

Characterization and comparative analysis of the complete mitochondrial genome of *Azygia hwangtsiyui* Tsin, 1933 (Digenea), the first for a member of the family Azygiidae

Yuan-An Wu^{1*}, Jin-Wei Gao^{1*}, Xiao-Fei Cheng¹,
Min Xie¹, Xi-Ping Yuan¹, Dong Liu¹, Rui Song¹

¹ Hunan Fisheries Science Institute, Changsha 410153, China

Corresponding author: Rui Song (ryain1983@163.com)

Academic editor: D. Gibson | Received 26 December 2019 | Accepted 4 May 2020 | Published 3 July 2020

<http://zoobank.org/4EE745AE-3269-4266-8CAD-FF4B67F969FB>

Citation: Wu Y-A, Gao J-W, Cheng X-F, Xie M, Yuan X-P, Liu D, Song R (2020) Characterization and comparative analysis of the complete mitochondrial genome of *Azygia hwangtsiyui* Tsin, 1933 (Digenea), the first for a member of the family Azygiidae. ZooKeys 945: 1–16. <https://doi.org/10.3897/zookeys.945.49681>

Abstract

Azygia hwangtsiyui (Trematoda, Azygiidae), a neglected parasite of predatory fishes, is little-known in terms of its molecular epidemiology, population ecology and phylogenetic study. In the present study, the complete mitochondrial genome of *A. hwangtsiyui* was sequenced and characterized: it is a 13,973 bp circular DNA molecule and encodes 36 genes (12 protein-coding genes, 22 transfer RNA genes, two ribosomal RNA genes) as well as two non-coding regions. The A+T content of the *A. hwangtsiyui* mitogenome is 59.6% and displays a remarkable bias in nucleotide composition with a negative AT skew (−0.437) and a positive GC skew (0.408). Phylogenetic analysis based on concatenated amino acid sequences of twelve protein-coding genes reveals that *A. hwangtsiyui* is placed in a separate clade, suggesting that it has no close relationship with any other trematode family. This is the first characterization of the *A. hwangtsiyui* mitogenome, and the first reported mitogenome of the family Azygiidae. These novel datasets of the *A. hwangtsiyui* mt genome represent a meaningful resource for the development of mitochondrial markers for the identification, diagnostics, taxonomy, homology and phylogenetic relationships of trematodes.

Keywords

gene arrangement, phylogenetic analysis, Trematoda

* Contributed equally as the first authors.

Introduction

The genus *Azygia* Looss, 1899 is an endoparasitic helminth found in the stomach and intestine of freshwater feral carnivorous fish (Frolova and Shcherbina 1975). This genus includes several species complexes and its type species is *Azygia lucii* (Müller, 1776), which is a parasite of numerous, but especially esocid and percid, fishes in Europe. Many researchers have added to our knowledge of this cosmopolitan species. To date, species of *Azygia* are frequently reported from the esophagus, stomach, and intestine of a wide range of predatory fishes from Asia, Europe and North America, including China, Japan, India, Russia, Germany, and North America (Tubangui 1928; Tsin 1933; Moravec and Sey 1989; Marcogliese and Cone 1996; Besprozvannykh 2005; Jadhav et al. 2011; Pallewad et al. 2015; Womble et al. 2016; Nagasawa and Katahira 2017).

Azygia hwangtsiyui Tsin, 1933 is a member of the family Azygiidae Odhner, 1911 and is often overlooked; it is parasitic in the gastrointestinal tract of species of the family Channidae Fowler, 1934 but caused only slight clinical signs, including malnutrition and weight loss. In China, *Azygia hwangtsiyui*-infected freshwater predatory fishes have been described from Shandong, Heilongjiang, Jiangsu, Fujian, Sichuan and Hunan Provinces (Tsin 1933; Zmejev 1936; Ma 1958; Tang and Tang 1964; Kiang 1965; Chen 1973; Wang 1985; Cheng 2011). It has a mainly inland distribution and utilizes freshwater snail species (e.g. *Vivipara quadrata* (Benson, 1842)) as intermediate hosts (Tang and Tang 1964) and develops into adults in the gastrointestinal tract of predatory fish species such as *Ophiocephalus argus* Cantor, 1842 and *Channa asiatica* (Linnaeus, 1758) (Tsin 1933; Besprozvannykh 2005).

Morphology is the most commonly used method for species identification and differentiation of metazoans and is widely adopted globally by parasitologists and taxonomists. A huge disadvantage of using morphological criteria, however, is that it is difficult to identify and distinguish closely related and cryptic species. Although the family Azygiidae was erected more than a century ago, its situation, and that of several species of *Azygia*, is still controversial and uncertain. Manter (1926) pointed out that *Azygia* is the only genus in the family then presenting systematic confusion: *Azygia longa* (Leidy, 1851) in North America may be a synonym of *A. lucii* in Europe (Manter 1926), and Van Cleave and Mueller (1934) reported that *Azygia acuminata* Goldberger, 1911 and *A. longa* should be considered conspecific. Nevertheless, due to the discovery of some life histories of members of *Azygia*, *A. lucii* and *A. longa* have been recognized as two distinct species (Szidat 1932; Sillman 1953; Sillman 1962).

Mitochondrial (mt) genome and nuclear ribosomal DNA sequences are effective molecular tools for taxonomic identification, phylogeny and biogeographical research (Bernt et al. 2013; Le et al. 2019). However, only a partial cytochrome oxidase subunit 1 protein sequence (AIY67834) of *Proterometra macrostoma* Horsfall, 1933 (Azygiidae) is currently available in GenBank. None of the mitochondrial genome data have been sequenced for a member of the family Azygiidae. Therefore, we determined the complete mitochondrial genome sequence of *A. hwangtsiyui* as a basis for the future definition of strain- and species-specific markers, and for assessing mitogenomics in resolving the interrelationships of trematodes.

Materials and methods

Sampling and DNA extraction

The specimens of flatworms were isolated from the stomach of their definitive host, in this case snakehead fish (*Ophiocephalus argus* (Cantor, 1842)) obtained from east Dongting Lake in Yueyang, Hunan province, China (29°22'N, 113°06'E). *Azygia hwangtsiyui* was morphologically identified according to the original and other descriptions (Tsin 1933; Tang and Tang 1964; Zhang et al. 1999; Besprozvannykh 2005), using a stereomicroscope and a light microscope. Furthermore, single samples were confirmed molecularly as *A. hwangtsiyui* based on sequencing of 1370 bp 28S rDNA sequence. The parasites were completely washed in water, preserved in 99% ethanol, and stored at 4 °C until genomic DNA extraction. Total genomic DNA extraction was performed from an intact specimen with the TIANamp Micro DNA Kit (Tiangen Biotech, Beijing, China), according to the manufacturer's instructions.

DNA amplification and sequencing

According to conserved regions of mitochondrial genes in other available digenea mitogenomes, six partial gene fragments for cytb, nad4, nad1, 16S, 12S and cox2 were amplified using six generic primers sets HWF1/HWR1 (for cytb), HWF3/HWR3 (for nad4), HWF5/HWR5 (for nad1), HWF7/HWR7 (for 16S), HWF9/HWR9 (for 12S), and HWF11/HWR11 (for cox2), respectively. On the basis of these obtained nucleotide sequences, *A. hwangtsiyui*-specific primers were designed for amplification and sequencing of the remaining mitogenome (Suppl. material 1: Table S1). All primers were designed to produce amplicons with overlaps of approximately 100 bp. PCR reactions were performed in a 50 µl reaction solution with the ingredient of 18.5 µl ddH₂O, 25 µl 2×PCR buffer (Mg²⁺, dNTP plus, Takara, Dalian, China), 1.5 µl of each primer (0.2–1.0 µM), 1 µl EX Taq polymerase (250U, Takara), and 2.5 µl DNA template. PCR amplification was compliant to the following amplification protocol: initial denaturation at 98 °C for 2 min, followed by 40 cycles 10 s at 98 °C, 15 s at 50 °C, 68 °C for 1 min/kb, and 10 min at 68 °C for a final extension. The amplified PCR products were purified with TIANgel Purification Kit (Tiangen Biotech, Beijing, China), and sequenced bidirectionally at Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China) based on the primer walking method using several specific primers (Suppl. material 1: Table S1).

Mitogenome annotation and analysis

According to sequence chromatograms, all raw fragments were quality-proofed using CHROMAS (<https://www.technelysium.com.au>) to remove ambiguity codes and low-quality bases. Whenever the quality was sub-optimal, sequencing was repeated until the amplicon is the consensus sequence. Before manual assembly of the entire mitochondrial genomic sequence, identification of all amplicons was performed by

BLASTN check (Altschul et al. 1990). The mt genome of *A. hwangtsiyui* was aligned against the mt genome sequences of other promulgated digenean mitogenomes utilizing multiple sequence alignment software MAFFT version 7.149 (Katoh and Standley 2013) to identify genetic boundary. Protein-coding genes (PCGs) were predicted with Open Reading Frame Finder (<https://www.ncbi.nlm.nih.gov/orffinder/>) adopting echinoderm and flatworm mitochondrial codes, and examining the nucleotide alignment against the reference mtDNA in trematode *Dicrocoelium chinense* Tang et Tang, 1978 (NC_025279.1). Whole tRNAs were inferred with the detection results of ARWEN (Laslett and Canback 2008) and MITOS web server (Bernt et al. 2013). Two rRNA (rrnL and rrnS) were founding by comparison with those of published fluke mitogenomes. Codon usage and relative synonymous codon usage (RSCU) for 12 PCGs of *A. hwangtsiyui* were computed by PHYLOSUITE (Zhang et al. 2020), and its operation results were imported into GGLOT2 program (Wickham 2016) to make figures of the RSCU. Tandem repeats in the non-coding regions were determined with Tandem Repeats Finder software version 4.09 (Benson 1999), and the prediction of their secondary structures were performed by the MFOLD web server (Zuker 2003). The annular diagram of *A. hwangtsiyui* mitogenome was plotted with mitochondrial genome data visualization tool MTVIZ (<http://pacosy.informatik.uni-leipzig.de/mtviz/mtviz>).

Phylogenetic analysis

For phylogenetic analyses, we utilized translated and concatenated amino acid sequences of twelve protein-coding genes for 49 Platyhelminthes including *A. hwangtsiyui* mitogenome determined in this study. Two tapeworm species, *Cloacotaenia megalops* (Nitzsch in Creplin, 1829) (NC_032295.1) and *Dibothriocephalus latus* (Linnaeus, 1758) (NC_008945.1) were included as outgroup taxa representing two different families. Species information including systematic positions and GenBank accession numbers is provided in Suppl. material 2: Table S2. The PHYLOSUITE program was used to extract twelve PCGs from the GenBank files, export fasta files with translated amino acid datasets, and align datasets in bulk using integrated applet MAFFT with normal-alignment mode. Phylogenetic analyses were performed using Bayesian Inference (BI) and Maximum Likelihood (ML) methods. Assessment of the best-fit evolutionary model for dataset was conducted via ModelGenerator v0.8527 (Keane et al. 2006). BI in MrBayes version 3.2.6 (Ronquist et al. 2012) was carried out under the MtRev matrix of amino acid substitution, and was analyzed with 1×10^7 metropolis-coupled Monte Carlo Markov Chain (MCMC) generations. Two independent runs with four simultaneous MCMC chains (one cold and three heated chains) were conducted for 1×10^7 million generations, sampling every 10,000 generations and discarding the initial 25% generations as burn-in. ML analysis in PHYLOSUITE was performed using MtART+I+G matrix with 1000 bootstrap replicates.

Results and discussion

General traits of the *Azygia hwangtsiyui* mitogenome

The entire *A. hwangtsiyui* mtDNA is 13,973 bp in length (GenBank accession number: MN844889) and comprised of 12 protein-coding genes (cox1-3, nad1-6, nad4L, cytb, and atp6), 22 tRNA genes, two rRNA genes (rrnL and rrnS), and two non-coding regions. The 12 protein-coding gene order arrangement is cox3-cytb-nad4L-nad4-atp6-nad2-nad1-nad3-cox1-cox2-nad6-nad5 (Fig. 1), which is identical to those of *Clinostomum complanatum* (Rudolphi, 1814), *Echinostoma hortense* Asada, 1926, and some species of the Fasciolidae (*Fasciola hepatica* Linneus, 1758, *Fasciola gigantica* Cobbold, 1856, and *Fasciola* sp. GHL-2014) (Liu et al. 2014a; Chen et al. 2016; Liu et al. 2016); the gene atp8 is similarly missing, as usual in trematode species. All genes are transcribed in the anticlockwise direction and encoded by H strand (Table 1), which is in accordance with other digeneans. The mt genome of *A. hwangtsiyui* has 22 intergenic spacers ranging from 1 to 15 bp and contains two overlapping nucleotides ranging from 1 to 40 bp (Table 1). Noteworthy, a 40 bp overlap between the nad4 and nad4L genes exists in the *A. hwangtsiyui* mitogenome, which is consistent with most helminths such as *Eurytrema pancreaticum* Janson, 1889 (Chang et al. 2016), *Hypoderma conoideum* (Bloch, 1782) (Yang et al. 2015), but shorter than that of *Schistosoma mekongi* Voge, Bruckner & Bruce, 1978 (64 bp; Littlewood et al. 2006). The nucleotide contents of T, C, A, G, in *A. hwangtsiyui* mitogenome are 42.8%, 12.0%, 16.8%, and 28.5%, respectively (Table 2). The whole A+T content of the mitogenome is 59.6%, which was markedly biased toward T over A (AT skew: -0.437), and G over C (GC skew: 0.408).

Protein-coding genes and non-coding regions

A total of 3364 amino acids was encoded by the *A. hwangtsiyui* mtDNA. The full scale of 12 concatenated protein-coding genes was 10126 bp, composed of 45.2% T, 11.5% C, 14.7% A, and 28.6% G. Average A+T content of concatenated 12 protein-coding genes was 59.9%, varying from 57.7% (cox2) to 64.8% (nad4L) (Table 2 and Suppl. material 2: Table S2). All 12 protein-coding genes of *A. hwangtsiyui* mt genome have a lower A+T percentage than those of *Trichobilharzia szidati* Neuhaus, 1952, *Calicophoron microbothrioides* Price & McIntosh, 1944, and some members of the Schistosomatidae Poche, 1907, but possess a higher A+T percentage than those of *Metagonimus yokogawai* Katsurada, 1912, and *Paragonimus westermani* Kerbert, 1878 (Suppl. material 3: Table S3) (Lee et al. 1987; Littlewood et al. 2006; Biswal et al. 2014; Semyanova et al. 2017; Oey et al. 2019). There is an obvious bias towards T over A (AT skew = -0.509), and G over C (GC skew = 0.425), and the coding strand is enriched with T and poor with A and especially C. For *A. hwangtsiyui*, the length of

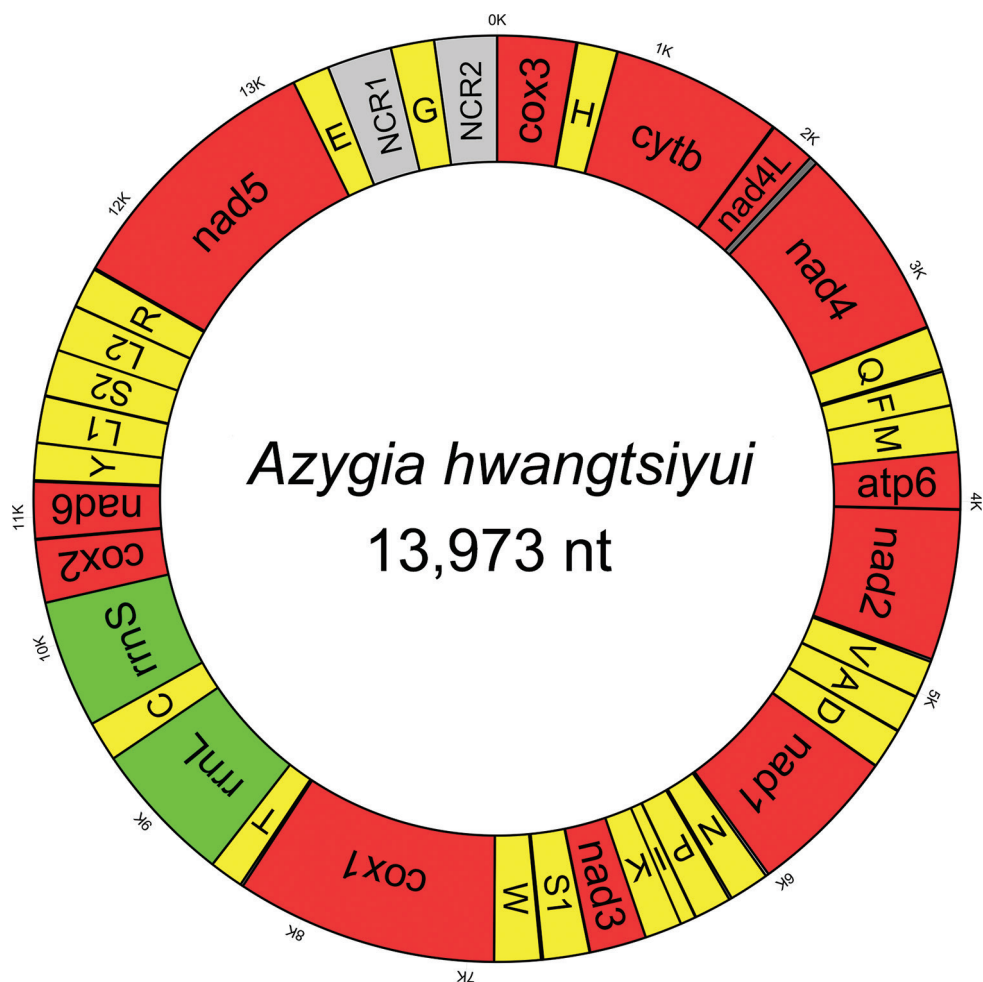


Figure 1. An annular diagram of the *Azygia hwangtsiyui* mitochondrial genome.

protein-coding genes was followed in the order: nad5 (1600 bp) > cox1 (1554 bp) > nad4 (1272 bp) > cytb (1110 bp) > nad1 (906 bp) > nad2 (861 bp) > cox3 (660 bp) > cox2 (582 bp) > atp6 (513 bp) > nad6 (444 bp) > nad3 (360 bp) > nad4L (264 bp). There are two non-coding regions (NCR1 and NCR2) in *A. hwangtsiyui* mitogenome, while the mt genome of *Paragonimus heterotremus* Chen et Hsia (1964), *C. complanatum*, *Fascioloides magna* (Bassi, 1875), and *T. szidati* have a single non-coding region (Chen et al. 2016; Ma et al. 2016; Semyanova et al. 2017; Qian et al. 2018). NCR1 and NCR2 of the *A. hwangtsiyui* mitogenome is partitioned into two parts by trnG, and accompanied by 70.5% and 57.6% A+T content, respectively. NCR1 and NCR2 have similar chemical base counts, 315 bp and 317 bp in size, respectively. While the NCR1 lacks distinguishing features and any tandem repeats, the NCR2 contains two typical tandem repeats, and each of tandem repeats sequence (120 bp) forms a hairpin-

Table 1. The organization of the mitochondrial genome of *Azygia hwangtsiyui*.

Gene	Position		Size	Intergenic nucleotides	Codon		Anti-codon	Strand
	From	To			Start	Stop		
cox3	1	660	660	–	ATG	TAG	–	H
trnH	666	729	64	+5	–	–	GTG	H
cytb	732	1841	1110	+2	ATG	TAG	–	H
nad4L	1848	2108	261	+6	ATG	TAG	–	H
nad4	2069	3340	1272	–40	ATG	TAG	–	H
trnQ	3345	3409	65	+4	–	–	TTG	H
trnF	3423	3488	66	+13	–	–	GAA	H
trnM	3490	3555	66	+1	–	–	CAT	H
atp6	3556	4068	513	–	ATG	TAG	–	H
nad2	4072	4932	861	+3	GTG	TAG	–	H
trnV	4946	5009	64	+13	–	–	TAC	H
trnA	5013	5076	64	+3	–	–	TGC	H
trnD	5081	5146	66	+4	–	–	GTC	H
nad1	5149	6054	906	+2	GTG	TAG	–	H
trnN	6070	6134	65	+15	–	–	GTT	H
trnP	6148	6212	65	+13	–	–	TGG	H
trnI	6216	6279	64	+3	–	–	GAT	H
trnK	6280	6348	69	–	–	–	CTT	H
nad3	6349	6708	360	–	ATG	TAA	–	H
trnS1	6712	6770	59	+3	–	–	GCT	H
trnW	6781	6842	62	+10	–	–	TCA	H
cox1	6843	8396	1554	–	TTG	TAG	–	H
trnT	8410	8474	65	+13	–	–	TGT	H
rrnL	8475	9449	975	–	–	–	–	H
trnC	9450	9506	57	–	–	–	GCA	H
rrnS	9507	10246	740	–	–	–	–	H
cox2	10247	10828	582	–	GTG	TAA	–	H
nad6	10834	11277	444	+5	GTG	TAG	–	H
trnY	11284	11352	69	+6	–	–	GTA	H
trnL1	11352	11416	65	–1	–	–	TAG	H
trnS2	11421	11490	70	+4	–	–	TGA	H
trnL2	11491	11555	65	–	–	–	TAA	H
trnR	11558	11617	60	+2	–	–	TCG	H
nad5	11626	13225	1600	+8	GTG	T	–	H
trnE	13226	13288	63	–	–	–	TTC	H
trnG	13604	13669	66	–	–	–	TCC	H

like secondary structure including a whole set of stems and loops (Suppl. material 4: Figure S1). Although tandem repeats are a segment of function-deficiency mitochondrial genome sequences, its hairpin-like secondary structures are widely perceived as regulating the replication and transcription of mitochondrial genome.

Codon usage, transfer RNAs, and ribosomal RNAs

For the *A. hwangtsiyui* mitogenome, codon ends in G or T were more continual than those ending in A or C. The most frequently used start codon in protein-coding genes was ATG (for six PCGs), secondly was GTG (for five PCGs), which resembles that of the most frequent extrapolated start codons for mitogenome protein-encoding genes

Table 2. Nucleotide contents of genes and the non-coding region within the mitochondrial genome of *Azygia hwangtsiyui*.

Regions	Size (bp)	T	C	A	G	AT (%)	GC (%)	AT skew	GC skew
atp6	513	50.5	11.3	12.9	25.3	63.4	36.6	−0.594	0.383
cox1	1554	44.0	13.0	15.9	27.1	59.9	40.1	−0.469	0.352
cox2	582	39.7	13.1	18.0	29.2	57.7	42.3	−0.375	0.382
cox3	660	44.8	11.4	15.9	27.9	60.7	39.3	−0.476	0.421
cytb	1110	44.4	13.5	15.8	26.3	60.2	39.8	−0.476	0.321
nad1	906	44.0	10.0	16.3	29.6	60.3	39.6	−0.459	0.493
nad2	861	46.2	11.3	11.8	30.7	58.0	42.0	−0.592	0.463
nad3	360	45.8	8.1	14.2	31.9	60.0	40.0	−0.528	0.597
nad4	1272	45.7	12.8	12.3	29.2	58.0	42.0	−0.577	0.391
nad4L	264	47.0	8.0	17.8	27.3	64.8	35.3	−0.450	0.548
nad5	1600	46.4	9.5	14.1	30.0	60.5	39.5	−0.535	0.519
nad6	444	45.9	11.9	14.2	27.9	60.1	39.8	−0.528	0.401
rrnL	975	37.4	14.1	22.5	26.1	59.9	40.2	−0.250	0.299
rrnS	740	36.6	14.5	21.6	27.3	58.2	41.8	−0.258	0.307
tRNAs	1349	35.3	14.5	21.7	28.5	57.0	43.0	−0.238	0.328
rRNAs	1715	37.1	14.2	22.1	26.6	59.2	40.8	−0.253	0.303
PCGs	10126	45.2	11.5	14.7	28.6	59.9	40.1	−0.509	0.425
Genome	13973	42.8	12.0	16.8	28.5	59.6	40.5	−0.437	0.408

of digenean species (Chen et al. 2016). The least-used start codon was TTG (only one PCGs). Likewise, the most-used terminal codon was TAG (for nine PCGs), followed by TAA (for two PCGs). Only one of 12 protein-coding sequence (nad5) was terminated with abbreviated T stop codon (Table 1). Although incomplete stop codons (T or TA) frequently occur in cestodes and nematodes, they were rarely presented in flukes other than *D. chinensis*, *Dicrocoelium dendriticum* (Rudolphi, 1819), and *Postharmostomum commutatum* (Dietz, 1858) (Liu et al. 2014b; Fu et al. 2019). The codon UUU (Phe, 10.17%), UUG (Leu, 8.17%), and GUU (Val, 7.19%) were the most frequently occurring codons in protein-coding genes. Leucine, valine, and phenylalanine are the most-used amino acids, with frequency of 15.96%, 13.38%, and 11.09%, respectively. The least-used codons were CGA (Arg, 0.06%) and GAC (Asp, 0.15%), and the least frequent utilized amino acid was glutamine (1.01%) (Suppl. material 5: Figure S2). As most of digenea mitogenome sequences, the mitogenome of *A. hwangtsiyui* possessed 22 commonly found tRNAs, with the exception of that of *P. westermani* Korean isolate (23 tRNAs), and *P. westermani* Indian isolate (24 tRNAs) (Biswal et al. 2014). In *A. hwangtsiyui*, tRNA-Gly (trnG) is located between NCR1 and NCR2 (Fig. 1). The size of ribosomal RNA genes (rrnL and rrnS) in mitochondrial DNA of *A. hwangtsiyui* are 975 bp and 740 bp, respectively (Table 2). The upstream and downstream of rrnL and rrnS are cascaded with trnT and cox2 genes, respectively, and are detached from each other by trnC, as in all reported platyhelminths to date (Littlewood et al. 2006, Le et al. 2016).

Gene arrangement

Comparative analysis of gene arrangement among 47 selected digenean taxa, two gene blocks (cox1-trnT-rrnL-trnC-rrnS-cox2-nad6 and cytb-nad4L-nad4-trnQ) are shared

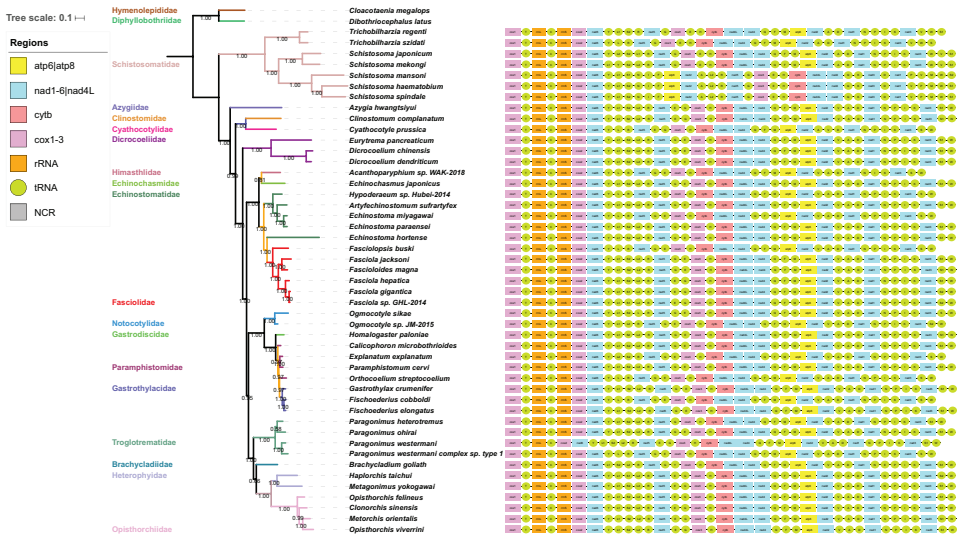


Figure 2. Phylogenetic relationships and gene arrangement of *Azygia huangtsiyui* with other selected digeneans based on translated mitochondrial proteins. The concatenated amino-acid sequence datasets of the 12 protein-coding genes were analyzed by Bayesian Inference (BI) and Maximum Likelihood (ML), utilizing *Cloacotaenia megalops* (NC_032295.1) and *Dibothriocephalus latus* (NC_008945.1) as the out-groups. Both ML and BI analyses constructed identical tree topologies.

by all selected taxa (Fig. 2). Disregarding *P. heterotremus* and members of the family Schistosomatidae and Fasciolidae Railliet, 1895, the gene order of the remaining digenean taxa is virtually identical with the exception of the translocation of trnE and trnG among the remaining members of selected digenean representatives in family level. Intriguingly, there is the translocation of trnE and trnG within different species of family Fasciolidae. The translocations of three tRNAs (trnS1, trnS2 and trnS) can be discovered even between taxa of the same subgroup. Gene order of the *Brachycladium goliath* (Van Beneden, 1858) mt genome (the only representative of family Brachycladiidae Faust, 1929) is nearly same as that of *P. westermani* (Troglotrematidae Ward, 1918) except for the relocations of trnY between trnG and cox3, and trnE to the position between trnN and trnP. The groups of Schistosomatidae show a massive gene reorganization of protein-coding genes and tRNAs compared with other sequenced digenean mitogenome, which is in accord with previous finding reported by Littlewood et al. (2006).

Mitogenome-derived phylogeny

To assess phylogenetic relationships among available flatworms, we utilized concatenated amino acid sequence dataset representing 12 protein-coding genes of *A. huangtsiyui*, 46 other digenean representatives, and two tapeworm species (*C. megalops* and *D. latus*) for analyzing molecular-based phylogeny. In this study, the topological structure is divided into two large clades: one consists of seven members of the family Schis-

tosomatidae; and the other clade comprises 40 members from 16 families including the family Azygiidae (*A. hwangtsiyui*) (Fig. 2). The topological structure shows that *A. hwangtsiyui* (Azygiidae) is identified as the most basal lineage of the Digenea, but separated from *C. complanatum* (Clinostomidae Lühe, 1901), and *Cyathocotyle prussica* Mühling, 1896 (Cyathocotylidae Poche, 1926). Phylogenetic analyses of all complete digenea mtDNAs confirmed taxonomic and previous phylogenetic assessments (Olson et al. 2003; Kostadinova and Pérez-del-Olmo 2014; Fu et al. 2019). The intricate structure and varying content of the family Azygiidae still awaits investigation of relationships based on a much wider taxon sampling and more mitogenome datasets.

Acknowledgments

This work was supported by the Earmarked Fund for China Agriculture Research System (CARS-45) and Important Research Project of Hunan Provincial Science and Technology Department (2016NK2176).

References

- Altschul SF, Gish W, Miller W, Myer EW, Lipman D (1990) Basic local alignment search tool (blast). *Journal of Molecular Biology* 215(3): 403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)
- Benson G (1999) Tandem repeats finder: a program to analyze DNA sequences. *Nucleic Acids Research* 27(2): 573–580. <https://doi.org/10.1093/nar/27.2.573>
- Bernt M, Donath A, Juhling F, Externbrink F, Florentz C, Fritzsche G, Putz J, Middendorf M, Stadler PF (2013) MITOS: improved de novo metazoan mitochondrial genome annotation. *Molecular Phylogenetics and Evolution* 69(2): 313–319. <https://doi.org/10.1016/j.ympev.2012.08.023>
- Besprozvannykh VV (2005) Life cycles of the trematode species *Azygia hwangtsiytii* and *A. robusta* (Azygiidae) in Primorsky Territory. *Parazitologiya* 39(4): 278–284.
- Biswal DK, Chatterjee A, Bhattacharya A, Tandon V (2014) The mitochondrial genome of *Paragonimus westermani* (Kerbert, 1878), the Indian isolate of the lung fluke representative of the family Paragonimidae (Trematoda). *PeerJ* 2: e484. <https://doi.org/10.7717/peerj.484>
- Chang QC, Liu GH, Gao JF, Zheng X, Zhang Y, Duan H, Yue DM, Fu X, Su X, Gao Y (2016) Sequencing and characterization of the complete mitochondrial genome from the pancreatic fluke *Eurytrema pancreaticum* (trematoda: Dicrocoeliidae). *Gene* 576(1): 160–165. <https://doi.org/10.1016/j.gene.2015.09.081>
- Chen CL (1973) An Illustrated Guide to the Fish Diseases and causative Pathogenic Fauna and Flora in the Hubei Province. Science Press, Beijing, 271 pp.
- Chen L, Feng Y, Chen HM, Wang LX, Feng HL, Yang X, Mughal MN, Fang R (2016) Complete mitochondrial genome analysis of *Clinostomum complanatum* and its comparison with selected digeneans. *Parasitology Research* 115(8): 3249–3256. <https://doi.org/10.1007/s00436-016-5102-6>

- Cheng YD (2011) Human and Animal Parasitic Helminthes in Hunan, China. Hunan Science and Technology Press, Changsha, 762 pp.
- Frolova EN, Shcherbina TV (1975) New species in genus *Azygia* Looss, 1899 (Trematoda, Azygiidae). *Parazitologiya* 9(6): 489–493.
- Fu YT, Jin YC, Liu GH (2019) The complete mitochondrial genome of the caecal fluke of poultry, *Postharmostomum commutatum*, as the first representative from the superfamily Brachylaimoidea. *Frontiers in Genetics* 10: 1–13. <https://doi.org/10.3389/fgene.2019.01037>
- Jadhav S, Anarse S, Borde SN (2011) Incidence of helminth parasites in fresh water fishes from Sina Kolegoan Dam, Dist. Osmanabad (MS) India. *Recent Research in Science and Technology* 3(3): 49–50.
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30(4): 772–780. <https://doi.org/10.1093/molbev/mst010>
- Keane TM, Creevey CJ, Pentony MM, Naughton TJ, McInerney JO (2006) Assessment of methods for amino acid matrix selection and their use on empirical data shows that ad hoc assumptions for choice of matrix are not justified. *BMC Evolutionary Biology* 6: 1–17. <https://doi.org/10.1186/1471-2148-6-29>
- Kiang TC (1965) Digenetic trematodes of the *Ophiocephalus argus*, *Cyprinus carpio*, and *Parasilurus asotus* in Chengtu I. *Journal of Sichuan University (Natural Science Edition)* 1(1): 115–122.
- Kostadinova A, Pérez-del-Olmo A (2014) The systematics of the Trematoda. *Advances in Experimental Medicine and Biology* 766: 21–44. https://doi.org/10.1007/978-1-4939-0915-5_2
- Laslett D, Canback B (2008) ARWEN: a program to detect tRNA genes in metazoan mitochondrial nucleotide sequences. *Bioinformatics* 24(2): 172–175. <https://doi.org/10.1093/bioinformatics/btm573>
- Le TH, Nguyen NTB, Nguyen KT, Doan HTT, Dung DT, Blair D (2016) A complete mitochondrial genome from *Echinochasmus japonicus* supports the elevation of Echinochasminae Odhner, 1910 to family rank (Trematoda: Platyhelminthes). *Infection Genetics and Evolution* 45: 369–377. <https://doi.org/10.1016/j.meegid.2016.09.024>
- Lee CC, Sheikumar AR, Jones A (1987) *Calicophoron microbothrioides* (Price and McIntosh, 1944) (Paramphistomidae: Paramphistominae) in Malaysian sambar deer (*Cervus unicolor*). *New Zealand Veterinary Journal* 35(11): 190–191. <https://doi.org/10.1080/00480169.1987.35442>
- Littlewood DTJ, Lockyer AE, Webster BL, Johnston DA, Le TH (2006) The complete mitochondrial genomes of *Schistosoma haematobium* and *Schistosoma spindale* and the evolutionary history of mitochondrial genome changes among parasitic flatworms. *Molecular Phylogenetics and Evolution* 39(2): 452–467. <https://doi.org/10.1016/j.ympev.2005.12.012>
- Liu GH, Gasser RB, Young ND, Song HQ, Ai L, Zhu XQ (2014a) Complete mitochondrial genomes of the ‘intermediate form’ of *Fasciola* and *Fasciola gigantica*, and their comparison with *F. hepatica*. *Parasites & Vectors* 7(1): 1–10. <https://doi.org/10.1186/1756-3305-7-150>
- Liu GH, Yan HB, Otranto D, Wang XY, Zhao GH, Jia WZ, Zhu XQ (2014b) *Dicrocoelium chinensis* and *Dicrocoelium dendriticum* (Trematoda: Digenea) are distinct lancet fluke species based on mitochondrial and nuclear ribosomal DNA sequences. *Molecular Phylogenetics and Evolution* 79: 325–331. <https://doi.org/10.1016/j.ympev.2014.07.002>

- Liu ZX, Zhang Y, Liu YT, Chang QC, Su X, Fu X, Yue DM, Gao Y, Wang CR (2016) Complete mitochondrial genome of *Echinostoma hortense* (Digenea: Echinostomatidae). Korean Journal of Parasitology 54(2): 173–179. <https://doi.org/10.3347/kjp.2016.54.2.173>
- Lockyer AE, Olson PD, Ostergaard P, Rollinson D, Johnston DA, Attwood SW, Southgate VR, Horak P, Snyder SD, Le TH, Agatsuma T, McManus DP, Carmichael AC, Naem S, Littlewood DTJ (2003) The phylogeny of the Schistosomatidae based on three genes with emphasis on the interrelationships of *Schistosoma* Weinland, 1858. Parasitology 126(3): 203–224. <https://doi.org/10.1017/S0031182002002792>
- Looss A (1899) Weitere Beiträge zur Kenntnis der trematoden-fauna Ägyptens, zugleich versuch einer natürlichen gliederung des genus *Distomum* Retzius. Zoologische Jahrbuecher Abteilung fuer Systematik Oekologie und Geographie der Tiere 12: 521–784. <https://doi.org/10.5962/bhl.part.2037>
- Ma CL (1958) Worm parasites from Taihu fishes: digenetic trematodes IV. *Azygia* (Azygiidae). Journal of East China Normal University (Natural Science) 1: 26–28.
- Ma J, He JJ, Liu GH, Leontovyc R, Kasny M, Zhu XQ (2016) Complete mitochondrial genome of the giant liver fluke *Fascioloides magna* (Digenea: Fasciolidae) and its comparison with selected trematodes. Parasites & Vectors 9: 1–7. <https://doi.org/10.1186/s13071-016-1699-7>
- Manter HW (1926) Some North American fish trematodes. Illinois Biology Monographs 10: 1–138. <https://doi.org/10.5962/bhl.title.50240>
- Marcogliese DJ, Cone DK (1996) On the distribution and abundance of eel parasites in Nova Scotia: influence of pH. Journal of Parasitology 82(3): 389–399. <https://doi.org/10.2307/3284074>
- Moravec F, Sey O (1989) Some trematodes of freshwater fishes from North Vietnam with a list of recorded endohelminths by fish hosts. Folia Parasitologica 36: 243–262.
- Nagasawa K, Katahira H (2017) A revised and updated checklist of the parasites of eels (*Anguilla* spp.) (Anguilliformes: Anguillidae) in Japan (1915–2017). Biosphere Science 56: 33–69.
- Oey H, Zakrzewski M, Narain K, Devi KR, Agatsuma T, Nawaratna S, Gobert GN, Jones MK, Ragan MA, McManus DP, Krause L (2019) Whole-genome sequence of the oriental lung fluke *Paragonimus westermani*. Gigascience 8: 1–8. <https://doi.org/10.1093/gigascience/giy146>
- Olson PD, Cribb TH, Tkach VV, Bray RA, Littlewood DTJ (2003) Phylogeny and classification of the Digenea (Platyhelminthes: Trematoda). International Journal for Parasitology 33(7): 733–755. [https://doi.org/10.1016/S0020-7519\(03\)00049-3](https://doi.org/10.1016/S0020-7519(03)00049-3)
- Pallewad S, Nanware SS, Bhure DB (2015) Incidence of infection of trematode genus *Azygia* Looss, 1899 parasitic in freshwater fish *Channa punctatus*. Biolife 3: 187–191.
- Qian L, Zhou P, Li WL, Wang HP, Miao TJ, Hu LG (2018) Characterization of the complete mitochondrial genome of the lung fluke, *Paragonimus heterotremus*. Mitochondrial DNA Part B-Resources 3(2): 560–561. <https://doi.org/10.1080/23802359.2018.1462119>
- Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(2): 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Semyenova S, Chrisanfova G, Mozharovskaya L, Guliaev A, Ryskov A (2017) The complete mitochondrial genome of the causative agent of the human cercarial dermatitis, the visceral

- bird schistosome species *Trichobilharzia szidati* (platyhelminthes: Trematoda: Schistosomatidae). Mitochondrial DNA Part B-Resources 2(2): 469–470. <https://doi.org/10.1080/23802359.2017.1347833>
- Sillman EI (1953) The life history of *Azygia longa* (Leidy, 1851) (Trematoda: Azygiidae). Journal of Parasitology 39: 1–15.
- Sillman EI (1962) The life history of *Azygia longa* (Leidy, 1851) (Trematoda: Digenea), and notes on *A. acuminata* Goldberger 1911. Transactions of the American Microscopical Society 81(1): 43–65. <https://doi.org/10.2307/3223942>
- Szidat L (1932) Über cysticerke Riesen cercarien, insbesondere *Cercaria mirabilis* M. Braun and *Cercaria splendens* n. sp., und ihre Entwicklung im Magen von Raubfischen zu Trematoden der Gattung *Azygia* Looss. Zeitschrift Für Parasitenkunde 4(3): 477–505. <https://doi.org/10.1007/BF02119996>
- Tang CC, Tang CT (1964) Studies on the life history of *Azygia anguillae* Ozaki 1924 and *Azygia hwanngsiyüi* Tsin 1933, with a discussion on the systematics of the genus. Acta Parasitologica Sinica 1: 137–152.
- Tsin SM (1933) A new species of trematode, *Azygia hwanngsiyüi*, from *Ophiocephalus argus*. Journal of Science National University of Shantung 1: 104–116.
- Tubanguí MA (1928) Trematode parasites of Philippine vertebrates. Philippine Journal of Science 36: 351–371.
- Van Cleave HJ, Mueller JF (1934) Parasites of Oneida Lake fishes, part III: A biological and ecological survey of the worm parasites. part IV: Additional notes on parasites of Oneida Lake fishes, including descriptions of new species. Roosevelt Wild Life Annals 3: 161–373. <https://doi.org/10.2307/3222099>
- Wang XY (1985) Catalogue of helminthes of fishes in Poyang Lake. Jiangxi Science 3(1): 34–48.
- Wickham H (2016) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York, 260 pp. <https://doi.org/10.1002/wics.147>
- Womble MR, Orélis-Ribeiro R, Bullard SAJPI (2016) New species of *Proterometra* (Digenea: Azygiidae) and its life cycle in the Chickasawhay River, Mississippi, USA, with supplemental observations of *Proterometra autraini*. Parasitology International 65(1): 31–43. <https://doi.org/10.1016/j.parint.2015.09.005>
- Yang X, Gasser RB, Koehler AV, Wang LX, Zhu KX, Chen L, Feng HL, Hu M, Fang R (2015) Mitochondrial genome of *Hypoderaeum conoideum* – comparison with selected trematodes. Parasites & Vectors 8: 1–8. <https://doi.org/10.1186/s13071-015-0720-x>
- Zhang D, Gao FL, Jakovlić I, Zou H, Zhang J, Li WX, Wang GT (2020) PhyloSuite: an integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. Molecular Ecology Resources 20(1): 348–355. <https://doi.org/10.1111/1755-0998.13096>
- Zhang JY, Qiu ZZ, Ding X J (1999) Parasites and Parasitic Diseases of Fishes. Science Press, Beijing, 735 pp.
- Zmejev GJ (1936) Les trematodes et les cestodes des poissons de l'Amour. Parazitologicheskii Sbornik 6: 405–436.
- Zuker M (2003) Mfold web server for nucleic acid folding and hybridization prediction. Nucleic Acids Research 31(13): 3406–3415. <https://doi.org/10.1093/nar/gkg595>

Supplementary material 1

Table S1

Authors: Yuan-An Wu, Jin-Wei Gao, Xiao-Fei Cheng, Min Xie, Xi-Ping Yuan, Dong Liu, Rui Song

Data type: molecular data

Explanation note: Primers for amplification and sequencing mitochondrial genome of *Azygia hwangtsiyui*.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/zookeys.945.49681.suppl1>

Supplementary material 2

Table S2

Authors: Yuan-An Wu, Jin-Wei Gao, Xiao-Fei Cheng, Min Xie, Xi-Ping Yuan, Dong Liu, Rui Song

Data type: molecular data

Explanation note: Information of the Digenea and the outgroups for which complete mitogenomes are available in GenBank.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/zookeys.945.49681.suppl2>

Supplementary material 3

Table S3

Authors: Yuan-An Wu, Jin-Wei Gao, Xiao-Fei Cheng, Min Xie, Xi-Ping Yuan, Dong Liu, Rui Song

Data type: molecular data

Explanation note: A+T content (%) for 12 protein-coding genes in the available 49 *Platyhelminthes* mitogenomes.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/zookeys.945.49681.suppl3>

Supplementary material 4

Figure S1

Authors: Yuan-An Wu, Jin-Wei Gao, Xiao-Fei Cheng, Min Xie, Xi-Ping Yuan, Dong Liu, Rui Song

Data type: molecular data

Explanation note: Secondary structure of tandem repeats in non-coding region 2 (NCR2) of *Azygia hwangtsiyui* mitochondrial genome.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/zookeys.945.49681.suppl4>

Supplementary material 5

Figure S2

Authors: Yuan-An Wu, Jin-Wei Gao, Xiao-Fei Cheng, Min Xie, Xi-Ping Yuan, Dong Liu, Rui Song

Data type: molecular data

Explanation note: Relative synonymous codon usage (RSCU) of *Azygia hwangtsiyui* mitochondrial genome.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/zookeys.945.49681.suppl5>

An annotated checklist and integrative biodiversity discovery of barnacles (Crustacea, Cirripedia) from the Moluccas, East Indonesia

Pipit Pitriana^{1,2,6}, Luis Valente^{1,3}, Thomas von Rintelen¹, Diana S. Jones⁴,
Romanus E. Prabowo⁵, Kristina von Rintelen¹

1 Museum für Naturkunde– Leibniz Institute for Evolution and Biodiversity Science, Invalidenstrasse 43, 10115 Berlin, Germany **2** Research Centre for Deep-sea, Indonesian Institute of Science (LIPI), Jl. Y. Syarmanual, Poka, Tlk. Ambon, Kota Ambon, Maluku, Indonesia **3** Naturalis Biodiversity Center, Understanding Evolution Group, Postbus 9517, 2300 RA Leiden, the Netherlands **4** The Western Australian Museum, 49 Kew Street, Welshpool WA 6106, Locked Bag 49, Welshpool DC WA 6986, Australia **5** Faculty of Biology, Universitas Jenderal Soedirman, Purwokerto, 53122, Indonesia **6** Institute of Geological Sciences, Freie Universität Berlin, Malteserstrasse 74-100 Building C and D, 12249 Berlin, Germany

Corresponding author: Pipit Pitriana (pipit.pitriana@mf.n.berlin)

Academic editor: Benny K.K. Chan | Received 13 August 2019 | Accepted 7 April 2020 | Published 3 July 2020

<http://zoobank.org/A91BFE95-C953-4B86-8710-74871CDFAC94>

Citation: Pitriana P, Valente L, von Rintelen T, Jones DS, Prabowo RE, von Rintelen K (2020) An annotated checklist and integrative biodiversity discovery of barnacles (Crustacea, Cirripedia) from the Moluccas, East Indonesia. ZooKeys 945: 17–83. <https://doi.org/10.3897/zookeys.945.39044>

Abstract

To contribute to the taxonomic knowledge of barnacles in this understudied area, the first checklist of barnacles from the Moluccas is presented, including additional information on morphology, distribution, and substrate as well as molecular data. The species of barnacles from the Moluccas have been determined using morphological analysis and DNA sequences. During 19 field trips conducted between January 2016 and September 2017, 1,513 specimens of 24 species of intertidal and one species of deep-sea barnacles were collected from 51 localities from the islands. Morphological and molecular analysis of the collected material detected members of three families of stalked barnacles and four families of acorn barnacles. In addition to sampling in the field, we also surveyed the literature on barnacles from the Moluccas. In total, our checklist comprises 97 species from the Moluccas including 23 new records, two of them yet to be described species. Results suggest that the Moluccas have a much higher diversity of barnacles than previously known, for example, from the reports of *Challenger* and *Siboga* expeditions. For further work,

routine application of molecular systematics could aid the detection of cryptic species, while increased sampling of more islands and a taxonomic revision of several groups would likely lead to an even higher number of species than currently known.

Keywords

Acorn barnacle, coral triangle, Indonesian biodiversity, new records, stalked barnacles, taxonomy

Introduction

Barnacles (Crustacea, Cirripedia) are an ancient, species-rich and abundant group of crustaceans with about 1,400 extant species (Newman and Abbott 1980). They have a worldwide distribution in tropical and temperate marine environments and at different depths and are adjusted to various lifestyles, from parasites of decapod crustaceans to free-living groups. Most cirripeds usually have two free-swimming planktonic larval stages consisting of distinctive nauplii and a unique non-feeding cyprid (Darwin 1852, 1854; Pochai et al. 2017). In the most abundant group, the Thoracica, adult specimens are permanently attached to various types of substrates, other living organisms (e.g., mangroves, corals, molluscs, other barnacles, sponges), rocks, and man-made materials such as cargo ships and concrete walls (Newman and Abbott 1980; Power et al. 2010). The Thoracica comprise the orders Cyprilepadiformes, Ibliformes, Lepadiformes, Scalpelliformes, and Sessilia (Buckeridge and Newman 2006).

The Indonesian Moluccas (or Spice Islands; Fig. 1) are part of the Coral Triangle, one of the most complex biogeographical and oceanographic areas on Earth. Although part of the global epicentre of marine biodiversity, knowledge of the barnacle fauna of the Moluccas is relatively sparse. The exploration of the natural history of the Moluccas dates back to the 17th century, starting with Georg Everhard Rumphius, and later, for example, Alfred Russel Wallace (Strack 1993; Lamoureux 1990). In the 19th and 20th centuries, there were approximately fifty scientific expeditions passing through or specifically targeting the area, such as the British *Challenger* (1872–1876), the Dutch *Siboga* (1899–1900) and the *Snellius* (1929–1930) expeditions (Lamoureux 1990). The most recent being the French *Karubar* expedition in 1991 (Crosnier et al. 1997).

Rumphius provided the first record of a barnacle (the stalked *Mitella* Oken, 1815 (= *Capitulum* Gray, 1825) found on a rock near the beach at Ambon Island) in his posthumously published ‘Amboinsche Rariteitkamer’ (Rumphius 1705). Indonesian and Moluccan barnacles were also studied by Darwin (1854), who assigned them to one of four geographical ‘provinces’, the third being the East Indian Archipelago. Moluccan barnacles have not been studied since Buckeridge (1994) examined some material from the *Karubar* expedition.

To contribute to the taxonomic knowledge of this understudied area, we herein present the first checklist of barnacles from the Moluccas, including additional information on morphology and molecular data, as well as distribution and substrate.

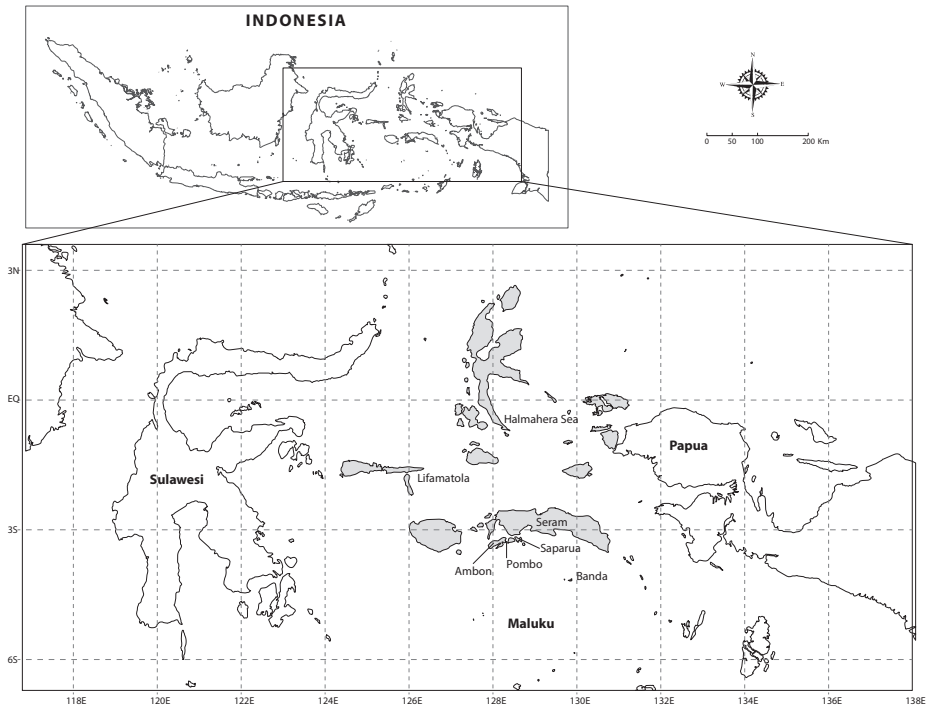


Figure 1. Map of the Moluccan Islands (Maluku in grey) in eastern Indonesia. Map modified from Short-house (2010).

Material and methods

Sampling

Specimens examined in this study were collected by the first author during 19 field trips between January 2016 and September 2017 to the intertidal zones of the Moluccan islands of Ambon, Saparua, Seram, Pombo, and Banda Neira (Fig. 1, Table 1, Suppl. material 1: Table S1). Deep-water barnacles (Table 1) from the Lifamatola Passage (250 m) and Halmahera Sea (250 m) were provided by Nurul Fitriya. Additional material used for the molecular analyses was collected from the island of Sulawesi in September to October 2017 (Suppl. material 1: Table S1). In total, 159 lots containing 1,513 specimens were collected from 51 Moluccan localities.

Barnacle specimens firmly attached to hard substrate (rocks, stone, concrete) were sampled using a chisel and hammer whereas those attached to softer substrate using a craft knife. Specimens were fixed and stored in 96% ethanol and transferred into 75% ethanol for long-term preservation.

Table 1. Annotated checklist tabulation of barnacle species from the Moluccas, Eastern Indonesia.

ORDER	Family	Genus / Species		Locality	Substrate	References
Suborder	Subfamily	No.	Name			
LEPADIFORMES Heteralepdomorpha	Heteralepadidae	1	<i>Heteralepas japonica</i> (Aurivillius, 1892)	Lifamatola Sea and Halmahera Sea	Deep-water cable to mooring	This study
		2	<i>Heteralepas ovalis</i> (Hoek, 1907)	West from Kei Islands		Hoek (1913)
		3	<i>Heteralepas tenuis</i> (Hoek, 1907)	South of Seram		Hoek (1913)
Lepadomorpha	Oxynaspididae	4	<i>Oxynaspis connectens</i> Broch, 1931	Kei Islands		Jones and Hosie (2016)
	Poecilasmatidae	5	<i>Glyptelasma carinatum</i> (Hoek, 1883)	Seram Sea		Hoek (1913)
		6	<i>Megalasma striatum</i> (Hoek, 1883)	East of Kei Islands		Hoek (1913)
		7	<i>Octolasmis orthogonia</i> Darwin, 1852	Tual anchorage		Hoek (1913)
		8	<i>Octolasmis weberi</i> (Hoek, 1907)	Kei Islands, Banda Sea		Hoek (1913), Jones and Hosie (2016)
		9	<i>Poecilasma kaempferi</i> Darwin, 1852	Banda Sea		Jones and Hosie (2016)
		10	<i>Temnaspis fissum</i> (Darwin, 1852)	Ternate anchorage		Hoek (1913)
	Lepadidae	11	<i>Conchoderma virgatum</i> Spengler, 1789	Banda Sea		Jones et al. (2001)
		12	<i>Dosima fascicularis</i> (Ellis & Solander, 1786)	Ambon	Floating in water at the beach	This study
		13	<i>Lepas anserifera</i> Linnaeus, 1767	Ambon, Saparua, Seram, Pombo	Mangrove, stone ship chart and ship wall, port pole, shell of <i>Megabalanus zebra</i>	This study
		14	<i>Lepas pectinata</i> Spengler, 1793	Banda Sea		Jones and Hosie (2016)
SCALPELLIFORMES	Calanticidae	15	<i>Calantica pollicipedoides</i> (Hoek, 1907)	East of Kei Islands		Hoek (1913)
		16	<i>Euscalpellum rostratum</i> (Darwin, 1852)	Kei Islands		Jones and Hosie (2016)
	Pollicipedidae	17	<i>Capirulum mitella</i> (Linnaeus, 1758)	Ambon, Saparua	Rocks, stone, wall of fortress, port pole and concrete wall	Rumphius (1705), this study
	Scalpellidae	18	<i>Compresoscalpellum inflatum</i> (Hoek, 1907)	West of Aru Island		Hoek (1913)
	Scalpellinae	19	<i>Scalpellum fissum</i> Hoek, 1913	West of Halmahera		Hoek (1913)
		20	<i>Scalpellum stearnsi</i> Pilsbry, 1890	Near Kei Islands		Hoek (1913)
		21	<i>Amigdoscalpellum vitreum</i> (Hoek, 1883)	South of Seram, South of Ambon		Hoek (1913)
	Arcoscalpellinae	22	<i>Anguloscalpellum pedunculatum</i> (Hoek, 1883)	Kei Islands		Hoek (1913)
		23	<i>Arcoscalpellum ciliatum</i> (Hoek, 1907)	South of Ambon		Hoek (1913)
		24	<i>Arcoscalpellum discolor</i> (Hoek, 1907)	Banda Sea		Hoek (1913)
		25	<i>Arcoscalpellum sculptum</i> (Hoek, 1907)	Banda Sea		Hoek (1913)
		26	<i>Arcoscalpellum sociabile</i> (Annandale, 1905)	Banda Sea		Jones et al. (2001)

ORDER	Family	Genus / Species		Locality	Substrate	References
Suborder	Subfamily	No.	Name			
SCALPELLIFORMES	Arcoscalpellinae	27	<i>Planoscalpellum hexagonum</i> (Hoek, 1907)	Banda Sea		Hoek (1913)
		28	<i>Teloscalpellum imbricatum</i> (Hoek, 1907)	Near Kei Islands		Hoek (1913)
		29	<i>Trianguloscalpellum balanoides</i> (Hoek, 1883)	Banda Sea		Hoek (1913), Shalaeva and Boxshall (2014)
		30	<i>Trianguloscalpellum diota</i> (Hoek, 1907)	Near Kei Islands		Hoek (1913)
		31	<i>Trianguloscalpellum hamulus</i> (Hoek, 1907)	Kei Islands		Hoek (1913)
		32	<i>Trianguloscalpellum hirsutum</i> (Hoek, 1883)	Moluccan Sea		Hoek (1913), Shalaeva and Boxshall (2014)
		33	<i>Trianguloscalpellum indicum</i> (Hoek, 1883)	Banda Sea		Hoek (1913), Shalaeva and Boxshall (2014)
		34	<i>Trianguloscalpellum moluccanum</i> (Hoek, 1883)	Banda Sea; West of Aru Island		Hoek (1913), Shalaeva and Boxshall (2014)
		35	<i>Trianguloscalpellum sessile</i> (Hoek, 1907)	Seram Sea		Hoek (1913)
		36	<i>Verum candidum</i> (Hoek, 1907)	Near Kei Islands		Hoek (1913), Jones and Hosie (2016)
	Meroscalpellinae	37	<i>Annandaleum japonicum</i> (Hoek, 1883)	Aru Island		Hoek (1913)
		38	<i>Annandaleum laccadivicum</i> (Annandale, 1906)	Kei Islands		Hoek (1913)
SESSILIA Verrucomorpha	Verrucidae	39	<i>Altiverruca navicula</i> (Hoek, 1913)	Between Seram and New Guinea, Kei Islands, Tanimbar Island		Hoek (1913), Buckeridge (1994)
		40	<i>Brochiverruca dens</i> (Broch, 1932)	Tanimbar Island		Buckeridge (1994)
		41	<i>Cristallinaverruca cristallina</i> (Gruvel, 1907)	Banda Sea		Jones and Hosie (2016)
		42	<i>Metaverruca recta</i> (Aurivillius, 1898)	Kei Islands		Hoek (1913)
		43	<i>Newmaniverruca albatrossiana</i> (Pilsbry, 1912)	East of Kei Islands, Tanimbar Island		Hoek (1913), Buckeridge (1994)
		44	<i>Rostratoverrucia intexta</i> (Pilsbry, 1912)	Kei Islands, Tanimbar Island		Hoek (1913), Buckeridge (1994)
		45	<i>Rostratoverrucia kruegeri</i> (Broch, 1922)	Kei Islands, Tanimbar Island		Jones et al. (2001), Buckeridge (1994)
		46	<i>Verruca capsula</i> Hoek, 1913	Between Seram and New Guinea		Hoek (1913)
Balanomorpha	Pachylasmatidae	47	<i>Hexelasma arafurae</i> Hoek, 1913	Kei Islands, Arafura Sea		Hoek (1913), Jones and Hosie (2016)
	Hexelasmatinae	48	<i>Hexelasma velutinum</i> Hoek, 1913	Kei Islands		Jones et al. (2001)
	Pachylasmatinae	49	<i>Pachylasma integrirostrum</i> Broch, 1931	Ambon		Jones et al. (2001)

ORDER	Family	Genus / Species		Locality	Substrate	References
Suborder	Subfamily	No.	Name			
Balanomorpha	Pachylamatinae	50	<i>Pseudoctomeris sulcata</i> (Nilsson-Cantell, 1932)	Ambon	Rocks, shell of <i>Tetracrita squamosa</i>	This study
	Chthamalidae	51	<i>Hexechamaesipho pilsbryi</i> (Hiro, 1936)	Ambon	Rocks	This study
	Notochthamalinae	52	<i>Nesochthamalus intertextus</i> (Darwin, 1854)	Ambon	Stone	This study
	Euraphiinae	53	<i>Euraphia hembeli</i> Conrad, 1837	Ambon	Rocks	This study
		54	<i>Microeuraphia withersi</i> (Pilsbry, 1916)	Kei Islands		Jones and Hosie (2016)
		55	<i>Microeuraphia</i> sp.	Seram Island	Stone, concrete wall at port	This study
	Chthamalinae	56	<i>Chthamalus malayensis</i> Pilsbry, 1916	Arafura Sea		Jones et al. (2001)
		57	<i>Chthamalus moro</i> Pilsbry, 1916	Ambon, Saparua, Seram, Pombo	Mangrove, stone, port pole, mollusc shell, shells of <i>Tetracrita squamosa</i> , <i>Tesseropora rosea</i> , and <i>Capitulum mitella</i>	This study
	Tetracritidae	58	<i>Tetracritella</i> (<i>Eotetracritella</i>) <i>costata</i> (Darwin, 1854)	Banda		Hoek (1913)
	Tetracritellinae	59	<i>Tetracritella divisa</i> (Nilsson-Cantell, 1921)	Ambon	Concrete wall at port	This study
		60	<i>Tetracritella karandei</i> Ross, 1971	Ambon	Stone, shells of <i>Capitulum mitella</i> and <i>Euraphia hembeli</i>	This study
	Tetracritinae	61	<i>Tesseropora rosea</i> (Krauss, 1848)	Ambon, Saparua	Stone, mollusc shell	This study
		62	<i>Tetracrita kuroshioensis</i> Chan, Tsang & Chu, 2007	Ambon, Saparua	Rocks, concrete wall at port	This study
		63	<i>Tetracrita squamosa</i> (Bruguère, 1789)	Ambon, Saparua	Stone, rocks, concrete bridge and wall at port, shipyard.	This study
	Newmanellinae	64	<i>Yamaguchiella coerulescens</i> (Spengler, 1790)	Banda, Kei Islands, Ambon, Saparua	Stone	Hoek (1913), Jones and Hosie (2016), This study
		65	<i>Neonrosella vitiata</i> (Darwin, 1854)	Ambon, Banda Neira, Saparua	Port pole, reef, stone	This study
		66	<i>Newmanella spinosus</i> Chan & Cheang, 2016	Ambon	Stone, reef surface	This study
	Archaeobalanidae	67	<i>Armatobalanus allium</i> (Darwin, 1854)	Banda Sea		Jones and Hosie (2016)
	Archaeobalaninae	68	<i>Armatobalanus cepa</i> (Darwin, 1854)	Aru Island		Jones and Hosie (2016)
		69	<i>Armatobalanus quadrivittatus</i> (Darwin, 1854)	Banda Sea		Jones and Hosie (2016)
		70	<i>Conopea dentifer</i> (Broch, 1922)	Kei Islands		Jones et al. (2001)
		71	<i>Conopea navicula</i> (Darwin, 1854)	Near Damar Island (South of Halmahera)		Hoek (1913)

ORDER	Family	Genus / Species		Locality	Substrate	References
Suborder	Subfamily	No.	Name			
Balanomorpha	Archaeobalanidae	72	<i>Membranobalanus cuneiformis</i> (Hiro, 1936)	Arafura Sea		Jones and Hosie (2016)
	Archaeobalaninae	73	<i>Solidobalanus auricoma</i> (Hoek, 1913)	Banda Sea, Ternate, Kei Islands		Hoek (1913), Jones et al. (2001)
		74	<i>Solidobalanus socialis</i> (Hoek, 1883)	Arafura Sea, Ternate, Kei Islands		Hoek (1913)
		75	<i>Sriatobalanus amaryllis</i> (Darwin, 1854)	Arafura Sea		Jones and Hosie (2016)
		76	<i>Sriatobalanus kruegeri</i> (Pilsbry, 1916)	Moluccas		Jones and Hosie (2016)
		77	<i>Sriatobalanus tenuis</i> (Hoek, 1883)	Kei Islands, Arafura Sea		Hoek (1913), Jones and Hosie (2016)
	Bryozobiinae	78	<i>Multatria terebratus</i> (Darwin, 1854)	Kei Islands		Hoek (1913), Jones and Hosie (2016)
		79	<i>Eoatria quinquevittatus</i> (Hoek, 1913)	Banda Sea, Ambon Island		Jones and Hosie (2016)
	Pyrgomatidae	80	<i>Cantellius euspinulosum</i> (Broch, 1931)	Ambon		Jones and Hosie (2016)
	Pyrgomatinae	81	<i>Cantellius gregarious</i> (Sowerby, 1823)	Banda Sea		Jones et al. (2001)
		82	<i>Cantellius pallidus</i> (Broch, 1931)	Banda Sea		Jones et al. (2001)
		83	<i>Galkinius indica</i> (Annandale, 1924)	Kei Islands		Jones and Hosie (2016)
		84	<i>Hoekia fornix</i> Ross & Newman, 1995	Moluccas		Jones and Hosie (2016)
		85	<i>Nobia grandis</i> Sowerby, 1839	Kei Islands		Jones et al. (2001)
		86	<i>Pyrgoma kuri</i> Hoek, 1913	Kei Islands		Hoek (1913)
	Balanidae	87	<i>Amphibalanus amphitrite</i> (Darwin, 1854)	Ambon, Saparua	Stone, mollusc shell, capitulum of <i>Lepas anserifera</i>	This study
	Amphibalaninae	88	<i>Amphibalanus reticulatus</i> (Utinomi, 1967)	Ambon	Stone, concrete wall at port	This study
		89	<i>Amphibalanus variegatus</i> (Darwin, 1854)	Ambon, Saparua	Stone, plastic	This study
		90	<i>Amphibalanus zhujiangensis</i> (Ren, 1989)	Ambon, Saparua, Seram	Stone, capitulum of <i>Lepas anserifera</i>	This study
		91	<i>Amphibalanus</i> sp.	Ambon, Seram	Stone, concrete wall at port	This study
	Balaninae	92	<i>Balanus arcuatus</i> Hoek, 1913	Banda		Hoek (1913)
		93	<i>Balanus hystrix</i> Hoek, 1913	Ambon		Hoek (1913)
		94	<i>Balanus longirostrum</i> Hoek, 1913	Bacan		Hoek (1913)
	Megabalaninae	95	<i>Megabalanus occator</i> (Darwin, 1854)	Near Obilatu Island	Coral	Kolosváry (1950)
		96	<i>Megabalanus tintinnabulum</i> (Linnaeus, 1758)	Ambon, Saparua	Concrete bridge at port, stone, reef surface	This study
		97	<i>Megabalanus zebra</i> (Darwin, 1854)	Ambon	Stone, capitulum of <i>Lepas anserifera</i>	This study

Morphological analysis

For detailed morphological analyses, all samples were studied at the Museum für Naturkunde in Berlin (**ZMB**), Germany. All specimens are deposited at the Museum Zoologicum Bogoriense (**MZB**; Suppl. material 1: Table S1), Research Center for Biology, Indonesian Institute of Sciences- **LIPI**, Indonesia. Barnacle species attached to other barnacle species were not separated (except for specimens dissected and measured) but were kept within the same glass container, enabling further morphological studies of different species attached to each other, e.g., with MicroCT scans.

Specimens were studied by the first author. All species were determined based on external shell morphology, including the pattern of the parietes, opercular plates, mouth parts, and arthropodal characters, as described by Darwin (1852, 1854), Hoek (1907, 1913), Southward and Newman (2003), Chan et al. (2007), Pérez-Losada (2008, 2014), Chan et al. (2009a), Tsang et al. (2015), and Chan et al. (2017).

Hard body parts (parietes and opercular plates) were separated from soft body parts using a scalpel. Shell plates were separated and cleaned with a bleach solution to remove any organic material, rinsed with fresh water, dried and observed under a stereo microscope (Leica M125) and photographed with a digital camera (Leica Microsystems M205C and Leica Z16 APo-A) (Fig. 2).

The mouthparts (labrum, palps, maxilla, maxillule, and mandible) were dissected using a scalpel, each was mounted on a glass slide and examined under a light microscope (Axioskop 20). The cirri were separated into couples of cirri I–VI and the penis, before being mounted on glass slides. The anatomy of these soft body parts was studied using a light microscope.

All measurements were made using digital callipers (accurate to 0.1 mm; Suppl. material 1: Tables S2–S26) generally following the method described in Beşir and Çınar (2012): basal length of shell, basal width, orifice length, orifice width, and carinal height. For stalked cirripeds, measurement of total height, capitular height, diameter of the base of the capitulum, carina and scutum distance, scutal length, scutal width, tergal length and tergal width were taken following the method described by Igić (2007). For deep-water barnacles, capitular height, capitular width, peduncular length, orifice height, number of crests, capitular thickness, and peduncular width were taken following the method described by Chan et al. (2009b).

Molecular phylogenetic analyses

We performed molecular phylogenetic analyses including new DNA sequences from our new samples from the Moluccas in combination with sequences of multiple barnacle specimens retrieved from GenBank. Our aim with the molecular analyses was not to provide a robust phylogeny of barnacles or to develop DNA barcodes for Moluccan barnacle taxa. Instead, our goal was to confirm the molecular taxonomic identity of the barnacles from the Moluccas with the published sequences in the GenBank, to examine whether they cluster near to congeneric or conspecific accessions. With this

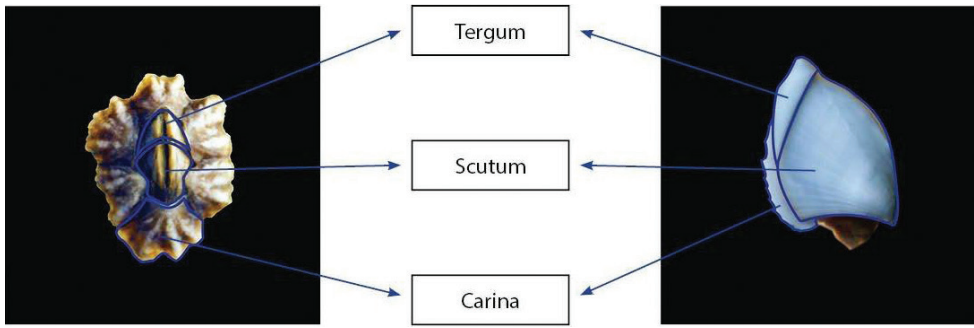


Figure 2. External morphology of barnacles showing the two parts of the operculum (tergum and scutum) and carina (without scale).

exercise we aimed to gain insights into the taxonomic positions of Moluccan barnacles in addition to those we may gain from morphology.

Genomic DNA was extracted from the adductor muscle tissue using CTab isolation buffer following the method of Doyle and Doyle (1987), as described at <http://www.geocities.com/CapeCanaveral/8431/CTab.html>. Tissue was ground and placed in 200 μ l CTab buffer and 5 μ l Proteinase K, homogenized by shaking, and incubated at 56 °C overnight. DNA was extracted from the lysate using a Qiagen BioSprint 96 using the manufacturer's protocol. The purified DNA was stored at –20 °C until required, and dilutions of 1 to 10 were used for the polymerase chain reaction (PCR).

We sequenced two gene fragments: cytochrome oxidase subunit I (COI), a rapidly evolving gene from the mitochondrial genome; and the 18S ribosomal RNA gene (18S), a slowly evolving and generally highly conserved gene from the ribosome. We chose these markers because of their contrasting evolutionary rates, but also because they have been widely used in barnacle phylogenetic analyses (Pérez-Losada et al. 2008, 2014; Tsang et al. 2014, 2015).

COI was sequenced using primers LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') (Folmer et al. 1994), and 18S using primers ai (5'-CCT GAG AAA CGG CTA CCA CAT C-3') and 7R (5'-GCA TCA CAG ACC TGT TAT TGC-3') (Whiting 2002). PCR was performed in 25 μ l volumes containing 17.8 μ l ddH₂O, 2.5 μ l 10 $\times$ Puffer, 1 μ l Mg (25mM), 0.5 μ l dNTP Mix, 0.5 μ l of each primer, and 0.2 μ l Taq Polymerase (2.00 units/25 μ l PCR) with 2 μ l DNA. The reaction conditions of PCR were 94 °C for 3 minutes before 35 cycles of amplification, with 94 °C for 30 sec, 55 °C for 1 minute and 72 °C for 60 sec, followed by a final extension at 72 °C for 5 minutes. PCR products were sent to Macrogen Europe for cycle sequencing of both strands of each gene.

Chromatograms were edited using CodonCode Aligner version 5.1.5 (<http://www.codoncode.com>) for COI and Geneious 11 (<http://www.geneious.com>) for 18S. All new DNA sequences generated for this study are deposited in GenBank under the accession numbers provided in Suppl. material 1: Table S1.

For comparison, 84 COI sequences and 88 18S sequences of related barnacles were downloaded from GenBank (accession numbers are provided in Figs 28, 29). Sequences were aligned using Muscle (Edgar 2004) as implemented in Geneious and later exported as nexus or fasta files.

Phylogenetic trees were reconstructed for each gene using both Maximum Likelihood (ML) and Bayesian Inference (BI). ML analyses were conducted with RAxML Black Box (Stamatakis et al. 2008) with 100 bootstrap replicates and under the GTR + I + G model of sequence evolution. Bayesian analyses were conducted in BEAST 2. As our goal was to obtain a topology and not dates for the branching events, we used a Bayesian relaxed lognormal clock with a rate of 1. Therefore, the ages obtained in the ultrametric trees emerging from this analysis are relative not absolute. Substitution model selection was performed in jModeltest (Posada 2008) using the Akaike information criterion, and GTR + I + G was identified as the best model for both genes. For each analysis, we ran two independent chains of between 10 and 40 million generations, with a birth-death tree prior. Convergence of chains and burn-ins were assessed with Tracer, runs combined using LogCombiner, and maximum clade credibility trees produced in Tree Annotator.

Genetic distances (K2P) were calculated by MEGA version X (Kumar et al. 2018).

Results

Checklist tabulation

This study provides the most comprehensive overview of barnacle species from the Moluccan islands (Table 1). The morphological analyses of the collected material revealed 24 intertidal species and one deep-water species from three families of stalked barnacles (Heteralepadidae: one genus and species; Lepadidae: two genera and species; Pollicipedidae: one genus and species) and four families of acorn barnacles (Pachylasmataidae: one genus and species; Chthamalidae: five genera and species; Tetraclitidae: five genera and eight species; Balanidae: two genera and seven species).

Including previous records from the literature, we found a total of 97 species from the Moluccan islands (Table 1) from the superorder Thoracica (free living or epizoic). Of these, 21 are new records and two (*Amphibalanus* sp. and *Microeuraphia* sp.) are currently unidentified species.

All specimens obtained from field work, except for one floating specimen, were attached to types of natural and artificial substrates (Table 1), and several smaller species, e.g., *Chthamalus moro*, were also attached to other larger barnacle species, e.g., *Megabalanus tintinnabulum*. In general, the smallest species was *C. moro* (basal length: 2.4–5.1 mm; basal width: 1.4–4.1; height 0.8–1.7 mm), and the largest *M. tintinnabulum* (basal length: 26.0–49.3 mm; basal width 29.0–43.1 mm; height 20.1–49.4 mm).

