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Revisited – the species of Tweeting vineyard snails, genus *Cantareus* Risso, 1826 (Stylommatophora, Helicidae, Helicinae, Otalini)

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Abstract

The generic allocation of *Helix subaperta* is clarified by using genetic data and morphological traits of the genital organs; its position within the hitherto monotypic genus *Cantareus* is corroborated. Further analysis of several specimens of *Cantareus apertus* from Algeria and Italy revealed that this taxon is composed of two species, *C. apertus* from Italy, and *C. koraegaelius* from Algeria. The morphological traits of the genital organs of all three species are discussed, and the definition of the genus *Cantareus* is amended. All three species confined to *Cantareus* are re-described, and the syntype specimen of *H. aperta* is illustrated.

Resumée

La répartition générique de *Helix subaperta* est clarifiée en utilisant des données génétiques et des traits morphologiques des organes génitaux; sa position au sein du genre *Cantareus*, jusque-là monotypique, est renforcée. Une analyse plus approfondie de plusieurs spécimens de *Cantareus apertus* d'Algérie et d'Italie a

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révélé que ce taxon est composé de deux espèces, *C. apertus* d'Italie et *C. koraegaelius* d'Algérie. Les traits morphologiques des organes génitaux des trois espèces sont étudiés et la définition du genre *Cantareus* est modifiée. Les trois espèces confinées à *Cantareus* sont à nouveau décrites et le spécimen de syntype de *H. aperta* est illustré.

Keywords
Algeria, Italy, cryptic species, genetic characterisation

Mots-clés
Algérie, Italie, Cantareus, espèce cryptique, caractérisation génétique

Introduction

The hitherto monotypic genus *Cantareus* is currently placed in the helicoid tribe Otalini G. Pfeffer, 1930 (http://www.molluscabase.org/aphia.php?p=taxdetails&id=994951) (Neiber and Hausdorf 2015; Razkin et al. 2015). This clade embraces 12 genera of helicoid snails, among them *Cornu* Born, 1778 and *Erctella* Monterosato, 1894. Both genera contain species possessing shells that resemble each other to some extent, and they exhibit a similar morphology of their genital organs. The Sicilian genus *Erctella* was previously reviewed and re-described by Colomba et al. (2011), and resulted in the resurrection of three valid, narrowly endemic species of the group on north-western Sicily.

The tribe originates from the Maghrebinian radiation centre (Korábek et al. 2019), and contains a considerable number of species, many of them only randomly known. One of these problematic species is the enigmatic *Helix subaperta* Ancey, 1893, which is endemic for a relatively small mountain ridge in the Kabylie in eastern Algeria, the Djudjura Mountains. It has a shell that shows character states typical for both aforementioned genera: it resembles *Cornu* in its colouration but shows no malleate pattern, and *Erctella* in the shell form and the considerably developed ribs on the surface of the adult shell. Thus, the starting point for this paper was to clarify the correct taxonomic position of this species. However, adding supplementary specimens, and using genetic data available from other studies revealed that there is another and completely overlooked species living in northern Africa, which turns out to be a member of *Cantareus*, the Tweeting vineyard snail.

Materials and methods

Taxon sampling

The specimens for this study were collected by the authors of the study, particularly by the senior author. Missing sequences for *Erctella* and Italian *Cornu* were added for the same specimens used by Colomba et al. (2011; 2015). Freshly sampled animals were preserved in 80 % EtOH. The analysed specimens were removed from their shells, the genital organs were isolated and fixed on a wax-bed. The situs as well as the details of the interior lumina were photographed. Tissue samples were taken from those speci-

Table 1. Taxa used in this study: family, species, locality, voucher, GenBank accession numbers for COI, 16S, H3, and 5.8S-ITS2-28S.

Species	Locality	Coordinates		Voucher	GenBank accession number					
		(N)	(E)		CO1	16S	H3	28S	5.8S-ITS2	Origin
Helix pomatia	Hannover-Anderten, N side of Mittelland Canal/ Lower Saxony	52.3586	9.8681	MN_2551-Hel/ MN_012	KR705053	KR705016	KR705127	KR705116	KR705093	Neiber and Hausdorf (2015)
Massylaea vermiculata	Makouda, Tizi Ouzou, DZ	36.7909	4.0659	NMBE 540544	MF564159	MF564112	MF564174	MF564128	MF564144	Bouaziz-Yahiatene et al. (2017)
	Beach between Agia Napa and Capo Greco, CY	34.9728	34.0427	NMBE 519919	MF564160	MF564113	MF564175	MF564129	MF564145	Bouaziz-Yahiatene et al. (2017)
Massylaea constantina	Draâ-Ben Khedda/ Tizi Ouzou, DZ	36.7318	3.9654	NMBE 534211_1	MF564164	MF564118	MF564181	MF564134	MF564150	Bouaziz-Yahiatene et al. (2017)
	Draâ-Ben Khedda/ Tizi Ouzou, DZ	36.7318	3.9654	NMBE 534211_2	MF564165	MF564119	MF564182	MF564135	MF564151	Bouaziz-Yahiatene et al. (2017)
Cantareus subapertus	Ighil Bourmi, DZ	36.4872	4.0613	NMBE 550458_1	MK883426	MK883301	MK883382	MK883375	MK883376	This work
	Ighil Bourmi, DZ	36.4872	4.0613	NMBE 550458_2	MK883427	MK883302	MK883383	MK883335	MK883377	This work
Cantareus koraegaelius	Tigzirt/ Tizi Ouzou, DZ	36.8901	4.1279	NMBE 534199	MK883424	MK883294	MK883384	MK883336	MK883378	This work
	Draa Ben Kheda/ Tizi Ouzou, DZ	36.7318	3.9654	NMBE 519923	MK883425	MK883295	MK883385	MK883337	MK883379	This work
	Djelfa, Algeria	34.6704	3.2504	MVHN-2013	-	KJ458491	-	-	KJ458589	Razkin et al. (2015)
Cantareus apertus	Marincola, Amantea, Calabria	39.1128	16.0797	NMBE 560941_1	MK883423	MK883300	MK883388	MK883338	MK883380	This work
	Marincola, Amantea, Calabria	39.1128	16.0797	NMBE 560941_2	MK883422	MK883296	MK883389	MK883339	MK883381	This work
	Palermo: Cefalú, Cocuzzola	38.0247	13.9417		KR921883	MK883297	MK883412	MK883345	GQ402427	Colomba et al. (2011, 2015, this work)
	Enna: Assoro, C. da Cernigliere	37.6331	14.4075		KR921884	MK883298	MK883413	MK883348	GQ402428	Colomba et al. (2011, 2015, this work)
	Enna: Assoro, C. da Cernigliere	37.6331	14.4075		KR921885	MK883299	MK883414	MK883368	GQ402429	Colomba et al. (2011, 2015, this work)
	Italy, Strada del Casone (Siena)	43.2363	11.4631	FGC 36599	KU869798	KU870009	-	-	-	Fiorentino et al. (2016)
	Italy, Strada del Casone (Siena)	43.2363	11.4631	FGC 36599	KU869799	KU870008	-	-	-	Fiorentino et al. (2016)
	Italy, Strada del Casone (Siena)	43.2363	11.4631	FGC 36599	KU869800	KU870006	-	-	-	Fiorentino et al. (2016)
Cornu aspersum	Draa Ben Kheda/ Tizi Ouzou, DZ	36.7318	3.9654	NMBE 519921	MK883429	MK883304	MK883387	MK883341	-	This work
	Ait Bouadou, Tizi Ouzou, DZ	36.5036	4.0546	NMBE 534201	MK883428	MK883303	MK883386	MK883340	-	This work
	Palermo: Cefalú, Mazzaforno	38.0267	13.9669		KR921888	MK883305	MK883392	MK883342	GQ402424	Colomba et al. (2011, 2015, this work)
	Palermo: Cefalú, Mazzaforno	38.0267	13.9669		KR921887	MK883307	MK883391	MK883343	GQ402425	Colomba et al. (2011, 2015, this work)
	Palermo: Cefalú, Mazzaforno	38.0267	13.9669		KR921886	MK883306	MK883390	MK883344	GQ402426	Colomba et al. (2011, 2015, this work)

Species	**Locality**	**Coordinates**		**Voucher**	**GenBank accession number**					
		(N)	**(E)**		**CO1**	**16S**	**H3**	**28S**	**5.8S-ITS2**	**Origin**
Erctella insolida	Trapani: San Vito lo Capo, Cala Mancina	38.1786	12.7186		KR921898	MK883332	MK883403	MK883363	GQ402457	Colomba et al. (2011, 2015, this work)
	Trapani: San Vito lo Capo, Cala Mancina	38.1786	12.7186		KR921899	MK883333	MK883404	MK883355	GQ402458	Colomba et al. (2011, 2015, this work)
	Trapani: San Vito lo Capo, Cala Mancina	38.1786	12.7186		KR921900	MK883334	MK883405	MK883356	GQ402459	Colomba et al. (2011, 2015, this work)
	Trapani: Custonaci, Monte Cofano	38.1075	12.6831		KR921896	MK883331	MK883399	MK883346	GQ402447	Colomba et al. (2011, 2015, this work)
	Trapani: Custonaci, Monte Cofano	38.1075	12.6831		KR921897	MK883330	MK883400	MK883347	GQ402448	Colomba et al. (2011, 2015, this work)
	Trapani: Custonaci, Monte Cofano	38.105	12.6725		-	MK883327	MK883408	MK883349	GQ402440	Colomba et al. (2011, 2015, this work)
	Trapani: Custonaci, Monte Cofano	38.105	12.6725		KR921893	MK883326	MK883409	MK883350	GQ402441	Colomba et al. (2011, 2015, this work)
	Trapani: Custonaci, Monte Cofano	38.105	12.6725		KR921894	MK883328	MK883396	MK883351	GQ402442	Colomba et al. (2011, 2015, this work)
	Trapani: Custonaci, Monte Cofano	38.105	12.6725		KR921895	MK883329	-	MK883352	GQ402443	Colomba et al. (2011, 2015, this work)
Erctella cephalaeditana	Palermo: Cefalú, La Rocca	38.0389	14.0264		KR921889	MK883308	MK883393	MK883357	GQ402430	Colomba et al. (2011, 2015, this work)
	Palermo: Cefalú, La Rocca	38.0389	14.0264		KR921890	MK883309	MK883411	MK883359	GQ402431	Colomba et al. (2011, 2015, this work)
	Palermo: Cefalú, La Rocca	38.0389	14.0264		KR921891	MK883310	MK883406	MK883358	GQ402432	Colomba et al. (2011, 2015, this work)
	Palermo: Cefalú, La Rocca	38.0389	14.0264		KR921892	MK883311	MK883394	MK883360	GQ402433	Colomba et al. (2011, 2015, this work)
Erctella mazzullii	Palermo: Monte Pellegrino	38.1633	13.3569		KR921909	MK883323	MK883401	MK883353	GQ402449	Colomba et al. (2011, 2015, this work)
	Palermo: Monte Pellegrino	38.1633	13.3569		KR921910	MK883324	MK883402	MK883374	GQ402450	Colomba et al. (2011, 2015, this work)
	Palermo: Monte Pellegrino	38.1633	13.3569		KR921911	MK883325	MK883418	MK883354	GQ402451	Colomba et al. (2011, 2015, this work)
	Palermo: Cinisi, Monte Pecoraro	38.1578	13.1283		KR921912	MK883319	MK883421	MK883365	GQ402454	Colomba et al. (2011, 2015, this work)
	Palermo: Cinisi, Monte Pecoraro	38.1578	13.1283		KR921913	MK883320	MK883419	MK883366	GQ402455	Colomba et al. (2011, 2015, this work)
	Palermo: Cinisi, Monte Pecoraro	38.1578	13.1283		KR921914	MK883321	MK883420	MK883367	GQ402456	Colomba et al. (2011, 2015, this work)
	Palermo: Sferracavallo	38.1953	13.2719		KR921901	MK883318	MK883415	MK883369	GQ402435	Colomba et al. (2011, 2015, this work)
	Palermo: Sferracavallo	38.1953	13.2719		KR921902	MK883312	MK883395	MK883370	GQ402436	Colomba et al. (2011, 2015, this work)
	Palermo: Sferracavallo	38.1953	13.2719		KR921903	MK883317	MK883407	MK883364	GQ402437	Colomba et al. (2011, 2015, this work)

Species	Locality	Coordinates		Voucher	GenBank accession number					
		(N)	(E)		CO1	16S	H3	28S	5.8S-ITS2	Origin
Erctella mazzullii	Palermo: Sferracavallo	38.1953	13.2719		KR921904	MK883322	MK883416	MK883371	GQ402438	Colomba et al. (2011, 2015, this work)
	Palermo: Sferracavallo	38.1953	13.2719		KR921905	MK883313	MK883417	MK883372	GQ402439	Colomba et al. (2011, 2015, this work)
	Palermo: Carini, Monte Columbrina	38.1583	13.2292		KR921906	MK883314	MK883397	MK883373	GQ402444	Colomba et al. (2011, 2015, this work)

mens and sequenced, the shells were photographed (in case they were not destroyed when extracting the animal). All shell photos were taken using a Leica M205 C microscope with the Leica DFC425 camera and the IMS Client (Imagic Bildverarbeitungs AG, Glattbrugg, Switzerland).

Acronyms

ANSP	Academy of Natural Sciences, Philadelphia, USA
NHMW	Natural History Museum Vienna, Austria
NMBE	Natural History Museum Bern, Switzerland
SMF	Senckenberg Research Institute Frankfurt am Main, Germany

Abbreviations

H	shell height
D	shell diameter
PH	peristome height
PD	peristome diameter

Table 2. The five markers used in this study.

Marker	Primer Name	Primer sequence	Reference
COI	LCO1490	5’-GGTCAACAAATCATAAAGATATTGG-3’	Folmer et al. (1994)
	HCO2198	5’-TAAACTTCAGGGTGACCAAAAAATCA-3’	
16S	16s F	5’-CGGCCGCCTGTTTATCAAAAACAT-3’	Palumbi et al. (1991)
	16s R	5’-GGAGCTCCGGTTTGAACTCAGATC-3’	
28S	LSU-2	5’-GGGTTGTTTGGGAATGCAGC-3’	Wade and Mordan (2000)
	LSU-4	5’-GTTAGACTCCTTGGTCCGTC-3’	
5.8S-ITS2-28S	ITS2ModA	5’-GCTTGCGGAGAATTAATGTGAA-3’	Bouaziz-Yahiatene et al. (2017)
	ITS2ModB	5’-GGTACCTTGTTCGCTATCGGA-3’	
H3	H3-F	5’-ATGGCTCGTACCAAGCAGAC(ACG)GC-3’	Colgan et al. (1998)
	H3-R	5’-ATATCCTT(AG GGCAT(AG) AT(AG)GTG-3’	

DNA Extraction, PCR amplification, and sequencing

Phylogenetic analysis

DNA was extracted from a piece of foot muscle tissue using Qiagen Blood and Tissue Kit (Qiagen cat nr. 69506) and the QIAcube extraction robot (Protocol 430, DNeasy Blood Tissue and Rodent tails Standard). Our phylogenetic hypotheses were reconstructed using five phylogenetic markers (mitochondrial COI (657 base pairs (bp)), 16S (374 bp) and nuclear 28S (528 bp), H3 (304 bp) and ITS2 (909 bp)), resulting in a length of 2772 bp (see Table 1).

The PCR included the following admixture: 2 µL template, 12.5 µL GoTaq (Promega) polymerase, 8.5 µL of nuclease-free water and 1 µL of both forward and reverse primer (10 µmol) respectively. In cases where the PCR signal was judged too weak, the reaction was repeated using 3 µL template DNA, 3 µL of the previous PCR product and 5.5 µL of nuclease-free water. The amount of GoTaq and primers stayed the same. The PCR was conducted using the following protocols: For COI, the admixture was first heated up to 95 °C for 1 minute (min), followed by 30 cycles of 30 seconds (s) at 95 °C, 30s at 52 °C and 30s at 72 °C, finishing with 3 min at 72 °C. For 16S, the protocol started with 2:30 min at 90 °C, followed by ten cycles of 30s at 92 °C, 30s at 44 °C and 40s at 72 °C, followed again by 30s at 92 °C, 40s at 48 °C and 40s at 48 °C. The protocol for 28S started with 1 min at 96 °C, then went into 35 cycles of 30s at 94 °C, 30s at 50 °C and 1 min at 72 °C, finishing with 10 min at 72 °C. The ITS2 protocol started with 1 min at 96 °C, followed by 35 cycles of 30s at 94 °C, 30s at 44 °C and 1 min at 72 °C, ending with 10 min at 72 °C. For H3 the admixture was first heated up to 95 °C for 3 min, followed by 40 cycles of 45s at 94 °C, 45s at 50 °C and 2 min at 72 °C, finishing with 10 min at 72 °C. The protocols for COI and H3 could be used for both markers. The PCR products were sequenced at the LGC Genomics GmbH (Berlin, Germany) and at Eurofins Genomics (Ebersberg, Germany) using their respective standard protocol. In total, 48 helicid specimens were used, chiefly from the genera *Cantareus*, *Cornu*, and *Erctella*. Five specimens, belonging to *Helix pomatia*, *Massylaea vermiculata*, and *Massylaea constantina* were used as outgroup. Sequences received from LGC and Eurofins were imported into the Geneious 5.4.7 software (Kearse et al. 2012). The forward and reverse sequences for each gene and individual were combined and edited. For each marker, sequences were aligned in Geneious using the MAFFT multiple sequence alignment plugin version 1.3.6 (based on MAFFT v7.308; Katoh et al. 2002, Katoh and Standley 2013), letting the program choose the appropriate algorithm. The sequence length of each alignment was standardized to the length mentioned above. The alignments were concatenated using the “Concatenate sequences or alignments” function in Geneious.

Topologies were estimated using two different phylogenetic methods: Maximum Likelihood (ML) and Bayesian inference (BI). The five markers were set as partitions in both of these methods, using a distinct model for the third codon in protein-coding genes (COI, H3). The Maximum Likelihood (ML) topology was estimated using the

RAxML 7.2.8 (Stamatakis 2014) plugin of Geneious with the GTR gamma Nucleotide model and 1'000 bootstrap replicates.

The Bayesian tree, which was used as a basis for the combined tree (Fig. 1), was reconstructed with MrBayes 3.2.6 (Huelsenbeck and Ronquist 2001) using the mixed substitution model (which incorporates model testing into the MCMC), invgamma rate variation, a Markov Chain Monte Carlo (MCMC) chain length of 10,000,000 generations, a subsampling frequency of every 4,000 generations, the first 100,000 generations were discarded as burn-in, four heated chains and a chain temperature parameter of 0.2. Calculations were performed on the UBELIx (http://www.id.unibe.ch/hpc), the HPC cluster at the University of Bern.

Molecular taxonomy

The Bayesian and RaxML reconstructions yielded the same topology for all species involved and are shown in Fig. 1.

All three genera treated here in the analysis split in monophyletic lineages, and the nodes on the generic level have high support values. The species *H. subaperta* turned out to be a member of *Cantareus* rather than of *Cornu*, as could be expected by the colour pattern of its shell. The specimens from northern Africa, which had been identified as *C. apertus* so far, form a well-supported (95/1) lineage separate from all Italian specimens available in the study. For this species, the nominal taxon name *Helix aperta* var. *globulosa* Bourguignat, 1863 from Constantine is available. It should be stressed that the specimen MVHN_2013 (Razkin et al. 2015) originates from Djelfa, a city in the southwest of Tizi Ouzou (shell not seen by the present authors). The Italian specimens of *C. apertus* show some genetic differentiation as they split into two major clades; interestingly, the two specimens from Amantea in Calabria (NMBE 560941) occur each in one of these lineages. The addition of nuclear markers in *Erctella* consolidated their topology on species level with high support values.

Taxonomic implications

Cantareus Risso, 1826

Cantareus Risso, 1826, Histoire naturelle des principales productions de l'Europe Méridionale, IV: 64.

Notes. In Table 3, the most important character states of the shells and the genital organs of the Otalini subgroup according to Razkin et al. (2015: 108, fig. 2) including the genera *Cantareus*, *Cornu*, and *Rossmaessleria* are shown. Within the Otalini, *Cantareus*, *Cornu*, and *Erctella* share the synapomorphy of a globular to slightly conical shell, other genera in the tribe tend to have more flattened shells (character 1). In all other shell traits, there is no apomorphy that discriminates between *Cantareus*, *Cornu*,

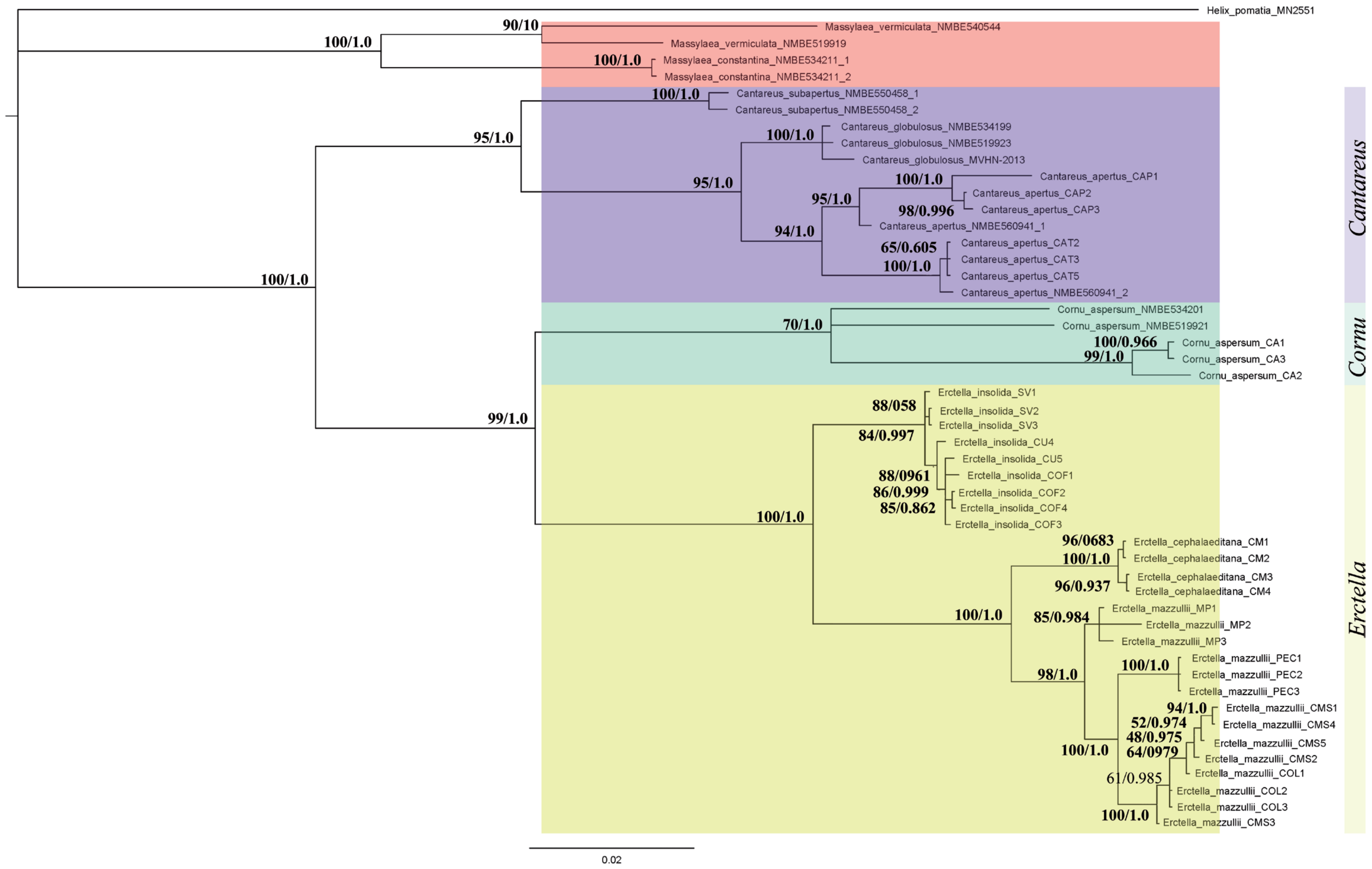


Figure 1. Combined RaxML and Bayesian tree of *Cantareus*, *Cornu*, and *Erctella*, using COI, 16S, H3, 28S, and ITS2.

Table 3. Character matrix including the genera Cantareus, Cornu, Erctella and Rossmaessleria. 1. Last whorl of the shell: 0: occupying more than two thirds of the shell height, 1: occupying less than two thirds of the shell height – 2. Teleoconch colour patterns: 0: none, 1: up to 5 spiral bands, 2: a reticulate pattern – 3. Teleoconch surface: 0: smooth, sometimes with longitudinal riblets and growth lines, 1: granulated, 2: with wrinkles, 3: strongly wrinkled and irregularly reticulated, 4: ribbed – 4. Penis form: 0: short, 1: elongate – 5. Epiphallus length: 0: as long as penis, 1: at least three times the length of penis – 6. Penial flagellum: 0: twice the length of the epiphallus, 1: clearly more than twice the length of the epiphallus – 7. Penial lumen: 0: with numerous crests; 1: smooth – 8. PP1 0: not shifted laterally, 1: shifted laterally, leaving a small pore as a connection between epiphallus and penis near its base – 9. PP2: 0: pp2 reduced to a septum, 1: reduced to a annular pad, 2: pp2 present – 10. Diverticulum: 0: as long as vesicle stem + vesicle, 1: slightly longer than vesicle stem + vesicle, 2: much longer (twice and more) than vesicle stem + vesicle, V: length variable – 11. Atrial stimulator: 0: small, 1: medium, 2: large.

	Cantareus apertus	*Cantareus koraegaelius*	*Cantareus subapertus*	*Cornu aspersum*	*Erctella insolida*	*Erctella mazzullii*	*Erctella cephalaeditana*	*Rossmaessleria scherzeri*
1	0	0	0	0	0	0	0	1
2	0	0	1	2	0	0/1	0	1
3	0	0	1	0	0	2	3	0/4
4	0	1	0	1	0	0	0	NA
5	0	0	1	0	0	0	0	0
6	0	0	0	1	0	0	0	1
7	0	1	1	NA	0	0	0	NA
8	1	1	1	1	1	1	1	0
9	0/2	2	2	1	1	1	1	2
10	1	2	1	0	0	2	1	V
11	1	2	2	2	0	0	0	0/1

and *Erctella* on generic level. On the level of the genital organs, the three genera share the synapomophy of the simple pore connecting epiphallus and penial chamber (character 8), while *Rossmaessleria* shows the plesiomophic state with two functional penial papillae. In *Cornu*, the flagellum is much longer than in the other genera. The phylogenetic value of this character state is not clear within the Otalini, within the Helicini, it is considered a plesiomorphy (Neubert 2014). Other character states like ratios in the bursa copulatrix complex (character 10). A massive atrial stimulator can be found in *Cantareus* and *Cornu*, while in *Erctella*, it is relatively small (character 11). Large and massive stimulators are found in many taxa of Helicidae, so a reduction of this system is here interpreted as an apomorphic character state.

Remarks. The change of the status of *Cantareus* from a monotypic to a polytypic genus causes some nomenclatorial problems. The type species of the genus is *Helix naticoides* Draparnaud, 1801 from France, which so far has been considered a synonym of *Helix aperta* Born, 1778, with the specimen preserved in the Born collection in the NHMW as the name bearing syntype of *aperta* (Fig. 2).The origin of Born's specimen is unknown, and there are almost no shell morphological differences to the Algerian lineage, which proved to represent a separate species (Fig. 1). The correct origin of Born's specimen could probably be clarified genetically by applying NGS methods using shell fragments of the syntype specimen, but this is beyond the scope of this paper.

Anticipating a north African origin of the syntype NHMW-MO 14005 by fixing its type locality in Algeria ends up in a chaotic rearrangement of species names in the group. For Europe, the name *naticoides* would be reactivated with its last use as an accepted species in 1850 (!). The north African species would then be named *apertus* contradicting 170 years of permanent use. By fixing the use of the name *Helix koraegaelia* Bourguignat in Locard, 1882, to the north African lineage, this problem is resolved, and the stability or universality of names used in zoology is guaranteed.

Cantareus apertus is well known for its protective behaviour, which gave the genus its name "*Cantareus*: the singer". Once disturbed (Fig. 23), the species is able to press the air in its lung cavity through the pneumostome producing a series of tweeking sounds (https://youtu.be/CWOhZWLkd4o).

Cantareus apertus **(Born, 1778)**

Figs 2–4, 21–24

Helix aperta Born, 1778, Index rerum naturalium Musei Caesarei Vindobonensis, I. Testacea: 399 [no type locality mentioned].

Helix naticoides: 1801, Draparnaud, Tableau des mollusques terrestres et fluviatiles de la France: 78–79 [France, la Provence, à Antibes, à Cannes].

Type material. Syntype *aperta*: NHMW-MO 14005.

Specimens examined. Italy: Foggia, Ordona, 41.313889N, 15.622222E, 12.10.2018, leg. G. Martucci (ex coll. Sparacio 5031/9), coll. Liberto (Fig. 3); Calabria, Amantea, Marincola, 39.112778N, 16.079722E, 7.10.2018, leg. W. Renda, NMBE 560941/2 (preserved), ex coll. Liberto (Fig. 4).

Description. Shell thick, medium sized if compared to other helicid species, with a depressed spire and a large last whorl occupying more than two thirds of the complete height of the shell; protoconch small, consisting of 1.5 smooth whorls; teleoconch consisting of approximately 4 whorls, separated by a deep, sometimes crenulated suture; basic colour of teleoconch greenish-brownish, often with longitudinal yellow streaks and a few scattered zig-zag markings; surface of teleoconch smooth, but also often covered by low longitudinal riblets; aperture almost perfectly rounded, enormously large, old specimens with an inconspicuous whitish lip; umbilicus always completely closed.

Genital organs: penis short, club-shaped, epiphallus short, of the same length as penis, mrp attaching in the distal third of epiphallus or even closer to penis; flagellum twice the length of the epiphallus; atrial and penial lumen with numerous crests, penial chamber lumen is wrinkled, pp2 a short broad papilla with a central perforation structured by thick annuli to almost completely reduced forming a septum; pp1 a blind papilla, in a central position inside the penial chamber, elongate, sometimes with a broadened tip; epiphallial pore in a lateral position; distal epiphallial lumen with six broad pilasters, the proximal lumen with elongated ridges.

Vagina short, stem of pedunculus thickened and short, diverticulum slightly longer than the vesicle stem + vesicle, longer than the flagellum; glandulae mucosae

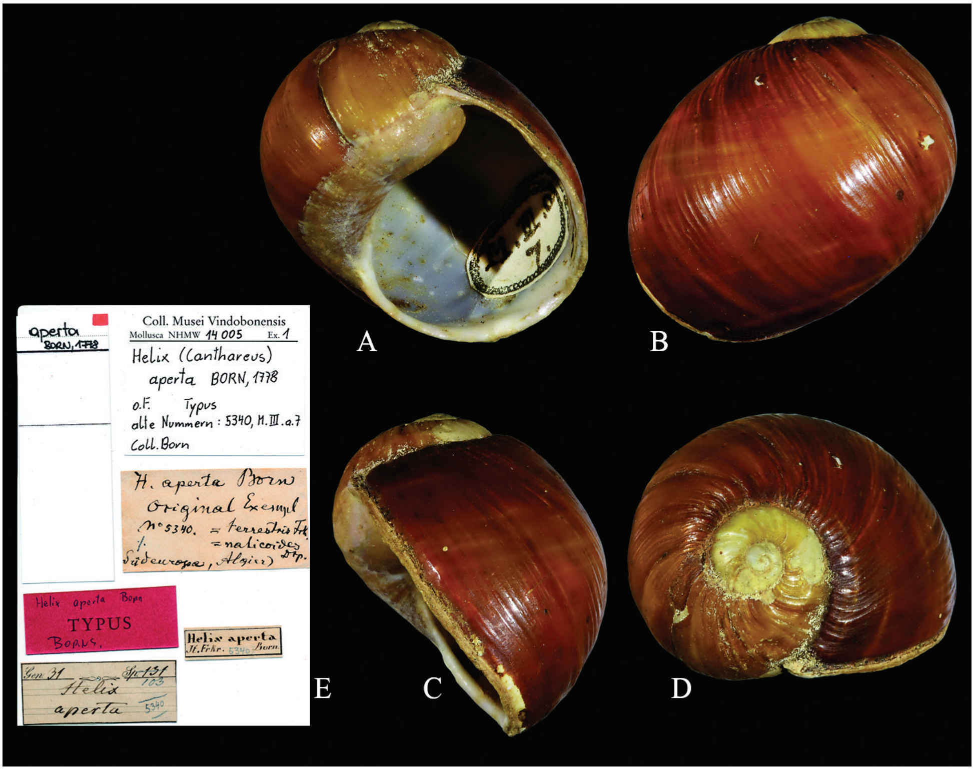


Figure 2. *Cantareus apertus*. Syntype of *Helix aperta* NHMW-MO 14005, shell diameter 28.75 mm. Shell in **A** frontal **B** dorsal **C** lateral and **D** apical views **E** labels of the syntype lot. Photographs NHMW, × 1.5.

longer than the dart sac, with a thickened basal part and two subsequent ramifications, tubules thin and weak, less than 10 tubules per stem; atrium with a medium sized stimulator flap.

Measurements. Syntype NHMW: H = 28.25 mm; D = 28.75 mm; PH = 22.3 mm; PD = 19.2 mm.

Distribution. South-eastern France including Corsica, Italy, Sicily, south-eastern Adriatic coast, Albania, western Greece; scattered found introduced on some Aegean Islands, and in Turkey, Muğla, Gökçebel (Örstan et al. 2005: 7).

Remarks. The anatomy of the genital organs of *C. apertus* has been investigated by several authors, for example Hesse (1919), Germain (1930) and Giusti et al. (1995). Schileyko (2006: 1801, fi.g. 2308) presented also details of the penial lumina. In his picture of the genital organs of an animal collected in the surroundings of Pisa, Italy, he misinterpreted the morphology of the epiphallial papilla (pp1) suggesting that it was a functional papilla as in many other helicid genera (the illustrated shell comes from Arles, France, and thus does not belong to the dissected specimen). Secondly, in his specimen, the penial papilla (pp2) was completely reduced, so only

Figure 3. *Cantareus apertus*. Italy, Foggia, Ordona **A** shell in frontal (left) and **B** lateral (right) view; genital anatomy: situs (**C**) and a section showing the male genital tract and the atrium (**D**). Photographs F. Liberto.

the perpendicular wall forming the basis of pp2 was left. This led to the misapprehension that in the genus *Cantareus*, this papilla is reduced, and only a "septum" is left in the place of the papilla.

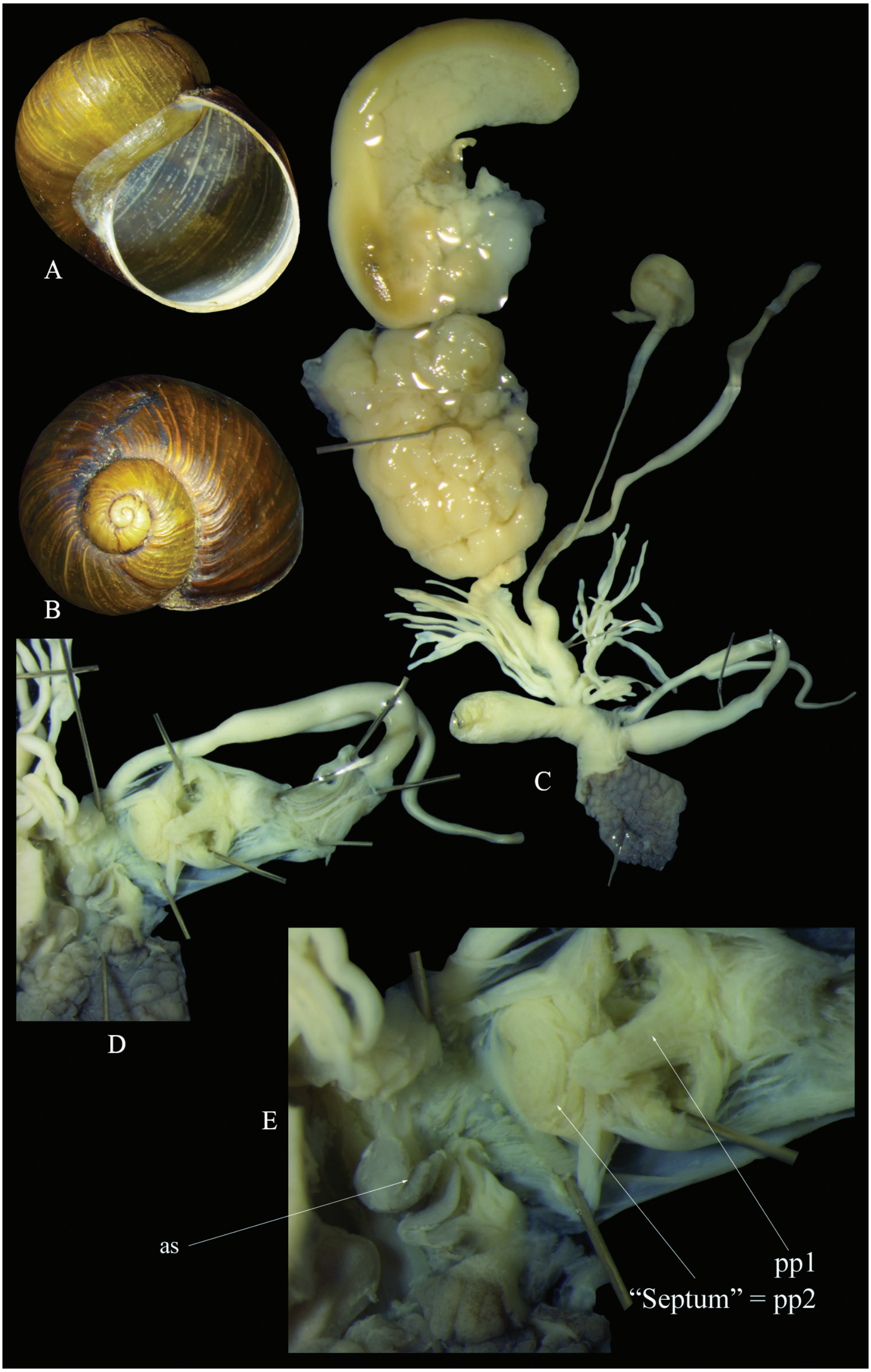


Figure 4. *Cantareus apertus*. Italy, Calabria, Amantea, Marincola, NMBE 560941 **A** shell in frontal and **B** lateral views **C** genital anatomy, situs **D, E** details showing the male genital tract and the atrium. Photographs E. Neubert, shell × 1.5.

***Cantareus subapertus* (Ancey, 1893)**

Figs 5–8, 10–14, 25

Helix subaperta: Ancey 1893, Bulletin de la Société Zoologique de France 18(3): 136–138 [la chaîne du Djurdjura, en Kabylie; published 20 June 1893].

Helix mazzuliopsis: Ancey 1893, Bulletin de la Société Zoologique de France 18(3): 136 [name mentioned in footnote; not an available name according to Article 11.6.1 as it has been published as a synonym and has not been treated as an available name before 1961].

Helix mazzulopsis: 1893, Pilsbry, Manual of Conchology (2)8(32): 238, pl. 46, figs 41, 42 [Jurjura Mts., Algeria; published 1 July 1893; lectotype designation by Baker (1963: 258)].

Type specimens. *Mazzulopsis*: lectotype ANSP 63133, paralectotype ANSP 459220. *subaperta*: 3 syntypes, NHMW 7861, NHMW 7862, NHMW 7863; paratypes SMF 75256/8, coll. Nägele ex Ancey, the original label of Ancey contains the additional information “Dra-el-Mizan, 1893”.

Specimens examined. Algeria, Kabylie: Tiguemounine (Ouacif), 1100 m alt. coll. Bouaziz; Ighil Bourmi (Ait Bouaddou), 950 m alt. NMBE 550458; ditto, le. F. Medjoub, NMBE 555649; Ait Houari (Assi Youcef), 1000 m alt. coll. Bouaziz; Tizi Guefres (Iferhounene), 1100 m alt coll. Bouaziz. The Senckenberg Research Institute houses > 30 shells of this species, all of them from “Kabylie” and/or “Djudjura”.

Description. Shell medium sized to large, thin, globose with a broad to relatively acute conical spire; protoconch whitish, large, with a diameter of up to 6 mm and 2.5 smooth whorls; basic shell colour olive yellowish with up to five separate brown spiral bands; teleoconch covered by a dense granulation, sometimes accompanied by very fine, deep spirals; teleoconch usually covered by irregularly arranged riblets of even ribs, usually stronger around the umbilical area; periostracum thick, often preserved on the shell in small patches; in eroded shells, ribs and riblets whitish; aperture large, elongate oval, slightly thickened forming a lip callus, with a parietal callus in fully adult specimens; aperture whitish inside, with the spiral bands shining through the thin shell; peristome sharp; umbilicus closed, periomphalum covered by a thickened calcareous layer.

Genital organs. Penis short, epiphallus reaching at least three times the length of penis, mrp attaching in the distal third of epiphallus; flagellum twice the length of the epiphallus; penial lumen smooth, pp2 a short broad papilla with a central perforation structured by thick annuli, pp1 a blind papilla, the epiphallial pore in a lateral position; distal epiphallial lumen with broad pilasters, the proximal lumen with elongated ridges.

Vagina short, stem of pedunculus thickened and short, diverticulum longer than the vesicle stem + vesicle, longer than the flagellum; glandulae mucosae longer than the dart sac, with a thickened basal part and two subsequent ramifications, tubules thin and weak, less than 10 tubules per stem; atrium dominated by a massive stimulator.

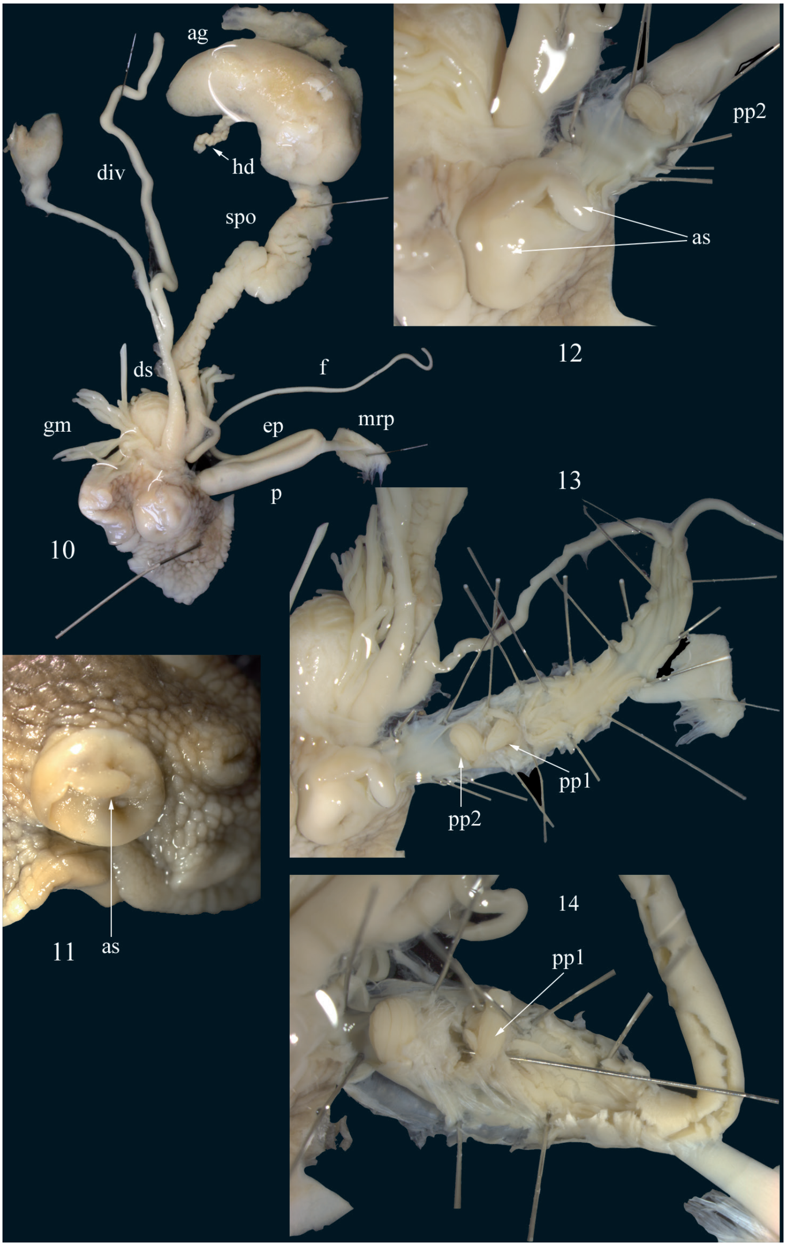
Measurements. Syntypes figured (n = 4): H = 26 mm; D = 27.5 mm; PH = 17.7 mm; PD = 15.4 mm.

Helix subaperta
Ancey
Djurdjura mount.
Kabylia

63133 = subaperta Anc
H. mazzulopsis Anc.
Types! Man. Conch. VIII, p. 238.
Mts Djurdjura
Conch. Sect. 1892.

Ward's Natural Science Establishment,
Helix mazzulopsis
Ancey
Djurdjura
16--26 College Ave., Rochester, N. Y.
63133
.35

Figures 5–9. *Cantareus subapertus* (Ancey, 1893). **5–7** syntypes *Helix subaperta* NHMW, Djurdjura, Kabylie ex Ancey **5** NHMW 7861, D = 23.44 mm **6** NHMW 7862, D = 23.51 mm **7** NHMW 7863, D = 29.44 mm **8** *Helix mazzulopsis* lectotype ANSP 63133, Jurjura Mts. Shell in frontal (**A**) lateral (**B**) and dorsal (**C**) view (**D, E**) labels **9** "*Helix aspersa*", original specimen of Iconographie (2) 3, pl. 69, fig. 359. Shell in frontal (**A**) lateral (**B**) ventral (**C**) and apical (**D**) views. Photographers **5–7** H. Wood, NHMW; photograph **8** E. Wildner, ANSP; photograph **9** E. Bochud, NMBE; all shells × 1.5.



Figures 10–14. *Cantareus subapertus*. Anatomical details of the genital organs; specimen collected at Ighil Bourmi, leg. H. Bouaziz- Yahiatene, NMBE 550458 **10** situs of the genital organs, 46 mm total length **11** partly everted genital atrium with the atrial stimulator **12** distal penial tube with pp2 **13** penis and epiphallus completely opened showing both papillae, and the internal structure of the penial chamber and the epiphallus **14** detail of the penial lumen; note: the needle represents the epiphallial canal, with pp2 bent upwards to show the ending of the canal. Abbreviations: ag = albumen gland; as = atrial stimulator; div = diverticulum; ds = dart sac; ep = epiphallus; f = flagellum; gm = glandulae mucosae; hd = hermaphroditic duct; mrp = musculus retractor penis; p = penis; pp1 = penial papilla 1; pp2 = penial papilla 2; spo = spermoviduct. All figures not to scale.

Distribution. As far as known, this species is restricted to the Djudjura Mts., where it inhabits quite high altitudes. It also occurs in the northern promontory of this mountain ridge.

Remarks. In the description of *Helix subaperta*, Ancey (1893) mentioned in a footnote that he already shared this species under the name *H. mazzuliopsis* with his correspondents. Pilsbry's shells (1893) were purchased from a shell dealer (see label in Fig. 8E) bearing the name *H. mazzulopsis*, which he consequently used! Moreover, Pilsbry remarks: "I have been unable to find any description or mention of this form in the literature"; thus, *H. mazzulopsis* cannot be considered an emendation or an incorrect subsequent spelling of *H. mazzuliopsis*. Strictly speaking, he introduced a new name, and corrected his error two years later in the "Index to Helices" (Pilsbry 1895: 120) with the note "For *H. mazzulopsis* read *H. subaperta*. Ancey's description appeared June 20; that in Man. Conch., July 1".

Cantareus koraegaelius **Bourguignat in Locard, 1882**

Figs 15–20

Helix aperta var. *globulosa*: Bourguignat 1863, Malacologie de l'Algérie, I: 96, pl. VII, figs 3 & 4 [environs de Constantine] [non *Helix* (*Helicogena*) *globulosa* A. Férussac, 1821, Tableau systématique de la famille des Limaçons, livr. 10: 28 (Quarto edition; Folio edition = page 32) (published 26 May 1821). There is no description but refers to plate 25, figs 3 & 4; this plate was published in livraison 5 (4 December 1819) nec *Helix globulosa* von Zieten, 1832, Die Versteinerungen Württembergs Heft 5: 38, pl. 29, fig. 3a-c].

Helix koraegaelia: Bourguignat in Locard 1882, Prodrome de malacologie française. [I]. Catalogue général des Mollusques vivants de France. Mollusques terrestres, des eaux douces et des eaux saumâtres 51: 302 [la Provence au nord, et le Sahara au sud jusqu'à l'Asie-mineure, embrassant la Corse, la Sardaigne, La Sicile, l'Italie, les îles Ioniennes, la Grèce et les îles de l'Archipel].

Type material. *Globulosa*: lost. *koraegaelia*: lectotype [sic!] MHNG-MOLL 117907 from Algeria; type locality: "Djemaa N'Saharidj" (= Djama-N-Saharidj) [Djemaa Saharidj, Mekla, 36.683484° 4.288257°].

Remarks. *Cantareus koraegaelius* is a species that is almost inseparable from its congener *C. apertus*. This also explains why Bourguignat recorded this species from the complete distribution area of the latter species (and including the Algerian lineage). All specimens left in Bourguignat's collection originating from the localities mentioned are syntypes of *Helix koraegaelia*. Thus, the type lots contain two different species. To unambiguously fix the use of this specific name, we herewith select the single specimen MHNG-MOLL 117907 from "Djemaa N'Saharidj" in Algeria as lectotype. This locality in Tizi Ouzou is very close to the places, where the anatomically and genetically well-known specimens (see below) have been recorded. The application of the name *H. koraegaelia* is herewith restricted to specimens exhibiting the character states as explained in this paper forming a new species.

