

# High species diversity of the soft coral family XenIIDae (Octocorallia, Alcyonacea) in the temperate region of Japan revealed by morphological and molecular analyses

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## Abstract

The soft coral family XenIIDae, commonly found in tropical and subtropical regions, consists of 20 genera and 162 species. To date, few studies on this family have been conducted in Japan, especially at higher latitudes. Although molecular phylogenetic analyses have recently been used to distinguish soft coral species, it is difficult to identify species and genera in this family due to the limited taxonomic indices and high morphological variation. In this study, we found a large XenIIDae community off the coast of Oshima Island (31°31.35'N, 131°24.27'E) at Miyazaki, Kyushu Island, located in the temperate region of Japan. The species composition and molecular phylogenetic relationships were investigated to uncover the species diversity of XenIIDae in this community. A total of 182 xeniid specimens were collected and identified to the species level, after which the samples were molecularly analyzed using a mitochondrial marker (ND2) and a nuclear marker (ITS) to infer the phylogenetic relationships. A total of 14 xeniid species were identified, including five undescribed species from five genera (*Anthelia*, *Heteroxenia*, *Symphodium*, *Xenia*, and *Yamazatum*). Miyazaki was identified as having the highest xeniid species diversity in Japan. The molecular phylogenetic trees inferred from each marker recovered very similar topologies: four genera (*Anthelia*, *Heteroxenia*, *Symphodium*, and *Yamazatum*) were monophyletic, whereas one (*Xenia*) was

polyphyletic. Thus, except for *Xenia*, the morphological characteristics used for traditional taxonomy well reflected the phylogeny of the Xeniidae at the genus level. On the other hand, our results show that further taxonomic revisions of *Xenia* are needed.

### Keywords

high latitude, Miyazaki, phylogeny, taxonomy, Xeniidae

## Introduction

Tropical marine animals, including zooxanthellate alcyonacean corals (i.e., soft corals) abundant in the southern part of the temperate region of Japan, due to the Kuroshio – a strong warm current running along the coast from the Ryukyu Archipelago to the mainland of Japan. However, studies looking into the zooxanthellate alcyonacean corals in Japan are limited, especially regarding the family Xeniidae Ehrenberg, 1828. Xeniidae comprises 20 genera and 162 species (Cordeiro et al. 2019), and is distributed mainly across the Red Sea and the Indian and Pacific Oceans. Additionally, a few species have been found in the south Atlantic (Kükenthal 1906) and Norwegian Sea (Koren and Danielssen 1883; Danielssen 1887; Grieg 1887; Jungersen 1892). Although 21 species from seven genera in this family have been recorded in Japan (Utinomi 1950, 1955, 1958; Imahara 1996; Benayahu 2010), its current species diversity remains unknown due to the lack of recent surveys.

The present study describes a large community of xeniids found around Oshima Island (31°31.35'N, 131°24.27'E) at Miyazaki, Kyushu Island. As xeniids are uncommon in Japan, this is an unusual community. Due to this area's higher latitude, coral reef structures are usually not formed, but there are over 100 zooxanthellate scleractinian coral species (Nishihira and Veron 1995). This area was occupied previously by zooxanthellate scleractinian corals, which were damaged drastically during the 1980s by outbreaks of the coral-eating gastropod *Drupella* spp. and the crown-of-thorns seastar *Acanthaster* sp. (Takayama and Shirasaki 1990). Currently, the area is occupied mainly by xeniids, which are known as pioneer alcyonaceans in ecological succession in tropical coral reefs (Benayahu and Loya 1987). Therefore, the ecological context of Oshima Island may represent an initial stage of secondary succession, following the drastic reduction of zooxanthellate scleractinian corals in the coral community. Identifying xeniid species diversity in this area is important to understand how coral communities change over time at higher latitudes.

Species identification difficulties are common among the anthozoans due to their limited key taxonomic characteristics and high morphological variation and plasticity. Recently, molecular phylogenetic analyses have been used to overcome such limitations. In particular, molecular phylogenetic data have been used frequently in scleractinian corals to revise taxonomy, identify cryptic species and describe new species (e.g., Budd et al. 2012; Huang et al. 2014a, b). In the alcyonacean corals, molecular phylogenetic analyses have also been applied to several families (France and Hoover 2002; McFadden and Hutchinson 2004; McFadden et al. 2009). For example, two genera,

*Sphaerasclera* McFadden & van Ofwegen, 2013 and *Parasphaerasclera* McFadden & van Ofwegen, 2013 and the family Parasphaerascleridae McFadden & van Ofwegen, 2013 were described based on the results of combined molecular phylogenetic and morphological analyses (McFadden and Ofwegen 2013). For xeniids, molecular phylogenetic analyses have been also performed at the genus level. Haverkort-Yeh et al. (2013) used molecular phylogenetic trees with mitochondrial (COI, mtMutS) and nuclear (ITS, ATP5 $\alpha$ ) markers, to show that *Anthelia* Lamarck, 1816, *Heteroxenia* K lliker, 1874, and *Sympodium* Ehrenberg, 1834 were genetically distinguishable from each other, whereas *Ovabunda* Alderslade, 2001 and *Xenia* Lamarck, 1816 were not. McFadden et al. (2014) also showed that *Anthelia*, *Cespitularia* Milne Edwards & Haime, 1850, and *Efflatounaria* Gohar, 1939 were genetically distant from all other xeniid genera, whereas *Ovabunda*, *Heteroxenia*, *Sansibia* Alderslade, 2000, and *Sarcothelia* Verrill, 1928 were paraphyletic with *Xenia* (COI, mtMutS, ND2, 28S rDNA). On the other hand, few molecular phylogenetic analyses have been performed in xeniids at the species level.

To date, the molecular data of xeniids indicate that mitochondrial ND2 marker is one of the best markers to infer the phylogenetic relationships among genera within many octocoral families (e.g., McFadden et al. 2006; McFadden et al. 2014), whereas nuclear ITS is a suitable marker to examine the octocorals' species-level relationships. In particular, the ITS marker has been used to investigate the relationships between closely related species in the soft coral genera such as *Alcyonium* Linnaeus, 1758 and *Pseudopterogorgia* K kenthal, 1919 (McFadden et al. 2001; McFadden and Hutchinson 2004; S nchez et al. 2007; Dorado and S nchez 2009). The present study aimed to investigate the current species diversity of the family XenIIDae around Oshima Island, Japan, and to clarify this family's taxonomic issues at the species level, through molecular phylogenetic analyses using ND2 and ITS markers.

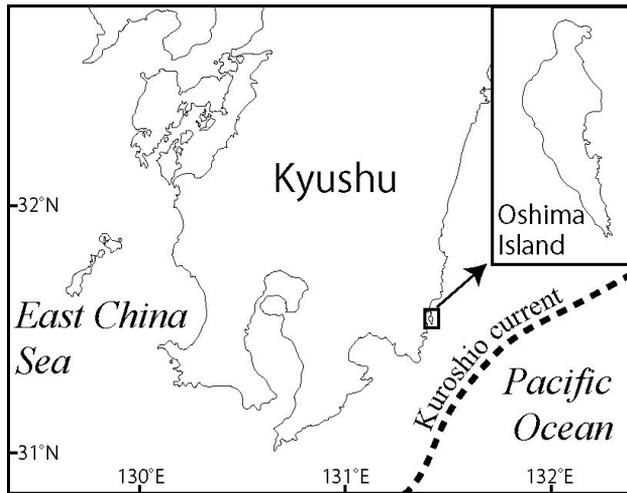
## Materials and methods

### Collection and identification of XenIIDae

Specimens of xeniids were collected around Oshima Island, Miyazaki, Japan (31°31.35'N, 131°24.27'E; Fig. 1) by SCUBA or snorkeling. A small piece of tissue (5–10 mm) from each specimen was put into CHAOS solution (sterile distilled water 100 ml, guanidine thiocyanate 50 g, N-lauroyl sarcosine sodium 0.5 g, 1M Tris pH8 2.5 mL, 2-mercaptoethanol 0.7 mL) (Fukami et al. 2004) for molecular analyses, and the remaining portions of specimens were preserved in 99% ethanol for morphological analyses.

### Species identification

For species identification, we first summarized the morphological characteristics for all species in the five genera we found in this study (*Xenia*, *Heteroxenia*, *Sympodium*,



**Figure 1.** Map of the sampling sites of specimens of Xeniididae.

*Yamazatum* Benayahu, 2010, and *Anthelia*) from original descriptions and related references to define the criteria for each species (Suppl. materials 1–5: Tables S1–S5), and used the summary to identify specimens at the species level. Table 1 shows a list of all specimens collected in this study. All specimens are deposited at Miyazaki University, Fisheries Sciences (MUFS) for coral collections (-C). Regarding specimen identification, the following morphological characteristics were measured or counted under stereo microscope: colony height, length and width of stalk, presence of branches, length and width of polyp, length and width of tentacle, length and width of pinnule, number of rows of pinnules, number of pinnules in the aboral row, sclerites form and sclerites size. In addition, microstructure of sclerites was observed by scanning electron microscope (SEM) (HITACHI Tabletop Microscope TM1000) as this morphological trait has been used recently to separate xeniid species (Janes and Mary 2012).

### DNA extraction, amplification, and sequencing

Tissue samples were kept in CHAOS solution for at least a week to dissolve proteins at room temperature. Total DNA was extracted from the CHAOS solution with tissue samples by conventional phenol/chloroform extraction method. We used the primers reported by McFadden et al. (2006) to amplify a fragment 5' end of the mitochondrial NADH-dehydrogenase subunit 2 gene (ND2) (16S647F: 5' -ACA CAG CTC GGT TTC TAT CTA CCA-3'; ND21418R: 5' -ACA TCG GGA GCC CAC ATA-3'). We also used two primers (1S: 5'-GGT ACC CTT TGT ACA CAC CGC CCG TCG CT-3'; 2SS: 5'-GCT TTG GGC GGC AGT CCC AAG CAA CCC GAC TC-3') (Wei et al. 2006) to amplify the internal transcribed spacer (ITS) of the nuclear riboso-

mal RNA gene. All PCR reactions contained 1  $\mu$ L of DNA solution, 1.6  $\mu$ L of 2.5 mM dNTP Mixture, 2  $\mu$ L of 10X *Ex Taq* buffer, 2  $\mu$ L of each 10 mM primer, *Ex taq* (TaKaRa) 0.08  $\mu$ L, and 11.32  $\mu$ L of sterile distilled water. Amplifications of these markers were performed (GeneQ PCR Thermal Cycler) with the following thermal profile; 35 cycles of 90 sec at 94 °C, 60 sec at 58 °C, 60 sec at 72 °C. Amplified fragments were checked on 1% agarose gel electrophoresis. All the PCR products were subjected to digest excess primers and inactivation of dNTP using Exonuclease I (TaKaRa) and Shrimp Alkaline Phosphatase (TaKaRa). These DNA sequences were determined by ABI3000 using a research contract service (Ltd. FASMAC).

### Sequence alignment and construction of phylogenetic trees

MEGA5 (Tamura et al. 2011) was used to manually align all the DNA sequences and to reconstruct phylogenetic trees. All indels were excluded from the analyses. Molecular phylogenetic trees were reconstructed using Neighbor-joining (NJ) method and maximum-likelihood (ML) method with model parameters (ND2: T92 + G, ITS: K2 + G) with 1000 bootstrap replicates. All the DNA sequences we obtained in this study were registered into DDBJ (accession nos. LC467016–LC467147).

## Results

### Identification of XenIIDae

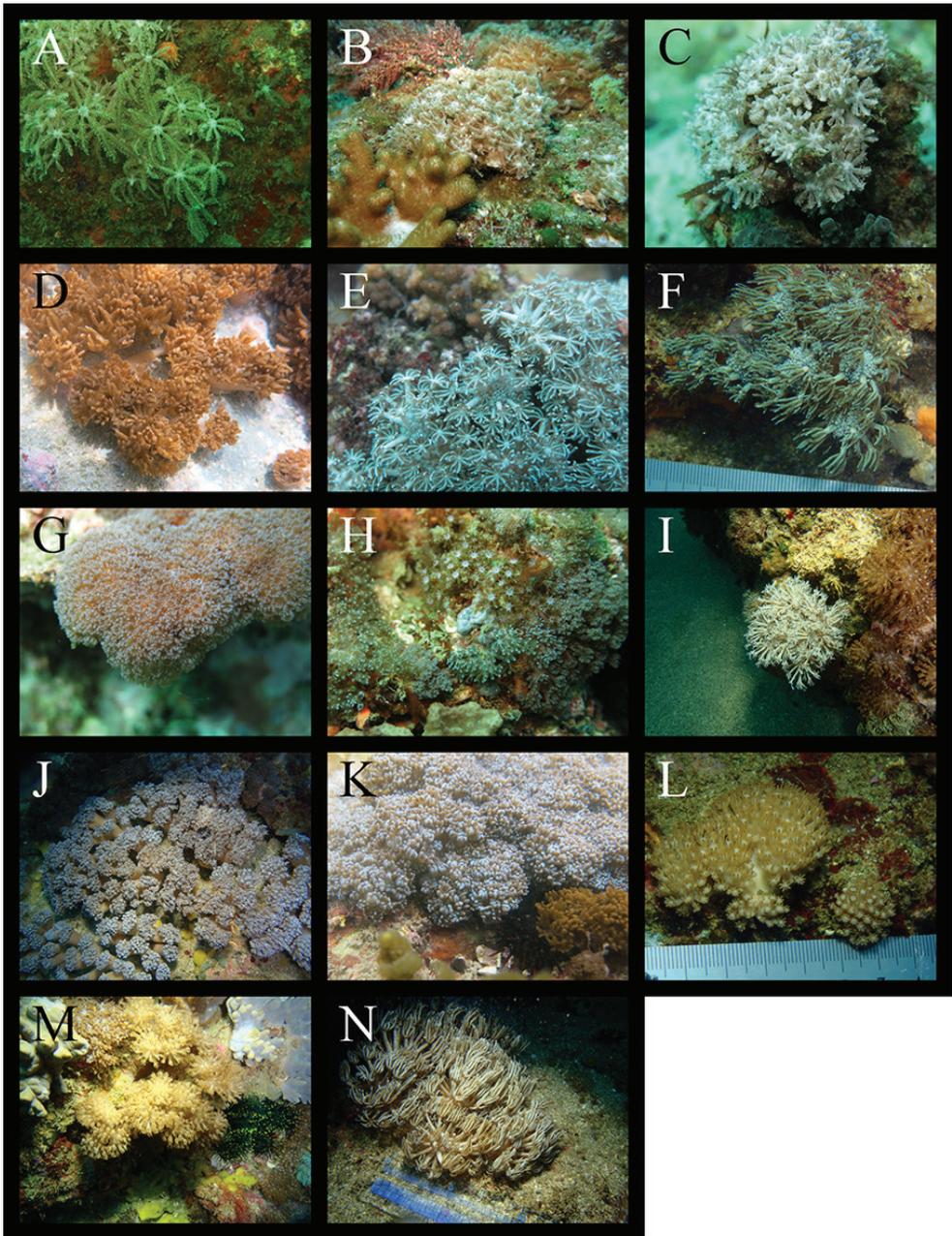
A total of 14 species from five genera in the family XenIIDae were identified: three species from *Anthelia*, three from *Heteroxenia*, two from *Sympodium*, five from *Xenia*, and one species from *Yamazatum* (Table 1). Since inconsistencies were found between the taxonomic morphological characteristics of some specimens and those of species described previously, those specimens were temporarily treated as either unidentified species (e.g., *Xenia* sp. 1), or closely related to specific species (e.g., *Heteroxenia* cf. *elisabethae*). Figures 2 and 3 show underwater photographs and optical microscope images of those species' sclerites. Among these, 12 species (*Xenia novaecaledoniae* Verseveldt, 1974, *X.* sp. 1, *X.* sp. 2, *Yamazatum* sp. 1, *Sympodium* sp. 1, *Sympodium* sp. 2, *Heteroxenia* cf. *elisabethae* K lliker, 1874, *H. medioensis* Roxas, 1933, *H. minuta* Roxas, 1933, *Anthelia* cf. *glauca* Lamarck, 1816, *A. rosea* Hickson, 1930, *A.* cf. *tosana* Utinomi, 1958) were first recorded in Japan. Additionally, we checked these species' sclerite microstructures (Fig. 4), as these have been used recently in the taxonomy of XenIIDae (Janes and Mary 2012). We observed that three out of five *Xenia* species (*X. plicata* Schenk, 1896, *X.* sp. 1, *X.* sp. 2) exhibited the typical genus microstructure (i.e., dendritic rods) whereas the remaining two species (*X. kuekenthali* Roxas, 1933 and *X. novaecaledoniae*) presented no sclerites. Further-

**Table 1.** Octocoral specimens for which partial ND2 and ITS sequences were obtained. MUFS-C: Miyazaki University, Fisheries Science for coral collections. NA: Not Analyzed.

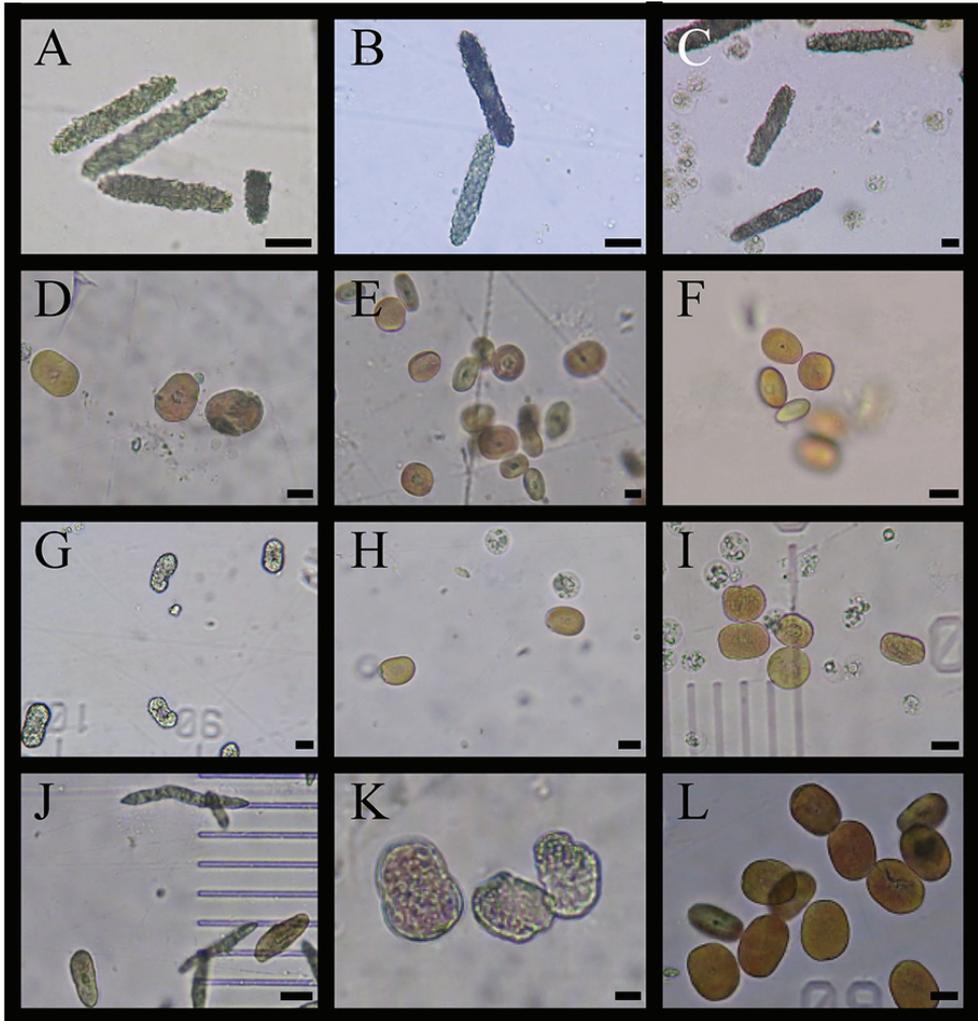
Family	Species	Specimen Catalog #	Date	Depth (m)	GenBank #		
					ND2	ITS	
Xeniidae	<i>Anthelia cf. glauca</i>	MUFS-COMO18	2012.7.2	4.3	LC467016	NA	
		MUFS-COMO67	2012.12.25	<10	LC467017	LC467102	
		MUFS-COMO70	2012.12.25	<10	LC467018	NA	
	<i>Anthelia rosea</i>	MUFS-COTUN6	2014.12.3	<15	LC467019	LC467103	
	<i>Anthelia cf. tosana</i>	MUFS-COMO13	2012.7.2	<5	LC467020	NA	
	<i>Heteroxenia cf. elisabethae</i>	MUFS-COSU2	2012.5.5	<1	LC467021	LC467104	
		MUFS-COSU3	2012.5.5	<1	LC467022	LC467105	
	<i>Heteroxenia medioensis</i>	MUFS-COOTUC4	2014.12.3	<15	LC467023	LC467106	
		MUFS-COOTUE3	2014.12.3	<15	LC467024	LC467107	
	<i>Heteroxenia minuta</i>	MUFS-COMO10	2012.7.2	3.7	LC467025	LC467108	
		MUFS-COMO12	2012.7.2	5.0	LC467026	LC467109	
		MUFS-COMO28	2012.8.31	<10	LC467027	LC467110	
	<i>Sympodium</i> sp. 1	MUFS-COMO63	2012.12.25	<10	LC467028	LC467111	
		MUFS-COOTUG2	2014.12.3	<15	LC467029	LC467112	
		MUFS-COOTUK16	2014.12.3	<15	LC467030	LC467113	
	<i>Sympodium</i> sp. 2	MUFS-COMO149	2013.7.30	<10	LC467031	LC467114	
	<i>Xenia</i> sp. 1	MUFS-COMO100	2012.12.25	<10	LC467032	LC467115	
		MUFS-COMO154	2013.7.30	<10	LC467033	LC467116	
		MUFS-COMO166	2013.7.30	<10	LC467034	LC467117	
		MUFS-COMO4	2012.7.2	<5	LC467035	LC467118	
		MUFS-COMO53	2012.12.25	<10	LC467036	LC467119	
		MUFS-COMO54	2012.12.25	<10	LC467037	LC467120	
		MUFS-COMO64	2012.12.25	<10	LC467038	LC467121	
		MUFS-COMO68	2012.12.25	<10	LC467039	LC467122	
		MUFS-COMO76	2012.12.25	<10	LC467040	LC467123	
		MUFS-COMO77	2012.12.25	<10	LC467041	LC467124	
		MUFS-COMO82	2012.12.25	<10	LC467042	LC467125	
		MUFS-COMO83	2012.12.25	<10	LC467043	LC467126	
		MUFS-COMO85	2012.12.25	<10	LC467044	NA	
		<i>Xenia kuekenthali</i>	MUFS-COMO11	2012.7.2	2.9	LC467045	NA
			MUFS-COMO3	2012.7.2	3.9	LC467046	LC467127
			MUFS-COMO87	2012.12.25	<10	LC467047	NA
		<i>Xenia novaecaledoniae</i>	MUFS-COMO152	2013.7.30	<10	LC467048	NA
			MUFS-COMO155	2013.7.30	<10	LC467049	NA
	MUFS-COMO5		2012.7.2	3.2	LC467050	LC467128	
	<i>Xenia plicata</i>	MUFS-COMO65	2012.12.25	<10	LC467051	LC467129	
MUFS-COKMG3		2014.12.3	<3	LC467052	NA		
MUFS-COMO148		2013.7.30	<10	LC467053	LC467130		
MUFS-COMO15		2012.7.2	4.6	LC467054	LC467131		
MUFS-COMO2		2012.7.2	4.8	LC467055	LC467132		
MUFS-COMO26		2012.8.31	<10	LC467056	NA		
MUFS-COMO40		2012.12.25	<10	LC467057	LC467133		
MUFS-COMO50		2012.12.25	<10	LC467058	LC467134		
MUFS-COMO69		2012.12.25	<10	LC467059	LC467135		
MUFS-COMO7		2012.7.2	4.0	LC467060	LC467136		
MUFS-COMO80	2012.12.25	<10	LC467061	LC467137			

Family	Species	Specimen Catalog #	Date	Depth (m)	GenBank #			
					ND2	ITS		
Xenidiidae	<i>Xenia</i> sp. 2	MUFS-COMO161	2013.7.30	<10	LC467062	NA		
		MUFS-COMO165	2013.7.30	<10	LC467063	LC467138		
		MUFS-COMO6	2012.7.2	4.2	LC467064	NA		
		MUFS-COMO8	2012.7.2	3.2	LC467065	LC467139		
		MUFS-COMO9	2012.7.2	3.9	LC467066	LC467140		
		MUFS-COSU1	2012.5.5	<1	LC467067	NA		
		MUFS-COSU4	2012.5.5	<1	LC467068	LC467141		
		MUFS-COSU5	2012.5.5	<1	LC467069	NA		
		MUFS-COSU6	2012.5.5	<1	LC467070	NA		
		<i>Yamazatum</i> sp. 1	MUFS-COMO1	2012.7.2	4.7	LC467071	LC467142	
			MUFS-COMO14	2012.7.2	3.7	LC467072	LC467143	
			MUFS-COMO147	2013.7.30	<10	LC467073	NA	
			MUFS-COMO162	2013.7.30	<10	LC467074	NA	
	MUFS-COMO42		2012.12.25	<10	LC467075	NA		
	MUFS-COMO45		2012.12.25	<10	LC467076	NA		
	Briareidae	<i>Briareum</i> sp.	MUFS-COMO48	2012.12.25	<10	LC467077	NA	
			MUFS-COMO73	2012.12.25	<10	LC467078	NA	
			MUFS-COMO89	2012.12.25	<10	LC467079	NA	
			MUFS-COMO17	2012.7.2	3.7	LC467080	NA	
Clavulariidae			<i>Clavularia</i> sp.	MUFS-COAK6	2012.6.5	<3	LC467081	NA
Alcyoniidae			<i>Cladiella pachyclados</i>	MUFS-COSU13	2012.5.5	<1	LC467082	LC467144
				MUFS-COSU14	2012.5.5	<1	LC467083	LC467145
				MUFS-COAK1	2012.6.5	<3	LC467084	LC467146
			<i>Cladiella sphaerophora</i>	MUFS-COAK5	2012.6.5	<3	LC467085	LC467147
				<i>Klyxum okinawanum</i>	MUFS-COMO150	2013.7.30	<10	LC467086
	MUFS-COMO164	2013.7.30	<10		LC467087	NA		
	<i>Klyxum</i> sp.	MUFS-COOTUD8	2014.12.3	<15	LC467088	NA		
		<i>Sarcophyton</i> sp.	MUFS-COAK7	2012.6.5	<3	LC467089	NA	
			MUFS-COSU16	2012.5.5	<1	LC467090	NA	
		<i>Simularia</i> sp.	MUFS-COAK2	2012.6.5	<3	LC467091	NA	
			MUFS-COAK3	2012.6.5	<3	LC467092	NA	
			MUFS-COAK4	2012.6.5	<3	LC467093	NA	
			MUFS-COAK8	2012.6.5	<3	LC467094	NA	
MUFS-COAK9			2012.6.5	<3	LC467095	NA		
Nephtheidae		<i>Dendronephthya rigida</i>	MUFS-COSS4	2012.5.29	<5	LC467096	NA	
			MUFS-COSS1	2012.5.29	<5	LC467097	NA	
	MUFS-COSS2		2012.5.29	<5	LC467098	NA		
	<i>Dendronephthya gigantea</i>	MUFS-COSS3	2012.5.29	<5	LC467099	NA		
		MUFS-COSU15	2012.5.5	<1	LC467100	NA		
		<i>Stereonephthya rubriflora</i>	MUFS-COAK10	2012.6.6	<10	LC467101	NA	
			<i>Stereonephthya japonica</i>					

more, we found that all three *Heteroxenia* species (*H. cf. elisabethae*, *H. medioensis* and *H. minuta*) exhibited similar microstructures to *Xenia* spp. None of these specimens presented sclerites, comprising aggregations of minute corpuscular-shaped microscleres (Alderslade 2001), which is a specific characteristic of *Ovabunda*, a genus related closely to *Xenia*. Two *Symphodium* species presented a very specific microstruc-



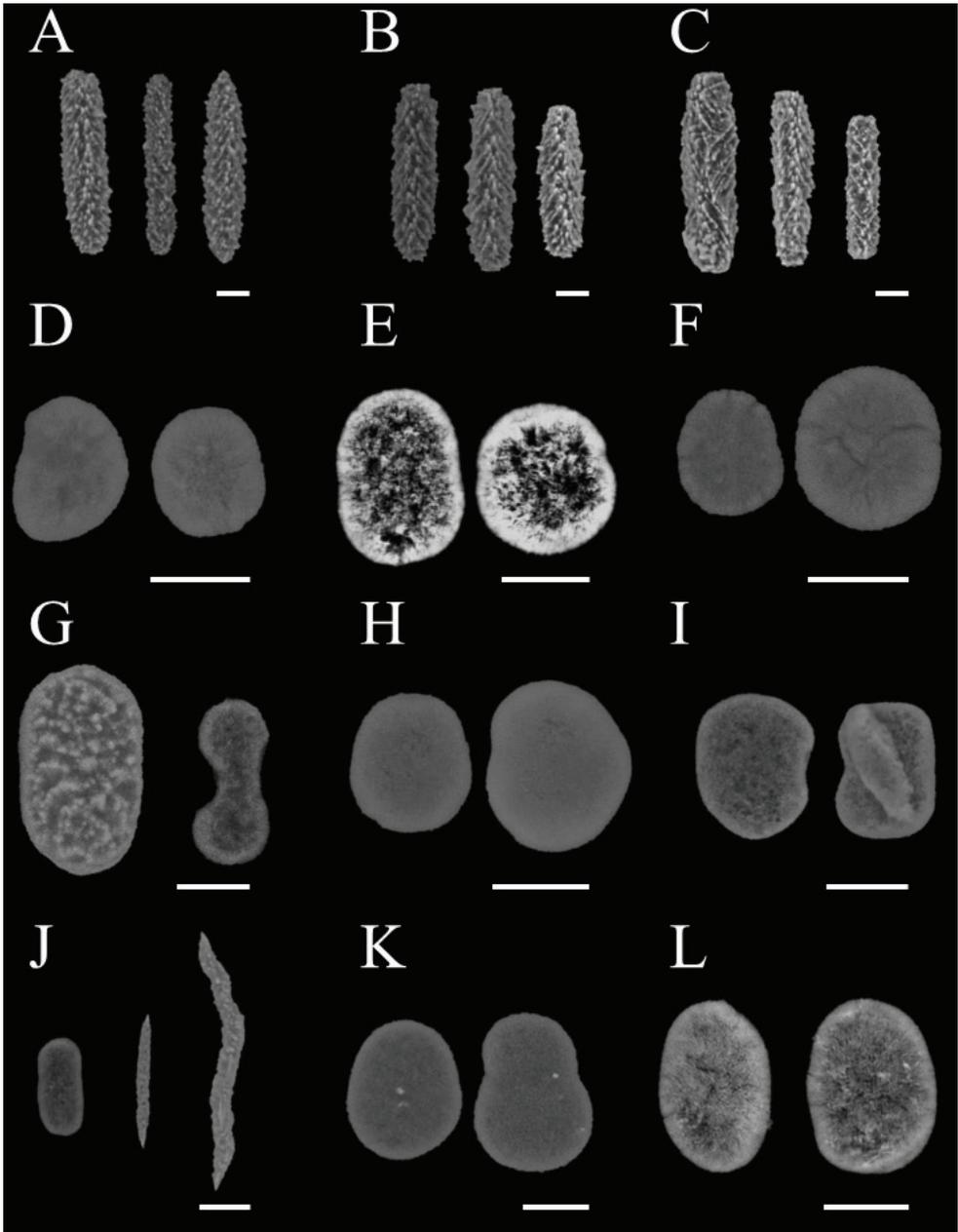
**Figure 2.** Living form of Xeniidae. **A** *Anthelia* cf. *glauca* **B** *A. rosea* **C** *A.* cf. *tosana* **D** *Heteroxenia* cf. *elisabethae* **E** *H. medioensis* **F** *H. minuta* **G** *Sympodium* sp. 1 **H** *S.* sp. 2 **I** *Yamazatum* sp. 1 **J** *Xenia* sp. 1 **K** *X.* sp. 2 **L** *X. novaecaledoniae* **M** *X. kuekenthali* **N** *X. plicata*.



**Figure 3.** Sclerites of Xenidiidae. **A** *Anthelia* cf. *glauca* **B** *A. rosea* **C** *A.* cf. *tosana* **D** *Heteroxenia* cf. *elizabethae* **E** *H. medioensis* **F** *H. minuta* **G** *Symphodium* sp. 1 **H** *S.* sp. 2. **I** *Yamazatum* sp. 1 **J** *Xenia* sp. 1 **K** *X.* sp. 2 **L** *X. plicata*. Scale bars: 10  $\mu$ m.

ture (see below). *Yamazatum* sp. 1 exhibited the typical sclerite architecture (crests on sclerites' surface) of this genus (Fig. 4I).

In the present study, *Xenia* sp. 1, *X.* sp. 2, *Yamazatum* sp. 1, *Symphodium* sp. 1 and *S.* sp. 2 were identified as undescribed species for the following reasons: *Xenia* sp. 1 shared common morphological characteristics with the genus *Xenia*, such as the colony shape and the presence of oval sclerites, but presented also with unique needlelike sclerites with many small spines (Fig. 4J), which have never been re-

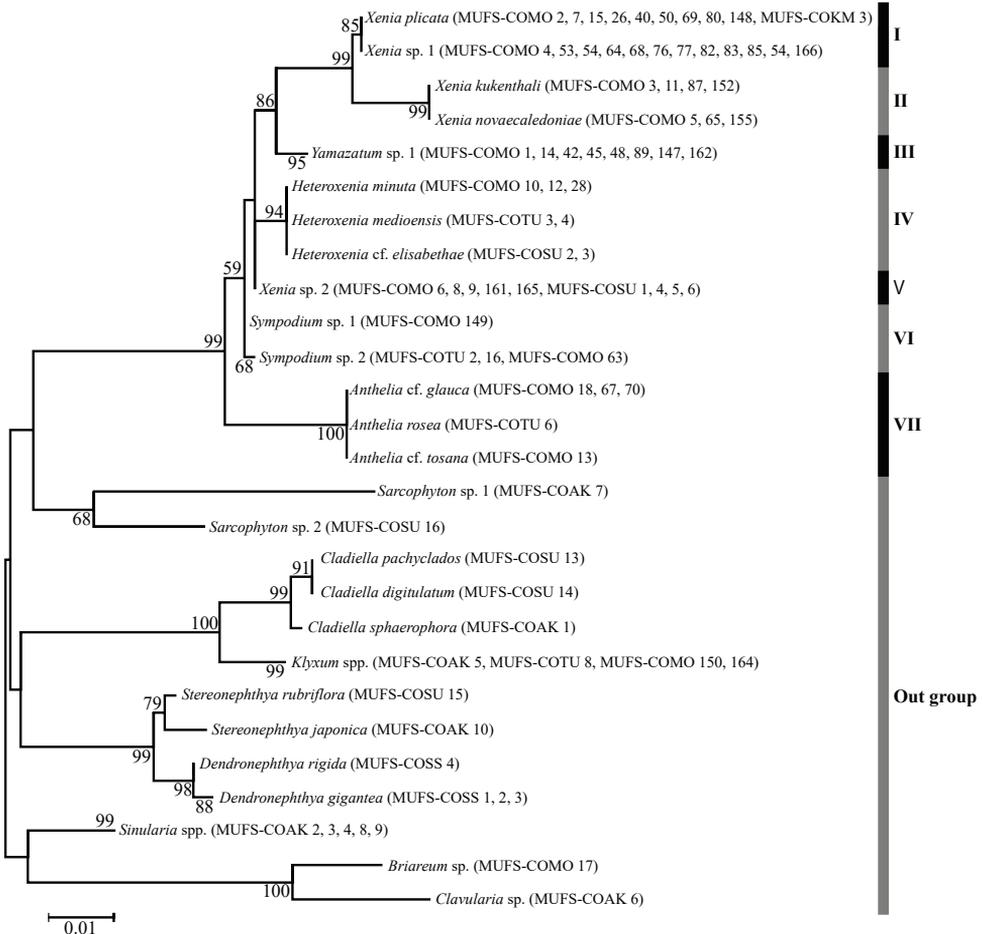


**Figure 4.** Scanning electron micrographs of sclerites of Xeniiidae. **A** *Anthelia* cf. *glauca* **B** *A. rosea* **C** *A.* cf. *tosana* **D** *Heteroxenia* cf. *elisabethae* **E** *H. medioensis* **F** *H. minuta* **G** *Sympodium* sp. 1 **H** *S.* sp. 2 **I** *Yamazatum* sp. 1 **J** *Xenia* sp. 1 **K** *X.* sp. 2 **L** *X. plicata*. Scale bar: 10  $\mu$ m.

ported in *Xenia*. *Xenia* sp. 2 was easily distinguishable from other *Xenia* species, as it presented many short branches extending from the top of colony, becoming hump-shaped (Fig. 2K). *Yamazatum* is a monotypic genus containing *Y. iubatum* Benayahu, 2010 and presenting two specific morphological characteristics: double-headed sclerites and a conspicuous crest on the sclerites' surface. *Yamazatum* sp. 1 presented a crest on the sclerites' surface (Fig. 4I) but lacked double-headed sclerites; in this species the sclerites were found only in the polyps, opposite to *Y. iubatum*, containing sclerites both in the surface and interior of the stalk layer and in the polyps. Furthermore, this species presents a branching stalk (Fig. 2I), opposite to *Y. iubatum*, which has a non-branching stalk. *Sympodium* sp. 1 and *S.* sp. 2 shared the common morphological characteristics of the genus *Sympodium*, such as a thin stolon-like sheet and no stalks in colony (Fig. 2G, H). However, both species found in this study presented unique sclerites, which differed from all eight known *Sympodium* species. *Sympodium* sp. 1 presented two types of sclerites; one a double-headed sclerite, typical from *Y. iubatum*, located in the polyps (Fig. 4G), and an oval sclerite with protrusions like a mountain range, located on the coenenchyme (Fig. 4G). *Sympodium* sp. 2 presented disk-shaped sclerites throughout the whole colony, with smooth surfaces and no protrusions (Fig. 4H). Under a light microscope the sclerites of *Sympodium* sp. 1 were mostly colorless, whereas those of *Sympodium* sp. 2 were light brown (Fig. 3G, H).

### Molecular phylogenetic analyses

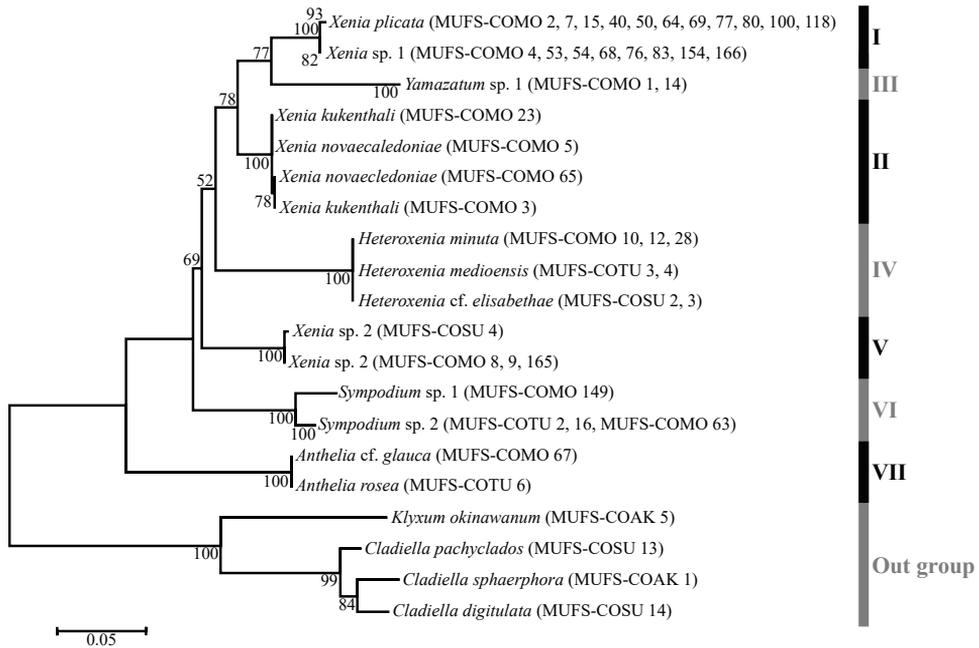
From the collected 14 species (78 samples), we obtained 673–707 bases of ND2 and 910–1039 bases of ITS. Molecular phylogenetic trees using the NJ and ML methods showed very similar topologies. Therefore, in this study, only ML trees for each marker are shown (Figs 5, 6). These trees showed that the family Xenidiidae was monophyletic in the Alcyonacea, and that the xeniid species were separated into seven clades. Clade I included *Xenia plicata* and *X.* sp. 1. Although the ND2 tree showed an absence of genetic differences between these two species (Fig. 5), the ITS tree showed that they were clearly separated from each other (Fig. 6). Clade II included *X. kuekenthali* and *X. novaecaledoniae*, and clade III included only one species, *Yamazatum* sp. 1. The ND2 tree showed that clade III formed a sister group with clades I and II with *Xenia* spp., whereas the ITS tree showed that clade III formed a sister group with only clade I. Clade IV contained all three *Heteroxenia* species (*H.* cf. *elisabethae*, *H. medioensis*, *H. minuta*). Clade V contained a single species *X.* sp. 2. Clades VI and VII contained *Sympodium* spp. and *Anthelia* spp., respectively. Thus, four genera (*Anthelia*, *Heteroxenia*, *Sympodium*, and *Yamazatum*) were monophyletic (clades III, IV, VI, VII) whereas *Xenia* was polyphyletic (clades I, II, V) because clades III and IV with *Heteroxenia* and *Yamazatum* were included within clades of *Xenia*.



**Figure 5.** Phylogenetic relationships of species in XenIIDae based on ND2 sequences. Numbers on main branches show percentages of bootstrap values (> 50%) in maximum likelihood analysis.

## Comparison between phylogenetic relationships and morphological characteristics

In the present study, except *Xenia*, all genera were monophyletic (clades III, IV, VI, VII). Therefore, the synapomorphy reflecting each of the four clades is consistent with the key morphological characteristics for each genus. On the other hand, only *Xenia* was polyphyletic (clades I, II, and V). Therefore, to determine the synapomorphy for each clade, the morphological characteristics of the species in these three clades were compared. In clade I, including *X. plicata* and *X. sp. 1*, the synapomorphy is a colony form 25–40 mm in height and without secondary branches. Clade II, including *X. novaecaledoniae* and *X. kuekenthali*, presented a colony form similar to clade I (typical and no secondary branches), but shorter (10–20 mm in height). It is noteworthy that, although the family XenIIDae is taxonomically defined as presenting oval sclerites, both



**Figure 6.** Phylogenetic relationships of species in Xeniidae based on ITS sequences. Numbers on main branches show percentages of bootstrap values (> 50%) in maximum likelihood analysis.

species in clade II lacked sclerites. Clade V, with just *X. sp. 2*, was characterized by a unique colony form, comprising a stalk measuring about 10 mm high and 20 mm in diameter, and many short branches extending from the top, becoming hump-shaped. This type of colony form has not been reported previously in the genus *Xenia*.

## Discussion

### High species diversity in the family Xeniidae in Miyazaki

The present study identified 14 species from five genera in the family Xeniidae around Oshima Island, Miyazaki, in Japan. Among these species, 12 (*Anthelia cf. glauca*, *A. rosea*, *A. cf. tosana*, *H. cf. elisabethae*, *H. minuta*, *H. medioensis*, *Sympodium* sp. 1, *S. sp. 2*, *Xenia novaecaledoniae*, *X. sp. 1*, *X. sp. 2* and *Yamazatum* sp. 1) were recorded in Japan for the first time, including five undescribed species (*Sympodium* sp. 1 and *S. sp. 2*, *Xenia* sp. 1, *Xenia* sp. 2 and *Yamazatum* sp. 1). On the other hand, two genera, *Fungulus* Tixier-Durivault, 1970 and *Cespitularia*, recorded previously in Japan (Utinomi 1977; Imahara 1991; Benayahu 1995, 2010) were not found in Oshima Island.

Miyazaki has the highest Xeniidae species diversity in Japan (Table 2; Suppl. material 6: Table S6). Taking together the results from the present study and those from two

**Table 2.** Distribution of XenIIDae by country. List of number of species and genera of the family XenIIDae, previously reported in the world. See Suppl. material 6: Table S6 for reference numbers in referece.

Location	Number of species	Number of genera	References
Philippines	42	5	15, 16, 26, 27, 29, 32
Indonesia	38	7	2, 12, 13, 16, 18, 21, 26, 28, 30, 32, 36
Red Sea	35	6	3, 10, 11, 12, 13, 16, 21, 24, 25, 26, 28, 30, 39, 40, 41, 44, 45, 46
Japan	32	8	4, 5, 14, 15, 16, 23, 32, 34, 35, 37, This study
Miyazaki Prefecture	14	5	This study
Nansei Islands	13	7	4, 5, 14, 15, 16, 37
Mainland of Japan (Honshu, Shikoku and Kyushu)	8	3	16, 23, 32, 34, 35
Australia	27	8	1, 13, 15, 16, 21, 30, 43
Tanzania	17	6	13, 16, 21, 30, 31
Taiwan	13	6	7, 8, 16, 32, 33
Seychelles	11	5	12, 17, 18
New Caledonia	7	3	15, 16, 18, 21, 42
Mozambique	6	4	13, 18, 21, 31, 32
Papua New Guinea	6	2	13, 21, 30, 31, 47
Fiji	6	2	1, 13, 21, 28
Palau	4	3	16, 18, 32
Malay	4	3	1, 16
Chagos Archipelago	4	3	1, 12, 21, 26, 31
Cargados Carajos	3	3	31
Norwegian Sea	3	2	9, 19, 20
Madagascar	2	2	12, 16
Kenya	2	2	30
Tonga	2	2	13, 21, 28
Republic of South Africa	2	2	21, 22
Sri Lanka	2	1	13
Singapore	1	1	6
Samoa	1	1	28
New Zealand	1	1	11
Hong Kong	1	1	38
Korea	1	1	16, 21, 31
Antarctic Ocean	1	1	13, 21, 22
Guam	1	1	18

previous reports (Imahara 1996; Benayahu 2010), eight genera and 32 species have been confirmed in Japan, the fourth highest XenIIDae diversity in the world (Table 2). Considering that the top three regions are tropical coral reef regions (Philippines, Red Sea, and Indonesia), XenIIDae has a relatively higher species diversity in Japanese waters than in the other regions listed in Table 2, despite its higher latitude. One reason behind this may be the larval supply from the tropics, brought by the strong warm Kuroshio Current that flows from the Philippines (with many coral reefs) up to Kyushu Island including Oshima Island, and the mainland of Japan.