Systematic account of the 25 species morphologically examined for this study

Class Hexanauplia Oakley, Wolfe, Lindgren & Zaharoff, 2013

Subclass Thecostraca Gruvel, 1905

Infraclass Cirripedia Burmeister, 1834 (= *Cirrhipèdes* Lamarck, 1806)

Superorder Thoracica Darwin, 1854

Order Lepadiformes Buckeridge & Newman, 2006

Suborder Heteralepadomorpha Newman, 1987

Family Heteralepadidae Nilsson-Cantell, 1921

Genus *Heteralepas* Pilsbry, 1907

Heteralepas japonica (Aurivillius, 1892)

Figure 3a–g, Table 1: species no. 1

Alepas japonica Aurivillius, 1892: 125; Aurivillius 1894: 28, pl. II figs 14, 15, pl. VIII figs 3, 7, pl. IX fig. 3.

Alepas indica Gruvel, 1901: 259; Gruvel 1905a: 162, fig. 179.

Heteralepas (Heteralepas) japonica: Pilsbry 1907a: 101.

Heteralepas (Heteralepas) japonica var. *alba* Krüger, 1911a: 34, pl. 1 fig. 2b.

Heteralepas (Heteralepas) dubia Broch, 1922: 288, fig. 38.

Heteralepas japonica: Pilsbry 1911a: 71, fig. 4; Zevina et al. 1992: 31, fig. 19; Chan et al. 2009a: 61; Chan et al. 2009b: 88–91, figs 2A–D, 3A–D, 4, 5.

Material examined. Deep-Sea: 32 specimens, MZB Cru Cir 050, Stn.23 Mooring Lifamatola, 250 m, coll. N. Fitriya, 9 Nov 2016; 13 specimens, MZB Cru Cir 051, Stn.39 Mooring Halmahera Seas, 250 m, coll. N. Fitriya, 13 Nov 2016; 27 specimens, MZB Cru Cir 052, Stn.56 MO2, 200 m, coll. N. Fitriya, 23 Nov 2016; 42 specimens, MZB Cru Cir 053, Stn.58 MO3, 250 m, coll. N. Fitriya, 25 Nov 2016.

GenBank accession numbers. COI gene (MK995372), 18S (MK981386).

Diagnosis. Capitulum rounded without hard valves and opercular plates, wall of capitulum tick with crest not more than two on the carinal region; cirrus I with filamentary appendage at the basal region; anterior rami shorter than posterior rami in cirri V–VI; caudal appendage present; maxillule strongly notched.

Description. Orifice slightly protuberant, crenulated, occupying one half to one third capitular length, parallel to or at oblique angle to capitulum; integument thick, chitinous; carinal margin sometimes with warty protuberances on slight keel; peduncle naked; colour of capitulum and peduncle yellowish (Fig. 3a–c). Cirrus I with anterior rami (19-segmented) shorter than posterior rami (25-segmented) and a filamentary appendage present at the basal region (Fig. 3d); cirri II – IV long, slender, anterior rami of cirri V and VI shorter than posterior rami; cirrus VI has a caudal appendage with 20-segmented and one fourth length of anterior ramus (Fig. 3e). Mandible with four large teeth excluding inferior angle (Fig. 3f); maxillule strongly notched with

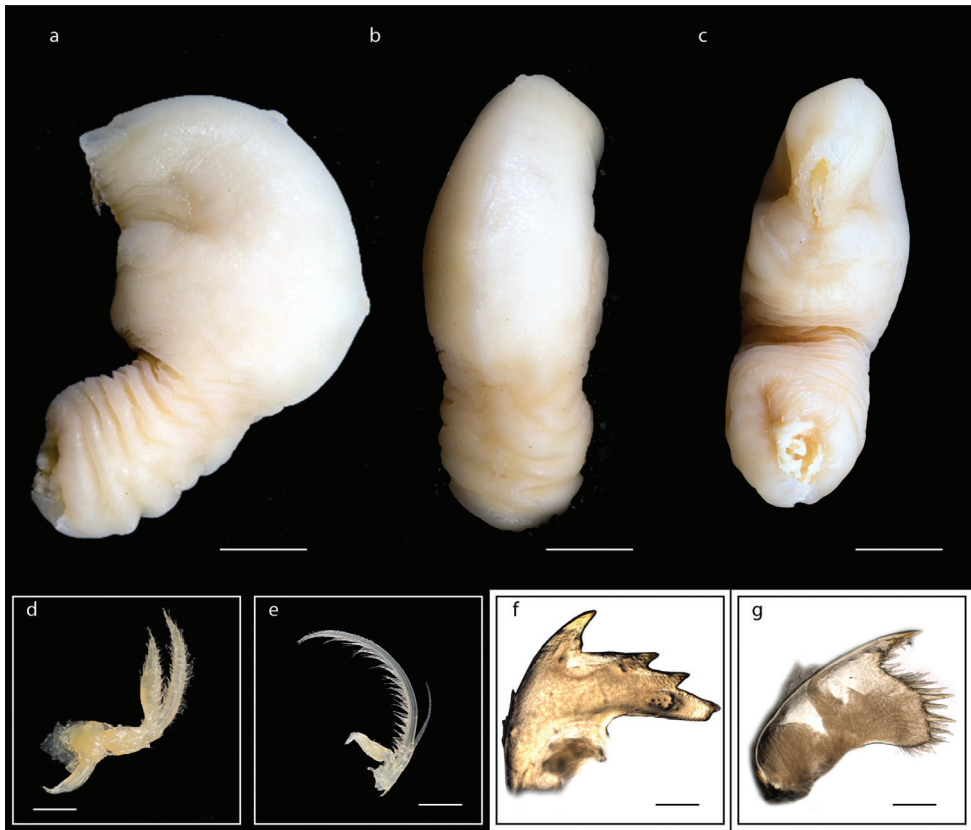


Figure 3. *Heteralepas japonica* (Aurivillius, 1892) (MZB Cru Cir 050- 2) **a** side view showing the capitulum and peduncle **b** upper view showing the capitulum and peduncle **c** side view showing the opening of operculum **d** cirrus I showing the filamentary appendage at the basal region **e** cirrus VI showing the caudal appendage **f** mandible **g** maxillule. Scale bars: 5 mm (**a–c**); 2 mm (**d**); 3 mm (**e**); 0.5 mm (**f–g**).

two big teeth on upper angle and blade-shaped setae on cutting margin (Fig. 3g); labrum concave, teeth numerous. Ranges of height of capitulum 11.9–18.6 mm, width 8.7–15.3 mm, thickness 7.0–13.3 mm; length of peduncle 7.7–27.6 mm and width 5.1–10.4 mm (measurements for 25 specimens are presented in Suppl. material 1: Table S2).

Distribution. *Heteralepas japonica* is widely distributed in Indo-west Pacific: Indian Ocean; Australia; Singapore, Malacca Str., Indonesia; Malay Archipelago; Vietnam; Condor Island; S China Sea; E China Sea; Taiwan, Philippines; S Japan; NE New Zealand; fouling hard rock substrata, crabs, gorgonians, antipatharians, deep-sea cables; 48–500 m (Jones and Hosie 2016). In this study, *Heteralepas japonica* was found attached to cable moorings in Lifamatola Sea and Halmahera Sea (a map with the occurrence of *Heteralepas japonica* in the Moluccas is shown in Suppl. material 1: Fig. S1).

Remarks. The external appearance of this species is extremely variable (Nilsson-Cantell 1927). After an extensive study of *H. japonica*, Nilsson-Cantell could not dis-

tinguish *H. japonica* and *H. indica* (Gruvel, 1901) and placed the latter in synonymy with *H. japonica*, and later authors have followed this suggestion (e.g., Broch 1931, Utinomi 1958). At the same time, Nilsson-Cantell (1927) also suggested that *H. nicobarica* Annandale, 1909, *H. gigas* Annandale, 1905 and *H. cygnus* Pilsbry, 1907 could be invalid species and future revision may synonymize some or all of them. Zullo and Newman (1964) pointed out the uncertainty surrounding the status of several of the species assigned to *Heteralepas* due to a lack of zoogeographic and morphological data, since extensive collections are unavailable. Furthermore, Foster (1978) suggested that a revision of the genus was called for since the variability of *H. japonica*, as noted by Foster and tabulated by Nilsson-Cantell (1927), encompasses characters which have been used to distinguish several different species by other authors (e.g., *H. dubia* Broch, 1922, *H. cornuta* Darwin, 1852, *H. indica* Gruvel, 1901, *H. lankestri* Gruvel, 1900).

Suborder Lepadomorpha Pilsbry, 1916

Family Lepadidae Darwin, 1852

Genus *Dosima* Gray, 1825

Dosima fascicularis (Ellis & Solander, 1786)

Figure 4a–c, Table 1: species no. 12

Lepas fascicularis Ellis & Solander, 1786: 197, tab. 15 fig. 6; Darwin 1852: 92, pl. 1 fig. 6.

Lepas fascicularis auriwillii Nilsson-Cantell, 1921: 238, fig. 40b.

Lepas cygnea Spengler, 1790: pl. 6 fig. 8.

Pentalasmis spirulicola, *P. donovani* Leach, 1818: 413.

Pentalasmis fascicularis: Brown 1844: pl. 51 fig. 2.

Lepas fasciculatus: Pilsbry 1907: 81, pl. IX fig. 6.

Lepas (Dosima) fascicularis: Weisbord 1979: 28, pl. 2 figs 10–11; Jones et al. 1990: 8.

Dosima fascicularis: Gray 1825: 100; Zevina 1982: 21, fig. 11.

Material examined. *Ambon Island*: 19 specimens, MZB Cru Cir 048, Tial, 3°38'10.2"S, 128°20'46.9"E, coll. Adin, 19 Sep 2017.

GenBank accession numbers. COI gene (MK995371), 18S (MK981385).

Diagnosis. The only pelagic barnacle with its own gas-filled float; plates very thin and paper-like; carina angle bent with a prominent umbo and expanded basal disk; cirri acanthopod.

Description. Five capitular plates, white, thin, delicate, wide interspaces between dark purple; base of carina almost round, not imbedded in membrane, distinct angle formed at sub-central carinal umbo peduncle short, naked (Fig. 4a); five filamentary appendages located at base of cirri on each side of body; caudal appendages small, smooth, summits rounded; mandible with five teeth (Fig. 4c); penis hirsute. Ranges of diameter of capitulum base 4.0–6.8 mm; capitular height 10.5–17.1 mm; total height 11.7–19.5 mm; scutal width 5.4–7.3 mm; scutal length 8.1–11.1 mm; tergal

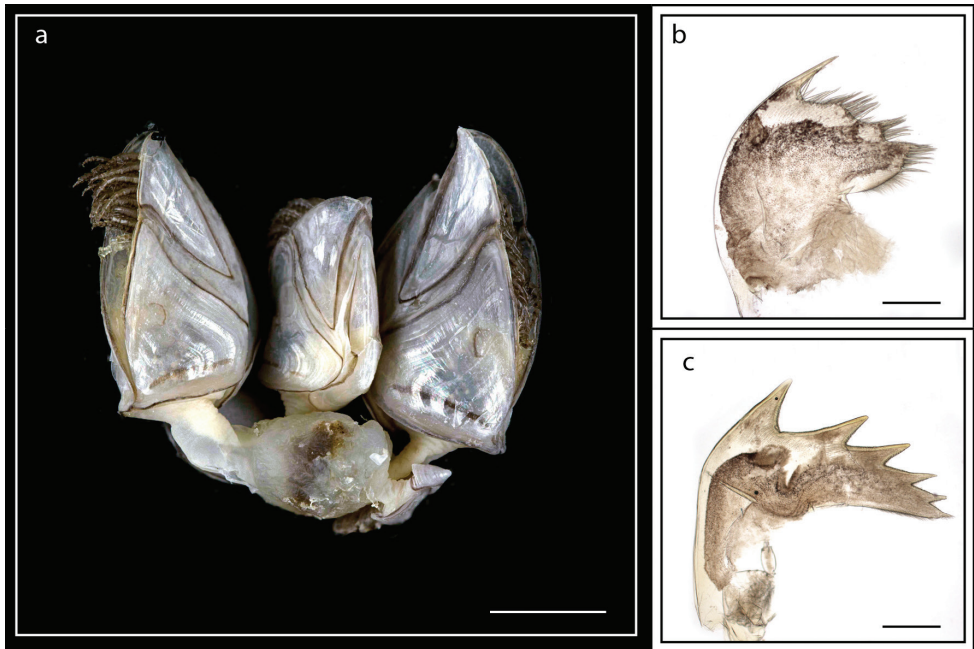


Figure 4. *Dosima fascicularis* (Ellis & Solander, 1786) (MZB Cru Cir 048-19) **a** side view showing the capitulum and peduncle **b** maxillule **c** mandible. Scale bars: 5 mm (**a**); 0.5 mm (**b–c**).

width 2.7–4.4 mm; tergal length 6.2–11.1 mm (measurements for six specimens are presented in Suppl. material 1: Table S3).

Distribution. *Dosima fascicularis* is cosmopolitan in tropical and temperate seas (Jones and Hosie 2016). While it has been reported found at New Zealand, South Africa and South America (Newman and Ross 1971). In the present study, *D. fascicularis* was found at Ambon Island at Tial (floating in water at the beach) (a map with the occurrence of *Dosima fascicularis* in the Moluccas is shown in Suppl. material 1: Fig. S2).

Remarks. *Dosima fascicularis* is the only pelagic barnacle that produces its own gas-filled float enabling it to sustain itself on the sea surface (Weisbord 1979). *Dosima* can also be distinguished from members of the genus *Lepas* by the distinct angle formed at the sub-central umbo of the carina, and by very thin and brittle plates (Hinojosa et al. 2006).

Genus *Lepas* Linnaeus, 1758

Subgenus *Anatifa* Bruguière, 1789

Lepas anserifera Linnaeus, 1767

Figure 5a–n, Table 1: species no.13

Anatifa striata Bruguière, 1789: pl. 166 fig. 3.

Pentalasmis anseriferus: Brown 1844: pl. 51 fig. 1.

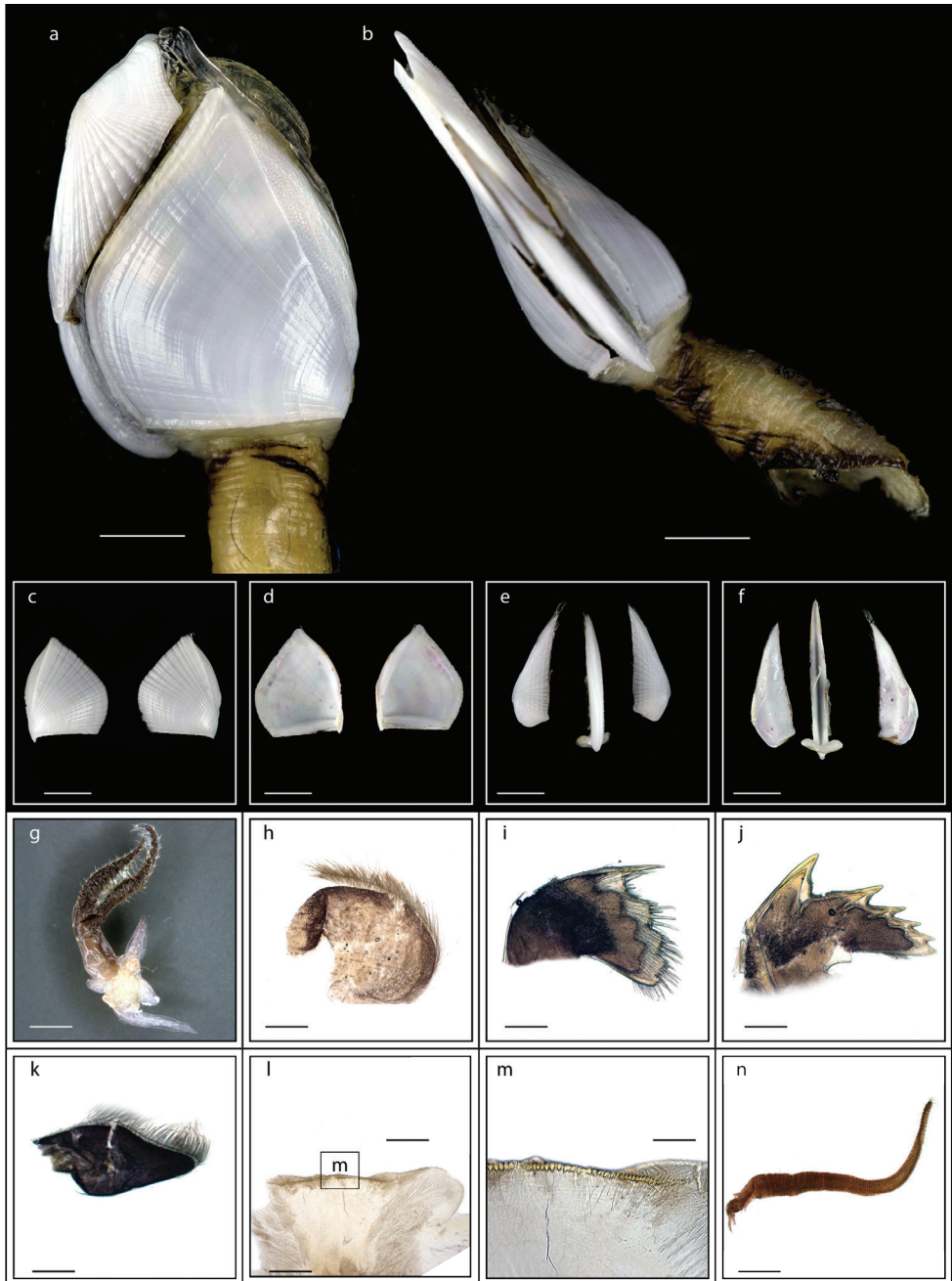


Figure 5. *Lepas anserifera* Linnaeus, 1767 (MZB Cru Cir 058-2) **a** side view showing the capitulum and peduncle **b** side view showing the carina **c** external view of scutum **d** internal view of scutum **e** external view of tergum and carina **f** internal view of tergum and carina **g** cirrus **h** maxilla **i** maxillule **j** mandible **k** mandibular palp **l** labrum **m** close up view on the teeth of labrum **n** penis. Scale bars: 4 mm (**a–b**); 3 mm (**c–f**); 1 mm (**g, n**); 0.5 mm (**h–m**).

Lepas anserifera Linnaeus, 1767: 1109; Darwin 1852: 81, pl. 1 fig. 4; Hoek 1907: 2; Hiro 1937a: 57, fig. 48; Utinomi 1949: 20; Stubbings 1967: 237; Newman 1971: 32, fig. 1; Dong et al. 1982: 73; Zevina et al. 1992: 14, fig. 6; Igic 2007: 37, fig. 10; Chan et al. 2009a: 45, fig. 34; Keable and Reid 2015: 266.

Material examined. *Ambon Island*: 5 specimens, MZB Cru Cir 056, Galala, 3°41'22.2"S, 128°10'52.6"E, coll. P. Pitriana & D. Tala, 6 Sep 2016; 8 specimens, MZB Cru Cir 057, Laha, 3°43'22.5"S, 128°05'02.5"E, coll. P. Pitriana & D. Tala, 5 Sep 2016; 74 specimens, MZB Cru Cir 058, Suli, 3°37'02.0"S, 128°16'31.6"E, coll. Adin, 19 Sep 2017; 93 specimens, MZB Cru Cir 059, Tial, 3°38'10.2"S, 128°20'46.9"E, coll. Adin, 19 Sep 2017. *Pombo Island*: 5 specimens, MZB Cru Cir 060, Pombo, 3°31'55.5"S, 128°22'28.8"E, coll. P. Pitriana & D. Tala, 8 Sep 2016. *Seram Island*: 13 specimens, MZB Cru Cir 061, Lepas Pantai Kawa, 2°57'32.5"S, 128°05'33.4"E, coll. P. Pitriana & D. Tala, 19 Sep 2017; 10 specimens, MZB Cru Cir 062, Desa Murnaten, 2°51'48.8"S, 128°20'32.3"E, coll. P. Pitriana & D. Tala, 20 Sep 2017; 10 specimens, MZB Cru Cir 063, Desa Kasie, 2°51'05.5"S, 128°32'54.1"E, coll. P. Pitriana & D. Tala, 20 Sep 2017; 10 specimens, MZB Cru Cir 064, Dermaga Pelita Jaya, 3°00'13.5"S, 128°07'09.2"E, coll. P. Pitriana & D. Tala, 21 Sep 2017. *Saparua Island*: 19 specimens, MZB Cru Cir 065, Negeri Mahu, 3°31'52.9"S, 128°41'12.4"E, coll. P. Pitriana & D. Tala, 11 Apr 2016.

GenBank accession numbers. COI gene (MK995373–MK995375), 18S (MK981387–MK981388).

Diagnosis. Capitulum with five completely calcified plates; surfaces striated with radiating lines; scuta with conspicuous growth lines; scutal margin of terga without notch, occluding margin of scutum strongly convex and swollen; carina apex extending to tergum, base of carina forked; filamentary appendages and caudal appendage present.

Description. Five capitular plates, closely approximate, white, slightly furrowed, terga sometimes strongly pectinated, occluding margin arched, protuberant (Fig. 5a, b). Scutum with well developed, strong internal umbonal tooth, left internal umbonal tooth small, or mere ridge; carina produced below base of scutum as fork, apex pointed (Fig. 5c–f); 5–6 filamentary appendages on each side, one on side of prosoma below base of pedicel of cirrus I, four others placed in pairs beneath basal segment of pedicel of cirrus I, lowest posterior filament of the four generally largest (Fig. 5g); caudal appendages small, smooth, curved, claw-like, tip pointed; mandible with five teeth excluding inferior angle, lower angle pectinate (Fig. 5j), labrum concave, toothed (Fig. 5l–m). Basal diameter of capitulum 2.0–7.2 mm; capitular height 8.1–14.8 mm; total height 14.2–31.9 mm. Scutal width 4.7–11.3 mm; scutal length 6.3–12.5 mm. Tergal width 4.7–11.3 mm; tergal length 4.3–9.6 mm (measurements for 25 specimens are presented in Suppl. material 1: Table S4).

Distribution. *Lepas anserifera* is a cosmopolitan, pelagic species occurring in tropical and temperate oceans (Jones et al. 2001). In this study, *Lepas anserifera* was found on the islands of Ambon (at Suli, Tial, Galala, Laha), Pombo, Seram (at Lepas Pantai Kawa, Desa Murnaten, Desa Kasie, Dermaga Pelita Jaya), and Saparua (at Negeri Mahu). *Lepas anserifera* was found attached to mangroves, stone ship charts and ship

walls, port poles, and shells of *Megabalanus zebra* (a map with the occurrence of *Lepas anserifera* in the Moluccas is shown in Suppl. material 1: Fig. S3).

Remarks. *Lepas anserifera* can be easily recognized by the presence and positions of the 5–6 filamentary appendages and the curved caudal appendages (Igić 2007).

Order Scalpelliformes Buckeridge & Newman, 2006

Suborder Scalpellomorpha Newman, 1987

Family Pollicipedidae Leach, 1817

Genus *Capitulum* Gray, 1825

***Capitulum mitella* (Linnaeus, 1758)**

Figure 6a–l, Table 1: species no.17

Lepas mitella Linnaeus, 1758: 668.

Pollicipes mitella: Sowerby 1833: fig. 2; Darwin 1852: 316, pl. VII fig. 3; Utinomi 1970: 339; Dong et al. 1982: 69; Zevina et al. 1992: 37, fig. 23.

Polylepas mitella: Blainville 1824: pl. 1 fig. 5.

Mitella mitella: Pilsbry 1907: 6; Annandale 1916: 128, pl. 12 fig. 1.

Capitulum mitella: Gray 1825: 101; Foster 1980: 209; Chan et al. 2009a: 85, fig. 70; Williamson 2014: 758, fig. 1D.

Material examined. *Ambon Island*: 10 specimens, MZB Cru Cir 023, Liang, 3°30'13.3"S, 128°20'34.1"E, coll. P. Pitriana & D. Tala, 30 Aug 2016; 2 specimens, MZB Cru Cir 024, Liang, 3°30'13.3"S, 128°20'34.1"E, coll. P. Pitriana & D. Tala, 7 Sep 2016; 2 specimens, MZB Cru Cir 025, Alang, 3°45'11.0"S, 128°01'23.1"E, coll. Adin, 20 Sep 2017; 4 specimens, MZB Cru Cir 026, Asilulu, 3°40'50.4"S, 127°55'27.6"E, coll. Adin, 20 Sep 2017; 3 specimens, MZB Cru Cir 027, Dermaga Tulehu, 3°35'21.8"S, 128°20'02.8"E, coll. P. Pitriana & D. Tala, 7 Sep 2017; 15 specimens, MZB Cru Cir 028, Tawiri, 3°42'10.1"S, 128°06'13.4"E, coll. P. Pitriana & D. Tala, 29 Mar 2016; 5 specimens, MZB Cru Cir 029, Tawiri, 3°42'10.1"S, 128°06'13.4"E, coll. P. Pitriana & D. Tala, 5 Sep 2016; 2 specimens, MZB Cru Cir 030, Laha, 3°43'22.5"S, 128°05'02.5"E, coll. P. Pitriana & D. Tala, 5 Sep 2017; 6 specimens, MZB Cru Cir 031, Morella, 3°31'06.5"S, 128°13'18.0"E, coll. Adin, 20 Sep 2017; 5 specimens, MZB Cru Cir 032, Ureng, 3°40'14.0"S, 127°56'47.6"E, coll. Adin, 20 Sep 2017; 5 specimens, MZB Cru Cir 033, Wakasih, 3°46'27.6"S, 127°56'36.6"E, coll. Adin, 20 Sep 2017. *Saparua Island*: 10 specimens, MZB Cru Cir 034, Teluk Saparua, 3°34'25.7"S, 128°39'25.8"E, coll. P. Pitriana & D. Tala, 8 Apr 2016; 11 specimens, MZB Cru Cir 035, Benteng Durstede, 3°34'32.8"S, 128°39'34.7"E, coll. P. Pitriana & D. Tala, 8 Apr 2016.

Genbank accession number. 18S (MK981390).

Diagnosis. Capitulum with more than 18 plates, all with apical umbones; lateral plates numerous and only one big plate under the rostrum; scales of the peduncle symmetrically arranged in close whorls.

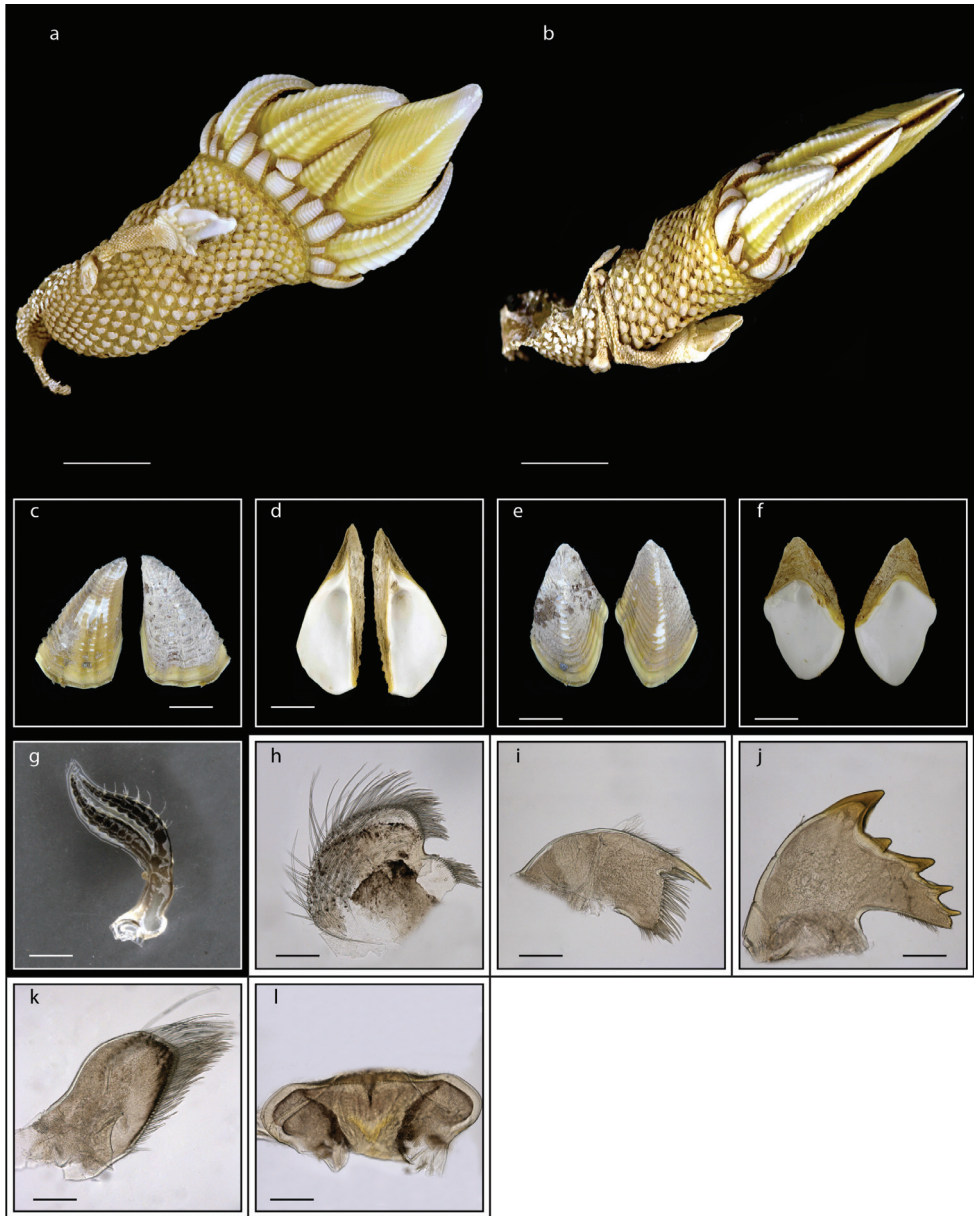


Figure 6. *Capitulum mitella* (Linnaeus, 1758) (MZB Cru Cir 026-4) **a** side view showing the capitulum and peduncle **b** side view showing the rostrum **c** external view of scutum **d** internal view of scutum **e** external view of tergum **f** internal view of tergum **g** cirrus I **h** maxilla **i** maxillule **j** mandible **k** mandibular palp **l** labrum. Scale bars: 6 mm (**a–b**); 2 mm (**c–f**); 1 mm (**g**); 0.5 mm (**h–l**).

Description. Capitulum fan-shaped, with eight large plates, basal ring of 18–25 smaller plates, all plates yellowish, umbos apical; peduncle covered by numerous yellowish, fine scales (Fig. 6a, b); mandible with five teeth (Fig. 6j); labrum concave, teeth lacking (Fig. 6l). Capitular diameter base ranges 4.5–20.4 mm; total height 10.7–47.1 mm; capitular

height 7.0–24.6 mm. Ranges of distance from rostrum to carina 5.8–27.3 mm and rostral height 5.5–24.2 mm (measurements for 25 are presented in Suppl. material 1: Table S5).

Distribution. Darwin (1852) reported *Capitulum mitella* from the Philippine Archipelago, Ambon, East Indian Archipelago and Madagascar. Chan et al. (2009) and Jones and Hosie (2016) reported the species as widely distributed in warmer parts of the Indo-Pacific region, from Madagascar to southern Japan. In this study, *C. mitella* was found on the islands of Ambon (at Ureng, Alang, Dermaga Liang, Asilulu, Doc. Tawiri, Morella, Wakasihu, Laha, and Tulehu) and Saparua (at Benteng Duurstede and Teluk Saparua). *Capitulum mitella* attach on rocks, stone, wall of fortress, port pole and concrete wall (a map with the occurrence of *Capitulum mitella* in the Moluccas is shown in Suppl. material 1: Fig. S4).

Remarks. *Capitulum mitella* is the famous Japanese goose barnacle or ‘kame-no-te’ (meaning the hand of the turtle, referring to its shape). This barnacle is edible and sold as an expensive seafood in Japan, China, Taiwan, and Korea, as well as in Portugal and Spain, where it is known as ‘percebes’.

Order Sessilia Lamarck, 1818

Suborder Balanomorphia Pilsbry, 1916

Family Pachylasmatidae Utinomi, 1968

Subfamily Pachylasmatinae Utinomi, 1968

Genus *Pseudoctomeris* Poltarukha, 1996

Pseudoctomeris sulcata (Nilsson-Cantell, 1932)

Figure 7a–h, Table 1: species no. 50

Octomeris sulcata Nilsson-Cantell, 1932: 8; Newman and Ross 1976: 40.

Pseudoctomeris sulcata: Poltarukha 1996: 988; Chan et al. 2009a: 156, fig. 131.

Material examined. *Ambon Island*: 4 specimens, MZB Cru Cir 073, Leahari, 3°42'45.3"S, 128°16'16.5"E, coll. P. Pitriana, 14 Jan 2016; 2 specimens, MZB Cru Cir 074, Hatu, 3°43'52.7"S, 128°02'51.4"E, coll. Adin, 20 Sep 2017.

Diagnosis. Shell with eight plates; compound rostrum; scutum and tergum fused; mandible tridentate; multi-jointed caudal appendage present.

Description. Shell externally white, internally black; eight plated, rostrum partially fused with rostrilaterals giving external appearance of six plates (Fig. 7a–b); basis membranous, calcareous; orifice rhomboidal; scutum and tergum thick (Fig. 7c, d); maxilla triangular, maxillule not notched (Fig. 7g), mandible with three teeth (Fig. 7h), labrum concave, with blunt teeth; cirrus VI with long, multi-segmented caudal appendages. Ranges of basal length 16.2–16.7 mm, basal width 11.5–15.0 mm, height 7.4–7.7 mm. Orifice length 5.5–7.9 mm, orifice width 4.3–6.1 mm (measurements for two specimens are presented in Suppl. material 1: Table S6).

Distribution. *Pseudoctomeris sulcata* was previously recorded from southern Japan, China, and Taiwan (Jones et al. 2001; Poltarukha and Zevina 2006). In this study, *P*



Figure 7. *Pseudoctomeris sulcata* (Nilsson-Cantell, 1932) (MZB Cru Cir 073-4) **a** upper view **b** side view **c** external view of scutum and tergum **d** internal view of scutum and tergum **e** cirrus I **f** cirrus III **g** maxillule **h** mandible. Scale bars: 4 mm (**a–b**); 2 mm (**c–d**); 1 mm (**e**); 2 mm (**f**), 0.5 mm (**g–h**).

sulcata was found on Ambon Island at Leahari and Hatu on rocks and shells of *Tetraclitia squamosa* (a map with the occurrence of *Pseudoctomeris sulcata* in the Moluccas is shown in Suppl. material 1: Fig. S2).

Remarks. Externally, the fused rostrum and rostrolaterals are six-plated, but the sutures are visible internally (Poltarukha 1996). Morphologically, *Pseudoctomeris sulcata* shows features of the scutum and tergum similar to those of representatives of the family Pachylasmatida. However, the species can be distinguished by its tridentate mandible and the presence of multi-jointed caudal appendages (Poltarukha 2006). A

previous molecular study showed that *P. sulcata* clustered together with members of the family Pachylasmatidae, not with members of the Chthamalidae (Chan et al. 2017). According to Chan et al. (2017), *P. sulcata* is an intertidal species of the Pachylasmatidae, previously believed to be an exclusive deep-sea taxon.

Superfamily Chthamaloidea Darwin, 1854

Family Chthamalidae Darwin, 1854

Subfamily Notochthamalinae Foster & Newman, 1987

Genus *Hexechamaesipho* Poltarukha, 1996

***Hexechamaesipho pilsbryi* (Hiro, 1936)**

Figure 8a–f, Table 1: species no. 51

Chthamalus pilsbryi Hiro, 1936: 227, fig. 3.

Euraphia pilsbryi: Newman & Ross, 1976: 41.

Hexechamaesipho pilsbryi: Poltarukha 1996: 989; Poltarukha 2006: 73–74; Chan et al. 2008: 320, fig. 3; Chan et al. 2009a: 149, fig. 125; Tsang et al. 2013: 188.

Material examined. *Ambon Island*: 20 specimens, MZB Cru Cir 054, Hila, 3°34'57.5"S, 128°05'31.9"E, coll. Adin, 20 Sep 2017; 1 specimen, MZB Cru Cir 055, Hatu, 3°43'52.7"S, 128°02'51.4"E, coll. Adin, 20 Sep 2017.

Diagnosis. Shell with six plates; surface grey with black spots scattered; scutum and tergum deeply interlock forming a sinuous line; cirri I and II with multi-cuspid setae.

Description. Surface of parietes grey or light brown in colour and spotted with black; orifice rhomboidal (Fig. 8a); basis calcareous; scutum and tergum strongly articulated, forming sinuous line; scutum elongated, triangular, tergal margin strongly articulated tergum narrow, basi-scutal angle almost 90° (Fig. 8b–c); cirrus II with multi-cuspid setae; mandible with three large teeth (Fig. 8f), labrum with row of large teeth. Basal length 8.9–17.0 mm, basal width 10.3–16.4 mm, height 1.0–3.7 mm. Orifice length 4.2–6.9 mm, orifice width 3.6–5.6 mm (measurements for ten specimens are presented in Suppl. material 1: Table S7).

Distribution. Previously, *Hexechamaesipho pilsbryi* was reported from Japan (Honshu, Shimoda, Wakayama, Okinawa); Taiwan (Turtle Island, Da Xiang Lang, Shi Ti Ping, Kenting); Philippines (Puerto Galera, Tiwi-Bicol, Boracay); Malaysia (Nexus Beach, Kota Kinnabalu, Sabah) (Tsang et al. 2013). In the present study, *H. pilsbryi* was collected from Hatu and Hila on Ambon Island (a map with the occurrence of *Hexechamaesipho pilsbryi* in the Moluccas is shown in Suppl. material 1: Fig. S2). A previous study of *H. pilsbryi* indicated that the distribution of this species bridges the junction of the Japan region and the Indo-Polynesian province of Briggs (Briggs 1974). Molecular results of *H. pilsbryi* analysed by Tsang et al. (2013) suggested that this species can be divided into two highly diverged lineages: (1) a northern lineage, predominantly distributed in Japan and Okinawa, and (2) a southern lineage, primar-

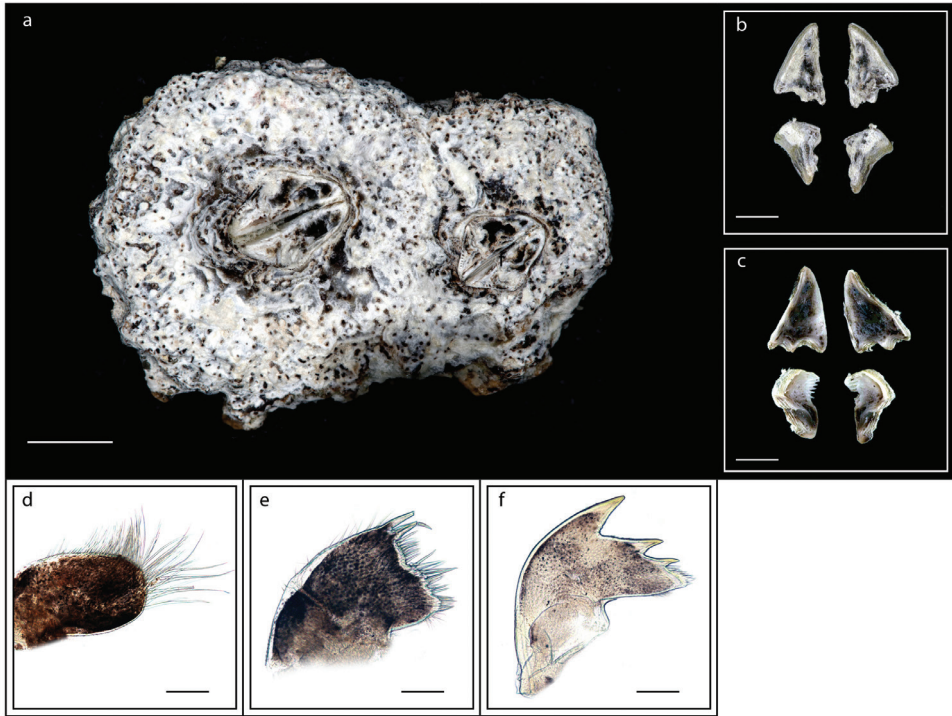


Figure 8. *Hexechamaesipho pilsbryi* (Hiro, 1936) (MZB Cru Cir 055) **a** upper view **b** external view of scutum and tergum **c** internal view of scutum and tergum **d** maxilla **e** maxillule **f** mandible. Scale bars: 4 mm (**a**); 2 mm (**b–c**); 0.5 mm (**d–f**).

ily distributed in Taiwan and Southeast Asia. Assuming that we have molecular data of *H. pilsbryi* in our samples, there is a probability that our samples from the Moluccas include members of the southern lineage.

Remarks. *Hexechamaesipho pilsbryi* was first identified from Japan as *Chthamalus pilsbryi* Hiro, 1936. However, due to the presence of three large teeth on the mandible, a characteristic of the subfamily Euraphiinae, the species was placed in the genus *Euraphia* (Nilsson-Cantell 1921). Later, due to the presence of multicuspidate setae on cirrus II, Poltarukha (1996) moved *E. pilsbryi* to the sub-family Notochthamalinae and determined a new genus, *Hexechamaesipho*, which had six parietes and a deeply interlocking scutum and tergum. Currently, *H. pilsbryi* is the only species in the genus.

Genus *Nesochthamalus* Foster & Newman, 1987

Nesochthamalus intertextus (Darwin, 1854)

Figure 9a–h, Table 1: species no. 52

Chthamalus intertextus Darwin, 1854: 467, pl. 19 figs 1a, b; Dong et al. 1982: 82; Pope 1965: 29, pl. I figs 1f, 3a–d.

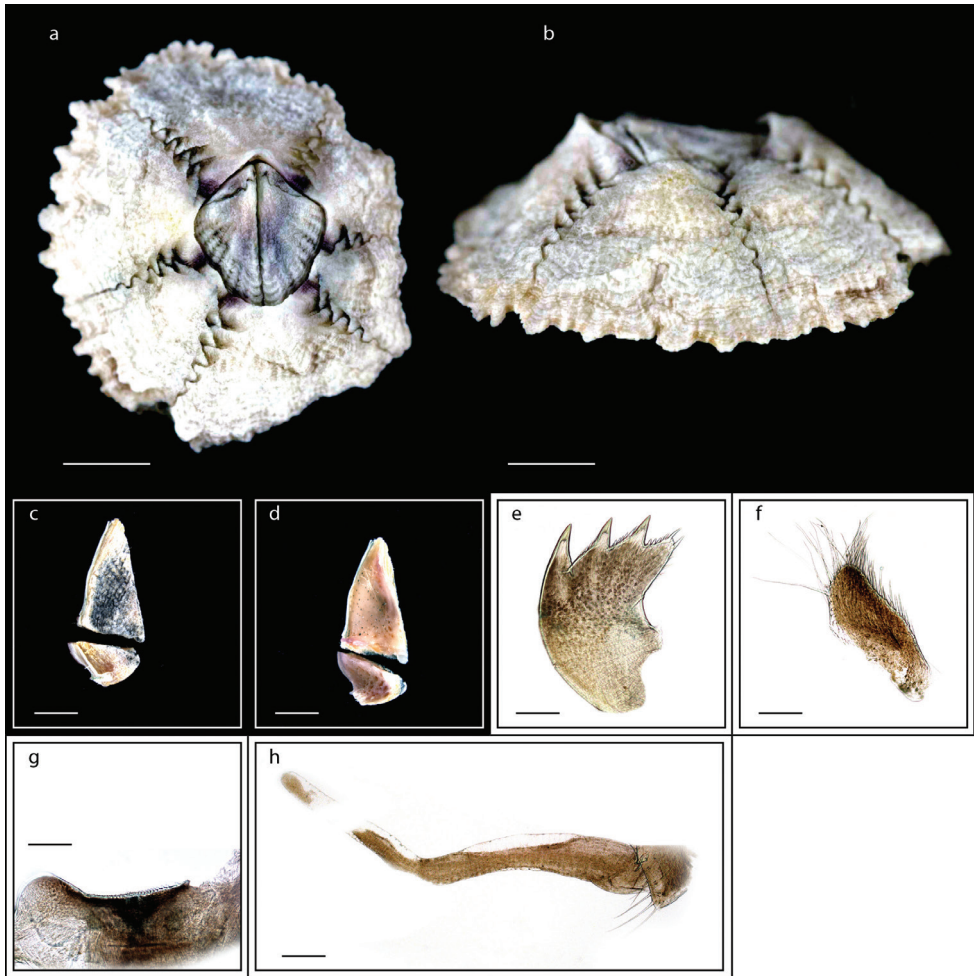


Figure 9. *Nesochthamalus intertextus* (Darwin, 1854) (MZB Cru Cir 070-5) **a** upper view **b** side view **c** external view of scutum and tergum **d** internal view of scutum and tergum **e** mandible **f** mandibular palp **g** labrum **h** penis. Scale bars: 3 mm (**a**); 0.75 mm (**c–d**); 0.25 mm (**e–g**); 1 mm (**h**).

Euraphia intertextus: Newman & Ross, 1976: 41; Zevina et al. 1992: 79, fig. 53.

Nesochthamalus intertextus: Foster & Newman, 1987: 326, fig. 3; Southward et al. 1998: 120, fig. 1D, 1H; Chan et al. 2009a: 147, fig. 124.

Material examined. *Ambon Island*: 5 specimens, MZB Cru Cir 070, Laha, 3°43'22.5"S, 128°05'02.5"E, coll. P. Pitriana & D. Tala, 7 Sep 2016; 5 specimens, MZB Cru Cir 071, Hila, 3°34'57.5"S, 128°05'31.9"E, coll. Adin, 20 Sep 2017.

GenBank accession numbers. COI gene (MK995376), 18S (MK981389).

Diagnosis. Shell depressed with large diamond-shaped orifice; scutum and tergum fused; external radii consist of oblique laminae arising on both sides of the sutures, standing nearly parallel to the parietes, interfolding with each other; cirri II and III with multi-cuspid setae.

Description. Shell with six plates, oval, flattened, colour of external shell white to pale grey, interior of shell violet; orifice rhomboidal; parietal sutures with conspicuous interlocking pattern (Fig. 9a, b); basis membranous with partial secondary calcification with age; opercular plates fused but separable (Fig. 9c, d); cirrus I with rami unequal; mandible with three large teeth (Fig. 9e), mandibular palp with long setae on exterior basal margin (Fig. 9f); labrum strongly dentate (Fig. 9g). Basal length 8.9–12.1 mm, basal width 6.9–10.3 mm, height 1.3–3.1 mm. Orifice length 3.0–4.9 mm, orifice width 2.6–3.9 mm (measurements for ten specimens are presented in Suppl. material 1: Table S8).

Distribution. *Nesochthamalus intertextus* is known from islands in the West and Central Pacific Ocean – Indonesia, New Guinea, Malaysia to Vietnam; China; Taiwan; Philippines; Japan; Hawaii; Pitcairn I (Pope 1965; Newman and Ross 1976; Chan et al. 2009; Jones and Hosie 2016). In this study, *N. intertextus* was found on Ambon Island at Laha and Hila on stone (a map with the occurrence of *Nesochthamalus intertextus* in the Moluccas is shown in Suppl. material 1: Fig. S2).

Remarks. *Nesochthamalus intertextus* can be distinguished by the conspicuous interlocking pattern exhibited by the parietal sutures and features of the basis, which is membranous in young specimens but becomes secondarily calcified with age, leaving a membranous centre only (Poltarukha 2008; Pope 1965).

Family Chthamalidae Darwin, 1854

Subfamily Euraphiinae Newman & Ross, 1976

Genus *Euraphia* Conrad, 1837

Euraphia hembeli Conrad, 1837

Figure 10a–c, Table 1: species no. 53

Chthamalus hembeli Darwin, 1854: 465, fig. 5a–5d; Pilsbry 1916: 324.

Euraphia hembeli Conrad, 1837: 261, pl.20 fig.6; Newman and Ross 1976: 41; Foster and Newman 1987: 330; Southward et al. 1998: 120, fig. 1E; Paulay and Ross 2003: 307; Jones 2012: 372; Pochai et al. 2017: 17.

Material examined. *Ambon Island:* 1 specimen, MZB Cru Cir 049, Asilulu, 3°40'50.4"S, 127°55'27.6"E, coll. Adin, 20 Sep 2017.

Diagnosis. Shell with interlocking teeth between plates; base with a true calcareous and complete secondary calcification; scutum higher than wide and interlocked but not concrescent with tergum.

Description. Shell with six plates, parietes symmetrical, calcareous, solid, separable, due to coarsely serrate sutures with interlocking toothed structure (Fig. 10a–c); colour yellowish or brownish grey, inner surface of parietes smooth, white with dark brown and pale violet horizontal striations around aperture; external surface of shell irregularly ribbed around basal margin, basis calcareous; orifice rhomboidal; tergum and scutum separable; scutum triangular, occluding margin with strong teeth; tergum

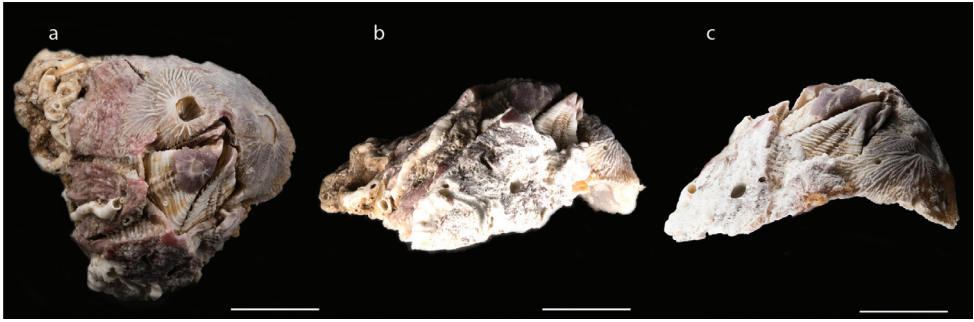


Figure 10. *Euraphia hembeli* Conrad, 1837 (MZB Cru Cir 049) **a** upper view of *E. hembeli* which is overgrown with other species of barnacles in its operculum **b** right side view **c** left side view. Scale bar: 16 mm.

strongly marked with 10–12 lateral depressor crests, scutal margin strongly articulated. Measurements for one specimen are presented in Suppl. material 1: Table S9.

Distribution. *Euraphia hembeli* has been recorded from the Mediterranean, West Africa, Indian Ocean: Ceylon; Andaman Sea, Cocos-Keeling Islands; Malay Archipelago (Sunda Islands); Pacific Ocean (Japan; Caroline Islands; Hawaiian Islands, California (Newman and Ross 1976; Jones 2012; Barrett and Freeman 2016; Pochai et al. 2017). In this study, *Euraphia hembeli* was found on Ambon Island at Asilulu on rocks (a map with the occurrence of *Euraphia hembeli* in the Moluccas is shown in Suppl. material 1: Fig. S2).

Remarks. *Euraphia hembeli* has a true calcareous basis and complete secondary calcification on its parietal wall and basis (Southward et al. 1998). It can be also distinguished from other species of the genus *Euraphia* by its size (up to 30 mm) and the presence of strong marked lateral depressor crests (between 10–12 in number) (Pochai et al. 2017).

Genus *Microeuraphia* Poltarukha, 1997

Microeuraphia sp.

Figure 11a–o, Table 1: species no. 55

Material examined. *Seram Island*: 2 specimens, MZB Cru Cir 138, Pantai Waimeteng-Piru, 3°04'15.3"S, 128°11'45.8"E, coll. P. Pitriana & D. Tala, 21 Sep 2017.

GenBank accession numbers. COI gene (MK995389, MK995390), 18S (MK981401, MK981402).

Diagnosis. Shell small with six thin plates; basis membranous; scutum and tergum remain articulated, scutum higher than wide; mandible tridentate; caudal appendage absent; one individual with two penises.

Description. Shell brownish (Fig. 11a, b), depressed (Fig. 11c); orifice diamond shaped (Fig. 11a, b); overlap of 'rostromedial' forming T junction (Fig. 11b); scutum and tergum triangular, tergal margins straight (Fig 11d, e); cirrus I with anterior ramus



Figure 11. *Microeuraphia* sp. (MZB Cru Cir 136-1) **a** upper view **b** lower view **c** side view **d** external view of scutum and tergum **e** internal view of scutum and tergum **f** cirrus I **g** cirrus II **h** cirrus III **i** cirrus IV **j** cirrus V **k** cirrus VI **l** penis **m** maxilla **n** maxillule **o** mandible. Scale bars: 3 mm (**a–c**); 0.5 mm (**d–l**); 0.25 mm (**m–o**).

longer than posterior (Fig 11f); mandible with smooth tridentate teeth (11o). Ranges of basal length 3.6–9.9 mm, basal width 3.0–9.1 mm, height 1.2–2.2 mm. Orifice of diamond shape with orifice length 1.5–4.5 mm, orifice width 0.7–3.6 mm (measurements for two specimens are presented in Suppl. material 1: Table S10).

Distribution. In this study, *Microeuraphia* sp. was found on Seram Island (at Pantai Waimeteng, Piru) (a map with the occurrence of *Microeuraphia* sp. in the Moluccas is shown in Suppl. material 1: Fig. S5).

Remarks. *Microeuraphia* sp. clustered as a unit, forming a well-supported clade in the COI tree (Fig. 29). Morphologically, one individual of this species exhibited two penises.

Family Chthamalidae Darwin, 1854**Subfamily Chthamalinae Darwin, 1854****Genus *Chthamalus* Ranzani, 1817*****Chthamalus moro* Pilsbry, 1916**

Figure 12a, b, Table 1: species no. 57

Chthamalus malayensis: Utinomi 1954: 18–21 (part.); Karande and Palekar 1963 (part.); Pope 1965 (part.); Newman and Ross 1976 (part.).

Chthamalus moro Pilsbry, 1916: 311; Nilsson-Cantell 1921: 277; Broch 1922: 307 (part.); Hiro 1937b: 49; Rosell 1972: 178; Dong et al. 1980: 125; Ren 1984: 153; Southward and Newman 2003: 798, fig. 2B; Chan et al. 2009a: 165, fig. 141.

non *Chthamalus moro* Broch, 1922: 307 (part.); Broch 1931: 56 (includes a euraphiid).

non *Chthamalus moro* Nilsson-Cantell, 1934: 50 (a euraphiid).

non *Chthamalus moro* Poltarukha, 2001b: 160 (= *C. malayensis*).

Material examined. *Ambon Island*: 2 specimens, MZB Cru Cir 036, Alang, 3°45'11.0"S, 128°01'23.1"E, coll. Adin, 20 Sep 2017; 2 specimens, MZB Cru Cir 037, Asilulu, 3°40'50.4"S, 127°55'27.6"E, coll. Adin, 20 Sep 2017; 10 specimens, MZB Cru Cir 038, Hila, 3°34'57.5"S, 128°05'31.9"E, coll. Adin, 20 Sep 2017; 7 specimens, MZB Cru Cir 039, Hatu, 3°43'52.7"S, 128°02'51.4"E, coll. Adin, 20 Sep 2017; 44 specimens, MZB Cru Cir 040, Mamala, 3°33'20.5"S, 128°11'32.8"E, coll. Adin, 20 Sep 2017; 38 specimens, MZB Cru Cir 041, Morella, 3°31'06.5"S, 128°13'18.0"E, coll. Adin, 20 Sep 2017; 25 specimens, MZB Cru Cir 042, Wakasihu, 3°46'27.6"S, 127°56'36.6"E, coll. Adin, 20 Sep 2017. *Pombo Island*: 4 specimens, MZB Cru Cir 043, Pombo, 3°31'55.5"S, 128°22'28.8"E, coll. P. Pitriana & D. Tala, 8 Sep 2016. *Saparua Island*: 32 specimens, MZB Cru Cir 044, Dermaga Ihamahu, 3°31'13.0"S, 128°41'14.9"E, coll. P. Pitriana & D. Tala, 11 Apr 2016; 31 specimens, MZB Cru Cir 045, Kulur, 3°29'48.5"S, 128°36'10.7"E, coll. P. Pitriana & D. Tala, 20 Sep 2016; 40 specimens, MZB Cru Cir 046, Waisisil, 3°34'48.6"S, 128°39'04.8"E, coll. P. Pitriana & D. Tala, 8 Apr 2016. *Seram Island*: 15 specimens, MZB Cru Cir 047, Desa Mur-naten, 2°51'48.8"S, 128°20'32.3"E, coll. P. Pitriana & D. Tala, 19 Sep 2017.

GenBank accession numbers. COI gene (MK995377–MK995388), 18S (MK981391–MK981400).

Diagnosis. Shell with six plates; rostrum and carina with radii; rostral lateral lacking radii; carinal lateral absent; base membranous; conical spines on cirrus I absent; basal guard on apex setae of cirrus II absent.

Description. Shell white to grey, surface with strong, radiating lines, orifice elliptical (Fig. 12a); parietes solid (Fig. 12b); scutum triangular, tergal margin straight; tergum triangular, scutal margin curved; conical spines on dorsal side of cirrus I absent, cirrus II with multi-cuspidate setae without basal guard. Basal length 2.4–5.1 mm, basal width 1.4–4.1 mm and height 0.8–1.7 mm. Orifice length 1.0–3.4 mm and orifice width 0.7–1.7 mm (measurements for 25 specimens are presented in Suppl. material 1: Table S11).

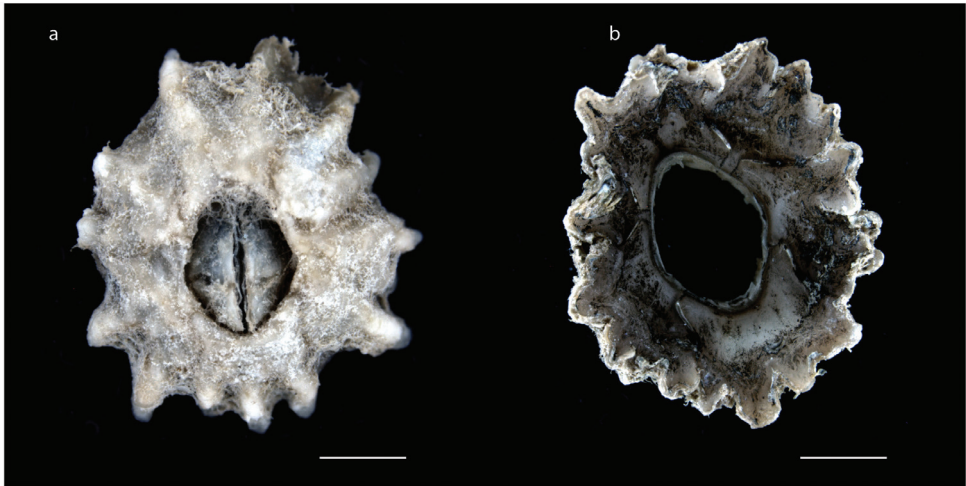


Figure 12. *Chthamalus moro* Pilsbry, 1916 (MZB Cru Cir 042-1) **a** upper view **b** lower view. Scale bar: 1 mm.

Distribution. *Chthamalus moro* is widely distributed in the Indo-Pacific-Indonesia, Philippines, Taiwan, Xisha Islands, Ryukyu Islands, Palau, Mariana Islands, Caroline Islands, Fiji, and Samoa (Southward and Newman 2003). In this study, *C. moro* was found on the islands of Ambon (at Hatu, Mamala, Alang, Asilulu, Hila, Morella, Wakasih), Pombo, Seram (at Murnaten), and Saparua (at Ihamahu, Kulur, and Waisisil) on mangroves, stone, port pole, mollusk shell, shells of *Tetracrita squamosa*, *Tesseropora rosea* and *Capitulum mitella* (a map with the occurrence of *Chthamalus moro* in the Moluccas is shown in Suppl. material 1: Fig. S3).

Remarks. Species of the genus *Chthamalus* are very difficult to distinguish in the field. *Chthamalus moro* has a stellate appearance and is smaller than *C. malayensis* (Southward and Newman 2003). In addition, conical spines on the dorsal side of cirrus I are absent and setae on cirrus II are without basal guards in *C. moro*.

Superfamily Tetracitoidea Gruvel, 1905

Family Tetracitidae Gruvel, 1903

Subfamily Tetracitellinae Newman & Ross, 1976

Genus *Tetracitella* Hiro, 1939

Subgenus *Tetracitella* Hiro, 1939

Tetracitella divisa (Nilsson-Cantell, 1921)

Figure 13a, b, Table 1: species no. 59

Tetracrita divisa Nilsson-Cantell, 1921: 362, fig. 83, pl. 3 fig. 11.

Tetracitella (*Tetracitella*) *divisa*: Ross and Perreault 1999: 6.

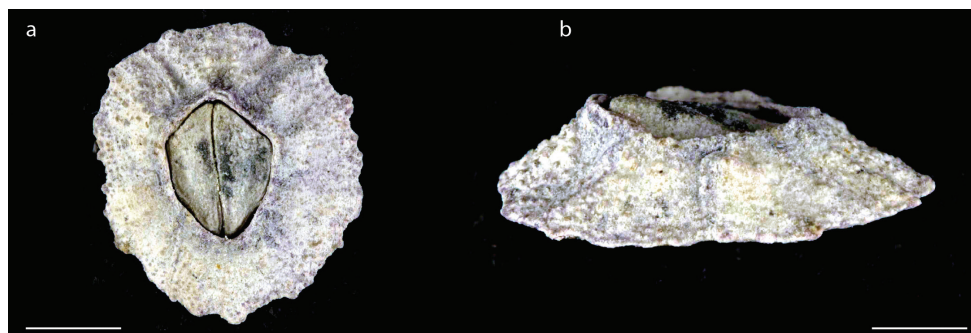


Figure 13. *Tetraclitella divisa* (Nilsson-Cantell, 1921) (MZB Cru Cir 120098) **a** upper view **b** side view. Scale bar: 4 mm.

Tetraclitella divisa: Ross 1968: 13; Dong et al. 1982: 111; Foster 1974: 45, figs 6E–F, 7E–F; Bacon et al. 1984: 86; Paulay and Ross 2003: 308; Chan et al. 2009a: 208, fig. 178.

Material examined. *Ambon Island*: 1 specimen, MZB Cru Cir 120, Laha, 3°43'22.5"S, 128°05'02.5"E, coll. P. Pitriana & D. Tala, 7 Sep 2016.

Diagnosis. Shell with four plates, flattened, not strongly articulated; radii tubiferous; summit of radii horizontal; tergal spur well separated from scutal margin.

Description. Shell depressed, covered by furry chitinous integument; shell plates with prominent radiating ribs; radii wide, porose, tubes running parallel to base of shell; colour of shell pale purplish; orifice diamond shaped (Fig. 13a, b); scutum triangular, tergal margin straight; tergum higher than wide, scutal margin straight, spur short; mandible with four teeth, second and third teeth bidentate; labrum with smooth cutting edge (measurements for one specimen are presented in Suppl. material 1: Table S12).

Distribution. *Tetraclitella divisa* was previously recorded from Western Africa, Java, Malaysia, Sumatra, Northern Australia, Singapore, South China Sea, China, Taiwan, Japan, the Pacific Ocean to Hawaii and Pitcairn (Jones and Hosie 2016). In this study, *T. divisa* was found on Ambon Island at Laha on a concrete wall at the port (a map with the occurrence of *Tetraclitella divisa* in the Moluccas is shown in Suppl. material 1: Fig. S2).

Remarks. *Tetraclitella divisa* exhibits a brooded phase to the cypris larval stage in the mantle cavity, whereas most other species release the first stage nauplius (Nilsson-Cantell 1921; Hiro 1939).

Tetraclitella karandei Ross, 1971

Figure 14a–g, Table 1: species no. 60

Tetraclitella (*Tetraclitella*) *karandei*: Ross & Perreault, 1999: 6.

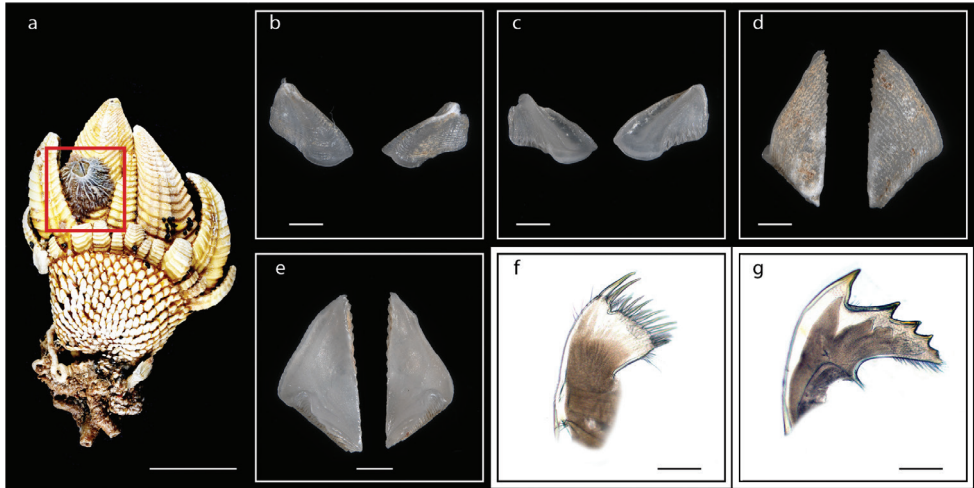


Figure 14. *Tetraclitella karandei* Ross, 1971 (MZB Cru Cir 122-2) on tergum of *Capitulum mitella* **a** upper view of *Tetraclitella karandei* on *Capitulum mitella* **b** external view of scutum **c** internal view of scutum **d** external view of tergum **e** internal view of tergum **f** maxillule **g** mandible. Scale bars: 15 mm (**a**); 1 mm (**b–e**); 0.25 mm (**f–g**).

Tetraclitella karandei Ross, 1971: 217, figs 2–3, 4A–J; Newmann and Ross 1979: 47; Chan et al. 2009a: 214, fig.184.

Material examined. *Ambon Island*: 10 specimens, MZB Cru Cir 121, Waitatiri, 3°37'04.0"S, 128°16'20.3"E, coll. P. Pitriana & D. Tala, 21 Sep 2017; 2 specimens, MZB Cru Cir 122, Asilulu, 3°40'50.4"S, 127°55'27.6"E, coll. Adin, 20 Sep 2017.

Diagnosis. Shell with four plates, tubiferous, not strongly articulated; radii tubiferous; summit of radii horizontal and elevated above the surface of the parietes; parietes with longitudinal ribs; scutum with nodose ornamentation.

Description. Shell with orifice diamond shaped, colour greyish (Fig. 14a); surface of parietes with chitinous coating and fine hairs, parietes with longitudinal ribs intercalated with lower secondary and tertiary ribs; radii broad, horizontally ridged from base to apex; scutum triangular, occluding and basal margins almost perpendicular, tergal margin straight, surface ornamentation nodose; tergum higher than wide, scutal margin straight, spur small; mandible with four teeth (Fig. 14g), labrum slightly notched, two small teeth on each cutting edge. Basal length 10.1–17.4 mm, basal width 8.2–18.2 mm, height 0.4–0.7 mm. Orifice length 3.8–5.6 mm, orifice width 2.7–5.2 mm (measurements for three specimens are presented in Suppl. material 1: Table S13).

Distribution. *Tetraclitella karandei* was previously recorded from India, Taiwan, the Philippine (Chan et al. 2009a). In this study, *T. karandei* was found on Ambon Island at Waitatiri and Asilulu on stone, on the shells of *Capitulum mitella* and *Euraphia hembeli* (a map with the occurrence of *Tetraclitella karandei* in the Moluccas is shown in Suppl. material 1: Fig. S2).

Remarks. *Tetraclitella karandei* can be distinguished by its radii, which are broad and have extended out and over the adjoining plates. The scutum is also unique because it has nodose ornamentation (Ross 1971).

Subfamily Tetraclitinae Gruvel, 1903

Genus *Tesseropora* Pilsbry, 1916

Tesseropora rosea (Krauss, 1848)

Figure 15a–e, Table 1: species no. 61

Conia rosea Krauss, 1848: 136.

Tetraclita rosea Darwin, 1854: 335, pl.10 fig. 3a–3d; Pilsbry 1916: 260, pl. 58 fig. 4.

Tesseropora rosea Newman & Ross, 1976: 47; Anderson and Anderson 1985: 89, figs 1–10; Jones and Anderson 1990: 13.

Material examined. *Ambon Island:* 6 specimens, MZB Cru Cir 075, Rutong, 3°42'23.7"S, 128°16'08.9"E, coll. P. Pitriana, 14 Jan 2016; 1 specimen, MZB Cru Cir 076, Leahari, 3°42'45.3"S, 128°16'16.5"E, coll. P. Pitriana, 14 Jan 2016; 25 specimens, MZB Cru Cir 077, Liang, 3°30'13.3"S, 128°20'34.1"E, coll. P. Pitriana & D. Tala, 7 Sept 2016. *Saparua Island:* 25 specimens, MZB Cru Cir 078, Dermaga Ihamahu, 3°31'13.0"S, 128°41'14.9"E, coll. P. Pitriana & D. Tala, 11 Apr 2016; 4 specimens, MZB Cru Cir 079, Kulur, 3°29'48.5"S, 128°36'10.7"E, coll. P. Pitriana & D. Tala, 20 Sep 2016; 10 specimens, MZB Cru Cir 080, Porto, 3°34'58.2"S, 128°36'58.2"E, coll. P. Pitriana & D. Tala, 20 Sep 2016.

GenBank accession number. COI gene (MK995370).

Diagnosis. Shell with four plates; wall of the parietes with a single row of parietal pore; orifice with traces of pink in colour; oral cone relatively broad; mouthparts relatively large.

Description. Shell steeply conical, whitish tinged pink, with longitudinal purple pinkish striations (Fig. 15a); four parietal plates with single row of large, square tubes, often eroded in upper areas giving pillared appearance (Fig. 15b, c); radii solid, well developed; orifice pentagonal in uneroded specimens, triangular in eroded specimens; basis mostly calcareous; scutum thick, articular furrow short, deep, articular ridge long, adductor ridge prominent, crests for lateral depressor faint; tergum with short, broad spur set close to basiscutal angle, wide articular furrow, carinal depressor crests prominent (Fig. 15d, e); maxillule with two large setae at the lateral angle; mandible with four teeth, labrum shallowly concave in shape, teeth on each side. Basal length 9.7–25.6 mm, basal width 9.7–24.5 mm, height 4.4–13.0 mm. Orifice length 2.9–7.8 mm, orifice width 2.3–6.9 mm (measurements for 15 specimens are presented in Suppl. material 1: Table S14).

Distribution. *Tesseropora rosea* was originally described from a specimen collected at Algoa Bay, South Africa (Krauss 1848; Darwin 1854) and has since been recorded



Figure 15. *Tesseropora rosea* (Krauss, 1848) (MZB Cru Cir 077-1) **a** upper view **b** lower view **c** side view **d** external view of scutum and tergum **e** internal view of scutum and tergum. Scale bars: 6 mm (**a–c**); 2 mm (**d, e**).

from Australia (SW and SE); Lord Howe Island and the Kermadec Islands (Jones 1990). In this study, *T. rosea* was found on Ambon Island (at Rutong, Leahari, and Liang) and Saparua Island (at Ihamahu, Kulur, and Porto) on stone and mollusc shells (a map with the occurrence of *Tesseropora rosea* in the Moluccas is shown in Suppl. material 1: Fig. S4).

Remarks. According to Anderson and Anderson (1985), *T. rosea* feeds in different ways, extending the cirral fan only in response to the fast water currents. Thus, *T. rosea* cannot survive in areas with a low current velocity. *Tesseropora rosea* exhibits a wide distribution although the species is represented by relatively few specimens.

Genus *Tetraclita* Schumacher, 1817

Tetraclita kuroshioensis Chan, Tsang & Chu, 2007

Figure 16a–g, Table 1: species no. 62

Tetraclita squamosa viridis: Hiro 1936b: 635.

Tetraclita squamosa squamosa: Utinomi 1968a: 178.

Tetraclita pacifica Chan et al., 2007a: 88, figs 4–6.

Tetraclita kuroshioensis Chan et al., 2007: 56; Chan et al. 2009a: 192, fig. 164; Pochai et al. 2017: 21, fig. 6.

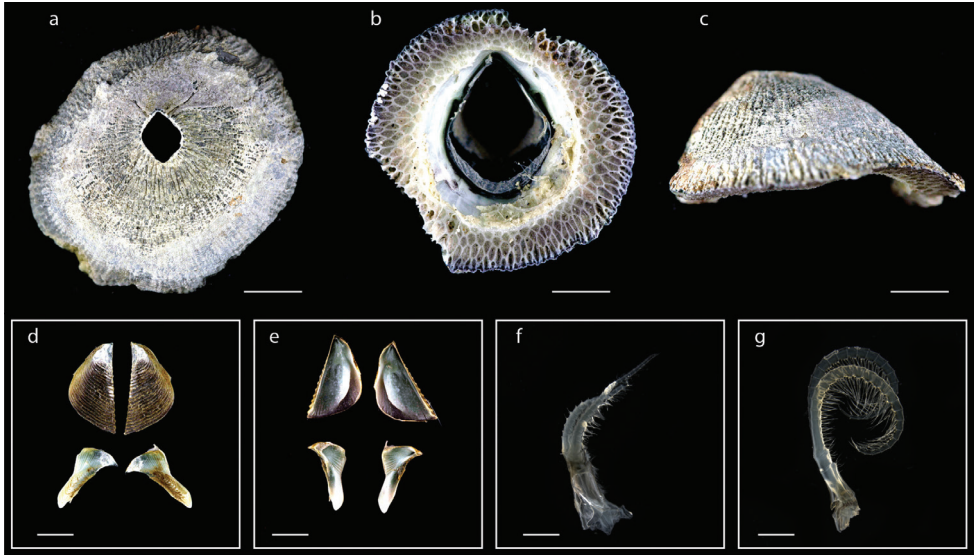


Figure 16. *Tetracrita kuroshioensis* Chan, Tsang & Chu, 2007 (MZB Cru Cir 097) **a** upper view **b** lower view **c** side view **d** external view of scutum and tergum **e** internal view of scutum and tergum **f** cirrus I **g** cirrus VI. Scale bars: 7 mm (**a–c**); 2 mm (**d–e**); 1 mm (**f, g**).

Material examined. *Ambon Island:* 1 specimen, MZB Cru Cir 097, Hatu, 3°43'52.7"S, 128°02'51.4"E, coll. Adin, 20 Sep 2017; 6 specimens, MZB Cru Cir 098, Ureng, 3°40'14.0"S, 127°56'47.6"E, coll. Adin, 20 Sep 2017. *Saparua Island:* 1 specimen, MZB Cru Cir 100, Dermaga Ihmahu, 3°31'13.0"S, 128°41'14.9"E, coll. P. Pitriana & D. Tala, 11 Apr 2016.

GenBank accession numbers. COI gene (MK995363, MK995364, MK995367), 18S (MK981375, MK9876, MK981379).

Diagnosis. Shell conical with four plates, tubiferous; radii solid; tergum broad, apex not beaked.

Description. Shell with four inseparable, multi-tubiferous plates, greyish black to purplish-grey or deep green to green, surfaces with mosaic scales pattern radiating randomly from base to apex, internal surface of parietes smooth, white with dark grey striations around aperture; radii solid (Fig. 16a–c); basis membranous; scutum larger than tergum, triangular, external surface with horizontal striations, occluding margin with fine teeth; tergum broad, higher than wide, apex not produced as beak, spur sharp, basi-scutal angle smaller than that of *Tetracrita squamosa* (Fig. 16d, e); external surface of operculum grey and yellowish-light brown, internal surface greyish-dusky green; mandible with four large teeth; maxillule not notched with eleven setae; labrum with five small teeth on each side; cirrus I possessing serrulate setae. Basal length 12.1–21.6 mm, basal width 18.1–21.8 mm, height 7.3–10.4 mm. Orifice length 3.2–5.3 mm, orifice width 2.4–4.2 mm (measurements for five specimens are presented in Suppl. material 1: Table S15).

Distribution. *Tetraclita kuroshioensis* was previously recorded from Japan, Taiwan, Palau, and Thailand (Chan et al. 2009a; Pochai et al. 2017). In this study, *T. kuroshioensis* was found on Ambon Island (at Hatu and Ureng) and Saparua Island (at Dermaga Ihamahu) on rocks and concrete wall of a port (a map with the occurrence of *Tetraclita kuroshioensis* in the Moluccas is shown in Suppl. material 1: Fig. S4).

