RESEARCH ARTICLE



Morphological and molecular characterisation, and phylogenetic position of X. browni sp. n., X. penevi sp. n. and two known species of Xiphinema americanum-group (Nematoda, Longidoridae)

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Abstract

Using ribosomal (18S, ITS1, ITS2, D2-D3 expansion segments of 28S rDNA) and mitochondrial (partial *cox*1 and *nad*4) DNA markers in a study of several populations of *Xiphinema americanum*-group from Europe and Morocco, two cryptic species *X. browni* **sp. n.** (formerly reported as *X. pachtaicum*) and *X. penevi* **sp. n.** were revealed. The species are described, illustrated and their phylogenetic relationships discussed. The first species is most similar to *X. parasimile* and is a member of *X. simile* species complex. The phylogenetic reconstructions inferred from three molecular markers (18S, D2-D3 28S rDNA and *cox*1) showed that *X. penevi* **sp. n.** is part of *X. pachtaicum*-subgroup and is closely related to *X. incertum, X. pachtaicum, X. parapachydermum, X. plesiopachtaicum, X. astaregiense* and *X. pachydermum.* Also, a separate "*X. simile*-subgroup", outside the *X. pachtaicum*-subgroup and so far consisting only of the parthenogenetic species *X. simile, X. parasimile, X. browni* **sp. n.** and probably *X. vallense* was formed. New primers for amplification and sequencing of part of the *nad*4 mitochondrial gene were designed and used.

Keywords

Bayesian Inference, Bulgaria, Cytochrome c oxidase subunit 1, Czech Republic, Morocco, Nicotinamide dehydrogenase subunit 4, phylogeny, ribosomal DNA, Slovakia

Introduction

The Xiphinema americanum-group is a well defined natural complex of species (Lamberti et al. 2000, Coomans et al. 2001, He et al. 2005b) with high significance to agriculture caused by the ability of several species to transmit economically important plant viruses (McFarlane et al. 2002), although there are controversial opinions defining the group (Archidona-Yuste et al. 2016). Even for experienced nematologists species delimitation within this group is challenging because they have rather similar morphology and metrics, and the existing keys (Lamberti et al. 2000, 2004) do not always allow species differentiation and identification. During the last decade wide usage of DNA sequencing in *Xiphinema* taxonomy including this group revealed the existence of a number of cryptic species (Gutiérrez-Gutiérrez et al. 2010, 2012, Archidona-Yuste et al. 2016). This was the case with several populations from the Czech Republic and Slovakia (Kumari et al. 2005, 2010b) originally identified as X. pachtaicum (Tulaganov, 1938) and one population from Morocco provisionally also determined as X. pachtaicum. The objectives of the present study were: i) to characterise populations from the Czech Republic, Slovakia and Morocco both morphologically and genetically; ii) to sequence populations of X. pachtaicum and X. parasimile Barsi & Lamberti, 2004 from Bulgaria for comparison; iii) to clarify phylogenetic relationships of identified species using ribosomal and mithochondrial DNA.

Material and methods

Sampling, nematode isolation and processing

The Xiphinema specimens examined originated from various localities in the Czech Republic (Kurdějov, Mohyla míru and Sokolnice, grapevines), Slovakia (Moča, grapevine), Bulgaria (Balgarene village, pear tree, Vinogradets vicinity, vineyard) and Morocco (Ifrane, holm oak tree). Details of the soil sampling, nematode isolation and processing for Czech and Slovakian populations are given in Kumari et al. (2005, 2010b). A decanting and sieving technique was used for extracting nematodes from soil samples from Bulgaria and Morocco. Xiphinema specimens recovered were heat killed at 55°C for two minutes, fixed in a 4% formalin, 1% glycerol solution, processed to anhydrous glycerol (Seinhorst 1959), and mounted on glass microscope slides. Drawings were prepared using an Olympus BX51 compound microscope with differential interference contrast (DIC). Photographs were taken using an Axio Imager.M2-Carl Zeiss compound microscope with a digital camera (ProgRes C7) and specialised software (CapturePro Software 2.8). Measurements were made using an Olympus BX41 light microscope, a digitising tablet (CalComp Drawing Board III, GTCO CalCom Peripherals, Scottsdale, AZ, USA), and computer Digitrak 1.0f programme (Philip Smith, Scottish Crop Research Institute, Dundee, UK).

DNA extraction, amplification and sequencing

Individual nematodes from Bulgaria, Morocco (DESS-preserved), Czech Republic and Slovakia (1M NaCl-preserved) were mounted on temporary slides containing glass beads and after taking measurements and photomicrographs the slides were dismantled, individual nematodes removed, and added in 0.25 M NaOH to digest overnight and thereafter heated to 99°C for 3 min. Afterwards 10 μ l of 0.25 M HCl, and 5 μ l each of 0.5 M Tris-HCl (pH 8) and 2% Triton X-100 were added and the mixture was incubated for another 3 min at 99°C (Stanton et al. 1998). Finally, the DNA suspension was cooled and the DNA was either used directly for PCR or stored at -20°C until template was needed for PCR reactions. Genomic DNA which was prepared by Kumari et al. (2010b) was also used in this study.

Six regions (18S, ITS1, ITS2, D2-D3 expansion segments of 28S, *cox*1 and *nad*4) of ribosomal and mitochondrial DNA were amplified and sequenced. Primer sequences and references to the primers are given in Table 1. The 18S gene of the Czech population was amplified by using primers SSU_F_04+SSU_R_09 (first fragment), SSU_F_22+SSU_R_13 (second fragment) and SSU_F_23+SSU_R_81 (third fragment). The 18S gene of other populations was amplified by using primer combination 988F+1912R (first fragment) and 1813F+2646R (second fragment).

Initially partial *nad4* gene was amplified with the primers CDF+CDR but only one specimen was amplified using these primers. A pair of new primers (nadpachF+nadpachR) was designed using online software PRIMER 3 (http://frodo. wi.mit.edu/) from the sequences which were amplified by (CDF+CDR). For final analysis all specimens and populations of *X browni* sp. n. from the Czech Republic and Slovakia were amplified and sequenced by using nadpachF + nadpachR primers.

The PCR reaction was performed in 25 µl total volume containing 1 PCR bead (GE Healthcare, Buckinghamshire, UK), 20.5 µl double distilled sterile water, 2.0 µl of each primer (10pmol/µl) (synthesized by Generi Biotech, Hradec Králové, Czech Republic), and 0.5 µl of DNA added as a template for PCR. A negative control (sterilized water) was included in all PCR experiments. The cycling profile for all ribosomal DNA and mtD-NA markers was as described by Kumari and Subbotin (2012) and by He et al. (2005a), respectively. All PCR reactions were performed in a DNA Engine PTC−1148 thermal cycler (Bio-Rad). Aliquots of PCR were analysed by gel electrophoresis and the remaining products were purified using High Pure Product Purification kit (Roche Diagnostics GmbH, Mannheim, Germany) and sequenced in both directions using each primer pair one forward and one reverse (Macrogen, Netherlands). SequencherTM 4.8 (Genes codes. Corp., Ann Arbor, MI, USA) was used to assemble and view each sequence and check for base-calling errors. Accession numbers of all sequences are given in Table 2.

Sequence and phylogenetic analyses

A BLAST (Basic Local Alignment Search Tool) search at NCBI (National Center for Biotechnology Information) was performed using the obtained sequences as queries

Gene	Primer name	Direction	Primer sequence 5' - 3'	Reference
18S	SSU_F_04	forward	GCT TGT CTC AAA GAT TAA GCC	Blaxter et al. (1998)
18S	SSU_R_09	reverse	AGC TGG AAT TAC CGC GGC TG	Blaxter et al. (1998)
18S	SSU_F_22	forward	TCC AAG GAA GGC AGC AGG C	Blaxter et al. (1998)
18S	SSU_R_13	reverse	GGG CAT CAC AGA CCT GTT A	Blaxter et al. (1998)
18S	SSU_F_23	forward	ATT CCG ATA ACG AGC GAG A	Blaxter et al. (1998)
18S	SSU_R_81	reverse	TGA TCC WKC YGC AGG TTC AC	Blaxter et al. (1998)
18S	988F	forward	CTC AAA GAT TAA GCC ATG C	Holterman et al. (2006)
18S	1912R	reverse	TTT ACG GTC AGA ACT AGG G	Holterman et al. (2006)
ITS1	pXb101	forward	TTG ATT ACG TCC CTG CCC TTT	Vrain et al. (1992)
ITS1	ChR	reverse	ACG AGC CGA GTG ATC CAC CG	Cherry et al. (1997)
ITS2	WDF	forward	AGA CAC AAA GAG CAT CGA CT	Kumari et al. (2009)
ITS2	pXb481	reverse	TTT CAC TCG CCG TTA CTA AGG	Vrain et al. (1992)
D2-D3	D2A	forward	ACA AGT ACC GTG AGG GAA AGT TG	Nunn (1992)
D2-D3	D3B	reverse	TCG GAA GGA ACC AGC TAC TA	Nunn (1992)
cox1	COIF	forward	GAT TTT TTG GKC ATC CWG ARG	He et al. (2005a)
cox1	XIPHR2	reverse	GTA CAT AAT GAA AAT GTG CCA	Lazarova et al. (2006)
nad4	CDF	forward	AAA AAG ATG GTA TTG GAG	Kumari and Cesare (2013)
nad4	CDR	reverse	GCA CAT GTA GAA GCT AGT	Kumari and Cesare (2013)
nad4	nadpachF	forward	ATA GAA GCA TTA CCA ACT A	This study
nad4	nadpachR	reverse	TAG TAC CAG AGG ATC AAT A	This study

Table 1. Primers used to amplify ribosomal and mitochondrial DNA.

to confirm their nematode origin and to identify the most closely related nematode sequences. Sequences revealing high similarity to those obtained here were included in the phylogenetic analyses of both ribosomal and mitochondrial gene regions (Neilson et al. 2004, Oliveira et al. 2004, He et al. 2005b, Gozel et al. 2006, Holterman et al. 2006; Lazarova et al. 2006, Kumari et al. 2009, Gutiérrez-Gutiérrez et al. 2010, Kumari et al. 2010a, Kumari et al. 2010b, De Luca and Agostinelli 2011, Gutiérrez-Gutiérrez et al. 2011a, Gutiérrez-Gutiérrez et al. 2011b, Meza et al. 2011, Sakai et al. 2011, Gutiérrez-Gutiérrez et al. 2012, Kumari and Subbotin 2012, Sakai et al. 2012, Kumari and Cesare 2013, Tzortzakakis et al. 2014, Getaneh et al. 2015, etc). Sequence numbers are presented in the trees. The multiple sequence alignments (MSA) of all datasets were performed using the GUIDANCE2 Server available at http://guidance.tau.ac.il/ (Sela et al. 2015). All three alignment algorithms (MAFFT, PRANK and ClustalW) were tested and the MSAs having highest alignment confidence scores were used for ITS phylogenetic reconstructions. Subsequently, the MSAs were manually optimised and trimmed using MEGA 6 (Tamura et al. 2013). The phylogenetic reconstructions were performed using the Bayesian Inference (BI) algorithm implemented in MrBayes 3.2.5. (Huelsenbeck and Ronquist 2001; Ronquist et al. 2012) using the General Time Reversible model plus Gamma distribution rates (GTR + G). The Bayesian MCMC tree searches were run using default heating parameters for 2 000 000 generations with a sample frequency of 1000 generations. The first 25% of the chains discarded as burning and the remaining 75% trees kept to summarise the tree topology, branch lengths,

Species		Xiphinema browni sp. n			X. pachtaicum	X. penevi	
							sp. n.
Country		Czech Republic		Slovakia	Bulgaria	Bulgaria	Morocco
Locality	Kurdějov	Mohyla míru	Sokolnice	Moča	Balgarene	Vinogradets	Ifrane
Isolate	NSB1	NSB2	NSB3	NSB4	NSB5	NSB6	NSB7
18S	KU250135	KU250136	KU250137	KU250138	KU250139	KU250140	KU250141
18S+ITS1	KU250142	KU250143	KU250144	NA	NA	NA	NA
5.8S+ITS2+28S	KU250145	KU250146	KU250147	KU250148	KU250149	NA	KU250150
D2/D3	KU250151	KU250152	KU250153	KU250154	KU250155	KU250156	KU250157
cox1	GU222424*	*	*	KU250158	NA	KU250159	NA
nad4	KU250160	KU250161	KU250162	KU250163	NA	NA	NA

Table 2. NCBI accession numbers of representative individual specimens for ribosomal and mitochondrial DNA.

* Kumari et al. (2010); NA = not acquired

and posterior probabilities (PP) of branch support. Convergence diagnostic values were calculated every 1000 generations with a predefined stop value equal to 0.01. A single strict consensus tree was visualised using FigTree v1.4.2 graphical viewer. Posterior probabilities values of \geq 0.80 were considered as credible support values for nodes.

Taxonomy

Xiphinema browni sp. n.

http://zoobank.org/E385F7F7-2C78-4D54-BC57-0D24EDD43CB8 Figures 1–8, 15–18

Xiphinema pachtaicum (Tulaganov, 1938) Kirjanova, 1951 apud Kumari et al. 2005, syn. n.

Measurements. See Tables 3-5.

Description. *Females.* Body slender C to open spiral shaped. Cuticle with fine transverse striae. Thickness of the cuticle at postlabial region 1–1.5 μ m, 1.5 rarely 2 μ m at mid-body and 2 μ m at post-anal region. Labial region set-off from the rest of the body by a constriction, expanded, rounded laterally, 5.0±1.1 (4–7) μ m high. Amphideal fovea hardly visible, funnel-shaped, its opening *c*. 5 μ m (50%) wide visible posterior the constriction level. Distance between first and second guide ring in specimens with retracted odonostyle 5–10 μ m long. Odontophore with moderately developed basal flanges 6.1±0.6 (5.5–7) μ m wide. A small vestigium observed occasionally in slender part of pharynx. Pharyngeal characters presented at Table 4. Dorsal pharyngeal gland nucleus 2 μ m diam. Ventrosublateral nuclei barely visible. Rectum 20.8 ± 1.5 (18–23) μ m, n=7, or *c* 1.3 times anal body diameter. Reproductive system amphidelphic, symbiont bacteria present in the ovaries. Separate uteri and ovejector present (Table 5), oviduct 90.5±13.0 (68–101) μ m; vagina bell-shaped 39.5% of the corresponding body width (33–50%, n=14), vulva post-equatorial. Numerous sperm observed in one female from Kurdějov (Figs 2B, 4B). Tail conical, dorsally convex, ventrally straight or slightly concave with narrowly rounded to pointed terminus. Two pairs of caudal pores.

Male. Very rare. One specimen found in Sokolnice population. Male similar to the female with posterior region more strongly curved. Lip region and tail shape as in females, differences were observed within body width and tail length, which reflected **a** and **c'** values. Spicules robust, slightly curved, lateral guiding piece 7 μ m long. Adanal pair preceeded by a row of 5 irregularly spaced supplements, the two anteriormost weakly developed. Tail conoid, ventrally straight, dorsally convex with pointed terminus, caudal pores not visible. The slide of the only male specimen, described by Kumari et al. (2005), was subsequently damaged.

Juveniles. The scatter diagram based on functional and replacement odontostyle, and body length revealed the presence of four juvenile stages (Fig. 8). Tail shape and length similar in all stages and females with **c'** slightly decreasing in successive stages (Kumari 2005, Fig. 3, Table 3).

Type locality and plant association. Kurdějov, Břeclav County, South Moravia, Czech Republic, associated with grapevine. Other localities: Mohyla míru, Brno-Venkov County, South Moravia, the Czech Repbulic, in the rhizosphere of apple trees; Sokolnice, Brno-Venkov County, South Moravia, the Czech Repbulic, in the rhizosphere of grapevine; Moča, Komárno County, Nitra, Slovak Republic, in the rhizosphere of grapevine.

Type material. The holotype, 9 paratype females and juveniles from all stages are deposited in the nematode collection of the Institute of Biodiversity and Ecosystem Research, Sofia, Bulgaria. Other paratypes deposited as follows: 15 females in the Crop Research Institute, Prague, the Czech Republic; 5 females in the USDA Nematode Collection, Beltsville, Maryland, USA; 5 females in the Nematode Collection of the Institute of Plant Protection, Bari, Italy; 5 females in the Wageningen Nematode Collection (WANECO), Wageningen, the Netherlands. The ribosomal and mtDNA sequences (18S rDNA, ITS1, ITS2, D2-D3, *cox1*, *nad*4) of *X. browni* sp. n. are deposited in GenBank (for accession numbers see Table 2).

Sequence and phylogenetic analyses. There was no sequence variation between populations for 18S and D2-D3, ITS1 and ITS2 rDNA regions of *X. browni* sp. n. Of all four populations studied *cox*1 region of three population from the Czech Republic (Kurdějov, Mohyla Míru, Sokolnice) were sequenced by Kumari et al. (2010b) and all populations were identical therefore only one population was submitted to Gen-Bank (accession number GU222424). The Slovakian population was sequenced in this study and it was identical to previously published sequence of Kurdějov the population identified as *X. pachtaicum* (GU222424, Kumari et al. 2010b). All four sequenced populations were also identical for *nad*4 part.

BLAST at NCBI using 18S and D2-D3 region sequences as queries revealed highest similarity (99 and 87%) to the corresponding sequences of *X. simile* Lamberti, Choleva & Agostinelli, 1983 from Serbia (AM086681) and two Spanish populations of *X. opisthohysterum* Siddiqi, 1961 (JQ990040 and KP268967), respectively. The es-



Figure I. *Xiphinema browni* sp. n. Female: Variations in: **A–C** Anterior end **D–F** Pharyngeal bulbus **G–I** Tail shape **A, D, G** Kurdějov (type population) **B, F, I** Mohyla míru **C, E, H** Sokolnice. Scale bars: 25 μm



Figure 2. *Xiphinema browni* sp. n. Female: Variations in genital system: **A**, **B** Anterior genital branch **C** Posterior genital branch **D**–**F** Region of vagina and uteri **A**, **B**, **D** Kurdějov (type population) **C**, **F** Mohyla míru **E** Sokolnice. Scale bars: 25 μm.

timated divergences (p-distance) between the 18S rDNA sequences of the new species and the closest species, *X. parasimile* from Bulgaria (this study) and *X. simile* from Serbia (AM086681) were 0.3 (6 nt) and 1.2% (21 nt), respectively. Again, the new D2-D3



Figure 3. *Xiphinema browni* sp. n. **A–C** Entire body (**A**, **C** females **B** male); Female: **D–F** Anterior ends **G–I** Pharyngeal bulbus **J–M** Tail shape variation **A**, **F**, **I**, **M** Mohyla míru **B**, **E**, **H**, **L** Sokolnice **C**, **D**, **G**, **J**, **K** Kurdějov (type population). Scale bars: (**A–C**) 400 μm; (**D–M**) 30 μm.



Figure 4. *Xiphinema browni* sp. n. Female: **A–D** Genital system (**B** uterus full with sperm) **E–G** Labial region (**E** Amphid **F** Female **G** Male) **H** Ovary with endosymbionts **I**, **K–N** Variations in vagina **J** Lateral field **A**, **B**, **H**, **I**, **J** Kurdějov **C**, **G** Sokolnice **D**, **K**, **M** Mohyla míru **E**, **F**, **L**, **N** Moča. Scale bars: 30 μm (**A–D**, **H–J**); 12 μm (**E–G**, **K–N**).



Figure 5. *Xiphinema browni* sp. n., Sokolnice. Male: **A** Anterior end **B** Pharyngeal bulbus **C** Posterior end **D** Spicules. Scale bars: 25 μm



Figure 6. *Xiphinema browni* sp. n., Kurdějov. Juveniles and female: **A–E** Anterior ends of first- to fourth-stage juveniles and female **F–J** Tails of first- to fourth- juvenile stages and female. Scale bar: 25 µm



Figure 7. *Xiphinema browni* sp. n. Kurdějov. Juveniles and female: **A–E** Anterior ends of first- to fourthstage juveniles and female **F–J** Tails of first to fourth juvenile stages and female (G1 and G2 – secondstage juvenile). Scale bar: 30μm.



Figure 8. Scatter plot of odontostyle (■) and replacement odontostyle (□) against body length of *Xiphinema browni* sp. n. juveniles and females from Kurdějov population.

Table 3. Morphometrics of *Xiphinema browni* sp. n (localities in the Czech Republic and Slovakia) and *X. pachtaicum* (Bulgaria). All measurements in micrometres, except ratios given as mean \pm standard deviation (range).

			X. pachtaicum				
Locality		Kurdějov	Sokolnic	ce	Mohyla míru	Moča	Balgarene
Plant host		grapevine	grapevin	ie	apple	grapevine	pear
n	Holotype	50 females	20 females	male	12 females	4 females	6 females
L	1904	2031±123	1886±89	1849	1972±90	1715±142	1735±232
		(1798–2408)	(1751–2099)		(1785–2079)	(1603–1922)	(1522–2015)
a	57.8	69.3±5.16	60.5±4.4	73.9	60.1±3.14	69.5±6.57	58.7±4.9
		(56.9-81.3)	(52.3–69.9)		(55.6–64.5)	(63.6–76.3)	(53.3–65.7)
b	6.7	7.3±0.76	6.9±0.38	6.9	7.0±0.32	8.2, 6.8	5.9±0.5
		(6.1-8.7)	(6.4–7.9)		(6.4–7.4)		(5.3-6.4)
С	64.8	69.9±6.22	65.8±5.71	54.4	64.9±3.41	61.6±8.72	58.2±8.3
		(54.7-83.0)	(56–79.6)		(58.5–70.3)	(53.4–73.9)	(50.9–66.3)
c'	1.9	1.78±0.12	1.82±0.14	1.89	1.8±0.08	1.8±0.17	1.7±0.1
		(1.53-2.07)	(1.61-2.13)		(1.6–1.9)	(1.5 - 1.9)	(1.6–1.8)
V/Spicule	56.1	55±1.30	55±1.71	29.0	55.4±1.15	55.5±1.16	58.6±1.4
length							
		(52.3–58.5)	(49–57)		(53.8–58.1)	(53.8–56.4)	(57.0-60.4)
Odontostyle	84	83±2.2	79±2.6	76	82±3.39	77±4.69	84.2±3.7
		(78–86)	(74–83)		(73–85)	(72-81)	(78-88.5)
Odontophore	43	42±1.69	41±0.91	38	43±1.88	38±3.30	48.9±2.1
		(38–48)	(39–43)		(39–46)	(35–42)	(46–51)
Oral	72	71±2.56	68±2.35	67	71±1.68	66±5.06	76.8±3.4
aperture to							
guide ring		(65–75)	(63–72)		(67–73)	(60–72)	(73–80)
Tail length	29	29±1.94	29±2.24	34	30±0.82	28±1.63	29.8±0.9
		(25–33)	(24–32)		(29–32)	(26–30)	(28–30)
Length of	8	8±1.28	8±1.22	10	8±0.68	8±1.41	8.7±1.0
hyaline							
part		(6–12)	(6–10)		(7–9)	(7-10)	(8-10)
Body diam.	8	8±0.58	8±0.51	9	9±0.43	8±0.50	8.8±0.2
at:							
- lip region		(8-10)	(8–9)		(8.5–10)	(7-8)	(8.5–9)
- guiding ring	22	20±0.67	19±0.49	19	22±1.44	19±1.41	21.5±1.0
		(19–21)	(19–20)		(19.5–24)	(18–21)	(20.5–23)
- base of	29	26±1.58	26±2.41	23	28±1.69	23.1, 23.8	26.5±1.2
pharynx							
		(22–32)	(19–20)		(25–30)		(25–28)
- mid body	33	29±2.78	31±2.58	25	34±2.66	25±1.89	28.9±2.1
		(25–38)	(26–37)		(29–38.5)	(22–26)	(26–32)
- anus	16	16±0.97	16±0.92	18	17±0.89	16±0.96	17.1±1.0
		(14–19)	(15–18)		(16–19)	(15–17)	(16–19)
- beginning	7.5	7±1.11	7±0.62	8		8±1.50	8.7±0.0
of							
hyaline part		(5–10)	(6–8)			(6–9)	(9–9)

sequence of *X. parasimile* from Bulgaria was most similar (p-distance = 4.6%), followed by the Serbian populations of *X. parasimile* (p-distance = 7.6–7.9%, calculated for D2 region only) and various populations of *X. simile* (14.1–14.7%). The partial *cox*1 sequences of *X. browni* sp. n revealed highest similarity to *X. simile* from Slovakia (AM086708). Surprisingly, these two species showed very high similarity 99% (2 nts difference) in *cox*1 sequences and higher dissimilarity in 18S rDNA (p-distance = 1.2%, 21 nts). Other authors (Gutiérrez-Gutiérrez et al. 2012) have also reported similar observation namely, 100% identity in *cox*1 part of two different species *X. duriense* Lamberti, Lemos, Agostinelli & D'Addabo, 1993 (JQ990053) and *X. opistohysterum* (JQ990054) and clear separation in D2-D3 28S sequences (or 96 % identity). Further, the *cox*1 sequences of *X. browni* sp. n. and the closest species *X. parasimile*, *X. simile* (GU222425, Czech Republic) and *X. pachtaicum* (HM921369, Spain) were translated to amino acids and aligned (Fig. 9). The estimated p-distances between *X. browni* sp. n. and the three species were 10.1%, 21.7% and 23.3%, respectively.

In all three phylogeny reconstructions (18S, D2-D3 and cox1) X. parasimile from Bulgaria was a sister species of X. browni sp. n. and both species were part of a well supported clade with other European populations of X. simile (Figs 10-12). The recently described species X. vallense Archidona-Yuste, Navas-Cortes, Cantalapiedra-Navarrete, Palomares-Rius & Castillo, 2016 presented only with D2-D3 and ITS1 rDNA sequences seems also to be evolutionary very closely related (Figs 11 and 13), however amplifying additional sequences for other molecular markers (e.g. 18S and cox1) could help to better clarify its relationships. The position of the new species in the phylogeny trees based on ITS1 and ITS2 sequences was unstable (Figs 13 and 14). The analyses resulted in various tree topologies when using different alignment algorithms and reconstruction methods (ML and BI) and because of the absence of homologous sequences from closely related species. In most cases X. browni sp. n. was part of a clade of European X. americanum-group species considered as group II in a previous publication (Archidona-Yuste et al. 2016). Due to insufficient number of nad4 sequences of species belonging to the X. americanum-group at NCBI no phylogenetic reconstructions are presented.

Diagnosis and relationships. *Xiphinema browni* sp. n. is characterised by a unique combination of traits: slender and medium sized body (1.6–2.41 mm) and odontostyle (73–85 μ m), lip region expanded, laterally rounded, separated from the rest of body by a constriction, post-equatorial vulva position (V=52–58 %), symbiotic bacteria present, female tail conical dorsally convex, with narrow rounded to pointed tip, 24–35 μ m long, (c=53.4–86.8; c'=1.5–2.1), and specific ribosomal and mtDNA sequences (Table 2). The alpha-numeric codes based on average values (ranges given in parentheses) using the polytomous key by Lamberti et al. (2004) are: A3 (2), B3 (2), C3 (4), D2 (1/3), E2 (3), F2 (1/3), G2, H1, I2 (1/3).

Species having similar morphometrics to *X. browni* sp. n. based on type populations are presented in Table 6. Recently described species *X. parasimile, X. parabrevicolle* Gutiérrez-Gutiérrez, Cantalapiedra-Navarrete, Decraemer, Vovlas, Prior, Palomares-Rius & Castillo, 2012, *X. parapachydermum* Gutiérrez-Gutiérrez, Cantalapie-

	Xipl	binema browni	sp. n.	Xiphinema pachtaicum	<i>Xiphinema</i> penevi sp. n.	<i>Xiphinema</i> Lazarova e	<i>parasimile</i> t al. (2008)
Locality	Kurdějov,	Sokolnice,	Mohyla míru,	Balgarene,	Ifrane,	Vinogradets,	Ralja, Serbia
Character	Czech Republic	Czech Republic	Czech Republic	Bulgaria	Morocco	Bulgaria	Paratypes
n	50	20	5	5	6	14	5
Pharynx length	278.0±17.9	272.0±12.1	271.4±20.1	303.5±13.5	274.4±29.2	250.5±21.0	282.2±2.1
(µm)	(236-309)	(247-297)	(234–294)	(291-317)	(229-308)	(233-311)	(260-310)
Bulbus length	60±3.48	59±3.02	60±2.88	75, 77, 80	68.4±2.7	59.8±3.5	61.6±4.8
(µm)	(53–69)	(56-67)	(56-63)		(65–72)	(55.5–68)	(56-63)
Bulbus width	13±1.30	13±1.21	14±1.14	15, 15, 16	13.6±0.8	12.0±0.6	14.4±0.5
(μm)	(9-16)	(11–16)	(12–15)		(12-14)	(11–13)	(14–15)
Bulbus length/	21.7± 1.7	21.8±1.6	22.3±1.3	25.6±07		24.2±2.3	21.8±0.7
Pharynx length	(17.4–27.1)	(19.5–27.1)	(20-24)	(25–26)		(19–28)	(20.7–22.6)
(%)						n=11	
DN* (%)	17.5±1.9	13.1±2.3	12.5, 13.0,	9.3, 10.3	11.4±1.4	16.7±3.3	16.5–17.7
	(15.3–21.1)	(12.7–17.3)	14.9		(9.9-12.9)	(13.6–18.6)	
	n=6	n=5				n=8	
DO* (%)	10.9±1.7	7.9±3.8	11.9±1.8	11.5, 12.0	12.1±1.6	11.1, 13.6	11.6-14.6
	(8.8-13.8)	(5.5–15.6)	(8.8-13.3)		(9.9-13.2)		
	n=6	n=5	n=5				
SVN1* (%)		53.9±1.6	55.6, 54.4	60.3	56.7±2.0		55.3-59.7
		(51.8-55.0)			(53.8–58.8)		
SVN2* (%)		53.2			58.7±2.9		57.3-60.1
					(55.4–61.0)		
SVO (%)	74.2±1.9	74.9±3.3	68.5, 71.1,	72.0, 74.4	75.4±2.4		
	(71.4–75.4)	(67.6–76.4)	71.8		(73.5–79.4)		
	n=4	n=5					
Glandularium	48.5±1.9	50.6±2.3	53, 48, 46	68, 70, 70	61.9±3.1	49.9±1.4	52.3±2.2
** (µm)	(46–52)	(48-51)			(57–65)	(48–52)	(52–56)
	n= 8	n=5			n=8	n=8	

Table 4. Pharyngeal characters of females of *Xiphinema americanum* group species studied from different localities.

Terminology adopted by Loof and Coomans (1972)*; and Andrássy (1998)**.

dra-Navarrete, Decraemer, Vovlas, Prior, Palomares-Rius & Castillo, 2012, *X. paratenuicutis* Gutiérrez-Gutiérrez, Cantalapiedra-Navarrete, Decraemer, Vovlas, Prior, Palomares-Rius & Castillo, 2012, *X. plesiopachtaicum* Archidona-Yuste, Navas-Cortes, Cantalapiedra-Navarrete, Palomares-Rius & Castillo, 2016 and *X. vallense* (Barsi and Lamberti 2004, Gutiérrez-Gutiérrez et al. 2012, Archidona-Yuste et al. 2016) have also been compared. Six of these species have non-European distribution (Table 6) whereas the others were described from and/or found mainly in Europe. *Xiphinema simile* was also included in the table comparing morphometrical data because of the close relationships based on sequence and phylogenetic analyses and its wide distribution in many European countries.

Based both on morphology and molecular data *X. browni* sp. n. is most similar with *X. parasimile*, *X. simile* and *X. vallense*. Morphologically, it can be distinguished from:

	Characters Locality	Anterior uterus	Posterior uterus	Ovejector	Vagina length	Pars distalis vaginae	Pars proximalis vaginae
	Kurdějov	42.1±5.7 (35–54) n=8	38.9±5.0 (31–43) n=7	26	12.9±1.4 (11–15) n=9	6.7±1.1 (5.5–8.5) n=6	13.7±0.6 (13–14) n=3
X. browni	Sokolnice	45.5±3.7 (38–46) n=4	46.0±4.0 (40–49) n=4	30.5		5, 6, 6	8.5, 10, 10
1	Mohyla míru	39, 40, 50	39, 41.5, 44	26, 33	12.5±1.0 (11–14) n=5	5,6	10, 10
X. penevi	Ifrane	52.2±9.0 (36–68)	52.3±4.3 (46–58)	26		8.9±0.3 (8–9)	10.6±1.2 (8–13)
X. pachtaicum	Balgarene	40, 48	42, 49, 50	37	13, 14, 15	9, 9	12, 12
ısimile	Vinogradets	33.1±0.4 (30–38) n=13	31.2±0.7 (24–39) n=13	29.4±4.3 (26–33.5) n=10	14.5±1.7 (13–15) n=17	7.4±0.5 (7–8) n=15	7.4±0.5 (7–9) n=19
X. parı	Ralja,Trešna paratypes	40.0±11.3 (27–46) n=3	-	-	14.5±1.05 (13–16) n=5	7.8±0.8 (7–8.5) n=3	8.75±0.3 (8.5–9) n=4
	Srebarna, Bulgaria	18.8±2.8 (14–21) n=6	18.5±2.4 (15–20) n=6	36.3±6.4 (29–41) n=3	14.8±1.3 (13–16) n=5	5.8±0.4 (5.5–6) n=8	9.5±0.9 (8.5–11) n=7
imile	Kalimok- Brashlen Bulgaria	21.8±1.9 (16.5–24) n=14	21.5±1.8 (19–24) n=14	43.1±3.1 (36.5–48) n=12	16.8±0.8 (15–18) n=15	6.4±0.65 (5.5–7) n=17	8.6±0.5 (8–10) n=17
X . s_i	Orlyane Bulgaria	21.75±2.2 (17–24) n=7	22.1±2.3 (19–26) n=7	43.8±4.2 (36–50) n=7	16.9±1.1 (15–18) n=8	6.05±0.6 (5.5–7) n=11	9.05±0.8 (8–10) n=10
	Kamen bryag Bulgaria	23.0±4.8 (18–30) n=5	24.2±4.15 (19–30) n=5	47.2±8.9 (37–60) n=5	15.9±1.8 (13–17) n=5	6.4±0.6 (6–7) n=5	9.8±0.8 (9–10) n=5

Table 5. Measurements of uteri (including ovejector), ovejector and vaginal parts. All measurements in micrometres presented as mean ± standard deviation (range).

