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A new genus and species of golden coral (Anthozoa, Octocorallia, Chrysogorgiidae) from the Northwest Atlantic

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Abstract

A new genus and species of unbranched golden coral, *Flagelligorgia gracilis*, is described based on several specimens collected off the southeastern coast of the United States. The genus is provisionally included in the family Chrysogorgiidae, pending molecular confirmation. *Flagelligorgia* morphologically resembles other unbranched chrysogorgiids, such as *Distichogorgia*, *Chalcogorgia*, *Helicogorgia* and *Radicipes*, to which it is compared. The type species is illustrated and its distribution mapped.

Keywords

Flagelligorgia gracilis, unbranched octocoral, biserial polyp arrangement, *Radicipes*, southeastern USA

Introduction

Chrysogorgiids form a polyphyletic family of octocorals (Pante et al. 2012), which is distributed in all oceans throughout the world (Watling et al. 2011). Most of its genera are in need of revision, some of which require reallocation into new families (Pante et al. 2012). In the course of doing a revision of the genus *Radicipes* Stearns,

1883 (see Cordeiro et al. in press), an unusual species was encountered that was similar to species in that genus that were already known, but was consistently different from them in several characters. Although no recently collected specimens were available for molecular analysis, sufficient alcohol-preserved specimens were present at the NMNH to describe the new species and the new genus in which it is placed.

Material and methods

All specimens are preserved in ethanol (70%) and deposited at the National Museum of Natural History. Reference material of all species of *Radicipes* was also examined (Cordeiro et al. in press). The terminology used in the description follows that of Bayer et al. (1983). Preparation of polyps for SEM to reveal sclerites includes short digestion (four seconds) of superficial coenenchyme, rinsing several times in distilled water, and drying at room temperature.

Abbreviations used in the text include: *Alb* – USFWS *Albatross*, L:W – length to width ratio of a sclerite, MCC – Monophyletic Chrysogorgiidae Clade *sensu* Pante et al. (2012), NMNH – National Museum of Natural History, Smithsonian Institution, USNM – United States National Museum (now known as the NMNH, but acronym still used for catalog numbers).

Taxonomy

Subclass Octocorallia Haeckel, 1866
Order Alcyonacea Lamouroux, 1812
Suborder Calcaxonia Grasshoff, 1999

Family Chrysogorgiidae Verrill, 1883

Chrysogorgidae Verrill 1883: 21.

Chrysogorgiidae: Versluys 1902: 2–4; Bayer 1956: F216; Bayer and Muzik 1976: 67–69 (key to genera); Bayer 1979: 876–878 (key to genera); Cairns 2001: 748–754 (synonymy); Pante and France 2010: 600 (key to genera); Watling et al. 2011: 68–74 (distribution maps of all genera); Pante et al. 2012: 1–12 (phylogenetic and bathymetric analyses).

Type genus. *Chrysogorgia* Duchassaing & Michelotti, 1864.

Diagnosis (after Cairns (2001) and Pante et al. (2012)). Calcaxonians having an unjointed, solid (non-spicular), concentrically layered scleroproteinous axis. The axial layers are usually smooth (not undulated) and thus not longitudinally grooved externally; the axis usually displays metallic or iridescent reflections. The colony may be branched or unbranched (flagelliform), arising from a root-like or discoidal, strongly

calcified holdfast. Polyps are contractile but not retractile, arranged in rows (uniserial, biserial or multiserial), but never in whorls. Sclerites predominantly flat, smooth scales, in some species warty rods and spindles.

Remarks. Fourteen genera have been assigned to the Chrysogorgiidae, but based on sequencing of three genes, Pante et al. (2012) have suggested that only six of these genera belong to Chrysogorgiidae *sensu stricto*, which they call the Monophyletic Chrysogorgiidae Clade, or MCC. They imply that the other genera may belong to as many as three other, as yet undescribed, families. The six genera of the MCC are keyed by Pante and France (2010) and their worldwide distributions plotted by Watling et al. (2011). They are further discussed by Pante et al. (2012) in the context of a phylogenetic analysis. *Flagelligorgia* is morphologically most similar to *Radicipes*, which is part of the MCC, but lacking material suitable for molecular analysis, the placement of *Flagelligorgia* in that family can only be a suggestion at this time. In fact, we did attempt to sequence four genes (*COI*, *mtMutS*, *28S* and *18S*) from specimens collected in 1964 with no results.

Distribution. Worldwide, 31–4492 m depths (Pante and Watling 2011: 6).

***Flagelligorgia* gen. n.**

http://zoobank.org/0C142EEB-5AD6-4EE5-A654-F0C5DD669E79

Type species. *Flagelligorgia gracilis*, here designated. Gender: feminine.

Diagnosis. Colonies unbranched (flagelliform), loosely coiled, attached by a holdfast; axis composed of undulating concentric layers (Figs 1C–E, 2D). Polyps arranged biserially. Body wall and coenenchymal scales elongate, smooth scales. Polyps lack an operculum.

Remarks. Until 1956 (Bayer 1956) the Chrysogorgiidae had been divided into three subfamilies, one being the Lepidogorgiinae Versluys, 1902, consisting of genera with an unbranched colony and lacking polyp opercula. However, the subfamily classification has been ignored for decades and is not supported by molecular evidence. Nonetheless, it is incumbent to compare *Flagelligorgia* to the four other unbranched chrysogorgiid genera. *Helicogorgia* Bayer, 1981, known only from the east coast of Africa at 66–775 m depth range, is unbranched but has its polyps arranged in a multiserial fashion on one side of the stem, a naked track displayed on the opposite side. Furthermore, its body wall scales are irregular plates and its coenenchymal scales are spindles. For all of these reasons *Helicogorgia* is easily distinguished from *Flagelligorgia*. Based on sequencing of three genes, Pante et al. (2012) indicate that *Helicogorgia* might constitute a family on its own, as sister to the Chrysogorgiidae. *Helicogorgia* was revised by Williams (1992), recognizing four species in the African coast.

Chalcogorgia Bayer, 1949, known only from its type locality off northwestern Cuba at 708 m depth, is also unbranched and has biserially arranged polyps, like *Flagelligorgia*, but differs in having eight triangular opercular scales on each polyp, and body wall scales shaped as irregularly shaped platelets. According to Pante et al. (2012:

8), based on "very limited data," *Chalcogorgia* was suggested to ally with *Helicogorgia*, and thus also outside of the MCC.

Distichogorgia Bayer, 1979, known only from the Blake Plateau off Florida at 814 m depth, is unbranched and has biserially arranged polyps, but differs in having unique body wall scales consisting of two fans of longitudinally arranged, blade-like scales, one on each side of the polyp, interspersed with tiny pointed sclerites, similar to its coenenchymals. Pante et al. (2012) did not report sequencing data for this genus, but based on its biserially polyps, they suggested an affinity with *Helicogorgia* and *Chalcogorgia* in a family separate from the Chrysogorgiidae.

Perhaps most similar to *Flagelligorgia* is the genus *Radicipes* Stearns, 1883, consisting of 9–10 species (see Cordeiro et al., in press), which occurs worldwide at depths of 241–3580 m. *Radicipes* is unbranched but differs from *Flagelligorgia* in having uniserially arranged polyps, and a body wall and coenenchymal sclerites that are rod-shaped to compressed rod-shaped. Furthermore, chrysogorgiids (MCC), including *Radicipes*, are characterized by having an axis formed by non-undulated (smooth) concentric layers (Bayer 1956), which is not the case for *Flagelligorgia*. Based on molecular evidence Pante et al. (2012) placed *Radicipes* firmly in the Chrysogorgiidae clade (MCC), as the sister genus to *Chrysogorgia*. Lacking molecular evidence for *Flagelligorgia* (all specimens are quite old), it is not possible at this time to determine if it is allied with *Radicipes* in the Chrysogorgiidae (based on similarity of body wall sclerites) or the other cluster of genera including *Helicogorgia, Chalcogorgia* and *Distichogorgia* (based on polyp arrangement), which may constitute an as yet undescribed family.

Distribution. Off Southeastern United States from North Carolina to Florida, 196–567 m depths.

Etymology. The name is a combination of *flagellum* (Latin for small whip) and *gorgia* (a common octocoral suffix and once the name of the order Gorgonacea, the name derived from the mythical female monster, the Gorgon), in reference to the flagelliform nature of the colony.

***Flagelligorgia gracilis* sp. n.**
http://zoobank.org/579269D2-B012-4C73-A854-E743F7154252
Figs 1–4

Material examined (Types). Holotype: *Alb*-2666, 1 specimen now in two pieces, USNM 49503. Paratypes: *Alb*-2601, 34°39'15"N, 33°30'10"W, 196 m, 1 colony, USNM 16607, 18 October 1885; *Alb*-2602, 34°38'30"N, 75°33'30"W, 227 m, 1 colony, USNM 16821, 18 October 1885; *Alb*-2666, 30°47'30"N, 79°49'W, 494 m, 33 colonies, SEM stubs 2364–2367, USNM 14458, 5 May 1886; *Alb*-2667, 30°53'N, 79°42'30"W, 499 m, 40 colonies, USNM 14457, 5 May 1886; *Gerda*-179, 27°41'N, 79°11'W, 549–567 m, 9 colonies, USNM 57315, 1 July 1963; *Megalopa*, 11.2 km SSE of Carysfort Reef, Florida Keys, 205 m, 9 July 1950, 1 colony, USNM 51956, 9 July

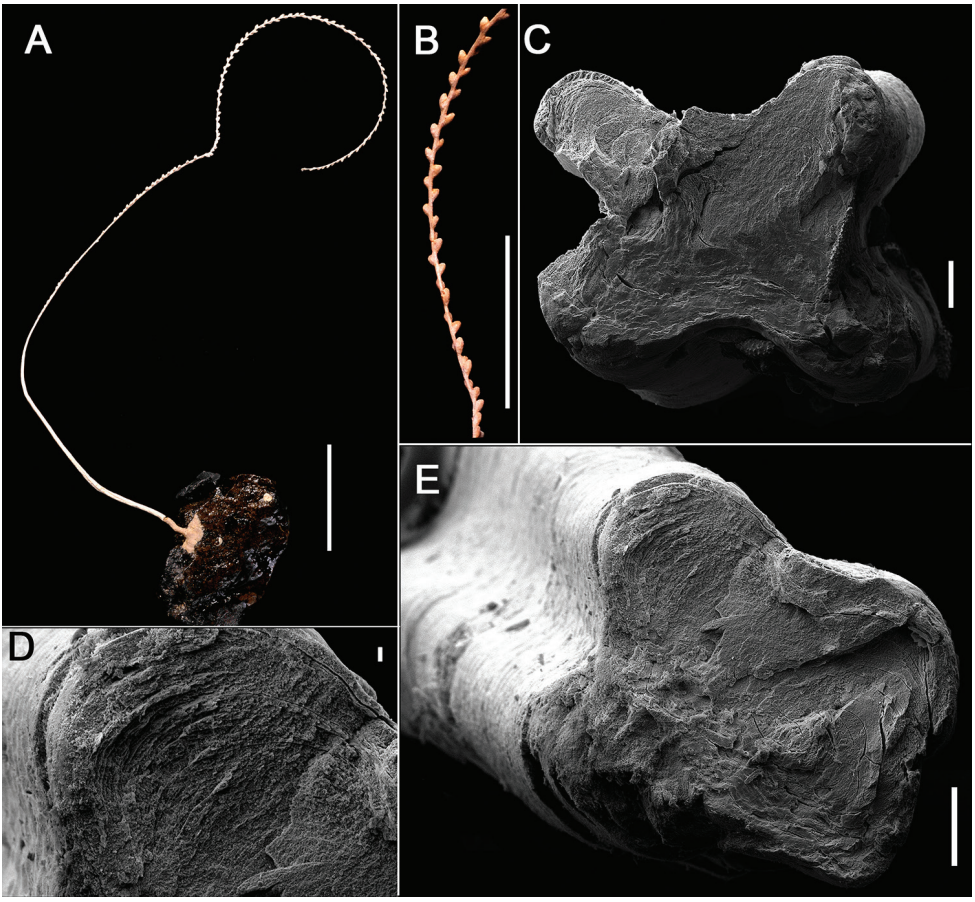

Figure 1. Diagnostic characters of *Flagelligorgia gracilis* sp. n. **A** general view of the holotype (USNM 49503) attached to a rocky substrate **B** detail of the holotype showing the biserial arrangement of polyps **C,E** cross-section of the stem of the holotype showing its four rounded longitudinal cords **D** detail of undulating concentric layers of the stem in cross-section. Scale bars: **A**: 10 mm, **B**: 5 mm, **C, E**: 0.1 mm, **D**: 0.01.

1950; *Pillsbury*-105, 31°00'N, 79°42'W, 388–403 m, 2 colonies, USNM 57316, 27 July 1964; *Pillsbury*-197, 27°59'N, 79°20'W, 567–586 m, 2 colonies, USNM 52913, 11 August 1964.

Type locality. 30°47'30"N, 79°49'W (continental slope off Georgia), 494 m depth.

Description. The colony is unbranched (flagelliform) and quite delicate, with an ascending clockwise spiral growth form (Fig. 1A, 2C). One of the largest specimens (the holotype) is 19 cm in length and only 1.1 mm in basal stem diameter. Colonies are attached to hard objects such as the deep-water coral *Lophelia pertusa* (Linnaeus, 1758), rocks, or echinoid tests, having a thin basal encrustation (holdfast) up to 7 mm in diameter; there are no root-like holdfasts. The axis is longitudinally grooved (Fig. 2D), resulting in four rounded longitudinal cords (1C, E), reflecting the underly-

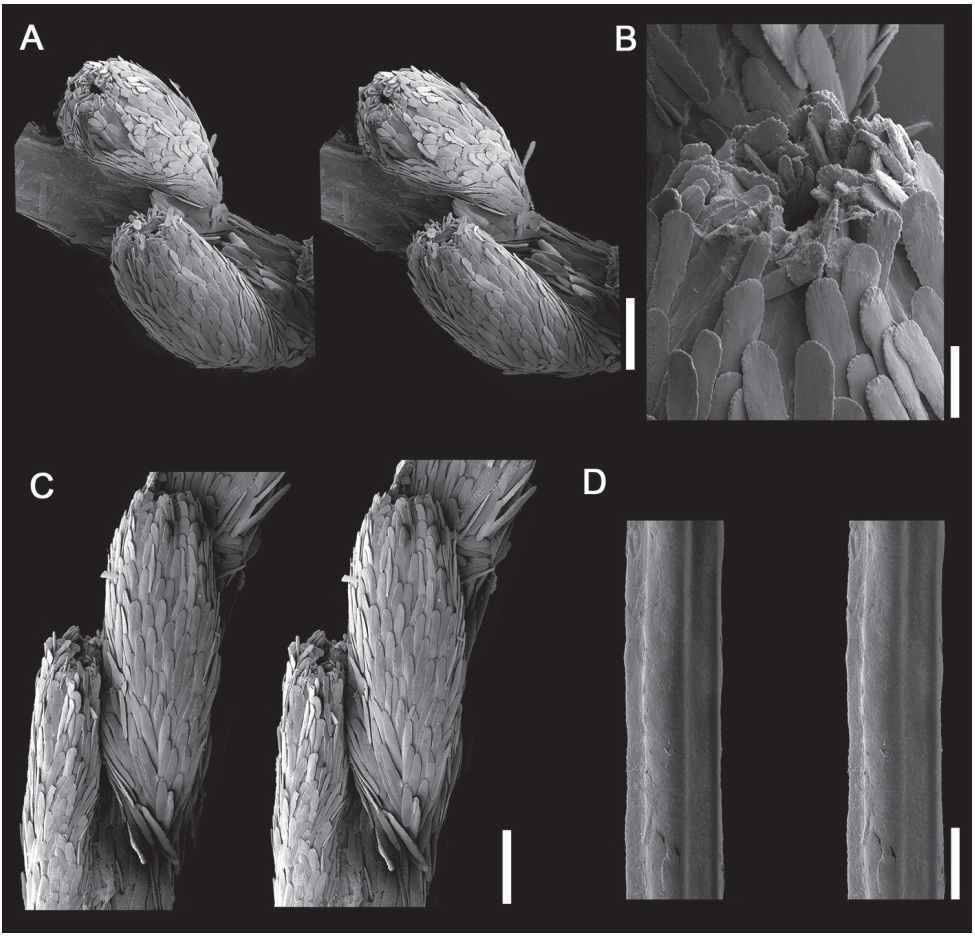

Figure 2. Detailed view of polyps and axis in *Flagelligorgia gracilis* sp. n. (USNM 14458) through Scanning Electron Microscopy. **A** stereo view of polyps **B** oral view of a polyp **C** stereo view of a polyp's abaxial side **D** stereo view of the axis. Scale bars: **A, C**: 0.2 mm, **B**: 0.04 mm, **D**: 0.01 mm.

ing undulated layers of scleroprotein (Fig. 1D), most easily seen in stem cross section. The axis is golden, the coenenchymal tissue usually pale brown to a dull yellow, in ethanol. Polyps are lacking from the proximal 45–50 mm of the stem, which is approximately 1/5–1/3 of the colony length, depending on its maturity (Fig. 1A). Polyps occur biserially, on opposite sides of the stem in alternating fashion (Figs 1B, 2A, C), and are relatively closely spaced such that 1.1–1.4 polyps occur per cm length. The polyps are 0.9–1.2 mm in length and are somewhat cigar-shaped (Fig. 2C), being slightly tapered distally; the greatest diameter (0.35–0.39 mm) is usually at mid-length. The body wall is covered with longitudinally oriented, elongate (L:W = 4.5–5.8), imbricating scales (Fig. 3B) that measure 0.17–0.24 mm in length. They are rounded distally, have smooth lateral edges, and are quite thin (e.g., 13–15 μm in thickness). Their outer and inner surfaces are smooth. Toward the end of the polyp are smaller scales

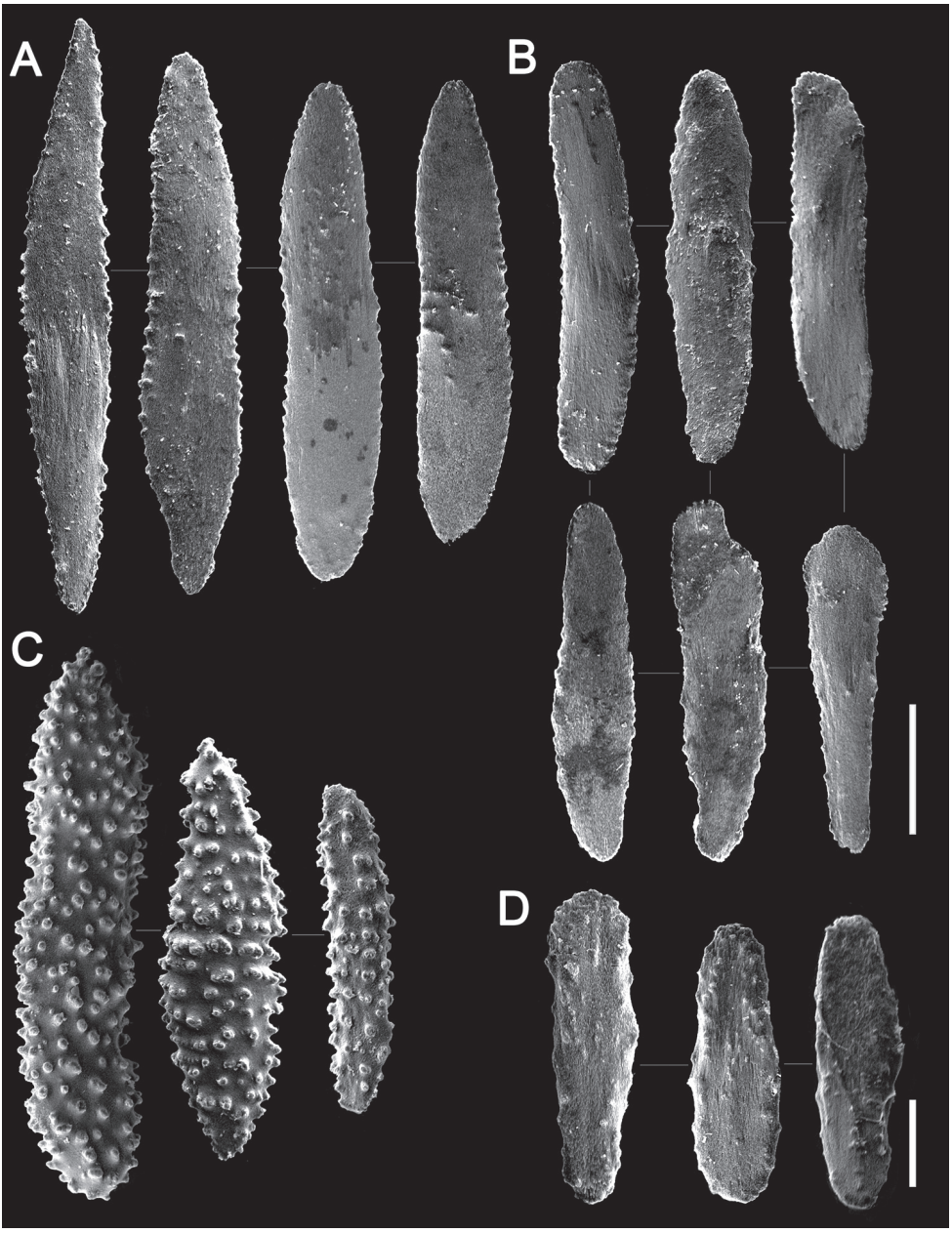

Figure 3. Sclerites of *Flagelligorgia gracilis* sp. n. (USNM 49503, holotype). **A** rods from coenenchyme **B** elongate rods from body wall **C** highly granular scales from first centimeters of the stem **D** tentacular scales. Scale bars: **A–C**: 0.05 mm, **D**: 0.02 mm.

associated with the tentacles (Figs 2B, 3D), similar in shape to the body wall scales but only 0.075–0.10 mm in length. Pinnular sclerites are virtually absent. Scales show concentric bands of interference colors in polarized light. There are no sclerites in the

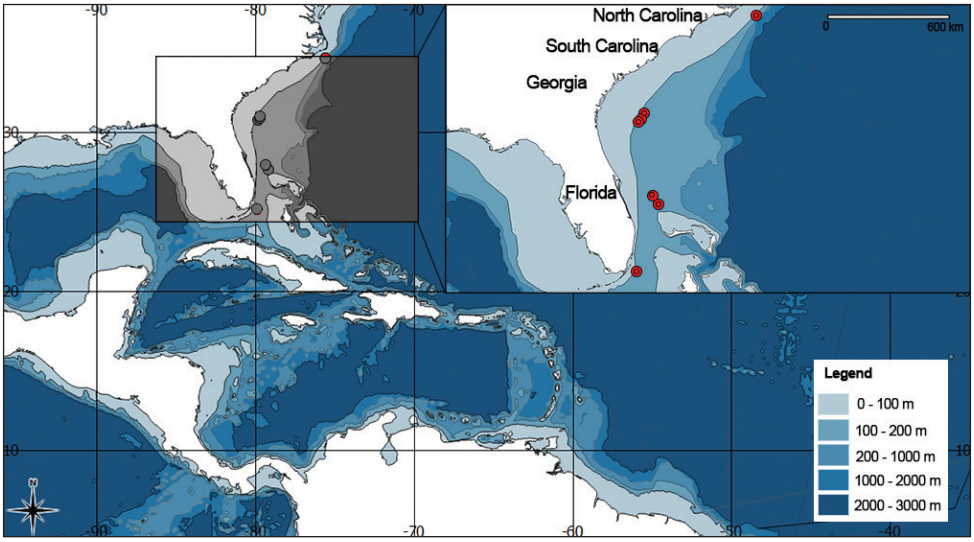

Figure 4. Distribution map of *Flagelligorgia gracilis* sp. n.

axial sheath of coenenchyme. The outer coenenchymal scales (Fig. 3A) are also longitudinally arranged on the stem, elongate (L:W = 4.8–7.9), and imbricate. They have pointed tips and their lateral edges are slightly serrate, each serration up to 5 µm in height; they are also quite thin, and their faces are also smooth. Coenenchymal scales on the first few centimeters of the stem are highly granular (Fig. 3C).

Remarks. As in other unbranched octocorals, such as species of *Radicipes*, some specimens (e.g., from USNM 14458, USNM 51956 and USNM 14457) host commensal ophiuroids. Other octocoral species found at the same stations at which *Flagelligorgia* were collected include: *Plumarella aurea* (Deichmann, 1936), *Plumarella pourtalesii* (Verrill, 1883), *Plumarella dichotoma* Cairns & Bayer, 2004, *Swiftia casta* (Verrill, 1883) and *Callogorgia americana* Cairns & Bayer, 2002.

Distribution. Southeastern coast of United States from off Outer Banks, North Carolina to off Carysfort Reef (near Key Largo, Florida) (Fig. 4), 196–567 m depths.

Etymology. Named *gracilis* (Latin for slender, gracile), in allusion to the very slender aspect of the colony.

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RESEARCH ARTICLE

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Molecular and morphological identification of *Biomphalaria* species from the state of São Paulo, Brazil

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Abstract

DNA barcoding and morphological characters were used to identify adult snails belonging to the genus *Biomphalaria* from 17 municipalities in the state of São Paulo, Brazil. The DNA barcode analysis also included twenty-nine sequences retrieved from GenBank. The final data set of 104 sequences of the mitochondrial cytochrome oxidase I (COI) gene was analyzed for K2P intraspecific and interspecific divergences, through tree-reconstruction methods (Neighbor-Joining, Maximum Likelihood and Bayesian inference), and by applying different models (ABGD, bPTP, GMYC) to partition the sequences according to the pattern of genetic variation. Twenty-seven morphological parameters of internal organs were used to identify specimens. The molecular taxonomy of *Biomphalaria* agreed with the morphological identification of specimens from the same collection locality. DNA barcoding may therefore be a useful supporting tool for identifying *Biomphalaria* snails in areas at risk for schistosomiasis.

Keywords

Biomphalaria, COI,DNA barcoding, morphological taxonomy, , schistosomiasis, species identification

Introduction

Brazil contains one of the richest faunas of freshwater snails of the genus *Biomphalaria* (Agostinho et al. 2005, Scholte et al. 2012). The state of São Paulo, in southeast Brazil, is of enormous epidemiological importance, as all the three Neotropical intermediate hosts of *Schistosoma mansoni* (Sambon, 1907), *Biomphalaria glabrata* (Say, 1818), *B. straminea* (Dunker, 1848), *B. tenagophila* (d'Orbigny, 1835), are distributed in streams, ponds, dams and reservoirs in this municipality. *Biomphalaria occidentalis* (Paraense, 1981), *B. peregrina* (d'Orbigny, 1835), *B. intermedia* (Paraense & Deslandes, 1962), *B. oligoza* (Paraense, 1975) and *B. schrammi* (Crosse, 1864) are also distributed in São Paulo state (Vaz 1989; Teles 2005; Ohlweiler et al. 2010).

Identification of *Biomphalaria* specimens to the species level and analysis of infection by *S. mansoni* are key elements of surveillance strategies for schistosomiasis control and elimination (PAHO 1968, WHO 2013). Shell morphology is of limited use for identifying different species of snails in this genus (Paraense 1966; Jarne and Théron 2001), and therefore the anatomical characters described by Paraense (1961, 1975, 2001) are used instead. However, identification of *Biomphalaria* solely based on morphological characters is constrained by phenotypic plasticity, the limited descriptions of cryptic species, and the difficulty in applying species-diagnostic characters to juvenile specimens (Carvalho et al. 2008; Teodoro et al. 2010). The issue of how useful molecular tools may be in the identification of *Biomphalaria* snails has become particularly important in recent years as there is consensus among malacologists that morphological identification using internal anatomical parameters is susceptible to error, especially when the snails being analyzed belong to complexes of morphologically similar species (Paraense 1972, 1974, 1988; Spatz et al. 1999; Vidigal et al. 2000). To overcome these limitations and difficulties associated with traditional taxonomy, various methodologies based on molecular markers have been developed.

PCR-RFLP analysis of mitochondrial and nuclear genes (Spatz et al. 1999; Vidigal et al. 1998, 2000; Caldeira et al. 2000, 2009), fingerprinting techniques using non-specific primers (Abdel-Hamid et al. 1999; Al-Quraishy et al. 2014) and sequence analysis of COI and r16RNA genes and ITS-1 and ITS-2 sequences (Woolhouse and Chandiwana 1989; Langand et al. 1998; Vidigal et al. 2000; Campbell et al. 2000; DeJong et al. 2003; Wethington et al. 2007; Tuan and Santos 2007; Tuan et al. 2012) have all produced results that allowed significant genetic differences in species and populations to be identified.

When used in conjunction with bioinformatics tools and sequence databases, DNA barcoding routinely facilitates the identification of biological species (Ratnasingham and Hebert 2007; Casiraghi et al. 2010). This technique is based on the polymorphism of a short region (approximately 600 bp long) of the mitochondrial cytochrome c oxidase 1 (COI) gene (Hebert et al. 2003). DNA barcode includes a series of strategies for delimiting species into molecular operational taxonomic units (MOTUs) using a combination of laboratory and bioinformatics methods (Fontaneto et al. 2013). The most important strategies for identifying MOTUs include analysis of intraspecific

and interspecific genetic distances, and analyses based on population and phylogenetic models. These approaches include (ABGD) (Puillandre et al. 2012) and the barcode index number (BIN) system (Ratnasingham and Hebert 2013), which use algorithms based on the partition of molecular data according distance methods, and the generalized mixed Yule coalescent (GMYC) method (Fujisawa and Barraclough 2013) and Bayesian Poisson Tree Processes (bPTP) method (Zhang et al. 2013).

DNA barcoding has been used to augment morphological identification of *Bulinus* in Africa (Kane at al. 2008; Stothard et al. 2013; Standley et al. 2014), and yielded better results than identifications based on shell characters. Although there are over 500 COI sequences in GenBank from snails of the genus *Biomphalaria* found in African and Neotropical regions, most DNA barcoding studies use African species. There is therefore a dearth of knowledge about the effectiveness of DNA barcoding in taxonomic identification of Neotropical species of *Biomphalaria* (Standley et al. 2011; Tuan et al. 2012).

Here, we investigate the utility of analysis of distributions of intraspecific and interspecific COI divergences based on genetic distances, tree reconstruction methods based on Bayesian inference, Maximum Likelihood (ML), and K2P-Neighbor-Joining (NJ) grouping of sequences, and the ABGD, GMYC and bPTP methods for delimitation of *Biomphalaria* species in conjunction with schistosomiasis field surveys.

Materials and methods

Experimental design

Planorbids were collected in 17 municipalities in the state of São Paulo, Brazil between May 2012 and January 2013 (Fig. 1). The collection points were georeferenced with a Garmin ETrex Summit® GPS (Table 1).

Samples were collected from freshwater habitats in the Paranapanema, Tietê, Ribeira do Iguape and Paraíba do Sul River basins and the northern coast of São Paulo that had been previously surveyed and classified according to the risk for schistosomiasis transmission as part of a program to monitor snails that are intermediate hosts of *S. mansoni* (*Biomphalaria*).

In accordance with the methods described in the Brazilian Ministry of Health Schistosomiasis Surveillance and Control Program (Ministry of Health 2008), snails were collected at sampling stations in each freshwater body and grouped into batches according to their origin. Most of the snails in each batch were then exposed to artificial light in the laboratory to determine whether they were infected with cercariae. At least two specimens from each batch were used for morphological analysis and at least two for the DNA barcode analysis.

DNA barcoding was applied to 75 adult snails taken from samples collected in the field. Only snails that did not have any parasite larvae in their digestive gland and ovotestis were used for molecular identification. Shells were removed by compress-

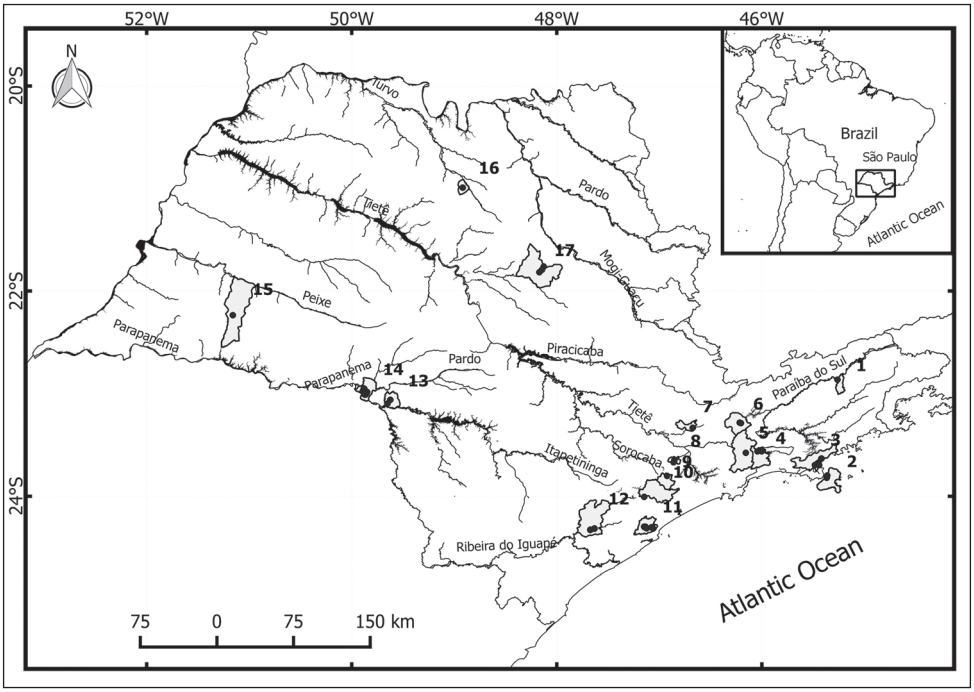

Figure 1. Locations of the 17 municipalities in São Paulo (Brazil) where the snails were collected. **1** Aparecida **2** Ilhabela **3** Caraguatatuba **4** Biritiba Mirim **5** Mogi das Cruzes **6** Santa Isabel **7** Franco da Rocha **8** Embu das Artes **9** São Lourenço da Serra **10** Juquitiba **11** Itariri **12** Juquiá **13** Ipaussu **14** Ourinhos **15** Martinópolis **16** Novais **17** Araraquara (coordinates are detailed in Table 1).

ing each snail between two slides. After removing the shell fragments, each crushed snail was transferred to a clean Petri dish. The portion of the cephalopodal mass corresponding to the foot was excised under a stereo microscope with forceps and scissors and used as starting material for isolation of total DNA. To maximize the efficiency of genomic DNA purification we used fresh material that had not been fixed. Each specimen was then dissected and identified to the species level based on the presence or absence of the renal ridge and the most informative characters of the male and female copulatory organs. DNA barcoding was carried out in a blind fashion, i.e., without prior knowledge of the general morphological characteristics identified in the animal.

An additional 118 adult specimens were taken from the same field samples (at least two per batch) and scored for 27 morphological characters used by Paraense (1975, 1981, 1984, 2001) in his descriptions of Neotropical species of the genus *Biomphalaria*. The soft parts were removed from the shell after placing the snails in 70°C for 40 seconds and then fixing them in Railliet-Henry's solution (distilled water 930 mL, sodium chloride 6 g, formalin 50 mL and glacial acetic acid 20 mL). After at least 24 hours of fixation, the specimens were dissected following Deslandes' (1951) protocols to examined the renal tube and reproductive system. Specimens were not anesthetized

Table 1. Collection localities, sample information, and GenBank accession numbers for COI sequences used in this study.

Sample Sites/ Country	Map locality	Municipality	Latitude (S)	Longitude (W)	COI sequence	GenBank accession number
São Paulo, Brazil	1	Aparecida	22°51'52.0"	45°15'46.0"	589, 588, 591	KF926184, KF926196, KF926186
	2	Ilhabela	23°49'17.5"	45°22'01.4"	564,555	KF926191, KF926187
			23°47'56.4"	45°21'44.0"	593,554	KF926213, KF926212
	3	Caraguatatuba	23°37'55.7"	45°25'08.7"	563	KF926218
			23°37'59.6"	45°25'11.4"	517	KF926105
			23°38'04.2"	45°25'14.7"	579, 580	KF926221, KF926217
			23°38'3.25"	45°25'14.3"	516	KF926106
			23°40'26.1"	45°26'54.3"	592	KF926215
			23°40'42.2"	45°27'18.5"	568	KF926214
			23°41'34.8"	45°26'58.1"	569	KF926216
			23°41'46.4"	45°28'57.9"	565, 571	KF926219, KF926220
			23°41'49.5"	45°26'30.8"	523	KF926222
	4	São Paulo	23°33'43.0"	45°59'66.0"	551	KF926204
			23°33'44.0"	46°02'35.0"	548, 549	KF926203, KF926205
	5		23°33'95.0"	46°09'24.0"	547	KF926202
	6	Santa Isabel	23°17'16.8"	46°12'16.1"	544	KF926174
			23°17'00.2"	46°12'59.1"	545, 546, 550, 552	KF926177, KF926189, KF926195, KF926190
	7	Franco da Rocha	23°20'02.0"	46°40'28.0"	-	
	8	Embu das Artes	23°38'50.5"	46°51'11.3"	524	KF926197
			23°40'08.5"	46°51'41.7"	640	KF926198
	9	Embu-Guaçu	23°48'11.0"	46°55'27.0"	630	KF926201
	10	Juquitiba	24°00'21.0"	47°08'52.0"	-	
	11	Itariri	24°17'53.6"	47°08'55.0"	537	KF926188
			24°17'55.0"	47°08'06.8"	536	KF926211
			24°18'26.3"	47°03'58.9"	618	KF926207
			24°18'39.9"	47°07'31.4"	503	KF926206
			24°18'11.8"	47°04'04.1"	532, 627, 534	KF926209, KF926185, KF926208
			24°18'13.5"	47°04'31.7"	535	KF926210

Sample Sites/ Country	Map locality	Municipality	Latitude (S)	Longitude (W)	COI sequence	GenBank accession number
São Paulo, Brazil	12	Juquiá	24°18'55.1"	47°37'58.6"	650, 651, 653	KT225577, KT225578, KT225579
			24°19'39.5"	47°40'25.0"	655	KT225580
	13	Ipaussu	23°05'39.6"	49°39'01.5"	756, 761, 755	KX354441-KX354442, KX354440
	14	Ourinhos	22°57'00.2"	49°52'33.1"	764	KX354435
			22°58'02.5"	49°52'27.1"	572, 543, 573	KF926181, KF926182, KF926183
			22°58'03.4"	49°52'28.9"	735, 733, 766	KX354437-KX354438, KX354433
			22°59'08.0"	49°50'59.9"	577	KF926192
			22°58'29.5"	49°53'29.4"	538, 578	KF926165, KF926193
			23°00'24.8"	49°51'48.7"	739	KX354444
			23°00'32.2"	49°52'21.9"	763, 765	KX354436, KX354434
			23°00'11.5"	49°51'41.4"	747	KX354443
			22°57'11.6"	49°52'41.9"	636, 540	KF926194, KF926166
			22°57'11.6"	49°52'41.9"	575, 542	KF926168, KF926167
			22°59'42.4"	49°52'27.6"	541	KF926178
	15	Martinópolis	22°14'04.4"	51°09'36.4"	581, 582	KX354445, KF926180
	16	Novais	20°59'30.0"	48°55'05.0"	570, 586, 587	KF926179, KF926169, KF926171
	17	Araraquara	21°45'37.9"	48°07'40.1"	595, 599, 601	KF926170, KF926173, KF926172
			21°47'30.3"	48°08'41.1"	594, 596	KF926199, KF926200
			21°48'57.1"	48°10'13.1"	602	KF926175
Argentina		*ARG_1, ARG_2, ARG_3, ARG_4*				JN621901, JN621902 JN621903, GU168593
Brazil		*BRA_1*				AF199090
		RS_*BRA_2*, RS_*BRA_3*, RS_*BRA_4*, MG_*BRA_5*, *BRA*_6, *BRA*_7, *BRA*_8, *BRA*_9				KF926107, KF926108 KF926109 AF199094 AF199091 , AF199092, AF199095 , AF199096
		RS_*BRA_10*				KX354439
		*BRA*_11				AF199084

Sample Sites/ Country	Map locality	Municipality	Latitude (S)	Longitude (W)	COI sequence	GenBank accession number
Brazil		RS_*BRA_12*, RS_*BRA_13*, RS_*BRA_14*, RS_*BRA_15*, RS_*BRA_16*				KF926155-KF926156 KX354446-KX354447 KX354448
		BRA_17 RS_BRA_18, RS_BRA_19				AF199089 EF433576, NC010220
Egypt		*EGY_2*, *EGY_1*				DQ084823 AF199111
Hong Kong		*HKG*				AF199085
M-Line *						AY380567
Puerto Rico		*PUR*				DQ084824
Venezuela		*VEN*				AF199093

*M-Line refers to a laboratory strain of *Biomphalaria glabrata* derived from a Puerto Rico pigmented snail and an albino Brazilian snail (Mulvey and Bandoni 1994).

prior to fixation to ensure that the procedure followed was the same as that used in our malacology laboratories.

The longitudinal renal ridge is considered the gold-standard character for differentiating *B. glabrata* (Paraense and Deslandes 1959) from other species in the genus, in which the ridge is absent. The anterior and posterior regions of the vagina were examined. The proportions for the diameter and length of the oviduct were based on the nidamental gland; for the diameter of the uterus, the cephalic portion of the nidamental gland was used, for the length of the uterus, the posterior region of the vagina; for the length of the spermathecal duct, the body of the spermatheca; and for the length of the anterior region of the vagina, the posterior region of the vagina. The relative proportions of the organs or structures were used for comparisons together with the shell and mantle pigmentation pattern.

DNA extraction, amplification and sequencing

DNA isolation was carried out with the DNeasy Tissue Kit (Qiagen®). A fragment of the COI gene (~600 bp) was amplified with the LCO/HCO primers (Folmer et al. 1994). Polymerase chain reaction (PCR) was carried out in a total volume of 50 µL and the following reaction mixture: 10-100 ng of DNA, 0.2 mM of each dNTP, 0.10 µM of each primer and 1 U of Taq DNA polymerase in the supplied reaction buffer. The cycling conditions consisted of an initial 3 min step at 95°C for denaturation; 25 cycles of 1 min at 95°C, 1 min at 47°C and 1 min 30 s at 72°C and a final extension step of 7 min at 72°C (Tuan et al. 2012). PCR products were purified with a Qiagen purification kit and then sequenced in the Biotechnology Center at the Butantan Institute in an ABI3100 automated sequencer (Applied Biosystems®).

Molecular data analysis

The electropherograms obtained from forward and reverse sequencing of each specimen were corrected using CHROMAS (Technelysium Pty Ltd.) and then aligned with CLUSTALX version 1.8 (Thompson et al. 1997). The aligned sequences were edited with BIOEDIT version 7.0 (Hall 1999), and the general polymorphism of the sequences was calculated in DNAsp version 5 (Librado and Rozas 2009).

The final alignment consisted of a matrix of 75 COI sequences from the collected specimens (36 *B. tenagophila*, 12 *B. occidentalis*, 10 *B. glabrata*, 9 *B. straminea*, 1 *B. intermedia*, 7 *B. peregrina)* and 29 COI sequences of *Biomphalaria* from other Neotropical areas that were retrieved from GenBank (Table 1).

Intraspecific and interspecific genetic distances (Kimura 1980) were calculated by pairwise comparison of the sequences of all the individuals using the Kimura 2-parameter (K2P) method with the MEGA 6 (Molecular Evolutionary Genetics Analysis) package (Tamura et al. 2013). Three tree-based methods were performed for phyloge-

netic reconstructions. The K2P distance matrix was used to reconstruct a Neighbor-Joining (NJ) tree. MEGA 6 was also used to perform Maximum Likelihood analysis. In the ML analysis, the GTR+I+G model of sequence evolution was chosen using the Akaike information criterion as implemented in MODELTEST 2.3 (Nylander 2004). The reliability of NJ and ML topologies was evaluated using bootstrap support with 1000 replicates. The parameters estimated by MODELTEST were also used in a Bayesian Markov-Chain Monte Carlo (MCMC) analysis in MRBAYES 3.1 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). Two simultaneous independent searches were run for 1.5 x 106 generations, with trees saved every 100 generations, and the first 1.500 sampled trees of each search discarded as "burn-in".