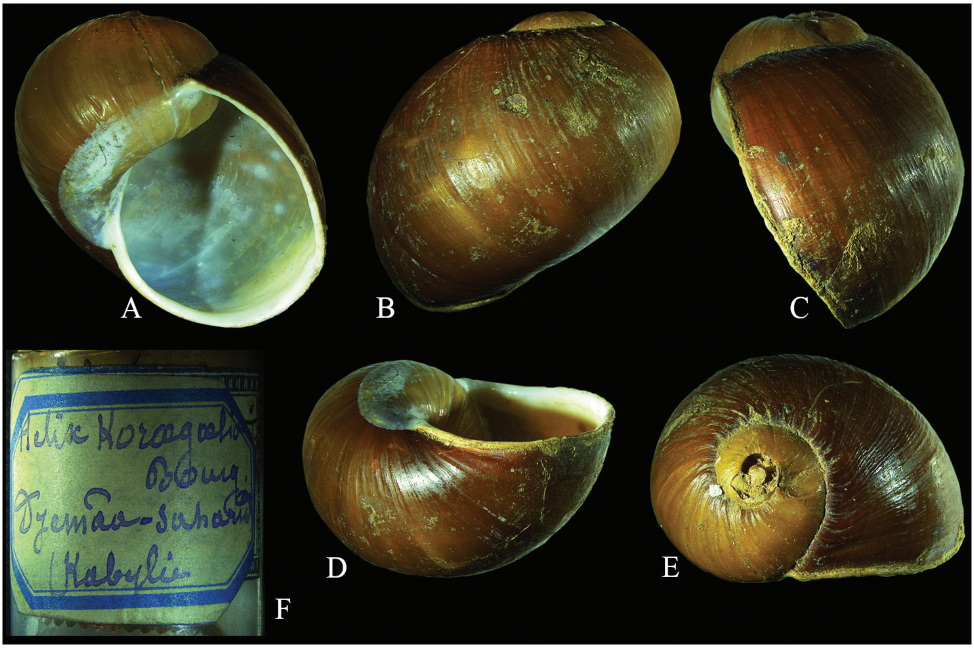


Figure 15. *Cantareus koraegaelius*. Lectotype of *Helix koraegaelia* MHNG-MOLL 117907, shell diameter 27.0 mm. Shell in **A** frontal **B** dorsal **C** lateral **D** ventral and **E** apical views **F** label. Photographs T. Inäbnit, NMBE, × 1.5.

Additional specimen examined. Algeria: Tigzirt/ Tizi Ouzou/ Kabylie, NMBE 534199/1 (specimen preserved and sequenced); Draa Ben Khedaa/ Tizi Ouzou/ Kabylie, NMBE 519923/1 (preserved and sequenced specimen).

Description. Shell thin, medium sized, with a relatively elevated spire and a large last whorl occupying more than half of the complete height of the shell; protoconch medium-sized, consisting of 1.5 smooth whorls; teleoconch with approximately four whorls, separated by a deep, crenulated suture; colour of teleoconch brownish, surface of teleoconch covered by low longitudinal riblets, which are more prominent below the suture, disappearing on the last whorl; aperture rounded, very large, with an inconspicuous whitish lip; umbilicus always completely closed.

Genital organs. penis elongate, club-shaped, epiphallus as long as penis, mrp attaching in the distal third of epiphallus or even closer to penis; flagellum twice the length of the epiphallus; penial lumen smooth; pp2 a broad acute conical papilla with a central perforation structured by thick annuli; pp1 a blind papilla with a slightly broadened apex, the epiphallial pore in a lateral position; atrial and penial lumen with numerous strong crests; distal epiphallial lumen with six broad pilasters, the proximal lumen with elongated ridges.

Vagina short, stem of pedunculus thickened and short, diverticulum extremely longer than the vesicle stem + vesicle, and almost three times longer than the flagellum; glandulae mucosae longer than the dart sac, with a thickened basal part and two

Figures 16–20. *Cantareus koraegaelius*. Shell and anatomical details of the genital organs of dissected and sequenced specimen NMBE 534199; specimen collected at Tigzirt, Tizi Ouzou, Kabylie **16** shell; shell diameter 28.1 mm **17** situs; situs length 57.5 mm **18** lumina of epiphallus, penial chamber, penial papillae and atrium **19** penial papillae **20** atrium with atrial stimulator. Photographs E. Neubert, shell × 1.5.

Figures 21–25. Pictures of living specimens of *Cantareus* species **21** *Cantareus apertus*: on the left a black specimen with brown shell from Roccapalumba, Sicily, Italy, 15.XI.2009 (Coll. F. Liberto 5532); on the right a yellow specimen with green shell from Prizzi, Sicily, Italy, 15.XI.2009 (Coll. F. Liberto 5545) **22** *Cantareus apertus*, Niscemi, Sicily Italy, 22.X.2016 **23** *Cantareus apertus*, Niscemi, Sicily, Italy, 22.X.2016, specimens defending themselves by emitting bubbles of slime and a series of tweeting sounds **24** *Cantareus apertus*, Foggia, Ordona, 41.313889N, 15.622222E, 12.10.2018, leg. G. Martucci (photo/collection I. Sparacio 5031/9) **25** *Cantareus subapertus*, Algeria, Parc National du Djudjura, 1700 m, 11.X.2008 (Photographs Vela Errol).

subsequent ramifications, tubules thin and weak, less than 10 tubules per stem; atrium dominated by a massive stimulator flap.

Measurements (of lectotype): H = 26.3 mm; D = 27.0 mm; PH = 21.8 mm; PD = 17.7 mm.

Distribution. the two genetically identified specimens originate from Eastern Algeria.

Remarks. The description of the genital organs is based on the specimen NMBE 534199 from Tigzirt; unfortunately, the other specimen from Draa Ben Khedaa was subadult with only partially developed genital organs.

It is almost impossible to define differences in shell morphology between this new species and *C. apertus*. In the two genetically identified specimens, the protoconch of *C. koraegaelius* seems to be larger than in *C. apertus*, and the shell colour is more or less uniformly brown without any yellowish or greenish streaks. However, the morphology of the genital organs is in fact different: the large triangular pp2 is strikingly different to all what is known so far from the Italian *C. apertus*, where pp2 is very short to almost completely reduced, so that a "septum" is left.

***Cantareus* species (?)**

Fig. 9

Helix aspersa: Kobelt 1888, Iconographie (2) 3: 9–10, pl. 69, figs 359 & 360 [non *Helix aspersa* O. F. Müller, 1774 [Gorges d'Isser bei Palestro].

Helix (*Cryptomphalus*) *aspersa*: Sacchi 1955, Italian Journal of Zoology 22(2): 638, pl. 3, fig. E [Palaestro].

This specimen was collected by Kobelt in the Gorge d'Isser; it lacks the malleation typical for *Cornu aspersum*, and thus is here considered to rather constitute a species in *Cantareus* than in *Cornu*. However, it also lacks the riblets on the teleoconch, but also has the typical granulation on the whorl exactly like in the specimens from the Djudjura Mts. This form might represent another species close to *C. subapertus*, but preserved specimens from the canyon are needed to decide about its status. This form might be a separate species endemic to the Gorge d'Isser.

Discussion

The main results of this work consist of the allocation of *H. subaperta* in the genus *Cantareus*, and the recovery of a third species in *Cantareus*, i.e., *C. koraegaelius*. The minute granulation of the teleoconch, which is a new shell morphological trait for *Cantareus*, can also be found in other Helicidae like for example *Helix* Linnaeus, 1758 (Neubert 2014), and thus represents a plesiomorphic character state above the species level.

Cantareus koraegaelius can almost be considered a cryptic species, because its shell does not deviate in any major trait from the shells of its sibling species, *C. apertus*

(Born, 1778). The separation between *C. apertus* and *C. koraegaelius* is mainly based on the clear genetic data, and all traits discussed to separate the shells of the two species have currently to be considered as first impressions. Only the shell morphological and anatomical study of a larger number of specimens from the Algerian clade can corroborate the stability of the characters discussed here. It also has to be proven whether or not *C. apertus* is also present in Algeria, which might well be possible. The distance between Tizi Ouzou and Constantine is > 200 km as the bird flies, so it can be estimated that *C. koraegaelius* constitutes a more widespread species than *C. subapertus*, which in fact seems to be a small-range endemic species restricted to submontane to alpine environments of the Djudjura Moutains.

The data presented here suggest the need for a more careful investigation of the phylogenetic relationships among the populations of *C. apertus* from Sicily and southern Italy. Recent studies on species with a wide Mediterranean distribution like *Rumina decollata* (Linnaeus, 1758), *Massylaea vermiculata* (O. F. Müller, 1774) and *Cornu aspersum* (O. F. Müller, 1774), have shown a remarkable genetic divergence (Prévot et al. 2013; Bouaziz-Yahiatene et al. 2017; Sherpa et al. 2018). This will also hold true for *C. apertus*, which is probably introduced by human activities to other Mediterranean areas like southern France and Greece.

Neubert and Bank (2006: 105) argue that the transformation of the papilla system represents a synapomophic character state for an "*Eobania* group" based on the state of knowledge of this time. Walther et al. (2016: 399) remark that this is wrong because *Massylaea* [= *Eobania*] is found on a cluster separate to the *Rossmaessleria*/*Cornu*/*Cantareus* lineage (Razkin et al. 2015: 108, fig. 2). Consequently, this transformation occurred convergently within Otalini. We fully concur with this statement, although we must note that Razkin et al. (2015: 114) also states "...Otalini in the concatenated-gene tree and Helicini in the nuclear rRNA tree were not supported by NJ analysis". The current research on the Otalini will hopefully include the missing genera, and deliver enough data to stabilise the phylogenetic structure of the tribe.

Acknowledgements

We are very grateful to Harriet Wood and Ben Rowson, Cardiff, who supplied photographs of the type specimens of *Helix subaperta* Ancey, 1893, and Ellen Wildner and Gary Rosenberg for the images of *H. mazzulopsis*. Ruud Bank, Groningen, is acknowledged for his review, information on publication dates, and nomenclatorial contributions. Bernhard Hausdorf is thanked for his valuable review of the paper. We thank Walter Renda, Amantea, Italy, and Giuseppe Martucci, Foggia, Italy, who supplied specimens of *Cantareus apertus* from southern Italy, and Errol Vela, Université de Montpellier, Montpellier, France, who supplied photographs of living *Cantareus subapertus*. Parts of this study were made possible by the financial support of the Naturhistorisches Museum der Burgergemeinde Bern (NMBE) and GBIF.ch hosted by the Centre Suisse de Cartographie de la Faune (CSCF), Neuchâtel, Switzerland.

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A new species of *Acerentulus* Berlese, 1908 (Protura, Acerentomata, Acerentomidae) from Bulgaria with a revised key to the *confinis* group

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Abstract

A new species, *Acerentulus bulgaricus* **sp. nov.**, belonging to the *confinis* group, is described from Bulgaria. This species is characterized by long foretarsal sensilla *a* and *b*, the posterior position of foretarsal seta *δ4*, the presence of seta *P1a* on abdominal tergites II–VII and seta *P3a* on abdominal tergite VII, possession of eight anterior setae on abdominal tergite VII and composed *spsm* pores on sternite VI. The new species differs from all members of the *confinis* group in possessing *P1a* setae on tergites II–VII. Otherwise it is similar in body chaetotaxy and porotaxy to three species of the *cunhai* group, *A. proximus*, *A. correzeanus* and *A. tuxeni*. The identification key to 22 *Acerentulus* species belonging to *confinis* group is revised.

Keywords

Chaetotaxy, Holarctic, identification key, porotaxy, proturans.

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Introduction

The proturan genus *Acerentulus* Berlese, 1908 is widely distributed over the Holarctic, reaching Southern America, Australia and New Zealand. According to the Catalogue of the World Protura (Szeptycki 2007), the genus comprises 40 species and 2 subspecies. *Acerentulus rapoporti* Condé, 1963, which was noted by Szeptycki (2007) as "species incertae sedis", was recently synonymized with *Andinentulus ebbei* (Tuxen, 1984) (Shrubovych et al. 2014a). Several species, noted as "species inquirendae" in Szeptycki (2007), are not placeable; type materials were lost for *Acerentulus americanus* Hilton, 1943 and *A. shensiensis* Chou & Yang, 1964, and *A. aubertoti* Condé, 1944 was described from a prelarva (see Tuxen 1955, Szeptycki 2007). Since Szeptycki (2007) additional species have been described, bringing the current total to 48 species (Wu and Yin 2007, Shrubovych et al. 2012, 2014b, Galli and Capurro 2013, Galli et al. 2017). Distributions and taxonomic differentiation between the 21 species within the *confinis* group was discussed previously (Shrubovych et al. 2012). The present paper contains a description of a new *Acerentulus* species from Bulgaria, which belongs to the *confinis* group. Only three species have been recorded from Bulgaria till now: *Acerentulus confinis* (Berlese, 1908), *A. gisini* Condé, 1952 and *A. traegardhi* Ionesco, 1937 (Szeptycki 2007) contrary to well-studied neighboring countries: 11 and 8 *Acerentulus* species were recorded in Serbia and in Romania respectively (Blesić and Mitrovski-Bogdanović 2012, Shrubovych and Fiera 2016). An identification key to the *confinis* group of species worldwide is updated and reorganized according to morphological characters.

Material and methods

Protura specimens, collected in Bulgaria from 2015 to 2018, were extracted from soil samples with Berlese-Tullgren funnels into 95% ethanol. All specimens were mounted on glass slides in Faure's medium (Dunger and Fiedler 1989). Additional material was analyzed in the collection of J. Rusek, deposited in the Institute of Soil Biology, Biology Centre of the Czech Academy of Sciences (**ISB**).

The holotype and other materials of D. Georgiev and C. Fiera are deposited in the collection of the Institute of Systematics and Evolution of Animals, Krakow, Poland (**ISEA**). One female paratype (ISB A-791.1) and materials of J. Rusek are deposited in the collection of the Institute of Soil Biology, Biology Centre, Czech Academy of Sciences. One female paratype (SMNH 90.1) is deposited in the collection of the State Museum of Natural History, Lviv, Ukraine (**SMNH**).

The morphological characteristics of the genus are given in Nosek (1973), Imadaté (1988), Szeptycki (1991), and Galli et al. (2018). Some new details concerning development and variability of chaetotaxy and porotaxy are added. Information on the taxonomy of *Acerentulus* species was taken from original descriptions or redescriptions of type materials in Nosek (1973). For description of morphological characters, the terminology used by Rusek et al. (2012), Szeptycki (1991) and Shrubovych (2014) was followed for this study. Abbreviations used in the description are as follows: Abd. = abdominal segments, Th. = thoracic segments, *al* = anterolateral pore, *sl* = sublateral pore, *psl* = posterosublateral pore, *psm* = posterosubmedial pore, *spm* = sternal posteromedial pore, *spsm* = sternal posterosubmedial pore.

Results

Acerentulus bulgaricus Shrubovych, sp. nov.

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Figs 1–6; Table 1

Material examined. ***Holotype*:** male (ISEA 6649): Bulgaria, Black Sea coast, near Tsarevo, Popska River, moss, soil and detritus, 42°10'31.7"N, 27°50'21.3"E, 16 m elev., 26.VI.2017, coll. D. Georgiev. ***Paratypes*:** 2 females (ISB A-791.1 and SMNH 90.1) same data as holotype. Other material: 10 females, 8 males, 1 preimago, 7 maturi juniores, 2 larvae II, 1 larva I, Bulgaria, St. Kirik and Yulita Monastery near Plovdiv, *Carpineto-Fagetum* forest, sample at decaying stump, 13.VI.1990, coll. J. Rusek; 4 females, 2 males, 1 preimago, 2 larvae II, Bulgaria, south foothills of Stara Planina, near Gurkovo town, mixed forest with *Robinia pseudoacacia* (L.) Gaerth., soil, 42°41'19.10"N, 24°45'09.08"E, 372 m elev., 30.VIII.2015, coll. C. Fiera; 2 females, Bulgaria, Sarnena Gora Mountains, near Kolena village, bank of stream, soil and detritus in roots of *Alnus glutinosa* (L.) Gaerth., 42°29'62"N, 25°41'28.61"E, 300 m elev., 15.VI.2017, coll. D. Georgiev; 1 maturus junior, 1 larva II, Bulgaria, Sarnena Gora Mountains, near Kolena village, *Pinus nigra* J. F. Arnold., soil and detritus, 42°24'03.1"N, 25°34'09.8"E, 296 m elev., 6.VI.2017, coll. D. Georgiev.

Diagnosis. Setae *P1a* present on tergites II–VII, absent on tergite I; setae *P3a* present on tergite VII. Abdominal tergites VI–VII with eight anterior setae. Sternites I–III without pores, sternites IV–V with 1+1 *spsm* pores, sternite VI with composed *spsm* pores (2+2 or 2+3 pores placed adjacent to each other), sternite VII with a *spm* pore. Foretarsal sensilla *a*, *b* and *c* long, setae β*1* and δ*4* thick, stout and sensilliform, δ*4* situated proximally to the level of sensillum *c'* base.

Description. Habitus is shown on Figure 1A. Head setae *l3*, *sd4* and *sd5* short thickened sensilla, additional seta *d6* lacking (Figs 1B, C, 5A, B), length ratio of posterior setae *d7*:*sd7* as 1.0:1.4 (Fig. 5A). Pseudoculus circular, with indistinct posterior extension, PR = 15–17 (Fig. 5B). Sensilla of maxillary palps slender, differing in length, dorsal (*d*) sensillum shorter than ventral (*v*) (Figs 1E, 5C). Labial palps with four-branched tuft of apical setae and a slender sensillum (Figs 1F, 5D). Maxillary gland with rounded calyx, long and slender posterior filament and bilobed posterior dilation (Figs 1D, 5E), CF = 4.4–5.5.

Foretarsus with *t1* claviform, *t3* leaf-like and the same length as *t1* (Figs 1H, 5F, J). All other sensilla slender, except the broadened sensillum *a'* (Figs 1H, 5J). Sensillum *a* long, reaching base of seta γ*3; b* and *c* long, extending past base of seta γ*3*, *b* slightly

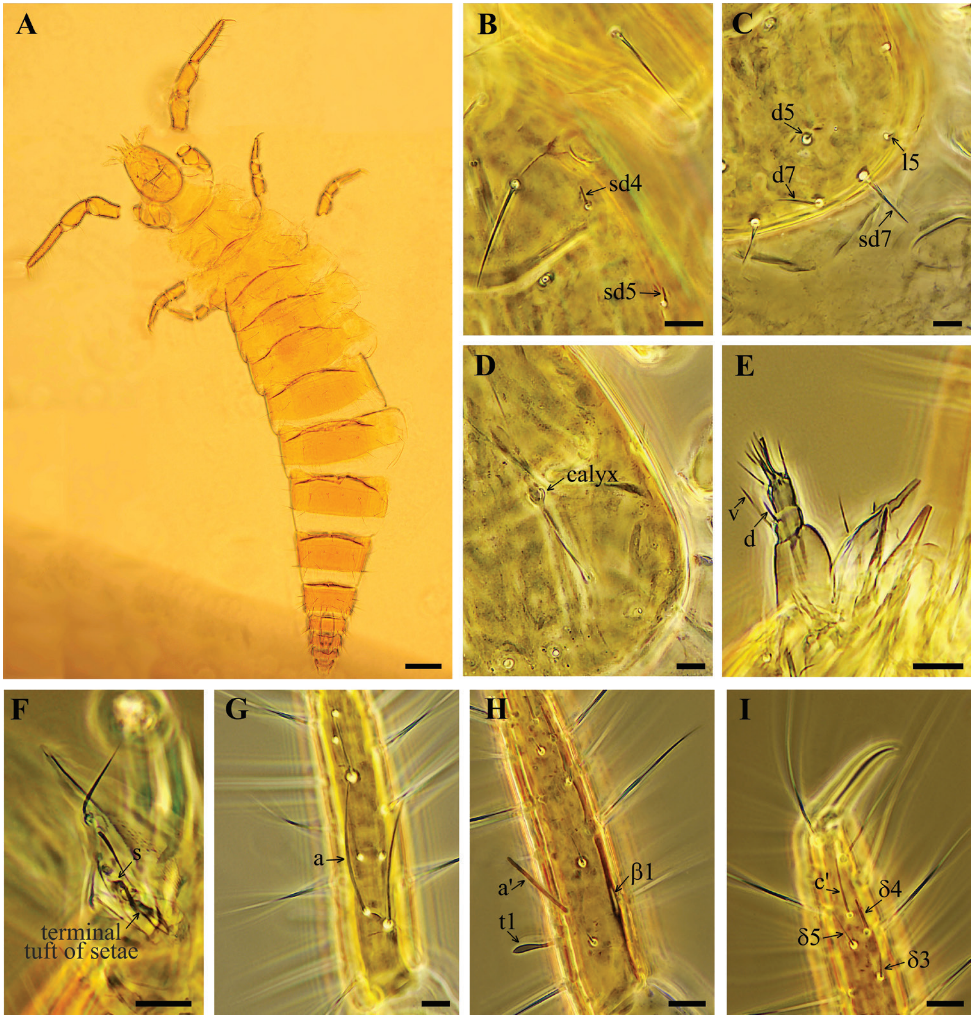

Figure 1. *Acerentulus bulgaricus* sp. nov.: Holotype **A** habitus **B** lateral part of head **C** hind part of head **D** maxillary gland **E** maxillary palpus **F** labial palpus **G–I** exterior view of foretarsus. Scale bars: 100 μm (**A**), 10 μm (**B–I**).

shorter than *c* (Figs 1G, 5F). Base of *d* close to *c*, near *t2* insertion; *a'* situated distal to *t1* insertion (Figs 5F, J). Relative length of sensilla: (*t1* = *t3*) < (*b'*= *c'*) < *a'*< *g* < *t2* < *e* < (*c* = *d*) < *f* < *b* < *a*. Setae β*1* and δ*4* sensilliform and thickened, each 7 μm long (Figs 1H, I, 5J). Seta δ*4* situated on the level of δ*5*, proximal to *c'* base (Figs 1I, 5J). Single pores situated near bases of sensilla *c* and *t3*. Claw long, without inner tooth, empodial appendage short. BS = 0.3, TR = 3.7–4.1, EU = 0.1.

Formula of chaetotaxy given in Table 1. Setae on nota strongly differing in length (Fig. 6A). Length ratio of pronotal setae *1*: *2* as 3.2: 1 (Figs 2A, 6A). Setae *P1a* and *P2a* on mesonotum and metanotum as small gemmate microchaetae, *P4* on metanotum sensilliform, short and thick (Figs 2B, C, D, E, 6A). Seta *P2a* situated close to *P3*. Length ratio

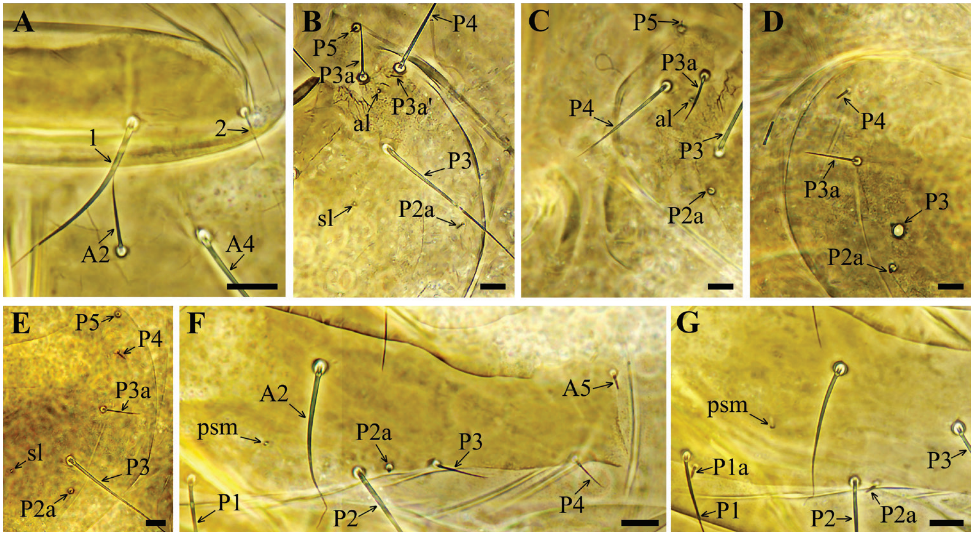

Figure 2. *Acerentulus bulgaricus* sp. nov. **A** part of pronotum **B** part of mesonotum **C** part of mesonotum **D** part of metanotum **E** part of metanotum **F** part of tergite I **G** part of tergite II. Figures **B, D** – paratype SMNH 90.1 **A, C, E, F, G** – holotype. Scale bars: 10 µm.

Table 1. Body chaetotaxy of *Acerentulus bulgaricus* sp. nov. Shrubovych.

	Dorsal		Ventral	
	Setae	Formula	Setae	Formula
Th. I	1, 2	4	A1, 2, M1, 2	4+4
			P1, 2, 3	6
Th. II	A2, 4, M	6	Ac, 2, 3, M	5+2
	P1, 1a, 2, 2a, 3, 3a, 4, 5	16	P1, 3	4
Th. III	A2, 4, M	6	Ac, 2, 3, 4, M	7+2
	P1, 1a, 2, 2a, 3, 3a, 4, 5	16	P1, 3	4
Abd. I	A1, 2, 5	6	Ac, 2	3
	P1, 2, 2a, 3, 4	10	P1, 1a	4
Abd. II–III	A1, 2, 5	6	Ac, 2	3
	P1, 1a, 2, 2a, 3, 4, 4a, 5	16	Pc, 1a, 2	5
Abd. IV–V	A1, 2, 5	6	Ac, 2	3
	P1, 1a, 2, 2a, 3, 4, 4a, 5	16	P1, 1a, 2, 3	8
Abd. VI	A1, 2, 4, 5	8	Ac, 2	3
	P1, 1a, 2, 2a, 3, 4, 4a, 5	16	P1, 1a, 2, 3	8
Abd. VII	A1, 2, 4, 5	8	Ac, 2	3
	P1, 1a, 2, 2a, 3, 3a, 4, 4a, 5	18	P1, 1a, 2, 3	8
Abd. VIII	A1, 4, 5	6	1, 2	4
	P1, 2, 2a, 3, 3a, 4, 4a, 5	16	1a	2
Abd. IX	1, 1a, 2, 2a, 3, 4	12	1, 2	4
Abd. X	1, 1a, 2, 2a, 3, 4	12	1, 2	4
Abd. XI	1, 3, 4	6	–	6
Abd. XII	–	9	–	6

of *P1*: *P2* on mesonotum as 1:1.2–1.4. Mesonotum with *sl* and *al* pores, metanotum with *sl* pores only (Figs 2B, E, 6A). Thoracic sterna without pores (Figs 3A, B, C, 6D, E). Setae *A2* on sterna and *M2* on prosternum short sensilliform and thickened (Figs 3A, 6D).

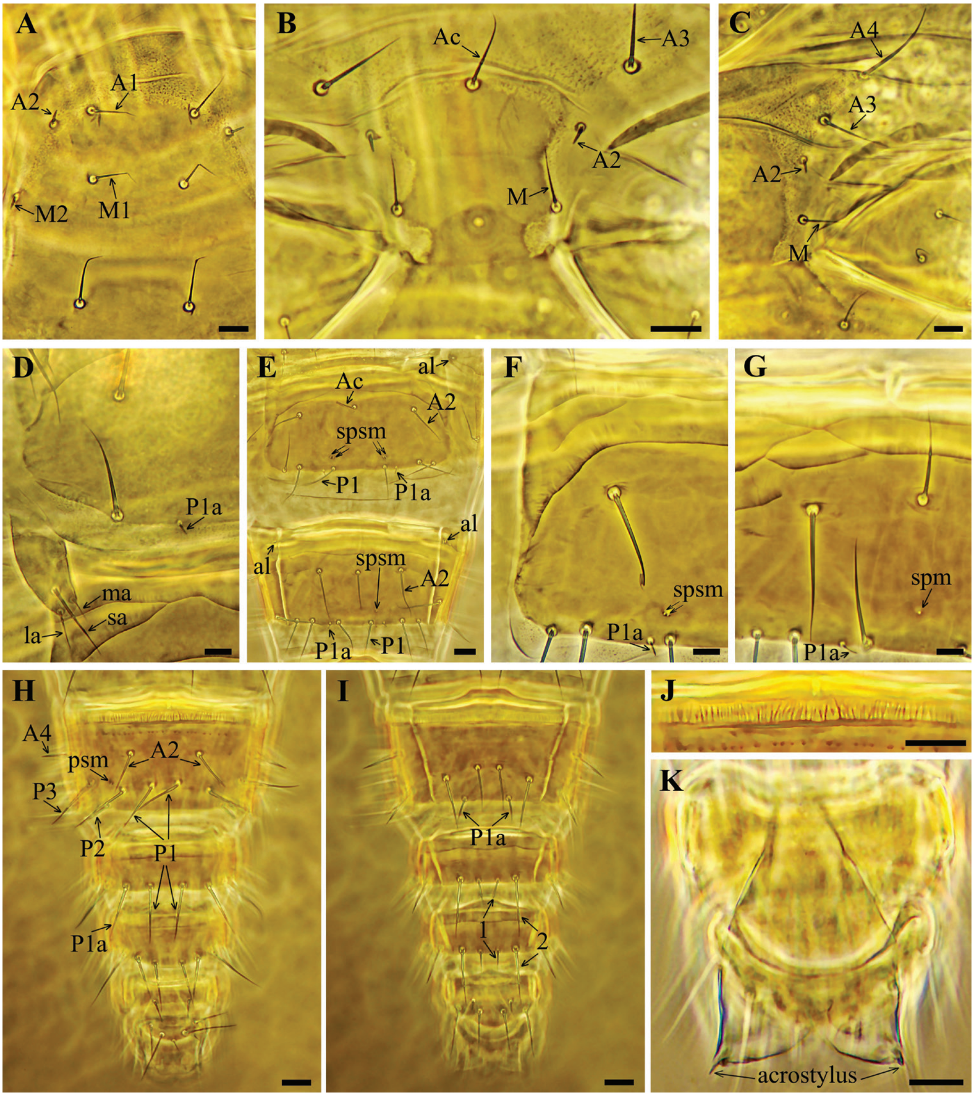

Figure 3. *Acerentulus bulgaricus* sp. nov. **A** prosternum **B** mesosternum **C** metasternum **D** part of sternite I **E** sternites VI–VII **F** part of sternite V **G** part of sternite VII **H** tergites VIII–XII **I** sternites VIII–XII **J** striate band on tergite VIII **K** female squama genitalis. Figures **B, D** – paratype ISB A-791.1 **A, C, E–K** – holotype. Scale bars: 10 μm.

Seta *P2a* on tergite I of same shape as *P1a* and *P2a* on nota, *P3* and *P4* short and setiform; *A5* a short thickened sensillum (Figs 2F, 6B). Accessory setae *P1a*, *P2a* and *P4a* on tergites II–VI short, sensilliform and thick, on tergite VII setae *P1a*, *P2a*, *P3a* and *P4a* thin and setiform (Figs 2G, 6C, F). Position of seta *P3* on tergites II–V anterior to other *P*-setae, on tergites I and VI–VII *P3* in the *P*-setae row (Figs 2F, G, 6B, C, F). Tergites II–VII each with a transverse connecting line in the anterior region (Fig. 6C, F). Pores *psm* on tergites I–VII, *al* on tergites II–VII, *psl* on tergites VI–VII (Figs

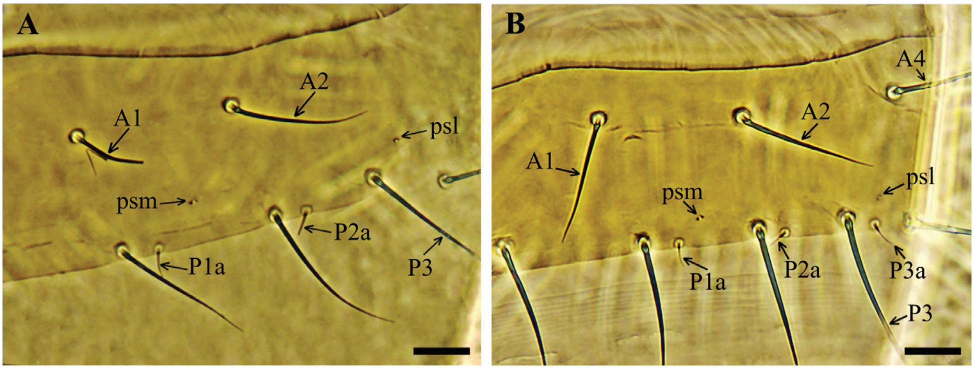

Figure 4. *Acerentulus bulgaricus* sp. nov.: maturus junior (Sarnena Gora. 6.VI.2017. coll. D. Georgiev). **A** part of tergite VI **B** part of tergite VII. Scale bars: 10 µm.

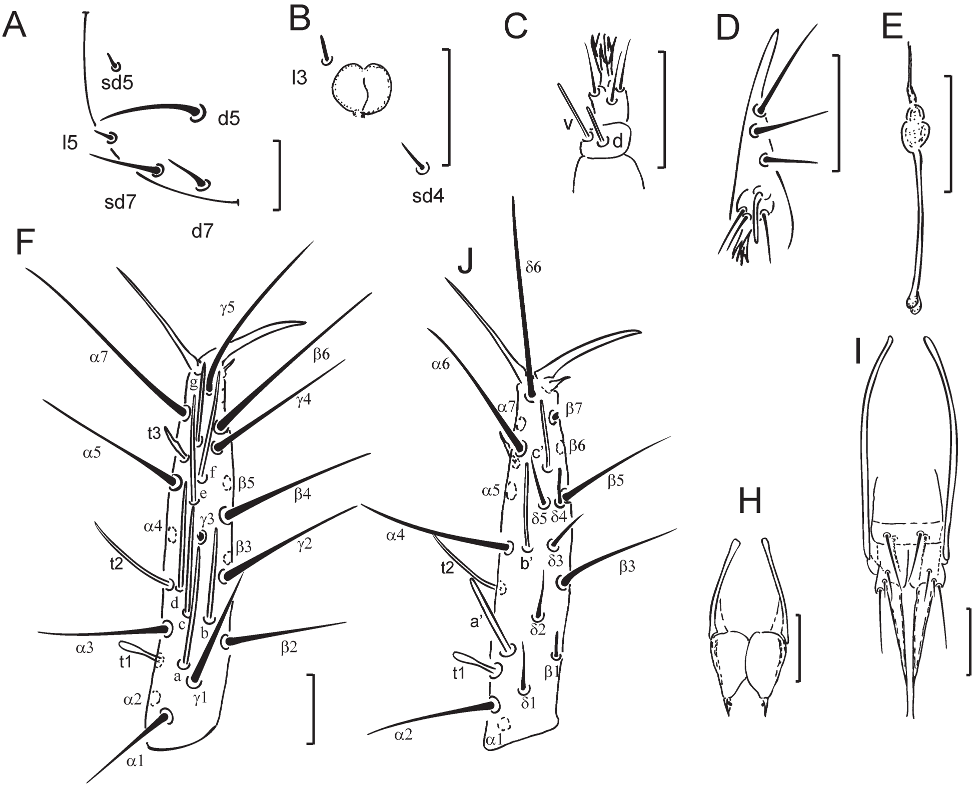

Figure 5. *Acerentulus bulgaricus* sp. nov.: **A** hind part of head **B** pseudoculus **C** maxillary palpus **D** labial palpus **E** maxillary gland **F** exterior view of foretarsus **G** interior view of foretarsus **H** female squama genitalis **I** male squama genitalis. Figure **H** – paratype SMNH 90.1 **A–G, I** – holotype. Scale bars: 20 µm.

2F, G, 6B, C, F). Abdominal legs I with 4 setae, abdominal legs II and III with 3 setae: medial apical (ma), lateral apical (la) and subapical (sa) (Figs 3D, 6I). Accessory setae on sternites I–VI the same length (4 µm) and sensilliform as on tergites (Figs 3F, 6H,

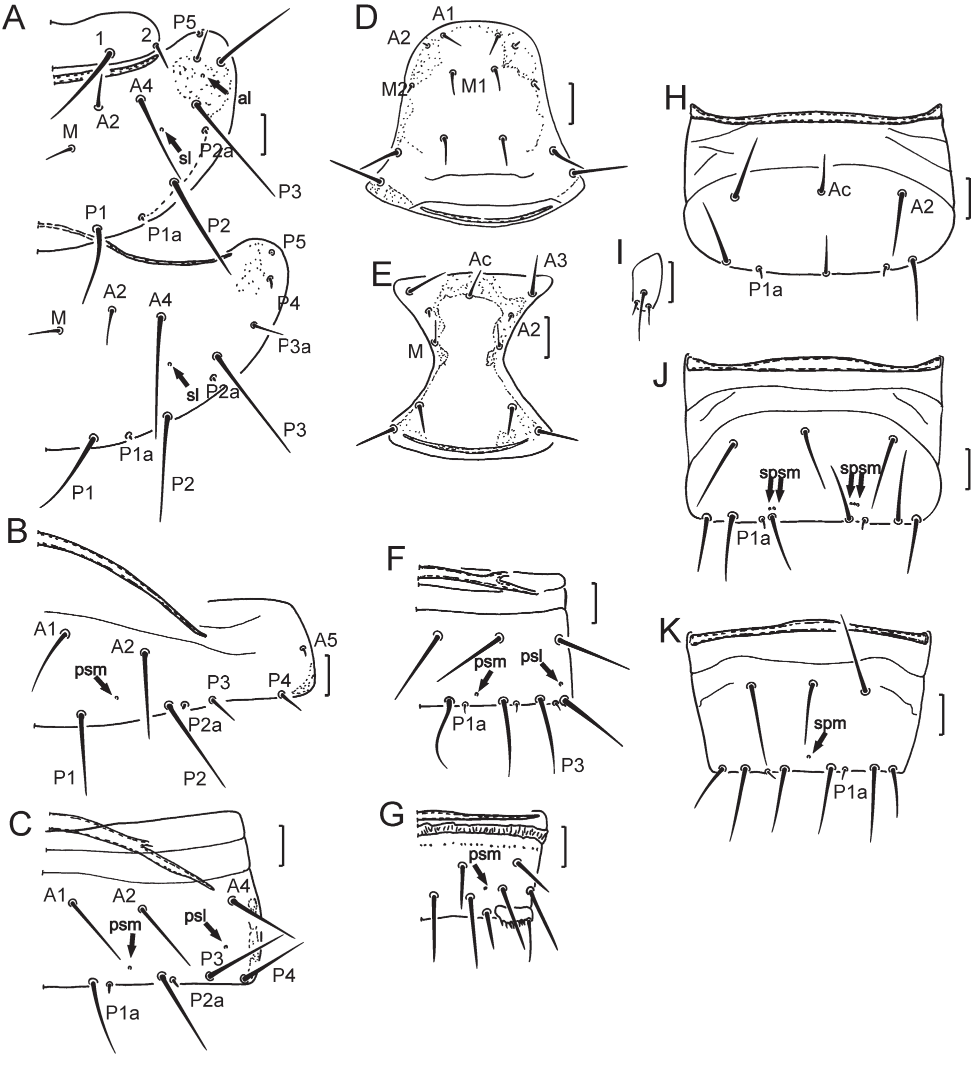

Figure 6. *Acerentulus bulgaricus* sp. nov.: Holotype **A** part of pronotum and mesonotum **B** part of tergite I **C** part of tergite VI **D** prosternum **E** mesosternum **F** part of tergite VII **G** part of tergite VIII **H** sternite III **I** abdominal leg of sternite III **J** sternite VI **K** sternite VII. Arrows show pores. Scale bar: 20 µm.

J). Accessory setae on sternite VII the same shape and length as on tergite VII (Figs 3G, 6K). Sternites II–III each with a connecting line anteriorly and with short lines in the anterolateral region (Fig. 6H); sternites IV–VI with two connecting lines, sternite VII with one connecting line (Figs 6J, K). Sternites I–III without pores. Sternites IV–V with 1+1 *spsm* pores (Fig. 3F), sternite VI with composed *spsm* pores (2+2 or 2+3) (Fig. 6J), sternite VII with single *spm* pore (Figs 3G, 6K).

Abdominal segment VIII with distinct striate band; tergite with a transverse row of small teeth and sternite with two rows of teeth (Figs 3J, 6G). Comb VIII with 10–12 small teeth (Fig. 6G). Pore *psm* without accompanying teeth. Posterior margin of sternite VIII and laterotergites smooth (Fig. 3I). Setae *1* and *1a* on tergites IX and X of equal length (Fig. 3H). Dorsal lobe of Abd. XII with single median pore, ventral lobe with 1+1 *sal* pores. Female squama genitalis with distinct distal prolongation on stylus and long pointed acrostylus (Figs 3K, 5H). Male squama genitalis with 5+5 setae (Fig. 5I).

Body measurements (18 adults) (in µm): maximum body length 1150, head 135–138, pseudoculus 8–9, posterior part of maxillary gland 25–30; posterior cephalic setae *d7* 15–16, *sd7* 20–23, *l5* 7; pronotal setae *1* 35–48, *2* 12–16; mesonotal setae *P1* 35–45, *P2* 45–55; foretarsus 112–115, claw 28–30, empodial appendage 3.

Chaetal variability. Asymmetrical absence of seta *A4* (5 specimens), and seta *A2* (2 specimens) on tergite VI, asymmetrical absence of seta *P1a* on tergite II (1 specimen), asymmetrical presence of additional small seta *P3a'* on mesonotum (1 specimen, Fig. 2B).

Remarks. This species belongs to the *confinis* group of *Acerentulus* species characterized by long foretarsal sensilla *a* and *b*; only *Acerentulus berruezanus* Aldaba, 1983 is characterized in possessing *P1a* setae on tergites I and VII (setae *P1a* absent on tergites II–VI). Other members of the *confinis* group are characterized by the absence of *P1a* setae on tergites I–VI. Three species from the *cunhai* group, *A. proximus* Szeptycki, 1997, *A. correseanus* Szeptycki, 1997 and *A. tuxeni* Rusek, 1966, have a similar chaetotaxy (setae *P1a* present on tergites II to VII, eight *A*-setae on tergite VII) and porotaxy (notal sterna and sternites I–III without pores, sternites IV–V with 1+1 *spsm* pores, sternite VII with a *spm* pore, sternite VI with two groups of *spsm* pores 2+2 or 2+3 in some males, except *A. tuxeni*, which has 1+1 *spsm* pores). However, as members of the *cunhai* group these three species have short sensilla *a* and *b*.

In preimago specimens the *A4* setae on tergite VII are absent symmetrically or asymmetrically. Setae *P1a* on tergites II–VII and *P3a* on tergite VII appear in the maturus junior stage (Fig. 4A, B). All pores on the tergites and sternites are present by the maturus junior stage, except for the posterosublateral pores (*psl*) on tergite VII, which appear in the preimago.

Discussion

In a previous key to the *confinis* group (Shrubovych et al. 2012) the presence of six or eight anterior setae on tergite VI was used as the first character to divide the species. In the current study we found this character to be quite variable, with frequent asymmetrical absence of setae *A4* and *A2* on tergite VI. Szeptycki (1991) confirmed a high degree of variability of these characters in *Acerentulus exiguus* Condé, 1944, *A. xerophilus* Szeptycki, 1979, *A. cunhai* Condé, 1950, *A. traegardhi* Ionescu, 1937 and *A. tuxeni* Rusek, 1966. Therefore, it can be difficult to decide how many setae are present on tergite VI. Seta *A4* on tergite VI appears mostly in the adult stage (Aldaba 1984, Imadaté 1988), and this may be a reason for high variability of this character. Therefore, we have improved the identification key by using presumably more stable characters that appear in earlier stages of acerentomid postembryonic development, such as the presence of accessory setae *P1a* and *P3a* on tergites and seta on sternite XI in the maturus junior stage. According to Szeptycki (1991) sternal porotaxy is a good taxonomic character. Sternal pores are easily visible and practically identical with adult porotaxy from the maturus junior stage. Foretarsal sensillum shapes, proportional lengths, and location of sensilla and setae are also stable characters from larva II to adult (Shrubovych and Rusek 2010).

Key to the *Acerentulus confinis* species group (valid from maturus junior stage)

1 Tergite VII without *P3a* seta **2**
– Tergite VII with *P3a* seta **7**
2 Sternite XI with 3+3 setae **3**
– Sternite XI with 2+2 setae .... ***A. halae* Szeptycki, 1997**
3 Foretarsal seta δ*4* in proximal position to base of *c'* **4**
– Foretarsal seta δ*4* in distal position to base of *c'* .... ***A. charrieri* Shrubovych, Schneider & D'Haese, 2012**
4 Base of foretarsal sensillum *a'* at level of seta *a3* insertion **5**
– Base of *a'* distal to seta *a3* insertion, at level of seta δ*2* insertion .... ***A. occultus* Szeptycki, 1979**
5 Foretarsal sensillum *a* slender **6**
– Foretarsal sensillum *a* broadened basally .... ***A. apuliacus* Rusek & Stumpp, 1988**
6 Foretarsal sensillum *a'* short, reaching to base of sensillum *t2* .... ***A. exiguus* Condé, 1944**
– Foretarsal sensillum *a'* long, reaching to base of sensillum *b'* .... ***A. carpaticus* Nosek, 1967**
7 Sternite XI with 3+3 setae **8**
– Sternite XI with 2+2 setae .... ***A. condei* Nosek, 1983**
8 Tergite VII without *Pla* seta **9**
– Tergite VII with *Pla* setae **12**
9 Foretarsal sensillum *a* slender **10**
– Foretarsal sensillum *a* broad .... ***A. ochsenhausenus* Rusek, 1988**
10 Tergite VIII with 16 posterior setae (*P1* setae present) **11**
– Tergite VIII with 14 posterior setae (*P1* setae absent) .... ***A. alpinus* Gisin, 1945**
11 Foretarsal sensilla *c* and *b* equal in length, foretarsus long (110–125 µm) .... ***A. gisini* Condé, 1952**
– Foretarsal sensillum *c* clearly longer than *b*, foretarsus shorter (about 80 µm) .... ***A. terricola* Rusek, 1965**
12 Foretarsal seta δ*4* in distal position to base of *c'* **13**
– Foretarsal seta δ*4* in proximal position to base of *c'* **15**
13 Tergite VI with *P3a* setae .... ***A. gigas* Szeptycki, 1997**
– Tergite VI without *P3a* seta **14**
14 Tergite I with *Pla* seta, foretarsal sensilla *b* and *c* long, surpassing base of seta γ*3*. .... ***A. berruezanus* Aldaba, 1983**
– Tergite I without *Pla* seta, foretarsal sensilla *b* and *c* short, reaching to base of seta γ*3* .... ***A. confinis maderensis* Tuxen, 1982**

15 Tergites II–VI with *Pla* setae .... ***A. bulgaricus* sp. nov.**

– Tergites II–VI without *Pla* seta.. **16**

16 Foretarsal sensilla *b* and *c* equal in length **17**

– Foretarsal sensilla *b* and *c* differing in length **20**

17 Foretarsal sensillum *a* long, surpassing base of seta *γ3*, foretarsus length about 80 µm .... ***A. setosus* Szeptycki, 1993**

– Foretarsal sensillum *a* short, not reaching base of seta *γ3*, foretarsus longer than 100 µm **18**

18 Foretarsal sensillum *c'* long, surpassing base of claw, sternite VI with simple *spsm* pores, foretarsus length about 115 µm .... ***A. sinensis* Wu &Yin, 2007**

– Foretarsal sensillum *c'* short, not reaching base of claw, sternite VI with composed *spsm* pores **19**

19 Sternite VII with seta *Pc*, foretarsus length about 100 µm .... ***A. xerophilus* Szeptycki, 1979**

– Sternite VII without seta *Pc*, foretarsus length about 120 µm............................. .... ***A. silvanus* Szeptycki, 1991**

20 Foretarsal sensillum *b* shorter than *c*.............................. ***A. palissai* Nosek, 1967**

– Foretarsal sensillum *b* longer than *c*.. **21**

21 Foretarsal sensillum *a* long, reaching to base of seta *γ3;* sensillum *d* long, surpassing base of *e*; length of foretarsus about 100 µm................. ***A. confinis* (Berlese, 1908)**

– Foretarsal sensillum *a* short, not reaching to base of seta *γ3;* sensillum *d* short, not passing base of *e*; foretarsus length more than 110 µm..... ***A. alni* Szeptycki, 1991**

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RESEARCH ARTICLE

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New species of the subgenus *Epiphragma* Osten Sacken from China (Diptera, Limoniidae)

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Abstract

Previously 12 species of the subgenus *Epiphragma* (*Epiphragma*) Osten Sacken, 1860 were known to occur in China. The following three species are described and illustrated as new to science: *E.* (*E.*) *acuminatum* **sp. nov.**, *E.* (*E.*) *henanensis* **sp. nov.**, and *E.* (*E.*) *longitubum* **sp. nov.**. *Epiphragma* (*E.*) *insigne* van der Wulp, 1878 is reported from China for the first time and is illustrated based on additional morphological characters. An updated key to the species of the subgenus *E.* (*Epiphragma*) from China is presented.

Keywords

Limnophilinae, morphology, description, key

Introduction

The subgenus *Epiphragma* (*Epiphragma*) Osten Sacken is the largest subgenus in the genus *Epiphragma*, with 115 known species, and is considered to be monophyletic (Ribeiro 2008). It is distributed worldwide with ten species from the East Palaearctic, 27 species from the Oriental Region, five species from the Nearctic Region, 64 species from the Neotropical Region, and 14 species from Australasian/Oceanian Region (Oosterbroek 2019). It is delimited by the following combination of characters: flagellum with two or more basal segments fused; wing broad, variegated with brown bands,

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spots; a single supernumerary cross-vein in cell C; posterior margin of tergite 9 with subtrigonal lobe on each side of median notch (Dienske 1987).

