – female **B1–B4, C** Head end with amphidial fovea **B1–B3** females, **B4** juvenile 4th stage (**B2** right and **B3** left) **C, D, E1, E2, F** Anterior ends of first- to fourth-stage juveniles **G–K** Pharyngeal bulb of female (**G**) and first- to fourth-stage juveniles (**H–K**).

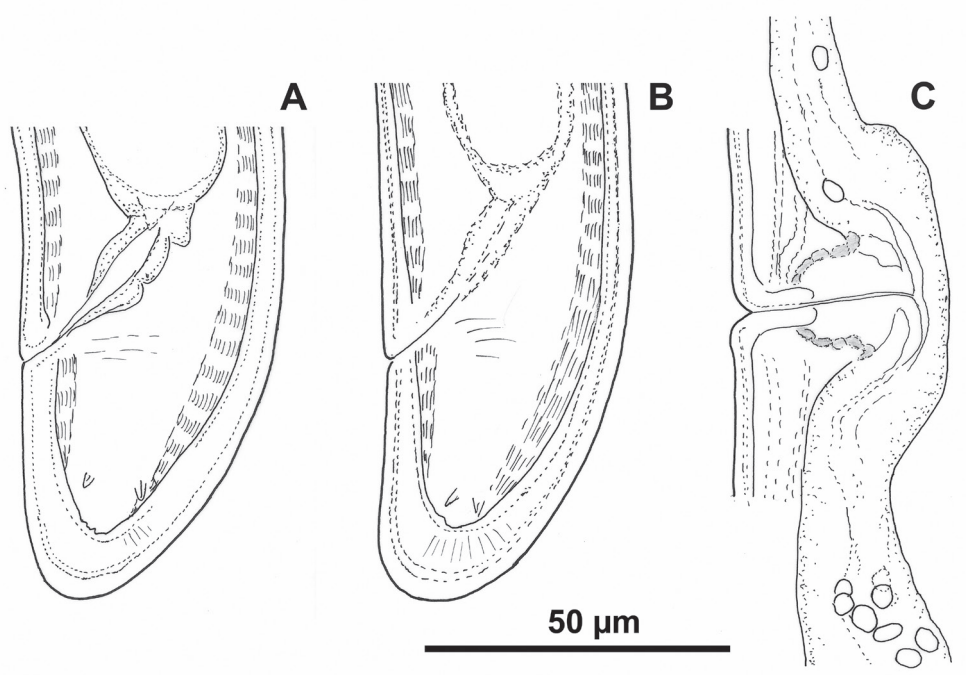


Figure 2. *Longidorus piceicola* Female from Bran locality: **A, B** Variations in tail shape **C** Vagina.

ventrally. Two pairs of caudal pores. Reproductive system didelphic, two branches of about equal size. Vagina occupies *ca* 50 % of corresponding body width; *pars distalis vaginae* and *pars proximalis vaginae* 13–15 µm and 15–19 µm long, respectively. Uteri short, anterior uterus 96.3 ± 13.5 (80–120) µm long, posterior 91.0 ± 10.5 (76–107) µm. Uteri shorter in Cernica population – anterior uterus 80.9 ± 7.0 (70–90) µm long and posterior 78.3 ± 8.3 (70–95) µm long. Sphincter between uterus and *pars dilatata oviductus* well developed. Sperm observed in both uteri of one female.

In the population from Cernica forest two females with reserve odontostyles have been observed (Table 3).

Male. Not found.

Juveniles (Figs 1C–F, H–K; 6A–D, F–I, K–N, 7).

General morphology similar to adult females. Body habitus similar in all stages, open C- to J-shaped. Tail of all juvenile stages conical, but becoming more rounded and c' decreasing in subsequent stages: tail of first stage juvenile elongate conoid with slightly digitate terminus, in the second stage – elongate conoid, in third – bluntly conoid, variable, with narrow to widely rounded terminus, in fourth – resembling that of female, bluntly conoid (Fig. 5). In several juveniles, the abnormalities in their development did not allow to assign them to a particular stage and the morphometrics are presented separately (Table 3). The lengths of functional and replacement odontostyles used to infer the developmental stages were in contradiction with other measurements

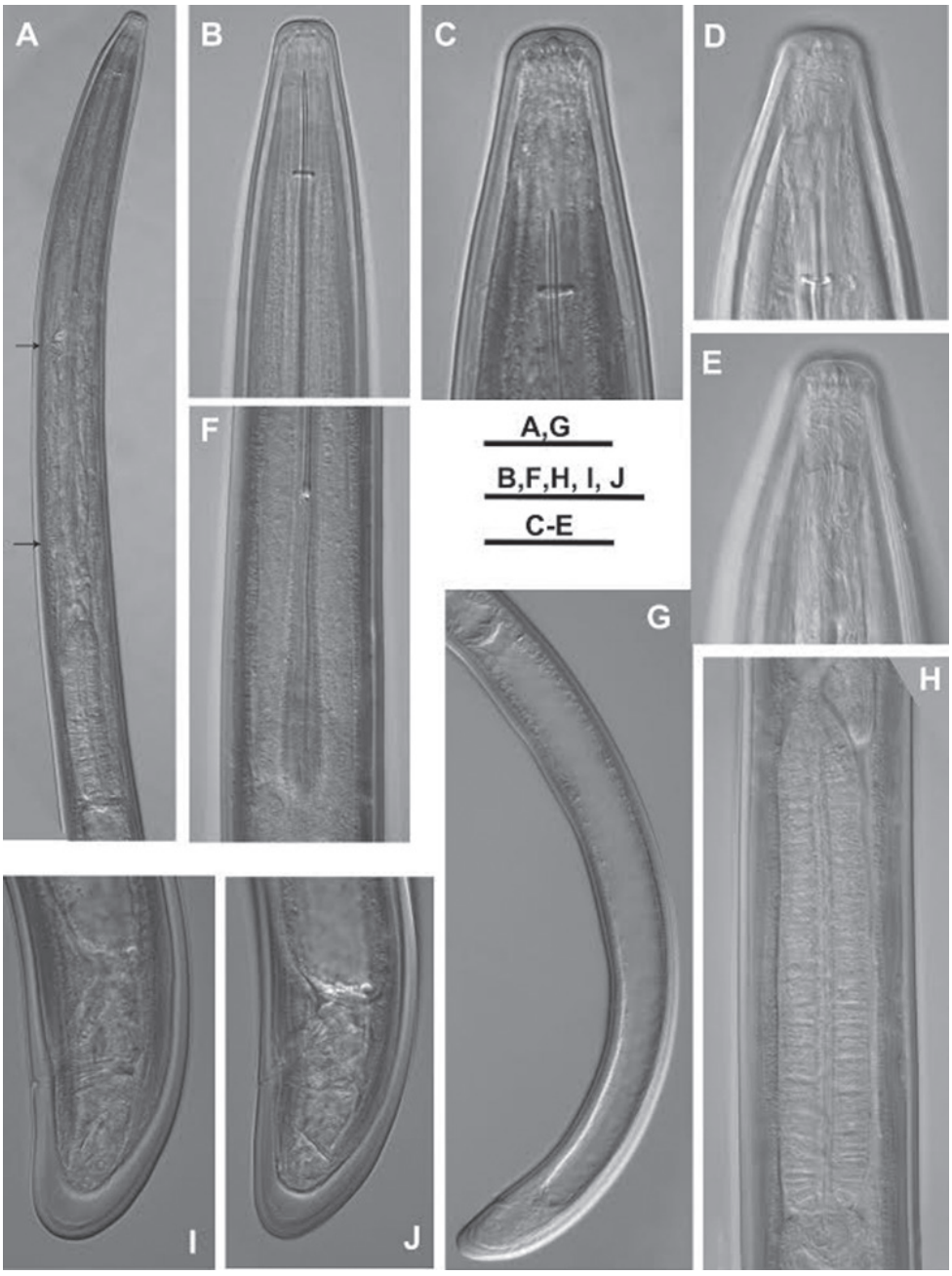


Figure 3. *Longidorus piceicola* Female from Bran locality: **A** Neck region, black arrows indicate nerve rings **B, C** Head end (different magnifications) **D, E** Amphideal fovea (right and left) **F** Odontophore **G** Prerectum **H** Pharyngeal bulb **I, J** Variations in tail shape. Scale bars: **A, G** 80 μm; **B, F, H, I, J** 40 μm; **C–E** 20 μm.

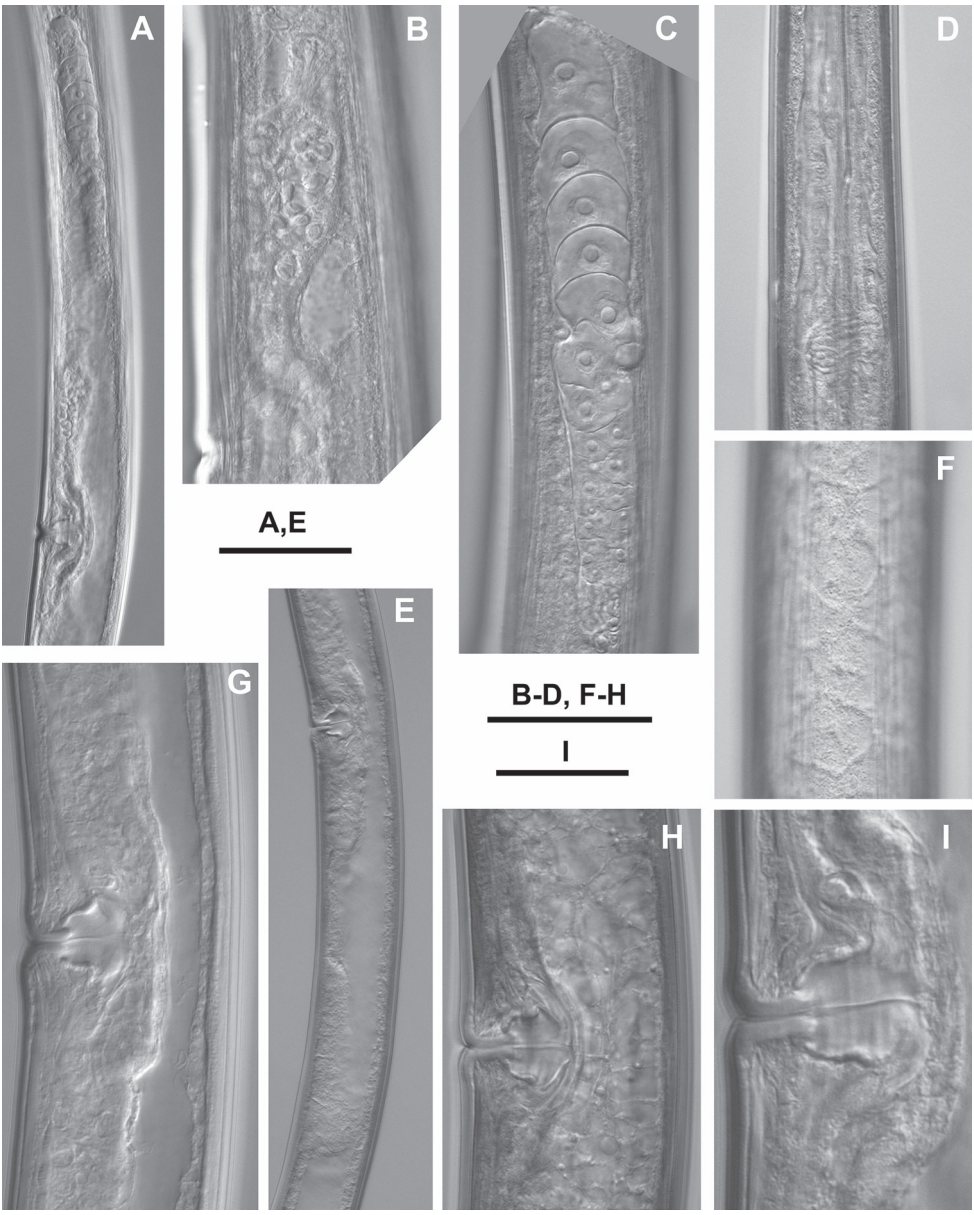


Figure 4. *Longidorus piceicola* Female from Bran locality: **A** Anterior genital branch **B** Uterus part with sperm **C** Ovary **D** Nerve ring **E** Posterior genital branch **F** Lateral field and epidermal glans **G–I** Variations in vagina (different magnifications). Scale bars: **A, E** 80 μm; **B–D, F–H** 40 μm; **I** 20 μm.

such as L, a, b, c etc. which were in correspondence with a different stage, or the functional odontostyle was in the ranges of one stage while the replacement one was not in the ranges of the next stage; in one occasion the length of replacement odontostyle was less than that of the replacement one (Table 3).

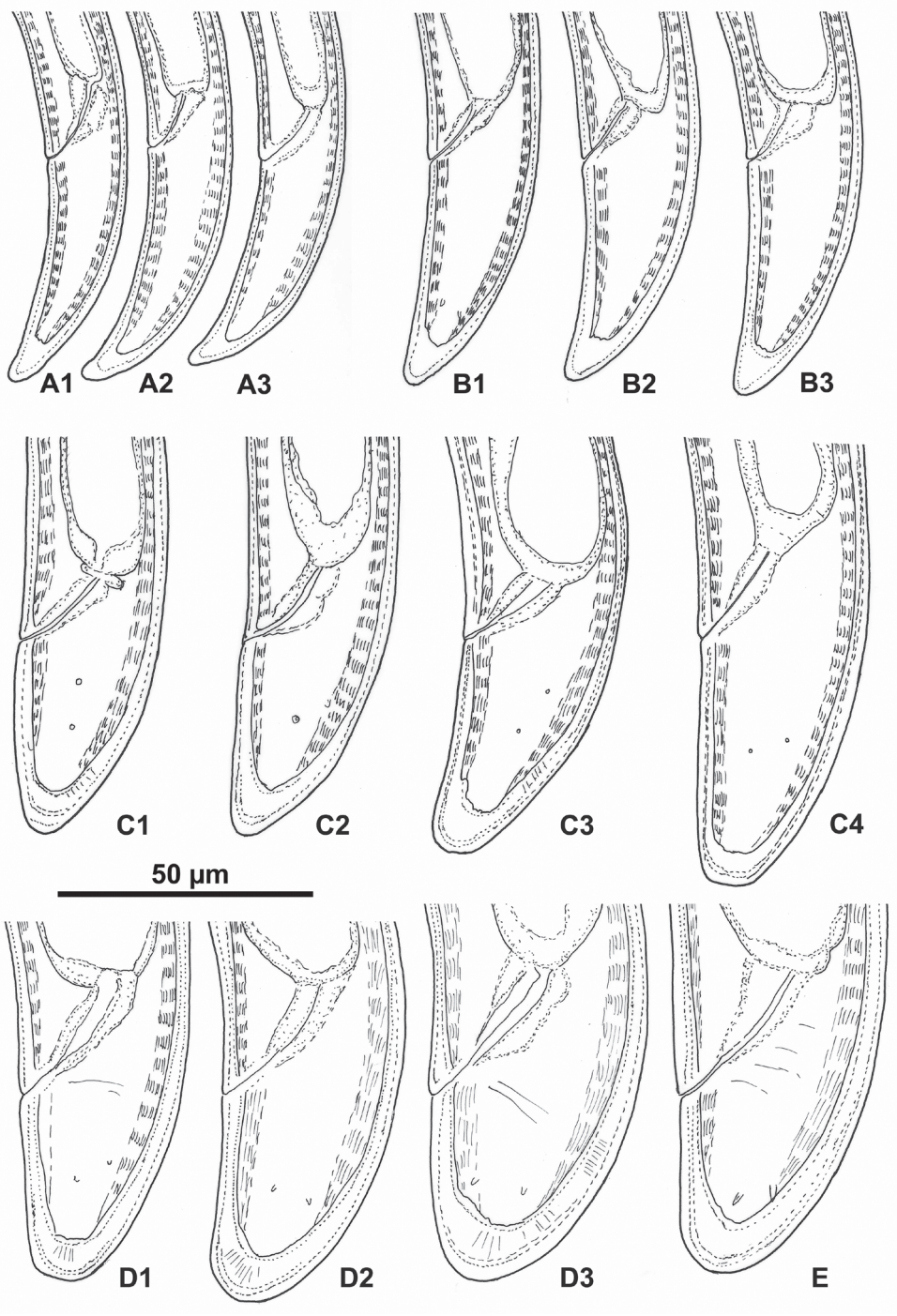


Figure 5. *Longidorus piceicola* Juveniles and female from Bran locality: Variations in tail shape of first (**A1–A3**), second (**B1–B3**), third (**C1–C4**), fourth (**D1–D3**) juvenile stages and female (**E**).

Table 1. Measurements of females and juveniles (J) of *Longidorus piceicola* from Bran, Braşov County, Romania (mean ± standard deviation, with range). All measurements in micrometers except for body length (mm).

Character	Females	J1	J2	J3	J4
n	9	6	4	8	3
L	4.90±0.47 4.05–5.64	1.32±0.11 1.15–1.47	1.83±0.16 1.63–2.02	2.62±0.13 2.38–2.81	3.21, 3.91, 3.22
a	84.6±8.0 71.1–97.3	55.4±4.6 47–60.8	59.3±5.5 53.5–65.9	67.6±3.6 62.3–71.9	73.6, 67.9, 75.5
b	9.9 ± 0.6 9.7–11.1	4.3±0.2 4.1–4.5	5.2±0.3 4.8–5.5	6.4±0.5 5.8–7.3	6.7, 8.1, 7.1
c	129.7±13.2 102.4–147.3	29.3±2.5 26.4–32.1	42.3±4.9 35–45.3	61.7±5.7 53.5–69.5	77.9, 108.4, 84.7
c’	0.97±0.06 0.89–1.10	2.8±0.3 2.6–3.2	1.9±0.2 1.64–2.15	1.45±0.1 1.38–1.58	1.23, 0.90, 1.18
V (%)	49.2±1.2 47.2–51.3	–	–	–	–
G1 (%)	6.7±0.7 5.8–7.8	–	–	–	–
G2 (%)	6.1±0.9 5.4–7.5	–	–	–	–
Developing gonad	–	16.2±1.2 15–17	28.3±7.2 20–33	33.3±2.1 31.5–37	–, 48, 45
d	2.63±0.1 2.45–2.8	2.6±0.2 2.5–2.7	2.7±0.3 2.5–3.0	2.37±1.0 2.5–2.8	2.9, 2.8, 2.9
d’	2.02±0.1 1.9–2.1	1.8±0.1 1.65–2.3	1.95±0.25 1.7–2.3	1.9±0.1 1.7–1.9	2.0, 2.1, 2.1
Odontostyle	155.5±5.2 147–163	95.8±1.2 82–90.3	100.7±3.0 97.5–105	118.4±3.7 115–125	130, 143, 142
Replacement odontostyle	–	103.7±3.5 99.5–110	115.4±6.0 109–123	137.8±2.7 134–143	151, 153, 154
Odontophore	77.7±3.4 71–82	47.5±1.4 46–50	55±4.2 50–60	62.9±2.9 60–68	75, 73, 73
Anterior end to guide ring	38.1±1.9 35–41	22.0±1.3 22–24	26±1.1 25–27	29.9±1.7 27–33	36, 37, 35
Bulbus length	118.5±7.9 105–130	65.9±4.5 59–69	71.8±3.4 75–83	91.1±3.6 86–97	104, 116, 101
Bulbus width	23.4±1.8 20–25	13.8±1.2 13–14	16.6±0.5 16–17	19.2±0.6 18–20	22, 22, 21
Pharynx	478.4±29.4 440.5 –528	307.6±12.3 290–319	352±12.9 338.5–364	409.3±22.9 374–447	480, 484, 455
Tail	38.2±1.8 35 – 42	45.4±4.2 42–51.5	43.5±2.9 40.5–47	42.7±4.2 36–48	41, 36, 38
Length of hyaline part	11.7±0.9 10–13	9.5±0.6 9–10	8.5±0.6 8–9	9.3±1.2 8–11	9.5, 12, 8
Body diameter at: – lip region	14.5±0.6 14–16	8.6±0.6 8–10	9.6±0.6 9–10	11.1±0.3 11–12	12, 14, –
– guide ring	29.2±1.6 27–32	15.3±0.7 14.5–16	18.5±1.3 28–31	21.1±1.2 19–23	25, 29, 26
– base of pharynx	48.4±3.3 44–55	22.8±0.6 23–24	29.2±1.3 28–31	36.2±2.3 32–40	39,47, 39
– mid–body/at vulva	58.7±5.4 53–71	23.8±0.8 23–25	30.9±1.8 29–33	38.9±2.7 33–41.5	44, 58, 43
– anus	39.7±3.5 35–46	16.1±0.6 15.5–17	23±1.6 22–25	29.5±2.4 25–32	34, 37, 30
– hyaline part	24.9±3.5 18–29	7.4±0.6 6.7–8.4	10.8±0.3 10.5–11	16.1±1.5 14–18	–, 25, 18

d, distance from the anterior end / body diameter at lip region.

d’, body diameter at guide ring / body diameter at lip region (Brown et al., 1994).

Table 2. Measurements of females and juvenile stages (J) of *Longidorus piceicola* from Cernica-Ilfov County, Romania (mean ± standard deviation, with range). All measurements in micrometers except body length (mm).

Character	Females	J1	J2	J3	J4
n	9	11	2	3	5
L	5.88±0.19 5.17–6.54	1.36±0.09 1.21–1.52	1.79, 2.16	3.29, 3.04, 2.81	3.95±0.47 3.6–4.7
a	95.2±11.5 73.8–105.5	58.96±4.9 53–66.8	64.1, 67.7	68.7, 67.7, 68.7	77±8.2 62.5–83.4
b	10.2±1.2 8.4–12.7	4.88±0.8 4.1–6.7		8.4, 9, 6.6	9±1.5 6.9–10.6
c	171.9±28.8 134.4–218.0	31.0±1.9 28.3–33.4	–	79, 71,64.3	102±8.1 89.8–109.8
c'	0.85±0.10 0.72–0.99	2.9±0.2 2.6–3.1	–	1.3, 1, 1.6	1±0.1 0.9–1.2
V (%)	48.1±0.98 47.1–50.6	–		–	–
G1 (%)	5.8±0.8 4.7–7.1	–	–	–	–
G2 (%)	5.4±0.7 4.4–6.4	–	–	–	–
d	2.9±0.1 2.7–3.1	2.9±0.2 2.6–3.3	2.78, 2.99	3, 3, 3.4	3±0.2 2.8–3.2
d'	1.8±0.1 1.7–1.9	1.9±0.2 1.5–2.2	1.8, 1.7	2, 2, 2	2±0.1 1.7–2
Anterior end to guide ring	42.2±1.8 40–45	22.8±1.4 21–26	25, 29	34.8, 34, 34	37.2±0.9 36–39
Odontostyle	155.4±5.4 150–165	86.9±2.7 82–90	97, 102	122, 124, 108	136.8±3.4 132–141.5
Bulbus length	135±4.9 126–141	72.7±4.3 65–78.5	71, 86	104, 104, 100	113.7±5.9 108–120
Bulbus width	24.7±2.0 22–29	12.5±0.9 11–14	15, 14.5	18, 21, 19	19.9±2.1 17–21
Replacement odontostyle	–	95.7±3.7 92–102	109, 111	142, 136, 132	157.3±6.7 150–165
Odontophore	78.1±4.9 70–83	52.5±4.8 48–65	55, 60	72, 60, 65	72.2±3.0 69–76
Oesophagus length	579.3±47.6 514–661	284.9±42.4 219–356	320, 366	393, 321,425	460.3±84.3 345–545
Tail	34.8±4.4 30–41.5	43.58±2.3 39–47	–	42, 43, 44	38.7±3.5 34–43
Length of hyaline part	12.3±1.1 11–14	9.5±0.9 8–11	10, 10	9, 10, 10	10±0.6 9.4–11.1
Body diameter at: – lip region	14.7±0.4 14–15	7.9±0.2 7.5–8	9, 10	11, 11,10	12.4±0.5 12–13
– guide ring	26.1±1.2 24–27	14.7±1.5 12–18	16, 16	20.5, 21, 20	23.16±0.6 22.5–24
– base of pharynx	53.6±6.1 45–62	21.9±0.9 20–23	25, 28.6	39, 38, 38	43.5±3.0 38.9–46.4
– mid–body/at vulva	63.2±4.5 58–70	23.2±1.4 21–26	28, 32	48, 45, 41	51.8±5.8 45–58
– anus	40.8±2.1 38–44	15.2±1.3 14–18	–	31, 30, 27	36±3.4 33.2–41.2
– hyaline part	27.8±1.9 25–31.5	7.4±0.6 7–8	7, 8	18, 16, 15	22±1.4 20–23

Table 3. Measurements of *Longidorus piceicola* females (f) from Cernica, and juveniles (j) from Bran, Braşov County, Romania showing different anomalies. All measurements in micrometers except body length (mm).

Character	f	f	j	j	j	j	j	j	j	j
No	1	2	1	2	3	4	5	6	7	8
L	5.95	5.86	4.73	2.34	2.72	2.71	2.62	1.14	3.66	2.71
a	99.1	97.7	93.0	63.6	77.7	61.9	67.0	32.6	75.1	63.8
b	9.5		10.3	5.6	6.0	5.9	7.1	2.9	7.9	6.2
c	220.3	172.4	98.8	60.5	65.2	61.9	61.4		–	70.3
c’	0.75	0.94	1.3	1.3	1.6	1.4	1.7		–	1.2
V	49.2	48.9	–	–	–	–	–	–	–	–
Developing gonade	–	–	65	–	–	–	22		27	
d	2.93	2.73	2.6	2.8	2.9	2.9	2.5		2.7	2.8
d’	1.79	1.80	1.4	2.0	2.0	2.0	1.8		1.9	1.8
Odontostyle	165	158	117	127	122	105	81	106	120	125
Replacement odontostyle	175	158	131	165	165	135	108	130	140	156
Odontophore	80	70	78.5	61	65	65	60		60	73
Anterior end to guide ring	41	41	35	30	32	33	25	26	32	32
Bulbus length	132	130	114	81	87	89	95	93	108	86
Bulbus width	23	23	22	19	18	20	17			
Pharynx	627	–	461	420	457	464	369	387	463	441
Tail	27	34	48	39	42	44	43		–	39
Length of hyaline part	11	8	10	9	9	6	9			
Body diameter at: - lip region	14	15	14	11	11	11			9	11.5
- guide ring	25	27	19.5	21	22	23	11		19	21
- base of pharynx	51	50	42	35	32	38	32	29	40	32
- mid-body/at vulva	60		51	37	35	44	39	35	49	43
- anus	60		38	29	26	32	24		–	32
- hyaline part	25	23	20	16	14	15			11	18

Sequences and phylogenetic analyses. The amplification of the ITS and the D2-D3 expansion domains of the 28S rRNA gene yielded fragments of 1646 and 756 bps, respectively, based on sequencing. The ITS sequences of *L. piceicola* from Romania were obtained for the first time in the present study. They showed 98 % similarity (962/984 identities, 9 gaps) when compared with the corresponding sequence of *L. intermedius* (KT308890) and 86 % with the ITS sequence of *L. elongatus* Hooper, 1961 (AJ549986, AJ549987). Intraspecific variation for the ITS sequences was low, with only two nucleotides difference and no indels.

D2-D3 rDNA sequences obtained from both Romanian populations were identical to each other and to the sequence of *L. piceicola* from Slovakia (AY601577, He et al. 2005). The phylogenetic relationships of *L. piceicola* with several related species is presented in Figure 8. *Longidorus intermedius* revealed sister relationships with *L. piceicola* and the sequences from both species formed a well-supported clade. In addition, five sequences of *L. intermedius* from Germany (AF480074, Rubtsova et al. 2001), Russia (KF242311 and KF242312, Subbotin et al. 2014), Spain (KT308868, Gutiérrez-Gutiérrez et al 2013 and JX445117, Archidona-Yuste et al. 2016), and the *L. piceicola*

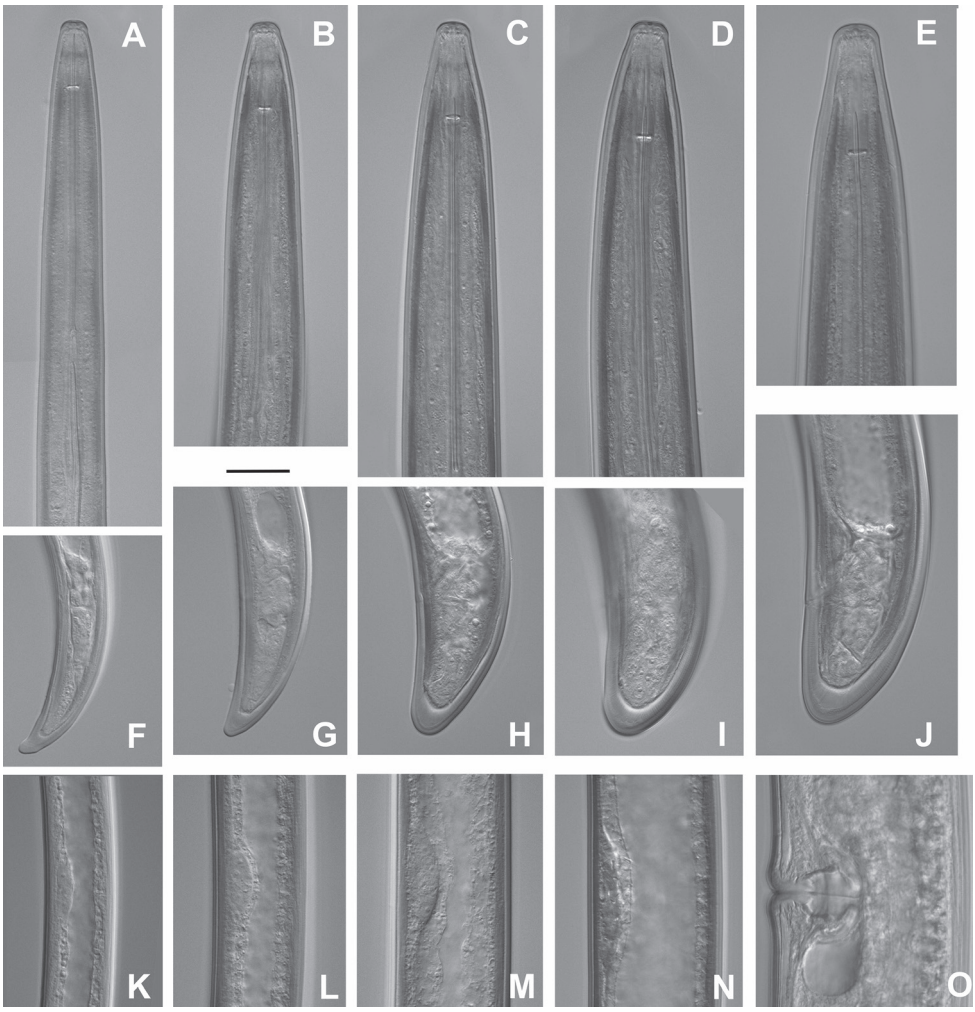
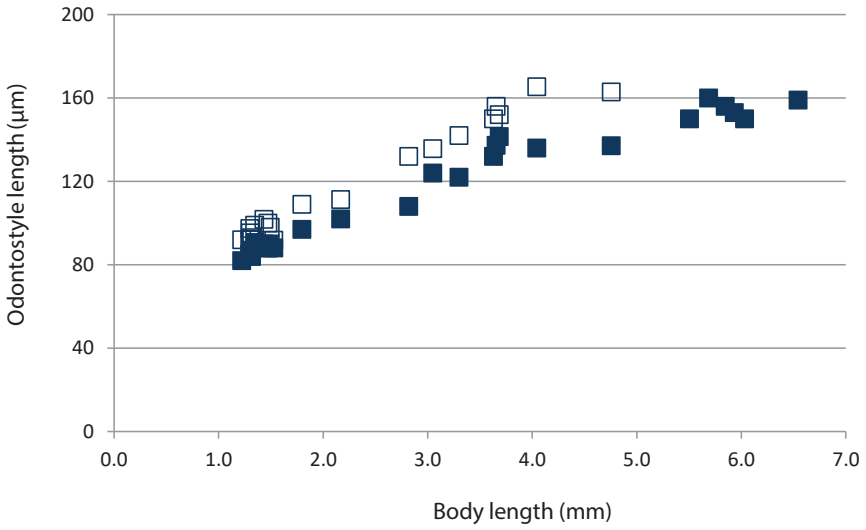


Figure 6. *Longidorus piceicola* Juveniles and female from Bran locality: **A–E** Anterior ends of first- to fourth-stage juveniles and female **F–J** Tails of first to fourth juvenile stages and female **K–M** Genital primordium of first to fourth juvenile stages. **O** Vagina. Scale bar: 20 μm.

sequence were realigned separately and pairwise distances estimated. A total of 737 positions was included in the dataset. The between species dissimilarities (p-distances) were 0.3–0.9 % (or 2–6 bp differences). Similarly, the intraspecific p-distances of *L. intermedius* from the three European countries were 0.4-0.9 % (i.e. 3–6 bp).

The SNPs analysis comparing all D2-D3 sequences of *L. piceicola* and *L. intermedius* revealed three parsimony-informative sites (i.e. nucleotide positions with transitions 89T/C, 134T/C and 297A/G) when compared to the reference sequence of *L. piceicola* (AY601577) (Table 4). The most similar sequence to the *L. piceicola* sequence was that of *L. intermedius* from Germany, revealing the highest similarity and only two interspecies differentiating nucleotides at positions 89 and 134 compared to the reference sequence (Table 4).

A)



B)

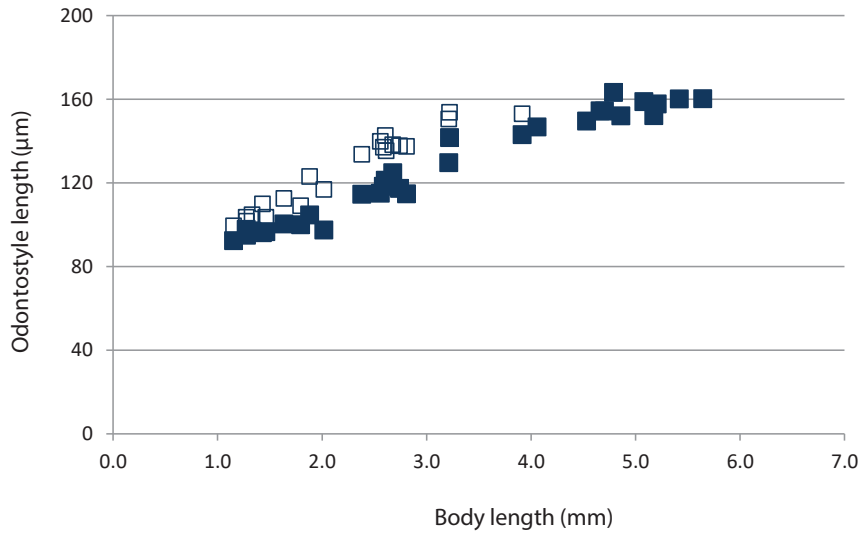


Figure 7. Scatter plot of odontostyle (■) and replacement odontostyle (□) against body length of *Longidorus piceicola* juveniles (J1 to J4) and females from **A** Cernica forest, Ilfov county and **B** Bran locality, Braşov county.

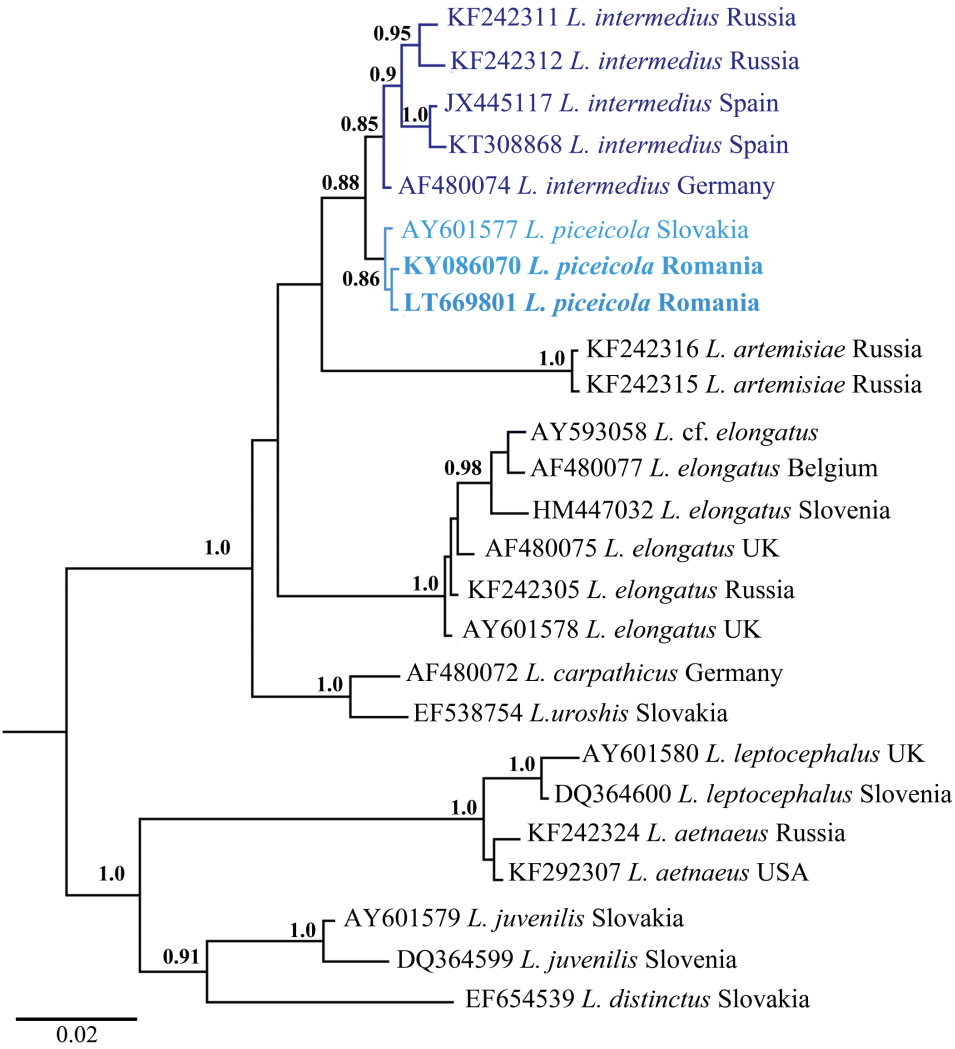


Figure 8. Phylogenetic tree using D2-D3 28S rDNA and inferred from a Bayesian analysis with GTR+G model and midpoint rooting. Posterior probabilities ≥ than 0.8 are presented.

Discussion

Morphologically, the specimens of *L. piceicola* from Romania are similar to the type-population from Slovakia (Lišková et al. 1997), except for the slightly longer body (av. 5.88 *vs* 5.19 mm) and shorter tail (av. 34.5 *vs* 42 µm, av. *c* = 172 *vs c* = 125) in the population from Cernica forest. Barsi and Lamberti (2001) described several *L. piceicola* populations from Bosnia and Herzegovina, Serbia and Montenegro. In comparison with those populations, the nematodes from Romania have a narrower lip region

Table 4. The variable positions in D2-D3 28S rDNA control region sequences of *Longidorus piceicola* and *L. intermedius*. The *L. piceicola* sequence from Slovakia (Acc. no AY601577) was used as a reference.

	SNPositions											
	89	129	**134**	197	255	285+1gap	285+2gap	**297**	310	413	514	584
AY601577 reference sequence	**T**	**C**	**T**	**A**	**C**	**–**	**–**	**A**	**T**	**G**	**G**	**C**
AY601577 *L. piceicola* Slovakia	.	.	.	.	.	–	–	.	.	.	.	.
KY086070 *L. piceicola* Romania 1	.	.	.	.	.	–	–	.	.	.	.	.
LT669801 *L. piceicola* Romania 2	.	.	.	.	.	–	–	.	.	.	.	.
AF480074 *L. intermedius* Germany	**C**	.	**C**	.	.	–	–	.	.	.	.	.
JX445117 *L. intermedius* Spain	**C**	.	**C**	.	T	A	T	**G**	G	.	S	.
KT308868 *L. intermedius* Spain	**C**	.	**C**	.	T	A	T	**G**	G	.	T	.
KF242312 *L. intermedius* Russia	**C**	T	**C**	.	.	–	–	**G**	.	T	.	T
KF242311 *L. intermedius* Russia	**C**	.	**C**	C	.	–	–	**G**	.	T	.	.

(avs. 14.5, 14.7 *vs* avs. 16–17 μm), a shorter odontostyle (avs. 155.4, 155.5 *vs* avs. 167–188 μm) and tail (av. 35 *vs* avs. 39–46 μm) in specimens from Bran population. Compared to subsequently recorded *L. piceicola* population from Poland, specimens from Romania have, again, a much shorter body (avs. 4.9, 5.2 *vs* av. 6.5 mm) and tail (avs. 34, 38 *vs* av. 47.4 μm).

The observed abnormalities (presence of reserve odontostyle) in females have been reported for other longidorids (Ferris et al. 2012) whereas atypical development in juveniles has not been recorded previously to such a great extent (*ca* 30 % of all juveniles studied from *L. decidua* forest were atypical). Ferris et al. (2012) hypothesized that "anatomical aberrations possibly are results from accidents in transcription of the genetic code or mutations which may or may not be mechanistically limiting to reproduction and therefore may or may not be maintained in the genome through either apomixis or amphimixis".

Longidorus piceicola was previously recovered in association with *P. abies*, *Abies alba* L., *Fagus sylvatica* L., *Carpinus betulus* L. and *Vitis vinifera* L. in Slovakia, West Balkans and Poland (Lišková et al. 1997, Barsi and Lamberti 2001, Kornobis and Peneva 2011, Skwiercz et al. 2015), and our findings in coniferous forest dominated by larch and mixed deciduous forest (*Fraxinus*, *Quercus* and *Tilia*) in Romania extend the geographical and plant association ranges further southeast.

Based on the molecular and morphological characterization *L. piceicola* is closely related to *L. intermedius*: however, it differs in having a much longer odontostyle (151–169 μm in the type population and reported range of 144–183 μm *vs* 105–118 μm and 97–121 μm, respectively), generally longer body (4.22–5.97 mm in the type population and reported range of 4.42–7.99 mm *vs* 3.6–4.5 mm and 3.11–5.4 mm, respectively) and bigger anterior end – guide ring distance (37–45 μm in the type population and a range of 34–46 μm *vs* 25–34 μm and 27–36 μm, respectively); a wider lip region (14–18 *vs* 11–12.5 μm), more ventromedian supplements (11 *vs* 5–7) in the males, and

four *vs* three juvenile stages (Lišková et al. 1997, Peneva et al. 2001, Barsi and Lamberti 2001, Kumari et al. 2006, Kornobis and Peneva 2011, Gutiérrez-Gutiérrez et al. 2013). Sequence and SNPs analyses of the D2-D3 rDNA region of *L. piceicola* and *L. intermedius* populations showed three transitions and four transversions that can be used to s differentiate between both species. Furthermore, *L. piceicola* was more frequently found in association with conifers, while *L. intermedius* occurred mainly in oak forests.

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Revision of the fish parasitic genus *Pleopodias* Richardson, 1910 (Isopoda, Cymothoidae), with the description of a new species and key to the genus

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Abstract

The cymothoid genus, *Pleopodias* Richardson, 1910, is revised and a new species from South Africa is recorded. *Pleopodias nielbrucei* **sp. n.** can be distinguished by large eyes covering majority of the cephalon (almost in contact), antennula bases wide apart, antenna extending to middle of pereonite 2, subtruncate pleotelson, pereopod 7 with numerous acute robust setae on the propodus as well as the carpus, and the uropod exopod longer than the endopod. The three known species, *Pleopodias diaphus* Avdeev, 1975; *P. elongatus* Richardson, 1910; and *P. vigilans* Richardson, 1911 are also redescribed. *Pleopodias nielbrucei* **sp. n.** differs from these known species in both morphological characters as well as geographical distribution. A key to the *Pleopodias* species is provided.

Keywords

External parasite, Indian Ocean, South Africa, *Pleopodias diaphus*, *Pleopodias elongatus*, *Pleopodias vigilans*, *Pleopodias nielbrucei*, *Pleopodias superatus*

Introduction

Depending on the genus or species, cymothoids can be located on the external surfaces or inside the branchial, buccal or body cavities of their fish host (Smit et al. 2014). The genus *Pleopodias* Richardson, 1910, is a small genus of fish-parasitic isopods which

has been rarely studied since it was founded. These cymothoids most likely occur on the external surfaces of their fish hosts and to date there are three recognised species. *Pleopodias diaphus* Avdeev, 1975 (*Pleopodias superatus* Williams & Williams, 1986 is the junior synonym of this species) is known only from Japan; *P. elongatus* Richardson, 1910 is known only from the Philippine Islands; and *P. vigilans* Richardson, 1911 has been reported only from the coast of Sudan.

While examining museum material housed in the Iziko South African Museum, Cape Town, a specimen collected off the coast of South Africa identified as belonging to the genus *Pleopodias* was observed. Several fish parasitic cymothoid genera from southern Africa have recently been revised (Hadfield et al. 2010, 2013, 2014, 2015), but this is the first record of *Pleopodias* from South Africa.

The *Pleopodias* species from South Africa differs from the three known species in both morphological characteristics as well as geographical distribution, Discovery of this specimen provided the opportunity to revise the genus, add a new species, and provide a key to all species of *Pleopodias*.

Methods

Pleopodias type material was borrowed or drawn at the respective museums. The *Pleopodias* specimen loaned from the Iziko South African Museum was collected from a RV Africana Cruise in 1988, off the coast of South Africa. Type material was not dissected and all isopods were processed according to the techniques in Hadfield et al. (2010, 2013). The cymothoid species descriptions were prepared in DELTA (DEscriptive Language for TAxonomy) format using a general Cymothoidae character set used previously (see Hadfield et al. 2016). Isopod classification follows that of Brandt and Poore (2003) and host nomenclature was sourced and verified from FishBase (Froese and Pauly 2017) and Catalog of Fishes (Eschmeyer 2017).

Abbreviations. MNHN –Muséum national d'Histoire naturelle, Paris; SAMC – South African Museum, Cape Town; USNM – National Museum of Natural History, Smithsonian Institution, Washington; TL – total length; W – width.

Taxonomy

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

Genus *Pleopodias* Richardson, 1910

Pleopodias Richardson, 1910: 25–26.—Barnard, 1936: 166–167.—Bruce, 1987: 87.—Trilles, 1994: 109.

Type species. *Pleopodias elongatus* Richardson, 1910, by monotypy.

Diagnosis. Body elongate; cephalon slightly immersed in pereonite 1, posterior margin not trilobed; eyes large and distinct. Rostrum folded back, lying between antennula bases, not concealing basal articles. Antennula long and narrow, extending past cephalon posterior margins; antenna longer than antennula, extending to or beyond pereonite 2, articles 3–6 elongate. Pereon most narrow at pereonite 1; pleon narrower than pereon with pleonites progressively getting narrower from pleonite 1 to 5; pleotelson elongate, 1.2–1.8 times longer than wide. Pereonite 2 shortest in length, pereonite 6 longest. Uropods long and narrow, extending beyond the posterior margin of the pleotelson. Pereopod 7 longer than other pereopods with the merus, carpus and propodus on pereonite 7 elongated.

Remarks. *Pleopodias* can be identified by the long antennae, with the antennula shorter than the antenna; body most narrow at pereonite 1; narrow pleon with the width of the pleonites decreasing from pereonite 1 to 5; pereopod 7 longer and more elongate than other pereopods; uropods extending past the posterior margin of the pleotelson; and a longer than wide pleotelson.

The original diagnosis for this genus was provided by Richardson (1910) but was based on the only species known at that time, *P. elongatus*. In his report on a *Pleopodias* sp. from the Andaman Islands, Barnard (1936) pointed out that of some of the generic characters used by Richardson to define the genus (i.e. antennula articles 2 and 3 expanded, and pleopods visible in dorsal view) were shared with *Anilocra* Leach, 1818 and thus not very informative. Bruce (1987) revised both *Pleopodias* and *Anilocra* simultaneously and was able to provide a number of differences between the two genera. According to Bruce (1987), *Pleopodias* has a narrow pleon, getting strongly narrower towards the posterior (not always narrower in *Anilocra*); antennula articles 4–8 are elongate (not in *Anilocra*); large robust setae on the maxilla and the medial lobe is distinct (acute, simple robust setae and the maxilla is partially fused in *Anilocra*); article 3 of the mandible palp is slim and longer than article 2 (article is stout and short in *Anilocra*); and pereopod 7 with more robust setae than observed in *Anilocra.* Bruce (1987) also included mouthpart and pleopod morphology in the generic diagnosis of *Pleopodias*, however, as not all species have these characters noted, we have refrained from adding them into the currently revised diagnosis.

Key to the species of the genus *Pleopodias*

This key is based on the morphological characters of the gravid female:

1 Uropod rami the same length; antennula bases close together or in contact... **2**

– Uropod exopod longer than endopod; antennula bases widely separated **3**

2 Antennula bases contiguous; antenna extending to middle of pereonite 3; pleotelson posterior margin deeply emarginate; eye size a third of cephalon width ..... ***P. diaphus***

– Antennula bases narrowly separated; antenna extending to posterior of pereonite 2; pleotelson posterior margin rounded; eye size a quarter of cephalon width ..... ***P. elongatus***

3 Antenna extending to posterior of pereonite 2; pleotelson posterior margin rounded, with caudomedial point; eye 0.4 times width of cephalon; pereopod 7 without robust setae..... ***P. vigilans***

– Antenna extending to middle of pereonite 2; pleotelson posterior margin subtruncate; eye 0.5 times width of cephalon; pereopod 7 with acute robust setae on propodus and carpus ..... ***P. nielbrucei* sp. n.**

Pleopodias diaphus Avdeev, 1975

Pleopodias diaphus Avdeev, 1975: 254–256, figs 1–11.—Bruce & Harrison-Nelson, 1988: 600.—Trilles, 1994: 109.—Yamauchi, 2009: 477–479, figs 7–8.
Pleopodias superatus Williams & Williams, 1986: 656, figs 62–68.

Material examined. Female holotype of *Pleopodias superatus* (26.5 mm TL, 10.4 mm W), caught in a shrimp net off Honshu Island, Japan, 11 April 1969 (USNM 231069).

Holotype. Non-ovigerous female, collected from the East-China Sea (Sea of Japan), from the body of *Diaphus coeruleus* (TINRO AGK 74190). *Paratypes*. Non-ovigerous females, same information as holotype (TINRO APK 74191–74195). Not examined.

Description. Female holotype of *Pleopodias superatus.* Length 26.5 mm, width 10.4 mm.

Body oval, 2.4 times as long as greatest width, dorsal surfaces smooth and polished in appearance, widest at pereonite 5, most narrow at pereonite 1, lateral margins subparallel. *Cephalon* 0.8 times longer than wide, visible from dorsal view, subtriangular. *Frontal margin* truncate, thickened and ventrally folded. *Eyes* oval with distinct margins; one eye 0.3 times width of cephalon, 0.4 times length of cephalon. *Pereonite 1* smooth, anterior border slightly indented, anterolateral angle narrowly rounded, extending to middle of the eye. Posterior margins of pereonites 1–4 smooth and straight, 5–7 slightly curved laterally, posterior margin of pereonite 7 produced medially. Coxae 2–3 narrow, with posteroventral angles rounded; 4–7 small and narrow, not extending past pereonite margin. Pereonites 1–5 increasing in length and width; 6–7 decreasing in length and width. Pereonite 7 partially overlapping pleonite 1. *Pleonites* posterior margin smooth, mostly concave. Pleonite 1 widest, visible in dorsal view. Pleonite 2 not overlapped by pereonite 7; posterolateral angles of pleonite 2 narrowly rounded. Pleonites 3–5 progressively getting smaller; pleonite 5 not overlapped by lateral margins of pleonite 4, posterior margin slightly concave. *Pleotelson* 1.8 times as long as anterior width, dorsal surface slightly depressed, lateral margins straight, posterior margin with medial notch.