Data for X parasimile and X. simile, Lazarova et al. (2008).

X. parasimile by its different lip region shape (expanded *vs* not expanded), somewhat longer odontostyle av. 79–83 (73–85) μm *vs* av. 70 (64–74) μm in the type population, avs. 69–70 (63–74) in Bulgarian populations and avs. 68–70 (67–72) μm in females from Romania (Barsi and Lamberti 2004, Lazarova et al. 2008, Bontă et al. 2012);



Figure 9. *Cox1* amino acid sequence alignment of *Xiphinema browni* sp. n. and the closest species *X. parasimile*, *X. simile* and *X. pachtaicum*.

- *X. simile* by its longer odontostyle av. 79–83 (73–85) *vs* av. 66 (62–69) in type population, avs. 68.5–70 (66–72.5) in other Bulgarian populations, 67.5 (65–70) μm in a population from Bosna and Herzegovina, and avs. 67–68 (61–73) μm in females from the Czech Republic (Lamberti et al. 1983, Barsi and Lamberti 2004, Kumari 2006, Lazarova et al. 2008). However, it should be noted that females from Serbia and Crete (odontostyle 71.5 (66–74) μm and 75–77 μm, respectively) have slightly overlapping values between *X. browni* sp. n. and *X. simile* for this character (Barsi and Lamberti 2004, Tzortzakakis et al. 2014). Further *X. browni* sp. n. differs from *X. simile* in the length and structure of uteri (in the new species separate uteri and ovejector present *vs* separate uteri not present), different tail shape (conoid *vs* bluntly conoid), and in the shorter bulbus (53–69 *vs* 76–92 μm) (Lazarova et al. 2008) (Table 4). Finally, *X. browni* sp. n. develops though 4 *vs* 3 juvenile stages in *X. simile*;
- X. vallense by the position of amphideal fovea aperture (posterior constriction level vs on the lips); higher lip region (4–7 μm vs 2–3.5 μm); presence of symbiont bacteria in ovaries vs ovaries without symbionts; somewhat higher c' values (c'=1.8 (1.53–2.07) vs c'=1.6 (1.4–1.7); the different tail shape (dorsoventral depression at hyaline region level not present vs present); shorter spicules in males (29 μm vs 38 μm).

Additionally, X. browni sp. n. can be differentiated from:

X. pachtaicum by the different vagina shape (bell-shaped vs funnel shaped, (Figs 16, 18) and shorter pars distalis vaginae, shorter pharyngeal bulb (53–69 vs 75–80 μm), more posterior location of the dorsal nucleus (DN=13–21% vs 9–10%) (Table 4), different tail shape in both sexes (conical vs subdigitate). Illustrations of selected features of the closest species X. pachtaicum, X. parasimile and X. penevi sp. n. are presented in Figs 15–18 for comparison.



Figure 10. Hypothesis of the phylogenetic relationships of *Xiphinema browni* sp. n., *X. parasimile, X. pachtaicum* and *X. penevi* sp. n. based on 18S rDNA inferred from a Bayesian analysis using GTR+G model and *Prionchulus punctatus* (Cobb, 1917) Andrássy, 1958, *Alaimus* sp. and *Tripylina* sp. as an outgroup. Posterior probabilities higher than 0.8 are presented. The sequence of *X. browni* from Moča was not included due to the shorter length.

X. paratenuicutis in having symbionts in its ovaries vs absent, males rare vs abundant, higher values for c' (1.8 (1.5–2.1) vs 1.4 (1.2–1.6), different location of dorsal nucleus (DN after beginning of the stronger cuticular lining of the bulbus vs before, see Fig. 1 D1-F and Fig. 2E in Gutiérrez- Gutiérrez et al. 2012);



Figure 11. Hypothesis of the phylogenetic relationships of *Xiphinema browni* sp. n., *X. parasimile, X. pachtaicum* and *X. penevi* sp. n. based on 28S rDNA inferred from a Bayesian analysis using GTR+G model and *Longidorus helveticus* Lamberti, Kunz, Grunder, Molinari, De Luca, Agostinelli & Radicci, 2001 and *L. poessneckensis* Altherr, 1974 as an outgroup. Posterior probabilities higher than 0.8 are presented.



Figure 12. Hypothesis of the phylogenetic relationships of *Xiphinema browni* sp. n. and *X. parasimile* based on *cox1* inferred from a Bayesian analysis using GTR+G model and *X. italiae* Mayl, 1953 and *X. diversicaudatum* (Micoletzky, 1927), Thorne, 1939 as an outgroup. Posterior probabilities higher than 0.8 are presented.

X. *plesiopachtaicum* by the position of the amphideal aperture (posterior *vs* at the constriction level); somewhat shorter bulbus (avs. 59–60 (53–69) *vs* av. 73 (60–86); shorter uteri (av. 81 *vs* av. 138 μm); higher c' values (c'=1.8 (1.53–2.07) *vs* c'=1.4 (1.3–1.7); and differently shaped vagina (bell-shaped *vs* funnel shaped).

For comparison between X. browni sp. n. and X. penevi sp. n. see below.

Etymology. The species is named after Prof Derek JF Brown, an outstanding nematologist, for his significant contributions to the knowledge of plant parasitic nematodes and the development of nematology in Bulgaria.



Figure 13. Hypothesis of the phylogenetic relationships of *Xiphinema browni* sp. n. based on ITS1 inferred from a Bayesian analysis using GTR+G model and *X. barense* Lamberti, Roca, Agostinelli, Bleve-Zacheo, 1986, *X. italiae* and *X. diversicaudatum* as an outgroup. Posterior probabilities higher than 0.8 are presented.

Xiphinema penevi sp. n.

http://zoobank.org/C98CE5B3-9BAE-423C-B887-9BFFFD489798 Figures 15–23

Measurements. See Tables 4, 5, 7.

Description. *Females.* Body open spiral to C shaped. Thickness of the cuticle at postlabial region 1 μ m, 1–1.5 μ m at mid-body and 2–2.5 μ m at post-anal region, outer cuticle layer not reaching the tail end. Labial region flat anteriorly, laterally rounded, set off from the rest of the body by constriction, 2.5–4 μ m high. Amphideal fovea hardly visible, its opening 4 μ m in a paratype specimen (40–47 % of the corresponding body width); Distance between first and second guide ring in specimens with retracted odonostyle, 2.5–5 μ m long. Odontophore with well developed flanges, 6–9 μ m wide, often a small vestigium located in odonthophore area. Pharyngeal characters presented at Table 4. Dorsal nucleus 2.5–3 μ m diam., ventrosublateral nuclei well vis-



Figure 14. Hypothesis of the phylogenetic relationships of *Xiphinema browni* sp. n., *X. parasimile, X. pachtaicum* and *X. penevi* sp. n. based on ITS2 inferred from a Bayesian analysis using GTR+G model and *X. italiae, X. diversicaudatum* and *X. vuittenezi* Luc, Lima, Weischer & Flegg, 1964 as an outgroup. Posterior probabilities higher than 0.8 are presented.

ible, 2–2.5 μ m. Prerectum indistinct, rectum 21.6±1.8 (19–24) μ m, n=8, c 1.3 of corresponding body width. Reproductive system amphidelphic, symbiotic bacteria present in the ovaries. Uteri short, ovejector not developed, only in one specimen a structure resembling ovejector was observed (Table 5); vagina c. 2/3 of the corresponding body width, *pars proximalis vaginae* with well developed wall. Tail conoid, dorsally convex, ventrally slightly concave, gradually narrowing to a pointed tip, two distinct pairs of caudal pores.

Male. Not found.

Juveniles. The scatter diagram based on functional and replacement odontostyle, and body length revealed the presence of four juvenile stages (Fig. 23). As in most species of the *X. americanum*-group there is a gradually decreasing of $\mathbf{c}^{\mathbf{c}}$ values with successive stages which reflects increasing body width while the tail length is more or less similar in juveniles and adults.

Type locality and plant association. Ifrane, Morocco, Quercus ilex L. forest.

Type material. The holotype, 7 paratype females and juveniles from all stages are deposited in the nematode collection of the Institute of Biodiversity and Ecosys-

				Non-European sp	ecies			European species
	X. penevi sp. n.	X. bricolensis	X. californicum	X. citricolum	X. intermedium	X. oxycaudatum	X. tenuicutis	X. plesiopachtaicum
Body L	1.69 (1.5–1.85)	1.9 (1.7–2.3)	2 (1.8–2.2)	1.6–1.8	1.6 (1.4–1.9)	1.6 (1.5–1.7)	1.8 (1.6–1.9)	1.9 (1.5–2.1)
я	61 (57.2–65.0)	56 (52–62)	60 (52–68)	45-46	43 (38–51)	47 (45–51)	46 (40–53)	64 (57.3–70.2)
c	57.7 (50.8–61.5)	57 (49–65)	63 (58–76)	44–50	47 (41–59)	51 (48–54)	61 (56–65)	71.1 (62.5–88.7)
Ŷυ	1.8 (1.6–1.9)	1.5(1.3-1.6)	1.6(1.3-1.9)	1.6 - 1.7	1.5(1.3-1.7)	1.6 (1.3–1.7)	1.5 (1.4–1.7)	1.4 (1.3–1.7)
Vulva [%]	57 (51–61.5)	52 (50-55)	51 (49–55)	52-54	52 (50-57)	52.5 (51–54)	51 (47–52)	57.3 (55.5–60)
Odontostyle L	77 (72–79)	87 (85–94)	90 (83–98)	78-86	76 (68–80)	82 (78–84)	76 (73–80)	83 (77–89)
Tail L	29 (26–32)	36 (31-41)	31 (27–36)	34–36	33 (31–38)	33 (27–35)	29 (26–32)	26 (23–28)
Length to GR	68 (66–71)	68 (61–76)	76 (66–83)	64–72	63 (58–67)	71 (66–75)	60 (55–64)	69 (63-76.5)
Lips width	8 (8–9)	11	10 (10–11)	12.5	10.5 (9.5–11)	10 (9–10)	9 (9–10)	9.5 (8.5–10.5)
J	9 (8–10)	(6-7)	6 (5-8.5)	12-14	10 (9–12)	9 (7–10)	8 (6.5–10)	8 (5.5–10)
Juvenile stages	4	۸.	4	ç		۸.		۰.
Males (number of VM supplements)	rare or absent	rare or absent 11	rare or absent 7	rare or absent 10	rare or absent 11	rare or absent 3	not found	Not found
				Euro	pean species			
	X. browni sp. n.	X. microstilum	X. pachtaicum	X. parasimile	X. paratenuicutis	X. simile	X. parapachydermum	X. vallense
Body L	2.03 (1.8-2.40)	2.6 (2.5–2.8)	1.88	1.99 (1.75–2.26)	2.01 (1.7–2.2)	1.9 (1.7–2.1)	1.78 (1.41–2.0)	2.0 (1.8–2.2)
я	69.3 (56.9-81.3)	86 (77–93)		70.5 (61.0–76.1)	61.1 (51.9–69.7)	71 (63–77)	64 (51.3–73.1)	68.9 (61.6–79.1)
c	69.9 (54.7-83.0)	74 (63–88)	72.3	59.9 (50.9–69.8)	68.8 (58.8–79.9)	67 (61–70)	60.3 (46.3–75.5)	73.4 (58.2–86.3)
°u	1.8 (1.53-2.07)	1.8 (1.6–2.0)	1.6	2.02 (1.79–2.28)	1.4(1.2-1.6)	1.7 (1.6–1.8)	1.8 (1.5–2.3)	1.6 (1.4–1.7)
Vulva [%]	55 (52.3–58.5)	57 (55–60)	60	55.5 (52.2–58.7)	56.8 (55–60)	53 (51–54)	59 (55–66)	57.5 (55–59.5)
Odontostyle L	79 (75–83)	74 (68–77)	83	69.7 (64.4–73.7)	75.2(71.5–83)	66 (62–69)	81 (70-87.5)	79 (73–85.5)
Tail L	29 (25–33)	35 (31–39)	26	33.3 (30.3–37.1)	29.4 (25–34.5)	29 (27–30)	28.8 (26.5–35.5)	27.8 (22.5–34)
Length to GR	71 (65–75)	63 (57–68)	78	62.6 (59.4–66.3)	63.2 (60–69)	51 (49–53)	70 (59.5–75.5)	69.5 (62–72.5)
Lips width	8 (8–10)	9 (9–10)	10	9.0 (8.4–9.7)	9.6 (9–10)	6-6) 6	8.8 (8–9.5)	8.5 (8–9)
J	8 (6–12)	10 (7–12)		8.2 (6-10)	8.4 (6.5–10.0)	7 (6–8)	9.3 (7–12.5)	7.6 (6.5–8.5)
Juvenile stages	4		4	4	4	\mathcal{C}	4	4
Males (number of supplements)	rare or absent 5	frequent 4–5	rare or absent 5–6	rare or absent 5	males abundant 5	rare or absent 3–5	Males abundant	Rare or absent $6, 7$

Table 6. Morphometric data of *Xiphinema americanum* species having similar morphometrics with the new species based on type populations.

tem Research, Sofia, Bulgaria. Other paratypes deposited as follows: 2 females in the USDA Nematode Collection, Beltsville, Maryland, USA; 2 females in the Nematode Collection of the Institute of Plant Protection, Bari, Italy; 1 female in the Wageningen Nematode Collection (WANECO), Wageningen, the Netherlands. Three ribosomal sequences (18S, ITS2 and D2-D3) of *X. penevi* sp. n. are deposited in GenBank (for accession numbers see Table 2).

Sequence and phylogenetic analyses. Sequences for three gene regions were obtained (18S, D2-D3 and ITS2). BLAST at NCBI using any of these sequences as queries revealed highest similarity to *X. pachtaicum* (99% for 18S, 6 nt difference), two populations of *X. incertum* Lamberti, Choleva & Agostinelli, 1983 from Spain (99% for D2-D3, 1 and 3 nt difference) and *X. pachtaicum* (90% for ITS2). In both 18S and D2-D3 phylogeny reconstructions *X. penevi* sp. n. was part of well supported clades with other species of *X. pachtaicum*, *S. parapachydermum* for 18S and *X. incertum*, *X. pachtaicum*, *X. parapachydermum* for 18S and *X. incertum*, *X. pachtaicum*, *X. parapachydermum*, *X. pachydermum* Sturhan, 1983 for D2-D3). In the phylogeny reconstruction based on ITS2 sequences, the species grouped with two other *X. pachtaicum* populations.

Diagnosis and relationships. Xiphinema penevi sp. n. is characterised by specific combination of traits: slender body of medium size (1.54–1.85 mm), lip region rounded laterally, flattened anteriorly, separated from the body by a constriction, odontostyle 72-79 µm long, post-equatorial vulva position (V=56-58%), symbiont bacteria present in ovaria, female tail 26-32 µm long (c=50.8-61.2 and c'=1.7-1.9), conoid dorsally convex ventrally slightly concave with pointed tip, and specific ribosomal sequences (18S and ITS2). The alpha-numeric codes based on average values (ranges given in parentheses) using the polytomous key by Lamberti et al. (2004) are: A2, B3, C3 (4), D1 (2), E2, F2 (1), G2, H1, I2 (1). Subsequently described species X. parasimile, X. parabrevicolle, X. parapachydermum, X. paratenuicutis (Barsi and Lamberti 2004, Gutiérrez-Gutiérrez et al. 2012) X. plesiopachtaicum, X. vallense and X. astaregiense (Archidona- Yuste et al. 2016) and X. browni sp. n. have been also compared. Species having most similar morphometrics with X. penevi sp. n. were: X. pachtaicum, X. plesiopachtaicum, X. browni sp. n., X. vallense and X. parasimile. Due to the close relationships based on phylogenetic analyses X. incertum, X. pachydermum Sturhan, 1983 and X. parapachydermum were also compared. Xiphinema penevi sp. n. can be differentiated morphologically from:

- *X. pachtaicum* by its shorter odontostyle av. 77 (72–79) *vs* 83 μm in holotype, av. 84 (78– 88.5) in the present study, 89 (85–97) in females from Ethiopia, and distance of oral aperture to guide ring (68 (66–71) *vs* 78 in holotype, 77 (73–80) in the present study; shorter pharyngeal bulb (65–72 *vs* 75–80 μm) in the present study; different tail shape (conoid with gradually pointed tip *vs* conoid, subdigitate), outer cuticular layer not reaching *vs* reaching tail tip. (Lamberti and Siddiqi 1977, Getaneh et al. 2015);
- *X. plesiopachtaicum* by the position of the amphideal fovea aperture (posterior *vs* at constriction level); its somewhat shorter odontostyle (72–79 *vs* 77–89 µm) and uteri



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Figure 15. *Xiphinema browni* sp. n., *X. penevi* sp. n. *X. pachtaicum* and *X. parasimile*. Female: **A–C** Anterior ends **D–F** Tail shapes **G–J** Pharyngeal bulbs **A, D, G** *X. browni* sp. n. **B, E, I** *X. penevi* sp. n. **C, F, J** *X. pachtaicum* **H** *X. parasimile*. Scale bars: 25 μm

(104 vs 138 μ m); different position of the dorsal nucleus (DN in front of or at the level of DO (beginning of cuticular lining of the bulb) vs DN below the level of DO); different tail shape (ventrally slightly concave vs straight), smaller values for **c** and larger for **c**' ratios (c=50.8–61.5 vs c=62.5–88.7; c'=1.7–1.9 vs c'=1.3–1.7);

X. vallense by the position of amphideal fovea (posterior constriction *vs* on the lips); its shorter body (L=1.69 (1.5–1.85) *vs* 2.01 (1.83–2.22), different position of dorsal

Α



Figure 16. *Xiphinema browni* sp. n., *X. penevi* sp. n. and *X. pachtaicum*. Female genital system comparison: **A**, **E** Posterior genital branch **B–D** Anterior genital branch **A**, **B** *X. browni* sp. n. **C** *X. penevi* sp. n. **D**, **E** *X. pachtaicum*. Scale bars: 25 μm



Figure 17. *Xiphinema browni* sp. n., *X. parasimile, X. pachtaicum* and *X. penevi* sp. n. Female and male: **A–D** Anterior ends **E–H** Labial region **I–K** Pharyngeal bulbs **L**, **M**, **R** Male tails **N–Q** Female tails **A, E, I, M, N** *X. browni* sp. n. **B, F, K, O, R** *X. parasimile* **C, G, J, L, P** *X. pachtaicum* **D, H, Q** *X. penevi* sp. n. Scale bars: 30 μm (**A–D, I–R**); 12 μm (**E–H**).



Figure 18. *Xiphinema browni* sp. n., *X. parasimile*, *X. pachtaicum* and *X. penevi* sp. n. Female: **A–D** Genital system **E–L** Vulval region **A**, **E**, **I** *X. browni* sp. n. **B**, **F**, **J** *X. parasimile* **C**, **D**, **G**, **K** *X. pachtaicum* **H**, **L** *X. penevi* sp. n. Scale bars: 30 μm (**A–H**); 12 μm (**I–L**).



Figure 19. *Xiphinema penevi* sp. n. Female. **A** Anterior end **B** Amphideal fovea outline **C** Variations in genital system: **CI** Anterior uterus and partim posterior genital branch **C2, C3** Region of vagina and uteri **D** Pharyngeal bulb **E** Tail. Scale bars: $25 \,\mu$ m.

nucleus (DN in front or at the level of DO *vs* DN below the level of DO); different tail shape (ventrally slightly concave *vs* straight) smaller values for **c** and larger values for **c**' ratios (c=50.8–61.5 *vs* c=58.2–86.3; c'=1.7–1.9 *vs* c'=1.4–1.7), longer hyaline part (8–10 μ m *vs* 6.5–8.5 μ m);

- X. browni sp. n. by its somewhat shorter body (L=1.69 (1.5–1.85) vs 2.03 (1.8–2.40) mm and longer bulbus (65–72 vs 53–69) μm; lower (2.5–4 vs 4–7 μm) and differently shaped lip region (not expanded vs expanded); different location of the dorsal nucleus (DN=9.9–12.9 % vs DN=12.7–21.1%); different vagina shape (funnel- vs bell-like Figs 16, 18);
- X. parasimile by its somewhat shorter body (L=1.69 (1.5–1.85) vs 1.99 (1.75–2.26) mm in type population and avs. 1.78 -1.82 (1.56–2.04) in females from Bulgaria), different lip region shape (laterally rounded vs not rounded), the different location of dorsal nucleus (DN 9.9–12.9 % vs 13.6–18.6 %), longer bulbus (65–72



Figure 20. *Xiphinema penevi* sp. n. Female. Variations in: **A–D** Anterior ends (B-holotype) **F, J–L** Vagina region **G–I** Tail shapes. Scale bars: 30 µm **(A, B, F–I)**; 12 µm **(C, D, J–L)**.



Figure 21. *Xiphinema penevi* sp. n. *Juveniles*: **A–E** Anterior ends of first- to fourth-stage juveniles and female **F–J** Tails of first to fourth juvenile stages and female (**F1** and **F2** tail of first stage juveniles). Scale bar: 25 µm.



Figure 22. *Xiphinema penevi* sp. n. *Juveniles and female*: **A–D** Neck region of first- to fourth-stage juveniles **E** Anterior end of female **F–J** Tails of first to fourth juvenile stages and female. Scale bar: 30μm.

vs 55.5–63 μ m) (Table 4); different vagina shape (funnel *vs* bell-like), structure of uteri (ovejector not present *vs* ovejector and separate uteri present) and length of uterus (36–68 vs 27–46 μ m in type population and 27–39 μ m in population from Bulgaria (Table 5); shorter tail (av. 29 (26–32) *vs* 33 (30.3–37.1) in the type population and 30–32 (27–35) in females from Bulgaria, c'=1.8 (1.6–1.9) *vs* 2.02 (1.79–2.28) in the type population and 2.0 (1.7–2.3) in females from Bulgaria) (Barsi and Lamberti 2004, Lazarova et al. 2008);

- X. incertum by its different tail shape (elongate conoid vs bluntly conoid, ventrally slightly concave vs straight) and larger c' values (c'=1.8 (1.6–1.9) vs c'=1.5 (1.4–1.7) in type material and 1.2 (0.9–1.3) in specimens from Spain, larger a values (a=61 (57–2-65) vs a=57 (56–58) in type population and a=49.7 (44.6–52.5) in the population from Spain and different vagina shape compared with females from Spain, this character not described for the type population (Lamberti et al. 1983, Gutiérres-Gutiérres et al. 2012);
- *X. pachydermum* by its shorter body (L=1.69 (1.5–1.85) mm vs 2.24 (2.08–2.44) mm), different location of dorsal nucleus (DN=10–13 % vs DN=15–20%), presence of symbiont bacteria in ovaria vs not present; males occurrence (not present vs abundant);

Characters		Females	Juveniles			
	Holotype	Paratypes	J1	J2	J3	J4
n		12	2	8	5	5
L	1726	1687±100 (1532–1846)	664, 602	777.6 ± 37 (702–816)	1049.0±54 (988–1126)	1318±38 (1292–1384)
a	62.1	61.0±2.6 (57.2–65.0)	40.6, 38.0	43.1±2.4 (40.2–47.0)	48.2±1.5 (46.7–49.8)	54.4 ± 3.6 (51–58)
b	5.8	6.1±1.1 (5.0-7.0)	3.8, 3.6	4.0±0.2 (3.6–4.2)	4.5±0.1 (4.4–4.6)	5, 6
С	55.1	57.7±3.9 (50.8–61.5)	20.9, 22.4	24.7 ± 2.5 (21.2–28.1)	33.4, 34.1	40.8±2.6 (38–43)
c'	2.0	1.8±0.1 (1.6–1.9)	2.9, 2.6	2.7±0.3 (2.4–3.1)	2.2, 2.3	2.1±0.2 (2-2)
V (%)	56.8	57.1±0.6 (55.9–58.1)				
G1(%)		11.2±0.5 (10.9–12.1)				
G2(%)		12.3±3.2 (9.2–19.5)				
Odontostyle	75	76.7 ± 2.1 (72-79)	36.5, 37	43.8±1.0 (43–45)	54.5±1.1 (53–56)	63.3±2.0 (60–65)
Replacement odontostyle			43, 46	56.5±1.8 (53–58)	66.2±1.4 (65–68)	75.0±1.6 (73–78)
Odontophore	50	47.7±1.8 (44–50)	28	33.6±1.2 (32–35)	37.6±1.9 (35–39)	43.9±1.6 (42–45)
Oral aperture to guide ring	71	68.0 ±0 .6 (66–71)	30.5, 33	38.5±1.2 (36–40)	49.0±1.4 (47–50)	55.6±3.9 (50–60)
Tail length	31	29.3±1.9 (26–32)	32, 27	32.0 ± 2.7 (29–35.5)	32, 33	31.9 ± 2.0 (30–34)
Length of hyaline part	9	8.4±0.7 (8–10)	4, 4	4.3±0.7 (3–5)	6, 6	6.9±0.7 (6–7)
Body diam. at: - lip region	9	8.3±0.3 (8–9)	7,7	7.1±0.5 (6–8)	7.3±0.7 (7–8)	7.8±0.2 (8–8)
- at guiding ring	21	20.6±0.5 (20–21)	12, 13	14.5 ± 0.6 (14–15)	16.2 ± 0.7 (15–17)	18.6±0.9 (18–20)
- at base of pharynx	25	24.0±1.0 (22–26)	15, 15	16.8±1.2 (15–18)	19.3±0.8 (18–20)	22.3±1.0 (21–23)
- at mid body/at vulva	28	27.6±1.4 (25–31)	16, 16	18.1±1.5 (16–20)	21.8±1.3 (21–24)	24.0±1.7 (22–26)
- at anus	16	16.2±0.7 (15–17)	11, 10	11.9 ± 0.8 (11–13)	25.2 ± 22.1 (13.5–58.4)	15.4±0.5 (15–16)
- at beginning of hyaline part	7	7.1±0.4 (7–8)	5, 4	4.2±0.3 (4-5)	5, 5	6.5±0.5 (6–7)

Table 7. Morphometrics of *Xiphinema penevi* sp. n. (females and juveniles) from *Q. ilex* Morocco. All measurements except ratios in micrometres given as mean ± standard deviation (range).



Figure 23. Scatter plot of odontostyle (■) and replacement odontostyle (□) against body length of *Xiphinema penevi* sp. n. juveniles and females from Morocco.

X. parapachydermum by its different tail tip (not so acute and not with dorso-ventral depression) and in having symbionts in its ovaries *vs* absent, males occurrence (not present *vs* abundant).

Etymology. The new species is named after Dr Lyubomir Penev, an internationally recognised publisher and authority in entomology and ecology as acknowledgement of his invaluable help and support provided to one of the authors (VP) in her research activities.

Xiphinema pachtaicum (Tulaganov, 1938) Kirjanova, 1951

Figures 15–18

Measurements. Tables 3–6.

Note. *Xiphinema pachtaicum* has been recorded from Bulgaria and data on its morphology are available in previous studies (Lamberti et al. 1983; Peneva and Choleva 1992); here we present additional morhometric data only for the population from Balgarene together with illustrations, LM micrographs and sequence data (Table 2). It is common and associated with a wide spectrum of cultivated and wild plants (Lamberti and Siddiqi 1977).

Xiphinema parasimile Barsi & Lamberti, 2004

Figures 15, 17, 18

Morphometric data and detailed description of *X. parasimile* from Bulgaria are reported previously (Lazarova et al. 2008). For the Vinogradets population two ribosomal and one mitochondrial DNA sequences were obtained (Table 2). *Xiphinema parasimile* has a limited distribution in Bulgaria (Lazarova et al. 2008).

Sequence and phylogenetic analyses

Three rDNA sequences were obtained for the Bulgarian *X. pachtaicum* population (18S, D2-D3 and ITS2) with BLAST showing identity or very high similarity to other *X. pachtaicum* populations available at NCBI (100% for 18S, 99/100% for D2-D3 and 98% for ITS2). Further, the DNA sequences of *X. parasimile* from Vinogradets (18S, D2-D3 and *cox1*) showed highest similarity to *X. simile* from Serbia (99% for 18S), various other populations of *X. simile* and *X. opisthohysterum* (88%, D2-D3) and 78% two *cox1* sequences – *X. pachtaicum* from the Czech Republic (GU222424) and *X. simile* from Slovakia (AM086708). The first one is the previously published sequence of *X. browni* sp. n. identified as *X. pachtaicum* (Kumari et al. 2010b). The D2 28S rDNA region was further compared to the Serbian population of *X. parasimile* (D2 part of sequences AM490214, AM490217, Barsi and De Luca 2008) and the alignment showing the different nucleotides is presented (Fig. 24). The p-distance calculated for D2 part only was 1.8–2.1% that might indicate that *X. parasimile* population from Bulgaria could represent a cryptic species.

Based on the phylogenetic analyses performed (Figs 11-15) both new species described are members of two well-supported species complexes - X. simile and X. pachtaicum. The first subgroup includes X. simile, X. parasimile X. browni sp. n. and probably X. vallense. All occur in Europe and X. simile has also been reported from Central Africa (Liškova and Brown 1996, Coomans and Heyns 1997, Barsi and Lamberti 2004, Kumari 2006, Repasi et al. 2008, Lazarova et al. 2008, Bontă et al. 2012). Whether some of these records represent X. simile or closely related species requires new investigations using morphological discrimination and molecular markers. So far, X. parasimile has been recorded from the Balkan region (Barsi and Lamberti 2004, Lazarova et al. 2008, Bontă et al. 2012). Xiphinema browni sp. n. (previously reported as X. pachtaicum) seems to occur in central European countries. The second group of closely related species consists of X. pachtaicum, X. penevi sp. n., X. incertum, X, parapachydermum, X. plesiopachtaicum, X. astaregiense and X. pachydermum. Again, one of these species (X. pachtaicum) has a much wider distribution in Europe, Asia and Africa (Lamberti and Siddiqi 1977, Fadaei et al. 2003, Getaneh et al. 2015). Xiphinema incertum has been reported from Bulgaria, Serbia, Croaita and Spain, all other species have limited distributions - X. plesiopachtaicum, X. pachydermum, X. parapachydermum, X. astaregiense, reported only from Spain, the latter three species being amphimictic, and X. penevi sp. n. so far found only in north-western Africa (Sturhan 1983, Lamberti et al. 1983, Barsi and Lamberti 2002, Gutiérrez- Gutiérrez, 2012).


Figure 24. Sequence alignment of D2 28S rDNA region of *Xiphinema parasimile* from Bulgaria (KU250156) and Serbia (AM490214 and AM490217).

Based on a hierarchical cluster analysis of morphometrics Lamberti and Ciancio (1993) distinguished five species subgroups, among them the X. pachtaicum-subgroup (IV) consisted of 8 species with five being described from Europe (X. fortuitum Roca, Lamberti & Agostinelli, 1987, X. incertum, X. madeirense, X. pachydermum and X. simile), one from North America (X. utahense Lamberti & Bleve-Zacheo, 1979), and one from Asia (X. opisthohysterum). Our analyses using ribosomal and mitochondrial DNA sequences currently available in GenBank and the two new species described in this study supports the delimitation of the "X. pachtaicum-subgroup", however it also includes X. incertum, X. pachtaicum, X. pachydermum and the recently described species X. parapachydermum, X. astaregiense, X. plesiopachtaicum and X. penevi sp. n. Phylogenetic reconstructions showed that X. madeirense, X. opisthohysterum, X. simile and X. uthahense are not part of this group, for X. fortutium no sequences are available. These results are in line with the findings of other recent studies on the X. americanum-group (Gutiérrez-Gutiérrez et al. 2012, Archidona-Yuste et al. 2016). Xiphinema simile (presented by two types of sequences for populations from Serbia and the Czech Republic in 18S rDNA and cox1 trees), X. parasimile and X. browni sp. n. formed a separate subgroup outside the X. pachtaicum-subgroup, so far consisting only of parthenogenetic species. Therefore we proposed this clade to be referred as the X. simile-subgroup. The recently described species X. vallense seems also evolutionary very closely related to this subgroup because of its high morphometric and DNA similarity, however amplifying additional sequences for other molecular markers (e.g. 18S and *cox*1) could help to clarify its relationships.