## Ecological succession in temperate coral communities

Alcyonacean corals (soft corals) have been known as pioneers in coral reefs (Benayahu and Loya 1987; Fabricius 1995), as well as negative indicators of the early developmental processes of the zooxanthellate scleractinian corals (Maida et al. 1995, 2001). Thus, alcyonacean corals play an important role for ecological succession in coral reefs. Around Oshima Island, zooxanthellate scleractinian corals were dominant until the 1980s, probably representing the late stage of ecological succession in the coral community. Subsequently, these corals were damaged by *Drupella* spp. and *Acanthaster* sp. (Takayama and Shirasaki 1990). Currently, many zooxanthellate alcyonacean corals inhabit the top of dead coral skeletons, which may represent the initial stage of the secondary ecological succession in this coral community. In fact, Endean (1976) reported that Alcyonacea attached onto dead coral skeletons after feeding damage by *Acanthaster* sp. One of the most dominant alcyonacean corals in Oshima Island is XenIIDae, which may be related to its faster growth, rapid colony migration and asexual reproduction (Benayahu and Loya 1985). Although no species diversity data pertaining to hard and soft corals are currently available from the time when hard corals were dominant, the fact that the three-dimensional structures constructed by the zooxanthellate scleractinian corals are gone, suggests that the biota in Oshima Island might have been dramatically different than the present one. Therefore, it would be worthwhile to continuously investigate the change of biota in this area, to understand the process of ecological succession of the benthic and coral community at this higher latitudinal region.

## Phylogeny and taxonomy of the XenIIDae

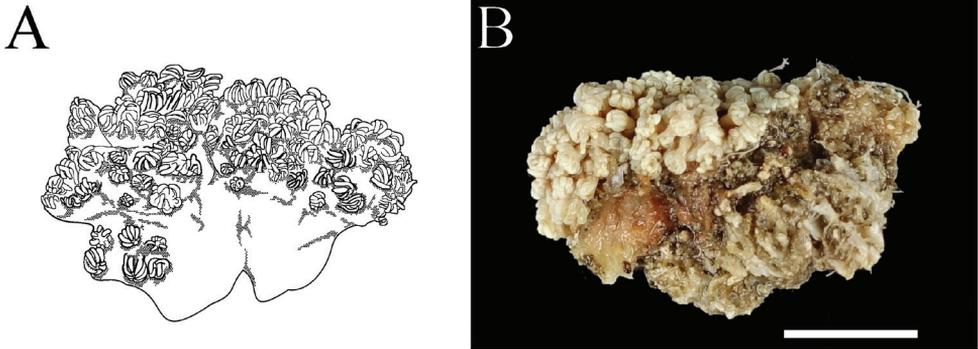
*Heteroxenia* and *Yamazatum* were monophyletic, although *Xenia* were closely related to both genera (Figs 5, 6). Although *Heteroxenia* presents dimorphic polyps composed of autozooids (normal polyps) and siphonozooids (i.e., no tentacles in polyps, but functional for inhalation and discharge of seawater), siphonozooids only develop when the colony is sexually mature (Gohar 1940; Fabricius and Alderslade 2001). Thus, *Heteroxenia* and *Xenia* can only be superficially distinguished during the breeding season, since during the non-breeding season *Heteroxenia* contains one type of polyp only (autozooids). The present study shows that *Xenia* and *Heteroxenia* can be clearly separated in the molecular trees, although some colonies of *Heteroxenia* were found not to form siphonozooids. These colonies were morphologically identified as *Heteroxenia*, based on the colony size and shape, the autozooids, the pinnules and the sclerites, despite the occurrence of dimorphic polyps. Although the presence or absence of siphonozooids, an important morphological characteristic for Alcyonacea's generic classification, was confirmed for *Xenia* and *Heteroxenia*, molecular phylogenetic analyses of all the 11 species of *Heteroxenia* are necessary to properly define the taxonomic position of this genus.

In the present study, the phylogenetic position of *Yamazatum* sp. 1 was ambiguous as this species formed a sister group with clade I in the ND2 tree (Fig. 5), and with both clades I and II in the ITS tree (Fig. 6). Currently, several xeniid genera, including *Yamazatum* are taxonomically classified based only on sclerite surface microstructure (*Bayerxenia* Alderslade, 2001; *Ingotia* Alderslade, 2001; *Ixion* Alderslade, 2001; *Orangaslia* Alderslade, 2001; *Ovabunda*; *Fasciclia* Janes, 2008; *Conglomeratusclera* Benayahu et al., 2018; *Caementabunda* Benayahu et al., 2018; and *Yamazatum*). Although most of these genera have never been analyzed molecularly, a recent molecular phylogenetic analysis revealed that *Ovabunda* belonged to the same clade as *Xenia* (Haverkort-Yeh et al. 2013; McFadden et al. 2014), which, in the present study, is also in the clade of *Yamazatum*. Therefore, detailed comparisons between molecular data and the sclerite microstructure will be needed for future xeniid taxonomic classification.

*Xenia* was polyphyletic, particularly due to *X.* sp. 2 (Figs 5, 6). Clade V with *X.* sp. 2 was closer to clade IV with *Heteroxenia* than other *Xenia* clades (clades I and II). *Xenia* sp. 2 exhibited slight but substantial differences from its congeners in terms of colony morphology, as their colony shapes lacked branching, exhibiting dome-shaped protrusions (Fig. 7). Considering that *Heteroxenia* presents specific characteristics that distinguish it from *Xenia*, such as dimorphic polyps, the species *X.* sp. 2 may be assigned to a new genus, although this requires further investigations into the morphological characteristics of other genera not observed in present study.

Two undescribed species, *S.* sp. 1 and *S.* sp. 2, were found in *Sympodium*, and presented different sclerites and microstructure types (Fig. 4) from their congeners. Currently, this genus has only eight species, *S. abyssorum* Danielssen, 1887, *S. caeruleum* (Ehrenberg, 1834), *S. fuliginosum* Ehrenberg, 1834, *S. hyalinum* Grieg, 1887, *S. norvegicum* Koren & Danielssen, 1883, *S. punctatum* May, 1898, *S. splendens* Thomson & Henderson, 1906 and *S. tamatavense* (Cohn, 1908). Their type localities are the Red Sea for *S. caeruleum* and *S. fuliginosum*, Norwegian Sea for *S. abyssorum*, *S. hyalinum* and *S. norvegicum*, Indian Ocean *S. punctatum* and *S. splendens*, and Madagascar for *S. tamatavense*. Except for *S. caeruleum*, all species have never been recorded in the Pacific region, probably due to the lack of research into this genus. Therefore, more species are likely to be found in the Pacific region in the future.

Studies on the species composition and biodiversity of alcyonacean corals have drawn considerably less attention than those on scleractinian corals, since alcyonacean corals do not form the same three-dimensional structures with their hard skeletons as scleractinian corals, and, therefore, provide less habitat for other animals. However, coral communities have been reported to shift from scleractinian corals to alcyonacean corals in the future, if ocean acidification persists (Inoue et al. 2013). Thus, further ecological and taxonomic studies of alcyonacean corals are needed. Although the current taxonomic classification of alcyonacean corals is still underdeveloped, this may be improved by further molecular analyses and accurate species identification will improve this situation.



**Figure 7.** *Xenia* sp. 2. **A** schema of *Xenia* sp. 2 **B** photo of a specimen of *Xenia* sp. 2 (MUFS-COMO9). Scale bar: 10 mm.

## Acknowledgements

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### Supplementary material 1

#### Table S1. Morphological features and key characters for species identification of *Xenia*

Authors: Tatsuki Koido, Yukimitsu Imahara, Hironobu Fukami

Data type: morphological data

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### Supplementary material 2

#### Table S2. Morphological features and key characters for species identification of *Yamazatum*

Authors: Tatsuki Koido, Yukimitsu Imahara, Hironobu Fukami

Data type: morphological data

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### Supplementary material 3

#### Table S3. Morphological features and key characters for species identification of *Heteroxenia*

Authors: Tatsuki Koido, Yukimitsu Imahara, Hironobu Fukami

Data type: morphological data

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Link: <https://doi.org/10.3897/zookeys.862.31979.suppl3>

## Supplementary material 4

### Table S4. Morphological features and key characters for species identification of *Sympodium*

Authors: Tatsuki Koido, Yukimitsu Imahara, Hironobu Fukami

Data type: morphological data

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Link: <https://doi.org/10.3897/zookeys.862.31979.suppl4>

## Supplementary material 5

### Table S5. Morphological features and key characters for species identification of *Anthelia*

Authors: Tatsuki Koido, Yukimitsu Imahara, Hironobu Fukami

Data type: morphological data

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## Supplementary material 6

### Table S6. List of references used in Table 2

Authors: Tatsuki Koido, Yukimitsu Imahara, Hironobu Fukami

Data type: reference data

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# Size variation and geographical distribution of the luminous earthworm *Pontodrilus litoralis* (Grube, 1855) (Clitellata, Megascolecidae) in Southeast Asia and Japan

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## Abstract

The luminous earthworm *Pontodrilus litoralis* (Grube, 1855) occurs in a very wide range of subtropical and tropical coastal areas. Morphometrics on size variation (number of segments, body length and diameter) and genetic analysis using the mitochondrial cytochrome c oxidase subunit 1 (COI) gene sequence were conducted on 14 populations of *P. litoralis* from Southeast Asia and Japan. Statistical inference on morphometric data revealed significantly different size variations in the body length and diameter among these 14 populations of *P. litoralis*. However, discordance between the morphometric and mitochondrial COI gene-based phylogenetic analyses was evident, where the size variations in *P. litoralis* showed a different pattern from the COI genetic differences. The update on the current distribution of *P. litoralis* is reported and revealed different aspects of the littoral habitat characteristics between Southeast Asia and Japan.

## Keywords

COI, habitat, morphometrics, phylogeny

## Introduction

Earthworms are considered as both ecosystem engineers (Jones et al. 1994) and keystone species (Blondel and Aronson 1995), and they function as decomposers, consumers, and food resources for animals (Lavelle et al. 1992). Earthworms are terrestrial oligochaetes (Annelida, Clitellata), except for a few semi-aquatic taxa, such as earthworms in the family Almididae and *Eiseniella tetraedra* (Savigny, 1826) in freshwater habitats, and *Pontodrilus litoralis* (Grube, 1855), *P. primoris* Blakemore, 2000, and *P. longissimus* Seesamut and Panha, 2018 in marine littoral habitats (Blakemore 2007; Seesamut et al. 2018).

*Pontodrilus litoralis* has a wide distribution in the tropical and subtropical coastal habitats of the Atlantic, Indian, and Pacific oceans. In Thailand, the first record of the littoral earthworm *P. litoralis* was from Khanom District, Nakhon Si Thammarat Province (Panha et al. 2007). Recently, Seesamut et al. (2018) re-examined the littoral earthworms in Thailand and described a new species, *P. longissimus*, based on distinct morphological characteristics and molecular genetic distances from *P. litoralis*. In Japan, the littoral earthworm was first discovered in Matsushima Bay, Miyagi Prefecture and described as *P. matsushimensis* by Iizuka (1898), but later this species was synonymized with the cosmopolitan *P. litoralis* (Easton 1984). Yamaguchi (1953) reported *P. matsushimensis* from Miyakojima in the Miyagi Prefecture, Misaki and Akashi in the Hyogo Prefecture, Ranshima (Hokkaido) and Fukuoka (Kyushu island). Subsequently, the distribution of this species in Japan was been further studied (Ohno 2003), with *P. litoralis* being recorded in more than 20 localities in Honshu, Shikoku, Kyushu, and Ryukyu (Oba et al. 2011, 2015). In addition, the occurrence of *P. litoralis* was also recorded on the beaches of Awaji Island, located between Honshu and Shikoku (Hara et al. 2016). Together, these reports indicate that *P. litoralis* is a cosmopolitan species and occurs in a very wide range of subtemperate and tropical coastal areas (Gates 1972; Easton 1984; Seesamut et al. 2018).

The study of body size can be helpful in identifying earthworm species, as morphometric characters have been represented as one of the keys for confirming their systematic positions (Chang et al. 2007; James et al. 2010). Morphometric analyses, which use mathematical definitions of size and shape, could be used as an addition to other evolutionary analyses, and the results of which could be interpreted in relation to developmental biology and genetics (Klingenberg 2002). Size variation has been studied in many earthworm species, in order to investigate their morphological variation and apply the results towards the identification of the earthworm species. Oboh et al. (2007) reported that populations of the terrestrial earthworm *Eudrilus eugeniae* from Lagos, Nigeria were separated into three distinct groups based on the statistical analysis of their morphometric parameters in terms of their body weight, length of clitellum, diameter of posterior and anterior ends, total body length, body size diameter, and total number of segments. In addition, the examination of body size and segment number

can be used to separate the terrestrial earthworms *Lumbricus terrestris* and *L. herculeus* into two distinct nominal species, which was also supported by DNA sequence analysis (James et al. 2010). The body size and coloration were also used to separate the *Amyntas wulinensis* species complex into three species (*A. lini*, *A. meishanensis*, and *A. wulinensis*) that were otherwise similar in morphological characters, and this was supported by DNA sequence analysis of the mitochondrial cytochrome c oxidase subunit 1 (COI) gene (Chang et al. 2007).

Many distribution records have reported size variation within *P. litoralis* (Gates 1972; Easton 1984; Seesamut et al. 2018). However, none of the studies have yet revealed whether the size variation indicates different species or only morphological variation within the same species. In addition, it is believed that there is only a single cosmopolitan species (*P. litoralis*), which led us to test this hypothesis based on their size variation coupled with a genetic analysis. The objective of this study, therefore, was to investigate the pattern between the size variations (number of segments, body length and diameter) and genetic (mitochondrial COI) variations in the littoral earthworm *P. litoralis* from 14 populations across Southeast Asia (Thailand, Myanmar, Vietnam, Malaysia, and Indonesia) and Japan. Moreover, we report new data on the distribution and habitat types used by this species.

## Materials and methods

### Field collection, preservation, and identification

From August 2011 to September 2018, samples of *P. litoralis* were collected throughout the coastal areas of both the east and west sides of the Thai-Malay Peninsula (Thailand and Malaysia) and Japan (Honshu, Kyushu, and Ryukyu islands). Moreover, samples from Myanmar, Vietnam, Malaysia, Singapore, and Indonesia were collected (Figs 1, 2). Both adult and juvenile stages of the worms were collected by digging suitable habitats, including sandy beaches at both low- and high-tide levels, estuaries, areas under seaweed debris, damp mud under stones, and areas with wet sand mixed with mud. The living specimens were washed with water, soaked in 30% (v/v) ethanol, photographed, and then killed in 30% (v/v) ethanol. Earthworm specimens were then fixed in 95% (v/v) ethanol for morphological and molecular analyses.

Coordinates of each locality were recorded using a GPS receiver, and salinity values were recorded using an ATAGO refractometer. For species identification, the specimens were carefully identified using the taxonomic literature of Gates (1972), Easton (1984), and Seesamut et al. (2018). Small adults (specimen length <50 mm) and juvenile stages of earthworms were observed under an OLYMPUS SZX16 stereomicroscope. Juveniles were identified by the position of male pores (segment XVIII) showing the inner wall of a longitudinal depression and the internal characters, such as prostate glands on XVIII and absent of nephridia on anterior segments.



**Figure 1.** Location and distribution of *P. litoralis* habitats (sampling sites) in Thailand, Malaysia, Myanmar, Singapore, Indonesia, and Vietnam (based on our field collections).

### Morphometric analysis

Fourteen populations of *P. litoralis* were selected based on being from different geographic regions (Table 1). At least nine adult worms from each population were then selected and this resulted in a total of 212 specimens used in the morphometric analysis. Only sexually mature earthworms, as determined by the presence of the clitellum, were measured and used to plot the frequency of the length distribution. Total body length, body size diameter, and total number of segments were measured and counted following Ng et al. (2017). Analysis of variance (ANOVA) and principal component analysis (PCA) were performed to assess the significant variation among the three morphometric characters. The mean length and diameter were calculated separately both within each locality and a country scale, and those mean differences were analyzed by one-way ANOVA. The clustering analysis (CA) of the sampling sites was performed to construct a dendrogram

**Table 1.** Sampling localities, GPS coordinates and number of specimens of *P. litoralis* used in the morphometric analysis.

	Locality	Latitude, Longitude	Number of adult samples
Thailand (TA)	1. Petchaburi (TA1)	12°49'36.2"N, 99°59'40.3"E	16
	2. Trat (TA2)	12°05'52.4"N, 102°21'27.9"E	20
	3. Chonburi (TA3)	12°50'25.1"N, 100°54'18.3"E	15
	4. Songkhla (TA4)	7°43'30.3"N, 100°22'55.4"E	18
Malaysia (MA)	5. Pulau Pinang (MA1)	5°28'06.7"N, 100°16'41.0"E	16
	6. Pahang (MA2)	3°48'25.0"N, 103°20'29.4"E	18
Myanmar (MY)	7. Dawei (MY1)	14°07'43.5"N, 98°05'50.1"E	10
Indonesia (IN)	8. Banten (IN1)	6°00'51.3"S, 106°40'38.4"E	13
Vietnam (VT)	9. Bến Tre (VT1)	9°48'11.0"N, 106°37'42.2"E	15
	10. Huế (VT2)	16°13'38.9"N, 108°04'58.4"E	16
	11. Nghệ An (VT3)	18°46'06.1"N, 105°45'31.0"E	16
Japan (JP)	12. Aichi (JP1)	34°48'00.2"N, 136°51'30.3"E	18
	13. Hiroshima (JP2)	34°17'45.0"N, 132°19'08.0"E	9
	14. Okinawa (JP3)	26°28'20.0"N, 127°49'54.1"E	12
Total			212

depicting the morphological relationship based on the three morphometric measurements, CA were tested based on complete linkage and Euclidean distances. All statistical analyses were performed using the MINITab software v. 18.1 (Minitab, Inc.).

## Molecular analysis

Three specimens were chosen from each of the same 14 populations as in the morphometric analysis resulting in the total of 42 samples used for the molecular analysis (Table 2). The total genomic DNA of each worm was extracted from a posterior body part using a Lysis Buffer for PCR (Takara) DNA extraction kit. The mitochondrial COI gene fragment was amplified using the Tks Gflex™ DNA Polymerase (Takara) and the universal primers (Folmer et al. 1994). Each PCR reaction was comprised of 1 µL of Tks Gflex DNA polymerase (1.25 unit/µL), 25 µL of 2x Gflex PCR buffer (Mg<sup>2+</sup>, dNTP plus), 1 µL each of 10 µM LCO1490 (forward) and HCO2198 (reverse) universal primer, 19.5 µL of sterilized distilled water and 2.5 µL of crude lysate (ca 500 ng/µL DNA) with Lysis buffer. Thermal cycling was performed at 94 °C for 2 min, followed by 35 cycles of 94 °C for 1 min, 48 °C for 1 min and 72 °C for 2 min and then a final 72 °C for 5 min. The concentration and quality of the amplicons were determined visually after coresolution through a 1% (w/v) agarose gel against a DNA standard marker in 1x TAE buffer and detected under UV transillumination.

For sequencing, the PCR products were directly sent to Macrogen Inc. (Japan) without purification. All COI sequences were aligned using the ClustalW algorithm in MEGA7 v. 7.0.18 (Thompson et al. 1994; Kumar et al. 2016) and manually checked by eye. The sequences were aligned, checked and compared with other sequences available in the GenBank databases at the National Center for Biotechnology Information (NCBI), obtained using the BLASTn similarity search tool (<http://www.ncbi.nlm.nih.gov>). Corrected genetic distances were calculated using the Kimura two-parameter

**Table 2.** Details of *P. littoralis* samples using DNA sequencing, and accession numbers of the COI sequences.

Locality	abbreviation	GenBank accession number
1. Petchaburi, Thailand(TA1)	TA1	MK642691
	TA1_A	MK714106
	TA1_B	MK714107
2. Trat, Thailand (TA2)	TA2	MK642690
	TA2_A	MK714108
	TA2_B	MK714109
3. Chonburi, Thailand (TA3)	TA3	MK642689
	TA3_A	MK714110
	TA3_B	MK714111
4. Songkhla, Thailand (TA4)	TA4	MK642688
	TA4_A	MK714112
	TA4_B	MK714113
5. Pulau Pinang, Malaysia (MA1)	MA1	MK642694
	MA1_A	MK714100
	MA1_B	MK714101
6. Pahang, Malaysia (MA2)	MA2	MK642693
	MA2_A	MK714102
	MA2_B	MK714103
7. Dawei, Myanmar (MY1)	MY1	MK642692
	MY1_A	MK714104
	MY1_B	MK714105
8. Banten, Indonesia (IN1)	IN1	MK642698
	IN1_A	MK714092
	IN1_B	MK714093
9. Bến Tre, Vietnam (VT1)	VT1	MK642687
	VT1_A	MK714114
	VT1_B	MK714115
10. Huế, Vietnam (VT2)	VT2	MK642686
	VT2_A	MK714116
	VT2_B	MK714117
11. Nghệ An, Vietnam (VT3)	VT3	MK642685
	VT3_A	MK714118
	VT3_B	MK714119
12. Aichi, Japan (JP1)	JP1	MK642697
	JP1_A	MK714094
	JP1_B	MK714095
13. Hiroshima, Japan (JP2)	JP2	MK642696
	JP2_A	MK714096
	JP2_B	MK714097
14. Okinawa, Japan (JP3)	JP3	MK642695
	JP3_A	MK714098
	JP3_B	MK714099

(K2P) model (Kimura 1980) as implemented in MEGA7. Phylogenetic reconstruction was performed using the maximum likelihood (ML) analysis in RAxML v. 8.1.20 (Stamatakis 2014), and 1,000 bootstraps were used to estimate the node reliability as bootstrap support values. Bootstrap values lower than 75% for each node were considered as insignificant (Okanishi et al. 2018). *Pontodrilus longissimus* was used as the outgroup (Accession number MK642683 and MK642684).

## Results

### Size variation of *P. litoralis*

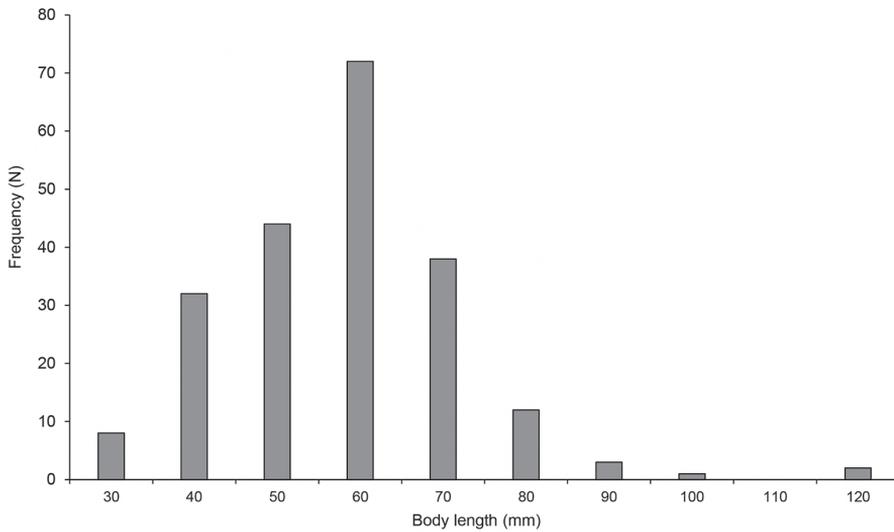
The measurement of 212 individuals of *P. litoralis* earthworms from all 14 sampling sites revealed a length range between 31.1–125.4 mm (Fig. 3). The length between 60.1–69.9 mm occurred at the highest frequency ( $n = 72$ ), followed by that between 50.0–59.6 mm ( $n = 44$ ) and 70.5–79.6 mm ( $n = 38$ ). Two specimens from JP2 were recorded as having a length  $>120$  mm. The relationship between the total number of segments and the body length of *P. litoralis* (Fig. 4), had a low correlation between them ( $R^2 = 0.0922$ ). The longest length of *P. litoralis* was 125.4 mm, found in Japan (JP2), while the shortest was 31.1 mm, found in Vietnam (VT2). The mean  $\pm$  S.D. and median length of *P. litoralis* were  $62.6 \pm 14.2$  mm and 63.1 mm, respectively. The highest number of segments in *P. litoralis* was 119, found in Thailand (TA1) and Myanmar (MY1), while the lowest was 81, found in Indonesia (IN1). The mean  $\pm$  S.D. and median of the total number of segments of mature *P. litoralis* were  $101.7 \pm 8.9$  and 102, respectively. In addition, the largest body diameter of *P. litoralis* was 4.08 mm, found in Japan (JP2), while the smallest was 1.21 mm, found in Vietnam (VT2). The mean  $\pm$  S.D. and median *P. litoralis* diameter were  $2.12 \pm 0.52$  mm and 2.02 mm, respectively.

The ANOVA analysis revealed a significant difference ( $p < 0.05$ ) in the mean length and diameter of *P. litoralis* among the 14 locations (Fig. 5). The JP2 population from Japan showed the highest mean body length ( $93.0 \pm 22.4$  mm) and diameter ( $3.39 \pm 0.6$  mm), while the VT2 population from Vietnam showed the lowest mean body length ( $39.5 \pm 5.4$  mm) and diameter ( $1.55 \pm 0.18$  mm). Moreover, the highest mean body length ( $73.6 \pm 16.4$  mm) and diameter ( $2.86 \pm 0.47$  mm) were found in all the Japanese populations (JP1, JP2, and JP3), while the lowest mean body length ( $52.4 \pm 14.2$  mm) and diameter ( $1.66 \pm 0.25$  mm) were found in all the Vietnamese populations (VT1, VT2, and VT3).

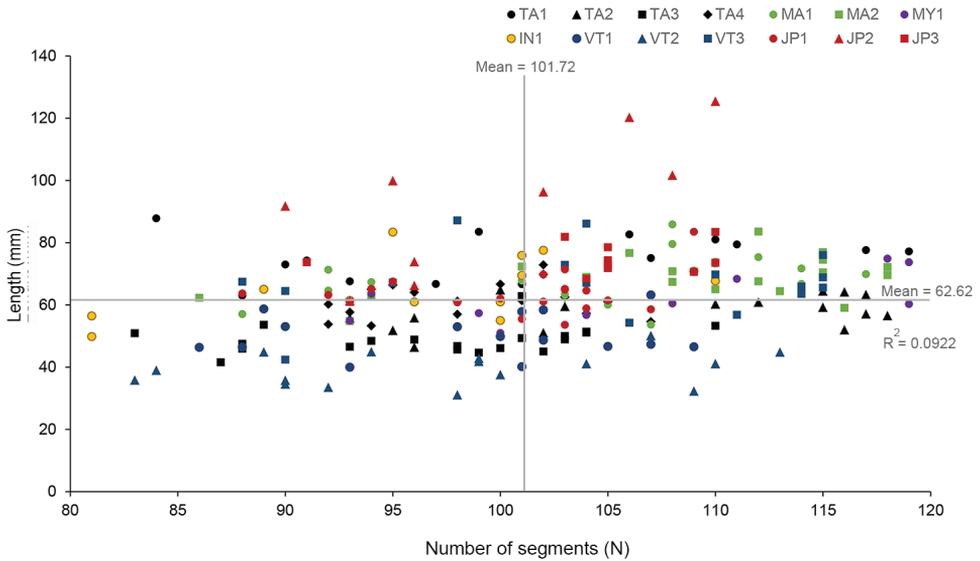
Cluster analysis of the 14 populations based on the three morphometric data revealed two clusters, one of which contained 13 populations and was further divided into two subclusters, and the other contained only the JP2 population from Japan (Figure 6). The PCA showing the first principal component (PC1) explained more than 60.6% of the variation in the dataset and had a variance (eigenvalue) of 1.8174. The second and third PCs (PC2 and PC3) had a variance (eigenvalue) of 0.8882 and 0.2944, respectively, which accounted for 29.6% and 9.8% of the data variability, respectively. The PC1 revealed that all the loadings were positive, whereas the PC2 showed both positive (number of segment) and negative loadings (body length and diameter). The loadings from the PC2 were less similar among themselves compared to the PC1. The PC1 had a large positive association with the body length and diameter as determined by loadings  $>0.5$ , so this PC1 primarily measured the size of the earthworms (Table 3). The scatter diagram of PC1 versus PC2 (Fig. 7) indicated that the size variation within populations of JP2 and VT2 were distinct from other populations.



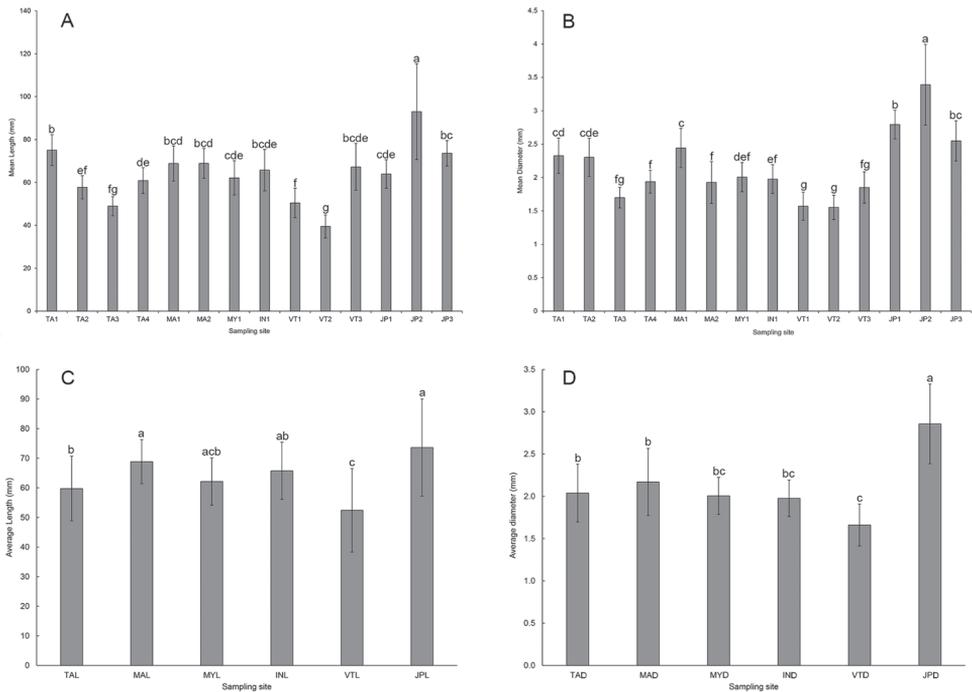
**Figure 2.** Location and distribution of *P. littoralis* habitats (sampling sites) in Japan (based on our field collections).



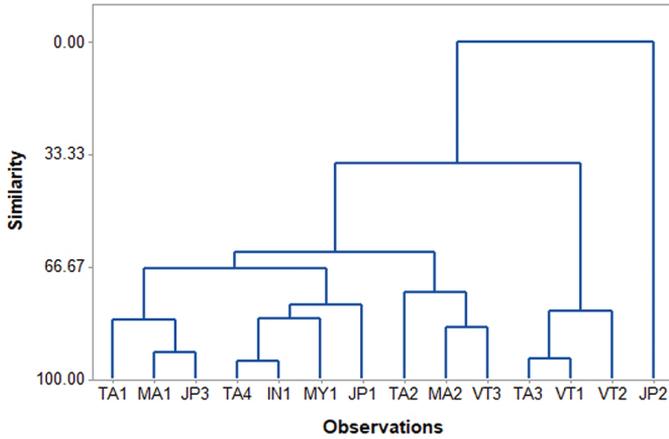
**Figure 3.** Histogram showing the length frequency distribution of the 212 *P. littoralis* samples from all 14 sampling sites.



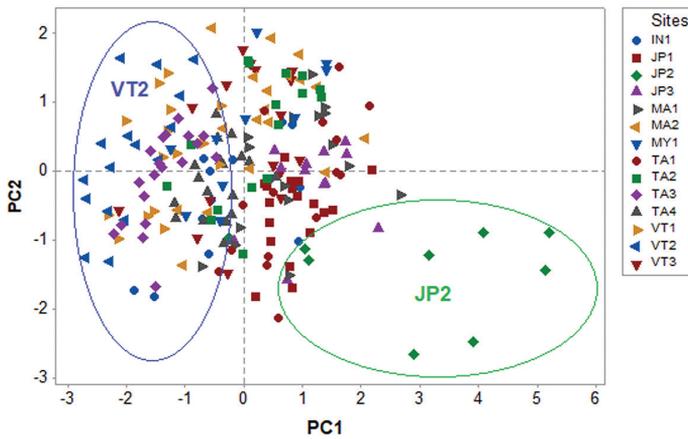
**Figure 4.** Scatter plot between the length and number of segments of *P. litoralis* (212 samples, 14 locations).



**Figure 5.** Mean (A, C) length and (B, D) diameter of *P. litoralis* samples within each (A, B) locality and (C, D) country sampled in this study. Sampling site codes are given in Table 1. Different letters above the bar indicate a significant difference ( $P < 0.05$ ; one-way ANOVA).



**Figure 6.** Cluster analysis based on the Euclidean distances among the 14 populations of *P. littoralis*. Sampling sites codes are given in Table 1.



**Figure 7.** PCA plot between PC1 and PC2 using the three morphometric variables (number of segments, body length, and diameter). Sampling sites codes are given in Table 1.

**Table 3.** PCA percentage of the explained variance and weights of morphometric ratios for the 14 populations of *P. littoralis*.

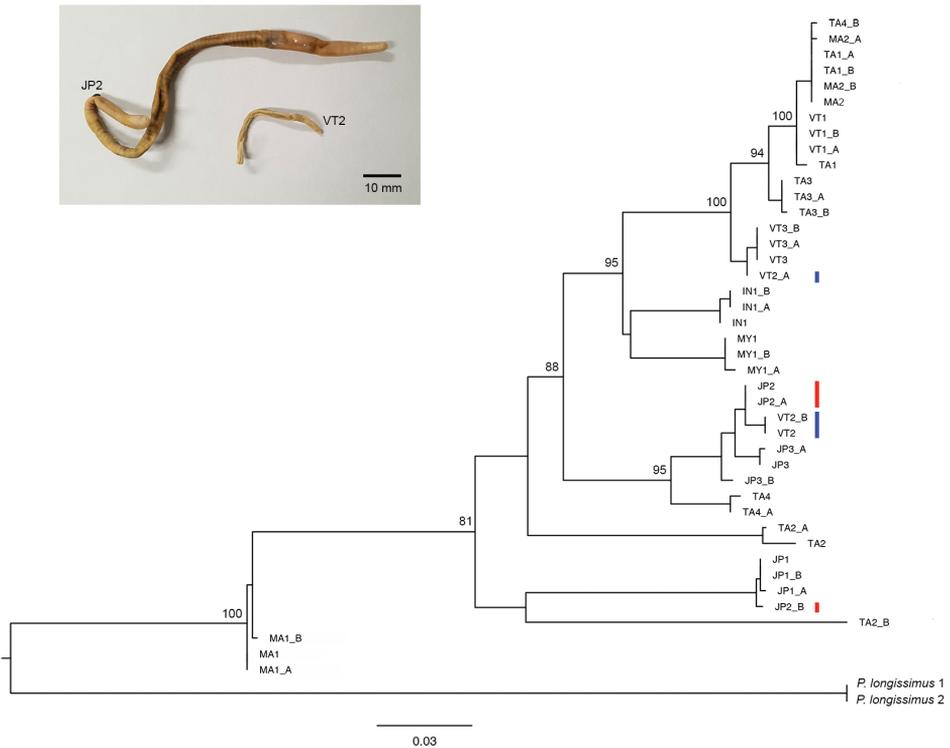
Variable	PC1	PC2	PC3
Length	0.675	-0.143	0.724
Diameter	0.638	-0.380	-0.670
Segment number	0.371	0.914	-0.165
Eigenvalue	1.8174	0.8882	0.2944
% total variance	60.6	29.6	9.8

## Genetic analysis

The COI DNA sequences (658 bp) from 42 individuals, three specimens from each of the 14 populations used in the morphometric analysis, were analyzed. The analysis yielded 158 variable (polymorphic) sites and 139 parsimony informative sites. No insertions, deletions, or stop codons were observed in any of the sequences. The K2P genetic distances among the 14 geographical locations within *P. litoralis* ranged from 0.3–12.8 % (Table 4). The highest divergence was estimated between TA1 and JP1; MA2 and JP1 (12.8%), while the lowest was estimated between TA1 and MA2 (0.3%). The genetic distance within group ranged from 0–9%. The highest was estimated in TA2 (9%), whereas the lowest was estimated in VT1 and VT3 (0.0%). The ML tree (Fig. 8) did not show any pattern congruent with the variation in the sizes of *P. litoralis* (Fig. 6). For instance, the analyses on the size variation between the shortest population (VT2) and the longest population (JP2) samples clearly showed a significant difference in their body length (39.5 mm and 93.0 mm for VT2 and JP2, respectively), and body diameter (1.55 mm and 3.39 mm for VT2 and JP2, respectively) ( $p < 0.05$ ), while the cluster analysis confirmed that the two clusters were separated, one contained 13 populations (included VT2) and the other contained only JP2. However, the genetic distance analysis showed a low genetic distance between VT2 and JP2 population (5.6%; Table 4) and the COI ML tree suggested a sister relationship between four samples from VT2 population (VT2, VT2\_B) and JP2 population (JP2, JP2\_A) (Fig. 8). The nucleotide sequences reported of *P. litoralis* in this study are deposited at GenBank under accession numbers as showing in Table 2.

**Table 4.** Between groups mean genetic distances corrected with the Kimura-2 parameter model among the 14 populations of *P. litoralis*. The bold values represent the genetic distance within group. Sampling site codes are given in Table 1.

	IN1	JP1	JP2	JP3	MA1	MA2	MY1	TA1	TA2	TA3	TA4	VT1	VT2	VT3
IN1	<b>0.002</b>													
JP1	0.102	<b>0.001</b>												
JP2	0.087	0.073	<b>0.072</b>											
JP3	0.079	0.108	0.043	<b>0.011</b>										
MA1	0.089	0.101	0.103	0.102	<b>0.002</b>									
MA2	0.064	0.128	0.100	0.083	0.112	<b>0.001</b>								
MY1	0.048	0.109	0.088	0.076	0.109	0.073	<b>0.002</b>							
TA1	0.067	0.128	0.099	0.082	0.111	0.003	0.074	<b>0.005</b>						
TA2	0.100	0.111	0.112	0.110	0.108	0.119	0.101	0.118	<b>0.090</b>					
TA3	0.064	0.125	0.093	0.075	0.106	0.018	0.068	0.017	0.113	<b>0.001</b>				
TA4	0.071	0.109	0.074	0.055	0.107	0.065	0.078	0.067	0.114	0.067	<b>0.067</b>			
VT1	0.067	0.127	0.098	0.081	0.109	0.005	0.074	0.004	0.116	0.013	0.066	<b>0.000</b>		
VT2	0.073	0.113	0.056	0.035	0.103	0.067	0.071	0.066	0.109	0.061	0.060	0.063	<b>0.050</b>	
VT3	0.067	0.119	0.088	0.072	0.102	0.030	0.060	0.030	0.105	0.024	0.066	0.026	0.052	<b>0.000</b>



**Figure 8.** ML phylogenetic tree of *P. litoralis* based on the mitochondrial COI gene (658 bp) with *Pontodrilus longissimus* as the outgroup. Only bootstrap values >70% are indicated at each node. Scale bar represents the number of nucleotide substitutions per site. The sample names correspond to those in Table 5. Photograph on the top left shows comparative size of the shortest and the longest samples in this molecular study. The longest population (JP2) is shown in red and the shortest population (VT2) is shown in blue.

### Distributions and habitats of *P. litoralis*

In Southeast Asia, *P. litoralis* was found scattered over the coastal areas in Thailand, Myanmar, Vietnam, Malaysia, Singapore, and Indonesia (Figures 1 and 2). The northernmost sampling site was at Nghê An Province, Vietnam (18°45'46.1"N, 105°45'23.54"E), whereas the southernmost site was in Banten, Indonesia (6°00'51.3"S, 106°40'38.4"E). In this study, we reported the first record of *P. litoralis* in Singapore despite only juveniles being collected from the beach in West Coast Park (1°17'45.0"N, 103°45'43.1"E). Among the localities in the subtropical areas, *P. litoralis* specimens were collected from various beaches in Japan, and the northernmost site was Matsushima Kaihin Koen in the Miyagi prefecture, where the synonym of *P. litoralis* (*P. matsushimensis*) was originally described from. In

**Table 5.** Salinity records (mean ‰ ± SD) and habitat characteristics of the sampling sites of *P. litoralis* in this study.

Locality	Collection time	Salinity (‰)	Habitat
Thailand	January 2015 – March 2018	19.29 ± 12.14	Salty mud margins of estuaries, brackish lakes, damp mud under stones, mangrove swamps, under the root of the tree near the shore, under the trash or leaf litter on the sandy beach, sanitary sewer emptying to the sandy beach
Myanmar	April 2016	18 ± 12.82	Estuaries, under the trash on the sandy beach
Malaysia	January 2016	15.94 ± 9.85	Estuaries, damp mud under stones and the beach, under the trash or leaf litter on the sandy beach
Vietnam	May 2018 – July 2018	19.38 ± 10.57	Estuaries, under the trash or leaf litter on the sandy beach
Indonesia	August 2017	12	Sanitary sewer emptying to the sandy beach
Singapore	December 2017	22	Under the root of the tree near the shore
Japan	August 2011 – September 2018	17.5 ± 9.85	Sand beach facing to the ocean (sand mixed with seaweed debris), estuaries

total, 29 localities were recorded in the distribution range of *P. litoralis* within Japan, including in Honshu, Kyushu, and the Ryukyu islands.

Based on field collections within Thailand and some parts of Southeast Asia, *P. litoralis* was found to occupy several types of habitats (Table 5; Fig. 9), such as estuaries, brackish habitats, damp mud under stones, under the trash or leaf litter on sandy beaches, mangrove swamps of the intertidal zone, sanitary sewer links, and freshwater channels between the mainland and the sea. However, collections of *P. litoralis* in the Japanese coastal areas showed that *P. litoralis* was abundant and mostly found in sandy beaches facing the ocean and lives in the sand mixed with seaweed debris (Fig. 10). Records of the salinity values during the field collections showed an average salinity between 12–22 ‰ (Table 5).

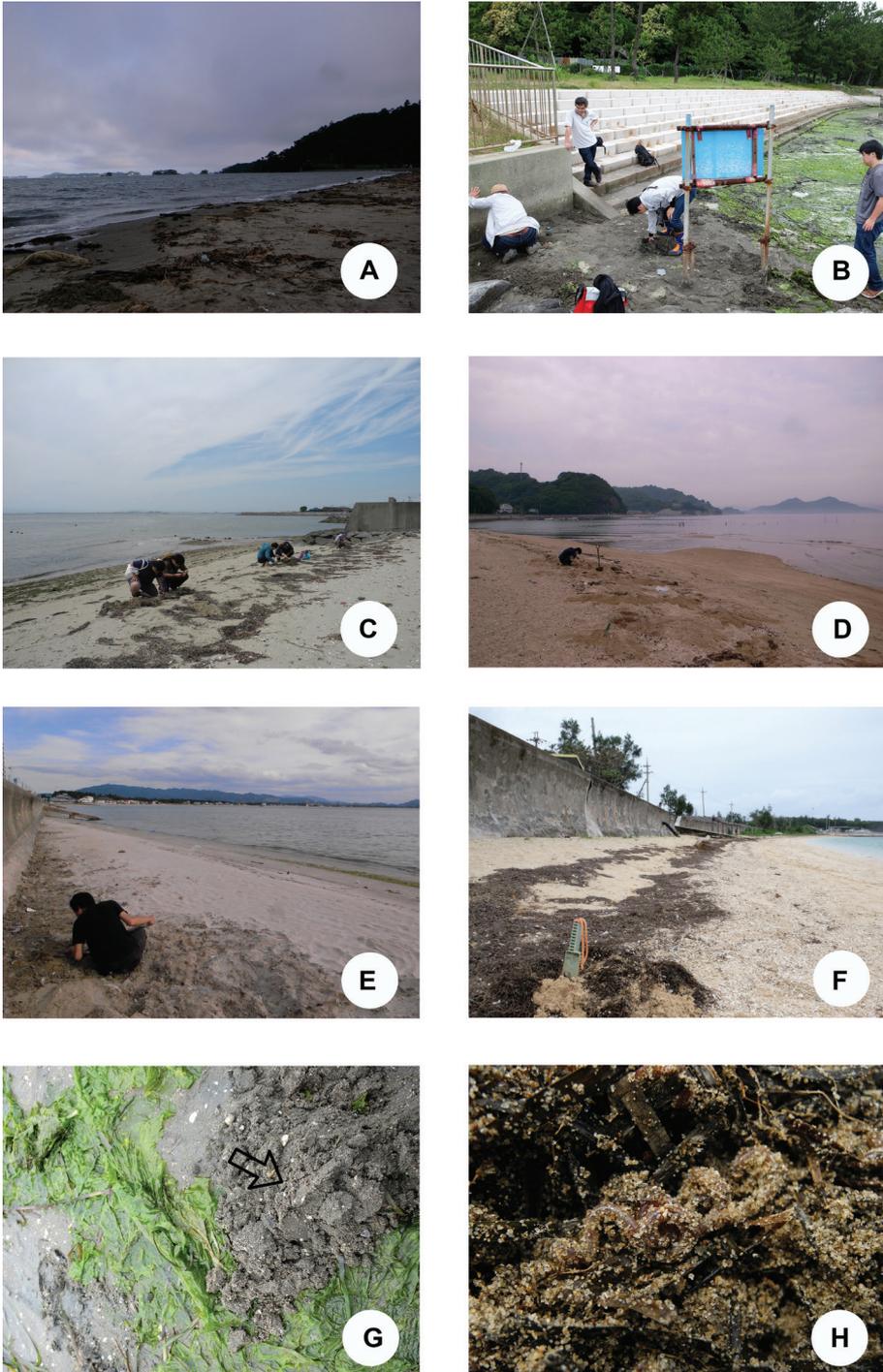
## Discussion

This study is the first attempt to integrate morphometric variations and molecular marker analyses together in the cosmopolitan littoral earthworm *P. litoralis*. The specimens investigated in this study were within the variation range previously reported by Jamieson (2001) (body length 32–120 mm, diameter 2–4 mm, and number of segments 78–120) and Seesamut et al. (2018) (body length 28–136 mm, diameter 1–5 mm, and number of segments 76–128).

