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Phylogenetic relationships within the Phyllidiidae (Opisthobranchia, Nudibranchia)

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Abstract

The Phyllidiidae (Gastropoda, Heterobranchia, Nudibranchia) is a family of colourful nudibranchs found on Indo-Pacific coral reefs. Despite the abundant and widespread occurrence of many species, their phylogenetic relationships are not well known. The present study is the first contribution to fill the gap in our knowledge on their phylogeny by combining morphological and molecular data. For that purpose 99 specimens belonging to 16 species were collected at two localities in Indonesia. They were photographed and used to make a phylogeny reconstruction based on newly obtained cytochrome oxidase subunit (COI) sequences as well as sequence data from GenBank. All mitochondrial 16S sequence data available from GenBank were used in a separate phylogeny reconstruction to obtain information for species we did not collect. COI data allowed the distinction of the genera and species, whereas the 16S data gave a mixed result with respect to the genera *Phyllidia* and *Phyllidiella*. Specimens which could be ascribed to species level based on their external morphology and colour patterns showed low variation in COI sequences, but there were two exceptions: three specimens identified as *Phyllidia* cf. *babai* represent two to three different

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species, while *Phyllidiella pustulosa* showed highly supported subclades. The barcoding marker COI also confirms that the species boundaries in morphologically highly variable species such as *Phyllidia elegans, P. varicosa,* and *Phyllidiopsis krempfi*, are correct as presently understood. In the COI as well as the 16S cladogram *Phyllidiopsis cardinalis* was located separately from all other Phyllidiidae, whereas *Phyllidiopsis fissuratus* was positioned alone from the *Phyllidiella* species by COI data only. Future studies on phyllidiid systematics should continue to combine morphological information with DNA sequences to obtain a clearer insight in their phylogeny.

Keywords
COI, Indonesia, mtDNA, nudibranch, phylogenetic relations, 16S

Introduction

Nudibranch gastropod molluscs have traditionally been classified with the Infraclass Opisthobranchia Milne Edwards, 1848, which consists of more than 6000 species (Yonow 2008). Although this taxon is not monophyletic and therefore is considered obsolete (Schrödl et al. 2011), taxonomic works still refer to "opisthobranchs" for practical reasons (e.g. Uribe et al. 2013) and Opisthobranchia is considered an "Informal Group" among the Heterobranchia (Wägele et al. 2014). These animals form, ecologically and morphologically, one of the most diverse groups of marine gastropods (Wägele et al. 2014). To avoid use of their misnomer, this well-known group of marine animals can also be referred to as sea slugs (Yonow 2015). Among these, the Nudibranchia Cuvier, 1817 form the largest order with an estimated number of more than 2000 species (Gosliner et al. 2008), although also estimates of nearly 3000 species are known (Vonnemann et al. 2005).

Much work has already been done to elucidate the phylogeny of the opisthobranchs by molecular analyses (e.g., Wollscheid and Wägele 1999, Grande et al. 2004a, 2004b, Vonnemann et al. 2005, Turner and Wilson 2008, Maeda et al. 2010, Pola and Gosliner 2010), but most of the phylogenetic relationships still remain unclear at family, genus, and species level, especially with regards to the nudibranchs. All nudibranch species and many other sea slugs are predators, which usually can be observed together with their prey (Behrens 2005, Pola and Gosliner 2010, van Alphen et al. 2011). Only rarely they are found together with potential predators such as sea anemones, mushroom corals, and pycnogonids (Piel 1991, Behrens 2005, van der Meij and Reijnen 2012, Mehrotra et al. 2015).

The present study aims to clarify the phylogenetic relationships within the Phyllidiidae Rafinesque, 1814, belonging to the Doridacea (Bouchet and Rocroi 2005). This family consists of more than 100 species divided over five genera: *Ceratophyllidia* Eliot, 1903, *Phyllidia* Cuvier, 1797, *Phyllidiella* Bergh, 1869, *Phyllidiopsis* Bergh, 1875, and *Reticulidia* Brunckhorst, 1990 (Bouchet 2015). The genera *Fryeria* JE Gray, 1853, and *Reyfria* Yonow, 1986, have been synonymised with *Phyllidia* (Valdés and Gosliner 1999).

Most nudibranchs of the family Phyllidiidae are commonly encountered on coral reefs, where they can easily be noticed because of their aposomatic colouration, which serves to deter possible predators from eating them (Ritson-Williams and Paul 2007). Nevertheless, only eight phyllidiid COI sequences can be found in GenBank, as well as two 18S sequences and 17 16S sequences. There are only a few published studies that incorporate even a single member of Phyllidiidae into a phylogenetic tree (e.g. Wollscheid-Lengeling et al. 2001) and even fewer deal with phylogenetic relationships among Phyllidiidae. Among the latter, most are using anatomical characters (Brunckhorst 1993, Valdés and Gosliner 1999, Valdés 2001, 2002) and only two are known to include a molecular and phylogenetic analysis (Valdés 2003, Cheney et al. 2014).

Phyllidiid slugs are characterized by their oval elongate and tough bodies, which generally possess hard notal tubercles on the dorsal side. Although their colouration is a main character used for their identification, many species cannot be identified based on colouration alone owing to their high intra-specific colour variation. Structure and pattern of the notal tubercles are important characters for identification. Other distinctive features of the Phyllidiidae are the retractile lamellate rhinophores, the compact digestive gland mass, and the triaulic reproductive system (Brunckhorst 1993). Another important character diagnosing the Phyllidiidae is the possession of numerous subdermal calcareous spicules of different microstructures (Chang et al. 2013). The Phyllidiidae have no jaws or radula and lack the dorsal, circumanal circlet of gills that is typical of other dorids (Brunckhorst 1993).

To study the phylogenetic relationships within the Phyllidiidae, a molecular analysis was performed based on DNA sequence data of the mitochondrial cytochrome oxidase I (COI) gene, combined with external morphological assessments of material collected in two areas in eastern Indonesia, the Raja Ampat islands (West Papua) and Ternate, off western Halmahera (Moluccas). Both locations are situated in the centre of maximum marine biodiversity, also known as the Coral Triangle (Hoeksema 2007). In earlier studies, high numbers of phyllidiid species were recorded from this area: 13 from the Bismarck Sea, Papua New Guinea (Domínguez et al. 2007), eleven from Ambon (Moluccas, Indonesia) (Yonow 2011), and eleven from the South China Sea (Sachidhanandam et al. 2000). Therefore, both of our areas were expected to show a high number of phyllidiid species that could be used for the present study.

Materials and methods

Sampling

Specimens were collected by SCUBA diving in West Papua by Gerard van der Velde in 2007, mostly in the coastal areas of Gam, Kri, Mansuar, and Batanta (Figures 1–2; see Hoeksema and van der Meij 2008). Additional specimens were mainly collected by Joris van Alphen and Nicole de Voogd, and also by Bert Hoeksema, Sancia van der Meij, and other expedition members (Hoeksema and van der Meij 2010) in

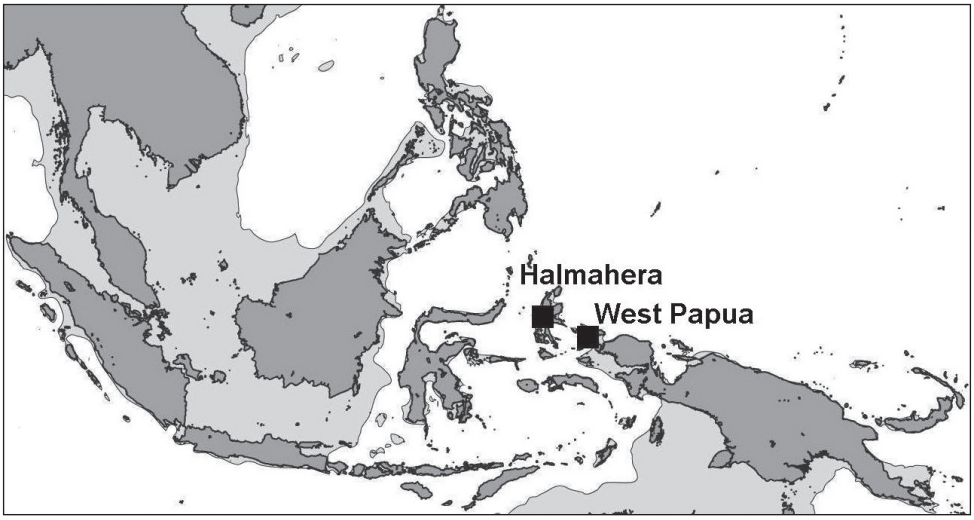

Figure 1. Location of field areas: Halmahera (including Ternate) and West Papua (including Raja Ampat).

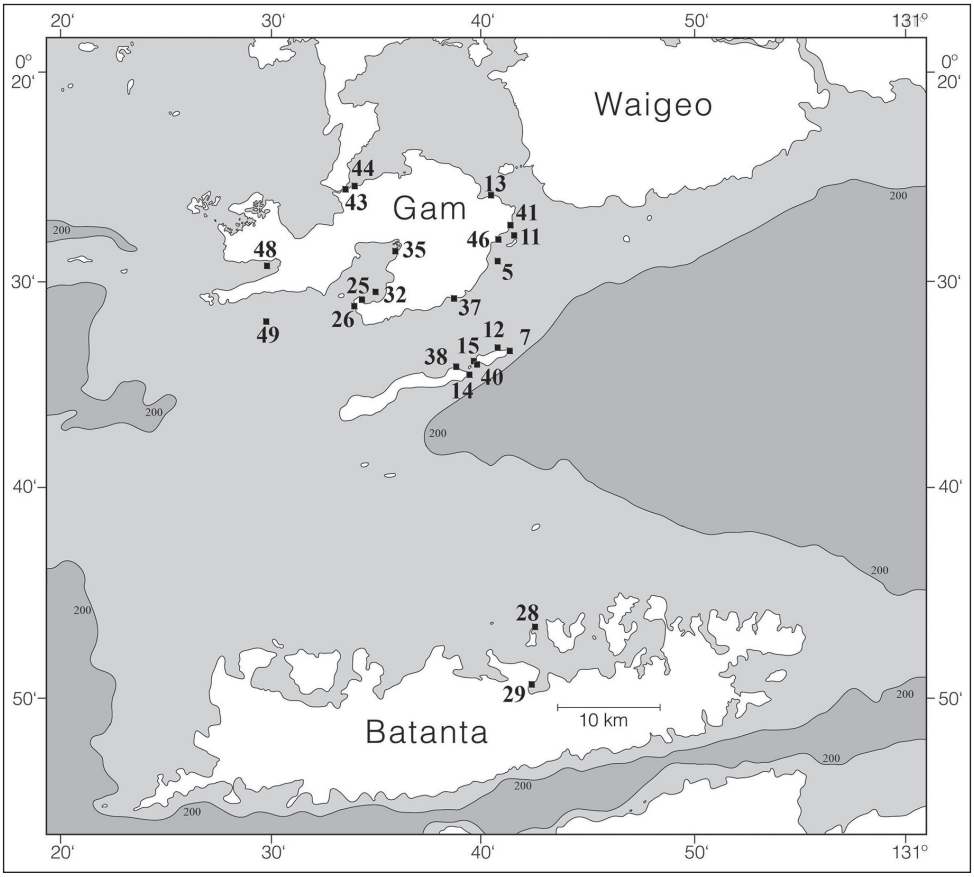

Figure 2. Raja Ampat sites where Phyllidiidae were sampled in 2007.

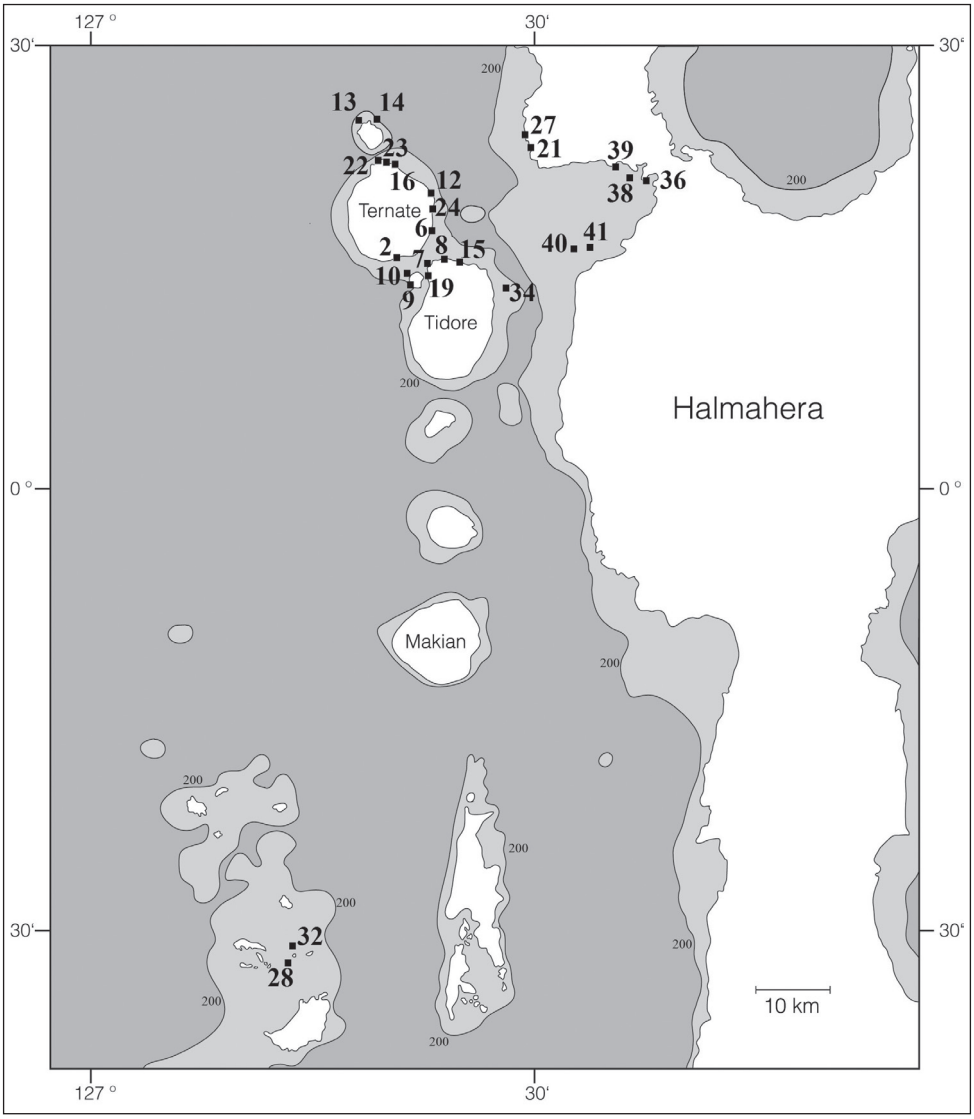

Figure 3. Halmahera and Ternate sites where Phyllidiidae were sampled in 2009.

2009 off Halmahera (northern Moluccas), especially around Ternate (Figures 1, 3). A locality list of the sampling stations is provided in Table 1. Collected slugs were first photographed and subsequently preserved in 96% ethanol (West Papua 2007). Halmahera specimens were transferred into fresh 96% ethanol and labelled in order to prepare them for DNA analysis. These have been deposited in the mollusc collection of Naturalis Biodiversity Center, Leiden (coded as RMNH.Mol.), with the exception of some specimens that dried out after sequencing (Table 1; Figures 5–15; Suppl. material 1: COI sequences).

Table 1. Information on analysed Phyllidiidae species: RMNH.MOL catalogue number or field code number in case voucher specimen became lost; Genbank number if available; collection site, station number (RAJ = Raja Ampat, TER=Ternate, Halmahera), coordinates.

RMNH.MOL or Field nr.	Genbank accession number	Species	Locality	Station	Coordinates
336464	KX235918	*Phyllidia babai*	Tanjung Ebamadu	TER08	N0°45'23.4", E127°24'26.5"
336575	KX235920	*Phyllidia* cf. *babai*	South Gam, shoal near mangroves	RAJ37	S0°31'08.2", E130°38'28.0"
336614	KX235919	*Phyllidia* cf. *babai*	Tanjung Ratemu (South of river)	TER27	N0°54'44.5", E127°29'09.9"
336573	KX235921	*Phyllidia coelestis*	Eastern entrance of passage	RAJ44	S0°25'44.3", E130°33'56.8"
336574	KX235922	*Phyllidia coelestis*	Wallace Lake	RAJ13	S0°26'31.1", E130°41'08.0"
58		*Phyllidia elegans*	Pulau Maka	TER13	N0°54'42.7", E127°18'32.9"
137		*Phyllidia elegans*	Pulau Pilongga, North	TER34	N0°42'49.8", E127°28'45.4"
156		*Phyllidia elegans*	Teluk Dodinga; Karang Ngeli West	TER40	N0°46'25.3", E127°32'22.0"
336475	KX073972	*Phyllidia elegans*	Tanjung Tabam	TER12	N0°50'05.1", E127°23'10.0"
336478	KX073973	*Phyllidia elegans*	Pulau Maka	TER13	N0°54'42.7", E127°18'32.9"
336488	KX073974	*Phyllidia elegans*	Tanjung Pasir Putih	TER16	N0°51'50.4", E127°20'36.7"
336514	KX073975	*Phyllidia elegans*	Dufadufa / Benteng Toloko	TER24	N0°48'49.1", E127°23'21.6"
336515	KX073976	*Phyllidia elegans*	Idem	TER24	N0°48'49.1", E127°23'21.6"
336554	KX073985	*Phyllidia elegans*	Passage	RAJ43	S0°25'45.2", E130°33'37.3"
336555	KX073990	*Phyllidia elegans*	Akber Reef	RAJ14	S0°34'15.2", E130°39'33.7"
336556	KX073988	*Phyllidia elegans*	Passage	RAJ43	S0°25'45.2", E130°33'37.3"
336557	KX073987	*Phyllidia elegans*	Idem	RAJ43	S0°25'45.2", E130°33'37.3"
336558	KX073984	*Phyllidia elegans*	Southwest Pulau Kri	RAJ40	S0°33'58.1", E130°39'46.2"
336559	KX073991	*Phyllidia elegans*	South Gam, shoal near mangroves	RAJ37	S0°31'08.2", E130°38'28.0"
336560	KX073983	*Phyllidia elegans*	Southwest Pulau Kri	RAJ40	S0°33'58.1", E130°39'46.2"
336561	KX073986	*Phyllidia elegans*	Passage	RAJ43	S0°25'45.2", E130°33'37.3"
336562	KX073989	*Phyllidia elegans*	Akber Reef	RAJ14	S0°34'15.2", E130°39'33.7"
336628	KX073977	*Phyllidia elegans*	Pulau Gura Ici, East	TER32	S0°01'17.3", E127°14'17.2"
336629	KX073978	*Phyllidia elegans*	Idem	TER32	S0°01'17.3", E127°14'17.2"
336631	KX073979	*Phyllidia elegans*	Pulau Pilongga, North	TER34	N0°42'49.8", E127°28'45.4"
336632	KX073980	*Phyllidia elegans*	Idem	TER34	N0°42'49.8", E127°28'45.4"

RMNH.MOL or Field nr.	Genbank accession number	Species	Locality	Station	Coordinates
336633	KX073981	*Phyllidia elegans*	Idem	TER34	N0°42'49.8", E127°28'45.4"
336649	KX073982	*Phyllidia elegans*	Teluk Dodinga; Karang Ngeli West	TER40	N0°46'25.3", E127°32'22.0"
336484	KX235923	*Phyllidia exquisita*	Tanjung Ngafauda	TER14	N0°54'38.3", E127°29'20.7"
336494	KX235924	*Phyllidia ocellata*	Southwest of Tobala	TER19	N0°44'56.6", E127°23'13.5"
336563	KX235926	*Phyllidia ocellata*	Southeast Gam, Friwen Wonda	RAJ11	S0°28'29.9", E130°41'54.8"
336564	KX235925	*Phyllidia ocellata*	Idem	RAJ11	S0°28'29.9", E130°41'54.8"
336565	KX235927	*Phyllidia picta*	South Gam, Shoal near mangroves	RAJ37	S0°31'08.2", E130°38'28.0"
336566	KX235929	*Phyllidia picta*	Passage	RAJ43	S0°25'45.2", E130°33'37.3"
336567	KX235928	*Phyllidia picta*	North Batanta, West Telok Gegenlol	RAJ29	S0°49'42.5", E130°42'42.0"
336619	KX235930	*Phyllidia* sp.	Pulau Popaco, East	TER28	S0°01'51.9", E127°14'01.8"
74		*Phyllidia varicosa*	Tanjung Pasir Putih	TER16	N0°51'50.4", E127°20'36.7"
336489	KX235931	*Phyllidia varicosa*	Idem	TER16	N0°51'50.4", E127°20'36.7"
336568	KX235942	*Phyllidia varicosa*	Northeast Pulau Mansuar	RAJ38	S0°34'05.0", E130°38'31.5"
336569	KX235941	*Phyllidia varicosa*	Idem	RAJ38	S0°34'05.0", E130°38'31.5"
336570	KX235943	*Phyllidia varicosa*	North Batanta, West Telok Gegenlol	RAJ29	S0°49'42.5", E130°42'42.0"
336571	KX235938	*Phyllidia varicosa*	South Gam, Eastern entrance Besir Bay, Cape Besir	RAJ25	S0°30'51.5", E130°34'11.5"
336572	KX235940	*Phyllidia varicosa*	Idem	RAJ25	S0°30'51.5", E130°34'11.5"
336604	KX235932	*Phyllidia varicosa*	East side Ternate Harbour (outside)	TER25	N0°46'55.3", E127°23'19.9"
336609	KX235933	*Phyllidia varicosa*	Pasir Lamo (West side)	TER26	N0°53'20.5", E127°27'34.2"
336612	KX235934	*Phyllidia varicosa*	Idem	TER26	N0°53'20.5", E127°27'34.2"
336617	KX235935	*Phyllidia varicosa*	Tanjung Ratemu (South of river)	TER27	N0°54'44.5", E127°29'09.9"
336621	KX235936	*Phyllidia varicosa*	Pulau Popaco E	TER28	S0°01'51.9", E127°14'01.8"
336637	KX235937	*Phyllidia varicosa*	Teluk Dodinga East; North of Pulau Jere	TER36	N0°50'47.8", E127°37'48.7"
336647	KX235939	*Phyllidia varicosa*	Teluk Dodinga, Karang Galiasa Kecil West	TER39	N0°51'09.1", E127°35'19.5"
336590	KX235944	*Phyllidiopsis fissuratus*	Yenweres Bay	RAJ46	S0°29'13.0", E130°40'23.6"
336589	KX235945	*Phyllidiella rudmani*	Southeast Gam, Friwen Wonda	RAJ11	S0°28'29.9", E130°41'54.8"
336434	KX235946	*Phyllidiella nigra*	Off Danau Laguna	TER02	N0°45'29.7", E127°20'59.2"
336471	KX235947	*Phyllidiella nigra*	Maitara Northwest	TER10	N0°44'32.0", E127°21'50.9"

RMNH.MOL or Field nr.	Genbank accession number	Species	Locality	Station	Coordinates
336472	KX235948	*Phyllidiella nigra*	Idem	TER10	N0°44'32.0", E127°21'50.9"
336501	KX235949	*Phyllidiella nigra*	Sulamadaha I	TER22	N0°52'03.6", E127°19'33.1"
336505	KX235950	*Phyllidiella nigra*	Sulamadaha II	TER23	N0°52'02.0", E127°19'45.8"
336576	KX235952	*Phyllidiella nigra*	South Gam, Eastern entrance Besir Bay, Pulau Bun	RAJ26	S0°30'59.3", E130°33'48.7"
336577	KX235951	*Phyllidiella nigra*	South Gam, Southeast Besir Bay	RAJ32	S0°30'45.2", E130°35'00.1"
75F		*Phyllidiella pustulosa*	North Batanta, West Telok Gegenlol	RAJ29	S0°49'42.5", E130°42'42.0"
336436	KX235953	*Phyllidiella pustulosa*	Off Danau Laguna	TER02	N0°45'29.7", E127°20'59.2"
336460	KX235954	*Phyllidiella pustulosa*	Desa Tahua	TER07	N0°45'09.1", E127°23'31.3"
336461	KX235955	*Phyllidiella pustulosa*	Idem	TER07	N0°45'09.1", E127°23'31.3"
336470	KX235956	*Phyllidiella pustulosa*	Northwest side of Maitara	TER10	N0°44'32.0", E127°21'50.9"
336474	KX235957	*Phyllidiella pustulosa*	Tanjung Tabam	TER12	N0°50'05.1", E127°23'10.0"
336495	KX235958	*Phyllidiella pustulosa*	Tanjung Ratemu (South of river)	TER21	N0°54'24.7", E127°29'17.7"
336508	KX235959	*Phyllidiella pustulosa*	Dufadufa / Benteng Toloko	TER24	N0°48'49.1", E127°23'21.6"
336510	KX235960	*Phyllidiella pustulosa*	Idem	TER24	N0°48'49.1", E127°23'21.6"
336578	KX235965	*Phyllidiella pustulosa*	South Gam, Southeast Besir Bay	RAJ32	S0°30'45.2", E130°35'00.1"
336579	KX235971	*Phyllidiella pustulosa*	South Gam, Besir Bay	RAJ35	S0°48'58.3", E130°59'16.6"
336580	KX235967	*Phyllidiella pustulosa*	Southwest Pulau Kri	RAJ40	S0°33'58.1", E130°39'46.2"
336581	KX235963	*Phyllidiella pustulosa*	South Gam, Besir Bay	RAJ35	S0°48'58.3", E130°59'16.6"
336582	KX235968	*Phyllidiella pustulosa*	Southwest Pulau Kri	RAJ40	S0°33'58.1", E130°39'46.2"
336583	KX235964	*Phyllidiella pustulosa*	South Gam, East entrance Besir Bay, Cape Besir	RAJ25	S0°30'51.5", E130°34'11.5"
336584	KX235961	*Phyllidiella pustulosa*	West Pulau Yeben Kecil	RAJ48	S0°29'20.6", E130°30'04.9"
336585	KX235969	*Phyllidiella pustulosa*	Southeast Gam, Desa Besir	RAJ41	S0°27'48.1", E130°41'14.6"
336586	KX235966	*Phyllidiella pustulosa*	Idem	RAJ41	S0°27'48.1", E130°41'14.6"
336587	KX235962	*Phyllidiella pustulosa*	South Gam, Eastern entrance Besir Bay, Cape Besir	RAJ25	S0°30'51.5", E130°34'11.5"
336588	KX235970	*Phyllidiella pustulosa*	West Pulau Yeben Kecil	RAJ48	S0°29'20.6", E130°30'04.9"
336453	KX235972	*Phyllidiopsis krempfi*	Kampung Cina / Tapak 2	TER06	N0°47'15.0", E127°23'25.0"

RMNH.MOL or Field nr.	Genbank accession number	Species	Locality	Station	Coordinates
336462	KX235973	*Phyllidiopsis krempfi*	Tanjung Ebamadu	TER08	N0°45'23.4", E127°24'26.5"
336466	KX235974	*Phyllidiopsis krempfi*	Idem	TER08	N0°45'23.4", E127°24'26.5"
336469	KX235975	*Phyllidiopsis krempfi*	West Maitara	TER09	N0°43'47.6", E127°21'44.7"
336512	KX235976	*Phyllidiopsis krempfi*	Dufadufa / Benteng Toloko	TER24	N0°48'49.1", E127°23'21.6"
336594	KX235979	*Phyllidiopsis krempfi*	Southwest Pulau Kri, Kuburan	RAJ15	S0°33'42.8", E130°39'40.4"
336595	KX235984	*Phyllidiopsis krempfi*	Southwest Pulau Kri	RAJ40	S0°33'58.1", E130°39'46.2"
336596	KX235983	*Phyllidiopsis krempfi*	Northwest Pulau Mansuar, Lalosi reef	RAJ49	S0°32'53.5", E130°29'51.1"
336597	KX235978	*Phyllidiopsis krempfi*	Southwest Pulau Kri, Kuburan	RAJ15	S0°33'42.8", E130°39'40.4"
336598	KX235980	*Phyllidiopsis krempfi*	North Batanta, North Pulau Yarifi	RAJ28	S0°46'46.7", E130°42'42.7"
336599	KX235982	*Phyllidiopsis krempfi*	East Kri, Sorido Wall	RAJ12	S0°33'13.2", E130°41'16.9"
336600	KX235981	*Phyllidiopsis krempfi*	Northeast Mansuar	RAJ38	S0°34'05.0", E130°38'31.5"
336650	KX235977	*Phyllidiopsis krempfi*	Teluk Dodinga; West Karang Ngeli	TER40	N0°46'25.3", E127°32'22.0"
336451	KX235985	*Phyllidiopsis shireenae*	Kampung Cina / Tapak 2	TER06	N0°47'15.0", E127°23'25.0"
336652	KX235986	*Phyllidiopsis shireenae*	Teluk Dodinga; East Karang Luelue	TER41	N0°46'32.8", E127°33'43.4"
336591	KX235987	*Phyllidiopsis xishaensis*	Southeast Gam, Pulau Kerupiar, Mike's Point	RAJ05	S0°30'57.1", E130°40'22.1"
336592	KX235988	*Phyllidiopsis xishaensis*	East Pulau Kri, Cape Kri	RAJ07	S0°33'22.2", E130°41'28.7"
336593	KX235989	*Phyllidiopsis xishaensis*	Eastern entrance of passage	RAJ44	S0°25'44.3", E130°33'56.8"
336640	KX235990	*Reticulidia fungia*	East Teluk Dodinga; North of Pulau Jere	TER36	N0°50'47.8", E127°37'48.7"
336455	KX235991	*Reticulidia halgerda*	Kampung Cina / Tapak 2	TER06	N0°47'15.0", E127°23'25.0"

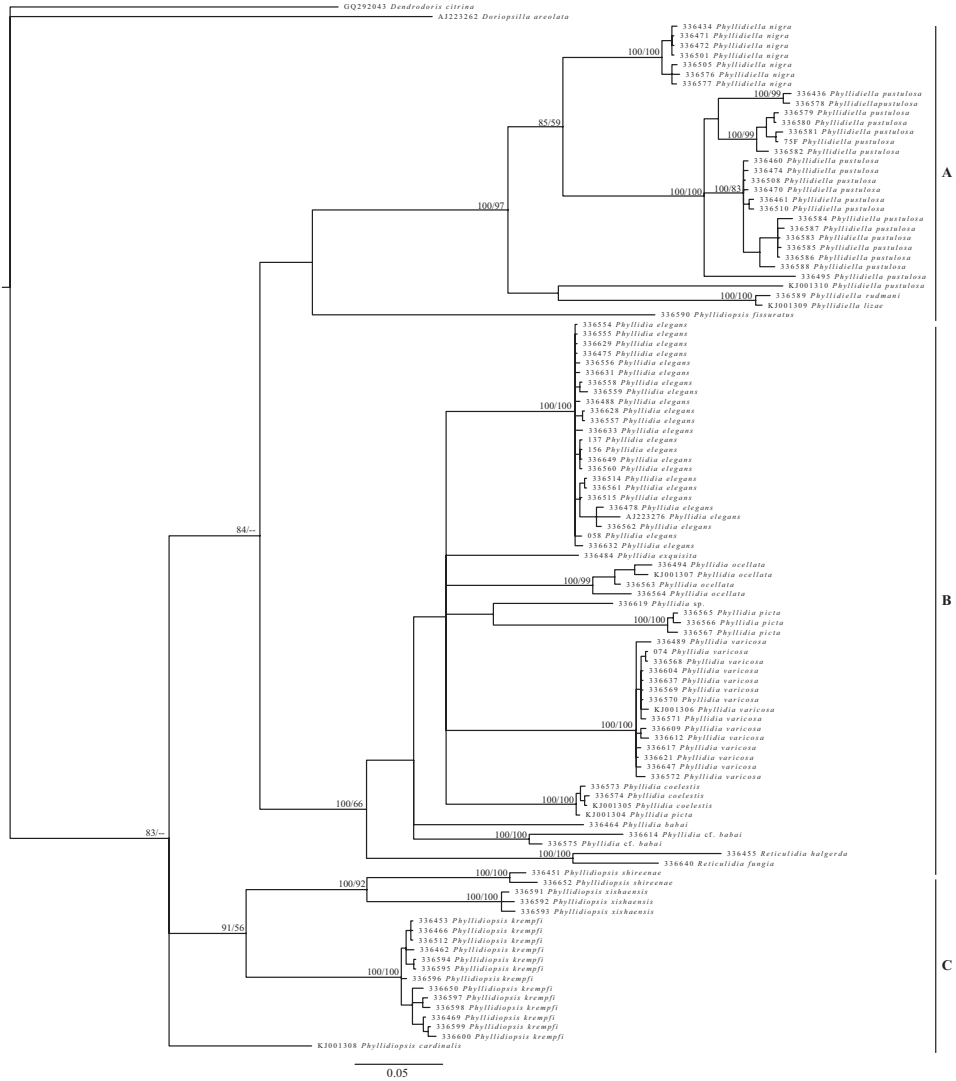

Figure 4. Phylogeny reconstruction of the Phyllidiidae based on COI gene sequence data of 109 specimens (including outgroups). Topology derived from Bayesian inference 50% majority rule, significance values are posterior probabilities / bootstrap values. Numbers refer to GenBank accession numbers / RMNH.Moll catalogue numbers.

Morphological study

Collected specimens were identified according to their external morphology using Brunckhorst (1993), Yonow et al. (2002), and Yonow (2011). In addition, field guides showing *in situ* photographs were used (Gosliner et al. 2008). All individuals except

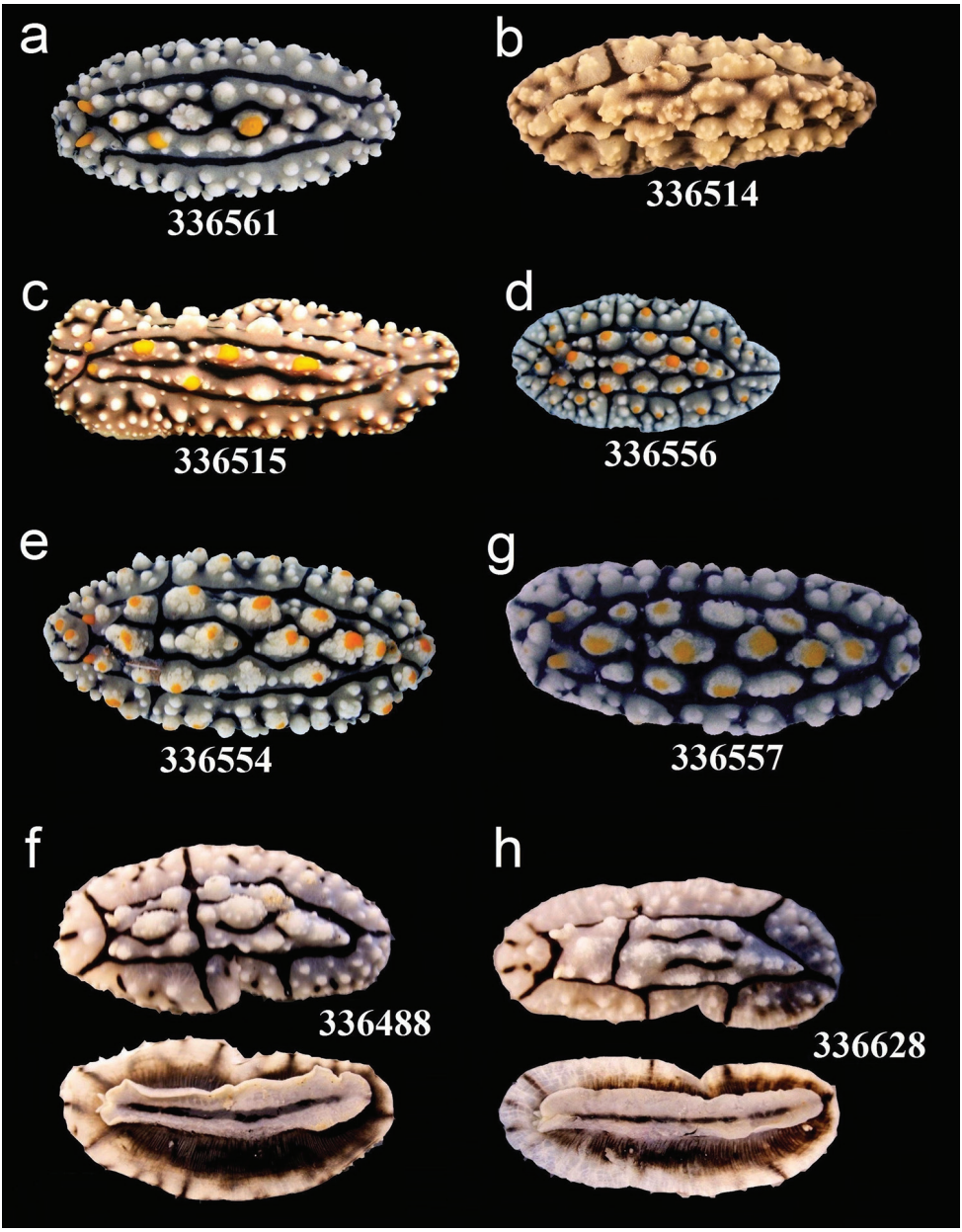

Figure 5. External morphology and colouration of Phyllidiidae specimens used for COI phylogeny reconstruction: *Phyllidia elegans*. Order of specimens (**a–h**) according to Figure 4 (**f**, **h** dorsal and ventral sides). Numbers refer to RMNH. Moll catalogue numbers.

for three could be identified to species level. All specimens were photographed alive or in the preserved state (Figures 5–15); these photos can be linked to the phylogeny reconstruction of the Phyllidiidae based on COI gene sequence data (Figure 4).

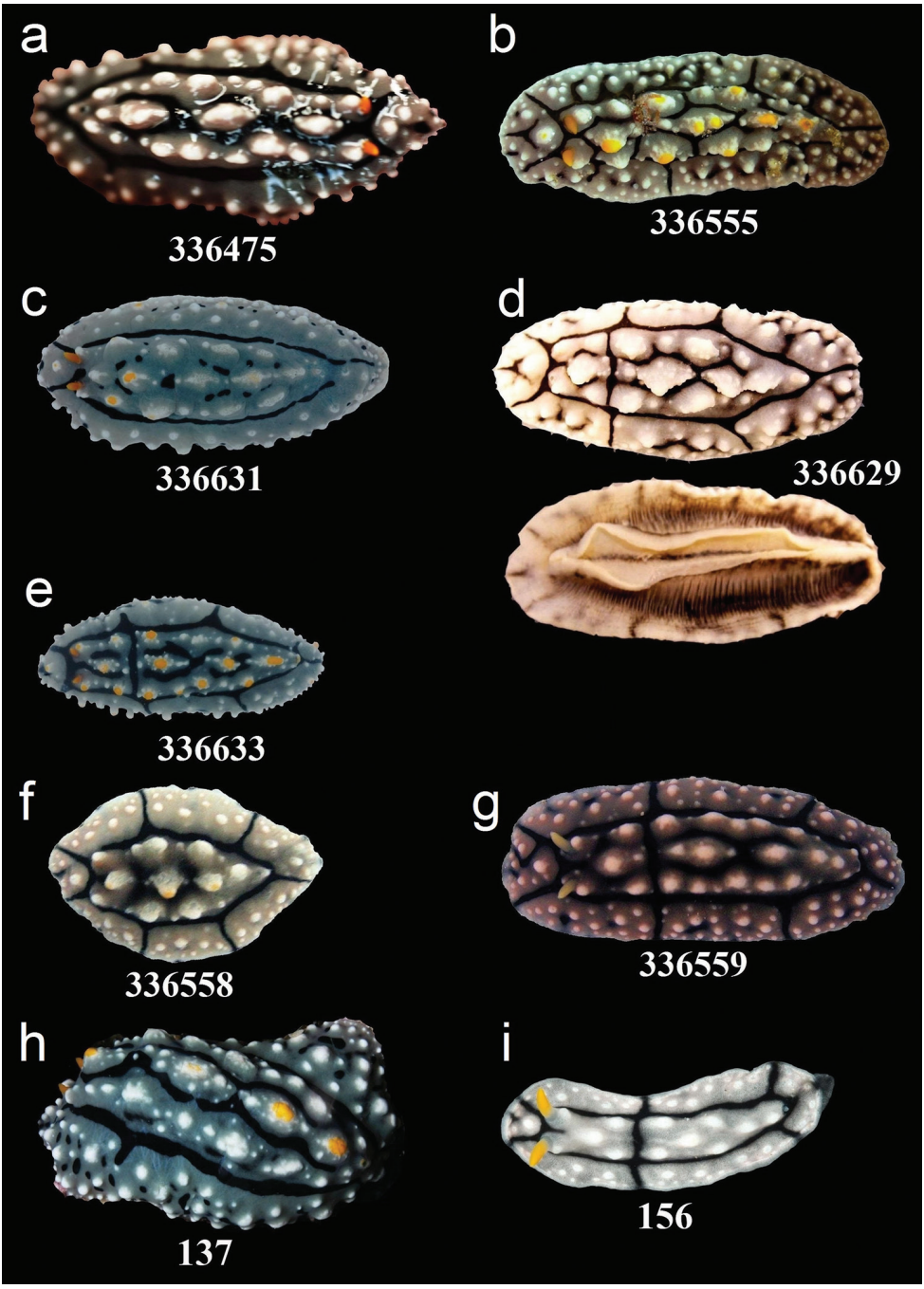

Figure 6. External morphology and colouration of Phyllidiidae specimens used for COI phylogeny reconstruction: *Phyllidia elegans*. Order of specimens (**a–i**) according to Figure 4 (**d** dorsal and ventral sides). Numbers refer to RMNH.Moll catalogue numbers and locality codes (137 and 156, dried-out).

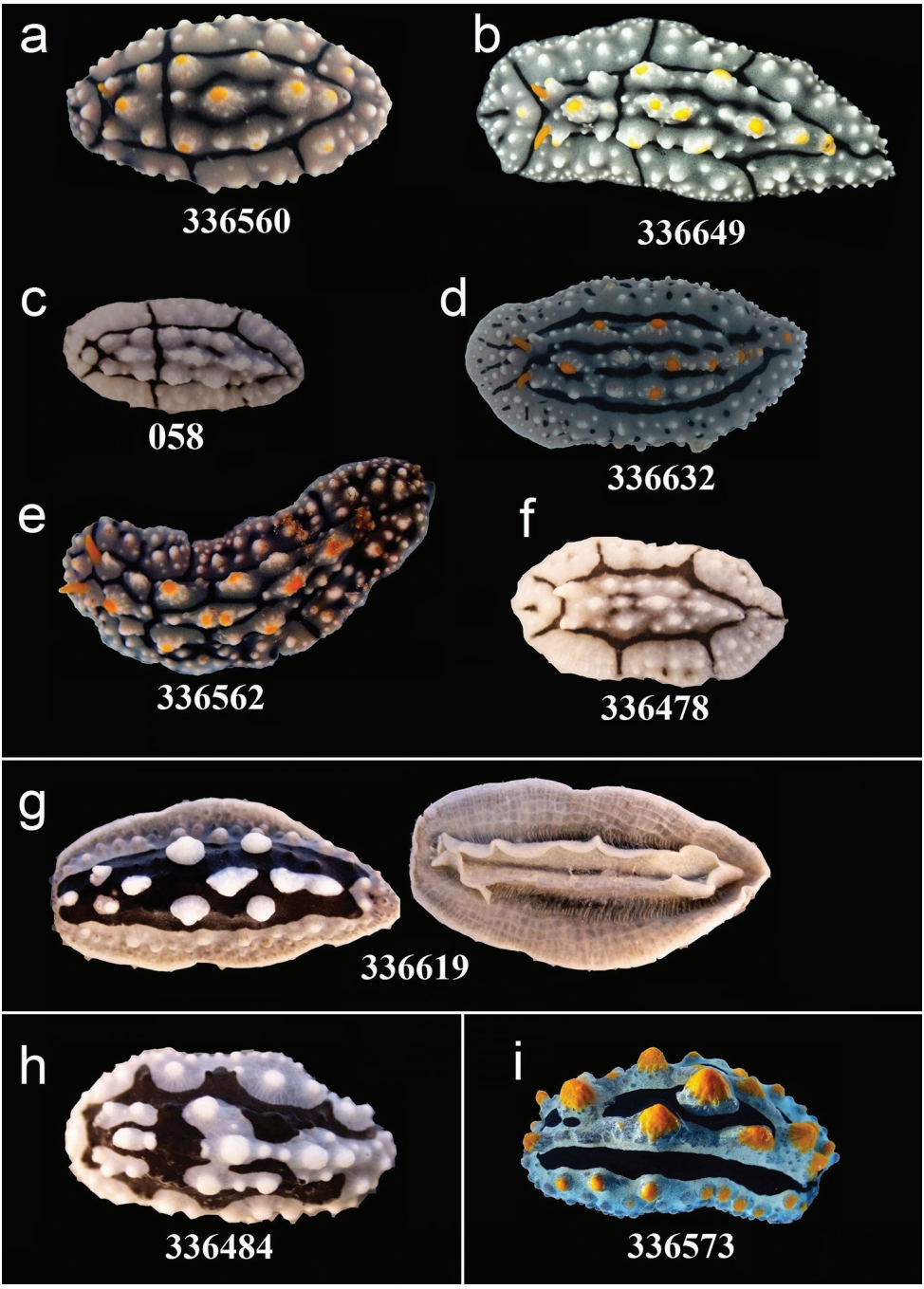

Figure 7. External morphology and colouration of Phyllidiidae specimens used for COI phylogeny reconstruction: *Phyllidia elegans* (**a–f**), *Phyllidia* sp. (**g** dorsal and ventral sides), *P. exquisita* (**h**), *P. coelestis* (**i**). Order of specimens (**a–i**) according to Figure 4. Numbers refer to RMNH.Moll catalogue numbers or locality code (058, dried-out).

