

A new species of *Pentamera* Ayres, 1852 from the Brazilian coast (Holothuroidea, Dendrochirotida, Phyllophoridae)

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Abstract

Pentamera paraibanensis **sp. n.** is described from more than 3000 specimens as a new species of Phyllophoridae from northeast Brazil, in the tropical southwestern Atlantic. It is distinguished from its congeners by the shape of the calcareous ring with moderate posterior processes, and by the tables in the body wall with a usually quadrilocular disc and a low, toothed spire composed of two pillars. The body is brown, with the tube feet in double rows per ambulacrum, and tentacles more lightly colored. The species was found inside rodoliths in large numbers. This paper contains a morphological description of the specimens, and an account of their habitat characteristics.

Resumo

Pentamera paraibanensis **sp. n.** é descrita com cerca de 3000 espécimes como uma nova espécie de Phyllophoridae proveniente do Nordeste do Brasil, no Atlântico Sul tropical. Distingue-se das outras espécies do gênero pela forma do anel calcário com processos posteriores moderados, e pelas torres da parede do corpo com um disco geralmente quadrilocular e uma espira curta composta por dois pilares, denteada no ápice. O corpo é marrom, os pés ambulacrais estão dispostos em fileiras duplas por ambulacro, e tentáculos tem coloração mais clara. A espécie foi encontrada em grande quantidade dentro de rodolitos. Esse trabalho contém uma descrição morfológica dos espécimes, e informações sobre as características de seu habitat.

Keywords

Sea cucumber, southwest Atlantic Ocean, taxonomy

Palavras-chave

Holotúria, Oceano Atlântico Sul, taxonomia

Introduction

The order Dendrochirotida contains most of the world's described holothuroids. In Brazilian waters, it is represented by the families Psolidae, Cucumariidae, Sclerodactylidae and Phyllophoridae (Tommasi 1974, Tommasi et al. 1988a, b, Martins et al. 2012, Ventura et al. 2012). Phyllophoridae (sensu Pawson and Fell 1965) have 10 to 25 tentacles, with tube feet scattered over the entire body or restricted to the radii and a calcareous ring complex with usually radial plates of medium to large size, subdivided into several pieces (Thandar 1990).

Östergren (1907) considered phyllophorids as a subfamily within the Cucumariidae, based on the arrangement of the tentacles. Later, Heding and Panning (1954) revised the family Phyllophoridae, which they subdivided into several subfamilies. Pawson and Fell (1965) regrouped the families of Dendrochirotida based on the shape of calcareous ring (simple or complex) and tentacle number. That review included within the Phyllophoridae the subfamilies Phyllophorinae, Semperiellinae and Thyoninae. Recently Smirnov (2012) considered Thyoninae as differing from other Phyllophoridae on the basis of the number of tentacles and the morphology of the calcareous ring complex. He raised its rank to family level. Several changes in the taxonomy of the Holothuroidea occurred through time, mainly in Dendrochirotida. However, many questions still remain unresolved until now. As more knowledge is produced, answers to some of these open questions will be provided.

Ayres (1852) erected the genus *Pentamera* to accommodate species with oval body, with tube feet in the radii and ossicles of the body wall that are smaller than those present in *Thyonidium* Dübén & Koren, 1846. *Pentamera* includes 19 species, most from the Pacific Ocean. In the Western Atlantic only the type species *Pentamera pulcherrima* Ayres, 1852 was hitherto recorded. Species of *Pentamera* are generally of small size with tube feet restricted to the radii and calcareous ring with moderate to long posterior processes and tables from body wall composed of two pillars (Cherbonnier 1951).

Only seven species of Phyllophoridae were known previously from the Southwestern Atlantic: *Pentamera pulcherrima* Ayres, 1852, *Euthyonidiella occidentalis* (Ludwig, 1875), *Neothyonidium parvum* (Ludwig, 1881), *Stolus cognatus* (Lampert, 1885), *Thyone pawsoni* Tommasi, 1972, *Thyone pseudofusus* Deichmann, 1930 and *Thyone montoucheti* Tommasi, 1971. All these species occur along the Brazilian coast.

Numerous specimens associated with rodoliths from the coast of Paraíba, northeast Brazil, represent a new species.

Abbreviations

- UFPB.ECH** Echinodermata Collection of Federal University of Paraíba
UFSITAB Echinodermata Collection from Federal University of Sergipe

Material and methods

The examined material is deposited in the Collection of Invertebrates Paulo Young, of the Department of Systematics and Ecology, Federal University of Paraíba (CIPY/DSE–UFPB), and in the Echinodermata Collection, of the Department of Biosciences, Federal University of Sergipe, Brazil. The methods used to study the specimens followed Rowe and Doty (1977), Pawson et al. (2010), and Thandar (1989). Tissue fragments for the extraction of ossicles were immersed in a 3% solution of sodium hypochlorite, washed five times in distilled water, and rinsed with absolute ethanol. Permanent slides were prepared and were studied by light microscope. Other samples were dried and mounted on metal stubs, coated with gold and observed with FEI Quanta 200F scanning electron microscope. Photographs of specimens were taken using a Canon Powershot A2000IS digital camera, and a Leica MZ12.5 stereomicroscope. Measurements were obtained from fixed specimens.

Systematics

Order Dendrochirotida Grube, 1840

Family Phyllophoridae Östergren, 1907

Subfamily Thyoninae Panning, 1949

Genus *Pentamera* Ayres, 1852

Diagnosis. Small to medium sized. Ten tentacles, with two ventral ones smaller. Tube feet only in the radii, elongated, slightly retractile. Calcareous ring with moderate to long posterior processes. Body wall with tables of two pillars or derivatives of these, sometimes accompanied by plates. Tube feet with large endplates and curved supporting tables varying from low to high spire. Tentacles with rods, plates or rosettes (modified after Deichmann 1941, Lambert 1998).

Remarks. The diagnosis has been modified to include the new species and the fact that the type and some other species have plates in the body wall in addition to tables. *Pentamera paraibanensis* sp. n. has posterior processes of medium size and rosettes in the tentacles.

***Pentamera paraibanensis* sp. n.**

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Figs 1, 2, 3

Type specimen. Holotype, UFPB.ECH-2229, João Pessoa, Paraíba State, Brazil, 7°05'01"S; 34°47'56"W, 10 m, associated to rhodoliths, 9 March 2006.

Type locality. João Pessoa, Paraíba State, Brazil, 7°05'01"S; 34°47'56"W, 10 m, associated with rhodoliths, 9 March 2006.

Other type material. Paratype, UFPB.ECH-2230, João Pessoa, Paraíba State, Brazil, 7°05'01"S; 34°47'56"W, 6 March 2006; Paratype, UFPB.ECH-2061, João Pessoa, Paraíba State, Brazil, 7°03'48"S; 34°45'10"W, 15 m, 21 March 2006; Paratype, UFPB.ECH-2048, João Pessoa, Paraíba State, Brazil, 07°05'05"S; 34°44'21"W, 12 m, 24 June 2005; Paratype, UFPB.ECH-2058, João Pessoa, Paraíba State, Brazil, 7°07'00"S; 34°43'54"W, 14 March 2006; Paratype, UFPB.ECH-2089, João Pessoa, Paraíba State, Brazil, 7°03'50"S; 34°47'19"W, 10 m, 21 March 2006.

Additional material. UFPB.ECH-2088, João Pessoa, Paraíba State, Brazil, 6°59'01"S; 34°47'23"W, 10 m, 6 spec., 7 March, 2006; UFPB.ECH-141, João Pessoa, Paraíba State, Brazil, 6°59'01"S; 34°47'23"W, 100 spec, 7 March 2006; UFPB.ECH-148, João Pessoa, Paraíba State, Brazil, 6°59'00"S; 34°46'41"W, 4 spec, 7 March 2006; UFPB.ECH-1684, João Pessoa, Paraíba State, Brazil, 6°59'01"S; 34°45'12"W, 20m, 1 spec., 7 March 2006; UFPB.ECH-145, João Pessoa, Paraíba State, Brazil, 7°01'02"S; 34°47'55"W, 86 spec, 6 March 2006; UFPB.ECH-149, João Pessoa, Paraíba State, Brazil, 7°01'00"S; 34°46'02"W, 2 spec., 6 March 2006; UFPB.ECH-140, João Pessoa, Paraíba State, Brazil, 7°03'50"S; 34°47'19"W, 165 spec., 21 March 2006; UFPB.ECH-143, João Pessoa, Paraíba State, Brazil, 7°03'50"S; 34°47'19"W, 400 spec., 7 March 2006; UFPB.ECH-150, João Pessoa, Paraíba State, Brazil, 7°03'48"S; 34°45'10"W, 31 spec., 21 March 2006; UFPB.ECH-153, João Pessoa, Paraíba State, Brazil, 7°03'49"S; 34°43'12"W, 31 spec., 21 March 2006; UFPB.ECH-204, João Pessoa, Paraíba State, Brazil, 7°04'24,4"S; 34°47'49"W, 6 m, 42 spec., June 2005; UFPB.ECH-858, João Pessoa, Paraíba State, Brazil, 7°7'25,2"S; 34°6'35,0"W, 23 spec.; UFPB.ECH-857, João Pessoa, Paraíba State, Brazil, 7°8'28,836"S; 34°46'34,118"W, João Pessoa, PB, Brazil, 1 spec., 4 October 2007; UFPB.ECH-2087, João Pessoa, Paraíba State, Brazil, 7°03'49"S; 34°47'19"W, 1 spec., 21 March 2006; UFPB.ECH-205, Picãozinho, North Point, João Pessoa, Paraíba State, Brazil, 1 spec., 12 June 2003; UFPB.ECH-2072, João Pessoa, Paraíba State, Brazil, 7°43'09"S; 34°45'00"W, 1 spec.; UFPB.ECH-2068, Coqueirinho Beach, Conde, Paraíba State, Brazil, 1 spec., 3 June 2008; UFPB.ECH-2059, João Pessoa, Paraíba State, Brazil, 07°07'00"S; 34°43'54"W, 1 spec., 11 March 2006; UFPB.ECH-2053, João Pessoa, Paraíba State, Brazil, 07°05'05"S; 34°44'21"W, 12m, 5 spec., 24 June 2005; UFPB.ECH-2057, Reefs in front of the yacht club, Bessa Beach, João Pessoa, Paraíba State, Brazil, 1 spec., 26 February 2006; UFPB.ECH-2049, João Pessoa, Paraíba State, Brazil, 7°05'01"S; 34°47'56"W, 50 spec., 9 March 2006; UFPB.ECH-2052, João Pessoa, Paraíba State, Brazil, 7°05'S, 10 m, 15 spec., 22 February 2006; UFPB.ECH-2037, Cabo Branco Beach, João Pessoa, Paraíba State,

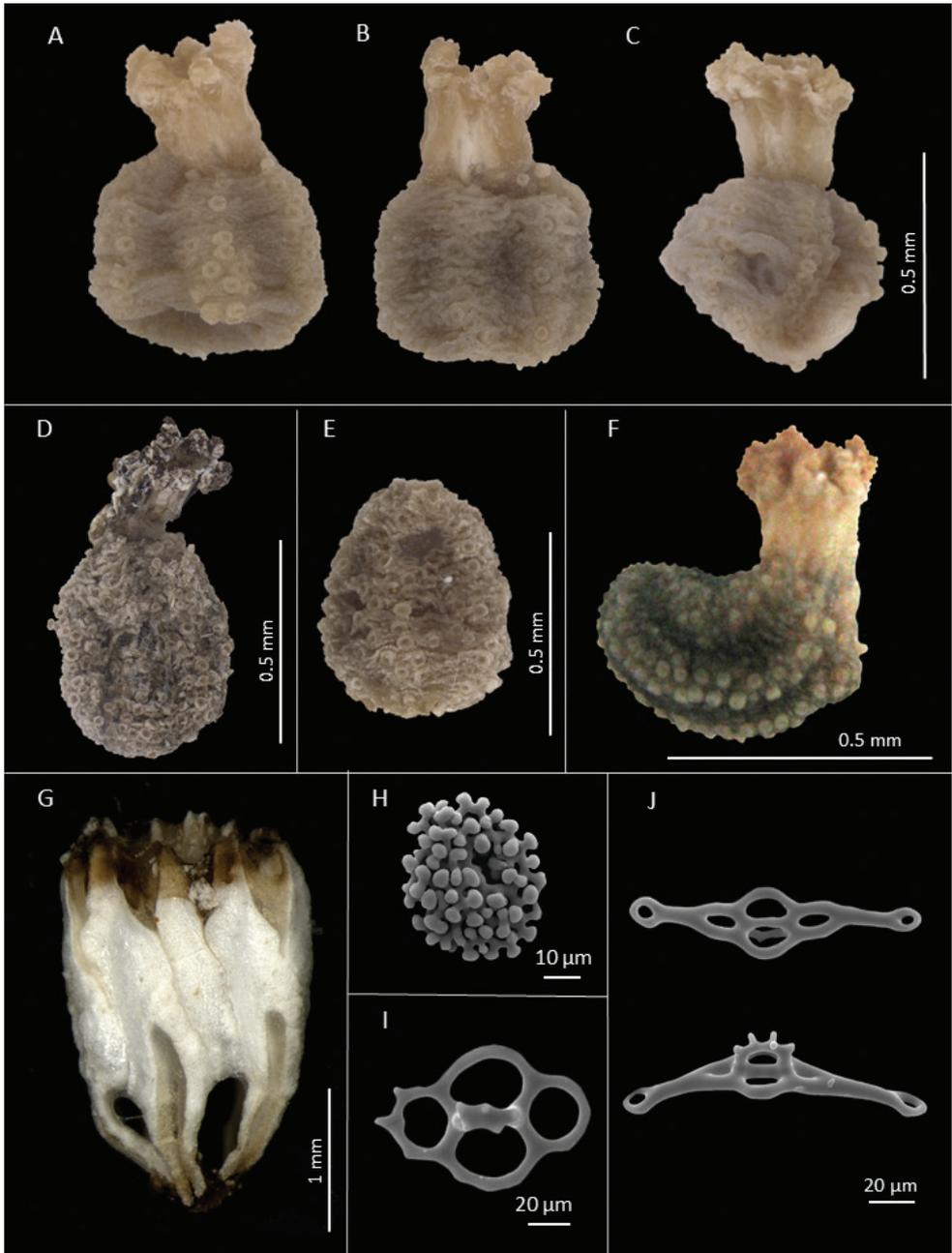


Figure 1. *Pentamera paraibanensis* sp. n. External view of holotype, **A** Dorsal **B** Ventral **C** Lateral **D** External view of specimen UFPB.ECH-2048, **E** External view of specimen UFPB.ECH-2058 **F** External view of specimen UFPB.ECH-2089. **G** Calcareous ring **H** Rosette of tentacles **I** Table from body wall **J** Support tables from tube feet.

Brazil, 1 spec., 17 September 2001; UFPB.ECH-2038, João Pessoa, Paraíba State, Brazil, 7°03'48"S; 34°45'W, 15m, 5 spec., 21 March 2006; UFPB.ECH-2033, João Pessoa, Paraíba State, Brazil, 7°01'02"S; 34°47'55"W, 10m, 13 spec., 6 March 2006; UFPB.ECH-2030, João Pessoa, Paraíba State, Brazil, 7°05'01"S; 34°47'56" W, 93 spec., 9 March 2006; UFPB.ECH-2031, João Pessoa, Paraíba State, Brazil, 7°05'59"S; 34°46'04"W, 10 m, 226 spec., 14 March 2006; UFPB.ECH-1683, João Pessoa, Paraíba State, Brazil, 7°05'05.1"S; 34°44'21"W, 12 m, 14 spec., 24 June 2005.

Diagnosis. Small body, reaching 7 mm, anterior and posterior ends slightly upturned. Color brown in life and in alcohol, tube feet light brown to white. Tube feet only in the radii. Tentacles ten, branched, two ventral ones smaller. Skin thin, smooth. Body wall ossicles comprise oval tables (with disc up to 64 μ m long) with four central holes, sometimes more elongated and also with smaller holes marginally and smooth multilocular plates; spire low, with two short pillars ending in 2–3 blunt teeth. Tube feet with supporting plates, curved support tables of variable height, and endplates. Tentacles with rosettes and rods. Introvert with rosettes.

Etymology. The species epithet is derived from the name of the State where it was collected (Paraíba State, Brazil).

Description of holotype. Specimen (female) small, globiform, slightly curved, length along the body 7 mm and breadth in mid-body 3 mm (Figure 1A, B, C). Preserved coloration brown, podia and tentacles light brown to white. Mouth upturned; anus terminal with five small papillae and five delicate anal teeth (Figure 3J). Tentacles extended, ten, well-branched, largest about 1.5–2 mm long, two ventral ones smaller. Tube feet restricted to radii, in double rows, longer in the ventral radii, shorter dorsally. Interambulacra usually naked, without papillae, warts or tubercles. Skin smooth, slightly translucent, with numerous small ossicles. Introvert thin, short, without tube feet.

Calcereous ring complex, not fragmented, with posterior processes elongated (Figure 1G). Radial plates longer than interradii, approximately 2 mm high and 0.5 mm wide, anterior part bifid, posterior processes with small pieces; interradii plates triangular anteriorly, with posterior margin convex, 1.3 mm high and 0.3 mm wide. Polian vesicle single, short, saccular, located slightly to left of ventral mesentery; stone canal thin, straight, elongated; madreporite well calcified, bean-shaped. Gonads in one tuft of several tubules attached anteriorly, unbranched but forming several saccules along the tubule, filling the entire body cavity, full of eggs in various stages of development. Longitudinal muscles thin; retractors also thin, more delicate. Respiratory trees confined to posterior quarter of body, with short branches.

Characteristic ossicles of body wall as oval tables with disc of usually four perforations and a low spire of two pillars (Figures 1I and 2D and H), 50–70 μ m long and 20–30 μ m high, ending in 2–3 teeth. Other body wall ossicles include some irregular smooth plates (Figure 3G). Tube feet ossicles of three types, supporting tables with curved disc with four central holes and 1–3 holes at ends, disc 128 μ m long and spire 30 μ m high (Figures 1J; 2F–G, and 3B–F). Elongate perforated plates, 99 μ m long and 40 μ m wide (Figure 2E and 3A), and rounded endplates with central perforations smaller than others, about 170 μ m in diameter (Figure 3H–I). Some large plates also occur

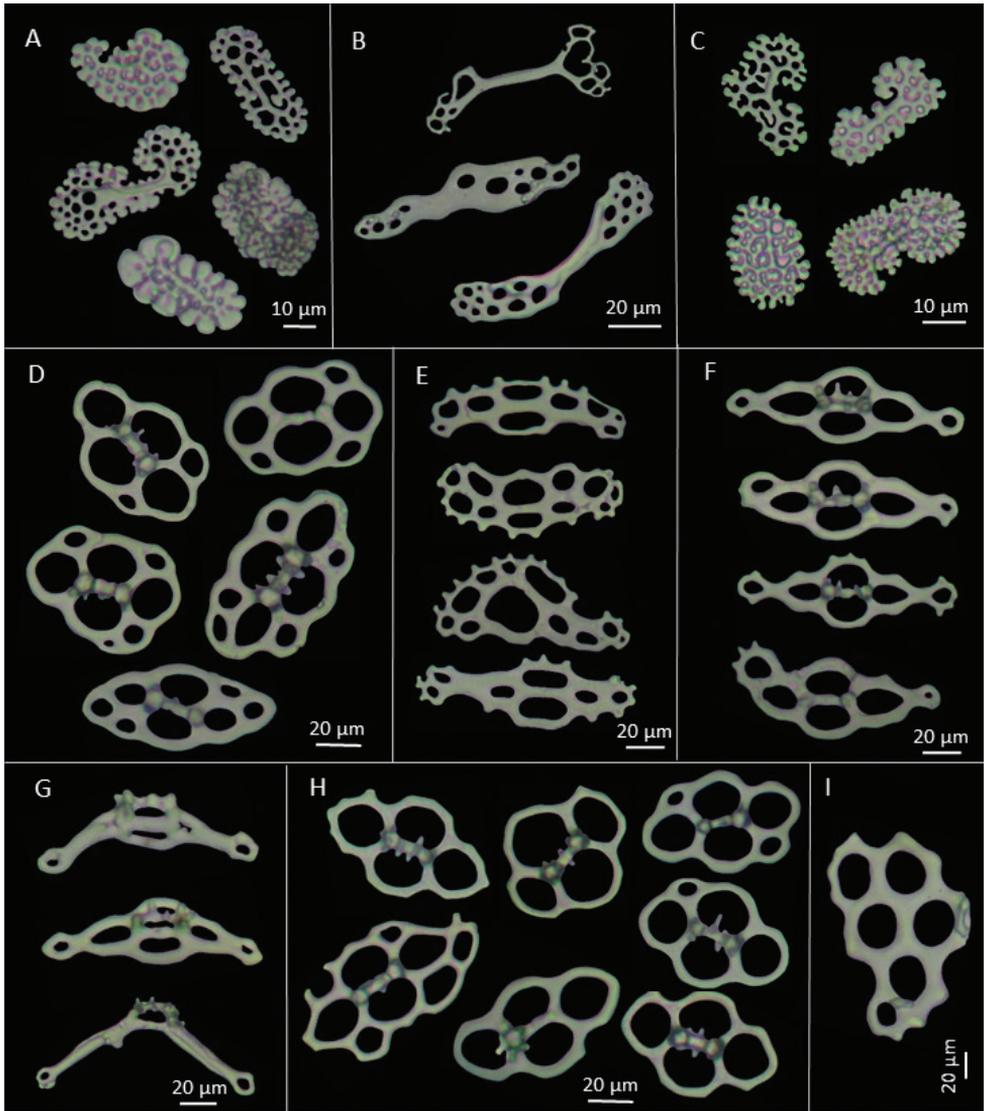


Figure 2. *Pentamera paraibanensis* sp. n. **A** Rosettes from tentacles **B** Rods from tentacles **C** Rosettes from introvert **D** Tables from dorsal body wall **E** Support plates from dorsal tube feet **F** Base of support tables from dorsal tube feet **G** Support tables from dorsal tube feet **H** Tables from ventral body wall, the more elongated table was found near the anus **I** Large plate of tube feet.

near the podia (Figure 2I). Introvert with rosettes only (Figure 2C). Tentacles with rods of various sizes, some delicate, with perforations at each end, some curved, others with four arms; irregular perforated plates, oblong, straight to slightly curved, medial perforations larger (Figure 2B) and rosettes similar to those of introvert (Figure 1H, 2A).

Morphometry. (See Table 1 below). Rosettes of tentacles are larger than those of introvert. In general, ossicles from dorsal surface larger than those from ventral surface.

Table 1. Ossicle morphometry of *Pentameru paraitbanensis* sp. n. SD, standard deviation; N, number of ossicles measured.

Tentacle				Introvert			
Rosette Length (µm)	Width (µm)	Rods Length (µm)	Width (µm)	Rosette Length (µm)	Width (µm)		
Mean	44.1	25.84	89.6	8.36	34.18	20.88	
SD	11.2	6.17	38.48	3.83	8.41	4.05	
N	40	40	40	40	40	40	

Anterior region dorsal								
	Table		Endplate Diameter (µm)	Height (µm)	Supporting tables		Supporting plates Length (µm)	Supporting plates Width (µm)
	Length (µm)	Width (µm)			Length (µm)	Width (µm)		
Mean	64.05	43.98	136.58	15.76	108.59	25.92	19.76	34.3
SD	6.38	4.11	18.14	3.28	9.64	3.39	3.58	5.65
N	40	40	20	40	30	30	30	20

Posterior region dorsal								
	Table		Endplate Diameter (µm)	Height (µm)	Supporting tables		Concave plates Length (µm)	Concave plates Width (µm)
	Length (µm)	Width (µm)			Length (µm)	Width (µm)		
Mean	60.43	42	120.86	16.84	99.58	30.5	20.09	55.65
SD	5.58	4.34	54	2.51	8.52	6.41	2.97	10.45
N	40	40	10	40	30	30	30	15

Anterior region ventral								
	Table		Endplate Diameter (µm)	Height (µm)	Supporting tables		Supporting plates Length (µm)	Supporting plates Width (µm)
	Length (µm)	Width (µm)			Length (µm)	Width (µm)		
Mean	60.78	42.21	114.33	15.85	109.17	25.28	19.88	26.95
SD	5.73	4.71	14.96	2.75	8.37	3.65	3.13	4.09
N	40	40	10	40	30	30	30	10

Posterior region ventral								
	Table		Endplate Diameter (µm)	Height (µm)	Supporting tables		Supporting plates Length (µm)	Supporting plates Width (µm)
	Length (µm)	Width (µm)			Length (µm)	Width (µm)		
Mean	58.14	40.57	140.46	18.37	108.08	26.51	17.47	28.06
SD	5.26	4.17	16.3	3.69	11.81	4.69	2.46	6.95
N	40	40	10	40	35	35	35	25

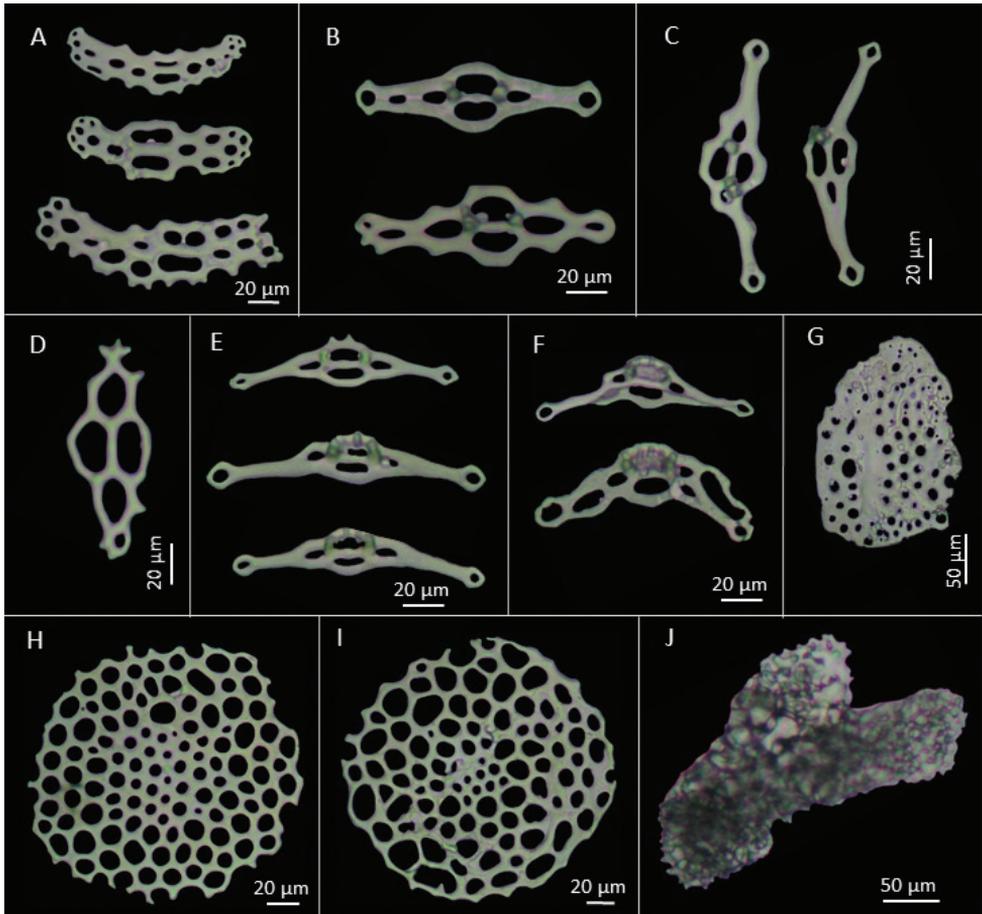


Figure 3. *Pentamera paraibanensis* sp. n. **A** Support plates from ventral body wall **B** Base of support tables from ventral tube feet **C** Curved base of support tables from ventral tube feet **D** Support plate of tube feet **E** Support tables from ventral tube feet **F** Curved support tables from ventral tube feet **G** Large plate from body wall near the anus **H** Endplate from dorsal tube feet **I** Endplate from ventral tube feet **J** Anal tooth.

Dorsally, tables of anterior region are larger than of posterior region but their spire is lower. Endplates are also larger anteriorly. Supporting tables of the podia are smaller in posterior region, but are wider and higher anteriorly. On ventral surface, the tables from anterior part are slightly larger than posteriorly. Endplates of the postero-ventral surface are larger than the anterodorsal surface, about 140 μm in diameter. The supporting tables are slightly larger and taller anteriorly, but wider posteriorly (108.08 \times 26.51 \times 17.47 μm). The supporting plates from the posterior region are larger, approx. 103 μm .

Description of paratypes. The paratypes are from 0.4 to 1 cm long. The ossicles of the body wall and other parts of the body are similar. Some tables are more elongated or have more than four perforations. The color varies from light to dark brown. Some specimens have their body dark brown and their tube feet light brown (Figures 1D–F).

Color variations. A total of 3225 specimens was examined, measuring 3–13 mm long and 3–3.5 mm wide in the mid part, and 1–2.5 mm at the ends were examined. In general, they all present a curved form, but some specimens are elongated or only slightly curved. The body wall is dark to light brown in color, sometimes with dark spots, the tube feet varying from whitish to yellowish, and the tentacles with translucent peduncles and brown to yellow branches. Most specimens present a brown coloration, with some dark brown spots and whitish tube feet.

Distribution. Bessa beach, reefs of Picãozinho, Cabo Branco beach, in Municipality of João Pessoa; Coqueirinho Beach, in municipality of Conde; with coordinates 6°59'01"S; 34°45'12"W and 7°43'09"S; 34°47'56"W, coast of Paraíba State, Brazil. Species found over the continental platform of the State of Paraíba, Brazil, up to 20 m deep.

Habitat. Most specimens were inside rhodoliths, but some samples were associated with *Halimeda* sp., were part of the phytal of *Hypneia* sp., or came from a rocky bottom.

Remarks. The new species seems to shed the calcareous ring when submitted to stress. Some specimens were without the tentacles and calcareous ring, and most of them presented tentacles and the calcareous ring totally extended outside the body. This seems a defense tactic of this animal. The specimens studied agree with the diagnosis of genus *Pentamera* as amended by Lambert (1998). They share the structure of the calcareous ring and the type of body wall ossicles with other species currently classified in the genus *Pentamera*. *Pentamera paraibanensis* sp. n. with its double row of tube feet, body wall with tables with two pillars, and with the shape of the supporting tables and endplates, has parallels with other species of the genus. *Pentamera paraibanensis* sp. n. has similar tables as those of *Pentamera pediparva* and *Pentamera constricta*, but differs from both in the moderate calcareous ring, height of spire of supporting tables of tube feet, presence of rods and rosettes in tentacles and only rosettes in the introvert. In addition, these species have stiff and rough skin, while *P. paraibanensis* sp. n. has soft and smooth skin. We also compared the new species with other species of the genus *Pentamera*, as well as with other species of Phylloporidae reported from the South Atlantic directly or through specialized literature (e.g., Cherbonnier (1951), Deichmann (1930, 1938, 1941), Lambert (1998), and Tommasi (1969).

The new species *P. paraibanensis* sp. n. differs of *Pentamera beebei* Deichmann, 1938 and *Pentamera zacaе* Deichmann, 1938 by the absence of high pillars of the body wall tables; from *Pentamera chierchiae* (Ludwig, 1887) by the absence of rods in the introvert and tables with spinous disc; from *Pentamera chiloensis* (Ludwig, 1887) by the absence of quadrangular base of tables from the body wall, with pillars ending in several teeth; from *Pentamera calcigera* Stimpson, 1851 it can be distinguished by the absence of a dense layer of plates and by the form of the tables from the body wall; from *Pentamera charlottae* Deichmann, 1938 by the absence of small tables from the body wall; from *Pentamera lissoplaca* (Clark, 1924) by the absence of diamond-shaped tables and diminutive tables in the body wall. *P. paraibanensis* sp. n. differs from *Pentamera trachyplaca* (Clark, 1924) by the absence of thick oval knobbed plates; from *Pentamera*

pseudocalcigera Deichmann, 1938 by the absence of star-shaped plates in the body wall; and from *Pentamera rigida* Lambert, 1998 it may be clearly distinguished by absence of large thick tables, knobbed plates in introvert and the shape of the calcareous ring.

Pentamera paraibanensis sp. n. distinguishes of the other Phyllophoridae species recorded to South Atlantic, *Euthyonidiella occidentalis* (Ludwig, 1875), *Neothyonidium parvum* (Ludwig, 1881), *Stolus cognatus* (Lampert, 1885), *Thyone pawsoni* Tommasi, 1972 and *Thyone pseudofusus* Deichmann, 1930 by the form of the calcareous ring, arrangement of the tube feet on the body, and set of ossicles from body wall.

Key to the *Pentamera* species

- 1 Small to moderate form, cylindrical, podia in 5 bands, oval to elongated tables with four central holes and a short spire 2-pillared 2
- Small to moderate form, U-shaped to curved body, podia in 5 bands, circular to triangular tables, more of four central holes and short to tall spire 2-pillared 6
- 2 Supporting tables of tube feet with a short to medium spire 3
- Supporting tables of tube feet with a tall spire.....
..... *Pentamera charlottae* Deichmann, 1938
- 3 Moderate calcareous ring, supporting tables with medium spire
..... *Pentamera paraibanensis* sp. n.
- Long calcareous ring, supporting tables with low spire..... 4
- 4 oval tables with four central holes, smooth margin, without knobs and a short spire 2-pillared..... *Pentamera citrea* (Semper, 1867)
- Oval tables with four central holes, with knobs and a short spire 2-pillared ... 5
- 5 Small oval buttons with 2 central and up to 8 marginal knobs in body wall, tentacles with round to oblong plates with perforations and knobs
..... *Pentamera montereyensis* Deichmann, 1938
- Body wall with thick oval, knobbed plates with meshwork of bumps covering one side, without buttons, tentacles with oblong reticulate plates
..... *Pentamera trachyplaca* (Clark, 1924)
- 6 Small to moderate form, curved, tapering to blunt ends, posterior processes of calcareous ring moderate to long, body wall without triangular ossicles 7
- Moderate form, tapering in the ends, long posterior processes of calcareous ring, body wall with triangular ossicles..... 8
- 7 Crowded layer of acorn-like cups with 2-pillared, tapering spire rising from a cup-shaped base *Pentamera zacaе* Deichmann, 1938
- Without a Crowded layer of acornlike cups with 2-pillared, tapering spire rising from a cup-shaped base 9
- 8 Large, oval to triangular plates, rarely star-shaped tables, supporting tables with moderate spire, introvert with oval plates with serrate edge and blunt spines on surface *Pentamera pseudocalcigera* Deichmann, 1938

–	Circular to triangular or star-shaped tables with a wide central spire, supporting tables with low bumpy spire, introvert with elongated to oval plates with numerous bumps and raised central holes	<i>Pentamera rigida</i> Lambert, 1998
9	circular to oval tables, with smooth margin, with four or more central holes and short to tall spire 2-pillared	10
–	Elongated tables, with wavy margin, with four or more central holes and short to tall spire 2-pillared	<i>Pentamera constricta</i> (Ohshima, 1915)
10	Introvert with plates and/ or rosettes.....	11
–	Introvert with tables.....	<i>Pentamera pediparva</i> Lambert, 1998
11	Ossicles of body wall in one layer.....	12
–	Ossicles of body wall in two layers	<i>Pentamera lissoplaca</i> (Clark, 1924)
12	Tentacles with rosettes and plates	13
–	Tentacles with plates only	14
13	Tentacles with rosettes only	<i>Pentamera chiloensis</i> (Ludwig, 1887)
–	Tentacles with rosettes and plates	<i>Pentamera pseudopopilifera</i> Deichmann, 1938
14	Tentacles with irregular to oval perforated plates	15
–	Tentacles with elongated diamond-shaped plates with two large central holes, some with bumps or low pillar arch ...	<i>Pentamera popuilifera</i> (Stimpson, 1864)
15	Tentacles with irregular perforated plates.....	<i>Pentamera pulcherrima</i> Ayres, 1852
–	Tentacles with oval perforated plates with a meshwork at center.....	<i>Pentamera calcigera</i> Stimpson, 1851

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Morphological and molecular evidence indicate *Dendronotus primorjensis* is a valid species that has priority over *D. dudkai* (Nudibranchia)

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Abstract

Morphological and molecular data of type material of the nudibranch mollusc *Dendronotus primorjensis* Martynov, Sanamyan, Korshunova, 2015 from the Sea of Japan are summarised and compared with those of *D. dudkai* Ekimova, Schepetov, Chichvarkhina, Chichvarkhin, 2016. The clear conclusion is that the latter is a junior synonym of *Dendronotus primorjensis*.

Keywords

Dendronotus, Gastropoda, Mollusca, Nudibranchia, new synonym

Introduction

Martynov et al. (2015a, b) described the nudibranch *Dendronotus primorjensis* from the north-west part of the Sea of Japan. The original description of *D. primorjensis* provided numerous diagnostic features including external characters, detailed scanning electron microscopy images of the radula, as well as remarks about its molecular phylo-

genetic relationships. The format of that publication precluded publication of lengthy molecular analyses, even though they were performed. Subsequently, Ekimova et al. (2016) described *Dendronotus dudkai* as new from exactly the same region in which the type material of *D. primorjensis* was collected. Nevertheless Ekimova et al. (2016) considered *Dendronotus primorjensis* a *nomen dubium* and presented weak evidence of its invalidity, including allegations that the type material of *D. primorjensis* “are lost if ever existed” (Ekimova et al. 2016: 31). Original type material of *Dendronotus primorjensis* (holotype and paratype) has been stored in the Zoological Museum of Moscow State University since 2014.

In this publication analysis of combined molecular and morphological data of the holotype and paratype of *Dendronotus primorjensis* demonstrate that it is a valid species and has taxonomic priority over *D. dudkai*, published one year later.

Material and Methods

Type material collection

Type specimens of *Dendronotus primorjensis* (ZooBank: <http://zoobank.org/2001DB-85-2005-4E6F-8A21-F15F9068EC7D>) have been described previously (Martynov et al. 2015a, b) and were used for both morphological and molecular examinations. The holotype of *D. primorjensis* (ZMMU Op-419) and the paratype (ZMMU Op-420) were collected in the Sea of Japan, Spokoinaya Bay, at the depth of 2–6.5 m (rocks and algae) on 25 Sept 2014, by T.A. Korshunova and A.V. Martynov using SCUBA diving. Photographs of the living animals were taken by T.A. Korshunova within a day of collection. In addition, staff members of the Diving Center Aquamax (Nakhodka) made additional photographic records immediately upon completion of the collecting dive. These photographs are permanently and publicly available since 30 September of 2014 (Diving Center Aquamax 2014). Afterwards, specimens were fixed in 75% ethanol for morphological study and in 99% ethanol for molecular investigations, and registered in the collection of the Zoological Museum of Moscow State University (ZMMU) under the registration numbers Op-419 and Op-420.

Morphological analysis

All specimens of *D. primorjensis* were examined with a stereomicroscope (MBS-9), a digital camera (Nikon D-90) with a set of extension rings (Kenko), and scanning electron microscope (CamScan Series II) for the original description. The pharynx of the preserved holotype *D. primorjensis* (ZMMU Op-419) was removed and processed with a weak solution of domestic bleach (NaOCl). The radula of the holotype was examined under SEM at the electron microscopy laboratory of the Biological Faculty of Moscow State University, and these were also published in the original description.

Molecular analysis

Small pieces of foot tissue of both specimens of *D. primorjensis* (ZMMU:Op-419 and ZMMU:Op-420) were used for DNA extraction with Diatom™ DNA Prep 100 kit (Iso-gene Lab.) according to the producer's protocols. Extracted DNA was used as a template for the amplification of partial sequences of the mitochondrial genes cytochrome *c* oxidase subunit I (COI) and 16S, and also the nuclear gene 28S (C1-C2 domain). The primers that were used for amplification are: LCO 1490 (GGTCAACAAATCAT-AAAGATATTGG, Folmer et al. 1994); HCO 2198 (TAAACTTCAGGGTGAC-CAAAAAATCA, Folmer et al. 1994); 16S arL (CGCCTGTTTAACAAAAACAT, Palumbi et al. 1991); 16S R (CCGRITYTGAAGCTCAGCTCACG, Puslednik and Serb 2008); 28S C1' (ACCCGCTGAATTTAAGCAT, Dayrat et al. 2001); and 28S C2 (TGAAGTCTCTCTTCAAAGTTCTTTTC, Le et al. 1993). Polymerase chain reaction (PCR) amplifications were carried out in a 20- μ L reaction volume, which included 4 μ L of 5x Screen Mix (Eurogen Lab), 0.5 μ L of each primer (10 μ M stock), 1 μ L of genomic DNA, and 14 μ L of sterile water. The amplification of COI and 28S was performed with an initial denaturation for 1 min at 95°C, followed by 35 cycles of 15 sec at 95°C (denaturation), 15 sec at 45°C (annealing temperature), and 30 sec at 72°C, with a final extension of 7 min at 72°C. The 16S amplification began with an initial denaturation for 1 min at 95°C, followed by 40 cycles of 15 sec at 95°C (denaturation), 15 sec at 52°C (annealing temperature), and 30 sec at 72°C, with a final extension of 7 min at 72°C. Sequencing for both strands proceeded with the Big Dye v3.1 sequencing kit (Applied Biosystems). Sequencing reactions were analysed using an ABI 3500 Genetic Analyser (Applied Biosystems). Protein-coding sequences were translated into amino acids for confirmation of the alignment. Both sequences of *D. primorjensis* (ZMMU:Op-419 and ZMMU:Op-420) were deposited in GenBank. Original data and publicly available sequences were aligned with the MUSCLE (Edgar 2004) algorithm. For phylogenetic reconstruction 31 specimens were used. All of the species and their sequences are listed in Table 1.

Two different phylogenetic methods, Bayesian Inference (BI) and Maximum Likelihood (ML) were used to infer evolutionary relationships. Separate analyses were conducted for the following data sets: resulting alignments are 641 bp for COI, 451 bp for 16S, 350 bp for 28S, and 1442 for the concatenated datasets. Evolutionary models for each data set were selected using MrModelTest 2.3 (Nylander et al. 2004) under the Akaike information criterion (Akaike 1974). Bayesian estimation of posterior probability was performed in MrBayes 3.2. Markov chains were sampled at intervals of 500 generations. Analysis was started with random starting trees and 10⁷ generations. Maximum likelihood-based phylogeny inference was performed in GARLI 2.0 (Zwickl 2006) with bootstrap in 1000 pseudo-replications. The program TRACER v1.6 was used to examine the convergence results. Additionally, Automatic Barcode Gap Discovery (ABGD) (Puillandre et al. 2012) was used to define species. The ABGD program is available from <http://www.wabi.snv.jussieu.fr/public/abgd/abgdweb.html>. COI and 16S FASTA alignments were analysed separately (excluding outgroups) using both proposed models: Jukes-Cantor (JC69) and Kimura (K80). The program Mega7 (Kumar et al. 2016) was used to calculate the uncorrected COI p-distances between all the sequences.

Table 1. List of specimens used for phylogenetic analyses.