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DATA PAPER



Free-living marine nematodes from San Antonio Bay (Río Negro, Argentina)

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Abstract

The dataset of free-living marine nematodes of San Antonio Bay is based on sediment samples collected in February 2009 during doctoral theses funded by CONICET grants. A total of 36 samples has been taken at three locations in the San Antonio Bay, Santa Cruz Province, Argentina on the coastal littoral at three tidal levels. This presents a unique and important collection for benthic biodiversity assessment of Patagonian nematodes as this area remains one of the least known regions. In total 7,743 specimens of free-living marine nematodes belonging to two classes, eight orders, 37 families, 94 genera and 104 species were collected.

Keywords

Nematoda, Enoplea, Chromadorea, South Atlantic

Introduction

This is the first study on nematodes performed on a sub-Antarctic salt marsh along the coast of Río Negro Province, Argentina. This site has a high biodiversity and was declared a Protected Natural Area N°2.670 of the province of Río Negro in 1993 as well as an international reserve of the hemispheric network of Shorebird Reserve within the Wetlands program for the Americas. However, it is also an urban center where economic, industrial and tourist activities take place. The objectives of the study were to collect, identify and discover the structure and diversity of the nematode community of San Antonio Bay. The coverage (Figure 1) of this dataset includes two classes: Chromadorea (76%) and Enoplea (24%); eight orders: Monhysterida (39%), followed by Enoplida (23%) and Chromadorida (19%) as main occurrences; and thirty-seven families (see Figure 1).

Taxonomic ranks

Kingdom: Animalia

Phylum: Nematoda

Class: Chromadorea, Enoplea

- **Order:** Monhysterida, Enoplida, Chromadorida, Desmodorida, Araeolaimida, Plectida, Rhabditida, Dorylaimida.
- Family: Xyalidae, Linhomoeidae, Monhysteridae, Sphaerolaimidae, Siphonolaimidae, Chromadoridae, Cyatholaimidae, Ethmolaimidae, Selachinematidae, Microlaimidae, Desmodoridae, Monoposthiidae, Coninckiidae, Comesomatidae, Diplopeltidae, Axonolaimidae, Leptolaimidae, Aegialoalaimidae, Haliplectidae, Ceramonematidae, Tarvaiidae, Tylenchidae, Mesorhabditidae, Criconematidae, Panagrolaimidae, Oncholaimidae, Oxystominidae, Ironoidae, Thoracostomopsidae, Tripyloididae, Anticomidae, Anoplostomatidae, Enchelidiidae, Trefusiidae, Lauratonematidae, Enoplidae, Dorylaimidae.
- Genera: Odontophora, Synodontium, Comesoma, Metasabatieria, Sabatieria, Coninckia, Campylaimus, Diplopeltula, Chromadora, Chromadorella, Chromadorina, Prochromadorella, Actinonema, Rhips, Dichromadora, Neochromadora, Spilophorella, Marylynnia, Paracantonchus, Paracyatholaimus, Pomponema, Paraethmolaimus, Gammanema, Halichoanolaimus, Latronema, Molgolaimus, Metachromadora, Onyx, Polysigma, Spirinia, Bolbolaimus, Microlaimus, Nudora, Desmolaimus, Metalinhomoeus, Terschellingia, Eleutherolaimus, Paralinhomoeus, Siphonolaimus, Diplolaimella, Diplolaimelloides, Halomonhystera, Monhystera, Sphaerolaimus, Omicronema, Paramonohystera, Promonhystera, Pseudosteineria, Rhynchonema, Theristus, Xyalidae gen.1, Ceramonema, Pselionema, Tarvaia, Haliplectus, Cyartonema, Dorylaimus, Chaetonema, Cephalanticoma, Enoplus, Epacanthion, Oxyonchus, Thoracostomopsidae gen.1, Conilia, Dolicholaimus, Syringolaimus, Halalaimus, Calyptronema, Eu-



Figure 1. Taxonomic coverage by class, order and family.

rystomina, Abelbolla, Adoncholaimus, Viscosia, Metoncholaimus, Oncholaimus, Lauratonema, Rhabdocoma, Trefusia, Trefusiidae gen.1, Bathylaimus, Tripyloides. **Species with higher occurrences:** Microlaimus globiceps, Paraethmolaimus dahli, Thalassomonhystera parva, Microlaimus decoratus, Diplolaimelloides oschei, Nudora crepidata, Viscosia macramphida, Chromadorina longispiculum, Diplolaimella gerlachi, Leptolaimus luridus.

Spatial coverage

General spatial coverage: San Antonio Bay, Río Negro Province, Argentina (Figure 2). For this study three sites were selected: "Ciudad" (A), located in the north of the bay; "Baliza Camino" (B), located off the mouth of the bay of San Antonio and "Banco Perdices" (C),



Figure 2. Spatial coverage. San Antonio Bay, Argentina. Sites: **A** "Ciudad" **B** "Baliza Camino" **C** "Banco Perdices". Levels = u, m, l..

located 12 km south of "Las Grutas". At each sampling site, three tidal levels were chosen: upper-littoral, high tide, salt-marsh habitat (u); middle littoral, mean tide, un-vegetated habitat (m) and low littoral, low tide, un-vegetated habitat (l) (Figure 3).

Coordinates: "Ciudad": Au = 40°43'40.2"S; 64°57'41.1"W; Am = 40°43'39.0"S; 64°57'41.6"W; Al = 40°43'39.0"S; 64°57'39.5"W. "Baliza Camino": Bu = 40°42'59.9"S; 64°50'46.8"W; Bm = 40°43'05.8"S; 64°50'58.5"W; Bl = 40°43'11.6"S; 64°51'14,6"W. "Banco Perdices": Cu = 40°47'00.8"S; 64°50'54.3"W; Cm = 40°47'05.6"S; 64°51'17.8"W; Cl = 40°46'51.9"S; 64°51'02.8"W.

Temporal coverage

12–14 February 2009.

Methods

Sampling description: At each site and level location, four replicates (20 ml) were sampled with a PVC syringe (60 ml, inner diameter 2.9 cm) and separated by a dis-



Figure 3. San Antonio Bay, Argentina. Views from the sampling sites. **A** "Ciudad" **B**, **C** "Baliza Camino" **D** "Banco Perdices".

tance of 5–10 m each: four for marine nematodes counts, two for organic matter and two for sediment analyses. Each sample was fixed in situ, with a solution of 5% formaldehyde in filtered sea water with the addition of Rose Bengal tint. Marine nematodes were extracted from samples using the elutriation/decantation LUDOX TM (colloidal silica polymer) method at a specific gravity of 1.15, quantifying only organisms passing through a 500 µm mesh and then retained by a 63 µm mesh. Samples were evaporated to anhydrous glycerol and permanent slides made (Somerfield and Warwick 1996). The taxonomic classification followed proposed by De Ley and Blaxter (2004). For the identification of species international keys (Platt and Warwick 1983, Platt and Warwick 1988, Warwick et al. 1998, Lorenzen 1994, Abebe et al. 2006) and taxonomical papers (Pastor de Ward 1978, 1980, 1984a, b, c, d, e, 1985, 1986, 1988, 1989, 1990, 1991, 1993, 1995a, b, 1996, 1998a, b, c, 1999, Pastor de Ward and Lo Russo 2009, Villares and Pastor de Ward 2012, Lo Russo et. al. 2012, Villares et al. 2013, Pastor de Ward et al. 2013, Lo Russo et. al. 2015) were used. Holotypes and paratypes are deposited in the Collection of Nematodes of the Centro Nacional Patagónico (CENPAT-CONICET), Chubut, Argentina recognized as National Service of Biological Data of Argentina and included in their web page from 2011 (http:// www.gbif.org/dataset/06df03fc-8973-490c-af74-089fffae9e24; http://www.gbif.org/ dataset/d592283b-b00e-4a39-9499-289842ccddf1).

Project details

Project title: "Comparación de comunidades de nematodos de marismas de San Antonio Oeste (río Negro) y San Julián (Sta. Cruz)". [Comparison of nematode marsh communities of San Antonio Oeste (Río Negro) and San Julián (Sta. Cruz)]. Doctoral thesis Universidad Nacional del Comahue (Lo Russo 2012).

"Diversidad funcional y producción secundaria de las comunidades de nematodos de las marismas de San Antonio (Río Negro) y de la ría de San Julián (Santa Cruz)". [Functional diversity and secondary production of nematode marsh communities of San Antonio (Río Negro) estuary and San Julián (Santa Cruz)". Doctoral thesis Universidad Nacional del Comahue (Villares 2014).

Personnel: Catalina Pastor de Ward (Project Director, meio-benthos specialist), Virginia Lo Russo and Gabriela Villares (field work, nematodes identification, data collection and analysis), Viviana Milano (grant-holding student, data input), Lidia Miyashiro (Darwin core data input), Renato Mazzanti (software engineer, data base manager).

Study extent description: The San Antonio Bay marine nematodes is a dataset that gives new insights on the taxonomic and geographic distribution of south Atlantic marine nematodes, covering an under-explored region of the southern Atlantic coasts. This is the first study on marine nematodes in this site. This dataset presents species occurrences and species richness of the individual free-living marine nematodes present at three coastal areas ("Ciudad", "Baliza Camino", "Banco Perdices") of the San Antonio Bay at three different tidal levels (upper, middle and low-littoral).

In total 7,443 specimens of free-living marine nematodes belonging to two classes, eight orders, 37 families, 94 genera and 140 species were collected.

Genera and Species	Family	Order	Class
Odontophora peritricha Wieser, 1956	Axonolaimidae	Araeolaimida	Chromadorea
Synodontium sp. 1	Axonolaimidae	Araeolaimida	Chromadorea
Comesoma sp. 1	Comesomatidae	Araeolaimida	Chromadorea
Metasabatieria sp. 1	Comesomatidae	Araeolaimida	Chromadorea
Sabatieria mortenseni (Ditlevsen, 1921)	Comesomatidae	Araeolaimida	Chromadorea
Sabatieria punctata (Kreis, 1924)	Comesomatidae	Araeolaimida	Chromadorea
Sabatieria wieseri Platt, 1985	Comesomatidae	Araeolaimida	Chromadorea
Coninckia sp. 1	Coninckiidae	Araeolaimida	Chromadorea
Campylaimus gerlachi Timm, 1961	Diplopeltidae	Araeolaimida	Chromadorea
Campylaimus sp. 2	Diplopeltidae	Araeolaimida	Chromadorea
Diplopeltula sp.1	Diplopeltidae	Araeolaimida	Chromadorea
Chromadora nudicapitata Bastian, 1865	Chromadoridae	Chromadorida	Chromadorea
Chromadorella sp.1	Chromadoridae	Chromadorida	Chromadorea
Chromadorina longispiculum Pastor de Ward, 1985	Chromadoridae	Chromadorida	Chromadorea
Prochromadorella sp. 1	Chromadoridae	Chromadorida	Chromadorea
Prochromadorella sp. 2	Chromadoridae	Chromadorida	Chromadorea
Actinonema sp. 1	Chromadoridae	Chromadorida	Chromadorea

Genera and Species	Family	Order	Class
Rhips sp. 1	Chromadoridae	Chromadorida	Chromadorea
Dichromadora sp. 1	Chromadoridae	Chromadorida	Chromadorea
<i>Neochromadora alejandroi</i> Lo Russo & Pastor de Ward, 2012	Chromadoridae	Chromadorida	Chromadorea
Neochromadora papillosa Pastor de Ward, 1865	Chromadoridae	Chromadorida	Chromadorea
Spilophorella paradoxa (De Man, 1888)	Chromadoridae	Chromadorida	Chromadorea
Marylynnia sp. 1	Cyatholaimidae	Chromadorida	Chromadorea
Marylynnia sp. 2	Cyatholaimidae	Chromadorida	Chromadorea
Marylynnia sp. 3	Cyatholaimidae	Chromadorida	Chromadorea
Paracanthonchus austrospectabilis Wieser, 1954	Cyatholaimidae	Chromadorida	Chromadorea
Paracanthonchus punctatus (Bastian, 1865)	Cyatholaimidae	Chromadorida	Chromadorea
Paracanthonchus sp. 1	Cyatholaimidae	Chromadorida	Chromadorea
Paracanthonchus sp. 2	Cyatholaimidae	Chromadorida	Chromadorea
Paracanthonchus sp. 3	Cyatholaimidae	Chromadorida	Chromadorea
Paracyatholaimus chilensis Gerlach, 1953	Cyatholaimidae	Chromadorida	Chromadorea
Paracyatholaimus sp. 1	Cyatholaimidae	Chromadorida	Chromadorea
Pomponema sp. 1	Cyatholaimidae	Chromadorida	Chromadorea
Paraethmolaimus dahli (Gerlach, 1953)	Ethmolaimidae	Chromadorida	Chromadorea
Gammanema sp. 1	Selachinematidae	Chromadorida	Chromadorea
Gammanema sp. 2	Selachinematidae	Chromadorida	Chromadorea
Halichoanolaimus sp. 1	Selachinematidae	Chromadorida	Chromadorea
Latronema sp. 1	Selachinematidae	Chromadorida	Chromadorea
Molgolaimus sp. 1	Desmodoridae	Desmodorida	Chromadorea
Molgolaimus sp. 2	Desmodoridae	Desmodorida	Chromadorea
Metachromadora sp. 1	Desmodoridae	Desmodorida	Chromadorea
Metachromadora spectans Gerlach, 1957	Desmodoridae	Desmodorida	Chromadorea
Onyx sp. 1	Desmodoridae	Desmodorida	Chromadorea
Polysigma sp. 1	Desmodoridae	Desmodorida	Chromadorea
Spirinia septentrionalis Cobb, 1914	Desmodoridae	Desmodorida	Chromadorea
Bolbolaimus sp. 2	Microlaimidae	Desmodorida	Chromadorea
Microlaimus conothelis (Lorenzen, 1973) Jensen, 1978	Microlaimidae	Desmodorida	Chromadorea
Microlaimus capillaris Gerlach, 1957	Microlaimidae	Desmodorida	Chromadorea
Microlaimus decoratus Pastor de Ward, 1991	Microlaimidae	Desmodorida	Chromadorea
Microlaimus globiceps De Man, 1880	Microlaimidae	Desmodorida	Chromadorea
Microlaimus sp. 2	Microlaimidae	Desmodorida	Chromadorea
Nudora besnardi (Gerlach, 1956)	Monoposthiidae	Desmodorida	Chromadorea
Nudora crepidata Wieser, 1954	Monoposthiidae	Desmodorida	Chromadorea
Desmolaimus sp. 3	Linhomoeidae	Monhysterida	Chromadorea
Desmolaimus sp. 4	Linhomoeidae	Monhysterida	Chromadorea
Metalinhomoeus gloriae Pastor de Ward, 1989	Linhomoeidae	Monhysterida	Chromadorea
Metalinhomoeus parafiliformis Pastor de Ward, 1989	Linhomoeidae	Monhysterida	Chromadorea
Metalinhomoeus typicus De Man, 1907	Linhomoeidae	Monhysterida	Chromadorea
Terschellingia longicaudata De Man, 1907	Linhomoeidae	Monhysterida	Chromadorea
<i>Terschellingia</i> sp. 2	Linhomoeidae	Monhysterida	Chromadorea
Eleutherolaimus sp. 1	Linhomoeidae	Monhysterida	Chromadorea
Paralinhomoeus visitus Pastor de Ward, 1989	Linhomoeidae	Monhysterida	Chromadorea

Genera and Species	Family	Order	Class
Siphonolaimus auratus Wieser, 1956	Siphonolaimidae	Monhysterida	Chromadorea
Diplolaimella gerlachi Pastor de Ward, 1984	Monhysteridae	Monhysterida	Chromadorea
Diplolaimella ocellata (Bütschli, 1874)	Monhysteridae	Monhysterida	Chromadorea
Diplolaimelloides oschei Meyl, 1954	Monhysteridae	Monhysterida	Chromadorea
Halomonhystera disjuncta (Bastian, 1865)	Monhysteridae	Monhysterida	Chromadorea
Thalassomonhystera parva (Bastian, 1865)	Monhysteridae	Monhysterida	Chromadorea
Sphaerolaimus pacificus Allgen, 1945	Sphaerolaimidae	Monhysterida	Chromadorea
Amphimonhystera sp. 2	Xyalidae	Monhysterida	Chromadorea
Cobbia macrodentata Lo Russo & Pastor de Ward, 2012	Xyalidae	Monhysterida	Chromadorea
Daptonema laxus Wieser, 1956	Xyalidae	Monhysterida	Chromadorea
Daptonema sp. 2	Xyalidae	Monhysterida	Chromadorea
Daptonema sp. 3	Xyalidae	Monhysterida	Chromadorea
Gonionchus sp. 1	Xyalidae	Monhysterida	Chromadorea
Linhystera sp. 1	Xyalidae	Monhysterida	Chromadorea
Metadesmolaimus sp. 3	Xyalidae	Monhysterida	Chromadorea
Metadesmolaimus sp. 4	Xyalidae	Monhysterida	Chromadorea
Metadesmolaimus sp. 5	Xyalidae	Monhysterida	Chromadorea
Omicronema sp. 1	Xyalidae	Monhysterida	Chromadorea
Paramonohystera sp. 4	Xyalidae	Monhysterida	Chromadorea
Promonhystera sp. 1	Xyalidae	Monhysterida	Chromadorea
Pseudosteineria sp. 1	Xyalidae	Monhysterida	Chromadorea
Rhynchonema separatum Lorenzen, 1975	Xyalidae	Monhysterida	Chromadorea
Rhynchonema sp. 1	Xyalidae	Monhysterida	Chromadorea
Rhynchonema sp. 2	Xyalidae	Monhysterida	Chromadorea
Rhynchonema sp. 3	Xyalidae	Monhysterida	Chromadorea
Theristus lorenzeni Pastor de Ward, 1985	Xyalidae	Monhysterida	Chromadorea
Theristus modicus Wieser, 1956	Xyalidae	Monhysterida	Chromadorea
Theristus sp. 2	Xyalidae	Monhysterida	Chromadorea
Theristus sp. 3	Xyalidae	Monhysterida	Chromadorea
Theristus sp. 4	Xyalidae	Monhysterida	Chromadorea
Theristus sp. 5	Xyalidae	Monhysterida	Chromadorea
Xyalidae gen. 1 sp. 1	Xyalidae	Monhysterida	Chromadorea
Ceramonema sp. 1	Ceramonematidae	Plectida	Chromadorea
Pselionema sp. 1	Ceramonematidae	Plectida	Chromadorea
Tarvaia sp. 1	Tarvaiidae	Plectida	Chromadorea
Haliplectus salicornius Pastor de Ward, 1984	Haliplectidae	Plectida	Chromadorea
Cyartonema flexile Cobb, 1920	Aegialoalaimidae	Plectida	Chromadorea
<i>Cyartonema</i> sp. 1	Aegialoalaimidae	Plectida	Chromadorea
Deontolaimus papillatus De Man, 1880	Leptolaimidae	Plectida	Chromadorea
Leptolaimus luridus Timm, 1963	Leptolaimidae	Plectida	Chromadorea
Leptolaimus puccinelliae Gerlach, 1959	Leptolaimidae	Plectida	Chromadorea
Mesorhabditis sp. 2	Mesorhabditidae	Rhabditida	Chromadorea
Macroposthonia sp. 1	Criconematidae	Rhabditida	Chromadorea
Panagrolaimus sp. 1	Panagrolaimidae	Rhabditida	Chromadorea
Tylenchus sp. 1	Tylenchidae	Rhabditida	Chromadorea

Genera and Species	Family	Order	Class
Dorylaimus sp. 1	Dorylaimidae	Dorylaimida	Enoplea
Chaetonema patagonica Lo Russo et al., 2015	Anoplostomatidae	Enoplida	Enoplea
Cephalanticoma sp. 1	Anticomidae	Enoplida	Enoplea
Enoplus benhami Ditlevsen, 1930	Enoplidae	Enoplida	Enoplea
Enoplus meridionalis Steiner, 1921	Enoplidae	Enoplida	Enoplea
Epacanthion bicuspidatum Lo Russo et al., 2012	Thoracostomopsidae	Enoplida	Enoplea
Oxyonchus sp. 1	Thoracostomopsidae	Enoplida	Enoplea
Thoracostomopsidae gen. 1 sp. 2	Thoracostomopsidae	Enoplida	Enoplea
Conilia divina Gerlach, 1956	Ironoidae	Enoplida	Enoplea
Dolicholaimus marioni De Man, 1888	Ironoidae	Enoplida	Enoplea
Syringolaimus smarigdus Cobb, 1928	Ironoidae	Enoplida	Enoplea
Halalaimus sp. 1	Oxystominidae	Enoplida	Enoplea
Halalaimus sp. 2	Oxystominidae	Enoplida	Enoplea
Halalaimus sp. 3	Oxystominidae	Enoplida	Enoplea
Halalaimus sp. 4	Oxystominidae	Enoplida	Enoplea
Thalassoalaimus sp. 2	Oxystominidae	Enoplida	Enoplea
Calyptronema keiense Wieser 1953	Enchelidiidae	Enoplida	Enoplea
Calyptronema maxweberi (De Man, 1922)	Enchelidiidae	Enoplida	Enoplea
Eurystomina sp. 1	Enchelidiidae	Enoplida	Enoplea
Abelbolla sp. 1	Enchelidiidae	Enoplida	Enoplea
Adoncholaimus sp. 2	Oncholaimidae	Enoplida	Enoplea
Oncholaimellus paracarlbergi Pastor de Ward, 1993	Oncholaimidae	Enoplida	Enoplea
Viscosia macramphida Chitwood, 1951	Oncholaimidae	Enoplida	Enoplea
Metoncholaimus sp. 1	Oncholaimidae	Enoplida	Enoplea
Metoncholaimus sp. 2	Oncholaimidae	Enoplida	Enoplea
Oncholaimus sp. 1	Oncholaimidae	Enoplida	Enoplea
Oncholaimus sp. 2	Oncholaimidae	Enoplida	Enoplea
Lauratonema sp. 1	Lauratonematidae	Enoplida	Enoplea
<i>Rhabdocoma</i> sp. 1	Trefusiidae	Enoplida	Enoplea
Trefusia litoralis (Allgén, 1932)	Trefusiidae	Enoplida	Enoplea
Trefusiidae gen. 1 sp. 1	Trefusiidae	Enoplida	Enoplea
Bathylaimus australis Cobb, 1894	Tripyloididae	Enoplida	Enoplea
Tripyloides amazonicus (Gerlach, 1957)	Tripyloididae	Enoplida	Enoplea

Quality control description: The geo-referencing of all specimens were recorded using a Garmin eTrex Legend GPS (WGS84 Datum) with an accuracy of less than 10 m and with at least 5 satellites. The taxonomic identification of specimens, scientific names, and their current accurate spelling were verified by C. Pastor de Ward, a free-living marine nematode specialist. Other post-validation procedures (including geographic coordinate format, congruence between collection and identification dates, absence of ASCII anomalous characters) were checked using the Darwin Test software (http://www.gbif.es/darwin_test/Darwin_Test_in.php).

Dataset description

Object name: Darwin Core Archive free-living marine Nematodes from San Antonio Bay (Río Negro, Argentina). Character encoding: UTF-8 Format name: Darwin Core Archive format Format version: 1.0 **Distribution:** http://ipt.cenpat-conicet.gob.ar:8081/resource?r=sao2009# Publication date of data: 2013-10-25 Language: English **Licenses of use:** This work is licensed under a Creative Commons CC0 1.0 License http://creativecommons.org/publicdomain/zero/1.0/legalcode **External datasets Object name:** Centro Nacional Patagónico (CENPAT-CONICET) **Distribution:** http://ipt.cenpat-conicet.gob.ar:8081/resource?r=sao2009# **Object name:** Ministerio de Ciencia y Tecnología de Argentina (Sistema Nacional de Datos Biológicos - SNDB) Distribution: GBIF: http://www.gbif.org/dataset/d592283b-b00e-4a39-9499-289842ccddf1 Formatted: English (U.K.) **Field Code Changed** Metadata language: English Date of metadata creation: 2014-08-27 Hierarchy level: Dataset

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RESEARCH ARTICLE



Protagonista lugubris, a cockroach species new to China and its contribution to the revision of genus Protagonista, with notes on the taxonomy of Archiblattinae (Blattodea, Blattidae)

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Abstract

The blattid genus *Protagonista* Shelford, 1908, which is tentatively assigned to the subfamily Archiblattinae (= Planeticinae), is reported from China for the first time with illustrations and description of *P. lugubris* Shelford, 1908. It is a wood-dwelling and potentially a wood-feeding species. The male and female genitalia of *Protagonista* are described and illustrated for the first time. The species *Protagonista pertristis* Hanitsch, 1923 is revived from the synonymy of *P. lugubris*, and the remaining three nominal species that were also considered as synonyms of *P. lugubris* are now recognized as synonyms of *P. pertristis*. In agreement with Princis (1965), we propose that *Eroblatta* Shelford, 1910, a genus closely related to *Protagonista*, should be placed in the subfamily Archiblattinae rather than Blattinae. However, the taxonomy of Archiblattinae is problematic and awaits revision. Photos and a key to species of *Protagonista* and *Eroblatta* are provided, including photos of the holotypes of the synonymized nominal species. In addition, although Planeticinae is the senior synonym of Archiblattinae, the priority of the latter should be maintained since it is in prevailing usage based on the Article 40.2 in ICZN 4th edition.

Keywords

Planeticinae, new record, synonym, taxonomy, Eroblatta

Introduction

The cockroach subfamily Archiblattinae (= Planeticinae) belongs to the family Blattidae and is distributed in southeast Asia. Archiblattinae has a controversial taxonomic history. Planeticidae was erected by Walker (1868) based on the genus *Planetica* Saussure, 1863. This was subsequently synonymized with the genus *Archiblatta* Snellen van Vollenhoven, 1862 by Saussure (1869). It was Kirby (1904) that then established the subfamily Archiblattinae (family: Blattidae) on the basis of the genus *Archiblatta*, making Planeticidae a synonym. Meanwhile, Kirby (1904) included *Catara* Walker, 1868 in this subfamily. Handlirsch (1930) raised Archiblattinae to Archiblattidae, which was assigned to Blaberoidea by Princis (1960).

Shelford (1908, 1910) established *Protagonista* and *Eroblatta*, respectively, and placed them in the subfamily Blattinae (note all cockroaches were included in Blattidae). Princis (1965) included the four genera listed above in Archiblattidae. Grandcolas (1996) synonymized Archiblattidae with Blattidae. Recently Roth (2003) consented to this placement according to the male and female subgenital plates of three genera (*Archiblatta*, *Catara*, *Protagonista*) out of the four, but listed *Eroblatta* under Blattinae. From then on, the subfamily Archiblattinae was accepted as comprised of 3 genera (*Archiblatta*, *Catara* and *Protagonista*).

The genus *Protagonista* was established by Shelford (1908). He described *Protagonista lugubris* from the Manson Mountains, Tonkin (i.e. northern Vietnam) without any description of the male genitalia and designated it as the type species of *Protagonista*. Later Hanitsch (1923, 1925, 1929, 1931) described another four species belonging to the genus *Protagonista* from Southeast Asia: *P. pertristis*, *P. fusca*, *P. aterrima* and *P. laeta*. But Bruijning (1948) synonymized these 4 species with *Protagonista lugubris* Shelford, 1908 according to the difference in the depth of color of the tegmina and the whole body, which has less taxonomic value. Until now, the genus *Protagonista* was only reported from Vietnam, Malaysia, Indonesia and Singapore.

Previous studies of the subfamily Archiblattinae are fairly limited. In this paper, we report one known species *P. lugubris* newly discovered from China, distributed in Hainan and Guangxi, of which the male and female genitalia are described in detail for the first time. A key to all species of *Protagonista* and the related genus *Eroblatta* is given. The taxonomic status of this subfamily and the genus *Eroblatta*, as well as the validity of the name Archiblattinae, are discussed. We also deal with the synonymy of *Protagonista lugubris* based on the examination of holotypes, geographical distribution and original descriptions.

Material and methods

The terminology for the body, male and female genitalia used in this paper mainly follows McKittrick (1964) and Roth (2003). Terminology of veins follows Haas and Kukalová-Peck (2001) with modification by Li and Wang (2015). The specimens are deposited in the College of Plant Protection, Southwest University, Beibei, Chongqing,

China (SWU), unless otherwise noted. Measurements are based on specimens examined. Widths of pronota and tegmina are based on their widest portion. The genital segments of the examined specimens were macerated in 10% NaOH and observed in glycerin jelly using a Motic K400 stereomicroscope. All drawings were made with the aid of a Motic K400 stereomicroscope. All specimens deposited in SWU were photographed using a digital camera (Canon EOS 50D) coupled with a macro lens (Canon EF 100mm f/2.8 USM). The photographs were processed in Helicon Focus software.

The terms of veins (abbreviations given in parentheses) are: subcosta (*Sc*), radius (*R*), radius anterior (*RA*), radius posterior (*RP*), media (*M*), cubitus anterior (*CuA*), cubitus posterior (*CuP*), anal (*A*), anal anterior (*AA*), anal posterior (*AP*). The terms of female genitalia (abbreviations given in parentheses) are: paraprocts (*pp.*), anterior arch (*a.a.*), paratergites (*pt.*), first valve (*v.I*), second valve (*v.II*), third valve (*v.III*), laterosternite IX (*ltst.IX*), basivalvula (*bsv.*), laterosternal shelf (*ltst.sh*), common oviduct opening (*c.o.o.*), spermathecal opening (*sp.o.*), and vestibular sclerite (*vst.s.*).

The terminology of Roth (2003) is used in describing the spines (armament) on the antero-ventral margin of the front femur, where type A refers to a row of stout or "heavy" spines which decrease gradually in size distad, terminating in two or three large spines, rarely up to five large terminal spines. The number of stout terminal spines are indicated by subscripts so that one or two terminal spines are Type A_1 or A_2 .

The standard barcoding sequences of the mitochondrial COI gene (658 bp) of *Protagonista lugubris* from Hainan and Guangxi are approved, which are deposited in GenBank under the accession numbers KU511283, KU511284, KU511285 and KU511286.

Taxonomy

Subfamily Archiblattinae Kirby, 1904 (1868), new record from China

- Planeticidae Walker, 1868: 25; Walker 1869: 121; Princis 1965: 386. Type genus: *Planetica* Saussure, 1863.
- Archiblattinae Kirby, 1904: 148, as a substitute name based on the synonymy of *Planetica* with *Archiblatta*; Princis 1965: 386; Roth 2003: 33. Type genus: *Archiblatta* Snellen van Vollenhoven, 1862.
- Archiblattidae: Handlirsch 1930: 836; Princis 1960: 439; Princis 1965: 386.

Genus Protagonista Shelford, 1908, new record from China

Protagonista Shelford, 1908: 158; Shelford 1910: 22; Hanitsch 1923: 443; Bruijning 1948: 117; Princis 1965: 388; Grandcolas 1996: 520; Roth 2003: 33. Type species: Protagonista lugubris Shelford, 1908.

Generic diagnosis. The genus *Protagonista* is remarkable on account of the shape of the pronotum (as long as broad, quadrangular, with rounded angles, sides not de-

flexed), and the pubescence on its pronotum and tegmina (after Shelford 1908). The other three genera of Archiblattinae differ from it by the apterous female and the unarmed or weakly-armed femur (*Archiblatta* and *Catara*) or by the tibia having three rows of spines (*Eroblatta*).

Description. Antennae slightly moniliform. Ocelli present. Pronotum as long as broad, quadrangular, with rounded angles, sides not deflexed and not covering vertex (Figs 1–6 appears to show it covering the vertex, however this is an artifact of the photo angle). Pronotum and tegmina with fine pubescence. Tegmina and hind wings fully developed in the male, exceeding the apex of the abdomen. Tegmina short and truncated in the female, hind wing vestigial to a small lobe. Styli present and cerci moderate. Legs slender; front femora Type A₂; hind tibia with 2 rows of spines along outer margin; hind metatarsus very long, considerably exceeding the remaining joints in length; the tarsal pulvilli present on the proximal four tarsomeres; arolia minute.

Distribution. Vietnam; Malaysia (Malacca State); Sumatra; Borneo; China (new record; Hainan, Guangxi).

Protagonista lugubris Shelford, 1908, new record from China

Figs 1-43, 45, 48-49

Protagonista lugubris Shelford, 1908: 158; Shelford 1910: 22; Hanitsch 1927: 40; Hanitsch 1929: 17; Bruijning 1948: 117.

Description. Male. Body slender, dark brown to black (Figs 1–2, 5–6, 49). Eyes black, ocelli yellowish white. Vertex and face dark brown to black. Clypeus yellowish or dark brown and the base of labrum pale, labial palpi and maxillary palpomeres brown. Antennae brown or black, apical joints creamy-white. Pronotum and tegmina dark brown or black and apex of tegmina brownish yellow or brown. Legs brown or black. Abdomen reddish brown with dark brown margins or uniformly black. Cerci brown or yellowish brown.