The following 12 species were previously known from China: Sichuan: *E.* (*E.*) *bicinctiferum* Alexander, 1935, *E.* (*E.*) *subobsoletum* Alexander, 1936, *E.* (*E.*) *sultanum* Alexander, 1938; Zhejiang, Hubei: *E.* (*E.*) *evanescens* Alexander, 1940; Taiwan: *E.* (*E.*) *divisum* Alexander, 1923, *E.* (*E.*) *kempi* Brunetti, 1913, *E.* (*E.*) *nymphicum* Alexander, 1928; Yunnan: *E.* (*E.*) *ancistrum* Mao & Yang, 2009, *E.* (*E.*) *breve* Mao & Yang, 2009, *E.* (*E.*) *elongatum* Mao & Yang, 2009, *E.* (*E.*) *mediale* Mao & Yang, 2009, *E.*(*E.*) *yunnanense* Mao & Yang, 2009. Of these 12 species, ten are endemic to China, *E.*(*E.*) *evanescens* being also known from Japan and *E.* (*E.*) *kempi* from Japan, India, and Sri Lanka. Here three new species are added to the fauna of China, as well as *E.* (*E.*) *insigne*, known previously only from Indonesia and Malaysia. An updated key to the species of the subgenus *E.* (*Epiphragma*) from China is presented.

Materials and methods

The specimens were studied and illustrated with a ZEISS Stemi 2000-c stereo microscope. Genitalic preparations were made by macerating the apical portion of the abdomen in cold 10% NaOH for 12–15 h. After examination in glycerin, genitalia were transferred to fresh glycerin and stored in a microvial pinned below the specimen. The type specimens of the new species are deposited in the Entomological Museum of China Agricultural University (CAU), Beijing, China.

Terminology of morphological features generally follows that of McAlpine (1981) except wing veins which follow Dienske (1987).

Taxonomy

Key to species of subgenus *Epiphragma* from China

1 Femora unpatterned (Fig. 7) **2**

– Femora patterned (Figs 1–6) **3**

2 Origin of Rs with short spur (Figs 12, 15); m-cu at basal 1/4 of cell *dm* .... ***E.* (*E.*) *divisum* (Alexander, 1923)**

– Origin of Rs without spur (Fig. 14); m-cu at basal 3/4 of cell *dm* (Fig. 14) .... ***E.* (*E.*) *insigne* (van der Wulp, 1878)**

3 Femora black or yellow with one subterminal ring **4**

– Femora yellow with two dark brown rings (Figs 1–6) **8**

4 Femora black with narrow yellow subterminal ring; verticils on antenna shorter than segments .... ***E.* (*E.*) *sultanum* (Alexander, 1938)**

– Femora yellow with darker subterminal ring; verticils on antenna longer than segments **5**

5 Femora with broad black subterminal ring; prescutum with narrow black median stripe obsolete for some distance before suture .... ***E.* (*E.*) *nymphicum* (Alexander, 1928)**

– Femora with pale brown subobsolete ring before tip; prescutum not as above ... **6**

6 Dark pattern of wing without narrow dark brown margin.. ***E.* (*E.*) *subobsoletum* (Alexander, 1936)**

– Dark pattern of wing with narrow dark brown margin **7**

7 R$_{2+3+4}$ nearly straight, in virtual longitudinal alignment with Rs; *m-cu* strongly sinuous .... ***E.* (*E.*) *evanescens* (Alexander, 1940)**

– R$_{2+3+4}$ not as above; *m-cu* straight........................***E.* (*E.*) *kempi* (Brunetti, 1913)**

8 Dark pattern of wing without narrow dark brown margin (Fig. 13) **9**

– Dark pattern of wing with narrow dark brown margin (Figs 12, 14, 15) **10**

9 Spur at origin of Rs almost obsolete; apical half of interbase like a slender rod bent at a 90-degree angle from thickened base... ***E.* (*E.*) *mediale* (Mao & Yang, 2009)**

– Spur at origin of Rs distinctly longer (Fig. 13); apical half of interbase finger-shaped (Figs 26, 27) ....***E.* (*E.*) *henanensis* sp. nov.**

10 Interbase very long, at least as long as gonocoxite..**11**

– Interbase relatively short, shorter than gonocoxite...**15**

11 Second ring of femora at tip (Figs 1, 2, 5, 6) **12**

– Second ring of femora near tip (Figs 3, 4) **14**

12 Aedeagus 4/5 as long as gonocoxite (Figs 38, 39) .... ***E.* (*E.*) *longitubum* sp. nov.**

– Aedeagus less than 4/5 as long as gonocoxite (Figs 20, 21) **13**

13 Crossvein *m-cu* at basal 1/4 of cell dm; interbase finger-shaped... ***E.* (*E.*) *elongatum* (Mao & Yang, 2009)**

– Crossvein *m-cu* at basal 1/3 of cell dm (Fig. 12); interbase S-shaped (Figs 20, 21) .... ***E.* (*E.*) *acuminatum* sp. nov.**

14 Base of interbase subtriangular; inner gonostylus with several setae at tip .... ***E.* (*E.*) *bicinctiferum* (Alexander, 1935)**

– Base of interbase triangular, connected with one small ellipsoidal hole; inner gonostylus without setae at tip ....***E.* (*E.*) *yunnanense* (Mao & Yang, 2009)**

15 Pronotum light brown without any black stripe; hyaline spots of wing rather small; median area of tergite 9 with small prominence; inner gonostylus setae, with tip curved into small spine ....***E.* (*E.*) *ancistrum* (Mao & Yang, 2009)**

– Pronotum brown with one black stripe; hyaline spots of wing rather large; median area of tergite 9 plane without small prominence; inner gonostylus with obtuse tip and several setae at base .... ***E.* (*E.*) *breve* (Mao & Yang, 2009)**

***Epiphragma* (*Epiphragma*) *acuminatum* sp. nov.**

http://zoobank.org/8D097E94-414F-4B15-8E28-99EF59F9637D
Figs 1, 2, 8, 12, 16–21

Diagnosis. Generally brownish yellow with pale gray pruinosity. Femora yellow with two dark brown rings, first at basal 2/3, second at tip, broader. Wing brownish hyaline with conspicuous brown pattern, chiefly spotted, with narrow dark brown margin, brownish hyaline areas large. Origin of Rs obtuse and curved with short spur; *m-cu* at basal 1/3 of cell *dm*. Interbase with long and slender rod beyond base, longer than gonocoxite, apex with sharp point; whole slender rod S-shaped. Aedeagus 1/2 as long as gonocoxite, sword-tip shaped.

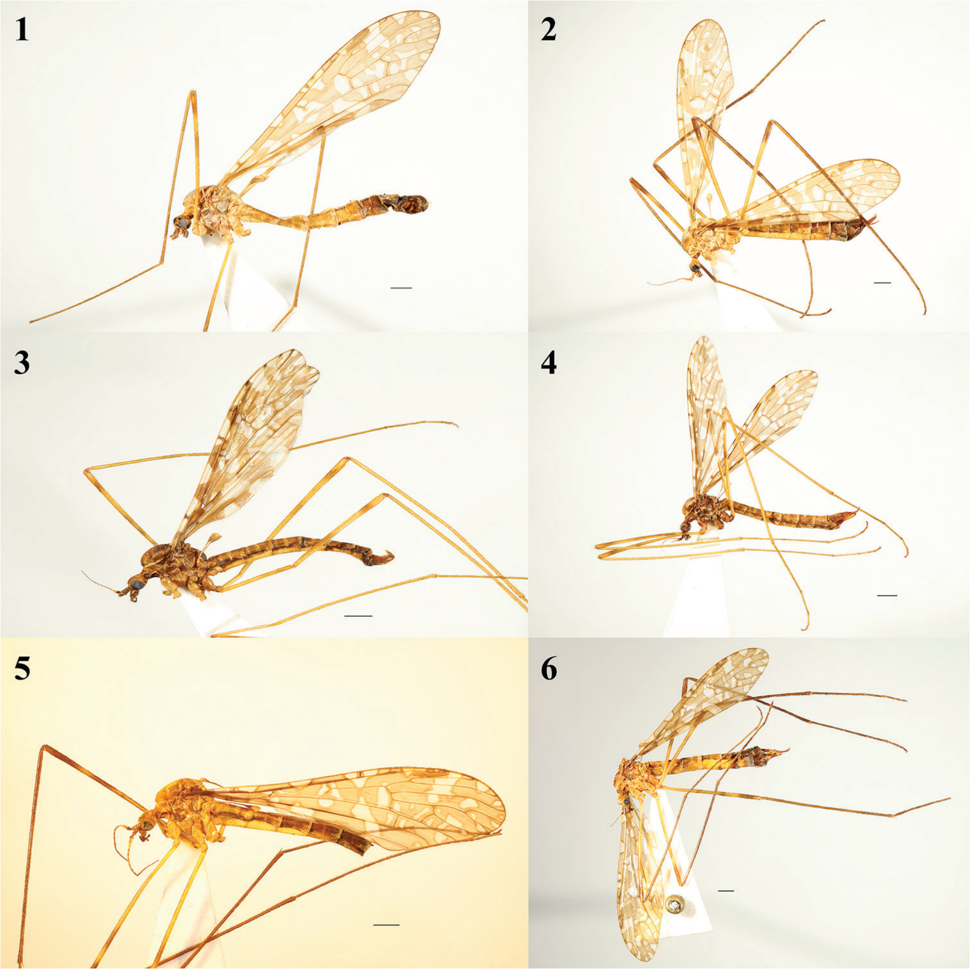
Description. Male. (n = 3): Body length 11.5–13.0 mm, wing length 12.0–14.0 mm, antenna length 2.5–2.7 mm. Head (Figs 1, 8). Brownish yellow with pale gray pruinosity except orbit pale yellow. One median tubercle between eyes. Setae on head black. Scape and pedicel brown, flagellum 13-segmented with two basal segments fused, fusion-segment yellow, succeeding segments brownish, flagellomeres cylindrical, apical segments elongate, with longer verticils. Proboscis brownish black with black setae; palpus black with black setae.

Thorax (Figs 1, 8). Generally brownish yellow with pale gray pruinosity. Pronotum and prescutum brownish yellow. Prescutum with four black stripes, intermediate pair long and almost extended to transverse suture. Scutum brownish black, intermediate brownish yellow, scutellum and mediotergite brownish yellow. Pleura brown, variegated by brownish yellow areas. Setae on thorax brown. Coxae and trochanters yellow; femora yellow with two dark brown rings, first at basal 2/3, second at tip, broader; tibiae brown with one spur; tarsi brown. Setae on legs brown except coxae with brownish yellow setae. Wing (Figs 1, 12). Brownish hyaline with conspicuous brown pattern, chiefly spotted, with narrow dark brown margin, brownish hyaline areas large. Base of wing brown, connected with two large ocellate circles with flattened tips; one circle along cord connected with second circle. Stigma solidly dark brown. Origin of Rs obtuse and curved with short spur; R_{2+3+4} longer than R_{2+3}; *m-cu* at basal 1/3 of cell *dm*. Halter 1.7–1.8 mm long, brownish black except base of stem yellow and apex of knob pale yellow. Setae on wings brownish yellow.

Abdomen (Fig. 1). Tergites 1–5 brownish yellow; tergites 6–9 brownish black. Sternites 1–5 grayish yellow, both sides brownish yellow; sternites 6–9 brownish black. Setae on abdomen brown. Hypopygium (Figs 16–21). Posterior margin of tergite 9 (Figs 16, 19, 20) with subtrigonal lobe on each side of median V-shaped notch. Base of outer gonostylus (Figs 17, 19–21) broad, tip curved into spine. Base of inner gonostylus (Figs 18–21) with setae, tip obtuse and curved up. Interbase (Figs 20, 21) with long and slender rod beyond base, longer than gonocoxite, apex with sharp point; whole slender rod S-shaped. Aedeagus (Figs 20, 21) half as long as gonocoxite, sword-tip shaped.

Female. (n = 19): Body length 12.6–14.0 mm, wing length 12.8–14.2 mm, antenna length 2.5–2.7 mm. Similar to male. Cerci reddish brown; hypovalves brown (Fig. 2).

Type Material. Holotype male (CAU), China: Ningxia, Jingyuan, Liupanshan, Liangdianxia, 2007.VI.28, Gang Yao (light trap). **Paratypes:** 2 males, 8 females (CAU), China: Ningxia, Jingyuan, Liupanshan, Liangdianxia, 2007.VI.28, Gang Yao (light trap); 2 females (CAU), China: Ningxia, Jingyuan, Liupanshan, Liangdianxia, 2007.VI.27, Gang Yao (light trap); 8 females (CAU), China: Ningxia Yinchuan Helanshan



Figures 1–6. 1, 2 *Epiphragma* (*Epiphragma*) *acuminatum* sp. nov. **3, 4** *Epiphragma* (*Epiphragma*) *henanensis* sp. nov. **5, 6** *Epiphragma* (*Epiphragma*) *longitubum* sp. nov. **1, 3, 5** male habitus, lateral view **2, 4, 6** female habitus, lateral view. Scale bars: 1 mm.

Suyukou, 2007. VII. 5, Gang Yao; 1 female (CAU), China: Ningxia, Jingyuan, Liupanshan, Liangdianxia, 2007.VII.15, Gang Yao (light trap).

Distribution. China (Ningxia).

Etymology. The specific name refers to the interbase narrowing to a slender point.

Remarks. This new species is somewhat similar to *E.* (*E.*) *sultanum* Alexander, 1938 from China (Sichuan) in having a similar shape of the hypopygium, but it can be separated from the latter in having the femora yellow with two dark brown rings, the origin of Rs obtuse and curved with a short spur, and *m-cu* at basal 1/3 of cell *dm*. In *E.* (*E.*) *sultanum*, the femora are black brown with a narrow yellow ring; the origin of Rs is obtuse and without a spur; and *m-cu* is at basal 1/2 of cell *dm* (Alexander 1938).

Epiphragma **(*Epiphragma*)** ***henanensis*** **sp. nov**

http://zoobank.org/18B3FC53-9530-4289-9C5E-0E276E1D26F4

Figs 3, 4, 9, 13, 22–27

Diagnosis. Generally brown with gray pruinosity. Vertex with one median brown line. Femora yellow with two brown rings, first at basal 2/3, second at before tip, longer than yellow tip. Wing brownish hyaline with conspicuous brown pattern, chiefly spotted, without narrow dark brown margin. Origin of Rs obtuse and sharp with long spur; *m-cu* at basal 1/2 of cell *dm*. Interbase with long and slender rod beyond base, almost as long as gonocoxite, apex with sharp point, whole slender rod finger-shaped. Aedeagus 1/2 as long as gonocoxite, sword-tip shaped.

Description. Male. (n = 27): Body length 8.5–10.5 mm, wing length 9.6–12.0 mm, antenna length 1.2–1.8 mm. Head (Figs 3, 9). Brownish yellow. Vertex with one brown median line. One median tubercle between eyes. Setae on head black. Scape and pedicel dark brown, flagellum 13-segmented with two basal segments fused, fusion-segment yellow, succeeding segments dark brown, flagellomeres cylindrical, apical segments elongate, with longer verticils. Proboscis brownish yellow with black setae; palpus black with black setae.

Thorax (Figs 3, 9). Generally dark brown with pale gray pruinosity. Pronotum brown with one brownish yellow stripe. Prescutum brownish with four dark brown stripes, intermediate pair long, with lateral, humeral and anterior parts brown. Scutum and scutellum brown. Mediotergite black brown. Pleura brown, variegated by dark brown areas. Setae on thorax black brown. Coxae pale brown; trochanters brownish yellow; femora yellow with two brown rings, first at basal 2/3, second near tip, longer than yellow tip; tibiae brownish yellow with one spur; tarsi brownish yellow with bright yellow tip. Setae on legs black brown. Wing (Figs 3, 13). Brownish hyaline with conspicuous brown pattern, chiefly spotted, without narrow dark brown margin. Base of wing brown, connected with two large ocellate circles with flattened tips; one circle along cord connected with second circle, each tip of vein with brown spot. Stigma solidly dark brown. Origin of Rs obtuse and sharp with long spur; R_{2+3+4} longer than R_{2+3}; *m-cu* at basal 1/2 of cell *dm*. Halter 1.1–1.6 mm long, brownish black except base of stem yellow and apex of knob brownish yellow. Setae on wings brownish yellow.

Abdomen (Fig. 3). Tergites brown with median brownish stripe, both sides with black brown lines. Sternites brownish yellow. Last three segments black brown. Setae on abdomen black. Hypopygium (Figs 22–27). Posterior margin of tergite 9 (Figs 22, 25, 26) with subtrigonal lobe on each side of median V-shaped notch. Tip of outer gonostylus (Figs 23, 25–27) abruptly slender, curved into spine. Inner gonostylus (Figs 24–27) apically obtuse with setae. Interbase (Figs 26, 27) with long and slender rod beyond base, almost as long as gonocoxite, apex with sharp point, whole slender rod finger-shaped. Aedeagus (Figs 26, 27) half as long as gonocoxite, sword-tip shaped.

Female. (n = 57): Body length 8.6–12.0 mm, wing length 9.5–12.3 mm, antenna length 1.2–1.8 mm. Similar to male. Cerci reddish brown; hypovalves reddish yellow (Fig. 4).

Type Material. Holotype male (CAU), China: Henan, Nanyang, Neixiang, Baotianman, 2004.VII.22, Kuiyan Zhang. **Paratypes:** 1 female (CAU), China: Shaanxi, Foping, Xigou, 2006.VII.27, Yajun Zhu; 2 females (CAU), China: Henan, Nanyang, Neixiang, Baotianman, 2004.VII.22, Hui Dong; 1 female (CAU), China: Henan, Nanyang, Neixiang, Baotianman, 2004.VII.23, Hui Dong; 1 female (CAU), China: Henan, Nanyang, Neixiang, Baotianman, 2008.VIII.10, Ding Yang; 1 female (CAU), China: Henan, Nanyang, Neixiang, Baotianman, 2008.VIII.11, Xingyue; 1 female (CAU), China: Hubei, Shennongjia, Liujiawuchang, 2007.VII.30, Qifei Liu (light trap); 1 female (CAU), China: Hubei, Shennongjia, Liujiawuchang, 2007.VII.31, Qifei Liu (light trap); 1 female (CAU), China: Hubei, Shennongjia, Ping Qian, 2007.VII.25, Qifei Liu (light trap); 1 female (CAU), China: Hubei, Shennongjia, Dalongtan, 2009.VI.27, Qifei Liu (light trap); 2 males, 1 female (CAU), China: Hubei, Shennongjia, Guanmenshan, 2009.VII.2, Qifei Liu (light trap); 20 males, 29 females (CAU), China: Hubei, Shennongjia, Qiangjiaping, 2009.VII.4, Qifei Liu (light trap); 1 male, 2 females (CAU), China: Hubei, Shennongjia, Qiangjiaping, 2009.VII.4, Liang Liang (light trap); 2 males (CAU), China: Hubei, Shennongjia, Caiqi, 2009.VII.14, Qifei Liu (light trap); 1 female (CAU), China: Hubei, Shennongjia, Caiqi, 2009.VII.14, Liang Liang (light trap); 1 male, 13 females (CAU), China: Hubei, Shennongjia, Yinyuhe, 2009.VII.18, Qifei Liu (light trap); 1 female (CAU), China: Hubei, Shennongjia, Yinyuhe, 2009.VII.18, Liang Liang (light trap).

Distribution. China (Henan, Shaanxi, Hubei).

Etymology. The species is named after the type locality Henan.

Remarks. This new species is somewhat similar to *E.* (*E.*) *elongatum* Mao & Yang, 2009 from China (Yunnan) in having a similar shape of the hypopygium, but it can be separated from the latter by the wing pattern without narrow dark brown margin, the origin of Rs with a long spur, and *m-cu* at basal 1/2 of cell *dm*. In *E.* (*E.*) *elongatum*, the pattern of the wing has a narrow dark brown margin; the origin of Rs has a short spur; and *m-cu* is at basal 1/4 of cell *dm* (Mao and Yang 2009).

***Epiphragma* (*Epiphragma*) *insigne* (van der Wulp, 1878)**

Figs 7, 10, 14, 28–33

Epiphragma (*Epiphragma*) *insigne* van der Wulp, 1878. Tijdschr. Ent. 21: 196. Type locality: Indonesia (Sumatra).

Diagnosis. Generally brown with gray pruinosity. Vertex with one median brown line. Femora yellow without ring. Wing brownish hyaline, with conspicuous brown pattern, chiefly spotted, without narrow dark brown margin; one long irregular hyaline band cross whole wing from tips of veins R_3, R_4, ending at cell A_1; hyaline areas large. Origin of Rs obtuse and curved without spur; R_{2+3+4} three times longer than R_{2+3}; *m-cu* at basal approximately 3/4 of cell *dm*. Interbase with base expanded, shorter than gonocoxite, but longer than aedeagus, apical rod slender, C-shaped, bent to sternite, tip curved into slender spine. Aedeagus very short and small.

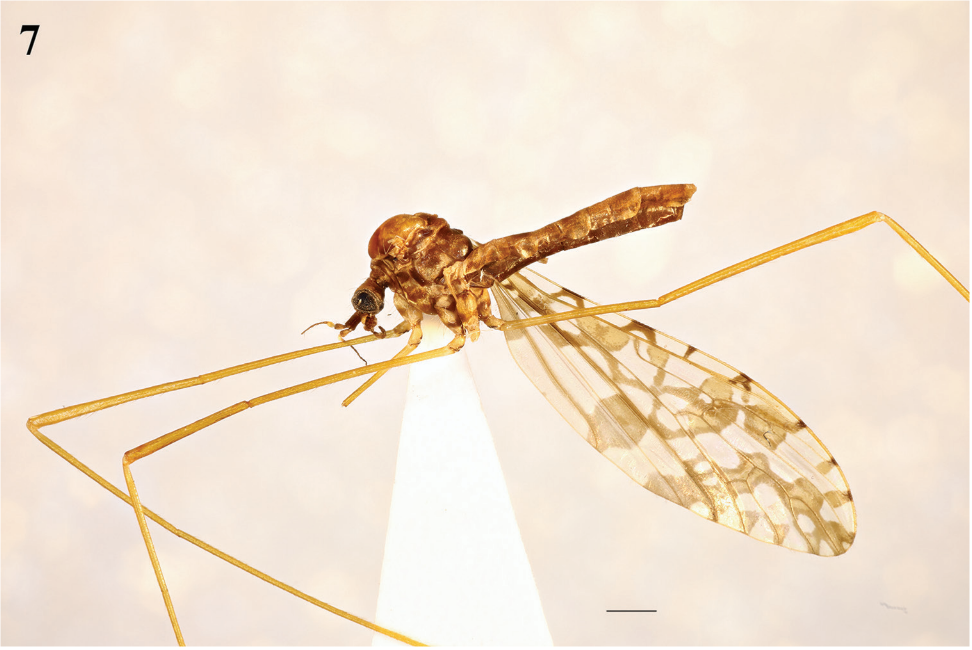
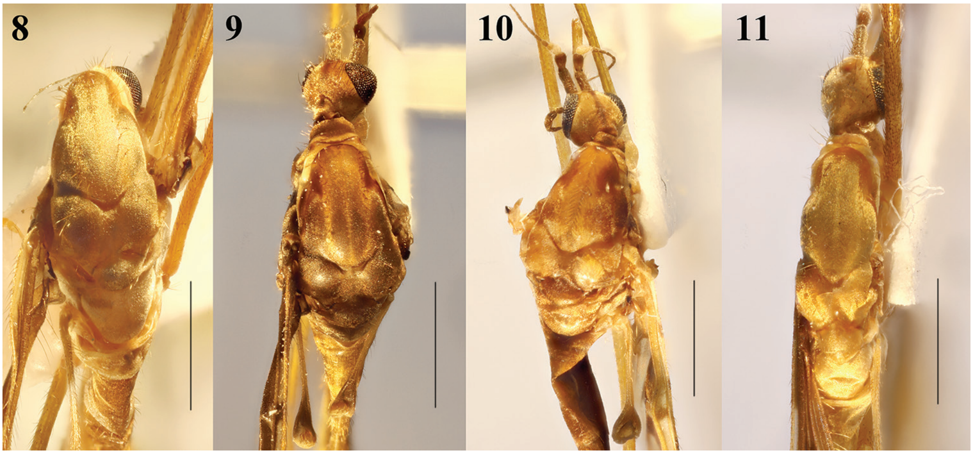
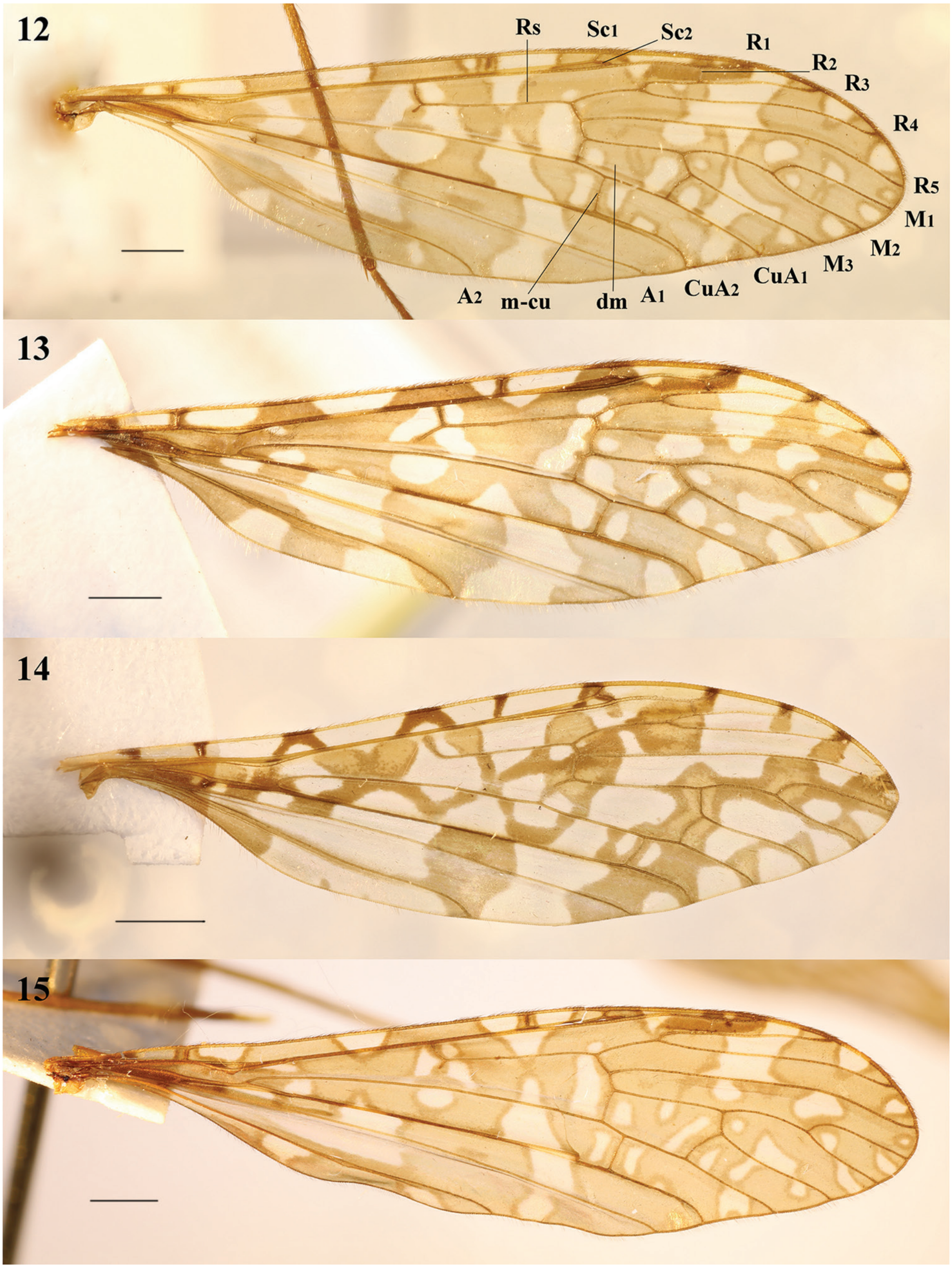


Figure 7. *Epiphragma* (*Epiphragma*) *insigne* (van der Wulp, 1878). Male habitus, lateral view. Scale bar: 1 mm.

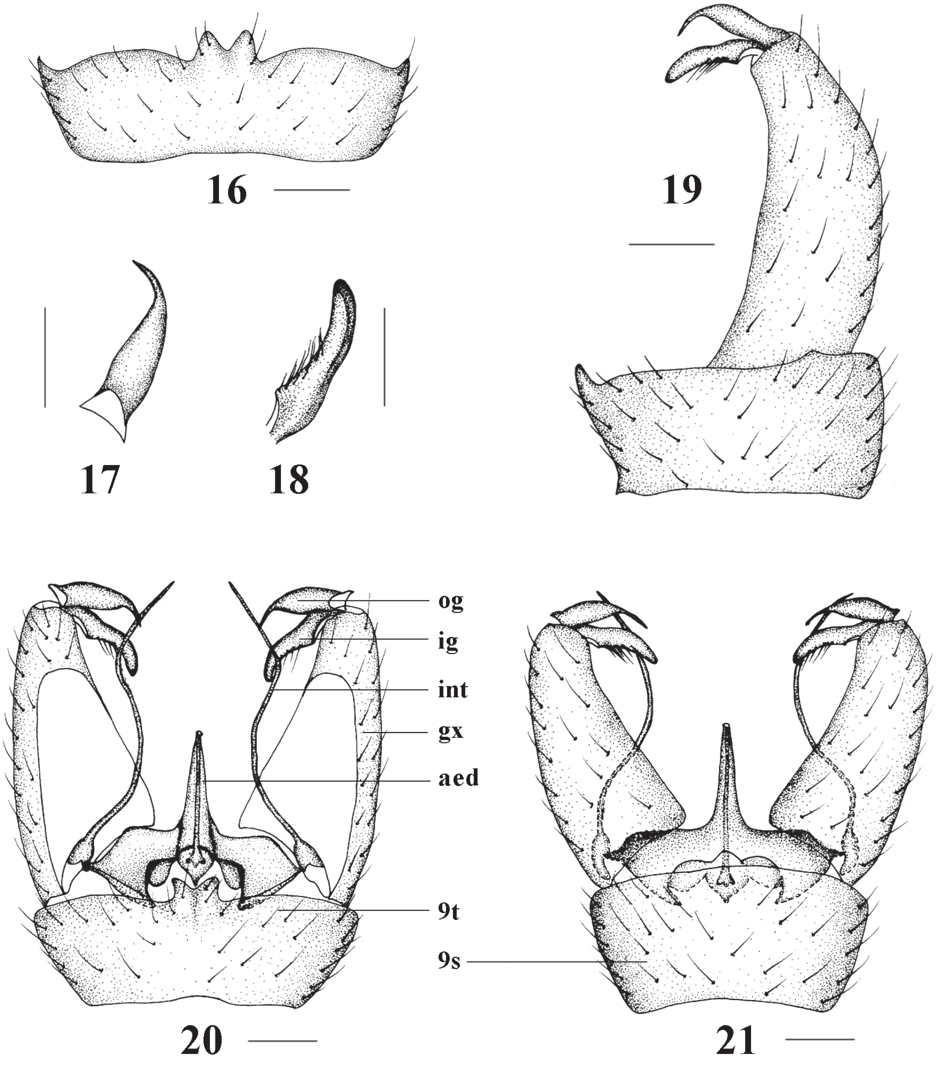


Figures 8–11. Head and thorax (male). dorsal view. **8** *Epiphragma* (*Epiphragma*) *acuminatum* sp. nov. **9** *Epiphragma* (*Epiphragma*) *henanensis* sp. nov. **10** *Epiphragma* (*Epiphragma*) *insigne* (van der Wulp, 1878) **11** *Epiphragma* (*Epiphragma*) *longitubum* sp. nov. Scale bars: 1 mm.

Description. Male. (n = 1): Body length 9.0 mm, wing length 9.8 mm, antenna length 1.7 mm. Head (Figs 7, 10). Brownish yellow. Vertex with one black median line. One median tubercle between eyes. Setae on head black. Scape and pedicel black brown, flagellum 13-segmented with two basal segments fused, fusion-segment yellow,



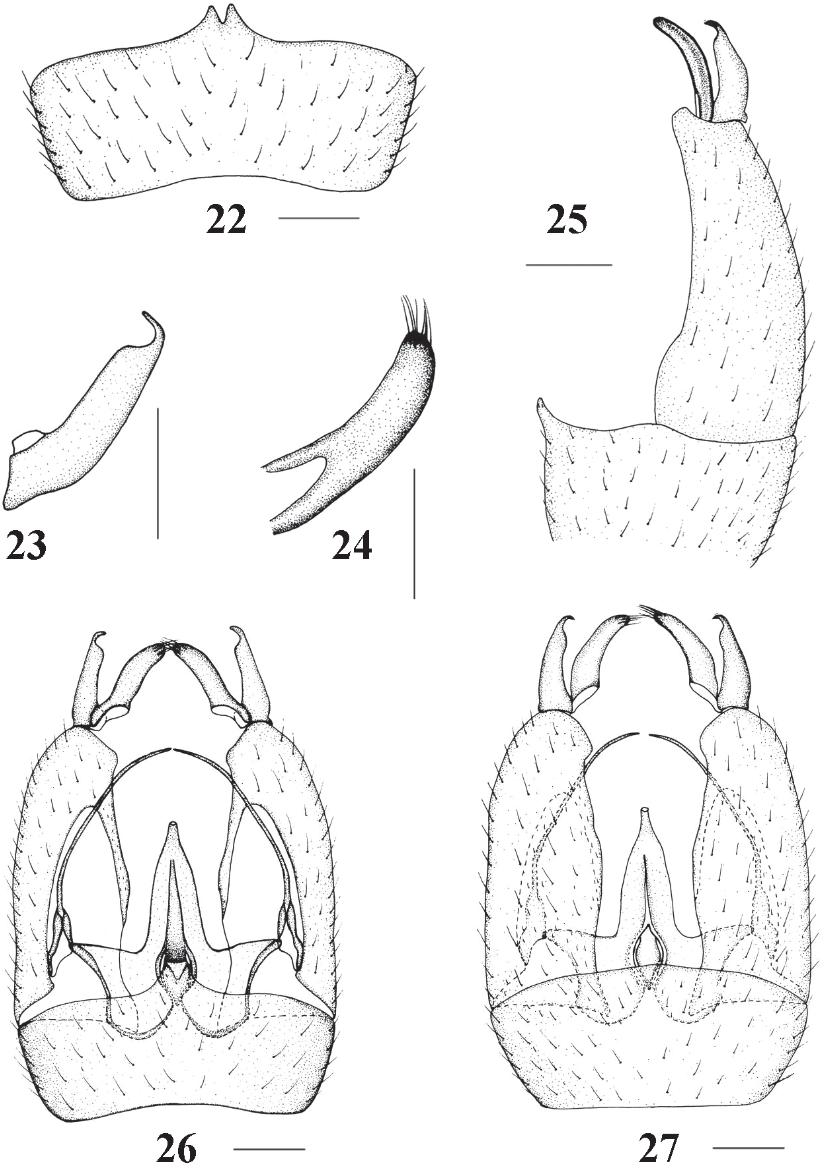
Figures 12–15. Wing (male). **12** *Epiphragma* (*Epiphragma*) *acuminatum* sp. nov. **13** *Epiphragma* (*Epiphragma*) *henanensis* sp. nov. **14** *Epiphragma* (*Epiphragma*) *insigne* (van der Wulp, 1878) **15** *Epiphragma* (*Epiphragma*) *longitubum* sp. nov. Scale bars: 1 mm.



Figures 16–21. *Epiphragma* (*Epiphragma*) *acuminatum* sp. nov. **16** Ninth tergite, dorsal view **17** outer gonostylus, lateral view **18** inner gonostylus, lateral view **19** hypopygium, lateral view **20** hypopygium, dorsal view **21** hypopygium, ventral view. Scale bars: 0.1 mm.

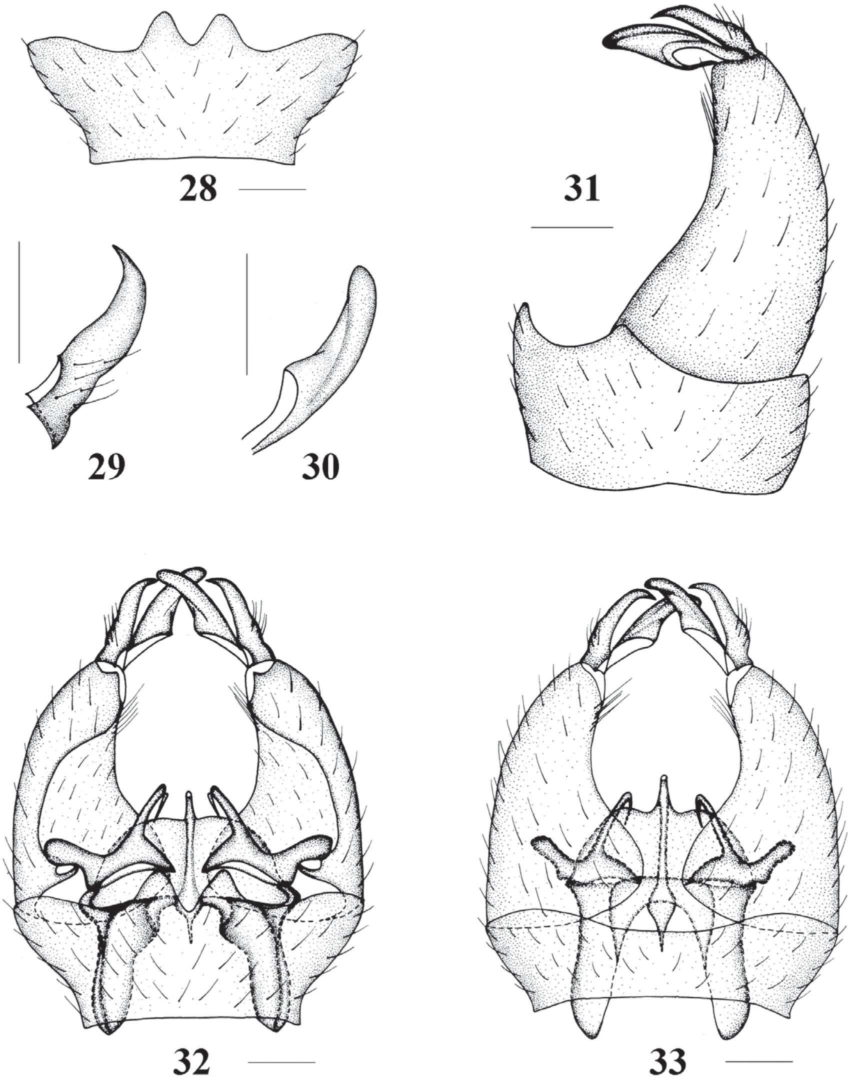
succeeding segments black brown, flagellomeres cylindrical, apical segments elongate, with longer verticils. Proboscis black brown with black setae; palpus blackish brown with black setae.

Thorax (Figs 7, 10). Generally brown with pale gray pruinosity. Pronotum brownish yellow with one dark blackish brown stripe. Prescutum brown with two brownish yellow stripes, one dark brown line at middle. Scutum yellow. Scutellum and mediotergite brownish yellow. Pleura brown, variegated by black brown areas. Setae on thorax brown. Coxae brownish yellow with one dark brown middle ring; trochanters yellow,



Figures 22–27. *Epiphragma* (*Epiphragma*) *henanensis* sp. nov. **22** Ninth tergite, dorsal view **23** outer gonostylus, lateral view **24** inner gonostylus, lateral view **25** hypopygium, lateral view **26** hypopygium, dorsal view **27** hypopygium, ventral view. Scale bars: 0.1 mm.

brown at tip; femora yellow without ring; tibiae yellow with one spur; tarsi yellow. Setae on wings brownish yellow. Wing (Figs 7, 14). Brownish hyaline, with conspicuous brown pattern, chiefly spotted, without narrow dark brown margin; base of wing brown; each tip of vein with brown spot; one long irregular hyaline band across whole wing from tips of veins R_3, R_4, ending at cell A_1; hyaline areas large. Origin of Rs obtuse



Figures 28–33. *Epiphragma* (*Epiphragma*) *insigne* (van der Wulp, 1878). **28** Ninth tergite, dorsal view **29** outer gonostylus, lateral view **30** inner gonostylus, lateral view **31** hypopygium, lateral view **32** hypopygium, dorsal view **33** hypopygium, ventral view. Scale bars: 0.1 mm.

and curved without spur; R_{2+3+4} three times longer than R_{2+3}; *m-cu* at basal approximately 3/4 of cell *dm*. Setae on wings brownish yellow. Halter 1.2 mm long, brownish yellow except base of stem yellow and apex of knob pale yellow. Setae on legs brownish yellow.

Abdomen (Fig. 7). Tergites brownish yellow except tergite 9 yellow. Sternites brown except sternite 9 yellow. Setae on abdomen brown. Hypopygium (Figs 28–33). Posterior margin of tergite 9 (Figs 28, 31, 32) with relatively large subtrigonal lobe on each side of median V-shaped notch. Base of outer gonostylus (Figs 29, 31–33) with setae, tip curved into spine. Inner gonostylus (Figs 30–33) relatively longer with tip obtuse. Interbase (Figs 32, 33) with base expanded, shorter than gonocoxite, but longer than aedeagus, apical rod slender, C-shaped, bent to sternite, tip curved into slender spine. Aedeagus (Figs 32, 33) very short and small.

Female. Unknown.

Material examined. male (CAU), China: Fujian, Meihuashan, 2006.VIII.31, Hui Dong.

Distribution. China (Fujian), Indonesia (Sumatra), Malaysia (Peninsular, Borneo: Sarawak, Sabah).

Remarks. This species is reported from China for the first time.

***Epiphragma* (*Epiphragma*) *longitubum* sp. nov**

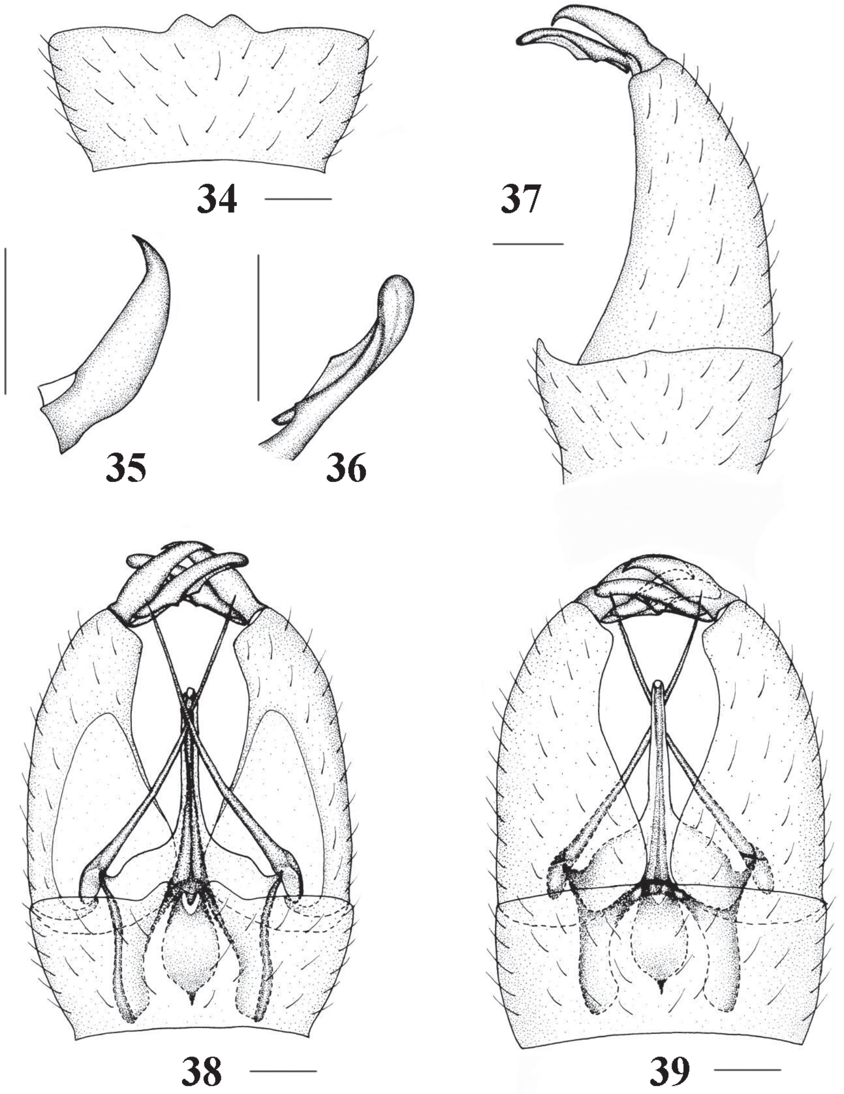
http://zoobank.org/533DE781-D30B-4811-BBD5-B8A50C08463E

Figs 5, 6, 11, 15, 34–39

Diagnosis. Generally brown with pale gray pruinosity. Vertex with one median black line. Femora yellow with two dark brown rings, first at basal 2/3, second at tip, broader. Wing brownish hyaline with conspicuous brown pattern, chiefly spotted, with narrow dark brown margin, brownish hyaline areas large. Origin of Rs obtuse and curved with short spur; *m-cu* at basal 1/3 of cell *dm*. Interbase with long and slender rod beyond base, little longer than gonocoxite, whole slender rod straight, tip spine-like. Aedeagus very stubby, 4/5 as long as gonocoxite.

Description. Male. (n = 1): Body length 12 mm, wing length 13 mm, antenna length 3 mm. Head (Figs 5, 11). Brownish yellow with pale gray pruinosity except orbit pale yellow. One median tubercle between eyes. Setae on head black. Scape and pedicel brown, flagellum 13-segmented with two basal segments fused, fusion-segment yellow, succeeding segments brown, flagellomeres cylindrical, apical segments elongate, with longer verticils. Proboscis brownish yellow with black setae; palpus blackish brown with black setae.

Thorax (Figs 5, 11). Generally brown with pale gray pruinosity. Pronotum brownish yellow with one dark blackish brown stripe. Prescutum brown with two brownish yellow stripes, one yellow line at middle. Scutum yellow. Scutellum and mediotergite brownish yellow. Pleura brown, variegated by light yellow areas. Setae on thorax brown. Coxae and trochanters yellow; femora yellow with two dark brown rings, first at basal 2/3, second at tip, broader; tibiae brown with one spur; tarsi brown. Setae on legs brown except coxae and trochanters with brownish yellow setae. Wing (Figs 5, 15). Brownish hyaline with conspicuous brown pattern, chiefly spotted, with narrow dark brown margin, brownish hyaline areas large. Base of wing brown, connected with two large ocellate circles with flattened tips; one circle along cord connected with second circle. Stigma solidly dark brown. Origin of Rs obtuse and curved with short spur; R_{2+3+4} longer than R_{2+3}; *m-cu* at basal 1/3 of cell *dm*. Halter 2.1 mm long, brown except base of stem and apex of knob pale yellow. Setae on wings brownish yellow.



Figures 34–39. *Epiphragma* (*Epiphragma*) *longitubum* sp. nov. **34** Ninth tergite, dorsal view **35** outer gonostylus, lateral view **36** inner gonostylus, lateral view **37** hypopygium, lateral view **38** hypopygium, dorsal view **39** hypopygium, ventral view. Scale bars: 0.1 mm.

Abdomen (Fig. 5). Tergites 1–3 brownish yellow; tergites 4–9 brownish black, with one brownish yellow middle line. Sternites 1–5 brownish yellow; sternites 6–9 brownish black. Setae on abdomen brownish yellow. Hypopygium (Figs 34–39). Posterior margin of tergite 9 (Figs 34, 37, 38) with flat and subtrigonal lobe on each side of median V-shaped notch. Tip of outer gonostylus (Figs 35, 37–39) curved into spine. Inner gonostylus (Figs 36–39) relatively longer, with tip obtuse and curved up. Interbase (Figs 38, 39) with long and slender rod beyond base, little longer than gonocoxite, whole slender rod straight, tip spine-like. Aedeagus (Figs 38, 39) very stubby, 4/5 as long as gonocoxite.

Female. (n = 5): Body length 11.0–14.0 mm, wing length 11.5–13.5 mm, antenna length 2.7–3.2 mm. Similar to male. Cerci and hypovalves reddish brown (Fig. 6).

Type Material. Holotype male (CAU), China: Guizhou, Tongren, Fanjingshan, 2002.VI.1, Ding Yang. **Paratypes:** 2 females (CAU), China: Guizhou, Tongren, Fanjingshan, 2002.VI.1, Ding Yang; 1 female (CAU), China: Guizhou, Tongren, Fanjingshan, 2002.V.29, Ding Yang; 2 females (CAU), China: Guizhou, Tongren, Fanjingshan, 2002.V.31, Ding Yang.

Distribution. China (Guizhou).

Etymology. The specific name refers to the long aedeagus.

Remarks. This new species is somewhat similar to *E.* (*E.*) *elongatum* Mao & Yang, 2009 from China (Yunnan) in having the similar femora and wing pattern, but can be separated from *E.* (*E.*) *elongatum* by the interbase rod being straight and the aedeagus stubby and 4/5 as long as the gonocoxite. In *E. elongatum*, the interbase rod is finger-shaped, and the aedeagus is slender and half as long as the gonocoxite (Mao and Yang 2009).

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RESEARCH ARTICLE

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A new species of *Synagoga* (Crustacea, Thecostraca, Ascothoracida) parasitic in an antipatharian from Green Island, Taiwan, with notes on its morphology

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Abstract

A new ascothoracidan species has been discovered off Taiwan in the north part of the west Pacific at SCUBA depths. Twelve specimens including both sexes of the new species, described herein as *Synagoga arabesque* **sp. nov.**, were collected from colonies of the antipatharian *Myriopathes* cf. *japonica* Brook, 1889. Three previously described species of *Synagoga*, morphologically the least specialized ascothoracidan genus, have been found as ectoparasites of antipatharians and an alcyonarian, whereas all other records of this genus have been based on specimens collected from the marine plankton. This is the second study of a new form of *Synagoga* to be based on more than a few mature specimens of a single sex or on a single juvenile. Furthermore, it is the second in which SEM has been used to document the fine-scale external morphology. The position of terminal pores in the anterior pairs of the lattice organs is different in *Synagoga arabesque* **sp. nov.** than those in *S. grygieri* Kolbasov & Newman, 2018 and *S. millipalus* Grygier & Ohtsuka, 1995. Species of *Synagoga* are small, host-specific predators or ectoparasites of antipatharians. This genus exhibits a major Tethyan reliction pattern.