Remarks. *Tetraclita kuroshioensis* and *T. squamosa* share great morphological similarity. However, DNA sequences separate the two species (Chan et al. 2007), which was confirmed in this study (Fig. 29). Morphologically, the shape of the tergum is definitive; that of *T. kuroshioensis* is broader and the apex blunter compared to *T. squamosa* (Chan et al. 2007).

Tetraclita squamosa (Bruguère, 1789)

Figure 17a–k, Table 1: species no. 63

Balanus squamosus Bruguère, 1789: 170, pl. 165 figs 9, 10.

Lepas fungites Spengler, 1790: 189.

Lepas porosa Gmelin, 1791: 3212; Wood 1815: pl. 9 fig. 4.

Tetraclita squamulosa Schumacher, 1817: 91.

Asemus porosus: Ranzani 1820: pl. 3 figs 32–35.

Conia porosa: Sowerby 1823: pl. 1.

Tetraclita porosa var. (3) *viridis* Darwin, 1854a: 329.

Tetraclita porosa viridis: Nilsson-Cantell 1921: 364.

Tetraclita squamosa squamosa: Pilsbry 1916: 251; Dong et al. 1982: 110, fig.

Tetraclita squamosa forma *viridis*: Broch 1922: 337.

Tetraclita squamosa viridis: Hiro 1936b: 635.

Tetraclita porosa perfecta Nilsson-Cantell, 1931a: 133, pl. II fig. 8a–e.

Tetraclita squamosa: Stebbing 1910: 570; Ren and Liu 1979: 339, pl. 1 figs 1–11;

Yamaguchi 1987: 344; Zevina et al. 1992: 45, fig. 30; Chan 2001: 625, fig. 8;

Chan et al. 2007a: 82, fig. 4; Chan et al. 2009a: 195, fig. 167; Pochai et al. 2017: 25, fig. 8.

Material examined. Ambon Island: 17 specimens, MZB Cru Cir 081, Alang, 3°45'11.0"S, 128°01'23.1"E, coll. Adin, 20 Sep 2017; 15 specimens, MZB Cru Cir 082, Dermaga Liang, 3°30'13.3"S, 128°20'34.1"E, coll. P. Pitriana & D. Tala, 30 Aug 2016; 2 specimens, MZB Cru Cir 083, Dermaga Tulehu, 3°35'21.8"S, 128°20'02.8"E, coll. P. Pitriana & D. Tala, 7 Sep 2016; 15 specimens, MZB Cru Cir 084, Doc Tawiri, 3°42'10.1"S, 128°06'13.4"E, coll. P. Pitriana & D. Tala, 29 Mar 2016; 2 specimens, MZB Cru Cir 085, Gudang Arang, 3°42'07.2"S, 128°09'43.7"E, coll. P. Pitriana & D. Tala, 5 Sep 2016; 5 specimens, MZB Cru Cir 086, Hila, 3°34'57.5"S, 128°05'31.9"E, coll. Adin, 20 Sep 2017; 17 specimens, MZB Cru Cir 087, Hutumuri, 3°41'47.6"S, 128°17'44.1"E, coll. P. Pitriana, 14 Jan 2016; 5 specimens, MZB Cru Cir 088, Leahari, 3°42'45.3"S, 128°16'16.5"E, coll. P. Pitriana, 14 Jan 2016; 6 specimens, MZB Cru Cir 089, Tawiri, 3°42'10.1"S, 128°06'13.4"E, coll. P. Pitriana & D. Tala, 5 Sep

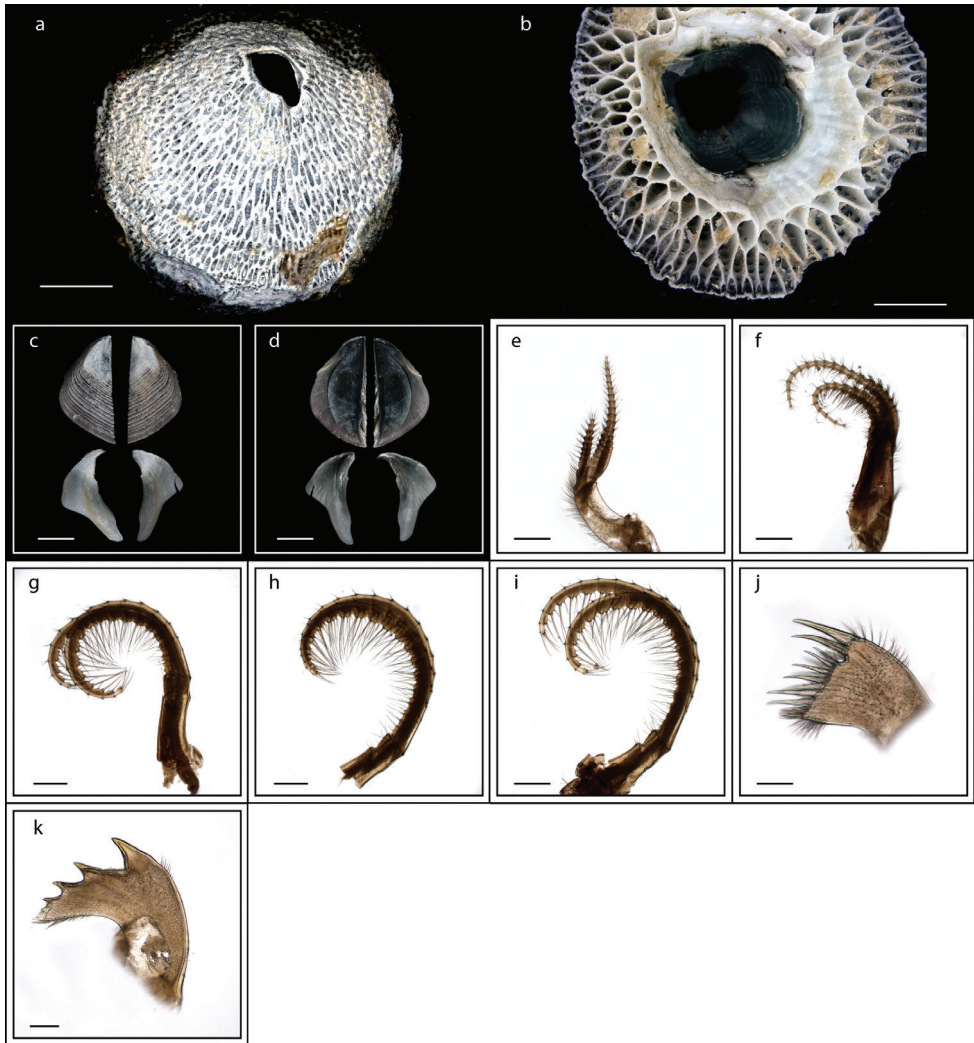


Figure 17. *Tetralita squamosa* (Bruguière, 1789) (MZB Cru Cir 081-3) **a** upper view **b** lower view **c** external view of scutum and tergum **d** internal view of scutum and tergum **e** cirrus I **f** cirrus II **g** cirrus III **h** cirrus IV **i** cirrus VI **k** maxillule **l** mandible. Scale bars: 8 mm (**a**, **b**); 2 mm (**c**, **d**); 1 mm (**e**, **f**); 2 mm (**g**–**i**); 0.5 mm (**j**, **k**).

2016; 3 specimens, MZB Cru Cir 090, Tulehu, 3°35'21.8"S, 128°20'02.8"E, coll. P. Pitriana & D. Tala, 7 Sep 2016; 7 specimens, MZB Cru Cir 091, Waai, 3°33'23.5"S, 128°19'33.9"E, coll. P. Pitriana & D. Tala, 7 Sep 2016; 2 specimens, MZB Cru Cir 092, Waai, 3°33'23.5"S, 128°19'33.9"E, coll. P. Pitriana & D. Tala, 31 Mar 2017; 45 specimens, MZB Cru Cir 096, Asilulu, 3°40'50.4"S, 127°55'27.6"E, coll. Adin, 20 Sep 2017; 40 specimens, MZB Cru Cir 099, Wakasih, 3°46'27.6"S, 127°56'36.6"E, coll. Adin, 20 Sep 2017. **Saparua Island:** 5 specimens, MZB Cru Cir 093, Benteng Durstede, 3°34'32.8"S, 128°39'34.7"E, coll. P. Pitriana & D. Tala, 8 Apr 2016; 6 specimens, MZB Cru Cir 094, Teluk Saparua, 3°34'25.7"S, 128°39'25.8"E, coll. P.

Pitriana & D. Tala, 8 Apr 2016; 5 specimens, MZB Cru Cir 095, Teluk Saparua, 3°34'25.7"S, 128°39'25.8"E, coll. P. Pitriana & D. Tala, 22 Sept 2016.

GenBank accession numbers. COI gene (MK995360–MK995362), 18S (MK981368–MK981373).

Diagnosis. Shell conical with four plates, tubiferous; radii solid; tergum narrow, concaved, apex beaked.

Description. Shell consisting of four fused, inseparable plates (Fig. 17a), parietes with eight rows of multi-tubiferous parietal tubes (Fig. 17b), external surface with longitudinal lines from base to apex, internal surface smooth, white with purplish grey striations close to aperture; orifice rhomboidal; basis membranous; shell greenish with brownish grey (Fig. 17a), external surface of operculum brownish grey, internal surface purplish grey; scutum triangular, larger than tergum, external surface with horizontal striations, occluding margin with very shallow teeth; tergum narrow, apex beaked, spur long, sharp (Fig. 17c, d); mandible with four large teeth, first tooth with three small spines, lower margin pectinate (Fig. 17k); maxillule notched with two large setae above notch, eleven small setae below notch (Fig. 17j); labrum with four large teeth on each side of notch; cirrus I with bidentate serrulate setae (Fig. 17e). Basal length 20.1–30.2 mm, basal width 19.3–28.3 mm, height 11.9–14.2 mm. Orifice length 4.4–7.9 mm, orifice width 3.4–7.2 mm (measurements for five specimens are presented in Suppl. material 1: Table S16).

Distribution. *Tetraclita squamosa* is widespread in the Indo-Pacific region, Australia, South China coast, and Taiwan (Newman 1978; Jones et al. 2001; Chan et al. 2009a). In this study, *T. squamosa* was found on Ambon Island (at Alang, Dermaga Liang, Dermaga Tulehu, Doc Tawiri, Gudang Arang, Hila, Hutumuri, Leahari, Tawiri, Tulehu, Waai, Asilulu, Wakasih) and Saparua Island (at Benteng Duurstede and Teluk Saparua) on stone, rocks, shipyards, concrete bridges and walls of the port (a map with the occurrence of *Tetraclita squamosa* in the Moluccas is shown in Suppl. material 1: Fig. S4).

Remarks. *Tetraclita squamosa* has characteristic green parietes (Yamaguchi 1987) and a wide distribution throughout the Indo-Pacific (Newman and Ross 1976). However, the taxonomy of *Tetraclita squamosa* has been confusing due to a high degree of morphological variation, and it is now considered a species complex. *Tetraclita squamosa* and *Tetraclita japonica* can be separated using characters such as the shape of the parietes, scutum geometry, and mandible structures (Darwin 1854; Pilsbry 1916). In addition, a key character for *T. squamosa* is the tergum with a beak on its apex (Chan et al. 2009a).

Subfamily Newmanellinae Ross & Perreault, 1999

Genus *Yamaguchiella* Ross & Perreault, 1999

Yamaguchiella coerulescens (Spengler, 1790)

Figure 18a–g, Table 1: species no. 64

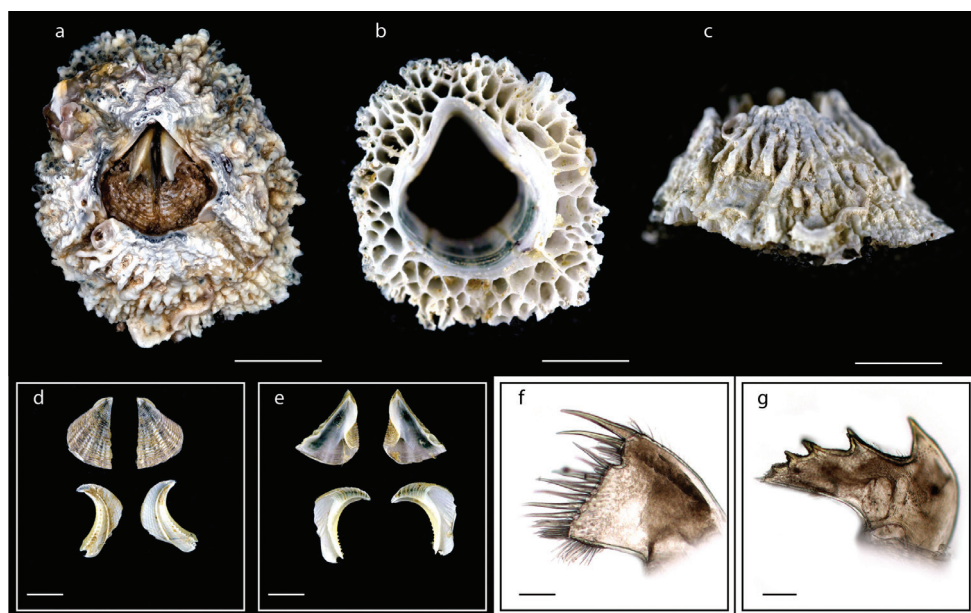


Figure 18. *Yamaguchiella coerulescens* (Spengler, 1790) (MZB Cru Cir 123-2) **a** Upper view **b** lower view **c** side view **d** external view of scutum and tergum **e** internal view of scutum and tergum **f** maxillule **g** mandible. Scale bars: 7 mm (**a–c**); 1 mm (**d, e**); 0.5 mm (**f, g**).

Lepas coerulescens Spengler, 1790: 191.

Tetraclita coerulescens: Darwin 1854: 342, pl. 11 figs 4a–d; Hoek 1883: 161, pl. 13 fig. 34; Pilsbry 1916: 259; Nilsson-Cantell 1938: 77; Newman and Ross 1976: 47; Dong et al. 1982: 111; Zevina et al. 1992: 48, fig. 31.

Yamaguchiella (*Yamaguchiella*) *coerulescens*: Ross and Perreault 1999: 5; Jones and Hosie 2016: 271; Chan et al. 2009a: 202, fig. 173.

Material examined. *Ambon Island*: 13 specimens, MZB Cru Cir 123, Gudang Arang, 3°42'07.2"S, 128°09'43.7"E, coll. P. Pitriana & D. Tala, 5 Sep 2016; 4 specimens, MZB Cru Cir 124, Dermaga Tulehu, 3°35'05.4"S, 128°19'43.3"E, coll. P. Pitriana & D. Tala, 7 Sep 2016; 5 specimens, MZB Cru Cir 125, Tulehu, 3°35'21.8"S, 128°20'02.8"E, coll. Adin, 19 Sep 2017; 14 specimens, MZB Cru Cir 126, Doc Tawiri, 3°42'10.1"S, 128°06'13.4"E, coll. P. Pitriana & D. Tala, 29 Mar 2016; 8 specimens, MZB Cru Cir 127, Tawiri, 3°42'10.1"S, 128°06'13.4"E, coll. P. Pitriana & D. Tala, 5 Sep 2016; 2 specimens, MZB Cru Cir 128, Galala, 3°41'22.2"S, 128°10'52.6"E, coll. P. Pitriana & D. Tala, 6 Sep 2016; 11 specimens, MZB Cru Cir 129, Waai, 3°33'23.5"S, 128°19'33.9"E, coll. P. Pitriana & D. Tala, 7 Sep 2016; 4 specimens, MZB Cru Cir 130, Pelabuhan Yos Sudarso, 3°41'36.5"S, 128°10'35.6"E, coll. P. Pitriana & D. Tala, 6 Sep 2016. *Saparua Island*: 1 specimen, MZB Cru Cir 131, Kulur, 3°29'48.5"S, 128°36'10.7"E, coll. P. Pitriana & D. Tala, 20 Sep 2016.

GenBank accession number. 18S (MK981381).

Diagnosis. Shell with the upper part tinged greenish-blue, longitudinally ribbed; radii moderately wide, with their summits oblique; scutum with a small adductor and extremely prominent articular ridge, united together and forms a small sub-cylindrical cavity; tergum with the spur not joined to the basi-scutal angle.

Description. Shell low conical to cylindro-conic (Fig. 18c) with four multi-tubiferous plates (Fig. 18b), parieties greenish or greyish with radiating lines (Fig. 18a); radii wide, summits oblique; basis calcareous, radii wide, tubiferous, summits oblique; orifice occluded wholly by scutum (Fig. 18a); scutum and tergum strongly articulated (Figs 18d, e); mandible with four teeth, the third teeth is tridentate (Fig. 18g); labrum with three large teeth on each side of cutting edge; penis with basidorsal point. Basal length 8.3–29.0 mm, basal width 8.5–27.8 mm, height 5.7–17.7 mm. Orifice length 4.2–11.5 mm, orifice width 3.2–11.8 mm (measurements for 25 specimens are presented in Suppl. material 1: Table S17).

Distribution. *Yamaguchiella coerulescens* was previously recorded from the Indo-west Pacific: the Indian Ocean, Bay of Bengal, Mergui Archipelago, Kei Islands, Banda Island, Malay Archipelago, Sulu Archipelago, Vietnam, China, Philippines, Goram Island, Palao Island, and Taiwan (Jones and Hosie 2016). In this study, *Y. coerulescens* was found on Ambon Island (at Tulehu, Dermaga Gudang Arang, Waai, Doc. Tawiri, Liang, Galala, Dermaga Yos Sudarso) and Saparua Island (at Kulur) on stone (a map with the occurrence of *Yamaguchiella coerulescens* in the Moluccas is shown in Suppl. material 1: Fig. S6).

Remarks. The subgenus *Yamaguchiella* was proposed by Ross and Perreault (1999) in honour of Toshiyuki Yamaguchi (Chiba University Japan), in appreciation of his contributions to the knowledge of recent and fossil barnacles.

Genus *Yamaguchiella* Ross & Perreault, 1999

Subgenus *Neonrosella* Jones, 2010

Neonrosella vitiata (Darwin, 1854)

Figure 19a–f, Table 1: species no. 65

Tetraclita vitiata Darwin, 1854: 340, pl. 11 fig. 3a–e.

Tetraclita (*Tetraclita*) *vitiata*: Rosell 1972: 214.

Newmanella vitiata: Ikeya and Yamaguchi 1993: 93; Jones et al. 1990: 14.

Yamaguchiella (*Rosella*) *vitiata*: Ross & Perreault, 1999: 5.

Yamaguchiella (*Neonrosella*) *vitiata*: Jones 2010: 214.

Neonrosella vitiata: Sukparangsi et al. 2019:4, figs 1–4.

Material examined. *Ambon Island*: 3 specimens, MZB Cru Cir 132, Liang, 3°30'13.3"S, 128°20'34.1"E, coll. P. Pitriana & D. Tala, 7 Sep 2016. *Banda Neira Island*: 1 specimen, MZB Cru Cir 133, Banda Neira, 4°31'22.8"S, 129°53'52.5"E, coll. P. Pitriana, 25 May 2016. *Saparua Island*: 4 specimens, MZB Cru Cir 134, Tuhaha, 3°32'38.1"S, 128°40'58.0"E, coll. P. Pitriana & D. Tala, 21 Sep 2016.

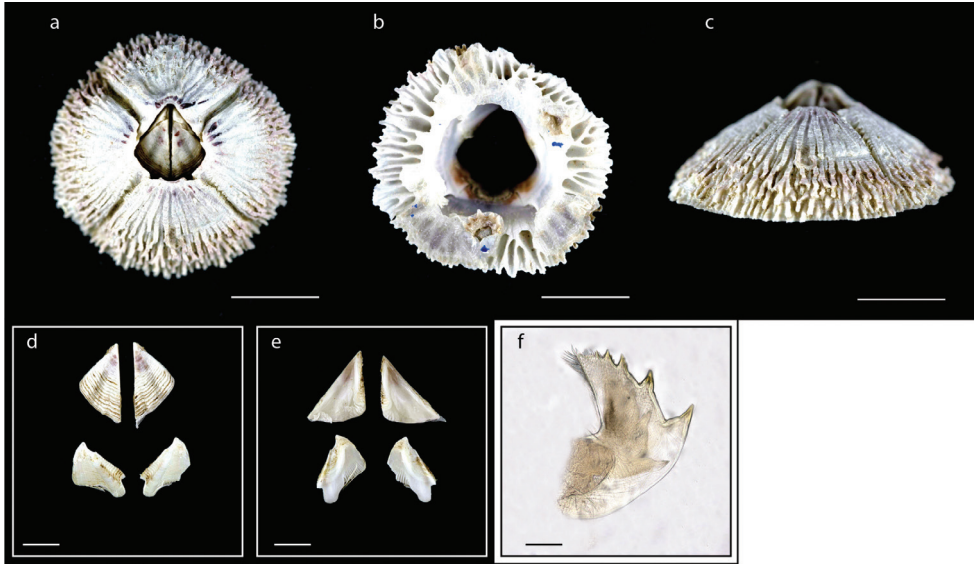


Figure 19. *Neonrosella vitiata* (Darwin, 1854) (MZB Cru Cir 132-3) **a** upper view **b** lower view **c** side view **d** external view of scutum and tergum **e** internal view of scutum and tergum **f** mandible. Scale bars: 8 mm (**a–c**); 1 mm (**d, e**); 0.5 mm (**f**).

GenBank accession number. 18S (MK981384).

Diagnosis. Parietes low with wall spreading; peritreme slightly toothed; base calcareous with two rows of irregular shape and size of parietal tubes; tergum with broad spur; lateral scutal depressor crests numerous and deep; five toothed mandibles; segments of posterior cirri with four pairs spines.

Synoptic description. Shell four plated, conical, whitish with spots of purple in upper part (Fig. 19a); parietal tubes irregular shape, size unequal (Fig. 19b); radii moderately wide, summits oblique, interior of irregularly branching ridges with solid interspaces (Fig. 19c); orifice trigonal; scutum and tergum coalesced, strongly articulated (Fig. 19d, e); tergum with broad spur, lateral tergal depressor crests on basal margin long, with numerous, deep crests, lateral scutal depressor crests numerous, deep; mandible with five teeth (Fig. 19f). Basal length 15.4–22.8 mm, basal width 13.8–22.8 mm, height 5.3–6.9 mm. Orifice length 5.2–6.9 mm, orifice width 4.6–5.4 mm (measurements for four specimens are presented in Suppl. material 1: Table S18).

Distribution. *Neonrosella vitiata* was previously recorded from the Indo-west Pacific, Indian Ocean, Nicobar Island to Australia, Indonesia, Malay Archipelago, Sulu Archipelago, Philippines, and the Pacific Ocean (Jones and Hosie 2016). Recently, *Neonrosella vitiata* also was discovered in the Andaman Sea of Thailand (Sukparangsi et al. 2019). In this study, *N. vitiata* was found on Ambon Island (at Liang), Banda Island and Saparua Island (at Tuhaha) on port poles, reef and stones (a map with the occurrence of *Neonrosella vitiata* in the Moluccas is shown in Suppl. material 1: Fig. S7).

Remarks. *Neonrosella vitiata* can be distinguished by its irregular parietal tubes, the shape of the terga, the five toothed mandibles and four pairs of spines on the segments of the posterior cirri (Darwin 1854).

Genus *Newmanella* Ross, 1969

Newmanella spinosus Chan & Cheang, 2016

Figure 20a–e, Table 1: species no. 66

Newmanella radiata: Chan et al. 2009: 199, fig. 170.

Newmanella sp. Tsang et al., 2015: 325, fig. 1A; 327 fig. 2.

Newmanella spinosus Chan & Cheang, 2016: 212, figs 9–15; Pochai et al. 2017: 20, fig. 5; Sukparangsi et al. 2019: 10, figs 5–8.

Material examined. *Ambon Island*: 5 specimens, MZB Cru Cir 072, Rutong, 3°42'23.7"S, 128°16'08.9"E, coll. P. Pitriana, 14 Jan 2016.

Diagnosis. Shell low conical to cylindro-conical; parietes discrete; base calcareous; radii broad; scutum with very deep depressor muscle crests; cirrus II and cirrus IV having numerous triangular spines; fourth and fifth teeth of mandible separated; cutting edge of maxillule below notch protruding; intromittent organ of penis lacking basi-dorsal point.

Description. Shell low conical, four plates externally greyish in colour, parietes with deep longitudinal, radiating lines from base to apex, internally with multiple rows of irregular parietal tubes (Fig. 20a, b); radii wide with horizontal striations, summits oblique (Fig. 20c); scutum triangular, external surface with horizontal striations, adductor ridge conspicuous; tergum high, narrow, basal margin with well-developed depressor muscle crests projecting beyond border; orifice pentagonal (Fig. 20d, e); basis calcareous, tubiferous, tubes in single layer; mandible with five teeth, the first tooth is the largest and separated from the rest, while the fifth tooth is the smallest and located at the middle of lower margin; labrum with V-shaped notch, two large teeth on the right side, five teeth on the left side of cutting margin; penis without basidorsal point, with few bundles of setae distally. Basal length 17.4–20.9 mm, basal width 15.9–20.5 mm, height 6.8–8.9 mm. Orifice length 5.3–7.3 mm, orifice width 5.0–6.7 mm (measurements for five specimens are presented in Suppl. material 1: Table S19).

Distribution. *Newmanella spinosus* was previously recorded from Japan, Taiwan, Philippines, and Thailand (Chan and Cheang 2016; Pochai et al. 2017). In the current study, this range is extended to Rutong (on stones and reef surface), Ambon Island (a map with the occurrence of *Newmanella spinosus* in the Moluccas is shown in Suppl. material 1: Fig. S2).

Remarks. *Newmanella spinosus* is morphologically close to *Newmanella radiata* but it can be distinguished by the morphology of the scutum, tergum, cirrus II, mandible and maxillule. *N. spinosus* also has numerous spines on its cirri, especially on cirrus II, which is different from *N. radiata* (Chan and Cheang 2016).

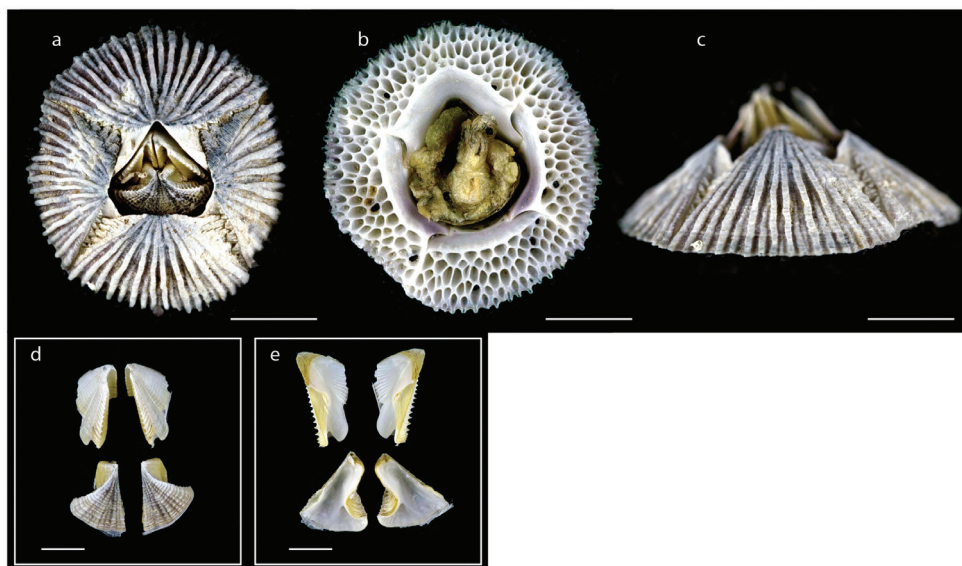


Figure 20. *Newmanella spinosus* Chan & Cheang, 2016 (MZB Cru Cir 072-1) **a** upper view **b** lower view **c** side view **d** external view of scutum and tergum **e** internal view of scutum and tergum. Scale bars: 7 mm (**a–c**); 2 mm (**d, e**).

Family Balanidae Leach, 1817

Subfamily Amphibalaninae Pitombo, 2004

Genus *Amphibalanus* Pitombo, 2004

Amphibalanus amphitrite (Darwin, 1854)

Figure 21a–h, Table 1: species no. 87

Balanus amphitrite Darwin, 1854: 240 (part.), pl. 5. figs 2a–d, i–k, m–o; Weltner 1897:264; Hoek 1913: 167; Pilsbry 1916: 89; Zevina et al. 1992: 89, fig. 61; Puspasari et al. 2001b: 7.

Balanus amphitrite var. (1) *communis* Darwin, 1854: 240, pl. 5 fig. 2e, h, l.

Balanus amphitrite communis: Nilsson-Cantell 1921: 311, fig. 64.

Balanus amphitrite forma *hawaiiensis* Broch, 1922: 314, fig. 56 (part.).

Balanus amphitrite forma *denticulata* Broch, 1927b: 133, fig. 14 (part.).

Balanus amphitrite hawaiiensis: Hiro 1937c: 432, figs 20, 21.

Balanus amphitrite cochinchinensis Nilsson-Cantell, 1938b: 43, fig. 11a–e.

Balanus amphitrite var. *fluminensis* Oliveira, 1941: 21, pl. 4 fig. 4, pl. 5 figs 1, 2, pl. 8 figs 1–5.

Balanus amphitrite var. *aeratus* Oliveira, 1941: 22, pl. 4 fig. 5, pl. 9 figs 1–4.

Balanus amphitrite herzi Rogers, 1949: 8, pl. 1 figs 6, 12–15.

Balanus amphitrite franciscanus Rogers, 1949: 9, pl. 1 figs 5, 7, 16–19.

Balanus amphitrite var. *columnarius* Tarasov & Zevina, 1957: 179, 184, fig. 68 a–e.

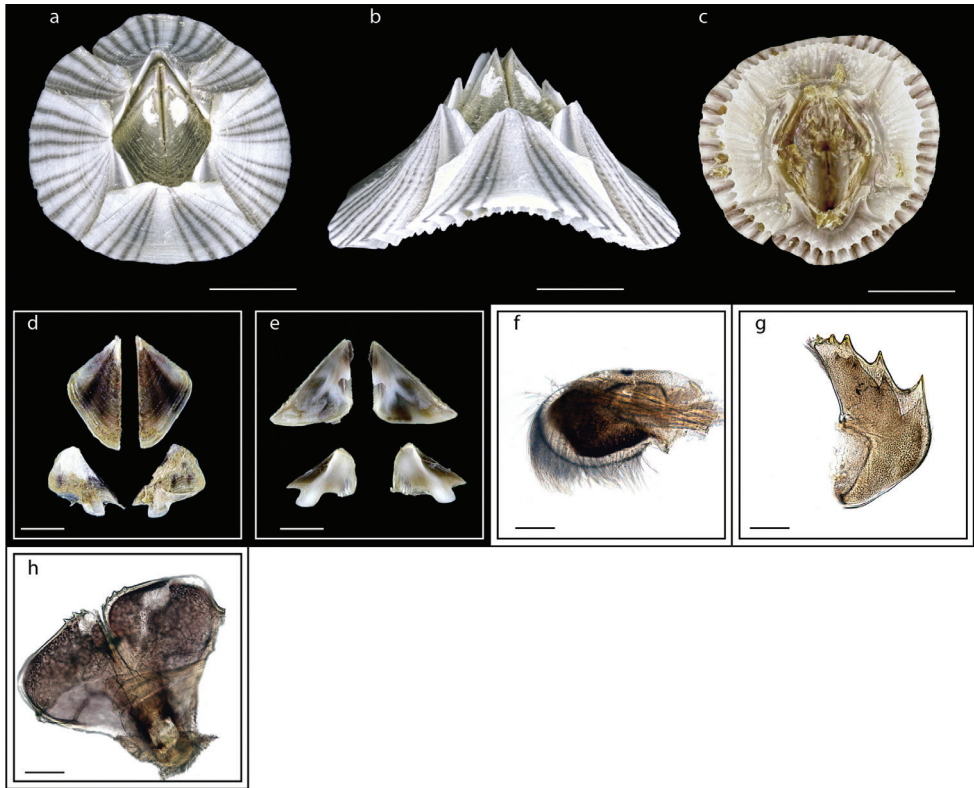


Figure 21. *Amphibalanus amphitrite* (Darwin, 1854) (MZB Cru Cir 005-4) **a** upper view **b** side view **c** lower view **d** external view of scutum and tergum **e** internal view of scutum and tergum **f** maxilla **g** mandible **h** labrum. Scale bars: 4 mm (**a–c**); 1 mm (**d, e**); 0.5 mm (**f–h**).

Balanus amphitrite denticulata Henry, 1959: 192, pl. 1 fig. 5, pl. 3 fig. 7, upper row right.

Balanus amphitrite amphitrite: Harding 1962: 274, pl. 1a–g, pl. 2a–k; Dong et al. 1982: 90, fig. A–E; Rosell 1981: 302.

Balanus amphitrite var. *hawaiiensis*: Stubbings 1963b: 15.

Amphibalanus amphitrite: Pitombo 2004: 263, 274, figs 2A, B, 7A, B, 8C; Chan et al. 2009a: 241; Chen et al. 2014: 1071; Shahdadi et al. 2014: 213; Pochai et al. 2017: 27, fig. 9; Xu 2017: 48.

Material examined. *Ambon Island*: 4 specimens, MZB Cru Cir 005, Galala, 3°41'22.2"S, 128°10'52.6"E, coll. P. Pitriana & D. Tala, 6 Sep 2016. *Saparua Island*: 4 specimens, MZB Cru Cir 007, Desa Mahu, 3°32'19.6"S, 128°41'17.3"E, coll. P. Pitriana & D. Tala, 11 Apr 2016; 5 specimens, MZB Cru Cir 008, Negeri Mahu, 3°31'52.9"S, 128°41'12.4"E, coll. P. Pitriana & D. Tala, 11 Apr 2016; 2 specimens, MZB Cru Cir 009, Tuhaha, 3°32'38.1"S, 128°40'58.0"E, coll. P. Pitriana & D. Tala, 21 Sep 2016.

Diagnosis. Primary parietal tubes with transverse septa; exterior of shell with longitudinal purple striations, horizontal striations absent; tergum short with wide spur; cirri III–VI with erect teeth below posterior angles of distal; cirrus III without complex setae.

Description. Shell six plated, conical, round, externally smooth, white with groups of well-spaced, dark purple vertical stripes, horizontal striations on shell surface absent (Fig. 21a, b), interior of parietes with single row of tubes (Fig. 21c); radii solid, wide; alae with summits moderately oblique (Fig. 21a, b); basis porous, calcareous; scutum externally striped, internally with prominent articular ridge 3/5 length of tergal margin, well separated from straight adductor ridge, occluding margin toothed, lateral depressor muscle pit small (Fig. 21d, e); tergum with spur wider than long, less than its own width from basi-scutal angle (Fig. 21d, e); mandible with four teeth (Fig. 21g); labrum multi-denticulate (Fig. 21h). Basal length 3.1–17.8 mm; basal width 2.8–17.6 mm; height 2.1–10.8 mm; orifice length 1.5–8.1 mm; orifice width 1.5–5.4 mm (measurements for 15 specimens are presented in Suppl. material 1: Table S20).

Distribution. *Amphibalanus amphitrite* is commonly found on beaches and in estuaries, lives attached to harsh natural substrate, such as bedrock, rocks, shells of molluscs, as well as the roots and trunks of mangrove trees. Many specimens also stick to artificial substrates, such as ship hulls and the walls and pillars of docks. *Amphibalanus amphitrite* is spread globally tropical and subtropical waters (Zullo et al. 1972; Henry and McLaughlin 1975; Chen et al. 2014). In this study, *A. amphitrite* was found on the islands of Ambon (at Galala) and Saparua (at Desa Mahu, Negeri Mahu and Tuhaha) on stone, mollusc shells and the capitulum of *Lepas anserifera* (a map with the occurrence of *Amphibalanus amphitrite* in the Moluccas is shown in Suppl. material 1: Fig. S4).

Remarks. *Amphibalanus amphitrite* is difficult to distinguish from two other members of the subgenus *Balanus*, i.e., *Balanus crenatus* Bruguière, 1789 and *Balanus trigonus* Darwin, 1854. However, *A. amphitrite* can usually be distinguished from the other species by the multi-denticulated labrum and also by the colour pattern of the parietes and sheath (Henry and McLaughlin 1975).

Amphibalanus reticulatus (Utinomi, 1967)

Figure 22a–o, Table 1: species no. 88

Balanus amphitrite var. (1) *communis* Darwin, 1854: 240, pl. 5 fig. 2e, h, l (part.).

Balanus amphitrite forma *communis*: Broch 1922: 314 (part.).

Balanus amphitrite forma *hawaiiensis* Broch, 1922: 314 (part.).

Balanus amphitrite communis: Hiro 1938a: 301, fig. 1a, b.

Balanus amphitrite cirratus: Zevina and Tarasov 1963: 89, fig. 10a–e.

Balanus amphitrite var. *variegatus*: Stubbings 1963a: 329, fig. 2a–e.

Balanus amphitrite variety: Southward and Crisp 1963: 43, fig. 23.

Balanus amphitrite tessellatus Utinomi, 1964: 52, pl. 26 fig. 11.

Balanus amphitrite var. *denticulata*: Karande & Palekar, 1966: 145, fig. 7, pl. 1 fig. 7, pl. 4 row 5 (part.).

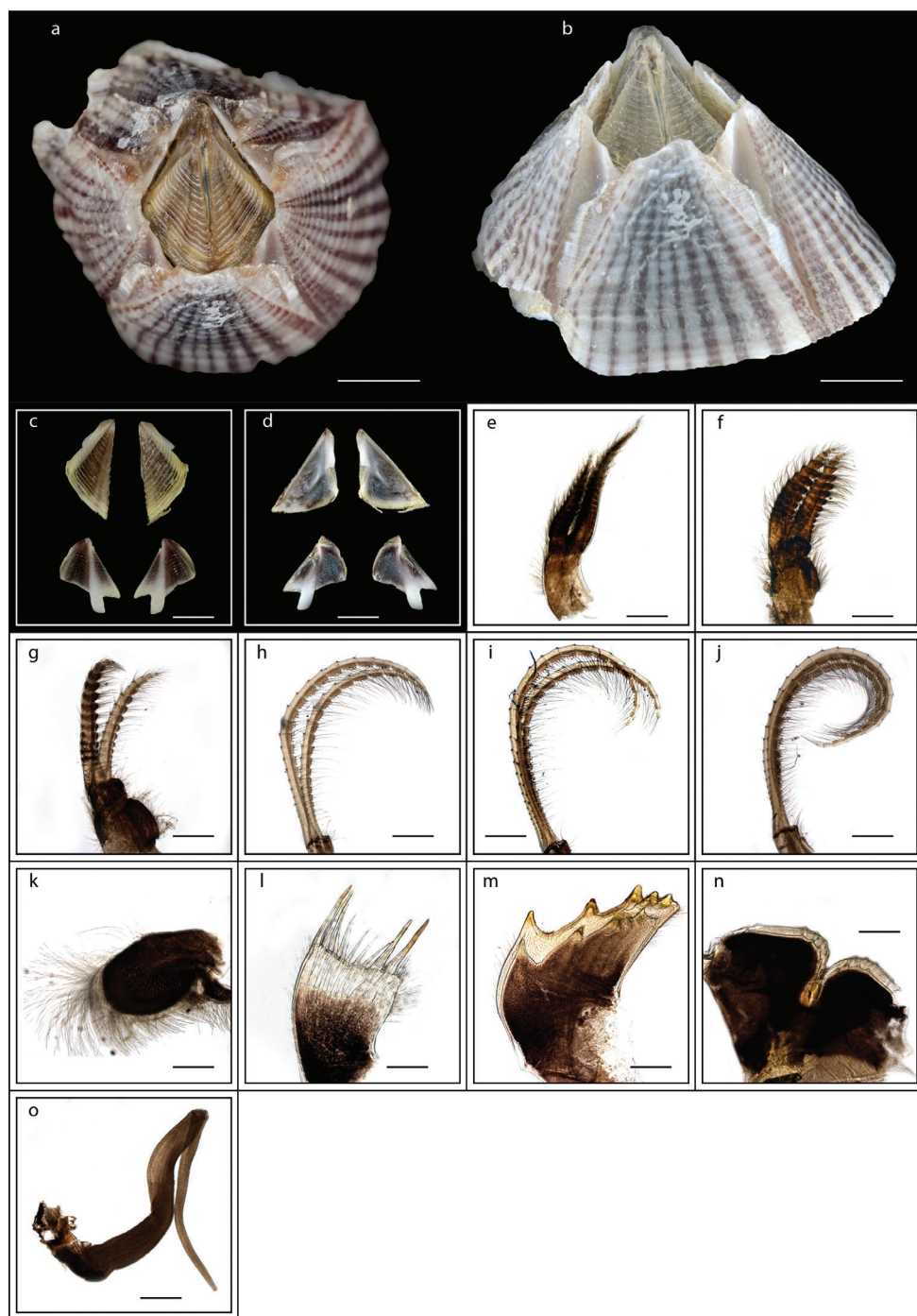


Figure 22. *Amphibalanus reticulatus* (Utinomi, 1967) (MZB Cru Cir 012-1) **a** upper view **b** side view **c** external view of scutum and tergum **d** internal view of scutum and tergum **e** cirrus I **f** cirrus II **g** cirrus III **h** cirrus IV **i** cirrus V **j** cirrus VI **k** maxilla **l** maxillule **m** mandible **n** labrum **o** penis. Scale bars: 4 mm (**a**, **b**); 1 mm (**c**–**g**); 2 mm (**h**–**j**); 2 mm (**o**); 0.5 mm (**k**–**n**).

Balanus variegatus tessellatus Utinomi & Kikuchi, 1966: 5.

Balanus amphitrite amphitrite: Stubbings 1967: 271, fig. 14d–f (part.).

Balanus reticulatus: Utinomi 1967: 216, figs 9a, b, 10a, b, 11a–e, pl. 6 figs 7, 8 (part.); Dong et al. 1982: 91, fig. A–C; Zevina et al. 1992: 92, fig. 63; Puspasari et al. 2001b.

Amphibalanus reticulatus: Pitombo 2004: 274; Chan, et al. 2009a: 234, fig. 200; Po-chai et al. 2017: 26, fig. 10; Xu 2017: 43, figs 10, 39.

Material examined. *Ambon Island*: 5 specimens, MZB Cru Cir 012, Yos Sudarso, 3°41'36.5"S, 128°10'35.6"E, coll. P. Pitriana & D. Tala, 6 Sep 2016.

Diagnosis. Primary parietal tubes with transverse septa; exterior of shell with longitudinal and horizontal striations; anterior margin of cirrus III with conical denticles, erect hooks below posterior angles of distal articles of rami present.

Description. Shell conic or cylindric; six parietal plates, externally smooth, white with groups of well-spaced purple, or purple-pink vertical stripes intersecting with transverse striations (Fig. 22a, b); parietes with single row of internal tubes; alae with summits moderately oblique; radii narrow, summits oblique; orifice toothed; basis calcareous, porous; scutum triangular, externally flat, internally adductor ridge conspicuous, short, low, well separated from prominent articular ridge; tergum flat, spur narrow, short, basiscutal angle acute, scutal margin straight; scutum triangular, occluding margin toothed; tergum flat, basiscutal angle acute, scutal margin straight (Fig. 22c, d); cirrus II with simple spinules or conic teeth on outer face near anterior margin (Fig. 22f); maxilla bilobed, dense setae on margin (Fig. 22k); maxillule not notched, setae on upper and lower margins (Fig. 22l); mandible with four teeth (Fig. 22m); mandibular palp with setulae on lower margin, pinnate setae on upper margin; labrum simple with four teeth and setulae on crest on each side of deep notch (Fig. 22n). Basal length 7.7–16.3 mm; basal width 2.9–15.5 mm; height 3.2–10.5 mm. Orifice length 3.5–8.9 mm; orifice width 2.5–7.1 mm (measurements for five specimens are presented in Suppl. material 1: Table S21).

Distribution. *Amphibalanus reticulatus* is native to the Indo-Pacific region and has been introduced by shipping to tropical-subtropical waters of the Eastern Pacific (Coles et al. 1999; Carlton et al. 2011). *A. reticulatus* can be found from Japan to the Malay Archipelago, east Asia from the Yellow Sea to Gulf of Siam, from Malaysia to southeast Africa, in the Mediterranean Sea, West Africa, the Southeast United States to the West Indies (Henry and McLaughlin 1975).

In this study, *A. reticulatus* was found on the islands of Ambon (at the port of Yos Sudarso) on stone and concrete wall of the port (a map with the occurrence of *Amphibalanus reticulatus* in the Moluccas is shown in Suppl. material 1: Fig. S2).

Remarks. *Amphibalanus reticulatus* can be confused with *A. amphitrite*. However, the shell of *A. reticulatus* exhibits clear vertical and horizontal striations, whilst *A. amphitrite* shows only vertical purple striations on all shell plates (Henry and McLaughlin 1975).

***Amphibalanus variegatus* (Darwin, 1854)**

Figure 23a–p, Table 1: species no. 89

Balanus amphitrite var. (8) *variegatus* Darwin, 1854: 241.*Balanus amphitrite* var. *stutsburi* Krüger, 1914: 437.*Balanus concavus sinensis* Broch, 1931: 63, fig. 23.*Balanus amphitrite rafflesia* Nilsson-Cantell, 1934a: 64.*Balanus amphitrite* var. *cirratus*: Pope 1945: 362, pl. 28 fig. 6, pl. 29 fig. 6, pl. 30 figs 13, 14.*Balanus amphitrite cirratus*: Skerman 1960: 610, figs 1, 3 (**non** *Balanus amphitrite cirratus* Darwin, 1854).*Balanus variegatus*: Harding 1962: 291, pl. 10 figs a–k; Zevina et al. 1992: 92, fig. 64.*Balanus variegatus* var. *cirratus*: Pope 1966: 179.*Balanus amphitrite*: Foster 1967: 83 (part.).287.*Balanus kondakovi*: Henry & McLaughlin 1975: 78 (part., New Zealand specimens; **non** *B. kondakovi* Tarasov & Zevina, 1957).*Balanus variegatus variegatus*: Foster 1979: 111, fig. 67, pl. 14b.*Balanus cirratus*: Ren & Liu, 1978: 145, figs 14, 15 (1–13), pl. 4 figs 15–20, pl. 5 figs 1–6.**non** *Balanus amphitrite variegatus*: Nilsson-Cantell 1934a: 60.**non** *Balanus variegatus*: Henry & McLaughlin 1975: 78, fig. 17, pls. 6, 7; Utinomi 1968b: 171 (= *B. cirratus*).*Amphibalanus variegatus*: Pitombo 2004: 274; Horikoshi and Okamoto 2005: 49, fig.3.**Material examined. Ambon Island:** 15 specimens, MZB Cru Cir 014, Waitatiri, 3°37'04.0"S ,128°16'20.3"E, coll. P. Pitriana & D. Tala, 19 Sep 2017. **Saparua Island:** 10 specimens, MZB Cru Cir 015, Teluk Saparua, 3°34'25.7"S, 128°39'25.8"E, coll. P. Pitriana & D. Tala, 22 Sep 2016.**GenBank accession numbers.** COI gene (MK995342–MK995345), 18S (MK981355).**Diagnosis.** Primary parietal tubes with transverse septa; exterior of shell with longitudinal and horizontal striations; anterior margin of cirrus III without conical denticles, erect hooks below posterior angles of distal articles of rami absent.**Description.** Shell steeply conical, tubular in crowded populations; six parietal plates, smooth, thin, brownish purple externally with longitudinal stripes crosshatched by transverse bands, single row of internal tubes (Fig. 23b); carina forming a spout-like projection; radii wide, summits oblique, pink-purple; alae with summits oblique; orifice toothed (Fig. 23a–c); sheath purple with white bands, vesicular; basis calcareous, porous; scutum externally with growth lines prominent, internally with articular ridge high, adductor ridge moderately long; tergum with spur furrow, externally purple, margins white, spur pointed, basal margin deeply excavated on either side of spur, depressor muscle crests prominent (Fig. 23d, e); cirrus III without conical denticles on anterior margin (Fig. 23h); maxilla without notch (Fig. 23l), mandible with four teeth

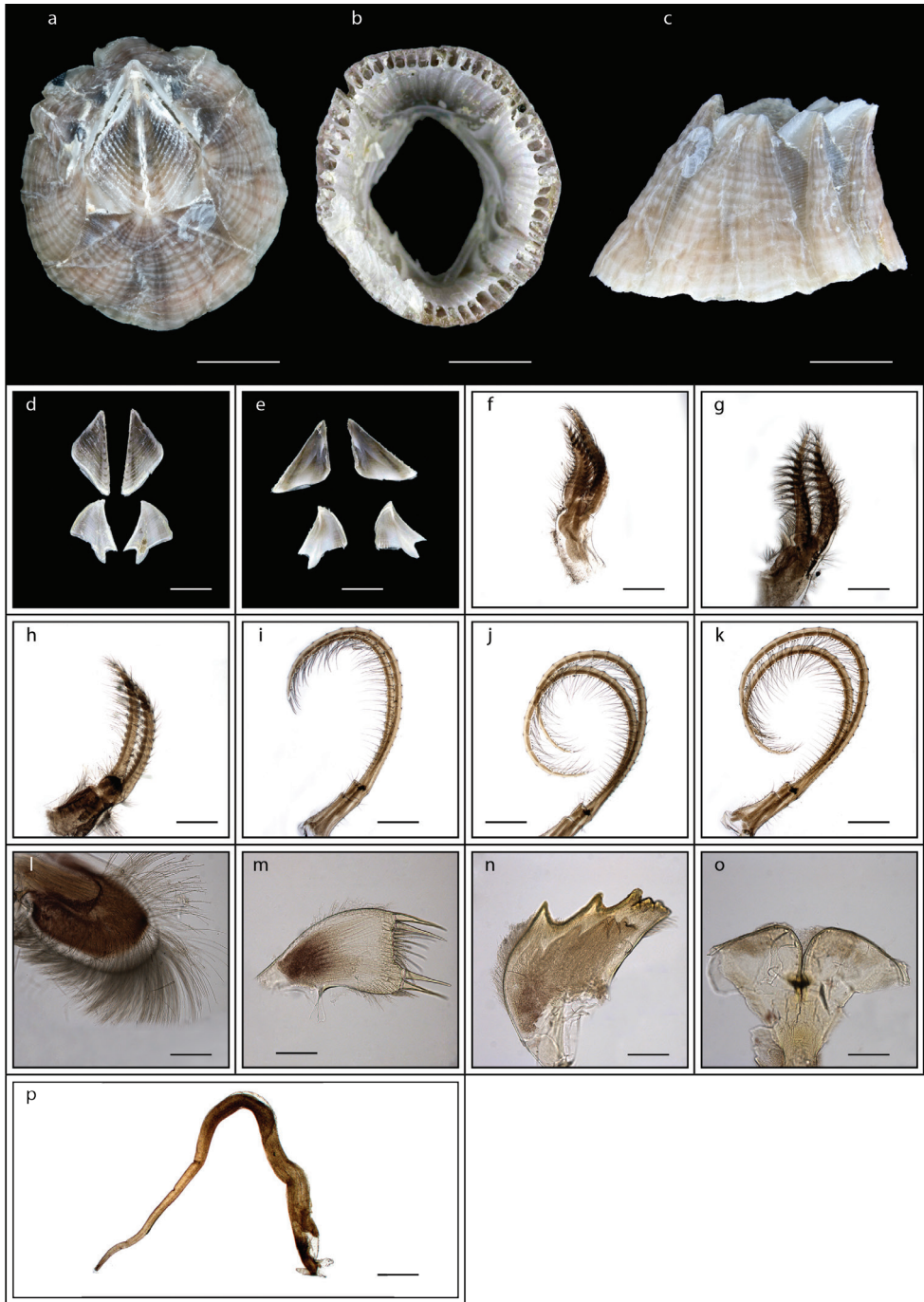


Figure 23. *Amphibalanus variegatus* (Darwin, 1854) (MZB Cru Cir 014-1) **a** upper view **b** lower view **c** side view **d** external view of scutum and tergum **e** internal view of scutum and tergum **f** cirrus I **g** cirrus II **h** cirrus III **i** cirrus IV **j** cirrus V **k** cirrus VI **l** maxilla **m** maxillule **n** mandible **o** labrum **p** penis. Scale bars: 3 mm (**a–c**); 1 mm (**d–h**); 2 mm (**i–k**); 2 mm (**p**); 0.5 mm (**l–o**).

(Fig. 23n), labrum notched, denticulate (Fig. 23o); penis with basidorsal point, with two apical setae (Fig. 23p). Basal length (8.3–11.8 mm, basal width 6.9–10.4 mm, height 4.3–8.4 mm; orifice length 4.8–11.8 mm; orifice width 3.4–5.3 mm (measurements for ten specimens are presented in Suppl. material 1: Table S22).

Distribution. *Amphibalanus variegatus* has been reported from the Indo-west Pacific: Bay of Bengal; Sumatra; New Zealand, Australia; Indonesia; Singapore; Vietnam; Gulf of Siam; Hong Kong; W Kyushu; Vladivostok; and is a common fouling species (Henry and McLaughlin 1975; Jones and Hosie 2016). In this study, *A. variegatus* was found on the islands of Ambon (at Waitatiri) and Saparua (at Teluk Saparua) on stones and a plastic bag (a map with the occurrence of *Amphibalanus variegatus* in the Moluccas is shown in Suppl. material 1: Fig. S4).

Remarks. *Amphibalanus variegatus* is a member of the *Balanus amphitrite* complex, whose members can be difficult to distinguish morphologically. *Amphibalanus variegatus* can be differentiated by its vesicular sheath, and from *A. reticulatus* by features of the tergum, armature of cirrus II and the lack of erect teeth below the posterior distal angles of cirri III–VI (Henry and McLaughlin 1975).

Amphibalanus zhujiangensis (Ren, 1989)

Figure 24a–j, Table 1: species no. 90

Balanus zhujiangensis Ren, 1989a: 467, fig. 2 (1–14).

Amphibalanus zhujiangensis: Pitombo 2004: 274; Puspasari et al. 2002: 235, figs 1A–G, 2A–H; Liu and Ren 2007: 501; Chan et al. 2009a: 238, fig. 204.

Material examined. *Ambon Island*: 10 specimens, MZB Cru Cir 016, Galala, 3°41'22.2"S, 128°10'52.6"E, coll. P. Pitriana & D. Tala, 6 Sep 2016; 3 specimens, MZB Cru Cir 017, Laha, 3°43'22.5"S, 128°05'02.5"E, coll. P. Pitriana & D. Tala, 5 Sep 2016; 6 specimens, MZB Cru Cir 018, Talake, 3°41'59.4"S, 128°10'19.2"E, coll. P. Pitriana & D. Tala, 5 Sep 2016. *Saparua Island*: 10 specimens, MZB Cru Cir 019, Desa Pia, 3°30'20.4"S, 128°36'55.0"E, coll. P. Pitriana & D. Tala, 21 Sep 2016; 7 specimens, MZB Cru Cir 020, Negeri Mahu, 3°31'52.9"S, 128°41'12.4"E, coll. P. Pitriana & D. Tala, 11 Apr 2016; 1 specimen, MZB Cru Cir 021, Desa Mahu, 3°32'19.6"S, 128°41'17.3"E, coll. P. Pitriana & D. Tala, 11 Apr 2016. *Seram Island*: 5 specimens, MZB Cru Cir 022, Desa Kasie, 2°51'05.5"S, 128°32'54.1"E, coll. P. Pitriana & D. Tala, 20 Sep 2017; 3 specimens, MZB Cru Cir 010, Lepas Pantai Kawa, 2°57'32.5"S, 128°05'33.4"E, coll. P. Pitriana & D. Tala, 19 Sep 2017.

GenBank accession numbers. COI gene (MK995334, MK995336, MK995337, MK995339), 18S (MK981347, MK981349, MK981350, MK981352).

Diagnosis. Primary parietal tubes without transverse septa; exterior of shell with longitudinal striations; scutum without adductor ridge, external surface scutum with row of pits; anterior margin of cirri III with conical denticles, erect hooks below posterior angles of distal articles of rami present; cirrus IV with erect hooks on posterodistal angles of articles.

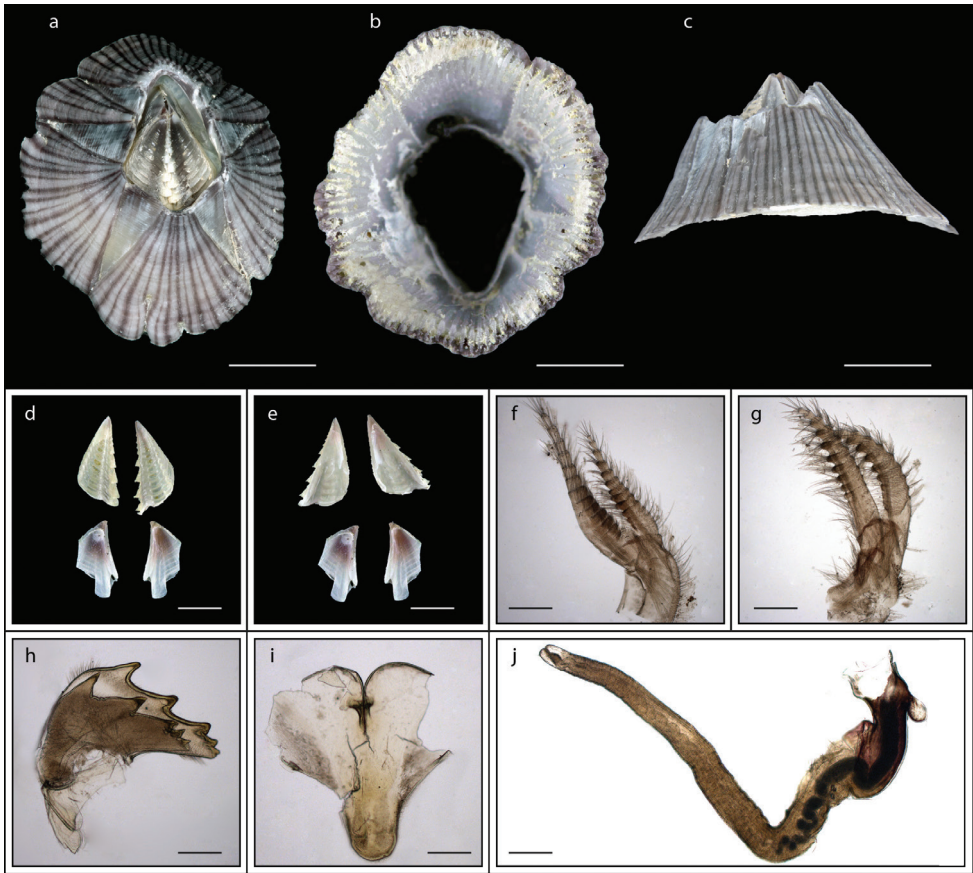


Figure 24. *Amphibalanus zhujiangensis* (Ren, 1989) (MZB Cru Cir 018-5) **a** upper view **b** lower view **c** side view **d** external view of scutum and tergum **e** internal view of scutum and tergum **f** cirrus I **g** cirrus II **h** mandible **i** labrum **j** penis. Scale bars: 6 mm (**a–c**); 1 mm (**d–g**); 0.5 mm (**h, i**); 2 mm (**j**).

Description. Shell six-plated, conic, purplish-white with longitudinal stripes of purple, not cross-hatched by transverse striations; parietes externally smooth, parietal tubes lacking transverse septa and subsidiary tubes; radii wide with slightly oblique summits; orifice rhomboidal, toothed (Fig. 24a–c); scutum trigonal, exterior of scutum with single row of pits extending down centre of valve, occluding margin toothed, inner surface smooth, adductor ridge lacking; tergum with carinal margin convex, spur furrow open, basal margin straight on both sides of spur (Fig. 24d, e); cirrus III without complex setae; cirri III–VI with erect hooks around posterior angle; first maxilla without notch, mandible with five teeth (Fig. 24h). Basal length 5.8–21.6 mm; basal width 4.8–19.2 mm; height 2.1–16.5 mm; orifice length 3.0–10.0 mm; orifice width 2.6–7.6 mm (measurements for eleven specimens are presented in Suppl. material 1: Table S23).

Distribution. *Amphibalanus zhujiangensis* was first recorded from the estuary of the Zhujiang River, South China Sea (Puspasari et al. 2002). Afterwards found on Okinawa Island, Japan and Taiwan (Chan et al. 2009a). In this study, *A. zhujiangensis* was found on the islands of Ambon Island (at Galala, Laha, and Talake), Saparua

Island (at Dusun Pia, Negeri Mahu, Desa Mahu), and Seram Island (at Desa Kasie, Lepas Pantai Kawa) on stone and capitulum of *Lepas anserifera* (a map with the occurrence of *Amphibalanus zhujiangensis* in the Moluccas is shown in Suppl. material 1: Fig. S8).

Remarks. The presence of a row of pits on the external surface of the scutum and the absence of an adductor ridge on the scutum are diagnostic for *A. zhujiangensis*. The species can be distinguished from *A. variegatus* by characters of the shell, cirri III and cirri IV; on *A. reticulatus* by characters of the shell and first maxilla. *Amphibalanus zhujiangensis* is distinct from *A. thailandicus* in lacking transverse septa in the longitudinal tubes and a notch on the first maxilla (Puspasari et al. 2002).

Amphibalanus sp.

Figure 25a–o, Table 1: species no. 91

Material examined. *Ambon Island:* 1 specimen, MZB Cru Cir 135, Talake, 3°41'59.4"S, 128°10'19.2"E, coll. P. Pitriana & D. Tala, 5 Sep 2016; 1 specimen, MZB Cru Cir 136, Waitatiri, 3°37'04.0"S, 128°16'20.3"E, coll. Adin, 19 Sep 2017. *Seram Island:* 2 specimens, MZB Cru Cir 137, Dermaga Pelita Jaya, 3°00'13.5"S, 128°07'09.2"E, coll. P. Pitriana & D. Tala, 21 Sep 2017.

GenBank accession numbers. COI gene (MK995349–MK995351, MK995353), 18S (MK981356–MK981358, MK981360).

Diagnosis. Primary parietal tubes with transverse septa, exterior of shell with longitudinal striations; orifice toothed; scutum without adductor ridge; anterior margin of cirri III with conical denticles, erect hooks below posterior angles of distal articles of rami present; cirrus IV with erect hooks on posterodistal angles of articles; basidorsal of penis absent.

Description. Shell six-plated, conical, whitish with dark purple transverse stripes (Fig. 25a, b); orifice slightly toothed; scutum trigonal, occluding margin toothed; tergum with closed spur furrow (Fig. 25c, d); mandible with five teeth (Fig. 25n); maxilla bilobed with dense setae only on lower margin (Fig. 25l). Basal length 7.4–12.2 mm, basal width 6.3–11.8 mm, height 5.5–9.4 mm; orifice length 3.6–8.3 mm; orifice width 2.6–5.9 mm (measurements for four specimens are presented in Suppl. material 1: Table S24).

Distribution. In this study, *Amphibalanus* sp. was found on Ambon (at Talake and Waitatiri) and Seram islands (at Dermaga Pelita Jaya) (a map with the occurrence of *Amphibalanus* sp. in the Moluccas is shown in Suppl. material 1: Fig. S9).

Remarks. In the molecular phylogeny, *Amphibalanus* sp. forms a well-supported clade in both, the COI and the 18S tree (Figs 28, 29). This species also has different maxilla than those of *A. amphitrite*, *A. reticulatus* and *A. variegatus*, which have setae on its upper and lower margins.

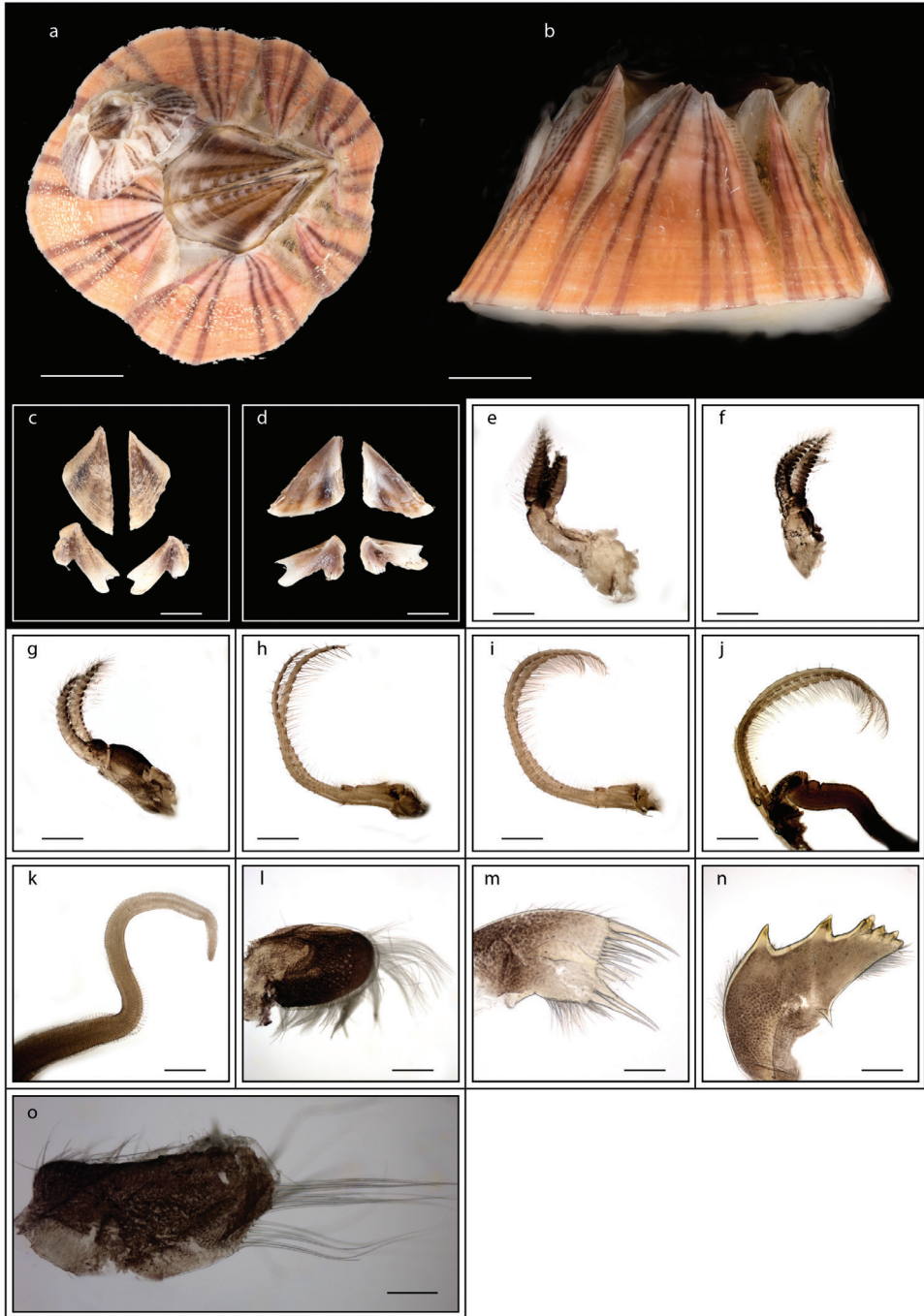


Figure 25. *Amphibalanus* sp. (MZB Cru Cir 135) **a** upper view **b** side view **c** external view of scutum and tergum **d** internal view of scutum and tergum **e** cirrus I **f** cirrus II **g** cirrus III **h** cirrus IV **i** cirrus V **j** cirrus VI **k** penis **l** maxilla **m** maxillule **n** mandible **o** mandibular palp. Scale bars: 3 mm (**a**, **b**); 1 mm (**c**–**g**); 2 mm (**h**–**k**); 0.5 mm (**l**–**o**).

Subfamily Megabalaninae Newman, 1979

Genus *Megabalanus* Hoek, 1913

Megabalanus tintinnabulum (Linnaeus, 1758)

Figure 26a–e, Table 1: species no. 97

Balani Rhumphius, 1705: 121, pl. 41 figs A, C, D.

Balanus tintinnabuliformis laevis Lang, 1772: 4.

Balanus cylindraceus unicum thalamum efformans, magnis ventricosus Gaultierus, 1742: un-numbered page, pl. 106, fig. H.

Glands de mer de la grande espèce Dezallier d'Argenville, 1742: 364, pl. 30 fig. A; 1757: 364, pl. 26 fig. A.

Lepas Tintinnabulum Linnaeus, 1758: 668; Chemnitz 1785 (part.): pl. 97 figs 830, 831 (non figs 828, 829).

Lepas calyciformis orientalis Ellis, 1758: 845, pl. 34 figs 8, 9.

Balanus tintinnabulum: Bruguière 1789 (part.): 165; Holthuis and Heerebout 1972: 24, pl. 1.

Lepas tintinnabulum: Wood 1815: 38, pl. 6 figs 1, 2.

Lepas spinosa Wood, 1815 (part.): pl. 7 fig. 4 (large shell only; small shells = *M. spinosus*).

Balanus tintinnabulum var. (1) *communis* Darwin, 1854: 195, pl. 1 figs a, b, f supra, pl. 2 figs 1 a, 1 c–e, 1 i, 1 k.

Balanus tintinnabulum var. *communis*: Gruvel 1905a: 21.

Balanus tintinnabulum tintinnabulum: Pilsbry 1916: 55, fig. 9, pl. 10 figs 1a–e; Dong et al. 1982: 86.

Balanus tintinnabulum antillensis Pilsbry, 1916: 63, pl. 13 figs 1, 2 e.

Balanus (Megabalanus) tintinnabulum forma *communis* Broch, 1931: 56.

Balanus tintinnabulum var. *tintinnabulum*; Oliveira 1941: 11, fig. 1, pl. 2 figs 1, 2, pl. 4 fig. 1, pl. 5 fig. 3, pl. 8 fig. 6.

Megabalanus antillensis Newman & Ross, 1976: 67.

Balanus (Megabalanus) tintinnabulum tintinnabulum: Ren & Liu, 1978: 121, fig. 1, pl. 1 figs 1–5.

non *Lepas tintinnabulum*: Spengler 1790: 180 [= *Megabalanus occator* (Darwin, 1854)]

non *Lepas tintinnabulum* var. a: Spengler 1790: 181 (*incertae sedis*).

non *Lepas tintinnabulum* var. b: Spengler 1790: 182 [= *Striatobalanus amaryllis* (Darwin, 1854)]

non *Lepas tintinnabulum*: Chemnitz 1785: pl. 97, figs 828, 829 [= *Austromegabalanus nigrescens* (Lamarck, 1818)].

non *Balanus tintinnabulum*: Chenu 1843: pl. 2 fig. 8, pl. 3 fig. 5, pl. 2 fig. 8 [= *Megabalanus ajax* (Darwin, 1854)]; pl. 3 fig. 5 [= *Megabalanus tulipiformis* (Darwin, 1854)].

non *Balanus tintinnabulum* var. *communis*: Krüger 1911a: 46, pl. 3 figs 31 a1–31 b2 [= *Megabalanus volcano* Pilsbry, 1916)].

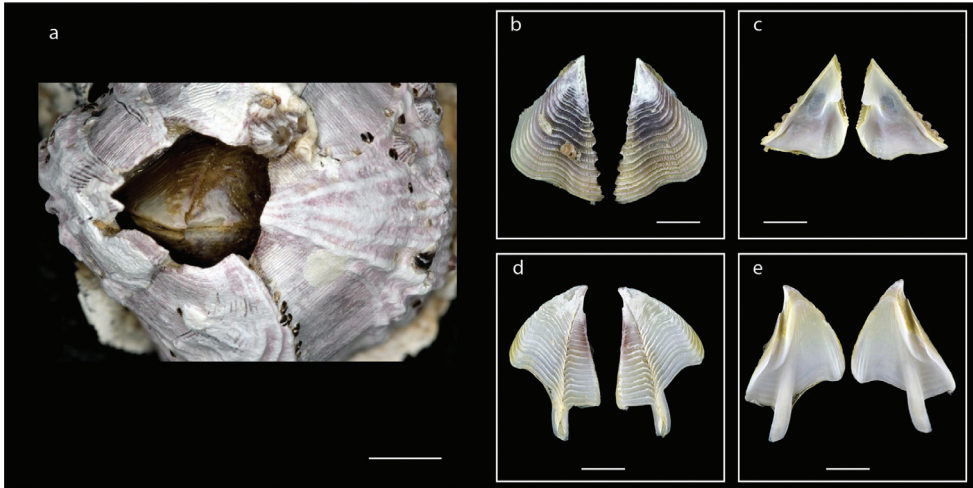


Figure 26. *Megabalanus tintinnabulum* (Linnaeus, 1758) (MZB Cru Cir 066-3) **a** upper view **b** external view of scutum **c** internal view of scutum **d** external view of tergum **e** internal view of tergum. Scale bars: 8 mm (**a**); 5 mm (**b–e**).

non *Balanus* (*Megabalanus*) *tintinnabulum*: Withers 1924: pl.6 figs 4–7 [= *Megabalanus linzei* (Foster, 1979)].

non *Balanus tintinnabulum antillensis* Pilsbry, 1927: 38, fig. 3 a–c [= *Megabalanus stultus* (Darwin, 1854)]

non *Balanus tintinnabulum tintinnabulum*: Linzey 1942: 279 [= *Megabalanus linzei* (Foster, 1979)].

non *Balanus tintinnabulum*: Foster 1967: 81, fig. 2a, b [= *Megabalanus linzei* (Foster, 1979)].

Megabalanus tintinnabulum: Newman & Ross, 1976: 68; Henry & McLaughlin 1986: 17, figs 1e, 2a, g, h, 3a–c, 5 a–l; Zevina et al. 1992: 99, fig. 67; Pitombo 2004: 175; Chan et.al 2009a: 259, fig. 224; Pochai et.al 2017: 28, fig.11.

Material examined. *Ambon Island*: 3 specimens, MZB Cru Cir 066, Laha, 3°43'22.5"S, 128°05'02.5"E, coll. P. Pitriana & D. Tala, 5 Sep 2016. *Saparua Island*: 1 specimen, MZB Cru Cir 067, Desa Pia, 3°30'20.4"S, 128°36'55.0"E, coll. P. Pitriana & D. Tala, 21 Sep 2016.

Diagnosis. Shell relatively large, lightly ribbed; radii wide; surface smooth without spines; tergum wider than scutum with spur narrow and long, crests for depressor muscle weakly to moderately well developed.

Description. Shell cylindrical to conical, parietes purplish, smooth, with longitudinal purple striations, tubiferous (Fig. 26a); radii wide, usually horizontally striated, summits horizontal, sutural edges with regular denticles; summits of alae oblique; orifice rhomboidal, moderately small to large, one-third to two-thirds basal diameter, sub-circular to subtriangular; scutum triangular, external surface with horizontal striations,

inner surface with conspicuous articular ridge; tergum triangular, frequently wider than scutum, external surface with horizontal striations, spur long, narrow, external surface with median furrow, scutal margin denticulate (Fig. 26b–e); mandible with five teeth, labrum with deep cleft, three teeth on each side. Basal length 26.0–49.2 mm, basal width 29.0–43.1 mm, height 20.1–49.4 mm. Orifice length 13.7–16.3 mm, orifice width 10.4–15.9 mm (measurements for four specimens are presented in Suppl. material 1: Table S25).

Distribution. *Megabalanus tintinnabulum* is a cosmopolitan species and widely distributed worldwide (Pochai et al. 2017). In this study, *M. tintinnabulum* was found on Ambon Island (at Laha) and in Saparua Island (at Desa Pia) on concrete bridge at the port, stones and reef surface (a map with the occurrence of *Megabalanus tintinnabulum* in the Moluccas is shown in Suppl. material 1: Fig. S4).

Remarks. The name *Megabalanus* was given by Hoek (1913), referring to the largest form of existing Balani. With the exception of *Balanus amphitrite*, Darwin (1854) considered *Balanus tintinnabulum* as the most difficult and variable species in the genus *Balanus* (Henry and McLaughlin 1986). *Megabalanus tintinnabulum* can be distinguished by its large shell plates and purple surface with irregular, unclear longitudinal stripes (Pochai et al. 2017).

Megabalanus zebra (Darwin, 1854)

Figure 27a–c, Table 1: species no. 98

Balanus tintinnabulum var. (4) *zebra* Darwin, 1854: 195. pl. 1 fig. g.

Balanus tintinnabulum zebra: Pilsbry 1916: 57, pl. 10 figs 2, 3; Stubbings 1967: 264; Dong et al. 1982: 86, fig. A–C.

Balanus tintinnabulum var. *zebra* Karande & Palekar, 1966: 143, pl. I, fig. 2.

Megabalanus zebra: Newman & Ross, 1976: 69; Henry and McLaughlin 1986: 47, figs 2f, 4j–k, 12e–l; Pitombo 2004: 275; Chan et al. 2009a: 265, fig. 232; Pitombo et al. 2017: 135, figs 2, 4, 5, 6, 7, 8.