Head with vertex punctate, with three smooth longitudinal stripes (Figs 13, 34). Eyes reniform and closer together than antennal sockets. Ocelli elliptical with distinct border. Face punctate, with some smooth interspaces and lines (Figs 13, 34). Antennae with numerous bristles, shorter than the body. Pronotum elongate, trapezoidal, with punctations and pubescence, margins thickened and raised, with three smooth longitudinal impressions and some smooth interspaces, disk not flat (Figs 7, 16, 28, 37). Both tegmina and hind wings fully developed, extending beyond the end of abdomen. Tegmina narrow with scattered erect pubescence; basal half sclerotized (Figs 8, 29), veins indistinct other than *Sc*, *R* and *CuP*; *CuP* ending at the middle of the hind margin, *A* almost invisible (Figs 17, 38). Hind wing with indistinct *Sc*, *M* bifurcated; *CuA* with 9 branches, of which four branch again (Figs 18, 39). Legs slender with dense pubescence. Tarsal claws symmetrical and unspecialized. First abdominal tergum specialized, with dense setae medially (Figs 9, 19, 30, 40).



Figures 1–6. *Protagonista lugubris* Shelford. **1–2** Male from Hainan: **1** dorsal view **2** ventral view **3–4** Female from Hainan: **3** dorsal view **4** ventral view **5–6** Male from Guangxi: **5** dorsal view **6** ventral view. Scale bars: 1.0 cm.

Supra-anal plate in ventral view symmetrical, hind margin convex with a weak medial indentation, two paraprocts large and nearly symmetrical (Figs 10, 20). Cerci conical and segmented (Figs 10, 31). Subgenital plate in dorsal view nearly symmetrical; styli modest, cylindroid (Figs 11, 21, 32, 42). Left phallomere consisting of three parts: L1, L2 and L3; sclerite L1 folded with a narrow and fingerlike terminus; sclerite L2 large, flat, and folded over posteriorly so that L2v lies on the ventral surface of the phallomere and L2d on the dorsal, sclerite L2d with a rough and curved margin, terminus acute, sclerite L2v with two acute ends posteriorly, of which (in dorsal view) the left one tapers towards the right and the other towards the left; sclerite L3 forming an elongate hook of which the curved part has a small spinous protuberance (Figs 12, 22, 33, 43) that is inconspicuous due to the observation angle. Right phallomere consisting of three parts: R1, R2 and R3; sclerite R1 expanding downward towards the left and with a serrate edge; R2, hook-like, expanding towards the left; the basal sclerite of R3 broad and slightly curved, joining with R2. The ventral phallomere (v.ph.) under



Figures 7–12. *Protagonista lugubris* Shelford, male from Hainan: **7** pronotum **8** tegmen **9** abdominal tergum 1, dorsal view **10** supra-anal plate and paraprocts, ventral view **11** subgenital plate, dorsal view **12** left phallomere and right phallomere. Scale bars: 1.0 mm.

the right phallomere, flat, posteriorly rounded, with a more or less sclerotized ventral surface (Figs 12, 22, 33, 43).

Female. Body black (Figs 3–4, 48). Eyes, ocelli and antennae similar to those of male. Vertex and face reddish brown. Labial palpi and maxillary palpomeres brown. Pronotum black. Abdominal terga black, but with the last segment brown. Abdominal sterna black and center reddish brown. Legs and cerci brown.

Vertex exposed, with 3 longitudinal shining stripes. Face punctuated. Tegmina short, just exceeding the metanotum, with punctures and scattered erect pubescence, heavily sclerotized with metallic shine. Hind wings much reduced. Legs slender, front femur Type A_2 . Each hind tibia with 2 rows of spines along outer margin. Hind meta-tarsus exceeding the remaining joints in length.

Supra-anal plate nearly symmetrical, roof shaped, the hind margin nearly blunt and round; paraprocts (*pp.*) broad and similar (Fig. 23). The juncture between the spermatheca plate and the anterior arch (*a.a.*) membranous and somewhat extensible (Fig. 23). First valve (*v.I*) falciform, sclerotized, with slender base and weakly sclerotized terminus (Figs 23, 24, 25); laterosternite IX (*ltst.IX*) large, fused to paratergites



Figures 13–22. *Protagonista lugubris* Shelford, male from Hainan: **13** head, frontal view **14** maxillary palps 3–5 **15** front femur **16** pronotum **17** tegmen **18** hind wing **19** abdominal tergum 1, dorsal view **20** supra-anal plate and paraprocts, ventral view **21** subgenital plate, dorsal view **22** left phallomere and right phallomere. Scale bars: 1.0 mm (**13, 15–16, 19–21**), 0.5 mm (**14, 22**), 2.0 mm (**17–18**).

(*pt.*); paratergites (*pt.*) slender (Fig. 23). Second valve (*v.II*) small and slender, basally fused, connecting to third valve (*v.III*) by membrane (Figs 23, 24, 26). Third valve larger than second valve but smaller than first valve, with weakly sclerotized and curved apex, basal portion fused and slightly raised (Figs 23, 24, 27). Anterior arch (*a.a.*) claviform, with tapering terminus (Fig. 23). Well developed basivalvula (*bsv.*) strongly sclerotized, fused with the anterolateral deflections of the spermatheca plate. Laterosternal shelf (*ltst.sh.*) flat, divided by common oviduct opening (*c.o.o.*). Subgenital plate in dorsal view symmetrical (Fig. 23).

Nymph. Body color, characters of pronotum and antennae similar to those of adults. Legs light brown. Cerci reddish brown.



Figures 23–27. *Protagonista lugubris* Shelford, female from Hainan, female genitalia: **23** posterior view and dorsal view of subgenital plate **24** valves and accessory sclerites, dorsal view **25** first valve, ventral view **26** second valve, ventral view **27** third valve, ventral view. Scale bars = 1.0 mm.

Infraspecific variation. The individual differences in morphological characters mainly involve: 1) the number and shape of smooth areas of pronotum (Figs 7, 16, 28, 37); 2) the dentate tine close to the largest tine of the serrated edge of sclerite *R1* sclerotized (Figs 12, 22) or not sclerotized (only one case, Figs 33, 43); 3) body color (Figs 1–2, 5–6). We provide pictures for detailed comparison (one male from Hainan, one male from Guangxi) illustrating the appearance of individual differences (Figs 1–2, 5–6, 7–12, 13–22, 28–33, 34–43). These infraspecific variations cannot separate the populations from each other into different species and the key morphological characters strongly suggest they are conspecific. However, their COI genes show a great genetic divergence among them: the standard barcoding sequence of one (Baisha) of the three Hainan populations has a distance of 3.0% and 3.1% from the other two (Wuzhishan, Baoting) respectively, and it is far distant (4.6%) from the Guangxi population, which in turn is very distinct from the two remaining Hainan populations (Wuzhishan, 6.1%; Baoting, 6.2%).

Male measurements (mm). Body length: 17.5–21.0. Total length including tegmen: 19.0-24.5. Pronotum length × width: $4.9-5.5 \times 5.5-6.5$. Tegmen length × width: $15.0-20.0 \times 5.0-6.5$.



Figures 28–33. *Protagonista lugubris* Shelford, male from Guangxi: 28 pronotum 29 tegmen 30 abdominal tergum 1, dorsal view 31 supra-anal plate and paraprocts broken, ventral view 32 subgenital plate, dorsal view 33 left phallomere and right phallomere. Scale bars: 1.0 mm (28, 30–33), 2.0 mm (29).

Female measurements (mm). Body length: 19.0-20.0. Pronotum length × width: $5.5-6.0 \times 6.0-6.5$. Tegmen length × width: $4.7-5.0 \times 4.7-5.0$.

Material examined. One male and one nymph, China: Hainan Prov., Baisha County, Yinggeling Natural Reserve, Nankai Station, in rotten wood, 21 April 2015, coll. Xinran Li (=Conlin McCat) and Zhiwei Qiu; one male, China: Hainan Prov., Mt. Diaoluoshan, 275m, 18°40.080'N, 109°53.998'E, 25 May 2014, coll. Shunhua Gui and Xinran Li (=Conlin McCat); one male, China: Hainan Prov., Baoting County, Maogan Township, 11–12 April 2015, coll. Qikun Bai; one female, China: Hainan Prov., Mt. Wuzhishan, 795m, 18–21 May 2014, coll. Shunhua Gui, Xinran Li (=Conlin McCat) and Jianyue Qiu; one male, China: Guangxi Aut. Reg., Guiping City, Longtan Park, 386 m, 23°31.140'N, 109°59. 510'E, 31 May–2 June 2014, coll. Shunhua Gui and Xinran Li (=Conlin McCat); one male and two females, China: Guangxi Aut. Reg., Fangchenggang City, Shangsi County, Shiwandashan Forest Park, 296 m, 28 June 2015, coll. Lu Qiu and Qikun Bai.

Distribution. China (new recored; Hainan, Guangxi); Vietnam.

Habitat. The adult *P. lugubris* were observed in shrubs at night by the collectors who also found the nymphs and adults in rotten wood. Their rugged pronotum with thickened and raised margins, which resembles that of Cryptocercinae and Panesthiinae is conducive to moving about in rotten wood. However, if *Protagonista* utilizes the wood tunneled by other organisms or if they bore the wood themselves is yet to be seen. Additionally, wood feeding has not been observed but is still a possibility in *Protagonista* and the other morphologically similar Archiblattinae.



Figures 34–43. *Protagonista lugubris* Shelford, male from Guangxi: 34 head, frontal view 35 maxillary palps 3–5 36 front femur 37 pronotum 38 tegmen 39 hind wing 40 abdominal tergum 1, dorsal view 41 supra-anal plate and paraprocts broken, ventral view 42 subgenital plate, dorsal view 43 left phallomere and right phallomere. Scale bars: 1.0 mm (34, 36–37, 40), 0.5 mm (35, 41–42), 2.0 mm (38–39).

Discussion

Validity of the name Archiblattinae. As explained in the introduction, the scientific names Archiblattinae and Planeticinae are synonyms. According the *Principle of Priority* in ICZN, Archiblattinae should be abandoned and the earlier name Planeticinae is

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valid although its type genus is no longer valid. But the name Planeticinae/–idae has been ignored for a long time and the substitute name Archiblattinae/–idae is in prevailing usage since Princis's (1965) catalogue; therefore the priority of the latter should be maintained based on the ICZN rule 40.2.

Taxonomic status of subfamily Archiblattinae and its genera. The subfamily Archiblattinae has a controversial taxonomic history since it was established. Although Kirby (1904) erected the subfamily Archiblattinae based on the genus Archiblatta, Shelford (1910), Hanitsch (1915) and Bruijning (1948) placed Archiblatta in Blattinae. Subsequent authors also have different suggestions on the taxonomic status of family Archiblattidae. Princis (1965) listed it as a family, but Grandcolas (1996) synonymized Archiblattidae with Blattidae and assigned Archiblatta and Catara to Blattidae. Roth (2003) suggested that Archiblatta, Catara and "?Protagonista" should be in their own subfamily because of the absence or greatly reduced femoral armament and reserved the subfamily Archiblattinae (Fam. Blattidae). Inward et al (2007) and Legendre et al (2015) found the subfamily Archiblattinae (Archiblatta) and Blattinae to be respectively monophyletic. Klass and Meier (2006) placed Archiblatta as sister to Polyzosteriinae + Blattinae. Djernæs et al. (2015) indicated that the structuring of Blattidae into the subfamily Polyzosteriinae (Drymaplaneta, Eurycotis), Archiblattinae (Archiblatta), and Blattinae (Periplaneta, Deropeltis) may be artificial since Archiblattinae were placed within Blattinae. In spite of the few studies on the Archiblattinae, our knowledge about the genera other than Archiblatta is still so lacking that it is reasonable to question their classification in Archiblattinae. There is uncertainty regarding the taxonomic status of Catara, Protagonista, and Eroblatta. When comparing the male genitalia of P. lugubris with those of Archiblatta hoeveni (illustrated by Klass 1997) and other species (e.g. Periplaneta americana, Periplaneta brunnea, Periplaneta ceylonica, Blatta orientalis, Neostylopyga rhombifolia, Melanozosteria nitida) in the subfamilies Blattinae and Polyzosteriinae, we find that the male genitalia of Archiblattinae and Blattinae are closer to each other than to those of Polyzosteriinae. Furthermore, *Protagonista* and *Archiblatta* are more similar to each other than they are to the genera of Blattinae. However, we failed to find independent, distinct morphological features separating the two taxa; thus the male genital differences between them might not be adequate as diagnostic characters in subfamily-group taxonomy. Our observations coincide, in a phylogenic sense, with Djernæs et al. (2015). However, Archiblattinae is easily distinguished from other blattid cockroaches by the special pronotum (hardened and rugose, sides thickened and not deflexed) and the special tibia which are extraordinarily cylindrical with sparse spines. Additionally, the cladistic results themselves are still in dispute. Yet we should not simply rely on the cladistic results solely to alter the classification, even if a widely accepted cladistic conclusion is demonstrated in the future. Therefore we propose to retain the validity of the subfamily Archiblattinae and the arrangement of the 4 genera mentioned above before a comprehensive taxonomic and phylogenetic study has been conducted. If done, this future study should on one hand confirm whether the subfamily Archiblattinae is monophyletic, and should on the other hand discern the relationships among the 4 genera and give an acceptable arrangement of them.



Figures 44–47. *Protagonista* species, holotypes and labels. 44 *P. lugubris*, male 45 *P. pertristis* stat. rev., female 46 *P. fusca*, now synonym of *P. pertristis*, male 47 *P. aterrima*, now synonym of *P. pertristis*, female. Scale bars: 5.0 mm.



Figures 48–49. 48 *Protagonista lugubris* Shelford, adult female from Longtan Park in Guiping, Guangxi, 2011.VIII.4, eating a wasp body near a light trap **49** *Protagonista lugubris* Shelford, adult male from Diaoluo Mt. in Lingshui, Hainan. Both photographed by XinRan Li (= Conlin McCat).

Synonymy of Protagonista lugubris. Hanitsch (1932) synonymized P. aterrima Hanitsch, 1929 with P. fusca Hanitsch, 1925. Bruijning (1948) synonymized 4 species (P. aterrima, P. fusca, P. pertristis Hanitsch, 1923 (Figs 44-47) and P. laeta Hanitsch, 1931) with P. lugubris Shelford, 1908, as he considered a slightly differing coloration not an important specific character that is coincident with Hanitsch (1932). We also consider the four nominal species P. aterrima, P. fusca, P. pertristis and P. laeta to be the same species, but separate from P. lugubris Shelford, 1908. We base this on evidence from holotypes, original descriptions and geographical distribution. They are all from a limited region (Sunda Shelf) far away from the localities of P. lugubris (Northern Vietnam and South China). We agree with Hanitsch's (1932) synonymy based on the holotypes: the distal half of coxae and the base of femora of P. aterrima are light testaceous, as is P. fusca. Meanwhile we indicate that Bruijning's (1948) viewpoint should be revised: P. aterrima, P. fusca, P. pertristis and P. laeta are identical indeed, but they are not synonyms of P. lugubris. Therefore P. aterrima, P. fusca and P. laeta are the junior synonyms of *P. pertristis*. *P. pertristis* is distinguished from *P. lugubris* by the following characters: 1) coxae of all the legs with the distal half and the base of femora are orange yellow on P. pertristis and P. fusca, but all the legs of P. lugubris are uniformly brown (Note: The character of difference in color depth of tegmina and the whole body has less taxonomic value, in accordance with Hanitsch (1932) and Bruijing (1948). However, the distinct coloration of part of the body (such as this case) should be the criteria for species differentiation.); 2) cerci of P. pertristis and P. fusca slender, yellow, but those of P. lugubris thicker and shorter, brown. In conclusion, the genus Protagonista is comprised of 2 species, P. lugubris Shelford, 1908 and P. pertristis Hanitsch, 1923, the latter with three junior synonyms: P. aterrima, P. laeta and P. fusca.

Taxonomic status of the genus *Eroblatta*. The genus *Eroblatta* was erected based on *Protagonista borneensis* Shelford, 1908 by Shelford (1910). The genera *Eroblatta* and *Protagonista* differed from each other in the spines on outer margin of tibiae according to his original description. *Eroblatta* has 3 rows of spines along outer margin of tibia,



Figure 50. Eroblatta borneensis (Shelford 1908), holotype and labels. Scale bar: 5.0 mm.

whereas there are only 2 rows in *Protagonista*. *Eroblatta* was also placed in Archiblattidae by Princis (1965) but Roth (2003) listed it under Blattinae without any explanation; within the period between these two publications (i.e. Princis 1965 and Roth 2003), works with respect to *Eroblatta* were absent, the change on its taxonomic arrangement is deemed to be simply an inadvertent error. After examining the holotype (Fig. 50) and according to original description, it is seen clearly that *Eroblatta borneensis* has the typical archiblattid pronotum (hardened and rugose with sides thickened and not deflexed) and the special tibia, which are extraordinarily cylindrical with sparse spines. Therefore *Eroblatta* is exactly, for the time being, a member of Archiblattinae.

Checklist of the synonyms of the species of Protagonista and Eroblatta

• .

Vietnam (Tonkin, type locality), South China
Sunda Shelf including Malay Peninsula (type locality)
Sarawak
Sumatra
Singapore
Borneo (Sarawak, type locality)

1	Tibia with 3 rows of spines along outer margin Eroblatta borneensi
_	Tibia with 2 rows of spines along outer margin (Protagonista)
2	Coxae of all legs with the distal half and the base of femora orange yellow
	Protagonista pertristi
_	All legs uniform in colour Protagonista lugubri

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Perisphaerinae Brunner von Wattenwyl and Hyposphaeria Lucas are valid names concealed by the unavailable names Perisphaeriinae and Perisphaeria Burmeister (Blattodea, Blaberidae)

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Abstract

The incorrect generic name *Perisphaeria* Burmeister, 1838 results from a mistake by Princis (1947). *Perisphaeria* is an incorrect spelling by Burmeister referring to *Perisphaerus* Serville, 1831. Princis (1947) incorrectly regarded *Perisphaeria* as proposed by Burmeister and as the senior synonym of *Hyposphaeria* Lucas, 1863, which is valid. The subfamilial name Perisphaeriinae based on *Perisphaeria* should be corrected to Perisphaerinae Brunner von Wattenwyl, 1865 based on *Perisphaerus*; but note Princis' Perisphaeriinae is based on his *Perisphaeria* Burmeister. The taxa involved are listed.

Keywords

cockroach, nomenclature, spelling, Perisphaerus, Perisphaera, Perisphaeriidae, Perisphaeridae

Introduction

Perisphaeriia and its type genus *Perisphaeria* are unavailable. *Perisphaeria* was never proposed as a new genus, but is an incorrect spelling of *Perisphaerus* Serville, 1831 by Burmeister (1838: 483). It is thus unavailable (ICZN 1999: Article 33.3), although it may be a Latinized spelling of *Périsphère* Serville, 1831, i.e. an unjustified emendation of *Perisphaerus*, by Burmeister (ICZN 1999: Article 33.2 and 33.5); the family name is therefore unavailable and should be corrected (ICZN 1999: Article 35.4.1). The genus *Perisphaeria* revised by Princis (1947: 51) refers to *Hyposphaeria* Lucas, 1863, which is valid but Princis regarded it as a junior synonym of *Perisphaeria* Burmeister of his own concept.

Perisphaeria was attributed to Burmeister by Princis (1947: 51). Here arises the question: did Burmeister establish such a genus or is it simply an unjustified emendation? Burmeister (1838: 483) added an asterisk to Perisphaeria with which he usually indicated a new taxon. However, it was normal practice for Burmeister to then provide an etymology for the proposed new name, but this was not the case for Perisphaeria, suggesting that this genus was not described as new. Secondly, Burmeister (1838: 484) included four subgenera in *Perisphaeria*, of which the first one (nominotypical subgenus) is *Perisphaeria* Serv., and doubted their ability to roll into a ball as described by Serville, whilst Serville (1831: 44) used this as a diagnostic character and as the etymology for his *Perisphaerus*. Thus Burmeister must have his *Perisphaeria* attributed to Serville was in fact Perisphaerus. Thirdly, Perisphaeria is not an emendation of Perisphaerus according to the rules of the Code (ICZN 1999: Article 33.2.1), i.e., there is no explicit statement of intention, Perisphaerus was not cited and replaced, and no similar case of treatment is found in the same publication. Therefore, Perisphaeria is deemed to be an incorrect subsequent spelling and thus unavailable (ICZN 1999: Article 33.3). It can be speculated that the asterisk annotation to *Perisphaeria* (Burmeister 1838: 483) was not only used by Burmeister to denote a new name, but also to denote names with new scope or definition, i.e. extending the scope of *Perisphaerus* to include species from both Asia and Africa. The species Burmeister (1838) described were from Africa and not Asia, from which Serville (1831: 44) described Perisphaerus armadillo; that is why he did not accept Serville's words. It is not impossible, however, that this asterisk is simply a mistake, especially when such a mark is absent next to the Perisphaeria in the key (Burmeister 1838: 481).

We speculate that *Perisphaeria* may be, in a practical but not a nomenclatural sense, a Latinized version of *Périsphère* Serville, 1831. In other words, an emendation of *Perisphaerus* rather than an incorrect spelling of *Perisphaerus*, because French names seemed to be more formal at that time. However, Serville (1831: 44) himself gave a Latinized (i.e. valid) version which was exactly *Perisphaerus*, just following PÉRISPHÈRE and followed by the Greek etymology; but such a change in spelling by Burmeister is not demonstrably intentional, thus not an emendation in nomenclatural sense (ICZN 1999: Article 33.2.1). Similarly, *Perisphaera* is also a Latinized version of *Périsphère*, being used by Serville (1839: 132) himself for the first time. The word ball is a feminine noun in

both French (sphère) and Latin (*sphaera*); therefore *Perisphaerus* is a result of incorrect Latinization by Serville (1831) when he Latinized *Périsphère* the moment he proposed it. Subsequently he realized that and re-Latinized the French name in a later paper (Serville 1839). However, *Perisphaerus* should remain in use rather than *Perisphaera* since incorrect Latinization is not to be corrected (ICZN 1999: Article 32.5.1).

No matter which name, or to be more accurate, which spelling-Perisphaerus, Perisphaera or Perisphaeria-the family name is based on, the name established by Brunner von Wattenwyl (1865: 303) should be corrected to Perisphaeridae (ICZN 1999: Article 32.5.3.3). Note also that Brunner v. W. (1865) proposed it at the rank of tribe, which should be corrected to Perisphaerini under current nomenclature. The situation is further complicated by Princis (1947: 51) who attributed Perisphaeria to Burmeister and regarded it as restricted to African species, whilst the Asian species were re-combined with Perisphaerus Serville. Princis (1960: 438) subsequently included Perisphaeria Burmeister in Perisphaeriidae and established a separate family, Derocalymmidae, to include Perisphaerus. As a result, the true type genus of Perisphaeriidae was transferred to another family, but one of its incorrect spellings and wrong authorship remained; Perisphaeriidae is thus no longer correct. Perisphaeriidae and Derocalymmidae are now considered synonyms (Roth 2003), whilst Princis' erroneous use of the genus-group names has become widespread since his catalogue (Princis 1964). It is noteworthy that Princis' Perisphaeriidae has the same spelling as Perisphaeriidae Brunner v. W. that was based on the incorrect spelling *Perisphaeria* Serville. These two names, identical in spelling and similar in scope, are inherently different.

Before Princis (1947), most authors were well aware of the concept of these generic names (spellings), and cited correct authorship (i.e. Serville) of Perisphaerus when referring to it under its incorrect spelling (Perisphaeria), though an exception is Fischer (1853: 94) who attributed Perisphaeria to Burmeister. However, he gave Perisphaera Serv. as a synonym, which suggests that Fischer understood well the concept of them but cited an incorrect spelling with its author instead of the original spelling with its author. Lucas (1863: 408) recognized Burmeister's Perisphaeria as the same name as Perisphaera Serville, but suggested that they cannot refer to each other, i.e., he disputed the extension of its scope. Lucas stated that Perisphaera should be restricted to the Asian species which can roll into a ball. Lucas proposed a new genus Hyposphaeria Lucas, 1863 to include African species that do not roll into a ball and included species described by Burmeister. Brunner v. W. (1865), Walker (1868), Saussure and Zehntner (1895) and Kirby (1904) all treated Perisphaeria Serville as valid and provided synonyms including Perisphaera and/ or Perisphaerus; it is inferred from this that they accepted Burmeister's spelling to be the valid name of the genus proposed by Serville, and that Perisphaeria and Perisphaeriidae were once in prevailing usage. Brunner v. W. (1865) and Walker (1868) did not mention Hyposphaeria, while Saussure and Zehntner (1895) agreed with Lucas and included only Asian 'rollable' species in Perisphaeria Serville. However, they did not adopt Hyposphaeria since they believed species in this genus should be placed in various genera. Kirby (1904) in his catalogue treated Hyposphaeria as a valid name and included four species in it, with H. stylifera (Burmeister, 1838) designated as type species.

In summary, *Perisphaerus* Serville, 1831 is a valid name and includes the 'rollable' species, mostly from the Oriental Region, with *Perisphaera* and *Perisphaeria* as unavailable spellings, although the latter spelling was once enlarged in its scope and widely used. *Hyposphaeria* Lucas, 1863 is a valid name and includes the African 'unrollable' species, of which the early ones were described under *Perisphaeria* by Burmeister (1838). This name was once incorrectly synonymized with the unavailable name *Perisphaeria* Burmeister by Princis (1947). Perisphaeridae Brunner v. W., 1865 is a valid family name based on *Perisphaerus* Serville. The Perisphaeriidae used by Princis has the same spelling but is not identical since this name was based on *Perisphaeria* Burmeister which is a consequence of his misunderstanding.

Princis' erroneous revision of the names mentioned above should be no longer adopted.

Serville signed his name as J. G. Audinet-Serville in his published works and some authors attributed the scientific names established by him to Audinet-Serville; however, cockroach taxonomists (e.g. those mentioned above) prefer Serville in citation and authorship of names, and as such this convention is followed to avoid ambiguity. The abbreviation Brunner v. W. for Brunner von Wattenwyl is used for brevity.

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Serville. [See: Audinet-Serville]

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Appendix

Synonymy and taxonomic inclusion of names mentioned

Family Blaberidae

Subfamily Perisphaerinae Brunner von Wattenwyl, 1865

- Perisphaeridae Brunner von Wattenwyl, 1865: 302, as a tribe, type genus *Perisphaeria* Serv..
- Perisphaeridae Walker, 1868: 168, type genus *Perisphaeria*. [not Perisphaeridae Brunner von Wattenwyl]
- Périsphaeriens: Saussure and Zehntner 1895: 1, as a tribe.
- Perisphaeriinae: Kirby 1904: 179.
- Derocalymmidae Princis, 1960: 438; Roth 2003: 102, as a synonym of Perisphaeriinae.
- Derocalymminae Princis, 1960: 438; Roth 2003: 102, as a synonym of Perisphaeriinae.
- Perisphaeriidae Princis, 1960: 438, type genus Perisphaeria Burmeister.

- Perisphaeriinae Princis, 1960: 438, type genus *Perisphaeria* Burmeister; Roth 2003: 41.
- Genera included (Roth 2003): Bantua, Compsagis, Cyrtotria, Derocalymma, Ellipsica, Elliptoblatta, Gymnonyx, Hostilia, Hyposphaeria (sic: Perisphaeria Burmeister), Laxta, Neolaxta, Perisphaerus, Pilema, Platysilpha, Poeciloblatta, Pseudoglomeris, Pseudocalolampra, Trichoblatta, Zuluia.

Genus Perisphaerus Serville, 1831

- *Perisphaerus* Serville, 1831: 44, type species *P. armadillo* Serville, 1831, by monotypy; Princis 1947: 51.
- *Perisphaeria*: Burmeister 1838: 483; Lucas 1863: 408; Brunner von Wattenwyl 1865: 303; Walker 1868: 168; Saussure and Zehntner 1895: 12, 32; Kirby 1904: 189.
- *Perisphaera*: Serville 1839: 132; Fischer 1853: 94, as a synonym of *Perisphaeria* Burmeister; Lucas 1863: 408; Brunner von Wattenwyl 1865: 303; Walker 1868: 168.
- Perisphaeria Burmeister: Fischer 1853: 94.
- Species included (Princis 1964; 1971; Roth 2003): aeneus, altus, armadillo, aterrimus, brunneri, contiguus, cotesianus, flavicornis, flavipes, flexicollis, glomeriformis, inaequalis, multipunctatus, pubescens, punctatus, rubescens, semilunatus.

Genus Hyposphaeria Lucas, 1863

Hyposphaeria Lucas, 1863: 408; Kirby 1904: 193, *H. stylifera* (Burmeister, 1838) was selected as type species.

Perisphaeria Burmeister: Princis 1947: 51.

Species included (Princis 1964; 1971; Roth 2003): aspera, basuto, bicolor, carlgreni, guillarmodi, hancocki, hirta, hirtula, micans, mucronata, nodosa, peringueyi, pilifera, pilosa, rudebecki, ruficornis, saussurei, saxicola, scabra, scabrella, stylifera, substylifera, verrucosa, virescens. RESEARCH ARTICLE



New cave-dwelling species of Tomoceridae from China, with a study on the pattern of mesothoracic bothriotricha in Tomocerinae (Collembola, Entomobryomorpha)

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Abstract

Two new troglobitic species of Tomoceridae are described from Guizhou and Guangxi provinces, China. *Tomocerus tiani* **sp. n.** resembles *Tomocerus kinoshitai* Yosii, 1954, *Tomocerus caecus* Yu & Deharveng, 2015 and *Tomocerus similis* Chen & Ma, 1997 but differs from them mainly in the body colour, the cephalic chaetotaxy and the number of manubrial pseudopores. *Monodontocerus* **sp. n.** is similar to *Monodontocerus mulunensis* Yu, Deharveng & Zhang, 2014 but is different from the latter in the body colour, the length of antennae, the number of ungual teeth and the chaetotaxy on Abd. III and Abd. IV. Special remarks are made on the mesothoracic bothriotricha in Tomocerinae.

Keywords

Tomocerus, Monodontocerus, troglobitic, Tomocerinae

Introduction

Since the discovery of *Tritomurus scutellatus* Frauenfeld, 1854 in Slovenian caves, troglobitic Tomoceridae have frequently been reported in Asia, Europe and North America. Besides the troglobitic genera *Tritomurus* Frauenfeld, 1854 and *Lethemurus* Yosii, 1970, some other main groups of Tomoceridae have also been found with cave dwellers, and several genera, i.e. *Monodontocerus* Yosii, 1955, *Plutomurus* Yosii, 1956 and *Aphaenomurus* Yosii, 1956 have mainly or usually been found in caves. However, despite of several highly troglomorphic species, for example *Tritomurus falcifer* Cassagnau, 1958 and *Tritomurus veles* Lukić, Houssin and Deharveng, 2010 which are both eyeless and have very elongated antennae and claws, other cave tomocerids do not exhibit distinct troglomorphic characters: most of them have short to moderate antennae, normal or slightly elongated claws and a full set of 6+6 eyes for Tomocerinae, only the sizes of eyes are usually smaller than those of the edaphic species, and the tenent hair is often pointed as in other cave Collembola.

Four cave tomocerids have previously been reported in China, including *Tomocerus caecus* Yu & Deharveng, 2015, *Monodontocerus absens* Yu, Deharveng & Zhang, 2014 and *Monodontocerus mulunensis* Yu, Deharveng & Zhang, 2014 from Guangxi Province, and *Monodontocerus trigrandis* Yu, Deharveng & Zhang, 2014 from Hunan Province. The present paper reports two new species of Tomoceridae discovered in caves in the south-west karst regions of China. Both of the new species have small eyes and pointed tenent hair, but neither of them is highly troglomorphic. *Tomocerus tiani* sp. n. bears some unusual characters, including the single mesothoracic bothriotrichum, which lead to a comprehensive examination of the different genera of Tomocerinae.

Materials and methods

Specimens were collected with aspirators and preserved in 99% ethanol. For detailed morphological study, specimens were cleared in Nesbitt's fluid and mounted in Marc André II solution. For some specimens the furca, the ventral tube and the legs were cut off from the trunk and mounted separately for detailed observation. The slide-mounted specimens were studied using a Nikon Ni microscope. Photos were taken using Nikon DS-Fi1 cameras mounted respectively on Nikon SMZ1000 stereomicroscope and Nikon Ni microscope.

Fjellberg (2007) is followed for maxillary lamellae numbering, Yu et al. (2014) for the pattern of cephalic dorsal chaetotaxy and Christiansen (1964) for body macrochaetotaxy. The description of the body chaetotaxy refers to one side only since in most case it is symmetric. The exact morphology of each chaeta was unclear due to shedding. The dental spines formula follows that of Folsom (1913), in which the dental spines are arranged from basal to distal, with a slash indicating the separation between basal and medial subsegments and the Roman numerals referring to spines that are noticeably larger. If not mentioned specially, all descriptions are based on fully developed individuals.

Abbreviations

Ant.	antennal segment	
PAO	postantennal organ	
Th.	thoracic segment	
Abd.	abdominal segment	

Institutional acronyms

NJAU Nanjing Agricultural University, Nanjing, China

Results

Tomocerus tiani Yu, sp. n.

http://zoobank.org/722C6CC5-F644-477E-91D4-5BFC6759A17B Figs 1A, B, 2, 3

Type-locality. China, Guizhou Province: Zunyi, Suiyang County, Wenquan Town, Guihua Village, Hejiao Cave, inside cave, 7 November 2008, Mingyi Tian leg.