The barcode gap analysis was performed with the ABGD (Puillandre et al. 2012), bPTP (Zhang et al. 2013) and GMYC methods (Fujisawa and Barraclough 2013). ABGD, bPTP and GMYC were run on the http://www.abi.snv.jussieu.fr/ public/ abgd/, http://species.h-its.org/ and http://species.h-its.org/gmyc/ web servers, respectively, using default parameters.

All the molecular analysis was performed on the 104 sequences (39 *B. tenagophila*, 23 *B. glabrata*, 13 *B. occidentalis*, 11 *B. straminea*, 12 *B. peregrina*, 1 *B. intermedia*, and 5 sequences from *B. tenagophila guaibensis*) (Table 1). *Biomphalaria oligoza* was excluded from the analysis because we were unable to amplify its DNA.

Results

Morphological analysis

The morphological identifications of the 118 adult snails that were studied are presented in Table 2. The results of morphological analysis revealed the following: **Shell:** the presence of a carina, the shape of the whorls and the shape of the shell aperture distinguished *B. tenagophila* and *B. occidentalis* from the other species in the group. **Renal tube:** The presence of renal ridge was observed in all the *B. glabrata* specimens studied. **Pigmentation of the mantle:** adult specimens of *B. tenagophila*, *B. glabrata* and *B. occidentalis* had more uniform pigmentation than the four other species studied, which had blotchy pigmentation. **Reproductive system:** the presence of a vaginal pouch in *B. tenagophila* and its absence in *B. occidentalis* differentiates these two species. *Biomphalaria straminea* and *B. intermedia* had marked variation in the posterior region of the vagina; in the former, the corrugation in this region was markedly wavy while in the latter it was swollen.

Biomphalaria peregrina differed from the species in the *B. straminea* complex (*B. straminea* and *B. intermedia*) in the width of the oviduct, the length of the uterus, the length of the spermathecal duct and the length of the anterior region of the vagina. *Biomphalaria intermedia* differed from *B. straminea* in the number of ovotestis diverticula, the length of the oviduct, the presence of an oviduct pouch, the number of prostate diverticula and the width of the uterus.

Table 2. Morphological characters used to identify 118 *Biomphalaria* specimens from the state of São Paulo.

Morphological character	*B. glabrata* (n= 9)	*B. tenagophila* (n= 56)	*B. occidentalis* (n= 18)	*B. oligoza* (n= 10)	*B. peregrina* (n= 9)	*B. intermedia* (n=10)	*B. straminea* (n= 6)
Carinate shell	Absent	Present	Present	Absent	Absent	Absent	Absent
Shape of the whorls on the shell	Rounded	Angular	Angular	Rounded	Rounded	Rounded	Rounded
Shell aperture	Rounded	Transverse, low or deltoid	Transverse, low or deltoid	Rounded	Rounded slightly to the right	Rounded	Rounded
Mantle pigmentation	Tends to be homogeneous	Tends to be homogeneous	Tends to be homogeneous	Spotted or blotchy	Spotted or blotchy	Spotted or blotchy	Spotted or blotchy
Longitudinal renal ridge	Present	Absent	Absent	Absent	Absent	Absent	Absent
Number of ovotestis diverticula	More than 100	More than 100	More than 100	18 to 37	More than 100	Around 60	More than 100
Shape of the ovotestis diverticula	Elongate, simple or subdivided	Elongate, simple or subdivided	Elongate, simple or subdivided	Bulging and simple	Elongate, simple or subdivided	Elongate, simple or subdivided	Elongate, simple or subdivided
Differentiation of the ovotestis diverticula	Weakly differentiated	Weakly differentiated	Weakly differentiated	Well differentiated	Well differentiated	Well differentiated	Well differentiated
Diameter of the oviduct	Narrow	Narrow	Narrow	Wide	Narrow	Wide	Wide
Length of the oviduct	Long	Long	Long	Short	Long	Long	Short
Appearance of the oviduct pouch	Clearly defined	Clearly defined	Clearly defined	Bulky	Clearly defined	Clearly defined	Bulky
Diameter of the uterus	Narrow	Narrow	Narrow	Wide	Wide	Narrow	Wide
Length of the uterus	Long	Long	Long	Short	Short	Long	Long
Length of the anterior region of the vagina	Long	Long	Long	Short	Short	Long	Long
Corrugation on the dorsal wall of the posterior region of the vagina	Absent	Absent	Absent	Absent	Absent	Present	Present
Type of vaginal Corrugation	-	-	-	-	-	Swollen	Strongly wavy
Vaginal pouch on the ventral wall of the posterior region of the vagina	Present	Present	Absent	Present	Present	Present	Absent
Shape of the vaginal pouch	Elongate	Bulging	_	Elongate	Elongate	Elongate	_
Appearance of the vaginal pouch	Clearly defined	Clearly defined	_	Discrete	Clearly defined	Discrete	_

Morphological character	***B. glabrata*** **(n= 9)**	***B. tenagophila*** **(n= 56)**	***B. occidentalis*** **(n= 18)**	***B. oligoza*** **(n= 10)**	***B. peregrina*** **(n= 9)**	***B. intermedia*** **(n=10)**	***B. straminea*** **(n= 6)**
Length of the spermathecal duct	Long	Long	Long	Short	Short	Long	Long
Shape of the prostate diverticula	Tree-like	Tree-like	Tree-like	Simple or subdivided	Tree-like	Tree-like	Tree-like
Number of prostate diverticula	Around 30	Around 30	Around 20	1 to 4	Around 13	Around 13	Around 20
Length of the penial sheath	Approx. the same length as the prepuce	Approx. the same length as the prepuce	Shorter than the prepuce	Approx. the same length as the prepuce	Longer than the prepuce	Approx. the same length as the prepuce	Longer than the prepuce
Diameter of the penial sheath	Narrow	Narrow	Narrow	Wide	Wide	Wide	Wide
Shape of the prepuce	Free end is wider	Free end is wider	Same diameter along its whole length	Same diameter along its whole length	Free end is wider	Free end is wider	Free end is wider
Seminal vesicle extensions	Finger-like	Finger-like	Finger-like	Nodular	Finger-like	Finger-like	Finger-like
Appearance of the seminal vesicle	Developed	Developed	Poorly developed	Poorly developed	Developed	Developed	Developed

Biomphalaria oligoza, *B. peregrina*, *B. intermedia* and *B. straminea* are differentiated by the number and shape of the ovotestis diverticula, appearence and size of seminal vesicle, the number and shape of the prostate diverticula, and the shape of the prepuce. The 27 morphological characters used to identify *Biomphalaria* are detailed in Table 2.

All these findings are in agreement with Paraense and Deslandes (1959), Paraense (1961, 1974, 1975, 1981, 1984).

Molecular analysis

The final alignment matrix for the 104 sequences consisted of 549 characters including 25% polymorphic, 21% parsimony-informative and 12 unique sites (Table 3).

The K2P sequence divergence for intraspecific comparisons ranged from 0.0% to 4.0%, while for interspecific comparisons the corresponding figure varied from 4.0% to 12% (Table 4). The greatest intraspecific genetic distances were observed between specimens of *B. peregrina* from SP and Rio Grande do Sul (southern Brazil) (4.0%) and specimens of *B. glabrata* from Rio Grande do Sul and Puerto Rico (3.9%).

The frequency distribution of the 104 analyzed sequences indicates that although there were some extreme pairwise distances (>3%) in *B. glabrata*, *B. tenagophila*, *B. peregrina* and *B. straminea*; intraspecific and interspecific divergences did not overlap (Fig. 2A). Nevertheless, a typical barcode gap was not observed in this dataset. A closer inspection of the distances for each taxonomic group shows that there is a clear barcode gap between *B. glabrata*, *B. straminea*, *B. peregrina* and *B. intermedia*. There was no clear barcode gap between closely related *B. tenagophila*, *B. t. guaibensis* and *B. occidentalis* (interspecific distance 3-4%) (Fig. 2 C, D, E, F).

The total number of MOTUs within the same taxon (Fig. 3) varied depending on the model used to partition the COI data (GMYC, bPTP or ABGD). Only bPTP recovered all seven groups identified by traditional morphology. GMYC revealed various sequences that were not consistent with morphological identifications: *B. peregrina* sequences from Rio Grande do Sul (BRA_10/KX354439) and São Paulo (756/KX354441), *B. straminea* sequences from Santa Isabel (SP) and Itariri (SP) (552/KF926190, 534/KF926185), one *B. intermedia* sequence (570/KF926179), two *B. tenagophila* sequences from Juquiá (SP) and four *B. glabrata* sequences from GenBank (RS_BRA_2/KF926107, RS_BRA_4/KF926109, BRA_6/AF199091 and PUR/DQ084824).

When run using the default settings, ABGD recovered five different subunits of *B. glabrata*. This result may be explained by the pronounced genetic variation in this species, but the possibility that these subgroups represent cryptic taxa cannot be ruled out.

The trees generated by the Bayesian, ML and NJ methods (Fig. 3) delineated six well supported groups (posterior probabilities and bootstrap values ≥90) congruent with the current classification of *Biomphalaria*. The only *B. intermedia* sequence appeared in a distinct branch supported by low Bayesian and bootstrap values.

Table 3. Sample size (N), number of haplotypes (H), haplotype diversity (Hd), nucleotide diversity (π, Nei 1987, equation 10.5) and average number of nucleotide differences (K, Tajima 1983, equation A3) calculated in DNAsp v.5 (Librado and Rozas 2009) for a 549 bp region of the COI gene in the six *Biomphalaria* species and one *Biomphalaria* subspecies.

Species	N	H	Hd	π	K
Biomphalaria	104	36	0.946	0.06805	
B. straminea	11	6	0.836	0.01199	6.582
B. occidentalis	13	1	0.000	0.00000	0.000
B. peregrina	12	6	0.848	0.01954	10.727
B. glabrata	23	10	0.862	0.01914	10.506
B. tenagophila	39	11	0.803	0.01222	6.707
B. t. guaibensis	5	1	0.000	0.00000	0.000
B. intermedia	1	1	-	-	-

Discussion

This study sought to determine the utility of DNA barcoding in delimiting species in freshwater snails of the genus *Biomphalaria*. The Bayesian, ML and NJ analyses (Fig. 3, Suppl. material 1) yielded trees with well-supported internal branches (≥90), resolving six out of the seven taxa as monophyletic groups.

The assessment of the potential of DNA barcode for species differentiation in *Biomphalaria* essentially revolves around the comparison of results of the morphological and molecular analysis of closely similar or taxonomically ambiguous species. In the case of the three taxa in the *B. tenagophila* complex, one character that is normally effective for specific identification is the vaginal pouch, which is present in *B. tenagophila* and *B. t. guaibensis* but not in *B. occidentalis*. (The anatomical features of these three taxa were illustrated by Tuan et al. 2012). Although we did not observe this in our material, in some specimens of *B. occidentalis* there is a slight projection of the ventral wall of the vagina (Paraense 1981), which raises questions regarding the distinctness of this taxon.

The intraspecific genetic distance within *B. tenagophila* showed values with a range from 0 to 3% (Table 4, Figs 2, 3). A high level of genetic divergence within this species was obtained for sequences associated with specimens collected in Juquiá (650,651,653), Itariri (535), Embu das Artes (524,535) and São Paulo (549, 551). Due to these values we could not assign a clear barcode gap between *B. tenagophila* and *B. occidentalis* and *B. t. guaibensis* (Fig. 2 b, d, f). However, the Bayesian tree inferred from COI data (Fig. 3), as well as the ABGD and both bPTP and GMYC analyses recovered these close related taxa as distinct groups. We suggest that in geographical areas where *B. tenagophila* species complex have the same geographical distribution.

The application of the 3-4% cutoff value for maximum intraspecific divergence may be appropriate for our dataset as 36% of the intraspecific comparisons reached

Table 4. Intraspecific and interspecific genetic distances (COI) generated using the Kimura 2-parameter model (K2P, Kimura 1980) in MEGA6 (Tamura et al. 2013).

Species 1	Species 2	Minimum distance	Mean distance	Maximum distance
		Intraspecific		
B. glabrata		0.00	0.03	0.04
B. tenagophila		0.00	0.02	0.03
B. straminea		0.00	0.01	0.03
B. occidentalis		0.00	0.00	0.00
B. peregrina		0.00	0.02	0.04
B. intermedia		0.00	0.00	0.00
B. t. guaibensis		0.00	0.00	0.00
		Interspecific		
B. glabrata	*B. tenagophila*	0.07	0.09	0.10
	B. straminea	0.07	0.09	0.10
	B. occidentalis	0.09	0.09	0.09
	B. peregrina	0.10	0.12	0.15
	B. intermedia	0.06	0.08	0.09
	B. t. guaibensis	0.07	0.09	0.09
B. tenagophila	*B. straminea*	0.08	0.09	0.10
	B. occidentalis	0.04	0.05	0.06
	B. peregrina	0.10	0.12	0.15
	B. intermedia	0.05	0.08	0.09
	B. t. guaibensis	0.04	0.04	0.05
B. straminea	*B. occidentalis*	0.09	0.09	0.10
	B. peregrina	0.09	0.01	0.10
	B. intermedia	0.05	0.05	0.06
	B. t. guaibensis	0.08	0.08	0.09
B. occidentalis	*B. peregrina*	0.10	0.11	0.13
	B. intermedia	0.08	0.08	0.08
	B. t. guaibensis	0.03	0.03	0.03
B. peregrina	*B. intermedia*	0.09	0.09	0.10
	B. t. guaibensis	0.10	0.12	0.13
B. intermedia	*B. t. guaibensis*	0.08	0.08	0.08

this value (Table 4). The highest values for intraspecific divergence (>3%) do not appear to be a consequence of geographic distance given that the greatest divergence in *B. tenagophila* was between closely proximal localities in São Paulo state (Fig. 3).

Biomphalaria glabrata and *B. tenagophila*, are differentiated by the renal ridge, which is present in the former and absent in the latter. Paraense and Deslandes (1959) described a false ridge that runs obliquely to the renal tube and is attached to the pneumostome, in specimens of *B. tenagophila* from Macaé, RJ. The presence of this false ridge in *B. tenagophila* may lead to incorrectly identify this species, particularly in juvenile specimens or specimens that have not been properly fixed. Five specimens

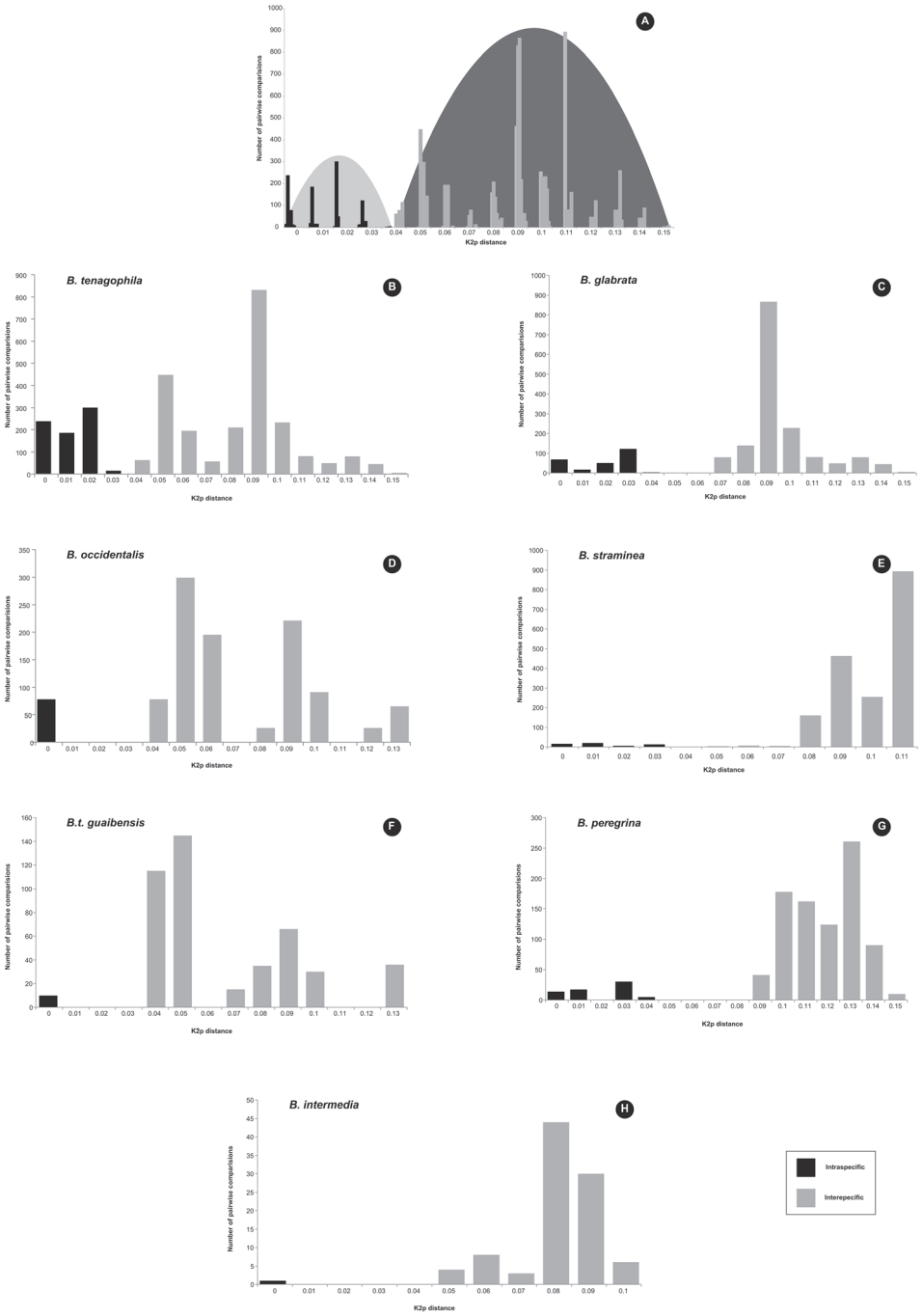

Figure 2. A histogram showing pairwise Kimura 2-parameter intraspecific and interspecific distances for 104 *Biomphalaria* cytochrome oxidase I sequences **B–H** pairwise distances between each species and the other taxa analyzed.

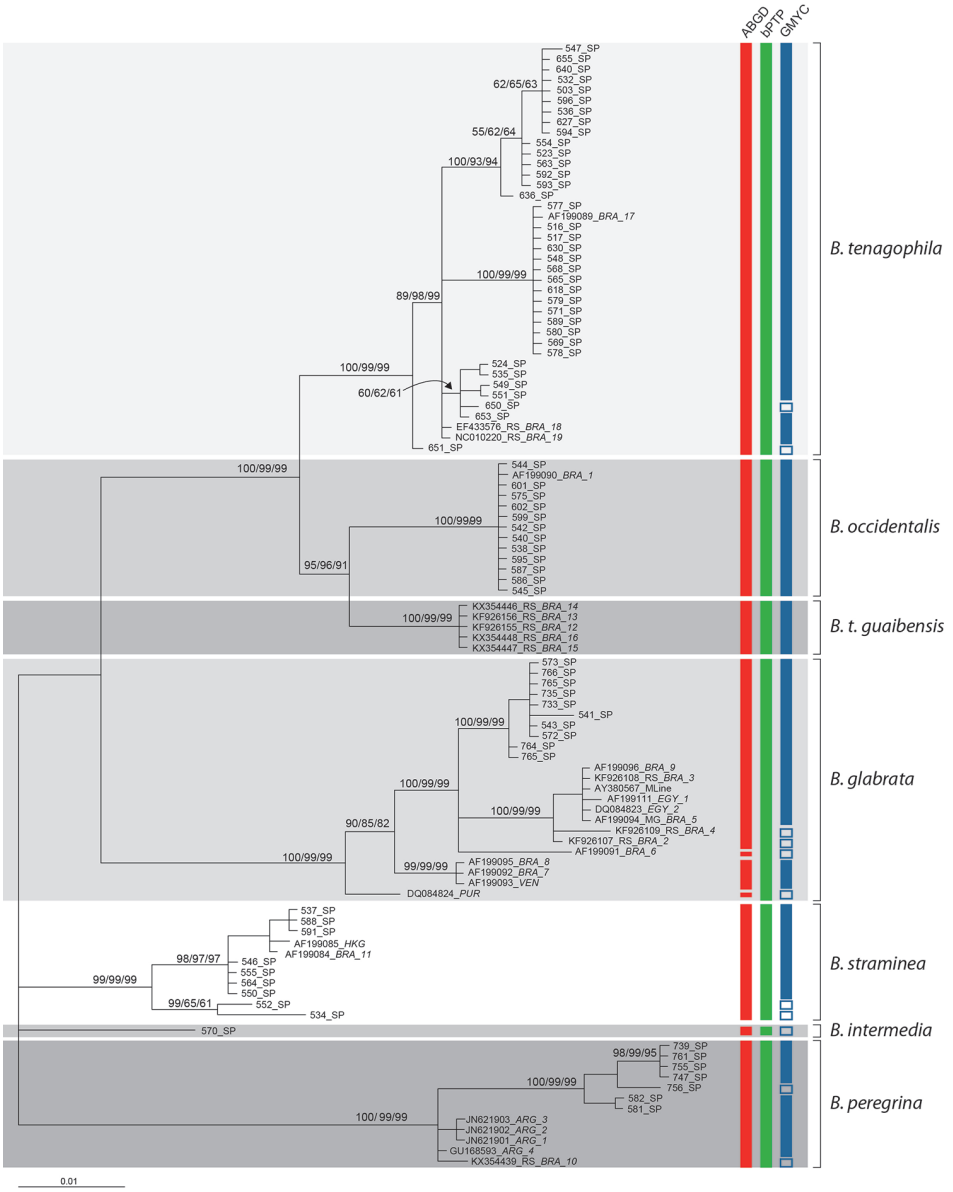

Figure 3. Bayesian phylogram. Support values for individual branches are given as Bayesian credibility/ML bootstrap/NJ bootstrap and are depicted above each node. The different shades of gray identify morphological species. The red, green and blue bars indicate species delimitations based on the distance-based (ABGD) and tree-based (bPTP and GMYC) models, respectively.

of *B. tenagophila* in our study (three from São Lourenço da Serra and two from São Paulo) had a membrane on the renal tubes similar to that described by Paraense and Deslandes. The genetic distance of 9% between *B. glabrata* and *B. tenagophila* observed

with both genetic distance and tree-based approaches show that DNA barcoding is an important tool for identifying these closely similar taxa.

The ABGD analysis partitioned *B. glabrata* into five distinct groups, while the GMYC analysis yielded a more cohesive group. Despite the pronounced COI divergence within *B. glabrata*, in all the specimens analyzed here the renal ridge has been considered a robust and consistent taxonomical character, suggesting that morphology is more effective than DNA barcode in this case. However, bBTP analysis and phylogenetic reconstruction supported *B. glabrata* as single and well supported MOTU, a result congruent with the morphological identification.

Another group of morphologically similar and frequently misidentified congeners includes *B. intermedia* and *B. straminea*; the latter a natural intermediate host of *S. mansoni*. Of the seventeen diagnostic characters common to *B. straminea* and *B. intermedia*, the degree of corrugation in the dorsal wall of the vagina has been used to these taxa as a species complex (Paraense 1975) . The vaginal corrugation, which is markedly wavy in *B. straminea* appears as swollen in *B. intermedia*. The large genetic divergence between *B. straminea* and *B. intermedia*, which was 9% greater than the intraspecific values in both species, indicates that these two species can be identified by DNA barcode. Note, however, that our study only included two of the three species in the *B. straminea* complex, as *B. kuhniana* does not occur in São Paulo state (Paraense 1988, Teodoro et al. 2010). In addition, we were unable to collect many specimens of *B. intermedia* owing to its rareness in São Paulo state.

Our findings show *Biomphalaria* species delimitation by phylogenetic approaches and bPTP yielded the same groups identified by traditional taxonomy. The use of DNA barcode to identify species in conjunction with *Biomphalaria* surveys requires the application of both evolutionary and bioinformatics criteria, making it a time-consuming approach that is dependent on specialist knowledge. Morphological identification also requires specialist knowledge. However, as shown in this study DNA barcoding can identify subtle (genetic) differences between intraspecific populations that are not detectable by traditional morphological study.

Furthermore, morphological identification of *Biomphalaria* species depends on subjective interpretation of anatomical variations, as these are measured in terms of relative rather than absolute sizes. We therefore agree with Hebert and Gregory (2005, p. 853), who stated that by reversing the logic of standard taxonomic approaches that *"operate in an a priori fashion—seeking…morphological discontinuities"*, DNA barcoding may, as *"a posteriori approach"*, direct the study of morphological variation in genetically divergent groups of *Biomphalaria*.

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Supplementary material 1

Figure S1

Author: Roseli Tuan

Data type: molecular data

Explanation note: ML and NJ trees generated with MEGA 6 software from the alignment of the 104 Biomphalaria COI sequences.

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RESEARCH ARTICLE

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A new species of the genus *Nicippe* from Japan (Crustacea, Amphipoda, Pardaliscidae)

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Abstract

A new species of the pardaliscid amphipod, *Nicippe recticaudata*, from off Cape Toi, Japan, is named and described. This is the first record of *Nicippe* Bruzelius, 1859 from the western Pacific coast of the Japanese archipelago. Additionally, nucleotide sequences of nuclear 28S ribosomal RNA and histone H3 as well as mitochondrial cytochrome *c* oxidase subunit I (COI) and 16S ribosomal RNA from the holotype and paratypes were determined. The morphological characteristics and the COI distance values enforced the distinctiveness of *N. recticaudata* **sp. n.** among the known *Nicippe* species. *Nicippe recticaudata* **sp. n.** closely resembles *N. tumida* Bruzelius, 1859 in having a two-dentate posterior margin of usoromite 1. However, the former is distinguished from the latter by the posterior margin of merus of pereopod 4 with 5–6 setae, anterior margin of merus of pereopod 5 with 9–10 setae, and telson with straight inner margin, tapering proximally. A key to the species of *Nicippe* is provided.

Keywords

COI, cryptic species, Gammaridea, *Nicippe tumida*, North Pacific

Introduction

The amphipod genus *Nicippe* Bruzelius, 1859 has been recorded from 35–1398 m deep, and currently consists of four species: *N. tumida* Bruzelius, 1859, *N. buchi* Andres, 1975, *N. rogeri* Lörz & Schnabel, 2015, and *N. unidentata* KH Barnard, 1932. Its type species

N. tumida has been diagnosed by the two-dentate posterior margin of urosomite 1. Originally, this species was described based on specimens collected from Drøbak, Norway (Bruzelius 1859). Later, this species has been recorded from North Atlantic, North Sea, North Pacific, East Pacific, Greenland, Mediterranean Sea, and European Fjords (Karaman 1974).

Contrary to the cosmopolitan cryptic species complex *N. tumida*, the other three species had been recorded from the type localities: *N. buchi* was collected from lava tubes off Lanzarote, North Atlantic Ocean (Andres 1975); *N. rogeri* from the central Chatham Rise, off east New Zealand (Lörz and Schnabel 2015); and *N. unidentata* was recorded from the Palmer Archipelago on the Antarctic Peninsula (Barnard 1932; Biswas et al. 2009).

Recently, unidentified specimens belonging to *Nicippe* were obtained from off Cape Toi, Miyazaki Prefecture, Japan, at a depth of 265–367 m. This is the first record of the genus from the western Pacific coast of the Japanese archipelago. Following a detailed examination of the specimens and their genetic data, these amphipods are described as a new species herein.

Material and methods

Sample

The present specimens were collected from off the southern tip of Kyushu (St-12) during a research cruise of the T/S *Toyoshio-Maru* (Hiroshima University) to Kyushu and the Nansei Islands, southwestern Japan in 2006 (Cruise # 2006-03). Specimens were collected with a sledge-net (mouth opening 145 cm × 15 cm, mesh opening 328 μm). The gear was towed along the bottom at a speed of 2 knots for 20 minutes. Samples were immediately fixed and preserved in 99% ethanol on-board ship. In the laboratory, specimens of *Nicippe* were sorted from amphipod samples under a stereomicroscope. For DNA extraction, muscle tissue was removed from the dorsal side of the pleon of each of three specimens.

Morphological observation

All appendages of the examined specimen were dissected in 70% ethanol and mounted in gum-chloral medium on glass slides under a stereomicroscope (Olympus SZ61). The specimens were examined using a light microscope (Olympus BH2) and illustrated with the aid of a camera lucida. The body length from the tip of the rostrum to the base of the telson was measured along the dorsal curvature to the nearest 0.1 mm. The specimens are deposited in the Tsukuba Collection Center of the National Museum of Nature and Science, Tokyo (NSMT) and the Zoological Collection of Kyoto University (KUZ).

PCR and DNA sequencing

The extraction of genomic DNA from pleon muscle followed Tomikawa et al. (2014). Primer sets for the PCR and cycle sequencing (CS) reactions used for histone H3 (H3) and 16S rRNA in this study were shown in Tomikawa et al. (2016). Those for the other regions were as follows: for 28S rRNA (28S), 28F (Hou et al. 2007) and 28SR (Tomikawa et al. 2012) (PCR and CS) with 28SF (Tomikawa et al. 2012), 28SFNici772 (5'-CCCGGATCGAAATCAGTAG-3'; this study) and 28SRNici679 (5'-CCATAAATTCGACACAGTAG-3'; this study) as internal primers for CS; and for cytochrome *c* oxidase subunit I (COI), LCO1490 and HCO2198 (PCR and CS) (Folmer et al. 1994).

The PCR reaction and DNA sequencing were performed using the modified methods mentioned in Nakano (2012) with the aid of a T100 Thermal Cycler. The PCR reaction mixtures were heated to 95°C for 5 min, then followed 35 cycles as below, and a final extension at 72°C for 6 min: 35 cycles for 28S, at 94°C (10 s), 50°C (20 s), and 72°C (1 min); for H3, at 94°C (10 s), 52°C (20 s), and 72°C (24 s); for COI, at 94°C (10 s), 46°C (20 s), and 72°C (42 s); and for 16S, at 94°C (10 s), 50°C (20 s), and 72°C (30 s). The sequencing mixtures were heated to 96°C for 2 min, followed by 40 cycles at 96°C (10 s), 50°C (5 s), and 60°C (54 s). The obtained sequences were edited using DNA BASER (Heracle Biosoft S.R.L.). The DNA sequences newly obtained in this study were deposited with the International Nucleotide Database Collaboration (INSDC) through the DNA Data Bank of Japan.

COI genetic diversity calculation

To calculate genetic diversity between the present specimens and the other *Nicippe* sample, one COI sequence (CMBIA134-11.COI-5P) of the amphipod identified as *N. tumida* was obtained from BOLD (Ratnasingham and Hebert 2007). The individual was collected from the eastern coast of the Pacific Ocean (Palos Verdes Peninsula, California, U.S.A.).

The COI sequences were manually aligned, because no indels were observed. Pairwise comparisons of uncorrected *p*-distances for three COI sequences obtained in this study (658 bp) and that obtained from BOLD (651 bp) were calculated using MEGA7.0.16 (Kumar et al. 2016). All missing positions were eliminated for each sequence pair.

Taxonomy

Family Pardaliscidae Boeck, 1871

Genus *Nicippe* Bruzelius, 1859

New Japanese name: Miko-yokoebi-zoku

***Nicippe recticaudata* sp. n.**
http://zoobank.org/811C54A4-0339-4043-809D-2E0715BA7C5A
Figures 1–7
New Japanese name: Toyotamamiko-yokoebi

Nicippe tumida: Ren 2012: 349–351, fig. 154.

Material examined. Holotype: male (8.4 mm), NSMT-Cr 25456 (Fig. 1A), off Cape Toi (31°14.54'N, 131°32.20'E–31°14.94'N, 131°31.46'E; 265–367 m deep), Japan, collected by K. Tomikawa, 29 May 2006. Paratypes: female (8.5 mm), NSMT-Cr 25457 (Fig. 1B), male (8.5 mm), NSMT-Cr 25458, male (10.4 mm), KUZ Z1807, male (6.8 mm), KUZ Z1808, data same as for holotype.

Diagnosis. Dorsal margin of urosomite 1 with 2 pointed teeth; posterior margin of merus of pereopod 4 with 5–6 setae; anterior margin of merus of pereopod 5 with 9–10 setae; telson with straight inner margin, tapering proximally.

Description of male (holotype, NSMT-Cr 25456). Head (Fig. 2A) without any trace of eyes or ommatidia; rostrum pointed; lateral cephalic corners angularly produced. Pereon segments 1–4 subequal in length, slightly shorter than segment 5; segment 6–7 longest, about 1.3 times length of previous segment. Pleonites 1–3 in length ratio of 1.0 : 1.3 : 1.3. Posteroventral corners of epimeral plates 1–3 each with prominent small tooth (Fig. 4G–I); ventral submargins of epimeral plates 2 and 3 with 2 setae, respectively. Urosomites 1–3 in length ratio of 1.0 : 1.0 : 1.2; urosomite 1 with 2 pointed teeth extending beyond posterior margin of its segment (Fig. 4J); urosomites 2 and 3 dorsally smooth.

Antenna 1 (Fig. 2B): length 0.6 × body length; peduncular articles 1–3 in length ratio of 1.0 : 0.6 : 0.3; posterodistal corner of peduncular article 1 with 2 long setae; accessory flagellum 3-articulate, article 1 long, length 4.8 × width; primary flagellum 38–articulate, length of article 1 longer than peduncular articles 2 and 3 combined, callynophore weakly developed.

Antenna 2 (Fig. 2C): length 0.9 × antenna 1, peduncular articles 3–5 in length ratio of 1.0 : 1.1 : 1.1; flagellum 29–articulate.

Mouthparts. Upper lip (Fig. 2D) with shallowly concave ventral margin, lobes symmetric. Mandibles (Fig. 2E–G): slightly asymmetric, incisor margins broad, straight, anterodosal corner rounded, anteroventral corner with a strong tooth; left lacinia mobilis (Fig. 2F) broad, about 0.8 × length of incisor, multi-dentate; right incisor (Fig. 2G) with 2 teeth on proximal to anterodorsal corner; right lacinia absent; accessory setal row of left and right mandibles each with 2 robust setae, and a proximal tuft of seta; molar absent; mandibular palp 3-articulate with length ratio of 1.0 : 3.6 : 2.7, article 2 with 12 setae, article 3 with 5 posterolateral and 3 apical setae. Lower lip (Fig. 2H) with broad outer lobes, inner lobes coalesced. Maxilla 1 (Fig. 2I) with inner and outer plate and palp; inner plate small with apical seta; outer plate subrectangular with 7 spine-teeth and 1 stout plumose seta, the lateral one strongest and longest; left and right palps symmetric, palp 2-articulate, article 1 lacking marginal setae, article 2 expanded distally,

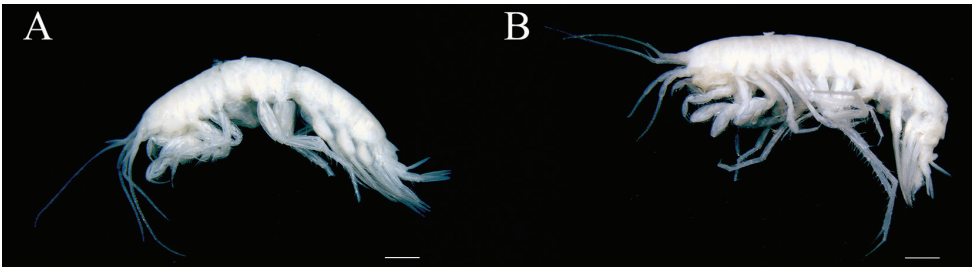

Figure 1. *Nicippe recticaudata* sp. n., habitus, lateral views. **A** holotype, male, 8.4 mm, NSMT-Cr 25456 **B** paratype, female, 8.5 mm, NSMT-Cr 25457. Scale bars: 1.0 mm.

with 8 robust and 8 slender setae on its apical margin. Maxilla 2 (Fig. 2J) with moderately slender inner and outer plates; inner plate bearing row of plumose setae on apical to medial margin; outer plate slightly longer than inner plate with apical plumose setae. Maxilliped (Fig. 2K) with inner and outer plates and palp; inner plate not reaching base of palp, with long plumose seta and short simple seta apically; outer plate narrowly rounded, reaching base of article 2 of palp, with setae along apical to medial margin; palp raptorial, 4-articulate, long, article 2 longest with inner marginal rows of setae, article 3 covered with 4 clusters of setae, article 4 slender with serrate inner margin.

Gnathopod 1 (Fig. 3A): coxa ovate with seta on anterodistal corner; basis long, expanded distally, anterior margin straight, posterior margin arched; ischium short, triangular, subequal in length to merus; carpus with short rounded lobe ventrally with long setae; propodus oval, about as wide as carpus, palm straight with long setae; dactylus slender, slightly curved, inner margin smooth with tooth near the base.

Gnathopod 2 (Fig. 3B): coxa rounded with seta on anterodistal corner, posteroproximal part with setae; compared to that of gnathopod 1, basis longer, more slender and straighter; carpal lobe stronger; dactylus similar, slightly shorter.

Pereopod 3 (Fig. 3C): coxa quadrate, posterior margin with setae; basis long with small setae on anterior and posterior margins; merus with 8 setae on posterior margin, 7 facial setae, and a cluster of setae comprising 3 setae on posterodistal corner; carpus and propodus with plumose and simple setae on posterior margin, and laterofacial setae; dactylus slender and weakly curved, length 0.7 × propodus.

Pereopod 4 (Fig. 3D): coxa rounded, ventral and posterior margins with setae; basis long with setae on anterior and posterior margins; merus with 5–6 setae on posterior margin, 7 laterofacial setae, and a cluster of setae comprising 3 setae on posterodistal corner; carpus and propodus with plumose and simple setae on posterior margin, and laterofacial setae; dactylus slender and weakly curved, length 0.6 × propodus.

Pereopod 5 (Fig. 4A): coxa bilobate, anterior lobe stronger and slightly longer with seta on ventral margin; basis posterior margin slightly convex with 2 small setae, posteroventral corner subquadrate with 2 minute setae; merus, carpus, and propodus in length ratio of 1.0 : 0.8 : 0.9; merus with 9 setae on anterior margin and 5 setae on submargin, anterodistal corner with 1 long and 3 relatively short setae; dactylus slender and almost straight, longer than on pereopods 3–4, 0.76 × length of propodus.

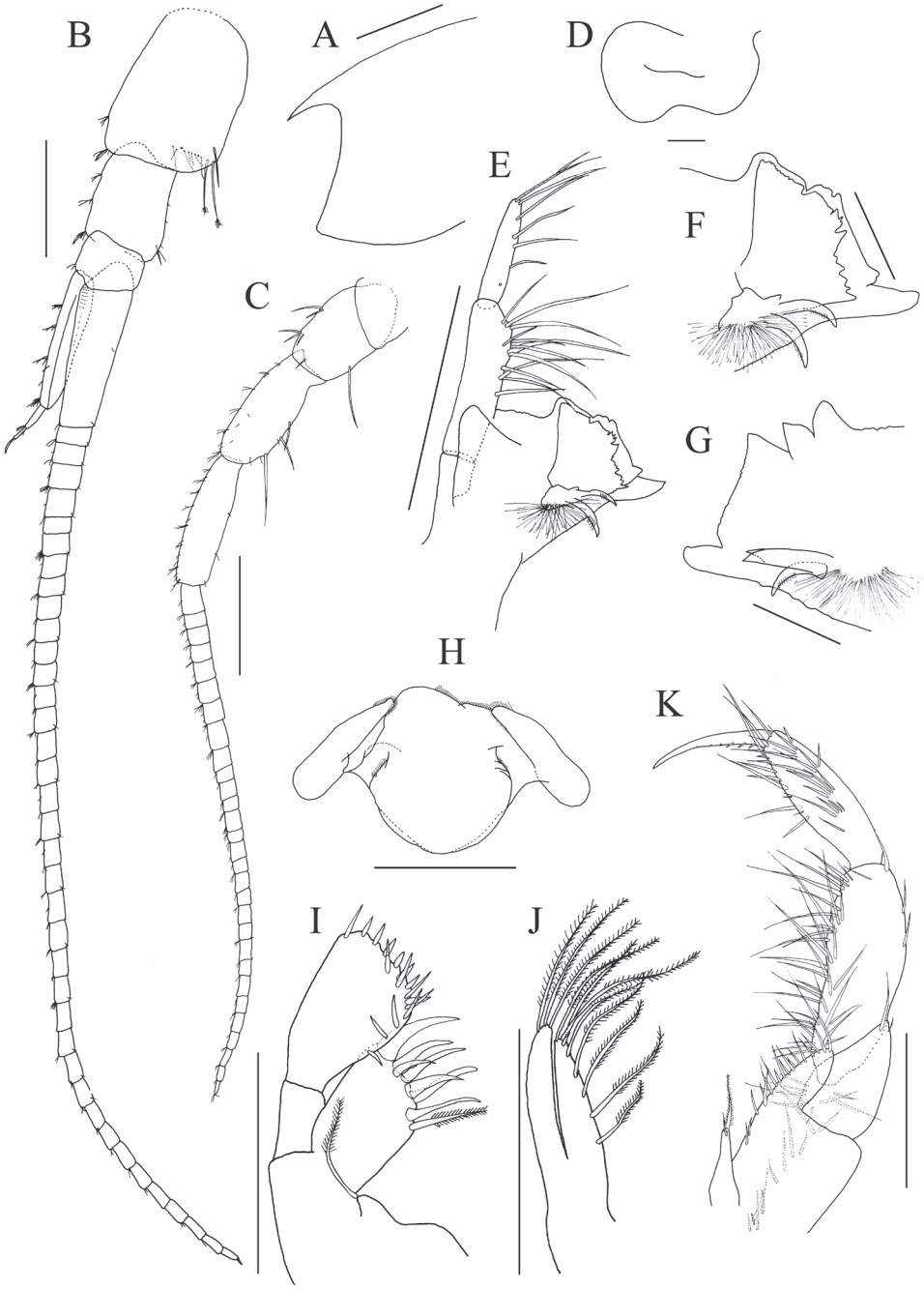

Figure 2. *Nicippe recticaudata* sp. n., holotype, male, 8.4 mm, NSMT-Cr 25456. **A** head, lateral view **B** antenna 1, lateral view, facial setae on accessory flagellum 1 omitted **C** antenna 2, lateral view **D** upper lip, anterior view **E** left mandible, medial view **F** distal part of left mandible, lateral view **G** distal part of right mandible, lateral view **H** lower lip, ventral view **I** maxilla 1, dorsal view **J** Maxilla 2, dorsal view **K** maxilliped, dorsal view. Scale bars: 0.5 mm (**A, E, H–K**), 1.0 mm (**B, C**), 0.1 mm (**D, F, G**).

