

A New Species in the *Marphysa sanguinea* Complex (Annelida, Eunicidae) from Hong Kong

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Zhi Wang, Yanjie Zhang, and Jian-Wen Qiu (2018) *Marphysa hongkongensa* n. sp. (Annelida: Eunicidae) is described based on samples from the intertidal zone of Tolo Harbour, Hong Kong. This new species belongs to the *Marphysa sanguinea* species complex. It is characterized by a subacicular hook from chaetiger 26-58 to the posterior end, branchiae with up to 5-10 filaments from chaetiger 14-35 to the posterior end, and four types of pectinate chaetae. Molecular analyses indicated that the cytochrome oxidase c subunit I gene and 16S RNA gene of *Marphysa hongkongensa* diverged from the corresponding sequence of the closest related species of *Marphysa* in GenBank by 19.5% and 12.1%, respectively. An identification key is provided for species in the *Marphysa sanguinea* complex along the Chinese coast.

Key words: Bait worm, Pectinate chaetae, Phylogeny, Polychaete, Tolo Harbour.

BACKGROUND

With 83 recognized species, *Marphysa* Quatrefages, 1866 is a large genus in the family Eunicidae (Polychaeta: Eunicida) (Read and Fauchald 2018). Species in this genus commonly inhabit intertidal shores, and are often used as bait in recreational fishing (Glasby and Hutchings 2010; Cole et al. 2018). *Marphysa* is characterized by the presence of three central antennae and two lateral palps (Glasby and Hutchings 2010), notopodial branchiae and the absence of peristomial cirri. Fauchald (1970) divided this genus into four groups according to the type of compound chaetae: Mossambica, with no compound chaetae (Group A); Sanguinea, with only compound spinigers (Group B); Aeana, with only compound falcigers (Group C); and Belli, with both compound spinigers and falcigers (Group D). Each of the four groups

can be further divided into two subdivisions: those with branchiae present only on a short anterior region (subdivision 1), and those with branchiae present over a long region of the body (subdivision 2) (Fauchald 1970). Glasby and Hutchings (2010) further identified the Teretiuscula group, which falls between Group A and Group B and is characterized by the presence of compound spinigerous subacicular chaetae in anterior segments and limbate chaetae in anterior and posterior segments.

The Sanguinea group (Group B) was named after the type species of this genus, *Marphysa sanguinea* (Montagu, 1813), based on specimens collected from the south coast of England. This species has been subsequently recorded from many parts of the world, including northern Europe, North America, South America, South Africa, Australia and Asia (Miura 1977; Yang and Sun 1988; Hutchings and Karageorgopolous 2003; Wu

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2013). Hutchings and Karageorgopolous (2003) and Hutchings et al. (2012) provided a detailed redescription of *M. sanguinea*. Subsequent studies showed that many records of *M. sanguinea* from outside its type locality were misidentified and actually represent new species (see Lewis and Karageorgopoulos 2008; Molina-Acevedo and Carrera-Parra 2015; Zanol et al. 2016 2017; Lavesque et al. 2017).

To date, seven species of *Marphysa* have been described from Chinese coastal waters: *M. sinensis* Monro, 1934; *M. orientalis* Treadwell, 1936; *M. tripectinata* Liu, Hutchings & Sun, 2017; *M. multiplectinata* Liu, Hutchings & Sun, 2017; *M. tribranchiata* Liu, Hutchings & Sun, 2017; *M. bulla* Liu, Hutchings & Kupryanova, 2018; and *M. maxidenticulata* Liu, Hutchings & Kupryanova, 2018. Among them, only *M. sinensis* Monro, 1934 belongs to the Belli group (Group D), and all the other six species belong to the Sanguinea group (Group B).