Antennula thinner and shorter than antenna, contiguous bases, consisting of 8 articles; peduncle articles 1 and 2 distinct and articulated; articles 2–3 expanded; extending to pereonite 2. *Antenna* consisting of 12 articles; extending to middle of pereonite 3. *Pereopod 1* basis 1.6 times as long as greatest width; ischium 0.7 times as long as basis; merus proximal margin without bulbous protrusion; carpus with straight proximal margin; propodus 1.2 times as long as wide; dactylus slender, 1.5 times as long as

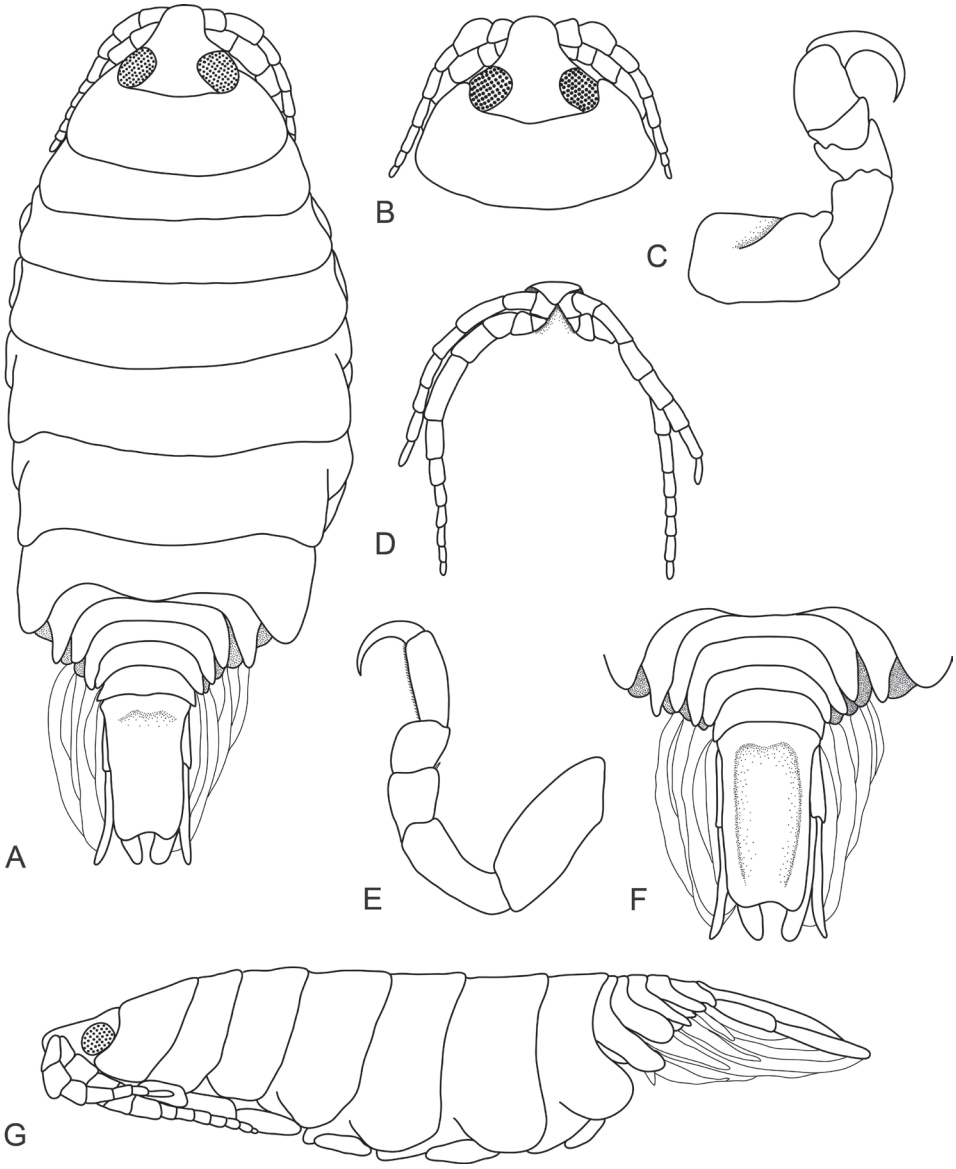

Figure 1. *Pleopodias diaphus* Avdeev, 1975 (USNM 231069), female (26 mm), (originally designated as the holotype of *Pleopodias superatus* Williams & Williams, 1986). **A** dorsal view **B** dorsal view of pereonite 1 and cephalon **C** pereopod 1 **D** ventral view of cephalon **E** pereopod 7 **F** dorsal view of pleon and pleotelson **G** lateral view.

propodus, 2.4 times as long as basal width. *Pereopod 7* longer than other pereopods, basis 2.2 times as long as greatest width; ischium 0.8 times as long as basis, without protrusions; merus proximal margin without bulbous protrusion, 1.2 times as long as wide, 0.5 times as long as ischium; carpus 0.9 times as long as wide, 0.4 times as long

as ischium, without bulbous protrusion; propodus with numerous acute robust setae, 2.6 times as long as wide, 0.8 times as long as ischium; dactylus slender, 0.8 times as long as propodus, 3.3 times as long as basal width. *Uropod* longer than the pleotelson, rami subequal. *Endopod* apically rounded. *Exopod* apically narrowly rounded.

Distribution. Sea of Japan (Avdeev 1975) and off Honshu, Japan (Williams and Williams 1986; Yamauchi 2009).

Hosts. Anterior to the dorsal fin of *Diaphus coeruleus* (Blue lantern fish) (Avdeev 1975).

Remarks. *Pleopodias diaphus* has an ovate shape, contiguous antennula bases, and an emarginated pleotelson posterior margin. The uropod rami are approximately the same length and the eyes are large (each eye approximately a third of the cephalon width).

It was originally described from Japan, with several drawings and a brief description in Russian. In 1986, Williams and Williams described a new species, *Pleopodias superatus* which shared many similarities with *P. diaphus*. After comparisons of the notched pleotelson as well as the antennae and somatic morphology, Bruce and Harrison-Nelson (1988) synonymised it with *P. diaphus*. Despite numerous attempts, the type specimens of *P. diaphus* could not be obtained for inclusion in the present study; however, the types do exist and the species is eminently recognisable from the original illustrations and is therefore not a *nomen dubium* or *species inquirenda*. Avdeev (1975) reported the types as immature females but both the size and drawings indicate they are still adult females (non-ovigerous) and therefore suitable for a valid species description. Both species (*P. diaphus* and *P. superatus*) are well illustrated, readily recognised, from the same region, and appear identical, thus the synonymy of the two species by Bruce and Harrison-Nelson (1988) is here upheld until a detailed redescription of the original type material of *P. diaphus* indicates otherwise.

As the type material for *P. diaphus* could not be obtained the redescription provided here is based on the holotype of *P. superatus* housed at USNM. This redescription includes updated measurements and characteristics which are comparable to the other *Pleopodias* species in this paper. This modern description of the type material of *P. superatus* will also aid future research into its current status as junior synonym of *P. diaphus*.

Pleopods and mouthparts of specimens identified as *P. diaphus* were drawn and described by Yamauchi (2009). The specimens Yamauchi (2009) examined largely conformed to the above description; however, the body size differed with the three more recent samples (12–27 mm in length) being more slender (3.4–3.9 times as long as wide).

Pleopodias elongatus Richardson, 1910

Pleopodias elongatus Richardson, 1910: 26–27, fig. 25.—Nierstrasz, 1931: 133.—Avdeev, 1975: 89, fig. 3.—Bruce, 1987: 89, fig. 3.

Material examined. *Holotype*. Ovigerous female (20 mm), off Matocot Point, Philippine Islands, 8 June 1908, 170 fathoms (= 311 m depth), station 5268, coll. U .S.

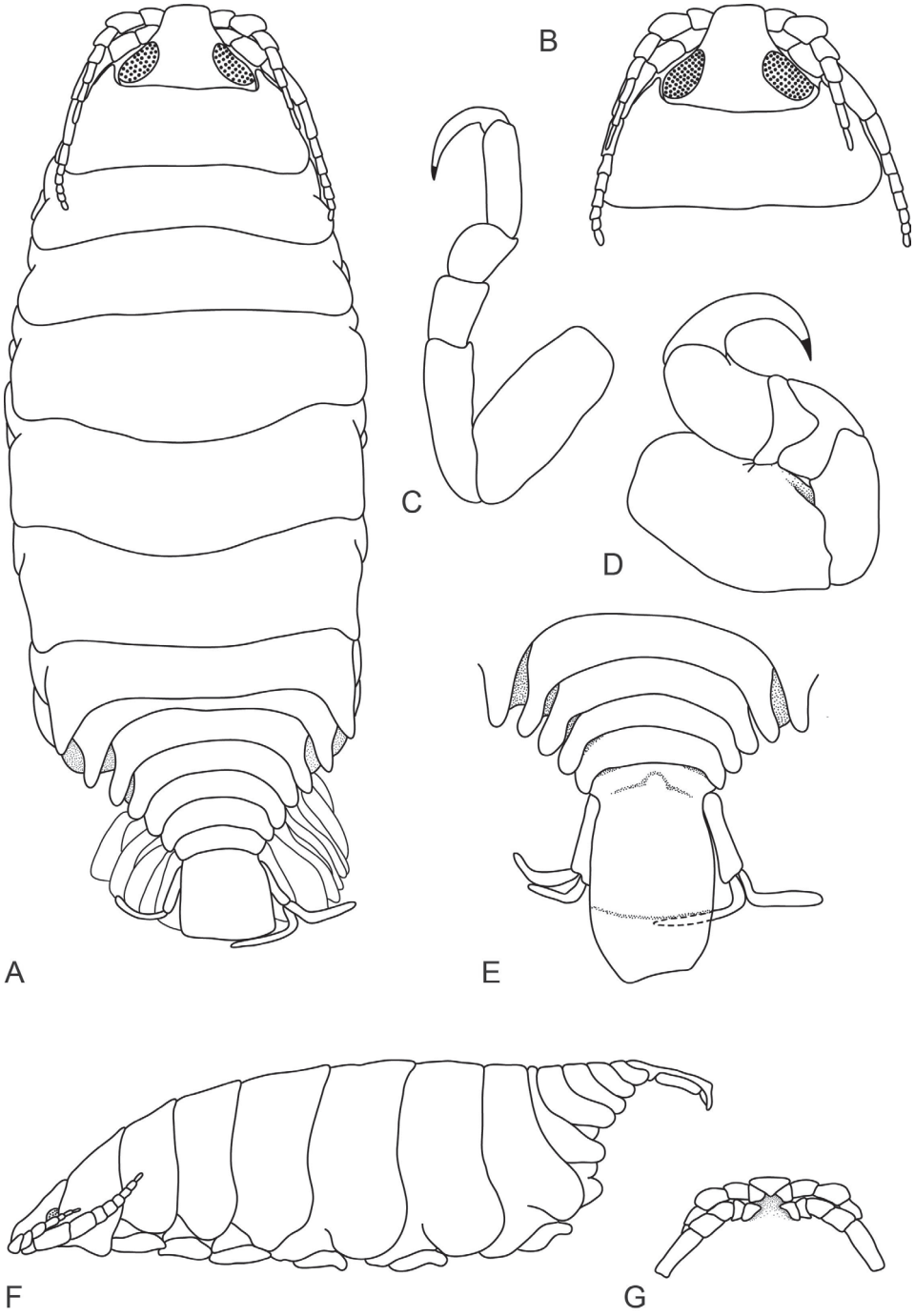

Figure 2. *Pleopodias elongatus* Richardson, 1910 (USNM 40917), female holotype (20 mm). **A** dorsal view **B** dorsal view of pereonite 1 and cephalon **C** pereonite 7 **D** pereopod 1 **E** dorsal view of pleon and pleotelson **F** lateral view **G** ventral view of cephalon.

Bureau of Fisheries *Albatross* Philippine Expedition 1907-08 (USNM 40917). Also noted: bottom third of the pleotelson folded in.

Description. *Female holotype.* Length 20 mm, width 7.5 mm.

Body elongate, 2.7 times as long as greatest width, dorsal surfaces smooth and polished in appearance, widest at pereonite 4 and pereonite 5, most narrow at pereonite 1, lateral margins subparallel. *Cephalon* 0.7 times longer than wide, visible from dorsal view, subtriangular. *Frontal margin* thickened, ventrally folded and truncate. *Eyes* oval with distinct margins, one eye 0.25 times width of cephalon; 0.4 times length of cephalon. *Pereonite 1* smooth, anterior border straight, anterolateral angle acute, anteriorly produced, extending to one third of the eye. Posterior margins of pereonites smooth and slightly curved laterally. Coxae 2–4 narrow, with posteroventral angles rounded; 5–7 small and narrow, not extending past pereonite margin, with posteroventral angles curved. Pereonites 1–4 increasing in length and width; 5–7 decreasing in length and width. *Pleonites* posterior margin smooth, mostly concave. Pleonite 1 widest, visible in dorsal view. Pleonite 1 and 2 not overlapped by pereonite 7; posterolateral angles of pleonite 2 rounded. Pleonites 3–5 progressively getting smaller; pleonite 5 not overlapped by lateral margins of pleonite 4, posterior margin straight. *Pleotelson* 1.6 times as long as anterior width, dorsal surface slightly depressed, lateral margins weakly convex, posterior margin rounded and damaged.

Antennula thinner than antenna, length longer than antenna, bases narrowly separated, consisting of 8 articles; peduncle articles 1 and 2 distinct and articulated; articles 2–3 expanded; extending to middle of pereonite 1. *Antenna* consisting of 11 articles; extending to middle of pereonite 2. *Pereopod 1* basis 1.6 times as long as greatest width; ischium 0.7 times as long as basis; merus proximal margin without bulbous protrusion; carpus with straight proximal margin; propodus 1.4 times as long as wide; dactylus moderately slender, 1.3 times as long as propodus, 2.4 times as long as basal width. *Pereopod 7* longer than other pereopods, basis 2.3 times as long as greatest width; ischium 0.8 times as long as basis, without protrusions; merus proximal margin without bulbous protrusion, 1.3 times as long as wide, 0.4 times as long as ischium; carpus 1.3 times as long as wide, 0.4 times as long as ischium, with slight bulbous protrusion; propodus 3.3 times as long as wide, 0.8 times as long as ischium; dactylus slender, 0.9 times as long as propodus, 3.7 times as long as basal width. *Uropod* longer than the pleotelson, rami subequal. *Endopod* apically slightly pointed. *Exopod* apically narrowly rounded.

Hosts. Not known.

Distribution. Philippine Islands (Richardson 1910; Bruce 1987).

Remarks. *Pleopodias elongatus* can be distinguished by the small eyes (each eye a quarter of the cephalon width), antennula bases narrowly separated, antenna extending to the middle of pereonite 2, rounded pleotelson, and uropod rami approximately the same length.

The only other species recorded from the Pacific is *P. diaphus* (from Japan). *Pleopodias elongatus* differs from *P. diaphus* in having antennula bases narrowly separated (*P. diaphus* bases in contact), shorter antenna (*P. diaphus* antenna extend to posterior

of pereonite 3), absence of robust setae on propodus of pereopod 7 (*P. diaphus* has numerous acute robust setae), a rounded pleotelson (*P. diaphus* pleotelson subquadrate with a deeply emarginated medial notch), and smaller eyes (*P. diaphus* eyes cover a third of the cephalon width).

Pleopodias vigilans **Richardson, 1911**

Pleopodias vigilans Richardson, 1911: 525–526.

Material examined. *Holotype.* Female (28 mm TL, 11 mm W), 9 July 1883, collected from the *Talisman*, St. DR71 (dredge), 640 m depth, coast of Sudan, MNHN-IU-2014-12188 (= MNHN-Is2460). Also noted: the specimen is black with the pleotelson folded in.

Description. *Female holotype.* Length 28 mm, width 11 mm.

Body elongate, 2.9 times as long as greatest width, dorsal surfaces slightly bumpy, widest at pereonite 4, most narrow at pereonite 1. *Cephalon* 0.6 times longer than wide, visible from dorsal view, subtriangular. *Frontal margin* thickened, ventrally folded and truncate. *Eyes* not clearly defined; one eye 0.4 times width of cephalon, 0.6 times length of cephalon. *Pereonite 1* smooth, anterior border slightly irregular, anterolateral angle narrowly rounded. Posterior margins of pereonites smooth and straight. Coxae 2–3 wide, with posteroventral angles rounded; 4–7 small and narrow, extending past pereonite margin. Pereonites 1–4 increasing in length and width; 5–7 decreasing in length and width. *Pleonites* posterior margin not smooth, mostly concave. Pleonite 1 widest, slightly visible in dorsal view. Pleonite 2 not overlapped by pereonite 7; posterolateral angles of pleonite 2 rounded. Pleonites 3–5 progressively getting smaller; pleonite 5 with posterolateral angles rounded, posterior margin produced medially. *Pleotelson* 1.2 times as long as anterior width, dorsal surface slightly depressed, lateral margins posteriorly narrow, posterior margin converging to rounded caudomedial point.

Antennula thinner and shorter than antenna, bases widely separated, consisting of 8 articles; peduncle articles 1 and 2 distinct and articulated; extending past the posterior margin of cephalon. *Antenna* consisting of 11 articles, extending to posterior of pereonite 2. *Pereopod 1* basis 1.6 times as long as greatest width; ischium 0.7 times as long as basis; merus proximal margin without bulbous protrusion; carpus with straight proximal margin; propodus 1.5 times as long as wide; dactylus slender, 1.8 times as long as propodus. *Pereopod 7* longer than other pereopods, basis 2.4 times as long as greatest width; ischium 0.6 times as long as basis, without protrusions; merus proximal margin without bulbous protrusion, 1.3 times as long as wide, 0.6 times as long as ischium; carpus 1.1 times as long as wide, 0.4 times as long as ischium, without bulbous protrusion; propodus 2.3 times as long as wide, 0.9 times as long as ischium; dactylus slender, 1.1 times as long as propodus, 3.7 times as long as basal width. *Uropod* longer than the pleotelson; peduncle 0.4 times longer than rami, peduncle lateral margin without setae, apices broadly rounded. *Endopod* apically rounded, 3.5 times as long

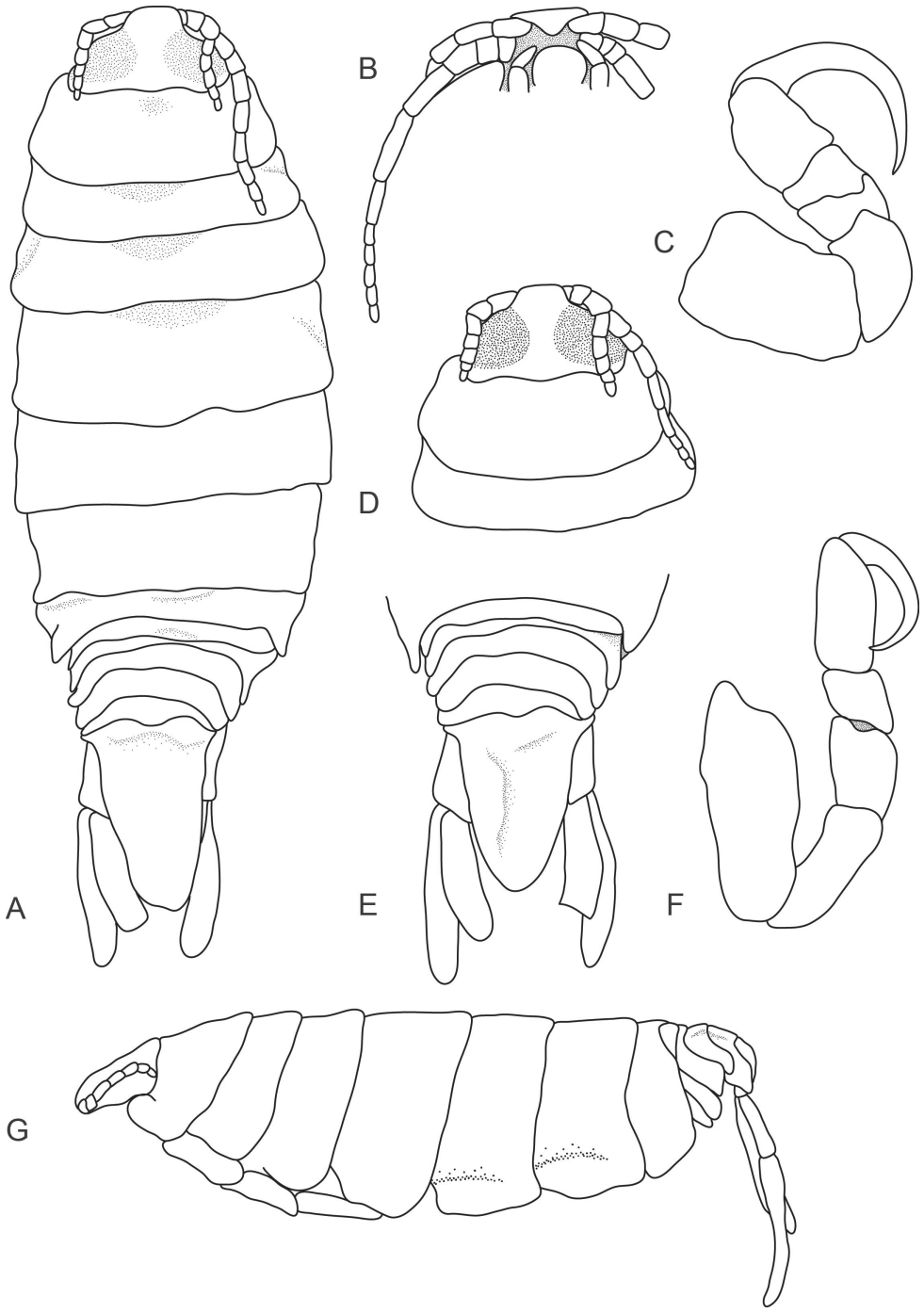

Figure 3. *Pleopodias vigilans* Richardson, 1911 (MNHN-IU-2014-12188), female holotype (28 mm). **A** dorsal view **B** ventral view of cephalon **C** pereopod 1 **D** dorsal view of pereonite 1, pereonite 2 and cephalon **E** dorsal view of pleon and pleotelson **F** pereopod 7 **G** lateral view.

as greatest width. *Exopod* extending beyond posterior of endopod, 5 times as long as greatest width, apically rounded.

Distribution. Sudan (Richardson 1911).

Hosts. Not known.

Remarks. *Pleopodias vigilans* can be identified by the antennula bases being widely separated, large eyes occupying majority of the cephalon, antenna extending to posterior of pereonite 2, uropodal exopod longer than endopod, and rounded pleotelson with a caudomedial point.

Only one specimen of this species has ever been collected. Due to the age and condition of the specimen, it was not possible to see some of the characters usually associated with *Pleopodias* (i.e. robust setae on pereopod 7 etc.).

No figures of the specimen were provided in the original description, and as no other collections have been made since, no drawings of the specimen have ever been produced. This redescription provides the first illustrated figures of *P. vigilans* and will help future identifications of this species. Fresh collections of this species could prove valuable in adding to this information, along with information on the mouthparts and pleopods of this species.

***Pleopodias nielbrucei* sp. n.**

http://zoobank.org/4063D2C2-A939-4E9C-8F1E-F759246443DD

Material examined. *Holotype.* Female (30mm TL; 9mm W), RV Africana Cruise 060 (34°46.6'S 18°02.5'E), Station A7033-060-14-03M, South Africa, 14 March 1988, 702m depth (SAMC A088881). *Paratype*. Male (20mm TL; 5 mm W), same info as holotype (SAMC A43478).

Description. *Female holotype.* Length 30 mm, width 9 mm.

Body narrow, 3.3 times as long as greatest width, dorsal surfaces smooth and polished in appearance, widest at pereonite 5, most narrow at pereonite 1, lateral margins subparallel. *Cephalon* 0.7 times longer than wide, visible from dorsal view, subtriangular. *Frontal margin* thickened, ventrally folded and truncate. *Eyes* oval with distinct margins, one eye almost 0.5 times width of cephalon; 0.6 times length of cephalon. *Pereonite 1* smooth, anterior border slightly indented, anterolateral angle narrowly rounded, extend to one third of the eye. Posterior margins of pereonites smooth and slightly curved laterally. Coxae 2–3 wide, with posteroventral angles rounded; 4–7 small and narrow, not extending past pereonite margin. Pereonites 1–5 increasing in length and width; 6–7 decreasing in length and width. *Pleonites* posterior margin smooth, mostly concave. Pleonite 1 widest, visible in dorsal view. Pleonite 2 not overlapped by pereonite 7; posterolateral angles of pleonite 2 narrowly rounded. Pleonites 3–5 similar in form to pleonite 2; pleonite 5 not overlapped by lateral margins of pleonite 4, posterior margin produced medially. *Pleotelson* 1.2 times as long as anterior width, dorsal surface slightly depressed, lateral margins straight, posterior margin subtruncate.

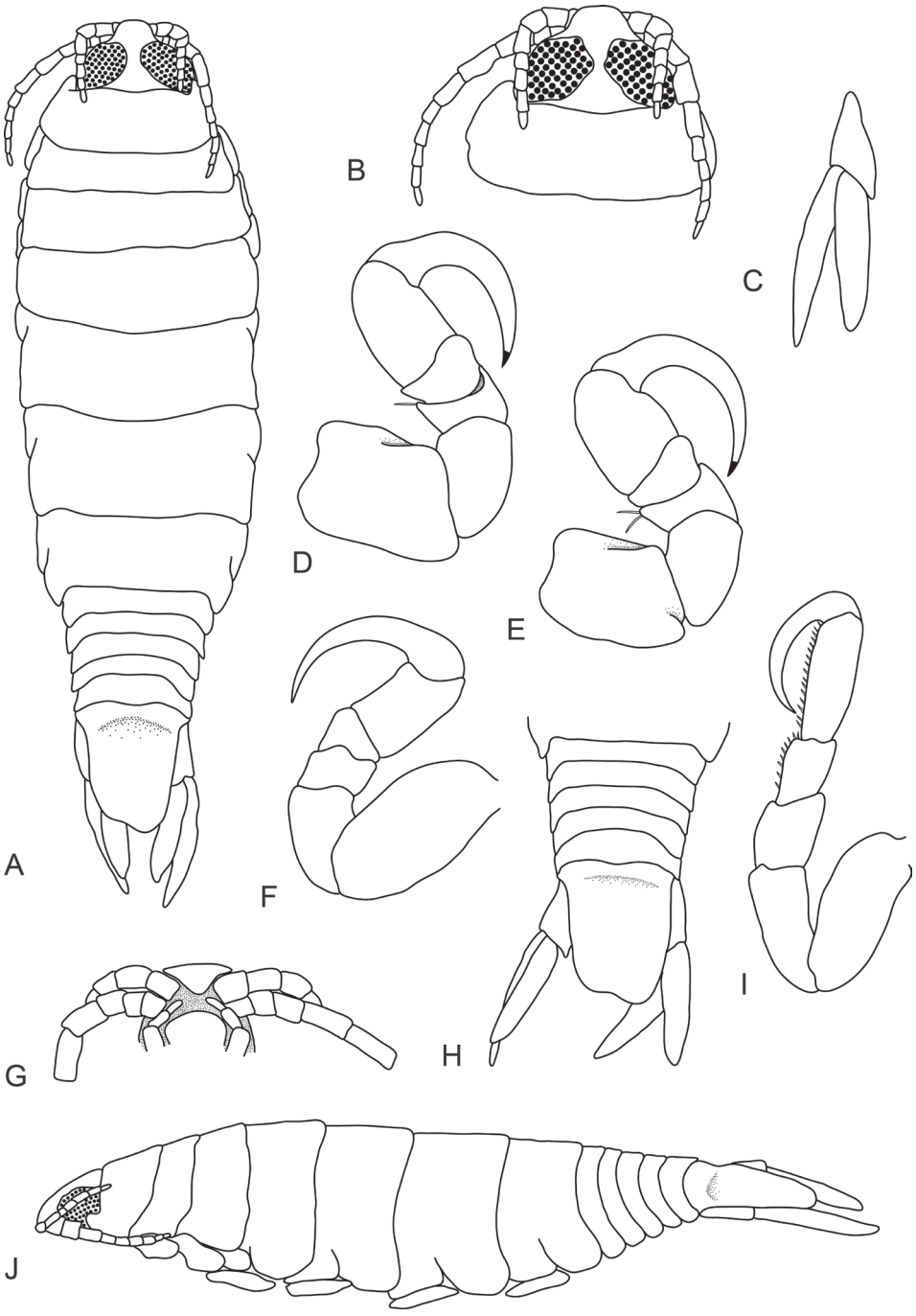

Figure 4. *Pleopodias nielbrucei* sp. n. (SAMC A088881), female holotype (30 mm). **A** dorsal view **B** dorsal view of pereonite 1 and cephalon **C** uropod **D** pereopod 1 **E** pereopod 2 **F** pereopod 6 **G** ventral view of cephalon **H** dorsal view of pleon and pleotelson **I** pereopod 7 **J** lateral view.

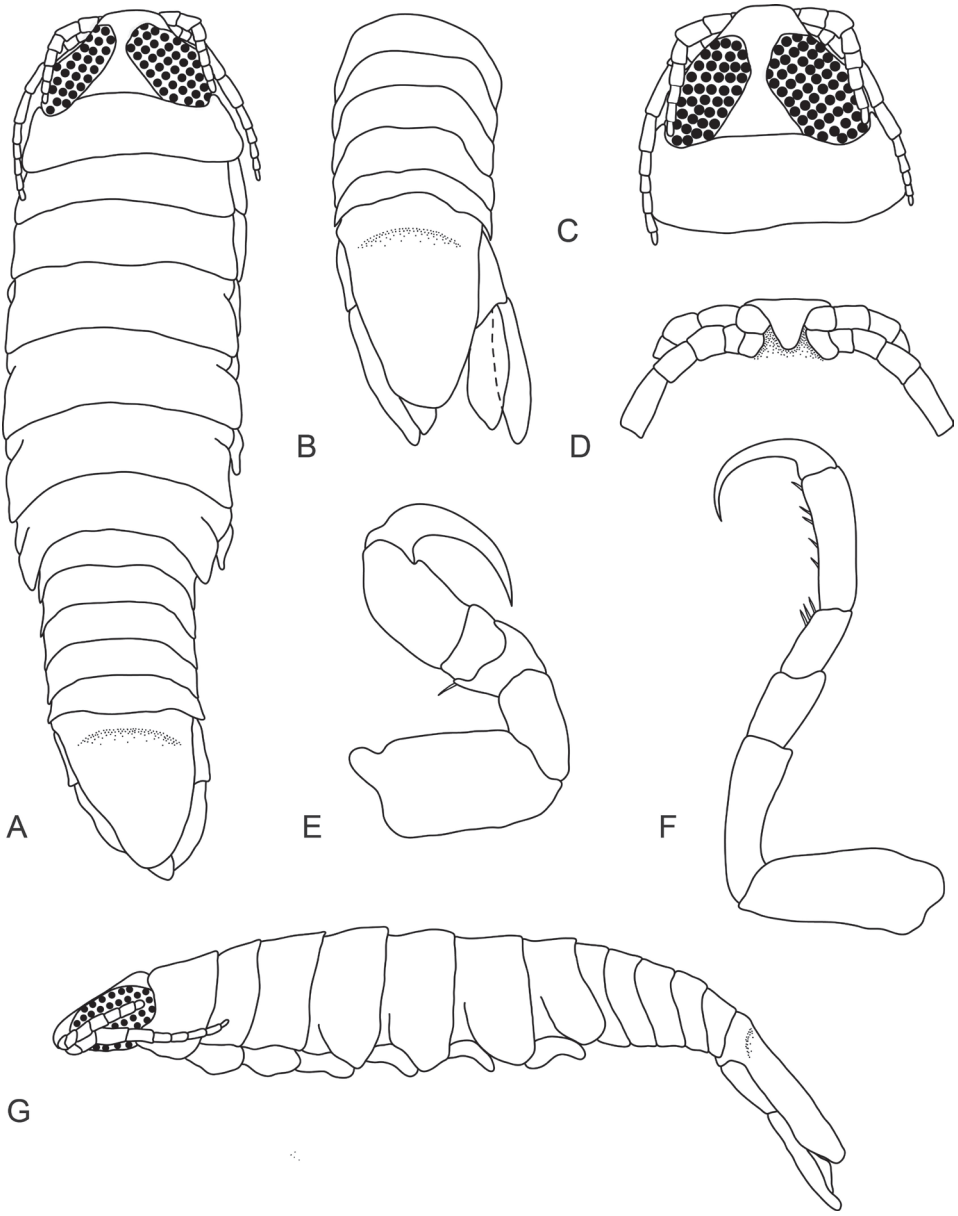

Figure 5. *Pleopodias nielbrucei* sp. n. (SAMC A43478), male paratype (20 mm). **A** dorsal view **B** dorsal view of pleon and pleotelson **C** dorsal view of pereonite 1 and cephalon **D** ventral view of cephalon **E** pereopod 1 **F** pereopod 7 **G** lateral view.

Antennula thinner and shorter than antenna, bases widely separated, consisting of 8 articles; peduncle articles 1 and 2 distinct and articulated; extending to anterior of pereonite 1. *Antenna* consisting of 11 articles, extending to middle of pereonite 2. *Pereopod 1* basis 1.4 times as long as greatest width; ischium 0.7 times as long as basis; merus with simple setae, proximal margin without bulbous protrusion; carpus with rounded proximal margin; propodus 1.5 times as long as wide; dactylus moderately slender, 1.7 times as long as propodus, 3.3 times as long as basal width. *Pereopod 2* propodus 1.4 times as long as wide; merus with simple setae; dactylus 1.7 times as long as propodus. *Pereopod 6* basis twice as long as greatest width, ischium 0.6 times as long as basis, propodus 1.7 times as long as wide, dactylus 1.8 times as long as propodus. *Pereopod 7* longer than other pereopods, basis 2.3 times as long as greatest width; ischium 0.8 times as long as basis, without protrusions; merus proximal margin without bulbous protrusion, 1.2 times as long as wide, 0.6 times as long as ischium; carpus with numerous acute robust setae, 1.4 times as long as wide, 0.5 times as long as ischium, without bulbous protrusion; propodus with numerous acute robust setae, 2.8 times as long as wide, as long as ischium; dactylus slender, as long as propodus, 3.5 times as long as basal width. *Uropod* longer than the pleotelson, peduncle 0.4 times longer than rami, peduncle lateral margin without setae. *Endopod* apically slightly pointed, 5 times as long as greatest width, terminating without setae. *Exopod* extending beyond posterior of endopod, apically narrowly rounded, terminating without setae.

Male paratype. Length 20 mm, width 5 mm.

Male similar to female but smaller. Body rectangular, body 3.5 times as long as wide. *Antennula* bases separated, consisting of 8 articles, extending to posterior margin of cephalon. *Antenna* consisting of 12 articles, extending to middle of pereonite 2. Eyes slightly separated, one eye almost 0.5 times width of cephalon; 0.7 times length of cephalon.

Etymology. Named in honour of Dr Niel Bruce, in recognition of his significant contribution to the taxonomy of isopods, specifically that of fish parasitic cymothoids.

Distribution. Off the coast of Cape Town, South Africa.

Hosts. Not known.

Remarks. *Pleopodias nielbrucei* sp. n. can be identified by the narrow body, large eyes covering majority of the cephalon (almost in contact), antennula bases wide apart, antenna extending to middle of pereonite 2, subtruncate pleotelson, pereopod 7 with numerous acute robust setae on the propodus as well as the carpus, and the uropodal exopod longer than the endopod.

This is the first named *Pleopodias* species from the southern hemisphere (not including the unknown *Pleopodias* sp. mentioned below). It differs from the other three known species in having larger eyes and a more elongate body, as well as a shorter and more quadrate pleotelson and antennula bases which are further apart than the other species. *Pleopodias nielbrucei* sp. n. also has a less graduated pleon (the pleonites do not decrease in width from pleonite 1 to 5 as prominently as *P. diaphus* and *P. elongatus*).

Pleopodias sp.

Pleopodias elongatus Barnard, 1936: 167–168, fig. 7f–g. (not *P. elongatus* Richardson, 1910). *Pleopodias* sp. Bruce, 1987: 87, figs 1–2.—Trilles, 1994: 109.

Material. Ovigerous female (14.5 mm TL), 232 km north of Port Hedland, Western Australia, 10 Oct 1982, 298-300 m depth, coll: L. Marsh & S. Slack-Smith on FRV Soela (WAM 607-80). Also noted: Specimen is crushed within the tube. Not examined.

Ovigerous female (15.5 mm TL), 370–419 fathoms, north of Andaman Islands (14°13'N; 93°40'E). Not examined.

Distribution. Andaman Islands and Australia (Barnard, 1936; Bruce, 1987).

Hosts. Not known.

Remarks. Bruce (1987) reported what appears to be an undescribed *Pleopodias* species; however, the Australian specimen is crushed and the whereabouts of Barnard's specimen is unknown. This species differs from *P. elongatus* (which it was originally identified as by Barnard) in having a sub-truncate and very narrow pleotelson, uropods which extend to the posterior margin of the pleotelson, antennula bases contiguous, a shorter rostrum, larger eyes, and a less laterally rounded pereonite 7. A tentative description of the Australian specimen was provided by Bruce (1987) but more specimens (in good condition) are required in order to describe the species.

Acknowledgments

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A new species of leech of the genus *Placobdella* (Hirudinida, Glossiphoniidae) from the American alligator (*Alligator mississippiensis*) in Mississippi, USA

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Abstract

To date, the only species of leech reported from the American Alligator, *Alligator mississippiensis* is *Placobdella multilineata*. Seven specimens of a previously undescribed species of *Placobdella* were collected from the feet and lower jaw of a single female alligator from the Pascagoula River Wildlife Management Area, George County, Mississippi. The new species was named *Placobdella siddalli* Richardson & Moser, **sp. n.**, in honor of the contributions of Dr. Mark Siddall to our understanding of the biology of leeches. *Placobdella siddalli* Richardson & Moser is similar to other papillated members of the genus *Placobdella*, but differs from *Placobdella ali* Hughes & Siddall, 2007, *Placobdella rugosa* (Verrill, 1874), *Placobdella multilineata* Moore, 1953, and *Placobdella papillifera* (Verrill, 1872) in coloration, papillation, ventral striping, and in the possession of a relatively large caudal sucker. In addition, molecular comparison of 626 nucleotides of CO-I between the new species and other papillated leeches (*P. ali*, *P. multilineata*, *Placobdella ornata*, *P. papillifera*, *P. rugosa*) revealed interspecific differences of 14.0–18.0% (88–113 nucleotides).

Keywords

Placobdella siddalli, *Alligator mississippiensis*, American Alligator, Glossiphoniidae, Hirudinea, Clitellata

Introduction

There are 22 recognized species of Glossiphoniid leeches in the genus *Placobdella* parasitizing birds, mammals, amphibians and reptiles (Moser et al. 2014). To date, the only species of leech reported from the American Alligator, *Alligator mississippiensis* (Daudin, 1802) Cuvier, 1807 is *Placobdella multilineata* Moore, 1953. *Placobdella multilineata* is a generalist parasite of reptiles having been reported from turtles, snakes, and alligators from throughout the southeastern United States and Mississippi River Valley as far north as Illinois and Iowa (Klemm 1985; Moser, Richardson, McAllister, et al. 2014). In addition, Saumure and Doody (1998) reported two specimens of *P. multilineata* from a three-toe Amphiuma, *Amphiuma tridactylum* Cuvier, 1827 from Louisiana. In the course of a routine parasitological survey of blood parasites of Mississippi alligators, seven specimens of a previously undescribed species of *Placobdella* were collected and are described herein.

Materials and methods

On 9 August 2015, seven specimens of a previously undescribed species of *Placobdella* were collected from the feet and lower jaw of a single female Mississippi alligator, approximately 1.2 m long, that was pole snared from Davis Eddy, a cypress swamp constituting an oxbow lake of the Pascagoula River in the Pascagoula River Wildlife Management Area, George County, Mississippi (30°54'11"N, 088°44'35"W). Six additional alligators examined from the same region were leech-free.

Leeches were relaxed, fixed and examined as described by Moser et al. (2006). Terminology for plane shapes follows Clopton (2004). Ranges are given followed by mean in parentheses. One specimen was mounted in Canada balsam following the techniques of Richardson and Barger (2006). Specimens were deposited in the Invertebrate Zoology Collection of the Peabody Museum of Natural History, Yale University, New Haven, Connecticut, USA (YPM IZ) and the Smithsonian Institution, National Museum of Natural History, Washington, District of Columbia, USA (USNM). The following specimens held in the collection of the Peabody Museum of Natural History, Yale University, New Haven Connecticut were examined in comparison to *Placobdella siddalli* Richardson & Moser, and caudal sucker diameter relative to body width and length was determined: *Placobdella ali* Hughes & Siddall, 2007 (YPM IZ 047497, N = 1; YPM IZ 058254, N = 1; and YPM IZ 058279, N = 1), *P. multilineata* (YPM IZ 083513, N = 8), *Placobdella ornata* (Verrill, 1827) Moore, 1901 (YPM IZ 048007, N = 1; YPM IZ 058351, N = 3, YPM IZ 058360, N = 1; YPM IZ 058371, N = 1; YPM IZ 058551, N = 1; and YPM IZ 058322, N = 2), *Placobdella papillifera* (Verrill, 1872) Moore, 1952 (YPM IZ 043493, N = 1; YPM IZ 043494, N = 7; and YPM IZ 043557, N = 3), *Placobdella parasitica* (Say, 1824) Moore, 1901 (YPM IZ 053088, N = 1; YPM IZ 058096, N = 1; YPM IZ 058091, N = 1; YPM IZ 058092, N = 1; YPM IZ 058093, N = 1; and YPM IZ 058094, N = 1), and *Placobdella rugosa* (Verrill, 1874) Moore,

1901 (YPM IZ 056679, N = 1; YPM IZ 056680, N = 2; YPM IZ 056681, N = 1; and YPM IZ 058081, N = 2).

Molecular analyses were conducted according to Richardson et al. (2010) as follows: For the proteinase K treatment step, tissue samples were taken from the caudal suckers of individual leeches and lysed overnight at 56°C. DNA was isolated with the DNeasy Blood & Tissue Kit from Qiagen (Cat. No. 69504), following the protocol given for the purification of total DNA from animal tissues (spin-column). DNA was eluted from the spin columns with 100 µl of buffer.

Polymerase Chain Reactions (PCR) were prepared using the Illustra PuRe Taq Ready-To-Go PCR beads from GE Health Care (Cat. No. 27-9559-01). Primers were purchased from Invitrogen and were comprised of two primers each for mitochondrial cytochrome c oxidase subunit I (CO-I) as specified by Light and Siddall (1999). Specifically, the CO-I primers were LCO1490 (5'GGTCAACAAATCATAAAGATATTGG 3') and HCO2198 (5'TAAACTTCAGGGTGACCAAAAAATCA 3'). Final volume of PCR reactions was 25 µl with three µl of leech genomic DNA added per reaction. DNA was amplified under the following PCR conditions: 94°C for five min.; 35 cycles of (94°C for 30 sec, 50°C for 30 sec, 72°C for 45 sec); 72°C for seven min. Following PCR, samples were cleaned up using a QIAquick PCR purification kit from Qiagen (Cat. No. 28104).

Purified PCR products were sequenced using the HCO2198 primer for the cytochrome c oxidase subunit I gene by the W. M. Keck Foundation Biotechnology Resource Laboratory at Yale University. The DNA sequences were aligned using Clustal W version 2 (Larkin et al. 2007) and checked manually using SeaView 4 (Gouy et al. 2010) and then analyzed using PAUP* 4.0b10 (Swofford 2002) and compared to other leech DNA sequences contained within Genbank. Uncorrected p distances were calculated using PAUP*.

Results

Species description

Placobdella siddalli **Richardson & Moser, sp. n.**
http://zoobank.org/9170DAD3-1657-4000-BF99-474E6DCDCB91
Figures 1–3; 5–6

Material examined. Holotype (YPM IZ 083857) Davis Eddy, a cypress swam constituting an oxbow lake of the Pascagoula River in the Pascagoula River Wildlife Management Area, George County, Mississippi (30°54'11"N, 088°44'35"W).

Paratypes (YPM IZ 083875-083876, YPM IZ 090164-090165; USNM 1422202-1422203) Davis Eddy, a cypress swam constituting an oxbow lake of the Pascagoula River in the Pascagoula River Wildlife Management Area, George County, Mississippi (30°54'11"N, 088°44'35"W).

Figure 1. Holotype specimen of *Placobdella siddalli* Richardson & Moser, YPM IZ 083857 **A** Dorsal surface **B** Ventral surface. Scale bar: 2 mm.

Morphological description. *External morphology*: (Fig. 1) Body elliptoid; length of preserved specimens 9.0–11.1 (9.8) mm, width at widest point (in center of body) 3.6–5.0 (4.5) mm. Dorsum base color beige with olive-green pigment spots. Dorsal papillae arranged in five rows (dorsomedial, two paramedial and two paralateral rows of unpigmented, stellate papillae) with repeating patterns of papillae size as follows: dorsomedial papillus of neural annulus large; paramedial papillae of neural annulus small; paralateral papillae of neural annulus large. Dorsomedial papillus of annulus posterior to neural annulus small; paramedial papillae of annulus posterior to neural annulus large; paralaterial papillae of annulus posterior to neural annulus small. Dorsomedial papillus of annulus anterior to neural annulus greatly reduced (sensillus); paramedial and paralateral papillae lacking on annulus anterior to neural annulus. Lateral papillae much less organized, not in distinct rows. Lateral region with alternating unpigmented and modestly pigmented sections (being characterized by small chromatophores). Anal opening located in furrow, one anteriad annulus from the caudal sucker. Beginning adjacent to the anus and commencing anteriad are two rows of

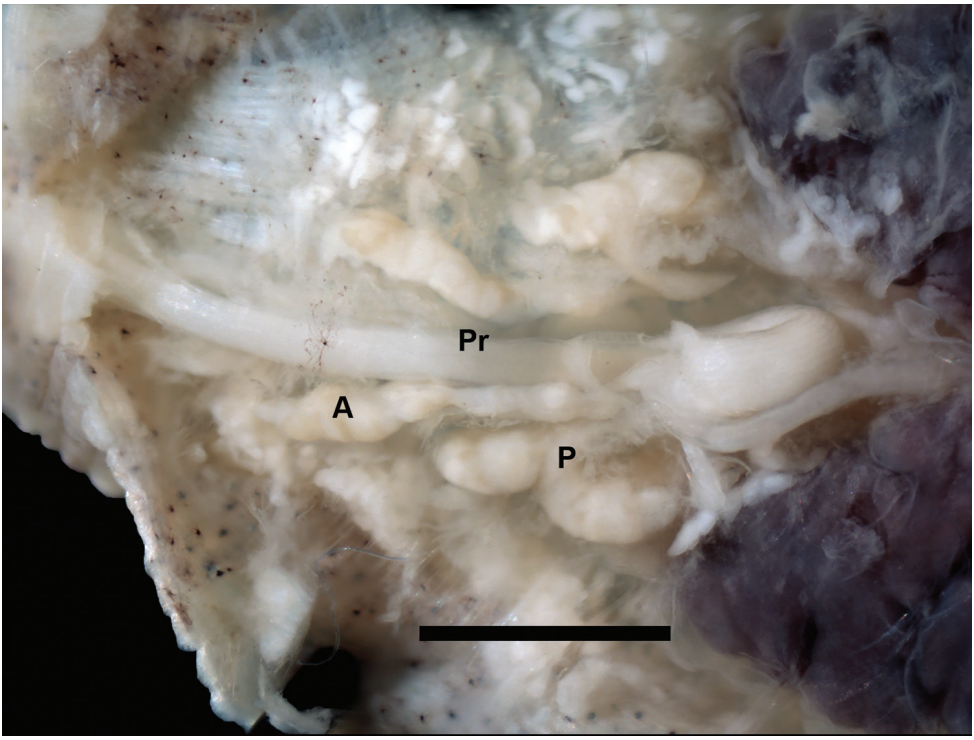

Figure 2. Internal anatomy of *Placobdella siddalli* Richardson & Moser, YPM IZ 083875. Dorsal view, anterior salivary gland (**A**), posterior salivary gland (**P**), proboscis (**Pr**). Scale bar: 1 mm.

three papillae, followed by two pairs of prominent paramedial papillae. Two pair of near-coalesced eyespots, typical of the genus *Placobdella*, within lateral unpigmented mask that extends posteriad into interrupted dorsal-medial pigment line that extends posteriorly to anus. Most pronounced pigmentation of dorsal-medial pigment line from genital region to anterior pair of prominent paramedial papillae. Caudal sucker orbicular, diameter 2.0–2.2 (2.1) mm; 18.64–22.9 (21.1) % of the length leech; dorsal surface with approximately three rows of papillae, the anterior-most of which is most prominent. Ventral surface of the whole body with scattered chromatophores, most concentrated in genital region and without stripes.

Internal morphology: (Figs 2 and 3) Proboscis pore on rim/lip of anterior sucker. Blunt-tipped muscular proboscis nearly uniformly cylindrical, only very modest enlargement at base. Two pair of discrete salivary glands. Anterior pair very narrowly doliform to oblong and slightly enlarged anteriad; ductal medially inserted into narrowly elliotoid posterior salivary glands; ductal of anterior salivary gland anastomoses with ductual of posterior salivary gland half-way between the posterior salivary gland and proboscis forming common duct. Esophagus extends from the base of the proboscis with one pair sac-like mycetomes. Seven pairs of diverticulated crop ceca, last pair extending posteriorly and diverticulated into four sections. Four pairs of intestinal ceca.

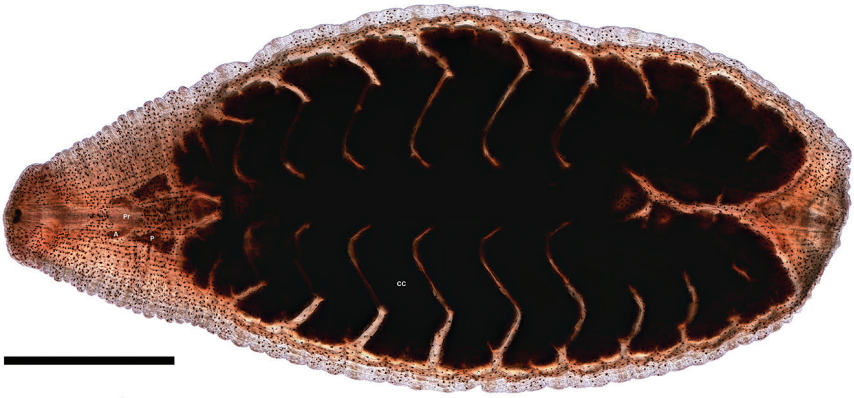

Figure 3. Paratype specimen of *Placobdella siddalli* Richardson & Moser, YPM IZ 083876 mounted in Canada balsam, crop ceca (**CC**), anterior salivary gland (**A**), posterior salivary gland (**P**), proboscis (**Pr**). Scale bar: 2 mm.

Reproductive system: Male and female gonopores in furrows and separated by two annuli. Six pair of testisacs.

Taxonomic summary. Type host. American Alligator, *Alligator mississippiensis* (Daudin, 1802) Cuvier, 1807

Type locality. Davis Eddy, a cypress swamp constituting an oxbow lake of the Pascagoula River in the Pascagoula River Wildlife Management Area, George County, Mississippi (30°54'11"N, 088°44'35"W).

Type material. YPM IZ 083857 (Holotype), YPM IZ 083875-083876, YPM IZ 090164-090165 (Paratypes), USNM 1422202–1422203 (Paratypes).

Etymology. The specific epithet *siddalli* is in honor of Dr. Mark Siddall in recognition of the profound advancements that he has contributed to our understanding of glossiphoniid leeches, particularly in regard to the taxonomic importance of preanal papillae.

Molecular description. Molecular characterization is based on sequence of 626 nucleotides of the mitochondrial Cytochrome c oxidase subunit I (GenBank KY780962). Molecular comparison of 626 nucleotides of CO-I revealed 100% identify between two specimens of *Placobdella siddalli* Richardson & Moser collected from the same host in Davis Eddy, George County, Mississippi (type locality; YPM IZ 083876, GenBank KY780962 and an interspecific difference of 14.0% to 15.7% (88 to 98 nucleotides) between *P. siddalli* Richardson & Moser and four specimens of *P. multilineata* from Louisiana, Mississippi, and Oklahoma. Additional intraspecific differences of 15.9% to 16.7% (99 to 105 nucleotides) were found between *P. siddalli* Richardson & Moser and four specimens of *P. rugosa* collected from the type locality (North Dakota; GenBank JX412986–JX412990); difference of 18.0% (113 nucleotides) between *P. siddalli* Richardson & Moser and three specimens of *P. ali* from the type locality (New York) and Connecticut (GenBank HM347040–HM347042);

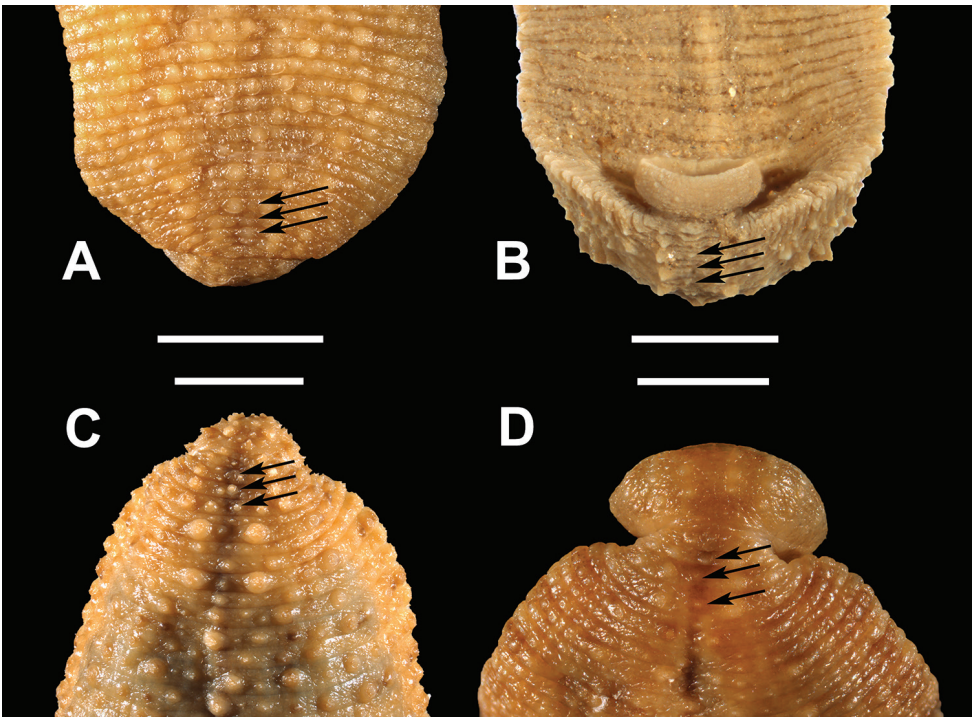

Figure 4. Dorsal surface, anal region. Medial row of small but distinct papillae (indicated by arrows) lying between the anus and commencement of prominent paramedial papillae, on **A** *Placobdella rugosa* (YPM IZ 083787) **B** *Placobdella ornata*, syntype (YPM IZ 000256) **C** *Placobdella ali* (YPM IZ 058254) **D** *Placobdella multilineata* (YPM IZ 083782). Scale bars: 3 mm (**A**), 1 mm (**B**), 4 mm (**C**), 2 mm (**D**).

differences of 16.9% to 17.3% (106 to 109 nucleotides) between *P. siddalli* Richardson & Moser and five specimens of *P. papillifera* (GenBank KC505241–KC505245) from its type locality (West River, New Haven, New Haven County, Connecticut); differences of 15.0% to 15.3% (94 to 96 nucleotides) between *P. siddalli* Richardson & Moser and five specimens of *P. ornata* (GenBank JQ812128–JQ812132) collected from the type locality (West River, New Haven County, Connecticut); and differences of 14.7% and 14.8% (92 to 93 nucleotides) between *P. siddalli* Richardson & Moser and five specimens of *P. parasitica* collected from the type locality (Minnesota; GenBank KF058895–KF058899).