According to the results of the one-way ANOVA, there was a significant difference in the body length and diameter among specimens from the different geographical sites. In addition, the PCA results supported that length and diameter had a higher influence than the number of segments in the 14 studied populations of *P. litoralis*. However, the phylogenetic tree did not show any congruent pattern with the size variation of the specimens analyzed in this study. For instance, in both the PCA and cluster



**Figure 9.** Photographs showing the habitats of *P. littoralis* in Thailand **A** Trat Province **B** Chonburi Province **C** Petchaburi Province **D** Chumphon Province **E** Songkhla Province **F** Satun Province **G** Petchaburi Province **H** Satun Province



**Figure 10.** Photographs showing the habitats of *P. litoralis* in Japan **A** Miyagi Prefecture **B** Kanagawa Prefecture **C** Aichi Prefecture **D** Hiroshima Prefecture **E** Fukuoka Prefecture **F** Okinawa Prefecture **G** Kanagawa Prefecture **H** Aichi Prefecture

analysis the longest (JP2) and the shortest samples (VT2) formed separate groups with statistical differences in their size, whereas a low genetic distance between the two samples from each respective population was detected, revealing that the size variation of *P. littoralis* was independent of the genetic (COI gene) differences.

Differences in the body length, diameter, and number of segments have also reported in other earthworms. The terrestrial earthworm *Metaphire peguana* (Rosa, 1890) from Penang and neighboring states of Malaysia revealed significant differences in their morphometric variations that were not matched by their genetic difference but rather were affected by the type of habitat (Ng et al. 2017). However, Heethoff et al. (2004) reported a strong correlation between the size of *Octolasion tyrtaeum* (Savigny, 1826) earthworms from Germany and Canada and their mitochondrial cytochrome c oxidase II (COII) sequences, showing that small and large individuals were genetically distinct.

This study is a comprehensive report on the occurrence, distribution and habitat characteristics of the luminous littoral earthworm, *P. littoralis*, in the coastal areas of Thailand, Japan (Honshu, Kyushu, and Ryukyu islands), and some parts of Southeast Asia (Myanmar, Vietnam, Malaysia, Singapore, and Indonesia) based on field collections. This survey supported the assumption that *P. littoralis* is widely distributed in subtropical and tropical coastal ecosystems (Gates 1972; Jamieson and Wampler 1979; Oba et al. 2015; Seesamut et al. 2018), and aligns with the worldwide distribution records (Easton 1984; Blakemore 2002).

In general, the distribution of earthworms is mostly affected by environmental factors, such as the temperature, organic matter content, and soil moisture (Johnston et al. 2014). This survey of *P. littoralis* habitats in Thailand and Southeast Asia revealed that the earthworms live in various habitat types with a relatively wide range of salinity and diverse sources of water. The earthworms were mostly found in the ecotone between the terrestrial and marine habitats, such as the mangrove swamps of the intertidal zone, sanitary sewer emptying to sandy beaches, estuaries, salty mud under stones near the shore, and under the trash or leaf litter on sand beaches. This indicated that *P. littoralis* mostly prefers to inhabit the ecotone between terrestrial and marine habitats. The earthworms were found to occupy the soil column that ranged from the top soil down to 30 cm deep, and on humid substrates in contact with tidal seawater, the level of which is an important factor governing the distribution of intertidal species (Penas and Gonzfilez 1983). In this survey, the habitats of *P. littoralis* in Japan, where the worms were collected, were mostly in sand mixed with seaweed debris on the sandy beaches facing the ocean, whereas we did not collect any littoral earthworms from this type of microhabitat in Southeast Asian shores.

In Japan, beach-cast seaweeds have been reported as important habitats and food for a diverse community of marine and terrestrial organisms, such as amphipods, isopods, and copepods (Okuda 2008). The habitats of *P. littoralis* in Japan are similar to those reported in Western Australia coastal areas, where the earthworms were recorded in high density within the wrack material, seaweed, and debris deposited on arid beaches, which provided a rich food resource and resulted in a high abundance of

earthworms (Blakemore 2007; Coupland and McDonald 2008). Carlo et al. (2012) reported the preference of *P. litoralis* to inhabit sites with an accumulation of macro-detritic matter that provided abundant organic matter contents and shade that helped to keep the soil surface cool during daytime. Moreover, the salinity of the *P. litoralis* habitats recorded in this study indicated that *P. litoralis* can survive a wide range of salinity between 1–33‰ (Seesamut et al. 2018), the upper bound of which is near the salinity of seawater in general (35‰; Schmidt et al. 2018). Taken together, we suggest that the habitat preference of *P. litoralis* is primarily determined by the abundance of organic matter contents but not the salinity.

In conclusion, although morphometric examinations of size variation could make reliable distinctions among different populations of *P. litoralis*, this distinction was not congruent with the phylogenetic relationship based on COI gene sequence analysis, reflecting that the size variation of *P. litoralis* did not correlate with their genetic (COI) differences. Thus, we propose that the food resource is the key factor underlying size variation in *P. litoralis*. Future analyses on the type of habitats, sand texture, and components of the food resources are necessary. Moreover, studies on salinity tolerance are needed to confirm the habitat preference of this littoral earthworm species.

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# *Zhezhoulinyphia* gen. nov. (Araneae, Linyphiidae) from Yunnan, China

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## Abstract

A new Linyphiinae genus *Zhezhoulinyphia* **gen. nov.** from Yunnan Province is described together with its two new species: *Z. caperata* **sp. nov.** (♂) and *Z. denticulata* **sp. nov.** (♂, ♀). *Centromerus yadongensis* Hu & Li, 1987 is transferred to *Zhezhoulinyphia* **gen. nov.** and a new combination, *Z. yadongensis* (Hu & Li, 1987), **comb. nov.** is proposed.

## Keywords

Gaoligong Mountain, morphology, Linyphiinae, sheet web spiders, taxonomy

## Introduction

Linyphiidae Blackwall, 1859, the second largest spider family, contains 4591 species in 611 genera, which accounts for approximately 10% of the total number of spiders worldwide (WSC 2019). Li and Lin (2016) listed 371 Chinese linyphiid species in 154 genera, including 69 species in 16 genera in the subfamily Linyphiinae. Yunnan is a biodiversity-rich spot in south China and about 9 genera and 39 species of linyphiid spiders have been described from there (Wunderlich and Song 1994; Xia et al. 2001; Liu and Chen 2010; Zhao and Li 2014; Zhao and Li 2017; Irfan and Peng 2018).

While examining the specimens collected from Gaoligong Mountain, *Zhezhoulinyphia* gen. nov. with two new species were identified and are described here.

## Material and methods

Specimens were collected by hand picking and beating shrubs and were kept in 75% ethanol. The type specimens are deposited at the College of Life Sciences, Hunan Normal University, Changsha, China. After dissection, epigyna were cleared in trypsin enzyme solution before examination and photography. The left male palp was used for description and illustration. Specimens were examined, measured with a Leica M205C stereomicroscope. Photos were taken with a digital camera Canon PowerShot G12 mounted on an Olympus BX53 and Leica MC170 HD mounted on a Leica M205C. Stacked focus images were generated using Helicon Focus software (3.10, free). A map was created using ArcMap 10.2, and then modified using Adobe Photoshop CS2 Extended. Leg measurements are given in the following order: total length (femur, patella + tibia, metatarsus, tarsus). All measurements are given in millimeters (mm).

Abbreviations used in the text and figures are as follows:

<b>AE</b>	anterior wall of epigyne;	<b>PC</b>	paracymbium;
<b>AER</b>	anterior eyes row;	<b>PER</b>	posterior eyes row;
<b>ALE</b>	anterior lateral eyes;	<b>PLE</b>	posterior lateral eyes;
<b>AME</b>	anterior median eyes;	<b>PME</b>	posterior median eyes;
<b>AME–AME</b>	distance between AME;	<b>PME–PME</b>	distance between PME;
<b>AME–ALE</b>	distance between AME and ALE;	<b>PME–PLE</b>	distance between PME and PLE;
<b>CO</b>	copulatory opening;	<b>PMP</b>	posterior median plate;
<b>CRL</b>	cymbial retrolateral lobe;	<b>R</b>	radix;
<b>DPE</b>	dorsal lobe of embolic plate;	<b>RA</b>	radical apophysis;
<b>DSA</b>	distal suprategular apophysis;	<b>S</b>	spermathecae;
<b>E</b>	embolus;	<b>ST</b>	suprategulum;
<b>EM</b>	embolic membrane;	<b>T</b>	tegulum;
<b>FD</b>	fertilization ducts;	<b>W</b>	wrinkle.
<b>P</b>	parmula;		

## Taxonomy

### Linyphiidae Blackwall, 1859

#### *Zhezhoulinyphia* gen. nov.

<http://zoobank.org/1250FBD2-CA7E-4C9A-93C3-39C8A27A8F0E>

**Type species.** *Zhezhoulinyphia denticulata* sp. nov.

**Etymology.** The species name comes from the Chinese word “褶皱 (Zhezhou)” meaning “wrinkle” and referring to parmula with wrinkles in epigyne. Gender feminine.

**Diagnosis.** *Zhezhoulinyphia* gen. nov. resembles *Diplostyla* Emerton, 1882, *Kaestneria* Wiehle, 1956, *Laetesia* Simon, 1908 and *Laperousea* Dalmas, 1917 in: Epigyne with parmula originating from posterior margin of posterior median plate with a socket ventrally (Fig. 7A–D; Ivie 1969, figs 105–108; van Helsdingen 1972, fig. 8; Irfan and Peng 2018, figs 5C, D, 6C, D;). Distal margin of radix semicircular with teeth as in *Laetesia* and *Laperousea* (Figs 4A, B, 5C–E, 6A, B; Millidge 1988, figs 145–146). It can be distinguished by the following characters: the embolus and embolic membrane arise from the dorsal side of the distal margin of radix (Figs 4A, B, D, 5C–E, 6A, B), whereas this arises from the lateral (inner) side of the embolic plate in *Kaestneria* and *Laetesia* (Millidge 1988, figs 145, 149; Zhao and Li 2014, figs 47B, 49B) and in *Diplostyla concolor* embolus arises near base of cymbium and extends parallel along with full length of cymbium (Ivie 1969, figs 107, 108); distal suprategular apophysis proximally broad with teeth, distal part strongly curved into inverse U-shaped, and almost touching distal margin of paracymbium (Figs 4A, B, D, 5G, 6A, B), whereas it is protruding upward with a notch in *Laetesia* (Millidge 1988, fig. 146); without a notch in *Kaestneria* (Tao et al. 1995, figs 77–78) and *Diplostyla concolor* (Ivie, 1969, fig. 108); parmula extending towards anterior margin first then folding backward, distal part with transverse wrinkles (Figs 7A–D, 9A–D, 10A, B), whereas parmula not folded and without transverse wrinkles both in *Kaestneria* and *Laetesia* (Millidge 1988, figs 145, 149; Zhao and Li 2014, figs 47B, 49B) and in *Diplostyla concolor*, epigyne parmula reduced, invisible in ventral view, ventral plate with very long and slender scape (Ivie, 1969, figs 105, 106).

**Description.** Large sized, 4.0–5.5. Male cephalic region strongly elevated, ocular area with spines, extending forward. AER procurved, PER slightly recurved. Chaetotaxy: 2–2–2–2. TmI 0.9–1.3, TmIV 0.5–0.75. Leg formula I–II–IV–III. Legs yellow without obvious patterns.

Male Palp: Femur almost equal to the collective length of patella and cymbium (Fig. 5A, B). Patella shorter than tibia, dorsally with a long spine (Figs 5A, B, 6A, B). Tibia with three retrolateral and a dorsal trichobothria (Figs 4A, B, 5G, 6A, B). Cymbium conical flask-shaped, with a cymbial retrolateral lobe (Figs 4B, 5B, G, 6B). Paracymbium large, distal arm broad with round tip (Figs 4B, 5F, 6B). Distal suprategular apophysis sclerotized, proximal end broad with teeth, strongly curved distally (Figs 4A–C, 5G, 6A, B). Radix long, proximal end with finger-shaped dorsal lobe of embolic plate; distal margin semicircular with teeth and a radical apophysis. Embolus and embolic membrane arise from dorsal side of distal semicircular serrated margin of radix (Figs 4A–C, 5C–E, 6A).

Epigyne (Figs 1A–D, 2A–C, 7A–D, 9A–D, 10A, B): Anterior wall of epigyne (AE) longer than wide, posterior margin with a big outgrowth; copulatory openings present inside atrium; parmula long, extending towards anterior margin first then folding backward, distal part with transverse wrinkles (number of wrinkles varies among different species), distal tip with a socket posteriorly. Vulva: posterior median plate cordiform; copulatory ducts long, arch-shaped; spermathecae U- or L-shaped, present mesally on posterior median plate; fertilization ducts long, extending mesally.

**Distribution.** Yunnan, Tibet (China) (Fig. 12).

***Zhezhoulinyphia caperata* sp. nov.**

<http://zoobank.org/4C3946E0-30E2-48F4-B560-0B7BCF6D7704>

Figs 1–3, 12

**Types.** Holotype female, **China, Yunnan Province:** Gongshan County, Bingzhonggluo Township, Chukuai, 27.97928°N, 98.47389°E, alt. 3725 m, 19 August 2006, Peng Hu (Hu060819). Paratypes: 2 females, Gongshan County, Dulongjiang Township, Maku, 27.68611°N, 98.29660°E, alt. 2097 m, 2 September 2006, Peng Hu (Hu060902).

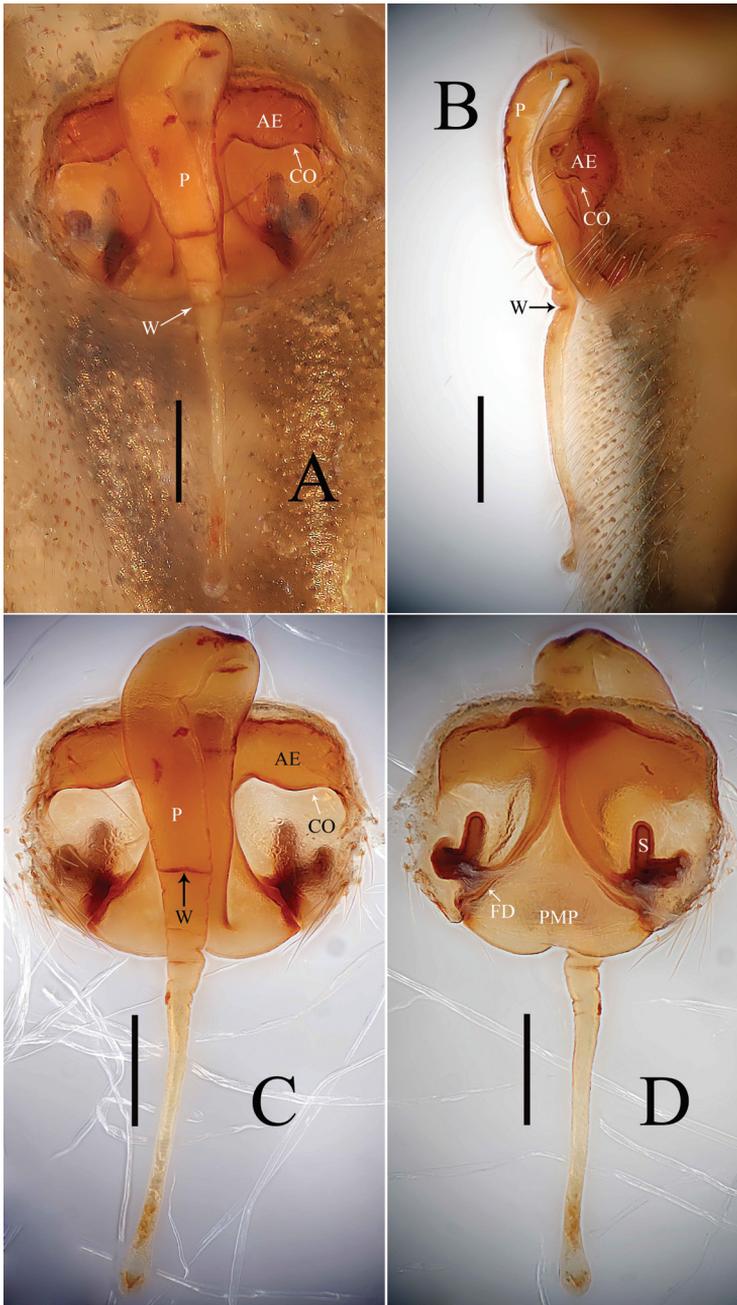
**Etymology.** The species name comes from the Latin adjective “*caperatus*”, meaning “wrinkled” and referring to parmula with wrinkles in epigyne.

**Diagnosis.** *Zhezhoulinyphia caperata* sp. nov. can be distinguished from *Z. denticulata* sp. nov. by having the anterior wall of epigyne wider than long, posterior margin without distinct outgrowth (Figs 1A–C, 2A, B), whereas it is longer than wide, with a big outgrowth in *Z. denticulata* sp. nov. (Figs 7A–D, 9A–D, 10A–C). Parmula with three transverse wrinkles in new species (Figs 1A–C, 2A, B), whereas there are seven to twelve in *Z. denticulata* sp. nov. (Figs 7A–D, 9A–D, 10A–C).

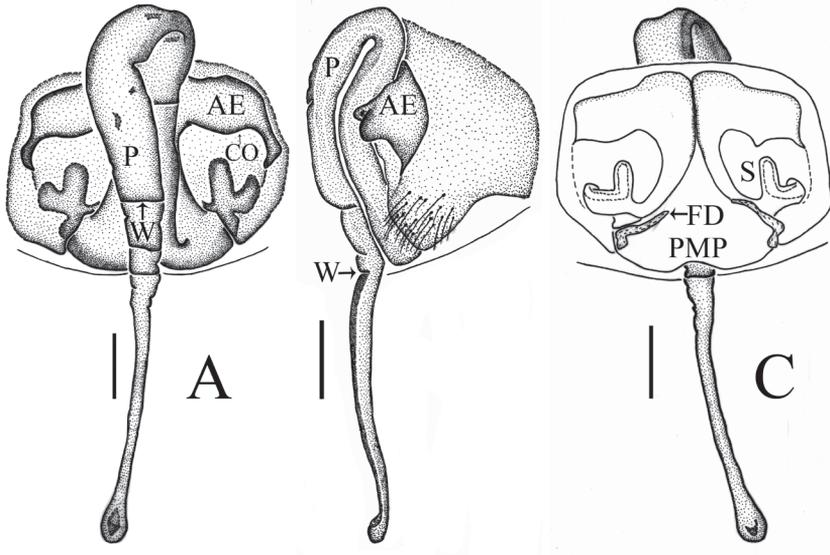
**Description.** Female (holotype): Total length: 5.35. Carapace 1.97 long, 1.54 wide, yellow to reddish, cephalic region slightly elevated with spine like hairs (Fig. 3A, B), fovea, cervical and radial grooves distinct (Fig. 3A); Clypeus 0.48 high. Sternum longer than wide, light yellow, provided with spine-like hairs; labium wider than long; maxillae long, distal end broader, with hairs (Fig. 3B). Chelicerae with 6 promarginal and 3 retromarginal teeth. AER recurved, PER straight, slightly wider (Fig. 3A). Eye sizes and interdistances: AME, 0.11; ALE, 0.16; PME, 0.13; PLE, 0.14; AME-AME, 0.06; PME-PME, 0.10; AME-ALE, 0.09; PME-PLE, 0.13; AME-PME, 0.15; ALE-ALE, 0.66; PLE-PLE, 0.71; ALE-PLE contiguous. Length of legs: I, 9.55 (2.68, 2.97, 2.62, 1.28); II, 8.65 (2.49, 2.68, 2.33, 1.15); III, 6.03 (1.83, 1.81, 1.56, 0.83); IV, 7.40 (2.21, 2.27, 1.99, 0.93). Leg formula I–II–IV–III. TmI, 1.02 and TmIV, 0.68. Tibial dorsal spine formula: 2–2–2–2. Abdomen 3.38 long, 2.25 wide, oval, grey, mid-dorsally with a black pattern with irregular white patches extending laterally, ventral side pale with irregular white patches (Fig. 3A, B). Epigyne (Figs 1A–C, 2A, B): Anterior wall of epigyne (AE) wider than long, with wave-like margin posteriorly; copulatory openings present inside atrium; parmula long, extending towards anterior margin first then folding backward, distal part with 3 transverse wrinkles, distal tip with a socket posteriorly. Vulva: posterior median plate broad and cordiform. Copulatory ducts long, arch-shaped; spermathecae L-shaped, present mesally on posterior median plate; fertilization ducts long and extending mesally.

**Male.** Unknown.

**Distribution.** China (Yunnan) (Fig. 12).



**Figure 1.** *Zhezhoulinyphia caperata* sp. nov., female holotype **A** epigyne, ventral view (before dissected from the body) **B** ditto, lateral view **C** ditto, ventral view (after dissected from the body) **D** vulva, dorsal view. Scale bars: 0.2 mm (**A–D**).



**Figure 2.** *Zhezhoulinyphia caperata* sp. nov., female holotype **A** epigyne, ventral view **B** ditto, lateral view **C** vulva, dorsal view. Scale bars: 0.2 mm (**A–C**).



**Figure 3.** *Zhezhoulinyphia caperata* sp. nov., habitus of female holotype **A** dorsal view **B** ventral view. Scale bars: 0.5 mm (**A–B**).

***Zhezhoulinyphia denticulata* sp. nov.**

<http://zoobank.org/CC3698D0-721A-4DB3-88F8-7CCB156FA036>

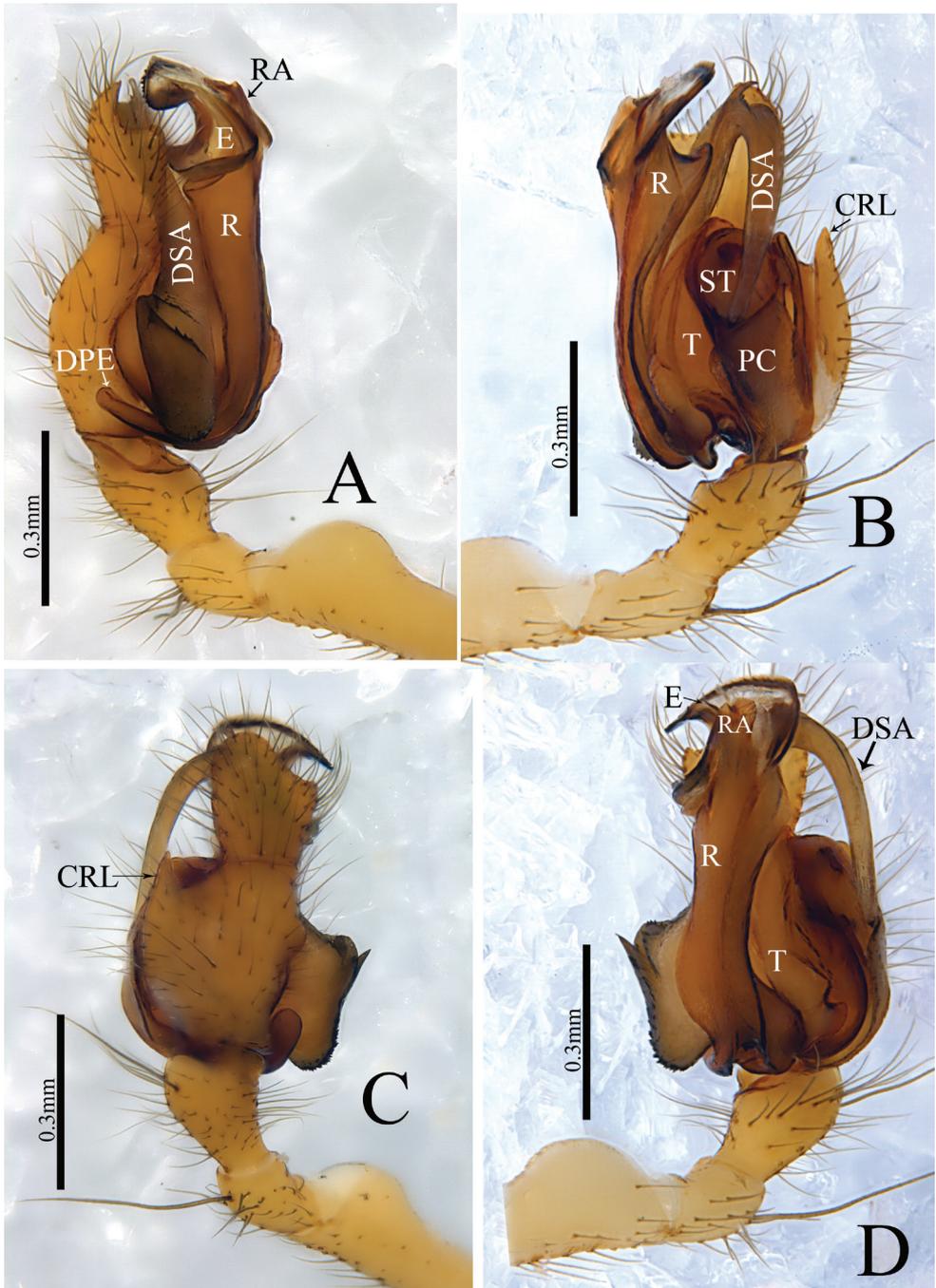
Figs 4–12

**Types.** Holotype male, **China, Yunnan:** Fugong County, Lishadi Township, Shibali, 27.10520°N, 98.77980°E, alt. 2530 m, 10 August 2005, Guo Tang (Tang–05–02). Paratypes: 2 males 2 females, same data as holotype (Tang–05–02); 2 females, Fugong County, Pihe Township, Yueliangtian Village, 26.56784°N, 98.90884°E, alt. 1520 m, 24 August 2005, Guo Tang (Tang–05–08); 6 males 1 female, Baoshan City, Nankang Yakou (National 320 Road), 24.43717°N, 98.46054°E, alt. 2186 m, 30 October 2003, Guo Tang (Tang031030); 1 female, Gongshan County, Qiqi Dongshaofang, 27.69521°N, 98.48514°E, alt. 3208 m, 29 September 2007, Xian-jin Peng (20071001); 1 female, Gongshan County, Qiqi Dongshaofang, 27.69521°N, 98.48514°E, alt. 3208 m, 29 September 2007, Xian-jin Peng (20070929); 1 female, Gongshan County, Cikai Township, Dabadi troops place north bank of Pula river, 27.78333°N, 98.51667°E, alt. 3030 m, 28 September 2002, Heng-mei Yan (Yan020928); 6 males 1 female, Nujiang Prefecture, Nujiang State Nature Reserve, No.12 bridge Camp area, 16.3 air km W of Gongshan, 27.71503°N, 98.50244°E, alt. 2775 m, 15–19 July 2000, Heng-mei Yan, D. H. Kavanaugh, Charles Griswold, Hong-bin Liang, Darrell Ubick and Da-zhi Dong (00–QD).

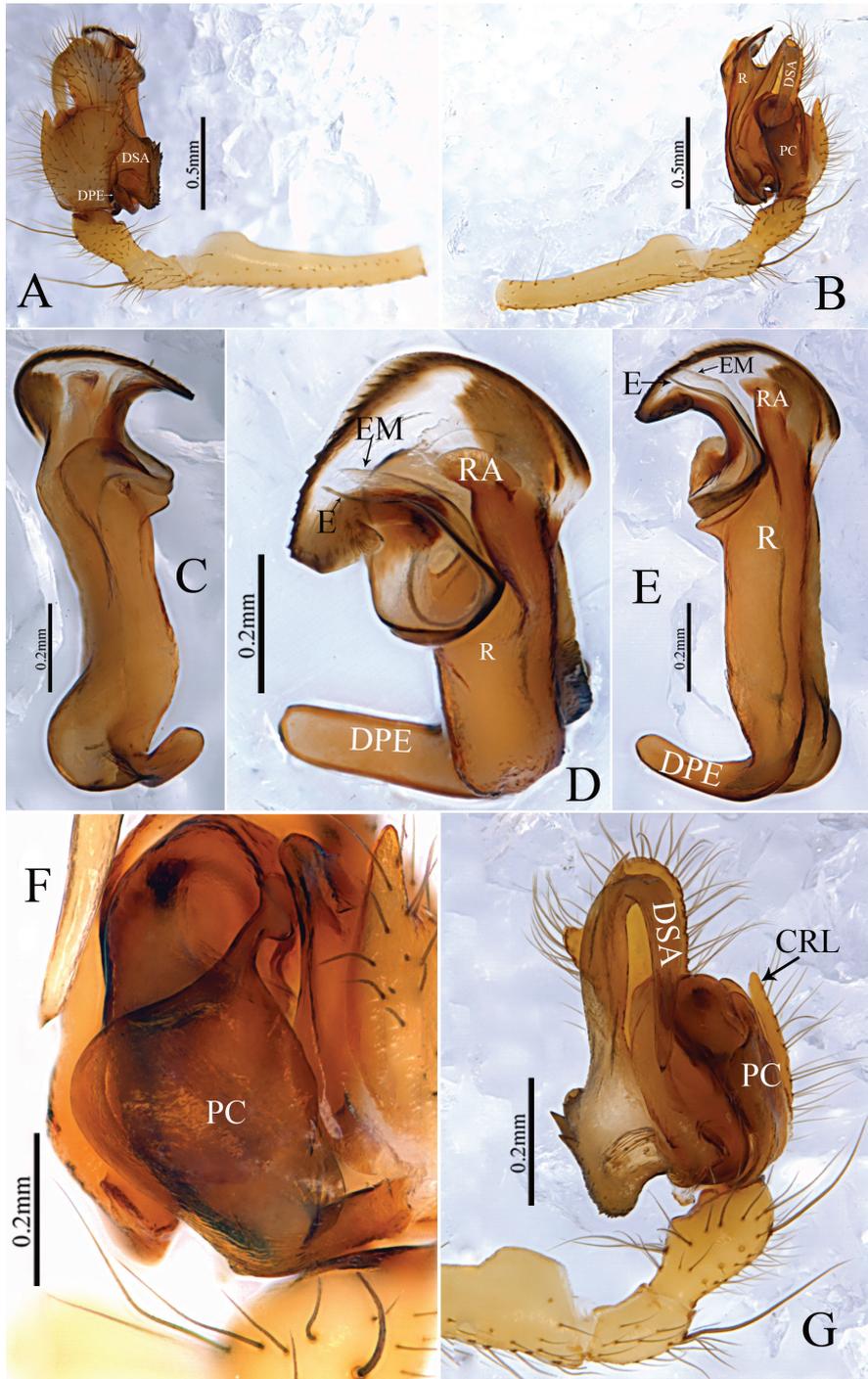
**Etymology.** The species name comes from the Latin adjective “*denticulus*”, meaning “teeth” and referring to the distal suprategular apophysis (DSA) with teeth in male palp.

**Diagnosis.** *Zhezhoulinyphia denticulata* sp. nov. can be distinguished from *Z. caperata* sp. nov. by having the anterior wall of epigyne longer than wide, with a big outgrowth (Figs 1A–C, 2A, B), whereas wider than long, posterior margin without distinct outgrowth in *Z. caperata* sp. nov. (Figs 7A–D, 9A–D, 10A–C). Parmula with seven to twelve transverse wrinkles in new species (Figs 1A–C, 2A, B), whereas there are three in *Z. caperata* sp. nov. (Figs 7A–D, 9A–D, 10A–C).

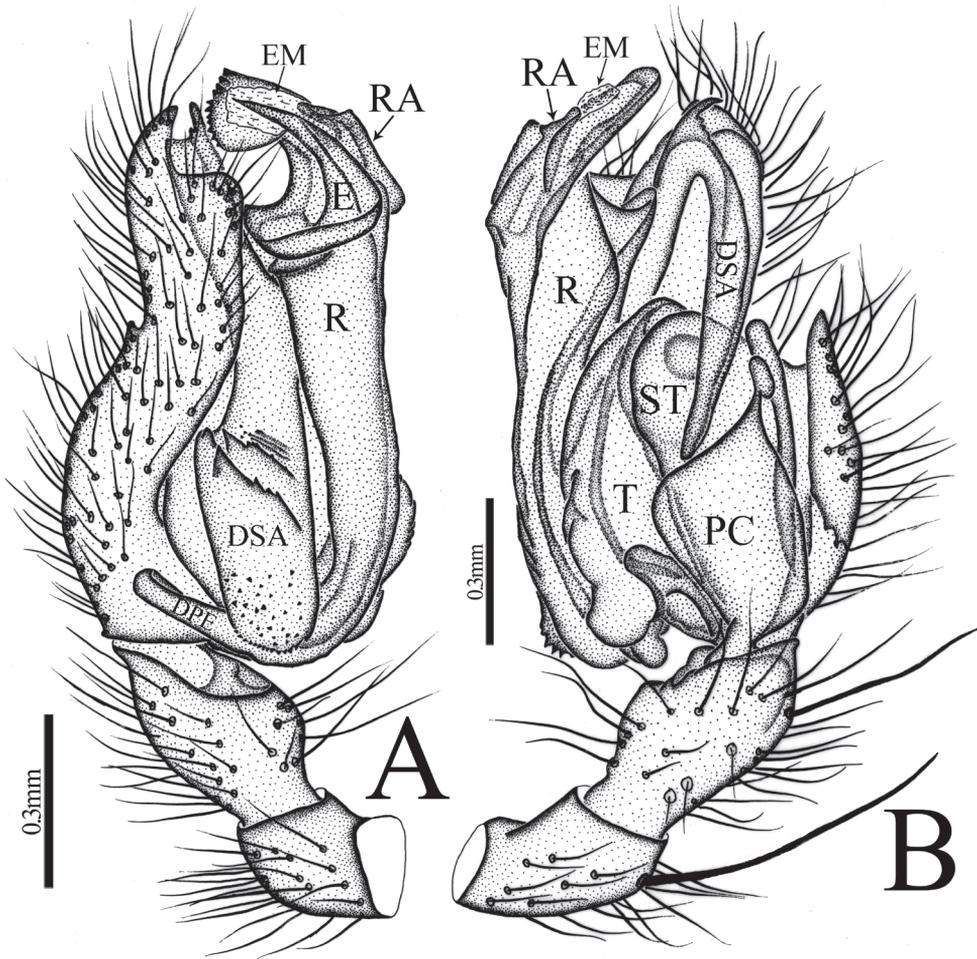
**Description.** Male (holotype): Total length: 4.24. Carapace 1.80 long, 1.43 wide, yellow, cephalic lobe 0.91 long, fovea, cervical and radial grooves distinct (Fig. 8A, B); Clypeus 0.59 high (Fig. 8B). Sternum longer than wide, brown, with spine-like hairs; labium wider than long; maxillae long, distal end broader with hairs (Fig. 8C). Chelicerae with three promarginal and three retromarginal teeth. AER recurved, PER straight, slightly wider. Eye sizes and interdistances: AME, 0.07; ALE, 0.10; PME, 0.11; PLE, 0.09; AME-AME, 0.07; PME-PME, 0.08; AME-ALE, 0.14; PME-PLE, 0.15; AME-PME, 0.16; ALE-ALE, 0.55; PLE-PLE, 0.59; ALE-PLE, 0.02. Length of legs: I, 12.63 (3.38, 3.88, 3.77, 1.60); II, 11.04 (3.16, 3.33, 3.23, 1.32); III, 7.21 (2.16, 2.16, 2.05, 0.84); IV, 8.93 (2.59, 2.63, 2.69, 1.02). Leg formula I–II–IV–III. TmI 1.25 and TmIV 0.74. Tibial dorsal spine formula: 2–2–2–2. Abdomen 2.44 long, 1.19 wide, cylindrical, grey, mid dorsally with a grey pattern and densely covered with white patches extending laterally, ventral side dark grey (Fig. 8A–C). Palp (Figs 4A–D, 5A–G, 6A, B); femur almost equal to collective length of patella and cymbium, distally expanded, dorsally with a row of fine spine like hairs (Fig. 5A, B); patella shorter than tibia, dorsally with a long spine (Fig. 5A, B, 6A, B); tibia conic, with three retrolateral



**Figure 4.** *Zhezhoulinyphia denticulata* sp. nov., male holotype, left palp **A** prolateral view **B** retrolateral view **C** dorsal view **D** ventral view. Scale bars: 0.3 mm (**A–D**).

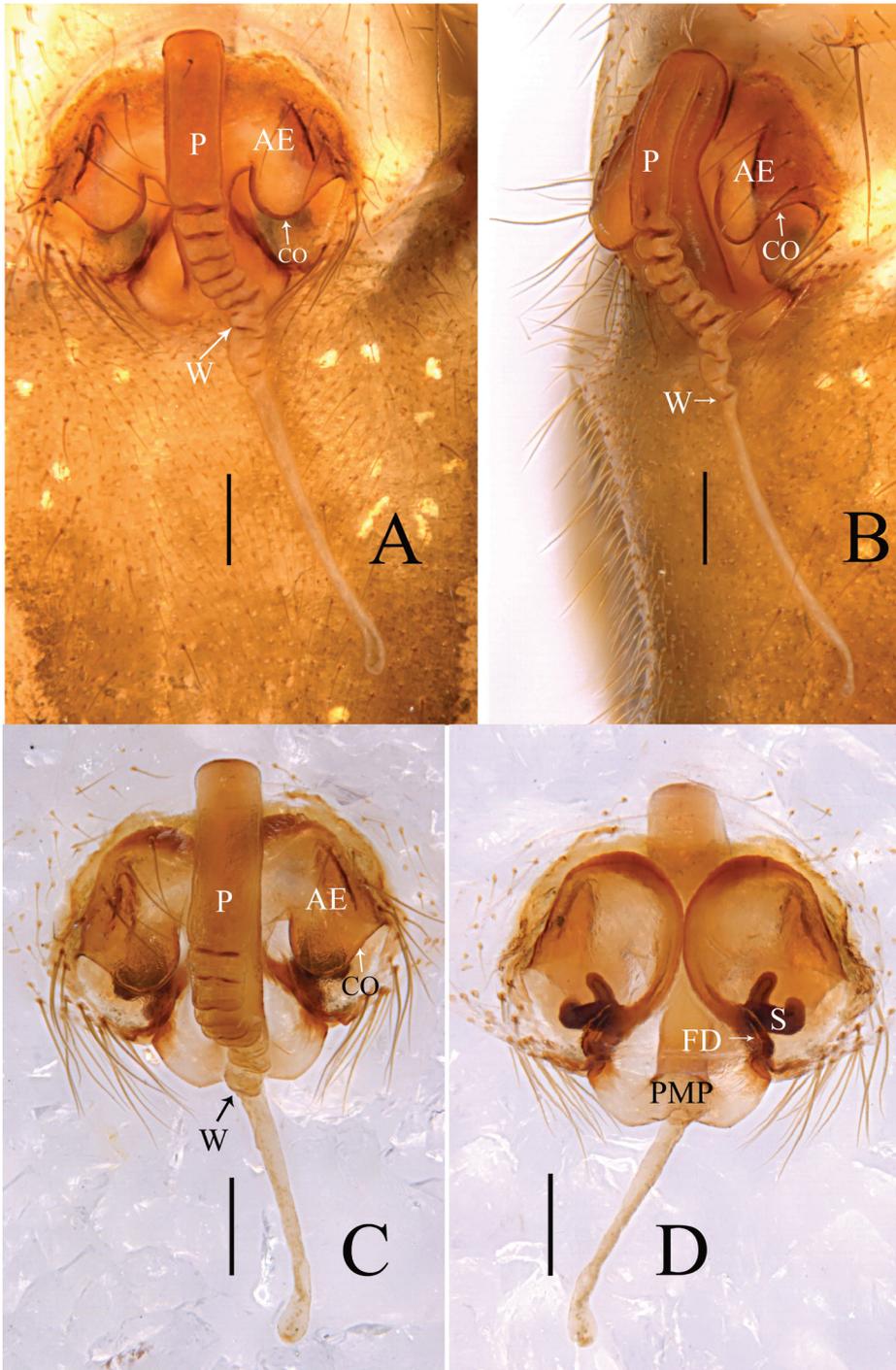


**Figure 5.** *Zhezhoulinyphia denticulata* sp. nov. **A, B** male holotype, left palp (Tang-05-02) **C-G** one of paratype male, left palp (00-QD).

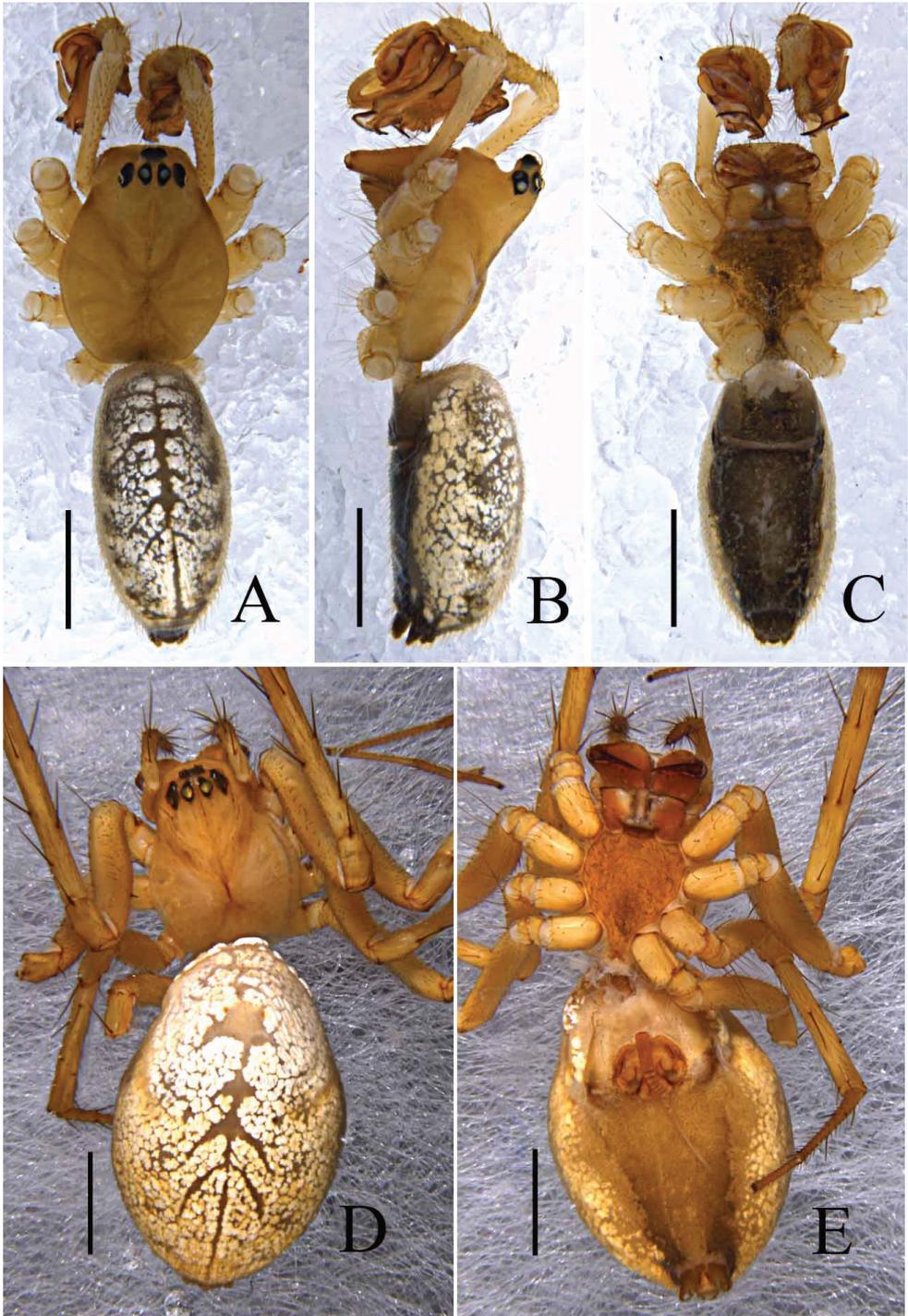


**Figure 6.** *Zhezhoulinyphia denticulata* sp. nov., male holotype, left palp **A** proteralateral view **B** retrolateral view. Scale bars: 0.3 mm (**A–B**).

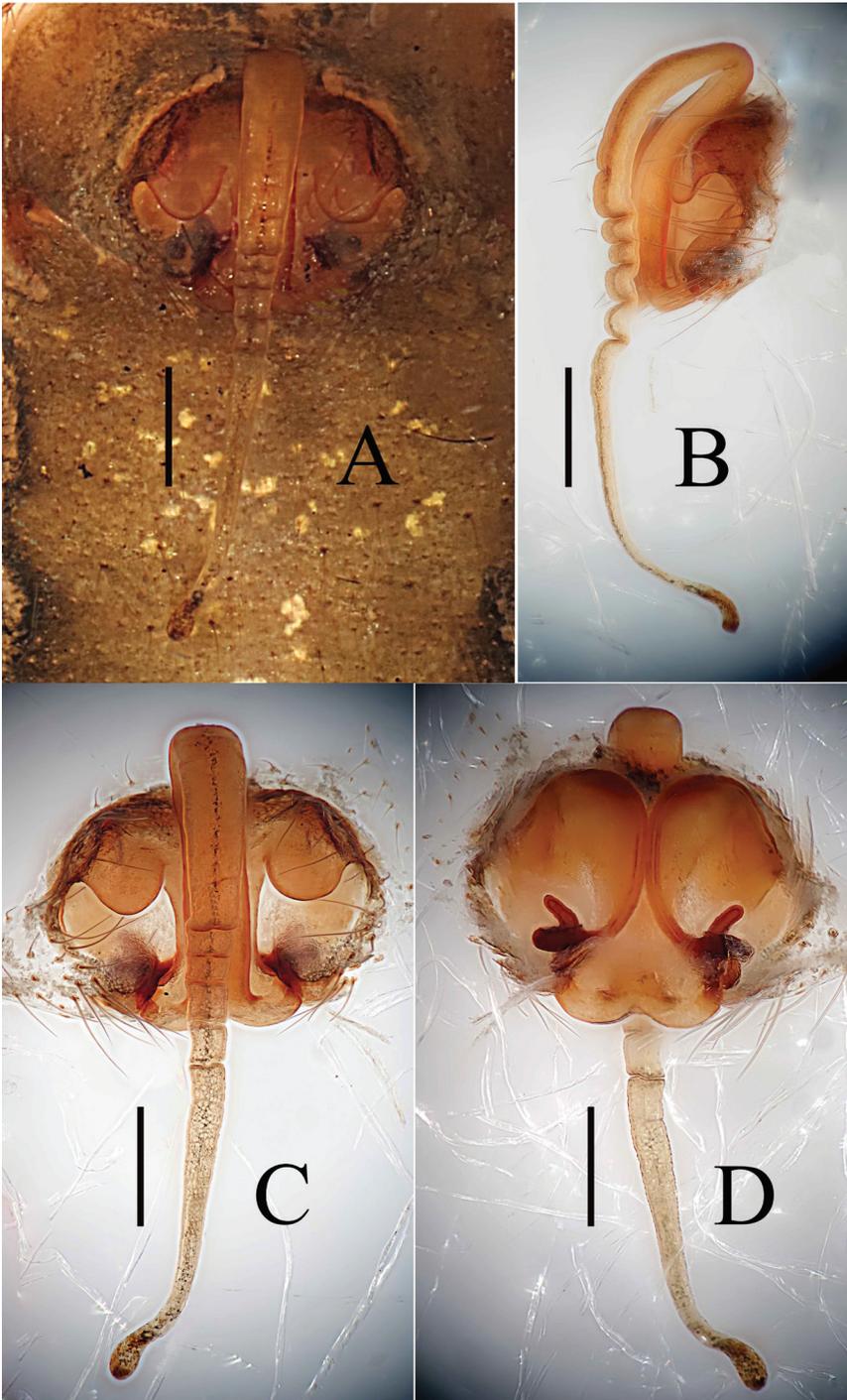
and a dorsal trichobothria, dorsally with a long spine (Figs 4A, B, 5G, 6A, B); cymbium conical, flask shaped, with a cymbial retrolateral lobe protruding upward (Figs 4B, 5B, G, 6B); paracymbium sclerotized, simple, distal arm longer than wide, tip pendulum-shaped (Figs 4B, 5F); distal suprategular apophysis proximally broad with teeth, distal part strongly curved into inverse U-shaped, and almost touches distal margin of paracymbium. Embolic division: dorsal lobe of embolic plate long, sclerotized, overlapping cymbium (Figs 4A, 5C–E, 6A); radix long, distal margin semicircular with teeth (Figs 4A, B, 5C–E, 6A, B); radical apophysis sclerotized, with blunt end (Figs 4A, B, 5C–E, 6A, B); embolic membrane and embolus arise from the dorsal margin of semicircular part (Figs 4A, B, 5C–E, 6A, B); embolus sclerotized, long, curved and almost touches serrated margin of radix (Figs 4A, 5C–E, 6A).