Seven species of *Marphysa* with non-Chinese type localities have been recorded from the mainland Chinese coast [namely *Marphysa sanguinea* (Montagu, 1813); *Marphysa depressa* Schmarda, 1861; *Marphysa stragulum* Grube, 1878; *Marphysa macintoshi* Crossland, 1903; *Marphysa gravelyi* Southern, 1921; *Marphysa mossambica* Peters, 1854; *Marphysa formosa* Steiner & Amaral, 2000] (Wu 1962; Wu et al. 1980; Meng et al. 1994; Yang and Sun 1988; Wu 2013). However, only the species names were mentioned in most of these publications; in Yang and Sun (1988), the descriptions were too brief to allow for species identification. Liu et al. (2017), based on the fact that most of these species were described from localities very distant from China, suggested that these species do not occur in Chinese waters, although they were unable to locate the specimens reported by Wu (2013) to be deposited at the Institute of Oceanology, Chinese Academy of Sciences, Qingdao. Four species of *Marphysa* have also been recorded from Hong Kong waters. Morton and Morton (1983) reported *M. sanguinea* as a common species in intertidal sedimentary rock crevices and *M. adenensis* Gravier, 1900 as a common species associated with subtidal corals. Shin (1980) reported *M. bellii* (Audouin & Milne Edwards, 1833) and *M. sanguinea* from the subtidal soft sediment of Tolo Harbour and Mirs Bay. Mak (1982) reported *M. adenensis* from the subtidal coral community in Hoi Ha Wan. Shin and Thompson (1982) reported *M. stragulum* from the soft sediment of Victoria Harbour. Similar

to most other *Marphysa* species recorded from the mainland Chinese coast, these records were not accompanied by detailed morphological descriptions, and the species were originally described from localities far from Hong Kong. In this study, we describe a new species in the *Marphysa sanguinea* complex based on specimens collected from Tolo Harbour, Hong Kong. We also sequenced the *COI* and 16S RNA genes of this new species and conducted phylogenetic analyses to assess its relationship with other *Marphysa* species based on the corresponding gene sequences deposited in public databases. A key to the Sanguinea group of *Marphysa* described from the Chinese coast is provided.

MATERIALS AND METHODS

Sampling

Twenty-four specimens of *Marphysa hongkongensa* n. sp. were collected from the intertidal zone of six soft shore beaches (Tolo Pond, Ting Kok West, Ting Kok East, Nai Chung, Sai Keng and Starfish Bay) inside Tolo Harbour (Table 1; Fig. S1) during surveys conducted from 2015 to 2018. Samples were fixed with either 10% formaldehyde in seawater and later transferred into 75% ethanol for preservation, or in 95% ethanol.

Morphological analysis

Morphological characteristics of all specimens were recorded under an Olympus SZX9 stereoscope (Table 1). Photographs showing the gross morphology were taken using a Canon 550D digital camera mounted on the stereoscope. Finer details of the parapodia and chaetae were captured using a True Chrome II camera mounted on a Motic BA210 compound microscope. Maxillary apparatuses and mandibles were dissected and treated with 10% sodium hypochlorite solution (Sigma-Aldrich, Belgium) for a few minutes to remove the tissue. All light stereo/microscopic photographs were taken at different foci and stacked into fully focused images using the software Helicon Focus 6 as described in Wang et al. (2018). Parapodia from anterior, middle and posterior parts of the holotype (SWIMS-ANN-18-012) were dissected for observation under a scanning electron microscope (SEM). The dissected parapodia were dehydrated in pure ethanol, dried after being treated with gradient

hexamethyldisilazane-ethanol solution (50%, 75% and 100%), observed and glued on NEM conductive carbon adhesive tape under a light microscope, then coated with gold and observed under a LEO 1530 FESEM scanning electron microscope.