Discussion

Placobdella siddalli Richardson & Moser most closely resembles *P. multilineata*, *P. ali*, and *P. rugosa*. Both *P. ali* and *P. rugosa* have faint but distinct brown pigmented lines corresponding to paralateral and paramedial papillae, that are lacking in *P. siddalli*

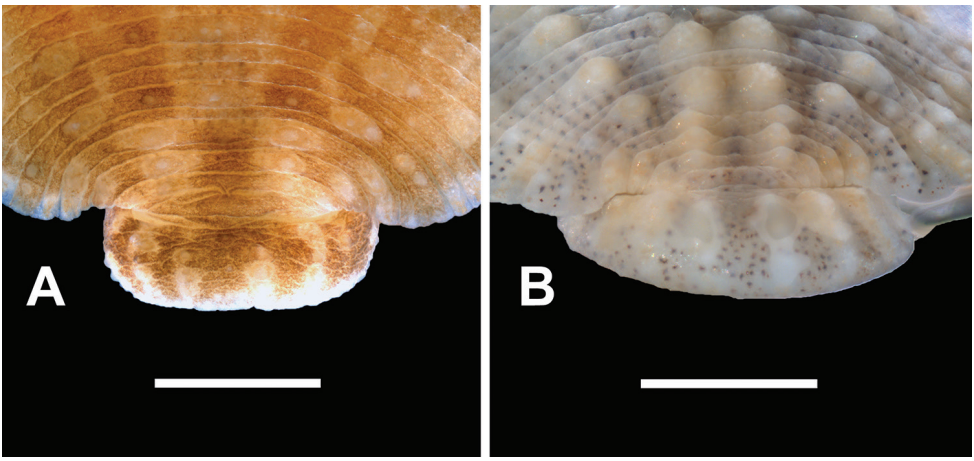

Figure 5. Dorsal surface, anal region, (note lack of papillae between anus and commencement of prominent paramedial papillae) of **A** *Placobdella papillifera* (YPM IZ 083792) **B** *Placobdella siddalli* Richardson & Moser (YPM IZ 083857). Scale bars: 1 mm.

Richardson & Moser. In *P. ali*, the dorso-medial line is unbroken, whereas it is broken in *P. siddalli* Richardson & Moser. Also *P. ali*, *P. multilineata*, *P. ornata*, and *P. rugosa*, have a medial row of small but distinct papillae, each lying between the anus and four prominent paramedial papillae (Fig. 4). Probably because of their diminutive size, these papillae have not previously been described although they are evident in Fig. 2 of Moser, Richardson, Hammond, and Lazo-Wasem (2012) and Fig. 3 of Moser, Richardson, Hammond, Govedich, and Lazo-Wasem (2012). These papillae are lacking in *P. siddalli* Richardson & Moser and *P. papillifera* (Fig. 5). *Placobdella ali* also exhibits ventral striping that is lacking in *P. siddalli* Richardson & Moser.

The relative diameter of the caudal sucker in comparison to body width and body length was found to be helpful in differentiating species of the genus *Placobdella* (Fig. 6). Table 1 gives relative size of the caudal suckers in comparison to body width and length for *P. ali*, *P. siddalli* Richardson & Moser, *P. multilineata*, *P. ornata*, *P. papillifera*, *P. parasitica*, and *P. rugosa*. The caudal sucker diameter of *P. siddalli* Richardson & Moser is 18% to 23% of the length of the body. This relative diameter is similar to that of *P. rugosa* and *P. papillifera*, but is greater than that of *P. ali* and *P. multilineata*, with the caudal sucker diameter to body-length ratio not overlapping. Likewise the diameter to body-length ratio of the caudal sucker of *P. siddalli* Richardson & Moser is larger than that of *P. ornata* and *P. parasitica*, overlapping only slightly. The caudal sucker diameter of *P. siddalli* Richardson & Moser is 40% to 54% of the width of the body. This relative diameter is greater than that of *P. ali* and *P. ornata*, with the caudal sucker diameter to body-width ratio not overlapping.

The unique color patterning, papillation and large relative size of the caudal sucker renders *P. siddalli* Richardson & Moser readily discernible from all described species in

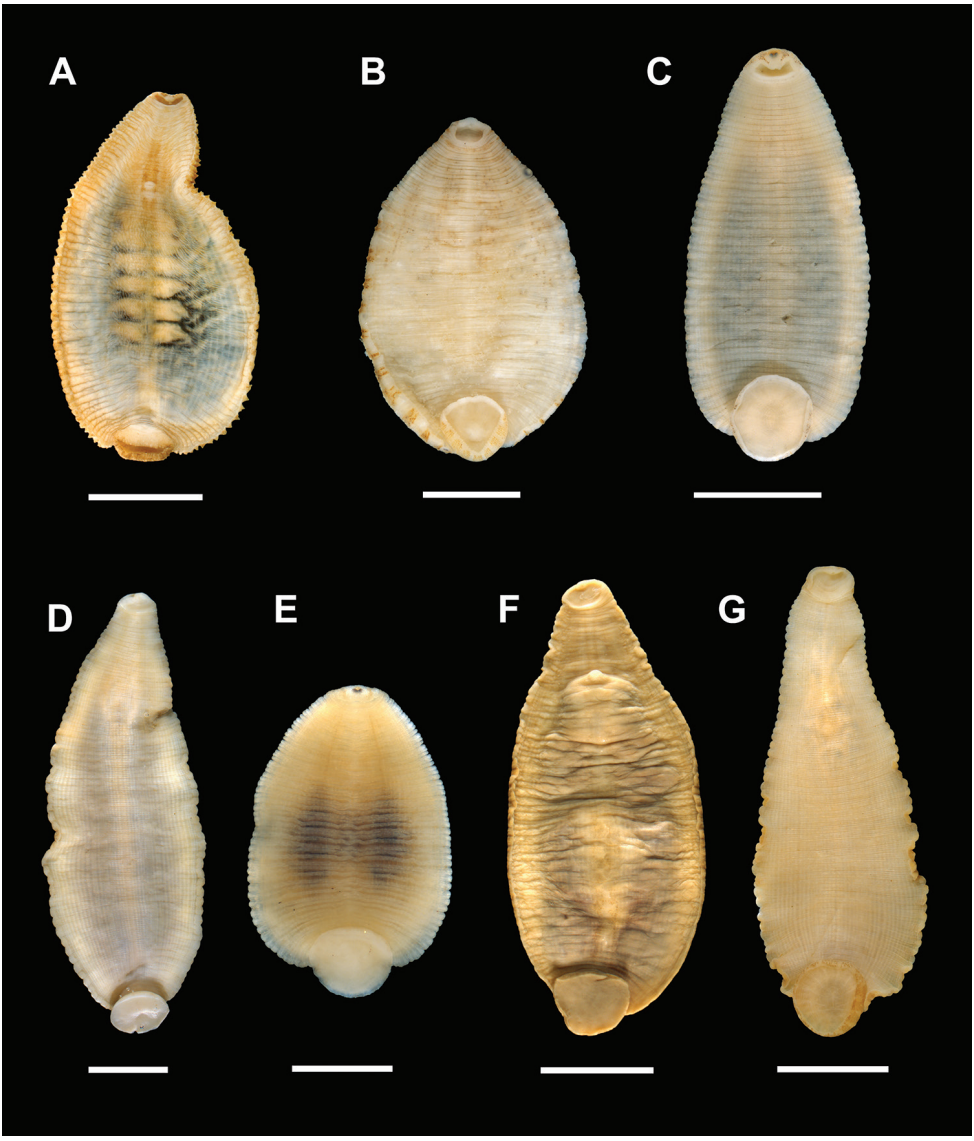

Figure 6. Ventral surface of various species of *Placobdella*. Note the diameter of the caudal sucker relative to body length and width of individuals. **A** *Placobdella ali* (YPM IZ 058279) **B** *Placobdella ornata* (YPM IZ 083847) **C** *Placobdella siddalli* Richardson & Moser (YPM IZ 083857) **D** *Placobdella multilineata* (YPM IZ 083850) **E** *Placobdella papillifera* (YPM IZ 083856) **F** *Placobdella parasitica* (YPM IZ 059092) **G** *Placobdella rugosa* (YPM IZ 056680). Scale bars: 10 mm (**A, F**), 2 mm (**B**), 3 mm (**C**), 5 mm (**D, G**), 1 mm (**E**).

the genus *Placobdella*. It is likely that further collection, and retrospective examination of museum holdings, of the papillated *Placobdella* will provide additional information on the distribution and host utilization patterns of this intriguing new species.

Table 1. Ratio of diameter of caudal sucker to body length and width for seven species in the genus *Placobdella*.

Species	Caudal sucker diameter/ Body length	Caudal sucker diameter/ Maximum body width
P. ali	0.12–0.17	0.27–0.33
P. siddalli	0.18–0.23	0.40–0.54
P. multilineata	0.11–0.15	0.32–0.47
P. ornata	0.13–0.19	0.23–0.38
P. papillifera	0.12–0.24	0.27–0.44
P. parasitica	0.11–0.19	0.32–0.49
P. rugosa	0.07–0.24	0.31–0.47

In the course of this study, two new taxonomic characters have been utilized for differentiation of species within the genus *Placobdella*: the presence or absence of a medial row of small but distinct papillae lying between the anus and 4 prominent paramedial papillae and the ratio of sucker diameter to body length and width. These characters may help provide resolution between other species in the genus *Placobdella*, as well as species representing other genera.

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A new *Paraleius* species (Acari, Oribatida, Scheloribatidae) associated with bark beetles (Curculionidae, Scolytinae) in Canada

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Abstract

Bark beetles (Scolytinae) are hosts to a broad diversity of mites (Acari), including several genera of Oribatida (Sarcoptiformes). Of these, *Paraleius* (Scheloribatidae) species are the most frequently collected oribatid mites associated with bark beetles. A new species was discovered while surveying the acarofauna of bark beetles in Eastern Canada and is described as *Paraleius leahae* **sp. n.** (Oribatida, Scheloribatidae). This species was collected from two host beetle species, *Hylastes porculus* Erickson and *Dendroctonus valens* LeConte, in Ontario, New Brunswick and Nova Scotia. The genus *Paraleius* is rediagnosed, *Metaleius* is considered a synonym of *Paraleius*, and the proposed synonymy of *Paraleius* with *Siculobata* is rejected. The three known species are *Paraleius leontonycha* (Berlese), *P. leahae* **sp. n.**, and *P. strenzkei* (Travé), **comb. n.** The barcode region of cytochrome oxidase subunit I (COI) was amplified from *P. leahae* **sp. n.**

Keywords

Mite, Acari, phoresy, COI, forest entomology, ecology

Introduction

A broad assemblage of wood-burrowing beetles (Cerambycidae, Buprestidae, Scolytinae), and associated mites, nematodes, and fungi reside under the bark of dead, dying or living trees. Bark beetles (Curculionidae, Scolytinae) are a diverse group of wood-borers

that feed and mate in the cambium or xylem of numerous tree species worldwide (Wood 1982). Mites are among one of the most diverse and common associates of scolytines, and in temperate forests some bark beetle species are associated with 15-20 mite species (Lindquist 1969).

Oribatid mites (Acari, Oribatida) dwell primarily in soil or forest litter, though many are found in arboreal habitats and a few occur in aquatic systems (Norton and Behan-Pelletier 2009). Several genera of oribatid mites are also found under tree bark in scolytine galleries, or dispersing phoretically on bark beetles (Moser and Roton 1971). Phoresy is relatively uncommon in oribatid mites, and typically phoretic species are not host specific and the association with their host appears to be passive (Norton 1980). *Paraleius leontonycha* (Berlese, 1910) (Scheloribatidae) is the most frequently encountered oribatid species on bark beetles, although it does not occur in high abundance or prevalence (Knee et al. 2013). *Paraleius leontonycha* is a broad host generalist with a Holarctic distribution, collected from 17 scolytine species and found in the galleries of 10 other bark beetle species (Knee et al. 2013, Ahadiyat and Akrami 2015). A recent survey of the mesostigmatic and oribatid mite fauna of bark beetles in eastern Ontario (Knee et al. 2013) uncovered a new species of the monotypic genus *Paraleius* Travé, 1960 associated with two scolytine species, *Hylastes porculus* and *Dendroctonus valens*. Herein, I propose and describe *Paraleius leahae* sp. n., including the barcode region of COI. I also provide a revised generic diagnosis for *Paraleius*, and resolve some taxonomic issues surrounding *Paraleius* and closely related genera.

Methods

Sampling and identifications

Bark beetle specimens collected with Lindgren funnel traps in eastern Ontario by Knee et al. (2013), and Eastern Canada by the Canadian Food Inspection Agency (CFIA) staff as part of the Invasive Alien Species Monitoring program, were examined for associated mites. Scolytines were identified to species using a dissecting microscope and keys from Bright (1976). The presence, abundance, and attachment location of oribatid mites was recorded. All mites were collected and preserved in 95% ethanol for later identification and/or molecular analysis. Specimens used for illustrations were mounted in Hoyer's medium on temporary cavity slides. Permanent slide-mounted mites were cleared in 85% lactic acid, mounted in polyvinyl alcohol medium (6371A, BioQuip Products, Rancho Dominguez, California, United States of America), and cured on a slide warmer at 40ºC for 3–4 days.

Oribatid collections at the Canadian National Collection of Insects, Arachnids, and Nematodes (CNC), State University of New York College of Environmental Science and Forestry (SUNY-ESF), and John Moser's collection at the United States Department of Agriculture (USDA) in Pineville, Louisiana were examined for *Paraleius* specimens.

Slide-mounted specimens were examined using a Leica DM2500 compound microscope and Leica ICC550 HD camera, with differential interference contrast illumination (DIC). Initial drawings of mites were made with pencil on paper using a camera lucida. These were later merged in Adobe Photoshop CS5 and redrawn in Adobe Illustrator CS5 using an Intuos 3 Graphics Tablet from WACOM Co., Ltd. (Saitama, Japan).

Morphological terminology used in this study follows that developed by F. Grandjean (see Travé and Vachon 1975 for references and Norton and Behan-Pelletier 2009 for overview). Notogastral setation follows the unideficient nomenclature detailed by R.A. Norton in Balogh and Balogh (1988). The following conventions for measurements are used: *prodorsal setae*, measured on permanent slide-mounted specimens (*ro*, rostral seta; *le*, lamellar seta; *in*, interlamellar seta; *ex*, exobothridial seta; *bo*, bothridial seta); *total length*, measured dorsally from tip of rostrum to posterior margin of the notogaster on specimens in cavity slides; *total width*, measured at widest part of the notogaster on specimens in cavity slides. Total length and width were measured only for the few mites that were stored in ethanol and not mounted on permanent slides, all other measurements were performed on five to seven slide mounted mites. Leg setation is presented as the number of setae per segment (including the famulus on tarsus I), with solenidial counts in parentheses, in the following order: trochanter–femur–genu–tibia–tarsus. All measurements are in micrometres (μm); lengths presented with mean followed by the range in parenthesis. Type specimens are deposited in the Canadian National Collection of Insects, Arachnids, and Nematodes, at Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada.

Molecular methods

Genomic DNA was extracted from whole specimens for 24 hours using a DNeasy Tissue kit (Qiagen, Inc., Santa Clara, California, United States of America). Following extraction, mites were removed from the extraction buffer, vouchers were-slide mounted, and genomic DNA was purified following the DNeasy Tissue kit protocol. PCR amplifications were performed in a total volume of 25 μl, with 14.7 μl ddH2O, 2.5 μl 10× ExTaq buffer, 0.65 μl 25 mM MgCl2, 1.0 μl of each 10 μM primer, 2.0 μl 10 mM dNTPs, 0.15 μl ExTaq DNA polymerase, and 3 μl genomic DNA template. Primer pairs PHF1 (5'–CWACAAAYCAYAAAGATATTGG–3') and PHR1 (5'–TAHACYTCHGGRTGVCCRAAAAAYCA–3') were used to amplify a 641 bp fragment of the 5'–end of COI. PCR amplification was performed on an Eppendorf ep Gradient S Mastercycler (Eppendorf AG, Hamburg, Germany), using the following protocol: initial denaturation cycle at 94 °C for 3 min, followed by 45 cycles of 94 °C for 45 s, primer annealing at 40 °C for 45 s, 72 °C for 1 min, and a final extension at 72 °C for 5 min. Amplified products and negative controls were visualized on 1% agarose electrophoresis gels, and purified using pre-cast E-Gel CloneWell 0.8% SYBR Safe agarose gels (Invitrogen, Carlsbad, California, United States of America).

Sequencing reactions followed the protocol of Knee et al. (2012), and sequencing was performed at the Agriculture and Agri-Food Canada, Ottawa Research and Development Centre, Core Sequencing Facility (Ottawa, Ontario, Canada). Sequence chromatograms were edited and contiguous sequences were assembled using Sequencher v5.3 (Gene Codes Corp., Ann Arbor, Michigan, United States of America). Sequence for *Paraleius leahae* sp. n. has been submitted to GenBank (KY402259).

Results and discussion

Family Scheloribatidae Grandjean, 1933

Genus *Paraleius* Travé, 1960

Type species. *Paraleius* (=*Oribella*) *leontonycha* (Berlese, 1910)

Revised diagnosis. Rostrum extended medially, forming narrow point; anterior border of notogaster convex; prodorsal setae long, thickened, attenuate, barbed; bothridium inserted dorsolaterally, close to lamella; bothridial seta capitate or fusiform; bothridium covered with numerous spicules; prolamella present; sublamella and translamella absent; pteromorphs absent; exobothridial seta (*ex*) medium sized and barbed; humeral porose organ (*Ah*) expressed as saccule; four pairs of saccules on notogaster; Ten pairs of medium sized notogastral setae; shallow sternal groove on ventral surface; solenidia of tibiae III and IV microcephalic (rounded vesicle) or not; eupathidia *p* of tarsus I smooth, seta *p* of tarsus II–IV with small bristles along one side; seta *s* of tarsus I with large barbs along ventral side, not eupathidial; leg pretarsi monodactylous or hetero-tridactylous with large curved median claw, lateral claws (if present) long and thin, resembling setae.

Remarks. Travé (1960) described *Paraleius* as closely resembling *Hemileius* Berlese, 1916 with the distinction of the following characters: rostrum extended medially, forming narrow point; bothridial seta capitate; sublamella absent; seta *ex* medium sized and barbed; *Ah* expressed as saccule; heterodactyl claws with pronounced central claw; solenidia of tibiae III and IV microcephalic. Travé's diagnosis lacked a few additional characters which have been included in the revised diagnosis above: notogaster anterior margin convex, bothridium inserted close to the lamella, numerous spicules on bothridium. To accommodate the new species herein described the description for three character states from Travé's original diagnosis were modified: bothridial seta shape, pretarsal dactyly, and solenidia of tibiae III and IV microcephalic or not.

While Weigmann (1969) treated *Paraleius, Metaleius* and *Siculobata* as distinct genera, he later (2006) considered *Paraleius* and *Metaleius* to be junior synonyms of *Siculobata* based on a shared lamellar complex. However, this complex is not identical: *Siculobata* has a rudimentary sublamella, while *Paraleius* and *Metaleius* lack a sublamella. The synonymization of these genera also overlooks several other distinct character states shared by *Paraleius* and *Metaleius* that *Siculobata* does not possess. These

include: rostrum with narrow medial point, anterior margin of notogaster convex, seta *ex* medium sized and barbed, *Ah* expressed as saccule, and bothridial seta inserted dorsolaterally close to lamella. Fredes and Martinez (2013) did not follow Weigmann's (2006) proposed synonymy and provided a diagnosis for *Siculobata sensu stricto* that excludes *Paraleius* and *Metaleius*. Based on their concepts and on the aforementioned shared character states, I also reject the synonymization of *Paraleius* with *Siculobata*, but synonymize *Metaleius* and *Paraleius*. Each of the latter genera has been monotypic to this point, so the revised diagnosis for *Paraleius* is based on *Paraleius leontonycha*, *Paraleius leahae* sp. n., and *Paraleius strenzkei* (Travé, 1960), comb. n.

In his checklist of the world oribatid mite fauna, Subías (2004) placed *Wallworkiella* Hammer, 1979 as a subgenus of *Paraleius*, with the single species *Paraleius* (*Wallworkiella*) *nasalis* (Hammer, 1979). No explanation or justification was provided by Subías. In an unpublished online update (Subías 2016), possibly following Weigmann's classification, he instead placed *Wallworkiella* as a subgenus of *Siculobata*. However, *Wallworkiella* differs from *Paraleius* by having five pairs of notogastral sacculi, homo-tridactylous tarsi, and inflated tarsal pulvilli. Additionally, *Wallworkiella* does not belong to *Siculobata* based upon the concept of Fredes and Martinez (2013). Clearly, the generic and species level relationships of Scheloribatidae require further research and revisions, but the demotion of *Wallworkiella* to subgeneric rank under either *Paraleius* or *Siculobata* is unsupported.

***Paraleius leahae* sp. n.**
http://zoobank.org/1B2E5D72-E272-4867-BE09-58C7EB727B46
Figs 1–6

Material examined. ***Type material.*** Holotype: adult female (vial CNC649357) on *Hylastes porculus* (female) collected in Westfield, Nova Scotia, Canada (44.40316, -64.97473), 28.v.2009, coll: W. Knee.

Paratypes (20): one female (vial CNC649359) with the same collection information as the holotype; female (vial CNC649361) on *H. porculus* (male), St. Stephen, Highway 1, New Brunswick (45.22321, -67.15371), 15.vi.2009, coll: W. Knee; female (vial CNC649362) on *H. porculus* (male), Bayside, Route 127, New Brunswick (45.20539, -67.14034), 15.vi.2009, coll: W. Knee; male (vial CNC649363) on *H. porculus* (female), Turner and Turner Mill, West Northfield, Nova Scotia, 1.vi.2009, coll: W. Knee; two females and two males (slides CNC649365–649368) on *H. porculus*, Algonquin Provincial Park (PP), Ontario (45.902, -77.605), 17.vi.2008, coll: W. Knee; one female and three males (slides CNC649371–649374) on *H. porculus*, Algonquin PP, Ontario (45.902, -77.605), 3.vi.2008, coll: W. Knee; two females (slides CNC649375, CNC649376) on *Dendroctonus valens*, Algonquin PP, Ontario (45.895, -78.071), 3.vi.2008, coll: W. Knee; three females and three males (slides CNC649378–649383) on *D. valens*, Algonquin PP, Ontario (45.895, -78.071), 28.v.2008, coll: W. Knee.

Other material. 67 slide mounted specimens from *D. valens*, and 22 from *H. porculus* collected in Algonquin PP, Ontario (45.895, -78.071), 2008–2009, coll: W. Knee; one slide mounted specimen from *D. valens*, and 70 from *H. porculus* collected in Algonquin PP, Ontario (45.902, -77.605), 2008–2009, coll: W. Knee.

Diagnosis adult. As for *Paraleius* (see above). Bothridial seta long and fusiform, covered with numerous spicules; carina *kf* present; tarsi monodactylous with prominent sickle shaped strongly hooked claw; solenidia of tibiae III and IV not microcephalic. Immatures unknown.

Description. ***Measurements.*** Total length female (n = 4) 453 (432–464), male (n = 7) 430 (423–440). Total width female (n = 4) 277 (255–296), male (n = 7) 274 (258–295).

Integument. Body cuticle red-brown. Notogastral surface and venter appear smooth, but with fine granulate structure at higher magnification (100x). Small microtubercles on epimeral surface (Fig. 4). Small microtubercles medially on subcapitulum between *h* setae.

Prodorsum (Figs 1, 3). Lamella narrow, about 63 long. Prolamella narrow, extending from base of seta *le* to slightly anterior to *ro*, about 47 long. All prodorsal setae long, thickened, attenuate, barbed, reaching beyond rostrum; *ro* 78 (63–85) and *le* 95 (91–102) directed anteriorly, *in* 127 (121–137) directed anterodorsally. Mutual distance of setal pairs *ro*, *le*, and *in* ~53, 54, and 56 respectively. Bothridial seta long 85 (79–89), fusiform, directed anterolaterally, spicules conspicuous on head and minute along stalk. Seta *ex* medium sized 55 (52–59) thick, attenuate and barbed.

Lateral aspect of podosoma (Figs 3, 4). Carina *kf* present. As for other scheloribatids pedotectum I large, visible from dorsal aspect. Pedotectum II smaller and less visible than pedotectum I. Circumpedal carina weakly curved, extending slightly posterior of acetabulum IV. Sublamellar porose area *Al* present. Humeral porose organ *Ah* (~14 length, 11 width) expressed as saccule. Gland opening *z* ventral to *ex*.

Notogaster (Figs 1, 3). Longer than wide, ratio approximately 1.3:1. Dorsophragma (*D*) small, oval, approximately 8 wide. Ten pairs of medium sized notogastral setae 29 (19–38), setiform, smooth. Four pairs of saccules present: *Sa* largest (~9 diameter of saccule), located lateral to seta *la*; S_1 (~7) lateral to *lp*; S_2 (~6) posterolateral to h_3; S_3 (~6) posterior to h_1. Lyrifissure *ia* posterolateral of seta c_2; *im* posterolateral of *lm*; *ih* anterolateral to p_3; *ips* posterolateral to p_3; *ip* lateral to p_1. Opisthonotal gland opening (*gla*) posterior of lyrifissure *im*.

Venter (Figs 2, 3). Epimeral setal formula 3-1-3-3. All epimeral setae setiform and slightly barbed except for smooth setae *2b*, *4a*, and *4b*. Setal lengths as follows: *1a*, *1b*, *1c* ~26, 30, 25, respectively; *2a*, *3a*, *3b*, *3c* ~ 29, 28, 26, 26; and *4a*, *4b*, *4c* ~ 18, 22, 24. Shallow sternal groove present, approximately 69 long. Genital plates nearly as wide as long, genital plates of female slightly larger than those of male; length to width in females 61x58 and in males 55x51. Four pairs of simple setiform genital setae 14–21 long. Single pair of simple setiform aggenital setae (19), three pairs of simple setiform adanal setae ad_1, ad_2, ad_3 ~24, 26, 27, and two pairs of simple setiform anal setae (23). Lyrifissure *iad* very close to anal plates, about midway between levels of setae ad_2 and ad_3.

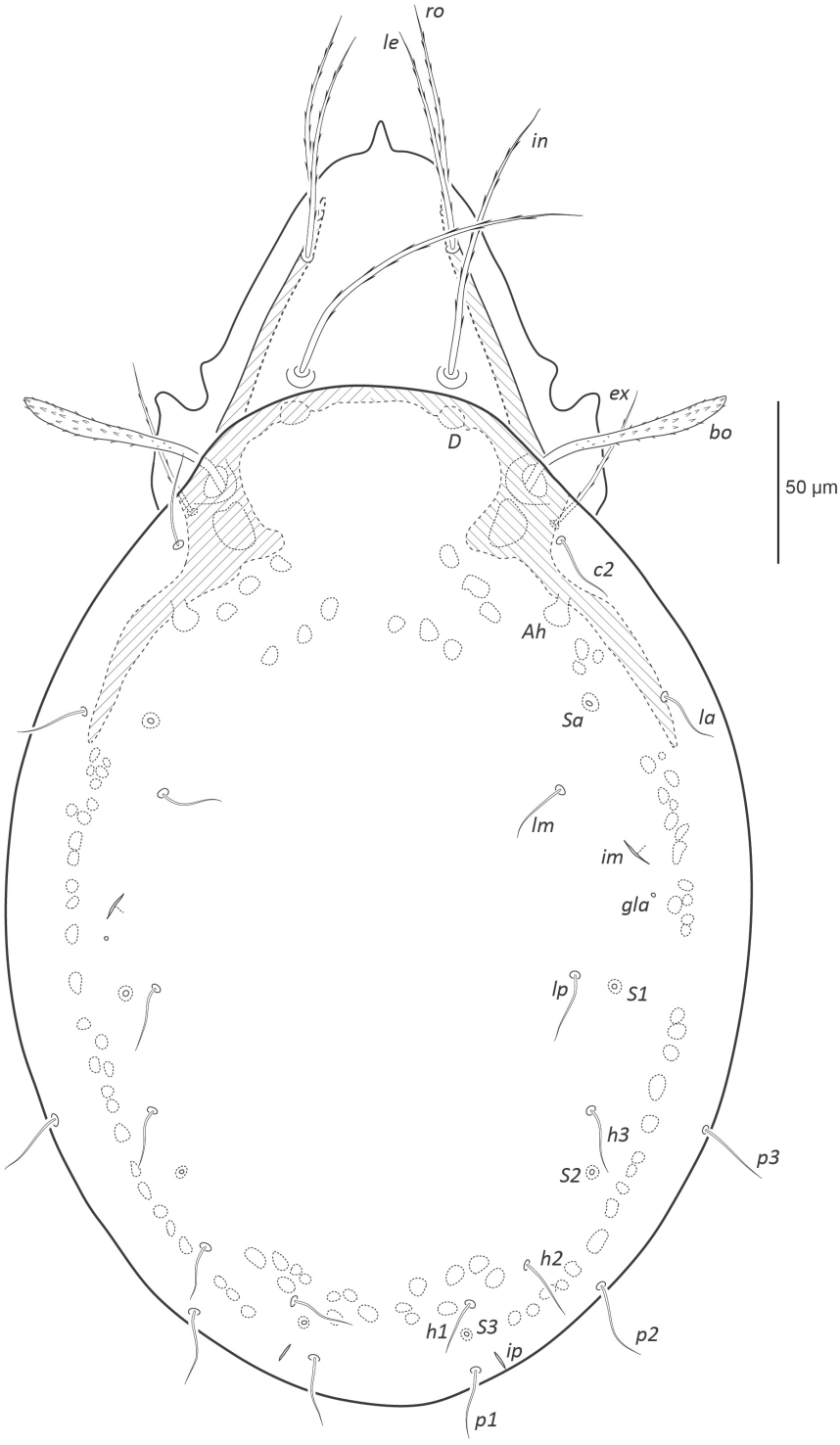

Figure 1. Female *Paraleius leahae* sp. n. dorsal view, legs omitted.

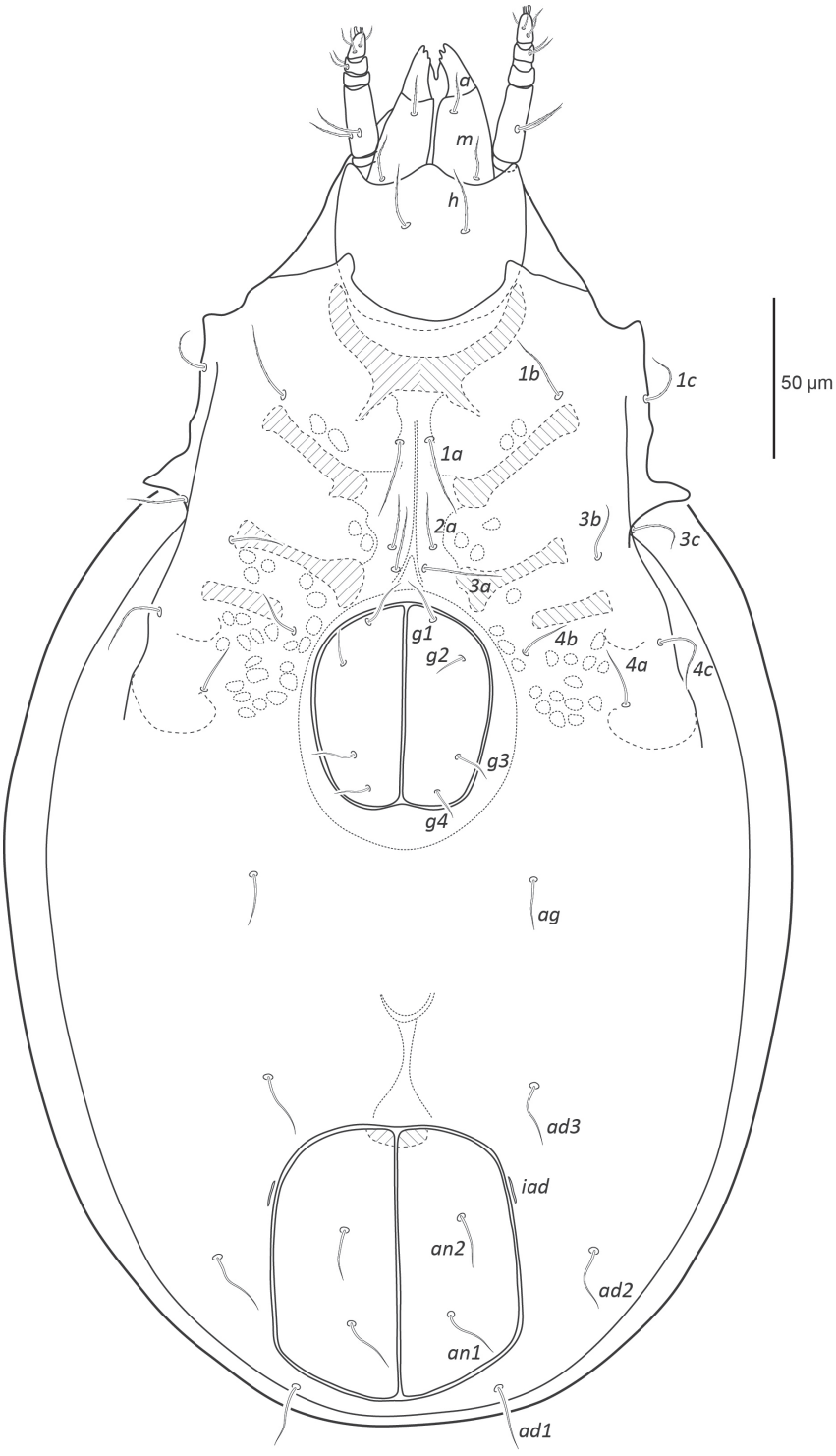

Figure 2. Female *Paraleius leahae* sp. n. ventral view, legs omitted.

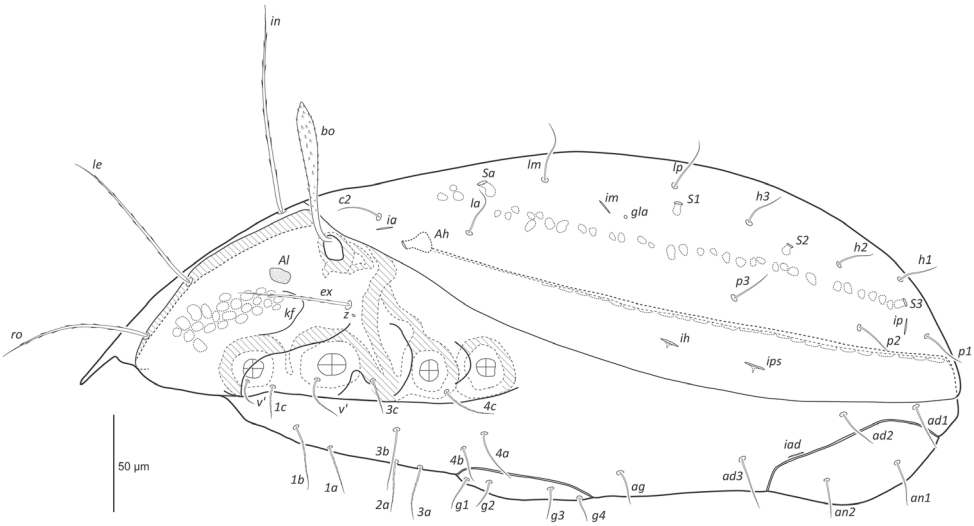

Figure 3. Female *Paraleius leahae* sp. n. lateral view, legs and gnathosoma omitted.

Figure 4. Female *Paraleius leahae* sp. n. photomicrograph of lateral view (DIC illumination), arrow indicating cuticular microtubercles.

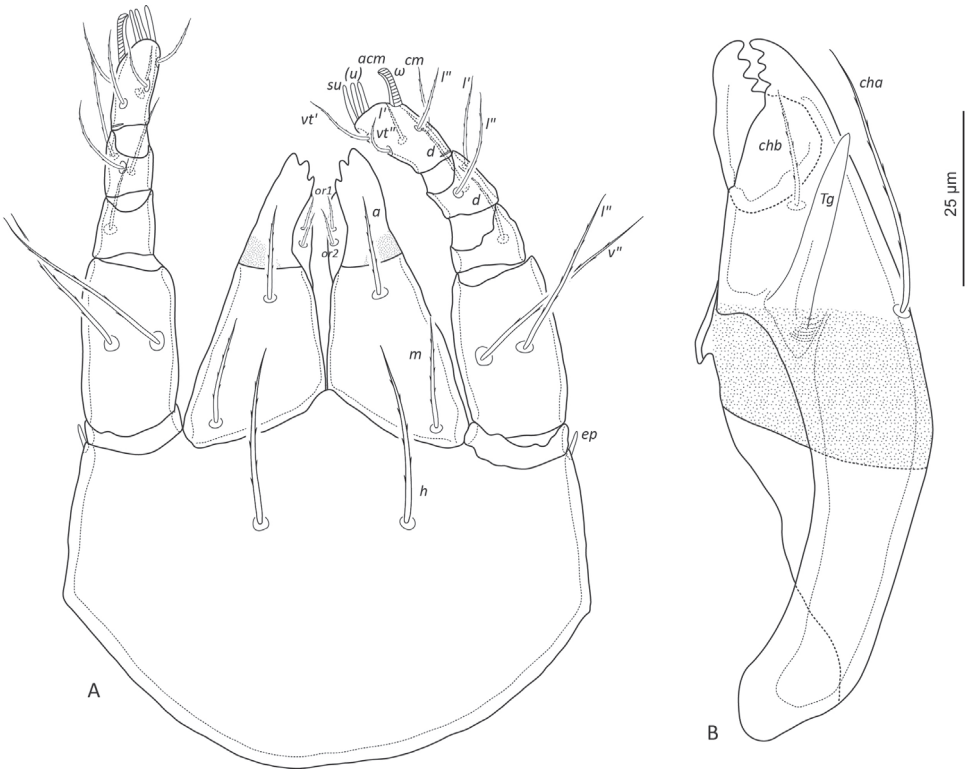

Figure 5. Female *Paraleius leahae* sp. n. **A** ventral view of subcapitulum **B** chelicerae, paraxial view.

Gnathosoma (Fig. 5). Subcapitulum wider than long; porose region on rutelli. Subcapitular setae setiform, barbed, *h* (32), *m* (18), *a* (18). Adoral setae (or_1, or_2) thin and barbed. Palp with setation 0-2-1-3-9(1), palpal solenidion ω and seta *acm* fused (~9), slightly curved near tip. Postpalpal setae (*ep*) simple, smooth and rounded. Chelicera 108 long, setae attenuate barbed; *cha* (44), *chb* (18), Trägårdh's organ (*Tg*) elongate triangular, rounded distally.

Legs (Fig. 6; Table 1). All tarsi monodactylous with prominent sickle shaped strongly hooked claw, claw surface smooth except for small bump along inner margin. Large porose areas present on femora I–IV, and on trochanters III and IV. Ventral porose region present distally on tibiae I–IV and proximally on tarsi I–IV; dorsal porose area present distally on tarsi I–IV. Setal formula same as *P. leontonycha*. Leg setation (solenidia) of leg I: 1–5–3(1)–4(2)–19(2); II: 1–5–2(1)–4(1)–15(2); III: 2–3–1(1)–3(1)–15; IV: 1–2–2–3(1)–12 (Table 1). All setae on trochanters and genua I–IV barbed. Seta *l'* on tibiae I, II barbed, all other setae on tibiae and tarsi I–IV with large barbs on one side, ventral setae with noticeably longer barbs than dorsal setae. Eupathidia *p* of tarsus I (~27), setae *p* of tarsi II–IV and *u* of tarsi I–IV with slight barbs unilaterally on ventral side. Famulus (8) short and blunt distally. Solenidia ω_1 on tarsus I baculiform, ω_1 and ω_2 on tarsus II ceratiform, all other solenidia piliform.

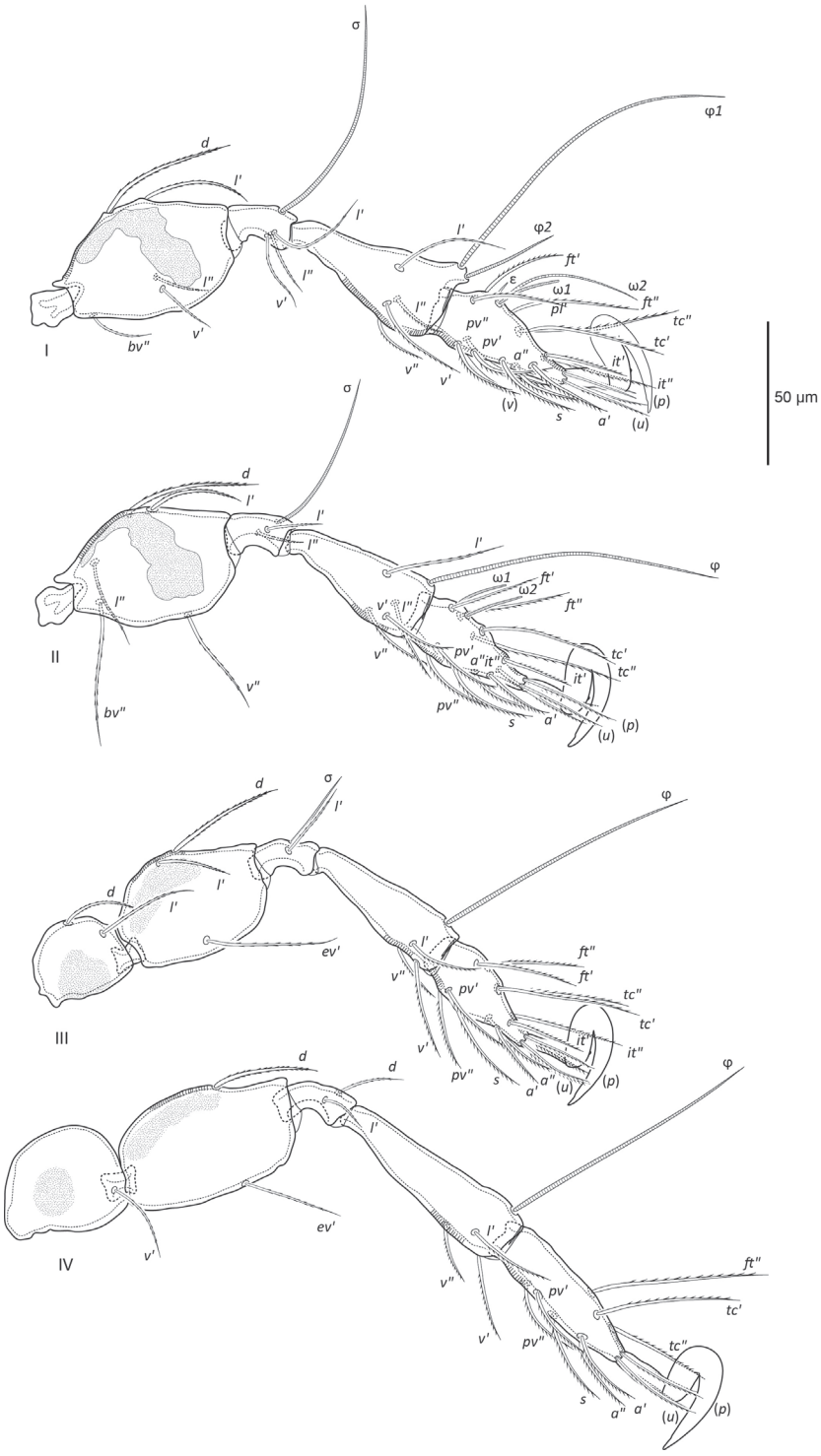

Figure 6. Female *Paraleius leahae* sp. n. legs; legs I, II paraxial view, legs III, IV antiaxial view.

Table 1. Leg setation and solenidia of adult *Paraleius leahae* sp. n., single prime (') indicates setae on anterior and double prime (") setae on posterior, seta in parenthesis indicates the presence of both setae.

Leg	Trochanter	Femur	Genu	Tibia	Tarsus
I	*v'*	*d, (l), v', bv"*	*(l), v', σ*	*(l), (v), φ$_1$, φ$_2$*	*(ft), pl', (tc), (it), (p), (u), (a), s, (pv), (v), ε, ω$_1$, ω$_2$*
II	*v'*	*d, (l), v", bv"*	*(l), σ*	*(l), (v), φ*	*(ft), (tc), (it), (p), (u), (a), s, (pv), ω$_1$, ω$_2$*
III	*d, l'*	*d, l', ev'*	*l', σ*	*l', (v), φ*	*(ft), (tc), (it), (p), (u), (a), s, (pv)*
IV	*v'*	*d, ev'*	*d, l'*	*l', (v), φ*	*ft", (tc), (p), (u), (a), s, (pv)*

Solenidia of tibiae III and IV not microcephalic. Bases of solenidia ω_1 and ω_2 on tarsus I positioned very close together.

Gender differences. No sexual dimorphism exists in external morphology, except for males being slightly smaller than females, their genital plates being slightly smaller proportionally than in females, and in the typical genitalic differences.

Genetics. There are no other sequences of *Paraleius* or *Metaleius* on GenBank; however, GenBank blast searches of the COI sequence (KY402259) of *P. leahae* sp. n. generally matches that of other poronotic brachypyline oribatid mites. Further analysis was not performed.

Etymology. This species is named after my wife and tireless supporter Leah Harper.

Remarks. *Paraleius leahae* sp. n. is most similar to *P. leontonycha* (Travé 1960, Wunderle et al. 1990), which has been collected from under tree bark, in the galleries of bark beetles, and is phoretic on numerous species of bark beetles (Vitzthum 1926, Wunderle et al. 1990, Knee et al. 2013). *Paraleius leahae* sp. n. differs from *P. leontonycha* by having a long fusiform bothridium; monodactylous tarsi; presence of carina *kf*; solenidia of tibiae III and IV not microcephalic.

Paraleius leahae sp. n. differs from *P.* (=*Metaleius*) *strenzkei* in having a long fusiform bothridial seta; monodactylous tarsi, medial claw large and strongly hooked; carina *kf* present; total length (432–464) of *P. leahae* females greater than *P. strenzkei* (310–360) (Travé 1960).

According to Grandjean (1959) microcephalic solenidia are found only in arboricolous or saxicolous species. *Paraleius leontonycha*, *P. leahae* sp. n. and *P. strenzkei* are arboricolous species, the former has microcephalic solenidia and the latter two species lack this feature. The tips of solenidia on tibiae III and IV are delicate and prone to breakage, so it is possible that they are microcephalic in *P. leahae*; however, I examined more than 100 specimens without finding microcephalic tips.

Distribution and biology

Paraleius leontonycha and *P. leahae* are quite similar morphologically, and it is possible that the latter has been misidentified as the former in the past. These two species are also ecologically similar in being corticolous and phoretic on bark beetles. The feeding biology of *P. leahae* and *P. leontonycha* is poorly understood, but fungal hyphae have been observed in the gut of slide mounted specimens of both species.

Paraleius leontonycha is the most commonly collected and widely distributed oribatid phoretic on bark beetles, however this species occurs infrequently and in low abundance (Norton 1980, Knee et al. 2013). *Paraleius leontonycha* has a Holarctic distribution; whereas, *P. leahae* has only been collected in Eastern Canada (Ontario, New Brunswick and Nova Scotia). *Paraleius* sp. and *P. leontonycha* collections at the CNC, SUNY-ESF, and the USDA were examined for *P. leahae* specimens. These collections contained material from across Canada (AB, BC, NB, NFLD, ON, QC), parts of the United States of America (AK, AZ, CA, LA, TX, UT, WI), parts of Europe (Croatia, Germany, Spain, Sweden, Switzerland), Mexico, Honduras, and Japan. All of the material examined from these collections represented *P. leontonycha*; no misidentified *P. leahae* were uncovered.

Typically the association between oribatid mites and their scolytine hosts is considered to be passive and with low host specificity (Norton 1980). *Paraleius leontonycha* is a host generalist, collected from 17 species of bark beetles (Knee et al. 2013, Ahadiyat and Akrami 2015). In contrast, *P. leahae* is a host specialist, collected from only two bark beetle species, *Hylastes porculus* and *Dendroctonus valens*. These two host species are not closely related species, but they are ecologically similar, as both species live in the stumps and roots of dead or dying conifers (Wood 1982). Multiple bark beetle species often occupy the same tree concurrently and occasionally their galleries cross, thus providing mites with an opportunity to transfer host species (Moser et al. 1971). *Paraleius leahae* shows a marked preference for only these two bark beetle species despite opportunities to switch host species. *Hylastes porculus* and *D. valens* are hosts to many species of mites; 16 other species of mites were collected from each of these host species in eastern Ontario including *P. leontonycha* (Knee et al. 2013). *Paraleius leahae* was the most abundant species collected out of the 33 species of mites collected from bark beetles in eastern Ontario using general lures (α-pinene and 95% ethanol) and Lindgren funnel traps (Knee et al. 2013). *Paraleius leahae* challenges the assumptions that bark beetle associated oribatid mites are uncommon and are not host specific.

Key to known *Paraleius* species

1 Tarsi monodactylous, central claw large sickle shaped and strongly hooked, hair-like lateral claws absent. Carina *kf* present. Long fusiform bothridial seta ..... ***Paraleius leahae* sp. n.**

– Tarsi hetero-tridactylous, large curved central claw, lateral claws hair-like. Carina *kf* absent. Capitate bothridial seta..... **2**

2 Central claw sickle shaped and strongly hooked. Solenidia of tibiae III and IV microcephalic. Total length approximately 435–480 μm..... ..... ***Paraleius leontonycha* (Berlese, 1910)**

– Central claw evenly curved, c-shaped. Solenidia of tibiae III and IV not microcephalic. Total length approximately 310–360 μm..... .....***Paraleius strenzkei* (Travé, 1960)**

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Two new Brazilian species of *Loxosceles* Heinecken & Lowe, 1832 with remarks on *amazonica* and *rufescens* groups (Araneae, Sicariidae)

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Abstract

The genus *Loxosceles* Heinecken & Lowe, 1832 has 91 representatives in the New World. Despite medical relevancy, the taxonomy of the genus is poorly understood. South American *Loxosceles* were divided into four groups of species: *laeta*, *spadicea*, *gaucho* and *amazonica*; this last one has a single species, *Loxosceles amazonica* Gertsch, 1967. More recently, the natural occurrence of *L. amazonica* in the New World has been questioned, due to the strong morphological resemblance and close phylogenetic relationship with Old World species, mainly with *Loxosceles rufescens* (Dufour, 1820). Herein, *L. amazonica* is rediagnosed and its morphological variation and natural distribution discussed. Two new species closely related to it from northeastern Brazil are also described, *Loxosceles willianilsoni* **sp. n.**, from the state of Rio Grande do Norte, and *Loxosceles muriciensis* **sp. n.**, from the state of Alagoas. The relationships of these new species with *L. amazonica* and *L. rufescens* are discussed.

Keywords

Alagoas, Brown recluse spider, Caatinga, Cave, Rio Grande do Norte

Introduction

Loxosceles Heinecken & Lowe, 1832 is a speciose spider genus with a core distribution in the New World (World Spider Catalog 2016). Several species are known also from Africa, Middle East, Mediterranean Europe and two species from China were recently described (World Spider Catalog 2016). Many species were reported as causing bites of importance to human health and several studies on their venom have been published (Gertsch 1967, Tambourgi et al. 2000, Isbister and Fan 2011). Despite this, the taxonomy of the genus is poorly understood. The most comprehensive works were done by Gertsch (1958, 1967) and Gertsch and Ennik (1983) who revised New World species. After these revisions, other species were sporadically described and more recently the African, Middle East and Asian species received more attention (Binford et al. 2008, Duncan et al. 2010, Lotz 2012, Planas and Ribera 2015, Wang 1994).

The South American *Loxosceles* were revised by Gertsch (1967), who created four groups of species: *laeta* with 26 species, *spadicea* with three species, *gaucho* with six species and *amazonica* with a single species. *Loxosceles amazonica* Gertsch, 1967 has been recorded from localities in the Amazon in Brazil, and Peru to northeastern Brazil. More recently, the natural distribution in the New World has been questioned, due to the strong morphological resemblance to the Old World species, mainly with *Loxosceles rufescens* (Dufour, 1820) (Binford et al. 2008; Duncan et al. 2010). Molecular analyses has also retrieved *L. amazonica* to be closely related to the Old World species (Binford et al. 2008; Duncan et al. 2010), therefore, *L. amazonica* origin and its relationship is still up for debate.

Herein, we describe two new species closely related to *L. amazonica* from northeastern Brazil. The relationship of these new species with *L. amazonica* and *L. rufescens* is discussed.

Materials and methods

The general format of the description follows Gertsch (1967). All measurements are in millimeters. Measurements of the legs and palp were taken from the dorsal aspect of the left side (unless appendages were lost or obviously regenerated) with a Mitutoyo® digital caliper, which had an error of 0.005 mm, rounded up to two significant decimals. Structures from the left side of the specimens were chosen for descriptions. When using structures from the right side, the figures were mirrored to show them as coming from the left side and allowing easy comparison. The copulatory organs of females were dissected and submitted to digestion by a commercial protein remover for contact lenses (with pancreatin) during some minutes in order to observe the internal structure; when necessary, they were also cleared with clove oil. A Leica LAS Montage and LAS 3D module mounted on a Leica M205C dissecting microscope were used for image capture and measurements of other spider structures.

Abbreviations

ALE anterior lateral eye,
ESEC Ecological Station,
FLONA National Forest,
PARNA National Park,
PLE posterior lateral eye,
PME posterior median eye.