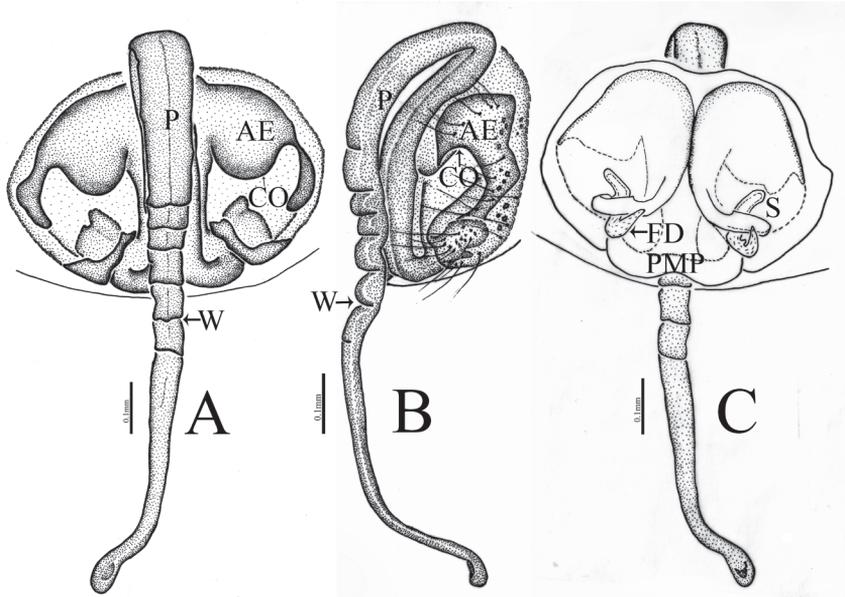
**Figure 7.** *Zhezhoulinyphia denticulata* sp. nov., female (one of paratype, Tang-05-02) **A** epigyne, ventral view (before dissected from the body) **B** ditto, lateral view **C** ditto, ventral view (after dissected from the body) **D** Vulva, dorsal view. Scale bars: 0.2 mm (**A-D**).



**Figure 8.** *Zhezhoulinyphia denticulata* sp. nov., habitus of male holotype (A–C) and female, (one of paratypes, Tang–05–02; D–E) A, D Dorsal view B Lateral view C, E Ventral view. Scale bars: 1 mm (A–D).



**Figure 9.** *Zhezhoulinyphia denticulata* sp. nov., female (one of paratype, Tang-05-08) **A** epigyne, ventral view (before dissected from the body) **B** ditto, lateral view **C** ditto, ventral view (after dissected from the body) **D** vulva, dorsal view. Scale bars: 0.1 mm (A–D).



**Figure 10.** *Zhezhoulinyphia denticulata* sp. nov., female (one of paratype, Tang–05–08) **A** epigyne, ventral view **B** ditto, lateral view **C** vulva, dorsal view. Scale bars: 0.1 mm (**A–D**).

Female (one of paratype, Tang–05–02): Total length: 4.46. Carapace 1.67 long, 1.46 wide, yellow, cephalic region slightly elevated with spine like hairs, fovea, cervical and radial grooves distinct (Figs 8A, 11A). Clypeus 0.59 high. Sternum longer than wide, light orange, with spine-like hairs; labium wider than long; maxillae long, distal end broader with hairs (Figs 8B, 11B). Chelicerae with three promarginal and three retromarginal teeth. AER recurved, PER straight, slightly wider. Eye sizes and interdistances: AME, 0.09; ALE, 0.15; PME, 0.12; PLE, 0.12; AME-AME, 0.05; PME-PME, 0.08; AME-ALE, 0.07; PME-PLE, 0.12; AME-PME, 0.11; ALE-ALE, 0.59; PLE-PLE, 0.63; ALE-PLE contiguous. Length of legs: I, 9.67 (2.68, 3.09, 2.65, 1.25); II, 8.75 (2.59, 2.76, 2.35, 1.05); III, 6.31 (1.94, 2.01, 1.57, 0.79); IV, 7.37 (2.23, 2.24, 2.04, 0.86). Leg formula I–II–IV–III. Tm I, 0.92 and Tm IV, 0.53. Tibial dorsal spine formula: 2–2–2–2. Abdomen 2.79 long, 1.64 wide, oval, grey, mid dorsally with a grey pattern and densely covered with white patches extending laterally, ventral side brown (Figs 8A, B, 11A, B). Epigyne (Figs 7A–D, 9A–D, 10A, B): Anterior wall of epigyne (AE), longer than wide, posterior margin with a big outgrowth; copulatory openings present inside the atrium; parmula long, extending towards anterior margin first then folding backward, distal part with seven to twelve transverse wrinkles (Figs 7A–D, 9A–D, 10A, B), distal tip with a socket posteriorly. Vulva: posterior median plate broad, cordiform; copulatory ducts long, arch-shaped; spermathecae U-shaped or L-shaped, present mesally on the posterior median plate; fertilization ducts long and extending mesally (Fig. 7D).

**Distribution.** China (Yunnan) (Fig. 12).



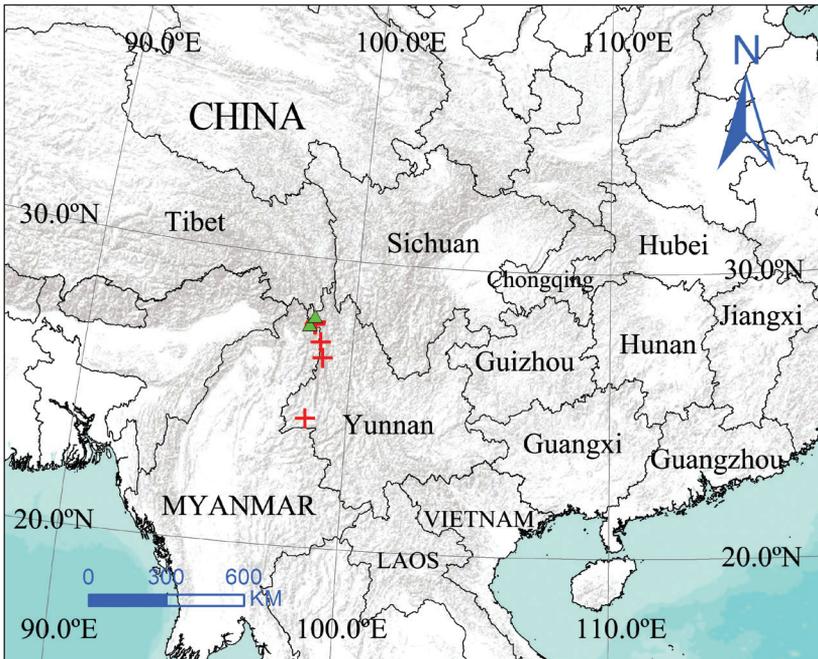
**Figure 11.** *Zhezhoulinyphia denticulata* sp. nov., habitus of female (one of paratypes, Tang-05-08) **A** dorsal view **B** ventral view. Scale bars: 1 mm (**A–D**).

***Zhezhoulinyphia yadongensis* (Hu & Li, 1987), comb. nov.**

*Centromerus yadongensis* Hu & Li, 1987: 343, fig. 21.1–4 (Df); Hu 2001: 494, fig. 328.1–3 (f).

**Remarks.** The epigyne of *Centromerus yadongensis* Hu & Li, 1987 shares the similar characters to the genus *Zhezhoulinyphia* gen. nov. such as: parmula extending towards the anterior margin first then folding backward, the distal part with transverse wrinkles, whereas it can be distinguished from *Zhezhoulinyphia caperata* sp. nov. and *Z. denticulata* sp. nov. by the number of the wrinkles in the parmula and the position of spermathecae (Figs 1, 2, 7, 9, 10; Hu 2001, figs 8–328). Based on the above-mentioned characters *Centromerus yadongensis* Hu & Li, 1987 is transferred to the genus *Zhezhoulinyphia* as *Z. yadongensis* com. nov. (Hu & Li, 1987).

**Distribution.** China (Tibet) (WSC 2019).



**Figure 12.** Distribution of *Zhezhoulinyphia caperata* sp. nov. (triangle) *Zhezhoulinyphia denticulata* sp. nov. (cross).

## Discussion

*Zhezhoulinyphia* gen. nov. can be identified as the member of the subfamily Linyphiinae by the following morphological characters: female palp with claw; male palp without tibial apophysis; maxillae long and parallel; all the tibiae with two dorsal spines (Merrett 1963).

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# On the genera *Qiongocera* and *Relictocera* (Araneae, Psilodercidae) from Southeast Asia

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## Abstract

Four new species are described in two psilodercid genera, *Qiongocera* Li & Li, 2017 and *Relictocera* Li & Li, 2017: *Qiongocera luxuan* Li & Li, **sp. nov.** (♂♀) from China, *Relictocera wugen* Li & Li, **sp. nov.** (♂♀) and *R. sigen* Li & Li, **sp. nov.** (♂) from Vietnam, and *R. qianzi* Li & Li, **sp. nov.** (♂♀) from Thailand. These genera were previously thought to be monotypic. In addition, one species of the genus *Merizocera* Fage, 1912 is transferred to *Relictocera*: *Relictocera mus* (Deeleman-Reinhold, 1995), **comb. nov.** (♂♀). The types of the new species are deposited in the Institute of Zoology, Chinese Academy of Sciences (IZCAS) in Beijing.

## Keywords

Cave, China, Ochyroceratidae, sexual dimorphism, Thailand, Vietnam

## Introduction

The spider family Psilodercidae Machado, 1951 was previously considered to be a subfamily of Ochyroceratidae Fage, 1912 by Machado (1951), and this was supported by Deeleman-Reinhold (1995). Psilodercids were elevated to family rank by Wunderlich (2004, 2008). Recently, phylogenetic analyses suggest that Psilodercidae are the sister group to the lineage encompassing Sicariidae Keyserling, 1880 and Scytodidae Blackwall, 1864 (Shao and Li 2018).

Psilodercidae comprises 120 named species in 11 genera (WSC 2019; Li and Quan 2017). Of these species, half of them belong to the genus *Althepus* Thorell, 1898. The other genera, such as *Flexicrurum* Tong & Li, 2007, *Luzonacera* Li & Li, 2017, *Qion-*

*gocera* Li & Li, 2017, *Relictocera* Li & Li, 2017, *Sinoderces* Li & Li, 2017, and *Thaiderces* Li & Li, 2017, have only recently been described (Tong and Li 2007; Liu et al. 2017). These haplogyne spiders are small web-weavers that are diverse in Southeast Asia. In total, 36 psilodercid species are reported from Thailand, 28 from Indonesia, 13 from China, 8 from Malaysia, 6 from Myanmar, 5 from Philippines, 5 from Laos and 3 from Vietnam (WSC 2019). The majority of the species are local endemics (WSC 2019).

While studying material from China, Thailand, and Vietnam, we found four new species belonging to two genera: *Qiongocera* and *Relictocera*, previously thought to be monotypic (Liu et al. 2017). The goal of this paper is to provide descriptions of the new species as well as new updated diagnoses for the two genera.

## Materials and methods

Types are deposited in the Institute of Zoology, Chinese Academy of Sciences (IZCAS) in Beijing. All specimens collected were preserved and observed in a 95% ethanol solution. The specimens were measured and examined under a Leica M205C stereomicroscope, and further morphological details were observed using an Olympus BX41 compound microscope. The male palp was dissected from the left side of the spider for further examination. The carapace measurements include the clypeus (except for *Relictocera* sp. which has a distinct clypeus). The length and width ratios were measured according to the length of the cymbium (including the cymbial protrusion) to its width. The internal genitalia of the female and the male palp were dissected and immersed in lactic acid for digestion. An Olympus C7070 wide zoom digital camera (7.1 megapixels) mounted on an Olympus SZX12 stereomicroscope was used to take photos at different focal plans. The photos were assembled with the image stacking software Helicon Focus 6.7.1 to generate high quality photos before further editing with Adobe Photoshop CC 2014. Leg measurements are given as total length (femur, patella, tibia, metatarsus, and tarsus). Leg segments were measured from their retrolateral side. All measurements are given in millimetres (mm). Terminology follows Li et al. (2014), Tong and Li (2007), and Deeleman-Reinhold (1995).

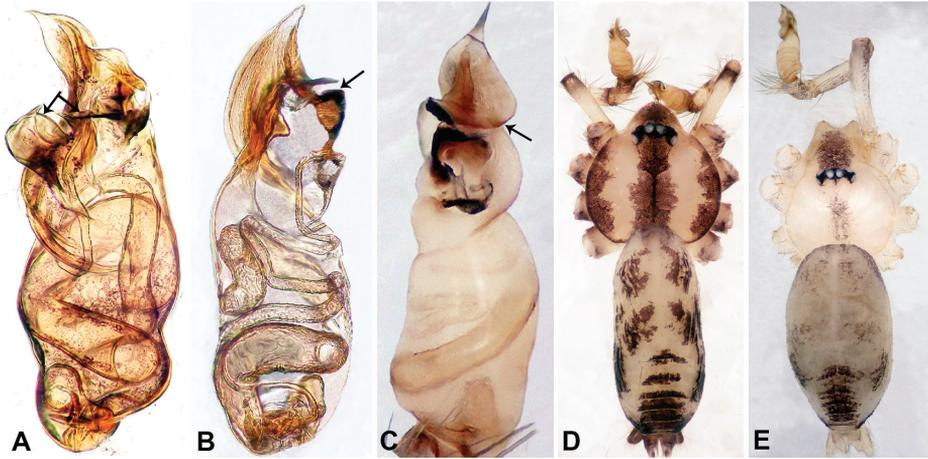
## Taxonomy

### Family Psilodercidae Machado, 1951

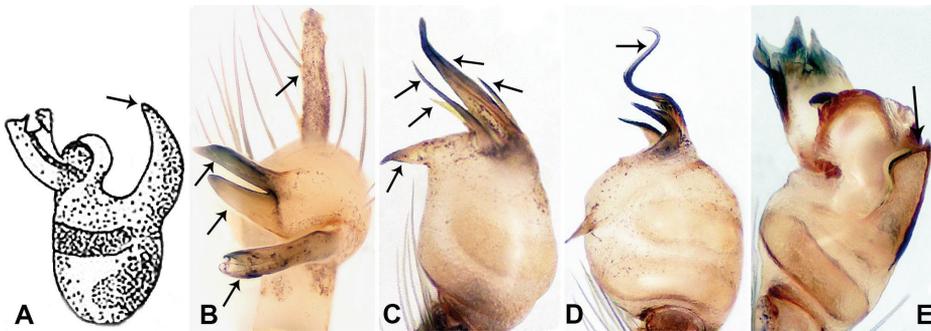
#### Genus *Qiongocera* Li & Li, 2017

**Type species.** *Qiongocera hongjunensis* Li & Li, 2017 from China, Hainan Province. Details and figures of the type species as in Fig. 1A, D, and Liu et al. (2017): figs 5, 6.

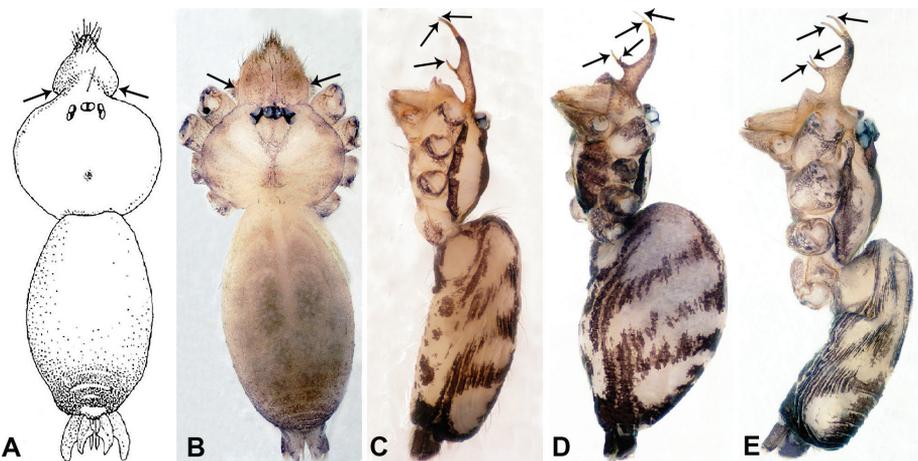
**Emended diagnosis.** *Qiongocera* resembles *Flexicrurum* but can be differentiated by the following combination of characters: 1) cymbium with a tilted protrusion (vs. cymbium with a strong lateral protrusion and with a small postero-lateral protrusion



**Figure 1.** Bulb and habitus of *Qiongocera hongjunensis* (A, D) and *Q. luoxuan* sp. nov. (B–C, E) A bulb, prolateral B bulb, retrolateral C bulb, ventral D–E habitus, dorsal.



**Figure 2.** Bulbs of *Relictocera mus* (A) *R. qiyi* (B) *R. wugen* sp. nov. (C) *R. sigen* sp. nov. (D) and *R. qianzi* sp. nov. (E). A retrolateral view; B–E ventral views.



**Figure 3.** Male habitus of *Relictocera mus* (A) *R. qianzi* sp. nov. (B) *R. qiyi* (C) *R. wugen* sp. nov. (D) *R. sigen* sp. nov. (E). A–B dorsal views; C–E lateral views.

bearing a strong seta; 2) laminar apophysis half the width and length of bulb (vs. bulbal apophysis length equals and width, 15 times shorter than that of bulb in *Flexicrurum*); 3) simple process on the distal end of bulb (vs. a complex processes with more than one extension in *Flexicrurum*); and 4) a short embolus (vs. a long embolus in *Flexicrurum*).

**Composition.** *Qiongocera hongjunensis* (the type species) and *Qiongocera luoxuan* Li & Li, sp. nov.

**Distribution.** Hainan Province, China.

### Key to species of *Qiongocera* (males only)

- 1 Bulb with laminar apophysis on distal part and with 2 unequal parts of a plier-like processes (Fig. 1A); body dark brown (Fig. 1D) ..... *Q. hongjunensis*  
 – Bulb with a distinct helical laminar apophysis extending from the distal half (Fig. 1C) and with a triangular process (Fig. 1B); body pale (Fig. 1E).....  
 ..... *Q. luoxuan* sp. nov.

### *Qiongocera luoxuan* Li & Li, sp. nov.

<http://zoobank.org/76C26986-8691-4315-B226-3E8FA56A11DA>

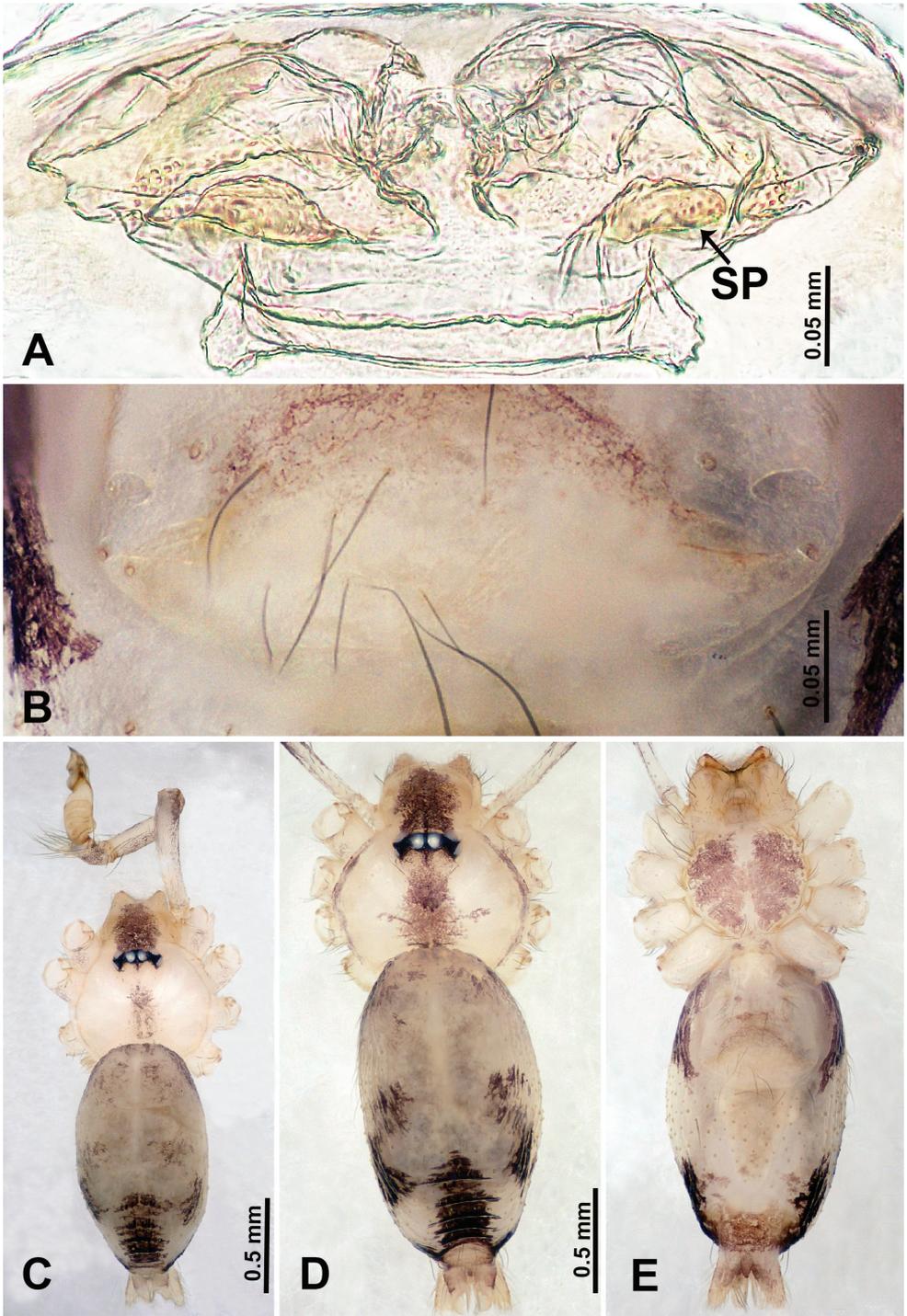
Figs 1B, C, E, 4, 5, 12A, 13

**Types. Holotype:** ♂ (IZCAS), China, Hainan Province, Dongfang City, Donghe Town, Yalong Village, Yalong-Huangxian Cave, 18°58.752'N, 108°53.308'E, 264 m, 15.XII.2014, Zhao Q. and Shao L. **Paratypes:** 1♂1♀ (IZCAS), same data as holotype.

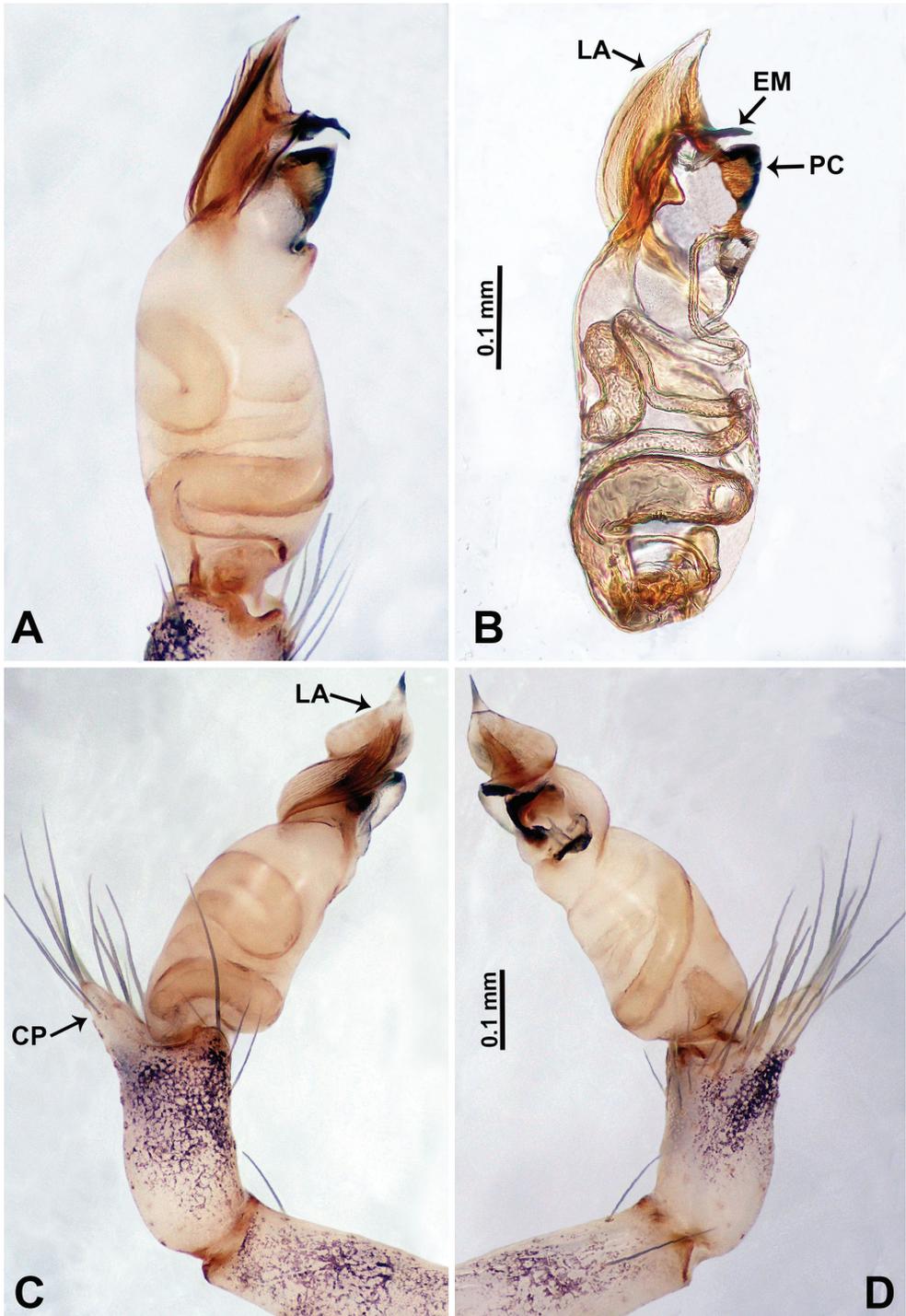
**Etymology.** The species name is a noun in apposition derived from the Chinese pinyin “luóxuán” (helical) and refers to the helical shape of the bulb in the distal half.

**Diagnosis.** Males of *Q. luoxuan* sp. nov. can be distinguished from *Q. hongjunensis* by the triangular process on the bulb (Fig. 5B) (vs. a plier-like process with 2 unequal parts in *Q. hongjunensis*); the distal half of the bulb helical (Fig. 5C, D) (vs. a rather simple pyriform bulb in *Q. hongjunensis*); females can be distinguished by the pair of thin, complex branches of the spermathecae that are convexly curved (Fig. 4A) (vs. a pair of bulging round spermathecae in *Q. hongjunensis*); the coloration and patterns of both male and female are relatively indistinct (Fig. 4C–E) (vs. a distinct dark brown coloration and pattern in *Q. hongjunensis*).

**Description. Male** (Holotype). Total length 2.65; carapace 1.09 long, 1.13 wide; abdomen 1.56 long, 0.78 wide. Carapace round and pale yellow with a faint longitudinal brown patch medially (Fig. 4C). Fovea shallow and brown. Anterior part of the thoracic region distinctly elevated. Chelicerae yellow. Cheliceral promargin with lamina bearing 3 triangular extensions, retromargin with 2 small teeth (Fig. 12A). Clypeus slanting, dark brown with two pale areas laterally. Endites pale yellow. Labium slanting, light brown. Sternum circular with brown complex pattern delimiting two pale oval areas anteriorly and posteriorly. Abdomen dorsum with several dark horizontal stripes concentrated posteriorly, venter with dark curves concentrated at the edges. Legs uni-



**Figure 4.** *Qiongocera luoxuan* sp. nov., male holotype and female paratype **A** internal genitalia, dorsal view **B** female epigastric area, ventral view **C** male habitus, dorsal view **D** female habitus, dorsal view **E** female habitus, ventral view. Abbreviation: SP = spermatheca.



**Figure 5.** *Qiongocera luoxuan* sp. nov., male holotype **A** palp, ventral view **B** palpal bulb, ventral view **C** palp, prolateral view **D** palp, retrolateral view. Abbreviations: CP = cymbial protrusion, EM = embolus, LA = laminar apophysis, PC = process of bulb.

formly brown; measurements: I 18.75 (5.45, 0.40, 5.77, 5.13, 2.00), II 14.02 (4.00, 0.40, 4.49, 3.53, 1.60), III missing, IV missing. Palp (Fig. 5A–D): femur slender, 5 times longer than patella; patella angled ventrally; tibia pale, 2 times shorter than femur, suffused with tiny sepia patches, strongly deflected prolaterally; cymbium pale, darker distally, with dorsal margin curved basally, with short, pale distal protrusion, conical in lateral view and directed at 45 °; length/width ratio = 2.0; bulb light yellow, proximal half roughly cylindrical, distal half twisted, forming 3 helical coils tapered apically, ending at a pointed laminar apophysis; triangular process adjacent to embolus short; embolus thin, sharp and darkened, extending from distal part of the bulb (Fig. 5B).

**Female** (Paratype). General features and coloration similar to that of male (Fig. 4D–E). Measurements: total length 2.60; carapace 1.00 long, 1.00 wide; abdomen 1.60 long, 0.80 wide. Leg measurements: I 12.85 (3.50, 0.40, 3.75, 3.40, 1.80), II missing, III 7.16 (2.00, 0.31, 2.19, 1.72, 0.94), IV 10.39 (3.20, 0.30, 3.30, 2.50, 1.09). Internal genitalia: one pair of sclerotized ovoid spermathecae surrounded by a pair of thin ducts with complex branches curving convexly, posteriorly with horizontal bar bearing droplet-shaped ducts (Fig. 4A).

**Distribution.** Known only from the type locality (Fig. 13).

### Genus *Relictocera* Li & Li, 2017

**Type species.** *Relictocera qiyi* Li & Li, 2017 from Vietnam, Thua Thien Hue Province. Details and figures of type species as shown in Figs 2B, 3C and Liu et al. (2017): figs 7, 8.

**Emended diagnosis.** *Relictocera* can be distinguished from *Luzonacera* by the following combination of characters: 1) bulb with numerous appendages (vs. bulb without appendages); 2) male with clypeal projection (vs. male without clypeal projection); 3) chelicera without promarginal teeth (vs. chelicera with 1 promarginal tooth); and 4) cheliceral promargin lamina with 3 triangular extensions (vs. lamina with 2 triangular extensions).

**Composition.** *Relictocera qiyi* (the type species), *R. sigen* Li & Li, sp. nov., *R. wugen* Li & Li, sp. nov., *R. qianzi* Li & Li, sp. nov., and *R. mus* (Deeleman-Reinhold, 1995).

**Distribution.** Vietnam and Thailand.

### Key to species of *Relictocera* (males only)

- 1 Bulb with pincer-like appendages; clypeus with a hairy snout-like projection; patella 2 times wider than cymbium ..... **2**
- Bulb with tentacle-like appendages; clypeus with a furcate projection; patella and cymbium equally wide ..... **3**
- 2 Appendages with pointed tips (Fig. 2A); clypeus with rounded base (Fig. 3A); carapace without pattern (Fig. 3A)..... ***R. mus***
- Appendages with blunt tips (Fig. 2E); clypeus with a straight base (Fig. 3B); carapace with distinct pattern (Fig. 3B)..... ***R. qianzi* sp. nov.**

- 3 Embolus straight..... 4  
 – Embolus spiralled (Fig. 2D)..... *R. sigen* sp. nov.  
 4 Bulb with 5 appendages (including embolus) (Fig. 2C); appendages of different length; clypeus with a quadrifurcate projection (Fig. 3D).....*R. wugen* sp. nov.  
 – Bulb with 4 appendages (including embolus) (Fig. 2B); appendages (except embolus) almost equal in length; clypeus with a trifurcate projection (Fig. 3C)..... *R. qiye*

***Relictocera wugen* Li & Li, sp. nov.**

<http://zoobank.org/0C9B73EC-29BF-44EC-9178-2E06E476D3CF>

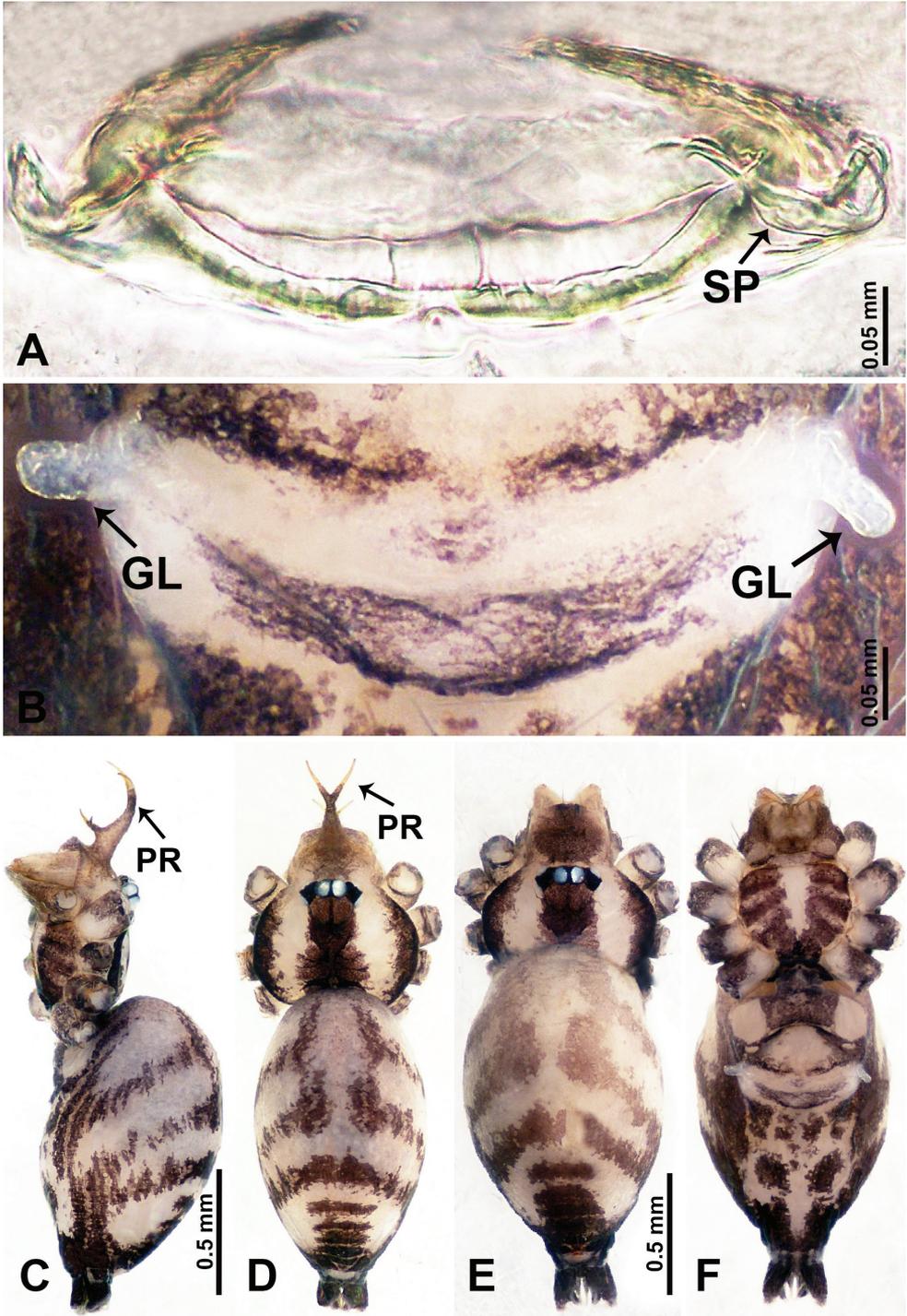
Figs 2C, 3D, 6, 7, 12B, 13

**Types. Holotype:** ♂ (IZCAS), Vietnam, Quang Binh Province, Phong Nha-Ke Bang National Park, outside of Botanical Garden, 17°32.895'N, 106°17.830'E, 261 m, 23.VIII.2015, Zhao Q., Li Y. & Chen Z. **Paratypes:** 1♂1♀ (IZCAS), same data as holotype.

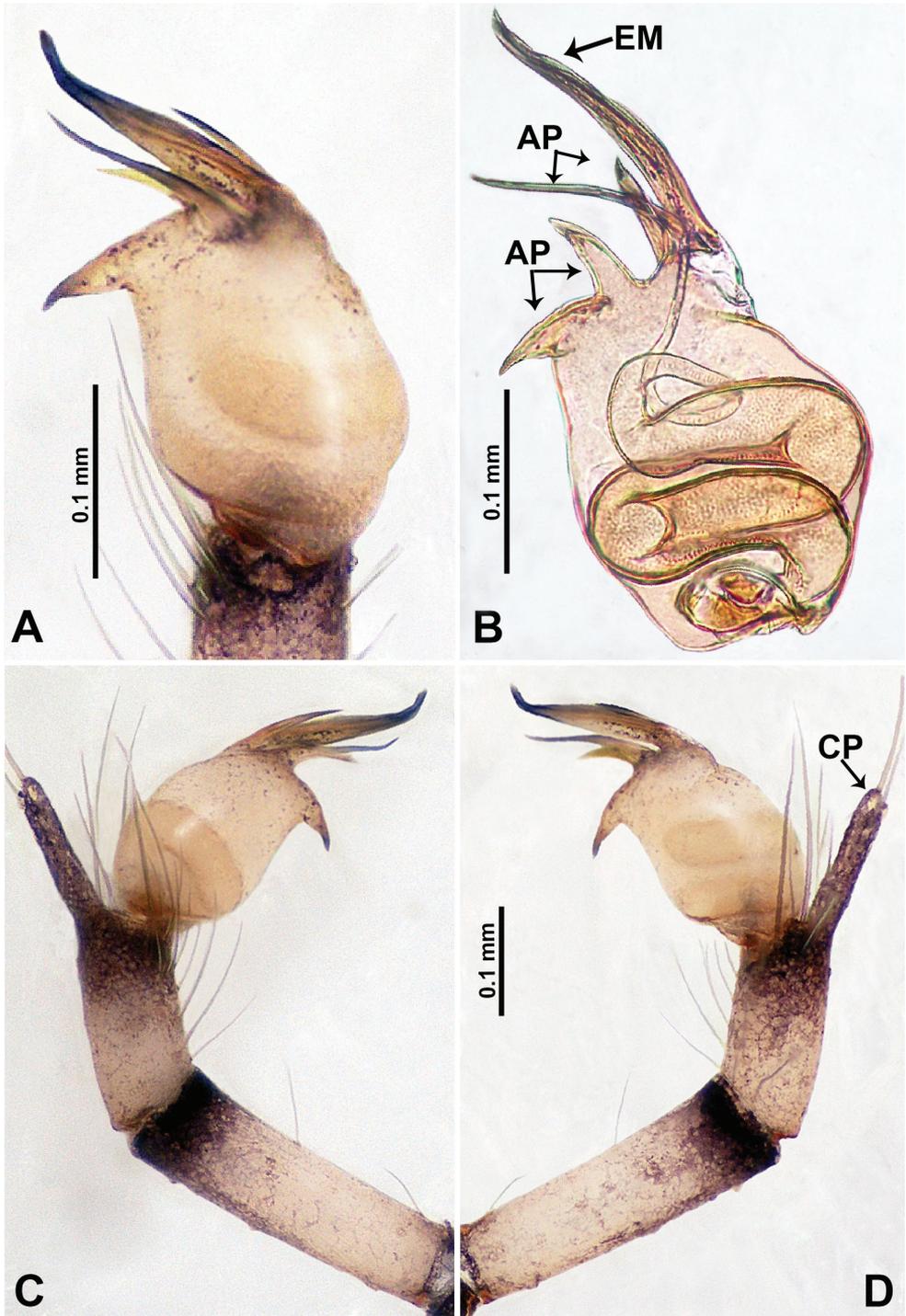
**Etymology.** The species name is a noun in apposition derived from the Chinese pinyin “wugen” (“five stripes”) and refers to the five appendages on the male bulb, including the embolus.

**Diagnosis.** Males of *R. wugen* sp. nov. can be distinguished from those of *R. sigen* sp. nov. by the nearly straight embolus (Fig. 7B) vs. a spiralled embolus Fig. 9B; *R. wugen* sp. nov. has 4 distinct and relatively long bulbal appendages (Fig. 7B) vs. 3 relatively short bulbal appendages (Fig. 9B); pyriform bulb (Fig. 7B) vs. globular bulb (Fig. 9B); carapace wider than long vs. longer than wide; females can be distinguished from *R. qianzi* sp. n. by a pair of slender and elongated spermathecae that curve upward vs. ovoid spermathecae flanked laterally by a pair of translucent ducts (Fig. 6A).

**Description. Male** (Holotype). Total length 1.70; carapace 0.70 long, 0.63 wide; abdomen 1.00 long, 0.69 wide. Carapace round, pale yellow, with 3 longitudinal dark brown bands; the middle band 2 times wider than the lateral band (Fig. 6D). Chelicerae yellow (Fig. 12B). Clypeus light brown, with a long, quadrifurcate medial projection (Fig. 6C, D). Endites brown. Labium brown with a pair of pale spots. Sternum with longitudinal white median band and pair of dark brown lateral stripes. Abdomen dorsally with longitudinal white band anteriorly and with 3 pairs of dark brown lateral stripes, posterior part with longitudinal dark brown stripes, anterior of ventrum with pair of circular pale lateral patches, posterior with dark brown lateral area and several longitudinal irregular dark brown spots (Fig. 6C, D). Legs uniformly brown; measurements: I 10.75 (2.75, 0.25, 3.25, 3.25, 1.25), II 7.50 (2.24, 0.25, 2.13, 2.13, 0.75), III 5.40 (1.60, 0.20, 1.50, 1.50, 0.60), IV missing. Palp (Fig. 7A–D): femur 5 times longer than patella; patella not swollen; tibia pale, 2 times shorter than femur, with dark distal ring; cymbium pale, distal half darker than proximal, with long and thin dark distal protrusion directed forward; length/width ratio = 3.13; bulb light yellow, oval, with 4 appendages; embolus straight, flattened, tapering apically; appendages (except embolus) differ in length, merging separately at distal part of bulb and adjacent to embolus (Fig. 7B).



**Figure 6.** *Relictocera wugen* sp. nov., male holotype and female paratype **A** internal genitalia, dorsal view **B** female epigastric area, ventral view **C** male habitus, retrolateral view **D** male habitus, dorsal view **E** female habitus, dorsal view **F** female habitus, ventral view. Abbreviations: **GL** = genitalic lobe, **PR** = clypeal protrusion, **SP** = spermatheca.



**Figure 7.** *Relictocera wugen* sp. nov., male holotype **A** palp, ventral view **B** palpal bulb, ventral view **C** palp, prolateral view **D** palp, retrolateral view. Abbreviations: AP = appendage of bulb, CP = cymbial protrusion, EM = embolus.

**Female** (Paratype). General features and coloration similar to those of male except for the absence of a quadrifurcate clypeal projection (Fig. 6E, F). Measurements: total length 2.50; carapace 0.75 long, 0.80 wide; abdomen 1.75 long, 0.80 wide. Leg measurements: I 6.54 (2.00, 0.16, 2.19, 1.19, 1.00), II 5.56 (1.56, 0.25, 1.50, 1.50, 0.75), III 3.90 (1.09, 0.16, 1.09, 1.09, 0.47), IV 6.25 (1.75, 0.25, 1.75, 1.75, 0.75). Epigastric furrow (slit) with pair of translucent lateral lobes (Fig. 6B). Internal genitalia: one pair of slender and elongated spermathecae curving anteriorly with pointed tips, bases separated by 3 times the width of the spacing of the of tips. (Fig. 6A).