Type-specimens. Holotype male (labelled 15cave15-1) and paratype juvenile (labelled 15cave15-2) on slide. Deposited in NJAU.

Diagnosis. Species similar to *Tomocerus kinoshitai* Yosii, 1954, with short antennae, multi-furcated dental spines and apically curved mucro. Body length approximately 3.0 mm, with purplish grey pigment all over; antennae approximately half as long as body; eyes small; terminal hair of maxillary outer lobe with a small basal denticle; Th. II with only one bothriotrichum; tenent hairs pointed; unguis with two teeth, baso-internal ridges at approximately 1/2 distance from base; manubrium with 12–17 pseudopores on each side; dental spines formula as 4/2, II; dens dorsally with only a few feather-like chaetae; mucro without intermediate tooth. Cave-dwelling species.

Description. Body length 2.9 mm. Body with uniform purplish grey pigment and unpigmented patches, appendages paler. Eye patches black (Fig. 1A). Types of scales and chaetae typical for Tomocerinae.

Antennae approximately half length of body. Length ratio of antenna segments as I:II:III+IV = 1.0:1.9–2.0:9.6–9.7. Only dorsal side of Ant. I and Ant. II scaled, Ant. III+IV unscaled. PAO not seen. Eyes 6+6, relatively small. Labral formula as 4/5, 5, 4. Distal edge of labrum with four curved spine-like papillae. Mandibular heads asymmetrical, the left one with four teeth and the right one with five, left molar plate distally with a tapered tooth (Fig. 2A). Maxillary lamella five without beard-like appendage. Maxillary outer lobe with trifurcate palp, one basal chaeta and four sublobal hairs; terminal hair with a small basal denticle (Fig. 2B). Both dorsal and ventral sides of head scaled. Cephalic dorsal macrochaetotaxy: anterior area: 2, 2; interocular area: 2, 4, central uneven macrochaeta absent; postocular area: 2+2; posterior area: 0. Pos-



Figure 1. Tomocerus tiani sp. n. and Monodontocerus cinereus sp. n. **A** and **B**, Tomocerus tiani sp. n. **A** appearance in alcohol (lateral view) **B** dorsal chaetae on dens (dorsal view, arrows pointing to feather-like chaetae) **C** Monodontocerus cinereus sp. n. **C** appearance in alcohol (lateral view). Scale bars: 1000 μ m (**A**, **C**), 50 μ m (**B**).

terior margin of head with ca. 70 small chaetae (Fig. 2C). Mentum with five chaetae, submentum with numerous chaetae.

Pattern of body chaetotaxy as in Fig. 2D. Number of bothriotricha as 1, 1/0, 0, 1, 2, 0, 0 on Th. II–Abd. VI. Macrochaetae densely arranged along anterior margin of Th. II (not shown in figure). Th. II with a row of macrochaetae behind anterior margin. Number of macrochaetae or large mesochaetae in the posterior row as 3, 3/3, 3, 4, 3, 4 (3 dorsal+1 lateral) from Th. II to Abd. V. Th. II with four central and one lateral macrochaetae, postero-central chaeta near pseudopore; Abd. III with two anterior macrochaetae; Abd. IV with one lateral macrochaeta; Abd. VI with numerous chaetae of moderate size. Most mesochaetae laterally and posteriorly on terga. Pseudopores near the axis of terga, number of them as 1, 1/1, 1, 1, 0, 0 from Th. II to Abd. VI.

Trochantero-femoral organ with 1, 1 small slender chaetae (Fig. 2E). Front, middle and hind tibiotarsus ventrally with 0, 0, 2 pointed spine-like chaetae (Fig. 2F). Each tibiotarsus with a distal whorl of 11 chaetae, ventral six as ordinary chaetae, dorsal five modified: tenent hair thin and pointed, approximately 0.33 times as long as inner edge of unguis; two accessory chaetae small, longer than pretarsal chaetae; two guard chaetae of same morphology and size as tenent hair. Unguis slender, with



Figure 2. *Tomocerus tiani* sp. n. **A** mandibular heads (dorsal view) **B** palp of left maxillary outer lobe (dorsal view); **C** cephalic dorsal chaetotaxy (dorsal view, circle: socket of chaeta, same as below) **D** dorsal chaetotaxy of Th. II–Abd. V (dorsal view, circle with a slash: pseudopore, wavy line: bothriotricha, same as below) **E** trochantero-femoral organ (inner view) **F** hind tibiotarsus (lateral view, showing spine-like inner chaetae) **G** hind claw (lateral view, t: tenent hair, a: accessory chaeta, g: guard chaeta, p: pretarsal chaeta, same as below).

baso-internal ridges at approximately 1/2 distance from base; lateral teeth pointed, of moderate size. Inner edge of unguis with one basal and one central minute teeth. Unguiculus rather slender, approximately 0.5–0.72 times as long as unguis, its inner edge with one corner tooth. Pretarsus chaetae 1+1 (Fig. 2G).



Figure 3. *Tomocerus tiani* sp. n. **A** tenaculum (anterior view) **B** right side of manubrium (dorsal view, showing prominent dorsal chaetae, T-shaped mark: socket of scale, same as below) **C** disto-external corner of manubrium (dorsal view) **D** basal and middle subsegments of dens (dorsal view, showing dental spines and prominent dorso-basal chaeta) **E** feather-like dental chaeta (lateral view) **F** mucro (lateral view).

Anterior face of ventral tube with scales, posterior face and lateral flaps unscaled, anterior face with ca. 25 chaetae on each side, posterior face with ca. 30 chaetae, each lateral flap with ca. 15 chaetae. Rami of tenaculum with 4+4 teeth, anterior face with one chaeta and without scale (Fig. 3A). Length ratio of furca segments as manubrium : dens : mucro = 2.5 : 3.6–3.7 : 1.0. Manubrium ventrally scaled, without chaetae, laterally with large round scales and 7–9 strong chaetae; dorsal chaetal stripe with ca. 200 chaetae of different sizes, including 2+2 pointed prominent chaetae; inner side of chaetal stripe with several scales; pseudopores 12–17 on each side (Fig. 3B); external corner chaeta as a microchaeta (Fig. 3C). Dens basally with a pointed prominent dorsal chaeta, without large modified inner scale or strong outer chaetae. Dental spines formula as 4/2, II; spines with moderate to large sized denticles around basal half (Fig. 3D). Dens dorsally with ordinary chaetae, swollen spine-like ciliated chaetae and a few feather-like chaetae (Figs 1B, 3E), ventrally with scales and several apical chaetae.

Mucro elongated, distally curved and multi-setaceous; both basal teeth with proximal lamellae, outer tooth with a toothlet; apical and subapical tooth subequal; two dorsal lamellae running from subapical tooth, outer lamella ending in inner basal tooth, inner lamella ending at base of mucro; without intermediate teeth (Fig. 3F).

Etymology. Named after the collector Prof. Mingyi Tian.

Remarks. Tomocerus tiani sp. n. is similar to Tomocerus caecus Yu & Deharveng, 2015, Tomocerus kinoshitai (materials from Changbai Mountain, China) and Tomocerus similis Chen & Ma, 1997 (type materials) in the length of antennae, the general pattern of chaetotaxy on Th. II, the number and position of spine-like tibiotarsal inner chaetae, the size of external corner chaeta on the manubrium, the type and general arrangement of dental spines and the shape of mucro, but it is clearly different from the first species in having eyes and pigment, and is different from the other two species mainly in the body colour, the cephalic chaetotaxy, the sharply pointed tibiotarsal strong inner chaetae and tenent hair, and the more slender unguiculus; besides, with similar body size, Tomocerus tiani sp. n. has more manubrial pseudopores than the three known species. The small basal denticle of the terminal hair of maxillary outer lobe is so far unique to Tomocerus tiani sp. n. and is useful for diagnosis if dissected and exposed carefully. The baso-internal ridges of unguis are located at approximately 1/2 distance from the base in Tomocerus *tiani* sp. n., whereas in most other species the distance between the ridges and the base is only 1/3 or less. The dorsal dental chaetae in Tomocerus tiani sp. n. is also characteristic, that the dense stripes of feather-like chaetae in most other tomocerids are almost replaced by ordinary chaetae and swollen serrated chaetae, leaving only a few feather-like ones. Similar condition was also reported in Tomocerus kinoshitai and Tomocerus caecus that some spine-like chaetae are present on dens (Yosii 1967, Yu and Deharveng 2015).

The juvenile specimen is almost identical to the adult in most characters, including the macrochaetotaxy, the number of teeth on claws and the dental spines formula. However, some characters on manubrium are distinctly different between juvenile and adult. In the juvenile specimen, there are ca. 80 dorsal chaetae on each side of manubrium, the number of pseudopores is only 4–5 on each side, and the external corner chaeta is as large as a mesochaeta in the dorsal chaetal stripe. These differences provide interesting information in the postembryonic development of manubrium in Tomocerinae, and indicate these characters are not suitable for the identification of immature specimens at different instars.

Monodontocerus cinereus Yu, sp. n.

http://zoobank.org/7C9C6A04-387B-4558-ADCC-B9C0CF7B8F2F Figs 1C, 4, 5

Type-locality. China, Guangxi Province: Hechi, Duan County, Chengjiang Township, Ganwan Village, Nongsi Cave, 23°56'24"N, 108°10'12"E, alt. ca. 270 m, inside cave, 25 July 2015, Jujian Chen, Xinhui Wang and Mingruo Tang leg.



Figure 4. Monodontocerus cinereus sp. n. A mandibular heads (dorsal view) B maxillary lamella 5 (dorsal view) C cephalic dorsal chaetotaxy (dorsal view) D dorsal chaetotaxy of Th. II–Abd. V (dorsal view)
E trochantero-femoral organ (inner view) F hind tibiotarsus (lateral view, showing spine-like inner chaetae)
G middle claw (lateral view).

Type-specimens. Holotype male (labelled 15cave6-1) and two paratypes female (labelled 15cave6-2 and -3) on slides, one paratype (labelled 15cave6) in alcohol. Deposited in NJAU.

Diagnosis. Typical *Monodontocerus* species with multi-furcated dental spines and single mucronal basal tooth. Body length approximately 4.0 mm, with light grey pigment all over; antennae slightly shorter than body; eyes small; chaetotaxy typical for the genus; tenent hair pointed; unguis with 2–4 teeth; manubrium with 28–35 pseudopores on each side; dental spines formula as 4, II/6, I, 3, I or 5, I/6, I, 3, I; mucro with 3–4 intermediate teeth. Cave-dwelling species.

Description. Body length 3.9–4.1 mm. Body colour uniformly light grey with unpigmented patches, appendages paler. Eye patches black. (Fig. 1C). Scales and chaetae of Tomocerinae type.

Antennae approximately 0.7–0.9 times as long as body. Length ratio of antenna segments as I:II:III:IV = 1.0:1.7–2.0:10.5–11.0:1.8. Only dorsal side of Ant. I and Ant. II scaled, Ant. III and Ant. IV unscaled. PAO not seen. Eyes 6+6, relatively small. Mouthparts typical for Tomocerinae. Labral formula as 4/5, 5, 4. Distal edge of labrum with four curved spine-like papillae. Mandibular heads asymmetrical, the left one with four teeth and the right one with five, left molar plate distally with a tapered tooth (Fig. 4A). Maxillary lamella five without beard-like appendage, basal teeth elongated (Fig. 4B). Maxillary outer lobe with trifurcate palp, one basal chaeta and four sublobal hairs. Both dorsal and ventral sides of head scaled. Cephalic dorsal macro-chaetotaxy: anterior area: 2, 2; interocular area: 2, 3, central uneven macrochaeta present; postocular area: 2+2; posterior area: 2. Posterior margin of head with 40–50 small chaetae (Fig. 4C). Mentum with five chaetae, submentum with numerous chaetae.

Pattern of body chaetotaxy as in Fig. 4D. Number of bothriotricha as 2 (1?), 1/ 0, 0, 1, 2, 0, 0 on Th. II–Abd. VI. Macrochaetae densely arranged along anterior margin of Th. II (not shown in figure). Th. II with a row of macrochaetae behind anterior margin. Number of macrochaetae or large mesochaetae in the posterior row as 3, 3/ 3, 3, 4, 2, 4 (3 dorsal+1 lateral) from Th. II to Abd. V. Th. II with three central macro-chaetae arranged approximately in a line, postero-central chaeta near pseudopore; Abd. III with two anterior macrochaetae; Abd. IV with two lateral macrochaetae; Abd. VI with numerous chaetae of moderate size. Most mesochaetae laterally and posteriorly on terga. Pseudopores near the axis of terga, number of them as 1, 1/ 1, 1, 1, 0, 0 from Th. II to Abd. VI.

Trochantero-femoral organ with 1, 1 small slender chaetae (Fig. 4E). Front, middle and hind tibiotarsus ventro-distally with 0, 0, 2 blunt spine-like chaetae (Fig. 4F). Each tibiotarsus with a distal whorl of 11 chaetae, ventral six as ordinary chaetae, dorsal five modified: tenent hair very small and pointed; two accessory chaetae subequal to tenent hair, longer than pretarsal chaetae; two guard chaetae thin and pointed, approximately three times as long as tenent hair. Unguis slender, with baso-internal ridges approximately 1/3 distance from base; lateral teeth pointed, of moderate size. Inner edge of unguis with one basal and 1–3 more distal teeth. Unguiculus slender,



Figure 5. *Monodontocerus cinereus* sp. n. **A** tenaculum (anterior view) **B** right side of manubrium (dorsal view) **C** disto-external corner of manubrium (dorsal view) **D** basal and middle subsegments of dens (showing dental spines) **E** mucro (lateral view).

approximately 0.6–0.7 times as long as unguis, its inner edge with 1–2 corner tooth. Pretarsus chaetae 1+1 (Fig. 4G).

Ventral tube with scales on both anterior and posterior faces, lateral flaps unscaled, anterior face with ca. 70 chaetae on each side, posterior face with ca. 160 chaetae, each lateral flap with ca. 100 chaetae. Rami of tenaculum with 4+4 teeth, anterior face with one chaeta and without scale (Fig. 5A). Length ratio of furca segments as manubrium : dens : mucro=3.7–3.8 : 5.1–5.3 : 1.0. Manubrium ventrally scaled, without chaetae, laterally with large round scales and 9–10 strong chaetae; dorsal chaetal stripe with ca. 280 chaetae of different sizes, without distinct prominent chaetae; dorsal scales mixed with chaetae in chaetal stripe; pseudopores 28–35 on each side (Fig. 5B); external corner chaeta as a small mesochaeta in chaetal stripe (Fig. 5C). Dens basally without large modified inner scale or strong outer chaetae. Dental spines formula as 4, II/6,

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I, 3, I or 5, I/6, I, 3, I, proximal spines enlarged gradually; each spine consisting of a strong trunk surrounded by several denticles near base (Fig. 5D). Dens dorsally with ordinary chaetae and feather-like chaetae, ventrally with scales. Mucro elongated and multi-setaceous; single basal tooth with proximal lamella; apical and subapical tooth subequal; two dorsal lamellae running from subapical tooth, outer lamella ending at basal tooth, inner lamella ending at base of mucro; outer lamella with 3–4 moderate sized intermediate teeth (Fig. 5E).

Etymology. Named for its light grey body colour, from the Latin *cinereus*, meaning ash-coloured.

Remarks. Within the genus, *Monodontocerus cinereus* sp. n. is more similar to *Monodontocerus mulunensis* Yu, Deharveng & Zhang, 2014 in the cephalic chaetotaxy, the pointed tenent hair and the number of mucronal intermediate teeth, but can be distinguished from the latter by having longer antennae, fewer teeth on unguis and more macrochaetae on Abd. III and Abd. IV. In alcohol the new species can be identified from other known species of *Monodontocerus* by the grey body colour.

Discussion

Tomocerus tiani sp. n. has only one bothriotrichum on Th. II, while there are two in most other *Tomocerus* species observed previously. The pattern of bothriotricha is significant for the taxonomy and phylogeny of Collembola (Szeptycki 1979, Soto-Adames et al. 2008). However, the exact pattern of mesothoracic bothriotricha in Tomocerinae has not been resolved though the number of them was commonly reported as either two (Yosii 1956, 1967, Fjellberg 2007) or one (Christiansen 1964, Chen and Ma 1997).

There are two main obstacles to determining the pattern of mesothoracic bothriotricha in Tomocerinae. Firstly, these long, thin and ciliated chaetae, as well as the macrochaetae, are easily lost during specimen collection and slide preparation, leaving only the sockets. The sockets of bothriotricha are usually characteristic for their round shape and small size, but sometimes can still be confused with the sockets of macro- or mesochaetae. Secondly, in case of two bothriotricha present they are arranged transversely, and the outer one is usually near the lateral margin of the tergum, thus could possibly be omitted when the margin is wrinkled or damaged. To avoid those disadvantages, we examined the specimens with almost intact coating of chaetae, most of which are pre-molting specimens with new chaetae under the old cuticle.

In the observed species, there are three main patterns of mesothoracic bothriotricha, which appear to be relevant to the generic division except for several species of *Tomocerus* and *Tomocerina*. Pattern A: all species of *Tomocerus ocreatus* complex (Zhang et al. 2014), *Tomocerus nigrus* Sun, Liang & Huang, 2006 and *Tomocerus jilinensis* Ma, 2011 have two mesothoracic bothriotricha, and the socket of the inner one is usually larger, thus is more similar to that of a macrochaeta (Fig. 6A). Pattern B: *Tomocerus kinoshitai*, *Tomocerus similis*, *Tomocerina annamitica* Yu, Man & Deharveng, 2016, *Tomocerus laxalamella* Lee, 1975 (materials from Changbai Mountain,



Figure 6. Dorsal view of the left antero-lateral corner of mesonotum (arrows directing anterior). **A** *To-mocerus* sp. (*ocreatus* complex) from China (b: bothriotricha, same as below) **B** *Tomocerus similis* from China (mb: macrochaeta at the location of outer bothriotricha, same as below) **C** *Tomocerina annamitica* from Vietnam **D** *Tomocerus laxalamella* from China (with bothriotricha-like macrochaeta two times magnified) **E** *Pogonognathellus* sp. from France. Scale bars: 50 μm (**A**, **B**, **C**, **E**), 100 μm (**D**).

China), *Tomocerina varia* Folsom, 1899 (materials from Changbai Mountain, China) and *Tomocerina tianshanensis* Ma, Chen & Christiansen, 2003 have only one bothriotrichum at approximately the place of the inner bothriotrichum in other *Tomocerus*, and a macrochaeta is present instead of the outer bothriotrichum (Fig. 6B, C, D). In the first two species this macrochaeta is longer and apically more tapered than the adjacent macrochaeta (Fig. 6B); in *Tomocerina annamitica* this macrochaeta is slender

(Fig. 6C), and its socket is very similar to that of a bothriotrichum; in the last three species the macrochaeta is large, rather elongated, pointed, and is more ciliated than other ordinary chaetae, forming a bothriotricha-like macrochaeta (Fig. 6D). Pattern C: in *Pogonognathellus heterochros* Wang, Yu & Zhang, 2013, *Pogonognathellus mai* Wang, Yu & Zhang, 2013 and *Pogonognathellus* sp. from France, there is only one bothriotrichum, and no distinctly special macro- or mesochaetae is present nearby (Fig. 6E).

This study has revealed several distinct patterns of mesothoracic bothriotricha in Tomocerinae, and has proved their taxonomic importance. However, since our study covered mostly Asian edaphic species, we have probably not exhausted the variability of this character among Tomocerinae. The exact pattern in *Monodontocerus* has not been successfully determined because of the lack of specimen with intact bothriotricha, though the sockets indicate pattern A more possible. For *Pogonognathellus* more examination is required since several species were described having two mesothoracic bothriotricha (Fjellberg 2007, Park et al. 2011). On the other hand, given the thoracic bothriotricha do not exist in the primary chaetotaxy (Szeptycki 1972, Yu et al. 2015), they could either be secondary elements or be transformed from certain primary chaetae. Tracing the postembryonic development of chaetotaxy will help to evaluate the true significance of thoracic bothriotricha for taxonomy, while study on the homology of chaetae will be a next step to review the current taxonomic system of Tomocerinae.

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RESEARCH ARTICLE



Description of a new species of the genus Monelata Förster, 1856 from China (Hymenoptera, Diapriidae)

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Abstract

A new species of the genus *Monelata* Förster, 1856, *Monelata truncata* **sp. n.**, is described and illustrated from Yunnan Province, China. This is the third Oriental species assigned to this genus. A key to Oriental species of the genus is provided.

Keywords

Hymenoptera, Diapriinae, Monelata, new species, Oriental Region, China

Introduction

Monelata Förster, 1856 belongs to the tribe Diapriini of the subfamily Diapriinae (Hymenoptera, Diapriidae). Currently the genus includes sixteen species, of which nine are found in the Palearctic Region, one in the Palearctic and Nearctic Regions, two each in the Nearctic, Afrotropical and Oriental Regions (Johnson 1992; Masner and Garcia 2002; Rajmohana 2006). Little is known of their biology, but it is thought that they are probably parasitizing some Diptera (Masner and Garcia 2002).

In the Oriental Region, Huggert (1982) found one new female species in India, *Monelata incisipennis* Huggert, 1982. Rajmohana and Narendran (2000) reported the

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second new species, also from India, *M. completa* Rajmohana & Narendran, 2000. Liu and Xu (2012) founded the males of *M. incisipennis* Huggert, 1982 from China. In this paper, one new species from Yunnan Province, China is described, *M. truncata* sp. n.. A key to the Oriental species of this genus is provided.

Materials and methods

Specimens were examined under a Leica MZ12.5 stereomicroscope. All photos were taken with a digital camera (Cool SNAP) attached to the Zeiss Stemi 2000-CS stereomicroscope and processed by using Image-Pro Plus software. Some holotypes of *Monelata* deposited in the Natural History Museum, London, UK (BMNH) were checked for this study.

Morphological terminology follows Masner and Garcia (2002). In the descriptions, abbreviations are as follows:

A1, A2, ... = the first, second, antennal segments, respectively; OL = the distance between inner edge of lateral ocellus and median ocellus; OOL = the shortest distance between lateral ocellus and compound eye; POL = the shortest distance between inner margins of two posterior ocelli; S2 = the second metasomal sterite. T2 = the second metasomal tergite. Measurements reported are relative, except for body length (head to abdominal tip, excluded the antennae and ovipositor) and fore wing length.

Taxonomy

Genus Monelata Förster, 1856

Monelata Förster, 1856: 123. Type species: *Diapria parvula* Nees von Esenbeck, designated by Ashmead (1893).

Monelata Förster: Ashmead 1893: 407; Dalla Torre 1898: 432; Kieffer 1910: 698; 1912: 5; 1916: 8; Prisner 1953: 452; Muesebeck and Walkley 1951: 676; 1956: 371; Pschorn-Walcher 1956: 58; Masner and Sundholm 1959: 165; Kozlov 1978: 594; Muesebeck 1979: 1143; Szabó 1979: 273; Masner and Garcia 2002: 93; Rajmohana 2006: 57.

For detailed generic synonymy see Johnson (1992).

Diagnosis. Body length 0.9–1.5 mm, smooth and shining. Female antenna 13-segmented, with A13 remarkably clavate. Male antenna 14-segmented, with A4 not sexually modified. Cervix of pronotum densely hairy, dense pronotal and propleural cushions confluent into complete hairy collar ventrally and dorsally. Notauli absent. Anterior scutellar pit absent. Metapleuron densely covered with appressed pilosity, pilosity continuing on metasternum, completely surrounding hind coxae. Propodeum

and petiole entirely carpeted with dense semi-hyaline pilosity. T2 with pilosity at base. S2 with large and dense anterior cushion.

Distribution. Afrotropical, Nearctic, Palearctic and Oriental Regions (Rajmohana 2006; Johnson et al. 2015).

Key to Oriental species of Monelata

1	Fore wing with apical margin deeply incised
_	Fore wing with apical margin round or truncate2
2	Forewing with apical margin round; head in dorsal view nearly as long as
	wide; mesosoma brown to reddish-brown
	<i>M. completa</i> Rajmohana & Narendran
_	Forewing with apical margin truncate (Fig. 3); head in dorsal view distinctly
	transverse; mesosoma black

Monelata incisipennis Huggert, 1982

Monelata incisipennis Huggert, 1982: 183. Monelata incisipennis Huggert: Rajmohana 2006: 57; Liu and Xu 2012: 460.

Material examined. 1 ♀, CHINA: Guangdong, Nankunshan, 2011.IX.27–29, Zaifu Xu (SCAU); 1 ♂, Hainan, Yinggeling, 2008.XI.16-20, Ya-li Tang (SCAU); 1 ♂, Guangxi, Maoershan, 2005.VIII.2-10, Bin Xiao (SCAU); 92 ♀♀ 48 ♂♂, Yunnan, Zhaotong, Yongshan, Huanghua, 2012.VIII–X, Shi-wen Yang (SCAU).

Biology. Unknown.

Distribution. China (Guangdong, Hainan, Guangxi, Yunnan); India (Rajmohana 2006).

Monelata truncata Hou & Xu, sp. n.

http://zoobank.org/2B503D40-A92F-4031-9730-8344634E40E4 Figs 1–5

Material examined. Holotype: ♀, CHINA: Yunnan, Zhaotong, Yongshan, Huanghua, 2012.VIII–X, Shi-wen Yang (SCAU). Paratypes: 22 ♀♀ 35 ♂♂, Yunnan, Zhaotong, Yongshan, Huanghua, 2012.VIII–X, Shi-wen Yang (SCAU).

Description. Holotype. *Female* (Figs 1–3). Body length 0.9 mm. Fore wing length 0.8 mm.

Color. Head black. Antenna dark brown, with A13 black. Mesosoma and metasoma black. Petiole and legs brown. Fore and hind wings hyaline, with veins brown.

Head. Head in dorsal view transverse, 0.75 times as long as wide. Relative proportion of length to width of antennal segment as follows: A1 (10.5 : 2.2); A2 (3.5 : 1.8);



Figures 1–3. *Monelata truncata* sp. n., ♀, holotype, habitus. I Lateral view 2 dorsal view 3 fore wing.

A3 (1.6 : 1.3); A4 (1.2 : 1.3); A5 (1.2 : 1.3); A6 (1.2 : 1.3); A7 (1.2 : 1.3); A8 (1.3 : 1.4); A9 (1.4 : 1.5); A10 (1.6 : 1.6); A11 (1.8 : 2.0); A12 (2.2 : 2.3); A13 (7.5 : 4.5). A1 slender, cylindrical, unarmed apically. A10 to A13 gradually enlarged. A13 ovoid,



Figures 4–5. Monelata truncata sp. n., *(*), paratype, habitus. 4 Lateral view 5 dorsal view.

remarkably clavate. Eye oval, 1.5 times as long as wide, 1.5 times as long as malar space. Posterior orbit of eye not sinuate. POL : OOL : OL = 1.5 : 4.0 : 1.5.

Mesosoma. Mesosoma as wide as head. Cervix densely hairy, dense pronotal and propleural cushions confluent into complete hairy collar ventrally and dorsally. Mesoscutum smooth, with three pairs of setae. Scutellar disc slightly converging posteriorly. Mesopleuron smooth. Metanotum with a median keel. Metapleuron covered with long hairs. Propodeum elongate, with a distinct median keel, pointed anteriorly. Posterior margin of propodeum excavate. Fore wing elongate, distinctly longer than mesosoma and metasoma; apical margin truncate (Fig. 3); apical margin with long fringes, 1/3 of wing width. Hind wing narrow, with fringes distinctly longer than wing width. Legs long and slender.

Metasoma. Petiole cylindrical, 1.5 times as long as wide. Petiole covered by dense, translucent, elongate setae. T2 enlarged, cover 0.8 length of gaster, 1.7 times as long as wide.

Male (Figs 4, 5). Body length 0.8 mm. Fore wing length 0.7 mm. Antenna fully brown. Relative proportion of length to width of antennal segment as follows: A1

(11.0: 2.0); A2 (3.2: 1.8); A3 (3.8: 1.9); A4 (3.8: 1.9); A5 (3.8: 2.0); A6 (3.8: 2.0); A7 (3.8: 2.0); A8 (3.8: 2.0); A9 (3.8: 2.0); A10 (3.8: 2.0); A11 (3.8: 2.0); A12 (4.0: 2.1); A14 (5.0: 2.1). Eye 1.4 times as long as wide, 2.3 times as long as malar space. POL : OOL : OL = 1.5: 4.0: 1.3. A4 not sexually modified. Other characteristics are similar to females.

Variation. *Females.* Body length 0.8–1.0 mm. Fore wing length 0.8–0.9 mm. *Males.* Body length 0.7–0.8 mm. Fore wing length 0.6–0.7 mm.

Distribution. China (Yunnan).

Etymology. The species name "*truncata*" is based on the truncate apical margin of fore wing.

Remarks. This is the third species of the genus *Monelata* in the Oriental Region, but it can be separated from the other two Oriental species, *M. incisipennis* Huggert and *M. completa* Rajmohana & Narendran by the following characteristics: fore wing with apical margin truncate (deeply incised in *M. incisipennis*, and round in *M. completa*); head in dorsal view distinctly transverse (nearly as long as wide in the latter two); and mesosoma black (reddish-brown to brown in the latter two).

According to the key and figure of Nixon (1980), *M. solida* (Thomson, 1858) in the Palearctic Region is "forewing faintly to hardly emarginate at apex", the new species is similar to *M. solida*. But after we checked the holotype of *M. solida* (Holotype Q, BMNH No. 9.688) in London, the apical margin of fore wing of *M. solida* is round as *M. completa*. The new species also can be separated from *M. solida* by head in dorsal view distinctly transverse (slightly wider than long in *M. solida*); female A7–A12 slightly wider than long, or as wide as long (distinctly wider than long in *M. solida*); A12 1.67 times as long as wide (2.27 times in *M. solida*).

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RESEARCH ARTICLE



A new species of *Hypoaspis* Canestrini (Acari, Mesostigmata, Laelapidae) associated with Oryctes sp. (Coleoptera, Scarabaeidae) in Iran

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Abstract

A new species of the genus *Hypoaspis* Canestrini, *Hypoaspis surenai* **sp. n.**, is described based on adult female specimens collected in association with *Oryctes* **sp**. (Coleoptera: Scarabaeidae) in Taft, Yazd province, Iran.

Keywords

Gamasina, Dermanyssoidea, taxonomy, Hypoaspis surenai sp. n., Hypoaspidinae, Taft, Yazd

Introduction

The mite family Laelapidae includes approximately 800 species of morphologically, ecologically and behaviourally very diverse dermanyssoid mites, including obligate and facultative parasites of vertebrates, insect paraphages, and free-living predators that inhabit soil-litter habitats and the nests of vertebrates and arthropods (Evans and Till 1966; Faraji and Halliday 2009; Lindquist et al. 2009; Joharchi et al. 2011; Joharchi et al. 2012a, b). Currently, the family is classified into approximately 144 genera, including *Hypoaspis* with 36 species.

Joharchi and Halliday (2011) treated *Hypoaspis sensu stricto* as a separate genus equivalent to *Hypoaspis* (*Hypoaspis*) of other authors (e.g., Evans and Till 1966; Karg 1979, 1982, 1993), and gave a diagnosis and comparison of diagnostic characters for the closely related genus *Coleolaelaps* Berlese. That concept of *Hypoaspis s.s.* is followed here. The most recent taxonomic work on the genus was by Joharchi et al. (2014), who clarified the diagnosis of the genus and reviewed species that occur in the Western Palaearctic Region. In Iran, *Hypoaspis s.s.* included 14 identified species prior to this study (Joharchi and Halliday 2011; Razavi Susan et al. 2014; Joharchi et al. 2014).

The ecological role of this genus is unknown. They may feed on exudates from the beetle's body or their eggs, or on other small invertebrates in the microhabitats created by the beetles (Costa 1971; Joharchi and Halliday 2011; Joharchi et al. 2014). This has not been established experimentally, and it will be necessary to do feeding experiments to establish the true ecological role of these mites. The purpose of this paper is to describe another species of *Hypoaspis s.s.* to increase our knowledge of the Iranian fauna of Laelapidae.

Materials and methods

Phoretic laelapids on beetles were collected from Taft, Yazd province, Iran, in 2015. Mites were removed from the beetles using an entomological pin. Specimens were cleared in Nesbitt's solution and mounted in Hoyer's medium (Walter and Krantz 2009). The line drawings and examination of the specimens were performed with an Olympus BX51 phase contrast microscope equipped with a drawing tube and figures were elaborated with Corel X-draw software, based on the scanned line drawings. Dorsal shield length and width were taken from the anterior to posterior margins along the midline, and at its broadest point, respectively. Length and width of the sternal shield were measured from the anterior border to the posterior margin at the full length and broadest point, respectively. Genital shield length and width were measured along the midline from the anterior border of the genital shield to the posterior margin of the genital shield, and at the maximum, respectively. Leg lengths were measured from base of the coxa to the apex of the tarsus, excluding the pre-tarsus. The nomenclature used for the dorsal idiosomal chaetotaxy is that of Lindquist and Evans (1965), the leg chaetotaxy is that of Evans (1963a), the palp chaetotaxy is that of Evans (1963b), and names of other anatomical structures mostly follow Evans and Till (1979). We use the terms "lyrifissures" to refer to slit-shaped sensilli, "gland pores" to refer to structures that we believe are the openings of secretory pores, and "poroids" for circular or ovalshaped cuticular openings of unknown function. The holotype (ARS-20150304-1a) and six paratypes (ARS-20150304-1b, ARS-20150304-1c, ARS-20150304-1d, ARS-20150304-1e, ARS-20150304-1f, ARS-20150304-1g) of the new species are deposited in the Acarological Collection, Department of Plant Protection, Yazd Branch, Islamic Azad University (YIAU). Two paratypes (ARS-20150304-1h, ARS-20150304-1i) are deposited in the Jalal Afshar Zoological Museum, College of Agriculture, University of Tehran, Iran (JAZM) and two paratypes (ARS-20150304-1k, ARS-20150304-1l) are also in the Australian National Insect Collection, CSIRO, Canberra, Australia (ANIC). All measurements in the descriptions are given in micrometres (μ m).