Species	Voucher	Locality	GenBank accession nos.		
			CO1	16S	28S
<i>Dendronotus primorjensis</i> Martynov et al., 2015 holotype	ZMMU:Op-419	Russia: Japan Sea	KX672010	KX672008	KX672006
<i>Dendronotus primorjensis</i> Martynov et al., 2015 paratype	ZMMU:Op-420	Russia: Japan Sea	KX672011	KX672009	KX672007
<i>Dendronotus dudkai</i> Ekimova et al., 2016	W195	Russia: Japan Sea	KT031811	KT031824	KT031841
<i>Dendronotus dudkai</i> Ekimova et al., 2016	W196	Russia: Japan Sea	KT031812	KT031825	KT031842
<i>Dendronotus dudkai</i> Ekimova et al., 2016	W197	Russia: Japan Sea	KT031813	KT031826	KT031843
<i>Dendronotus dudkai</i> Ekimova et al., 2016	W198	Russia: Japan Sea	KT031814	KT031827	KT031844
<i>Dendronotus dudkai</i> Ekimova et al., 2016	W199	Russia: Japan Sea	KT031815	KT031828	KT031845
<i>Dendronotus dudkai</i> Ekimova et al., 2016	W201	Russia: Japan Sea	KT031816	KT031829	KT031837
<i>Dendronotus dudkai</i> Ekimova et al., 2016	W202_1	Russia: Japan Sea	KT031817	KT031830	KT031838
<i>Dendronotus dudkai</i> Ekimova et al., 2016	W202_2	Russia: Japan Sea	KT031818	KT031831	KT031840
<i>Dendronotus dudkai</i> Ekimova et al., 2016	W203	Russia: Japan Sea	KT031819	KT031832	KT031839
<i>Dendronotus dalli</i> Bergh, 1879	ZMMU:Op-295	Russia: Kamchatka	KM397001	KM397083	KM397042
<i>Dendronotus dalli</i> Bergh, 1879	ZMMU:Op-330	Russia: Kamchatka	KM396999	KM397081	KM397040
<i>Dendronotus frondosus</i> (Ascanius, 1774)	ZMMU:Op-380	Norway	KM396976	KM397056	KM397017
<i>Dendronotus frondosus</i> (Ascanius, 1774)	ZMMU:Op-324	Russia: Barents Sea	KM396980	KM397062	KM397021
<i>Dendronotus kalikal</i> Ekimova et al., 2015	ZMMU:Op-284.3	Russia: Kamchatka	KM396988	KM397070	KM397029
<i>Dendronotus kalikal</i> Ekimova et al., 2015	ZMMU:Op-349.1	Russia: Kamchatka	KM396986	KM397068	KM397027
<i>Dendronotus kamchaticus</i> Ekimova et al., 2015	ZMMU:Op-246.2	Russia: Kamchatka	KM396989	KM397072	KM397030
<i>Dendronotus kamchaticus</i> Ekimova et al., 2015	ZMMU:Op-247.1	Russia: Kamchatka	KM396991	KM397073	KM397032
<i>Dendronotus lacteus</i> (W. Thompson, 1840)	ZMMU:Op-288	Russia: Barents Sea	KM396975	KM397059	KM397016
<i>Dendronotus lacteus</i> (W. Thompson, 1840)	ZMMU:Op-335	Russia: Barents Sea	KM396973	KM397057	KM397014
<i>Dendronotus niveus</i> Ekimova et al., 2015	ZMMU:Op-269	Russia: White Sea	KM396996	KM397078	KM397037

Species	Voucher	Locality	GenBank accession nos.		
			CO1	16S	28S
<i>Dendronotus niveus</i> Ekimova et al., 2015	ZMMU:Op-279	Russia: Barents Sea	KM396995	KM397077	KM397036
<i>Dendronotus patricki</i> Stout et al., 2011	SIO-BIC M12133	USA: California	HQ225828	HQ225829	–
<i>Dendronotus regius</i> Pola & Stout, 2008	CASIZ179492	Philippines	HM162708	HM162629	–
<i>Dendronotus robustus</i> Verrill, 1870	ZMMU:Op-343	Russia: Barents Sea	KM397002	KM397084	KM397043
<i>Dendronotus robustus</i> Verrill, 1870	ZMMU:Op-344	Russia: Barents Sea	KM397003	KM397085	KM397044
<i>Doto coronata</i> (Gmelin, 1791)	CASIZ176278	South Africa	HM162734	HM162657	–
<i>Doto koenneckeri</i> Lemche, 1976	CASIZ178247	Portugal	HM162735	HM162658	–
<i>Marionia arborescens</i> Bergh, 1890	CAS:177735	Philippines	KP226855	KP226859	–
<i>Tritonia challengeriana</i> Bergh, 1884	CASIZ171177	Atlantic Ocean: Bouvet Island	HM162718	HM162643	–

Results

Morphological characters of *Dendronotus primorjensis*

The holotype of *D. primorjensis* (ZMMU Op-419, Fig. 1A, B) and paratype (ZMMU Op-420, Fig. 1C, D) possess the following morphological features: body elongate, high, laterally compressed. Living length 21–35 mm. Oral veil narrow with 7–8 large branched appendages. Branched lateral papilla at middle of rhinophoral sheaths. Five appendages of rhinophoral stalks, 8–11 rhinophoral lamellae. 5–6 pairs of highly branched dorsolateral appendages, decreasing in size and branching towards tail. Digestive gland branches penetrate most dorsolateral appendages as well as rhinophoral sheaths. Dorsolateral appendages with long primary stalk, secondary branches, and elongate tertiary branches (Fig. 1A–D). Dorsal surface tuberculate. Foot narrow, rounded in front, narrowed towards tail. Reproductive opening placed laterally on right side at level of first pair of dorsolateral appendages. Anal opening on right side between first and second pairs of dorsolateral appendages. Colour non-uniformly reddish brown with few opaque white stripes between dorsolateral processes (holotype, Fig. 1A), more uniformly olive almost without white pigment (paratype, Fig. 1C), or almost lacking general pigmentation. Dorsum, dorsolateral appendages, and upper sides of foot with small scattered whitish and yellowish dots. 7–12 lip papillae. Dorsal processes of the jaws inclined posteriorly at approximately 47° to the longitudinal axis of the jaw body and 0.48 of its length; denticles present. Radular formula 37 × 8–9.1.9–8 (holotype) (Fig. 1E, F). Central tooth strong, distinctly denticulate in both anterior and posterior parts of radula, with up to 14 denticles (Fig. 1E, F)

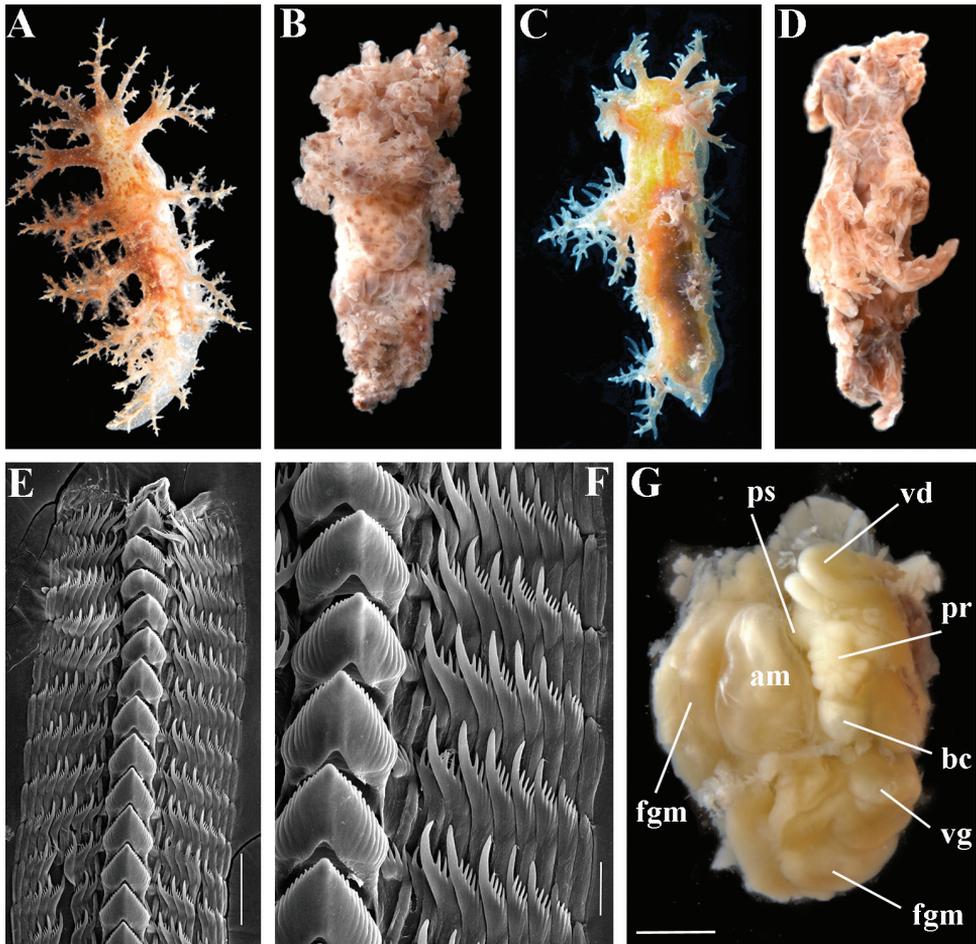


Figure 1. *Dendronotus primorjensis*, type material from Zoological Museum MSU: **A** holotype ZMMU Op-419, live, 35 mm in length, dorsal view **B** fixed holotype ZMMU Op-419 **C** paratype ZMMU Op-420, live, 21 mm in length **D** fixed paratype ZMMU Op-420 **E** radula of the holotype ZMMU Op-419, posterior part, SEM; **F** same, details **G** reproductive system of the holotype ZMMU Op-419. Abbreviations: **am** ampulla; **bc** bursa; **fgm** female gland mass; **pr** prostate; **ps** penial sheath; **vd** vas deferens; **vg** vagina. Scale bars **E** = 100 μ m **F** = 30 μ m **G** = 1 mm. Photos and SEM images by T.A. Korshunova and A.V. Martynov (Figures A, E, and F were published as part of the original description by Martynov et al. 2015a).

bearing deep furrows. Lateral teeth narrow, with relatively long curved cusp, bearing 3–6 distinct denticles (Fig. 1F). Outermost lateral teeth almost devoid of denticles. Reproductive system triaulic (Fig. 1G), ampulla twice folded. Prostate in holotype rounded, consists of no less than 19 alveolar glands. Distal part of vas deferens relatively short, entangled, and expanded to oval penial sheath and relatively long and curved conical penis. Vagina moderate in length. Bursa copulatrix large, rounded, elongated, seminal receptaculum placed distally (nomenclature of the seminal reservoirs according to Stout et al. 2011).

Morphological characters of *Dendronotus dudkai*

These characters are taken directly from Ekimova et al. (2016: 35, 37, shortened):

“Body elongate, laterally compressed [the range of the lengths of specimens was not recorded in the description of *D. dudkai* by Ekimova et al. (2016), and only from the figure legends is it possible to estimate that length can be up to 28 mm]. Oral veil with 6–12 large, secondary branched cerata. 5–10 short lip papillae. Rhinophoral sheaths with long stalk and 4–5 crown secondary branched appendages. Lateral papillae moderate in size with small secondary branches. Rhinophores with 8–10 lamellae. 6–8 pairs of highly branched dorsolateral processes, size and degree of branching decrease towards the tail. Secondary branches long and rounded, tertiary branches short and sometimes pointed. General colour pattern varies from beige to dark-brown. Background colour translucent-white or light yellow. A lot of spots, stripes and dots on dorsal side of the body, cerata, rhinophoral sheath processes and upper parts of foot. Their colour varies from yellow to dark-brown. Some specimens covered with dots of golden or white opaque pigment. This pigment locates also in low body papillae and small tubercles. All specimens possess well-visible white stripes between pairs of cerata. Dorsal processes of jaws about 2.5 times shorter than jaw body. Inclined posteriorly at about 45°. Masticatory process about one-third as long as jaw body, slightly curved at base and become transparent and subulate posteriorly. Masticatory border with a single row of denticles. Radula formula up to $32 \times 7-8.1.7-8$. Rachidian tooth bears 12–18 sharp denticles with thin furrows on both sides of the reduced cusp. Lateral teeth slightly curved, bear 4–8 well-defined denticles. Reproductive system triaulic. Ampulla wide and sinuous. Prostate concentric ring-shaped, consists of 12–14 oval alveolar glands. Distal part of vas deference winding expand into wide, muscular portion. Penis slightly curved. Oviduct connects through insemination duct into female gland complex. Vagina long, convoluted, rounded seminal receptaculum, small bursa copulatrix” [according to the updated nomenclature by Stout et al. 2011, receptaculum = bursa and vice versa].

Phylogenetic analysis

Brief molecular results, including the genetic distances between *Dendronotus primorjensis* and closely related species were provided in two previous studies (Martynov et al. 2015a, b). In this study an extended molecular analysis is provided for a detailed comparison of the molecular data between *D. primorjensis* and *D. dudkai*.

Phylogenetic analyses were separately performed for COI, 16S, and 28S genes, and three concatenated nucleotide datasets from the holotype and paratype of *D. primorjensis*, available *Dendronotus* specimens from GenBank, and an outgroup consisting of four species of *Doto*, *Tritonia*, and *Marionia* (Table 1). Trees of both Bayesian Inference (BI) and Maximum Likelihood (ML) were used to infer phylogenetic trees. All single-gene trees as well as concatenated trees revealed very low divergence between *D. primorjensis*

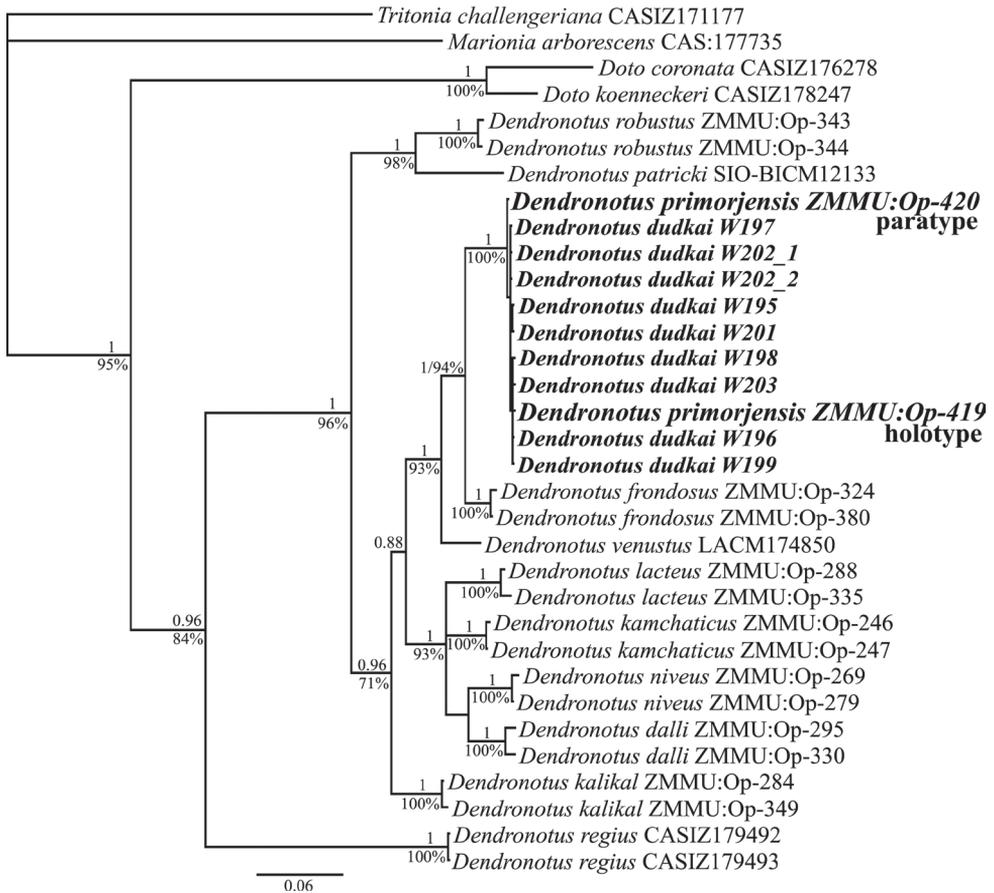


Figure 2. Phylogenetic tree based on combined molecular data (COI + 16S + 28S) represented by Bayesian Inference. Numbers above branches represent posterior probabilities from BI. Numbers below branches indicate bootstrap values for Maximum Likelihood.

and *D. dudkai* specimens. A phylogenetic tree based on combined molecular data is represented in Figure 2. The combined data set including the three loci was presented in a sequence alignment of 1442 codon positions. The General Time Reversal model with invariant sites and gamma distribution (GTR+I+G) was selected as the best model for three nucleotide datasets. The topologies of phylogenetic trees inferred from two methods (ML and BI) and three datasets were identical. *Dendronotus primorjensis* and *D. dudkai* specimens are clustered in a single clade with maximum support (PP = 1, BS = 100%).

The ABGD analysis of the COI data set run with two different models revealed eleven potential species each:

D. regius (CASIZ179492, CASIZ179493);

D. lacteus (ZMMU:Op-288, ZMMU:Op-335);

D. kamchaticus (ZMMU:Op-246, ZMMU:Op-247);

- D. niveus* (ZMMU:Op-269, ZMMU:Op-279);
D. dalli (ZMMU:Op-295, ZMMU:Op-330);
D. kalikal (ZMMU:Op284, ZMMU:Op349);
D. venustus (LACM174850);
D. primorjensis (ZMMU:Op-419, ZMMU:Op-420) together with *D. dudkai* (W195, W196, W197, W198, W199, W201, W202_1, W202_2, W203);
D. frondosus (ZMMU:Op-380, ZMMU:Op-324);
D. patricki (SIO-BICM12133);
D. robustus (ZMMU:Op-343; ZMMU:Op-344).

The prior maximal distance ranged between 0.001 and 0.059.

The ABGD analysis of the 16S data set run with two different models revealed ten potential species each:

- D. regius* (CASIZ179493, CASIZ179492);
D. robustus (ZMMU: Op344, ZMMU:Op343);
D. patricki (SIO-BIC M12133);
D. kalikal (ZMMU:Op349, ZMMU:Op349);
D. venustus (LACM:174852.1);
D. frondosus (ZMMU:Op324, ZMMU:Op380) together with *D. primorjensis* (ZMMU:Op-419, ZMMU:Op-420) together with *D. dudkai* (W195, W196, W197, W198, W199, W201, W202_1, W202_2, W203);
D. niveus (ZMMU:Op269, ZMMU:Op279);
D. lacteus (ZMMU:Op288, ZMMU:Op335);
D. kamchaticus (ZMMU:Op247, ZMMU:Op246);
D. dalli (ZMMU:Op295, ZMMU:Op330).

The prior maximal distance ranged between 0.001 and 0.021.

Results for genetic distances between *Dendronotus primorjensis*, *D. frondosus*, *D. venustus*, *D. kalikal*, and *D. kamchaticus* have been described previously (Martynov et al. 2015b). Uncorrected COI p-distances between the holotype and paratype of *D. primorjensis* and other species of the genus *Dendronotus* are listed in Table 2. The COI p-distances of *D. primorjensis* – *D. dudkai* specimens range from 0 to 0.31%, indicating a strong overlap in the molecular dataset. Thus, the results unmistakably indicate that *D. primorjensis* and *D. dudkai* belong to the same species, and *D. dudkai* is here regarded as a junior synonym.

Discussion

According to the morphological and molecular data presented above there are no species-level differences between *D. primorjensis* (Figs 1 and 2; Table 2) and *D. dudkai* (Ekimova et al. 2016: 33–37). Molecular analysis robustly places the holotype of *D.*

Table 2. Uncorrected COI p-distances (%) between holotype and paratype of *Dendronotus primorjensis* and other species of the genus *Dendronotus*.

Species	<i>Dendronotus primorjensis</i> ZMMU:Op-419 holotype	<i>Dendronotus primorjensis</i> ZMMU:Op-420 paratype
<i>Dendronotus primorjensis</i> paratype	0.16	-
<i>Dendronotus dudkai</i> KT031811	0.16	0.31
<i>Dendronotus dudkai</i> KT031814	0.16	0.31
<i>Dendronotus dudkai</i> KT031816	0.16	0.31
<i>Dendronotus dudkai</i> KT031819	0	0.16
<i>Dendronotus dudkai</i> KT031812	0	0.16
<i>Dendronotus dudkai</i> KT031813	0	0.16
<i>Dendronotus dudkai</i> KT031815	0	0.16
<i>Dendronotus dudkai</i> KT031817	0	0.16
<i>Dendronotus dudkai</i> KT031818	0	0.16
<i>Dendronotus frondosus</i> KM396976	6.71	6.55
<i>Dendronotus venustus</i> HM162709	8.11	7.96
<i>Dendronotus kalikai</i> KM396986	11.2	11.4
<i>Dendronotus kamchaticus</i> KM396989	11.86	11.7
<i>Dendronotus albopunctatus</i> GQ292064	12.01	11.86
<i>Dendronotus niveus</i> KM396996	12.32	12.17
<i>Dendronotus dalli</i> KM397001	13.88	13.73
<i>Dendronotus lacteus</i> KM396975	14.51	14.35
<i>Dendronotus patricki</i> HQ225828	14.7	14.5
<i>Dendronotus robustus</i> KM397002	14.8	14.7
<i>Dendronotus regius</i> HM162708	16.8	16.7

primorjensis inside the clade of *D. dudkai* (Fig. 2). Both the external and internal morphologies, as well as radular and reproductive features, do not demonstrate any significant differences between the two. Other differences between the original descriptions of *D. primorjensis* and *D. dudkai* are discussed below.

Since *D. frondosus* is a closely related species to *D. primorjensis* (= *D. dudkai* syn. n.) (Fig. 2) and, according to Ekimova et al. (2016), co-occurs with *D. primorjensis* in the Sea of Japan, it is important to discuss distinguishing morphological characters between these two species as well as their biogeographical patterns. In the first description of *D. primorjensis* “an absence of the large amount of the white pigment” was described as a potential distinguishing external character from *D. frondosus* and the eastern Pacific related species *D. venustus* (Martynov et al. 2015a: 60). This statement was challenged by Ekimova et al. (2016: 31, 35) who claim that “all specimens possess well-visible white stripes between pairs of cerata.” However, surprisingly, on the figures of *D. dudkai* (Ekimova et al. 2016: fig. 9A, C) no white pigment is visible, thus in full agreement with the data on both the holotype and paratype of *D. primorjensis* (Martynov et al. 2015: 60, Fig. 5A–G and present study, Fig. 1A, C). The colour pat-

terns of *D. frondosus* and *D. venustus* vary considerably (Robilliard 1970; pers. obs.) and *D. primorjensis* is potentially expected to have similar variations. However, to date no specimens of *D. primorjensis* with really large amounts of white pigment between the dorsolateral appendages have been reported (as previously documented for *D. frondosus* and *D. venustus*).

Ekimova et al. (2016: 37) claim that “*D. frondosus* is differentiated from *D. dudkai* by the absence of denticles on the masticatory process of jaws” but this is in error: in Robilliard’s review on the genus *Dendronotus*, the presence of denticles has been indicated for *D. frondosus sensu lato* (“A small number of relatively large, black denticles adorns the masticatory margin”, Robilliard 1970: 443). Furthermore, since Robilliard’s study refers to a mixture of *D. frondosus s. str.* and *D. venustus* MacFarland, 1966 (but anyway not *D. primorjensis*) we have studied a topotype of *D. frondosus s. str.* from the North Atlantic (Norway) to clarify the situation. Species identity was confirmed using both morphological and molecular data: the topotype of *D. frondosus s. str.* demonstrated several small but distinct denticles on the jaws. In the original description of *D. venustus*, numerous denticles (between 27 and 40) on the masticatory processes were also described (MacFarland 1966: 277). Thus, the presence of denticles on the masticatory processes of the jaws cannot serve as a diagnostic character for *D. primorjensis* (= *D. dudkai* syn. n.) despite claims by Ekimova et al. (2016).

Instead, it is suggested here that the number of denticles on the central teeth is a better diagnostic, and is higher in *D. primorjensis* (commonly more than 12, in holotype 14, reported range 12–18) than in *D. frondosus* (commonly up to 12, rarely up to 14 denticles in the topotype specimens from North Atlantic, pers. obs., reported range 8–12). The original description of the *D. primorjensis* is accompanied by two detailed SEM images of the radula (see Martynov et al. 2015a: fig. 5). On these images up to 14 denticles of the central radular tooth can be recognized, whereas according to Ekimova et al. (2016), the central tooth of *D. frondosus* from the Sea of Japan bears a maximum of 12 denticles. Therefore, a larger number of denticles on the central teeth of *D. primorjensis* distinguishes it from the Japan Sea’s *D. frondosus*, but was not discussed by Ekimova et al (2016). No other species of *Dendronotus* with a similar radula have been so far reported from the Sea of Japan. The discussion of the origin of *D. frondosus* in the Sea of Japan in the Ekimova et al. (2016: 38–39) mainly concentrates on the idea that *D. frondosus* could be a natural amphiboreal species. However, the fact that the only Japan Sea *D. frondosus* specimens recorded in Ekimova et al. (2016) were found exclusively in the vicinity of the Institute of Marine Biology FEB RAS (Vladivostok) indicates a high probability of anthropogenic introduction of *D. frondosus*. On the contrary, *D. primorjensis* (= *D. dudkai* syn. n.) has a broad distribution in the Sea of Japan.

The number of lobules present in the prostate of *D. dudkai* are described by Ekimova et al. (2016: 31) as being among the “most important diagnostic characters” for the genus *Dendronotus*. This is inaccurate, as Ekimova et al. (2016: 32) indicate 16–30 prostatic lobules for *D. frondosus*, whereas 12–14 lobules are described for *D. dudkai*

(Ekimova et al. 2016: 37). However, the holotype of *D. primorjensis* possesses no less than 19 lobules in the prostate clearly implying that variability of numbers of prostatic lobules in the genus *Dendronotus* can be significant, and therefore cannot be species-diagnostic. The number of prostate lobules has recently been shown in Korshunova et al. (2016) to be an unreliable character in several *Dendronotus* species.

Unfortunately, the description of *D. dudkai* contains a number of problems that prevent the repetition of their results obtained by molecular phylogenetic analysis. Despite the presence of a molecular analysis that was based on “additional material” (i.e. non-type specimens) no molecular data is available for the type material. According to their Table 1 and “Type material” section (Ekimova et al. 2016:19–21, 33–35) five specimens were listed as type material for *D. dudkai*, but the molecular data for only one paratype (ZMMU Lc-40366) was registered at GenBank; however, this voucher number for this paratype of *D. dudkai* does not exist in GenBank: specimen W203 was registered at GenBank instead of specimen ZMMU Lc-40366, but specimen W203 is completely absent in the “Material” list or Table 1. In addition, no illustrations of the holotype of *D. dudkai* were provided nor were characters of the holotype mentioned in the description. Thus, the analysis of Ekimova et al. (2016) leads to the conclusion that there is no evidence that description of *D. dudkai* is based on the primary type, i.e. the holotype. According to the Recommendations 73A, 73B, and 73C of ICZN (1999) the “author who establishes a new nominal species-group taxon should designate its holotype in a way that will facilitate its subsequent recognition”, “an author should designate as holotype a specimen actually studied by him or her, not a specimen known to the author only from descriptions or illustrations in the literature”, and “an author who establishes a new nominal species-group taxon should publish at least the following data concerning the holotype, if they are relevant and known to the author, including: 73C.1. its size or the size of one or more relevant organs or parts.” Thus, the absence of the holotype’s morphological (and molecular data), and even its measurements in the original description of Ekimova et al. (2016) do not allow anyone to recognize it.

Conclusions

The morphological and molecular data provided in this publication and two previous studies (Martynov et al. 2015 a, b) conclusively demonstrate that *D. primorjensis* is a valid species as per current ICZN regulations (ICZN 1999). The integrative taxonomy (Dayrat 2005) presented here indicate that *D. primorjensis* and *D. dudkai* are one and the same species, and that *D. primorjensis* has taxonomic priority over *D. dudkai* published one year later. The species epithet *D. dudkai* is therefore to be considered a junior synonym of *D. primorjensis*.

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Descriptions of two new species of the genus *Camaena* from Guangxi, China (Gastropoda, Stylommatophora, Camaenidae)

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Abstract

The sinistral *Camaena* species are mainly distributed in southern China and northern Vietnam. There is a total of eight species or subspecies of sinistral *Camaena* recorded at present. By systematically collecting specimens in Guangdong, Guangxi and Yunnan in southern China and the northern areas in Vietnam, two new species, *Camaena linyunensis* Zhou & Lin, **sp. n.** and *Camaena detianensis* Zhou & Lin, **sp. n.** have been discovered. These new species are here characterised based on the comparison of shells, their reproductive system, the molecular phylogenetic analyses of the mitochondrial genes COI and 16S, and the nuclear gene ITS2. Detailed descriptions of the morphological characters, the DNA sequences, and the habitat of the two new species are given. Differential comparisons with related species are provided as well as a key to the sinistral species of *Camaena*.

Keywords

Camaena detianensis sp. n., *Camaena linyunensis* sp. n., camaenid species, molecular phylogeny, taxonomy

Introduction

The genus *Camaena*, which contains large dextral or sinistral shell, was established in 1850 by Albers, with the type species *Helix cicatricosa* Müller, 1774. The common features of shell include a large protoconch, a scar-like protuberance or malleation on surface, tawny colouring, and multiple red or brown spiral bands.

The classification of this genus is confused historically, which is especially true for *Camaena cicatricosa* (Müller, 1774) in the sinistral group. Most taxonomists divided the sinistral group, which is distributed in southern China and northern Vietnam into three species, *C. cicatricosa*, *C. habni* (Mabille, 1887), and *C. seraphinica* (Heude, 1890). The taxonomic statuses of *C. habni* and *C. seraphinica* are relatively stable. *Camaena habni* contained two subspecies, *C. h. habni* and *C. habni broti* (Dautzenberg & d’Hamonville, 1887). Some scholars considered *C. habni broti* as a synonym of *C. habni* (e.g. Pilsbry 1891). The classification of *C. cicatricosa* is most confused, and different western scholars divided it into five distinct subspecies or variations on the basis of diverse shell, *C. c. cicatricosa*, *C. c. ducalis* (Ancey, 1885), *C. c. inflata* (Möllendorff, 1885), *C. c. oblecta* (Fischer, 1898), and *C. c. connectens* (Dautzenberg & Fischer, 1906). However, due to lack of fresh specimens, further research on the histological anatomy and molecular biology have not been done. The taxonomic status of the sinistral group has always been controversial, and scientific names have been revised repeatedly. Previously, the Chinese scholars disagreed with this classification, and the name *C. cicatricosa* was used (e.g. Chen and Gao 1987). Ding et al. (2016) clarified the phylogenetic relationships and taxonomic status of the sinistral group with the help of comparative shell morphology, genital anatomy, and molecular phylogeny, and recognised it to contain four species, *C. cicatricosa*, *C. inflata* (Möllendorff, 1885), *C. oblecta* (Fischer, 1898) and *C. connectens* (Dautzenberg & Fischer, 1906). In addition, they described one new species *C. poyuensis* (Zhou, Wang & Ding, 2016). Thus, the number of species within the sinistral *Camaena* group now reached eight species or subspecies.

On the basis of the above work, the authors have studied a large number of specimens collected in Guangdong, Guangxi and Yunnan in southern China and the northern areas in Vietnam during 2013–2016, and discovered two new species according to shell morphology, reproductive system, and molecular biology. The details including morphological characteristics, DNA sequences, and habitat of these two new species are described herein.

Material and methods

This study is based on material collected by the authors from several sites in China (Fig. 1). The longitude and latitude were recorded using a GPS. The live adults were drowned in water for 12–24 hours, and then killed in hot water. Soft body parts were preserved in 75% or 95% ethanol and stored at -20°C. Empty shells were cleaned and preserved at room temperature. Samples have been deposited in the State Key Laboratory of Molluscan Quarantine and Identification, FJIQBC.

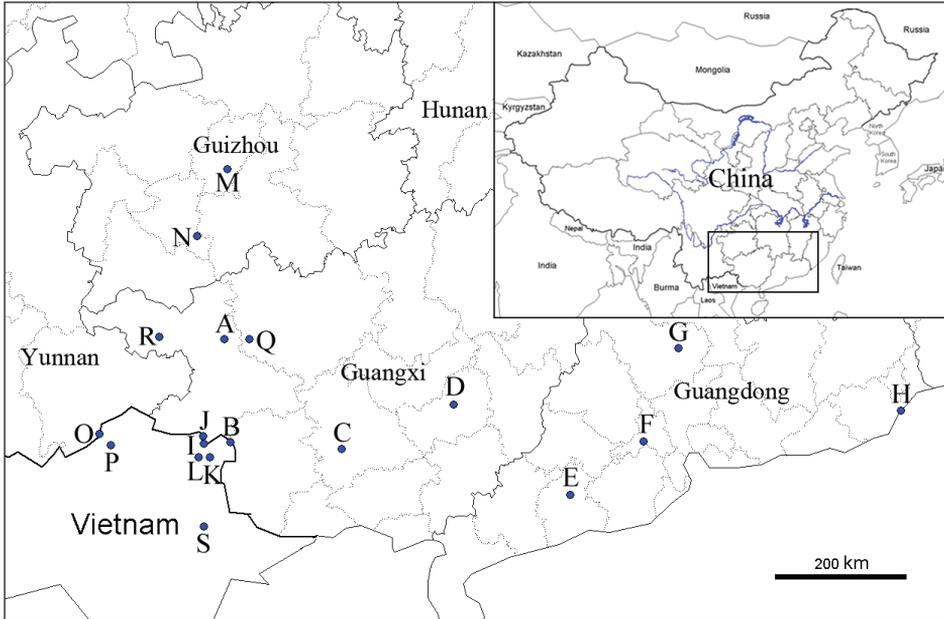


Figure 1. Map of locations of *Camaena* species. *C. linyunensis* sp. n.: **A** Kasuo, Linyun, Guangxi, China. *C. detianensis* sp. n.: **B** Detian Falls, Daxin, Guangxi, China. *C. cicatricosa*: **C** Nanning, Guangxi, China **D** Guiping, Guangxi, China **E** Yangchun, Guangdong, China **F** Gaoming, Canton, Guangdong, China **G** Yingde, Guangdong, China **H** Shantou, Guangdong, China. *C. obtecta*: **I** Buhaitun, Jinxi, Guangxi, China **J** Longbang, Jingxi, Guangxi, China **K** Longo coc tan, Quang-Huyen, Vietnam **L** Cao Bang, Vietnam (type locality). *C. inflata*: **M** Qianlin park, Guiyang, Guizhou, China **N** Ziyun, Anshun, Guizhou, China. *C. connectens*: **O** Tianbao, Malipo, Yunnan, China **P** Ha Giang, Vietnam (type locality). *C. poyuensis*: **Q** Poyue, Bama, Hechi, Guangxi, China. *C. seraphinica*: **R** Dingan, Tianlin, Guangxi, China (type locality). *C. habni*: **S** Huu Lien Nature Reserve, Lang-Son, Vietnam.

Shells were measured to 0.1 mm using electronic calipers. Standard shell parameters were taken following Kerney and Cameron (1979). All adult specimens of each species were measured. Only sexually matured specimens were dissected for the examination of reproductive system. Genitalia were dissected under a dissecting microscope (ZEISS Stemi 2000); three specimens of each species were dissected. Terminology for reproductive system follows Gómez (2001). All drawings were traced with the aid of a Canon 550D digital camera.

Approximately 0.02–0.04 g of foot muscle tissue was used for DNA extraction. The muscle tissue was bathed in sterile water for 3–6 hours to remove residual alcohol. Genomic DNA was isolated using Qiagen DNeasy Blood & Tissue kit (Qiagen, Beijing) on more than three specimens per species, examined by agarose gel electrophoresis, and stored at -20°C for further use. The partial mitochondrial cytochrome c oxidase subunit 1 (COI) and 16S rRNA (16S), and the internal transcribed spacer 2 (ITS2) region of nuclear ribosomal DNA were amplified by PCR using the primer pairs, reaction systems and amplification conditions listed in Table 1. The PCR products were analysed by 1.2% agarose gel electrophoresis.

Table 1. Primer pairs and PCR conditions used in the analyses of the COI, 16S rRNA, and ITS2 genes of *Camaena*.

Gene	COI
Primer pairs (5'-3')	LCO:GGTCAACAAATCATAAAGATATTGG HCO:TAAACTTCAGGGTGACCAAAAAATCA
Reaction systems	25ul Taq PCR MasterMix×2; 1ul each primer; 2ul DNA; 16ul ddH ₂ O
Cycling conditions	94°C: 30s; 94°C: 10s, 45°C: 50s, 72°C: 1min, 40 cycles; 72°C: 10min.
Reference	Folmer et al. 1994
Gene	16S
Primer pairs (5'-3')	16SAR:CGCCTGTTTATCAAAAACAT 16SBR:CCGGTCTGAACTCAGATCACGT
Reaction systems	25ul Taq PCR MasterMix×2; 1ul each primer; 2ul DNA; 16ul ddH ₂ O
Cycling conditions	94°C: 30s; 94°C: 10s, 45°C: 50s, 72°C: 1min50s, 40 cycles; 72°C: 10min.
Reference	Palumbi et al. 1991
Gene	ITS2
Primer pairs (5'-3')	FYIT2:CATCGACATCTTGAACGCACAT RYIT2:TCCCAAAACAACCCGACTCCT
Reaction systems	25ul Taq PCR MasterMix×2; 1ul each primer; 2ul DNA; 16ul ddH ₂ O
Cycling conditions	94°C: 30s; 94°C: 10s, 55°C: 30s, 72°C: 1min30s, 40 cycles; 72°C: 10min.
Reference	Ding et al. 2016

After sequencing, raw sequences were proof-read on chromatograms and aligned into contigs using BioEdit 7.2 (Hall 1999). ITS2 sequences were annotated by using HMMer (Eddy 1998) and ITS2 Database (Koetschan et al. 2010). Sequence alignments were generated using ClustalW implemented in MEGA 5 (Tamura et al. 2011). A total of 165 sequences of COI, 16S, and ITS2 were used in this study, 36 sequences of which were newly generated and deposited in GenBank (Table 2), and the rest referenced in Ding et al. (2016). Pairwise *p*-distances between taxa were calculated using MEGA5. For phylogenetic analysis, the three sequenced data sets were concatenated into one, with a length of 1,619 bp. The concatenated alignment contained 39 unique sequences, which were used for subsequent analysis. Neighbor Joining (NJ), Maximum Parsimony (MP), and Maximum Likelihood (ML) analyses based on COI+16S+ITS2 combined data set were performed using MEGA5 with default settings. *Bradybaena sequiniana* (Heude, 1885) and *Cornu aspersum* (Müller, 1774) were used as outgroups. The node support values were assessed by bootstrap resampling (Felsenstein 1985) using 1000 replicates.

Abbreviations used

COI	cytochrome c oxidase subunit 1 gene;
16S	16S rRNA gene;
ITS2	internal transcribed spacer 2 region of nuclear ribosomal DNA;
NJ	Neighbor Joining;

Table 2. Sampling information and GenBank accession numbers of some species.

Sampling	Locality	Collection date	Coordinates	Accession number		
				COI	16S rRNA	ITS2
<i>Camaena lingyunensis</i> sp. n.	Kasuo, Lingyun, Guangxi, China	2014.04.24	24°17'47.33"N, 106°39'6.53"E	KX345077	KX345083	KX345089
				KX345078	KX345084	KX345090
				KX345079	KX345085	KX345091
<i>Camaena detianensis</i> sp. n.	Detian Falls, Daxin, Guangxi, China	2013.05.21	22°51'29.54"N, 106°43'13.51"E	KX345074	KX345080	KX345086
				KX345075	KX345081	KX345087
				KX345076	KX345082	KX345088
<i>Camaena habni</i>	Huu Lien Nature Reserve, Lang-Son, Vietnam	2016.06.22	21°44'53.28"N, 106°22'57.96"E	KX621263	KX621257	KX621269
				KX621264	KX621258	KX621270
				KX621265	KX621259	KX621271
<i>Camaena oblecta</i>	Longo coc tan, Quang-Huyen, Vietnam	2016.06.20	22°41'19.86"N, 106°26'16.50"E	KX621260	KX621254	KX621266
				KX621261	KX621255	KX621267
				KX621262	KX621256	KX621268

- ML** Maximum Likelihood;
- FJQBC** Fujian Entry-Exit Inspection & Quarantine Bureau, Fuzhou, Fujian, China;
- MNHN** Muséum National d'Histoire Naturelle, Paris, France.
- MP** Maximum Parsimony;

Results

Molecular analysis

Molecular analysis was based on DNA sequences of 53 specimens in the genus *Camaena* from 18 localities. In this study a total of 165 sequences of COI, 16S and ITS2 was used. There were 36 sequences from *C. lingyunensis* sp. n., *C. detianensis* sp. n., *C. oblecta* (Fischer, 1898) (distributed in Longo coc tan, Quang-Huyen, Cao Bang, Vietnam) and *C. habni* (distributed in Huu Lien Nature Reserve, Huu Lung, Lang-Son, Vietnam) listed in Table 2. The rest of the sequences and geographical information from five sinistral *Camaena* (*C. cicatricosa*, *C. oblecta*, *C. inflata*, *C. connectens* and *C. poyuensis*), two dextral *Camaena* (*C. menglunensis* and *C. jingpingensis*) and the out-group (*Bradybaena sequiniana* and *Cornu aspersum*) were taken from a previous article (Ding et al. 2016). The sequence alignment was based on lengths of 601 bp (COI), 428 bp (16S) and 590 bp (ITS2), respectively.

Inter- and intraspecific *P*-distances from the three genes of eight species were calculated and listed in Table 3. According to the results of target gene COI, the *p*-distances between *C. lingyunensis* sp. n. and other seven sinistral *Camaena* were 0.098–0.178, and the *p*-distances between *C. detianensis* sp. n. and other seven sinistral *Camaena* were 0.073–0.189. These numbers significantly exceed the interspecific differentiation standard of terrestrial molluscs, the limit of *p*-distance 0.03 (average 0.03, generally between 0.00–0.06) (Criscione and Köhler 2014).

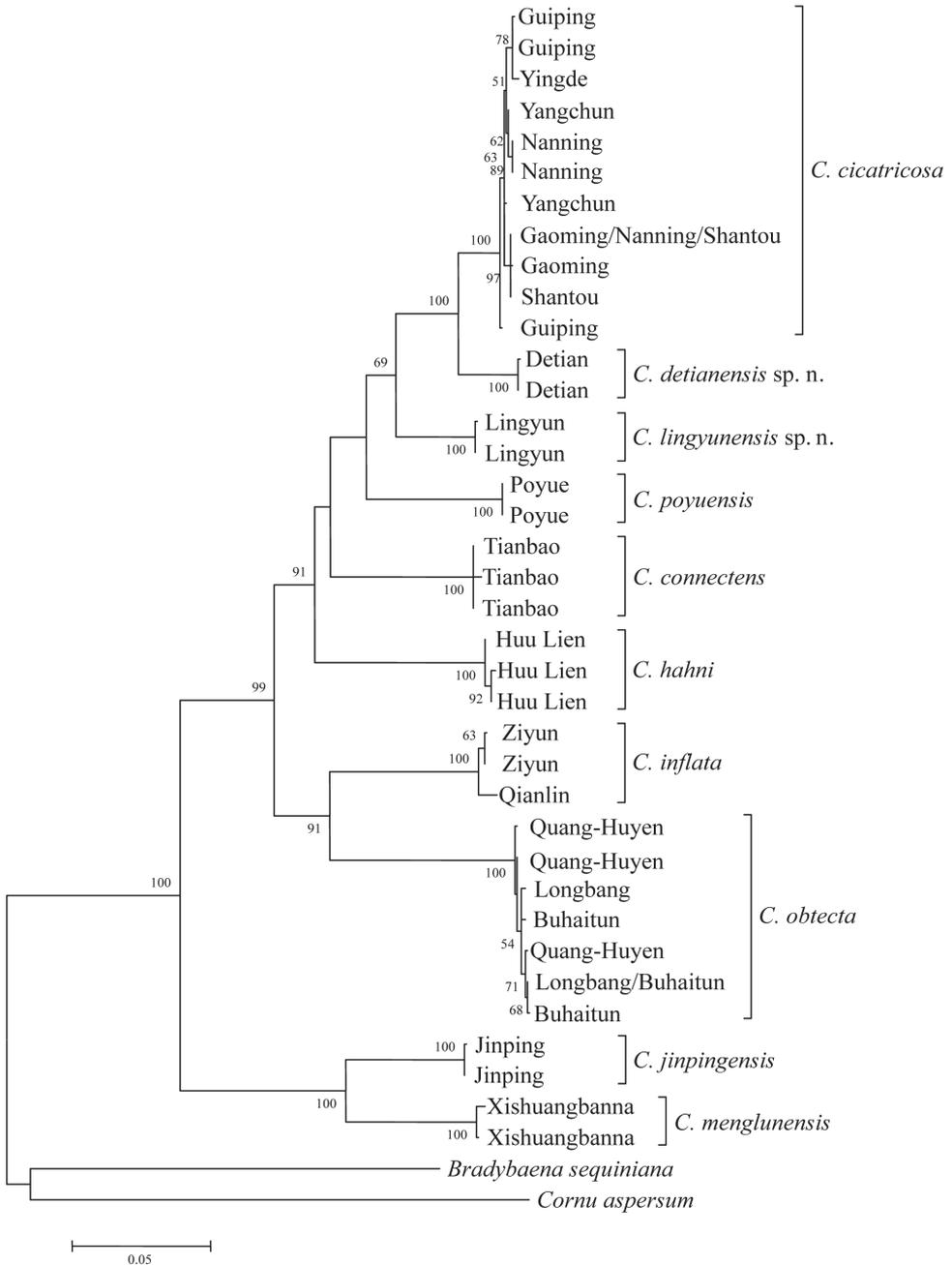


Figure 2. Maximum Likelihood tree based on analysis of the concatenated dataset of COI, 16S, and ITS2 sequences. Numbers beside nodes indicate bootstrapping support (%) for main clades.