Molecular analysis

Specimens of *M. hongkongensa* n. sp. (SWIMS-ANN-18-012, SWIMS-ANN-18-022) were preserved in 95% ethanol for DNA extraction. Genomic DNA was extracted from a small piece of tissue in the pharynx from each specimen using a DNeasy blood & tissue kit (QIAGEN). The primers ACOIAF (CWAATCAYAAAGATATTGGAAC) and COIEU-R (TCDGGRTGDCCAAARAATCA) were used for amplifying the mitochondrial cytochrome oxidase I (*COI*) gene (Zanol et al. 2010). The primers 16SAR-L (CGCCTGTTTATCAAAAACAT) and 16SBR-H (CCGGTCTGAACTCAGATCACGT)

were used to amplify the mitochondrial 16S rRNA gene (Struck et al. 2006). PCR products were purified using a Zymoclean™ Gel DNA Recovery Kit and sequenced using Sanger sequencing at BGI Hong Kong.

Phylogenetic analysis was conducted as described in Zhang et al. (2015 2017) using the *COI* and 16S rRNA sequences of *M. hongkongensa* n. sp. and other species of *Marphysa* available in GenBank. Several sequences from specimens collected from Chinese waters that were not included in the original species description were confirmed by Yubin Liu (Table 2). The corresponding sequences of several species in other genera of the family Eunicidae were used as the outgroup (Table 2). The *COI* and 16S rRNA sequences were aligned using the Muscle algorithm in the Mesquite software (Edgar 2004), and the online Gblocks Server was applied to remove the unaligned sequences and highly divergent regions. Molecular evolution

Table 1. Major morphological characteristics and sampling information for type specimens of *Marphysa hongkongensa* n. sp.

Catalog No.	Length to chaetiger 10 (mm)	Width of chaetiger 10 (mm)*	Total chaetigers	First branchiae on chaetiger	Maximum No. of branchia filaments	First subacicular hook on chaetiger	MxII-teeth No. (L + R)	Collection date	Locality†	Fixation††
SWIMS-ANN-18-012	4.7	3.0	215	21	6	34	5 + 6	2016.7.8	Ting Kok West	Ethanol
SWIMS-ANN-18-013	7	2.3	230	22	6	39	5 + 5	2015.1.30	Ting Kok West	Ethanol
SWIMS-ANN-18-014	5.2	2.8	209	25	7	45	5 + 5	2016.7.22	Nai Chung	Ethanol
SWIMS-ANN-18-015	4.2	2	127**	15	6	29	6 + 6	2016.7.22	Nai Chung	Ethanol
SWIMS-ANN-18-016	7	3	81**	27	7	36	5 + 6	2016.8.6	Tolo Pond	Ethanol
SWIMS-ANN-18-017	9	3.8	110**	25	7	26	5 + 6	2016.8.6	Tolo Pond	Ethanol
SWIMS-ANN-18-018	6	3.5	79**	19	7	29	5 + 6	2016.8.6	Tolo Pond	Ethanol
SWIMS-ANN-18-019	5.3	3.3	190	22	5	31	5 + 5	2016.8.6	Tolo Pond	Ethanol
SWIMS-ANN-18-020	5.7	4.3	233	23	7	41	5 + 6	2016.8.6	Tolo Pond	Ethanol
SWIMS-ANN-18-021	5.3	3.5	288	35	10	39	5 + 5	2016.8.6	Tolo Pond	Ethanol
SWIMS-ANN-18-022	3.8	1.8	213	20	6	29	5 + 5	2016.8.6	Tolo Pond	Ethanol
SWIMS-ANN-18-023	5.2	2.3	174	20	7	30	5 + 6	2016.9.3	Ting Kok East	Formalin
AM W.50930	3.5	1.3	129	18	6	27	5 + 6	2017.3.29	Ting Kok East	Ethanol
AM W.50931	3.3	1.5	147	17	4	27	5 + 6	2017.3.29	Ting Kok East	Ethanol
AM W.50932	11	4.5	151**	26	7	44	5 + 6	2017.6.22	Starfish Bay	Ethanol
AM W.50933	6.8	4	109**	22	7	58	6 + 5	2017.6.24	Tolo Pond	Ethanol
AM W.50934	4.7	2.7	109**	22	8	38	*** + 5	2017.6.24	Tolo Pond	Ethanol
AM W.50935	6.3	4.2	108**	25	7	41	5 + 6	2017.6.24	Tolo Pond	Ethanol
AM W.50936	4	2	155	22	5	28	5 + 6	2017.7.21	Ting Kok West	Ethanol
AM W.50937	5.7	3.5	140**	25	7	37	5 + 5	2017.7.21	Ting Kok West	Ethanol
AM W.50938	3.2	2.3	197**	22	5	32	5 + 6	2017.8.8	Sai Keng	Ethanol
AM W.50939	9.2	3	162**	24	8	41	5 + 5	2018.3.19	Ting Kok East	Ethanol
AM W.50940	7	2.5	217	18	5	32	5 + 5	2018.3.19	Ting Kok East	Ethanol
AM W.50941	5.5	2	147	24	5	58	5 + 6	2018.3.19	Ting Kok East	Ethanol