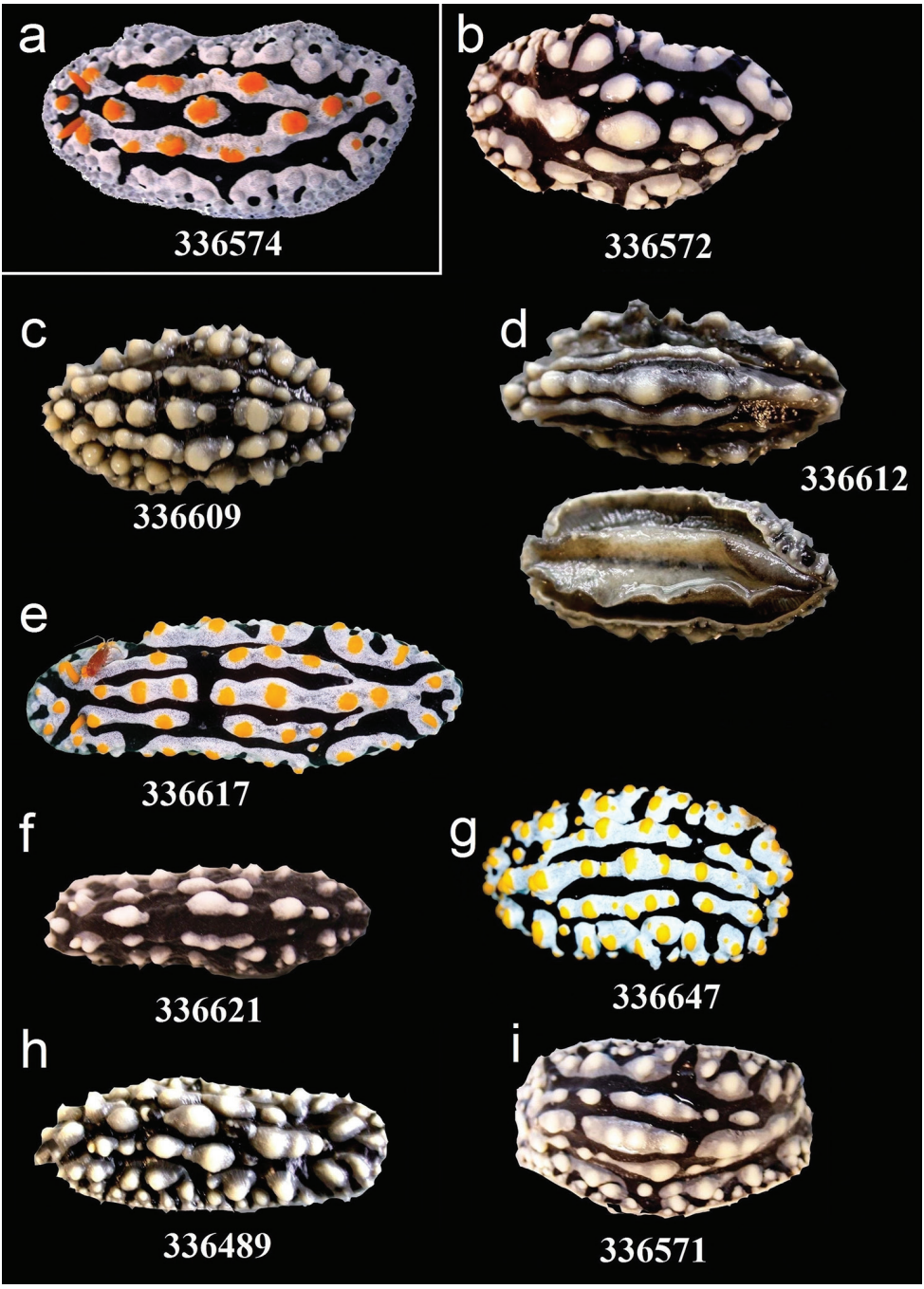

Figure 8. External morphology and colouration of Phyllidiidae specimens used for COI phylogeny reconstruction: *Phyllidia coelestis* (**a**), *P. varicosa* (**b–i**). Order of specimens (**a–i**) according to Figure 4 (**d** dorsal and ventral sides). Numbers refer to RMNH.Moll catalogue numbers.

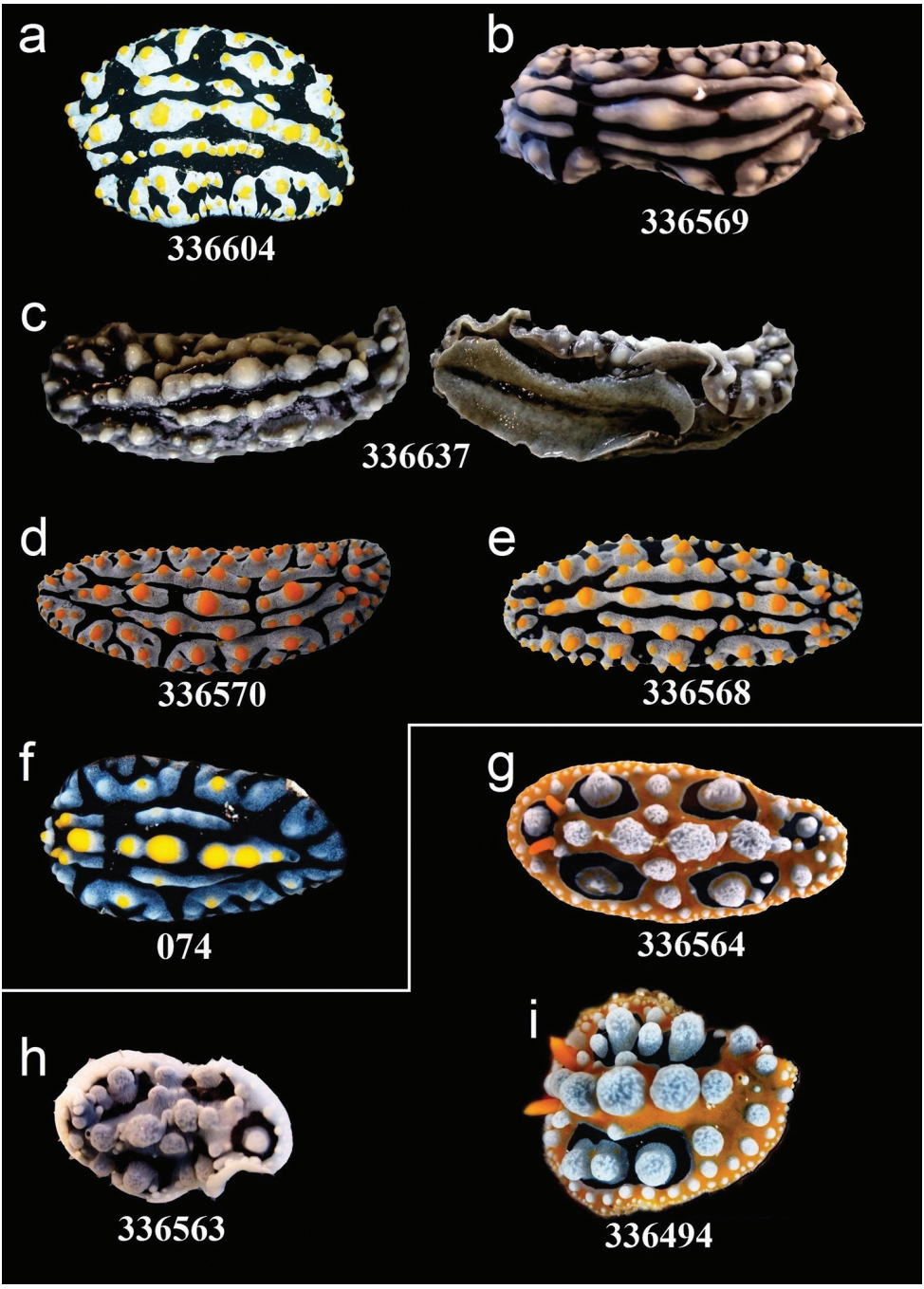

Figure 9. External morphology and colouration of Phyllidiidae specimens used for COI phylogeny reconstruction: *Phyllidia varicosa* (**a–f**), *P. ocellata* (**g–i**). Order of specimens (**a–i**) according to Figure 4 (**c** dorsal and ventral sides). Numbers refer to RMNH.Moll catalogue numbers or locality code (074, dried-out).

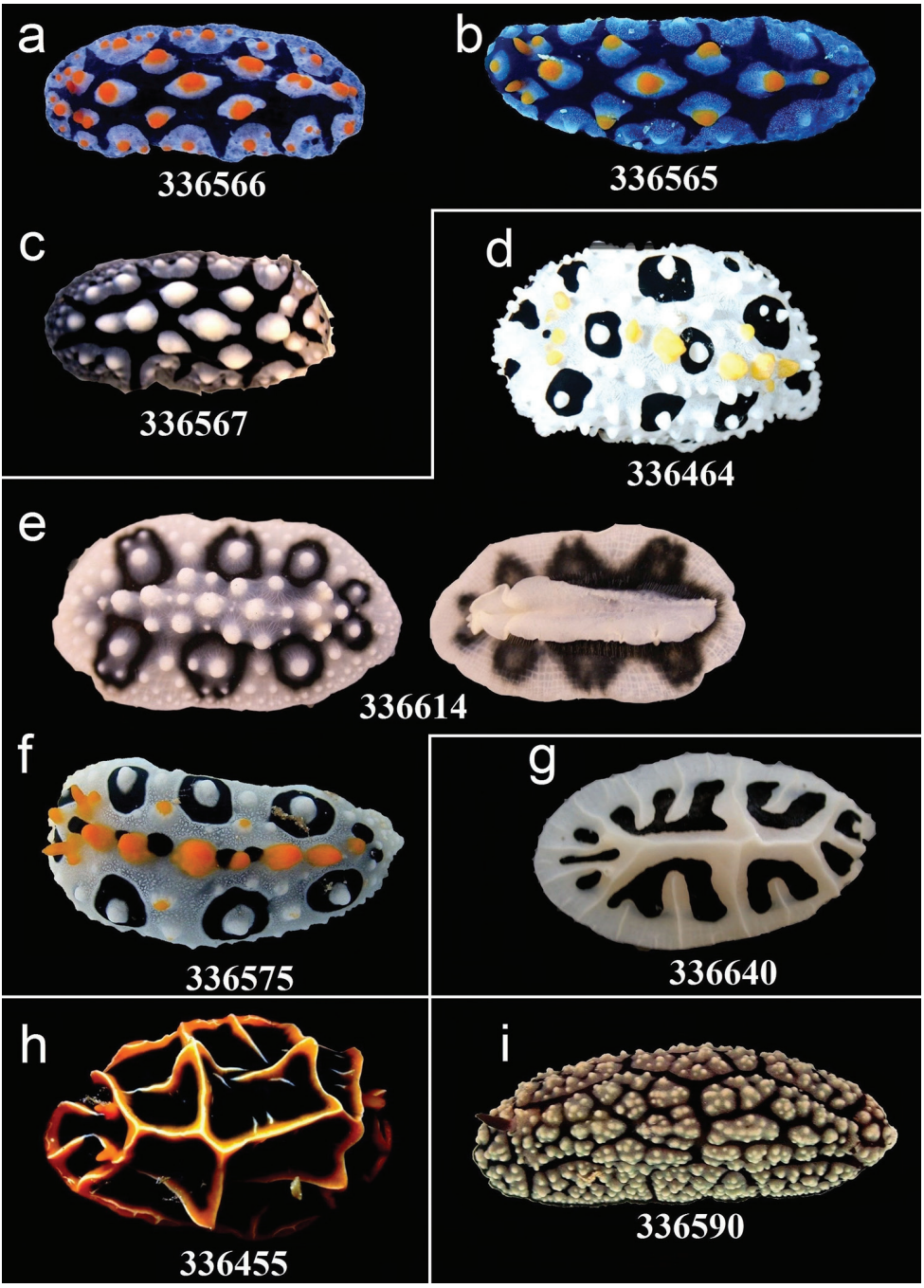

Figure 10. External morphology and colouration of Phyllidiidae specimens used for COI phylogeny reconstruction: *Phyllidia picta* (**a–c**), *Phyllidia babai* (**d**), *Phyllidia* cf. *babai* (**e–f**), *Reticulidia fungia* (**g**), *Reticulidia halgerda* (**h**), *Phyllidiopsis fissuratus* (**i**). Order of specimens (**a–i**) according to Figure 4 (**e** dorsal and ventral sides). Numbers refer to RMNH.Moll catalogue numbers.

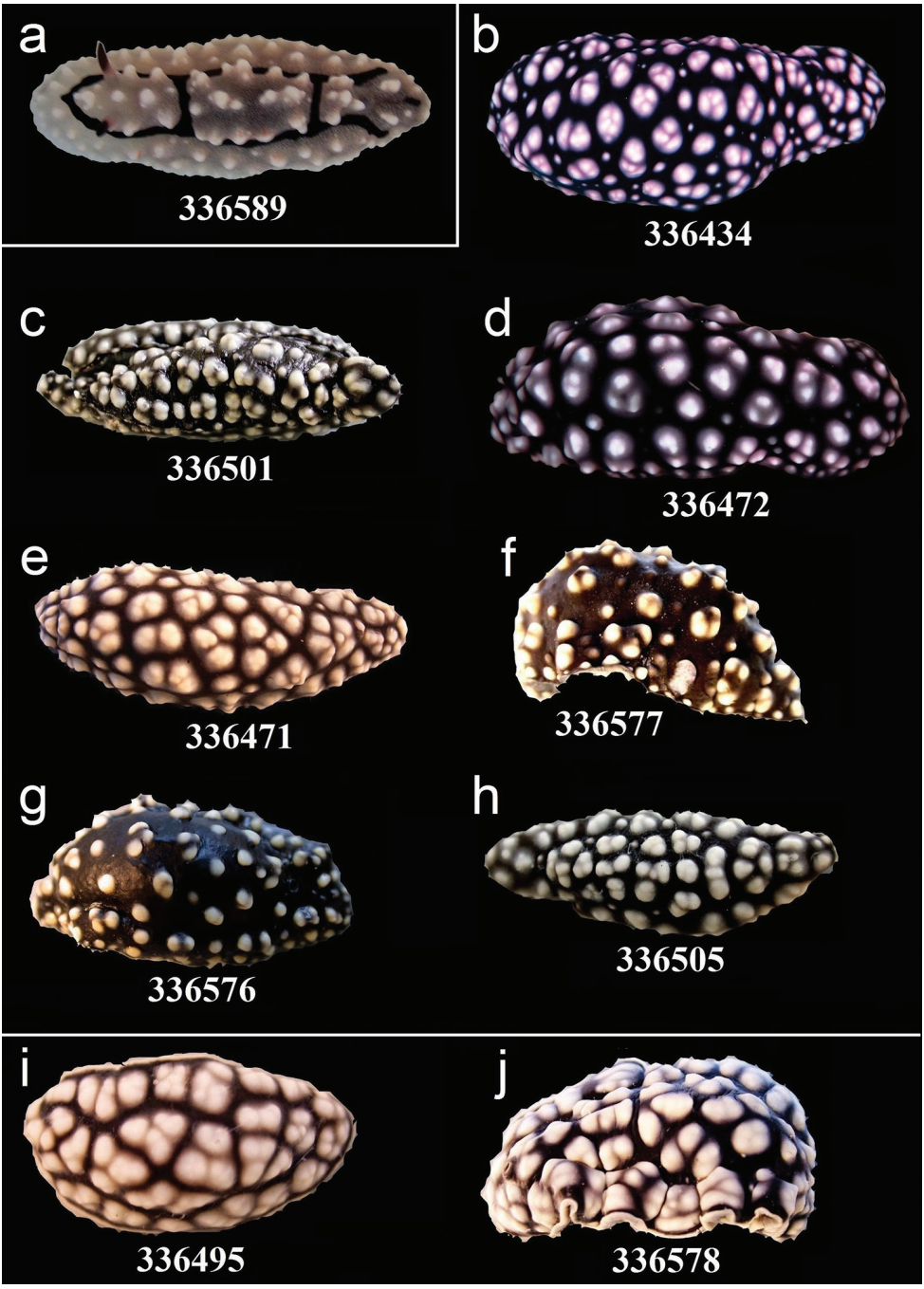

Figure 11. External morphology and colouration of Phyllidiidae specimens used for COI phylogeny reconstruction: *Phyllidiella rudmani* (**a**), *Phyllidiella nigra* (**b–h**), *Phyllidiella pustulosa* (**i–j**). Order of specimens (**a–j**) according to Figure 4. Numbers refer to RMNH.Moll catalogue numbers.

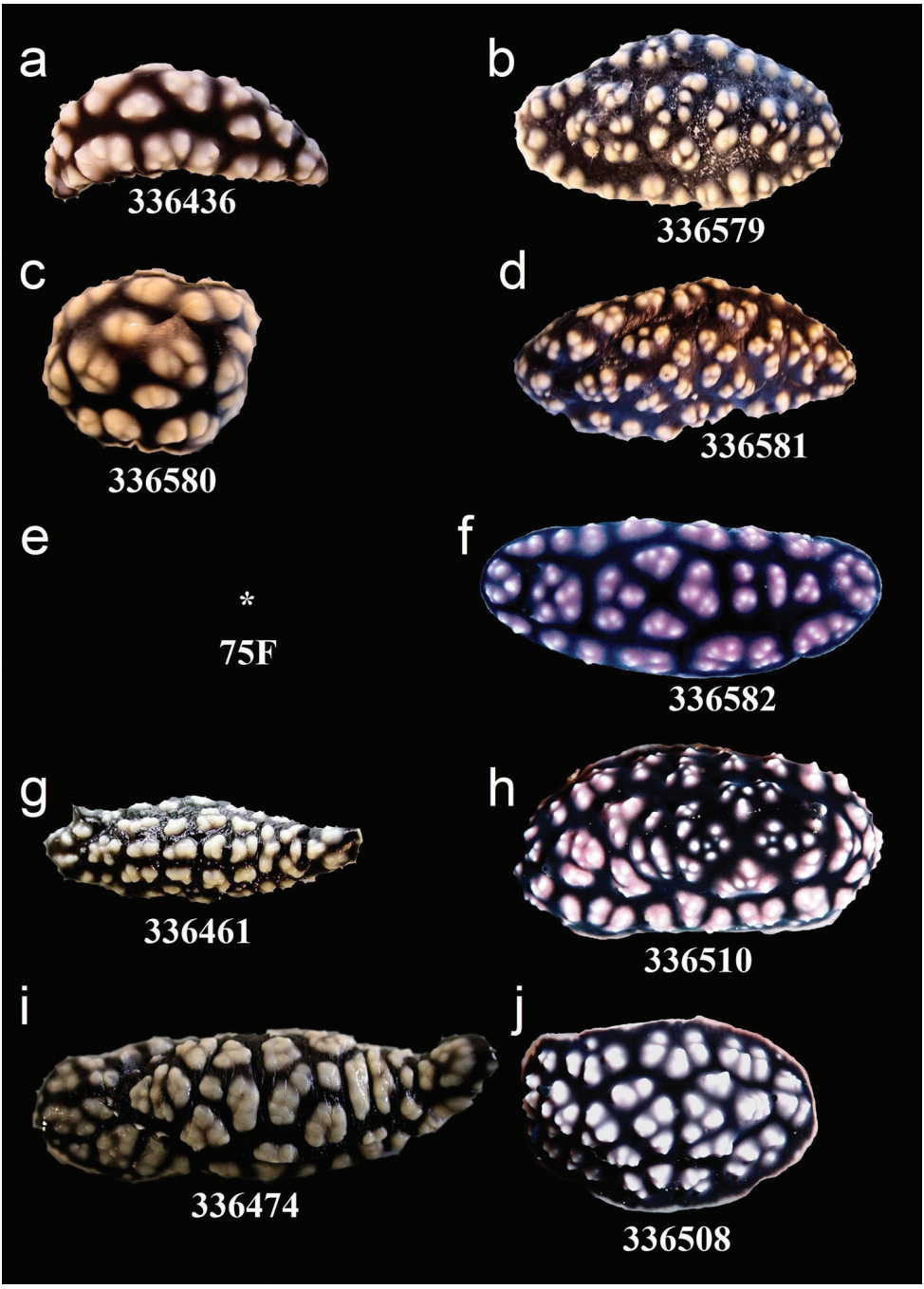

Figure 12. External morphology and colouration of Phyllidiidae specimens used for COI phylogeny reconstruction: *Phyllidiella pustulosa*. Order of specimens (**a–j**) according to Figure 4. Numbers refer to RMNH.Moll catalogue numbers or locality code (75F, dried-out).

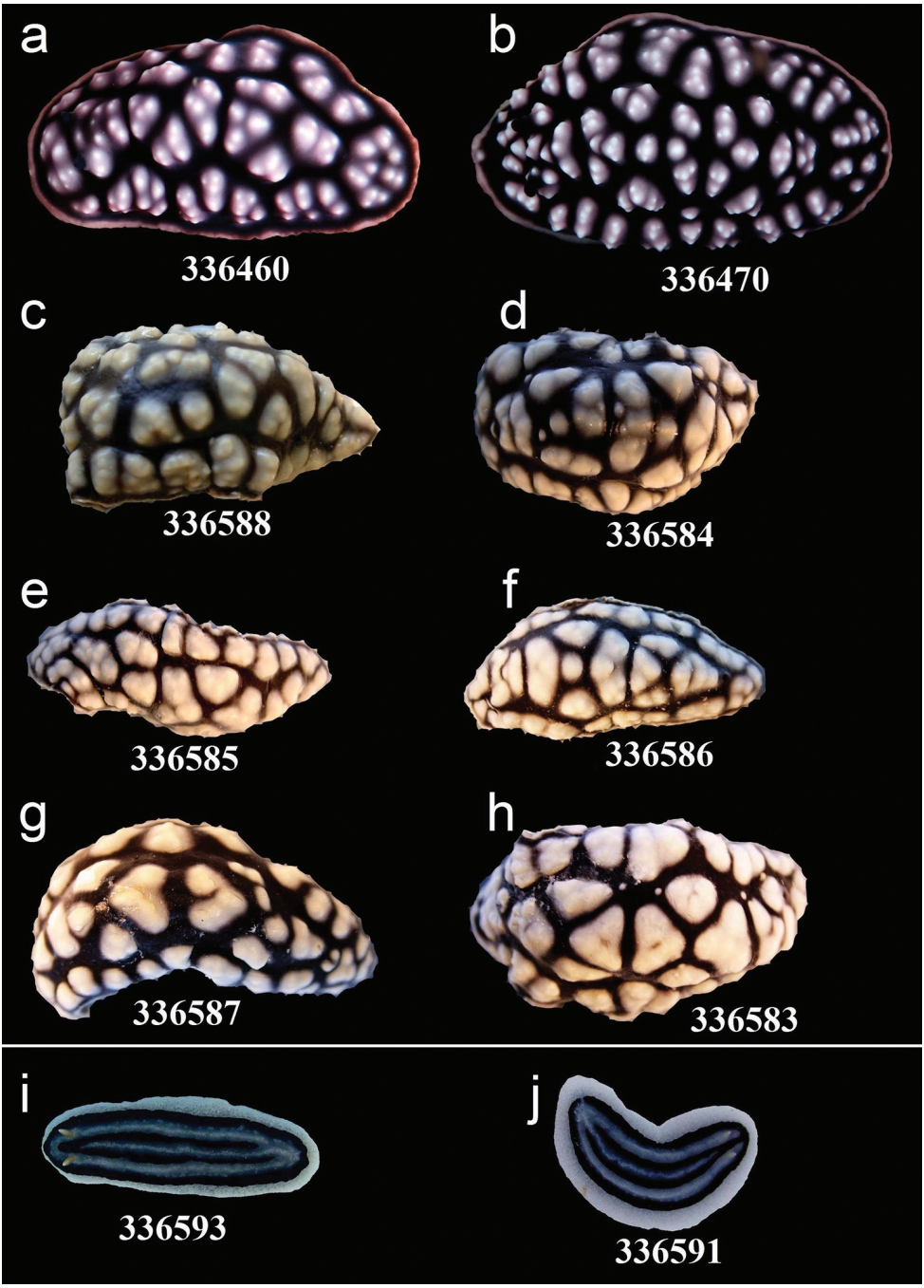

Figure 13. External morphology and colouration of Phyllidiidae specimens used for COI phylogeny reconstruction: *Phyllidiella pustulosa* (**a–h**), *Phyllidiopsis xishaensis* (**i–j**). Order of specimens (**a–j**) according to Figure 4. Numbers refer to RMNH.Moll catalogue numbers.

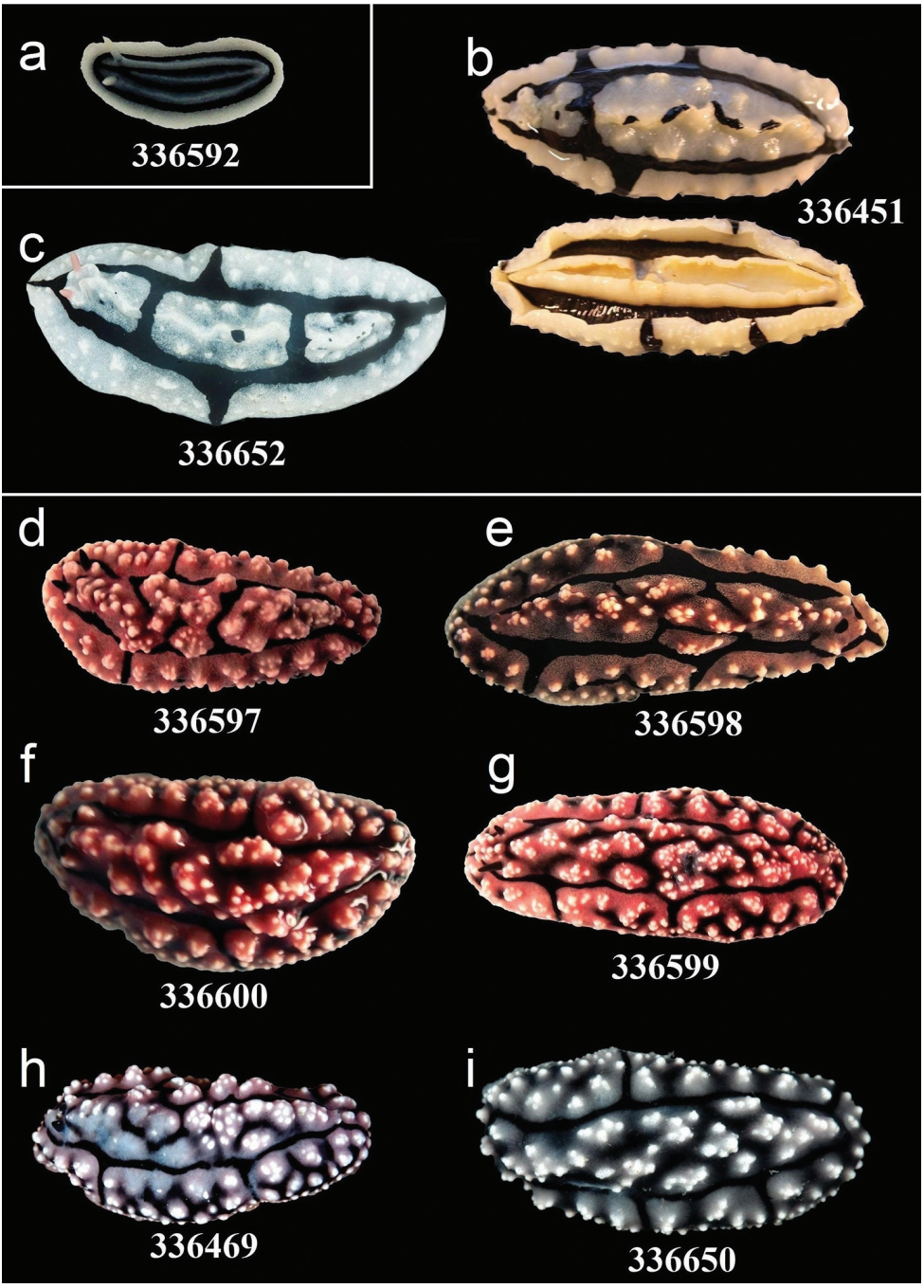

Figure 14. External morphology and colouration of Phyllidiidae specimens used for COI phylogeny reconstruction: *Phyllidiopsis xishaensis* (**a**), *Phyllidiopsis shireenae* (**b–c**), *Phyllidiopsis krempfi* (**d–i**). Order of specimens (**a–i**) according to Figure 4 (**c** dorsal and ventral sides). Numbers refer to RMNH.Moll catalogue numbers.

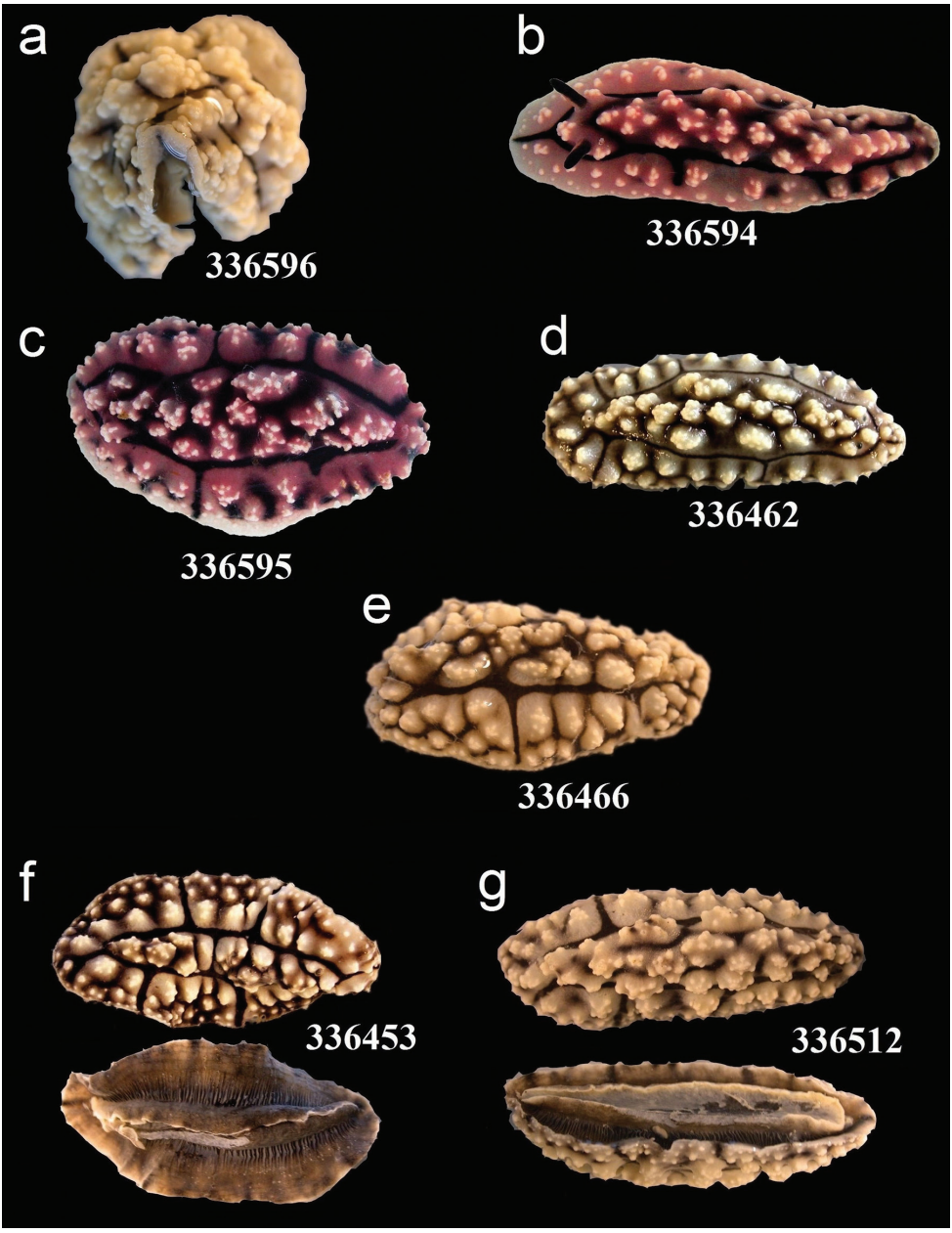

Figure 15. External morphology and colouration of Phyllidiidae specimens used for COI phylogeny reconstruction: *Phyllidiopsis krempfi*. Order of specimens (**a–g**) according to Figure 4 (**f**, **g** dorsal and ventral sides). Numbers refer to RMNH.Moll catalogue numbers.

DNA extraction

For each species encountered in the field surveys one or more individuals were chosen for DNA analysis as well as from the morphologically distinct unidentified specimens, resulting in a total of 99 samples (Table 1). DNA was extracted from tissue of small foot fragments with the DNeasy Blood & Tissue Kit (Qiagen, Germany) according to the manufacturer's protocol. DNA was eluted in DEPC treated water. The quality of the extracted DNA was tested by agarose gel (0.7%) electrophoresis.

PCR amplification, purification, and sequencing

Extracted DNA was used for Polymerase Chain Reaction (PCR) to amplify fragments of the mitochondrial gene COI (cytochrome *c* oxidase subunit 1). The primers used for the amplification of the COI gene were: 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). Thermal cycling conditions used for the amplification of the COI gene were: initial denaturing at 94 °C for 3 min followed by 38 amplification cycles of denaturation at 94 °C for 15 sec, primer annealing at 50 °C for 30 sec, and elongation at 72 °C for 1 min. A final elongation step at 72 °C for 5 min was performed. After checking by agarose (1%) electrophoresis if the PCR resulted the unique PCR fragments of the expected size (approximately 658 bp), the fragments were purified using the GeneJET PCR Purification Kit (Thermo Scientific, Landsmeer, NL). Purified PCR products were sequenced with corresponding primers.

Sequence alignment and phylogenetic analyses

The quality of the sequences was checked using Chromas Lite (Technelysium Pty Ltd.). Subsequently the sequences were edited in MEGA 6 (Tamura et al. 2013) and analysed by BLAST searches (http://www.ncbi.nlm.nih.gov). COI sequences of *Dendrodoris citrina* (Cheeseman, 1881) and *Doriopsilla areolata* Bergh, 1880 were collected from GenBank and used as outgroups. Additional COI sequences of *Phyllidia coelestis* Bergh, 1905, *Phyllidia elegans* Bergh, 1869, *Phyllidia ocellata* Cuvier, 1804, *Phyllidia picta* Pruvot-Fol, 1957, *Phyllidia varicosa* Lamarck, 1801, *Phyllidiella lizae* Brunckhorst, 1993, *Phyllidiella pustulosa* (Cuvier, 1804), *Phyllidiopsis cardinalis* Bergh, 1875 were obtained from GenBank (Table 2).

The newly obtained COI sequences and the sequences from GenBank were aligned using the Guidance server (Clustal W; Penn et al. 2010), resulting in an alignment score of 1.000. There were no unreliable columns. Prior to the model-based phylogenetic analysis, the best-fit model of nucleotide substitution was identified by means of the Akaike Information Criterion (AIC) calculated with jModeltest (Posada 2008), resulting in TVM+I+G as the most suitable model. Phylogenetic reconstructions were

Table 2. Mitochondrial COI sequences of Phyllidiidae (and outgroups) obtained from GenBank.

Species	Accession number	Reference	Collection locality
Dendrodoris citrina	GQ292043	Shields et al. (2009 unpubl.)	Ross Sea, Antarctica?
Doriopsilla areolata	AJ223262	Thollesson (2000)	Cadiz, Andalusia, Spain
Phyllidia coelestis	KJ001305	Cheney et al. (2014)	Lizard I., Queensland Australia
Phyllidia elegans	AJ223276	Thollesson (2000)	Tab I., Papua New Guinea
Phyllidia ocellata	KJ001307	Cheney et al. (2014)	Mooloolaba, Queensland, Australia
Phyllidia picta	KJ001304	Cheney et al. (2014)	Lizard I., Queensland Australia
Phyllidia varicosa	KJ001306	Cheney et al. (2014)	Lizard I., Queensland Australia
Phyllidiella lizae	KJ001309	Cheney et al. (2014)	Lizard I., Queensland Australia
Phyllidiella pustulosa	KJ001310	Cheney et al. (2014)	Lizard I., Queensland Australia
Phyllidiopsis cardinalis	KJ001308	Cheney et al. (2014)	Mooloolaba, Queensland, Australia

carried out with Bayesian inference in MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) using the most complex GTR+I+G model of nucleotide substitution. Bayesian inference coupled with Markov Chain Monte Carlo techniques (MCMC; six chains) were run for 5,000,000 generations with a sample tree saved every 1000 generations. The burnin was set to 25%. Likelihood scores stabilized at 0.007476. Consensus trees were visualized in FigTree v.1.3.1 (Rambaut 2009). A maximum likelihood analysis (GTR+I+G; 1000 bootstraps) was carried out with Phyml 3.1 (Guindon et al. 2010) using the Seaview platform (Gouy et al. 2010).

Initial phylogenetic analyses showed high intraspecific variation on the COI region between specimens identified as *Phyllidiella pustulosa*. Tests to estimate the average evolutionary divergence over sequence pairs between and within groups were carried out in MEGA 6.06. *Phyllidia elegans*, *P. varicosa*, *Phyllidiella nigra* (van Hasselt, 1824), *P. pustulosa*, and *Phyllidiopsis krempfi* Pruvot-Fol, 1957 were used as representatives for each of the species groups, because of the larger number of available sequences for these species. The *Phyllidiella pustulosa* sequence from GenBank (KJ001310) was excluded from this analysis: based on its position in the phylogeny reconstruction the identification of this specimen as *P. pustulosa* is doubtful. The web version of ABGD (Automatic Barcode Gap Discovery, Puillandre et al. 2012) was used to estimate the genetic distance corresponding to the difference between a speciation process versus intra-specific variation in *Phyllidiella pustulosa*. Runs were performed using the default range of priors (pmin = 0.001, pmax = 0.10) using the JC69 Jukes-Cantor measure of distance. The analysis involved 20 nucleotide sequences with a total of 588 positions in the final dataset.

All available mitochondrial 16S sequences of Phyllidiidae on GenBank (Tholesson 2000, Wolfscheid-Lengeling et al. 2001, Valdés 2003, Cheney et al. 2014, Shields et al. unpublished) were used for a phylogeny reconstruction based on this marker, which allowed us to study the phylogenetic position of 17 phyllidiid species including two species (*Phyllidia rueppelii* (Bergh, 1869) and *Phyllidiopsis sphingis* Brunckhorst, 1993) for which no COI data were available. *Doriopsilla albopunctata* (JG Cooper, 1863) was used as outgroup (Table 3). The sequences were aligned using the Guidance server

Table 3. 16S sequences of Phyllidiidae obtained from GenBank.

Species	Accession number	Reference	Collection locality
Doropsilla albopunctata	AF430354	Valdés (2003)	Baja California, Mexico
Phyllidia coelestis	AF430361	Valdés (2003)	Lifou I., New Caledonia
Phyllidia coelestis	KJ018917	Cheney et al. (2014)	Lizard I., Queensland Australia
Phyllidia elegans	AF430362	Valdés (2003)	Lifou I., New Caledonia
Phyllidia elegans	AJ225201	Thollesson (2000)	Tab I., Papua New Guinea
Phyllidia ocellata	AF430363	Valdés (2003)	Lifou I., New Caledonia
Phyllidia picta	KJ018916	Cheney et al. (2014)	Lizard I., Queensland Australia
Phyllidia rueppelii	AF430358	Valdés (2003)	Hurghada, Egypt
Phyllidiella lizae	AF430365	Valdés (2003)	Lifou I., New Caledonia
Phyllidiella lizae	KJ018918	Cheney et al. (2014)	Lizard I., Queensland Australia
Phyllidiella pustulosa	AF249232	Wollscheid-Lengeling et al. (2001)	Great Barrier Reef, Australia
Phyllidiella pustulosa	AF430366	Valdés (2003)	Lifou I., New Caledonia
Phyllidia varicosa	AF430364	Valdés (2003)	Lifou I., New Caledonia
Phyllidiopsis cardinalis	AF430367	Valdés (2003)	Lifou I., New Caledonia
Phyllidiopsis sphingis	AF430368	Valdés (2003)	Lifou I., New Caledonia
*Phyllidiopsis xishaensis**	AF430369	Valdés (2003)	Lifou I., New Caledonia
Reticulidia fungia	AF430370	Valdés (2003)	Lifou I., New Caledonia
Reticulidia halgerda	AF430371	Valdés (2003)	Lifou I., New Caledonia

* Re-identification according to Yonow (pers. comm.)

(ClustalW; Penn et al. 2010), resulting in an alignment score of 0.996281. All unreliable columns (confidence score below 0.93) were removed. Prior to the model-based phylogenetic analysis, the best-fit model of nucleotide substitution was identified by means of the Akaike Information Criterion (AIC) calculated with jModeltest (Posada 2008), resulting in TVM+I+G. Because of the unavailability of TVM in MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003), we used the most complex GTR+I+G model of nucleotide substitution. Bayesian inferences coupled with MCMC techniques (six chains) were run for 3,000,000 generations, with a sample tree saved every 1000 generations and the burnin set to 25%. Likelihood scores stabilized at a value of 0.005654. Consensus trees were visualized in FigTree v.1.3.1 (Rambaut 2009). A maximum likelihood analysis (GTR+I+G; 1000 bootstraps) was carried out with Phyml 3.1 (Guindon et al. 2010) using the Seaview platform (Gouy et al. 2010).

Results and discussion

Position of genera

The reconstruction based on COI (Figure 4) is derived from the Bayesian inference 50% majority rule consensus. This topology is congruent with the one resulting from

the maximum likelihood analysis. Three large groupings can be discerned (indicated as A, B, and C in Figure 4), albeit with low support for the higher taxonomic levels. The support values in the distal branches are high. The genera *Phyllidia, Phyllidiella, Phyllidiopsis*, and *Reticulidia* are retrieved in distinct clades, with *Reticulidia* as a sister clade to *Phyllidia*. *Phyllidiopsis fissuratus* Brunckhorst, 1993 formed a separate lineage basal to *Phyllidiella* species (albeit without support). *Phyllidiopsis cardinalis* does not cluster with its congeners, but instead forms a separate lineage in the Phyllidiidae.

The 16S phylogeny reconstruction is also derived from the Bayesian inference 50% majority rule consensus of the trees remaining after the burnin. There are low support values in the basal part of the tree and high support values in the distal phylogenetic branches (Figure 17). The Bayesian inference topology is congruent with the topology resulting from the maximum likelihood analysis. The outgroup *Doriopsilla albopunctata* is separated by a long branch. Within the overall clade four main groupings can be distinguished: *Phyllidiella*, *Phyllidiopsis*, and *Reticulidia*, and a mixed clade of *Phyllidiella* and *Phyllidia*. Based on this analysis only the genus *Reticulidia* is monophyletic. *Phyllidiopsis cardinalis* does not cluster with any of the other analysed taxa, and holds a separate position in the phylogeny reconstruction. The latter is in accordance with the COI reconstruction (Figure 4).

The arrangement of the four phyllidiid genera based on the molecular data (Figures 4, 16a) is similar to that of Brunckhorst (1993) that was based on morphological and anatomical data (Figure 16b). The only exception is the position of the genus *Fryeria*. Brunckhorst (1993) distinguished *Fryeria* from *Phyllidia* based on the position of the anus and other anatomical features. *Phyllidia picta* (with its synonyms *Fryeria picta* (Pruvot-Fol, 1957), *Fryeria menindie* Brunckhorst, 1993, *Phyllidia menindie* (Brunckhorst, 1993)) was included in our analyses which, according to Brunckhorst, should belong to the genus *Fryeria*. Valdés and Gosliner (1999) synonymized both genera, which was later followed by Valdés (2003) and Cheney et al. (2014). The present reconstruction based on COI (Figure 16a) reconfirms the inclusion of *Fryeria* in the genus *Phyllidia*.

The cladogram of the genera based on 16S mtDNA sequence data collected by Valdés (2003) (Figure 16c) is roughly similar to the cladogram based on COI, except for the different positions of *Phyllidiopsis* and *Phyllidiella*. The cladogram based on morphological and anatomical data as shown by Valdés (2002; Figure 16d) is different from the other proposed classifications (Figures 16a–c). Brunckhorst (1993) considered *Ceratophyllidia* a sister group to all the other genera (Figure 6b). Valdés (2002; Figure 16d) distinguished two larger groupings within the Phyllidiidae; *Ceratophyllidia* and *Phyllidiopsis* as one group and *Phyllidia*, *Phyllidiella*, and *Reticulidia* as the other group. *Phyllidia* and *Phyllidiella* in turn formed a sister group of *Reticulidia* (Figure 16d). The cladogram by Brunckhorst (1993) and our cladogram based on COI (Figure 4) both show that *Phyllidiella* is a sister clade of *Reticulidia* and *Phyllidia*. In contrast, *Phyllidiella* is not a sister group of *Phyllidia* but to all the other genera grouped together in the cladogram of Valdés (2003).

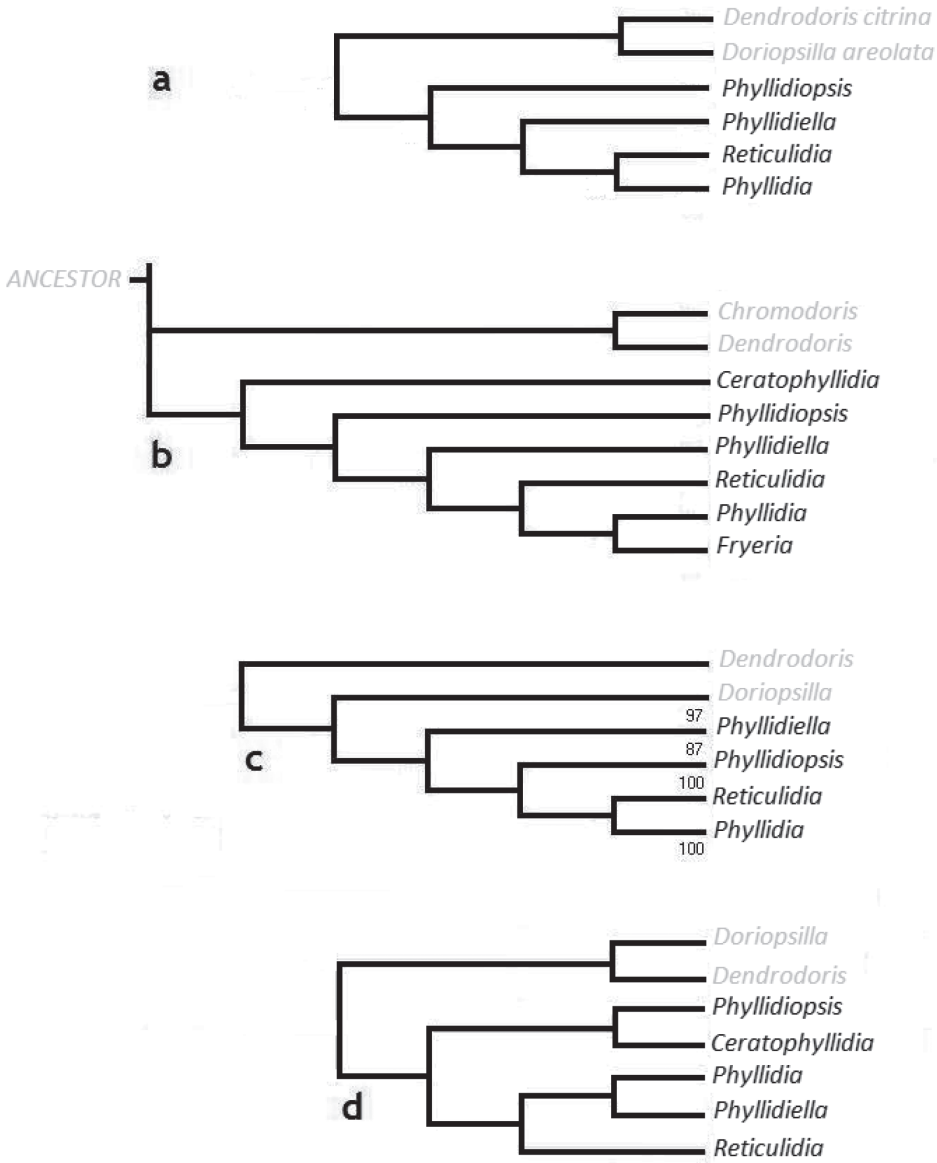

Figure 16. a Cladogram based on COI gene sequence data showing topology of four genera of Phyllidiidae **b** Cladogram according to Brunckhorst (1993) based on morphological data showing topology of six genera of Phyllidiidae **c** Cladogram based on 16S mtDNA sequence data showing topology of four genera of Phyllidiidae (Valdés 2003) **d** Cladogram based on morphological data (Valdés 2002) showing topology of five genera of Phyllidiidae.

Unfortunately no *Ceratophyllidia* specimens were available to complete our analysis at genus level. Up to this point the phylogenetic position of the genus *Ceratophyllidia* remains unclear, and additional molecular analyses are necessary to establish its position.