The phylogenetic analysis showed that NJ, MP, and ML trees have the mostly same topological structure, and indicated that phylogenetic analyses in this research was relatively correct and reliable, and can be applied in genetic relationship research

and systematic classification. The support degree of each species on ML tree (Fig. 2) all reached 100, and eight clades contained described and published species (including 2 dextral species as contrast), and another two clades included two new taxon. In this study, the genus *Camaena* was clearly divided into sinistral and dextral groups. From the tree structure, branch length and comparison of the known species, the phylogenetic tree supported *C. lingyunensis* sp. n. and *C. detianensis* sp. n. as new species. Moreover, the two new species have closer genetic relationship with *C. cicatricosa*, all of three have semi-open or open umbilicus.

Systematics

Camaenidae Pilsbry, 1895

Camaena Albers, 1850

Type species. *Helix cicatricosa* Müller, 1774, subsequent designation by Martens, 1860.

Camaena lingyunensis Zhou & Lin, sp. n.

<http://zoobank.org/9A26F678-0F74-42FC-A37A-610668FDEBB7>

Figs 3A, 4A, 5A, Table 3

Holotype. [FJIQBC 19280] Shell height 29.0 mm, shell width 52.5 mm, height of aperture 21.3 mm, width of aperture 27.0 mm, 24 April 2014, collected from the type locality.

Paratype. [FJIQBC 19281–19293] 13 specimens: 2 empty adult shells, 11 live snails including 9 adults and 2 juveniles. Results of adult measurements: shell height 24.0–34.0 (27.40 ± 2.96) mm, width 49.8–59.5 (53.00 ± 2.77) mm, height of aperture 18.0–25.0 (20.75 ± 1.74) mm, width of aperture 22.0–31.0 (26.35 ± 2.57) mm, 24 April 2014, collected from type locality.

Type locality. Kasuo, Lingyun, Guangxi, China (24°17'47.33"N, 106°39'6.53"E).

Etymology. The name of the new species refers type locality.

Description. *Shell.* Shell sinistral, large, slightly thin, semi-translucent, hard and fragile, flat globose. 4.75 whorls, the upper whorls increasing fast and slightly convex. Spire relatively low. Body whorl rapidly expanded, convex, with a weakly obtuse angulated margin at periphery. Shell fawn with countless light chestnut spiral bands. Spiral bands slender and dense below the periphery of body whorl, forming wide area of bands. Growth lines dense and thick on the surface. Apex quite blunt. Growth lines on protoconch visible when using 15× magnification. Suture line shallow. Aperture lunate, slightly descending in front view. Peristome reflected, sharp and white. Columellar lip reflected, slightly covering the umbilicus. Inner lip attached to the body whorl, forming translucent, thin and smooth callus. Umbilicus open and round. The first whorl can be seen through the umbilicus. Hump beside umbilicus absent.

Soft body. Foot hazel. Tentacles darker. White band from the head to the neck.

Table 3. Inter and intraspecific *P*-distances of sinistral *Camaena* species.

<i>p</i> -distance								
	<i>C. lingyunensis</i> sp. n.		<i>C. detianensis</i> sp. n.		<i>C. habni</i>		<i>C. cicatricosa</i>	
	within	between	within	between	within	between	within	between
COI	0.000 -0.002	0.098 -0.178	0.000 -0.002	0.073 -0.189	0.000 -0.004	0.119 -0.183	0.000 -0.017	0.073 -0.168
16S	0.000 -0.005	0.046 -0.145	0.000 -0.002	0.013 -0.137	0.000 -0.002	0.094 -0.153	0.000 -0.016	0.013 -0.137
ITS2	0.000	0.008 -0.045	0.000	0.000 -0.044	0.002 -0.004	0.025 -0.068	0.000 -0.006	0.000 -0.048
	<i>C. poyuensis</i>		<i>C. connectens</i>		<i>C. inflata</i>		<i>C. obtecta</i>	
	within	between	within	between	within	between	within	between
COI	0.000 -0.003	0.104 -0.177	0.000	0.108 -0.179	0.000 -0.019	0.155 -0.185	0.000 -0.010	0.154 -0.189
16S	0.002 -0.007	0.062 -0.160	0.000 -0.002	0.086 -0.157	0.000 -0.007	0.093 -0.141	0.000 -0.007	0.122 -0.160
ITS2	0.000	0.025 -0.057	0.006 -0.015	0.008 -0.068	0.000	0.008 -0.042	0.000 -0.002	0.008 -0.055

Reproductive system. Penis slightly swollen, short. Epiphallus long and thick. Penis retractor muscle very slender and long. Flagellum medium length, thick basally, tapering distally. Vas deferens long and thin. Vagina thick and slightly short. Bursa copulatrix oval. Pedunculus of bursa copulatrix quite long, expanded at basal half, while smooth and slender at the end. Inner penial wall supporting transverse, smooth, and dense pilasters proximally and several longitudinal, thin, curly, and widely-spaced pilasters distally. Verge conical and smooth, with eight transverse wrinkles basally. An obvious longitudinal crack on the verge, and six smooth and longitudinal pilasters with wide space in the crack. Verge opens laterally.

Ecology. This species was found on limestone in Lingyun county of Guangxi province. It generally inhabits mountaintops with clouds and mists, but cannot be found at the foot of the mountain.

Remarks. The key characters of *C. lingyunensis* sp. n. and the other eight sinistral *Camaena* species are presented in an identification key. This species is clearly different from other species, with a more oblate shape, lower spire, thinner and more fragile shell, and lighter colouration. The umbilicus of the new species is fully open, and the first whorl can be seen from the umbilicus, which significantly is distinguished from *C. poyuensis* (Zhou, Wang & Ding, 2016) and *C. obtecta* (Fischer, 1898) without an umbilicus, *C. inflata* and *C. connectens* (Dautzenberg & Fischer, 1906) with a narrow umbilicus, and *C. cicatricosa* and *C. detianensis* sp. n. with a semi-open umbilicus. The new species is similar with another sinistral snail, *C. seraphinica*, which also has a fully open umbilicus, but with a relatively higher and arched spire, wide and beautiful red spiral bands, and colour spots and no forged trace on the surface. Furthermore, the base region colour of the body whorl around the umbilicus is white. Differently, the

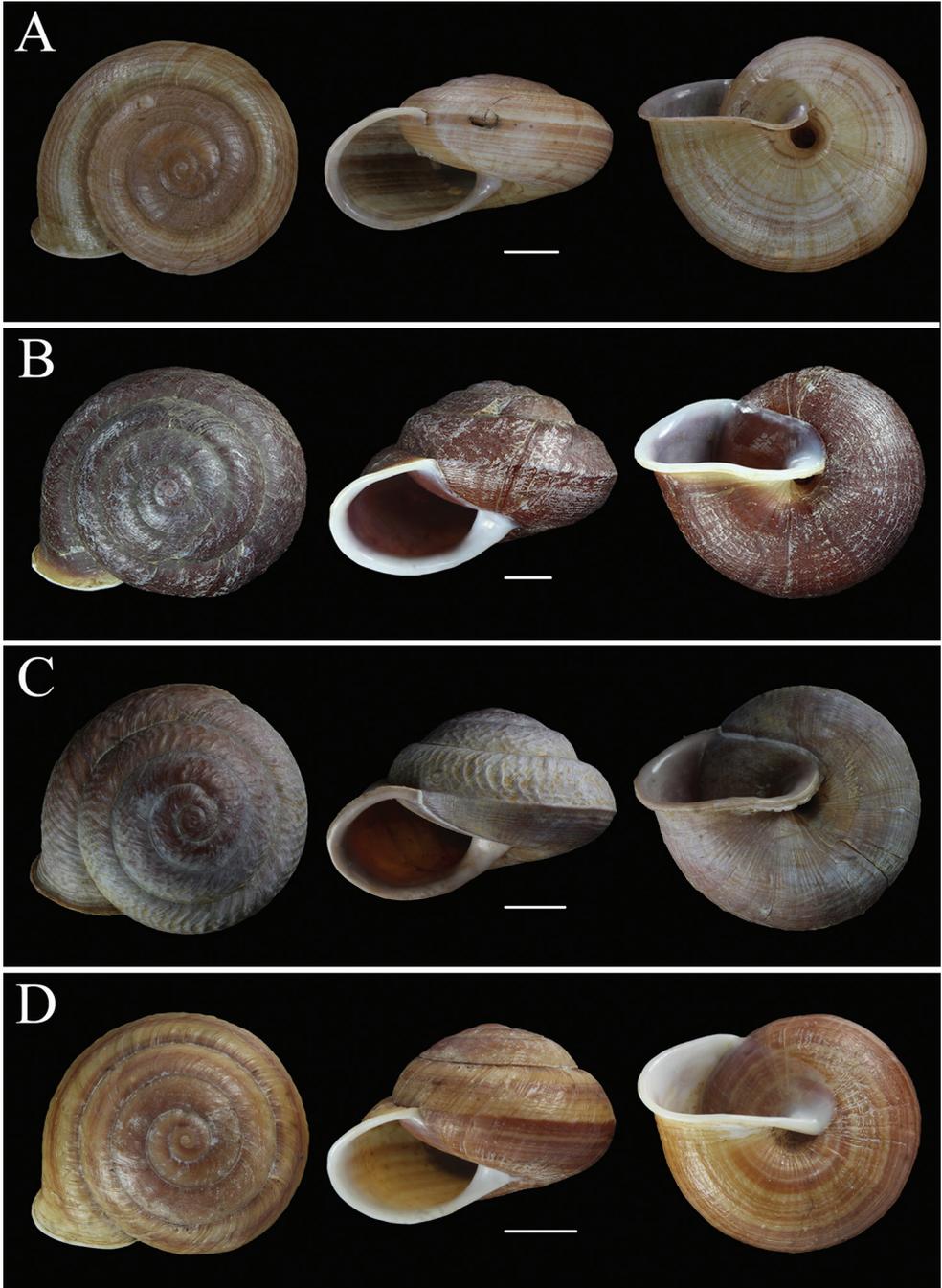


Figure 3. Photographs of shells. **A** *Camaena lingyunensis* sp. n. (holotype, FJIQBC 19280, Kasuo, Lingyun, Guangxi, China) **B** *Camaena detianensis* sp. n. (holotype, FJIQBC 18472, Detian Falls, Daxin, Guangxi, China) **C** *Camaena bahni* (FJIQBC 19300, Huu Lien Nature Reserve, Lang-Son, Vietnam) **D** *Camaena cicatricosa* (FJIQBC 18505, Guiping, Guangxi, China). Scale bars 10 mm.

new species has a flat and low spire with an inconspicuous forged trace, and contains countless slender spiral bands. The base region colour of the body whorl is the same as the shell surface with slender spiral bands.

Some sinistral *Camaena* species have the same features as the new species on penial wall, such as *C. habni*, *C. poyuensis* and *C. inflata* (Ding et al. 2016), while the verge of these species is significantly different from the new species. The surface of verge of *C. habni*, *C. poyuensis* and *C. inflata* all contains transverse or longitudinal microgrooves, but in the new species it is conical and smooth, and does not have any microgrooves except for a longitudinal crack, from which six longitudinal and smooth pilasters can be seen. There are six longitudinal and deep cracks on verge of *C. poyuensis*, but the cracks cannot be riven.

COI gene *p*-distances between this new species and the other seven sinistral species are 0.098–0.178 (Table 3). On phylogenetic tree, *C. lingyunensis* sp. n. is adjacent to *C. cicatricosa* and *C. detianensis* sp. n. However, the shells of the three species were greatly distinct as follows: (1) the fawn shell color of *C. lingyunensis* sp. n. is much lighter than the dark tawny shell color of *C. cicatricosa* and brown shell color *C. detianensis* sp. n. (2) The spire of *C. lingyunensis* sp. n. is low and flat, while the spires of the latter two are relatively high and arched (3) the *C. lingyunensis* sp. n. has countless light chestnut and slender spiral bands on the body whorl while *C. detianensis* sp. n. has no spiral bands (4) the umbilicus of *C. lingyunensis* sp. n. is fully open, while that of the latter two are semi-open.

***Camaena detianensis* Zhou & Lin, sp. n.**

<http://zoobank.org/5F5B2C7E-954C-4644-8644-9873877959C5>

Figs 3B, 4B, 5B, Table 3

Holotype. [FJQBC 18472] Shell height 38.5 mm, shell width 61.4 mm, height of aperture 22.5 mm, width of aperture 26.2 mm, 21 May 2013, collected from the type locality.

Paratype. [FJQBC 18473–18482] 10 specimens: all live snails including 4 adults and 6 juveniles. Shell height 34.2–40.4 (38.46±2.49) mm, width 55.0–62.5 (59.60±2.41) mm, height of aperture 19.6–23.9 (22.44±1.67) mm, width of aperture 24.2–28.1 (25.86±1.58) mm, 21 May 2013, collected from holotype locality.

Type locality. Detian Falls, Daxin, Guangxi, China (22°51'29.54"N, 106°43'13.51"E).

Etymology. Named for the type locality, adjective.

Description. *Shell.* Shell sinistral, large, thick, solid, non-transparent and depressed-globular. 5.5 whorls, the front whorls increasing and convex rapidly. Spire arched. Body whorl expanding with an acute carina at periphery and a shallow groove-like depression above and below the carina, obviously near the aperture. Apex quite blunt. Growth lines on protoconch visible when using 15× magnification. Suture line deep. Surface dark brown and slightly red with obvious malleation. Spiral bands absent. The upper part of the periphery of body whorl with countless thick growth lines, convex, and the lower part smooth. Spire ribs below the periphery relatively obvious.

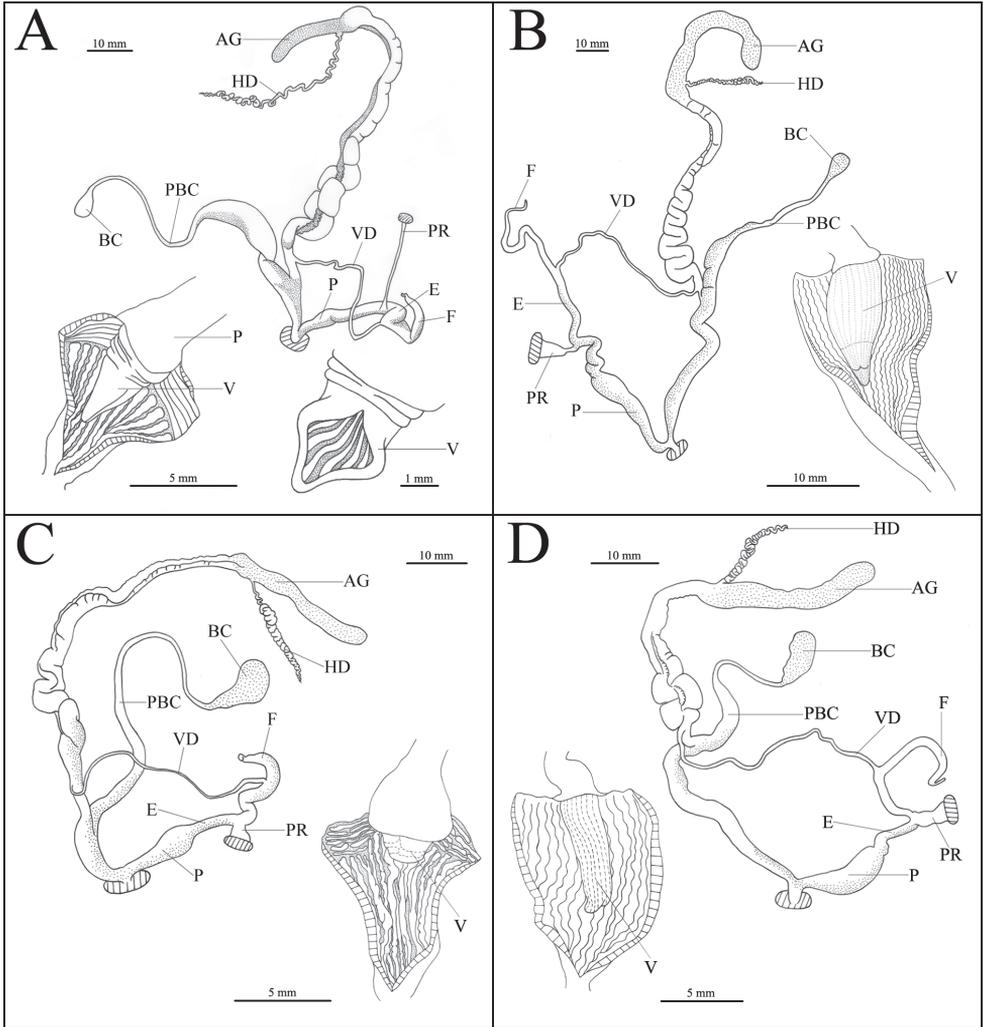


Figure 4. Reproductive system. **A** *Camaena linyunensis* sp. n. (holotype, FJIQBC 19280, Kasuo, Lingyun, Guangxi, China) **B** *Camaena detianensis* sp. n. (holotype, FJIQBC 18472, Detian Falls, Daxin, Guangxi, China) **C** *Camaena bahni* (FJIQBC 19301, Huu Lien Nature Reserve, Lang-Son, Vietnam) **D** *Camaena cicatricosa* (FJIQBC 18505, Guiping, Guangxi, China). Abbreviations: V, verge; AG, albumen gland; BC, bursa copulatrix; E, epiphallus; F, flagellum; HD, hermaphroditic duct; P, penis; PR, penis retractor muscle; PBC, pedunculus of bursa copulatrix; VD, vas deferens.

Aperture lunate. Peristome reflected, white and not sharp. Columellar margin reflected. Umbilicus partly covered by reflected columellar lip. Inner lip attached to the body whorl tightly, forming translucent, smooth and thick callus. Umbilicus obvious and round. A hump beside umbilicus absent.

Soft body. Foot and tentacles are dark brown, and the head color is relatively light.

Reproductive system. Penis slightly long, slender and smooth. Epiphallus long and thin. Penis retractor muscle short, wide and flat. Flagellum very slender and long. Va-

gina smooth and slender. Vas deferens slender. Bursa copulatrix oval. Pedunculus of bursa copulatrix quite long, expanded at the base. Inner penial wall supporting several longitudinal, dense, and curly pilasters with narrow space. Verge long conical with many shallow, longitudinal and narrow pilasters. A shallow transverse microgrooves at the apical 1/3, surrounding the verge. A harelip-like crack at the end of verge. Verge opens terminally.

Ecology. The species was found on limestone in Daxin county of Guangxi province. It generally lives in primeval forest or forest with a good ecological environment, and cannot be found in gardens near the forest. The population density of this new species in field is relatively low.

Remarks. The key characters of *C. detianensis* sp. n. and the other eight sinistral *Camaena* are presented in the identification key. The carina and groove above and below the carina of *C. detianensis* sp. n. are typical features, which are different from other species of *Camaena* except for *C. habni*. There is no obvious groove-like depression above and below the periphery of *C. habni*.

The differences of shell between the new species and *C. cicatricosa* were as follows: (1) *C. detianensis* sp. n. is quite large, and the largest width can reach 62.5 mm, while the maximum width of shell of *C. cicatricosa* is less than 50 mm (2) The shell of this species is dark brown without spiral bands, while the shell of *C. cicatricosa* is yellowish brown, and contains many annular spiral bands (3) There is an acute carina at periphery of body whorl of the new species, and groove-like depression above and below the carina, but *C. cicatricosa* has no obvious carina.

The new species has similar appearance with *C. habni*, both of which have semi-open umbilicus and a distinct carina at periphery of body whorl, but there still exist differences (1) *C. habni* is relatively small, and the width of shell of mature specimen is generally between 45.0–47.0 mm. While the new species is very large, and the width of shell is generally between 55.0–62.5 mm (2) Compared to the new species, *C. habni* has a sharper carina, but there is no obvious groove-like depression above and below the carina (3) The growth lines and spire ribs of *C. habni* are thicker, and the shell surface is very rough, while that of the new species is relatively finer (4) The new species has thicker callus, while *C. habni* has thinner callus.

A dissection of reproductive system shows that the pilasters of penis wall and verge shape of *C. detianensis* sp. n. is similar to that of *C. cicatricosa*, both of which have longitudinal and curly pilasters on the penis wall with narrow spaces, and the verge is conical with many longitudinal pilasters, while *C. habni* has longitudinal and transverse pilasters on the penis wall, and verge is semicircle and small. There is an annular microgroove on verge, and a harelip-like crack at the end of verge in the new species, which is a diagnostic feature differing from other sinistral *Camaena* species.

COI gene *p*-distances between this new species and other seven sinistral species were 0.073–0.189 (Table 3). On the phylogenetic tree, this new species and *C. cicatricosa* are mutually sister groups, and *p*-distance of the two species is 0.073–0.086. According to above information, it is reasonable that the species is recognized as a new species (Criscione and Köhler 2014).



Figure 5. Ecological photographs of snails. **A** *Camaena lingyunensis* sp. n. (Kasuo, Lingyun, Guangxi, China) **B** *Camaena detianensis* sp. n. (Detian Falls, Daxin, Guangxi, China) **C** *Camaena bahni* (Huu Lien Nature Reserve, Lang-Son, Vietnam) **D** *Camaena cicatricosa* (Yangchun, Guangdong, China).

Key to the sinistral *Camaena* species

- 1 Umbilicus open 2
- Umbilicus closed 4
- 2 Completely open umbilicus 5
- Not completely open umbilicus 3
- 3 Semi-open umbilicus and no hump beside the umbilicus 6
- Narrow umbilicus and a hump beside the umbilicus..... 8
- 4 No hump beside the umbilicus; many transverse microgrooves and few longitudinal deep groove on surface of verge.....
..... *C. poyuensis* (Zhou, Wang & Ding, 2016)
- A hump beside the umbilicus; many irregular curly grooves on surface of verge *C. oblecta* (Fischer, 1898)
- 5 No malleation on shell; a wide red band at periphery of body whorl.....
..... *C. seraphinica* (Heude, 1890)
- Malleation on shell and countless chestnut slender spiral bands, forming a wide area of bands below periphery of body whorl *Camaena lingyunensis* sp. n.

- 6 Acute carina at periphery of body whorl7
 – No obvious carina at periphery of body whorl
 *Camaena cicatricosa* (Müller, 1774)
 7 Shell medium size; no obvious groove-like depression above and below the
 carina at periphery of body whorl; verge short and semicircle without a hare-
 lip-like crack *Camaena habni* (Mabille, 1887)
 – Shell very large; shallow groove-like depression above and below the carina at
 periphery of body whorl; verge long and conical with a harelip-like crack.....
 *Camaena detianensis* sp. n.
 8 Shell globose and thick; verge with transverse deep wrinkles basally and dense
 longitudinal microgrooves apically *C. inflata* (Möllendorff, 1885)
 – Shell depressed-globose; verge with longitudinal deep wrinkles only
 *C. connectens* (Dautzenberg & Fischer, 1906)

Discussion

In the present study, two new species of sinistral *Camaena* were identified based on shell structure and colouration, reproductive system morphology, and molecular characteristics. *Camaena linyunensis* sp. n. can be distinguished from other sinistral camaenids by the flat, thin, fragile, semi-translucent, and light coloured shell, especially the unique flat globose shape. The large shell, thick callus, acute carina at periphery of the body whorl and groove-like depression above and below the carina are key features of *C. detianensis* sp. n.

Genetic distance has been generally used for classification and determination of Camaenidae, such as the Australian camaenid *Kimberleytrachia* (0.055–0.161, Criscione and Köhler 2014), the Japanese camaenid *Luchuhadra* (0.003–0.205, Kamada et al. 2007), and the Taiwanese camaenid *Satsuma* (0.006–0.150, Wu et al. 2008). The *p*-distance between *C. linyunensis* sp. n. and the other seven sinistral *Camaena* was significant, 0.098–0.178, as well as between *C. detianensis* sp. n. and the other seven sinistral *Camaena*, 0.073–0.189. All attain interspecific differentiation, and molecular phylogenetic analyses also support these two new species.

Camaena detianensis sp. n. and *C. cicatricosa* are closer in phylogeny and reproductive system dissection besides the shell morphology. While *C. detianensis* sp. n. and *C. habni* have the similar shell. *Camaena habni broti* (Dautzenberg & d'Hamonville, 1887) once was regarded as a subspecies of *C. habni*, which is distributed in Nuy-Dong-Nay, Lang-Son, Vietnam. Due to the lack of specimens of *C. habni broti* we did not compare it with the new species. However, from the pictures of syntypes it can be seen that *C. habni broti* (MNHN-IM-2000-1848) and *C. habni* (MNHN-IM-2000-1906) have the same size, morphology, and geographical distribution. Actually, Pilsbry considered *C. habni broti* as a synonym of *C. habni* in 1891. In the article of Ding et al. (2016), *C. c. ducalis* (Ancey, 1885), a subspecies of *C. cicatricosa*, was not revised due to lack of specimens. In this research, molecular comparison was not

conducted either. According to literature records, *C. c. ducalis* was named based on a single specimen collected from Kouy-Yang-Fou (nowadays Guiyang), Guizhou. No further specimens were confirmed or recorded since its publication. The shell of *C. c. ducalis* (Ancey, 1885) with narrow umbilicus is quite large, shell width is 74 mm, but the maximum sinistral snail *C. detianensis* sp. n. is 62.5 mm. Some scholars have made great efforts to collect *C. c. ducalis* (Ancey, 1885), but failed (Ding et al. 2016). It is possible that this species has died out. On the other hand, *C. seraphinica* demonstrates great differences from the other sinistral *Camaena* by possessing a non-malleated surface, and white shell background with few wide bands; these characters are closer to Bradybaenidae in shell.

During the gradual lifting from the north Vietnam to the mid-west of Guangxi and then the Yunnan-Guizhou Plateau, the biological and geographical climate conditions changed complicatedly. The limestone landform is widely distributed in these areas and the complex environment has provided helpful conditions for life and reproduction of land snails. These areas have become the hot spots in research on biodiversity of land snails, and many new species have been found in recent years (Páll-Gergely et al. 2016; Páll-Gergely et al. 2015; Schileyko 2011; Nordsieck 2007). In our opinion, as research progresses, more and more new species of *Camaena* will be found in this area. Hence, a phylogenetic research based on morphology and molecular biology of *Camaena* is essential and urgent.

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MonotomidGen – A matrix-based interactive key to the New World genera of Monotomidae (Coleoptera, Cucujoidea)

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Abstract

A matrix-based Lucid™ key is presented for the twelve genera of Monotomidae (Coleoptera: Cucujoidea) represented in the New World. A general overview is given for the features and technical specifications of an original interactive key for the identification of these genera. The list of terminal taxa included with the key provides a current summary of monotomid generic diversity for the Nearctic and Neotropical regions.

Keywords

interactive key, data matrix, identification, morphology, LUCID, minute clubbed beetles

Introduction

Matrix-based (also known as interactive, multi-access, multi-entry, or filter-style) keys offer vast advantages over traditional dichotomous identification keys. Some advantages include: freedom to follow more than a single path, ability to use only subsets of characters, integration of non-traditional (e.g., biology, distribution) and overlapping characters, effective use of multi-state characters, and inclusion of numerous graphics (Penev et al. 2009, 2012). These keys have been used successfully

to overcome the challenges of identification of many groups of organisms, including various flies (Lyons and Dikow 2010; Ceretti et al. 2012), thrips (Mound et al. 2012), aphids (Favret and Miller 2012), and beetles (Lawrence et al. 2010; Lord et al. 2011; Nearn et al. 2016). However, many other challenging groups could use such powerful identification tools.

With 117 described species in twelve genera, the New World Monotomidae (also known as the “minute clubbed beetles”) are a small group of mostly mycophagous and predaceous beetles within the superfamily Cucujoidea. The worldwide generic diversity was last reviewed by Sen Gupta (1988). Since that review, nine new genera have been described, including one from the New World (Pakaluk and Ślipiński 1993; Pal 1996; Sen Gupta and Pal 1995). The North American genera were briefly reviewed and an identification key was provided by Bousquet (2002a). Many Nearctic genera have been reviewed relatively recently (Bousquet 1990, 2002b, 2003a, b, c; Bousquet and Laplante 1999). Despite these reviews, identification remains difficult, especially to non-specialists who are unfamiliar with the diagnostic characters. Since Monotomidae are important components of forest ecosystems as predators of scolytine bark beetles (Gregoire et al. 1985), vectors of fungal pathogens in trees (Hinds 1972), and pollinators (Jenkins et al. 2015), non-specialists frequently encounter them and need to make confident determinations. In addition, some monotomids, such as *Rhizophagus parallelocollis*, *Monotoma longicollis*, *M. spinicollis*, *M. johnsoni*, *M. picipes*, and others (Kuschel 1979; Bousquet 1990; Bousquet and Laplante 1999; Jelinek 2007), are being spread worldwide through human commerce or expanding their native range (Peck and Thomas 1998), and their effects on ecosystems will remain undocumented until they are identified. However, monotomid identifications have been complicated by the inaccessibility of taxonomic literature, lack of a recent, synthetic, genus-level treatment, and inadequacy of available graphics (habitus photographs, electron micrographs, and illustrations) to interpret many diagnostic features.

To address these issues, an interactive matrix-based identification key was developed for the twelve described genera of New World Monotomidae. This key is based on a matrix of 46 characters derived from morphometrics, discrete anatomical features, distributional data, and ecology. Included are illustrations of diagnostic features and dorsal and ventral photomicrographs of reliably determined representatives of each genus. Complete taxonomic coverage was possible for some genera, allowing inclusion of photomicrographs and morphometric data for all known species.

Project description

Taxonomic coverage

This key covers 12 of the 12 genera belonging to the family Monotomidae that are currently known to occur in the New World (Bousquet 2009).

List of the terminal taxa included in the current version of the identification key (last update November 2016)

Aneurops Sharp, 1900; *Bactridium* LeConte, 1861; *Crowsonius* Pakaluk & Ślipiński, 1993; *Europs* Wollaston, 1854; *Hesperobaenus* LeConte, 1861; *Leptipsius* Casey, 1916; *Macreurops* Casey, 1916; *Monotoma* Herbst, 1793; *Phyconomus* LeConte, 1861; *Pycnotomina* Casey, 1916; *Rhizophagus* Herbst, 1793; *Thione* Sharp, 1899.

Photomicrographs of terminal taxa

Each genus included in the key has at least one associated dorsal and ventral photomicrograph. For most genera, multiple photomicrographs were provided in order to illustrate the range of intrageneric diversity. All photomicrographs represent either type specimens, authoritatively identified museum material, or material determined by the first author (TCM). Illustrative shots of important characters are provided within the key, and larger dorsal and ventral habitus photographs are included within the Fact Sheets section of the website.

Characters used in the key

General features

Characters used for identification were derived from existing literature (Sen Gupta 1988; Bousquet 2009) but then confirmed and scored from specimens in the University of Georgia Collection of Arthropods (UGCA) and the Smithsonian Institution National Museum of Natural History (NMNH). Anatomical terminology follows that of Bousquet (2009) and Sen Gupta (1988), the most comprehensive morphological treatments of Monotomidae to date.

The data matrix forming the foundation for this key is based on 46 anatomical, distributional, and ecological characters. These features are encoded into characters with a range of two to eight possible states. Most characters refer to external anatomical features of the adult form that are easily visible without preparation or dissection. Because multi-access keys provide users with greater flexibility than dichotomous keys, hard-to-view and rarely available features also are included. For example, ecological characters are provided for unusual cases when such information is available. The key includes several hind wing characters, usually visible only after dissection and preparation, because they are very valuable for separating genera. In addition, five morphometric characters are included. The diagnostic range values for these characters were based on measurements of multiple species within each genus, including measurements from as many reliably identified specimens as was reasonable. By measuring many diverse individuals representing each genus, more accurate estimates of

the range in sizes was possible for these morphometric characters. The morphological characters are sorted by body part (head, mouthparts, thorax, scutellum, legs, hind wing, and abdomen; available via the “Subsets” button on the Lucid Player control bar, and sorted by default) allowing the user to easily focus on particular regions or preparations of a specimen. To quickly narrow some identifications, distributional characters are included.

Most characters are accompanied by supporting images and clarifying explanations within the key, as well as in the “Glossary of Terms” section (see below) of the website.

List of the characters used in the key

GENERAL: length (mm); ratio of body length: greatest body width; body shape (lateral view); dorsal surface of the body (setation); elytral color; biology (habitat, known host associations, etc.); geographic distribution

HEAD: ratio of head length: greatest head width (including eyes); head constriction (presence/absence); ratio of temple length: longitudinal length of eye; antennal cavity on ventral side of head (presence/absence); eyes (number of facets); antennal club (number of apparent segments); antennal club (whether distinct)

MOUTHPARTS: maxillary palps (size of second segment); labial palps (size of second segment); mandibular dentation (number of teeth); mandibular cavity (presence/absence)

THORAX: ratio of pronotal length along midline: greatest pronotal width; pronotal disc (vestiture); pronotal disc (shape); lateral margin of pronotum (smooth/crenulate); pronotal disc (impressions); pronotal microsculpture (presence/absence); pronotal puncture density (center of disc only); anterior angles of pronotum (whether projecting); procoxal cavities externally (shape); procoxal cavities (degree of separation); procoxal trochantins (exposure); scutellar microsculpture (presence/absence); scutellar setation (presence/absence); mesocoxal cavities (degree of separation)

LEGS: number of metatarsomeres of male

WINGS: elytral setigerous punctures (arrangement); setigerous punctures on epipleural fold (number of rows); hind wing (presence/absence); number of anal veins; r-m cross (degree of development); subcubital fleck (presence/absence)

ABDOMEN: intercoxal process of abdominal ventrite (shape); metacoxal bead or femoral line (presence/absence); metacoxal bead or femoral line (length of production); first abdominal ventrite of male (special modifications); puncture rows on abdominal ventrites two-four (presence/absence); number of rows of punctures on abdominal ventrites two-four; punctures on abdominal ventrites two-four (size/shape)

Software technical specifications

Application: Lucid Builder 3.5 (available at www.lucidcentral.org, see website for exact technical specifications and features list)

Key version: 1.0

Requirements for use: Java-enabled browser and internet connectivity

License for use of the key: Creative Commons Attribution License (CC-BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Web location: <http://www.monotomidae.com/MonotomidGen.html>

Data resources

The data underpinning the Lucid Key (Lucid Key files) reported in this paper are deposited in the Dryad Data Repository at <http://dx.doi.org/10.5061/dryad.q9p4j>.

Website features

Genus fact sheets

<http://monotomidae.com/facts.html>

Each of the twelve genera represented in the key are treated and figured with dorsal and ventral habitus images. For each genus, informational sections about the following subjects are provided to assist identifications: Taxonomy, Diagnosis, Biology, Distribution, List of Species (photographed and not-photographed), and Suggested References.

Resources

<http://monotomidae.com/resources.html>

An anatomical atlas, glossary of terms, and guide to diagnosing the beetle family Monotomidae are included here. The anatomical atlas illustrates many of the characters used in the identification key and includes an illustration of the dorsal and ventral habitus of *Monotoma producta*, as well as a wing illustration of *Rhizophagus sayi*. The glossary of terms (<http://monotomidae.com/glossary.html>) provides clarifying definitions and explanations of all terms included in the interactive key, listed alphabetically, drawn from Nichols (1989), Lawrence et al. (2011), and Nearn et al. (2016). The diagnosis page (<http://monotomidae.com/whatis.html>) discusses characters that could diagnose a beetle as belonging to Monotomidae. It also provides photographs of taxa that are commonly misidentified as Monotomidae.

References

<http://monotomidae.com/references.html>

A list of useful monotomid references is given. Links are provided to available PDFs or websites of these references when not in violation of copyright restrictions.

Conclusions and future work

During development of this identification resource, several problems became apparent. First and foremost, nearly all monotomid genera included herein require modest or extensive taxonomic work. For the Nearctic region, the problem is not as serious because most genera, with the exception of *Bactridium*, have been at least partially treated within the last 25 years (e.g., Bousquet 1990, 2002a, 2002b, 2003a, 2003b, 2003c; Bousquet and Laplante 1999). *Bactridium* requires extensive work and is currently undergoing revision by TCM. In addition, most other genera represented in the Nearctic harbor some undescribed species (e.g., *Monotoma*, *Aneurops*, and *Rhizophagus*). As new types of data are examined, some currently recognized polymorphic species may be recognized as species complexes. The Neotropical fauna has been far less studied; numerous undescribed species and potentially even genera exist. Species identification in this region almost always requires comparison with type material. Even genus-level identifications of Neotropical specimens should be confirmed by a specialist, though this key will narrow down options for tentative determinations considerably.

Second, the relationships between monotomid genera are poorly understood. No phylogenetic analyses of any kind have been performed for this family. Thus, some morphological characters currently used to delimit genera require investigation to test their success in characterizing monophyletic groups. Some monotypic North America genera (e.g., *Pycnotomina*, *Macreurops*, and *Phyconomus*) should especially be targeted, as they may represent highly autapomorphic lineages nested within other genus-level clades.

Pending completion of a number of alpha taxonomic studies and phylogenetic analyses of the family, it will be possible to update this key to include species as the terminal units, and to more rigorously define the genera, as supported by additional characters. In the meantime, the numerous habitus images and illustrations should provide enough resources for confident genus-group determinations, and the other resources provided within MonotomidGen should facilitate approximate species identification.

This key provides a flexible, powerful, and media-rich information resource for any scientist or non-professional who needs to identify monotomid beetles. In addition, it provides a framework upon which to build future identification resources for this family. Eventually, a worldwide resource for identification of monotomid beetles should be completed to identify the species being transported around the world through human activities. This will allow for quicker identifications and therefore, quicker documentation of the spread of newly adventive species. Taxonomic resources of broader scope such as MonotomidGen can assist those tasked with discovering and identifying these anthropogenic species introductions.

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A taxonomic review of the Hydraenidae in South Korea (Coleoptera)

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Abstract

A taxonomic study of the South Korean Hydraenidae is presented. Eight species in two genera are recognized, one of which is reported for the first time in the Korean peninsula, *Ochthebius marinus* (Paykull). It was also found that *Hydraena riparia* Kugelann and *O. inermis* Sharp previously recorded in South Korea were incorrect identifications of *H. puetzi* Jäch and *O. lobatus* Pu, respectively. Habitus and SEM photographs, line drawings of aedeagus, distribution maps, keys, and redescriptions of the species are provided.

Keywords

Coleoptera, Hydraenidae, South Korea, taxonomy

Introduction

The Hydraenidae, relatively small water beetles with unique habitus, are usually found at margins of running water, slowly flowing water, and stagnant water in the vicinity of lotic biotopes (Jäch et al. 2016). Approximately 1600 species in 42 genera have been recorded in the world (Ślipiński et al. 2011); 941 species in nine genera in the Palearctic (Jäch and Skale 2015); 100 species in seven genera in China, 35 species in three genera in Japan; and 11 species in three genera in the Far East of Russia (Jäch and Skale 2015).

Members of the Hydraenidae are characterized by combination of the following features: separation of the gula and submentum by confluent genae; presence of an anterior plate-like premento-hypopharyngeal extension; large labrum with a deep medial incision in some species; and palpigerae connected by a transverse sclerotized bar (Beutel et al. 2003).

Kwon and Suh (1986) first recorded *Hydraena riparia* Kugelann in South Korea. Later, Lee (1995) added two species (*Ochthebius inermis* Sharp and *O. satoi* Nakane) with descriptions and habitus illustrations. Recently, Jäch and Delgado (2014) found that a marine littoral species of *Neochthebius granulatus* (Satô) previously recorded by Park and Ahn (2008) was an incorrect identification of *O. abni* Jäch and Delgado and *O. parki* Jäch and Delgado. Jäch and Skale (2015) added four species [*H. miyatakei* Satô, *H. puetzi* Jäch, *O. hasegawai* Nakane and Matsui, and *O. lobatus* Pu]. Accordingly, nine species in two genera have been recorded in South Korea.

In this paper, one species is reported for the first time in the Korean peninsula, *O. marinus* (Paykull). It was also found that *H. riparia* and *O. inermis* previously recorded in South Korea were incorrect identifications of *H. puetzi* and *O. lobatus*, respectively. Habitus and scanning electron microscopy photographs, keys, redescriptions, and diagnostic characters with illustrations of the species are provided.

Material and methods

To identify South Korean hydraenid species more reliably, they were compared with voucher specimens in the Natural History Museum (NHM, London, United Kingdom), Naturhistorisches Museum (NMW, Wien, Austria) and Ehime University Museum (EUMJ, Matsuyama, Japan). The specimens used in this study are deposited in Chungnam National University Insect Collection (CNUIC, Daejeon, Korea). Habitus and scanning electron microscope (SEM) photographs were prepared based on a former study (Lee and Ahn 2015). See Jäch and Delgado (2014) for detailed descriptions of *O. abni* and *O. parki*. The terms of taxonomic characters and measurements of specimens mainly followed Perkins (2001) and Jäch et al. (2016). The geographical subdivision of China and Russia was based on the standards of Löbl and Löbl (2015).

Results

Hydraenidae Mulsant, 1844

Key to the genera of South Korean Hydraenidae

- 1 Anterior margin of mentum (Fig. 8) dentate; maxillary palpi longer than antenna.....*Hydraena*
- Anterior margin of mentum (Fig. 18) not dentate; maxillary palpi shorter than antenna.....*Ochthebius*

Genus *Hydraena* Kugelann, 1794

Hydraena Kugelann, 1794: 578. Type species *Hydraena riparia* Kugelann, 1794.

Diagnosis. Labral-mandibular interlocking device present. Mentum (Fig. 8) with acute median projection on anterior part. Sensilla various and complex. Features of the secretion delivery system specialized (Jäch et al. 2000).

Key to the subgenera of South Korean *Hydraena*

- | | |
|---|--|
| 1 | Body brown to dark blue; longitudinal median genal suture and longitudinal inner genal carina complete <i>Hydraena</i> |
| – | Body yellowish brown; longitudinal median genal suture and longitudinal inner genal carina incomplete or absent <i>Hydraenopsis</i> |

Subgenus *Hydraena* Kugelann, 1794

Hydraena Kugelann, 1794: 578. Type species: *Hydraena riparia* Kugelann, 1794. See Jäch and Skale (2015) for more detailed synonymy and references.