Material examined. *Ambon Island*: 4 specimens, MZB Cru Cir 068, Galala, 3°41'22.2"S, 128°10'52.6"E, coll. P. Pitriana & D. Tala, 6 Sep 2016; 4 specimens, MZB Cru Cir 069, Laha, 3°43'22.5"S, 128°05'02.5"E, coll. P. Pitriana & D. Tala, 5 Sep 2016.

Diagnosis. Parietes reddish purple with strong longitudinal white ribs; radii and sheath dark purple to reddish brown; scutum with narrow tergal segment slightly inflected; tergum approximately as wide as scutum, crest for depressor muscle prominent.

Synoptic Description. Shell conic, six-plated; parietes smooth, purple with well-developed white ribs and dark purple interspaces; radii wide, summits horizontal, white with dark purple spots on proximal side (Fig. 27a); orifice rhomboidal; scutum triangular, external surface with the intersecting horizontal and longitudinal striations; tergum triangular, white with purple spots in some areas, scutal margin slightly curved, basal

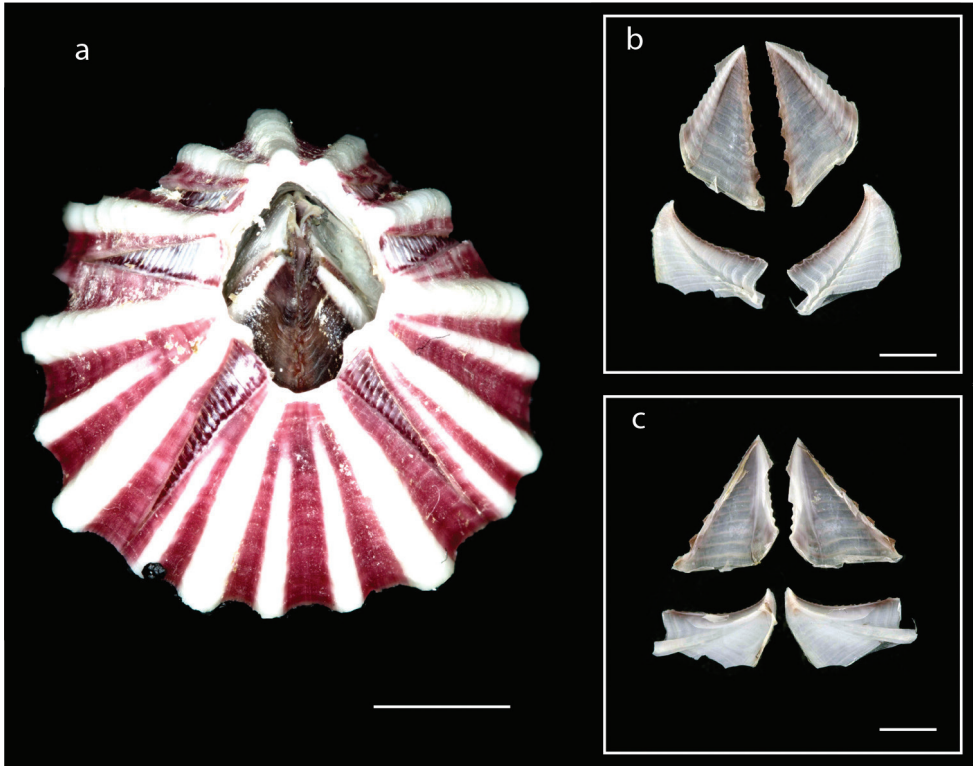


Figure 27. *Megabalanus zebra* (Darwin, 1854) (MZB Cru Cir 068-3) **a** upper view **b** external view of scutum and tergum **c** internal view of scutum and tergum. Scale bars: 4 mm (**a**); 2 mm (**b–c**).

margin inclined, spur short (Fig. 27b, c); mandible with five teeth, labrum with deep cleft, three teeth on each side. Basal length 5.4–19.0 mm, basal width 5.0–20.0 mm, height 4.4–13.1 mm. Orifice length 3.1–7.6 mm, orifice width 1.7–6.2 mm (measurements for eight specimens are presented in Suppl. material 1: Table S26).

Distribution. *Megabalanus zebra* is a well-known fouling species of ship hulls, floating structures, moveable oil platforms, etc., and has been widely recorded from the Atlantic Ocean; W Africa; Indo-west Pacific: Indian Ocean; Australia; Thailand; China; Philippines; Taiwan (Pilsbry 1896, 1916; Stubbings 1961; Karande and Pakelar 1966; Foster and Willan 1979; Chan et al. 2009a; Jones and Hosie 2016; Pitombo et al. 2017). In this study, *Megabalanus zebra* was found on Ambon Island (at Galala and Laha) on stones and the capitulum of *Lepas anserifera* (a map with the occurrence of *Megabalanus zebra* in the Moluccas is shown in Suppl. material 1: Fig. S1).

Remarks. *Megabalanus zebra* can be distinguished from other species in the *Megabalanus* group by, for example, the inflection of the tergal segment of the scutum and the position of the spur (Henry and McLaughlin 1986). *M. zebra* can also be differentiated by an intermediate shape of the tergum and scutum compared to *M. tintinnabulum* and *M. coccopoma* (Pitombo et al. 2017).

Molecular results

In total, we produced 120 new sequences for this study (COI = 62 sequences, 18S = 58 sequences; Suppl. material 1: Table S1; Figs 28, 29). We downloaded a total of 172 sequences from GenBank (COI = 84 sequences, 18S = 88 sequences). The final COI alignment used for phylogenetic analyses was 641 bp long, and included 156 sequences from 50 species (Fig. 29). The 18S alignment was 1918 bp long and included 154 sequences from 83 species (Fig. 28).

In general, support values (bootstrap and posterior probability) were low for both markers, with the majority of internal nodes receiving support values below 50% bootstrap or 0.5 posterior probability. However, there are several highly supported nodes throughout (> 70% bootstrap; > 0.85 posterior probability), which allow us to gain insights into the evolutionary history of the group. In general, and as expected, COI (Fig. 29) provided higher resolution at terminal nodes but low resolution at deeper nodes (rapidly evolving marker), whereas 18S (Fig. 28) provided higher resolution than COI at basal nodes (slowly evolving marker). The trees resulting from the BEAST and RaxML analyses were fully congruent, with no highly supported relationships being favoured in one analysis but not the other. The main purpose of our phylogenetic analyses was to find out where the new accessions from the Moluccas sequenced for this study are retrieved on the barnacle tree, and to see whether putative species are retrieved as monophyletic. We therefore show multiple accessions per species on the trees. The trees resulting from RaxML analyses and the concatenated analyses are given in the Suppl. material (Suppl. material 1: Figs S10–S13).

Molecular study of Moluccan barnacles

The vast majority of new samples from the Moluccas produced in this study matched sequences from the same species that are available on GenBank. For example, DNA sequence of our *Heteralepas japonica* matched the sequence of *H. japonica* EU884146.1 and EU884169.1 from Chan, et al. (2009c); and our *Nesochathamalus intertextus* matched the sequence of *N. intertextus* JX083869.1 from Perez-Losada, et al. (2012). This applies to all species for which we have new sequences. The only exception is *Chthamalus moro*, for which one of our samples in the 18S tree does not match the GenBank samples of that species. However, for this particular case, the support values of that clade in the tree are very low, therefore the odd positioning is not strongly supported (that clade is essentially a polytomy).

Two taxa for which we sequenced multiple accessions, but for which we could not assign a species name, were retrieved in positions on the tree that lead us to propose these may constitute new unidentified species. The first one is *Amphibalanus* sp., clustering as a unit in both COI and 18S trees (Figs 28, 29; Suppl. material 1: Figs S10–S13). The other was *Microeuraphia* sp., which formed well supported and separated clades in the COI tree, and was clustered in the same unresolved clade in 18S (Figs 28, 29; Suppl. material 1: Figs S10–S13).

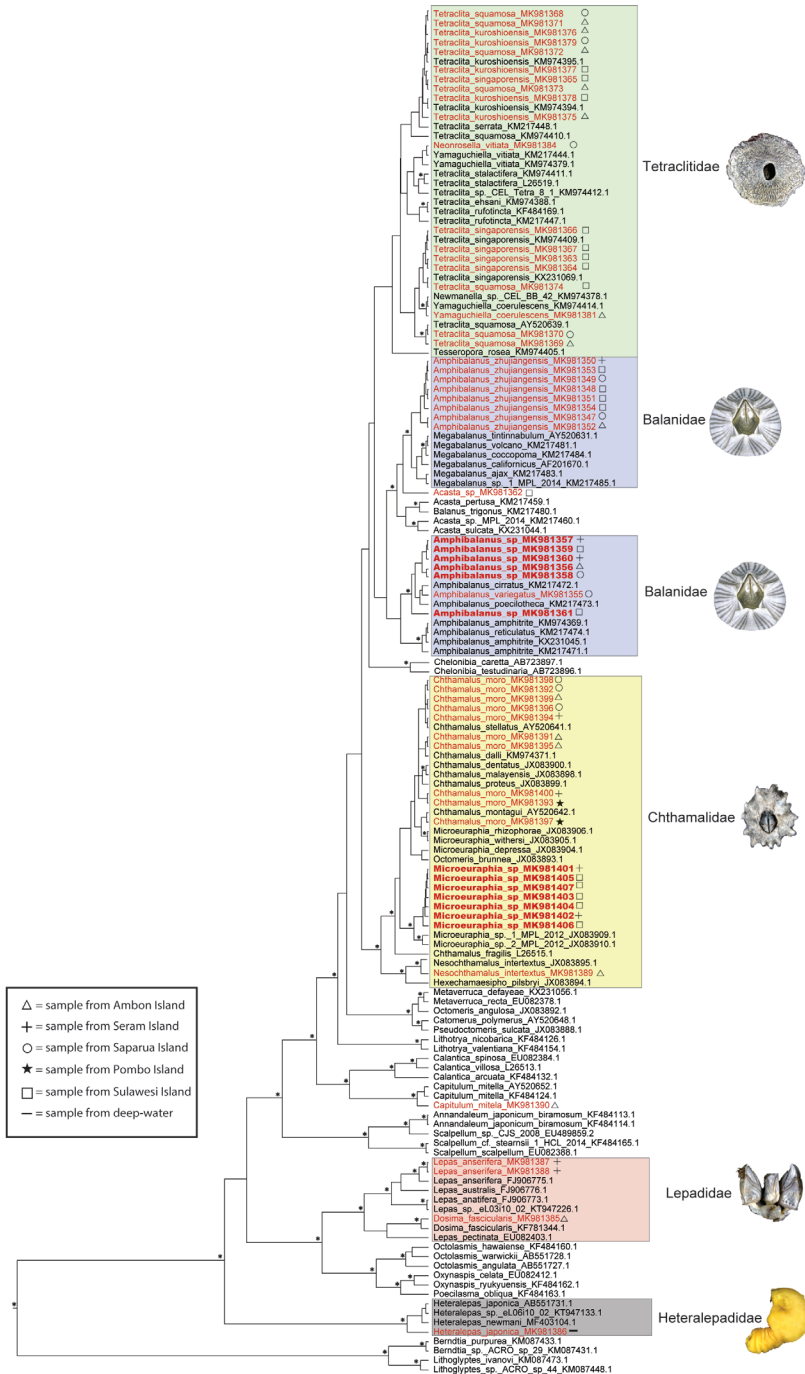


Figure 28. Bayesian phylogeny of 18S gene sequences. High Bayesian posterior probabilities (≥ 0.85) are indicated by an asterisk at the respective node. Families with relevance for this study are highlighted by coloured rectangles. Sample labels in red indicate sequences newly generated for this study. Species names in bold indicate potential new species.

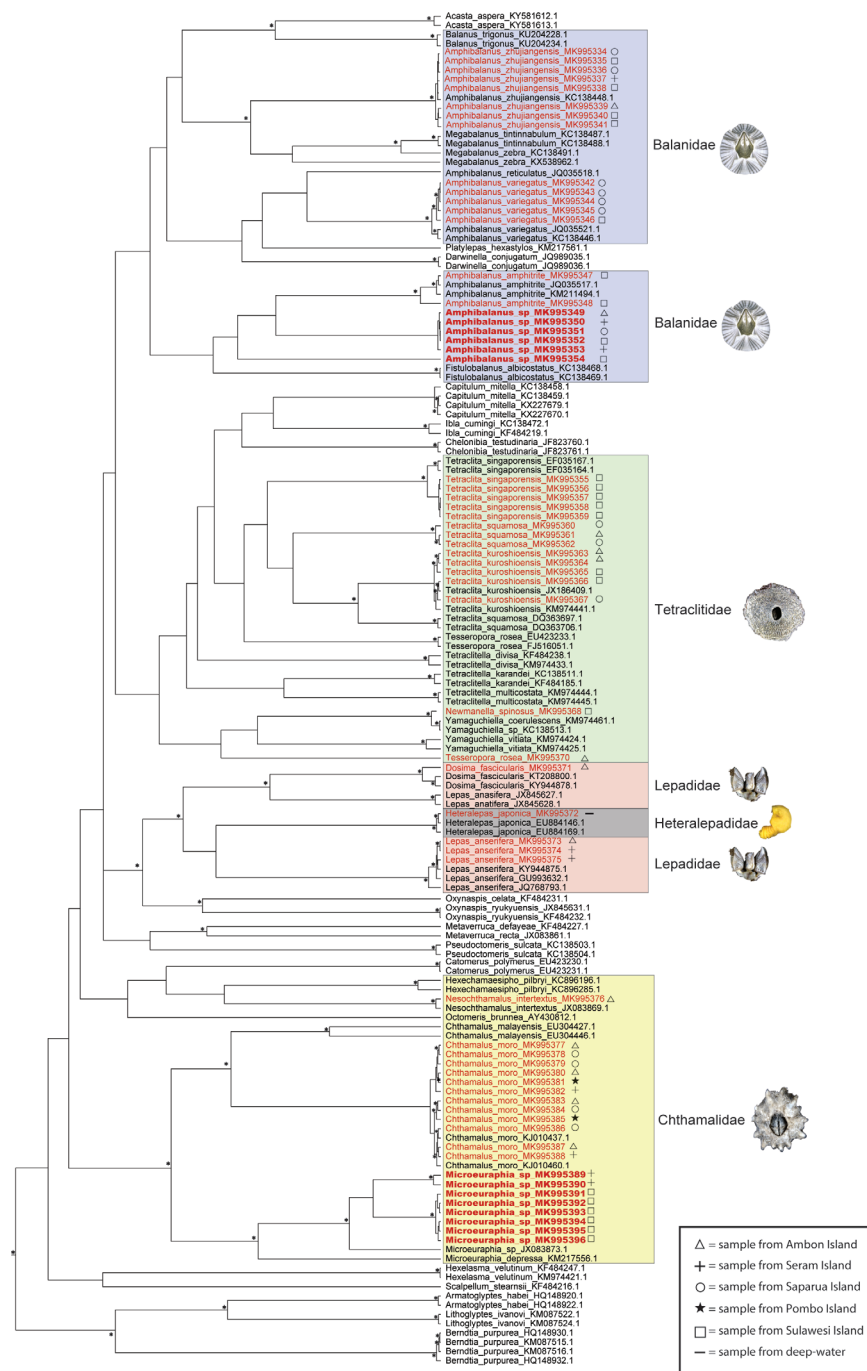


Figure 29. Bayesian phylogeny of COI gene sequences. High Bayesian posterior probabilities (≥ 0.85) are indicated by an asterisk at the respective node. Families with relevance for this study are highlighted by coloured rectangles. Sample labels in red indicate sequences newly generated for this study. Species names in bold indicate potential new species.

The K2P distances within *Microeuraphia* sp. were $1.74\% \pm 0.51\%$ for the COI sequences. The K2P distances between *Microeuraphia* sp. and other species ranged from 10.90% to 22.70%; and overall averaged distances between the species and other species were 13.82% (Suppl. material 1: Table S27). Whilst for *Amphibalanus* sp. the K2P distances within the species were $0.22\% \pm 0.13\%$ for the COI sequences. The K2P distances between *Amphibalanus* sp. and other species ranged from 13.34% to 18.33%; and overall averaged distances between the species and other species were 14.37% (Suppl. material 1: Table S28).

Discussion

This checklist lists 97 species, including 23 new records the Moluccas and two of which still await their species descriptions. The past record on barnacles from these islands dates back to the *Challenger* (1872–1876) and *Siboga* (1899–1900) expeditions (Hoek 1913). Later, Kolosváry (1950) only mentioned some balanids living in corals collected during *Snellius* Expedition (1929–1930), which contrasts reports from other groups numerous collected during the same expedition, such as in decapod crustaceans and Foraminifera.

Hoek (1913) listed a total of 210 species from the Malay Archipelago that were collected during the *Challenger* and *Siboga* expeditions. Among these, 45 species were found in the Moluccas. However, the majority of the Moluccan species listed by Hoek (32 species) were deep-water barnacles found at depths of 204–2,798 m, while 10 species barnacles were found at depths of 9–90 m, and only three inshore species were recorded: *Temnaspis fissum* (Darwin, 1851) from Ternate; *Yamaguchiella coerulescent* (Spengler, 1790) and *Tetraclitella costata* (Darwin, 1854) from Banda Island. In contrast, sampling for this study focused on inshore habitats with only two deep-sea locations. In consequence to the different sampling approaches, we found 24 inshore species and only one deep-sea species among the new samples.

A comparison of the number of species previously recorded from Ambon, Seram, and Banda by Hoek (1913) and Jones (2001, 2016) with those recorded in this study indicates that species diversity for each island has been heavily underestimated. On Ambon, for example, seven species were previously known compared to the 24 species listed here. For other smaller islands such as Saparua and Pombo, no barnacle species was previously recorded. Given the size of the Moluccan Archipelago, with ca. 1,000 islands, many of which have never been sampled despite including relatively large islands such as Haruku, Buru, Yamdena or Wetar, a much higher number of species can be expected in the Moluccas.

The molecular results also indicate that the barnacle fauna of the region is understudied. In addition to evidence for two potentially new species (see above), the generic assignment of some described species is also challenged. For example, *Amphibalanus zhujiangensis* was found to be more closely related to *Megabalanus* than to other *Amphibalanus* species, suggesting the need to conduct in-depth research on this species

to clarify its taxonomy. However, we must caution against over interpretation of our phylogenetic trees, because the markers we used revealed low node support overall.

The molecular phylogeny failed to reveal any biogeographic pattern of barnacles from the Moluccas, which is not surprising given the limited scope of sampling. These points all underline again the necessity of a more comprehensive approach to sampling in the region as well as the need to explore more molecular markers for a truly integrative taxonomy of barnacles, not just in the Moluccas.

Acknowledgements

The first author would like to thank Prof. Frank Riedel from the Freie Universität Berlin for his general support. Daniel Josep Tala, Ading, Nurul Fitriya, Doni Nurdi-ansah, M. Masrur Islami, and Frilla R. Saputra (all LIPI) for supporting the sampling of barnacles in Indonesia; Bani Wahyudi and Abdul Basit for their support and comments on picture editing. Thanks to Robert Schreiber, Philipp Vogt, Christine Zorn, Bernhard Schurian, Lukas Kirschey, Dr. Christian Mitgutsch, and Anke Sängler (all MfN Berlin) for supporting the molecular, morphological, and logistic work progress at the Museum für Naturkunde Berlin. We would like to thank the head of crustacean collections at MZB (Dr. Daisy Wowor) for providing museum numbers for all samples and her general support of our crustacean research. We would like to thank Prof. William Anderson Newman, Prof. Benny Kwok Kan Chan, and Meng-Chen Yu for valuable comments on the manuscript.

This study was funded by the Ministry of Research, Technology and Higher Education, the Republic of Indonesia within the Program for Research and Innovation in Science and Technology (RISET-Pro), World Bank Loan No. 8245-ID.

Barnacle samples were collected by the first author with permit numbers as follows: 488/IPK.3/KP/V 2016 for Banda Island; 694/IPK.3/AP/IX/2016 for the islands of Ambon and Pombo; 737/IPK.3/AP/IX/2016 for Saparua Island; B-671/IPK.3/KP.01.06/IX72017 for South Sulawesi; B-684/IPK.5/KP.01.06/IX/2017 for Seram Island.

References

- Anderson DT, Anderson JT (1985) Functional morphology of the balanomorph barnacles *Tesseropora rosea* (Krauss) and *Tetraclitella purpurascens* (Wood) (Tetraclitidae), Marine and Freshwater Research. CSIRO 36(1):87–113. <https://doi.org/10.1071/MF9850087>.
- Barrett PH, Freeman RB. (2016) The Works of Charles Darwin Vol 12: A Monograph on the sub-class Cirripedia (1854), Vol II, Part 1. Routledge, Oxford, 448 pp. <https://doi.org/10.4324/9781315477176>
- Beşir C, Çınar ME (2012) Thoracic cirripeds (Thoracica: Cirripedia) from Antalya Bay (Eastern Mediterranean). Journal of Black Sea/Mediterranean Environment 18(2): 251–270.

- Briggs JC (1974) Marine Zoogeography. McGraw-Hill Book Company, New York, 475 pp. <https://doi.org/10.2307/1442613>
- Broch H (1931) Papers from Dr Th. Mortensen's Pacific Expedition 1914–1916. LVI. Indomalayan Cirripedia. Videnskabelige Meddelelser fra dansk naturhistorisk Forening i Kjøbenhavn, 91: 1–146.
- Buckeridge JS (1994) Cirripedia Thoracica: Verrucomorpha of New Caledonia, Indonesia, Wallis, and Futuna Islands. In: Résultats Des Campagnes MUSORSTOM Volume 12. Muséum national d'Histoire naturelle, Paris, 87–125.
- Buckeridge JS, Newman WA (2006) A revision of the Iblidae and the stalked barnacles (Crustacea: Cirripedia: Thoracica), including new ordinal, familial and generic taxa, and two new species from New Zealand and Tasmanian waters. Zootaxa 1136(1): 1–38. <https://doi.org/10.11646/zootaxa.1136.1.1>.
- Carlton JT, Newman WA, Pitombo FB (2011) Barnacle invasions: introduced, cryptogenic, and range expanding Cirripedia of North and South America, in In the wrong place-alien marine crustaceans: Distribution, biology and impacts. Springer, 159–213. https://doi.org/10.1007/978-94-007-0591-3_5
- Chan BKK, Tsang LM, Chu KH (2007) Morphological and genetic differentiation of the acorn barnacle *Tetraclita squamosa* (Crustacea, Cirripedia) in East Asia and description of a new species of *Tetraclita*. Zoologica Scripta. Wiley Online Library 36(1): 79–91. <https://doi.org/10.1111/j.1463-6409.2007.00260.x>.
- Chan BKK, Prabowo RE, Lee K-S (2009a) Crustacean fauna of Taiwan: barnacles, volume I- Cirripedia: Thoracica excluding the Pyrgomatidae and Acastinae. National Taiwan Ocean University, Keelung, Taiwan, 297 pp.
- Chan BKK, Tsang LM, Shih FL (2009b) Morphological and genetic differentiations of the stalked barnacle *Heteralepas japonica* Aurivillius, 1892, with description of a new species of *Heteralepas* Pilsbry 1907 from the Philippines. Raffles Bulletin of Zoology Supplement 20: 83–95.
- Chan BKK, Tsang LM, Shih FL (2009c). Genetic differentiation of the *Heteralepas japonica* species complex in the Philippines and Taiwan waters, with description of a new species, *Heteralepas profundus* sp. nov. Raffles Bulletin of Zoology supplement no. 20:81–93.
- Chan BKK, Cheang CC (2016) First discovery of a new species of *Newmanella* Ross, 1969 (Balanomorpha: Tetraclitidae) in the western Pacific, with a note on the new status of *Neonrosella* Jones, 2010. Zootaxa. Magnolia Press 4098(2): 201–226. <https://doi.org/10.11646/zootaxa.4098.2.1>.
- Chan BKK, Corbari L, Rodriguez MPA, Tsang LM (2017) Molecular phylogeny of the lower acorn barnacle families (Bathylasmatidae, Chionelasmatidae, Pachylasmatidae and Waiakalasmataidae) (Cirripedia: Balanomorphia) with evidence for revisions in family classification. Zoological Journal of the Linnaean Society 180(3): 542–555. <https://doi.org/10.1093/zoolinnean/zlw005>.
- Chen HN, Tsang LM, Chong VC, Chan BK (2014) Worldwide genetic differentiation in the common fouling barnacle, *Amphibalanus amphitrite*. Biofouling 30(9): 1067–1078. <https://doi.org/10.1080/08927014.2014.967232>.
- CodonCode Aligner version 5.1.5 (2020) (<http://www.codoncode.com>)

- Coles SL, DeFelice RC, Eldredge LG, Carlton JT (1999) Historical and recent introductions of non-indigenous marine species into Pearl Harbor, Oahu, Hawaiian Islands. *Marine Biology* 135(1): 147–158. <https://doi.org/10.1007/s002270050612>
- Crosnier A, Richer De Forges B, Bouchet P (1997) La campagne KARUBAR en Indonésie, au large des îles Kai et Tanimbar. In: Résultats Des Campagnes MUSORSTOM Volume 16: Campagne Franco-Indonésienne KARUBAR. Muséum national d'Histoire naturelle, Paris, 9–26. CTab isolation buffer (<http://www.geocities.com/CapeCanaveral/8431/CTab.html>)
- Darwin C (1852) A monograph of the subclass Cirripedia with figures of all the species. The Lepadidae; or, pedunculated cirripedes. 400 pp., 10 pls. (Ray Society, London. 1851 published 1852)
- Darwin C (1854) A Monograph on the Sub-class Cirripedia: The Balanidae (or sessile cirripedes) the Verrucidae, etc., etc., etc. (Vol. 2). Ray society, London, 684 pp.
- Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small amounts of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.
- Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic acids research* 32(5): 1792–1797. <https://doi.org/10.1093/nar/gkh340>
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3(5): 294–299.
- Foster BA (1978) The Marine Fauna of New Zealand: Barnacles (Cirripedia: Thoracica). New Zealand Oceanographic Institute Memoir 69: 1–160.
- Foster BA, Willan RC (1979) Foreign barnacles transported to New Zealand on an oil platform. *New Zealand Journal of Marine and Freshwater Research* 13(1): 143–149. Geneious 11 (<http://www.geneious.com>) <https://doi.org/10.1080/00288330.1979.9515788>
- Henry DP, McLaughlin PA (1975) The barnacles of the *Balanus amphitrite* complex (Cirripedia, Thoracica). *Zoologische Verhandelingen* 141(1): 1–254. <http://www.repository.naturalis.nl/record/317846>
- Henry DP, McLaughlin PA (1986) The recent species of *Megabalanus* (Cirripedia: Balanomorpha) with special emphasis on *Balanus tintinnabulum* (Linnaeus) sensu lato. *Rijksmuseum van Natuurlijke Historie*. <http://www.repository.naturalis.nl/record/317744>
- Hinojosa IA, Boltana S, Lancellotti D, Macaya EC, Ugalde P, Valdivia N, Vasquez N, Newman WA, Thiel M (2006) Geographic distribution and description of four pelagic barnacles along the south east Pacific coast of Chile—a zoogeographical approximation. *Revista Chilena de Historia Natural* 79(1): 13–27. <https://doi.org/10.4067/S0716-078X2006000100002>
- Hiro F (1939) Studies on the Cirripedian fauna of Japan. IV Cirripeds of Formosa (Taiwan) with some geographical and ecological remarks on the littoral forms. *Memoirs of the College of Science, Kyoto University, Series B*, 15(2): 245–284.
- Hoek PP (1907) The Cirripedia of the Siboga Expedition: A. Cirripedia Pedunculata. EJ Brill, Leiden, 127 pp. <https://doi.org/10.5962/bhl.title.63641>
- Hoek PP (1913) The Cirripedia of the Siboga-Expedition: B. Cirripedia Sessilia. EJ Brill, Leiden, 275 pp.
- Igić L (2007) Cirripedia of Adriatic. *Studia Marina* (24): 168.

- Jones DS, Anderson JT, Anderson DT (1990) Checklist of the Australian Cirripedia. Technical Reports of the Australian Museum 3: 1–38. <https://doi.org/10.3853/j.1031-8062.3.1990.76>
- Jones DS, Hewitt MA, Sampey A (2001) A checklist of the Cirripedia of the South China Sea. *Raffles Bulletin of Zoology*. National University of Singapore 48: 233–308.
- Jones DS (2012) Australian barnacles (Cirripedia: Thoracica), distributions and biogeographical affinities. *Integrative and comparative biology*, 52(3): 366–387. <https://doi.org/10.1093/icb/ics100>
- Jones DS, Hosie AM (2016) A checklist of the barnacles (Cirripedia: Thoracica) of Singapore and neighbouring waters. *Raffles Bulletin of Zoology* 34: 241–311.
- Karande AA, Palekar VC (1966) The sessile barnacles (Cirripedia) of the Bombay coast. *Journal of the Bombay Natural History Society* 63(1): 139–151.
- Kolosváry G (1950) On some balanids living in corals, collected by the Snellius Expedition in 1930. *Zoologische Mededelingen Leiden* 30: 289–296. <http://www.repository.naturalis.nl/document/150224>
- Krauss F (1848) Die sudafrikanischen Mollusken. Ein Beitrag zur Kenntniss der Mollusken des Kap- und Natallandes und zur geographischen Verbreitung derselben, mit Beschreibung und Abbildung der neuen Arten. (Stuttgart.)
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution* 35:1547–1549. <https://doi.org/10.1093/molbev/msy096>
- Lamoureux CH (1990) Maluku: Its Place in the History of Science. The Center for Southeast Asian Studies. <http://hdl.handle.net/10125/4049>
- Liu JY, Ren X (2007) Crustacea Cirripedia Thoracica. *Fauna Sinica: Invertebrata*, 42. Science Press, Beijing, China, 633 pp.
- Newman WA, Ross A (1971) Antarctic Cirripedia: Monographic Account Based on Specimens Collected Chiefly Under the United States Antarctic Research Program, 1962–1965. Vol. 14. American Geophysical Union, Washington D.C., 257 pp.
- Newman WA, Ross A (1976) Revision of the balanomorph barnacles; including a catalog of the species. *Memoirs of San Diego Society of Natural History*, 9: 1–108.
- Newman WA, Abbott DP (1980) Cirripedia: the barnacles. *Intertidal Invertebrates of California* 504–535. Available at: <http://decapoda.arthroinfo.org/pdfs/31753/31753.pdf>
- Nilsson-Cantell CA (1921) Cirripeden-studien. Zur Kenntnis der biologische, anatomie und systematik dieser gruppe, *Zoologiska Bidrag Från Uppsala* 7: 75–395. <https://doi.org/10.5962/bhl.title.10682>
- Nilsson-Cantell CA (1927) Some barnacles in the British Museum (Nat. Hist.). *Proceedings of the Zoological Society of London* 1927(3): 743–790. <https://doi.org/10.1111/j.1469-7998.1927.tb07432.x>
- Pérez-Losada M, Harp M, Høeg JT, Achituv Y, Jones D, Watanabe H, Crandall KA (2008) The tempo and mode of barnacle evolution. *Molecular phylogenetics and evolution* 46(1): 328–346. <https://doi.org/10.1016/j.ympev.2007.10.004>
- Pérez-Losada M, Høeg JT, Crandall KA, Achituv Y (2012) Molecular phylogeny and character evolution of the chthamaloid barnacles (Cirripedia: Thoracica). *Molecular phylogenetics and evolution* 65(1): 329–334. <https://doi.org/10.1016/j.ympev.2012.06.004>

- Pérez-Losada M, Høeg JT, Simon-Blecher N, Achituv Y, Jones D, Crandall KA (2014) Molecular phylogeny, systematics and morphological evolution of the acorn barnacles (Thoracica: Sessilia: Balanomorphia). *Molecular phylogenetics and evolution* 81: 147–158. <https://doi.org/10.1016/j.ympev.2014.09.013>
- Pilsbry HA (1896) On a collection of barnacles. *Proceedings of the Academy of Natural Sciences, Philadelphia* 48: 208. <https://doi.org/10.1080/00222939608680419>
- Pilsbry HA (1916) The sessile barnacles (Cirripedia) contained in the US National Museum; including a monograph of the American species. *Bulletin of the United States National Museum* 93: 1–366. <https://doi.org/10.5479/si.03629236.93.1>
- Pitombo FB, Gobin J, Abreu NMN, Jute A (2017) A Cryptic Invasion in the Western Atlantic: Presence of the Fouling Barnacle *Megabalanus zebra* (Darwin, 1854) (Crustacea, Cirripedia) in the Caribbean Sea. *Zootaxa* 4237(1): 131–153. <https://doi.org/10.11646/zootaxa.4237.1.7>
- Pochai A, Kingtong S, Sukparangsi W, Khachonpisitsak S (2017) The diversity of acorn barnacles (Cirripedia, Balanomorphia) across Thailand's coasts: The Andaman Sea and the Gulf of Thailand. *Zoosystematics and Evolution* 93: 13–34. <https://doi.org/10.3897/zse.93.10769>
- Poltarukha OP (1996) Composition, phylogeny and position in system of the subfamily Notochthamalinae (Crustacea, Chthamalidae). *Zoologicheskii Zhurnal* 75(7): 985–994.
- Poltarukha OP, Zevina GB (2006) Barnacles (Cirripedia, Thoracica) of the north-eastern Atlantic. Biogeography of the North Atlantic seamounts. KMK Scientific Press Ltd, Moscow, 162–176.
- Pope EC (1965) A review of Australian and some Indomalayan Chthamalidae (Crustacea: Cirripedia). *Proceedings of the Linnean Society of New South Wales* 90(1): 10–77.
- Posada D (2008) jModelTest: phylogenetic model averaging. *Molecular biology and evolution*. Oxford University Press 25(7): 1253–1256. <https://doi.org/10.1093/molbev/msn083>
- Power AM, Klepal W, Zheden V, Jonker J, McEvilly P, von Byern J (2010) Mechanisms of adhesion in adult barnacles. In: von Byern J., Grunwald I. (eds) *Biological Adhesive Systems*. Springer, Vienna, 153–168. <https://doi.org/10.1007/978-3-7091-0286-2>
- Puspasari IA, Yamaguchi T, Ross A (2002) New record of *Balanus zhujiangensis* (Cirripedia, Balanidae) from Okinawa, Japan. *Journal of Crustacean Biology*. 22(2): 235–240. <https://doi.org/10.1163/20021975-99990230>
- Ross A (1971) Studies on the Tetraclitidae (Cirripedia: Thoracica). A new tetraclitellan from India. *Transactions of the San Diego Society of Natural History* 16(8): 215–224. <https://doi.org/10.5962/bhl.part.15459>
- Ross A, Perreault RT (1999) Revision of the Tetraclitellinae and description of a new species of *Newmanella* Ross from the tropical western-Atlantic Ocean (Cirripedia: Tetraclitoidea). *Sessile Organisms* 15(2): 1–8. https://www.jstage.jst.go.jp/article/sosj1996/15/2/15_2_1/_pdf. https://doi.org/10.4282/sosj.15.2_1
- Rumphius GE (1896) 1705. D, Amboinsche Rariteitkamer. Francois Halma, Amsterdam, 552 pp.
- Shalaeva K, Boxshall G (2014) An illustrated catalogue of the scalpellid barnacles (Crustacea: Cirripedia: Scalpellidae) collected during the HMS “Challenger” expedition and deposited in the Natural History Museum, London. *Zootaxa* 3804(1): 1–63. <https://doi.org/10.11646/zootaxa.3804.1.1>

- Shorthouse DP (2010) SimpleMappR, an online tool to produce publication-quality point maps. [Retrieved from <http://www.simplemappR.net> Accessed August 21, 2018].
- Southward AJ, Burton RS, Coles SL, Dando PR, DeFelice R, Hoover J, Parnell PE, Yamaguchi T, Newman WA (1998) Invasion of Hawaiian shores by an Atlantic barnacle. *Marine Ecology Progress Series*, 165, 119–126. <https://www.jstor.org/stable/24827260>. <https://doi.org/10.3354/meps165119> <https://doi.org/10.3354/meps165119>
- Southward AJ, Newman WA (2003) A review of some common Indo-Malayan and western Pacific species of *Chthamalus* barnacles (Crustacea: Cirripedia). *Journal of the Marine Biological Association of the United Kingdom* 83(4): 797–812. <https://doi.org/10.1017/S0025315403007835h>
- Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap algorithm for the RAxML web servers. *Systematic biology* 57(5): 758–771. <https://doi.org/10.1080/10635150802429642>
- Strack HL (1993) Results of the Rumphius Biohistorical Expedition to Ambon (1990). Part 1. General account and list of stations. *Zoologische Verhandlungen* 289(1): 1–72.
- Stubbings HG (1961) Some Cirripedia from the Persian Gulf. *Annals and Magazine of Natural History*, (13)4 (39): 171–176. <https://doi.org/10.1080/00222936108655798>
- Stubbings HG (1967) The Cirriped Fauna of Tropical West Africa. *Zoology* 15(6): 227–319. <https://doi.org/10.5962/bhl.part.27518>
- Sukparangsi W, Pochai A, Wongkunanusorn C, Khachonpisitsak S (2019) Discovery of *Neon-rosella vitiata* (Darwin) and *Newmanella spinosus* Chan & Cheang (Balanomorpha, Tetraclitidae) from the Andaman Sea, eastern Indian Ocean. *ZooKeys* 833: 1–20. <https://doi.org/10.3897/zookeys.833.30689>
- Tsang LM, Chan BKK, Williams GA, Chu KH (2013) Who is moving where? Molecular evidence reveals patterns of range shift in the acorn barnacle *Hexechamaesipho pilsbryi* in Asia. *Marine Ecology Progress Series* 488: 187–200. <https://doi.org/10.3354/meps10385>
- Tsang LM, Chu KH, Nozawa Y, Chan BKK (2014) Morphological and host specificity evolution in coral symbiont barnacles (Balanomorpha: Pyrgomatidae) inferred from a multi-locus phylogeny. *Molecular phylogenetics and evolution* 77: 11–22. <https://doi.org/10.1016/j.ympev.2014.03.002>
- Tsang LM, Chu KH, Achariy Y, Chan BKK (2015) Molecular phylogeny of the acorn barnacle family Tetraclitidae (Cirripedia: Balanomorpha: Tetraclitoidea): Validity of shell morphology and arthropodal characteristics in the systematics of Tetraclitid barnacles. *Molecular phylogenetics and evolution* 82: 324–329. <https://doi.org/10.1016/j.ympev.2014.09.015>
- Utinomi H (1958) Studies on the Cirripedian Fauna of Japan. VII. Cirripeds from Sagami Bay. *Publications of the Seto Marine Biological Laboratory*, 6(3): 281–311. Figs 1–10. <https://doi.org/10.5134/174591>
- Weisbord NE (1979) Lepadomorph and Verrucomorph barnacles (Cirripedia) of Florida and adjacent waters, with an addendum on the Rhizocephala. *Bulletin American Paleontological* 72(297): 143–166.
- Whiting MF (2002) Mecoptera is paraphyletic: multiple genes and phylogeny of Mecoptera and Siphonaptera. *Zoologica scripta* 31(1): 93–104. <https://doi.org/10.1046/j.0300-3256.2001.00095.x>

- Yamaguchi T (1987) Changes in the barnacle fauna since the Miocene and the infraspecific structure of *Tetraclita* in Japan (Cirripedia: Balanomorpha). *Bulletin of Marine Science* 41: 337–359.
- Zullo VA, Newman WA (1964) Thoracic Cirripedia from a SE Pacific Guyot. *Pacific Science* 18: 355–72.
- Zullo VA, Beach DB, Carlton JT (1972) New barnacle records (Cirripedia, thoracica). *California Academy of Sciences* 4 (39): 65–74.

Supplementary material I

Tables S1–S28, Figs S1–S13

Authors: Pipit Pitriana, Luis Valente, Thomas von Rintelen, Diana S. Jones, Romanus E. Prabowo, Kristina von Rintelen

Data type: species data

Explanation note: **Fig. S1** The occurrence of *Heteralepas japonica* in the Moluccas (red dots); **Fig. S2** The occurrence of *Amphibalanus reticulatus*, *Dosima fascicularis*, *Euraphia hembeli*, *Hexechamaesipho pilsbryi*, *Megabalanus zebra*, *Nesochthamalus intertextus*, *Newmanella spinosus*, *Pseudoctomeris sulcata*, *Tetraclitella divisa* and *Tetraclitella karandei* in the Moluccas (the red dot indicates that the species occurs on the coastline of the island); **Fig. S3** The occurrence of *Chthamalus moro* and *Lepas anserifera* in the Moluccas (the red dots indicate that the species occurs on the coastline of the islands); **Fig. S4** The occurrence of *Amphibalanus amphitrite*, *Amphibalanus variegatus*, *Capitulum mitella*, *Megabalanus tintinnabulum*, *Tesseropora rosea*, *Tetraclita kuroshioensis* and *Tetraclita squamosa* in the Moluccas (the red dots indicate that the species occurs on the coastline of the islands); **Fig. S5** The occurrence of *Microeuraphia* sp. in the Moluccas (the red dot indicates that the species occurs on the coastline of the island); **Fig. S6** The occurrence of *Yamaguchiella coerulescens* in the Moluccas (the red dots indicate that the species occurs on the coastline of the islands); **Fig. S7** The occurrence of *Neonrosella vitiata* in the Moluccas (the red dots indicate that the species occurs on the coastline of the islands); **Fig. S8** The occurrence of *Amphibalanus zhujiangensis* in the Moluccas (the red dots indicate that the species occurs on the coastline of the islands); **Fig. S9** The occurrence of *Amphibalanus* sp. in the Moluccas (the red dots indicate that the species occurs on the coastline of the islands); **Fig. S10** Bayesian phylogeny of concatenated COI and 18S gene sequences. Values next to nodes are support values; **Fig. S11** ML phylogeny of concatenated COI and 18S gene sequences. Values next to nodes are support values; **Fig. S12** ML phylogeny of 18S gene sequences. Tips coloured in red are the new samples sequenced for this manuscript. Values next to nodes are support values; **Fig. S13** ML phylogeny of COI gene sequences. Tips coloured in red are the new samples sequenced for this manuscript. Values next to nodes are support values; **Table S1**. Table of sample information for DNA samples sequenced for this study; **Table S2**. Measurements for *Heteralepas*

japonica (N = 25); **Table S3.** Measurements for *Dosima fascicularis* (N = 6); **Table S4.** Measurements for *Lepas anserifera* (N = 25); **Table S5.** Measurements for *Capitulum mitella* (N = 25); **Table S6.** Measurements for *Pseudotomeris sulcata* (N = 2); **Table S7.** Measurements for *Hexechamaesipho pilsbryi* (N = 10); **Table S8.** Measurements for *Nesochthamalus intertextus* (N = 10); **Table S9.** Measurements for *Euraphia hembeli* (N = 1); **Table S10.** Measurements for *Microeuraphia* sp. (N = 2); **Table S11.** Measurements for *Chthamalus moro* (N = 25); **Table S12.** Measurements for *Tetracitella divisa* (N = 1); **Table S13.** Measurements for *Tetracitella karandei* (N = 3); **Table S14.** Measurements for *Tesseropora rosea* (N = 15); **Table S15.** Measurements for *Tetracitella kuroshioensis* (N = 5); **Table S16.** Measurements for *Tetracitella squamosa* (N = 5); **Table S17.** Measurements for *Yamaguchiella coerulescens* (N = 25); **Table S18.** Measurements for *Neonrosella vitiata* (N = 4); **Table S19.** Measurements for *Newmanella spinosus* (N = 5); **Table S20.** Measurements for *Amphibalanus amphitrite* (N = 15); **Table S21.** Measurements for *Amphibalanus reticulatus* (N = 5); **Table S22.** Measurements for *Amphibalanus variegatus* (N = 10); **Table S23.** Measurements for *Amphibalanus zhujiangensis* (N = 11); **Table S24.** Measurements for *Amphibalanus* sp. (N = 4); **Table S25.** Measurements for *Megabalanus tintinnabulum* (N = 4); **Table S26.** Measurements for *Megabalanus zebra* (N = 8); **Table S27.** Kimura 2-parameter (K2P) distances of COI sequences between species for *Microeuraphia* sp.; **Table S28.** Kimura 2-parameter (K2P) distances of COI sequences between species for *Amphibalanus* sp.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/zookeys.945.39044.suppl1>

Redescription of holotypes of four *Alopecosa* species (Araneae, Lycosidae) from China

Jia Tang¹, Xiang Xu¹, Haiqiang Yin¹, Yuri M. Marusik^{2,3,4}, Zongguang Huang¹

1 College of Life Science, Hunan Normal University, Changsha 410081, Hunan, China **2** Institute for Biological Problems of the North RAS, Portovaya Str. 18, Magadan 685000, Russia **3** Department of Zoology and Entomology, University of the Free State, Bloemfontein 9300, South Africa **4** Zoological Museum, Biodiversity Unit, University of Turku, FI-20014, Finland

Corresponding author: Haiqiang Yin (yinhaiqiang@hunnu.edu.cn), Yuri M. Marusik (yurmar@mail.ru)

Academic editor: G. Blagoev | Received 21 March 2020 | Accepted 26 April 2020 | Published 3 July 2020

<http://zoobank.org/2CDE5115-76CB-4C2A-A05E-FACFE64C38F4>

Citation: Tang J, Xu X, Yin HQ, Marusik YM, Huang ZG (2020) Redescription of holotypes of four *Alopecosa* species (Araneae, Lycosidae) from China. ZooKeys 945: 85–97. <https://doi.org/10.3897/zookeys.945.52287>

Abstract

The holotypes of four species of *Alopecosa* Sundevall, 1833 described from China, *A. disca* Tang, Yin & Yang, 1997 (♀); *A. orbisaca* Peng, Yin, Zhang & Kim, 1997 (♀); *A. wenxianensis* Tang, Yin & Yang, 1997 (♂), and *A. xilinensis* Peng, Yin, Zhang & Kim, 1997 (♀), are reexamined. Detailed descriptions, illustrations, remarks, and a distribution map of the three valid species are given. *Alopecosa xilinensis* **syn. nov.** is found to be junior synonym of *Alopecosa licenti* (Schenkel, 1953).

Keywords

Lycosinae, new synonymy, redescription, wolf spiders

Introduction

Alopecosa Simon, 1885, with 162 valid named species (WSC 2020), is the third largest genus in Lycosidae. Only *Pardosa* C.L. Koch, 1847 (542 species) and *Arctosa* C.L. Koch, 1847 (171) have more species (WSC 2020). *Alopecosa* is considered as globally distributed genus, known from all biogeographical realms, but most of its species oc-

cur in the Palaearctic. The genus is relatively poorly studied: over a third of all species (67) are known by a single sex, and *Alopecosa* has never been revised at a wide scale. There are only regional reviews of the genus in Europe (Lugetti and Tongiorgi 1969), the Nearctic region (Dondale and Redner 1979), and Japan (Tanaka 1992). The lack of the studies on *Alopecosa* is partly caused by the brief original descriptions, some of which lack figures, and by difficult access to type material in numerous museums. Currently, 42 *Alopecosa* species are known in China (WSC 2020). Of them, 17 are known by a single sex and 12 are known by a single taxonomic entry (WSC 2020). Leading up to a regional revision of *Alopecosa* from East Asia, we redescribe all available types of poorly known species deposited in Chinese institutions. The goal of this paper is to provide detailed illustrated redescription of four species deposited in the Hunan Normal University.

Material and methods

Specimens were examined under an Olympus SZX16 stereomicroscope and an Olympus BX53 compound microscope. Photographs were taken with a Canon PowerShot G12 digital camera mounted on an Olympus BX53 compound microscope. Both the male palps and female genitalia were examined, photographed, and illustrated after being dissected. All morphological measurements are calculated using a stereomicroscope (LEICA M205C) and given in millimeters. Eye diameters are taken at the widest point. Promarginal and retromarginal teeth on the chelicerae are given as the first, second, third, etc., from the base of the fang to the distal groove. Leg measurements are given as total length (femur, patella, tibia, metatarsus, tarsus). Measurements of the holotypes are from the original description. All specimens examined in this study are deposited in the College of Life Sciences, Hunan Normal University (HNU).

Terminology in the present paper follows Zyuzin (1993) and Nadolny (2018). The abbreviations used in the present paper are as follows:

Ag	accessorial gland;	PLE	posterior lateral eye;
ALE	anterior lateral eye;	PME	posterior median eye;
AME	anterior median eye;	PME–PLE	distance between PME and PLE;
AME–AME	distance between AMEs;	PME–PME	distance between PMEs;
AME–ALE	distance between AME and ALE;	Sb	septal base;
At	atrium;	Sd	sperm duct;
Cd	copulatory duct;	Sp	spermatheca;
Em	embolus;	Ss	septal stem;
Et	tip of embolus;	STL	sternum length;
Fd	fertilization duct;	STW	sternum width;
Ho	hood;	Sy	synembolus;
Ma	median apophysis;	Ta	tegular apophysis.
Pa	palea;		

Taxonomy

Family Lycosidae Sundevall, 1833

Genus *Alopecosa* Simon, 1885

Type species. *Araneus fabrilis* Clerck, 1757 from Sweden.

Alopecosa disca Tang, Yin & Yang, 1997

Figures 1, 2, 10

Alopecosa disca Tang, Yin & Yang in Yin et al. 1997: 64, fig. 27a–d (♀); Song et al. 1999: 317, fig. 187E (♀, republication of the original figures).

Type. Holotype ♀ (HNU, Lyco-Alop-0004-001): CHINA, Gansu Province, Lanzhou City, Yuzhong County, Temple Majiamiao, 13.VII.1981, leg. Yingqiu Tang. Temple Majiamiao: 35°51'N, 104°7.12'E (information supplied by present authors).

Diagnosis. Epigyne of this species is similar to that of *A. chagyabensis* Hu & Li, 1987, a species known from Xizang Autonomous Region of China. Both species are lacking anterior hood, have base of septum width much longer than septal stem length, both have distinct copulatory opening, but can be separated by septum width/length ratio 2.5 in *A. disca* and 1.8 in *A. chagyabensis*. Two species well differ by the shape of the endogyne (compare Fig. 1F–I and fig. 64-2 in Hu 2001).

Description. Body (Fig. 1A) length 16.0, carapace 7.9 long, 5.0 wide, abdomen 8.8 long, 5.8 wide (data from original description by Yin et al. 1997: 64). Carapace dark reddish brown. Cervical and radial grooves distinct, darker than body color. Fovea linear, short, but a little thick. Head region wide, with anterior margin almost 4/5 width of thorax region. Anterior eye row straight, almost as wide as median one, posterior row widest. Eye sizes and inter-distances (Fig. 1A, B): AME 0.30, ALE 0.27, PME 0.49, PLE 0.47; AME–AME 0.19, AME–ALE 0.2, PME–PME 0.49, PME–PLE 0.67. Clypeus height 0.23. Chelicerae black-brown, with three promarginal and two retromarginal teeth (Fig. 1C). Both labium and endites black brown, with reddish brown distal parts. Sternum (Fig. 1D) brown, with metallic luster. STL 3.52, STW 2.82 (Fig. 1D). Palp and legs reddish brown, robust, without any distinct annuli. Leg measurements: I 18.30 (5.00, 6.60, 3.80, 2.90); II 16.70 (4.80, 5.80, 3.60, 2.50); III 15.00 (4.50, 5.00, 3.60, 1.90); IV 20.40 (6.10, 7.00, 5.00, 2.30) (data from original description by Yin et al. 1997: 64), leg formula 4123. Dorsum of abdomen (Fig. 1A) dark brown mixed yellowish brown. Cardiac mark distinct, dark brown and large. Posterior half of abdomen with 4 or 5 chevrons (because the type specimen has been wrinkled, chevron patterns showed in color photos of the present paper are not clearer than those showed in line drawings of the original paper). Venter of abdomen grey yellowish in the middle and dark grey laterally.

Epigyne (Figs 1E–I, 2) wider than long. Atrium and anterior hood absent. Septum weakly sclerotized, with short stem (*Ss*) and broad base (*Sb*); stem shorter than base



Figure 1. *Alopecosa disca* Peng, Yin, Zhang & Kim, 1997, female. **A** Habitus, dorsal view **B** eyes, front view **C** chelicera, ventral view **D** sternum, ventral view **E** epigyne **F** vulva **G** vulva, showing the detailed left half **H** vulva, moved slightly from the normal ventral view **I** vulva, showing the detailed right half. Abbreviations: *Ag* accessorial gland, *Cd* copulatory duct, *Sb* septal base, *Sp* spermatheca, *Ss* septal stalk. Scale bars: 1 mm (**A**, **B**, **D**); 0.5 mm (**C**); 0.1 mm (**E**–**I**).

height. Spermathecae with clavate head, slender and curved stalk, and accessorial gland (*Ag*) situated at the place near copulatory duct. Copulatory ducts short, slightly twisted.

Male. Unknown.

Distribution. Only known from the type locality, Gansu, China (Fig. 10).

Alopecosa orbisaca Peng, Yin, Zhang & Kim, 1997

Figures 3, 4, 10

Alopecosa orbisaca Peng et al. 1997: 41, figs 1–5 (♀); Yin et al. 1997: 70, fig. 30a–e (♀, republication of the original figures); Song et al., 1999: 317, fig. 187M (♀, republication of the original figure).

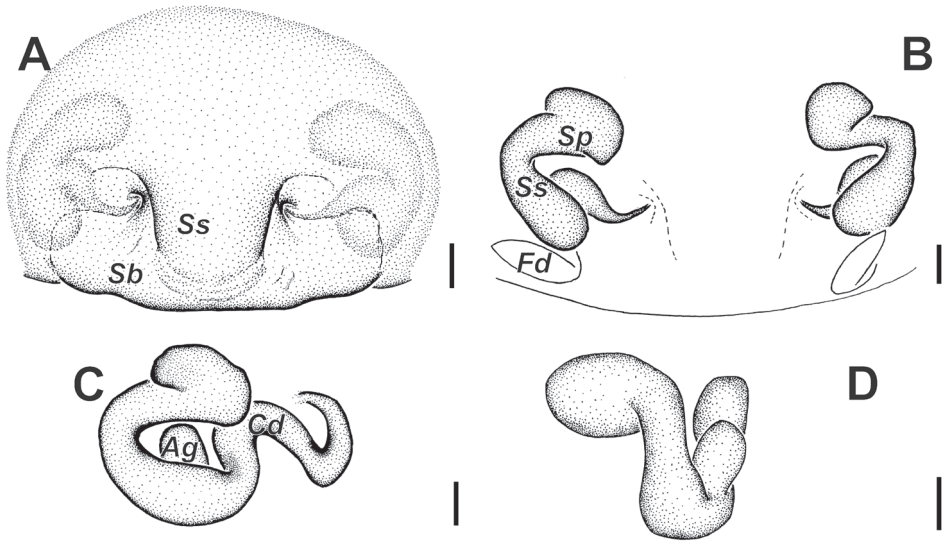


Figure 2. *Alopecosa disca* Peng, Yin, Zhang & Kim, 1997, female. **A** Epigyne **B** vulva **C** vulva, showing the detailed left half **D** vulva, showing the detailed right half. Abbreviations: **Ag** accessory gland, **Cd** copulatory duct, **Fd** fertilization duct, **Sb** septal base, **Sp** spermatheca, **Ss** septal stalk. Scale bars: 0.1 mm (**A–D**).

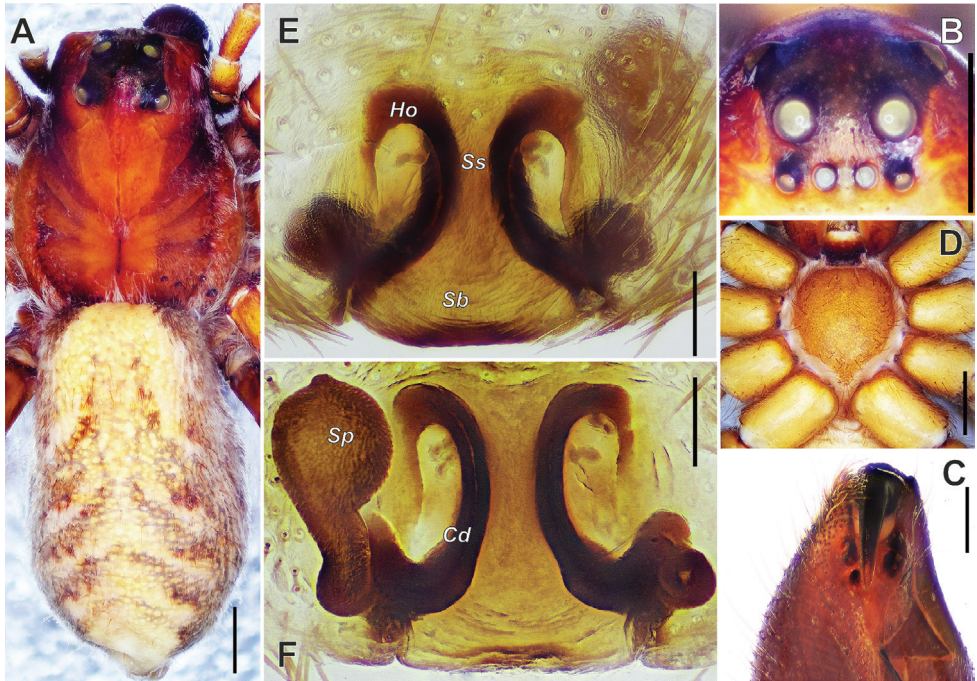


Figure 3. *Alopecosa orbisaca* Peng, Yin, Zhang & Kim, 1997, female. **A** Habitus, dorsal view **B** eyes, front view **C** chelicera, ventral view **D** sternum, ventral view **E** epigyne **F** vulva (right spermatheca lost). Abbreviations: **Cd** copulatory duct, **Ho** hood, **Sb** septal base, **Sp**-spermatheca, **Ss** septal stalk. Scale bars: 1 mm (**A, B, D**); 0.5 mm (**C, E, F**).

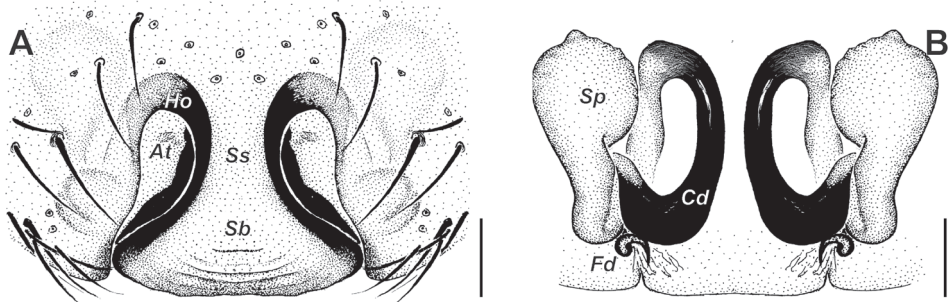


Figure 4. *Alopecosa orbisaca* Peng, Yin, Zhang & Kim, 1997, female. **A** Epigyne **B** vulva. Abbreviations: *At* atrium, *Cd* copulatory duct, *Fd* fertilization duct, *Ho* hood, *Sb* septal base, *Sp* spermatheca, *Ss* septal stalk. Scale bars: 0.1 mm.

Type. Holotype ♀ (HNU, *Lyco-Alop*-0003-001): CHINA, Qinghai Province, Xining City, 36°36'N, 101°48'E, 1978.

Diagnosis. The female of this species is similar to that of *Alopecosa zyuzini* Logunov & Marusik, 1995 in having a pair of separate anterior hoods and similar shape of septum. *Alopecosa orbisaca* can be separated from similar species by having large size (carapace 4.0 vs 2.85–3.58 in *A. zyuzini*), and wider stem of septum (septum wider than hood vs narrower than hood) (compare Fig. 3E and fig. 64-2 in Hu 2001).

Description. Holotype female. Body (Fig. 3A) length 9.20, carapace 4.00 long, 3.50 wide, abdomen 5.2 long, 3.20 wide (data from original description in Peng et al., 1997: 41). Carapace brown, with median pattern orange brown, short and about 1/4 width of thorax region. Cervical and radial grooves black brown, distinct. Pair of paraxial longitudinal bands brown, each band about 1/4 width of thorax region. Pair of lateral longitudinal bands also brown, however each band half of width of paraxial longitudinal band. Fovea long, linear, black. Cephalic region wide, with anterior margin about 3/4 width of thorax region. Anterior eye row straight, as wide as median one, posterior row widest. Eye sizes and interdistances (Fig. 3A, B): AME 0.14, ALE 0.16, PME 0.3, PLE 0.18; AME-AME 0.13, AME-ALE 0.11, PME-PME 0.35, PME-PLE 0.51. Clypeus height 0.19. Chelicerae black, with 3 promarginal (the first one largest and the third one smallest) and 2 retromarginal teeth (Fig. 3C). Labium with the base black and the distal part yellow and endites black-brown. Sternum yellow-brown, STL 1.94, STW 1.81 (Fig. 3D). Palps and legs reddish brown, robust, no distinct annulus except for femur of palp with dark stripes dorsally. Leg measurements: I 9.80 (2.90, 3.20, 2.00, 1.70); II 8.70 (2.80, 2.70, 1.90, 1.30); III 8.70 (2.80, 2.70, 1.90, 1.30); IV 11.30 (3.10, 3.50, 2.80, 1.90) (data from original description by Peng et al. 1997: 41). Abdomen yellow-brown, with large, pale cardiac and 5 brown chevron patterns dorsally (Fig. 3A). Venter of abdomen grey brown.

Epigyne (Figs 3E, F, 4) wide than long, with septum as long as wide, stem gradually widening toward the base, lateral margins of septum strongly sclerotized; anterior part of atrium with a pair of hoods (*Ho*); anterior part of septum (or stem, *Ss*) about

1.5 times wider than hoods. Spermatheca (*Sp*) divided distinctly into head and stalk, both of them with not smooth surface; a strong twist present at the connecting part between spermatheca and copulatory duct (*Cd*); copulatory duct long, ear-shaped.

Male. Unknown.

Distribution. Only known from the type locality, Qinghai, China (Fig. 10).

***Alopecosa wenxianensis* Tang, Yin & Yang, 1997**

Figures 5–7, 10

Alopecosa wenxianensis Tang, Yin & Yang in Yin et al. 1997: 75, fig. 33a–e (♂); Tang et al. 1998: 91, fig. 2a–e (♂); Song et al. 1999: 318, fig. 188J (♂, republication of the original figure).

Type. Holotype ♂ (HNU, Lyco-Alop-0001-001): CHINA, Gansu Province, Longnan City, Wenxian County (32°54'N, 104°6'E), Guanjiagou, 2.V.1992, leg. Yingqiu Tang.

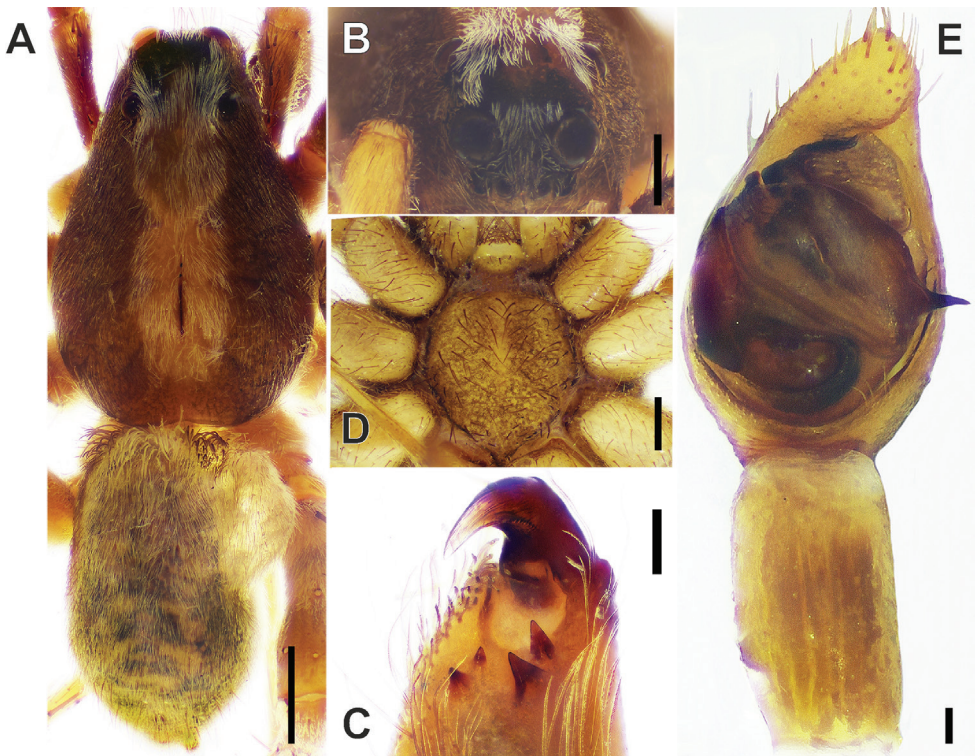


Figure 5. *Alopecosa wenxianensis* Tang, Yin & Yang, 1997, male. **A** Habitus, dorsal view **B** eyes, front view **C** chelicera, ventral view **D** sternum, ventral view **E** palp, ventral view (showing complete tibia). Scale bars: 1 mm (**A**); 0.5 mm (**B, D**); 0.1 mm (**C, E**).

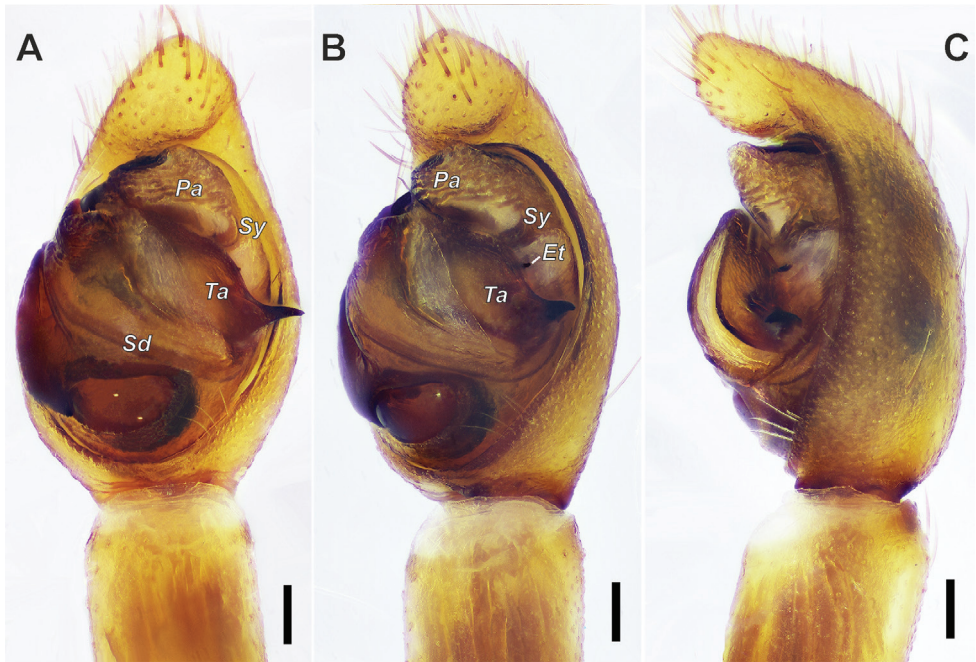


Figure 6. *Alopecosa wenxianensis* Tang, Yin & Yang, 1997, male. **A** Palp, ventral view **B** palp, ventral-retrolateral view **C** palp, retrolateral view. Abbreviations: **Et** tip of embolus, **Pa** palea, **Sd** sperm duct, **Sy** synembolus, **Ta** tegular apophysis. Scale bars: 0.1 mm.

Diagnosis. Male palp of this species (Figs 5E, 6, 7) is similar to that of *A. xinjiangensis* Hu & Wu, 1989 by having sharply pointed tegular apophysis and differs by having relatively shorter tip of cymbium, rounded anterior edge of the tegular apophysis (vs almost straight) and tip of tegular apophysis located in mid part of the bulb (vs anterior 1/3) (Hu and Wu 1989: figs 162.5–6; Marusik et al. 2007: fig. 3).

Description. Body (Fig. 5A) length 7.9, carapace 4.4 long, 3.0 wide, abdomen 3.5 long, 2.2 wide (after Yin et al. 1997: 75). Carapace dark-brown, with median pattern yellow-brown and about 1/3 width of thorax region. Cervical groove and radial grooves distinct, slightly darker than body color. A pair of lateral longitudinal bands dark brown, each about 2/3 width of thorax region. Cephalic region with anterior margin about 1/3 width of thoracic part. Anterior eye row slightly recurved, slightly narrower than median one, posterior row widest. Ocular area covered with white setae; eye sizes and inter-distances (Fig. 5A, B): AME 0.12, ALE 0.13, PME 0.31, PLE 0.25; AME-AME 0.12, AME-ALE 0.1, PME-PME 0.31, PME-PL 0.38. Clypeus height 0.09. Chelicerae brown, with blackish-grey patterns, and with 2 small promarginal and 2 large retromarginal teeth (Fig. 5C). Labium and endites yellow-brown. Sternum brown, with marginal lines darker, STL 1.3, STW 1.04 (Fig. 5D). Palp and legs brown, with black and gray patterns. Leg measurements: I 10.2 (2.8, 3.4, 2.5, 1.5); II 9.80 (2.6, 3.4, 2.3, 1.5); III 10.0 (2.5, 3.3, 2.6, 1.6); IV 13.5 (3.7, 4.2, 3.8, 1.8) (after Yin et al., 1997: 75). Dorsum of abdomen dark brown, densely covered with setae. Cardiac mark about 1/3 abdomen length, black

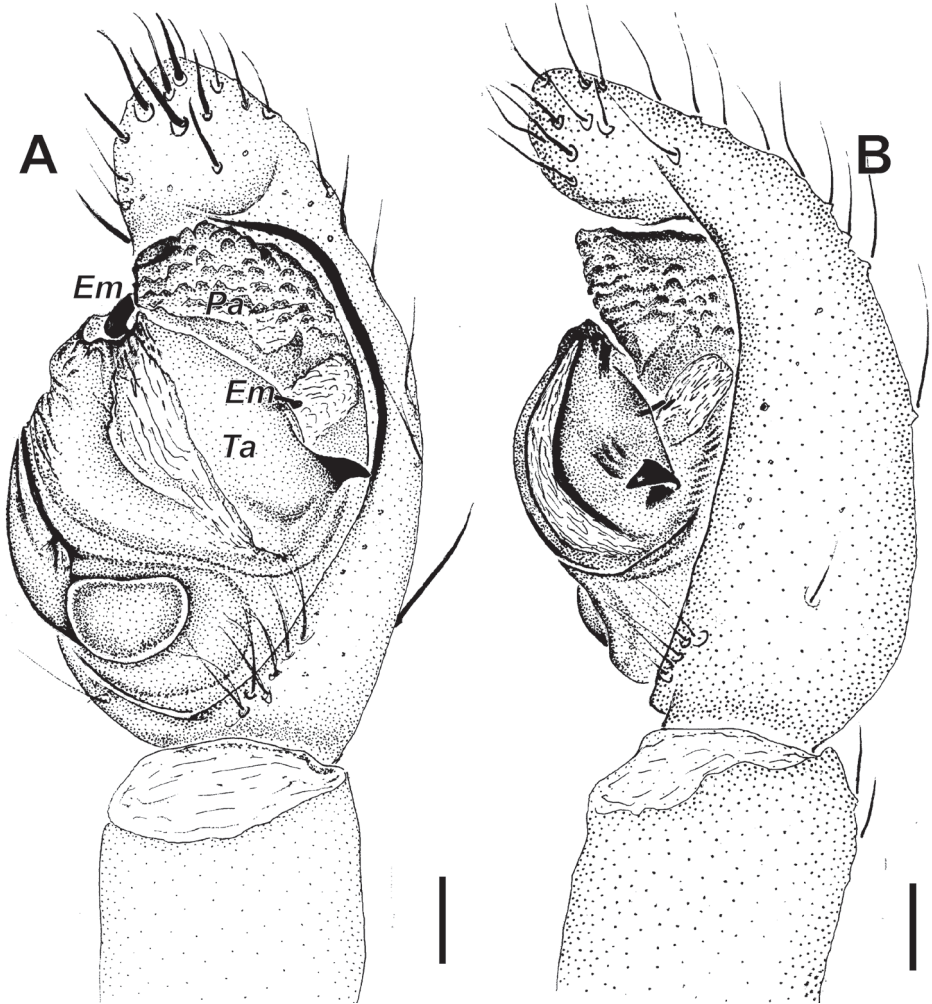


Figure 7. *Alopecosa wenshanensis* Tang, Yin & Yang, 1997, male. **A** palp, ventral view **B** same, retrolateral view. Abbreviations: Em-embolus; **Ta**-tegular apophysis; **Pa**-palea. Scale bars: 0.1 mm.

brown, with several yellow-brown transversal or oblique stripes on the sides of it. Venter of abdomen yellow-brown medially, and with irregular grey-black dots laterally.

Palp (Figs 5E, 6, 7). Cymbium brown, about 1.5 times longer than tibia, ca 1.8 times longer than wide, with the tip slightly swollen and covered with some strong setae. Bulb 1.25 times longer than wide; tegulum inclined at ca 50° angle and sperm duct (*Sd*) at about 20°; tegular apophysis sharply pointed, with smoothly rounded anterior margin, tip directed at right angle to the axis of cymbium and located in the middle part of the bulb; palea subequal in size to the subtegulum, with almost undeveloped short and rounded synembolus (*Sy*); embolus hidden by the tegular apophysis and only tip visible in ventral view.

Female. Unknown.

Distribution. Known only from the type locality, Gansu, China (Fig.10).

Remarks. Because the abdomen is wrinkled, several transversal or oblique stripes on the dorsum shown in Figure 5A are less clear than those shown in line drawings of the original description.

***Alopecosa licenti* (Schenkel, 1953)**

Figures 8, 9

Tarentula licenti Schenkel 1953: 77, fig. 36 (♀).

Tarentula argentata Schenkel 1963: 306, fig. 174a, b (♂).

Tarentula fenestrata Schenkel 1963: 311, fig. 177 (♀).

Tarentula fenestrata pseudobarbipes Schenkel 1963: 312, fig. 178 (♀).

Tarentula davidi Schenkel 1963: 313, fig. 179 (♀).

Tarentula orbiculata Schenkel 1963: 315, fig. 180 (♀).

Tarentula bipennis Schenkel 1963: 316, fig. 181 (♀).

Alopecosa xilinensis Peng, Yin, Zhang & Kim 1997: 42, figs 6–9 (♀); Yin et al. 1997: 76, fig. 34a–d (♀, republication of figures from Peng et al. 1997); Song et al. 1999: 318, fig. 188D (♀, copy of fig. 30c in Yin et al. 1997), syn. nov. For complete list of references, see WSC (2020).

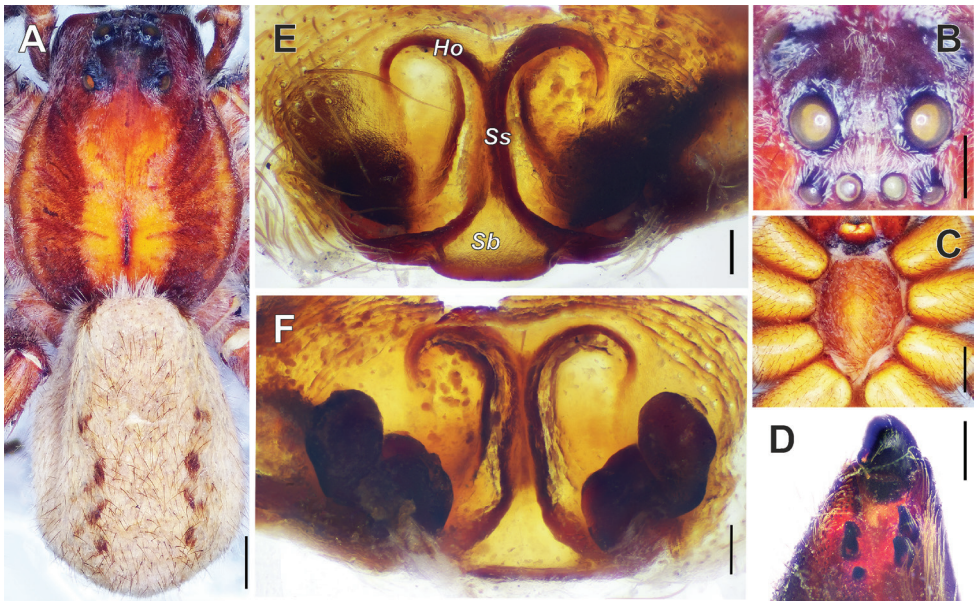


Figure 8. *Alopecosa licenti* (Schenkel, 1953), female. **A** Habitus, dorsal view **B** eyes, front view **C** Sternum, ventral view **D** Chelicera, ventral view **E** Epigyne **F** Vulva. Abbreviations: *Ho*-hood; *Sb*-septal base; *Ss*-septal stem. Scale bars: 1 mm (**A–C**); 0.5 mm (**D**); 0.1 mm (**E, F**).