*Width data without parapodia; **Number of chaetigers in anterior fragment; ***Teeth on left Mx II broken in this specimen. †GPS coordinates of sampling sites: Ting Kok West (22°28'18"N, 114°12'56"E); Nai Chung (22°25'56"N, 114°15'22"E); Tolo Pond (22°26'18"N, 114°11'20"E); Ting Kok East (22°28'06"N, 114°13'02"E); Starfish Bay (22°25'56"N, 114°14'41"E); Sai Keng (22°25'11"N, 114°16'07"E).

††Formalin means fixed with 10% formaldehyde then transferred to 75% ethanol; Ethanol means 95% ethanol.



Fig. 1. *Marphysa hongkongensa* n. sp. A-E, I-J, paratype (AM W.50939); F-H, paratype (AM W.50940). (A) living worm, dorsal view; (B) anterior end, dorsal view; (C) anterior end, ventral view; (D) anterior end, lateral view, labels represent chaetiger numbers; (E) anterior parapodia, lateral view, labels represent chaetiger numbers; (F) posterior parapodia, lateral view; (G) pygidium, dorsal view, showing pygidium and long pygidial cirri (*lpc*); (H) pygidium, ventral view, showing short (*spc*) and long pygidial cirri (*lpc*); (I) dissected maxillary apparatuses, dorsal view; (J) mandible, ventral view. *Abbr.* *bf*, branchial filament; *bl*, buccal lip; *dc*, dorsal cirrus; *la*, lateral antenna; *lpc*, long pygidial cirrus; *ma*, median antenna; *pa*, palps; *pt*, peristomium; *py*, pygidium; *spc*, short pygidial cirrus; *vc*, ventral cirrus. Scale bars: A = 2 mm; B-J = 1 mm.

models for the *COI* and 16S genes and their concatenated sequences were evaluated using jModeltest2 based on the Akaike Information Criterion (AIC) (Darriba et al. 2012), which resulted in the selection of the GTR+G model as the best model for the 16S gene and the GTR+I+G model as the best model for the *COI* gene and their concatenated sequences. Phylogenetic analyses were conducted using the Maximum Likelihood (ML) method implemented in the RaxmlGUI 1.5 beta software based on 1, 000 replicates.

RESULTS

SYSTEMATICS

Order Eunicida

Family Eunicidae Berthold, 1827

Genus *Marphysa* Quatrefages, 1866

Type species: *Marphysa sanguinea* (Montagu, 1813). Type locality: southern England.

Marphysa hongkongensa n. sp.

(Figs. 1-4, Tables 1-2)

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Materials examined: 24 type specimens collected from the intertidal zone of six beaches in Tolo Harbour, Hong Kong during 2015-2018 (Table 1). Holotype: SWIMS-ANN-18-012. Paratypes: SWIMS-ANN-18-013-SWIMS-ANN-18-023; AM W.50930-AM W.50941.