**Distribution.** Known only from the type locality (Fig. 13).

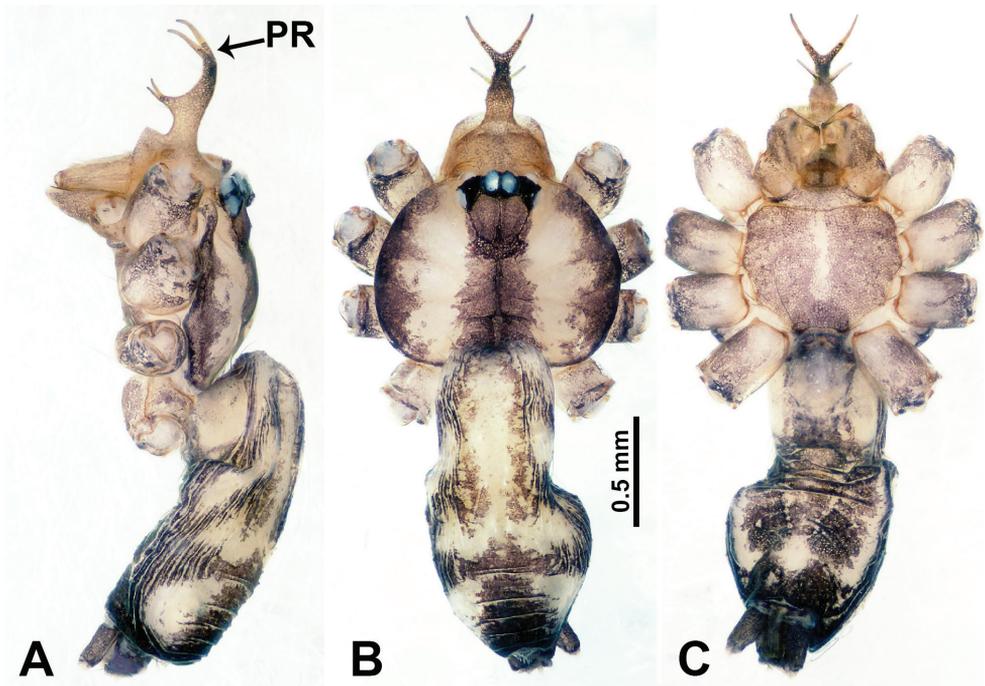
***Relictocera sigen* Li & Li, sp. nov.**

<http://zoobank.org/6462B938-880A-4C6A-BDFE-64058181721C>

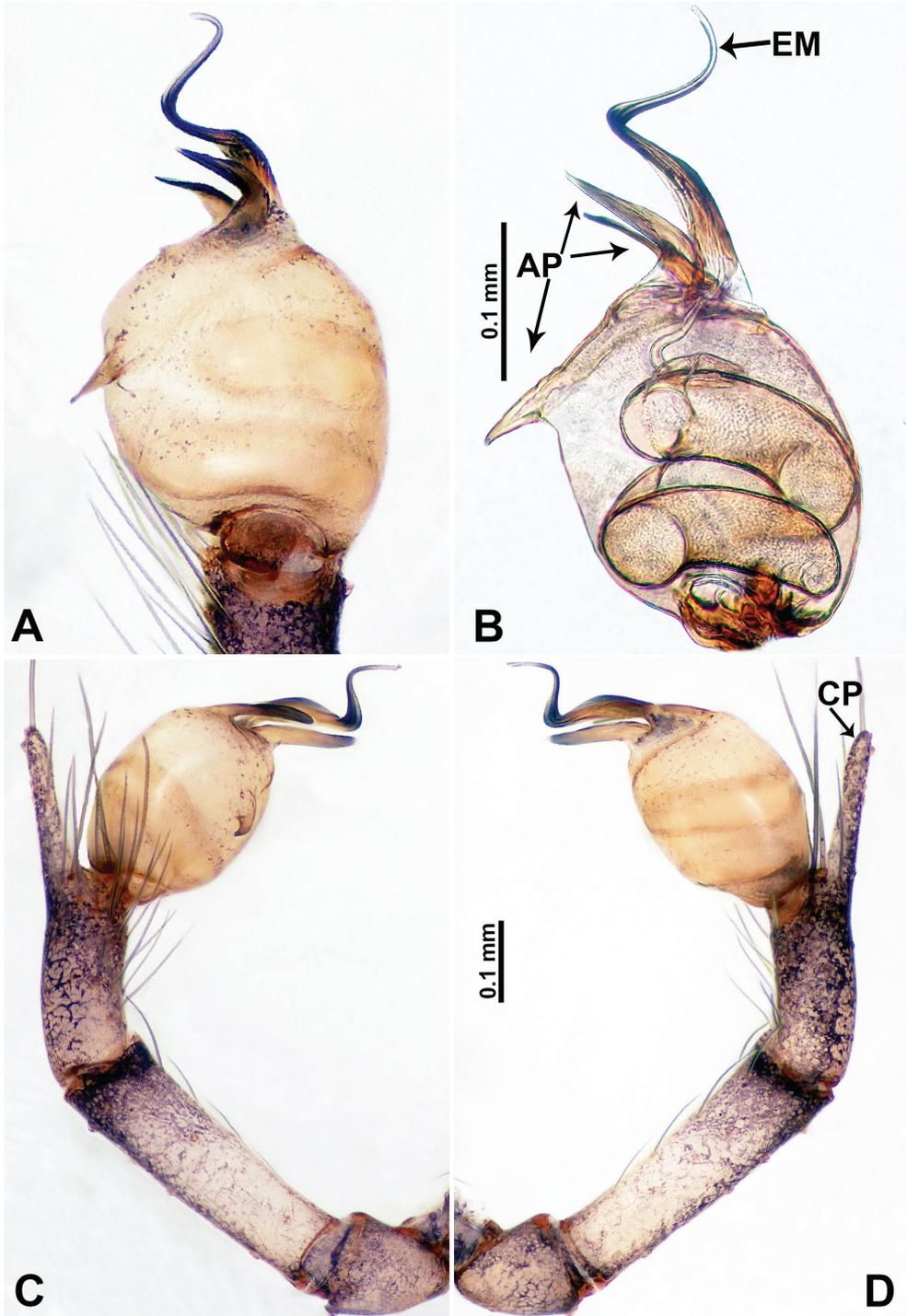
Figs 2D, 3E, 8, 9, 12C, 13

**Types. Holotype:** ♂ (IZCAS), Vietnam, Ninh Binh Province, Cuc Phuong National Park, Palace Cave, 20°21.350'N, 105°36.282'E, 523 m, 19.VIII.2015, Zhao Q., Li Y. & Chen Z.

**Etymology.** The species name is a noun in apposition derived from the Chinese pinyin “sigen” (“four strips”) and refers to the four appendages on the male bulb, including the embolus.



**Figure 8.** *Relictocera sigen* sp. nov., male holotype **A** male habitus, retrolateral view **B** male habitus, dorsal view **C** male habitus, ventral view. Abbreviation: PR = clypeal projection.



**Figure 9.** *Relictocera sigen* sp. nov., male holotype **A** palp, ventral view **B** palpal bulb, ventral view **C** palp, prolateral view **D** palp, retrolateral view. Abbreviations: **AP** = appendage of bulb, **CP** = cymbial protrusion, **EM** = embolus.

**Diagnosis.** See diagnosis for *R. wugen* sp. nov.

**Description. Male** (Holotype). Total length 2.28; carapace 1.00 long, 1.20 wide; abdomen 1.28 long, 0.64 wide. Carapace round, yellow, with 3 longitudinal dark brown bands; the middle band 2 times wider than the lateral band (Fig. 8B). Chelicerae yellow (Fig. 12C). Clypeus pale brown, with quadrifurcate medial projection (Fig. 8A, B). Endites pale brown. Labium brown with 2 large pale spots (Fig. 8C). Sternum brown with median longitudinal pale band not reaching the posterior margin (Fig. 8C). Abdomen dorsally with anterior longitudinal pale-yellow band and pair of lateral dark brown stripes, posteriorly with longitudinal dark brown stripes, venter pale anteriorly, posterior part with scattered dark brown patches. Legs uniformly brown; measurements: I (3.51, 0.40, 5.77, 6.73, missing), II 12.70 (3.53, 0.32, 3.85, 4.00, 1.00), III 9.40 (2.60, 0.40, 2.80, 2.80, 0.80), IV 15.39 (4.49, 0.32, 4.49, 4.81, 1.28). Palp (Fig. 9A–D): femur 5 times longer than patella; patella not swollen; tibia pale, 2 times shorter than femur, dark distally; cymbium almost completely dark, with long and thin distal protrusion directed forward, slightly oblique; length/width ratio=3.33; bulb light yellow, globose, with 3 appendages (except embolus); embolus coiled, tapering apically; trifurcate appendages almost equal in length but distinctly shorter than embolus, emerging separately at distal part of bulb, except for one prolateral appendage directed perpendicular to other appendages (Fig. 9B).

**Female.** Unknown.

**Distribution.** Known only from the type locality (Fig. 13).

***Relictocera qianzi* Li & Li, sp. nov.**

<http://zoobank.org/02E7AC89-2B89-4108-B450-F9DBAD1D30F4>

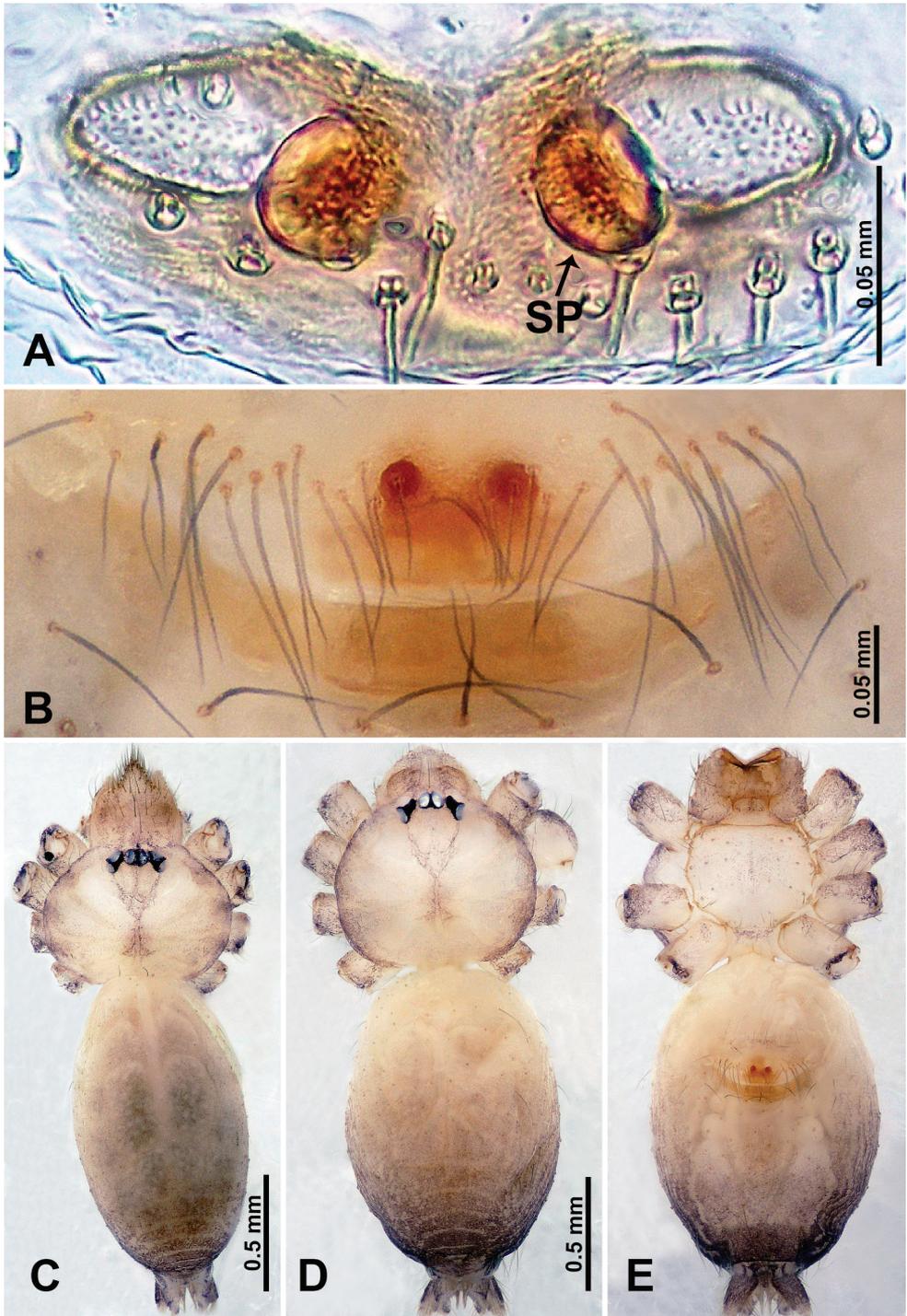
Figs 2E, 3B, 10, 11, 12D, 13

**Types. Holotype:** ♂ (IZCAS), Thailand, Krabi Province, Ao Luk District, Petch Cave, 8°23.578'N, 98°46.437'E, 56 m, 11.X.2015, Zhao Q., Zhou G. & Chen Z. **Paratypes:** 1♂1♀ (IZCAS), same data as holotype.

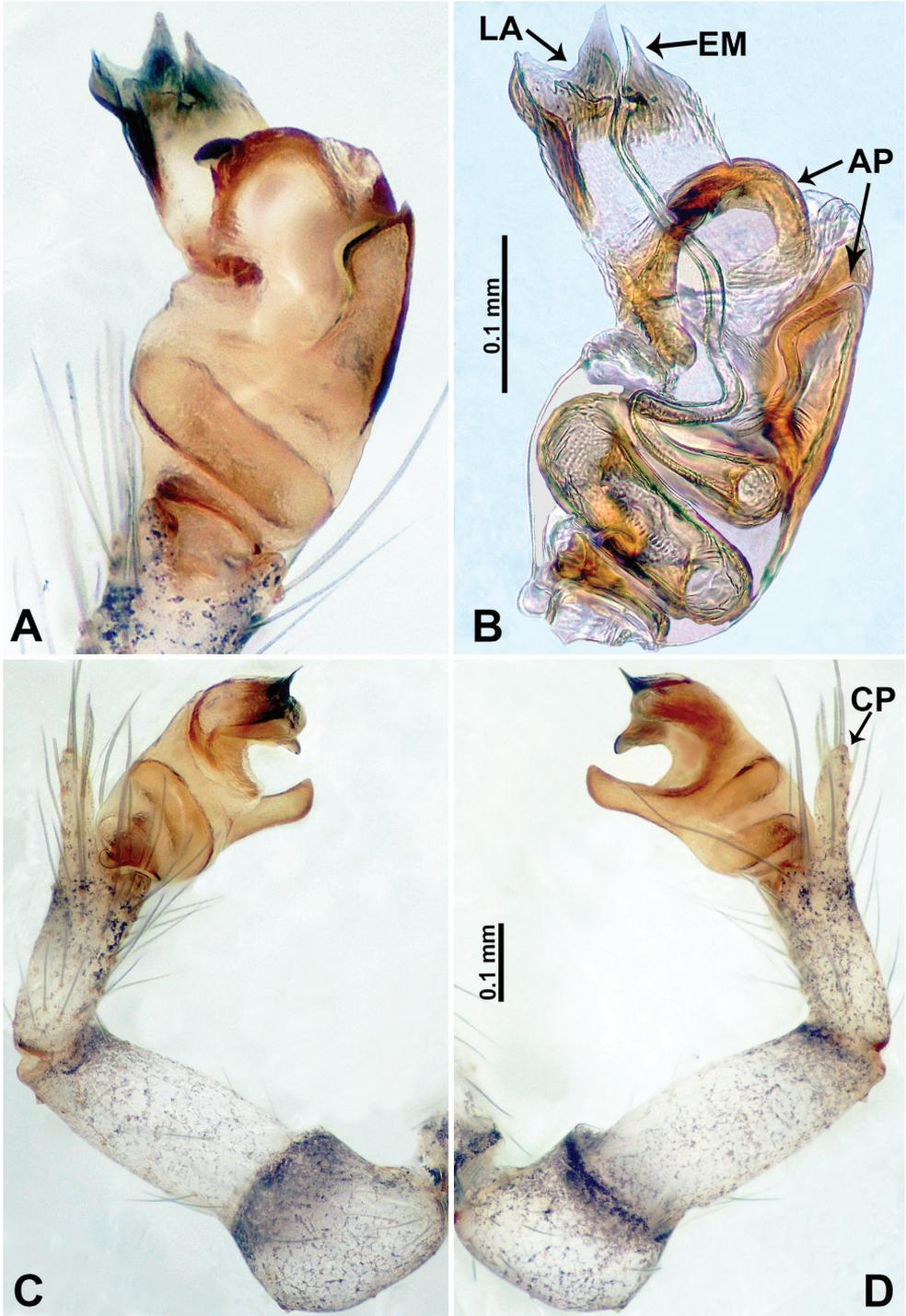
**Etymology.** The species name is a noun in apposition derived from the Chinese pinyin “qiánzǐ” (pincer) and refers to the unique structure of the bulb appendages which are similar to the gripping jaws of a pincer (Fig. 11C, D).

**Diagnosis.** Males of *R. qianzi* sp. nov. can be distinguished from all other congeners by the bulb bearing two unequal parts, a strongly swollen palpal patella, and a partly swollen tibia (Fig. 11C–D). Males can be easily distinguished from *R. mus* by the blunt tips of the bulbal appendages (vs. sharply pointed tips); a hairy snout-like clypeus with a straight base (vs. a spade-shaped clypeus with a distinct rounded base in *R. mus*); carapace with trident pattern medially (vs. absence of pattern); the female can be distinguished from other species by the pair of ovoid spermathecae flanked laterally with a pair of translucent ducts (vs. a pair of horizontal ducts bearing round spermathecae).

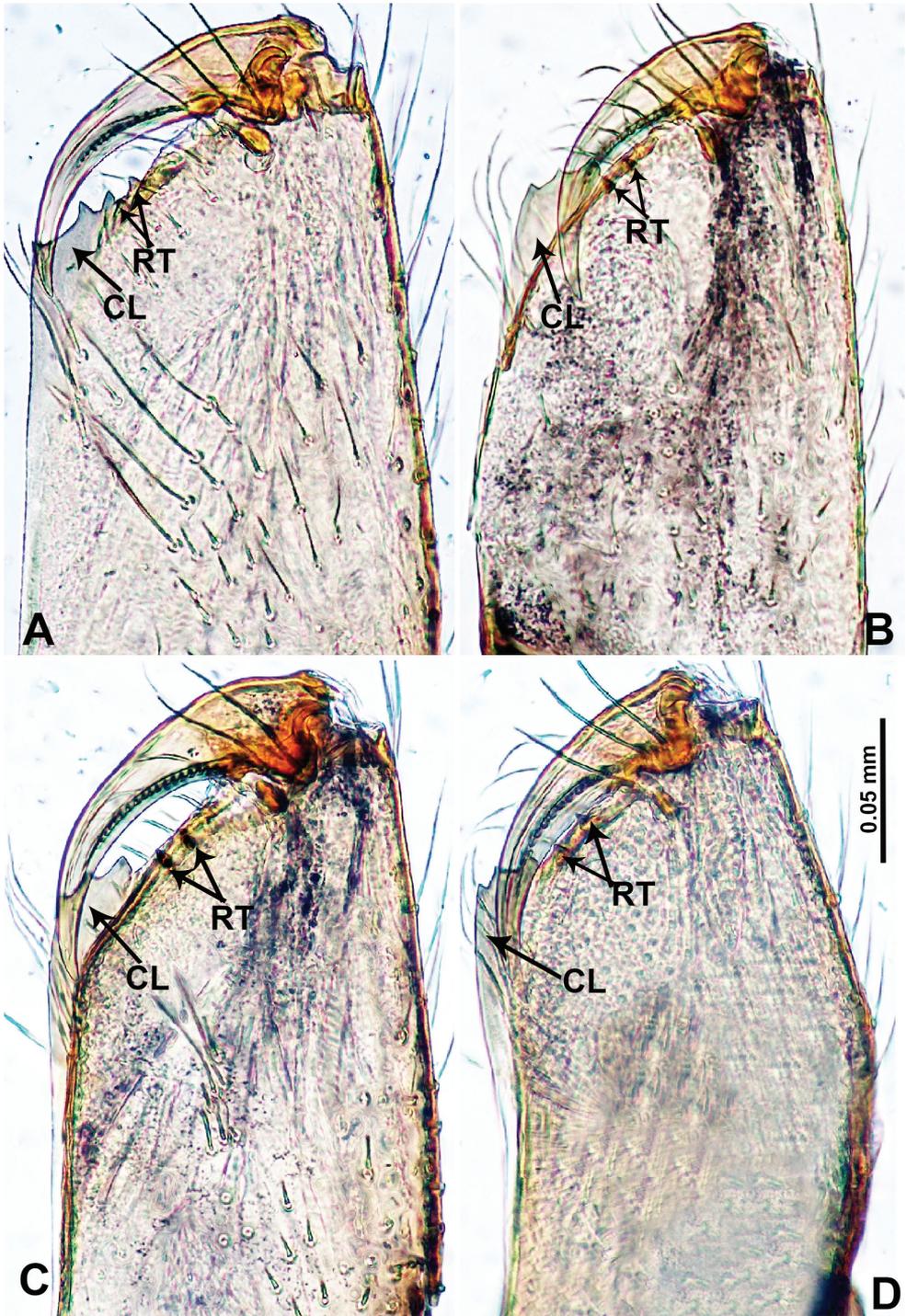
**Description. Male** (Holotype). Total length 2.70; carapace 1.10 long, 1.10 wide; abdomen 1.60 long, 0.90 wide. Carapace round and pale yellow, medially with light brown trident pattern, laterally slightly darker (Fig. 10C). Chelicerae yellow



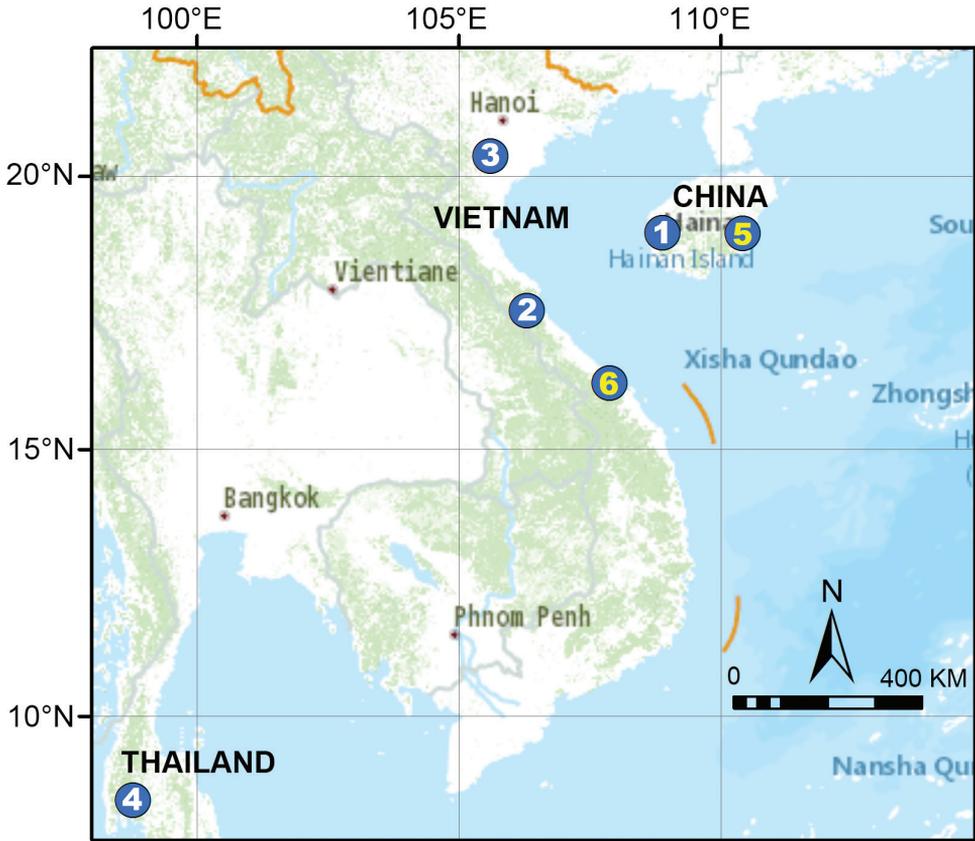
**Figure 10.** *Relictocera gianzi* sp. nov., male holotype and female paratype **A** internal genitalia, dorsal view **B** female epigastric area, ventral view **C** male habitus, dorsal view **D** female habitus, dorsal view **E** female habitus, ventral view. Abbreviation: SP = spermatheca.



**Figure 11.** *Relictocera qianzi* sp. nov., male holotype **A** palp, ventral view **B** palpal bulb, ventral view **C** palp, prolateral view **D** palp, retrolateral view. Abbreviations: **AP** = appendage, **CP** = cymbial protrusion, **EM** = embolus, **LA** = laminar apophysis.



**Figure 12.** Cheliceral retromargin **A** *Qiongcera luoxuan* sp. nov. **B** *Relictocera wugen* sp. nov. **C** *R. sigen* sp. nov. **D** *R. qianzi* sp. nov. Abbreviations: **CL** = cheliceral lamina, **RT** = retromarginal teeth.



**Figure 13.** Distribution of *Qiongocera* and *Relictocera* species in China (Hainan), Vietnam, and Thailand. White numbers indicate new species and yellow numbers indicate previously known species. **1** *Qiongocera luoxuan* sp. nov. **2** *Relictocera wugen* sp. nov. **3** *R. sigen* sp. nov. **4** *R. qianzi* sp. nov. **5** *Qiongocera hongjunensis* **6** *Relictocera qi yi*.

(Fig. 12D). Clypeus brown, with hairy snout-like projection. Endites pale brown. Labium brown, delimiting 2 oval paler areas. Sternum yellow. Abdomen with dorsal longitudinal pale band, with shaded lateral patches, posterior part with a few dark brown stripes, anterior part of ventrum pale, posterior part with dark brown shading on the margin. Legs uniformly brown; measurements: I missing, II missing, III 10.48 (3.00, 0.31, 2.97, 3.20, 1.00), IV missing. Palp (Fig. 11A–D): femur slender, 2 times longer than patella; patella strongly swollen, as long as wide, 2 times wider than cymbium; tibia 2/3 the length of femur, basally swollen (length/width ratio = 3.0); cymbium pale, slightly yellowish distally, with distal protrusion pale, slightly curved upward; length/width ratio = 3.00; bulb brown, bifurcate; laminar apophysis branched; embolus located distally, adjacent to laminar apophysis, ribbon-like with a protruding tip and a pair of irregular pincer-like appendages adjacent to the embolus (Fig. 11C, D).

**Female.** General features and coloration similar to those of the male (Fig. 10D, E). Measurements: total length 2.46; carapace 0.90 long, 1.09 wide; abdomen 1.56 long, 0.94 wide. Leg measurements: I 16.73 (4.00, 0.40, 4.81, 5.77, 1.75), II 11.68 (3.20, 0.32, 3.21, 3.75, 1.20), III missing, IV missing. Epigastric area with two small, round red spots medially, anterior to epigastric furrow; posterior margin of epigastric slit thick, slightly sclerotized (Fig. 10B, E). Internal genitalia: one pair of sclerotized ovoid spermathecae flanked laterally by 2 larger translucent ducts, spermathecae separated by one spermatheca diameter (Fig. 10A).

**Distribution.** Known only from the type locality (Fig. 13).

***Relictocera mus* (Deeleman-Reinhold, 1995), comb. nov.**

Figs 2A, 3A

*Merizocera mus* Deeleman-Reinhold 1995: 42, figs 94–103 (♂♀)

**Diagnosis.** Diagnostic features are discussed under *R. qianzi* sp. nov.

**Description.** Described by Deeleman-Reinhold (1995).

**Distribution.** Thailand.

**Remarks.** Although we did not examine the type material of *Merizocera mus*, figures of this species in Deeleman-Reinhold (1995) are congruent with the features of the *Relictocera*.

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# A key to the flat grass scale genus *Nipponaclerda* (Hemiptera, Coccoomorpha, Aclerididae)

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## Abstract

The flat grass scale genus *Nipponaclerda* comprises four species, native to Central and East Asia. *Nipponaclerda biwakoensis* has been introduced to the United States and is considered a serious pest of *Phragmites australis*, the common reed. Heavy infestations of *N. biwakoensis* in coastal marshes of Louisiana have coincided with extensive die-off of reeds. In this article, dichotomous identification keys to the genera of Aclerididae and to the species of *Nipponaclerda* are provided, allowing for accurate identification of species found in the native and invasive range.

## Keywords

Identification, invasive species, Louisiana, pest, scale insect, *Phragmites australis*

## Introduction

The genus of flat grass scale insects, *Nipponaclerda* McConnell (Hemiptera, Coccoomorpha, Aclerididae), comprises four species native to Central and East Asia, one of which has been introduced to the United States. This small group is receiving attention due to the recent discovery of *Nipponaclerda biwakoensis* (Kuwana) (the roseau cane scale) in Louisiana, U.S.A. (Knight et al. 2018). The detection of invasive populations of *N. biwakoensis* in Louisiana coincided with the discovery of extensive die-off of *Phragmites australis* (Cav.) Trin. Ex Steud. (i.e., common reed, roseau cane). In 2016,

surveyors found large stands of dead or dying *P. australis* in Plaquemines Parish, Louisiana (Knight et al. 2018), part of the Mississippi River Delta (MRD). This sparked concern due to the reeds' importance to marsh ecosystems and coastal infrastructure. Losses of *Phragmites* in the MRD could have substantial, negative economic repercussions. Roseau cane is the dominant emergent plant in the MRD; it builds, maintains, and protects marsh soils and provides habitat for diverse wildlife. Dense stands of roseau cane help reduce wave action, which shields coastal regions from damaging storm surges and hurricanes. The marshes also shelter a network of shipping channels as well as oil and gas infrastructure in this region.

Presently, *N. biwakoensis* is the only member of this genus to be considered a pest. Heavy infestations in Louisiana reached over 2,000 individuals per stem in 2016–2017 (Knight et al. 2018). In China it is considered a pest of *P. australis*, which is cultivated for the paper industry (Brix et al. 2014). Natural enemies help keep populations at low abundance in the native range, and in Japan and China *N. biwakoensis* served as a model for studies about parasitism by hymenopterans and predation from birds (Kaneko 1995; 2004; 2005; Xiong et al. 2010).

Aclerids feed primarily on grasses (Poaceae), which include some of the most important commodities such as maize, rice, wheat, and sugarcane. But damaging infestations of acclerids are uncommon on commodities (McConnell 1954), which explains why acclerids have received minimal attention in the taxonomic literature, where efforts skew toward larger groups with more significant agricultural impact. Thus, Acleridae is an especially difficult group to identify to species. The only key ever published to differentiate between species of *Nipponaclerda* was by Borchsenius (1960), which can be used to separate *N. biwakoensis* from *N. turanica* (Archangelskaya) (reproduced in Wang 1994). Considering the potential economic impact of *N. biwakoensis*, a history of introduction to the United States, and the possibility of discovering additional species, it is important to have a tool that allows for accurate identification of species in this genus.

## Materials and methods

Specimens of *N. biwakoensis* collected from Louisiana and Texas were slide-mounted following the protocol of the Systematic Entomology Laboratory (USDA, ARS) at Beltsville, Maryland (<http://www.ars.usda.gov/Main/docs.htm?docid=9832>). DNA extractions were performed on some specimens prior to slide-mounting (see Knight et al. 2018); others were prepared without having an extraction performed. Specimens are deposited in the United States National Museum of Natural History's Collection of Cocomorpha, housed in Beltsville, Maryland. Observations were made using a Zeiss Axio Imager.M2 (Carl Zeiss Inc., Thornwood, NY, U.S.A.). Diagnostic information for species of *Nipponaclerda* was drawn from published descriptions and illustrations (Borchsenius 1960; Kuwana 1907; McConnell 1954; Wang and Zhang 1994; Zhang 1998).

### **Institutional abbreviations**

- C DFA** California State Collection of Arthropods at California Department of Food and Agriculture  
**USNM** The Smithsonian Institute, United States National Museum of Natural History, Entomology Collection, Coccoomorpha

### **Taxonomy**

#### ***Nipponaclerda* McConnell, 1954**

*Nipponaclerda* McConnell 1954: 107.

**Type species.** *Aclerda biwakoensis* Kuwana by monotypy and original designation.

**Comments.** *Nipponaclerda* are classified within the subfamily Aclerinae Cockerell (Hodgson and Millar 2002), with *Aclerda* Signoret and *Lecanaclerda* Hodgson & Millar. Members of this genus are similar to species of *Aclerda* but can be distinguished in the adult female stage by the poor development of anal ring setae, which are few in number, shorter than the anal plate, and never extend beyond the body apex (Borchsenius 1960). In adult females of *Aclerda*, these setae are numerous, long, and usually protrude beyond the posterior body margin; however, these setae fail to extend beyond the margin in *A. pasquieri* Balachowsky, *A. sinaloaensis* McConnell, and *A. subterranea* Signoret. Additionally, dorsal conical pores (referred to as invaginated setae in McConnell 1954) can be found in species of *Aclerda* but are absent from *Nipponaclerda*. There are no discernable differences between the immature instars of these two genera but adult males of *Nipponaclerda* can be differentiated by the encircling marginal setae, absence of dorsal invaginated conical pores, and reduction in the number of anal ring setae (McConnell 1954). The adult male stage has only been described for *N. biwakoensis*.

The list of host genera reported in ScaleNet (<http://scalenet.info/>; last accessed 19 March 2019) for *Nipponaclerda* species includes: *Agropyron*, *Bambusa*, *Fargesia*, *Phragmites*, *Sorghum* (Poaceae), and *Juncus* (Juncaceae) (Borchsenius 1960; García Morales et al. 2016; McConnell 1954; Wang 1994; Wang and Zhang 1994; Zhang 1998).

### **Species list**

- Nipponaclerda biwakoensis* (Kuwana, 1907: 187)  
*Nipponaclerda leptodermis* Wang & Zhang, 1994: 94  
*Nipponaclerda triumphpha* Zhang, 1998: 7  
*Nipponaclerda turanica* (Borchsenius, 1950: 156)

### Key to genera of Acleridae based on adult females\*

- 1 Legs and antennae fully developed; pregenital disc-pores present across abdominal segments medially ..... *Lecanaclerda*
- Legs and antennae absent or very reduced; pregenital disc-pores absent ..... 2
- 2 Caudal region of abdomen with sclerotized cone; anal cleft absent; most abundant dorsal tubular duct bilocular ..... 3
- Caudal region of abdomen without sclerotized cone; anal cleft clearly present; most abundant dorsal tubular duct without internal divisions or loculi..... 4
- 3 Thorax with pair of sclerotized brachial plates extending laterally onto dorsum from near spiracles ..... *Kwazulaclerda*
- Thorax without pair of sclerotized brachial plates extending laterally onto dorsum from near spiracles ..... *Rhodesaclerda*
- 4 Anal ring usually bearing about 10–20 anal ring setae, each longer than length of anal plate, often extending beyond posterior body margin; dorsal conical pores (invaginated setae of McConnell 1954) present..... *Aclerda*
- Anal ring bearing 2 groups of 3–5 setae, each much shorter than length of anal plate, or anal ring setae inconspicuous, never extending beyond body margin; dorsal conical pores (invaginated setae of McConnell 1954) absent .... *Nipponaclerda*

### Key to *Nipponaclerda* based on adult females

- 1 Marginal tuberculate setae present; few multilocular disc pores associated with spiracles only; macrotubular ducts present..... 2
- Marginal tuberculate setae absent; numerous multilocular disc pores arranged in submarginal ring and associated with spiracles; macrotubular ducts absent ..... 3
- 2 Marginal tuberculate setae arranged in continuous unbroken ring; multilocular disc pores variable in number, ranging from 4–20 just anterior to spiracles, rarely absent; microtubular ducts at anterior end of body numerous, arranged in 6–10 irregular rows ..... *N. biwakoensis* (Kuwana)
- Marginal tuberculate setae interrupted at anal cleft, not forming continuous unbroken ring around margin; multilocular disc pores numbering greater than 20 just anterior to spiracles; microtubular ducts at anterior end of body few, scattered, arranged in 1–4 irregular rows..... *N. turanica* (Borchsenius)
- 3 Vestigial legs present on mesothorax between anterior and posterior spiracles, represented by small tubercle with approximately 7 short setae; atrium of spiracles relatively small with about 2 rows of multilocular disc pores; submarginal patch of microtubular ducts present on dorsum of head ..... *N. triumpha* Zhang
- Vestigial legs entirely absent; atrium of spiracles relatively large with approximately 4 rows of multilocular disc pores; microtubular ducts absent from dorsum of head..... *N. leptodermis* Wang & Zhang

\* The key to genera is adapted from Hodgson and Millar (2002), with the fourth couplet revised to reflect traits shared among all species of *Nipponaclerda*.

***Nipponaclerda biwakoensis* (Kuwana, 1907)**

**Material examined.** UNITED STATES • 11 ♀; Louisiana, Plaquemines Parish, Venice, West Bay South End; 29°7.5'N, 89°17.2'W; 1 March 2017; R. Diaz leg.; *Phragmites australis*; USNM • 2 immatures; same collection data as for preceding; 1 March 2017; R. Diaz leg.; *Phragmites australis*; USNM • 2 ♀; Texas, Jefferson County, Port Arthur, near J.D. Murphree WMA; 29°53.2'N, 94°2.2'W; 11 July 2018; I.A. Knight leg.; *Phragmites australis*; USNM • 1 ♀; quarantine interception at California, San Diego, originating from Japan; 2 April 1959; L. Widman leg.; *Phragmites* sp. or rush; CDFR • 3 ♀; quarantine interception at California, Los Angeles, San Pedro, originating from Japan; 22 March 1960; M.F. Brown, Jr. leg.; *Phragmites communis* (= *australis*); USNM • 1 ♀; quarantine interception at California, Stockton, originating from Japan; 25 May 1961; R.E. DeVol leg.; *Phragmites communis* (= *australis*); USNM • same collection data as for preceding; 3 ♀; 25 May 1961; R.E. DeVol leg.; *Phragmites communis* (= *australis*); CDFR • 2 ♀; quarantine interception at Hawaii, originating from Japan; 25 March 1960; L. Chilson leg.; *Phragmites communis* (= *australis*) stems; USNM. CHINA • 2 ♀; Taiwan, Taichung County; 24°19.2'N, 12°33.5'E; 22 August 2018; S-G. Syu and J-L. Jhu leg.; *Phragmites australis*; USNM • 3 ♀; Hong Kong Special Administrative Region, Tin Shui Wai, Hong Kong Wetland Park, fresh water marsh; 22°28.1'N, 11°0.4'E; 7 August 2018; B. Brown leg.; *Phragmites australis*; USNM. JAPAN • 2 ♀; Fukuoka, Nishi-Ku, Motooka; 33°35.5'N, 13°13.9'E; 25 October 2018; H. Tanaka leg.; *Phragmites australis*; USNM • 3 ♀, syntype; Omi; August 1902; S.I. Kuwana leg.; on rush; USNM • 5 ♀, syntype; Tokio [Tokyo]; 16 April 1906; S.I. Kuwana leg.; *Phragmites communis* (= *australis*); USNM • 4 immatures; same collection data as for preceding • 1 ♀; Tokyo; 18 October 1953; R. Takahashi leg.; *Phragmites*; USNM.

**Notes.** Adult females of *N. biwakoensis* are similar in appearance to *N. turanica* but the two can be easily distinguished by the traits mentioned above, and in addition by the pattern of sclerotization and by the types of setae located on the anal plate and posterior margin. The posterior end is heavily sclerotized in *N. turanica*, whereas in *N. biwakoensis*, moderate sclerotization is more evenly distributed along the marginal rim, becoming more heavily sclerotized in mature females. *N. biwakoensis* possesses some tuberculate setae on the anal plate and bears only tuberculate or spine-like setae on the posterior body margin. In contrast, only flagellate setae are present on the anal plates of *N. turanica* and several flagellate setae fall on the posterior body margin. *N. biwakoensis* was well-illustrated by McConnell (1954); additional illustrations were published by Kuwana (1907; 1932) and Wang (1994).

Several natural enemies of *N. biwakoensis* are reported in the literature. The parasitoids *Astymachus japonicus* Howard, *Boucekiella depressa* Hoffer, *Platencyrtus aclerus* Xu (Hymenoptera: Encyrtidae), and *Aprostocetus* sp. (Hymenoptera: Eulophidae) have been reported from the native range (Kaneko 2004; Xu and Wang 2003), which includes China, Japan, and South Korea (García Morales et al. 2016). Knight et al. (2018) reported *Neastymachus japonicus* Tachikawa, *B. depressa*, and *Astymachus* sp. (Hymenoptera: Encyrtidae) parasitizing populations in Louisiana, U.S.A.

It is difficult at present to determine the origin and timing of invasion to the United States. Specimens of *N. biwakoensis* were encountered in quarantine interceptions originating from Japan three times in California and once in Hawaii, between 1959 and 1961. But populations have only been collected from the U.S. since 2016 (Knight et al. 2018), suggesting their establishment is a recent event. Infestations have been found in Louisiana and eastern Texas on *Phragmites australis*, which appears to be the primary host for this species. *N. biwakoensis* was also reported on species of *Agropyron* and *Juncus* (Wang 1994), but these host records should be confirmed through further sampling.

## Acknowledgements

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# *Aphaenogaster illyrica*, a new species from the mountains of the Balkan Peninsula (Hymenoptera, Formicidae)

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## Abstract

*Aphaenogaster illyrica* **sp. nov.**, a member of the *A. subterranea* species group, is described from Dinaric Alps of Slovenia and Croatia, from Golešnica Mt. in north Macedonia, Osogovo-Belasica Massif of southwestern Bulgaria, and from Kerkini Mts. of Greek Macedonia. It is characterised by large body size, moderately sculptured head, elevated mesonotum, and long propodeal spines. Its habitat preferences are discussed. A key to the *Aphaenogaster graeca* complex is provided.

## Keywords

*Aphaenogaster subterranea* species group, Mediterranean Region, Superficial Subterranean Habitat, taxonomy

## Introduction

*Aphaenogaster* Mayr, 1853 is a worldwide genus, which includes 226 species and subspecies. Among them, 128 species and subspecies are known from the Palearctic Region (Bolton 2018) and 43 from Europe (Borowiec 2014, Borowiec and Salata 2014,

2018, Salata and Borowiec 2016, Borowiec et al. 2019, Gómez et al. 2018). Schulz (1994) proposed the division of some west Palearctic taxa of *Aphaenogaster* into species groups. Since then, several taxonomists introduced new species groups or redefined those proposed by Schulz (1994) (e.g., Kiran et al. 2008, Boer 2013, Borowiec and Salata 2014, Salata and Borowiec 2018, Alicata and Schifani 2019). One of the most characteristic and easily distinguished group is formed by taxa defined by Schulz (1994) as the *Aphaenogaster subterranea* species group. Twelve species were included, and recently one more taxon (*Aphaenogaster subterranea fiorii* Emery) was transferred to this group by Salata and Borowiec (2018). However, most recently Alicata and Schifani (2019) distinguished a new species group comprising taxa formerly assigned to the *A. subterranea* group. In general, most of species belonging to the *A. subterranea* group are characterised by yellowish red to dark brown body colouration, mostly medium-sized body length, weakly sculptured and partly smooth head and mesosoma, with head dorsal surface being from completely sculptured and matt to partly smooth and shiny, scape reaching over the occipital margin of head, middle funicular segments at most 1.5 times as long as wide, and head rectangularly rounded in frontal view.

The material from the broader Mediterranean region that was recently studied showed that this group is more speciose and comprises several morphologically cryptic or subcryptic species, especially from the complex of species close to *A. subterranea* (Latreille, 1798), which is now under detailed study (Borowiec, Csösz, Galkowski & Salata unpublished data). One of the morphospecies of the newly studied material, collected from the mountains of the Balkan Peninsula in Bulgaria, Croatia, Greece, North Macedonia, and Slovenia, differs from all known European taxa of the *A. subterranea* group. Its separation, based on strong morphological features, is possible, and we present its description below.

## Materials and methods

Ant material of the new species, for which we have more detailed data, was sampled either by hand sampling (Bulgarian sample from 2013 and Slovenian sample from 2018) or by applying surface pitfall traps (Bulgarian samples from 2002, 2009, and 2014) or subterranean pitfall traps sensu Wong and Guénard (2017) (Slovenian sample from 2003). In the latter, pitfall traps were glass jars with a saturated salt solution as a fixative, baited with rotting meat and fermented cheese in a vial, as described by Giachino and Vailati (2010). We set them in the so-called Superficial Subterranean Habitat (Juberthie et al. 1980; 1981) among the limestone rocks and soil at depths of 30–50 cm and left them for approximately half a year (from autumn 2002 to May 2003).

A total of 48 specimens was examined. Additionally, we examined 31 specimens of the most closely related species *A. graeca* Schulz, endemic to the Olympus Massif and adjacent mountain ranges Pieria and Kato Olympus. Specimens were compared using standard methods of comparative morphology. For measurement purposes we randomly chose ten specimens, which represented the geographical and morphological

variation of the species. The same method was applied to the specimens of *A. graeca*. Photographs were taken using a Nikon SMZ 1500 stereomicroscope, Nikon D5200 photo camera, and Helicon Focus software. All provided label data for the holotype are in original spelling; a vertical bar (|) separates data on different rows and double vertical bars (||) separate labels. All locality points that did not have latitude-longitude information with a paratype were georeferenced using online mapping resources.

### Repository abbreviations:

- BFUS** Biological Faculty, University of Sofia, Bulgaria;  
**DBET** Department of Biodiversity and Evolutionary Taxonomy, University of Wrocław, Poland;  
**BFUL** Biotechnical Faculty, Department of Biology, University of Ljubljana, Slovenia;  
**MNHW** Museum of Natural History, University of Wrocław, Wrocław, Poland;  
**PW** coll. P. Werner, Prague, Czech Republic.

### Measurements: all measurements are given in mm.

- EL** eye length; measured along the maximum vertical diameter of eye;  
**EW** eye width; measured along the maximum horizontal diameter of eye;  
**HL** head length; measured in straight line from mid-point of anterior clypeal margin to mid-point of posterior cephalic margin in full-face view;  
**HS** arithmetic mean of HL and HW;  
**HW** head width; measured in full-face view directly above the eyes;  
**ML** mesosoma length; measured as diagonal length from the anterior end of the neck shield to the posterior margin of the propodeal lobe;  
**PEH** petiole height; the chord of ventral petiolar profile at node level is the reference line perpendicular to which the maximum height of petiole is measured;  
**PEL** petiole length; length of the petiolar node, measured in lateral view from petiolar spiracle to dorso-caudal corner of caudal cylinder;  
**PEW** petiole width; maximum width of petiole in dorsal view;  
**PNW** pronotum width; maximum width of pronotum in dorsal view;  
**PPH** postpetiole height; maximum height of postpetiole in lateral view measured perpendicularly to a line defined by the linear section of the segment border between dorsal and ventral petiolar sclerite;  
**PPL** postpetiole length; maximum length of postpetiole in lateral view;  
**PPW** postpetiole width; maximum width of postpetiole in dorsal view;  
**PSL** propodeal spine length; measured from the centre of the propodeal spiracle to the tip of the propodeal spine in lateral view;  
**SDL** spiracle to declivity length; minimum distance from the centre of the propodeal spiracle to the propodeal declivity;  
**SL** scape length; maximum straight-line length of scape excluding the articular condyle.

**Indices:**

- HI** (head index).  $HW/HL \times 100$ .  
**SI1** (scape index 1).  $SL/HL \times 100$ .  
**SI2** (scape index 2).  $SL/HW \times 100$ .  
**MI** (mesosoma index).  $ML/PNW \times 100$ .  
**EI** (eye index).  $(EW+EL)/(HW+HL) \times 100$ .  
**PEI** (petiole index).  $PEL/PEH \times 100$ .  
**PPI** (postpetiole index).  $PPL/PPH \times 100$ .  
**PSI** (propodeal spine index).  $PSL/SDL \times 100$ .

We list the Mediterranean species considered to be members of the *Aphaenogaster subterranea* species group, as defined by Schulz (1994) and Boer (2013), below. However, *A. crocea* André, *A. fiorii* Emery, *A. faureli* Cagniant, *A. hesperia* Santschi, *A. sicula* Emery, and the recently described *A. trinacriae* Alicata & Schifani are excluded from the list as they are considered members of a closely related, but distinct group (Alicata and Schifani 2019).