Keywords

Ascothoracida, black corals, lattice organs, live observations, morphology, parasitic crustaceans, SEM, taxonomy, ultrastructure

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Introduction

Species of Ascothoracida are crustacean ecto-, meso-, and endoparasites of cnidarians (Scleractinia: Zoantharia, Antipatharia, and Alcyonacea) and echinoderms (Asteroidea, Crinoidea, Echinoidea, and Ophiuroidea). Currently this taxon is comprised of more than 100 described species assigned to two orders (Grygier1987a, 1996): Laurida, species of which are parasites of anthozoans except for those of *Waginella* Grygier, 1983a, which are ectoparasites of crinoids, and Dendrogastrida, species of which are parasites of non-crinoid echinoderms. Ascothoracidans are normally dioecious, the larger females being accompanied by smaller, sometimes dwarf, cypridiform males (Grygier and Fratt 1984; Grygier 1985a, 1987b, 1991a, b; Kolbasov 2007). However, members of Petrarcidae and possibly Ctenosculidae are simultaneous hermaphrodites (Okada 1938; Grygier 1983b, c). The life cycle of ascothoracidans includes up to six naupliar instars, one or two instars of a specialized ascothoracid larva, juveniles, and adults, but in a few species the naupliar phase is condensed or even omitted (Høeg et al. 2014). Depending on species, the larval stages may be free-swimming or brooded.

Members of the family Synagogidae represent the most generalized or basal group of ascothoracidans. Adult ascothoracidans belonging to such genera as *Synagoga* Norman, 1888, *Waginella* Grygier, 1983a, and *Sessilogoga* Grygier, 1990b are characterized by a bivalve carapace enclosing the whole body; the head bearing a pair of W-shaped, six-segmented prehensile antennules and an oral cone enclosing piercing mouthparts; the trunk consists of eleven segments, including six thoracomeres with biramous thoracopods, a genital somite bearing a (sexually dimorphic, vestigial in females) penis, three limbless abdominal somites, and the telson, with a pair of furcal rami.

Six described species and one unnamed ascothoracid larva are currently assigned to the genus *Synagoga*: the type species *S. mira* Norman, 1888 (see also Norman 1913) from the Bay of Naples; *S. normani* Grygier, 1983a from East Africa, *S. paucisetosa* Grygier, 1990a and *S. bisetosa* Grygier, 1990a (the latter only tentatively attributed to this genus) from the bathyal Atlantic, *S. millipalus* Grygier & Ohtsuka, 1995 from off Okinawa, *S. grygieri* Kolbasov & Newman, 2018 from the Azores and Cape Verde Islands, and "McKenzie's larva" (Grygier 1988) from the eastern Indian Ocean (Table 2). Most of the descriptions were based on single individuals, and not always mature ones. Only *S. grygieri* was described on the basis of a number of specimens of both sexes (Kolbasov and Newman 2018), while *S. mira* was based on a few males. Furthermore, hosts have only been recorded for *S. mira* (the antipatharian *Parantipathes larix* (Esper 1788)), *S. normani* (an unidentified species of the alcyonarian *Dendronephthya* Kükenthal, 1905), and *S. grygieri* (the antipatharian *Antipathella wollastoni* (Gray 1857)).

The present study is the second, after that of *S. grygieri*, to describe a new species of *Synagoga* based on a number of specimens of both sexes with the extensive use of scanning electron microscopy (SEM) to document the fine-scale external morphology.

Materials and methods

The ascothoracidans belonging to the new species *Synagoga arabesque* sp. nov. were collected alive from the two colonies of the antipatharian *Myriopathes* cf. *japonica* Brook, 1889. The colonies were first photographed and then collected alive in situ into sealed plastic bags (to prevent the escape of parasites) by GAK using SCUBA at depth of 35 m (Fig. 1A), at Green Island (Lyudao), Taiwan. Host specimens were transported in a portable ice box filled with seawater to the Green Island Marine Research Station, Biodiversity Research Center, Academia Sinica within 2 hrs of collection and subsequently maintained in an aquarium at 23–25 °C. Each colony was examined for crustacean

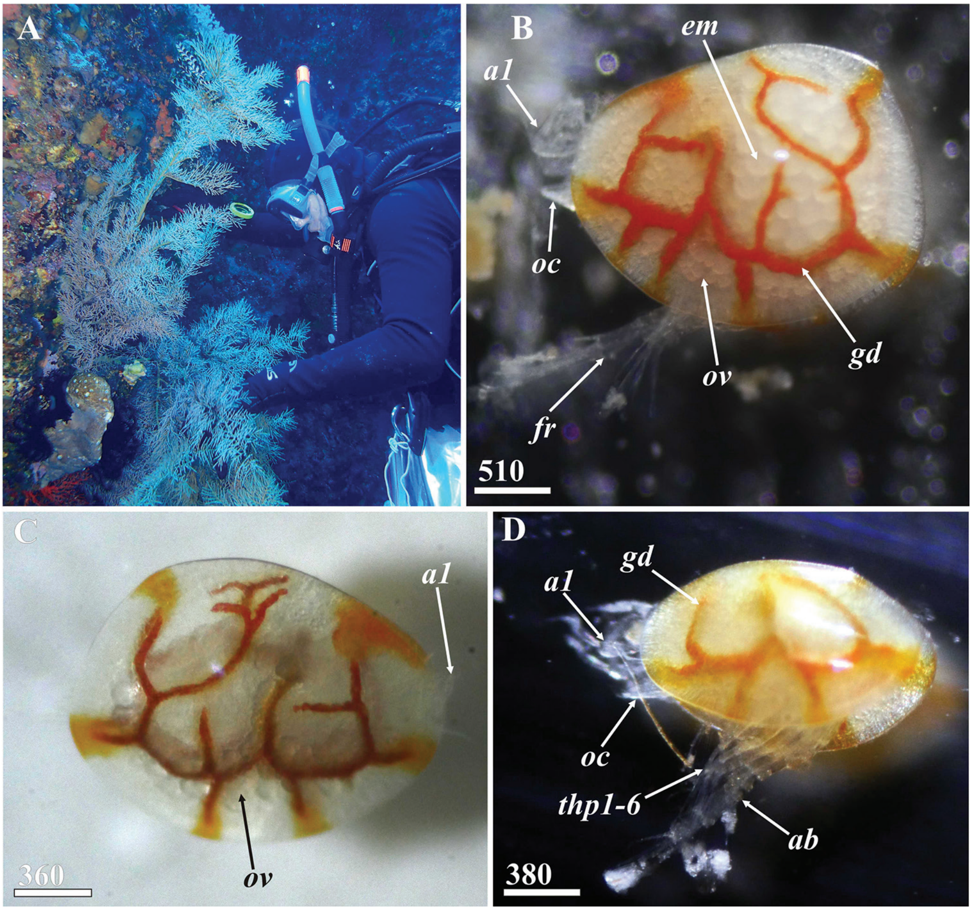

Figure 1. Collection and natural coloration of living specimens of *Synagoga arabesque* sp. nov. **A** Collection of living specimens of *Synagoga* from black coral *Myriopathes* sp. **B** mature female with outstretched antennules, oral cone and abdomen, lateral view, left side **C** young female, lateral view, right side **D** male with outstretched antennules, oral cone, thoracopods and abdomen, lateral view, left side. Abbreviations: *a1* – antennule, *ab* –abdomen, *em* – embryos, *fr* – furcal rami, *gd* – gut diverticulum, *oc* – oral cone, *ov* – ovary, *thp1-6* – thoracopods I-VI. Scale bars: in µm.

parasites using stereomicroscope. The seawater from the sealed plastic bags was filtered through a sieve and the sample was also examined under the stereomicroscope. The ascothoracidans thereby discovered were fixed one-two days later in 100% alcohol, formalin, and glutaraldehyde, after digital photography using a Lumix (Panasonic) GH4 camera equipped with a Leica DG Macro-Elmarit 45 mm f2.8 lens and the same camera body affixed to an Olympus SZ61 dissecting microscope. Two females (holotype and paratype) and two males (paratypes) were dissected and mounted in glycerol on glass slides. They were examined and illustrated using a WILD Heerbrugg M20-35369 light microscope. Line drawings were also made using oil immersion, Nomarsky differential interference contrast, and a drawing tube on an Olympus BX 51 microscope. For SEM, three non-type females and two non-type males were post-fixed in 2% OsO4 for 2 h, dehydrated in acetone and critically-point dried with CO_2. Dried specimens were sputter-coated with platinum-palladium and examined on a JEOL JSM-6380LA scanning electron microscope operating at voltages of 15–20 kV at the University of Moscow. Resulting photographs were touched up using CorelDraw X3 Graphics Suite.

Systematics

Subclass Ascothoracida Lacaze-Duthiers, 1880
Order Laurida Grygier, 1987a
Family Synagogidae Gruvel, 1905

Genus *Synagoga* Norman, 1888

Type species. *Synagoga mira* Norman, 1888

Synagoga arabesque sp. nov.

http://zoobank.org/3BE6E08C-6AF5-45A9-946D-7DA1AF2BD63D
Figs 1–18

Type locality. Gongguan harbor, Green Island (Ludao), ca. 33 km off the southeastern coast of Taiwan, 22°41.438'N, 121°29.678'E, 35 m depth, 08 and 09 September 2017.

Material examined. Twelve specimens of the new species, *Synagoga arabesque* sp. nov. (five males and seven females), were collected from two colonies of the antipatharian *Myriopathes* cf. *japonica*. Slides of the holotype female Mg 1243, and three paratypes (female, Mg 1244 and two males, Mg 1245) are deposited in the Zoological Museum of Moscow State University in Moscow, Russian Federation. The remaining two undissected paratypes (female and male) are deposited in alcohol in the Biodiversity Research Museum, Biodiversity Research Center, Academia Sinica, Taipei, Taiwan (ASIZCR000412). The other four SEM specimens and two undissected specimens in alcohol have been retained by the first author for further study and comparison with other synagogids.

Diagnosis. Diagnoses for both adult females and males are provided for the new species, and a full list of interspecific differences is given in Table 2.

Females: carapace oval, slightly elongated in posterio-dorsal direction, up to 2.3 mm long and 2.0 mm high, with projecting posterio-dorsal tip. Massive setae (spines) of fourth antennular segment with row of dense, conspicuous denticles along anterior edge and rare, tiny denticles on posterior edge; fifth segment with 6–9 large setae; concave margin of antennular claw serrate in middle part. Exopod of second segment of thoracopod I with seven setae. Telson spines ca. 1/3 of blade length of furcal ramus; inner surface of furcal ramus with eight setae. Gut diverticulum red-orange, W-shaped, with numerous branches; dorsal, ventral, anterior and posterior branches terminate with light orange, wide areas at the edge of carapace.

Males: carapace ellipsoidal, up to 1.5 mm long and 0.9 mm high, with slightly projecting posterio-dorsal tip. Massive setae (spines) of fourth antennular segment differing slightly in length, with anterior and posterior rows of small denticles; fifth segment with 4–6 large setae; other characters of antennules similar to those in female. Exopod of second segment of thoracopod I with eight setae. Telson spines ca. 1/3 of blade length of furcal ramus; inner surface of furcal ramus with six setae. Gut diverticulum red-orange, W-shaped, with short anterior, posterior, and two ventral branches; branches terminate with light orange wide areas at edge of carapace.

Etymology. From French *arabesque* borrowed from Italian *arabesco* - foliate ornament, used in the Islamic world, referring to the complex ornament of gut diverticula in carapace valves. The name *arabesque* has no appropriate equivalent in Latin and is used in this context as an arbitrary combination of letters (sensu ICZN Article 11.3) to avoid using the word in the vernacular.

Relation to host and behavior. Animals were seen freely swimming from one branch of the antipatharian colony to another and represent small predators rather than ectoparasites. All live specimens of *Synagoga* were collected after washing the colonies. Animals were quite motile and moved in a Petri dish by jumping. To accomplish these jumping movements, they bent and unbent their developed abdomen with furca, while thoracopod beating was used for slow swimming.

Description. Living specimens of both sexes semitransparent, light colored, but with bright red-orange gut diverticula; rounded embryos brooded inside female mantle cavity visible through carapace (Figs 1, 2). Abdomen and antennules often extending out of carapace during movements (Fig. 1B–D).

Female (Figs 1–4, 8–12): Carapace oval, up to 2.3 mm long and 2.0 mm high, bivalved (Figs 1B, C, 2A–C, 8A, B), valves joined and hinged along dorsal margin (Fig. 17A). Dorsal and posterior margins of valves feebly convex, meeting at slightly produced posterio-dorsal angle; anterior and ventral margins rounded (Figs 1B, C, 2A–C, 8B).Exterior of carapace smooth, lacking setae but covered with small pores (Figs 8A, 17A, B, 18A–D). Right and left gut diverticula (Figs 1B, C, 2A–C) lying within respective carapace valve, resembling letter "W"; short main branch descending toward ventral margin and bifurcating, with anterior branch shorter than posterior and numerous simple and bifid small branches extending from them in various directions; dorsal, ventral, anterior and posterior small branches terminated with light orange, wide areas at edge of carapace (Fig. 1B, C). Inner surface of carapace valves with cuticular lining or mantle (Fig. 8B–F). Small, narrow pit on inner surface of anterior part of each valve (Figs 2A, C, 8E). Anterior pit of carapace infundibuliform, with wide entrance and long, narrowed internal part (Figs 2D, 8E); cuticle of pit wrinkled, with circular folds, small pores and volcano-shaped papillae (Fig. 8E, F). Body situated within mantle cavity (Figs 2B, 8A); oval brood chamber for embryos in posterior portion of each valve (Fig. 2B). Cuticular armament of mantle similar to that in *S. grygieri* (see Kolbasov and Newman 2018). Main cuticular structures of mantle arrayed along its margin: anterior and ventral sides with submarginal underlying folder consisting of dense row of cuticular projections forming fringe or palisade (Fig. 8C); anterior, ventro-posterior and posterior sides of mantle bearing long setae with short setules, these being absent ventro-anteriorly (Fig. 8B–D).

Body proper consisting of unsegmented head and segmented thorax and abdomen. Head bearing W-shaped prehensile antennules followed by large ventral oral cone formed of mouth parts surrounded by labrum (Fig. 2B). Frontal filament complex (Fig. 8D) originating on mantle rather than body proper, ~380 µm long and trifid, with anterior ramus longest (ca. 350 µm) and densely covered by long, setiform protrusions; ampuliform, short basal ramus (ca. 70 µm) with smooth cuticle; and small, thin posterior ramus (ca. 40 µm).

Thorax consisting of six segments (Figs 2B, 9A, B), each with pair of biramous natatory thoracopods described in detail below. Dorsal sides of segments (II–VI) covered with thin setae (Fig. 9A, B). Posterio-ventral angles of sixth thoracic segment formed as small triangular projections or epaulets, their surface covered by rounded plaques (Fig. 9B).

Abdomen U-shaped, five-segmented, including telson (Figs 2B, 8A, 9B). First segment with vestigial penis on ventral side (Figs 2E, 9B, C), an unpaired process – 140–190 µm long, its distal part bearing ctenoid scales (Fig. 9C). Second segment trapezoid, bigger than either third or fourth. Last body segment (telson) cylindrical, ca. 300 µm long, its posterio-ventral margin bearing fringe of ctenoid scales and pair of conspicuous telson spines (Figs 2B, 9B, D) approximately 190 µm long with row of nine or ten sharp denticles along their dorsal margins. Furcal rami unsegmented (Figs 2B, F, 9B), approximately 410–560 µm long, thus approximately 2.5–2.9 times longer than telson spines; ventral margin with one medial, one subdistal and two distal setae, rarely with long setules (Figs 2F, 9B, E, G); proximal half of ventral margin armed with large, sharp denticles and ctenoid scales (Figs 2F, 9D, F, H). Inner subdorsal margin of each ramus with row of eight long natatory setae with long setules (Figs 2F, 9F, H); row of dense ctenoid scales along inner side of dorsal margin (Fig. 9F–H).

Extendable, prehensile antennules subchelate, folded into W-shape, consisting of six segments with complex of intrinsic and extrinsic flexor and extensor muscles (Figs 2B, 3A, B, 10A). First segment rectangular, narrowing somewhat distally, without setae. Second segment irregularly rectangular, with dense, thin omniserrate setae along postaxial/ventral margin (Figs 3A, B, 10A, C). Third segment equilaterally triangular, narrowing toward ventral margin; preaxial/dorsal margin curved, densely covered by

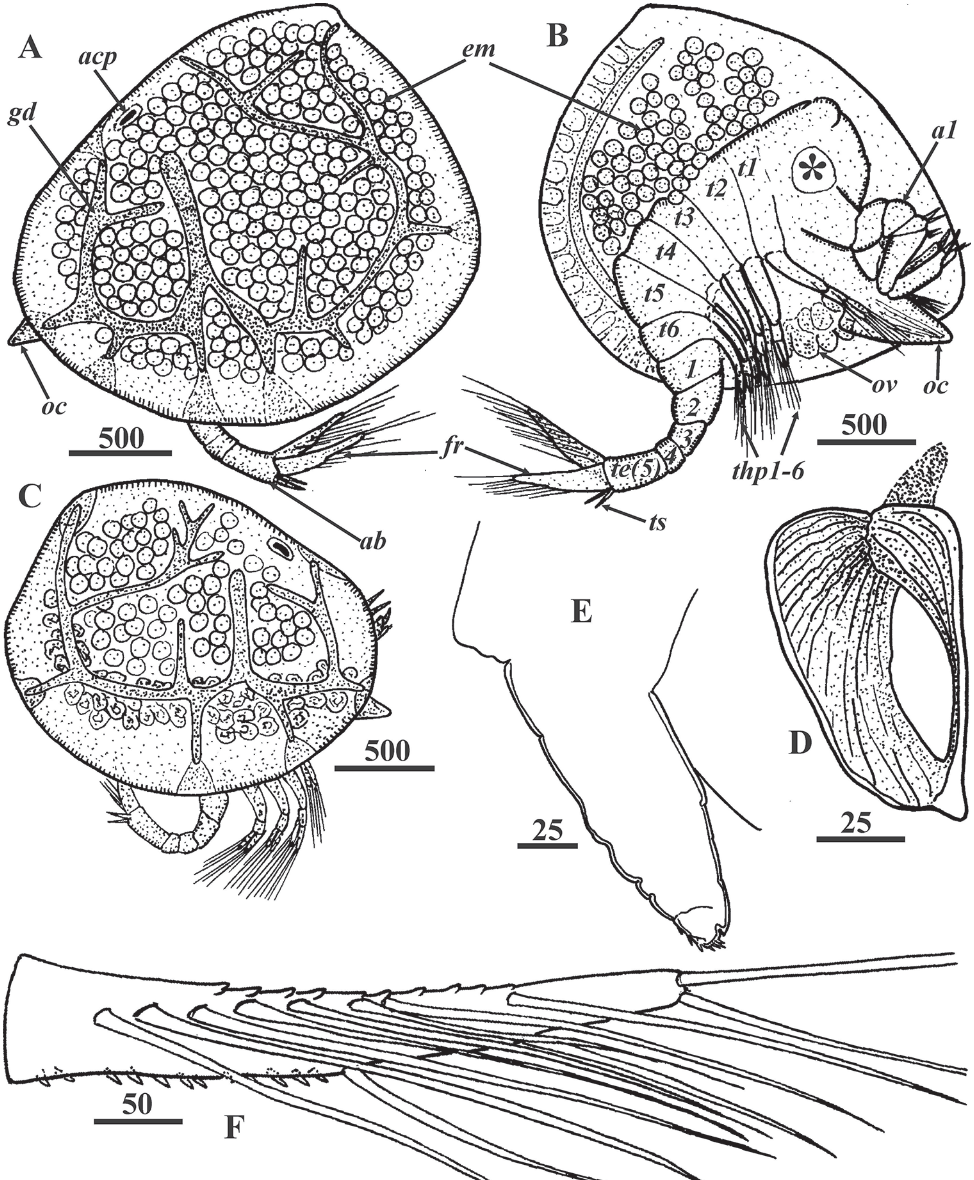

Figure 2. *Synagoga arabesque* sp. nov., female. General morphology **A, B, D–F** holotype **C** paratype Mg 1244 **A** general view lateral, left side **B** General view lateral, right valve of carapace removed, segments of thorax (*t1-6*) and abdomen (*1-5*), entrance of gut diverticulum and adductor muscle indicated by asterisk **C** General view lateral, right side **D** Anterior carapace pit, ventral end below **E** Rudimentary penis **F** furcal ramus, inner side. Abbreviations: *a1* – antennules, *ab* – abdomen, *acp* – anterior carapace pit, *em* – embryos, *fr* – furcal rami, *gd* – gut diverticulum, *oc* – oral cone (pyramid), *ov* – ovary, *t1-6* – segments of thorax, *te(5)* – fifth abdominal segment(telson), *thp1-6* – thoracopods I–VI, *ts* – telson spines. Scale bars: in μm.

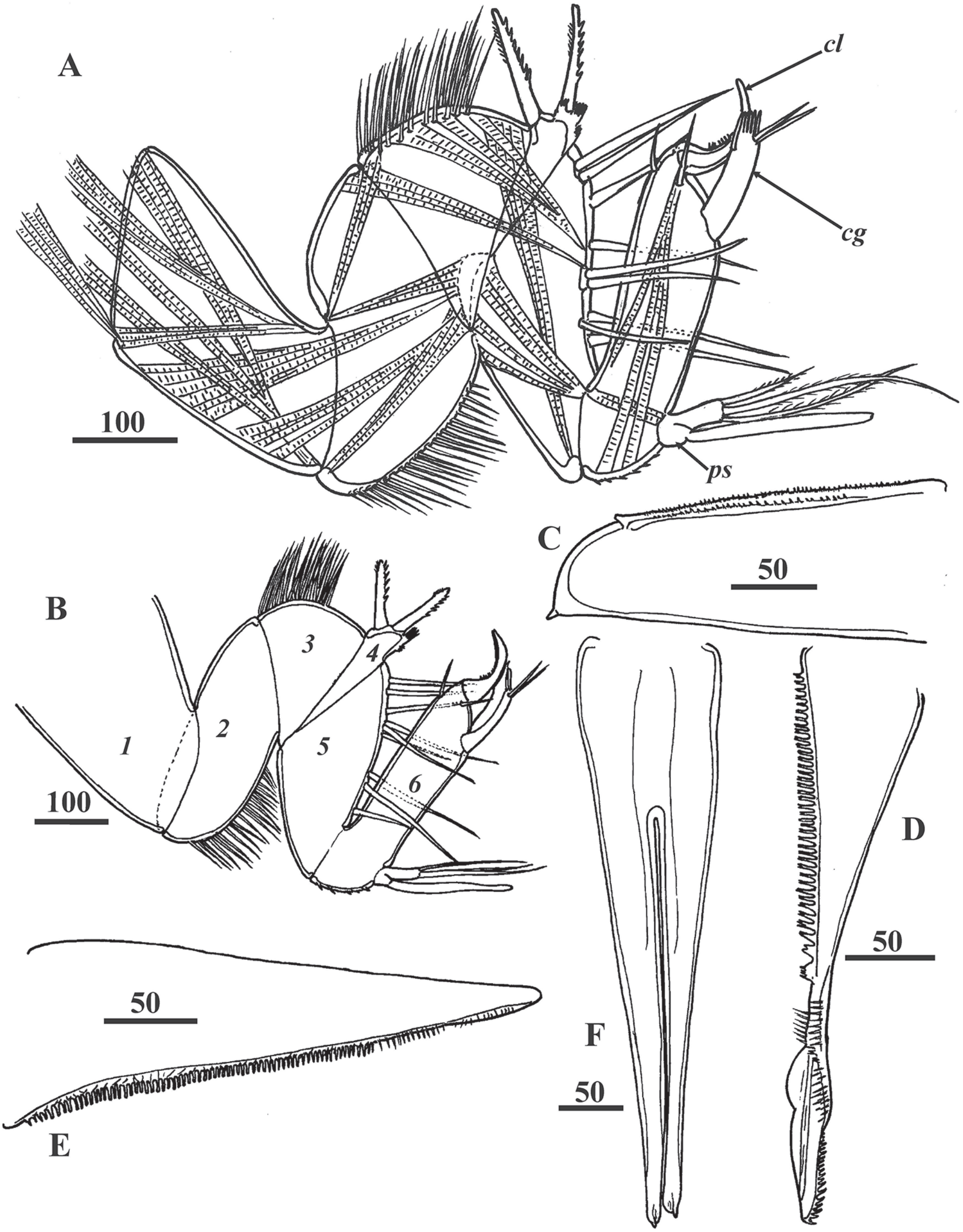

Figure 3. *Synagoga arabesque* sp. nov., female, holotype. Head appendages **A** right antennule with musculature **B** left antennules, segments numbered **C** medial languette **D** mandible **E** maxillule **F** maxillae. Abbreviations: *cg* – claw guard, *cl* – claw, *ps* – proximal sensory process. Scale bars: in µm.

thin omniserrate setae (Figs 3A, B, 10A). Fourth segment rectangular, trapezoid (appearing triangular in folded antennules, Fig. 3A, B), very narrow, narrowing towards dorsal margin, with two massive and denticulate setae ('spines') armed with row of dense, conspicuous denticles along anterior edge and, rarely, tiny denticles on posterior edge; these two spines sitting on dorsal projection apex bearing ctenoid scales with sharp denticles (Figs 3A, B, 10A, F). The two spines form a fork to accept movable claw. Fifth segment conical, forming a palm against which sixth segment can fold in order to grasp host tissue, with 6–9 strong, simple setae along upper margin (Figs 3A, B, 10A). Sixth segment longer than fifth segment and armed with sensory and grasping structures (Figs 3A, B, 10A, D, E, G–I). Short proximal sensory process on lower margin at base of sixth segment (Figs 3A, B, 10A, G), with 3 terminal setae, middle one setulated and longest, and 1 thick, blunt sub-basal seta (at least this seta probably an aesthetasc). Curved claw on distal end of sixth segment apparently with muscles attached (Figs 3A, B, 10A, D, E); concave margin of claw serrate, with sharp tiny denticles in middle part (Fig. 10D, E); three small setae at base of claw, two lateral on inner and outer surfaces and one on anterior dorsal margin (Figs 3A, B, 10D, H). Relaxed claw sheathed by grooved claw guard (Figs 3A, B, 10D, E, H, I), latter approximately 110 µm long, with wide flange on inner side, thin, membranous, apical ctenoid hood (Fig. 10I) and four small terminal setae including two longer and one tiny subapical (Fig. 10E) and 1 tiny apical seta (Fig. 10I). Cuticle on sides of antennular segments bearing dense small ctenoid scales (Fig. 10B).

Oral cone prominent, approximately 600–650 µm long; distal end often protruding outside carapace (Figs 1B, D, 2A–C, 11A); formed by cone-shaped labrum surrounding piercing mouth parts (Fig. 11A). Posterior margins of labrum free, unfused (Fig. 11A). Tuft of long, thin simple setae in middle of anterior face of labrum; dense, small ctenoid scales on external cuticle (Fig. 11A). Mandibles in form of lanceolate stylets, approximately 350 µm long (Figs 3D, 11B); cutting edge of each bearing approximately 80–90 sharp, complex teeth with four tips (quadrifid), length of teeth increasing towards middle part of blade, with row of small setae paralleling them (Fig. 11D); neck of mandible lacking denticles or teeth but bearing small simple setae; distal part with row of 16–20 curved teeth on posterior margin (Figs 3D, 11B). Maxillules consisting of a wide basal half and narrow distal half (Figs 3E, 11B, C); cutting edge bearing numerous denticles with serrate margin and cuticular setiform projections, these denticles being massive in proximal part and thin and elongate in middle and distal parts (Fig. 11B, C, E); tip with thin, curved setiform projections (probably setae, Fig. 11F). Maxillae (Figs 3F, 11C) thin, fused at bases, with row of thin, needle-shaped denticles along inner cutting edge at distal end (Fig. 11G); tips not distinctly bifid, not harpoon-shaped, with apical projection and adjacent tiny process (probably seta, Fig. 11G). Unpaired process or medial languette (fused paragnaths?) originating from between paired mouth parts, with sharp tip and two rows of denticles on anterior margin (Fig. 3C).

All thoracopods natatory and biramous (Figs 4, 12). Seminal receptacles found in lateral proximal parts of coxae of thoracopods II–V (Fig. 4B–E), consisting of ampuliform sacs with proximal parts converging but external opening(s) not observed; thoracopods II with four seminal receptacles, thoracopods III and IV each with three and thoracopod V with one. Thoracopodal setation summarized in Table 1. First thoracopod (Fig. 4A) slightly separated from others, with elongate protopod comprised of

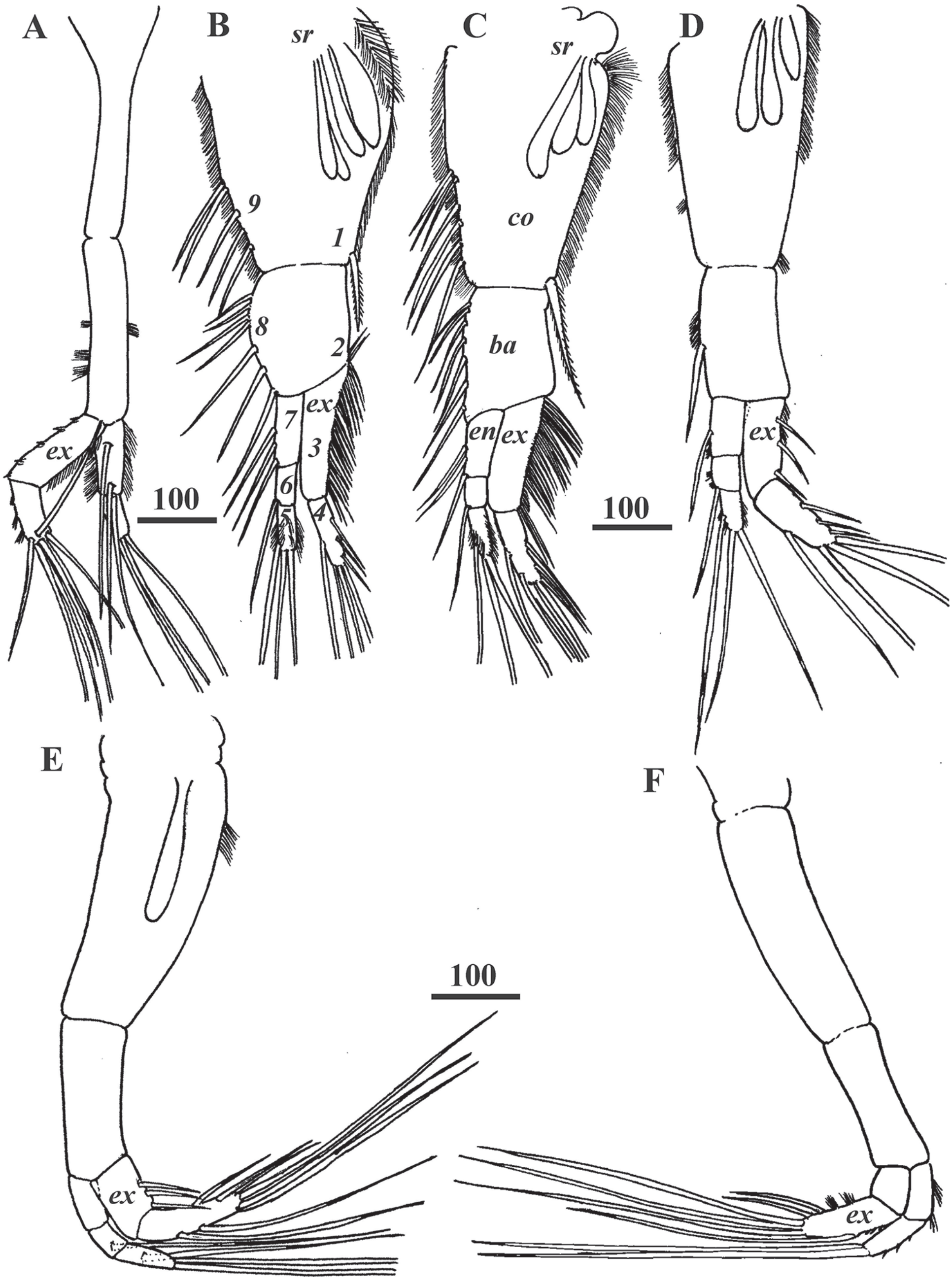

Figure 4. *Synagoga arabesque* sp. nov., female, holotype. Left (**A, B, D–F**) and right (**C**) thoracopods I–VI respectively. Ampuliform seminal receptacles are situated in upper outer parts of coxae of thoracopods II–V (**B–E**). Numbers indicating positions for setal counts in description (1–9) are shown for thoracopod II (**B**). Abbreviation: *ba* – basis, *co* – coxa, *en* – endopod, *ex* – exopod, *sr* – seminal receptacles. Scale bars: in μm.

Table 1. Thoracopodal setation in *Synagoga arabesque* sp. nov. (ignoring tiny setae). Roman numerals indicate thoracopods I–VI. Positions 1–9 are indicated in Figs 4B and 7C. Question marks indicate that the position in question was obscured. Parentheses in thoracopods I and VI are used for the 2-segmented (instead of 3-segmented) endopods.

	Position on thoracopods								
	1	2	3	4	5	6	7	8	9
♀									
I	0	0	0	7	3	(3)		0	0
II	1	0	30?	7	3	1	3	7	5
III	1	0	24?	7	3	1	3	7	7
IV	0	0	3	7	3	1	1	1	0
V	0	0	3	8	3	1	0	0	0
VI	0	0	0	6	2	(0)		0	0
♂									
I	0	0	0	8	4	(3)		0	0
II	1	0	16?	6	3	1	2	3	1
III	1	0	20?	7	3	1	1	3	1
IV	0	0	8	6	4	1	0	0	0
V	0	0	1	7	3	1	0	0	0
VI	0	0	0	6	2	(0)		0	0

coxa and basis and two-segmented exopod and endopod; margins of basis with tufts of short setae; segments of exopod with ctenoid scales and small denticles, inner margin of basal segment lined with dense thin, small setae; seven long, plumose setae situated at distal end of second segment; basal segment of endopod bearing three long, plumose setae, margins being lined with dense thin, small setae; distal segment with three terminal plumose setae. Thoracopods II-V with three-segmented endopods and two-segmented exopods (Figs 4B–E, 12A–E). Coxae of thoracopods II and III (Figs 4B, C, 12B, C) with large, distal seta in position "1" (see Table 1 for further explanation) and row of plumose setae along inner edge (position "9"); these setae absent on coxae of thoracopods IV and V (Fig. 4D, E). Number of setae on rami of thoracopods II and III much more numerous than on thoracopods IV and V. Protopod of thoracopod VI (Figs 4F, 12F) narrow; coxa and basis without setae; both rami two-segmented with long, plumose terminal setae on distal segments; two tufts of thin, small setae on basal segment of endopod and distal segment of exopod. Surface of all thoracopods bearing conspicuous ctenoid scales (Fig. 12).

Male (Figs 1D, 5–7, 13–16): Carapace bivalved, ellipsoidal, up to 1.5 mm long and 0.9 mm high, with slightly produced posterio-dorsal tip (Figs 5A, B, 13A). Dorsal margin almost straight; anterior, ventral and posterior margins rounded. Exterior of carapace smooth, lacking setae but covered with small pores (Figs 13A, 17E, F, 18E–H). Conspicuous deep pit with curved lumen opening on inner surface of anterior part of each valve (Fig. 5A, B). Gut diverticulum of simplified W-shape in comparison to female (Figs 1D, 5A, B), with 4 short lateral branches extending from anterior, posterior and ventral parts and terminated with light orange, widened areas at edge of carapace.

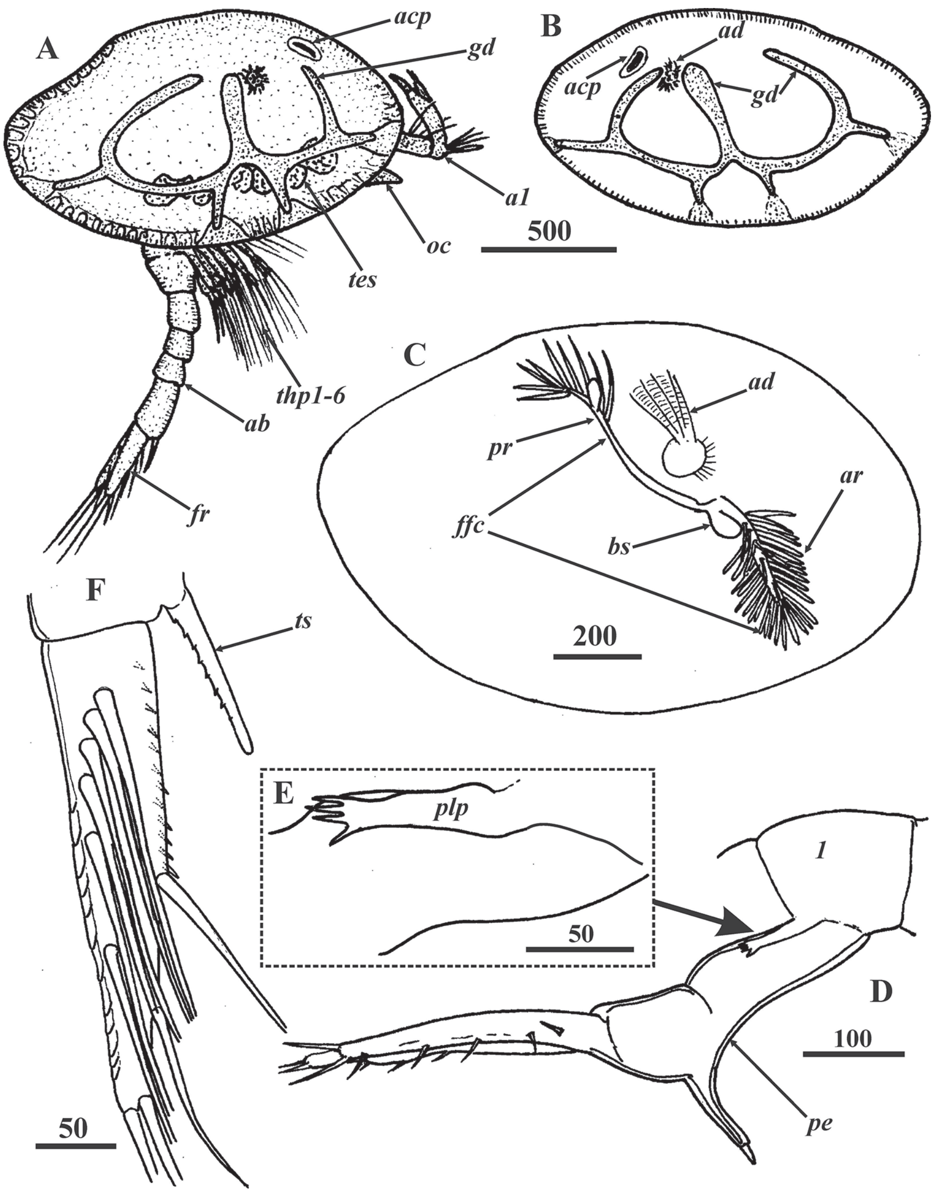

Figure 5. *Synagoga arabesque* sp. nov., male. General morphology **A, C–E** one paratype Mg 1245; **B** other paratype Mg 1245 **A** general view lateral with outstretched antennules, oral cone, thoracopods and abdomen, right side **B** general view lateral, left side, anterior end left **C** left valve of carapace with frontal filament complex, inner side, anterior end right **D** first abdominal (seventh trunk) segment with penis, lateral view **E** Enlarged part of basal shaft of penis with pleural process of first abdominal segment (*plp*) **F** Distal part of telson with telson spine and furcal ramus (inner side). Abbreviations: *a1* – antennule, *ab* – abdomen, *acp* – anterior pit of carapace, *ad* – adductor muscle, *ar* – anterior ramus of frontal filament complex, *bs* – basal ramus of frontal filament complex, *ffc* – frontal filament complex, *fr* – furcal rami, *gd* – gut diverticulum, *oc* – oral cone, *pe* – penis, *plp* – pleural process of first abdominal segment, *pr* – posterior ramus of frontal filament complex, *tes* – testis, *thp1-6* – thoracopods I–VI, *ts* – telson spine. Scale bars: in µm.

Lobed testis within each carapace valve along lower part of gut diverticulum (Fig. 5A). Cuticular armament of mantle is similar to that of female (Fig. 13C–E). Edge of mantle forming thin marginal fold adjacent to margin of carapace and consisting of dense, tiny cuticular projections (Figs 13E, 15A). Anterior, ventral and posterior sides with submarginal underlying folder consisting of dense row of cuticular projections forming fringe or palisade, these projections longer in posterior side (Fig. 13C–E); anterior, ventroposterior and posterior sides of mantle bearing setae with short setules (Fig. 13C, E).

Frontal filament long, trifid, more complex or less reduced than in female, with well-developed anterior and posterior rami covered by long, setiform cuticular projections (Figs 5C, 12B, F). Anterior ramus thicker and shorter than posterior, approximately 200–250 μm long; medial (basal) ramus short (50–80 μm), ampulliform, with smooth cuticle; posterior ramus longest, approximately 470 μm.

Body of male resembling that of female (Figs 5A, 13B): head bearing similar W-shaped antennules and well-developed oral cone; trunk consisting of 6 thoracic and 5 abdominal segments (Figs 5A, 13B, 14B); telson spines of same proportions and morphology (Figs 5F, 14E). Furcal rami resembling these of female in many details (Fig. 14E–H) but differ in having fewer long natatory setae on inner subdorsal margin (six instead of eight, Fig. 5F). Unlike in females, epaulets of sixth thoracic segment more strongly developed (Fig. 14A).

Condition of penis considerably different between male and female, tergite of penis-bearing first abdominal segment with conspicuous pair of long (approximately 100 μm), posteriorly directed pleural processes with four sharp terminal extensions that are absent in females (Figs 5D, E, 14B). Penis complex, approximately 600 μm long, ~ 4 times longer than supporting segment, and consisting of three parts: basal, medial and distal (Figs 5D, 14B, C). Basal shaft cylindrical, approximately 160 μm long. Medial part swollen, ~ 136 μm long, with unpaired thin process ~ 110 μm long extending from anterior side, tip of process (Fig. 5D) covered by thin layer of epicuticle. Distal part consisting of two rami originating from medial part and narrowing toward tips (Figs 5D, 14B, C). Cuticular setiform projections 10–20 μm long with apical pore (not setae) present along anterior margin of each ramus (Figs 5D, 14B, C). Tip of each ramus terminating in pair of these projections (Fig. 14C).

Antennules of male resembling those of female (Figs 6A, B, 15) but relatively thinner and longer with respect to body size. Second and third segments with dense, thin setae in same positions as in female. Two massive spines of fourth segment armed with row of conspicuous denticles along both anterior and posterior edges (Fig. 15A). Fifth segment with 4–6 rather than 6–9 setae on anterior margin (Figs 6A, B, 15B). Sensory and grasping structures of sixth segment of same morphology as in females, but ctenoid scales denser in lateral surfaces of segment (Figs 6B, 15C, D).

Oral cone and mouth parts similar to those of female (Figs 6C–G, 16), consisting of labrum (Figs 6G, 16A–C) enclosing an unpaired medial languette (Fig. 6D) and paired mouth parts, mandibles (Figs 6E, 16C, D), maxillules (Figs 6F, 16C) and maxillae (Figs 6G, 16C, E); tips of maxillules bifid, not harpoon-shaped, apical projection and adjacent process slightly larger than in females (Figs 6G, 16E). Thoracopodal setation of male (Table 1, Fig. 7) similar to that of female (Fig. 4) but showing

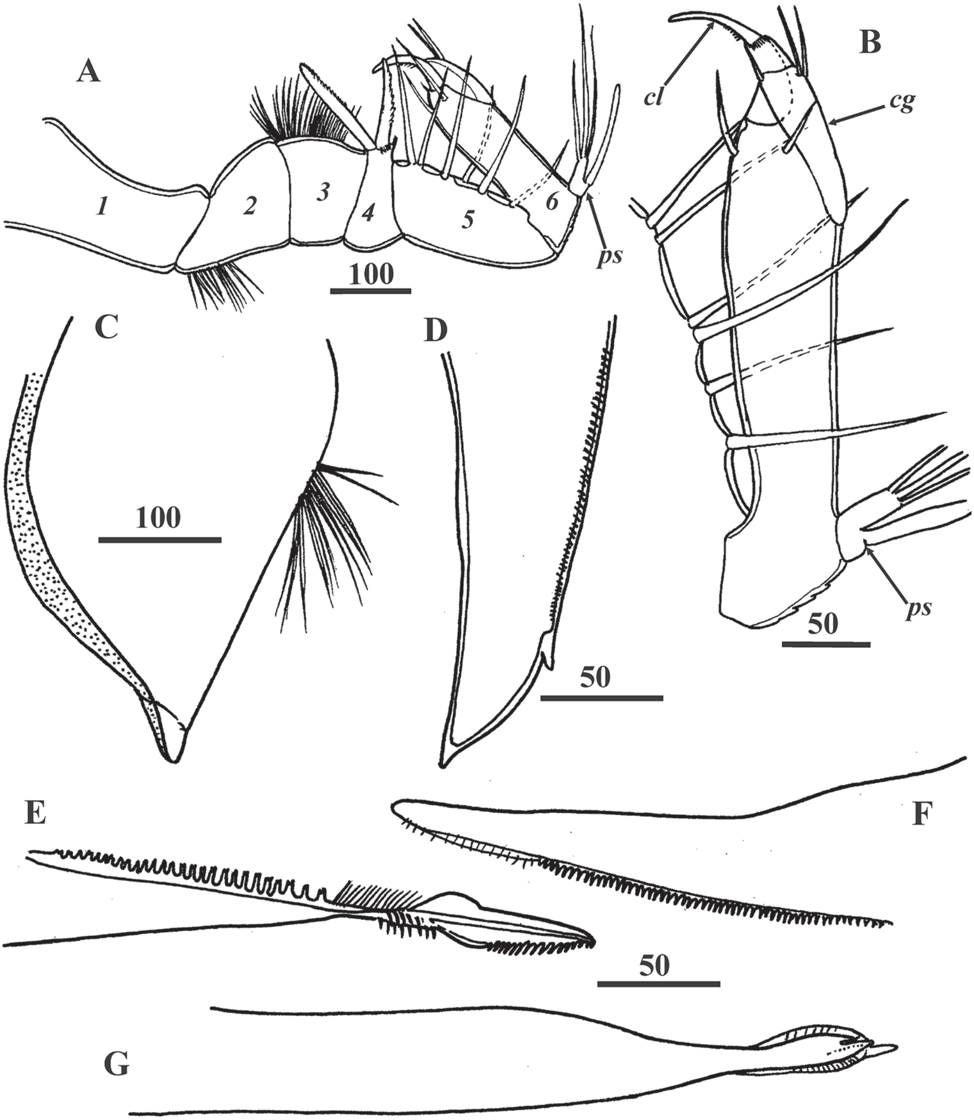

Figure 6. *Synagoga arabesque* sp. nov., male, paratype Mg 1245. Head appendages **A** left antennule, segments numbered **B** fifth and sixth antennular segments of right antennule **C** labrum lateral, anterior margin right **D** medial languette **E** mandible **F** maxillule **G** maxilla lateral. Abbreviations: *cg* – claw guard, *cl* – claw, *ps* – proximal sensory process. Scale bars: in μm.

some differences (only thoracopod VI have same setation); distal segment of exopod of thoracopod I with eight rather than seven setae (Fig. 7A), coxae of thoracopods II and III and bases of thoracopods II–IV have fewer setae along inner margins (Fig. 7B–D).

Lattice organs. (Figs 17, 18): both sexes with five pairs of trough-like slits along hinge line of carapace (lattice organs: *lo1-5*, Figs 17, 18), situated co-linearly in two groups: anterior pairs 1–2 and posterior pairs 3–5. Those of both female and male are of similar morphology and arrangement and are therefore described together.

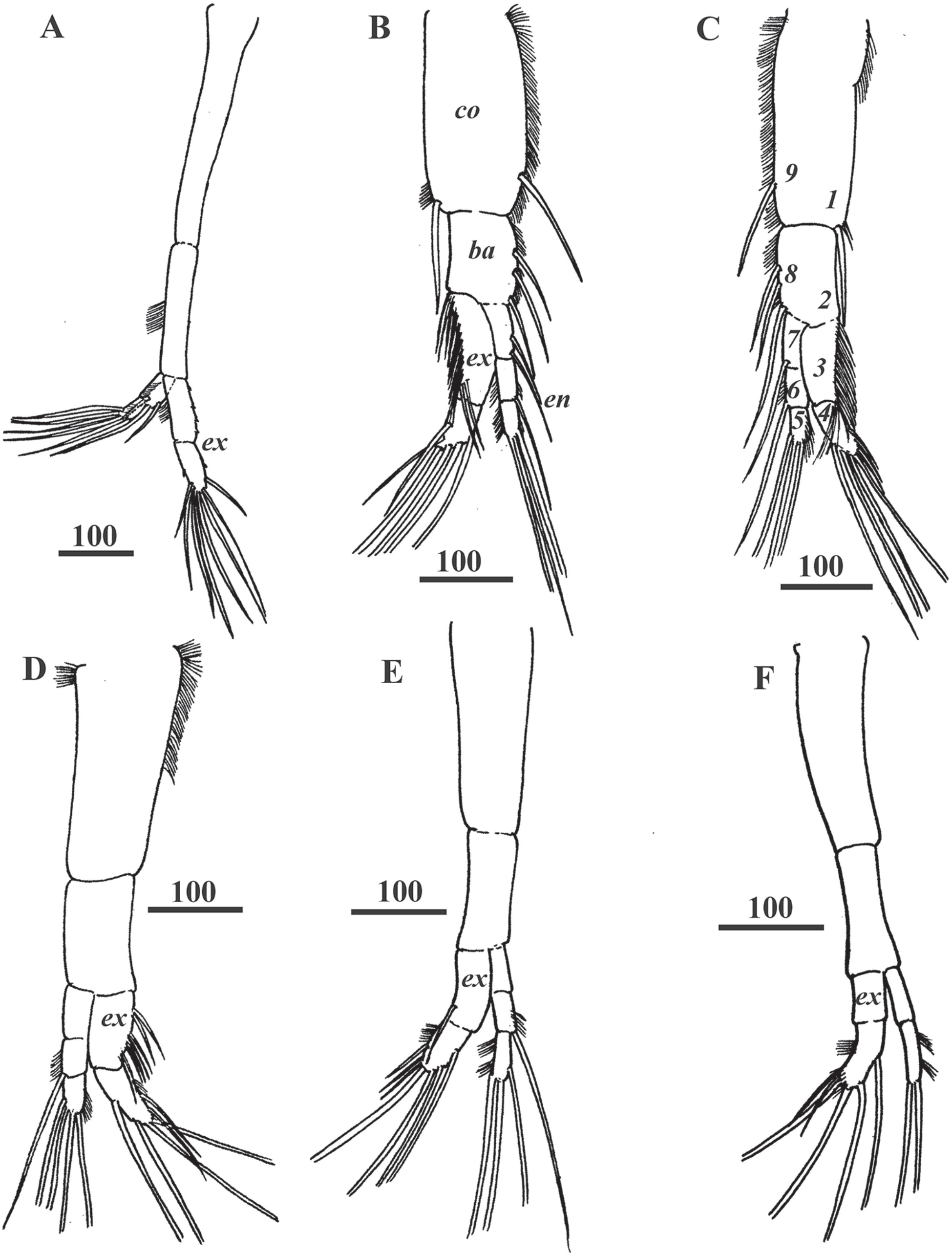

Figure 7. *Synagoga arabesque* sp. nov., male, paratype Mg 1245. Right thoracopods I–VI respectively (**A–F**). Numbers indicating positions for setal counts in description (1–9) are shown in thoracopod III (**C**). Abbreviations: *ba* – basis, *co* – coxa, *en* – endopod, *ex* – exopod. Scale bars: in µm.