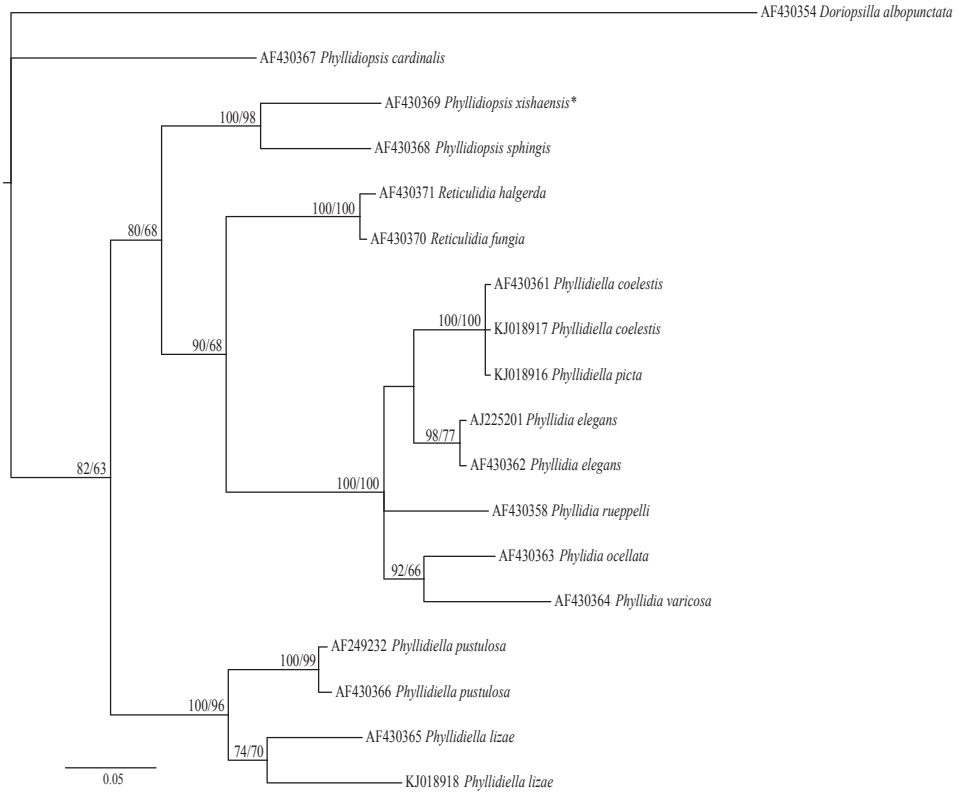

Figure 17. Phylogeny reconstruction of the Phyllidiidae based on 16S mtDNA of 17 specimens of 14 species (including outgroup). Topology derived from Bayesian inference 50% majority rule, significance values are posterior probabilities/bootstrap values. Numbers refer to GenBank accession numbers. *Re-identification according to Yonow (pers. comm.)

Species level analysis

Species level analysis was mainly based on COI (Figure 4). Four nominal species were sequenced in the genus *Phyllidiella*. *Phyllidiella nigra* formed a highly supported clade. In the clade containing *P. pustulosa* much variation is visible indicating larger genetic differences among individuals. The ABGD analysis shows that four Molecular Operational Taxonomic Units (MOTUs) are present in *Phyllidiella pustulosa*, suggesting the presence of cryptic species or, alternatively, high intraspecific variation. The *P. pustulosa* of Cheney et al. (2014) falls in between the group consisting of *P. nigra* and *P. pustulosa* on one side and *P. rudmani* Brunckhorst, 1993 on the other and probably represents another species. Our specimen of *P. rudmani* clustered with the specimen identified as *P. lizae* in Cheney et al. (2014). *Phyllidiella rudmani* and *P. lizae* resemble each other (Brunckhorst 1993) and hence it is possible that the species identified as *P. lizae* in Cheney et al. (2014) is in fact *P. rudmani*.

Specimens of seven nominal *Phyllidia* species were sequenced. Sequences of 25 individuals of *Phyllidia elegans* (including one from GenBank) formed a highly supported clade, just like the clades containing *P. ocellata*, *P. picta*, and *P. varicosa. Phyllidia coelestis* was also retrieved as a highly supported clade. An individual identified as *P. picta* by Cheney et al. (2014) was part of this group suggesting that it should probably be identified as *P. coelestis*. Brunckhorst (1993) already noticed the close similarity between the two species but still confused them (Yonow 1996), and hence identification errors are likely to occur. Individuals identified as *Phyllidia babai* Brunckhorst, 1993 and *P.* cf. *babai* were retrieved in two different clades. Specimens 336464 and 336614 differ in 75 base pairs, 336464 and 336575 by 68 base pairs and 336614 and 336575 by 32 base pairs. Differences based on COI suggest that they represent two, or possibly three, different species. The genus *Reticulidia* was retrieved as a sister group of *Phyllidia.*

Material of four nominal species in the genus *Phyllidiopsis* was sequenced, with additional data of one species from GenBank (*P. cardinalis*). *Phyllidiopsis fissuratus* clusters basal to *Phyllidiella*, without support. *Phyllidiopsis shireenae* Brunckhorst, 1990 and *P. xishaensis* (Lin, 1983) cluster as sister species, in highly supported clades. *Phyllidiopsis krempfi* also formed a clear group. *Phyllidiopsis cardinalis* does not cluster with any of the phyllidiid genera based on either the 16S or the COI analysis. This result suggests that *P. cardinalis* should be separated from the other *Phyllidiopsis* species, but further morphological analyses are needed to confirm this outcome. Brunckhorst (1993) noted that *P. cardinalis* is the type species of the genus *Phyllidiopsis*, and that it has a unique and complex coloration totally different from that of any other known phyllidiid species, as well as a different anatomy, especially in the foregut. Valdés (2003) states "Additionally, the genus *Phyllidiopsis* is not monophyletic when molecular characters are used, because *Phyllidiopsis cardinalis* is at the base of the Phyllidiidae clade, and not nested with the other members of *Phyllidiopsis*". Surprisingly, in the analysis of Cheney et al. (2014), based on a concatenated dataset of 16S and COI mtDNA, *P. cardinalis* was retrieved in a highly supported clade with several species of *Phyllidiella* and *Phyllidia*.

Variation within *Phyllidiella pustulosa*

Phyllidiella pustulosa is the only species in the COI cladogram (Figure 4) in which highly supported subclades can be discerned. To estimate the average evolutionary divergence within *Phyllidiella pustulosa* the base differences were compared per site for all grouped sequences of the species *Phyllidia elegans* (n = 24), *P. varicosa* (n = 15), *Phyllidiella nigra* (n = 7), *P. pustulosa* (n = 20), and *Phyllidiopsis krempfi* (n = 13) (Tables 4–5).

The genetic variation on the barcoding marker COI is much higher within *Phyllidiella pustulosa* (3.9%) than within the other four species, which showed genetic variations between 0.6 and 1.2% (Table 4). The interspecific genetic variation (involving three different genera) ranges between 10.5 and 18.9% (Table 5). The congeners *Phyl-*

Table 4. Estimates of average evolutionary divergence (p-distance) over sequence pairs within groups, in percentages.

Species	Distance (%)
Phyllidia elegans	0.7
Phyllidia varicosa	0.7
Phyllidiella nigra	0.6
Phyllidiella pustulosa	3.9
Phyllidiopsis krempfi	1.2

Table 5. Estimates of average evolutionary divergence (p-distance) over sequence pairs between groups, in percentages.

	Distance (%)				
Species	***Phyllidia elegans***	***Phyllidia varicosa***	***Phyllidiella nigra***	***Phyllidiella pustulosa***	***Phyllidiopsis krempfi***
Phyllidia elegans					
Phyllidia varicosa	12.1				
Phyllidiella nigra	15.8	15.5			
Phyllidiella pustulosa	18.3	18.9	10.5		
Phyllidiopsis krempfi	15.8	16.4	14.6	17.2	

lidiella nigra and *P. pustulosa* differ by 10.5%, and the congeners *Phyllidia elegans* and *P. varicosa* differ by 12.1%. The observed levels of genetic variation within *Phyllidiella pustulosa* (Table 4) and between the five species (Table 5) call for additional studies on possible cryptic speciation in *P. pustulosa*.

Conclusions

The barcoding marker COI works well to separate the different species in the Phyllidiidae, and confirms that the species boundaries in highly variable species, such as *Phyllidia elegans*, *P. varicosa,* and *Phyllidiopsis krempfi*, are correct as presently understood. However, a multi-locus approach, preferably including nuclear markers, is needed to improve the resolution for the higher taxonomic levels. With the exception of a few species that are difficult to place (*Phyllidiopsis fissuratus*, *Phyllidiopsis cardinalis*) the studied genera (*Phyllidia*, *Phyllidiella*, *Phyllidiopsis*, and *Reticulidia*) were retrieved as separate genera within the family. Additional representatives of *Ceratophyllidia* are needed to indicate the position of this genus within the Phyllidiidae. The observed groupings within *Phyllidiella pustulosa* suggest that multiple (cryptic) species could be present in this species, for which further analyses are needed including morphological data and multiple markers. Chang and Willan (2015) indicated that at least nine clades could be recognized in *Phyllidiella pustulosa* that could be separated slightly according

to morphological characters. We recommend that future studies combine DNA sequences with morphological characters, which can easily be done by adding pictures of the specimens to avoid increasing confusion in the identification of specimens.

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Supplementary material 1

COI sequences of lost Phyllidiidae specimens

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Data type: Adobe PDF file

Explanation note: COI sequences of Phyllidiidae specimens that dried out after sequencing (numbers and localities are indicated in Table 1.

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RESEARCH ARTICLE

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A new species of *Nebalia* (Crustacea, Leptostraca) from coral reefs at Pulau Payar, Malaysia

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Abstract

A new species of Leptostraca, *Nebalia terazakii* **sp. n.** is described and figured. The species was sampled from the coral reefs of Pulau Payar Marine Park, Langkawi, Malaysia. There are 32 existing species of *Nebalia* but *Nebalia terazakii* sp. n. can be distinguished from the other known species of *Nebalia* by the following combination of characters: the rostrum is 1.89 times as long as wide and the eyes have no dorsal papilla or lobes. Article 4 of the antennular peduncle has one short thick distal spine. The proximal article of the endopod of maxilla 2 is shorter than the distal, a feature peculiar to *Nebalia terazakii* **sp. n.**, the exopod of maxilla 2 is longer than article 1 of the endopod, the posterior dorsal borders of the pleonites 6 to 7 are provided with distally sharp denticles, anal plate with prominent lateral shoulder and finally, the terminal seta of the caudal rami is 1.17 times the length of the entire rami.

Keywords

Nebalia, new species, Leptostraca, coral reefs, Pulau Payar, Malaysia

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Introduction

The leptostracan genus *Nebalia* was thought to contain only a few species, but with rather a wide range of distribution. However, when Dahl (1985, 1990) re-examined specimens from the European Shelf and the Southern Oceans, he managed to solve some of the long outstanding problems on the taxonomy of *Nebalia*. According to Dahl (1985) the taxonomy of the European species was in a state of confusion and the synonymy so interwoven. Two new species were described for the European Shelf (Dahl 1985), and for the Southern Oceans Dahl (1990) described four new species from specimens previously referred to as *Nebalia longicornis* Thomson, 1879.

Since then many new species of *Nebalia* from various areas namely the Atlantic coasts (Haney et al. 2001; Moreira et al. 2003, 2009), Mediterranean Sea (Ledoyer 1997; Moreira et al. 2007, 2012; Kocak and Moreira 2015), Mexico (Escobar-Briones and Villalobos-Hiriart 1995), Red Sea (Wagele 1983), Africa (Kensley 1976; Olesen 1999, Bochert and Zettler 2012), Pakistan (Kazmi and Tirmizi 1989), New Caledonia (Ledoyer 2000), Hong Kong (Lee and Bamber 2011), South Korea (Song et al. 2012; Song and Min 2016) and California (Martin et al. 1996; Vetter 1996; Haney and Martin 2000, 2005) have been described. The present finding brings the total of the existing *Nebalia* species to 33.

As part of the study on the biodiversity of marine invertebrate fauna around Malaysia (Othman and Morino 1996, 2006; Othman and Toda 2006; Othman and Azman 2007; Gan et al. 2010; Lim et al. 2010, 2015; Azman and Othman 2012, 2013; Chew et al. 2014; Tan et al. 2014) a new species of *Nebalia* from Pulau Payar Marine Park has been discovered. The area has extensive coral reefs and was gazetted a National Park and near the northern entrance of the Straits of Malacca, within the Langkawi group of islands. Pulau Payar is situated 15 km south of the main Langkawi island and 20km off mainland Peninsular Malaysia.

Materials and methods

The animals were sampled using a baited trap. The trap consists of a clear 500 ml screw-cap wide mouthed polythene jars with a dozen 8 mm holes on the bottle cap. Fresh fish used as bait were wrapped in cheese cloth. Animals caught were fixed in 4% formaldehyde sea water solution and later transferred into glycerol. Drawings were made using a camera lucida on a Zeiss Axioscope light microscope. The specimens were dissected and appendages and mouthparts mounted onto slides in glycerol.

Type materials were deposited in the South China Sea Research and Repository Centre, Institute of Oceanography and Environment, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia.

Results

Order Leptostraca Claus, 1880
Family Nebaliidae Samouelle, 1819
***Nebalia* Leach, 1814**

***Nebalia terazakii* sp. n.**
http://zoobank.org/E31AE970-6D53-487F-9EC3-472804F76537
Figs 1–5

Material examined. Holotype: female, post ovigerous, 2.2 mm carapace length and 5.3 mm total length, Ref UMTCrus 00478, sample no 3272; paratypes, 12 adult females, Ref UMTCrus 00479, sample no 3272; 6 adult females, Ref UMTCrus 00480, sample no 3274; 5 adult females, Ref UMTCrus 00481, sample no 3276; 4 adult females and 32 juveniles, Ref UMTCrus 00482, sample no 3277.

Type locality. Pulau Payar, Kedah, Malaysia 6°03'48.0"N, 100°02'28.9"E; baited trap on coral reef, 12.9.1995.

Description of holotype. Body robust (Fig. 1a). Carapace about 1.5 times as long as wide, almost reaching posterior margin of pleonite 4, dorsally convex, anterior and posterior margin rounded.

Rostrum (Fig. 1a, d) prominent, 1.89 times as long as wide, slightly broader near base, sides almost parallel from proximal end to about midway then tapering to rounded distal end, ca. 0.3 times of carapace length, extending beyond eye and anterior margin of carapace; narrow in lateral, upper margin convex, lower margin flattened.

Compound eye without papilla (Fig. 1e), ommatidial part occupying 0.67 length of eyestalk. Eye stalk with small and pointed supraorbital spine present at posterior margin, tip not reaching posterior border of cornea.

Antennule (Fig. 1a) extending to about 0.4 times length of carapace. Peduncle 4-articulate (Fig. 2a), article 1 short, 0.2 times length of article 2, naked. Article 2, three times as long as wide with two plumose setae on mid-anterior margin, row of six plumose setae on lateral margin and 13 setae antero-distally. Article 3 half length of article 2, slightly longer than wide with two setae on disto-lateral margin and an antero-distal cluster of setae. Article 4, 0.7 times length of article 3, width same as length, with one distal stout spine, two rows each of four setae on inner lateral margin near spine. Outer lateral margin with two setae and long distal seta behind the scale. Antennular scale elliptical (Fig. 2a'), 2.5 times as long as wide, with rows of setae on anterior distal margin. Antennular flagellum slightly longer than peduncle and composed of 10 articles.

Antenna (Fig. 1a) extending beyond posterior margin of carapace. Peduncle 3-articulate (Fig. 2b), article 1, 1.59 times as long as wide, naked. Article 2, 0.86 times as long as article 1, 1.94 times as long as wide, and with one seta on anterior margin about midway. Article 3 (Fig. 2b') longer than article 2, with four short setae and one plumose seta on proximal inner margin and with several rows of setae along medial anterior margin; (1) six simple setae, (2) five short spine along proximal half, (3) six

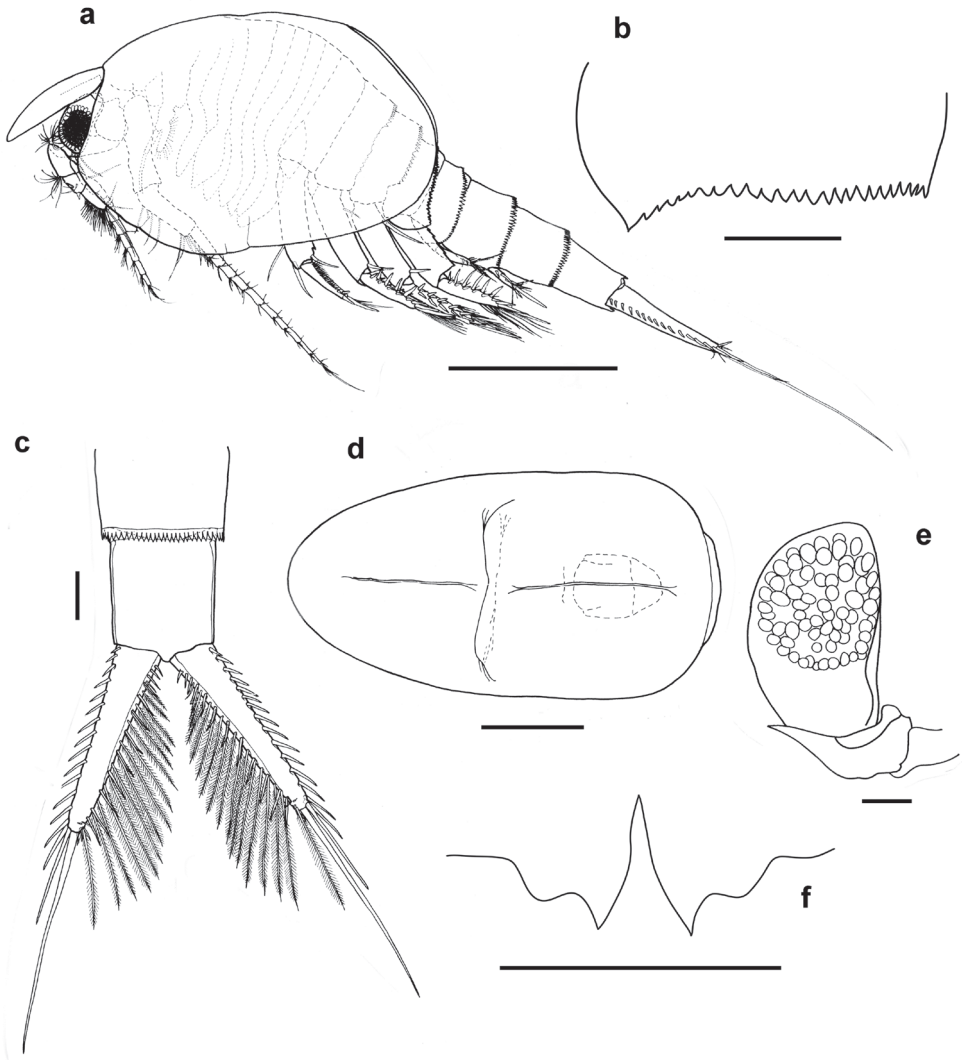

Figure 1. *Nebalia terazakii* sp. n., female, **a** body, lateral **b** epimeron of pleopod 4, lateral **c** caudal furca, dorsal **d** rostrum, dorsal **e** eye, lateral **f** anal scale, ventral. Scale bars: **a** = 1.0 mm, **b–f** = 0.2 mm.

longer simple setae, (4) three thin setae, (5) six long setae; terminal row of four spines, increasing distally in length, the distal most next to four simple setae and one long spine, one long plumose seta on posterior margin about midway, cluster of about 10 plumose setae along distal inner margin, two short setae on postero-distal margin. Flagellum well developed, composed of 11 articles; each article with five terminal setae of various lengths.

Mandible (Fig. 2c) well developed. Mandibular palp three-articulate, article 2 equal in length as article 3, and with sub-terminal seta and another seta midway on lateral margin. Article 3 cylindrical, with marginal setae-row covering anterior mar-

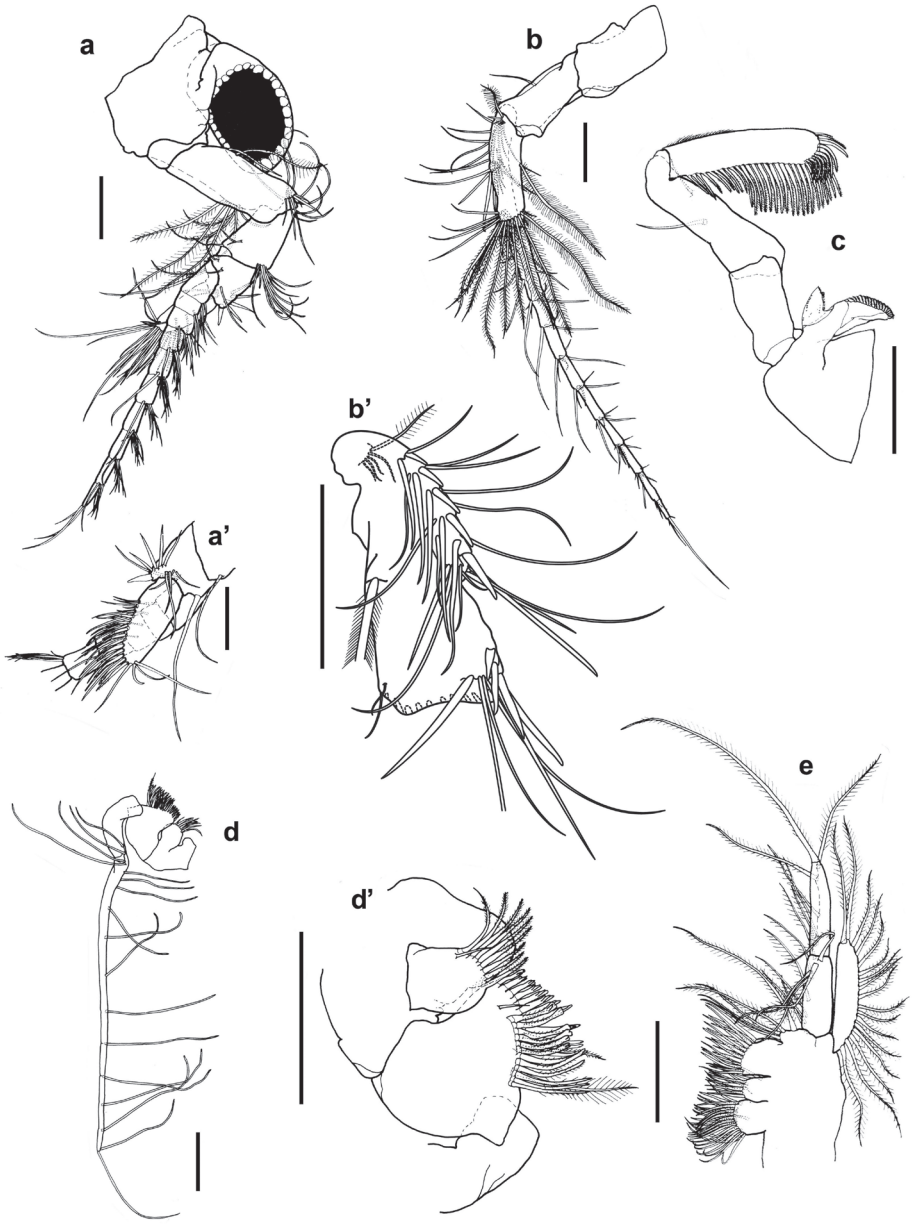

Figure 2. *Nebalia terazakii* sp. n., female, **a** antennule, lateral **a'** antennule scale **b** antennae, lateral **b'** antennae, article 3, medial border **c** mandible, anterior: **d** maxilla 1, anterior **d'** maxilla 1 endite, anterior **e** maxilla 2, anterior. Scale bars: 0.2 mm.

gin beginning small distance from proximal margin, all setae equal in length, weakly plumose, beginning with length about as wide as article, doubling in length about 2/5 from proximal end, posterior margin with minute hairs covering about midway from

proximal end. Article 1, 0.6 times length of article 3, 1.95 times as long as wide, naked. Molar process three times as long as wide, slightly shorter in length than article 1 of palp. Distal margin with rows of teeth forming grinding surface. Incisor process broad basally with acute terminal process and with minute teeth along inner and outer face.

Maxilla 1 (Fig. 2d) with distal endite as long as proximal one and carrying row of plumose setae and two rows of sculptured setae on inner medial margin (Fig. 2d'). Inner medial margin of proximal endite lobed into two parts, with upper one bearing row of nine weakly plumose setae. Palp very long, about 4.6 times longer than combined length of both endites, and with 16 widely spaced long setae along its length and a terminal seta.

Maxilla 2 (Fig. 2e) protopod with four endites, endites 1 to 3 armed with many rows of short weakly plumose setae, endite 4 with five relatively longer plumose setae. Endopod two-articulate, article 1, 0.83 times length of article 2 and with nine plumose setae on medial margin. Article 2 with six plumose setae on medial margin and one weakly plumose terminal seta 1.67 times combined length of articles 1 and 2. Exopod slightly longer than article 1 of endopod and with one terminal and 16 weakly plumose setae spreading from proximal to distal outer margin.

Thoracopods leaf-like, all eight thoracopods with endopods extending beyond distal margin of exopods, and with terminal article of endopods showing traces of sheded brood pouch setae. Thoracopod 1 (Fig. 3a), exopod elliptical in shape, 2.3 times as long as wide, extending to middle of sub-terminal article of endopod, with 15 weakly plumose setae along outer margin equally spaced from distal to proximal end. Endopod five-articulate with two rows of plumose setae and one row of spines along inner margin from proximal to distal end of article 2, tuft of smooth setae also present near proximal end of endopod. Epipod bilobed and elongated, 3.5 times as long as wide and reaching distal 2/3 of exopod. Thoracopods 2 (Fig. 3b) and 3 (Fig. 3c), exopod triangular in shape with broadest part 1.45 times as long as wide, extending to 0.33 times of sub-terminal article of endopod, and with six to ten plumose setae on outer-lateral margin from 1/3 way of proximal end to its distal end. Endopod four-articulate with row of weakly plumose setae on inner medial margin extending from proximal end of endopod to sub-terminal article, row of shorter setae and row of spines extending from proximal end to about 0.67 times of endopod length. Row of seven plumose setae also present near distal end. Epipod bilobed, slightly broarder than that of thoracopod 1 and 3.2 and 2.8 times as long as wide for thoracopods 2 and 3, respectively. Thoracopod 4 (Fig. 3d) similar to thoracopods 2 and 3 except that endopod three-articulate and exopod extends to proximal end of sub-terminal article of endopod. Thoracopods 5 (Fig. 4a) and 6 (Fig. 4b) similar to preceeding thoracopods except having broarder epipods with 2.3 times as long as wide for thoracopod 5 and 2.1 times as long as wide for thoracopod 6. Endopod of thoracopod 5, four-articulate whereas thoracopod 6, three-articulate. Exopods extend to 0.67 length of sub-terminal article of endopod. Thoracopod 7 (Fig. 4c) similar to preceeding thoracopod except endopod is two-articulate and exopod extend to about 0.67 times of terminal segment of endopod. Distal lobe of epipod much broader, about 1.8 times as long as wide. Thoracopod 8 (Fig. 4d) endopod five-articulate and with row of smooth setae extending from proximal end to distal end of sub-terminal article.

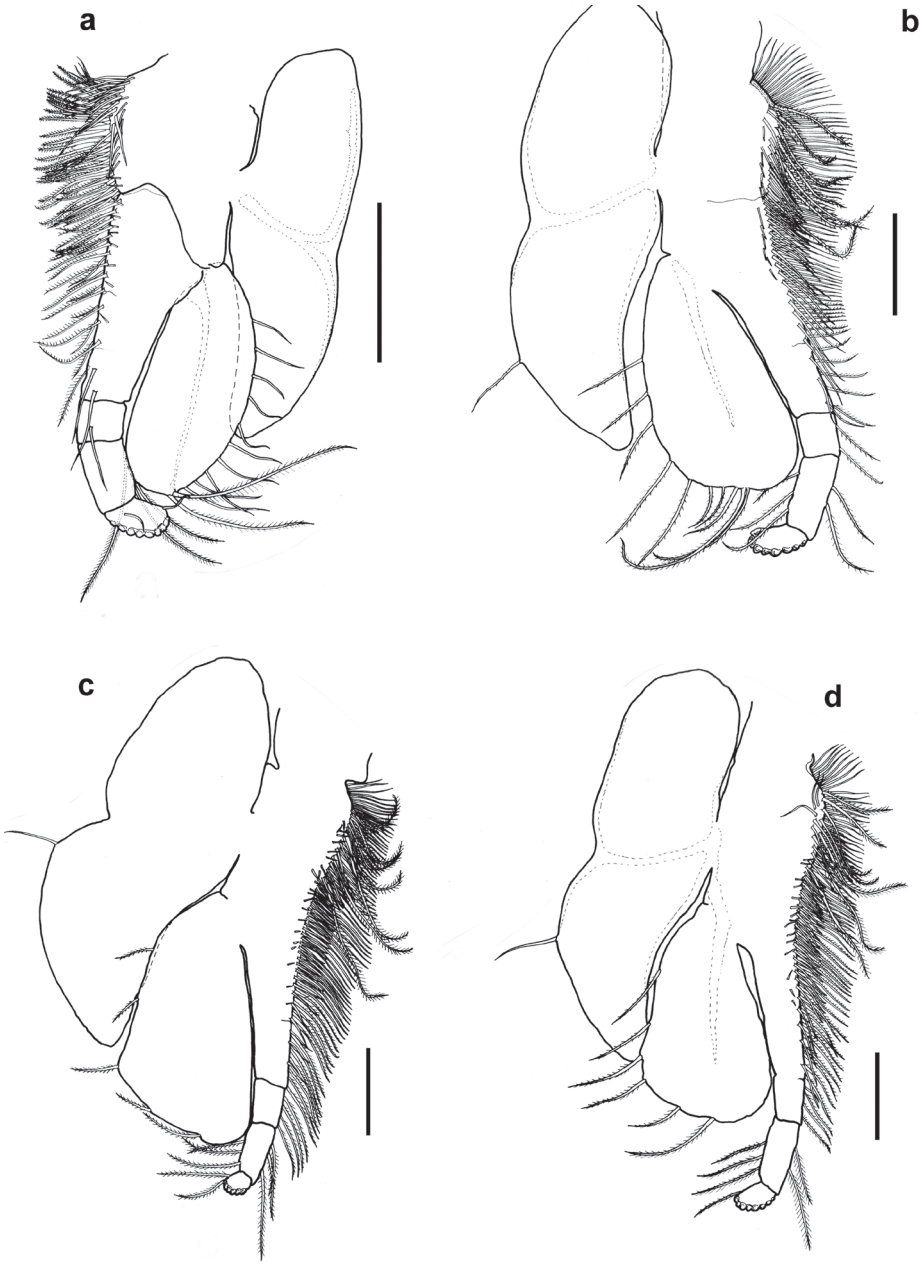

Figure 3. *Nebalia terazakii* sp. n., female, **a** thoracopod 1, dorsal **b** thoracopod 2, dorsal **c** thoracopod 3, dorsal **d** thoracopod 4, dorsal. Scale bars: 0.2mm.

A row of plumose setae extends from proximal end of endopod to distal end of subterminal article. Exopod oblong 2.7 times as long as wide with three long setae on outer margin. Epipod narrow, 3.4 times as long as wide and extends 0.25 times of exopod.

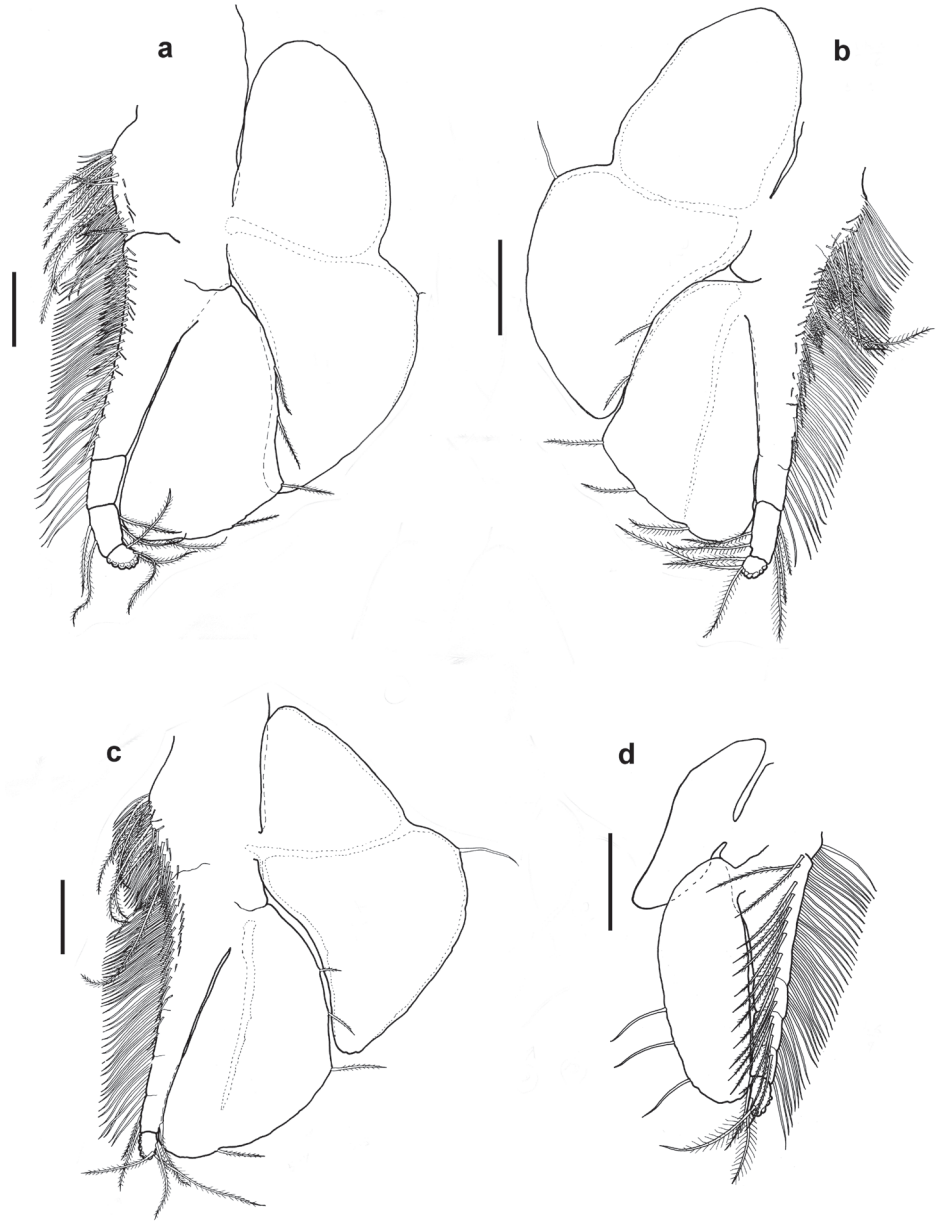

Figure 4. *Nebalia terazakii* sp. n., female, **a** thoracopod 5, dorsal **b** thoracopod 6, dorsal **c** thoracopod 7, dorsal **d** thoracopod 8, dorsal. Scale bars: 0.2 mm.

Posterior margins of pleonites 3 to 7 serrated throughout their lengths, denticles pointed along dorsal margins changing to blunt along lateral margins of pleonites 3 to 6, denticles of pleonite 7 pointed all through. Epimeron of pleon 4 with margin evenly serrated and with acutely pointed posterolateral corner (Fig. 1b).

Pleopod 1 (Fig. 5a), composed of protopod, exopod and endopod. Protopod measuring 1.7 times as long as wide, broadest at proximal end tapering at distal end, with one seta on outer margin 1/3 from proximal end, two setae on inner margin same distance from proximal end and two small setae close to endopod and one stout long distolateral seta reaching to 0.67 times of exopod. Endopod, two-articulate, 0.85 times as long as protopod and 1.5 times longer than exopod, and with long terminal spine half length of endopod, reticulum present. Exopod with comb-row of short trifid setae on outer margin, long plumose setae along inner margin and 4 stout spines on distal margin, terminal spine of which by far largest.

Pleopod 2 (Fig. 5b), protopod 1.7 times as long as wide and with pair of setae on inner lateral margin 1/4 way from proximal end, pair of short setae on distal margin near endopod, stout seta on distal margin near exopod and two setae on outer lateral margin. Endopod two-articulate, subequal in length as protopod with plumose setae along outer and medial margins and terminal spine half as long as endopod, reticulum present. Exopod 0.8 times length of endopod with six pairs of robust setae and single plumose seta in between on outer margin, three terminal setae and row of long plumose setae on medial margin.

Pleopod 3 (Fig. 5d) protopod 1.7 times as long as wide, with pair of setae each on posterior and anterior lateral margins 1/3 way from proximal end, seta on outer margin 1/3 way from proximal end, distal margin with pair of plumose setae near endopod and stout seta near exopod reaching 0.4 times of exopod. Endopod two-articulate, subequal in length as protopod, with plumose setae along outer and medial margins and terminal spine almost half as long as endopod, reticulum present. Exopod 0.7 times length of endopod and with five pairs of stout seta and single plumose seta in between and three terminal stout seta and row of long plumose setae along medial margin.

Pleopod 4 (Fig. 5e) protopod rectangular, 1.3 times as long as wide, outer margin serrated and with row of five setae 1/4 way from the proximal margin, inner margin with pair of setae 1/4 way from proximal end, inner distal margin with single plumose seta. Endopod two-articulate, 1.3 times as long as protopod, with plumose setae along outer and medial margins, terminal spine 0.6 times as long as endopod, rectangular shaped reticulum present. Exopod 0.8 times length of endopod and with seven pairs of stout setae and single plumose seta in between, three terminal stout setae and row of long plumose setae along medial margin.

Pleopod 5 (Fig. 5c) uniramous, two-articulate, distal article 3.7 times as long as wide, with five stout spines along distolateral and terminal border, increasing in length distally, about 25 simple setae along medial and distal border.

Pleopod 6 (Fig. 5f) uniramous, single article, 2.6 times as long as wide, with five very strong lateral and distal stout spines, distal most spine slightly longer than pleopod, with circlet of sharp teeth surrounding base. Lateral border with six setae, medial border with four setae and three distal setae.

Anal somite, anal plate and uropods (Fig. 1c), anal somite (pleonite 8) short, marginally longer than wide, slightly longer than pleonite 7. Anal plates (Fig. 1f) with convex medial margin and with long, acute points over medial part of scale, lateral margin

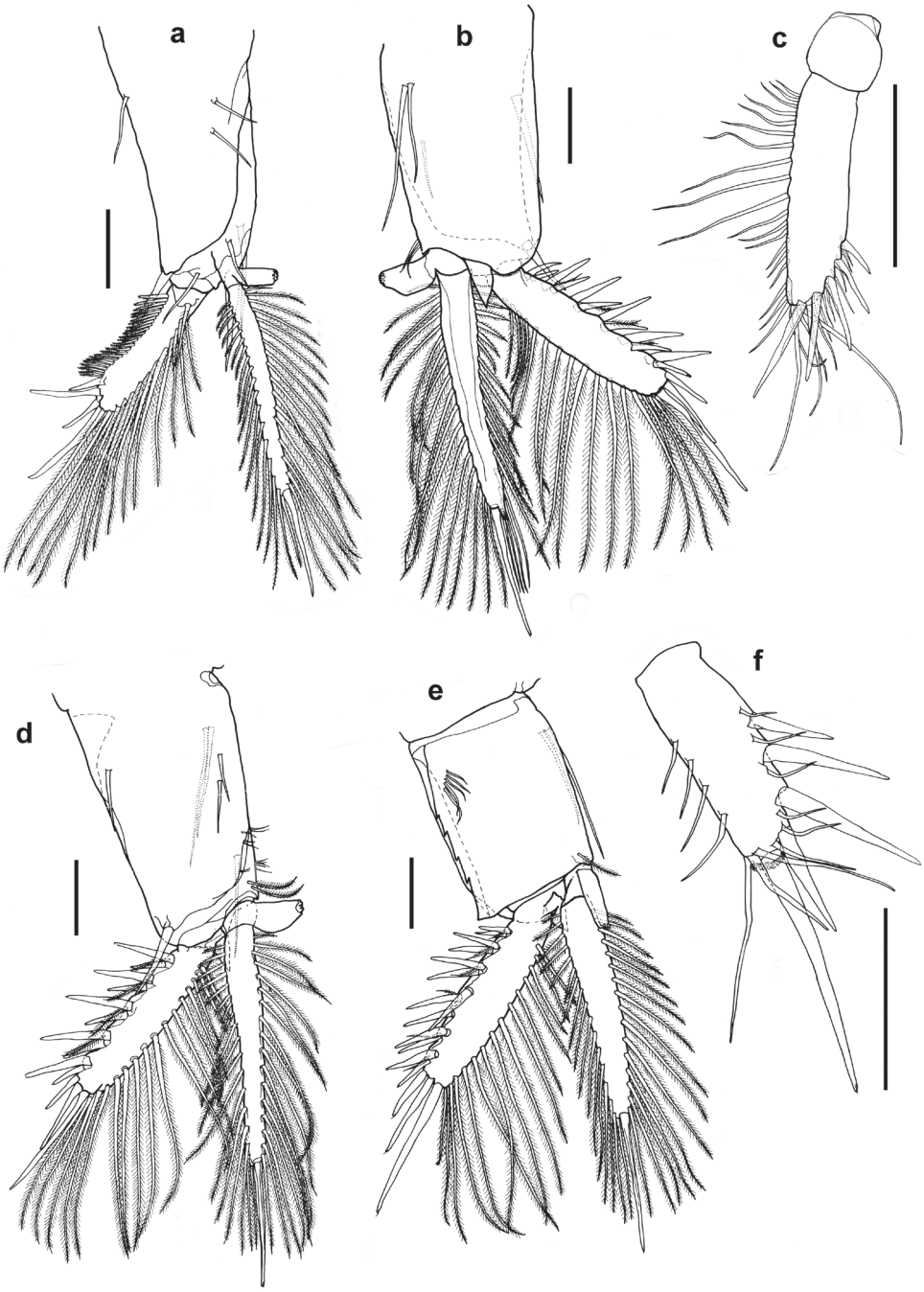

Figure 5. *Nebalia terazakii* sp. n., female, **a** pleopod 1, anterior **b** pleopod 2, posterior **c** pleopod 5, anterior **d** pleopod 3, anterior **e** pleopod 4, anterior **f** pleopod 6, anterior. Scale bars: 0.2 mm.

with prominent and narrow shoulder. Uropods, about 0.9 times as long as combined pleonite 7 and anal somite, slightly tapering distally, with about 16 to 18 robust setae along lateral margin progressively increasing in length from proximal to distal end. Along lateral inner margin of uropod, about 12 to 14 similar setae as well as 15 to 18 long plumose setae. Terminal spine of uropod about 1.17 times length of uropod.

Remarks. Dahl (1985) revised the Leptostracans of the European Shelf and described a new genus with *Sarsinebalia typhlops* (G.O. Sars, 1870), formerly *Nebalia typhlops*, and relegated the species *Nebalia geoffroyi* Milne-Edwards, 1928 as a junior synomym of *N. herbstii* Leach, 1814. Martin et al. (1996) expounded *Nebalia pugettensis* (Clark, 1932) as *nomen nudum* and replaced it with *Nebalia hessleri* Martin, Vetter & Cash-Clark, 1996. Walker-Smith and Poore (2001) revised the classification of the Leptostraca using phylogenetic analysis and reducing the number of species in the genus from 41 to 32 known species. Currently, there are 33 described species of *Nebalia* as in Table 1.

Nebalia terazakii sp. n. differs from the other known species of *Nebalia* in the following combination of characters: the rostrum is 1.9 times as long as wide, the eyes have no dorsal papilla or lobes, article 4 of the antennule peduncle has only one short thick distal spine, the armature of the external lateral side of the antennal peduncle article 3 has distribution and appearance of spines and setae which differs from other known species, article 1 of the endopod of maxilla 2 is peculiarly short, about 0.83 times as long as article 2, the exopod of maxilla 2 is longer than article 1 of the endopod, the posterior dorsal borders of the pleonites 6 to 7 are provided with distally sharp denticles, anal plate with prominent lateral shoulder and finally, the terminal seta of the caudal rami is 1.17 times the length of the entire ramus. In all other known species of *Nebalia* the proximal article of maxilla 2 is longer than the distal article, however, in *Nebalia terazakii* sp. n. the distal article of maxilla 2 is longer than the proximal, a feature peculiar to *N. terazakii* sp. n.

Nebalia terazakii sp. n., when compared with recently described species from the Asian and Southeast Asian regions (Table 2), shows differences in the length to width ratio of the rostrum. The ratios for all species are >2, except *N. melanophthalma* and *N. terazakii* sp. n. which are 1.73 and 1.89 respectively. The area occupied by the ommatidial part of the eye is similar for *N. terazakii* sp. n., *N. dolsandoensis*, *N. melanophthalma* and *N. moretoni*, however, in *N. koreana* and *N. pseudotroncosoi* the area is larger and smaller respectively. Another feature which differs between the species is the number of thick spines on the article 4 antennular peduncle. In *N. melanophthalma* and *N. terazakii* sp. n. there is one thick spine whereas there are >1 for the rest of the species. Article 1 of maxilla 2 endopod is shorter than article 2 in *N. terazakii* sp. n. whereas in all other species articles 1 is longer than 2. Denticles on pleonite 6 to 7 are acutely shaped in *N. melanophthalma* and *N. terazakii* sp. n. but square to rounded in the others. The anal plate shoulder of *N. terazakii* n.sp is prominent and this distinguishes it from the other species mentioned. The uropod and combined pleonite 7 and anal somite length ratios vary between 0.7 and 1.0. The terminal spine to uropod length ratio shows similarity between *N. terazakii* sp. n. and *N. koreana* but differs greatly from *N. dolsandoensis* and *N. moretoni*.

Table 1. List of existing species of *Nebalia*, type locality and references.

Species	Type locality	Reference
N. abyssicola Fage, 1929	Monaco	Moreira et al. (2012)
N. antarctica Dahl, 1990	Antarctic, Ross Sea	Dahl (1990)
N. biarticulata Ledoyer, 1997	Marseille, France	Ledoyer (1997)
N. bipes (Fabricius, 1730)	Greenland	Dahl (1985)
N. borealis Dahl, 1985	Norway	Dahl (1985)
N. brucei Olesen, 1999	Zanzibar, Tanzania	Olsen (1999)
N. cannoni Dahl,1990	South Georgia	Dahl (1990)
N. capensis Barnard, 1914	Cape Town, S. Africa	Kensley (1976)
N. clausi Dahl, 1985	Adriatic Sea, Italy	Dahl (1985)
N. dahli Kazmi & Tirmizi, 1989	Karachi, Pakistan	Kazmi and Tirmizi (1989)
N. daytoni Vetter, 1996	San Diego, California	Vetter (1996)
N. deborahae Bochert & Zettler, 2012	Namibia & Angola	Bochert and Zettler (2012)
N. dolsandoensis Song & Min, 2016	Dolsand Island, S. Korea	Song and Min (2016)
N. falklandensis Dahl, 1990	Falkland Is.	Dahl (1990)
N. gerkenae Haney & Martin, 2000	Monterey Bay, California	Haney and Martin (2000)
N. helbstii Leach, 1814	British coast	Dahl (1985)
N. hessleri Martin, Vetter & Cash-Clark, 1996	Southern California	Martin et al. (1996)
N. ilheoensis Kensley, 1976	South-western Africa	Kensley (1976)
N. kensleyi Haney & Martin, 2005	Marin County, California	Haney and Martin (2005)
N. kocatasi Moreira, Kocak & Katagan, 2007	Izmir Bay, Turkey	Moreira et al. (2007)
N. koreana Song, Moreira & Min, 2012	Dolsando Island, S. Korea	Song et al. (2012)
N. lagartensisi Escobar-Briones & Villalobos-Hiriart, 1995	Yucatan Peninsula, Mexico	Escobar-Briones and Villalobos-Hiriart (1995)
N. longicornis Thomson, 1879	South Island, New Zealand	Dahl (1990)
N. marerubi Wagle, 1983	Red Sea	Wagle (1983)
N. mediterranea Kocak & Moreira, 2015	Aegean Sea Turkey, N. Cyprus	Kocak and Moreira (2015)
N. melanophthalma Ledoyer, 2000	Noumea, New Caledonia	Ledoyer (2000)
N. mortoni Lee & Bamber, 2011	Hong Kong	Lee and Bamber (2011)
N. patagonica Dahl, 1990	Magellan region	Dahl (1990)
N. pseudotroncosoi Song, Moreira & Min, 2012	South coast of Korea	Song et al. (2012)
N. schizophthalma Haney, Hessler & Martin, 2001	North Atlantic, Gay Head	Haney et al. (2001)
N. strausi Risso, 1826	Channel Is, Guernsey	Dahl (1985)
N. terazakii sp. n.	Pulau Payar, Malaysia	Present study
N. troncosoi Moreira, Cacbelos & Dominguez, 2003	Galicia, Iberian peninsula	Moreira et al. (2003)

Nebalia terazakii sp. n. is most similar to *N. brucei* in that both species have a broad rostrum with a similar length to width ratio, the antennular armatures on peduncle article 4 are each armed with a single spine, the antennular scales are both elliptical, the epimerons of the pleopod 4 are pointed and the lateral margins of the anal plates are both with prominent shoulder. However, these two species can be distinguished from one another in that the antennular flagellum has 12 articles in *N. brucei*, whereas it is 10 in *N. terazakii* sp. n. The armature of the external lateral side of the antennal peduncle article 3, differ in the distribution and appearance of spines and setae between

Table 2. Comparison of some diognostic characters of *Nebalia* females from the Asian and Southeast Asian regions.