Description: Live worms light green with iridescence; middle part with red branchial filaments on dorsal side (Fig. 1A). Preserved specimens beige. Complete specimens 23-147 mm long, 129-288 chaetigers; anterior prostomial margin to chaetiger 10 3.3-7.0 mm; chaetiger 10 (without parapodia) 2.2-5.3 mm wide (Table 1).

Prostomium with two dorsoventrally flattened buccal lips and an anterior notch between them (Fig. 1B-D). Two palps and three antennae slender, attached on short palpiphore, arranged in a more or less curved line on the posterior margin

Table 2. DNA sequences with GenBank accession numbers used for the phylogenetic analysis

Taxa	Accession Number		References
	<i>COI</i>	16S	
<i>Lysidice ninetta</i> Audouin & H Milne Edwards, 1833	GQ497564	GQ478169	Zanol et al. 2010
<i>Leodice rubra</i> (Grube, 1856)	GQ497528	GQ478132	Zanol et al. 2010
<i>Nicidion angeli</i> (Carrera-Parra & Salazar-Vallejo, 1998)	GQ497550	GQ478161	Zanol et al. 2010
<i>Nicidion</i> cf. <i>hentscheli</i> (Augener, 1931)	GQ497551	GQ478164	Zanol et al. 2010
<i>Marphysa fauchaldi</i> Glasby & Hutchings, 2010	KX172165	-	Zanol et al. 2016
<i>Marphysa mossambica</i> (Peters, 1854)	JX559751	JX559747	Direct Submission*
<i>Marphysa kristiani</i> Zanol, da Silva & Hutchings, 2016	KX172142	-	Zanol et al. 2016
<i>Marphysa fallax</i> Marion & Bobretzky, 1875	-	GQ478160	Zanol et al. 2010
<i>Marphysa bellii</i> (Audouin & Milne Edwards, 1833)	KT307661	AY838835	Aylagas et al. 2016; Struck et al. 2006
<i>Marphysa disjuncta</i> Hartman, 1961	GQ497549	GQ478159	Zanol et al. 2010
<i>Marphysa californica</i> Moore, 1909	GQ497552	GQ478162	Zanol et al. 2010
<i>Marphysa bifurcata</i> Kott, 1951	KX172178	-	Zanol et al. 2016
<i>Marphysa tripectinata</i> Liu, Hutchings & Sun, 2017	KY315333	-	Liu et al. 2017 [†]
<i>Marphysa bulla</i> Liu, Hutchings & Kupriyanova, 2018	KY328276	-	Liu et al. 2018 ^{††}
<i>Marphysa maxidenticulata</i> Liu, Hutchings & Kupriyanova, 2018	KY328278	-	Liu et al. 2018 ^{†††}
<i>Marphysa victori</i> Lavesque, Daffe, Bonifácio & Hutchings, 2017	MG384998	MG385001	Lavesque et al. 2017
<i>Marphysa</i> sp.	KY129891	-	Direct Submission
<i>Marphysa viridis</i> Treadwell, 1917	GQ497553	GQ478163	Zanol et al. 2010
<i>Marphysa graveleyi</i> Southern, 1921	KP258205	-	Direct Submission
<i>Marphysa sanguinea</i> (Montagu, 1813)	KR916872	GQ478157	Lobo et al. 2016; Zanol et al. 2010
<i>Marphysa mullawa</i> Hutchings & Karageorgopoulos, 2003	KX172173	-	Zanol et al. 2016
<i>Marphysa regalis</i> Verrill, 1900	GQ497562	GQ478165	Zanol et al. 2010
<i>Marphysa brevitentaculata</i> Treadwell, 1921	GQ497548	GQ478158	Zanol et al. 2010
<i>Marphysa hongkongensa</i> n. sp. Holotype	MH598525	MH598527	This study
<i>Marphysa hongkongensa</i> n. sp. Paratype	MH598526	MH598528	This study

*Zanol (28-Aug-2016). [†]This sequence was labeled as *Marphysa* sp. 1 in GenBank; ^{††}This sequence was labeled as *Marphysa* sp. 2 in GenBank; ^{†††}This sequence was labeled as *Marphysa* sp. 3 in GenBank.

of the prostomium. All five prostomial appendages approximately the same length, 1.5 times as long as prostomium (Fig. 1B-D). Peristomium approximately 3 times as long as segment 2, with notched anterior margin on ventral side (Fig. 1C).