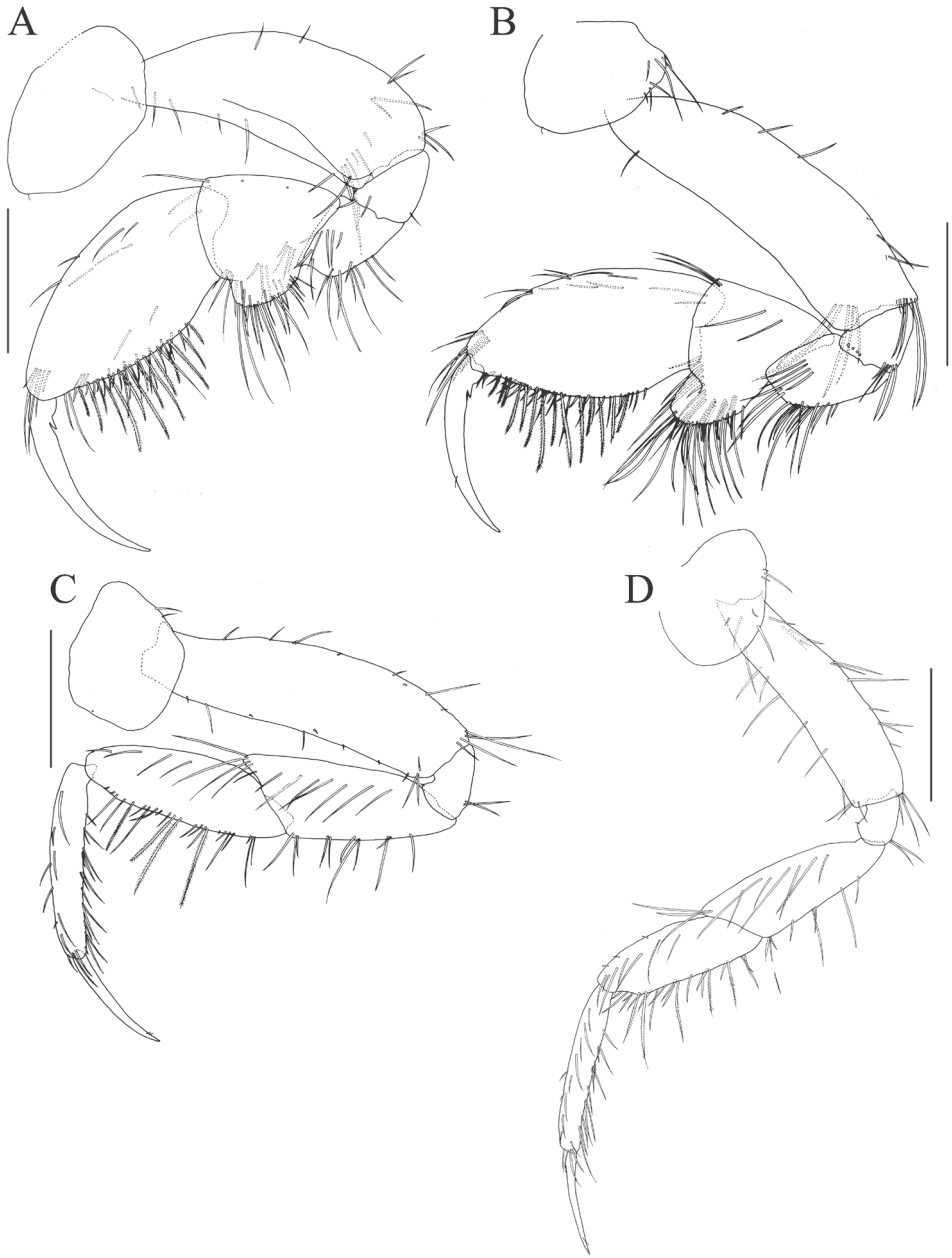

Figure 3. *Nicippe recticaudata* sp. n., holotype, male, 8.4 mm, NSMT-Cr 25456. **A** gnathopod 1, lateral view **B** gnathopod 2, lateral view **C** pereopod 3, lateral view **D** pereopod 4, lateral view. Scale bars: 0.5 mm.

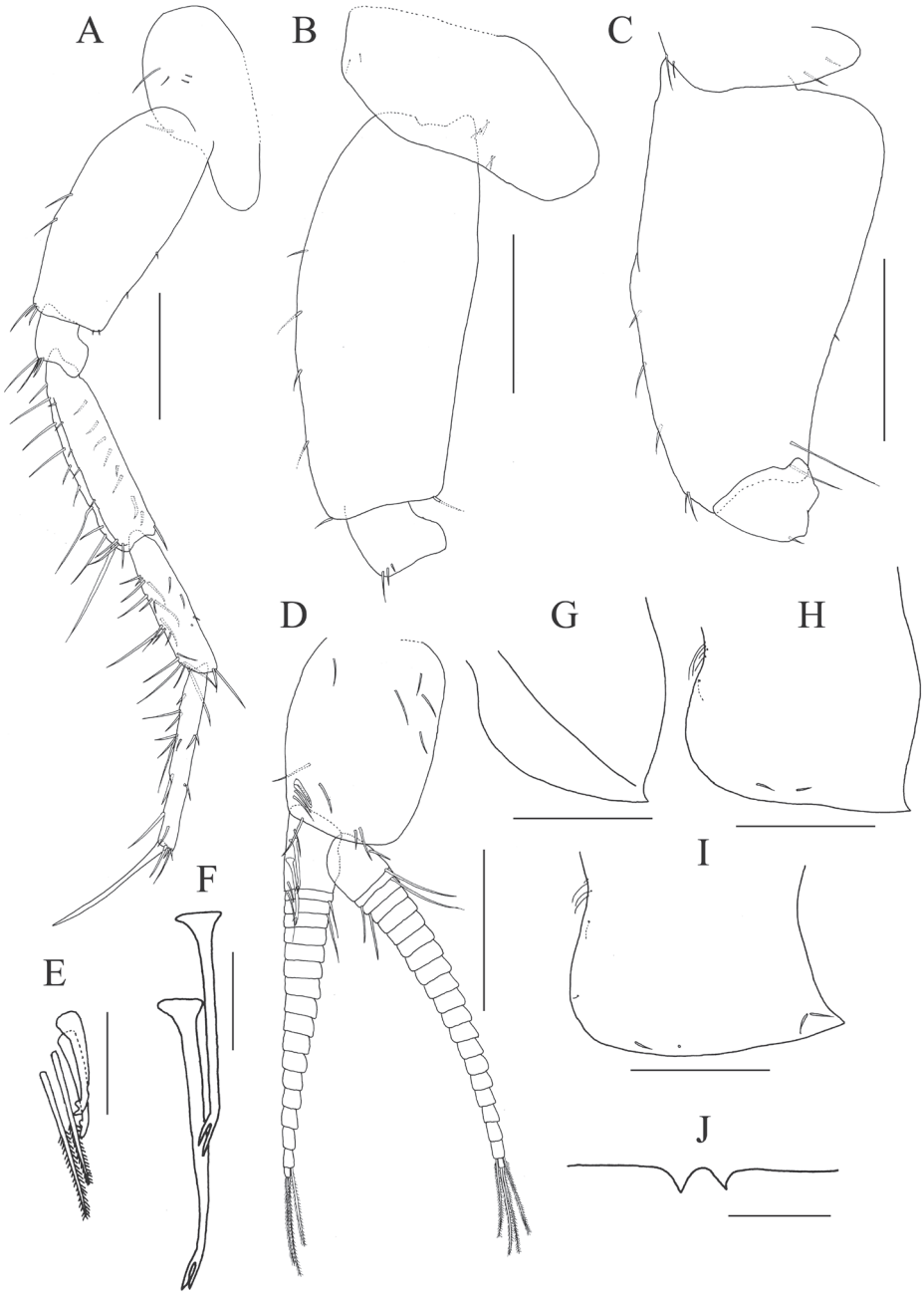

Figure 4. *Nicippe recticaudata* sp. n., holotype, male, 8.4 mm, NSMT-Cr 25456. **A** pereopod 5, medial view **B** coxa–ischium of pereopod 6, lateral view **C** coxa–ischium of pereopod 7, lateral view **D** pleopod 2, medial view, some setae on rami omitted **E** retinacula and associate setae on peduncle of pleopod 2, medial view **F** bifid plumose setae (clothes-pin setae) on inner basal margin of inner ramus of pleopod 2, medial view **G–I** epimeral plates 1–3, respectively, lateral views **J** dorsal margin of urosomite 1. Scale bars: 0.5 mm (**A–D, G–I**), 0.1 mm (**E, F, J**).

Pereopod 6 (Fig. 4B): coxa shallowly bilobate, anterior lobe slightly stronger; basis posterior margin slightly convex without setae, posteroventral corner subquadrate with seta.

Pereopod 7 (Fig. 4C): coxa ventral margin shallowly concave, anterior margin and ventral submargin each with 2 setae; basis expanded more strongly than pereopods 5–6, distally tapering, posteroproximal margin convex, anterodistal margin with setae, posteroventral corner slightly produced into a small rounded lobe, armed with 2 long setae.

Coxal gills on gnathopod 2 and pereopods 3–5 broad, longer than those of bases; gill on pereopod 6 ovate, length 0.5 × basis; gill on pereopod 7 slender, length 0.2 × basis.

Pleopods 1–3 (Fig. 4D) each with paired retinacula (Fig. 4E) on inner distal margin of peduncle, bifid setae (clothes-pin setae) (Fig. 4F) on inner basal margin of inner ramus; inner and outer rami of each pleopod consisting of 18 and 21 articles, respectively.

Uropods. Uropod 1 (Fig. 5A): peduncle slightly longer than rami, distolateral peduncular tooth very strong; outer ramus somewhat longer than inner ramus, outer and medial margins of outer ramus with 6 and 4 robust setae, respectively; outer and medial margins of inner ramus with 4 and 8 robust setae, respectively; both rami with stout setae apically. Uropod 2 (Fig. 5B): distolateral peduncular tooth short; outer ramus slightly longer than inner ramus, outer and medial margins with 6 and 7 robust setae, respectively; inner ramus with 6 outer and 4 medial robust setae, respectively; both rami with a stout seta apically. Uropod 3 (Fig. 5C): peduncle setose on outer margin, length 0.5 × outer ramus; outer ramus 1-articulate, somewhat longer than inner ramus; outer and medial margins of inner ramus each with plumose seta; medial margins of both rami with traces of setae.

Telson (Fig. 5D) length 2.0 × width, cleft for 88% of length in V-shape with straight inner margins of incision, each lobe with 4 setae laterally; apex of each lobe incised, lateral part of apex slightly longer than medial part, with small robust seta, lobes slightly tapering distally.

Description of female (paratype, NSMT-Cr 25457). *Antenna 1* (Fig. 6A): length 0.6 × body length; article 1 of accessory flagellum short, length 2.4 × width; primary flagellum 43-articulate, callynophore weakly developed.

Antenna 2 (Fig. 6B): length 0.7 × antenna 1; flagellum 18-articulate.

Gnathopod 1 (Fig. 6C): anterior and posterior submargins of basis with many setae.

Gnathopod 2 (Fig. 6D): anterior margin and posterior submargin of basis with many setae.

Pereopods 6 and 7 (Fig. 7A, B): similar to those of holotype; merus–propodus slender, with setae on anterior and posterior margins, and facial setae; dactylus slender, slightly curved inward.

Telson (Fig. 7C): length 1.9 × width, cleft for 90% of length, incision wider than that of male, each lobe with 6–7 setae laterally.

Variation. Callynophore on antenna 1 well developed (3 males, NMST-Cr 25458, KUZ Z1807, Z1808); peduncular article 5 of antenna 2 longer than 1.5 × article 4 (3 males, NMST-Cr 25458, KUZ Z1807, Z1808); posterior margin of merus of pereopod 4 with 5–6 setae; anterior margin of merus of pereopod 5 with 9–11 setae.

Coloration. Color in life unknown; faded in preservative (Fig. 1).

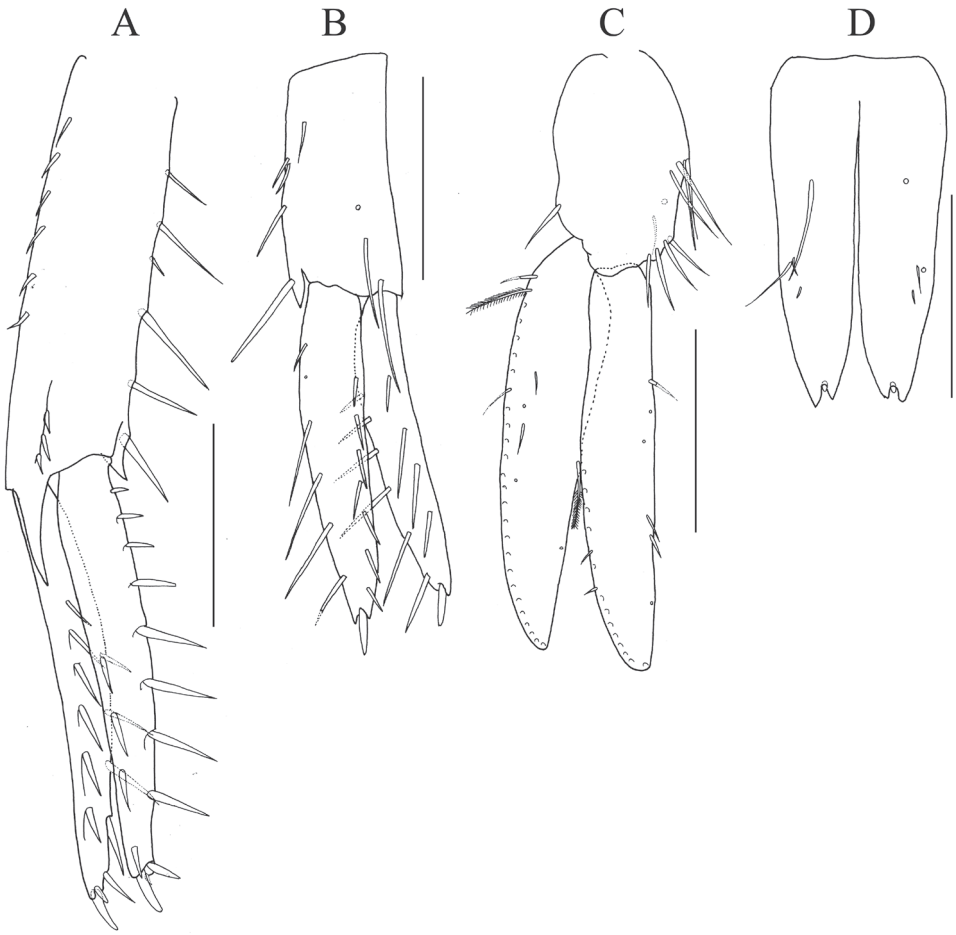

Figure 5. *Nicippe recticaudata* sp. n., holotype, male, 8.4 mm, NSMT-Cr 25456. **A** uropod 1, dorsal view **B** uropod 2, dorsal view **C** uropod 3, ventral view **D** telson, dorsal view. Scale bars: 0.5 mm.

Etymology. The specific name is a compound adjective derived from the Latin words *rectus*, and *caudatus* referring to the fact that the inner margin of the telson of this species is straight, a diagnostic character of the species.

Sequences and COI genetic distances. In total nine nucleotide sequences were determined: holotype (NSMT-Cr 25456), four sequences, 28S (LC214961; 1336 bp), H3 (LC214963; 328 bp), COI (LC214958; 658 bp), and 16S (LC214956; 407 bp); paratype (NSMT-Cr 25457), four sequences, 28S (LC214962; 1336 bp), H3 (LC214964; 328 bp), COI (LC214959; 658 bp), and 16S (LC214957; 407 bp); and paratype (KUZ Z1807), one sequence, COI (LC214960; 658 bp).

The obtained three COI sequences (LC214958–LC214960) were completely identical to each other. Based on the 651 bp aligned sequences, the COI uncorrected *p*-distance between *N. recticaudata* and the eastern North Pacific "*N. tumida*" was 17.1%.

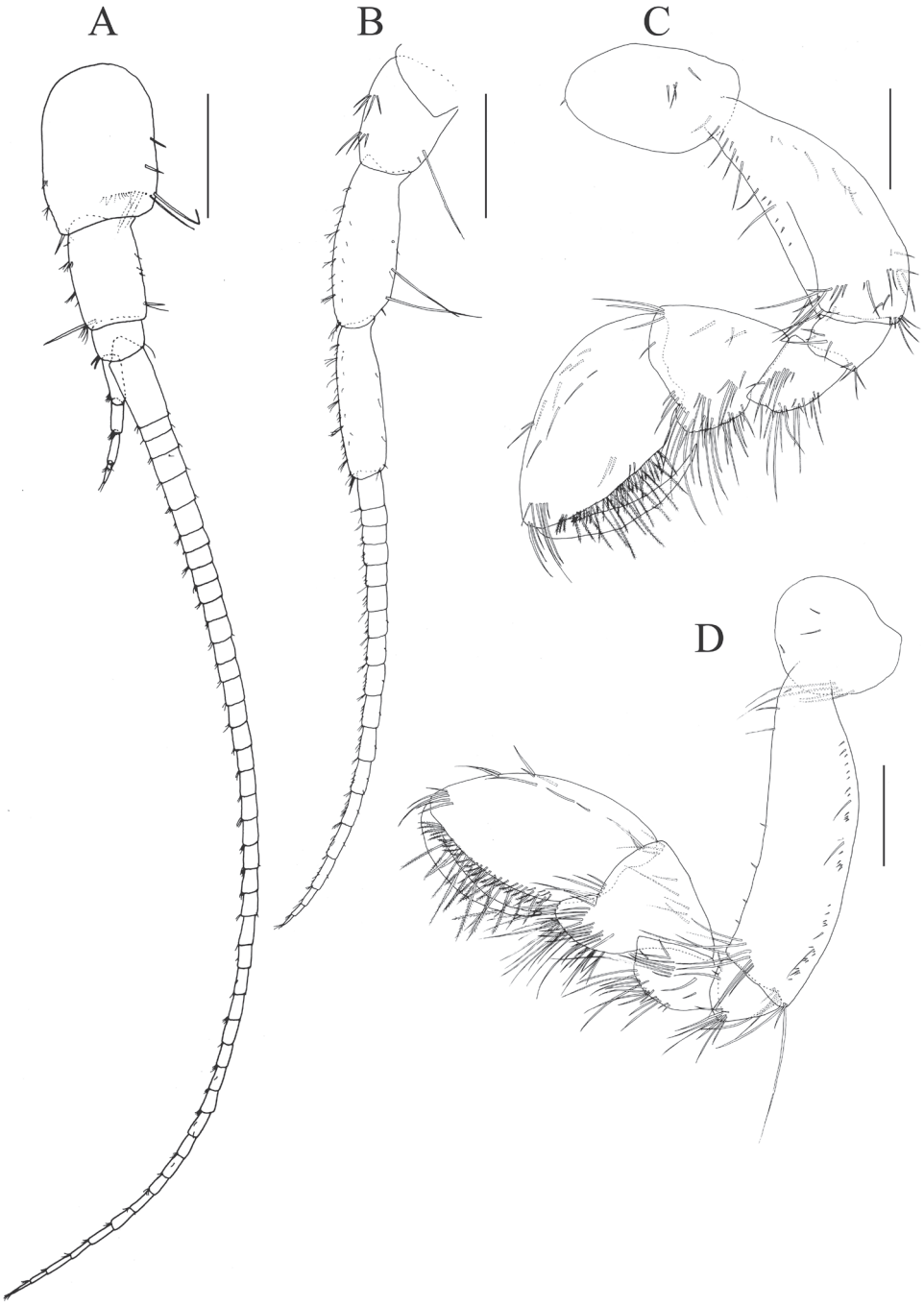

Figure 6. *Nicippe recticaudata* sp. n., paratype, female, 8.5 mm, NSMT-Cr 25457. **A** antenna 1, lateral view **B** antenna 2, lateral view **C** gnathopod 1, medial view **D** gnathopod 2, medial view. Scale bars: 0.5 mm.

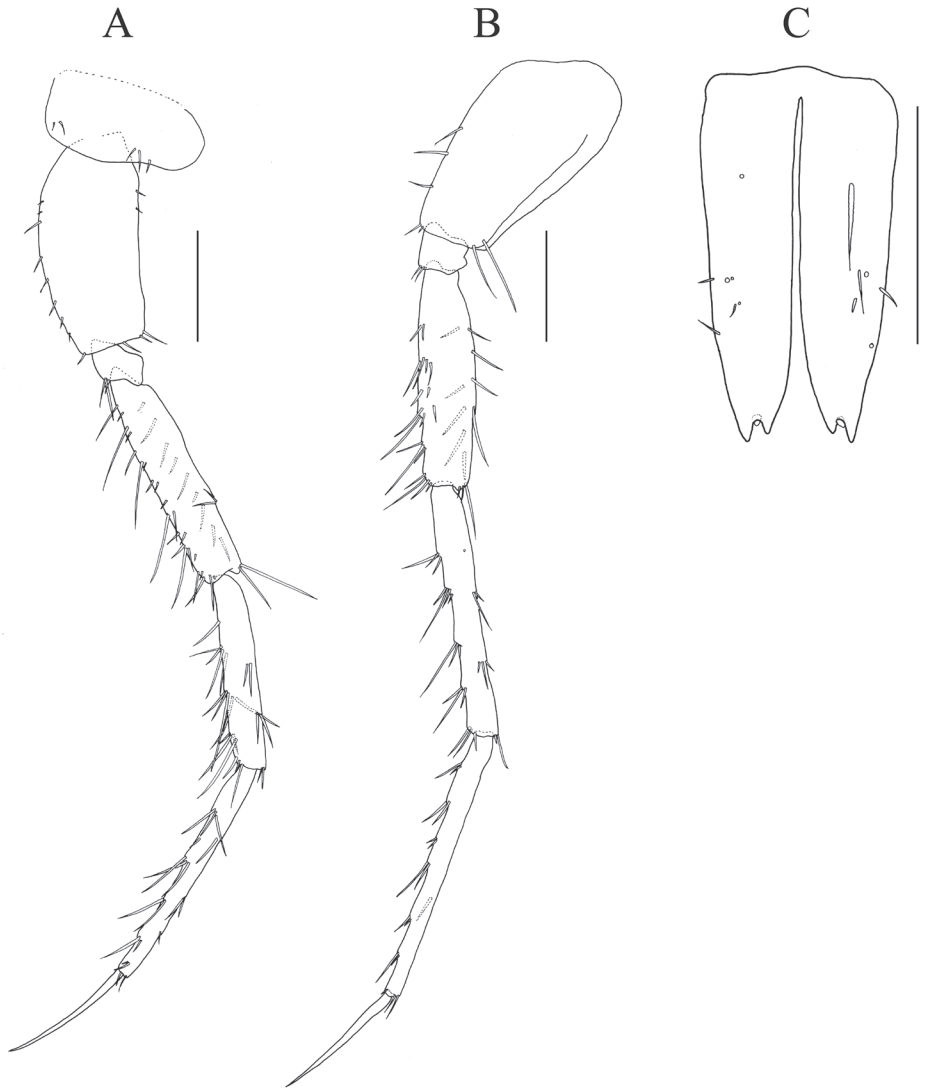

Figure 7. *Nicippe recticaudata* sp. n., paratype, female, 8.5 mm, NSMT-Cr 25457. **A** pereopod 6, lateral view **B** pereopod 7, lateral view, coxa lacking **C** telson, dorsal view. Scale bars: 0.5 mm.

Remarks. Although the present specimens showed two characteristics in the degree of callynophore of male antenna 1 and the length of peduncular article 5 of male antenna 2, the obtained genetic data revealed that these morphological variants (specimens with strongly developed callynophore and long peduncular article 5 of antenna 2, e.g., KUZ Z1807 vs specimens with weakly developed callynophore and short peduncular article 5 of antenna 2, e.g., NSMT-Cr 25456) shared completely identical COI sequences, and thus they were identified as the same species. The character states of the well developed callynophore of antenna 1 and the elongate peduncular article 5

of antenna 2 were observed only in males of *N. recticaudata*. Males with these characteristics might be regarded as fully mature individuals.

According to the conventional classification of *Nicippe* species, this species would be identified as *N. tumida* based on the possession of the two-dentate posterior margin of urosomite 1. However, *Nicippe recticaudata* clearly differs from the "true" *N. tumida* defined by Bruzelius (1859) and Sars (1895) in the following three characteristics (character states of *N. tumida* in parentheses): posterior margin of merus of pereopods 4 with 5–6 setae (more than 10); anterior margin of merus of pereopod 5 with 9–10 setae (more than 20); and telson with straight inner margin, tapering proximally (sinuous inner margin, weakly expanding proximally). Moreover, the calculated COI genetic distance (17.1%) between *N. recticaudata* specimens and the *N. tumida* sample from California (Northeastern Pacific) revealed that the new species is genetically diverged from the Californian population of *N. tumida*; 3.5–4% COI distances have been proposed as the threshold for amphipod species discrimination (Witt et al. 2006; Rock et al. 2007; Hou et al. 2009). The *N. tumida* individuals inhabiting Californian waters were once reported to possess the morphological characteristics resembling those of *N. recticaudata* (Barnard 1959). Contrary to their morphological similarities, therefore, the present COI data highlighted that the population from Northwestern Pacific (*N. recticaudata*), and that inhabiting Northeastern Pacific ("*N. tumida*" in Californian waters) are different species. In summary, the present *tumida*-like Japanese population of *Nicippe* is considered as distinctive, and thus was described as a new species of the genus.

A *Nicippe* specimen identified as *N. tumida* was recorded from the East China Sea (Ren 2012). However, its description as well as figures clearly shows the diagnostic characteristics of *N. recticaudata*, and thus the Chinese sample in Ren (2012) definitely belongs to the present new species. As well, *N. tumida* has been recorded from around the Japanese Archipelago, e.g., from Sea of Japan and Sea of Okhotsk (Gurjanova 1951; Bulycheva 1957). Because these previous records lacked the detailed descriptions related to the diagnostic characters of *N. recticaudata*, their identities remain unclear. A worldwide systematic revision is essential to elucidate the cryptic species diversity in *N. tumida*.

Key to species of *Nicippe* modified from Lörz and Schnabel (2015)

1 Dorsal margin of urosomite 1 smooth ..... ***N. buchi***
– Dorsal margin of urosomite 1 dentate..... **2**
2 Dorsal margin of urosomite 1 with 2 teeth **3**
– Dorsal margin of urosomite 1 with 1 tooth..... **4**
3 Posterior margin of merus of pereopod 4 with many (more than 10) setae; anterior margin of merus of pereopod 5 with numerous (more than 20) setae; telson with sinuous inner margin, weakly expanding proximally.... ***N. tumida***
– Posterior margin of merus of pereopod 4 with 5–6 setae; anterior margin of merus of pereopod 5 with 9–10 setae; telson with straight inner margin, tapering proximally..... ***N. recticaudata***

4 Pereonites 1 and 7 the widest, pereonites 2–6 shorter, subequal; uropod 3 peduncle with unarmed distodorsal lobe ..... ***N. rogeri***

– Pereonites 1–4 subequal, shorter than 5–7 with segment 5 the longest; uropod 3 peduncle with 3 pointed distodorsal process ..... ***N. unidentata***

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RESEARCH ARTICLE

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The genus *Anaphothrips* with one new species from China (Thysanoptera, Thripidae)

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Abstract

A key to six species of *Anaphothrips* known from China is provided, together with distribution information. *Anaphothrips dentatus* **sp. n.** is described and illustrated from Sanjiang Plain in northeastern China, based on one male and five apterous females. This species is unusual in having the posterior margin of tergite VIII with a craspedum of small teeth rather than long microtrichia.

Keywords

Anaphothrips, China, new species, thrips

Introduction

Currently, 81 species are described in the genus *Anaphothrips* (ThripsWiki 2017), most being associated with species of Poaceae. A key to distinguish the genus from similar genera in China was provided by Mirab-balou et al. (2012), and five species have been recorded in this genus from China (Mirab-balou et al. 2012). The diagnosis of the genus includes the following character states: antennae 8- or 9-segmented, sense cone on segment IV forked, on segment III forked or simple; pronotum without long setae; metafurcal spinula absent; all tarsi 2-segmented; abdominal tergite VIII posterior margin with or without comb, some species with craspedum; sternites without discal setae; male abdominal sternites usually with pore plate (Mound and Masami 2009).

Sanjiang Plain (45°01.08'–48°27.93'N, 130°13.17'–135°05.43'E) is located in Northeast China, with a total area of approximately 108.9 thousand square kilometers. It is the largest area of freshwater marsh wetland (Fig. 1) in China, but from which no species of thrips has previously been reported. The thrips diversity of this region was investigated from 2012 to 2014, and two species from the genus *Anaphothrips* were discovered, *A. obscurus* (Müller) and the new species described in this paper.

Materials and methods

The slide preparation method followed Zhang et al. (2006). Descriptions and measurements were conducted using a Nikon Eclipse 80i microscope; photographs were taken using an ISH500 camera with ISCapture software and were processed with the software of Adobe Photoshop CS6. All measurements described in this paper are in micrometers. One paratype of the new species is deposited in the Insect Collection, South China Agricultural University (**SCAU**); other specimens examined are deposited in the Insect Collection of Jilin University (**JLU**), Changchun City, Jilin Province, China.

Taxonomy

Anaphothrips beijingensis Mirab-balou, Chen & Tong

Anaphothrips beijingensis Mirab-balou, Chen & Tong, 2012: 719.

Distribution. China (Beijing).

Anaphothrips dentatus sp. n.

http://zoobank.org/947ABA6E-BA6B-4B94-96B4-E5F8C509E0CD
Figs 2–14

Specimens examined. Holotype: Female (apterous), China, Heilongjiang Province, Sanjiang Plain (47°35.08'N, 133°31.42'E), 18.vii.2013, from grasses (Jun Wang). Paratypes: 1 male and 1 female same data as holotype; 3 females, same locality and habitat as holotype, 2.vii.2014, from grasses (Jun Wang).

Diagnosis. Both sexes apterous. Body brown, but head and thorax paler, legs yellow, antennal segments I, III–V yellow, segments II, VI–IX brown. Head wider than long slightly, projecting in front of eyes; ocelli reduced. Antennae 9-segmented, segments III–IV with sense cone forked. Pronotum almost smooth; metanotum median setae far apart and arising on posterior third of sclerite. Abdominal tergite VIII posterior margin with craspedum formed of small teeth. Male tergite IX with two pairs of

Figure 1. The habitats of *Anaphothrips dentatus* sp. n. (Photo taken on 9.viii.2012 by Jun Wang).

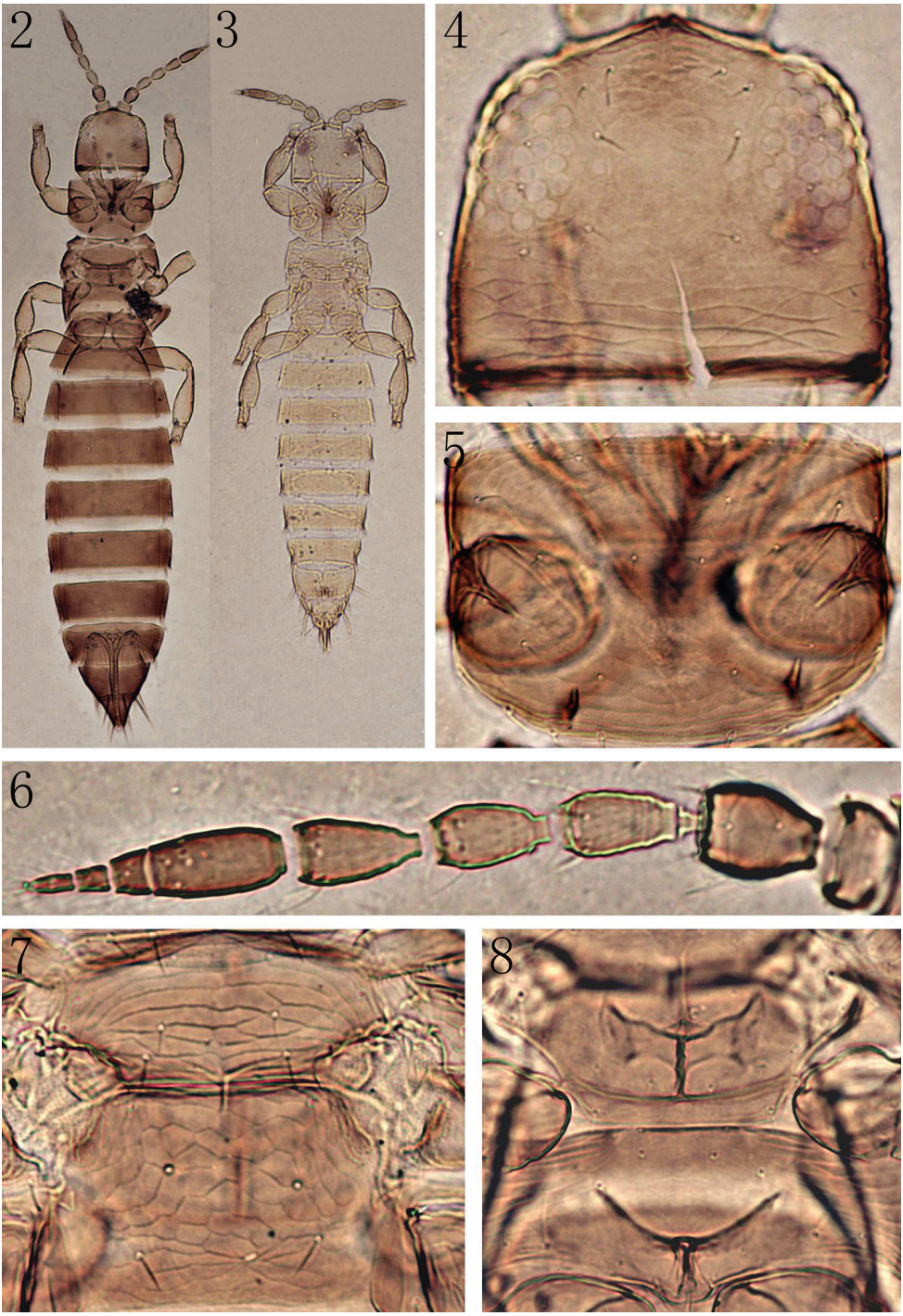
stout median thorn-like setae near posterior margin; sternites III–VII with C-shaped pore plate slightly wider than distance between posteromarginal seta S1.

Description. Apterous female (Fig. 2). Body uniformly brown, head and thorax paler; legs yellow; antennal segments I, III–V yellow, segments II, VI–IX brown.

Head (Fig. 4) 0.9 times as long as wide, projecting in front of eyes, dorsal surface sculptured with irregular transverse reticulations, but almost smooth between eyes; ocelli reduced, three pairs of ocellar setae present, seta III longer; three pairs of postocular setae present; antennae 9-segmented (Fig. 6), segment III with pedicel, segments III–IV with small forked sense cone and rows of microtrichia, a complete transverse suture present between segments VI and VII.

Pronotum almost smooth (Fig. 5), without long setae; prosternal ferna undivided (Fig. 9). Mesonotum sculptured with transverse reticulations (Fig. 7), a pair of campaniform sensilla close to anterior margin, a pair of median setae and a pair of anterior external setae present, a pair of setae arising close to posterior margin. Metanotum sculptured with polygonal reticulations (Fig. 7), paired anteromarginal setae and paired campaniform sensilla present, paired median setae far apart and arising on posterior third of sclerite. Mesofurcal spinula present, metafurca without spinula (Fig. 8).

Abdominal tergites II–VIII with sculpture laterally (Fig. 10), one pair of median setae, two pairs of campaniform sensilla, two pairs of lateral setae and a pair of lateral marginal setae present, a pair of posteroangular setae arising at posterior margin far from the posterior angles; tergite VIII with spiracles occupying less than half of lateral margins, posterior margin with craspedum formed of small teeth; tergite IX with a pair



Figures 2–8. *Anaphothrips dentatus* sp. n. (**2, 4–8** female **3** male). **2** Female **3** Male **4** Head. **5** Pronotum. **6** Antenna **7** Meso- and metanotum **8** Meso- and metasternum.

of mid–dorsal setae and two pairs of campaniform sensilla, SI and S2 close to posterior margin longer than tergite X; tergite X divided longitudinally with a pair of campaniform sensilla and two pairs of long setae close to posterior margin (Fig. 13). Sternites II–VII reticulated laterally and without discal setae, sternite II with two pairs of posteromarginal setae, III–VII with three pairs (Fig. 11); sternite VIII with three pairs of setae laterally. Pleurotergites III–VII with posteromarginal setae, posterior margin with lobes (Fig. 11).

Measurements (holotype). Body length 1258. Head length 125, width 146; antennae length 238; antennal segments I–IX length(width): 20(28), 32(25), 37(17), 30(17), 31(18), 36(19), 8(11), 8(7), 12(4); antennal terminal setae 17; eyes length 66; diameter of ocelli 6, distance of posterior ocelli 37; ocellar setae I–III: 7, 7, 13; postocular setae I–III: 9, 13, 6. Pronotum median length 130, width 171; setae length 5–14. Mesonotum anterior median setae 8. Metanotum anteromarginal setae 12, median setae 14. Abdominal pelta median setae 11; tergite IX S1 65, S2 80; tergite X median setae 62; sternites II–VII posteromarginal setae 6–33.

Apterous male (Fig. 3). Similar to female but smaller and paler; tergite IX with two pairs of stout median thorn-like setae near posterior margin, setae I 1.7 times as long as II (Fig. 14); sternites III–VII with C-shaped pore plate slightly wider than distance between posteromarginal setae S1 (Fig. 12).

Measurements (paratype male). Body length 1003. Head length 120, width 141; antennae length 202; antennal segments I–IX length(width): 17(25), 27(22), 34(15), 26(15), 27(15), 30(15), 7(10), 6(7), 11(4); antennal terminal setae 14; eyes length 59; diameter of ocelli 5, distance of posterior ocelli 36; ocelli setae I–III: 9, 7, 11; postocular setae I–III: 7, 9, 8. Pronotum median length 106; width 152; setae length 3–11. Mesonotum anterior median setae 8. Metanotum anteromarginal setae 9, median setae 12. Abdominal tergite I median setae 12; tergite IX stout median thorn–like setae I 19, II 11, setae S1 53, S2 56; tergite X median setae 63; sternites II–VII posteromarginal setae 7–28; sternites III–VII pore plate thickness (T) 5–9, width (W) 48, 45, 47, 48, 36.

Distribution. China (Heilongjiang Province).

Etymology. The Latin word *dentatus* derived from tooth-shaped craspedum on abdominal tergite VIII posterior margin.

Remarks. The new species belongs to a small group of *Anaphothrips* in which abdominal tergite VIII posterior margin has a craspedum that is tooth-shaped not ciliate. It is similar in appearance to the description by Pitkin (1978) of the Australian species *A. moundi*, but can be distinguished from the latter by the following features: antennal segment II brown, sensorium on III forked; abdominal tergite VIII with craspedum of teeth longer; male abdominal sternites pore plate only slightly wider than the distance of posteromarginal setae S1. The morphological characteristics of *A. moundi* are provided by Mound and Masumoto (2009). The new species is also similar to *A. obscurus* in appearance, but can be distinguished by the following characters: both sexes apterous; ocelli reduced; head wider than long; abdominal tergite VIII with tooth-shaped craspedum; male sternites III–VII with C-shaped pore plates only slightly wider than the distance of posteromarginal setae S1.

Figures 9–14. *Anaphothrips dentatus* sp. n. (**9–11, 13** female; **12, 14** male). **9** Ventral view of prothorax **10** Tergites II–III **11** Sternites IV–V **12** Sternites IV–V. T: thickness; W: width **13** Tergites VIII–X **14** Tergites IX–X.

***Anaphothrips floralis* Karny**
Figs 15–16

Anaphothrips floralis Karny, 1922: 109; Zhang and Tong 1992: 73.

Description. Macropterous female. Body and legs yellow; antennal segment I yellow, segments II–IV and base of V light brown but segment II darker, segments V–VIII brown. Head with ocellar setae III arising at outer tangent between fore and hind ocelli; antennae 8-segmented (Fig. 15), segments III–IV with sensorium forked. Fore wing (Fig. 16) upper vein with eight basal setae and four distal setae, lower vein with 6–11 setae. Metanotum reticulate in mid line, campaniform sensilla absent. Abdominal tergite VIII posterior margin with complete comb.

Distribution. China (Guangdong); Vietnam.

Specimens examined. 1 female (macropterous), 1 male (macropterous), China, Guangdong Province, suburb of Guangzhou, 4.xi.1976, form *Allium tuberosum* (Weiqiu Zhang).

***Anaphothrips obscurus* (Müller)**
Fig. 17

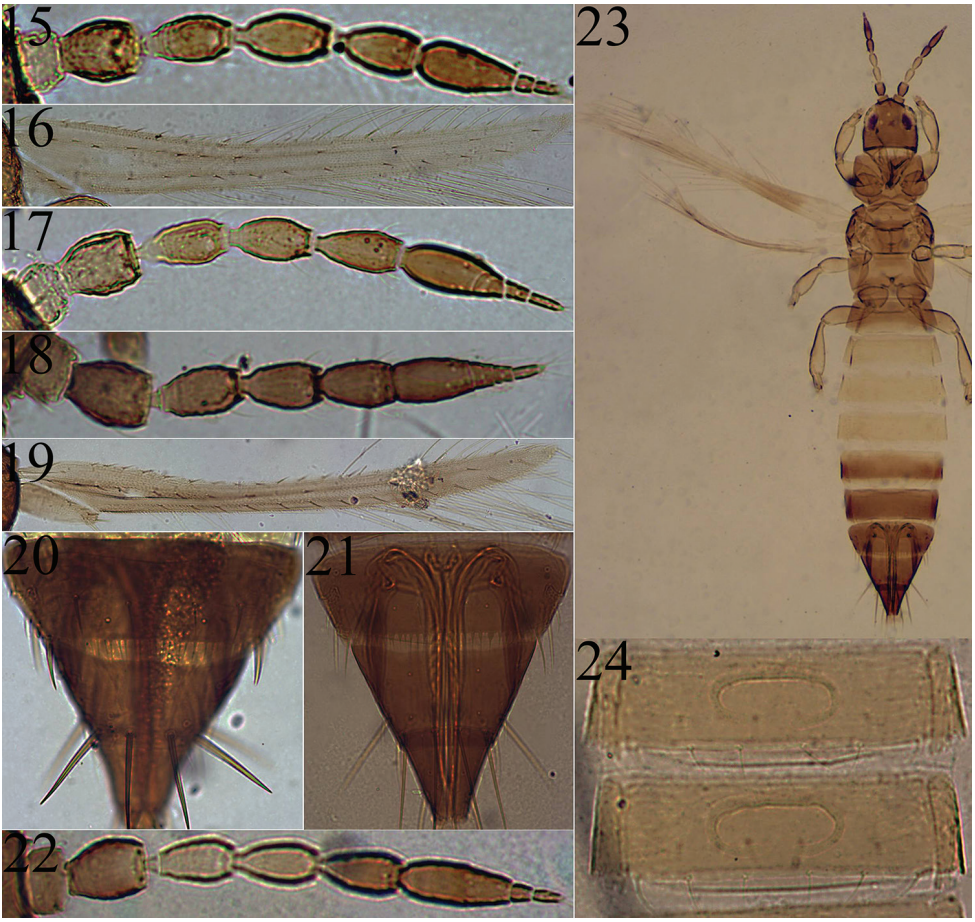
Thrips obscura Müller, 1776: 96.

Description. Macropterous female. Body and legs yellow; antennal segment I yellow, segments II-IV light brown but segment II darker, segments V-IX brown but segment V paler slightly; fore wing weakly shaded. Head wider than long, dorsal surface sculptured with irregular transverse reticulations behind eyes; ocellar setae I present, ocellar setae III out of ocellar triangle just anterior to hind ocelli. Antennae 9-segmented (Fig. 17), segments III–IV with sense cone forked. Fore wing upper vein with 7–8 basal setae and 2–3 distal setae, lower vein with 8–9 setae. Abdominal tergites with microtrichia laterally, tergite VIII posterior margin with comb of microtrichia.

Micropterous male. Similar to macropterous female, but wing shorter than thorax width (Mound and Masumoto 2009).

Distribution. Widespread around the world.