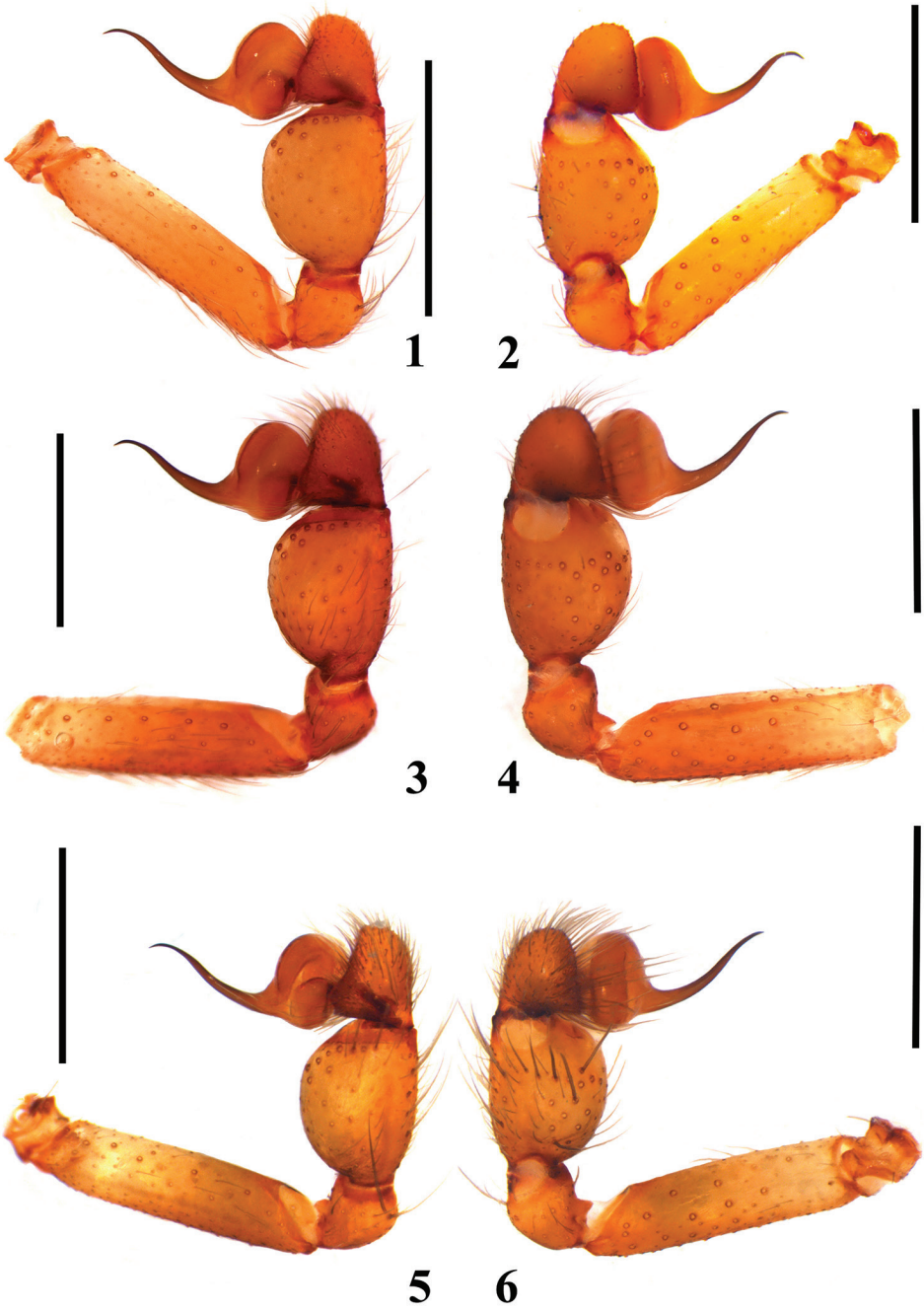
The examined specimens are deposited at **MNRJ**, Museu Nacional, Rio de Janeiro, and **AMNH**, American Museum of Natural History, New York. Geographical coordinates are denoted as primary sources between round brackets, secondary sources (Google Earth) between square brackets. The coordinates from the secondary source were obtained from the center of the municipality cited in the specimen label and are in DMS (Degrees, Minutes and Seconds) format rounded off to minutes. Maps were made with SimpleMappr, an online tool used to produce maps (Shorthouse 2010).

Taxonomy

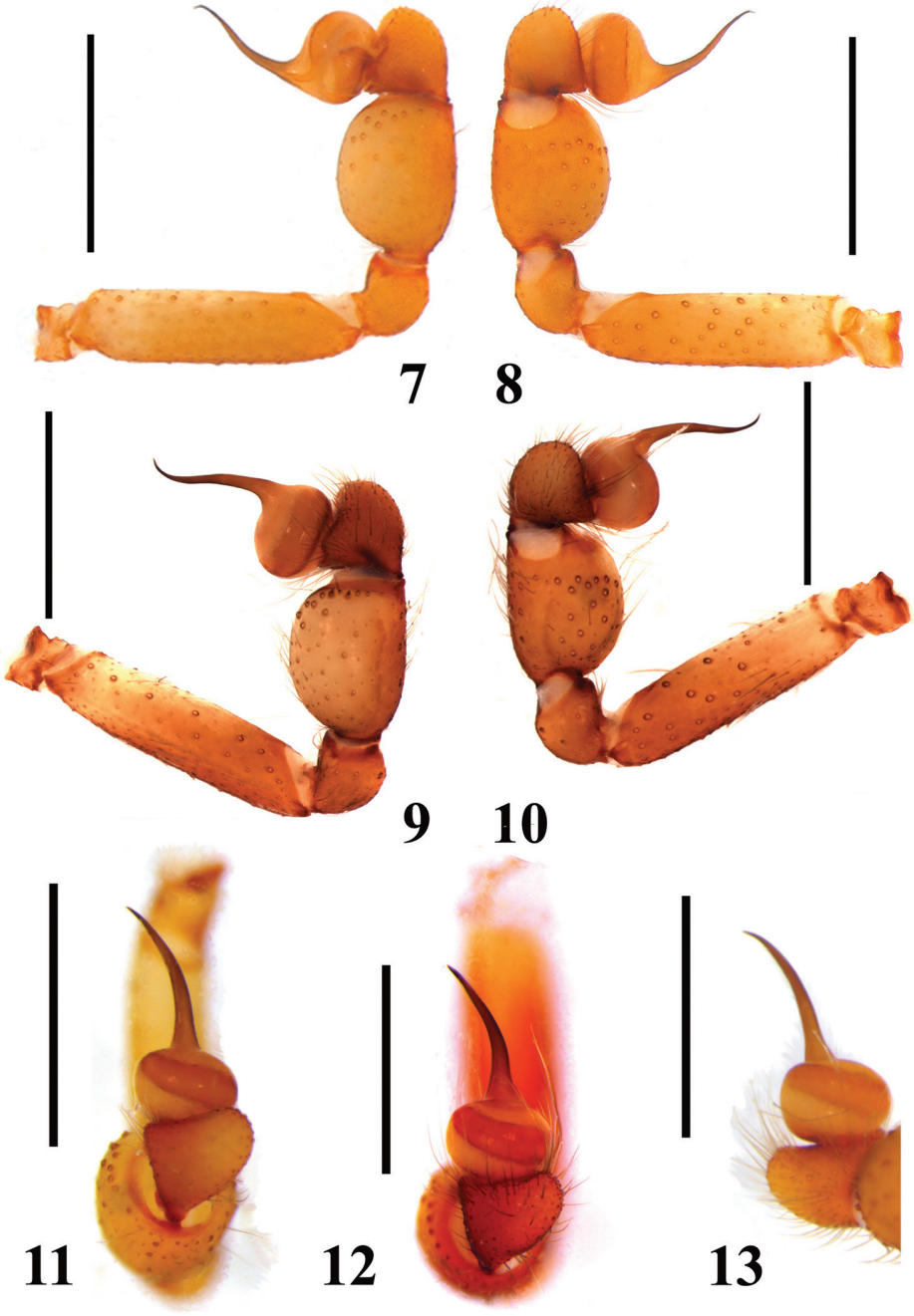
***Loxosceles amazonica* Gertsch, 1967**
Figs 1–51, 78–79

Loxosceles amazonica Gertsch, 1967: 143, pl. 4, figs 7–10, pl. 5, figs 6–7 (female holotype examined (AMNH), Brazil, state of Mato Grosso, Santa Isabel, Araguaia river, Mato Grosso side, 15–25 July 1957, B. Malkin col., receptacles not in the vial); Lucas, Cardoso and Moraes 1986: 130, figs 3–4; Duncan et al. 2010: 241, fig. 3; World Spider Catalog 2016.

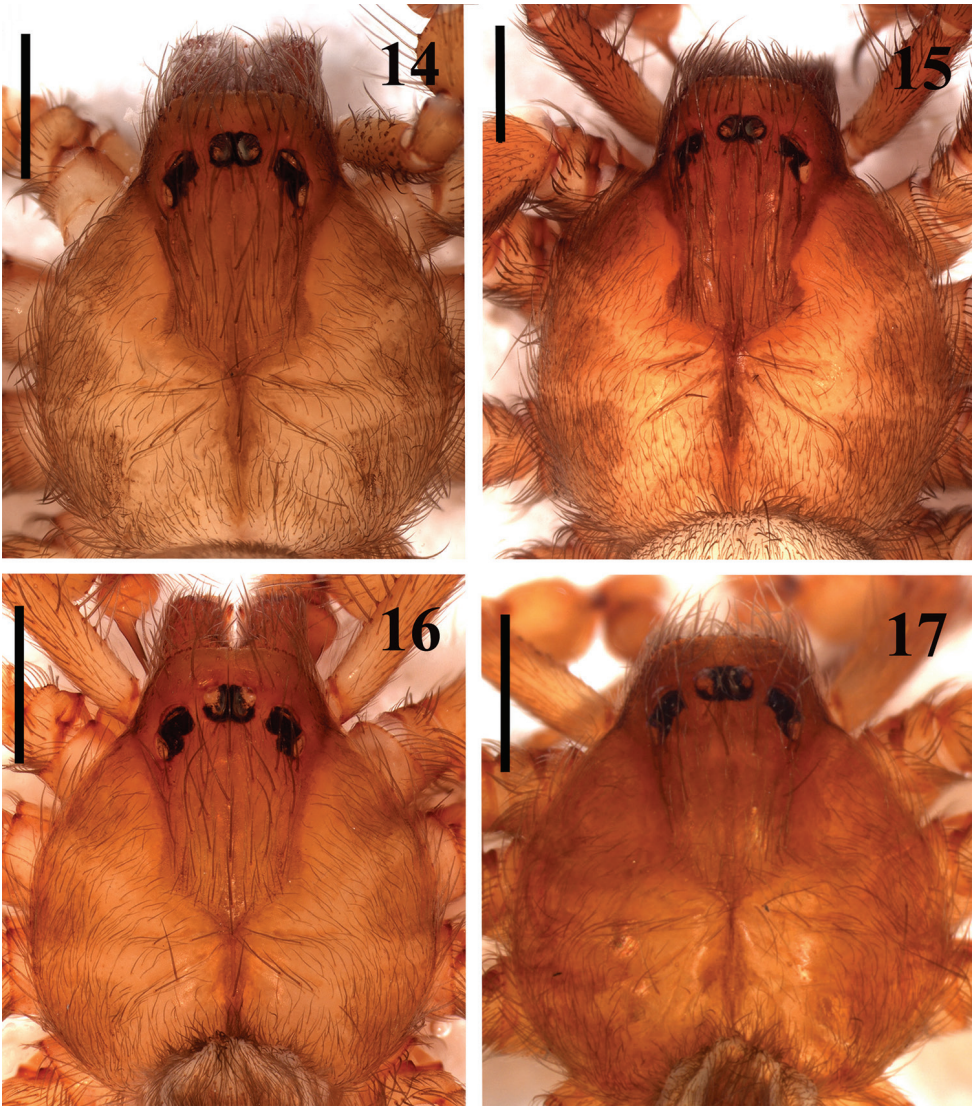
Material examined (Table 3). BRAZIL: *Piauí*, Serra Branca, Parque Nacional Serra da Capivara, São Raimundo Nonato [9°00'S, 42°41'W], 1 male, 1 female and 11 immatures, R. M. Gonçalves Andrade col. (MNRJ 6927); *Rio Grande do Norte*: Serra Negra do Norte, ESEC Seridó (6°34'S, 37°15'W), 2 females and 5 males, C. S. Fukushima, K. C. T. Riciluca and N. M. Gonçalves col., 14 March 2014, ref. Ser 8, 12, 2, 7, 9, 10, 33, respectively (MNRJ 6928); 1 female, under tree bark, during the night, C. S. Fukushima col., 14 March 2014, ref. C28 (MNRJ 6929); 1 female, C. S. Fukushima col., 14 March 2014, inside tree trunk, during the day, ref. C44 (MNRJ 6930); 1 male, C. S. Fukushima col., 14 March 2014, ref. C41 (MNRJ 7303); Açu, FLONA de Açu (5°34'S, 36°56'W), 1 female, under old house debris, during the night, L. Monteiro col., 30 October 2014, ref. L72 (MNRJ 6931); 1 female, under tree bark, during the day, C. S. Fukushima col., 30 October 2014, ref. C599 (MNRJ 6932); 1 female, near Carnaúba trees, during the day, K. C. T. Riciluca col., 26 March 2014, ref. K137 (MNRJ 6933); 1 female, in a vacated old house during the night, C. S. Fukushima col.,



Figures 1–6. *Loxosceles amazonica*, male palpal bulbs. **1–2** Serra Negra do Norte, ESEC Seridó, state of Rio Grande do Norte, Brazil (MNRJ 6928, ref. Ser 7), left palp. **1** retrolateral **2** prolateral **3–4** Açu, FLONA de Açu, state of Rio Grande do Norte, Brazil (MNRJ 6939), left palp **3** retrolateral **4** prolateral **5–6** Martins, state of Rio Grande do Norte, Brazil (MNRJ 7306), right palp (mirrored) **5** retrolateral **6** prolateral. Scale bars: 1mm.

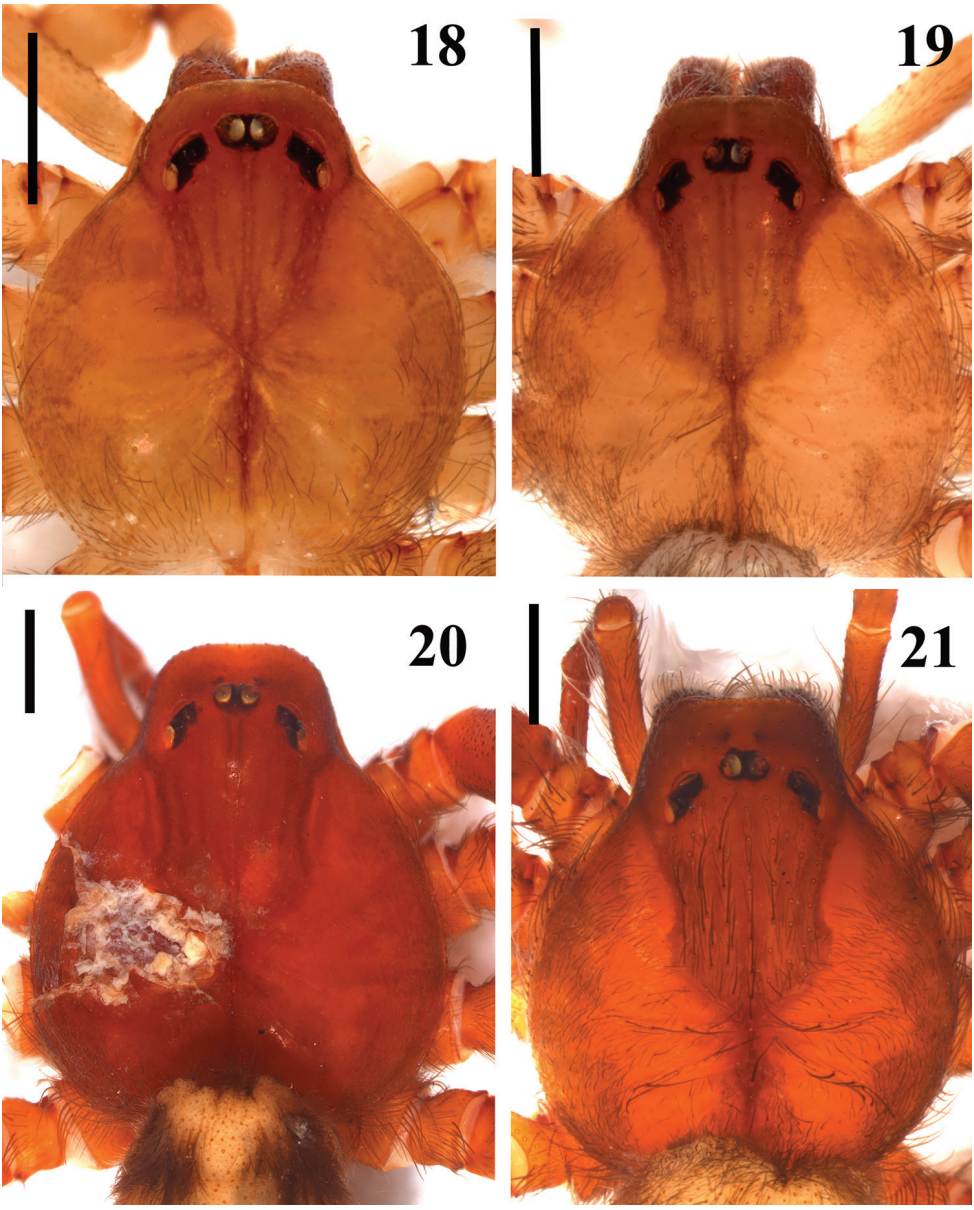


Figures 7–13. *Loxosceles amazonica*, male palpal bulbs, left palp. **7–8** Santa Quitéria, state of Ceará, Brazil (MNRJ 6950) **7** retrolateral **8** prolateral **9–10** São Raimundo Nonato, state of Piauí, Brazil (MNRJ 6927, ref. GSB11A-17) **9** retrolateral **10** prolateral. **11–13** dorsal **11** Açu, FLONA de Açu, state of Rio Grande do Norte, Brazil (MNRJ 6936) **12** Serra Negra do Norte, ESEC Seridó, state of Rio Grande do Norte, Brazil (MNRJ 6928, ref. Ser 7) **13** Santa Quitéria, state of Ceará, Brazil (MNRJ 6950). Scale bars: 1mm.



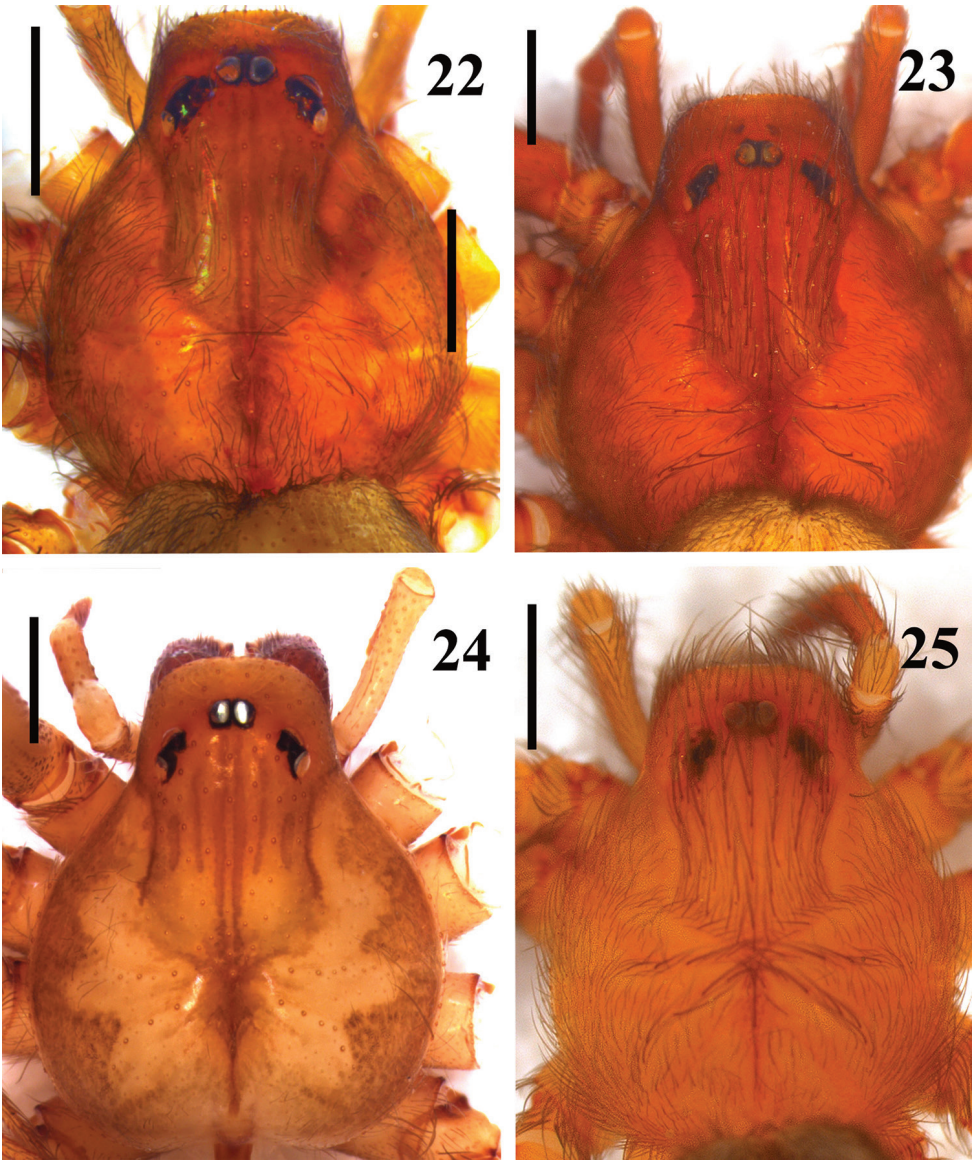
Figures 14–17. *Loxosceles amazonica*, male carapace. **14–15** Açu, FLONA de Açu, state of Rio Grande do Norte, Brazil **14** MNRJ 6935 **15** MNRJ 6936 **16** Serra Negra do Norte, ESEC Seridó, state of Rio Grande do Norte, Brazil (MNRJ 6928, ref. Ser 7) **17** Martins, state of Rio Grande do Norte, Brazil (MNRJ 6947). Scale bars: 1mm.

23 March 2014, ref. C163 (MNRJ 6934); 1 male, under roof tiles, C. S. Fukushima col., 23 March 2014, ref. C167g (MNRJ 6935); 1 male, under roof tiles, C. S. Fukushima col., 23 March 2014, ref. C167o (MNRJ 6936); 1 male, under roof tiles, C. S. Fukushima col., 30 October 2014, ref. C631 (MNRJ 6937); 1 male, in fallen Carnaúba tree, during the night, N. M. Gonçalves col., 25 March 2014, ref. N186 (MNRJ 6938); 1 male, under roof tiles, during the night, C. S. Fukushima col., 23 March 2014, ref.



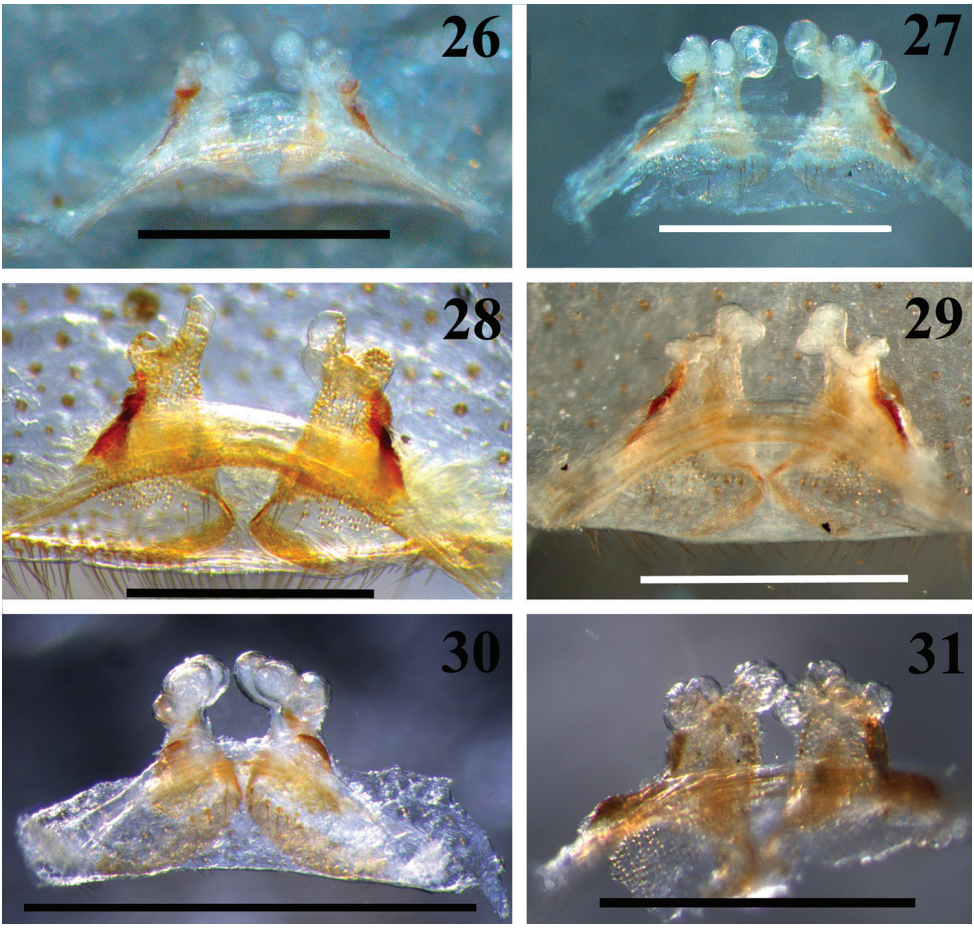
Figures 18–21. *Loxosceles amazonica*, carapace. **18–19** Male **18** São Raimundo Nonato, state of Piauí, Brazil (MNRJ 6927, ref. GSB11A-17) **19** Santa Quitéria, state of Ceará, Brazil (MNRJ 6950) **20–21** Female **20** holotype, Santa Isabel, state of Mato Grosso, Brazil (AMNH) **21** Açu, FLONA de Açu, state of Rio Grande do Norte, Brazil (MNRJ 7305). Scale bars: 1mm.

XXXI (MNRJ 6939); 1 female, K. C. T. Riciluca col., March 2014, ref. K133 (MNRJ 7305); Martins (6°04'S, 37°54'W), 1 female, Mirante-Casa de Pedra cave track, during the night, C. S. Fukushima col., 20 March 2014, ref. C144 (MNRJ 6940); 1 female, near Casa de Pedra cave, during the day, N. M. Gonçalves col., 19 March 2014, ref.



Figures 22–25. *Loxosceles amazonica*, carapace, female. **22** Martins, state of Rio Grande do Norte, Brazil (MNRJ 7304) **23** Serra Negra do Norte, ESEC Seridó, state of Rio Grande do Norte, Brazil (MNRJ 6928, ref. Ser 8) **24** Santa Quitéria, state of Ceará, Brazil (MNRJ 6952) **25** São Raimundo Nonato, state of Piauí, Brazil (MNRJ 6927, ref. GSB11A-17). Scale bars: 1mm.

N81 (MNRJ 6941); 1 female, Mirante-Casa de Pedra cave track, during the day, N. M. Gonçalves col., 20 March 2014, ref. N91 (MNRJ 6942); 1 female, under fallen tree, near grange of Sr. Clesinho, during the day, A. P. L. Giupponi col., 23 October 2014, ref. A132 (MNRJ 6943), 1 female, near Casa de Pedra cave, under rock, during



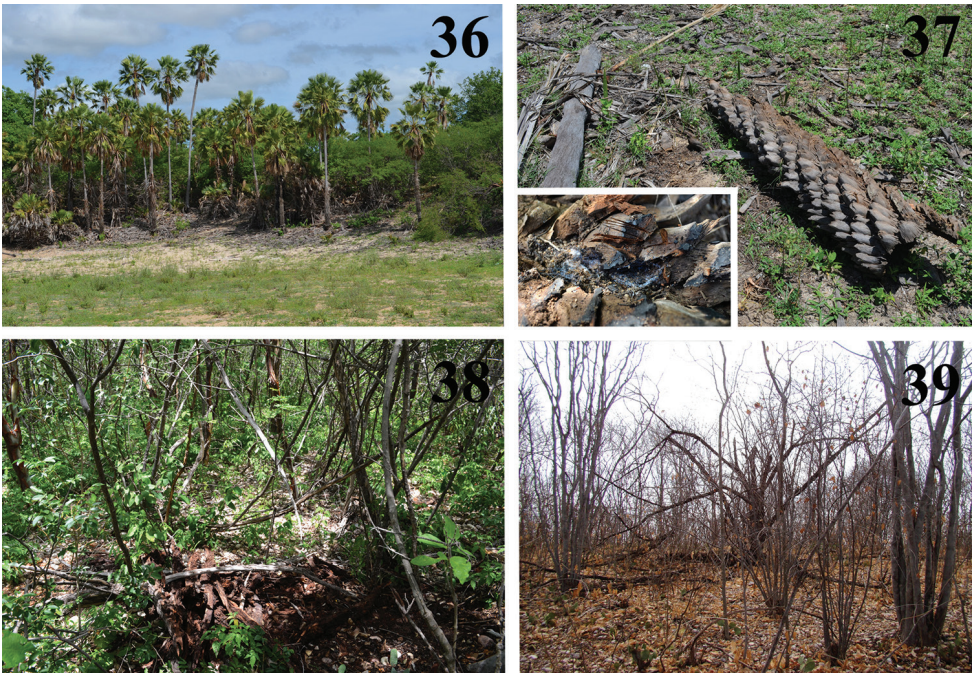
Figures 26–31. *Loxosceles amazonica*, seminal receptacles. **26** Martins, state of Rio Grande do Norte, Brazil (MNRJ 6942) **27** São Raimundo Nonato, state of Piauí, Brazil (MNRJ 6927, ref. GSB11A-17) **28** Serra Negra do Norte, ESEC Seridó, state of Rio Grande do Norte, Brazil (MNRJ 6928, ref. Ser 8) **29** Açu, FLONA de Açu, state of Rio Grande do Norte, Brazil (MNRJ 6931) **30** Santa Quitéria, state of Ceará, Brazil (MNRJ 6952) **31** Macaíba, state of Rio Grande do Norte, Brazil (MNRJ 6949). Scale bars: **27–29** 1 mm; **26, 30–31** 0.5 mm.

the night, C. S. Fukushima col., 23 October 2014, ref. C495 (MNRJ 6944); 1 male, in a ravine near Casa de Pedra cave, during the night, K. C. T. Riciluca col., 19 March 2014, ref. K59 (MNRJ 6945); 1 male, near Casa de Pedra cave, during the day, C. S. Fukushima col., 19 March 2014, ref. C103 (MNRJ 6946); 1 male, in a ravine, C. S. Fukushima col., 19 March 2014, ref. C116 (MNRJ 6947); 1 female, near Casa de Pedra cave, C. S. Fukushima col., 23 October 2014, ref. C497; 1 male, Mirante-Casa de Pedra cave track, C. S. Fukushima col., 20 March 2014, ref. C148 (MNRJ 7306); Macaíba, Escola Agrícola de Jundiaí (5°53'S, 35°21'W), 1 male (MNRJ 6948) and 1 female (MNRJ 6949), in a tree trunk during the night, C. S. Fukushima and W. Pes-

Figures 32–35. *Loxosceles amazonica*, habitus. **32–34** Female **32** Martins, state of Rio Grande do Norte, Brazil **33** Açu, FLONA de Açu, state of Rio Grande do Norte, Brazil **34** Macaíba, state of Rio Grande do Norte, Brazil **35** Male. Açu, FLONA de Açu, state of Rio Grande do Norte, Brazil (MNRJ 6936). Photos C. S. Fukushima.

soa col., 13 September 2013 (ref. AV046, AV047, respectively); *Ceará*, Santa Quitéria (4°19'S, 40°09'W), 1 male and 1 immature male, D. R. Pedroso col., 3–12 February 2014 (MNRJ 6950); 1 male, 1 female and 9 immatures, Gruta W13, SAD'69, Camp 1, F. Pellegatti & D. R. Pedroso col., 3–13 February 2014 (MNRJ 6952).

Diagnosis. Males of *L. amazonica* resemble those of *Loxosceles rufescens*, *Loxosceles bentejui* Planas & Ribera, 2015, *Loxosceles foutadjalloni* Millot, 1941, *Loxosceles guayota* Planas & Ribera, 2015, *Loxosceles hupalupa* Planas & Ribera, 2015, *Loxosceles lacta* Wang, 1994, *Loxosceles mahan* Planas & Ribera, 2015, *Loxosceles tazarte* Planas & Ribera, 2015, *Loxosceles tibicena* Planas & Ribera, 2015, *Loxosceles willianilsoni* sp. n., and *Loxosceles muriciensis* sp. n. by incrassated palpal tibia, longer than cymbium (Figs 1–2). They differ from those of *L. hupalupa*, *L. mahan* and *L. tazarte* by having shorter embolus (Figs 1–2), and entire pars cephalica as well as carapace border dark brown (Fig. 14), best seen in live specimens. From those of *L. rufescens*, *L. bentejui*, *L. foutadjalloni*, *L. guayota*, *L. lacta*, *L. tibicena*, *L. willianilsoni* sp. n. and *L. muriciensis* sp. n., they can be distinguished by having embolus with a mild retrolateral curvature along its length (Fig. 11). Females of *L. amazonica* resemble those of *L. rufescens*, *L. bentejui*, *L. foutadjalloni*, *L. hupalupa*, *L. lacta*, *L. mahan*, *L. tazarte*, *L. tibicena*, *L. willianilsoni* sp. n. and *L. muriciensis* sp. n.

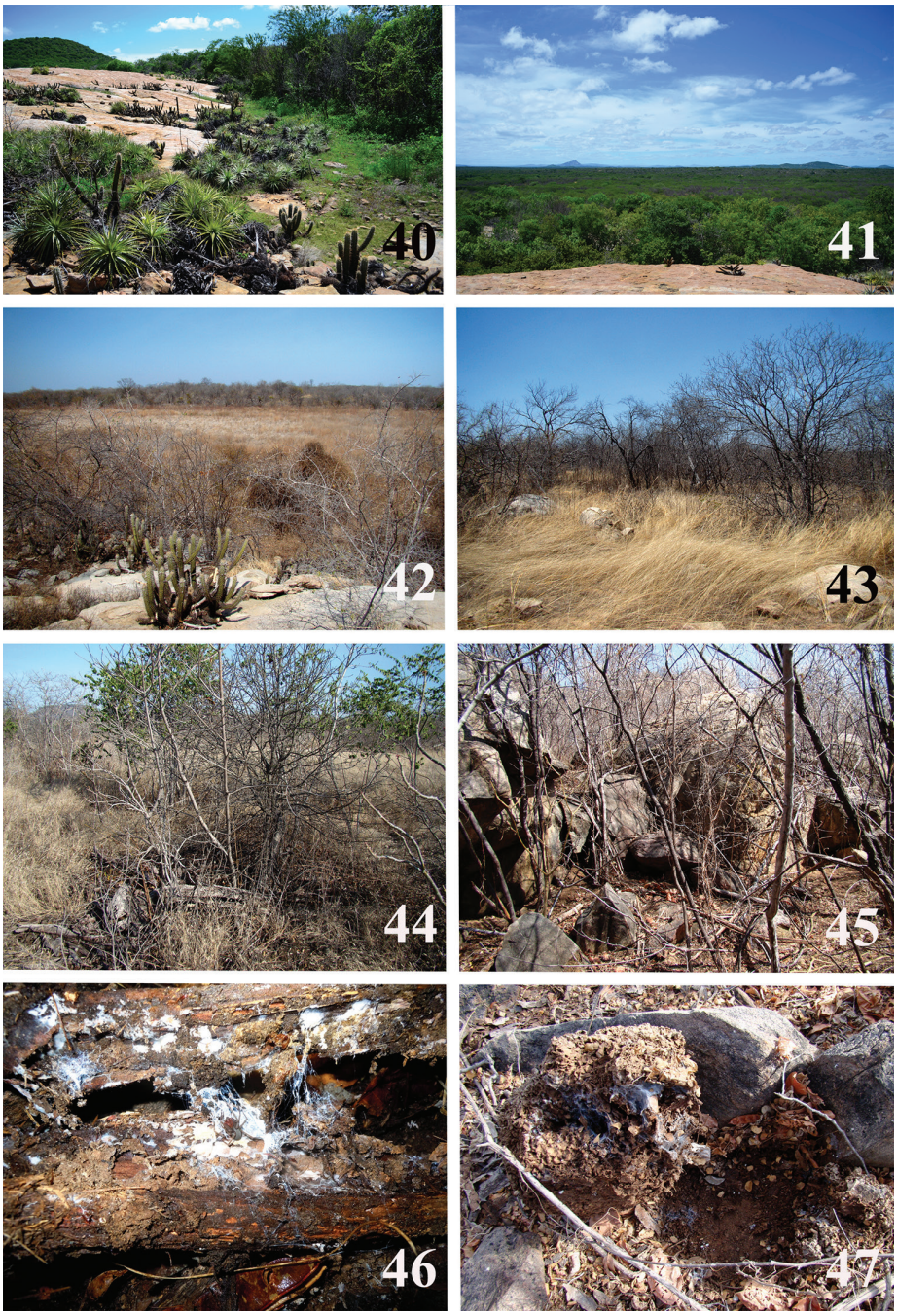


Figures 36–39. *Loxosceles amazonica* habitats in FLONA de Açu, Açu, state of Rio Grande do Norte, Brazil **36** Carnaúba trees **37** fallen Carnaúba tree, in detail web of *L. amazonica* **38** caatinga vegetation in rainy season **39** caatinga vegetation in dry season. Photos C. S. Fukushima.

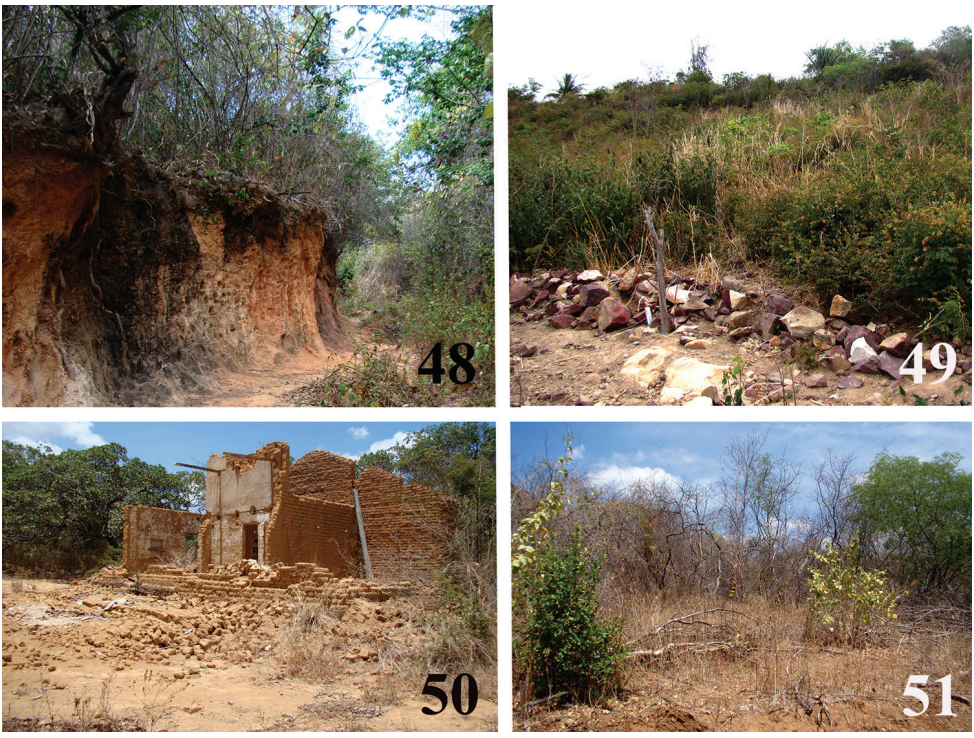
by having spermathecae with large seminal receptacles and dark sclerotized lateral bands (Fig. 26). Females of *L. amazonica* can be distinguished from all these species by a cluster of globular lobes on apex of seminal receptacles (Figs 26–31). Additionally, *L. amazonica* males and females can be distinguished from *L. mahan*, *L. tazarte*, *L. bentejui*, *L. guayota*, *L. tibicena* and *L. hupalupa* by lacking a conspicuous dark V-mark posteriorly on pars cephalica.

Natural history. Despite its specific epithet, *L. amazonica* specimens were found in areas covered by caatinga (Figs 36–47), a semi-arid vegetation found in northeastern Brazil (Fig. 78). At FLONA de Açu, specimens were found under rocks and tree bark, and also under or inside fallen trees, especially carnaúbas (*Copernicia prunifera* Miller) (Figs 36–39). They were also found at vacant old houses inside an area of conservation unit, and under house debris near the FLONA's base.

The ESEC Seridó is located on a *sui generis* region of the state of Rio Grande do Norte characterized by a hyper-xerophilous, arboreal-shrubby caatinga, with irregular precipitation of 500 to 800 mm/year (Varella-Freire 2002). Specimens of *L. amazonica* were found throughout different landscapes of the ESEC (Figs 40–43). They were found under rocks and tree bark in shaded areas (Fig. 44), inside termite nests (Fig. 47) or cracks of rocky outcrops (Fig. 45), under fallen trees (Fig. 46) or under house debris near ESEC's base.



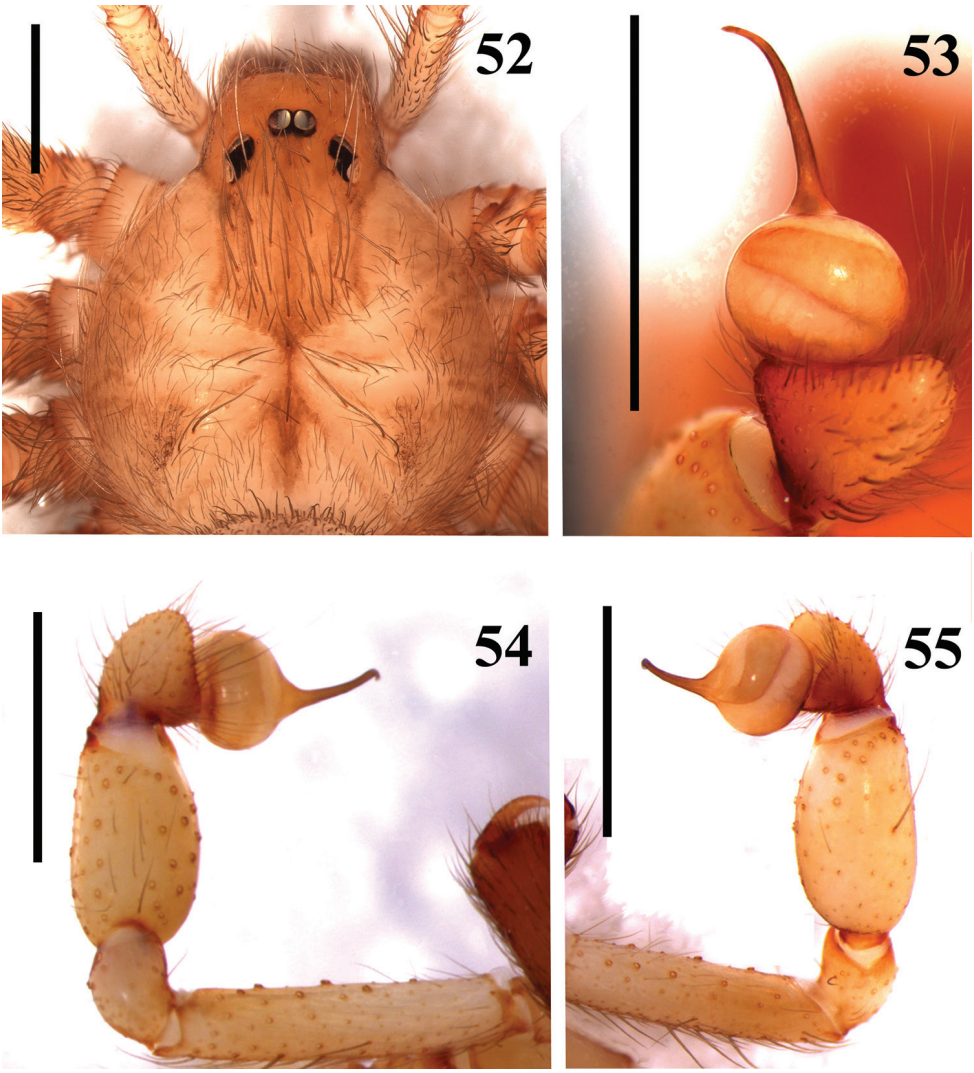
Figures 40–47. *Loxosceles amazonica* habitats in ESEC Seridó, Serra Negra do Norte, state of Rio Grande do Norte, Brazil. **40** large rocky outcrops **41** hyper-xerophilous, arboreal-shrubby caatinga in rainy season **42** dry temporary lagoon **43** grass areas over neosoil **44** fallen dead tree trunk in shaded area **45** small rocky outcrops **46** web of *L. amazonica* inside rotten tree trunk **47** web of *L. amazonica* inside termite nest. Photos C. S. Fukushima.



Figures 48–51. *Loxosceles amazonica* habitats in Martins, state of Rio Grande do Norte, Brazil **48** ravine in a humid area near town **49** under rocks at Mirante-Casa de Pedra cave trail **50** under debris of old house in rural area **51** in caatinga vegetation close to Casa de Pedra cave. Photos C. S. Fukushima.

Specimens of *L. amazonica* were also found in Martins, state of Rio Grande do Norte, "a brejo de altitude" region, i.e. an area covered by humid forest surrounded by arid caatinga (Pereira Filho and Montingelli 2011), usually over mountains and hillsides with an elevation of more than 500 m (Ruiz-Esparza 2009) and that receives more than 1,200 mm of orographic rains (Prado 2003, in Ruiz-Esparza 2009). We found specimens of *L. amazonica* in ravines near the town (Fig. 48), in a trail on the top on the hill (Fig. 49) and under old house debris close to more humid and higher areas (about 700 m a.s.l.) (Fig. 50), as well as under rocks and tree bark near Casa de Pedra cave, in a lower region with caatinga vegetation (about 300 m a.s.l.) (Fig. 51). No specimens were found inside Casa de Pedra cave.

Spermatheca variation (see Fig. 79). Specimens vary in number and size of globular lobes on spermatheca apex and seminal receptacles proportions. Specimens from Martins and Macaíba in the State of Rio Grande do Norte (Figs 26 and 31, respectively), São Raimundo Nonato, state of Piauí (Fig. 27) and Santa Quitéria, state of Ceará (Fig. 30) have three to six lobes in each spermatheca, more or less similar in size. The seminal receptacles of specimens of these areas are slightly short and trapezoid. On the other hand, specimens of ESEC Seridó and FLONA de Açu, both in the state



Figures 52–55. *Loxosceles willianilsoni* sp. n., male holotype (MNRJ 6953). **52** carapace **53–55** left palpal bulb **53** dorsal **54** prolateral **55** retrolateral. Scale bar 1mm.

of Rio Grande do Norte (Figs 28 and 29, respectively) have four to five lobes, usually one of them larger than the others. The seminal receptacles are slightly longer, with a triangular shape.

It is not clear how these genitalic traits vary along the distribution of *L. amazonica* or if these variations reflect a higher diversity in *amazonica* lineage. Variation in the morphology of palps and spermatheca of other *Loxosceles* species has already been noted, such as in *L. rufescens* (Brignoli 1969). However, Duncan et al. (2010) recovered a monophyletic group of specimens that morphologically resemble *L. rufescens*, within which there are divergent clusters of specimens and populations, but with genetic

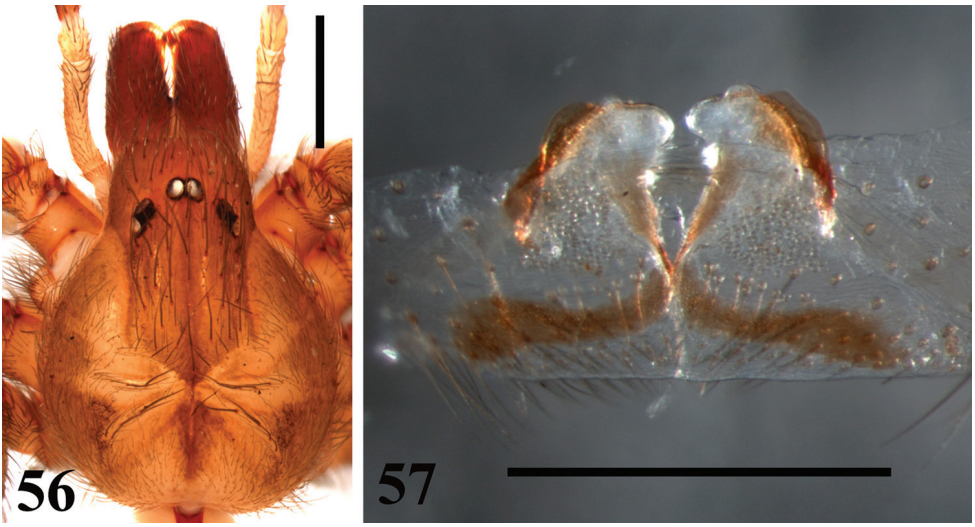
distances high enough to be considered as cryptic species. In the same way, the slight morphological variations in *L. amazonica* could correspond to separated species, only detectable through a molecular approach, which was beyond the scope of this study.

***Loxosceles willianilsoni* sp. n.**
http://zoobank.org/DE5FF5FD-1637-461A-ACBD-93A670CC6E1F
Figs 52–69, 78–79

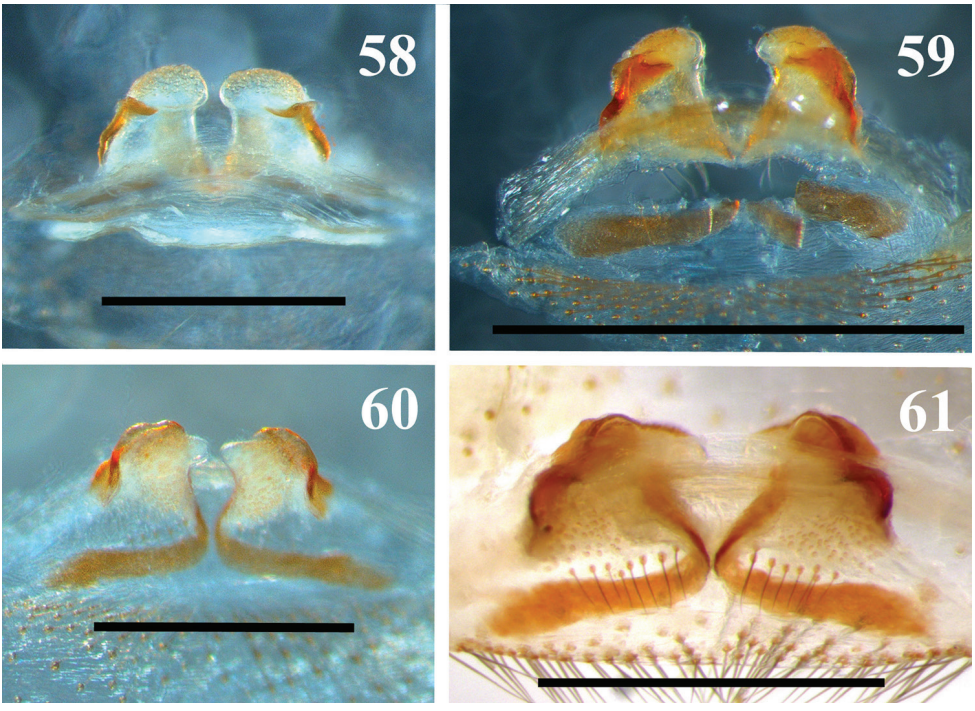
Material examined (Table 3). Male holotype (MNRJ 6953) and female paratype (MNRJ 6954), BRAZIL: *Rio Grande do Norte*, Martins, Casa de Pedra cave (06°05'S, 37°55'W), C. S. Fukushima col., 2014.

Other material examined (Table 3). Casa de Pedra cave (06°05'S, 37°55'W), 319 m a.s.l., 1 female, A. P. L. Giupponi col., 2014, ref. A100 (MNRJ 6955); 1 female, N. M. Gonçalves col., 2014, ref. N60 (MNRJ 6956); 1 female, N. M. Gonçalves col., 2014, ref. N63 (MNRJ 6957); 1 female, C. S. Fukushima col., 2014, ref. C92 (MNRJ 6958); 1 female, C. S. Fukushima col., 2014, ref. C481 (MNRJ 6959); 1 male, N. M. Gonçalves col., 2014, ref. N59 (MNRJ 6960); 1 male, A. P. L Giupponi col., 2014, ref. A107 (MNRJ 6961); 1 male, C. S. Fukushima col., ref. C76 (MNRJ 6962); 1 male, K. C. T. Riciluca col., 2014, ref. K33 (MNRJ 6963); 1 male, A. P. L. Giupponi col., 2014, ref. A102 (MNRJ 6964); 1 male, C. S. Fukushima col., 2014, ref. C64 (MNRJ 6965); 1 male, C. S. Fukushima col., 2014, ref. C72 (MNRJ 6966), 1 female, C. S. Fukushima col, 2014, ref. C479 (MNRJ 6951).