Taxonomy

Genus Hypoaspis Canestrini

Hypoaspis Canestrini, 1884: 1569.

Type species. Gamasus krameri G. & R. Canestrini, 1881, designated by Berlese (1904).

The short diagnosis below is summarised from the detailed diagnosis in Joharchi and Halliday (2011).

Short diagnosis. Dorsal shield oval, without lateral incisions, bearing 35–40 pairs of setae including one or more pairs of Zx setae; some opisthonotal setae greatly elongated, especially Z4 (at least three times as long as J4); post-anal seta distinctly shorter than para-anals; hypostomal setae h3 distinctly longer than other hypostomal setae; tarsus II with two subterminal blunt spines (setae *al1* and *pl1*).

Hypoaspis surenai sp. n.

http://zoobank.org/11977B75-8434-4596-A481-782332BE2541 Figures 1–7

Type material. Holotype, female, **Iran**, Yazd Province, Taft, Kahduiyeh, 31°16'N, 53°43'E, alt. 1496 m a.s.l, 04March 2015, A. Shahedi coll., on adult females of *Oryctes* sp. (Coleoptera: Scarabaeidae). Paratypes: ten females same data as holotype.

Description of the female. *Dorsal idiosoma* (Fig. 1). Length 796–802, width at level of r5, 446–450. Dorsal shield oval, without lateral incisions, length 778–785, width at level of r5, 420–426 (n= 11), shield without distinct reticulate ornamentation over whole surface, only with weak reticulation, more distinct in opisthonotal region (Fig. 1). Dorsal shield with 37 pairs of smooth and pointed setae, 21 pairs on podonotal shield (*j1–6*; *z1–6*; *s1–6*; *r4–5* and including a supernumerary pair near *s6*), plus *r2*, *r3* and *r6* off the shield in the soft skin, 16 pairs on the opisthonotal shield (*J1–5*, *Z1*, *Z2*, Z4, Z5, *S1–5*), including two pairs of *Zx* setae between *J* and *Z* setae, seta *Z3* absent (Fig. 1); *Z4* longest (322–330) and slightly wavy, *s5* (219–225), *s4* (198–207), *z4* (232–245) and *j3* (222–230) also long, *j1* (74–75) and *z1* (30–31) short; *j4* (128–132) long enough to reach *J2* (100–108) but reaching past *J1* (118–123); *J4* (98–100) long enough to reach *J5* (27–29); *Z5* (178–180) and *S5* (136–139) also



Figures 1–7. *Hypoaspis surenai* sp. n., female 1 dorsal idiosoma 2 ventral idiosoma 3 epistome 4 Subcapitulum 5 chelicera 6 femur and genu II 7 femur and genu IV.
long. Seven pairs of setae in R series on the lateral area of weakly sclerotised cuticle surrounding shield; R7 elongate (182–191) and appearing wavy. Shield with 12 pairs of pore-like structures, apparently including four pairs of gland pores and eight pairs of poroids; lyrifissures near the base of *j1* large and slit-like, others smaller and ovoid.

Ventral idiosoma (Fig. 2). Tritosternum with paired pilose laciniae (141-143), columnar base 30-32 long, 20-21 wide; pre-sternal area weakly reticulated. Sternal shield (length 138-148) narrowest between coxae II (138-148), widest between coxae II-III (198-200), with slightly concave anterior margin and irregular posterior margin, with three pairs of long, smooth pointed setae (st1 52-54, st2 82-84, st3 74-79), st2 and st3 reaching well past base of next posterior setae, and two pairs of lyrifissures, one pair adjacent to st1, the other between st2 and st3, lateral and central surface of sternal shield with weak reticulation. Metasternal platelets absent, metasternal setae st4 (45-47) and metasternal poroids located on weakly sclerotised cuticle. Endopodal plates II/III completely fused to sternal shield, endopodal plates III/IV roughly triangular and curved. Genital shield tongue-shaped, length 278-280, maximum width 118-120, posterior margin rounded, surface with reticulate ornamentation, genital setae st5 (50-52) on edge of the shield. Circular paragenital poroids located on weakly sclerotised cuticle close to st5. Anal shield rounded triangular, length 87-89, width 87-89, para-anal (39-41) and post-anal (38-40) setae equal in length, cribrum small, a pair of circular lateral gland pores flank anal shield. Opisthogaster with one pair of oval metapodal plates (22-23 long × 5-7 wide) and 11 pairs of smooth setae on the weakly sclerotised cuticle; Jv1, Jv2 70-72, Jv3 89-92, Jv4 158-160, Zv1 50-52, Zv2 91-93, Zv3, Zv4 100-105, UR 124-126, Jv5 242-250 very long and wavy. Exopodal plates behind coxa IV long and narrow. Peritrematal shield free posteriorly, with large protrusion on outer margin opposite coxae II-III bearing two pairs of pore-like structures (apparently one lyrifissure 'ip', and one gland pore 'gp'; see Fig. 2), poststigmatal section conspicuous and narrow, with three pairs of pore-like structures of post-stigmatal pores (apparently two lyrifissures 'ip', and one gland pore 'gp'; see Fig. 2), peritreme extending from posterior margin of coxa III to near mid level of coxa I.

Gnathosoma. Epistome irregularly denticulate laterally, apical section smooth with minute denticles in some specimens (Fig. 3). Hypostomal groove with six rows of 6–11 denticles, and smooth anterior and posterior transverse lines. Hypostome with four pairs of setae, internal posterior hypostomal setae h3 longest (109–110), h1 (54–55), h2 (45–47), palpcoxal pc (52–54) (Fig. 4). Corniculi robust and horn-like, reaching mid-level of palp femur. Palp setal numbers: trochanter 2, femur 5, genu 6, tibia 12, tarsus 15, all setae smooth and pointed, palp tarsal apotele two-tined. Internal malae complex, with two pairs of lobes, inner lobes narrow, with serrated edges, outer lobes narrow, pointed, shorter than inner lobes, with serrated edges (Fig. 4). Fixed digit of chelicera with 15 small teeth, the one level with the pilus dentilis largest (Fig. 5), pilus dentilis short and robust, dorsal seta short, semi-erect, movable digit with two large subterminal teeth, arthrodial membrane a rounded flap with a corona and cheliceral lyrifissure indistinct.

Legs. Legs II and III shortest (564–570, 604–610), I and IV both longer (702– 711, 872–880) (excluding pretarsus). Chaetotaxy normal for free-living Laelapidae. Leg I: coxa 0-0/1, 0/1-0, trochanter 1-0/1, 1/2-1, femur 2-3/1, 2/3-2, genu 2-3/2, 3/1-2, tibia 2-3/2, 3/1-2. Leg II: coxa 0-0/1, 0/1-0, trochanter 1-0/1, 0/2-1, femur 2-3/1, 2/2-1 (macrosetae *pd1* 184–190, *pd2* 94–97, Fig. 6), genu 2-3/1, 2/1-2 (*pd1* 84–86 and *pd2* 100–103 longer, Fig. 6), tibia 2-2/1, 2/1-2. Leg III: coxa 0-0/1, 0/1-0, trochanter 1-1/1, 1/1-0, femur 1-2/1, 1/0-1 (macroseta *ad1* 124–128; *ad2* longer 37–43), genu 2-2/1, 2/1-1 (*ad1* 57–59 and *pd1* 90–94 longer), tibia 2-1/1, 2/1-1 (ventral setae all thicker). Leg IV: coxa 0-0/1, 0/0-0, trochanter 1-1/1, 0/1-1, femur 1-2/1, 1/0-1 (macroseta *ad1* 200–207, *ad2* longer 90–92, Fig. 7), genu 2-2/1, 3/0-1 (*ad1* 84–86 and *pd1* 60–62 longer, Fig. 7), tibia 2-2/1, 3/1-2. Tarsi II–IV with 18 setae 3-3/2, 3/2-3 + *mv*, *md*. On tarsus II, *al1*, *pl1* and all ventral setae thicker. Tarsus IV with three macrosetae, *ad2* (164–169), *pd2* (100–107) and *pd3* (142– 147) and *pl3* thick. All pre-tarsi with a pair of claws and a long thin membranous ambulacral stalk.

Genital structures. Insemination ducts opening on posterior margin of coxa III, sacculus indistinct, apparently unsclerotised.

Males & immature. Unknown.

Etymology. The species is named in memory of Surena (died 53 BC) was a Parthian spahbed ("General" or "Commander") during the 1st century BC.

Remarks. According to the key to species of *Hypoaspis s.s.* occurring in the Western Palaearctic Region provided by Joharchi et al. (2014), *Hypoaspis surenai* most resembles *H. pentodoni* Costa, 1971 but has the following unique character states for the genus: 21 pairs of long smooth, pointed setae on the podonotal shield, including a supernumerary pair near s6 (x) and r2, r3, r6 off the shield; 16 pairs of smooth and long setae on the opisthonotal shield including two pairs of *Zx* setae between the *J* and *Z* setae, seta *Z3* absent; three long macrosetae on tarsus IV (*ad2*, *pd2* and *pd3*); one macroseta on each of femora II–IV and seta *ad1* on genu IV being only slightly longer than the remaining setae on the segment.

Discussion

Fifteen species regarded to belong to *Hypoaspis s.s.* had been reported from Iran until now (including the new species): *Hypoaspis alborzensis* Razavi Susan & Joharchi, 2014; *H. campestris* (Berlese, 1887) *sensu* Bregetova, 1977; *H. elegans* Joharchi et al. 2014; *H. integer* Berlese, 1911; *H. krameri* (G. & R. Canestrini, 1881); *H. larvicolus* Joharchi & Halliday, 2011; *H. maryamae* Joharchi & Halliday, 2011; *H. melolonthae* Joharchi & Halliday, 2011; *H. neokrameri* Costa, 1971; *H. pentodoni* Costa, 1971; *H. phyllognathi* Costa, 1971; *H. polyphyllae* Khanjani & Ueckermann, 2005; *H. rhinocerotis* Oudemans, 1925; *Hypoaspis surenai* sp. n.; *H. terrestris* (Leonardi, 1899).

Almost all of the species of *Hypoaspis s.s.* occurring in Iran are associated with Coleoptera, especially with a wide variety of species in the family Scarabaeidae, while a

few have been collected in soil. Most of these species have been collected on only a few occasions, so it is difficult to draw any firm conclusions about their host specificity. The question of host or microhabitat specificity of the species cannot be analysed in detail until all of the available collections are re-examined to confirm the identifications.

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RESEARCH ARTICLE



Resolving the taxonomic conundrum in *Graphoderus* of the east Palearctic with a key to all species (Coleoptera, Dytiscidae)

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Abstract

The Holarctic diving beetle genus *Graphoderus* (Dytiscinae, Aciliini) contains relatively few and well-known species but these may still be difficult to identify based on external characters. A taxonomic problem in the eastern Palearctic was discovered that relates to the Palearctic *G. zonatus* (Hoppe, 1795) and the Nearctic *G. perplexus* Sharp, 1882. Based on qualitative and quantitative characters, especially on male genitalia which have been poorly studied in the past, it is shown that eastern Palearctic specimens identified by previous authors as either of the two species in fact belongs to a third species. The synonymized name *G. elatus* Sharp, 1882, is reinstated as a valid species (**stat. n.**) and a lectotype is designated from the mixed syntype series. The male genitalia of all known *Graphoderus* species have been examined and an illustrated identification key to the genus is provided. The three species in the complex of focus, *G. elatus*, *G. zonatus* and *G. perplexus* are found to have allopatric distributions; *G. perplexus* in the Nearctic region, *G. zonatus* in the west Palearctic region and eastwards to the Yenisei-Angara river and *G. elatus* east of the Yenisei-Angara river. All previous records of either *G. zonatus* or *G. perplexus* in the east Palearctic, east of the Yenisei-Angara river turned out to be misidentified *G. elatus*. This conclusion also brings with it that dimorphic females, thought only to be present in the single subspecies *G. zonatus verrucifer* (CR Sahlberg, 1824), proved to be present also in a second species, *G. elatus*. The dimorphic female forms is either with dorsally smooth elytra and

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pronotum or conspicuously granulated elytra and wrinkly pronotum. As has been shown in *G. z. verrucifer* there is a correlation between the occurrence of granulate female forms in a population and an increase in the number of adhesive discs on pro- and mesotarsus in males within *G. elatus*.

Keywords

Graphoderus, east Palearctic, Nearctic, *G. zonatus*, *G. perplexus*, *G. elatus*, male genitalia, Yenisei-Angara river, dimorphic females

Introduction

The genus *Graphoderus* Dejean, 1833 consists of medium sized (10–16 mm) diving beetles of the family Dytiscidae. Adults are dorsally testaceous to rufous with black irrorations and except for one species they all have two transverse black bands across the pronotum (Nilsson and Holmen 1995, Larson et al. 2000). Both adults and larvae are found in ponds, smaller lakes, bogs or wetlands (Nilsson and Holmen 1995, Larson et al. 2000). Larvae feed mainly on planktonic microcrustaceans but also on larvae and pupae of mosquitoes (Culicidae) (Galewski 1975, 1990). Adults are predatory on crustaceans, insects and worms (Galewski 1990). Oviposition, and larval and pupal development takes place from April or May to October, and in some years there is also a partial second generation (Galewski 1990, Hilsenhoff 1993, Nilsson and Holmen 1995, Foster 1996). Adults overwinter in aquatic habitats, under *Sphagnum* in bogs or hidden in bottom sediments of water-bodies which do not dry out (Galewski 1990, Hilsenhoff 1993). Males have enlarged protarsomeres I–III forming a palette with adhesive discs and mesotarsomeres I–III may be with or without adhesive discs (Nilsson and Holmen 1995).

Current diversity and state of affairs

Currently, *Graphoderus* is regarded as consisting of eleven species and two subspecies, all distributed in the Holarctic realm (Nilsson 2001, 2015). The Nearctic species of *Graphoderus* were recently treated by Larson et al. (2000) who listed five species: *G. liberus* (Say, 1825), *G. perplexus* Sharp, 1882, *G. occidentalis* Horn, 1883, *G. fascicollis* (Harris, 1828) and *G. manitobensis* Wallis, 1933. The first three are transcontinental whereas the latter two are more restricted in distribution to east-central North America (Larson et al. 2000). Nilsson and Holmen (1995) treated the four west Palearctic species: *G. austriacus* (Sturm, 1834), *G. cinereus* (Linnaeus, 1758), *G. bilineatus* (De Geer, 1774) and *G. zonatus* (Hoppe, 1795) with the latter divided into the subspecies *G. z. zonatus* (Hoppe, 1795) and *G. z. verrucifer* (CR Sahlberg, 1824), following Nilsson (1986). How far the distribution of these species extends into the east Palearctic is somewhat uncertain, but all reach at least west Siberia (Nilsson 2003a, Nilsson and Hájek 2015). *Graphoderus austriacus* and *G. zonatus* are regarded as extending further into Far East Russia where they meet the two exclusively east Palearctic species: *G. adamsii* (Clark, 1864) and *G. bieneri* Zimmermann, 1921 (Lafer 1989).

This order was shaken when Nilsson et al. (1999) reported the Nearctic species *G. perplexus* to also occur in the east Palearctic and we started to discover additional *G. perplexus*-like specimens from the east Palearctic.

Graphoderus species in the spotlight

One of the species, *G. bilineatus*, has received increased attention after it was put on the EU list of species in Annex II under the Habitats Directive. EU member states were required to designate special areas of conservation for the species in Annex II and report on their conservation status. Environmental agencies in several EU countries have since made focused inventories of this species to get better data on its occurrence, distribution and abundance (Hájek 2004, Hendrich and Balke 2005, Kalniņš 2006, Hendrich and Spitzenberg 2006, Sierdsema and Cuppen 2006, Cuppen et al. 2006, Naturvårdsverket 2011, Hendrich et al. 2011, Iversen et al. 2013, Przewoźny et al. 2014, Scheers 2015). Results of the inventories have also contributed to new studies investigating the distribution of *G. bilineatus* in neighboring countries (Kálmán et al. 2011, Csabai et al. 2015).

Another *Graphoderus* species which has received substantial attention lately is G. zonatus and in particular its subspecies G. zonatus verrucifer, as its females are dimorphic with one morph dorsally smooth like the male and the other morph with a peculiar wrinkly pronotum and roughly granulated elytra (Nilsson 1986, Nilsson and Holmen 1995, Bergsten et al. 2001, Härdling and Bergsten 2006, Karlsson-Green et al. 2013, 2014). The two morphs co-occur in varying proportions in different populations and it was shown by Bergsten et al. (2001) that the proportion of the granulate morphs in a population was significantly correlated with suction cup characteristics of the male's pro- and mesotarsal palettes. With a higher proportion of the granulated female morph male pro- and mesotarsal palettes got wider, the three large suction cups got larger and the smaller suction cups got smaller and more numerous (Bergsten et al. 2001). The female granulate morph was interpreted as an antagonistic character evolved in an arms race with male suction cups over aspects in the mating, e.g. frequency and timing (Arnqvist and Rowe 2005). The antagonistic nature of the dorsal female sculpture to the function of the mechanically working male suction cups was inferred mathematically from first principles by Bergsten and Miller (2007) and later shown empirically (Karlsson-Green et al. 2013). Both theoretical (Härdling and Bergsten 2006) and empirical (Karlsson-Green et al. 2014) work has been conducted to try to understand the role of selection and drift for the distinct morphs to be able to co-occur over time.

The taxonomic conundrum in the east Palearctic

In 1882, Sharp (1882) described a *Graphoderus* species with Holarctic distribution, *Graphoderus elatus* Sharp, 1882, based on material from both Canada and east Siberia.

Sharp (1882) separated this species from the Nearctic species G. perplexus, described in the same work, based on the number of adhesive discs on pro- and mesotarsus. Both species were distinguished from the Palearctic species G. zonatus by being narrower in front and having wider epipleura. Horn (1883) was not convinced that the male tarsal characters given by Sharp (1882) indicated separate species and made G. fascicollis, G. perplexus and G. elatus synonyms of the Palearctic G. cinereus. Zimmermann (1920) disagreed with Horn (1883) and instead placed G. perplexus and G. elatus as synonyms of G. zonatus. We will refer to this "species group" (G. zonatus, G. perplexus and G. elatus) which might not be closely related, as the zonatus-species complex and it is distinguished from other Graphoderus species in that the black pronotal bands mostly neither reach the anterior nor the posterior pronotal margins (Nilsson and Holmen 1995, Larson et al. 2000). Further, Gschwendtner (1937) mentioned that G. perplexus and G. elatus were not particularly different from G. zonatus. Instead Wallis (1939) was the one who separated G. perplexus and G. elatus from G. zonatus by the same characters which Sharp (1882) used - wider epipleura and a body shape more narrowed in front. Also, Wallis (1939) specified differences between G. zonatus and G. perplexus/G. elatus in the bifurcation of the chitinous ring enclosing the genitalia. However, after having examined over 100 males, he proposed that G. perplexus and G. elatus could not be separated from each other based on the number of adhesive discs and he synonymized them as the same species with *perplexus* as the valid name. Zaitsev (1953) (English translation (Zaitsev 1972)) described the Russian fauna of Dytiscidae in 1953 and did not mention G. perplexus as occurring in the Palearctic region. However Zaitsev (1972) put Sharp's G. elatus type from east Siberia as a synonym of G. zonatus and also reported G. zonatus to be distributed in North America. Later, in the treatment of the Russian Far East Dytiscidae by Lafer (1989), the G. elatus type from east Siberia was not referred to at all, instead Lafer only mentioned G. zonatus from the zonatus-species complex to occur in the Palearctic. Yet, in 1999 Nilsson et al. (1999) studied newly collected material from Kamchatka (Russian Far East) and identified the material of a Graphoderus species as G. perplexus based on the number of tarsal adhesive discs which was significantly fewer than the material from Urup, Kuril Islands, instead identified as G. zonatus (Nilsson et al. 1997). This resurrected the old hypothesis by Sharp (1882) of a Graphoderus species occurring in both the Nearctic and east Palearctic realms, now under the name G. perplexus. This was also transferred to the world catalogue (Nilsson 2001, 2015) and the Palearctic catalogue (Nilsson 2003a, Nilsson and Hájek 2015). There is however a problem with using the number of tarsal suction cups as a sole distinguishing character because of its variation and correlation with female counter-adaptions (Bergsten et al. 2001). The number of suction cups was used by Nilsson (1986) to delimit the two subspecies G. zonatus zonatus and G. z. verrucifer but only based on Swedish material. In Sweden the two subspecies could be delimited geographically in that south of a diagonal line across southern Sweden no granulate females were known (Nilsson 1986). On average the number of both pro- and mesotarsal suction cups were higher in the northern subspecies (G. z. verrucifer) but the variation overlapped (Nilsson 1986). Populations of G. z. verrucifer occur as well in Italy but these populations were earlier considered to be

a variety of *G. cinereus* (var. *bertolinii* Seidlitz, 1887), which Pederzani (1986) instead reported to belong to a relict population of *G. verrucifer* (CR Sahlberg, 1824), new to Italy. The same year Nilsson (1986) changed the status of *G. verrucifer* to a subspecies of *G. zonatus*. Yet, how to delimit the subspecies of *G. zonatus* in the eastward extension of the distribution across Russia and Siberia to Japan is poorly understood (Nilsson and Holmen 1995). Both subspecies of *G. zonatus* are listed for Mongolia and all records of *G. zonatus* from China and Japan are considered to be *G. z. zonatus* whereas all western and eastern Siberian as well as Far East Russian *G. zonatus* are considered to be *G. z. verrucifer* (Nilsson 2003a, Nilsson and Hájek 2015). The number of tarsal suction cups in the Kamchatka material is in fact partly within the documented range for *G. z. zonatus* (Nilsson 1986, Nilsson et al. 1999) which was probably excluded only on the basis of a presumed more southern distribution.

In summary then, there is an unresolved taxonomic conundrum in the east Palearctic. Is there really a partly Holarctic *Graphoderus* species spanning both sides of Beringia? Are there other distinguishing characters apart from the number of males' suction cups to shed light on the *zonatus*-species complex? We are especially interested in evaluating the diagnostic power of the male genitalia because the genitalia in *Graphoderus* have not been as extensively used as in e.g. Agabini due to their partly soft-tissue nature (Nilsson and Holmen 1995). There is no reason to believe that it is less informative than the genitalic characters in the sister-group *Acilius* Leach, 1817 (Bukontaite et al. 2014) which recently helped to solve a taxonomic confusion in the Nearctic region (Bergsten and Miller 2006). If there is no Holarctic *Graphoderus* how should we treat *G. elatus* based on the syntype series including both continents? In the updated world catalogue (Nilsson 2015), *G. elatus* is listed with a "?" as a synonym under *G. perplexus* and in the updated Palearctic catalogue (Nilsson and Hájek 2015) *G. perplexus* is listed as occurring in far east Russia and Nearctic but without the synonym *G. elatus*.

The aim of this study is to 1) resolve the taxonomic conundrum of the *zonatus*species complex in the east Palearctic, 2) evaluate the usefulness of the male genitalia for species identification and delimitation in *Graphoderus* and 3) to provide an identification key and iconography with habitus and male genitalia images of all *Graphoderus* species.

Methods

Examined material came from the following collections referred to by their abbreviation:

BMNH	The Natural History Museum, London, United Kingdom;
CNC	Canadian National Collection of Insects, Ottawa, Ontario, Canada;
IRCW	University of Wisconsin (Ex. Coll W. Hilsenhoff), Madison, Wisconsin, USA;
NHRS	Swedish Museum of Natural History, Stockholm, Sweden;
OMNH	Osaka Museum of Natural History, Osaka, Japan;
SYSU	Biological Museum of Sun Yat-sen University, Guangzhou, China;

ZIN Zoological Institute, Russian Academy of Sciences, St Petersburg, Russia;ZMUM Zoological Museum, Moscow State University, Moscow, Russia.

Measurements

Genitalia were prepared from 57 male specimens of Nearctic *G. perplexus* and from sixteen males of the *G. perplexus*-like specimens from east Palearctic, shown in this paper to be *G. elatus* Sharp, 1882. Six measurements were then taken (in micrometers, μ m) from photographs of the genitalia, with focus at the anterior lobes of the penis. The camera was an infinity X, mounted on an Olympus SZX12 stereomicroscope using the program DeltaPix InSight v4.0.9. The measurements were; PW = penis width (at midway between apex and base), PL = penis length, PCLL = penis central lobe length, PLLL = penis lateral lobe length, PCLWb = penis central lobe width at base and PCLWt = penis central lobe width at level of lateral lobe apex (Fig. 1a). Another five measurements were also taken from seventeen males and ten females of *G. elatus* from the east Palearctic; TL = total body length, EL = elytral length, MEW = maximum elytral width, PrL = pronotal length and PrWb = pronotal width at base (Fig. 1b). Total body length (TL) was also measured for the 57 Nearctic male specimens of *G. perplexus*.

Four ratios, PL/TL; PW/PL; PLLL/PCLL and PCLWt/PCLWb, were calculated from the measurements to test the hypothesis that there were no differences in ratios between the populations from the two continents. The ratios were tested with separate, independent 2-group Mann-Whitney tests with default settings in R version 3.2.2 2015-08-14 (R Team Core 2015). Bonferroni correction, a multiple-test correction, was used to compensate for testing several measurements from the same individuals against each other (Dunn 1961). Boxplots of the ratios with median and ± 25% of the ratio values (whiskers showing minimum and maximum ratios), were also made in R.

The number of adhesive discs on pro- and mesotarsus was counted for seventeen male specimens of Nearctic *G. perplexus* and fourteen males of *G. elatus* from the east Palearctic, excluding males from populations with or possibly with granulated females. The average number of adhesive discs from left and right pro- and mesotarsus of each specimen was calculated and the numbers were tested against the hypothesis that there were no differences between the populations from the two continents. This was tested with independent 2-group Mann-Whitney tests with default settings in R, in which also boxplots were made. The tests were repeated, this time including males from populations with granulated females which did not alter the results (not shown).

Images

Habitus photographs of the species in dorsal view were taken with a Canon EOS 5D DSLR, and a Canon 100mm 2.8L Macro lens mounted on a motorized rail (Stackshot)



Figure I. Explanations of measurements for penis (**a**) and body (**b**). PW = penis width, PL = penis length, PCLL = penis central lobe length, PLLL = penis lateral lobe length, PCLWb = penis central lobe width at base and PCLWt = penis central lobe width at level of lateral lobe apex, TL = total body length, EL = elytral length, MEW = maximum elytral width, PrL = pronotal length and PrWb = pronotal width at base.

from Cognisys. The photos were stacked in Zerene Stacker v1.04 and edited in Digital Photo Professional v3.13.20 and Adobe Photoshop CS5. Photographs of the male genitalia in dorsal and lateral view (slightly dorsolateral to avoid penis apex being hidden by tips of the parameres) were taken with the same infinity X camera and Olympus SZX12 stereomicroscope as above. The photographs shown in Figure 7, 10 and 11 were taken with a Leica M125 stereomicroscope + Canon EOS 550D digital camera in the Sackler Bioimaging Laboratory of the Natural History Museum, London. They were stacked using Helicon Focus software. The photographs of the genitalia in dorsal view were edited in Adobe Photoshop CS5 and the photographs of the lateral view were used as aid to make line drawings of each species penis in Adobe Illustrator CS5. The final plates were made in Adobe Photoshop CS5.

Results

Examination of male genitalia in the *zonatus*-species complex revealed that the lateral view of the central lobe at the penis' trifid apex was highly diagnostic to separate *G. zonatus* from *G. perplexus* and *G. elatus* (Fig. 2). In *G. zonatus*, in lateral view the dorsal margin of the central penis lobe forms an even convex curve (Fig. 2x). In *G. perplexus* and *G. elatus* the same view shows a concave shape (Fig. 2v, l). The shape was identical in both subspecies of *G. zonatus*, including material from the population in the Italian Alps, monomorphic for the granulated female morph. Examination of east Palearctic material based on this character showed that all material east of the Yenisei river and its headwater tributary Angara river was not *G. zonatus* although it had often been misidentified as *G. zonatus* (Zaitsev 1972, Lafer 1989, Mori and Kitayama 1993, Nilsson 1995, Nilsson et al. 1999). As well, all records from the northernmost peninsula on Hokkaido in Japan of *G. zonatus* are based on misidentifications (Mori and Kitayama 1993). The easternmost record of *G. zonatus* we have studied is a male taken by RB Angus in 1970 at Dachnaya, just west of Irkutsk Lat. 52.1220°N Lon. 104.0840°E.

Reported *G. zonatus* from Sakhalin and Kuril Islands (Nilsson et al. 1997, 1999) are based on misidentifications as shown by reexamination of the material in Nilsson's collection donated to NHRS in 2013. Records of *G. zonatus* from northeast China are with all certainty also based on misidentifications (Nilsson 1995), as we found material from Inner Mongolia, Heilongjiang and Qinghai to have the concave shape of the central penis lobe. This shape was also identified in beetles from northeast Mongolia, Onon river, but the distribution of *G. zonatus* in the north-central part of Mongolia (Shaverdo et al. 2008) is more uncertain. Apart from the male genitalia, *G. zonatus* can also be distinguished from *G. perplexus* and *G. elatus* by having narrower epipleura posteriorly.

Granulate females and adhesive discs

The realization that true *G. zonatus* could be distinguished by the shape of male genitalia in lateral view and based on this also could be inferred not to occur east of Yenisei-Angara river brought about an enticing novelty. *G. zonatus* was no longer the only *Graphoderus* species with dimorphic females, one morph of which had elytra granulated and pronotum wrinkled and the other morph which had smooth elytra like the males. Granulated females have been reported east of the Yenisei-Angara river, e.g. from the Kuril Island Urup, which has been seen as evidence for the subspecies *G. zonatus verrucifer* (Nilsson et al. 1997). But our examinations of the same Kuril material showed that the males from that population were not *G. zonatus*. We have also found granulated females from Inner Mongolia (leg. Li, Chunyuan and Chaoqun) and Yakutsk with a male from the latter population rejecting the identity as *G. zonatus*. As well, in the material from North Sakhalin we found females with smooth elytra, however the male from the same population had a higher number of suction cups indicating that female dimorphism can occur within the same population. Closer comparison of the granulated females showed



Figure 2. Genitalia in dorsal view (photo), with detailed lateral view of penis apex (line drawing) of all *Graphoderus* species. **a–b** *G. adamsii* **c–d** *G. austriacus* **e–f** *G. bieneri* **g–h** *G. bilineatus* **i–j** *G. cinereus* **k–l** *G. elatus* [dorsal view, processed by E. Binkiewicz] **m–n** *G. fascicollis* **o–p** *G. liberus* **q–r** *G. manitobensis* **s–t** *G. occidentalis* **u–v** *G. perplexus* [dorsal view, processed by E. Binkiewicz] **w–x** *G. zonatus.*

a somewhat more irregular granulated structure on the elytra, more elongated convexities of the granules and a stronger tendency towards forming longitudinal lines in the east Palearctic material (Fig. 3) but this difference is so far based on too few granulate females to be considered conclusive. Just as predicted by the arms race hypothesis males from granulated populations showed a higher number of adhesive discs on the tarsi. Males from the North Sakhalin and Urup populations had 59-66 protarsal suction cups and 29-31 mesotarsal suction cups. This should be compared with 28-47 protarsal and 14-20 mesotarsal suction cups from localities without granulated females. Granulated *Graphoderus* females have never been found in the Nearctic region but females of the Nearctic species may sometimes have wrinkles on the pronotum.

Quantitative morphometrics

As Nearctic *G. perplexus* and east Palearctic *G. elatus* have a similar concave shape of the male central penis lobe in lateral view, various characteristics of the male genitalia were quantified to test for con- or heterospecificity (see Methods). In particular we had from initial examination noted that the penis, as well as the entire genitalic package with parameres, seemed to be notably longer in the east Palearctic specimens (compare Fig. 2k with 2u). Two separate Mann-Whitney tests showed that *G. elatus* from the east Palearctic are distinguished from Nearctic *G. perplexus* in the male genitalia by significant difference in the ratio PL/TL (relative penis length, P < 0.001) (Fig. 4a) and in PW/PL (penis shape, P < 0.001) (Fig. 4b) (Table 1). Two separate Mann-Whitney tests also showed that Nearctic *G. perplexus* had significantly fewer adhesive discs on both pro- and mesotarsus (P < 0.001) (Fig. 5). Figure 6 illustrates the difference of pro- and mesotarsus between *G. perplexus* and *G. elatus*.