Specimens examined. 3 female (macropterous), 5 female (micropterous), China, Heilongjiang Province, Sanjiang Plain, 2.vii.2014, from grasses (Jun Wang). 1 female (micropterous) China, Heilongjiang Province, Sanjiang Plain, 9.viii.2012, from grasses (Jun Wang). 1 female (micropterous), China, Ningxia, Pingluo, 24.vii.1987, from *Polygonum* (Caixia Yang).



Figures 15–24. *Anaphothrips* of China. *A. floralis* (**15–16**). **15** Antennae **16** Fore wing **17** *A. obscurus* antennae. *A. populi* (**18–20**) **18** Antennae **19** Fore wing **20** Abdominal tergites VIII–X. *A. sudanensis* (**21–23** female; **24** male) **21** Abdominal tergites VIII–X **22** Antennae **23** Body **24** Sternites IV–V.

Anaphothrips populi Zhang & Tong

Figs 18–20

Anaphothrips populi Zhang & Tong, 1992: 71.

Description. Macropterous female. Body brown; antennae brown, segment I paler; all leg brown, tarsi paler; fore wing and clavus pale. Head about 0.7 times as long as wide. Ocellar setae 3 pairs, setae III arising at anterior margin of ocellar triangle almost as long as I and II. Antennae 9-segmented (Fig. 18), segments III and IV with forked sense cone. Fore wing (Fig. 19) upper vein with 8–9 basal setae and 3–4 distal setae, lower vein with 11–13 setae; clavus with 5 short setae. Abdominal tergites III–VII with irregular transverse sculpture laterally; tergite VIII (Fig. 20) posterior margin with comb of long microtrichia.

Remarks. This species is similar to *A. incertus* in appearance but can be distinguished from the latter by the following features: antennal segments III–IV brown; segment VI with incomplete suture in distal third; fore wing upper vein with 8–9 basal setae and 3–4 distal setae, lower vein with 11–13 setae.

Distribution. China (Henan, Gansu).

Specimens examined. Paratype: 1 female (macropterous), China, Henan Province, Baiquan, Baoding Mountain, 21.vi.1979, from *Populus* (Shengfu Shi).

Anaphothrips sudanensis Trybom

Figs 21–24

Anaphothrips sudanensis Trybom, 1911: 1; Zhang and Tong 1992: 73.

Description. Macropterous female. Body bicolored (Fig. 23), generally brown but abdominal segments III–V yellow; antennal segments I–II and V–VIII brown, segments III–IV yellow; legs yellow; fore wing pale but with dark cross band close to base. Head wider than long slightly. Antennae 8-segmented (Fig. 22), segments III–IV with forked sense cone. Pronotum weakly sculptured. Fore wing upper vein with six basal setae and five distal setae, lower vein with six setae; Abdominal tergite VIII (Fig. 21) posterior margin with comb of long microtrichia.

Macropterous male. Similar to female, but stergites III–VIII with large C-shaped pore plate (Fig. 24).

Distribution. China (Hubei, Hunan, Jiangsu, Zhejiang, Fujian, Taiwan, Guangdong, Hainan, Guangxi, Sichuan, Guizhou, Yunnan); worldwide in tropical and subtropical countries.

Specimens examined. 1 female (macropterous), China, Guangdong Province, Guangning, Baoding mountain, 16.vii.2014, from *Pelargonium hortorum* (Zhaohong Wang). 1 male (macropterous), China, Guangdong Province, Guangning, Baoding Mountain, 16.vii.2014, from *Phyllanthus urinaria* (Zhaohong Wang).

Key to Chinese species of *Anaphothrips*

(* not examined)

1 Antennae clearly 8-segmented (Fig. 15) **2**

– Antennae 9-segmented (Fig. 17), segment VI with complete oblique or transverse suture **4**

2 Female body distinctly bicolored (Fig. 23), mainly dark brown, antennal (Fig. 22) segments III–IV and abdominal segments III–V (or VI) yellow, male color various ..... ***A. sudanensis***

– Female body brown or yellow, not distinctly bicoloured **3**

3 Head with ocellar setae III arising outside of ocellar triangle, anterior to hind ocelli; fore wing upper vein with 8–11 setae, lower vein with 7–9 setae; metanotum reticulate, campaniform sensilla present ..... ***A. beijingensis****

– Head with ocellar setae III arising at outer tangent between fore and hind ocelli; fore wing upper vein with about 12 setae, lower vein with 6–11 setae; metanotum reticulate in mid line, campaniform sensilla absent .... ***A. floralis***

4 Tergite VIII (Fig. 13) posterior margin with tooth-shaped craspedum; male sternal pore plates (Fig. 12) only slightly wider than distance between posteromarginal setae S1 ..... ***A. dentatus* sp. n.**

– Tergite VIII (Fig. 21) posterior margin with comb of long microtrichia **5**

5 Body yellow; antennal (Fig. 17) segment I yellow, segments II–IV light brown, segments V–VIII dark brown; microptera or else fore wing upper vein with 7–8 basal setae and 2–3 distal setae, lower vein with 8–9 setae..... ***A. obscurus***

– Body brown; antennae (Fig. 18) brown, segment I paler; fore wing (Fig. 19) upper vein with 8–9 basal setae and 3–4 distal setae, lower vein with 11–13 setae..... ***A. populi***

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RESEARCH ARTICLE

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Addition of a new *Quedius* Steph. (Coleoptera, Staphylinidae) species to the biodiversity of Albertan mixedwood forest, Canada

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Abstract

Quedius (*Raphirus*) *spencei* Jacobs and Bergeron, new species, (Coleoptera: Staphylinidae), is described based on specimens from two localities (type locality: 35 km. E Dixonville, Alberta, Canada) in the Boreal Forest. Male genitalia are illustrated, compared with congeners (*Q. rusticus* Smetana and *Q. simulator* Smetana) in the *Aenescens* species group, and included in a slightly modified key to the species of *Quedius*.

Keywords

Quedius spencei, *Raphirus*, EMEND

Introduction

The genus *Quedius* Stephens currently contains 92 species in America north of Mexico (Newton et al. 2001; Smetana and Webster 2011). Taxonomy of this genus has been revised three times by Horn (1871), Casey (1915) and Smetana (1971a, 1971b, 1973, 1976, 1978, 1981). The Alberta fauna of the genus *Quedius* is particularly diverse with 29 species (Bousquet et al. 2013) and has been included in many biodiversity studies in boreal forest (Bergeron et al. 2013; Buddle et al. 2006; Gandhi et al. 2004;

Gandhi et al. 2001; Hammond et al. 2001, 2004; Jacobs et al. 2007a, 2007b; Pohl et al. 2008; Pohl et al. 2007). J. Jacobs detected a specimen of an undescribed species while identifying flight intercept trap samples from a central Albertan study on saproxylic beetles (Cobb et al. 2011). Further specimens (fifteen) of this same species were found from pitfall trap samples collected by C. Bergeron from a northwestern Albertan forest biodiversity study (Bergeron et al. 2011). In an effort to assess the impact of alternative forest management practices on the boreal mixedwood forest ecosystem, the Ecosystem Management Emulating Natural Disturbance (EMEND) research site is subject to long term intensive arthropod sampling. Therefore, this species has the potential to contribute important information about ecological processes and modern forest management techniques. Furthermore, the fact that this species was collected from two sites separated by c. 260 km suggests that it may be widely distributed at least in the boreal mixedwood forest of Alberta. Combined with the increasing popularity of ground dwelling and saproxylic beetles in ecological impact assessment (Langor and Spence 2006), and the widespread use of pitfall and flight intercept traps in such studies, it is very likely that this species will be collected again. For these reasons, as well as taxonomical interest, we describe the new species in this paper.

Classification

In North America, the genus *Quedius* is divided into 6 subgenera. *Quedius spencei* is included in the subgenus *Raphirus* Stephens with 23 other species characterized by large eyes and a usually broad and narrowly bilobed labrum (Smetana 1971a). This new species belongs to the *Aenescens* group which is characterized by the two additional setiferous punctures between the anterior frontal punctures and the placement of the last puncture of sublateral row distinctly behind the level of the lateral puncture. The following description is based on the terms used by (Smetana 1971a) for the similar species *Quedius rusticus* Smetana and *Quedius simulator* Smetana.

Methods

Measurements and ratios

The width of the head was measured along the widest part including the eyes. The length was measured along the midline, from the base of the head to the apex of clypeus. These measurements were used to determine the width to length ratio (w:l) for the head. The length of the eyes (from anterior to posterior margin) and the length of the temples (from posterior margin of the eyes to the neck) were measured as viewed dorsally. These values were used to determine the temple to eye ratio (t:e). The width of the pronotum was measured along the widest segment separating the lateral margins of the pronotum and the length is measured along the midline from the anterior

to the posterior margin. These measurements were used to calculate w:l ratio for the pronotum. The lateral length of the elytra was measured between the humeri and the posterior elytral angle. This measurement was used to define the elytra at sides to pronotum at midline ratio.

Description

Quedius (*Raphirus*) *spencei* Jacobs & Bergeron, sp. n.
http://zoobank.org/7458C3CE-78E3-4AFE-9060-01FE507CCB7D
Figs 1, 2A

Description. Habitus as in Fig. 1. Piceous to piceous black, elytra and abdominal tergites of some specimens brownish. Palpi, antennae and legs piceous to brownish with tibia distinctly darker than rest of leg. Head, pronotum and elytra with bronze luster. Head rounded, slightly transverse (1.07–1.09 w:l). Eyes large, considerably longer than the length of the temples in dorsal aspect (0.31–0.32 t:e). Two additional setiferous punctures between anterior frontal punctures, posterior frontal puncture situated somewhat closer to posterior margin of eye than posterior margin of head (similar to *Q. rusticus*). Surface of head with very fine and dense microsculpture consisting of transverse lines. Antennae with first 3 segments darker and elongate (longer than wide), third segment slightly shorter than second, segments 4-11 densely pubescent. Segments 4 and 5 are slightly longer than wide, sixth barely longer than wide, and segments 7–10 quadrate to slightly transverse. Pronotum as long as wide (1.00, w:l), broadly arcuate at base and moderately narrowed in front. Chaetotaxy of pronotum similar to other species in the *Aenescens* group with three punctures in each dorsal row, sublateral rows with last puncture situated distinctly behind level of large lateral puncture; microsculpture similar to head. Scutellum impunctate. Elytra at sides only barely longer than pronotum at midline (1.07–1.10). Punctation and pubescence of elytra fine and moderately dense (as in *Q. rusticus*), interspaces smooth without distinct microsculpture. Punctation of abdominal tergites finer than punctuation of elytra and usually a little denser on bases of first three or four visible abdominal tergites. Pubescence brownish with a single large piceous bristle originating from the lateral apex of first four visible abdominal tergites, usually with a second bristle on the second to fourth visible tergites (as in *Q. rusticus* and *Q. simulator*).

Male. Sixth visible sternite with a moderately shallow, obtuse triangular emargination in the middle of apical margin, with a slightly impressed, smooth, narrow triangular area anteriad of the emargination, less than twice the depth of the emargination. Aedeagus with paramere extended to the tip of narrow, sharp median lobe. Paramere slightly narrowed posteriad of base, expanded to maximum width one-third from apex, at which point margins obtusely angle toward narrowed apex (Fig. 2A; pm, a, b). Paramere with several short and two long apical bristles, with two additional long bristles on lateral margins near apex. Sensory peg setae on dorsal surface of paramere arranged

Figure 1. Dorsal habitus of *Quedius spencei*, sp. n.

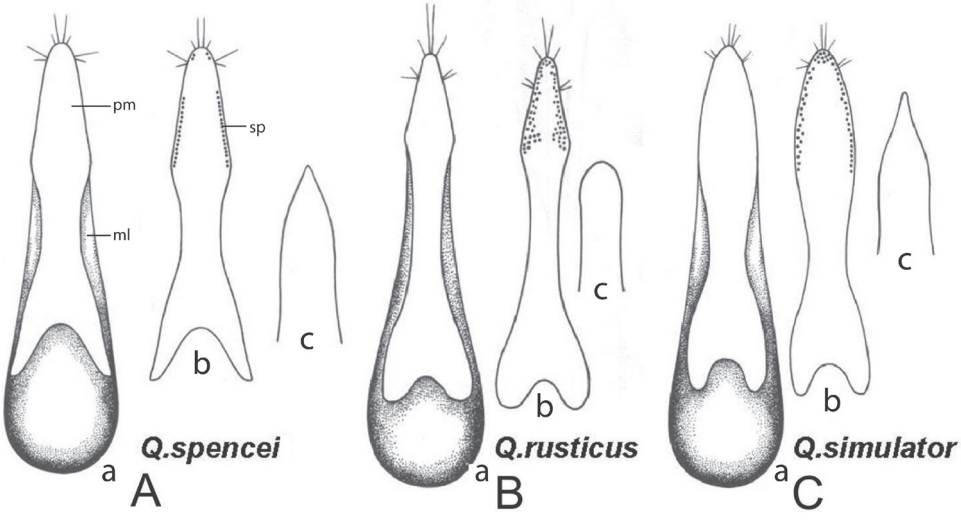

Figure 2. Male genitalia of **A** *Q.spencei* **B** *Q.rusticus*, and **C** *Q.simulator* **a** aedeagus, ventral aspect, ml: median lobe, pm: paramere **b** paramere, dorsal aspect, sp: sensory peg setae c) apex of median lobe, ventral aspect. Illustrations of *Q.rusticus* and *simulator* from Smetana (1971a).

in a single row on lateral margins, terminated distinctly anteriad of apex, with one to three additional peg setae on each side of apex (Fig. 2A; b, sp).

Length: 5.6–5.8mm.

Type material. Type material is deposited in the Strickland Entomological Museum at the University of Alberta (UASM) and at the Canadian National Collection of Insects, Arachnids and Nematodes in Ottawa (CNC). See supplementary table for more information on each specimen.

Holotype (♂) // CAN:AB: EMEND, 56°46'13"N; 118°22'28"W, Coll: C. Bergeron 2003 // HOLOTYPE, *Quedius spencei* Jacobs & Bergeron, // CB1802 // UASM# 212610. The holotype is pointed with genitalia stored in plastic vial. The right foreleg is missing tibia and tarsus and the right hind leg is missing the three last tarsal segments. Left maxillary palp is broken.

Paratypes: 7 ♂♂, same labels as holotype but CB0954 // UASM# 212609; CB1555 // UASM# 212608; CB1555 // UASM# 212606; CB0956 // UASM# 212607; CB0955 // UASM# 212611; CB2034 // CNC# 615416; CB2036 // CNC# 615417; and 3 ♂♂ CAN:AB: Slave Lake, 55°17'52"N; 115°05'29"W, Coll. T.Cobb 2003 // PARATYPE, *Quedius spencei*, Jacobs & Bergeron // tpc02841 // CNC# 615418; tpc02320 // CNC# 615419; tpc3220 // CNC# 615420.

Type locality. EMEND research site, 35 km east of Dixonville, Alberta, Canada

Geographical distribution. Known specimens were collected from central Alberta, Slave Lake (N55°17.86', W115°05.49') and north-western Alberta, near Dixonville (N56°46,22', W118°22.47'). Probably more widely distributed.

Collection notes. The specimens from Dixonville were collected in pitfall traps operating from the second week of May until the last week of June 2003 in old-growth spruce-fir forest. The Slave Lake specimens were collected from a flight intercept trap in May 2003 in a former conifer forest two years following harvesting; regenerating with aspen trees. *Quedius spencei* seems to live in conifer forest.

Comparison and diagnostic features. *Quedius spencei* is similar in general habitus to *Quedius rusticus* Smetana, but shares characteristics of the male genitalia with *Quedius simulator* Smetana: shape of the apex of the median lobe acute (Fig. 2A, c), and paramere of the male genitalia does not exceed the apex of the median lobe. However, *Q. spencei* differs from *Q. simulator* in other characters of the aedeagus: paramere (Fig. 2A; pm, a, b) with margins of apical half obtusely angulate, reaching maximum width one-third from apex; base of paramere deeply emarginate (Fig. 2A, b); and sensory peg setae (Fig. 2A, b, sp) on each lateral margin in a single row. Additionally, similar to *Q. rusticus* this species is lighter in coloration than *Q. simulator,* with elytra usually brownish, and apical margins of tergites and apex of abdomen paler. Also, similar to *Q. rusticus*, the posterior frontal puncture of head is removed from the hind margin of eyes.

Etymology. The specific epithet is an eponym, a singular Latin noun, genitive case, based on the surname of our mentor and friend, John R. Spence, who has dedicated his career to the study of arthropod biodiversity, particularly ecological and taxonomic aspects of gerrid bugs and carabid beetles, and to community aspect of forest invertebrates. He has mentored many students, putting emphasis on species level identifications, and has greatly influenced the field of entomology in Canada.

Key to selected species of *Quedius* (modified from Smetana 1971a)

21(22) Paramere with the apex considerably exceeding the apex of median lobe, which is broadly arcuate (Fig. 2B, c) ..... ***Q. rusticus* Smetana**

22(21) Paramere with the apex about even with the apex of median lobe, which is more or less acute (figs 136, 137, Smetana 1971a).

22a(22b) Paramere with margins of apical half obtusely angulate (Fig. 2A, a,b). Coloration usually lighter, punctation of abdomen moderately dense. Length 5–6mm.....***Q. spencei* sp. n.**

22b(22a) Paramere with margins of apical half broadly rounded (Fig. 2C, a,b). Coloration usually darker, punctation of abdomen usually very dense.

23(24) Paramere with the apical part long and spatulate, the narrow and more or less parallel-sided middle part short (Fig. 2C, a,b). Head and pronotum usually with darker metallic luster; punctation of abdomen usually finer and more dense. Length 5–7mm ..... ***Q. simulator*** **Smetana**

24(23) Paramere with the apical part short, lancet-like, the narrow and more less parallel-sided middle part long (fig. 137, Smetana 1971a). Head and pronotum usually with lighter metallic luster; punctation of abdomen usually less fine and dense. Length 5–7mm ..... ***Q. aenescens*** **Mäklin**

Acknowledgements

We thank the following: John R. Spence University of Alberta, for mentorship, support and inspiration; Aleš Smetana, for access to specimens at the CNCI and sharing his knowledge on *Quedius* taxonomy; George E. Ball, University of Alberta for comments on early versions of this paper; Gregory R. Pohl and Dustin J. Hartley, Natural Resources Canada for taxonomic support; Danny Shpeley from the Strickland Entomological Museum for guidance in capturing images; S. Odsen from Fuse Consulting Ltd. for her participation in imaging the dorsal habitus; Tyler P. Cobb, Royal Alberta Museum, for collection of specimens and funding provided to him from foothills model forest; and Daishowa Marubeni International Ltd., Canadian Forest Products, Manning Diversified Forest Products, Alberta Agriculture and Forestry, and NSERC for funding to J.A.C. Bergeron.

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Supplementary material 1

Locality table for the holotype and paratype specimens of *Quedius spencei* Jacobs & Bergeron, sp. n.

Authors: Jenna M. Jacobs, J. A. Colin Bergeron

Data type: occurence

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RESEARCH ARTICLE

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Faunistic notes on Cryptophagidae and Latridiidae of Talassemtane National Park, Western Rif, Morocco, with the description of a new species (Coleoptera, Cucujoidea)

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Abstract

In order to contribute to the knowledge of beetles (Coleoptera) of the mountainous region of Morocco, Talassemtane National Park (Western Rif, Chefchaouen district, Morocco) was surveyed. This is an exceptional protected area of the Mediterranean Intercontinental Biosphere Reserve (RIBM). The checklist was made using different traps combined with active periodical searches during 2013–2015. A total of 153 beetles belonging to 19 species from four subfamilies (Cryptophagidae: Cryptophaginae and Atomariinae; Latridiidae: Latridiinae and Corticariinae) was collected. *Dienerella* (*Cartoderema*) *talassemata*, a new species (Coleoptera: Latridiidae) was compared to other morphologically related species. One species is recorded for the first time for North Africa; three species are new records for Morocco. In addition, amongst the species listed, three are endemic to Morocco: *Dienerella talassemtana* **sp. n.**, *Caenoscelis humifera* and *Dienerella besucheti*.

Keywords

Coleoptera, Cryptophagidae, *Dienerella* (*Cartoderema*) *talassemtana* sp. n., Latridiidae, Morocco, Rif, Talassemtane National Park

Introduction

Created in October, 2004, Talassemtane National Park (PNTLS) is located in the western area of the calcareous ridge of the Rif mountain range. The park constitutes a unique territory containing natural landscapes of great heritage value at a national level. Its position at the boundary line between Europe and Africa, its climatic and geological characteristics, and its paleogeographic history, have given rise to unique fauna and flora. As such, the national park is included in the Mediterranean Intercontinental Biosphere Reserve (RBIM), intended for the conservation of the most emblematic natural areas of northern Morocco and southern Spain. Talassemtane National Park harbours more than 750 plant species (56 endemic) belonging to 103 families (Meda 2008). It also lodges about 40 species of mammals and more than 100 species of birds; reptiles and amphibians are represented by about 30 species with a rate of endemism reaching 27% (Meda 2008). Aquatic macro-invertebrates comprise 180 species of which 48 are endemic. Obviously, the invertebrates of PNTLS have been less studied than plants and vertebrates, with only a few of studies to date on Diptera, Simuliidae (Belqat et al. 2001), ants (Taheri et al. 2014), and water beetles (Benamar et al. 2011). To tackle the lack of information on invertebrates of Moroccan protected areas and to enrich the list of the Rifian entomofauna and especially that of PNTLS, a study of the Coleoptera was carried out. This study was developed within the framework of the Agronomic Research Program for Development (PRAD). In addition to the checklist, the objective of this study is to detect species related to the forest stands in order to establish a management policy that aims to improve their conservation. In this paper, we treat only the families Cryptophagidae and Latridiidae.

Cryptophagidae is a moderately large group of small-sized beetles (1–6 mm long) containing more than 1,000 described species belonging to approximately 50 genera. Cryptophagidae has a worldwide distribution, and as indicated by Crowson (1980), some groups have amphipolar distribution. Both adults and larvae are commonly found on mold, fungi, under bark as well as in decaying vegetation and nests of social hymenoptera, birds and mammals (Otero et al. 2001; Lyubarsky and Perkovsky 2011). Latridiidae is a moderately large family with approximately 500 species which is represented in all major biogeographic regions. They are minute (1-3 mm) and often live in moldy stored food and decomposed plant materials. They seem to be spore feeders (Pal and Ghosh 2007).

Materials and methods

Study site

PNTLS covers an area of 64,601 ha. Two-thirds of the park are located within the province of Chefchaouen and one-third belongs to the province of Tetouan (Fig. 1). It is mainly formed by the southern area of the Rifian calcareous ridge and includes the

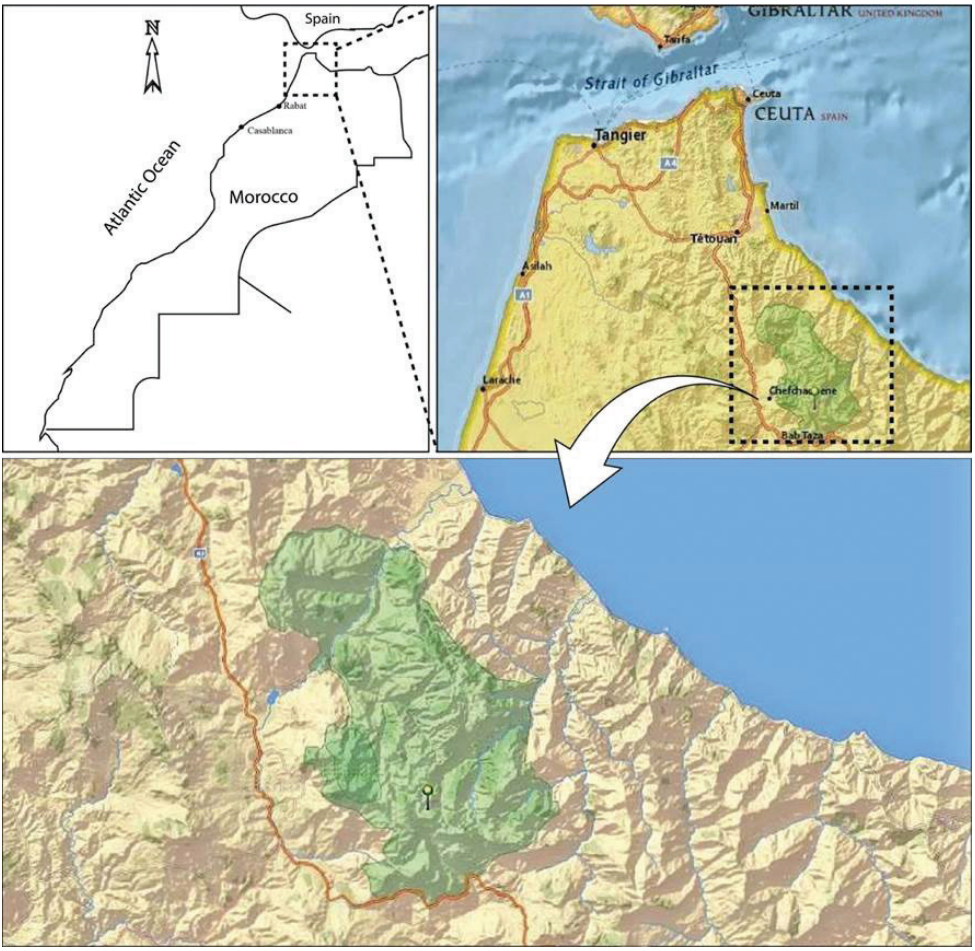

Figure 1. Map of Morocco showing the location of Talassemtane National Park. The green cropped area indicates the limits of the Park.

highest summits of western Rif. The climate of PNTLS is characterized by being the wettest zone of North Africa. The park is characterized by two climax forest stands, formed by an endemic and relict fir species, *Abies maroccana* Trab. The Talassemtane fir forest is the largest and extends over 2,300 ha. Our survey was carried out in pure fir (*Abies alba*) forests (in the highest zones), as well as in fir-oak (*Quercus ilex*) forests.

Methods

Terminology and measurements of new species follow an earlier paper (Otero 1997). Morphological structures were measured under a Leica M205C stereomicroscope equipped with an analysis system Application Suite.

Data collection

The records of trap contents were performed once every 15 days, for 7 months (April–October), during 2013 and 2014. Window flight traps, and in particular the multidirectional PolytrapTM, were used as they are probably the best suited for inventorying saproxylophagous beetles in temperate forest. The output of these traps and their selective power are rather enlightening, as far as the capture of Coleoptera is concerned, compared to other arthropods (Bouget and Brustel 2009). The multidirectional window flight traps were primed with ethanol, which serves as an attractant for numerous saproxylophagous beetles (Bouget and Brustel 2009). Two other types of traps were used in the checklist; pitfall traps were installed at ground level to intercept ground fauna, and yellow-coloured traps were used in open environments to attract flower-dwelling species. All traps were installed in 20 unsystematic plots in the fir grove.

Active searching was done during April 2013, August 2013, October 2013, June 2014, September 2014 and November 2015, most often during the installation of the protocol or the placement of traps. Many active methods were then used such as barking dead trees, raising stones, beating or sifting.

Abbreviations

L	length;
WL	width/length ratio;
E	eccentricity of the eyes, which is calculated as width/half of the length (width is measured across the widest part of a line joining the anterior and posterior limit of the eye; length is the maximum length of the eye);
L	length in dorsal view;
W	width;
Ø	diameter.
CBT	coll. H. Brustel, Toulouse, France.
COCC	coll. O. Courtin, Castres, France.
CWR	coll. W. Rucker, Neuwied, Germany.
MNHN	Museum National d'Histoire Naturelle, Paris, France.
USCO	Universidad de Santiago de Compostela, coll. J.C. Otero, Santiago de Compostela, Spain.

Results

In total, 153 beetles belonging to 19 species from four subfamilies (Cryptophagidae: Cryptophaginae and Atomariinae; Latridiidae: Latridiinae and Corticariinae) were

collected. *Dienerella* (*Cartoderema*) *talassemata* represents a new species (Coleoptera: Latridiidae) for the Palaearctic Region. One species is recorded for the first time for the north of Africa: *Cryptophagus cylindrellus* C. Johnson, 2007. Three species are new records for Morocco: *Cryptophagus pallidus* Sturm, 1845; *Cryptophagus uncinatus* Stephens, 1830 and *Atomaria* (*Atomaria*) *nigripennis* (Kugelann, 1794). In addition, amongst the species listed, two others are endemic to Morocco: *Caenoscelis humifera* Esser, 2008 and *Dienerella* (*Cartoderema*) *besucheti* Vincent, 1994.

Family Cryptophagidae
Subfamily Cryptophaginae

***Caenoscelis humifera* Esser, 2008**

Caenoscelis humifera Esser, 2008: 7

Examined material. Morocco, Rif, Talembote, Sapinière de Talassemtane, 10-13. XI.2015, 1 ex (leg. H. Brustel). Mts Rif, Al-Hoceima, Torres-de-Alcatá, Steilküste, 27.XII.2001, 1 ex; Jbel Tazzeka, Taza, Gorges du Zireg, 5.I.2002, 1 ex (leg. Esser)

Distribution. Endemic to Morocco (Esser 2008; Otero 2013).

***Cryptophagus cylindrellus* C. Johnson, 2007**

Cryptophagus cylindrellus C. Johnson, 2007: 66

Examined material. Morocco, Rif, Sapinière de Talassemtane, 22. V.2014, 1 ex.; 15.VI.2014, 1 ex; 19.VI.2014, 2 exx; 3.X.2014, 3 exx (leg. H. Brustel).

Distribution. Throughout Europe and Turkey (Johnson et al. 2007; Otero 2013). First mention for North Africa.

***Cryptophagus dentatus* (Herbst, 1793)**

Cryptophagus dentatus (Herbst, 1793:15)

Examined material. Morocco, Rif, Sapinière de Talassemtane, 19.VII.2012, 1 ex; 4. V.2013, 1 ex; 9. V.2013, 1 sp; 24. V.2013, 1 ex; Talembote, Sapinière de Talassemtane, 30. X.2015, 1 ex; 10-13.XI.2015, 3 exx (leg. H. Brustel).

Distribution. Europe, North Africa, Central Asia and North America (Johnson et al. 2007; Otero and Johnson 2013).

Cryptophagus jakowlewi Reitter, 1888

Cryptophagus jakowlewi Reitter, 1888: 424

Examined material. Morocco, Rif, Sapinière de Talassemtane, 4.V.2013, 8 sp.; 9.V.2013, 4 exx; 26.IX.2013, 1 sp; 31.X.2013, 4 exx; 30.IV.2014, 2 exx; 15.VI.2014, 7 exx; 19.VI.2014, 24 exx; 18.X.2014, 1 sp; 15.X.2016, 2 exx; Talembote, Sapinière de Talassemtane, 7.V.2015, 1 ex; 10-13.XI.2015, 8 exx (leg. H. Brustel).

Distribution. Europe, North Africa, Caucasus, Asia Minor, Central Asia and Eastern Siberia (Johnson et al. 2007; Otero 2013).

Cryptophagus pallidus Sturm, 1845

Cryptophagus pallidus Sturm, 1845: 69

Examined material. Morocco, Rif, Talembote, Sapinière de Talassemtane, 10-13. XI.2015, 3 exx (leg H. Brustel).

Distribution. Throughout Europe, North Africa (Algeria, Lebanon and Tunisia), Iran, Israel, Lebanon and Turkey (Johnson et al. 2007; Otero 2013).

First mention for Morocco.

Cryptophagus pubescens Sturm, 1845

Cryptophagus pubescens Sturm, 1845: 103

Examined material. Morocco, Rif, Sapinière de Talassemtane, 15.VI.2014, 1 ex (leg H. Brustel).

Distribution. Europe, Caucasus and North Africa (Johnson et al. 2007; Otero 2013).

Cryptophagus punctipennis C.N.F. Brisout de Barneville, 1863

Cryptophagus punctipennis Brisout de Barneville, 1863: 63

Examined material. Morocco, Rif, Talembote, Sapinière de Talassemtane, 10-13. XI.2015, 5 exx (leg. H. Brustel).

Distribution. Cosmopolitan species (Johnson et al. 2007; Otero 2013).

***Cryptophagus scanicus* (Linnaeus, 1758)**

Cryptophagus scanicus (Linnaeus, 1758: 357)

Examined material. Morocco, Rif, Talembote, Sapinière de Talassemtane, 7.V.2015, 2 exx (leg. H. Brustel).

Distribution. Holarctic (Johnson et al. 2007; Otero 2013).

***Cryptophagus uncinatus* Stephens, 1830**

Cryptophagus uncinatus Stephens, 1830: 75

Examined material. Morocco, Rif, Sapinière de Talassemtane, 30.IV.2014, 1 ex (leg. H. Brustel).

Distribution. Throughout Europe, North Africa (Algeria) and Turkey (Johnson et al. 2007; Otero 2013).

First record for Morocco.

Family Latridiidae
Subfamily Atomariinae

***Atomaria* (*Atomaria*) *nigripennis* (Kugelann, 1794)**

Atomaria (*Atomaria*) *nigripennis* (Kugelann, 1794: 578)

Examined material. Morocco, Rif, Talembote, Sapinière de Talassemtane, 10-13. XI.2015, 9 exx (leg H. Brustel).

Distribution. Europe and North Africa (Tunisia) (Johnson et al. 2007; Otero 2011).

First record for Morocco.

***Atomaria* (*Atomaria*) *pallidipennis* Holdhaus, 1903**

Atomaria (*Atomaria*) *pallidipennis* Holdhaus, 1903: 364

Examined material. Morocco, Rif, Sapinière de Talassemtane, 4. V.2013, 1 sp.; 30.IV.2014, 1 ex (leg. H. Brustel).

Distribution. Europe and North Africa (Johnson et al. 2007; Otero 2011).

Atomaria (*Atomaria*) *pusilla* (Paykull, 1798)

Atomaria (*Atomaria*) *pusilla* (Paykull, 1798: 295)

Examined material. Morocco, Rif, Talembote, Sapinière de Talassemtane, 10-13. XI.15, 1 ex (leg. H. Brustel).

Distribution. Europe, Caucasus, Mongolia, Turkey, Iran, Afghanistan, Central Asia, North Africa, Madeira and the United States (Johnson et al. 2007; Otero 2013).

Subfamily Latridiinae

Cartodere (*Aridius*) *nodifer* (Westwood, 1839)

Cartodere (*Aridius*) *nodifer* (Westwood, 1839: 155)

Examined material. Morocco, Rif, Sapinière de Talassemtane, 19.VI.2014, 1 ex (leg H. Brustel).

Distribution. Cosmopolitan species (Johnson 2007).

Dienerella (*Cartoderema*) *besucheti* Vincent, 1994

Dienerella (*Cartoderema*) *besucheti* Vincent, 1994: 77

Examined material. Morocco, Rif, 12 km W Bab Berret, 35°01'06"N, 05°00'40"W, 11.IV.2013, 2 exx (det. W. Rücker) (leg. H. Brustel).

Distribution. Endemic to Morocco (Johnson 2007; Vincent 1994).

Dienerella (*Cartoderema*) *talassemtana* sp. n.

http://zoobank.org/69EF0F36-5247-43D4-99F0-3DC957E3AEBE
Fig. 2A–E

Description. Body length: 1.16–1.31 mm. Body elongated, narrow and superficially depressed. Reddish grey-brown or testaceous-brown colour; lighter appendages. Body (Fig. 2A) glabrous. Lacking metathoracic wings. **Head** rough and transverse (WL= 1.4); slightly narrower (including the eyes) than the pronotum in its anterior region. Clypeus short, as wide as the head, separated from the frons by a slightly arcuate suture and from the labrum by a concave suture. Labrum visible from top view. Temples short, oblique and visible from top view. Eyes hemispherical and slightly

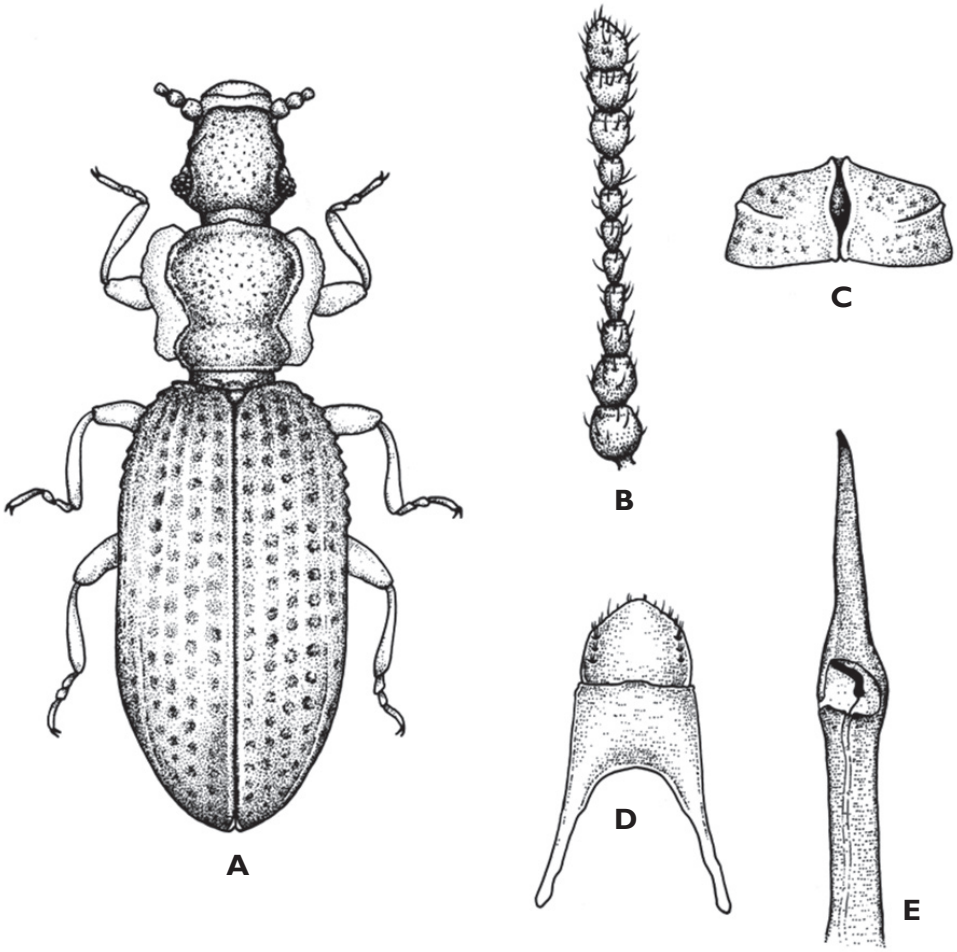

Figure 2. *Dienerella* (*Cartoderema*) *talassemtana* sp. n. **A** dorsal habitus **B** antennae **C** elytral suture **D** tergite V masculine **E** aedeagus.

protruding (E=1), not surpassing the margin of the pronotum, made up of few (15 to 20) facets. Eye facets with small diameter (Ø= 6–8 μm). Antennae (Fig. 2B) short (L= 0.314 mm), reaching the pronotum constriction. Antennomere I spherical, wider and 1.4 longer than the II; subequal from III to VIII. The last three form an extended club; X as long as IX; XI truncate distally, 1.3 times longer than the previous one. **Pronotum** 1.1 times longer than wide. Anterior and posterior margins straight; lateral margins rounded. Strongly constricted in the basal third. Rough surface with

marked (Ø= 10–15 µm) and thick puncturation. Tibiae with a small spine on the apex. **Elytra** together oval, elongated; 1.8 times longer than wide; rounded base. Lateral margins rounded and denticulate in their anterior half. Provided with 8 rows of large (Ø= 24–30 µm) and ordered punctures (4 dorsal and 2 lateral). Space between suture and fourth elytral interval slightly convex, not excavated behind the scutellar shield. Slight depression in the last third of the dorsal area of the elytra. Fifth striae higher, forming a curved lining that slightly separates near the middle area and joins to the suture in the posterior area; fourth striae higher in the anterior two thirds. Elytral suture bearing a rhomboid opening in the posterior area (Fig. 2E). Anterior coxae almost adjacent; intermediate separated and posterior widely separated. Tergite V masculine (Fig. 2D). **Aedeagus** (Fig. 2E). Aedeagus with very elongated apex, not widened. Internal sac as in Fig. 2E.

Type material. Morocco. Holotype (m). Morocco, Rif, Talembote, P.N. Talassemtane, 10-13.XI.2015 (leg. H. Brustel) (USCO).

Paratypes. 1 m. Morocco, Rif, P.N. Talassemtane, 13.XI.2015 (leg O. Courtin), (COCC). 1 f. Morocco, Rif, Talembote, P.N. Talassemtane, 10-13.XI.2015 (leg. H. Brustel) (MNHN). 1 f. Morocco, Rif, Talembote, P.N. Talassemtane, 10-13.XI.2015 (leg. H. Brustel), (CBT). 1 f. Morocco, Rif, Talembote, P.N. Talassemtane, 10-13. XI.2015 (leg. H. Brustel), (CWR).

Distribution. Morocco.

Etymology. This species is named in reference to the region in which the type material was collected.

Differential diagnosis

In order to distinguish the species of *Dienerella* Reitter of the group *elongata* (Curtis), the following table may be useful (from Rücker 1998, modified)

1 Posterior margin of tergite V in males with distinct apical widening (Fig. 3A). Lanceolate aedeagus (Fig. 3D). L=1.4–1.5mm..... ***D. huguettae*** **Vincent**

– Posterior margin of tergite V masculine without distinct apical widening. Aedeagus with very elongated apical end.....**2**

2 Posterior margin of tergite V masculine rounded (Fig. 3C, D.....**3**

– Posterior margin of the tergite V masculine slightly pointed (Figs 2D, 3B) ... **4**

3 Aedeagus with very elongated and narrow apical end; armour of internal sac as in Fig. 3H. L=1.3–1.7 mm ..... ***D. clathrata*** **(Mannerheim)**

– Aedeagus with very elongated and narrow apical end; C-shaped armour of internal sac (Fig. 3G) ..... ***D. besucheti*** **Vincent**

4 Aedeagus with elongated and spatulate apex (Fig. 3F); armour of internal sac as in Fig. 3F. L=1.2–1.4 mm ..... ***D. separanda*** **(Reitter)**

– Aedeagus with very elongated and not widened apex (Figs. 2E); armour of internal sac as in Fig. 2E. L= 1.16–1.31 mm..... ***D. talemsattana*** **sp. n.**

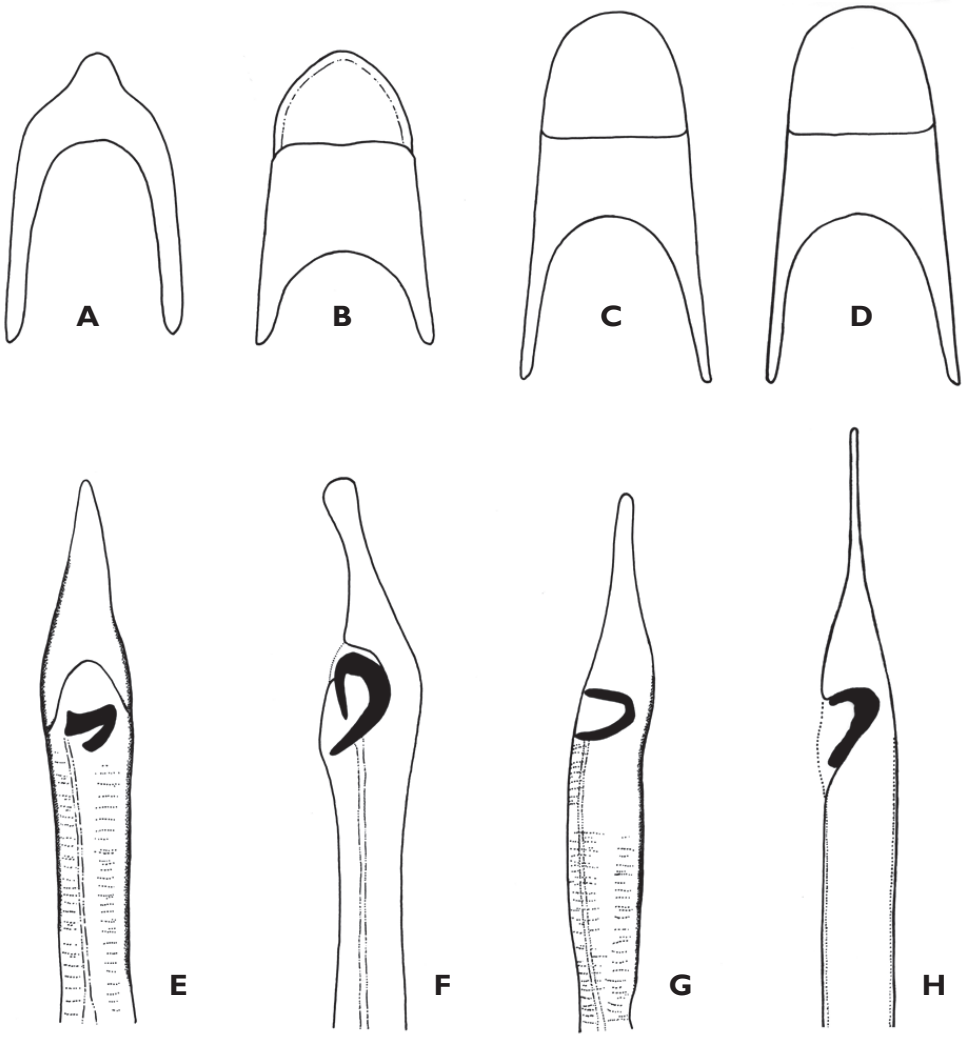

Figure 3. Tergite V masculine and aedeagus: **A, E** *Dienerella huguetae* Vincent **B, F** *Dienerella separanda* (Reitter) **C, G** *Dienerella besucheti* Vincent **D, H** *Dienerella clathrata* (Mannerheim).