Material examined. *Holotype* ♀ of *Alopecosa xilinensis* Peng et al., 1997 (HNU, Lyco-Alop-0002-001) from CHINA, Inner Mongolia Autonomous Region, Xilinhot, 44°N, 116°6'E, 13–15.VII.1987, leg. Jiafu Wang; 3 ♀, Inner Mongolia, VII.1983, leg. Lita Wu.

Remarks. Copulatory ducts in the original description of the species are known short and stout (Peng et al. 1997: 42). In fact, they are long and slender, and the short and stout parts are stalks of the spermathecae (Figs 8F, 9B).

Comments. This species has the most synonyms of any Chinese *Alopecosa* due to variations of the shape of the epigyne. Some individuals have very narrow septum (Schenkel 1953: fig. 36; Schenkel 1963: figs 177, 179; Yin et al. 1997: fig. 29b; Zhu and Zhang 2011: fig. 183A) and some have slightly wide septum (Schenkel 1963: figs 178, 180, 181; Song 1986: fig. 14). The septum has a smooth posterior margin (Schenkel 1953: fig. 36; Schenkel 1963: figs 179–181; Yin et al. 1997: fig. 29b; Zhu

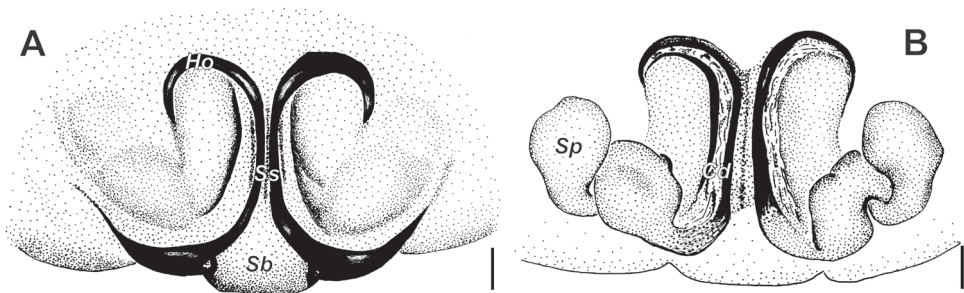


Figure 9. *Alopecosa licenti* (Schenkel, 1953), female. **A** Epigyne **B** vulva. Abbreviations: *Cd*-copulatory duct; *Ho*-hood; *Sb*-septal base; *Sp*-spermathecae; *Ss*-septal stem. Scale bars: 0.1 mm.

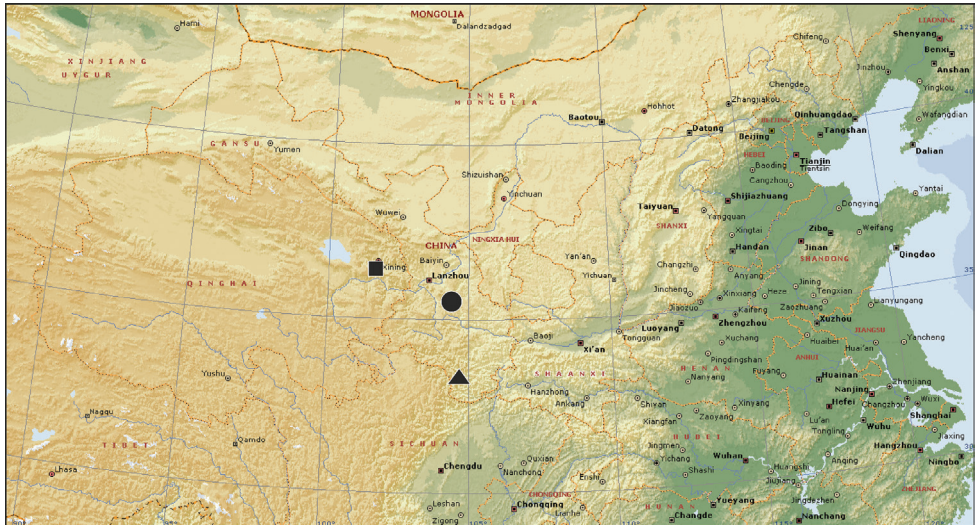


Figure 10. Type localities of *Alopecosa disca* (circle), *Alopecosa orbisaca* (square), and *Alopecosa wenxianensis* (triangle).

and Zhang 2011: fig. 183) in some specimens. But in others, the posterior margin of the septum has a lip-shaped protrusion (Schenkel 1963: figs 177–178). The holotype of *A. xilinensis* has the same narrow septum as shown by Schenkel (1963: fig. 177) or by Yin et al. (1997: fig. 29b). It also has the same posterior margin of septum as shown by Schenkel (1963: fig. 178). The vulva of holotype of *A. xilinensis* is the same as figured by Song (1986: fig. 15), Yin et al. (1997: fig. 29c), and Zhu and Zhang (2011: fig. 183b). Accounting for the similarity of the epigyne and vulva of the holotypes of *A. xilinensis* and *A. licenti*, we consider these names to be synonyms.

Distribution. The species has a rather wide distribution in China, known from Gansu to Heilongjiang and south to Sichuan (Zhu and Zhang 2011; Li and Lin 2016). Besides China, this species is known from Tuva, Khabarovsk and Maritime provinces in Russia (Mikhailov 2013), Mongolia, and also Korea (WSC 2020).

Acknowledgements

We are grateful to Ping Liu for her help in preparing for the specimens. We also thank Huizhuan Sheng and Yun Liang for their help in examining specimens and taking photographs. This study was supported by the National Natural Sciences Foundation of China (31772423/31471963/31372160) and the Key Project of Hunan Provincial Department of Education (19A320).

References

- Dondale CD, Redner JH (1979) Revision of the wolf spider genus *Alopecosa* Simon in North America (Araneae: Lycosidae). The Canadian Entomologist 111: 1033–1055. <https://doi.org/10.4039/Ent1111033-9>
- Hu JL (2001) Spiders in Qinghai-Tibet Plateau of China. Henan Science and Technology Publishing House, Zhengzhou, 658 pp. [in Chinese]
- Hu JL, Wu WG (1989) Spiders from agricultural regions of Xinjiang Uygur Autonomous Region, China. Shandong University Publishing House, Jinan, 435 pp.
- Li SQ, Lin YC (2016) Species Catalogue of China. Volume 3. Animalia. Invertebrata (I). Arachnida: Araneae. Science Press, Beijing, 578 pp. [in Chinese]
- Lugetti G, Tongiorgi P (1969) Ricerche sul genere *Alopecosa* Simon (Araneae-Lycosidae). Atti della Società Toscana di Scienze Naturali (B) 76: 1–100.
- Marusik YM, Fritzen NR, Song DX (2007). On spiders (Aranei) collected in central Xinjiang, China. Arthropoda Selecta 15 (3): 259–276.
- Mikhailov KG (2013). The spiders (Arachnida: Aranei) of Russia and adjacent countries: a non-annotated checklist. Arthropoda Selecta, Supplement 3: 1–262.
- Nadolny AA (2018) Lost and found: *Alopecosa krynickii* (Araneae: Lycosidae) in the Crimea. Zootaxa 4394 (4): 594–599. <https://doi.org/10.11646/zootaxa.4394.4.11>

- Peng XJ, Yin CM, Zhang YJ, Kim JP (1997) Five new species of the family Lycosidae from China (Arachnida: Araneae). *Korean Arachnology* 13 (2): 41–49.
- Schenkel E (1953) Chinesische Arachnoidea aus dem Museum Hoangho-Peiho in Tientsin. *Boletim do Museu Nacional do Rio de Janeiro (Nova Série, Zoologia)* 119: 1–108.
- Schenkel E (1963) Ostasiatische Spinnen aus dem Muséum d'Histoire naturelle de Paris. *Mémoires du Muséum National d'Histoire Naturelle de Paris (A, Zoologie)* 25: 1–481.
- Song DX (1986) A revision of the Chinese wolf spiders of the genus *Alopecosa* described by Schenkel (Araneae: Lycosidae). *Sinozoologia* 4: 73–82.
- Song DX, Zhu MS, Chen J (1999) The spiders of China. Hebei University of Science and Technology Publishing House, Shijiazhuang, 640 pp.
- Tanaka H (1992) Lycosid spiders of Japan VIII. The genus *Alopecosa* Simon. *Sonoda Women's College Studies* 26: 315–340.
- Tang YQ, Yin CM, Yang JT (1998) Three new species of family Lycosidae from Gansu, China (Araneae). *Journal of Lanzhou University Natural Sciences* 34: 90–93.
- World Spider Catalog (2020) World Spider Catalog. Natural History Museum Bern. Version 20.0. <https://wsc.nmbe.ch/> [Accessed on: 2020-2-10]
- Yin CM, Peng XJ, Xie LP, Bao YH, Wang JF (1997) Lycosids in China (Arachnida: Araneae). Hunan Normal University Press, 317 pp. [in Chinese]
- Zhu MS, Zhang BS (2011) Spider Fauna of Henan: Arachnida: Araneae. Science Press, Beijing, 558 pp. [in Chinese]
- Zyuzin AA (1993) Studies on the wolf spiders (Araneae: Lycosidae). I. A new genus and species from Kazakhstan, with comments on the Lycosinae. *Memoirs of the Queensland Museum* 33: 693–700.

Taxonomy of the *Cryptopygus* complex. III. The revision of South African species of *Cryptopygus* and *Isotominella* (Collembola, Isotomidae)

Mikhail B. Potapov^{1,2}, Charlene Janion-Scheepers^{3,4}, Louis Deharveng⁵

1 Senckenberg Museum of Natural History Görlitz, Am Museum 1, 02826, Görlitz, Germany **2** Moscow State Pedagogical University, Kibalkich str., 6, korp. 3, Moscow 129278, Russia **3** University of Cape Town, Department of Biological Sciences, Rondebosch, 7701, South Africa **4** Iziko Museums of South Africa, 25 Queen Victoria Street, Cape Town, 8001, South Africa **5** Institut de Systématique, Évolution, Biodiversité ISYEB-UMR 7205-CNRS, MNHN, UPMC, EPHE, Muséum national d'Histoire naturelle, Sorbonne Universités, 45 rue Buffon, F-75005, Paris, France

Corresponding author: Mikhail B. Potapov (mpnk-abroad@yandex.ru)

Academic editor: W. M. Weiner | Received 8 March 2020 | Accepted 24 April 2020 | Published 3 July 2020

<https://zoobank.org/CCD1B83E-2624-41CD-BD0A-E1C57EDCA4DC>

Citation: Potapov MB, Janion-Scheepers C, Deharveng L (2020) Taxonomy of the *Cryptopygus* complex. III. The revision of South African species of *Cryptopygus* and *Isotominella* (Collembola, Isotomidae). ZooKeys 945: 99–127. <https://doi.org/10.3897/zookeys.945.51860>

Abstract

Species of the genera of the *Cryptopygus* complex in South Africa are morphologically revised. Five new species of the genus *Cryptopygus* Willem, 1902 **s. s.** and one new species of the genus *Isotominella* Delamare Deboutteville, 1948 are described. *Cryptopygus abulbus* **sp. nov.** and *C. bulbus* **sp. nov.** have only one chaeta on the anterior side of dens and no chaetae on the anterior side of manubrium, the latter species being characterized by the presence of a bulb at apex of antennae; *C. inflatus* **sp. nov.** shows a rare combination of eight ocelli on each side of the head with a tridentate mucro; *C. longisensillus* **sp. nov.** has five long s-chaetae on the fifth abdominal segment; *C. postantennalis* **sp. nov.** is unique by having a very long and slender postantennal organ with strong inner denticles; *Isotominella laterochaeta* **sp. nov.** is the second member of the genus and differs from the type species by many more anterior chaetae on the manubrium and the presence of chaetae on ventral side of metathorax. The genera are discussed and a key to all species of the *Cryptopygus* complex recorded in South Africa is given. The focus is on the Western Cape Province where the complex is the most diverse and sampling more complete than in other provinces of South Africa.

Keywords

endemism, South Africa, springtails, taxonomy

Introduction

Although poor compared to Europe, the diversity of South Africa Collembola is increasingly better understood due to international collaborations since 2008 (Janion et al. 2011a, b, 2012; Janion-Scheepers et al. 2013, 2015; Potapov et al. 2011). The family Isotomidae, which include the dominant Collembola in soil and moss habitats of South Africa, has been particularly little studied, and currently consists of 19 confirmed described species (Janion-Scheepers et al. 2015). From samples collected over the last decade, especially in the Western Cape Province of South Africa, we found a large diversity of *Cryptopygus* species (Janion-Scheepers et al. 2011), which had not been recorded so far. Previous South African records of this genus include *Cryptopygus caecus* by Paclt (1959), now known as *Mucrosomia caeca* (Wahlgren, 1906), while Barra (1997) described *Cryptopygus riebi* Barra, 1997, now a synonym of *Pauropygus caussaneli* (Thibaud, 1996). We describe here the first five *Cryptopygus* species of South Africa and a new species of the genus *Isotominella*, monotypic up to this date. To summarize our data, we provide a key to all species of the *Cryptopygus* complex for South Africa. This is our second contribution to the taxonomy of the family Isotomidae of the country (Potapov et al. 2011) and the third piece of work on the *Cryptopygus* complex (Potapov et al. 2013; Potapov et al. 2017).

Material and methods

Abbreviations:

A, B, C, D, E	papillae of labial palp following Fjellberg (1999)
Abd.	abdominal segments
accp	s-chaeta(e) situated near or within p-row of chaetae
Ant.	antennal segments
bms	basal micro s-chaeta(e)
Mac1, Mac2	Macrochaeta(e)
MNHN	Muséum national d'Histoire Naturelle
ms	micro s-chaeta(e) or ms-chaeta(e)
MSPU	Moscow State Pedagogical University
PAO	postantennal organ
s	macro s-chaeta(e) or s-chaeta(e)
SAMC	South African Museum, Cape Town
SMNG	Senckenberg Museum of Natural History Görlitz
Th.	thoracic segments.

Abbreviations on figures are given in associated legends. Nomenclature used follows Potapov (2001) and Fjellberg (1999).

Type material of the new species described below are deposited at the Iziko South African Museum, Cape Town (South Africa, **SAMC**), Senckenberg Museum of Natu-

ral History, Görlitz (Germany, **SMNG**), Moscow State Pedagogical University (Russia, **MSPU**), and Muséum national d'Histoire Naturelle, Paris (France, **MHNH**).

Specimens were mounted on cavity or flat microscope slides using Gisin's solution or Marc André II mounting liquid, respectively. Specimens were studied using a Leica DM2500 microscope, while drawings were made using camera lucida.

Taxonomic part

In our understanding, the *Cryptopygus* complex includes all taxa of Anurophorinae s. l. having Abd. V well separated from Abd. IV and fused with Abd. VI, and lacking any special apomorphy (e.g., presence of true spines at the end of abdomen, loss of PAO, furca, etc.). Both genera studied below belong to the *Cryptopygus* complex.

Key to South African species of the *Cryptopygus* complex

- 1 Abdominal tip with several foil-chaetae (chaetae with a cluster of cilia near the tip, Fig. 60)..... *Hemisotoma thermophila* s. l. (Axelson, 1900)
- Abdominal tip without foil-chaetae..... 2
- 2 S-chaetae in p-row of chaetae on Abd. I–III..... 3
- S-chaetae in mid-tergal position on Abd. I–III..... 6
- 3 Furca long: dens with more than 30 anterior chaetae..... 4
- Furca of medium size: dens with less than 20 anterior chaetae..... 5
- 4 Mucro falciform. Anterior side of manubrium with 4+4 chaetae. Without ocelli..... *Arlea tridens* Barra, 1997
- Mucro bidentate. Anterior side of manubrium with 1+1 chaetae. 1+1 ocelli....
..... *Proisotomodes bipunctatus* (Axelson, 1903)
- 5 Number of s-chaetae on Th. III–Abd. II 1/1,1. Pleural fold on mouth cone with two finger-like processes (fig. 20 in Potapov et al. 2013). Only on sea littoral..... *Pauropygus caussaneli* (Thibaud, 1996)
- Number of s-chaetae on Th. III–Abd. II 3/2,2. Pleural fold on mouth cone without finger-like processes (Fig. 52)..... *Isotominella laterochaeta* sp. nov.
- 6 Mucro with 2 teeth, dens not crenulated on posterior side..... 7
- Mucro with 3 or more teeth, dens crenulated on posterior side..... 9
- 7 Manubrium without chaetae on anterior side..... 8
- Manubrium with 1+1 chaetae on anterior side.....
..... *C. antarcticus* complex, several species
- 8 Antennal segment IV with bulb (Figs 9, 10), tenaculum with one chaeta.....
..... *C. bulbosus* sp. nov.
- Antennal segment IV without bulb (Figs 17, 18), tenaculum with two chaetae..... *C. abulbus* sp. nov.
- 9 Blind, mucro with 3 teeth and 2 lateral basal spines.....
..... *Mucrosomia caeca* (Wahlgren, 1906)
- With ocelli, mucro with 3 teeth and without lateral basal spines..... 10

- 10 PAO slender and long (twice as long as width of Ant. I), with strong inner denticles (Fig. 27) *Cryptopygus postantennalis* sp. nov.
- PAO normal (at most slightly longer than width of Ant. I), without strong inner denticles 11
- 11 Macrochaetae and s-chaetae on Abd. V short.... *Cryptopygus inflatus* sp. nov.
- Macrochaetae and s-chaetae on Abd. V long.....
..... *Cryptopygus longisensillus* sp. nov.

Cryptopygus Willem, 1902

Type species. *Cryptopygus antarcticus* Willem, 1902.

Diagnosis. A genus of the *Cryptopygus* complex. Medial s-chaetae in mid-tergal position from Th. II to Abd. III. Number of s-chaetae 4,3/2,2,2,3,5. Foil chaetae at the end of abdomen absent.

Discussion. Here we follow our simplified characterisation of the genus proposed formerly (Potapov et al. 2017) that is an expanded version of diagnosis of Rusek (2002). The latter diagnosis offers to be within the limits of the definite number of ocelli (6+6), “*antarcticus*-like” furca and clavate tibiotarsal hairs. We presume a wide variation of the main characters: the mucro may be absent, bidentate or tridentate; the manubrium with or without anterior chaetae; the dens short and smooth to long and crenulated; the ocelli absent to their full set, i.e. 8+8; tenent tibiotarsal hairs clavate or pointed; number of ms-chaetae from 1,0/0,0,0 to 1,1/1,1,1; maxillary outer lobe with changeable shape of palp and number of sublobal hairs. In our view, the splitting of *Cryptopygus* to groups, subgenera or genera will be probably necessary, but calls for preliminary morphological revisions and expanded molecular data of known species.

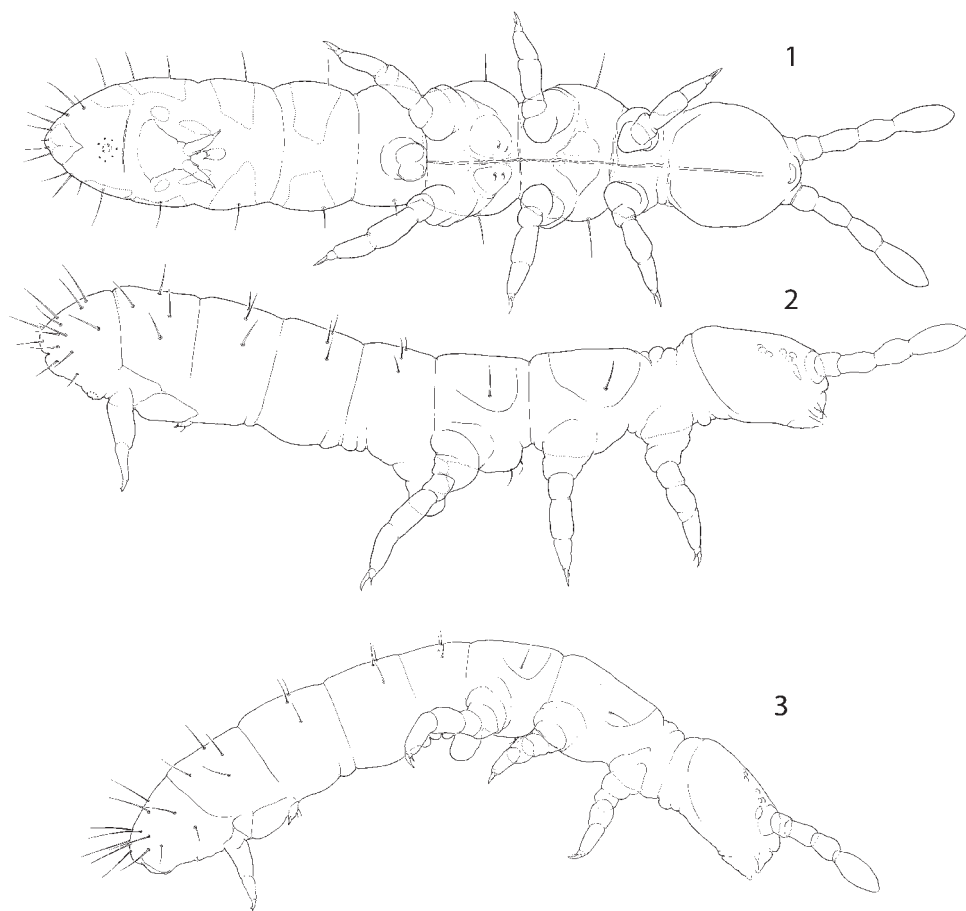
Species closely related to *C. antarcticus*, i.e., belonging to *Cryptopygus* sensu Rusek, have also been found in this study in South Africa (see below) but their status remain unsolved because of the taxonomic and molecular complexity of this group (Deharveng, 1981; Stevens et al. 2006; McGaughan et al. 2010).

Cryptopygus bulbosus sp. nov.

<http://zoobank.org/BDBE2CF-1565-4A84-A15F-5E51CB5C857E>

Figures 1, 2, 4, 6–14

Type material. Holotype and thirteen paratypes: SOUTH AFRICA • Western Cape, Cederberg Wilderness Area, Wolfberg Crag; 32.471507S, 19.278397E; 19 Feb 2011; C. Janion-Scheepers leg.; litter, Tullgren extraction; RSA11_CED002, deposited at SAMC • six paratypes on four slides and seven paratypes in ethanol; same locality • sixteen paratypes; Western Cape, Cederberg Wilderness Area; 32.310167S, 19.175183E; Oct. 2008; C. Janion-Scheepers leg.; pitfall trap; RSA08_CED001. Four paratypes deposited in ethanol at SAMC, five paratypes on three slides deposited at SMNG, seven paratypes on three slides at MSPU.



Figures 1–3. Appearance and macrochaetotaxy of *C. bulbus* sp. nov., ventral (1) and lateral (2) views, and *C. abulbus* sp. nov. (3).

Other material. SOUTH AFRICA • Western Cape, Mont Rochelle Nature Reserve, Franschoek; 33.902967S, 19.158950E; 06 Oct. 2008; C. Janion-Scheepers leg.; *Erica* site with *Erica-Protea* (Ericaceae and Protacea) mixed litter, litter trap; MR644, MR648 • Western Cape, Kogelberg Nature Reserve; 34.328083S, 18.962250E; 29 Aug. 2008; C. Janion-Scheepers leg.; *Erica* Site, Litter trap (K467), with *Galenia* litter.

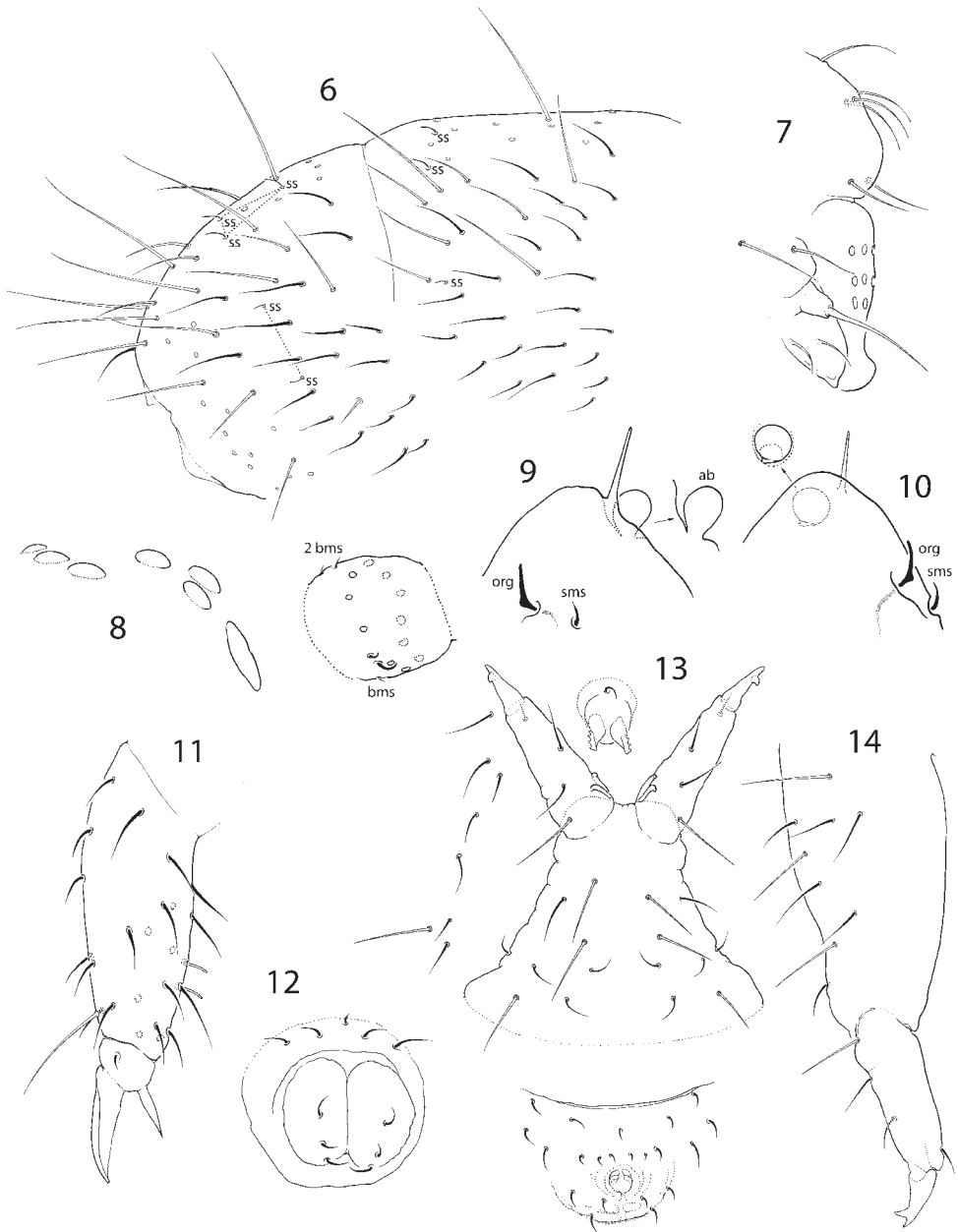
Diagnosis. With a globular retractile bulb on Ant. IV. Organite on Ant. IV chili-like. 6+6 ocelli. Maxillary palp simple. Two sublobal hairs. Anterior side of manubrium without chaetae. Tenaculum with one chaeta.

Description. Body size 0.7–0.9 mm, habitus as in Figs 1, 2. Body with rather regular blue pigmentation, slender. Abd. V well separated from Abd. IV and fused with Abd. VI (Fig. 4). Cuticle “smooth”, with orthogonal primary granulation. Ocelli 6+6 arranged in anterior and posterior groups (Fig. 8), three in each. PAO more than twice as long as ocellus, 0.6–0.7 as long as width of Ant. I and 1.0–1.3 as long as inner unguis length. Maxillary head with unmodified lamellae. Maxillary outer lobe with two sublobal hairs, maxillary palp simple. Labral formula as 2/5,5,4, edge of la-



Figures 4–5. Macrochaetotaxy and s-ms-pattern of *C. bulbus* sp. nov. (4) and *C. abulbus* sp. nov. (5). Abbreviations: ms ms-chaeta, ss s-chaeta.

brum not reduced (Fig. 7). Labium with five usual papillae (A–E) and labial formula A1B3C0D4E6, guard chaetae e7 and b4 absent, three proximal and four basomedian chaetae. Ventral side of head with 4+4 chaetae. Ant. I with two ventral s-chaetae (s) and three small bms, two dorsal and one ventral, Ant. II with three bms and one latero-distal s, Ant. III with one bms and five distal s (including one lateral), without additional s-chaetae. S-chaetae on Ant. IV weakly differentiated. Organite long, of chili-like shape, set apart from subapical micro s-chaeta (Figs 9, 10). A globular retractile bulb embedded at tip of antennae, near pin-chaeta (Figs 9, 10).



Figures 6–14. *C. bulbus* sp. nov. **6** Abd. IV and fused Abd. V and VI **7** labrum, lateral view **8** ocelli, PAO and Ant. I **9**, **10** apical part of Ant. IV, different views **11** tibiotarsus and claw of Leg III in adult male **12** ventral tube, ventral view **13** furcal area, ventral view **14** furca, lateral view. Abbreviations: ab apical bulb, org organit, sms subapical ms-chaeta, bms basal ms-chaeta, ss s-chaeta.

Common chaetae often slightly serrated at the posterior part of Abd. V. S-formula as 4,3/2,2,2,3,5 (s), 1,0/1,0,0 (ms) (Fig. 7). Tergal s-chaetae much shorter than common chaetae and well distinguishable (Fig. 4). Medial s-chaetae on Th. II–Abd. III situated in

mid-tergal position. On Abd. V, three dorsal s-chaetae (al, accp1, accp2) and two lateral ones slightly shorter (Fig. 6). Macrochaetae smooth and short, 1,1/3,3,3 in number, medial ones on Abd. VI 1.6–2.0 times longer than dens and 2.8–4.1 times longer than mucro. Foil chaetae at the tip of abdomen absent. Axial chaetotaxy as 5–6,4/3,3(4),3–4,4–6. Th. I and II without ventral chaetae, Th. III with 2+2 ventral chaetae (Fig. 1).

Unguis of normal shape, without teeth. Empodial appendage 0.5–0.7 as long as unguis. Tibiotarsi without additional chaetae on Leg I and II (21 chaetae), and with few ones on Leg III (>25), adult males with short thickened spurs on tibiotarsi III (Fig. 11). Tibiotarsal tenent hairs clavate, 1,2,2 on Tibiotarsi 1,2,3. Ventral tube with 3+3 laterodistal and 4–6 posterior chaetae, anteriorly without chaetae (Fig. 12). Tenaculum with 4+4 teeth and one chaeta. Anterior furcal subcoxa with 5–7, posterior one with three chaetae. Anterior side of manubrium without chaetae, posterior side with 4+4 laterobasal and 8–10 chaetae on main part, without lateral chaetae (Figs 13, 14). Dens short, without crenulation, with one rigid and short anterior and three posterior chaetae. Mucro bidentate. Ratio manubrium : dens : mucro = 2.5–4.1 : 1.7–2.2 : 1.

Etymology. The name is derived from the presence of apical bulb on Ant. IV.

Distribution and ecology. Currently known to occur in the southern part of the Western Cape Province of South Africa, including Kogelberg, Franschoek and the Table Mountain area (Cape Town). All specimens were collected from leaf litter in indigenous vegetation.

Discussion. The species differs from other representatives of the *Cryptopygus* complex, if not from all Isotomidae of the Southern Hemisphere, by the presence of a globular bulb at tip of the antennae. The taxonomical value of this character is not fully clear. In the Northern Hemisphere several unrelated genera also possess an apical bulb which is embedded on the tip of the antennae, for example *Anurophorus* Nicolet, 1842 (most of the species), *Sibiracanthella* Potapov & Stebaeva, 1994, *Tuvia* Grinbergs, 1962 and *Vertagopus* Börner, 1906 (few species). The antennal bulb of *C. bulbosus* sp. nov. is set apart from the apex, which is unlike in the aforementioned taxa. The only exception found was in specimens observed from Orangetree (Cape Town), where the apical bulb was less developed.

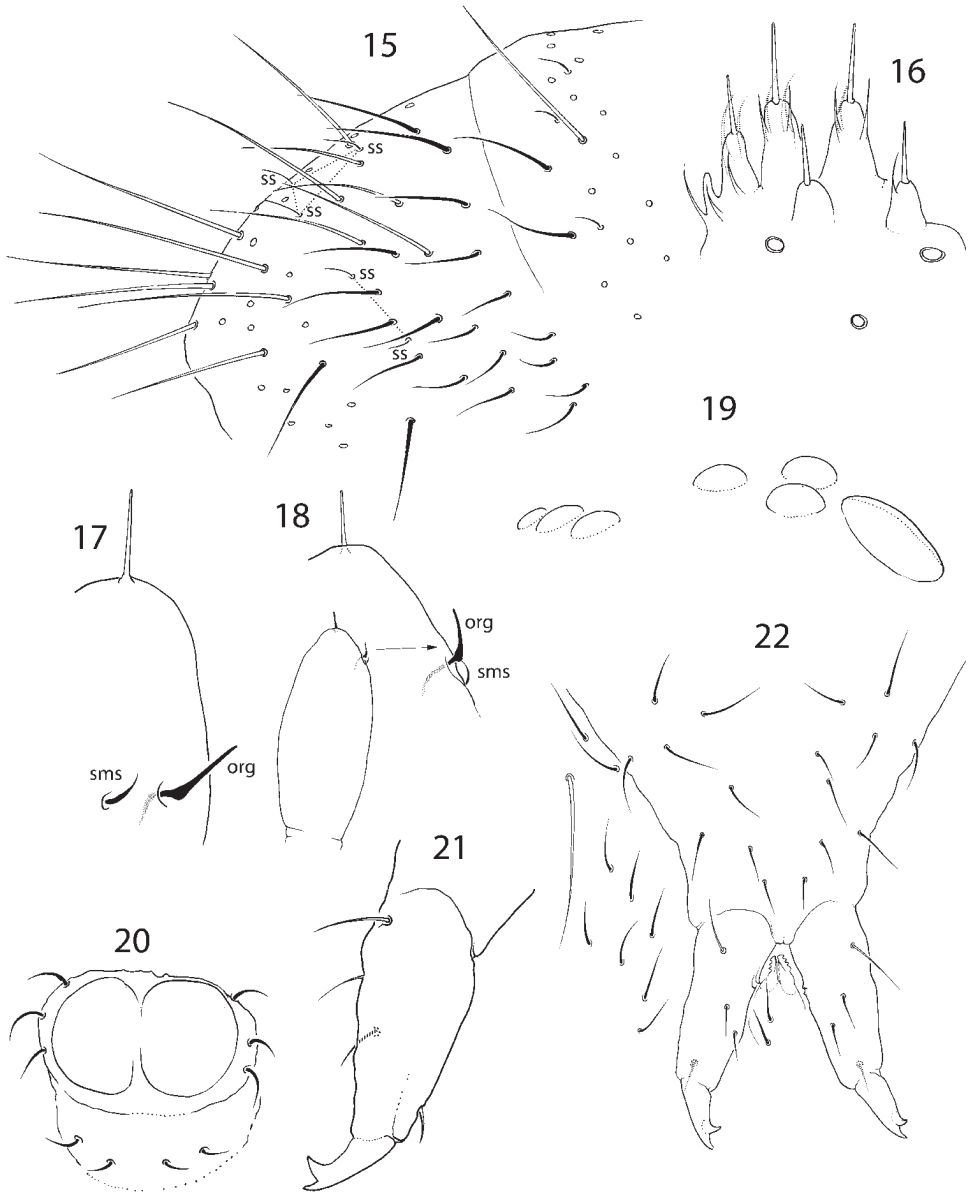
For other differences of the new species from congeners see the Discussion of *C. abulbus* sp. nov.

***Cryptopygus abulbus* sp. nov.**

<http://zoobank.org/EE9161B6-9521-407C-ADA5-1742B864573E>

Figures 3, 5, 15–22

Type material. Holotype and eighteen paratypes: SOUTH AFRICA • Western Cape, Stellenbosch, Jonkershoek Nature Reserve; 33.986883S, 18.955350E; 30 July 2009; C. Janion-Scheepers leg.; litter trap (J2_32), Holotype and eight paratypes deposited on four slides at SAMC, four paratypes on two slides deposited at SMNG, four paratypes on two slides deposited at MSPU, two paratypes on one slide at MNHN.



Figures 15–22. *C. abulbus* sp. nov. **15** Abd. IV and fused Abd. V and V **16** labial palp **17, 18** apical part of Ant. IV, different views **19** ocelli and PAO **20** ventral tube, ventral view **21** furca, lateral view **22** furcal area, ventral view. Abbreviations: org organite, sms subapical ms-chaeta, ss s-chaeta.

Diagnosis. Without globular retractile bulb on Ant. IV. Organite on Ant. IV chili-like. 6+6 ocelli. Maxillary palp simple. Two sublobal hairs. Anterior side of manubrium without chaetae. Tenaculum with two chaetae.

Description. Body size 0.6–0.7 mm. Body with regular blue pigmentation, slender (Fig. 3). Abd. V well separated from Abd. IV and fused with Abd. VI (Fig. 15). Cuticle with orthogonal granulation. Ocelli 6+6 arranged as three in anterior and three in posterior group (Fig. 19). PAO more than twice as long as ocellus, 0.6–0.7 as long as width of Ant. I and 0.8–1.1 mm as long as inner unguis length. Maxillary outer lobe with two sublobal hairs, one individual with one sublobal hair on one side was found. Maxillary palp simple. Labral formula as 2/5,5,4. Labium with five usual papillae (A–E, Fig. 16) and labial formula as in *C. bulbosus* sp. nov. Ventral side of head with 4+4 postlabial chaetae. Ant. I with eleven common chaetae, two ventral s-chaetae (s) and three small basal micro s-chaetae (bms), two dorsal and one ventral, Ant. II with three bms and one latero-distal s, Ant. III with one bms and five distal s (including one lateral). S-chaetae on Ant. IV weakly differentiated. Organite long, of chili-like shape, set apart from subapical micro s-chaeta (Figs 17, 18). Tip of antennae without retractile bulb.

Common chaetae slightly (under very high magnification) serrated at the posterior part of abdomen. S-formula as 4,3/2,2,2,3,5 (s), 1,0/1,0,0 (ms) (Figs 5,15). Tergal s-chaetae much shorter than common chaetae and well distinguishable. Medial s-chaetae on Th. II–Abd. III situated in mid-tergal position. Macrochaetae smooth and short, 1,1/3,3,3 in number, medial ones on Abd. VI 1.6–2.0 times longer than dens and 2.8–4.1 times longer than mucro. Foil chaetae at the tip of abdomen absent. Axial chaetotaxy as 6–8,5/3,3,3,5–6. Th. I and II without chaetae, Th. III with 2+2 ventral chaetae.

Unguis without teeth. Empodial appendage 0.5–0.7 as in *C. bulbosus* sp. nov. Tibiotarsi without additional chaetae on Leg I and II (21 chaetae), and with a few additional ones on Leg III, about 26 chaetae. Tibiotarsal tenent hairs clavate, 1,2,2 on Tibiotarsi 1,2,3. Ventral tube with 3+3 laterodistal and four posterior chaetae, anteriorly without chaetae (Fig. 20). Tenaculum with 4+4 teeth and two chaetae. Anterior furcal subcoxa with 5–7, posterior one with three chaetae. Anterior side of manubrium without chaetae, posterior side with 4+4 laterobasal and 8–9 chaetae on main part, without lateral chaetae. Dens short, without crenulation, with one rigid and short anterior and three posterior chaetae (Figs 21, 22). Mucro bidentate. Ratio manubrium : dens : mucro = 3.3–3.8 : 1.7–2.3 : 1.

Etymology. The name is derived from the absence of apical bulb on Ant. IV to stress the difference from *C. bulbosus* sp. nov.

Distribution and ecology. Currently known from indigenous vegetation in the Jonkershoek Nature Reserve, Stellenbosch.

Discussion. Unlike *C. bulbosus* sp. nov., the new species has no antennal bulb. Nevertheless, the two species form a rather well-defined group differing from almost all congeners by a simple maxillary palp, two sublobal hairs, chili-shaped organite on Ant. IV, and the absence of chaetae on the anterior side of the manubrium. Concerning the last character, only *C. nivicolus* (Salmon, 1965) and *C. sverdrupi* Lawrence, 1978 also lack this pair of chaetae, which is common to other species of the genus. Both mentioned species are inhabitants of Antarctic polar deserts and can hardly be conspecific to *C. abulbosus* sp. nov. found in dry sites in a subtropical climate. These two Antarctic

species are very dark and have two clavate tenent hairs (vs. one in *C. abulbus* sp. nov.) on tibiotarsi I. In addition, *C. nivicolus* has no mucro (vs. present in the new species) while *C. sverdrupi* has very small PAO (more than twice longer than ocellus in *C. abulbus* sp. nov.). Recently, *Gressitacantha terranova* Wise, 1967 was moved to *Cryptopygus* (Greenslade, 2015) adding another *Cryptopygus* species without anterior chaetae on the manubrium. The differences between *C. abulbus* sp. nov. and *C. terranovus* are more numerous than those from *C. nivicolus* and *C. sverdrupi* (in furca, arms of abdomen, length of macrochaetae, and others).

***Cryptopygus postantennalis* sp. nov.**

<http://zoobank.org/B6CDC3CA-C778-4B6B-A5EF-BE57FE61F6D6>

Figures 23, 26–31

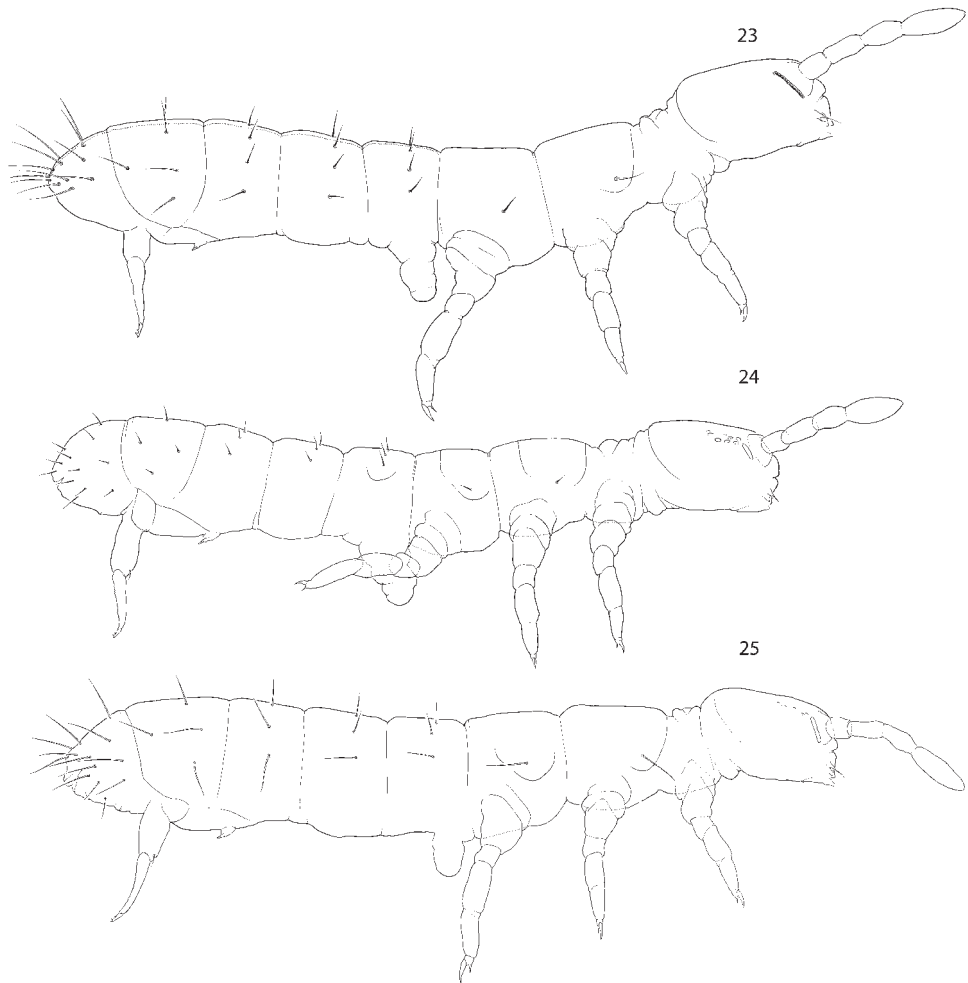
Type material. Holotype and three paratype: SOUTH AFRICA • Western Cape, Kogelberg Biosphere Reserve; 34.332650S, 18.950900E; 04 Oct. 2011; C. Janion-Scheepers leg.; Afromontane forest, litter/wood, Tullgren-Berlese extraction; RSA11_KOG007. Deposited on two slides at SAMC.

Other material. SOUTH AFRICA • Western Cape, Haarwegskloof, Swellendam; 34.335968S, 20.325094E; 18 July 2017; O. Cowan leg. Deposited at MSPU.

Diagnosis. 1+1 ocelli. PAO very long and slender, with large inner denticles. MS-formula 1,0/0,0,1 (ms). All s-chaetae of Abd. V in one dorsal group. Anterior side of manubrium with 1+1 chaetae. Dens of medium length. Mucro tridentate.

Description. Body size 0.8 mm (only one adult female could be measured). Body mostly white, slender, with rare, scattered pigmentation and a distinct black eyespot (Fig. 23). Abd. V well separated from Abd. IV and fused with Abd. VI (Fig. 31). Cuticle with orthogonal and hexagonal granulation. One rudimentary ocellus, with a concentration of pigmentation. PAO very long and slender (Fig. 27), at least five times as long as ocellus, twice as long as width of Ant. I and 2.8 as long as inner unguis length. PAO constricted, with large “inner denticles”. Maxillary head with unmodified lamellae. Maxillary outer lobe with four sublobal hairs, maxillary palp bifurcate. Labral formula as 4/5,5,4, edge of labrum not reduced. Labium with five usual papillae (A–E), guard chaetae e7 present, three proximal and four basomedian chaetae. Ventral side of head with 4+4 chaetae. Ant. I with two ventral s-chaetae (s), distal one much shorter than proximal one, and three basal micro s-chaetae (bms), of which a long dorsal one (Fig. 28), Ant. II with three bms and one latero-distal s, Ant. III with one bms and five distal s (including one lateral), without additional s-chaetae. S-chaetae on Ant. IV weakly differentiated. Organite hook-like, close to subapical micro s-chaeta, which is long and bent (Fig. 29).

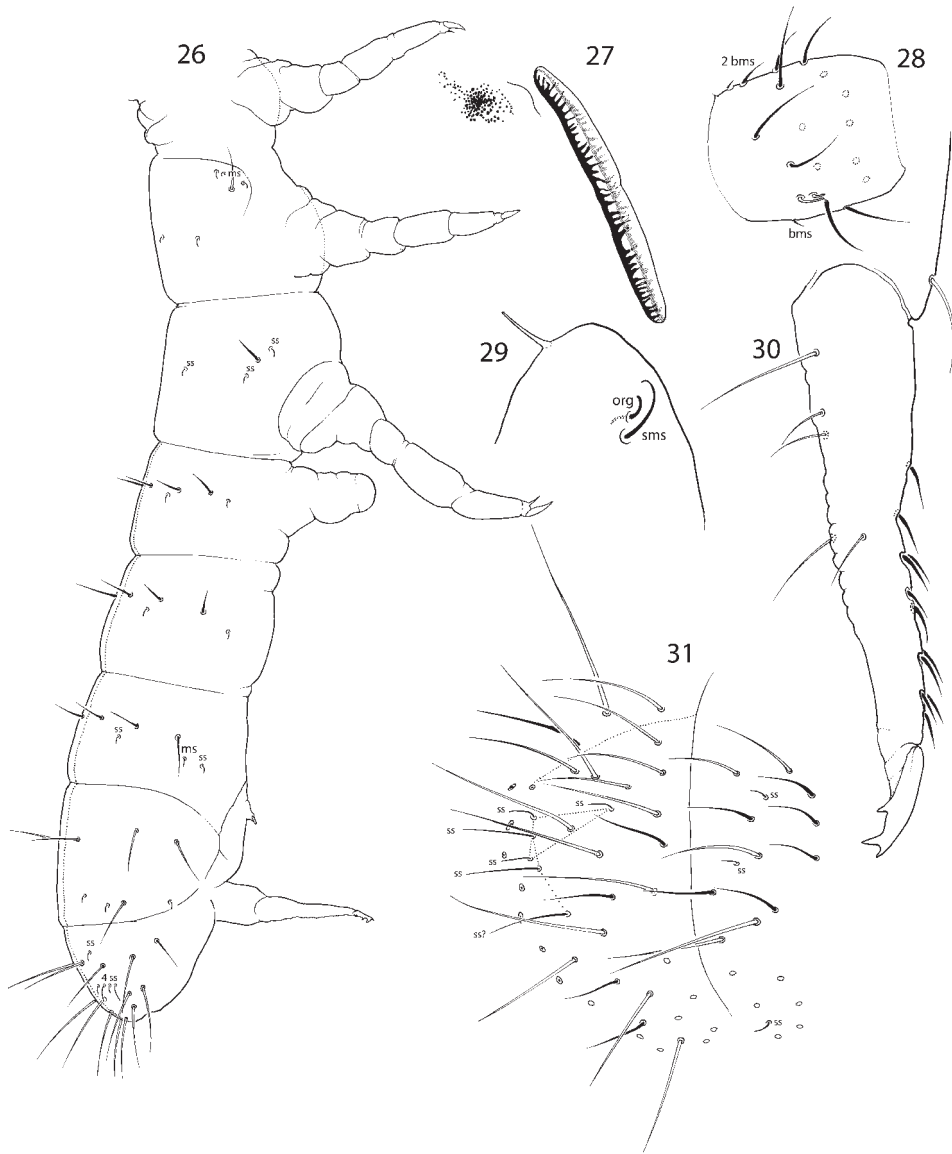
Common chaetae often slightly serrated at the posterior part of Abd. V. S-formula as 4,3/2,2,2,3,5(6?) (s), 1,0/0,0,1 (ms) (Fig. 26). Ms on Abd. III as large as s-chaetae. Tergal s-chaetae much shorter than common chaetae and well distinguishable. Medial s-chaetae on Th. II–Abd. III situated in mid-tergal position. On Abd. V, three dorsal



Figures 23–25. Appearance and macrochaetotaxy of *C. postantennalis* sp. nov. (23), *C. inflatus* sp. nov. (24), and *C. longisensillus* sp. nov. (25).

s-chaetae (triplet: al, accp1, accp2) short. Two lateral s-chaetae long, migrated to dorsal side and integrated to dorsal triplet (Fig. 31). One additional thin chaeta of unclear nature present on lateral side of Abd. V (notated as “ss?” in Fig. 31 and not shown in Fig. 26). Macrochaetae smooth and long, 1,1/3,3,3 in number, medial ones on Abd. VI more or less the same length as the dens and 3.8 times longer than mucro. Foil chaetae at the tip of abdomen absent. Axial chaetotaxy for abdomen 4–5,4–5,4–5, ca. 8 (difficult to observe in most individuals). All thoracic segments without ventral chaetae.

Unguis of normal shape, without teeth. Empodial appendage 0.5–0.6 as long as unguis. All tibiotarsi with additional chaetae: 2–3 chaetae on Legs I and II, and about five on Leg III. Tibiotarsal tenent hairs not clavate. Ventral tube with 3+3 laterodistal and 5–6 posterior chaetae, anteriorly without chaetae. Tenaculum with 4+4 teeth and



Figures 26–31. *C. postantennalis* sp. nov. **26** macrochaetotaxy and s-ms-pattern **27** PAO and eye area **28** Ant. I **29** apical part of Ant. IV **30** dens, lateral view **31** Abd. IV and V. Abbreviations: org organit, sms subapical ms-chaeta, ss s-chaeta, bms basal ms-chaeta, ms ms-chaeta.

two chaetae. Anterior furcal subcoxa with ten, posterior one with five chaetae (from one individual we could observe). Anterior side of manubrium with 1+1 chaetae. Dens of medium length, with crenulation, with 12 anterior and five posterior chaetae (Fig. 30). Mucro tridentate. Ratio manubrium : dens : mucro = 2.7–3.0 : 3.6–3.9 : 1.

Etymology. The species is named after its remarkable PAO.

Distribution and ecology. Known only from type locality and Swellendam, from indigenous vegetation (fynbos and Afromontane forest).

Discussion. The new species has a unique PAO with large inner denticles visible at any magnification. All s-chaetae of Abd. V (al, accp1, accp2, accp3, accp4) are in one dorsal group, while in other species of *Cryptopygus* the triplet 'al+accp1+accp2' and duplet 'accp3+accp4' are placed in dorsal and lateral positions, respectively. The ms-formula 1,0/0,0,1 (vs. 1,1/1,1,1 or 1,0,1,0,0) is also unique.

***Cryptopygus inflatus* sp. nov.**

<http://zoobank.org/AE4D008B-A24F-446B-9C24-F90F1B3FA613>

Figures 24, 32–36

Type material. Holotype and 21 paratypes: SOUTH AFRICA • Western Cape, Cederberg Wilderness Area, Wolfberg Cracks; 32.471507S, 19.278397E; 19 Feb. 2011; C. Janion-Scheepers leg.; litter, Tullgren extraction; RSA11_CED002. Holotype and three paratypes deposited on two slides at SAMC, six paratypes on three slides deposited at SMNG, four paratypes on two slides deposited at MSPU, and eight paratypes on four slides at NMHN. Ten paratypes in ethanol deposited at SAMC.

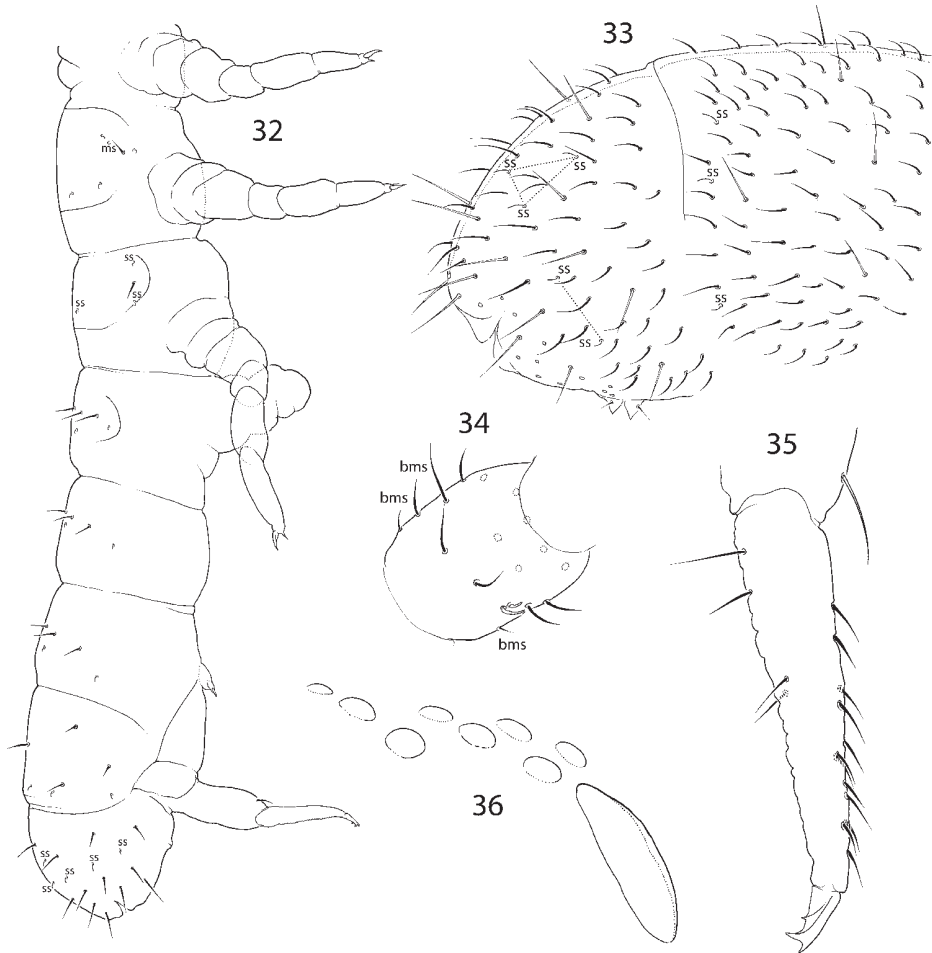
Other material. SOUTH AFRICA • Western Cape, Cederberg Wilderness Area; 32.310167S, 19.175183E; Oct. 2008, C. Janion-Scheepers leg.; pitfall trap, RSA08_CED001.

Form with two chaetae on the basal part of the posterior side of the dens (see text below) from: South Africa, Northern Cape, Ezeljacht farm, 20 km from Sutherland, 32.4105S, 20.57747E, 1550m asl, 16 Jul. 2007, litter of shrub, C. Janion-Scheepers leg, RSA09_SUT001.

Form with three chaetae on the basal part of the posterior side of the dens (see text below) from: South Africa, Western Cape, Mont Rochelle Nature Reserve, Franschoek, 33.902967S, 19.158950E, 06 Oct. 2008, Litter trap with *Galenia* litter (MR510), C. Janion-Scheepers leg.

Diagnosis. 8+8 ocelli. Macrochaetae short. Anterior side of manubrium with 1+1 chaetae. Dens of medium length. Mucro tridentate.

Description. Body size 0.6–0.9 mm. Body grey, Abd. V well separated from Abd. IV and fused with Abd. VI, slightly swollen (Figs 24, 33). Cuticle unmodified. Ocelli 8+8 (Fig. 36). PAO more than three times as long as ocellus, about as long as width of Ant. I and 1.8–2 times as long as inner unguis length. Maxillary head with unmodified lamellae. Maxillary outer lobe with four sublobal hairs, maxillary palp bifurcate. Labral formula as 3/5,5,4, edge of labrum not reduced. Labium with five usual papillae (A–E), guard chaetae e7 present, three proximal and four basomedian chaetae. Ventral side of head with 4+4 chaetae. Ant. I with two ventral s-chaetae (s) and three small basal micro s-chaetae (bms), two dorsal and one ventral (one dorsal large) (Fig. 34), Ant. II with three bms and one latero-distal s, Ant. III with one bms and five distal s (including one lateral), without additional s-chaetae. S-chaetae on Ant. IV weakly



Figures 32–36. *C. inflatus* sp. nov. **32** macrochaetotaxy and s-ms-pattern **33** Abd. IV and fused Abd. V and VI **34** Ant. I **35** dens, lateral view **36** ocelli and PAO. Abbreviations: ss s-schaeta, bms basal ms-schaeta, ms ms-schaeta.

differentiated. Organite and subapical micro s-schaeta of normal shape, small and set together as normal.

Common chaetae smooth. S-formula (Fig. 32) as 4,3/2,2,2,3,5 (s), 1,0/0,0,0 (ms). Tergal s-schaetae much shorter than common chaetae and well distinguishable. Medial s-schaetae on Th. II–Abd. III situated in mid-tergal position. On Abd. V all s-schaetae short subequal. Macrochaetae short, 1,1/3,3,3 in number, medial ones on Abd. VI 0.3–0.4 times longer than dens and 0.3–0.7 times longer than mucro. Foil chaetae at the tip of abdomen absent. Axial chaetotaxy as 8,7/4,4,4,7 (based on one individual). All thoracic segments without ventral chaetae.

Unguis of normal shape, without teeth. Empodial appendage about 0.6 as long as unguis. Tibiotarsi without additional chaetae on Leg I and II (21 chaetae), and

with several chaetae on Leg III (>26). Tibiotarsal tenent hairs pointed. Ventral tube with 4+4 laterodistal and 5–6 posterior chaetae, anteriorly without chaetae. Tenaculum with 4+4 teeth and two chaetae. Anterior furcal subcoxa with 12–13, posterior one with 5–6 chaetae. Anterior side of manubrium with 1+1 chaeta, posterior side with 4+4 laterobasal and about 22 chaetae on main part, with a pair of lateral chaetae (Fig. 35). Dens normal, with crenulation, with 11–12 anterior and six posterior chaetae (three basal and two at middle and one subapical and very small). Mucro tridentate. Ratio manubrium : dens : mucro = 4.4–5.7 : 4.3–4.7 : 1.

Etymology. The name is derived from the swollen posterior part of abdomen.

Distribution and ecology. Known from mostly dry, mountainous areas, from the Northern Cape (Sutherland), the Cederberg Wilderness area, and Franschhoek (not as dry as previous two sites).

Discussion. Considering all species in the *Cryptopygus* complex, the combination of 8+8 ocelli and tridentate mucro is unique. The only species resembling our species is *Proisotoma* (*Isotomina*) *pseudominuta* Schött, 1927, described from Cameroon. However, this species has clavate tenent hairs and a shorter dens, while *Cryptopygus inflatus* sp. nov. does not have clavate tenent hairs.

Some variation exists in the material examined and two forms of unclear status can be recognized; both have an ms on Abd. I (1,0/1,0,0) while *C. inflatus* sp. nov. does not. These two forms differ as follows:

- a. Specimens from Sutherland (SUT002) have an ms and two chaetae on the basal part of the posterior side of the dens.
- b. Specimens from Mont Rochelle (MR510) have an ms on Abd. I and three chaetae on the basal part of the posterior side of the dens like in *C. inflatus* sp. nov.

***Cryptopygus longisensillus* sp. nov.**

<http://zoobank.org/EDBC3A2C-5B34-439A-BF2F-9C8868BF8991>

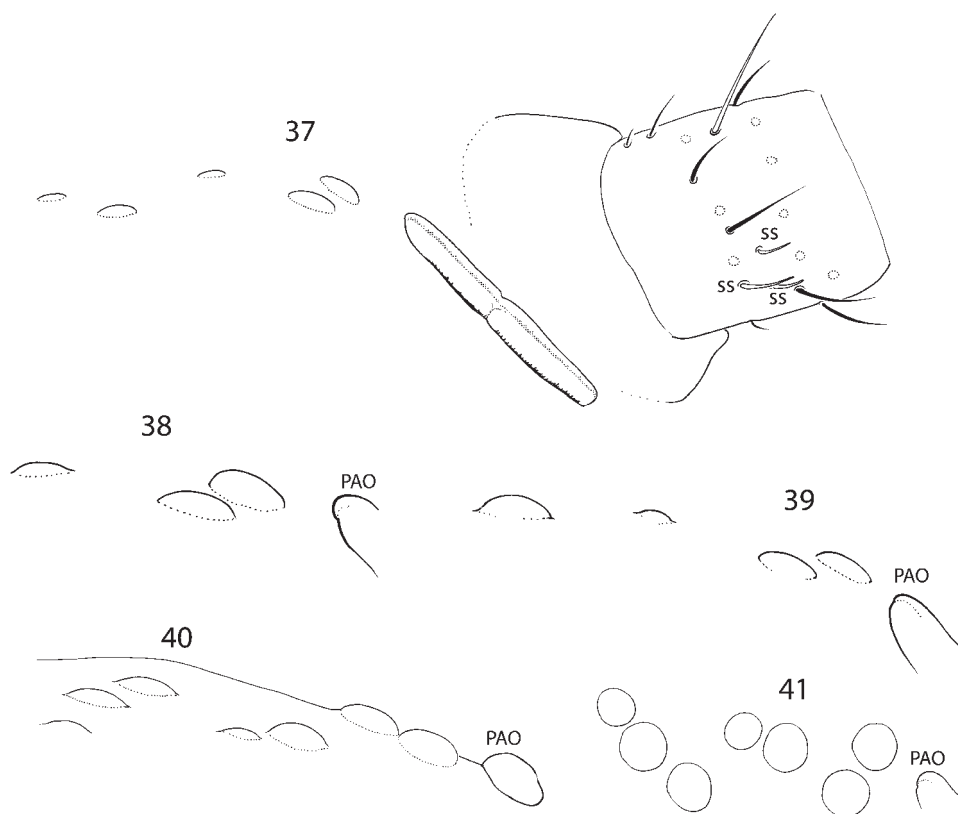
Figures 25, 37–46

Type material. Holotype and six paratypes: SOUTH AFRICA • Northern Cape, Ezeljacht farm, 14 km from Sutherland; 32.4105S, 20.57747E; 1550 m asl; 16 July 2009; C. Janion-Scheepers leg.; shrub litter; RSA09_SUT002. Holotype and five paratypes on four slides deposited at SAMC, one paratype on one slide deposited at MSPU.

Other material. Several specimens from SOUTH AFRICA • Western Cape, Jonkershoek Nature Reserve; 33.989350S, 18.957433E; 30 July 2009 and 12 Aug. 2010; C. Janion-Scheepers leg.; litter trap (32).

Diagnosis. Anterior side of manubrium with 1+1 chaetae. Dens of medium length. Mucro tridentate. All s-chaetae of Abd. V elongated.

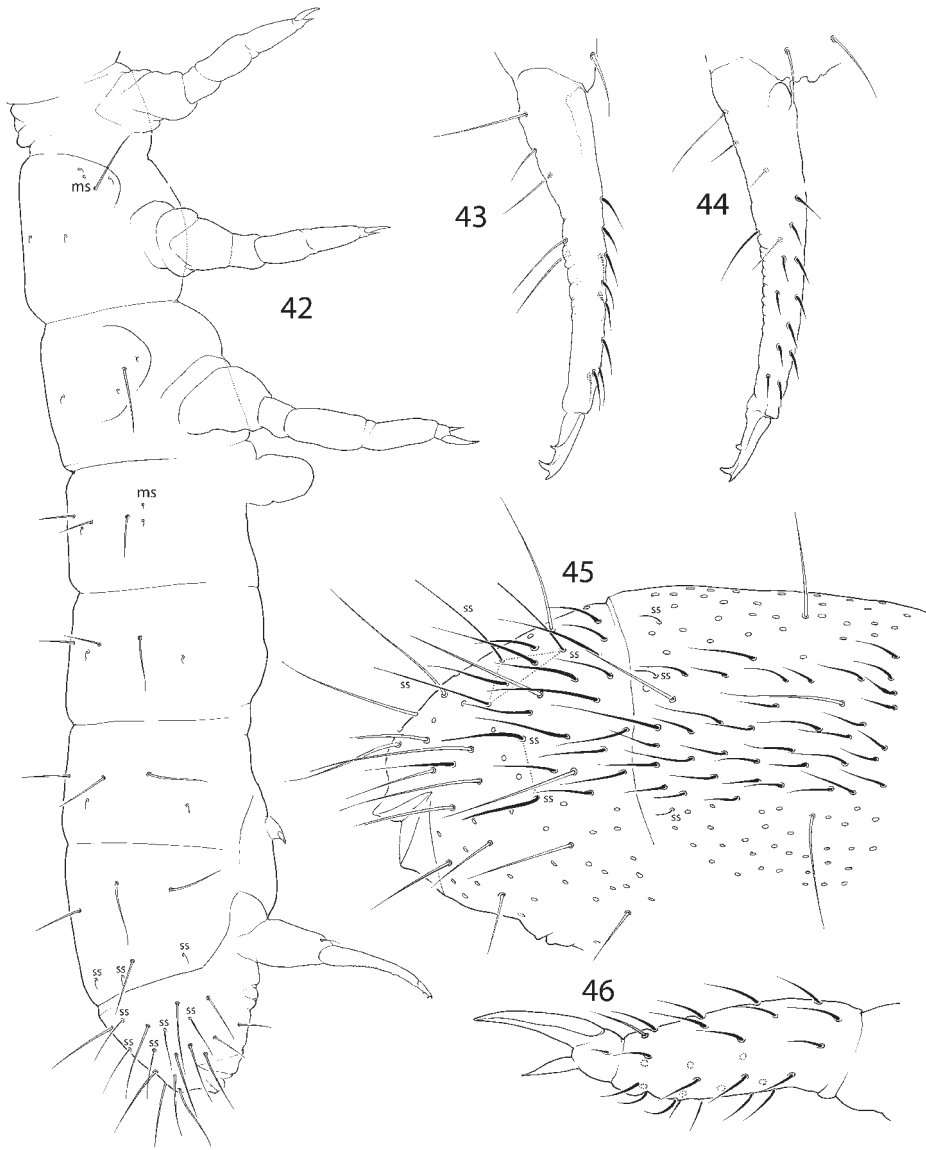
Description. Body size 0.9–1.3 mm (Fig. 25). Body pale, with scattered black granules of pigmentation, more concentrated on head, eye spots and posterior of trunk. Body tubular. Abd. V well separated from Abd. IV and fused with Abd. VI



Figures 37–41. Variation of number of ocelli in *C. longisensillus* sp. nov. **37** 5+5 ocelli **38** 3+3 ocelli **39** 4+4 ocelli **40, 41** 7+7 ocelli.

(Fig. 45). Cuticle with thin hexagonal primary granulation (“smooth”). Ocelli range from three to seven on each side (Figs 37–41) (see Discussion). PAO rather narrow, sharply constricted, with small inner denticles, longer than width of Ant. I (1.1–1.3) and inner unguis length (1.3–1.4). Maxillary head without modified lamellae. Maxillary outer lobe with four sublobal hairs, maxillary palp bifurcate. Labral formula as 4/5,5,4, edge of labrum not reduced. Labium with five usual papillae (*A–E*), guard chaetae *e7* present, three proximal and four basomedian chaetae. Ventral side of head with 4–5+4–5 chaetae. Ant. I with three ventral s-chaetae (*s*) and two small basal micro s-chaetae (*bms*), dorsal and ventral, the former set together with long chaeta-like micro s-chaeta, Ant. II with three *bms* and one latero-distal *s*, Ant. III with one *bms* and six distal *s* (including two lateral), without additional s-chaetae. S-chaetae on Ant. IV weakly differentiated. Organite pin-like.

Common chaetae rather long, smooth. S-formula as 4,3/2,2,2,3,5 (*s*), 1,0/1,0,0 (*ms*) (Fig. 42). Tergal s-chaetae short (apart from Abd. V) and well different from common chaetae. Medial s-chaetae on Th. II–Abd. III situated in mid-tergal position, on Abd. I–III between Mac1 and Mac2. Abd. V with five s-chaetae arranged with three



Figures 42–46. *C. longisensillus* sp. nov. **42** macrochaetotaxy and s-ms-pattern **43, 44** dens, lateral (**43**) and anterior (**44**) views **45** Abd. IV and fused Abd. V and VI **46** tibiotarsus and claw of Leg III. Abbreviations: ss s-chaeta, ms ms-chaeta.

dorsal ones (al, accp1, accp2), long and slender, and two lateral ones (accp3, accp4), long and slightly thickened so hardly distinguishable from common chaetae (Fig. 45). Macrochaetae smooth and long, 1,1/3,3,3 in number (Th. II–Abd. III), medial ones on Abd. VI 0.7–0.9 as long as dens and 2.8–3.7 times longer than mucro. Foil chaetae at the tip of abdomen absent. Axial chaetotaxy abundant 10–11,8–9/4–6,4–6,4–6. Thorax (incl. Th. III) without ventral chaetae.

Unguis of normal shape, without lateral and inner teeth. Empodial appendage about half as long as unguis. Tibiotarsi with additional chaetae, at whole with 23–24 ones on Leg I and II, and more than 25 on Leg III (Fig. 46). Tibiotarsal tenent hairs pointed. Adult males with stick-like thin spurs on Tibiotarsi III. Ventral tube with 3+3 laterodistal and six posterior chaetae, anteriorly without chaetae. Tenaculum with 4+4 teeth and a chaeta. Anterior furcal subcoxa with 13–15, posterior one with 4–6 chaetae. Anterior side of manubrium with 1+1 chaetae. Posterior side of manubrium with 12–14+12–14, including 5+5 on basolateral flaps. Dens with 10–12 anterior chaetae (Figs 43, 44). Posterior side of dens crenulated and with five chaetae (three basal and two at the middle). Mucro tridentate. Ratio manubrium : dens : mucro = 2.8–3.4 : 3.8–5.1 : 1.

Etymology. The name is derived from the very long s-chaetae on Abd. V.

Distribution and ecology. *Cryptopygus longisensillus* sp. nov. is currently known from Sutherland (Northern Cape) and Jonkershoek, Stellenbosch (Western Cape).

Discussion. The species belongs to a group of forms having ocelli, tridentate mucro and rather long dens (*C. insignis* Massoud & Rapoport, 1968, *C. patagonicus* Izarra, 1972, *C. quadrioculatus* (Wise, 1970), *C. tricuspis* Enderlein, 1909) (Table 1). Two species, *C. insignis* and *C. tricuspis* have 1+1 chaetae (vs. 0+0 in *C. longisensillus* sp. nov.) on ventral side of Th. III and common s-chaetae (vs. elongated in new species) on Abd. V. Due to insufficient descriptions the morphology of *C. patagonicus* and *C. quadrioculatus* is less understood. Both species differ from *C. longisensillus* sp. nov. in fewer chaetae (eight and nine vs. 10–12) on anterior and more chaetae (six vs. five) on posterior sides of dens. *C. quadrioculatus* shows specific position of ocelli with one anterior and one posterior at a distance.

Cryptopygus longisensillus sp. nov. shows a unique pattern of s-chaetae on Abd. V not found before in the genus: all five s-chaetae elongated while s-chaetae of dorsal triplet thin, two lateral ones slightly thickened, representing a s-pattern of type “4” according to the classification of Potapov et al. (2017). The variability in the number of ocelli is unusual: among six individuals of one population we recorded variants with three, four, five, and seven ocelli. Two ocelli near the PAO are always well visible, while the other ones are only recognizable by weak swellings of the cuticle. The nature of this variability is not fully clear and calls for further study. The only similar case is known in *C. insignis* (fig. 4A in Massoud and Rapoport 1968). In the specimens from South Africa, Western Cape, Jonkershoek Nature Reserve, six posterior chaetae on dens were found. This variation also calls for further study.

Table 1. Some key characteristics of *Cryptopygus* species having ocelli, tridentate mucro, and long dens.

Species	Ocelli	Chaetae on anterior side of dens	Chaetae on posterior side of dens	s-chaetae on Abd. V
<i>C. insignis</i> , Argentina	3+3	13	six	short
<i>C. patagonicus</i> , Argentina	5+5	eight	six	?
<i>C. quadrioculatus</i> , sub-Antarctic (South Georgia)	2+2	nine	six	?
<i>C. tricuspis</i> , sub-Antarctic (Kerguelen)	2+2	13	six	short
<i>C. longisensillus</i> sp. nov., South Africa	(3+3 to 7+7)	10–12	five	long

Morphological remarks on *C. insignis* Massoud & Rapoport, 1968

We had an opportunity to study two type specimens of this species kept in MNHN (Paris) labelled as “Lago Menendez 16.III.1959”. It was possible to observe the following characters: s-formula as 4,3/2,2,2,3,5 (s), 1,0/0,0,0 (ms), five s-chaetae on abd.V short, as common for the genus (unlike in *Cryptopygus longisensillus* sp. nov.); four prelabral chaetae, maxillary palp bifurcate; e7 present on labial palp; ventral tube with 4+4 laterodistal chaetae; Ant. I with three s-chaetae (s) and three basal micro s-chaetae, with long and short in dorsal group (like in *Cryptopygus longisensillus* sp. nov.).

Cryptopygus antarcticus complex

Material. SOUTH AFRICA • Western Cape, Kogelberg Nature Reserve; 34.331833S, 18.952467E; 04 Oct. 2011, C. Janion-Scheepers leg.; litter (*Mimetes* sp.), Tullgren extraction; RSAII_KOG010 • Western Cape, Orangetkloof, Table Mountain National Park; 33.983883S, 18.403050E; 24 Oct. 2011, C. Janion-Scheepers leg.; indigenous vegetation litter, Tullgren extraction; RSAII_OK026 • Western Cape, Orangetkloof, Table Mountain National Park; 33.975917S, 18.407667E; 24 Oct.2011; C. Janion-Scheepers leg.; indigenous vegetation litter, Tullgren extraction; RSAII_OK029 • South Africa, Western Cape, Table Mountain National Park, Cape Point, Platboom; 34.336017S, 18.447633E; 06 Aug. 2009; Tullgren extraction; RSA09_PEN008) • South Africa, Western Cape, Outeniqua, 33.887583S, 22.424067E, Afromontane Forest leaf litter Tullgren extraction (OUTF38), 14.ii.2013, A. Liu leg.; SAF-627 • Prince Albert: Swartberg South: Swartberg south slope; 12 March 2019; L. Deharveng & A. Bedos leg.; moss, moss on rock, Berlese; SAF-614, • Prince Albert, Swartberg North: Swartberg crest, 12 March 2019; L. Deharveng, C. Janion-Scheepers & A. Bedos leg.; moss, moss on rock, Berlese; SAF-401 • Constantia: Orange Kloof; 09 Jan. 2012; L. Deharveng & A. Bedos leg.; restio, litter, litter and humus, Berlese.