Lattice organs straight, each trough containing one short, modified seta (so-called crest) with terminal pore at free distal end (Figs 17D, 18B), terminal pore maybe hidden by debris, shrinkage or trough. Normally, each trough has oblique and rounded

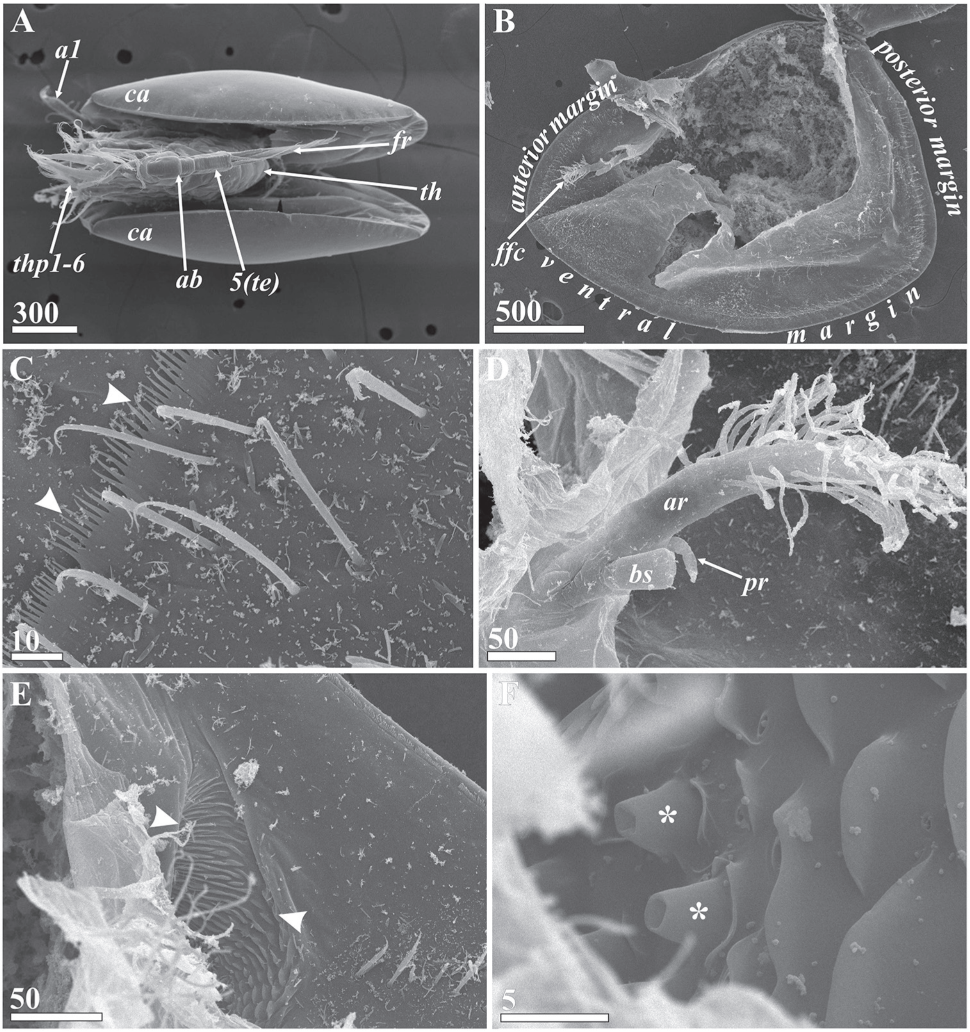

Figure 8. *Synagoga arabesque* sp. nov., female. General morphology, inner structures of carapace and mantle (SEM) **A** general view ventral **B** right valve of carapace, inner surface, mantle at place of body attachment (entrance of gut diverticulum and adductor muscle) destroyed **C** enlarged detail of mantle surface near anterior margin, submarginal fold of mantle with cuticular fringe (indicated by arrowheads) **D** frontal filament complex, anterior end left **E** Entrance of anterior pit of carapace (indicated by arrowheads) **F** surface of anterior pit of carapace (cuticular papillae indicated by asterisks). Abbreviations: *a1* – antennules, *ab* – abdomen, *ar* – anterior ramus of frontal filament complex, *bs* – basal ramus of frontal filament complex, *ca* – carapace (valve), *ffc* – frontal filament complex, *fr* – furcal ramus, *pr* – posterior ramus of frontal filament complex, *te* – telson, *th* – thorax, *thp1-6* – thoracopods I–VI. Scale bars: in µm.

ends; distal part of crest lies at rounded end (Figs 17C, D, 18B–D). Cuticle of crests smooth, not perforated by small pores. Anterior lattice organs situated just posterior to point of divergence of carapace valves (Fig. 17A, E). *Lo1* 15 µm long in female (Fig. 17B, C) and 10–11 µm long in male (Fig. 17F, G), with posterior terminal pore,

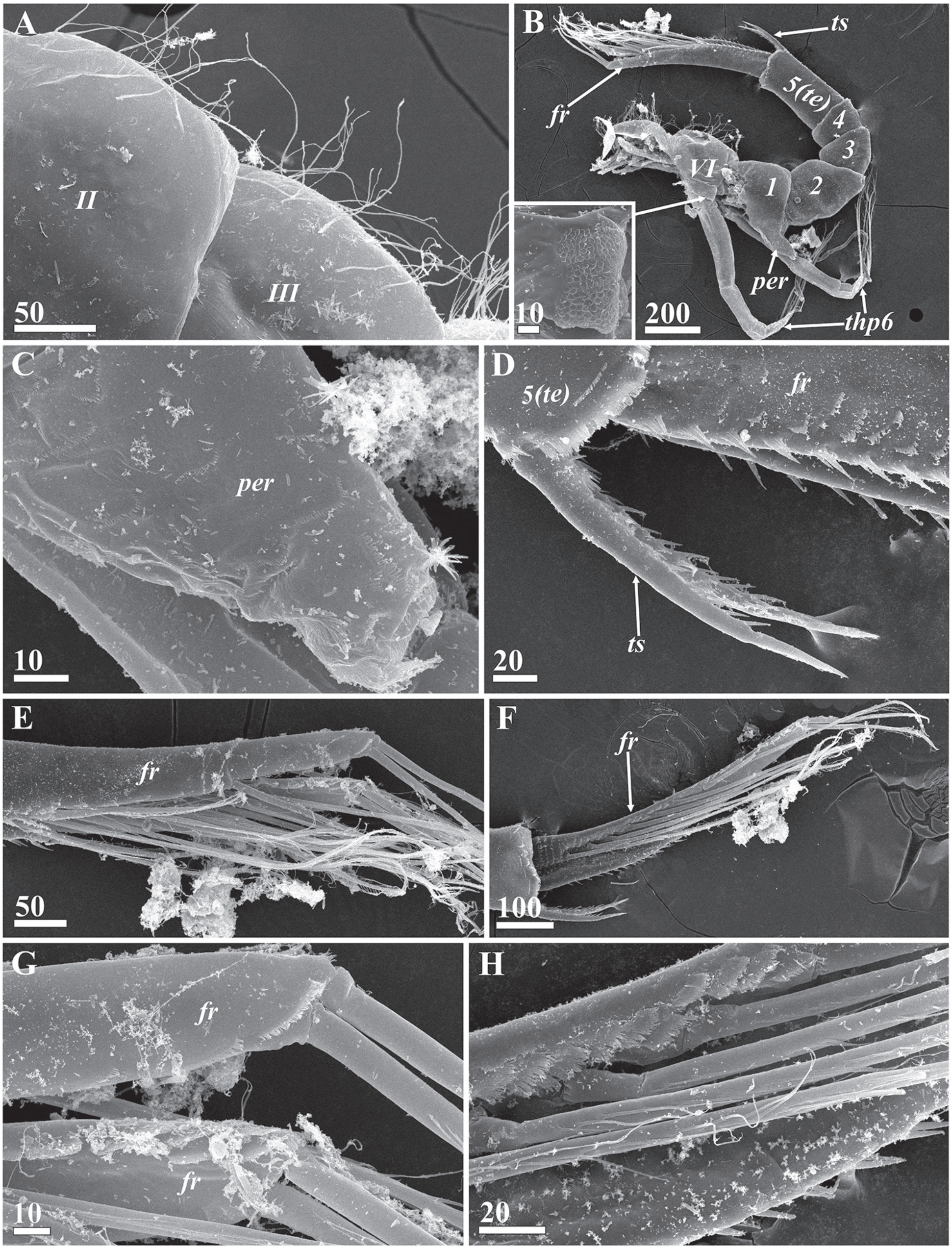

Figure 9. *Synagoga arabesque* sp. nov., female. Morphology and structures of thorax and abdomen (SEM) **A** dorsal surface of thoracic segments 2 and 3 **B** posterior part of thorax (segments numbered in Roman numerals) and abdomen (segments numbered in Arabic numerals), enlarged small epaulet in rectangle area in lower left angle **C** penis rudiment **D** telson spines **E** distal halves of furcal rami **F** furcal ramus, inner surface **G** terminal ends of furcal rami **H** Enlarged basal part of furcal ramus showing setation and sculpture on inner surface. Abbreviations: *fr* – furcal ramus, *per* – rudimentary penis, *te* – telson, *ts* – telson spines, *thp6* – thoracopod VI. Scale bars: in µm.

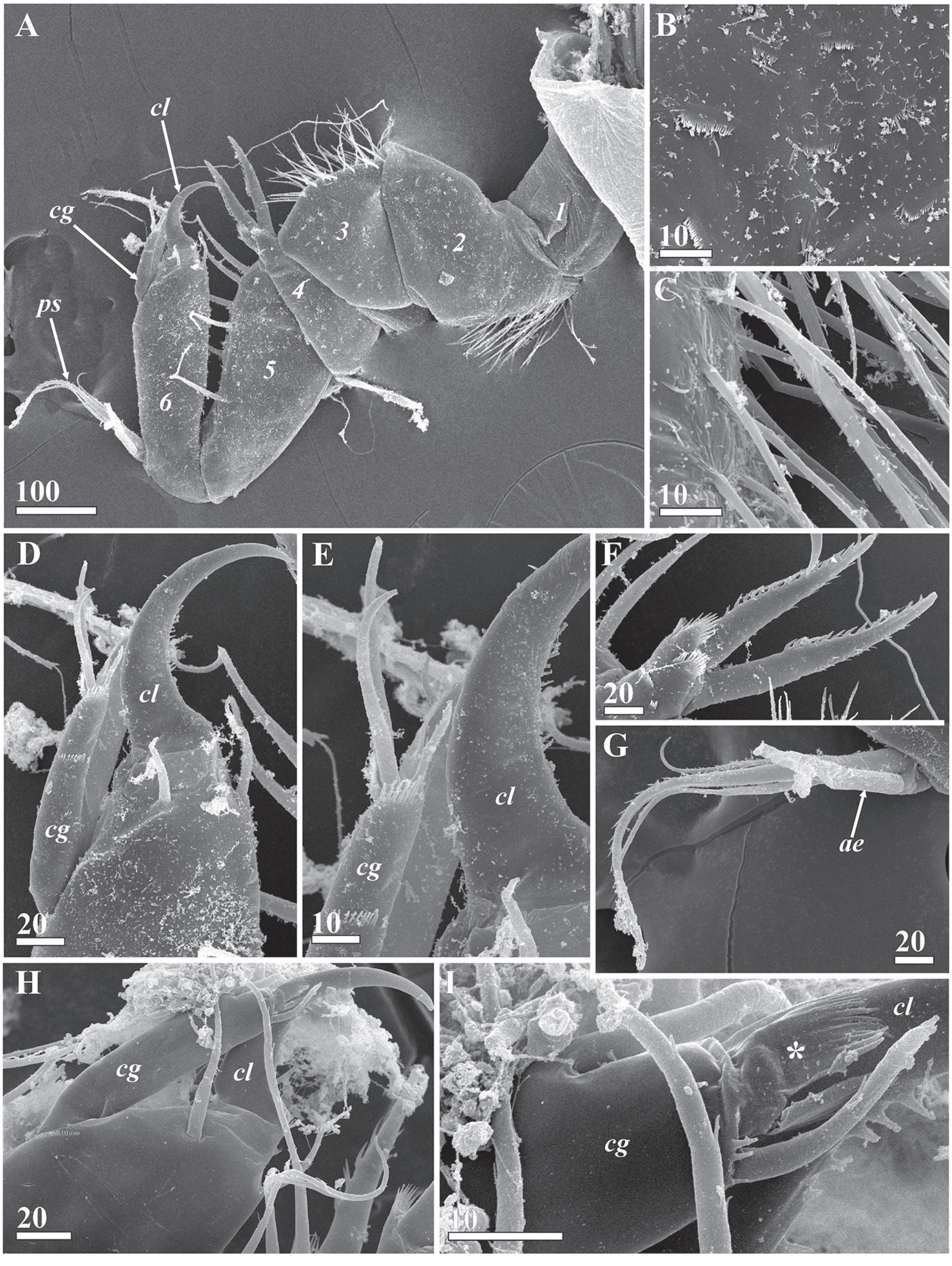

Figure 10. *Synagoga arabesque* sp. nov., female. Morphology of antennules (SEM) **A** right antennule, lateral view, inner surface, segments numbered **B** Ctenoid scales of second segment **C** omniserrate setae on postaxial/ventral surface of second segment **D** Claw sheathed by claw guard, inner side of sixth segment **E** Junction between claw and claw guard showing their microsculpture, inner side **F** Spines of fourth segment forming 'fork' to accept claw of sixth segment **G** proximal sensory process of sixth segment **H** claw sheathed by claw guard, outer side of sixth segment **I** junction between claw and claw guard showing their microsculpture, terminal ctenoid fold of claw guard sheathed claw indicated by asterisk, outer side. Abbreviations: *ae* – aesthetasc, *cg* – claw guard, *cl* – claw, *ps* – proximal sensory process. Scale bars: in µm.

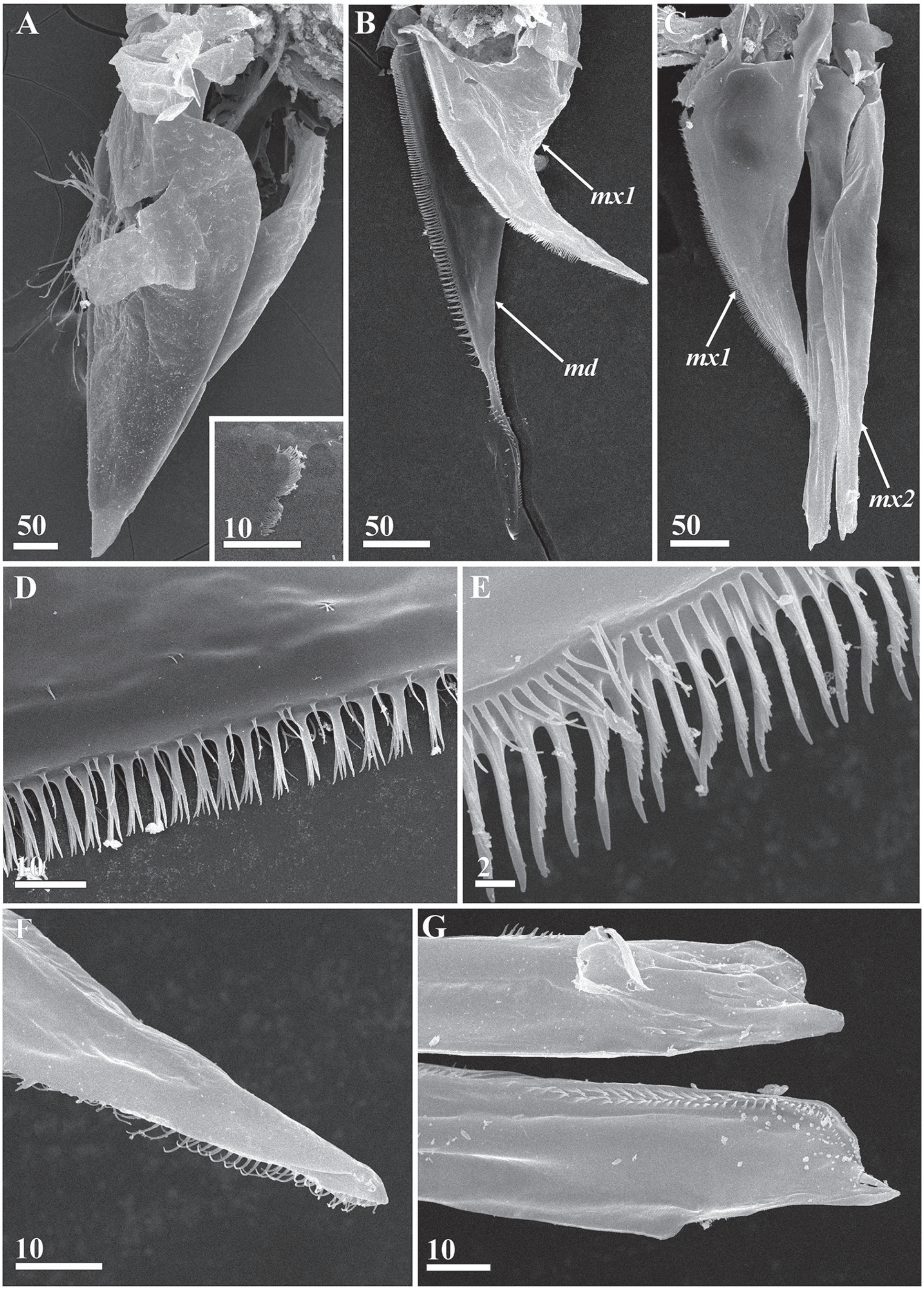

Figure 11. *Synagoga arabesque* sp. nov., female. Mouth parts (SEM) **A** labrum, posterio-lateral view, anterior margin left, enlarged ctenoid scales in rectangle area in lower right angle **B** mandible and maxillule (tip of mandible partially embedded in glue) **C** maxillule and maxillae **D** spines and setae along cutting (posterior) margin of mandible, middle part **E** spines and setiform projections along cutting (posterior) margin of maxillule, middle half **F** tip of maxillule **G** tips of maxillae. Abbreviations: *md* – mandible, *mx1* – maxillule, *mx2* – maxilla. Scale bars: in µm.

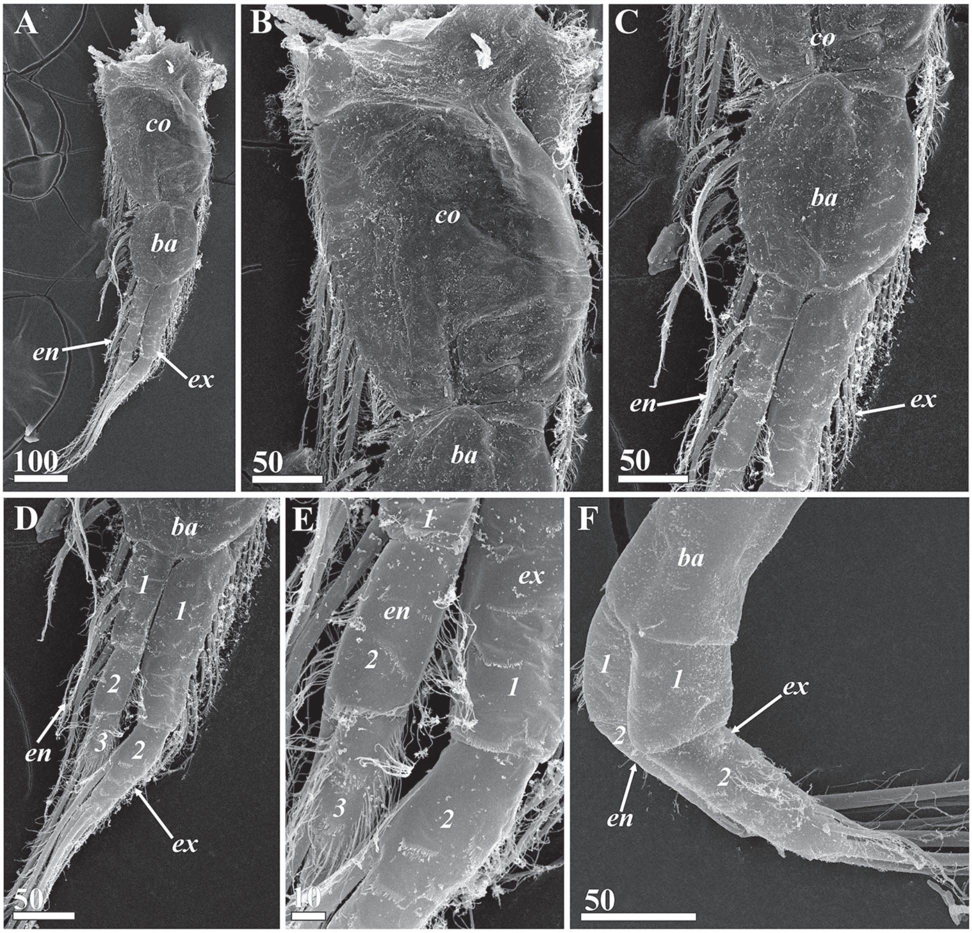

Figure 12. *Synagoga arabesque* sp. nov., female. Thoracopods (**A–E** – left thoracopod II, **F** – left thoracopod VI, SEM) **A** general view **B** surface and setation of coxa **C** surface and setation of basis **D** setation of rami, segments numbered **E** enlarged segments (numbered) of rami showing microsculpture **F** basis and rami, ramal segments numbered. Abbreviations: *ba* – basis, *co* – coxa, *en* – endopod, *ex* – exopod. Scale bars: in µm.

located 5–6 µm from hinge line (Fig. 17B, F). *Lo2* 100 µm behind first pair in female (Fig. 17B) and 80 µm behind in male (Fig. 17F), 16 µm long in female (Fig. 17D) and 10 µm long in male (Fig. 17H), with anterior terminal pore, located 9–10 µm from hinge line (Fig. 17B, D). Posterior lattice organs situated somewhat anterior to point of divergence of carapace valves, near their apices (Figs 17A, E, 18A, E), 530–550 µm behind anterior organs in mature female (Fig. 17A), 370–380 µm behind in male (Fig. 17E). *Lo3* 14–15 µm long in female (Fig. 18B) and 12 µm long in male (Fig. 18F), with anterior terminal pore, located 5–6 µm from hinge line. *Lo4* 25–28 µm behind *lo3* in female and 40–45 µm behind in male (Fig. 18A, E), 17–18 µm long in female (Fig. 18C) and 13–14 µm long in male (Fig. 18G), with posterior terminal pore, located 7–8 µm from hinge line (Fig. 18A). *Lo5* 45–50 µm

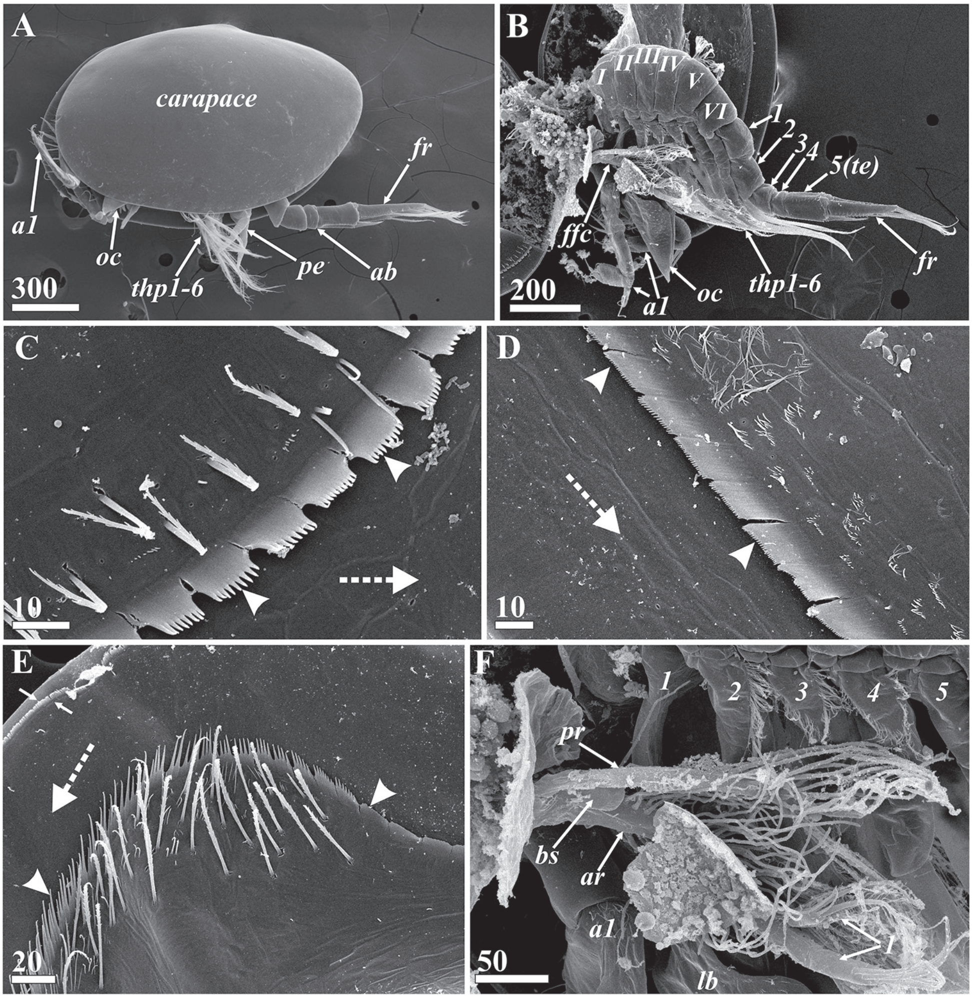

Figure 13. *Synagoga arabesque* sp. nov., male. General morphology, mantle structures (SEM) **A** general view lateral, left side **B** Inner body (prosoma), lateral view (thoracic segments numbered in Roman numerals, abdominal segments in Arabic numerals) **C** submarginal fold of mantle with cuticular fringe (indicated by arrowheads) near anterior margin (anterior direction indicated by dotted arrow) **D** submarginal fold of mantle with cuticular fringe (indicated by arrowheads) in middle part on ventral side of valve of carapace (anterior direction indicated by dotted arrow) **E** submarginal fold of mantle with cuticular fringe and setiform projections (indicated by arrowheads) at posterior end of valve of carapace (thin marginal fold indicated by small arrows, anterior direction indicated by dotted arrow) **F** frontal filament complex (thoracopods numbered). Abbreviations: *a1* – antennules, *ab* – abdomen, *ar* – anterior ramus of frontal filament complex, *bs* – basal ramus of frontal filament complex, *ffc* – frontal filament complex, *fr* – furcal rami, *lb* – labrum, *oc* – oral cone, *pe* – penis, *pr* – posterior ramus of frontal filament complex, *te* – telson, *thp1-6* – thoracopods I–VI. Scale bars: in µm.

behind *lo4* in female and 40 µm behind in male (Fig. 18A, E), 17 µm long in female (Fig. 18D) and 14 µm long in male (Fig. 18H), with posterior terminal pore, located 10–15 µm from hinge line.

Table 2. Main diagnostic characters of species of the genus *Synagoga* (modified from Kolbasov and Newman 2018). The finding of *S. normani* on alcyonarian *Dendronephthya* is questioned, because all other congeners attributed to hosts were found on antipatharians.

Species characters	***S. mira* Norman, 1888**	***S. normani* Grygier, 1983**	***S.* sp. of Grygier 1988**	***S. bisetosa* Grygier, 1990**	***S. millipalus* Grygier & Ohtsuka, 1995**	***S. paucisetosa* Grygier, 1990**	***S. grygieri* Kolbasov & Newman, 2018**	***S. arabesque* sp. nov.**
Location, host, number and size of specimens	Naples, depth unknown, on *Parantipathes larix*, several males, 4×3 mm	Mombasa, 20 m, on *Dendronephthya* (?), single female, 1.73×1.32 mm	off West Australia, in plankton, host unknown, juvenile, 0.79×0.53 mm	outside Gibraltar, ca. 2000 m, host unknown, single immature female, probably male (has male penis), 2.8×2.2 mm	off Okinawa, between 575 m and surface, host unknown, single male, 1.7×1.25 mm	equatorial mid-Atlantic, 3459 m, host unknown, single male, 2.04×1.64 mm	Azores & Cape Verde Is, 20–40 m, on *Antipathella wollastoni*, males & females (app. 3+7), (1.8×1.25mm – male, 2.5×2.0 mm – female)	Green Island, off east Taiwan, 35 m, on *Myriopathes* sp., males & females (6+6), (1.5×0.9 mm – male, 2.3×2.0 mm – female)
shape of 4th segment of a1	Subtriangular (anteriorly acuminate)	Triangular (posteriorly acuminate)	Rectangular	Rectangular	Triangular (posteriorly acuminate)	Triangular (posteriorly acuminate)	Rectangular in male, triangular (posteriorly acuminate) in female	Rectangular in male and female
armament at base of massive setae of 4th segment of a1	Many very short spines	Very short, single spine	Absent	Absent	Single spine	Single spine	Both sexes with several very short spines – ctenoid scales	Both sexes with several very short spines – ctenoid scales
relative sizes of 5th and 6th segments of a1	approximately equal	5th slightly shorter	5th longer	approximately equal	5th shorter	5th shorter	ca. equal	5th shorter
number of setae on anterior margin of 5th segment of a1	15	5	5	10	3	3	9 (female) 8 (male)	6–9 (female) 4–6 (male)
number of setae on 2ndexopodal segment of T1	18	9	11	10	5	5	7 (female) 9 (male)	7 (female) 8 (male)
length of telson spine	Median length	Median length	Median length	Short	Very long	Very long	Median length	Short
number of medial setae on inner face of furcal ramus	~14	5 or 6	3	13	3	3	6	8 (female) 6 (male)
gut diverticula	Low, frilly W-shape without major bifurcations	W-shaped with anterior and posterior arms bi- and tri-furcate respectively	None mentioned	Rounded W	Not observed	Rounded W	W-shaped with numerous branches in females. Males with less elaborate W-shape	W-shaped with numerous branches in females. Males with less elaborate W-shape
position of aesthetasc seta of proximal sensory process of a1	Subbasal?	Subbasal	Basal	Terminal	Basal	Subbasal	Subbasal	Subbasal
number and size of terminal setae on ramus of male penis	?	Inapplicable	Inapplicable	2 tiny	4 long	Few (3?) medium	2 medium	2 medium

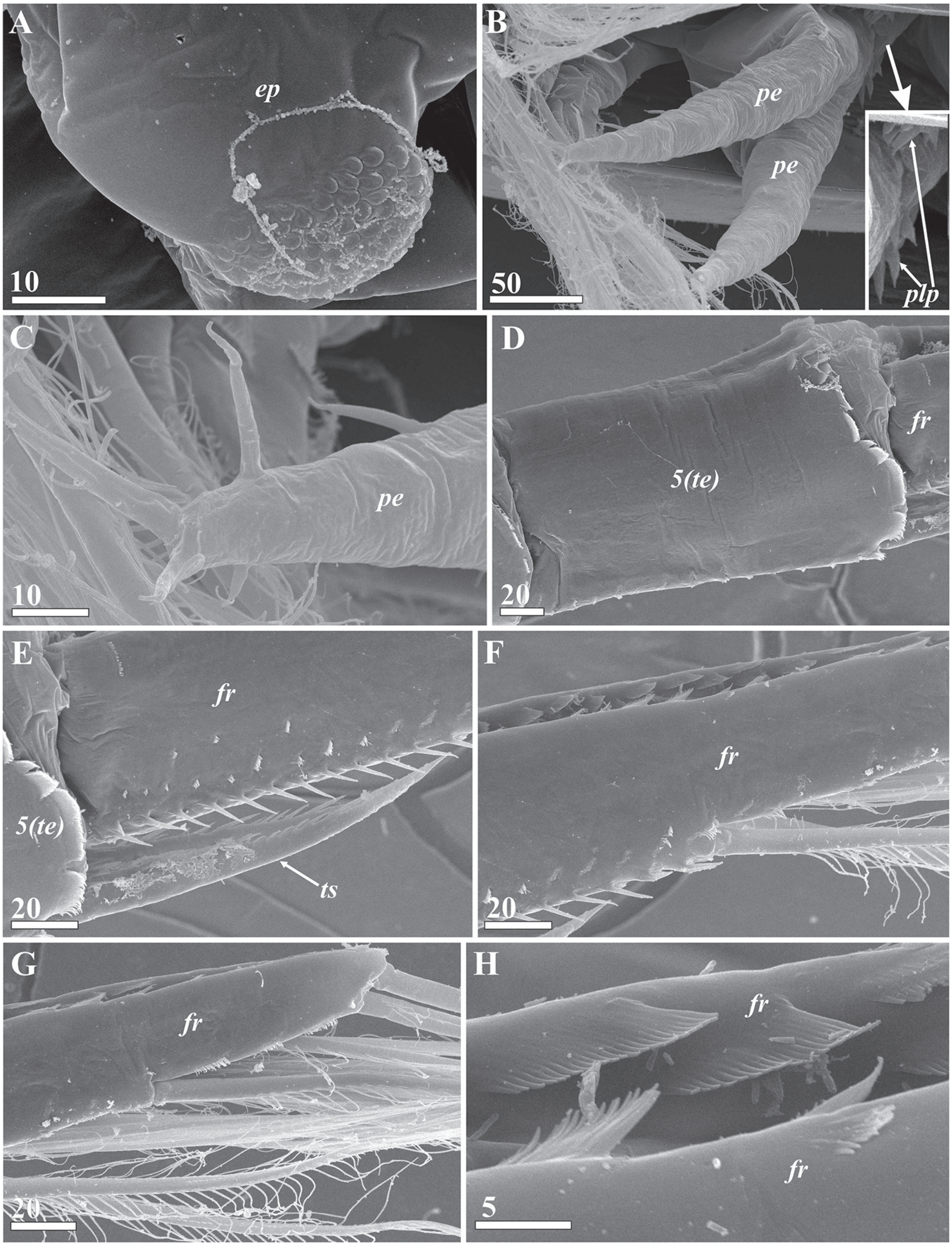

Figure 14. *Synagoga arabesque* sp. nov., male. Morphology of epaulet, penis, telson and furcal rami (SEM) **A** epaulet of thoracic segment 6 **B** rami of penis; enlarged terminal parts of pleural processes of first abdominal segment in rectangle area **C** tip of ramus of penis **D** telson, lateral side **E** telson spines and base of furcal ramus **F** middle parts of furcal rami **G** terminal parts of furcal rami **H** ctenoid scales on dorsal sides of furcal rami. Abbreviations: *ep* – epaulet, *fr* – furcal rami, *pe* – penis, *plp* – pleural process of first abdominal segment, *te* – telson, *ts* – telson spines. Scale bars: in µm.

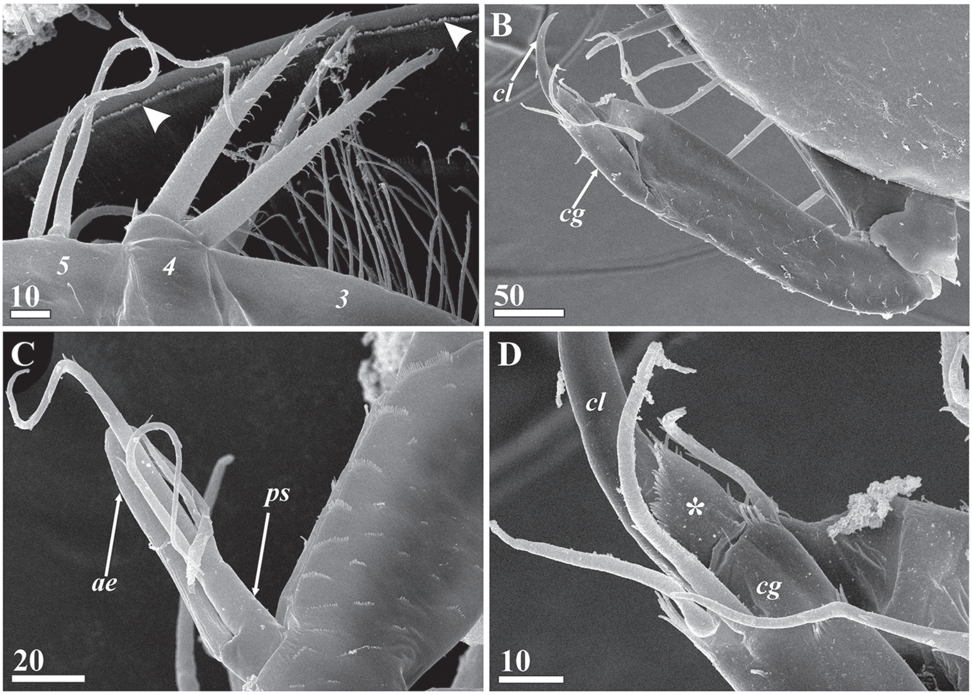

Figure 15. *Synagoga arabesque* sp. nov., male. Morphology of antennules (SEM) **A** spines of fourth segment forming 'fork' to accept claw of sixth segment (antennular segments numbered, marginal fold of mantle indicated by arrowheads) **B** sixth segment, left antennules, outer surface **C** proximal sensory process of sixth segment **D** junction between claw and claw guard showing their microsculpture, terminal ctenoid fold of claw guard sheathed claw indicated by asterisk, outer side. Abbreviations: *ae* – aesthetasc, *cg* – claw guard; *cl* – claw; *ps* – proximal sensory process. Scale bars: in µm.

Comparison. Having both sexes of *S. arabesque* available makes it possible to compare this species with all other described species of *Synagoga*. The main characters used for comparison are given in Table 2. Owing to a lack of detailed description, no meaningful comparison with the juvenile "McKenzie's larva" from the eastern Indian Ocean (cf. Grygier 1988) can be made. Only one species, *S. millipalus* represented by a single male, found in the Pacific Ocean off Okinawa, Japan. It differs in having fewer setae on the fifth antennular segment (three instead of four-six) and on the inner side of the furcal ramus (three instead of six), and also relatively longer telson spines (Grygier and Ohtsuka 1995). Only a single species, *S. normani* (based on a female), is known from the western Indian Ocean (Grygier 1983a). It has fewer setae on the fifth antennular segment (five instead of 6–9) and on the inner side of the furcal ramus (five or six instead of eight), and more setae on the second exopodal segment of thoracopod I (nine instead of seven). Four species inhabit the Atlantic and adjacent seas, these are *S. mira*, *S. bisetosa*, *S. paucisetosa* and *S. grygieri* (Norman 1888; Grygier 1983a, 1990a; Kolbasov and Newman 2018). The new species differs from *S. mira* (Norman 1888; Grygier

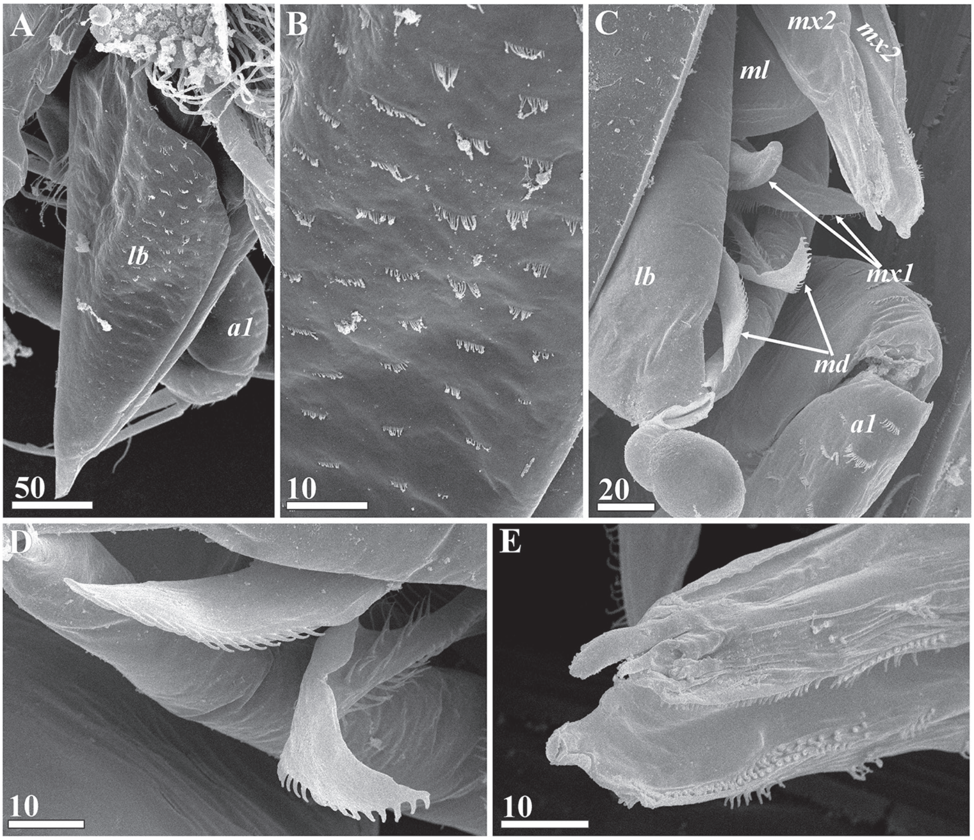

Figure 16. *Synagoga arabesque* sp. nov., male. Mouth parts (SEM) **A** labrum, posterio-lateral view, anterior margin left **B** lateral surface of labrum **C** distal part of oral cone with exposed tips of mouth parts **D** tips of mandibles **E** tips of maxillae. Abbreviations: *a1* – antennules, *lb* – labrum, *md* – mandible, *ml* – medial languette, *mx1* – maxillule, *mx2* – maxilla. Scale bars: in μm.

1983a) by having smooth, unscalloped edges of the gut diverticula, fewer setae on the fifth antennular segment (4–9 instead of 15), the second exopodal segment of thoracopod I (seven(eight) instead of 18) and the inner side of the furcal ramus (eight(six) instead of 14). It can be distinguished from *S. bisetosa* (Grygier 1990a) by having fewer setae on the fifth antennular segment (four-nine instead of ten), the second exopodal segment of thoracopod I (seven(eight) instead of ten) and the inner side of the furcal ramus (eight(six) instead of 13). The new species differs from *S. paucisetosa* (Grygier 1990a) in having more setae on the fifth antennular segment (four-nine instead of three) and the inner side of the furcal ramus (eight(six) instead of three); it also has relatively shorter telson spines. Finally, it can be distinguished from *S. grygieri* (Kolbasov and Newman 2018) by fewer setae on the fifth antennular segment of males (four to six instead of eight) and more setae on the inner side of the furcal ramus of females (eight instead of six); it also has relatively shorter fifth antennular segment and telson spines.

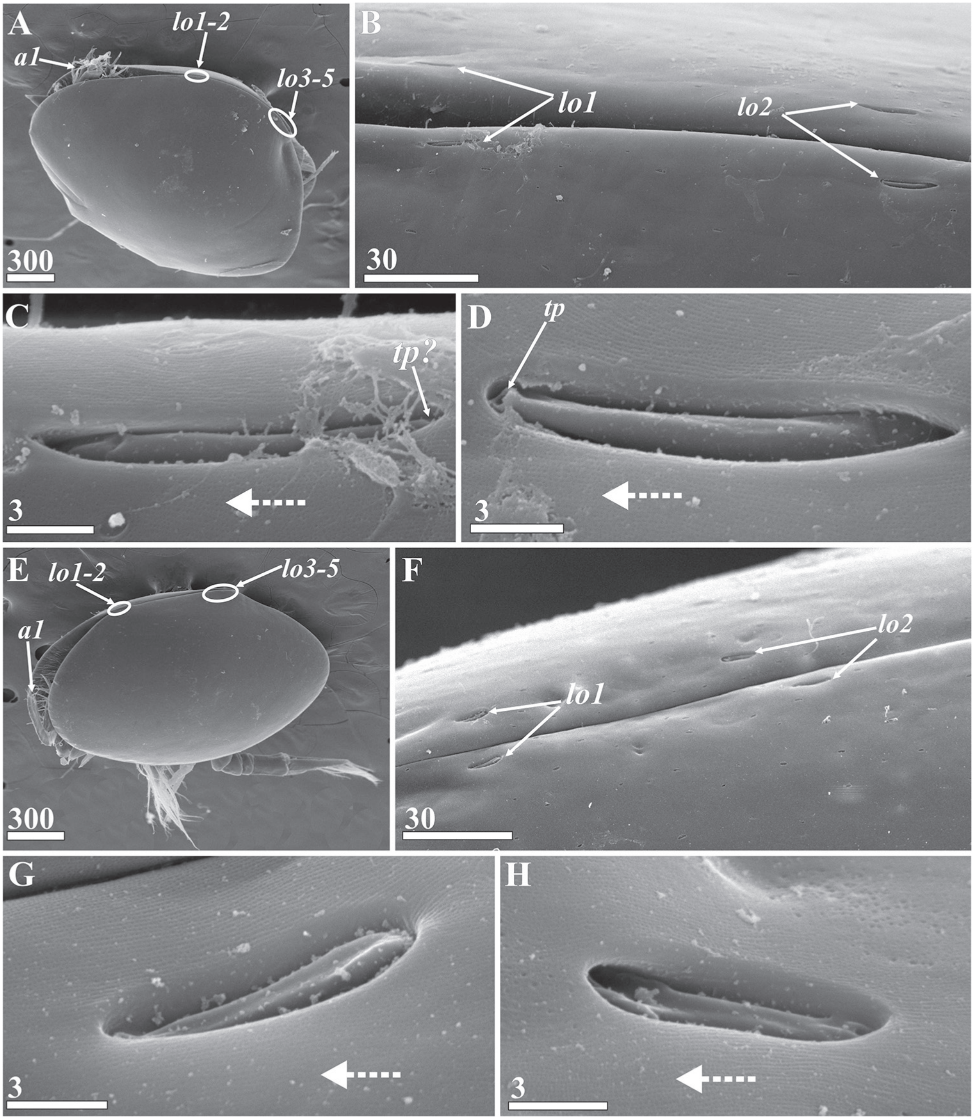

Figure 17. *Synagoga arabesque* sp. nov. Lattice organs, with dotted arrows indicating anterior direction (SEM) **A–D** female **E–H** male **A, E** general view, dorsolateral view, locations on carapace of anterior (1, 2) and posterior (3–5) pairs of lattice organs indicated by oval outlines **B, F** anterior lattice organs (1, 2) **C, G** left lattice organs 1 (first pair) **D, H** left and right lattice organs 2 (second pair). Abbreviations: *a1* – antennules, *lo1-5* – lattice organs, *tp* – terminal pore of lattice organ. Scale bars: in µm.