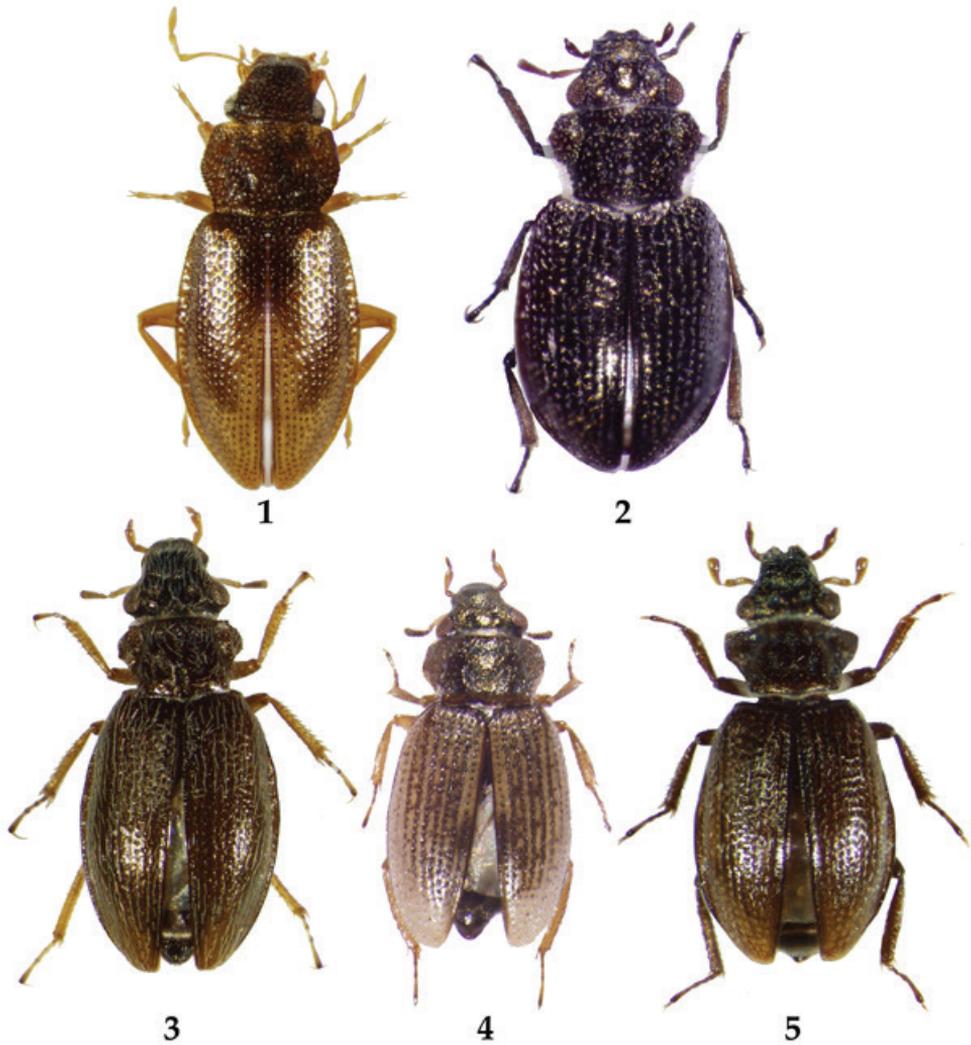
Diagnosis. Lateral margin of labrum (Fig. 6) abruptly constricted posteriorly. Setae of labial palpomere 2 closely set. Anterior apex of modified longitudinal hypomeral carina with antennal pocket setae. Elytra (Fig. 11) with 15 or more striae. Mesoventral process (Fig. 14) obtuse, between 100° and 130° angle. Sternite III (Fig. 17) without pubescence behind coxal pits. Sternite VII (Fig. 17) pubescent with semicircular glabrous posterior region. Gonocoxite with a pair of subapical tufts (Jäch et al. 2000).

***Hydraena (Hydraena) puetzi* Jäch, 1994**

Figs 1, 6–17, 50, 55

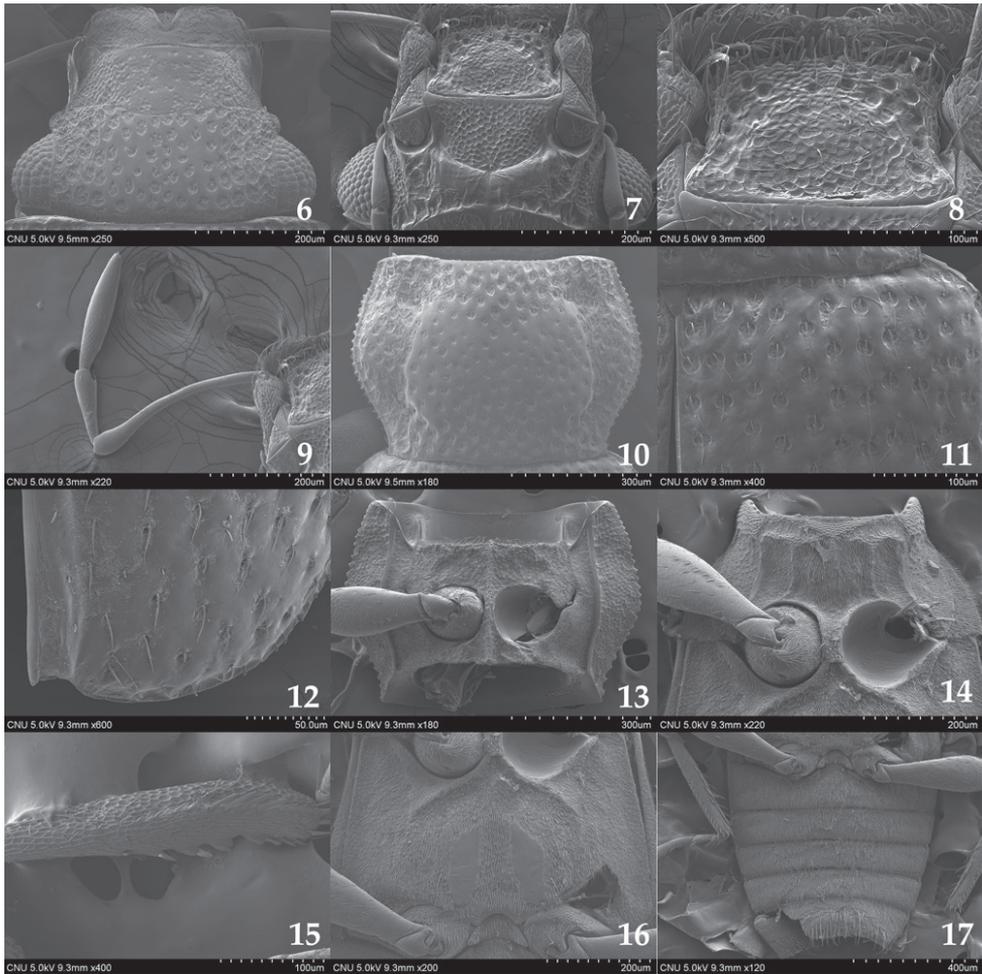
Hydraena (s. str.) puetzi Jäch, 1994: 43; Jäch and Skale 2015: 139.

Specimens examined. SOUTH KOREA: Gangwon Prov.: 1♀, Goseong-gun, Ganseong-eub, Jinburyeong, N38°15'59" E128°23'04" , 640m, 21.VII.2004, KM Yang, JS Park, leaf litter; 1♂ 2♀♀, Hongcheon-gun, Nae-myeon, Changchon-ri, Unduryeong-hill, 10.VIII.2012, SW Jeong, mountain stream; 1♂, Hongcheon-gun, Seo-myeon, Bangok-ri, Bangokgyo, 17.IX.2009, DH Lee, mountain stream (1♂, on slide); 1♂ 3♀♀, Pyeongchang-gun, Daegwanryeong-eub, Yucheon-ri, Haewon-temple, 15.IX.2009, YJ Park, under stone on stream margin (1♂, on slide); 1♀, Pyeongchang-gun, Jinbu-myeon, Dongsan-ri, Mt. Odaesan, Sangwon-temple, 16.VIII–15.IX. 2001, SJ Park, CW Shin, FIT; 1♂, same data as former except for, 8.V.2004, DH



Figures 1–5. Habitus. **1** *Hydraena puetzi*, 2.2 mm **2** *Ochthebius hasegawai*, 1.8 mm **3** *O. lobatus*, 2.2 mm **4** *O. marinus*, 2.0 mm **5** *O. satoi*, 1.8 mm.

Lee, stream; 1♂, same data as former except for, 8.V.2004, DH Lee, stream; 9♂♂ 11♀♀, same data as former except for, Mt. Odaesan, 22.V.2012, DH Lee, springfed pool; 1♂, Samcheok-si, Gagok-stream, 25.VI.1985. SH Lee; 3♀♀, Yeongwol-gun, Suju-myeon, Mt. Baehyang-san, 17.VII.2010, SW Jeong, mountain stream; Gyeongbuk Prov.: 1♀, Uljin-gun, Buk-myeon, Mt. Eungbongsan, 6.VI.1995, SH Lee; 1♂, Uljin-gun, Seo-myeon, Wangpi-ri, Golan-bridge, N36°54'27.1" E129°14'34.2" , 385m, 26.IV.2012, DH Lee, mountain stream; Gyeonggi Prov.: 1♀, Gapyeong-gun, Buk-myeon, Jeongmok-ri, Garim-bridge, 16.IX.2010, SW Jeong, stream; 1♂, Paju-si, Jangdan-myeon, Nosang-ri, 21.IX.2012, SW Jeong, stream.



Figures 6–17. *Hydraena puetzi*. **6** head (dorsal aspect) **7** head (ventral aspect) **8** mentum (ventral aspect) **9** maxillary palpus (ventral aspect) **10** pronotum (dorsal aspect) **11** anterior part of elytron (dorsal aspect) **12** posterior part of elytron (dorsal aspect) **13** prosternum (ventral aspect) **14** mesoventrite (ventral aspect) **15** mesotibia in male (dorsal aspect) **16** metaventricle (ventral aspect) **17** abdominal sternites (ventral aspect).

Published South Korean records. *Hydraena puetzi*: Jäch and Skale (2015: 103). *Hydraena riparia*: Kwon and Suh (1986: 99); Kim et al. (1994: 134); Lee (1995: 13); Han et al. (2007: 271); Han et al. (2008: 263); Cho and Park (2010: 96) [misidentification].

Redescription. Length 2.0–2.3 mm. Head black; pronotum and elytra brown to dark blue; ventral surface dark brown. Head (Fig. 6) trapezoidal, widest cross eyes, ventral side (Fig. 7) with sparse setae. Anterior margin of labrum (Fig. 6) nearly straight except antero-medial part deeply excised. Clypeus (Fig. 6) with relatively small punctures; antero-medial margin broadly rounded; antero-lateral part

protruded. Frontoclypeal suture (Fig. 6) bisinuate. Mentum (Fig. 8) subquadrate, widest at posterior corner, with large punctures on anterior and lateral parts; anterior margin broadly rounded, with a row of thick setae; posterior part protruded laterally. Submentum (Fig. 7) semicircular; antero-medial margin nearly straight, dentate; antero-lateral margin protruded and acute. Antenna with nine antennomeres; 1 longest, approx. 2.5 times as long as 2; 2 approx. 6.0 times as long as 3, bulbous at basal part; 3 bulbous at apical part; 4 dish-shaped; 5–9 clubbed and with pubescence. Maxillary palpomere (Fig. 9) 1 smallest; 2 longest, approx. 3.0 times as long as 3, few setae present on ventral part, with transverse imbricate reticulation except apical part; 3 bulbous at apical part, few setae present on dorsal and ventral parts, with imbricate reticulation except anterior third; 4 approx. 2.0 times as long as 3, many setae present on dorsal and ventral parts, apical part acute, with imbricate reticulation except anterior to middle. Pronotum (Fig. 10) hexagonal, widest at middle and narrowest posterior part, medial part protruded and flattened; anterior margin sinuate; antero-median margin straight; anterior and posterior corners rectangular; lateral margin rounded and serrated. Elytra (Figs 11, 12) widest at middle; antero-lateral and postero-lateral parts serrated. Prosternum (Fig. 13) transverse, with longitudinal carina on medial part; anterior and posterior corners acute. Mesoventrite (Fig. 14) reverse pentagonal, with two vertical carina on each side of midline. Mesotibia (Fig. 15) serrate on apico-lateral part, with thick spines. Metaventrite (Fig. 16) flattened, with two glabrous parts medially. Sternite VII (Fig. 17) with many long setae on medial part in female. Male terminal sternite semicircular; postero-medial part bifid; posterior part rounded and with many setae. Median lobe of aedeagus (Fig. 50) longer than paramere, strongly curved at middle; a seta present on anterior third; apical part acute and protruded; flagellum bisinuate. Left paramere (Fig. 50) as long as right; apical part long-oval; many long setae present on apical margin. Apical part of right paramere (Fig. 50) semicircular; many long setae present on apical margin.

Distribution. Korea, China (Liaoning, Shandong), Russia (Far East).

Biological note. They are found under leaves or stones in margins of mountain streams. Some specimens were collected from submerged branch and leaves in seepage on small cliff.

Remarks. Kwon and Suh (1986) first recorded this species as *H. riparia* in Korea. After that, many entomologists [Kim et al. (1994); Lee (1995); Han et al. (2007); Han et al. (2008); Cho and Park (2010)] reported the species, only in the local fauna without any taxonomic comments. After studying the specimen (1♂, Samcheok-si, Gagok-stream, 25.VI.1985. SH Lee) previously examined by Kwon and Suh (1986), we found that it had been incorrectly identified and actually is *H. puetzi*. This species can be distinguished from *H. riparia* by left paramere (Fig. 7A) parallel at middle, and apical part of right paramere (Fig. 7A) semicircular, with compact long setae on apex.

Subgenus *Hydraenopsis* Janssens, 1972

Hydraenopsis Janssens, 1972: 254. Type species: *Hydraenopsis vietnamensis* Janssens, 1972.

Diagnosis. Transverse anterior inner gular carina absent; mesosternal intercoxal process protruding from mesosternal disc at an angle of 150°–180°; at least one of the two parameres shifted (Jäch et al. 2000).

***Hydraena (Hydraenopsis) miyatakei* Satô, 1959**

Fig. 55

Hydraena miyatakei Satô, 1959: 62; *Hydraena (Hydraenopsis) miyatakei*: Jäch and Skale 2015: 143.

Specimens examined. SOUTH KOREA: Gangwon Prov.: 1♀, Pyeongchang-gun, Baesujang, 29 VII 1994, SH Lee.

Published South Korean record. *Hydraena (Hydraenopsis) miyatakei*: Jäch and Skale (2015: 143).

Distribution. Korea, China (Jilin, Liaoning, Shandong), Japan, Russia (Far East).

Remarks. Only one specimen of this species was collected, not suitable for description or illustration.

Genus *Ochthebius* Leach, 1815

Figs 18, 19, 22, 23, 29, 30, 31, 37, 38, 45, 46

Ochthebius Leach, 1815: 95. Type species: *Helophorus marinus* Paykull, 1798.

Diagnosis. Body (Fig. 26) with distinct pronoto-elytral angle. Head (Fig. 27) with a transverse groove separating clypeus and frons, the latter on each side with an interocular pit-like depression and a short longitudinal depression or groove on postero-medial part. Anterior margin of labrum truncate, sometimes with a small medial emargination. Pronotum (Figs 21, 30, 37, 45) with a narrow hyaline membranous cuticula, middle pronotal portion raised, and with longitudinal or transversal groove. Elytra (Figs 22, 31, 38, 46) with rows of punctures (Chiesa 1959; Hansen 1987; Jäch 1992).

Key to the species of South Korean *Ochthebius*

- 1 Pronotum widest at anterior third to three seventh part, protruded laterally.... 2
- Pronotum widest at middle part, rounded laterally 5
- 2 Elytra (Fig. 31) with long setae *O. lobatus*
- Elytra (Figs 22, 38, 46) with short setae 3

- 3 Maxillary palpomere 3 (Fig. 20) approx. 4.0 times as long as 4... ***O. basegawai***
 – Maxillary palpomere 3 (Figs 36, 44) approx. 2.0 times as long as 4..... **4**
 4 Medial part of metaventrite (Fig. 41) with pubescence; apical part of distal lobe of aedeagus acute..... ***O. marinus***
 – Medial part of metaventrite (Fig. 48) without pubescence; apical part of distal lobe of aedeagus round..... ***O. satoi***
 5 Pronotal longitudinal groove distinct (Jäch and Delgado 2014: Fig. 6); distal lobe of aedeagus curved in lateral view (Jäch and Delgado 2014: Figs 8, 15h)..... ***O. abni***
 – Pronotal longitudinal groove indistinct (Jäch and Delgado 2014: Fig. 7); distal lobe of aedeagus bisinuate in lateral view (Jäch and Delgado 2014: Figs 13, 15a) ***O. parki***

Subgenus *Ochthebius* Leach, 1815

Figs 22, 27, 29, 31, 37

Ochthebius Leach, 1815: 95. Type species: *Helophorus marinus* Paykull, 1798.
 See Jäch and Skale (2015) for more detailed synonymy and references.

Diagnosis. Lateral margin of pronotum (Figs 21, 30, 37, 45) with hyaline membranous cuticula. Marginal elytral ridge (Fig. 39) disappearing posteriorly. Pedicel (Fig. 19, 35) not enlarged distally (Hansen 1987; Jäch 1992).

Ochthebius (*O.*) *abni* Jäch & Delgado, 2014

Ochthebius (*s. str.*) *abni* Jäch & Delgado, 2014: 85; Jäch and Skale 2015: 153.

Specimens examined. Holotype: 1♂ (CNUIC), with labels as follows: “KOREA: Gyeongbuk prov.: Kugae, 6 VII 1991, K. J. Ahn, *ex.*, rock crevice., Holotype *Ochthebius abni* sp. n. Jäch and Delgado 2014”. Paratypes: 1♂1♀, same data as holotype. 3♂♂ 2♀♀, same data as holotype.

Published South Korean records. *Ochthebius abni*: Jäch and Delgado (2014; 85); Jäch and Skale (2015: 153). *Neochthebius granulatus*: Park and Ahn (2008: 2506) [misidentification].

Diagnosis. See Jäch and Delgado (2014).

Distribution. Korea.

***Ochthebius (O.) hasegawai* Nakane & Matsui, 1986**

Figs 2, 18–25, 51, 56

Ochthebius (s. str.) hasegawai Nakane & Matsui, 1986: 81; Jäch 1998: 186; Jäch and Skale 2015: 156.

Ochthebius mamagri Shatrovsky, 1989: 263.

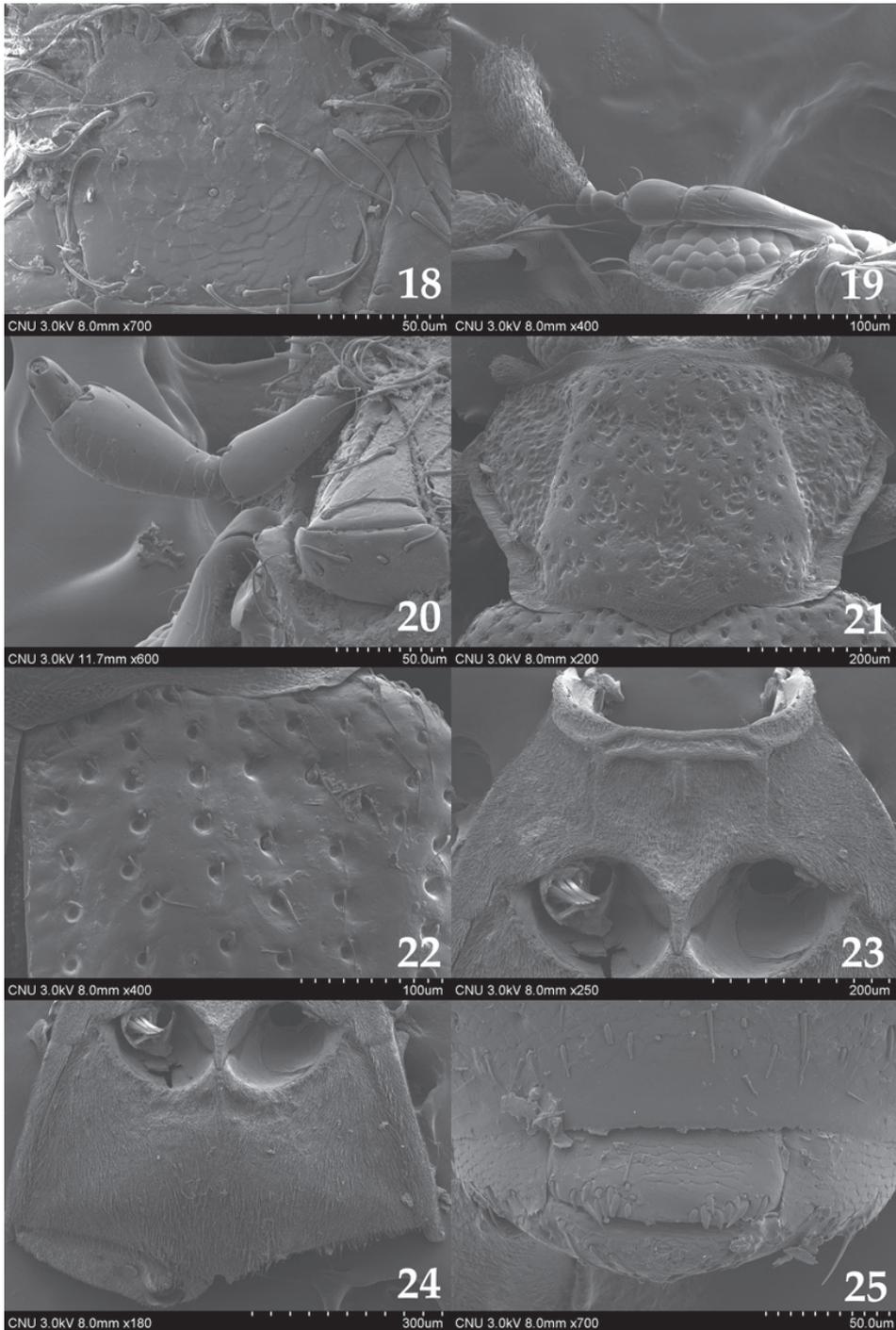
Specimens examined. SOUTH KOREA: Chungnam Prov.: 1 ♀, Nonsan-si, Beolgok-myeon, Sajeong-ri, N36°11'41.77" E127°16'18.77" 118 m, 24 V 2014, IS Yoo, under stone in stream; Gyeongbuk Prov.: 6 ♂♂ 8 ♀♀, Uljin-gun, Giseong-myeon, Dacheon-ri, Giseong-stream 17 VII 1995, SH Lee, mountain stream (3 ♂♂ 2 ♀♀, on slides); Gyeonggi Prov.: 1 ♀, Gapyeong-gun, Buk-myeon, Dodae-ri, Seungcheon-temple, 5 VII 2013, SW Jeong, HJ Park, stream.

Published South Korean record. *Ochthebius hasegawai*: Jäch and Skale (2015: 156).

Redescription. Length 1.6–2.0 mm. Body dark blue to black. Mentum (Fig. 18) 1.2 times as wide as long and with sparse setae; protruded and widest at anterior third; with a row of long setae on anterior margin. Anterior gena without long setae on posterior part in ventral view. Antenna (Fig. 19) with nine antennomeres; 1 longest, approx. 6.0 times as long as 2, two long setae present on lateral part; 2 widest at base; 3 bulbous at apical part; 4 semicircular; 5–9 clubbed and with pubescence. Maxillary palpomere (Fig. 20) 1 small, approx. 2.0 times as long as wide; 2 bulbous at apical part, 2.0 times as long as 1; 3 largest, bulbous at apical part, slightly longer than 2; 4 slender and paralleled, 0.25 times as long as 3. Pronotum (Fig. 21) reverse trapezoidal, widest at anterior fourth, with indistinct longitudinal groove on medial part, two small oval grooves on anterior part, relatively large oval groove on posterior part; anterior margin bisinuate; antero-medial margin protruded; anterior corner rectangular; lateral margin protruded at anterior third; posterior corner obtuse; postero-medial part rounded. Elytra (Fig. 22) widest at middle, with setae. Mesoventrite (Fig. 23) pentagonal, with T-shaped carina on anterior part; anterior margin transverse. Metaventrite (Fig. 24) with pubescence on medial part. Sternite VIII (Fig. 25) with more or less long setae on posterior part. Male terminal sternite (Fig. 25) semicircular and with a row of compact setae on posterior part. Median lobe of aedeagus (Fig. 51) long, slender, very weakly curved at middle; apical part acute; distal lobe slender, slightly acute apically. Paramere (Fig. 51) very short, with long setae on apical part.

Distribution. Korea, Japan, Russia (Far East).

Biological note. The specimens were collected under boulders in a fast-flowing stream.



Figures 18–25. *Ochthebius hasegawai*. **18** mentum (ventral aspect) **19** antennae (lateral aspect) **20** maxillary palpus (ventral aspect) **21** pronotum (dorsal aspect) **22** anterior part of elytron (dorsal aspect) **23** mesoven- trite (ventral aspect) **24** metaventr- ite (ventral aspect) **25** male terminal sternite (ventral aspect).

***Ochthebius (O.) lobatus* Pu, 1958**

Figs 3, 26–33, 52, 56

Ochthebius (s. str.) lobatus Pu, 1958: 256; Jäch, 1995:178; 2003: 351; Jäch and Skale, 2015: 157.

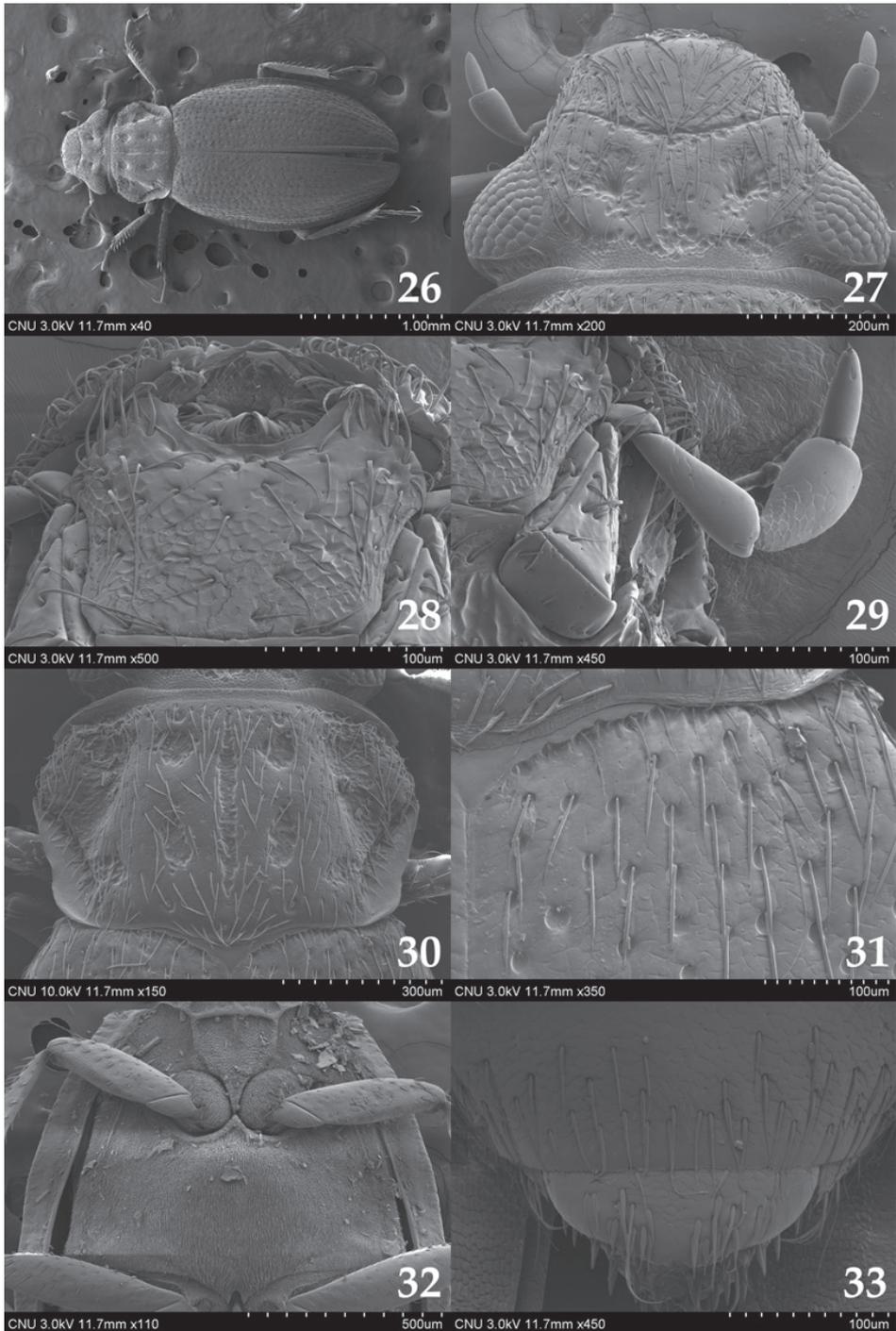
Specimens examined. SOUTH KOREA: Gangwon Prov.: 4♂♂ 2♀♀, Samcheok-si, Wondeok-eub, Wolcheon-ri, Gagok-steam, 1 V 1994, SH Lee, beside stream (4♂1♀, on slides); 5♂♂ 7♀♀, same data as former except for, 1 VI 1995; 1♀, same data as former except for, 25 VI 1995; Gyeongbuk Prov.: 2♂♂ 3♀♀, Uljin-gun, Namdae-stream, 17 VII 1995, SH Lee.

Published South Korean records. *Ochthebius lobatus*: Jäch and Skale (2015: 157). *Ochthebius inermis*: Lee (1995: 13) [misidentification].

Redescription. Length 2.2–2.6 mm. Head dark brown; pronotum and elytra brown to dark brown; ventral surface mostly brown. Dorsum (Figs 27, 30, 31) with whitish long setae. Mentum (Fig. 28) 1.2 times as wide as long and with sparse setae, widest anterior third; antero-lateral part protruded. Anterior gena without long setae on posterior part in ventral view. Antenna with nine antennomeres; 1 longest, approx. 6.0 times as long as 2, two long setae present on lateral part; 2 bulbous at medial part; 3 bulbous at apical part; 4 semicircular; 5–9 clubbed and with pubescence. Maxillary palpomere (Fig. 29) 1 small, approx. 2.0 times as long as wide; 2 bulbous at apical part, 3.5 times as long as 1; 3 bulbous at apical part, as long as 2; 4 slender and parallel-sided, 0.5 times as long as 3. Pronotum (Fig. 30) reverse trapezoidal, widest at anterior three seventh, with distinct longitudinal groove on medial part, two rounded grooves on anterior part, two oval grooves on posterior part; anterior margin bisinuate; antero-median margin straight; anterior corner rectangular; posterior corner obtuse; postero-medial part rounded. Elytra (Fig. 31) widest at middle, with long setae. Mesoventrite (Fig. 32) pentagonal with T-shaped carina on anterior part; anterior margin transverse. Metaventrite (Fig. 32) with pubescence on medial part. Sternite VIII (Fig. 33) with more or less long setae on posterior part. Male terminal sternite semicircular with few spines on posterior part. Median lobe of aedeagus (Fig. 52) weakly slender, curved; subapical part vented, inflated at anterior third; apical part acute with few setae; distal lobe cylindrical, approx. 0.25 times as long as median lobe. Paramere (Fig. 52) slender, shorter than median lobe, with few setae on apical part.

Distribution. Korea, China (Chongqing, Jilin, Liaoning, Sichuan, Yunnan).

Remarks. Lee (1995) first reported *O. inermis* Sharp in South Korea but we found that this was a misidentification of *O. lobatus*, based on our examination of his voucher specimens (4♂♂ 2♀♀, Samcheok-si, Wondeok-eub, Wolcheon-ri, Gagok-steam, 1 V 1994, SH Lee, ex. beside stream). This species can be distinguished from *O. inermis* by elytra brown and distal lobe of aedeagus robust.



Figures 26–33. *Ochtthebius lobatus*. **26** body (dorsal aspect) **27** head (dorsal aspect) **28** mentum (ventral aspect) **29** maxillary palpus (dorsal aspect) **30** pronotum (dorsal aspect) **31** anterior part of elytron (dorsal aspect) **32** meso– and metaventrite (ventral aspect) **33** sternites VIII–IX (ventral aspect).

***Ochthebius (O.) marinus* (Paykull, 1798)**

Figs 4, 34–41, 53, 56

Helophorus marinus Paykull, 1798: 245.*Ochthebius pallidus* Mulsant, 1844: 61.*Ochthebius holmbergi* Mannerheim, 1853: 166.*Ochthebius subabruptus* Rey, 1885: 23.*Ochthebius (s. str.) marinus* Jäch, 1992: 112; 2003: 330; Jäch and Skale 2015: 157.

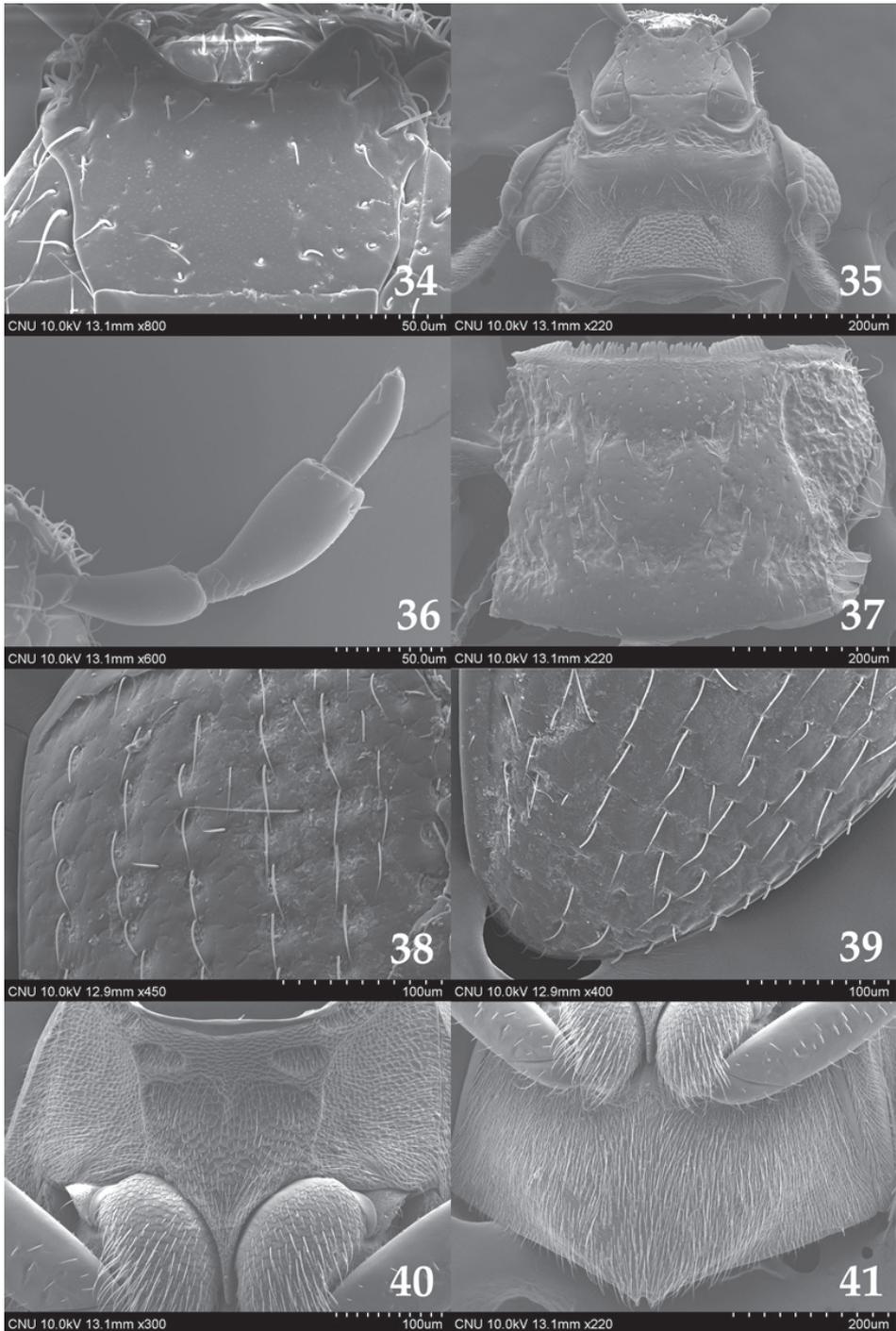
Specimens examined. SOUTH KOREA: Gyeonggi Prov.: 2♂♂ 3♀♀, Ansan-si, Danwon-gu, Daebudo-dong, Dongju-salt farm, N37°14'05.60" E126°36'19.55" 4 m, 7 XI 2013, DH Lee, IS Yoo, SG Lee, saline pond near salt farm (1♂, on slide).

Redescription. Length 1.7–2.0 mm. Head metallic dark green; pronotum mostly pale yellowish brown and medial part brown; elytra pale yellow and rows of serial punctures dark brown; ventral surface mostly dark brown. Mentum (Fig. 34) as long as wide, widest anterior third and with sparse setae; antero-medial part broadly excised; antero-lateral part protruded. Anterior gena (Fig. 35) with long setae on posterior part in ventral view. Gula (Fig. 35) with spiny microreticulation. Antenna (Fig. 35) with nine antennomeres; 1 longest, approx. 6.0 times as long as 2, with two long setae on lateral part; 2 widest at base; 3 bulbous at apical part; 4 dish-shaped; 5–9 clubbed with pubescence. Maxillary palpomere (Fig. 36) 1 small, approx. 2.0 times as long as wide; 2 bulbous at apical part, 4.0 times as long as 1; 3 largest, bulbous at apical part, slightly longer than 2; 4 slender and paralleled, 0.5 times as long as 3. Pronotum (Fig. 37) reverse trapezoidal, widest at anterior third, with indistinct longitudinal groove on median part; anterior margin nearly straight; anterior corner rectangular; lateral margin protruded at anterior third; posterior corner obtuse; postero-medial part rounded. Elytra (Figs 38, 39) widest at middle, with setae. Mesoventrite (Fig. 40) pentagonal, with transverse thick carina on anterior part; antero-lateral part with two grooves. Metaventrite (Fig. 41) with pubescence on medial part. Sternite VIII with more or less long setae on posterior part. Male terminal sternite semicircular and with long setae on posterior margin. Median lobe of aedeagus (Fig. 53) slightly curved; apical part acute; distal lobe approx. 0.1 times as long as median lobe. Paramere (Fig. 53) shorter than median lobe; apical part oval with setae.

Distribution. Asia: Korea, China (Beijing, Heilongjiang), Russia (East Siberia); Europe: Denmark, Estonia, Finland, France, Great Britain, Germany, Ireland, Latvia, The Netherlands, Norway, Poland, Russia, Spain, Sweden; North America.

Biological note. The specimens were collected in saline pond (salinity 32.84 ‰) with algae and plentiful vegetation near salt farm. They were found with *Hydroglyphus coreanus* Lee & Ahn (Dytiscidae), *Berosus lewisius* Sharp, *B. spinosus* (Steven), *Enochrus simulans* (Sharp), and *Paracymus aeneus* (Germar) (Hydrophilidae).

Remarks. This species is recorded for first time in Korea.



Figures 34–41. *Ochthebius marinus*. **34** mentum (ventral aspect) **35** head (ventral aspect) **36** maxillary palpus (ventral aspect) **37** pronotum (dorsal aspect) **38** anterior part of elytron (dorsal aspect) **39** posterior part of elytron (dorsal aspect) **40** mesoventrite (ventral aspect) **41** metaventrite (ventral aspect).

***Ochthebius (O.) parki* Jäch & Delgado, 2014**

Ochthebius (s. str.) parki Jäch & Delgado, 2014: 88; Jäch and Skale 2015: 158.

Specimens examined. Holotype: 1♂ (CNUIC), with labels as follows: “Geoje City, Gabae-ri, 1 VII 2000, K.-J. Ahn, H.-J. Kim, M.-J. Jeon, on barnacles, Holotype *Ochthebius parki* sp. n. Jäch and Delgado 2014”. Paratypes: 2♂♂, same data as holotype. **SOUTH KOREA:** Chungnam Prov.: 1♂, Boryeong-si, Ungcheon-eup, Doksan-ri, Holmoi beach, 6 IX 2003, K J Ahn, J S Park, under seaweeds; Gangwon Prov.: 1♀, Sokcho-si, Dongmyeong-dong, N38°12'48.0" E128°36'05.9" -1 m, 28 V 2012, JH Song, under stone in beach; Gyeongbuk Prov.: 3♂♂ 4♀♀, Yongyeon [=Pohang-si, Buk-gu, Heunghae-eub, Yonggok-ri], 20 VII 1991, K. J. Ahn, , in rock crevice; Gyeonggi Prov.: 1♂1♀, Incheon-City, Is. Baekryeongdo, 7 VIII 2000, C.-W. Shin, on rock (near sea); Gyeongnam Prov.: 5♂♂ 2♀♀, Geoje City, Gabae-ri, 1 VII 2000, K.-J. Ahn, H.-J. Kim, M.-J. Jeon, *ex* barnacles; 2♂♂ 3♀♀, Is. Geojedo, Dongbu-myeon, Gabae-ri, 30 VI 2001, S.-J. Park, inside barnacles; 1♂1♀, Koje, Kabae, Korean Marine Biological Laboratory, 3 VII 1998, J-Y Lyu, H-J Kim, empty barnacle; 1♂1♀, Köje-City, Gabae-ri, 30 VII 1999, K.-J. Ahn, on rock; Jeju Prov.: 1♂, Gwagji beach [=Jeju-si, Aewol-eub, Gwakji-ri, Gwakji beach], 3 VII 1991, K. J. Ahn, , on rock with clam; 2♂♂ 1♀, Namjeju-gun, Seongsan-eub, Ilchulbong, 11 VI 2005, S.I. Lee, S.J. Park, K.J. Ahn, M.J. Jeon, D.H. Lee, *ex* stones; Jeonnam Prov.: 2♂♂ 5♀♀, Jindo, Imhoe-myeon, Geumgab beach, 21 VIII 2001, S.-J. Park, on barnacles; 3♂♂, Wando, Sinji-myeon, Myeongsasimri beach, 23 VIII 2001, K.-J. Ahn, J.-H. Ahn, on barnacles.

Published South Korean records. *Ochthebius parki*: Jäch and Delgado (2014: 88); Jäch and Skale (2015:158). *Neochthebius granulatus*: Park and Ahn (2008: 2506) [misidentification].

Diagnosis. See Jäch and Delgado (2014).

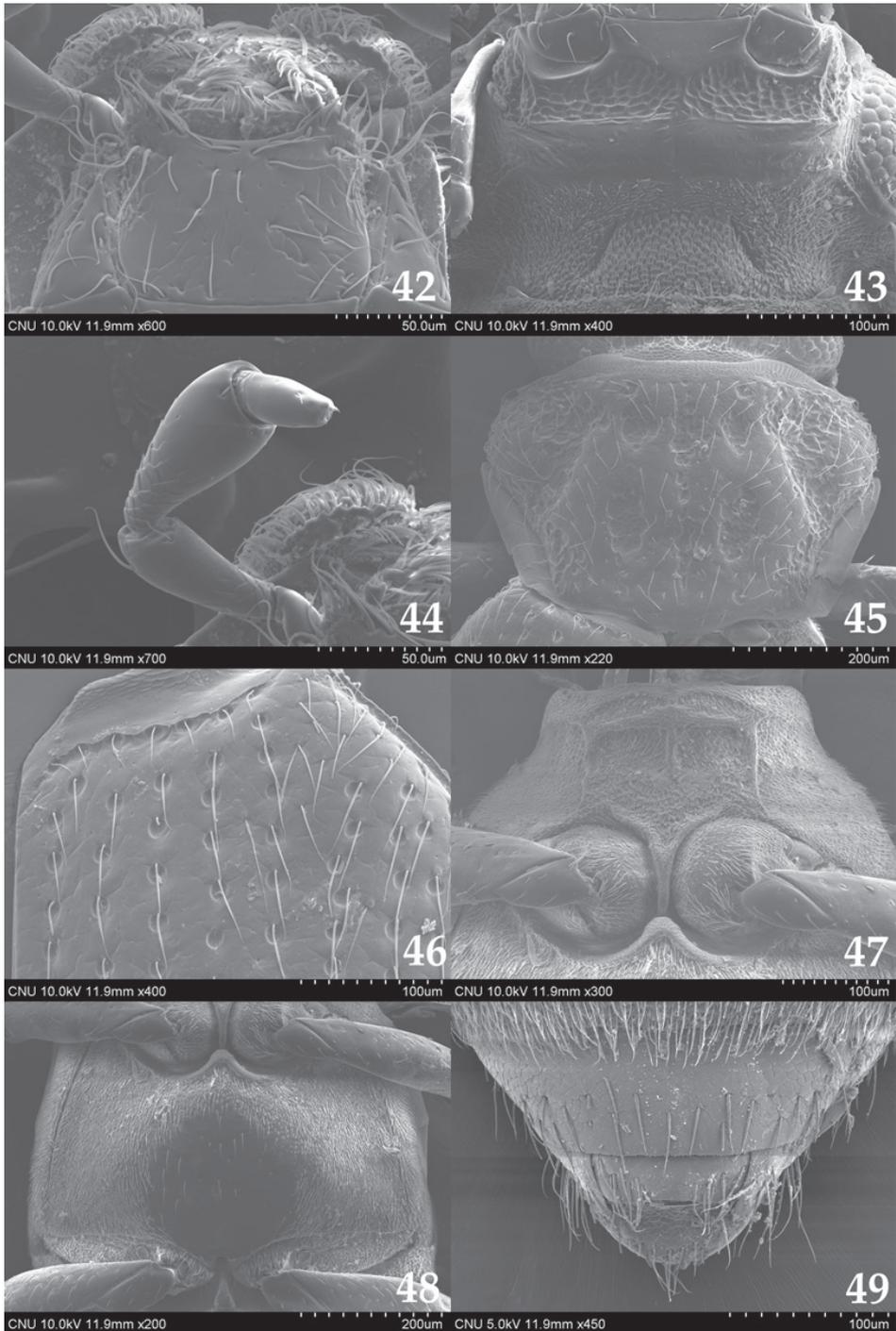
Distribution. Korea.