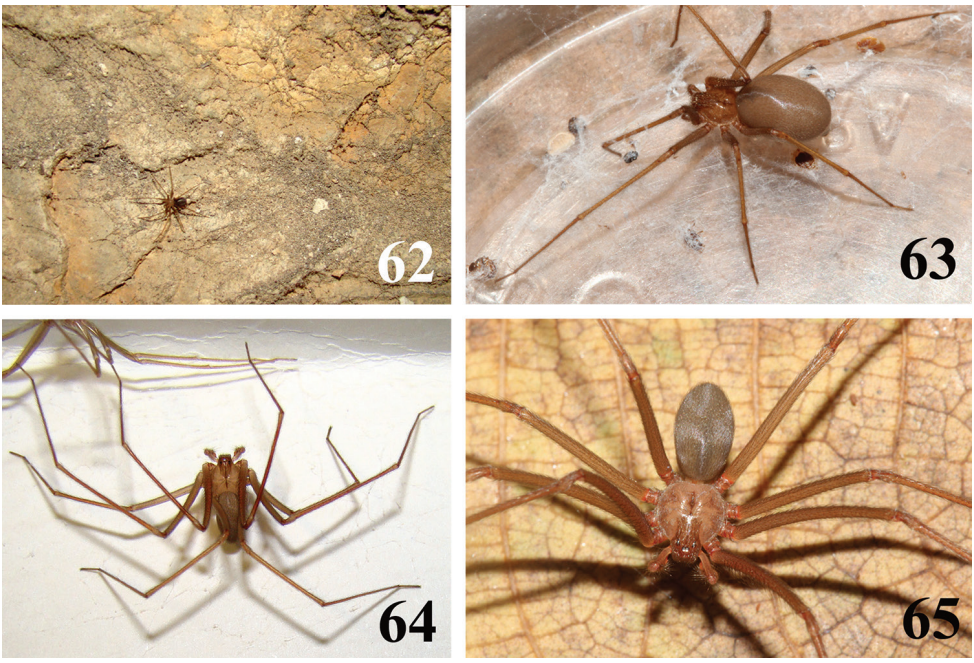
Diagnosis. Males of *Loxosceles willianilsoni* sp. n. resemble those of *L. amazonica, L. rufescens*, *L. bentejui*, *L. foutadjalloni*, *L. guayota*, *L. hupalupa*, *L. lacta*, *L. mahan*, *L. tazarte*, *L. tibicena*, and *L. muriciensis* sp. n. by incrassated palpal tibia, longer than cymbium (Fig. 54). They differ from those of *L. hupalupa*, *L. mahan* and *L. tazarte* by having shorter embolus (Fig. 54), and entire pars cephalica as well as carapace border dark brown (Fig. 52), best seen in live specimens. From those of *L. amazonica, L. rufescens*, *L. bentejui*, *L. foutadjalloni*, *L. guayota*, *L. lacta*, *L. tibicena*, and *L. muriciensis* sp. n. they can be distinguished by having straight embolus with a strong curvature on its apex (Fig. 53). Additionally, males of *L. willianilsoni* sp. n. differ from those of all these species except *L. foutadjalloni*, *L. guayota*, and *L. muriciensis* sp. n. by having leg I at least eight times as long as carapace (Table 1). Females of *L. willianilsoni* sp. n. resemble females of *L. amazonica, L. rufescens*, *L. bentejui*, *L. foutadjalloni*, *L. hupalupa*, *L. lacta*, *L. mahan*, *L. tazarte*, *L. tibicena*, and *L. muriciensis* sp. n. by having spermathecae with large seminal receptacles and dark sclerotized lateral bands (Fig. 57). Females of *L. willianilsoni* sp. n. can be distinguished from all these species by the combination of the following characters: spermathecae with dark sclerotized lateral bands almost reaching their apex, which has no lobes and no constriction forming a neck (Figs 57–61), leg I at least 6.5 times as long as carapace (Table 2). Additionally, *L. willianilsoni* sp. n. males and females can be distinguished from *L. mahan*, *L. tazarte*, *L. bentejui*, *L. guayota*, *L. tibicena* and *L. hupalupa* by lacking a conspicuous dark V-mark posteriorly on pars cephalica.



Figures 56–57. *Loxosceles willianilsoni* sp. n., female paratype (MNRJ 6954). **56** carapace **57** seminal receptacles. Scale bar: 1 mm.



Figures 58–61. *Loxosceles willianilsoni* sp. n., seminal receptacles variation. **58** MNRJ 6957 **59** MNRJ 6956 **60** MNRJ 6959 **61** MNRJ 6951. Scale bars: **58–60** 1 mm; **61** 0.5 mm.



Figures 62–65. *Loxosceles willianilsoni* sp. n., habitus. **62** specimen walking inside Casa de Pedra cave **63** female **64** male **65** carapace pattern, male. Photos **62, 64** C. S. Fukushima; **63, 65** R. Bertani.

Description. *Male* holotype (MNRJ 6953). Total length 7.39. Carapace 3.16 long, 2.74 wide. Eye sizes and interdistances: ALE 0.15, PME 0.21, PLE 0.18, PME-PLE 0.02, PME-ALE 0.15; clypeus 0.26. Leg formula II, I, IV, III. Legs length: leg I: femur 7.47, patella 0.98, tibia 8.37, metatarsus 8.85, tarsus 1.77, total 27.44; II: 8.29, 1.11, 9.88, 10.95, 1.85, 32.08; III: 6.40, 1.09, 6.23, 7.64, 1.30, 22.66; IV: 7.12, 1.05, 7.08, 8.38, 1.52, 26.15. Palp: femur 1.46 long, 0.31 wide; patella 0.49 long, 0.33 wide; tibia 0.88 long, 0.48 wide; cymbium 0.43 long, 0.42 wide. Labium 0.71 long, 0.38 wide. Sternum 1.78 long, 1.50 wide. Femur I 2.4 times as long, tibia I 2.7 times as long and leg I 8.7 as long as carapace. Palpal femur four times longer than wide, tibia 1.8 times longer than wide, cymbium oval (Fig. 54). Bulb suboval and approximately same size as cymbium. Embolus straight, with a strong curvature on apex, approximately 1.3 times longer than bulb length in retrolateral view, without carina (Fig. 53). Cephalic region of carapace covered by many long setae (Fig. 52). Entire pars cephalica as well as carapace border dark brown (Fig. 52). Legs and palps light brown, covered by short greyish setae on the femora and patellae (Fig. 64). Endites, coxae and sternum light brown. Labium dark brown.

Female paratype (MNRJ 6954): As in male, except: Total length 8.72. Carapace 2.99 long, 2.39 wide. Eye sizes and interdistances: ALE 0.14, PME 0.17, PLE 0.16,

Figures 66–69. *Loxosceles willianilsoni* sp. n. habitat in Martins, state of Rio Grande do Norte, Brazil **66** Casa de Pedra cave **67** entrance of the cave **68–69** caatinga vegetation surrounding the cave **68** dry season **69** rainy season. Photos C. S. Fukushima.

PME-PLE 0.02, PME-ALE 0.19; clypeus 0.35. Leg formula II, I, IV, III. Legs length: leg I: femur 5.25, patella 1.17, tibia 5.93, metatarsus 5.88, tarsus 1.24, total 19.47; II: 5.96, 1.14, 6.40, 6.32, 1.50, 21.32; III: 4.76, 1.00, 4.22, 4.80, 1.19, 15.97; IV: 5.32, 1.15, 4.89, 5.96, 1.40, 18.72. Palp: femur 0.98 long, 0.21 wide; patella 0.28 long, 0.25 wide; tibia 0.70 long, 0.20 wide; tarsus 1.06 long, 0.16 wide. Labium 0.53 long, 0.44 wide. Sternum 1.63 long, 1.38 wide. Femur I 1.8 times as long, tibia I 2.0 times as long and leg I 6.5 as long as carapace. Palpal femur 4.7 times longer than wide, tibia 3.5 longer than wide, tarsus not incrassate. Spermathecae with enlarged seminal receptacles; without transversal plate; and presence of dark sclerotized lateral bands almost reaching the apex (Fig. 57). Palps pale brown, except by darker tibiae and metatarsi. Endites pale brown.

Etymology. This species is named after the biology student Willianilson Pessoa, in honor of his friendship and support during expeditions in Rio Grande do Norte. This name is masculine in gender.

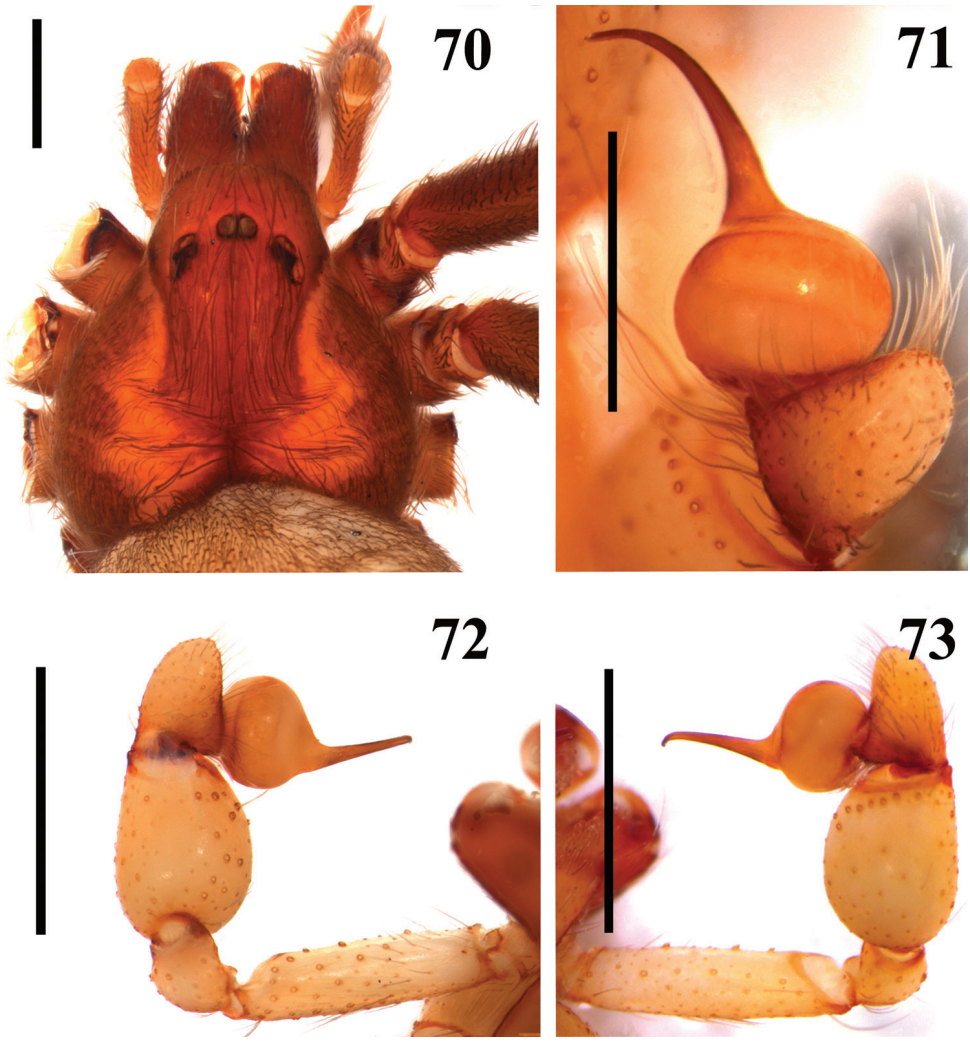
Natural history. Specimens were found inside Casa de Pedra cave walking on walls, in webs inside wall cracks or under loose stones on the cave ground. This calcarian cave is very large regarding regional patterns and has turistic use (Ferreira et al. 2010). Apparently, specimens of *L. willianilsoni* sp. n. are found only inside the cave.

***Loxosceles muriciensis* sp. n.**
http://zoobank.org/CC85E3A6-44F7-4C7C-BCBD-EA9002A7309F
Figs 70–79

Material examined (Table 3). Male holotype (MNRJ 6967) and female and male paratypes (MNRJ 6968), BRAZIL: *Alagoas*, Murici, Estação Ecológica de Murici (9°15'S, 35°48'W), 23.1°C, 84% URA, under the bark of a large burnt tree, R. Bertani, D. R. M. Ortega and R. H. Nagahama col., 13 August 2006.

Diagnosis. Males of *L. muriciensis* sp. n. resemble those of *L. amazonica, L. rufescens*, *L. bentejui*, *L. foutadjalloni*, *L. guayota*, *L. hupalupa*, *L. lacta*, *L. mahan*, *L. tazarte*, *L. tibicena* and *L. willianilsoni* sp. n. by incrassated palpal tibia, longer than cymbium (Fig. 72). Males differ from those of *L. hupalupa*, *L. mahan* and *L. tazarte* by having shorter embolus (Fig. 72), and entire pars cephalica as well as carapace border dark brown (Fig. 70), best seen in live specimens. Males of *L. muriciensis* sp. n. differ from those of *L. amazonica, L. rufescens*, *L. bentejui*, *L. foutadjalloni*, *L. guayota*, *L. lacta*, *L. tibicena*, and *L. willianilsoni* sp. n. by having straight embolus with a mild curvature on apex, forming a hook (Fig. 71). Additionally, males of *Loxosceles muriciensis* sp. n. differ from males of all these species except *L. foutadjalloni*, *L. guayota* and *L. willianilsoni* sp. n. by having leg I at least eight times as long as carapace (Table 1). Females of *L. muriciensis* sp. n. resemble those of *L. amazonica, L. rufescens*, *L. bentejui*, *L. foutadjalloni*, *L. hupalupa*, *L. lacta*, *L. mahan*, *L. tazarte*, *L. tibicena*, and *L. willianilsoni* sp. n. by having spermathecae with large seminal receptacles and dark sclerotized lateral bands (Fig. 75). Females of *L. muriciensis* sp. n. can be distinguished from all these species by the following combination of characters: spermathecae with dark sclerotized lateral bands almost reaching their apex, which has two well-developed lobes, and no constriction forming a neck (Fig. 75); leg I more than five times as long as carapace (Table 2). Additionally, *L. muriciensis* sp. n. males and females can be distinguished from *L. mahan*, *L. tazarte*, *L. bentejui*, *L. guayota*, *L. tibicena* and *L. hupalupa* by lacking a conspicuous dark V-mark posteriorly on pars cephalica.

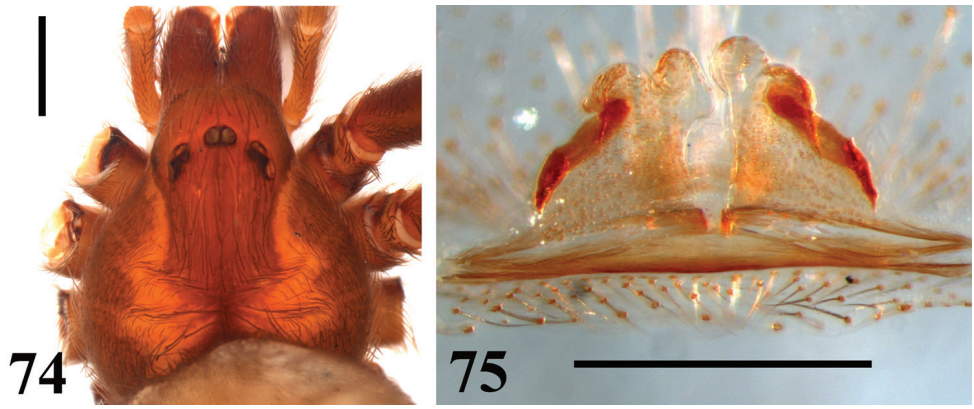
Description. *Male* holotype (MNRJ 6967). Total length 5.46. Carapace 2.21 long, 2.10 wide. Eye sizes and interdistances: ALE 0.12, PME 0.16, PLE 0.16, PME-PLE 0.02, PME-ALE 0.12; clypeus 0.30. Leg formula II, I, IV, III. Legs length: leg I: femur 4.73, patella 0.90, tibia 5.20, metatarsus 5.65, tarsus 1.42, total 17.9; II: 5.15, 0.95, 5.13, 6.39, 1.45, 19.07; III: 4.21. 0.70. 3.73. 4.37. 0.93. 13.94; IV: 4.77. 0.69. 4.55. 5.55. 1.15. 16.71. Palp: femur 1.12 long, 0.30 wide; patella 0.46 long, 0.35 wide; tibia 0.70 long, 0.55 wide; cymbium 0.50 long, 0.35 wide. Labium 0.49 long, 0.33 wide. Sternum 1.23 long, 1.16 wide. Femur I 2.2 times as long, tibia I 2.4 times as long and leg I 8.1 as long as carapace. Palpal femur 3.7 times longer than wide, tibia 1.3 times longer than wide, cymbium oval (Fig. 72). Bulb suboval and larger than cymbium. Embolus straight, with a mild curvature on apex, approximately 1.6 times longer than bulb length in retrolateral view, without carina (Fig. 71). Cephalic region of carapace covered by many long setae (Fig. 70). Entire pars cephalica as well as carapace border dark brown (Fig. 70). Legs and palps light brown, covered by short



Figures 70–73. *Loxosceles muriciensis* sp. n., male holotype. **70** carapace **71–73** right palpal bulb **71** dorsal (mirrored) **72** prolateral (mirrored) **73** retrolateral (mirrored). Scale bars: **70, 72–73** 1mm;**71** 0.5mm.

greyish setae on the femora and patellae. Endites, coxae and sternum light brown. Labium dark brown.

Female paratype (MNRJ 6968): As in male, except: Total length 8.65. Carapace 2.98 long, 2.80 wide. Eye sizes and interdistances: ALE 0.15, PME 0.21, PLE 0.20, PME-PLE 0.05, PME-ALE 0.17; clypeus 0.40. Leg formula II, I, IV, III. Legs length: leg I: femur 4.51, patella 1.13, tibia 4.50, metatarsus 4.35, tarsus 1.45, total 15.94; II: 5.05, 1.06, 5.33, 3.41, 1.30, 16.15; III: 4.25, 1.05, 3.55, 4.30, 1.02, 14.17; IV: 4.55, 0.62, 4.50, 3.45, 1.22, 14.34. Palp: femur 1.20 long, 0.25 wide; patella 0.37 long, 0.31 wide; tibia 0.71 long, 0.25 wide; tarsus 1.07 long, 0.17 wide. Labium 0.58 long, 0.50 wide. Sternum 1.84 long, 1.40 wide. Femur I 1.5 times as long, tibia I 1.5



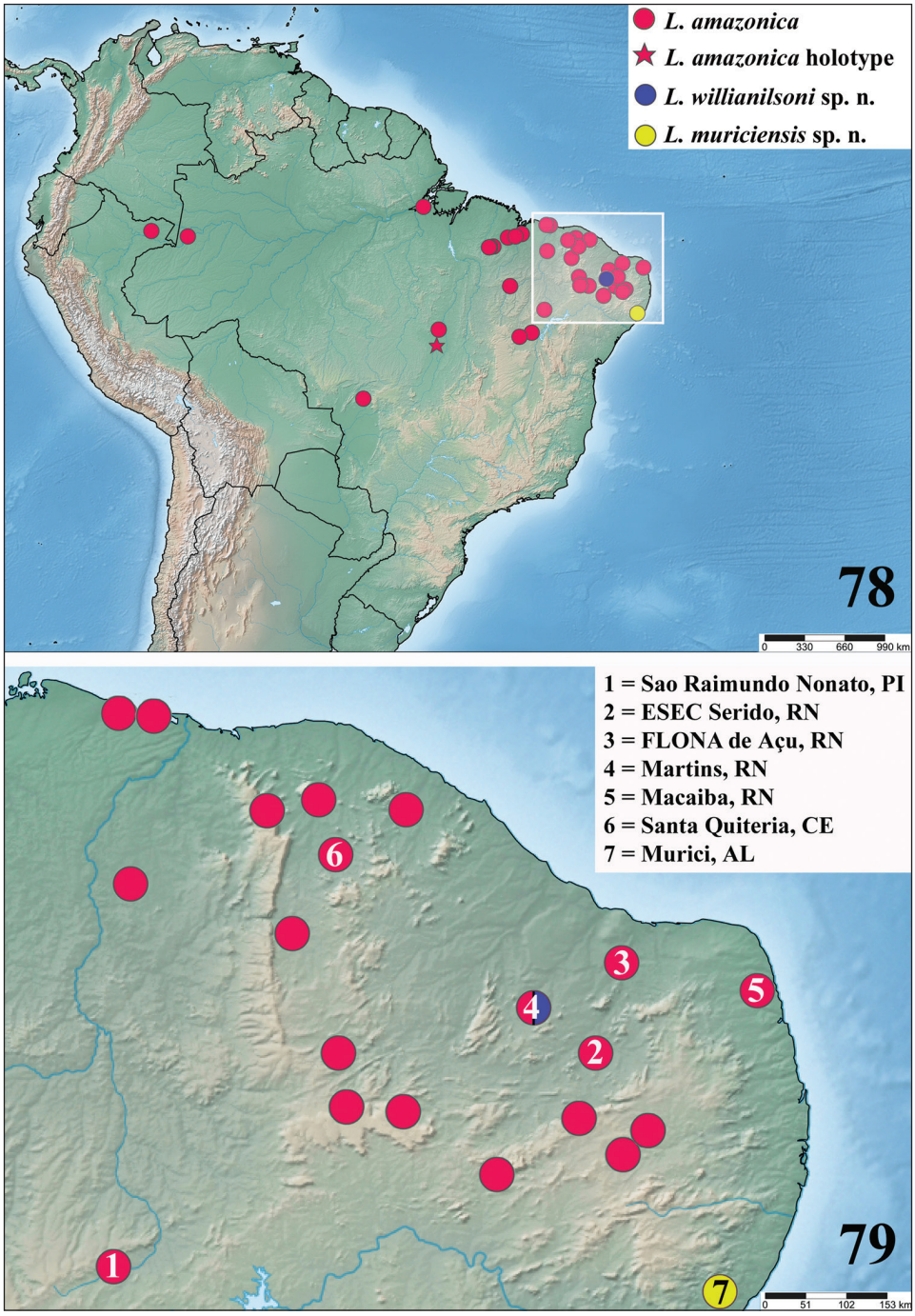
Figures 74–75. *Loxosceles muriciensis* sp. n., female paratype. **74** carapace **75** seminal receptacles. Scale bars: 1 mm.

Figures 76–77. *Loxosceles muriciensis* sp. n. male holotype, habitus. **76** overall aspect **77** carapace pattern. Photos R. Bertani.

times as long and leg I 5.3 as long as carapace. Palpal femur 4.8 times longer than wide, tibia 2.8 longer than wide, tarsus not incrassate. Spermathecae with enlarged seminal receptacles; without transversal plate, lacking a constriction near apex forming a neck; presence of two well-developed lobes on apex and dark sclerotized lateral bands almost reaching apex (Fig.75). Palps brown, except by pale patellae and femora. Endites pale brown.

Etymology. The specific name refers to the type locality, Estação Ecológica de Murici, state of Alagoas, Brazil and is neutral in gender.

Natural history. The few specimens of *L muriciensis* sp. n. were found inside a burnt tree in an Atlantic rainforest conservation unit in the state of Alagoas. The ESEC Murici is one of the last and largest remnants of the northeastern Atlantic rainforest and it is inserted in a biodiversity hotspot known as the "Pernambuco Endemism Center" (Nemésio and Santos Junior 2014).



Figures 78–79. 78 Map showing records of *L. amazonica*, *L. willianilsoni* sp. n. and *L. muriciensis* sp. n. Area inside rectangle represented on Figure 79. Records of *L. amazonica* include also those from Azevedo et al. (2014), Gertsch (1967) and Silveira (2015). **79** Expanded map showing the records of the illustrated specimens of *L. amazonica*, *L. willianilsoni* sp. n. and *L. muriciensis* sp. n.

Table 1. *Loxosceles* spp. of *rufescens* group, males. Carapace and leg I measurements. Data from (1) Gertsch (1967), (2) Lotz (2012), (3) Planas and Ribera (2015). Legs differentiated by less than 0.5 mm are in bold. AL = state of Alagoas, AM = state of Amazonas, CE = state of Ceará, PI = state of Piauí, RN = state of Rio Grande do Norte.

Taxon	Locality	Specimen	Carapace	Leg I	Leg I / Carapace	Leg Formula
L. amazonica [1]	Gurupá (AM), Brazil	paratype	4	19.5	4.88	2, **4**, **1**, 3
L. amazonica	FLONA Açu (RN), Brazil	MNRJ 6936	3.87	22.11	5.72	2, **4**, **1**, 3
L. amazonica	ESEC Seridó (RN), Brazil	MNRJ 7303	3.26	17.57	5.39	2, 4, 1, 3
L. amazonica	Martins (RN), Brazil	MNRJ 6947	3.12	16.94	5.43	2, 4, 1, 3
L. amazonica	Macaíba (RN), Brazil	MNRJ 6948	2.86	15.95	5.58	**2**, **4**, 1, 3
L. amazonica	São Raimundo Nonato (PI), Brazil	MNRJ 6927	2.62	16.39	6.25	2, **4**, **1**, 3
L. amazonica	Santa Quitéria (CE), Brazil	MNRJ 6950	2.76	20.81	7.54	2, **4**, **1**, 3
L. willianilsoni sp. n.	Martins (RN), Brazil	holotype	3.16	27.44	8.69	2, 1, 4, 3
L. muriciensis sp. n.	Murici (AL), Brazil	holotype	2.21	17.9	8.12	2, 1, 4, 3
L. rufescens [1]	Rome, Italy	AMNH	3	20.1	6.70	2, **4**, **1**, 3
L. foutadjalloni [2]	Guinea	lectotype	4	45.9	11.48	2, 1, 4, 3
L. mahan [3]	Canary Islands	holotype	2.89	17.37	6.01	2, **4**, **1**, 3
L. tazarte [3]	Canary Islands	holotype	2.34	15.42	6.59	2, **1**, **4**, 3
L. bentejui [3]	Canary Islands	holotype	2.91	20.63	7.09	2, 1, 4, 3
L. tibicena [3]	Canary Islands	holotype	2.63	20.19	7.68	2, 4, 1, 3
L. guayota [3]	Canary Islands	holotype	3.62	34.78	9.61	2, 1, 4, 3
L. hupalupa [3]	Canary Islands	holotype	2.51	19.51	7.77	2, 4, 1, 3

Discussion

In his revision of the South American *Loxosceles* species, Gertsch (1967) proposed four species groups for the thirty species he recognized. The only group with a single species is *amazonica* with the species *L. amazonica* described in the same paper (Gertsch 1967). This author approximated *L. amazonica* to the *gaucho* group due to the carapace marked with dark lateral bands and some incrassated segments of male palps. On the other hand, the presence of spermathecae with free receptacles with rounded lobes, not closely tied by a transverse band, resembles *laeta* species (Gertsch 1967). Despite *L. amazonica* having characteristics of both South American groups *gaucho* and *laeta*, in some genitalic features it closely resembles species of the *rufescens* group from the Paleartic fauna (Gertsch 1967). Due to these special characteristics, *L. amazonica* was considered to have group status by Gertsch (1967).

After Gertsch's revision (1967), only scattered descriptions of new species of *Loxosceles* were published. A more embracing work was done by Binford et al. (2008), which proposed the first phylogenetic relationship hypothesis concerning representative *Loxosceles* species. In that work, besides morphological similarity, a molecular proximity was detected between *L. amazonica* and *L. rufescens* (Binford et al. 2008). The ubiquitous species *L. rufescens*, associated or not with the Chinese species *L. lacta*, was presented as the sister-group of *L. amazonica* in analyses with different types and combinations of

Table 2. *Loxosceles* spp. of *rufescens* group, females. Carapace and leg I measurements. Data from (1) Gertsch (1967), (2) Lotz (2012), (3) Planas and Ribera (2015). Legs differentiated by less than 0.5 mm are in bold. * = Legs 2 and 4, and legs 4 and 1 have difference in length less than 0.5 mm. AL = state of Alagoas, CE = state of Ceará, MT = state of Mato Grosso, PI = state of Piauí, RN = state of Rio Grande do Norte.

Taxon	Locality	Specimen	Carapace	Leg I	Leg I / Carapace	Leg Formula
L. amazonica	Santa Isabel (MT), Brazil	holotype	4.17	19.04	4.57	2, **4**, **1**, 3
L. amazonica	Açu (RN), Brazil	MNRJ 6933	3.82	17.32	4.53	2, **4**, **1**, 3
L. amazonica	Serra Negra do Norte (RN), Brazil	MNRJ 6928	3.89	17.60	4.52	**2**, **4**, **1**, 3*
L. amazonica	Martins (RN), Brazil	MNRJ 6940	3.83	17.63	4.60	2, **4**, **1**, 3
L. amazonica	Macaíba (RN), Brazil	MNRJ 6949	3.45	14.06	4.08	Missing legs 3 and 4
L. amazonica	São Raimundo Nonato (PI), Brazil	MNRJ 6927	3.08	12.48	4.06	**2**, **4**, **1**, 3*
L. amazonica	Santa Quitéria (CE), Brazil	MNRJ 6952	2.86	16.56	5.79	2, 4, 1, 3
L. willianilsoni sp. n.	Martins(RN), Brazil	paratype	2.99	19.47	6.52	2, 1, 4, 3
L. muriciensis sp. n.	Murici (AL), Brazil	paratype	2.98	15.94	5.34	2, 1, 4, 3
L. rufescens [1]	Alto Douro, Portugal	AMNH	3.2	15.4	4.81	2, 4, 1, 3
L. foutadjalloni [2]	Guinea	paralectotype	3.9	26.8	6.87	2, 1, 4, 3
L. mahan [3]	Canary Islands	paratype	2.97	12.97	4.37	**2**, **4**, 1, 3
L. tazarte [3]	Canary Islands	paratype	2.88	14.65	5.09	2, **4**, **1**, 3
L. bentejui [3]	Canary Islands	paratype	3.35	16.78	5.01	2, **4**, **1**, 3
L. tibicena [3]	Canary Islands	paratype	3.35	18.43	5.50	2, **4**, **1**, 3
L. hupalupa [3]	Canary Islands	paratype	3.71	23.09	6.22	Missing leg 4

datasets (Binford et al. 2008). The authors considered two possible explanations for the strong evidence of a close relationship between these species. In one explanation, the *rufescens* lineage would be old, with the ancestors of both species pre-dating the split of the continents; in the other, the lineage would be younger and it was suggested to be a natural dispersion from South America to Africa after the continent split occurred. According to the authors, the great genetic divergence found between *L. amazonica* and *L. rufescens* and the species diversity of the *rufescens* group in the Old World makes the human-mediated transportation explanation unlikely (Binford et al. 2008). However, the divergence date between *L. amazonica* and *L. rufescens* estimated by Binford et al. (2008) is too young for the presence of the most recent ancestor on Gondwana. Binford et al. (2008) also stated that the current range of *L. amazonica* and *L. rufescens*, northeastern South America and North Africa respectively, is compatible either with the Gondwana ancestor explanation or with dispersal through temporary land corridors after continental split. Thus, the distinction between ancient vicariance and more recent dispersion to explain the relationship of both species would require the inclusion of more species of these related areas in a more extensive analysis (Binford et al. 2008).

A more detailed study of the diversity of the northwestern African *Loxosceles* species and new molecular phylogenetic analyses including *L. rufescens* and *L. amazonica*

Table 3. Localities of all the material studied. F = female, J = juvenile, M= male, MJ= immature male.

Species	Quantity	Number	Locality	Coordinates
L. amazonica	1M, 1F, 11J	MNRJ 6927	PARNA Serra da Capivara, São Raimundo Nonato, Piauí, Brazil	[9°00'S, 42°41'W]
	5M, 2F	MNRJ 6928	ESEC Seridó, Serra Negra do Norte, Rio Grande do Norte, Brazil	(6°34'S, 37°15'W)
	1F	MNRJ 6929		
	1F	MNRJ 6930		
	1M	MNRJ 7303		
	1F	MNRJ 6931	FLONA de Açu, Açu, Rio Grande do Norte, Brazil	(5°34'S, 36°56'W)
	1F	MNRJ 6932		
	1F	MNRJ 6933		
	1F	MNRJ 6934		
	1M	MNRJ 6935		
	1M	MNRJ 6936		
	1M	MNRJ 6937		
	1M	MNRJ 6938		
	1M	MNRJ 6939		
	1F	MNRJ 7305		
	1F	MNRJ 6940	Martins, Rio Grande do Norte, Brazil	(6°04'S, 37°54'W)
	1F	MNRJ 6941		
	1F	MNRJ 6942		
	1F	MNRJ 6943		
	1F	MNRJ 6944		
	1M	MNRJ 6945		
	1M	MNRJ 6946		
	1M	MNRJ 6947		
	1M	MNRJ 7306		
	1F	MNRJ 7304		
	1M	MNRJ 6948	Macaíba, Rio Grande do Norte, Brazil	(5°53'S, 35°21'W)
	1F	MNRJ 6949		
	1M, 1MJ	MNRJ 6950	Santa Quitéria, Ceará, Brazil	(4°19'S, 40°09'W)
	1M, 1F, 9J	MNRJ 6952		
L. willianilsoni sp. n.	1M	MNRJ 6953	Casa de Pedra cave, Martins, Rio Grande do Norte, Brazil	(06°05'S, 37°55'W)
	1F	MNRJ 6954		
	1F	MNRJ 6955		
	1F	MNRJ 6956		
	1F	MNRJ 6957		
	1F	MNRJ 6958		
	1F	MNRJ 6959		
	1M	MNRJ 6960		
	1M	MNRJ 6961		
	1M	MNRJ 6962		
	1M	MNRJ 6963		
	1M	MNRJ 6964		
	1M	MNRJ 6965		
	1M	MNRJ 6966		
	1F	MNRJ 6951		
L. muriciensis sp. n.	1M	MNRJ 6967	Murici, Alagoas, Brazil	(9°15'S, 35°48'W)
	1F, 1M	MNRJ 6968		

was done by Duncan et al. (2010). Once again, *L. amazonica* was recovered in the clade including the northwestern African *Loxosceles* species. However, there was no agreement that *L. amazonica* was the sister-group of the monophyletic *L. rufescens* lineage nor the basal taxa of the northwestern African clade. The lack of resolution inside the northwestern African clade, the existence of African male specimens very similar morphologically to *L. amazonica* and the fact that the most recent common ancestor of *L. amazonica* and *L. rufescens* was found by Binford et al. (2008) to be too young to be explained by Gondwanan vicariance were considered by Duncan et al. (2010) to indicate that *L. amazonica* is derived from within northwest Africa *Loxosceles* and dispersed recently from one continent to other. They proposed that the split of the continents did not influence the distribution of the common ancestor *L. amazonica* and *L. rufescens* (Duncan et al. 2010). They considered *L. amazonica* as a species that can be easily introduced by human transport and suggested the trade between Brazil and Africa in 16th century could explain the dispersal of *L. amazonica* from Africa to South America (Duncan et al. 2010). They also considered the absence of other species related to *L. amazonica* in South America as further evidence supporting an African origin of this species.

The discovery of two new species, herein described, closely related to *L. amazonica* in northwestern Brazil, throw a new light on this discussion. It is very unlikely that *L. amazonica* came from Africa about 500 years ago and in so little time speciated into two more different species. Another point that contradicts the argument that *L. amazonica* was introduced in South America is the large distribution of the species (Fig. 78). It is very improbable that such a reclusive spider would disperse to many natural localities throughout northwestern Brazil in such a short period of time, reaching remote localities in central western Brazil such as the type locality, an indigenous village difficult to access even nowadays. Furthermore, specimens of *L. amazonica* as well specimens of *L. willianilsoni* sp. n. and *L. muriciensis* sp. n. were found in natural environments (Figs 39–47, 66) inside and outside four Conservation Units in three Brazilian states. Moreover, if *L. amazonica* is an invasive species as proposed by Duncan et al. (2010), their presence in larger cities in southeastern and southern Brazil would also be expected, as invasive species are normally introduced by means of human activities and benefited by urban environments, normally forming large populations. Even though they can be found in disturbed environments in northwestern Brazil, they are found in natural conditions and are not found in urban areas in localities more to the South.

The question on the origin of *L. amazonica* and *L. rufescens* lineages is, therefore, open to discussion. A way to test the origin and evolution of *L. amazonica* lineage would be to collect *L. amazonica* specimens from different parts of northern, northwestern and central western Brazil as well as other South American countries, and determine the genetic divergence among the different populations.

As demonstrated by Duncan et al. (2010), the *amazonica* group is recovered in the middle of *rufescens* lineage. Therefore, it makes no sense to use the group name *amazonica,* and *L. amazonica*, *L. willianilsoni* sp. n. and *L. muriciensis* sp. n. should be referred as belonging to *rufescens* group.

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RESEARCH ARTICLE

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Phylogeny of the genus *Yumtaax* Boucher (Coleoptera, Passalidae, Proculini): Taxonomic and evolutionary implications with descriptions of three new species

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Abstract

Yumtaax Boucher (Coleoptera: Passalidae) is an endemic genus from the temperate sierras of Mexico and includes six narrowly distributed species. *Yumtaax* species have been assigned to several genera of Passalidae throughout history, and a phylogenetic approach is necessary to understand species delimitation and interspecific relationships. This study reconstructed the molecular phylogeny of six *Yumtaax* morphotypes using parsimony and Bayesian analysis of DNA sequence data from the ribosomal nuclear gene region 28S and the mitochondrial gene regions 12S and cytochrome oxidase I (COI) in addition to morphological characters. Analyses recovered two well-supported *Yumtaax* clades (the *Yumtaax laticornis* and *Yumtaax imbellis* clades) that are possible sister lineages. One synapomorphic morphological character state and the geographic isolation of the group provide corroborative evidence for monophyly. Molecular phylogenetic analyses and traditional morphological examinations also resulted in the discovery of two undescribed *Yumtaax* species and the discovery of two separate evolutionary lineages (cryptic species) within *Yumtaax recticornis*. As a result we describe three new species (*Yumtaax veracrucensis* Beza-Beza, Reyes-Castillo & Jameson, **sp. n.**, *Yumtaax cameliae* Beza-Beza, Reyes-Castillo & Jameson, **sp. n.**, and *Yumtaax jimenezi* Beza-Beza, Reyes-Castillo & Jameson, **sp. n.**), redescribe two species (*Yumtaax recticornis* [Burmeister 1847] and *Yumtaax laticornis* [Truqui 1857]), and provide a key to all nine *Yumtaax* species. This study is one of two studies to use molecular data to evaluate the evolutionary relationships of a genus of Bess Beetles (Passalidae), an ecologically important insect group exhibiting low morphological variability and heretofore lacking molecular phylogenetic study.

Keywords
Passalidae, phylogeny, species description, *Yumtaax*

Introduction

Yumtaax Boucher (Coleoptera: Passalidae: Proculini) is an endemic genus of the southern and eastern Sierra Madre (Boucher 2006). As other members of the family Passalidae, these beetles feed on rotten wood and are important in the process of nutrient cycling in forests (Cano and Schuster 2012). Due to competition for this food resource with other Passalidae and resulting resource partitioning, *Yumtaax* species specialize on feeding in the periphery of large logs or on twigs and branches with a diameter less than 15 cm (Castillo and Reyes-Castillo 1984). Species of *Yumtaax* are associated with high altitude habitats such as cloud and pine-oak forests (Castillo and Reyes-Castillo 1984, Boucher 2006).

Yumtaax was described by Boucher (2006) for six species previously considered by Castillo and Reyes-Castillo (1984) as part of the genus *Petrejoides* Kuwert: *Yumtaax recticornis* (Burmeister, 1847), *Yumtaax laticornis* (Truqui, 1857), *Yumtaax imbellis* (Casey, 1897), *Yumtaax nebulosus* (Castillo & Reyes-Castillo, 1984) (Fig. 1A), *Yumtaax mazatecus* (Castillo & Reyes-Castillo, 1984), and *Yumtaax olmecae* (Castillo & Reyes-Castillo, 1984). Boucher (2006) considered this a morphologically and biogeographically cohesive group that deserved generic status based on the dorsal mesotibial ridge that is elevated at the middle and setose on its dorsal edge (Fig. 2). *Yumtaax* species exhibit low morphological variability (Castillo and Reyes-Castillo 1984), rendering a traditional morphological phylogenetic approach of limited utility. A traditional morphological approach in combination with molecular data are needed to define species and reconstruct the phylogeny of the genus. Molecular data have historically proven useful in the family Passalidae (Villatoro 1997, Archila 2009, Beza-Beza et al. 2011, Jiménez-Ferbans et al. 2016), and these data are essential for species delimitation and phylogeny reconstruction in the absence of strong morphological data. Although passalids are a potentially informative group for understanding the dynamics of New World cloud forests (Beza-Beza et al. 2011, Schuster and Cano 2006), a strong phylogenetic hypothesis is needed for such applications. The aims of this study are to: (1) test the monophyly of *Yumtaax* and (2) reconstruct the phylogenetic relationships among *Yumtaax* species.

Taxonomic history

Species currently considered members of *Yumtaax* have been assigned to several genera of Passalidae throughout history, and circumscription of the genus is unclear. *Yumtaax recticornis,* the type species of the genus (Boucher 2006), was originally described in *Passalus* Fabricius (Burmeister 1847) and was subsequently transferred to the passalid genera

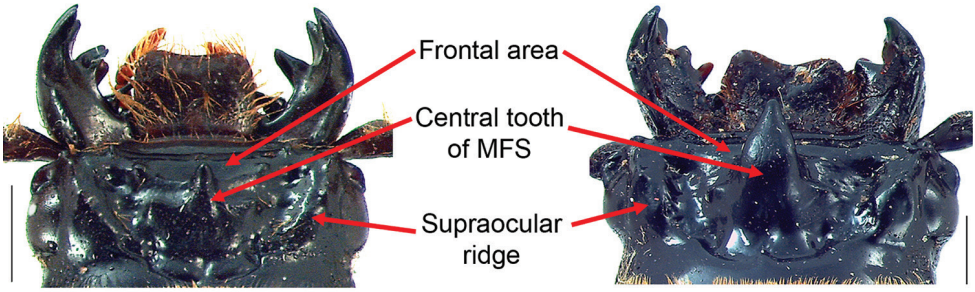
Soranus (Kaup, 1871), *Popilius* Kaup (Gravely, 1918), *Petrejoides* Kuwert (Reyes-Castillo, 1970), and finally *Yumtaax* (Boucher, 2006). *Yumtaax laticornis* was described by Truqui (1857) as part of *Passalus* Fabricius, but was subsequently transferred to the proculine genera *Pseudacanthus* Kaup (Kaup, 1871) and *Petrejoides* Kuwert (Reyes-Castillo, 1970). *Yumtaax imbellis* was described in the passalid genera *Soranus* (Casey, 1897) but was later transferred to the genera *Popilius* (Hincks & Dibb, 1935) and *Petrejoides* (Reyes-Castillo, 1970). The remaining three species (*Yumtaax nebulosus*, *Yumtaax olmecae*, and *Yumtaax mazatecus*) were considered part of *Petrejoides* (Castillo & Reyes-Castillo, 1984). Subsequently, the aforementioned six species were transferred to the new genus *Yumtaax* by Boucher (2006). This classification instability clearly illustrates a lack of consistent morphological circumscription for both the genera and species of Passalidae.

Using traditional morphology-based taxonomic methods, Reyes-Castillo (1970) was the first author to recognize shared characters among *Yumtaax* species, grouping *Y. imbellis, Y. laticornis,* and *Y. recticornis* in the genus *Petrejoides* along with *Petrejoides tenuis* Kuwert, *Petrejoides jalapensis* (Bates), and *Petrejoides orizabae* Kuwert. In a subsequent revision of *Petrejoides,* Castillo and Reyes-Castillo (1984) proposed three species groups, two of which included species currently considered *Yumtaax* (Boucher, 2006). The monotypic "laticornis species group" included *Yumtaax laticornis* and the "recticornis species group" included *Yumtaax recticornis*, *Y. imbellis*, *Y. nebulosus*, *Y. olmecae*, and *Y. mazatecus* along with *Petrejoides tenuis* (Castillo & Reyes-Castillo, 1984).

The morphological characters of the *Y. laticornis* species group include a short frontal area (Fig. 1B), dorsal mesotibial ridge elevated at the middle (described as "quilla dorsal de la tibia II corta" by Castillo and Reyes-Castillo 1984) (Fig. 2), presence of the infraocular ridge (Fig. 3), and the striatopunctatus-type mesofrontal structure (MFS) (Castillo and Reyes-Castillo 1984). The morphological characters of the *Y. recticornis* species group include the short frontal area (Fig. 1A), dorsal mesotibial ridge that is elevated at the middle (Fig. 2), and the central tooth of MFS short (Fig. 1A) (long in *P. tenuis*) (Castillo and Reyes-Castillo 1984). Morphological character states shared by *Yumtaax* species (*P. laticornis* species group + *P. recticornis* species group [- *P. tenuis*]) include the short frontal area (Fig. 1A, B) and the dorsal mesotibial ridge elevated at the middle (Boucher 2006).

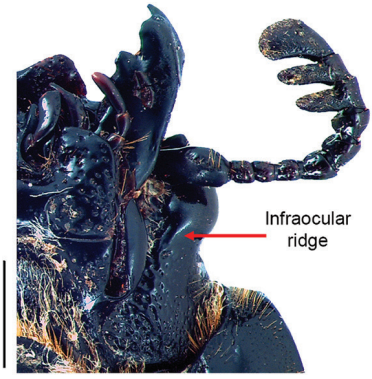
Relationships of the genus *Yumtaax*

The tumultuous nomenclatural history of *Yumtaax* species is due in part to the lack of molecular phylogenetic study of generic relationships in Passalidae. Most phylogenetic studies in the family have concentrated on the resolution of deeper relationships (subfamily and tribal level) (e.g., Fonseca 1987, Gillogly 2005, Boucher 2006, Fonseca et al. 2011) or have addressed the phylogeny of genera using morphology alone (e.g., Marshall 2000, Schuster et al. 2003, Boucher 2015, Jiménez-Ferbans and Reyes-Castillo 2015). The most complete generic-level phylogenetic analysis of Passalidae is that of Boucher (2006), who conducted a phylogenetic analysis of the tribe Proculini based on 51 morphological characters. Based on this analysis, Boucher placed *Yumtaax* within the tribe



1A *Yumtaax nebulosus* 1B *Yumtaax cameliae*

2



3

Figures 1–3. Morphological structures for *Yumtaax* species. **1** Head structures for identification of *Yumtaax* species. Central tooth of the mesofrontal structure (MSF) short (**1A**) versus long (**1B**). Eye size large in *Yumtaax nebulosus* (distal edge of the eye surpasses the distal edge of the canthus); eye size reduced in *Y. cameliae* (distal edge of the eye does not surpass the distal edge of the canthus). Scale bars: 1 mm **2** Mesotibia showing dorsal ridge elevated at the middle and setose (lateral view) of *Yumtaax cameliae* sp. n. **3** Head, ventral view, of *Yumtaax cameliae* sp. n. showing the infraocular ridge. Scale bar: 1.5 mm

Proculini and hypothesized that *Yumtaax* is sister to *Spurius* Kaup (*Yumtaax* + *Spurius*), that the *Yumtaax* + *Spurius* clade is sister to *Popilius sensu* Boucher (2006), and that this clade (*Yumtaax* + *Spurius* + *Popilius*) is sister to *Petrejoides sensu* Boucher (2006).

Research design and methods

Taxon selection

To address the hypothesis that *Yumtaax* is a monophyletic group, seven operational taxonomic units (OTUs) were included. *Yumtaax recticornis sensu lato* (*Y. recticornis s. l.*)

(type species of the genus) was represented by two OTUs: *Y. recticornis* from Veracruz (*Yumtaax recticornis* VM) and *Y. recticornis* from Oaxaca (*Yumtaax recticornis* OM). The remaining OTUs were *Y. laticornis* sensu Castillo and Reyes-Castillo (*Yumtaax* LM), *Y. imbellis*, *Y. mazatecus*, and two undescribed OTUs (Suppl. material 1). Although these undescribed OTUs possessed morphological characters that place them within *Yumtaax* (dorsal mesotibial ridge elevated at the middle and setose in its dorsal edge; Fig. 2), they were morphologically distinct from the remaining OTUs. These were referred to as the *Yumtaax* "calcahualco" morphotype (*Yumtaax* CM) and the *Yumtaax* "lacortadura" morphotype (*Yumtaax* LCM). Two species of *Yumtaax* (*Y. olmecae*, and *Y. nebulosus*) were not sampled in this study. Regarding outgroup selection, a broad phylogenetic sampling of passalids was used to test the monophyly of *Yumtaax* and address sister group relationships. Exemplar species from the proculine genera *Chondrocephalus* Kuwert, *Heliscus* Zang, *Odontotaenius* Kuwert, *Oileus* Kaup, *Popilius* Kaup, *Spurius*, *Petrejoides*, *Vindex* Kaup, and *Verres* Kaup were chosen based on the phylogenetic relationships proposed by Boucher (2006) (Suppl. material 1). The genus *Passalus* (Passalinae: Passalini) was used to root all members of Proculini (Suppl. material 1).

Specimen acquisition, DNA extraction, and amplification

Both freshly collected and museum specimens were used (Suppl. material 1). Adults and larvae were field-collected by opening rotting logs with an axe and actively searching for tunnels, adults, and larvae. Specimens were stored in 95% ethanol and kept at a cool temperature. Muscle tissue was obtained from the right hind legs of specimens. DNA was extracted using the protocol detailed in Tagliavia et al. (2011) with two modifications. In order to more fully macerate tissue, legs were ground to a fine powder using the modified reciprocating saw approach described in Alexander et al. (2007). Additionally, 240 µl of lysis buffer with detergent was used in the first step instead of 80 µl of lysis buffer per mg of ground leg.

Two mitochondrial gene regions were used: the 5' end of the small ribosomal subunit 12S rRNA and cytochrome oxidase 1 (COI). The 12S region has been shown to be useful for distinguishing clades at various taxonomic levels within Passalidae (Archila 2009, Beza-Beza 2009), and COI has been used to study relationships at species and population levels within scarabaeoids specifically (Monaghan et al. 2005) and the identification of animal species in general (International Barcode of Life Project 2009). The nuclear 28S D2 ribosomal subunit was also utilized. This region has been used in numerous Coleoptera studies (e.g., Smith et al. 2006, Monaghan et al. 2007, Wild and Maddison 2008, Ocampo et al. 2010). The 12S and 28S regions were amplified using the following primer combinations: 12S (12S 2F/SR-N-14594); 28S (28SF/28SR) (Table 1). The COI region was amplified as two segments (C1-J-1751/C1-N-2191, C1-J-2183/TL2-M3014) (Table 1). When these primer combinations failed, internal primers were used to target smaller fragments. These internal primer combinations for COI included: C1-J-1751/C1-N-2191, C1-J-2183/MaryLiz4, and C1-J-2441/TL2-M3014 (Table 1).

Table 1. Primers used in this study. Asterisk indicates that primers were slightly modified.

Gene region	Name	Primer Sequence	Reference
12S	12S 2F	5' TACTATGTTWMGACTTATCC 3'	Kambhampati and Smith 1995*
	SR-N-14594	5' AAACTAGGATTAGATACCC 3'	Kambhampati and Smith 1995
COI	C1-J-1751	5' GGATCACCTGATATAGCATTCCC 3'	Simon et al.1994
	C1-J-2183	5' CAACATTTATTTTGATTTTTTGG 3'	Simon et al.1994
	C1-J-2441	5' CCAACAGGAATTAAAATTTTTAGATGATTAGC 3'	Simon et al.1994
	C1-N-2191	5' CCCGGTAAAATTAAAATATAAACTTC 3'	Simon et al.1994
	Mary Liz4	5' GATGAATTWGCTAAATTACTCC 3'	Moore et al. 2015
	TL2-N-3014	5' TCCAATGCACTAATCTGCCATATTA 3'	Simon et al.1994
28S	28SF	5' CCCSSGTAATTTAAGCATATTA 3'	Whiting 2001
	Yoshi	5' CGGTTTCACGTACTCTTGAAC 3'	Moore et al. 2015
	Charmander	5' GTTCAAGAGTACGTGAAACCG 3'	Moore et al. 2015
	Toad	5' CTACWGGGGGAGAAGTGCAC 3'	Moore et al. 2015
	Squirtle	5' GTGCACTTCTCCCCCWGTAG 3'	Moore et al. 2015
	Peach	5' CTAGACTCCTTGGTCCGTGTTTC 3'	Moore et al. 2015
	Bulbasaur	5' GAAACACGGACCAAGGAGTCTAG 3'	Moore et al. 2015
	28SR	5' TCGGAAGGAACCAGCTAC 3'	Whiting et al. 1997*

Internal primer combinations for 28S included: 28SF/Yoshi, Charmander/Toad, Squirtle/Peach, Bulbasaur/28SR (Table 1) (Moore et al. 2015). The 12S and 28S regions were amplified with 10 µl reactions including: 1 µl 10× Klentaq (DNA Polymerase Technology, St. Louis, MO) reaction buffer (final concentration 1×), 1 µl DNTPs (0.25 µM), 1 µl each primer (1 µM each primer), 1 µl DNA template, 0.05 µl Klentaq LA polymerase, and 5 µl DI water. Ten µl COI reactions included: 1 µl 10× Klentaq reaction buffer (1x), 1 µl DNTPs (0.25 mM), 1 µl each primer (1 µM each primer), 2 µl DNA template, 0.05 µl Klentaq LA polymerase, and 4 µl DI water. Betain PCR enhancer was added at a final concentration of 1.1 M when these standard reactions failed. The following cycling parameters were used: 1) 94°C for 2 minutes, 2) 94°C for 40 seconds, 3) variable annealing temperatures (see Table 2) for 40 seconds, 68°C for various time intervals (see Table 2). Steps 2-3 were repeated for 25 cycles. All amplicons were sequenced at the University of Chicago Comprehensive Cancer Center DNA Sequencing and Genotyping Facility.

Sequence contigs were constructed using CLC MAIN WORKBENCH (CLC bio, Aarhus, Denmark). If samples required amplification with internal primers (see above) sequences were assembled using GENEIOUS R9.1 (Kearse et al. 2012). Uninterpretable sequences were cropped or discarded using the GENEIOUS default trimming tool. Sequences were then aligned with MEGA version 5 (Tamura et al. 2011). The Muscle algorithm with default settings (Edgar 2004) was used first, followed by Clustal W (Higgins et al. 1994) alignment with default settings. Alignments were then checked by eye and manually modified if necessary. Sections of missing data were replaced with Ns.

Table 2. Annealing temperatures and extension times used in this study.