*Aphaenogaster graeca* Schulz, 1994, endemic to Greece

[Holotype (CASENT0911129) and paratypes (CASENT0917360, FOCOL0516, FOCOL1838, FOCOL1839, FOCOL1840) images examined, AntWeb, photos by Will Ericson, Kate Martynova, Christiana Klingenberg available on AntWeb.org].

*Aphaenogaster holtzi* (Emery, 1898), eastern Turkey

[Syntype worker images examined, AntWeb, CASENT0904178, photos by Will Ericson, available on AntWeb.org].

*Aphaenogaster illyrica* sp. nov.

*Aphaenogaster lesbica* Forel, 1913, endemic to Greece

[Syntype worker examined].

*Aphaenogaster maculifrons* Kiran & Aktaş, 2008, western Turkey

[Paratype worker examined].

*Aphaenogaster subterranea* (Latreille, 1798), described from France, recorded from almost the whole western Palearctic Region, probably a complex of cryptic species

[Topotype workers examined, the same locality and series from which neotype has been designated].

*Aphaenogaster subterranea ichnusa* Santschi, 1925, France, Italy, and Spain

[Syntype worker images examined, AntWeb, CASENT0913132, photos by Zach Lieberman, available on AntWeb.org].

## Taxonomy

### *Aphaenogaster illyrica* sp. nov.

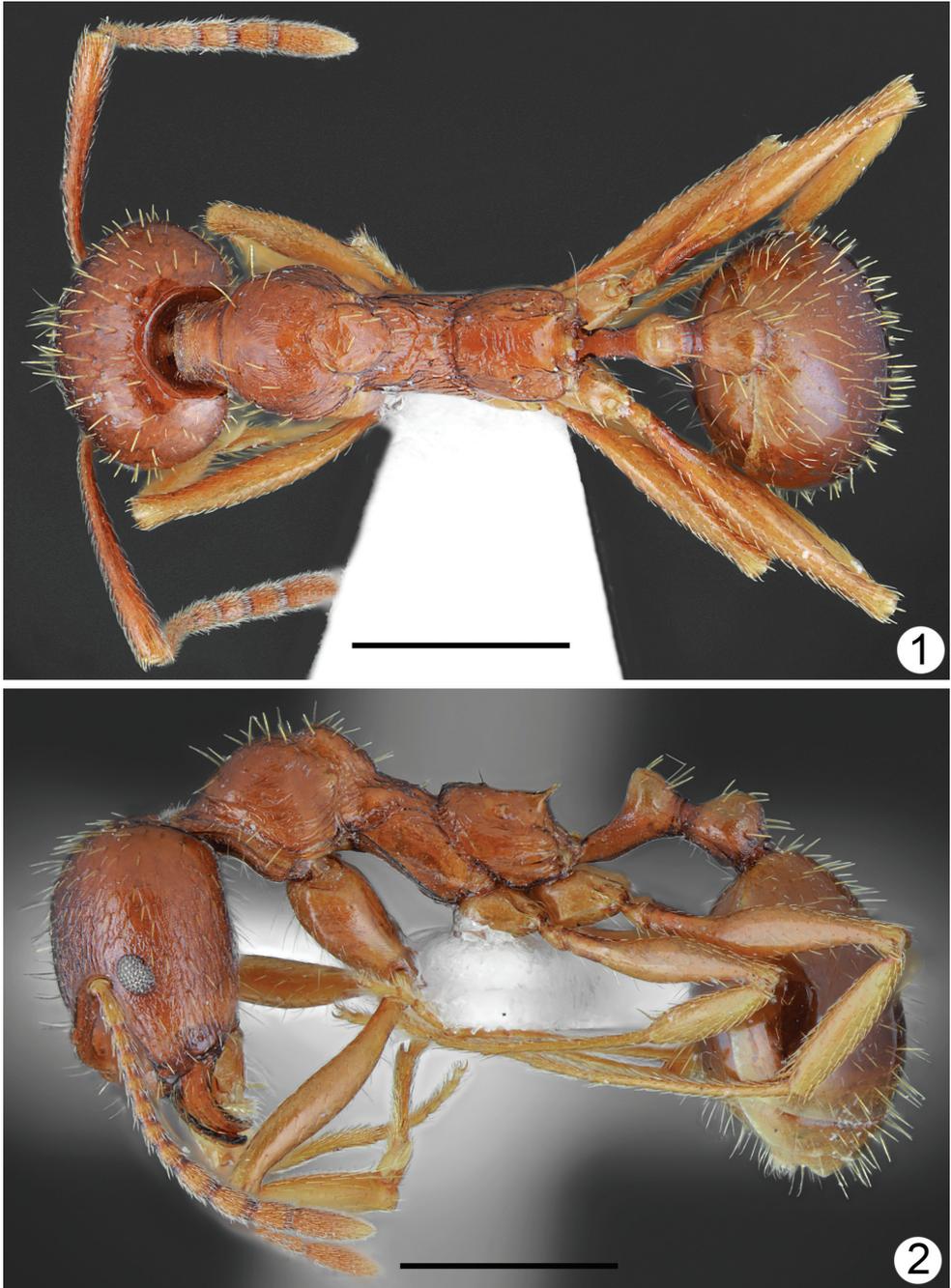
<http://zoobank.org/52996364-A884-45B3-BB10-8C3FC41BDEA6>

Figures 1–8

**Material examined. Holotype worker: SLOVENIA:** Mt. Velika Milanja | MSS | Volovja reber | Ilirska Bistrica, SLO | 45.593N, 14.313E, 1060 m | 23.05.2003, leg. S. Polak (MNHW, holotype no. CASENT0872099).

**Paratypes: BULGARIA:** 5 workers (CASENT0872100-CASENT0872104): Maleshevska Mt., Strumyani distr., Dobri Laki vill., 41.58484N, 22.98138E, 650 m, soil traps, along Lebnitsa river, beech and alder trees, 30.07.-20.08.2002, leg. S. Lazarov, T. Ljubomirov (BFUS); 1 worker (CASENT0872105): Belasitsa Mt., Petrch district, Belasitsa hut, 41.370N, 23.187E, 690 m, beech forest, 28.03.2009, leg. R. Bekchiev (BFUS); 15 workers (CASENT0872106-CASENT0872120): Belasitsa Mt., Petrch district, Kamena vill., 41.360N, 23.074E, 500 m, beech forest, along Kamenishka river, soil traps, June 2009, leg. R. Kostova; 02.05.2013, direct sampling, leg. A. Lapeva-Gjonova (BFUS, DBET); 6 workers (CASENT0872121-CASENT0872126): Slavyanka Mt., Sandanski district, Goleshovo vill., 41.42139N, 23.625N, 1094 m, 16.08.2014, leg. A. Lapeva-Gjonova (BFUS); **CROATIA:** 9 workers (CASENT0872127-CASENT0872135): Oltari, Mt. Senjsko bilo, 7 km NW of Krasno, 44.84604N, 15.00298E, 02.06.1992, leg. A. Schulz, K. Vock (DBET, PW); **GREECE:** 3 workers (CASENT0872136- CASENT0872138): [Macedonia] Kerkini Mts., Ano Poroia, 41.28563N, 23.03598E, 28.5.1984, V. Vohralik lgt. (PW, DBET); **NORTH MACEDONIA:** 4 workers (CASENT0872139-CASENT0872142): Golešnica Mts., 2 km S of Aldinci, 41.80189N, 21.42848E, 9.7.2010, 1420 m, V. Vohralik lgt. (DBET, PW); **SLOVENIA:** 1 worker (CASENT0872143): Mt. Velika Milanja, MSS, Volovja reber, Ilirska Bistrica, SLO, 45.593N, 14.313E, 1060 m, 23.05.2003, leg. S. Polak (DBET); 2 workers (CASENT0872144-CASENT0872145): Mt. Velika Milanja, MSS, Volovja reber, Ilirska Bistrica, SLO, 45.593N, 14.313E, 1060 m, 05.10.2018, leg. G. Bračko (BFUL).

**Differential diagnosis.** The sculpture of head and mesosoma, head shape, scape length, and length of funicular segments place this species into the *Aphaenogaster subterranea* species group. *Aphaenogaster illyrica* differs from other members of this group in the combination of the following features: mesonotum clearly raised above the surface of pronotum, long and thin propodeal spines, as long as or longer than 0.7 length of the first segment of funiculus, elongated mesosoma, large body size (ML more than 1.64 mm, HW more than 1.02 mm), anterolateral sides of pronotum regularly convex, without setose angulations or tubercles, and yellowish brown to rusty brown body colour. In most of the other members of the group (i.e., *A. lesbica* Forel, 1913 from Lesbos, *A. maculifrons* Kiran & Aktaç, 2008 from the western Turkey, *A. subterranea* (Latreille, 1798)), pronotum and mesonotum form a regular convexity, without mesonotum raised above the surface of pronotum, propodeal spines are shorter, not



**Figures 1, 2.** *Aphaenogaster illyrica*, holotype **1** dorsal **2** lateral. Scale bars: 1 mm.

longer than half length of the first segment of antennal funiculus, ML is less than 1.60 mm, and HW less than 1.0 mm.

*Aphaenogaster illyrica* most closely resembles *A. graeca* Schulz, 1994 from Mount Olympus (see Table 1) in morphometric data and general body shape. The new species



**Figures 3, 4.** *Aphaenogaster illyrica*, holotype **3** head and antennae **4** head sculpture. Scale bars: 1 mm (**3**), 0.5 mm (**4**).

**Table 1.** Measurements and indices of *Aphaenogaster illyrica* and *A. graeca*. Values are given as arithmetic mean  $\pm$  standard deviation (minimum–maximum); n = number of workers; all measurements in mm.

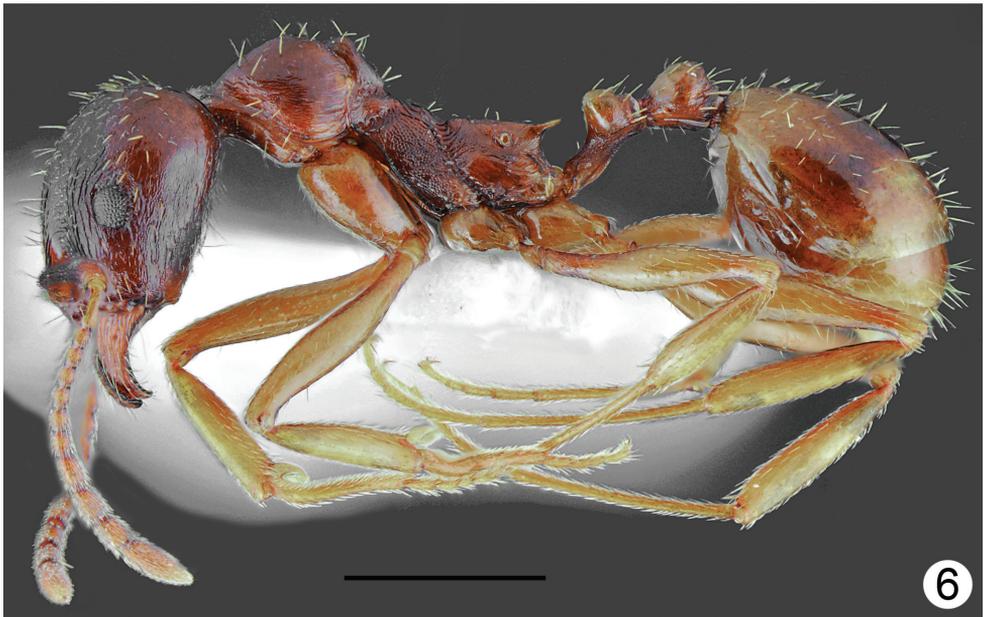
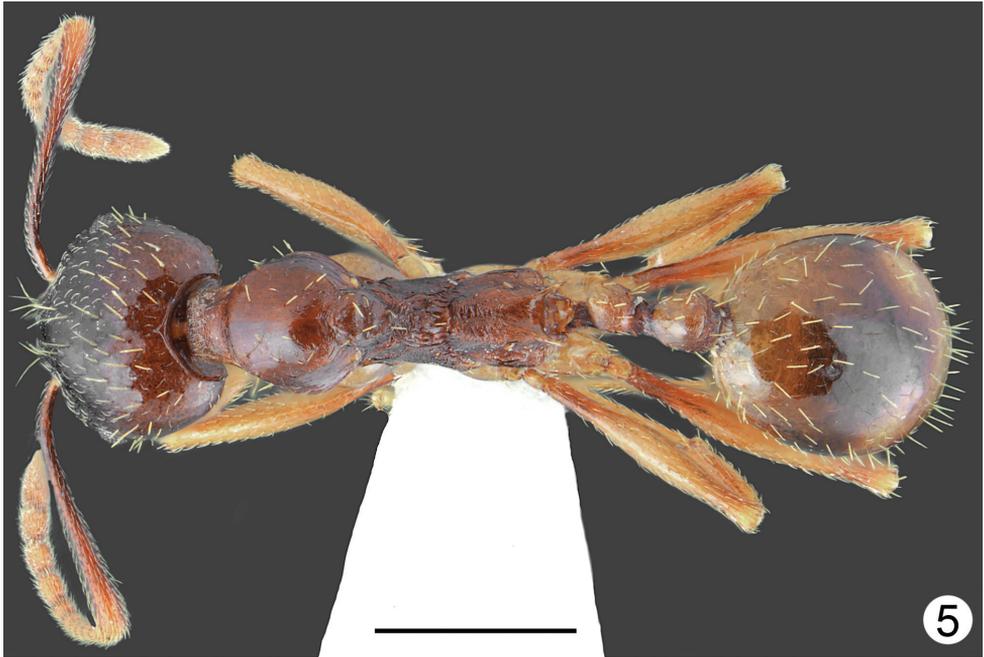
Measurements and indices	<i>Aphaenogaster illyrica</i> n = 10	<i>Aphaenogaster graeca</i> n = 10
HL	1.319 $\pm$ 0.06 (1.218–1.432)	1.373 $\pm$ 0.1 (1.18–1.48)
HW	1.132 $\pm$ 0.07 (1.021–1.23)	1.156 $\pm$ 0.1 (0.93–1.27)
HS	1.226 $\pm$ 0.07 (1.119–1.331)	1.265 $\pm$ 0.1 (1.055–1.375)
SL	1.337 $\pm$ 0.07 (1.235–1.448)	1.367 $\pm$ 0.07 (1.24–1.46)
EL	0.218 $\pm$ 0.01 (0.197–0.247)	0.217 $\pm$ 0.02 (0.18–0.24)
EW	0.167 $\pm$ 0.009 (0.152–0.181)	0.148 $\pm$ 0.02 (0.11–0.17)
ML	1.758 $\pm$ 0.07 (1.646–1.909)	1.882 $\pm$ 0.1 (1.63–2.0)
PSL	0.313 $\pm$ 0.03 (0.263–0.378)	0.333 $\pm$ 0.05 (0.26–0.42)
SDL	0.203 $\pm$ 0.01 (0.181–0.23)	0.224 $\pm$ 0.04 (0.18–0.31)
PEL	0.551 $\pm$ 0.03 (0.51–0.609)	0.6 $\pm$ 0.07 (0.48–0.67)
PPL	0.378 $\pm$ 0.02 (0.346–0.395)	0.342 $\pm$ 0.03 (0.28–0.37)
PEH	0.36 $\pm$ 0.02 (0.329–0.395)	0.38 $\pm$ 0.03 (0.33–0.41)
PPH	0.358 $\pm$ 0.02 (0.329–0.378)	0.356 $\pm$ 0.03 (0.3–0.4)
PNW	0.722 $\pm$ 0.04 (0.658–0.79)	0.757 $\pm$ 0.07 (0.64–0.88)
PEW	0.266 $\pm$ 0.01 (0.246–0.283)	0.273 $\pm$ 0.03 (0.22–0.3)
PPW	0.337 $\pm$ 0.02 (0.296–0.366)	0.328 $\pm$ 0.04 (0.22–0.36)
HI	85.8 $\pm$ 2.2 (82.2–89.4)	84.1 $\pm$ 2.7 (78.8–86.9)
SI1	101.3 $\pm$ 2.9 (96.2–106.7)	99.8 $\pm$ 3.0 (95.9–105.1)
SI2	118.1 $\pm$ 6.0 (109.2–127.4)	119.0 $\pm$ 7.9 (111.8–133.3)
MI	243.6 $\pm$ 8.5 (227.1–255.0)	249.3 $\pm$ 9.4 (227.3–257.6)
EI	15.7 $\pm$ 0.7 (14.6–16.9)	14.4 $\pm$ 1.3 (12.3–16.7)
PEI	154.0 $\pm$ 5.0 (145.5–161.9)	157.3 $\pm$ 8.7 (145.5–169.2)
PPI	105.1 $\pm$ 8.1 (93.8–114.3)	96.5 $\pm$ 5.3 (89.7–103.2)
PSI	156.4 $\pm$ 6.4 (150.0–166.7)	148.9 $\pm$ 6.8 (135.4–159.1)

differs from *A. graeca* in having a brighter and more uniform body colouration (yellowish brown to rusty brown vs. dark brown), weaker head sculpture, which fades laterad, less distinctly sculptured pronotum especially at sides, propodeum smooth and lacking longitudinal rugae on almost of whole lateral surface, and absence of long rugae at the base of the first gaster tergite (Figs 9–12).

Stout members of the *A. splendida* species group, i.e., *A. festae* Emery, 1915 and its relatives with the mesonotum raised clearly above the surface of pronotum, clearly differ in the yellowish body, short propodeal spines directed distinctly upwards, and elongate segments 2–4 of antennal funiculus, always 1.5 times or more longer than wide.

We also recognise several yet undescribed members of the *A. subterranea* group, which will be a subject for further, more advanced studies. *Aphaenogaster illyrica* is most similar to an undescribed species collected on the island of Cephalonia, especially in its long propodeal spines and mesonotum slightly raised above the surface of pronotum, but the undescribed form differs in having a distinctly microreticulated and dull dorsal and occipital parts of the head surface and dorsum of pronotum, as well as in the anterolateral corners of pronotum bearing setose tubercles.

**Description of worker.** Measurements: see Table 1.



**Figures 5, 6.** *Aphaenogaster illyrica*, paratype from Kamena, Bulgaria **5** dorsal **6** lateral. Scale bars: 1 mm.

**Body colouration.** Head, mesosoma, petiole and postpetiole yellowish brown to rusty brown, frons and area lateral of frontal carinae darker brown. Gaster from yellowish to mostly brown, first tergite yellowish anteriorly and yellowish brown posteriorly, but without distinct border between paler and darker parts, or completely brown.

Mandibles yellowish-brown, legs yellow, antennal scapes ochraceous brown with yellowish apex, funiculus ochraceous-yellow (Figs 1, 2). **Head.** Approximately 1.2 times as long as wide, lateral margins in frontal view almost parallel behind eyes and evenly rounded at the posterior cephalic corners, posterior margin straight (Fig. 3). Anterior margin of clypeus shallowly emarginated. Eyes small, approximately 0.16 times as long as lateral margin of head, placed in the middle of lateral margin of head (Fig. 4). Scape approximately 1.2 times as long as head width, at base twice narrower than at apex, then gradually widened, without preapical constriction. Funiculus approximately 1.4 times as long as scape, first segment elongated, 2.6 times as long as wide at apex, 0.9 times as long as two subsequent segments combined, segments 2–6 short, 1.2–1.4 times as long as wide, segments 8–10 approximately 1.6 times as long as wide, last 4 segments forming an indistinct club, as long as basal funicular segments 1–7 combined. Mandibles elongate, with distinct striation and with some elongate punctures but shiny, masticatory margin with 7–9 teeth. Clypeus in the middle microreticulated, with short and thin median keel and few indistinct, longitudinal rugae, laterally with distinct longitudinal rugae. Frontal carinae moderately elongate, not reaching half-length of head, subparallel, frontal triangle with median keel and smooth laterally. Frons along the middle with single elevated keel, on sides with 2–3 longitudinal rugae, interspaces microreticulated, moderately shiny. Antennal cavities margined by regular, circular rugae. Central part of head dorsum between eyes with mostly sparse, partly longitudinal and partly irregular rugae, extending to 2/3 length of head, area between rugae microreticulated and moderately dull. Posterior part of head dorsum microreticulated, slightly dull, occiput smooth and shiny. Antennal scape with thin, longitudinal rugae. **Mesosoma.** Distinctly elongate. Promesonotum in dorsal view approximately 1.7 times as long as wide, pronotum strongly convex in profile. Anterolateral sides of pronotum convex, setose angulations or tubercles absent. Anterior part of mesonotum angulate or bituberculate, protruding distinctly above the level of posterior part of pronotum, thus promesonotal outline with distinct emargination in profile. Propodeum elongate, approximately 1.26 times as long as wide. Propodeal spines long, thin, at base only twice wider than at apex, acute apically, run only slightly upwards (Fig. 2). Dorsal part of pronotum with diffused microreticulation and only with median line smooth and shiny to mostly smooth and shiny, sides of pronotum with sparse, thin, mostly longitudinal rugae and diffused microreticulation between rugosities but appear shiny. Elevated part of mesonotum dorsally shiny with diffused microreticulation, laterally microreticulated with few rugae, posterior part of mesonotum rugose dorsally and granulate laterally (Fig. 2). Anterior surface of propodeum with short longitudinal rugae, laterally at least anteriorly smooth and shiny, posteriorly with few longitudinal rugae, dorsally with transverse or more or less longitudinal and around spiracles with irregular rugae. **Petiole.** Elongate with long peduncle, its anterior face deeply concave, node subangulate. Ventral margin of petiole in the middle straight, shallowly concave before apex, without spine or angulation. In dorsal view, petiole constricted at base then weakly divergent, almost parallel before petiolar node, then slightly globular. Base and ventral side distinctly microreticulated but without rugae, on sides and dorsally



**Figures 7, 8.** *Aphaenogaster illyrica*, paratype from Kamena, Bulgaria **7** head and antennae **8** head sculpture. Scale bars: 1 mm (**7**), 0.5 mm (**8**).



**Figures 9, 10.** *Aphaenogaster graeca*, worker from Mt. Olympus **9** dorsal **10** lateral. Scale bars: 1 mm.

with diffused microreticulation to smooth and shiny, posterior faces with few rugae. **Postpetiole.** In lateral view rounded or slightly depressed at apex, in dorsal view approximately as long as wide with regularly rounded sides (Fig. 1). Base and ventral side distinctly microreticulated but without rugae, on sides and dorsally with diffused microreticulation to smooth and shiny, posterior faces with few rugae. **Gaster.** Shiny, with indistinct, diffused microreticulation, basal part of first tergite without or with very short longitudinal rugae. **Setosity.** Head in frontal view with short, light yel-

low, sparse setae. Entire dorsum of mesosoma and anterior margins of pronotum with sparse, short to moderately long, erect setae, the longest setae from shorter to approximately as long as propodeal spines. Petiolar node, postpetiole and gaster with short standing pilosity, the longest setae in large specimens shorter and in small specimens as long as propodeal spines.

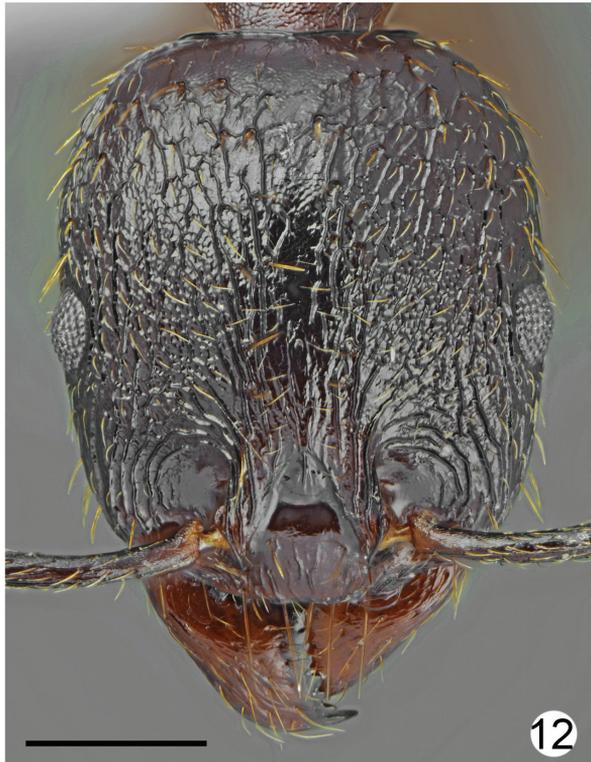
**Gyne and male.** Unknown.

**Range of the morphological variability.** Variability within the geographic populations of the new species *A. illyrica* is mostly in size of propodeal spines and distinctness of microreticulation of head occiput, dorsal part of pronotum, and mesopleuron. Variability between geographically distant populations is more distinct but features overlap. Specimens from Slovenia (terra typica) have the stoutest head (largest HI) while in samples from Croatia and Bulgaria head is less stout. Microreticulation on the dorsal surface of the head and on the dorsal part of the pronotum in specimens from Slovenia and Croatia is more distinct than in those from Bulgaria, and similarly the northern populations have more distinct longitudinal rugae on the sides of the pronotum. In contrast, reticulation of mesopleuron in Bulgarian samples is distinct on the whole surface while in some specimens from Croatia and Slovenia reticulation of the mesopleuron is partly diffused. In specimens of similar sizes, the propodeal spines are shorter and directed more or less upwards in northern populations, while in Bulgarian populations they are longer and almost in the prolongation of the upper edge of the propodeum, not or very slightly directed upwards.

**Etymology.** Named after Illyria, a historical region in the western part of the Balkan Peninsula inhabited by the Illyrians and the ancient Roman Prefecture of Illyricum. All localities of *Aphaenogaster illyrica* are within the area of this region.

**Distribution.** All known records of *Aphaenogaster illyrica* are restricted to the mountainous areas of the Balkan Peninsula, from the altitudes of 500 m to 1420 m a.s.l. Its range stretches from the Dinaric Alps in southern Slovenia and western Croatia to Osogovo-Belasica Massif in southwestern Bulgaria and the adjacent Kerkini Mts. in Greece and to Golešnica Mt. in North Macedonia. This distribution area is much larger compared to the area of the sister species *Aphaenogaster graeca*, whose distribution range is limited to the massif of Mount Olympus and adjacent mountain ranges (Fig. 15).

**Biological notes.** Details on the new species habitat are available only from the Bulgarian and Slovenian records. In Bulgaria, *A. illyrica* was mostly collected in beech forests in wet sites, close to streams, on silicate (Belasitsa and Maleshevska Mts.) and limestone (Slavyanka Mt.) rocks. This differs quite dramatically from the Slovenian site, where the ants were found in a large karstic depression (karstic doline) situated in the sub-montane karst grassland, partly covered with sparse trees and shrubs. This area is characterised by harsh winters and relatively wet summers. Due to the strong and almost permanent winds, the upper part of the soil is often dry. The specimens collected in 2003 were found in subterranean pitfall traps set in soil at the depth of 30–50 cm among the limestone rocks in the so-called Superficial Subterranean Habitat (SSH) or “Milieu Souterrain Superficiel” (MSS), as originally described (Juberthie et al. 1980; 1981). SSH is a hypogean environment, generally formed by the fragmentation of the



**Figures 11, 12.** *Aphaenogaster graeca*, worker from Mt. Olympus **11** head and antennae **12** head sculpture. Scale bars: 1 mm (**11**), 0.5 mm (**12**).



**Figures 13, 14.** Habitat of *A. illyrica* **13** locality Velika Milanja, Slovenia **14** locality Kamena, Bulgaria.



**Figure 15.** Distribution of *Aphaenogaster illyrica* (red circles) and *Aphaenogaster graeca* (black circles).

bedrock and accumulation of debris, which contains a wide network of air-filled epikarstic spaces, small voids and fissures (Culver and Pipan 2009; Giachino and Vailati 2010) and represents a transition zone between surface soils and deeper subterranean habitats such as caves (Culver and Pipan 2009). A presence of a rare species *A. cardenai* Espadaler, 1981, was already reported from SSH in the Iberian Peninsula (Ortuño et al. 2014). In 2018, we found few scattered workers at the same site while digging in the stony ground to the depth of approximately 50 cm.

*Aphaenogaster illyrica* can be characterised as a ground-dwelling species. The records of *A. illyrica* well above 1000 m a.s.l. or those from beech forests at lower al-

titudes indicate that it tolerates lower temperatures, which is relatively rare in other species of the genus.

**Comments.** Recently published papers (Borowiec and Salata 2017, Alicata and Schifani 2019) indicate that the *A. subterranea* group is very diverse and comprise several undescribed taxa. The Balkans appears to be the most species-rich region and is in need of further investigation. Results presented in this publication are a preliminary attempt to systematise our knowledge about this group, and *Aphaenogaster illyrica* and *A. graeca* compose a distinct complex within the *A. subterranea* group. Therefore, we decided to describe the new species in a separate paper. Other undescribed forms, mentioned in the publication, will be a subject of further study. Because the *A. subterranea* group consists of mixture of species of uncertain taxonomic status and several undescribed morphotaxa, we can provide only a generic key to the *subterranea* group with features focused on the *graeca* complex.

**A key to the *Aphaenogaster graeca* complex (within *A. subterranea* group)**

- 1 Metanotal groove absent or very shallow (Maghreb, Canary Islands, Siculo-Maltese archipelago, Southern Italy) ..... ***A. crocea* group, sensu Alicata and Schifani (2019)**
- Metanotal groove present, deep and narrow (Mediterranean Region)..... **2**
- 2 Pronotum and mesonotum form regular convexity, mesonotum not raised above the surface of pronotum, propodeal spines short, not longer than half length of the first segment of antennal funiculus, mesosoma short..... ***A. subterranea* complex**
- Mesonotum clearly raised above the surface of pronotum, propodeal spines long and thin, as long as or longer than 0.7 length of the first segment of funiculus, mesosoma elongated..... **3**
- 3 Base of the first gaster tergite with distinct rugae, body brown to dark brown, head distinctly darker than mesosoma, head sculpture strong, posterior part of head dorsum with sculpture only slightly reduced but still distinct, lateral surface of propodeum, at least partly, with strong longitudinal rugae..... ***A. graeca***
- Base of the first gaster tergite smooth, body uniformly yellowish-brown to rusty-brown, head sculpture weaker, posterior part of head dorsum with sculpture at least partly reduced, lateral surface of propodeum smooth or with few gentle longitudinal rugae ..... ***A. illyrica* sp. nov.**

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# Ten newly recorded species of xyleborine ambrosia beetles (Coleoptera, Curculionidae, Scolytinae, Xyleborini) from Thailand

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## Abstract

Ten species of ambrosia beetles of the tribe Xyleborini, *Amasa beesoni* (Eggers, 1930), *Amasa opalescens* (Schedl, 1937), *Amasa cylindrotomica* (Schedl, 1939), *Arixyleborus hirsutululus* Schedl, 1969, *Beaverium latus* (Eggers, 1923), *Cnestus protensus* (Eggers, 1930), *Coptodryas quadricostata* (Schedl, 1942), *Cryptoxyleborus confusus* Browne, 1950, *Cryptoxyleborus percuneolus* (Schedl, 1951) and *Cyclorhipidion vigilans* (Schedl, 1939), are recorded here for the first time in Thailand. Diagnostic characters, illustrations, distribution and biological data are provided for each species. *Xylosandrus ramulorum* (Schedl, 1957), **stat. res.** is removed from synonymy with *Amasa cylindrotomica* and reinstated as a valid species.

## Keywords

Diversity, Oriental region, reinstated species, southern Thailand, xyleborines

## Introduction

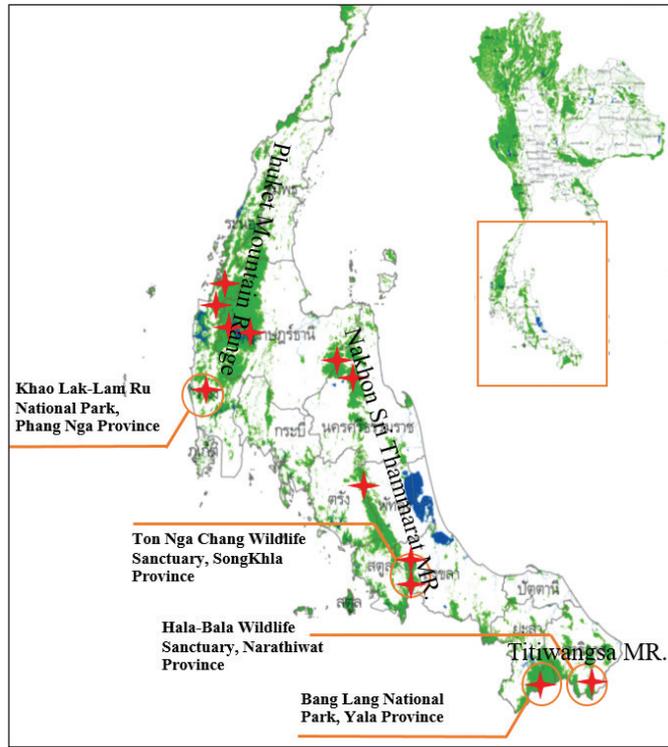
The Scolytinae is a subfamily of bark and wood-boring weevils which includes more than 6000 species (Alonso-Zarazaga and Lyal 2009), and is of considerable economic importance in both temperate and tropical regions. The majority of species attack dying or dead trees; a few, economically important species attack healthy or apparently

healthy trees and can cause die-back or mortality. The Xyleborini is one of the largest tribes of Scolytinae with more than 1100 described species (Hulcr et al. 2015), and many more undescribed. All are inbreeding ambrosia beetles. They are wood-borers intimately associated with symbiotic ambrosia fungi upon which both adults and larvae feed in gallery systems constructed in the xylem (Beaver et al. 2014, Kirkendall et al. 2015). The female alone is responsible for gallery construction. The eggs are laid loose in the gallery, and the larvae develop freely in the maternal gallery feeding upon the ambrosia fungi growing on its walls. The sex ratio is strongly biased towards females, and sib-mating occurs within the maternal gallery prior to the emergence of the new generation via the original entrance hole (Kirkendall et al. 2015).

The first checklist of the Scolytinae of Thailand was that of Beaver and Browne (1975), which listed 33 species of Xyleborini, the majority collected in the north of the country. Further species of Xyleborini have been added by Beaver (1990, 1999, 2010), Beaver and Hulcr (2008), Sittichaya (2012), Sittichaya et al. (2012) and Beaver et al. (2014), bringing the current total number of xyleborine species recorded in Thailand to 146 (Beaver et al. 2014). The present paper records 10 further species of Xyleborini collected in southern Thailand, with diagnostic characters, collecting localities and information on host plants and biology where available. We expect many further species to be collected in this region. The scolytine fauna of the south of Thailand is continuous with the species-rich fauna of Malaysia and Indonesia, whilst that of Thailand north of the Isthmus of Kra on the Thai-Malay Peninsula at about 11–13°N is more similar to that of the rest of South-East Asia (Cambodia, Laos, Myanmar, Vietnam) (Beaver et al. 2014).

## Material and methods

Specimens were collected from three forest complexes in peninsular southern Thailand over a thirteen to fifteen month trapping period. Ethanol-baited flight intercept traps were placed in 12 study sites in 10 conservation areas in the Titiwangsa Mountain Range, Nakhon Sri Thammarat Mountain Range and Phuket Mountain Range (Fig. 1). In the Titiwangsa Mountain Range, at the Hala-Bala Wildlife Sanctuary, 10 traps were deployed at one site from 1 May 2014 to 30 May 2015. In the Nakhon Sri Thammarat Mountain Range, 10 traps were placed at each of five sites from 1 October 2013 to 31 December 2014. In the Phuket Mountain Range, 10 traps were placed at each of five sites from 1 April 2014 to 30 April 2015. At each site, the traps were deployed in a transect line, 100 m apart, in mature forest 1 km from the surrounding agricultural areas or secondary forest. At the Bang Lang National Park in Yala Province, hand collecting from logs and branches was carried out for one week from 1–7 February 2014. Trapped beetles were sorted and identified using a Leica stereomicroscope EZ4 and Leica S8 APO (Leica Microsystems Pte Ltd, Germany). Photographs were taken with a Canon 6D digital Camera with a Canon MP-E 65mm macro photo lens (Canon, Tokyo, Japan) and StackShot-Macrorail (Cognisys Inc, Michigan, USA). The photos



**Figure 1.** Peninsular Thailand showing forest covered areas (green), and conservation areas in which the beetles were trapped (stars). Labels indicate study areas where newly recorded species were captured. (Modified from <http://new.forest.go.th/land/>)

were then combined with Helicon Focus 6.8.0. (Helicon Soft, Ukraine), and all photos were improved with Adobe Photoshop CS6 (Adobe Systems, California, USA). Plant names and classification follow <http://www.theplantlist.org/>. All specimens of the newly recorded species are currently deposited in the collection of W. Sittichaya at Prince of Songkla University. Duplicate specimens will subsequently be deposited in other museum collections.

## New records

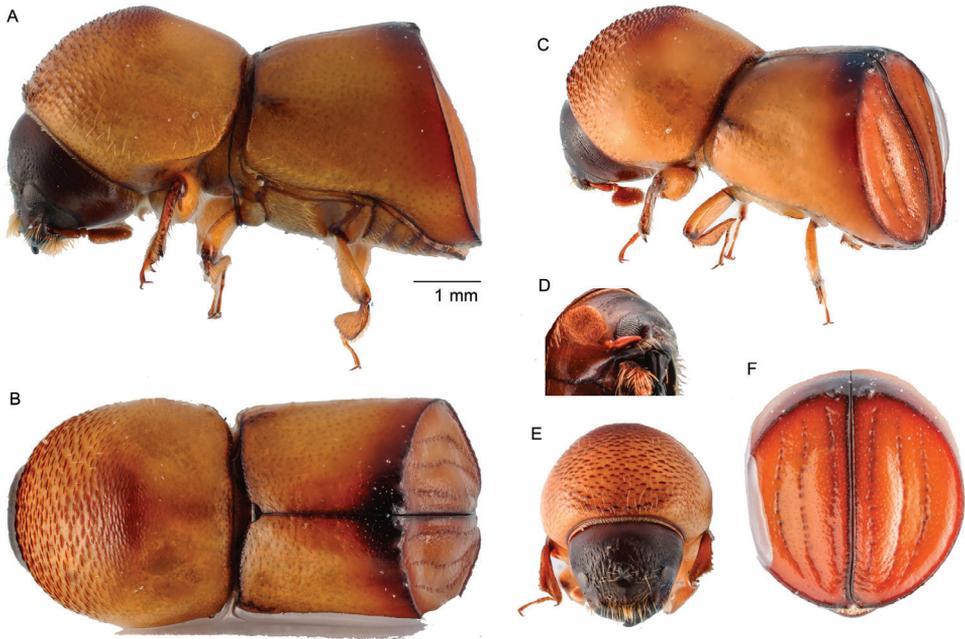
### Genus *Amasa* Lea, 1893: 322

#### *Amasa beelsoni* (Eggers, 1930)

Fig. 2A–F

*Pseudoxyleborus beelsoni* Eggers 1930: 207.

*Amasa beelsoni* (Eggers): Wood 1984: 223.



**Figure 2.** *Amasa beelsoni* (Eggers, 1930) **A** lateral view **B** dorsal view **C** posterolateral view **D** antenna **E** front **F** declivity.

**Diagnosis.** Large, 4.60–4.79 mm ( $N = 2$ ) long; stout, 2.0–2.1 times longer than wide; body smooth, shining nearly glabrous, yellowish brown to dark brown in color; eye completely divided; antennal club with sutures obscured (type 5; Hulcr et al. 2007); pronotum from dorsal view rounded (type 1; Hulcr et al. 2007), anterior margin broadly round, anterior half finely asperate, 1.1 times wider than long; elytra 1.1 times longer than pronotum, elytral disc punctures very fine, confused, never seriate, lateral sides subparallel, widest on declivital summit, declivital summit at first interstriae bearing a pair of small flattened teeth, declivital face shining, striae impressed, interstriae finely, densely punctate.

**Material examined.** THAILAND, Khao Lak-Lam Ru National Park, Phang Nga Province, 8°39'22.4"N 98°17'31.6"E, tropical rainforest, ethanol-baited trap, 01.v.2015 (1), 01.iv.2015 (1), (W. Sittichaya).

**Distribution.** Indonesia, Malaysia, Myanmar. New to Thailand.

**Biology.** Recorded from *Dimocarpus longan* Lour and *Xerospermum intermedium* Radlk. (Sapindaceae) and possibly with a fixed association with this family (Browne 1961). The gallery system, as in other *Amasa* species, comprises a short radial tunnel leading to a single, large, flat brood chamber, extending in the longitudinal plane (Browne 1961).

**Remarks.** This species can be distinguished from all other *Amasa* recorded in Thailand by the completely divided eye and the small teeth at the apex of the elytral disc on the first interstriae.

***Amasa cylindrotomica* (Schedl, 1939)**

Fig. 3A–E

*Pseudoxyleborus cylindrotomicus* Schedl, 1939: 40.*Xyleborus cylindrotomicus* (Schedl): Schedl 1942: 6.*Xylosandrus cylindrotomicus* (Schedl): Wood 1989: 177.*Amasa cylindrotomica* (Schedl): Dole and Cognato 2010: 525.Synonyms: *Xyleborus semitruncatus* Schedl, 1942: 35. Synonymy: Schedl 1951: 79; Wood 1989: 177.*Xyleborus truncatellus* Schedl, 1951: 79. Synonymy: Kalshoven 1959: 95.*Xyleborus jucundus* Schedl, 1954: 138 (new name for *Xyleborus truncatellus* Schedl, 1951 non Schedl 1949). Synonymy: Kalshoven 1959: 95.

**Diagnosis.** Small, 2.1 mm ( $N = 1$ ) long; stout, 2.0 times longer than wide; body shining, nearly glabrous, yellowish brown to brown in color; eye deeply emarginate; antennal club with sutures obscured (type 5; Hulcr et al. 2007); pronotum from dorsal view round (type 1; Hulcr et al. 2007) front broadly convex, from lateral view round near (type 1; Hulcr et al. 2007), anterior half of pronotum densely, finely asperate, base very finely punctate; elytra 1.07 times as long as pronotum, sides subparallel, widest at declivital summit, declivity dull, glabrous, stria punctures seriate, first stria straight, second and third laterally diverging, interstriae shagreened, two times broader than striae.

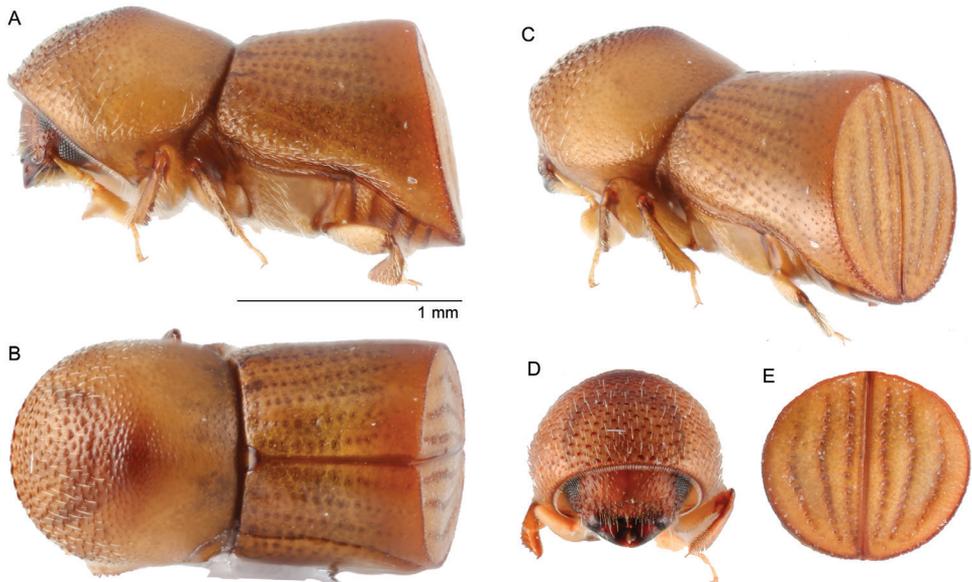
**Material examined.** THAILAND, Ton Nga Chang Wildlife Sanctuary, Songkhla Province, 6°59'32.1"N 100°08'57.8"E, tropical rainforest, ethanol-baited trap, 01.ii.2014 (1) (W. Sittichaya).

**Distribution.** Indonesia (Java, Sumatra). New to Thailand.

**Biology.** Recorded only from *Syzygium aromaticum* Merr & LM Perry (clove) (Myrtaceae).

**Remarks.** This species can be distinguished from all other *Amasa* recorded in Thailand by its small size (2.1 mm long) and stout appearance; elytra approximately as long as the pronotum, and elytral declivity dull, glabrous.

It should be noted that the Afrotropical species, *Xyleborus ramulorum* Schedl, 1957, included as a synonym of *A. cylindrotomica* by Wood (1989), Wood and Bright (1992), and Dole and Cognato (2010) is a different species. *Xyleborus ramulorum* was described and figured from three specimens collected by Schedl in what was then Belgian Congo (Schedl 1957). Schedl (1963) gave some additional biological information, and figured the gallery system. Nunberg (1963) re-examined the holotype in the Royal Museum for Central Africa, Tervuren, and provided additional morphological characters. He also noted (Nunberg 1963) that the holotype was badly damaged, and that Schedl had evidently retained undamaged paratypes in his own collection. Browne (1965) transferred the species to *Xylosandrus* Reitter, 1913. Wood (1989) synonymised this Afrotropical species with the Oriental species, *Xylosandrus cylindrotomicus* Schedl, 1939, without providing any reason for the synonymy. Dole and Cognato (2010) accepted the synonymy without examining type material, and listed the species as a



**Figure 3.** *Amasa cylindrotomica* (Schedl, 1939) **A** lateral view **B** dorsal view **C** posterolateral view **D** front declivity. **E** declivity.

synonym of *Amasa cylindrotomica* (Schedl). Examination of an undamaged paratype (NHMW) clearly indicates that the species should be returned to *Xylosandrus* as a distinct species. *Xylosandrus ramulorum* **stat. res.** shares numerous characteristics with *Xylosandrus* including: mesonotal mycangial tuft present but unlike other *Xylosandrus* the mycangium opening is on the pronotal disc rather than the pronotal base; truncate antennal club with segment 1 encircling the anterior face (type 1; Hulcr et al. 2007); pronotal anterior margin serrate; pronotum from lateral view with disc as long or longer than anterior slope (type 7; Hulcr et al. 2007); and elytral declivity truncate with 5 granulate striae on declivital face, interstriae also granulate. By comparison, *Amasa* species have the following characteristics (Hulcr and Smith 2010; Smith et al. in prep.): mesonotal mycangial tuft absent; flat antennal clubs with segment 1 never encircling the anterior face (types 3,4,5; Hulcr et al. 2007), pronotal anterior margin never serrate; pronotum from lateral view basic or robust (types 1 and 5; Hulcr et al. 2007); elytral declivity truncate with no more than 3 punctate striae on declivital face. Ventral characters, including the separation of the procoxae and protibia shape and sculpturing, were not described by Schedl and are not visible on the card mounted paratype. Based on the characteristics listed above, *Xyleborus ramulorum* is here transferred to *Xylosandrus* where it shares features with the Asian species included in the *Xylosandrus s.l.* clade (Dole and Cognato 2010): *X. beasoni* Saha, Maiti & Chakraborti, 1992, *X. borealis* Nobuchi, 1981, *X. brevis* (Eichhoff, 1877), *X. discolor* (Blandford, 1898), *X. diversipilosus* (Eggers, 1941), *X. jaintianus* (Schedl, 1967), *X. subsimilis* (Eggers, 1930) and *X. subsimiliformis* (Eggers, 1939).