***Ochthebius (O.) satoi* Nakane, 1965**

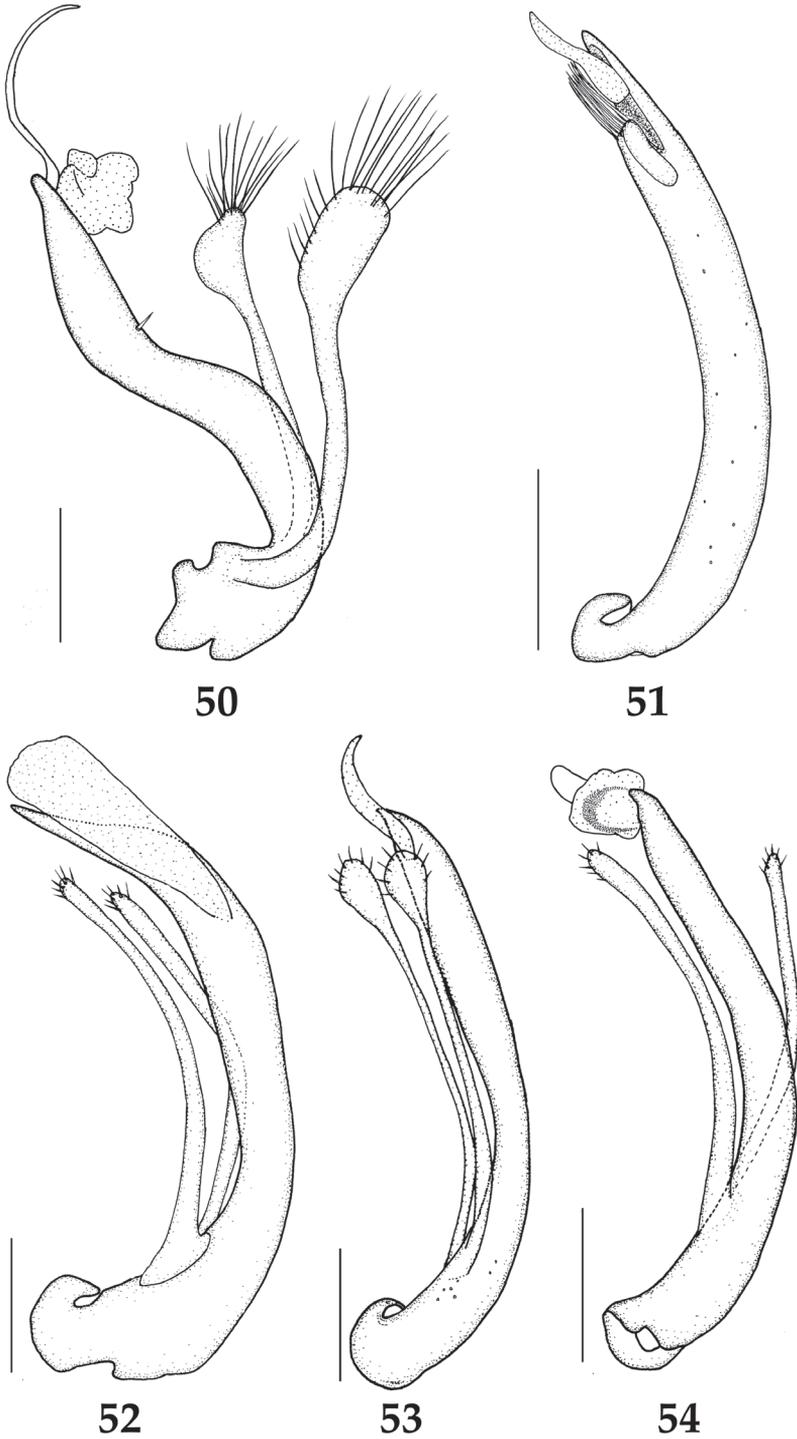
Figs 5, 42–49, 54, 56

Ochthebius (s. str.) satoi Nakane, 1965: 51; Jäch 1991: 77; 1998: 177; 2003: 328; Jäch and Skale 2015: 159.

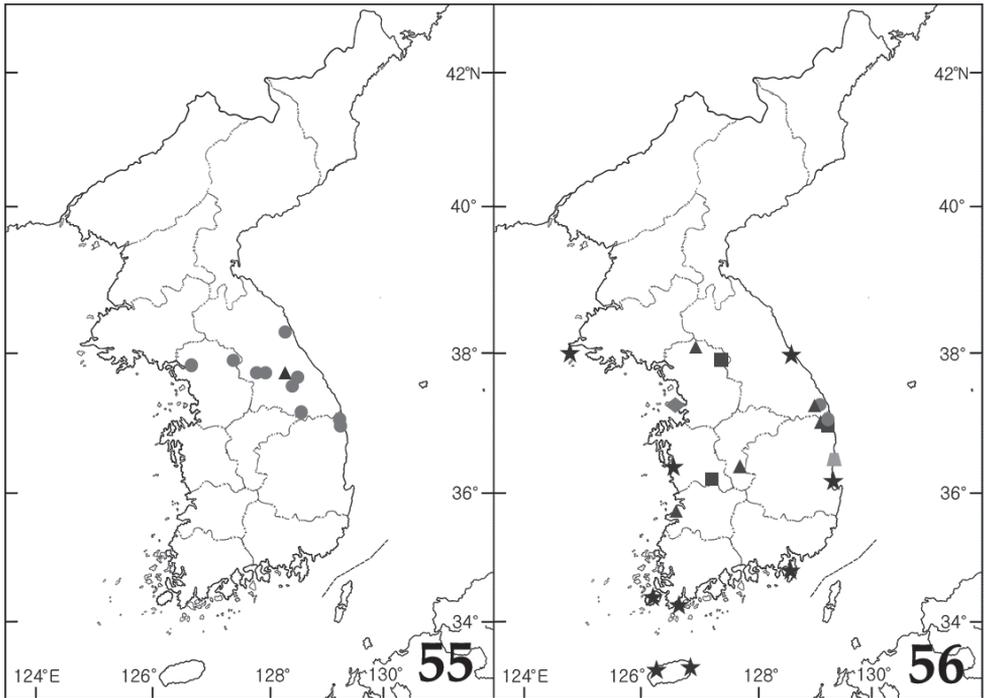
Specimens examined. **SOUTH KOREA:** Chungbuk Prov.: 2♂♂ 4♀♀, Okcheon-gun, Okcheon-eub, Gyodong-ri, 4 V 1990, SH Lee, beside stream; Gangwon Prov.: 3♂♂ 5♀♀, Samcheok-si, Wondeok-eub, Wolcheon-ri, Gagok-steam, 1 VI 1995, SH Lee, beside stream (3♂♂ 1♀, on slides); Gyeongbuk Prov.: 10♂♂ 7♀♀, Uljin-gun, Giseong-myeon, Dacheon-ri, Giseong-stream 17 VII 1995, SH Lee, *ex*. mountain stream (2♂♂ 2♀♀, on slides); 2♂♂ 1♀, Uljin-gun, Wonnam-myeon, Gilgok-ri, Maehwa-stream, beside stream (2♂♂, on slides); Gyeonggi Prov.: 1♀, Yeoncheon-



Figures 42–49. *Ochtthebius satoi*. **42** mentum (ventral aspect) **43** gena and gula (ventral aspect) **44** maxillary palpus (dorsal aspect) **45** pronotum (dorsal aspect) **46** anterior part of elytron (dorsal aspect) **47** mesoventrite (ventral aspect) **48** metaventrite (ventral aspect) **49** male terminal sternite (ventral aspect).



Figures 50–54. Aedeagus (lateral aspect). **50** *Hydraena puetzi* **51** *Ochthebius hasegawai* **52** *O. lobatus* **53** *O. marinus* **54** *O. satoi*. Scale bars 0.1 mm.



Figures 55–56. Distribution maps. **55** *Hydraena puetzi* (circle), *H. miyatakei* (triangle) **56** *Ochthebius abni* (trapezoid), *O. lobatus* (circle), *O. marinus* (diamond), *O. parki* (star), *O. satoi* (triangle), *O. hasegawai* (square).

gun, Jangnam-myeon, Wondal-ri, Samicheon-bridge, 5 VII 2014, SW Jeong, stream; Jeonbuk Prov.: 1 ♀, Buan-gun, Byeonsan-myeon, Junggye-ri, Jikso-fall, 11 VII 2014, SW Jeong, mountain stream.

Published South Korean records. *Ochthebius satoi*: Lee (1995: 14); Jäch and Skale (2015: 159).

Redescription. Length 1.6–1.8 mm. Head dark brown; pronotum and elytra yellowish brown; ventral surface mostly brown. Mentum (Fig. 42) slightly longer than wide and with sparse setae; antero-lateral part protruded. Anterior gena (Fig. 43) without long setae on posterior part in ventral view. Antenna with nine antennomeres; 1 longest, approx. 6.0 times as long as 2, two long setae present on lateral part; 2 bulbous at middle; 3 oval, widest at middle; 4 dish-shaped; 5–9 clubbed and with pubescence. Maxillary palpomere (Fig. 44) 1 small, approx. 2.0 times as long as wide; 2 bulbous at apical part, 3.0 times as long as 1; 3 largest, bulbous at apical part, slightly longer than 2; 4 slender and paralleled, 0.5 times as long as 3. Pronotum (Fig. 45) reverse trapezoidal, widest at anterior third, with indistinct longitudinal groove on medial part, two small oval grooves on anterior part, relatively large oval groove on posterior part; anterior margin bisinuate; antero-medial margin straight; anterior corner rectangular; lateral side protruded at anterior third; posterior corner obtuse; postero-medial part rounded. Elytra (Fig. 46) widest at middle, with setae. Mesoventrite (Fig. 47) pentagonal, with

T-shaped carina on anterior part; anterior margin transverse. Metaventricle (Fig. 48) without pubescence on medial part. Metatrochanter with a row of setae. Sternite VIII (Fig. 49) with more or less long setae on posterior part. Male terminal sternite (Fig. 49) semicircular, with sparse setae, relatively long setae on posterior part. Median lobe of aedeagus (Fig. 54) slender, curved; apical part strongly vented; distal lobe rounded. Paramere (Fig. 54) nearly parallel-sided, apical margin rounded, with few setae.

Distribution. Korea, Japan, China (Henan, Jilin, Liaoning, Nei Mongol, Shaanxi, Shandong, Taiwan), Russia (Far East).

Discussion

This study revises the number and taxonomic status of hydraenid species known to occur in South Korea. In total, eight species are recognized. Two species (*O. abni* and *O. parki*) are apparently endemic to the South Korean fauna (Jäch and Delgado 2014). Of the remaining species, five are widespread in the East Palearctic region (*H. puetzi*, *H. miyatakei*, *O. lobatus*, *O. hasegawai*, *O. satoi*) and one occurs in the Holarctic region (*O. marinus*).

Diversity of South Korean Hydraenidae is very low compared to other adjacent countries, such as China (82 endemic species out of 100 species), Japan (29 endemic species out of 35 species), and the Far East Russia (no endemic species out of 11 species) (Jäch 2003; Jäch and Díaz 2003; 2004; 2005; 2006; 2012; Jäch and Delgado 2014; Jäch and Skale 2015). They show high diversity and endemism because of their small size and limited dispersal abilities (Jäch and Balke 2008). Further collecting efforts in the Korean Peninsula will probably add more hydraenid species to the Korean fauna such as the Palearctic species, *H. riparia* Kugelann and *O. angusi* Jäch, and more species.

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Review of the genus *Harnischia* Kieffer from China (Diptera, Chironomidae), with description of one new species

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Abstract

The genus *Harnischia* Kieffer, 1921 from China is reviewed and one new species, *Harnischia parallela* Yan & Wang, **sp. n.**, is described and illustrated as adult male. *Harnischia okilurida* Sasa is recognized as a new synonym of *H. longispuria* Wang & Zheng. The pupae of *H. fuscimana* Kieffer and *H. curtilamellata* Malloch are redescribed from material collected in China, and an unplaced pupa is described. Key to male adults and pupae of known species of *Harnischia* from China is given.

Keywords

Harnischia, Chironomidae, China, new species, key

Introduction

The genus *Harnischia* is characterized by the morphology of the hypopygium, which is bearing a vestigial superior and inferior volsella. Previously erected by Kieffer (1921), this genus is treated as a group within *Tendipes* (*Cryptochironomus*) by Goetghebuer (1937–54). Townes (1945) proposed dividing the genus into two subgenera (*Harnischia* and *Cladopelma*). Beck and Beck (1969) also treated the genus as two subgenera. Recently, Sæther (1977) elevated *Cladopelma* to the genus level. The *Harnischia* genus

has a worldwide distribution (Cranston and Martin 1989; Ashe and Cranston 1990; Chaudhuri and Chattopadhyay 1990; Oliver et al. 1990; Wang et al. 1993; Sasa and Kikuchi 1995; Sasa and Ogata 1999; Wang 1999; Sæther et al. 2000; Wang 2000; Chaudhuri et al. 2001; Kawai et al. 2002, Makarchenko et al. 2005; Yan and Wang 2011; Paasivirta 2014; Murray 2015).

Wang (1999) recorded six species from China and recognized *H. longispuria*, which is erected by Wang et al. (1993) as a synonym of *H. curtilamellata*. After re-examination of the type specimen, *H. longispuria* has been treated as a valid species (Wang 2000, Yan and Wang 2011). To date, seven *Harnischia* species (*H. angularis*, *H. cultrata*, *H. curtilamellata*, *H. fuscimana*, *H. japonica*, *H. longispuria* and *H. turgidula*) of the genus have been recorded in China.

In this paper the genus *Harnischia* from China is reviewed and a description of the male adult of *Harnischia parallela* Yan & Wang, sp. n., is given. *Harnischia okilurida* Sasa is recognized as a new synonym of *H. longispuria* Wang & Zheng. The pupae of *H. fuscimana* Kieffer and *H. curtilamellata* Malloch are redescribed from China, and an unplaced pupa is described. Key to male adults and pupae of known species of *Harnischia* from China is provided.

Material and methods

Morphology and terminology follow Sæther (1980). The material examined consists of slide-mounted following the procedures outlined by Sæther (1969). Measurements are given as the ranges followed by a mean when four or more measurements are made. The specimens examined in this study are deposited in the collection of the College of Life Sciences, Tianjin Normal University, China (BDN).

Taxonomy

Harnischia Kieffer, 1921

Harnischia: Kieffer 1921: 273; Beck and Beck 1969: 296; Sæther 1971: 350; 1977: 89; Pinder and Reiss 1986: 326; Sasa 1989: 83; Cranston et al. 1989: 382; Langton 1991: 275; Wang, 1995: 169; Rufer and Ferrington 2007: 82.

Type species. *Harnischia fuscimana* Kieffer, 1921.

Diagnostic characters. **Adult male.** The characters of superior and inferior volsellae vestigial and gonostylus broad and short, not attenuated from junction with gonocoxite will easily differentiate *Harnischia* adults from other genera in the subfamily Chironominae. **Pupae.** The medially interrupted hook row, pattern of armature on the tergites, absence of posterolateral comb on segment VIII and brush-like thoracic horn will differentiate the pupae from all the other chironomids.

***Harnischia angularis* Albu & Botnariuc, 1966**

Fig. 1

Harnischia angularis: Albu and Botnariuc 1966: 54; Wang 1999: 169; Wang 2000: 644; Makarchenko et al. 2005: 410.

Harnischia hamata: Wang et al. 1993: 461; Wang 1999: 169. **Syn. n.**

Type locality. Romania.

Material examined. CHINA: 1♂, Yunnan Province, Huaping Country, Xinzhuan Town, Liangma River, 30.05.1996, sweep net, X. Wang; 1♂, Ningxia, Yinchuan City, 26.07.1987, Wang; 2♂♂, Xinjiang, Buerjin Hotel, 1.09.2002, light trap, H. Tang.

Diagnostic characters. Frontal tubercles absent. R_1 without microtrichia. Tibia of front leg with a subapical seta; posterior margin of tergite IX narrowed, anal point elongated, swollen medially, sharp and slender, without lateral setae. Anal tergite bands V-shaped, abruptly interrupted medially; gonocoxite with a projection in inner distal parts, bearing 5 strong setae and covered with microtrichia. Gonostylus slightly swollen distally, with hook-like apical teeth at the apex.

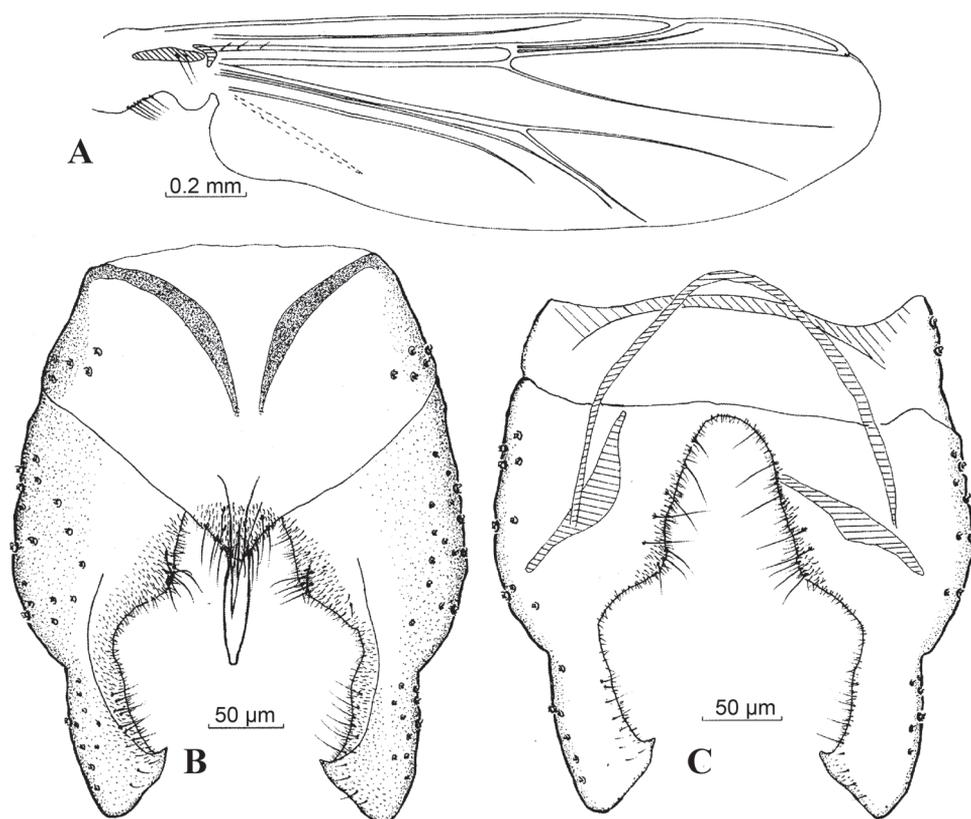


Figure 1. *Harnischia angularis*. **A** Wing; Hypopygium: **B** (dorsal) **C** (ventral).

Distribution. China (Yunnan, Ningxia, Xinjiang); Russian Far-East; Germany, Yugoslavia, Romania, Italy.

Remarks. The species can be easily distinguished by gonostylus with strong hook-like apical teeth. We agree with Wang (1999), who considered *H. hamata* Wang & Zheng as a synonym of *H. angularis* Albu & Botnariuc.

***Harnischia cultriata* Wang, 1999**

Fig. 2

Harnischia cultriata Wang 1999: 172.

Type locality. China (Gansu).

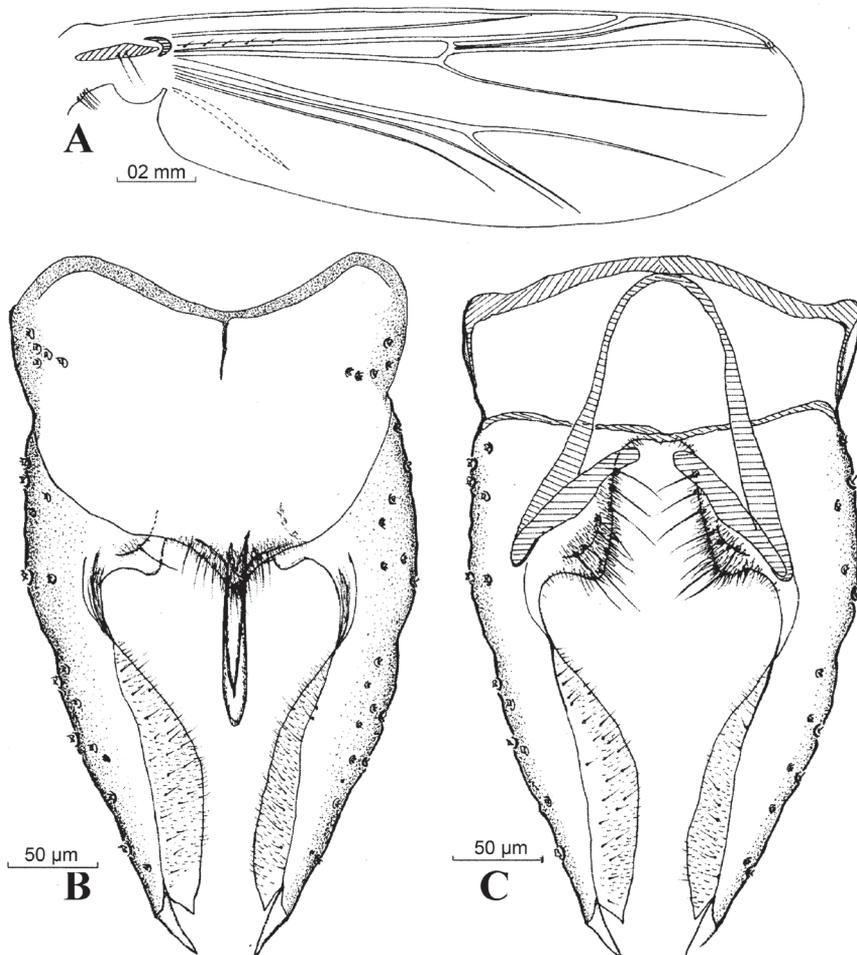


Figure 2. *Harnischia cultriata*. **A** Wing; Hypopygium: **B** (dorsal) **C** (ventral).

Material examined. Holotype: ♂, (BDN no. 03438), CHINA: Gansu Province, Tianshui City, Dangchuan Country, 1450 m, 8. viii. 1993, sweeping net. W. Bu.

Diagnostic characters. Frontal tubercles absent. R_1 without microtrichia. Tergite IX with shoulder-like posterior margin. Anal point slightly swollen in the distal 1/3, sharp at the apex, with median ridges but without lateral setae. Anal tergite bands Y-shaped, slightly flat at the bottom. Superior volsella present, bearing 4 strong setae and microtrichia. Gonocoxite with an obvious projection in distal portion of inner margin, bearing 2–3 strong setae and microtrichia; Gonostylus swollen- to knife-like at the middle, with sharp teeth at the apex.

Distribution. China (Gansu).

Harnischia curtilamellata (Malloch, 1915)

Figs 3, 4

Chironomus curtilamellata Malloch 1915: 474.

Chironomus pseudosimplex Goetghebuer 1923: 116; Edwards 1929: 390.

Chironomus (Cryptochironomus) monilis Freeman 1954a: 19.

Chironomus (Cryptochironomus) atrofasciatus Freeman 1954b: 177.

Harnischia pseudosimplex Wang S. et al. 1977: 231.

Harnischia curtilamellata: Townes 1945: 166; Sæther 1971: 347; 1977a: 88; Pinder 1978: 124; Freeman and Cranston 1980: 351; Hashimoto et al. 1981: 22; Sasa and Kikuchi 1986: 20; Sasa and Kawai 1987: 18; Ashe and Cranston 1990: 285; Cranston and Martin 1989: 270; Wang X. et al. 1993: 462; Makarchenko et al. 2005: 410; Dutta et al. 1996: 272; Wang 1999: 170; Wang 2000: 644; Chaudhuri et al. 2001: 351.

Type locality. America (Michigan).

Material examined. CHINA: 3♂♂, Tianjin City, Xian River, 12.06.1985, Xinhua Wang; 1♂, Jiangxi Province, Poyang Lake, Nanjishan Natural Conservation area, 12.06.2004, sweep net, Chuncai Yan. 2♂♂, Hubei Province, Hefeng Watershed, 1200 m, 17.07.1999, light trap, Bingchun Ji; 1♂, Hunan Province, Zhuzhou City, Central South Forestry University, 17.07.1995, Winjun Bu; 1♂, Guangxi Province, Jinxiu County, 1.06.1990, Xinhua Wang; 1♂, Hainan Province, Ledong Li Autonomous County, Jianfengling, 17.04.1985, light trap, Leyi Zheng; 2♂♂, Guizhou Province, Guiyang City, Huaxi area, 1050 m, 23.07.1995, light trap, Wenjun Bu; 1♂, Guizhou Province, Daozhen County, Dashaha Natural Conservation area, Fairy Cave, 600 m, 30.05.2004, light trap, Hongqu Tang; 1♂, Guizhou Province, Jiangukou County, Fanjingshan Natural Conservation area, Black Creek, 3.06.2002, light trap, Bingchun Ji; 9♂♂, Yunnan Province, Dalier Seaside, 2000 m, 23.05.1996, light trap, Xinhua Wang; 3♂♂, Yunnan Province, Eryuan County, Niujie Town, Futian, Meici River, 2262–2332 m, 14.5°C, 24.05.1996, light trap, Changfa Zhou and Beixin Wang; 1♂, Yunnan Province, Lijiang City, Shigu Town, Chongjiang Ri-

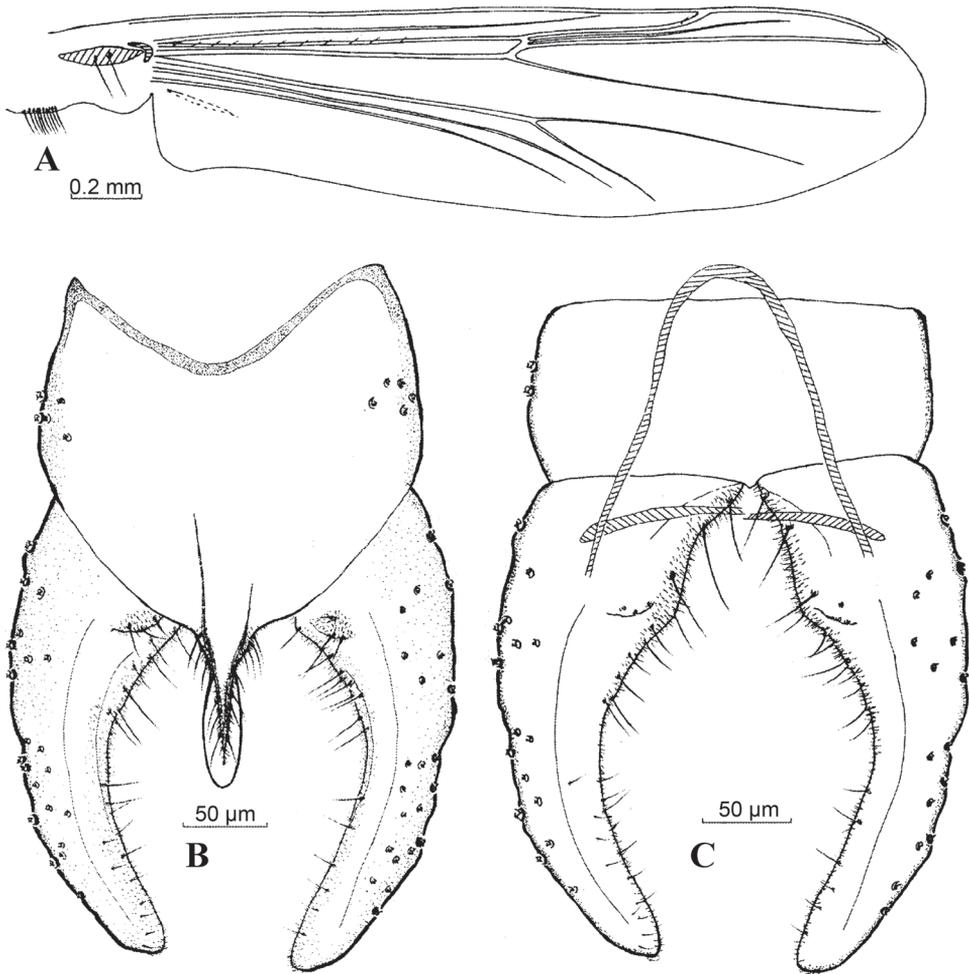
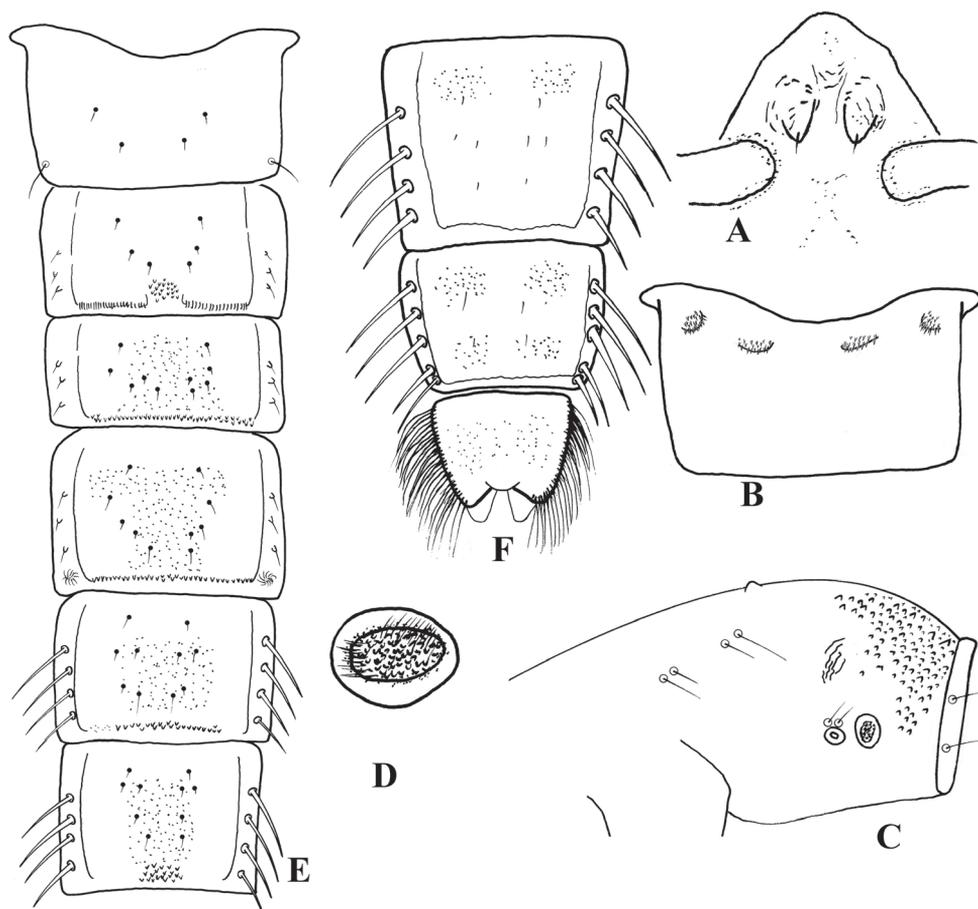


Figure 3. *Harnischia curtilamellata*. **A** Wing; Hypopygium: **B** (dorsal) **C** (ventral).

ver, 25.05.1996, light trap, Changfa Zhou; 1 ♂, Yunnan Province, Wuding County, Mashan Stream, 1.06.1996, Xinhua Wang; 1 ♂, Yunnan Province, Zhongdian Martyr Cemetery, 3150 m, 14.07.2001, Ruilei Zhang; 28 ♂♂, Taiwan, Taibei City, Kuandu Wetland, 20.10.1988, Kanjin Ma.

Diagnostic characters. Male. (Fig. 3A–C) Frontal tubercles elliptical or rounded, sometimes absent. Anal point wide, shrunken and slender at the base, distally swollen like a bubble, semi-transparent, with median ridges, bearing lateral setae and microtrichia. Phallapodeme long and slender. Gonostylus widest at the base, curved in the middle and gradually tapering distally, round and blunt in the apex. **Pupa.** (Fig. 4A–F) Sternite I with a pair of spinose anterolateral and anteromedian tubercles on each side; tergites III–VI with narrow, posterior robust spines; hook row medially interrupted; segment VIII without comb or spurs.



Figures 4. *Harnischia curtilamellata*. Pupae. **A** frontal apotome **B** sternite I **C** thorax, lateral view **D** basal ring **E** tergites I–VI **F** tergite VII–VIII and anal lobe.

Materials examined. CHINA: 1P, Guangxi Zhuang Autonomous Region, Guilin City, Dingjiang County, Yangjiangtou town 6.04.2015. Wenbin Liu. 1P, CHINA: Shandong Province, Jining City, Weishan County, Weishan Island 4.10.2015. Wenbin Liu. 5P, CHINA: Guangdong Province, Maoming City, Linchen River 27.11.2013. Jun Liu.

Description. Pupa [n = 8] Total length 3.2–4.9 mm. Cephalothorax brown; abdomen pale brownish.

Cephalothorax (Fig. 4A, C–D). Frontal setae 20–30 μm long, fine, arising subapically from conical, 50–100 μm long cephalic tubercles. Thoracic horn plumose, with numerous fine branches; basal ring oval. Thorax granulose dorsally, more densely granulose anteriorly. Prealar tubercle low, rounded. Scutal tubercle prominent. Wing sheath without nose; pearl row absent. Two short precorneals, 2 short anteprenotals and 4 dorsocentrals present. Lengths of dorsocentrals (μm): 50–75, 38–50, 56–63, 25–40.

Abdomen (Fig. 4B, E–F). Tergite I (Fig. 4E) bare; II with posteromedian group of small points; III–VI with narrow, transverse, posterior band of robust spines; VII with a pair of anterior patches fine shagreen; VIII with a pair of anterior and posterolateral patches of shagreen. Hook row widely interrupted medially. Conjunctions bare. Pedes spurii A present on segment IV; pedes spurii B present on segment I. Sternite I (Fig. 4B) with a pair of spinose anterolateral and anteromedian tubercles each sides. Segment VIII without posterolateral comb or spurs. Segment II–IV with 3 strong L setae situated on tubercles; V–VII with 4 LS setae, VIII with 5 LS setae.

Anal lobe (Fig. 4 F) 1.44–2.20 × as long as broad, with complete fringe of 31–51 lamelliform setae. Genital sac 125–220 μm long, extending beyond anal lobe.

Distribution. China (Tianjin, Jiangxi, Hubei, Hunan, Guangxi, Hainan, Guizhou, Yunnan, Taiwan); Japan; Thailand; India; Russian Far–East; Europe; North America; Africa region (South Africa, Sudan, Sebegal, Zaire); Australian Region (Australia).

Remarks. The Chinese specimens mainly agree with the description of Langton (1991), but the color of spinose anteromedian tubercles of sternite I is lighter than from Europe. Based on original descriptions and figures, the record of *Harnischia pseudosimplex* Goetghebuer in China (Hubei province; Wuhan City) by Wang S. et al. (1977: 231, fig. II: 15) should be *Harnischia curtilamellata* (Malloch).

Harnischia fuscimana Kieffer, 1921

Fig. 5

Harnischia fuscimana: Kieffer 1921b: 69; Goetghebuer 1937–54: 48; Sæther 1971: 348; Wang S. et al. 1977: 231; Fittkau and Reiss 1978: 432; Wang 2000: 644; Makarchenko et al. 2005: 410.

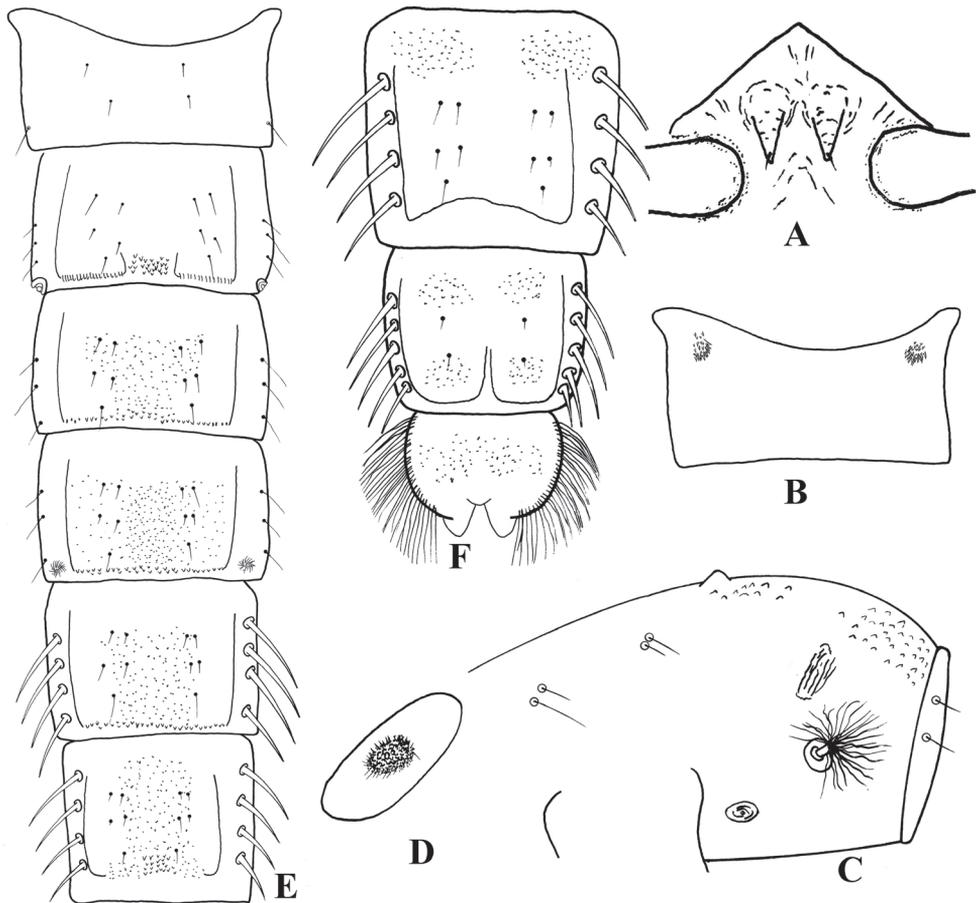
Type locality. Poland

Diagnostic characters. **Male.** Body pale green. Anal point taper-shaped and tapering towards the sharp apex, bearing lateral setae and microtrichia. Gonostylus thick, blade-shaped projection. Superior and inferior volsellae absent. The conjunction of gonostylus and gonocoxite shrunken and obviously tapered. Gonostylus short, with a weak projection at base, almost as long as gonocoxite. **Pupa.** (Fig. 5A–F) Sternite I with only spinose anterolateral tubercles; hook row medially interrupted; segment VIII without comb or spur.

Materials examined. CHINA: 1P, Guangdong Province, Maoming City, Baowei town 10.12.2012. Hongqu Tang.

Description. **Pupa** [n = 1] Total length 6.55 mm. Cephalothorax brown; abdomen pale brownish.

Cephalothorax (Fig. 5A, C–D). Frontal setae 45 μm long, fine, arising subapically from conical, 130 μm long cephalic tubercles. Thoracic horn (Fig. 5C) plumose, with numerous fine branches; basal ring oval. Thorax granulose dorsally, more densely granulose anteriorly. Prealar tubercle low, rounded. Scutal tubercle prominent. Wing



Figures 5. *Harnischia fuscimana*. Pupae. **A** frontal apotome **B** sternite I **C** thorax, lateral view **D** basal ring **E** tergites I–VI **F** tergite VII–VIII and anal lobe.

sheath without nose; pearl row absent. Two short precorneals, 2 short antepronotals and 4 dorsocentrals present.

Abdomen (Fig. 5B, E–F). Tergite I (Fig. 5E) bare; II with posteromedian group of small points; III–VI with transverse, posterior band of spinules; VII with a pair of anterior patches fine shagreen; VIII with a pair of anterior and posterolateral patches of shagreen. Hook row widely interrupted medially. Conjunctives bare. Pedes spurii A present on segment IV; pedes spurii B present on segment I and II. Sternite I (Fig. 5B) with spinose anterolateral tubercles. Segment VIII without posterolateral comb or spur. Segment II–IV with 3 strong L setae; V–VII with 4 LS setae, VIII with 5 LS setae.

Anal lobe (Fig. 5F) $1.74 \times$ as long as broad, with complete fringe of 70 lamelliform setae. Genital sac $300 \mu\text{m}$ long, extending beyond anal lobe.

Distribution. China (Hubei), Russian Far East; Afghanistan; Lebanon; Europe (Gemary, Poland, Yugoslavia, Romania, France, Belgium, Spain, Italy).

Remarks. The species was recorded in China by Wang S. (1977). The Chinese specimens of pupal stages mainly agree with the description of Langton (1991), but fringe setae of anal lobe of specimens from China (70) more than from Europe (45–59).

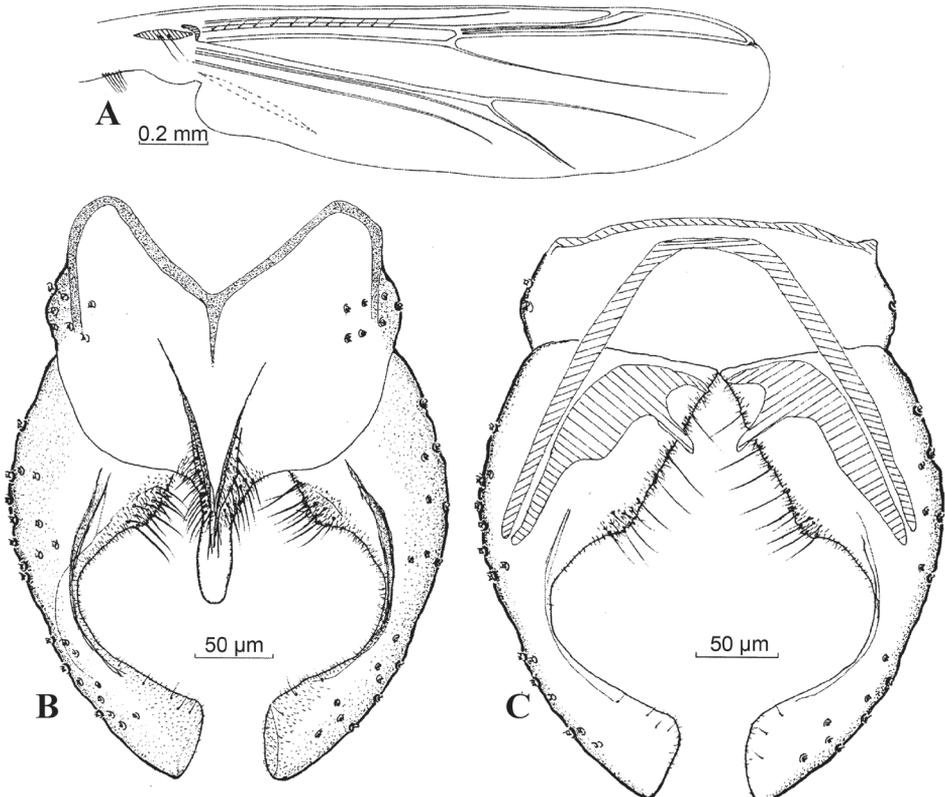
***Harnischia japonica* Hashimoto, 1984**

Fig. 6

Harnischia japonica: Hashimoto 1984: 262; Wang et al. 1993: 461; Sasa et al. 1988: 32; Sasa 1990a: 31; Sasa 1993: 72; Wang 1999: 172; Wang 2000: 644; Makarchenko et al. 2005: 410.