Discussion

Morphology of both sexes including external ultrastructure, as well as sexuality, host specificity, and biogeography of the genus *Synagoga* have been recently discussed in detail (Kolbasov and Newman 2018). In the present study we are providing new data on the structure of the lattice organs and anterior sensory pits of carapace, host specificity

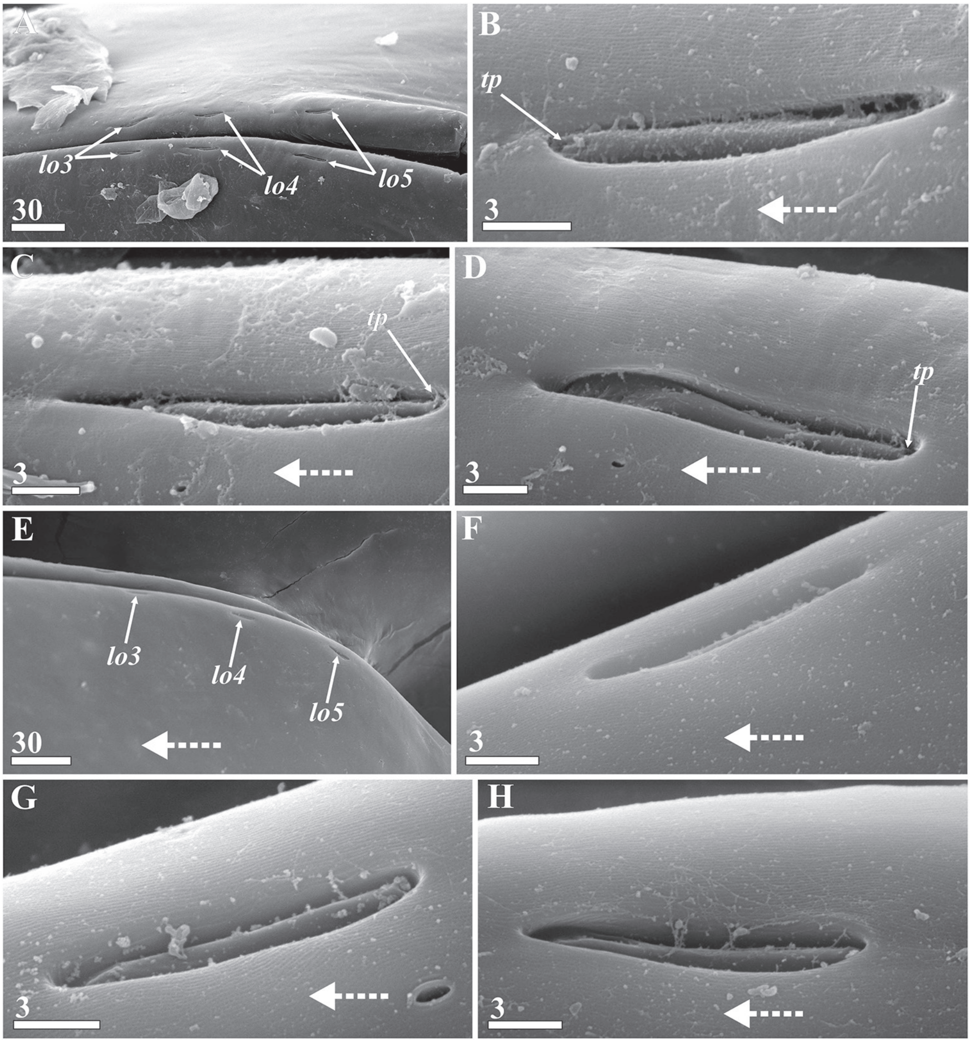

Figure 18. *Synagoga arabesque* sp. nov. Posterior (3–5) pairs of lattice organs, with dotted arrows indicating anterior direction (SEM) **A–D** female **E–H** male **A, E** posterior pairs (3–5) of lattice organs **B, F** left lattice organs 3 (third pair) **C, G** left lattice organs 4 (fourth pair) **D, H** left lattice organs 5 (fifth pair). Abbreviations: *lo3-5* lattice organs, *tp* – terminal pore of lattice organ. Scale bars: in µm.

and biogeography of *Synagoga*. Developed anterior sensory pits (Figs 1A, 5A, B, 8E, F) are found on the inner side of valves in adult stages of both sexes of genera *Synagoga* and *Sessiligoga* (Grygier 1990b; Grygier and Ohtsuka1995; Kolbasov and Newman 2018; unpublished data). They are considered as possibly homologous to the pair of large anterio-ventral pores found externally on the ventral faces of the carapace valves of both sexes of both species of *Waginella*, *Waginella sandersi* (Newman 1974) and *Waginella metacrinicola* (Okada 1926), as well as two undescribed species of this genus (Newman 1974; Grygier 1990c; Itô and Grygier 1990; unpublished data). A chemosensory function was putatively proposed for these structures (Kolbasov and Newman

2018). Small pores and conspicuous volcano-shaped papillae observed on the surface of the canal of these pits in *S. arabesque* sp. nov. (Fig. 8E, F) may also be evidence in favor of chemosensory function.

In adults of both sexes of *S. grygieri* and *Synagoga arabesque* sp. nov. and the male of *S. millipalus*, all five pairs of lattice organs are situated co-linearly along the hinge line of the carapace valves, i.e., parallel to the hinge. A fully co-linear arrangement of the lattice organs has been considered plesiomorphic for ascothoracidans and also for all thecostracans (Jensen et al. 1994; Høeg and Kolbasov 2002; Celis et al. 2008; Kolbasov and Newman 2018). Apart from both *S. grygieri* and *S. millipalus* having the anterior terminal pore in *lo1* and posterior terminal pore in *lo2*, the new species has the posterior terminal pore in *lo1* and the anterior terminal pore in *lo2*. Thus, only posterior pairs of lattice organs (*lo3*, *lo4*, *lo5*) share the same position of terminal pores in the studied species of the genera *Synagoga* and *Sessilogoga* (Grygier and Ohtsuka 1995; Kolbasov and Newman 2018; herein; unpublished data). Species of both *Synagoga* and *Sessilogoga* share anterior terminal pores in *lo3* and posterior terminal pores in *lo4* and *lo5*. This is opposite to the condition in most thecostracans, which have a posterior terminal pore in *lo3* (e.g., Jensen et al. 1994; Kolbasov et al. 1999; Høeg et al. 2004; Celis et al. 2008), and thus represents a potential synapomorphy of these two genera (unpublished data). The different position of terminal pores of the lattice organs even within congeners (terminal pores of anterior lattice organs in *Synagoga*) shown here for the first time might be evidence that the configuration of lattice organs in ascothoracidans is not constant, at least in adult stages.

Four of the seven described species of *Synagoga* are attributed to particular hosts (Table 2) and three of them (*S. mira*, *S. grygieri*, and *Synagoga arabesque* sp. nov) were found on antipatharians. This may be evidence of the host specificity of *Synagoga* as exclusive ectoparasites or small predators of black corals. Therefore, we consider the attribution of *S. normani* to the alcyonarian host *Dendronephthya* as a possible misinterpretation. Grygier (1983a) described a single isolated female of *S. normani* 'collected by P. Hutchence from alcyonacean coral, *Dendronephthya* sp.' in Mombasa harbor and forwarded to him. We suspect that this record *Dendronephthya* may be of a non-specific substrate rather than an actual specific host for this species.

Synagoga arabesque sp. nov. is the second species of the genus found in the north part of the west Pacific after *S. millipallus*. Despite this fact, the new species resembles *S. grygieri* recently described from the Atlantic Ocean, Macaronesia (Kolbasov and Newman 2018; Table 2 herein) in many details. This may indicate that both *Synagoga arabesque* sp. nov. and *S. grygieri* evolved from a common ancestor and that the genus *Synagoga* exhibits the major Tethyan reliction pattern that is also characteristic of some ascothoracidans and barnacles (Newman and Ross 1971; Newman and Tomlinson 1974; Foster 1981; Kolbasov 2009; Kolbasov et al. 2015). Currently, studies of diversity of Ascothoracida are still based mainly on morphological approaches, future directions can involve molecular techniques to examine cryptic diversity and population genetics of Ascothoracida (see approaches in Chai et al. 2017; Chang et al. 2017; Ma et al. 2019; Jung et al. 2018)

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Six years of fruit fly surveys in Bangladesh: a new species, 33 new country records and discovery of the highly invasive *Bactrocera carambolae* (Diptera, Tephritidae)

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Abstract

We engaged in six years of snap-shot surveys for fruit flies in rural environments and ten protected forest areas of Bangladesh, using traps baited with male lures (cue-lure, methyl eugenol, zingerone). Our work has increased the recorded number of species of Tephritidae in the country from seven to 37. We summarize these surveys and report eight new country occurrence records, and a new species (*Zeugodacus madhupuri* Leblanc & Doorenweerd, **sp. nov.**) is described. The highlight among the new records is the discovery, and significant westward range extension, of *Bactrocera carambolae* Drew & Hancock, a major fruit pest detected in the Chattogram and Sylhet Divisions. We rectify the previously published erroneous record of *Bactrocera bogorensis* (Hardy), which was based on a misidentification of *Zeugodacus diaphorus* (Hendel). We also report the occurrence in Bangladesh of nine other Tephritidae, the rearing of three primary fruit fly parasitoids from *Zeugodacus*, and records of non-target attraction to fruit fly lures.

Keywords

Dacini, Indian subcontinent, pest species, range extension, taxonomy

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Introduction

The Dacini is a very diverse group of fruit flies, with 939 described species, including 83 pests of cultivated fruit and cucurbits (e.g., Doorenweerd et al. 2018). Of these, 118 are known to occur on the Indian subcontinent (David and Ramani 2011; Drew and Romig 2013; David et al. 2016, 2017; Leblanc et al. 2018b). Fruit fly surveys in rural environments of Bangladesh, initiated in 2013, increased the published number of known species from seven to 27 (Leblanc et al. 2013, 2014; Hossain and Khan 2013; Khan et al. 2015, 2017). While Drew and Romig (2013) could not confirm the presence of *Bactrocera dorsalis* (Hendel) on the Indian subcontinent, variation in color pattern and preliminary molecular data from Bangladesh and African populations suggested that *B. dorsalis* is widespread on the subcontinent and that the species described as *B. invadens* Drew, Tsuruta & White is conspecific with *B. dorsalis* (Leblanc et al. 2013). That same year, *B. philippinensis* Drew & Hancock was declared a synonym of *B. papayae* Drew & Hancock (Drew and Romig 2013). Soon after *B. papayae*, along with *B. invadens*, were declared conspecific with *B. dorsalis*, with formal designation of synonyms (Schutze et al. 2015a, 2015b), leaving *B. carambolae* Drew & Hancock as a distinct species, based on genetic differences, morphological differences in aedeagus, wing shape and color pattern, non-random assortative mating with *B. dorsalis*, and significant differences in pheromone composition (Wee and Tan 2007; Schutze et al. 2012, 2013, 2015b; Tan et al. 2013). With this revised status, *B. dorsalis* is now widespread across tropical Asia, and introduced to most of Africa and several islands in the Pacific, while *B. carambolae* has been restricted to a smaller range in South-East Asia (Fig. 1) and introduced to South America. To generate a complete inventory of the economic species and assess the diversity of fruit flies in the protected forest areas of Bangladesh, we surveyed for fruit flies during 2013–2018 with a focus on rural areas and report here cumulative results from these surveys, focusing on previously unpublished new records. Using a morphological and molecular approach, we discovered numerous new country records, including the highly invasive *B. carambolae*, and a new species of *Zeugodacus* Hendel is described here.

Material and methods

Collecting and curation

Starting in 2013, we periodically maintained a series of traps (described in Leblanc et al. 2015a) separately baited with male lures plus a 10×10 mm piece of dichlorvos (DVVP) insecticide strip to kill trapped flies. Cue-lure and methyl eugenol were included as commercially available plugs (Scentry Biologicals, Billings, Montana) whereas zingerone lure, also used in the surveys since 2016, was prepared by dipping dental cotton wicks in zingerone (= vanillylacetone) (Sigma-Aldrich) melted over a hot plate and allowed to solidify in the wicks. We deployed traps at 383 sites through-

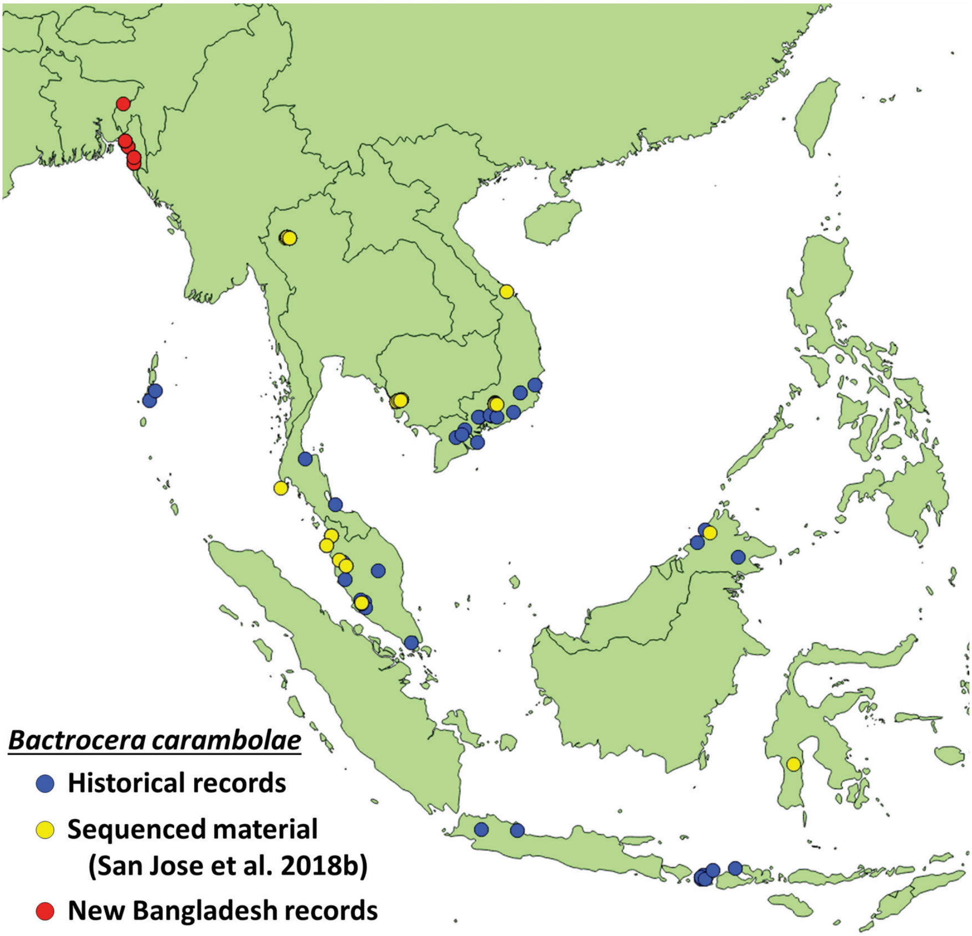


Figure 1. Distribution of *Bactrocera dorsalis* and *B. carambolae* in Asia, including the new records of *B. carambolae* in Bangladesh and range expansion recorded in San Jose et al. (2018b).

out the country for periods ranging from one to 14 days, either as individual sites scattered over rural areas or as series of 11–26 sites, about 50 m apart, concentrated in selected rural areas and in 10 different protected forest areas (Nishorgo Support Project 2007) (Fig. 2, Table 1). Sampled flies were stored in 95% ethanol in a -20 °C freezer, to preserve DNA for analysis. All flies were identified by the first three authors, using available keys (Drew and Romig 2013, 2016). Before drying flies for double-mounting, we pinned them through the scutum with a minuten pin and soaked them in ethyl-ether for 3–12 hours to fix and preserve their natural coloration. We photographed specimens using a Nikon D7100 camera attached to an Olympus SZX10 microscope and used Helicon Focus Pro ver. 6.7.1 to merge pictures taken at a range of focal planes. To measure specimens, we used an ocular grid mounted on an Olympus SZ30 dissecting microscope.

Table 1. Checklist and distribution of Dacine fruit flies of Bangladesh, including previously known species, new country occurrence records, and number of specimens collected in the surveys (2013–2018) reported in this paper.

Taxa	Male Lure	Barishal	Chattogram							Dhaka			Khulna	Mymensingh	Rajshahi	Rangpur	Sylhet		
		Rural sites (6)	Chimbuk Hills (25)	Chunati (26)	Fashiakali (15)	Kaptai (24)	Sita-Kunda (23)	Tesara Range (25)	Rural sites (46)	Bhawal (15)	Madhu-pur (15)	Rural sites (49)	Rural sites (25)	Rural sites (7)	Rural sites (16)	Rural sites (13)	Lawacho-ra (22)	Rema-Kalenga (11)	Rural sites (20)
Bactrocera																			
B. abbreviata (Hardy) *	ZN								1										
B. bhutaniae Drew & Romig [7]	CL		5																4
B. carambolae (Drew & Hancock) * [1]	ME [5]			38	95		25		8									1	
B. correcta (Bezzi) [1]	ME				1				563			3							
B. digressa Radhakrishnan	CL, ZN									3		1			10				
B. dorsalis (Hendel) [1, 5]	ME [5]	19	219	552	548	150	172	216	2811	516	567	2373	825	375	2414	684	50	56	330
B. latifrons (Hendel) [2]	Latilure [6]											11							
B. nigrifacia Zhang, Chi & Chen	CL	1	1			3	1					28	9	3	2	16			3
B. nigrofemoralis White & Tsuruta	CL									1									
B. pendleburyi (Perkins) *	ZN			2	3		3												
B. propinqua (Hardy & Adachi)	CL		3	6	5	2	6	4		5	1	1							3
B. rubigina (Wang & Zhao)	CL, ZN		28	953	658	11	664	50	56	711		106				63	80	73	29
B. syzygii White & Tsuruta *	ZN		35	20	31	2	102	11	16			2							2
B. tuberculata (Bezzi) [1]	ME		1	1	10		4		3			13							4
B. zonata (Saunders) [1]	ME			2			1		1	2		48	63	2	69	11			1
Dacus																			
D. ciliatus Loew [3]																			
D. longicornis (Wiedemann) [3]	CL	3			1	5	12	9	25	16	1	71	10	1	23	7		1	40
Zeugodacus																			
Z. apicalis (de Meijere) *	CL								1										
Z. atrifacies (Perkins) *	CL						23												
Z. caudatus (Fabricius) [4]	CL							4			9	1		2	45	33			
Z. cilifer (Hendel)	CL							1				1							
Z. cucurbitae (Coquillett) [3]	CL, ZN	141	5	13	10	2	83	64	550	8	14	982	308	198	334	236			67
Z. diaphorus (Hendel) * [8]	CL													1	3	8			
Z. diversus (Coquillett) [3]	ME [6]											40							9
Z. hochii (Zia) [3]	CL [5]			1					1		9							2	
Z. incisus (Walker) *	CL			4	32		29	1				1				1			
Z. infestus (Enderlein) *	CL						1												
Z. tau (Walker) [3]	CL	273	434	30	11	10	33	69	383	15	115	672	143	191	82	169	1	1	103
Z. madhupuri Leblanc & Doorenweerd **	CL										4								
NEUROPTERA: CHRYSOPIDAE																			
Ankylopteryx anomala (Brauer) *	ME		5	14	13	251	53	88	1	2	5016	2					77	103	1

* New country record for Bangladesh. ** New species described in this paper. [1] Polyphagous fruit pest. [2] Oligophagous fruit pest. [3] Cucurbit fruit pest. [4] Cucurbit flower pest. [5] Zingerone attraction also known, but not recorded in Bangladesh. [6] Bangladesh specimens cited here collected by hand. [7] Previously reported as *Bactrocera* sp (possibly *B. bhutaniae*). [8] Previously reported as *Bactrocera* species 45 (likely *B. propinqua*). [8] Erroneously recorded as *Bactrocera bogorensis* in Leblanc et al. (2014).

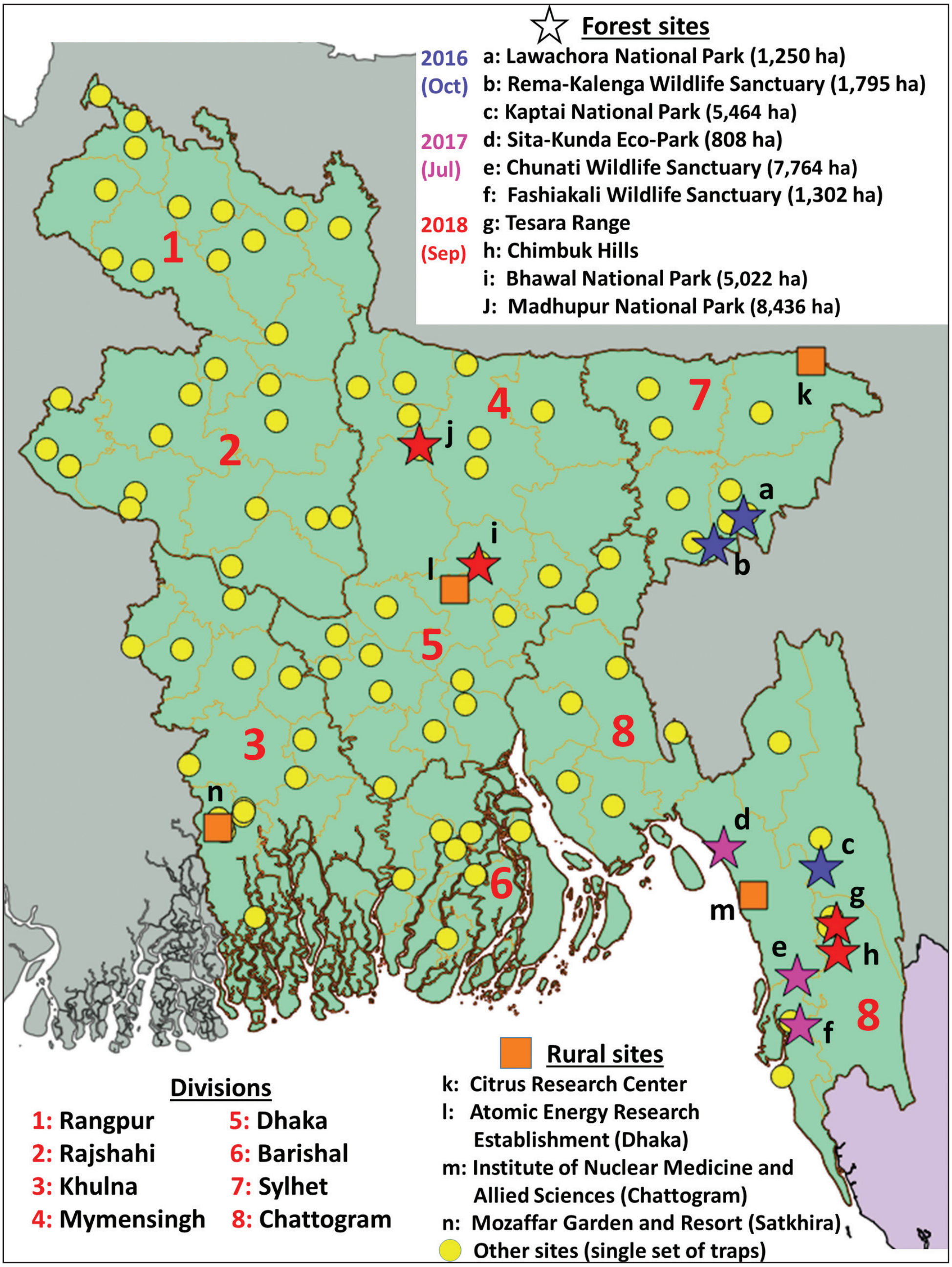


Figure 2. Trapping locations in the various Bangladesh surveys (2013–2018).

We also reared parasitoids and hyperparasitoids from readily available, heavily fly-infested snake gourd (*Trichosanthes cucumerina*) collected at the AERE campus (Dhaka). Infested gourds were weighed and placed on a cloth-covered small bowl (to collect excess juice from decay), over moist sawdust (as a pupation media) in a fine nylon netted cage. Pupae were separated from the sawdust using fine-meshed sieve and placed in a petri dish inside a very fine-netted plastic cage to collect emerged fruit flies and parasitoids.

Morphological terms and taxonomic assignment

Morphological terminology used in the descriptions follows White (1999) and assignment of species to genera follows Doorenweerd et al. (2018). The genus *Zeugodacus*, of which a new species is described in this paper, is treated as separate from *Bactrocera* Macquart and *Dacus* Fabricius, based on recent molecular-based phylogenetic assessments (Krosch et al. 2012; Virgilio et al. 2015; Dupuis et al. 2017; San Jose et al. 2018a). Despite recent efforts to reassign species to subgenera (e.g., Hancock and Drew 2018 a, b), the understanding of higher relationships of species within Dacini is still in state of flux, and a number of traditionally recognized subgenera and species complexes (Drew and Romig 2013) are demonstrated to be polyphyletic groups of convenience defined on the basis of highly homoplastic morphological characters and male lure relations (e.g., Leblanc et al. 2015b; San Jose et al. 2018a; Catullo et al. 2019). For this reason, we have not attempted to include subgenera in the country's species list.

DNA extraction, PCR and sequencing

Methods for DNA extraction, PCR primers and conditions, and Sanger sequencing follow those of San Jose et al. (2018a). We attempted to amplify and sequence regions of the Cytochrome C Oxidase I (COI) and Elongation Factor 1-alpha (EF1-alpha) genes. It has previously been shown that COI cannot be used to differentiate *Bactrocera dorsalis* from *B. carambolae* (San Jose et al. 2018b). However, we found that there are five diagnostic single nucleotide polymorphisms (SNP's) that separate *B. dorsalis* from *B. carambolae* in the 762 base-pair (bp) fragment of EF1-alpha that we used for multimarker phylogenetic studies (San Jose et al. 2018a). We therefore sequenced this segment to confirm or refute the identity of *B. carambolae*. For *Zeugodacus madhupuri*, we attempted to amplify a large section of 1540 bp of COI as well as EF1-alpha, but we only successfully amplified the COI-3P region. Amplified regions of COI-5P proved to be nuclear pseudogenes after sequencing (data not shown), and EF1-alpha did not yield any PCR product, possibly due to degradation of the template DNA. We aligned newly generated sequences with the published data of EF1-alpha or COI, respectively, from San Jose et al. (2018a) and performed maximum likelihood analyses using IQTree (Nguyen et al. 2015). We allowed IQTree to determine the substitution model via its integrated modeltest and ran a standard maximum likelihood analyses with 1000 ultrafast bootstraps and 1000 Sh-aLRT bootstraps. We consider branches with support values >95% for ultrafast bootstraps and >80% for Sh-aLRT bootstraps as well supported. Resulting trees were optimized for publication using FigTree 1.4.3 and Adobe Illustrator. Data from this study are available from the BOLDSYSTEMS Digital Repository: https://doi.org/10.5883/DS-BANG01.

Estimating biodiversity

We used EstimateS software (Colwell 2013) to generate species accumulation curves. We estimated species diversity with the incidence-based Chao 2 algorithm, which does not include abundance in its extrapolation, thereby avoiding abundance bias in our data related to how strongly each species is attracted the lures and controlling for the predominance of a few agricultural pests in the samples. Diversity estimations were done comparing forest and rural sites, and the individual protected forest areas separately, with 100 randomizations without replacement for confidence intervals. It is understood that diversity estimates are underestimations, because they are based solely on species attracted to the male lures used in our sampling.

Abbreviations

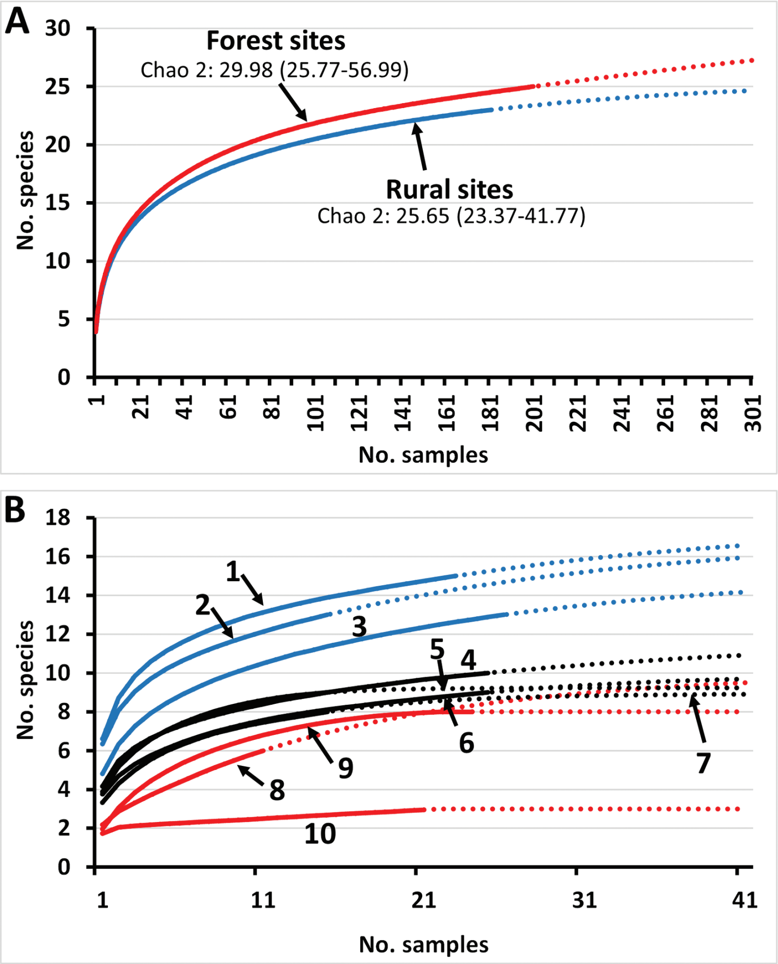
AERE	Atomic Energy Research Establishment, Dhaka, Bangladesh
UHIM	University of Hawaii Insect Museum, Honolulu, HI, USA
USDA	United States Department of Agriculture
WFBM	William F. Barr Entomological Museum, University of Idaho, Moscow, ID, USA

Results

Between April 2013 and September 2018, we collected a total of 23,939 specimens of Dacine fruit flies, representing 29 species (Table 1), among 1012 samples (372 cue-lure, 357 methyl eugenol, 271 zingerone; and a few others hand-collected or bred from fruit) across 383 sites (Fig. 2). We report a number of new country occurrence records, including a major pest species in need of management attention (*Bactrocera carambolae*) and describe a new species, increasing the number of species of Tephritidae in Bangladesh from 27 (Leblanc et al. 2013, 2014; Hossain and Khan 2013; Khan et al. 2015, 2017) to 37 (29 Dacini and eight from other tribes).

Biodiversity and species accumulation curves

Rural sites were dominated by three pest species: *Bactrocera dorsalis* (61.3% of specimens captured), *Zeugodacus cucurbitae* (Coquillett) (17.5%), and *Z. tau* (Walker) (12.6%). Forest sites also yielded large numbers of *B. dorsalis* (39.1%), as well as the non-economically important *B. rubigina* (Wang & Zhao) (41.4%), whereas cucurbit pests were less common (2.6% *Z. cucurbitae* and 9.2% *Z. tau*). The Chao 2 algorithm estimated overall number of species is 30.0 in forest sites and 25.7 in rural sites (Fig. 3A). Among the surveyed protected forests (Fig. 3B), the highest diver-



	Sites	Chao 2 mean (and 95% CI)		Sites	Chao 2 mean (and 95% CI)
1	Sita-Kunda	17.87 (15.34-39.47)	6	Chimbuk Hills	9.96 (9.07-22.46)
2	Fashiakhali	17.2 (13.46-51.34)	7	Madhupur	8.93 (8.07-21.06)
3	Chunati	15.16 (13.25-31.4)	8	Rema-Kalenga	10.09 (6.45-43.4)
4	Tesara Range	11.92 (10.17-31.2)	9	Kaptai	8 (8-9.99)
5	Bhawal	9 (9.44-10.1)	10	Lawachora	3 (3-4.48)

Figure 3. Species accumulation curves for species collected in the rural and forest sites through the whole sampling period (2013–2018) (**A**) and for the individually surveyed protected forest areas (**B**). Estimates of species numbers based on the Chao 2 estimator, with the 95% confidence interval ranges). Data used to generate these curves and estimates include two species not yet definitely identified and not included on Table 1.

sity was collected in three locations in the Chattogram District: Sita-Kunda Eco-Park, (15 species), Fashiakali Wildlife Sanctuary (12 species) and Chunati Wildlife Sanctuary (12 species), with the estimated Chao 2 number of species ranging from 15 to 18. Most other sites had a moderate diversity of 6–10 species, with estimated numbers of

8–12 species. For unclear reasons, only three species were collected in Lawachora National Park, possibly due to trapping done during the tail end of the rainy season (October 2016) and/or its relatively small size and geographic isolation from other forested areas within a densely populated environment dominated by agriculture. Paradoxically, the smallest sampled protected area, Sita-Kunda Eco-Park (808 ha) yielded the highest species diversity. The estimated total number of species in Bangladesh, based on the Chao 2 algorithm from a species accumulation curve including all sites, is 37.5 species, relatively few compared to the 118 species known to occur in the Indian subcontinent. A higher estimate might have been attained had species not attracted to lures been more actively collected and had access to the Chattogram Hills tracts forests not been severely restricted due to security concerns.

Bactrocera carambolae new to Bangladesh

We collected 167 specimens of *B. carambolae* among 55 methyl eugenol samples, mostly in protected forest sites in the Chattogram District (Table 1, Fig. 2). One specimen morphologically consistent with *B. carambolae* was collected further north, in the Rema-Kalenga Wildlife Sanctuary (Sylhet Division) (UHIM molecular voucher ms07278), but its identity could not be confirmed molecularly because the amplification of EF1-alpha failed repeatedly, possibly due to degradation. All specimens are morphologically consistent with the diagnostic features of *B. carambolae*: subapical spots on fore femora, costal band slightly overlapping and expanded beyond apex of R_{2+3}, presence of narrow transverse black band across anterior margin of tergum III, widening to cover lateral margins (Drew and Hancock 1994; Drew and Romig 2013) (Fig. 4). Species identity of a selection of nine specimens was further confirmed through sequencing of a region of EF1-alpha with five diagnostic SNP's that differentiate *B. carambolae* from *B. dorsalis* (Genbank accession numbers MG683467, MG683640, MN413902–MN413909, MN418232–MN418240; Supplementary material 1: Figure S1). The discovery of *B. carambolae* in Bangladesh is a significant westward extension of the known distribution (Fig. 1) of this polyphagous fruit pest (>74 known fruit hosts in 26 families in Asia (Allwood et al. 1999)), until recently known to occur from southern portions of Vietnam, Thailand and Cambodia, Peninsular Malaysia, Java, Borneo, and south to Lombok (Indonesia), as well Andaman Island and South America (introduced in 1975). The origin of this pest species in forest habitats in south-eastern Bangladesh is enigmatic. It may reflect a relatively recent introduction, not detected during the 2013–2015 surveys which included agricultural environments. Alternatively, it may represent the extreme natural western range of its populations, possibly in expansion, if the species is demonstrated to be widespread across southern Myanmar. Recently, *B. carambolae* was demonstrated to occur in northwestern Thailand (Fig. 1) (San Jose et al. 2018b). Clearly, additional surveys to delimitate the range of this invasive pest in Bangladesh and Myanmar, focusing on trapping and host fruit surveys in agricultural environments, are of paramount priority.

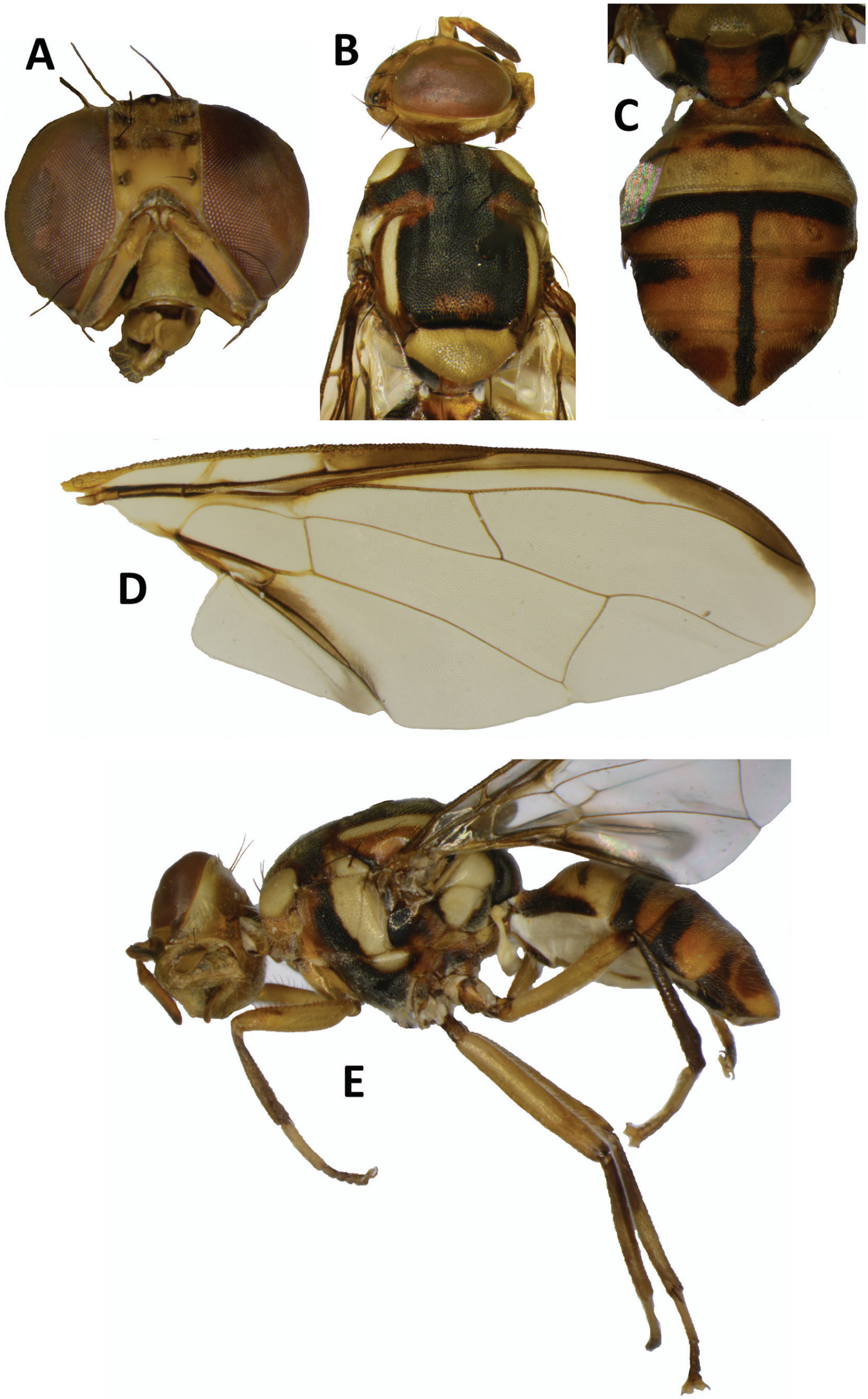


Figure 4. *Bactrocera carambolae* collected in Bangladesh **A** head **B** head and scutum **C** abdomen **D** wing **E** lateral view.

***Zeugodacus* (*Sinodacus*) *madhupuri* Leblanc & Doorenweerd, sp. nov.**

http://zoobank.org/A992E0B1-F808-4744-9FE1-9ABD7F674FDF
Figs 6A–D; 7A–C

Holotype. Male. Labelled: "Bangladesh, Tangail District, Madhupur National Park, 24.702375N, 90.086325E, 5–13-ix-2018, M. Aftab Hossain, FFBn-316, cue-lure", labelled as molecular voucher ms08804. Deposited at UHIM. **Paratypes**: One male. Labelled: "Bangladesh, Tangail District, Madhupur National Park, 24.704048N, 90.077770E, 5–13-ix-2018, M. Aftab Hossain, FFBn-311, cue-lure". Deposited at WFBM. Two males labelled: "Bangladesh, Tangail District, Madhupur National Park, 24.703023N, 90.078774E, 5–13-ix-2018, M. Aftab Hossain, FFBn-312, cue-lure", labelled as molecular vouchers ms08805 and ms08806. Deposited at UHIM.

Differential diagnosis. *Zeugodacus madhupuri* is similar to the Indian *Zeugodacus* (*Sinodacus*) *brevipunctatus* (David & Hancock) (David et al. 2017), but differs in that the fuscous medial band and lateral markings on the abdomen are pale and less extensive than in *Z. brevipunctatus*, dark marking on legs are fulvous rather than fuscous, and *Z. madhupuri* consistently has two pairs of equally well-developed scutellar setae. *Zeugodacus brevipunctatus*, along with most other species of subgenus *Sinodacus* Zia has only one pair of scutellar setae (Hancock and Drew 2018a).

Molecular diagnostics. We obtained COI-3P sequences for three specimens, aligned them with the available COI-3P sequences from San Jose et al. (2018a) and performed maximum likelihood analyses. The full tree is available in Supplementary material 2: Figure S2, and a subset of *Z. madhupuri* and its closest relatives is shown in Figure 5. Based on our reference dataset, the new species is most similar to *Z. hengsawadae* (Drew & Romig) and *Z. heinrichi* (Hering) at around -11% pairwise distance and can be diagnosed reliably using COI. Note however that *Zeugodacus brevipunctatus* was not represented in our COI dataset.

Description of adult. ***Head*** (Fig. 6A, B). Vertical length 1.65–1.95 mm. Frons, of even width, 1.11–1.23 times as long as broad; fulvous with anteromedial hump covered by short red-brown hairs; orbital setae large-sized and dark fuscous and strong: one pair of superior and three pairs of inferior fronto-orbital setae present, the most anterior pairs nearly contiguous; lunule yellow. Ocellar triangle black. Vertex fuscous. Face fulvous to yellow with a broad transverse black band at mid height; length 0.58–0.75 mm. Genae fulvous, with or without a faint fuscous subocular spot; red-brown seta present. Occiput fulvous and yellow along eye margins; with two pairs of large occipital dorsal setae and lateral occipital rows with 5–8 light to dark setae. Antennae with scape, pedicel and first flagellomere fulvous and arista black (fulvous basally); length of segments: 0.20–0.25 mm; 0.25–0.35 mm; 0.83–0.93 mm.

Thorax (Fig. 6B). Scutum fulvous with very narrow median and lateral faint fuscous longitudinal lines. Pleural areas fulvous except well-defined or faint red-brown area narrowly along anterior margin of mesopleural stripe, over most of anepimeron, and above mid coxae. Yellow markings as follows: postpronotal lobes (narrowly fulvous anteriorly); notopleura (notopleural callus); broad mesopleural (anepisternal)

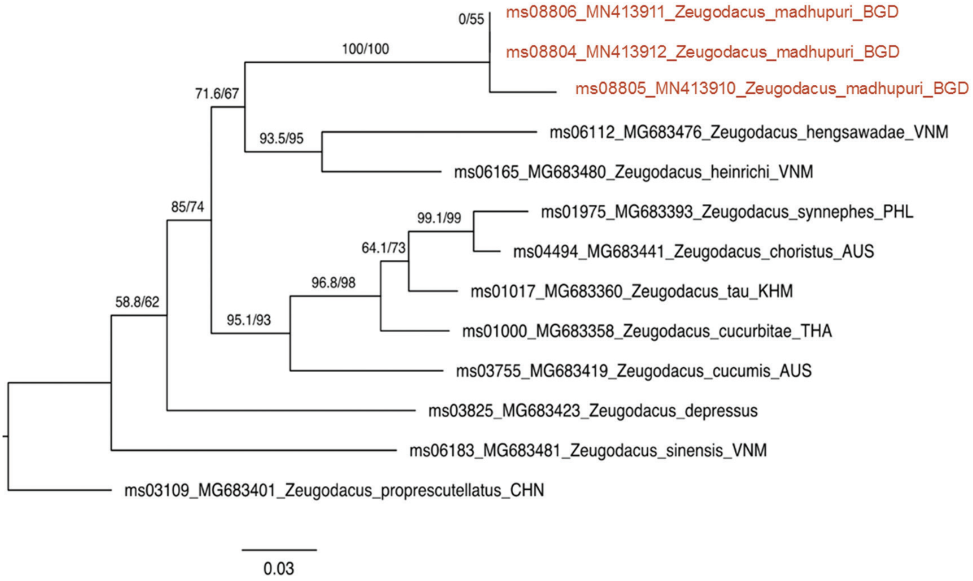


Figure 5. Maximum likelihood tree of COI-3P sequences of *Zeugodacus madhupuri* sp. nov. and its closest relatives in COI. Taxa names include UHIM 'ms' molecular voucher numbers, GenBank accessions and ISO three letter country codes. The record of *Zeugodacus hengsawadae* was published as *Zeugodacus* nr. *tau* in San Jose et al. (2018a). The scale bar indicates substitutions per site; values on the branches indicate ultrafast bootstrap support values and Sh-aLRT bootstrap values, respectively.

stripe, reaching level of anterior notopleural seta dorsally, continuing to katepisternum as a broad transverse spot, anterior margin straight or slightly convex; anatergite (with or without posterior margin narrowly red-brown); anterior 75% of katatergite (remainder fulvous); a narrow medial postsutural vitta and two narrow lateral postsutural vittae tapering posteriorly ending before or faintly reaching intra-alar setae, and prolonged narrowly anteriorly beyond notopleural suture. Postnotum fulvous. Scutellum yellow except for very narrow black basal band. Setae: four scutellar (both pairs well developed); prescutellar absent; one intraalar; one posterior supraalar; anterior supraalar absent; one mesopleural; two notopleural; four scapular; all setae well developed and red-brown. A weakly to well-developed postpronotal seta present in some specimens.

Legs (Fig. 7B). Fore coxae basally and posteriorly dark fuscous and anteroapically fulvous. Mid and hind coxae predominantly dark fuscous. Trochanters fulvous. Femora fulvous, with basal half of mid femora and three-fifths of hind femora yellow; tibiae fulvous with apical black spur on mid tibiae; tarsi fulvous to yellow.

Wings (Fig. 7A). Length 6.78–7.78 mm; basal costal (bc) and costal (c) cells fuscous; microtrichia in outer corner of cell costal only; remainder of wings with a pale fulvous tint except fuscous subcostal cell, broad fuscous costal band overlapping con-

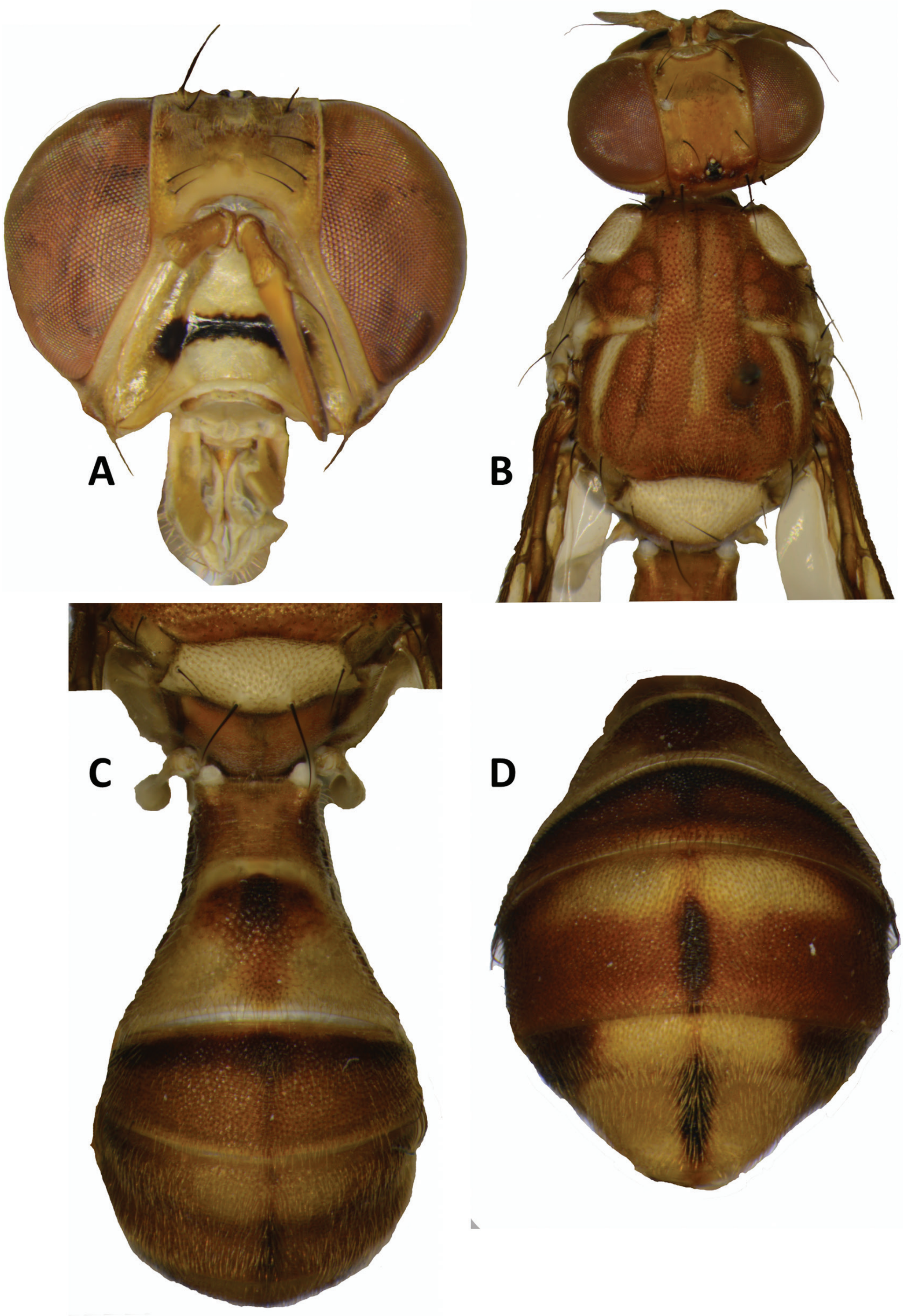


Figure 6. *Zeugodacus madhupuri* **sp. nov. A** head **B** head and scutum **C–D** abdomen.

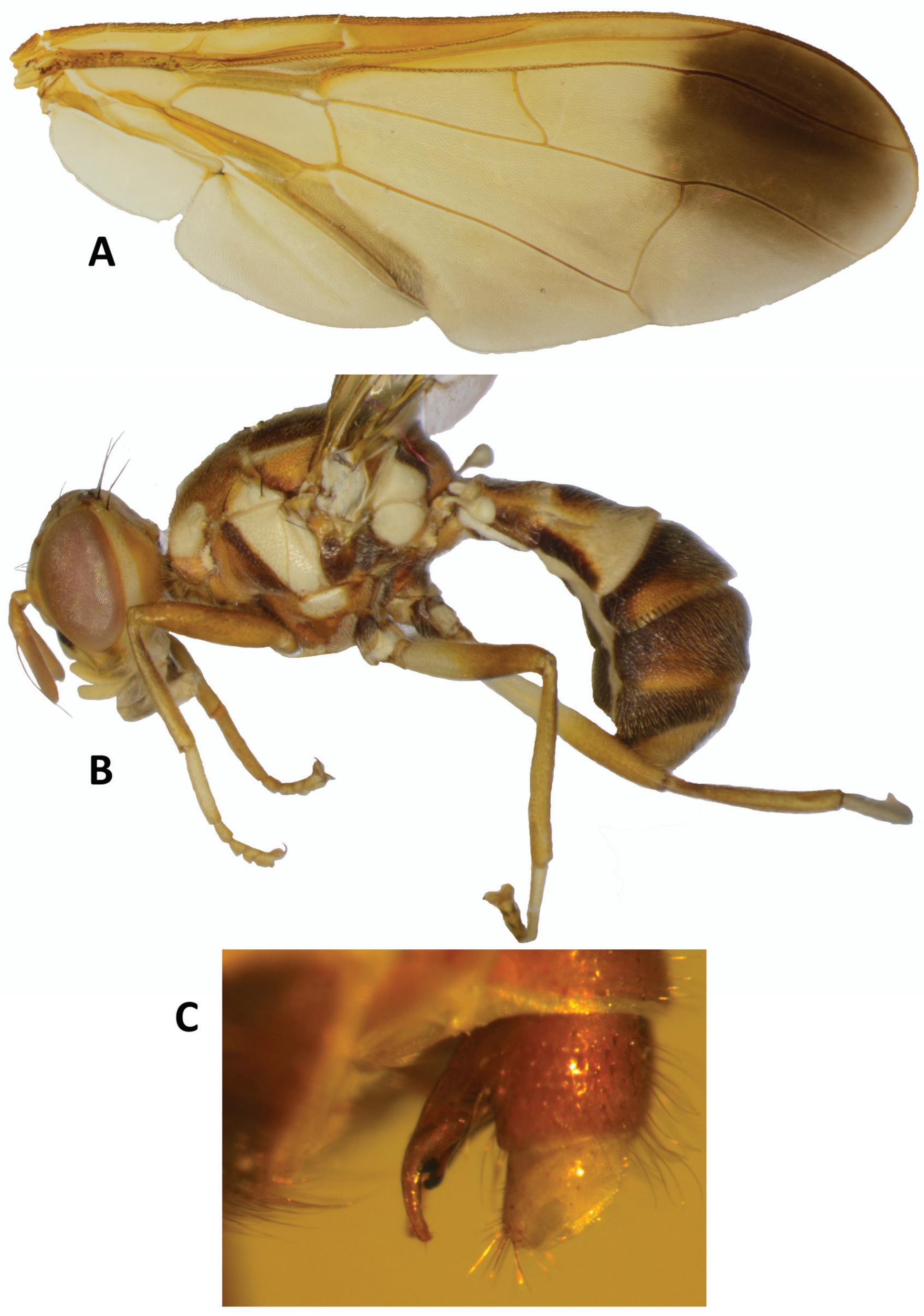


Figure 7. *Zeugodacus madhupuri* sp. nov. **A** wing **B** lateral view **C** terminal abdominal segment.

fluent with R_{4+5}, a large dark fuscous apical spot from apex of R_{2+3}, and englobing apical portions of veins R_{4+5} and M (from interception with dm-cu), a broad fuscous anal streak ending at apex of A_1 + CuA_2; dense aggregation of microtrichia around A_1 + CuA_2; supernumerary lobe well developed.