Gene region	Primer combination	Annealing temperature (°C)	Extension time at 68° C (minutes: seconds)
12S	12S 2F/SR-N-14594	45	1:30
COI	C1-J-1751/C1-N-2191	47	1:30
	C1-J-2183/TL2-N-3014	44	2:30
	C1-J-2183/MaryLiz4	51	1:30
	C1-J-2441/TL2-N-3014	50	1:00
28S	28SF/28SR	52	2:30
	28SF/Yoshi	49	1:15
	Toad/Charmander	52	0:30
	Peach/Squirtle	56	1:15
	Bulbasaur/28SR	53	1:15

Phylogenetic analysis

Phylogenetic inference using maximum parsimony and Bayesian optimality criteria was conducted for each locus independently (COI, 12S, and 28S) and the total combined dataset (COI+12S+28S). Maximum parsimony bootstrap analyses were conducted using PAUP 4.0 (Swofford 2002) and included 1000 bootstrap replicates, each involving a heuristic search with 100 random additions. Clades with bootstrap support higher than 80% were considered well supported (Baum and Smith 2013). Bayesian analyses were performed using MR. BAYES 3.2 (Ronquist and Huelsenbeck 2003). Each analysis included 4 independent runs of one million generations, with trees sampled every 1,000 trees generations. For the COI and 28S datasets, 500,000 extra generations were run after the first one million generations until the split frequency reached less than 0.00. A concatenated alignment of the three loci was assessed with PARTITION FINDER v.1.1.0 (Lanfear et al. 2012), which suggested treating each locus as a separate partition with the GTR+I+G model applied to the C01 and 28S partitions and the GTR+G model applied to the 12S partition. For the mitochondrial datasets (12S and COI), the genetic code Bayes function (lset code) was set to invertebrate mitochondrial (lset code=invertmt). The first 100 trees of each run were discarded the remaining 901 trees for each run of the 12S and combine dataset were then used to create a 50% majority-rule consensus tree of posterior probability values. The remaining 1,401 trees for each run of the 28S and COI dataset were then used to create a 50% majority-rule consensus tree of posterior probability values Clades with Bayesian posterior probabilities equal to or higher than 0.95 were considered well supported (Baum and Smith 2013).

Species delimitation and species descriptions

The species status of each OTU was evaluated using two criteria. In order to be considered a species, an OTU must (1) be morphologically distinctive and (2) the molecular phylogeny must provide either evidence of its status as an evolutionary lineage or not provide

contrary evidence. Species are segments of evolutionary lineages which can be diagnosed by a variety of criteria ("The General Lineage Concept"; de Queiroz 1998, Hey 2006), among them morphological distinctiveness. We view morphological distinctiveness alone as a sufficient criterion for species diagnosis, with the supporting phylogenetic data (when present) as confirmation. The taxa we diagnose represent working hypotheses and future workers should test these hypotheses with additional criteria (Carstens et al. 2013).

Type specimens for the six described *Yumtaax* species were examined in order to properly associate species names. Species descriptions used the morphological terminology of Reyes-Castillo (1970) and Castillo and Reyes-Castillo (1984) with the following modifications: total body length was measured from the anterior apex of the left mandible to the posterior apex of the left elytrum. Head width was defined as the distance between the posterior tubercles of the supraorbital ridge. Eyes were considered large if the distal edge of the eye projected beyond the distal edge of the canthus (e.g., Fig. 1A; *Y. nebulosus*), moderately reduced if the distal edge of the eye was subequal to the canthus (e.g., Figs 1B, 9C; *Y. cameliae*), and greatly reduced if the distal edge of the eye did not surpass the distal edge of the canthus (e.g., Fig. 7C; *Y. laticornis*). Using the terminology of Reyes-Castillo (1970), borders or edges of structures and sutures are described as concave (curved posteriorly), straight, or convex (curved anteriorly).

Results

COI data partition

The COI mitochondrial data partition consisted of 1140 aligned characters of which 450 (39%) were variable. Of the variable characters, 366 (81%) were parsimony-informative. Because parsimony and Bayesian analyses provided concordant tree topologies, bootstrap support (BS) and Bayesian posterior probabilities (PP) are shown on a Bayesian 50% majority-rule consensus tree (Suppl. material 2). The COI dataset does not support a monophyletic *Yumtaax*. However, there was support for two separate *Yumtaax* clades: a strongly supported clade comprising *Y.* CM, *Y. mazatecus*, *Y.* LM, and *Y. recticornis* VM (= "*Y. laticornis* clade") (1.0 PP, 95 BS) and a marginally supported clade comprising *Y.* LCM, *Y. imbellis*, and *Y. recticornis* OM (= "*Y. imbellis* clade") (0.98 PP, 55 BS). Both clades were placed in a polytomy including species from *Chondrocephalus*, *Petrejoides*, *Verres*, *Heliscus*, *Oileus*, *Odontotaenius*, and *Popilius*.

12S data partition

The 12S mitochondrial data partition consisted of 356 aligned characters of which 111 (31%) were variable. Of the variable characters, 82 (73%) were parsimony-informative. Because parsimony and Bayesian analyses provided concordant tree topologies, bootstrap support (BS) and Bayesian posterior probabilities (PP) are shown on a

Bayesian 50% majority-rule consensus tree (Suppl. material 3). The 12S dataset does not support a monophyletic *Yumtaax*. Similar to the COI dataset, there was support for two separate *Yumtaax* clades: a strongly supported clade comprising *Y.* CM, *Y. mazatecus*, *Y.* LM, and *Y. recticornis* VM (= "*Y. laticornis* clade") (1.0 PP, 89 BS) and a poorly supported clade comprising *Y.* LCM, *Y. imbellis*, and *Y. recticornis* OM (= "*Y. imbellis* clade") (0.63 PP, <50 BS). Both clades are placed in a polytomy including another clade comprising species from *Chondrocephalus*, and *Petrejoides*.

28S nuclear data partition

The 28S data partition consisted of 1083 aligned characters of which 184 (16%) were variable. Of the variable characters, 74 (40%) were parsimony-informative. Because the parsimony and Bayesian analyses provided concordant tree topologies, bootstrap support (BS) and Bayesian posterior probabilities (PP) are shown on a Bayesian 50% majority-rule consensus tree (Suppl. material 4). Analysis of the 28S dataset did not provide support for a monophyletic *Yumtaax*. However, most *Yumtaax* OTUs (6 out of 7) were placed within a poorly supported clade (0.71 PP, <50 BS); *Y.* CM was placed sister to *P. orizabae* with maximum Bayesian and parsimony support. This relationship was also the only strongly supported conflict between the mitochondrial and nuclear 28S datasets (Suppl. material 4).

Total combined data

The Bayesian 50% majority rule consensus phylogram resulting from analysis of the combined mitochondrial (COI, 12S) and nuclear (28S) dataset (Fig. 4) supports a monophyletic *Yumtaax* with marginal Bayesian support (0.95 PP) and no parsimony support (< 50 BS). Similar to results from the mitochondrial partitions (12S and COI, Suppl. material 2–3), there was strong support for two *Yumtaax* clades (*Y. laticornis* clade and *Y. imbellis* clade). Support for a clade comprising *Y. laticornis* (=*Y.* CM), *Y. mazatecus*, *Y. cameliae* sp. n. (=*Y.* LM), and *Y. jimenezi* sp. n. (=*Y. recticornis* VM) was strong (0.99 PP, 99 BS) (=*Y. laticornis* clade). Support for a clade comprising *Y. veracrucensis* sp. n. (=*Y.* LCM), *Y. imbellis*, and *Y. recticornis (=Y. recticornis* OM) (= *Y. imbellis* clade) was strong (1.0 PP, 96 BS). The *Yumtaax* clade was placed sister to a clade including species from *Odontotaenius*, *Petrejoides*, *Heliscus*, *Spurius*, and *Popilius*.

Discussion

Monophyly of *Yumtaax*

The monophyly of *Yumtaax* was supported by the total combined molecular data set examined in this study; however monophyly of *Yumtaax* was not supported when

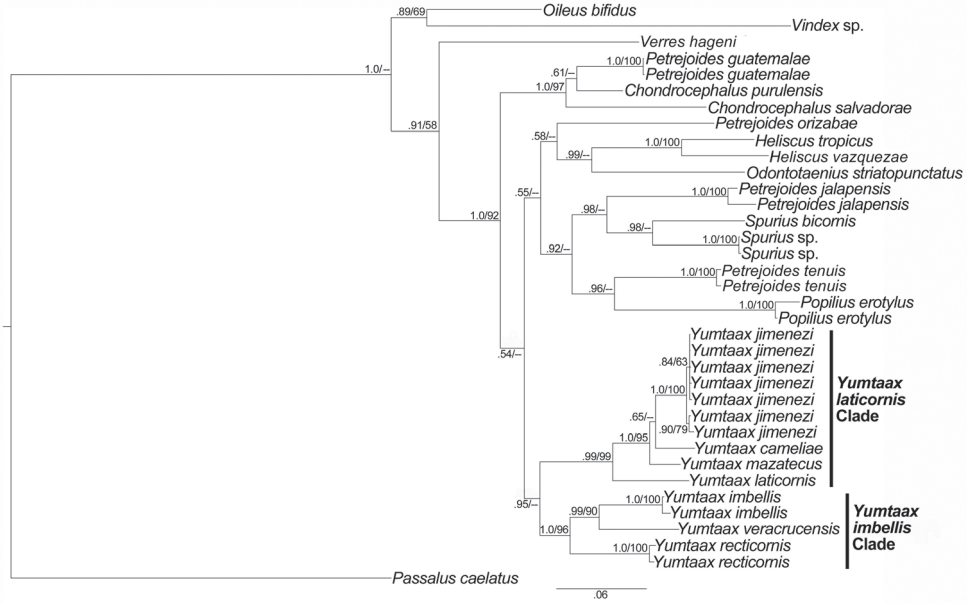


Figure 4. 50% majority-rule consensus of Bayesian posterior probabilities resulting from analysis of the combined data. Bayesian posterior probabilities (PP) (> 0.50) and bootstrap support (BS) (> 50) are noted. *Yumtaax veracrucensis* sp. n. = *Yumtaax* LCM; *Yumtaax recticornis* = *Yumtaax recticornis* OM; *Yumtaax laticornis* = *Yumtaax* CM; *Yumtaax cameliae* sp. n. = *Yumtaax* LM; *Yumtaax jimenezi* sp. n. = *Yumtaax recticornis* VM.

genes were analyzed individually. The 12S and COI data strongly support two *Yumtaax* clades and do not provide strong evidence for the non-monophyly of *Yumtaax*. The 28S dataset does not support a monophyletic *Yumtaax* whereas the combined data suggest a monophyletic *Yumtaax* (Fig. 4). However, a morphological character state and geographic distribution (endemic to the temperate sierras of Mexico) both suggest that *Yumtaax* species form a monophyletic group. Evaluation of all *Yumtaax* OTUs revealed that a dorsal mesotibial ridge elevated at the middle (Fig. 2) is a character state unique to this group. This contrasts with a mesotibial ridge that is elevated for the entire length of the tibia and that is observed in the remaining 16 species of this study. Boucher (2006) also considered this dorsal mesotibial ridge elevated at the middle a synapomorphy for *Yumtaax*, and thus far, the dorsal mesotibial ridge elevated at the middle has not been observed in any other species of Passalidae (approximately 930 spp.) (Boucher 2006).

A combined consideration of the molecular phylogenetic, morphological, and geographic data suggests that the best working hypothesis is of *Yumtaax* as a valid, monophyletic genus. Further study should include both broader taxon sampling (including the genera *Petrejoides sensu* Boucher, *Popilius sensu* Boucher, and the excluded species of *Yumtaax* [*Y. nebulosus*, *Y. olmecae*]) and data from additional gene regions (particularly nuclear). For the remainder of this work, *Yumtaax* is treated as a monophyletic genus.

Yumtaax species delimitation

Based on combined morphological, molecular, and geographic data, we provide evidence that the *Yumtaax* OTUs analyzed in this study include seven distinctive species: *Y. imbellis*, *Y.* LCM, *Y. recticornis* OM, *Y.* CM, *Y. mazatecus*, *Y.* LM, and *Y. recticornis* VM (Fig. 4, Suppl. material 2, 3). Analysis of the combined molecular dataset recovered clear evidence for three independent evolutionary lineages (= species) corresponding to *Y. imbellis*, *Y. recticornis* OM, and *Y. recticornis* VM (Fig. 4, Suppl. materials 2, 3, 4). Although the lineage status of *Y.* LCM, *Y.* CM, *Y. mazatecus*, and *Y.* LM could not be established due to the lack of multiple samples per OTU, the phylogeny does not provide evidence that these are *not* lineages. For instance, each individual sample of these OTUs is genetically distinguishable from other *Yumtaax* lineages (has a non-zero branch length) and does not render *Y. imbellis*, *Y. recticornis* OM, or *Y. recticornis* VM paraphyletic. We treat each of these OTUs as species (see species diagnoses below).

Molecular and morphological data both suggest that *Y. recticornis s. l.* is composed of two independent lineages. First, eye size in *Y. recticornis s. l.* is geographically dependent; populations from Veracruz have reduced eyes whereas those from Oaxaca have large eyes (Castillo and Reyes-Castillo 1984). Molecular data reveal that these morphotypes form separate lineages: *Y. recticornis* VM is part of the *Y. laticornis* clade, and *Y. recticornis* OM is part of the *Y. imbellis* clade (Fig. 4). Based on examination of the type specimen of *Y. recticornis,* the name should be assigned to *Y. recticornis* OM; this species is distributed exclusively in Sierra Madre del Sur in Mexico. *Yumtaax recticornis* VM is an unnamed species that it is currently known exclusively from the transverse neo-volcanic system in Mexico. Within the *Y. imbellis* clade, molecular (Fig. 4) and morphological (see diagnosis below) data suggest that *Y.* LCM is a distinct species currently known only in the transverse neo-volcanic system in Mexico. Based on examination of type specimens in the genus *Yumtaax*, this morphotype also represents an undescribed species. Close examination of the type specimen of *Y. laticornis* indicated that this name should be applied to the *Y.* CM morphotype rather than the *Y.* LM morphotype (*Yumtaax laticornis sensu* Castillo and Reyes-Castillo 1984). Based on the type specimen, we re-circumscribe *Y. laticornis* and describe a new species for *Y.* LM.

Due to geographic isolation and morphological differences we follow Castillo and Reyes-Castillo (1984) and consider *Y. nebulosus* and *Y. olmecae* as independent, evolutionary lineages (= species) within the *Yumtaax* genus.

Species descriptions of *Yumtaax*

As a result of this work, we describe three new species: *Y. veracrucensis* sp. n. (= *Y.* LCM), *Y. cameliae* sp. n. (= *Y.* LM), and *Y. jimenezi* sp. n. (= *Y. recticornis* VM). In order to stabilize nomenclature, we re-circunscribe two species (*Y. recticornis* and *Y. laticornis*). *Yumtaax recticornis s. l.* is composed of two unrelated and heretofore cryptic species (*Y. recticornis* OM and *Y. recticornis* VM). Close examination of the *Y. recticornis*

lectotype and one paralectotype indicates that the name *Y. recticornis* corresponds to our *Y. recticornis* OM. Redescription of this species is necessary to re-circumscribe *Y. recticornis sensu stricto* (*Y. recticornis s. s.*) and distinguish it from the *Y. recticornis* VM lineage (= *Y. jimenezi*). Close examination of the *Y. laticornis* holotype indicates that this name corresponds to *Y.* CM rather than to *Y. laticornis sensu* Castillo and Reyes-Castillo (1984) (= *Y.* LM). A redescription of the holotype of *Y. laticornis* is provided to clarify this finding. As such, the genus *Yumtaax* includes nine species that can be separated morphologically using the following key.

Key to the species of *Yumtaax*

(Modified from Castillo and Reyes-Castillo 1984, Schuster 1991)

1 Apex of central tooth of mesofrontal structure (MFS) (viewed from above) extends beyond frontoclypeal suture (Fig.1B) **2**

1 Apex of central tooth of mesofrontal structure (MFS) (viewed from above) not reaching the frontoclypeal suture (Fig. 1A) **4**

2 Mesofrontal structure (MFS) dorsally with scarce micro-punctures (Fig 6C). Body length 17.5–20.0 mm. Aedeagus elongated (Fig. 6E, F). Mexican Transvolcanic Belt..... ***Y. veracrucensis* Beza-Beza, Reyes-Castillo & Jameson, sp. n.**

– Mesofrontal structure (MFS) dorsally impunctate. Body length >22.0 mm. Aedeagus globose (e.g., Fig. 8F, G, H) **3**

3 Scutellum punctate. Mesofrontal structure (MFS) of the falsus type (Fig. 7C). Distal edge of the eye not surpassing distal edge of the canthus (Fig. 7D). Brachypterous. Pico de Orizaba ..... ***Y. laticornis* (Truqui)**

– Scutellum impunctate. Mesofrontal structure of the striatopunctatus type (Fig. 8C). Distal edge of the eye subequal to the canthus (Fig. 8D). Macropterous. Puerto del Aire, Veracruz ..... ***Y. cameliae* Beza-Beza, Reyes-Castillo & Jameson, sp. n.**

4 Frons with central longitudinal ridge. Southeastern Sierra Madre, Oaxaca Highlands ..... ***Y. mazatecus* (Castillo & Reyes-Castillo)**

– Frons without central longitudinal ridge **5**

5 Mesofrontal structure (MFS) with dorsal groove (Fig. 9A in Castillo and Reyes-Castillo 1984). Dorsal anterior profile of elytra straight **6**

– Mesofrontal structure (MFS) without dorsal grove (Fig. 8A in Reyes-Castillo 1984). Dorsal anterior profile of elytra V-shaped **7**

6 Femur I without longitudinal anterior-ventral groove. Union of elytral striae 1–10 with a row of fine punctures. Dorso-lateral surface of the pronotum punctate. Body length 16.5–19.0 mm. Eastern Sierra Madre ..... ***Y. nebulosus* (Castillo & Reyes-Castillo)**

– Femur I with longitudinal anterior-ventral groove. Union of elytral striae 1–10 with >1 rows of punctures. Dorso-lateral surface of the pronotum im-

punctate. Body length 24.0–27.0 mm. Sierra Madre del Sur, Guerrero..... ..... ***Y. olmecae*** **(Castillo & Reyes-Castillo)**

7 Infraocular ridge absent (not as in Fig. 3). Clypeus vertical. Sierra Madre del Sur, Guerrero..... ***Y. imbellis*** **(Casey)**

– Infraocular ridge present (Fig. 3). Clypeus inclined. Distribution Sierra Madre del sur (Sierra Juarez, Oaxaca); or Transverse neo-volcanic system (Veracruz) **8**

8 Clypeal surface concave. Frontoclypeal suture concave. Central tooth of mesofrontal structure (MFS) largely free from frontal ridges (Fig. 5C). Distal edge of the eye projected beyond distal edge of the canthus (Fig. 5D). Aedeagus elongated (Figs. 5F, G, H). Sierra Juarez, Oaxaca..... ..... ***Y. recticornis*** **(Burmeister)**

– Clypeal surface flat. Frontoclypeal suture straight. Central tooth of mesofrontal structure (MFS) fused with frontal ridges (Fig. 9C). Distal edge of the eye subequal to the canthus (Fig. 9D). Aedeagus globose (Fig. 9E, F, G). Transverse neo-volcanic system .... ***Y. jimenezi*** **Beza-Beza, Reyes-Castillo & Jameson, sp. n.**

Yumtaax recticornis **(Burmeister, 1847)**

Passalus recticornis Burmeister, 1847: 508–509.
Soranus recticornis (Burmeister) [comb. n. by Kaup 1871: 105, 108].
Popilius recticornis (Burmeister) [comb. n. by Gravely 1918: 24, 26].
Petrejoides recticornis (Burmeister) [comb. n. by Reyes-Castillo 1970: 125].
Yumtaax recticornis (Burmeister) [comb. n. by Boucher 2006: 348].

Material examined. 105 specimens (lectotype, paralectotype, and 103 non-type specimens). *Lectotype* ♂. MEXICO: WB zoologie S. Nr. 812123. (MLU Halle). *Paralectotype* 1 ♀. MEXICO: no data (MLU Halle).

Non-type specimens (103 total). MEXICO: 1 ♀, Oaxaca, Amatepec (1.6 km N), alt. 1840 m, bosque mesófilo de montaña, II-28-1988 (*Reyes*, *Boucher*, *C. Castillo*). 1 ♂, Carretera Tuxtepec-Oaxaca (87 km), III-21-1967 (*R. MacGregor*); 1 ♀, (88 km), alt. 2350 m, IV-4-1986 (*A. Ibarra*); 1 ♂, 1 ♀, (119 km), X-6-1973 (*J. Mateu*); 4 ♂, 2 ♀, (153 km), alt. 2800 m, X-7-1973 (*J. Mateu*). 1 ♀, Cerro Pelón (2.8 km), V-18-1980 (*C. Castillo*, *M. L. Castillo*, *G. Quintero*, *E. Rivera*); 3 ♀, (11.4 km NE), alt. 2170 m (*P. Reyes et al.*). 1 ♀, Comaltepec, Brecha 60 (unknown locality), V-18-1980 (*C. Castillo*, *M. L. Castillo*, *G. Quintero*, *E. Rivera*); 3 ♂, 2 ♀, (4.5 km), V-18-1980 (*C. Castillo*, *M. L. Castillo*, *G. Quintero*, *E. Rivera*). 1 ♂, 4 ♀, Comaltepec, Galera San Isidro (800 m), alt. 2000 m, V-17-1980 (*C. Castillo*, *M. L. Castillo*, *G. Quintero*, *E. Rivera*); 3 ♂, 5 ♀, (3.6 km), alt. 2160 m, V-17-1980 (*C. Castillo*, *M. L. Castillo*, *G. Quintero*, *E. Rivera*). 1 ♀, La Esperanza, alt. 1670 m, V-16-1980 (*C. Castillo*, *G. Quintero*, *M. L. Castillo*, *E. Rivera*); 4 ♂, 3 ♀, (3.5 km N), alt. 1670 m, bosque mesófilo de

montaña, III-1-1988 (*Reyes*, *Boucher*, *Castillo*); 1 ♀ (4 km), alt. 1800 m, V-20-1980 (*C. Castillo*, *M. L. Castillo*, *G. Quintero*, *E. Rivera*); 5 sex unknown, (6.8 km), alt. 1820 m, II-25-1984 (*P. Reyes et al.*); 1 sex unknown, (13.1 km), alt. 2030 m, II-25-1984 (*P. Reyes et al.*); 3 sex unknown, (14.1 km), alt. 1985 m, II-25-1984 (*P. Reyes et al.*); 2 sex unknown, (103.1 km), alt. 2030 m, II-25-1984 (*P. Reyes et al.*). 1 sex unknown, San Juan Lachao Viejo, km 85 de Sola de Vega (N 16°13.220' W 97°08.913), alt. 1858-1870 m, bosque mesófilo de montaña, VIII-6-2004 (*K. Araya*). 30 sex unknown, San Miguel Talea de Castro (8 km SE) (N 17°19.620' W 96°17.403), alt. 2082 m, VII-22-2007 (*O. Francke*, *H. Montaño*, *A. Valdéz*, *C. Santibañez*, *A. Ballesteros*). 3 ♂, 4 ♀, Sierra de Juárez, alt. 2000 m, VI-2-1995 (*G. Nogueira*). 2 ♂, 1 ♀, Totontepec (3.4 km S), alt. 1940 m, bosque mesófilo de montaña, II-29-1988 (*Reyes*, *Boucher*, *Castillo*). 3 ♂, 3 ♀, Valle Nacional (32 miles S), V-21/24-1971 (*H. Howden*); 1 ♀, (105-117 km E), IV-1964 (*C. R. Rotger*).

Diagnosis. *Yumtaax recticornis* is a small (18.0–21.0 mm), macropterous species and is a member of the *Y. imbellis* clade (Fig. 4). This species is diagnosed by the following character combination: the clypeus is inclined (shared with *Y. jimenezi*, *Y. imbellis*, *Y. mazatecus*, *Y. nebulosus*, *Y. olmecae*; clypeus vertical in *Y. veracrucensis*, *Y. laticornis*, *Y. cameliae*), surface concave (flat in other members of *Yumtaax*), and the anterior border is concave (shared with *Y. olmecae*; flat in other members of *Yumtaax*); mesofrontal structure (MFS) of the "falsus" type (see Reyes-Castillo 1970) (shared with all members of *Yumtaax* except *Y. cameliae* that has a MFS of the "striatopunctatus" type) with the central tooth largely free (shared with *Y. veracrucensis*, *Y. laticornis*, *Y. cameliae*, *Y. mazatecus*; fused with frontal ridges in *Y. jimenezi*, *Y. imbellis*, *Y. nebulosus*, *Y. olmecae*), central tooth directed dorsally (shared with *Y. imbellis*, *Y. nebulosus*, *Y. olmecae*; directed dorsally and anteriorly in *Y. jimenezi*, *Y. mazatecus*; directed anteriorly in *Y. veracrucensis*, *Y. laticornis*; elevated in the posterior half bending abruptly forward in the anterior half in *Y. cameliae*), central tooth not reaching the anterior border of the frontoclypeal suture (shared with *Y. jimenezi*, *Y. imbellis*, *Y. mazatecus*, *Y. nebulosus*, *Y. olmecae*; reaching the clypeus in *Y. laticornis*, *Y. cameliae*, *Y. veracrucensis*); and large eyes (shared with *Y. imbellis*; eyes moderately reduced in *Y. veracrucensis*, *Y. cameliae*, *Y. jimenezi*, *Y. nebulosus*, *Y. olmecae*; strongly reduced in *Y. laticornis*, *Y. mazatecus*).

Dimensions (mm) (n = 12). Total length 18.0–21.0 (χ = 19.0); elytral length 10.0–11.5 (χ = 11.0); pronotal length 3.5–5.0 (χ = 4.5); pronotal width 5.5–6.0 (χ = 5.5); humeral width 5.5–6.5 (χ = 6.0).

Redescription of lectotype (Fig. 5). Head (Fig. 5C). Labrum: anterior border concave, dorsal surface smooth and glabrous medially, punctate and setose apicolaterally, apically, and basally; anterior edge excavated. Clypeus: inclined, rectangular, concave, shiny, and smooth. Frontoclypeal suture: concave and opaque; external tubercles rounded, directed anteriorly and laterally. Frontal area: inclined, concave, smooth and shiny; frontal ridges present without inner tubercles. Frontal fossae: punctate and setose. Mesofrontal structure (MFS): of the "falsus" type (see Reyes-Castillo 1970); base subparallel and as wide as the lateral ridge of MFS; center horn short with apex rounded, largely free and directed anteriorly and dorsally (Fig. 5D), not reaching the

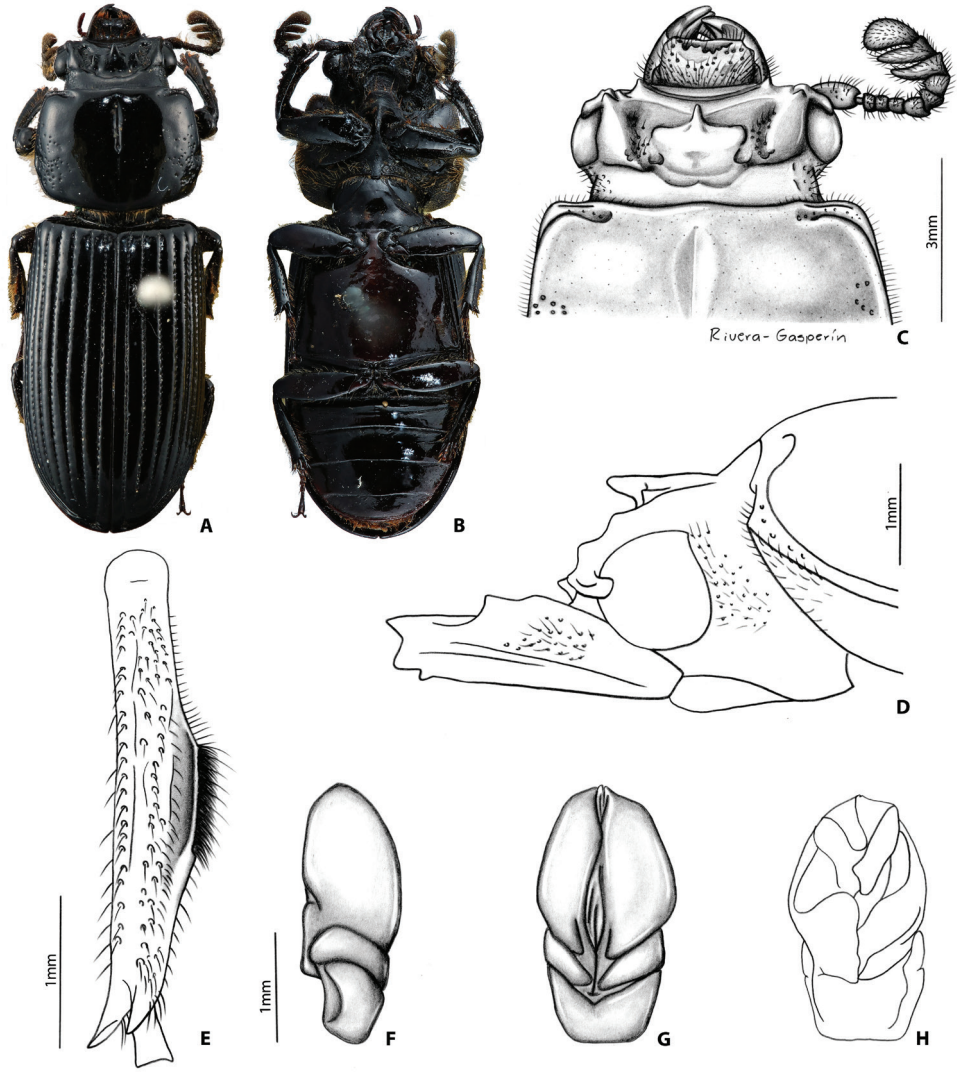


Figure 5. *Yumtaax recticornis* (Burmeister), lectotype: **A** dorsal habitus **B** ventral habitus **C** dorsal view of pronotum and head **D** lateral view of pronotum and head **E** lateral view of right mesotibia **F** lateral view of aedeagus **G** ventral view of aedeagus **H** dorsal view of aedeagus.

posterior margin of clypeus, dorsally without micro-punctures; base of center horn wide, narrowing gradually until apex; dorsal fossa present at the base of MFS. Occipital fossa: shallow posteriorly and deeper laterally, connected with the frontal fossae. Posterior occipital sulcus biconcave. Supraorbital ridge: bituberculate, anterior tubercle larger than posterior tubercle; posterior half of supraorbital ridge not bifurcated. Canthus: with apex rounded, covering less than 1/3 of eye, not expanded distally. Eyes large (distal edge of the eye surpassing distal edge of the canthus), width = 0.5 mm (each eye).

Head width (between posterior tubercles of the supraorbital ridge) = 3.0 mm. Ratio of sums of both eye widths/total head width = 0.36; postocular area punctate and setose. Ligula: tridentate, central tooth surpassing apex of lateral teeth, lateral teeth rounded; setose punctures present in discal area; posterior border convex. Mentum: lateral lobes rounded and wide, with setose punctures. Basomedial portion protruding ventrally; anterior border at middle convex; basal fossae present and rugose. Hypostomal process: with lateral depression; separated from mentum by a distance shorter than the width of the anterior width of hypostomal process. Infraocular ridge (e.g., Fig. 3): short and narrow anteriorly. Mandible: with 3 apical teeth; internal tooth of left mandible bidentate (teeth 1 and 2 of internal tooth fused); dorsal tooth occupies less than half length of the mandible. Pronotum: anterior angles rounded. Anterior fossae of marginal sulcus punctate. Lateral fossae with scattered punctures. Marginal groove lacking punctures. Prosternum: opaque; prosternellum with anterior half and lateral edges opaque and posterior half and middle area shiny. Scutellum: smooth and glabrous. Mesosternum: with lateral areas opaque. Metasternum: with setae anterolaterally, lacking punctures in lateral margins of metasternal disc. Lateral fossae wide across metathorax, with setose punctures. Elytra: anterior border straight. Meeting point of striae 1-10 (see Reyes-Castillo 1970) with one line of punctures. Wings: well developed. Legs (Fig. 5E): femur I with longitudinal anteroventral groove weakly developed, not reaching distal end of femur, posteroventral half pubescent; setae long, dense, reddish. Abdomen: last sternite with marginal groove complete (Fig. 5B). Aedeagus (Fig. 5F, G, and H) (description based on non-type material): dorsal view phallus elongated (longer than wider). In ventral view distal edges of the phallus more or less at the same level of distal edges of parameres.

Variation. The paralectotype and other specimens vary from the lectotype by the following characteristics: frontoclypeal suture varies from opaque to shiny, from concave to almost straight; frons and clypeus inclined to nearly vertical (always concave); concavity of frons and clypeus vary from strongly developed to nearly flat. Internal tubercles strongly developed or absent; frontal ridges always present, but not always terminating in internal tubercles; ratio of eyes and head width varies from 0.32–0.57; supraocular ridge weakly developed or absent. Occipital sulcus biconcave to concave in a few individuals. Small portion of individuals with frontal ridges fused to the base of the central horn of MFS (apex of the central horn always free).

Distribution. The lectotype and paralectotype are labeled "Mexico" (Burmeister 1847). Castillo and Reyes-Castillo (1984) suggested the Sierra de Juarez in Oaxaca, Mexico, as the possible type locality. The species is known only from cloud forest (1424-2986 m elevation) in Oaxaca, Mexico.

Remarks. Originally, this species was thought to be widely distributed across the Sierra Madre Oriental, the Mexican Transvolcanic Belt, and Sierra Madre del Sur (Reyes-Castillo 1970, Castillo and Reyes-Castillo 1984, Boucher 2006). Our phylogenetic analysis (Fig. 4), as well as close examination of morphological characters, provide evidence that *Y. recticornis s. l.* is composed of at least two cryptic species: *Y. recticornis* ("*Y. recticornis* OM" in the *Y. imbellis* clade, Fig. 4 and Suppl. materials 2–4) and *Y. jimenezi* sp. n.

("*Y. recticornis* VM" in the *Y. laticornis* clade, Fig. 4 and Suppl. materials 2–4). Based on comparison with the lectotype and one paralectotype of *Y. recticornis*, this name should be applied to *Y. recticornis* OM. The following character states provide evidence that *Y. recticornis* OM is conspecific with Burmeister's concept of *Y. recticornis*: large eye size (distal edge of the eye surpassing distal edge of the canthus), shape of the central tooth of the MFS (base subparallel and as wide as the lateral ridge of the MFS; center horn short with apex rounded, largely free and directed anteriorly and dorsally [Fig. 5D]), and shape of the frons and clypeus concave (rather than straight as in *Y. jimenezi*). Castillo and Reyes-Castillo (1984) suggested that eye size variation among *Y. recticornis s. l.* was dependent upon the locality of the population (populations in Oaxaca possess large eyes; populations in Veracruz possess small eyes). Characters of the internal tooth of the left mandible and aedeagus are described based on the paralectotype and non-type material because the mandibles of the lectotype are closed (thus making it impossible to determine the state of this character in this specimen).

***Yumtaax veracrucensis* Beza-Beza, Reyes-Castillo & Jameson, sp. n.**
http://zoobank.org/E98FFCB6-66DD-4281-9DEF-400E0063359B

Material examined. Seven type specimens (two males, four females, and two sex unknown).

Holotype ♂. MEXICO: Veracruz, Municipio de Coatepec, Reserva de La Cortadura, 1895–1900 msnm, bosque mesófilo de montaña, colecta en un tronco podrido, interior del bosque, V-2-2005 (*P. Reyes-Castillo*) (IEXA).

Paratypes. MEXICO: 1 ♂, 3 ♀. Veracruz, Municipio de Coatepec, Reserva de La Cortadura, 1895-1900 msnm, bosque mesófilo de montaña, colecta en un tronco podrido, interior del bosque, V-2-2005 (*P. Reyes-Castillo*) (IEXA, CFBB). Chiconquiaco: 1 ♂. Veracruz. Congr. La Guacamaya, X-6-2008 (*P. Rojas*); 2 sex unknown Near La Parra, IX-17-1995 (*J. Bueno*); One paratype is molecular voucher CB0035 (CFBB).

Diagnosis. *Yumtaax veracrucensis* is a small (17.5–20.0 mm), macropterous species that is a member of the *Y. imbellis* clade (Fig. 4). This species is diagnosed by the following character combination: the clypeus is vertical (shared with *Y. laticornis*, *Y. cameliae*; inclined in other members of *Yumtaax*) and with the anterior border straight (shared with other members of *Yumtaax* except for *Y. recticornis* and *Y. olmecae* that have a concave anterior border); mesofrontal structure (MFS) of the "falsus" type (see Reyes-Castillo 1970) (shared with all members of *Yumtaax* except *Y. cameliae* which has the MFS of the "striatopunctatus" type) with the central tooth largely free (shared with *Y. recticornis*, *Y. laticornis*, *Y. cameliae*, *Y. mazatecus*; fused with frontal ridges in *Y. jimenezi*, *Y. imbellis*, *Y. nebulosus*, *Y. olmecae*), directed anteriorly (shared with *Y. laticornis*; directed dorsally in *Y. recticornis*, *Y. imbellis*, *Y. nebulosus*, *Y. olmecae*; directed dorsally and anteriorly in *Y. jimenezi*, *Y. mazatecus*; elevated in the posterior half bending abruptly forward in the anterior half in *Y. cameliae*), and reaching the frontoclypeal suture (shared with *Y. laticornis*, *Y. cameliae*; not reaching the clypeus in other members of *Yumtaax*); and reduced eyes (shared with *Y. cameliae*, *Y. jimenezi*, *Y.*

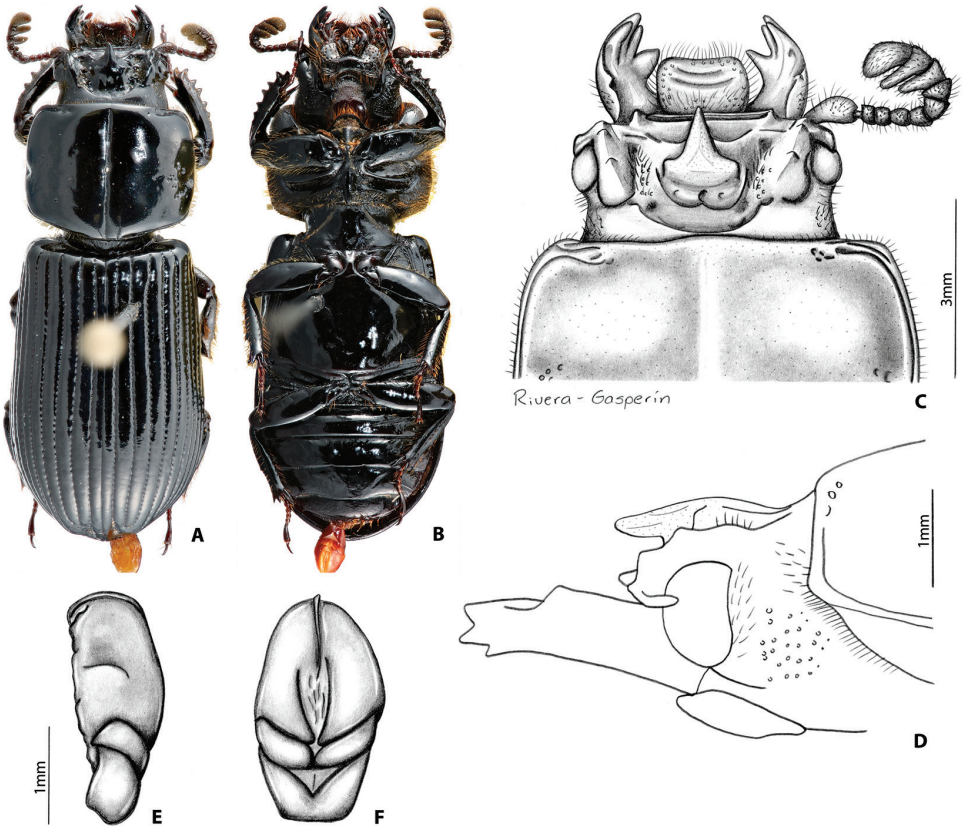


Figure 6. *Yumtaax veracrucensis* Beza-Beza, Reyes-Castillo & Jameson, sp. n., holotype: **A** dorsal habitus **B** ventral habitus **C** dorsal view of pronotum and head **D** lateral view of pronotum and head **E** lateral view of aedeagus **F** ventral view of aedeagus.

nebulosus, *Y. olmecae*; large in *Y. recticornis*, *Y. imbellis*; strongly reduced in *Y. laticornis*, *Y. mazatecus*).

Dimensions (mm) (n = 4). Total length 17.5–20.0 (χ = 19.0); elytral length 10.5–11.5 (χ = 11.0); pronotal length 4.0–5.0 (χ = 4.5); pronotal width 5.0–6.5 (χ = 6.0); humeral width 5.0–6.5 (χ = 6.0).

Description of holotype (Fig. 6). Head (Fig. 6C). Labrum: anterior border concave, dorsal surface smooth and glabrous medially, punctate and setose apicolaterally, apically, and basally; anterior edge excavated. Clypeus: vertical, rectangular, flat, shiny, and smooth. Frontoclypeal suture: straight, and opaque; external tubercles rounded, directed anteriorly and laterally. Frontal area: horizontal, flat, smooth and shiny, frontal ridges weak finishing in inner tubercles; inner tubercles smaller than external tubercles. Frontal fossae: punctate and setose. Mesofrontal structure (MFS): of the "falsus" type (see Reyes-Castillo 1970); base subparallel, slightly narrower than MFS' lateral ridges; center horn long with apex acute, largely free and directed anteriorly (Fig. 6D), surpass-

ing posterior margin of clypeus, dorsally with sparse micro-punctures; base of the center horn wide, narrowing gradually until apex; dorsal fossa present at the base of MFS. Occipital fossa: shallow posteriorly and deeper laterally connected with the frontal fossae. Posterior occipital sulcus sinuate. Supraorbital ridge: bituberculate, tubercles of similar size; posterior half of supraorbital ridge not bifurcated. Canthus: with apex rounded, almost oblique, covering 1/3 of the eye, not expanded distally. Eyes: reduced (distal edge of the eye not reaching the distal edge of the canthus), width = 0.3 mm (each eye). Head width = 3.0 mm. Ratio of sums of both eyes widths/total head width = 0.2; postocular area punctate and setose. Ligula: tridentate, central tooth surpassing apex of lateral teeth; lateral teeth rounded; setose punctures present in discal area; posterior border convex. Mentum: lateral lobes rounded and wide, with setose punctures. Basomedial portion protruding ventrally; anterior border at the middle convex; basal fossae present with setose punctures. Hypostomal process: without lateral depression; separated from the mentum by a distance shorter than the width of the anterior width of the hypostomal process. Infraocular ridge (e.g., Fig. 3): short, weak, and wide anteriorly. Mandible: with 3 apical teeth; internal tooth in left mandible tridentate; dorsal tooth occupies at least half length of the mandible. Pronotum: anterior angles rounded. Anterior fossae of marginal sulcus punctate. Lateral fossae without punctures. Marginal groove lacking punctures. Prosternum: opaque; prosternellum with anterior and lateral edges rugose and opaque, anteriorly and posteriorly shiny. Scutellum: smooth and glabrous. Mesosternum: with anterior-lateral areas opaque. Metasternum: with setae anterolaterally, lacking punctures in lateral margins of metasternal disc. Lateral fossae wide posteriorly with setose punctuations. Elytra: anterior border straight. Meeting point of striae 1-10 (see Reyes-Castillo 1970) with one line of punctures. Wings: well developed. Legs: femur I with longitudinal anteroventral groove weakly developed, not reaching the distal part of the femur, posteroventral half pubescent; setae long, sparse, reddish. Abdomen: last sternite with marginal groove incomplete (Fig. 6B). Aedeagus (Fig. 6E, F): in dorsal view phallus elongated (longer than wider). In ventral view distal edges of the phallus more or less at the same level of distal edges of parameres.

Variation. Paratypes vary from the holotype by the following characteristics: internal tubercles weak to obsolete; frontal fossae glabrous or setose; ratio of eyes to head width vary between 0.19 and 0.22; basal fossae of mentum strong, opaque and glabrous or shiny and with setose punctures; infraocular ridge weak or absent; femur I with longitudinal antero-ventral groove weakly developed to obsolete.

Etymology. This species is named after its home state of Veracruz in Mexico.

Distribution. This species is known from cloud forest between around 1900 m in the transverse neo-volcanic system, Mexico. The surrounding states and areas in which this species is distributed have been well-collected, and *Y. veracrucensis* has only been found at three localities in Veracruz, Mexico: La Cortadura Natural Reserve near Coatepec; Chiconquiaco (near La Parra); and the road between Las Minas and Xalapa; Chiconquiaco; Congr. La Guacamaya (19°45'51.4"N, 96°48'1.7"W).

Remarks. Specimens of *Y. veracrucensis* were originally identified as *P. orizabae* and were collected in Reserva La Cortadura in Coatepec, Veracruz, Mexico. Based on

our phylogenetic analysis, *Y. veracrucensis* (*Y.* LCM) and *Y. imbellis* are potential sister species (Fig. 4; PP 0.99/BS 90). Molecular distinctiveness and form of the dorsal ridge in tibia II (as in all species of *Yumtaax*) provide support that this is a distinct species within the genus *Yumtaax*.

Yumtaax laticornis (Truqui, 1857)

Passalus laticornis Truqui, 1857: 262, 316.
Pseudacanthus laticornis (Truqui) [comb. n. by Kaup 1871: 72, 74].
Petrejoides laticornis (Truqui) [comb. n. by Reyes-Castillo 1970: 125].
Yumtaax laticornis (Truqui) [comb. n. by Boucher 2006: 348].

Material examined. Holotype and 31 non-type specimens.

Holotype ♂. MEXICO: Jacale, 1708 (*Sallé*) (BMNH).

Non-type specimens (31 total): 2 ♂, 20 ♀, 9 unknown. MEXICO: Veracruz, Calcahualco, Tecuanapa, bosque mesófilo, alt. 2200 m, VI 1992 (*Capistrán, Delgado*) (IEXA; CFBB).

Diagnosis. *Yumtaax laticornis* is a large (24.5–33.0 mm) brachypterous species and is part of the *Yumtaax laticornis* clade (Fig. 4). This species is diagnosed by the following character combination: the clypeus is vertical (shared with *Y. veracrucensis*, *Y. cameliae*; inclined in other members of *Yumtaax*) and the anterior border is straight (shared with other members of *Yumtaax* except for *Y. recticornis* and *Y. olmecae* with concave anterior border of clypeus); mesofrontal structure (MFS) of the "falsus" type (see Reyes-Castillo 1970) (shared with all members of *Yumtaax* except *Y. cameliae* which has the MFS of the "striatopunctatus" type), with the central tooth largely free (shared with *Y. recticornis*, *Y. veracrucensis*, *Y. cameliae*, *Y. mazatecus*; fused with frontal ridges in *Y. jimenezi*, *Y. imbellis*, *Y. nebulosus*, *Y. olmecae*), directed anteriorly (shared with *Y. veracrucensis*; directed dorsally in *Y. recticornis*, *Y. imbellis*, *Y. nebulosus*, *Y. olmecae*; directed dorsally and anteriorly in *Y. jimenezi*, *Y. mazatecus*; elevated in the posterior half bending abruptly forward in the anterior half in *Y. cameliae*), and reaching the frontoclypeal suture (shared with *Y. veracrucensis*, *Y. cameliae*; not reaching the clypeus in other members of *Yumtaax*); eyes are strongly reduced (shared with *Y. mazatecus*; eyes large in *Y. recticornis*, *Y. imbellis*; eyes moderately reduced in *Y. veracrucensis*, *Y. cameliae*, *Y. jimenezi*, *Y. nebulosus*, *Y. olmecae*); and the scutellum is punctate (smooth in other members of *Yumtaax*).

Dimensions (mm) (n = 19). Total length 24.5–33.0 (χ = 29.5); elytral length 14.0–17.5 (χ = 16.5); pronotal length 6.0–9.0 (χ = 8.0); pronotal width 8.0–11.0 (χ = 10.0); humeral width 7.0–10.0 (χ = 9.0).

Redescription of holotype (Fig. 7). Head (Fig. 7C). Labrum: anterior border concave, dorsal surface smooth and glabrous medially, punctate and setose apicolaterally, apically, and basally; anterior edge excavated. Clypeus: vertical, rectangular, flat, shiny, and smooth. Frontoclypeal suture: straight, and shiny; external tubercles

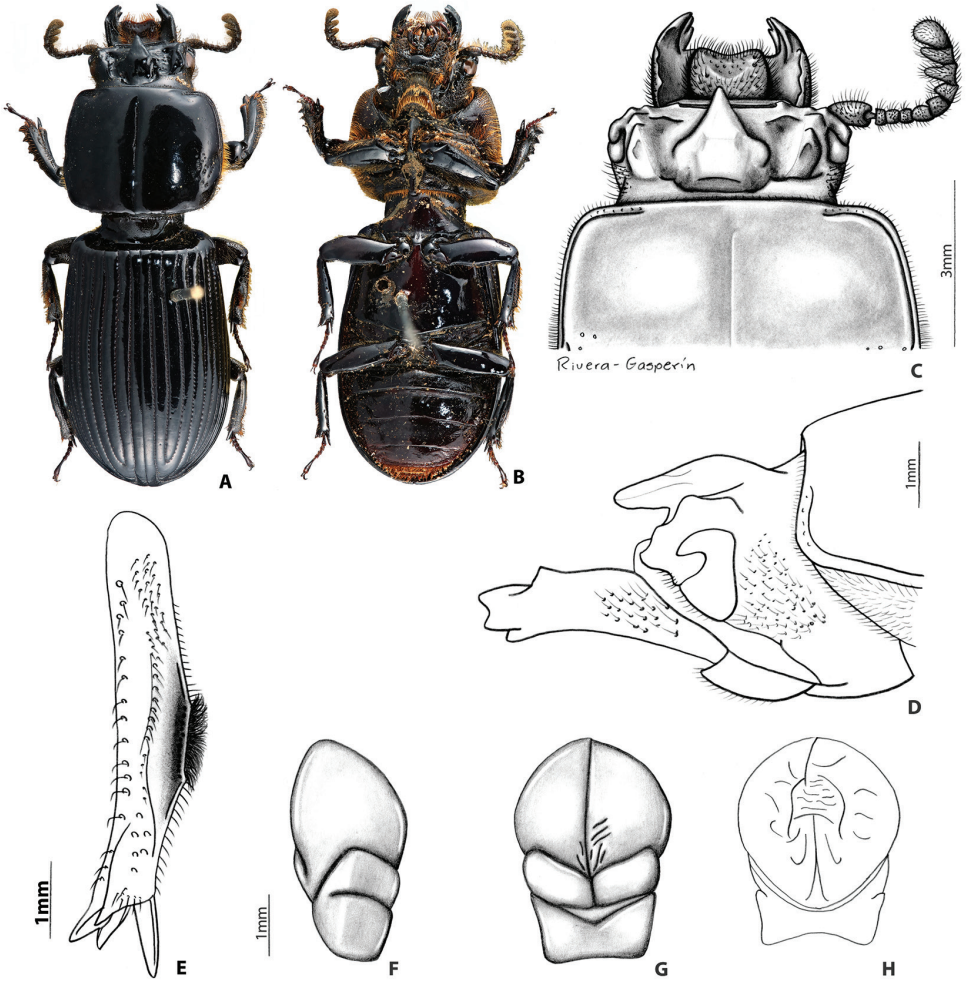


Figure 7. *Yumtaax laticornis* (Truqui), holotype: **A** dorsal habitus **B** ventral habitus **C** dorsal view of pronotum and head **D** lateral view of pronotum and head **E** lateral view of right mesotibia **F** lateral view of aedeagus **G** ventral view of aedeagus **H** dorsal view of aedeagus.

rounded weak, directed anteriorly. Frontal area: vertical, flat, smooth and shiny, frontal ridge absent. Frontal fossae: punctate and glabrous. Mesofrontal structure (MFS): of the "falsus" type (see Reyes-Castillo 1970); base subparallel and narrower than MFS' lateral ridge; center horn long with apex acute, largely free and directed anteriorly (Fig. 7D), surpassing posterior margin of clypeus, dorsally without micro-punctures; base of center horn wide narrowing gradually until apex; dorsal fossa absent at the base of MFS. Occipital fossa: shallow posteriorly and deeper laterally, not connected with the frontal fossae. Posterior occipital sulcus concave. Supraorbital ridge: bituberculate, tubercles of similar size; posterior half of supraorbital ridge bifurcated. Canthus: with apex rounded, covering more than 1/3 of the eye, expanded distally. Eyes: strongly

reduced (distal edge of the eye shorter than the distal edge of the canthus), width = 0.4 mm (each eye). Head width (between posterior tubercles of the supraorbital ridge) = 5.0 mm. Ratio of sums of both eyes widths/total head width = 0.16; postocular area punctate and setose. Ligula: tridentate, central tooth surpassing apex of lateral teeth, lateral teeth rounded; setose punctures present in discal area; posterior border straight. Mentum: lateral lobes rounded and wide, with setose punctures. Basomedial portion protruding ventrally; anterior border at middle straight; basal fossae present, with setose punctures. Hypostomal process: with lateral depression; separated from mentum by a distance larger than the wide of the anterior width of hypostomal process. Infraocular ridge (e.g., Fig. 3): short and wide anteriorly, narrow posteriorly. Mandible: with 3 apical teeth; internal tooth of left mandible bidentate; dorsal tooth occupies more than half length of the mandible. Pronotum: anterior angles rounded. Anterior fossae of marginal sulcus punctate. Lateral fossae without punctures. Marginal groove with punctures. Prosternum: opaque. Prosternellum with anterior half and lateral edges opaque and posterior half and middle area shiny. Scutellum: punctate and glabrous. Mesosternum: with anterior-lateral areas opaque. Metasternum: with setae anterolaterally, lacking punctures in lateral margins of the metasternal disc. Lateral fossae wide glabrous posteriorly with setose punctures anteriorly. Elytra: anterior border straight. Meeting point of striae 1-10 (see Reyes-Castillo 1970) with one line of punctures. Wings: reduced. Legs (Fig. 7E): femur I with longitudinal anteroventral groove strongly developed, reaching the distal end of the femur, posterioventral half pubescent; setae short, dense, reddish. Abdomen: last sternite with marginal groove complete (Fig. 7B). Aedeagus (Fig. 7F, G, and H) (Description based on non-type material): dorsal view phallus globose (wider than long). Ventral view lateral edges of the phallus surpassing the laterodistal edges of the parameres.