Enicmus brevicornis (Mannerheim, 1844)

Enicmus brevicornis (Mannerheim, 1844: 102)

Examined material. Morocco, Rif, Sapinière de Talassemtane, 9.IV-4. V.2013, 1 ex; 24.V-4.VI.2013, 1 ex; 4. VI-20.VI. 2013, 2 exx; 20.VI.4.VII. 2013, 1 ex; 31.VII-15. VIII.2013, 11 exx (det. W. Rücker); 15. VIII.2013, 11 exx; 31. VIII.2013, 1 ex; 10.IX-26.IX. 2013, 1 ex (det. W. Rücker); 31. X.2013, 4 exx; 10. IV-30.IV.2014, 1 ex; 30.IV.2014, 1 ex; 15.V.2014, 1 ex; 30.VI-15.VII. 2014, 1 ex (det. W. Rücker);

15.VII.2014, 1 ex; Talembote, Sapinière de Talassemtane, 10-13.XI.15, 1 ex, (leg. Y. Benyahia)

Distribution. Europe, North Africa, Iran and Turkey (Johnson 2007).

Revelieria genei (Aubé, 1850)

Revelieria genei (Aubé, 1850: 333)

Examined material. Morocco, Rif, 12 km W Bab Berret, 11.IV.2013, 35°01'06"N, 05°00'40"W, 11.IV.2013, 1 ex (det. W. Rücker) (leg H. Brustel).

Distribution. France, Great Britain, Italy, Spain, Algeria, Morocco, Tunisia, Israel, Turkey (Johnson 2007).

Subfamily Corticariinae

Corticaria illaesa Mannerheim, 1844

Corticaria illaesa Mannerheim, 1844: 33

Examined material. Morocco, Rif, Talembote, Sapinière de Talassemtane, 10-13. XI.2015, 1 ex (leg. H. Brustel).

Distribution. Widespread but sporadic throughout the Mediterranean region (Johnson 2007).

Corticaria inconspicua Wollaston, 1860

Corticaria inconspicua Wollaston, 1860: 260

Examined material. Morocco, Rif, Talembote, Sapinière de Talassemtane, 10-13. XI.2015, 1 ex, Sapinière de Talassemtane, 10.IV.2013, 1 ex (det. W. Rücker); Sapinière de Tazaot, 35°15'50"N 05°06'14"W, 1 ex (det. W. Rücker) (leg. H. Brustel).

Distribution. Europe, North Africa and Cyprus (Johnson 2007).

Conclusion

This brief survey allows us to contribute to the Moroccan faunistic checklist with some new data:

Dienerella (*Cartoderema*) *talassemtana* (Coleoptera: Latridiidae) represents a new species from Morocco and the the Palaearctic Region. In addition, amongst the species

listed, two other species are apparently endemic to Morocco: *Caenoscelis humifera* Esser, 2008 and *Dienerella* (*Cartoderema*) *besucheti* Vincent, 1994. One species is mentioned for the first time for North Africa: *Cryptophagus cylindrellus* C. Johnson, 2007.

Three species are new records to Morocco: *Cryptophagus pallidus* Sturm, 1845; *Cryptophagus uncinatus* Stephens, 1830 and *Atomaria* (*Atomaria*) *nigripennis* (Kugelann, 1794).

This research effort must continue in the future to improve our knowledge on the Moroccan entomofauna. It is also especially interesting for the definition of the local biodiversity hot spot and the selection of suitable taxa to establish a red list of saproxylic beetles in Maghreb.

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We would like to thank the project financers (Agronomic Research Program for Development (PRAD), who developed this joint action of the French Ministry of Foreign affairs, the Moroccan Ministry of Agriculture and Fisheries and the High Commissioner for Water, Forests and the Fight against Desertification (HCEFLCD). We also thank Wolfgang Rücker who studied the first Latridiidae of this project; Olivier Courtin, El Hassan Dahmani, Salwa El Antry, Noureddin Maatouf and Lionel Valladares for field trip assistance and data collection; Moustafa Naima, for having guided us along the Moroccan roads and Mohamed Guenbour for participating in the installation, recording and monitoring of the traps.

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RESEARCH ARTICLE

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New species and new records of *Manota* Williston from Colombia, Brazilian Amazonia, and Costa Rica (Diptera, Mycetophilidae)

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Abstract

The following five species are described as new: *Manota clava* **sp. n.** (Colombia), *Manota multilobata* **sp. n.** (Colombia), *Manota perplexa* **sp. n.** (Costa Rica), *Manota setilobata* **sp. n.** (Colombia) and *Manota subaristata* **sp. n.** (Colombia). In addition, new records for the following 11 species are presented: *Manota acuminata* Jaschhof & Hippa, 2005 (Costa Rica), *Manota arenalensis* Jaschhof & Hippa, 2005 (Costa Rica), *Manota corcovado* Jaschhof & Hippa, 2005 (Costa Rica), *Manota costaricensis* Jaschhof & Hippa, 2005 (Costa Rica), *Manota diversiseta* Jaschhof & Hippa, 2005 (Colombia, Brazilian Amazonia, Costa Rica), *Manota minutula* Hippa, Kurina & Sääksjärvi, 2017 (Brazilian Amazonia), *Manota multisetosa* Jaschhof & Hippa, 2005 (Costa Rica), *Manota parva* Jaschhof & Hippa, 2005 (Colombia, Costa Rica), *Manota pisinna* Hippa & Kurina, 2013 (Brazilian Amazonia), *Manota spinosa* Jaschhof & Hippa, 2005 (Colombia) and *Manota squamulata* Jaschhof & Hippa, 2005 (Costa Rica). Distribution patterns include (1) species known only locally in Costa Rica or Colombia, (2) distributions connecting Central America to west Andes lowlands, and (3) north-west Neotropical components, extending from Central America to Brazilian Amazonia. The possible biogeographical and taxonomical context of *Manota* species with a widespread distribution is considered.

Keywords

Diptera, *Manota*, Neotropical region, new species, Sciaroidea, taxonomy

Introduction

The monophyletic subfamily Manotinae of Mycetophilidae is represented by four extant genera in the world fauna, but only *Manota* Williston (type species *Manota defecta* Williston) has almost cosmopolitan distribution, with the highest diversity in tropical areas (e.g. Kurina and Hippa 2015 and references therein). The other three genera are restricted either only to the Oriental region (in case of *Paramanota* Tuomikoski and *Promanota* Tuomikoski) or to the Oriental and Australasian regions (in case of *Eumanota* Edwards) (Søli 2002, Papp 2004, Hippa and Ševčík 2010, Hippa et al. 2005, 2016). The Oriental region could be an original area of distribution of the manotines (Jaschhof et al. 2011). *Manota* is considered to be the sister group of a clade including the other three genera (Hippa et al. 2005, Ševčík et al. 2013). Members of *Manota* have a unique habitus, including small size, yellowish to brownish coloration, and considerably reduced wing venation, which makes them easily recognizable in samples (for characteristic general facies, see, e.g., Hippa and Kurina 2012, Hippa et al. 2017).

The last 15 years revealed an explosion in the number of described *Manota* species. The number of species of the genus in the world increased from 28 (Bechev 2000) to 271 (Hippa et al. 2017). In the Neotropical region, this number moved from three species (Papavero 1978) to 67 species known to date, viz. 32 species from Peru (Hippa et al. 2017), 27 species from Costa Rica (Jaschhof and Hippa 2005), 21 species from Ecuador (Hippa and Kurina 2013), 8 species from French Guyana (Hippa and Kurina 2013), four species from Mexico (Hippa and Huerta 2009), two from Brazil (Enderlein 1911, Lane 1948), and one species from each St. Vincent, Lesser Antilles (Williston 1896), Nicaragua (Hippa and Kurina 2013) and Argentina (Hippa and Kurina 2013). Most of the species are known from their type localities only. The genus was mentioned as occurring in Colombia by Oliveira and Amorim (2016); the details on the species are being dealt with in this paper. A considerable number of species from the Atlantic Forest of Brazil are to be described soon, increasing the diversity of the genus in the region.

The aim of this study is to increase the knowledge of the genus *Manota* in the Neotropical region by describing new species and by giving new records based on material collected in Colombia, Brazilian Amazonia, and Costa Rica.

Materials and methods

The Colombian material was collected by Malaise traps within the framework of a collection project leaded by Dr. M. Sharkey (National Science Foundation Grant DEB-0205982; see also Oliveira and Amorim 2016). This project resulted in a huge amount of material, including fungus gnats, of which only a few genera have been worked to date (e.g., Oliveira and Amorim 2012, 2016, Kurina and Oliveira 2015). The Costa Rican material was collected by Malaise traps or sweeping from rainforest near the Soltis Center for Research and Education, San Isidoro. The material from Brazil comes

from Malaise traps at the Reserva Ducke, in Manaus, State of Amazonas, and at the State of Roraima, close to the border with Venezuela.

All the material was initially stored in ethyl alcohol. In most cases, the hypopygium was detached from the specimen and macerated in warm 20% potassium hydroxide (KOH). Several specimens, especially those collected in Colombia, were faded after being more than a decade in alcohol. After macerating in KOH and washing in distilled water, the hypopygium was stained with Chlorazol Black and thereafter mounted in "Euparal" between two pieces of coverslip, which allowed a study from both sides under a compound microscope. These preparations are now attached to a normal microscope slides by two strips of adhesive tape across their edges and are easily detached when needed, together to the remainder of the body, which was not macerated, but dehydrated and mounted in "Euparal" under a coverslip.

The morphological terminology follows mainly Søli et al. (2000), while the term "parasegment" is used in accordance with Jaschhof and Hippa (2005). The terminology of the hypopygium follows Hippa and Papp (2007), but the term aedeagus is used here instead of tegmen. The terminology of hypopygium is explained in Figs 1–5. The mid tibial organ is an area of tightly placed setae basoventrally on the mid tibia (Jaschhof and Jaschhof 2010). The hind tibial organ is a similar area apicoventrally on the hind tibia (Jaschhof et al. 2011). Wing length was measured from wing base to wing tip. Description of colour was made from specimens on slides under a stereomicroscope; when available, additional specimens in ethanol were used to confirm sclerite colours. In slides, medial part of the scutum, scutellum and abdominal tergites appear somewhat darker due to their curvature on slide, while they are unicolorous in ethanol preserved specimens. Illustrations were made with the aid of a drawing tube attached to a Leitz Diaplan compound microscope. The slide mounting was done under a Leica MZ16 stereomicroscope; compound microscopes Leica DM 2500 and Leica DM 6000 B were used for final identification of species.

The material is deposited in the following collections:

IAvH Alexander von Humboldt Biological Resources Research Institute, Bogota, Colombia;
IZBE Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences (formerly Institute of Zoology and Botany), Tartu, Estonia;
MNCR InBio collection, Museo Nacional de Costa Rica, San José, Costa Rica;
MZUSP Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil.

Taxonomy

Manota Williston

Manota Williston, 1896: 260. Type-species, *M. defecta* Williston (mon.).
Aphanizophleps Enderlein, 1911: 201. Type-species, *A. coxata* Enderlein (orig. des.).

References. Hippa et al. 2005 (phylogeny); Jaschhof and Hippa 2005 (identification key to Costa Rican species); Hippa and Huerta 2009 (new species from Mexico); Hippa and Kurina 2013 (new species from Ecuador, French Guyana, Nicaragua, Argentina and Peru); Oliveira and Amorim 2014 (catalogue of Neotropical Mycetophilidae); Hippa et al. 2017 (new species from Peru).

***Manota clava* sp. n.**
http://zoobank.org/7B2C94AD-B520-4D59-B86F-B9099E7FB946
Figs 1A–D, 6

Types. *Holotype.* Male, COLOMBIA, Risaralda, SFF Otún Quimbaya Cuchilla Camino, 04°43'N, 75°35'W, 2050 m, Malaise trap, 03–19.i.2003, G. López Leg. M. 3702 (on slide, IAvH).

Diagnosis. Laterotergite setose; anterior basalare non-setose; sternite 9 posteriorly broadly concave and laterally free from gonocoxa; parastylar lobe large and apically broadened; gonocoxa without a remarkable posterolateral lobe; gonostylus subrectangular, posterolaterally drawn out; two juxtagonostylar megasetae, ventral one flame-shaped and pointed, dorsal one bilobed.

Description. Male. **Colour.** Head brown, face somewhat paler. Antenna light brown, including scape and pedicel. Clypeus and mouthparts pale yellow. Thorax light brown. Legs yellowish. Wing with light brownish tinge because of microtrichia; halter brownish with blackish knob. Abdomen with tergites dark brown to blackish, sternites light brown to yellowish. All vestiture pale, yellowish or brownish, thicker setae and trichia seeming darker than finer ones. **Head.** Antennal flagellomere 4 ca. 2 times as long as wide. Palpomere 3 of maxillary palpus with apicomesial thumb-like extension, with 3 apically curved sensilla; palpomere 4 with parasegment; palpomere 5 ca. 1.1 times longer than palpomere 4. Number of strong postocular setae, 9. **Thorax.** Anepisternum with 52 setae; anterior basalare and preepisternum 2 non-setose; laterotergite with 13 setae; metepisternum with 9 setae. **Legs.** Mid and hind tibial organs absent. **Wing.** R_1 meeting C within basal half of costal margin; sclerotized part of M_2 extending to level of tip of R_1; wing length, 2.4 mm. **Hypopygium** (Fig. 1A–D). Sternite 9 ca. 2/3 as long as gonocoxa with delimited lateral margins, broadly concave posteriorly, anteriorly incised; posterior half covered with setae which are slightly stronger than adjacent ventral setae of gonocoxa, anterior half non-setose. Ventral medial margin of gonocoxa simple. Parastylar lobe large, apically broadened, club-like, with 3-5 fine posteriorly directed setae medially. No paraapodemal lobe observable. Posterolateral part of gonocoxa not drawn into a remarkable lobe but bearing many long curved setae. Dorsal medial margin of gonocoxa simple, medially bulging, posteromedially almost right-angled. Ventrally from posteromedial corner, there are two plate-like lobes on different levels: more dorsal lobe bears 6 strong apically curved setae laterally, more ventral lobe bears aggregation of fine setae at anteromedial corner and one strong seta at posteromedial corner. Two juxtagonostylar megasetae present, the more anterior

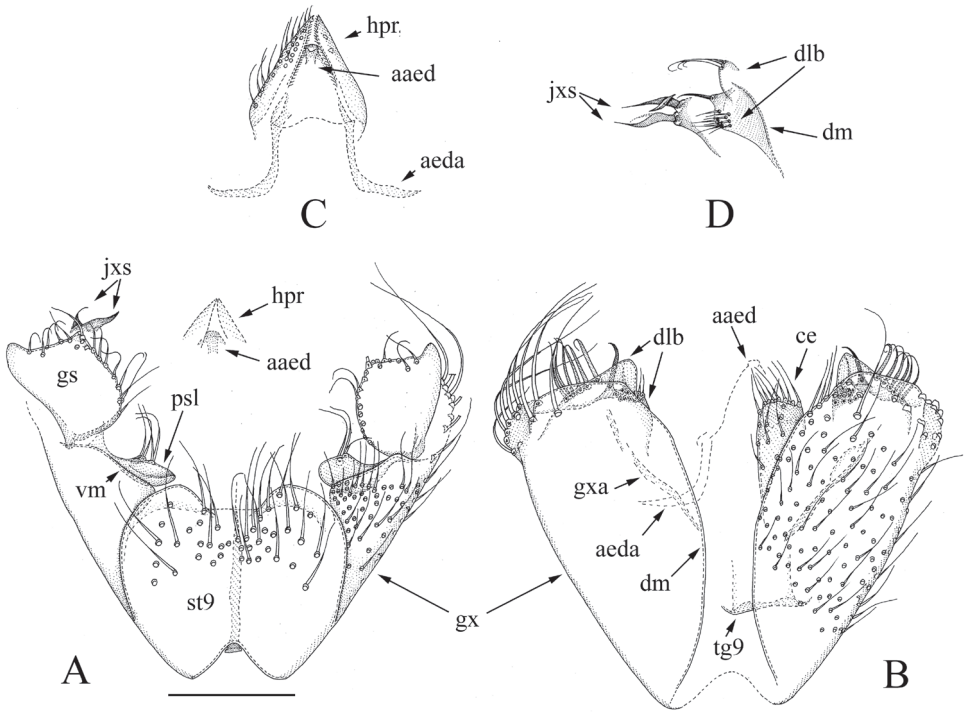

Figure 1. *Manota clava* sp. n. (holotype). **A** Hypopygium, ventral view **B** Hypopygium, dorsal view **C** Aedeagus and hypoproct, ventral view **D** Juxtagonostylar megasetae with associated parts, mediodorsal view. Scale bar 0.10 mm. Abbreviations: aaed = apex of aedeagus, aeda = aedeagal apodeme, ce = cercus, dlb = plates posteriorly at dorsal medial margin of gonocoxa, dm = dorsal medial margin of gonocoxa, gs = gonostylus, gx = gonocoxa, gxa = gonocoxal apodeme, hpr = hypoproct, jxs = juxtagonostylar megaseta, psl = parastylar lobe, st9 = sternite 9, tg9 = tergite 9, vm = ventral medial margin of gonocoxa.

and ventral one flame-shaped and pointed, with obscurely discernible seta-like branch marked by broken line in Fig. 1D, the more posterior and dorsal one bilobed, both arising from a large common basal body which is as long as megasetae. Gonostylus sub-rectangular, posterolateral corner drawn out into a rounded lobe, posterior and medial margin with short setae. Aedeagus broadly subtriangular, with lateral shoulders, the apex curved ventrally. Aedeagal apodemes directed laterad. Hypoproct extending posteriorly to apex of gonostyli, each side with ca. 25 normal setae on ventral surface. Cerci medially separated.

Female. Unknown.

Discussion. The setose laterotergite, non-setose anterior basalare, sternite 9 laterally free from gonocoxa, and gonocoxa without a remarkable posterolateral lobe group together *Manota clava* sp. n. with *M. caribica* Jaschhof & Hippa, 2005 (Costa Rica) and *M. micula* Hippa & Kurina, 2013 (Ecuador, Peru). All three species have also the sternite 9 posteriorly broadly concave and a similar aggregation of setae on plate-like lobe ventrally from dorsal medial margin of gonocoxa. Parastylar lobe is distinct be-

tween all three species: large, apically broadened with 3–5 posterior setae in *M. clava*, large, subtriangular with three posterior setae in *M. micula* and small, stout with 2–3 setae posteroapically in *M. caribica*. The gonostylus of *M. clava* is subrectangular and posterolaterally drawn out while it is oval or almost circular in case of the two other species. *Manota clava* and *M. micula* have the juxtagonostylar megasetae complex with transverse and leaf-like expansions, while they are simple and pointed in *M. caribica*.

Etymology. The specific epithet is Latin, *clava* [club or mace], referring to the prominent club-shaped parastylar lobe, and is a noun used as in apposition.

Manota multilobata sp. n.

http://zoobank.org/DF26817D-97B1-408E-B9F9-875D7F5688C9
Figs 2A–C, 6

Types. *Holotype.* Male, COLOMBIA, Valle de Cauca, PNN Farallones de Cali Cgto., La Meseta, 03°34'N, 76°40'W, 2,200 m, Malaise trap, 27. viii–10.ix.2003, S. Sania & M. Losso col., M 4570 (on slide, IAvH).

Diagnosis. Laterotergite non-setose; anterior basalare non-setose; sternite 9 posteriorly broadly and deeply concave, anterior half fused to gonocoxa; parastylar lobe transversally oblong, with *ca* 20 setae; gonocoxa drawn into a short and broad posterolateral lobe; gonostylus widening apically, somewhat sunken into gonocoxa; two juxtagonostylar megasetae, ventral one flame-shaped, dorsal one twisted; two and one apically setose finger-like lobes anteriorly and posteriorly from juxtagonostylar megasetae, respectively.

Description. Male. **Colour**. Head brown, face somewhat paler. Antenna light brown, including scape and pedicel. Clypeus and mouthparts yellowish. Thorax light brown. Legs yellowish, basal third of femur 3 infuscated. Wing with brownish tinge because of microtrichia; halter yellow with blackish knob. Abdomen with tergites brownish, sternites somewhat lighter. All vestiture pale, yellowish or brownish, thicker setae and trichia seeming darker than finer ones. **Head**. Antennal flagellomere 4 ca. 2.1 times as long as wide. Palpomere 3 of maxillary palpus with apicomesial thumb-like extension, its curved sensilla not discernible; palpomere 4 with parasegment; palpomere 5 not measurable on holotype. Number of strong postocular setae 9. **Thorax**. Anepisternum with 26 setae; anterior basalare, preepisternum 2 and laterotergite non-setose; metepisternum with 8 setae. **Legs**. Mid and hind tibial organs absent. **Wing**. R_1 meeting C within basal half of costal margin; sclerotized part of M_2 extending to level of tip of R_1; wing length, 2.5 mm. **Hypopygium** (Fig. 2A–C). Sternite 9 broad, extending to the middle of gonocoxa, anterior half fused to gonocoxa, posterior half free, posterior margin broadly and deeply concave, anterior margin shallowly and angularly incised, laterally with bare narrow area, medially setose with setae similar to the adjacent ventral setae of gonocoxa. Ventral medial margin of gonocoxa simple. Posterior margin with two long setae having prominent sockets. Parastylar lobe transversally oblong, well exposed in ventral view, bearing ca 20 setae. No paraapodemal lobe observable. Posterolateral part of gonocoxa not drawn into a remarkable lobe.

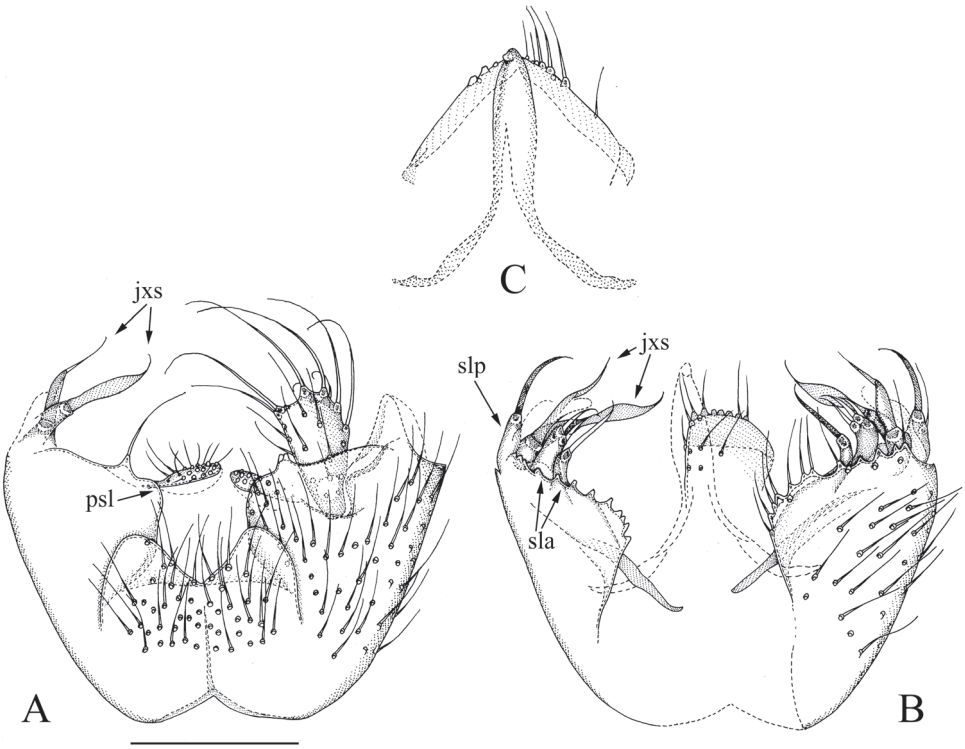

Figure 2. *Manota multilobata* sp. n. (holotype). **A** Hypopygium, ventral view **B** Hypopygium, dorsal view **C** Aedeagus and hypoproct, ventral view. Scale bar 0.10 mm. Abbreviations: jxs = juxtagonostylar megaseta, psl = parastylar lobe, sla = setigerous finger-like lobe anteriorly from juxtagonostylar megasetae, slp = setigerous finger-like lobe posteriorly from juxtagonostylar megasetae.

Dorsal medial margin of gonocoxa simple, bulging medially, contiguous with the dorsal posterior margin. Two juxtagonostylar megasetae arising from separate basal bodies, dorsal megaseta somewhat twisted, with a basal body ca. one fourth of seta's length, ventral megaseta flame-shaped, with a basal body slightly less than seta's length. Two finger-like lobes anteriorly from juxtagonostylar megasetae: more anterior lobe subequal to basal body of dorsal juxtagonostylar megaseta with one seta apically, more posterior lobe subequal to basal body of ventral juxtagonostylar seta with three setae apically. Posteriorly from the juxtagonostylar megasetae, a lobe, subequal to basal body of ventral juxtagonostylar seta, bearing one strong apical seta and one weak subapical seta. Gonostylus somewhat sunken into gonocoxa, apically widening, with 5 strong and long setae at posterior and posterolateral margins, other setosity similar to that on gonocoxa ventrally. Aedeagus elongate, narrowly subtriangular, the lateral sides slightly concave, apex curved ventrally. Hypoproct extending posteriorly to level of apex of gonostyli, each side with 6 setae on apical third ventrally. Cerci broad, medially separated.

Female. Unknown.

Discussion. *Manota multilobata* sp. n. groups together with *M. setilobata* sp. n. by having the non-setose anterior basalare, non-setose laterotergite, indistinct or short posterolateral lobes of the gonocoxa, and the megasetae and aggregations of setae at the dorsal medial margin of the gonocoxa all placed far posteriorly. Both species have the obovate gonostylus, which is somewhat sunken into the gonocoxa, and have 4–5 strong apical and subapical setae deviating from other setae, similar arrangement of small setose lobes around juxtagonostylar setae, and sternite 9 basally fused with the gonocoxa. The species differ as follows: 1) in *M. multilobata* there are two finger-like lobes close together anteriorly from the juxtagonostylar megaseta, the more anterior one with one, the more posterior one with three strong setae (in *M. setilobata* there is a plate-like lobe anteriorly bearing one seta widely separated from a posterior group of several setae), 2) in *M. multilobata* posteriorly from the juxtagonostylar megasetae there is a finger-like lobe with one strong and one weak seta (in *M. setilobata* a flat lobe with numerous fine setae), 3) in *M. multilobata* the gonocoxa is drawn into a short and broad posterolateral lobe (in *M. setilobata* it is drawn into a short and narrow lobe), and 4) in *M. multilobata* sternite 9 has the posterior margin broadly v-shaped incised (in *M. setilobata* there is narrow and deep medial cleft).

Etymology. The specific epithet is Latin, *multilobata* [many-lobed], referring to the setigerous lobes dorsally on the gonostylus (adjective).

Manota perplexa sp. n.

http://zoobank.org/7FB344E8-9876-4EDD-8253-AD0167D702C8
Figs 3A–D, 6

Types. *Holotype.* Male, COSTA RICA, San Isidro de las Peñas Blancas, Texas A&M Soltis Center, Malaise trap, 400 m, 10°23'00"N, 84°36'58"W, 20.iv–26. v.2010, Wendy Porras col. (on slide, MNCR).

Diagnosis. Laterotergite non-setose; anterior basalare non-setose; sternite 9 laterally entirely fused to gonocoxa, posterior margin free with protruding posterolateral corners; parastylar lobe indistinct; gonocoxa with a large plate-like lobe bearing four simple megasetae medioventrally from dorsal medial margin and anteriorly from the juxtagonostylar setae; gnostylus subtriangular, with prominent lateral angle; two juxtagonostylar megasetae, anterior one simple and pointed, posterior one bifurcated.

Description. Male. **Colour.** Head brown, face somewhat paler. Antenna light brown, scape, pedicel and two basal flagellomeres slightly paler. Clypeus and mouthparts yellowish. Thorax light brown. Legs yellowish. Wing with light brownish tinge because of microtrichia; haltere yellow with brown knob. Abdomen with tergites dark brown to blackish, sternites yellowish. All vestiture pale, yellowish or brownish, thicker setae and trichia seeming darker than the finer ones. **Head.** Antennal flagellomere 4 ca. 1.8 times as long as wide. Palpomere 3 of maxillary palpus with apicomesial thumb-like extension, with three apically curved sensilla; palpomere 4 with parasegment; palpomere 5 ca. as long as palpomere 4. Nine strong postocular setae. **Thorax.**

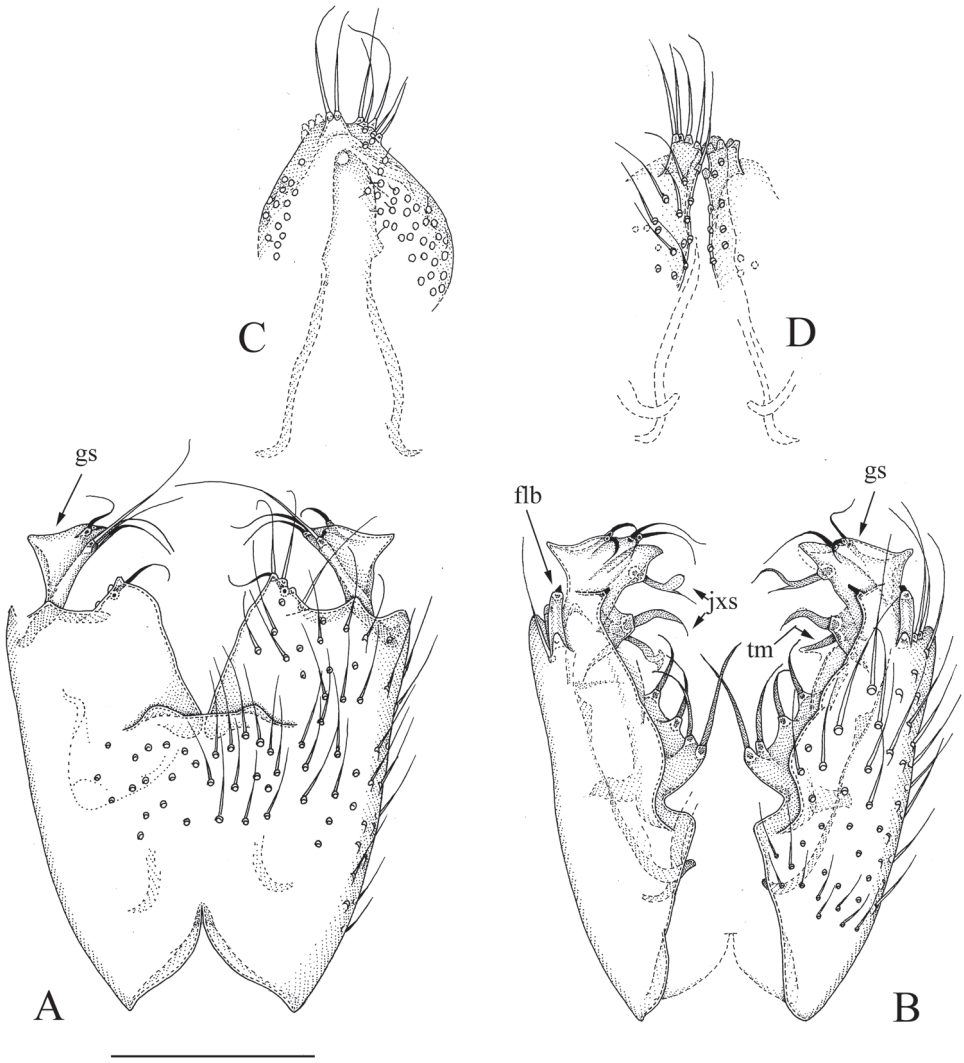

Figure 3. *Manota perplexa* sp. n. (holotype). **A** Hypopygium, ventral view **B** Hypopygium dorsal view **C** Aedeagus and hypoproct, ventral view **D** Cerci with associated parts, dorsal view. Scale bar 0.10 mm. Abbreviations: flb = finger-like lobe, gs = gonostylus, jxs = juxtagonostylar megasetae, tm = twisted megaseta.

Anepisternum with 46 setae; anterior basalare, preepisternum 2 and laterotergite nonsetose; metepisternum with 15 setae on anterior part. **Legs.** Mid and hind tibial organs absent. **Wing.** R_1 meeting C within basal half of costal margin; sclerotized part of M_2 extending to level of tip of R_1; wing length 1.6 mm. **Hypopygium** (Fig. 3A–D). Sternite 9 laterally entirely fused to gonocoxa, posterior margin free with protruding posterolateral corners; setae similar to adjacent ventral setae of gonocoxa. Ventral medial margin of gonocoxa simple, posteromedial corner drawn into a lobe, posterolateral part of gonocoxa not drawn into a lobe. Parastylar lobe indistinct, apparently

represented by one seta by ventral medial margin of gonocoxa. No paraapodemal lobe observable. Dorsal medial margin of gonocoxa with a transverse shallow incision medially. A large plate-like lobe bearing four simple megasetae medioventrally from dorsal medial margin and anteriorly from the juxtagonostylar setae, anteriormost with its own basal body ca. 1/3 longer than others. Two juxtagonostylar megasetae present, more anterior one pointed, slightly curved simple megaseta, arising from a basal body which is shorter than the megaseta, more posterior one bifurcate, one of the branches whip-like, the other flat and dilated, arising subapically from a very prominent basal body which is longer than the megaseta itself and ca. as long as gonostylus. Ventrally from the more anterior juxtagonostylar megaseta there is an apically twisted megaseta. Dorsally at the posterior margin of gonocoxa a long finger-like lobe apically bearing a seta (in holotype the seta is broken on both sides). Gonostylus subtriangular, with prominent lateral angle, with one very strong and 2–3 weaker setae at posteromedial corner, and with one curved strong seta at posterior margin. Aedeagus narrowly subtriangular, the apex curved ventrally, otherwise the details not visible in the mount. Hypoproct posteriorly extending to the base of gonostyli, with ca. 35 ventral setae on each side. Cerci medially separated, with their apical parts narrowed.

Female. Unknown.

Discussion. In the key to Costa Rican species by Jaschhof and Hippa (2005), *M. perplexa* sp. n. would run into couplet 13, because of non-setose laterotergite and absence of posterolateral lobes of gonocoxa. Due to the structure of male genitalia, *M. perplexa* is clearly different from the two included species, viz. *M multisetosa* Jaschhof & Hippa and *M. tapantiensis* Jaschhof & Hippa. *Manota perplexa* is distinguished e.g. by the lack of well-developed parastylar lobe and the presence of complicated pattern of lobes and strong setae dorsally at the medial margin of gonocoxa as well as by laterally with the gonocoxa fused sternite 9. The complex dorsomedial armature of gonocoxa is unique and easily distinguishes *M. perplexa* from any other described *Manota* species.

Etymology. The specific epithet is Latin, *perplexa* [confused, complicated or ambiguous], referring to the very complex gonostylus and its juxtapositional structures (adjective).

Manota setilobata **sp. n.**

http://zoobank.org/23635692-B4C3-431D-859D-A382FBD9B89D
Figs 4A–F, 6

Types. *Holotype.* Male, COLOMBIA, Risaralda, SFF Otún Quimbaya Cuchilla Camino, 04°43'N, 75°35'W, 2050 m, Malaise trap, 08–24.v.2003, G. López Leg. M. 3673 (on slide, IAvH). *Paratype.* Male, same as holotype except 04–17.ii.2003, M. 3694 (on slide, IAvH).

Diagnosis. Laterotergite non-setose; anterior basalare non-setose; sternite 9 posteriorly and anteriorly deeply incised, posterior third laterally free; parastylar lobe indistinct; posterolateral part of gonocoxa drawn into a narrow lobe; dorsomedial margin of

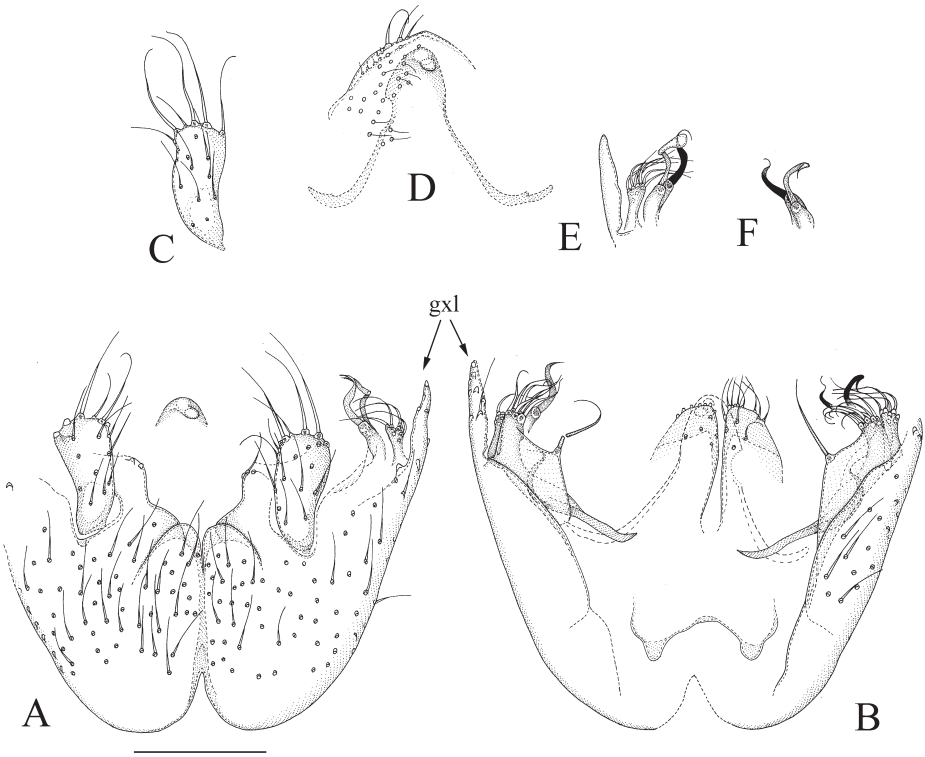

Figure 4. *Manota setilobata* sp. n. (**A, B, D, E** and **F** holotype, **C** paratype). **A** Hypopygium, ventral view **B** Hypopygium, dorsal view **C** Right gonostylus, ventral view **D** Aedeagus and hypoproct, ventral view **E** Left side juxtagonostylar megasetae with associated parts, dorsal view **F** Right side juxtagonostylar megasetae. Scale bar 0.10 mm. Abbreviation: gxl = posterolateral lobe of gonocoxa.

gonocoxa with a large plate-like lobe bearing one strong seta at posteromedial corner; gonostylus elongated subquadrangular, slightly sunken into gonocoxa; two juxtagonostylar megasetae, both twisted, the more dorsal one apically flattened and dilated; posteriorly from the juxtagonostylar megasetae a narrow flat apically setose lobe.

Description. Male. **Colour.** Head brown, face somewhat paler. Antenna light brown, including scape and pedicel. Clypeus and mouthparts yellowish. Thorax light brown. Legs yellowish. Wing with light brownish tinge because of microtrichia; haltere yellow with blackish knob. Abdomen with tergites dark brown to blackish, sternites light brown to yellowish. All vestiture pale, yellowish or brownish, thicker setae and trichia seeming darker than finer ones. **Head.** Antennal flagellomere 4 ca. 2.3 times as long as wide. Palpomere 3 of maxillary palpus with apicomesial thumb-like extension, with three apically curved sensilla; palpomere 4 with parasegment; palpomere 5 missing in both known specimens. Number of strong postocular setae, 10. **Thorax.** Anepisternum with 29–33 setae; anterior basalare, preepisternum 2 and laterotergite non-setose; metepisternum with 3–5 setae. **Legs.** Mid and hind tibial organs absent.

Wing. R_1 meeting C within basal half of costal margin; sclerotized part of M_2 extending to level of tip of R_1; wing length, 2.4 mm. **Hypopygium** (Fig. 4A–F). Sternite 9 ca. 2/3 as long as gonocoxa, anterior 2/3 laterally fused to gonocoxa, posterior 1/3 free, posterior and anterior margins with deep incisions which separate the sclerite almost into two halves, covered with setae similar to adjacent ventral setae of gonocoxa. Ventral medial margin of gonocoxa simple. Parastylar lobe not identifiable with certainty, possible fused with gonocoxa and in Fig. 4A comprising the part visible between the posterior margin of sternite 9 and the gonostylus. No paraapodemal lobe observable. Posterolateral part of gonocoxa drawn into a narrow lobe. Dorsomedial margin of gonocoxa simple. In a more ventral level, a large plate-like lobe bearing one strong seta at posteromedial corner. Two juxtagonostylar megasetae present, both twisted, the more dorsal one apically flattened and dilated, both arising from basally fused basal bodies which are as long as the megasetae. Dorsally from juxtagonostylar megasetae, a flat apically setose lobe, connected with a thin, one seta bearing plate-like lobe anteriorly from it. Posteriorly from the juxtagonostylar megasetae a narrow flat apically setose lobe. Gonostylus elongated subquadrangular, slightly sunken into gonocoxa, with 4–5 strong and long setae at posterior margins, other setosity similar to that on gonocoxa ventrally, dorsal side non-setose. Aedeagus subtriangular, lateral sides slightly concave, apex curved ventrally. Hypoproct extending posteriorly over apex of gonostyli, each side with 4–5 strong setae apically and ca. 30 fine setae on ventral surface. Cerci medially separated.

Female. Unknown.

Discussion. *Manota setilobata* sp. n. resembles *M. multilobata* sp. n. For a more detailed discussion on distinguishing characters, see above.

Etymology. The specific epithet is Latin, *setilobata* [with seta-bearing lobes], referring to the apically setose lobes dorsally on the gonocoxa (adjective).