Mandibles slightly longer than Mx I plus carriers (Fig. 1I-J). Mx I approximately 2.4 times as long as carriers. Mx II edge serrated, with teeth on left and right sides well matched. Mx III single, arched, slightly smaller than right Mx IV. Mx IV paired, both attached with basal lamellae; left Mx IV smaller than the right one. Mx V paired, flat, left one slightly smaller than right one (Fig. 1I-J). Maxillary formula: I = 1 + 1, II = 5-6 + 5-6, III = 7 + 0, IV = 4 + 8, V = 1 + 1.

Parapodia commencing from segment 3 (Fig. 1B-D). First parapodia located below the middle line of body wall, but gradually positioned dorsally in following segments (Fig. 1D-F). Notopodial cirri tapering, approximately as long as neuropodial cirri, longer than acicular lobes in anterior chaetigers, and similar in length with acicular lobes in posterior chaetigers (Fig. 2). Neuropodial cirri conical, similar in size with notopodial cirri in first few chaetigers, but inflated in middle and posterior chaetigers (Fig. 2).

Branchiae pectinate, commencing from anterior (15th-35th chaetiger) to near end. Branchiae single filament in anterior parapodia, pectinate with up to 5-10 filaments in middle

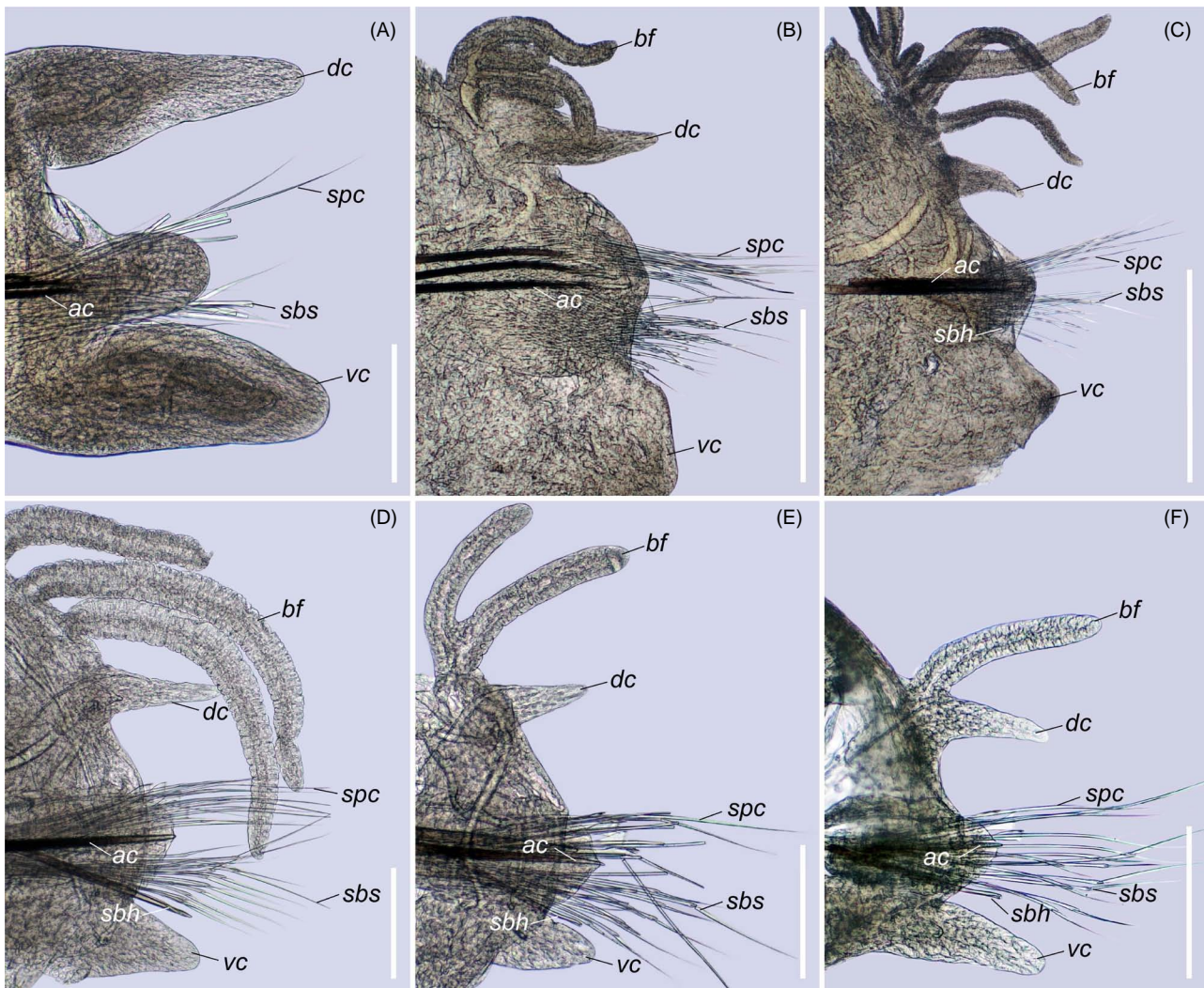


Fig. 2. *Marphysa hongkongensis* n. sp. parapodia, left side, anterior views. A-C, paratype (SWIMS-ANN-18-014); D-F, paratype (SWIMS-ANN-18-023). (A) chaetiger 2; (B) chaetiger 30; (C) chaetiger 70; (D) chaetiger 143; (E) chaetiger 153; (F) chaetiger 163. Abbreviations: ac, acicula; bf, branchial filament; dc, dorsal cirrus; sbh, subacicular hook; sbs, subacicular spinigers; spc, supra-acicular capillary; vc, ventral cirrus. Scale bars: A, D-F = 200 μ m; B-C = 500 μ m.