Type locality. Japan.

Material examined. CHINA: 1 ♂, Fujian Province, Wuyi Mountain Natural Conservation area, 24.04.1993, light trap, Xinhua Wang; 3 ♂♂, Shandong Province, Yantai City, Mouping, Kunyu Mountain, Dianhou, 24.08.1987, Hongyang Li; 2 ♂♂, Guangxi Province, Longsheng County, Sanmen Town, 27.05.1990, Xinhua Wang. KOREA: 1 ♂, 20.05.2000, Dr. T.S. Chon.



Figures 6. *Harnischia japonica*. **A** Wing; Hypopygium: **B** (dorsal) **C** (ventral).

Diagnostic characters. Ventral tergites I-IV each terminal with brown band. R_1 without microtrichia. Tergite IX with shoulder-like posterior margin. Anal point slightly swollen in the distal 1/3, round and blunt at the apical and with median ridges, bearing lateral setae and microtrichia, stretching to the middle of tergite IX. Anal Tergite bands Y-shaped. Inner margin of gonocoxite with a small protrusion in distal, bearing setae and microtrichia. Gonostylus slender at the base, swollen and truncated apically.

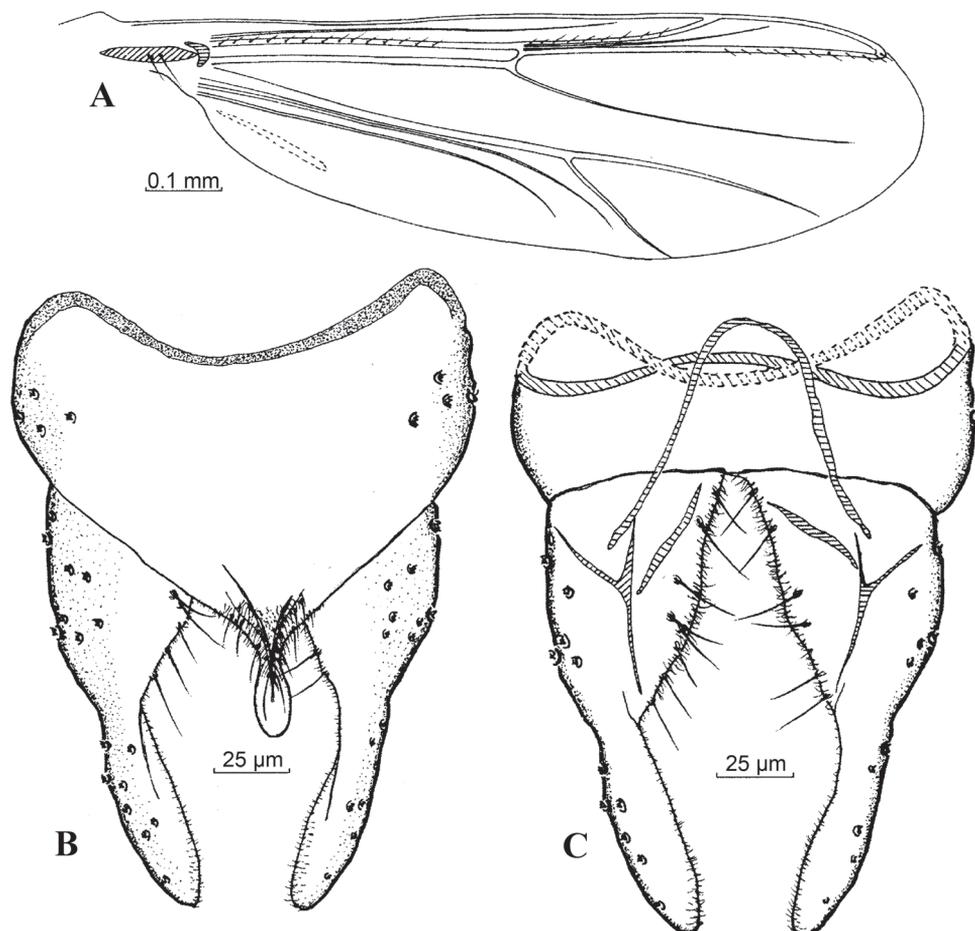
Distribution. China (Fujian, Shandong, Guangxi); Korea; Japan; Russian Far East.

***Harnischia longispuria* Wang & Zheng, 1993**

Fig. 7

Harnischia longispuria: Wang and Zheng 1993: 459; Wang 1999: 170; Wang 2000: 644.

Harnischia okilurida: Sasa 1993: 127 **Syn. n.**



Figures 7. *Harnischia longispuria*. **A** Wing; Hypopygium: **B** (dorsal) **C** (ventral).

Type locality. China (Hainan).

Material examined. CHINA: Holotype, ♂ (BDN No. 05224), Hainan Province, Ledong Li Autonomous County, Jianfeng town, 17.05.1988, light trap, Leyi Zheng. JAPAN: Holotype (*Harnischia okilurida* Sasa), ♂ (No. 246: 10), at the side of a dam of Yona River, Lake Nawagaike, 20.05.1993, insect net.

Diagnostic characters. Thorax yellow with dark brown spots; AR 0.98, frontal tubercles absent; posterior margin of tergite IX triangular and cone-like; anal point constricted at base and swollen distally, with median ridges, bearing setae and microtrichia; anal tergite bands V-shaped, slightly flat at bottom; a fusion of gonostylus and gonocoxite obviously constricted, gonostylus straight, rod-like, both sides almost parallel, round and blunt at apex, without conspicuous short setae in inner margin.

Distribution. China (Hainan); Japan.

Remarks. Wang (1999) treat *H. longispuria* Wang & Zheng as a synonym of *H. curtilamellata* (Malloch). After re-examining, the specimens of *H. longispuria* Wang & Zheng, (which should be *H. okilurida* Sasa), it currently should be a valid species. However, *H. okilurida* Sasa (December 1993) described later than *H. longispuria* Wang & Zheng (October 1993), is consequently considered as a new synonym of *H. longispuria* Wang & Zheng.

***Harnischia parallela* Yan & Wang, sp. n.**

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Fig. 8

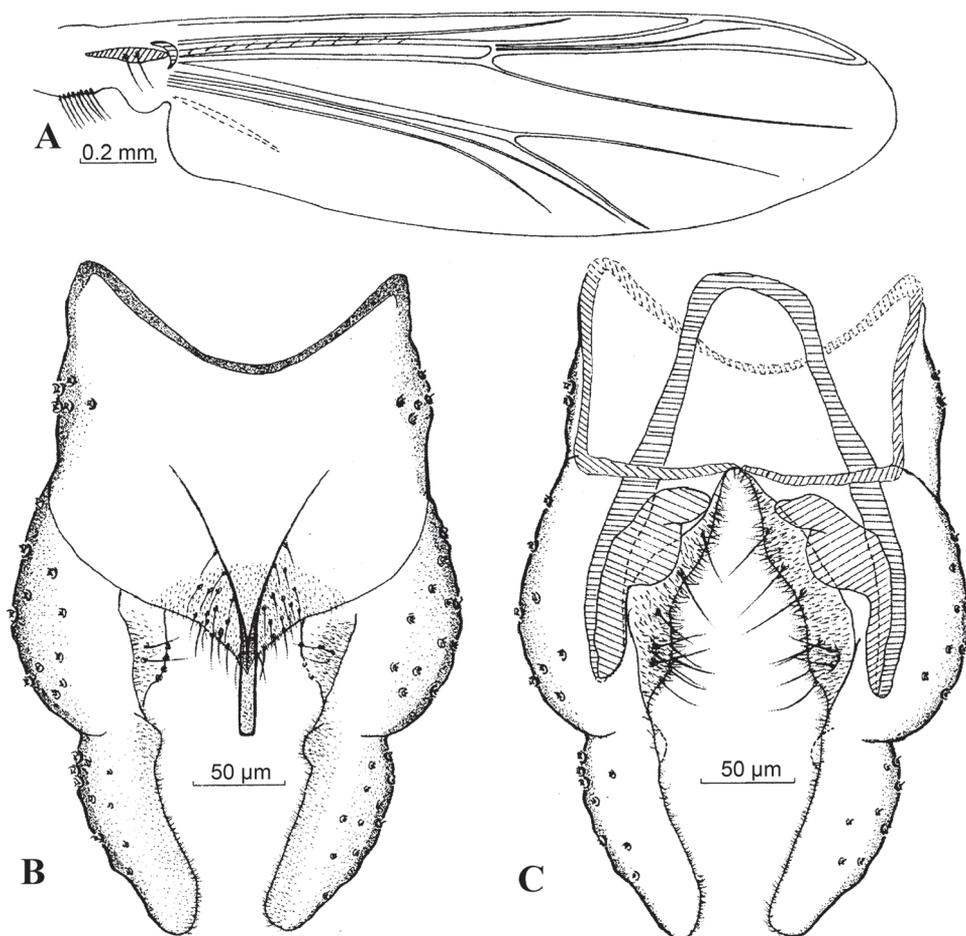
Diagnostic characters. The new species is distinguished by the following combination of characters: R_1 without microtrichia; front tibia with a subapical seta; tergite IX broadly triangular, with 26 setae (13 on each side); anal point parallel-sided, distinctly extended basally as a V-shaped form; without lateral and dorsal setae; phallapodeme wide and large; gonocoxite blunt and rounded apically, with 2 broad dorsal lobes; gonostylus with a small dorsal basal lobe bearing setae.

Type material. Holotypes: ♂ (BDN No. 24949), CHINA: Xinjiang burqin hotel (49.41°N, 86.59°E), 1.09.2002, Light trap, H. Tang; Paratype: 3 ♂♂ (BDN No. 24900, 24915, 24968), as holotypes.

Etymology. The specific name, from Latin *parallela*, refers to anal point parallel-sided.

Description. Male imago [n = 4, unless otherwise stated] Total length 3.55–3.68, 3.63 mm; wing length 1.83–1.98, 1.93 mm; total length / wing length 1.86–1.94, 1.88; wing length /length of profemur 2.38–2.54, 2.47.

Coloration. Thorax yellow brown, with dark brown spots. Femora of front legs yellow green, tibia dark brown, tarsus 1 dark brown except for yellow brown in basal 1/2 yellow brown, tarsi 2–4 dark brown; femora and tibiae of mid and hind legs yellow green, tarsi 1–4 yellow brown to dark brown, tarsi 5 black brown. Abdomen. Tergites I–IV yellow brown, each terminal with light brown, tergites V–VIII; hypopygium dark brown.



Figures 8. *Harnischia parallela* Yan & Wang, sp. n. **A** Wing; Hypopygium: **B** (dorsal) **C** (ventral).

Head. AR: 2.06–2.24, 2.17. Ultimate flagellomere 660–740, 710 μm . Frontal tubercles absent. Temporal setae 12–16, 14, including 3–4, 3 inner verticals; 4–5, 5 outer verticals; and 5–7, 6 postorbitals. Clypeus with 13–16, 15 setae. Tentorium 100–130, 117 μm long, 28–33, 31 μm wide. Palpomere lengths (μm): 40–45, 43(3); 42–50, 47 (3); 122–150, 137(3); 163–170, 165(3); 235–245(2); palp segment 5th / 3rd: 1.57–1.69 (2).

Thorax. Anteprepronotum with 2–5, 4 setae, acrostichals 7–7, 7, dorsocentrals 9–11, 10, prealars 4–4, 4. Scutellum with 6–9, 7 setae.

Wing (Fig. 8A). VR: 1.11–1.13, 1.12. R with 8–11, 10 setae. R₁ without setae. R₄₊₅ with 1–2, 1 seta. Brachiolum with 2–2, 2 setae. Squama with 7–11, 9 fringed setae.

Legs. Front tibia with a subapical seta, 82–90, 87 μm . Mid legs with 2 spur, 15–20, 18 μm and 22–28, 26 μm , comb with 20–28, 25 teeth, 8–10, 9 μm long. Spurs of

Table 1. Lengths (in μm) and proportions of adult male legs in *H. parallela* Yan & Wang, sp. n. (n=4).

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅	LR
P ₁	730–820, 783	440–480, 460	1000–1020 1010 (3)	530–550, 540 (3)	370–390, 383 (3)	270–270, 270 (3)	130–150, 140 (3)	2.13–2.27, 2.18 (3)
P ₂	700–770, 745	580–650, 625	390–430, 413	190–200, 198	140–160, 150	100–110, 103	80–90, 85	0.63–0.67, 0.66
P ₃	800–900, 858	760–830, 810	580–640, 617 (3)	300–320, 313 (3)	260–280, 273 (3)	150–170, 160 (3)	110–110, 110 (3)	0.76–0.77, 0.77 (3)

hind tibia 18–22, 20 mm and 25–32, 29 mm long, comb with 50–60, 54 teeth, 9–10, 10 mm long. Tarsus I of mid leg with 2–4, 3 sensilla chaetica. Lengths (in μm) and proportions of thoracic legs as in Table 1.

Hypopygium (Fig. 8B–C). Tergite IX broadly triangular at base, bearing 26–32, 29 setae. Laterosternite IX with 4–6, 5 setae. Anal point 58–70, 65 mm long, parallel-sided, without dorsal and lateral setae. The basal ridge of anal point stretched towards the middle of tergite IX. Anal tergite bands transversally extended and concave medially. Phallapodeme 108–120, 116 mm long. Transverse sternapodeme 35–60, 47 mm long. Gonocoxite 130–147, 138 mm long, with two broad dorsal lobes, one basal and one distal, distal lobe with 8 strong setae; basal inner margin with 4 stout setae; gonostylus 117–120, 119 mm long, blunt and rounded apically, with a distinct basal inner protrusion, rows of setae along inner margin absent. HR: 1.12–1.24, 1.18; HV: 3.03–3.12, 3.05.

Distribution. China (Xinjiang).

Remarks. *H. parallela* Yan & Wang, sp. n. can be easily be separated from all other members of the *Harnischia* genus the morphological characters of: anal tergite band (transversally concave medially); anal point (parallel-sided); gonocoxite (bearing two broad dorsal lobes: 1 basal and 1 distal); gonostylus (with a small dorsal lobe at base).

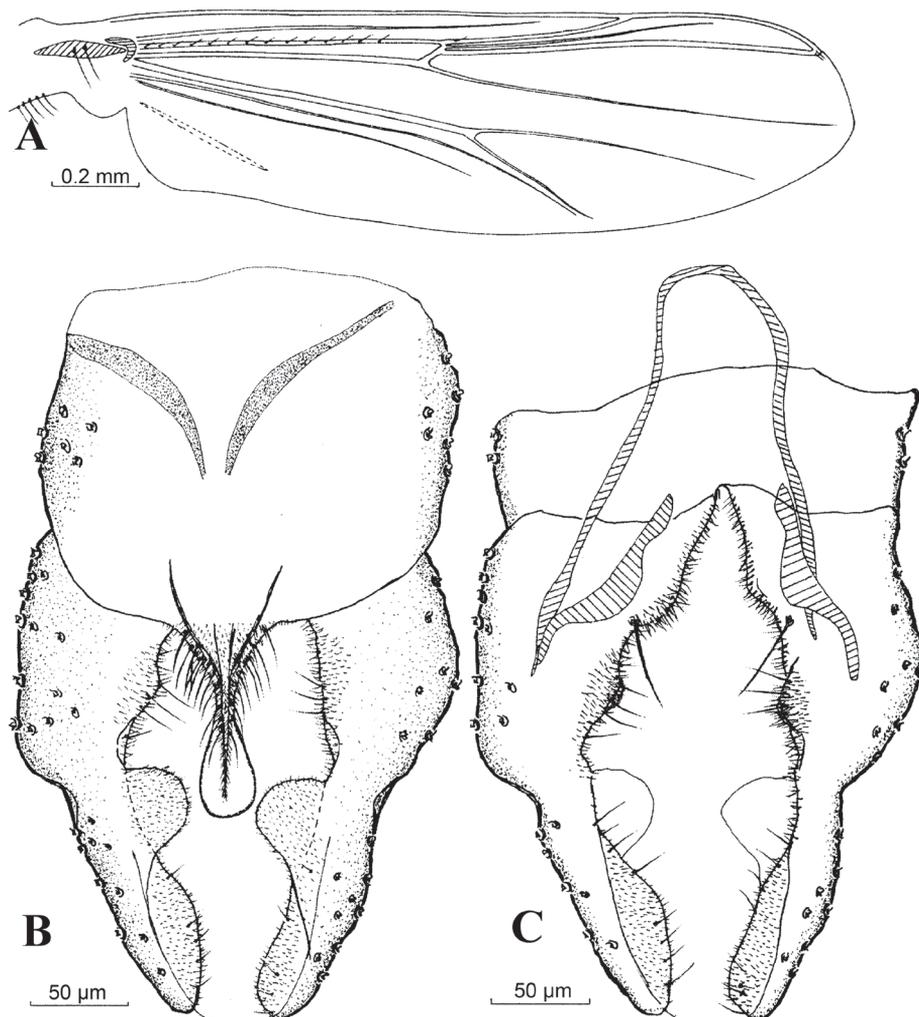
Harnischia turgidula Wang & Zheng, 1993

Fig. 9

Harnischia turgidula: Wang and Zheng 1993: 460; Wang 1999: 172; Wang 2000: 644; Makarchenko et al. 2005: 410.

Type locality. China (Guangdong).

Material examined. CHINA: 4 ♂♂, Hunan Province, Yizhang County, Mang Mountain Natural Conservation area, Elevation 1200 m, 22.07.2004, Light trap, C. Yan; 4 ♂♂, Hunan Province, Yizhang County, Mang Mountain Natural Conservation area, Elevation 1280 m, 22.07.2004, Sweep net, Yan; 1 ♂, Guangdong Province, Fengkai County, Heishiding Mountain Natural Conservation area, 20.04.1988,



Figures 9. *Harnischia turgidula*. **A** Wing; Hypopygium: **B** (dorsal) **C** (ventral).

Sweep net, Wang; 1 ♂, Guangxi Province, Longsheng, 26.05.1990, Wang; 1 ♂, Yunnan Province, Dali City, Yinqiao Town, Elevation 2000 m, 21.05.1996, Water net, Wang.

Diagnostic characters. AR 1.73–2.09. Frontal tubercles absent or small. R_1 without microtrichia. Front tibia with a subapical long seta; tergite IX shoulder-like at the posterior margin. Anal point constricted in the middle and swollen at the apex, with median ridges; anal tergite bands “V” shaped, no fusion in the middle; gonocoxite with a degenerated and small projection in inner distal, bearing setae and microtrichia; gonostylus with a swelling bubble-like protrusion at base and carry microtrichia.

Distribution. China (Hunan, Guangdong, Yunnan); Russian Far East.

***Harnischia* sp.1**

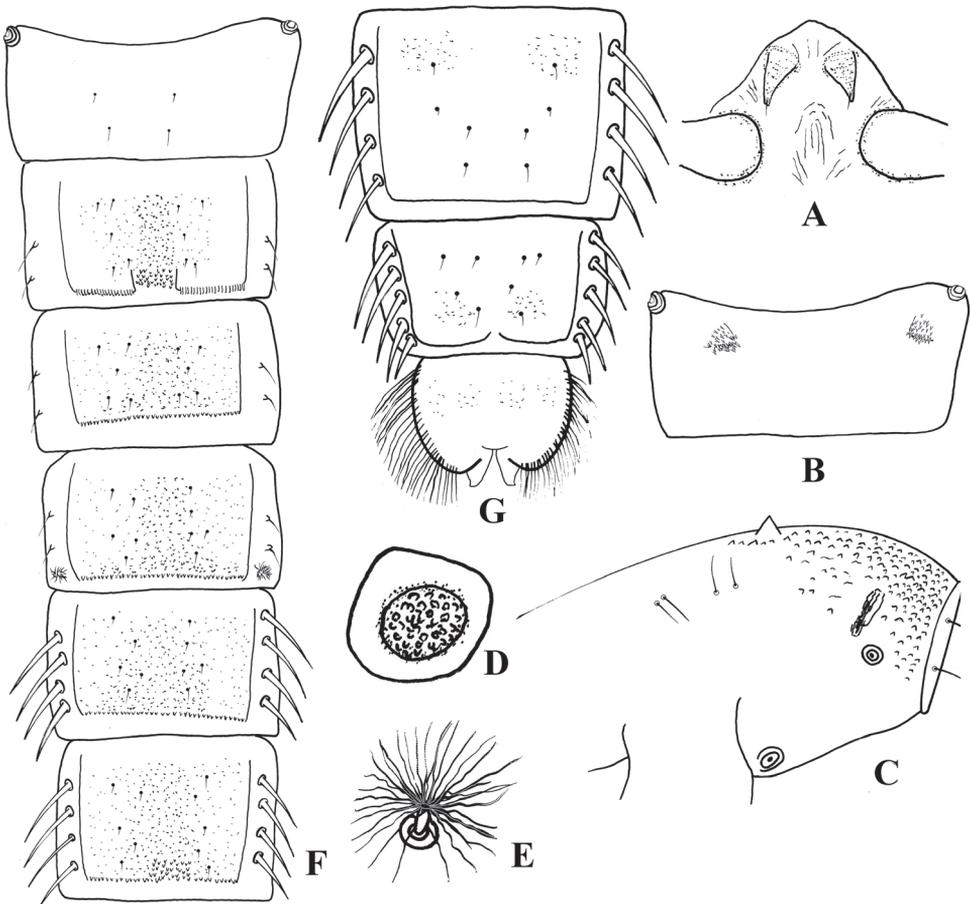
Fig. 10

Diagnostic characters. Pupal stage: sternite I with a pair of spinose anterolateral tubercles, dark and developed; tergites III–VI with narrow, posterior robust spines; hook row medially interrupted; segment II–IV with 2 L setae situated on tubercles; segment VIII without comb or spur.

Materials examined. CHINA: 2P, Jiangxi Province, Shangrao City, Poyang County, Poyang Lake 12.05.2015. W. Liu.

Description. *Pupa* [n = 2] Total length 5.8–6.0 mm. Exuviae brown.

Cephalothorax (Figs 10A, C–E). Frontal setae 25–30 μm long, fine, arising subapically from conical, 125–150 μm long cephalic tubercles. Thoracic horn plumose, with numerous fine branches (Fig. 10E); basal ring oval (Fig. 10D). Thorax granulose



Figures 10. *Harnischia* sp.1. Pupae. **A** frontal apotome **B** sternite I **C** thorax, lateral view **D** basal ring **E** thorax horn **F** tergites I–VI **G** tergite VII–VIII and anal lobe.

dorsally, more densely granulose anteriorly. Prealar tubercle low and rounded. Scutal tubercle prominent. Wing sheath without nose; pearl row absent. Two short precorneals, 2 short anteprenotals and 4 dorsocentrals present. Lengths of dorsocentrals (μm): 100–105, 80–105, 75–90, 65–90.

Abdomen (Fig. 10B, F–G). Tergite I (Fig. 10F) bare; II with posteromedian group of small points; III–VI with narrow, transverse, posterior band of robust spines; VII with a pair of anterior patches fine shagreen; VIII with a pair of anterior and posterolateral patches of shagreen. Hook row widely interrupted medially. Conjunctives bare. Pedes spurii A present on segment IV; pedes spurii B present on segment I. Sternite I (10 B) with a pair of spinose anterolateral tubercles, dark and well developed. Segment VIII without posterolateral comb or spur. Segment II–IV with 2 strong L setae situated on tubercles; V–VII with 4 LS setae, VIII with 5 LS setae.

Anal lobe (Fig. 10G) 1.75–1.94 \times as long as broad, with complete fringe of 68–72 lamelliform setae. Genital sac 300 μm long, extending beyond anal lobe.

Remarks. This species can easily be separated from other known pupa of *Harnischia* species by the following characters: sternite I with a pair of spinose anterolateral tubercles, dark and well developed; tergites III–VI with narrow posterior robust spines; hook row medially interrupted; segment II–IV with 2 L setae situated on tubercles; anal lobe with complete fringe of 68–72 lamelliform setae.

Key to male adults of known *Harnischia* species from China

- | | | |
|---|--|--|
| 1 | Gonostylus with apical tooth | 2 |
| – | Gonostylus without apical tooth | 3 |
| 2 | Inner margin of gonocoxite with obvious projection; gonostylus longer than gonocoxite, swollen and knife-like at the middle; apical teeth straight | <i>H. curltriata</i> Wang |
| – | Inner margin of gonocoxite with inconspicuous projection; gonostylus shorter than gonocoxite, not swollen at the middle; apical teeth hooked..... | <i>H. angularis</i> Albu & Botnariuc |
| 3 | Gonostylus with inner basal projection | 4 |
| – | Gonostylus without inner basal projection..... | 6 |
| 4 | Gonocoxite protrudes into vesicular-shape at base | <i>H. turgidula</i> Wang & Zheng |
| – | Gonocoxite slightly protruding, not bulb-like..... | 5 |
| 5 | Anal point taper-shaped, and the apex of anal point sharp | <i>H. fuscimana</i> Kieffer |
| – | Anal point parallel-sided, the apex of anal point broad and blunt | <i>H. parallela</i> Yan & Wang, sp. n. |
| 6 | Gonostylus with swollen in the apex..... | <i>H. japonica</i> Hashimoto |
| – | Gonostylus parallel-sided or moderately slender apically | 7 |

- 7 Junction of the gonostylus and gonocoxites not as above; anal point swollen in the middle, with median ridges..... *H. curtilamellata* (Malloch)
 – Junction of the gonostylus and gonocoxite shrunken; anal point not swollen in the middle *H. longispuria* Wang & Zheng

Key to pupae of known *Harnischia* species from China

- 1 Sternite I with 1 pair of spinose anterolateral tubercles (Fig. 5B) 2
 – Sternite I with 2 pairs of spinose tubercles on each side, 1 anterolateral and 1 anteromedian (Figs 4B, 10B) *H. curtilamellata* (Malloch)
 2 Pairs of spinose anterolateral tubercles on sternite I brown; segment II-IV with 3 strong L setae *H. fuscimana* Kieffer
 – Pairs of spinose anterolateral tubercles on sternite I dark and well-developed; segment II-IV with 2 L setae situated on tubercles *Harnischia* sp.1

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The blowflies of the Madeira Archipelago: species diversity, distribution and identification (Diptera, Calliphoridae s. l.)

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Abstract

Knowledge on the taxonomic diversity and distribution of blowflies from the Madeira Archipelago is updated. New and interesting findings are reported for poorly studied islands and islets of this archipelago, together with a brief analysis of the diversity of Macaronesian Calliphoridae s. l. Seven blowfly species were collected during this study, including the first records of *Calliphora vicina* Robineau-Desvoidy, 1830, *Chrysomya albiceps* (Wiedemann, 1819), *Lucilia sericata* (Meigen, 1826), *Pollenia rudis* (Fabricius, 1794) and *Stomorhina lunata* (Fabricius, 1805) from Porto Santo, and of *C. vicina*, *L. sericata* and *S. lunata* from Desertas Islands. The presence of *Calliphora loewi* Enderlein, 1903 in Madeira Laurisilva forest is discussed and its first instar larva is redescribed, revealing important differences in relation to its original description. An identification key to the adult Madeiran blowflies is provided for the first time.

Keywords

Calliphora loewi, island diversity, key, larval description, Laurisilva, Macaronesia

Introduction

Blowflies in the broad sense (Diptera: Calliphoridae, Mesembrinellidae, Rhiniidae) (Kutty et al. 2010, Marinho et al. 2016) are conspicuous flies that can be found in both synanthropic and natural ecosystems. Some species exhibit beautiful metallic colours, ranging from green to violet, while others have a more cryptic colouration. Nevertheless, due to their moderate size and frequent association with domestic and human-disturbed environments, blowflies are usually familiar to people.

There are 115 blowfly species in Europe (Rognes and Baz 2007, Rognes 2013), 12 of these being Rhiniidae, once a subfamily of Calliphoridae *s. l.* but recently raised to the rank of family (Kutty et al. 2010). Recent molecular studies also point to a similar status for the Polleniinae, but this taxon is still treated as a subfamily of Calliphoridae *s. str.* (e.g., Kutty et al. 2010, Singh and Wells 2013, Marinho et al. 2016, Zhang et al. 2016). Adult blowflies have a wide variety of life habits: some species feed on pollen and nectar, playing a role in pollination (Rognes 1998, Pérez-Bañón et al. 2007), while most are usually associated with faeces, animal corpses and other decaying organic matter (Norris 1965, Heath 1982, Rognes 1998). A considerable number of species are parasites or predators of earthworms (*Bellardia* Robineau-Desvoidy, 1863, *Onesia* Robineau-Desvoidy, 1830 and *Pollenia* Robineau-Desvoidy, 1830 species), snails (*Angioneura* Brauer & Bergenstamm, 1893, *Eurychaeta* Brauer & Bergenstamm, 1891 and *Melinda* Robineau-Desvoidy, 1830 species), or parasites of bird nestlings (*Protocalliphora* Hough, 1899 and *Trypocalliphora* Peus, 1960 species) (Rognes 1998). Many blowfly species play a key ecological role as natural agents of decomposition (Erzinçlioglu 1985) and are also important in human and veterinary medicine as transmitters of pathogens and as agents of myiasis (Zumpt 1965, Greenberg 1971, Erzinçlioglu 1985, Rognes 1998). In the Rhiniidae, however, the immature morphology and life cycles are unknown for almost all species, though a predatory/parasitic behaviour in nests of social insects and on orthopteran egg-masses has been either suggested or confirmed (Peris 1952, Rognes 1998, Bharti and Bharti 2016).

The catalogue of Iberian Diptera (Carles-Tolrá 2002), which also includes information on the Canarian, Azorean and Madeiran islands, is a landmark in the study of these insects by providing valuable information on the taxonomic diversity of Diptera at both the national and regional level. Furthermore, the comparison of checklists and associated scientific literature presented in the catalogue clearly highlights disparities in knowledge on many families between the different regions. For example, the poor knowledge of the Portuguese fauna, with only 19 blowfly species recorded (13 Calliphoridae and 6 Rhiniidae) contrasts with the amount of information available for Spain, where 48 blowfly species (41 Calliphoridae and 7 Rhiniidae) are known to oc-

cur (Martínez-Sánchez et al. 2002). New findings of blowflies have been reported for continental Portugal (Prado e Castro and García 2009, Prado e Castro et al. 2010) and Spain (Carles-Tolrá 2007) in the last decade, but no significant changes were recorded for the fauna of the Madeira Archipelago. In Portugal, including the Azores and Madeira archipelagos, there is still scarce information on the taxonomic diversity of blowflies due to a lack of national experts and severe limitations in funding for biodiversity inventorying and monitoring programmes targeting invertebrates.

The checklist of Madeiran terrestrial biodiversity (Borges et al. 2008) reports nine species, and recently, *C. loewi* was found for the first time in the archipelago (Prado e Castro et al. 2016). Earlier studies on blowflies from the Madeira Archipelago are scarce and all species records are restricted to the main island (Báez and Santos-Pinto 1975, Rognes 1987, Báez 1990). Herein, an updated checklist is provided of the Calliphoridae and Rhiniidae of the Madeira Archipelago, including new data on the distribution and abundance of seven species. The presence of *Calliphora loewi* Enderlein in Madeira Laurisilva is discussed and its first instar larva is redescribed, showing significant differences in relation to the original description. Finally, a key is provided to enable the identification of adults of all species so far reported from the archipelago.

Material and methods

Study area

The Madeira Archipelago is located in the North Atlantic, nearly 600 km from the African coast (Morocco), between latitudes 32°24' and 33°07'N and longitudes 16°16' and 17°16'W. The archipelago consists of three groups of volcanic islands and islets, namely Madeira, Porto Santo and Desertas. Madeira is the largest (~ 740 km²) and highest (1862 m) island and also presents the highest diversity of habitat types, including the largest surviving area of Laurisilva forest in Macaronesia. Laurisilva is a relict laurel forest native to the Macaronesian archipelagos of Azores, Canaries and Madeira, which during the Tertiary covered a considerable area of the western Mediterranean Basin (Aguiar et al. 2004). Madeira Island has a considerable number of laurel forest fragments in pristine condition (Neves et al. 1996), which led Madeiran Laurisilva to be classified as a UNESCO World Natural Heritage site (IUCN 1999). Furthermore, mostly due to the unique biodiversity associated with Laurisilva, the Madeira Archipelago was included in one of the most important global biodiversity hotspots, the Mediterranean hotspot (Médail and Quézel 1999, Myers et al. 2000). Porto Santo and Desertas have drier environmental conditions than Madeira. On these islands, the dominant plant cover is of herbaceous communities with some thermophilous shrub species, but there are also a considerable number of areas lacking vegetation and affected by soil erosion.

Protected areas cover a large fraction of the archipelago, aiming to maintain and protect its native biodiversity from a number of human-related threats (Martin et al. 2008, Silva et al. 2008; see also <http://www.pnm.pt/>). Nevertheless, despite the recognized vulnerability of several endemic terrestrial arthropod species (Martin et al. 2008, Crespo et al. 2014), very few conservation actions specifically targeting this animal group have been carried out.

Sampling and laboratory work

A sampling programme encompassing different habitat types in all islands of Madeira Archipelago was carried out during the springs and summers of 2011 and 2012 (Table 1). At each site, a combination of sampling techniques (direct sampling, pitfall trapping, vegetation sweeping) were applied to provide an inventory of species of several groups of terrestrial arthropods. Detailed information on the sampling methodology was provided by Serrano et al. (2014). The samples were sorted in the Entomology Laboratory of the Faculty of Sciences, University of Lisbon, Portugal, where adult blowfly specimens were identified to species level using Olympus SZX7 stereomicroscopes and several taxonomic resources (González-Mora and Peris 1988, González-Mora 1989, Peris and González-Mora 1991, Szpila 2012). *Pollenia rudis* specimens were identified, using specific literature (Rognes 1987, 1992), with a Leica M80 at the laboratory of the Department of Environmental Sciences and Natural Resources, University of Alicante, Spain. All identified adult specimens are deposited in the entomological collection of the Department of Animal Biology, University of Lisbon, Portugal.

The unexpected finding of larviposition by *Calliphora loewi* in Madeira Laurisilva (Prado e Castro et al. 2016) allowed for a detailed study of its first instar larval morphology. A revision of the first instar larval morphology of forensically important European Calliphorinae species was recently published by Szpila et al. (2014); for a few species, including *C. loewi*, the authors were unable to access original material and had to base their conclusions on information from the literature (Erzinçlioglu 1985). The study of the first instar larva of *C. loewi* was carried out in Poland (Chair of Ecology and Biogeography Laboratory, Faculty of Biology and Environmental Protection, Nicolaus Copernicus University, Toruń) after extraction from dissected, gravid females. Abdomens of females were detached from the rest of the body and boiled in a 5% KOH solution for 5 minutes. Each abdomen was dissected by slicing the membrane between the tergites and sternites, enabling intact first instars to be pulled out of the female oviduct. The larvae were slide-mounted in Hoyer's medium for study under a light microscope. A Nikon 8400 digital camera, mounted on a Nikon Eclipse E200 microscope, was used for documentation of larval morphology. The larval specimens are housed in the entomological collection of the Chair of Ecology and Biogeography, Nicolaus Copernicus University, Toruń, Poland.

The classification and the taxonomic terminology used in the key follow Rognes (1991), while the terminology used in the description of the first instar larva morphology follows Szpila et al. (2014) and Grzywacz et al. (2014).

Table 1. Information on sampling dates and localities (site name, habitat type, and geographic coordinates) in the Madeira Archipelago.

Island group	Site name	Habitat type	Latitude (N)	Longitude (W)	Dates	
Madeira	Abobreiras	Heathland	32°43'13"	16°51'37"	30/V-13/VI/2011	
	Achadas da Cruz	<i>Eucalyptus</i> plantation	32°50'42"	17°12'25"	26/V-9/VI/2011	
	Bica da Cana	<i>Pinus</i> plantation	32°44'47"	17°03'25"	26/VI-9/VII/20110	
	Calheta 1	Heathland	32°45'28"	17°08'48"	27/V-10/VI/20110	
	Calheta 2	<i>Eucalyptus</i> plantation	32°45'06"	17°09'14"	27/V-10/VI/20110	
	Dunas da Piedade	Dune system	32°44'49"	16°39'27"	2-16/V/20110	
	Funduras	Laurisilva	32°44'58"	16°47'30"	31/V-14/VI/20110	
	Galhano 1	Laurisilva	32°48'07"	17°09'57"	4-18/VII/20120	
	Galhano 3	Laurisilva	32°47'48"	17°10'30"	5-19/VII/20120	
	Ilhéu do Farol	Coastal vegetation	32°43'43"	16°39'27"	18/V-1/VI/20110	
	Loreto	<i>Pinus</i> plantation	32°46'41"	17°12'36"	24/VI-9/VII/20110	
	Miradouro das Voltas 1	Laurisilva	32°48'28"	16°57'00"	2-16/VI/20110	
	Miradouro das Voltas 2	Laurisilva	32°48'15"	16°56'48"	2-16/VI/20110	
	Miradouro das Voltas Ps	<i>Pseudotsuga</i> plantation	32°48'43"	16°57'04"	2-16/VI/20110	
	Miradouro das Voltas Seq	<i>Sequoia</i> plantation	32°48'24"	16°56'47"	2-16/VI/20110	
	Montado dos Pessegueiros 2	Laurisilva	32°47'40"	17°05'12"	3-17/VII/20120	
	Montado dos Pessegueiros 3	Laurisilva	32°47'44"	17°05'07"	3-17/VII/20120	
	Pico das Pedras L	Laurisilva	32°46'08"	16°54'42"	31/V-14/VI/20110	
	Pico das Pedras Ps	<i>Pseudotsuga</i> plantation	32°46'33"	16°53'48"	31/V-14/VI/20110	
	Ponta de S. Lourenço E	Coastal vegetation	32°44'56"	16°41'30"	3-17/V/20110	
	Parque eólico	Semi-natural meadow	32°44'45"	16°43'29"	2-16/V/20110	
	Ponta de S. Lourenço W	Coastal vegetation	32°44'50"	16°41'55"	3-17/V/20110	
	Portela	<i>Eucalyptus</i> plantation	32°44'45"	16°49'23"	3-17/VI/20110	
	Porto Moniz	<i>Eucalyptus</i> plantation	32°50'46"	17°10'37"	24/V-7/VI/20110	
	Prazeres	<i>Pinus</i> plantation	32°45'58"	17°11'33"	24/VI-9/VII/20110	
	Ribeira da Cruz	Laurisilva	32°49'34"	17°12'35"	26/V-9/VI/20110	
	Santana	<i>Pinus</i> plantation	32°48'09"	16°51'57"	25/VI-10/VII/20110	
Desertas	Bugio N	Coastal vegetation	32°24'52"	16°28'40"	27/IV-19/V/2011	
	Bugio S	Coastal vegetation	32°24'38"	16°28'23"	27/IV-19/V/2011	
	Castanheira N	Coastal vegetation	32°33'52"	16°32'12"	26/IV-10/V/20110	
	Castanheira S	Coastal vegetation	32°33'11"	16°31'47"	26/IV-10/V/20110	
	Eira	Coastal vegetation	32°30'50"	16°30'10"	27/IV-11/V/20110	
	Doca	Coastal vegetation	32°31'03"	16°30'41"	26/IV-10/V/20110	
	Ilhéu Chão N	Coastal vegetation	32°35'10"	16°32'43"	28/IV-18/V/20110	
	Ilhéu Chão S	Coastal vegetation	32°34'52"	16°32'25"	28/IV-18/V/20110	
	Porto Santo	Fonte da Areia	Dune system	33°04'54"	16°21'18"	21/VI-5/VII/20110
		Ilhéu da Cal N	Coastal vegetation	33°0'41"	16°23'07"	22/VI-6/VII/20110
Ilhéu da Cal S		Coastal vegetation	33°00'7"	16°23'01"	22/VI-6/VII/20110	
Ilhéu do Farol N		Coastal vegetation	33°03'19"	16°17'04"	20/VI-4/VII/20110	
Ilhéu do Farol S		Coastal vegetation	33°03'13"	16°16'43"	20/VI-4/VII/20110	
Ilhéu do Ferro		Coastal vegetation	33°02'16"	16°24'28"	21/VI-5/VII/20110	
Pico Ana Ferreira		<i>Pinus</i> plantation	33°02'36"	16°22'24"	21/IV-5/V/20110	
Pico Branco Cup		<i>Cupressus</i> plantation	33°05'40"	16°17'55"	23/IV-7/V/20110	
Pico Branco mead		Semi-natural meadow	33°05'29"	16°18'23"	23/IV-7/V/20110	
Pico do Castelo		<i>Pinus</i> plantation	33°04'51"	16°19'55"	22/IV-6/V/20110	
Pico do Facho Cup		<i>Cupressus</i> plantation	33°05'02"	16°19'17"	22/IV-6/V/20110	
Pico do Facho Pin		<i>Pinus</i> plantation	33°04'58"	16°19'28"	22/IV-6/V/20110	
Pico Juliana		<i>Cupressus</i> plantation	33°05'33"	16°19'20"	23/IV-7/V/20110	

Results

The blowfly species of the Madeira Archipelago

Four-hundred and seventy (470) specimens of six Calliphoridae and one Rhiniidae species were collected during this study. The occurrence of blowfly species is reported for the first time from Porto Santo and Desertas. Detailed information associated with the specimens collected in the Madeira Archipelago (sampling date, location, geographic coordinates, habitat-type and number and sex of specimens) is presented under “Material examined” and in Table 1. An updated list of blowfly species from the Madeira Archipelago is presented in Table 2, while the spatial distribution of new species records is shown in Figure 1.

Calliphora loewi Enderlein, 1903

Figs. 2J, 3A

Material examined. MADEIRA: Galhano 3 (20 females); Montado dos Pessegueiros 2 (1 female); Montado dos Pessegueiros 3 (3 females).

Remarks. *Calliphora loewi* is a carrion-breeder present in the Holarctic and in a small part of the Oriental Region (Schumann 1986, Verves 2005). It is found in forests of northern and central Europe (Smith 1986, Byrd and Castner 2010), being common in alpine regions. In North America it is found in Alaska, Canada and in the northern continental United States (Rognes 1991, Tantawi et al., in press). Throughout its range, this species is generally not found in urban and disturbed areas (Byrd and Castner 2010). Although widespread, *C. loewi* is rarely recorded, and usually in low abundance, in carcasses of large vertebrates, demonstrating a preference for small animal remains (Szpila et al. 2014). In Madeira, *C. loewi* is restricted to a few native forest areas at high altitude (1000–1300 m) (Fig. 1). This species was recently collected for the first time in Madeira (Prado e Castro et al. 2016).