Manota subaristata sp. n.

http://zoobank.org/E5250B84-994F-4B05-B780-ACE895044332
Figs 5A–C, 6

Types. *Holotype.* Male, COLOMBIA, Valle de Cauca, PNN Farallones de Cali Cgto., La Meseta, 03°34'N, 76°40'W, 2200 m, Malaise trap, 27.viii–10.ix.2003, S. Sania & M. Losso col., M 4570 (on slide, IAvH). *Paratypes.* 2 males, same as holotype (on slides, MZUSP); 1 male, same as holotype except 24.xii.2003–27.i.2004, M 4564 (on slide, IAvH); 1 male, same as holotype except 27.i–10.ii.2004, M 4563 (on slide, IAvH); 1 male, same as holotype except 10–25.ii.2004, M 4555 (on slide, IZBE); 1 male, COLOMBIA, Huilla, PNN Cueva de los Guácharos, Alto el Mirador, 01°38'N, 76°06'W, 1980 m, Malaise trap, 6-21.iv.2002, J. Fonseca col., M 3127 (on slide, IAvH); 1 male, COLOMBIA, Cauca, PNN Gorgona, El Saman, 02°58'N, 78°11'W, 5 m, Malaise trap, 28.ix–22.x.2001, H. Torres col., M 2457 (on slide, IZBE); 1 male, same as previous except 06–23.iii.2002, R. Duque col., M 3088 (on slide, IAvH); 1 male, COLOMBIA,

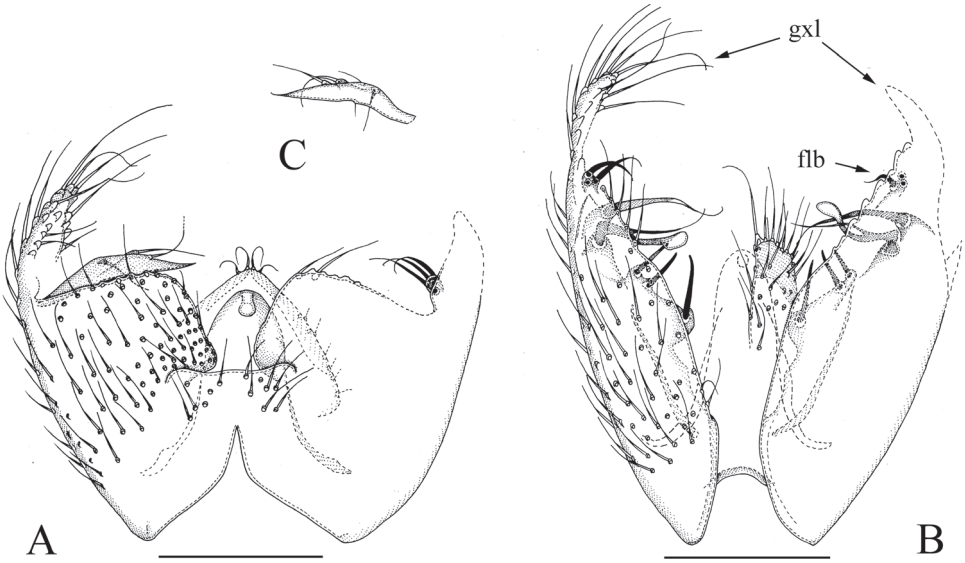

Figure 5. *Manota subaristata* sp. n. (**A** and **C** holotype **B** paratype). **A** Hypopygium, ventral view **B** Hypopygium, dorsal view **C** Right gonostylus, ventral view. Scale bar 0.10 mm. Abbreviations: flb = finger-like setigerous lobe, gxl = posterolateral lobe of gonocoxa.

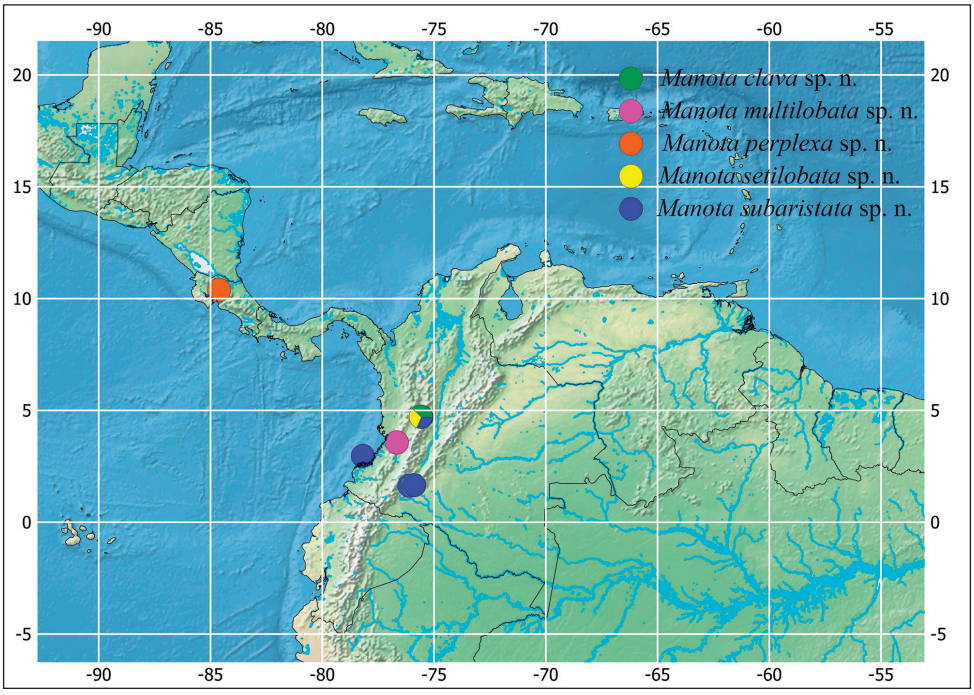

Figure 6. Distribution of the new species of *Manota*.

Risaralda, SFF Otún Quimbaya, El Molinillo, 04°43'N, 75°34'W, 2200 m, Malaise trap, 17.ii–4.iii.2003, G. López col., M 3696 (on slide, IAvH).

Diagnosis. Laterotergite non-setose; anterior basalare non-setose; sternite 9 laterally fused to gonocoxa except for posterior fifth; parastylar lobe indistinct; posterolateral part of gonocoxa drawn into a lobe; dorsomedial margin of gonocoxa with a plate-like lobe bearing one anterior and two posterior simple megasetae; gonostylus in dorsal and ventral view narrow, crescent-shaped; two juxtagonostylar megasetae, more dorsal one subbasally geniculate and apically bifurcate, more ventral one simple, slightly flattened whip-like; posteriorly from juxtagonostylar megasetae a short finger-like lobe with 3–4 strong setae.

Description. Male. **Colour**. Head brown, face somewhat paler. Antenna light brown, including scape and pedicel. Clypeus and mouthparts yellowish. Thorax brown. Legs yellowish. Wing with brownish tinge because of microtrichia; halter yellow with blackish knob. Abdomen with tergites brown to dark brown, sternites somewhat lighter. All vestiture pale, yellowish or brownish, thicker setae and trichia seeming darker than finer ones. **Head**. Antennal flagellomere 4 ca. 1.6–1.7 times as long as wide. Palpomere 3 of maxillary palpus with apicomesial thumb-like extension, with 3 apically curved sensilla; palpomere 4 with parasegment; palpomere 5 ca. 1.4–1.5 times longer than palpomere 4. Number of strong postocular setae 9–11. **Thorax**. Anepisternum with 42–47 setae; anterior basalare, preepisternum 2 and laterotergite non-setose; metepisternum with 8–14 setae. **Legs**. Mid and hind tibial organs absent. **Wing**. R_1 meeting C within basal half of costal margin; sclerotized part of M_2 extending to level of tip of R_1; wing length, 1.9–2.3 mm. **Hypopygium** (Fig. 5A–C). Sternite 9 laterally fused to gonocoxa except for posterior 1/5, extending to middle of gonocoxa, posterior margin slightly concave, anterior margin deeply incised. Posterior 1/3 of sternite 9 setose, otherwise non-setose, setae similar to adjacent ventral setae of gonocoxa. Medioventral margin of gonocoxa roundly angled. Parastylar not identifiable, apparently membranous and covered by gonocoxa, possibly with one seta visible at the gonocoxal margin in Fig. 5A. No paraapodemal lobe observable. Posterolateral part of gonocoxa drawn into a lobe ca. 1/3 length of gonocoxa. Mediodorsal margin of gonocoxa simple. A plate-like lobe with one anterior and two posterior simple megasetae medioventrally from dorsal medial margin and anteriorly from juxtagonostylar setae. Two juxtagonostylar megasetae present: more dorsal megaseta subbasally geniculate, apical part bifurcate, one of the branches whip-like, the other apically flattened and dilated; the more ventral megaseta simple, slightly flattened whip-like. Posteriorly from juxtagonostylar megasetae a short finger-like lobe with 3–4 strong setae. Gonostylus in dorsal and ventral view narrow, crescent-shaped (in some slides apically pointed), with 3–4 setae dorsally near lateral margin and 1 ventral seta near the medial margin. Aedeagus elongate subtriangular, without lateral shoulders, apex curved ventrally. Hypoproct extending posteriorly to level of base of gonostyli or slightly over, with ca. 25 ventral setae on each side. Cerci medially separate, apically slightly widened.

Female. Unknown.

Discussion. *Manota subaristata* sp. n. is similar to *M. aristata* Hippa & Kurina, 2013 in having the dorsal juxtagonostylar megaseta with a long whip-like branch. *Manota subaristata*, however, has the megaseta subbasally geniculate, arising from a separate basal body, while it is basally straight and arising from apical half of the common basal body with the ventral juxtagonostylar seta in *M. aristata*. *Manota subaristata* has 3–4 strong setae on a finger-like setose lobe posteriorly from the juxtagonostylar megaseta, which are absent in *M. aristata*. By the latter character, the species resembles *M. acutisty*lus Jaschhof & Hippa, 2005, but the megasetae at the dorsal medial margin of gonocoxa in *M. subaristata* are longer and there are two of them in the posterior group, not three as in *M. acutistylus* (see also the discussion for *M. aristata* in Hippa and Kurina 2013: 109). In *M. subaristata*, the juxtagonostylar megasetae are subequal in length, while *M. acutistylus* has the dorsal juxtagonostylar megaseta remarkably shorter, ca. half of the length of ventral one. All these three species have the gonostylus apically tapering in dorsal and ventral view, and sternite 9 largely fused to the gonocoxa, and may compose together a small clade within the Neotropical diversity of the genus.

Etymology. The specific epithet is Latin, formed from the specific epithet of *M. aristata* by the prefix *sub-* [somewhat], as a reference to the similarity of the two species (adjective).

New records

Manota acuminata Jaschhof & Hippa, 2005

Studied material. COSTA RICA. 3 males, San Isidro de las Peñas Blancas, Texas A&M Soltis Center, Malaise trap, 400 m, 10°23'00"N, 84°36'58"W, 20.iv–26.v.2010, Wendy Porras col. (on slides, 1 male MZUSP, 2 males MNCR).

Remarks. The species was earlier known from Costa Rica (Jaschhof and Hippa 2005), Ecuador (Hippa and Kurina 2013) and Peru (Hippa et al. 2017), hence widespread at the north-western corner of South America and Central America.

Manota arenalensis Jaschhof & Hippa, 2005

Studied material. COSTA RICA. 1 male, San Isidro de las Peñas Blancas, Texas A&M Soltis Center, Sweeping, 420 m, 10°23'00"N, 84°36'58"W, 13–18.viii.2010, D. Ament col. (on slide, MZUSP); 2 males, San Isidro de las Peñas Blancas, Texas A&M Soltis Center, Malaise trap, 400 m, 10°23'00"N; 84°36'58"W, 20.iv–26.v.2010, Wendy Porras col. (on slides, MNCR).

Remarks. *Manota arenalensis* was earlier known only from Costa Rica (Jaschhof and Hippa 2005).

Manota corcovado Jaschhof & Hippa, 2005

Studied material. COSTA RICA. 1 male, San Isidro de las Peñas Blancas, Texas A&M Soltis Center, Sweeping, 420 m, 10°23'00"N, 84°36'58"W, 13–18.viii.2010, D. Ament col. (on slide, MNCR).

Remarks. The terminalia of the specimen studied here slightly differs from those figured by Jaschhof and Hippa (2005: fig. 16): three internal megasetae on the gonocoxa subapically (= position IV by Jaschhof and Hippa 2005) are more smoothly outlined, the gonostylus is slightly wider and the apical re-curved seta on gonostylus is pointed instead of being blunt. These differences are here considered to be within intraspecific variation or are differently exposed due to different position at the slide-mounting. The large posterolateral lobes of the gonocoxa, the number and arrangement of megasetae at ventromedial margin of the gonocoxa, the shape of juxtagonostylar megasetae and sternite 9 are identical to the specimens of the original description of *M. corcovado*. The species was earlier known only from Costa Rica (Jaschhof and Hippa 2005).

Manota costaricensis Jaschhof & Hippa, 2005

Studied material. COSTA RICA. 1 male, San Isidro de las Peñas Blancas, Texas A&M Soltis Center, Malaise trap, 420 m, 10°23'00"N, 84°36'58"W, 15.vi–10.vii.2010, Wendy Porras col. (on slide, MNCR).

Remarks. The species is known only from Costa Rica (Jaschhof and Hippa 2005).

Manota diversiseta Jaschhof & Hippa, 2005

Studied material. COLOMBIA. 1 male, Amazonas, PNN Amacayacu, Matamata, 03°41'N, 70°15'W, 150 m, Sweeping, 23.x.2000, A. Parente col., M 3552 (on slide, IAvH); 1 male, Vaupés, Estación Biológica Mosiro-Itajura (Caparú), Igapo, 01°04'S 69°31'W, 60 m, Malaise trap, 25.ii–04.iii.2003, J. Pinzón Leg. M 3627 (on slide, MZUSP). BRAZIL. 1 male, State of Amazonas, Manaus, Reserva Ducke, Igarapé Ipiranga, 2°53'S, 59°58'W, 31.xii.2002, Malaise trap, J. Vidal col. (on slide, MZUSP). COSTA RICA. 3 males, San Isidro de las Peñas Blancas, Texas A&M Soltis Center, Malaise trap, 400 m, 10°23'00" N, 84°36'58" W, 20.iv–26.v.2010, Wendy Porras col. (in alcohol, MNCR); 6 males, same data as previous except 15.vi–10.vii.2010 (4 in alcohol, MZUSP; 2 on slides, MNCR and MZUSP); 1 male, same data as previous except sweeping, 13–18.viii.2010, D. Ament col. (on slide, IZBE).

Remarks. Having been described from Costa Rica (Jaschhof and Hippa 2005), the species has subsequently been recorded from Ecuador, French Guyana (Hippa and Kurina 2013) and Peru (Hippa et al. 2017).

Manota minutula Hippa, Kurina & Sääksjärvi, 2017

Studied material. BRAZIL. 1 male, State of Amazonas, Manaus, Reserva Ducke, Igarapé Barro Branco, 2°59'30"S, 59°57'25"W, 12–22.vii.2004, Malaise trap, A. Henriques col. (on slide, MZUSP).

Remarks. The species was earlier known only from the Iquitos area in Peru (Hippa et al. 2017). We have not been able to see much material from Manaus and this is the only species identified from the state of Amazonas, a species shared with other areas of the Amazon Basin.

Manota multisetosa Jaschhof & Hippa, 2005

Studied material. COSTA RICA. 1 male, San Isidro de las Peñas Blancas, Texas A&M Soltis Center, Malaise trap, 420 m, 10°23'00"N, 84°36'58"W, 15.vi–10.vii.2010, Wendy Porras col. (on slide, MNCR).

Remarks. *Manota multisetosa* was earlier known only from Costa Rica (Jaschhof and Hippa 2005) and Ecuador (Hippa and Kurina 2013).

Manota parva Jaschhof & Hippa, 2005

Studied material. COLOMBIA. 2 males, Chocó, PNN Utría Boroboro, 06°01'S 77°20'W, 10 m, Malaise trap, 01–05.vii.2000, B. Brown Leg. M 3310 (on slide, 1 male IAvH, 1 male MZUSP); 1 male, Risaralda, SFF Otún Quimbaya El Molinillo, 04°43'N, 75°34'W, 2200 m, Malaise trap, 03–14.i.2003, G. López Leg. M. 3701 (on slide, IZBE); 1 male, same data as previous except 17.ii–04.iii.2003, M. 3696 (on slide, IZBE); 1 male, Cauca, PNN Gorgona, El Saman, 02°58'N, 78°11'W, 5 m, Malaise trap, 11.xi.2001–18.i.2002, H. Torres col., M 2791 (on slide, IAvH); 1 male, Nariño, R.N. La Planada, Parcela Olga, 01°15'N, 78°15'W, 1,850 m, Malaise trap, 16.vii–02.ix.2001, G. Oliva col., M 665 (on slide, MZUSP). COSTA RICA. 3 males, San Isidro de las Peñas Blancas, Texas A&M Soltis Center, Malaise trap, 420 m, 10°23'00"N, 84°36'58"W, 15.vi–10.vii.2010, Wendy Porras col. (on slides, 2 males MNCR, 1 male MZUSP,); 3 males, same data as previous except 20.iv–26.v.2010 (on slides, MNCR); 2 males, same data as previous except 13–20.iv.2010 (on slides, MZUSP); 1 male, same data as previous except sweeping, 18.viii.2010 (on slide, MZUSP).

Remarks. *Manota parva* was earlier known only from Costa Rica (Jaschhof and Hippa 2005) and Ecuador (Hippa and Kurina 2013). The additional records make it one of the widespread species at the north-west corner of South America and Central America.

Manota pisinna Hippa & Kurina, 2013

Studied material. BRAZIL. 1 male, State of Roraima, Caracarai (Vila Caicubi, Trilhada do Bacaba), 00°58'36.5"S, 62°06'08.7"W, Malaise trap #2, 10.ix.2011, Biffi, G. & Prado, L.R. cols. (on slide, MZUSP)

Remarks. Having been described from French Guyana (Hippa and Kurina 2013), the species has subsequently been recorded from Peru (Hippa et al. 2017). Without the Peruvian record, the species would represent a typical Guyana Shield distribution. However, its presence in Iquitos makes it probably another widespread species at least in north-west South America.

Manota spinosa Jaschhof & Hippa, 2005

Studied material. COLOMBIA. 1 male, Vaupés, Estación Biológica Mosiro-Itajura (Caparú), Igapo, 01°04'S 69°31'W, 60 m, Malaise trap, 17–24.xi.2003, J. Pinzón Leg. M 4434 (in alcohol, IAvH); 1 male, same data as previous except 24.xi–01.xii.2002, M 4437 (on slide, MZUSP).

Remarks. The species was earlier known from Costa Rica (Jaschhof and Hippa 2005) and Peru (Hippa et al. 2017). This distribution is the same of that of *M. parva*, *M. acuminata*, *M. diversiseta*, and probably *M. squamulata*.

Manota squamulata Jaschhof & Hippa, 2005

Studied material. COSTA RICA. 1 male, San Isidro de las Peñas Blancas, Texas A&M Soltis Center, Sweeping, 420 m, 10°23'00"N, 84°36'58"W, 13–18. viii.2010, D. Ament col. (on slide, MNCR).

Remarks. Having been described from Costa Rica (Jaschhof and Hippa 2005), the species has subsequently been recorded from Ecuador (Hippa and Kurina 2013).

Discussion

A distribution map of the species described in this paper is depicted in Fig. 6, while maps in Figs 7–8 sum up the distribution of other recorded species including earlier data from Jaschhof and Hippa (2005), Hippa and Kurina (2013), and Hippa et al. (2017). Among the new species, only *M. subaristata*, sp. n. is known from more than one locality, all of them in Colombia. The other four new species are known only from the type locality. The species previously described accumulate more records that suggest some distribution patterns.

There are general patterns known for the Neotropical region (Amorim and Pires 1996, Amorim 2009) in which the fauna of north-western South America, including

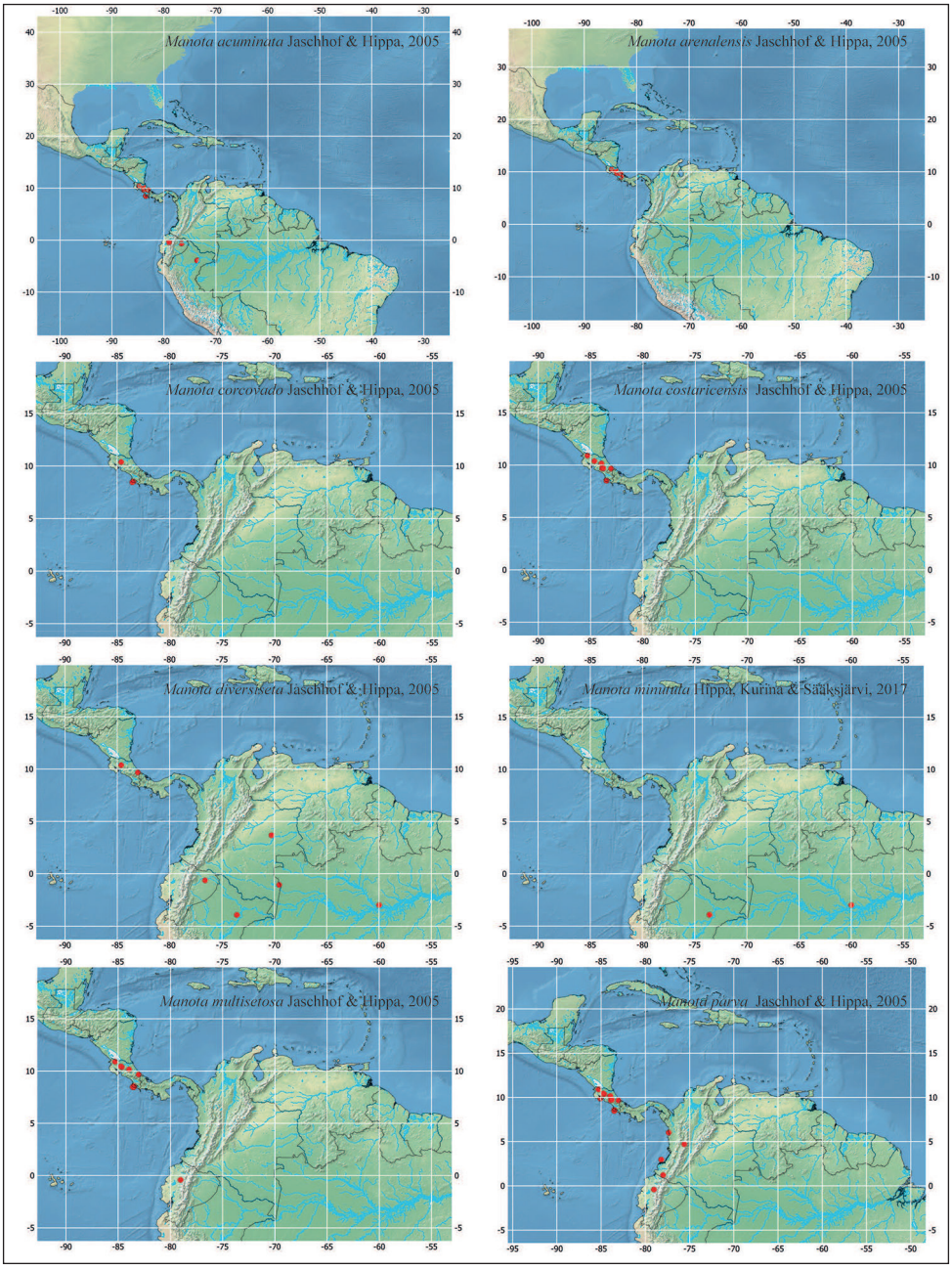

Figure 7. Distribution of described *Manota* species recorded in this paper.

Amazon basin elements, connects to that of Central America. For some groups, as e.g. monkeys and some sciarids, as *Rhynchosciara* (Amorim and Pires 1996), the patterns refer to species restricted to smaller areas in lowlands on both sides of the Andes, Central America and Mexico, as well as in the Brazilian Amazon.

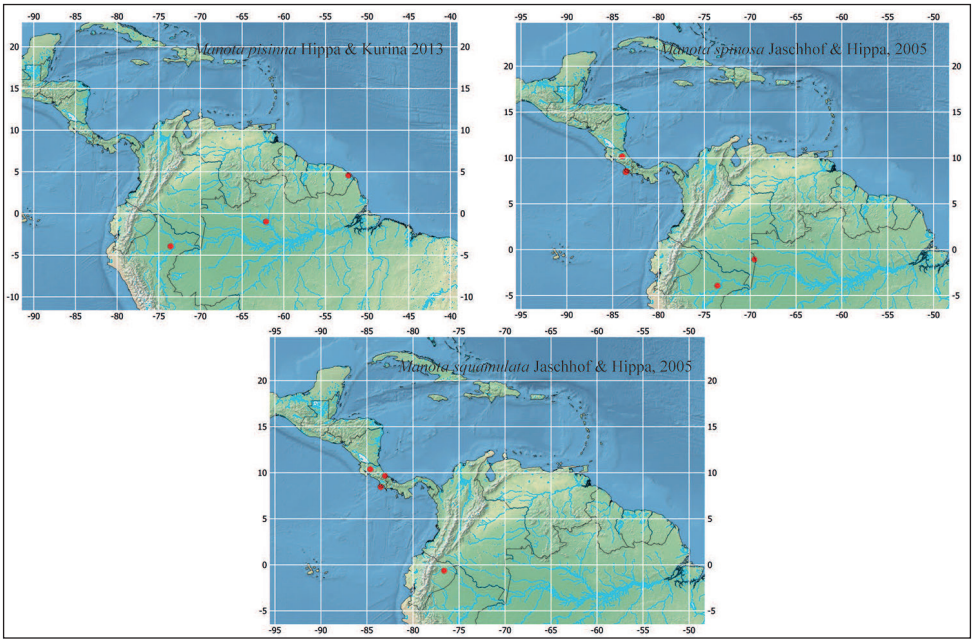

Figure 8. Distribution of described *Manota* species recorded in this paper.

The observed *Manota* distribution patterns show individual species with a considerably wide distribution, which fit in this larger pattern—named as North-west Neotropical (Amorim 2009). In some cases, the species distribution is slightly more restricted and connects populations of lowlands in the Chocó region of Colombia, west to the Andes, to populations in Central America. This is a quite well-known pattern and in the genus *Manota* it is the case of *M. multisetosa* and *M. parva*. Future collections may show that this is either a real pattern or that these species actually have wider distributions and they just were still not found in other parts of South America. In other cases, species as *M. acuminata*, *M. diversiseta*, *M. spinosa*, and *M. squamulata*, present in Costa Rica, are also found east of the Andes, including Iquitos, at the west of the Amazon basin. This fits into an important biogeographical component, a triangular area delimited by the Andes, the Solimões river at the north, and Madeira-Mamoré rivers at the southeast. Some of the above-mentioned patterns can be discerned also in other groups like phorids of the genera *Apocephalus* Coquillett and *Dohrniphora* Dahl (Brown 2002, Brown and Kung 2007).

Although the patterns sometimes are obvious, explanations can be more complex. Nominal species distributions that enclose areas of different endemism may correspond to: (1) secondary expansion of younger species with prior local distribution; (2) lack of response of older species to barriers that affected the younger groups; or (3) clades with undetected, cryptic species. This cannot be answered for the *Manota* species in question. A phylogeographic study would be useful to verify whether populations of these widespread species at the extremes of their distribution are beyond the threshold of genetic differentiation, often used to recognized separate species. This kind of problem

of insect species is hard to distinguish using only morphological features, as they can involve also "hidden" molecular divergence. This aspect has been addressed in a number of recent papers (e.g. Laamanen et al. 2003, Meier et al. 2006, Bickford et al. 2007, Tan et al. 2010, Rohner et al. 2014). If these taxonomical entities in *Manota* presently called species actually correspond to clades of more local species, the genus would show even more intensely its condition of an open-ended taxon (Bickel 2009). This would put *Manota* even closer to *Megaselia* as one of the most diverse and taxonomically complex genera in flies.

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RESEARCH ARTICLE

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Review of the genus *Roeslerstammia*, with a new species from China (Lepidoptera, Roeslerstammiidae)

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Abstract

The new species *Roeslerstammia tianpingshana* **sp. n.** is described from Hunan, China as the first record of the genus in the country. Examination of two enigmatic Indian species, *R. metaplastica* Meyrick, 1921 and *R. hemiadelpha* Meyrick, 1922, revealed that the latter is a synonym of the former. The male and female genitalia of *R. metaplastica* are described and illustrated for the first time. A checklist for the genus is given.

Keywords

Genitalia, Hunan, India, morphology, new species, taxonomy

Introduction

The family Roeslerstammiidae (= Amphitheridae) includes 57 species, mostly Australian, with one genus (*Roeslerstammia* Zeller, 1839) extending from Europe to Japan, and several genera (e.g., *Agriothera* Meyrick, 1907 and *Telethera* Meyrick, 1913) in the Oriental tropics (Heppner 2008). Two species of *Agriothera* were recorded from mainland China (Huang et al. 2008). The genus *Roeslerstammia* is widespread through the Palaearctic region and Kyrki (1983) indicated seven species in a tentative checklist. However, three species have been synonymized, so that currently only four species, *R. erxlebella* (Fabricius, 1787) and *R. pronubella* ([Denis & Schiffermüller], 1775) from

Europe to Japan, and *R. metaplastica* Meyrick, 1921 and *R. hemiadelpha* Meyrick, 1922 from India are included in the genus (Heppner 2005). Until now no species of the genus *Roeslerstammia* have been recorded from China.

In May, 2009, an unknown species of *Roeslerstammia* was collected at Tianpingshan, in the northern part of Hunan Province, China. As a result of examination of morphological characters such as the wing markings, wing venation, and genitalia, it was concluded that it was a new species of *Roeslerstammia*, and it is described here. In addition, two enigmatic species of *Roeslerstammia* from India (Punjab) described by Meyrick, which have never been examined since the original descriptions, are also investigated in order to clarify their identities on the basis of examination of the genitalia.

Materials and methods

Field surveys (light trap) were conducted in Tianpingshan (1,500 m), Badagongshan National Nature Reserve, Hunan Province, China on May 26–27, 2009 and on August 12–14, 2014. As for the two Indian species of the genus *Roeslerstammia*, syntype specimens deposited in the collection of the Natural History Museum (BMNH) were examined. Interocular index (= vertical eye diameter/inteocular distance) of Davis (1975) was calculated for description of eye size. Wing venations were examined after scales were removed and wings were stained with acetocarmine and embedded in Canada balsam on slide. Male and female genitalia were examined after the abdomen was macerated for about 5 minutes in 10% KOH heated in a boiling water bath. SEM photographs of the head were taken using HITACHI SU1510.

Abbreviations

BMNH The Natural History Museum, London.
ELKU Entomological laboratory, Faculty of Agriculture, Kyushu University, Fukuoka.
HUNAU Hunan Agricultural University, Changsha.

Taxonomy

***Roeslerstammia metaplastica* Meyrick**
Figs 1–3

Roeslerstammia metaplastica Meyrick, 1921: 439, lectotype here designated; Kyrki 1983: 322; Heppner 2005: 27.
Roeslerstammia hemiadelpha Meyrick, 1922: 553, lectotype here designated; Kyrki 1983: 322. **syn. n.**
Roeslerstammia hemidelpha (!): Heppner 2005: 26. Misspelling.

Type material. Lectotype ♂ (here designated), "Murree [Hills, Punjab] / 7500 ft/ June 18 / Dutt Coll", "Presented by / R.L.E.Ford. / B.M.1949–487.", "*Roeslerstammia metaplastica* Meyrick / det.T.B.Flecher", "♂" (Fig.1A, B), in BMNH. B.M. Genitalia slide No. 29548.

Paralectotype ♀, same labels as lectotype except for "♀" (Fig.1C, D) , in BMNH. B.M. Genitalia slide No. 29549.

Lectotype ♂of *Roeslerstammia hemiadelpha* Meyrick, 1922 (here designated), "Murree Hills / Punjab / 7500 ft / May 1920 / Dutt Coll", "Presented by / R.L.E.Ford. / B.M.1949–487.", "*Roeslerstammia hemiadelpha* Meyrick / det.T.B.Flecher", "in Cop /C" "♂" (Fig.1E, F) , in BMNH. B.M. Genitalia slide No. 29550.

Paralectotype ♀of *Roeslerstammia hemiadelpha* Meyrick, 1922, same labels as lectotype except for "♀" (Fig.1G, H), in BMNH. B.M. Genitalia slide No. 29551.

Diagnosis. Distinguished from the other species by the narrow triangular creamy-white tornal spot of the forewing. In the male genitalia, the uncus is rectangular, apically bilobed and broad; the valva has a short sacculus terminating in a blunt process; the phallus is short, sinuate, and tapered toward the apex. In the female genitalia, the ductus bursae is slender, nearly straight; the corpus bursae is long-ellipsoidal, with a strongly sclerotized sword-shaped signum.

Description. ***Male*** (Fig. 1A, E).

Forewing length 5.3 mm. Wing expanse 11.3 mm.

Head vertex, including between antennae, with raised pale yellow hairs; frons smooth, ochreous with golden luster, laterally pale yellow along eyes. Eyes moderate, interocular index *ca* 0.8. Antenna filiform, *ca* 0.9× as long as forewing; scales in flagellar segments near the middle of the antenna somewhat raised; scape pale yellow on basal half and dark brown on distal half; flagellum dark brown on basal 2/3, white on distal third, densely ciliate with sensory hairs ventrally. Labial palpus slightly upcurved, relatively long *ca* 2.2 × as long as horizontal eye diameter, 3rd segment as long as 2nd, entirely smooth, 2nd pale yellow, 3rd pale yellow with dark brown laterally.

Thorax tegula pale yellow (scales partly removed); mesonotum dark brown with metallic luster. Fore- and midlegs pale yellow, tarsomeres brown distally; hindleg pale yellow. Forewing, lanceolate, dark brown with metallic blue or golden luster; a narrow indistinct oblique marking present at basal 2/3 near costa, a line along fold from base, terminating in a narrow triangular creamy-white tornal spot; fringe dark brown. Hindwing dark brown, darker near apex; fringe dark brown.

Abdomen pale brown with golden luster, terminally with pale yellow tufts.

Male genitalia (Fig. 2). Uncus rectangular, apically broad and bilobed. Tegumen broad, slightly shorter than uncus. Gnathos consisting of two slender arms united medially with a membranous part. Valva rectangular; sacculus short, about 1/3 length of valva, terminating in a blunt process; a small pad of long hair scales near the base ventrally. Vinculum broad ventromedially; saccus cylindrical, as long as dorsal part of tegumen. Phallus, short, sinuate, tapered toward apex, with indistinct minute spine-like cornuti.

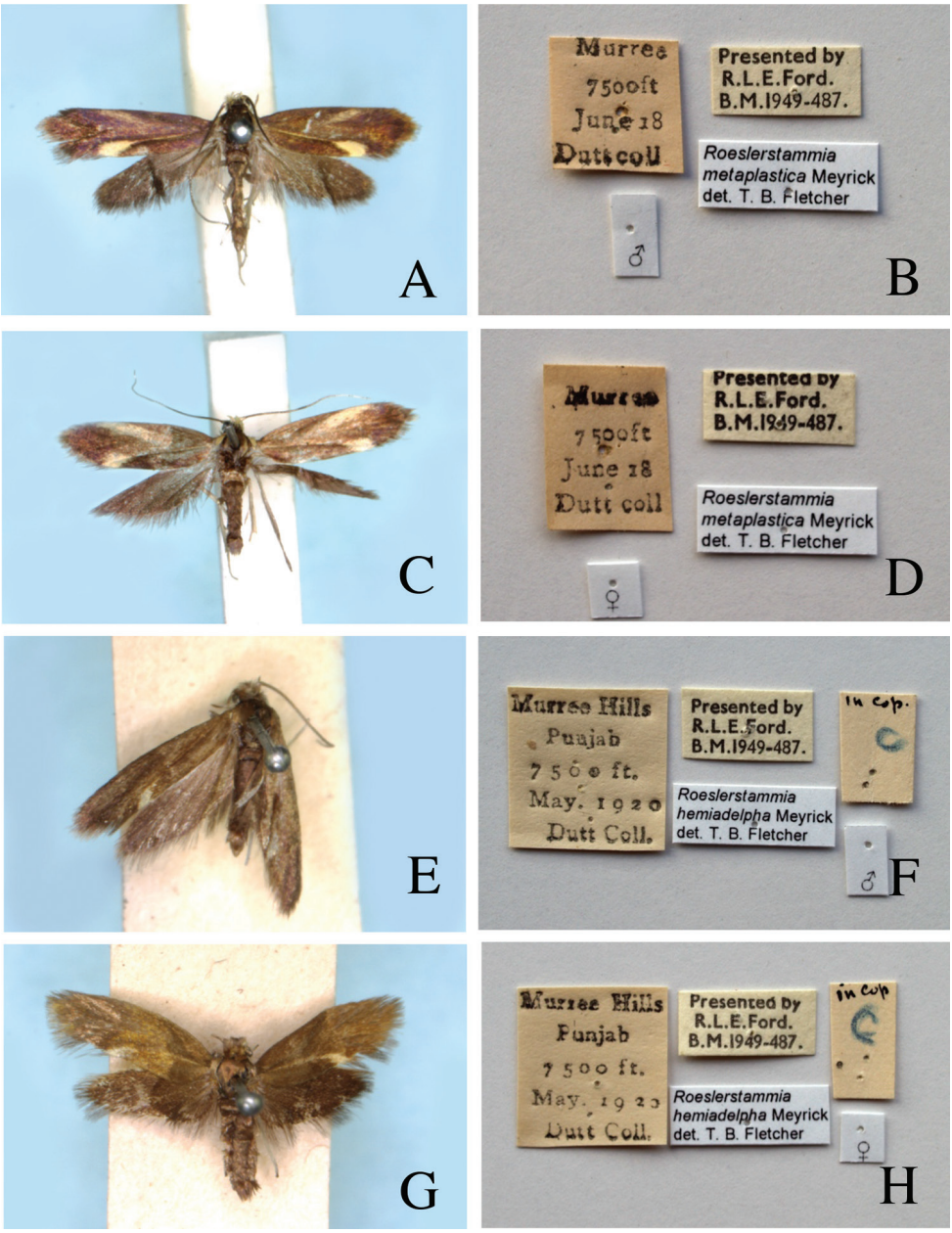

Figure 1. Type series of *Roeslerstammia* spp. from India. **A** Lectotype of *R. metaplastica* Meyrick, 1921, male **B** *Ditto*, labels **C** Paralectotype of *R. metaplastica* Meyrick, 1921, female **D** *Ditto*, labels **E** Lectotype of *R. hemidelpha* Meyrick, 1922, male **F** *Ditto*, labels **G** Paralectotype of *R. hemidelpha* Meyrick, 1922, female **H** *Ditto*, labels.

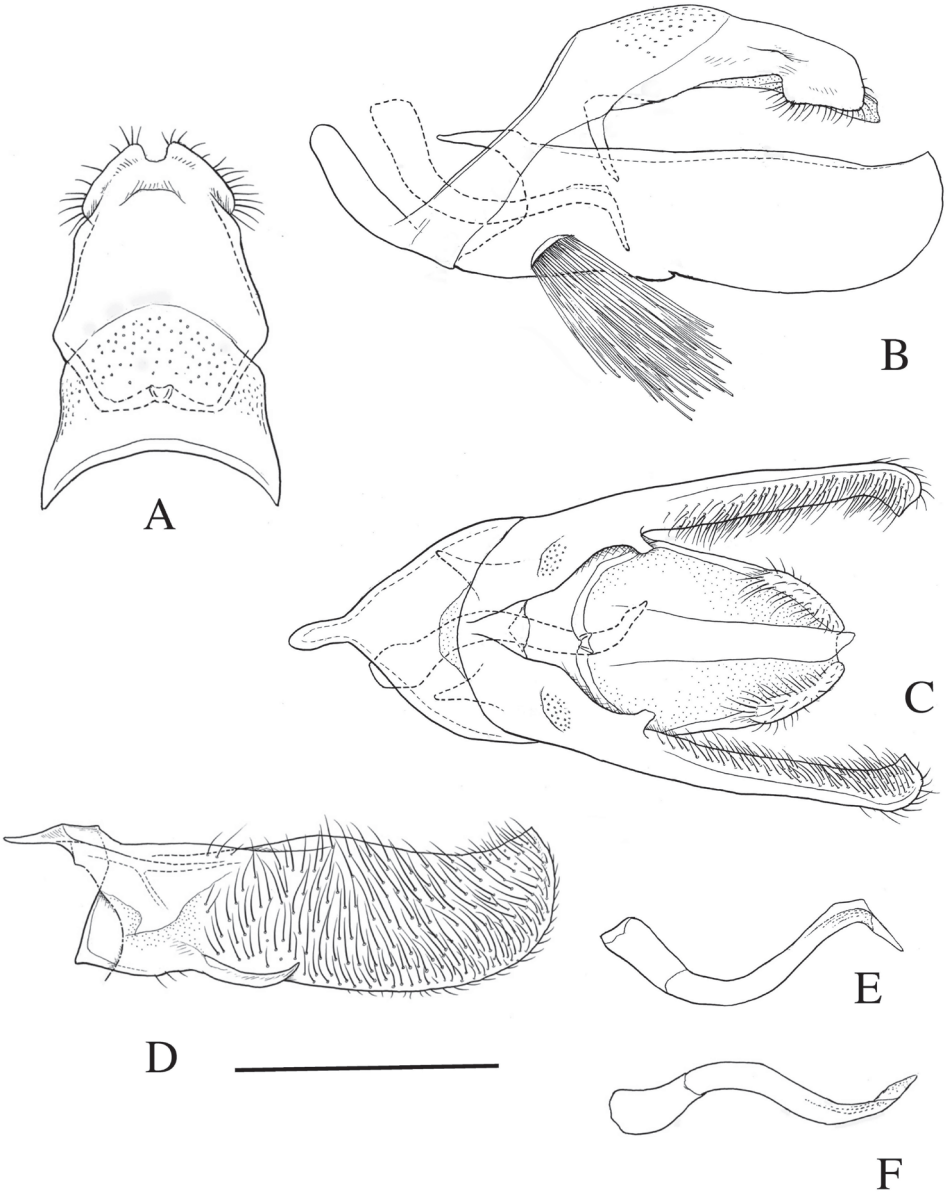

Figure 2. Male genitalia of *Roeslerstammia metaplastica* Meyrick, 1921, lectotype. **A** Dorsum (uncus and tegumen), dorsal view **B** Genitalia without phallus, lateral view **C** *Ditto* ventral view **D** Right valva, inner view **E** Phallus, lateral view **F** *Ditto,* dorsal view. Scale bar: 0.5 mm.

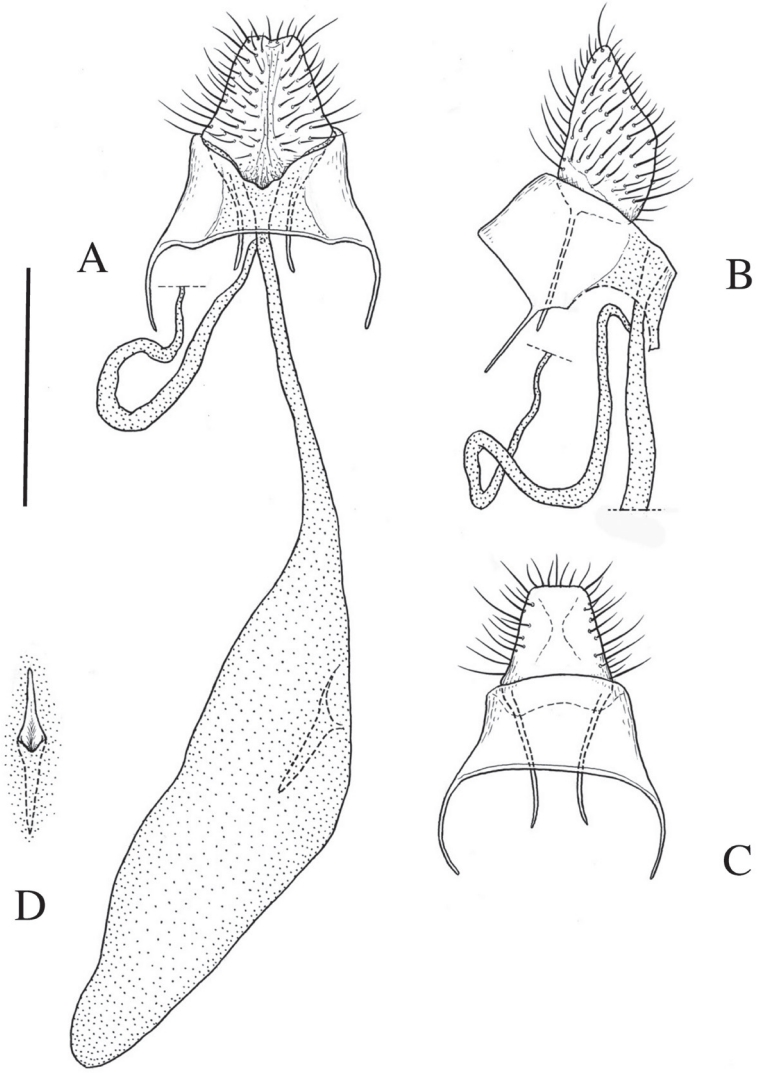

Figure 3. Female genitalia of *Roeslerstammia metaplastica* Meyrick, 1921, paralectotype. **A** Terminalia and bursa copulatrix, ventral view **B** Terminalia, lateral view **C** *Ditto*, dorsal view. **D** Signum. Scale bar: 0.5 mm.