Diagnosis of the “antarcticus complex”. 4+4 to 6+6 ocelli. Manubrium with 1+1 chaetae on anterior side. Dens stout, not crenulated, with 4–6 anterior and 3–4 posterior chaetae. Mucro bidentate. Clavate tibiotarsal hairs present.

Distribution and ecology. Currently known from the indigenous vegetation from the larger Table Mountain National park area, and the Outeniqua (George) area.

Discussion. So far several subspecies and species resemble typical antarctic species *C. antarcticus* Willem, 1902 (*C. antarcticus maximus* Deharveng, 1981, *C. antarcticus travei* Deharveng, 1981, *C. antarcticus reagens* (Enderlein, 1909), *C. quinqueoculatus* Izarra, 1970, *C. hirsutus* Denis, 1931, *C. badasa* Greenslade, 1995, *C. araucanus* Massoud & Rapoport, 1968) and combine a group which is named “antarcticus complex” by us. In fact, they all almost fit to the strict diagnosis of the genus *Cryptopygus* proposed by Rusek (2002). This complex, often recorded just under the name ‘*C. antarcticus*’, is widely distributed in the sub-Antarctic (Deharveng 1981, Stevens et al. 2006) and less in temperate zones of the Southern Hemisphere (South America: Mari Mutt

and Bellinger 1990; Australia: Greenslade, 1994; New Zealand: Babenko and Minor 2015), with *C. hirsutus* extending into Costa Rica. In South Africa, we have also found the forms with clavate tenent hairs on the tibiotarsi and the characteristic ‘*antarcticus*-like’ furca and so then belonging to ‘*C. antarcticus*’ complex. They differ from typical *C. antarcticus* at least by having fewer ocelli (three anterior and one or two posterior) and lighter pigmentation. So far, the following forms were found:

- micro s-chaetae 10\000, 4+4 ocelli Kogelberg (RSAII_KOG010), Table Mountain (RSAII_OKO26), and Cape Point (RSA09_PEN008).
- micro s-chaetae 10\100, 4+4 ocelli RSAII_OKO29 (Table Mountain)
- micro s-chaetae 10\100, 5+5 ocelli. This form fits descriptions of *C. quinqueoculatus* (Patagonia) well. OUT_F_38 (Outeniqua)
- micro s-chaetae 11\111, 4+4 ocelli SAF-401 (Table Mountain), SAF-614; SAF-627.

Reliable taxonomical decision on their status cannot be made at present and preliminary study of all known and unknown forms is underway.

***Isotominella* Delamare Deboutteville, 1948**

Type species. *Isotominella geophila* Delamare Deboutteville, 1948

Diagnosis. The genus belongs to the *Cryptopygus* complex. Ocelli absent. Medial s-chaetae in posterior position from Th. II to Abd. IV. Number of s-chaetae 3,3/2,2,2,2,3. Foil chaetae at the end of abdomen absent.

Discussion. The genus *Isotominella* was described from Ivory Coast and was subsequently given a detailed diagnosis by Jordana et al. (2009) based on material of *I. geophila* from Algeria. The taxon was considered a member of the *Cryptopygus* complex and was said to differ from related genera mainly by the crenulation, which is developed only in the proximal half of the dens. According to Jordana et al. (2009), in other genera of the complex this crenulation is either absent (i.e., dens is “smooth”), or extends further along the posterior side of dens, as in *Hemisotoma* Bagnall, 1949.

In our view, the crenulation is a flexible character within the genera of the *Cryptopygus* complex and depends on the length of furca, which can vary highly within a large genus, e.g., in *Cryptopygus* s. str., and particularly, among its representatives in South Africa (Figs 14, 30, 35, 43). Two species of the genus *Isotominella*, *I. geophila* and *I. laterochaeta* sp. nov. share a remarkable s-chaetotaxy, particularly, the posterior position of medial s-chaetae on body tergites, a reduced s-formula (3,3/2,2,2,2,3), and differentiation of s-chaetae on Abd. V with two long dorsal and one short lateral s-chaetae. The mouth parts of the two species are uncommon: the terminal ‘sensilla’ of papilla A and B are rod-like, the number of basolateral chaetae of labium are increased, the labrum has two prelabral chaetae, the number of sublobal hairs of maxillary outer lobe is reduced (three in the new species and two in *I. geophila*), and the maxillary head is modified. The presence of 7–9 basolateral chaetae on the labium (vs. commonly five as

determined by Fjellberg 1999) in *I. laterochaeta* sp. nov. have not been recorded so far for Isotomidae. This remarkable feature is less pronounced in *I. geophila* in which this number is variable (five or six). Six basolateral chaetae on the labium were found in the genus *Pauropygus* (Potapov et al. 2013) in *P. projectus* Potapov et al., 2013, *P. caussaneli*, *P. pacificus* Potapov et al., 2013, which also shows the posterior position of the s-chaetae on tergites. The two genera share other important characters and are probably closely related (Potapov et al. 2013). Labral chaetae are normally pressed to the labrum in Isotomidae, while they are projected forward in the new species (the character is unclear for *I. geophila*). *Isotominella* also resembles the blind genera *Cylindropygus* Deharveng et al., 2005 (Europe) and *Dagamaea* Yosii, 1965 (East Asia and North America) but reliably differs by the posterior (vs. mid-tergal) position of the s-chaetae on the body tergites. A table to compare all the genera of the complex *Cryptopygus* is given by Jordana et al. (2009).

***Isotominella laterochaeta* sp. nov.**

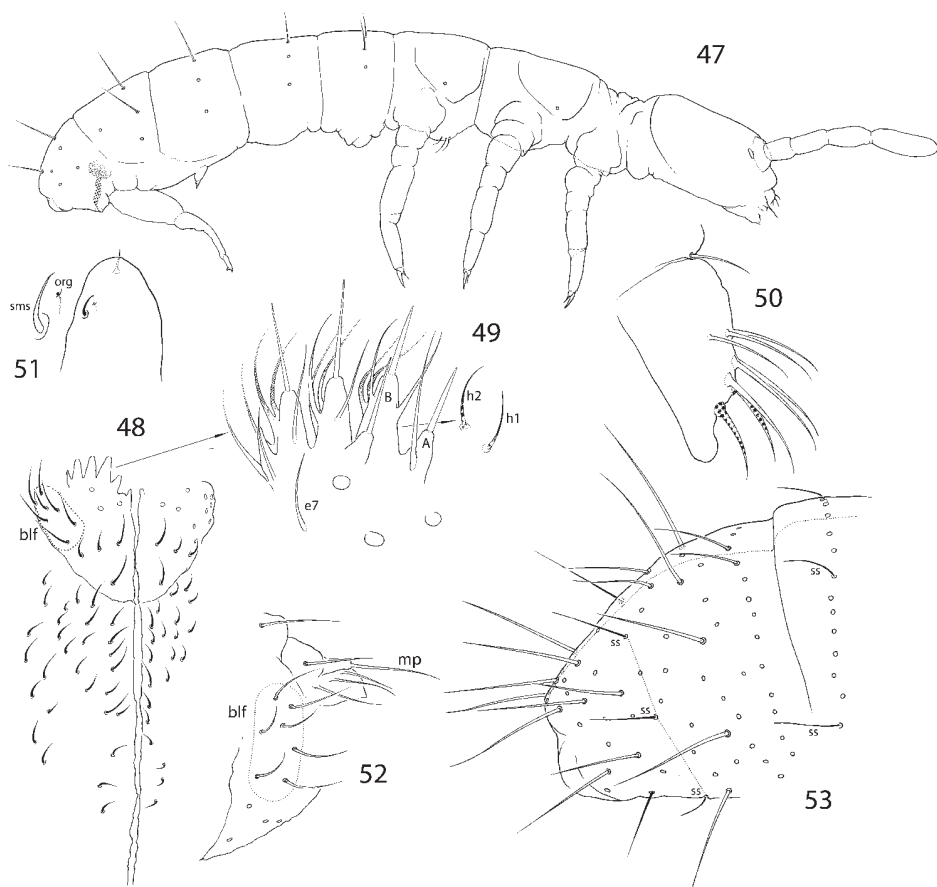
<http://zoobank.org/DD3D202C-F6D0-4912-A1BE-DFD11557771B>

Figures 47–59

Type material. Holotype and eight paratypes: South Africa • Western Cape, Platboom, Cape Point National Park; 34.336017S, 18.447633E; 14 Nov. 2010; L. Deharveng and A. Bedos leg.; soil; SAF 318. Holotype and two paratypes on three slides deposited at SAMC, three paratypes on three slides at NMHN, and three paratypes on three slides at MSPU.

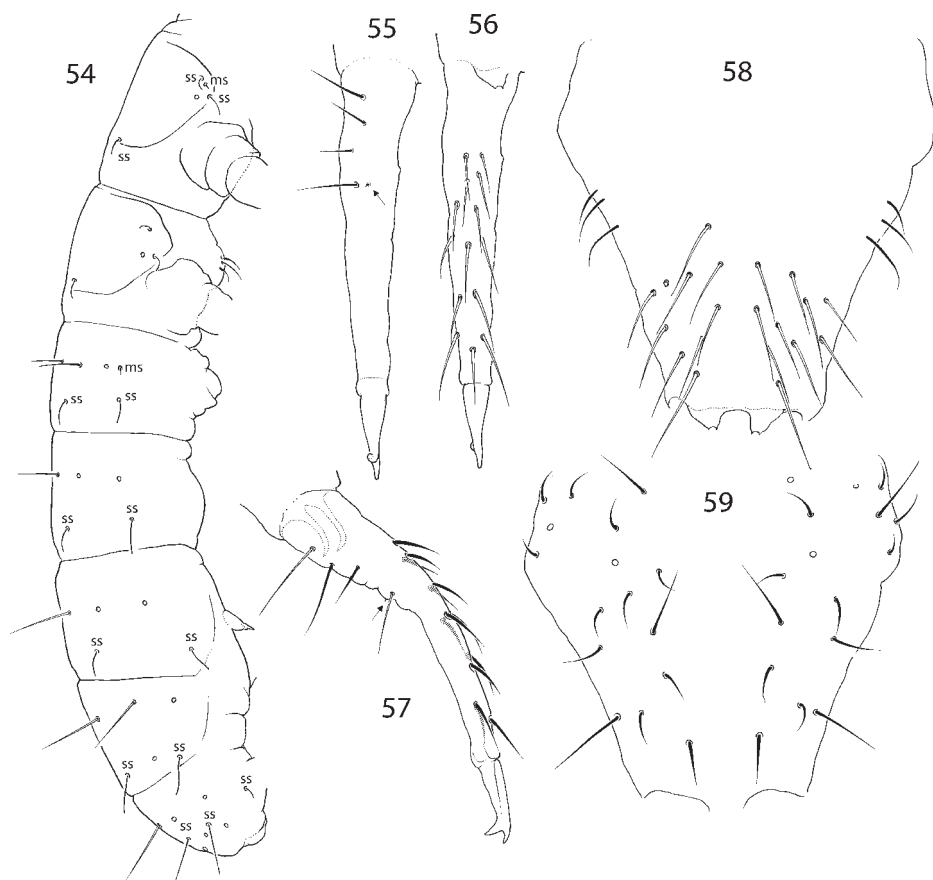
Diagnosis. Blind. Labium with 7–9 basolateral chaetae. Chaetae on ventral side of Th. III present. Labial palp with 16 guard chaetae. Three sublobal hairs on maxillary outer lobe. Anterior side of manubrium with 9–10+9–10 chaetae. Dens with 12–14 anterior and four posterior chaetae. Mucro bidentate.

Description. Body size 0.8–1.0 mm. White, without pigmentation, appearance as *Mucrosomia caeca* (Fig. 47). Mouth cone projected forward. Abd. V well separated from Abd. IV and fused with Abd. VI (Fig. 53). Cuticle “smooth”. Without ocelli. PAO small, not constricted, less than a half of width of Ant. I and 0.5–0.7 as long as inner unguis length. Maxillary head with slender lamellae and thin capitulum. Maxillary outer lobe with three sublobal hairs, maxillary palp simple (Fig. 52). Labral formula as 2/5,5,4, edge of labrum not reduced. Chaetae of labrum conspicuously projected forward (Fig. 50). Labral chaetae of middle and distal rows thicker than chaetae of proximal row. Inner chaetae of distal row shifted to more proximal position and integrated to middle row resulting the impression of 5,7,2 formula. Up to ten clypeal chaetae. Labium with five papillae (A–E), 16 guard chaetae (guard chaetae e7 present), and three proximal chaetae (Fig. 49). Terminal ‘sensilla’ of papillae A and B rod-like. All inner guard chaetae (b3, b4, d3, d4, e2, e3, e5, e6) strongly curved. With two curved and small accessory hypostomal chaetae (h1, h2), main hypostomal chaeta (H) absent (Fig. 49). Basal part of labium with four basomedian and 7–9



Figures 47–53. *Isotominella laterochaeta* sp. nov. **47** appearance and macrochaetotaxy (some macrochaetae lost) **48** ventral side of head **49** labial palp (hypostomal chaetae shown separately) **50** labrum, lateral view (chaetae of distal row marked, three, three, and two chaetae shown for proximal, middle, and distal rows, respectively) **51** apical part of Ant. IV **52** basal parts of labium and maxillary outer lobe, lateral view **53** posterior edge of Abd. IV and fused Abd. V and VI. Abbreviations: A and B papillae of labial palp, blf basolateral field of labium, e7 guard chaeta e7, mp maxillary palp, h1 and h2 hypostomal chaetae, org organite, sms subapical ms-chaeta, ss s-chaeta, ms ms-chaeta.

basolateral chaetae (Figs 48, 52). Ventral side of head with numerous chaetae (up to 13 on one side along ventral line), with numerous chaetae at base of labium (Fig. 48). Normal s-chaetae and basal micro s-chaetae hardly differentiated on antennae, their number difficult to ascertain. Ant. I with many chaetae and at least two ventral s-chaetae (s) mixed with normal chaetae (or thinner s-chaetae). Ant. III with five distal s (including one lateral), without additional s-chaetae. S-chaetae on Ant. IV weakly differentiated. Organite small, rudimental, close to subapical micro s-chaeta, which is long and bent (Fig. 51).



Figures 54–59. *I. laterochaeta* sp. nov. **54** macrochaetotaxy and s-ms-pattern **55–57** dens, posterior (**55**), anterior (**56**), and lateral (**57**) views **58, 59** manubrium, anterior (**58**) and posterior (**59**) views. Abbreviations: ss s-chaeta, ms ms-chaeta.

Common chaetae long. S-formula as 3,3/2,2,2,2,3 (s), 1,0/1,0,0 (ms) (Fig. 54). Tergal s-chaetae longer than common chaetae, less distinguishable on Th. II and III. Medial s-chaetae on Th. II–Abd. III situated in p-row of chaetae. On Abd. V, two dorsal s-chaetae (accp1, accp2) long, one lateral s-chaeta short (Fig. 53). Macrochaetae smooth and long, 1,1/3,3,3,4 in number. Macrochaetae on Abd. V longer than on Abd. VI: 0.8–0.9 and 0.6–0.7 as long as the dens and 3.3–3.5 and 2.3–2.8 times longer than mucro on Abd. V and VI, respectively. One thin macrochaetae (possibly additional s-chaeta) present in latero-ventral position on Abd. V. Foil chaetae at the tip of abdomen absent. Axial chaetotaxy for Th. II–Abd. IV 14–16,7–8/5–6, 5–6, 5–6, ca. 6. Th. III with 2–4+2–4 ventral chaetae.

Unguis of normal shape, without inner tooth and with two lateral teeth. Lateral teeth often asymmetrical in size and position. Empodial appendage thin, without la-

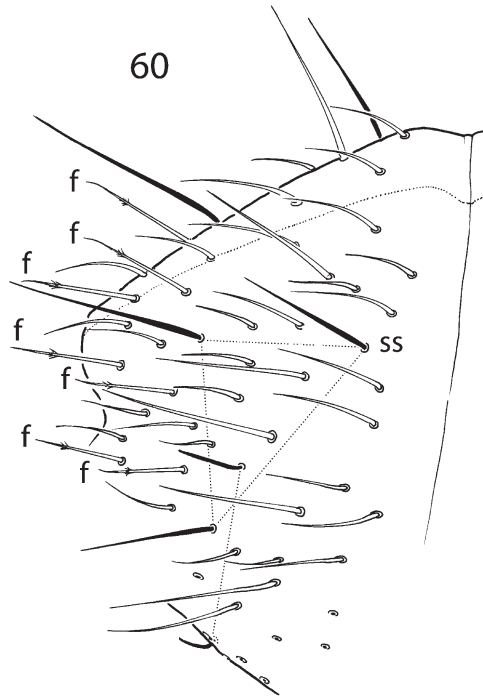


Figure 60. *Hemisotoma thermophila*, Abd. V–VI. Abbreviations: f foil chaeta, ss s-chaeta.

mellae, 0.6–0.7 as long as unguis. All tibiotarsi with many additional chaetae: Tibiotarsal tenent hairs not clavate. Ventral tube with 5–8+5–8 laterodistal and 6–9 posterior chaetae (with two larger in distal position), anteriorly without chaetae. Tenaculum with 4+4 teeth and two chaetae (rarely more). Anterior furcal subcoxa with 19–23, posterior one with 11–15 chaetae. Anterior side of manubrium with 9–10+9–10 chaetae arranged in two symmetrical group (Fig. 58), with distal pair thickened. Posterior side of manubrium with 11–14+11–14 chaetae on main part, 4+4 laterodistal and 2(3)+2(3) lateral chaetae (Fig. 59). Dens of medium length, with weak crenulation at the middle, with 12–14 anterior and four posterior chaetae (Figs 55–57). One minute chaeta-like process often seen close to distal chaeta. Mucro bidentate. Ratio manubrium : dens : mucro = 3.0–3.9 : 3.2–4.3 : 1.

Etymology. The name is derived from its many lateral chaetae on the basal part of labium.

Distribution and ecology. Known only from type locality (Cape Point National Park).

Discussion. *Isotominella laterochaeta* sp. nov. and *I. geophila* (type species of the genus) differ by the number of anterior chaetae on the manubrium (9–10+9–10 vs. 1–4+1–4), chaetae on ventral side of Th. III (present vs. absent), number of guard chaetae on labial palp (16 vs. 13–14), and number of sublobal hairs (three vs. two)

on maxillary outer lobe. Minute chaeta placed at the middle of posterior side of dens, numerous postlabial chaetae, and absence of hypostomal chaeta H are unique to *I. laterochaeta* sp. nov. The two last characters are unknown for *I. geophila*.

General discussion and conclusions

The six new springtails from South Africa described here, five *Cryptopygus* and one *Isotominella*, bring the total number of Isotomidae to 25 species for the country. Our study is a first contribution to the knowledge of the rich fauna of *Cryptopygus* of this high endemism country. The five new species described are strongly dissimilar with each other and belong to different groups within the genus, suggesting different colonization events. In addition, local geographic speciation is suspected within several of these species, as reflected in the forms of unclear status presented in species discussions. They call for more detailed variability analyses on more extensive material in order to establish the taxonomical value of the morphological differences that were detected, to reconstruct the distribution patterns of the recognized forms, and to better understand the origin of this local geographical diversification.

Acknowledgements

We are grateful to Cape Nature and SanParks for collection permits. This work was partly funded by the SA (NRF) / Russia (RFBR) Joint Science and Technology Research Collaboration project no. 19-516-60002 (FRBR) and no. 118904 (NRF) awarded to M. Potapov and C. Janion-Scheepers respectively, SA-France (Protea I and II) awarded to L. Deharveng. We are also indebted to Dr. Enrique Baquero and an anonymous reviewer for their critical comments.

References

- Axelson WM (1900) Vorläufige Mitteilung über einige neue Collembolen-Formen aus Finnland. Meddelanden af Societas pro Fauna et Flora Fennica 26: 105–123. <https://doi.org/10.5962/bhl.part.26726>
- Axelson WM (1903) Weitere Diagnosen über neue Collembolen-Formen aus Finnland. Acta Societatis pro Fauna et Flora Fennica 25: 3–13.
- Babenko A, Minor M (2015) *Austrodontella monticola* sp. nov., a new species of Collembola from montane New Zealand. Zootaxa 3974: 122–128. <https://doi.org/10.11646/zootaxa.3974.1.9>
- Bagnall RS (1949) Contribution towards a knowledge of the Isotomidae (Collembola) VII–XV. Annals and Magazine of Natural History 12: 81–96. <https://doi.org/10.1080/00222934908653961>

- Barra JA (1997) Nouveaux Collemboles Entomobryomorphes des sables littoraux (partie terrestre) de la Province du Natal (Rép. Sud Africaine) (Insecta: Collembola). *Journal of African Zoology* 111: 465–480.
- Börner C (1906) Das System der Collembolen nebst Beschreibung neuer Collembolen des Hamburger Naturhistorischen Museums. *Mitteilungen aus den Naturhistorischen Museum in Hamburg* 23: 147–188.
- Deharveng L (1981) Collemboles des Iles Subantarctiques de l’Océan Indien. *Biologie des sols. Comité National Française des Recherches Antarctiques* 48: 33–109.
- Deharveng L, Potapov M, Bedos A (2005) *Cylindropygus ferox* gen. n., sp. n.: A new member of the *Cryptopygus* complex (Collembola, Isotomidae) from central France. *Journal of Natural History* 39: 2179–2185. <https://doi.org/10.1080/00222930500061213>
- Delamare DC (1948) Recherches sur les Collemboles Termitophiles et Myrmécophiles (Écologie, Éthologie, Systématique). *Archives de Zoologie Expérimentale et Générale* 85: 261–425.
- Denis JR (1931) Contributo alla conoscenza del Microgenton di Costa Rica II. *Bollettino del Laboratorio di zoologia generale e agraria della R. Scuola superiore d’agricoltura in Portici* 25: 69–170.
- Enderlein G (1909) Die Insekten des antarctisches Gebietes. *Deutsche Südpolar Expedition, 1901–1903* 10: 361–458.
- Fjellberg A (1999) The labial palp in Collembola. *Zoologischer Anzeiger* 237: 309–330.
- Greenslade P (1994) *Zoological Catalogue of Australia: Protura, Collembola, Diplura*. (Ed. W.W.K. Houston). Melbourne, CSIRO Australia 22: 19–138.
- Greenslade P (1995) Collembola from the Scotia Arc and Antarctic Peninsula including descriptions of two new species and notes on biogeography. *Polskie Pismo Entomologiczne* 64: 305–319.
- Greenslade P (2015) Synonymy of two monobasic Anurophorinae genera (Collembola: Isotomidae) from the Antarctic Continent. *New Zealand Journal of Zoology* 38: 134–141. <https://doi.org/10.1080/00779962.2015.1033810>
- Grinbergs A (1962) Über die Collembolenfauna der Sowjetunion. II. Neue Collembolen aus der Tuvischen ASSR. *Latvijas Entomologs* 5: 59–67.
- Izarra DC (1970) Tres nuevas especies de Colembolos de Siera de la Ventana (Provincia de Buenos Aires, Argentina). *Physis* 29: 393–397.
- Izarra DC (1972) Fauna Collembologica de Isla Victoria (Provincia de Neuquen, Argentina). III. Familias Isotomidae y Entomobryidae. *Physis* 31: 373–382. *ca. South African Journal of Science* 107: Art. #582. <https://doi.org/10.4102/sajs.v107i11/12.582>
- Janion C, Bedos A, Deharveng L (2011b) The genus *Ectonura* Cassagnau, 1980 in South Africa (Collembola: Neanuridae: Neanurinae), with a key to South African Neanurinae. *ZooKeys* 136: 31–45. <https://doi.org/10.3897/zookeys.136.1744>
- Janion C, Deharveng L, Weiner WM (2013) Synonymy of *Spicatella* Thibaud, 2002 with *Delamarephorura* Weiner and Najt, 1999, and description of two new species (Collembola: Tullbergiidae). *Raffles Bulletin of Zoology* 61: 657–663. [http://lkc.nhm.nus.edu.sg/nus/pdf/PUBLICATION/Raffles%20Bulletin%20of%20Zoology/Past%20Volumes/RBZ%2061\(2\)/61rbz657-663.pdf](http://lkc.nhm.nus.edu.sg/nus/pdf/PUBLICATION/Raffles%20Bulletin%20of%20Zoology/Past%20Volumes/RBZ%2061(2)/61rbz657-663.pdf).

- Janion C, D'Haese C, Deharveng L (2012) A new species and first record of the genus *Triacanthella* Schaffer, 1897 (Collembola, Hypogastruridae) for Africa. *ZooKeys* 163: 57–68. <https://doi.org/10.3897/zookeys.163.2298>
- Janion C, Bedos A, Bengtsson J, Deharveng L, Jansen van Vuuren B, Leinaas HP, Liu A, Malmström A, Porco D, Chown SL (2011a) Springtail diversity in South Africa. *South African Journal of Science* 107: Art. #582. <https://doi.org/10.4102/sajs.v107i11/12.582>
- Janion-Scheepers C, Deharveng L, Bedos A, Chown SL (2015) Updated list of Collembola species currently recorded from South Africa. *ZooKeys* 503: 55–88. <https://doi.org/10.3897/zookeys.503.8966>
- Jordana R, Hamra-Kroua S, Baquero E (2009) Redescription of *Isotominella geophila* Delamare Deboutteville, 1948 from Algeria (Collembola, Entomobryomorpha, Isotomidae), A second world record for an Ivory Coast species. *Zootaxa* 2169: 63–68. <https://doi.org/10.11646/zootaxa.2169.1.6>
- Lawrence PN (1978) *Cryptopygus sverdrupi* n. sp. A new species of Collembola (Isotomidae) from Sverdrupfjella, Antarctica, with notes on the related species in five genera. *Norwegian Journal of Entomology* 25: 51–55.
- Mari Mutt JA, Bellinger PF (1990) Flora & Fauna Handbook 5. A Catalog of the Neotropical Collembola, Including Nearctic Areas of Mexico. Sandhill Crane Press, Gainesville, 237 pp.
- Massoud Z, Rapoport EH (1968) Collembolles Isotomides d'Amérique du Sud et de l'Antarctique. *Biologie de l'Amérique Australe* 4: 307–337.
- McGaughan A, Stevens MI, Holland BR (2010) Biogeography of circum-Antarctic springtails. *Molecular Phylogenetics and Evolution* 57: 48–58. <https://doi.org/10.1016/j.ympev.2010.06.003>
- Nicolet H (1842) Recherches pour servir à l'histoire des Podures. *Neue Denkschriften der Allgemeinen Schweizerischen Gesellschaft für die gesammten Naturwissenschaften* 6: 1–88.
- Paclt J (1959) Collembola. *South African Animal Life. Results of the Lund University Expedition in 1950–1951* 6: 24–78.
- Potapov M (2001) Synopses on Palaearctic Collembola (Vol. 3). Isotomidae. *Abhandlungen und Berichte für Naturkunde Görlitz* 73: 1–603.
- Potapov MB, Stebaeva SK (1994) *Sibiracanthella* and *Sahacanthella* new genera of Anurophorinae (Collembola, Isotomidae) with anal spines from continental Asia. *Miscellanea Zoologica* 17: 129–139.
- Potapov M, Gao Y, Deharveng L (2013) Taxonomy of the *Cryptopygus* complex. I. *Pauropygus*—a new worldwide littoral genus (Collembola, Isotomidae). *ZooKeys* 304: 1–16. <https://doi.org/10.3897/zookeys.304.4083>
- Potapov M, Janion C, Deharveng L (2011) Two new species of *Parisotoma* (Collembola: Isotomidae) from the Western Cape, South Africa. *Zootaxa* 2771: 17–24. <https://doi.org/10.11646/zootaxa.2771.1.2>
- Potapov M, Janion-Scheepers C, Deharveng L (2017) Taxonomy of the *Cryptopygus* complex. II. Affinity of austral *Cryptopygus* s.s. and *Folsomia*, with the description of two new *Folsomia* species (Collembola, Isotomidae) *ZooKeys* 658: 131–146. <https://doi.org/10.3897/zookeys.658.11227>
- Rusek J (2002) Do we have *Cryptopygus*-representatives in Europe? *Pedobiologia* 46: 302–310. <https://doi.org/10.1078/0031-4056-00137>

- Salmon JT (1965) Two new genera of Antarctic Collembola. *Pacific Insects* 7: 468–472.
- Schött H (1927) Kamerunischen Collembolen. *Linköping* 5: 1–40.
- Stevens MI, Greenslade P, Hogg ID, Sunnucks P (2006) Examining southern hemisphere springtails: could any have survived glaciation of Antarctica? *Molecular Biology and Evolution* 23: 874–882. <https://doi.org/10.1093/molbev/msj073>
- Thibaud JM (1996) Étude des Collemboles (Hexapoda) interstitiels des sables littoraux de Mauritanie. *Annales de la Société entomologique de France (N.S.)* 32: 475–479.
- Wahlgren E (1906) Antarktische und Subantarktische Collembolen gesammelt von der Schwedischen Sudpolarexpedition. *Wissenschaftliche ergebnisse der Schwedischen sudpolarexpedition 1901–1903* 5: 1–22.
- Willem V (1902) Les collemboles recueillis par l'expédition antarctique belge. *Annals of the society for Entomology of Belgium* 45: 260–262.
- Wise KAJ (1967) Collembola (Springtails). *Antarctic Research Series* 10: 123–148. <https://doi.org/10.1029/AR010p0123>
- Wise KAJ (1970) Collembola of South Georgia. *Pacific Insects Monograph* 23: 183–208.
- Yosii R (1965) On some Collembola of Japan and adjacent countries. *Contributions from the Biological Laboratory Kyoto University* 19: 1–71.

Egg parasitoids of *Arboridia apicalis* (Nawa, 1913) (Hemiptera, Cicadellidae), a leafhopper pest of grapevines in Japan, with description of a new species of *Anagrus* Haliday, 1833 (Hymenoptera, Mymaridae)

Serguei V. Triapitsyn¹, Tetsuya Adachi-Hagimori², Paul F. Rugman-Jones¹,
Natsuko Kado³, Nobuo Sawamura³, Yutaka Narai³

1 Department of Entomology, University of California, Riverside, California, USA **2** Organization for Promotion of Tenure Track, University of Miyazaki, Miyazaki, Japan **3** Shimane Agricultural Technology Center, Izumo, Shimane, Japan

Corresponding author: Tetsuya Adachi-Hagimori (tadachi@cc.miyazaki-u.ac.jp)

Academic editor: Andreas Köhler | Received 9 March 2020 | Accepted 4 May 2020 | Published 3 July 2020

<http://zoobank.org/04BAF072-83CE-4630-B101-4356A1688F77>

Citation: Triapitsyn SV, Adachi-Hagimori T, Rugman-Jones PF, Kado N, Sawamura N, Narai Y (2020) Egg parasitoids of *Arboridia apicalis* (Nawa, 1913) (Hemiptera, Cicadellidae), a leafhopper pest of grapevines in Japan, with description of a new species of *Anagrus* Haliday, 1833 (Hymenoptera, Mymaridae). ZooKeys 945: 129–152. <https://doi.org/10.3897/zookeys.945.51865>

Abstract

Several species of egg parasitoids (Hymenoptera: Mymaridae and Trichogrammatidae) of the leafhopper pest of grapevines in Japan, *Arboridia* (*Arboridia*) *apicalis* (Nawa) (Hemiptera, Cicadellidae), were reared and identified for the first time. Using a combination of genetic and morphological evidence, *Anagrus* (*Anagrus*) *arboridiae* Triapitsyn & Adachi-Hagimori, **sp. nov.** (Mymaridae) is described and illustrated from Honshu Island (Shimane Prefecture) and Kyushu Island (Miyazaki Prefecture). It is shown to be different from *Anagrus* (*Anagrus*) *japonicus* Sahad and *A. flaviapex* Chiappini & Lin, to which it is most similar; the latter species was originally described from China and is newly recorded here from Okinawa Island, Japan. Mitochondrial and nuclear ribosomal DNA sequence data provide clear evidence for the separation of *A. arboridiae* from *A. flaviapex*, *A. japonicus*, and some other members of the *Anagrus* (*Anagrus*) *atomus* (L.) species group. Two other species of *Anagrus* Haliday, *A. (Anagrus) avalae* Soyka and *A. atomus*, are also identified in Japan from eggs of the leafhoppers *Edwardsiana ishidae* (Matsumura) and *Eurhadina ?betularia* Anufriev, respectively. An updated key to females of the Japanese species of *Anagrus*

is given. *Oligosita pallida* Kryger (a new record for Japan), *Oligosita* sp., and an *Aphelinoidea* (*Aphelinoidea*) sp. (Trichogrammatidae) were the other, although much less abundant, apparent egg parasitoids of *A. apicalis* in Shimane Prefecture, mainly in non-organic vineyards.

Keywords

Aphelinoidea sp., egg parasitoid, grapevine pest, identification key, natural enemy, *Oligosita* spp., taxonomy

Introduction

The leafhoppers *Arboridia* (*Arboridia*) *apicalis* (Nawa) (Fig. 1a) and *A. (Arboridia)* *suzukii* (Matsumura) (Hemiptera, Cicadellidae) have been recorded in Japan as pests of cultivated grapes, *Vitis* spp. (Vitaceae) (Tanaka et al. 1986; Sakagami 2003; Yamada 2003). Feeding by the leafhopper adults and nymphs causes stippling, a characteristic damage to the grape leaves (Fig. 1b–f); also, when abundant, particularly in the organic vineyards on Honshu Island, adult *A. apicalis* are a significant nuisance to the pickers at harvest by getting into peoples' faces. Both *A. apicalis* and *A. suzukii* are native to the Eastern Palearctic and Northeastern Oriental regions, with more or less similar distributions: that of *A. apicalis* includes Japan (throughout), Korean Peninsula, Far East of Russia, mainland China and Taiwan (Guglielmino et al. 2012; Song and Li 2013, 2015; Oh et al. 2015; Dmitriev 2019) while *A. suzukii* occurs in Japan (Honshu, Kyushu, and Shikoku Islands), Korean Peninsula, Far East of Russia, mainland China, and Taiwan (Oh et al. 2015; Dmitriev 2019). *Arboridia apicalis* is a polyphagous leafhopper feeding on various deciduous trees and vines, such as cherry, grape, hawthorn, apple, pear, peach, mulberry, maple (Yamada 2003; Guglielmino et al. 2012; Song and Li 2015; Dmitriev 2019). The known host plants of *A. suzukii*, besides grape, are apple, pear, Manchurian cherry (Dmitriev 2019), and also Japanese chestnut; this leafhopper is a pest in the Japanese vineyards mainly in Kyushu Island, causing damage particularly to leaf edges whereas *A. apicalis* damages the entire leaf more uniformly (Yamada 2003, illustrated by Sakagami (2003)). Both species overwinter as adults in dropped leaves, weeds, cracks in bark, and under cover of buildings. On Honshu Island, *A. apicalis* has three generations per year, although, in greenhouses, it develops earlier and can have four generations per year (Yamada 2003).

Egg parasitoids of *Arboridia* spp. have been unknown in Japan, yet elsewhere several species of Mymaridae and Trichogrammatidae (Hymenoptera: Chalcidoidea) were reported from eggs of other species of this genus (Noyes 2019). In particular, *Anagrus* (*Anagrus*) *turpanicus* Triapitsyn & Hu was described recently from eggs of the invasive leafhopper pest of grapes in Xinjiang Uyghur Autonomous Region of China, *Arboridia kakogawana* (Matsumura) (Hu and Triapitsyn 2016). Interestingly, *A. kakogawana* is native to Japan but it has not been reported there as a pest of cultivated grapes. One of the possible explanations for that, requiring further investigation, could be a possibility that the holotype female of *A. kakogawana*, collected (without indication of a host plant association) in Kakogawa (near Akashi), Hyogo Prefecture, Honshu Island (Mat-

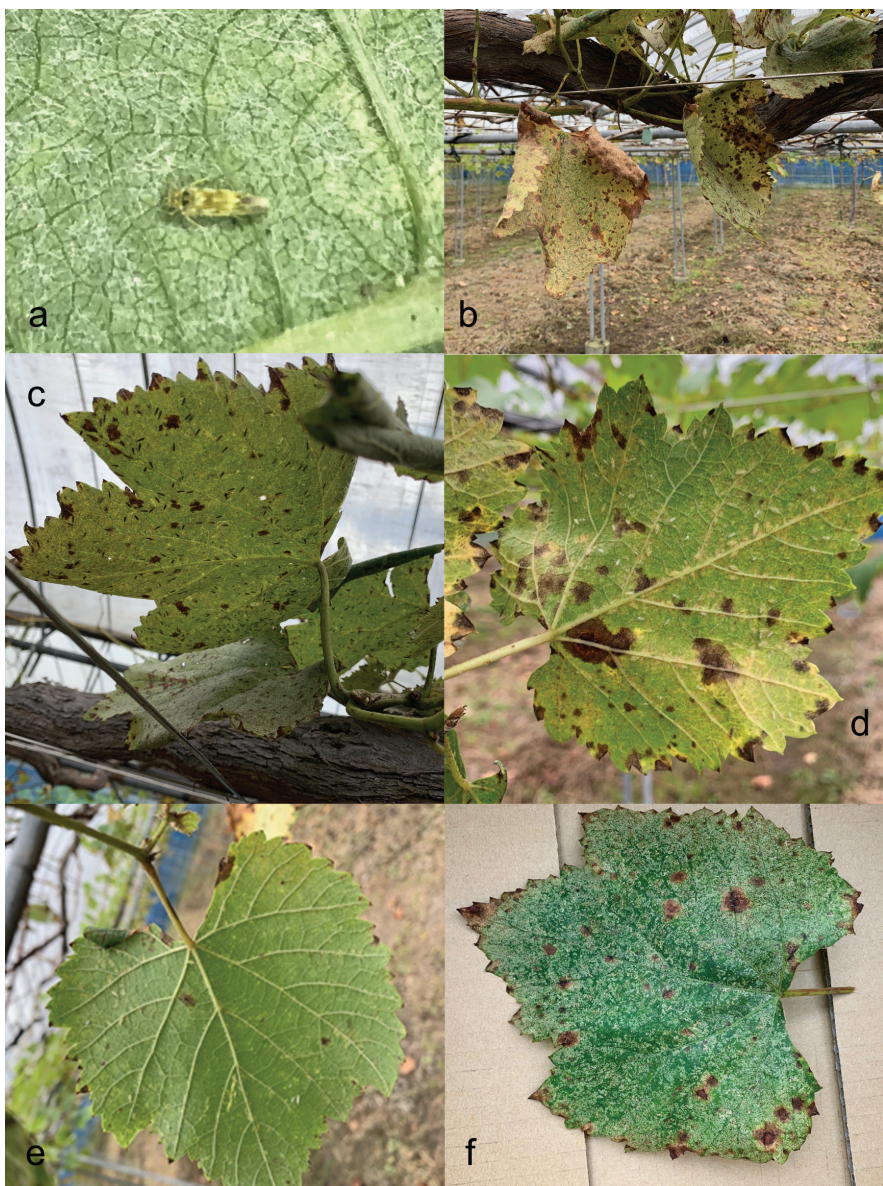


Figure 1. *Arboridia (Arboridia) apicalis* and its damage to cultivated grapevines in Japan **a** adult (Takayama, Gifu Prefecture, Honshu Island) **b** heavy damage to grape leaves in a covered vineyard (Shimane Prefecture, Honshu Island, also **c–f**) **c** numerous adults on the underside of a grape leaf **d** nymphs on the underside of a grape leaf **e** light damage to a grape leaf by a few nymphs **f** heavy damage to a grape leaf.

sumura 1932), might not be conspecific with the males from Korea, on whose genitalic characters the current recognition of this species is based upon (Dworakowska 1970). According to our observations, eggs of *A. apicalis* are laid singly, embedded in the soft

tissues of grapevines, such as leaf veins. In the commercial vineyards of Japan, many of which are covered from at least above to control temperature and humidity, grape leafhoppers are usually controlled by insecticides (e.g., Arai and Toyama 2018) so finding organic vineyards, which are not common, was essential to the success of this survey. Because, according to the personal observations of the first author in other countries, grape leafhoppers from several genera, including *A. kakogawana*, prefer to feed on the leaves inside the vines, where it is more shady, it appears that the practice of covering the vines in many Japanese vineyards (and thus providing favorable shady conditions) might enhance damage to the entire vine, including peripheral foliage.

As the first step towards the establishment of a biological control-based integrated pest management (IPM) of these grape leafhoppers in Japan, we identified egg parasitoids of *A. apicalis* collected mainly from organic vineyards.

Materials and methods

Specimen collection

Both adults and nymphs of *A. apicalis* were collected in Japan by sweeping from the grapevines at several vineyards in Shimane Prefecture, Honshu Island, as well as from the organic vineyard in Aya, Miyazaki Prefecture, Kyushu Island (26°06'38"N, 129°41'16"E, 52 m). *Arboridia suzukii* was not present in these sites. Parasitized eggs, which turn dark orange as the parasitoid larva develops and then pupates, were documented by dissections in Petri dishes of the heavily leafhopper-infested grape leaves from the organic Oku-Izumo vineyard (Black Olympia cultivar) in Unnan, Shimane Prefecture. In early October 2019, leaves and vines infested with *A. apicalis* were collected from one organic and two non-organic vineyards in Shimane Prefecture and also in the above-mentioned organic vineyard in Miyazaki Prefecture; these were placed in tightly taped carton boxes. To collect adult parasitoids and host leafhoppers (both adults and nymphs), two clear glass vials were inserted through the holes into each of the boxes, one on the top and the other on the side near the top of the box, the latter to maximize exposure to daylight coming through the window. The vials were also exposed to a constant light source in the laboratory, and the emerging individuals were collected in 80% ethanol at least once almost daily (except for some weekends) for at least 30 days. Both host leafhoppers and parasitoids were sorted to morphospecies and identified by the first author at the second author's laboratory at the University of Miyazaki Kibana Campus, Miyazaki City. The parasitoids were stored at -20 °C until they were shipped to the first author's laboratory at the Entomology Research Museum, University of California at Riverside, California, USA (UCRC). These specimens were used both for molecular analyses and taxonomic studies (as type material of the new species of *Anagrus* Haliday described below).

Voucher specimens of the grape leafhoppers resulting from this study are deposited in the insect collections of the Entomological Laboratory, Faculty of Agriculture, Ky-

ushu University, Fukuoka, Japan (**ELKU**), Illinois Natural History Survey, University of Illinois at Urbana-Champaign, Champaign, Illinois, USA (**INHS**) and the **UCRC**. Their identities were confirmed by Dmitry A. Dmitriev (INHS).

Taxonomic studies

Morphological identifications of the *Anagrus* sp., made by the first author, were based mainly on females because males of many species of *Anagrus* are often similar and difficult to determine beyond a species group.

For the taxonomic description of the new species, the morphological terms of Gibson (1997), with some modifications made by Triapitsyn (2015), were used. All measurements (as length or length and width for the wings) are given in micrometers (μm). Abbreviations used in the description and key are:

- F** funicle segment of the female antenna or flagellomere of the male antenna;
mps multi-porous plate sensillum or sensilla on the antennal flagellar segments (= longitudinal sensillum or sensilla, or sensory ridge(s)).

Specimens from ethanol were dried using a critical point drier, then point-mounted and labeled. Selected specimens were dissected and slide-mounted in Canada balsam. Slide mounts were examined under a Zeiss Axioskop 2 plus compound microscope (Carl Zeiss Microscopy, LLC, Thornwood, New York, USA) and photographed using the Auto-Montage system (Syncroscopy, Princeton, New Jersey, USA). Photographs were retouched where necessary using Adobe Photoshop (Adobe Systems, Inc., San Jose, California, USA).

Specimens of the parasitoids examined are deposited in the collections with the following acronyms:

- CNC** Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa, Ontario, Canada;
ELKU Entomological Laboratory, Faculty of Agriculture, Kyushu University, Fukuoka, Japan;
UCRC Entomology Research Museum, Department of Entomology, University of California, Riverside, California, USA.

DNA extraction, amplification, and sequencing

DNA was extracted from two individual female wasps of the new species described herein using the “HotSHOT” method of Truett et al. (2000), in a total volume of 80 μL . This non-destructive method allowed for the recovery and slide-mounting of each specimen following extraction; each slide was then labeled with the assigned P. F. Rugman-Jones’ primary molecular voucher PR number and UCRC database UCRC ENT number. The polymerase chain reaction (PCR) was employed to amplify

the “barcoding” region of the mitochondrial cytochrome c oxidase subunit I gene (COI) and the internal transcribed spacer 2 (ITS2) region of nuclear ribosomal RNA (rRNA). Amplification and sequencing of the COI were performed using the same protocols as described in Triapitsyn et al. (2018, 2019a, b) for some other species of *Anagrus*. It was only possible to sequence the ITS2 after cloning the amplicon as described in Triapitsyn et al. (2019b). For each specimen, two insert-positive clones were subsequently sequenced.

In addition to the specimens of the newly described species, the same methods were used to extract, amplify, and sequence the DNA from two individual females of *Anagrus* (*Anagrus*) *japonicus* Sahad, which represent the voucher specimens of the study by Adachi-Hagimori et al. (unpublished) (P. F. Rugman-Jones’ molecular vouchers PR19-501 [UCRC_ENT 005517343] and PR19-502 [UCRC_ENT 005517344]), and also from one female of *A.* (*Anagrus*) *flaviapex* Chiappini & Lin (data given below under “Comments”). Both species belong to the *atomus* species group of the nominate subgenus of *Anagrus*; all three specimens were recently collected in Okinawa Island, Ryukyu Islands, Japan.

All sequences generated in this study were deposited in GenBank (Benson et al. 2008).

Genetic analysis

The COI sequences generated from the five specimens in this study (GenBank accessions MT396446–MT396450) were combined with 45 others retrieved from GenBank, each of which was a unique haplotype identified in three earlier studies of members of the *Anagrus* ‘*atomus*’ species complex, which is altogether comprised of six nominal species (Zanolli et al. 2016 [34 haplotypes, KM677212–KM677245]; Nugnes et al. 2017 [11 haplotypes from KX691520–KX691551]). One further sequence, from *A.* (*Anagrus*) *incarnatus* Haliday [MK024811], a member of the *incarnatus* species group, was also retrieved for use as an outgroup, and the combined sequence data were subsequently aligned using MAFFT version 7.050 (Katoh and Standley 2013) and the Q-INS-i algorithm with default settings. This resulted in an aligned COI dataset containing 51 terminal taxa and 587 nucleotide positions. The only gaps in the matrix were the result of 147 missing bases at the 5’ end of many of the sequences retrieved from GenBank. Genetic variation among our sequences was estimated by calculating uncorrected p-distances between all possible sequence pairs, using MEGA version 6 (Tamura et al. 2013). Gapped positions (i.e. at the 5’ end) were removed for each sequence pair in the analysis. A neighbor-joining (NJ) tree based on those p-distances was subsequently constructed, again using MEGA. Branch support was estimated using a bootstrap procedure with 1000 replicates.

Since phylogenetic inference from ITS2 is typically problematic due to large inter-specific differences that make alignment of this region difficult and somewhat ambiguous, ITS2 sequences were examined “by eye” to corroborate the differentiation of our specimens based on COI. Furthermore, BLAST searches of the NCBI database were performed to assess their similarity to other members of the ‘*atomus*’ species complex.

Results

Taxonomy

Mymaridae

Anagrus (Anagrus) arboridiae Triapitsyn & Adachi-Hagimori, sp. nov.

<http://zoobank.org/915C3341-6BD3-4E69-A6FD-8B47FD77792E>

Figures 2–4

Type material. *Holotype* ♀ (Fig. 3a, b, d), deposited in ELKU, on slide (Fig. 3c) labeled: 1. “JAPAN: Honshu Island Shimane Prefecture, Unnan Oku-Izumo vineyard, 35°17'20"N, 132°55'46"E, 155 m (organic Black Olympia table grapes heavily infested with *Arboridia apicalis* (Nawa) in a covered vineyard), leaves collected 4.x.2019, N. Kado, N. Sawamura, T. Adachi-Hagimori, S. V. Triapitsyn Emerged 6.x.2019, S. V. Triapitsyn”; 2. [magenta] “*Anagrus (Anagrus) arboridiae* Triapitsyn & Adachi-Hagimori HOLOTYPE ♀”; 3. “Det. by S. V. Triapitsyn 2019”; 4. [barcode database label/unique identifier] “UCRC [bold] UCRC_ENT 005517345”.

Paratypes. JAPAN: Honshu Island, Shimane Prefecture, Unnan, Oku-Izumo vineyard, 35°17'20"N, 132°55'46"E, 155 m (organic Black Olympia table grapes heavily infested with *A. apicalis* in a covered vineyard), collected 4.x.2019, N. Kado, N. Sawamura, T. Adachi-Hagimori, S. V. Triapitsyn, emerged from grape leaves: 5.x.2019, S. V. Triapitsyn [4 ♀♀, 6 ♂♂, ELKU, UCRC: 2 ♀♀, 5 ♂♂ on points and 2 ♀♀, 1 ♂ on slides (including 1 ♀ on slide, P. F. Rugman-Jones' molecular voucher PR19-504, UCRC_ENT 005517353)]; 6.x.2019, S. V. Triapitsyn [15 ♀♀, 10 ♂♂, CNC, ELKU, UCRC: 8 ♀♀, 6 ♂♂ on points and 7 ♀♀, 4 ♂♂ on slides (including 1 ♀ on slide, P. F. Rugman-Jones' molecular voucher PR19-503, UCRC_ENT 005517352)]. Kyushu Island, Miyazaki Prefecture, Higashimorokata, Aya, Kitamata, 32°00'32"N, 131°14'26"E, 86 m (Katsuki Wines LLC organic vineyard, Chardonnay wine grapes lightly infested with *A. apicalis* in a covered vineyard), leaves collected 30.x.2019, T. Adachi-Hagimori, S. V. Triapitsyn, emerged 6.xi.2019, S. V. Triapitsyn [1 ♀, 1 ♂ on slides, UCRC].

Other (non-type) material examined. JAPAN: Honshu Island, Shimane Prefecture, Unnan, Oku-Izumo vineyard, 35°17'20"N, 132°55'46"E, 155 m (organic Black Olympia table grapes heavily infested with *A. apicalis* in a covered vineyard), collected 4.x.2019, N. Kado, N. Sawamura, T. Adachi-Hagimori, S. V. Triapitsyn: sweeping on vines [1 ♀]; emerged from grape leaves: 5.x.2019, S. V. Triapitsyn [10 ♀♀, 10 ♂♂]; 6.x.2019, S. V. Triapitsyn [27 ♀♀, 22 ♂♂]; 7.x.2019, N. Kado [35 ♀♀, 29 ♂♂]; 8.x.2019, N. Kado [27 ♀♀, 18 ♂♂]; 9.x.2019, N. Kado [24 ♀♀, 12 ♂♂]; 10.x.2019, N. Kado [6 ♀♀, 6 ♂♂]; 11.x.2019, N. Kado [12 ♀♀, 7 ♂♂]; 13.x.2019, N. Kado [7 ♀♀]; 15.x.2019, N. Kado [1 ♀♀, 3 ♂♂]. Kyushu Island, Miyazaki Prefecture, Higashimorokata, Aya, Kitamata, 32°00'32"N, 131°14'26"E, 86 m (Katsuki Wines LLC organic vineyard, Chardonnay wine grapes lightly infested with *A. apicalis* in a covered vineyard), leaves collected 30.x.2019, T. Adachi-Hagimori, S. V. Triapitsyn: emerged 31.x.2019, S. V. Triapitsyn [1 ♂]; emerged 2.xi.2019, T. Adachi-Hagimori

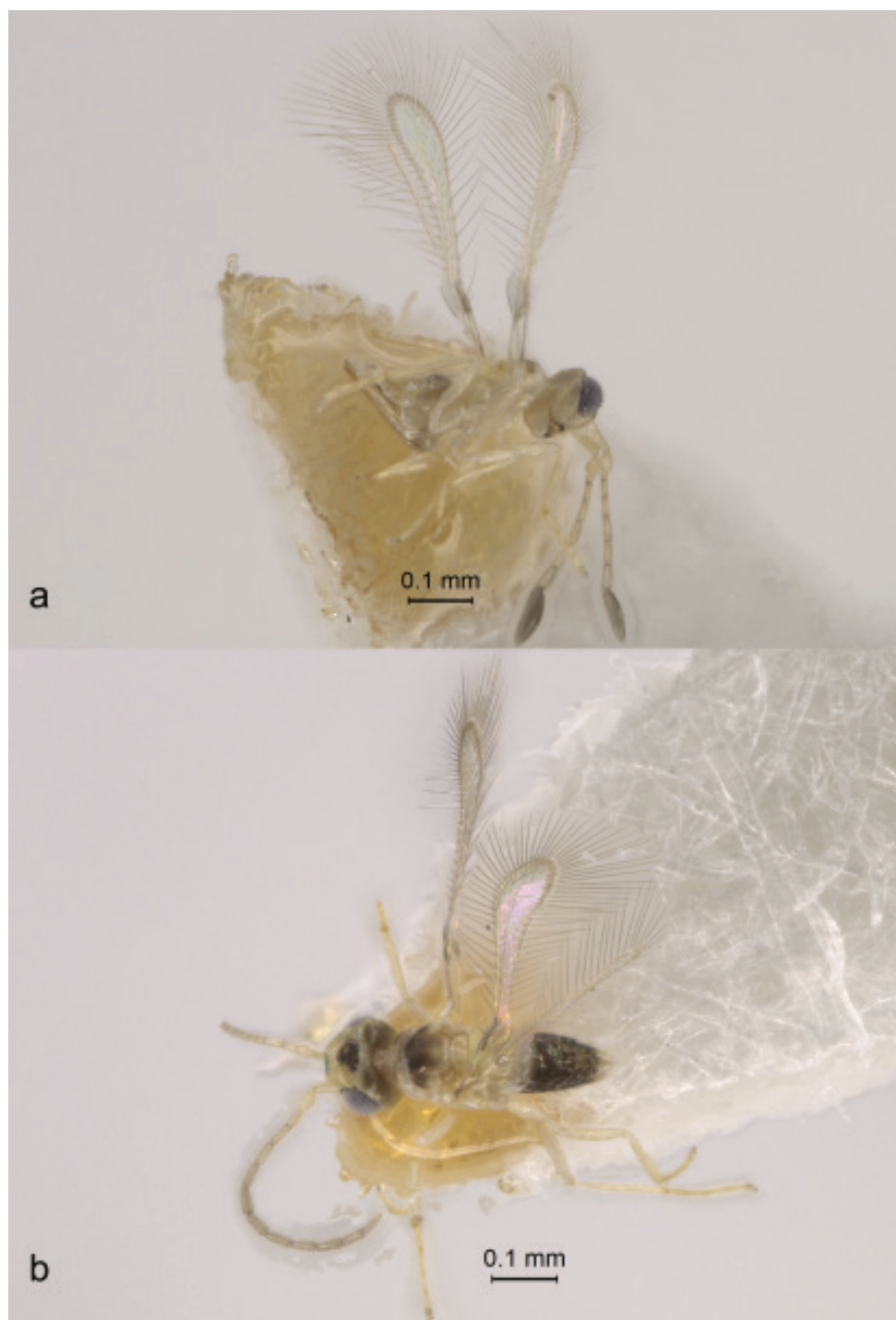


Figure 2. *Anagrus arboridiae* sp. nov. (paratypes from Unnan, Shimane Prefecture, Honshu Island, Japan) **a** habitus of female (lateral view) **b** habitus of male (dorsal view).

[1 ♂]; emerged 4.xi.2019, T. Adachi-Hagimori [2 ♀♀]; emerged 2.xi.2019, T. Adachi-Hagimori [1 ♂]; emerged 5.xi.2019, S. V. Triapitsyn [1 ♀]. All in 80% ethanol in a freezer [UCRC].

Diagnosis. The new species is a member of the *atomus* species group of *Anagrus* (*Anagrus*) as defined by Chiappini et al. (1996). Female antenna (Fig. 3a) with mps on F3 (1), F4 (1), F5 (1 or 2), F6 (2), and clava (3); midlobe of mesoscutum without adnotaular setae; fore wing disc with a distinct subapical bare area (Figs 3b, 4b); ovipositor (Fig. 3d) 2.3–2.5× length of protibia. Male genitalia (Fig. 4c) with hooked digiti.

Morphologically, *A. arboridiae* is most similar to *A. flaviapex*, to which its female specimens with a more or less distinct bare area on the fore wing disc key in Triapitsyn (2015) and Li et al. (2018). Both taxa have one mps on F3 of the female antenna (Chiappini and Lin 1998). However, male genitalia of *A. flaviapex* have cone-shaped, straight digiti (Triapitsyn 1999) and body of the female is mostly brown (Triapitsyn 2015). Besides *A. arboridiae*, the three other known species within the nominate subgenus of *Anagrus* with hooked digiti of male genitalia and three mps on the clava of female antenna, the European *A. vilis* Donev and the Afrotropical *A. scassellatii* Paoli and *A. sensillatus* Viggiani & Jesu (Chiappini and Mazzoni 2000), were assigned to the *atomus* species group by them and also by Triapitsyn (2015). More recently, Nugnes et al. (2017) placed these, without providing much of a justification and based solely on the hooked shape of the digiti of male genitalia, in a separate *vilis* species group of *Anagrus* (*Anagrus*). However, based on other morphological features of both sexes *A. arboridiae* is not related to *A. vilis* and either of them are not related to the two aforementioned Afrotropical species. Moreover, in males of *A. japonicus* from Okinawa Island, shape of the digiti is of an intermediate state between the strongly hooked (such as in *A. arboridiae* and *A. vilis*) and the straight ones (like in most other species within the *atomus* species group): these are more or less cone-shaped in dorsoventral view but notably curved in lateral view (Adachi-Hagimori et al. unpublished). Because the hooked shape of the digiti is likely to have evolved independently in the different lineages within the *atomus* species group *sensu lato*, we agree with the conclusion made by Chiappini and Mazzoni (2000) that by itself this feature should not be used for defining separate species groups without support of any additional characters. Thus, we do not recognize the *vilis* species group proposed by Nugnes et al. (2017) as a separate entity from the *atomus* species group.

Females of *A. arboridiae* are also similar to those *A. japonicus* that sometimes have an mps on F3, but the former always have distinct light brown and brown patches on the mesoscutum and the basal gastral terga respectively, which the latter species lacks.

An updated key to females of the Japanese species of *Anagrus* is provided below, as the latest key by Triapitsyn et al. (2019a) is already outdated after the addition of four species herein which are new to the fauna of Japan.

Description. Female (holotype and paratypes). Body length of dry-mounted, critical point-dried paratypes 400–500 µm, and of the slide-mounted paratypes 415–520 µm. Body (Fig. 2a) and appendages mostly pale yellow except funicle, occiput and almost all of mesoscutum (except posteriorly) light brown, transverse trabecula and

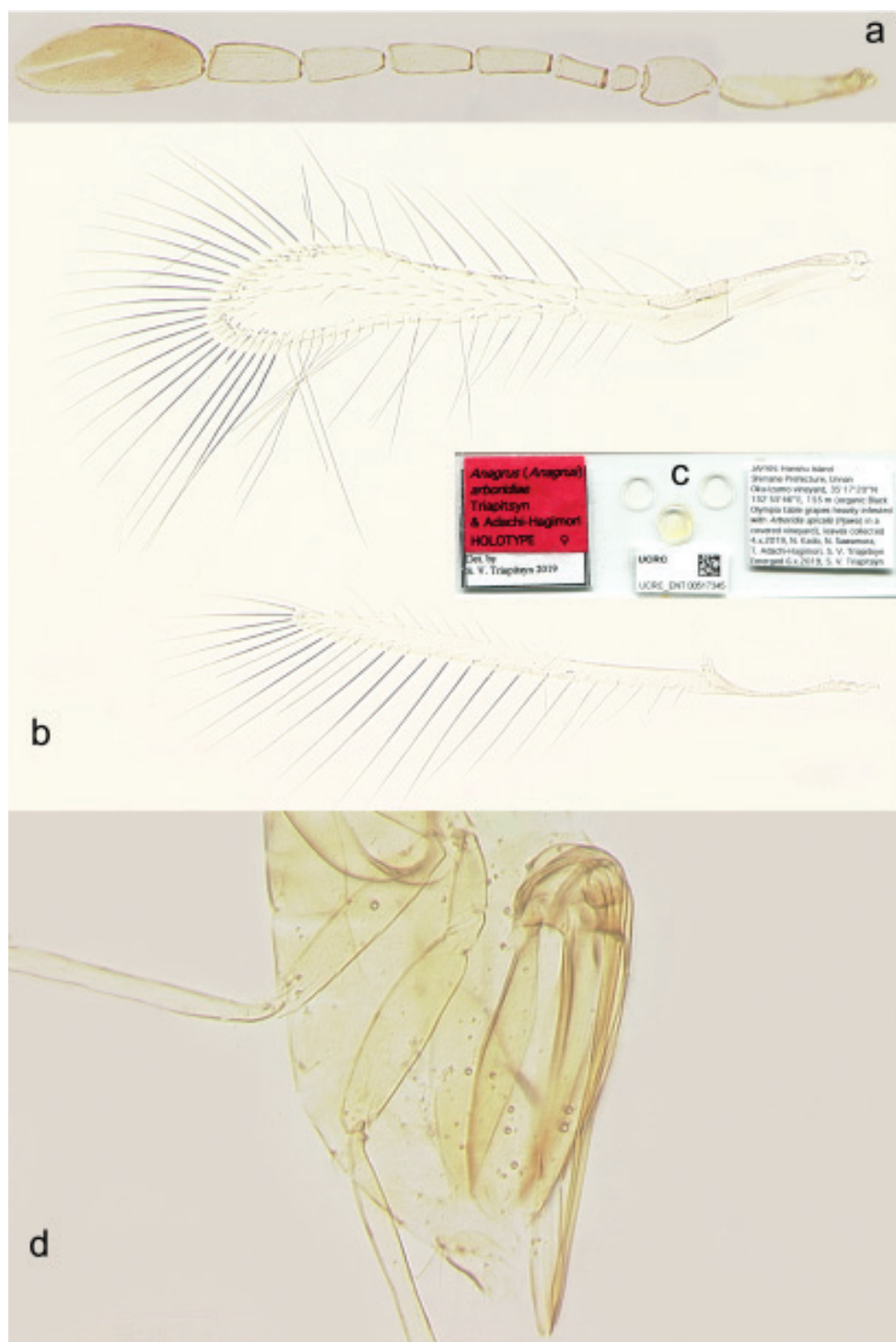


Figure 3. *Anagrus arboridiae* sp. nov. female (holotype) **a** antenna **b** fore and hind wings **c** slide **d** gaster.

stematicum dark brown, clava and two basal gastral terga brown; wings almost hyaline (slightly infumate). Antenna (Fig. 3a) with scape $3.4\text{--}4.1\times$ as long as wide, with cross-ridges, $2.0\text{--}2.3\times$ length of pedicel; F1 at most slightly longer than wide, almost half of pedicel length; F2 usually notably shorter than following funicular segments but occasionally approximately as long as F3 or even slightly longer (when F3 lacks mps), F4 usually slightly longer than F5 but occasionally subequal to F5, F6 the longest funicular segment; mps on F3 (usually one but often none), F4 (one or two); F5 (one), and F6 (two); clava with three rather short mps (just a little longer than half length of clava), $2.7\text{--}3.3\times$ as long as wide, usually slightly longer (but occasionally approximately as long as) than combined length of F5 and F6. Midlobe of mesoscutum without adnotaular setae. Fore wing (Fig. 3b) $6.1\text{--}7.0\times$ as long as wide, longest marginal seta $2.5\text{--}2.6\times$ maximum wing width; distal macrochaeta ca. $4\times$ length of proximal macrochaeta; disc with several rows of setae in addition to admarginal rows of setae (single complete row originating behind apex of venation and two or three irregular rows in the broadest part of disc), leaving a distinct subapical bare area at posterior margin. Hind wing (Fig. 3b) $21\text{--}25\times$ as long as wide, longest marginal seta $7.4\text{--}9.0\times$ maximum wing width; disc mostly bare except for an almost complete row of microtrichia along posterior margin and 1–3 additional microtrichia at apex. Ovipositor (Fig. 3d) extending anteriorly almost to mesophragma in slide-mounted specimens and at most barely exerted beyond apex of gaster posteriorly (by at most $0.06\times$ total ovipositor length). Second valvifers (= external plates of ovipositor), e.g., Chiappini et al. (1996), each with 1 seta. Ovipositor $1.9\text{--}2.1\times$ length of protibia ($2.05\times$ in the holotype).

Measurements (μm) of the holotype (as length or length: width). Body: 545; mesosoma 197; gaster 251; ovipositor 212. Antenna: scape 75; pedicel 38; F1 13; F2 27; F3 39; F4 43; F5 42; F6 48; clava 100. Fore wing 463; 74; longest marginal seta 185. Hind wing 424; 20; longest marginal seta 148.

Male (paratypes). Body length of dry-mounted, critical point-dried paratypes $330\text{--}460\text{ }\mu\text{m}$, and of the slide-mounted paratypes $400\text{--}520\text{ }\mu\text{m}$. Body color mainly as in female except most of mesoscutum brown and most of gaster dark brown (light brown basally) (Fig. 2b); flagellum light brown. Antenna (Fig. 4a) with scape $2.6\text{--}3.2\times$ as long as wide, F1 usually at least slightly shorter than following flagellomeres but occasionally either much shorter than F2 or approximately as long as F2. Fore wing (Fig. 4b) $5.9\text{--}6.4\times$ as long as wide. Genitalia (Fig. 4c) length $86\text{--}90\text{ }\mu\text{m}$; digiti hooked.

Etymology. This new species is named after the host leafhopper genus.

Distribution. Palaearctic region: Japan (Honshu and Kyushu Islands).

Host. Cicadellidae: *Arboridia* (*Arboridia*) *apicalis* (Nawa). Only *A. apicalis* was present on the grapevines both in Aya, Miyazaki Prefecture and in Unnan, Shimane Prefecture, with any other leafhoppers being absent, so this host association of *A. arboridiae* is obvious.

Biology. In the dissected parasitized eggs of *A. apicalis* in the organic vineyard in Unnan, Shimane Prefecture, *A. arboridiae* was observed to develop as a solitary endoparasitoid. Other aspects of its biology are unknown and thus would require further investigations.

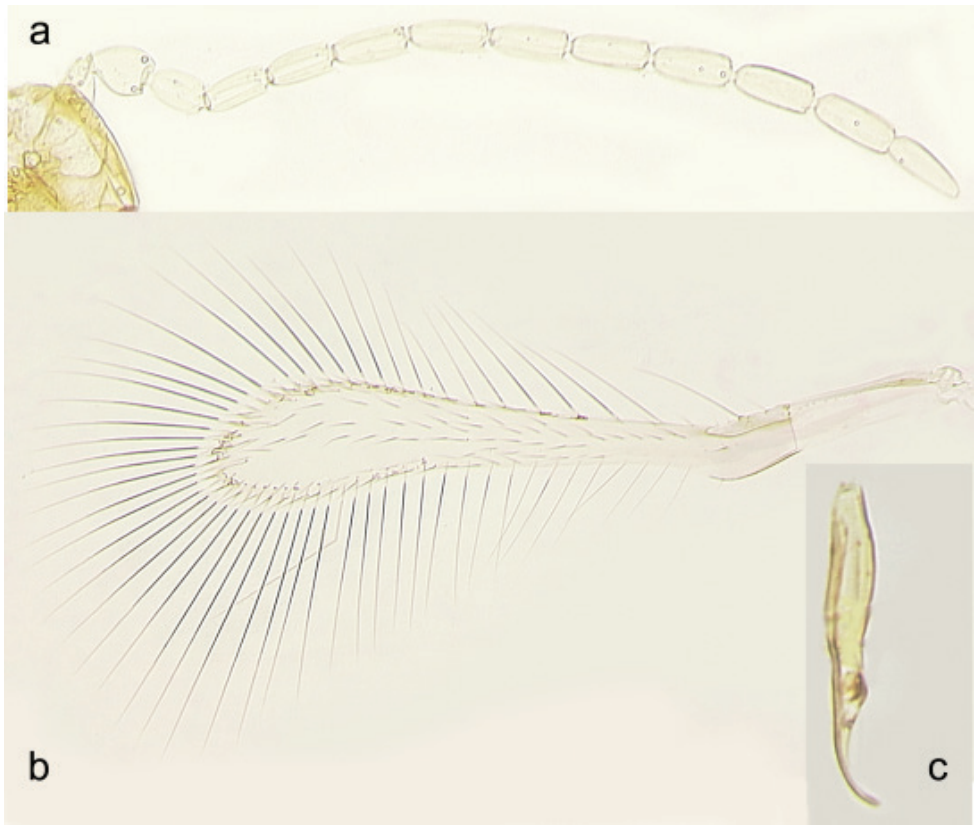


Figure 4. *Anagrus arboridiae* sp. nov. male (paratypes from Unnan, Shimane Prefecture, Honshu Island, Japan) **a** antenna **b** fore wing **c** genitalia (lateral view).

Remarks. The following species of *Anagrus* are newly recorded for the fauna of Japan.

Anagrus (Anagrus) flaviapex: Japan, Ryukyu Islands, Okinawa Prefecture, Okinawa Island, Itoman, Makabe, Okinawa Prefectural Agricultural Research Center (26°06'37.9"N, 129°41'16.1"E, 52 m), okra (organic experimental plot), 15–18.x.2019, S.V. Triapitsyn, T. Adachi-Hagimori, T. Uesato, Malaise trap [1 ♀, UCRC; P. F. Rugman-Jones' molecular voucher PR19-505, UCRC_ENT 005517351]. This species has an Oriental and Eastern Palaearctic distribution but is known from different leafhopper and possibly also planthopper hosts (Chiappini and Lin 1998; Triapitsyn 1999, 2015).

The following dry-mounted specimens of *Anagrus* were found in T. Tachikawa's collection during the first author's visit of ELKU in October 2019 and consequently borrowed, some of them slide-mounted, and then identified. They represent new and interesting host associations for the respective taxa:

Anagrus (Anagrus) atomus (L.): Japan, Shikoku Island, Ehime Prefecture, Kihoku, 24.iii.1966, emerged 28.iii.1966 from eggs of *Eurhadina ?betularia* Anufriev (a tentative identification of the host per the original label in Japanese) [4 ♀♀, ELKU];

Anagrus (Anagrus) avalae Soyka: Japan, Honshu Island, Aomori Prefecture, Hirosaki City, vi.1964, R. Tsugawa, from eggs of *Edwardsiana ishidae* (Matsumura) [13 ♀♀, 4 ♂♂, ELKU].

Key to females of the Japanese species of *Anagrus*

- 1 Ocelli on a stemmaticum 3
- Ocelli not on a stemmaticum (*Anagrus (Anagrella)* Bakkendorf) 2
- 2 F2 approximately 1.5× F1 length
..... *Anagrus (Anagrella) brevis* Chiappini & Lin
- F2 at least 4.0× F1 length *Anagrus (Anagrella) hirashimai* Sahad
- 3 Frenum with triangular paramedial plates widely separated from each other; metafemur short, less than 2× trochanter length, trochantellus incision almost half-way between coxa-trochanter and femur-tibia articulations (*Anagrus (Paranagrus)* Perkins) 4
- Frenum with triangular paramedial plates very close to each other; metafemur long, more than 2× trochanter length, trochantellus incision ca. one-third way between coxa-trochanter and femur-tibia articulations (*Anagrus (Anagrus)* Haliday [*sensu stricto*]) 5
- 4 Ovipositor projecting beyond apex of gaster by approximately 1/3 of its total length; ovipositor length: protibia length ratio at least 3.5
..... *Anagrus (Paranagrus) perforator* (Perkins)
- Ovipositor not projecting or at most slightly projecting beyond apex of gaster; ovipositor length: protibia length ratio at most 2.5
..... *Anagrus (Paranagrus) optabilis* (Perkins)
- 5 Clava with 3 mps (*atomus* species group) 6
- Clava with 5 mps (*incarnatus* species group) 10
- 6 Fore wing length: width ratio more than 10
..... *Anagrus (Anagrus) frequens* Perkins
- Fore wing length: width ratio less than 10 7
- 7 Body light brown or brown (at most frenum, propodeum and apex of gaster yellow) 8
- Body pale yellow, yellow, or greyish yellow (at most parts of mesoscutum and 2 basal gastral terga light brown or brown) 9
- 8 F3 without mps; gaster uniformly colored... *Anagrus (Anagrus) atomus* (L.)
- F3 with 1 mps; 2 apical gastral terga contrastingly yellow
..... *Anagrus (Anagrus) flaviapex* Chiappini & Lin
- 9 Body pale yellow except mesoscutum mostly light brown and 2 basal gastral terga contrastingly brown
..... *Anagrus (Anagrus) arboridiae* Triapitsyn & Adachi-Hagimori, sp. nov.
- Body yellow or greyish yellow, with 2 basal gastral terga concolorous with the rest of metasoma *Anagrus (Anagrus) japonicus* Sahad
- 10 Midlobe of mesoscutum with adnotaular setae 11
- Midlobe of mesoscutum without adnotaular setae 12

- 11 Body light yellow except mesoscutum partially a little darker; each external plate of ovipositor (second valvifer) with 2 setae *Anagrus (Anagrus) avalae* Soyka
- Body brown; each external plate of ovipositor with 3 setae..... *Anagrus (Anagrus) subfuscus* Foerster
- 12 Fore wing approximately 6.3× as long as wide..... *Anagrus (Anagrus) takeyanus* Gordh
- Fore wing at least 7.0× as long as wide 13
- 13 F2 the longest funicular segment ... *Anagrus (Anagrus) incarnatus* Haliday
- F2 at least slightly shorter than following funicular segments *Anagrus (Anagrus) rugmanjonesi* Triapitsyn & Adachi-Hagimori

Trichogrammatidae

Oligosita pallida Kryger, 1919

Figure 5

Oligosita pallida Kryger, 1919: 318–319. 2 syntype ♀♀ [Zoological Museum, Natural History Museum of Denmark, University of Copenhagen, Copenhagen, Denmark (ZMUC)] (not examined). Type locality: Gentofte, Denmark.

Oligosita pallida Kryger: Viggiani 1987: 543–546 (synonymy, type material, diagnosis, distribution, host associations, illustrations); Liu and Li 2019: 1125 (key), 1126–1127 (taxonomic history, list of synonyms, distribution, hosts).

Oligosita sp.: Triapitsyn, 1998: 83 (reared from eggs of an *Arboridia* sp. in Turkmenistan).

Material examined. JAPAN, Honshu Island, Shimane Prefecture: Izumo, Taisha (emerged from leaves of non-organic table grape infested with *A. apicalis* in a covered vineyard): 35°21'11"N, 132°40'59"E, 8 m, Shine Muscat grapes: collected 25.ix.2019, N. Kado, N. Sawamura, emerged 2–6.x.2019, S. V. Triapitsyn, N. Kado [2 ♂♂, UCRC]; collected 30.ix.2019, N. Kado, N. Sawamura, emerged 6.x.2019, S. V. Triapitsyn [1 ♀, UCRC]; collected 2.x.2019, N. Kado, Y. Narai, T. Adachi-Hagimori, S. V. Triapitsyn, emerged 3–5.x.2019, S. V. Triapitsyn, N. Kado [3 ♀♀, UCRC]. 35°21'21"N, 132°41'22"E, 3 m, Watanabe vineyard, Delaware grapes, collected 1.x.2019, N. Kado, N. Sawamura: emerged 3.x.2019, S. V. Triapitsyn, N. Kado [2 ♀♀, 1 ♂, UCRC]; emerged 4.x.2019, S. V. Triapitsyn, N. Kado [1 ♀, UCRC]. Unnan, Oku-Izumo vineyard, 35°17'20"N, 132°55'46"E, 155 m (organic Black Olympia table grapes heavily infested with *A. apicalis* in a covered vineyard), leaves collected 4.x.2019, N. Kado, N. Sawamura, T. Adachi-Hagimori, S. V. Triapitsyn, emerged 17.x.2019, N. Kado [1 ♀, UCRC].

Other material examined. FRANCE, Gironde, Sainte Colombe, M. van Helden, in vineyards: 44°52'N, 00°02'W [3 ♀♀, UCRC]; Pitray, 44°54'N, 00°02'W [1 ♀, UCRC]. IRAN: Isfahan, vi.2000, S. Hesami, from grape leafhopper [1 ♀, UCRC]. Khorasan, Fazd Village, 10.x.1994, J. Vafabaksh, from grape leaves [1 ♀, UCRC].