Species	Rostrum length/ width	Area occupied by ommatidial part of eye	Antennule peduncle article 4: no. of thick spines	Maxilla 2 endopod: article 1/ article 2 length	Pleonites 6-7: shape of dorsal denticles	Anal plate shoulder	Uropod length/ pleonite 7 + anal somite	Terminal spine length/ urosome
N. dolsandoensis	2.14	0.67	4	1>2	round	present	0.7	1.69
N. koreana	2.35	0.85	5	1>2	round	none	0.8	1.15
N. melanophthalma	1.73	0.67	1	1>2	acute	none	1.0	na
N. mortoni	2.37	0.67	4	1>2	square	none	1.0	1.7
N. pseudotroncosoi	2.27	0.5	2	1>2	round to pointed	none	0.9	na
N, terazakii sp. n.	1.89	0.67	1	1<2	acute	prominent	0.9	1.17

the two species. Length ratio of maxilla 2 endopod article 1 and 2 is 1.39 in *N. brucei* whereas in *N. terazakii* sp. n. it is 0.83. The terminal spine of uropod is about 1.17 times the length of uropod in *N. terazakii* sp. n. whereas it is 0.70 in *N. brucei*.

Etymology. The species is named after the late Professor Dr. Makoto Terazaki, Ocean Research Institute, University of Tokyo, Japan.

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The first geophilid centipedes from Malesia: a new genus with two new species from Sumatra (Chilopoda, Geophilidae)

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Abstract

A new genus *Sundageophilus* is here described for two new species of geophilid centipedes (Chilopoda: Geophilidae) from Sumatra, Indonesia. Both *S. bidentatus* **sp. n.** and *S. poriger* **sp. n.** feature a minute body size (less than 1 cm long with 31–35 pairs of legs), a similar structure of the maxillae, elongated forcipules, and few coxal organs. *Sundageophilus bidentatus* is unique among geophilids because the ultimate article of the forcipule is armed with two conspicuous denticles, one dorsal to the other, instead of a single one or none. The two species of *Sundageophilus* are the first genuine Geophilidae ever found in Malesia, and among the very few representatives of this family in the entire south-eastern Asia.

Keywords

Chilopoda, forcipules, Geophilidae, Southeast Asia, *Sundageophilus*

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Introduction

The diversity of geophilomorph centipedes (Geophilomorpha) in south-eastern Asia is still largely unexplored. In comparison with other areas, including nearby tropical regions, these soil arthropods have remained notably under-sampled in the entire Indochina, Malesia, and Papuasia. Many naturalistic expeditions reached these lands and islands between the late 19th and early 20th centuries, but they provided only few specimens and records (Attems 1914). Some advances were subsequently contributed by various taxonomists (Chamberlin 1920, 1939, 1944; Attems 1930a, 1938, 1953; Verhoeff 1937; Würmli 1972; Lewis 1991); however, to date all records from this broad area are based on a small number of samples from a few sparse localities. Additionally, records are biased towards epigeic and larger-bodied species. For a recent overview see Bonato and Zapparoli (2011).

The situation is especially unsatisfactory for the large island of Sumatra, when compared with the neighbouring Malay peninsula and the other Malesian islands. To the best of our knowledge, all records of geophilomorph centipedes from Sumatra derive from half a dozen papers (Pocock 1894; Silvestri 1895, 1919; Attems 1930b; Chamberlin 1944; Lewis 1991) and concern specimens collected in no more than a dozen localities. Most of the species hitherto recorded belong to Mecistocephalidae, Oryidae and Gonibregmatidae, which are relatively large and conspicuous geophilomorphs, the only small sized exception is a species of Ballophilinae (Table 1). No concrete evidence has been obtained to date for Sumatra and even for Malesia as a whole, for the presence of species of Geophilidae, which is by far the richest and most widespread family of geophilomorph centipedes in the world (Bonato and Zapparoli 2011).

Two new species of Geophilomorpha are described from Sumatra. They are representatives of a new lineage of minute animals that have hitherto escaped the attention of myriapodologists and have evolved a previously unknown pattern of forcipular denticles. They are the first Geophilidae ever found in Malesia, and among the very few representatives of this family recorded in the entire south-eastern Asia, from Indochina to Papuasia.

Material and methods

Specimens were found in soil samples collected in Sumatra, along a gradient including secondary rainforests, jungle rubber agroforests, rubber, and oil palm plantations. Sampling has been carried out within the interdisciplinary project "Ecological and socioeconomic functions of tropical lowland rainforest transformation systems (Sumatra, Indonesia) – EFForTS". For details on the study region and experimental design see Drescher et al. (2016). Specimens were extracted from soil cores by heat (Kempson et al. 1963) and fixed in 70% ethanol.

The specimens were examined by light microscopy (Leica DMLB) according to standard protocols for geophilomorphs, by placing them in ethylene glycol in

Table 1. Species of Geophilomorpha hitherto recorded from Sumatra and published sources of records. For the taxonomic names and classification, we referred to Bonato et al. (2016). Published taxonomic names different from the current ones are given in squared parentheses.

Species	**Source/s**
Mecistocephalidae	
Tygarrup sp. (at least one species, possibly more than one)	Pocock 1894 [*Mecistocephalus spissus*] Silvestri 1895 [*Mecistocephalus spissus*] possibly Attems 1930a [*Mecistocephalus spissus*]
Mecistocephalus sp. (at least two species)	Haase 1887 [*Mecistocephalus punctifrons*] Pocock 1894 [*Mecistocephalus punctifrons*] Silvestri 1895 [*Mecistocephalus punctifrons*] Attems 1914 [*Mecistocephalus insularis*] Silvestri 1919 [*Lamnonyx insularis* varietas *orientalis*; *Lamnonyx cephalotes* varietas *subinsularis*] Verhoeff 1937 [*Mecistocephalus verrucosus*] Lewis 1991 [*Mecistocephalus verrucosus*]
Oryidae	
Orphnaeus brevilabiatus (Newport,1845)	Silvestri 1895 Attems 1930a Chamberlin 1944
Schendylidae Ballophilinae	
Ballophilus pedadanus Chamberlin, 1944	Chamberlin 1944 Lewis 1991
Gonibregmatidae	
Geoporophilus angustus Silvestri, 1919	Silvestri 1919
Geoporophilus aporus Attems, 1930	Attems 1930a
Himantosoma porosum Pocock, 1891	Silvestri 1895 [later described as *Himantosoma typicum* varietas *tridivisum*; Silvestri 1919]

temporary mounts (Pereira 2000). The head was detached from the trunk for some specimens only. Measurements were taken using a micrometre eyepiece. Digital photographs were taken using a camera (Leica DFC420) attached to the microscope and assembled using an image stacking software (CombineZP; Hadley 2008). Line-drawings were produced manually from the photographs. For the morphological terminology, we followed Bonato et al. (2010).

To evaluate whether similar or possibly related species were already recorded in south-eastern Asia, the entire taxonomic and faunistic literature on centipedes was browsed to retrieve all published records from that area.

Abbreviations

LIPI Indonesian Institute of Science, Cibinong, Indonesia;
PD Department of Biology, University of Padova, Italy.

Taxonomy

***Sundageophilus* gen. n.**
http://zoobank.org/11CB8320-AC3E-4657-B217-AB6E30459855

Diagnosis. Relatively small geophilids, less than 1 cm long; cephalic plate distinctly elongate, without frontal line; clypeus with two pairs of setae on the anterior medial part, without a distinct clypeal area; intermediate part of labrum bearing stout tubercles, lateral parts far apart from each other and bearing bristles; first maxillae without lappets; second maxillary coxosternite with anterior margin entire and concave, without anterior projections, neither statuminia nor other distinctly sclerotized parts associated with the metameric pores; second maxillary pretarsus in shape of an elongate claw, more than 3.5 times as long as wide at the basis, sub-conic and slightly bent, with a small sub-basal dorsal bulge; forcipular tergite subtrapezoidal; forcipular coxosternite relatively elongate, the exposed part as wide as or only slightly wider than long, the anterior margin slightly projecting anteriorly, with two very short denticles and a narrow notch inbetween; coxopleural sutures complete, entirely ventral, straight and subparallel for most of their length; chitin-lines absent or hardly distinct; forcipules relatively elongate, the trochanteroprefemur is more than 1.4 times as long as wide, the tarsungulum more than 2.5 times as long as wide; forcipular intermediate articles distinct, without denticles; tarsungulum with at least a distinct basal denticle; trunk metasternites longer than wide, without obvious "carpophagus" pit; whenever present, a single sub-circular, posterior pore-field on all metasternites of the trunk; leg claws with at most a pair of accessory spines, shorter than mid-length of the pretarsus, similar to each other in length; ultimate leg-bearing segment with an entire pleuropretergite, without sulci; ultimate metasternite sub-trapezoid, the setae distributed almost uniformly in the female, unknown in the male; coxopleuron with at least two coxal organs, opening through independent pores on the ventral side; telopodite of the ultimate leg pair approximately 1.8–2.0 times as long as that of the penultimate pair; anal pores distinct.

Etymology. From "Sunda", the name in use for the south-eastern part of the continental shelf of Asia, including Sumatra and other islands, and "Geophilus", which is used in many other names of genera of geophilids.

Type species. *Sundageophilus bidentatus* sp. n.

***Sundageophilus bidentatus* sp. n.**
http://zoobank.org/96F28A97-E612-4D89-A8CC-99A8F270A5BC

Diagnosis. A *Sundageophilus* species with cephalic plate ca. 1.4–1.5 times as long as wide; first maxillary telopodite apparently composed of a single article; a distinct denticle on the distal part of the trochanteroprefemur; tarsungulum with two basal denticles, one dorsal to the other; 33 or 35 pairs of legs; no ventral pore-fields along the trunk; ultimate metasternite 1.7–1.8 times as wide as long, anteriorly ca. 2.0 times as

wide as posteriorly, lateral margins slightly convex; two coxal pores on each coxopleuron, along the lateral margin of the metasternite; telopodites of the ultimate pair conspicuously swollen in the female, unknown in the male, apparently composed of only five articles because of the indistinct articulation between trochanter and prefemur; some articles of the ultimate leg pair with disto-ventral bulges covered with denser, longer setae, and a tuft of tiny spines replacing the pretarsus.

Material examined. *Holotype*. ♀ with gonopods partially developed, body length 6.9 mm, head width 220 μm; some legs broken and missing, including one of the ultimate pair; originally entire, subsequently divided in three pieces, (i) head, (ii) forcipular segment and leg-bearing segments 1–16, (iii) leg-bearing segments 17–33 and postpedal segments; collected Nov. 2013, by B. Klarner; in ethanol, LIPI macrosoil-BO4a13_chilo144.

Type locality. Indonesia, Sumatra, Bukit Duabelas, oil palm plantation, research site BO4, 02°03'02"S, 102°45'12"E, ca. 30 m a.s.l., from upper soil layer (0–5 cm).

Other specimens examined. 1 ♀, from Bukit Duabelas, secondary rainforest, research site BF2, 01°58'55"S, 102°45'03"E, ca. 80 m a.s.l., from upper soil layer (0–5 cm), same date and collector as holotype, PD5768; 6 ♀♀, from Bukit Duabelas, secondary rainforest, research site BF3, 01°56'34"S, 102°34'53"E, ca. 90 m a.s.l., from upper soil layer (0–5 cm), same date and collector as holotype, LIPI macrosoil-BF3a13_chilo178–183; 1 ♀, from Harapan, secondary rainforest, research site HF3, 02°10'30"S, 103°19'58"E, ca. 60 m a.s.l., from upper soil layer (0–5 cm), same date and collector as holotype, LIPI macrosoilHF3c13_chilo17; 1 specimen, sex unknown because body posterior part missing, from Harapan, jungle rubber agroforest, research site HJ2, 01°49'32"S, 103°17'39"E, ca. 80 m a.s.l., from upper soil layer (0–5 cm), same date and collector as holotype, PD5767.

Etymology. "*bidentatus*" means "with two teeth" and refers to the presence of two distinct basal denticles on each forcipular tarsungulum.

Description. *Description of holotype* (♀, LIPI macrosoilBO4a13_chilo144). See also Figs 1A, 1C, 2, 3.

General features. Body remarkably narrow, almost uniformly wide for most part of the trunk, only slightly narrowing posteriorly. Legs relatively short, most of them ca. 0.2 mm long. Colour (in ethanol) almost uniformly pale yellow, but cephalic plate, forcipular condyles, tarsungula, and leg pretarsi darker.

Cephalic capsule. Cephalic plate subrectangular, ca. 1.4–1.5 times as long as wide, ca. 1.1 times as wide as the forcipular tergite, the lateral margins slightly convex; scutes approximately isometric and up to 10 μm in the anterior half of the cephalic plate, distinctly elongate longitudinally and up to 18 μm long in the posterior half; frontal line absent; setae up to ca. 30 μm long. Clypeus ca. 1.5–1.6 times as wide as long, with lateral margins complete; uniformly areolate, the scutes being up to 10 μm wide, without a distinct clypeal area; a total of 4 setae arranged in two pairs, one anterior to the other. Pleurites uniformly areolate, without setae. Both the intermediate and the lateral parts of the labrum separated from the clypeus by complete sulci; the intermediate part ca. 2.5 times as wide as long, the lateral parts far apart from each other.

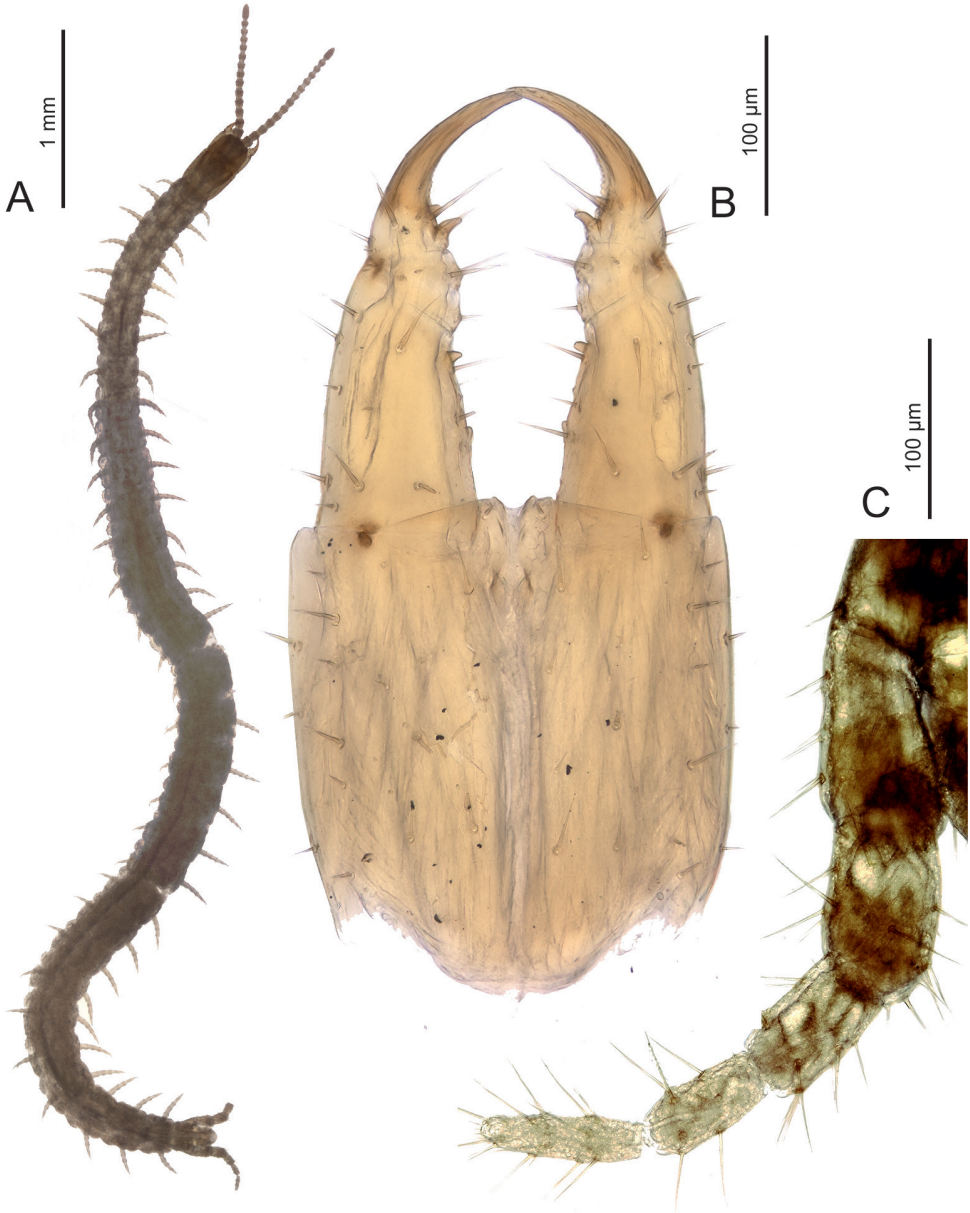

Figure 1. *Sundageophilus bidentatus* sp. n.: **A** entire body, dorsal view **B** forcipular segment, ventral view **C** ultimate left leg, dorsal view. LM photos of holotype LIPI macrosoilBO4a13_chilo144 (**A, C**) and PD5768 (**B**).

Antennae. Slender, ca. 3.6 times as long as the head width. Intermediate articles up to ca. 1.2 times as long as wide. Article XIV ca. 2.2 times as long as wide, ca. 1.9–2.0 times as long as article XIII and slightly narrower than the latter. Setae gradually denser

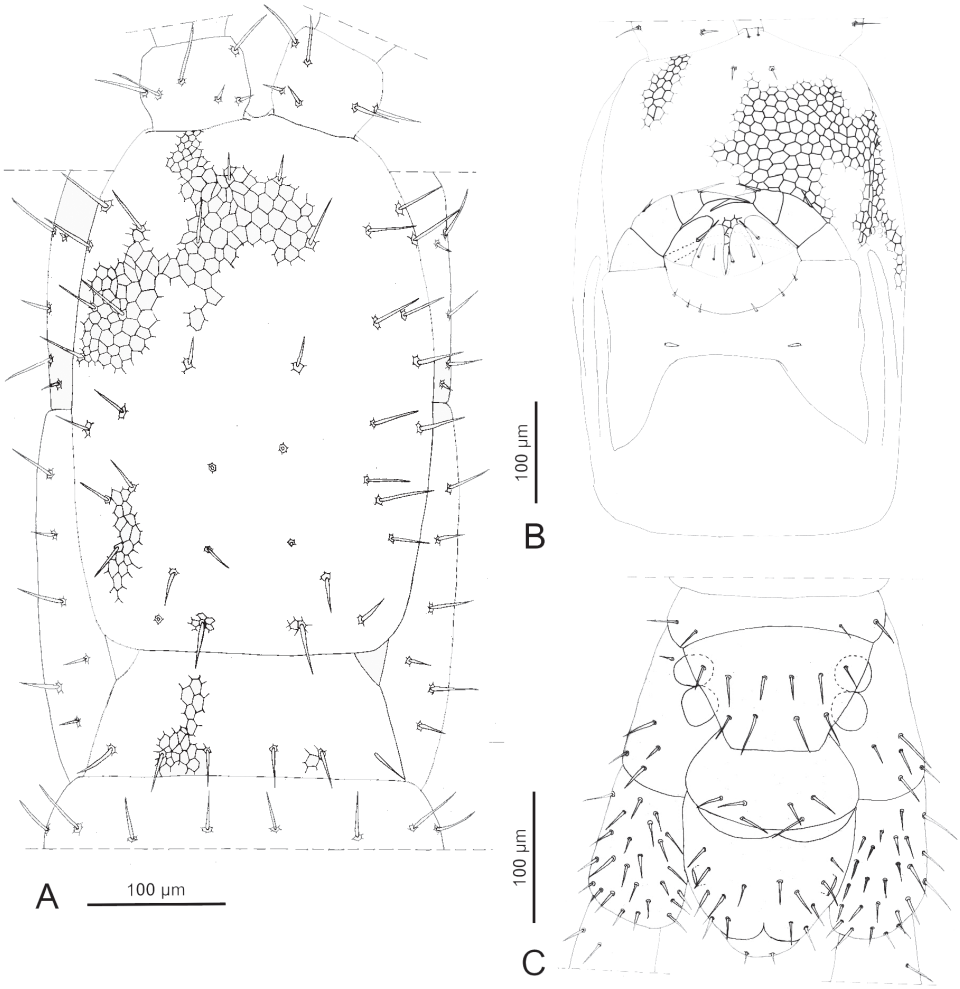

Figure 2. *Sundageophilus bidentatus* sp. n.: **A** head and forcipular segment, dorsal view, antennal articles II–XIV and tips of forcipules omitted, areolation only partially drawn **B** head, ventral view, areolation only partially drawn **C** ultimate leg-bearing segment and postpedal segments, ventral view, ultimate legs partially omitted. Line drawings based on LM photos of holotype LIPI macrosoilBO4a13_chilo144.

and shorter from the basal articles to the distal ones, both ventrally and dorsally, in particular up to 40 µm long on article I but less than 20 µm long on article XIV. Apical sensilla ca. 8 µm long, spear-like, without projections, only gently narrowing at nearly the mid-length. Club-like sensilla ca. 10 µm long, only on article XIV, grouped on the distal parts of both the internal and external sides. Longitudinal rows of 1–3 propioceptive spine-like sensilla at the bases of the antennal articles: two rows on articles I–III, approximately dorsal and ventral; three rows on articles IV–XIV, approximately dorsal, ventro-internal and ventro-external; rows reduced to 0–1 spine on antennal articles VI, X and XIV. A single sensillum, similar to the apical ones, ca. 5 µm long, on

Figure 3. *Sundageophilus bidentatus* sp. n.: left forcipule, dorsal (**A**) and ventral (**B**) views. LM photos of holotype LIPI macrosoilBO4a13_chilo144.

both dorso-external and ventro-internal position, close to the distal margin of articles V, IX and XIII.

First maxillae. Coxosternite entire, without mid-longitudinal sulcus, without setae. Coxal projection sub-triangular, longer than wide, bearing 2 setae. Telopodite apparently composed of a single article, with 1 seta. Lappets apparently lacking.

Second maxillae. Anterior margin of coxosternite concave, without anterior projections. Coxosternite with setae only close to the anterior margin; neither statuminia

nor other distinctly sclerotized parts associated to the metameric pores. Telopodite composed of three articles, only slightly narrowing towards the tip, with some distal setae; pretarsus in shape of an elongate claw, more than four times as long as wide at the basis, sub-conic and slightly bent, with a small dorsal bulge.

Forcipular segment. Tergite subtrapezoidal, ca. 2.1 times as wide as long, partially covered both by the cephalic plate and the tergite of the first leg-bearing segment, with lateral margins strongly converging anteriorly, posteriorly ca. 0.8 times as wide as the subsequent tergite. Pleurites with sclerotized scapular ridge. Exposed part of the coxosternite ca. as wide as long; anterior margin slightly projecting anteriorly with intermediate part shallowly concave, with a pair of stout, shallow denticles; coxopleural sutures complete, entirely ventral, straight and subparallel for most of their length; chitin-lines apparently absent. Basal distance between the forcipules ca. 0.1–0.2 of the maximum width of the coxosternite. Forcipular trochanteroprefemur ca. 1.6 times as long as wide, the internal side much shorter than the external side, with two mesal denticles, the distal denticle obviously longer than the basal one and slightly bent basalwards. Forcipular intermediate articles distinct, with a mesal shallow bulge each. Tarsungulum ca. 2.8–2.9 times as long as wide, both the external and the internal profiles uniformly curved, but for a mesal moderate basal bulge bearing two denticles, one dorsal to the other; the dorsal denticle longer than all other denticles and distinctly bent basally, not so the ventral denticle; a groove along the internal side of most part of the ungulum, between a dorsal scalloped ridge and a ventral entire ridge. Poison calyx not elongate, in the forcipular intermediate articles.

Leg-bearing segments. A total of 33 leg-bearing segments. Metatergite 1 slightly wider than the subsequent one, without pretergite. No paratergites. Metasternites longer than wide, without obvious "carpophagus" pit¸ without glandular pore-fields. Legs of the first pair only slightly smaller than the subsequent ones. Leg claws simple, uniformly bent; a pair of accessory spines, shorter than mid-length of the pretarsus, similar to each other in length.

Ultimate leg-bearing segment. Pretergite separated by sulci from pleurites. Metatergite sub-trapezoid, ca. 1.3 times as wide as long, lateral margins convex and distinctly converging posteriorly, posterior margin slightly convex. Metasternite sub-trapezoid, ca. 1.7–1.8 times as wide as long, anteriorly ca. 2.0 times as wide as posteriorly, lateral margins slightly convex and converging backwards; setae distributed uniformly. Coxopleuron ca. 1.7–1.8 times as long of the metasternite; setae distributed uniformly. Coxal organs of each coxopleuron opening through two independent pores, which are approximately aligned along the lateral margin of the metasternite, similar in size, ca. 25–30 μm wide. The telopodite ca. 6–7 times as long as wide, ca. 2.3 times as long and ca. 1.7 times as wide as the penultimate telopodite; six articles, conspicuously swollen, especially prefemur and femur with a disto-ventral bulge each; setae sparse, denser and longer on the ventral distal part of the articles, up to 50 μm long. Pretarsus lacking; a tuft a variously long spines surrounding the tip.

Postpedal segments. Genital pleurosternite entire. Gonopods appearing as a pair of basally wide, short laminae. Anal organs relatively large and anal pores exposed.

Complementary description of mouthparts of another specimen (PD5768)

Labrum. A row of ca. eight very stout tubercles along the posterior margin of the intermediate part. A row of bristles along the posterior margins of the lateral parts.

Mandibles. A single pectinate lamella, with ca. 15–20 teeth, on each mandible.

Intraspecific variability. Considering a total of nine complete specimens, all females with variously developed gonopodal lamina, the body length varied in the range of 5.7–8.1 mm (measured ± 0.1 mm), the maximum width of the cephalic plate varied in the range of 180–235 μm (measured ± 5 μm) and the number of leg-bearing segments was 33 in four specimens and 35 in five specimens. Some variation was found between specimens also in the elongation of antennae (length/width ratio of the longest intermediate article 1.0–1.2; length/width ratio of article XIV 1.8–2.2) and the forcipules (length/width ratio of trochanteroprefemur 1.5–1.7), the shape of the forcipular denticles (denticles on the trochanteroprefemur more or less pronounced and bent; Fig. 1B), the elongation of the metasternite of the ultimate leg-bearing segment (width/length ratio 1.7–2.0) and the shape of gonopodal lamina (either an entire bilobate lamina or apparently two paired laminae).

***Sundageophilus poriger* sp. n.**

http://zoobank.org/690B5097-63CA-44FB-9B8B-6EBFFA2EA9AA

Diagnosis. A *Sundageophilus* species with cephalic plate ca. 1.3 times as long as wide; first maxillary telopodite composed of two articles; no distinct denticles on the trochanteroprefemur; tarsungulum with a single basal denticle; approximately 31 pairs of legs; ventral pore-fields from the first to the penultimate leg-bearing segment; ultimate metasternite ca. 1.5–1.6 times as wide as long, anteriorly ca. 2.6 times as wide as posteriorly, lateral margins almost straight; four coxal pores on each coxopleuron, of which two along the lateral margin of the metasternite; legs of the ultimate pair not swollen in the female, unknown in the male, composed of six articles including distinct trochanter and prefemur, without disto-ventral bulges and without additional dense ventral setae; pretarsus of the ultimate leg pair similar to the other leg claws.

Material examined. *Holotype*. ♀ with gonopods developed, body length 5.8 mm, head width 190 μm; one leg of the ultimate pair damaged; originally entire, subsequently divided into three pieces, (i) head, (ii) forcipular segment, (iii) leg-bearing segments 1–31 and postpedal segments; collected Nov. 2013 by B. Klarner; in ethanol, LIPI macrosoilHJ2c13_chilo165.

Type locality. Indonesia, Sumatra, Harapan, jungle rubber agroforest, research site HJ2, 01°49'32"S, 103°17'39"E, ca. 80 m a.s.l., from upper soil layer (0–5 cm).

Other specimens examined. 1 ♀, from Bukit Duabelas, jungle rubber agroforest, research site BJ3, 02°03'47"S, 102°48'04"E, ca. 90 m a.s.l., from upper soil layer (0–5 cm), same date and collector as holotype, PD5771; 1 specimen, sex unknown because both anterior and posterior parts missing, from Bukit Duabelas, jungle rubber agroforest,

research site BJ5, 02°08'36"S, 102°51'05"E, ca. 50 m a.s.l., from upper soil layer (0–5 cm), same date and collector as holotype, PD5770.

Etymology. "*poriger*" means "bearing pores" and refers to the presence of glandular pore-fields on the ventral side of the trunk.

Description. *Description of holotype* (♀, LIPI macrosoilHJ2c13_chilo165). See also Figs 4, 5C.

General features. Body distinctly narrowing posteriorly. Legs relatively short, most of them ca. 0.2 mm long. Colour (in ethanol) almost uniformly pale yellow, but forcipular tarsungula darker.

Cephalic capsule. Cephalic plate subrectangular, ca. 1.3 times as long as wide, ca. as wide as the forcipular tergite, the lateral margins slightly convex; scutes approximately isometric and up to 10 µm in the anterior half of the cephalic plate, indistinct in the posterior half; frontal line absent; setae up to ca. 30 µm long. Clypeus ca. 1.3–1.4 times as wide as long, with lateral margins complete; uniformly areolate, the scutes being up to 10 µm wide, without a distinct clypeal area; two pairs of setae, closed to anterior margin, one anterior to the other. Intermediate part of labrum bearing ca. 8 short tubercles; lateral parts of labrum far apart from each other, separated from the clypeus by complete sulci.

Antennae. Slender, ca. 3.7 times as long as the head width. Intermediate articles up to ca. 1.2 times as long as wide. Article XIV ca. 1.9 times as long as wide, ca. 2.1 times as long as article XIII and slightly narrower than the latter. Setae gradually denser and shorter from the basal articles to the distal ones, both ventrally and dorsally, in particular up to 25 µm long on article I but less than 15 µm long on article XIV. Apical sensilla ca. 10 µm long, spear-like, without projections, only gently narrowing at around the mid-length. Club-like sensilla ca. 10 µm long, only on article XIV, grouped on the distal parts of both the internal and external sides. Longitudinal rows of 1–3 proprioceptive spine-like sensilla at the bases of the antennal articles: two rows on articles I–III, approximately dorsal and ventral; three rows on articles IV–XIV, approximately dorsal, ventro-internal and ventro-external; rows reduced to 0–1 spine on antennal articles VI, X and XIV. A single sensillum, similar to the apical ones, ca. 5 µm long, on both dorso-external and ventro-internal position, close to the distal margin of articles V, IX and XIII.

First maxillae. Coxosternite without setae. Coxal projection sub-triangular, longer than wide, with a seta. Telopodite composed of two articles, with a seta on the distal one. Lappets lacking.

Second maxillae. Anterior margin of coxosternite entire and concave, without anterior projections. Coxosternite with few setae; neither statuminia nor other distinctly sclerotized parts associated with the metameric pores. Telopodite composed of three articles, only slightly narrowing towards the tip, with some distal setae; pretarsus in shape of an elongate claw, ca. 5 times as long as wide at the basis, sub-conic and slightly bent.

Forcipular segment. Tergite subtrapezoidal, ca. 1.8 times as wide as long, contiguous to the cephalic plate and partially covered by the tergite of the first leg-bearing segment, with lateral margins strongly converging anteriorly, posteriorly ca. 0.9 times as wide as the

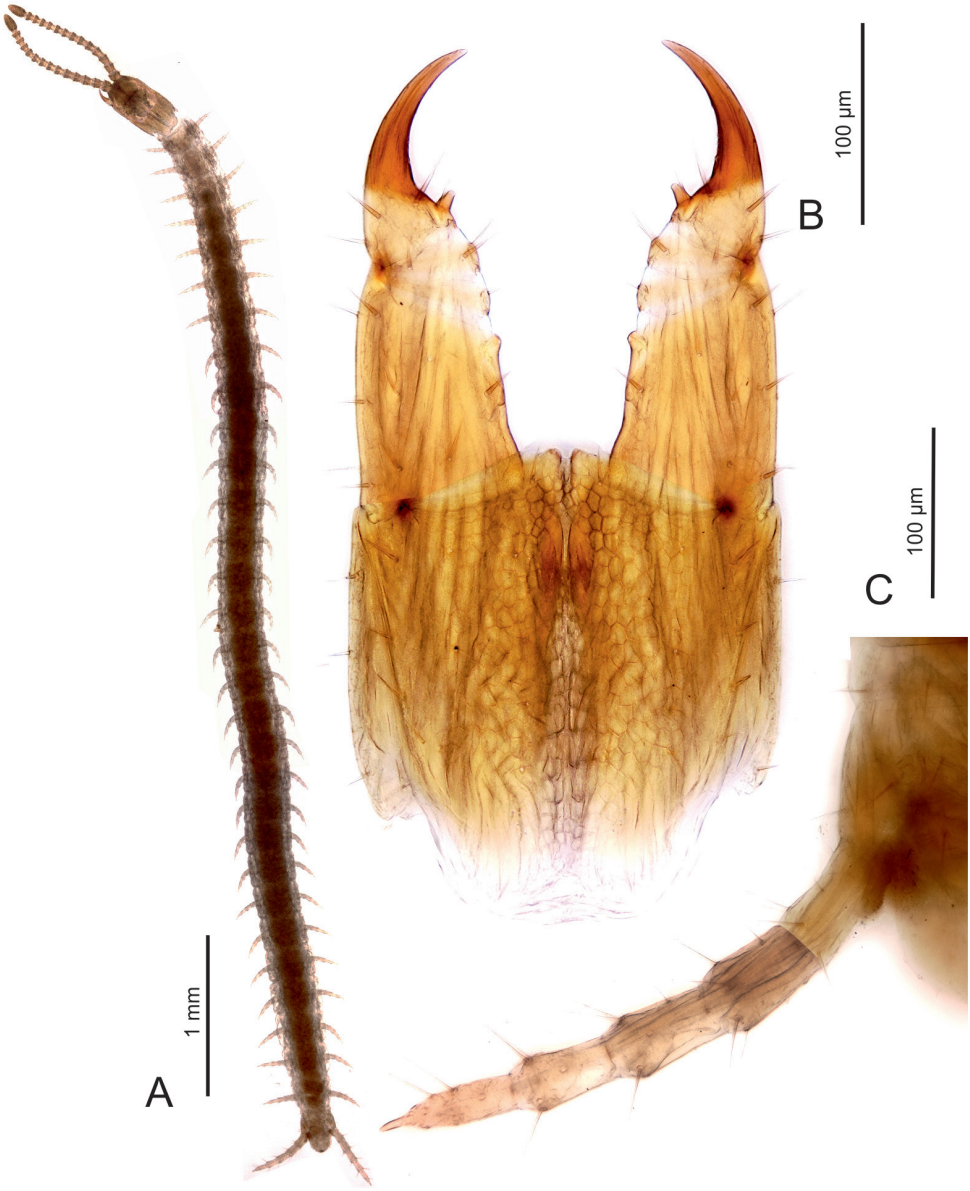

Figure 4. *Sundageophilus poriger* sp. n.: **A** entire body, dorsal view **B** forcipular segment, ventral view **C** ultimate left leg, dorsal view. LM photos of holotype LIPI macrosoilHJ2c13_chilo165.

subsequent tergite. Pleurites without distinctly sclerotized scapular ridges. Exposed part of the coxosternite ca. 1.1 times as wide as long; anterior margin slightly projecting anteriorly with intermediate part shallowly concave, with short sclerotized denticles; coxopleural sutures straight and subparallel for most of their length; chitin-lines indistinct. Basal distance between the forcipules ca. 0.2 of the maximum width of the coxosternite. Forcipular tro-

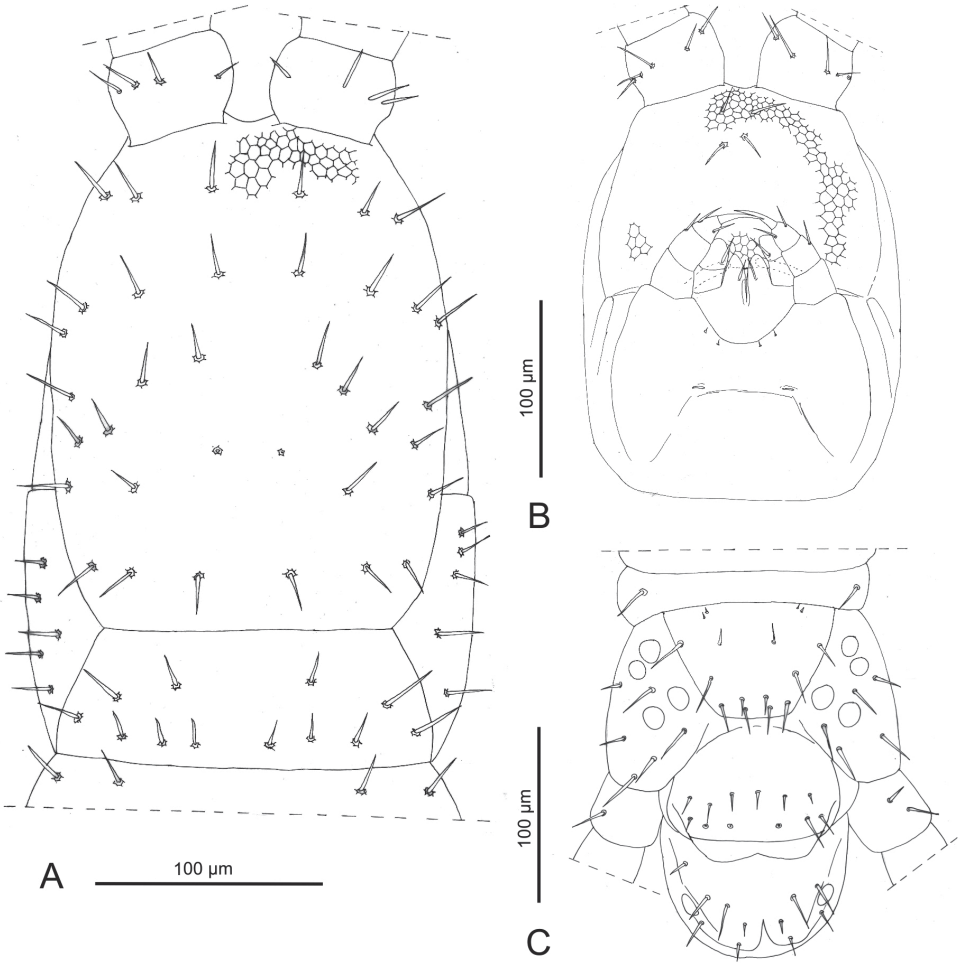

Figure 5. *Sundageophilus poriger* sp. n.: **A** head and forcipular segment, dorsal view, antennal articles II–XIV omitted, areolation only partially drawn **B** head, ventral view, areolation only partially drawn **C** ultimate leg-bearing segment and postpedal segments, ventral view, ultimate legs partially omitted. Line drawings based on LM photos of PD5771 (**A, B**) and holotype LIPI macrosoilHJ2c13_chilo165 (**C**).

chanteroprefemur ca. 1.5–1.6 times as long as wide, the internal side much shorter than the external side, without denticles, only a distal shallow bulge. Forcipular intermediate articles distinct, without denticles. Tarsungulum ca. 2.5–2.6 times as long as wide, both the external and the internal profiles uniformly curved, but for a mesal basal bulge bearing a sub-conic denticle. Poison calyx not elongate, in the forcipular intermediate articles.

Leg-bearing segments. 31 leg-bearing segments. Metatergite 1 slightly wider than the subsequent one, without pretergite. No paratergites. Metasternites longer than wide, without obvious "carpophagus" pit, with pore-fields from the first to the penultimate leg-bearing segment. A single, sub-circular, posterior pore-field on each metas-

ternite. Leg claws simple, uniformly bent; at least a posterior accessory spine, much shorter than mid-length of the pretarsus.

Ultimate leg-bearing segment. Pleuropretergite without sulci. Metatergite sub-trapezoid, ca. 1.1 times as wide as long, lateral margins convex and distinctly converging posteriorly, posterior margin slightly convex. Metasternite sub-trapezoid, ca. 1.5–1.6 times as wide as long, anteriorly ca. 2.6 times as wide as posteriorly, lateral margins almost straight and converging backwards; setae denser in the posterior part. Coxopleuron ca. 1.7–1.8 times as long as the metasternite; setae distributed uniformly. Coxal organs of each coxopleuron opening through four independent pores, of which two are approximately aligned along the lateral margin of the metasternite, the largest ca. 12 µm wide. The telopodite ca. seven times as long as wide, ca. 1.6 times as long and ca. 1.2 times as wide as the penultimate telopodite; six articles, not swollen; setae sparse, uniformly distributed, up to 50 µm long. Pretarsus claw-like, approximately as long as that of the penultimate legs, apparently without accessory spines.

Postpedal segments. Genital pleurosternite entire. Gonopods appearing as a short bilobate lamina. Anal organs relatively large and anal pores exposed.

Intraspecific variability. The body length, which is 5.8 mm in the holotype, is estimated to be shorter in the other two specimens, which being damaged cannot be measured accurately. The two specimens with complete trunks are both females with 31 leg-bearing segments.

Discussion

Taxonomical remarks

The two new species are confidently recognised as belonging to the family Geophilidae. The combination of a number of characters (pattern of areolation on the clypeus, structure of projections on the labrum, shape of the second maxillary pretarsus and structure of female gonopods) is diagnostic of the Geophilidae in the perception of both traditional taxonomy (e.g., Edgecombe et al. 2011) and recent cladistic analysis (Bonato et al. 2014).

The two species are here described in a new genus because they do not fit the diagnosis of any other known geophilid genus (Table 2) and their morphology does not suggest any obvious relation with other genera. Some characters (elongation of the head, of the second maxillary pretarsus and of the forcipular segment, and number of coxal pores) suggest that *Sundageophilus* may be close to other mainly tropical genera such as *Schizotaenia* Cook, 1896, *Alloschizotaenia* Brölemann, 1909 and *Schizonampa* Chamberlin, 1914, or even to the temperate genus *Plateurytion* Attems, 1909. However, the second maxillae of all species belonging to the latter genera are invariably described and/or illustrated with a medial constriction and distinct sclerotized ridges (so-called statuminia) or rims bordering the metameric pores, also in the smallest species similar in body size to *Sundageophilus*. The elongation of the head, the second maxillary pretarsus, and the forcipular segment is common in two other poorly known

genera, namely *Schizonium* Chamberlin, 1955 from South America and *Cephalodolichus* Verhoeff, 1938 from South Africa, but they differ from *Sundageophilus* at least by the denticulate forcipular coxosternite and the densely setose metasternite of the ultimate leg-bearing segment.

The two new species are similar to each other in the minute body size, the head and the forcipules distinctly elongate, the second maxillae provided with very slender claws, as well as in other characters. Nevertheless, uniting the two species in a single genus should be taken as a preliminary, parsimonious arrangement. Actually, we cannot rule out the possibility that most similarities between the two species comprise convergent adaptive characters or shared ancestral conditions. As a matter of fact, body miniaturization evolved independently in different lineages of geophilids (Bonato et al. 2015), as happened with the elongation of the head and the forcipules. On the other hand, second maxillae with unusually elongate claws are common in other genera of Geophilidae that are mainly distributed in tropical regions (Table 2), and they evolved independently at least in one species of *Geophilus*, *G. oweni* Bollman, 1887 (Crabill 1954).

Morphological remarks

The forcipules of *Sundageophilus bidentatus* are unusual in comparison with those of other geophilomorphs: two conspicuous denticles, one dorsal to the other, emerge at the basis of each tarsungulum (Figs 1B, 3).

The forcipules of the geophilomorphs show great diversity in number, size and pattern of denticles (Bonato et al. 2014). The tarsungulum, in particular, is either smooth or provided with a single denticle, which emerges in a sub-basal position on the inner side, sometimes slightly dorsal (Maruzzo and Bonato 2014). In addition to this single basal denticle, other projections are found very rarely; however, in distantly related lineages belonging to all three major clades of geophilomorphs (Bonato et al. 2014): in the mecistocephalid *Takashimaia* Miyosi, 1955, *Anarrup* Chamberlin, 1920 and some species of *Mecistocephalus* Newport, 1843; in the schendyloid *Dinogeophilus* Silvestri, 1909, *Falcaryus* Shinohara, 1970 and some species of *Ityphilus* Cook, 1899; in the geophiloid *Dignathodon* Meinert, 1870 and *Damothus* Chamberlin, 1960. In most of these cases, additional projections emerge distal to the sub-basal denticles, longitudinally aligned along the tarsungulum. Paired sub-basal denticles, one dorsal to the other, are found only in some mecistocephalids (*Anarrup* and some species of *Mecistocephalus*; Bonato and Minelli 2009); however, they are closer to each other and much less conspicuous than those found in *S. bidentatus*.

Biogeographical remarks

The discovery of two geophilid species inhabiting Sumatra is quite unexpected when confronting the known global distribution of the Geophilidae. Up to now, the south-

Table 2. Major differences between the species of *Sundageophilus* gen. n. and all known genera of Geophilidae from south-east Asia and Australasia. Notes: * = number counted on a single or few specimens only.

Genus/Species	General features	Clypeus	Labrum	Second maxillae					Forcipule	Leg-bearing segments		Ultimate pair of legs				
	head and forcipules: distinctly elongate	clypeal area: distinctly present	lateral parts: almost touching each other	coxosternite: statuminia: distinctly present	coxosternite: anterior margin: deeply angulated	coxosternite: anterior projections: distinctly present	pretarsus: much elongate	pretarsus: distinctly stout	tarsungulum: a second denticle flanking the basal denticle	number of leg pairs	anterior metasternites: pore-fields present	coxopleuron: ventral pores opening into pits	coxopleuron: all ventral pores close to metasternite	telopodite: number of articles	telopodite: distinctly swollen in females	pretarsus: shape
Sundageophilus bidentatus sp. n.	+	-	-	-	-	-	+	-	+	33–35	-	-	+	6	+	group of spines
Sundageophilus poriger sp. n.	+	-	-	-	-	-	+	-	-	31*	+	-	-	6	-	claw
Geomerinus Brölemann, 1912	+	+	-	-	+	-	-	-	-	71*	-	-	-	5	-	claw
Javaenia Chamberlin, 1944	-	?	-	?	+	-	?	?	-	41–45 *	+	+	?	?	-	claw
Maoriella Attems, 1903	+	+	+	-	-	-	+	-	-	41–91	+	+	+	6	-	claw
Pachymerellus Chamberlin, 1920	-	-	-	-	+	-	-	-	-	47–65	+	-	+	6	-	claw
Pachymerinus Silvestri, 1905	+	+	-	-	+	-	-	+	-	47–81	-	-	-	6	?	claw
Queenslandophilus Verhoeff, 1925	+	+	+	+	+	-	-	-	-	37–75	-	-	-	6	-	claw
Ribautia Brölemann, 1909	+	+	-	+	+	+	-	-	-	31–125	+	+/-	+/-	6	-	claw/spine
Sepedonophilus Attems, 1909	+	+	-	+	+	+	-	+	-	49–79	-	-	-	6	-	claw
Steneurytion Attems, 1909	+	+	+	-	+	-	+	-	-	37–53	-	-	-	6	-	claw
Tuoba Chamberlin, 1920	-	-	-	-	-	-	-	-	-	39–73	+	+	+	6	-	claw
Zelanoides Chamberlin, 1920	+	+	-	?	?	?	?	?	-	33–41	-	-	+	6	-	claw

eastern Asia singled out as a major gap in the almost worldwide occurrence of this family, which is the richest and most widespread among the geophilomorph centipedes (e.g., Bonato and Zapparoli 2011).