*Amasa opalescens* (Schedl, 1937)

Fig. 4A–E

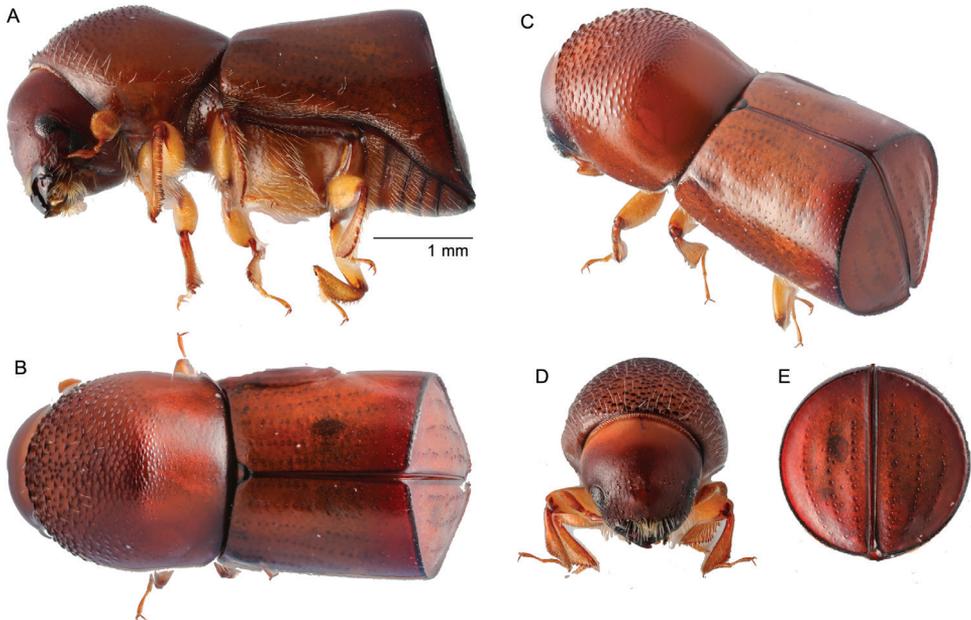
*Xyleborus opalescens* Schedl, 1937: 550*Amasa opalescens* (Schedl): Wood and Bright 1992: 683.

**Diagnosis.** Large, 4.5 mm long ( $N = 1$ ); moderately stout, 2.5 times longer than wide; body shining, declivity opalescent, brown in color; eye deeply emarginated, almost completely divided; antennal club with first segment smaller than second (type 4; Hulcr et al. 2007); pronotum from dorsal view round (type 1; Hulcr et al. 2007), from lateral view tall (type 2; Hulcr et al. 2007), anterior margin round, armed with 6 small asperities, anterior portion of pronotum densely asperate, base shagreened; elytra 1.24 times longer than pronotum, lateral sides subparallel, widest at declivital summit, disc shining, striae minutely punctate, interstriae four times broader than striae, broadest at declivital summit, declivital margin unarmed, declivity smooth, opalescent, striae punctures large, irregularly spaced.

**Material examined.** Thailand, Bang Lang National Park, Yala Province, Thailand-Malaysia border, 5°48'51.8"N 101°17'14.7"E, ex. small branches of unknown tree, 01.ii.2014 (1).

**Distribution.** 'Borneo', East and West Malaysia, Vietnam. New to Thailand.

**Biology.** Recorded from *Eugenia* sp. and *Tristania* sp. (Myrtaceae), and possibly with a fixed association with this family (Browne 1961).



**Figure 4.** *Amasa opalescens* (Schedl, 1937) **A** lateral view **B** dorsal view **C** posterolateral view **D** front **E** declivity.

**Remarks.** This species can be distinguished from all other *Amasa* recorded in Thailand by its large size (4.5 mm), moderately stout form (2.5 times longer than wide), declivital summit entirely carinate without teeth on first interstriae, declivity smooth, subshining, opalescent, striae punctures large, irregularly spaced, and eye deeply emarginated, almost completely divided.

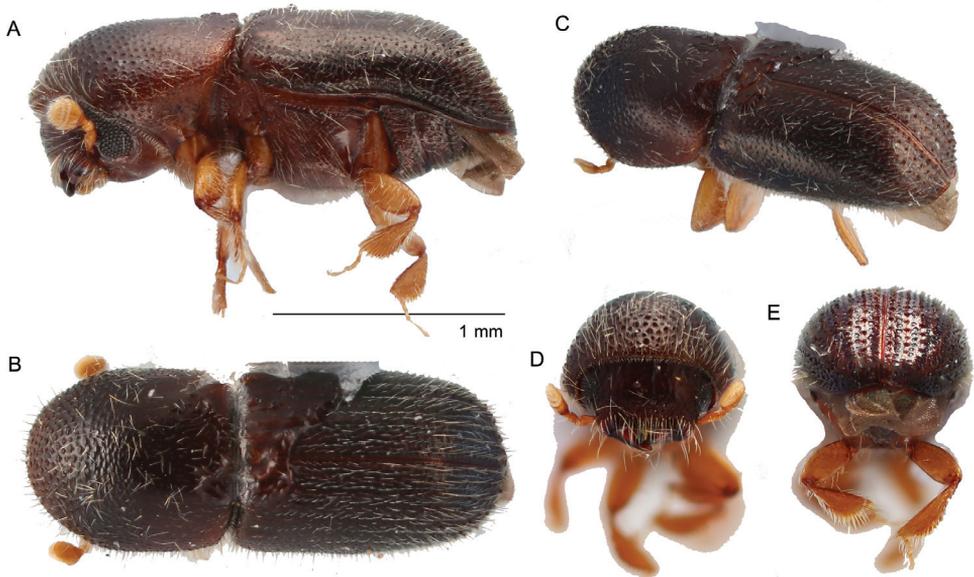
*Arxyleborus* Hopkins, 1915: 59.

*Arxyleborus hirsutulus* Schedl, 1969

Fig. 5A–E

*Arxyleborus hirsutulus* Schedl, 1969: 212.

**Diagnosis.** Small, 2.0 mm ( $N = 1$ ) long; 2.27 times longer than wide; pronotum shining, elytra densely setose, dark brown to black in color; pronotum from dorsal view with sides parallel, weakly elongate and rounded frontally (type 7; Hulcr et al. 2007), from lateral view elongate with low summit (type 7; Hulcr et al. 2007); elytra 1.25 times longer than pronotum, disc weakly convex, apical three-fourths rugose, striae and interstriae covered with small equally size granules, never forming striae furrows and interstitial ridges, elytra densely covered with setae, setae increasing in density towards apex, posterolateral carina oblique, granulate.



**Figure 5.** *Arxyleborus hirsutulus* Schedl, 1969 **A** lateral view **B** dorsal view **C** posterolateral view **D** front view **E** declivity.

**Material examined.** THAILAND, Hala-Bala Wildlife Sanctuary, Narathiwat Province, lowland tropical rainforest, 5°47'44"N, 101°50'07"E, 01.iii.2015 (1), ethanol-baited trap (W. Sittichaya).

**Distribution.** Philippines; imported to Japan from Borneo and Indonesia (Maluku). New to Thailand.

**Hosts.** *Anisoptera* sp., *Dipterocarpus* sp., *Dryobalanops* sp., *Shorea* spp. (Dipterocarpaceae), *Artocarpus* sp. (Moraceae), and an unidentified species of Sapotaceae (Ohno 1990).

**Remarks.** This species can be distinguished from all other *Arixyleborus* recorded in Thailand by the elytral striae and interstriae covered with small equally sized granules and without strial furrows and interstitial ridges, elytra densely setose with the setae increasing in density toward the apex.

### *Beaverium* Hulcr & Cognato, 2009: 25

#### *Beaverium latus* (Eggers, 1923)

Fig. 6A–F

*Xyleborus latus* Eggers, 1923: 177.

*Terminalinus latus* (Eggers): Wood 1986: 267.

*Beaverium latus* (Eggers): Hulcr and Cognato 2009: 26.

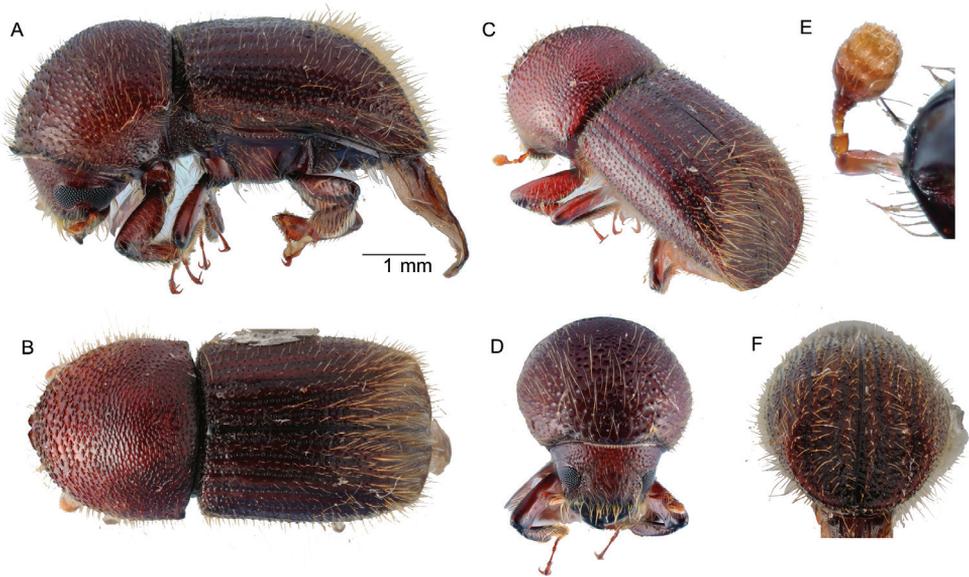
**Diagnosis.** Large, 6.6 mm long ( $N = 1$ ); stout, 2.2 times longer than wide; body covered with golden setae, setae longer on declivity, reddish brown to dark brown in color; pronotum from dorsal view conical (type 0; Hulcr et al. 2007), anterior half densely asperate, asperities robust, base asperate, anterior margin armed with two medium serrations, from lateral view appearing rounded and robust (type 5; Hulcr et al. 2007); elytra 1.35 times longer than pronotum, disc flat, weakly impressed, declivital posterolateral margins carinate, declivity flat, densely covered with long erect golden hair-like setae.

**Material examined.** THAILAND, Hala-Bala Wildlife Sanctuary, Narathiwat Province, lowland tropical rainforest, 5°47'44"N, 101°50'07"E, 01.v.2015 (1), ethanol-baited trap (W. Sittichaya).

**Distribution.** 'Borneo', East and West Malaysia, Indonesia (Sumatra). New to Thailand.

**Biology.** Recorded from *Maranthes corymbosa* Blume (Chrysobalanaceae), *Shorea balanocarpoides* Symington, *S. leprosula* Miq., *Shorea* sp. (Dipterocarpaceae), *Intsia palembanica* Miq. (Fabaceae), *Castanopsis inermis* (Lindl.) Benth. & Hook.f., *Lithocarpus sundaicus* (Blume) Rehder (Fagaceae) (Browne 1961).

**Remarks.** This species can be distinguished from all other *Beaverium* recorded in Thailand by the body brown to dark brown in color, declivital posterolateral margins carinate, declivity flat, and densely covered with long golden setae.



**Figure 6.** *Beaverium latus* (Eggers, 1923) **A** lateral view **B** dorsal view **C** posterolateral view **D** front **E** antenna **F** declivity.

### *Cnestus* Sampson, 1911: 383

#### *Cnestus protensus* (Eggers, 1930)

Fig. 7A–F

*Xyleborus protensus* Eggers, 1930: 201.

*Cnestus protensus* (Eggers): Schedl 1958: 145.

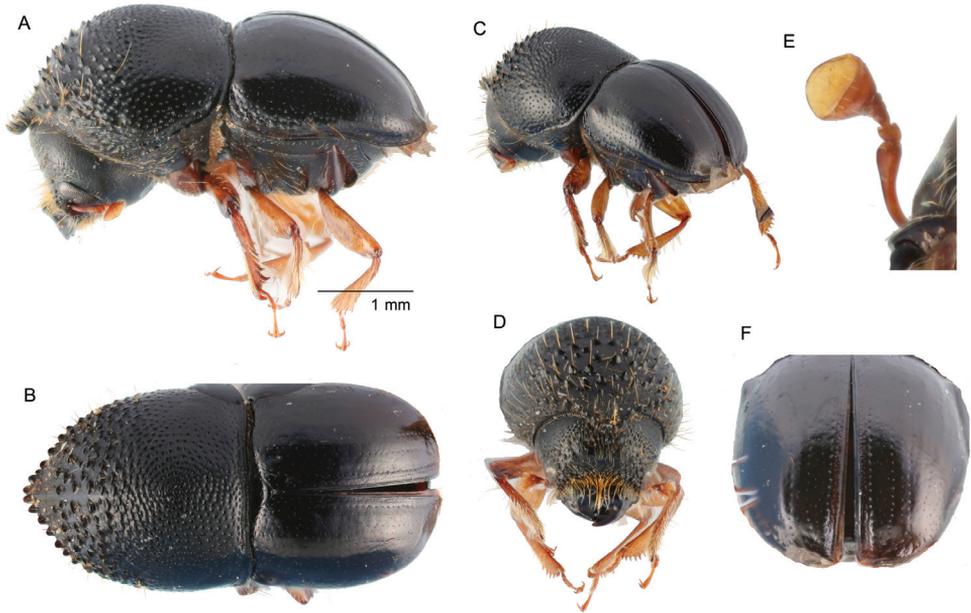
**Diagnosis.** Large, 4.0 mm long ( $N = 1$ ); stout, 2.0 times longer than wide; body strongly shining, glabrous, black in color; pronotum from dorsal view conical frontally (type 6; Hulcr et al. 2007), pronotal apex strongly produced, armed with numerous strong serrations, anterior portion of pronotum strongly asperate, lateral margins parallel from the base to the middle, base densely coarsely punctate; mesonotal mycangial tuft absent on pronotal base; elytra round, elytral declivity strongly rounded and convex.

**Material examined.** THAILAND, Khao Lak-Lam Ru National Park, Phang Nga Province, 8°39'22.4"N, 98°17'31.6"E, tropical rainforest, ethanol-baited trap, 01.xii.2014 (1) (W. Sittichaya).

**Distribution.** India (Meghalaya), Indonesia (Java). New to Thailand.

**Biology.** Unknown. *Cnestus* species, as far as is known, are twig and shoot-borers, and the gallery system is typical of such species with a short radial or circumferential gallery running to the middle of the stem, and longitudinal branches up and down the stem in which the brood develop (Browne 1961, Hulcr and Cognato 2013).

**Remarks.** This species can be distinguished from all other *Cnestus* recorded in Thailand by the strongly produced pronotal apex armed with strong serrations, prono-



**Figure 7.** *Cnestus protensus* (Eggers, 1930) **A** lateral view **B** dorsal view **C** posterolateral view **D** front **E** antenna **F** declivity.

tal base without a mycangial tuft, elytral declivity strongly rounded and convex. This species most closely resembles *C. nitidipennis* (Schedl), and can be distinguished by the distinctly larger size, much larger, coarser and more numerous pronotal apical serrations, punctures on pronotal base clearly coarser and denser, and sides of pronotum parallel for approximately half of the total length.

#### *Coptodryas* Hopkins, 1915: 54

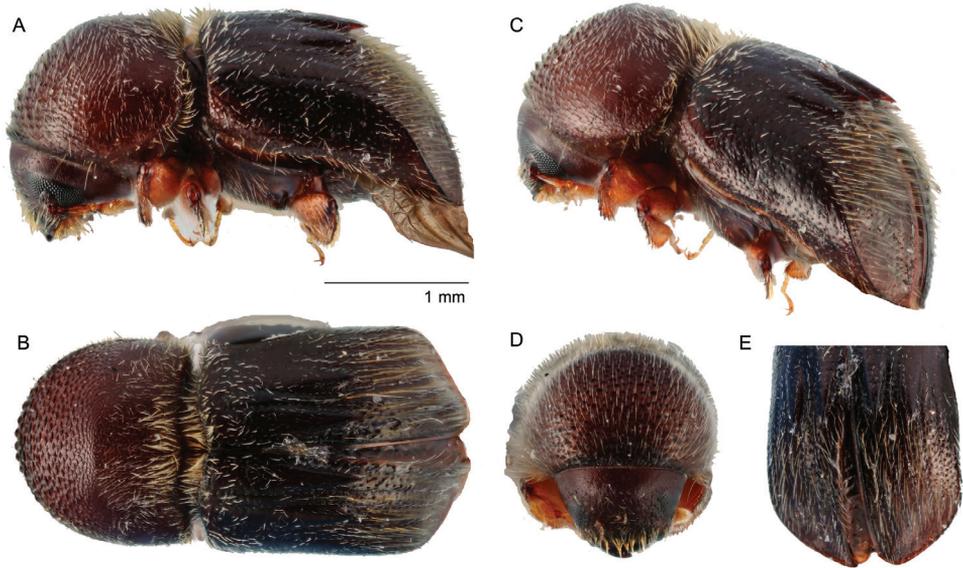
#### *Coptodryas quadricostata* (Schedl, 1942)

Fig. 8A–E

*Xyleborus quadricostatus* Schedl, 1942: 30.

*Coptodryas quadricostata* (Schedl): Wood and Bright, 1992: 826.

**Diagnosis.** Moderately sized, 3.0 mm ( $N = 1$ ) long; stout 2.0 times longer than wide; body moderately setose, brown to dark brown in color; pronotum from dorsal view round and robust (type 5; Hulcr et al. 2007), from lateral view round (type 1; Hulcr et al. 2007), anterior margin with a row of serrations, anterior half asperate, base shagreened, mesonotal mycangial tuft present along the base; elytra 1.53 times longer than pronotum, base covered with elytral mycangial tuft of setae, disc shining, covered with long golden setae, striae and interstriae 1,3,5 deeply depressed, interstriae 2,4



**Figure 8.** *Coptodryas quadricostata* (Schedl, 1942) **A** lateral view **B** dorsal view **C** posterolateral view **D** front **E** declivity.

weakly elevated from middle of elytral and narrower behind forming horizontal sharp spines and extending beyond declivital summit, declivity densely covered with long soft hair-like striae and shorter interstriae.

**Material examined.** THAILAND, Hala-Bala Wildlife Sanctuary, Narathiwat Province, lowland tropical rainforest, 5°47'44"N, 101°50'07"E, 01.i.2015 (1), ethanol-baited trap (W. Sittichaya).

**Distribution.** 'Borneo', East and West Malaysia, Indonesia (Java). New to Thailand.

**Biology.** Recorded from *Campnosperma* sp. (Anacardiaceae), *Shorea leprosula* Miq., *S. parvifolia* Dyer (Dipterocarpaceae), *Elaeocarpus* sp. (Elaeocarpaceae), and *Garcinia* sp. (Clusiaceae). Browne (1961) notes that the species attacks small branches (1–5 cm diameter). The gallery system usually encircles the stem, and has 1–2 longitudinal branches in which the larvae develop (Browne 1961).

**Remarks.** This species can be distinguished from all other *Coptodryas* recorded in Thailand by the declivital summit with four sharp spines extending beyond the summit.

### *Cryptoxyleborus* Wood & Bright, 1992: 828

#### *Cryptoxyleborus confusus* Browne, 1950

Fig. 9A–D

*Cryptoxyleborus confusus* Browne, 1950: 644.



**Figure 9.** *Cryptoxyleborus confusus* Browne, 1950 **A** lateral view **B** dorsal view **C** posterolateral view **D** front.

**Diagnosis.** Small, 2.0 mm long ( $N = 1$ ); very elongate, 3.3 times longer than wide; body nearly glabrous, light brown in color; pronotum elongated basic shape (type 7; Hulcr et al. 2007), anterior margin rounded, convex, armed with a row of five serrations, anterior half of pronotum asperate, asperities tiny, pronotal base shagreened; elytra 1.71 times longer than pronotum, 1.87 times longer than wide, elytra abruptly tapering at apical third, interstriae reticulate-punctate, punctures shallow, very dense at the base, area of dense punctures broader at suture, discal punctures confused.

**Material examined.** THAILAND, Ton Nga Chang Wildlife Sanctuary, Songkhla Province, 6°59'32.1"N 100°08'57.8"E, tropical rainforest, ethanol-baited trap, 01.iv.2014 (1) (W. Sittichaya).

**Distribution.** Brunei Darussalam, East and West Malaysia, Indonesia (Sumatra). New to Thailand.

**Biology.** Recorded from several species of *Shorea* (Dipterocarpaceae) (Browne 1961, Beaver and Hulcr 2008). Browne (1961) notes that the gallery system differs from the usual pattern found in the genus. A surface brood chamber is constructed between bark and wood in which most of the larvae develop. However, there are also more deeply penetrating tunnels into the wood.

**Remarks.** This species is closely related to *C. vestigator* Schedl, which has the elytra more strongly posteriorly tapered and is more strongly shining in appearance. *Cryptoxyleborus confusus* seems to be somewhat morphologically variable and DNA could show that the species is not monophyletic.

***Cryptoxyleborus percuneolus* (Schedl, 1951)**

Fig. 10A–E

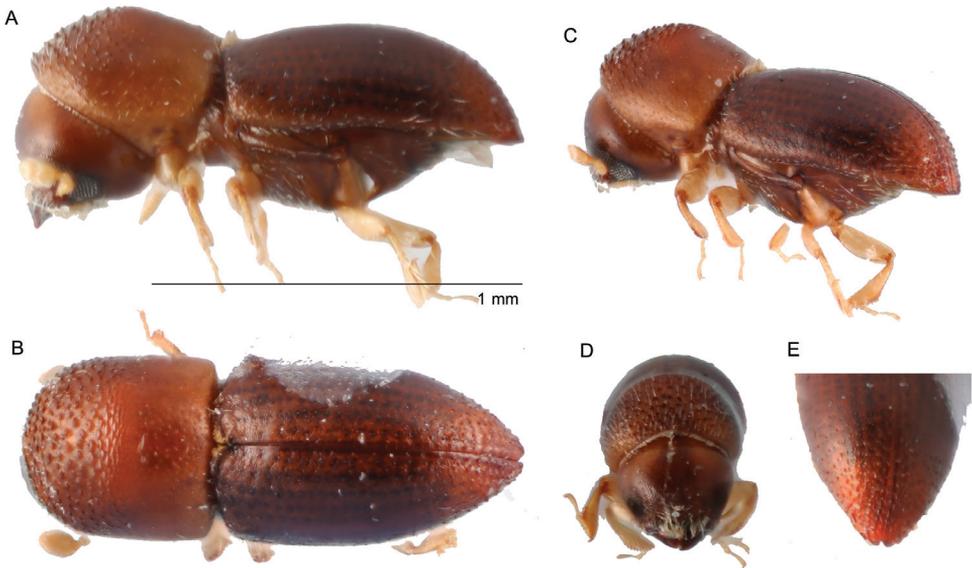
*Xyleborus percuneolus* Schedl, 1951: 85.*Xyleborinus percuneolus* (Schedl): Wood and Bright 1992: 809.*Cryptoxyleborus percuneolus* (Schedl): Beaver and Hulcr 2008: 145.

**Diagnosis.** Minute, the smallest *Cryptoxyleborus* species, 1.4 mm long; elongate, 2.55 times longer than wide; body dull, glabrous except for mycangial tuft along elytral base, red brown to brown in color; antennal club approximately circular, first segment smaller than second (type 4; Hulcr et al. 2007), pronotum from dorsal view elongated basic shape (type 7; Hulcr et al. 2007), anterior margin round, armed with minute serrations, pronotal disc alutaceous, shagreened, from lateral view elongate, with low summit (type 7; Hulcr et al. 2007); elytra stout, 1.75 times longer than pronotum, base sinuate without mycangial pits, disc shining, interstriae distinctly seriate punctate, declivital striae and interstriae granulate, never bearing hooked tubercles, elytra gradually tapering from midpoint to apex.

**Material examined.** THAILAND, Ton Nga Chang Wildlife Sanctuary, Songkhla Province, 6°59'32.1"N, 100°08'57.8"E, tropical rainforest, ethanol-baited trap, 01.vi.2015 (1) (W. Sittichaya).

**Distribution.** Indonesia (Java), Malaysia (Sabah). New to Thailand.

**Biology.** Like other species of *Cryptoxyleborus*, its hosts are probably confined to trees of the family Dipterocarpaceae (Beaver and Hulcr 2008). However, no host trees have yet been recorded. One gallery system investigated in an undetermined tree com-



**Figure 10.** *Cryptoxyleborus percuneolus* (Schedl) **A** lateral view **B** dorsal view **C** posterolateral view **D** front **E** declivity.

prised an unbranched entrance tunnel leading to a single terminal brood chamber enlarged in the longitudinal plane, with multiple tunnels extending further into the wood (Beaver and Hulcr 2008).

**Remark.** This species can be distinguished from all other *Cryptoxyleborus* recorded in Thailand by its minute size (1.4 mm), elytral base sinuate and lacking mycangial pits.

***Cyclorhipidion* Hagedorn, 1912: 355**

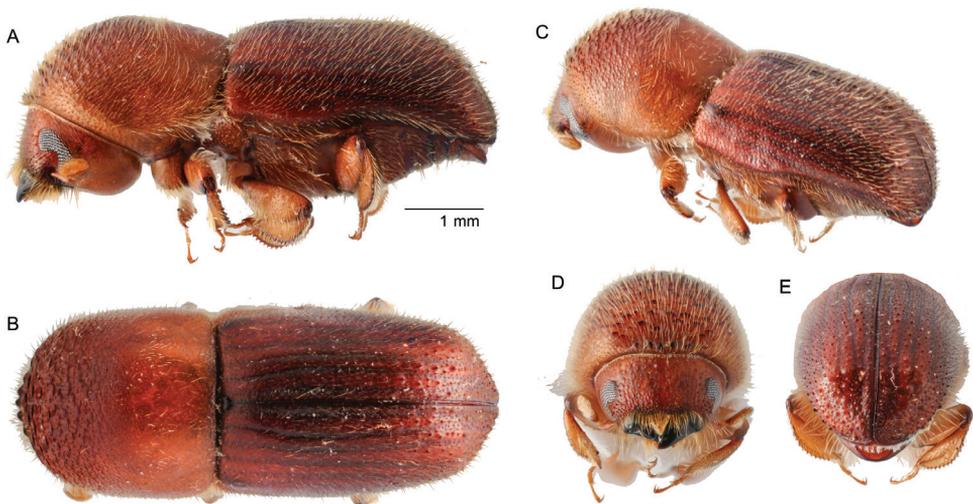
***Cyclorhipidion vigilans* (Schedl, 1939)**

Fig. 11A–E

*Xyleborus vigilans* Schedl, 1939: 43.

*Cyclorhipidion vigilans* (Schedl): Wood and Bright 1992: 704.

**Diagnosis.** Large, 5.5 mm long ( $N = 5$ ); elongate, 2.45–2.48 times longer than wide; pronotum with less vestiture than elytra, elytra densely covered with interstitial setae, density of vestiture varies; brown to dark brown in color; pronotum from dorsal view basic shape, anterior margin subquadrate, sides parallel (type 3; Hulcr et al. 2007), 1.55 times longer than wide, from lateral view lateral view rounded, robust (type 5; Hulcr et al. 2007), anterior margin extended anteriorly, armed with 4–6 medium sized serrations, anterior half of pronotum asperate, base shagreened; elytra elongate, gradually tapering from base posteriorly, discal striae punctate, clearly impressed, interstriae two times broader than striae, covered with fine hair-like setae, setae longer posteriorly, discal striae and interstriae 1–3 laterally diverging from suture at basal third, declivital



**Figure 11.** *Cyclorhipidion vigilans* (Schedl, 1939) **A** lateral view **B** dorsal view **C** posterolateral view **D** front **E** declivity.

striae irregularly punctate, interstria granulate, posterolateral carina granulate, extending from apex to interstriae 7.

**Material examined.** THAILAND, Ton Nga Chang Wildlife Sanctuary, Songkhla Province, 6°59'32.1"N, 100°08'57.8"E, tropical rainforest, ethanol-baited trap, 01.i.2014 (1), 01.iii.2014 (1), 01.iv.2015 (3) (W. Sittichaya).

**Distribution.** East and West Malaysia, Indonesia (Java). New to Thailand.

**Biology.** Recorded only from 'kalapa tjoeng' (*Horsfieldia glabra* (Reinw. ex Blume) Warb.) (Myristicaceae) (Schedl 1939).

**Remarks.** This species can be distinguished by the large size, and anterior margin of pronotum extended anteriorly and armed with 4–6 medium sized serrations; elytra elongate tapering from base to angularly rounded apex, discal striae 1–3 impressed, interstriae 2 widened and outwardly curved in middle of disc, interstriae 3 correspondingly narrowed, interstriae granulate on upper part of declivity. This species is similar to species in the genus *Fortiborus*, but the body is densely covered with long hairs and the lower part of the eye is larger than the upper part.

## Discussion

The xyleborine fauna of Thailand is the most well-known and diverse in South-East Asia with 146 species previously recorded. The ten additional records presented here illustrate both the richness of this fauna and how much remains to be discovered, particularly in the south of the country. Three of the species we reported were already known from South-East Asia, while the remaining seven are shared with the Indo-Malayan fauna. We have not included a key to the xyleborines of Thailand in this paper because a monograph of the tribe in East and South-East Asia is currently being prepared (Smith, Beaver, Cognato, in prep.). This will include a key to the xyleborines of the whole of this region. Most xyleborine species have broad distributions, and this large-scale monograph will provide the necessary information and tools to identify genera and species, and assist in the recognition of new taxa. This publication reports new records of Xyleborini found during an intensive survey of Thai forests. Additional new records of species from other tribes will be covered in future papers as further study is necessary.

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## A revised annotated checklist of louse flies (Diptera, Hippoboscidae) from Slovakia

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### Abstract

The list of all known locality and host records from the literature on louse flies from Slovakia are summarized, with the addition of new collection data. New locality data are provided for five species. Three species are added to the Slovakian list: *Icosta minor* (Bigot in Thomson, 1858), which was erroneously cited for Moravia instead of Slovakia in the previous checklist, and *Ornithophila metallica* (Schiner, 1864) and *Ornithomya chloropus* (Bergroth, 1901), which were overlooked from the last checklist. As a result, the louse fly fauna of Slovakia increases to 19 species: 12 autochthonous species and seven rare, non-native species only occasionally imported to Slovakia or migrating to the country with their hosts. This is by far the largest regional fauna of Hippoboscidae in Central Europe, and matches the richest southern European faunas. In total, 78 host-parasite associations concerning 46 bird-host species from eight orders and nine species of mammals, including humans, have been found from a literature review in Slovakia. Two host-parasite associations are reported from Slovakia for the first time: *Ornithomya avicularia* (Linnaeus, 1758) on *Prunella modularis* (Linnaeus, 1758) (Aves: Prunellidae) and *Lipoptena fortisetosa* Maa, 1965 on *Homo sapiens* Linnaeus, 1758 (Mammalia: Hominidae).

**Keywords**

Faunistics, literature review, louse flies, parasite-host associations

**Introduction**

Flies in the family Hippoboscidae, known as ‘louse flies’ or ‘keds’, belong among the Diptera and are a group of obligate parasites of mammals and birds (Rahola et al. 2011). All species are macrolarviparous, with females retaining the larva in the uterus until the end of the third instar; the three larval instars feed on secretions from the maternal accessory glands. The stage at which larviposition occurs represents a prepupal larva (e.g., Mehlhorn 2016). The larva (or pupa) is deposited in birds’ nests or on the hair of a mammalian host, but sometimes it is deposited on the ground by the female (e.g., Halos et al. 2004). Adults of both sexes are blood sucking and are known to act as vectors of many infectious agents, such as protozoa, bacteria, helminths, and possibly also viruses (e.g., Baker 1967, Kečera 1983, Halos et al. 2004, Liu et al. 2016, Skvarla and Machtinger 2019). Some species are host-specific, whereas others feed on a wide array of hosts (e.g., Ibáñez-Bernal et al. 2015, Mehlhorn 2016, Veiga et al. 2018).

Worldwide, more than 213 louse fly species are known (e.g., Maa 1963, Dick 2006, Rahola et al. 2011): 57 species from the Afrotropical region (e.g., Hutson and Oldroyd 1980, Oboňa et al. 2016), 26 from East Palaearctic Asia and Japan (e.g., Maa 1967, Mogi and Sawada 2002, Matyukhin et al. 2017), 9 from China, 8 from the eastern part of Russia (e.g., Soós and Hůrka 1986, Sun 1999), 6 from the Oriental and Australasian regions (e.g., Paramonov 1954, Amiot and Ji 2015, Farrow 2016), and 31 species have been reported from the Nearctic and Neotropic regions (e.g., Bequaert 1942, 1954, 1965, Savage et al. 2019).

From Europe, 30 species of Hippoboscidae are known (Petersen 2004, Pape et al. 2015). The species composition of the hippoboscid fauna in Slovakia is relatively well known; however, the investigation of Hippoboscidae in Slovakia is still far from complete. Scattered older published data on louse flies by Thalhammer (1899) and Brancsik (1910) are mentioned by Povolný and Rosický (1955). Subsequently, Povolný and Balát (1956), Dyk and Schanzel (1964), Čepelák (1974, 1982, 1985, 1986, 1987, 1988, 1992, 1993, 1994a, 1994b), Čepelák and Čepelák (1991), Chalupský and Macháček (1977), Chalupský (1980, 1986), Křištofík and Štefan (1980), Chalupský and Povolný (1983), Hubálek et al. (1988), Křištofík (1998), Kočišová et al. (2007), Roháček (1995, 2009), Straka (1981, 2001, 2005a, 2005b, 2010, 2011, 2016), Straka and Majzlán (2008, 2010, 2014, 2016), and Kočišová (2015) published additional information on the hippoboscid fauna of Slovakia. The most recent checklist of the family Hippoboscidae from Slovakia (Sychra 2009) comprised 16 species.

**Materials and methods**

Samples of Hippoboscini, Lipoptenini, and Ornithomyini came from unidentified material in the collections of the Laboratory and Museum of Evolutionary Ecology,

Department of Ecology, University of Presov (LMEE PO); of the Department of Entomology, Silesian Museum, Opava (SMOC); and of the Department of Biology and Wildlife Diseases, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno (VFU).

The material was identified using Povolný and Rosický (1955), Chalupský and Povolný (1983), Hutson (1984), Ducháč and Bádř (1998). The systematics and nomenclature follow Hutson and Oldroyd (1980), Kutty et al. (2010), Petersen et al. (2007), and Evenhuis et al. (2016).

## Results

### Tribe Hippoboscini

#### *Hippobosca equina* Linnaeus, 1758

**Published records:** Ladomirov (Ladomér), Rožňava (Rozsnyó), Slovenské Nové Mesto (S.A. Ujhely), Stropkov (Sztropkó – Olyka) (Thalhammer 1899); Trenčín (Trencsén) (Brancsik 1910, Chalupský 1986); Kečovo, Remetské Hámre, Rožňava (Povolný and Rosický 1955, Chalupský 1986); Bratislava, Kurinec, Staré Hory, Šaštín – Stráže, Veľké Leváre (Krištofik and Štefan 1980, Chalupský 1986); Kráľovský Chlmec, Plešivecká planina (plain), Silica, Zádiel (Chalupský and Povolný 1983); Chľaba (Čepelák 1986); Dolné Štitáre, Svorad, Žibrica (Čepelák and Čepelák 1991); Veľký Lysec (Čepelák 1992); Hunták (Čepelák 1993); Krivín (Čepelák 1994b); Bratislava-Lamač, Devínska Nová Ves, Horné Orešany, Jakubov, ostrov Kopač (island), Malacky, Štúrovo (Krištofik 1998); “Veľká Fatra” (Straka 2005b); Bábsky les (forest) (Straka and Majzlán 2010); Obručná, Radzovce (Straka 2016); Burdov, Leliansky les (forest) (Straka and Majzlán 2016).

**Published host records:** main host: *Equus caballus* Linnaeus, 1758 (see Povolný and Rosický 1955, Krištofik and Štefan 1980, Chalupský and Povolný 1983, Krištofik 1998); occasional hosts: *Accipiter gentilis* (Linnaeus, 1758) (see Krištofik and Štefan 1980), *Bos taurus* Linnaeus, 1758 (see Povolný and Rosický 1955, Chalupský and Povolný 1983), *Canis familiaris* Linnaeus, 1758 (see Povolný and Rosický 1955), *Capreolus capreolus* (Linnaeus, 1758) (see Povolný and Rosický 1955, Chalupský and Povolný 1983), *Homo sapiens* Linnaeus, 1758 (see Povolný and Rosický 1955, Krištofik and Štefan 1980, Krištofik 1998).

**Material examined:** Báb, 48°18'21.6"N, 17°53'16.5"E, 150 m a.s.l., 11.6.2007, 1 female, malaise trap, O. Majzlán leg. (LMEE PO); Drienovec, 48°37'04.4"N, 20°55'29.9"E, 200 m a.s.l., 1.10.2015, 1 female, from human, S. Greš leg. (LMEE PO); Kamenica nad Hronom nr. Štúrovo, 47°49'30"N, 18°43'03"E, 105 m a.s.l., 17.5.1984, 1 female, J. Roháček leg. (SMOC); Muránska planina NP, Poludnica res.-Suchý dol, 48°45'26"N, 20°02'32"E, 480 m a.s.l., 6.9.2011, 1 female (Fig. 1), sweeping over pasture meadow, J. Roháček leg. (SMOC); Muránska planina NP, Muráň castle env., 48°45'03"N, 20°02'54"E, 625 m a.s.l.; 4.5.2015, 1 male, sweeping undergrowth of steppe forest, J. Roháček leg. (SMOC); Cerová vrchovina PLA, Gemer-



**Figure 1.** Female of *Hippobosca equina* Linnaeus, 1758 from Muránska planina NP (J Roháček).

ský Jablonec – Vodokáš 1 km N, 48°13'00"N, 19°59'42"E, 280 m a.s.l., 6.9.2017, 1 male, sweeping over steppe meadow, J. Roháček leg. (SMOC); Cerová vrchovina PLA, Tachty 2.2 km SW, Gortva valley, 48°08'41"N, 19°54'51"E, 320 m a.s.l., 13.9.2018, 1 female, netted from forest margin vegetation, J. Roháček leg. (SMOC).

**Comments:** *H. equina* is a Palearctic and West Oriental species. It is relatively large, once common, presently disappearing from Central Europe. An ectoparasite of livestock (preferably horses and donkeys) and dogs, but also attacks humans (Krištofík 1998). Previously published Slovak records are from the years 1953–1977, with a single record from 2007 (Straka and Majzlán 2010). Its recent occurrence is strongly affected by a decline of traditional horse and sheep farming in the monitored area (e.g., Bezák and Petrovič 2006). Classified as endangered (EN) in Slovakia (Jedlička and Stloukalová 2001).

### ***Hippobosca longipennis* Fabricius, 1805**

**Published records:** Kečovo (Chalupský 1980, Chalupský and Povolný 1983, Chalupský 1986); Bábsky les (Straka and Majzlán 2010).

**Published host records:** *Canis familiaris* (see Chalupský 1980, Chalupský and Povolný 1983).

**Comments:** A rare and non-native species in Central Europe, distributed in the Mediterranean and Afrotropical regions. Up until now, only two individuals have been recorded in Slovakia, in 1953 and 2007 (Chalupský 1980, Straka and Majzlán 2010). It is an ectoparasite of dogs; occasionally it can occur also on other predatory mammals or ungulates (Chalupský 1980). Occasionally, it can be imported via human activities (e.g., through breeding of particular species of mammal).

### *Hippobosca variegata* Megerle, 1803

**Published records:** Gabčíkovo (Povolný and Rosický 1955, as *H. maculata*, Chalupský 1980, Chalupský 1986).

**Published host records:** *Canis familiaris* (see Povolný and Rosický 1955, Chalupský 1980).

**Comments:** A rare and non-native species in Central Europe, distributed in the Afrotropical region. The only record from Slovakia is from 1951. It is an ectoparasite of cattle and domestic equines (Povolný and Rosický 1955). It can be occasionally imported through human activities (e.g., through breeding of particular species of mammals).

## Tribe Lipoptenini

### *Lipoptena cervi* (Linnaeus, 1758)

**Published records:** Mníchova Lehota (Barátszabadi), Omšenie (Nagysziklás) (Brancsik 1910); Dlhá Ves, Timoradz (Povolný and Rosický 1955); Blatnická dolina (valley in Velká Fatra) (Dyk and Schanzel 1964); Chlába, Hlboké, Jovsa, Kamienka, Kúty, Lozorno (Krištofík and Štefan 1980, Chalupský 1986); Gabčíkovo, Modrá, Nitra, Plášťovce, Plešivec, Podunajské Biskupice, Silická planina (plain), Šaštín – Stráže, Timoradza (Chalupský and Povolný 1983, Chalupský 1986); Burdov (Čepelák 1986); Topoľčany, (Chalupský 1986); Hrášková Lúka (Čepelák 1987); Hrdovická (Čepelák 1988); Silická planina (plain) (Hubálek et al. 1988); Bartošov prameň (well), Dolné Štitáre, Gáborka, Haranč, Hrnčiarovce, Hunták (Čepelák and Čepelák 1991); Veľký Lysec (Čepelák 1992); Nebrová (Čepelák 1994a); Nové Mesto nad Váhom, Podunajské Biskupice, Stará Lesná, Šaštín – Stráže, Veľká Fatra – Gaderská dolina (valley) (Krištofík 1998); Klín, Rozsutec (Straka 2001); Hrochoť – Beňova dolina (valley) (Roháček 2009); Žalostiná (Straka 2010); Vršatské bradlá (cliff), Záhorská Bystrica (Straka 2011); “Nitrické vrchy” (Straka and Majzlán 2014).

**Published host records:** main hosts: *Capreolus capreolus* (see Dyk and Schanzel 1964, Krištofík and Štefan 1980, Chalupský and Povolný 1983, Krištofík 1998); *Cervus elaphus* Linnaeus, 1758 (see Dyk and Schanzel 1964, Krištofík and Štefan 1980, Chalupský and Povolný 1983, Hubálek et al. 1988); occasional hosts: *Capra hircus* Linnaeus, 1758 (see Povolný and Rosický 1955); *Homo sapiens* (see Krištofík and Štefan 1980, Krištofík 1998); *Rupicapra rupicapra* (Linnaeus, 1758) (see Krištofík 1998); *Turdus philomelos* C. L. Brehm, 1831 (see Chalupský and Povolný 1983).

**Material examined:** Diviacka Nová Ves, 48°44'58.9"N, 18°29'29.5"E, 280 m a.s.l., 4.9.2012, 1 male, from a human, J. Oboňa leg. (LMEE PO); Drienovec, 48°37'04.4"N, 20°55'29.9"E, 200 m a.s.l., 1.10.2015, 2 males, 1 female, from human, S. Greš leg. (LMEE PO); Stará Lesná, 49°08'11.3"N, 20°17'47.5"E, 750 m a.s.l., 8.9.2017, 1 male, from human, P. Manko leg. (LMEE PO); Tvrdošín (Skorušické vrchy), 49°22'19.5"N, 19°31'57.4"E, 750 m a.s.l., 23.9.2017, 1 female, from human, J. Šlapák leg. (LMEE PO); Východná, 49°04'04.2"N, 19°53'57.0"E, 780 m a.s.l., 15.9.2017, 1 male, from car, A. Šestáková leg. (LMEE PO); Nová Sedlica env., 49°03'22.1"N, 22°31'03.1"E, 505 m a.s.l., 1.10.1997, 4 females, sweeping undergrowth of deciduous forest, J. Roháček leg. (SMOC); Muránska planina NP, Šiance res., top plateau, 48°46'11"N, 20°04'14"E, 1000 m a.s.l., 7.9.2011, 1 male, the same, 4.9.2012, 1 male, J. Roháček leg. (SMOC); Muránska planina NP, Pohronská Polhora 5.9 km E, Kučalach Mt., 48°44'51"N, 19°52'27"E, 1060 m a.s.l., 10.10.2014, 2 females, sweeping undergrowth of beech-fir forest, J. Roháček leg. (SMOC); Muránska planina NP, Šarkanica res., 48°42'45"N, 19°59'19"E, 580 m a.s.l., 29.9.2017, 1 female, sweeping undergrowth of deciduous forest in ravine, J. Roháček leg. (SMOC); Cerová vrchovina PLA, Gemerský Jablonec – Vodokáš 1 km N, 48°13'00"N, 19°59'42"E, 280 m a.s.l., 27.9.2017, 1 male, the same, 1.11.2017, 3 females, sweeping undergrowth of oak-beech forest (Fig. 2), J. Roháček leg. (SMOC).

**Comments:** A relatively frequent species in Central Europe, widespread in the Palearctic region and introduced to the Nearctic region. It is an ectoparasite of Cervidae, and also attacks human beings (Křištofík 1998).

### *Lipoptena fortisetosa* Maa, 1965

**Published records:** Rozhanovce (Kočišová et al. 2007).

**Published host records:** *Capreolus capreolus* (Kočišová et al. 2007).

**Material examined:** Bogliarka, 49°16'37.1"N, 21°08'52.3"E, 450 m a.s.l., 15.7.2017, 1 female, from human, P. Manko leg. (LMEE PO); Lažany, 49°02'20.2"N, 21°05'40.8"E, 380 m a.s.l., 7.2017, 17 males, 13 females, 21.6.2017, 1 female, 5.9.2017, 4 males, 2 females, all from human, P. Manko leg. (LMEE PO); Levočská (dolina) valley, 49°04'08.3"N, 20°36'17.5"E, 780 m a.s.l., 12.8.2017, 1 female, from a human, A. Šestáková leg. (LMEE PO); Magurka – Oravská Magura (Oravská priehrada), 49°23'19.6"N, 19°31'34.3"E, 850 m a.s.l., 29.7.2017, 1 male, 1 female, 650 m a.s.l., 17.7.2017, 1 male, all from human, J. Šlapák leg. (LMEE PO); Prešov env. (near "pri Kríži"), 48°59'57.0"N, 21°13'03.7"E, 300 m a.s.l., 9.9.2017, 1 male, from a human, J. Oboňa leg. (LMEE PO); Stráne pod Tatrami, 49°09'26.7"N, 20°21'59.9"E, 750 m a.s.l., 8.9.2017, 1 male, from a human, J. Oboňa leg. (LMEE PO); Tvrdošín (Skorušické vrchy), 49°22'19.5"N, 19°31'57.4"E, 700 – 750 m a.s.l., 7.2016, 1 female, 10.7.2017, 1 male, 22.7.2017, 1 male, 3 females, 26.7.2017, 1 male, 31.8.2017, 1 female, all from human, J. Šlapák leg. (LMEE PO); Cerová vrchovina PLA, Tachty 2.2 km SW, Gortva valley, 48°08'41"N, 19°54'51"E, 320 m a.s.l., 13.9.2018, 2 females, netted from forest margin vegetation, J. Roháček leg. (SMOC).