Female (Fig. 1C, G).

Forewing length 5.5 mm. Wing expanse 12.1 mm.

Similar to male but differs as follows: scales in flagellar segments near the middle of the antenna not raised; flagellum without dense sensory hairs ventrally. Forewing with the oblique marking on costa broader, but indistinct in "*R. hemiadelpha*".

Female genitalia (Fig. 3). Papillae anales broad and truncate in ventral view, nearly rectangular in lateral view. Apophysis posterioris slender, 0.7 × as long as papilla analis. Apophysis anterioris slender and moderate in length, 0.6 × as long as eighth tergite. Eighth tergite weakly sclerotized, dorsal posterior margin nearly straight. Ostium bursae situated at posterior margin of eighth abdominal segment, posterior margin weakly emarginate at middle. Ductus bursae slender, nearly straight. Ductus seminalis attached to ductus bursae near ostium. Corpus bursae long-ellipsoidal, with a strongly sclerotized sword-shaped signum.

Host plant. Unknown.

Distribution. India (Punjab).

Remarks. Meyrick (1921) described *R. metaplastica* from the "Murree Hills, Punjab" based on five specimens collected by Dutt in June. Subsequently, Meyrick (1922) described *R. hemiadelpha* based on six specimens, which were collected by the same collector in the same locality in May 1920. Meyrick distinguished his two species on the basis of external characters such as coloration and wing markings (see Fig. 1). Since then, these specimens have not been studied again and they have been regarded as two distinct species following Meyrick. Both the male and female genitalia of some syntypes of *R. metaplastica* and *R. hemiadelpha* were examined, but there were no differences in the shape of male or female genitalia between the two taxa. Therefore, it was concluded that the latter is a junior synonym of the former. Of the original syntype series, only one male and female of each taxon was found in BMNH: both males are selected as lectotypes.

***Roeslerstammia tianpingshana* sp. n.**
http://zoobank.org/C03D3623-B273-49E8-ACDF-C23364922139
Figs 4–9

Type material. Holotype male, "Tianpingshan (1,500 m)/ Badagongshan/ Hunan, China/ 26–27.v.2009/ G.H. Huang & M. Li", in HUNAU. Paratypes 3 males, same label as holotype, in HUNAU; 1 female, same label as holotype, in ELKU.

Diagnosis. Distinguished from other *Roeslerstammia* species by the triangular white marking on the costa of the forewing. In the male genitalia, the uncus is triangular, apically narrow and bilobed; the valva has a blunt process on the median part of the costa; the phallus is long, its basal half straight, distal half strongly upcurved and sinuate. In the female genitalia, the ductus bursae is stout, the caudal part upcurved; the corpus bursae is ellipsoidal, with a thorn-shaped signum.

Description. ***Male*** (Figs 4A, 5D).

Forewing length 7.3 mm in holotype, 6.7–7.1 mm in paratypes.

Wing expanse 15.3 mm in holotype, 13.5–14.3 mm in paratypes.

Head vertex, including between antennae, with raised blackish brown hairs anteriorly, yellow hairs posteriorly; frons smooth, ochreous with golden luster, laterally

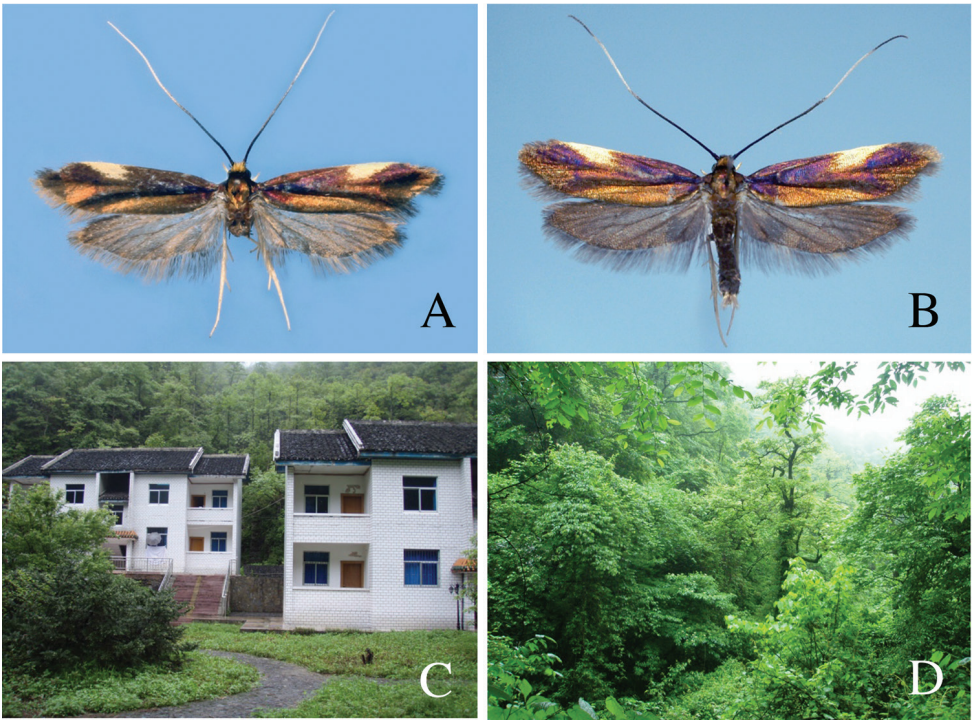

Figure 4. Adult and type locality of *Roeslerstammia tianpingshana* sp. n. **A** Holotype male **B** Paratype female **C** Type locality of *R. tianpingshana* sp. n., Tianpingshan, Hunan, China **D** Vegetation near the type locality.

along eyes blackish brown with a metallic blue lustre (Fig. 5D). Eyes relatively large, interocular index *ca* 1.0. Antenna filiform, 0.8 >(apical part lost)× as long as forewing; scales in flagellar segments near the middle of antenna somewhat raised (Fig. 7B); scape blackish brown with metallic blue lustre; flagellum dark brown on basal 1/3, white on distal 2/3 (apical part lost). Labial palpus slightly upcurved, relatively long *ca* 2.3 × as long as horizontal eye diameter, 3rd segment slightly longer than 2nd; entirely smooth and terminally acute, pale yellow (Fig. 5D).

Thorax tegula dark brown with metallic blue luster; mesonotum dark brown with metallic blue or golden luster. Foreleg dark brown, partly mixed with pale yellow; midleg pale yellow with dark brown tibial spurs; hindleg pale browish gray dorsally, tibia with pale yellow hairs ventrally. Forewing lanceolate, apex narrowly rounded, dark brown with metallic blue or golden luster; a clear triangular creamy white marking present at basal 2/3 of costa; fringe dark brown; veins R4 and R5 stalked, R5 reaching to costa (Fig. 6). Hindwing dark brown, darker near apex; fringe dark brown. Hindwing with frenulum consisting of a long bristle.

Abdomen dorsal part pale brown with golden luster, terminally with pale yellow tufts of long hairs. Ventral part similar to dorsal part, terminally with creamy yellow smooth scales.

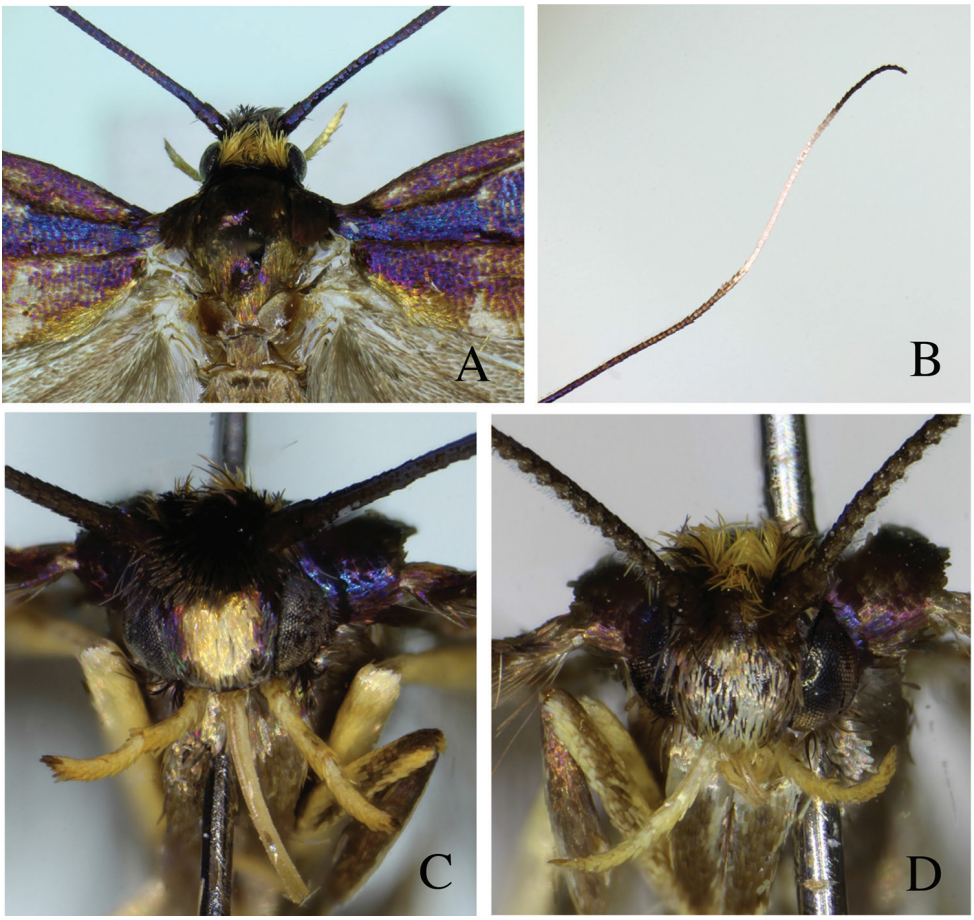

Figure 5. Head and thorax of *Roeslerstammia tianpingshana* sp. n. **A** Paratype female, dorsal view **B** *Ditto*, distal half of antenna **C** *Ditto*, head, frontal view **D** holotype, head, frontal view.

Male genitalia (Fig. 8). Uncus triangular, apically narrow and bilobed. Tegumen broad, as long as uncus. Gnathos consisting of two slender arms united medially with a circular plate. Valva broad basally and narrowed distally, with a blunt process on median part of costa; sacculus short, about 1/3 length of valva, terminating in an indistinct projection; a small pad of long hair scales near the base ventrally. Vinculum narrow ventrally; saccus cylindrical, as long as dorsal part of tegumen. Phallus long, basal half straight, distal half upcurved and sinuate, with a band of minute spine-like cornuti.

Female (Fig. 4B).

Forewing length 7.6 mm. Wing expanse 15.8 mm.

Similar to male but differs as follows: antenna filiform, 1.1× as long as forewing. Scales on antennal flagellar segments not raised; flagellum dark brown on basal half and apical 1/7, white on distal half to near apex. Hindwing with frenulum of two slender bristles.

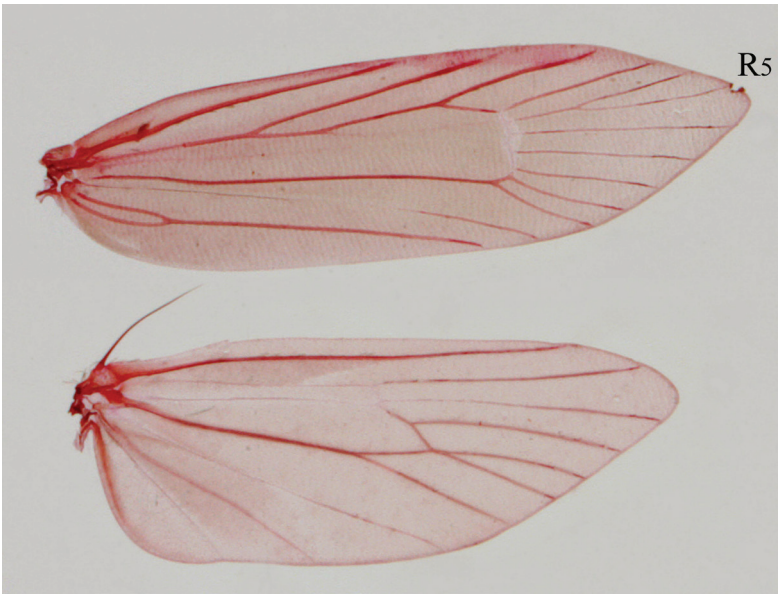

Figure 6. Wing venation of *Roeslerstammia tianpingshana* sp. n., male.

Female genitalia (Fig. 9). Papillae anales narrow and apically pointed in ventral view, nearly triangular in lateral view. Apophysis posterioris slender, 0.7 × as long as papilla analis. Apophysis anterioris short and basally broad, 0.3 × as long as eighth tergite. Eighth tergite strongly sclerotized, dorsal posterior margin weakly emarginate at middle. Ostium bursae situated on anterior margin of eighth abdominal segment, posterior margin nearly straight. Ductus bursae stout, caudal part upcurved. Ductus seminalis attached to ductus bursae near ostium. Corpus bursae ellipsoidal, with a thorn-shaped signum.

Etymology. The specific name, an adjective, is derived from the type locality.

Host plant. Unknown. The larvae of *Roeslerstammia* are first leafminers and then become skeletonizers and mainly feed on plants of Betulaceae (*Alnus*, *Betula*) and Malvaceae (*Tilia*) (e.g., Kyrki 1983, Heppner 2005). Recently Hirowatari et al. (2012) recorded Facaceae (*Fagus*) as the hostplant. The type locality of *R. tianpingshana* is located in a deciduous forest where *Fagus lucida* Rehder & Wilson is the dominant tree species. Although we carried out a survey for immature stages of the new species, no information on the hostplant was obtained.

Distribution. China (Hunan Province).

Remarks. On 26–27 May 2009, a total of four males and one female of the new species were collected in a light trap at Tianpingshan (1,500 m), Badagongshan National Nature Reserve, Hunan Province, China. On 12–14 August 2014, we ran a light trap in the same locality, but did not obtain additional material. The adults of *R. pronubella* and *R. erxlebella* are known to fly in spring and summer (Agassiz 1996, Huemer and Segerer 2001, Hirowatari et al. 2012) but we could not confirm the voltinism of the new species.

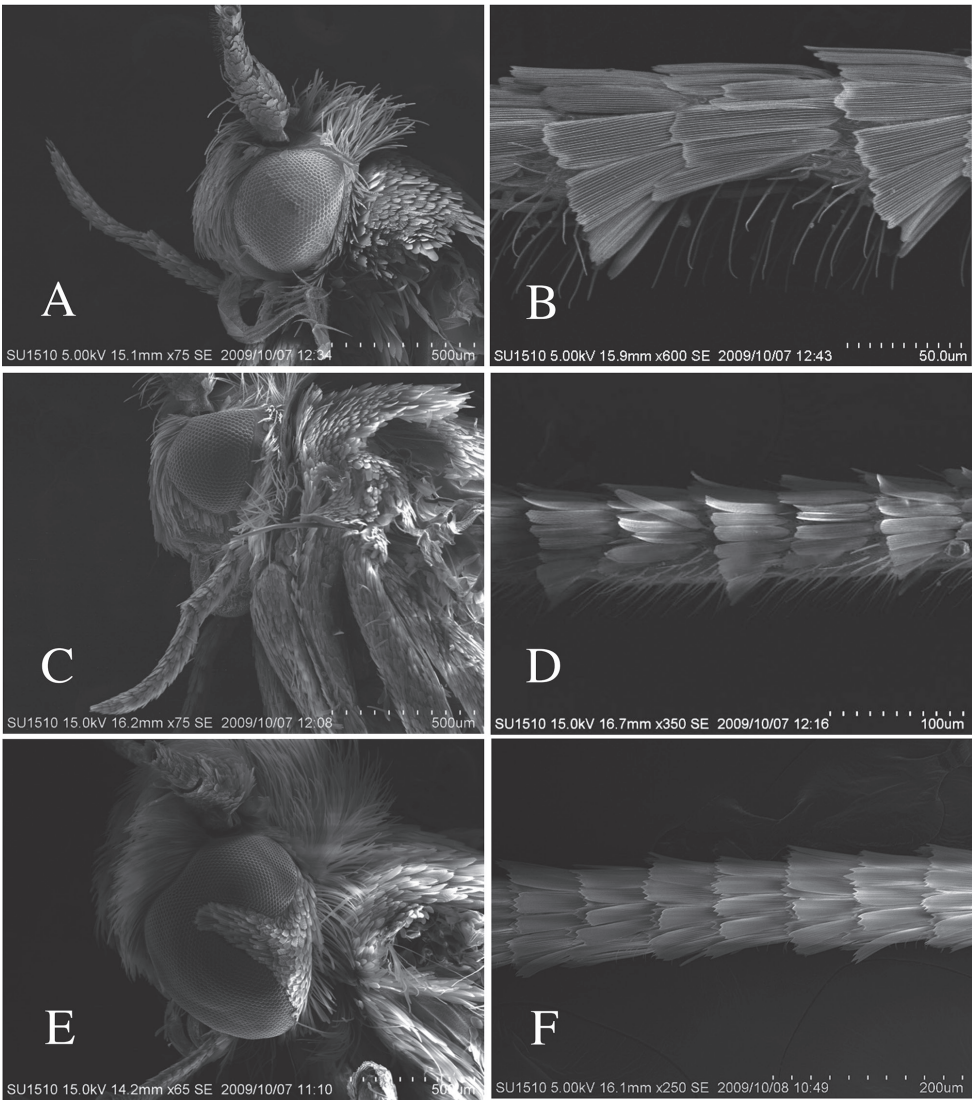

Figure 7. SEM images of head and antenna in Roeslerstammidae. **A–B** *Roeslerstammia tianpingshana* sp. n., male **C–D** *R. pronubella* male, Japan. **E–F** *Telethera blepharacma* Meyrick, 1913, male, Japan. **A, C, E** Lateral aspect of the head **B, D, F** Antenna, lateral aspects.

Discussion

In the present study, it is confirmed that the new species has all of the following diagnostic characters of Roeslerstammiidae Bruand, [1851] (= Amphitheridae Meyrick, 1913) proposed by Kyrki (1983): forewing R5 reaches to costa, the tegumen is bilobed, the gnathos is present, the valva has a small pad of long hair scales on the outside near the base, the phallus (= aedeagus) is a slender usually curved tube.

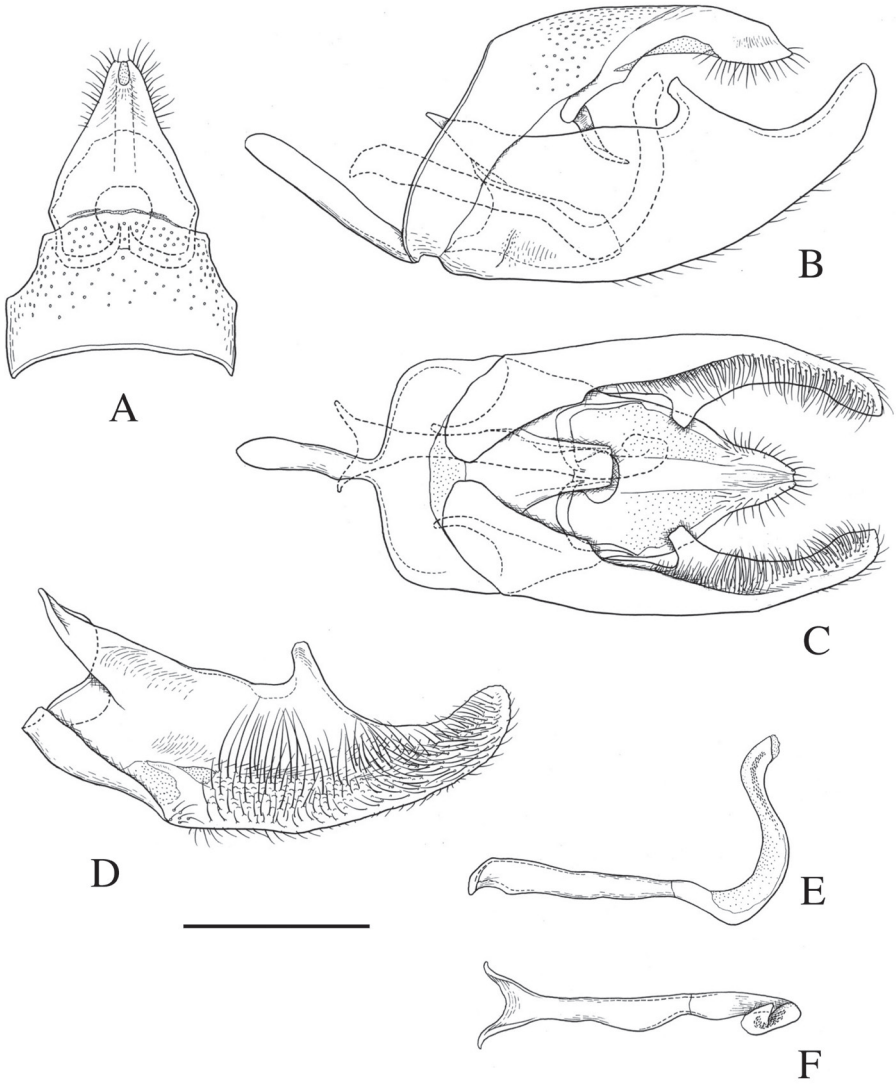

Figure 8. Male genitalia of *Roeslerstammia tianpingshana* sp. n., holotype. **A** Dorsum (uncus and tegumen), dorsal view **B** genitalia without phallus, lateral view (basal long hair scales of valva removed) **C** *Ditto* ventral view **D** Right valva, inner view **E** Phallus, lateral view **F** *Ditto*, dorsal view. Scale bar 0.5 mm.

Although Heppner (2005) insisted on the validity of Amphitheridae, Nieukerken and Karsholt (2006) concluded that when the type genera of Amphitheridae and Roeslerstammiidae are considered to belong to the same family, the senior name Roeslerstammiidae is the valid family-group name. They regarded that Roeslerstamiidae should be a justified emendationof the originally proposed name Röslertammidae, an incorrect original spelling (following article 32.5.3 of ICZN 1999). In some species

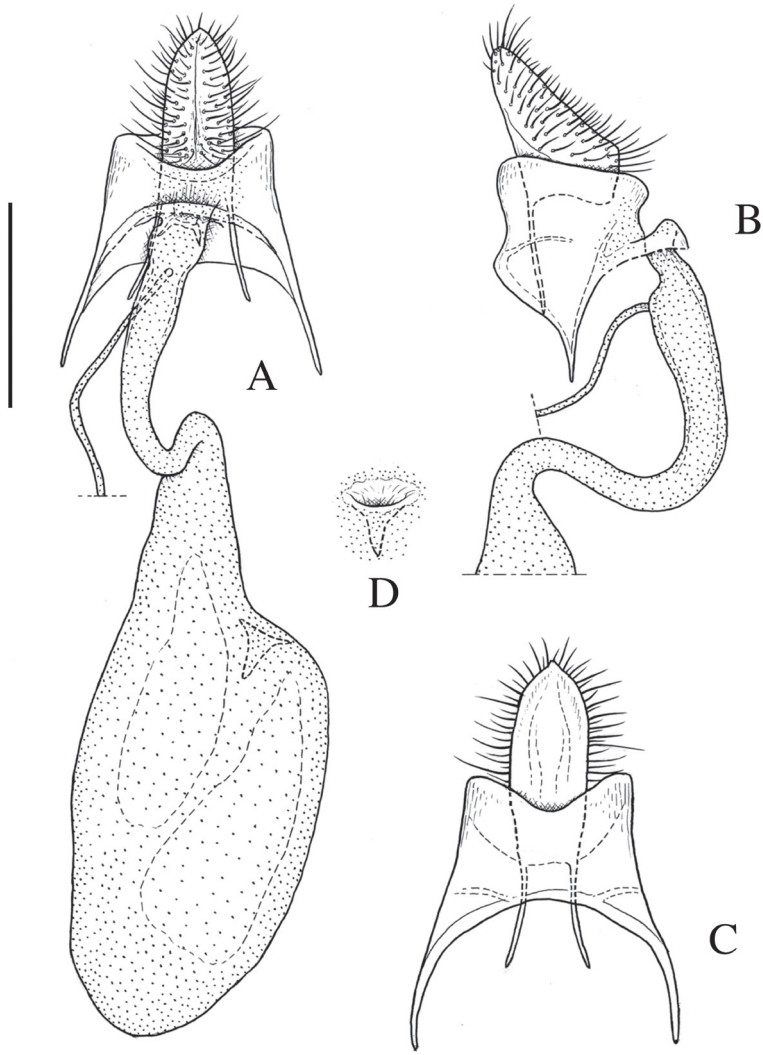

Figure 9. Female genitalia of *Roeslerstammia tianpingshana* sp. n., paratype. **A** Terminalia and bursa copulatrix, ventral view **B** Terminalia, lateral view **C** *Ditto*, dorsal view **D** Signum. Scale bar 0.5 mm.

of the Roeslerstammiidae *sensu lato*, such as *Telethera* Meyrick, the compound eyes are divided into two parts by scales, extending from the posterior margin (Fig. 7E) (Moriuti 1978, 1987). In the new species (Fig. 7A), the compound eyes are of normal type, as seen in the other *Roeslerstammia* species such as *R. pronubella* (Fig. 7C). Kyrki (1983) pointed out in the redescription of *R. erxlebella* that the scales on the flagellar segments near the middle of the male antenna are somewhat raised, giving them a serrated appearance. This condition may be one of the synapomorphies of *Roeslerstammia* because it is also distinct in *R. tianpingshana* (Fig. 7B) and *R. metaplastica* (=*R. hemiadelpha*).

Among species of *Roeslerstammia*, *R. tianpingshana* is unique in having a blunt process on the median part of the costa of the valva in the male genitalia. In the other species, there is no such process on the costa, but instead a small process is present on the ventral part of the valva. Moriuti (1972) and Kyrki (1983) regarded the small spine-like process present in *R. pronubella* and *R. erxlebella* as the terminal part of the sacculus. In *R. metaplastica*, a blunt process is present on the sacculus, while in *R. tianpingshana*, it is represented by an indistinct projection. If these processes are homologous, the presence of them may be one of the synapomorphies of the genus. In addition, *R. pronubella* and *R. erxlebella* are considered to be closely related in having a protruding sacculus which is approximately 2/3 the length of the valva. In *R. tianpingshana*, the shape and size of the male phallus and female ductus bursae (Figs 8 and 9) correspond well. In the other *Roeslerstammia* species, the shapes of the male phallus are variable and species-specific, but their shapes indicate no clear relationship to the female ductus bursae.

In Japan, *R. nitidella* and *R. bella* were described by Moriuti (1972) focusing on the presence or absence of yellow markings on the hindwing, but they are treated as synonyms of *R. pronubella* and *R. erxlebella*, respectively. Currently it is known that the yellow marking on the hindwings in these species is variable and they are separable by genital characters only (Agassiz 1996, Huemer and Segerer 2001, Hirowatari et al. 2012). As for the Indian species, in the original description of *R. metaplastica*, Meyrick (1921) noted that an elongate spot on the costa is variable in development. Nevertheless, he pointed out many differences in coloration and wing markings between *R. metaplastica* and *R. hemiadelpha*. Thus, the coloration and wing markings of *Roeslerstammia* species tend to be variable and external differences between the two taxa described by Meyrick are attributable to the individual variation or seasonal forms of the same species.

Check list

A check list of the genus *Roeslerstammia* is provided, based on Heppner (2005). Heppner (2005) treated Japanese representatives of both *R. pronubella* and *R. erxlebella* as distinct subspecies, *R. pronubella nitidella* Moriuti and *R. erxlebella bella* Moriuti. However, as Hirowatari et al. (2012) noted, there is no unique feature in Japanese specimens either in the wing markings or in the genitalia, and thus Moriuti's species are treated as synonyms.

ROESLERSTAMMIA Zeller, 1839	
erxlebella (Fabricius, 1787) (*Alucita*)	Denmark
fuscocuprella (Haworth, 1828) (*Tinea*)	England
chrysitella (Treitschke, 1833) (*Oecophora*)	[Austria?]
aeneella (Duponchel, [1839]) (*Adela*)	France
erxlebeniella Zeller, 1839, emend.	
bella Moriuti, 1972	Japan

durulguensis Budashkin & Kostjuk, 1993	Russia
pronubella ([Denis & Schiffermüller], 1775) (*Tinea*)	Austria
transcaucasica Toll, 1958	Georgia
nitidella Moriuti, 1972	Japan
metaplastica Meyrick, 1921	India
hemidelpha Meyrick, 1922, **syn. n.**	India
tianpingshana **sp. n.**	China

Acknowledgements

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RESEARCH ARTICLE

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A new species of *Hyphessobrycon* (Characiformes, Characidae) from the upper Guaviare River, Orinoco River Basin, Colombia

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Abstract

Hyphessobrycon klausanni **sp. n.** is described from small drainages of the upper Guaviare River (Orinoco River Basin) in Colombia. It differs from all congeners by having a wide, conspicuous, dark lateral stripe extending from the anterior margin of the eye across the body and continued through the middle caudal-fin rays, and that covers (vertically) three or four horizontal scale rows. It also differs by having an orange-yellow stripe extending from the anterosuperior margin of the eye to the caudal peduncle above the lateral line in life. It differs from all other species of *Hyphessobrycon* that have a similar dark lateral stripe: *H. cyanotaenia*, *H. loretoensis*, *H. melanostichos*, *H. nigricinctus*, *H. herbertaxelrodi*, *H. eschwartzae*, *H. montogoi*, *H. psittacus*, *H. metae*, *H. margitae*, *H. vanzolinii*, and *H. peruvianus* in having only three or four pored scales in the lateral line, 21 to 24 lateral scales and six teeth in the inner premaxillary row. *Hyphessobrycon klausanni* differs from *H. loretoensis* in having seven to eight maxillary teeth (vs. three to four) and in having a longer caudal peduncle (12.4–17.0% SL vs. 4.6–8.0% SL). Additionally *Hyphessobrycon klausanni* can be distinguished from the other species of *Hyphessobrycon* with a dark lateral stripe from the Orinoco River Basin (*H. metae* and *H. acaciae*) in having two teeth in the outer premaxillary row (vs. three to four) and 10 branched pectoral–fin rays (vs. 11 to 12). It further differs from *H. metae* by the length of the snout (17.6–22.8% HL vs. 9.9–15.2% HL) and by the length of the caudal peduncle (12.4–17.0% SL vs. 7.3–11.8% SL).

Resumen

Hyphessobrycon klausanni **sp. n.** es descrita de pequeños drenajes del alto río Guaviare (cuenca del río Orinoco) en Colombia. Se diferencia de todos los congéneres por tener una amplia franja lateral oscura lateral que se extiende desde el margen anterior del ojo a través del cuerpo y se continúa hasta la base de la aleta caudal y que cubre (verticalmente) tres o cuatro escamas laterales, además difiere por tener una franja de color naranja-amarillo que se extiende desde el margen anterosuperior del ojo hasta el pedúnculo caudal por encima de la línea lateral en vida. Se diferencia de *H. cyanotaenia*, *H. loretoensis*, *H. melanostichos*, *H. nigricinctus*, *H. herbertaxelrodi*, *H. eschwartzae*, *H. montogoi*, *H. psittacus*, *H. metae*, *H. margitae*, *H. vanzolinii*, y *H. peruvianus* por tener solo 3 a 4 escamas con poros en la línea lateral, 21 a 24 escamas laterales y 6 dientes en la fila interna del premaxilar. *Hyphessobrycon klausanni* se diferencia de *H. loretoensis* por presentar 7 a 8 dientes maxilares (vs. 3 a 4) y la longitud del pedúnculo caudal (12.4–17.0% LE vs. 4.6–8.0% LE). Además se puede distinguir de las demás especies con banda lateral oscura presentes en la cuenca del rio Orinoco (*H. metae* y *H. acaciae*) por tener dos dientes en la fila externa del premaxilar (vs. 3 a 4) y 10 radios ramificados en la aleta pectoral (vs. 11 a 12), además se separa de *H. metae* por la longitud de hocico (17.6–22.8% LC vs. 9.9–15.2% LC) y por la longitud del pedúnculo caudal (12.4–17.0% LE vs. 7.3–11.8% LE).

Keywords

New taxon, Neotropical Ichthyology, Guaviare River, diversity

Palabras clave

Nuevo taxón, Ictiología Neotropical, río Guaviare, diversidad

Introduction

Hyphessobrycon Durbin, 1908, with 147 valid species (Eschmeyer et al. 2016), is a member of the subfamily Tetragonopterinae in Characidae (Mirande 2010). In addition to being one of the largest characid genera, it is found in all major drainages of the Neotropics, from southern México to the La Plata River in Argentina (García-Alzate et al. 2013a). In morphological and molecular phylogenetic analyses Mirande (2010) and Oliveira et al. (2011) respectively, state that *Hyphessobrycon* is paraphyletic. The genus was proposed by Durbin in Eigenmann (1908) as a subgenus of *Hemigrammus*, the type species of which is *Hemigrammus unilineatus* (Gill, 1858) described from the Island of Trinidad. *Hyphessobrycon* is defined by the following combination of non-exclusive characters: lateral line incompletely pored, adipose fin present, few or no teeth present on the maxilla, third infraorbital bone not in contact with the preopercle, premaxilla with two rows of teeth, with five or more on each side of the inner row, and caudal fin not covered with scales (the character that supposedly differentiates it from *Hemigrammus*). Within *Hyphessobrycon*, species have been grouped primarily by similarities of colour pattern; some of which were proposed merely as artificial operational assemblages to aid species identification (Géry 1977), whereas others represent potential monophyletic groups, as is the case for the Rosy Tetra species group (Castro Paz et al. 2014).

Twenty-two species of *Hyphessobrycon* have been identified from the different hydrographic drainages in Colombia (García-Alzate et al. 2015) and thirteen species are found in streams of the Orinoco River Basin: *H. acaciae*, *H. albolineatum*, *H. bentosi*, *H. diancistrus*, *H. epicharis*, *H. fernandezi*, *H. heterorhabdus*, *H. mavro*, *H. metae*, *H. niger*, *H. otrynus*, *H. sweglesi*, and *H. saizi*. The objective of this paper is to describe a new species of *Hyphessobrycon* from the upper Guaviare River drainage, which is part of the Orinoco River Basin in Colombia.

Materials and methods

Fishes were captured using seines and were preserved in situ in 10% formalin and later stored in 70% ethanol. Counts and measurements follow Fink and Weitzman (1974). Measurements were made with digital callipers to 0.1mm precision and are expressed as percentages of standard (**SL**) and head length (**HL**). In count ranges, values for the holotype are indicated with an asterisk (*) and number of individuals after the meristic counts in parentheses. Counts and measurements were taken on the left side of specimens when possible. Osteological observations were made on cleared and stained adult specimens (**CS**) prepared according to Taylor and Van Dyke (1985). Bone nomenclature follows Weitzman (1962) and Vari (1995). Type specimens are deposited in the University of Atlántico-Caribbean Region, Dept. Biology, Museum Collection, Barranquilla, Colombia (**UARC-IC**), Auburn University Natural History Museum Fish Collection, Auburn, Alabama (**AUM**), the Ichthyology Laboratory at the Universidad del Quindío, Armenia, Colombia (**IUQ**) and Museo Javeriano de Historia Natural "Lorenzo Uribe, S. J.", Bogotá D. C. (**MPUJ**). In the list of paratypes, the number of individuals is given in parentheses immediately after the catalog number. Institutional abbreviations are as listed at http://www.asih.org/node/204, except UARC-IC. We performed a Principal Components Analysis (**PCA**) of morphometric characters of *Hyphessobrycon metae*, *H. acaciae*, *H. mavro*, *H. niger*, and *H. klausanni* and the Burnaby method was used to eliminate the influence of overall size, with the Past program, version 3.0 for Windows (Hammer et al. 2001). Species for which no specimens were available, such as *Hyphessobrycon montogoi*, *H. margitae*, *H. vanzolinii*, *H. lucenorum*, *H. psittacus*, and *H. peruvianus* were included in the comparisons using their original descriptions. The abbreviation masl means meters above sea level.

Comparative material examined

***Hyphessobrycon acaciae*:** COLOMBIA, Meta: IUQ 2796, holotype, Morichal del Estero, Puerto López, Meta, ~ 4°04'N; 72°57'W, 10 Sep. 1994. Paratypes: MPUJ 393, 28, collected with holotype; IUQ 2795, 2 CS, collected with holotype; IUQ 2433, 49, Laguna Hacienda La Cabaña, Inspección de Surinera, San Carlos, Acacías, ~03°55'N;

73°50'W, 7 Jan. 2009; IUQ 2492, 17, Acacías Creek on road to Vista Hermosa-Puerto Lucas, 03°06'51"N; 73°45'44"W, 259 masl, 08 Jan. 2009; IUQ 2793, 4 CS, Laguna Hacienda La Cabaña, Inspección de Surinera, San Carlos, Acacías, 07 Jan. 2009. MPUJ 2604, 52, Laguna El Retiro, Inspección La Loma, Acacías, 11 Oct. 2006. ***H. metae*:** Colombia, Meta: CAS 61751, Holotype, Río Meta in Barrigona, Orinoco River drainage, 1914; IAvH-P 3014, 57, Caño Muco, Hacienda San Francisco, Puerto Gaitán-Gaviotas road, 3°13'49"N; 73°52'39"W; IavH-P 6151, Vichada, Las Galapagitas Creek near camp in La Sabana, Puerto Carreño, Vichada, 30 Apr. 1990. Venezuela, Guárico: MCNG 32469, 19, Aguaro Guariquito National Park, San José River, 13 Jan. 1995. ***H. mavro*:** Colombia, Vichada: IUQ 2791, Holotype, Payara Creek, tributary of Negro Creek, Puerto Carreño, ~6°12'N; 67°28'W, 27 Apr. 2005; IMCN 3751, 21, Paratypes, collected with holotype; IUQ 1964, 2 CS, collected with holotype. ***H. niger*:** Colombia, Meta: IUQ 2792, holotype, Mojaculo Creek, Vereda Dinamarca, Acacías, Meta, 03°53'20.6"N; 73°28'30"W, 03 Apr. 2008; MPUJ 5039, 28, Paratypes, collected with holotype; IUQ 2794, 2 CS, collected with holotype. ***H. agulha*:** Colombia, Amazonas, Leticia: IAvH-P 8345, 19, tributary of Matamata Creek Mar. 2001; IAvH-P 8335, 37, tributary of Matamata Creek, 18 Mar 2001; IAvH-P 8332, 4, tributary of Matamata Creek, 02 Jul 2001; IAvH-P 8333, 52, tributary of Purité River, 25 Mar 2001; IAvH-P 9025, 85, Sufragio in front of Zafire Station, 15 Dec 2002; IAvH-P 9046, 14, stream, tributary of Calderón River, 45 minutes north of Zafire Station, 11 Dec 2002; IAvH-P 9071, 38, tributary of Calderón River, 45 min. north of Zafire Station, 12 Dec 2002; IAvH-P 9407, 25, stream 2, tributary of Purité River three hours from Salados Varios, Amacayacu National Park, 25 Ma. 2003; Perú, Madre de Dios: MUSM 23173, 9, stream at km 43 Tambopata; MUSM 23173, 1 CS, stream at km 43 Tambopata; MUSM 25315, 9, stream at km 43 Tambopata; MUSM 25315, 1 CS, stream at km 43 Tambopata. ***H. compressus*:** México: FMNH 4641, holotype, Obispo, Vera Cruz; FMNH 4662, 1 CS paratypes, collected with holotype; BMNH 1905.12.6.4-5, 2 paratypes, Obispo, Vera Cruz; IBUAM-P 8538, 2, Trinitaria, Flor de Café, Chris, 3 Jul. 1993; ANSP 124774, 12, Río Usumacinta almost connected to Pasión, near Sayache, 18 Aug. 1996; ANSP 124774, 3 CS, Río Usumacinta almost connected to Pasión, near Sayache, 18 Aug. 1961. ***H. herbertaxelrodi*:** Brazil: MCP 30829, 5, stream tributary to the Caibi River, at its mouth, Mato Groso, 18 Jan 2002; MCP 30829, 2 CS, Brazil, stream tributary to the Caibi River, at its mouth, Mato Grosso, 18 Jan 2002. ***H. heterorhabdus*:** CAS 44415, Syntype, 1, 16.9 mm de SL, Brazil, Para, 1894; ICNMNH 5063, 10, 17.8-23.9 mm SL, Colombia, Amazonas, Puré River, Leticia 02° 07'05"S; 69°37'50"W, 8 Jan 2000; MCP 41577, 5, 19.5-23.6 mm SL, Brazil, Para, Igarapé Acuí, 01°35'46"S; 48°44'26"W, 21 Oct 2006; IUQ 1961, 3 CS, 28.3-34.6 mm SL, Colombia, Puré River, Leticia, Amazonas, 02°07'05'S; 69°37'50"W, 8 Jan 2000; IUQ 1963, 1 CS, 33.1 mm SL, Brazil, Para, Igarapé Acuí Igarapçe Acuí, 01°35'46"S; 48°44'26"W. ***H. loretoensis*:** MUSM 20179, 2, Perú, Loreto, upper Amazon, Morona River drainage, Anazo Creek, 7 May 2002; MUSM 23172, 4, Perú, Loreto, upper Amazon, Abanico del Pastaza, Corrientes River drainage, Platanoyacu River, 27 Oct 2004; MUSM 23233, 5, 20.7-30.2 mm SL,

Perú, Loreto, upper Amazon, Abanico del Pastaza, Corrientes River drainage, Platanoyacu River, 27 Oct 2004. ***H. melanostichos*:** MCP 39808, 5, Brazil, Doze de Outubro River between Comodro and Vilhena, 12°35'46"S; 60°00'30"W, 14 Jul 2004. ***H. nigricinctus*:** MUSM 26791, 1, 32.04 mm SL, Perú, Cusco, Quispicanchi, Camanti Cuenca Araza, San Lorenzo River, Ilahuala Creek, 26 Oct 2005; MUSM 26786, 5, Perú, Cusco, Quispicanchi, Camanti, Araza River drainage, San Lorenzo River, Ilahuala Creek, 26 Oct 2005. ***H. notidanus*:** MCP 38676, 2 paratypes, 24.8-25.7mm SL, Brazil, Doze de Outubro River between Comodoro and Vilhena, 12°58'39"S; 60°00'30"W, 14 Jul 2004; MCP 38676, 1 CS, Brazil, Doze de Outubro River between Comodoro and Vilhena, 12°58'39"S; 60°00'30"W, 14 Jul 2004. ***H. taphorni*:** Perú, Madre de Dios: MUSM 42391, holotype, Paiche pool, Aguajal Baja, Madre de Dios, Tambopata River, 12°29'4.2"S; 68°57'9.09"W, 22 Feb 2004; MUSM 22042, 95, paratype, collected with holotype; AUM 56757, 2 paratypes, Aguajal de Aguas Negras, mining pond, Tambopata River, Madre de Dios River drainage, 12°38'9.84'' S; 69°25'35.8"W, 21 Jan 2004; AUM 56758, 2 paratypes, Aguajal Este, Tambopata River, Madre de Dios River drainage, 20 Feb. 2004; MUSM 5562, 60 paratypes; Sandoval Lake, Las Piedras, Tambopata River drainage, 12°36'17.76"S; 69°02'40.2"W, 23 Jan. 1990; MUSM 5581, 4 paratypes, Sandoval Lagoon, Las Piedras, Tambopata River drainage, 12°36'17.76'' S; 69°02'40.29"W, 24 Jan 1990; MUSM 5588, 80 paratypes, Tambopata, Las Piedras, Quebrada 2 km from Sandoval Lagoon, 12° 36'17"S; 69°03'23"W, 23 Jan.1990; MUSM 9584, 11 paratypes, Sandoval Lake, Tambopata, Madre de Dios River drainage, 12°36'17.76"S; 69°02'40.29"W, 15 May. 1996; MUSM 21703, 15 paratypes, Aguajal Aguas Negras, Aguajal Satélite, Tambopata River, Madre de Dios River drainage, 12°39'22"S; 69°26'28"W, 22 Jan. 2004; MUSM 21824, 58 paratypes, Aguajal de Aguas Negras, mining pond, Tambopata River, Madre de Dios River drainage, 12°38'9.84"S; 69°25'35.8"W, 21 Jan 2004; MUSM 21994, 272 paratypes, Aguajal Este, Tambopata River, Madre de Dios River drainage, 20 Feb. 2004; IUQ 3032, 2 CS paratypes; Aguajal Aguas Negras, Agujal Satélite, Tambopata River, Madre de Dios River drainage, 12°39'22"S; 69°26'28"W, 22 Jan. 2004. ***H. eschwartzae*:** Perú, Madre de Dios: MUSM 42392, holotype, La Cachuela, Madre de Dios River, Tambopata, 12°16'38.2"S; 69°09'8.12"W, 8 Jul. 2003; AUM 51350, 12 paratypes, Río Planchón, crossing the bridge of the Interoceanic highway, 36.3 km N of Puerto Maldonado, Río Inambari, 01 Aug. 2010; AUM 51374, 16 paratypes, Río Buyuyoc, at bridge of Interoceanic highway, 91.1km N of Puerto Maldonado, 01 Aug. 2010; MUSM 22474, 111 paratypes, collected with holotype; MUSM 3684, 16 paratypes, Tambopata River, Quebrada 500 m from campsite, Sandia, Puno, 26 Aug. 1992; MUSM 9771, 26 paratypes, Madre de Dios River, km 29, Tambopata, 12°42'26.22"S; 69°26'54.03"W, 22 May. 1996; MUSM 21221, 143 paratypes, Quebrada at km 14 on road to San Juan, Manuripe Alegría River drainage, 12°0'57.41"S; 69°03'43.39"W; MUSM 22893, 30 paratypes; Quebrada El Planchón (km 40), Tambopata, 12°16'34.94"S; 69°09'8.12"W, 13 Jul, 2003; IUQ 3033, 3 CS paratypes, Quebrada at km 14, road to San Juan, Manuripe Alegría River drainage, 11 Jul 2003. ***H. oritoensis*:** Colombia, Putumayo, Orito: IUQ 1574, Holo-

type, Quebrada La Palma, La Palma ranch, Vereda Calimonte, 29 Jun. 1998, IUQ 139, 6 Paratypes, collected with the holotype. IUQ 1575, 2 CS, paratypes, collected with the holotype; MBUCV-V 33737, 2 paratypes, collected with the holotype; MCNG 55844, 2, paratypes, collected with the holotype. ***H. paucilepis*:** Venezuela, Lara state: IUQ 1897, Holotype, Los Quediches Reservoir, overflow channel, 03 Sep. 1987; MBUCV-V 23710, 3 paratypes, collected with holotype; IUQ 1898, 1 CS paratype, collected with holotype; MBUCV-V 903, 4 CS paratypes, Los Quediches Reservoir, overflow channel; MBUCV-V 23706, 42 paratypes, Los Quediches Reservoir, overflow channel, MBUCV-V 6933, 6 paratypes, Burere, Carora-Cabimas highway. CPUCLA 532, 5 paratypes, Ciénaga de Puricaure Carora - El Venado highway, about 1 km from Puricaure, Quebrada Arriba road, 10°06'26.0"N; 70°28'93"W. ***H. vilmae*:** MCP 38881, 5, 28.0-30.6 mm SL, Brazil, Papagaio River in front of public beach, Mato Grosso 13°33'35"S; 58°24'31"W, 13 Jul 2004.