While many geophilid species in different genera are known living in temperate Asia, Australia and many Pacific islands, only a few claims have been published so far for the entire area comprising Indochina, Malesian islands, and New Guinea, and all these putative records have turned out to refer to misclassified representatives of different families. In particular, the species of *Geoporophilus* Silvestri, 1919 and *Himantosoma* Pocock, 1891 recorded from Sumatra (Table 1) had been originally described as geophilids but they are actually belonging to Gonibregmatidae (Edgecombe et al. 2011). Also, two species of uncertain identity described from Laos (*Luangana varians* Attems, 1953 and *Brachygeophilus robustus* Attems, 1953) had been originally classified between the geophilids, but the described morphological characters are actually inconsistent with Geophilidae, but consistent with Gonibregmatidae. Around Sumatra, the closest undisputable records of Geophilidae are from the Himalayas (*Geophilus intermissus* Silvestri, 1935) and from Bismark and Solomon Islands (*Tuoba sydneyensis* (Pocock, 1891)) (Silvestri 1935, 1936, Jones 1998). However, records of Linotaeniidae, which are morphologically distinct but most probably derived within the Geophilidae (Bonato et al. 2014), are known from northern Laos and Vietnam (species of *Strigamia* Gray, 1843; Attems 1953, Bonato et al. 2012, Maruzzo and Bonato 2014) and apparently also from Java (*Javaenia bataviana* Chamberlin, 1944; Chamberlin 1944; Würmli 1972).

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RESEARCH ARTICLE

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Geographic distribution of *Gryllotalpa stepposa* in south-eastern Europe, with first records for Romania, Hungary and Serbia (Insecta, Orthoptera, Gryllotalpidae)

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Abstract

Described from the steppe zones north of the Black Sea, Caucasus, and central Asia, *Gryllotalpa stepposa* Zhantiev was recently recorded from a few localities in Greece, R. Macedonia, and Bulgaria. In May 2015, several specimens were collected from Ivrinezu Mare in Romania, which suggested a continuous distribution area of the species, stretching from the central Balkans to central Asia. Thus, to reveal its actual range of occurrence, a survey of several Orthoptera collections became mandatory and, as expected, a large number of misidentified specimens of *Gryllotalpa stepposa* were discovered, providing new data on the species distribution in south-eastern Europe, including also the first records of this mole cricket in Serbia and Hungary. Here a full locality list is presented of this species west of Ukraine and Moldova and the current geographic distribution of the genus *Gryllotalpa* in the Balkans is revised. A key for distinguishing the mole crickets in south-eastern Europe and a distribution map for this region are presented.

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Keywords
Distribution, *Gryllotalpa*, Orthoptera, south-eastern Europe

Introduction

Although the Orthoptera fauna of south-eastern Europe, including the Balkan Peninsula, is comparatively well explored, several faunistic and taxonomic issues remain and most of these address one of the most fragmentary known groups: the crickets. In the past decades, during the extensive work of exploring the Orthoptera fauna in this area, Tettigonioids and Acridoids received the highest attention, while the Grylloids were neglected, most likely due to their elusive, nocturnal way of life.

The mole crickets form a particular group within the Grylloidea. Family Gryllotalpidae includes eight genera with more than 100 species, excluding the fossil/extinct ones (Eades et al. 2016). These insects are adapted to living underground, having reduced ovipositor, fore legs highly modified for digging and hind legs fully losing their jumping ability during the ontogenesis (e.g., Gorochov 1995).

Gryllotalpa Latreille is a subcosmopolitan genus, missing only from the northernmost areas of Asia, whole south America and the boreal areas of north America. The *Gryllotalpa gryllotalpa* species group occurs throughout Europe, from Britain to Iran and central Asia (Gorochov 1993; Broza et al. 1998; Ingrisch et al. 2006). Fifteen species are known within the *Gryllotalpa gryllotalpa* group, out of which 12 are found in Europe: *G. gryllotalpa* (Linnaeus, 1758) – present all over Europe, excepting the southernmost areas; *G. septemdecimchromosomica* Ortiz, 1958 – present in the Iberian Peninsula, southern France, Tuscany and Umbria in Italy; *G. vinae* Bennet-Clark, 1970 – in southern France; *G. sedecim* Baccetti & Capra, 1978 and *G. octodecim* Baccetti & Capra, 1978 – in north-western Italy, south-eastern France and Sardinia; *G. quindecim* Baccetti & Capra, 1978 – in south Italy and Sicily, *G. viginti* Baccetti & Capra, 1978 – in north-western Italy (Liguria); *G. cossyrensis* Baccetti & Capra, 1978 – in Pantelleria island; *G. vigintiunum* Baccetti, 1991 – in Sardinia; *G. krimbasi* Baccetti, 1992 – in Greece; *G. stepposa* Zhantiev, 1991 – in the Balkan Peninsula, Moldova, south Ukraine, the southern part of the steppic zone of European Russia, the Caucasus, central Asia, Saudi Arabia; and *G. unispina* Saussure, 1874 – along the coasts of the Black and Caspian Sea and in central Asia (Bacetti and Capra 1978, Zhantiev 1991, Broza et al. 1998, Ingrisch 2006).

The mole crickets excavate two different types of tunnels: vertical burrows, used for hiding from predators, overwintering and molting, and horizontal tunnels for feeding, mating and escaping predators (Jafari et al. 2015). Males stridulate in the evening and at night from a special acoustic chamber, usually cylindrical, with one to several openings extended upward; five types of song are known: calling, precopulation/courtship, territorial, aggressive, and remonstrative (Zhantiev et al. 2003). In some species, the females also sing with a secondarily developed stridulatory apparatus on the upper side of some tegminal veins (Ragge and Reynolds 1998). In the genus *Gryllotalpa*,

both oscillographic analysis and song frequency can be used when separating species, e.g. *Gryllotalpa gryllotalpa* and *G. vineae* (Ragge and Reynolds 1998); *G. gryllotalpa*, *G. unispina* and *G. stepposa* (Zhantiev et al. 2003); *G. tali* Broza, Blondheim & Nevo and *G. marismortui* Broza, Blondheim & Nevo (Broza et al. 1998). Other characters used in species discrimination are: male genitalia, wing venation, number of teeth in the stridulatory file, morphometrics, number of chromosomes and even cuticular hydrocarbons (Broza et al. 1998; Ingrisch et al. 2006).

In the present paper light is shed on the distribution of *Gryllotalpa* species in south-eastern Europe, based on extensive material from this area. Recent data revealed that four species of *Gryllotalpa* occur in south-eastern Europe and the Balkan Peninsula: *G. gryllotalpa*, *G. stepposa*, *G. unispina* and *G. krimbasi*. In order to distinguish these, several morphological traits are used and included in a key.

Materials and methods

Gryllotalpa specimens were found while actively searching in moist ground, preferably near a water source. The easiest and most efficient way was the collection of specimens attracted to black Ultra Violet fluorescent tubes and Mercury vapor light lamps. The material preserved in the following collections was revised:

MGAB	"Grigore Antipa" National Museum of Natural History, Bucharest, Romania
UBB	Museum of Zoology, Patrimony Department, Babeș-Bolyai University, Cluj-Napoca, Romania
NMNHS	National Museum of Natural History, Bulgarian Academy of Sciences, Sofia, Bulgaria
HMB	Collection of the Zoological Department of the History Museum, Blagoevgrad, Bulgaria
HNHM	Hungarian Natural History Museum, Budapest, Hungary
ZZDBE	Zoological Collection of the Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Serbia
MNHM	Macedonian Museum of Natural History, Skopje Macedonia
CC	Dragan Chobanov's personal collection

Data from public collections from Macedonia are already published (Chobanov and Mihajlova 2010). A database with all the studied material is available as a Suppl. material 1. Specimens were identified according to the general and genital morphology, venation of tegmina and structure of the stridulatory file. Distribution of chromosomal forms (number of chromosomes in the karyotype of certain populations) from earlier published sources was also taken into account (compare References).

Photos used in the key were taken with a Canon EOS 6D DSLR camera and a Canon MP-E 65 mm lens. For genitalia microphotographs, the camera was mounted to a Leica 205C Stereomicroscope.

Key for the identification of south-east European species of *Gryllotalpa*:

1 Space between the proximal and next dorsal inner spine on hind tibiae wide, larger than the space between the distal spines (Fig. 1A). Tegmina with weak light-colored veins (Fig. 1D). Apical part of epiphallus shovel-like widened; its tip usually convex and flattened (Fig. 1I).....***G. unispina***

– Space between all dorsal inner spines on hind tibiae approximately equal (Fig. 1B, C). Tegmina with strong dark-colored veins (Fig. 1E, F). Apical part of epiphallus widened or not but the tip concave, dorso-ventrally thick and humped, forming a longitudinal ventral slot (Fig. 1J, K) **2**

2 Epiphallus short and wide (less than 2× longer than its widest part), apically more flattened, with a shallow ventral slot (Fig. 1K). Distal part of the median vein (♂) opposite to the radial branch 1 (transverse radio-cubital vein) weak and poorly visible (Fig. 1H) ..... ***G. gryllotalpa***

– Epiphallus long and slender (its length 2–2.3× larger than its widest part and over 3× the width of apex), apically thicker, with a deep slot (Fig. 1J). Distal part of the median vein (♂) opposite to the radial branch 1 (transverse radio-cubital vein) well visible, dark (Fig. 1G) **3**

3 Male karyotype 2n=14, 15 or 16 (hybrids?). Poorly distinguished morphologically from the following species (according to our own measurements, differences in epiphallus proposed by Ingrisch et al. 2006 are unreliable)..... .....***G. stepposa***

– Male karyotype 2n=19..... ***G. krimbasi***

Discussion

Until recently only *Gryllotalpa gryllotalpa*, *G. unispina*, and *G. krimbasi* were known to occur in the Balkans. The latter was recently described from Greece (Baccetti 1992) following the results by Krimbas (1956; 1960) and relying on a male karyotype with 19 chromosomes, but without giving any structural characteristics and even using a nymph as a holotype. This chromosome number corresponds to the male karyotype of *Gryllotalpa unispina*. Later on, Ingrisch et al. (2006) redescribed *Gryllotalpa krimbasi* using specimens from the distribution range of the 19–chromosome form (central Greece). According to new material observed, *Gryllotalpa krimbasi* is hardly distinguishable morphologically from *G. stepposa* using both general morphology and the male phallic complex. The differences in the ratio length: width of epiphallus (proposed by Ingrisch et al. 2006, using the drawings of *Gryllotalpa stepposa* by Zhantiev 1991) are considered unreliable after measurements implemented for this study, due to a clear overlap. On the other hand, *Gryllotalpa krimbasi* (as well as *G. stepposa*) differs well from *G. unispina* in the shape of male genitalia, body shape, number of spines on the hind tibia etc. Both species differ also in their habitat preferences. While *Gryllotalpa unispina* is a halophilous species distributed along the northern and eastern Black

Figure 1. Inner part of hind tibia: **A** *Gryllotalpa unispina* **B** *G. stepposa* **C** *G. gryllotalpa*. Dorsal view of male tegminae: **D** *Gryllotalpa unispina* **E** *G. stepposa* **F** *G. gryllotalpa*. Distal part of the median vein (♂): **G** *Gryllotalpa stepposa* **H** *G. gryllotalpa*. Epiphallus: **I** *Gryllotalpa unispina* **J** *G. stepposa* **K** *G. gryllotalpa*. Locations: *Gryllotalpa unispina* – Letea; *G. stepposa* – Şura Mare; *G. gryllotalpa* – Paşcani (Romania). Scale bars 1 mm.

Sea coast, surroundings of the Caspian Sea, central and south-western Asia (Zhantiev 1991; Gorochov 1993), *G. krimbasi* prefers inland humid habitats and seemingly avoids saltings.

In 1939, Steopoe, following the works of Voinov (1912), shows that a "14 chromosomes form" with variations of 15 and 16 chromosomes is present in Romania and named it "Romanian form". He points out the differences of the metaphasic chromosomes between the so called Romanian form, the typical form of *Gryllotalpa gryllotalpa* (2n=12) and the "Naples form" with 15 chromosomes. According to Zhantiev (1991), *G. stepposa* also has a karyotype with 14 chromosomes (and occasionally 15 or 16) and such forms were found in southern Turkey (Kushnir 1956), on the Greek mainland and some Aegean Islands (e.g., Krimbas 1956; data in Willemse 1984). Recently, morphological examination revealed that the typical *Gryllotalpa stepposa* occurs in the Republic of Macedonia (Chobanov and Mihajlova 2010) and Bulgaria (Chobanov

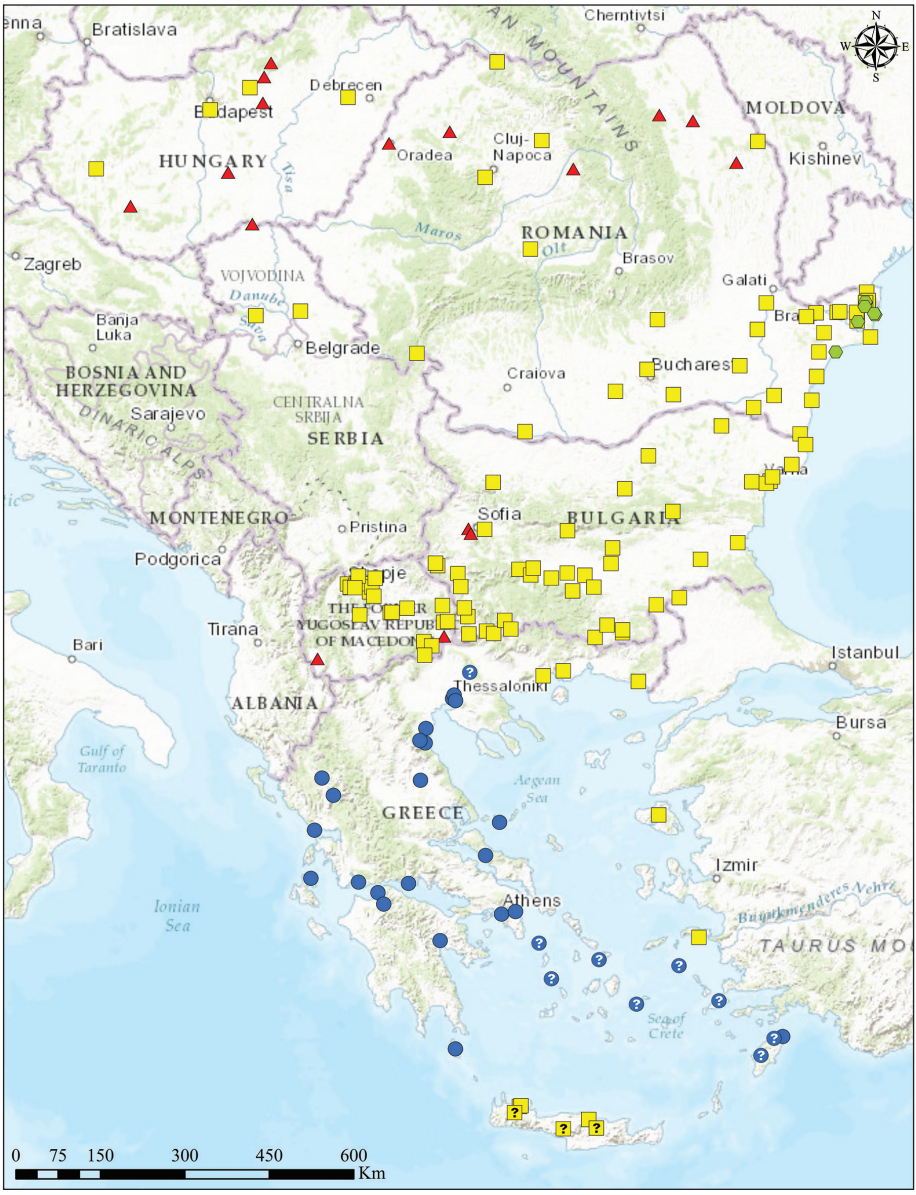

Figure 2. Geographic distribution of *Gryllotalpa* species in south-eastern Europe: yellow squares – *G. stepposa*; red triangles – *G. gryllotalpa*; green hexagons – *G. unispina*; blue dots – *G. krimbasi* (although some localities are not confirmed by karyology studies, we considered them for *G. krimbasi*, based on indirect data from its distribution and nearby records).

2009; 2011). With the current study we prove that the range of this species is significantly wider, covering Romania (thus making the connection with the range of the species in Moldova and Ukraine), all the territory of Bulgaria and eastern Macedonia

(as high as 1000–1200 m asl), north-eastern Greece (on the territory of the district of east Macedonia and Thrace), the lowland of northern (possibly also central and south) Serbia, and some areas of Hungary (Figure 1). Its occurrence in eastern Croatia and partly in Bosnia and Herzegovina is expected, and even its discovery in Slovakia and eastern Austria would not be surprising.

With the present data, *Gryllotalpa stepposa* almost entirely replaces *G. gryllotalpa* on the Balkan Peninsula. In the south and west, *Gryllotalpa stepposa* borders *G. krimbasi* in Greece: the ranges of both species border approximately in the lower courses of Vardar (Axios) or Strouma (Strimon) rivers. Thus, both taxa are possibly direct competitors and exclude each other. The western border of the range of *Gryllotalpa stepposa* is unclear for the moment. In the north (Croatia, Serbia, Hungary, and north Romania), *Gryllotalpa stepposa* meets *G. gryllotalpa* (compare map in Baccetti and Capra 1978), thus the taxonomic identity of all published *G. gryllotalpa* data from this region is uncertain. The patchy and scarce distribution of *Gryllotalpa gryllotalpa* in Bulgaria (only a single locality known close to the border with Serbia) and Republic of Macedonia (two isolated localities in the south) suggests recent expansion of *G. stepposa* in the west and north and replacement of *G. gryllotalpa*, whose current occurrences may represent remnants from a former wider range. In the valley of Drin River and the connected plain of Ohrid Lake (extreme south-western Macedonia), only *Gryllotalpa gryllotalpa* was found; thus, this area may represent the southernmost border of its population, linking its range in northern Italy and central Europe through the northern Adriatic coast.

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Supplementary material 1

Occurence records of the studied *Gryllotalpa* species

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Data type: occurence

Explanation note: The occurence records of *Gryllotalpa* material preserved in the collections: MGAB, UBB, NMNHS, HMB, HNHM, ZZDBE, MNHM, CC.

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RESEARCH ARTICLE

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A new species of *Octaspidiotus* (Hemiptera, Diaspididae) from China

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Abstract

Adult females of a new species of armored scale insect, *Octaspidiotus shanghaiensis* **sp. n.** are described and illustrated from specimens collected in China. A key is provided for the all described species of *Octaspidiotus*.

Keywords

Taxonomy, Sternorrhyncha, armored scale insect, China

Introduction

Scale insects (Hemiptera: Coccoidea) are sap-sucking parasites which are small (generally less than 5 mm) and cryptic in their habitats (Gullan 1997), with at least 30 families and approximately 8000 species (García et al. 2016). Containing more than 2500 described species, Diaspididae is the largest species-rich family in the Coccoidea (García et al. 2016). Adult diaspidid females are sessile and permanently reside on their host plants (Gullan 1997). Adult females have the complete loss of the legs, the reduction of the antennae to a single segment and the modification of the abdomen into a specialized pygidium for forming the test, and these characteristics are the primary recognition features for these insects (Andersen 2010; Balachowsky 1948). Armored scale insects are important agricultural pests and have colonized a diverse set of plant species. They are distributed on every continent except Antarctica (Andersen 2010).

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Although the family classification is controversial, the Aspidiotinae and the Diaspidinae are the two major subfamilies. The genus *Octaspidiotus* was established as a member of the former subfamily by MacGillivray (1921), with *Aspidiotus subrubescens* Maskell as its type species. However, two species that he transferred from *Aspidiotus* are not now included in this genus. Since then, many additional species were described and added to *Octaspidiotus* by other authors (Borchsenius 1966; Tang 1984; Tang and Chu 1983; Takagi 1984).

Takagi (1984) showed that *O. corticoides* (Green) was not a member of *Octaspidiotus* because the distinguishing characteristics were invalid. Currently, this genus is comprised of 14 valid species, eight of which are known to occur in China (García et al. 2015; Tang 1984; Takagi 1984). There are only two species recorded from Oceania, the other 12 species being distributed throughout East Asia.

Recently, one new species of *Octaspidiotus* was discovered from China. It was described and illustrated in this paper, bringing the number of recognized species in the genus to 15, of which nine species are recorded from China. A key to all known species of *Octaspidiotus* is provided.

Materials and methods

In this paper, the terminology described by Henderson (2011) has been used. This publication also includes illustrations for most of the species treated herein. All measurements are presented in micrometers (μm). Measurements were made using NIT-Elements D tools.

The abbreviations L_1, L_2 and L_3 are short for the median, second, and third pygidial lobes, respectively.

All specimens have been deposited in the Entomological Museum, Northwest A & F University, Yangling, Shaanxi, China (NWAFU).

Taxonomy

Genus *Octaspidiotus* MacGillivray, 1921

Metaspidiotus Takagi, 1957: 35. Junior synonym.

Type species. *Aspidiotus subrubescens* Maskel, 1892.

Generic diagnosis. Adult female. Body is oval to rounded; derm membranous except pygidium. **Cephalothorax.** Antennae with 1 seta. No trilocular pores associated to the spiracles. **Pygidium.** With 3-4 pairs of lobes, never bilobed. Median lobes (L_1) well-developed, with notches on both margins or only present on the outer margin. Second lobes (L_2) smaller than L_1, with notches on both laterals or only present on the outer lateral. Third lobes (L_3) similar to L_2. Fourth lobes (L_4) small and pointed

apically, only present in *O. subrubescens*. Marginal setae occurring on dorsal bases of L_2 and L_3, lanceolate, broadened and flattened. Plates are well-developed, fimbriate on the outer margin in most species, occurring laterally and even extended to the abdominal segment IV. Paraphyses absent on pygidial margin. **Ducts.** Dorsum has one-barred type macroducts, that are aligned in some species. Ventral microducts are scattered. **Anal opening** is toward the apex of the pygidium, more or less elongate. Vulvar opening situated anterior to anal opening. **Perivulvar pores** are quinquelocular, present or absent, if present, in four groups.

Remarks. This genus is very close to *Aspidiotus* Bouché, 1833 and *Oceanaspidiotus* Takagi, 1984 in terms of pygidial lobes and pygidium, but can be distinguished by the form of the dorsal marginal setae occurring on L1 and L2 which are lanceolate, broadened and flattened, while these setae in the other two genera are simply thickened.

***Octaspidiotus shanghaiensis* sp. n.**
http://zoobank.org/07E3AD76-AF7A-4130-92CC-0C7895FF0A0F
Figures 1–7

Material examined. Holotype: 1 adult female: CHINA: Changfeng Park, Shanghai City, 11. IV. 2015, Hongliang Li (NWAFU).

Paratypes. 3 adult females: same data as the holotype (NWAFU).

Diagnosis. Description, n = 4. Adult females. **Field characters:** adult female scale nearly oval, flat, dark greyish in colour; exuviae nearly central.

Slide-mounted: Adult female not pupillarial, 810–952 um long (holotype 905 μm long); 756–883 μm wide (holotype is 881 μm in the widest part of the body). Body outline oval, derm membranous except for pygidium (Figure 1). **Cephalothorax.** Antennae each with 1 seta (Figure 2), distance between antennae is 164.3 μm. Prespiracular pores absent (Figure 3). **Pygidium** (Figure 5). The pygidium has three pairs of lobes: L_1 are well-developed, a small mesal notch is present on or near the apex, and a relative larger notch is present on or near the apex of the outer margin. L_1 is 6.7–7.2μm wide and the distance of two lobes of L_1 is 1.5–2.1μm wide. Median lobes separated by a space 0.2–0.3 times the width of L_1. L_2 smaller than L_1, with one notch on the outer margin. L_3 similar to L_2, but smaller. Lanceolate setae on L_2 and L_3 shorter than these lobes themselves. **Plates** (Figure 5 and 7) one pair of pointed plates between L_1, not extending to the apex of the lobe; 2 pairs of plates between L_1 and L_2, apically fringed with few fine bifurcated; with 3 pairs of plates similar in size and shape between L_2 and L_3; with 6–7 pairs of plates lateral to L3. **Ducts (**Figure 4 and 5**)**. Dorsal macroducts 1-barred-shaped. No marginal macroduct between median lobes. One marginal macroduct between L_1 and L_2, two between L_2 and L_3, and 3–4 present between L3. Dorsal submarginal macroducts about the same size as marginal macroducts which are 30–35 μm long. Total dorsal macroducts on dorsum in submarginal and marginal areas of pygidium on each side of body 32–44 (44 in holotype). Dorsal macroducts on abdomen segment IV shorter than on pygidium, with 5–6 macroducts on margin

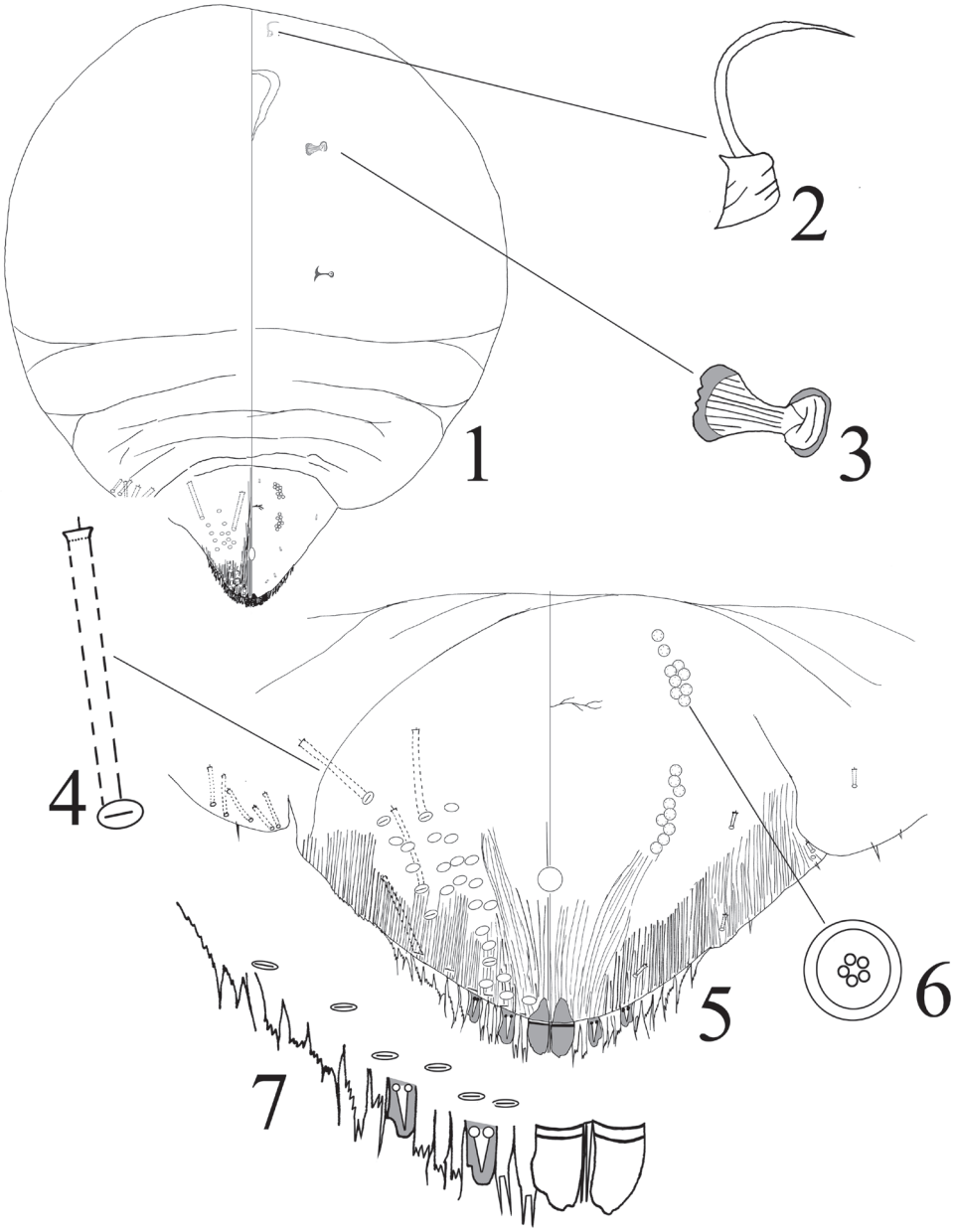

Figure 1–7. *Octaspidiotus shanghaiensis* sp. n. adult female: **1** habitus **2** detail of antenna **3** detail of anterior spiracle **4** dorsal 1-barred duct **5** pygidium **6** quinquelocular pores **7** detail of the end of pygidium margin.

of abdomen segment IV. Ventral microducts are fewer and more scattered than the dorsal macroducts. **Anal opening** (Figure 5) 22.4–25.5 μm long in diameter, located 46.2–48.7 μm between the base of the anal opening and the base of L_1. **Perivulvar**

pores (Figure 5 and 6) present in an arc, divided in four groups, 9–12 anterolaterally and 8–9 posterolaterally.

Remarks. This species is similar to *O. cymbidii* Tang, 1984 in the body shape and the pygidial lobes, but can be distinguished by the following characters (those for *O. cymbidii* in parentheses): 1) without marginal macroduct on abdomen segment III (with 3–4); 2) the three plates between L_2 and L_3 all equally shaped (the third plate is narrower than the first and the second plates); 3) L_1 is separated by a space 0.2–0.3 times the width of each median lobe (by a space 0.5 times the width of each L_1); 4) without marginal macroducts between L_1 (present).

Host. *Echinochloa crusgalli* (L.)

Etymology. The specific epithet is named after Shanghai, the type locality.

Distribution. China (Shanghai).

Key to the adult females *Octaspidiotus* MacGillivray

*denotes Chinese species

1 With 3 pairs lobes on pygidium, L_4 absent **2**

– With 4 pairs lobes on pygidium, L_4 present as small, pointed, sclerotized processes ..... ***O. subrubescens* (Takahashi)**

2 Lanceolate marginal setae occurring on dorsal bases of L_2 and L_3 not extending to the apex of L_2 and L_3, respectively **3**

– Lanceolate marginal setae occurring on dorsal bases of L_2 and L_3 more-or-less extending to the apex of L_2 and L_3, respectively **13**

3 All lobes hippocrepiform, without notches on margin of L_1 ..... ***O. bituberculatus* Tang***

– Lobes normal, with notches on margin of L_1 ..... **4**

4 With notches on outer margin of L_1 ..... ***O. australiensis* Kuwana**

– Notches present on both margins of L_1 ..... **5**

5 Three plates occurring between L_2 and L_3 are not equal in width **6**

– Three plates occurring between L_2 and L_3 are equal in width **10**

6 Plates between L_1 bifurcate or pointed apically; distance between L_1 narrower than 1/2 of each lobe of L_1; with 6 plates occurring lateral to L_3 ..... ***O. cymbidii* Tang***

– Plates between L_1 fringed; distance between L_1 no less than 1/2 of each lobe of L1; with no less than 7 plates occurring on the outer lateral to L_3 ..... **7**

7 With notches on both margins of L_3; both second and third plates between L_2 and L_3 narrower than first plates between L_2 and L_3 ..... ***O. rhododendronii* (Tang)***

– With notches on outer margin of L_3, without notches on mesal margin of L_3; Second or third plates between L_2 and L_3 narrower than first plates between L_2 and L_3 ..... **8**

8 Second plates between L_2 and L_3 narrower than first and third plates between L_2 and L_3 ..... **9**

– Third plates between L_2 and L_3 narrower than first and second plates between L_2 and L_3 ..... ***O. yunnanensis*** **(Tang & Chu)***

9 With 22–24 perivulvar pores and 35–42 dorsal macroducts on pygidium .... ***O. tamarindi*** **(Green)**

– With 43–60 perivulvar pores and 54–65 dorsal macroducts on pygidium .... ***O. tripurensis*** **Takagi**

10 With notches on mesal margin of L_2; distance between L_2 and L_3 equal to 1/5 of each lobe of L1; plates between L_1 bifurcate or pointed apically .... ***O. shanghaiensis*** **sp. n.***

– With notches on both margins of L_2; distance between L_2 and L_3 more than 1/3 of each lobe of L_1; plates between L_1 fringed **11**

11 Body strongly sclerotized at maturity **12**

– Body remaining membranous ..... ***O. nothopanacis*** **(Ferris)***

12 Number of perivulvar pores less than 30; with 7 plates occurring on the outer side of L_3 ..... ***O. stauntoniae*** **(Takahashi)***

– Number of perivulvar pores more than 30; with 8 plates occurring on the outer side of L_3 ..... ***O. calophylli*** **(Green)**

13 With notches on outer margin of L_2 and L_3; with no more than 7 plates occurring on the outer side of L_3 ..... ***O. pinicola*** **(Tang)***

– With notches on both margin of L_2 and L_3; with no less than 8 plates occurring on the outer side of L_3 ..... **14**

14 With more than 80 dorsal macroducts and 32–47 perivulvar pores .... ***O. multipori*** **(Takahashi)**

– With less than 80 dorsal macroducts and 23–29 perivulvar pores .... ***O. machili*** **(Takahashi)***

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RESEARCH ARTICLE

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First description of the male of *Hiranetis atra* Stål and new country records, with taxonomic notes on other species of *Hiranetis* Spinola (Hemiptera, Heteroptera, Reduviidae, Harpactorinae)

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Abstract

The male of *Hiranetis atra* Stål, 1872 is described and illustrated for the first time. In addition, this paper illustrates the female and provides new country records for this species. Photographs of all extant types of species of *Hiranetis* Spinola, 1840 are presented with taxonomic notes on the other two species of the genus.

Keywords

Costa Rica, Ecuador, *Graptocleptes*, Harpactorini, *Hiranetis braconiformis*, *Hiranetis membranacea*, wasp-mimicking bug

Introduction

Harpactorinae is the largest subfamily of Reduviidae and is represented by the tribes Apiomerini and Harpactorini in the Neotropical region (Gil-Santana et al. 2015). Harpactorini is the most diversified Reduviidae group with more than 53 recognized genera in the Neotropical region (McPherson and Ahmad 2011, Forero 2011, 2012,

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Swanson 2012, Gil-Santana 2015, Gil-Santana et al. 2015). The only outdated key to American Harpactorini genera is that of Stål (1872). However, Maldonado and Lozada (1992) presented a key to Neotropical wasp-mimetic Harpactorinae genera, which in their view helps to quickly sort out specimens from unidentified material, although this is a somewhat artificial way of grouping genera. Maldonado and Lozada (1992) considered six Neotropical Harpactorini genera to be wasp-mimetic: *Acanthischium* Amyot & Serville, 1843, *Graptocleptes* Stål, 1866, *Hiranetis* Spinola, 1840, *Myocoris* Burmeister, 1835, *Neotropiconyttus* Kirkaldy, 1909 and *Xystonyttus* Kirkaldy, 1909. They regarded *Neotropiconyttus* as resembling braconids, while all others somewhat resembled ichneumonid wasps. Although *Coilopus* Elkins, 1969 was described as a wasp-mimicking genus (Elkins 1969), Maldonado and Lozada (1992) considered it akin to bees and did not include this genus in their key. Gil-Santana (2015) has updated this key, including all these seven genera, and also *Parahiranetis* Gil-Santana, 2015. Forero and Giraldo-Echeverry (2015) further proposed that a Vespidae (*Mischocyttarus* sp.) was the hymenopteran mimetic model of *Coilopus vellus* Elkins, 1969.

Recently, Gil-Santana et al. (2013) showed that *Hiranetis coleopteroides* (Walker, 1873) was in fact a species of *Graptocleptes* and a junior synonym of *G. bicolor* (Burmeister, 1838). Therefore, three species are currently included in *Hiranetis*: *H. atra* Stål, 1872, *H. braconiformis* (Burmeister, 1835) and *H. membranacea* Spinola, 1840 (Maldonado 1990, Gil-Santana et al. 2013).

Champion (1898) considered *Hiranetis* spp. to resemble various Ichneumonidae and Braconidae (Hymenoptera), while Haviland (1931) recorded a Müllerian mimicry association among species of *Graptocleptes*, and an association between *Xystonyttus* and ichneumonid wasps. Hogue (1993) cited a similar association among species of *Graptocleptes* and *Hiranetis*.

In a review of *Alabagrus* Enderlein, 1920 (Hymenoptera: Braconidae), Leathers and Sharkey (2003) argued that many species of this genus belong to a Neotropical, presumably mimetic complex, with thousands of other species, including 1,300 species of Braconidae in other genera, more than 1,000 species of Ichneumonidae, several hundred species of Reduviidae (e.g. *Hiranetis*) and unknown numbers of species in other orders. Some of the Reduviidae, the 'braconiformes clade', have wings, shape and physical proportions that are very similar to some braconids (Leathers and Sharkey 2003). These authors presented a photo of a specimen in lateral view, identified as *Hiranetis* nr. *braconiformis* (Burmeister, 1835), to illustrate their assertion.

Hespenheide (2010) recorded examples of mimicry of braconids by *Agrilus* Curtis, 1825 (Coleoptera: Buprestidae). In Panama, species of *Agrilus* share a braconid-like color pattern with the orders Coleoptera, Diptera and Hymenoptera, and with six species of Reduviidae, including *Hiranetis* nr. *braconiformis* and five other undetermined species.

Most authors have only mentioned or taken into consideration the pattern of yellowish or straw-colored hemelytra with a median transverse black band, in relation to the alleged mimicry between Harpactorini and certain Ichneumonidae and Braconidae, as models (Champion 1898, Haviland 1931, Maldonado and Lozada 1992, Hogue 1993, Leathers and Sharkey 2003, Hespenheide 2010). On the other hand,

Gil-Santana (2015) has emphasized that other wasp-mimicking Harpactorini, like *Parahiranetis salgadoi* Gil-Santana, 2015, show a pattern of darkened to reddish general colouration with yellowish 'pterostigmata' on the hemelytra, which is similar to the coloration also exhibited by several other species of Ichneumonidae and Braconidae. This pattern was also observed for instance in *Graptocleptes bicolor* and *G. haematogaster* (Stål, 1860). Another common feature among all these Harpactorini species with a darkened general coloration on the hemelytra, including in *H. atra* and *Graptocleptes sanguiniventris* (Stål, 1862), is a yellowish band on the femora (Gil-Santana 2015).

Sexual dimorphism has been recorded in several species of Harpactorini. In addition to the bigger size and larger abdomen of females, which is common in many other insects, males in several genera have larger eyes and/or the thickening of the third antennal segment in its basal portion. The latter has been considered to be among the diagnostic features at genus level (Stål 1872, Champion 1898, Gil-Santana et al. 2013, Martin-Park et al. 2012).

Champion (1898) recorded that the males of *Hiranetis braconiformis* present thickening of the third antennal segment at its base and, apparently based only on this species, stated that this was a feature belonging to *Hiranetis*.

In the present paper, the male of *Hiranetis atra* is described and illustrated for the first time. In addition, this paper illustrates the female and provides new country records for this species. Photographs of all extant types of species of *Hiranetis* and taxonomic notes on the other two species of the genus are presented.

Material and methods

Photographs of the type specimens of *Hiranetis atra*, which are deposited at the Swedish Royal Natural History Museum (**NRM**), Stockholm, Sweden, were made by Dr Gunvi Lindberg (NRM). The other extant types and additional specimens were directly examined. The respective depositories and curators, who kindly allowed me to examine them, are the following: "Museum für Naturkunde der Humboldt-Universität zu Berlin" (**ZMHB**), Berlin, Germany, Dr Jürgen Deckert, and "Muséum National d'Histoire Naturelle" (**MNHN**), Paris, France, Dr Éric Guilbert.

Dissections of the male genitalia were made removing the pygophore from the abdomen with a pair of forceps and then clearing it in KOH solution for 24 hours. The dissected structures were studied and photographed in glycerol. Drawings were made using a *camera lucida*. Images of external and genital structures by the author were taken with digital cameras (Nikon D5200® with a Nikon® Macro Lens 105 mm, Sony DSC-W830® and Sony DSC-HX400V®). The vestiture (setation) was omitted in the ink drawings showing some genital structures (Figs 7–8) in order to make more clear the shape and/or structure of these areas. General morphological terminology mainly follows Schuh and Slater (1995). Terminology applied to male genital characteristics follows mainly those used by Gil-Santana et al. (2013). Measurements are in millimeters (mm).

Taxonomy

Hiranetis Spinola, 1840

Hiranetis Spinola, 1840: 112–113 [description]; Stål 1859: 367 [key], 371 [citation, species included]; Stål 1866: 294 [key]; Stål 1872: 69 [diagnosis, key], 82–83 [catalog]; Walker 1873a: 64 [key] ; Walker 1873b: 129 [catalog]; Lethierry and Severin 1896: 178 [catalog]; Champion 1898: 280 [comments]; Wygodzinsky 1949: 40 [catalog]; Elkins 1969: 459 [citation]; Putshkov and Putshkov 1985: 46 [catalog]; Maldonado 1990: 218 [catalog]; Maldonado and Lozada 1992: 165 [key]; Froeschner 1999: 206 [catalog]; Forero 2011: 15 [checklist]; Gil-Santana et al. 2013: 348, 358 [citations], 359 [separation from *Graptocleptes*]; Gil-Santana 2015: 29, 30 [citations], 35, 36 [separation from *Graptocleptes* and *Parahiranetis*], 37 [key].

Type species. *Hiranetis membranacea* Spinola, 1840: 113–114, by monotypy.

Diagnosis. General appearance: wasp-mimetic. Head gibbous, large, as long as wide across eyes, densely covered with long setae on ventral and postocular portions; postantennal tubercles very short to almost imperceptible, acute or rounded; legs elongated, slender; fore femur slightly longer than head and pronotum together, thicker basally. Hemelytra long, surpassing the abdomen by about half of the length of the membrane.

Hiranetis atra Stål, 1872

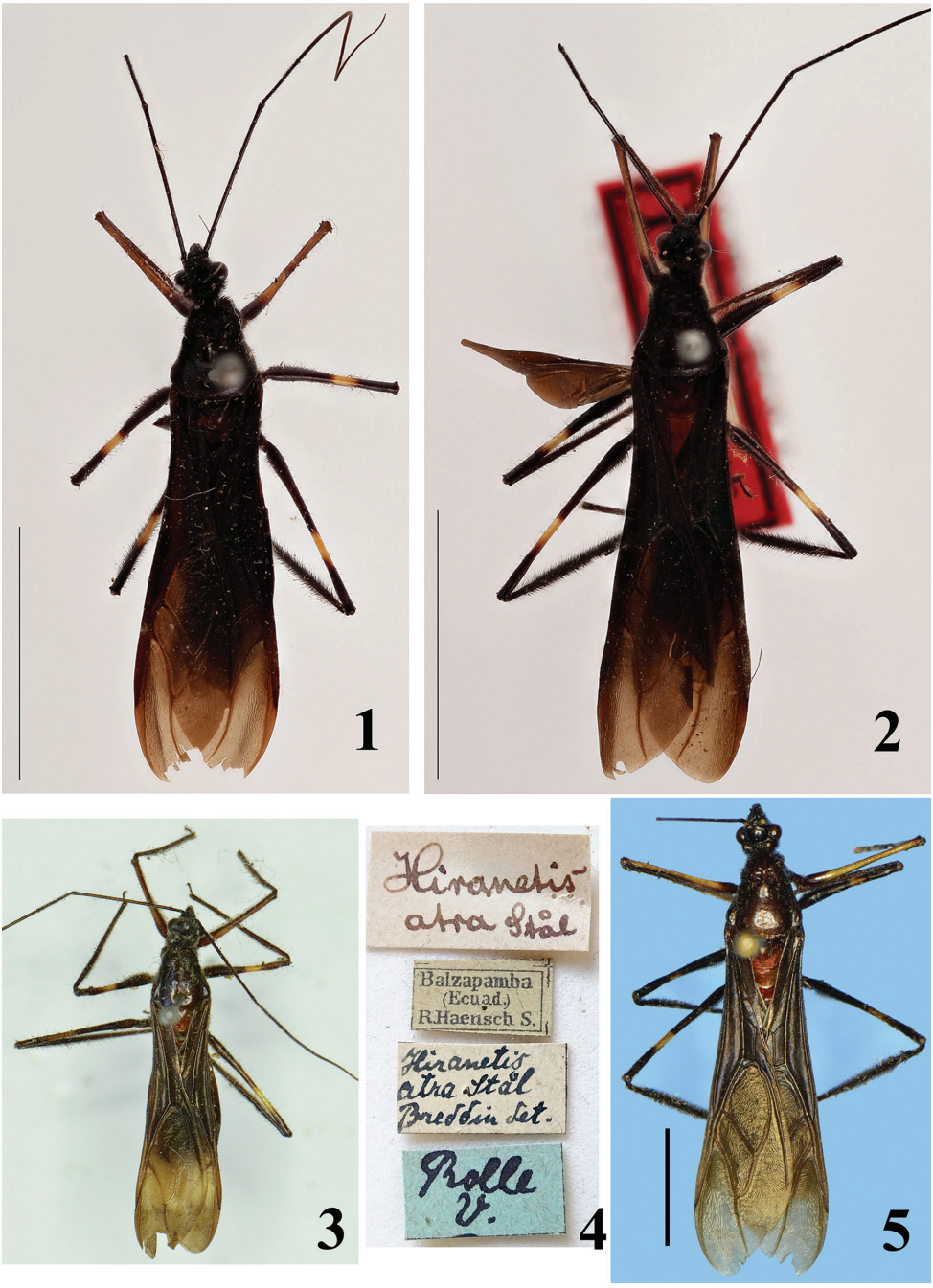
Figures 1–17

Hiranetis atra Stål, 1872: 82–83 [description]; Lethierry and Severin 1896: 178 [catalog]; Wygodzinsky 1949: 40 [catalog]; Maldonado 1990: 218 [catalog]; Gil-Santana et al. 2013: 348 [citation]; Gil-Santana 2015: 36 [citation].

Notes. *Hiranetis atra* was first described based on one or more female specimens from Bogotá, Colombia (Stål 1872), without any further descriptions of the species. It is noteworthy that although the type locality of *H. atra* might really be "Bogota", it is possible that the real locality of collecting of the specimens had been different. In the 19th century, "Bogotá" was just the shipping denomination for the commercial trade, including specimens going to Europe (Forero 2006).

Although no figures of *H. atra* have so far been published, the Swedish Royal Natural History Museum (NRM) has made photos of its type available, and these can be freely accessed at: http://www2.nrm.se/en/het_nrm/a/hiranetis_atra.html.

Based on these photos, Gil-Santana (2015) stated that *H. atra* would have very small yellowish markings like dots in hemelytra, at a site where some other wasp-mimicking Harpactorini have larger yellowish 'pterostigmata'.