No statistical significance were found in the ratios PLLL/PCLL (relative extension of central penis lobe to lateral lobes, Mann-Whitney test, P = 0.8051) or in PCLWt/PCLWb (anterior narrowing of central penis lobe, Mann-Whitney test, P = 0.2628) (Table 1). The four genitalia ratio tests were conducted using Bonferroni correction to adjust alpha to 0.0125 (0.05/4). The results clearly reject the hypothesis that *G. perplexus* and *G. elatus* are the same species.

Table 1. Results of independent 2-group Mann-Whitney tests. P-values for ratios and the average number of adhesive discs on pro- and mesotarsus between *G. perplexus* and *G. elatus*, representing the number of specimens in each test, and representing the outcome value from each test (**W**).

Ratio or tarsus	W	G. perplexus	G. elatus	P-value
PL/TL	901	57	16	< 0.001
PW/PL	55	57	16	< 0.001
PLLL/PCLL	475	57	16	0.8051
PCLWt/PCLWb	500	56	15	0.2628
Average protarsus	210	17	14	< 0.001
Average mesotarsus	195	16	13	< 0.001



Figure 3. Female dimorphism in *G. zonatus* (**a–b**) and *G. elatus* (**c–d**). **a, c** granulated female elytra **b, d** smooth female elytra.



Figure 4. Boxplots with the variation in PL/TL (**a**) and PW/PL (**b**). Ratios for *G. elatus* and *G. perplexus* in penis length over total body length (PL/TL) and penis width over penis length (PW/PL), the box represents median ± 25% and whiskers show minimum and maximum values.



Figure 5. Boxplots with the variation in number of adhesive pro- (**a**) and mesotarsal (**b**) discs. Number of adhesive discs for *G. elatus* and *G. perplexus*, the box represents median $\pm 25\%$ and whiskers show minimum and maximum numbers.

Taxonomy

Graphoderus elatus Sharp, 1882, new status

Graphoderus elatus Sharp, 1882: 695 (original description);
Graphoderus cinereus sensu Horn (1883) (in part);
Graphoderus zonatus sensu Zimmermann (1920) (in part), Gschwendtner (1937) (in part), Kamiya (1940), Balfour-Browne (1946), Zaitsev (1972), Lafer (1989), Zeng (1989), Mori and Kitayama (1993), Nilsson (1995), Nilsson et al. (1997);
Graphoderus zonatus zonatus sensu Nilsson (2003a) (in part), Nilsson and Hájek (2015) (in part);



Figure 6. Adhesive discs on pro- (a, c) and mesotarsus (b, d). a-b G. perplexus c-d G. elatus.

Graphoderus zonatus verrucifer sensu Nilsson et al. (1997), Nilsson (2003a) (in part), Nilsson and Hájek (2015) (in part);

Graphoderus perplexus sensu Wallis (1939) (in part), Larson (1975) (in part), Nilsson et al. (1999), Nilsson (2001) (in part), Nilsson (2003a) (in part), Nilsson (2015) (in part), Nilsson and Hájek (2015) (in part).

Type locality. Russia > East Siberia > Amurland.

Type material. Lectotype \Diamond (BMNH), by present designation. Labeled: "Eastern Siberia 995 elatus. Sharp Coll. 1905-313. Data in NHRS JLKB 000023379. Lecto-type *Graphoderus elatus* Sharp, 1882 Des. S. Holmgren et al., 2015". Paralectotype \Diamond (BMNH). Labeled: "Red River. Am. Bor. 995 var. Paralectotype. Sharp Coll. 1905-313. Data in NHRS JLKB 000023380. *Graphoderus perplexus* Sharp 1882 Det. J. Bergsten, 2015".

Lectotype justification. Sharp (1882) based his description of *Graphoderus elatus* on two male specimens, one from "Eastern Siberia (Amurland)" and the other from "North America (Red River)". He gave *G. elatus* the number 995. These two syntype specimens, present in Sharp's collection (BMNH), were studied and genitalia extracted. Both are pinned through cards, these cards being mounted on longer pins. The Siberian specimen is labeled on the face of the card, in Sharp's handwriting "Eastern Siberia 995 elatus", while the North American specimen is labeled "Red River. Am. Bor. 995 var". The syntype specimen from Red River Am. Bor. we consider conspecific with *G. perplexus*. The Lectotype of *G. perplexus* (designated by Larson 1975) was studied at BMNH but is a female so the shape of male genitalia could not be compared. The characters Sharp (1882) used to distinguish *G. elatus* from *G. perplexus* are unconvincing as already argued by Wallis (1939). The syntype specimen from Siberia has the concave outline of the penis' apex central lobe in lateral view. The penis is also of the longer type (Fig. 2k) and belongs to what we initially called *G. perplexus*-like specimens from the east Palearctic. We therefore designate the Siberian specimen as lectotype of *Graphoderus elatus* Sharp, 1882, and have so labeled it. What we initially called *G. perplexus*-like specimens from the east Palearctic belong to *Graphoderus elatus* sharp, 1882, and have so labeled it. What we initially called *G. perplexus*-like specimens from the east Palearctic belong to *Graphoderus elatus* which is here reinstated as a valid species (stat. n.). The American specimen although belonging to *G. perplexus*, is a paralectotype of *G. elatus*. As Sharp's description of *G. elatus* is very short and was based on a mix of two species we provide a redescription and documentation (Fig. 7) based on the designated lectotype followed by a discussion of the intraspecific variations as here interpreted from all examined material (Table 2).

Description of the Lectotype ♂. Body length 14.0 mm; maximum elytral width 8.3 mm.

Head (Fig. 7a) dorsally testaceous; basal black band extending between eyes, retracted under anterior of pronotum, extending apically to eyes with no apparent separation from eyes; V-shaped black marking anteriorly. Head ventrally testaceous; clypeus testaceous; maxillary palpi yellow with apical palpomere piceous distally. Labial palpi yellow with apical palpomere darkened along its inner margin. Antenna testaceous with antennomeres more or less piceous in distal half.

Pronotum (Fig. 7a) 2.1 mm long; maximum width 6.5 mm; testaceous; transverse black bands separated from anterior margin by testaceous band and from posterior margin by narrow testaceous, more piceous band; black bands do not reach sides of pronotum; anterior black band at edges with narrow posteriorly directed projections; posterior black band with narrow lateral portions projecting from basal margin of band. Elytron 10.5 mm long; smooth, yellow with black irrorations; irrorations reduced along margins of elytra; sutural midline black; scutellum piceous.

Ventral side (Fig. 7b) testaceous-rufous, darkened due to age so that minor variations in its color not apparent. Forelegs testaceous; midlegs partly testaceous, mesotibia and mesotarsus rufous-testaceous with golden setae along edge; metatrochanter and metafemur testaceous; metatibia rufous with golden setae along edge; metatarsus rufous-piceous with long golden setae along edge. Elytral epipleuron testaceous; broad anteriorly, gradually tapering along edges of abdomen but relatively broad also in first part of posterior half.

Protarsal claws similar in size and shape, shorter than protarsomere V; mesotarsal claws similar in size and shape. Posterior metatarsal claws almost three times as long as anterior metatarsal claws. Protarsomeres I-III enlarged with three larger adhesive discs basally and about 32 smaller discs distally. Mesotarsomeres with two more or less regular rows of seven discs, left mesotarsus with one additional smaller disc on mesotarsomere I and a second on mesotarsomere II and right one with only one extra disc, on mesotarsomere II.

Penis in dorsal view (Fig. 7c) about 2.8 mm long; width 0.55 mm; apex trifid with three distinct lobes. Penis in lateral view (Fig. 7d) with lateral apical lobes fairly slender; central penis lobe sclerotized along edge, longer than side lobes, its dorsal margin concave (Fig. 7e). Parameres with external margins straighter medially, their apices convergent (Fig. 7f). Lappets of aedeagal ring sclerite short and wide, their outer apical margins rounded (Fig. 7g).



Figure 7. The designated lectotype for *G. elatus* Sharp, 1882 (BMNH). **a** head and pronotum (2.1 mm long, maximum width 6.5 mm) in dorsal view **b** ventral view (body length 14.0 mm) **c** penis in dorsal view (2.8 mm, long 0.55 mm width) **d** penis in lateral view **e** central penis lobe with concave dorsal margin **f** entire genitalia with parameres surrounding the penis **g** lappets of aedeagal ring.

le 2. Studied material of <i>Graphoderus elatus</i> . Sex, catalog number (ID), deposition, locality information, latitude (Lat.), etor of the studied <i>G. elatus</i> specimens. δ =male, 2 =female.	longitude (Lon.), date collected and	
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Sex	Catalog ID	Museum	Locality	Lat.	Lon.	Date	Collectors
4	NHRS-JLKB000023379	BMNH	Russia, Amurland, Siberia				
60	NHRS-JLKB000040578	NHRS	Russia, Kamchatka, Ponds inland from the bay between Cape Zheltyi (south) and Cape Ilya (north)	51.5583°N	157.709°E	1999-07-27	Minakawa & Kurowski
۴0	NHRS-JLKB000040579	NHRS	Russia, Kamchatka, Ponds inland from the bay between Cape Zheltyi (south) and Cape Ilya (north)	51.5583°N	157.709°E	1999-07-27	Minakawa & Kurowski
0+	NHRS-JLKB000040580	NHRS	Russia, Kamchatka, Ponds inland from the bay between Cape Zheltyi (south) and Cape Ilya (north)	51.5583°N	157.709°E	1999-07-27	Minakawa & Kurowski
0+	NHRS-JLKB000040581	NHRS	Russia, Kamchatka, Ponds inland from the bay between Cape Zheltyi (south) and Cape Ilya (north)	51.5583°N	157.709°E	1999-07-27	Minakawa & Kurowski
0+	NHRS-JLKB000040582	NHRS	Russia, Kamchatka, Ponds inland from the bay between Cape Zheltyi (south) and Cape Ilya (north)	51.5583°N	157.709°E	1999-07-27	Minakawa & Kurowski
50	NHRS-JLKB00000054	NHRS	Russia, Kamchatka, Ponds inland from the bay between Cape Zheltyi (south) and Cape Ilya (north)	51.5583°N	157.709°E	1999-07-27	Minakawa & Kurowski
60	NHRS-JLKB000040583	NHRS	Russia, Kamchatka, Elizovo, 12km S	53.0283°N	158.6454°E	1997-07-09	Kholin
60	NHRS-JLKB000040584	NHRS	Russia, Kamchatka, Elizovo, 12km S	53.0283°N	158.6454°E	1997-07-09	Kholin
60	NHRS-JLKB000040591	NHRS	Russia, Kamchatka, Elizovo, 12km S	53.0283°N	158.6454°E	1997-07-09	Kholin
0+	NHRS-JLKB000040585	NHRS	Russia, North Sakhalin, Val river env.	52.493°N	142.683°E	2002-07-29	Minakawa
60	NHRS-JLKB000040586	NHRS	Russia, North Sakhalin, Val river env.	52.493°N	142.683°E	2002-07-29	Minakawa
0+	NHRS-JLKB000040587	NHRS	Russia, Lopukhovaya, Urup, Kuril islands	45.7965°N	149.9002°E	1995-08-29	Oberg
60	NHRS-JLKB000040588	NHRS	Russia, Lopukhovaya, Urup, Kuril islands	45.7965°N	149.9002°E	1995-08-29	Oberg
60	NHRS-JLKB000040589	NHRS	Russia, Lopukhovaya, Urup, Kuril islands	45.7965°N	149.9002°E	1995-08-28	Oberg
60	NHRS-JLKB000040590	NHRS	Russia, Lopukhovaya, Urup, Kuril islands	45.7965°N	149.9002°E	1995-08-28	Oberg
0+	NHRS-JLKB000023362	NHRS	Japan, Horonobe-chô, Teshio gun, Hokkaido	45.0172°N	141.8491°E	1999-10-30	Kamite
50	NHRS-JLKB000023363	NHRS	Japan, Horonobe-chô, Teshio gun, Hokkaido	45.0172°N	141.8491°E	1999-10-30	Kamite
0+	NHRS-JLKB00000061	NHRS	Japan, Horonobe-chô, Teshio gun, Hokkaido	45.0172°N	141.8491°E	2009-09-13	Nakajima
60	NHRS-JLKB000023364	NHRS	Russia, Shimanovsk, Amur region	52.0011°N	127.6842°E	1975-06-20 - 29	Zolotukhin
۴0	NHRS-JLKB000000951	ZMUM	Russia, Lake Kenon, Chita region	52.0402°N	113.3856°E	1973-08-06	Berlov
40	NHRS-JLKB000000952	ZMUM	Russia, Lake Kenon, Chita region	52.0402°N	113.3856°E	1971-08-06	Berlov

Sex	Catalog ID	Museum	Locality	Lat.	Lon.	Date	Collectors
50	NHRS-JLKB000000953	ZMUM	Russia, Lake Kenon, Chita region	52.0402°N	113.3856°E	1973-08-06	Berlov
0+	NHRS-JLKB000023365	HNMO	Japan, Wakasakanai, Toyotomi	45.1059°N	141.6328°E	1987-08-01	Mori
۴0	NHRS-JLKB000023369	HNMO	Japan, Wakasakanai, Toyotomi	45.1059°N	141.6328°E	1987-07-31	Mori
0+	NHRS-JLKB000023370	HNMO	Japan, Wakasakanai, Toyotomi	45.1059°N	141.6328°E	1987-07-31	Mori
50	NHRS-JLKB000023366	HNMO	Japan, Wakasakanai, Toyotomi	45.1059°N	141.6328°E	1993-07-25	Hayashi
۴0	NHRS-JLKB000023367	HNMO	Japan, Wakasakanai, Toyotomi	45.1059°N	141.6328°E	1993-07-25	Hayashi
0+	NHRS-JLKB000023368	HNMO	Japan, Wakasakanai, Toyotomi	45.1059°N	141.6328°E	1993-07-25	Hayashi
۴0	NHRS-JLKB000023371	HNMO	Japan, Bakkaimura, Yuukuru	45.3103°N	141.6207°E	1992-08-22	Kitayama
۴0	NHRS-JLKB000023372	HNMO	Japan, Sarobetsu, Wakasakanai	45.0853°N	141.8197°E	1992-08-21	Kitayama
0+	NHRS-JLKB000023373	HNMO	Japan, Sarobetsu, Wakasakanai	45.0853°N	141.8197°E	1992-08-21	Kitayama
0+	NHRS-JLKB000023374	HNMO	Japan, Sarobetsu, Wakasakanai	45.0853°N	141.8197°E	1992-08-21	Kitayama
0+	NHRS-JLKB000023375	BMNH	Russia, Yakutsk, 18 km E of river Lena, Siberia	61.4372°N	131.0155°E	1970-07-21	Angus
۴0	NHRS-JLKB000023376	BMNH	Russia, Yakutsk, 18 km E of river Lena, Siberia	61.4372°N	131.0155°E	1970-07-21	Angus
۴0	NHRS-JLKB000023377	BMNH	China, Gangca, Qinghai Hu, Qinghai N	37.2952°N	100.1797°E	2013-06-05	Angus, Jia & Zhang
۴0	NHRS-JLKB000023378	BMNH	China, Gangca, Qinghai Hu, Qinghai N	37.2952°N	100.1797°E	2013-06-05	Angus, Jia & Zhang
۴0		BMNH	China, Gangca, Qinghai Hu, Qinghai N	37.2952°N	100.1797°E	2013-06-05	Angus, Jia & Zhang
۴0		BMNH	China, Gangca, Qinghai Hu, Qinghai N	37.2952°N	100.1797°E	2013-06-05	Angus, Jia & Zhang
۴0		SYSU	China, Gangca, Qinghai Hu, Qinghai N	37.2952°N	100.1797°E	2013-06-05	Angus, Jia & Zhang
۴0		SYSU	China, Gangca, Qinghai Hu, Qinghai N	37.2952°N	100.1797°E	2013-06-05	Angus, Jia & Zhang
0+		BMNH	China, Gangca, Qinghai Hu, Qinghai N	37.2952°N	100.1797°E	2013-06-05	Angus, Jia & Zhang
0+		SYSU	China, Gangca, Qinghai Hu, Qinghai N	37.2952°N	100.1797°E	2013-06-05	Angus, Jia & Zhang
۴0		SYSU	China, Gangca, Qinghai Hu, Qinghai N	37.2952° N	100.1797° E	2013-06-05	Angus, Jia & Zhang
۴0	NHRS-JLKB000023381	ZIN	Mongolia, Onon river	48.5941°N	110.8558°E	1987-08-29	Dulma
۴0	NHRS-JLKB000023382	ZIN	Mongolia, Onon river	48.5941°N	110.8558°E	1987-08-29	Dulma
۴0	NHRS-JLKB000023383	ZIN	Russia, Indigirka river	69.5267°N	146.6575°E	1891-07-16	Cherskiy
۴0		ZIN	Russia, Indigirka river	69.5267°N	146.6575°E	1891-07-16	Cherskiy
۴0		ZIN	Russia, Indigirka river	69.5267°N	146.6575°E	1891-07-16	Cherskiy
50	NHRS-JLKB000023384	ZIN	Russia, Verkhoyansk	67.8181°N	134.0181°E	1885-05 & 07	Bung & Tol.
50		BMNH	China, Lesser Kingan, Mts China	49.0892°N	127.5374°E		Weymarn

Sex	Catalog ID	Museum	Locality	Lat.	Lon.	Date	Collectors
۴0		BMNH	China, Lesser Kingan, Mts China	49.0892°N	127.5374°E		Weymarn
0+		NSYSU	China, Nei Mongol, Hulunber, Huihe			2013-07-22	Li, Chunyuan & Chaoqun
50		NSAS	China, Inner Mongolia (Nei Mongol), Xing'an near entry-exit inspection of border between China and The Republic of Mongolia			2014-07-24	Jia
0+		SYSU	China, Inner Mongolia (Nei Mongol), Xing'an near entry-exit inspection of border between China and The Republic of Mongolia			2014-07-24	Jia

† Lectotype.



Figure 8. Distribution map of *G. elatus* based on examined specimens. The lectotype is imprecisely marked in former Amur Region.

Intraspecific variation. Body length between 13.9 and 16.3 mm; maximum elytral width between 8.0 and 9.6 mm. Pronotum length 1.8 to 2.5 mm long; width 5.3 to 7.2 mm; smooth in males; either deeply wrinkled (when also elytra granulated) or smooth in females; in smooth specimens either shining with anterior row of impressed punctures very distinct, or matt with puncture-row less distinct; anterior black band of pronotum mostly continuous, sometimes thin and weak or non-continuous; shape of posterior black band of pronotum varies, separated from posterior margin by testaceous band which sometimes is partly piceous. Elytron between 10.4 and 12.7 mm long; smooth in males; smooth or granulated in females.

Male posterior metatarsal claws almost three times as long as anterior metatarsal claws; female posterior metatarsal claws less than twice as long as anterior metatarsal claws which are slightly curved apically. Protarsomeres I-III enlarged in males with three larger adhesive discs basally and 28-66 smaller discs distally; mesotarsomeres in males with irregular rows of 14-31 adhesive discs; in populations with granulated females, number of adhesive discs in males are in upper range. Penis in dorsal view between 2.4 and 2.8 mm long; width between 0.6 and 0.7 mm. Shape of lappets in aedeagal ring sclerite variable which also applies to outer apical margin.

Distribution. (Fig. 8) The distribution covers Russia, east of the Yenisei-Angara river to the pacific coast, north to the East Siberian Sea and south to Qinghai in China

and Hokkaido in Japan. Specimens from the following regions in east Palearctic were examined: Russia: Yakutia, Kamchatka, Chita Region, Amur Region, Kuril Islands, North Sakhalin, Verkhoyansk and Indigirka. Japan: Hokkaido. Mongolia: Onon river. China: Heilongjiang, Inner Mongolia and Qinghai.

Key to Graphoderus species

As the resolved situation in the east Palearctic means that there are no species in common between Nearctic and Palearctic the key is constructed with a first dichotomy between the continents for ease of use. In order for both males and females to be identifiable, each key step has multiple characters and characters of the pronotal black bands are included as they are often very useful albeit not always absolutely trustworthy. Mesotarsal formula, e.g. 6-4-4, refers to six adhesive discs on mesotarsomere I, four on mesotarsomere II and four on mesotarsomere III. Note that there are errors in the *Graphoderus* genitalia figured in Larson et al. (2000); 190c labeled as *G. perplexus* is more likely *G. fascicollis* and 190b labeled as *G. fascicollis* is possibly *G. manitobensis*, and true *G. perplexus* genitalia does not seem to be included in the figure.

1	Nearctic species
_	Palearctic species
2	Head and pronotum yellow to reddish brown with no defined black mark-
	ings (Fig. 9h); body length 10.4 to 12.4 mm; male genitalia simple with
	barely trifid apex (Fig. 20–p)G. liberus
_	Head with black V-shaped markings and pronotum with two well-defined
	black bands (Fig. 9g, i-k); body length larger, up to 15.7 mm; penis with
	distinct trifid apex (Fig. 2m–n, q–v)
3	Posterior black band of pronotum not reaching posterior margin, or some-
	times separated from margin by a piceous-reddish area, anterior black band
	separated from anterior margin (Fig. 9k); male protarsus with 25-35 adhe-
	sive discs, male mesotarsus with 13–20 discs; male penis deeply trifid, invagi-
	nations separating lateral lobes from central lobe distinctly deeper than width
	of lateral lobes of penis apex, as in Figure 2u-vG. perplexus
_	Posterior black band of pronotum contiguous with posterior margin, anterior
	black band of pronotum contiguous or not with anterior margin; male tarsal
	discs various, mesotarsus with 0, 12 or 25-30 discs; trifid apex of male penis
	shallower, invaginations not deeper than width of lateral lobes of penis apex
	(Fig. 2m–n, q–t)
4	Anterior black band of pronotum contiguous with anterior margin (Fig. 9j); fe-
	male pronotum with weak corrugated sculpture; male mesotarsus not dilated and
	lacking adhesive discs; male protarsal claws different in shape and size, posterior
	claw with sinuate ventral margin and about 2/3 length of anterior claw; male
	parameres very long, at least 1/4th longer than penis (Fig. 2s) G. occidentalis

_	Anterior black band of pronotum mostly separated from anterior margin by a more or less evident reddish area; female pronotum with conspicuous cor-
	rugated sculpture; male mesotarsus dilated with adhesive discs on ventral sur- face; male protarsal claws equal or anterior claw only slightly longer than pos-
	terior which does not have a sinuate ventral margin; male parameters shorter, not more than $1/5$ th longer than penic (Fig. 2m. g)
5	Metanepisterna ("metasternal wing") broad, width between 0.48 and 0.60
	mm; female elytron at shoulder with less pronounced striolate punctures;
	much shorter than lateral lobes (Fig. $2m-n$)
_	Metanepisterna ("metasternal wing") narrower, width between 0.30 and 0.41
	mm; female elytron at shoulder with pronounced strioles; male mesotarsus
	with 25–30 discs in four rows; central penis lobe of trifid apex about as long
	as lateral lobes (Fig. 2q–r)
6	Posterior black band of pronotum narrow, equal to only $1/3$ to $1/2$ of medial
	yellow area, contiguous with posterior margin (Fig. 9d); epipleuron broader
	at level of abdominal ventrite II than at level of ventrite I, body pear-shaped
	due to posteriority widened epipieura; male genitaria as in Figure 2g-n
_	Posterior black band of pronotum broad, equal to at least 1/2 of medial vel-
	low area, or if narrower then not contiguous with posterior margin; epipleu-
	ron evenly tapering from base to apex, body not overly "pear-shaped"7
7	Ventral side of body mostly piceous; metatibia and metatarsus dark brown
	to black; female pronotum with conspicuous corrugated sculpture; anterior
	black band of pronotum continuous with anterior margin, in males this band
	is narrow and equal to about 1/3 of medial yellow band (Fig. 9a); male geni-
	talia as in Figure 2a–b. East Palearctic
_	babitus darker: female proportum with or without conspicuous corrugated
	sculpture: anterior black hand of pronotum continuous or not with anterior
	margin, if continuous in males broader then 1/3 of medial vellow band. East
	or west Palearctic
8	Transverse black bands of pronotum contiguous with anterior and posterior
	margin, respectively (Fig. 9b); anterior mesotarsal claw longer than posterior
	claw, strongly in males weakly in females; female pronotum with weak cor-
	rugated sculpture; male mesotarsus not dilated and without discs; trifid apex
	of male penis very shallow, parameters very long, at least 1/4th longer than
	Anterior and posterior black bands of proportion continuous or pot with mar
_	gins: mesotarsal claws of same length in both seves: female proportium cor-
	rugated or not; male mesotarsus dilated and with adhesive discs on ventral
	surface; male penis apex moderate to deeply trifid and parameters shorter,
	maximum 1/5th longer than penis (Fig. 2e–f, i–l, w–x)

9 Transverse black bands of pronotum not contiguous with anterior and posterior margin, separated by narrow bands or rarely almost contiguous; female elytra granulated or not; male mesotarsus with 14-60 adhesive discs that are small and usually in irregular rows10 Posterior black band of pronotum contiguous with posterior margin, anterior transverse band contiguous with anterior margin or narrowly separated by rufous area (Fig. 9e); female elytra never granulated; male mesotarsus with 12–14 discs that are larger and in two regular rows......11 10 Epipleura rather wide at level of abdominal ventrites I-III (Fig. 10f-h); central lobe of male trifid apex in lateral view concave (Fig. 2l). East Palearctic, east of Yenisei-Angara river G. elatus Epipleura narrower at level of abdominal ventrites I-III (Fig. 10a-b); central lobe of male trifid apex in lateral view convex (Fig. 2x). Palearctic, west of 11 Minimum distance between meso- and metacoxae almost same as width of metaventral process between mesocoxae (Fig. 11a); female posterior metatarsal claw about 1.8 the length of anterior claw, which is not strongly curved apically; male mesotarsus with 12 discs in two rows ventrally, formula 4-4-4; male penis apex less deeply trifid and central lobe in lateral view more abruptly raised (Fig. 2e-f). East Palearctic...... G. bieneri Minimum distance between meso- and metacoxae clearly less than width of metaventral process between mesocoxae (Fig. 11b); female posterior metatarsal claw about 1.5 the length of anterior claw which is strongly curved apically; male mesotarsus with 14 discs in two rows, formula 6-4-4; male penis deeply trifid and central lobe in lateral view describing a long evenly convex curve (Fig. 2i-j). Palearctic G. cinereus

Discussion

The within species variation in the shape and extension of the transverse black bands on the pronotum in *G. zonatus* (Nilsson 1986) was observed to exist in *G. perplexus* and *G. elatus* as well. Rarely, *G. zonatus* specimens are found where the basal black band reaches all the way to the posterior margin. Initially, we were struck by the very thin black bands in several specimens of *G. elatus*, and while this color pattern seems more common in *G. elatus* than in either *G. zonatus* or *G. perplexus* we have in some specimens found the same coloration in the latter species too. We therefore consider this character as less reliable for species diagnosis within the *zonatus*-species complex. We also examined the shape of the bifurcation in the chitinous ring around the male genitalia, which Wallis (1939) indicated as diagnostic. We found it to be informative, probably significantly so if the shape had been quantified and tested statistically but we observed within species variation in all three species. It has also been seen in *G. zonatus* that the coloration of elytra can probably be connected to the habitat e.g. darker speci-



Figure 9. Habitus photographs of all *Graphoderus* species in dorsal view. **a** *G. adamsii* **b** *G. austriacus* **c** *G. bieneri* **d** *G. bilineatus* **e** *G. cinereus* **f** *G. elatus* **g** *G. fascicollis* **h** *G. liberus* **i** *G. manitobensis* **j** *G. occidentalis* **k** *G. perplexus* **l** *G. zonatus.*



Figure 10. Ventral view showing the epipleural width. **a-b** *G. zonatus* **c-e** *G. perplexus* and **f-h** *G. elatus*. Specimens from Sweden (**a**), France (**b**), USA (**c** Lectotype of *G. perplexus*), Canada, Quebec (**d**), Red River Am. Bor. (**e** paralectotype of *G. elatus*), Amurland Russia (**f** Lectotype *G. elatus*), Gangca China (**g**), "Manchuria" Weymarn coll. (**h**). The species differ in the epipleural width especially at level of abdominal ventrites I-III.

mens have more often been found in dystrophic water (Nilsson 1986) and this might indicate that specific coloration may also be an inferior character to separate species. Instead the great interspecific variation in the male genitalia, especially at the top of the penis, is commonly used to separate species within Dytiscidae (Nilsson and Holmen 1995, Larson et al. 2000, Miller 2001, Bergsten and Miller 2006), and we found this as the most informative character (Fig. 2). The shape in lateral view of the central lobe at the penis' trifid apex was a qualitative non-overlapping character we found the most reliable to separate *G. zonatus* from *G. perplexus*/*G. elatus*. The male penis (and parameres) were significantly longer relative to body length and also had a more elongate shape as measured by a length to width ratio in *G. elatus* compared to *G. perplexus*. Though significant, variation was overlapping for these characters (Fig. 4). Although not quantified, the epipleura are also in general wider in *G. elatus* not only compared to *G. zonatus*, but also compared to *G. perplexus* (Fig. 10).

Since *G. elatus* was described by Sharp (1882) it has mostly been treated as a synonym of different species (Horn 1883, Zimmermann 1917, Wallis 1939) rather than a valid species. Wallis (1939) was the one who concluded that its diagnostic character was not enough to retain both *G. perplexus* and *G. elatus*, but neither the size nor the shape of the male genitalia had been considered (Sharp 1882, Wallis 1939). However, when Nilsson et al. (1999) later identified five adult specimens from Kamchatka as *G. perplexus*, they somewhat misleadingly stated this to be the first Palearctic record of the species despite the fact that in synonymizing *G. elatus* with *G. perplexus* Wallis (1939) defined *G. perplexus* as a species occurring in both Nearctic and east Siberia



Figure 11. Ventral view showing meso- and metathorax of *G. bieneri* (**a**) and *G. cinereus* (**b**). To indicate the relative distances between the mesocoxae (1) and the meso- and metacoxae (2).

of Russia. It was not until the compilation of the world catalogue two years later that Nilsson (2001) brought back the somewhat forgotten name *G. elatus*. As Nilsson's collection now is housed at the NHRS, we have re-examined all *Graphoderus* material from Kamchatka (Nilsson et al. 1999) and Urup, Kuril Islands (Nilsson et al. 1997) as well as material from North Sakhalin collected by Minakawa in 2002. All the males proved to have the concave shape of the central penis lobe that separates *G. elatus* and *G. perplexus* from *G. zonatus* (Fig. 2) and the genitalia also proved to be larger than *G. perplexus*, fitting into the diagnosis of *G. elatus*.

From our results we here propose a strict allopatric distribution of the three species in the zonatus complex. We propose that 1) G. perplexus only occurs in the Nearctic region, 2) G. elatus only occurs in the east Palearctic region, east of the Yenisei-Angara river and 3) G. zonatus occurs in the Palearctic region from central and north Europe through Turkey, Caucasus and eastwards up to the Yenisei-Angara river. It is likely that there is a contact zone where G. zonatus and G. elatus meet, but whether any hybridization occurs is unknown. All Graphoderus zonatus records from Amur, Chita, Verkhoyansk, Indigirka, Kamchatka, Sakhalin and Kuril Islands are Graphoderus elatus. Also material from Magadan, Khabarovsk and Primorsky Kray reported by Lafer (1989) is most likely G. elatus (not examined). Material we have examined from Yakutia has also been G. elatus and records referred to East Siberia are probably mostly G. elatus but could refer to G. zonatus. The material we studied from northeast Mongolia was G. elatus and the reported G. zonatus from the north-central and western part of the country (Shaverdo et al. 2008) might be misidentified G. elatus. Likewise, the newly collected material from Gangca in China was G. elatus as well as material from Heilongjiang and Inner Mongolia, but G. zonatus may occur in the more western parts

of China. What was thought to be *G. zonatus* in Japan was first discovered on Hokkaido by Matsumoto in 1986 (Mori and Kitayama 1993). We here conclude that all material from Japan referred to *G. zonatus* is instead *G. elatus*.

Our easternmost record of true G. zonatus was collected outside Irkutsk, just west of the Yenisei-Angara river. Strikingly this is also the easternmost record of several other west Palearctic aquatic beetles like *Helophorus granularis* (Linnaeus, 1761), H. strigifrons Thomson, 1868 and H. pumilio Erichson, 1837 (Angus 2011). The sister species pair in the *Agabus lineatus* Gebler, 1848 species group is also separated east and west of Yenisei-Angara river (Nilsson 2003b). As well, the Palearctic Colymbetes paykulli Erichson, 1837 and the Holarctic C. dahuricus Aubé, 1837 are separated in this area (Drotz et al. 2015). All other examined "G. zonatus" from the Palearctic, east of the Yenisei turned out to be G. elatus but it is important to note that the number of adhesive discs on male pro- and mesotarsus are not reliable characters to separate G. zonatus from G. elatus. In the populations of G. elatus from Urup, Inner Mongolia (leg. Li, Chunyuan and Chaoqun) and Yakutsk (18 km E. of river Lena) the females had granulated elytra and the males from Urup had a significant larger number of adhesive discs. In the material examined from Kamchatka, Hokkaido, Inner Mongolia (leg. Jia), Gangca and Heilongjiang females had smooth elytra and males a lower number of adhesive discs. Yet our examined material from North Sakhalin indicates that the two morphs can exist within the same population. However, the question arises whether these dorsal female forms could have arisen twice, completely independent of each other or if they are sister-species and inherited the polymorphism from a common ancestor. Based on the male genitalia it seems more likely that G. elatus and G. perplexus are sister-species, which would require either a loss of the polymorphism in G. perplexus or independent gains in G. zonatus and G. elatus. We are inclined to believe that a loss of polymorphism is more likely than independent gains, but phylogenetic studies (in progress) are first required to establish sister-species relationship in Graphoderus.