Variation. The non-type material differs from the holotype in the following characters: internal tubercles obsolete to strongly developed; frontal ridges obsolete to strongly developed; frontal area glabrous to sparsely setose; ratio of eyes versus head width varies from 0.13-0.23; pronotum laterally with or without strong punctures, even at the individual level (right vs left side of the pronotum); prosternelum completely opaque or opaque and shiny.

Distribution. This species is known from cloud forest (bosque mesófilo, 2200 m elevation) at Orizaba Peak, Veracruz, Mexico. In the original description, Truqui (1857) cited one specimen collected by Sallé from Jacal near the Orizaba Volcano. This locality corresponds to El Jacal, Coscomatepec, Orizaba Peak (Reyes-Castillo 2011).

MEXICO: Veracruz: Calcahualco (Tecuanapa, road from Calcahualco to the Pico de Orizaba), Jacale, Pico de Orizaba.

Remarks. Castillo and Reyes-Castillo (1984) redescribed *Y. laticornis* without examining type specimens. We compared two specimens of *Y. laticornis* determined by Castillo and Reyes-Castillo with the holotype specimen designated by M. E. Bachus at The Natural History Museum, London. Close examination of the holotype and results of the phylogenetic analysis (Fig. 4 and Suppl. materials 2–4) provide evidence that *Y. laticornis* is not conspecific with *Y. laticornis sensu* Castillo and Reyes-Castillo (1984) (*Y.*

LM in Suppl. materials 2–4). Specimens described as *Y. laticornis* by Castillo and Reyes-Castillo (1984) correspond with *Y. cameliae* sp. n. (*Y.* LM in Suppl. materials 2–4), and the holotype of *Y. laticornis* corresponds with *Y.* CM (Suppl. materials 2–4). The overall length of the holotype specimen is 30.0 mm, and this falls within the size range for *Y. laticornis* (25.0–33.0 mm), but not within the range for *Y. cameliae* (22.5–25.5 mm). Furthermore, based on distribution and biogeography, *Y. cameliae* has been collected only in the type locality where suitable habitat for the species occurs. This area is geographically isolated from the distribution area of *Y. laticornis*. *Yumtaax laticornis*' inclusion in the *Y. laticornis* clade (is strongly supported (PP 1.0/BS 100) (Fig. 4).

***Yumtaax cameliae* Beza-Beza, Reyes-Castillo & Jameson, sp. n.**
http://zoobank.org/FAEEDD32-CA2C-4FD5-99D8-3A4A73DDC04F

Material examined. 22 type specimens.

Holotype ♀. MEXICO: Veracruz, Acultzingo, Puerto del Aire, 2400 msnm, bosque mesófilo de montaña, encinar tronco 4, VII-16-80 (*C. Castillo*) (IEXA).

Paratypes (21 total). 1 ♂, 7 ♀ with same label data as holotype. MEXICO: 3 ♀, Veracruz, Acultzingo, VI-I-1963 (*G. Halffter*) (IEXA). 1 ♀, Acultzingo, Puerto del Aire, 2400 msnm, bosque mesófilo de montaña, encinar tronco 4, VII-17-80 (*C. Castillo*) (IEXA, CFBB). 2 ♂, 3 ♀, Acultzingo, Puerto del Aire, 2400 msnm, bosque mesófilo de montaña, encinar tronco 4, VIII-16-80 (*C. Castillo*) (IEXA, CFBB). 4 ♀, Acultzingo, Puerto del Aire, 2400 msnm, bosque mesófilo de montaña, encinar tronco 4, VIII-17-80 (*C. Castillo*) (IEXA, CFBB).

Diagnosis. *Yumtaax cameliae* is a medium sized (22.5–25.5 mm), macropterous species that is part of the *Y. laticornis* clade (Fig. 4). This species is diagnosed by the following character combination: the clypeus is vertical (shared with *Y. laticornis*, *Y. veracrucensis*; inclined in other members of *Yumtaax*) and with the anterior border straight (shared with other members of *Yumtaax* except for *Y. recticornis* and *Y. olmecae* with concave anterior border of clypeus); mesofrontal structure (MFS) of the "striatopunctatus" type (see Reyes-Castillo 1970) (MFS of the "falsus" type in other members of *Yumtaax*), with the central tooth largely free (shared with *Y. recticornis*, *Y. laticornis*, *Y. veracrucensis*, *Y. mazatecus*; fused with frontal ridges in *Y. jimenezi*, *Y. imbellis*, *Y. nebulosus*, *Y. olmecae*), elevated in the posterior half and bending abruptly forward in the anterior half (directed dorsally *Y. recticornis*, *Y. imbellis*, *Y. nebulosus*, *Y. olmecae*; directed dorsally and anteriorly in *Y. jimenezi*, *Y. mazatecus*; directed anteriorly in *Y. veracrucensis*, *Y. laticornis*), reaching the clypeus (shared with *Y. veracrucensis*, *Y. cameliae*; not reaching the clypeus in other members of *Yumtaax*); and moderately reduced eyes (shared with *Y. veracrucensis*, *Y. jimenezi*, *Y. nebulosus*, *Y. olmecae*; large in *Y. recticornis*, *Y. imbellis*; strongly reduced in *Y. laticornis*, *Y. mazatecus*).

Dimensions (mm) (n = 4). Total length 22.5–25.5 (χ = 24.0); elytral length 13.5–14.0 (χ = 14.5); pronotal length 6.0–7.0 (χ = 6.5); pronotal width 7.0–9.5 (χ = 8.5); humeral width 7.0–8.0 (χ = 7.5).

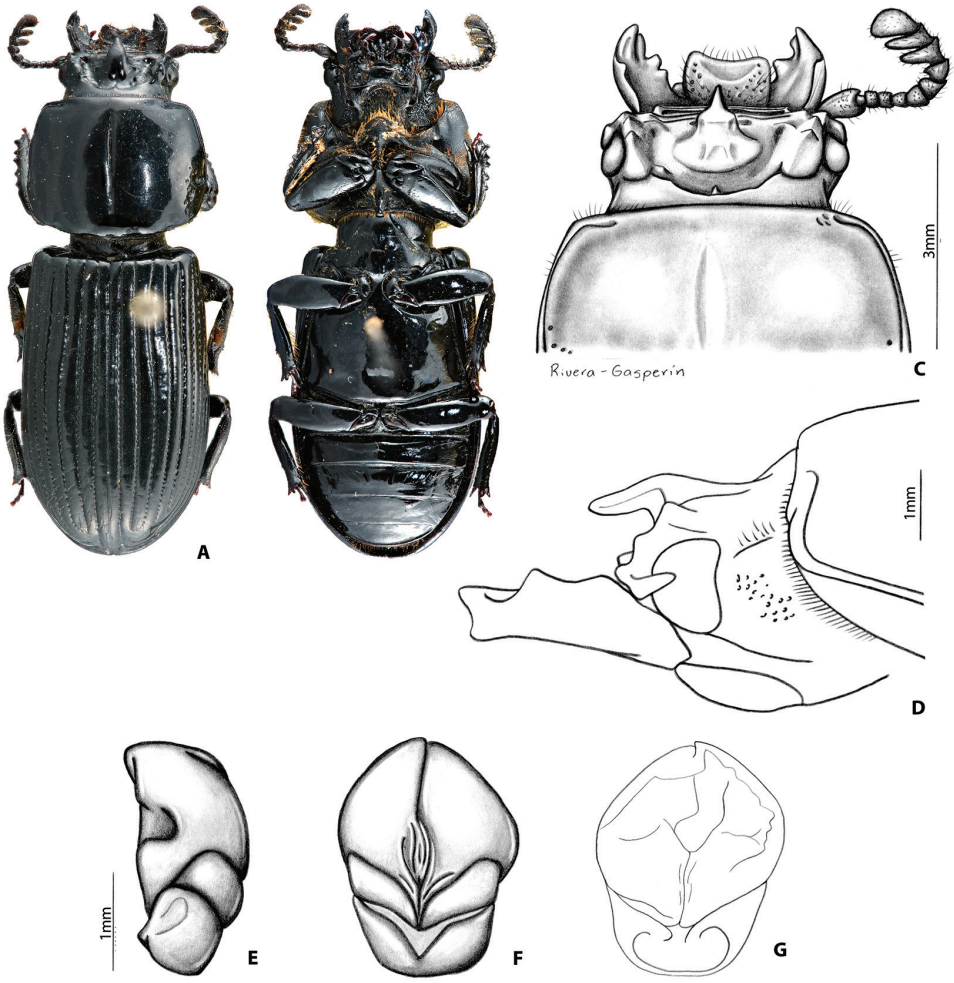


Figure 8. *Yumtaax cameliae* Beza-Beza, Reyes-Castillo & Jameson, sp. n., holotype: **A** dorsal habitus **B** ventral habitus **C** dorsal view of pronotum and head **D** lateral view of pronotum and head **E** lateral view of aedeagus **F** ventral view of aedeagus **G** dorsal view of aedeagus.

Description of holotype (Fig. 8). Head (Fig. 8C). Labrum: anterior border concave, dorsal surface smooth and glabrous medially, punctate and setose apicolaterally, apically, and, basally; anterior edge excavated. Clypeus: vertical, rectangular, flat, shiny, and smooth. Frontoclypeal suture: straight, and shiny; external tubercles rounded, weak, directed dorsally and anteriorly. Frontal area: inclined, flat, smooth, and shiny; frontal ridges absent without inner tubercles. Frontal fossae: impunctate and glabrous. Mesofrontal structure (MFS): of the "striatopunctatus" type (see Reyes-Castillo 1970); base subparallel and narrower than MFS' lateral ridge; center horn long with apex acute, largely free elevated in the posterior half bending abruptly forward in the anterior half (Fig. 8D), reaching the posterior margin of clypeus, dorsally without micro-

punctures; base of center horn wide, not narrowing in the posterior half and narrowing abruptly in the anterior half until apex; dorsal fossa present at base of MFS. Occipital fossa: shallow posteriorly and deeper laterally connected with the frontal fossae. Posterior occipital sulcus concave. Supraorbital ridge: bituberculate, tubercles of similar size; posterior half of supraorbital ridge not bifurcated. Canthus: with apex rounded, covering less than 1/3 of the eye, not expanded distally. Eyes moderately reduced (distal edge of the eye more or less at the distal edge of the canthus), width = 0.6 mm (each eye). Head width (between posterior tubercles of the supraorbital ridge) = 4.3 mm. Ratio of sums of both eyes widths/total head width = 0.27; postocular area punctate and setose. Ligula: tridentate, central tooth surpassing apex of lateral teeth; lateral teeth rounded; glabrous punctures present in discal area; posterior border straight. Mentum: lateral lobes rectangular and wide, with setose punctures. Basomedial portion protruding ventrally; anterior border at middle convex; basal fossae present. Hypostomal process: without lateral depression; separated from mentum by a distance shorter than the width of the anterior width of hypostomal process. Infraocular ridge (Fig. 3): short and wide anteriorly, narrow posteriorly. Mandible: with 3 apical teeth; internal tooth of left mandible bidentate; dorsal tooth occupies half of length of the mandible. Pronotum: anterior angles rounded. Anterior fossae of marginal sulcus punctate. Lateral fossae impunctate. Marginal groove lacking punctures. Prosternum: opaque; prosternellum with anterior half and lateral edges opaque and posterior half and middle area shiny. Scutellum: smooth and glabrous. Mesosternum: with anterolateral areas opaque. Metasternum: with setae anterolaterally, lacking punctures in lateral margins of metasternal disc. Lateral fossae wide posteriorly with setose punctures. Elytra: anterior border straight. Meeting point of striae 1-10 (see Reyes-Castillo 1970) with one line of punctures. Wings: well developed. Legs: femur I with longitudinal anteroventral groove strongly developed, reaching distal end of femur, posteroventral half pubescent; setae long, sparse, reddish. Abdomen: last sternite with marginal groove complete and opaque laterally (Fig. 8B). Aedeagus (Fig. 8E, F, G) (based on male paratype): in dorsal view phallus globose (wider than long). In ventral view distal edges of phallus surpassing the distal edge of the parameres.

Variation. Paratypes vary from the holotype in the following characters: internal tubercles obsolete to strongly developed; frontal ridges obsolete to strongly developed; frontal area glabrous to setose; ratio of eyes versus head width varies from 0.19-0.31; central area of the ligula always punctate, occasionally setose; pronotum with lateral fossae with or without strong punctures, even at the individual level (right vs left side of the pronotum); prosternellum shiny (one specimen of the type series) or opaque in anterior half; terminal sternite with lateral areas of the marginal groove opaque or not.

Etymology. The species is named *Y. cameliae,* honoring Passalidae researcher Camelia Castillo whose research (Castillo and Reyes-Castillo 1984) provided a better understanding of *Yumtaax*.

Distribution. This species is known only from the type locality in Veracruz, Mexico. It was collected in a small patch of oak forest (bosque mesófilo de montaña) surrounding the Puerto del Aire village at 2400 m elevation.

Remarks. Specimens of *Y. cameliae* were originally identified as *Y. laticornis* (Castillo and Reyes-Castillo 1984). Close examination of the *Y. laticornis* holotype (see "Remarks" for *Y. laticornis*) and distribution of the holotype suggested that *Y. laticornis sensu* Castillo and Reyes-Castillo and *Y. laticornis* Truqui do not correspond to the same species.

***Yumtaax jimenezi* Beza-Beza, Reyes-Castillo & Jameson, sp. n.**
http://zoobank.org/C8313A69-5326-49BF-829B-53343207F53E

Material examined. 27 type specimens.

Holotype ♂. MEXICO: Veracruz, Calcahualco, Tecuanapa. Bosque mesófilo, alt. 2400 m V-2/3-1992 (*Capistrán and Delgado*) (IEXA).

Paratypes (26 total). MEXICO: Veracruz: 10 ♀, 15 unknown sex, Calcahualco, Tecuanapa, bosque mesófilo, alt. 2400 m, V-2/3-1992 (*Capistrán and Delgado*). 5 ♂, 8 ♀, 34 sex unknown, Calcahualco, Tecuanapa, bosque mesófilo, alt. 2400 m, V-1992 (*Capistrán and Delgado*). 14 ♀, Calcahualco, Tecuanapa, bosque mesófilo, alt. 2200 m, VI-1992 (*Capistrán and Delgado*). 1 ♂, Calcahualco, Dos Caminos, II-29-1992, alt. 1415 m, bosque de encino-pino, dentro de *Quercus* sp. (*R. Novelo, F. Capistrán and L. Delgado*). 1 ♀, 2 sex unknown, Calcahualco, Nueva Vaquería (1 km before), II-28-1992, alt. 2700 m, bosque de pino-encino, en tronco (*R. Novelo, F. Capistrán and L. Delgado*). 2 ♀, Veracruz, Calcahualco, 1 km antes de Nueva Vaquería, 2700 m, VI-1992, (*L. Delgado and Capistrán*) (CFBB, IEXA). 1 ♂, Cosautlan, Los Laureles, alt. 2680 m, VIII-27-1999 (*J. P. Lumaret*). 4 sex unknown, Orizaba, Sallé, Mex. Collection (*Sallé*) (BMNH). 1 sex unknown, Mexico (*Truqui*) (BMNH).

Diagnosis. *Yumtaax jimenezi* is a small (18.5–23.0 mm) macropterous species, and it is part of the *Y. laticornis* clade (=Fig. 4). This species is diagnosed by the following character combination: clypeus is inclined (shared with *Y. recticornis*, *Y. imbellis*, *Y. mazatecus*, *Y. nebulosus*, *Y. olmecae*; vertical in *Y. laticornis*, *Y. cameliae*, *Y. veracrucensis*) and with the anterior border straight (shared with other members of *Yumtaax* except for *Y. recticornis* and *Y. olmecae* that have the anterior border of clypeus concave); MFS of the "falsus" type (see Reyes-Castillo 1970) (shared with all members of *Yumtaax* except *Y. cameliae* which has the MFS of the "striatopunctatus" type), with the central tooth that is not free (fused with frontal ridges) (shared with *Y. imbellis*, *Y. nebulosus*, *Y. olmecae*; largely free in *Y. recticornis*, *Y. veracrucensis*, *Y. laticornis*, *Y. cameliae*, *Y. mazatecus*), directed dorsally and anteriorly (shared with *Y. mazatecus*, directed dorsally *Y. recticornis*, *Y. imbellis*, *Y. nebulosus*, *Y. olmecae*; directed anteriorly in *Y. veracrucensis*, *Y. laticornis*; elevated in the posterior half bending abruptly forward in the anterior half in *Y. cameliae*), and not reaching the clypeus (shared with *Y. recticornis*, *Y. imbellis*, *Y. mazatecus*, *Y. nebulosus*, *Y. olmecae*; reaching the clypeus in *Y. laticornis*, *Y. cameliae*, *Y. veracrucensis*); and moderately reduced eyes (shared with *Y. veracrucensis*, *Y. cameliae*, *Y. nebulosus*, *Y. olmecae*; large in *Y. recticornis*, *Y. imbellis*; strongly reduced in *Y. laticornis*, *Y. mazatecus*).

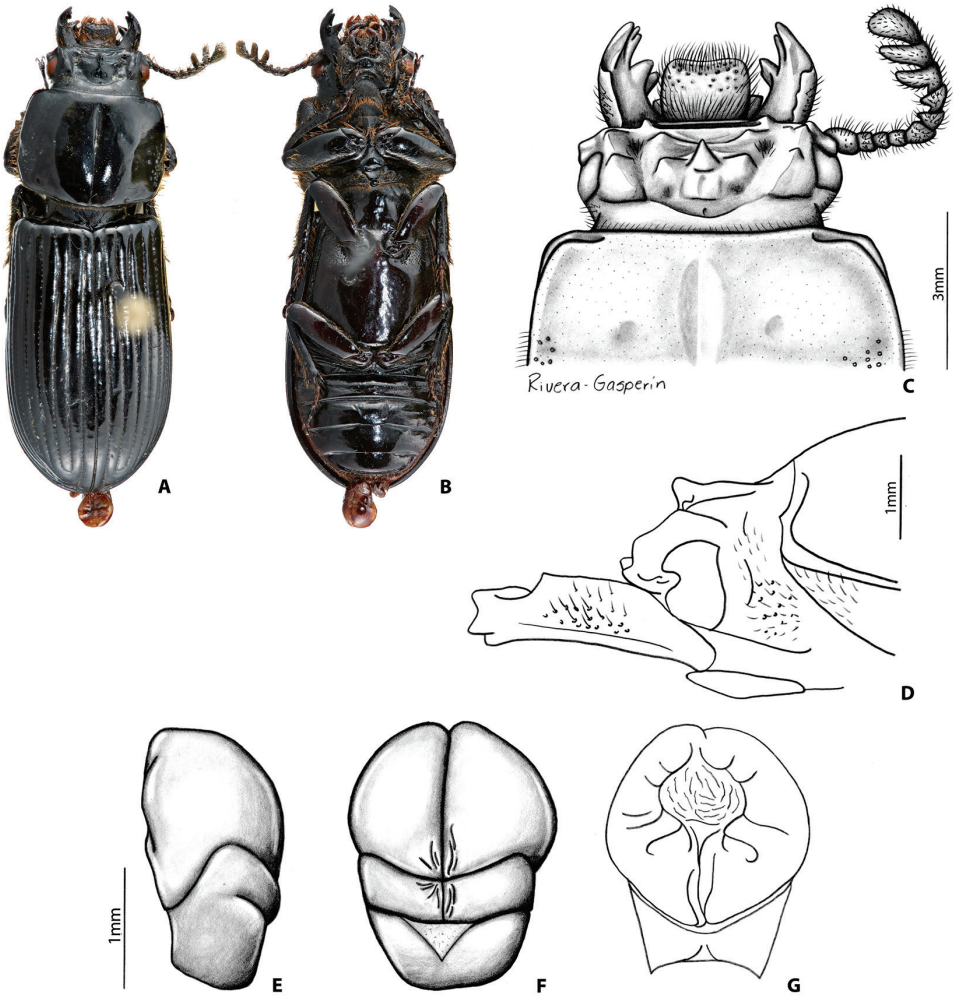


Figure 9. *Yumtaax jimenezi* Beza-Beza, Reyes-Castillo & Jameson, sp. n., holotype: **A** dorsal habitus **B** ventral habitus **C** dorsal view of pronotum and head **D** lateral view of pronotum and head **E** lateral view of aedeagus **F** ventral view of aedeagus **G** dorsal view of aedeagus.

Dimensions (mm) (n = 12). Total length 18.5–23.0, (χ = 20.5); elytral length 11.5–14.0, (χ = 12.5); pronotal length 4.0–6.0, (χ = 5.5); pronotal width 5.5–7.0, (χ = 6.5); humeral width 5.5–7.0, (χ = 6.5).

Description of holotype (Fig. 9). Head (Fig. 9C). Labrum: anterior border concave, dorsal surface smooth and glabrous medially, and punctate and setose in the apicolaterally, apically, and basally; anterior edge excavated. Clypeus: inclined, rectangular, shiny, and smooth. Frontoclypeal suture: straight, and shiny. External tubercles rounded and directed dorsally. Frontal area: inclined, smooth, and shiny, frontal ridges present finishing in inner tubercles. Frontal fossae: punctate and setose. Mesofrontal

structure (MFS): of the "falsus" type (see Reyes-Castillo 1970); base subparallel and narrower than the MFS' lateral ridge; center horn short with apex rounded, not free (fused with frontal ridges) and directing dorsally (Fig. 9D), not reaching the posterior margin of clypeus (Fig. 9C, D), dorsally without micro-punctures; base of the center horn narrow not narrowing down along its length (central tooth tubercle like shape [Fig. 9C, D]); dorsal fossa present at the base of MFS. Occipital fossa: shallow posteriorly and deeper laterally not connected to frontal fossae. Posterior occipital sulcus sinuate. Supraorbital ridge: bituberculate, tubercles of similar size; posterior half of supraorbital ridge not bifurcated. Canthus: with apex rounded covering less than 1/3 of the eye, expanded distally. Eyes: reduced (distal edge of the eye more or less at the distal edge of the canthus), width = 0.5 mm (each eye). Head width = 3.5 mm. Ratio of sums of both eyes widths/total head width = 0.24; postocular area punctate and setose. Ligula: tridentate, with central tooth surpassing apex of lateral teeth, lateral teeth rounded; setose punctures present in discal area; posterior border convex. Mentum: lateral lobes rounded and wide, with setose punctures. Basomedial portion protruding ventrally; anterior border at middle convex; basal fossae absent. Hypostomal process: without lateral depression; separated from mentum by a distance shorter than the wide of the anterior width of hypostomal process. Infraocular ridge absent. Mandible: with 3 apical teeth; internal tooth of left mandible bidentate; dorsal tooth occupies less than half length of the mandible. Pronotum: anterior angles rounded. Anterior fossae of marginal sulcus impunctate. Lateral fossae with heavy punctures. Marginal groove lacking punctures. Prosternum: opaque. Prosternellum anterior half opaque and lateral edges and posterior half shiny. Scutellum: smooth and glabrous. Mesosternum: with anterolateral areas opaque. Metasternum: with setae in anterolaterally, without punctures in lateral margins of metasternum disc. Lateral fossae wide across the metathorax, with setose punctures. Elytra: anterior border straight. Meeting point of striae 1-10 (see Reyes-Castillo 1970) with one line of punctures. Wings: well developed. Legs: femur I with longitudinal anteroventral groove weakly developed in the proximal half and strongly developed on the distal end of the femur, posteroventral half pubescent; setae long, sparse, reddish. Abdomen: last sternite with marginal groove complete (Fig. 9B). Aedeagus (Fig. 9E, F, G): in dorsal view phallus globose (wider than long). In ventral view distal edges of phallus surpassing the distal edge of the parameres.

Variation. Frontoclypeal suture can be from opaque to shiny; internal tubercles from strongly to weakly marked but always present; ratio of eyes and head with varies from 0.18-0.32; supraocular ridge from weak to absent; hypostomal process with weak lateral depression to lateral depression absent; prosternellum varies from anterior half opaque and lateral edges and posterior half shiny to anterior half and lateral edges opaque and posterior half and middle shiny to completely opaque; femur I longitudinal anterior-ventral groove from weak in the proximal half to absent; femur I longitudinal anterior-ventral groove from strongly developed in the distal half to absent.

Etymology. This species is named in honor of Passalidae worker Dr. Larry Jiménez-Ferbans who assisted in collecting trips supporting this study.

Distribution. This species is known from cloud forest (bosque mesófilo) at 2400 m elevation from the state of Veracruz, Mexico.

MEXICO: Veracruz: Calcahualco (Tecuanapa, Dos Caminos, Nueva Vaquería [1 km before]).

Remarks. *Yumtaax jimenezi* is a cryptic, widespread species that has been confused with *Y. recticornis*. Previously, *Y. recticornis s. l.* was thought to be broadly distributed in Mexico from the Sierra Madre Oriental in the Mexican Transvolcanic Belt and Sierra Madre del Sur (Reyes-Castillo 1970, Castillo and Reyes-Castillo 1984, Boucher 2006). Phylogenetic analysis (Fig. 4 and Suppl. materials 2–4) and close examination of morphology provide evidence that *Y. recticornis s. l.* comprises two cryptic species [*Y. recticornis* (= *Y. recticornis* OM) and *Y. jimenezi* (*Y. recticornis* VM)].

These species are distinguished by eye size (small in *Y. jimenezi* and large in *Y. recticornis*), shape of the central tooth of the MFS (center horn short with apex rounded, not free [fused with frontal ridges] and directed dorsally [Fig. 9D] in *Y. jimenezi;* center horn short with apex rounded, largely free and directed anteriorly and dorsally [Fig. 5D] in *Y. recticornis*), and the shape of the surface of the frons and clypeus (concave in *Y. recticornis* versus flat in *Y. jimenezi*). Interestingly, the reduced eye size in *Y. jimenezi* results in the distal expansion of the canthus. Based on seven exemplars, phylogenetic analysis (Fig. 4) strongly supports *Y. jimenezi* as a unique lineage (1.0 PP/100 BS).

Conclusions

A single, unique synapomorphy (dorsal mesotibial ridge elevated at the middle), molecular phylogenetic analysis, and distributional affinities collectively support the hypothesis of *Yumtaax* monophyly.

Yumtaax species, as with most Passalidae, exhibit a high degree of morphological conservatism, rendering traditional systematics studies quite challenging. Cryptic species, such as those revealed in this study, are likely to be discovered by employing molecular data and careful consideration of morphological characters. Further studies, ideally those that include significant additional molecular phylogenetic data, are needed to rigorously evaluate the Passalidae species boundaries and evolutionary history.

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Schuster (Guatemala City, Guatemala). Vouchers and type specimens are deposited at the Instituto de Ecología, A. C. (IEXA); The Natural History Museum, London (BMNH); Martin-Luther-Universitat, Halle, Germany (MLU); Wichita State University Collection, Wichita, KS (WICH); and the Cristian Beza-Beza collection at UVG (CFBB). We are grateful to several Passalidae experts for critical discussions and assistance during this research: Enio Cano, Jack Schuster, Larry Jimenez, and Allan Gillogly. Many thanks to members of the Jameson Biodiversity Lab (Wichita State University, Wichita, KS): Oliver Keller, Matt Moore, and David Wickell. Thanks to Sara Rivera-Gasperín for species illustrations. Thanks to Dr. Duane McKenna and the McKenna lab at the University of Memphis for manuscript edits. Funding for this research was provided by the Wichita State University Department of Biological Sciences; the Harvard University Museum of Comparative Zoology; and NSF DBI 0743783 to M.L. Jameson and collaborators.

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Supplementary material 1

Table S1

Authors: Cristian Fernando Beza-Beza, James Beck, Pedro Reyes-Castillo, Mary Liz Jameson

Data type: specimen data

Explanation note: Voucher specimens for taxa included in the molecular analysis (species, depository, preservation method, collection data, and GenBank DNA sequence accession numbers).

Supplementary material 2

Figure S1

Authors: Cristian Fernando Beza-Beza, James Beck, Pedro Reyes-Castillo, Mary Liz Jameson

Data type: molecular data

Explanation note: 50% majority-rule consensus of Bayesian posterior probabilities resulting from analysis of the COI data. Bayesian posterior probabilities (PP) (> 0.50) and bootstrap support (BS) (> 50) are noted. *Yumtaax veracrucensis* sp. n. = *Yumtaax* LCM; *Yumtaax recticornis* = *Yumtaax recticornis* OM; *Yumtaax laticornis* = *Yumtaax* CM; *Yumtaax cameliae* sp. n. = *Yumtaax* LM; *Yumtaax jimenezi* sp. n. = *Yumtaax recticornis* VM..

Supplementary material 3

Figure S2

Authors: Cristian Fernando Beza-Beza, James Beck, Pedro Reyes-Castillo, Mary Liz Jameson

Data type: molecular data

Explanation note: 50% majority-rule consensus of Bayesian posterior probabilities resulting from analysis of the 12S data. Bayesian posterior probabilities (PP) (> 0.50) and bootstrap support (BS) (> 50) are noted. *Yumtaax veracrucensis* sp. n. = *Yumtaax* LCM; *Yumtaax recticornis* = *Yumtaax recticornis* OM; *Yumtaax laticornis* = *Yumtaax* CM; *Yumtaax cameliae* sp. n. = *Yumtaax* LM; *Yumtaax jimenezi* sp. n. = *Yumtaax recticornis* VM.

Supplementary material 4

Figure S3

Authors: Cristian Fernando Beza-Beza, James Beck, Pedro Reyes-Castillo, Mary Liz Jameson

Data type: molecular data

Explanation note: 50% majority-rule consensus of Bayesian posterior probabilities resulting from analysis of the 28S data. Bayesian posterior probabilities (PP) (> 0.50) and bootstrap support (BS) (> 50) are noted. *Yumtaax veracrucensis* sp. n. = *Yumtaax* LCM; *Yumtaax recticornis* = *Yumtaax recticornis* OM; *Yumtaax laticornis* = *Yumtaax* CM; *Yumtaax cameliae* sp. n. = *Yumtaax* LM; *Yumtaax jimenezi* sp. n. = *Yumtaax recticornis* VM.

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Frit flies of Turkey with descriptions of two new species and new records (Diptera, Chloropidae)

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Abstract

Faunistic records for 88 frit flies species from southwestern Turkey (Muğla province) and from Samsun (north Turkey) are given. Two species, *Dicraeus civeleki* **sp. n.**, and *Meromyza samsunensis* **sp. n.**, are described as new to science. Altogether, nine genera (*Calamoncosis*, *Eribolus*, *Gaurax*, *Incertella*, *Speccafrons*, *Trachysiphonella*, *Chloropsina*, *Eutropha*, and *Lagaroceras*) and 46 species are recorded for the first time from Turkey.

Keywords

Acalyptratae, *Dicraeus*, Diptera, *Meromyza*, Turkey

Introduction

Frit flies (Diptera, Chloropidae) are small to medium sized flies, adult body length 1.5–5.0 mm, rarely larger, with reduced bristling. Body colour very variable, most species are entirely black, and often with metallic sheen (subfamily Oscinellinae, Siphonellopsinae, Rhodesiellinae), whereas some species are yellow with black, red or

brown longitudinal stripes on the scutum (subfamily Chloropinae). The adults occur in various marshy habitats, in deciduous woods, in damp meadows and in open areas. Chloropid larvae have varied feeding habits. Many species are phytophagous, and some of those damage cereals and other grasses. There are also saprophagous species, a few species that have been bred from fungi, and some predaceous species.

The family Chloropidae has not been an object of focused investigation in Turkey. Only two species, *Scoliophthalmus civeleki* and *Elachiptera bimaculata*, were included in the first Turkish checklist of Diptera (Koçak and Kemal 2009). Nartshuk (2012) summarized all published historical data, identified several specimens from Turkey, and published a more complete list in which she listed 64 species from 31 genera and 4 subfamilies. Koçak and Kemal (2013) took over the list of species from Nartshuk (2012) but forgot to include the work of Deeming and Al-Dhafer (2012) with the first record of *Rhodesiella fedtshenkoi* from Turkey. Kubik et al (2016) described *Tricimba dursuni* from Turkey as new to science. Two other species described as new to science in the current paper and 46 species recorded for the first time from Turkey increasing the total number of known Turkish species to 114.

Materials and methods

The studied material, unless stated otherwise, was collected between 2011–2015 by M. Barták and Š. Kubík, and it is deposited in the collection of the Czech University of Life Sciences, Prague. It originates from southwestern and northern Turkey, mainly from the Muğla province and, to a lesser extent, also from the city of Samsun (Samsun province). The specimens were collected by Malaise traps (MT) and yellow and white pan water traps (PT), or they were swept from vegetation (SW). Most of the specimens were originally preserved in alcohol and were dried and mounted later on using the method described by Barták (1997). The genitalia of the described species here were macerated in 10 % KOH (24 hours, room temperature) and later stored together with the specimens on plastic tags and fixed with butyl-methacrylate copolymer of methyl-methacrylate, xylene. The genitalia and individual species were photographed using a Nikon D300 digital camera mounted on a Nikon SMZ-U microscope and images were edited with the computer software NIS-Elements 3.0. On average, each final image is a stack from 15 layers. Images were improved using the software Adobe Photoshop, genitalia served as models for outline of hand drawn illustrations; details were added by direct observation of the genitalia.

The morphological terms used here follow Merz and Haenni (2000). The distribution of species, unless stated otherwise, was taken from Nartshuk (2012, 2013). The species recorded here with for the first time from Turkey are marked by an asterisk and males, females are abbreviated M, F, respectively.

List of species

Subfamily: Siphonellopsinae

***Apotropina longepilosa* (Strobl, 1893)**

Material examined. Samsun, University campus, 22.vi–4.vii.2014, 3M.

Distribution. Widely distributed in the southern Palaearctic Region, from Europe to the Russian Far East and Mongolia.

***Siphonellopsis lacteibasis* Strobl, 1906**

Material examined. Turkey: Akyaka, river bank + salty meadow, 37°03'16''N, 28°19'57"E, 16.–27.v.2011, 2M and 1F.

Distribution. From southern Europe and North Africa to Central Asia.

Subfamily: Rhodesiellinae

***Rhodesiella fedtshenkoi* Nartshuk, 1978**

Material examined. Turkey: 8 km S of Çine, river bank, 68 m, 37°32'34"N, 28°03'46"E, 21.ix.2012, 6M and 5F; Turkey: Toparlar, lowland forest, 36°58'39"N, 28°39'30"E, sweeping, 5.–7.5.2013, 4M and 2F.

Distribution. the species was described from Kyrgyzstan and further recorded from Japan, Yemen, Saudi Arabia, Tunisia, Greece, Macedonia and Cyprus. Deeming and Al-Dhafer (2012) recorded this species from Turkey for the first time.

***Scoliophthalmus civeleki* Deeming, 2006**

Material examined. Turkey: Akyaka, pasture, 4 m, 37°03'09"N, 28°20'17"E, 23.–27. ix.2012, 2M.

Distribution. originally described and hitherto known only from Turkey.

****Scoliophthalmus trapezoides* Becker, 1903**

Material examined. Turkey: Akyaka, pasture, 4 m, 37°03'09"N, 28°20'17"E, 23.–27. ix.2012, 1M.

Distribution. described from Egypt and further recorded from Kenya, Uganda, Tanzania, Zambia, Mozambique, Senegal, Burkina Faso, Nigeria, Cameroun, South Africa, Yemen, Saudi Arabia, Israel and Cyprus.

Subfamily: Oscinellinae

***Aphanotrigonum bicolor* Nartshuk, 1964**

Material examined. Turkey: Akyaka, forest, 37°03'16"N, 28°19'35"E, 30.4.–9.5.2013, 30 m, 2M; Turkey: Akyaka, 40 m, forest, SW + PT, 37°03'21"N, 28°19'09"E, 16.–27.v.2011, 1M; Samsun, University campus, 22.vi–4.vii.2014, 4M.

Distribution. southern Palaearctc Region, from Hungary to Central Asia.

***Aphanotrigonum femorellum* (Collin, 1946)**

Material examined. Turkey: Akyaka, pasture, 37°03'19"N, 28°20'07"E, 28.4.–8.5.2013, 6 m, 5M and 2F; Turkey: Akyaka, pasture, 4 m, 37°03'09"N, 28°20'17"E, 23.–27.ix.2012, 14M and 18F; Samsun, University campus, 22.vi–4.vii.2014, 1M.

Distribution. a widely distributed but rare Palaearctic species, known from Europe and North Africa to Oman and Mongolia.

****Aphanotrigonum inerme* Collin, 1946**

Material examined. Turkey: Akyaka, salty meadow, SW + PT, 37°02'53"N, 28°19'39"E, 28.4.–9.5.2013, 3M; Turkey: Toparlar, lowland forest, 36°58'39"N, 28°39'30"E, sweeping, 5.–7.5.2013, 2M.

Distribution. West Palaearctic species.

****Aphanotrigonum parahastatum* Dely-Draskovits, 1981**

Material examined. Turkey: Gökçeova Gölü, lake shore, 1 750 m, 37°03'42.52"N, 28°48'28.42"E, 20.ix.2012, 12M and 14F; Turkey: 8 km S of Çine, river bank, 68 m, 37°32'34"N, 28°03'46"E, 21.ix.2012, 18M and 7F; Turkey: Muğla, University, campus, PT, 700 m, 37°09'42"N, 28°22'21"E, 21.–24.ix.2012, 10M and 14F,: Samsun, University campus, 22.vi–4.vii.2014, 1M.

Distribution. a mediterranean species, known from the North Africa, Greek mainland, French mainland, Crete and Bulgaria.

****Calamoncosis duinensis* (Strobl, 1909)**

Material examined. Turkey: Akyaka, river bank + salty meadow, 37°03'16"N, 28°19'57"E, 16.–27.v.2011, 2M and 1F; Turkey: Akyaka, pasture, 4 m, 37°03'09"N, 28°20'17"E, 23.–27.ix.2012, 3M; Turkey: Akyaka, salty meadow, SW + PT,

37°02'53"N, 28°19'39"E, 28.4.–9.5.2013, 2M and 3F; Turkey: Toparlar, lowland forest, 36°58'39"N, 28°39'30"E, sweeping, 5.–7.5.2013, 4M.

Distribution. a widely distributed Palaearctic species.

Conioscinella frontella (Fallén, 1820)

Material examined. Turkey: Muğla, University campus, MT, 720 m, 37°09'42"N, 28°22'13"E, H. Kavak, 26.v.–26.vi.2015, 2M.

Distribution. a widely distributed Palaearctic species, known from Europe to Israel and Mongolia.

**Dicraeus* (*Dicraeus*) agropyri Nartshuk, 1964

Material examined. Turkey: 13km NE of Muğla, pasture/pine wood, 1200 m, 37°14'50"N, 28°30'E, 23.–27.vi.2015, 2M.

Distribution. the species is known from Russia East, Russia South, Ukraine and East Palaearctic.

**Dicraeus beschovskii* Nartshuk, 2010

Material examined. Turkey: Akyaka, pasture, 37°03'19"N, 28°20'07"E, 28.4.–8.5.2013, 6 m, 2M; Turkey: Toparlar, lowland forest, 36°58'39"N, 28°39'30"E, sweeping, 5.–7.5.2013, 2M and 1F, Turkey: Muğla, University campus, MT, 720 m, 37°09'42"N, 28°22'13"E, H. Kavak, 26.v.–26.vi.2015, 2M.

Distribution. described and hitherto known only from Greece.

Dicraeus raptus (Holiday, 1838)

Material examined. Turkey: 12km SW of Muğla, *Ferula communis*, 660 m, 37°07'40"N, 28°16'28"E, 23.v. 2011, 1M; Turkey: Muğla, University campus, MT, 720 m, 37°09'42"N, 28°22'13"E, H. Kavak, 26.v.–26.vi.2015, 2M.

Distribution. this species was recorded from West Europe and from the Crimea.

Dicraeus tibialis (Macquart, 1835)

Material examined. Turkey: Muğla, University campus, MT, 720 m, 37°09'42"N, 28°22'13"E, H. Kavak, 26.v.–26.vi.2015, 2M and 1F.

Distribution. Holarctic species.

***Elachiptera bimaculata* (Loew, 1858)**

Material examined. Turkey: Toparlar, lowland forest, 36°58'39"N, 28°39'30"E, sweeping, 5.–7.5.2013, 2M; Turkey: Akyaka, pasture, 37°03'19"N, 28°20'07"E, 28.4.–8.5.2013, 6 m, 3M; Samsun, University campus, 22.vi–4.vii.2014, 1M.

Distribution. southern Europe, Canary Islands, Madeira, Israel.

****Elachiptera brevipennis* (Meigen, 1830)**

Material examined. Samsun, University campus, 22.vi–4.vii.2014, 1M.

Distribution. widely distributed in the West Palaearctic Region.

***Elachiptera cornuta* (Fallén, 1820)**

Material examined. Turkey: Toparlar, lowland forest, 36°58'39"N, 28°39'30"E, sweeping, 5.–7.5.2013, 1M; Samsun, University campus, 22.vi–4.vii.2014, 1M.

Distribution. widely distributed in the Palaearctic Region.

****Elachiptera graeca*** Becker, 1910

Material examined. Turkey: Akyaka, pasture, 4 m, 37°03'09"N, 28°20'17"E, 23.–27.ix.2012, 4M and 2F; Turkey: Akyaka, pasture, 37°03'19"N, 28°20'07"E, 28.4.–8.5.2013, 6 m, 8M and 4F.

Distribution. Mediterranean species

***Elachiptera rufifrons* Duda, 1932**

Material examined. Turkey: Akyaka, pasture, 4 m, 37°03'09"N, 28°20'17"E, 23.–27.ix.2012, 2M.

Distribution. southern Eurasian species, known from Spain to China.

****Elachiptera sarda* Nartshuk, 2009**

Material examined. Turkey: Akyaka, pasture, 4 m, 37°03'09"N, 28°20'17"E, 23.–27.ix.2012, 4M and 3F; Turkey: 8 km S of Çine, river bank, 68 m, 37°32'34"N, 28°03'46"E, 21.ix.2012, 2M and 1F; Turkey: Akyaka, pasture, 37°03'19"N, 28°20'07"E, 28.4.–8.5.2013, 6 m, 2M and 2F; Turkey: Samsun, University campus, 22.vi–4.vii.2014, 3M.

Distribution. this species was described from Italia, Sardegna and further known from the Balearic Islands.

***Eribolus hungaricus* Becker, 1910**

Material examined. Turkey: Akyaka, pasture, 4 m, 37°03'09"N, 28°20'17"E, 23.–27.ix.2012, 3M and 1F; Turkey: Akyaka, pasture, 37°03'19"N, 28°20'07"E, 28.4.–8.5.2013, 6 m, 3M and 2F.

Distribution. widely distributed West Palaearctic species.

***Gaurax fascipes* Becker, 1910**

Material examined. Turkey: Samsun, University campus, 22.vi–4.vii.2014, 1M.

Distribution. widely distributed West Palaearctic species.

***Gaurax niger* Czerny, 1906**

Material examined. Samsun, University campus, 22.vi–4.vii.2014, 1M.

Distribution. widely distributed West Palaearctic species.

Hapleginella laevifrons (Loew, 1858)

Material examined. Turkey: 11km E of Muğla, pine wood + meadow, 1310m, 37°12'45"N, 28°27'42"E, 23.v.2011, 1M.

Distribution. Eurasian species

***Incertella zuercheri* (Collin, 1946)**

Material examined. Turkey: Akyaka, pasture, 4 m, 37°03'09"N, 28°20'17"E, 23.–27.ix.2012, 6M and 2F; Turkey: 8 km S of Çine, river bank, 68 m, 37°32'34"N, 28°03'46"E, 21.ix.2012, 2M and 2F.

Distribution. widely distributed Palaearctic species.

***Lasiambia albidipennis* (Strobl, 1893)**

Material examined. Turkey: Muğla, University campus, YPWT, 720 m, 37°09'42"N, 28°22'13"E, 26.–27.vi.2015, 1M; Turkey: 4 km N of Yatagan, *Foeniculus* flowers, 460 m, 37°22'12"N, 28°09'22"E, 30.vi.2015, 2F; Turkey: Akyaka, salty meadow, 2 m, 37°01'49"N, 28°20'01"E, 22.vi.–1.vii.2015, 1M.

Distribution. this species is known from southern Europe, Kazakhstan, and Asia Minor.

Lasiambia brevibucca (Duda, 1933)

Material examined. Turkey: Akyaka, pasture, 4 m, 37°03'09"N, 28°20'17"E, 23.–27.ix.2012, 1M.

Distribution.this species is known from Europe, Turkey and Iran.

**Lasiambia coxalis* (von Roser, 1840)

Material examined. Turkey: Muğla, University campus, YPWT, 720 m, 37°09'42"N, 28°22'13"E, 26.–27.vi.2015, 2M.

Distribution. widely distributed Palaearctic species.

**Lasiambia fycoperda* (Becker, 1910)

Material examined. Turkey: Muğla, University campus, 700 m, 37°09'41"N, 28°22'21"E, Malaise trap, edge of pine wood, xi.2012–iii.2013, 4M and 2F.

Distribution. this species is known from Southern Europe

Lasiochaeta pubescens (Thalhammer, 1898)

Material examined. Turkey: Akyaka, pasture, 4 m, 37°03'09"N, 28°20'17"E, 23.–27.ix.2012, 15M and 12F; Turkey: Akyaka, river bank + salty meadow, 37°03'16"N, 28°19'57"E, 16.–27.v.2011, 10M and 5F; Turkey: Akyaka, pasture, 37°03'19"N, 28°20'07"E, 28.4.–8.5.2013, 6 m, 4M and 2F; Samsun, University campus, 22.vi–4.vii.2014, 12M and 24F.

Distribution. common and widely distributed species in the southern Palaearctic Region, from Azores and Madeira to Afghanistan, recently spreading as north as England and Northern Germany.

**Lipara rufitarsis* Loew, 1858

Material examined. Turkey: Akyaka, salty meadow, SW + PT, 37°02'53"N, 28°19'39"E, , 28.4.–9.5.2013, 1M Turkey: Toparlar, lowland forest, 36°58'39"N, 28°39'30"E, sweeping, 5.–7.5.2013, 2M.

Distribution. widely distributed Holarctic species.

***Lipara similis* Schiner, 1854**

Material examined. Turkey: Toparlar, lowland forest, 36°58'39"N, 28°39'30"E, sweeping, 5.–7.5.2013, 2M.

Distribution. widely distributed Palaearctic species.

***Oscinimorpha arcuata* (Duda, 1932)**

Material examined. Turkey: Akyaka, 40 m, forest, SW + PT, 37°03'21"N, 28°19'09"E, 16.–27.v.2011, 2M and 6F.

Distribution. West Palaearctic species.

***Oscinimorpha longirostris* (Loew, 1858)**

Material examined. Turkey: Akyaka, pasture, 37°03'19"N, 28°20'07"E, 28.4.–8.5.2013, 6 m, 2M; Turkey: Akyaka, river bank + salty meadow, 37°03'16"N, 28°19'57"E, 16.–27.v.2011, 12M and 10F.

Distribution. mediterranean species, known from the Canary Islands, southern Europe, and North Africa to Israel.

***Oscinimorpha minutissima* (Strobl, 1900)**

Material examined. Turkey: Akyaka, pasture, 37°03'19"N, 28°20'07"E, 28.4.–8.5.2013, 6 m. 3M; Turkey: Muğla, 700 m, University campus, SW + PT, 37°09'42"N, 28°22'21"E, 29.iv.–10.v.2013, 2M.

Distribution. this species is known from North Africa and West Palaearctic Region.

***Oscinimorpha novakii* (Strobl, 1893)**

Material examined. Samsun, University campus, 22.vi–4.vii.2014, 1M; Turkey: Akyaka, river bank + salty meadow, 37°03'16"N, 28°19'57"E, 16.–27.v.2011, 10M and 6F.

Distribution. mediterranean species, known from the Canary Islands, southern Europe to Israel.

***Polyodaspis splendida* Nartshuk, 2012**

Material examined. Turkey: 8 km S of Çine, river bank, 68 m, 37°32'34"N, 28°03'46"E, 21.ix.2012, 4M and 3F; Turkey: 4 km N of Yatagan, *Foeniculus* flowers, 460 m, 37°22'12"N, 28°09'22"E, 30.vi.2015, 1M

Distribution. this species is known only from Turkey.

***Polyodaspis sulcicollis* (Meigen, 1838)**

Material examined. Turkey: 11km E of Muğla, pine wood + meadow, 1310m, 37°12'45"N, 28°27'42"E, 1.v.2013, 3M and 1F; Turkey: Samsun, University campus, 22.vi–4.vii.2014, 5M and 6F; Turkey: 4 km N of Yatagan, *Foeniculus* flowers, 460 m, 37°22'12"N, 28°09'22"E, 30.vi.2015, 3M and 4F; Turkey: 8 km S of Çine, river bank, 68 m, SW + YPWT, 37°32'34"N, 28°03'46"E, 28.–30.vi.2015, 5M and 6F

Distribution. this species is distributed in Europe, the mediterranean subregion, and in Palaearctic Asia eastwards to Yakutia and Mongolia.

***Sabroskyina aharonii* (Duda, 1933)**

Material examined. Turkey: Akyaka, salty meadow, 2 m, 37°03'N, 28°20'E, 23.–27.ix.2012, 1M; Turkey: 8 km S of Çine, river bank, 68 m, 37°32'34"N, 28°03'46"E, 21.ix.2012, 5M; Turkey: Toparlar, lowland forest, 36°58'39"N, 28°39'30"E, sweeping, 5.–7.5.2013, 6M and 5F; Turkey: Dalyan, orchard, 4 m, 36°49'37"N, 28°39'39"E, 24.ix.2012, 32M and 43F;

Distribution. the species was previously known from Turkey to Pakistan and Israel, Africa from Egypt to Chad, Seychelles, and Cape Verde Islands.

****Speccafrons genavensis* Merz, 2008**

Material examined. Turkey: Akyaka, river bank + salty meadow, 37°03'16"N, 28°19'57"E, 16.–27.v.2011, 3M and 2F; Turkey: Akyaka, pasture, 4 m, 37°03'09"N, 28°20'17"E, 23.–27.ix.2012, 4M and 2 F; Turkey: 8 km S of Çine, river bank, 68 m, 37°32'34"N, 28°03'46"E, 21.ix.2012, 2M and 2F.

Distribution. described and hitherto known only from Switzerland.

****Trachysiphonella carinifacies* Nartshuk, 1964**

Material examined. Turkey: Akyaka, river bank + salty meadow, 37°03'16"N, 28°19'57"E, 16.–27.v.2011, 3M and 2F; Turkey: Akyaka, pasture, 4 m, 37°03'09"N, 28°20'17"E, 23.–27.ix.2012, 4M and 2F.

Distribution. the species was described from Kazakhstan and further recorded from Mongolia, Tajikistan, Saudi Arabia, Yemen and Greece.

**Trachysiphonella recurva* Deeming & Al–Dhafer, 2012

Material examined. Turkey: 13km NE of Muğla, pasture/pine wood, 1200m, 37°14'50"N, 28°30'E, 23.–27.vi.2015, 1M; Turkey: 8 km S of Çine, river bank, 68 m, SW + YPWT, 37°32'34"N, 28°03'46"E, 28.–30.vi.2015, 1M and 1F.

Distribution. this species was described from Yemen and further recorded from Oman and Saudi Arabia.

**Trachysiphonella ruficeps* (Macquart, 1835)

Material examined. Turkey: 8 km S of Çine, river bank, 68 m, 37°32'34"N, 28°03'46"E, 10.–12.ix.2014, 3M and 2F; Turkey: Toparlar, lowland forest, 36°58'39"N, 28°39'30"E, sweeping, 5.–7.5.2013, 2M and 1F; Turkey: Akyaka, pasture, 37°03'19"N, 28°20'07"E, 28.4.–8.5.2013, 6 m, 4M and 2F

Distribution. this species is distributed in Palaearctic Region.

**Tricimba albiseta* Dely–Draskovits, 1983

Material examined. Turkey: Akyaka, river bank + salty meadow, 37°03'16"N, 28°19'57"E, 16.–27.v.2011, 1M; Turkey: Akyaka, pasture, 37°03'19"N, 28°20'07"E, 28.4.–8.5.2013, 6 m, 3M.

Distribution. this species is known from Europe.

Tricimba humeralis (Loew, 1858)

Material examined. Turkey: Muğla, University campus, YPWT, 720 m, 37°09'42"N, 28°22'13"E, 26.–27.vi.2015, 2M and 11F

Distribution. widely distributed species, recorded from the southern Palaearctic Region and the Afrotropical Region.

**Tricimba hungarica* Dely–Draskovits, 1983

Material examined. Turkey: Muğla, University, campus, PT, 700 m, 37°09'42"N, 28°22'21"E, 21.–24.ix.2012, 1M.

Distribution. this species is known only from Hungary, Czech Republic and Ukraine.

***Tricimba lineella* (Fallén, 1820)**

Material examined. Turkey: 8 km S of Çine, river bank, 68 m, 37°32'34"N, 28°03'46"E, 21.ix.2012, 1M; Turkey: Samsun, University campus, 22.vi–4.vii.2014, 1M.

Distribution. widely distributed Palaearctic species.