**Figure 2.** Female of *Lipoptena cervi* (Linnaeus, 1758) from Cerová vrchovina PLA (J Roháček).

**Comments:** A relatively frequent species in Slovakia, distributed in the eastern Palearctic region. Previously often confused with *Lipoptena cervi*. *L. fortisetosa* has a western boundary of distribution in Central Europe. It is an ectoparasite of Cervidae, and also attacks human beings (Ducháč and Bádr 1998). *Homo sapiens* is here newly recorded as a (common) host of *L. fortisetosa* in Slovakia.

### *Melophagus ovinus* (Linnaeus, 1758)

**Published records:** Štúrovo (Povolný and Rosický 1955); Silica (Povolný and Rosický 1955, Hubálek et al. 1988, Chalupský 1986); Hažín, Kôprová dolina (valley), Šahy (Chalupský and Povolný 1983, Chalupský 1986); Poprad (Kočišová 2015).

**Published host records:** main host: *Ovis aries* Linnaeus, 1758 (see Povolný and Rosický 1955, Chalupský and Povolný 1983, Hubálek et al. 1988); occasional hosts: *Canis familiaris* and *Homo sapiens* (see Povolný and Rosický 1955), and *Equus caballus* (Kočišová 2015).

**Comments:** It is an ectoparasite of Bovidae, especially sheep (including lambs) (Chalupský and Povolný 1983). *Melophagus ovinus* is strongly affected by a decline of traditional horse and sheep farming in the monitored area (e.g., Bezák and Petrovič 2006).

### *Melophagus rupicaprinus* Rondani, 1879

**Published records:** Námestovo (Chalupský 1980, Chalupský 1986); Tatranská Kotlina (Chalupský and Povolný 1983, Chalupský 1986).

**Published host records:** *Ovis aries* (see Chalupský 1980), *Rupicapra rupicapra* (see Chalupský and Povolný 1983).

**Comments:** A relatively rare species in Central Europe. In Slovakia it is listed as endangered (EN) (Jedlička and Stloukalová 2001). Until now, only two records from 1951 and 1980 have been published from Slovakia (Chalupský 1980, Chalupský and Povolný 1983). It is an ectoparasite of mammals, collected mainly from *R. rupicapra* and, secondarily, sheep (Chalupský and Povolný 1983). *Rupicapra rupicapra* has an isolated population (*R. rupicapra* ssp. *tatica*) in the Tatra Mountains in the north of the country, where *M. rupicaprinus* are found. Because it is isolated at the edge of its distribution range, this population of *M. rupicaprinus* is very vulnerable and like many other marginal populations, it could disappear rapidly and suddenly.

## Tribe Olfersiini

### *Crataerina pallida* (Olivier in Latreille, 1811)

**Published records:** no localities (Povolný and Rosický 1955); Banská Bystrica (Krištofík and Štefan 1980, Chalupský 1986); Vrútky (Straka 1981); Suchý (Čepelák 1985, Chalupský 1986); Bratislava (Krištofík 1998).

**Published host records:** *Apus apus* (Linnaeus, 1758) (see Povolný and Rosický 1955, Krištofík and Štefan 1980, Straka 1981, Krištofík 1998).

**Comments:** A frequent louse fly species in Central Europe, widespread in the Palearctic region. A common ectoparasite of the bird species *Apus apus*, *Delichon urbicum* (Linnaeus, 1758) and (infrequently) of species from other birds, most frequently on young individuals (Krištofík 1998).

Even if the hosts are still widespread, their population density has declined over the past decades, and therefore the parasites will also suffer (BirdLife International 2018).

***Icosta ardeae* (Macquart, 1835)**

**Published records:** Bohel'ov (Krištofík and Štefan 1980, Chalupský 1986).

**Published hosts:** *Ardea purpurea* Linnaeus, 1766 (Krištofík and Štefan 1980), *Ixobrychus minutus* (Linnaeus, 1766) (Krištofík and Štefan 1980).

**Comments:** A relatively rare species in Central Europe, widespread in the tropics and subtropics of the Old World. In Slovakia it is listed as vulnerable (VU) (Jedlička and Stloukalová 2001). Until now, only two records from 1977 have been published from Slovakia. *Icosta ardeae* is an ectoparasite of birds belonging to several different families; it is common on Ardeidae, and rarely found on species from other families (Krištofík and Štefan 1980). The host *Ardea purpurea* is mainly found in the SW part of the country, and although *Ixobrychus minutus* has a wider distribution, it is also more common in SW Slovakia. The rarity of this parasite in Slovakia is mainly due to its occurrence on the edge of its range (and hosts' ranges) in the country.

***Icosta minor* (Bigot in Thomson, 1858)**

**Published records:** Patince (Chalupský and Macháček 1977, Chalupský 1986).

**Published host records:** *Passer montanus* (Linnaeus, 1758) (see Chalupský and Macháček 1977).

**Comments:** A relatively small, rare and non-native species in Central Europe, distributed in the Afrotropical region and the Mediterranean Basin. In Slovakia it was erroneously listed as vulnerable (VU) (Jedlička and Stloukalová 2001), even though it is not a native species in the country. An ectoparasite on various species of Passeriformes, less frequently found on species from other bird orders (Chalupský and Macháček 1977). The only known record from Slovakia is from 1974 (Chalupský and Macháček 1977, Chalupský 1980). It was mistakenly cited as occurring in the Czech Republic by Chalupský and Povolný (1987, 1997) and Sychra (2006, 2009).

***Olfersia fumipennis* (Sahlberg, 1886)**

**Published records:** "Slovakia" (Povolný and Balát 1956, as *Lynchia palustris*, Chalupský 1986).

**Published host records:** "eagle" (Povolný and Balát 1956).

**Comments:** A rare and non-native species in Central Europe, distributed mainly in the Nearctic and Neotropical regions. It is an ectoparasite of birds, mainly birds of prey (Chalupský 1980). The only known record from Slovakia is from 1904. That individual was originally misidentified as *Ornithophila metallica* by A. Wimmer (see Povolný and Balát 1956). Also, Povolný and Balát (1956) mentioned this specimen mistakenly under the name *Lynchia palustris*, which is in fact a synonym of *Icosta albipennis* from America (Chalupský 1980).

***Ornithoica turdi* (Olivier in Latreille, 1811)**

**Published records:** Košice, Podunajské Biskupice (Povolný and Rosický 1955, Chalupský 1986); Podunajské Biskupice (Chalupský 1980, Chalupský and Povolný 1983).

**Published host records:** *Emberiza citrinella* Linnaeus, 1758 (see Povolný and Rosický 1955); *Fringilla coelebs* Linnaeus, 1758 and *Sitta europaea* Linnaeus, 1758 (see Povolný and Rosický 1955, Chalupský and Povolný 1983).

**Comments:** A relatively small species distributed in the Afrotropical region and southern Palaearctic, with a recent increase in records from Central Europe (Droz and Haenni 2011). In Slovakia, where it reaches the northernmost limit of its known distribution, it has been recorded only from a few individuals collected in 1953 (Povolný and Rosický 1955, Chalupský and Povolný 1983), and it was listed as vulnerable (VU) by Jedlička and Stloukalová (2001). It is an ectoparasite of birds, mainly small Passeriformes. It is less frequently found on species from other bird orders (Krištofík 1998).

***Ornithophila metallica* (Schiner, 1864)**

**Published records:** Jakubov (Krištofík 1998).

**Published host records:** *Saxicola rubetra* (Linnaeus, 1758) (see Krištofík 1998).

**Comments:** A rare and non-native species in Central Europe, distributed in southern parts of the Palaearctic, Afrotropical, Oriental and Australasian regions. The only known record from Slovakia is from 1993 (Krištofík 1998). It was, however, overlooked and not listed in the most recent checklist (Sychra 2009). It is an ectoparasite of birds, mainly small Passeriformes but also species from other bird orders (Krištofík 1998).

***Pseudolynchia canariensis* (Macquart in Webb & Berthelot, 1839)**

**Published records:** Devín (Povolný and Balát 1956, Chalupský 1986)

**Published host records:** *Pandion haliaetus* (Linnaeus, 1758) (Povolný and Balát 1956, Chalupský 1980).

**Comments:** A relatively rare and non-native species in Central Europe, widespread (subcosmopolitan) in the tropical and subtropical belts. In Slovakia it has been listed as vulnerable (VU) (Jedlička and Stloukalová 2001), despite not being a native species. An ectoparasite on species of many bird families but preferentially associated with Columbidae, including the domestic pigeon (Chalupský 1980). The only known record from Slovakia is from 1949 (Povolný and Balát 1956).

***Stenopteryx hirundinis* (Linnaeus, 1758)**

**Published records:** Vyhne (Vihnye) (Thalhammer 1899); Trenčín (Trencsén) (Brancsik 1910, Chalupský 1986); Devínska Nová Ves (Povolný and Rosický 1955, Chalupský 1986); Bratislava (Krištofík 1998).

**Published host records:** *Delichon urbicum* (see Povolný and Rosický 1955; Krištofík 1998); *Hirundo rustica* Linnaeus, 1758 (see Thalhammer 1899).

**Comments:** A frequent Central European species, widespread in the Palaearctic region. A common ectoparasite of the bird species *Delichon urbicum*, *Hirundo rustica*, *Ptyonoprogne rupestris* (Scopoli, 1769), *Riparia riparia* (Linnaeus, 1758), and (more rarely) of species from other bird species, most frequently found in nests (Krištofík 1998). *Stenopteryx hirundinis* might suffer from the decline of its hosts (BirdLife International 2018).

## Tribe Ornithomyini

### *Ornithomya avicularia* (Linnaeus, 1758)

Fig. 3

**Published records:** Snina (Szinna) (Thalhammer 1899); Súľov (Čepelák 1974), Bratislava, Čalovec, Čičov, Jarok, Lozorno, Plešivec, Sása (Krištofík and Štefan 1980, Chalupský 1986); Kečovo, Šurany, Vtáčnik (Povolný and Rosický 1955, Chalupský and Povolný 1983, Chalupský 1986); Dražovce, Nitra (Čepelák and Čepelák 1991); Rača (Čepelák 1982, Chalupský 1986); Ivánka pri Dunaji, Nitra, Rača, Sládkovičovo (Chalupský 1986); Uličské Krivé (Roháček 1995); Bratislava, Brzotín, Gbelce, Kiarov, Kňažia, Kostolište, Košice – Šaca, Košická Nová Ves, Limbach, Mojš, Oravský Podzámok, Pavlovce nad Váhom, Pezinok, Plavecký Mikuláš, Podunajské Biskupice, Svätý Jur, Šiatorská Bukovinka, Závod (Krištofík 1998); Bábsky les (Straka and Majzlán 2010).

**Published host records:** *Accipiter gentilis* (Linnaeus, 1758), *Acrocephalus arundinaceus* (Linnaeus, 1758), *A. melanopogon* (Temminck, 1823), *A. scirpaceus* (Hermann, 1804), *Anser anser* (Linnaeus, 1758) (see Krištofík 1998); *Anthus trivialis* (Linnaeus, 1758) (see Krištofík and Štefan 1980; Krištofík 1998); *Aquila pomarina* C. L. Brehm, 1831 (see Krištofík 1998); *Carduelis chloris* (Linnaeus, 1758) (see Chalupský and Povolný 1983); *Circus pygargus* (Linnaeus, 1758), *Coccothraustes coccothraustes* (Linnaeus, 1758), *Emberiza cia* Linnaeus, 1766, *Erithacus rubecula* (Linnaeus, 1758) (see Krištofík 1998); *Falco tinnunculus* Linnaeus, 1758 (Krištofík and Štefan 1980); *Ficedula albicollis* (Temminck, 1815), *Fringilla coelebs* Linnaeus, 1758 (see Krištofík 1998); *Homo sapiens* (Krištofík and Štefan 1980); *Lanius collurio* Linnaeus, 1758, *L. excubitor* Linnaeus, 1758, *Locustella luscinioides* (Savi, 1824), *Monticola saxatilis* (Linnaeus, 1766), *Oriolus oriolus* (Linnaeus, 1758), *Panurus biarmicus* (Linnaeus, 1758) (see Krištofík 1998); *Passer domesticus* (Linnaeus, 1758) (see Krištofík and Štefan 1980); *Passer montanus* (Linnaeus, 1758) (see Krištofík 1998); *Pernis apivorus* (Linnaeus, 1758) (see Thalhammer 1899); *Phoenicurus ochruros* (S. G. Gmelin, 1774), *Pica pica* (Linnaeus, 1758) (see Krištofík 1998); *Picus canus* J. F. Gmelin, 1788 (see Chalupský and Povolný 1983); *Saxicola rubetra* (Linnaeus, 1758) (see Krištofík 1998); *Strix aluco* Linnaeus, 1758 (see Chalupský and Povolný 1983); *Turdus merula* Linnaeus, 1758 (see Čepelák and Čepelák 1991; Krištofík 1998); *Turdus pilaris* Linnaeus, 1758 (see Krištofík 1998).

**Material examined:** Diviacka Nová Ves, 48°44'58.9"N, 18°29'29.5"E, 280 m a.s.l., 15.8.2012, 1 female, on a family house, J. Oboňa leg. (LMEE PO);



**Figure 3.** Female of *Ornithomya avicularia* (Linnaeus, 1758) (M Deml).

Drienovec, 48°37'04.4"N, 20°55'29.9"E, 200 m a.s.l., 15.9.2015; 1 female, from *Prunella modularis* (Linnaeus, 1758), S. Greš leg. (LMEE PO).

**Comments:** A frequent louse fly species in Central Europe, widespread in the Palearctic region. A common ectoparasite of birds from the order Passeriformes and other orders, most frequently find in more individuals per host (Krištofík 1998). *Prunella modularis* is here recorded as a new host of *O. avicularia* in Slovakia.

### *Ornithomya biloba* Dufour, 1827

**Published records:** Omšenie (Nagysziklás) (Brancsik 1910, as *Ornithomyia tenella*); Čaradská pustatina (Krištofík and Štefan 1980, Chalupský 1986); Humenné (Chalupský and Povolný 1983, Chalupský 1986); Bašovce, Bernolákovo, Bodíky, Klúčovec, Podunajské Biskupice (Krištofík 1998).

**Published host records:** *Hirundo rustica* (see Krištofík 1998, Krištofík and Štefan 1980); *Riparia riparia* (see Chalupský and Povolný 1983).

**Material examined:** Gbelce, 47°51'29.4"N, 18°30'17.9"E, 120 m a.s.l., 21.4.2009, 1 male, 27.4.2009, 1 male, 28.4.2009, 1 male, 2.5.2009, 2 males, all from *Hirundo rustica*, O. Sychra leg. (VFU).

**Comments:** A Palaearctic species, common in Central Europe; its distribution range is incompletely known. It is an ectoparasite mainly of *Delichon urbicum*, *Hirundo rustica*, *Riparia riparia* and, less often, of species from other bird orders (Krištofík 1998).

### ***Ornithomya chloropus* (Bergroth, 1901)**

**Published records:** Kalinovo, Košice (Povolný and Rosický 1955, Chalupský 1986).

**Published host records:** *Regulus regulus* (Linnaeus, 1758) (see Povolný and Rosický 1955); without host record (Chalupský 1986).

**Comments:** A Palaearctic species distributed in the northern and middle belts of the region. It is an ectoparasite mainly of Passeriformes, but also of species of other bird orders (Povolný and Rosický 1955). The only known record from Slovakia is from 1953, and it was “hidden” in the figure legend in Povolný and Rosický (1955). It was incorrectly doubted by Chalupský (1980) and was not listed in the last (Sychra 2009) or all previous checklists (Chalupský and Povolný 1987, 1997, Sychra 2006).

### ***Ornithomya fringillina* Curtis, 1836**

**Published records:** Bohel'ov, Kamienska, Pilsko, Rovinka (Krištofík and Štefan 1980, Chalupský 1986); Kalinovo (Chalupský and Povolný 1983, Chalupský 1986); Brodské, Čičov, Gbelce, Jakobov, Oravský Podzámok, Svätý Jur (Krištofík 1998); Krasín (Straka 2005a); Lutovský Drieňovec (Straka and Majzlán 2008); “Nitrické vrchy” (Straka and Majzlán 2014).

**Published host records:** *Acrocephalus arundinaceus*, *A. schoenobaenus* (Linnaeus, 1758) (see Krištofík 1998); *Ardea purpurea* (see Krištofík and Štefan 1980); *Parus caeruleus* Linnaeus, 1758 (see Krištofík and Štefan 1980; Krištofík 1998); *Parus major* Linnaeus, 1758, *Riparia riparia* (see Krištofík and Štefan 1980); *Sitta europaea*, *Sylvia atricapilla* (Linnaeus, 1758) (see Krištofík 1998); *Troglodytes troglodytes* (Linnaeus, 1758) (see Krištofík and Štefan 1980).

**Comments:** A Palaearctic species distributed in the northern and middle belts of the region. It is an ectoparasite mainly of Passeriformes, but also parasitizes species of other bird orders (Krištofík 1998).

## **Discussion**

We have critically evaluated all available data on the occurrence of the family Hippoboscidae in Slovakia, and published data are completed with new collection data and unpublished localities. We confirmed 19 species as recorded from the country, which has one of the richest hippoboscid faunas in Europe. Out of 19 total species, 12 are native. While seven species (*Crataerina pallida*, *Lipoptena cervi*, *L. fortisetosa*,

*Ornithomya avicularia*, *O. biloba*, *O. fringillina*, and *Stenepteryx hirundinis* are widespread, three species (*Icosta ardeae*, *Melophagus rupicaprinus*, and *Ornithomya chloropus*) are known only from a few records, and the occurrence of the last two species (*Hippobosca equina* and *Melophagus ovinus*) is strongly affected by a decline of traditional horse and sheep farming in the monitored area (e.g., Bezák and Petrovič 2006). Species *S. hirundinis* and *C. pallida* might suffer from the decline of its hosts (BirdLife International 2018). Another seven species (*Hippobosca longipennis*, *H. variegata*, *Icosta minor*, *Olfersia fumipennis*, *Ornithoica turdi*, *Ornithophila metallica*, and *Pseudolynchia canariensis*) have been recorded from Slovakia based on very few records, due to occasional introduction with their hosts. These species can be introduced naturally due to migrating hosts (e.g., *Icosta minor*, *Olfersia fumipennis*, *Ornithophila metallica*, and *Pseudolynchia canariensis*) or imported together with domestic animals (e.g., *Hippobosca longipennis*, *H. variegata*).

Previous records of three species were omitted from the most recent checklist (Sychra 2009):

- 1) a single record of *Icosta minor* from Slovakia (Chalupský 1980; Chalupský and Macháček 1977) had been erroneously cited as being from Moravia (Czech Republic) in previous checklists (Chalupský and Povolný 1987, 1997; Sychra 2006, 2009);
- 2) *Ornithophila metallica* was reported from Slovakia by Krištofik (1998), and
- 3) *Ornithomya chloropus* (Bergroth, 1901) was reported from Slovakia by Povolný and Rosický (1955) in a note “hidden” in the illustration legend, but these records were omitted from all versions of the regional checklist (Chalupský and Povolný 1987, 1997; Sychra 2006, 2009), possibly due to Chalupský (1980), who doubted its occurrence in Slovakia.

Altogether, 78 host-parasite associations have so far been recorded for Slovakian Hippoboscidae (Table 2). The hosts of the 19 species of louse flies recorded in Slovakia belong to 46 species of birds from eight orders (Accipitriformes, Anseriformes, Apodiformes, Ciconiiformes, Falconiformes, Passeriformes, Piciformes, Strigiformes) and nine species of mammals, including humans. The host records of *Prunella modularis* for *O. avicularia* and *Homo sapiens* for *L. fortisetosa* are here recorded from Slovakia for the first time.

The species composition of the hippoboscid fauna of Slovakia is relatively well known, and is, in comparison with other European countries, unexpectedly diverse. In Table 1, a list of European hippoboscid faunas is given, based on Petersen (2004) and Pape et al. (2015) and supplemented with data from relatively recent regional checklists (Büttiker 1998, Chandler 1998, Müller 1999, Beuk 2001, Draber-Monko 1991, Pape et al. 1995, Papp 2001, Carles-Tolrá and Báez 2002, Sychra 2009, Pohjoismäki and Kahanpää 2014, present paper).

The comparison of species richness of Hippoboscidae across Europe's best studied countries for Diptera surprisingly showed that in Slovakia, the fauna of this group is

not only distinctly more diverse than in all surrounding Central European countries, but even comparable with the faunas of much larger and more southern countries, such as Spain or Italy (including their insular areas), which also comprise 19 species (Table 1). However, raising any hypotheses about a possible latitudinal pattern in hippoboscid species richness would require much more thorough data on the continental scale.

We have noted that a number of country occurrences are missing in Fauna Europaea (Petersen 2004, Pape et al. 2015) when compared with the above checklists, most markedly for Spain and its adjacent islands (seven species missing) and Great Britain (six species missing). In contrast, some species listed in Fauna Europaea are missing in national checklists: e.g., *I. minor* is present in Fauna Europaea for Italy (Petersen 2004, Pape et al. 2015) but is absent in the national checklist (Pape et al. 1995); similarly, *O. chloropus* is present in Fauna Europaea for Hungary but is missing in the checklist of this country (Papp 2001). The national checklist of Spain (Carles-Tolrá and Báez 2002) also includes the species *Crataerina nigriventris* Gil Collado, 1932, which was wrongly referred to as *C. nigriventris* (Strobl, 1906) although it was originally described by Gil Collado (1932). However, according to Schneider-Orelli (1937), it is only an aberrant form of *C. melbae* (Rondani, 1879) and, therefore, it is omitted from the list in Table 1.

Judging from the occurrences of Hippoboscidae in other European countries, the list of Slovak species of the family is obviously not yet complete, despite its richness. At least the following two species can be expected in Slovakia: *Crataerina melbae* and *Pseudolynchia garzettae* (Rondani, 1879), both of which parasitize bird species living in Slovakia and are known from Italy and Switzerland, and Great Britain and Italy, respectively (see Table 1). In addition, the introduction of additional, more exotic hippoboscid species, like *Olfersia spinifera* (Leach, 1817) (known from G. Britain) or *Ornithomya rupes* Hutson, 1981 (recorded from Switzerland) to Slovakia cannot be excluded. In conclusion, more than 20 species of louse flies can be expected to occur in Slovakia, including both native residents and sporadic introductions.

Several species of the family Hippoboscidae can interfere with human life and interests, as ectoparasites of some domestic animals, occasionally parasitizing also humans. These are mainly *Lipoptena cervi*, *L. fortisetosa*, and relatively rare species *Hippobosca equina* and *Melophagus ovinus*. Damage is caused by direct bloodsucking and the venomous saliva of the louse flies, which can lead to permanent loss of blood and to animal wasting (especially ovine wasting), reduced milk and wool production, damage to wool caused by the parasite's faeces, etc. (Hutyra and Marek 1952). A secondary consequence of ectoparasitism by keds is the constant discomfort and scratching by the parasitised host (Hase 1927). Louse flies are also known as possible vectors of various diseases (Baker 1967, Kečera 1983, Oyieke and Reid 2003, Halos et al. 2004, Reeves et al. 2006, Martinković et al. 2012). A few of the above-mentioned species, especially *L. cervi* and *L. fortisetosa*, may bite humans in forest environments.

In Slovakia, species of the family Hippoboscidae have not received sufficient attention, even though they are among the most abundant ectoparasites in some localities. Therefore, it is important to pay attention to this group and maintain an accurate

**Table 1.** Hippoboscid faunas of selected European countries (see Petersen 2004, Pape et al. 2015), supplemented with data from relative recent checklists.\*

			Spain incl. islands	Italy incl. islands	Switzerland	Great Britain	Czech Republic	Slovakia	Finland	Germany	Hungary	Poland	The Netherlands
Hippoboscini	<i>Hippobosca</i>	<i>equina</i>	+	+	+	+	+	+	+	+	+	+	+
		<i>longipennis</i>	+	+		+		+			+		
		<i>variegata</i>						+					
Lipoptenini	<i>Lipoptena</i>	<i>ariana</i>											
		<i>capreoli</i>											
		<i>cervi</i>	+	+	+	+	+	+	+	+	+	+	+
		<i>couturieri</i>	+										
	<i>Melophagus</i>	<i>fortisetosa</i>			+		+	+		+			+
		<i>ovinus</i>	+	+	+	+	+	+	+	+	+	+	+
Olfersiini	<i>Crataerina</i>	<i>rupicaprinus</i>		+	+			+		+			
		<i>acutipennis</i>	+										
		<i>melbae</i>	+	+	+								
		<i>obtusipennis</i>											
	<i>Icosta</i>	<i>pallida</i>	+	+	+	+	+	+	+	+	+	+	+
		<i>ardeae</i>			+	+	+	+	+			+	
		<i>massonati</i>											
	<i>Olfersia</i>	<i>minor</i>	+	+		+		+					
		<i>fumipennis</i>	+				+	+	+				
		<i>spinifera</i>				+							
		<i>turdi</i>	+	+			+	+				+	+
		<i>gestroi</i>	+	+									
		<i>metallica</i>	+	+	+	+	+	+	+	+		+	+
<i>canariensis</i>		+	+			+	+						
<i>Pseudolynchia</i>	<i>garzettae</i>		+		+								
	<i>Stenopteryx</i>	<i>hirundinis</i>	+	+	+	+	+	+	+	+	+	+	
Ornithomyiini	<i>Ornithomya</i>	<i>avicularia</i>	+	+	+	+	+	+	+	+	+	+	
		<i>biloba</i>		+	+	+	+	+		+	+	+	
		<i>chloropus</i>	+	+	+	+	+	+	+	+	+	+	
		<i>fringillina</i>	+	+	+	+	+	+	+	+	+	+	
		<i>rupes</i>	+		+								
<b>number of species</b>			19	19	15	15	19	10	12	12	11	12	

\* Spain incl. islands (Carles-Tolrá and Báez 2002), Italy incl. islands (Pape et al. 1995), Switzerland (Büttiker 1998), Great Britain (Chandler 1998), Czech Republic and Slovakia (Sychra 2009, present paper), Finland (Pohjoismäki and Kahanpää 2014), Germany (Müller 1999), Hungary (Papp 2001), Poland (Draber-Monko 1991) and The Netherlands (Beuk 2001).

overview of the species living in our territory, including monitoring of the occurrence of non-native species migrating with their hosts or imported with domestic animals. From a wider perspective, verified and accurate information on the diversity and distribution of louse flies in Slovakia can contribute to knowledge of this parasitic group from a global point of view.

**Table 2.** Systematic overview of host-parasite associations recorded for Slovakian Hippoboscidae.

Parasite sp.	Hosts	Order	Family	Species	
<i>Hippobosca equina</i>	Aves	Accipitriformes	Accipitridae	<i>Accipiter gentilis</i>	
	Mammalia	Carnivora	Canidae	<i>Canis familiaris</i>	
		Cetartiodactyla	Bovidae	<i>Bos taurus</i>	
		Cetartiodactyla	Cervidae	<i>Capreolus capreolus</i>	
		Perissodactyla	Equidae	<i>Equus caballus</i>	
		Primates	Hominidae	<i>Homo sapiens</i>	
<i>Hippobosca longipennis</i>	Mammalia	Carnivora	Canidae	<i>Canis familiaris</i>	
<i>Hippobosca variegata</i>	Mammalia	Carnivora	Canidae	<i>Canis familiaris</i>	
	Aves	Passeriformes	Turdidae	<i>Turdus philomelos</i>	
		Mammalia	Cetartiodactyla	Bovidae	<i>Capra hircus</i> , <i>Rupicapra rupicapra</i>
			Cetartiodactyla	Cervidae	<i>Capreolus capreolus</i> , <i>Cervus elaphus</i>
			Primates	Hominidae	<i>Homo sapiens</i>
<i>Lipoptena cervi</i>	Mammalia	Cetartiodactyla	Cervidae	<i>Capreolus capreolus</i>	
Primates		Hominidae	<i>Homo sapiens</i>		
<i>Lipoptena fortisetosa</i>	Mammalia	Cetartiodactyla	Cervidae	<i>Capreolus capreolus</i>	
	Mammalia	Carnivora	Canidae	<i>Canis familiaris</i>	
		Cetartiodactyla	Bovidae	<i>Ovis aries</i>	
		Perissodactyla	Equidae	<i>Equus caballus</i>	
		Primates	Hominidae	<i>Homo sapiens</i>	
<i>Melophagus ovinus</i>	Mammalia	Cetartiodactyla	Bovidae	<i>Ovis aries</i> , <i>Rupicapra rupicapra</i>	
<i>Melophagus rupicaprinus</i>	Mammalia	Cetartiodactyla	Bovidae	<i>Ovis aries</i> , <i>Rupicapra rupicapra</i>	
<i>Crataerina pallida</i>	Aves	Apodiformes	Apodidae	<i>Apus apus</i>	
<i>Icosta ardeae</i>	Aves	Passeriformes	Hirundinidae	<i>Ardea purpurea</i>	
	Aves	Pelecaniformes	Ardeidae	<i>Isobrychus minutus</i>	
<i>Icosta minor</i>	Aves	Passeriformes	Passeridae	<i>Passer montanus</i>	
<i>Ornithoica turdi</i>	Aves	Passeriformes	Emberizidae	<i>Emberiza citrinella</i>	
	Aves		Fringillidae	<i>Fringilla coelebs</i>	
	Aves		Sittidae	<i>Sitta europaea</i>	
<i>Ornithophila metallica</i>	Aves	Passeriformes	Muscicapidae	<i>Saxicola rubetra</i>	
<i>Pseudolynchia canariensis</i>	Aves	Accipitriformes	Pandionidae	<i>Pandion haliaeetus</i>	
<i>Stenoteryx hirundinis</i>	Aves	Passeriformes	Hirundinidae	<i>Delichon urbica</i> , <i>Hirundo rustica</i>	
<i>Ornithomya avicularia</i>	Aves	Accipitriformes	Accipitridae	<i>Accipiter gentilis</i> , <i>Aquila pomarina</i> , <i>Circus pygargus</i> , <i>Pernis apiivorus</i>	
		Anseriformes	Anatidae	<i>Anser anser</i>	
		Falconiformes	Falconidae	<i>Falco tinnunculus</i>	
		Passeriformes	Prunellidae	<i>Prunella modularis</i>	
			Acrocephalidae	<i>Acrocephalus arundinaceus</i> , <i>A. melanopogon</i> , <i>A. scirpaceus</i>	
			Corvidae	<i>Pica pica</i>	
			Emberizidae	<i>Emberiza cia</i>	
			Fringillidae	<i>Carduelis chloris</i> , <i>Coccothraustes coccothraustes</i> , <i>Fringilla coelebs</i>	
			Laniidae	<i>Lanius collurio</i> , <i>L. excubitor</i>	
			Locustellidae	<i>Locustella luscinioides</i>	
			Motacillidae	<i>Anthus trivialis</i>	
			Muscicapidae	<i>Erithacus rubecula</i> , <i>Ficedula albicollis</i> , <i>Phoenicurus ochruros</i> , <i>Saxicola rubetra</i>	
			Oriolidae	<i>Oriolus oriolus</i>	
			Paradoxornithidae	<i>Panurus biarmicus</i>	
		Passeridae	<i>Passer domesticus</i> , <i>P. montanus</i>		
		Turdidae	<i>Monticola saxatilis</i> , <i>Turdus merula</i> , <i>T. pilaris</i>		
		Strigiformes	Strigidae	<i>Strix aluco</i>	
Mammalia	Primates	Hominidae	<i>Homo sapiens</i>		
<i>Ornithomya biloba</i>	Aves	Passeriformes	Hirundinidae	<i>Hirundo rustica</i> , <i>Riparia riparia</i>	
<i>Ornithomya chloropus</i>	Aves	Passeriformes	Sylviidae	<i>Regulus regulus</i>	
<i>Ornithomya fringillina</i>	Aves	Passeriformes	Acrocephalidae	<i>Acrocephalus arundinaceus</i> , <i>A. schoenobaemus</i>	
			Paridae	<i>Parus caeruleus</i>	
			Hirundinidae	<i>Riparia riparia</i>	
			Sittidae	<i>Sitta europaea</i>	
			Sylviidae	<i>Sylvia atricapilla</i>	
			Troglodytidae	<i>Troglodytes troglodytes</i>	
Pelecaniformes	Ardeidae	<i>Ardea purpurea</i>			

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# A new species of *Fannia* (Diptera, Fanniidae) from Yunnan, China

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## Abstract

A new species of the genus *Fannia* (Diptera, Fanniidae) is described from Yunnan, China, namely *Fannia baihualingensis* sp. nov. The male habitus as well as terminalia are documented with focus-stacked photographs. A detailed comparison of new species with related species is provided.

## Keywords

Description, *Fannia posticata*-group, male terminalia

## Introduction

The Fanniidae (Diptera, Muscoidea) are cosmopolitan flies with over 400 described species. Around 160 species have been found from China, including one species of *Euryomma* Stein, two species of *Piezura* Rondani, and 157 species of *Fannia* Robineu-Desvoidy. Of these species, 61.25% of them (i.e., 98 species) are endemic to China.

The *Fannia posticata*-group was established by Chillcott (1961), originally as the *Fannia pretiosa*-group. Wang et al. (2010) reviewed the *F. posticata*-group and expanded it to include 21 species.

Yunnan is the highest biodiversity hotspot in China. In this study, we describe a new species of the *Fannia posticata*-group from Yunnan, *Fannia baihualingensis* sp. nov., and provide an extensive documentation of the adult male of this species.

## Materials and methods

Terminology follows McAlpine (1981) and Stuckenberg (1999). Methods for the preparation of terminalia and illustrations follow Zhang et al. (2013). All type specimens of the new species are deposited in the Museum of Beijing Forestry University, Beijing, China (MBFU).

Abbreviations used throughout the text are as follows:

acr acrostichal seta,  
ad anterodorsal seta,  
av anteroventral seta,  
d dorsal seta,  
p posterior seta,  
pd posterodorsal seta,  
pv posteroventral seta.

## Taxonomy

### *Fannia baihualingensis* sp. nov.

<http://zoobank.org/DA934C3A-C351-424A-8421-30EC259742D1>

Figures 1, 2

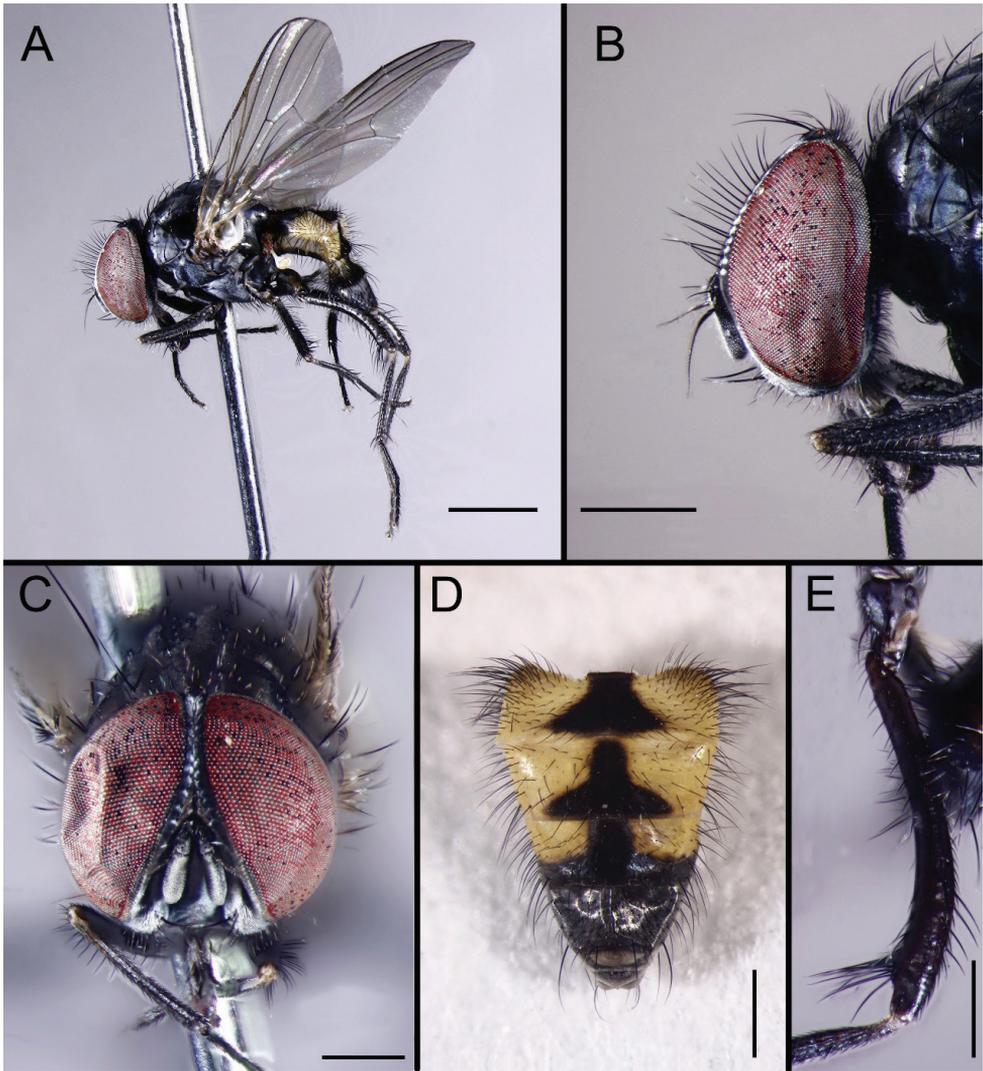
**Material examined.** Holotype ♂: China: Yunnan, Gaoligong, Baihualing, 25.VII.2015, Coll. L.P. Yan & C. Wang (MBFU).

Paratypes 2 ♂, same data as holotype (MBFU).

**Diagnosis.** *Fannia baihualingensis* can be readily identified by the following character states: distinctly projecting lower calypter; hind coxa bare on posterior surface; hind femur arcuate, with clump of long black setae on swollen part; hind tibia with two av, hook-like projection on lower margin of cercus curved outward; surstylus very long and slender; bacilliform process absent.

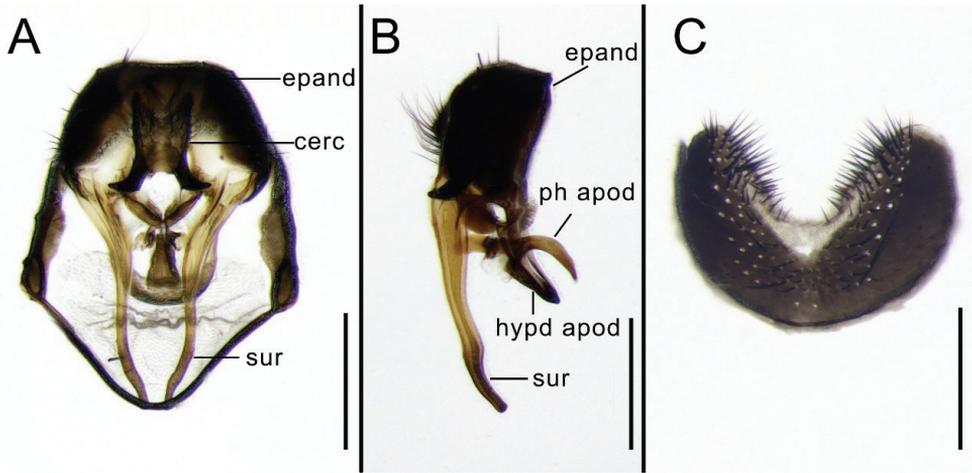
**Description.** Male. Body length 5.00–6.50 mm (2 specimens measured). Eye bare. Fronto-orbital plate and parafacial with grayish-silvery pollinosity. Frons slightly narrower than the distance between two posterior ocelli at narrowest point, frontal vitta black, frontal setae 7–9, stout. Postocular setae in 1 row, without occipital seta behind the postocular setae on vertex. Parafacial bare, at middle about 3/4 as wide as the width of postpedicel. Antenna grayish black, postpedicel 2 × longer than wide, arista black and short plumose, slightly swollen in basal part. Epistoma not projecting beyond vibrissal angle, vibrissal angle behind frontal angle in profile, subvibrissal setae in 1 row, lateral with one 1 of short setae. Proboscis stout. Palpus black, claviform, longer than the length of prementum.

Thorax ground color black, without distinct vitta. Postpronotal lobe gray. Presutural acr biserial, hair-like, only prescutellar pairs slightly stout, dorsocentrals 2+3,



**Figure 1.** *Fannia baihualingensis* sp. nov. from Yunnan, China, male. **A** Habitus, lateral view **B** Head, lateral view **C** Head, anterior view **D** Abdomen, dorsal view **E** hind femur, anterior view. Scale bars: 1.00 mm (**A**); 0.50 mm (**B–E**).

intra-alars 2, supra-alars 2, postpronotals 2, notopleurals 2. Katepisternal setae 1+1, katepisternum without ventral spine. Scutellum black, with 3 pairs of lateral, 2 pair of discal, and 2 pair of apical setae. Calypters white, the lower one slightly projecting beyond the upper one. Wing brownish; veins brown; tegula dark brown; basicosta brownish-yellow; costal spine inconspicuous; node of Rs bare on ventral and dorsal surfaces; vein  $R_{4+5}$  straight; crossveins without obvious cloud; haltere yellow but brown in basal part. Legs entirely black. Fore femur with complete d, pd, and pv rows, fore tibia with 1 pd seta; mid coxa with spin-like setae, mid femur with complete ad row,



**Figure 2.** *Fannia baihualingensis* sp. nov. from Yunnan, China, male. **A** Terminalia, ventral view **B** Terminalia, lateral view **C** Sternite 5, ventral view. Abbreviations: cerc = cercus; epand = epandrium; hypd apod = hypandrial apodeme; ph apod = phallapodeme; sur = surstylus. Scale bar: 0.25 mm.

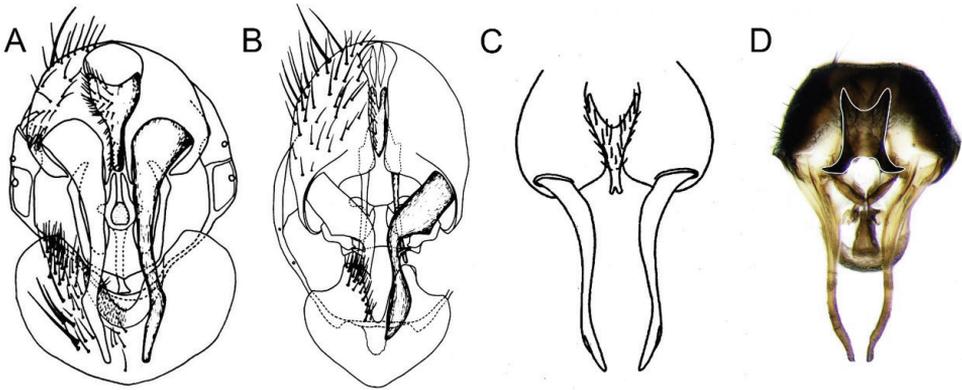
becoming gradually shorter and denser towards apex, pv row complete, in 1 row, ad rows weak, 5 setae strong in distal part, mid tibia slightly swollen in distal 2/3, with 1 ad and 1 pd; hind coxa bare on posterior surface, hind femur curved and arcuate, swollen at apex below, the swollen part with a clump of long black setae on postero-ventral (Fig. 1E), antero-ventral surface with a complete series of setae, (the apical 4 longer and stronger), hind tibia with 2 av.

Abdomen long, depressed and flattened. Syntergite 1+2 dark in basal part. Syntergite 1+2 to tergite 4 largely yellow with 1 median inverted black triangular vitta (Fig. 1D). Distal half of tergite 4 and all of tergite 5 black gray-pollinose, each tergite with long lateral marginal setae. Sternite 5 profoundly indented on posterior margin and covered with setae (Fig. 2C). Cercus slightly rounded, the hook-like projection on its lower margin curved outward. Surstylus very long and slender (Fig. 2A), slightly arcuate on apical half. Bacilliform process absent.

Female: Unknown.

**Remarks.** According to the keys by Chillcott (1961), Hennig (1955), and Wang et al. (2010) and the detailed description by Chillcott (1961: 142), Pont (1977: 19), and Hennig (1955:42), *F. baihualingensis* sp. nov. resembles *F. arcuata*, *F. curvipes*, *F. fasciculata*, *F. gilvitaris*, and *F. anteroventralis* due to the strongly arcuate hind femur. *Fannia gilvitaris* and *F. anteroventralis* has a black ground-color. The abdomen of *F. baihualingensis* sp. nov. is yellow in lateral part. *Fannia anteroventralis* has a black haltere at apex, *F. baihualingensis* sp. nov. has a yellow haltere at apex.

Compared with *F. arcuata*, *F. curvipes*, and *F. fasciculata*, only the hind tibia of *F. arcuata* has a complete ad row. *Fannia curvipes*, *F. fasciculata*, and *Fannia baihualingensis* sp. nov. are all only with one ad. The male terminalia of *F. arcuata* is also very different from those of the other three species (Fig. 3B).



**Figure 3.** Male terminalia of *Fannia* spp. **A** *F. curvipes* Malloch (adapted from Chillcott 1961: fig. 88) **B** Male terminalia, *F. arcuata* Chillcott (adapted from Chillcott 1961: fig. 89) **C** Male terminalia, *F. fasciculata* (Loew) (adapted from Hennig 1955: pl. 4, fig. 75) **D** Male terminalia, *Fannia baihualingensis* sp. nov.

The male terminalia of *F. curvipes*, *F. fasciculata*, and *Fannia baihualingensis* sp. nov. are very similar, especially the shape of surstylus. However, the new species can be identified by the shape of the cercus. In *F. curvipes* the cercal plate is very slender in its apical half and prolonged into an upcurved process (Fig. 3A). The cercus of *F. fasciculata* is slender and bifurcate at the apex (Fig. 3C). The cercus of *F. baihualingensis* sp. nov. is slightly rounded, with the hook-like projection on its lower margin strongly curved outward (Fig. 3D).

*Fannia baihualingensis* sp. nov. can also be distinguished from *F. fasciculata* by some external characters, such as the number of av on hind tibia: the new species has only two av, while *F. fasciculata* has four or five.

**Etymology.** The new species is named after its type locality, Baihualing.

**Distribution.** Known only from the type locality in Yunnan, China.

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