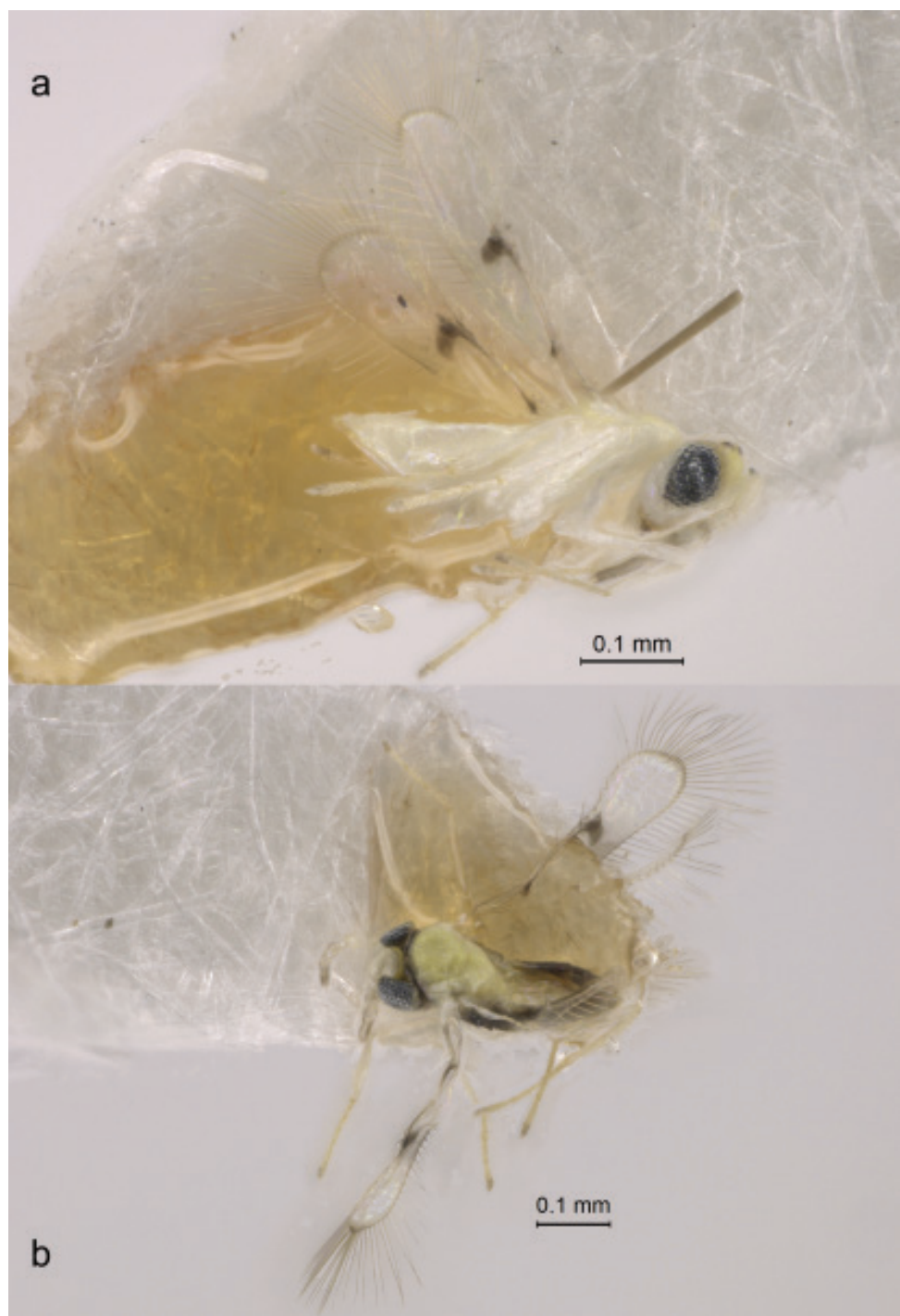


Figure 5. *Oligosita pallida* (Taisha, Izumo, Shimane Prefecture, Honshu Island, Japan) **a** habitus of female (lateral view) **b** habitus of male (dorsolateral view).

TURKMENISTAN, Ashgabat, 24.viii.1993, S. N. Myartseva, from eggs of *Arboridia* (*Arboridia*) *hussaini* (Ghauri) on grape [1 ♀, UCRC].

Distribution. Palaearctic region: China (Liu and Li 2019), Croatia (Bakkendorf 1971 [for *Oligosita tominici* Bakkendorf, a synonym of *O. pallida* (Viggiani 1987)]), Czech Republic, Denmark, Hungary, Iran, Italy, Netherlands, Turkey, UK (Noyes 2019), France, Japan, and Turkmenistan (new records).

Hosts. Cicadellidae: *Arboridia* (*Arboridia*) *apicalis* (Nawa), *A.* (*Arboridia*) *hussaini* (Ghauri) (Triapitsyn 1998 [as *Arboridia* sp. for *Oligosita* sp.]) (new records), as well as *A.* (*Arboridia*) *adanae* (Dlabola) (Viggiani 1987; Yiğit and Erkiliç 1987), *A.* (*Arboridia*) *kermanshah* Dlabola (Mostaan and Akbarzadeh 1995), *Edwardsiana rosae* (L.) (Viggiani 1987), *Zygina* (*Zygina*) *eburnea* Fieber (Bakkendorf 1971 [as *Erythroneura eburnea* for *O. tominici*]), and *Zygina* (*Zygina*) *rhamni* Ferrari (Viggiani 1987).

Remarks. Females of this distinctive species can be recognized by the uniformly pale color of the body (Fig. 5a), while in males the sides of metasoma and sometimes mesosoma are dark (Fig. 5b). Other distinguishing features of both sexes include a rather long clava of the antenna and a characteristic fore wing with a distinctive dark spot behind the apex of venation (Fig. 5), as described and illustrated by Kryger (1919) and Viggiani (1987).

Other examined female specimens of an *Oligosita* sp. in UCRC, collected from grape leaves in Iran, have several small dark spots on the sides of metasoma; thus, we cannot positively identify them as *O. pallida* without availability of a thorough assessment of intraspecific variation of body color in this species.

Oligosita sp.

Figure 6a

Material examined. JAPAN, Honshu Island, Shimane Prefecture, Izumo, Taisha, 35°21'21"N, 132°41'22"E, 3 m, 5.x.2019, N. Kado, N. Sawamura, T. Adachi-Hagimori, S. V. Triapitsyn (sweeping upon leaves of non-organic Delaware table grapes infested with *A. apicalis* in a covered Watanabe vineyard) [1 ♀, UCRC].

Distribution. Palaearctic region: Japan (Honshu Island).

Host. Cicadellidae: *Arboridia* (*Arboridia*) *apicalis* (Nawa). This tentative host association will need to be confirmed by experimental work using sentinel eggs of this leafhopper.

Remarks. Because taxonomy of the genus *Oligosita* Walker is in flux and no working keys are available, we could not positively identify this specimen to the species. Its body color is more or less light brown except dorsum of mesosoma is mostly yellow (Fig. 6a).

Aphelinoidea (*Aphelinoidea*) sp.

Figure 6b

Material examined. JAPAN, Honshu Island, Shimane Prefecture, Unnan, Oku-Izumo vineyard, 35°17'20"N, 132°55'46"E, 155 m, leaves of organic Black Olympia table



Figure 6. Habitus in lateral view **a** *Oligosita* sp. female (Taisha, Izumo, Shimane Prefecture, Honshu Island, Japan) **b** *Aphelinoidea* (*Aphelinoidea*) sp. male (Unnan, Shimane Prefecture, Honshu Island, Japan).

grapes heavily infested with *A. apicalis* in a covered vineyard collected 4.x.2019, N. Kado, N. Sawamura, T. Adachi-Hagimori, S. V. Triapitsyn, emerged 5.x.2019, S. V. Triapitsyn [1 ♂, UCRC].

Distribution. Nearctic region: USA (Nebraska); Palaearctic region: Japan (Honshu Island).

Host. Cicadellidae: *Arboridia* (*Arboridia*) *apicalis* (Nawa). This tentative host association will need to be confirmed by experimental work using sentinel eggs of this leafhopper.

Remarks. This specimen is similar to *Aphelinoidea* (*Aphelinoidea*) *waterhousei* (Blood & Kryger) which belongs to the *semifuscipennis* species group of the nominate subgenus of the genus *Aphelinoidea* Girault, formerly placed in the synonymized subgenus *Aphelinoidea* (*Diaclava* Blood & Kryger) (Triapitsyn 2018). Like *A. waterhousei* from England, UK, known from the single male specimen, the one from Japan (Fig. 6b) has apical segment of the clava contrastingly darker than the light-colored basal segment, and the marginal vein of its fore wing is notably thickened while the stigmal vein is short, inconspicuous. But because the fore wing of the specimen from Japan is notably wider and its marginal setae are relatively shorter (ca. one-third of the greatest fore wing width), it is unlikely to be *A. waterhousei*; rather, it is more similar to the unidentified male specimen from Nebraska, USA which has the same features, as illustrated by Triapitsyn (2018: 78, figs 93–97).

Molecular analyses

Sequences of the COI gene provided strong evidence that *A. arboridiae* is distinct from *A. flaviapex* and also from members of the *A. atomus* species complex for which comparative sequence data are available. The two *A. arboridiae* specimens (both from Unnan, Shimane Prefecture, Honshu Island) shared a single haplotype which, based on uncorrected p-distance, differed from the nearest taxon, *A. japonicus*, by 5%, and from *A. flaviapex* by 7.3% (Fig. 7; Table 1). In turn, these three species were at least 6.4% divergent from previously sequenced members of the *A. atomus* species complex, which in the Palaearctic region includes *A. atomus*, *A. (Anagrus) lindberginae* Nugnes & Viggiani, *A. (Anagrus) nepetellae* Viggiani & Nugnes, and *A. (Anagrus) parvus* Soyka (Fig. 7; Table 1). Intraspecific variation in the cloned ITS2 sequences was minimal and largely restricted to the length of microsatellite repeat regions. In contrast, the five

Table 1. Genetic divergence between *Anagrus arboridiae* sp. nov., *A. japonicus*, *A. flaviapex*, and other members of the *A. atomus* species group including those in the *A. atomus* species complex*, based on DNA sequences of the mitochondrial COI gene. Diagonal element shows intraspecific variation (only when more than one sequence was considered). Average pairwise uncorrected p-distances calculated using MEGA 6.06.

	<i>A. arboridiae</i>	<i>A. japonicus</i>	<i>A. flaviapex</i>	<i>A. atomus</i> *	<i>A. incarnatus</i> §
<i>A. arboridiae</i>	0.000				
<i>A. japonicus</i>	0.050	0.005			
<i>A. flaviapex</i>	0.073	0.074	–		
<i>A. atomus</i> *	0.073	0.071	0.064	0.034	
<i>A. incarnatus</i> §	0.073	0.078	0.082	0.080	–

**Anagrus atomus* species complex as considered by Zanolli et al. (2016) and Nugnes et al. (2017); §outgroup

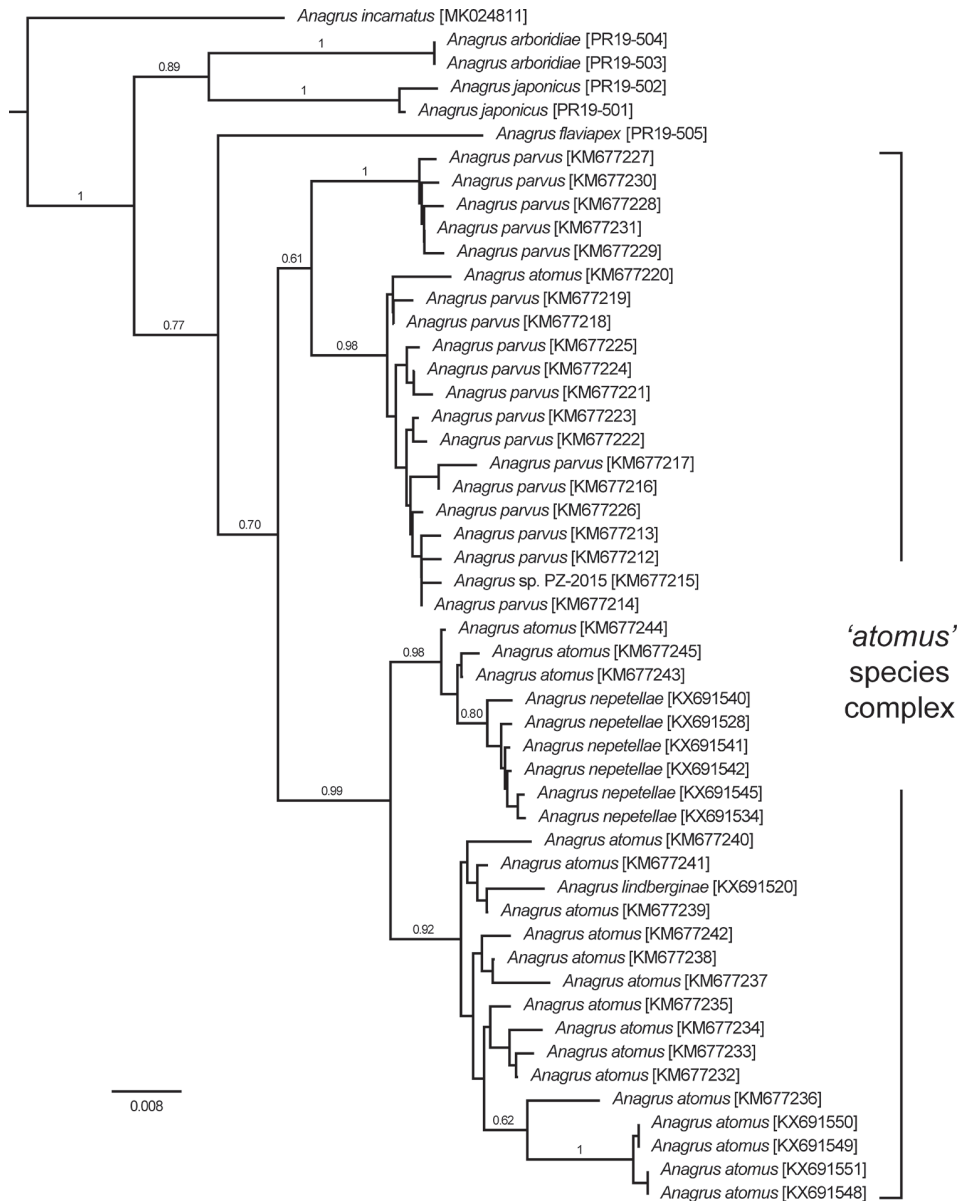


Figure 7. Relationship of *Anagrus arboridiae* sp. nov. with other members of the *A. atomus* species group for which reliable DNA sequences are available, based on a 587 bp fragment of COI. Optimal NJ tree with the sum of branch length = 0.34117946. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches and the tree is drawn to scale, with branch lengths indicating uncorrected p-distance. Analyses conducted in MEGA 6.06.

specimens in this study showed clear interspecific differences between *A. arboridiae*, *A. flaviapex*, and *A. japonicus* in both length (~517, ~591 and ~534bp, respectively) and nucleotide constitution (see GenBank accessions MT414958–MT414966).

Discussion

The results of this study are well within the expected composition of the genera of the parasitoids, as outside of Japan members of both *Anagrus* and *Oligosita* are known to parasitize eggs of other species of *Arboridia*; however, at species level the parasitoids turned out to be mostly different. Besides the above-mentioned *A. turpanicus* (parasitizing eggs of the invasive *A. kakogawana* in Xinjiang, China), which unlike *A. arboridiae* belongs to the *incarnatus* species group of the nominate subgenus of *Anagrus* (Hu and Triapitsyn 2016), *A. (Anagrus) atomus* (L.) from the *atomus* species group of the same subgenus had been recorded in Khorasan Province of Iran from eggs of *Arboridia kermanshah*, along with an *Oligosita* sp. (Triapitsyn 1998), and later was studied by Hesami et al. (2004, 2009). Indeed, *Oligosita pallida* had been previously reported as a very effective natural enemy of the same host leafhopper in the Iranian province of West Azerbaijan in the absence of insecticide treatments (Mostaan and Akbarzadeh 1995). Both *A. atomus* and *O. pallida* were also recorded as egg parasitoids of *A. (Arboridia) adanae* in Turkey (Viggiani 1987; Yiğit and Erkiliç 1987). An *Oligosita* sp. was identified in Turkmenistan from eggs of *Arboridia* sp. on grape (Triapitsyn 1998); it is shown here to belong to *O. pallida* parasitizing eggs of *A. (Arboridia) hussaini*. Because taxonomy of the speciose and very difficult genus *Oligosita* is in flux worldwide, few previous species identifications can be trusted beyond the generic ones and thus most, especially those outside of Europe, need confirmation.

Of note is the fact that in Japan, most of the obtained *Oligosita* spp. from Shimane Prefecture emerged from table grape leaves collected in non-organic vineyards where no *A. arboridiae* were present, whereas in the organic vineyard in the same prefecture *A. arboridiae* was abundant while only one specimen of *O. pallida* was collected. That perhaps could be due to the longer developmental times of *Oligosita* spp. versus *Anagrus* spp. and the fact that the former spin a cocoon at pupal stage and the latter do not (Chiappini 2002), thus presumably making the immature stages of these trichogrammatids more resistant to insecticides. That assumption, however, needs to be further investigated by a thorough experimental work.

These results could be of importance for other agricultural crops as well because *A. apicalis* is also known to feed on very common, aforementioned fruit trees in Japan. Furthermore, knowledge of the egg parasitoids of *Arboridia* spp. in Japan would be important for the potential classical biological control programs against *A. kakogawana* in other countries of Asia and Europe within the Palearctic region, where it has established recently as an invasive pest of grapes (Chireceanu et al. 2019).

Acknowledgments

We thank Dmitry A. Dmitriev (INHS) for identification of the grape leafhoppers, Vladimir V. Berezovskiy (UCRC) for mounting specimens, as well as grape growers in Shimane Pre-

fecture and Yoshitada Katsuki (Katsuki Wines LLC, Kitamata, Aya, Miyazaki Prefecture) for allowing us to collect specimens at their properties. We are also grateful to Toshiharu Mita (ELKU) for the loan of specimens to SVT and Takumi Uesato (Okinawa Prefectural Agricultural Research Center, Makabe, Itoman, Okinawa, Japan) who kindly helped with collecting by a Malaise trap on Okinawa Island. SVT's visit to Japan in October–November 2019 was sponsored by the Short-term Invitational Fellowship for Research in Japan from the Japan Society for the Promotion of Science (2nd Recruitment for 2019).

References

- Adachi-Hagimori T, Triapitsyn SV, Uesato T (unpublished) Egg parasitoids (Hymenoptera: Mymaridae) of *Amrasca biguttula* (Ishida) (Hemiptera: Cicadellidae) from Okinawa Island, a pest of okra in Japan. *Journal of Asia-Pacific Entomology* (submitted, in review).
- Arai T, Toyama M (2018) Occurrence of *Arboridia apicalis* (Nawa) (Hemiptera: Cicadellidae) under reduced insecticide management and reflective sheet mulching in grapevine cultivar 'Shine Muscat'. *Bulletin of the NARO, Fruit Tree and Tea Science* 2: 33–41. [In Japanese]
- Bakkendorf O (1971) Description of *Oligosita tominici* n. sp. [Hym., Trichogrammatidae] and notes of *Anagrus atomus* (L.) and *Anaphes autumnalis* Foerster [Hym. Mymaridae]. *Entomophaga* 16(4): 363–366. <https://doi.org/10.1007/BF02370918>
- Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL (2008) GenBank. *Nucleic Acids Research* 36: D25–D30. <https://doi.org/10.1093/nar/gkm929>
- Chiappini E (2002) *Anagrus* and *Oligosita*: different strategies adopted to develop in the same host egg (Hymenoptera: Mymaridae, Trichogrammatidae). In: Melika G, Thuróczy C (Eds) *Parasitic Wasps: Evolution, Systematics, Biodiversity and Biological Control. International Symposium: "Parasitic Hymenoptera: taxonomy and biological control"* (14–17 May 2001, Kőszeg, Hungary). *Agroinform Kiadó és Nyomda Kft., Budapest*, 261–266.
- Chiappini E, Lin N-q (1998) *Anagrus* (Hymenoptera: Mymaridae) of China, with descriptions of nine new species. *Annals of the Entomological Society of America* 91(5): 549–571. <https://doi.org/10.1093/aesa/91.5.549>
- Chiappini E, Mazzoni E (2000) Differing morphology and ultrastructure of the male copulatory apparatus in species-groups of *Anagrus* Haliday (Hymenoptera: Mymaridae). *Journal of Natural History* 34(8): 1661–1676. <https://doi.org/10.1080/00222930050117549>
- Chiappini E, Triapitsyn SV, Donev A (1996) Key to the Holarctic species of *Anagrus* Haliday (Hymenoptera: Mymaridae) with a review of the Nearctic and Palearctic (other than European) species and descriptions of new taxa. *Journal of Natural History* 30: 551–595. <https://doi.org/10.1080/00222939600770301>
- Chireceanu C, Nedelcea D, Seljak G (2019) First record of the Japanese grape leafhopper *Arboridia kakogawana* (Matsumura, 1932) (Hemiptera: Cicadellidae) from Romania. *Bulletin OEPP/EPPD Bulletin* 49(2): 391–397. <https://doi.org/10.1111/epp.12585>
- Dmitriev DA (2019) 31 Interactive keys and taxonomic databases. <https://doi.org/10.1155/2019/8750643> [last accessed 6 November 2019]

- Dworakowska I (1970) On the genus *Arboridia* Zachv. (Auchenorrhyncha, Cicadellidae, Typhlocybinae). Bulletin de l'Académie Polonaise des Sciences, Série des Sciences Biologiques 18(10): 607–615.
- Gibson GAP (1997) Chapter 2. Morphology and terminology. In: Gibson GAP, Huber JT, Woolley JB (Eds) Annotated Keys to the Genera of Nearctic Chalcidoidea (Hymenoptera). NRC Research Press, Ottawa, 16–44.
- Guglielmino A, Xu Z, Bückle C, Dong W (2012) Description of a new species of *Arboridia* (Hemiptera: Auchenorrhyncha: Cicadellidae: Typhlocybinae) from China. Florida Entomologist 95(4): 877–881. <https://doi.org/10.1653/024.095.0409>
- Hesami S, Seyedoleslami H, Ebadi R (2004) Biology of *Anagrus atomus* (Hym., Mymaridae), an egg parasitoid of the grape leafhopper *Arboridia kermanshah* (Hom., Cicadellidae). Entomological Science 7: 271–276. <https://doi.org/10.1111/j.1479-8298.2004.00073.x>
- Hesami S, Seyedoleslami H, Hatami B (2009) Impact of overwintering refugia of *Anagrus atomus* on egg parasitism of grape leafhopper, *Arboridia kermanshah*. Plant Protection Journal, Islamic Azad University, Shiraz Branch 1(1): 94–106.
- Hu H-y, Triapitsyn SV (2016) *Anagrus turpanicus* sp. n. (Hymenoptera: Mymaridae) from China, an egg parasitoid of *Arboridia kakogawana* [sic] (Hemiptera: Cicadellidae). Zootaxa 4161(4): 573–578. <https://doi.org/10.11646/zootaxa.4161.4.9>
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30: 772–780. <https://doi.org/10.1093/molbev/mst010>
- Kryger JP (1919 [1918]) The European Trichogramminae. Entomologiske Meddelelser 12(2): 257–354.
- Li Q, Hu H, Triapitsyn SV, Yi L, Lu J (2018) *Anagrus dmitrievi* sp. n. (Hymenoptera, Mymaridae), an egg parasitoid of *Zyginidia eremita* (Hemiptera, Cicadellidae), a pest of maize in Xinjiang, China. ZooKeys 736: 43–57. <https://doi.org/10.3897/zookeys.736.20883>
- Liu S, Li C (2019) A new species, first report of a male and new distributional records for five species of *Oligosita* Walker (Hymenoptera: Trichogrammatidae) from China. Journal of Forestry Research 30(3): 1123–1130. <https://doi.org/10.1007/s11676-018-0749-8>
- Matsumura S (1932) A revision of the Palaearctic and Oriental Typhlocybid-genera with descriptions of new species and new genera. Insecta Matsumurana 6(3): 93–120.
- Mostaan M, Akbarzadeh G (1995) Studies on the egg parasitoid of the Grapevine leafhopper (*Arboridia kermanshah* Dlab.). In: Wajnberg E (Ed.) *Trichogramma* and Other Egg Parasitoids: Proceedings of the 4th International Symposium held in Cairo (Egypt), October 4–7, 1994. INRA Ed., Paris (Les Colloques, n°73), 201–202.
- Noyes JS (2019) Universal Chalcidoidea database. World Wide Web electronic publication, the Natural History Museum, London. <http://www.nhm.ac.uk/chalcidoids> [last accessed 11 November 2019]
- Nugnes F, Bernardo U, Viggiani G (2017) An integrative approach to species discrimination in the *Anagrus atomus* group *sensu stricto* (Hymenoptera: Mymaridae), with a description of a new species. Systematics and Biodiversity 15(6): 582–599. <https://doi.org/10.1080/14772000.2017.1299811>

- Oh S, Choe K-R, Jung S (2015) Two new species of the genus *Arboridia* Zachvatkin (Hemiptera: Auchenorrhyncha: Cicadellidae: Typhlocybinae) from Korea. *Zootaxa* 3918(3): 446–450. <https://doi.org/10.11646/zootaxa.3918.3.9>
- Sakagami Y (2003) Grape [plate]. In: Sakagami Y, Kudo A (Eds) A handbook of diseases and insect pests of fruit trees (Vol. 2). Pears, grape, persimmon, chestnut and fig (Revised Edition). Japan Plant Protection Association, Komagome-1-chome, Toshima-ku, Tokyo, 103 pp. [In Japanese]
- Song Y-H, Li Z-Z (2013) Some new species and new record of the genus *Arboridia* Zachvatkin (Hemiptera: Cicadellidae: Typhlocybinae) from six provinces of China. *Zootaxa* 3613(3): 229–244. <https://doi.org/10.11646/zootaxa.3613.3.2>
- Song Y-H, Li Z-Z (2015) Review of the genus *Arboridia* Zachvatkin (Hemiptera: Cicadellidae: Typhlocybinae), with description of two new species from China. *Zootaxa* 3990(4): 584–592. <https://doi.org/10.11646/zootaxa.3990.4.7>
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* 30: 2725–2729. <https://doi.org/10.1093/molbev/mst197>
- Tanaka F, Kondo A, Henmi T (1986) Catches of the grape leafhopper, *Arboridia apicalis* Nawa (Homoptera: Cicadellidae), on yellow cylindrical sticky trap. *Japanese Journal of Applied Entomology and Zoology* 30(4): 305–307. [In Japanese] <https://doi.org/10.1303/jjaez.30.305>
- Triapitsyn SV (1998) *Anagrus* (Hymenoptera: Mymaridae) egg parasitoids of *Erythroneura* spp. and other leafhoppers (Homoptera: Cicadellidae) in North American vineyards and orchards: a taxonomic review. *Transactions of the American Entomological Society* 124(2): 77–112.
- Triapitsyn SV (1999) Description of the male of *Anagrus flaviapex* (Hymenoptera: Mymaridae), with new distribution and host records. *Entomological News* 110(4): 237–239.
- Triapitsyn SV (2015) Taxonomy of the genus *Anagrus* Haliday (Hymenoptera: Mymaridae) of the world: an annotated key to the described species, discussion of the remaining problems, and a checklist. *Acta Zoológica Lilloana* 59: 3–50.
- Triapitsyn SV (2018) Review of the Palearctic *Aphelinoidea* (Hymenoptera: Trichogrammatidae), with focus on the species described by Ś. Nowicki. *Israel Journal of Entomology* 48(2): 33–81.
- Triapitsyn SV, Adachi-Hagimori T, Rugman-Jones PF, Barry A, Abe A, Matsuo K, Ohno K (2019[a]) Egg parasitoids of the tea green leafhopper *Empoasca onukii* (Hemiptera, Cicadellidae) in Japan, with description of a new species of *Anagrus* (Hymenoptera, Mymaridae). *ZooKeys* 836: 93–112. <https://doi.org/10.3897/zookeys.836.32634>
- Triapitsyn SV, Rugman-Jones PF, Tretiakov PS, Luft Albarracin E, Moya-Raygoza G, Querino RB (2019[b]) Molecular, morphological and biological differentiation between *Anagrus virilai* sp. n., an egg parasitoid of the corn leafhopper *Dalbulus maidis* (Hemiptera: Cicadellidae) in the New World, and *Anagrus incarnatus* from the Palaearctic region (Hymenoptera: Mymaridae). *Neotropical Entomology* 48(1): 87–97. <https://doi.org/10.1007/s13744-018-0606-7>

- Triapitsyn SV, Rugman-Jones PF, Tretiakov PS, Shih H-T, Huang S-H (2018) New synonymies in the *Anagrus incarnatus* Haliday ‘species complex’ (Hymenoptera: Mymaridae) including a common parasitoid of economically important planthopper (Hemiptera: Delphacidae) pests of rice in Asia. *Journal of Natural History* 52(43–44): 2795–2822. <https://doi.org/10.1080/00222933.2018.1552333>
- Truett GE, Heeger P, Mynatt RL, Truett AA, Walker JA, Warman ML (2000) Preparation of PCR Quality Mouse Genomic DNA with Hot Sodium Hydroxide and Tris (HotSHOT). *BioTechniques* 29: 52–54. <https://doi.org/10.2144/00291bm09>
- Viggiani G (1987) Notes on *Oligosita pallida* Kryger and on other species of the group *minima* Girault (Hymenoptera: Trichogrammatidae). *Redia* 70: 543–547.
- Yamada K (2003) Grape. In: Sakagami Y, Kudo A (Eds) *A Handbook of Diseases and Insect Pests of Fruit Trees* (Vol. 2). Pears, Grape, Persimmon, Chestnut and Fig (Revised Edition). Japan Plant Protection Association, Komagome-1-chome, Toshima-ku, Tokyo, 105 pp. [In Japanese]
- Yiğit A, Erkişiç L (1987) Studies on egg-parasitoids of grape leafhopper, *Arboridia adanae* Dlab. (Hom.: Cicadellidae), injurious in Southern Anatolia vineyards. In: *Proceedings of the First Turkish National Congress of Entomology* (13–16 October 1987, Izmir), 35–42. [In Turkish]
- Zanolli P, Martini M, Mazzon L, Pavan F (2016) Morphological and molecular identification of *Anagrus* ‘*atomus*’ group (Hymenoptera: Mymaridae) individuals from different geographic areas and plant hosts in Europe. *Journal of Insect Science* 16(1): 38, 1–14. <https://doi.org/10.1093/jisesa/iew017>

A new species of *Queskallion* Smetana, 2015 (Coleoptera, Staphylinidae, Quediina) from China

Yanpeng Cai¹, Xiaoyan Li⁴, Hongzhang Zhou^{2,3}

1 Morphological Laboratory, Guizhou University of Traditional Chinese Medicine, Guiyang, 550025, Guizhou, China **2** Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences, 1 Beichen West Rd., Chaoyang District, Beijing 100101, China **3** University of the Chinese Academy of Sciences, 19A Yuquan Rd., Shijingshan District, Beijing, 100049, China **4** Hebei Key Laboratory of Animal Diversity, Langfang Normal University Aiminxidao 100, Anci Area, Langfang 065000, Hebei Province, China

Corresponding author: Hongzhang Zhou (zhouhz@ioz.ac.cn)

Academic editor: A. Brunke | Received 21 April 2020 | Accepted 20 May 2020 | Published 3 July 2020

<http://zoobank.org/8209A13B-1E39-40DF-867D-3DA7DCB39248>

Citation: Cai Y, Li X, Zhou H (2020) A new species of *Queskallion* Smetana, 2015 (Coleoptera, Staphylinidae, Quediina) from China. ZooKeys 945: 153–161. <https://doi.org/10.3897/zookeys.945.53480>

Abstract

A new species, *Queskallion saetosum* **sp. nov.**, is described herein from Sichuan Province, China. It is diagnosed from a closely related species, *Q. tangi* Smetana, 2015. Color images and line drawings of the adult of the new species, as well as its genitalia are provided. In addition, a checklist of species, an updated key to species and a geographical distribution map of all known species in the genus *Queskallion* Smetana are included.

Keywords

Checklist, key, morphology, rove beetle, taxonomy

Introduction

The genus *Queskallion* was established by Smetana (2015) in the subtribe Quediina sensu stricto (Brunke et al. 2016), with *Queskallion tangi* Smetana, 2015 as the type species by original designation. It is a small genus including only five species, known from China, Myanmar and Nepal.

The genus *Queskallion* is mainly characterized by the second and third segments of the antenna. In addition to the usual long setae, they have numerous short setae and the surface between the setae is slightly granulose, not quite shiny, therefore visually not obviously contrasting with the dull granulose surface of the following segments bearing dense appressed pubescence; the pronotum with three to five additional setiferous punctures on the posterior lateral area well behind the large lateral puncture; and the surface of the elytra with characteristic semigranulose microsculpture giving it a greasy appearance (Smetana 2015).

Examination of rove beetle specimens collected from Sichuan Province uncovered the new species. This study aims to describe the new species, distinguish it from congeners and provide up-to-date information on the distribution of the genus (Fig. 3).

Material and methods

Specimens were relaxed in warm water (60 °C) for 5–8 hours for dissection of the abdominal segments VIII–X and the genitalia. After examination, the dissected body parts were glued back to the mounting cards for future study. Observation, dissection and measurements were performed using a stereo microscope (Zeiss SteREO Discovery V20). Images of the adult and genitalia were captured with an AxioCam MRc 5 camera attached to a Zeiss Axio Zoom V16 Fluorescence Stereo Zoom Microscope, and photomontage was performed in Zen 2012 (blue edition) imaging software (<https://www.zeiss.com.cn/microscopy/products/microscope-software/zen.html>). Inkscape V0.91 was used to make the line drawings. The abdominal tergites and sternites were entirely flattened for the line drawings to make the illustrations more distinguishable among species. Species distribution data were compiled within Microsoft Excel using both published records and specimen label data. The distribution map was produced with the aid of DIVA-GIS 7.5 (Hijmans et al. 2011).

The specimens examined, including types, were deposited in the Institute of Zoology, Chinese Academy of Sciences (IZ-CAS).

Morphological terminology followed Smetana and Davies (2000) and Smetana (2015).

The following abbreviations are used in the text:

BL	body length (from apex of clypeus to apex of abdominal tergite VIII);
BW	body width (maximal body width, usually equal to EW);
HL	head length (from base of clypeus to neck constriction);
HW	head width (maximal head width, including eyes);
PL	pronotal length (along midline of pronotum);
PW	pronotal width (maximal pronotal width);
EL	elytral length (maximal elytral length);
EW	elytral width (maximal elytral width);
ESL	elytral suture length (from apex of scutellum to apex of elytral suture);
AW	abdominal width (maximal width of abdomen);
HEL	(head) eye length;
HTL	(head) temporal length.

Taxonomy

***Queskallion* Smetana, 2015: 399.**

Type species. *Queskallion tangi* Smetana, 2015, by original designation.

Checklist of *Queskallion* Smetana species

Queskallion dispersepunctatum (Scheerpeltz, 1965: 209)

Distribution: China (Yunnan Province), Myanmar, Nepal.

Queskallion montanum Smetana, 2015: 410

Distribution: China (Gansu and Sichuan Provinces).

Queskallion saetosum sp. nov.

Distribution: China (Sichuan Province).

Queskallion schuelkei Smetana, 2015: 408

Distribution: China (Yunnan Province).

Queskallion seronatum Smetana, 2015: 411

Distribution: Nepal.

Queskallion tangi Smetana, 2015: 407

Distribution: China (Xizang Autonomous Region).

Key to the species of *Queskallion* Smetana

(updated from Smetana (2015) to include the new species)

- 1 Last puncture of dorsal rows on pronotum shifted considerably posteriad toward posterior third of pronotal length, each dorsal row with four punctures. Paramere of aedeagus quite narrow and elongate; sensory peg setae on underside not numerous, most situated near apex of paramere..... ***Q. dispersepunctatum* (Scheerpeltz)**
- Last puncture of dorsal rows on pronotum not shifted posteriad, situated before or at middle of pronotal length, each dorsal row with three punctures. Paramere of aedeagus of different shape, in general much wider, sensory peg setae on underside numerous and located differently..... **2**
- 2 Sensory peg setae on underside of paramere arranged into groups, forming certain characteristic figures **3**
- Sensory peg setae on underside of paramere widely spread over most of the fusiform part of paramere, not forming any kind of discernible figure **4**
- 3 Paramere of aedeagus slightly narrower than median lobe (Fig. 2G), underside with sensory peg setae arranged into one characteristic inversed Y-shaped figure (Figs 1D, 2E). Female tergite X with apical margin forming distinctive M-shaped indentation (Fig. 2H)..... ***Q. saetosum* sp. nov.**
- Paramere of aedeagus with subapical portion slightly dilated laterally, becoming wider than median lobe, underside with sensory peg setae arranged into

- two S-like figures. Female tergite X with apical margin deeply and arcuately emarginated *Q. tangi* Smetana
- 4 Apical portion of median lobe of aedeagus parallel-sided with apex slightly emarginated, aedeagus in general relatively short *Q. schuelkei* Smetana
- Apical portion of median lobe of aedeagus at least slightly narrowed toward arcuate apex, aedeagus in general longer 5
- 5 Aedeagus in general quite narrow, elongate, apical portion of paramere moderately dilated, elongate-oval in shape *Q. seronatum* Smetana
- Aedeagus in general broader, less elongate, apical portion of paramere markedly dilated, broadly-oval in shape *Q. montanum* Smetana

***Queskallion saetosum* sp. nov.**

<http://zoobank.org/DA7B0E5D-4B45-4BC4-AF10-1CCE5D81AA02>

Figures 1, 2

Material examined. *Holotype*: CHINA • ♂; Sichuan Province, Baoxing County, Longdong, Ruobigou; alt. 1600 m; 10 August 2003; Xiaodong Yu (IZ-CAS) leg. *Paratypes*: 3 ♂♂, 1 ♀; same locality as holotype; 10–13 August 2003; Xiaodong Yu (IZ-CAS) leg.

Diagnosis. This new species is very similar to *Q. tangi* Smetana in all characters, but it can be distinguished from the latter by having the male paramere of the aedeagus slightly narrower than the median lobe, underside with sensory peg setae arranged into one characteristic inversed Y-shaped figure, female tergite X with apical margin forming distinctive M-shaped indentation; whereas the latter has male paramere of aedeagus with subapical portion slightly dilated laterally becoming wider than median lobe, underside with sensory peg setae arranged into two S-like figures, female tergite X with apical margin deeply and arcuately emarginated.

Description. Head dark brown to blackish brown; pronotum, scutellum and elytra dark brown; abdomen dark brown, each tergite with posterior margin slightly paler; head, pronotum and abdomen strongly iridescent; antennae dark brown, labrum yellowish-brown, mandibles dark brown, maxillary and labial palpi dark brown; legs dark brown.

BL = 8.5 mm, BW = 1.7 mm, HL/PL/EL = 1.00: 1.52: 1.80, HW/PW/EW/AW = 1.00: 1.60: 1.84: 1.55.

Head (Fig. 1A) obtusely quadrangular, nearly as wide as long, HW/HL = 1.05; eye moderately large and slightly convex, in dorsal view tempora shorter than length of eye, gradually narrowed posteriad, HEL/HTL = 1.50; no additional setiferous punctures between anterior frontal setiferous punctures; posterior frontal setiferous puncture situated distinctly behind level of posteriomedial margin of eye, about midway between posteriomedial margin of eye and nuchal constriction of head; temporal setiferous puncture situated closer to posterior margin of eye than to nuchal constriction, with some small setiferous punctures behind and below it; one basal setiferous puncture situated closer to nuchal constriction than to posterior frontal setiferous puncture; head with very fine and dense microsculpture of transverse waves and meshes. Antenna

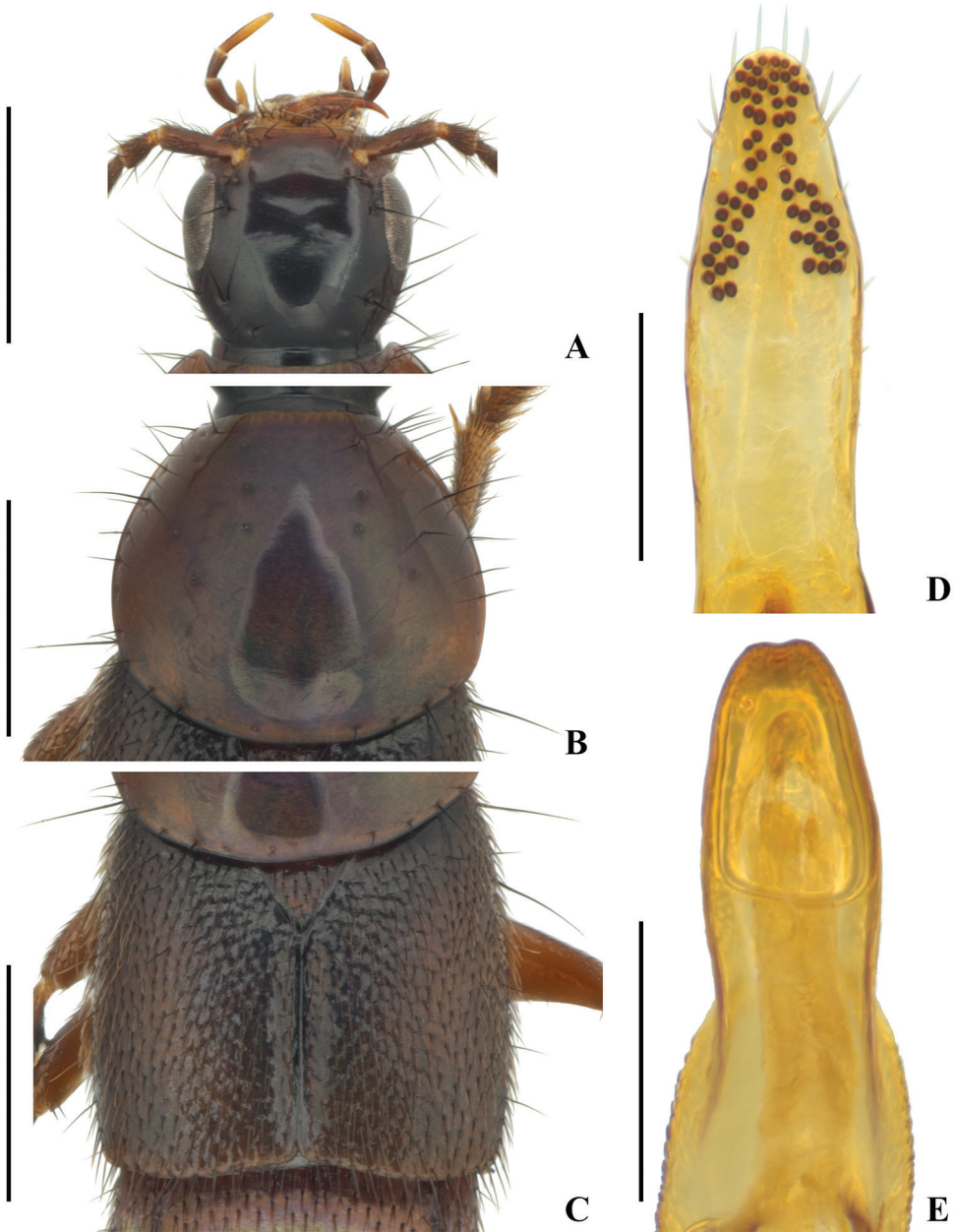


Figure 1. *Q. saetosum* sp. nov., morphology **A** head **B** pronotum **C** elytra **D** underside of paramere **E** apical portion of median lobe, parameral view. Scale bars: 1 mm (**A–C**); 0.20 mm (**D, E**).

moderately long, with segment I longer than segment II or III, segment III longer than segment II, segments IV–XI slightly longer than wide.

Pronotum (Fig. 1B) large and broad, wider than long, $PW/PL = 1.10$, distinctly narrowed anteriorly, posteriolateral and posterior margins continuously and broadly

rounded, lateral margins not explanate; three setiferous punctures in each dorsal and sublateral row, one additional setiferous puncture situated between each dorsal and sublateral row, distinctly behind level of last dorsal and sublateral row puncture, last sublateral row puncture situated behind level of large lateral setiferous puncture; one smaller additional setiferous puncture before each large lateral puncture; surface of pronotum with microsculpture similar to that of head, but even finer.

Scutellum (Fig. 1C) with dense setiferous punctures, surface with very fine and dense microsculpture of transverse waves.

Elytra (Fig. 1C) broad, slightly wider than long, $EW/EL = 1.08$, $ESL/EL = 0.52$, nearly parallel-sided laterally, each elytron with surface covered with dense setiferous punctures, transverse interspaces between punctures about as wide as diameter of punctures; surface between punctures with semigranulose microsculpture. Wings fully developed.

Abdominal tergite II finely punctate; setiferous punctures of other tergites finer and sparser than those of elytra, distinctly becoming sparser toward posterior margin of each tergite, and generally becoming so toward apex of abdomen; tergite VII with whitish apical seam of palisade setae.

Male with first four segments of foretarsus moderately dilated, sub-bilobed, each heavily covered with tenent setae ventrally, segment II slightly narrower than apex of tibia. Tergite VIII with basal ridge complete, slightly arched backward in middle, surface without long seta; sternite VIII (Fig. 2A) with basal ridge complete, slightly sinuate, with two long setae on each side, apical margin with very shallow and narrow medioapical emargination, a very small triangular area in front of the emargination impunctate; sternite IX (Fig. 2B) with basal portion long and curved, with a moderately deep arcuate medioapical emargination, with two long setae on each side of the emargination apically; tergite X (Fig. 2C) with basal side broadly and deeply concave, apical margin rounded, vaguely protruded. Aedeagus in lateral view (Fig. 2F) with apex of paramere not quite reaching that of median lobe, median lobe bent toward parameral side, without any process at apex; in parameral view (Fig. 2G) with paramere slightly narrower than median lobe, apical $1/4$ gradually narrowed, forming rounded apex, median lobe wide at base, slightly narrowed in middle, distinctly constricted at about apical $1/5$, apex subtruncate, with an inconspicuous medioapical emargination (Figs 1E, 2D); apical portion of paramere with four moderately long apical setae, and two similar subapical setae on each lateral side below apex, underside with numerous sensory peg setae arranged into one characteristic inversed Y-shaped figure at apex (Figs 1D, 2E).

Female first four segments of fore tarsus similar to those of male, but less dilated; sternite VIII with basal ridge inconspicuous, with 2 long setae on each side; tergite X (Fig. 2H) with basal side broadly and deeply concave, with subtriangular area in middle more strongly sclerotized and pigmented, apical margin incomplete, forming distinctive M-shaped indentation.

Distribution. *Queskallion saetosum* sp. nov. is at present known only from the type locality in central Sichuan Province (Fig. 3), China, at an altitude of 1600 m. The specimens were collected from stacks of withered grass on the roadside in August.

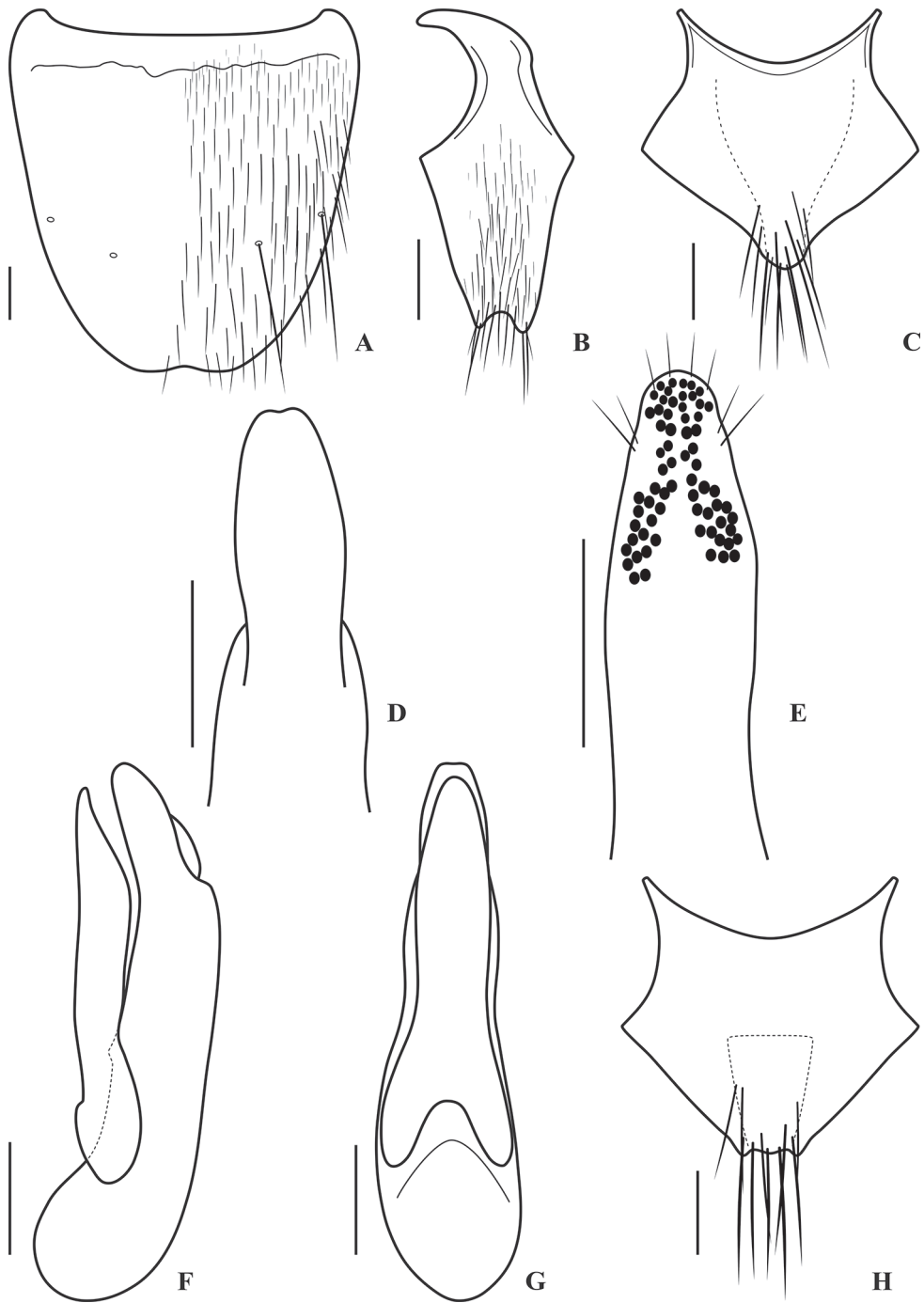


Figure 2. *Q. saetosum* sp. nov., morphology **A** male sternite VIII **B** male sternite IX **C** male tergite X **D** apical portion of median lobe, parameral view **E** underside of paramere **F** aedeagus, lateral view **G** aedeagus, parameral view **H** female tergite X. Scale bars: 0.20 mm.

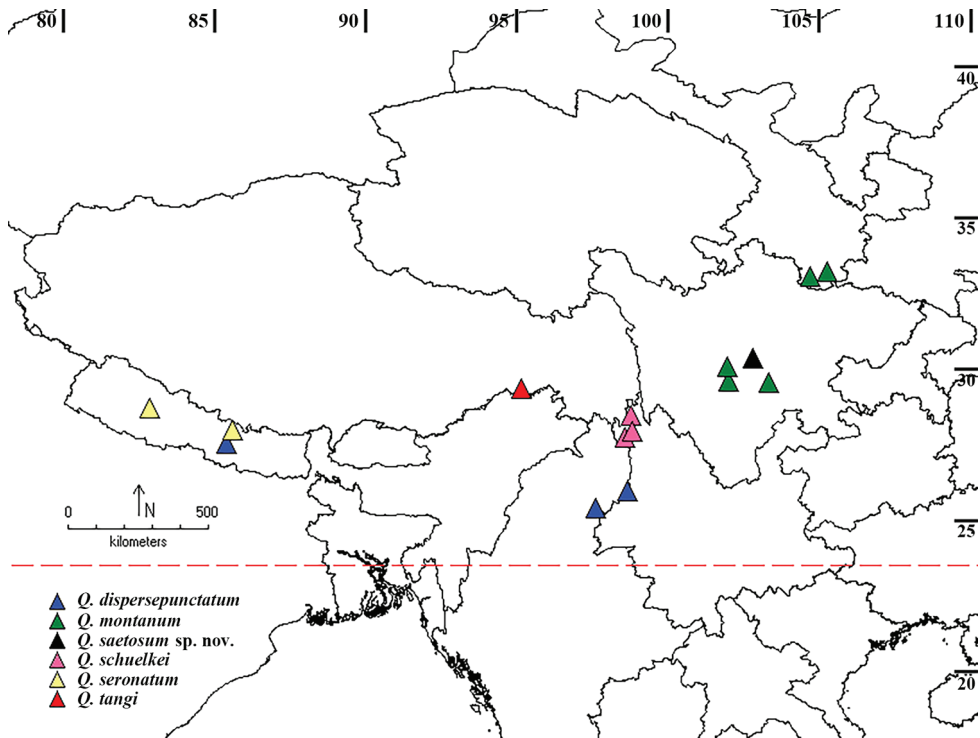


Figure 3. Known distribution of *Queskallion* species in China, Myanmar and Nepal.

Etymology. The specific name is from the Latin adjective *saetosus*, *-a*, *-um* (bristly), referring to the additional setae on the pronotum.

Acknowledgements

We want to thank the editors and the reviewers for reviewing this manuscript and giving a lot of constructive suggestions, and also, we are very grateful to Dr Xiaodong Yu (IZ-CAS) for the field investigation and the collection of the type materials of this new species. This project was supported by the National Natural Science Foundation of China (No. 31760629), Guizhou Provincial Department of Education Youth Science and Technology Talent Growth Project (黔教合KY字[2017]175) and the Natural Science Foundation of Hebei Province (C2019408016).

References

Brunke A, Chatzimanolis S, Schillhammer H, Solodovnikov A (2016) Early evolution of the hyperdiverse rove beetle tribe Staphylinini (Coleoptera: Staphylinidae: Staphylininae) and

- a revision of its higher classification. *Cladistics* 32(4): 427–451. <https://doi.org/10.1111/cla.12139>
- Hijmans RJ, Guarino L, Rojas E (2011) DIVA-GIS, version 7.5. A geographical information system for the analysis of biodiversity data. Available from: <http://www.diva-gis.org> [Accessed 18 January 2020]
- Scheerpeltz O (1965) Wissenschaftliche Ergebnisse der Schwedischen Expedition 1934 nach Indien and Burma. Coleoptera, Staphylinidae (except Megalopsidiinae et Steninae). *Arkiv för Zoologi* 17(2): 93–371.
- Smetana A (2015) Contributions to the knowledge of the Quediina (Coleoptera: Staphylinidae: Staphylinini) of China. Part 55. Genus *Queskallion* gen. nov. *Studies and Reports Taxonomical Series* 11(2): 99–419.
- Smetana A, Davies A (2000) Reclassification of the north temperate taxa associated with *Staphylinus* sensu lato, including comments on relevant subtribes of Staphylinini (Coleoptera: Staphylinidae). *American Museum Novitates* 3287: 1–88. [https://doi.org/10.1206/0003-0082\(2000\)287<0001:ROTNNT>2.0.CO;2](https://doi.org/10.1206/0003-0082(2000)287<0001:ROTNNT>2.0.CO;2)

Distribution of trichodorid species in mainland China with description of *Trichodorus hangzhouensis* sp. nov. (Nematoda, Triplonchida)

Xuqing Li^{1,2}, Munawar Maria¹, Ruihang Cai¹, Eda Marie Barsalote¹,
Vlada Peneva³, Jingwu Zheng^{1,4}

1 Laboratory of Plant Nematology, Institute of Biotechnology, College of Agriculture & Biotechnology, Zhejiang University, Hangzhou 310058, Zhejiang, China **2** Hangzhou Academy of Agricultural Sciences, Hangzhou 310024, China **3** Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Gagarin Street, 1113 Sofia, Bulgaria **4** Key Lab of Molecular Biology of Crop Pathogens and Insects, Ministry of Agriculture, Hangzhou 310058, China

Corresponding author: Jingwu Zheng (jwzheng@zju.edu.cn)

Academic editor: Sergei Subbotin | Received 24 January 2020 | Accepted 29 March 2020 | Published 3 July 2020

<http://zoobank.org/2823D647-6277-4F58-833B-F71E5A7304D2>

Citation: Li X, Maria M, Cai R, Barsalote EM, Peneva V, Zheng J (2020) Distribution of trichodorid species in mainland China with description of *Trichodorus hangzhouensis* sp. nov. (Nematoda, Triplonchida). ZooKeys 945: 163–189. <https://doi.org/10.3897/zookeys.945.50424>

Abstract

Seven trichodorid species including a new one (*Trichodorus hangzhouensis* sp. nov., *T. nanjingensis*, *T. pakistanensis*, *T. cedarus*, *Paratrichodorus porosus*, *Nanidorus renifer* and *N. minor*) were recovered from the rhizosphere of different hosts in 13 provinces of China. Each of the recovered species was characterized based on morphology and molecular data using rRNA gene sequences. *Trichodorus hangzhouensis* sp. nov. is characterized by its males having medium-sized onchiostyle (46–49 µm) and three ventromedian cervical papillae (CP) anterior to the secretory-excretory (S-E) pore, CP1 located opposite the anterior part of isthmus, S-E pore opposite the isthmus or anterior end of pharyngeal bulb, spicules slightly ventrally curved, relatively small, 33.2 (32.0–34.5) µm long, wider slightly marked capitulum, lamina partially striated without bristles at striation; and females having rounded triangular sclerotized vaginal pieces with tips directed towards vulva, 1.5–2.0 µm sized, at about 1 µm apart, vulva pore-like in ventral view. Phylogenetic analysis based on D2-D3 28S rRNA gene sequences differentiated the new species among *Trichodorus* species from Europe, Asia and USA which formed a large clade. A review of the distribution of *Trichodorus*, *Nanidorus* and *Paratrichodorus* species revealed that *T. cedarus*, *T. nanjingensis*, *T. pakistanensis* and *P. porosus* are the most widespread species recorded from different provinces of China. This is the first extensive study of trichodorid species occurring in China.

Keywords

18S rRNA gene, D2-D3 28S rRNA gene, ITS2 region, host association, morphology, new species, phylogeny, taxonomy, Trichodoridae

Introduction

Stubby root nematodes of the family Trichodoridae Thorne, 1935 are polyphagous root ectoparasites and have a global distribution (Decraemer and Robbins 2007). They cause damage to a wide range of crops and natural vegetation by directly feeding on root hairs and epidermal cells. Additionally, some of the species have the ability to transmit plant pathogenic viruses (e.g., Tobacco Rattle Virus (TRV), Pea Early Browning Virus (PBV) and Pepper Rinspot Virus (PRV)) which has stimulated experts to focus on the family Trichodoridae (MacFarlane et al. 2002, Decraemer et al. 2013, 2019). Currently, the family Trichodoridae contains 117 species and six genera (Xu and Zhao 2019, Decraemer et al. 2019). The three didelphic genera (*Trichodorus* Cobb, 1913 (67 spp.), *Paratrichodorus* Siddiqi, 1974 (29 spp.), and *Nanidorus* Siddiqi, 1974 (6 spp.) contain the virus vector species while the rest three monodelphic genera (*Monotrichodorus* Andr  ssy 1980 (5 spp.), *Allotrichodorus* Rodriguez-M, Sher & Siddiqi, 1978 (8 spp.), and *Ecuadorus* Siddiqi, 2002 (2 spp.) contain few species which are not considered as potential pathogens (Decraemer et al. 2019, Subbotin et al. 2019).

Previously, morphological identification alone rendered difficulties due to the mixed species complexes, phenotypic variation (such as shape of some sclerotized structures, e.g., stylet, male spicules, vagina with its sclerotized pieces), overlapping diagnostic characters and uniformity in general appearance; however, DNA-based strategies have made it possible to overcome the limitations of the morphological approach only and provided useful insights into trichodorid taxonomy (Subbotin et al. 2019). In recent years several nematologists have successfully applied rRNA genes sequence (18S, 28S, and ITS) analyses for studying the phylogenetic relationships of trichodorid species (Duarte et al. 2010, Zhao et al. 2013, Pedram et al. 2015, 2017, Decraemer et al. 2019). The importance of sequence-based studies for species identification and the lack of molecular data of known species from China has led us to compile a detailed report of trichodorids occurring in the country.

The agricultural land of China represents 10% of the total arable land in the world. About 75% of the lands are cultivated areas used for food production such as rice, wheat, potatoes, tea, soybean, various fruits, tea and sugarcane (Guo 2008). Over the past years, there have been preliminary surveys of trichodorid nematodes in China associated with some of the above-mentioned crops, however many of the occurrence records of these nematodes were incomplete or overlooked. Yin and Feng (1981) reported *Trichodorus* species from the southern provinces of Guangdong and Hunan, however, the first official trichodorid survey started with Liu and Cheng (1990). Gradually, several studies on the distribution of trichodorids in China have been published

with records from Fujian, Yunnan, Zhejiang and Guangdong provinces reporting the occurrence of six *Trichodorus*, two *Nanidorus* and two *Paratrichodorus* species (Xu and Decraemer 1995, Zheng et al. 2004, Zhao et al. 2005, Chi et al. 2011).

Considering the potential importance of trichodorids in China, an extensive survey of various biotopes was carried out during the recent years. The objectives of the present study are to: i) characterize morphologically and molecularly recovered trichodorid species including one new *Trichodorus* species; ii) evaluate the phylogenetic relationships of the new species with other members of the genera based on their 18S rRNA, D2-D3 expansion domain of 28S rRNA and ITS2 of rRNA gene sequences and iii) summarize the geographic distribution of *Trichodorus*, *Nanidorus* and *Paratrichodorus* species in China, in addition, providing a comprehensive list of the past records and present findings of trichodorid nematodes.

Materials and methods

Soil sampling, nematode extraction, and morphological identification

Two-thousand and fifty-two soil samples have been collected from 13 provinces of China. Nematodes were extracted from soil samples using a modified Baermann funnel method and modified Cobb' sieving and flotation-centrifugation method (Jenkins 1964). For morphological studies, nematodes were killed by hot formalin solution and processed to glycerine according to Seinhorst (1959) as modified by De Grisse (1969). Morphological observation, measurements, and photomicrographs were made using a Leica CTR 5000 compound microscope with differential interference contrast (DIC). Measurements were expressed as mean $\pm$ standard deviation (range). Species diagnoses were made following the polytomous key of Decraemer and Baujard (1998). Original descriptions were used for species added to trichodorid genera.

DNA Extraction, PCR, and sequencing

DNA was extracted from single specimens of all seven recovered species as described by Zheng et al. (2003). Four sets of primers (synthesized by Invitrogen, Shanghai, China) were used in the PCR analyses to amplify the partial 18S, D2-D3 region of 28S and ITS2 region of rRNA gene. The 18S rRNA gene was amplified with the forward primer A (5'-AAA GAT TAA GCC ATG CAT G-3') (Boutsika et al. 2004) and the reverse primer S3 (5'-AGT CAA ATT AAG CCG CAG-3') (Waite et al. 2003). The D2-D3 region of 28S rDNA gene was amplified with the forward primer D2A (5'-ACA AGT ACC GTG AGG GAA AGT TG-3') and the reverse primer D3B (5'-TCG GAA GGA ACC AGC TAC TA-3') (De Ley et al. 1999). The ITS2 region was amplified as two partially overlapping fragments, for the first fragment, the forward primer 18S (5'-TTG ATT ACG TCC CTG CCC TTT-3') (Vrain et al. 1992) and the reverse primer ITS2B (5'-GCT GCG TTC TTC ATC GAT-3') (Boutsika et al. 2004) were used, and

for the second fragment, the forward primer ITSA (5'-ATC GAT GAA GAA CGC AGC-3') (Boutsika et al. 2004) and the reverse primer 26S (5'-TTT CAC TCG CCG TTA CTA AGG-3') (Vrain et al. 1992) were used. PCR conditions were as described by Ye et al. (2007). PCR products were separated on 1.5% agarose gels and visualized by staining with ethidium bromide. PCR products of sufficiently high quality were purified for cloning and sequencing by Invitrogen, Shanghai, China.

Phylogenetic analyses

The partial sequences of 18S, D2–D3 fragment of 28S and ITS2 of rRNA gene of *Trichodorus hangzhouensis* sp. nov. were compared with those of other species of fam. Trichodoridae available in GenBank using the BLAST homology search program. The sequence data sets used in this study were selected based on previously published studies (Zeng et al. 2014, Decraemer et al. 2019, Subbotin et al. 2019) and were used in phylogenetic analyses. Three separate 18S, 28S and ITS2 datasets were prepared. Multiple sequence alignment of each dataset was made using the Q-INS-i algorithm of MAFFT V.7.205 (Katoh and Standley 2013). The sequence alignments were edited by BioEdit (Hall 1999). The best fitted model of DNA evolution was obtained using jModelTest V.2.1.7 (Darriba et al. 2012) with the Akaike information criterion (AIC). General time-reversible model with invariable sites and a gamma-shaped distribution (GTR + I + G) was used for the 28S, 18S and ITS2 rRNA genes to reconstruct the phylogenies. Bayesian analysis was used to infer a phylogenetic tree by MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003).

Model parameters were unlinked and the overall rate was allowed to vary across partitions. The number of generations for the total analysis was set to 10 million, with the chain sampled every 1000 generations and the burn-in value was 25%. The Markov chain Monte Carlo method within a Bayesian framework was used to estimate the posterior probabilities of the phylogenetic trees using the 50% majority rule (Larget and Simon 1999). Posterior probabilities (PP) were given on appropriate clades. The consensus trees were visualized using FigTree V1.4.3 (Stöver and Müller 2010).

Taxonomy

Trichodoridae Thorne, 1935

Trichodorus hangzhouensis sp. nov.

<http://zoobank.org/F0E3BA04-6CB9-4333-9477-1E2DCB844DB7>

Description (Figs 1–3, For measurements see Table 1). **Male.** Body cylindrical with posterior end slightly curved ventrally. Cuticle slightly swollen upon fixation, 2.0–2.5 µm thick at mid-body. Lip region dome-shaped with double papillae (composed of outer labial and cephalic papillae). Amphidial aperture post-labial, slit-like, amphidial

fovea cup-shaped. Stoma narrow, refractive strengthening rods 4–5 μm long. Nerve ring surrounding the anterior part of isthmus. Slender mid part of pharynx gradually widening to form a pharyngeal bulb. Five pharyngeal gland nuclei visible, the first ventrosublateral pair obscure. Pharyngeal bulb offset from intestine. Cardia conoid, difficult to observe. Three ventromedian CP present anterior to the secretory-excretory pore (S-E), the latter opposite isthmus or anterior end of pharyngeal bulb. CP1 situated opposite the end of pharyngostom to mid-isthmus, distance of CP1-CP2, CP2-CP3 and CP3-SE becomes gradually shorter. Lateral cervical pores not clearly seen. Reproductive system typical of the genus, i.e., with a single anterior outstretched testis, short germinal zone, seminal vesicle packed with large round sperm cells with fibrillar structure and a sausage-shaped central nucleus. Spicules paired, relatively short 33.2 (32.0–34.5) μm , in holotype 34.5 μm , slightly ventrally curved. Capitulum widened, slightly marked, lamina partially striated, tapers gradually to the distal end, no bristles at striation. Gubernaculum having a keel-like thickening and proximal end visible between spicules (Fig. 1E). Three ventromedian precloacal supplements (SP) present. The posterior-most one (SP1) at the level of spicule capitulum, the SP2 slightly less than, or equal to one spicule length anterior to the SP1. The anterior most (SP3), 1.0–1.5 times spicule length apart from SP2. Cloacal lip rounded; slightly protruded, post-cloacal papillae not prominent. Tail short, conoid, less than one cloacal diameter long with one pair of subterminal subventral pores.

Female. Body straight or slightly curved upon heat relaxation. Anterior region similar to that of male except for secondary male characteristics. S-E pore located opposite isthmus or anterior part of pharyngeal bulb. Reproductive system didelphic amphidelphic with reflexed ovaries. Two finely granular oviduct cells at the tip of reflexed ovary, sperm round in shape distributed in the distal part of the uteri. Vagina well developed, *pars proximalis vaginae* barrel shaped in lateral optical view extending less than half corresponding body diameter. Sclerotized vaginal pieces (= *pars refringes vaginae*) rounded triangular with tips directed towards vulva, pieces 1.5–2.0 μm sized, at about 1.0 μm distance from each other, vulva pore-like in ventral view. Copulatory plug observed in uterus of two specimens. One pair of sublateral body pores almost opposite the vulva. Tail terminus conoid to rounded, anus subterminal, caudal pores subventral, immediately posterior to anus.

Type host and locality. The new species was detected in association with three plants, i.e., *Eriobotrya japonica* (Thunb.) Lindl., *Morus alba* L. and *Toona sinensis* (A. Jussieu) M. Roemer from the botanical garden of Huajiachi Campus, Zhejiang University, Hangzhou, Zhejiang Province, P. R. China. The specimens from *E. japonica* were regarded as a type population. The geographical position of the sampling site is: 120°19'06"E, 30°25'67"N.

Type material. *Holotype* male, 8 male and 11 female *paratypes* (slide nos. ZJU-29-01-ZJU-29-19) deposited in the Nematode Collection of Zhejiang University, Hangzhou, China, and 2 male and 13 female paratypes deposited in Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Bulgaria (slide nos. PNT 102-104).

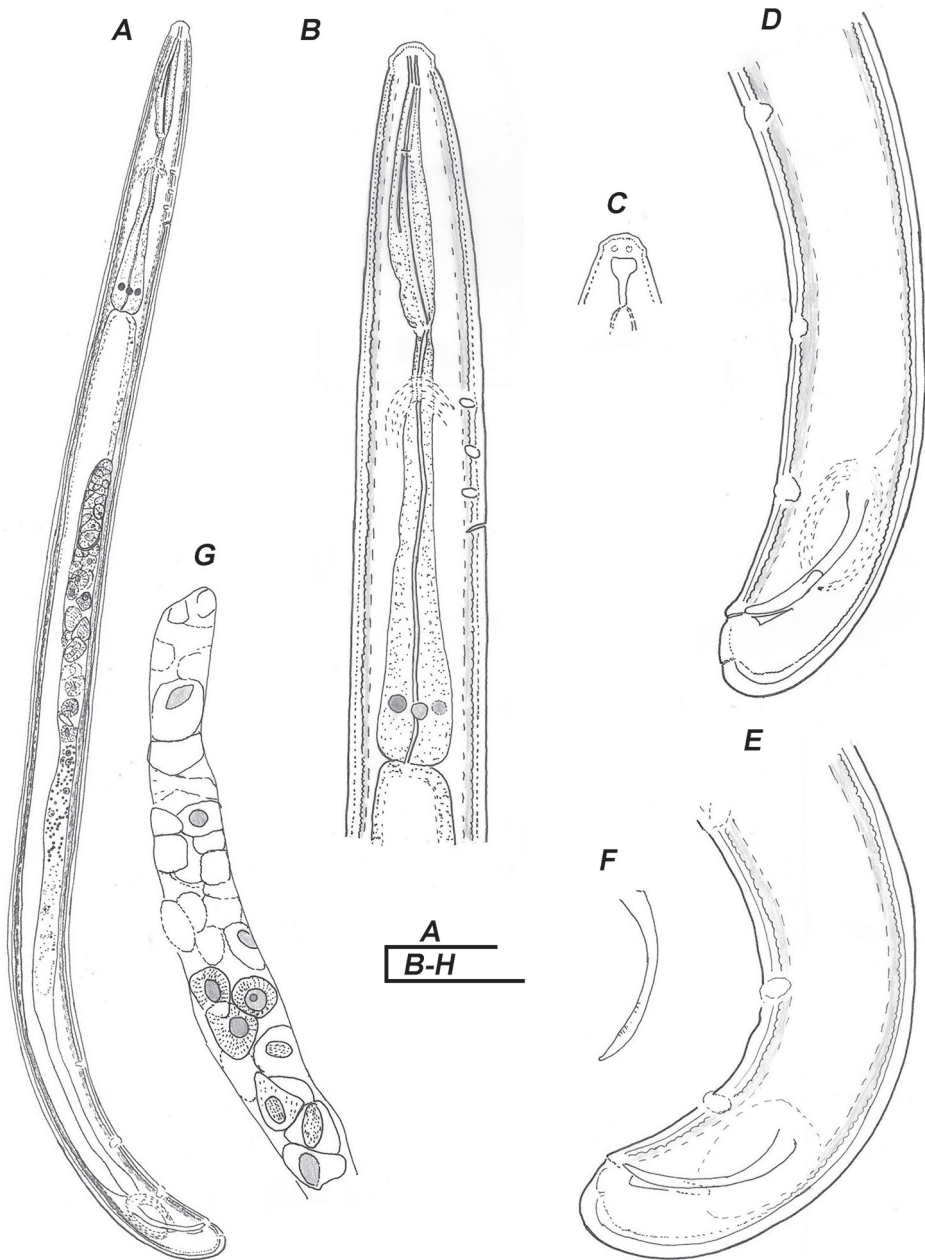


Figure 1. Line drawings of *Trichodorus hangzhouensis* sp. nov., paratypes, Male **A** entire body **B** pharyngeal region **C** surface view **D, E** posterior end **F** spicule **G** germinal zone of testis. Scale bars: 50 μ m.

Molecular profiles and phylogenetic analysis. The new species was molecularly characterized and newly obtained sequences were deposited in the GenBank with the accession numbers HM106498, MF979178 for 18S, MF979185–MF979186, HM106497 for 28S and MF979181, HM106496, MF979182 for ITS2 of rRNA

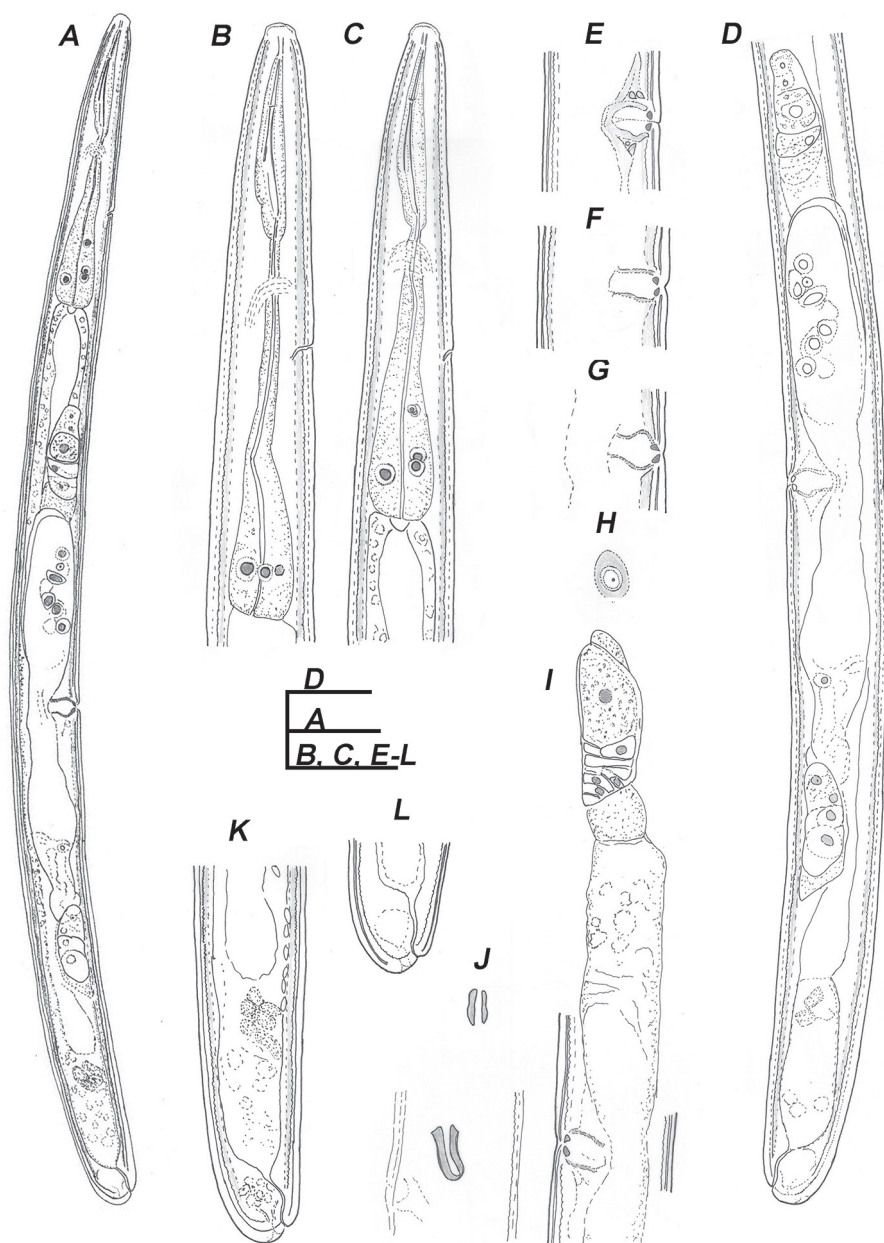


Figure 2. Line drawings of *Trichodorus hangzhouensis* sp. nov., paratypes, Female **A** entire body **B**, **C** pharyngeal region **D** genital branches **E–G** vulval region, lateral view **H** vulva, ventral view **I** anterior genital branch **J** copulatory plugs **K**, **L** posterior ends. Scale bars: 50 μ m.

gene. The available sequences of trichodorid taxa (accession numbers of 18S, D2–D3 region of 28S and ITS2 rRNA gene sequences in Suppl. material 1: Table S1) were selected to reconstruct the phylogenetic trees.