Taxonomy

***Hyphessobrycon klausanni* sp. n.**
http://zoobank.org/B648B8AE-BE21-44B9-A5A5-D2BD5CEF9EE9
Figures 1–4

Holotype. UARC-IC 539, 23.1 mm SL, male, Colombia, Meta, Mapiripán County, upper Guaviare River drainage, Caño Claro, 03°07'05.1"N; 72°30'14.8"W; 209 masl; **Paratypes**. UARC-IC 540, five, 22.1–24.2 mm SL, collected with holotype; UARC-IC 541, two CS, 20.2–22.3 mm SL, collected with holotype; UARC-IC 542, eight, 20.1–23.3 mm SL, Mapiripán county, upper Guaviare River drainage, Caño La División, 03°07'05.8"N; 72°32'36.7"W, 209 masl; UARC-IC 543, two, 25.1–28.4 mm SL, El Castillo County, upper Guaviare River drainage, Caño Hondo, 03°33'08.6"N; 73°47'17.9"W, 209 masl; UARC-IC 544, six, 22.1–23.4 mm SL, Mapiripán County, upper Guaviare River drainage, Caño División 2, 03°07'03.3"N; 72°32'32.5"W, 221 masl; MPUJ 7857, eight, 25.4–31.4 mm SL, El Castillo County, Caño Hondo, upper drainage of Guaviare River, 03°33'08.6"N; 73°47'17.9"W.

Diagnosis. *Hyphessobrycon klausanni* sp. n. can be diagnosed from all other species of *Hyphessobrycon*, except of *H. cyanotaenia*, *H. loretoensis*, *H. melanostichos*, *H. nigricinctus*, *H. herbertaxelrodi*, *H. eschwartzae*, *H. montogoi*, *H. psittacus*, *H. metae*, *H. margitae*, *H. vanzolinii* and *H. peruvianus*, by having a wide, conspicuous, dark lateral stripe extending from the anterior margin of the eye across the head and body and continuing through the middle caudal-fin rays to their tips. It differs from the species excepted above in having a wider lateral stripe that covers three or four horizontal scale rows (vs. stripe covering just one or two scales rows); in having an orange-yellow stripe extending from the anterodorsal margin of the eye to the caudal peduncle above the dark lateral stripe in life (vs. red lateral stripe extending from the anterodorsal margin of the eye to the caudal peduncle above the dark lateral stripe in live *H. heterorhabdus*,

Figure 1. *Hyphessobrycon klausanni* sp. n., UARC-IC 539, holotype, male, 23.1 mm SL, Mapiripán County, upper Guaviare River drainage, Caño Claro, Meta, Colombia.

H. amapaensis, *H. eschwartzae* and *H. montagi*); by having fewer pored lateral line scales (three to four vs. five to10); fewer lateral scales (21 to 24 vs. 29 to 34); and in having more teeth on the inner premaxillary row (six vs. five). It differs from *H. loretoensis* in having seven to eight maxillary teeth (vs. three to four) and in having a longer caudal peduncle (12.4–17.0% SL vs. 4.6–8.0% SL). Additionally *Hyphessobrycon klausanni* can be distinguished from the other species of *Hyphessobrycon* with a dark lateral stripe from the Orinoco River Basin (*H. metae* and *H. acaciae*), in having: two teeth in the outer premaxillary row (vs. three to four see Fig. 2) and 10 branched pectoral-fin rays (vs. 11 to 12). It further differs from *H. metae* by the length of the snout (17.6–22.8% HL vs. 9.9–15.2% HL) and by the length of the caudal peduncle (12.4–17.0% SL vs. 7.3–11.8% SL).

Description. Morphometric data shown in Table 1. Body compressed, moderately thick, greatest depth between pelvic-fin insertions and dorsal-fin origin. Dorsal profile of head straight from upper lip to vertical through middle of orbit; then convex to dorsal-fin origin. Dorsal-fin base convex, then slightly concave to adipose-fin origin, then straight to base of upper caudal-fin lobe. Ventral profile of head convex from lower lip to insertion of anal fin, anal-fin base straight and then slightly concave to base of lower caudal-fin lobe.

Head and snout long, jaws equal, mouth terminal, lips soft and flexible, outer row of premaxillary teeth not exposed. Premaxilla with long, sharp lateral process over ethmoids, with two rows of teeth: the external row with one (2) or two* (23), all tricuspid; inner row with six* (25) tricuspid teeth the gradually diminish in size away from symphysis. Maxillary long and narrow, posterior margin straight, anterior margin convex, ventral margin reaching anterior border of third infraorbital, with seven* (10) or eight (15) tricuspid and conical teeth. Dentary with convex ventral margin, four (25) frontal multicuspid teeth followed by six* (15) or eight (10) smaller conical teeth (Fig. 2).

Scales cycloid. Lateral line with three (10) or four* (15) pored scales. Lateral scale series with 21 (10), 22 (6) or 24* (9) scales, including those with pores. Transverse

Table 1. Morphometric and meristic data of *Hyphessobrycon klausanni* sp. n. Standard length given in mm. Mean given in parentheses. SD = Standard deviation.

	Holotype	Paratypes	SD
Standard length	23.1	20.1–28.4 (22.2)	1.2
Total length	27.5	25.8–29.3 (27.5)	0.9
Percentages of SL			
Body depth	29.4	29.4–35.1 (32.5)	1.5
Snout-dorsal fin distance	50.2	49.7–56.5 (53.7)	2.5
Snout-pectoral fin distance	32.0	25.7–33.6 (30.2)	2.2
Snout-pelvic fin distance	44.2	42.8–53.7 (47.7)	3.5
Snout-anal fin distance	57.1	57.1–69.2 (62.0)	3.3
Dorsal fin-hypural distance	47.2	47.1–53.5 (50.4)	2.0
Dorsal-fin length	29.9	29.2–35.0 (31.9)	2.1
Pectoral-fin length	38.5	35.0–45.0 (39.2)	3.1
Pelvic-fin length	26.0	23.2–29.9 (25.9)	2.1
Caudal peduncle depth	21.2	17.5–24.5 (20.8)	2.2
Caudal peduncle length	15.2	15.1–20.9 (17.9)	1.8
Head length	22.5	20.3–25.3 (21.5)	1.3
Dorsal-anal fin distance	10.0	8.7–12.7 (10.0)	1.1
Dorsal-pectoral fin distance	15.2	12.2–17.0 (14.4)	1.2
Anal-fin length	28.6	27.2–31.3 (29.6)	1.5
Percentages of HL			
Snout length	18.2	17.6–22.8 (20.4)	1.8
Orbital diameter	36.4	30.1–45.0 (37.9)	4.9
Postorbital distance	39.4	36.6–53.1 (43.5)	4.3
Maxilla length	39.4	23.9–49.2 (35.6)	7.4
Interorbital distance	39.4	29.5–41.5 (25.8)	3.4
Mandible superior distance	27.3	22.5–30.4 (26.4)	2.5
MERISTICS			
Lateral scales	24	20–24	
Pored lateral-line scales	4	3–4	
Scales from lateral line to dorsal fin	5	5	
Scales from lateral line to anal fin	4	4	
Scales from lateral line to pelvic fin	3	3	
Predorsal scales	9	9–10	
Dorsal-fin rays	ii, 9	ii, 9	
Anal-fin rays	iii, 20	iii, 19–20	
Pelvic-fin rays	ii, 7	ii, 7	
Pectoral-fin rays	ii, 10	ii, 10	

scales rows five* (25) between dorsal-fin origin and lateral line, not including the predorsal series just in front of first dorsal-fin ray. Four (25) horizontal scale rows from anal-fin origin to lateral line. Three (25) horizontal scale rows between pelvic-fin origins and lateral line. Predorsal scales nine*(18) or 10 (7). Five scales in single row along anterior anal-fin base. Fin rays: Dorsal ii, 9 (25). Anal iii, 19 (18) or 20* (7). Pelvic ii,

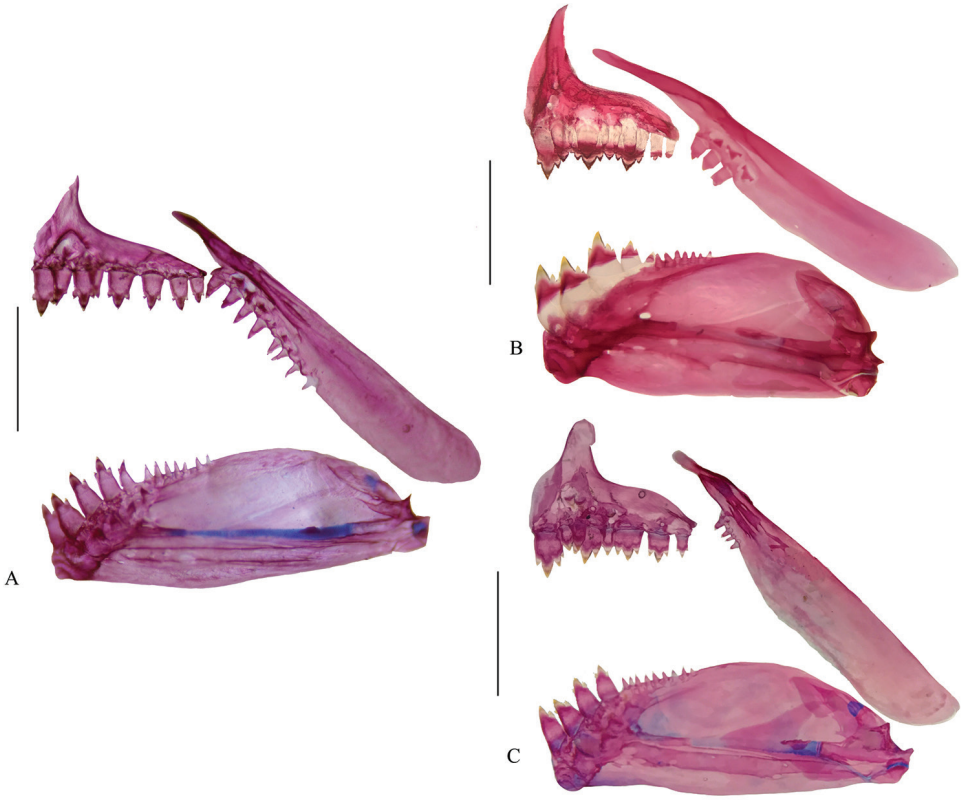

Figure 2. Upper and lower jaws of **A** *Hyphessobrycon klausanni* sp. n., UARC-IC 541, 20.2 mm SL **B** *H. metae* MCNG 32469, 19.9 mm SL and **C** *H. acaciae*, IUQ 2795 Paratypes, 28.6 mm SL. Scale bar: 1 mm.

7 (25). Pectoral ii, 10 (25). Caudal 10+10 (2) principal and 10 (2) procurrent. Caudal fin bifurcate, upper and lower lobes similar in size, pointed. Total vertebrae 32–33.

Sexual dimorphism. Males have hooks on anal-fin and pelvic–fin rays. Anal fin with pair of rows of eight to ten small hooks along third simple ray followed by two to eight pairs of hooks on first to fifth branched rays. Pelvic fins with two to ten pairs of hooks on branched rays, located above internal branch of ray, each segment of branched rays with pair of hooks.

Color in alcohol. Opercular and humeral spots absent. Dorsal part of head and body to dorsal fin dark brown, then yellow on sides and light yellow ventrally. Base color divided by conspicuous, wide (three to four horizontal scale rows), dark lateral stripe from anterior margin of eye through middle caudal-fin rays. Pectoral, pelvic and anal fins hyaline. Dorsal, anal and caudal fins with dark margins. Anal fin with dark pigment concentrated on interradial membranes.

Color in life. Body greenish-yellow, predorsal area orange-yellow, preventral area silvery-yellow, upper and lower margins of eye red and black respectively, dorsal area of head orange-yellow, ventral area greenish-yellow with great concentration of mel-

Figure 3. Live colours of *Hyphessobrycon klausanni* sp. n. Paratype, UARC–IC 540, 22.4 mm SL.

anophores on infraorbitals, preopercle and opercle. Wide, black, lateral stripe from anterior part of eye along sides through middle caudal-fin rays, covering at least half of body height near midbody. Iridescent orange stripe present above black stripe from eye to upper caudal-fin lobe. Lower lobe of caudal fin with orange iridophores at bases of rays. Bases of dorsal-fin rays orange, base of caudal and pelvic fins greenish–yellow. Adipose fin light orange (Fig. 3).

Distribution. *Hyphessobrycon klausanni* is known only from the type locality in the upper Guaviare River Orinoco Basin in Colombia (Fig. 4).

Etymology. Research leading to the discovery and recognition of this species was partially funded by Mr. Klaus-Peter Lang from Oberhausen, Germany. To commemorate the 80th birthday of his mother, this species is dedicated to and named for his father "Klaus" and his mother "Anni".

Remarks. Principal component analysis (PCA) detected morphological differences among *Hyphessobrycon klausanni* and *H. acaciae*, *H. mavro*, *H. metae* and *H. niger*. For the first component, upper jaw length, postorbital length and dorsal-pectoral fin distance were the most important variables. For the second component, orbital diameter, caudal peduncle length and snout length were most important. The first component explained 43.71% of total variation, and combined with the second this rose to 85.78% (Fig. 5, Tables 2 and 3).

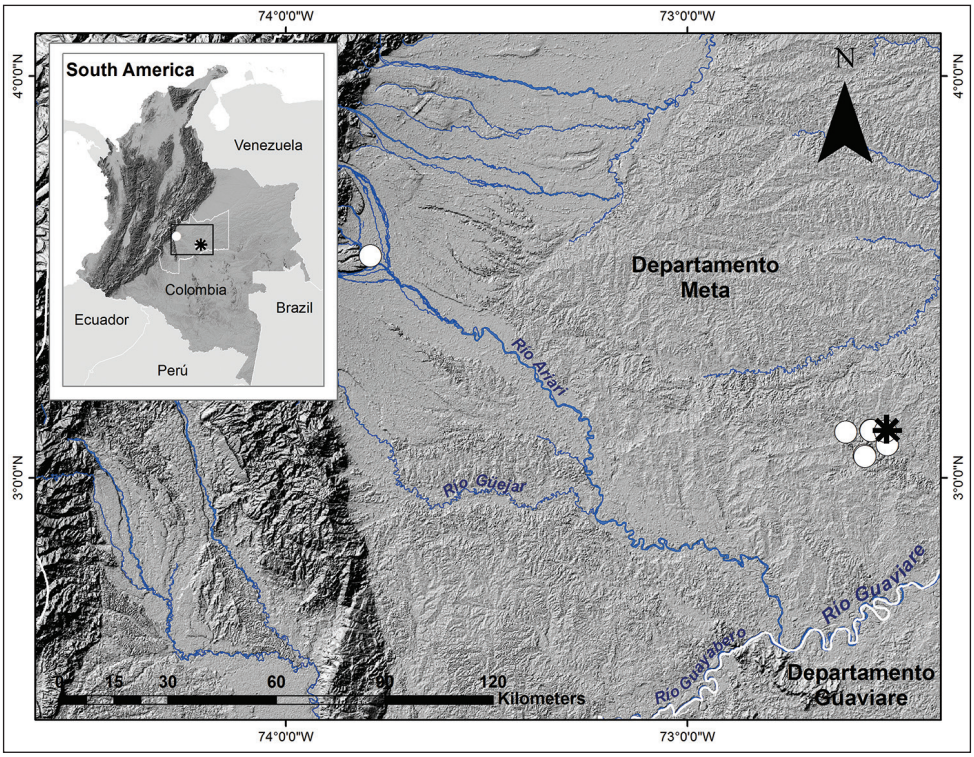

Figure 4. Distribution of *Hyphessobrycon klausanni* sp. n. in the upper tributaries of Guaviare River drainage. Type locality indicated by an asterisk.

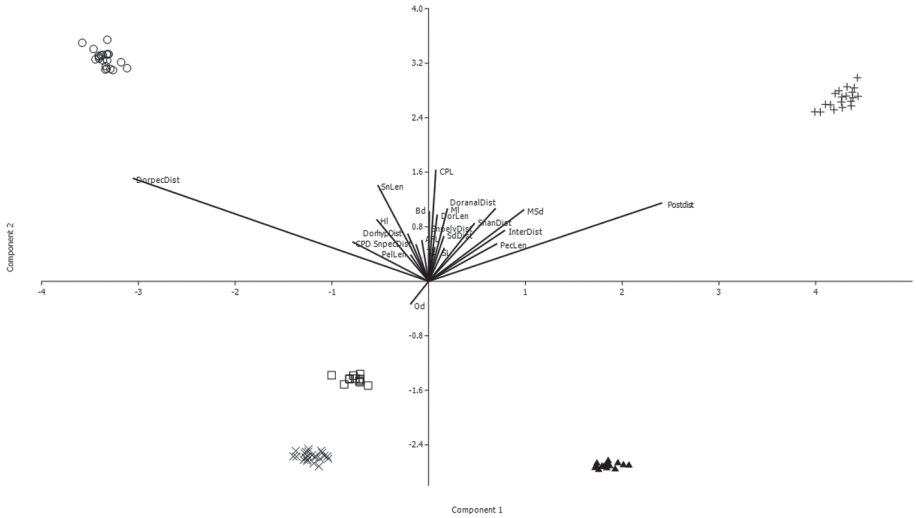

Figure 5. Principal component analysis for morphological data of *Hyphessobrycon klausanni* sp. n. (□), *H. acaciae* (+), *H. mavro* (▲), *H. metae* (○) and *H. niger* (x); component 1 on X axis and component 2 plotted on Y axis. Abbreviations same as Table 1.

Table 2. Eigenvalue for principal components (PC) between *Hyphessobrycon klausanni* sp. n., *H. acaciae*, *H. mavro*, *H. metae* and *H. niger*.

PC	Eigenvalue	Percentage variance
1	742.872	43.712
2	715.071	42.076
3	143.989	8.4725
4	0.887396	5.2216
5	0.0177261	0.1043
6	0.012861	0.075676
7	0.0106605	0.062728
8	0.00831635	0.048935
9	0.00719985	0.042365
10	0.00544939	0.032065
11	0.00483203	0.028432
12	0.00454893	0.026767
13	0.00389345	0.02291
14	0.00330895	0.01947
15	0.00202874	0.011937
16	0.00152486	0.0089725
17	0.00134239	0.0078988
18	0.00118167	0.0069531
19	0.00103976	0.0061181
20	0.00091383	0.0053771
21	0.000677078	0.003984
22	0.000411058	0.0024187
23	0.000201424	0.0011852

Ecological note. *Hyphessobrycon klausanni* lives in shallow (0.30–1.5 m) well-oxygenated (6.39–7.68 mg/l) streams with transparent waters flowing (0.063 m/s) over different types of substrates (rocks, sand, gravel and decomposing organic material). The temperature range was narrow, 25.5–26°C but pH varied from moderately acidic to basic (6.47–8.7). *Hyphessobrycon klausanni* was found near shore among aquatic vegetation, tree roots and fallen logs. Other species found at the sites included: *Hemigrammus barrigonae*, *Ctenobrycon spilurus*, *Tyttocharax metae*, *Moenkhausia oligolepis*, *M. chrysargyrea*, *M. lepidura*, *Chrysobrycon guahibo*, *Ochmacanthus orinoco*, *Farlowella vittata*, *Ancistrus triradiatus*, *Centromochlus reticulatus* and *Anablepsoides* sp. Stomach content analysis (n = 4) revealed a diet of aquatic and terrestrial invertebrates: Coleoptera (Dytiscidae), Ephemeroptera, Hymenoptera (Formicidae), larvae of Chironomidae and others not identified due to the degree of fragmentation.

Discussion

Although in our opinion the artificial groupings based on pigmentation patterns proposed by Géry (1977) lack a systematic basis upon which to evaluate relationships

Table 3. Eigenvector for first four principal components (PC) between *Hyphessobrycon klausanni* sp. n., *H. acaciae*, *H. mavro*, *H. metae* and *H. niger*. Abbreviations same as Table 1.

	PC 1	PC 2	PC 3	PC 4
SL	0.036926	0.11125	0.053717	0.15823
TL	0.014453	0.11428	0.12973	0.1757
Bd	0.0014034	0.2336	0.19898	0.10276
Sndorsalfin	0.035809	0.15093	0.19889	0.077996
Snpectoralfin	-0.029764	0.12309	0.14127	-0.055979
Snpelvicfin	0.00039923	0.17469	0.19359	-0.035305
Snanalfin	0.10796	0.19393	0.084414	-0.03285
Dorfinhyp	-0.049318	0.15887	0.12493	0.34098
Doranafin	0.020214	0.22269	-0.017732	0.24495
Dorpecfin	0.16087	0.1257	0.18137	0.021098
Dfl	-0.042492	0.088703	-0.12982	0.0055815
Pecfl	-0.17901	0.13165	-0.31013	0.37798
Pelfl	0.016911	0.3717	0.12014	-0.010603
Anfl	-0.12234	0.20629	-0.10574	0.209
CDP	0.15708	0.24308	0.21375	0.093422
CPL	-0.69393	0.34328	-0.0388	-0.34286
HL	-0.016057	0.13764	-0.11393	0.29254
SnL	-0.11936	0.32028	0.22983	-0.24192
ED	-0.043155	-0.076829	0.19662	0.4488
PostOL	0.54766	0.26111	-0.013493	-0.21286
ML	0.044176	0.24261	-0.56997	0.079634
IW	0.17908	0.1701	-0.10416	-0.15303
UJL	0.22414	0.23888	-0.40194	-0.10821

among species of *Hyphessobrycon*, some recent contributions use these criteria to segregate groups of species (Lima et al. 2014, García-Alzate et al. 2015, Ohara and Lima 2015, Zarske 2015) in an attempt to clarify the alpha taxonomy that forms the basis of our systematic study of this group. Among these species groups we find those defined by the presence of a dark longitudinal stripe (group e, Géry 1977: 470) which is further subdivided into the *Hyphessobrycon agulha*-group: including species with the lower half of the body all dark, especially above the anal fin, an usually horizontally elongate humeral spot that is more or less united with an asymmetrical, broad lateral stripe (Géry 1977: 470), the *H. agulha*-group was recently defined by Ohara and Lima (2015: 568) to include species that have a broad, relatively diffuse lateral stripe (typically more discernible ventrally, posterior to the midbody) and a humeral blotch that may or may not coalesce with the stripe, included the following species in this group: *H. agulha*, *H. herbertaxelrodi*, *H. metae*, *H. peruvianus*, *H. mutabilis* and *H. loretoensis* (in the latter two, the humeral blotch may or may not coalesce with the stripe), which also includes the following species: *H. clavatus*, *H. lucenorum*, *H. vanzolinii*, *H. margaritae* and *Hyphessobrycon klausanni* sp. n. Also, Lima et al. (2014) define the *Hyphessobrycon*

heterorhabdus-group: to include species that share a well-defined, elongated humeral blotch, which is continuous with a midlateral, well-defined dark stripe that becomes blurred towards the caudal peduncle and also have a longitudinal red stripe, extending along the body above the midlateral line, as well as on the upper half of the eye red in living specimens that includes: *H. heterorhabdus*, *H. amapaensis*, *H. eschwartzae* and *H. montagi*. *Hyphessobrycon klausanni* is similar to *H. metae*, with which it shares a well-developed broad midlateral stripe and a well-developed humeral blotch. As mentioned by Ohara and Lima (2015), broad phylogenetic studies on the genus and related genera are necessary to evaluate its putative relationships, but these two species groups could be a starting point. As mentioned in the introduction, the genus *Hyphessobrycon* is paraphyletic and the new species described in this paper is not a member of the genus *Hyphessobrycon sensu stricto* (García–Alzate et al. 2013 a, b) which includes the type species *H. compressus* and related species in Central America and some from the Colombian Chocó region: *H. colombianus*, *H. sebastiani*, *H. condotensis*, *H. chocoensis* and *H. ecuadorensis*. A phylogenetic reconstruction is needed that includes the type species and related Central American species to begin to unravel the phylogenetic relationships of the species of *Hyphessobrycon sensu lato*.

Rapid taxonomic description of the many as yet unnamed fish species of the Neotropical ichthyological biodiversity is urgently needed given the accelerated rate of extirpation caused by human impacts in many aquatic ecosystems in the Orinoco River Basin. The loss of habitat for fish species is caused by many different human activities such as dam construction, urban water pollution, mining, poor agricultural and animal husbandry practices, the introduction of non-native species and overfishing (Barletta et al. 2012). The Guaviare River drainage in Colombia is an area of high priority for conservation (Machado-Allison et al. 2010), and even with greater recent efforts to sample this system, our knowledge of its fish fauna remains poor, categorized as medium (30–38%) by Portocarrero-Aya et al. (2014). In this contribution a new species, *Hyphessobrycon klausanni*, is described which shows that new taxa will continue to be discovered as more and better inventories are carried out in the upper Guaviare. This also confirms that the Guaviare River drainage remains a priority area for conservation and ichthyological exploration, with regards to species richness and endemism. However, the streams of the upper Guaviare are being impacted by water extraction, and conversion of riparian forests into cattle pastures and monoculture crops such as oil palms. These impacts will undoubtedly be reflected as changes in the composition, richness and ecosystem functions of the fish community and in some cases may eventually lead to the extinction of some species before they have even been discovered or described.

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DATA PAPER

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Demersal and pelagic species of fish and squid from the Patagonian shelf

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Abstract

The dataset contains 2007 records of occurrence of 39 species of fish and 2 species of squid distributed on the Patagonian continental shelf and slope. This dataset describes a new and revised version of the original data published through OBIS with individual morphometrics. Specimens are representative of pelagic, demersal, demersal-pelagic, demersal-benthic and benthic habits and they were collected by commercial fishing vessels in autumn (May–June, 2001, 51 catches), winter (July–August, 2001, 38 catches) and summer (January-February, 2002, 112 catches). The sampling was carried out with bottom trawls at a depth range of 73–370 m. The survey was located between 39°–52°S and 55°–65°W.

Keywords

Occurrence, Patagonia, teleosts, elasmobranches, cephalopods, Southwestern Atlantic Ocean, demersal habitat, pelagic habitat

Data published through

GBIF: http://arobis.cenpat-conicet.gob.ar:8081/resource?r=argentina-fishes

Introduction

The Patagonian continental shelf, in the Atlantic margin of South America, is characterized by a remarkable productivity accounted by its oceanographic and bathymetric features, promoting 'hotspots' of biodiversity and attracting marine top predators, such as sea birds and marine mammals. The objective of this dataset is to provide basic information (location and depth capture, and individual morphometrics) of potential prey species of marine top predators from the Patagonian shelf, representative of pelagic, demersal, demersal-pelagic, demersal-benthic and benthic habits as useful information for ecological and biogeographical studies.

Taxonomic coverage

General taxonomic coverage description: All specimens were identified to species level. The dataset included 39 marine species of fish, representative of half the best-known fish species from the Argentine continental shelf (55.7 %, Cosseau and Perrota 2013), and 2 species of squid, that were captured during three seasons, autumn (May-June, 2001), winter (July-August, 2001) and summer (January–February, 2002). Autumn and summer records presented a high diversity of species (80.5 and 78 %, respectively), while only 46% of the species were present in winter (Figure 1).

Taxonomic ranks

Kingdom: Animalia
Phylum: Chordata
Class: Chondrichthyes
Order: Chimaeriformes, Carcharhiniformes, Squaliformes, Torpediniformes, Rajiformes.
Family: Callorhinchidae, Scyliorhinidae, Squalidae, Torpedinidae, Rajidae
Genera: Callorhinchus, Schroederichthys, Squalus, Discopyge, Bathyraja, Dipturus, Psammobatis.
Species: *Callorhinchus callorhynchus*, *Schroederichthys bivius*, *Squalus acanthias*, *Discopyge tschudii*, *Bathyraja brachyurops*, *Bathyraja albomaculata*, *Bathyraja scaphiops*, *Bathyraja macloviana*, *Dipturus chilensis*, *Psammobatis scobina*, *Psammobatis normani*.
Class: Actinopterygii

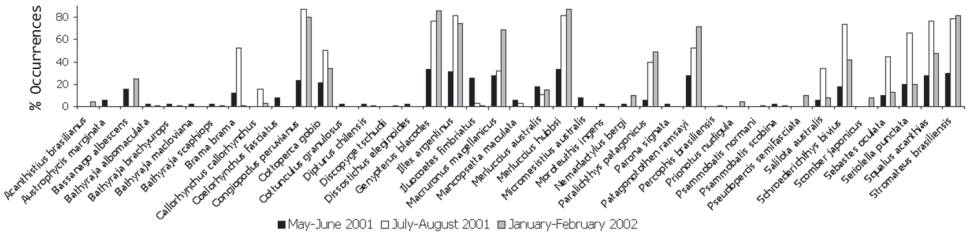

Figure 1. Distribution of occurrences of species during three seasons. Occurrence was calculated in each season as: (number of localizations where the species was present/ total number of localizations)*100.

Order: Anguilliformes, Gadiformes, Ophidiiformes, Pleuronectiformes, Perciformes, Scorpaeniformes.

Family: Congridae, Macrouridae, Moridae, Merlucciidae, Gadidae, Ophidiidae, Paralychthydae, Achiropsettidae, Scombridae, Centrolophidae, Stromateidae, Pinguipedidae, Carangidae, Serranidae, Bramidae, Cheilodactylidae, Bovichtidae, Nototheniidae, Scorpaenidae, Congiopodidae, Triglidae, Zoarcidae, Psychrolutidae.

Genera: Bassanago, Coelorhynchus, Salilota, Austrophycis, Macruronus, Merluccius, Micromesistius, Genypterus, Paralichthys, Mancopsetta, Scomber, Seriolella, Stromateus, Pseudopercis, Parona, Acanthistius, Brama, Nemadactylus, Cottoperca, Dissostichus, Patagonotothen, Sebastes, Congiopodus, Prionotus, Iluocoetes, Cottunculus.

Species: *Bassanago albescens*, *Coelorhynchus fasciatus*, *Salilota australis*, *Austrophycis marginata*, *Macruronus magellanicus*, *Merluccius hubbsi*, *Merluccius asutralis*, *Micromesistius australis*, *Genypterus blacodes*, *Paralichthys patagonicus*, *Mancopsetta maculata*, *Scomber japonicus*, *Seriolella punctata*, *Stromateus brasiliensis*, *Pseudopercis semifasciata*, *Parona signata*, *Acanthistius patachonicus*, *Brama brama*, *Nemadactylus bergi*, *Cottoperca gobio*, *Dissostichus eleginoides*, *Patagonotothen ramsayi*, *Pseudopercis*, *Sebastes oculata*, *Congiopodus peruvianus*, *Prionotus nudigula*, *Iluocoetes fimbriatus*, *Cottunculus granulosus*.

Phylum: Mollusca

Class: Cephalopoda

Order: Oegopsida

Family: Ommastrephidae, Onychoteuthidae.

Genera: Illex, Moroteuthis.

Species: *Illex argentinus*, *Moroteuthis ingens*.

Spatial coverage

General spatial coverage: The Argentine continental shelf, in the Atlantic margin of South America (Figure 2), is bounded by the line of the coast and the continental slope and is the most extensive submerged plain in the world, with an area of 930.000 km^2

(Cousseau and Perrotta 2013). It is characterized by gentle slopes and low-relief. The shelf waters are of sub-Antarctic origin diluted by continental flows and modified by exchanges of mass and heat with the atmosphere. The main distribution of the species of this dataset is:

- The internal and external sectors of the shelf off the Province of Buenos Aires (35° S) to Patagonia (48° S).
- The southern part of the Patagonian-Fuegian shelf and Malvinas/Falkland Islands, along the outer shelf to the North, up to approximately 42° S.
- Deeper waters near the continental slope.

Coordinates: 52°S and 39°S Latitude; 65°W and 55°W Longitude.
Temporal coverage: May 11, 2001–June 26, 2001, July 21, 2001–August 31, 2001, January 11, 2002–February 27, 2002.

Methods

Study extent description

The sampling area was located on the Patagonian continental shelf (Figure 2), within 52 to 39°S and 65 to 55°W. This is the largest submerged plain in the Southern Hemisphere (930.000 km^2), characterized by relatively shallow depth (mostly 150 m deep). Productive zones are associated with major water mass transitions, currents nutrient flow connected to upwelling and bathymetric features (Cosseau and Perrota 2013). The different water masses promote the recycling of nutrients accounting for its high productivity (Podesta et al. 1991, Carreto et al. 1995). The productivity of this area generates 'hotspots' of biodiversity and foraging of marine top predators is regularly observed (Croxall and Wood 2002, Campagna and Croxall 2003, Bastida and Rodríguez 2005). The specimens of this work were obtained through autumn (May-June, 2001), winter (July-August, 2001) and summer (January-February).

Sampling description

Specimens of fish and squid (commercial target and by catch) were taken daily by commercial fishing vessels operating with bottom trawls during autumn (May-June, 2001, 51 catches), winter (July-August, 2001, 38 catches) and summer (January-February, 2002, 112 catches). The fishing company provided the associated data of each fishing haul: date, hour, location (decimal Latitude and Longitude of the position while pulling the net) and depth of the catch (the maximum depth reached by the net). Specimens were frozen on board, and identified at species level at the Ichthyology laboratory of Centro Nacional Patagónico, Puerto Madryn, Argentina.

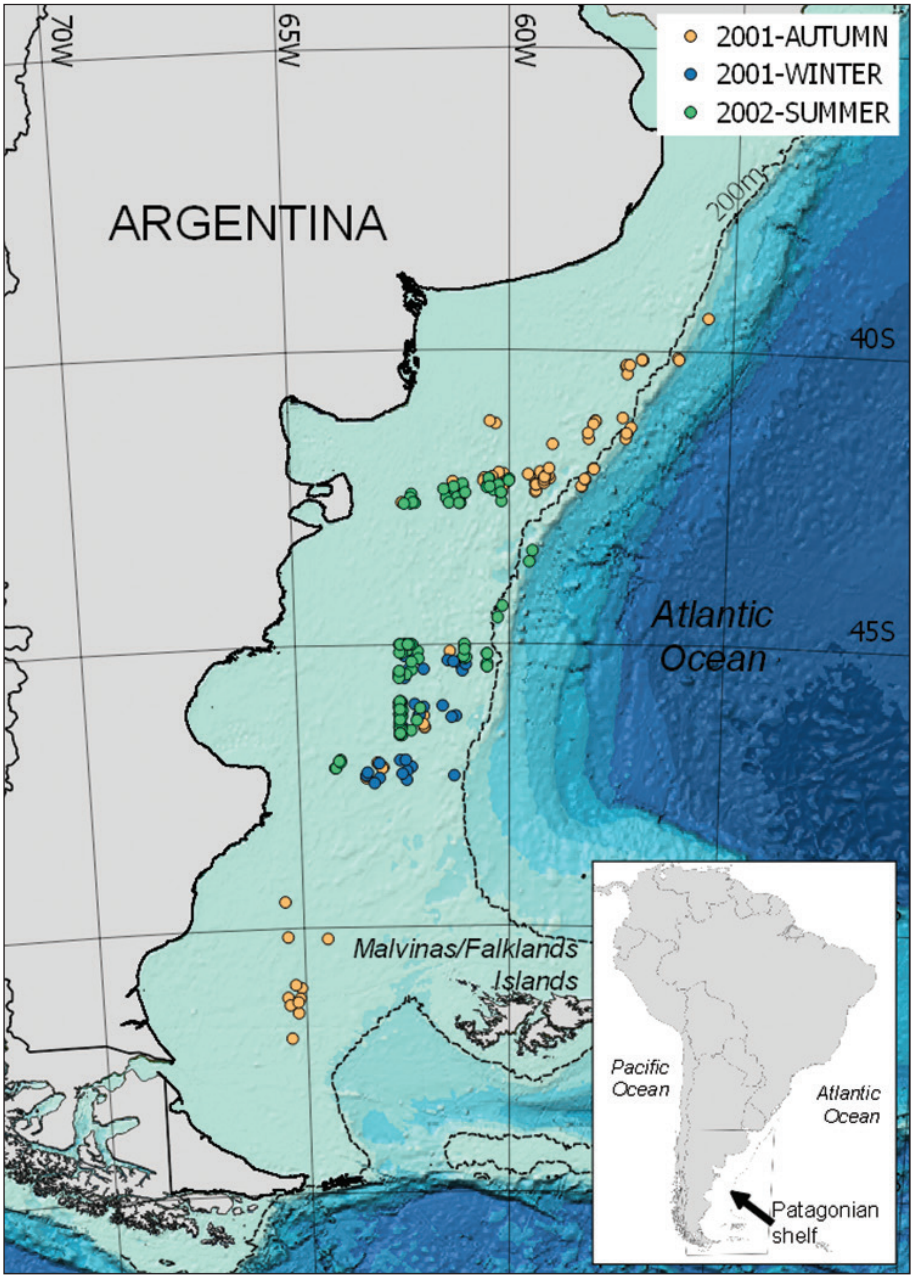

Figure 2. Spatial coverage (sampling locations).

The taxonomical identification of species was made by the specialists Dr A. Gosztonyi and Dr M. Re and the scientific names and their current accurate spelling were also reviewed using suitable literature (Brunetti et al. 1998; Cousseau and Perrotta 2013) and the WoRMS web site (http://www.marinespecies.org/).

Sex (when possible) and morphometric measures were taken for each specimen: wet mass (g) and wet mass of viscera (g, empty stomach); total and standard length (cm) for fish; mantle, head and fin length and width (cm) for squid; left and right fin length (cm) and maximum fin width (cm) for skates.

Method step description

Step1: Sampling locality and depth were recorded in each season.
Step2: Specimens were sent to the lab for species and sex identification and morphometric measurements.

Datasets

Dataset description

The star schema used to arrange the data has a DwC Event Core (seasons and catches) and two extensions: Occurrence (species) and ExtendedMeasurementOrFacts (individual morphometrics) (http://bdj.pensoft.net/articles.php?id=10989). The Darwin Core terms included in each file are:

Event core: type, eventID, parentEventID, samplingProtocol, eventDate, locationID, waterBody, locality, minimumDepthInMeters, decimalLatitude, decimalLongitude.
Occurrence (extension): modified, institutionCode, collectionCode, basisOfRecord, occurrenceID, catalogNumber, sex, occurrenceStatus, eventID, identifiedBy, scientificNameID, scientificName, kingdom, phylum, class, order, family, genus, specificEpithet, scientificNameAuthorship.
ExtendedMeasurementOrFacts (extension): occurrenceID, measurementType, measurementTypeID, measurementValue, measurementUnit, measurementUnitID, measurementDeterminedDate, measurementDeterminedBy.

Object name: Darwin Core Archive Demersal and pelagic species of fish and squid from the Patagonian shelf
Character encoding: UTF-8
Format name: Darwin Core Archive format
Format version: 1.0
Distribution: http://arobis.cenpat-conicet.gob.ar:8081/archive.do?r=argentina-fishes

Publication date of data: 2016-11-25
Language: English

Metadata language: English
Date of metadata creation: 2016-09-07
Hierarchy level: Dataset

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