Abdomen (Figs 6C, D, 7C). Elongate oval and petiolate; terga free; pecten present on tergum III; posterior lobe of surstylus long (Fig. 7C); abdominal sternum V with a shallow concavity on posterior margin. Tergum I as long as wide and sterna I and II longer than wide. Tergum I fulvous with apical margin narrowly yellow. Tergum II yellow with a median fulvous heart-shaped marking with an inner anteromedial fuscous marking. Terga III–V fulvous with fuscous along basal margin of tergum III, a narrow median longitudinal band reaching apex of tergum V, narrowly along lateral margins of terga III–IV, and as broad lateral markings on tergum V, anterior to ceromata, and with pale fulvous along base of tergum IV and on tergum V medially, anterior to ceromata (shining spots), which are also pale fulvous.

Etymology. The species name is an adjective that refers to the Madhupur National Park, where all specimens were collected.

Other new records

Bactrocera abbreviata (Hardy) new country record: One specimen collected in zingerone-baited trap, in October 2016, at the Institute of Nuclear Medicine and Allied Sciences (Chattogram). Known from the Philippines, China and Thailand (Drew and Romig 2013). Doorenweerd et al. (2018) noted that it may be conspecific with and junior synonym to *B. bipustulata* Bezzi, known from Sri Lanka and southern India. It has been bred in Thailand from *Chionanthus ramiflorus* and *Olea salicifolia* (family Oleaceae) (Allwood et al. 1999).

Bactrocera pendleburyi (Perkins) new country record: Eight specimens in six zingerone samples in forest sites of Chattogram District (Sita-Kunda, Chunati, Fashiakhali). Previously known from Peninsular Malaysia and Thailand, its presence in Bangladesh is a significant range extension. A non-pest species bred from *Symplocos cochinchinensis*, *S. racemosa* (Symplocaceae), and *Gmelina arborea* (Verbenaceae) (Allwood et al. 1999). A closely related species with entirely fulvous femora, *B. clarifemur* Leblanc & Doorenweerd, was recently described from Vietnam (Leblanc et al. 2018a). All Bangladesh specimens belong to *B. pendleburyi*.

Bactrocera syzygii White & Tsuruta new country record: Formerly known only from a small series of specimens bred from rose-apple (*Syzygium jambos*) in Sri Lanka (Drew and Romig 2013), it was recently recorded from India (David et al. 2017). The use of zingerone-baited traps collected large numbers in Sri Lanka (Leblanc et al. 2018b), Bangladesh (Table 1), and as far east as Vietnam (Leblanc et al. 2018a) and south as Sarawak and Sulawesi (CD, unpublished), consistent with the widespread distribution of rose-apple.

Zeugodacus apicalis (de Meijere) new country record: One specimen collected in cue-lure in Chattogram District (Rangunia Upazila) in November 2014. Widespread from China south to Sulawesi, it breeds on flowers of *Trichosanthes wawraei* (Cucurbitaceae) (Drew and Romig 2016). The Bangladesh new record is a significant westward extension.

Zeugodacus atrifacies (Perkins) new country record: A total of 23 specimens collected in 12 cue-lure samples, all in Chunati Wildlife Sanctuary. This species is widespread, from Bhutan east to Vietnam and south to Sarawak. This validates the record from India cited by Norrbom et al. (1999) that could not be confirmed by Drew and Romig (2013).

Zeugodacus diaphorus (Hendel) new country record and correction: 12 specimens in five cue-lure samples, all in rural sites. This species was erroneously identified as *B.* (*Sinodacus*) *bogorensis* (Hardy) in Leblanc et al. (2014), based on a comparison with specimens (of *Z. diaphorus*) in the UHIM, erroneously identified as *B. bogorensis* by Elmo Hardy. The record was published before keys to species (Drew and Romig 2016) became available and before we had access to a specimen of *Z. diaphorus* from Vietnam for molecular comparison. The record of *Z. diaphorus*, a species widespread from Sri Lanka and India East to Taiwan and South to Java is far more plausible than of *Z. bogorensis*, a species known only from Indonesia (Drew and Romig 2013). We therefore do not believe that *Z. bogorensis* is present in Bangladesh.

Zeugodacus incisus (Walker) new record: 67 specimens collected from 31 cue-lure samples, almost all in protected forest. Widespread from India to Vietnam and South to Peninsular Malaysia.

Zeugodacus infestus (Enderlein) new record: A single specimen collected at cue-lure in Sita-Kunda Eco-Park in July 2017. Significant range extension of a common species previously known from Vietnam, Thailand, Peninsular Malaysia Java, and Sumatra.

Bactrocera bhutaniae Drew & Romig confirmation of record: We confirm the identity of *Bactrocera* sp. (possibly *B. bhutaniae*) in Leblanc et al. (2014) as belonging to this species.

Bactrocera propinqua (Hardy & Adachi) confirmation of record: We confirm that the species previously reported as *Bactrocera* species 45 (likely *B. propinqua*) (Leblanc et al. 2013, 2014) as belonging to this species.

Other Tephritidae:

Diarrhegma modestum (Fabricius) (Acanthonevrini) was originally collected in Dhaka by Hossain and Khan (2013), and more recently in rural areas of Rajshahi District.

Khan et al. (2017) recorded the following bamboo-shoot fruit flies in Dhaka: *Felderimyia gombokensis* Hancock & Drew, *Rioxoptiolona dunlopi* (van der Wulp), and *R. vaga* (Wiedemann) (all Acanthonevrini), and *Acroceratitis distincta* (Zia), *A. ceratitina* (Bezzi) and *Gastrozona soror* (Schiner) (all Gastrozonini). *Gastrozona soror* was also collected by hand in rural areas of Feni District (Chattogram).

Tephraciura basimacula (Bezzi) (Tephrellini) new record: one specimen was hand-collected at the AERE, in Dhaka. This species, also known from southern India and Sri Lanka, breeds in flowerheads or seedpods of Acanthaceae (Hancock 2010).

Non-target records

A total of 5626 specimens of *Ankylopteryx anomala* (Brauer) (Neuroptera, Chrysopidae) were collected in methyl eugenol traps, almost all in the forested areas. This species is widespread across tropical Asia, from Sri Lanka to Taiwan, and its attraction to methyl eugenol is well documented (Leblanc et al. 2015c). Two unidentified moths in the family Crambidae were caught in two separate methyl eugenol traps in Sita-Kunda Eco-Park. These may be real instances of attraction, because attraction of two species of flower-visiting crambids was demonstrated in Hawaii (Leblanc et al. 2009). Likewise, six specimens of one unidentified bee species in the genus *Amegilla* (subgenus *Zonamegilla*) (Hymenoptera, Apidae) (Fig. 8A, B) were collected in zingerone-baited traps in Sita-Kunda (three specimens in three traps), Chimbuk Hills (3 specimens in one trap) and Chunati (one specimen in one trap), and one specimen of the same species entered a methyl eugenol trap in Chattogram. The zingerone attraction record is credible and worth further investigation. One specimen of *Amegilla calceifera* (Cockerell) was also caught in a zingerone trap in Nepal in 2017 (LL, unpublished). The single specimen in the methyl eugenol trap may be accidental, though honeybee attraction to that lure has been reported (Leblanc et al. 2009).

New records of fruit fly parasitoids

Psyttalia fletcheri (Silvestri) (Hymenoptera, Braconidae) new record: One kilogram of infested snake gourd yielded 427 fruit fly puparia, from which emerged 81 specimens of *Zeugodacus cucurbitae*, 286 specimens of *Z. tau*, and 43 specimens of *P. fletcheri*. A laboratory colony of this species was established at AERE in preparation for a pilot area-wide control program. Adult *P. fletcheri* are fed with a 10% sugar solution and honey, and oviposit in third instar larvae of *Z. cucurbitae* and *Z. tau*. *Psyttalia fletcheri* was rarely observed in commercially cultivated crop fields, likely due to the frequent applications of pesticides and low prevalence of alternate wild host fruits surrounding the fields.

Spalangia sp. and *Pachycrepoideus vindemmiae* (Rondani) (Hymenoptera, Pteromalidae) new records: Two 5 kg bottle gourds (*Lagenaria siceraria*) each infested with larvae of *Z. cucurbitae* and *Z. tau*, yielded 383 puparia and seven *Spalangia* and 387 pupae and nine *P. vindemmiae*, respectively. Laboratory colonies of both parasitoids were established at AERE. Hosts determined to be suitable for both species are puparia of *Z. cucurbitae*, *Z. tau*, *B. dorsalis*, and *B. zonata* with a preference for *Z. cucurbitae* by *Spalangia* and for *B. dorsalis* by *P. vindemmiae*.

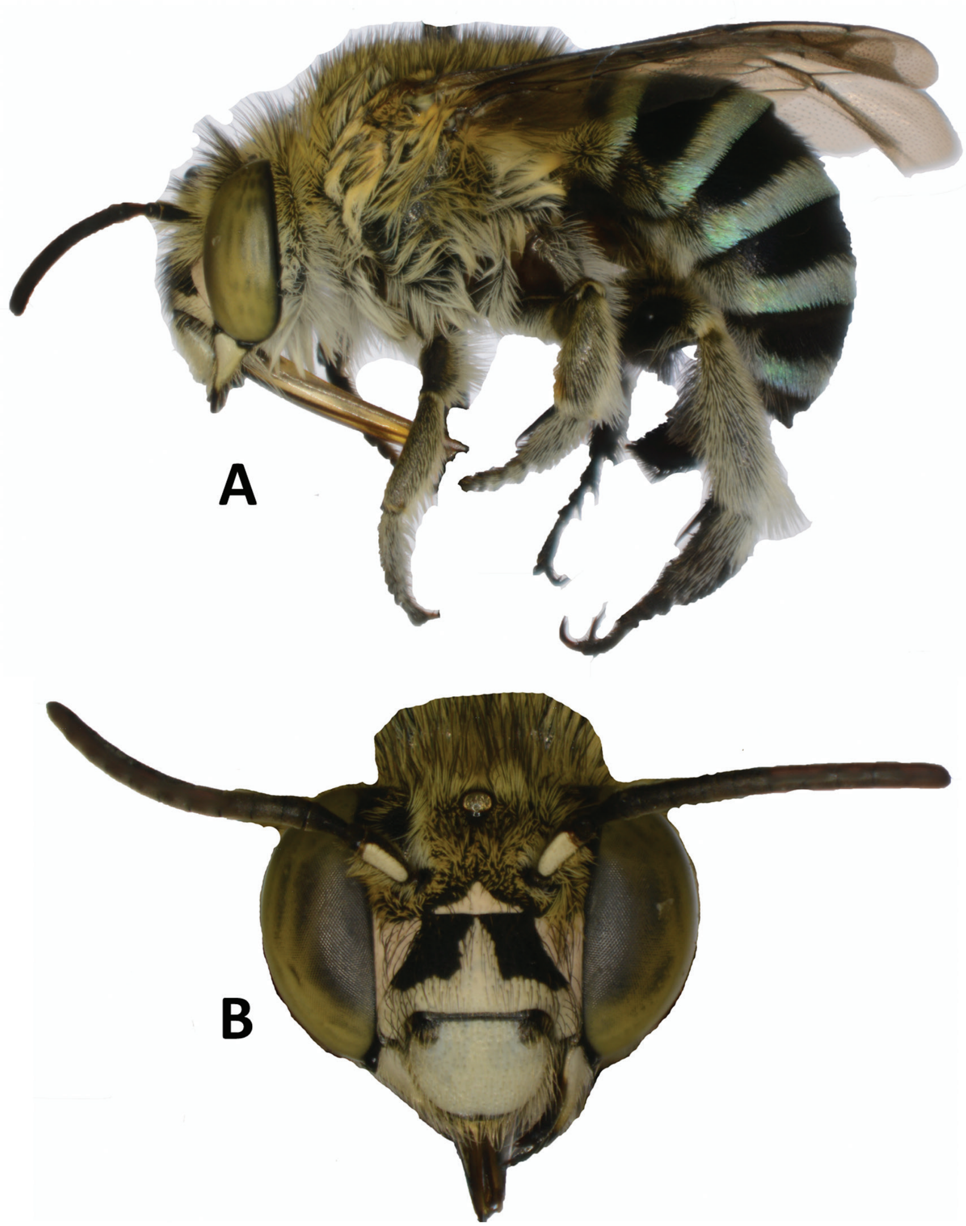


Figure 8. *Amegilla* (*Zonamegilla*) sp. (Hymenoptera: Apidae) collected in zingerone traps in Bangladesh. **A** Face **B** lateral view.

Acknowledgements

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Supplementary material 1

Figure S1

Authors: Luc Leblanc, M. Aftab Hossain, Camiel Doorenweerd, Shakil Ahmed Khan, Mahfuza Momen, Michael San Jose, Daniel Rubinoff

Data type: statistical data

Explanation note: Maximum likelihood tree based on elongation factor 1 alpha sequences from (San Jose et al. 2018) with newly generated *Bactrocera carambolae* sequences added.

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Supplementary material 2

Figure S2

Authors: Luc Leblanc, M. Aftab Hossain, Camiel Doorenweerd, Shakil Ahmed Khan, Mahfuza Momen, Michael San Jose, Daniel Rubinoff

Data type: statistical data

Explanation note: Maximum likelihood tree based on cytochrome c oxidase I sequences from (San Jose et al. 2018) with newly generated *Zeugodacus madhupuri* sequences added, taxa names colored orange.

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RESEARCH ARTICLE

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A new species of *Monstrilla* (Copepoda, Monstrilloida) from the plankton of a large coastal system of the northwestern Caribbean with a key to species

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Abstract

The genus *Monstrilla* Dana, 1849 is the most diverse of the copepod order Monstrilloida. Monstrilloid copepods are endoparasites of benthic polychaetes and molluscs; adult individuals are free-living, non-feeding reproductive forms that briefly become part of the zooplankton community, where they are occasionally captured by plankton nets. Monstrilloid copepods are frequently found during routine plankton samplings of coastal and estuarine habitats, but they are rarely found in large numbers. The western sector of the Caribbean Sea is known to harbor a diverse monstrilloid fauna. The analysis of zooplankton samples obtained during nine years from Chetumal Bay, a large embayment of the Mexican Caribbean coast, yielded a male monstrilloid that was found to represent a new species. It is herein described following upgraded standards and compared with its congeners. A key to males and females of the *Monstrilla* species known from the northwestern Caribbean is also provided.

Keywords

estuaries, crustaceans, parasitic copepods, taxonomy, tropical zooplankton

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Introduction

Monstrilloid copepods are protelean parasites of benthic invertebrates; most juvenile stages are endoparasitic and adult individuals are free-living, non-feeding reproductive forms that briefly become part of the zooplankton community, where they are occasionally captured by plankton nets (Suárez-Morales 2011). As parasites they cause a strong inflammatory response in its hosts (Suárez-Morales et al. 2010). Because of their rarity in the plankton and taxonomic complexity, there are large geographic areas in which the monstrilloid copepod fauna remains largely unknown (Suárez-Morales 2011, 2015). According to Suárez-Morales (2011), the regions with the highest number of monstrilloid records are the North Atlantic (32 species), followed by the northwestern Caribbean Sea and the Gulf of Mexico (24), the region around Indonesia, Malaysia, the Philippines, and Japan (20+), the Mediterranean-Black Sea region (19), and the Brazilian-Argentine coasts (16). In the Caribbean Sea, most records, particularly of the genus *Monstrilla* Dana, 1849, are from its westernmost area, the Mexican Caribbean coast (Suárez-Morales and Gasca 1992; Suárez-Morales 1994, 1995, 1996, 1998, 2003).

At more than 2500 km^2, Chetumal Bay is the largest estuarine lagoonal system of the Mexican Caribbean coast. It is a priority protection area for the conservation of the Caribbean manatee, both nationally and internationally (Morales-Vela et al. 2003).

A large set of 607 zooplankton samples was obtained over a period of nine years (1990–1997, 2015, 2016). Different zooplankton groups have been studied in Chetumal Bay, including medusae (Suárez-Morales and Segura-Puertas 1995), appendicularians and chaetognaths (Gasca and Castellanos 1993), fish and crustacean larvae (Gasca et al. 1994), planktonic copepods (Ruíz-Pineda et al. 2016), as well as zooplankton biomass variations (Vásquez-Yeomans et al. 2012). Despite these intense sampling efforts, no monstrilloid copepods have previously been obtained in this lagoonal system. During a zooplankton haul performed in July 1997 at station 12, an adult male specimen of the genus *Monstrilla* was collected. After its taxonomic analysis, this monstrilloid was found to represent a new species which is herein described following upgraded standards (Grygier and Ohtsuka 1995, 2008) and compared with its known congeners. A key to the species of *Monstrilla* known from the Mexican Caribbean is also provided.

Material and methods

Zooplankton samples were obtained monthly in 1997 by performing daytime surface trawls at each of 13 sampling stations in Chetumal Bay on the southern coast of the Mexican Caribbean (Fig. 1). A standard 1.2 m long plankton net was used having a 0.45 m diameter mouth and 0.1 mm filtering mesh. The volume of filtered water was estimated with a digital flowmeter. The filtered volume values fluctuated between 132 and 232 m^3.

The new species is herein described in full following the current upgraded descriptive standards in monstrilloid taxonomy (Grygier and Ohtsuka 1995, 2008). The morphologic terminology follows Huys and Boxshall (1991). The holotype specimen

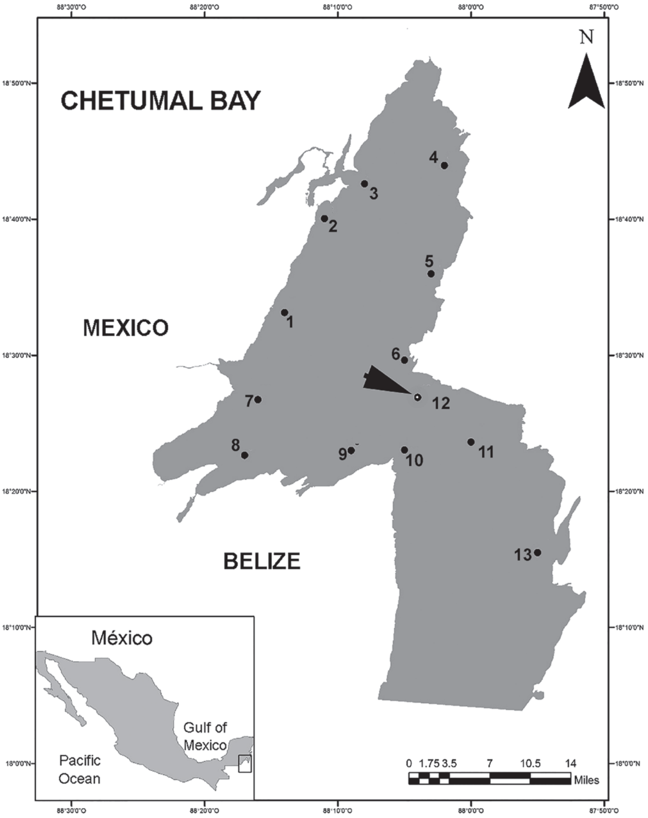

Figure 1. Surveyed area in Chetumal Bay showing zooplankton sampling sites.

was deposited in the collection of zooplankton held at El Colegio de la Frontera Sur (ECOSUR), Chetumal, Mexico (ECO-CHZ), where it is available for consultation.

Taxonomy

Subclass Copepoda Milne-Edwards, 1840
Order Monstrilloida Dana, 1849
Family Monstrillidae Dana, 1849
Genus *Monstrilla* Dana, 1849

***Monstrilla chetumalensis* sp. nov.**
http://zoobank.org/87550E34-4F19-465A-BBDF-42BD18CF4C1D
Figures 2–4, Table 1

Material examined. Holotype adult male (ECO-CH-Z-10330), Chetumal Bay, near Mexico-Belize international border (18°26'54"N; 88°04'00"W) on 27 July 1997 by I. Castellanos-Osorio. Specimen partially dissected, cephalothorax and urosome in a vial, ethanol-preserved. Appendages including antennules and legs 1–4 mounted on semipermanent slide with glycerine, sealed with acrylic varnish.

Etymology. The epithet of the new species is a toponym that refers to Chetumal Bay, the type locality of this species.

Diagnosis. Small-sized male *Monstrilla* (0.73 mm), with body divided in relatively short, robust prosome, pedigerous somites 2–4 tapering posteriorly, and slender urosome. Cephalothorax with low, rounded medial rostral projection, with both dorsal and ventral cuticular ornamentation. Antennule 5-segmented geniculate antennules. Geniculation between segments 4 and 5. Fifth pedigerous somite separated from preceding somite. Posterolateral margins produced and partially overlapping succeeding somite, visible in lateral and dorsal views. Somite with two small rounded ventral processes visible in lateral view. Legs 1–4 with outer sea on basis; exopods and endopods 3-segmented. Leg 5 absent. Genital somite with dorsal field of transverse striations; ventral genital complex represented by short shaft with distal laterally diverging lappets with rugose anterior surface, branches with dorsally directed apical spiniform processes, probably representing opercular flaps; lappets connected medially by dentate margin. Caudal rami with four subequally long caudal setae.

Description of adult male holotype. Body shape and tagmosis as usual in male *Monstrilla* (Huys and Boxshall 1991; Suárez-Morales 1993, 1996, 2003) (Fig. 2A, B). Total body length of holotype individual 0.69 mm, measured from anterior end of cephalothorax to posterior margin of anal somite. Cephalothorax representing 47.5% of total body length. Succeeding pedigerous somites 2–4 each with pair of biramous swimming legs; pedigerous somites 2–4 combined accounting for 31% of total body length in dorsal view. Cephalic region wide, bilaterally protuberant in dorsal view, narrower than cephalothorax; outer margin of cephalic protuberances corrugate. Pair of small dorsal pit setae present between antennulary bases; ventral anterior surface also with two pit setae (1, 2 in Figs 2A, 3A, respectively). Forehead moderately produced, weakly rounded, with coarsely rugose anterior margin and field of transverse striations on dorsal anterior surface; no other cephalic ornamentation discernible on dorsal anterior surface (Fig. 2A). Cephalothorax robust, 0.36 mm long, representing 47.5% of total body length; dorsal surface with scattered dorsal pores (Fig. 2A). Midventral oral papilla moderately protuberant (Fig. 2B), located at about proximal 1/3 (0.31) along ventral surface of cephalothorax. Pair of relatively small lateral pigment cups moderately developed, separated by length of less than one eye diameter, weakly pigmented; ventral cup slightly larger than lateral cups. Preoral ventral surface with low, wide-based rounded process protruding between antennulary bases (arrowed in Fig. 2B); nipple-like cuticular processes surrounded by striated surface (Fig. 3A).

Table 1. Armature of legs 1–4 including basis, exopods, and endopods. (Roman numerals indicate spiniform elements, Arabic numbers indicate setiform elements, set from inner to outer positions).

	Basis	Exopod	Endopod
Leg 1	1-0	0–I;0–1;2,2,I	1-0;1-0;2,2,I
Legs 2–4	1-0	0–I ;1-0;2,2,1, I	1-0;1-0; 2,2,1, I

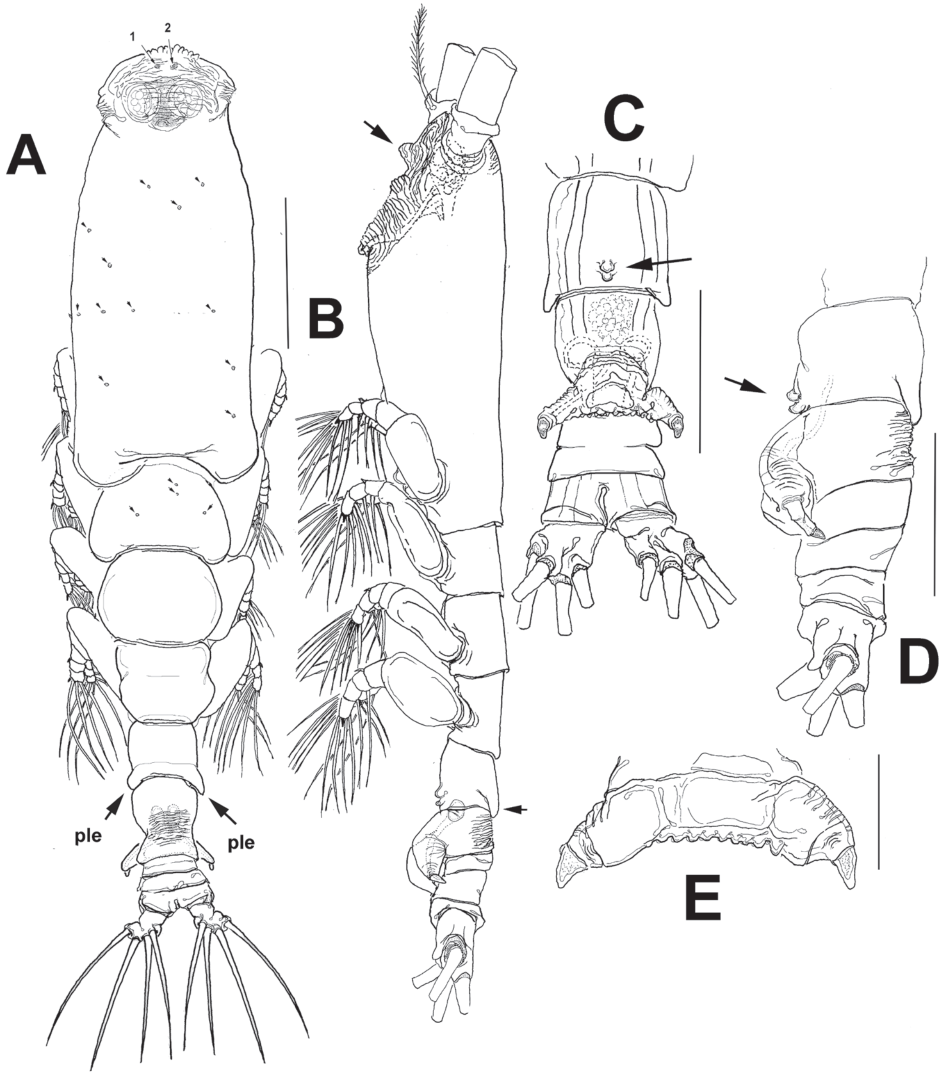

Figure 2. *Monstrilla chetumalensis* sp. nov., male holotype **A** habitus dorsal view **B** habitus lateral view, arrow indicates medial ventral protuberance **C** urosome ventral view; arrow indicates ventral globular processes on fifth pedigerous somite **D** urosome lateral view; arrow indicates ventral globular processes on fifth pedigerous somite **E** genital complex with lappets, ventral view. Scale bars: 100 µm. (**A–C**)

Antennule relatively robust (Fig. 3B), 5-segmented; length = 0.53 mm, representing 38% of total body length, and 75% of cephalothorax length, with segments 1–4 separated by complete sutures. Intersegmental division between segments 3 and 4 lacking suture, division marked by constriction; segment 4 being longest: geniculation between segments 4 and 5 (Fig. 3B). Armature, using terminology of Grygier and Ohtsuka (1995) for female monstrilloid antennular armature of segments 1–4 complemented with nomenclature by Huys et al. (2007) for elements on male fifth antennule segment, antennulary segment (1) element 1 present on first; element setiform, setulated, distinctively long, reaching well beyond distal margin of second segment. (2) elements $2d_{1,\,2}$, $2v_{1–3}$, and IId expressed on second segment. (3) third segment with elements 3, IIId, and IIIv with setal element 3 setiform, pinnate, remarkably long, reaching beyond proximal half of succeeding fourth segment. (4) Segment four bearing normally developed elements $4d_{1,\,2}$ and $4v_{1–3}$ as well as setae IVd, IVm, and IVv; elements of group $4v_{1–3}$ short, spiniform, except for long, setiform, spinulose element $4v_3$. Slender aesthetasc 4aes in ventral position. (5) terminal segment armed as follows (sensu Huys et al. 2007): elements 1–7 present on anterior margin, with three branched setal elements A–C (Fig. 3C); segment with small apical aesthetasc (element 2 in Fig. 3C). Terminal segment lacking unusual features or ornamentation (Fig. 3C).

Legs 1–4 with smooth intercoxal sclerites of rectangular, smooth. Bases with straight inner margins; outer basal setal sparsely setulose on legs 1–4; on leg 3, outer basal seta about twice as long as and slightly thicker than in other legs. Endopods and exopods of triarticulated, outer margins of exopods smooth. All elements setiform and biserially plumose except for outer spines on first segments and outer apical spiniform seta on third exopodal segments displaying and third exopodal segments displaying sparsely spinulose inner margin and smooth outer margin (Fig. 4A–D). Armature of legs 1–4 as:

Urosome consisting of fifth pedigerous, genital somite (carrying genital complex), two short, free postgenital somites divided by incomplete dorsal suture, and short anal somite (Fig. 2B–D). Fifth pedigerous somite with ventrally produced proximal half; dorsal surface smooth. Distal half of fifth pedigerous somite with pair of small medial rounded processes visible in lateral view (arrowed in Fig. 2C, D). Posterolateral margins of fifth pedigerous somite produced, partially overlapping succeeding genital somite both dorsally and laterally (“ple” in Fig. 2A, arrow in Fig. 2B). Genital somite slightly shorter than fifth pedigerous somite; genital complex of type I (Suárez-Morales and McKinnon 2014), represented by short, robust ventrally expanded shaft; complex with short, widely divergent lappets tapering distally into apical subtriangular opercular process (Fig. 2C–E). Lappets with rugose anterior surface, branches connected medially by wide dentate margin. Anal somite 1.3 times as long as genital somite. Caudal rami subquadrate, approximately 1.1 times as long as wide and about 0.7 times as long as anal somite. Each ramus armed with four subequally long caudal setae (Fig. 2A).

Remarks. The new species differs from the males of other known congeners in several respects. Firstly, there are only a few other male *Monstrilla* with divergent genital lappets that point backwards and end in a subtriangular or spiniform opercular process. The male of *Monstrilla chetumalensis* sp. nov. most closely resembles the Indian species *Monstrilla lata* Desai & Bal, 1962. Both have similar body proportions, cephalothorax ornamentation, and paired, divergent genital lappets, each with a short distal opercular structure. However, in *M. chetumalensis*, the lappets are strongly curved and have an inverted U-shape (Desai and Bal 1962, figs 4, 5). Moreover, the antennulary armature differs strongly between these two species, particularly in the size and number of setal elements of segments 1–4 (Desai and Bal 1962: fig.3). In addition, *M. lata* has six caudal setae (Desai and Bal 1962: fig. 5) vs four in the new species (Fig. 2A). In *M. lata* the fifth pedigerous somite has a weak concavity in its proximal half (Desai

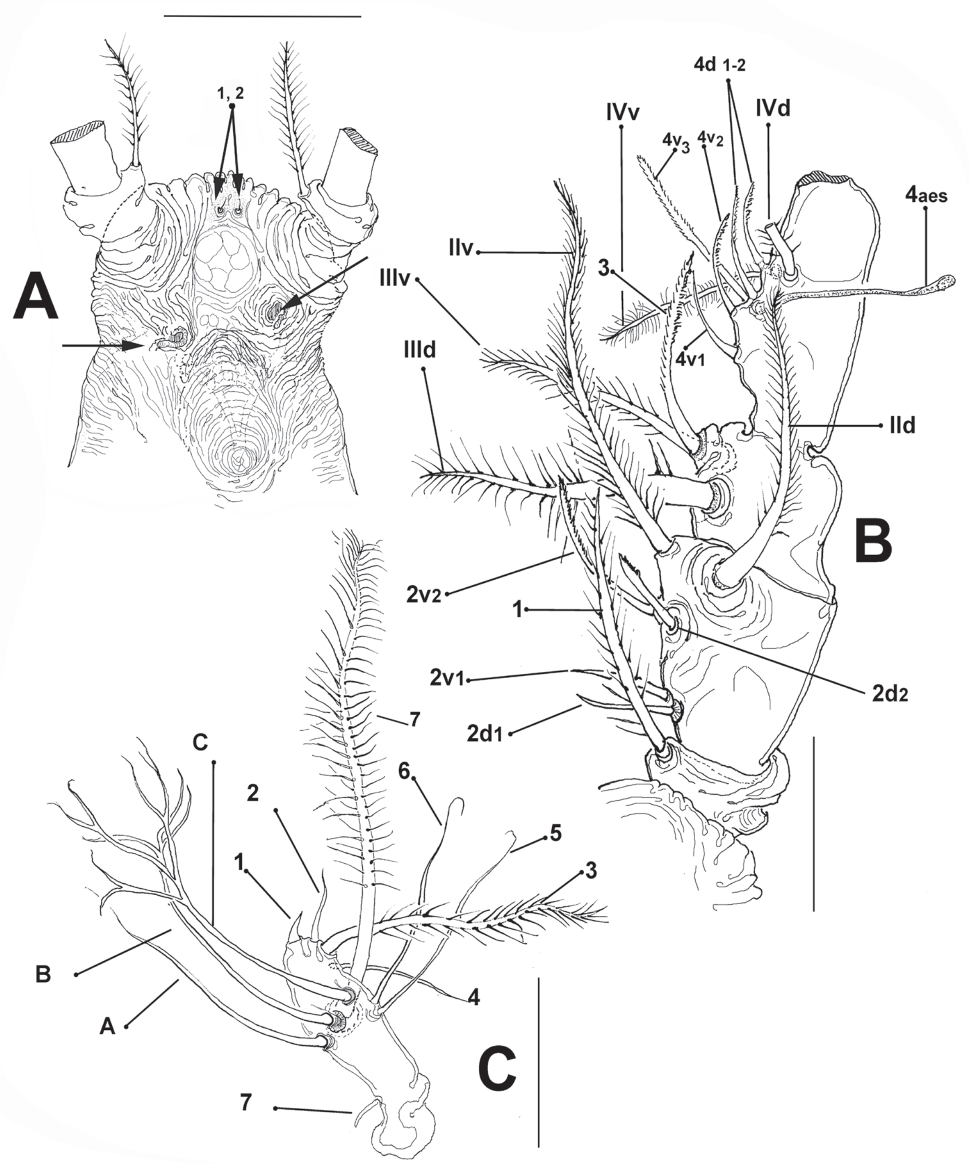

Figure 3. *Monstrilla chetumalensis* sp. nov., male holotype (**A–E, C**) **A** anterior part of cephalosome ventral view; arrow indicates nipple-like processes; s = sensilla **B** antennule segments 1–4 in dorsal view showing setal elements (sensu Grygier and Ohtsuka 1995) **C** fifth antennulary segment with setal elements (sensu Huys et al. 2007) **D** urosome lateral view **E** genital complex with lappets ventral view. Scale bars: 200 μm (**A, B**), 100 μm (**C, D**), 50 μm (**E**).

and Bal 1962: fig.4), whereas the same structure is ventrally produced in *M. chetumalensis* (Fig. 2D). The genital complex of the new species shares some features with *M. papilliremis* Isaac, 1975 from South Africa. Both have divergent lappets with distal subtriangular opercular structures pointing backwards (Isaac 1975); however, in

M. papilliremis, the lappets are medially connected by a smooth margin with a medial notch and also have an inverted U-shape, thus diverging from the conditions observed in the genital complex of *M. chetumalensis*. In two other well-known species of the genus, *M. longicornis* Thompson, 1890 and *M. longiremis* Giesbrecht, 1893, the genital lappets are also divergent, connected medially by a smooth, straight margin (Sars 1921; Huys and Boxshall 1991; Suárez-Morales 2010) and thus diverging from the dentate condition observed in *M. chetumalensis* sp. nov. In addition, both *M. longicornis* and *M. longiremis* have a 1-segmented leg 5 (see Sars 1921; Suárez-Morales 2010), which is absent in the new species. Backwardly directed genital lappets, as those observed in *M. chetumalensis* sp. nov., were reported in male *M. longicornis* by Huys and Boxshall (1991) and also in male *M. longiremis* by Suárez-Morales (2010). Secondly, the presence of small rounded ventral processes on the fifth pedigerous somite have been reported previously only in the Caribbean *M. marioi* Suárez-Morales, 2003 (Suárez-Morales 2003: fig. 4), but in this species the process involves three small lobes (Suárez-Morales 2003: fig. 4) instead of two observed in *M. chetumalensis* sp. nov. (Fig. 2D). The distinctive characters observed in our male specimen appear to be enough evidence to support its assignment as a new species.

Also, we considered the resemblance of the described male with males of the recently described genus *Caromiobenella* Jeon, Lee & Soh, 2018, which is known from males only. It has been recognized (Grygier and Ohtsuka 2008) that the type species of *Monstrilla*, *M. viridis* Dana, 1849 should be redescribed from a neotype in order to clearly define the genus delimitation and clarify the status of related genera. Morphological comparison shows that *M. chetumalensis* sp. nov. is assignable to *Monstrilla*, as it clearly diverges from *Caromiobenella* in the following characters:

In male *Caromiobenella* branched antennulary setae are absent from the fifth antennulary segment (Jeon et al. 2018: fig. 2C), but in the new species setae A and B of thee fifth antennulary segment are branched (Fig. 3C). The cephalothoracic ornamentation in *Caromiobenella* spp. includes two pairs of large dorsal crater-like depressions and pitted sensilla (Jeon et al. 2018: fig. 1A); these structures are absent in *M. chetumalensis* in which the cephalothoracic ornamentation is represented mostly by a conspicuous pattern of striations on its ventral surface and a few scattered dorsal pores (Fig. 2A, B). In addition, the two outermost setae on the third exopodal segments of legs 1–4 are serrate along the outer margin and smooth along the inner margin in *Caromiobenella* (Jeon et al. 2018: fig. 3C–E). In *M. chetumalensis* a distinct condition was observed: these exopodal setae are either smooth or serrate along the inner margin (Fig. 4A–D). Also, species of *Caromiobenella* have five or six caudal setae (Jeon et al. 2018), whereas only four caudal seta are present in the new species (Fig. 2A). According to Jeon et al. (2018), the presence of a type 3 male antennule (see Huys and Boxshall 1991; Suárez-Morales 2011), with a modified fifth segment bearing distal transverse serrate ridges on the inner distal margin, is a diagnostic character to species of *Caromiobenella*. The new species has an unmodified (type I) antennule. In *Caromiobenella* the genital complex is represented by a robust shaft

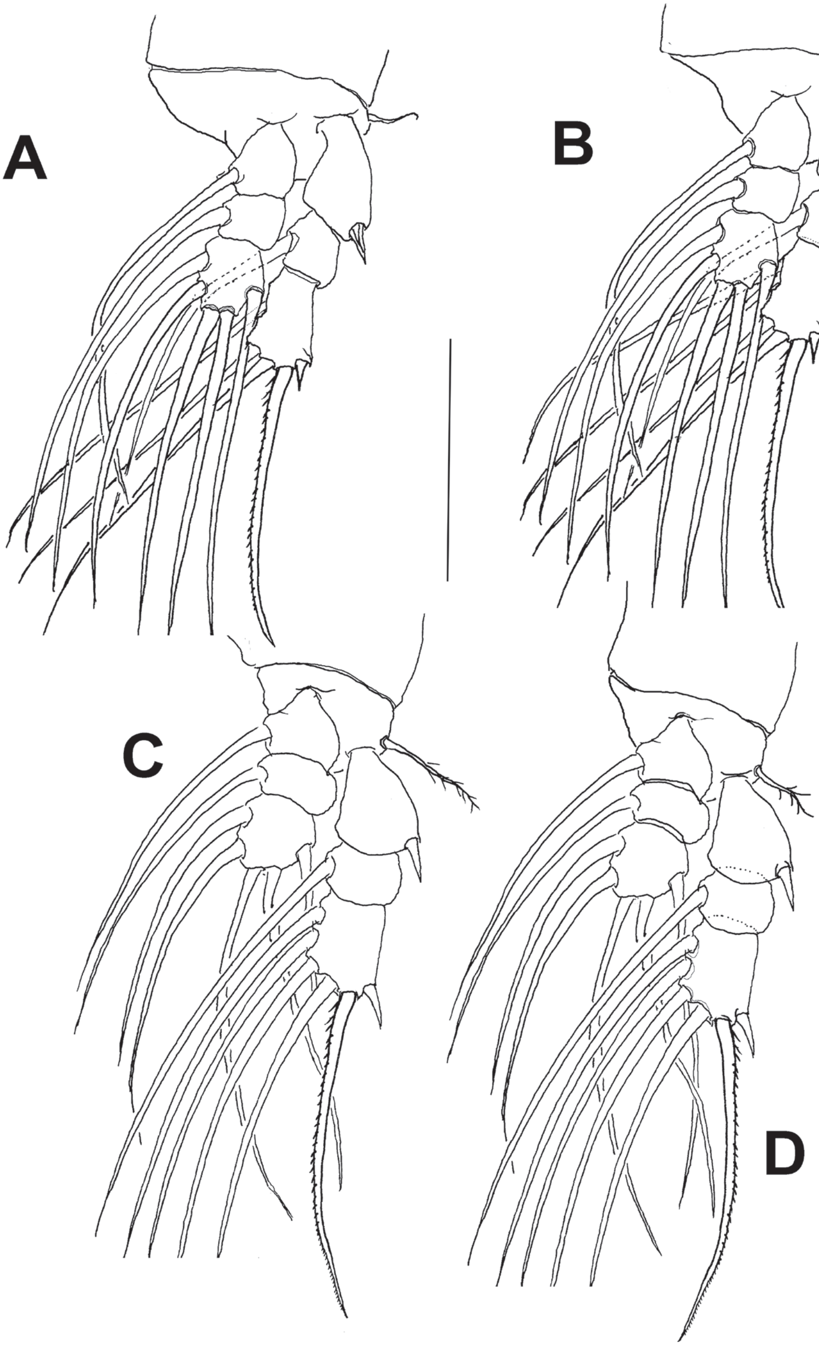

Figure 4. *Monstrilla chetumalensis* sp. nov., male holotype **A** leg 1 **B** leg 2 **C** leg 3 **D** leg 4. Scale bars: 100 µm.

and short, subtriangular non-divergent distal lappets with a medial smooth protrusion (Jeon et al. 2018: fig.7C) and paired medial opercular flaps. In *M. chetumalensis* the genital complex is also robust and short, but lappets are strongly divergent and are medially joined by a dentate margin. No such medial opercular flaps were observed in the new species; these are probably represented by the terminal structures on the tip of each lappet.

Currently, the are eight species of *Monstrilla* recorded from different coastal or reef areas of the Mexican Caribbean: *M. reidae* Suárez-Morales, 1993a (male) from Bahia de la Ascensión, *M. mariaeugeniae* Suárez-Morales and Islas-Landeros, 1993 (female) from off Puerto Morelos reef zone, *M. ciqroi* (Suárez-Morals, 1993b) (female) from Bahia de la Ascension, *M. barbata* Suárez-Morales & Gasca, 1992 (female) (see Suárez-Morales et al. 2006), *M. elongata* Suárez-Morales, 1994 (both sexes) (see Suárez-Morales 1996) from Puerto Morelos reef zone, *M. globosa* Suárez-Morales, 2003 (male) and *M. marioi* Suárez-Morales, 2003 (male), and *M. rebis* Suárez-Morales, 1993b (female) from Bahia de la Ascension (see Suárez-Morales and Gasca 1992; Suárez-Morales 1993a, 1993b, 1996, 2003; Suárez-Morales and Islas-Landeros 1993; Suárez-Morales et al. 2006).

Key to the female *Monstrilla* of the Mexican Caribbean

1 Antennules indistinctly segmented, slender or robust................................**4**
– Antennules distinctly 4-segmented..**2**
2 With irregularly rugose medial rostral process ....***M. barbata* Suárez-Morales & Gasca, 1992**
– Medial rostral process absent..**3**
3 A Fifth leg with small rounded protuberance adjacent to inner lobe............. ....***M. ciqroi* (Suárez-Morales, 1993b)***
– Fifth leg with inner margin of fifth leg smooth... ....***M. rebis* Suárez-Morales, 1993b**
4 Antennule with straight anterior and posterior margins; fifth leg with 1 lobe armed with 2 setae, inner margin smooth ....***M. elongata* Suárez-Morales, 1994**
– Antennule with rounded protuberances along anterior and posterior margins; fifth leg with 1 lobe armed with 2 setae and with strong spiniform process on inner margin ....***M. mariaeugeniae* Suárez-Morales and Islas-Landeros, 1993**

Key to male *Monstrilla* of the Mexican Caribbean

1 Fifth legs absent **2**
– Fifth legs present, with 1 lobe armed with single seta ....***M. elongata* Suárez-Morales, 1994****

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* originally described as *Monstrillopsis*; assigned to *Monstrilla* by Suárez-Morales et al. (2006).

** Male described as *M. wandelii tropica* by Suárez-Morales (1996)

2 Genital complex with lappets directed backwards; fifth pedigerous somite with two small globular processes........................***M. chetumalensis* sp. nov.**

– Genital complex with lappets not directed backwards **3**

3 Genital complex with inverted U-shaped lappets tapering into acute points... ....***M. marioi* Suárez-Morales, 2003**

– Genital complex with different structure, elongate, cylindrical, rod-like, with paired globular processes in terminal position **4**

4 Fifth pedigerous somite with short digitiform ventral process; apical antennulary element 6_1 bifurcate ....***M. globosa* Suárez-Morales, 2003**

– Fifth pedigerous somite lacking ventral process; apical antennulary element 6_1 not bifurcate ....***M. reidae* Suárez-Morales, 1993a**

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RESEARCH ARTICLE

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Discovery of a new ant species of the elusive termitophilous genus *Metapone* in Singapore (Hymenoptera, Formicidae, Myrmicinae), with the first detailed description of male genitalia of the genus

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Abstract

A new species of the rare ant genus *Metapone*, *Metapone murphyi* **sp. nov.**, is described based on museum material consisting of a single nest series (workers, queens, and males) collected from a decayed coconut palm stump on Pulau Sakra, previously an offshore island south of mainland Singapore. Workers can be distinguished from other named congeners mainly by the following characters: 1) subpetiolar lamella subrectangular; 2) short median longitudinal ventral subpetiolar edge and roundly obtuse posteroventral subpetiolar angle; 3) outer margin of posterior subpetiolar face in posteroventral view forming a continuous, U-shaped, translucent, laminate carina; and 4) petiole subtrapezoidal in dorsal view with extended blunt tooth-like posterolateral corners. Detailed description and illustrations of male genitalia of the genus are given for the first time. The key to Asian species of *Metapone* is updated to include the new species.

Keywords

Museum specimens, rare genus, taxonomy, nest series

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Introduction

The myrmicine genus *Metapone* Forel, 1911 is known from tropical Africa, Madagascar, Sri Lanka, the Philippines, archipelagic Southeast Asia, Melanesia east to Fiji and Eastern Australia (Guénard et al. 2017). From Asia and Southeast Asia, nine nominal species have been reported: *M. bakeri* Wheeler, 1916 (Philippines, Luzon), *M. balinensis* Taylor & Alpert, 2016 (Indonesia, Bali), *M. greeni* Forel, 1912 (Sri Lanka), *M. hewitti* Wheeler, 1919 (Malaysia, Sarawak), *M. jacobsoni* Crawley, 1924 (Indonesia, Sumatra), *M. javana* Taylor & Alpert, 2016 (Indonesia, Java), *M. quadridentata* Eguchi, 1998 (Malaysia, Sabah), *M. sauteri* Forel, 1912 (Taiwan), and *M. wallaceana* Taylor & Alpert, 2016 (Indonesia, Lombok). There are no past records from continental Southeast Asia (Taylor and Alpert 2016). This genus is rarely collected by myrmecologists, and generally presumed to be termitophilous, since colonies are usually encountered in close association with termite nests (Taylor and Alpert 2016).

We herein describe a new species, *Metapone murphyi* sp. nov., from Singapore, based on a nest series which was found stored in ethanol as part of the Zoological Reference Collections (ZRC), in the Lee Kong Chian Natural History Museum (National University of Singapore). Multiple queens (five of eight), two workers (two of three; one headless), and two males were dry mounted for morphological examination. The present paper provides the detailed description of the male genitalia of *Metapone* for the first time: this information will be valuable for future comparative studies on this understudied genus. In addition, the existing key to Asian species of *Metapone* (Taylor and Alpert 2016) is also updated to include the new species.

Materials and methods

Morphological observations were made using an Olympus SZX16 stereomicroscope, while measurements were made using micrometres (ocular graticule) on the same microscope. Genitalia of one male were slide-mounted according to the preparation steps described in Yamada and Eguchi (2016), and examined with a Nikon Eclipse E600 microscope.

Measurements and indices

Measurements in mm, mostly adapted from dimensions in Taylor and Alpert (2016):

TL	Total aggregate outstretched length of the ant from mandibular apex to gastral apex in lateral view.
HL	Maximum length of head capsule excluding the mandibles, measured in full-face view in a straight line from the mid-point of the occipital margin to the limits of clypeal projection.