Conclusions

From qualitative and quantitative characters of the male genitalia the earlier synonymized name *G. elatus* Sharp, 1882 is reinstated as a valid species and a lectotype has been designated. We propose an allopatric distribution of species in the *zonatus*-species complex where *G. perplexus* is Nearctic, *G. elatus* only occurs in east Palearctic, east of the Yenisei-Angara river and *G. zonatus* occurs only west of the same river. All previous *G. zonatus* records from east of this river are misidentified *G. elatus*. Surprisingly, there are now two *Graphoderus* species with distinctly dimorphic females, *G. zonatus* and *G. elatus*. Finally, by providing male genitalia illustrations together with a new identification key to all *Graphoderus* species we hope to aid future identification work and taxonomic endeavors in the group.

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RESEARCH ARTICLE



Notes on Nilothauma Kieffer from Oriental China, with descriptions of three new species (Diptera, Chironomidae)

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Abstract

Three new species of *Nilothauma* Kieffer are described and figured from Oriental China: *N. angustum* **sp. n.** based on the male only, *N. aristatum* **sp. n.** based on the male, pupa and larva, and *N. bilobatum* **sp. n.** based on the male and pupa. In addition, new distribution records are given for *N. japonicum* Niitsuma, *N. nojirimaculatum* Sasa, *N. hibaratertium* Sasa, and *N. acre* Adam & Sæther. A key to known males of *Nilothauma* Kieffer in China is provided.

Keywords

Nilothauma, new species, new records, Oriental China, key

Introduction

The genus *Nilothauma* Kieffer, 1921 is represented by 43 species: six species occurring in the Palaearctic region, four in the Nearctic region, 16 in the Neotropical region (not including *N. aleta* Roback and *N. duena* Roback due to the uncertain status), six species in the Oriental region, 11 species in the Afrotropical region, two species in the Australasian region, and two species occurring both in the Palaearctic and Oriental regions (Adam and Sæther 1999; Mendes and Andersen 2009; Qi et al. 2014). From China, five species have been recorded: *N. japonicum* Niitsuma, *N. nojirimaculatum* Sasa, *N. acre* Adam & Sæther, *N. quatuorlobum* Yan, Tang & Wang, and *N. pandum* Qi, Lin, Wang & Shao; all in the Oriental part of the country. No adult information is available on the genus from Palearctic parts of China.

In the present paper, we present new material of *Nilothauma* from Oriental China. Three species are described as new to China, and new distributional records are given for *N. acre* Adam & Sæther, *N. hibaratertium* Sasa, *N. japonicum* Niitsuma and *N. nojirimaculatum* Sasa. We also present an identification key to males of *Nilothauma* in China.

Materials and methods

Descriptions of morphological characters are based on slide-mounted specimens in Euparal. Terminology for morphology and abbreviations follow Sæther (1980) and Adam and Sæther (1999).

Most of the specimens examined here are deposited in the College of Life Science, Taizhou University (LTZU) and partial in Nankai University (LNKU). The holotype specimens of three new species are deposited in the Ecology Department, Jinan University (EJNU).

Taxonomy

Nilothauma angustum sp. n.

http://zoobank.org/6AEAD9D5-C373-4D0A-822A-5449C7A62C06 Figs 1-11

Type material. Holotype: male (EJNU), CHINA: Yunnan, Ximeng City, Mengsuo Lake, 22°38.689'N, 99°35.631'E, Alt. 1090m, 27.viii.2014, Tang HQ, light trap. Paratype: 1 male (LTZU), as holotype.

Diagnosis. The adult male of *N. angustum* sp. n. can be distinguished from all other known species of the genus by the following combination of characters: wing with four partially connected dark markings; anterior T IX projection extensively microtrichiose, divided into two lobes, each with apical simple setae forming a fan-like structure; posterior T IX projection extensively microtrichiose, nearly parallel-sided, setose, with long anterolateral arms; anal point broadly lanceolate, microtrichiose along the median ridge and the apical margin; median volsella with microtrichia and two apical setae; gonostylus peaked apically.

Etymology. From the Latin *angustus* (narrow), referring to the male hypopygium with apically narrowed gonostylus.


Figures 1–11. *Nilothauma angustum* sp. n., male. I wing 2 thorax, lateral view 3 legs 4 foretibial apex 5 mid tibial apex 6 hind tibial apex 7 hypopygium, dorsal view (left) and ventral view (right) 8 anterior anal tergal projection 9 posterior anal tergal projection 10 anal point 11 posterior margin of anal tergite.

Description. Male imago (n = 2).

Total length 2.1–2.2 mm. Wing length 0.9–1.1 mm. Total length/wing length 2.1–2.2. Wing length/length of profemur 2.1–2.2.

Coloration. Generally yellow, thorax (Fig. 2) yellow except scutum, pre-episternum, scutellum and postnotum dark brown, abdomen yellowish brown. Wing with 4 partially connected dark markings (Fig. 1). Foreleg yellow with both ends of femur, apex of tibia, apical 1/3 of $ta_{1,}$ and ta_{2-5} brown; mid leg with sub-apex of femur and sub-base of tibia brown; hind leg with sub-apex of femur brown (Fig. 3).

Head. AR 0.18–0.20. Temporals 7–11, uniserial. Clypeus with 17–19 setae. Tentorium 80–108 μ m long, 11–15 μ m wide; stipes 50–65 μ m long, 6–8 μ m wide. Lengths of palpomeres 1–5 (in μ m): 28–30, 25–30, 58–63, 85–105, 125–130. Palpomere 3 with 2 sensilla clavata; Pm₅/Pm₃ 2.1–2.2.

Thorax (Fig. 2). Antepronotal lobe much reduced. Dorsocentrals 9–10, uniserial; acrostichals 7–9, biserial; prealars 2. Scutellum with 2 setae.

Wing (Fig. 1). VR 1.5–1.6. Brachiolum with 1 seta, R with 9–13 setae, R_1 with 6–9 setae, R_{445} with 10–11 setae.

Legs (Fig. 3). Spur of fore tibia 63–65 μ m long including 30–35 μ m long scale (Fig. 4). Spur of mid tibia 20–25 μ m long (Fig. 5) including 13–15 μ m long comb. Spurs of hind tibia 18–25 μ m and 28–30 μ m long, respectively; comb 15–20 μ m long (Fig. 6). Width at apex of fore tibia 28–30 μ m, of mid tibia 33–35 μ m, of hind tibia 30–34 μ m. Lengths and proportions of legs in Table 1.

Hypopygium (Fig. 7). Tergite IX with 2 dorsal projections. Anterior projection (Fig. 8) microtrichiose, 60–63 μ m long, 20–23 μ m wide at base, split into 2 lobes; each 40–45 μ m long, 10–12 μ m wide at base, 5–6 μ m wide at apex, with simple apical setae, together forming fan-like structure. Posterior projection (Fig. 9) extensively microtrichiose, 37–40 μ m long, 35–40 μ m wide at base, 17–20 μ m wide at apex, nearly parallel-sided, apically rounded with 8 setae; long anterolateral arms present. Anal point (Fig. 10) broadly lanceolate, 30–35 μ m long, 25–28 μ m wide at base, 28–30 μ m wide at middle, with microtrichia along median ridge and apical margin. Posterior margin of tergite IX (Fig. 11) with 8–10 setae located to each side of anal point. Laterosternite IX with 3 setae. Phallapodeme 28–30 μ m long. Transverse sternapodeme rounded medially without median elongation. Gonocoxite 78–80 m long. Superior volsella 30–35 μ m long, slender, clubshaped with 4 apical setae, without microtrichia. Median volsella 8–10 μ m long, with 2 apical setae and microtrichia. Inferior volsella 53–58 μ m long, pointed apically, with microtrichia and 6–7 apically cleft setae. Gonostylus 88–90 μ m long, apically narrowed and peaked, with row of 4–5 split distal-median setae. HR 0.86–0.89, HV 2.3–2.5.

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅	LR	BV	SV
P ₁	438-500	325-360	438-500	155-195	130-150	105-110	70-80	1.4	2.5-2.6	1.7
P_2	450-500	325-338	185-190	80-100	60-75	50-63	50-60	0.56-0.58	3.5-4.4	3.6-4.1
P ₃	488-538	488-538	270-320	135-165	140-165	100-108	70-78	0.55-0.60	2.7-2.8	3.4-3.6

Table 1. Lengths (μ m) and proportions of legs of *Nilothauma angustum* sp. n., male (n = 2).

	N. angustum sp. n.	N. flabellatum	N. kakumense	
Anterior T IX projection	with simple setae	with apically branched setae	with apically widened and unbranched setae	
Main part of posterior T IX projection	with microtrichia	without microtrichia	without microtrichia	
Median volsella	with microtrichia	without microtrichia	without microtrichia	
Anal point	without distal-median knob	with distal-median knob	with distal-median knob	
Transverse sternapodeme	without median elongation	with median elongation	without median elongation	

Table 2. Comparison of male hypopygial characters in *N. angustum* sp. n., *N. flabellatum* Adam & Sæther and *N. kakumense* Adam & Sæther.

Female imago, pupa and larva. Unknown.

Remarks. The male hypopygium is similar to those of *N. flabellatum* Adam & Sæther, 1999 and *N. kakumense* Adam & Sæther, 1999 as the anterior T IX projection has long apical setae forming fan-like structures. The differences between these three species are given in Table 2.

Distribution. Oriental China (Yunnan Province).

Biological note. The males were collected at the bank of Mengsuo Lake by light trap, where the nutrient levels are relatively high (conductivity $39-42 \ \mu s/cm$, chlorophyll-a $10.5-11.1 \ \mu g/l$). The co-occurring dominant species are eutrophic taxa, such as *Kiefferulus* sp., *Polypedilum nubeculosum* (Meigen), *Polypedilum sordens* (van der Wulp), and *Tanytarsus oscillans* Johannsen.

Nilothauma aristatum sp. n.

http://zoobank.org/53489B41-D9EC-4AA2-AC3B-B6001B819231 Figs 12–24

Type material. Holotype: male with pupal exuviae (EJNU), CHINA: Anhui Province, Huangshan Nature Conservation Reserve, stream in Huang Mountain, 30°04.317'N, 118°09.320'E, Alt. 520 m, 4.v.2014, Tang HQ, light trap. Paratypes: 1 male (LTZU), CHINA: Zhejiang Province, Lin-An City, Tianmu Mountain, 16.vii.2012, Lin XL, hand net; male with larval and pupal exuviae (LTZU), reared by Lin XL, as previous; 3 pupal exuviae (EJNU), CHINA: Guangdong Province, Dongguan City, Yinping Nature Conservation Reserve, 22°53.772'N, 114°14.086'E, 17.iv.2012, Tang HQ, hand net.

Diagnosis. The adult male of *N. aristatum* sp. n. can be distinguished from other known *Nilothauma* species by the anterior T IX projection with plumose setae; the anal point broadly lanceolate with microtrichia along the median ridge; the superior volsella slender with a lateral spur, and one lateral and 2–3 apical setae, without microtrichia. The pupa is characterized by the relatively short frontal setae (1.5–2.0 times as long as the major axis of basal ring); and the anal comb of abdominal segment VIII consisting of a main spur and a single accessory spine. The larva cannot be reliably separated from those of other species.



Figures 12–17. *Nilothauma aristatum* sp. n., male. 12 wing 13 foretibial apex 14 mid tibial apex 15 hind tibial apex 16 hypopygium, dorsal view (left) and ventral view (right) 17 posterior margin of anal tergite.

Etymology. From Latin *aristatus* (aristate), referring to the male hypopygium with a lateral spur on the superior volsella.

Description. Male imago (n = 2).

Total length 3.0–3.5 mm. Wing length 1.4–2.1 mm. Total length/wing length 1.7–2.2. Wing length/length of profemur 1.9–2.6.

Coloration. Entirely pale yellow. Wing without any marking (Fig. 12).

Head. AR 0.16–0.21. Temporals 6. Clypeus with 10–13 setae. Tentorium 145–170 μ m long, 21–23 μ m wide. Stipes 85–90 μ m long, 8–10 μ m wide. Lengths of palpomeres 1–5 (μ m): 30–32, 30–40, 70–80, 130–140, 155–160. Palpomere 3 with 2 sensilla clavata, longest 10 μ m long. Pm₃/Pm₃ 1.9–2.3.

Thorax. Dorsocentrals 5-7, acrostichals 10-15, prealars 2-3, scutellars 1-2.

Wing. VR 1.4. Brachiolum with 1 seta, R with 13–15 setae, R_1 with 11 setae, R_{4+5} with 3–4 setae.

Legs. Spur of foretibia 68–75 μ m long including 30–43 μ m long scale (Fig. 13). Spur of mid tibia 30–38 μ m long (Fig. 14) including 16–25 μ m long comb. Spurs of hind tibia 30–38 μ m and 37–40 μ m long, respectively (Fig. 15); comb 15–28 μ m long. Width at apex of foretibia 40–50 μ m, of mid tibia 45–50 μ m, of hind tibia 43–54 μ m. Lengths and proportions of legs in Table 3.

Hypopygium (Fig. 16). Tergite IX with 2 dorsal projections. Anterior projection completely divided into 2 oval lobes; each 35–37 μ m long, 12–13 μ m wide at middle, with 8–10 plumose setae 50–63 μ m long. Posterior projection 10–12 μ m long, 10–13 μ m wide at base, 5–6 μ m wide at apex, apically rounded, with 5 setae 13–20 μ m long. Anal point very broadly lanceolate, 50–60 μ m long, 18–20 μ m at base, 25–27 μ m at middle, with microtrichia along median ridge. Posterior margin of tergite IX (Fig. 17) with 9–11 setae. Laterosternite IX with 3 setae. Phallapodeme 37–40 μ m long. Superior volsella 45–50 μ m long, with lateral spur, and one lateral and 2–3 apical setae, without microtrichia. Median volsella 10–13 μ m long, bearing 2 apical setae and microtrichia. Inferior volsella 78–90 μ m long, curved dorsally, pointed apically, with microtrichia and 5 apically branched setae. Gonostylus 110–130 μ m long, with 8 split median setae in distal 1/3. HR 1.02–0.92, HV 2.69–2.73.

Pupa (n = 4).

Total length 3.5–4.4 mm. Exuviae pale brown with anal comb on abdominal segment VIII yellowish brown.

Cephalothorax. Frontal seta short, $30-50 \mu m \log (n = 2)$. Basal ring small, stomalike, with major axis $20-25 \mu m \log n$, minor axis $5-8 \mu m$ high. Frontal setae 1.8-2.0 times as long as major axis of basal ring. Thorax pebbled and rugose dorsally.

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅	LR	BV	SV
P_1	725-788	538-575	725-775	375-450	310-375	260-288	125-150	1.4	1.7-1.9	1.7-1.8
P ₂	625	450-525	275-350	140-150	100-125	70-75	60-75	0.61-0.67	3.5-3.7	3.3-3.9
P ₃	750-800	725-800	375-488	200-250	200-250	150-163	85-88	0.52-0.61	2.8-2.9	3.3-3.9

Table 3. Lengths (μ m) and proportions of legs of *Nilothauma aristatum* sp. n., male (n = 2).



Figures 18–24. *Nilothauma aristatum* sp. n., pupa (**18–21**) and larva (**22–24**). **18** dorsal view of the abdomen (male) **19** anal comb of abdominal segment VIII, showing combs of both sides **20** ventral view of abdomen I–VIII **21** dorsal view of abdomen IX (female) **22** antenna **23** mandible **24** mentum.

Abdomen (Fig. 18). Tergite I without spinulation; T II–VI extensively spinulated; T VII with anterior and posterior bands of spines; T VIII with anterolateral and median bands of spines; tergite T IX with median spinulation in female (Fig. 21), but without any spinulation in male. S I–II without spinulation; S III–IV with anterior spinulation; sternite IV with weak anterolateral spinulation; S V with weak anterolateral and caudolateral spinulation; S VI–VIII with anterolateral and median spinulation, occasionally anterolateral spinulation merged to median in S VIII (Fig. 20). T II with row of 70–78 caudal hooklets with posterior groups of points behind each end. Conjunctives III/IV and IV/V with rows of spinules. Pedes spurii B weakly developed on segment II. Anal comb of segment VIII (Fig. 19) composed of main spur 20–30 μ m long and single accessory spine 7.5–17.5 μ m long. Segment I without L-setae; segments II–III each with 3 L-setae on each side; segment IV with 2 L-setae and 1 LS-seta on each side; segments V–VIII each with 4 LS-setae on each side. Anal lobe 200–240 μ m long, 2.4–2.6 times as long as broad, with 35–48 lateral setae, dorsal seta located near distal 1/3.

Larva (n = 1).

Total length 5 mm. Head capsule about 300 µm long, about 260 µm wide.

Coloration. Red color in fresh specimens, head pale yellow. Mentum and postoccipital margin brown.

Antenna (Fig. 22). Lengths of antennal segments 1–6 (μ m): 28, 10, 4, 13, 4, 4. AR 0.8. Basal segment with ring organ situated in distal 1/6; antennal blade 25 μ m long, extending to apex of segment 4; segment 6 hair-like, almost as long as segment 5.

Mandible (Fig. 23). Total length 85 μ m. Apical tooth 40 μ m long; 4 inner teeth small, arising from common base. Seta subdentalis 30 μ m long, reaching middle of apical tooth.

Mentum (Fig. 24). Width 55 μ m. Two pale median teeth and 7 pairs of gradually decreasing lateral teeth present. Ventromental plate 65 μ m wide.

Female imago. Unknown.

Remarks. The male is similar to that of Oriental species *N. acre* Adam & Sæther, 1999 in having the wing unmarked, the anterior T IX projection with plumose setae, the anal point lanceolate, and the superior volsella slender with a lateral spur and one lateral and two three apical setae. It differs from it as the anal point bears microtrichia along the median ridge, the superior volsella is relatively long compared to the median volsella (length ratio, Svo/Mvo > 4.0) and the inferior volsella has simple setae only. In *N. acre*, the anal point is bare, length of Svo/Mvo is around 2.0 and the inferior volsella has apically split setae.

The pupa of *N. aristatum* sp. n. will key to "*N.* sp. Australia" in Adam and Sæther (1999), but may be separable by the relatively short frontal setae. The ratio of the length of the frontal seta to the length of the major axis of basal ring is 1.8-2.0 in *N. aristatum* sp. n., but 4.6-6.5 in the latter. The larva of *N. aristatum* sp. n. somewhat resembles that of *N. japonicum* Niitsuma, 1985, but it remains uncertain because of a paucity of data.

Distribution. Oriental China (Anhui, Guangdong and Zhejiang Provinces).

Biological note. The larva and pupa of *N. aristatum* sp. n. are found in first-, or second-order streams. The water is relatively clean and cold (water temperature 15°C-20°C, pH 7.80-7.88, DO% 90.6-93.4, DO 8.09-9.36 mg/l, and conductivity 25-34 μ s/cm). The co-existing dominant species of chironomids are *Eukiefferiella* spp., *Rheotanytarsus* spp., *Rheocricotopus* spp., and *Parametriocnemus* spp. Some steno-thermic species, such as *Heleniella* sp. and *Pagastia* sp., are frequently observed in the pupal exuviae samples.

Nilothauma bilobatum sp. n.

http://zoobank.org/191CECE5-B1B0-4BE0-A649-67F7EAB2B4CE Figs 25-40

Type material. Holotype: male with associated pupal exuviae (EJNU), CHI-NA: Guangxi Zhuang Autonomous Region, Guilin City, Qingshitan Reservoir, 25°31.640'N, 110°13.499'E, Alt. 235 m, 26.viii.2014, Long Term Ecology Research Group (LTER), light trap. Paratypes: 2 males with pupal exuviae as holotype (EJNU); 1 male and 1 female pupa (EJNU), CHINA: Guangdong Province, Shantou City, Nan'ao county, Shen-Ao Reservoir, 23°28.390'N, 117°06.683'E, Alt. 61m, 17.iv.2015, Tang HQ, light trap.

Diagnosis. The male of *N. bilobatum* sp. n. can be distinguished from other *Ni-lothauma* species by the following combination of characters: anterior T IX projection bearing simple setae only; anal point broadly lanceolate with microtrichia; superior volsella with a lateral spur, a main lobe bearing 4–5 apical setae, and a blunt-tipped lobe bearing a terminal seta, without microtrichia. The pupa can be separated from others by the following characters: relatively short frontal setae (as long as or slightly longer than the major axis of basal ring); and anal comb of abdominal segment VIII consisting of a main spur and 2–3 accessory spines.

Etymology. From Latin *bi*- (two) and *lobatus* (lobate), referring to the male hypopygium with two lobes in the superior volsella.

Description. Male imago (n = 4).

Total length 2.4–3.1 mm. Wing length 1.2–1.6 mm. Total length/wing length 1.6–2.7. Wing length/length of profemur 2.0–2.5.

Coloration. Generally pale yellow. Wing without any marking. Foreleg entirely yellowish brown; mid and hind legs with femora and tibiae pale yellow, and tarsus yellowish brown.

Head. AR 0.18–0.19. Temporals 7–10. Clypeus with 12–13 setae. Tentorium 100–125 μ m long, 15–25 μ m wide. Stipes 120–130 μ m long, 5–8 μ m wide. Lengths of palpomeres 1–5 (μ m): 18–25, 33–37, 55–65, 100–125, 123–165. Palpomere 3 with 2 sensilla clavata; Pm₃/Pm₃ 2.2–2.5.

Thorax. Dorsocentrals 9–11, acrostichals 6–10, prealars 2–3, scutellars 2.

Wing (Fig. 25). VR 1.3–1.6. Brachiolum with 1 seta, R with 11–13 setae, R_1 with 8–11 setae, R_{4+5} with 13–17 setae.



Figures 25–32. *Nilothauma bilobatum* sp. n., male. **25** wing **26** foretibial apex **27** mid tibial apex **28** hind tibial apex **29** hypopygium, dorsal view (left) and ventral view (right) **30** anal point **31** posterior margin of anal tergite **32** median volsella.

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅	LR	BV	SV
P_1	625-650	475-513	663-700	300-360	280-300	210-240	120-130	1.3-1.4	1.8-1.9	1.6-1.9
P_2	530-650	390-475	230-290	90-130	83-100	48-70	45-70	0.59-0.66	3.8-4.2	3.7-4.0
P ₃	600-725	600-700	290-400	180-210	175-210	120-150	75-100	0.48-0.60	2.7-2.8	3.3-4.1

Table 4. Lengths (μ m) and proportions of legs of *Nilothauma bilobatum* sp. n., male (n = 4).

Legs. Spur of foretibia 60–80 μ m long including 28–38 μ m long scale (Fig. 26). Spur of mid tibia 22–25 μ m long including 15–23 μ m long (Fig. 27). Spurs of hind tibia 27–35 μ m and 33–47 μ m long, respectively (Fig. 28); comb 17–24 μ m long. Width at apex of foretibia 34–42 m, of mid tibia 41–52 m, of hind tibia 48–50 m. Lengths and proportions of legs in Table 4.

Hypopygium (Fig. 29). Tergite IX with 2 dorsal projections. Anterior projection completely divided into 2 oval lobes; each 35–55 μ m long, 8–10 μ m wide at middle, with 12–15 simple setae 30–50 μ m long. Posterior projection 28–32 μ m long, 50–65 μ m wide at base, 8–15 μ m wide at apex, apically rounded, with 11–13 setae 20–25 μ m long. Anal point (Fig. 30) very broadly lanceolate, 35–50 μ m long, 13–20 μ m at base, 15–20 μ m at middle, with microtrichia. Posterior margin of tergite IX (Fig. 31) with 4–6 setae. Laterosternite IX with 3 setae. Phallapodeme 38–50 μ m long. Transverse sternapodeme medially triangular, but without median elongation. Gonocoxite 100–120 μ m long. Superior volsella 30–38 μ m long, trifid; with lateral spur, main lobe bearing 4–5 apical setae, and blunt-tipped lobe terminating in seta; without microtrichia. Median volsella (Fig. 32) 20–30 μ m long, with microtrichia and 4–6 apical setae. Inferior volsella 80–94 μ m long, pointed apically, microtrichiose, with 7–8 simple apically split setae. Gonostylus 130–160 μ m long, with 7–10 simple median setae in distal 1/3. HR 0.63–0.88, HV 1.5–2.4.

Pupa (n = 4).

Total length 5.0–5.6 mm. Exuviae yellow with posterior antepronotum and anal comb on abdominal segment VIII brown.

Cephalothorax (Fig. 33). Frontal apotome smooth. Frontal seta short, $38-40 \ \mu m$ long (n = 2). Basal ring oval with major axis $30-40 \ \mu m$ long, the posterior usually with 2–3 small tubercles. Frontal seta 1.0–1.2 times as long as major axis of basal ring. Thorax with one patch of small granules on each side of median suture.

Abdomen (Fig. 34–35). T I without spinulation; T II–V extensively spinulated; T VI–VII with anterior and posterior bands of spinules; T VIII with anterolateral and median spinulation; T IX with median spinulation in female pupa (Fig. 34), but without any spinulation in male. Anterior spinulation on T II–VIII consisting of somewhat large spinules. S I–III and IX without spinulation; S IV–VI with weak posterolateral spinulation; S VII–VIII with weak anterolateral and strong median spinulation, occasionally these merging into extensive spinulation in S VIII (Fig. 39, 40). Tergite II with row of 60–85 caudal hooklets. Conjunctives III/IV and IV/V with rows of spinules. Pedes spurii B distinct on segment II. Anal comb of segment VIII (Fig. 36–38) composed of main spur 30–50 μm long, and 2 or 3 accessory spines 10–30 μm long.



Figures 33–40. *Nilothauma bilobatum* sp. n. pupa. **33** frontal apotome **34** female abdomen, dorsal view **35** abdominal segment VIII, showing variation of tergal spinulation **36–38** anal comb of abdominal segment VIII, showing variation **39** the ventral view of abdomen IV–VIII **40** the ventral view of abdomen VIII, showing variation of sternal spinulation.

Anal lobe 250–280 μ m long, 1.8–2.2 times as long as broad, with 41–50 lateral setae, dorsal setae located near the distal margin of disc.

Female imago and larva. Unknown.

Remarks. The male of *N. bilobatum* sp. n. is similar to that of *N. mirabile* (Townes, 1945) as the superior volsella has a lateral spur and two setigerous lobes, but separable by the anterior T IX projection bearing simple setae only and the anal point covered with microtrichia. In *N. mirabile*, the anterior projection has apically plumose setae and the anal point is bare. The pupa of *N. bilobatum* sp. n., as well as that of *N. aristatum* sp. n., will key to "*N.* sp. Australia" in Adam and Sæther (1999). The pupa resembles that of *N. aristatum* sp. n., rather than that of *N.* sp. Australia, in having relatively short frontal setae (1.0–1.2 times as long as the major axis of basal ring), but differs in the anal comb of abdominal segment VIII consisting of a main spur and 2–3 accessory spines. In *N. aristatum* sp. n., the anal comb has a main spur and a single accessory spine.

Distribution. Oriental China (Guangxi Zhuang Autonomous Region and Guangdong Province).

Biological note. The material was collected from two relatively eutrophic reservoirs (conductivity 24–65 μ S/cm, dissolved oxygen 6.6–8.3 mg/l). The adults of the following species also occurred from there: *Glyptotendipes tokunagai* Sasa, *Dicroten-dipes pelochloris* (Kieffer), *Tanytarsus oscillans* Johannsen, *Cladotanytarsus paratridorsus* Wang & Guo, and *Polypedilum masudai* (Tokunaga).

Nilothauma acre Adam & Sæther

Nilothauma acre Adam & Sæther, 1999: 69.

Material examined. 2 males (LNKU), Jiangxi Province, Qianshan County, 13.vi.2004, Yan CC, light trap; 4 males (LTZU), Zhejiang Province, Taishun County, Wuyanling Natural Conservation Reserve, 1.viii.2005, Qi X, light trap; 1 male (LTZU), Zhejiang Province, Lin-An City, Tianmu Mountain, 16.vii.2012, Lin XL, sweep net.

Remarks. This species was described from Fujian Province in China for the first time by Adam and Sæther (1999).

Distribution. Oriental China (Fujian, Jiangxi, and Zhejiang Provinces).

Nilothauma hibaratertium Sasa

Nilothauma hibaratertia Sasa, 1993: 73. Tosayusurika simantofea Sasa, Suzuki & Sakai, 1998: 52 Nilothauma hibaratertium Sasa: Adam and Sæther 1999: 71.

Material examined. 1 male (EJNU), Yunnan Province, Mengla County, Menglun Town, Luosuo River at Xishuang Banna Tropical Botanical Garden, 29.viii.2014, Tang

HQ, light trap; 2 males (EJNU), Anhui Province, Huangshan Nature Conservation Reserve, Fuxi stream, 25.v.2012, Tang HQ, light trap; 1 male (EJNU), Guangdong Province, Jiangmen City, Beifengshan Nature Conservation Reserve, 7.vii.2012, Tang HQ, light trap; 2 males (LTZU), Zhejiang Province, Jiangshan City, 12.viii.2012, Lin XL, sweep net; 1 male (LTZU), Zhejiang Province, Linan City, Tianmu Mountain, 16.vii.2012, Lin XL, sweep net; 1 male (EJNU), Fujian Province, Longqishan Nature Conservation Reserve, 14.xi.2012, Tang HQ, light trap; 2 males (EJNU), Fujian Province, Meihuashan Nature Conservation Reserve, 16.xi.2012, Tang HQ, light trap; 1 male (EJNU), Hainan Province, Bawangling Nature Conservation Reserve, 30.iv.2012, Tang HQ, light trap.

Remarks. *N. hibaratertium* has never been described sufficiently, especially in the coloration of the adult. Examination of fresh specimens showed that the foreleg of the adult has distinct dark markings on the base and sub-apex of femora, and the apices of tibia and tarsomere 1. This is the first record of *N. hibaratertium* from the Oriental region; previously, this species has only been recorded from Palaearctic Japan (Yamamoto and Yamamoto 2014).

Distribution. Oriental China (Yunnan, Anhui, Guangdong, Zhejiang, Fujian, and Hainan Provinces); Palaearctic Japan.

Nilothauma japonicum Niitsuma

Nilothauma japonicum Niitsuma, 1985: 230. Kribioxenus jintuprimus Sasa, 1990: 32. Nilothauma jintuprima (Sasa): Sasa and Kikuchi, 1995: 34.

Material examined. 1 male (LTZU), Zhejiang Province, Linhai City, Sanjiang wetland,01.VI.2010, Li YF, sweep; 1 male (EJNU), Hainan Province, Jianfengling Nature Conservation Reserve, 29.iv.2012, Tang HQ, sweep net.

Remarks. So far this species has been recorded from Thailand, Zhejiang and Hainan Province in China, as well as Palaearctic Japan (Adam and Sæther 1999; Yan et. al. 2005; Yamamoto and Yamamoto 2014).

Distribution. Oriental China (Zhejiang, Hainan province); Thailand; Palaearctic Japan.

Nilothauma nojirimaculatum Sasa

Nilothauma nojirimaculatum Sasa, 1991: 82.

Material examined. 1 male (EJNU), Hainan Province, Diaoluoshan Natural Conservation Reserve, 27.iv.2012, Tang HQ, light trap; 1 male (EJNU), Guangdong Province, Conghua City, Yugongdong Reservoir, 19.iii.2014, Tang HQ, light trap; 1 male

(EJNU), Guangdong Province, Conghua City, Dongkeng Reservoir, 18.x.2014, Tang HQ, light trap.

Remarks. This species was described from Palaearctic Japan and later recorded from Hainan in China (Adam and Sæther 1999).

Distribution. Oriental China (Hainan and Guangdong Provinces); Palaearctic Japan.

Key to males of the genus Nilothauma Kieffer in China

1	T IX with one dorsal projection	<i>japonicum</i> Niitsuma
_	T IX with two dorsal projections	
2	Wing with dark markings	
_	Wing without any marking	
3	Anterior T IX projection with microtrichia	N. angustum sp. n.
_	Anterior T IX projection without microtrichia N. 1	nojirimaculatum Sasa
4	Superior volsella with one lateral spur or spinose brand	ch5
_	Superior volsella without spur or spinose branch	8
5	Anterior T IX projection undivided N. quatuorlobu	<i>m</i> Yan, Wang & Tang
_	Anterior T IX projection divided into two lobes	6
6	Anal point without microtrichia	acre Adam & Sæther
_	Anal point with microtrichia	7
7	Superior volsella with two lobes and one lateral spur	N. bilobatum sp. n.
_	Superior volsella with one lateral spur, without lobes	N. aristatum sp. n.
8	Anal point with microtrichia N. pandum Q	i, Lin, Wang & Shao
_	Anal point without microtrichia	V. <i>hibaratertium</i> Sasa

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