Calliphora vicina Robineau-Desvoidy, 1830

Figs. 2I, 3B

Material examined. MADEIRA: Abobreiras (1 female); Achadas da Cruz (1 male); Calheta 1 (1 female); Calheta 2 (1 female); Ponta de São Lourenço E (1 male); Ilhéu do Farol (1 male); Funduras (1 female); Galhano 1 (1 female); Miradouro das Voltas 1 (1 female); Miradouro das Voltas 2 (1 female); Miradouro das Voltas Ps (1 female); Miradouro das Voltas Seq (2 females); Montado dos Pessegueiros 2 (1 female, 1 male); Montado dos Pessegueiros 3 (1 female, 2 males); Pico das Pedras L (1 female); Pico das Pedras Ps (2 females); Portela (1 female); Porto Moniz (2 females); Ribeira da Cruz

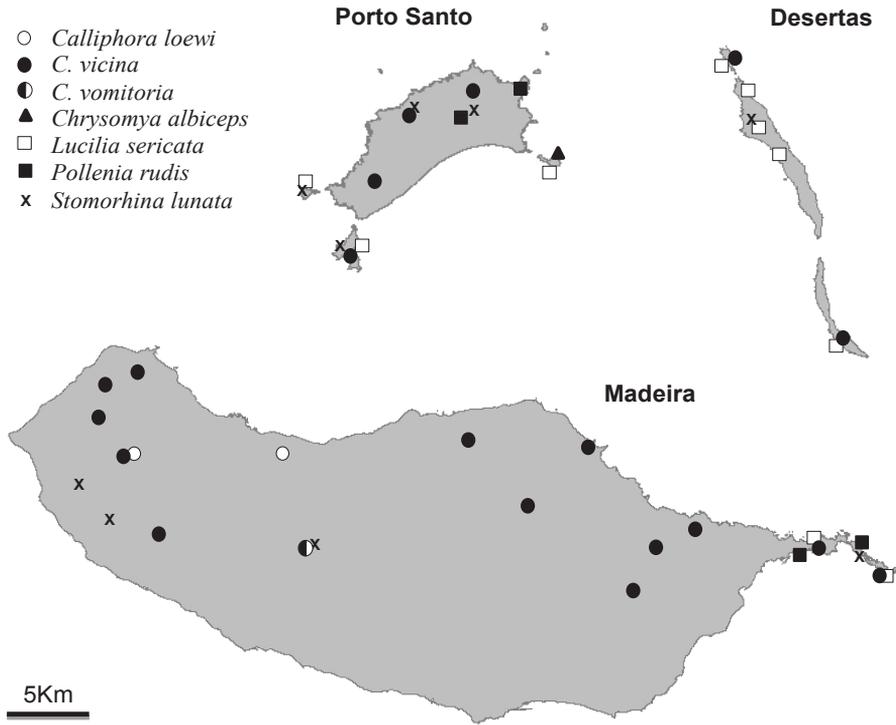
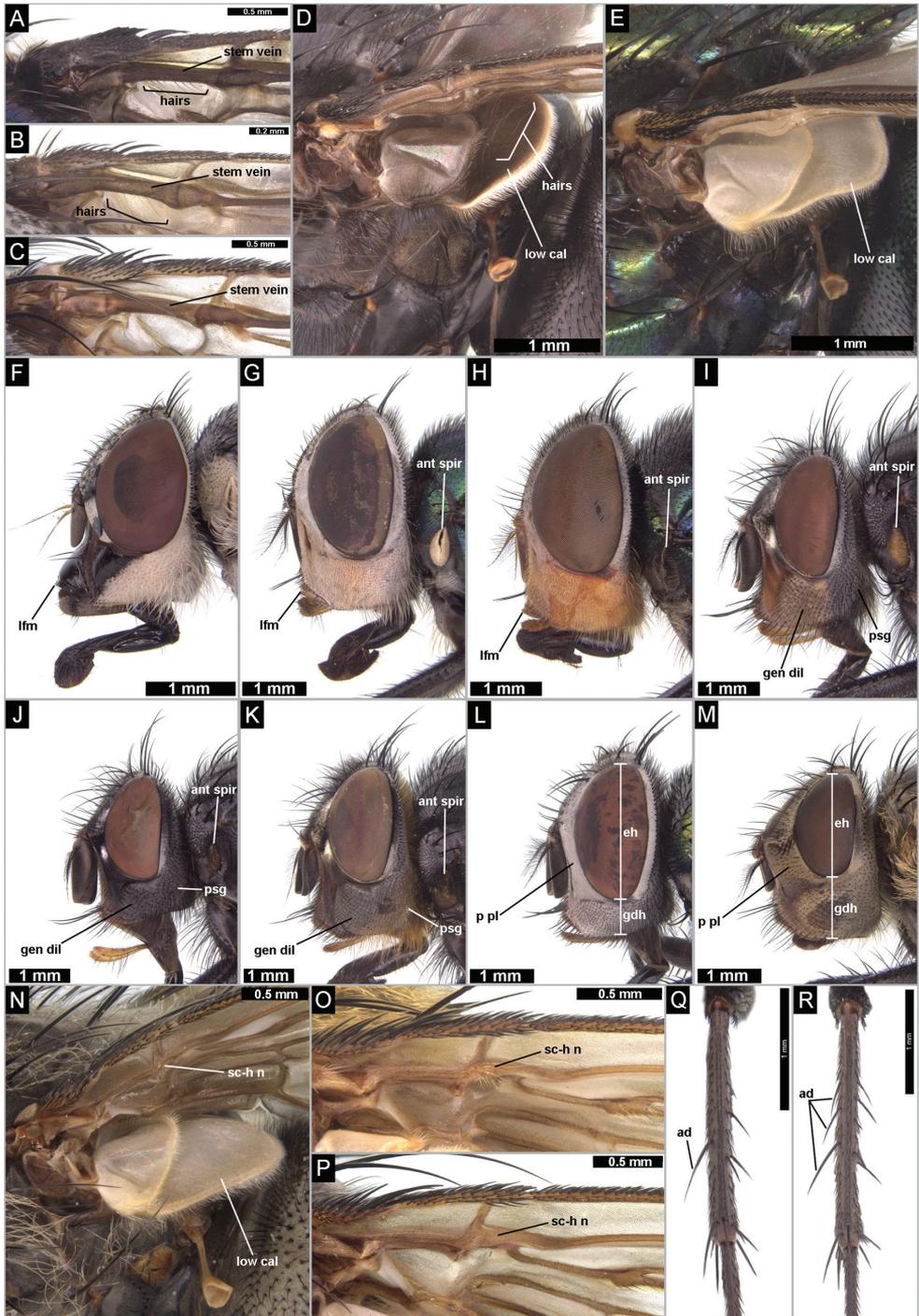


Figure 1. Blowfly records collected during the present study in the islands and islets of the Madeira Archipelago. Species records are plotted on a simplified representation of the archipelago to ease understanding.

Table 2. Updated list of the Calliphoridae and Rhiniidae of the Madeira Archipelago. New records are indicated by a full black circle. M – Madeira Island, PS – Porto Santo Island and surrounding islets, D – Desertas islands (Ilhéu Chão, Deserta Grande and Bugio).

Species	M	PS	D
<i>Calliphora loewi</i> Enderlein, 1903	X		
<i>Calliphora vicina</i> Robineau-Desvoidy, 1830	X	•	•
<i>Calliphora vomitoria</i> (Linnaeus, 1758)	X		
<i>Chrysomya albiceps</i> (Wiedemann, 1819)	X	•	
<i>Chrysomya megacephala</i> (Fabricius, 1794)	X		
<i>Lucilia sericata</i> (Meigen, 1826)	X	•	•
<i>Pollenia angustigena</i> Wainwright, 1940	X		
<i>Pollenia pediculata</i> Macquart, 1834	X		
<i>Pollenia rudis</i> (Fabricius, 1794)	X	•	
<i>Stomorphina lunata</i> (Fabricius, 1805)	X	•	•



(1 female); Santana (1 female); PORTO SANTO: Ilhéu da Cal S (1 female, 1 male); Fonte da Areia (1 female, 1 male); Pico Ana Ferreira (7 females, 1 male); Pico do Facho Cup (1 female); Pico Juliana (1 female); DESERTAS: Bugio N (12 females, 5 males); Bugio S (7 females, 1 male); Ilhéu Chão N (1 female, 1 male).

Remarks. *Calliphora vicina* is a cosmopolitan species, widely distributed all over the world and closely connected with human activity (Zumpt 1965, Greenberg 1971, González-Mora 1989, Martínez-Sánchez et al. 2002), being commonly found in urban areas (Erzinçlioglu 1985, Rognes 1998, Szpila et al. 2014). The adults are attracted to faeces, meat and fruits, while the larvae are mainly necrophagous, usually developing in carrion (Zumpt 1965, Greenberg 1971). This species is widespread in a variety of habitat types in the Madeira Archipelago (Table 1).

Calliphora vomitoria (Linnaeus, 1758)

Figs. 2K, 3C

Material examined. MADEIRA: Bica da Cana (1 female).

Remarks. This common carrion-breeder is distributed throughout the Holarctic Region and is also present in the Oriental and Australasian regions (Erzinçlioglu 1985). It is more rural in its distribution than *C. vicina* (Smith 1986), frequenting natural and cultivated forests and showing a preference for more shaded habitats (Szpila et al. 2014). *Calliphora vomitoria* was found only in Madeira, in a pine plantation with low human disturbance.

Chrysomya albiceps (Wiedemann, 1819)

Figs. 2G, 3D

Material examined. PORTO SANTO: Ilhéu do Farol S (1 female).

Figure 2. Diagnostically important characters of Madeiran blowflies (Diptera, Calliphoridae s. l.): **A** *Chrysomya albiceps*, basal part of wing, dorsal surface, showing haired stem vein **B** *Stomorbina lunata*, basal part of wing, dorsal surface, showing haired stem vein **C** *Calliphora vicina*, basal part of wing, dorsal surface, showing bare stem vein **D** *Calliphora vomitoria*, thorax, upper and lower calypters **E** *Lucilia sericata*, thorax, upper and lower calypters **F** *Stomorbina lunata*, female, head, lateral view **G** *Chrysomya albiceps*, female, head, lateral view **H** *Chrysomya megacephala*, female, head, lateral view **I** *Calliphora vicina*, female, head, lateral view **J** *Calliphora loewi*, female, head, lateral view **K** *Calliphora vomitoria*, female, head, lateral view **L** *Lucilia sericata*, female, head, lateral view **M** *Pollenia rudis*, female, head, lateral view **N** *Pollenia rudis*, thorax, upper and lower calypters **O** *Pollenia pediculata*, basal part of wing, ventral surface, showing haired node of subcostal and humeral veins **P** *Pollenia rudis*, basal part of wing, ventral surface, showing bare node of subcostal and humeral veins **Q** *Pollenia angustigena*, mid tibia **R** *Pollenia rudis*, mid tibia. Abbreviations: **ad**, anterodorsal seta; **ant spir**, anterior spiracle; **eh**, eye height; **gen dil**, genal dilation; **gdh**, genal dilation height; **lfm**, lower facial margin; **low cal**, lower calypter; **p pl**, parafacial plate; **psg**, postgena; **sc-h n**, node subcosta-humeral vein.

Remarks. *Chrysomya albiceps* can be found from the southern Palaearctic Region (southern Europe, Arabia, India) through to Africa (Zumpt 1965) and the Americas, where since its introduction it has rapidly expanded north (Guimarães et al. 1978, Baumgartner and Greenberg 1984). In Europe it is very abundant in the Iberian Peninsula (Martínez-Sánchez et al. 2002, Prado e Castro et al. 2012) and is expanding towards central Europe, having reached France, Switzerland and Austria (Grassberger et al. 2003), the Ukraine (Verves 2004) and Poland, from where its current northernmost records are known (Szpila et al. 2008, Michalski and Szpila, in press). Our record from Ilhéu do Farol is the first for Porto Santo. *Chrysomya albiceps* normally breeds in carrion; newly-hatched first instar larvae feed on exudations of decomposing flesh, but the second and third larval stages are facultative predators, feeding also on the larvae of other blowfly species (Zumpt 1965).

Chrysomya megacephala (Fabricius, 1794)

Figs. 2H, 3E

Records. MADEIRA: Funchal (Báez 1990).

Remarks. *Chrysomya megacephala* is widely distributed over the Oriental and Australasian regions, also occurring in many neighbouring parts of the Palaearctic Region (Zumpt 1965). It was introduced in South America (Guimarães et al. 1978), probably from southern Africa (Baumgartner and Greenberg 1984), and into the United States (Greenberg 1988). In Europe it is known from Spain (Martínez-Sánchez et al. 2001), Portugal (Prado e Castro and García 2009), Malta (Ebejer 2007) and from the archipelagos of Madeira (Báez 1990) and the Canaries (Báez et al. 1981). *Chrysomya megacephala* is normally a faeces and carrion breeder (Zumpt 1965), considered a dangerous dipteran vector of pathogens (Wells 1991) and a major pest of fish products (Wall et al. 2001).

Lucilia sericata (Meigen, 1826)

Figs. 2L, 3F

Material examined. MADEIRA: Dunas da Piedade (1 male); Ilhéu do Farol (1 female); PORTO SANTO: Ilhéu da Cal S (1 male); Ilhéu do Farol N (1 female, 5 males); Ilhéu do Farol S (2 females); Ilhéu do Ferro (1 female); DESERTAS: Bugio N (130 females, 30 males); Bugio S (116 females, 34 males); Castanheira N (13 females, 5 males); Castanheira S (3 females); Doca (10 females, 5 males); Eira (1 female); Ilhéu Chão N (1 female); Ilhéu Chão S (1 female).

Remarks. A very common fly in temperate areas of the Holarctic Region (Zumpt 1965). It is practically cosmopolitan, widespread throughout the major zoogeographical regions (Smith 1986, Rognes 1991), and is directly connected to human activity (Martínez-Sánchez et al. 2002). *Lucilia sericata* was found in all island groups of the Madeira Archipelago where it was occasionally recorded in high abundance. The adults are attracted mainly to carrion or open wounds (usually with necrotic tissues) (Zumpt 1965).

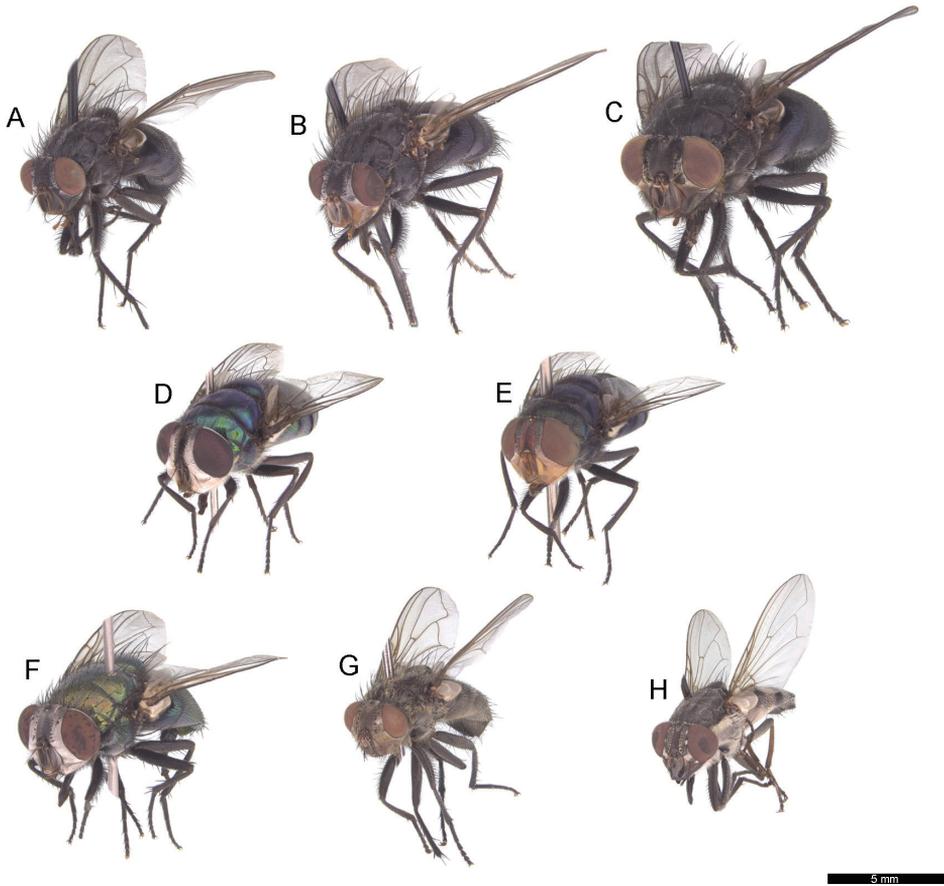


Figure 3. Habitus in antero-lateral view of Madeiran blowflies: **A** *Calliphora loewi* **B** *Calliphora vicina* **C** *Calliphora vomitoria* **D** *Chrysomya albiceps* **E** *Chrysomya megacephala* **F** *Lucilia sericata* **G** *Pollenia rudis* **H** *Stomorphina lunata*.

Pollenia angustigena Wainwright, 1940

Records. MADEIRA: Madeira (Rognes 1987).

Remarks. This species is cited from most countries of Europe and from the Nearctic Region (Rognes 1991). In Europe, *P. angustigena* can be found from the southern parts of Norway and Finland to Italy and the Iberian Peninsula (Martínez-Sánchez et al. 2002). In the Iberian Peninsula the species seems to be restricted to high altitudes (from 300 m to above 1200 m) (Martínez-Sánchez et al. 1998). No specimens were caught in this study but the species was previously cited from Madeira (Rognes 1987, Martínez-Sánchez and Rognes 2008). Adults of this fly are active mostly from March to October with peaks in early spring in the northern parts of its range (Rognes 1991). As the rest of *Pollenia* spp., it is a predator on earthworm species (Rognes 1987). The egg and first instar larva of this species were described by Grzywacz et al. (2012) and

Szpila (2003), respectively, and their morphology is significantly different compared to that of necrophagous blowflies.

Pollenia pediculata Macquart, 1834

Records. MADEIRA: Madeira (Rognes 1987).

Remarks. This species is distributed throughout most zoogeographical regions, being widespread in Europe. *Pollenia pediculata* was first cited from Madeira by Rognes (1987), but no specimens were obtained in the present study. The adults are active mainly in summer (Martínez-Sánchez et al. 2001). The larvae are predators on earthworms, including the lumbricid *Eisenia rosea* (Savigny) (Rognes 1987). The early immature stages were described by Szpila (2003) and Grzywacz et al. (2012).

Pollenia rudis (Fabricius, 1794)

Figs. 2M, 3G

Material examined. MADEIRA: Parque eólico (2 females); Ponta de São Lourenço W (1 female); PORTO SANTO: Pico Branco Cup (6 females); Pico Branco mead (4 females); Pico do Castelo (4 females).

Remarks. This is the most common species in the genus, being widespread in the Palaearctic, Nearctic and Oriental regions. In Porto Santo it was found in different habitat types (Table 1). *Pollenia rudis* uses earthworms as larval hosts and is active all year round, particularly during spring (Rognes 1987, Martínez-Sánchez et al. 1998). The immature stages were described by Yahnke and George (1972), Szpila (2003) and Grzywacz et al. (2012).

Stomorhina lunata (Fabricius, 1805)

Figs. 2F, 3H

Material examined. MADEIRA: Bica da Cana (2 females); Loreto (3 females); Prazeres (1 female); Parque eólico (1 female); Ponta de São Lourenço W (1 female); PORTO SANTO: Ilhéu da Cal N (4 females); Ilhéu da Cal S (2 females); Ilhéu do Ferro (1 female); Fonte da Areia (7 females); Pico do Facho Pin (1 female); DESERTAS: Castanheira N (1 female).

Remarks. *Stomorhina lunata* is distributed almost worldwide, being absent only from the Neotropical and Australasian regions (Rognes 2013). It is common in Europe including all of the Mediterranean area, and is widely distributed throughout the Iberian Peninsula (González-Mora and Peris 1988). It is known as a predator on egg-masses of the locust *Schistocerca gregaria* (Forskål, 1775) (Rognes 1998) and a parasite

in nests of ants (Bharti and Bharti 2016). Adults are commonly found on flowers (González-Mora and Peris 1988).

Key to the adult blowflies of the Madeira Archipelago

- 1 Stem-vein with a row of weak setulae on dorsal surface (Fig. 2A, B).....2
 – Stem-vein bare on dorsal surface (Fig. 2C).....4
 2 Lower facial margin strongly protruded (Fig. 2F); arista bare on ventral side (Fig. 2F); abdomen with a yellow pattern on black background.....
 *Stomorbina lunata*
 – Lower facial margin not protruded (Fig. 2G, H); arista always with hairs dorsally and ventrally (Fig. 2G, H); abdomen lustrous, bluish or green, with dark transverse strips on posterior margins of segments3
 3 Anterior spiracle light-coloured, white-yellowish (Fig. 2G)
 *Chrysomya albiceps*
 – Anterior spiracle dark, brownish (Fig. 2H)..... *Chrysomya megacephala*
 4 Lower calypter black or at least darkly infuscate, with numerous long hairs on dorsal surface (Fig. 2D).....5
 – Lower calypter white-yellowish and bare on dorsal surface (Fig. 2E, N)7
 5 Facial ridge, lower facial margin and anterior part of genal dilation yellowish-red (Fig. 2I); anterior spiracle (Fig. 2I) and basicosta light-coloured, brownish..... *Calliphora vicina*
 – Facial ridge, lower facial margin and anterior part of genal dilation black (Fig. 2J, K); anterior spiracle and basicosta black (Fig. 2J, K)6
 6 Hairs on posterior part of genal dilation and postgena black (Fig. 2J)
 *Calliphora loewi*
 – Hairs on posterior part of genal dilation and postgena orange (Fig. 2K).....
 *Calliphora vomitoria*
 7 Parafacial plates narrow and bare, with white dusting (Figs 2L, 3F); height of genal dilation distinctly shorter than half of eye height (Fig. 2L); body lustrous green (Fig. 3F)..... *Lucilia sericata*
 – Parafacial plates broad and densely haired, with brown-yellowish dusting (Fig. 2M); height of genal dilation distinctly longer than half of eye height (Fig. 2M); body dark, abdomen with grey tessellated pattern.....8
 8 Node at junction of humeral crossvein and subcostal vein with a bundle of several light-coloured hairs (Fig. 2O) *Pollenia pediculata*
 – Node at junction of humeral crossvein and subcostal vein bare (Fig. 2N, P) .
9
 9 Mid tibia with one anterodorsal seta (Fig. 2Q)..... *Pollenia angustigena*
 – Mid tibia with two or three anterodorsal setae (Fig. 2R)
 *Pollenia rudis*

Redescription of the first instar larva of *Calliphora loewi*

Calliphora loewi Enderlein, 1903

Material examined. MADEIRA: Galhano 3 (15 first instar larvae). The first instar larvae (Fig. 4A) were obtained from 13 gravid females collected in Galhano (Prado e Castro et al. 2016).

Diagnosis. The first instar larvae of *C. loewi* from Madeira possess the general habitus characteristic of most Calypttratae, being divided into a bilobed pseudocephalon (pc), three thoracic segments (t1–t3), seven abdominal segments (a1–a7), and an anal division (ad) that carries the posterior spiracles.

Redescription. Body length: 1.4–5.1 mm. *Pseudocephalon.* Antennal complex with small antennal dome situated on basal ring, antennal dome slightly longer than height of basal ring; maxillary palpus located on anterior surface of pseudocephalic lobe and readily visible under a light microscope as a flat disc clearly distinguished from the surrounding cuticular surface; oral ridges present from lateral margins of functional mouth opening to ventral and lateral surfaces of pseudocephalon; functional mouth opening with two lateral tufts of numerous cirri. *Cephaloskeleton.* As in other necrophagous blowflies; consisting of unpaired labrum (lb), paired mouthhooks (mh), unpaired and H-shaped intermediate sclerite (is) and basal sclerite with parastomal bars (pb), vertical plates (vp) and ventral and dorsal cornua (vc, dc) (Figs 4B, C); each mouthhook an L-shaped bar, with tip equipped with 6 strong, pointed teeth directed ventrally, teeth arranged in one row with one tooth situated in front of row (well visible in ventral view); basal part of mouthhook also strongly sclerotized and equipped with a lateral arm (Figs 4B, C); labrum with readily differentiated sharp and curved apical part, ventral incision separating apical and basal parts of labrum indistinct (Fig. 4C); epistomal sclerite [“posterior expansion of labrum” in Szpila et al. (2014)] flat in lateral view (Fig. 4C); parastomal bars (pb) long and slender; intermediate sclerite (is) and crossbeam of intermediate sclerite broad; vertical plate (vp) as broad as width of ventral cornua (Fig. 4B); dorsal cornua slightly longer than ventral cornua (Fig. 4B); dorsal bridge present (Fig. 4B). *Thoracic segments.* Anterior spinose band on first thoracic segment broad (Fig. 4A), with spines arranged in 5–7 rows dorsally and 12–14 rows ventrally; anterior spinose bands of second and third thoracic segments with homogenous conical, slightly flattened spines, tip of spines slightly curved. *Abdominal segments.* Anterior spinose bands complete on segments a1–a5, narrowly interrupted dorsally on segment a6; segment a7 with anterior spinose band on ventral and ventro-lateral surfaces and with several spines on lateral surface; posterior spinose band on segment a1 reduced to two small groups of spines situated ventro-laterally, on a2 posterior spines only on ventral and ventro-lateral surfaces, segment a3 with narrow posterior spinose band interrupted dorsally, segments a4–a7 with complete posterior spinose band. *Anal division.* Anal pads rounded and slightly protruding, anal tuft with several spines dorsally; circle of hair-like spines around spiracular field complete; anterior spinose band developed only on ventral and ventro-lateral surfaces.

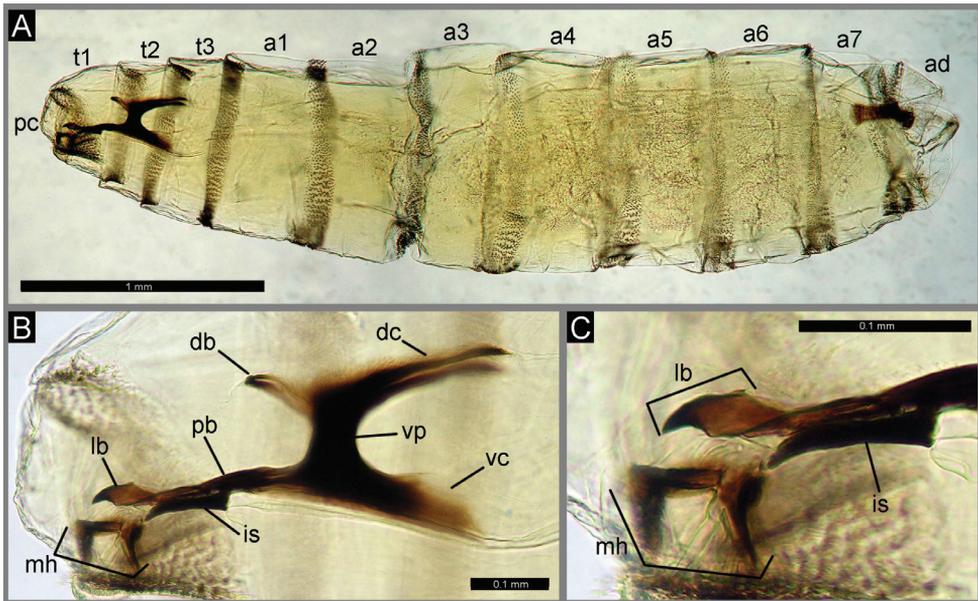


Figure 4. First instar larva of *Calliphora loewi* from Madeira: **A** Habitus in lateral view **B** Cephaloskeleton in lateral view **C** Anterior part of cephaloskeleton in lateral view. Abbreviations: **a1-a7**, abdominal segments; **ad**, anal division; **db**, dorsal bridge; **dc**, dorsal cornua; **is**, intermediate sclerite; **lb**, labrum; **mh**, mouthhook; **pb**, parastomal bar; **pc**, pseudocephalon; **t1-t3**, thoracic segments.

Comparison with original description. The comparison of first instar larval specimens from Madeira with the original description (Erzinçlioğlu 1985) points to several discrepancies. Erzinçlioğlu (1985) described the anterior spinose band on a5 of *Calliphora loewi* as interrupted dorsally. Szpila et al. (2014) used this character for separation of *C. loewi* larvae from those of *C. vicina* and *Cynomyia mortuorum* (Linnaeus, 1761), where the anterior spinose band on a5 is complete. However, the first instar larvae of *C. loewi* from Madeira also possess a complete anterior spinose band on a5. This character seems to be variable and cannot be treated as reliable. Serious discrepancies between the material from Madeira and the British larvae studied by Erzinçlioğlu (1985) were also found in the morphology of the labrum in the cephaloskeleton. Larvae from Madeira possess a massive labrum with broad basal part and well differentiated apical part. Additionally, the apical part of the labrum is noticeably curved downward. The cephaloskeleton of *C. loewi* larvae in the schematic illustration provided by Erzinçlioğlu (1985, fig. 28) possesses an elongated labrum (labelled as “median tooth”), without clear differentiation between an apical part and a basal part. Unfortunately, at this stage it is not possible to state that these differences between Madeira specimens and the original description result from interpopulation variation or inaccuracy of observation, as the larval material analyzed for the original description is unavailable (Szpila et al. 2013). Future studies on the variation of *C. loewi* larval morphology across the species distribution range will help to clarify this issue.

Discussion

The effective conservation of insect diversity is, to some extent, hindered by the lack of knowledge of species taxonomy as well as by poor data on species distribution, abundance and sensitivity to habitat change (e.g., Cardoso et al. 2011, New 2012). Consequently, major efforts have been made during the last decade to overcome these obstacles, leading to the production of updated catalogues of species and the identification of conservation priorities for many countries and regions in the world (e.g., Martín et al. 2008, Verdú and Galante 2009, Van Swaay et al. 2010).

This recent survey of the calliphorid diversity on the islands and islets of the Madeira Archipelago allowed the collection of valuable information concerning species taxonomy, distribution and ecology, including the first report of blowfly species from Porto Santo and Desertas islands (Table 2). The number of Calliphoridae *s. l.* species known from the Madeira Archipelago is presently ten, but there is a considerable difference in calliphorid species richness between islands: Madeira (10 spp.), Porto Santo (5 spp.) and Desertas (3 spp.). The lower number of blowfly species recorded from Porto Santo and Desertas is not only due to geographic and ecological determinants (e.g. lower surface area, lower habitat diversity), but also a result of undersampling. Our blowfly survey was the first carried out in Porto Santo and Desertas. These islands have traditionally been disregarded by taxonomic experts, who have focused their sampling efforts exclusively on the main island when visiting the archipelago. Consequently, the present knowledge of Madeiran biodiversity is biased and efforts should be addressed to develop inventory programmes exploring the taxonomic biodiversity of also Porto Santo and Desertas.

This study presents evidence that the species found in the Madeira Archipelago use different habitats: *C. vicina* was the most widespread blowfly, occurring in a variety of habitat types (natural, semi-natural and forest plantations) and ranging from thermophilous coastal areas to native forest patches in valleys and mountainous areas. Three other species (*L. sericata*, *P. rudis*, *S. lunata*) were more common in coastal areas and inland forest plantations, while the two remaining *Calliphora* species (*C. loewi* and *C. vomitoria*) were restricted to a few forest locations at higher altitudes. A similar pattern of calliphorid species distribution was found in the Canaries, where *C. vicina* was considered an “extreme habitat generalist”, *L. sericata* seemed to be restricted to open habitats at low altitudes and *C. splendens* Macquart, 1839 and *C. vomitoria* were rare and confined to forest habitats (Hanski 1977).

The finding of *Calliphora loewi* in the Madeira Archipelago was surprising, since in Europe the species is known to occur mainly in forest ecosystems at much higher latitudes (Smith 1986, Byrd and Castner 2010) and is absent from the Azores and the Canary Islands (Arechavaleta et al. 2010, Borges et al. 2010). It is presumed that the Madeiran populations of *C. loewi* are relictual due to their isolation from the nearest mainland populations and considering the historical biogeography of Lau-

risilva, a laurel forest type once distributed throughout the western Mediterranean and which is now restricted to Macaronesia (Neves et al. 1996). Furthermore, the finding that the Madeiran populations of *C. loewi* are strictly associated with pristine Laurisilva patches at high altitudes also seems to support their relictual origin: the two Laurisilva patches where *C. loewi* was found, Montado dos Pessegueiros and Galhano, are well preserved, have minimal human disturbance, and show similar plant compositions (Neves et al. 1996). Like *C. loewi*, the Canarian endemic *C. splendens* is also restricted to native forest areas at higher altitudes (above 400 m) being absent from disturbed, low altitude areas where introduced calliphorids dominate (Hanski 1977, Báez 1988).

The number of calliphorid species so far recorded for the Madeira Archipelago remains low, but it is similar to numbers reported from the Azores (nine species) and Canary Islands (ten species) (Báez and Oromí 2010, Martínez-Sánchez 2010). The low species richness of these oceanic archipelagos is in part due to the difficulties faced in overcoming the ecological barrier posed by the ocean, even for insects with a good flight capacity such as blowflies. Moreover, according to Hanski (1977), the most likely explanation for the impoverished calliphorid faunas of Madeira and the Canaries is the intense interspecific competition due to low carrion density and diversity in these isolated ecosystems, which severely constrains species richness and abundance of the carrion breeding species.

During the last decades we have witnessed a significant increase in the number of blowfly introductions in oceanic archipelagos worldwide due to human-assisted dispersal (Hanski 1977, Báez et al. 1981, Báez 1990, Peck et al. 1998, Jensen et al. 2013). The dramatic increase in the frequency of transport of human commodities to islands (Pombo et al. 2010) is a serious challenge for local authorities that have to deal with the consequences of undesired introductions of pathogens, agricultural and forest pests, and disease vectors such as calliphorid species of veterinary or medical importance (Heath and Bishop 2006). The checklists of Calliphoridae *s. l.* of the Madeira, Azores and Canaries archipelagos show that, as a result of human activities, introduced species are well represented, with six species being common to the three archipelagos and widespread in each of them (Martínez-Sánchez and Rognes 2008, Báez and Oromí 2010, Martínez-Sánchez 2010). For example, the introduction of *Chrysomya megacephala* in Tenerife was followed by its expansion within this island and to other Canary Islands (Gran Canaria, Fuerteventura and Lanzarote), and it was later found also in Madeira (Báez et al. 1981, Báez 1990). The increase in blowfly species introductions has led to the increased homogenization of the faunas of the Macaronesian islands, but its consequences for native biodiversity remain unstudied (Hanski 1977). Thus, efforts should be made to implement monitoring programmes to evaluate the conservation status of native blowfly species in Macaronesia, particularly the Canarian endemic *Calliphora splendens* and the recently discovered *C. loewi*, and assess their vulnerability to introduced species.

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Description of a new species of *Parotocinclus* (Siluriformes, Hypoptopomatinae) from the rio Tapajós basin

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Abstract

A new species of *Parotocinclus* is described from three small tributaries of the rio Tapajós basin, Mato Grosso State, Brazil. The new species can be distinguished from its congeners by presenting the following characters: (1) a triangular dark blotch at the anterior base of the dorsal fin, (2) the absence of an adipose fin but presence of one small platelet at typical adipose-fin region, (3) the abdomen completely covered by dermal plates, (4) a pectoral girdle totally exposed, (5) a single series of bicuspid teeth, and (6) the higher number of bicuspid premaxillary and dentary teeth.

Keywords

Biodiversity, Cascudinhos, freshwater, Neotropical fish, taxonomy

Introduction

The subfamily Hypoptopomatinae currently includes the tribes Otothyriini, Neoplecostomini, and Hypoptopomatini (*sensu* Lujan et al. 2015) and is one of the most diversified and widespread groups of the Neotropical family Loricariidae, with about 210 species according to Eschmeyer and Fong (2016). Within Otothyriini, the genus *Parotocinclus* initially described by Eigenmann and Eigenmann (1889) as a subgenus of *Hisonotus* Eigenmann & Eigenmann, 1889 to include the species *Otocinclus maculicauda* Steindachner, 1887, presents 28 species (Eschmeyer 2016).

Recently, as a result of molecular studies, the genus *Parotocinclus* was recognized as non-monophyletic with several species more related to species assigned to other Otothyriini genera, mainly *Hisonotus*, than to the type species *Parotocinclus maculicauda* (e.g. Cramer et al. 2011; Roxo et al. 2014; Silva et al. 2016). This genus is distributed through almost all hydrographic systems in South America from the Guyana Shield drainages and Amazon Shield tributaries to the coastal drainages of eastern and southeastern Brazil, including the rio São Francisco basin (Sarmiento-Soares et al. 2009; Lehmann et al. 2014, 2015).

Lehmann et al. (2014, 2015) proposed that several species included in *Parotocinclus*, namely: *P. amazonensis* Garavello, 1977; *P. britskii* Boeseman, 1974; *P. collinsae* Schmidt & Ferraris, 1985; *P. eppleyi* Schaefer & Provenzano, 1993; *P. halbothi* Lehmann, Lazarotto & Reis, 2014; *P. longirostris* Garavello, 1988; *P. polyochrus* Schaefer, 1988; and *P. variola* Lehmann, Schwambach & Reis, 2015, could be part of a new genus. These authors suggested that this possible new genus could be diagnosed by (1) the presence of a triangular dark blotch at the anterior base of the dorsal fin; (2) a canal cheek plate on the ventral surface of head elongated posteriorly and contacting the cleithrum; and (3) the head and snout being elongated and with a Y-shaped, white or cream colored mark dorsally.

Here, we recognized a new Otothyriini species as a result of collection efforts in the rio Tapajós basin. The new species is described below in the genus *Parotocinclus*, but we will not be surprised if this new entity is reallocated into a new genus in a close future.

Material and Methods

Measurements and counts were taken from the left side. The measurements followed Boeseman (1968) with modifications suggested by Armbruster and Page (1996), except for the folded dorsal-fin length. Furthermore, the following measurements were added: anal-fin spine length, lower caudal spine length, suborbital depth and mandibular ramus. Meristics followed Carvalho and Reis (2009) and Schaefer (1997). All measurements were taken point to point with digital calipers to the nearest 0.1 mm. All samples analyzed are deposited at the **LBP** – Laboratório de Biologia e Genética de Peixes, São Paulo State, Brazil; and **MZUSP** – Museu de Zoologia da Universidade de São Paulo, São Paulo State, Brazil. Abbreviations used throughout the text followed Carvalho and Reis (2009). One specimen of the new species was cleaned and double-stained (c&s) according to the method of Taylor and Van Dyke (1985).

Results

Parotocinclus dani sp. n.

<http://zoobank.org/637C26FF-4E1D-4DA6-A810-F41FCEB1C976>

Fig. 1, Table 1

Holotype. MZUSP 120737, 27.3 mm SL, municipality of Peixoto de Azevedo, Mato Grosso State, small tributary of rio Peixoto de Azevedo, drainage of rio Teles Pires, rio Tapajós basin, 10°23'10"S, 54°18'22"W, 18 August 2007, coll. JLO Birindelli, AL Netto-Ferreira & LM Souza.

Paratypes. All from Brazil, Mato Grosso State, rio Tapajós basin. MZUSP 96785, 126, 17.8–26.7 mm SL, collected with holotype. LBP 22089, 1, 26.9 mm SL, 1 c&cs, 27.3 mm SL, collected with holotype. MZUSP 96194, 18, 16.7–24.7 mm SL, municipality of Paranaíta, rio Teles Pires, 09°27'31"S, 56°29'19"W, 30 September 2007, coll. LM Souza, AL Netto-Ferreira. MZUSP 96225, 5, 17.3–24.1 mm SL, municipality of Paranaíta, rio Teles Pires, 09°25'44"S, 56°32'36"W, 29 September 2007, coll. LM Souza, AL Netto-Ferreira.

Diagnosis. The new species *Parotocinclus dani* can be distinguished from all congeners, except *P. amazonensis*, *P. bidentatus*, *P. britskii*, *P. eppleyi*, *P. longirostris*, *P. polyochrus*, and *P. variola* by one character proposed by Lehmann et al. (2014, 2015): the presence of a triangular dark blotch at the anterior base of the dorsal fin, Fig. 2a (*vs.* absence Fig. 2b). The new species can be distinguished from *P. amazonensis*, *P. britskii*, *P. collinsae*, *P. eppleyi*, *P. halbothi*, *P. longirostris*, *P. polyochrus*, and *P. variola* by the absence of an adipose fin but presence of one small platelet at typical adipose-fin region, Fig. 3 (*vs.* presence of a poorly developed to well-developed adipose fin); from *P. bahiensis*, *P. cearensis*, *P. cesarpintoi*, *P. jumbo*, *P. prata*, *P. robustus*, and *P. spilosoma* by the abdomen completely covered by dermal plates (*vs.* abdomen totally exposed or with few small and dispersed platelets); from *P. cearensis*, *P. cesarpintoi*, *P. jumbo*, *P. prata*, *P. robustus*, *P. spilosoma*, and *P. spilurus* by having the pectoral girdle totally exposed (*vs.* the pectoral girdle medially covered by skin and exposed only laterally); from *P. bidentatus* by the presence of a single series of bicuspid teeth (*vs.* the presence of a series of unicuspid teeth behind the series of bicuspid teeth of the dentary and premaxilla), and by the higher number of bicuspid premaxillary teeth 15–25, mode 21 (*vs.* 6–12, mode 9) and bicuspid dentary teeth 15–22, mode 21 (*vs.* 4–10, mode 7).

Description. Morphometric and meristic data shown in Table 1. Small size Loricariidae, holotype 27.3 mm SL; paratypes 20.7–27.3 mm SL. Dorsal profile in lateral view straight from snout tip to anterior portion of parieto-supraoccipital, slightly convex to dorsal-fin origin. Dorsal-fin base straight, slightly concave and descending from posterior end of dorsal-fin base to caudal peduncle. Ventral profile in lateral view slightly concave from snout tip to anal-fin origin, slightly convex from anal-fin base to caudal-fin origin. In dorsal view body progressively narrowing posteriorly from cleithrum to caudal peduncle and anteriorly to snout tip. Greatest body depth at dorsal-fin origin. Cross-section of body between pectoral and pelvic fins dorsally

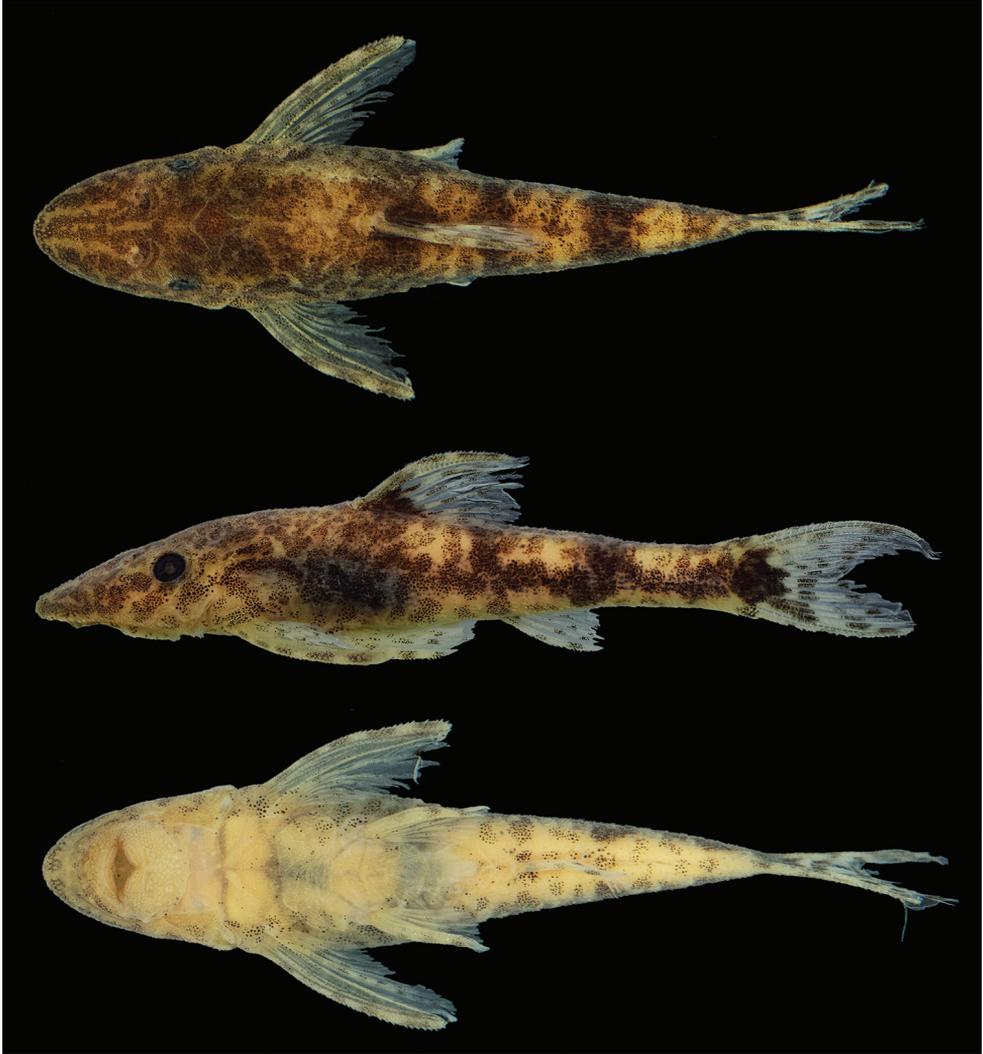


Figure 1. *Parotocinclus dani*, MZUSP 120737, 27.3 mm SL, holotype from small tributary of rio Peixoto de Azevedo, rio Tapajós basin, municipality of Peixoto de Azevedo, Mato Grosso State, Brazil.

upper. Maxillary barbel present and poorly developed. Teeth slender and bicuspid; medial cusp larger than lateral cusp. Left premaxillary teeth 15–25 (mode 21). Left dentary teeth 15–22 (mode 21).