Subfamily: Chloropinae

***Assuania thalhammeri* (Strobl, 1893)**

Material examined. Turkey: Toparlar, lowland forest, 8 m, 36°59'27"N, 28°38'50"E, 24.ix.2012, 2M; Turkey: 8 km S of Çine, river bank, 68 m, 37°32'34"N, 28°03'46"E, 10.–12.ix.2014, 4M; Turkey: 8 km S of Çine, river bank, 68 m, 37°32'34"N, 28°03'46"E, 21.ix.2012, 4M and 3F.

Distribution. south Palaearctic species, known from southern Europe and North Africa to Afghanistan.

***Camarota curvipennis* (Latreille, 1805)**

Material examined. Samsun, University campus, 22.vi–4.vii.2014, 2M.

Distribution. This species is known almost from all Europe (except the northern parts), the Caucasus, southern part of Palaearctic Asia and North Africa.

***Cetema neglectum* Tonnoir, 1921**

Material examined. Turkey: Akyaka, pasture, 37°03'19"N, 28°20'07"E, 28.4.–8.5.2013, 6 m, 2M; Samsun, University campus, 22.vi–4.vii.2014, 1M.

Distribution. this species is known only from Europe and Turkey.

***Chlorops figuratus* (Zetterstedt, 1848)**

Material examined. Samsun, University campus, 22.vi–4.vii.2014, 1M.

Distribution. widely distributed Palaearctic species.

***Chlorops freidmani* Nartshuk, 2012**

Material examined. Turkey: Akyaka, pasture, 37°03'19"N, 28°20'07"E, 28.4.–8.5.2013, 6 m, 4M and 2F; Turkey: Toparlar, lowland forest, 36°58'39"N, 28°39'30"E, sweeping, 5.–7.5.2013, 5M and 3F.

Distribution. this species is known only from Turkey.

***Chlorops geminatus* Meigen, 1830**

Material examined. Samsun, University campus, 22.vi–4.vii.2014, 1M.

Distribution. this species is distributed in Palaearctic Region.

***Chlorops hypostigma* Meigen, 1830**

Material examined. Samsun, University campus, 22.vi–4.vii.2014, 1M; Turkey: Toparlar, lowland forest, SW + YPWT, 8 m, 36°59'27"N, 28°38'50"E, 22.–24.vi.2015, 2M and 1F;

Distribution. Palaearctic Region.

***Chlorops interruptus* Meigen, 1830**

Material examined. Samsun, University campus, 22.vi–4.vii.2014, 3M.

Distribution. this species is known from Palaearctic Region.

***Chlorops limbatus* Meigen, 1830**

Material examined. Turkey: Akyaka, pasture, 37°03'19"N, 28°20'07"E, 28.4.–8.5.2013, 6 m, 2M; Turkey: Akyaka, river bank + salty meadow, 37°03'16"N, 28°19'57"E, 16.–27.v.2011, 8M and 6F.

Distribution. widely distributed Palaearctic species.

***Chlorops pumilionis* (Bjerkander, 1778)**

Material examined. Turkey: Muğla, 700 m, University campus, SW + PT, 37°09'42"N, 28°22'21"E, 29.iv.–10.v.2013, 1F; Turkey: 15km SW of Muğla, damp valley nr.brook, 630 m, 37°06'31"N, 28°15'31"E, 23.v.20111M.

Distribution. Eurasian species, known from Europe to Mongolia.

***Chlorops serenus* Loew 1866**

Material examined. Turkey: Akyaka, forest, 37°03'16"N, 28°19'35"E, 30.4.–9.5.2013, 30 m, 1M.

Distribution. this species is known from West Palaearctic Region.

***Chloropsina lucens* (Becker, 1910)**

Material examined. Turkey: Akyaka, river bank + salty meadow, 37°03'16"N, 28°19'57"E, 16.–27.v.2011, 1F; Turkey: Akyaka, pasture, 4 m, 37°03'09"N, 28°20'17"E, 23.–27.ix.2012, 1F; Turkey: Akyaka, salty meadow, SW + PT, 37°02'53"N, 28°19'39"E, 28.4.–9.5.2013, 1F; Turkey: Akyaka, pasture, 4 m, 37°03'09"N, 28°20'17"E, 8.–14.ix.2014, 1M.

Distribution. this species was described and hitherto known only from Greece.

*** *Cryptonevra diadema* (Meigen 1830)**

Material examined. Turkey: Akyaka, salty meadow, SW + PT, 37°02'53"N, 28°19'39"E, 28.4.–9.5.2013, 1M; Turkey: 8 km S of Çine, river bank, 68 m, 37°32'34"N, 28°03'46"E, 21.ix.2012, 3M.

Distribution. species widely distributed in North Africa and Palaearctic Region.

***Cryptonevra flavitarsis* (Meigen, 1830)**

Material examined. Turkey: Akyaka, salty meadow, SW + PT, 37°02'53"N, 28°19'39"E, 28.4.–9.5.2013, 2M and 1F; Turkey: Akyaka, pasture, 37°03'19"N, 28°20'07"E, 28.4.–8.5.2013, 6 m, 3M and 2F; Turkey: Akyaka, river bank + salty meadow, 37°03'16"N, 28°19'57"E, 16.–27.v.2011, 4M; Turkey: Toparlar, lowland forest, SW + YPWT, 8 m, 36°59'27"N, 28°38'50"E, 22.–24.vi.2015, 4M and 2F.

Distribution. Europe and Kazakhstan.

***Cryptonevra nigritarsis* (Duda, 1933)**

Material examined. Turkey: Akyaka, river bank + salty meadow, 37°03'16"N, 28°19'57"E, 16.–27.v.2011, 1M.

Distribution. Palaearctic distributed species.

***Diplotoxa messoria* (Fallén, 1820)**

Material examined. Turkey: Akyaka, pasture, 37°03'19"N, 28°20'07"E, 28.4.–8.5.2013, 6 m, 1M.

Distribution. Holarctic species; in the Palaearctic Region known from the British Isles to Far East Russia.

***Eurina ducalis* A. Costa, 1885**

Material examined. Turkey: Toparlar, lowland forest, 36°58'39"N, 28°39'30"E, sweeping, 5.–7.5.2013, 4M and 4F; Turkey: Akyaka, pasture, 37°03'19"N, 28°20'07"E, 28.4.–8.5.2013, 6 m, 4M and 4F.

Distribution. this species is known from Central and South Europe, Syria and Israel.

***Eurina lurida* Meigen, 1830**

Material examined. Turkey: Akyaka, pasture, 37°03'19"N, 28°20'07"E, 28.4.–8.5.2013, 6 m, 1M.

Distribution. widely distributed Palaearctic species known also from Near East.

***Eurina triangularis* Becker, 1903**

Material examined. Turkey: Akyaka, pasture, 37°03'19"N, 28°20'07"E, 28.4.–8.5.2013, 6 m, 1M.

Distribution. this species is known from North Africa (Egypt) and Israel.

***Eutropha fulvifrons* (Haliday, 1833)**

Material examined. Turkey: Akyaka, salty meadow, SW + PT, 37°02'53"N, 28°19'39"E, 28.4.–9.5.2013, 3M

Distribution. the species is known in Near East and West Palaearctic Region.

***Lagaroceras megalops* Becker, 1903**

Material examined. Turkey: 8 km S of Çine, river bank, 68 m, 37°32'34"N, 28°03'46"E, 10.–12.ix.2014, 2M

Distribution. this species is known from Near East (Egypt), Ethiopia, Mozambique and South Africa.

***Lasiosina albipila* (Loew, 1866)**

Material examined. Turkey: Akyaka, pasture, 4 m, 37°03'09"N, 28°20'17"E, 23.–27.ix.2012, 3M and 1F; Turkey: Akyaka, river bank + salty meadow, 37°03'16"N, 28°19'57"E, 16.–27.v.2011, 2M and 1F.

Distribution. Palaearctic species.

***Lasiosina aurea* Dely-Draskovits, 1981**

Material examined. Turkey: 8 km S of Çine, river bank, 68 m, 37°32'34"N, 28°03'46"E, 21.ix.2012, 1M; Turkey: Akyaka, pasture, 37°03'19"N, 28°20'07"E, 28.4.–8.5.2013, 6 m, 2M; Turkey: Toparlar, lowland forest, 36°58'39"N, 28°39'30"E, sweeping, 5.–7.5.2013; Turkey: Toparlar, lowland forest, 8 m, 36°59'27"N, 28°38'50"E, 24.ix.2012, 3M and 3F.

Distribution. this species was described from Israel.

***Lasiosina cinctipes* (Meigen, 1830)**

Material examined. Turkey: Samsun, University campus, 22.vi–4.vii.2014, 4M.

Distribution. Palaearctic species.

***Lasiosina emiliae* Dely-Draskovits, 1982**

Material examined. Turkey: Akyaka, salty meadow, SW + PT, 37°02'53"N, 28°19'39"E, 28.4.–9.5.2013, 2M.

Distribution. this species was known earlier from Kazakhstan, Kirghizia, Tajikistan, and Uzbekistan.

***Lasiosina herpini* (Guérin-Méneville, 1843)**

Material examined. Turkey: Samsun, University campus, 22.vi–4.vii.2014, 1M.

Distribution. Transpalaearctic species.

***Lasiosina immaculata* Becker, 1912**

Material examined. Turkey: Akyaka, pasture, 4 m, 37°03'09"N, 28°20'17"E, 23.–27.ix.2012, 4M.

Distribution. this species was known earlier from Europe and Near East.

***Lasiosina lindbergi* (Duda, 1933)**

Material examined. Turkey: Toparlar, lowland forest, 36°58'39"N, 28°39'30"E, sweeping, 5.–7.5.2013, 5M.

Distribution. Mediterranean species, known from Bulgaria, the North Africa and Corsica.

***Lasiosina paralittoralis* Dely-Draskovits, 1981**

Material examined. Turkey: Akyaka, pasture, 4 m, 37°03'09"N, 28°20'17"E, 8.–14.ix.2014, 8M and 4F; Turkey: Toparlar, lowland forest, 8 m, 36°59'27"N, 28°38'50"E, 24.ix.2012, 6M and 8F.

Distribution. this species was described from Israel.

***Meromyza eduardi* Hubicka, 1966**

Material examined. Turkey: Akyaka, salty meadow, 2 m, 37°01'49"N, 28°20'01"E, 22.vi.–1.vii.2015, 2M and 1F.

Distribution. this species was known earlier from Estonia, Lithuania and Poland.

***Meromyza filippovi* Ozerov, 2009**

Material examined. Turkey: Toparlar, lowland forest, 8 m, 36°59'27"N, 28°38'50"E, 24.ix.2012, 4M and 1F.

Distribution. this species is known only from European part of Turkey.

***Meromyza meigeni* Nartshuk, 2006**

Material examined. Turkey: 13km NE of Muğla, pasture/pine wood, 1200m, 37°14'50"N, 28°30'E, 23.–27.vi.2015, 6M and 9F.

Distribution. this species was described from Slovenia and further known from Bulgaria, Albania, Macedonia and Bosnia.

***Meromyza pluriseta* Peterfi, 1961**

Material examined. Turkey: Gökçeova Gölü, lake shore, 1 750 m, 37°03'42.52"N, 28°48'28.42"E, 20.ix.2012, 6M; Turkey: 8 km S of Çine, river bank, 68 m, 37°32'34"N, 28°03'46"E, 21.ix.2012, 4M and 2F.

Distribution. Palaearctic species.

***Meromyza nigriventris* Macquart, 1835**

Material examined. Samsun, University campus, 22.vi–4.vii.2014, 1M.

Distribution. Holarctic species: in the Palaearctic Region it is widely distributed from the British Isles to Japan; in North America it is known only from the West.

***Phyladelphus thalhammeri* Becker, 1910**

Material examined. Turkey: Akyaka, pasture, 4 m, 37°03'09"N, 28°20'17"E, 8.–14.ix.2014, 6M and 4F; Turkey: 8 km S of Çine, river bank, 68 m, 37°32'34"N, 28°03'46"E, 10.–12.ix.2014, 5M and 4F;

Distribution. Mediterranean species.

***Thaumatomyia notata* (Meigen, 1830)**

Material examined. Turkey: Akyaka, forest, 37°03'16"N, 28°19'35"E, 30.4.–9.5.2013, 30 m, 4M and 8F; Turkey: Toparlar, lowland forest, 36°58'39"N, 28°39'30"E, sweeping, 5.–7.5.2013, 2M and 8F; Turkey: Akyaka, pasture, 37°03'19"N, 28°20'07"E, 28.4.–8.5.2013, 6 m, 1M and 6F; Samsun, University campus, 22.vi–4.vii.2014, 4M and 8F.

Distribution. widespread species, recorded from the Palaearctic, Afrotropical, and Oriental Regions.

***Thaumatomyia sulcifrons* (Becker, 1907)**

Material examined. Turkey: 4 km N of Yatagan, *Foeniculus* flowers, 460 m, 37°22'12"N, 28°09'22"E, 30.vi.2015, 1M and 2F.

Distribution. South Palaearctic species, known from the Canary Islands to China.

Descriptions of new species

Oscinellinae

***Dicraeus civeleki* sp. n.**

http://zoobank.org/DB8D5B04-4123-4738-9552-5D377D1C4D75

Figs 1–5

Type material. Holotype male, Turkey: Akyaka, salty meadow, 2 m, 37°01'49"N, 28°20'01"E, 22.vi.–1.vii.2015. Holotype is in good condition, abdomen on plastic tags together with the specimen. Paratype: 1M same data.

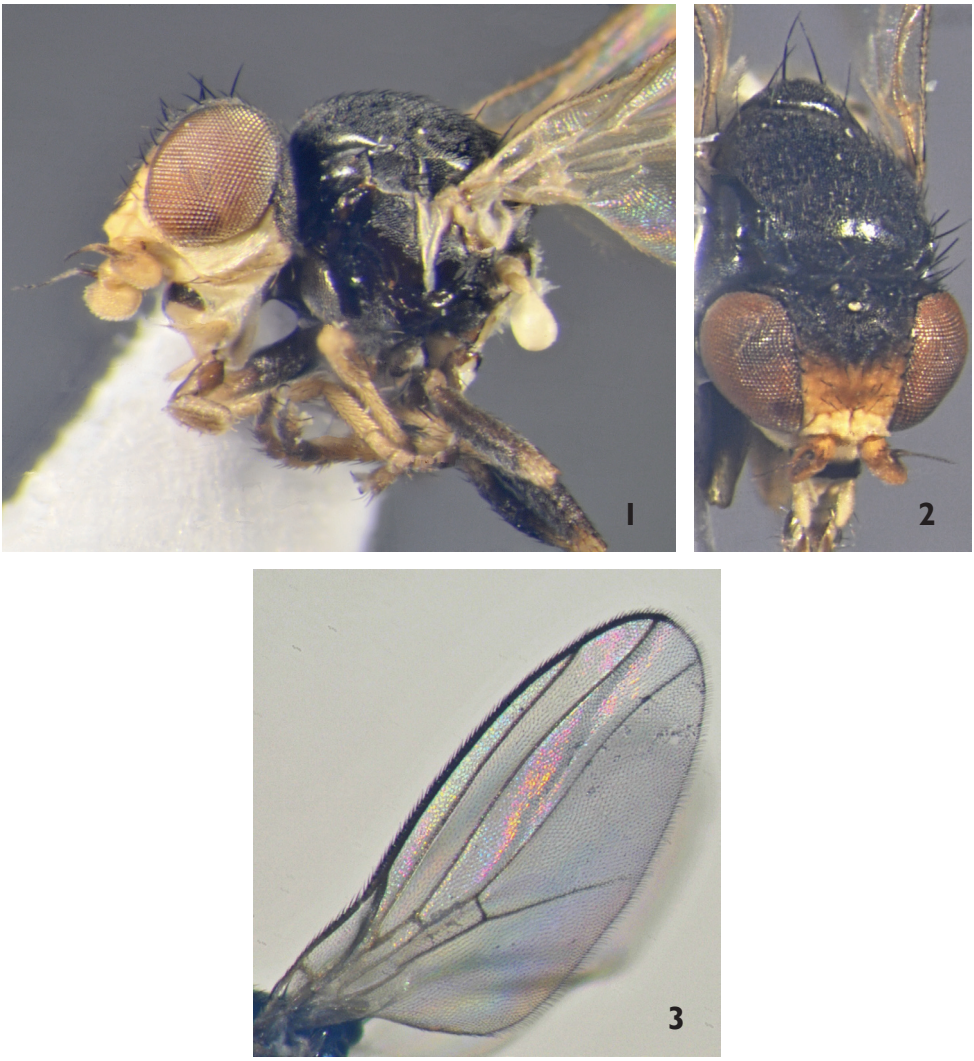

Figure 1–3. *Dicraeus civeleki* sp. n. (holotype): **1** body (abdomen missing), lateral view **2** body (abdomen missing), dorsal view **3** wing.

Diagnosis. Grey dusted black species with yellow face, anterior part of frons, antennae, palpus, fore and mid tibia. Costal vein reaches one-fourth the way between R_{4+5} and M_{1+2}.

Description. *Male*. Frons longer than wide, yellow on anterior third and black on posterior portion, ocellar triangle black, 2/3 length of frons. Face and gena yellow. Gena wider than first flagellomere with a row of black peristomal setulae. Palpus yellow with black setulae. Antenna yellow, first flagellomere round and yellow, arista short pubescent. Occiput black. Setae and setulae of head black.

Thorax black with grey microtrichosity, entirely covered with black setulae. Scutellum round triangular with long apical convergent setae and a pair of subapicals 2/3 length of

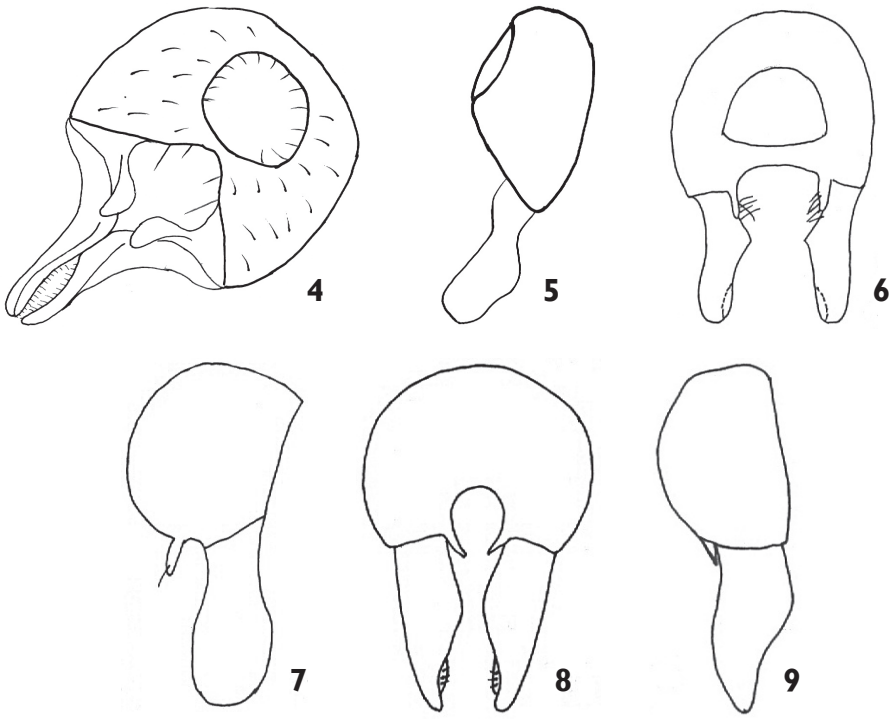

Figure 4–9. 4–5 *Dicraeus civeleki* sp. n. (holotype): **4** epandrium posterior view **5** epandrium lateral view **6–7** *Dicraeus beschovski*: **6** epandrium posterior view **7** epandrium lateral view (after Nartshuk 2010) **8–9** *Dicraeus sabroskyi*: **8** epandrium posterior view **9** epandrium lateral view (after Nartshuk 2010).

apical ones. Anterior portion of pleura shining, anepisternum and katepisternum partly microtrichose. Chaetotaxy: 2 postpronotal, 1 + 2 notopleural, two postalar and one prescutellar setae. Wing clear with whitish yellow veins. Costal vein reaches one-fourth the length between R_{4+5} and M_{1+2} (Fig 3). Halter whitish yellow. Legs: fore coxa, fore and mid tibia yellow, all femora and hind tibia black.*Abdomen* brown with a narrow yellow band on tergites. Male genitalia (Figs 4–5): epandrium black, surstylus brownish yellow with several long setae at base. Apex of surstylus broad and straight. Cercus broad and orthogonally curved, not pointed.

Body length: 2 mm.

Female: unknown.

Remarks. The species belongs to subgenus *Oedesiella* Becker based on the structure of the male genitalia: cerci long and wide apart, surstyli longer than epandrium. Cerci wide and curved, not narrow, straight and pointed, surstylus with wide and straight apex, not narrowed as in *D. sabroskyi* Beschovski, 1977 (Figs 8–9) and not rounded as in *D. beschovski* Nartshuk, 2010 (Figs 6–7).

Etymology. Named in honour of Prof. Hasan Civelek, our colleague and dipterologist from Muğla University, Turkey.

Chloropinae

***Meromyza samsunensis* sp. n.**
http://zoobank.org/F6774AC1-8773-4C3B-9927-A02419668DCA
Figs 10–15

Type material. Holotype male, Turkey: Samsun, University campus, 22.vi–4.vii.2014. Holotype is in good condition, abdomen on plastic tags together with the specimen. Paratypes: 2M and 2F same data.

Diagnosis. Species with black palpus on apical half, first flagellomere 1.5 times as long as wide, red grey microtrichose stripes on scutum and hind femur nearly four times thicker than tibia. *Meromyza samsunensis* has anterior process of postgonite widened laterally forming distinct longitudinal rib; upper half parallel and curved, lower half concave. This character is hardly visible in lateral view (Fig 15). In *M. femorata*, the anterior process of postgonite is flat, wide and with three to four smooth spinules on the surface (Fig 16).

Description. *Male* (Figs 10–11). Ground colour yellow. Frons produced anteriorly, produced region of frons same width of first flagellomere. Ocellar triangle occupying two-thirds of frons, shining, rugose on apical portion and black on ocellar tubercle only, with one row of black interfrontal setae along sides. First flagellomere 1.5 times as long as wide, yellow, darkened on dorsal portion and with long pale setulae. Arista yellow, nearly bare. Genal as wide as first flagellomere. Vibrissal angle obtuse. Palpus black on apical half and yellow basally.

Thorax: Scutum with red grey microtrichose stripes, midstripe reaching scutellum and scutellum with small red mark. Pleura with red marks except small black mark on anepisternum. Wing hyaline with whitish yellow veins. Halter whitish yellow. Legs yellow, fore tarsus darkened. Hind femur strongly swollen, nearly four times as thick as tibia (Fig 12).

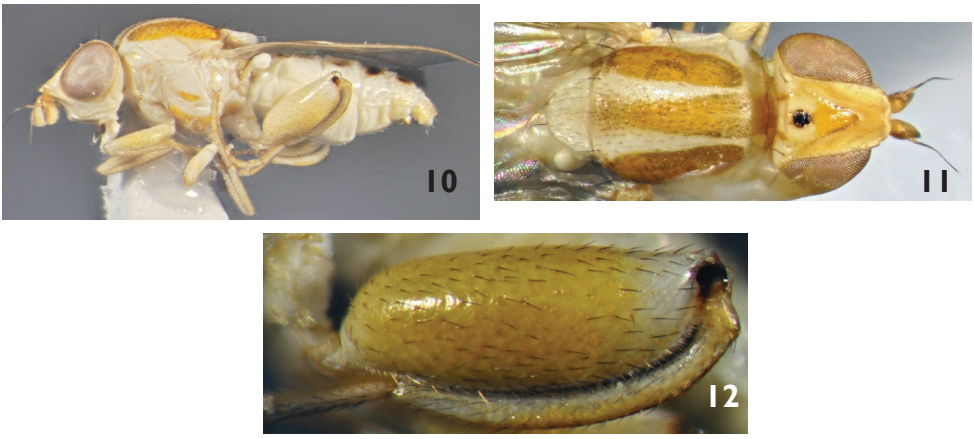

Figure 10–12. *Meromyza samsunensis* sp. n. (paratype): **10** body lateral view **11** body dorsal view **12** hind femora, lateral (dorsal) view.

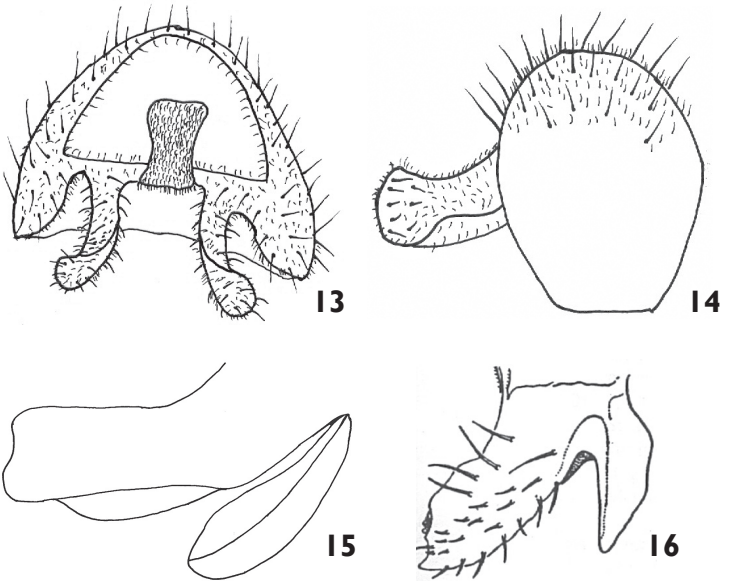

Figure 13–16. *Meromyza samsunensis* sp. n. (holotype): **13** epandrium, posterior view **14** epandrium, lateral view **15** postgonite, lateral view **16** *Meromyza femorata*: postgonite, lateral view (after Nartshuk and Fedoseeva, 2011).

Abdomen: yellow with dark midstripe and small spots on tergites 2–5. Male genitalia (Figs 13–14): epandrium yellow, with long curved surstylus evenly covered with small setulae. The upper half of anterior process of postgonite is parallel and curved, lower half concave. Posterior process enlarged (Fig 15).

Body length 3.5–4.0 mm.

Remarks. New species has elongated first flagellomere. The character is rear in *Meromyza*, only two species have elongated first flagellomere: *Meromyza mirabilis* Fedoseeeva, 1974 and *Meromyza longicornis* (Frey, 1921). *Meromyza mirabilis* has first flagellomere 1.5 times as long as wide (similar to *M. samsunensis* sp. n) but palpus is yellow and stripes on the scutum are brown. *Meromyza longicornis* has first flagellomere 2.5 times as long as wide and hind femur 3 times as wide as hind tibia. *M. samsunensis* sp. n is similar to *Meromyza femorata* Macquart, 1835 in having red stripes on the scutum with median stripe reaching the scutellum, palpus black on apical half, and hind femur strongly swollen. The main difference between both species is in the shape of postgonite.

Etymology. the species epithet refers to the location where the holotype was collected (the city of Samsun).

Comments

The new species may be included in the key to Palaearctic species of the genus *Meromyza* Meigen (Nartshuk and Fedoseeva, 2011) by the following modification:

123 (124) Hind femur strongly thickened, at least 4 times as wide as hind tibia. Stripes of scutum rufous **123a**

123a Anterior process of postgonite flat, wide and with three to four smooth spikes on surface (Fig. 16)..... ***M. femorata***

123b Anterior process of postgonite widened laterally forming distinct longitudinal rib; upper half parallel and curved, lower half concave. (Fig. 15)..... ..... ***M. samsunensis* sp. n.**

124 (123) Hind femur moderately thickened, less than 3 times as wide as hind tibia. Stripes of scutum mostly dark; if rufus, anterior margin of anterior process of postgonite sharply narrowed and projecting.

Discussion

Altogether 114 species of the family Chloropidae are known at the present time from Turkey. Nine genera (*Calamoncosis*, *Eribolus*, *Gaurax*, *Incertella*, *Speccafrons*, *Trachysiphonella*, *Chloropsina*, *Eutropha*, and *Lagaroceras*) and 46 species are recorded here for the first time. Two species (*Dicraeus civeleki* sp. n. and *Meromyza samsunensis* sp. n.) are described. Based on comparisons with the Chloropidae fauna of some adjacent countries, it seems as though the number of Chloropidae species in Turkey is in fact much larger: Bulgaria (Beschovski 1985) with 144 species, Israel with more than 100 species (Kaplan 1977), 51 species from Greece (Nartshuk 2010) and 394 species are known to occur in Europe (Nartshuk 2013).

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RESEARCH ARTICLE

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A new species of *Oxynetra* from Mexico (Hesperiidae, Pyrginae, Pyrrhopygini)

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Abstract

Oxynetra aureopecta **sp. n.** is described from the Sierra Madre Oriental of east-central Mexico. Visually similar to Mesoamerican *O. hopfferi* Staudinger, 1888 in having five orange bands on the abdomen above, it is diagnosed by orange forecoxae and palpi beneath, narrower forewing hyaline bands and a prominent 6% difference in the COI DNA barcode sequence. It is the northernmost representative of the *hopfferi* species group that also includes *O. stangelandi* Grishin & Burns, 2013, characterized by a single-banded abdomen and currently known only from the Area de Conservación Guanacaste in northwestern Costa Rica. Both *O. hopfferi* and *O. stangelandi* possess white forecoxae and ventral palpi. This new discovery brings the total number of *Oxynetra* C. & R. Felder, 1862 species to five.

Resumen

Oxynetra aureopecta **sp. n.** se describe de la Sierra Madre Oriental, en el centro-este de México. Visualmente similar a la especie mesoamericana *O. hopfferi* Staudinger, 1888 en tener cinco bandas de color naranja en el abdomen anterior, se diagnostica por forecoxae y palpos naranja debajo, bandas hialinas más estrechas en la ala anterior, y una diferencia destacada de 6% en la secuencia de código de barras de ADN COI. Es el representante más septentrional del grupo de especies *hopfferi* que también incluye *O. stangelandi* Grishin & Burns, 2013, que se caracteriza por una banda en el abdomen y actualmente conocida solamente desde el Área de Conservación Guanacaste en el noroeste de Costa Rica. Tanto *O. hopfferi* y *O. stangelandi* poseen forecoxae y cara ventral de palpos blancos. Este nuevo descubrimiento eleva el número total de especies de *Oxynetra* C. & R. Felder a cinco.

Keywords
Biodiversity, mimicry, skipper butterflies, *Prunus*

Palabras clave
Biodiversidad, mimetismo, mariposa hesperido, *Prunus*

Introduction

Butterflies are loved for the colorful patterns of their wings. However, the lack of scales resulting in wing transparency sometimes is more appealing than colors. Most prominently known in the Clearwings (Nymphalidae: Ithomiini) and some Satyrs (Nymphalidae: Haeterini) (Warren et al. 2016), transparent wings are rarely found in Skippers (Hesperiidae). *Oxynetra* C. & R. Felder, 1862, from the Pyrrhopygini tribe, is perhaps the foremost example. These arctiid moth mimics (Grishin et al. 2013) are some of the most unusually patterned Hesperiidae, with broad hyaline discal (and sometimes subapical) bands on the wings and frequently orange-banded abdomens.

Oxynetra is a neotropical genus (type species *O. semihyalina* C. & R. Felder, 1862) of four species. South American *O. semihyalina* and *O. confusa* Staudinger, 1888 possess larger scale-free areas on the wings. In addition to the discal band, they have forewing subapical hyaline spots, which are larger and rounder in *O. semihyalina*. Their sexes are similar, but females have rounder wings. Mesoamerican *O. hopfferi* Staudinger, 1888, and *O. stangelandi* Grishin & Burns, 2013 lack the subapical hyalinity and their discal bands are narrower. *Oxynetra hopfferi* is characterized by its striking five-banded abdominal pattern, while *O. stangelandi* has a single complete abdominal band, as does *O. semihyalina*. The two Mesoamerican species (the *hopfferi* group) are extreme in sexual dimorphism: females lack hyalinity altogether and have black wings, sometimes with white fringes. Due to such extremism, a female of *O. hopfferi* was originally described not only as a separate species, but also in a distinct genus: *Dis annulatus* Mabille, 1889.

Unlike *O. semihyalina* and *O. confusa*, *O. hopfferi* is very rare in collections; we know only 12 male and 4 female specimens world-wide (Warren et al. 2016). *Oxynetra stangelandi* is currently known only from the type series of 10 specimens (4 males and 6 females) reared from Area de Conservación Guanacaste (ACG) in northwestern Costa Rica. Its caterpillars feed on *Prunus annularis* Koehne, adding a new family, Rosaceae, to those eaten by Pyrrhopygini in ACG (Grishin et al. 2013). The *hopfferi* group is comprised of cloud forest species recorded from above 1000 m in elevation. Its unique wasp-like appearance creates the potential for cryptic species to be overlooked within *O. hopfferi*.

Materials and methods

Specimens were examined from the following collections: Los Angeles County Museum of Natural History, Los Angeles, CA, USA (**LACM**); National Museum of Natural

History, Smithsonian Institution, Washington, DC, USA (**USNM**); Colección Nacional de Insectos "Dr. Alfredo Barrera Marin", Museo de Historia Natural y Cultura Ambiental de la Ciudad de México, Mexico (**CNIABM**); McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, University of Florida, Gainesville, FL, USA (**MGCL**); Natural History Museum, London, UK (**BMNH**); Museum für Naturkunde, Berlin, Germany (**ZMHB**); Senckenberg Museum für Tierkunde, Dresden, Germany (**MTD**); O. H. H. Mielke, Curitiba, Paraná, Brazil, together with the collection of Departamento de Zoologia, Universidade Federal do Paraná, Curitiba, Brazil (**OM-DZUP**) and Instituto Nacional de Biodiversidad, Santo Domingo de Heredia, Costa Rica (**INBio**). Wing venation terminology follows Steinhauser (1981). Length measurements are in metric units and were made from photographs of specimens taken next to a scale and magnified on a computer screen. Photographs of specimens were taken with Nikon E5000 and Nikon D200, D800 cameras through a Nikkor 105 mm f/2.8G AF-S VR Micro lens. Images were assembled and edited in Photoshop CS5.1.

Legs, crumbs and pieces of muscle tissue from the thorax of dissected specimens (plucked from the abdomen attachment site), or a distal part of an abdomen (dropped into lysis buffer, and after overnight incubation at 56°C transferred into 10% KOH for genitalia dissection) were used to extract genomic DNA with the Macherey-Nagel (MN) NucleoSpin tissue kit following the manufacturer's protocol. The lysis buffer volume was scaled down to 70 µl for legs and volumes of subsequent reagents were proportionally reduced. Genomic DNA was eluted in a total volume of 40–100 µl MN BE buffer (concentration of DNA as measured by Promega QuantiFluor® dsDNA System was from near 0 to 20 ng/µl, mostly around 1 ng/µl, depending on specimen age and storage conditions) and was stored at -20°C. PCR was performed using Invitrogen AmpliTaq Gold 360 master mix in a 20 µl total volume containing less than 1 ng of template DNA (usually 0.5–1 µl of DNA extract) and 0.5 µM of each primer. The following pairs of primers were used: sCOIF (forward, 5'-ATTCAACCAATCATAAAGATATTGG-3') – Meg-mCOIR (reverse, 5'-CCAGTWCCTGYACCATTTTCTAC-3'), and VenmCOIF (forward, 5'-GCATTCCCTCGTATAAATAATA-3') – sCOIR (reverse, 5'-TAAACTTCTGGATGTCCAAAAAATCA-3'), to amplify the barcode in two overlapping segments. The PCR reaction was cleaned by enzymatic digestion for the whole barcode amplifications, ID tag amplification, and sequences amplified in more than two segments, with 4 µl Shrimp Alkaline Phosphatase (20 U/µl) and 1 ul Exonuclease I (1 U/µl) from New England Biolabs. For sequences obtained in two segments, due to the frequent presence of primer dimers and other short nonspecific PCR products, Agencourt Ampure XP beads or Invitrogen E-Gel EX Agarose Gels (followed by Zymo gel DNA recovery kit) were used to select the DNA products of expected length. Sequences were obtained with primers used in PCR. Sanger sequencing was performed with Applied Biosystems Big Dye Terminator 3.1 kit on ABI capillary instrument in the DNA Sequencing Core Facility of the McDermott Center at UT Southwestern. The resulting sequence traces were proof-

read in FinchTV (http://www.geospiza.com/Products/finchtv.shtml). Sequences and accompanying specimen data were submitted to GenBank and received accession numbers KT272397 and KT272398.

Additional DNA sequences were downloaded from GenBank (https://www.ncbi.nlm.nih.gov/genbank) or BOLD (http://www.boldsystems.org). Many of these sequences have been reported in Janzen et al. (2011) and photos of specimens are available from the Area de Conservación Guanacaste (ACG) online database (Janzen and Hallwachs 2014) and BOLD database (Ratnasingham and Hebert 2007) to confirm or suggest identifications. Sequences were aligned manually since they matched throughout their length without insertions or deletions. The Phylogeny.fr server (http://www.phylogeny.fr) was used with the Hamming distance model (Dereeper et al. 2008) to compute evolutionary distances from aligned DNA sequences and dendrograms were built with BioNJ (Gascuel 1997). Maximum Likelihood analysis was performed using RAxML (version 7.0.4) under several substitution models, such as GTRCAT, GTRGAMMA, and GTRGAMMAI (Stamatakis 2006). Rapid RAxML bootstrap values (-x option, and "-f a" for complete analysis) were computed to judge the confidence of tree nodes. Bayesian Inference was performed with MrBayes v3.2.1 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003). Models with 1, 2 and 6 states were used (nst = 1, 2, 6), with optimized fraction of invariant positions (propinv), gamma distribution parameter (gamma) or both (invgamma). The COI alignment was treated as a single partition, or analyzed as three partitions by codon position. Generations were carried out until convergence (standard deviation of split frequencies less than 0.01) and the first 25% were discarded as "burn in." Posterior probabilities of nodes computed by MrBayes were used as the indicators of confidence.

Results

Working in the Colección Nacional de Insectos, "Dr. Alfredo Barrera Marin" (CNIABM), ADW found and photographed a damaged (missing two wings and distal ends of antennae) *Oxynetra* specimen from the R. Müller collection, the only *Oxynetra* specimen known from Mexico (Veracruz: Presidio). It was assumed that it is probably *O. hopfferi* near its northern distribution limits. Upon further analysis, differences from typical Costa Rican and Panamanian *O. hopfferi* were noticed, including the orange "chest" and palpi below, narrower forewing hyaline band and the lack of a ventral hindwing postdiscal white spot in cell CuA_2-2A. A second specimen from Mexico, very similar to the Müller specimen and collected 300 kilometers to the northwest (Hidalgo: Puerto Caballo), identified as "*Oxynetra hopfferi*," surfaced when NVG was browsing the Hesperiidae collection of the Los Angeles County Museum of Natural History (LACM). The morphological differences from *O. hopfferi* were consistent with the Müller specimen, and the DNA COI sequence of the LACM specimen revealed a remarkable 6% difference (about 40 different base pairs) from *O. hopfferi* and 4.7% (31 base pairs) from *O. stangelandi*. Despite the five-banded abdomen and the presence

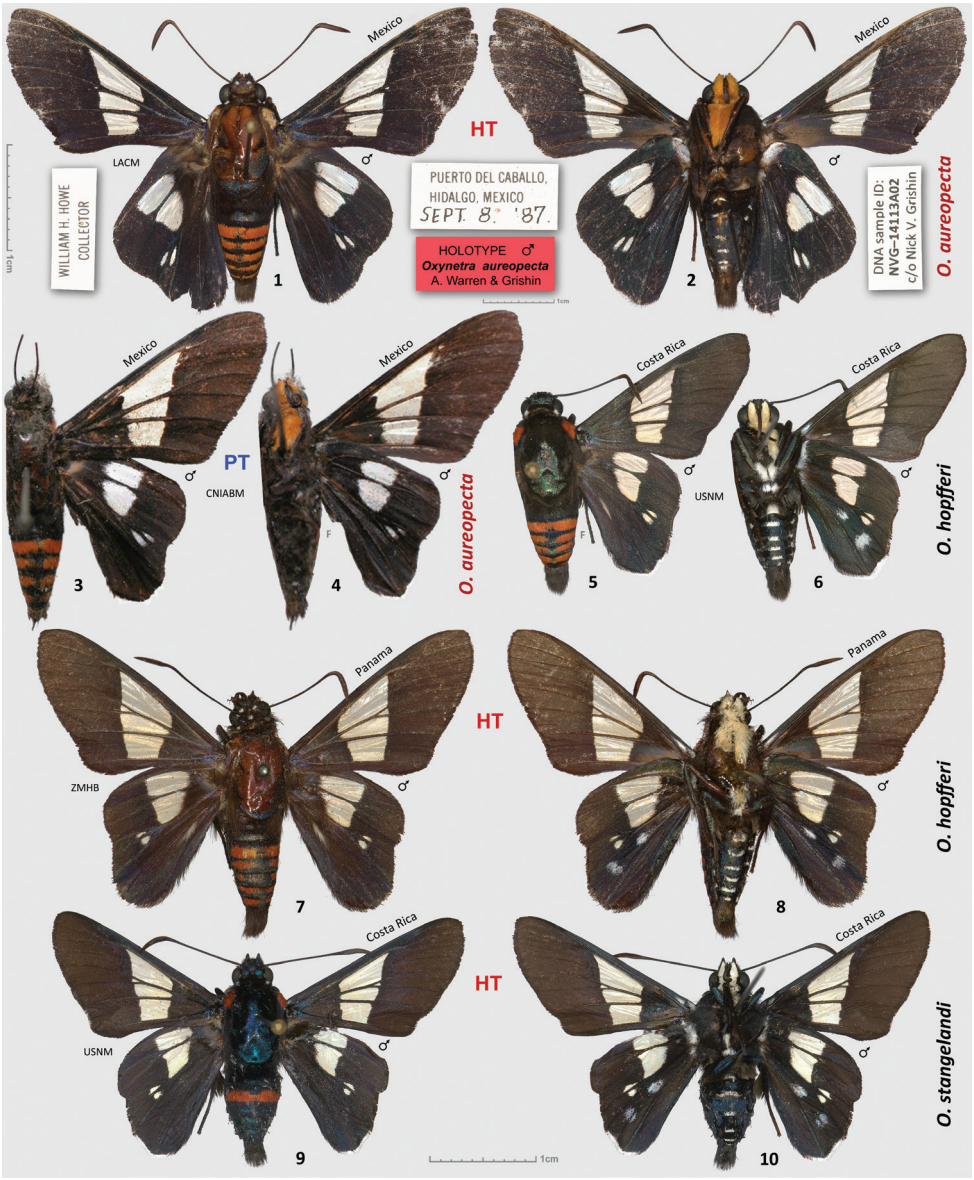
of a white streak on the dorsal hindwing posteriad of vein 2A shared with *O. hopfferi*, but not with O. *stangelandi*, it appears that the Mexican *Oxynetra* might be more distant from both *O. hopfferi* or *O. stangelandi*, a sister-species pair differing by about 3% (about 20 base pairs) in the COI barcode (Grishin et al. 2013). We therefore describe the Mexican taxon as a distinct species.

***Oxynetra aureopecta* A. Warren & Grishin, sp. n.**
http://zoobank.org/9325E7C1-E690-4E38-9709-BC83613B4D18
Figs 1–4, 11 part

Description. Male (Figs 1–4): right forewing length 21.8 mm (holotype). Hindwing (HW) narrow and elongate; forewing (FW) extending well beyond it. Outer wing margin slightly concave at cell CuA_2–1A+2A of FW and at cells between veins M_1 and M_3 of HW. Dorsal and ventral FW (including fringe) brownish-black with blue-purple metallic sheen, and with a hyaline band from anterior edge of discal cell (where it is widest) to vein 1A+2A; band divided into three aligned parts by dark-scaled veins CuA_1 and CuA_2; its outer edge does not extend beyond the origin of vein M_3; the hyaline wedge at the very base of cell M_3–CuA_1 is either very small (holotype) or lacking (paratype). Dorsal HW concolorous with FW (except fringe around tornus white), with two median, large, aligned, hyaline spots in cell Sc+R1–Rs (oval) and in discal cell (roughly triangular), separated by vein Rs, and suggesting continuation of FW band; smaller, postmedian pair of hyaline streak-like spots in proximal ends of cells M_3–CuA_1 and CuA_1–CuA_2; a small patch of white hair-like scales in the discal part of cell 2A-3A. Ventral HW similar to dorsal, but with wing base white and with a diffuse patch of white scales in discal part of cell CuA2–1A+2A. Antenna black; nudum (missing on paratype) medium brown, 20 segments. Head and body primarily brownish-black with a blue-purple sheen, marked as follows: two small white spots at base of antenna and one small spot at dorsoposterior margin of eye; first and second segments of palpi orange ventrally; forecoxae orange; white patches at posterior margin of each sternite and on sides of abdomen; large orange spot on anterior half of tegula; five orange bands across terga III to VII. Genitalia not dissected. Female unknown.

Barcode sequence of the holotype. Genbank Accession KT272397, voucher NVG-14113A02, 658 base pairs:

AACTTTATATTTTATATTTGGAATTTGAGCAGGAATAATTG-
GAACTTCATTAAGATTACTAATTCGAACTGAATTAGGTACCCCCG-
GATCTTTAATTGGAAATGATCAAATTTACAATACTATCGTAACAGCT-
CATGCATTTATTATAATTTTTTTTTATAGTTATACCTATTATAATTG-
GAGGATTTGGAAATTGATTAATTCCTTTAATATTAGGAGCACCAGA-
TATAGCTTTTCCTCGTATAAATAATATAAGATTTTGATTATTACCCC-
CATCTTTAACTCTTTTAATTTCAAGAAGAACTGTAGAAAATGGTGTTG-
GAACTGGATGAACAGTTTATCCCCCCCTCTCTTCTAATATTGCTCAT-
CAAGGGGCCTCAGTTGATTTAGCTATTTTTTCTCTTCATTTAGCAG-



Figures 1–10. Males of *Oxynetra*. **1–4** *O. aureopecta* sp. n. holotype (**1–2**) and paratype (**3–4**), data in text **5–6** *O. hopfferi*, Costa Rica: Puntarenas, Monteverde, 1997, voucher 97-ZFuentes-055 [USNM] **7–8** *O. hopfferi* holotype, Panama: Chiriqui [ZMHB] **9–10** *O. stangelandi* holotype, Costa Rica: Guanacaste, eclosed on 19.VIII.2002, voucher 02-SRNP-23284 [USNM]. Dorsal and ventral surfaces are shown on odd- and even-numbered figures, respectively. Labels are shown for the holotype of the new species and are reduced 1.5 times compared to specimens: smaller scale bar above the top labels refers to labels, and larger scale bars refer to specimens. Pinholes and some imperfections have been removed to emphasize actual wing patterns.

GAATTTCTTCAATTTTAGGAGCTATTAATTTTATTACAACAATTAT-
TAATATACGAATTAAAAATTTATCTTTTGATCAAATACCTCTTTTTG-
TATGAGCAGTAGGAATTACTGCATTACTATTATTATTATCTTTACCT-
GTATTAGCAGGTGCTATTACTATACTTTTAACAGATCGAAATATTAA-
TACTTCTTTTTTTTGACCCAGCAGGTGGAGGAGATCCTATTTTATAT-
CAACATTTATTT

Types. Holotype ♂ (Figs 1–2) with the following four rectangular labels: white, printed and handprinted - || PUERTO DEL CABALLO, | HIDALGO, MEXICO | SEPT. 8. '87. ||; white, printed - || WILLIAM H. HOWE | COLLECTOR ||; red, printed - || HOLOTYPE ♂ | *Oxynetra aureopecta* | A. Warren & Grishin ||; white printed - || DNA sample ID: | NVG-14113A02 | c/o Nick V. Grishin ||. The holotype is in the Los Angeles County Museum of Natural History, Los Angeles, CA, USA (LACM). Paratype ♂ (Figs 3–4) from MEXICO: Veracruz, Presidio, R. Müller Coll., in CNIABM.

Type locality. MEXICO: Hidalgo: Puerto del Caballo, elevation about 1020 m, GPS approximately 21°10', –98°55'.

Etymology. The name of this new species refers to its orange "chest", including palpi beneath and forecoxae, which is the most obvious diagnostic character. The name is an adjective.

Distribution and habitat. *Oxynetra aureopecta* is known only from the holotype and one paratype, both males, from Puerto del Caballo, Hidalgo, and Presidio, Veracruz, which are about 300 km from each other in eastern Mexico. Puerto del Caballo is situated at about 1020 m in the central Sierra Madre Oriental, along Hwy. 85, about 4.5 air km southwest of the San Luis Potosí border. This area is comprised of cloud forest vegetation, near the transition at lower elevations to tropical deciduous forest. The Presidio, Veracruz area has been extensively modified, and very few forested areas remain; material labeled from Presidio includes species typical of tropical deciduous and cloud forest habitats. The similar *O. hopfferi* and *O. stangelandi* are both cloud forest denizens, the latter reported to use *Prunus annularis* (Rosaceae) as a larval foodplant (Grishin et al. 2013). Various *Prunus* species are likely present in the Puerto del Caballo area, including *P. samydoides* Schlecht., *P. salicifolia* HBK. and *P. microphylla* (Kunth) Hemsl. (Standley 1920, Pennington and Sarukhán 2005), which could serve as foodplants for *O. aureopecta*.

Diagnosis. This new species belongs to *Oxynetra* because it has the traits of the genus as defined by Evans (1951). In particular, "F end cell upright, convergent with termen at tornus" (Evans, 1951). By the COI DNA barcode, this species groups within *Oxynetra* as a sister to the *O. hopfferi* and *O. stangelandi* clade, in accord with similarities in appearance to these two species, and away from the *O. semihyalina* and *O. confusa* clade (Fig. 11). A combination of the following characters identifies males of *O. aureopecta*: (1) orange "chest", i.e., forecoxae and palpi beneath (males of both *O. hopfferi* and *O. stangelandi* have white forecoxae and palpi); (2) no postdiscal white spot on ventral hindwing in cell CuA_2-2A (the other two species possess this white spot in addition to the discal spot in that cell); (3) narrower forewing hyaline band barely extending distad the base of M_3-CuA_1 cell, and very small (or lacking) spot at the base

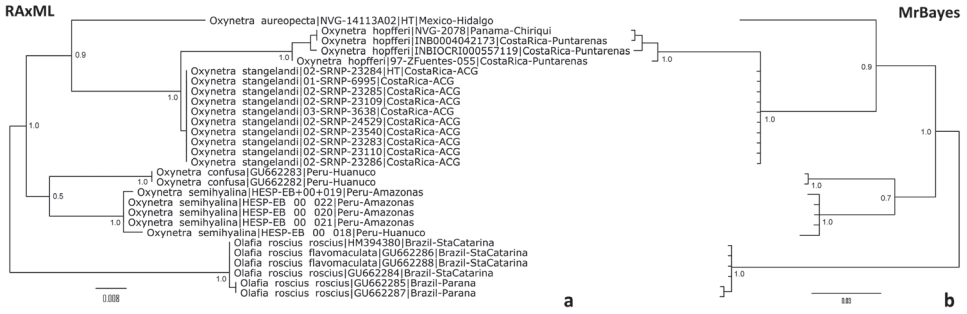


Figure 11. COI DNA barcode trees of *Oxynetra* species. The trees are obtained by **a** RAxML under "GTRGAMMA" model; and **b** MrBayes under "propinv" model with 2 states (see Materials and Methods) and show identical topology. The taxa are arranged in the same sequence in both trees. The trees are rooted with *Olafia* Nemésio, 2005 sequences. Bootstrap fractions (a) and posterior probabilities (b) are shown (except for nodes within species). Sequences with NVG- voucher codes were obtained in this work. For other sequences, ACG voucher codes (with -SRNP- and -ZFuentes-, Janzen & Hallwachs 2014), INBio voucher codes (starting with INB, Grishin et al. 2013), GenBank accessions (starting from GU and HM, http://genbank.gov/), or Ernst Brockmann collection voucher codes (with HESP-EB) are indicated for each sequence. The general locality of each specimen is indicated.

of this cell (the band prominently extends distad of M_3-CuA_1 cell and the hyaline spot at the base of this cell is more prominent in the other two species); (4) longer (and narrower), streak-like spots in hindwing cells M_3-CuA_1 and CuA_1-CuA_2 (the spots, in particular the one in cell CuA_1-CuA_2, are rounder in the other two species); (5) five orange bands on the abdomen above, similarly to *O. hopfferi* (only a single complete band is present in *O. stangelandi*, Fig. 9); (6) a weakly developed white streak of a few hair-like scales near the anal fold on dorsal hindwing (the streak is absent in *O. stangelandi*, Fig. 9, but is well-defined in *O. hopfferi*, Figs 5, 7); **(7)** DNA COI barcode 6.1% and 4.7% different from that of *O. hopfferi* and *O. stangelandi*, respectively. Characters (1) and (3) appear to be the most easily observed. The female of *O. aureopecta* is unknown but may be mostly black similar to females of the other two species.

Discussion. The description of *O. aureopecta* adds a fifth species to *Oxynetra*, and confirms the occurrence of the genus in Mexico. While the damaged paratype specimen in CNIABM has been examined by many researchers, its authenticity has been questioned since it was apparently the only specimen of the genus labeled from Mexico (De la Maza et al. 1991, Warren 2000). Thus, the discovery of the holotype specimen in the LACM, in much better condition than the paratype—and nearly identical in appearance—confirms the provenance of *Oxynetra* in Mexico. Based on the known distribution of *O. aureopecta* in cloud forest habitats of the Sierra Madre Oriental, we suspect that the species might be endemic to Mexico.

However, *Oxynetra* species remain unknown from Guatemala, El Salvador, Honduras and Nicaragua. Given the rarity of species in the *hopfferi* group- the only group of the genus thus far known to occur in Mesoamerica, it may be that the genus has merely gone undetected in those countries (significant rearing efforts were necessary

to detect the presence of *O. stangelandi* in northwestern Costa Rica). Therefore, much more fieldwork must be conducted before the overall distributions of *Oxynetra* species in Mesoamerica can be defined.

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