parapodia (Fig. 2B), reducing to 1 in last several chaetigers.

Aciculae black with paler tip, approximately three per parapodia in anterior chaetigers, two per parapodia in middle chaetigers, and one per parapodia in posterior chaetigers. Supracicular chaetae with limbate capillaries and pectinates. Capillaries present from first chaetiger to near pygidium, numbering up to 20 in anterior chaetigers. Pectinate chaetae commencing from first few chaetigers to near end, four types: a) isodont with 8-15 fine and short teeth, lateral teeth longer and usually straight, plate symmetrical or slightly asymmetrical, distributed in anterior chaetigers, 1-3 per parapodia (Fig. 3G, J); b) isodont with 15-23 fine and short teeth, lateral teeth longer and usually incurved, plate asymmetrical, distributed from middle to posterior chaetigers, 1-7 per parapodia (Fig. 3I, K, O-P); c) anodont with approximately 15 median teeth, teeth length approximately $\frac{1}{4}$ - $\frac{1}{3}$ plate width, plate asymmetrical, distributed in posterior chaetigers, 1-3 per parapodia (Fig. 3L, P); d) anodont with 7-13 large teeth, three medial teeth larger or similar in size with adjacent teeth, teeth length approximately $\frac{1}{2}$ of plate width, plate asymmetrical, distributed in posterior chaetigers, 1-2 per parapodia (Fig. 3M-P). Pectinates arranged in rows in posterior chaetigers, with asymmetrical short and fine-toothed isodonts (type b) in anterior two rows, median- and large-toothed anodonts in posterior row (types c and d); Median-toothed anodonts (type c) in near middle chaetigers (anterior part of posterior chaetigers), approximately three in posterior row and gradually replaced by 1-2 large-toothed anodonts (type d) in posterior chaetigers (Fig. 3L, O-P).