Dorsal fin ii,7; its origin slightly posterior to pelvic-fin origin; when depressed reaching beyond vertical line through anal-fin insertion. Tip of branched dorsal-fin rays reaching vertical line slightly posterior of anal-fin origin. Dorsal-fin spinelet V-shaped, laterally extended; dorsal-fin locking mechanism functional. Pectoral fin i,6; tip of longest pectoral-fin ray almost reaching vertical line through center of horizontal pelvic-fin length when depressed. Pectoral axial slit present between pectoral-fin base

Table 1. Morphometric and meristic data for *Parotocinclus dani* (N = 18).

	Holotype	Range	Mean	SD
SL	27.3	20.7–27.3	24.0	1.93
Percent of SL				
Predorsal length	48.8	45.9–50.1	47.7	1.24
Preanal length	66.2	63.3–67.1	65.6	1.07
Head length	38.0	36.4–46.9	39.1	2.26
Cleithral width	23.4	12.7–26.8	22.4	4.84
Dorsal-fin spine length	25.8	24.0–30.6	26.4	1.68
Base of dorsal fin length	12.2	12.2–15.1	14.0	0.86
Thorax length	17.5	11.6–21.3	14.4	2.16
Pectoral-fin spine length	28.6	27.1–32.8	29.8	1.47
Abdomen length	22.4	19.8–23.5	21.4	1.19
Pelvic-fin spine length	16.7	16.2–21.0	17.9	1.33
Postanal length	26.7	23.6–29.1	26.7	1.26
Caudal peduncle depth	8.4	8.4–9.4	8.8	0.29
Anal width	13.3	12.5–13.8	13.2	0.44
Snout-opercle length	28.0	20.0–30.8	26.8	4.29
Anal-fin spine length	16.2	15.1–18.8	17.0	1.04
Lower caudal spine length	29.7	22.1–30.3	27.1	2.41
Percent of HL				
Head width	62.3	48.0–65.7	59.8	3.91
Head depth	44.9	36.4–49.0	43.6	2.42
Snout length	53.3	43.0–55.6	51.6	2.69
Interorbital width	40.5	31.6–40.8	38.4	2.15
Orbital diameter	12.5	9.7–17.0	14.1	2.06
Suborbital depth	17.8	15.4–20.6	18.7	1.35
Mandibular ramus	10.5	6.9–11.2	9.2	1.40
Meristics				
Lateral plates	24	24–26	25	–
Premaxillary teeth	22	15–25	21	–
Dentary teeth	21	15–22	21	–

rounded and ventrally flat; cross-section of caudal peduncle ellipsoid, round laterally, flat dorsally and ventrally.

Top of head in parieto-supraoccipital region and between orbits convex; superior margin of orbits elevated. Eyes moderately small (9.7–17.0% of HL), and dorsolaterally positioned. Snout pointed and rounded in dorsal view. Nostril small. Body and almost all head plates covered with minute, uniformly sized and evenly distributed odontodes. Absence of tufts of hypertrophied odontodes at posterior medial portion of parieto-supraoccipital or crests on head. Dorsal and ventral anterior margin of snout covered with larger odontodes compared to rest of head. Lips moderately developed and rounded; lower lip far from reaching pectoral girdle. Papillae uniformly distributed on base of dentary and premaxilla, getting smaller distally. Lower lip larger than

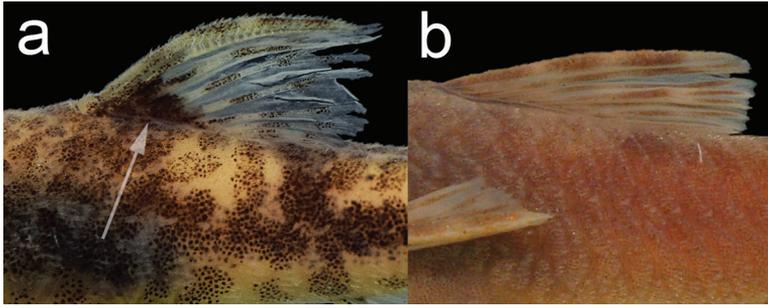


Figure 2. Photographs showing **a** the presence of a triangular dark blotch at the anterior base of the dorsal fin in *P. dani* (arrow), holotype, MZUSP 120737, 27.3 mm SL; and **b** absence of the triangular dark blotch in *P. prata*, holotype, MZUSP 68359, 38.2 mm SL. Photo: CH Zawadzki.

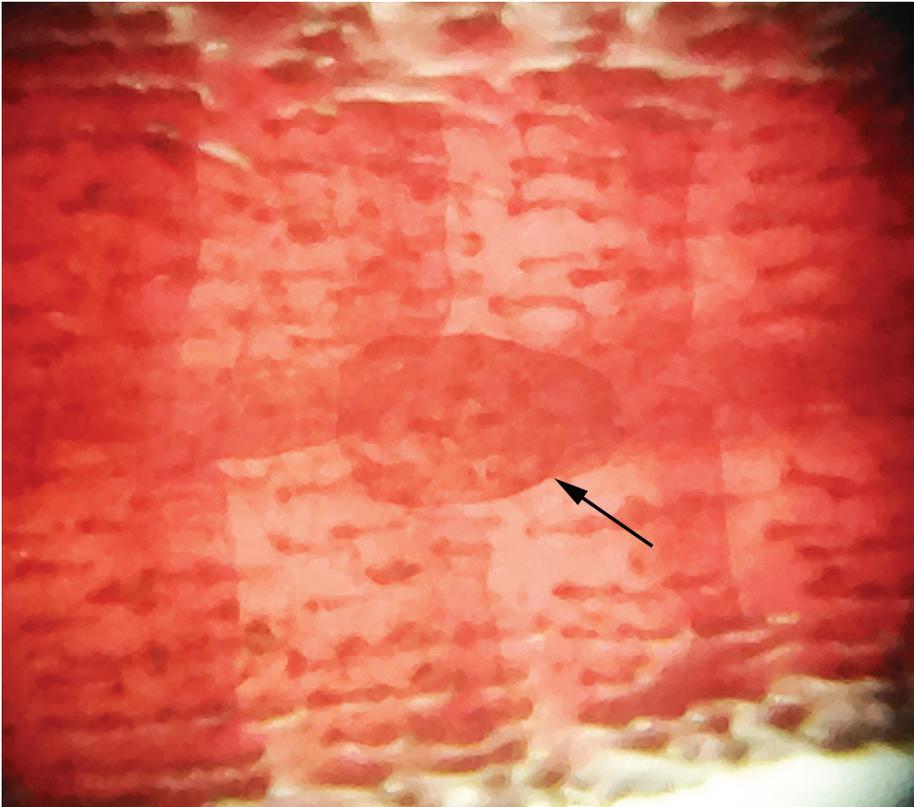


Figure 3. Picture showing (arrow) the single dorsal plate in the caudal peduncle at typical adipose-fin region of *Parotocinclus dani*, LBP 22089, 27.3 mm SL.

and lateral process of cleithrum. Lateral margin of pectoral spine possessing odontodes increasing in size posteriorly. Pelvic fin i,5; tip not exceeding anal-fin origin when depressed. Males with flap along dorsal margin of unbranched pelvic-fin ray, absent

in females. Anal fin i,5; tip of unbranched anal-fin ray reaching 7th to 9th plate from anal-fin origin. Adipose-fin absent but with small unpaired plates in typical adipose fin region. Caudal fin i,14,i; distal margin forked. Lateral plate series formed by 24–26 (mode 25) plates. Lateral line with one or two unperforated plates in line of pores along mid length of body, terminating in two plates preceding last lateral plate. Abdomen completely covered by dermal plates. Cleithrum partly enclosed by ventral lamina of coracoids.

Color in alcohol. Background color dark yellowish-brown in dorsal portion of body and yellowish tan in ventral portion. Dorsal surface of head dark brownish, except for striking V-shaped yellowish tan mark from rostral plate passing through nares to orbital margins. Irregular and conspicuous dark brownish longitudinal stripe along lateral line. Four dark brownish bars crossing dorsum, reaching longitudinal stripe on sides of trunk: first below dorsal-fin origin, second at end of dorsal-fin base, third at adipose fin region, and fourth more inconspicuous at end of caudal peduncle. Dorsal, pectoral, and pelvic fins with dark chromatophores, forming irregular sets of bands: five on dorsal and pectoral fins, three to four on pelvic-fin, and four on anal fin. Dorsal-fin with triangular dark blotch at anterior base. Unpaired plates in typical adipose-fin region yellowish tan. Caudal-fin hyaline, except for one black spot at its origin extending to ventral lobe, and two almost inconspicuous bands. Entire body covered with irregularly distributed chromatophores.

Sexual dimorphism. Adult males can be distinguished from females by presenting two characters: (1) presence of a papilla at urogenital opening (*vs.* papilla absent in females), and (2) unbranched pelvic-fin ray supporting a dermal flap on proximal dorsal surface (*vs.* dermal flap absent in females).

Distribution. The new species is known from three drainages of rio Tapajós in Mato Grosso State, Brazil (Fig. 4). Two from the rio Teles Pires, in the municipality of Paranaíta and from a small tributary of rio Peixoto de Azevedo, in the municipality of Peixoto de Azevedo.

Etymology. The specific name “dani” is in honor of Daniela Fernandes Roxo, FF Roxo’s sister.

Discussion. Lehmann et al. (2014, 2015) proposed that the species *P. amazonensis*, *P. britskii*, *P. collinsae*, *P. eppleyi*, *P. halbothi*, *P. longirostris*, *P. polyochrus*, and *P. variola* should be part of a new genus of Otothyirini based on the following synapomorphies (1) presence of a triangular dark blotch at the anterior base of the dorsal fin, (2) canal cheek plate on the ventral surface of the head elongated posteriorly and contacting the cleithrum, and (3) head and snout elongated and with a Y-shaped, white or cream colored mark dorsally. The first character is apparently conserved and may help to diagnose a new genus within Otothyirini. However, it is also present in *P. bidentatus* (see the holotype picture in Gauger and Buckup 2005, Fig. 5). The second character is absent in *P. dani* and the third character is present not only in *P. dani* and species of this possible new genus proposed by Lehmann et al. (2014, 2015), but also in species of *Hisonotus* – e.g., *H. acuen* and *H. chromodontus*, species of *Curculionichthys* – e.g., *C. luteofrenatus*, *C. parsi*, and species of *Epactionotus* – e.g., *E. bilineatus*, *E. itaimbezinho*

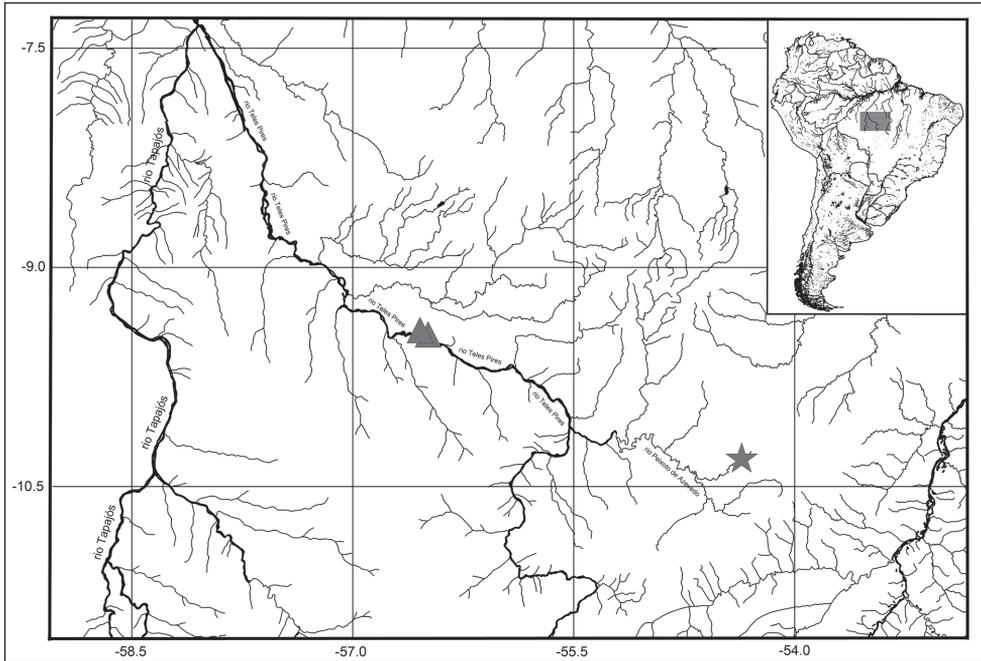


Figure 4. Map of the distribution of *Parotocinclus dani*. Star = holotype locality, affluent of the rio Peixoto de Azevedo. Triangles = paratype localities at the rio Teles Pires. All are drainages of the rio Tajajós basin, Mato Grosso State, Brazil.

and *E. gracilis*. Given the above information, it is clear that new analyses are necessary to recognize this putative new genus more accurately.

Carvalho and Datovo (2012) described a new Otothyrid species, *Hisonotus bockmanni*, from small tributaries of the rio Teles Pires, drainages of the rio Tapajós. This species lacks an adipose fin as the new species *P. dani*, and presents several small platelets at typical adipose-fin region. Furthermore, *H. bockmanni* shows a triangular dark blotch at the anterior base of the dorsal-fin suggesting that this species may also be part of the new genus proposed by Lehmann et al. (2014, 2015). However, we could not examine the clear and stained specimens of *H. bockmanni* to verify if this species presents the first character proposed by Lehmann et al. (2014, 2015), i.e., a canal cheek plate on the ventral surface of the head elongated posteriorly and contacting the cleithrum. *Hisonotus bockmanni* and the new species *P. dani* could be part of the same monophyletic genus and may be closely related. Notwithstanding, *H. bockmanni* can be distinguished from its congeners by the presence of the following characters of coloration pattern proposed by Carvalho and Datovo (2012): (1) the snout with unpigmented, rostrocaudally elongate ellipse anterior to each naris; (2) the dark-brown pigmented pre-dorsal region with five unpigmented white spots arranged as an anteriorly chevron-shaped blotch with three spots anteriorly of dorsal-fin and two posterior spots lateral to and coequal with insertion of dorsal-fin spine; and (3) the caudal-fin lacking pigments on half of membrane and rays.

Comparative material

- Corumbataia cuestae* Britski, 1997: LBP 3688, 3, 28.5–29.9 mm SL, municipality of Botucatu, São Paulo State, upper rio Paraná basin.
- Curculionichthys insperatus* (Britski & Garavello, 2003): LBP 1316, 4 (1 c&s), 23.9–27.7 mm SL, municipality of Botucatu, São Paulo State, rio Tietê basin; LBP 1344, 2, 22.9–24.9 mm SL, municipality of Botucatu, São Paulo State, rio Tietê basin.
- Hisonotus bocaiuva* Roxo, Silva, Oliveira & Zawadzki, 2013: MZUSP 112204, 24.2 mm SL, holotype, municipality of Bocaiúva, Minas Gerais State, rio São Francisco basin; LBP 9817, 9, 4 c&s, 18.3–23.2 mm SL, municipality of Bocaiúva, Minas Gerais State, rio São Francisco basin.
- Hisonotus bockmanni* Carvalho & Datovo, 2012: MZUSP 116430, 2, 16.3–18.5 mm SL, municipality of Paranaitá, Mato Grosso State, rio Tapajós basin.
- Hisonotus francirochai* (Ihering, 1928): LBP 5026, 1, 34.6 mm SL, municipality of Rio Claro, São Paulo State, rio Tietê basin.
- Hisonotus notatus* Eigenmann & Eigenmann, 1889: LBP 3472, 20, 21.0–34.3 mm SL, municipality of Macaé, Rio de Janeiro State, Coastal Drainage.
- Lampiella gibbosa* (Miranda Ribeiro, 1908): LBP 2652, 8, 27.6–34.2 mm SL, municipality of Campinhos, Paraná State, rio Ribeira de Iguape.
- Microlepidogaster dimorpha* Martins & Langeani, 2011: LBP 10683, 2, 28.8–35.6 mm SL, municipality of Uberaba, Minas Gerais State, upper rio Paraná basin.
- Otothyris travassosi* Garavello, Britski & Schaefer, 1998: LBP 1971, 13, 14.0–27.2 mm SL, municipality of Canavieiras, Bahia State, Coastal Drainages.
- Otothyropsis marapoama* Ribeiro, Carvalho & Melo, 2005: LBP 4698, 6, 23.9–36.3 mm SL, municipality of Marapoama, São Paulo State, rio Tietê basin.
- Parotocinclus amazonensis* Garavello, 1977: MZUSP 10145, holotype, 14.6 mm SL, municipality of Coari, Amazonas State, rio Amazonas basin.
- Parotocinclus* cf. *bahiensis* (Miranda Ribeiro, 1918): LBP 7182, 3, 27.9–35.6 mm SL, municipality of Lençóis, Bahia State, Coastal Drainages.
- Parotocinclus longirostris* Garavello, 1988: MZUSP 36891, holotype, 27.8 mm SL, municipality of Manaus, Amazonas State, Amazon basin.
- Parotocinclus maculicauda* (Steindachner, 1877): LBP 2869, 15, 20.2–44.7 mm SL, municipality of Miracatu, São Paulo State, rio Ribeira de Iguape basin, LBP 3181, 1, 40.3 mm SL, municipality of Tapiraí, São Paulo State, rio Ribeira do Iguape basin.
- Parotocinclus prata* (Ribeiro, Melo & Pereira, 2002): MZUSP 68359, holotype, 37.5 mm SL, municipality of Presidente Olegário, Minas Gerais State, ribeirão Quiricó; LBP 11683, 3, 18.6–29.6 mm SL, municipality of Claro de Minas, Minas Gerais State, rio São Francisco.
- Pseudotothyris obtusa* (Miranda Ribeiro, 1911): LBP 898, 17, 23.6–30.9 mm SL, municipality of Cajati, São Paulo State, rio do Queimado.
- Schizolecis guntheri* (Miranda Ribeiro, 1918): LBP 2123, 21, 28.4–36.3 mm SL, municipality of Parati, Rio de Janeiro State, Coastal Drainages, LBP 3546, 77, 20.9–35.8 mm SL, municipality of Ubatuba, São Paulo State, Coastal Drainages.

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Mammals in the MZNA Vertebrate Collection of University of Navarra, Spain

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Abstract

In this paper five datasets are described that provide information about records of mammals in the Vertebrate Collection of the Museum of Zoology of the University of Navarra (MZNA-VERT). The datasets contain 3,466 records belonging to 20 species of mammals sampled across the transition zone between the Atlantic and Mediterranean biogeographical regions (north Iberian Peninsula). The datasets include both distributional data (georeferenced records) and basic biometric data of most of the vouchered specimens stored in the museum facilities. The samples originated mainly within research projects and PhD theses carried out in the former department of Zoology and Ecology of the University of Navarra between 1982 and 2011. The Darwin Core Archive Format datasets are accessible through GBIF.

Keywords

Mammals, occurrence, specimens, biometry, biogeographical regions, Iberian Peninsula

Introduction

Natural History Museums collections are valuable worldwide as they have been documenting biodiversity for centuries, providing primary data for biodiversity research and conservation (Krishtalka and Humphrey 2000, Ponder et al. 2001). Data stored

in museums have been used in a variety of studies investigating climate change, invasive species or changes in biodiversity (Shaffer et al. 1998, Suarez and Tsutsui 2004, Powney and Isaac 2015), but also in education, bringing science close to wider public (Jones 2013), although there are large amounts of locked data still waiting for digitization (Ariño 2010). Moreover, as museums often store sizable numbers of specimens, they are quite useful for taxonomical verification and reuse of metadata, which is most important for groups subject to systematic challenge (Berendsohn et al. 2010, Schilthuizen et al. 2015).

The Museum of Zoology of the University Navarra (MZNA) is a university museum founded in 1980 as the repository of fauna samples originated during research and instructional activities of the former Department of Zoology and Ecology (now integrated into the Department of Environmental Biology). The Museum is a data provider for the Global Biodiversity Information Facility (GBIF) and is an Affiliate to the International Commission on Zoological Nomenclature (ICZN). The Museum is also in charge of the curation and management of the Natural History Collections of the School of Sciences of the University of Navarra. MZNA stores more than two million specimens from a variety of taxa, from invertebrates to vertebrates (e.g. insects, springtails, nematodes, fish and mammals, among many other groups). In this paper we describe the datasets of mammals from the Vertebrate Collection of the MZNA that have been recently shared via GBIF, providing information about more than 3,000 accessions from direct captures and observations. Most of the collections consist of voucher-specimens, a specimen type that is crucial for verifying species identity (Rocha et al. 2014, Schilthuizen et al. 2015). The provenance area is an intensely sampled 10,000 km² zone sitting astride three biogeographical regions, ideally suited for studies on biogeographical transitions and their shifts. Thus, the release of these data series would be of interest to anyone working on environmental change as they allow comparisons between current and past distributional patterns of the biodiversity of mammals at both local and global scales.

General description

Purpose: the Vertebrate Collection of the Museum of Zoology of University of Navarra (2016) contains several subsets of data including mammals, birds and fish. We introduce here data compiled about mammals in the following datasets (Table 1):

Mammals in MZNA-VERT: project “Human impacts in rivers of Navarra” (AHER)

Mammals in MZNA-VERT: biology of *Arvicola sapidus* in Navarra. PhD project, Juan Manuel Garde (ARSA)

Mammals in MZNA-VERT: project “Loza” (BDLZ)

Mammals in MZNA-VERT: project “CAS” (CAS)

Mammals in MZNA-VERT: project “Biodiversity of mammals in Pamplona” (DVPA)

Table 1. Overview of the datasets.

Dataset	Records	Species	Latitude	Longitude	Temporal coverage	Sampling method	Measured specimens
AHER	2278	18	42.76 to 42.8	-1.52 to -1.39W	2001–2003	Trapping, pitfall, pellet analyses	212
ARSA	447	6	42.01 to 42.39N	-1.71 to -1.43W	1983–1992	Trapping, pellet analyses	321
BDLZ	220	8	42.83 to 42.84N	-1.73 to -1.71W	2007	Trapping	2
CAS	201	9	41.84 to 43.37N	-2.51 to -0.67W	1982–1983	Trapping	200
DVPA	342	15	42.76 to 42.87N	-1.73 to -1.56W	2011	Trapping, pellet analyses	2

Project details

Project citation

AHER: Actuaciones humanas en ríos de Navarra. Su incidencia en la conservación de la biodiversidad. Carmen Escala (Principal investigator, data collector), David Galicia (author).

ARSA: not receive funding.

BDLZ: not receive funding.

CAS: Efecto de la explotación y repoblación forestal sobre la fauna del suelo. Rafael Jordana (Principal investigator), Carmen Escala (author).

DVPA: Trabajo de campo para el estudio sobre las especies potenciales en la zona de Pamplona de especies de pequeños mamíferos. Carmen Escala (Principal investigator), David Galicia (author), Enrique Baquero (author).

Data digitization financial support: Acceso en línea a las colecciones de Ciencias Naturales de la Universidad de Navarra – I and II. Arturo H. Ariño (Principal investigator).

Funding

The following institutions supported the projects that produced the field data compiled in MZNA-VERT: Gobierno de Navarra (AHER), Comisión Asesora de Investigación Científica y Tecnológica, Ministerio de Educación y Ciencia (CAS), Ayuntamiento de Pamplona (DVPA). In addition, two grants of the Ministry of Science and Education provided partial support for the data digitization (2005–2006 and 2009–2010).

Study extent description

All data were collected in the province of Navarra, a 10,391 km² region situated in the north of the Iberian Peninsula (Figure 1), between the western end of the Pyrenees and the Ebro basin. Location and topography ensure a wide range of local climates

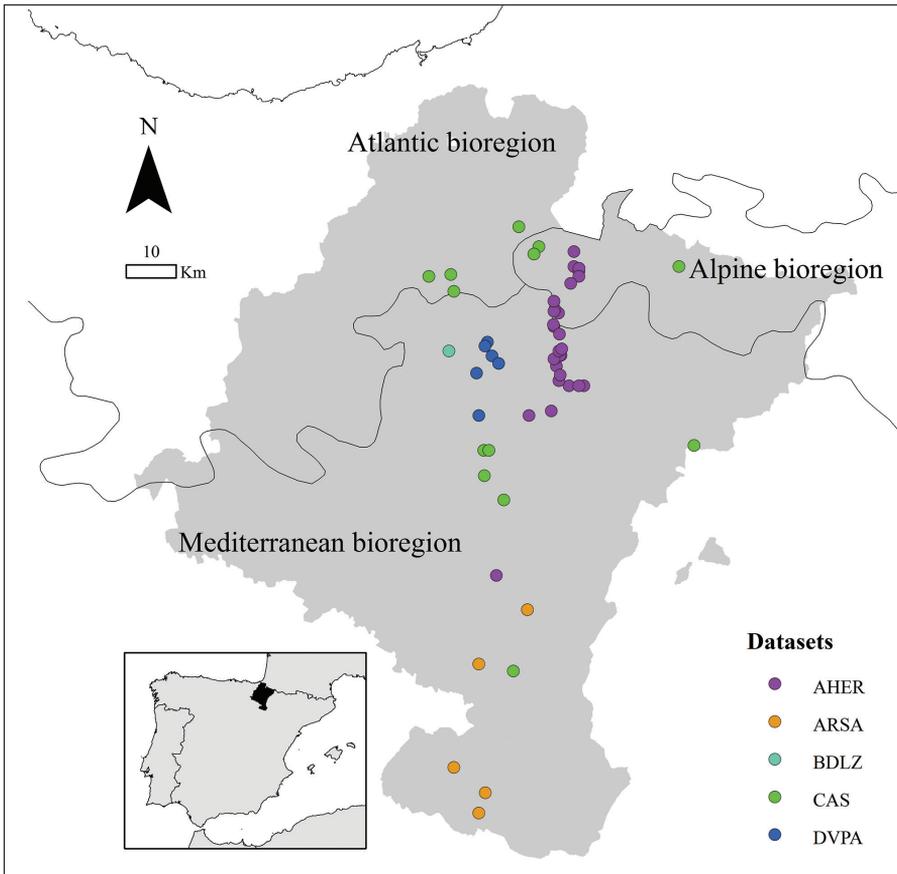


Figure 1. Study area: Navarra (inset, within Spain) and localities for the five datasets (AHER, ARSA, BDLZ, CAS, DVPA).

in Navarra, varying from the oceanic to mediterranean climate (Loidi and Bascónes 1995). The northern half of Navarra belongs to the Eurosiberian region and the rest to the Mediterranean region, which is characterized by summer drought (Rivas-Martínez 1987). The high diversity of flora and fauna in Navarra derives from this sharp transition between bioregions over a relatively small distance of less than 160 km along a north-south axis (Loidi and Bascónes 1995).

Design description

The datasets compile all data provided by separate projects carried out in Navarra by research within the former department of Zoology and Ecology (now Environmental Biology). The resources of these datasets are research projects (BDLZ, CAS, DVPA) and PhD theses (AHER, ARSA).

Taxonomic coverage

General taxonomic coverage: the datasets include information approximately 3,466 records of the orders Carnivora, Rodentia, and Soricomorpha (Figure 2). However, most of the datasets contain data of small mammals belonging to the families Soricidae and Cricetidae. Twenty species of small mammals are represented in the datasets.

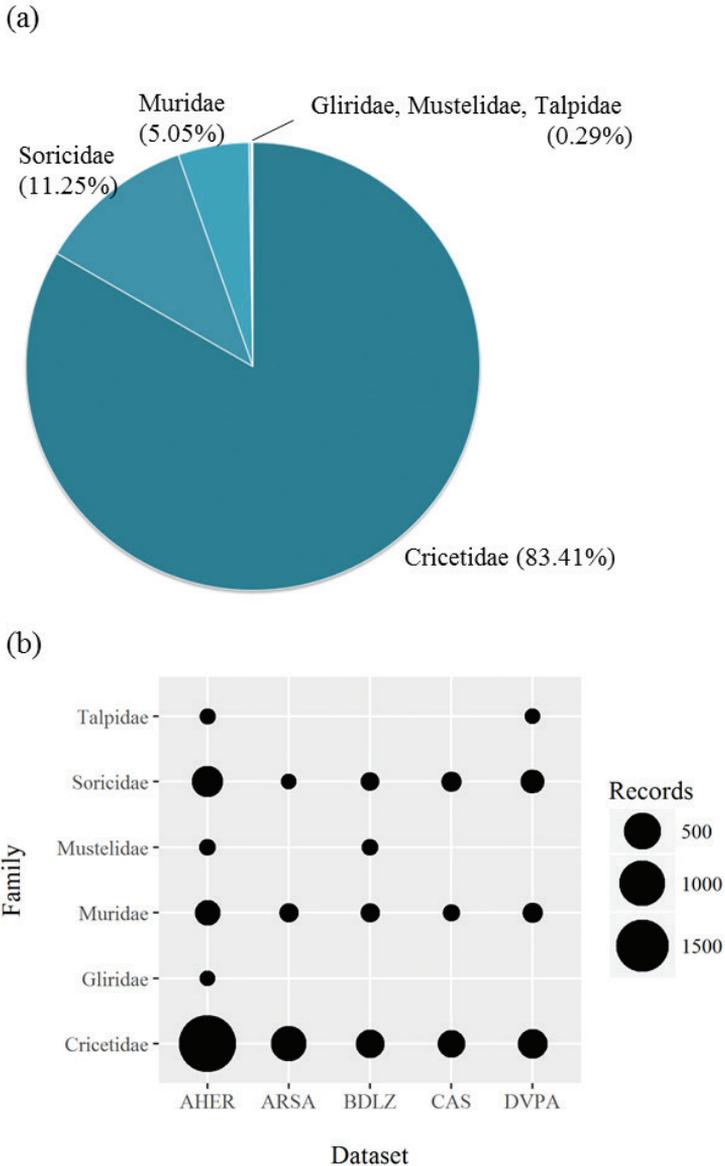


Figure 2. Taxonomic coverage of the datasets by families. Blob size proportional to number of records of each taxon in each dataset.

Taxonomic ranks

Kingdom: Animalia

Phylum: Chordata

Class: Mammalia

Order: Carnivora, Rodentia, Soricomorpha

Family: Cricetidae, Gliridae, Muridae, Mustelidae, Soricidae, Talpidae

Genus: *Apodemus*, *Arvicola*, *Crocidura*, *Eliomys*, *Microtus*, *Mus*, *Mustela*, *Myodes*, *Neomys*, *Rattus*, *Sorex*, *Suncus*, *Talpa*

Species: *Apodemus flavicollis* (Yellow-necked field mouse), *Apodemus sylvaticus* (Long-tailed field mouse), *Arvicola sapidus* (Southern water vole), *Crocidura russula* (Greater white-toothed shrew), *Eliomys quercinus* (Garden dormouse), *Microtus agrestis* (Field vole), *Microtus duodecimcostatus* (Mediterranean pine vole), *Microtus gerbei* (Pyrenean pine vole), *Microtus lusitanicus* (Lusitanian pine vole), *Mus domesticus* (House mouse), *Mus spretus* (Western Mediterranean mouse), *Mustela nivalis* (Least weasel), *Myodes glareolus* (Bank vole), *Neomys fodiens* (Eurasian water shrew), *Rattus norvegicus* (Brown rat), *Rattus rattus* (Black rat), *Sorex coronatus* (Miller's shrew), *Sorex minutus* (Eurasian pygmy shrew), *Suncus etruscus* (Etruscan shrew), *Talpa europaea* (European mole).

Spatial coverage

General spatial coverage: Navarra, north of Spain (Figure 1).

Coordinate box: 41°84'N to 43°37'N Latitude; -2°51'W to -0°67'W Longitude.

Temporal coverage

1982-2011 (see Figure 3).

Methods

All projects were carried out in different areas of Navarra (Spain, Figure 1) and under different sampling designs (see sampling description per dataset).

Study extent per dataset

AHER: The Erro river basin is located in the north of Navarra in the western side of the Pyrenees. Its climate conditions strongly vary from the source of the Erro River, influenced by Pyrenean climate, to its mouth river, where the Mediterranean climate rules.

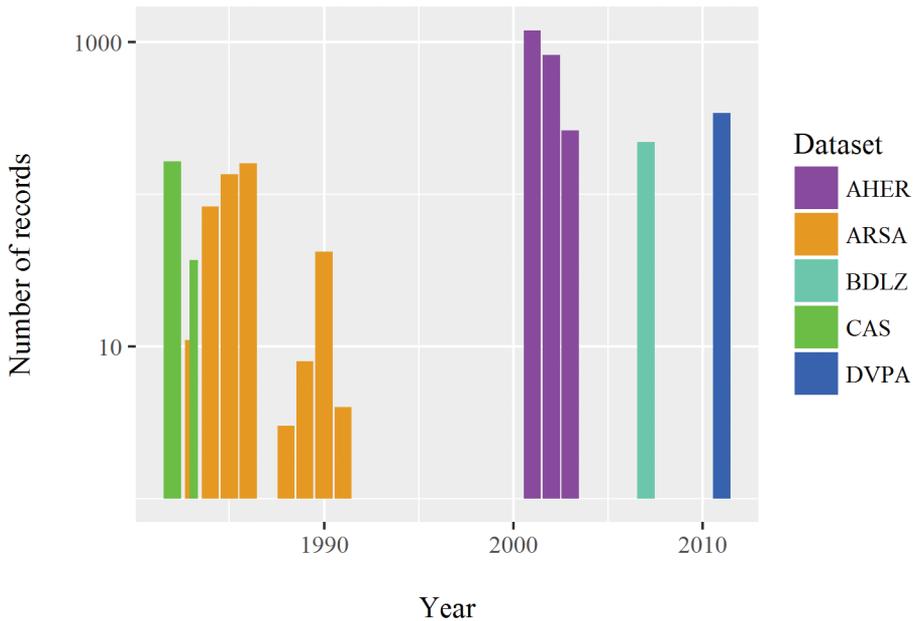


Figure 3. Temporal distribution of the records by dataset. Number of records in log scale.

Rainfall decreases while temperature increases from north to south. The river traverses a wide range of landscapes, which have a positive effect on the flora and fauna diversity. The headwater's area and the middle section of the basin are dominated by oak and beech forests, pastures and forestry exploitation mostly consisting of pine stands. The lower section is dominated by crops and cattle grazing fields (Rivas-Martínez 1987).

ARSA: Ribera Alta is a geographic area located in the south of Navarra dominated by an agricultural landscape mixed with natural vegetation coverage consisting mainly of Mediterranean scrub patches and black pine repopulations (Rivas-Martínez 1987). Surveys took place at the Aragón's riverbank as it passes through the Mélida municipality. The bank has a riparian forest including elms, black poplars, ashes and willow trees. Reed beds in the area provide shelter and a source of food for the water vole.

BDLZ: The Loza pond is situated in Pamplona basin in the centre of Navarra. Pools and ponds play an important role in biodiversity conservation as they afford a stopover plot for migrant birds. Loza stands out as being one of the few semi-natural still water environments of the northern sector in Navarra, and makes one of the first rest places for incoming birds from the north of Europe through the west Pyrenees route. Land cover in Loza is characterised by prairies, scrubland, reed bed patches and some black poplar as well. Crops and pine repopulations enclose the pond (Arizaga et al. 2009).

CAS: This collection is part of a wider project that encompasses the study of soil fauna. Here, only the information related to mammal samplings is reported. In each locality one or two types of habitats were studied: Bardenas Reales (Mediterranean

maquis and Aleppo pine stands), Sansoain (holm oak forest and black pine repopulation), Carrascal (Mediterranean scrub), Bigüezal (Scots pine forest and meadow), Beunza (Japanese larch stands), Quinto Real (Japanese larch stands and meadow) and Irati (beech forest).

DVPA: Pamplona, pop. approx. 200,000, is a city located in the middle of Navarra. The urban area includes several parks which constitute a refuge for wild fauna, especially birds and small mammals. Pamplona is traversed by the Arga River which plays a key role as a natural corridor for fauna. Six sampling locations were chosen to study the small mammal community of the wider Pamplona municipality. Azoz and Zolina are two small villages near Pamplona that were checked for pellets. Azoz is situated in the north near Ezcaba Mountain while Zolina lies to the south of the city. Like Pamplona they are surrounded by crop fields although there are hills covered by pines and oaks. Trapping campaigns took place in the four main biotopes that can be found in Pamplona and its surroundings: riparian forest of Arga River, pine repopulation, Mediterranean scrubland and crop field.

Sampling description per dataset

AHER: The project was conducted between 2001 and 2003. Fifteen sites were sampled seasonally during two consecutive years. Sherman traps (7.5×9×23 cm) baited with bread and oil were used. Each sampling event consisted of two nights with an average sampling effort of 160 traps per night. Specimens were sexed and identified in the field when possible. Some of the specimens captured were transported to the laboratory for further studies. In order to reduce the bias associated with single-type sampling method, eleven sites were additionally sampled using pitfall traps in order to get a better knowledge of the small mammal community. Each sampling site was composed of six cylindrical jars (11.5 cm diameter and 13.5 cm depth) which were active from May to November 2001. Finally, Barn owl pellets, information about footprints and other mammal trails were collected during the sampling period.

ARSA: The main sampling effort occurred from October 1984 to December 1986 once or twice a month. Five sampling sites were selected but most of the material comes from Mérida, specifically from the riparian forest around the Aragón river. Traps were placed in areas where activity of *A. sapidus* was observed (e.g. scats). Each trapping event lasted one day and traps were checked twice a day, at dawn and twilight. Traps were not baited to avoid attracting other species. All specimens captured were brought to the laboratory for further studies.

BDLZ: Sampling was conducted between February and October in 2007 and consisted of live-trapping sessions. Sherman traps (7.5×9×23 cm) were placed along a habitat gradient varying from pastures to scrubland following a line-transect. The distance between traps was approximately 10 metres. Only half of the traps were baited in order to avoid bias due to the baiting. Each sampling event lasted four nights. Traps

were placed on the first night and checked for animals during the next four days. Total sampling effort was 800 traps-night. Specimens were sexed, identified in the field using external morphology and then released. In addition, a few barn owl pellets were collected within the study area during the sampling period.

CAS: Sampling took place between 1982 and 1983. Snap traps were used for sampling the small mammal community. They were active for 15 days. All captured specimens were brought to the laboratory for further studies.

DVPA: The project was carried out in 2011 and consisted of live-trapping samplings and barn owl pellet analyses. Live trapping campaigns were conducted in four locations from June to September (2011). Sherman traps (7.5×9×23 cm) were baited with bread and oil and placed along a line-transect. Each sampling event consisted of two nights and the average sampling effort made was 80 traps per night. Specimens were sexed and identified in the field and then released. Specimens found dead were brought to the laboratory. Barn owl pellets were found in Azoz and Zolina. Churches and barns were checked for barn owl pellets in these villages. When found, they were transported to the laboratory for later identification of the remains of prey items.

Method description

All data provided by the different projects were systematically incorporated to MZNA database (Ariño 1991) and given unique catalogue numbers.

In the case of data obtained from the analyses of barn owl pellets (in AHER, BDLZ, and DVPA datasets), all pellets were processed as follows: pellets were frozen at -20°C for bug removal. Afterwards, they were dissected separating skulls, mandibles and other bones from the rest of remains (e.g. fur, broken bones or insect's remains). Specimens were identified using a stereoscopic microscope and appropriate literature (Gosálbez 1987). Finally, specimens were placed in zip plastic bags with their unique catalogue number and stored in the museum facilities.

All datasets involved trapping surveys. Most specimens were brought to the laboratory for further studies (AHER, ARSA, CAS) or released except for individuals found already dead (BDLZ, DVPA). Some specimens from the datasets were measured and prepared in the laboratory (Table 1). Measurements were taken following rules from Comisión de Biometría (1972). Next, the skin of dead individuals was removed, cleaned with soap and dried using sodium borate, mounted on cardboard and stored in MZNA facilities labeled with their unique catalogue number. Afterwards, skull, mandibles and in some cases skeleton parts were obtained and preserved dry in zip-lock plastic bags with their unique catalogue number. Generally, after preparing the skin, specimens were boiled and flesh was separated from the bones, also using a KOH 0.1% solution if needed. ARSA dataset also contains tissues (e.g. crystalline, testicles, ovaries and histological preparations in slides) conserved in ethanol (ETOH 70%) from specimens of *A.sapidus*.

Quality control description

All specimens were deposited in the Museum of Zoology of the University of Navarra (MZNA, Pamplona, Spain) within its Vertebrate Collection.

All the species were sexed and identified in the field (when possible) and the taxonomic identity of each specimen brought to the laboratory was verified by experienced researchers using suitable literature (Gosálbez 1987).

All datasets have been standardized to Darwin Core standards. First, we checked errors and inconsistencies in the data following the guidelines by Chapman (2005). Scientific names were checked and synonyms exchanged for valid names according to Mammal Species of the World and the atlas of mammals from the Iberian Peninsula (Wilson and Reeder 2005, Palomo et al. 2007). All coordinates in UTM/MGRS were transformed to the geographic system. The uncertainty of coordinates was calculated in metres using the point-radius method. Finally, we checked locality consistency by visual inspection overlapping them against an administrative map of Navarra using GIS (ESRI 2015). All doubtful records were checked and corrected.

Data resources

The datasets are deposited at GBIF, the Global Biodiversity Information Facility, MZNA (2016) Mammals in MZNA-VERT: project “Human impacts in Navarra’s rivers”. v1. University of Navarra, Museum of Zoology. Dataset/Occurrence. http://www.gbif.es/ipt/resource?r=mzna_vert_mast_aher&v=2.1 <http://doi.org/10.15470/gzw8bz>

MZNA (2016) Mammals in MZNA-VERT: biology of *Arvicola sapidus* in Navarra. PhD project, Juan Manuel Garde. v1. University of Navarra, Museum of Zoology. Dataset/Occurrence. http://www.gbif.es/ipt/resource?r=mzna_vert_mast_arsa&v=1.0 <http://doi.org/10.15470/zqgojj>

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MZNA (2016) Mammals in MZNA-VERT: project “CAS”. v1.1. University of Navarra, Museum of Zoology. Dataset/Occurrence. http://www.gbif.es/ipt/resource?r=mzna_vert_mast_cas&v=1.1 <http://doi.org/10.15470/swjzkt>

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Datasets descriptions

Object name

Darwin Core Archive Mammals in MZNA-VERT: project human impacts in rivers of Navarra

Darwin Core Archive Mammals in MZNA-VERT: biology of *Arvicola sapidus* in Navarra. PhD project. Juan Manuela Garde

Darwin Core Archive Mammals in MZNA-VERT: project Loza

Darwin Core Archive Mammals in MZNA-VERT: project CAS

Darwin Core Archive Mammals in MZNA-VERT: project biodiversity of mammals in Pamplona

Character encoding: UTF-8

Format name: Darwin Core Archive format

Format version: AHER (v2.1), ARSA (v1.0), BDLZ (1.2), CAS (v1.1) and DVPA (v1.0).

Distribution: all datasets can be found in <http://datos.gbif.es/collectory/public/show/co82>

Publication date of data: 2016-03-22 (ARSA, DVPA), 2016-04-14 (AHER, BDLZ, CAS).

Language: English

Licenses of use: These datasets are made available under a Creative Commons Attribution Non Commercial (CC-BY-NC) 4.0 License

Date of metadata creation: 2016-03-22 (AHER, ARSA, CAS, DVPA), 2016-04-06 (BDLZ)

Hierarchy level: Dataset

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