Figures 1–5. *Hiranetis atra* Stål, females. **1–2** syntypes, dorsal view, photos: Gunvi Lindberg. Copyright Swedish Museum of Natural History, Stockholm (NRM). Scale bars: 10 mm. **1** "type", **2** "paratype" **3–4** specimen from Ecuador deposited in ZMHB **3** dorsal view **4** labels **5** specimen from Costa Rica, deposited in MNHN, dorsal view. Scale bar: 5.0 mm.

However, Dr Gunvi Lindberg (NRM) subsequently provided new figures (Figs 1–2) and the information that both "type" and the "paratype" of *H. atra* have hemelytra completely dark. It seems that the apparent small dot on the hemelytra is likely to be some form of fouling, like mycelium.

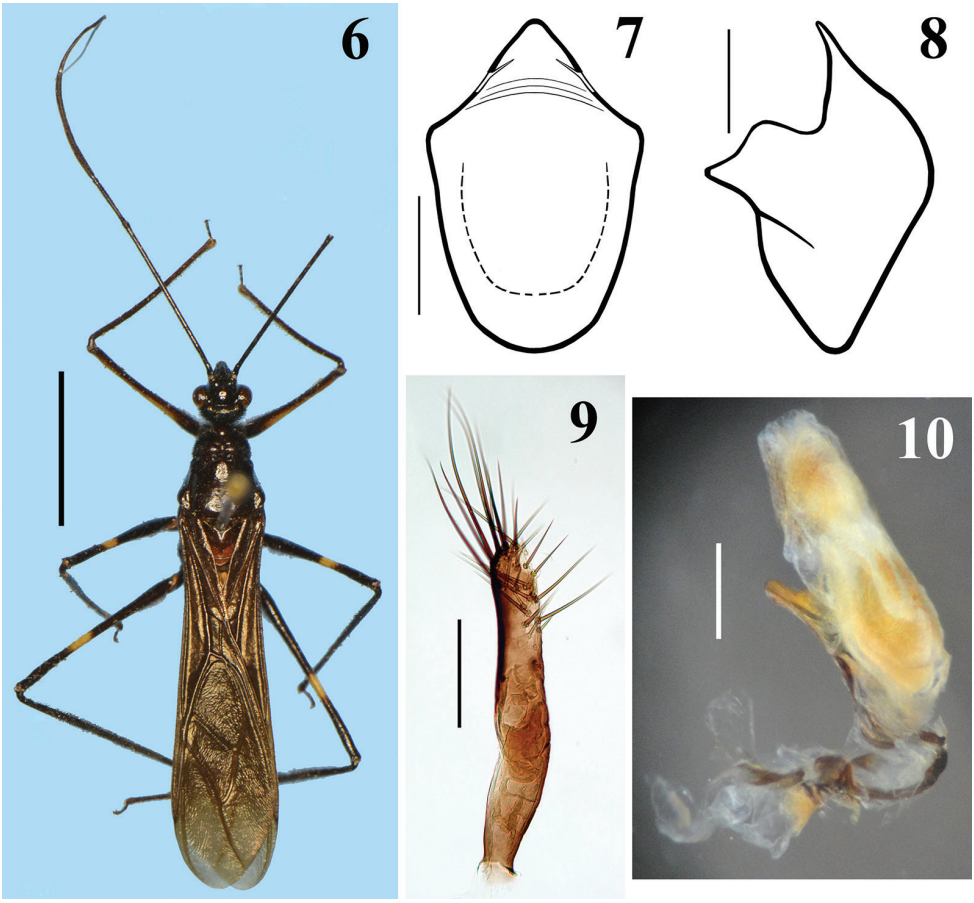
On the other hand, because the original description (Stål 1872) did not mention the number of types or designate a holotype, as was generally done at that time, it is better to consider all the type specimens to be syntypes.

In addition to the male and female from Costa Rica that are described below, an additional female from Ecuador (Figs 3–4) was examined at ZMHB, where it is deposited.

Material examined. One male and one female, each with a green label with the same information: "Museum Paris, Costa Rica, Paul Serre, 1920" (MNHN). One female, labels: *Hiranetis atra* Stål / Balzapamba, (Ecuad.), R.Haensch S. / *Hiranetis atra* Stål, Breddin det. / k[?]olle v. [green label] (ZMHB).

Diagnosis. *Hiranetis atra* can be readily separated from other species of the genus by its general coloration, which is mostly blackish, especially the hemelytra, which are completely dark (Figs 1–3, 5–6), while the other species have the pattern of yellowish or straw-colored hemelytra, with a median transverse band and dark apex.

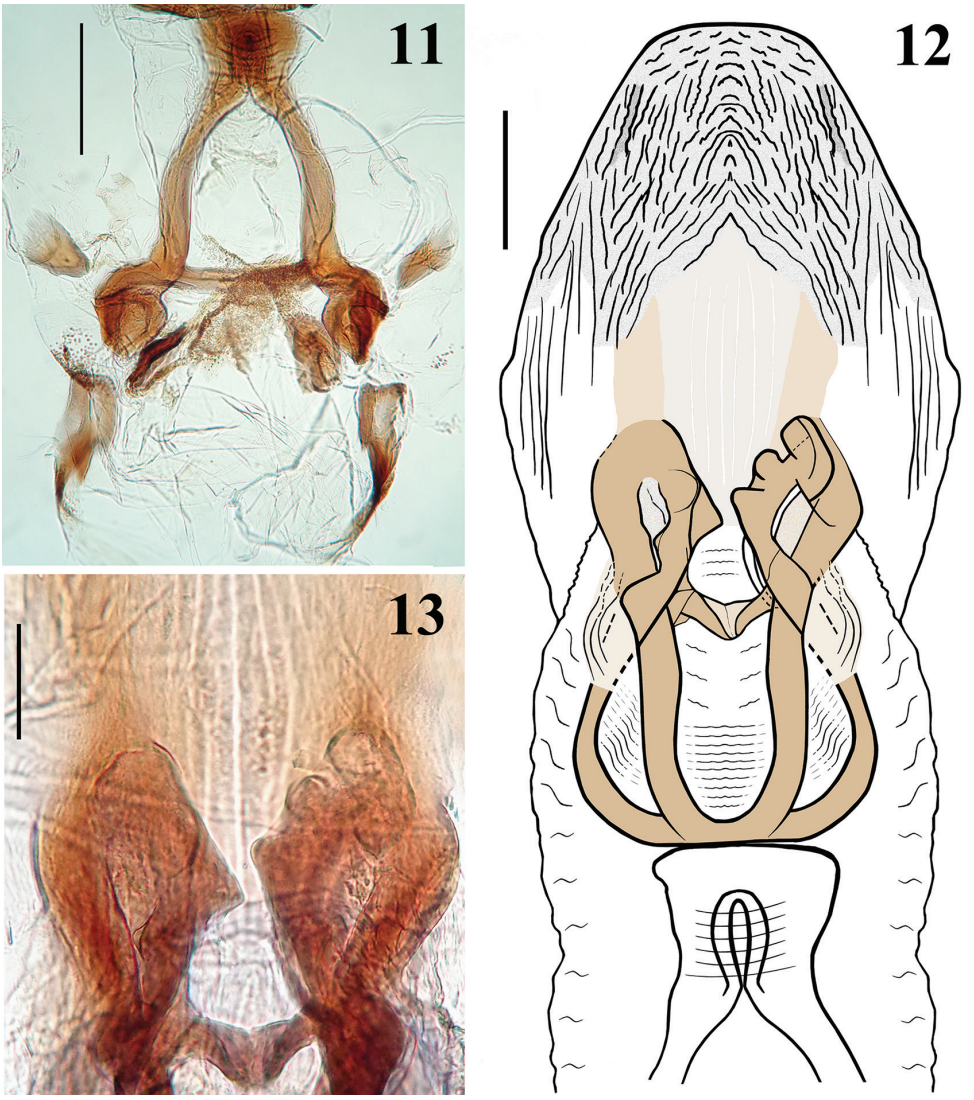
Description. MALE. Figures 6–17. Measurements (mm): Total length: to tip of abdomen: 12.1; to tip of hemelytra: 16.2; head: total length (lateral view): 1.9; maximum width across eyes: 1.9; interocular space: 1.0; antennal segments: I: 5.5; II: 1.7; III (very bent; approximately): 6.9; IV: 2.0; labium segments: II [first visible]: 1.4; III: 1.1; IV: 0.3. Thorax: pronotum: fore lobe length: 0.7; hind lobe: length: 2.0; width at posterior margin: 2.8. Legs: fore legs: femur: 5.4; tibia: 5.5; tarsus: 0.7; mid legs: femur: 4.8; tibia: 6.2; tarsus: 0.7; hind legs: femur: 6.5; tibia: 9.1; tarsus: 0.8. Abdomen: length: 6.3; maximum width: 2.3. COLORATION: general coloration black (Fig. 6). Head, including antennae and labium, blackish, eyes brownish-black. Thorax blackish, with exception of metanotum, which is reddish-brown. Hemelytra blackish. Legs mostly blackish; fore femur with dorsal surface, except at base and extreme apex, pale yellowish, and with a lighter-colored subbasal portion ventrally; mid and hind femora with yellowish annulus situated somewhat distally to their midportion (Fig. 6). Abdominal segments II and III (first two visible) reddish; sternite IV almost completely reddish, except on posterolateral portion, including connexivum at this area, where it is blackish; sternite V mostly reddish but blackish on posterior and lateral portion, including connexivum. Tergites IV and V, and remaining segments, including pygophore and parameres, blackish. STRUCTURE and VESTITURE: Integument mostly shiny, smooth. Head gibbous, large, as long as wide across eyes; integument shiny, with sparse long and short, straight or somewhat curved blackish setae; the latter much denser, forming pubescence of long blackish thick setae on postocular portion and gula; almost completely glabrous between eyes. Labium curved, with scattered and somewhat curved blackish setae. Antennal segments I and II straight, the former approximately three times longer than head, with shiny and smooth integument and sparse short darkened setae; segments II-IV with opaque and somewhat rugose integument; segment II, except at basis, covered with very numer-



Figures 6–10. *Hiranetis atra* Stål, male from Costa Rica, deposited in MNHN. **6** dorsal view **7–8** pygophore without parameres. **7** ventral view **8** lateral view **9** paramere. **10** phallus, lateral view. Scale bar: (**6**): 5.0 mm; (**7–8, 10**): 0.5 mm; (**9**): 0.2 mm.

ous darkened short setae, with some longer intermixed setae and some very thinner elements at distal portions (interpreted as trichobothria); segment III thickened in basal half, curved; III and IV covered with dense, very short and somewhat lighter-colored pubescence, with short darkened setae scattered on segment III and few of these on segment IV; the latter is thinner than the other segments and moderately curved. Postantennal tubercles small and somewhat acuminate. Eyes globose, glabrous, projecting laterally, prominent in dorsal view, reaching dorsal margin of head at interocular sulcus in approximately its midportion; not reaching ventral margin of head, which is far from inferior margin of the eye. Interocular sulcus thin and moderately deep. Ocelli elevated, much closer to eyes than to each other. Collum thin. Thorax with shiny integument; prothorax covered with very numerous blackish thick setae on forelobe, anterior portions of propleura and hind lobe; the latter with sparse

long setae at dorsal portion or, almost glabrous, except on midline, where thinner, somewhat shorter and light yellowish to whitish setae form a faint midlongitudinal line on hind lobe. Transverse sulcus not very deep, interrupted before middle by a pair of submedian shallow carina. Midlongitudinal sulcus on forelobe of pronotum becoming abruptly deeper at transverse sulcus to form a depression; posteriorly to the latter, a blunt short rounded prominence; disc of hind lobe smooth; lateral longitudinal sulci well marked at posterior half to posterior two-thirds of hind lobe of pronotum. Humeral angle elevated, rounded at lateral margin; median portion of posterior margin of pronotum with some long thin darkened setae. Scutellum elevated at disc, pointed posteriorly, with scattered thin dark long setae. Posterior portion of propleura, mesopleura, metapleura and thoracic sterna with long darkened setae, which are shorter and thinner at center of mesosternum and metasternum. Legs: coxae with numerous long dark setae on distal half, ventrally, and some longer thinner light-colored elements, while the basal third and lateral portions are almost completely glabrous; trochanters densely covered with long setae ventrally and with some scattered even longer thinner setae, which are lighter-colored on forelegs and dark on mid and hind legs. Fore femur subequally longer than head and pronotum together; all femora thicker basally and slightly subapically too, covered with scattered few long and strong dark setae and with a dense group of long and thick setae and some thinner and even longer setae on ventral portion of the basal enlarged portion of femora; these setae are lighter on fore femora and darker on the others. Fore tibiae somewhat curved, with uniform thickness, except at apex, which is somewhat enlarged, and where there is a dorsal spur and a mesal comb. Mid and hind tibiae straight and somewhat thickened at basal half. All tibiae with scattered long thick blackish setae; fore and mid tibiae covered with shorter dark setae on ventral surface, which become progressively more numerous towards apex, where they also covers lateral and dorsal surfaces; hind tibiae, except at base, densely covered with short dark setae, which are somewhat longer in the slightly enlarged basal half. Tarsi with moderately long dark setae. Hemelytra long, surpassing abdomen by about half length of membrane; corium with curved scattered adpressed short dark setae, which are much more numerous over costal and subcostal veins, becoming less numerous on distal half of corium, including over those veins; membrane glabrous. Abdomen: elongate; spiracles rounded; sternites with shiny integument and sparse long thin setae, which are light on reddish portions and dark on the blackish segments, and thicker, longer and more numerous on parts adjacent to genitalia and on the latter too. There is also a fusiform grouping of whitish minute short setae on midlateral portions of sternite V. MALE GENITALIA (Figs 7–17): pygophore: blackish, subpentagonal in ventral view, with a subtriangular rounded apex (medial process) (Fig. 7); lateral to the latter, a somewhat deep and rounded emargination (Fig. 8); between anterior and genital opening, a very well sclerotized bridge that has a conspicuous median dorsal rounded prominence; long, thick and dark setae ventrally (on exposed surface), somewhat more numerous on lateroapical portions. Parameres symmetrical, rod-like in shape; somewhat curved in basal half and straight towards apices, which are rounded, blackish, glabrous in basal



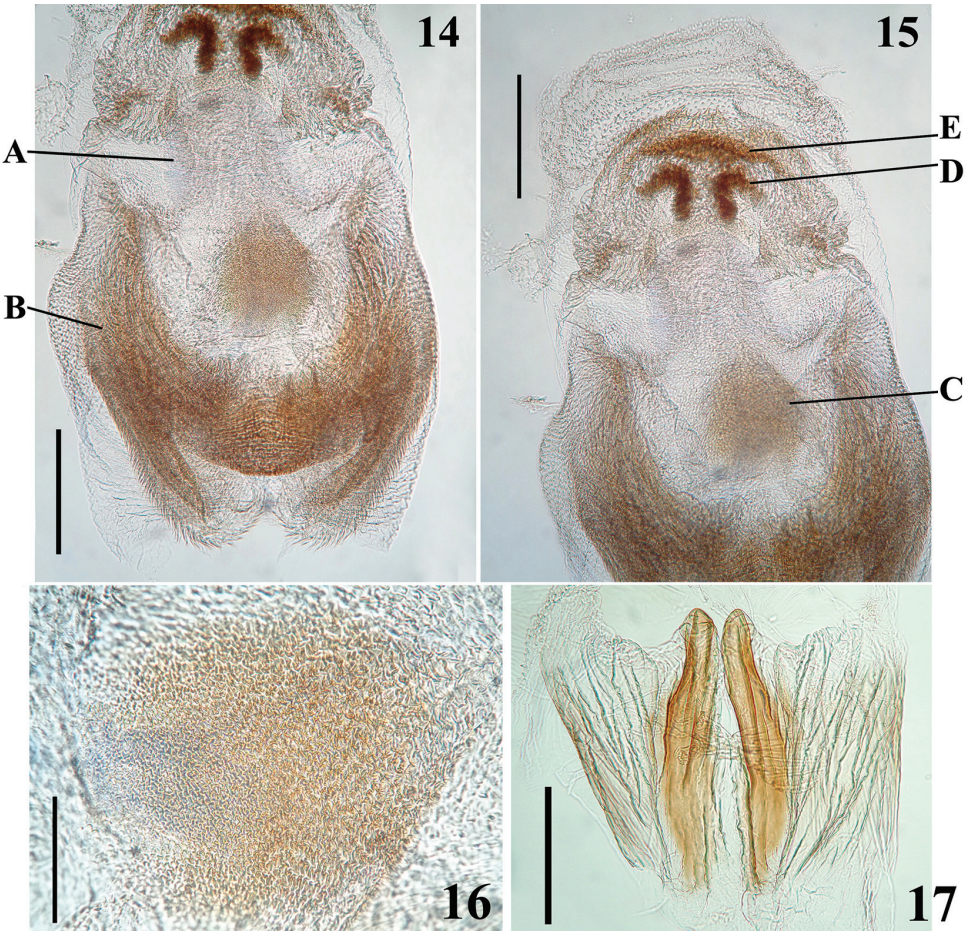
Figures 11–13. *Hiranetis atra* Stål, male genitalia, dorsal view. **11** articulatory apparatus **12** basal plate extension (pedicel), phallothecal dorsal plate and struts **13** struts apices. Scale bar: (**11**): 0.3 mm; (**12**): 0.2 mm; (**13**): 0.1 mm.

two-thirds and with long setae in apical third; those at apicomedial margins even longer (Fig. 9). Phallus somewhat elongate when not inflated (Fig. 10); articulatory apparatus with basal plate arms and bridge narrow, forming a subrectangular set, except in apical portion, where the arms are curved (Fig. 11); basal plate extension (pedicel) moderately short, slightly expanded towards apex and somewhat more sclerotized than the arms and basal bridge (Fig. 11). Dorsal phallothecal plate weakly sclerotized, flat, suboval in shape, with numerous longitudinal thin grooves at apical

half; apical margin almost transverse, straight (Fig. 12). Struts with curved lateral arms and parallel somewhat curved median arms which are expanded at apex into a pair of asymmetrical sub oval/subsquared structures; there is a medial bridge joining the bases of the latter (Figs 12–13). Endosoma wall mostly minutely spiny, with a small smooth semi-oblong dorsal prolongation at midportion (Figs 14–15). After endosoma extension, seven processes were observed: 1 - a larger U to M-shaped basal process formed by diffuse thickening (Fig. 14); 2 - a median subspherical process, situated between the upper arms of the basal process, formed by minute tooth-like thickenings (Figs 14–16); 3 - a pair of elongate apical-median flat, longitudinally striated and somewhat curved and moderately sclerotized processes, wrapped in a smooth portion (not minutely spiny, but with fine longitudinal grooves) of endosoma wall, all of which lying dorsally to the other subapical processes described next (Fig. 17); 4 - a pair of small sclerotized thickened curved processes, located below the next process (Fig. 15); 5 - a transverse thickening above the pair of processes described previously (Fig. 15). The spiny endosoma wall above the latter process has larger and more sclerotized elements (Fig. 15).

FEMALE (from Costa Rica): Measurements (mm): Total length: to tip of abdomen: 16.5; to tip of hemelytra: 21.0; head: total length (lateral view): 2.3; maximum width across eyes: 2.2; interocular space: 1.2; antennal segments: I: 6.3; II: 2.3; III-IV: absent; labium segments: II [first visible]: 1.6; III: 1.3; IV: 0.5. Thorax: pronotum: fore lobe length: 0.9; hind lobe: length: 2.5; width at posterior margin: 3.5. Legs: fore legs: femur: 6.0; tibia: 6.0; tarsus: 0.7; mid legs: femur: 4.9; tibia: 6.5; tarsus: 0.7; hind legs: femur: 7.2; tibia: 10.0; tarsus: 0.9. Abdomen: length: 9.5; maximum width: 3.8. Similar to male (Fig. 5). Anterior half of stridulitrum lighter-colored, reddish; sternite IV completely reddish; sternite V almost completely reddish, except on posterolateral portion, including connexivum in this area, where it is blackish; mid-anterior portion of sternite VI somewhat reddish.

Comments. Since all the specimens studied here have hemelytra that are completely darkened without any yellowish markings (Figs 1–3, 5–6), the mistake in the statement of Gil-Santana (2015), who alleged the presence of small yellowish markings on the hemelytra, is confirmed. Because the features of females examined are in accordance with the description (Stål 1872) and with those of the syntypes of *H. atra* (Figs 1–2), they were considered conspecific. Similarly, the male collected together with the female from Costa Rica was considered as belonging to the same species too. The variation in size, in which the male was shown to be smaller than the female measured here, may or may not be due to sexual dimorphism. This would be clarified if or when more specimens of both sexes are examined in the future. Additional data might also show whether the eyes of the males are or are not larger in this species, since it was not possible to ascertain this through the single observation made here. Although the third antennal segments were absent in the female that was directly compared with the male that had been collected together with it (from Costa Rica; Figs 5–6), the other females recorded here (Figs 1–3) show uniform thickness in this



Figures 14–17. *Hiranetis atra* Stål, male genitalia, dorsal view. **14–15** endosoma, without dorsal apical-median process. (A: semi-oblong dorsal prolongation at midportion of endosoma wall; B–E: endosoma processes, B: U to M-shaped basal process; C: median subspherical process; D: small sclerotized thickened curved process; E: transverse thickening) **16–17** endosoma processes **16** median. **17** dorsal apical-median. Scale bar: (**14–15**): 0.3 mm; (**16**): 0.1 mm; (**17**): 0.3 mm.

segment, while the male presented thickening in the basal half of this segment (Fig. 6). This form of sexual dimorphism has been recorded in several genera of Harpactorini (Stål 1872, Champion 1898, Gil-Santana et al. 2013, Martin-Park et al. 2012) and in another species of *Hiranetis*, *H. braconiformis* (Champion 1898). The minor differences in coloration between the male and female examined were probably due to intraspecific variation, as already recorded in other species of *Hiranetis* (Spinola 1840, Herrich-Schäffer 1848, Champion 1898). On the other hand, they are in accordance with the Stål's concise description of *H. atra*, including the coloration of the abdomen, which he defined as reddish in its basal half. The total length (measured to the

tip of the abdomen) of the female described by Stål was 22 mm, i.e. very similar to that of the female specimen examined here (21 mm).

The importance of the male genitalia for distinguishing species within Harpactorini genera has previously been recorded, e.g. in *Aristathlus* Bergroth, 1913 (Forero et al. 2008), *Atopozelus* Elkins, 1954 (Elkins 1954a), *Atrachelus* Amyot & Serville, 1843 (Elkins 1954b), *Ischnoclopius* Stål, 1868 (Hart 1975) and *Zelus* Fabricius, 1803 (Hart 1972, 1986, 1987, Zhang 2012). For the latter, which is a very speciose genus, studying the male genitalia for taxonomic purposes was shown to be so important that "while males of most species [of *Zelus*] can be readily identified based on characters of the genitalia, identification of females is less straightforward" (Zhang 2012). In all of these studies, the main structures that were shown to be important or that had attributes at a specific level were the medial process of the pygophore, the dorsal phallothecal plates and the struts. The endosoma contents, such as its processes, were not examined or recorded in most of these studies. Although other authors have provided records regarding endosomal structures, most of these studies relate to a single species or very few species in different genera of Neotropical Harpactorini, e.g. *Aristathlus* spp. (Forero et al. 2008), *Graptocleptes bicolor* (Gil-Santana et al. 2013) and *Pronozelus schuhi* Forero, 2012 (Forero 2012). This impedes comparative appraisal between the studies for taxonomic purposes.

There are no previous studies describing the male genitalia of any species of *Hiranetis*, but there is one study on a species of *Graptocleptes* (*G. bicolor*; Gil-Santana et al. 2013). This genus has been considered to be closely related to *Hiranetis* (Stål 1872, Champion 1898, Gil-Santana 2015). The male genitalia of *H. atra* showed similarities to those of *G. bicolor*, such as: pygophore with a subtriangular rounded apex (medial process); parameres similar in shape and somewhat similar in vestiture; dorsal phallothecal plate suboval in shape, with apical margin almost transverse, straight; and endosoma wall mostly minutely spiny. On the other hand, the shape of the struts is quite different, and the pattern observed in *H. atra* (Figs 12–13) may possibly be revealed as characteristic of this species, since the struts pattern has been shown to be useful with regard to the taxonomy of other Neotropical Harpactorini (e.g. Hart 1972, 1986, 1987, Zhang 2012). Interestingly, however, asymmetry on the apical portion of the median arms of the struts was recorded in the present study (Figs 12–13). No similar previous record could be found. If more specimens were to be observed in the future, it would be possible to ascertain whether this was an isolated anomaly or a real feature of the species. Thus, at least for the moment, and as stated in all the studies previously cited, the features of the male genitalia of *H. atra* that should specially be taken into consideration for future comparative purposes are the subtriangular rounded medial process of the pygophore (Fig. 7), the suboval shape of the dorsal phallothecal plate, with an apical margin that is almost transverse (Fig. 12), and the shape and "design" of the struts (Figs 12–13).

Distribution. Colombia (Stål 1872, Maldonado 1990).

New records. Costa Rica, Ecuador.

Hiranetis braconiformis **(Burmeister, 1835)**

Myocoris braconiformis Burmeister, 1835: 226 [description]; Burmeister 1838: 107 [redescription]; Stål 1866: 295 [citation]; Walker 1873b: 129 [catalog]; Wygodzinsky 1949: 40 [catalog].

Hiranetis braconiformis; Stål 1859: 371 [citation]; Stål 1872: 82 [redescription]; Lethierry and Severin 1896: 178 [catalog]; Champion 1898: 281 [included comments on color and morphological features], Tab. XVII [Figures 8, 8a, 9]; Maldonado 1990: 218 [catalog]; Maldonado and Lozada 1992: 165 [citation]; Froeschner 1999: 206 [catalog]; Gil-Santana et al. 2013: 348 [citation].

Myocoris pompilodes Burmeister, 1838: 106 [description]; Champion 1898: 281 [as a junior synonym of *H. braconiformis*].

Hiranetis pompilodes; Stål 1859: 371 [citation]; Stål 1872: 82 [diagnosis]; Champion 1898: 281 [as a junior synonym of *H. braconiformis*]; Wygodzinsky 1949: 40 [catalog, as a valid species].

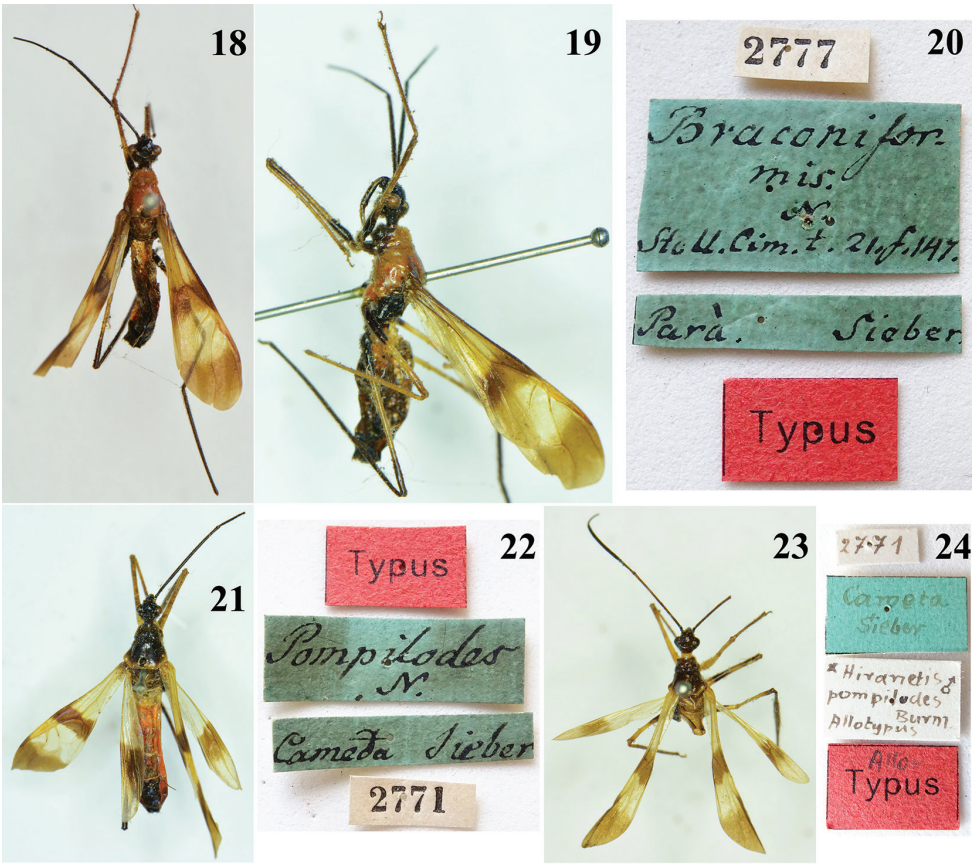
Myocoris pompiloides [*sic*]: Walker 1873b: 129 [catalog]; Maldonado 1990: 218 [catalog, as a junior synonym of *H. braconiformis*].

Hiranetis pompiloides [*sic*]: Lethierry and Severin 1896: 178 [catalog]; Maldonado 1990: 218 [catalog, as a junior synonym of *H. braconiformis*].

Material examined. *Myocoris braconiformis*, female, "typus", labels: 2777 / *Braconiformis*, N., Stoll. Cim. t. 21.f.147 [green label] / Pará, Sieber [green label] / Typus [red label]; *Myocoris pompilodes*, female, "typus", labels: 2771 / *Pompilodes*, N. [green label] / Cameta, Sieber [green label] / Typus [red label]; *Myocoris pompilodes*, male, "allotypus", labels: 2771 / * *Hiranetis pompilodes* Burm., ♂, Allotypus / Cameta, Sieber [green label] / Allo-Typus [red label] (ZMHB).

The female "typus" of *Hiranetis braconiformis* (Burmeister), described from "Para" (Burmeister 1835, 1838), is deposited in ZMHB (Figs 18–20). This region ("Pará") is today a state in the northern region of Brazil, within the Amazonian region of South America.

The female "typus" and a male "allotypus" of *H. pompilodes* (Burmeister), from "Cameta", are also deposited in ZMHB (Figs 21–24). Because the original description (Burmeister 1838) did not designate a holotype, it is better to consider all the type specimens to be syntypes. In the male type, the distal portion of the abdomen is missing (Fig. 23). Although Burmeister (1838) had mentioned that "Cameta" was in "South Brazil", the only locality with this name in Brazil is the municipality of "Cametá" in the same northern state of Pará, from which *H. braconiformis* was described. It is possible that all these specimens were collected in the same region (Pará) and even during the same period, since on all the labels, the name "Cameta" was followed by the name "Sieber" and at least those of the female syntype were apparently handwritten by the same person (Figs 20, 22). As a matter of fact, Friedrich Wilhelm Sieber was a servant and preparator of Johann Centurius Count von



Figures 18–24. 18–20 *Myocoris braconiformis* Burmeister, female, "Typus", deposited in ZMHB **18** dorsal view **19** lateral view **20** labels. **21–24** *Myocoris pompilodes* Burmeister, type specimens deposited in ZMHB **21**–**22** female, "Typus" **21** dorsal view **22** labels **23–24** male "allotypus" **23** dorsal view **24** labels.

Hoffmannsegg, who obtained permission from the King of Portugal to send him to Brazil to collect insects. Leaving Lisbon in 1801, Sieber went to the province of Pará, where he remained 12 years, collecting in different parts of this province, including Cametá (Papavero 1971). Friedrich W. Sieber did not collect in other regions of Brazil and remained in Amazonia throughout this period (Papavero 1971), which reinforces the preceding assertion.

All of these points may be important in ascertaining the type locality of these taxa and are particularly relevant because *H. pompilodes* was subsequently considered by Champion (1898) to be a junior synonym of *H. braconiformis*.

With the exception of the mention of the length, the descriptions of *H. braconiformis* and *H. pompilodes* emphasized only their coloration (Burmeister 1835, 1838). Stål (1872) stated that the two taxa were very similar and *H. pompilodes* differed from

H. braconiformis through the coloration of the thorax (blackish, except at its margin), coxae, trochanters and basal portion of fore femora (yellowish and not blackish).

Champion (1898) considered *H. pompilodes* to be a junior synonym of *H. braconiformis*. He reported that he had examined "a long series" of *H. braconiformis*, stating that it varied in "the colour of the pronotum and also to a certain extent in that of the femora. In many of the specimens the pronotum is entirely rufo-testaceous (*braconiformis*, Burm.); but in others (...) it is partly or entirely black, the basal margin or a subtriangular patch on the disc behind being pale in some examples (*pompilodes*, Burm.)." Champion (1898) also recorded variation in coloration of the femora, which are sometimes narrowly (fore femora) to broadly (mid and hind femora) black basally; "the hind pair have the apex broadly, and rarely a median ring, fuscous or black", while the mid femora are often infuscate apically. In his figures of a pair of this species, he highlighted the variation in color amongst specimens from the same locality.

The fact that Champion (1898) recorded the color variation among specimens from the same locality, which had been attributed by Burmeister (1838) and Stål (1872) to *H. braconiformis* and *H. pompilodes*, may be considered to be arguments in favor of both the historical evidence that the types of these taxa must have been collected in the same region (Brazilian state of Pará) and the assumption that they belong to the same species as stated by Champion (1898).

On the other hand, Champion (1898) apparently did not examine any type specimens of these taxa, or any specimen from Brazil. He also did not mention how many specimens formed his "long series", or whether there might be any other sexual differences besides the third antennal segment thickened at its base. Moreover, he did not take into account any features other than coloration when commenting on the synonymy between *H. braconiformis* and *H. pompilodes*.

Subsequently, Wygodzinsky (1949) still listed *H. pompilodes* as a valid species in his catalogue, while Maldonado (1990) considered it to be a junior synonym of *H. braconiformis*.

Distribution. Brazil (state of Pará, Amazonian region) (Burmeister 1835, 1838), Mexico, Guatemala, Costa Rica, Panama and Guyana (Champion 1898).

***Hiranetis membranacea* Spinola, 1840**

Hiranetis membranacea Spinola, 1840: 113–114 [description]; Lethierry and Severin 1896: 178 [catalog]; Wygodzinsky 1949: 40 [catalog]; Maldonado 1990: 218 [catalog]; Gil-Santana et al. 2013: 348 [citation].

Myocoris membranaceus; Herrich-Schäffer 1848: 43 [redescription], Tab. CCLXI [Figure 811].

Myocoris barbipes Burmeister, 1838: 107 [description]; Stål 1866: 295 [citation]; Stål 1872: 82 [as a junior synonym of *H. membranacea*]; Walker 1873b: 129 [catalog, as a valid species]; Lethierry & Severin 1896: 178 [catalog, as a junior synonym of *H. membranacea*]; Maldonado 1990: 218 [catalog, as a junior synonym of *H. membranacea*].

Hiranetis barbipes; Stål 1859: 371 [citation in text, with footnote: "= *membranaceus* Spin.; H. Sh."]; Wygodzinsky 1949: 40 [catalog, as a junior synonym of *H. membranacea*].

Material examined. *Myocoris barbipes*, female, "typus", labels: 2772 / *barbipes*, two unrecognizable markings, &, ♀. / Bras. r. Olf. [green label] / Typus [red label] (ZMHB).

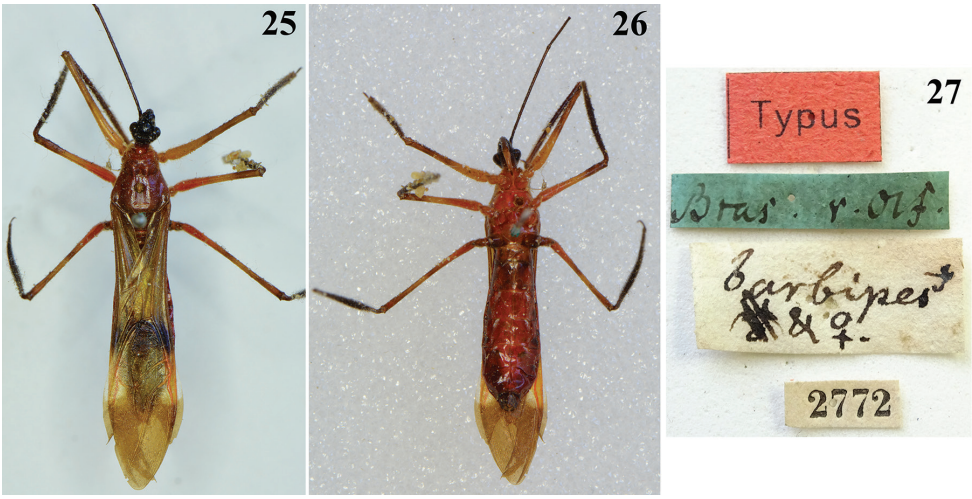
The description of *Hiranetis* mentioned some structural features, and also that their tibiae are all hairy (Spinola 1840). This was followed by the description of *H. membranacea*, based on one or more females and males from Brazil, without ascribing any specific locality to the specimens described. The antennae, body and legs of this species were recorded as black; the hemelytra as entirely membranous, blackish, often slightly darker at their base to their end, but all transparent: a large yellow spot, on three-quarters of their length on outer edge, and a smaller, hyaline, also on the external borders. Single measurements were attributed to the species ("m. long 9. lign. Larg. 2. Lign."; approximately 20.3 and 4.5 mm, respectively).

After making this short description, Spinola (1840) commented that *H. membranacea* did not seem to be rare in South America and often showed variation: 1 - in the coloration of the thorax and abdomen, which were black, brown or even testaceous; 2 - in the legs, which could have yellowish annulus or be entirely yellowish; 3 - in the coloration of the hemelytra, which could be lighter-colored or hyaline, even in the basal portion, in some specimens; 4 - in the size, which could be half of or a third smaller.

However, he concluded by stating that the intermediary specimens that he had at hand left no doubt in his own mind regarding the unity of the species.

Unfortunately, it was not possible to locate any type specimen of *H. membranacea*. The material described by Massimiliano Spinola (1780–1857) is in his collection, which is deposited in the "Museo Regionale di Scienze Naturali", Turin, Italy (Schuh and Slater 1995). More than a decade ago, when looking for a type of other species described by M. Spinola (see Forero and Gil-Santana 2003), I contacted its [former] curator, Dr Mauro Daccordi, who kindly donated the catalogue of Spinola's hemipterological collection (Casale 1981), clarifying that all extant specimens were listed there. There is no reference to any specimens of *Hiranetis membranacea* in it. It is worth mentioning that after M. Spinola's death (1857), his hemipterological collection remained in Tassarolo Castle until its acquisition by Museo Regionale di Scienze Naturali of Turin in 1979 (Casolari and Moreno 1980, Casale 1981). Taking into account "the precarious conditions the Collection was exposed for over a century" (Casale 1981), the types of *H. membranacea* can be considered lost. Nonetheless, at the end of 2015, I contacted the current curator of the Museum, Dr Marinella Garzena, who also kindly confirmed that no specimens of this species are present there. Therefore, it must be assumed that no type specimens of *Hiranetis membranacea* Spinola exist anymore.

Myocoris barbipes was considered to be the largest species among several other species that were included in *Myocoris* Burmeister, 1835, at that time (Burmeister 1838). This species was recorded as coming from "Rio Janeiro" (Burmeister 1838). Its female "typus" is deposited in ZMHB (Figs 25–27). Regarding its type locality, "Rio Janeiro" (Burmeister 1838) may correspond to the current municipality of Rio de Janeiro or, because of the historical scenario at the beginning of the nineteenth century, more likely it should be extended to the state of Rio de Janeiro or even to some of the contiguous states in southeastern Brazil as they are currently delimited. In fact, the handwritten de-



Figures 25–27. *Myocoris barbipes* Burmeister, female, Typus", deposited in ZMHB. **25** dorsal view **26** ventral view **27** labels.

scription on the green label attached to the type seems to read "Bras. r. Olf." (Fig. 27). It is known that in 1816, Ignaz Franz Werner Maria von Olfers arrived in Rio de Janeiro with the Legation of Prussia to study Brazilian nature. He collected extensively in trips from the state of Rio de Janeiro to the contiguous states of Minas Gerais and São Paulo, and back to Rio de Janeiro, in the years 1818 to 1820. His collection, including insects, was then sent to museums in Vienna and Berlin (Papavero 1971).

Herrich-Schäffer (1848) provided a figure (habitus) and a short diagnosis of *H. membranacea* (as *Myocoris membranaceus*). The diagnosis referred only to color features: [general coloration] red; antenna, head, femora apices, tibiae and tarsi black; hemelytra pale yellowish with a median band and apex dark. He then commented on the variation in coloration and size, as had previously been recorded by Spinola (1840) for this species.

In a footnote, Stål (1859) mentioned *Hiranetis barbipes* ("= *membranaceus* Spin.; H. Sh."). On the other hand, in Stål (1872), *Myocoris barbipes* was set as a junior synonym of *H. membranacea*. There was no mention of the reasons for attributing synonymy to these two species. However, with exception of Walker (1873), this was adopted in all the subsequent catalogues (Lethierry and Severin 1896, Wygodzinsky 1949, Maldonado 1990).

Stål (1872) recorded features of structure and vestiture in his diagnosis of the species of *Hiranetis*. However, it is unlikely that these will be helpful in ascertaining better characteristics of *H. membranacea*, so as to remove doubts regarding the validity of the synonymy that he proposed, and/or to provide better knowledge about the diagnostic features of all taxa discussed here. Firstly because he reported that he had examined a single specimen of *H. membranacea* from "Brasilia" [i.e., country of Brazil], which he stated was deposited in the Museum of Stockholm ["Mus. Holm."]. Taking into consideration all the historical data on types of *H. membranacea*, there is

no evidence that this specimen could be a type. In this case, it becomes clear that he did not examine the type of *M. barbipes* that is still extant and is deposited in Berlin (ZMHB; Figs 25–27), even though he placed *M. barbipes* as a junior synonym of *H. membranacea*. Similarly, regarding *H. braconiformis*, he also cited "Mus. Holm.", thus denoting that he probably used other specimen(s) but not the type (also deposited in ZMHB; Figs 18–20) to define the features of the latter species. Secondly, among those features, some are common to other species of *Hiranetis* and coincide with the diagnosis of the genus, or may even be common to species of other genera. Some other features are known to vary among specimens and the possibility of inter-individual variation was probably not taken into consideration at that time. Thirdly, as discussed below, the recorded variations in *H. membranacea* (Spinola 1840) and *H.* [cf.] *braconiformis* (Champion 1848), the similarities in coloration between them and the absence of records of other or better features of each of them when they were originally described (Spinola 1840, Burmeister 1835, 1838) make any identification imprecise. This compromises the diagnosis of *H. membranacea* and *H. braconiformis* furnished by Stål (1872), because it seems that he did not examine any type specimens of these species.

Distribution. Brazil (Spinola 1840, Burmeister 1838, Herrich-Schäffer 1848, Maldonado 1990).

Discussion

Hiranetis atra can be separated from the other species of the genus by its coloration, which is predominantly blackish, including the hemelytra, which are entirely dark (Figs 1–3, 5–6).

Otherwise, while all other currently valid species have the pattern of yellowish or straw-colored hemelytra, with a median, transverse band and a dark apex (Figs 18–19, 21, 23, 25), the limits or validity of these species are uncertain. It is possible that they could be variations of a single species or could be two or more species.

Taking in account the variation in *H. membranacea*, in relation to its description by Spinola (1840), as commented on above, it is possible that among the specimens of the type series, more than a single species could have been present. Unfortunately, this hypothesis is no longer verifiable, because these specimens have been lost.

As discussed above, the previous statements regarding synonymies between taxa of *Hiranetis* (Stål 1872, Champion 1898) needs to be better reviewed, because none of them were based on examination of type specimens and they took in account only coloration (Champion 1898) or a few structural features that were not mentioned in the original descriptions, with feeble or no taxonomic value, verified in only a few specimens (Stål 1872). Moreover, the reliability of the identification of the specimens studied by these authors (Stål 1872, Champion 1898) may be considered doubtful.

A better record of size, including possible sexual variation, and studies on structural features, particularly the male genitalia, and possibly a molecular approach, could

help or be determinant in defining the taxonomy of *H. braconiformis*, *H. barbipes*, *H. membranacea*, and *H. pompilodes*.

However, it seems that such studies on the type specimens will be impossible. In addition to the loss of types of *H. membranacea*, two of the extant types are females and in the only male, the distal portion of the abdomen is missing, and consequently the genitalia is no longer available for examination.

Therefore, in order to resolve the taxonomy of *Hiranetis* spp. a taxonomic review of the group should be done in the future, including the study of a new series of specimens.

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RESEARCH ARTICLE

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Socially-parasitic *Myrmica* species (Hymenoptera, Formicidae) of Himalaya, with the description of a new species

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Abstract

A new socially-parasitic species, *Myrmica latra* **sp. n.** is described based on a queen and male from Indian Himalaya. Its queen differs from other species by the distinctly narrower petiole and postpetiole, blunt and non-divergent propodeal spines, and a darker body colour. The taxonomic position of the three known Himalayan socially-parasitic *Myrmica* species is discussed, and *M. ereptrix* Bolton 1988 is transferred to the *smythiesii* species-group. It is supposed that *M. nefaria* Bharti 2012 is a temporary social parasite, but *M. ereptrix* and *M. latra* **sp. n.** are permanent social parasites, and a key for their identification is provided.

Keywords

Ants, taxonomy, social parasitism, *Myrmica latra* sp. n., *M. ereptrix*, *M. nefaria*

Introduction

More than 100 years ago, Wheeler (1910) proposed the classification of socially parasitic ants and divided them into four large groups: temporary social parasites, slave-makers, degenerate slave-makers and permanent (or true, workerless = inquilines) social parasites. Basically, a similar classification, but with different terminology was developed by Forel (1922, 1923) (see also Wilson 1971; Buschinger 1986, 1990, 2009, Hölldobler and Wilson 1990).

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All socially-parasitic ant species have characteristic morphological features that, taken collectively, were termed as the "inquiline syndrome" by Wilson (1971, 1984) (see also Arnoldi 1930, 1933, Kutter 1973, Bolton 1988, Dowes 1990, Hölldobler and Wilson 1990, Radchenko and Elmes 2003, 2010). The principal features are: reduced size of gynes and males, a widened petiole and especially postpetiole, and the presence of a plate-like tooth or lobe on the ventral surfaces of the petiole and postpetiole. Secondary features for many *Myrmica* social parasites in comparison to free-living species are: much greater body pilosity, spurs on the middle and hind tibiae that are reduced or completely absent, venation in the forewing of alates that is often atypical, and 12-segmented antennae in the males of some species (instead of 13).

The first true socially-parasitic *Myrmica* species, *M. myrmicoxena* Forel, 1895, was discovered in 1869, in a nest of *M. lobicornis* Nylander, 1846, but was not formally described and named until much later, at the end of the 19th century (Forel 1895). A total of 21 species of "true" and putative socially-parasitic *Myrmica* ants have been described from the Holarctic. Some of these species were placed originally in "satellite genera" that have since been synonymised with *Myrmica*; for the taxonomic history of the other generic names see Bolton (1988) and Radchenko and Elmes (2003, 2010). As a result of synonymy only 15 of these names are currently recognized as valid species: eight species from Europe and Algeria, three from North America, two from Siberia and East Asia, and two from the Himalaya (see Radchenko and Elmes 2003, 2010, Francoeur 2007, Bharti 2012, Csösz 2012, Chen et al. 2016).

Recently, the lead author of this paper discovered a queen and a male in Himalaya that possess the typical parasitic *Myrmica* features. Based on differential morphological diagnosis we describe these as a new species *Myrmica latra* sp. n. Additionally, we have compiled a key for the identification of all three known Himalayan socially-parasitic *Myrmica* species.