HW	Maximum width of head in full-face view, across eyes when applicable.
CpL	Maximum clypeal length as measured from the anterior to posterior clypeal margins.
EL	Maximum eye length measured across its maximum diameter in lateral view.
MSL	Length of mesosoma in lateral view, measured as a straight line approximately parallel to the dorsal outline of the mesosoma, from the furthest anterodorsal point of the pronotum (including anterior slope) to the posterior limit of the propodeum.
PML	Maximum length of promesonotal disc in dorsal view, measured along the midline originating from anterior margin of promesonotal disc (excluding anterior slope of promesonotum).
PMW	Maximum width of promesonotal disc in dorsal view.
PDW	Maximum width of propodeum in dorsal view.
PetL	Maximum length of petiolar node in dorsal view, including posterolateral extensions if present.
PetW	Maximum width of petiolar node in dorsal view, including posterolateral extensions if present.
PetH	Maximum height of petiolar node, measured as a direct straight line connecting its dorsal and ventral extremities in lateral view, including the subpetiolar extension.
PpetL	Maximum length of postpetiolar dorsum (excluding helcium) in lateral view, including antero- and/or posterolateral extensions of the postpetiolar disc if present.
PPetW	Maximum width of postpetiolar dorsum, measured in dorsal view.
PPetH	Maximum height of postpetiole, measured as a direct straight line connecting its dorsal and ventral extremities.
GW	Maximum width of first gastral segment, measured in dorsal view.
CI	Cephalic Index, HW/HL × 100.
CpI	Clypeal Index, CpL/HL × 100.
PMI	Promesonotal Index, PML/MSL × 100.
REL	Relative Eye Length, EL/HW × 100.

Additional measurements applicable to gyne mesosoma:

PnL	Length of pronotal disc, measured along the midline originating from anterior margin of promesonotal disc (excluding anterior slope of pronotum).
PnW	Maximum width of pronotal disc, measured across the widest part of the disc in dorsal view.
ScL	Length of mesoscutum, measured along the midline of the scutum in dorsal view.
ScW	Width of mesoscutum, measured across the widest part of the sclerite in dorsal view.

Specimen depositories, collections, and their abbreviations:

MNHAH	Museum of Nature and Human Activities, Hyogo, Japan.
SKYC	Seiki Yamane Collection, Japan.
ZRC	Zoological Reference Collection, Lee Kong Chian Natural History Museum, Singapore.

Additional *Metapone* material examined

Types. *Metapone quadridentata* Eguchi, 1998. Three paratypes from East Malaysia (Borneo), Sabah, Poring, Kinabalu were examined. Two paratype workers (SKYC) and one paratype queen (MNHAH).

Source images of the new species for focus stacking were taking using a Canon EOS Kiss X9 digital camera, attached to a Nikon AZ100 stereomicroscope (for worker, queen, and male bodies, excluding male genitalia), and a Nikon Eclipse E600 microscope (for male genitalia). Focus-stacked images were produced using Helicon Focus Pro 7.0.2 (Helicon Soft Ltd., http://www.heliconsoft.com/), and improved with the retouching function of the same software. Colour balance and contrast were adjusted using GIMP 2.8 (The GIMP Development Team, http://www.gimp.org). Two paratype workers of *M. quadridentata* were imaged on a Dun Inc™ Passport II macrophotography imaging system, using a Canon MP-E 65 mm lens. Focus-stacked images of the two paratypes were produced using Zerene Stacker (Zerene Systems LLC, http://zerenesystems.com/cms/stacker). The final images were further adjusted, annotated, and scale bars added using Adobe Photoshop CS6. All specimens of the new species examined are deposited in the Lee Kong Chian Natural History Museum, under the Zoological Reference Collection (ZRC).

High resolution image plates are available at https://doi.org/10.5061/dryad.9776725

Taxonomic accounts

Metapone murphyi sp. nov

http://zoobank.org/6FC954CB-057C-4430-8F32-63825B750A64
Figs 1–23

Types. ***Holotype:*** Worker. SINGAPORE, Pulau Sakra (1.2592°N, 103.7042°E), decayed coconut stump, 7.March.1981, D.H. Murphy leg., colony code: DHM-81-Metapone, depository catalogue number: ZRC_ENT_00000878 (ZRC). ***Paratypes*:** Two workers (1 headless), 8 alate queens, 2 males, same colony as the holotype, depository catalogue number: ZRC_HYM_0000016.01–11 (ZRC).

Diagnosis. *Worker*. (1) Body, small-sized, monomorphic with broad size variation (HL 0.98–1.24, HW 0.75–0.93). (2) Head subrectangular, with lateral sides nearly entirely straight and parallel; (3) anterior margin of rostrate projection of clypeus faintly crenate; (4) petiolar node in lateral view subrectangular, in dorsal view subtrapezoidal, slightly wider than long, with posterolateral corners extended to form tooth-like projections; (5) subpetiolar lamella small and subrectangular, dorsoventrally slightly higher than anteroposteriorly wide, thin and translucent; (6) short median longitudinal ventral subpetiolar edge meeting the broadly rounded lateral outline of posterior subpetiolar face at a roundly obtuse posteroventral subpetiolar angle; (7) outer margin of posterior subpetiolar face forming a continuous, U-shaped, translucent, laminate carina in posteroventral view. (8) Head and mesosoma mostly densely striated longitudinally and shining.

Queen. (1) Body relatively small-sized (HL 1.28–1.33, HW 0.85–0.93); (2) posterolateral corners of head faintly striated and shining; (3) petiolar node subrectangular in lateral view, elongate-subtrapezoidal in dorsal view, more than twice as long as its anterior margin; (4) lateral face of petiolar node substriate-reticulate with few long diagonally-placed carinae; (5) subpetiolar lamella, subpetiolar edge and posterior subpetiolar face as in the worker.

Worker measurements. Holotype worker (small worker): TL 4.60, HL 0.98, HW 0.75, CpL 0.38, EL 0.10, MSL 1.18, PML 0.60, PMW 0.55, PDW 0.45, PetL 0.30, PetW 0.35, PetH 0.50, PpetL 0.33, PpetW 0.40, PpetH 0.43, GW 0.75, CI 77, CpI 38, PMI 51, REL 13.

Paratype worker (large worker; ZRC_HYM_0000016.01), body from petiole to gaster missing: HL 1.24, HW, 0.93, CpL 0.49, EL 0.18, MSL 1.60, PML 0.85, PMW 0.70, PDW 0.55, CI 75, CpI 40, PMI 53, REL 19.

Queen measurements. Four paratype queens (n = 4): TL 6.4–6.9, HL 1.28–1.33, HW 0.85–0.93, CpL 0.5–0.53, EL 0.38–0.40, MSL 1.90–2.05, PML 1.30–1.40, PMW 0.75–0.88, PDW 0.50–0.55, PetL 0.48–0.55, PetW 0.40, PetH 0.65–0.75, PpetL 0.40, PpetW 0.58–0.63, PpetH 0.53–0.58, GW 0.80–0.90, CI 67–70, CpI 38–40, PMI 65–68, REL 43–44, PnL 0.65–0.70, PnW 0.70–0.83, ScL 0.60–0.68, ScW 0.75–0.80.

Male measurements. One paratype male (n = 1): TL 4.5, HL 0.90, HW 0.93, CpL 0.40, EL 0.43, MSL 1.60, PML 1.20, PMW 0.80, PDW 0.50, PetL 0.25, PetW 0.40, PetH 0.58, PpetL 0.33, PpetW 0.50, PpetH 0.43, GW 0.85, CI 103, CpI 44, PMI 75, REL 46, PnL 0.60, PnW 0.75, ScL 0.75, ScW 0.75.

Description. *Worker.* Body small-sized (HL 0.98–1.24, HW 0.75–0.93), monomorphic with distinct variation in size. Head in full-face view subrectangular; posterior margin very weakly concave; posterolateral corners round; lateral margins almost entirely straight. Eye elongate-elliptical, relatively small, larger in large worker in proportion to head length, positioned far behind the midlength of head, ventrad to antennal scrobe, close to the level of the posterior end of antennal scrobe; REL 13 in holotype, 19 in a paratype (large worker). Mandible large and dorsoventrally high in lateral view; masticatory margin with four robust teeth roughly uniform in size; basal angle well-developed. Clypeus large; median portion of clypeus extended forward, forming a short rostrate projection; the projection slightly narrowed anteriorly, with anterior margin faintly convex, lateral margin broadly concave, and anterolateral angles subdentate. Frontal carinae parallel, well developed, partly overhanging antennal scrobe

Figures 1–4. *Metapone murphyi*, holotype **1** head in full-face view **2** body in lateral view **3** waist segments in lateral view **4** mesosoma and waist in dorsal view.

which accommodates antennal scape. Antenna 11-merous; scape short, roughly reaching the midlength of head when laid backward, flattened and dilated apically, with anterior part of leading edge forming a translucent lamella; funiculus flattened; apical 3 antennomeres forming a club. Mesosoma elongate rectangular in lateral view, with dorsal outline almost entirely straight. Promesonotal suture absent; anterior margin of promesonotal disc in dorsal view broadly and roundly convex, in lateral view extended to form a short overhang above pronotal neck; lateral margins of promesonotal disc in dorsal view almost entirely straight and parallel; humeral corner roundly angulate. Mesothoracic spiracle in lateral view almost spherical, in dorsal view slightly protruding from the lateral outline of mesosoma. Metanotal groove present as a thin but distinct sulcus which is slightly convex anteriorly in dorsal view. Propodeum in dorsal view a little narrower than promesonotum; lateral margins almost parallel in the anterior half and then converging posterad; posterior margin almost straight; propodeal junction in lateral view with angle round and obtuse. Petiolar node in lateral view subrectangular, longer than high, in dorsal view subtrapezoidal, almost as long as wide, with ante-

Figures 5–8. *Metapone murphyi* **5–7** large paratype worker (ZRC_HYM_0000016.01) **5** head in full-face view **6** body in lateral view (waist segments and gaster missing) **7** mesosoma in dorsal view **8** paratype worker, headless (ZRC_HYM_0000016.02) waist segments in lateral view.

rior margin straight or weakly concave, posterior margin weakly concave, and lateral margins almost straight and diverging posterad; anterolateral corner in dorsal view obtusely angulate; posterolateral corner in dorsal view angulate and weakly produced posterad; in posterior view lateral margins slightly converging from dorsum to base without distinctly curving inwards near the dorsum. Subpetiolar lamella subrectangular, thin and translucent in a paratype (large worker; Fig. 8), or with a large central opening in the holotype (Fig. 3), with anteroventral corner broadly round, with posteroventral corner narrowly round and slightly produced posteroventrad; the ventral subpetiolar edge in lateral view short, forming an obtuse, rounded angle (posteroventral subpetiolar angle) with outer margin of posterior subpetiolar face; outer margin of posterior subpetiolar face in posteroventral view forming a continuous, U-shaped, translucent and laminate carina. Postpetiole in dorsal view globular, broader than long, slightly broader than petiole, with anterior margin weakly concave; sub-postpetiolar process in lateral view moderately and triangularly produced. Legs short and stout; femora flattened anteroposteriorly, high dorsoventrally; anteroventral part forming a groove which is margined with carinae and partly accommodates tibia; protibia with a tuft of short hairs anterior to large pectinate spur and a short spine behind the spur; meso- and metatibia each with a much smaller pectinate spur and two large spinose setae anterior to the spur; basitarsus stout; probasitarsus posteroapically with three large spinose setae and one small spinose seta; meso- and metabasitarsus anteroapically with four large spinose setae.

Dorsum and anterior half of lateral face of head, and antennal scrobe densely striate and shining; mandible striate-punctate and shining; posterolateral corners and ventral face of head mostly smooth and shining. Dorsum and lateral face of mesosoma densely striated and largely shining; interspaces in metapleuron and lateral face of propodeum faintly punctate. Petiole with lateral face weakly striate-punctate and shiny, and dorsal face largely smooth and shining. Postpetiole with lateral face shagreened, and dorsal face largely smooth and shining. Gaster largely smooth and shining; anterior part of tergites II–IV faintly and transversely imbricate.

Dorsum of head bearing very short, sparse, decumbent hairs, with a few longer erect hairs; ventral and lateral faces of head with numerous short decumbent hairs; posterior border of hypostoma and ventral margin of mandible with linearly-arranged long bristle-like setae. Rostrate projection of clypeus with several long bristle-like setae beneath the anterior margin. Dorsum of mesosoma bearing very short, sparse, decumbent hairs, with a few longer erect hairs; lateral face of mesosoma almost hairless; propodeal declivity with many long erect hairs. Petiolar node with dense and long decumbent hairs on upper part of anterior face, with sparse standing hairs on dorsal face; each postero-dorsolateral corner with a long erect hair. Postpetiole and gaster with abundant standing and subdecumbent hairs of roughly uniform length, with sparse longer erect hairs.

Entire body generally brown in colour; head darker than remainder of body; antenna and legs more yellowish; tip and joint of antenna paler than remainder of antenna. Whole body darker and more reddish in large worker than in small worker.

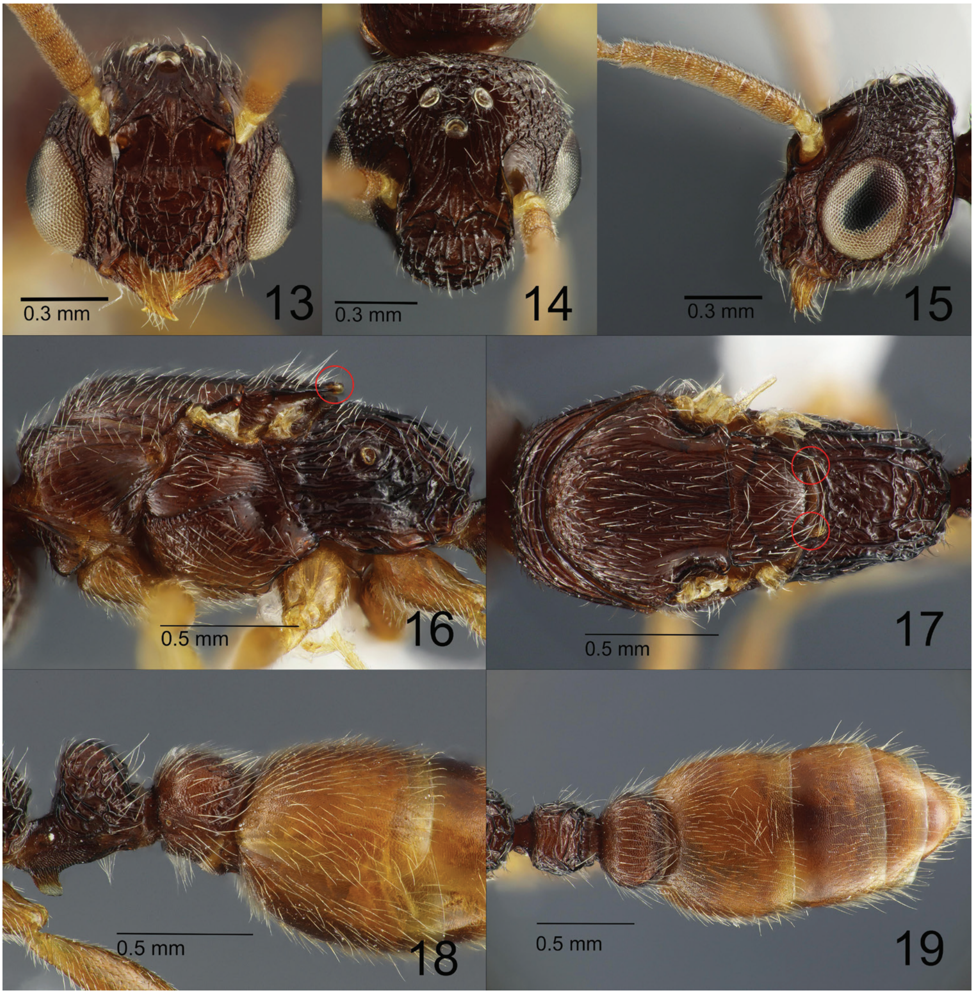
Queen. Body similar in size to the large worker (HL 1.28–1.33, HW 0.85–0.93). Head in full-face view subrectangular, more elongate than in the worker (CI 67–70 in the queen, 75–77 in the worker); posterior margin weakly and broadly concave; posterolateral corners rounded, slightly more angulate than in the worker; lateral margins almost entirely straight and parallel. Eye very large and elongate, in full-face view located behind the midline of head, very weakly convex and protruding from the lateral outline of head. Ocelli very small; median ocellus located around the level of the posterior ends of frontal carinae. Mandible as in the worker. Clypeus as in the worker; rostrate projection a little more slender in the queen than in the worker. Frontal carina, antennal scrobe, and antenna as in the worker. Mesosoma elongate rectangular, more slender than in the worker, in lateral view with dorsal outline weakly and broadly convex. Anterior margin of pronotal disc in dorsal view broadly and roundly convex, in lateral view extended to form a short overhang above pronotal neck; lateral margins of the disc almost straight or slightly concave, weakly diverging posterad; humeral corner roundly angulate. Mesoscutum wider than long, slightly longer than pronotal disc; notaulus absent; parapsidal signum weakly present; transscutal articulation recognized as a transverse weakly curved scutoscutellar sulcus spanning almost the entire posterior mesoscutal margin; axilla distinctly separated from mesoscutellum by a curved

Figures 9–12. *Metapone murphyi*, paratype alate queen (ZRC_HYM_0000016.03) **9** Head in full-face view **10** body in lateral view **11** waist segments in lateral view **12** mesosoma and waist in dorsal view.

sulcus; posterior margin of mesoscutellum roundly convex. Mesopleuron distinctly divided into upper and lower part by a weakly sinuate sulcus. Propodeum in dorsal view longer than wide, with anterior margin broadly and roundly concave, with lateral margins almost parallel in the anterior 3/5 and then converging posterad, with posterior margin roundly convex; dorsal outline of propodeal dorsum in lateral view entirely downward-sloping; propodeal junction in lateral view roundly and weakly produced over posterior propodeal face. Petiolar node in lateral view subrectangular, longer than high, in dorsal view elongate subtrapezoidal, less than twice as long as wide, with anterior margin straight or weakly concave, posterior margin weakly concave, and lateral margins slightly sinuate and diverging posterad; anterolateral corner in dorsal view almost right-angled; posterolateral corner in dorsal view angulate and weakly produced posterad; subpetiolar lamella, subpetiolar edge and posterior subpetiolar face as in the worker. Postpetiole as in the worker; subpostpetiolar process in lateral view weakly and bluntly extending anteroventrad. Legs as in the worker.

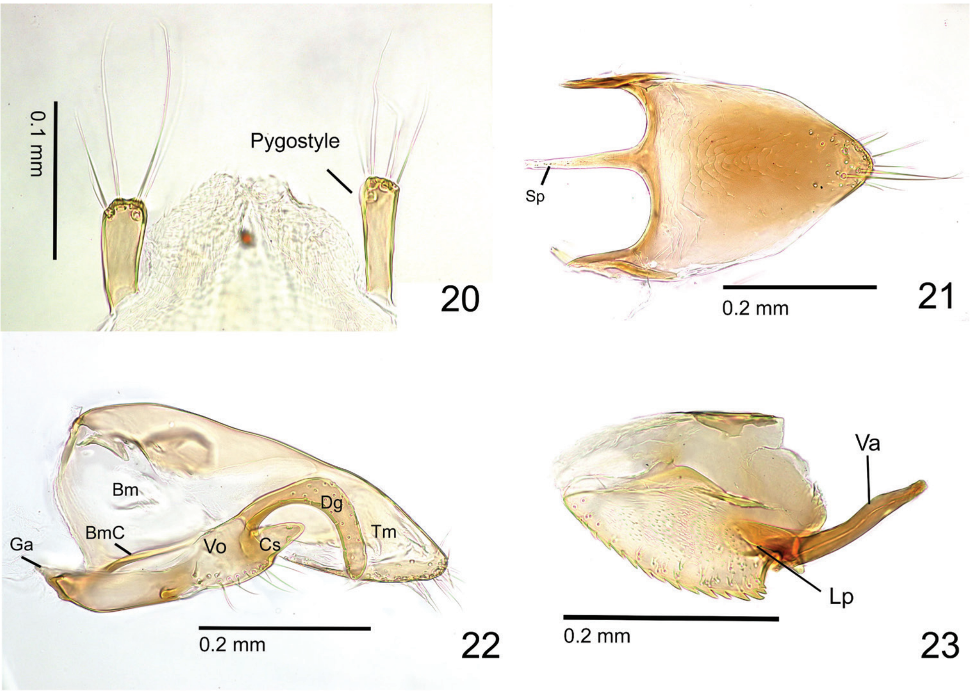
Sculpture, pilosity, and body colour as in the worker.

Male. Body small-sized (HL 0.90, HW 0.93). Head in full-face view (Fig. 13) subovate, with posterior margin very roundly convex, in anterodorsal view (Fig. 14) subtriangular, with posterolateral corners and anterior margin broadly round, in lateral view relatively high dorsoventrally (Fig. 15). Occipital carina distinct laterally, less distinct dorsally. Eye very large, in full-face view with posteriormost end reaching or slightly surpassing the midlength of head; in full-face view lateral outline of eye evenly convex and protruding from the lateral outline of head. Ocelli developed more than in the queen; median ocellus located around the level of the posterior ends of frontal carinae (Fig. 14); the distance between the median and lateral ocelli a little shorter than that between lateral ocelli. Mandible with distinct basal and masticatory margins; masticatory margin 3-toothed, with a large apical tooth followed by slightly smaller preapical tooth and minute 3rd tooth. Anterior clypeal margin as a whole roughly M-shaped (Fig. 13); median part of clypeus extended forward to form a short rostrate process; rostrate process narrowed anteriorly, partly overhanging mandible, with an almost straight anterior margin, and lateral margin carinate and weakly sinuate; clypeus in lateral view broadly and evenly convex anterodorsad. Frontal carina short and lamellate; antennal scrobe short but deep. Antenna 12-merous; scape very short and small; 2nd and 3rd antennomeres greatly reduced and extremely small; 3rd antennomere shorter than 2nd. Mesosoma in dorsal view elongate sub-elliptical, widest at the level of posteriormost ends of pronotum, in lateral view elongate-rectangular. Anterior margin of pronotal disc in dorsal view broadly and roundly convex, extended to form a short overhang above pronotal neck; lateral margins of the disc in dorsal view broadly and roundly convex; humeral corner present but indistinct. Mesoscutum in dorsal view a little wider than long, with anterior margin strongly and roundly convex; notaulus and parapsidal signum absent; transscutal articulation recognized as a transverse weakly curved scutoscutellar sulcus spanning almost the entire posterior mesoscutal margin; axilla distinctly separated from mesoscutellum by a curved sulcus; posterior margin of mesoscutellar disc with a pair of posteriorly-pointing short tooth-like extensions. Mesopleuron distinctly divided into upper and lower parts by a weakly sinuate sulcus. Propodeum in dorsal view longer than wide, with anterior margin broadly and roundly concave; lateral margins almost straight, converging posterad; posterior margin roundly convex; dorsal outline of propodeal dorsum roundly down-sloping; propodeal junction in lateral view angulate. Petiolar node in lateral view slightly higher than long, with parabolic dorsal outline, in dorsal view sub-oval, broader than long, with weakly concave anterior and posterior margins; subpetiolar lamella small, semi-translucent and subrectangular, a little higher than long, with round anteroventral corner and strongly produced posteroventral corner; ventral subpetiolar edge and posteroventral subpetiolar angle reduced; posterior subpetiolar face in posteroventral view recognized as an isosceles triangle. Postpetiole in dorsal view subelliptical, nearly twice as wide as long, slightly longer than and wider than petiolar node, with roundly convex lateral margins. Gaster in dorsal view sub-elliptical, tapering apicad. Legs moderately long; pro- and meso- femora distinctly longer than pro- and meso-tibiae respectively, but metatibiae almost as long as metafemora; femora each with weak apicoventral lamellate flange; protibiae each with one large pectinate ventroapical spur; mid- and metatibiae each with one simple ventroapical spur; pretarsal claw simple and strongly curved, such that its apex is directed almost perpendicularly upwards from its base; arolium small.



Figures 13–19. *Metapone murphyi*, paratype male (ZRC_HYM_0000016.10) **13** head in full-face view **14** head in subdorsal view **15** head in lateral view **16** mesosoma in lateral view **17** mesosoma in dorsal view **18** metasoma in lateral view **19** metasoma in dorsal view. Posterior tooth-like extensions of the mesoscutellar disc are encircled in red.

Pygostyle digitiform, with 4–5 long hairs on its apex. Abdominal sternite IX (subgenital plate) 1.7 times as long as wide (including spiculum); posterior disc subparabolic, with posterolateral corner weakly produced; outer face of apical part of disc with several hairs; spiculum long, 0.4 times as long as entire length of the sternite IX (spiculum length measured from the apex to the transverse line spanning the posteriormost points of each side of the anterolateral margin of the posterior disc). Cupula well-developed, 1.6 times as wide as long in dorsal view. Telomere in lateral view subparabolic, distinctly longer than high, without clear articulation to basimere. Gonostipital arm well-developed, with acute apex; lower basimere with conspicuous oblique carina (BmC in



Figures 20–23. Male genitalia of *Metapone murphyi*, paratype male (ZRC_HYM_0000016.11) **20** Pygostyle in dorsal view **21** abdominal sternite IX in ventral view **22** paramere and volsella, right side, inner view **23** penisvalva, right side, in outer view. Abbreviations: Bm basimere; BmC carina of lower basimere; Cs cuspis; Dg digitus; Ga gonostipital arm; Lp lateral apodeme of penisvalva; Sp spiculum; Tm telomere; Va valvura; Vo volsella.

Fig. 22). Cuspis well developed, forming subtriangular lobe in lateral view with 2 hairs near apex. Digitus in lateral view elongate-digitiform, entirely hooked ventrad, with blunt apex; mesal face of the apical part with very short hairs (sockets are recognized in Fig. 22); ventral margin of volsella with 12–14 long hairs. Valviceps in lateral view a little longer than wide, gently tapering posterad, forming a broadly rounded apex; anteroventral corner weakly produced; ventral margin serrated with 15 denticles.

Head mostly rugoso-reticulate, with interspaces smooth and shining; dorsal face of rostrate projection rugoso-areolate, with interspaces microsculptured and shining. Mandible carinate, with punctate and shiny interspaces. Dorsum of pronotum and anterior border of mesoscutum irregularly reticulate; the remainder of mesoscutum, mesoscutellum and upper part of mesopleuron longitudinally rugoso-reticulate; lateral face of pronotum longitudinally rugose. Propodeum coarsely rugoso-reticulate; interspaces with sparse punctures and shining. Anterior face of petiole and subpetiolar process finely microreticulate and weakly shining; dorsal and lateral faces of petiolar node rugoso-reticulate, with interspaces microsculptured and shining. Postpetiole and gastral tergites I–III (excluding anterior borders) finely microreticulate and nearly matte.

Head, dorsal and lateral faces of mesosoma, petiole, postpetiole, and gaster covered by short standing hairs (Figs 13–19).

Figures 24–27. *Metapone quadridentata*, paratype, large worker **24** head in full-face view **25** body in lateral view **26** waist segments in lateral view **27** body in dorsal view.

Body except gaster deep reddish brown; gaster lighter orange-brown. Mandible mostly light brown, becoming yellowish toward masticatory margin. Legs and antennae light yellowish-brown, becoming paler toward the apex.

Etymology. The specific name is dedicated to Prof. D.H. ('Paddy') Murphy, who collected a huge number of ants (including the type series of *M. murphyi*) and other insects in Singapore, many of which make up a substantial and invaluable part of the ZRC, collectively referred to informally as 'the Murphy Collection'.

Habitat. The offshore island where *M. murphyi* was collected, Pulau Sakra, used to be mostly forest and swampland, before being merged with other islands in close vicinity and developed for establishment of oil refining facilities after 1985.

Remarks. The worker of *M. murphyi* (hereafter referred to as MM-w) is most similar in morphology to that of *M. quadridentata* Eguchi, 1998 (MQ-w) (Figs 24–31), but is distinguishable from the latter by the following features:

1) Slope of propodeal declivity in dorsal view weakly concave and depressed medially in MQ-w, but weakly convex and almost entirely flat in MM-w.
2) Petiolar node in posterior view with lateral margins sharply curving inwards from the dorsum of the node then downwards to the base of the node in MQ-w, but slightly converging from the dorsum to the base without distinctly curving inwards in MM-w.

Figures 28–31. *Metapone quadridentata*, paratype, small worker **28** head in full-face view **29** body in lateral view **30** waist segments in lateral view **31** body in dorsal view.

3) Posteroventral corner of subpetiolar lamella strongly produced posteroventrad in MQ-w (Figs 26, 30), but only slightly in MM-w (Figs 3, 8).
4) Mesothoracic spiracle of large worker more elongate-oval in MQ-w (Fig. 25), but more spherical in MM-w (Fig. 6).
5) Propodeal lobes present as low but distinct and near-translucent lamellate flanges in MQ-w, but absent in MM-w.

The queen of *M. murphyi* (hereafter referred to as MM-q) is also most similar in morphology to that of *M. quadridentata* Eguchi, 1998 (MQ-q), but distinguishable from the latter by the following features:

1) MQ-q is distinctly larger in size than MM-q (MQ-q: TL 10.1, HL 1.91, MSL 3.04; MM-q: TL 6.4–6.9, HL 1.28–1.33, MSL 1.90–2.05). Body also stouter in MQ-q than in MM-q.
2) Posterior corner of propodeum in dorsal view forming a smoothly round angle which clearly separates lateral and posterior margins of propodeum in MQ-q, but not forming a distinct angle in MM-q.
3) Anterior margin of petiolar node in dorsal view weakly convex, with rounded anterolateral corner in MQ-q, but straight or weakly concave, with angulate corner in MM-q.

4) Subpetiolar lamella in lateral view distinctly longer than high, with anteroventral and posteroventral corners roundly angulate and separating anterior, ventral and posterior margins in MQ-q, but slightly longer than high, with anterior, ventral and posterior margins forming a continuous rounded outline in MM-q.
5) In MQ-q, posteroventral subpetiolar angle (including outer margin of posterior subpetiolar face) in lateral view with the apex slightly extended as a sharply-pointed process; in MM-q, posteroventral subpetiolar angle obtuse and not pointed.
6) Dorsal outline of propodeum largely flat, with posterior half slightly down-sloping in MQ-q, but broadly convex, and evenly, though weakly, down-sloping in MM-q.
7) PpetL/PetL = 0.90 in MQ-q, but 0.73–0.84 in MM-q.
8) Dorsum of pronotal neck with dense hairs in MQ-q, but with sparse or almost no hairs in MM-q.
9) Anterior margin of pronotal disc in lateral view not extended over the pronotal neck in MQ-q, but extended slightly over the pronotal neck forming a short overhang in MM-q.

The male of *M. murphyi* (MM-m) is distinguishable from that of the known male-based species, *M. hewitti* (MH-m), by the following characters; see also Wheeler (1919) and Taylor and Alpert (2016):

1) Masticatory margin of mandible with 4 subequal teeth in MH-m, but with 3 teeth of different sizes in MM-m.
2) 2nd antennomere (pedicel) distinctly broader than long in MH-m, but globular and almost as broad as long in MM-m.
3) Petiolar node in lateral view cuboidal, with dorsal outline nearly flat in MH-m, but with parabolic dorsal outline in MM-m.
4) Subpetiolar lamella subtriangular in MH-m, but subrectangular, with round anteroventral corner and strongly produced posteroventral corner in MM-m.
5) Posteroventral subpetiolar angle in lateral view obtuse, with a low apex in MH-m, but reduced and indistinct in MM-m.

Modification to the key to the Asian species of *Metapone*

The key to the Asian species of *Metapone* given by Taylor and Alpert (2016) is herein partly modified below; as a result of these modifications the couplet “8(2)” in the original key becomes couplet “9(2)”.

5 Posterior face of subpetiolar process relatively large, with a distinct translucent framing lamella in lateral view **6**
– Posterior face of subpetiolar process reduced and relatively small; in lateral view posteroventral subpetiolar angle either obtuse, or indistinct with the reduced posterior face transiting directly into the broadly-rounded profile of the ventral subpetiolar edge **8**

6 Posteroventral subpetiolar angle in lateral view distinct, slightly extended as a pointed process 7

– Ventral subpetiolar edge in lateral view forming an obtuse, rounded angle (posteroventral subpetiolar angle) with outer margin of posterior subpetiolar face .... ***M. murphyi* sp. nov**.

7 Anteroventral subpetiolar extension relatively large, longitudinally subrectangular with slightly rounded corners; outline of posterior subpetiolar face in lateral view steeply sloping and broadly concave, curved downwards to meet apex of posteroventral subpetiolar angle (Sri Lanka, gyne and worker) .... ***M. greeni* Forel**

– Anteroventral subpetiolar extension relatively small, subtrapezoidal with rounded corners, in lateral view its posterolateral corner extended slightly posterad to form a hook-like structure; outline of posterior subpetiolar face in lateral view broadly and weakly convex .... ***M. quadridentata* Eguchi**

8 Anterior border of median clypeal projection subtended by a minute parallel groove bearing approximately 6 stout, pale bristle-like hairs directed anterad. Anteroventral subpetiolar extension relatively large and subrectangular, posteriorly inclined; posteroventral subpetiolar angle obtuse (Indonesia, Bali; gyne only) .... ***M. balinensis* Taylor & Alpert**

– Anterior border of anteromedian clypeal projection a single shallowly concave edge, without accompanying groove or hairline. Anteroventral subpetiolar extension small, roughly right-angled-subtriangular; posteroventral subpetiolar angle vestigial and almost absent (Indonesia, Lombok; gyne only) ... . ***M. wallaceana* Taylor & Alpert**

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We would like to thank Prof. Yoshiaki Hashimoto (Museum of Nature and Human Activities, Hyogo) and Prof. Seiki Yamane (Kagoshima University) for access and permission to examine *M. quadridentata* paratypes, enabling critical morphological comparisons. Eguchi's research activity was supported by Asahi Glass Foundation (Leader: Katsuyuki Eguchi; FY2017–FY2020).

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A new species of *Petrolisthes* (Crustacea, Anomura, Porcellanidae) inhabiting vermetid formations (Mollusca, Gastropoda, Vermetidae) in the southern Caribbean Sea

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Abstract

Petrolisthes virgilius **sp. nov.** from the Caribbean Sea of Colombia is described. The new species resembles *P. tonsorius* morphologically but differs from it principally by its color and habitat. *Petrolisthes tonsorius* is brown or blueish brown and occurs under intertidal boulders strongly exposed to water movement. *Petrolisthes virgilius* **sp. nov.** is pale brown to beige and lives exclusively in intertidal areas dominated by vermetid snails, exposed to heavy wave action. The entangled tubular shells of vermetids are cemented to each other and to a hard substrate like beach rock, forming a microhabitat for the new crab species and other porcellanids of the genera *Neopisosoma* and *Clastotoechus*. Large genetic distances between DNA sequences of the mitochondrial 16S rDNA gene from *P. virgilius* **sp. nov.** and *P. tonsorius* confirmed that they comprise different species. *Petrolisthes virgilius* **sp. nov.** is the 53rd member of the West Atlantic porcellanid fauna.

Keywords

West Atlantic, *Petrolisthes virgilius* sp. nov., *Petrolisthes tonsorius*, ecological differences, color morphs, vermetid formations, mitochondrial marker

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Introduction

The porcellanid fauna of the western Atlantic has been studied intensively in the last 60 years. With the last additions by Ferreira and Tavares (2017, 2019) and Werding and Hiller (2017), consisting of three new species of *Pachycheles* Stimpson, the number of western Atlantic species rose to 52. Werding (1978) found individuals of *Petrolisthes* inhabiting vermetid formations in the Colombian Gulf of Urabá, and assigned them to *P. tonsorius* Haig, 1960, but warned that coloration and habitat of the crab specimens were atypical. *Petrolisthes tonsorius* inhabits the rocky intertidal of both the Caribbean and the tropical East Pacific and exhibits a brown to dark-brown color (Fig. 1a), sometimes blueish (Fig. 1b). The individuals found inhabiting vermetid formations were pale brown to beige (Fig. 2). Morphological re-examination of this color morph, and genetic distances estimated between DNA sequences of the 16S rDNA gene from this new form and *P. tonsorius* confirmed that they comprise different species. Here we describe the new form as *Petrolisthes virgilius* sp. nov.

Materials and methods

Material of *Petrolisthes virgilius* sp. nov. collected in the Colombian regions of Santa Marta and the Gulf of Urabá was used for morphological examination and molecular analyses. Type material was deposited in the collection of the Museo de Historia Natural Marina de Colombia (INV CRU), INVEMAR (Institute of Marine and Coastal Research of Colombia, Santa Marta). Specimens were sexed and measured by using a stereoscope with a micrometer. Measurements are given in mm and correspond to carapace length, followed by carapace width.

DNA was extracted from the chelipeds or walking legs of seven specimens of the new species (3 from Santa Marta and 4 from the Gulf of Urabá) using the DNeasy Blood & Tissue Kit (Qiagen), following the manufacturer´s protocol for animal tissues. A 540 bp fragment of the ribosomal 16S rDNA was amplified using primers 16Sar (CGCCTGTTTATCAAAAACAT) and 16Sbr (CCGGTCTGAACTCAGATCACGT) (Palumbi 1996), and trimmed to 496 bp. Double-stranded amplifications were performed in 12 ml volume reactions containing 2.5 µl of Taq buffer (5×), 1.7 µl of dNTP mix (8 mM), 0.6 µl of each primer (10µM), 1.2 µl of $MgCl_2$ (25mM), 0.2 µl of GoTaq Flexi DNA *Taq* Polymerase (Promega), 1 µl of DNA template, and 4.8 µl of ddH_20. Thermal cycling conditions consisted of an initial denaturation step at 96 °C for 3 min, followed by 30 cycles of 95 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min. An extension step at 72 °C for 5 min followed the last cycle. PCR products were cleaned using the ExoSap-IT kit (USB Corporation) following the manufacturer´s protocol. Clean PCR products were cycle-sequenced in both directions using the BigDye Terminator v. 3.1 Cycle Sequencing Kit, and electrophoresed in an Applied Biosystems 3130 Genetic Analyzer.

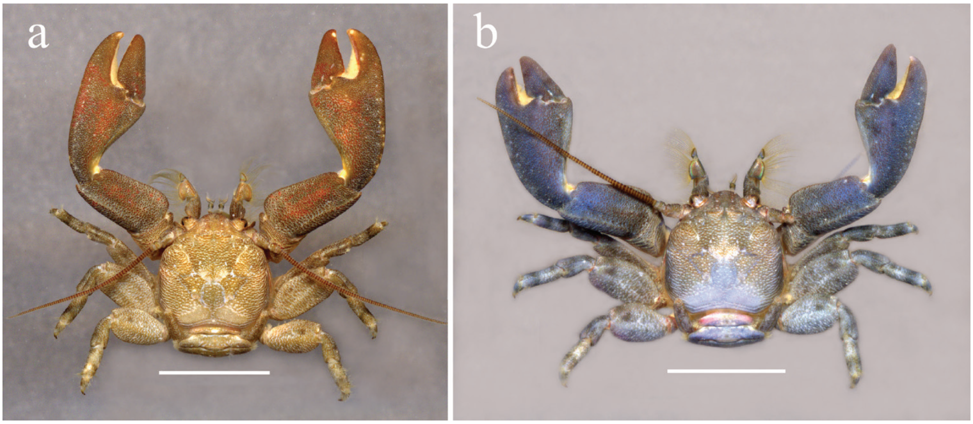

Figure 1. *Petrolisthes tonsorius*, Venezuela, Isla Cubagua. **a** Brownish color morph **b** Blueish color morph. Scale bars: 3.7 mm (**a**); 3.2 mm (**b**).

The 16S rDNA sequences of *P. virgilius* sp. nov. were compared to two sequences of *P. tonsorius* from the Venezuelan Caribbean and the Colombian Pacific, published by Hiller et al. (2006) (GenBank accession numbers DQ444960 and DQ444959). The BioEdit Sequence Alignment Editor (Hall 1999) was used to trim primer regions from DNA sequences. MAFFT v. 7 (Katoh and Standley 2013) was used to align the sequences of *P. virgilius* sp. nov. and *P. tonsorius*. Genetic distances within and between these species were estimated using Kimura's two-parameter model (K2P; Kimura 1980) implemented in MEGA v. 7.0 (Kumar et al. 2016). GenBank accession numbers of the 16S rDNA sequences of *P. virgilius* sp. nov. are MN275526–MN275532.

Systematics

Family Porcellanidae Haworth, 1825

Petrolisthes virgilius sp. nov.

http://zoobank.orgE0018D07-579A-4A53-84D1-2EF333D59098
Figs 2, 3a–e

Petrolisthes tonsorius Werding, 1978: 220 (not *P. tonsorius* Haig, 1960: 85–88)

Material. Holotype: male, INV CRU8404, Colombia, Chocó, Gulf of Urabá, Triganá, Napú, coll. J. Lazarus, 05 Dec. 2010; 4.6 × 4.5 mm.

Paratypes: 2 males, 2 females (1 ovigerous), INV CRU8405, same collection data as holotype. Size of males is 4.2 × 3.7 mm and 3.3 × 3.2 mm; size of females is 5.3 × 5.2 mm (ovigerous) and 4.3 × 4.0 mm (Fig. 3).

Figure 2. *Petrolisthes virgilius* sp. nov., Colombia, Santa Marta. Scale bar: 2.4 mm.

Sizes of largest male and female reported by Werding (1978) are, respectively, 8.0 × 8.1 mm and 6.5 × 6.1 mm.

Diagnosis. Carapace subquadrate, its margins subparallel posterior to epibranchial angle, nearly smooth, covered anteriorly with few flattened granules; no epibranchial spine; front narrow, triangular, with deep median groove; carpus 1½ times as long as wide, surface granulate, anterior margin with a broad, rounded lobe, separated through an indentation from a shallow distal lobe; manus with a longitudinal ridge; fingers blunt, outer margin convex, forming a rounded crest along entire length; merus of walking legs unarmed.

Description. Carapace about as long as broad, subquadrate, lateral margins subparallel posterior to epibranchial angles; nearly smooth, covered anteriorly with few flattened granules and posteriorly with light plications; grooves marking the various regions distinct. No epibranchial spine. Front narrow, triangular, strongly produced, with a deep median groove extending between protogastric lobes; no supraocular spine; inner orbital angle not produced. Orbits rather shallow; outer orbital angle produced into a small tooth. Eyes moderately large. Carapace naked. Basal segments of antennae thick, granulate, first movable segments with a marked crest produced to distal edge, second massive and cylindrical, flagellum about 1½ as long as carapace.

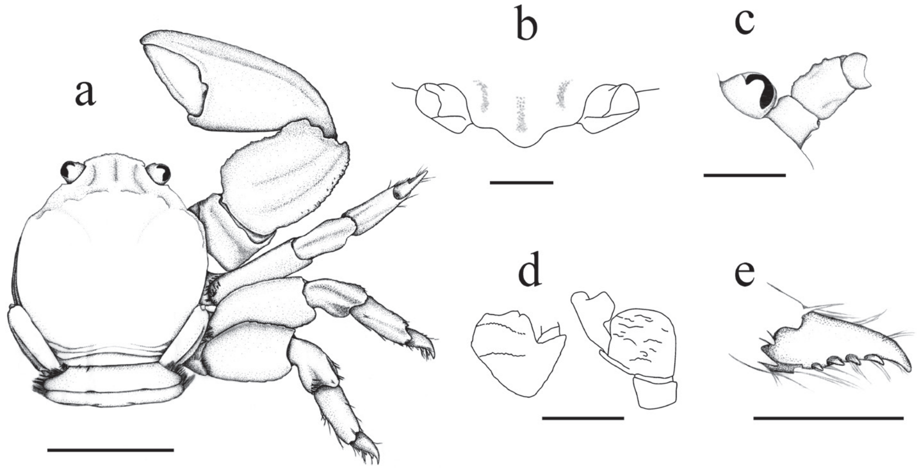

Figure 3. *Petrolisthes virgilius* sp. nov., female (ovigerous) paratype, INV-CRU 8405, Colombia, Chocó, Gulf of Urabá, Triganá, Napú. **a** Dorsal view **b** rostrum, frontal view **c** orbit with basal segments of antenna **d** basal segment of antennular peduncle **e** dactylus of last right walking leg. Scale bars: 5.0 mm (**a**); 1.0 mm (**b–e**).

Chelipeds broad, naked, covered with small flattened granules on dorsal surface, smooth ventrally. Merus with a small granular lobe on anterior margin, inner distal edge not produced. Carpus ca. 1½ times as long as broad, surface granulate, anterior margin produced into a broad, rounded lobe extending 2/3 of its length, and separated through an indentation from a shallow distal lobe; dorsal surface with a broad, longitudinal ridge. Posterior border convex, forming a granulate crest, ending distally in a rounded tooth. Chelae subequal, moderately large. Manus with a longitudinal ridge. Fingers blunt, pollex frequently longer than dactylus, outer margin convex, forming a rounded crest along entire length; dactylus longitudinally notched. Gape without pubescence.

Walking legs compact, with scattered simple setae. Merus unarmed, broad, flattened; carpus and propodus naked, crested above, dactylus with four movable spinules on inner border.

Telson with seven plates.

Coloration. The overall coloration of *P. virgilius* sp. nov. is pale brown to beige, the brown coloration prevailing on chelipeds and frontal half of carapace (Fig. 2). Carpus and manus of chelipeds frequently show scattered, dark-brown spots.

Ecology. *Petrolisthes virgilius* sp. nov. was found exclusively in intertidal formations of vermetid snails exposed to strong waves. The new species shares this habitat with *Neopisosoma angustifrons* (Benedict, 1901), *N. neglectum* Werding, 1986, and *Clastotoechus nodosus* (Streets, 1872). The latter species can also be found in other intertidal fouling incrustations in heavily wave-exposed rocky shores (Werding 1986).

Distribution. Colombia, Santa Marta and Gulf of Urabá regions.

Etymology. The new species is named after Dr. Virgilio Galvis Ramírez MD for his support and interest in our research on marine crabs, and for his contributions to medical sciences in Colombia.

Molecular analysis. The 496 bp alignment consisting of seven 16S rDNA sequences of *Petrolisthes virgilius* and two of *P. tonsorius* revealed two haplotypes within the new species, which differ only by one nucleotide. One haplotype was more frequent than the other and was shared by two individuals from Santa Marta and three from the Gulf of Urabá. The other haplotype was shared by two individuals from Santa Marta. The average of genetic distance within the new species was 0.2%. Distances between *P. virgilius* and *P. tonsorius* ranged between 9.6% and 10.2%. The average distance between populations of *P. tonsorius* from the Pacific and Caribbean was 4.8%.

Remarks. *Petrolisthes virgilius* can be distinguished morphologically from *P. tonsorius* and similar species by the marked granulation of carapace and extremities, the marked indentation of the anterior margin of the cheliped's carpus, and the accentuated crests of the chelipeds.

Discussion

The new species belongs to one of the morphological lines in the diverse and worldwide distributed genus *Petrolisthes*. This morphological line is strictly American and includes species lacking spines and dentations in carapace and extremities. In the West Atlantic this group is represented by two trans-isthmian species, *P. tridentatus* Stimpson, 1859, and *P. tonsorius* Haig, 1960, and by *P. quadratus* Benedict, 1901, *P. gertrudae* Werding, 1996, *P. hispaniolensis* Werding & Hiller, 2005, and now *P. virgilius*. Besides the two trans-isthmian species, the East Pacific members of this group are numerous, and are morphologically represented by the tropical *P. galapagensis* Haig, 1960, and a number of subtropical, warm-temperate and temperate species (see Haig 1960). Despite the close resemblance between *P. virgilius* and *P. tonsorius*, which led Werding (1978) to cautiously consider them conspecific, the large 16S genetic distance and distinguishing color and habitat confirms that they are different species. The genetic distance is double to that between individuals of *P. tonsorius* from each side of the Isthmus of Panama and surpasses by far the distance of 1.5% found by Hiller et al. (2006) for the trans-isthmian *P. armatus* Gibbes, 1850. The high molecular divergence between *P. virgilius* and *P. tonsorius* seems to be accompanied by species-specific coloration. However, intraspecific variation in color in *P. tonsorius* from each side of the Isthmus of Panama overlaps and therefore, in this case, color does not distinguish populations from each ocean. The crabs from both the East Pacific and West Atlantic display carapace and extremities that vary from brown to blueish. Haig (1960) described *P. tonsorius* from preserved specimens collected in the Galápagos Islands and surmised that they were blueish-colored in life. She wrote (p. 87): "After more than twenty years in alcohol, a few specimens show a dark blue-violet on the metabranchial regions of the carapace, ringed by a darker line of the same color; this shade of blue is also present on the eyestalks, movable segments of the antennae, walking legs, telson of the abdomen, and palps of the maxillipeds". Color and color pattern have been taxonomically reliable characters for distinguishing cryptic species of porcellanids (Hiller et al. 2006; Hiller and Werding 2007) and other anomurans like hermit crabs (Malay et al. 2012; Negri et al. 2014) and squat lobsters (Macpherson and Machordom 2001; Cabezas et al. 2011). However, in some species complexes in Anomura color may not vary interspecifically (Werding and Hiller 2017) or it can vary intraspecifically (Rodríguez-Flores et al. 2018).

Petrolisthes virgilius is ecologically unique compared to its morphological allies, typically occurring under intertidal boulders moderately to highly exposed to water movement. The only other species in association with living organisms is *P. gertrudae*, occasionally found on *Zoanthus sociatus* (Ellis, 1768) (see Werding 1996). The vermetid conglomerates where *P. virgilius* was found in Santa Marta and the Gulf of Urabá are cemented to a beachrock platform exposed to strong waves, which provide the vermetids and associated fauna with oxygen and nutrients. This singular vermetid habitat provides shelter to other porcellanid species of genera *Neopisosoma* Haig, 1960, and *Clastotoechus* Haig, 1960. The new species seems to have evolved in tight association to intertidal vermetid formations exposed to extremely strong water movement.

Including the new species, the genus *Petrolisthes* now comprises 111 species and the family Porcellanidae 305 species.

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