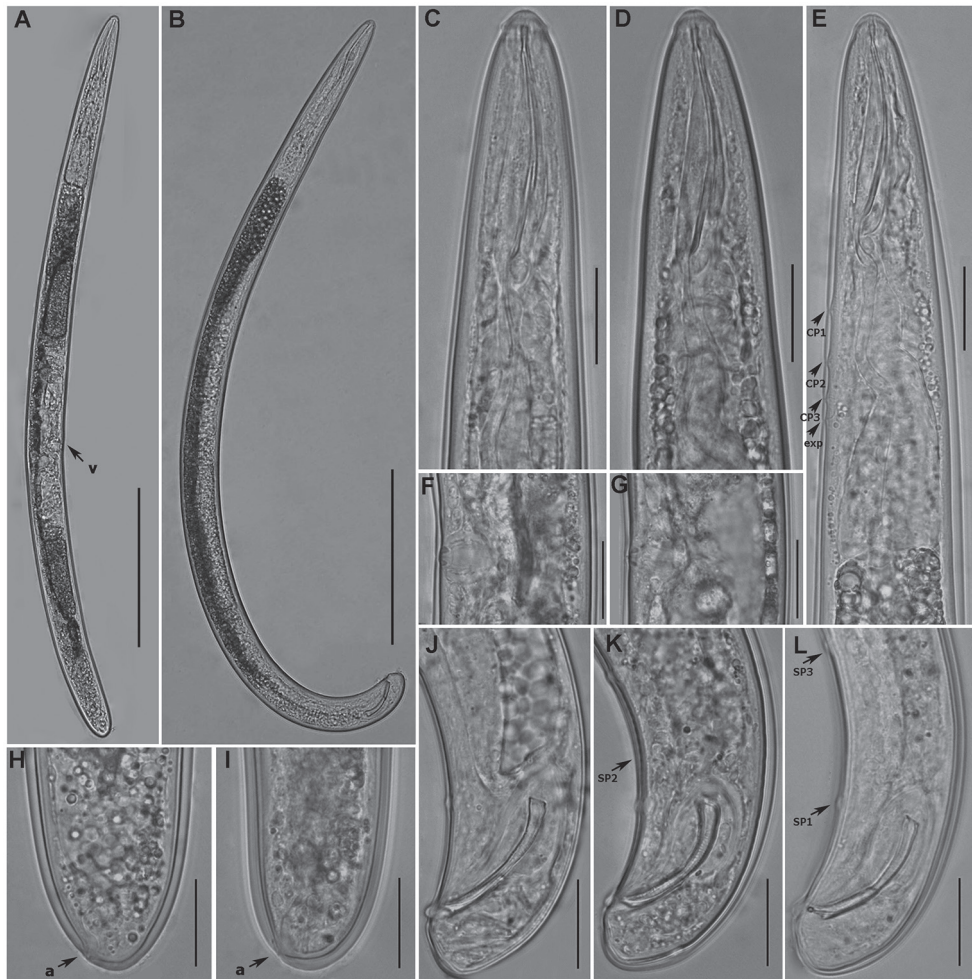


Figure 3. Light photomicrographs of *Trichodorus hangzhouensis* sp. nov., paratypes **A** female entire body, arrow points vulva **B** male entire body **C, D** anterior region of female **E** pharyngeal region of male, arrows pointing position of cervical papillae (CP) and excretory pore (exp) **F, G** vulval region **H, I** female tail, arrows pointing position of anus (a) **J–L** male tail, arrows pointing position of male tail supplements (SP). Scale bars: 100 μ m (**A, B**) 20 μ m (**C–L**).

The 18S rRNA gene tree (Fig. 7) revealed that *T. hangzhouensis* sp. nov. occupied a basal placement in an unsupported clade including *T. primitivus* (de Man, 1880) Micoletzky, 1922 (KY119675–76, AF036609), *T. similis* Seinhorst, 1963 (AJ439584–85, AJ439522) and *T. obtusus* Cobb, 1913 (KT282335). The pairwise sequence identity of new species with the aforementioned species is 97–99% with 16–24 nucleotide differences.

In the 28S phylogenetic tree (Fig. 8), *T. hangzhouensis* sp. nov. formed a clade with four species distributed in Spain: *T. giennensis* Decraemer, Roca, Castillo, Peñ-Santiago & Gomez-Barcina, 1993 (JQ716452), *T. illiplaensis* Decraemer, Palomares-Rius, Cantalapiedra-Navarrete, Landa, Duarte, Almeida, Vovlas & Castillo, 2013 (JQ716462),

Table 1. Measurements of *Trichodorus hangzhouensis* sp. nov. males and females. All measurements are in µm and in the form: mean ± SD (range).

Characters	Holotype	Paratypes	
	Male	Males	Females
n		8	11
L	708	689.9 ± 30.4 (628–727)	686.2 ± 29.8(646–744)
Body diameter	33	33.8 ± 2.2 (29–36)	40.4 ± 3.3 (36.0–47.0)
Pharynx	145	140.8 ± 8.3 (125–146)	143.3 ± 15.0 (117–171)
Onchiostyle	48	48.1 ± 1.2 (46–49)	49.3 ± 1.8 (48–52)
Onchium	27	26.9 ± 2.3 (24–28)	29.4 ± 1.9 (27–31)
Onchiophore	22	21.6 ± 0.7 (21–23)	22.1 ± 0.9 (2–23)
Pharyngostom	53	50.5 ± 2.8 (47–54)	54.6 ± 2.1 (52–56)
Ant. end to S-E pore	99	100.0 ± 4.0 (96.5–108)	96.8 ± 8.2 (87–114)
Ant. genital branch	–	–	174.2 ± 6.8 (163–185)
Post. genital branch	–	–	170.1 ± 15.5 (137–193)
a	21.3	20.5 ± 1.1 (19.0–21.6)	17.1 ± 1.5 (14.5–19.4)
b	4.9	4.9 ± 0.4 (4.3–5.6)	4.8 ± 0.6 (3.8–6.0)
V/T	64.5	65.8 ± 1.4 (63.9–68.4)	57.1 ± 2.0 (53.0–60.0)
Length of vagina	–	–	15.6 ± 1.4 (14–19)
CP1-CP2	12	10.9 ± 1.9 (8–12.5)	–
CP2-CP3	9	7.9 ± 1.7 (5–9)	–
CP3 to S-E pore	8	6.0 ± 1.5 (4–8)	–
Spicules	34.5	33.2 ± 1.0 (32–34.5)	–
Gubernaculum	15	14.7 ± 1.5 (11.5–17)	–
Cloaca to SP1	23	25.7 ± 3.0 (22.5–32)	–
SP1-SP2	24	31.3 ± 4.4 (24–37)	–
SP2-SP3	37.0	38.0 ± 4.0 (33–45)	–

T. onubensis Decraemer, Palomares-Rius, Cantalapiedra-Navarrete, Landa, Duarte, Almeida, Vovlas & Castillo, 2013 (JQ716454–55) and *T. paragiennensis* Decraemer, Palomares-Rius, Cantalapiedra-Navarrete, Landa, Duarte, Almeida, Vovlas & Castillo, 2013 (JQ716461); three species occurring in Iran – *T. orientalis* De Waele & Hashim, 1983 (KY115140); *T. persicus* De Waele & Sturhan, 1987 (KX348138); *T. zanzanensis* Asghari, Eskandari, Tanha Maafi & Decraemer, 2018 (KY115138); one species from Israel *T. minzi* De Waele & Cohn, 1992 (KP259801) and an undescribed *Trichodorus* species (KM212949) from the USA. The pairwise sequence identity of new species with the aforementioned species is 88–91% with 73–86 nucleotide differences.

In ITS2 tree (Fig. 9), *T. hangzhouensis* sp. nov. shared the same clade with *T. pakistanensis* Siddiqi, 1962 (GU645896, GU645897, GU645899, JN123384) and the clade of these two species was in sister relation to a clade including *T. gilanensis* Maafi & Decraemer, 2002 (KY115164–KY115165), *T. nanjingensis* Liu & Cheng, 1990 (GU645800, GU645804, GU645893, GU645895), *T. viruliferus* Hooper, 1963 (JN123391) and *T. sparsus* Szczygiel, 1968 (JN123388). The pairwise sequence identity of the new species with the aforementioned species is 94–98% with 4–9 nucleotide differences.

The other known *Trichodorus*, *Nanidorus* and *Paratrichodorus* species sequenced during this study clustered with their respective species available through GenBank database, thus supporting their identity.

Diagnosis and relationships. The new species is characterized by the male having a relatively short onchiostyle (46–49 μm) and 3 ventromedian cervical papillae anterior to the S-E pore, CP1 located opposite isthmus, distance of CP1-CP2, CP2-CP3 and CP3-S-E becoming gradually shorter, S-E pore located opposite isthmus or anterior end of pharyngeal bulb, pharynx offset, spicules relatively short, slightly curved, 33.2 (32.0–34.5) μm long, with wider slightly marked capitulum, lamina partially striated and tapering gradually to the distal end, bristles at striation absent, three ventromedian preloacal supplements; female with barrel shaped vagina, vaginal sclerotized pieces medium-sized (1.5–2.0 μm), rounded triangular with tips directed towards vulva, slightly separated from each other (c. 1.0 μm), vulva pore-like in ventral view.

The species-specific codes sensu Decraemer and Baujard (1998) for this new species are as follows, for the male: F3-D3-P2-A1(2)-B2-C1-E0-G1-H2-I1-J2-K3-L2-M4-N1-O5; for female: D1-C1-L2- K2-A1(2)-B2-E2 -F1 G1-H3-I2(3)-J1-M1-N1-O1-P1-Q1-R2-S5. Based on male prime diagnostic characters for fam. Trichodoridae (F = number of ventromedian preloacal supplements, D = number of ventromedian cervical papillae, P = body habitus) and female (D = type of genital system, C = vulva position, K = size of vaginal sclerotized pieces, L = position of vaginal sclerotized pieces) the new species male belongs to group 12 while the female falls in the category group 1 of subgroup 1–7 as described by Decraemer and Baujard (1998). Among several *Trichodorus* species of group 12 (for male) and group 1, subgroup 1–7 (for female) the new species comes close to *T. cedarus* Yokoo, 1964, *T. guangzhouensis* Xie, Feng & Zhao, 2000, *T. reduncus* Siddiqi & Sharma, 1995, *T. tricaulatus* Shishida, 1979 and *T. yokooi* Eroshenko & Teplyakov, 1975. It can be differentiated as follows:

T. cedarus – by having a different position of SP1 (at the level of spicules capitulum vs posterior), shorter spicules length (32.0–34.5 vs 36–53 μm), shape of vulva (pore-like vs slit-like) and shape of vagina (barrel vs pear);

T. guangzhouensis – by having a longer onchiostyle in females (48–52 vs 36.4–41.6 μm), striations on spicule (present vs absent), different spicule shape (without constriction vs with constriction) and shape of vulva in ventral view (pore-like vs a longitudinal slit);

T. reduncus – by having a longer onchiostyle in males (46–49 vs 36–40 μm) and female (48–52 vs 37–40 μm), striations on spicules (present vs absent), different proximal part of gubernaculum (not hooked vs hooked) position of vaginal pieces (close vs widely separated) and shape of vulva (pore-like vs a small transverse slit);

T. tricaulatus – by having different position of SP1 (at the level of spicule capitulum vs outside it), spicule bristles (absent vs present), longer onchiostyle in both males (49.3 (46–49) vs 42.6 (39–51) μm) and females (49.3 (48–52) vs 42.2 (39–44) μm), type of pharyngo-intestinal junction (pharyngeal bulb offset vs pharynx overlapping intestine ventrally) and shorter spicules (33.2 (32.0–34.5) vs 39 (36–53) μm);

T. yokooi – by having striation on spicule (vs absent), shorter onchiostyle both in males (46–49 vs 57–82 μm) and females (48–52 vs 62–77 μm) and spicule (32.0–34.5 vs 38–46 μm).

Etymology. The species name is derived from the name of the city where the new species was recovered.

Distribution of trichodorid species in mainland China

The geographical distribution of trichodorids recovered in 13 different provinces of China including Beijing, Shandong, Shanxi, Henan, Jiangsu, Anhui, Hunan, Chongqing, Zhejiang, Fujian, Yunnan, Hainan and Guangdong (based on 2054 examined soil samples) is mapped in Fig. 4. Eighty-five trichodorid populations were recovered in this study while ninety-three populations are listed in previous records from China (Table 2). In this study, three known species of *Trichodorus* (*T. nanjingensis*, *T. pakistanensis*, *T. cedarus*) with one new species, one *Paratrichodorus* species (*P. porosus* (Allen, 1957) Siddiqi 1974), and two *Nanidorus* species (*N. renifer* Siddiqi, 1974; and *N. minor* (Colbran, 1956) Siddiqi, 1974) have been identified (Figs 5, 6; Tables 3, 4), while *T. paracedarus*, Yokoo, 1964; *T. rinae* Yokoo, 1964; *T. guangzhouensis* and *P. pachydermus* Siddiqi, 1974 were reported in the past (Xu and Decraemer 1995, Xie et al. 2000, Zhao et al. 2005, Yan et al. 2005). In our study 14, 16, 5, 30, 9 and 8 soil samples contain respectively *T. nanjingensis*, *T. pakistanensis*, *T. cedarus*, *P. porosus*, *N. renifer* and *N. minor* accounts for 16.4%, 18.8%, 5.9%, 35.3%, 10.6% and 9.4% of the total trichodorid populations detected. Combined records of past and present reports revealed that the most frequently found *Trichodorus* species are *T. nanjingensis*, *T. pakistanensis* and *T. cedarus*, while *P. porosus* showed remarkably high frequency of



Figure 4. Distribution map of trichodorid species in China (stars indicate the occurrence of trichodorid taxa).

Table 2. The occurrence of *Trichodorus*, *Paratrachodorus* and *Nanidorus* in China.

Provinces (bold) /Localities	Species	Host	References
Jiangsu			
Nanjing	<i>T. nanjingensis</i>	<i>Prunus persica</i>	Liu and Cheng 1990
Suzhou	<i>T. nanjingensis</i>	<i>Prunus salicina</i>	Decraemer and Cheng 1994
Linbao	<i>T. nanjingensis</i>	<i>Malus domestica</i>	Decraemer and Cheng 1994
Ganyu	<i>T. cedarus</i>	<i>Malus pumila</i>	Xu and Decraemer 1995
Wuxi	<i>T. cedarus</i>	<i>Prunus persica</i>	Xu and Decraemer 1995
Nanjing	<i>T. paracedarus</i>	<i>Lycopersicon esculentum</i>	Xu and Decraemer 1995
Lianyungang	<i>T. paracedarus</i>	<i>Prunus yedoensis</i>	Xu and Decraemer 1995
Nantong	<i>P. porosus</i>	<i>Marus alba</i>	Liu 1994
Zhejiang			
Hangzhou	<i>T. nanjingensis</i>	<i>Bambusa glaucescens</i>	Zheng et al. 2004
Hangzhou	<i>T. pakistanensis</i>	<i>Metasequoia glyptostroboides</i>	Zheng et al. 2004
Changxing	<i>T. cedarus</i>	<i>Pyrus pyrifolia</i>	Xu and Decraemer 1995
Huzhou	<i>P. porosus</i>	<i>Bambusa vulgaris</i>	Xie et al. 2007
Fuyang	<i>P. porosus</i>	<i>Camellia japonica</i>	Zheng et al. 2004
Ningbo	<i>T. cedarus</i>	<i>Hedera helix</i>	This study
Ningbo	<i>P. porosus</i>	<i>Ilex chinensis</i>	This study
Ningbo	<i>N. renifer</i>	<i>Myrica rubra</i>	This study
Ningbo	<i>N. renifer</i>	<i>Myrica rubra</i>	This study
Ningbo	<i>N. renifer</i>	<i>Myrica rubra</i>	This study
Hangzhou	<i>T. nanjingensis</i>	<i>Morus alba</i>	This study
Hangzhou	<i>T. hangzhouensis</i> sp. nov.	<i>Albizia julibrissin</i>	This study
Hangzhou	<i>T. cedarus</i>	<i>Magnolia denudata</i>	This study
Hangzhou	<i>T. cedarus</i>	<i>Osmanthus fragrans</i>	This study
Hangzhou	<i>P. porosus</i>	<i>Eriobotrya japonica</i>	This study
Hangzhou	<i>T. hangzhouensis</i> sp. nov. + <i>P. porosus</i>	<i>Firmiana simplex</i>	This study
Hangzhou	<i>T. hangzhouensis</i> sp. nov.	<i>Toona sinensis</i>	This study
Hangzhou	<i>T. hangzhouensis</i> sp. nov.	<i>Eriobotrya japonica</i>	This study
Hangzhou	<i>T. nanjingensis</i>	<i>Pyrus</i> sp.	This study
Hangzhou	<i>T. nanjingensis</i>	<i>Cinnamomum camphora</i>	This study
Hangzhou	<i>T. cedarus</i>	<i>Cryptomeria fortunei</i>	This study
Hangzhou	<i>T. nanjingensis</i> + <i>P. porosus</i>	<i>Albizia julibrissin</i>	This study
Hangzhou	<i>T. nanjingensis</i>	<i>Rosa rugosa</i>	This study
Hangzhou	<i>T. pakistanensis</i>	<i>Magnolia grandiflora</i>	This study
Hangzhou	<i>T. cedarus</i>	<i>Pseudotsuga sinensis</i>	This study
Hangzhou	<i>N. renifer</i>	<i>Azalea</i> sp.	This study
Hangzhou	<i>P. porosus</i>	<i>Gmelina hainanensis</i>	This study
Fuyang	<i>P. porosus</i>	<i>Camellia japonica</i>	This study
Linan	<i>P. porosus</i>	<i>Prunus pseudocerasus</i>	This study
Taizhou	<i>P. porosus</i>	<i>Quercus acutissima</i>	This study
Yuyao	<i>N. renifer</i>	<i>Myrica rubra</i>	This study
Yuyao	<i>N. renifer</i>	<i>Myrica rubra</i>	This study
Lishui	<i>P. porosus</i>	<i>Citrus reticulata</i>	This study
Jinhua	<i>N. renifer</i>	<i>Rosa chinensis</i>	This study

Provinces (bold) /Localities	Species	Host	References
Fenghua	<i>P. porosus</i> + <i>N. renifer</i>	<i>Acer truncatum</i>	This study
Fenghua	<i>P. porosus</i> + <i>N. renifer</i>	<i>Acer palmatum</i>	This study
Hainan			
Anding	<i>P. pachydermus</i>	<i>Saccharum officinarum</i>	Ding et al. 2015
Danzhou	<i>P. pachydermus</i>	<i>Saccharum officinarum</i>	Ding et al. 2015
Unknown	<i>T. pakistanensis</i>	<i>Saccharum officinarum</i>	This study
Danzhou	<i>N. minor</i>	<i>Solanum melongena</i>	This study
Danzhou	<i>N. minor</i>	<i>Melia azedarach</i>	This study
Danzhou	<i>N. minor</i>	<i>Lactuca sativa</i>	This study
Chongqing			
Beipei	<i>T. pakistanensis</i>	<i>Trachycarpus fortunei</i>	This study
Yunnan			
Kunming	<i>T. nanjingensis</i>	<i>Pyrus</i> sp.	Zhao et al. 2005
Kunming	<i>T. rinae</i>	<i>Pyrus</i> sp.	Zhao et al. 2005
Kunming	<i>T. cedarus</i>	<i>Pyrus</i> sp.	Zhao et al. 2005
Kunming	<i>P. porosus</i>	<i>Pyrus</i> sp.	Zhao et al. 2005
Hekou	<i>T. cedarus</i>	<i>Musa</i> sp.	Du et al. 2010
Kunming	<i>N. minor</i>	<i>Hydrangea macrophylla</i>	Lin et al. 2009
Chengong	<i>N. minor</i>	<i>Prunus persica</i>	This study
Dabanqiao	<i>N. minor</i>	<i>Pyrus</i> sp.	This study
Dabanqiao	<i>N. minor</i>	<i>Pyrus</i> sp.	This study
Luliang	<i>P. porosus</i>	<i>Pyrus</i> sp.	This study
Luliang	<i>P. porosus</i>	<i>Pyrus</i> sp.	This study
Majie	<i>P. porosus</i>	<i>Pyrus</i> sp.	This study
Majie	<i>P. porosus</i>	<i>Pyrus</i> sp.	This study
Majie	<i>P. porosus</i>	<i>Solanum tuberosum</i>	This study
Kunming	<i>P. porosus</i>	<i>Sapindus delavayi</i>	This study
Kunming	<i>P. porosus</i>	<i>Corylus chinensis</i>	This study
Kunming	<i>P. porosus</i>	<i>Diospyros kaki</i>	This study
Kunming	<i>P. porosus</i>	<i>Sophora japonica</i>	This study
Kunming	<i>P. porosus</i>	<i>Quercus variabilis</i>	This study
Kunming	<i>P. porosus</i>	<i>Abies holophylla</i>	This study
Kunming	<i>T. pakistanensis</i>	<i>Acer truncatum</i>	This study
Kunming	<i>N. minor</i>	<i>Juglans regia</i>	This study
Kunming	<i>P. porosus</i>	<i>Prunus persica</i>	This study
Xundian	<i>P. porosus</i>	<i>Pinus massoniana</i>	This study
Guangdong			
Shenzhen	<i>P. porosus</i>	<i>Litchi chinensis</i>	Wang et al. 1996
Unknown	<i>T. cedarus</i>	<i>Salix babylonica</i>	Chi et al. 2011
Unknown	<i>P. porosus</i>	<i>Magnoliaceae glanca</i>	Chi et al. 2011
Unknown	<i>N. renifer</i>	<i>Magnoliaceae glanca</i>	Chi et al. 2011
Guangzhou	<i>T. guangzhouensis</i>	<i>Lactuca sativa</i>	Xie et al. 2000
Gaozhou	<i>P. pachydermus</i>	<i>Musa paradisiaca</i>	Yan et al. 2005
Guangzhou	<i>P. porosus</i>	<i>Osmanthus fragrana</i>	This study
Beijing			
Nankou farm	<i>T. nanjingensis</i>	<i>Malus domestica</i>	Wang et al. 1996
Nankou farm	<i>T. nanjingensis</i>	<i>Mains baccata</i>	Hao et al. 1998
Unknown	<i>T. nanjingensis</i>	<i>Malus domestica</i>	Zheng et al. 2004

Provinces (bold) /Localities	Species	Host	References
Nankou farm	<i>T. pakistanensis</i>	<i>Malus domestica</i>	Wang 1993
Nankou farm	<i>T. pakistanensis</i>	<i>Malus domestica</i>	Wang et al. 1994
Unknown	<i>P. porosus</i>	<i>Vitis</i> sp.	Liu 1994
Shisanling	<i>T. nanjingensis</i>	<i>Prunus persica</i>	This study
Shisanling	<i>T. nanjingensis</i>	<i>Juglans regia</i>	This study
Shisanling	<i>T. nanjingensis</i>	<i>Malus pumila</i>	This study
Fenghuangling	<i>T. nanjingensis</i>	<i>Pyrus</i> sp.	This study
Zhiwuyuan	<i>T. nanjingensis</i>	<i>Malus micromalus</i>	This study
Zhiwuyuan	<i>T. nanjingensis</i>	<i>Prunus persica</i>	This study
Zhiwuyuan	<i>T. nanjingensis</i>	<i>Cotoneaster multiflorus</i>	This study
Unknown	<i>T. nanjingensis</i>	<i>Prunus blireana</i>	This study
Xiangshan	<i>T. nanjingensis</i>	<i>Prunus armeniaca</i>	This study
Hebei			
Zhuolu	<i>P. porosus</i>	<i>Vitis vinifera</i>	Wang et al. 1996
Xingtang	<i>P. porosus</i>	<i>Vitis vinifera</i>	Wang et al. 1996
Shandong			
Linyi	<i>P. porosus</i>	<i>Malus pumila</i>	Liu 1994
Fujian			
Zhangzhou	<i>T. pakistanensis</i>	<i>Litchi chinensis</i>	Xu and Decraemer 1995
Fuzhou	<i>T. pakistanensis</i>	<i>Dimocarpus longan</i>	Liu and Zhang 1999
Putian	<i>T. pakistanensis</i>	<i>Dimocarpus longan</i>	Liu and Zhang 1999
Fuzhou	<i>T. pakistanensis</i>	<i>Canarium album</i>	Zhang et al. 2002
Xiamen	<i>T. pakistanensis</i>	<i>Ficus carica</i>	Pan et al. 2000
Nan'an	<i>N. minor</i>	<i>Myrica rubra</i>	Zhang and Chen 1994
Fuzhou	<i>T. pakistanensis</i>	<i>Dimocarpus longan</i>	This study
Fuzhou	<i>T. pakistanensis</i>	<i>Eriobotrya japonica</i>	This study
Fuzhou	<i>T. pakistanensis</i>	<i>Citrus reticulata</i>	This study
Fuzhou	<i>T. pakistanensis</i>	<i>Ilex chinensis</i>	This study
Fuzhou	<i>P. porosus</i>	<i>Citrus reticulata</i>	This study
Fuzhou	<i>T. pakistanensis</i>	<i>Dimocarpus longan</i>	This study
Xiamen	<i>T. pakistanensis</i>	<i>Xylosma congestum</i>	This study
Xiamen	<i>P. porosus</i> + <i>N. minor</i>	<i>Dimocarpus longan</i>	This study
Xiamen	<i>P. porosus</i>	<i>Dimocarpus longan</i>	This study
Xiamen	<i>T. pakistanensis</i>	<i>Dimocarpus longan</i>	This study
Zhangzhou	<i>P. porosus</i>	<i>Litchi chinensis</i>	This study
Zhangzhou	<i>T. pakistanensis</i>	<i>Dimocarpus longan</i>	This study
Zhangzhou	<i>T. pakistanensis</i>	<i>Dimocarpus longan</i>	This study
Zhangzhou	<i>T. pakistanensis</i>	<i>Dimocarpus longan</i>	This study
Zhangzhou	<i>T. pakistanensis</i>	<i>Litchi chinensis</i>	This study
Zhangzhou	<i>T. pakistanensis</i>	<i>Dimocarpus longan</i>	This study
Anhui			
Huangshan	<i>P. porosus</i>	<i>Boehmeria nivea</i>	Liu and Cheng 1990
Shexian	<i>T. pakistanensis</i>	<i>Boehmeria nivea</i>	Xu and Decraemer 1995
Hunan			
Changsha	<i>P. porosus</i>	<i>Averrhoa carambola</i>	Wang et al. 1996
Changsha	<i>P. porosus</i>	<i>Pinus massoniana</i>	This study

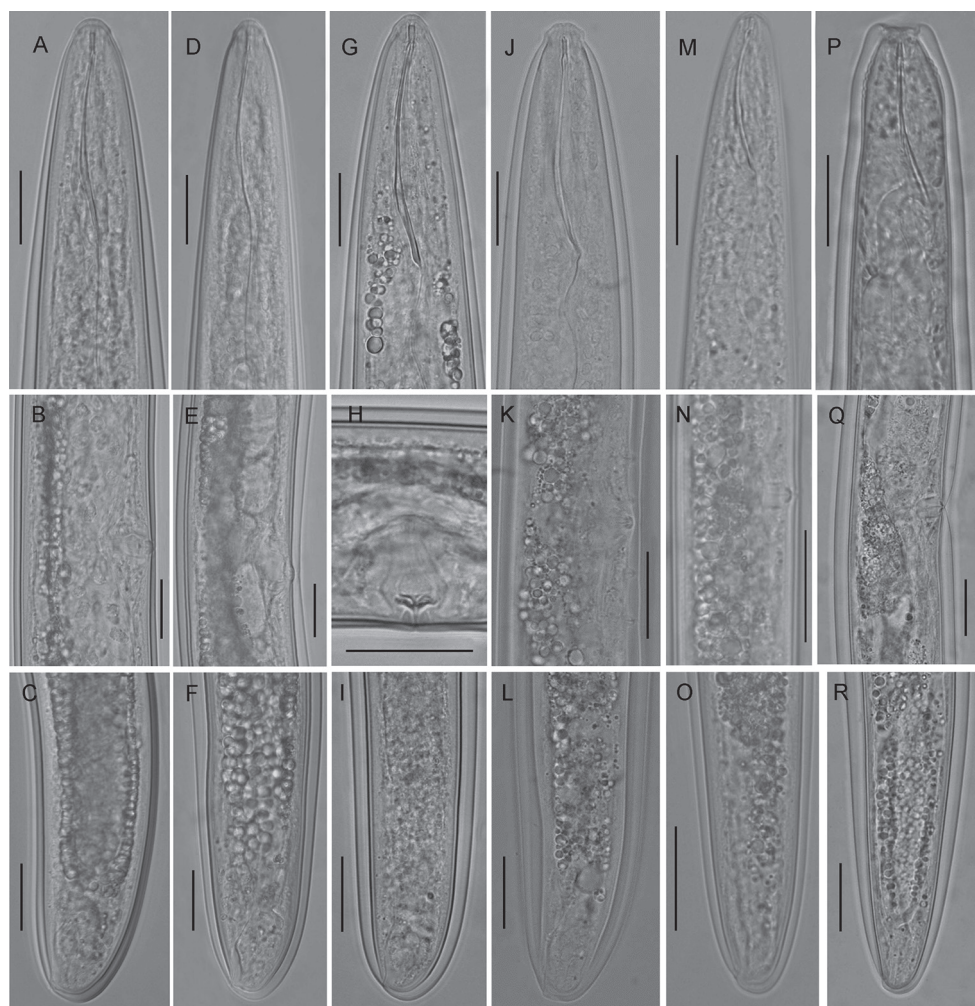


Figure 5. Photomicrographs of females of *Trichodorus*, *Paratrichodorus* and *Nanidorus* species **A–C** *T. nanjingensis* Liu & Cheng, 1990 **D–F** *T. pakistanensis* Siddiqi, 1962 **G–I** *T. cedarus* Yokoo, 1964 **J–L** *P. porosus* **M–O** *N. renifer* Siddiqi, 1974 **P–R** *N. minor* (Colbran, 1956). Scale bars: 20 μ m (**A–R**).

occurrence in China. In general, trichodorids have been recovered from many localities in China but some species are geographically concentrated in some areas, e.g., *T. nanjingensis* is recorded in high percentage in the northern area (Beijing), *T. pakistanensis* in the southeastern region (Fujian), *T. cedarus* in eastern regions (e.g., Zhejiang), and *P. porosus* is very common in Yunnan and Zhejiang provinces. Other species were found in a relatively few numbers.

Two of the recorded species are known to transmit tobra viruses (*N. minor* and *P. pachydermus*) (MacFarlane et al. 2002).

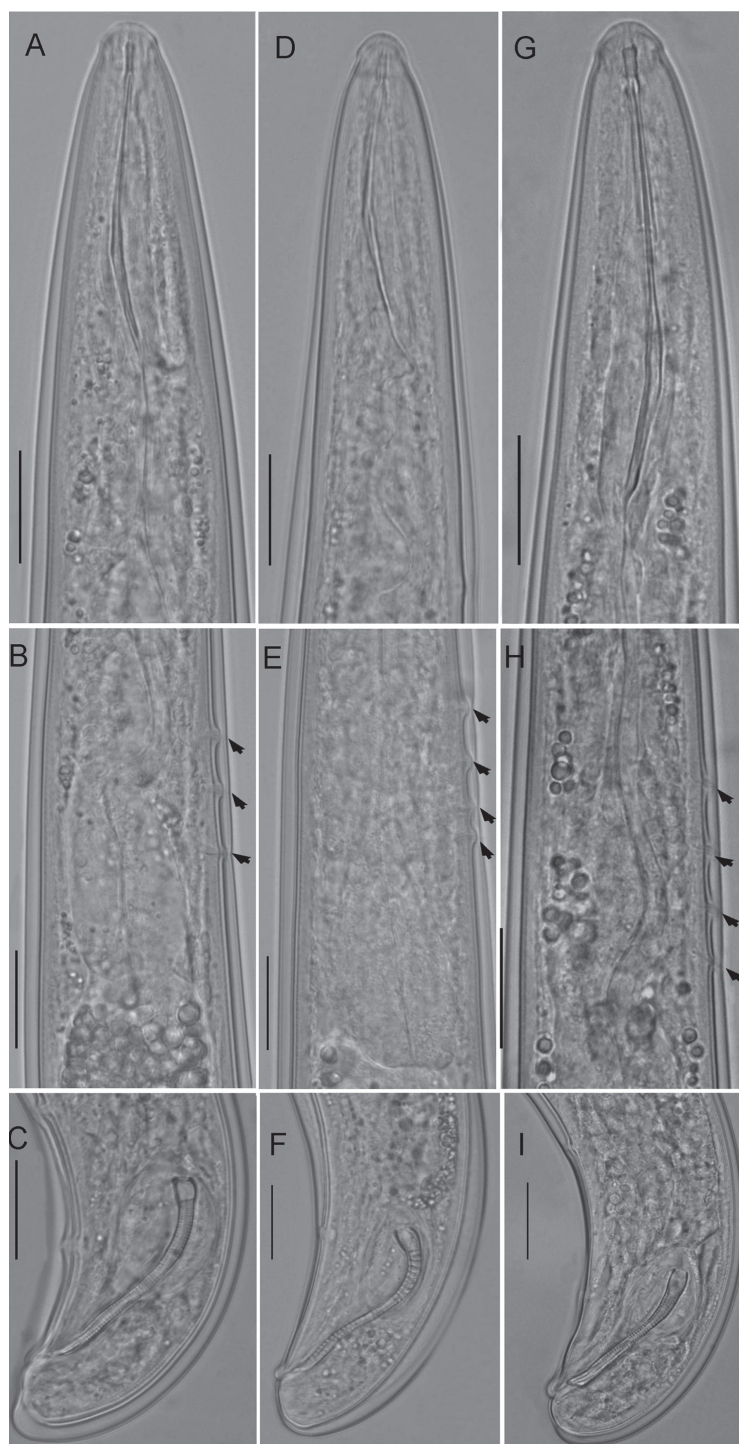


Figure 6. Photomicrographs of *Trichodorus* species, Males **A–C** *T. nanjingensis* Liu & Cheng, 1990 **D–F** *T. pakistanensis* Siddiqi, 1962 **G–I** *T. cedarus*. Scale bars: 20 μm (**A–I**).

Table 3. Measurements of females of *Trichodorus*, *Paratrichodorus* and *Nanidorus* species from China (all measurements in μm).

Species	<i>T. nanjingensis</i>	<i>T. pakistanensis</i>	<i>T. cedarus</i>	<i>P. porosus</i>	<i>N. renifer</i>	<i>N. minor</i>
Location	Beijing	Fuzhou Fujian Province	Hangzhou Zhejiang Province	Xiamen Fujian Province	Jinhua Zhejiang Province	Danzhou Hainan Province
n	10	11	13	25	19	6
L	1099.1 $\pm$ 117.7 (836–1284)	1051.7 $\pm$ 91.2 (883.5–1175)	749.2 $\pm$ 56.5 (680–843)	731.9 $\pm$ 113.4 (498.5–898)	538.1 $\pm$ 36.8 (466.5–607.5)	645.8 $\pm$ 16.9 (632–678)
Body diam	52.9 $\pm$ 7.7 (389–63)	49.2 $\pm$ 5.9 (39–59)	45.9 $\pm$ 4.3 (34–52)	48.0 $\pm$ 6.6 (36–58)	24.3 $\pm$ 2.9 (20.6–29.3)	31.6 $\pm$ 3.6 (27–35)
Pharynx	170.6 $\pm$ 16.7 (155–214)	159.9 $\pm$ 11.1 (139–172)	176.7 $\pm$ 10.8 (161–197)	143.9 $\pm$ 19.0 (103–173)	111.4 $\pm$ 10.3 (91–133)	129.7 $\pm$ 2.6 (126–133)
Onchiostyle	54.3 $\pm$ 4.5 (47–63)	50.7 $\pm$ 2.1 (47–54)	66.2 $\pm$ 2.3 (62–70)	53.5 $\pm$ 1.7 (49–57)	36.3 $\pm$ 1.7 (33–41)	35.3 $\pm$ 0.7 (34.5–36.5)
Ant. end to S-E pore	139.7 $\pm$ 7.9 (137–154)	116.5 $\pm$ 11.6 (110–134)	122.8 $\pm$ 8.0 (111–135)	–	105	–
Ant. genital branch	217.2 $\pm$ 35.5 (172–274)	185.8 $\pm$ 35.5 (141–265)	186.1 $\pm$ 34.2 (141–266)	122.6 $\pm$ 22.4 (91–153)	114.2 $\pm$ 16.7 (69.5–136)	120.2 $\pm$ 31.7 (90.5–154)
Post. genital branch	224.6 $\pm$ 30.9 (160–269)	172.6 $\pm$ 39.8 (132–262)	178.5 $\pm$ 26.5 (144–221)	149.0 $\pm$ 32.0 (104–212)	105.3 $\pm$ 14.3 (67–133)	98.5 $\pm$ 25.4 (70–119)
a	21.1 $\pm$ 2.9 (15.6–25.7)	21.6 $\pm$ 2.8 (18.2–26.6)	16.5 $\pm$ 1.8 (14.1–20.3)	15.2 $\pm$ 1.5 (12.4–19.2)	22.4 $\pm$ 2.1 (17.7–25.5)	20.7 $\pm$ 2.4 (17.9–23.6)
b	6.5 $\pm$ 0.9 (4.7–7.7)	6.6 $\pm$ 0.8 (5.6–8.4)	4.2 $\pm$ 0.3 (3.8–4.6)	5.1 $\pm$ 0.6 (4.2–6.3)	4.9 $\pm$ 0.5 (4.1–6.0)	5.0 $\pm$ 0.1 (4.9–5.2)
V	56.1 $\pm$ 2.4 (50.3–57.9)	56.6 $\pm$ 0.9 (55.1–57.8)	57.7 $\pm$ 1.1 (55.8–59.7)	54.8 $\pm$ 2.2 (50.9–57.7)	56.4 $\pm$ 1.7 (53.7–59.1)	56.3 $\pm$ 0.0 (52.5–58.8)
Length of vagina	18.8 $\pm$ 1.7 (16–21)	17.1 $\pm$ 0.9 (15–18)	18.5 $\pm$ 1.6 (17–22)	8.6 $\pm$ 1.3 (6–10)	6.1 $\pm$ 0.7 (5–7)	11
Size of vaginal pieces	2	2.3 $\pm$ 0.2 (2–3)	2	1	1.5 $\pm$ 0.1 (1–2)	2

Table 4. Measurements of males of *Trichodorus* species from China (all measurements in μm).

Species	<i>T. nanjingensis</i>	<i>T. pakistanensis</i>	<i>T. cedarus</i>
Location	Beijing	Fuzhou Fujian Province	Hangzhou Zhejiang Province
n	16	9	14
L	1011.7 $\pm$ 122.9 (731–1163)	933.7 $\pm$ 91.4 (812–1074)	765.3 $\pm$ 54.3 (650–862)
Body diam	48.7 $\pm$ 9.1 (35.5–60)	43.7 $\pm$ 6.3 (34–53)	42.8 $\pm$ 4.2 (36.9–49.1)
Pharynx	160.6 $\pm$ 14.6 (132–190)	159.9 $\pm$ 11.5 (147–177)	174.5 $\pm$ 7.3 (160–187.5)
Onchiostyle	54.2 $\pm$ 3.4 (49–59)	50.9 $\pm$ 2.5 (48–54)	67.7 $\pm$ 1.5 (65–70)
Ant. end to S-E pore	127.6 $\pm$ 12.8 (113–142)	124.8 $\pm$ 8.5 (113–135)	127.0 $\pm$ 7.2 (117–142)
CP1-CP2	12.3 $\pm$ 1.4 (11–15)	11.8 $\pm$ 2.9 (8–17)	10.1 $\pm$ 1.7 (7–14)
CP2-CP3		15.1 $\pm$ 0.7 (14–16)	8.9 $\pm$ 1.5 (5–10)
CP3 to S-E pore (CP2 to S-E pore for <i>T. nanjingensis</i>)	14.2 $\pm$ 3.4 (7–19)	6.4 $\pm$ 1.0 (5–8)	7.6 $\pm$ 3.8 (2.5–17)
a	21.1 $\pm$ 2.5 (17.0–25.3)	21.7 $\pm$ 3.3 (17.0–26.8)	18.0 $\pm$ 1.9 (15.1–22.2)
b	6.3 $\pm$ 1.0 (4.5–8.7)	5.9 $\pm$ 0.5 (4.8–6.4)	4.4 $\pm$ 0.3 (4.1–4.9)
T	61.0 $\pm$ 4.2 (56.7–68.0)	61.7 $\pm$ 3.8 (57.1–69.5)	64.6 $\pm$ 3.8 (57.4–69.6)
Spicules	48.3 $\pm$ 2.7 (43–53)	54.4 $\pm$ 2.8 (48.5–57.2)	44.6 $\pm$ 2.5 (40–48)
Gubernaculum	21.3 $\pm$ 1.7 (19–24)	15.7 $\pm$ 1.6 (12–18)	20.3 $\pm$ 1.3 (19–22)
Cloaca to SP1	27.7 $\pm$ 3.4 (21.5–34)	37.5 $\pm$ 3.6 (33–44)	27.5 $\pm$ 2.0 (24–30.5)
SP1-SP2	35.5 $\pm$ 4.2 (27–40)	45.2 $\pm$ 5.8 (39.5–59)	40.2 $\pm$ 5.2 (30.5–51)
SP2-SP3	50.3 $\pm$ 10.6 (30.5–65)	51.8 $\pm$ 8.3 (36–65)	44.9 $\pm$ 4.0 (38–50)

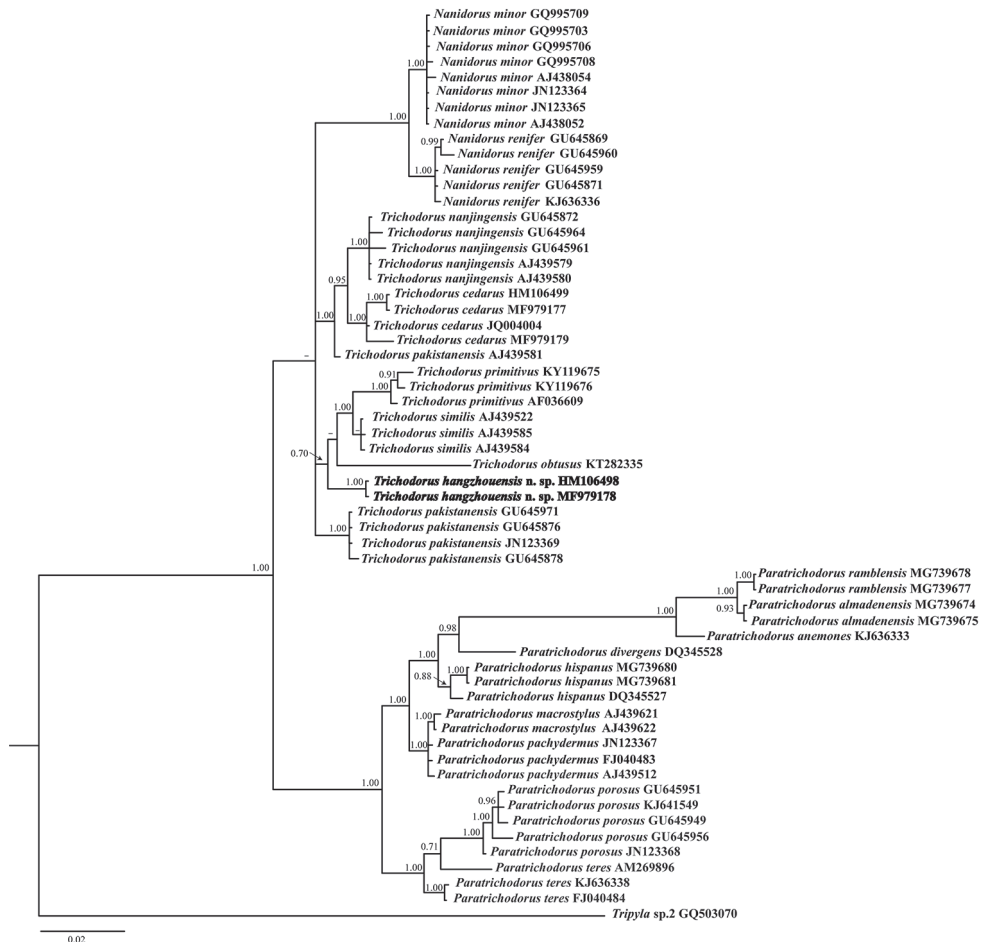


Figure 7. Phylogenetic relationships of *Trichodorus hangzhouensis* sp. nov. and other trichodoridae species based on partial 18S rRNA gene sequences. The Bayesian tree was inferred under the general time-reversible model of sequence evolution with correction for invariable sites and a gamma-shaped distribution (GTR+I+G). *Tripyla* sp. served as an outgroup species. Posterior probability values exceeding 70% are given on appropriate clades.

Discussion

Among trichodoridae, *Trichodorus*, *Nanidorus* and *Paratrachodorus* are cosmopolitan genera, species of those genera have been reported from all the continents except Antarctica (Decraemer and Robbins 2007). Regional endemicity has been observed for *Trichodorus* and *Paratrachodorus* species. Subbotin et al. (2019) stated that Californian populations of *Trichodorus* may be endemic originating in the same region, and hypothesized that this is an apparent centre of speciation, in addition to the Iberian Peninsula (Decraemer et al. 2013) and Irano-Anatolian region (Pedram et al. 2015,

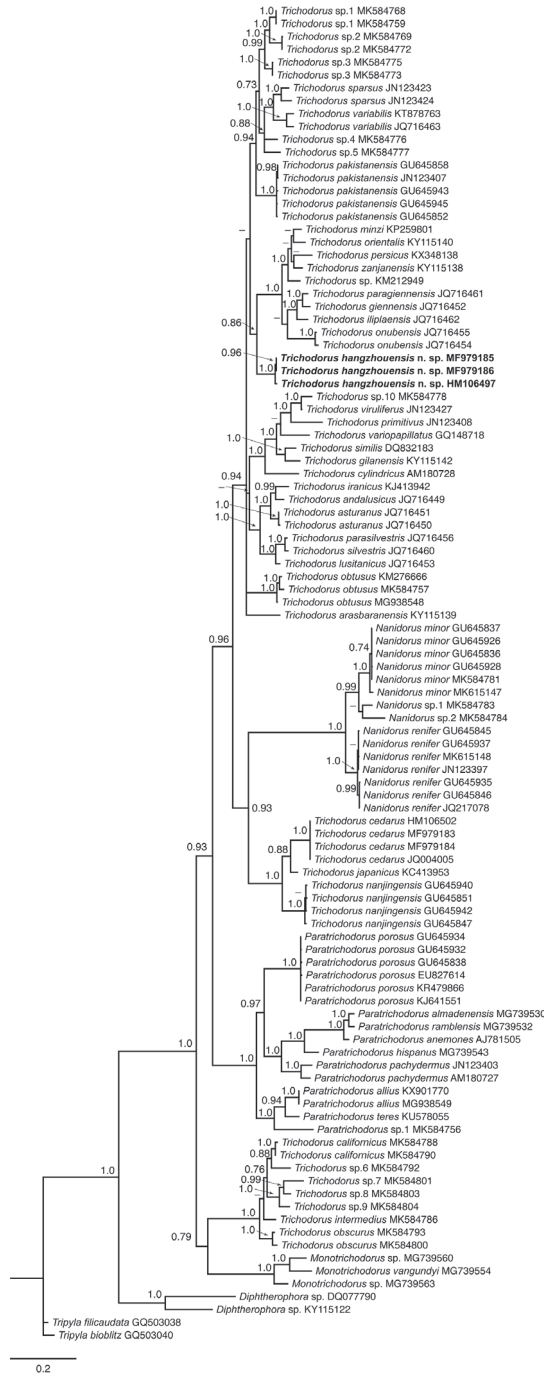


Figure 8. Phylogenetic relationships of *Trichodorus hangzhouensis* sp. nov. and other trichodoridae species based on D2-D3 28S rRNA gene sequences. The Bayesian tree was inferred under the general time-reversible model of sequence evolution with correction for invariable sites and a gamma-shaped distribution (GTR+I+G). *Tripylina tamaki* served as an outgroup species. Posterior probability values exceeding 70% are given on appropriate clades.

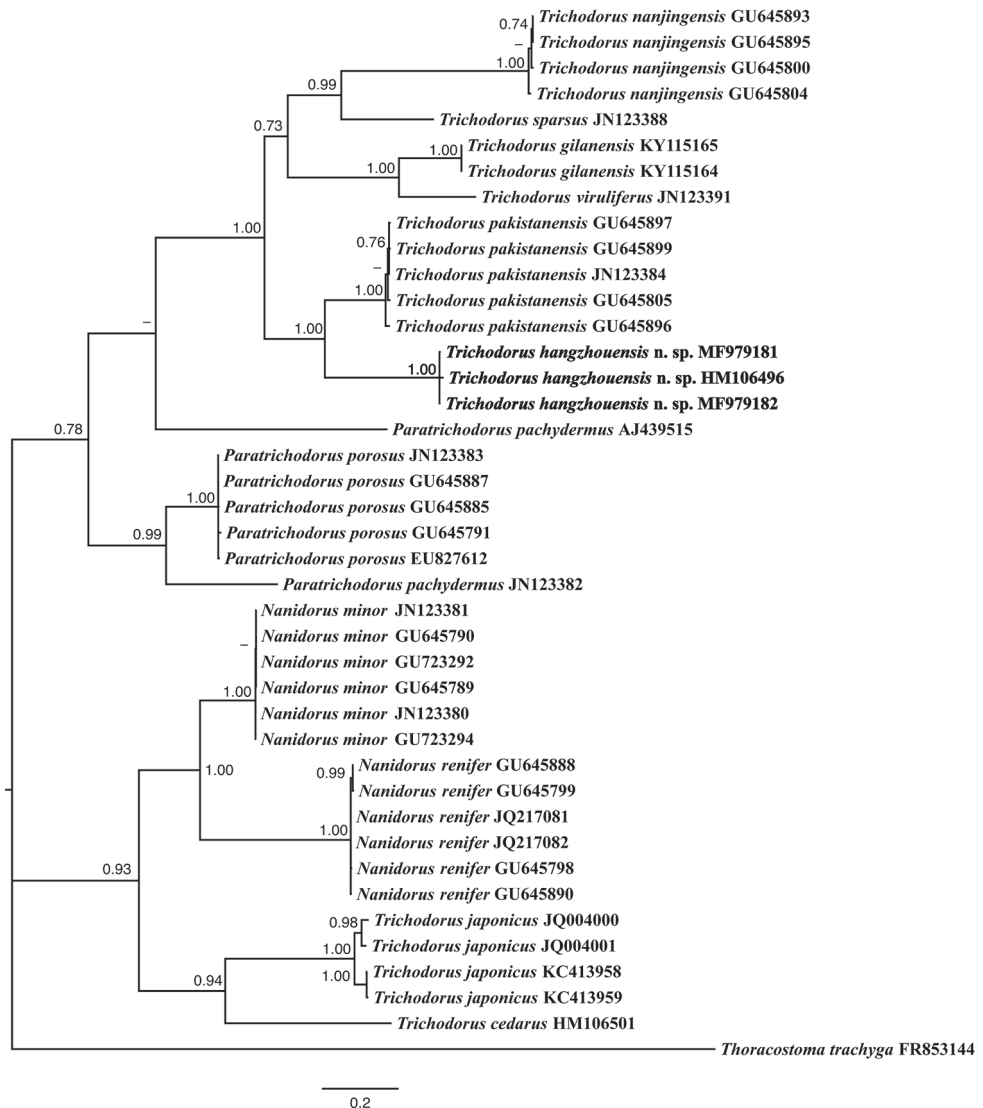


Figure 9. Phylogenetic relationships of *Trichodorus hangzhouensis* sp. nov. and other trichodorid nematodes based on ITS2 sequences. The Bayesian tree was inferred under the general time-reversible model of sequence evolution with correction for invariable sites and a gamma-shaped distribution (GTR+I+G). *Thoracostoma trachyga* served as an outgroup species. Posterior probability values exceeding 70% are given on appropriate clades.

2017, Asghari et al. 2018). The distribution of trichodorids throughout Asia (except for Iran) is not well documented but in the distribution data of EPPO 2014, trichodorids are either present or widespread in Afghanistan, Bahrain, China, India, Indonesia, Japan, Korea, Turkey, and Uzbekistan. Taxonomic and faunistic records presently

list 10 trichodorids (six *Trichodorus* spp., two *Paratrichodorus* spp. and two *Nanidorus* spp.) from China, which represents a comparatively low diversity, and possibly reflects the relatively few studies conducted; so far about half of the territory of the country has been observed for this nematode group with different intensity of sampling. *Trichodorus hangzhouensis* sp. nov. seems to represent another endemic for China in addition to *T. nanjingensis* and *T. guangzhouensis* which are reported only for this country so far.

The D2-D3 region of the 28S rDNA gene has been shown to be of importance in trichodorid molecular taxonomy (Subbotin et al. 2019). The phylogenetic analysis inferred from this gene sequences revealed four highly supported clades. The first is the largest one and consists of *Trichodorus* species from Europe, Asia and the USA including *T. hangzhouensis* sp. nov., and corresponds to the Clade I according to Subbotin et al. (2019), the second clade includes *Nanidorus* species and *Trichodorus* species from Asia (corresponds to the Clade II (Subbotin et al. 2019)), the third clade consists of *Paratrichodorus* species distributed in USA, Europe and Asia (Clade III in Subbotin et al. 2019), the fourth clade includes *Trichodorus* and *Monotrichodorus* from the USA (Clade IV and V according to the same authors). These results are consistent also with other previously published studies (Asghari et al. 2018, Decraemer et al. 2019). It is interesting to mention that the three species occurring in southeastern Asia (*T. cedarus*, *T. nanjingensis* and *T. japonicus*) form a highly supported subclade within Clade II, while the new species is part of another phylogenetically more distant group (Clade I). However, in the ITS2 tree the position of *T. nanjingensis* differs substantially.

In addition, the trichodorid species molecularly characterized during this study (*T. nanjingensis*, *T. cedarus*, *T. pakistanensis*, *P. porosus*, *N. minor*, *N. renifer*) clustered with the known species from different countries; these results further validated their identity. It is also noted that the position of *T. hangzhouensis* sp. nov. differs more or less in the phylogenetic trees based on the different gene sequences, and this could be also caused by the incomplete sequence data for a given species.

All present, and most previous, phylogenetic reconstructions inferred from three different gene sequences (18S, D2D3 28S and ITS2) showed that *Nanidorus* and *Paratrichodorus* species each formed highly supported clades. *Trichodorus* species studied molecularly so far take three different positions based on D2D3 28S r RNA gene sequences: i) the large part containing only *Trichodorus* species and forming Clade I sensu Subbotin et al. (2019); ii) three species of southeastern Asian origin clustering together with *Nanidorus* species and iii) Californian species forming a highly supported clade.

All the three aforementioned genera occur in China. From our observations, trichodorids seem not host specific and can be found in various types of ecosystems. The occurrence of *Trichodorus*, *Nanidorus* and *Paratrichodorus* recovered from soils in China is quite low (4.1%) compared to trichodorid occurrence in other countries such as Great Britain (22%), Italy (9.6%), Iran (7%), Belgium (19.6), Portugal (32.6%) and Slovak Republic (33%) (Alphey and Boag 1976, Roca and Lamberti 1985, De Waele and Sturhan 1987, De Waele and Coomans 1991, Almeida 1993, Lišková and Sturhan 1999).

In the past surveys concerning the stubby root nematodes, this group is reported being generally in somewhat low densities (Aballay and Eriksson 2006). The low density

of trichodorids populations in the soil could be related to the sampling strategies (depth and intensity of sampling) or studied plants (crops or natural vegetation). Boag (1981) suggested that the distribution of trichodorids is correlated with soil moisture, particle size structure and seasonal fluctuation of temperature. De Waele and Coomans (1991) also recognized that geographic distribution of certain trichodorid species may be influenced by their habitat and found a relatively high presence of populations in soil with a pH <5.5.

In conclusion, this study provides a morphological and molecular characterization of *T. hangzhouensis* sp. nov. and three known trichodorid species together with updated records of this group in China. Among 164 populations recovered in China, the highest number of records is for *P. porosus* (42.6%) followed by *T. nanjingensis* (39.6%). The systematics and diagnostics of trichodorid nematodes are important because of regulatory and management issues attributed to this group of nematodes being vectors of tobra viruses. Thus, updated descriptions based on sufficient examination material and accurately identified specimens, coupled with molecular analysis are necessary for better understanding of the current distribution and host association of this complex group of nematodes.

Acknowledgments

This research was supported by the National Natural Science Foundation of China (No: 31371921) and a Sino-Bulgaria government cooperation project (No. 14-7 and ДНТС Китай 01/03).

References

- Aballay E, Eriksson B (2006) Trichodorid nematodes in the central area of Chile. *Nematologia mediterranea* 34: 43–48. <https://journals.flvc.org/nemamed/article/view/86885>
- Allen MW (1957) A review of nematode genus *Trichodorus* with descriptions of ten new species. *Nematologica* 2: 32–62. <https://doi.org/10.1163/187529257X00635>
- Almeida MT (1993) Characterization and ecology of Portuguese populations of *Trichodorus* spp. and *Paratrichodorus* spp. (Nematoda: Trichodoridae). Ph.D. Thesis, Universidade do Minho, Braga, Portugal, 567 pp.
- Alphey TJW, Boag B (1976) Distribution of trichodorid nematodes in Great Britain. *Annals of Applied Biology* 84: 371–381. <https://doi.org/10.1111/j.1744-7348.1976.tb01780.x>
- Asghari R, Eskandari A, Tanha Maafi Z, Decraemer W (2018) Characterisation of a new species of *Trichodorus* Cobb, 1913 (Triplonchida: Trichodoridae) from Iran based on morphological and molecular data. *Systematic Parasitology* 95: 257–269. <https://doi.org/10.1007/s11230-018-9780-6>
- Boag B (1981) Observations on the population dynamics and vertical distribution of trichodorid nematodes in a Scottish forest nursery. *Annals of Applied Biology* 984: 63–469. <https://doi.org/10.1111/j.1744-7348.1981.tb00778.x>

- Boutsika K, Brown DJE, Phillips MS, Blok VC (2004) Molecular characterisation of the ribosomal DNA of *Paratrichodorus macrostylus*, *P. pachydermus*, *Trichodorus primitivus* and *T. similis* (Nematoda: Trichodoridae). *Nematology* 6: 641–654. <https://doi.org/10.1046/j.1365-3059.2003.00938.x>
- Chi YL, Zhang WD, Wu CK, Shao XY, Liao L, Chen QW (2011) Investigation of Longidoridae and Trichodoridae in the landscape plants of Guangdong. *Plant Quarantine* 25: 88–89.
- Cobb NA (1913) New nematode genera found inhabiting fresh water and non brackish soils. *Journal of the Washington Academy of Sciences* 9: 217–343. <https://doi.org/10.5962/bhl.part.20323>
- Colbran RC (1956) Studies of plant and soil nematodes. 1. Two new species from Queensland. *Queensland Journal of Agricultural Science* 13: 123–126.
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772. <https://doi.org/10.1038/nmeth.2109>
- De Grisse AT (1969) Redescription ou modifications de quelques techniques utilisées dans l'étude de nématodes phytoparasitaires. *Mededelingen Rijksfaculteit Landbouw Wetenschappen Gent* 34: 315–359.
- De Ley P, Félix MA, Frisse LM, Nadler SA, Sternberg PW, Thomas WK (1999) Molecular and morphological characterisation of two reproductively isolated species with mirror-image anatomy (Nematoda: Cephalobidae). *Nematology* 2: 591–612. <https://doi.org/10.1163/156854199508559>
- De Man JG (1880) Die einheimischen, frei in der reinen Erde und im süßen Wasser lebenden Nematoden. *Tijdschrift nederlandshe dierkundige Vereeniging* 5: 1–104.
- De Waele D, Coomans A (1991) Occurrence and ecology of trichodorid nematodes in Belgium. *Revue de Nématologie* 14: 127–132.
- De Waele D, Cohn E (1992) Trichodoridae from Israel, with a description of *Trichodorus minzi* n. sp. *Fundamental and applied Nematology* 15: 201–207.
- De Waele D, Hashim Z (1983) *Trichodorus orientalis* n. sp. (Nematoda: Trichodoridae) from Jordan and Iran. *Systematic Parasitology* 6: 63–67. <https://doi.org/10.1007/BF00752618>
- De Waele D, Sturhan D (1987) *Trichodorus persicus* n. sp. (Nematoda: Trichodoridae) from Iran. *Systematic Parasitology* 10: 79–83. <https://doi.org/10.1007/BF00009103>
- Decraemer W, Baujard PA (1998) Polytomous key the identification of species of the family Trichodoridae Thorne, 1935 (Nematoda: Triplonchida). *Fundamental and applied Nematology* 21: 37–62.
- Decraemer W, Cantalapiedra-Navarrete C, Archidona-Yuste A, Varela-Benavides I, Gutiérrez-Gutiérrez C, Castillo P, Palomares-Rius JE (2019) Integrative taxonomy unravels cryptic diversity in the *Paratrichodorus hispanus*-group complex and resolves two new species of the genus and the molecular phylogeny of the family (Nematoda: Trichodoridae). *Zoological Journal of the Linnean Society* 3: 656–692. <https://doi.org/10.1093/zoolinnean/zly059>
- Decraemer W, Cheng H (1994) On the occurrence of an inner stylet in adult trichodorids and additional information on *Trichodorus nanjingensis* Liu & Cheng, 1990. *Fundamental and applied Nematology* 17: 503–507. https://horizon.documentation.ird.fr/exl-doc/pleins_textes/fan/40820.pdf

- Decraemer W, Palomares-Rius JE, Cantalapiedra-Navarrete C, Landa BB, Duarte I, Almeida T, Vovlas N, Castillo P (2013) Seven new species of *Trichodorus* (Diphtherophorina, Trichodoridae) from Spain, an apparent centre of speciation. *Nematology* 15: 57–100. <https://doi.org/10.1163/156854112X645598>
- Decraemer W, Robbins RT (2007) The who, what and where of Longidoridae and Trichodoridae. *Journal of Nematology* 39: 295–297. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2586508/pdf/295.pdf>
- Decraemer W, Roca F, Castillo P, Peña-Santiago R, Gomez-Barcina A (1993) Trichodoridae from southern Spain, with description of *Trichodorus giennensis* n. sp. (Nemata: Trichodoridae). *Fundamental and Applied Nematology* 16: 407–500.
- Ding XF, Wang X, Li X, Li ZP (2015) Investigation and identification of parasitic nematodes extracted from rhizosphere of sugarcane in Hainan Island. *Sugarcane and Cane Sugar* 5: 17–22.
- Duarte IM, de Almeida MTM, Brown DJF, Marques I, Neilson R, Decraemer W (2010) Phylogenetic relationships based on SSU rDNA sequences, among the didelphic genera of the family Trichodoridae from Portugal. *Nematology* 12: 171–180. <https://doi.org/10.1163/156854109X461721>
- Du Y, He WZ, Hao WG, Li SG, Duan LH, Zhou J (2010) Description of *Trichodorus cedrus*, a new record on banana. *Plant protection* 36: 170–172.
- EPPO (2014). EPPO Global Database. <https://gd.eppo.int>
- Eroshenko AS, Teplyakov AA (1975) A new species of ectoparasitic nematode in *Trichodorus* from coniferous forest in the Far East. *Parasitology* 9: 545–547. [In Russian]
- Guo HB (2008) Cultivation of lotus (*Nelumbo nucifera* Gaertn. ssp. *nucifera*) and its utilization in China. *Genetic Resources and Crop Evolution* 56: 323. <https://doi.org/10.1007/s10722-008-9366-2>
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.
- Hao YJ, Zhai H, Wang SH (1998) Study on the pathogenesis of *Trichodorus nanjingensis* to *Mains baccata*. *Journal of Fruit Science* 15: 26–29.
- Hooper DJ (1963) *Trichodorus viruliferus* sp. nov. (Nematoda: Dorylaimida). *Nematologica* 9: 200–204. <https://doi.org/10.1163/187529263X00368>
- Jenkins WT (1964) A rapid centrifugal-flotation technique for separating nematodes from soil. *Plant Disease Reporter* 48: 692.
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780. <https://doi.org/10.1093/molbev/mst010>
- Larget B, Simon DL (1999) Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. *Molecular Biology and Evolution* 16: 750–759. <https://doi.org/10.1093/oxfordjournals.molbev.a026160>
- Larsson A (2014) AliView: a fast and lightweight alignment viewer and editor for large datasets. *Bioinformatics* 30: 3276–3278. <https://doi.org/10.1093/bioinformatics/btu531>
- Lin LF, Li WF, Hu XQ, Liu CG (2009) Identification of the nematodes causing the stubby root disease in rhizospheric soils of *Hydrangea macrophylla*. 35(2): 131–133.

- Li X, Guo K, Zhang Y, Yan X, Zheng J (2010) First Report of the Stubby Root Nematode, *Paratrichodorus minor*, in Mainland China. *Plant Disease* 94: 376. <https://doi.org/10.1094/PDIS-94-3-0376A>
- Lišková M, Sturhan D (1999) The occurrence and distribution of *Trichodorus* and *Paratrichodorus* spp. (Nematoda: Trichodoridae) in the Slovak Republic. *Nematology* 1(6): 631–636. <https://doi.org/10.1163/156854199508586>.
- Liu GK, Zhang SS (1999) Identification of parasitic nematodes on longan in Fujian, China. *Journal of Fujian Agricultural University* 28: 59–65.
- Liu RJ (1994) Further studies with redescrptions on *Paratrichodorus porosus* (Allen, 1957) Siddiqi, 1974 in China. *Journal of Laiyang Agricultural College* 11: 37–41.
- Liu RJ, Cheng HR (1990) Investigation and identification of Nematoda: Trichodoridae. *Journal of Nanjing Agriculture University* 13: 50–54.
- Maafi ZT, Decraemer W (2002) Description of *Trichodorus gilanensis* n. sp. from a forest park in Iran and observations on *Paratrichodorus tunisiensis* (Siddiqi, 1963) Siddiqi, 1974 (Nematoda: Diphtherophorina). *Nematology* 4(1): 43–54. <https://doi.org/10.1163/156854102760082195>
- MacFarlane SA, Neilson R, Brown DJF (2002) Nematodes. *Advances in Botanical Research* 36: 169–198. [https://doi.org/10.1016/S0065-2296\(02\)36063-4](https://doi.org/10.1016/S0065-2296(02)36063-4)
- Micoletzky H (1922) Die freilebende Erd-Nematoden. *Archiv für Naturgeschichte A*. 87: 1–650.
- Pan CS, Zheng JW, Zhou XP, Neilson R, Brown DJF (2000) Preliminary assessment of the occurrence of longidorid and trichodorid nematodes (Nematoda: Longidoridae and Trichodoridae) in Xiamen, Fujian Province, China. *Russian Journal of Nematology* 8: 153–159.
- Pedram M, Pourjam E, Panahandeh Y, Soleymanzadeh M, Atighi MR, Roshan-Bakhsh A, Ehsani A (2015) Additional data on Iranian trichodorids (Triplonchida: Trichodoridae) and proposal for a new synonymy. *Nematology* 17: 1185–1191. <https://doi.org/10.1163/156854111-00002933>
- Pedram M, Roshan-Bakhsh A, Pourjam E, Atighi MR, Decraemer W, Bagheri A (2017) Additional data on Iranian trichodorids (Triplonchida: Trichodoridae) and first record of a rare species, *Trichodorus variabilis* Roca, 1998. *Nematology* 19: 121–129. <https://doi.org/10.1163/156854111-00003036>
- Roca F, Lamberti F (1985) Distribution of Longidoridae, Xiphinemidae and Trichodoridae. In: Alphey TJW (Ed.) *Atlas of Plant Parasitic Nematodes of Italy*. Dundee, UK. Scottish Crop Research Institute, 36–44.
- Rodriguez-M R, Sher SA, Siddiqi MR (1978) Systematics of the monodelphic species of Trichodoridae (Nematoda: Diphtherophorina) with description of a new genus and four ne species. *Journal of Nematology* 10: 141–152.
- Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574. <https://doi.org/10.1093/bioinformatics/btg180>
- Seinhorst JW (1959) A rapid method for the transfer of nematodes from fixative to anhydrous glycerine. *Nematologica* 4: 67–69. <https://doi.org/10.1163/187529259X00381>
- Seinhorst JW (1963) A redescription of the male of *Trichodorus primitivus* (de Man, 1880), and a description of a new species *Trichodorus similis*. *Nematologica* 9: 125–130. <https://doi.org/10.1163/187529263X00232>

- Shishida Y (1979) Studies on nematodes parasitic on woody plants. 1. Family Trichodoridae (Thome, 1935) Clark, 1961. Japanese Journal of Nematology 9: 28–44.
- Siddiqi MR (1962) *Trichodorus pakistanensis* n. sp. (Nematoda: Trichodoridae) with observations on *T. porosus* Allen, 1957, *T. mirzai* Siddiqi, 1960, and *T. minor*, Colbran, 1956, from India. Nematologica 8: 193–200. <https://doi.org/10.1163/187529262X00422>
- Siddiqi MR (1974) Systematics of the genus *Trichodorus* Cobb, 1913 (Nematoda: Dorylaimida), with description of three new species. Nematologica 19: 259–278.
- Siddiqi MR (2002) *Ecuadorus equatorius* gen. n., sp. n. and *Nanidorus mexicanus* sp. n. (Nematoda: Trichodoridae). International Journal of Nematology 12: 197–202.
- Siddiqi MR, Sharma SB (1995) *Trichodorus reduncus* sp. n. and *Tylenchorhynchus* (*Divittus*) *dispersus* sp. n. associated with groundnut in Vietnam. Afro-Asian journal of Nematology 5: 48–52.
- Stöver BC, Müller KF (2010) TreeGraph 2: combining and visualizing evidence from different phylogenetic analyses. BMC Bioinformatics 11: 7. <https://doi.org/10.1186/1471-2105-11-7>
- Subbotin SA, Del Prado Vera CI, Inserra RN, Chizhov VN, Decraemer W (2019) Molecular characterisation of some stubby root nematodes (Nematoda: Trichodoridae) from the USA and other countries. Nematology 22: 39–52. <https://doi.org/10.1163/15685411-00003279>
- Szczygiel A (1968) *Trichodorus sparsus* sp. n. (Nematoda: Trichodoridae). Bulletin de l'Académie polonaise des Sciences. Classe II 16: 695–698.
- Vrain TC, Wakarchuk DA, Levesque AC, Hamilton RI (1992) Intraspecific rDNA restriction fragment length polymorphism in the *Xiphinema americanum* group. Fundamental and Applied Nematology 15: 563–573.
- Waele D, Coomans A (1991) Occurrence and ecology of trichodorid nematodes in Belgium. Revue de Nématologie 14: 127–132.
- Waite IS, O'Donnell AG, Harrison A, Davies JT, Colvan SR, Ekschmitt K, Dogan H, Wolters V, Bongers T, Bongers M, Bakonyi G, Nagy P, Papatheodorou EM, Stamou GP, Bostrom S (2003) Design and evaluation of nematode 18S rDNA primers for PCR and denaturing gradient gel electrophoresis (DGGE) of soil community DNA. Soil Biology and Biochemistry 35: 1165–1173. [https://doi.org/10.1016/S0038-0717\(03\)00177-9](https://doi.org/10.1016/S0038-0717(03)00177-9)
- Wang S (1993) Occurrence of *Trichodorus pakistanensis* in apple orchards and its relation to growth vigor of trees. Deciduous Fruits 1: 13–15.
- Wang S, Chiu WF, Yu C, Li C, Robbins RT (1996) The occurrence and geographical distribution of longidorid and trichodorid nematodes associated with vineyards and orchards in China. Russian Journal of Nematology 4: 145–153.
- Wang SH, Miao ZQ, Zhang JF (1994) Incidence of apples stubby root disease and species identification of pathogenetic nematodes. Acta Phytopathologica Sinica 24: 62–66.
- Xie H, Feng ZX, Zhao LR (2000) Description of *Trichodorus guangzhouensis* n. sp. (Nematoda: Trichodoridae). Acta Phytopathologica Sinica 43: 444–448.
- Xie QY, Zhang HB, Zheng W, Wang JW, Zheng JW (2007) Investigation on species of plant parasitic nematodes in bamboo seedlings exported from Huzhou. Plant Quarantine 21: 49–50.
- Xu J, Decraemer W (1995) *Trichodorus* species from China, with a description of *T. paracedarus* n. sp. (Nematoda: Trichodoridae). Fundamental and applied Nematology 18: 455–464.
- Xu YM, Zhao ZQ (2019) Longidoridae and Trichodoridae (Nematoda: Dorylaimida and Triplonchida). 79, 149 pages. Lincoln, N.Z. Landcare Research.

- Yan XN, Zheng FC, Lin MS (2005) Investigation and identification of nematode parasites of banana in Guangdong and Guangxi. *Chinese Journal of Tropical Agriculture* 25: 4–8.
- Ye W, Giblin-Davis RM, Braasch H, Morris K, Thomas WK (2007) Phylogenetic relationships among *Bursaphelenchus* species (Nematoda: Parasitaphelenchidae) inferred from nuclear ribosomal and mitochondrial DNA sequence data. *Molecular Phylogenetics and Evolution* 43: 1185–1197. <https://doi.org/10.1016/j.ympev.2007.02.006>
- Yin G, Feng Z (1981) Preliminary investigations on nematode parasites of agricultural crops. *Acta Phytopathologica Sinica* 8: 116–126.
- Yokoo T (1964) On the stubby root nematodes from the western Japan. *Agriculture Bulletin Saga University* 20: 57–62.
- Zeng Y, Ye W, Yang L, Huang Y, Zhao K, Zhang Z, Liang H, Kerns J (2014) Morphological and molecular characterization of two isolates of *Paratrichodorus porosus* from Shenzhen, China. *Helminthologia* 51: 323–330. <https://doi.org/10.2478/s11687-014-0248-1>
- Zhang SS, Chen YB (1994) Studies on nematodes of Bayberry (*Myrica rubra*). *Journal of Fujian University (Natural Sciences Edition)* 23: 172–177.
- Zhang SS, Xiao RF, Lin NQ, Ai HM (2002) Identification of parasitic nematode species from Chinese olive in Fujian, China. *Journal of Fujian Agriculture and Forestry University (Natural Science Edition)* 3: 445–451.
- Zhao LR, Xie H, Wu MT, Zhong GQ, Feng ZX (2005) Description of Trichodoridae nematodes from rhizosphere of pear trees in Kunming region. *Journal of South China Agricultural University* 26: 59–61.
- Zhao L, Xie H, Gu J, Xu C (2013) Description of *Trichodorus japonicus* n. sp. (Triplonchida: Trichodoridae) from Japan. *Nematology* 15: 783–793. <https://doi.org/10.1163/15685411-00002718>
- Zheng J, Jiang L, Liang D, Brown DJF (2004) Three trichodorid species (Nematoda: Trichodoridae) occurring in China. *Helminthologia* 41: 39–44.
- Zheng J, Subbotin SA, He S, Gu J, Moens M (2003) Molecular characterisation of some Asian isolates of *Bursaphelenchus xylophilus* and *B. mucronatus* using PCR-RFLPs and sequences of ribosomal DNA. *Russian Journal of Nematology* 11: 17–22.

Supplementary material I

Table S1. Sequences of nematode species used for the phylogenetic analyses

Authors: Xuqing Li, Munawar Maria, Ruihang Cai, Eda Marie Barsalote, Vlada Peneva, Jingwu Zheng

Data type: species data

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/zookeys.945.50424.suppl1>