Materials and methods

The queen and male of *Myrmica latra* sp. n. were collected by handpicking from nests of *M. aimonissabaudiae* Menozzi, 1939, located under stones. Taxonomic analysis was conducted on a Nikon SMZ 1500 stereo zoom microscope with maximum magnification of 112.5×. For digital images, an MP (Micro Publisher) digital camera was used on the same microscope with AUTO-MONTAGE software (Syncroscopy, Division of Synoptics, Ltd.). Later, images were cleaned with HELICON FILTER 5. The holotype and paratype of new species have been deposited in PUAC (Punjabi University Patiala Ant Collection at Department of Zoology and Environmental Sciences, Punjabi University, Patiala, Punjab, India) and can be uniquely identified with specimen-level codes affixed to each pin (PUAC1569803 and PUAC1569804). Measurements were recorded in millimetres on Nikon SMZ 1500 stereo zoom microscope fitted with ocular micrometer. The comparative morphometric data of the species are listed in Tables 1 and 2.

Morphological terminology for measurements (accurate to 0.01 mm) and indices are as follows (see Fig. 1):

HL maximum length of head in dorsal view, measured in a straight line from the anterior point of clypeus (including any carina or rugae, if they protrude beyond the anterior margin) to the mid-point of occipital margin

HW maximum width of head in dorsal view behind the eyes

FW minimum width of frons between the frontal carinae

FLW maximum distance between the outer borders of the frontal lobes

SL maximum straight-line length of scape from its apex to the articulation with condylar bulb

AL (= WL-Weber's length) diagonal length of the alitrunk (=mesosoma) (seen in profile) from the most antero-dorsal point of alitrunk/mesosoma to posterior margin of propodeal lobes

AH height of alitrunk (= mesosoma), measured from upper level of mesonotum perpendicularly to the level of lower margin of mesopleuron in profile view

PL maximum length of petiole in dorsal view, measured from the posterodorsal margin of petiole to the articulation with propodeum; the petiole should be positioned so that measured points lay on the same plane

PW maximum width of petiole in dorsal view

PH maximum height of petiole in profile, measured from the uppermost point of the petiolar node perpendicularly to the imaginary line between the anteroventral (just behind the subpetiolar process) and posteroventral points of petiole

PPL maximum length of postpetiole in dorsal view between its visible anterior and posterior margins

PPW maximum width of postpetiole in dorsal view

PPH maximum height of postpetiole in profile from the uppermost to lowermost point, measured perpendicularly to the tergo-sternal suture

ESL maximum length of propodeal spine in profile, measured along the spine from its tip to the deepest point of the propodeal constriction at the base of the spine

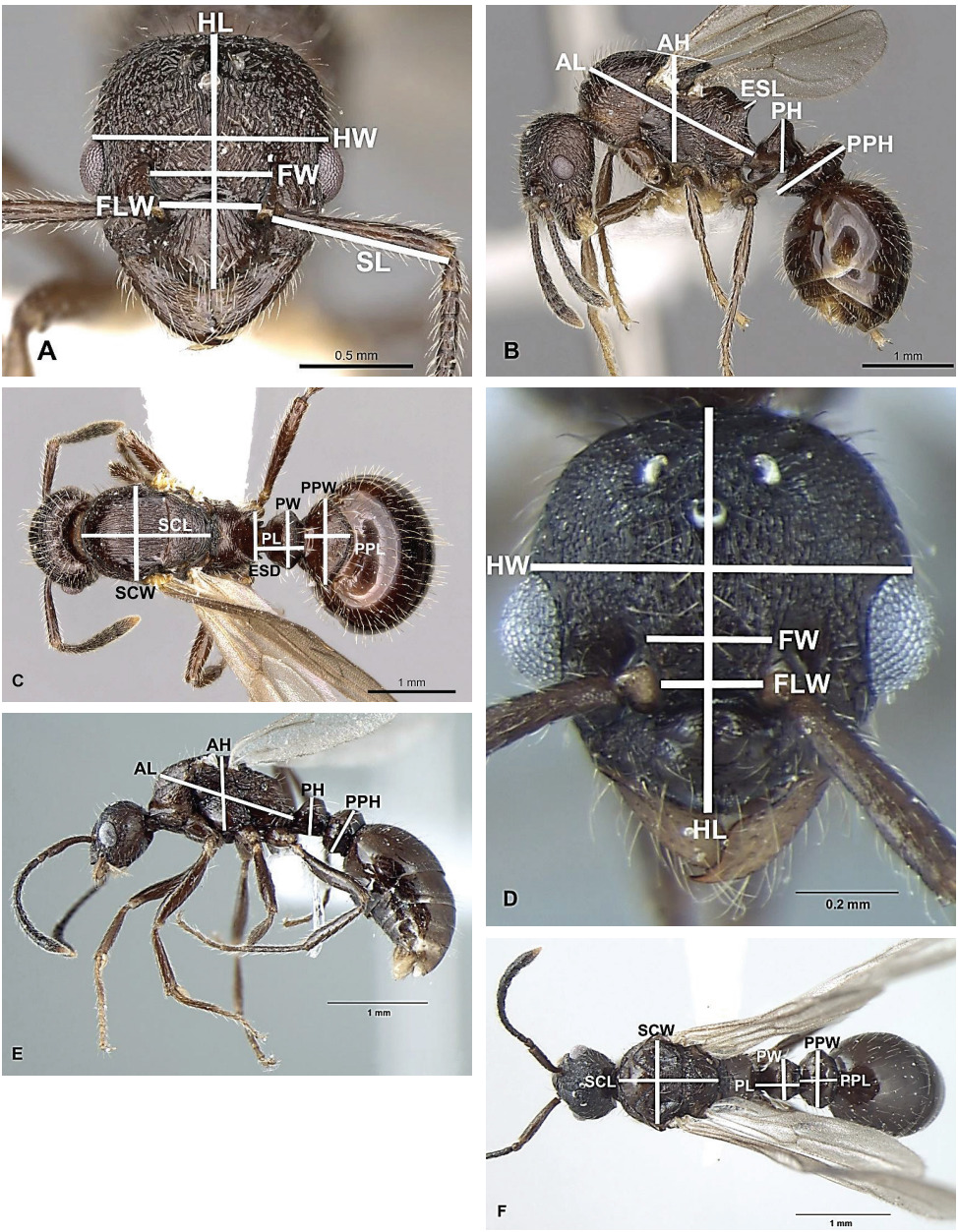
ESD distance between the tips of propodeal spine in dorsal view

SCW maximum width of scutum in dorsal view

SCL length of scutum+scutellum in dorsal view

Indices

Cephalic (**CI**) = HL/HW
Frontal (**FI**) = FW/HW
Frontal-lobe (**FLI**) = FLW/FW
Scape-1 (**SI_1**) = SL/HL



Figures 1. Illustrations: **A** Head (queen) **B** Profile (queen) **C** Dorsum (queen) **D** Head (male) **E** Profile (male) **F** Dorsum (male).

Scape-2 (**SI_2**) = SL/HW
Petiolar-1 (**PI_1**) = PL/PH
Petiolar-2 (**PI_2**) = PL/HW
Petiolar-3 (**PI_3**) = PW/HW

Petiolar-4 (**PI_4**) = PL/PW
Postpetiolar-1 (**PPI_1**) = PPL/PPH
Postpetiolar-2 (**PPI_2**) = PPH/PPW
Postpetiolar-3 (**PPI_3**) = PPW/PW
Postpetiolar-4 (**PPI_4**) = PPW/HW
Postpetiolar-5 (**PPI_5**) = PPL/PPW
Propodeal spine-length (**ESLI**) = ESL/HW
Propodeal spine-distance (**ESDI**) = ESD/ESL
Alitrunk (=mesosomal) (**AI**) = AL/AH
Scutum (**SCI**) = SCL/SCW.

Although the abbreviations of index names have been used in numerous publications (e.g. Radchenko and Elmes 2003) in our experience, many readers find it more convenient to use an explicit description of the ratios, i.e. PPW/PW or PPW/HW instead PPI_3 or PPI_4, etc.

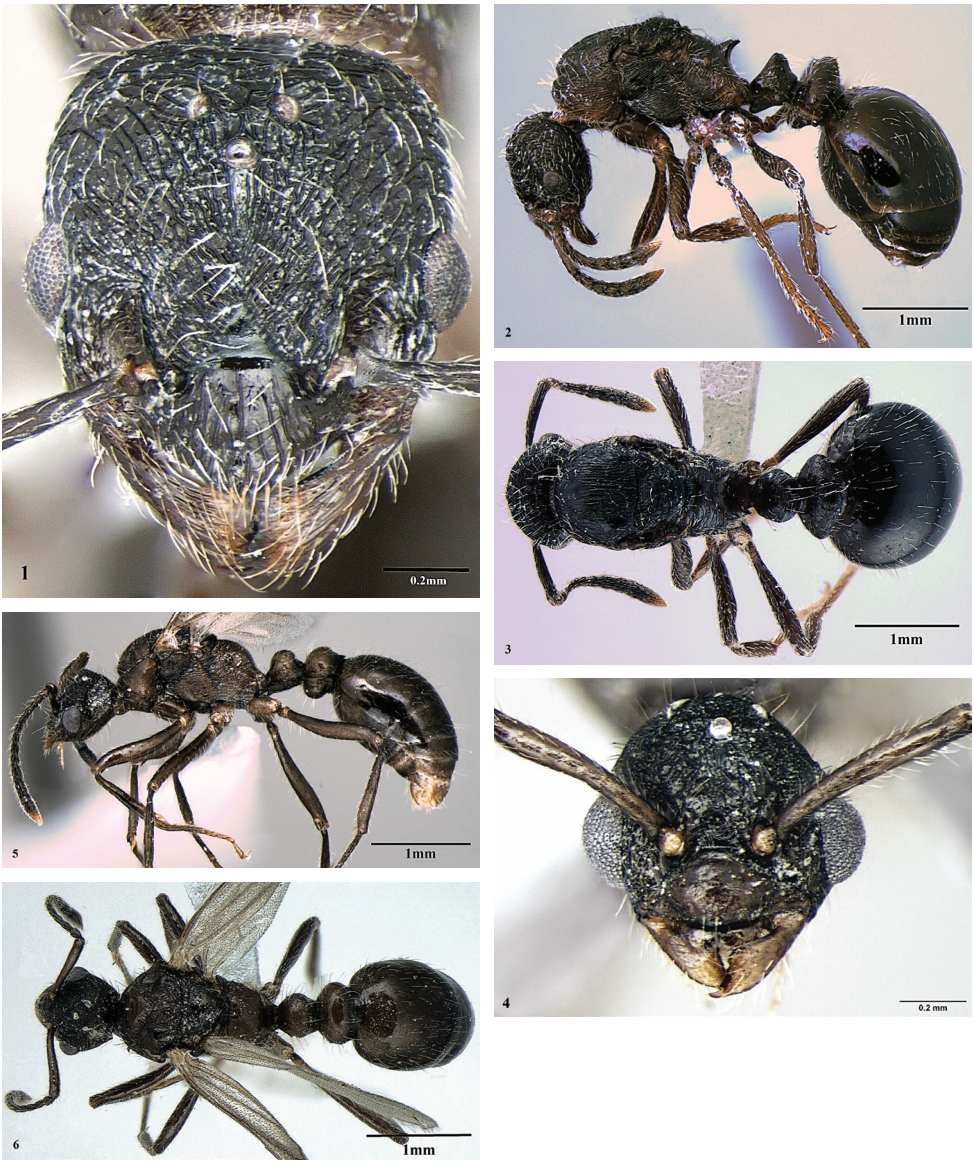
Taxonomy

***Myrmica latra* sp. n.**
http://zoobank.org/834B2826-B346-46F2-BA03-5AC2A112D87F
Figs 2–7, Tables 1–2

Type-material. ***Holotype*** (PUAC1569803) queen, pinned, point-mounted, "India, Himachal Pradesh: Prounthi, 31.1043, 77.6487, 2260m, Hand picking, 14 July 2013, Joginder Singh leg.". ***Paratype*** (PUAC1569804) male (alate), pinned, point-mounted, "India, Himachal Pradesh, Roggling, 32.5514, 76.9704, 2740m, 12 July 2015, Pawanpreet Kaur leg." [PUAC]. Nest understone in ground covered with low vegetation and scattered *Pinus* and *Cedrus* trees.

Description. ***Queen*** (Figs 2–4, Tables 1–2). Head somewhat longer than broad, with slightly convex sides and occipital margin and widely rounded occipital corners. Anterior clypeal margin convex, but not strongly prominent and not notched medially. Upper latero-ventral corners of head somewhat angulate, but not strongly pointed (seen in profile). Eyes situated slightly in front of midlength of sides of head, Ocelli well developed. Right mandible with 7 teeth, left mandible with 6, apical tooth the largest, preapical one smaller, and other ones uniform and small. Frontal carinae curved outwards to merge with rugae, which surround antennal sockets. Frons wide, frontal lobes converging anteriorly, so that width of frons somewhat wider than distance between frontal lobes. Antennae 12-segmented, with 5-segmented club, scape slender, gradually and feebly curved at the base, without any trace of lobe or carina, shorter than head width, only slightly surpassing occipital margin.

Mesosoma of moderate length, mesonotum feebly convex, scutum not overlapping pronotum, antero-lateral corners of pronotum visible from above, propodeal lobes



Figures 2–7. *Myrmica latra* sp. n. **2** Head (queen) **3** Profile (queen) **4** Dorsum (queen) **5** Head (male) **6** Profile (male) **7** Dorsum (male).

rounded apically. Propodeal dorsum almost flat (seen in profile). Propodeal spines quite short, widened at the base, thick, not pointed, but narrowly rounded at tips, directed upward (at an angle ca. 45°) and backward, not diverging when seen from above. Metapleural glands moderately large, with conspicuous orifice dorsally on bulla.

Petiole and postpetiole distinctly widened, while less in width in comparison to other Himalayan socially-parasitic *Myrmica* species. Petiole high, with short but dis-

Figures 8–10. *Myrmica ereptrix*. **8** Head (queen) **9** Profile (queen) **10** Dorsum (queen).

tinct peduncle, slightly longer than wide (in other Himalayan socially-parasitic *Myrmica* it is distinctly shorter than wide); its anterior surface concave, node dorsum narrowly rounded; ventral process quite small, widely rounded on tip and directed mostly forward and slightly downward. Postpetiole high, more than 1.5 times higher than petiole, and 1.75 times higher than its length, quite thick and with rather widely rounded dorsum, its anterior surface convex, posterior one almost straight (seen in profile); ventral process well developed, subtriangular, narrowly rounded apically. Spurs of middle and hind tibiae well developed and pectinate.

Head dorsum with coarse longitudinal rugosity and reticulation, diverging postero-laterally. Vertex and occiput with transverse rugosity and reticulation; surface between rugae finely punctate, but appearing shiny. Frontal triangle deep, smooth and shiny. Clypeus longitudinally rugose, surface between rugae finely punctate. Mandibles coarsely longitudinally rugose.

Pronotum longitudinally rugo-reticulate and transverse dorsally. Scutum densely longitudinally rugose, only its anterior part smooth and shiny. Anterior part of scutellum with short longitudinal rugae, its posterior part transversely-concentrically rugose. Propodeal dorsum with finer transverse rugae, its declivity smooth and shiny. Mesopleurae and sides of

Figures 11–16. *Myrmica nefaria.* **11** Head (queen) **12** Profile (queen) **13** Dorsum (queen) **14** Head (male) **15** Profile (male) **16** Dorsum (male).

propodeum longitudinally rugose, only posterior part of anepisternum smooth and shiny. Petiolar node and postpetiolar dorsum transversely rugose. Whole surface of mesosoma between rugae densely while not coarsely punctate, appears dull. Gaster very smooth, polished.

Table 1. Measurements of the Himalayan socially-parasitic *Myrmica* species.

Measurements (in mm)	*M. latra* sp. n.		*M. ereptrix*	*M. nefaria*					
	holotype queen	paratype male	holotype gyne	gynes (n=63)			males (n=4)		
				mean±SD	min	max	mean±SD	min	max
HL	1.23	0.795	1.20	1.13±0.02	1.10	1.17	0.76±0.02	0.74	0.78
HW	1.08	0.63	1.06	1.01 ± 0.01	0.99	1.02	0.69±0.03	0.66	0.71
FW	0.57	--	0.56	0.53 ± 0.01	0.52	0.55	--	--	--
FLW	0.54	--	0.57	0.51 ± 0.02	0.49	0.53	--	--	--
SL	0.90	0.675	0.82	0.87 ± 0.03	0.82	0.92	0.54±0.03	0.50	0.56
PL	0.57	0.40	0.46	0.51 ± 0.02	0.48	0.52	0.39±0.03	0.36	0.42
PW	0.54	0.42	0.65	0.60 ± 0.03	0.58	0.66	0.39±0.02	0.38	0.41
PH	0.54	0.40	0.58	0.54 ± 0.01	0.53	0.54	0.39±0.02	0.38	0.41
PPL	0.48	0.375	0.49	0.45 ± 0.02	0.41	0.49	0.38±0.03	0.36	0.42
PPW	0.87	0.60	0.98	0.95 ± 0.02	0.91	0.97	0.57±0.04	0.53	0.60
PPH	0.84	0.55	0.88	0.81 ± 0.04	0.78	0.89	0.50±0.03	0.47	0.53
ESL	0.21	--	0.19	0.21 ± 0.01	0.19	0.21	--	--	--
ESD	0.48	--	0.56	0.54 ± 0.03	0.46	0.57	--	--	--
AL	2.04	1.47	1.96	1.77 ± 0.02	1.74	1.78	1.35±0.01	1.35	1.36
AH	1.17	0.90	0.96	1.09 ± 0.03	1.06	1.14	0.87±0.005	0.87	0.88
SCW	1.17	0.996	1.06	1.03 ± 0.02	1.00	1.06	0.84±0.03	0.81	0.86
SCL	1.56	1.11	1.54	1.21 ± 0.03	1.14	1.25	0.96±0.03	0.93	0.98

Whole body with whitish hairs. Head dorsum, margins and ventral surface with abundant semi-erect to erect straight whitish hairs of various length, anterior clypeal margin with long setae, mandibles with quite long curved hairs, scape and 7 basal funicular segments with abundant semi-erect to subdecumbent long hairs and shorter pilosity, segments of club with very dense subdecumbent pilosity.

Mesosoma, waist and gaster with numerous long and curved erect hairs, combined with shorter suberect to subdecumbent straight hairs.

Whole body brownish-black, mandibles, antennae, legs (especially tibia and tarsi) and sides of pronotum lighter, brownish.

Male (Figs 5–7, Table 1–2). Head distinctly longer than broad, suboval, gradually narrowing behind and in front of eyes, occipital margin convex. Upper latero-ventral corners of head somewhat angulate, but not strongly pointed (seen in profile). Frons somewhat raised up anteriorly and gradually sloping to the level of central ocellus. Clypeus convex, its anterior margin very feebly convex, not prominent and not notched medially. Eyes large in comparison to queen, situated in front of midlength of sides of head, ocelli quite prominent. Mandibles with well-developed apical and smaller preapical teeth, followed by 6 minute blunt denticles. Antennae 13-segmented, with 5-segmented club; scape long, longer than six basal funicular segments and head width, surpassing occipital margin.

Mesosoma long and low, ca. 1.6 times longer than height, scutum and scutellum convex, forming regular arch, scutellum does not project dorsally above scutum when

Table 2. Morphometric indices of the Himalayan socially-parasitic *Myrmica* species.

Indices	*M. latra* sp. n.		*M. ereptrix*	*M. nefaria*					
	holotype queen	paratype male	holotype gyne	gynes			males		
				mean±SD	min	max	mean±SD	min	max
HL/HW (CI)	1.14	1.26	1.13	1.12±0.02	1.11	1.15	1.10±0.01	1.10	1.12
FW/HW (FI)	0.53	--	0.53	0.53±0.02	0.51	0.54	--	--	--
FLW/FW (FLI)	0.95	--	1.02	0.96 ± 0.05	0.94	1.02	--	--	--
SL/HL (SI_1)	0.73	0.85	0.68	0.77 ± 0.04	0.75	0.81	0.71±0.06	0.68	0.77
SL/HW (SI_2)	0.83	1.07	0.77	0.86 ± 0.04	0.83	0.90	0.78±0.02	0.76	0.79
PL/PH (PI_1)	1.05	1.00	0.79	0.94 ± 0.06	0.91	1.00	0.99 ± 0.14	0.88	1.08
PL/HW (PI_2)	0.53	0.64	0.43	0.50 ± 0.02	0.48	0.51	0.56 ± 0.02	0.55	0.59
PW/HW (PI_3)	0.50	0.67	0.61	0.59 ± 0.04	0.58	0.65	0.56 ± 0.02	0.54	0.58
PL/PW (PI_4)	1.06	0.95	0.71	0.82±0.03	0.77	0.87	0.99±0.10	0.89	1.10
PPL/PPH (PPI_1)	0.57	0.68	0.56	0.55 ± 0.06	0.49	0.58	0.76 ± 0.11	0.68	0.84
PPH/PPW (PPI_2)	0.96	0.92	0.90	0.88±0.02	0.86	0.90	0.88 ± 0.00	0.88	0.88
PPW/PW (PPI_3)	1.61	1.43	1.51	1.52±0.08	1.46	1.68	1.46 ± 0.04	1.40	1.46
PPW/HW (PPI_4)	0.81	0.95	0.92	0.94 ± 0.04	0.92	0.98	0.82 ± 0.03	0.80	0.85
PPL/PPW (PPI_5)	0.55	0.63	0.50	0.53±0.02	0.49	0.55	0.67±0.05	0.63	0.72
ESL/HW (ESLI)	0.19	--	0.18	0.21 ± 0.00	0.21	0.21	--	--	--
ESD/ESL (ESDI)	2.20	--	2.95	2.61±0.43	2.38	3.00	--	--	--
AL/AH (AI)	1.74	1.63	2.04	1.78±0.06	1.66	1.83	1.69±0.02	1.67	1.70
SCL/SCW (SCI)	1.33	1.48	1.45	1.01±0.10	0.86	1.11	1.13±0.02	1.12	1.16

seen in profile. Propodeum gradually rounded, without tubercles, length of its dorsal surface subequal to posterior one, propodeal lobes rounded apically. Petiole with short peduncle, strongly concave anterior surface and widely rounded node dorsum. Postpetiole short and high, ca. 1.5 times higher than length, with evenly rounded dorsum, its sternite looks like a rather long widely rounded ventral plate. Ventral process on petiole small, tooth-like. Both petiole and postpetiole obviously widened. Spurs of middle and hind tibiae well developed and pectinate.

Wing venation almost typical to the genus, e.g. forewing with closed cell *mcu*, open cell *3r*, vein 2+3RS reduced proximally so that cells *1+2r* and *rm* only partly separated.

Head dorsum with irregular short coarse rugae, sides of head and vertex with reticulation. Mandibles smooth, only sparsely punctate, appearing shiny overall. Sides of pronotum mostly smooth, but with fine longitudinal slightly sinuous rugulosity posteriorly. Anterior part of scutum between Mayrian furrows smooth and shiny, its posterior part and scutellum irregularly rugulo-punctate. Anepisternum with irregular fine rugulosity, katepisternum and sides of propodeum coarsely longitudinally rugulose and with fine reticulation; propodeal dorsum and declivity shagreened, somewhat shiny. Petiolar node and postpetiole with fine superficial microsculpture, but appearing more or less shiny. Gaster smooth and shiny.

Whole head surface with numerous long erect to suberect, often curved long hairs and shorter subdecumbent pilosity. Scape and basal funicular segments with subde-

cumbent to suberect hairs, club segments with subdecumbent short pubescence. Mesosoma and waist with abundant, quite long suberect to erect hairs, gaster with similar long hairs and sparse short subdecumbent pilosity. Legs with numerous subdecumbent, quite long hairs. Whole body and appendages brownish.

Workers. unknown.

Remarks. The queen of *M. latra* sp. n. differs from the known non-parasitic Himalayan *Myrmica* species by possessing characteristic features of the "inquiline syndrome", particularly by the distinctly widened petiole and postpetiole, presence of the well-developed ventral lobe on the petiole and postpetiole, and also by the presence of more hair on the body. Although *M. latra* shares these features with two already described socially-parasitic Himalayan species, *M. ereptrix* Bolton, 1988 and *M. nefaria* Bharti, 2012, it differs from both by in following characters: *M. latra* has a relatively less-widened petiole and postpetiole, its head is twice as wide as the petiole: PW/HW = 0.50 compared to PW/HW = 0.58-0.65 in the two other species; PPW/HW = 0.81 in *Myrmica latra* versus a ratio > 0.92 in the other two species. The petiole in *M. latra* sp. n. is nearly as long as wide (PL/PW = 1.06), but in the other two it is distinctly wider than long (PL/PW ≤ 0.85); the ratios PPL/PPW are 0.55 *vs.* ≤ 0.55, respectively. Other differences include the ventral processes on the petiole and postpetiole in *M. latra* being distinctly smaller than in *M. ereptrix* (compare Figs 3 and 9); its propodeal spines are blunt and not divergent, while in both *M. ereptrix* and *M. nefaria* they are pointed and distinctly divergent (compare Figs 3 and 9, 12); the spur on the middle tibiae in *M. ereptrix* is strongly reduced, while in the other species it is well developed and pectinate; the body colour of *M. latra* sp. n. is darker than in two other species.

The male of *M. latra* sp. n. well differs from all the known males of the species of the *smythiesii*-group (see also Discussion, below) by the much wider petiole and postpetiole, as well as by the distinctly higher postpetiole, its sternite gives the appearance of rather long and widely rounded ventral plate. Thus, in *M. latra* PW/HW = 0.67, PPW/HW = 0.95 and PPL/PPH = 0.68, but these ratios in the non-parasitic species from the *smythiesii*-group (*M. bactriana* Ruzsky, 1915, *M. fortior* Forel, 1904 and *M. ruzskyana* Radchenko et Elmes, 2010) are: PW/HW < 0.40, PPW/HW < 0.60 and PPL/PPH > 0.80 (our unpublished data).

While the male of *M. latra* morphologically resembles the male of *M. nefaria* (the males of *M. ereptrix* are unknown), it differs by its longer head (HL/HW = 1.26 *vs.* 1.10–1.12) that is distinctly narrowed posteriorly (compare Figs 5 and 14); by the distinctly longer scape that is longer than the head width in *M. latra*: SL/HL = 0.85, SL/HW = 1.07 *vs.* SL/HL = 0.68–0.77 and SL/HW = 0.76–0.79; by the wider petiole and postpetiole (PW/HW = 0.67, PPW/HW = 0.95 *vs.* 0.54–0.58 and 0.80–0.85). Additionally, the head dorsum in *M. latra* has short irregular rugae, but in *M. nefaria* males, the head dorsum has longitudinal rugae; posterior part of scutum has longitudinal rugae *vs.* transversal rugosity; its propodeum is gradually rounded, without teeth or tubercles, but in *M. nefaria* propodeum is distinctly angulated with short teeth. Finally, the forewing venation of the male of *M. latra* sp. n. is almost typical for the genus *Myrmica* and resembles that of *M. ereptrix* (see

above and Bolton 1988), but in some males of *M. nefaria* it is modified (see Bharti 2012). However, it should be remembered that the forewing venation in different specimens of the same species, especially in social parasites, may be quite variable so not too much reliance should be placed on this feature (see Arnoldi 1930, 1933; Bolton 1988; our own observations).

Etymology. From the Latin adjective *latra*, meaning robber or thief.

Ecology. Both queen and male were collected from nests of *M. aimonissabaudiae* built under stones. The ground is covered with low vegetation, and scattered *Pinus* and *Cedrus* trees. The recorded nest temperature and humidity at site one, where queen was collected was 18 °C and 76%, whereas at site two, where male was collected, the recorded nest temperature was 19 °C and humidity 66%.

Key for identification of the socially-parasitic Himalayan *Myrmica* species

Queens

1 Petiole and postpetiole narrower, PW/HW = 0.50, PPW/HW = 0.81, petiole nearly as long as wide, PL/PW = 1.06 (Figs 3, 4). Propodeal spines blunt and not divergent (Figs 2–4). Body colour darker, blackish-brown ..... ***M. latra* sp. n.**

– Petiole and postpetiole wider, PW/HW = 0.58-0.65, PPW/HW > 0.90, petiole distinctly wider than length, PL/PW ≤ 0.87 (Figs 9, 10, 12, 13). Propodeal spines pointed and distinctly divergent (Figs 10, 12). Body colour lighter, reddish-brown **2**

2 Head dorsum with longitudinal rugae and reticulation (Fig. 11). Dorsal surface of propodeum with divergent longitudinal rugae (Fig. 13). Middle and hind tibiae with well-developed pectinate spur (Fig. 12). Petiole somewhat longer, PL/PH > 0.90, PL/PW = 0.77–0.87; mesosoma relatively higher, AL/AH =1.66–1.83 ..... ***M. nefaria* Bharti**

– Head dorsum with longitudinal, somewhat divergent rugae, reticulation present only on vertex and temples (Fig. 8). Dorsal surface of propodeum transversally rugose (Fig. 10). Hind tibiae with well-developed pectinate spur, but spur on middle tibiae strongly reduced, short and simple (Fig. 9). Petiole somewhat shorter, PL/PH = 0.79, PL/PW = 0.71; mesosoma relatively lower, AL/AH =2.04 ..... ***M. ereptrix* Bolton**

Males (males of *M. ereptrix* are unknown)

1 Head longer, HL/HW = 1.26, distinctly narrowed posteriorly above eyes; head dorsum with short irregular rugae (Fig. 5). Scape longer, SL/HL = 0.85, SL/HW = 1.07. Petiole and postpetiole wider, PW/HW = 0.67, PPW/HW = 0.95; posterior part of scutum with longitudinal rugae (Fig. 7). Propodeum gradually rounded, without teeth or tubercles (Fig. 6).....***M. latra* sp. n.**

– Head shorter, HL/HW = 1.10–1.12, gradually arched above eyes; head dorsum with longitudinal rugae (Fig. 14). Scape shorter, SL/HL = 0.68–0.77, SL/HW = 0.76–0.79. Petiole and postpetiole narrower, PW/HW = 0.54–0.58, PPW/HW = 0.80-0.85; posterior part of scutum with transversal rugosity (Fig. 16). Propodeum distinctly angulated and with short teeth (Fig. 15) .....***M. nefaria* Bharti**

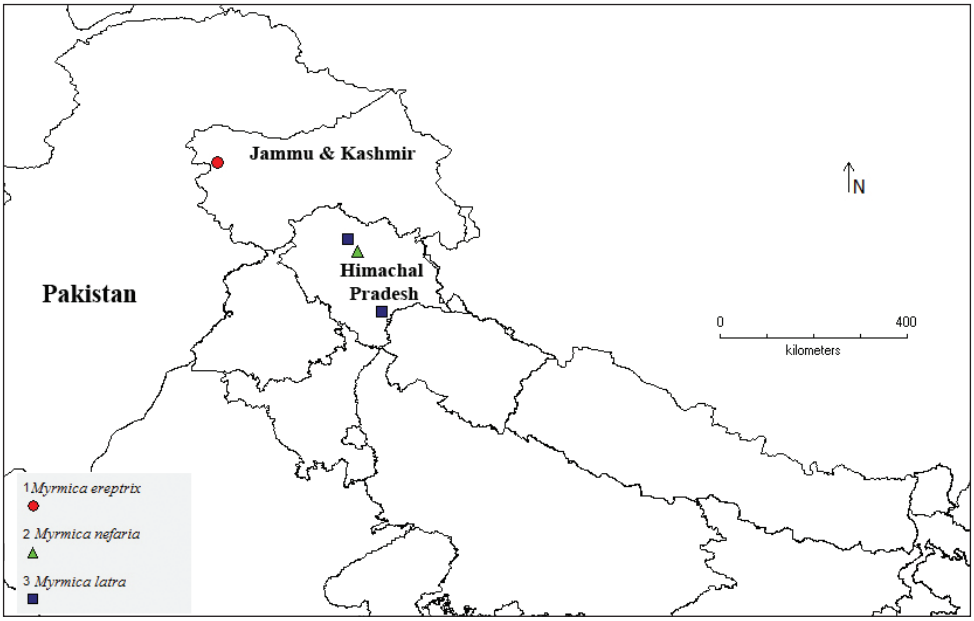
Discussion

There are two questions that need to be addressed: first, why have we described this queen and male that were collected from different nests as the same species? Secondly, why have we described them as social parasites?

The second question is more easily answered: both castes possess a combination of features known as the "inquiline syndrome" (discussed above) and by these features they significantly differ from all known free-living Himalayan *Myrmica* species. This species is most unlikely to occur elsewhere, given that the *Myrmica* fauna of the Himalayan region is almost completely isolated from the fauna of adjacent regions (Radchenko and Elmes 2001, 2010). If *M. latra* is a social parasite then the queen well differs from those of the two known Himalayan socially-parasitic species, *M. ereptrix* and *M. nefaria*, while the male differs significantly from those of *M. nefaria* (males of *M. ereptrix* are unknown).

We have decided to describe the queen and male as the same species, despite coming from different nests, because the putative host colonies were of the same species, *M. aimonissabaudiae,* living in the same general region at similar altitudes albeit the two sites were 173 km apart (see Map 1). *M. aimonissabaudiae* is now known to host two socially parasitic species (*M. ereptrix* and *M. latra*) and while there is no reason why it should not host several more species (e.g. as in the case of *M. sabuleti* Meinert in Europe) the simplest hypothesis this queen and male belong to the same species. With our present knowledge, we do not wish to create an extra name, which might be synonymised later.

Furthermore, it is always better to avoid the description of a new taxon based on a single specimen, especially, if it is collected in isolation (e.g. in a pitfall trap), but in this case the specimens were collected from a nest of same host species and both male and female differ from already known species of the genus. To date, eight *Myrmica* species have been described based on a single queen (Emery 1907, Bernard 1967, Bolton 1988, Radchenko and Elmes 1999) or worker (Forel 1902, Radchenko et al. 2008, Radchenko and Elmes 2009), but no valid *Myrmica* species have been described based on males. Therefore, we have designated the queen as the holotype and male as a paratype. If, in the future, queens of *M. latra* are found with males in the same host nest (or collected *in copula* in a mating swarm) and the males are distinctly different from the paratype male of *M. latra* described here, then the specimen in question would be validated as a separate species. Additionally, it is quite logical to designate the queen as holotype, as male-based taxonomy in the genus *Myrmica* is much less developed than



Map 1. Geographical distribution of socially parasitic species in Himalaya.

the female-based one, and in many cases correct identification of a single male is nearly impossible (see Radchenko and Elmes 2010, Czechowski et al. 2012).

The present concept of species-groups in the genus which is based on morphology, was outlined by Radchenko (1994) and further improved by Radchenko and Elmes (2001, 2010), and currently in the absence of a complete molecular phylogeny, this concept is quite useful to indicate the degree of relatedness between species. Although, a molecular phylogeny based on a sample of *Myrmica* species (Jansen et al. 2010) mostly complemented the morphological species-group concept (Radchenko and Elmes 2010). However, in the light of present findings, we ought to reconsider the *rugosa* and *smythiesii* species groups.

The molecular genetic analysis published by Jansen et al. (2010) did not support the separation of the *rugosa* and *smythiesii* species groups. In the above mentioned analysis, three of the *rugosa*-group species were analyzed (*M. rugosa* Mayr, 1865, *M. aimonissabaudiae* Menozzi, 1939, and *M. rupestris* Forel, 1902) along with *M. wittmeri* Radchenko et Elmes, 1999 (a quite peculiar species in some characters that was tentatively placed in the *smythiesii*-group). Besides, the material of "*M. rugosa*" was collected in Kyrgyzstan, well outside the limits of known geographic distribution of this species, either this was a typing error in the paper or the specimens were misidentified. Moreover, in the above cited phylogenetic analysis, the American *M. wheeleri* Weber, 1939 (that quite well differs morphologically from the Himalayan species) is grouped with the species of "*rugosa*-group". Thus, these intriguing results indicate that there are still a lot of taxonomic problems within the supra-specific taxonomy of the Himalayan *Myrmica*, until a molecular

analysis with inclusion of many more species is carried out; the morphological species-groups still have some usefulness.

Morphologically, female castes of the *rugosa* and *smythiesii* groups share several diagnostic features (e.g.: scape very smoothly curved at the base, not angled and with no trace of a lobe or carina; frontal lobes slightly curved, frons wide and frontal lobes not extended; anterior clypeal margin is convex and prominent, without a medial notch). The main difference is the shape of the frontal carinae: in the *rugosa*-group they merge with the rugae that extend to the occipital margin, do not curve outwards and do not merge with rugae that surround antennal sockets, but in the *smythiesii*-group frontal carinae curve outwards to merge with the rugae that surround the antennal sockets. In addition, males of the *rugosa*-group have a relatively short scape, SL/HL < 0.60, but those of the *smythiesii*-group have much longer scape – SL/HL > 0.70 but unfortunately males are unknown for some species in this group: *M. wittmeri*, *M. bactriana* Ruzsky and *M. ruzskyana* Radchenko & Elmes. If the *rugosa*- and *smythiesii*- species groups are quite closely related, then, taking into account the length of scape in males, species placed in the latter group are obviously more evolved, because a short scape is a plesiomorphic state not only for *Myrmica*, but for ants as a whole (see Radchenko and Elmes 2001, 2010, Radchenko et al. 2007, Dlussky and Radchenko 2009).

Regarding the Himalayan social parasites: when Bolton (1988) described *M. ereptrix* from a single gyne found in the nest of *M. aimonissabaudiae*, the present species-group concept in the genus *Myrmica* was not fully established, and he placed this species in the *rugosa*-group. Later, Radchenko and Elmes (2001, 2003, 2010) erroneously subscribed to his viewpoint. However, based on the diagnostic features of species-groups (discussed above), now we formally transfer *M. ereptrix* to the *smythiesii*-group (*M. nefaria* and *M. latra* are also placed in this group, see also Bharti 2012), while the host species of *M. ereptrix* and of *M. nefaria* belong to the *rugosa*-group. Generally, the social parasites of *Myrmica* are phylogenetically close to their hosts (Jansen et al. 2010) and we may only suppose that these parasites evolved at the same time when the *smythiesii*-group was diverging from the *rugosa*-group (Bharti 2012).

Probably, *M. nefaria* is a temporary social parasite as all its castes were found in the host colony and in the right circumstances may potentially form free-living colonies (as in the case of *M. vandeli* Bondroit, 1920 in Europe (see Elmes et al. 2003, Radchenko and Elmes 2003, 2010). At the moment, we can only speculate on the life-style of the other two species, most probably they are obligate social parasites.

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Mulloidichthys flavolineatus flavicaudus Fernandez-Silva & Randall (Perciformes, Mullidae), a new subspecies of goatfish from the Red Sea and Arabian Sea

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Abstract

The number of goatfish species has increased recently, thanks in part to the application of molecular approaches to the taxonomy of a family with conservative morphology and widespread intraspecific color variation. A new subspecies *Mulloidichthys flavolineatus flavicaudus* Fernandez-Silva & Randall is described from the Red Sea and Arabian Sea, including Socotra and Gulf of Oman. It is characterized by a yellow caudal fin, 25–28 gill rakers, and 37–38 lateral-line scales and it is differentiated from nominal subspecies *M. flavolineatus flavolineatus* by 1.7% sequence divergence at the mitochondrial cytochrome b gene. The morphometric examination of specimens of *M. f. flavolineatus* revealed variation in head length, eye diameter, and barbel length, in western direction from the Hawaiian Islands, South Pacific, Micronesia, and the East Indies to the Indian Ocean. The population of *Mulloidichthys f. flavicaudus* **subsp. n.** in the Gulf of Aqaba differs from that of the remaining Red Sea by shorter barbels, smaller eyes, shorter head, and shorter pelvic fins. We present a list of 26 endemic fishes from the Gulf of Aqaba and discuss the probable basis for the endemism in the light of the geological history of this region.

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Keywords
cytb, marine fish, glacial refugia, phylogeography, taxonomy, vicariance

Introduction

The goatfish *Mulloidichthys flavolineatus* was described by Lacepède (1801) based on a manuscript written by Dr. Philibert Commerçon (Commerson in English). There is no type specimen and no record of the type locality (Bauchot et al. 1985). It is almost certainly Mauritius, where Commerson spent several years collecting biological specimens, including many fishes. Fricke (1999: 309) designated a neotype for *M. flavolineatus* from nearby Réunion, but it was later considered invalid by him (Fricke 2000: 639) as "not sufficiently in accordance with Article 75b and Article 75d of the International Code of Zoological Nomenclature." We designate and describe a neotype in the present paper (Fig. 1) collected and photographed in Mauritius by the second author. We also illustrate a live individual from the island (Fig. 2).

Mulloidichthys flavolineatus is presently regarded as the most wide-ranging species of the family Mullidae, from the northern Red Sea (Ben-Tuvia and Kissil 1988) to the Pitcairn Islands (Nichols 1923; Randall 1999). Such a broad distribution might be expected from the unusually large size attained by the postlarvae at settlement, 60 to 80 mm SL (Randall 2005). It is also unusual for such a common and widespread species to have only two junior synonyms, *Mulloides samoensis* Günther, 1874, type locality, Upolu, Samoa Islands, and *Upeneus preorbitalis* Smith & Swain, 1882, type locality, Johnston Atoll.

Figure 1. Color photograph of the neotype of *Mulloidichthys flavolineatus flavolineatus* from Mauritius, BPBM 20135, 162 mm SL. Photo by John E. Randall.

Figure 2. Underwater photograph of *Mulloidichthys flavolineatus flavolineatus* (aprox. 230 mm SL) from Mauritius, the type locality of the subspecies. Photo by John E. Randall.

Figure 3. Underwater photography of *Mulloidichthys flavolineatus flavolineatus* from O'ahu, Hawai'i. Photo by John E. Randall.

Like other goatfishes, this species uses the pair of sensory barbels on its chin to locate prey, mainly in sedimentary substrata, as seen in Fig. 3 of an adult in the Hawaiian Islands and one in the Red Sea (Fig. 4). Randall (2005: 292) summarized the prey

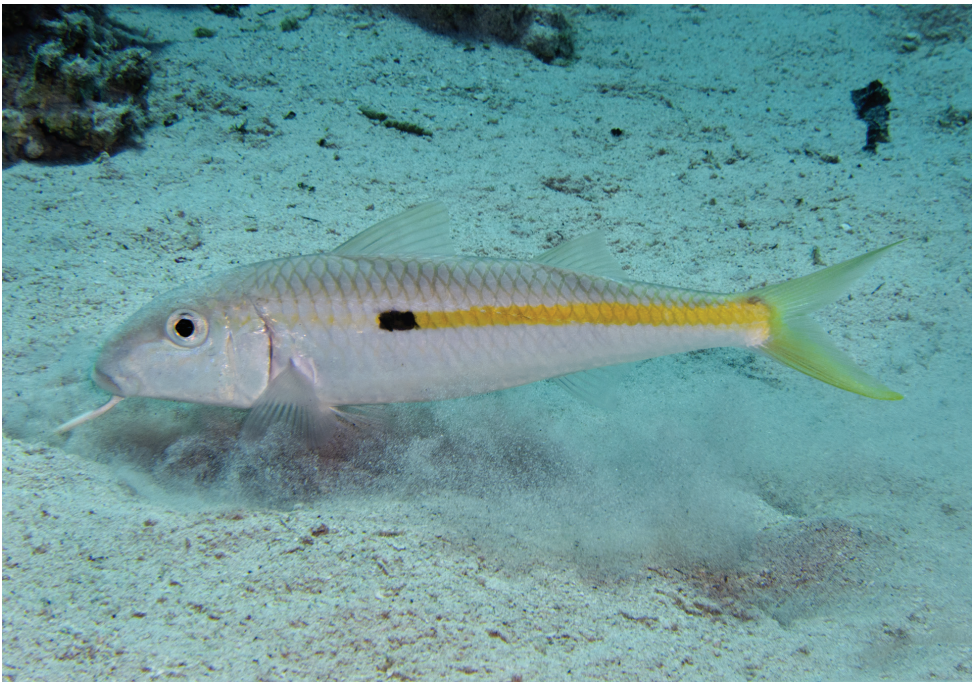

Figure 4. Underwater photographs of *Mulloidichthys flavolineatus flavicaudus* subsp. n. from Dahab in the Gulf of Aqaba. Photo by Sergey V. Bogorodsky.

of specimens from the Hawaiian Islands as small crabs, shrimps, polychaete worms, small bivalve mollusks, hermit crabs, crab megalops, heart urchins, small gastropods, amphipods, foraminifera, and unidentified eggs. During periods of inactivity, the fish may be seen hovering in aggregations a short distance above the bottom (Fig. 5) or in groups resting on sand (Fig. 6).

Myers (1999: 159) reported spawning in Palau over shallow sandy areas near the reef's edge for several days following new moon. Females in the Mariana Islands may be mature as small as 123 mm in SL, and males as small as 112 mm. The spawning season is December to September, with peaks from March to April. Large aggregations of silvery postlarvae settle out between March and June to shallow water on reef flats where they are often caught in seines or throw nets.

We, and surely others, have noticed that the population of *Mulloidichthys flavolineatus* in the Red Sea has only yellow caudal fin (Fig. 7), whereas in most of the Indian Ocean and in the Pacific, the caudal fin is usually gray but occasionally also yellow. This goatfish should not be confused with *M. vanicolensis* (Valenciennes, 1831), which also has a yellow caudal fin (lead fish of the three of Fig. 8), as well as yellow dorsal, anal, and pelvic fins, whereas pelvic and dorsal fins are whitish in *M. flavolineatus*. The geographic distribution of the two color morphs of *M. flavolineatus* matches the distribution of two distinct mitochondrial lineages with 1.7% divergence at the cytochrome b (*cytb*) gene (Fernandez-Silva et al. 2015).

Figure 5. School of *Mulloidichthys flavolineatus flavolineatus* in Maui, Hawai'i. Photo by John E. Randall.

Figure 6. School of *Mulloidichthys flavolineatus flavolineatus* in Maui, Hawai'i resting on the bottom. Photo by John E. Randall.

Figure 7. School of *Mulloidichthys flavolineatus flavicaudus* subsp. n. at Eilat, Gulf of Aqaba. Photo by John E. Randall.

Figure 8. Underwater photography of two *Mulloidichthys flavolineatus flavicaudus* subsp. n. and one *M. vanicolensis* (left) in the Saudi Red Sea off Jeddah. Photo by Hagen Schmid.

Figure 9. *Mulloidichthys ayliffe* with one individual of *M. flavolineatus flavicaudus* subsp. n. at Socotra. Photo by Hajnalka Kovacs.

Figure 10. *Mulloidichthys flavolineatus flavicaudus* subsp. n. in Fahal Island in the Gulf of Oman. Photo by Richard Field.

Figure 11. School of *Mulloidichthys flavolineatus* in Oman, some fish with white caudal fins and some fish with yellow caudal fins. Photo by John E. Randall.

The caudal fin continues to be yellow from the Red Sea into the Gulf of Aden and Socotra, as shown by Fig. 9, where a few individuals of *Mulloidichthys flavolineatus* have mixed with a school of *M. ayliffe*. Uiblein (2011) described the latter in a review of the species of *Mulloidichthys* of the Western Indian Ocean. It mimics and often schools with the snapper *Lutjanus kasmira*. It is an amazing example of parallel evolution with *M. mimicus* Randall & Guézé, 1980 of the Marquesas Islands and Line Islands in the Central Pacific, which closely mimics the stripe pattern of *L. kasmira* and forms aggregations with it.

Across the Arabian Sea to the south coast of Oman aggregations of *Mulloidichthys flavolineatus* in Oman and Maldives include many individuals with yellowish caudal fin mixed with a few gray-tailed and yellow-tailed fish (Figs 10, 11 and 12). Elsewhere, caudal fins are predominantly white or light gray, although we have observed that the color of the caudal fin in individuals from South Africa to French Polynesia and Hawaiian Islands may vary from hyaline gray (predominantly) to yellow (occasionally).

Figure 12. School of *Mulloidichthys flavolineatus* in South Ari Atoll in the Maldives, with some fish with whitish caudal fins in the background and other fish with caudal fins with different shades of yellow in the front. Photo by Rainer Kretzberg.

Methods

Measurements and counts

Type specimens were deposited at the Bernice P. Bishop Museum, Honolulu, HI, U.S.A. (BPBM); the California Academy of Sciences, San Francisco, CA, U.S.A. (CAS); the Museum of the Hebrew University of Jerusalem, Israel (HUJ); the Senckenberg Museum, Frankfurt, Germany (SMF); and the U.S. National Museum of Natural History (NMNH). These were the primary sources of goatfish specimens examined in this study.

Lateral-line counts begin with the first pored scale completely posterior to the upper end of the gill opening and end at the base of the caudal fin (three pored scales continue onto the caudal fin). Counts of gill rakers were made on the first gill arch; they include all rudiments.

Lengths of specimens are given as standard length (SL), measured from the median anterior point of the upper lip to the base of the caudal fin (posterior end of the hypural plate); body depth is taken vertically from the base of the first dorsal-fin spine where it emerges from the body (not the internal base); body width is the maximum

width measured just posterior to the gill openings; head length (HL) from the front of the upper lip to the posterior end of the opercular membrane, and snout length from the same anterior point to the nearest fleshy edge of the orbit; orbit diameter is the greatest fleshy diameter, and interorbital width the least fleshy width; upper-jaw length is taken from the front of the upper lip to the end of the maxilla; barbel length is the maximum straight length; caudal-peduncle depth is the least depth, and caudal-peduncle length the horizontal distance between verticals at the rear base of the anal fin and the caudal-fin base; length of fin spines and rays of the dorsal and anal fins are measured from where they emerge from the body to their tip; caudal-fin length is the horizontal length from the posterior end of the hypural plate to a vertical at the tip of the longest ray; caudal concavity is the horizontal distance between verticals at the tips of the shortest and longest rays; pectoral-fin length is measured from the base of the uppermost ray; pelvic-fin length is measured from the base of the pelvic spine to the tip of the longest soft ray. Proportional measurements in the text are rounded to the nearest 0.05.

Only meristic characters and measurements that vary between *M. f. flavolineatus* and *M. f. flavicaudus* subsp. n. were applied in the diagnoses and comparisons: the number of gill rakers, lateral-line scale counts, barbel length, eye diameter and head length. We also compared the length of the pectoral and pelvic fins, but these did not show differences between *M. f. flavolineatus* and *M. f. flavicaudus* subsp. n.

Because goatfishes present allometric changes in body form (Uiblein and Heemstra 2010) during ontogeny, in the current study we only included fish > 73 mm and <288 mm.

Genetic methods

During a previous phylogeographic survey of *M. flavolineatus* we obtained *cytb* sequences from 217 specimens sampled at nineteen sites throughout the Red Sea, the Arabian Sea, the Indian Ocean and the Pacific Ocean. To elucidate phylogenetic relationships we sequenced an additional fragment of the mitochondrial genome, the ATP synthetase 8 and ATP synthetase 6 (ATPase-8 and ATPase-6) regions, from individuals representative of the *cytb* diversity. We also sequenced an individual of *M. vanicolensis* and one of *M. pfluegeri* to use as outgroups. Briefly, DNA was extracted from fin clips and Polymerase Chain Reactions (PCR) were carried out using the primers L8331 (5'-AAA GCR TYR GCC TTT TAA GC-3') and H9236 (5'-GTT AGT GGT CAK GGG CTT GGR TC-3') (Meyer 1993). We carried out PCRs in a 15 µl volume containing 5 to 20 ng of template DNA, 0.1 µM of each primer and 5 µl of BioMix Red™ (Bioline Inc., Springfield, NJ, U.S.A.) in deionized water. PCRs were carried out with an initial denaturation step of 95 °C for 4 min, 35 cycles of denaturation (95 °C for 30 s), annealing (52 °C for 30 s) and extension (72 °C for 45 s), followed by a final extension step of 72 °C for 10 min. To clean PCR products we treated them

with 0.75 units of Exonuclease I and 0.5 units of Fast Alkaline Phosphatase (ExoFAP; Thermo Fisher Scientific, Waltham, MA, U.S.A.) per 7.5 µL of PCR product, at 37 °C for 15 min, followed by deactivation at 85 °C for 15 min. We cleaned all PCR products using ExoSAP (USB, Cleveland, Ohio) and then sequenced them in the forward direction (and reverse direction, where appropriate) using a genetic analyzer ABI 3130XL (Applied Biosystems, Foster City, California) at the Hawai'i Institute of Marine Biology EPSCoR Sequencing Facility. The *ATPase-8* and *ATPase-6* sequences were aligned, edited, and trimmed to a common length using GENEIOUS PRO vers. 4.8.4 (Drummond et al. 2012), and the sequences were deposited in Genbank (accession numbers: KT960949–KT960972). We concatenated this alignment with the *cytb* sequences from the same specimens and applied Bayesian methods for phylogenetic reconstruction in BEAST *vers.* 1.8.0 (Drummond et al. 2012), based on Yule models of speciation and a strict molecular clock (1% per myr as per Bowen et al. 2001). We also applied Neighbor-joining distance and Maximum-Likelihood tree-building methods for phylogenetic reconstruction using MEGA (Tamura et al. 2013) and the RaxML web server at http://embnet.vital-it.ch/raxml-bb/ (Varsamos et al. 2005), respectively. Support for the trees was evaluated by bootstrapping over 1,000 replicates.

Data resources

The data underpinning the analysis reported in this paper are deposited in the Dryad Data Repository at http://dx.doi.org/10.5061/dryad.f54m5

Results

***Mulloidichthys flavolineatus flavicaudus* Fernandez-Silva & Randall, subsp. n.**
http://zoobank.org/779C9D55-B037-4548-A717-F5C33BC1ACD5
Figures 4, 7–10, 13 (holotype), 14; Tables 1–4.
Yellowtail Goatfish

Mulloides flavolineatus (non Lacepède, 1801): Dor 1984: 161 (Red Sea listed); Ben-Tuvia and Kissil 1988: 3 (Gulf of Aqaba); Goren and Dor 1994: 44 (Red Sea listed); Debelius 1998: 112 (Egypt).

Mulloidichthys flavolineatus (non Lacepède, 1801): Randall 1995: 239 (Oman); Khalaf and Disi 1997: 117 (Jordan); Zajonz et al. 2000: 155 (Socotra); Lieske and Myers 2004: 123 (Mangrove Bay, El Quseir); Golani and Bogorodsky 2010: 35 (Red Sea listed); Field 2013: 47 (Gulf of Oman).

Holotype. SMF 35486 [ex SMF 24824], 142 mm SL, Red Sea, Sudan, Sanganeb Atoll (19°39'N; 37°14'E), April 1991, coll. F. Krupp, V. Neumann & T. Paulus.

Figure 13. Preserved holotype of *Mulloidichthys flavolineatus flavicaudus* subsp. n., SMF 35486 [ex SMF 24824], 142 mm SL, Sanganeb Atoll, Sudan, Red Sea. Photo by John E. Randall.

Paratypes. SMF 24818, 6: 106–125 mm SL, Red Sea, Sudan, Sanganeb Atoll (19°39'N; 37°14'E), April 1991, coll. F. Krupp, V. Neumann & T. Paulus; USNM 221124, 181 mm SL, Red Sea, Egypt, off "ancient site" of Koseir along reef in cove ca. 5 km north of Koseir town (26°8'N; 34°16'E), 8 Jan 1965, coll. H. A. Fehlmann; HUJ 9985, 3: 73–93.5 mm SL, Red Sea, Gulf of Suez, El Kura, a few km south of Dahab (28°12'04"N; 34°28'49"E); CAS 237352, 4: 107–147 mm SL, Red Sea, Saudi Arabia, Thuwal, inner Fesar (22°13'50"N; 39°01'43"E), 18 April 2014, coll. P. Saenz-Agudelo; BPBM 41246 [ex CAS 237352], 102 mm SL, same data as preceding.

Non-type material. Gulf of Suez: HUJ 5635, 107 mm SL, A-Tur. **Gulf of Aqaba:** BPBM 19843, 4: 114–122 mm SL, Nuweiba; CAS 58876, 252 mm SL, Eilat; CAS 206715, 133 mm SL, Eilat; CAS 206726, 198 mm SL, Eilat; CAS 206736, 167 mm SL, Eilat; HUJ 5905, 2: 128–144 mm SL, Eilat; HUJ 20216, 4: 107–110 mm SL (poor condition), Eilat; HUJ 8315, 169 mm SL, Ras Muhammed; HUJ 8543, 108 mm SL, Ras Muhammed; HUJ 8658, 2: 164–235 mm SL, Nabq; HUJ 8645, 3: 159–177 mm SL, Sanafir Island; HUJ 11663, 109 mm SL, Eilat; HUJ 8642, 243 mm SL, Nabek. **Sudan:** SMF 24821, 203 mm SL, Sanganeb Atoll; SMF 24823, 13: 97.5–161 mm SL, Sanganeb Atoll.

Diagnosis. Body elongate, the depth at first dorsal-fin origin 4.1–4.5 in SL; head moderately compressed, the length 3.0–3.3 in SL; snout long, slightly blunt. Barbels usually not reaching a vertical at posterior margin of preopercle, their length 4.1-5.0 in SL. Eye diameter 10.3–13.5 in SL. Pectoral-fin rays 16–18. Gill-raker counts 25–28 (usually 26 or 27); lateral-line scales 37–38. Caudal fin yellowish to yellow. [Diagnosis based on the Red Sea proper population, i.e. excluding the Gulf of Aqaba, see remarks].

Description. Meristics are provided in Tables 1 & 2 and measurements as % of SL in Table 4 and Fig. 14. Below, morphometric ratios are given as ratios of SL for the holotype and in parentheses for selected paratypes (n=7), except where indicated.

Table 1. Lateral-line scale counts of *M. flavolineatus* subspp. In bold, counts for the holotype of *M. f. flavicaudus* subsp. n.

		37	**38**	**39**	**40**	**mean**
M. f. flavicaudus subsp. n.	Gulf of Aqaba	12	10	1		37.5
	Red Sea off Sudan & Saudi Arabia	**18**	5			37.2
†	Maldives	2	4			37.7
M. f. flavolineatus	Islands of Western Indian Ocean ‡	5	5	4		37.9
	East Indies §	4	24	5		38.0
	Islands of Oceania (except Hawai'i) |	3	26	7		38.1
	Hawaiian Islands & Johnston Atoll	2	16	6	1	38.2

† Both subspecies may overlap and interbreed in Maldives
‡ Chagos Archipelago and Mauritius
§ Indonesia, Papua New Guinea, Philippines and Solomon Islands
| Wake, Minami-Tori Shima, Mariana Islands, Marquesas Islands, Phoenix Islands, Samoa Islands and Rapa

Table 2. Total gill-raker counts of *M. flavolineatus.* In bold, counts for the holotype of *M. f. flavicaudus* subsp. n.

		25	**26**	**27**	**28**	**29**	**30**	**mean**
M. f. flavicaudus subsp. n.	Gulf of Aqaba	3	6	8	2	4		26.9
	Red Sea off Sudan & Saudi Arabia	4	**5**	11	2			26.5
†	Maldives		1	3	2	0		27.2
M. f. flavolineatus	Islands of Western Indian Ocean ‡			4	2	2		27.8
	East Indies §		3	11	12	7		27.7
	Islands of Oceania (except Hawai'i) |			12	18	11	3	28.4
	Hawaiian Islands & Johnston Atoll			2	3	10	3	28.8

† Both subspecies may overlap and interbreed in Maldives
‡ Chagos Archipelago and Mauritius
§ Indonesia, Papua New Guinea, Philippines and Solomon Islands
| Wake, Minami-Tori Shima, Mariana Islands, Marquesas Islands, Phoenix Islands, Samoa Islands and Rapa

Body elongate, its depth at first dorsal-fin origin 4.1 (4.2–4.5), and maximum width 6.7 (6.5–7.3), head length 3.2 (3.0–3.3, n=27), snout length 6.9 (6.8–7.7), orbit diameter 13.0 (10.3–13.5, n=27), barbel length 4.5 (4.2–5.1, n=27), caudal-peduncle length 4.8 (4.6–5.1), caudal-peduncle depth 11.6 (11.4–12.0), pelvic-fin length 4.9 (4.7–5.3), pectoral-fin length 4.8 (4.7–5.1), longest anal ray 7.5 (7.2–7.7), longest dorsal spine 4.8 (4.6–5.1), longest dorsal ray 7.2 (7.2–7.8).

Mouth small, maxilla not reaching a vertical at front of orbit, upper-jaw length 12.3 (12.2–13.9) in SL; jaws with small conical teeth, in two rows with teeth more irregularly placed between both rows; no teeth on the vomer and palatines; anterior nostril small, elliptical, two-thirds eye diameter in front of eye; posterior nostril small, elliptical, at dorsoanterior corner of orbit; opercular spine flat, at mid-eye height.

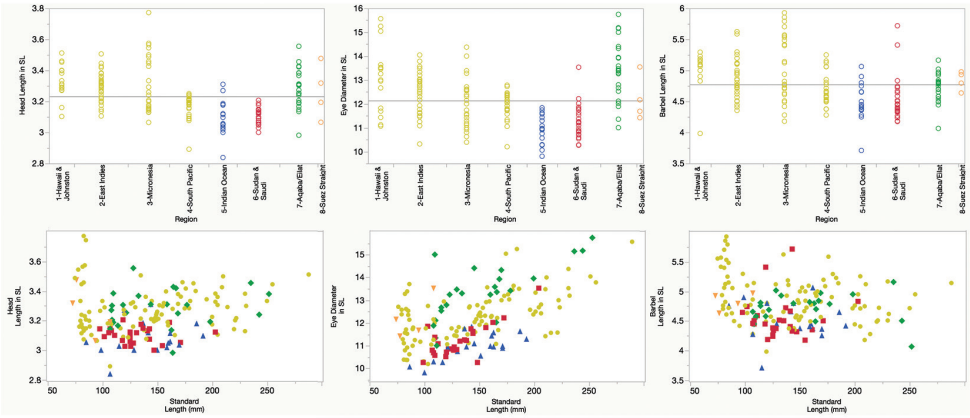

Figure 14. Comparison of head length, eye diameter, and barbel length in SL among regions and against SL in *Mulloidichthys flavolineatus flavolineatus* and *M. f. flavicaudus* subsp. n. Below, measurements against SL. These colors identify the region of origin of each individual following the scheme in the upper panel. These are the same data as in Table 3.

Table 3. Comparison of Head Length, Eye Diameter, and Barbel Length in subspecies of *M. flavolineatus*. Ranges and mean values (in brackets) are given for each ratio.

	Locality	Standard length (mm) and number of specimens	Head length in standard length	Eye diameter in standard length	Barbel length in standard length
M. f. flavicaudus subsp. n.	Gulf of Aqaba	107–252 (n=23)	3.0–3.6 (3.3)	11.0–15.8 (13.4)	4.1–5.2 (4.7)
	Red Sea ‡	97.5–203 (n=28)	3.0–3.2 (3.1)	10.2–13.5 (11.3)	4.2–4.8 (4.5)
†	Maldives	85.5–144 (n=6)	2.8–3.3 (3.2)	10.1–11.8 (10.7)	3.7–4.8 (4.4)
M. f. flavolineatus	Indian Ocean §	120–192 (n=12)	3.0–3.3 (3.1)	10.5–11.7 (11.0)	4.2–5.1 (4.5)
	East Indies |	98–255 (n=34)	3.1–3.5 (3.3)	10.3–14.0 (12.5)	4.3–5.6 (4.9)
	Micronesia ¶	75–230 (n=26)	3.1–3.8 (3.3)	10.4–14.4 (11.9)	4.2–5.9 (5.0)
	South Pacific #	81–198 (n=26)	2.9–3.4 (3.2)	10.2–12.8 (11.9)	4.3–5.2 (4.7)
	Hawaiian Is. ††	83–288 (n=16)	3.1–3.7 (3.3)	10.4–15.6 (12.9)	4.0–6.0 (5.1)

† Both subspecies may overlap and interbreed in Maldives
‡ Off Sudan and Saudi Arabia
§ Chagos Archipelago and Mauritius
| Indonesia, Papua New Guinea, Philippines, and Solomon Islands
¶ Wake, Minami-Tori Shima and Mariana Islands
# Marquesas Islands, Phoenix Islands, Samoa Islands and Rapa
†† Including Johnston Atoll

Scales very finely ctenoid; head fully scaled; scales on the base of caudal fin, other fins without scales; dorsal fin behind the vertical at fourth lateral line scale, origin of second dorsal above 18th (17th in some paratypes) scale. Pored scales on lateral line with many branching tubules.

Table 4. Proportional measurements of type specimens of *Mulloidichthys flavolineatus flavicaudus* subsp. n. and of comparative material of *M. f. flavolineatus* as percentages of the standard length.

	***M. f. flavicaudus* subsp. n.**								***M. f. flavolineatus***			
	Sudan								**Mauritius**			**Johnston Atoll**
	Holotype								**Neotype**			
	SMF 35486	SMF 24818-1	SMF 24818-2	SMF 24818-3	SMF 24818-4	SMF 24818-5	SMF 24818-6	USNM 221124	BPBM 20135	BPBM 41252–1	BPBM 41252–2	BPBM 7520
Standard length (mm)	142	125	120	118	111	108	106	181	162	120	135	166
Body depth	24.3	23.1	23.2	24.1	22.8	22.1	22.2	22.1	22.2	22.1	22.0	25.1
Body width	14.9	14.9	15.3	14.4	14.5	13.6	13.9	15.1	13.1	12.3	9.6	14.2
Head length	31.3	33.2	32.6	32.3	31.9	32.1	30.9	32.1	30.6	31.5	32.2	33.2
Snout length	14.4	14.4	13.9	13.5	14.7	13.2	13	14.4	14.5	13.1	13.5	15.2
Orbit diameter	7.7	9.3	9.5	9.0	8.8	9.2	7.7	8.6	9.2	8.5	8.6	9.3
Interorbital width	7.8	7.5	7.2	7.4	7.8	7.0	7.0	7.5	9.0	7.6	6.9	8.0
Upper-jaw length	8.1	7.0	8.0	8.0	7.8	7.2	7.6	8.2	8.3	8.4	8.1	7.8
Barbel length	22.3	23.4	24.0	22.5	21.8	22.5	–	21.0	19.8	20.8	22.2	20.0
Caudal-peduncle least depth	8.6	8.6	8.3	8.6	8.5	8.4	8.7	8.8	9.2	8.8	9.2	9.2
Caudal-peduncle length	24.3	22.4	20.1	23.0	23.1	24.9	21.8	25.2	23.3	30.5	20.3	23.4
Snout to origin of first dorsal fin	41.1	39.2	39.6	39.3	40.4	37.3	27.3	38.6	40.6	38.9	40.1	42.0
Snout to origin of second dorsal fin	68.1	67.0	66.6	67.3	66.5	63.8	65.1	65.5	65.9	62.8	63.2	68.8
Preanal-fin length	69.4	65.6	67.5	68.9	66.5	66.4	65.7	67.7	67.0	64.8	6.7	67.4
Prepelvic-fin length	33.2	33.2	35.2	34.9	32.8	34.3	33.0	33.5	32.9	31.3	31.8	32.7
Second dorsal-fin base	12.8	12.5	11.7	12.3	12.2	11.5	12.1	13.1	11.8	10.8	11.1	12.4
Anal-fin base	9.9	10.5	9.1	10.6	9.6	9.9	10.7	9.7	9.0	10.4	9.4	10.4
First dorsal-fin base	16.7	18.8	19.9	21.8	17.6	16.6	17.4	17.2	15.3	17.3	16.0	19.0

Pectoral-fin base	4.9	5.1	5.2	5.2	5.7	4.7	4.9	5.6	5.0	5.2	4.8	5.4
Longest dorsal spine	20.8	19.9	21.3	21.8	19.7	18.7	19.0	21.3	20.6	20.2	21.2	20.1
Longest dorsal ray	14.6	14.1	14.1	13.4	14.3	14.5	13.2	13.4	14.3	15.4	15.2	14.3
Longest anal ray	13.9	13.4	13.5	13.7	13.2	12.9	13.6	13.8	13.3	14.7	14.4	13.7
Caudal-fin length	28.1	27.1	26.8	–	26.6	24.9	25.0	–	26.3	27.5	28.6	25.9
Caudal concavity	19.1	18.1	19.1	–	16.4	16.9	16.5	–	16.7	19.7	20.6	16.8
Pectoral-fin length	20.8	20.3	20.6	21.5	21.2	19.9	20.7	19.8	20.0	21.3	–	21.7
Pelvic-fin length	20.6	20.3	20.4	21.0	20.1	19.0	21.1	19.8	20.3	22.1	21.1	21.0

* Two specimens of BPBM 41252 (103 and 111 mm), from Mauritius were damaged and not included in the table.

Color. Color in life silvery white to yellowish, slightly darker over lateral line; margin of each scale on upper half of body darker than scale. Yellow stripe on side of body at level of eye, from posterior margin of orbit to caudal-fin base, bordered by a narrow whitish stripe (stripe sometimes slightly blue); the stripe usually containing a black spot above posterior part of pectoral fins (under the first dorsal fin), sometimes faint due to fading, stripe anterior to spot occasionally indistinct; barbels white; dorsal fins usually transparent, sometimes first dorsal fin with yellowish tinge; pectoral, anal, and pelvic fins whitish, translucent; caudal fin yellowish or yellow. Color when fresh often pink and all fins yellow. Uniformly creamy white in preservative.

Etymology. *Mulloidichthys f. flavicaudus* subsp. n. is named in reference to the yellow color of the caudal fin, in contrast to the whitish gray color of the caudal fin of *M. f. flavolineatus*.

Distribution. *Mulloidichthys f. flavicaudus* subsp. n. is restricted to the NW Indian Ocean biogeographic province, where it ranges from various locations in the Red Sea (including the Gulf of Aqaba), the Gulf of Tadjoura, the Gulf of Aden, and Socotra (Fig. 9). *M. f. flavicaudus* subsp. n. has extended its range to Oman (Fig. 11) and probably to the Maldives (Fig. 12), where it has encountered the western distribution of *M. f. flavolineatus*. Underwater photographs of fish with yellow and gray caudal fins suggest overlap and interbreeding by the two subspecies. Carpenter et al. (1997) included *M. flavolineatus* in their catalog of fishes of the Arabian Gulf. They did not cite any voucher specimens, and the photo they used is from Mauritius.

Remarks. The population of *M. f. flavicaudus* subsp. n. in the Gulf of Aqaba differs from that in the Red Sea proper by having smaller eyes (11.0–15.8 in SL) and shorter head (3.0–3.6) (Tables 1, 2 and 3). It occasionally also has higher lateral-line scales counts (37–38, occasionally 39) and higher gill-raker counts (25–29).

Comparisons. *Mulloidichthys f. flavicaudus* subsp. n. differs from its nominal subspecies *M. f. flavolineatus* in having 25–28 (usually 26 or 27) gill-raker counts (26–30, usually 27–29, in *M. f. flavolineatus*), usually 37–38 lateral-line scales (37–40 in *M. f. flavolineatus*) and a yellow caudal fin (white to light gray in *M. f. flavolineatus*). Also, the eyes are smaller in *M. f. flavicaudus* subsp. n. (10.3–13.5 in SL) than in *M. f. flavolineatus* (9.8–15.6 in SL).

***Mulloidichthys flavolineatus flavolineatus* (Lacepède, 1801)**

Yellowstripe Goatfish

Figures 1, 2, 3, 5, 6, 14; Tables 1–4

Mullus flavolineatus Lacepède, 1801: 384, 406 (locality unknown, no types known).

Mulloidichthys flavolineatus (Lacepède 1801): Randall and Anderson 1993: 20 (Maldives); Randall et al. 1997: 208 (Great Barrier Reef); Kuiter 1998: 117 (Maldives: in part, upper photo); Anderson 2005: 57 (Maldives); Allen et al. 2007: 122

(Christmas Island); Okamura and Okamoto 1997: 373 (Japan); Randall 2007: 260 (Hawaiian Islands); Myers 1999: 159, Pl. 74H (Micronesia); Matsunuma et al. 2011: 142 (Malaysia); Uiblein 2011: 59 & 69, Pl. 1 (description, color images); Allen and Erdmann 2012: 504 (Philippines).

Neotype. BPBM 20135, 162 mm SL, Indian Ocean, Mauritius, East Coast, Oyster Bay (19°43'S; 63°21'E), 1 November 1973, coll. J.E. Randall.

Non-type material. Hawaiian Islands: BPBM 28726, 83 mm SL, Kona Coast, South Kohala; BPBM 4087, 288 mm SL, Laysan; BPBM 4086, 180 mm SL, Laysan; BPBM 4088, 2: 139–230 mm SL, Lisiansky; BPBM 25457, 130 mm SL, Oʻahu, Waiʻanae coast; BPBM 25674, 175 mm SL, Oʻahu; BPBM 1749, 183 mm SL, Oʻahu; BPBM 1750, 173 mm SL, Oʻahu; BPBM 15308, 152 mm SL, Midway Atoll; BPBM 25517, 119 mm SL, Midway Atoll; USNM 147073, 158 mm SL, Midway Atoll. **Johnston Atoll**: BPBM 4090, 85 mm SL; BPBM 4091, 93 mm SL; BPBM 7520, 166 mm SL; **Philippines**: USNM 327877, 107 mm SL, Sorsogon, Gubat Bay; USNM 405724, 209 mm SL, W Luzon, Port Matalvi; USNM 147062, 222 mm SL, Mindoro, Varadero Bay; USNM 147066, 169 mm SL, W Luzon, Zambales; USNM 322272, 3: 138-155 mm SL, Babuyan, Maybag Island; USNM 147069, 2: 171–176 mm SL, Batangas, Maricaban; USNM 84231, 128 mm SL, Mindanao, Zamboanga; USNM 84232, 139 mm SL, Mindanao, Zamboanga; USNM 147070, 164 mm SL, Palawan, Candaraman; USNM 147072, 145 mm SL, Tulayan Island, Jolo; USNM 145294, 2: 98–100 mm SL; USNM 147065, 231 mm SL, Sulu, Siasi Island; USNM 147076, 135 mm SL, Sulu, Simaluc Island, Tawi Tawi. **Indonesia**: USNM 147067, 221 mm SL, Moluccas, Bouru Island; USNM 147064, 3: 195–205 mm SL, Moluccas, Makian I; USNM 405723, 200 mm SL, Moluccas, Makian Island; USNM 267514, 2: 102–126 mm SL, Mentawai Islands, Pulau Siburu; USNM 267503, 2: 155–156 mm SL, Mentawai Islands, Pulau Siburu; USNM 147058, 203 mm SL, Sulawesi, Talisse Island; USNM 87989, 255 mm SL, Sumatra, Poeloe Toekus; USNM 75887, 250 mm SL, Borneo, Tandjoeng, Setebah. **Cocos-Keeling**: SU 35630, 200 mm SL, Cocos-Keeling I. **Papua New Guinea**: USNM 267499, 203 mm SL, Trobriand Kuia Islands; USNM 267515, 2: 116–120 SL, New Britain, Rabaul. **Solomon Islands**: USNM 382371, 205 mm SL, Santa Cruz Islands. **Micronesia:** BPBM 77, 8: 75–230 mm SL, Guam; BPBM 4089, 3: 209–220 mm SL, Wake Island; BPBM 24628, 12: 79–160 mm SL, Chuuk, Puluwat Atoll. **Japan**: BPBM 7086, 2: 88–108 mm SL, Minami-Tori Shima; BPBM 7087, 210 mm SL, Minami-Tori Shima. **South Pacific:** BPBM 27868, 5: 81–119 mm SL, Samoan Islands, Tutuila Island; BPBM 27906, 3: 84–107 mm SL, Samoan Islands, Tutuila Island; BPBM 15299, 16: 81–159 mm SL, Phoenix Islands, Orona Atoll; BPBM 12937, 165 mm SL, Rapa; BPBM 2136, 198 mm SL, Marquesas Islands, Nuku Hiva. **Western Indian Ocean**: USNM 229036, 9: 129–192 mm SL, Chagos Archipelago, Salomon Atoll; CAS 237312, 2: 137–144 mm SL, Maldives, Faafu Atoll; BPBM 34673, 2: 107–115 mm SL, Maldives, N Malé Atoll; CAS 35383, 2: 85.5–142 mm SL, Maldives, Malé Atoll; BPBM 41252, 2: 120–135 mm SL, Mauritius, Oyster Bay.

Diagnosis. Body elongate, the depth at first dorsal-fin origin 4.0–4.6 in SL; head moderately compressed, the length 2.9–3.8 in SL; snout long, slightly blunt anteriorly. Barbels usually not reaching a vertical at posterior margin of preopercle, their length 3.7–6.0 in SL. Eye diameter 10.1–15.6 in SL. Pectoral-fin rays 16–18. Gill-raker counts 27–29 (rarely 26 or 30); lateral-line scales 37–40 (usually 38). Caudal fin varying from usually white or light gray to occasionally yellowish or yellow.

Color. Silvery white to yellowish, slightly darker over lateral line, margins of each scale on upper half of body darker than scale. Yellow stripe on side of body at level of eye, beginning from posterior margin of orbit and ending at caudal-fin base, bordered by two whitish narrow stripes (sometimes slightly blue); the stripe usually containing a black spot above posterior part of pectoral fins (under the first dorsal fin), sometimes faint due to fading, stripe anterior to spot occasionally indistinct; barbels white; dorsal fins usually transparent, sometimes first dorsal fin with yellowish tinge; pectoral, anal, and pelvic fins whitish, translucent; caudal fin varying from usually white or light gray to occasionally yellowish or yellow. Sometimes body color pattern of broad irregular red-brown bars, especially at night. When fresh, body color can turn pink and all fins yellow. Uniformly creamy white in preservative.

Distribution. *Mulloidichthys f. flavolineatus* is wide-ranging from East Africa north to the Maldives and Chagos Archipelago and east to the Hawaiian, Marquesas and Pitcairn Islands, north to the Ryukyu and Bonin Islands and south to Lord Howe Island, New Caledonia and Rapa Island (Randall 2002, Uiblein 2011) (Fig. 15).

Genetics. The parsimony-based haplotype networks constructed with mtDNA *cytb* sequences from 217 *M. flavolineatus* specimens revealed a separation between individuals from the NW Indian Ocean (including the Red Sea, the Gulf of Aden and Oman) and individuals in the rest of the Indian Ocean and the Pacific Ocean (Fig. 16). Corrected genetic distance was 1.7%, with seven diagnostic mutations (Fernandez-Silva et al. 2015).

We obtained a concatenated alignment of a 715-bp segment of the *cytb* gene and a 731-bp segment of the *ATPase-8* and *ATPase-6* genes of the mitochondrial genome

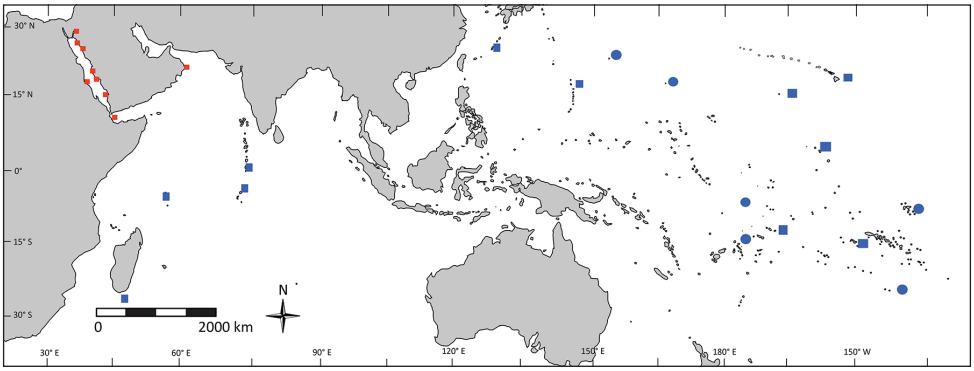

Figure 15. Distribution map of *Mulloidichthys flavolineatus* surveyed in this study. Red symbols denote locations of specimens of *M. f. flavicaudus* subsp. n. and blue symbols denote locations of specimens of *M. f. flavolineatus*. Squares indicate locations included in the genetic surveys. Circles indicate locations of specimens for which only morphological analyses were carried out.

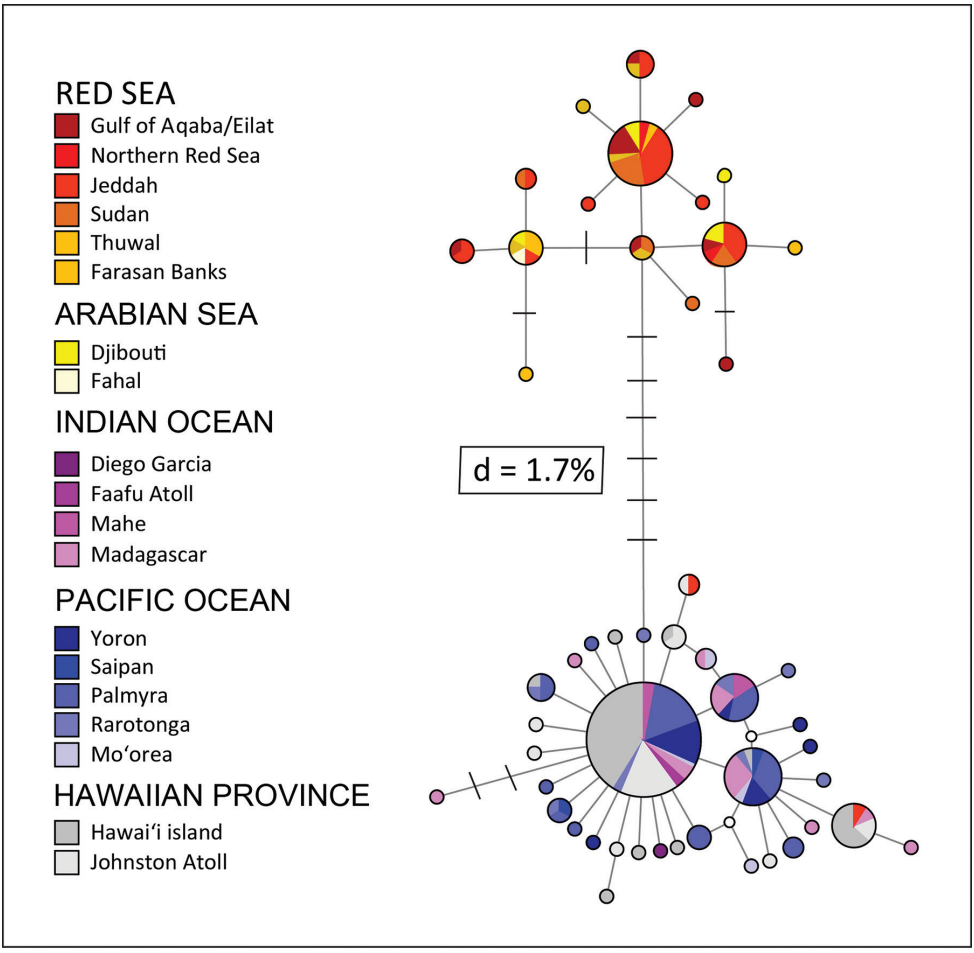

Figure 16. Median-joining haplotype network based on mitochondrial *cytochrome b* sequence data (715 bp) from 217 *Mulloidichthys flavolineatus* individuals sampled across the Red Sea, Arabian Sea, Indian Ocean and Pacific Ocean. Each circle represents a haplotype, with size proportional to its total frequency. Branches separated by black crossbars represent a single nucleotide change, whereas open circles indicate unsampled haplotypes; colors indicate collection location as in the embedded key. The network depicts two distinct clades separated by seven mutational steps (corrected sequence divergence, d = 1.7%; Kimura 1980) (From Fernandez-Silva et al. 2015).

from seven individuals from the Red Sea (Jeddah) and five from the Pacific (Hawai'i and Okinawa). Phylogenetic reconstructions based on Bayesian inference (Fig. 17) revealed a genetic break and the presence of two well-supported monophyletic clades (posterior probability = 1): one with sequences from the Red Sea and one with the haplotypes from the Pacific. Reconstructions based on the Maximum-Likelihood and Neighbor-Joining methods were in agreement with this topology but clades had lower statistical support (results not shown).

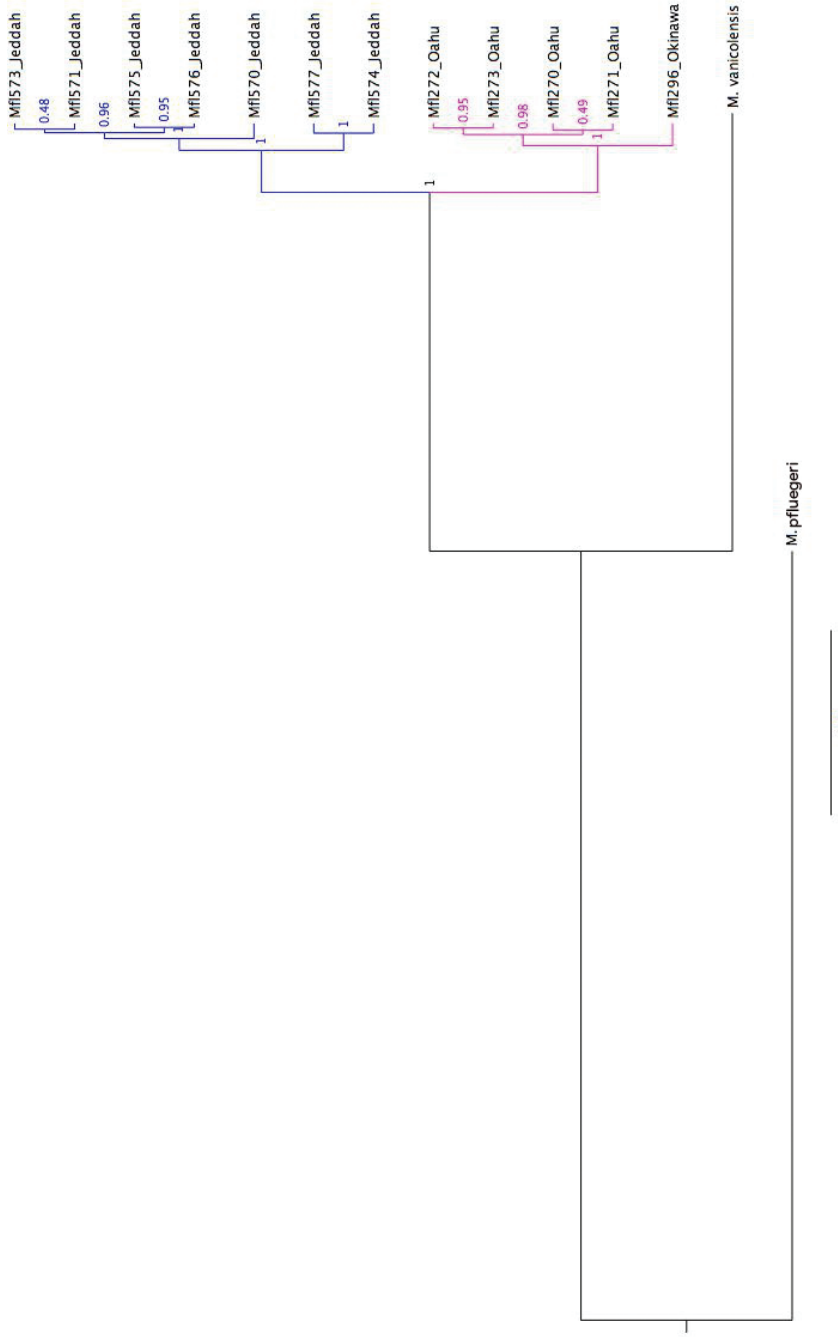

Figure 17. Bayesian-inference based phylogenetic tree showing relationships among mtDNA concatenated haplotypes of segments of the *cytb* and *ATPase-8* & *ATPase-6* genes from seven individuals of *Mulloidichthys flavolineatus flavicaudus* subsp. n. from the Red Sea (Jeddah), five individuals of *M. f. flavolineatus* from the Pacific (O'ahu in Hawai'i and Okinawa) and two *Mulloidichthys* spp. as outgroups. The nodes show posterior probabilities. Branch lengths are according to estimated divergence time (note that the branch leading to *M. pfluegeri* was reduced by 50%).

Discussion

Higher gill-raker and lateral-line counts, smaller eyes and stable yellow coloration of the caudal fin in *M. flavolineatus* from the Red Sea are characters in alignment with the genetic isolation of a mitochondrial lineage in the NW Indian Ocean biogeographic province (as per Kulbicki et al. 2013) and support the subspecies designation of *M. f. flavicaudus* subsp. n.

Some ichthyologists, notably Gill (1999), have questioned the validity of subspecies in marine fishes, especially in reference to wide-ranging Indo-Pacific species. One could argue that the existence of subspecies should be demonstrated by intermediates between two isolated populations before they could be labeled as subspecies. Divisions of populations into two or more populations have resulted from the change in sea level caused by the variation in the size of the polar ice caps. The Indian Ocean was isolated from the Pacific, and the Red Sea from the Indian Ocean when the ice caps were very large. We assume that the yellow-tailed population of *Mulloidichthys flavolineatus* arose as a subspecies when the Red Sea was isolated, approximately half a million years ago assuming a molecular clock of 2% divergence per million years (as per Bowen et al. 2001). This population persisted in isolation through several Pleistocene glacial cycles (Fernandez-Silva et al. 2015) and over time extended out to Socotra, Oman and possibly Maldives, where it entered into secondary contact with the Indo-Pacific population. In the second author's book *Coastal Fishes of Oman* (Randall 1995), a single individual of *M. flavolineatus* is illustrated as Figure 620. It has a yellowish caudal fin. He wrote in the brief species account, "fins whitish, the caudal fin often yellowish." The underwater photograph of *M. flavolineatus* of Fig. 11 taken on the south coast of Oman shows caudal fins varying from pale greenish gray (the green part from the sea color) to a few all yellow. This photograph suggests that the two subspecies of *M. flavolineatus* may overlap and interbreed, hypotheses to be confirmed with genetic methods. The geographic extension of the yellow-tailed subspecies in the understudied Western Indian Ocean warrants further investigation.

Notably, the age of split of the *Mulloidichthys flavolineatus* subspecies is older than the radiation that gave rise to *M. vanicolensis*, *M. mimicus*, *M. dentatus* (Gill, 1862) and *M. martinicus* (Cuvier, 1829) less than 350,000 years ago (unpublished results).

It is remarkable that individuals of *Mulloidichthys f. flavicaudus* subsp. n. from the Gulf of Aqaba have consistently smaller eyes, longer head, and longer barbels than fish from the Red Sea proper (Fig. 14). Pelvic fins are also shorter in the Gulf of Aqaba (mean length in SL = 5.17) than in the rest of the Red Sea (4.40 in SL). However, both populations extensively share *cytb* haplotypes and the analyses of haplotype frequencies do not support genetic differentiation, although this comparison is based on mitochondrial markers only (Fernandez-Silva et al. 2015). In the northern tip of the Gulf of Aqaba, *M. f. flavicaudus* was among the 11 most common species on the shallow sandy habitat, but all specimens were juveniles or subadults (maximum length: 15 cm TL) (Golani 1993; Golani and Lerner 2007). The Gulf of Aqaba has remarkably high endemism. Twenty-six species of fishes, including the goatfish *Up-*

Table 5. Endemic fishes of the Gulf of Aqaba.

Endemic fishes of the Gulf of Aqaba	Remarks
Amblyeleotris neglecta Jaafar & Randall 2009	
Cabillus nigrostigmus Kovačić & Bogorodsky 2013	Known from Sharm el Moya, close to the entrance of the Gulf of Aqaba
Callionymus profundus Fricke & Golani 2013	Deep-water species
Chromis pelloura Randall & Allen 1982	
Cirrhilabrus blatteus Springer & Randall 1974	
Evoxymetapon moricheni Fricke, Golani & Appelbaum–Golani 2014	
Gymnapogon melanogaster Gon & Golani 2002	
Gymnothorax baranesi Smith, Brokovich & Einbinder 2008	
Hetereleotris psammophila Kovačić & Bogorodsky 2014	Recently photographed at Safaga
Heteronarce bentuviai (Baranes & Randall 1989)	
Limnichthys marisrubri Fricke & Golani 2012	
Myxomyrophis longirostris Hibino, Kimura & Golani 2014	
Paragunnellichthys springeri Dawson 1970	Formally endemic to Gulf of Aqaba, known from Sharm el Moya, close to the entrance
Parascolopsis baranesi Russell & Golani 1993	
Pseudogramma megamyctera Randall & Baldwin 1997	Reported from West Papua (Allen and Erdmann 2012); a record probably represented by a similar undescribed species
Scorpaenodes steinitzi Klausewitz & Fröiland 1970	A specimen identified as *S. steinitzi* collected from Djibouti, but no voucher available for confirmation
Stalix davidsheni Klausewitz 1985	
Suculentophichthus nasus Fricke, Golani & Appelbaum–Golani 2015	
Symphysanodon disii Khalaf & Krupp 2008	
Syngnathus safina Paulus 1992	
Thamnaconus erythraeensis Bauchot & Maugé 1978	
Tomiyamichthys dorsostigma Bogorodsky, Kovačić & Randall 2011	
Upeneus davidaromi Golani 2001	
Uropterygius genie Randall & Golani 1995	Known at Ras Mohammed, close to the entrance of the Gulf of Aqaba
Uropterygius golanii McCosker & Smith 1997	Known at Ras Mohammed, close to the entrance of the Gulf of Aqaba
Vanderhorstia opercularis Randall 2007	

eneus davidaromi, are known to the Gulf of Aqaba only (Table 5). Although further research may result in range extensions for some of these fishes to the Northern Red Sea, the number of endemics is very high for an area of only 160 × 24 km. Environmental differences could explain this isolation. The Gulf of Aqaba is much deeper

(1850 m) than the Red Sea to the south, and seawater temperature is considerably lower (20–27°C) and salinity higher (40–41‰) than in the Red Sea proper (25–31°C; 37–41‰) (Oren 1962). Moreover, the Gulf may have acted as a glacial refuge for reef fauna during Pleistocene low sea level stands, when most of the Red Sea was too saline for coral reef development. Geological and paleoclimatic research suggest that during these periods the Gulf of Aqaba, owing to rainfall and fluvial intake, maintained lower salinity levels and that environmental conditions were favorable to sustain coral reefs and associated fauna (DiBattista et al. 2016). Therefore, the Gulf of Aqaba served as a refuge for marine life from the harsh marine environment to the south. Parapatric speciation processes reinforced by selection may account for the elevated endemism in the region (Golani 1993; Por 2008; Tikochinski et al. 2013).

Our range-wide phylogeographic survey of *Mulloidichthys flavolineatus* (Fernandez-Silva et al. 2015) indicated the genetic isolation of the Hawaiian population (including Johnston Atoll) from the remainder of the Indo-Pacific. Uiblein (2011) indicates that Pacific Ocean *M. flavolineatus* have shorter barbels than those in the Indian Ocean, but he includes the Hawaiian Islands with the rest of the Pacific in this study. We found the Hawaiian population has shorter barbels, shorter head, smaller eyes, higher gill-raker counts, and higher lateral-line scale counts than all other populations examined, and that there is a range of variation as we move from Hawaiʻi to other islands of Oceania, the West Indies, the Western Indian Ocean, and the Red Sea (Tables 1–3, and Fig. 14).

Fernandez-Silva et al. (2015) listed four Red Sea endemic species of Mullidae: *Parupeneus forsskali* (Fourmanoir & Guézé, 1976), *Upeneus davidaromi*, *U. niebuhri* Guézé, 1976, and *U. pori* Ben-Tuvia & Golani, 1989, but inclusion of the latter to the Red Sea endemics is a mistake, as this species is also reported from Oman, Madagascar and South Africa.

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