Subacicular chaetae compound spinigers and subacicular hooks (Fig. 2D-F). Compound spinigers commencing from first chaetiger to near pygidium, with long, tapered blade bearing unilateral fine serration (Fig. 3C, F). Subacicular hooks amber in colour, commencing from anterior chaetiger (26th-58th) to near end and inferior to bundle of spinigers, one per parapodia; slightly thinner than aciculae; Most subacicular hooks unidentate, thin and bidentate ones only present in last few parapodia (Figs. 2D-F; 3D-E).

Pygidium round, dorsally positioned, with two pairs of tapering pygidial cirri attached at the ventral edge, one pair 2 x and another pair half of pygidial diameter (Fig. 1G-H).

Variations in morphological characters: The complete specimens vary in length from 2.3 cm for

147 segments to 14.7 cm for 288 segments (Table 1). The first pair of branchia occurs in chaetiger 15-35, being more posterior in larger individuals. The maximum number of branchial filaments varied between 5-10. In complete specimens, the last pair of branchia is present in the last 8th-10th chaetiger with only one filament. Most of the specimens have a maxilla formula of Mx II = 5 + 5-6 (21 specimens) or Mx II = 6 + 5-6 (2 specimens); but in one specimen the left Mx II was broken and the right has five teeth. Subacicular hooks commence from parapodia 26-58, but all of these parapodia carry only one subacicular hook. Hooded bidentate subacicular hooks are only present on terminal posterior chaetigers, which explains why these hooks are missing in the several incomplete specimens.

Distinction from closely related species: *Marphysa hongkongensis* n. sp. resembles the other 25 *Marphysa* species belonging to Group B2 by having compound spinigers but no compound falcigers, and having branchiae in middle and posterior segments. This species can be distinguished from the type species *M. sanguinea* (Montagu, 1813) by having unidentate and unhooded subacicular hook from anterior to middle chaetigers, hooded bidentate hook only present in terminal posterior chaetigers and subacicular limbate capillaries absent. Of the seven species that have been considered to be valid in Chinese waters (Liu et al. 2017 2018), *M. sinensis* Monro, 1934 belongs to the Belli group (Group D1) by having both compound spinigers and falcigers. *M. orientalis* Treadwell, 1936 has pectinate chaetae from more posterior chaetigers (193th vs. 1st chaetiger), fewer teeth in Mx II (3 + 3 vs. 5-6 + 5-6), and a smaller maximal number of branchial filaments (3 vs. 5-10). *M. multipectinata* Liu, Hutchings & Sun, 2017 has pectinate chaetae first present from more posterior chaetigers (70th vs. 1st chaetiger), fewer teeth in Mx II (3 + 3 vs. 5-6 + 5-6), and a smaller maximal number of branchial filaments (3-5 vs. 5-10). *M. tribranchiata* Liu, Hutchings & Sun, 2017 has pectinate chaetae first present from more posterior chaetigers (20th vs. 1st chaetiger), fewer teeth in Mx II (4 + 4 vs. 5-6 + 5-6), and a smaller maximal number of branchial filaments (2-3 vs. 5-10). *M. tripectinata* Liu, Hutchings & Sun, 2017 has subacicular hook from more posterior chaetigers (170th vs. 26th-58th chaetiger), and has only one pair of pygidial cirri. *M. bulla* Liu, Hutchings & Kupriyanova, 2018 has fewer teeth in Mx II (4 + 4 vs. 5-6 + 5-6), subacicular hooks from more posterior chaetigers

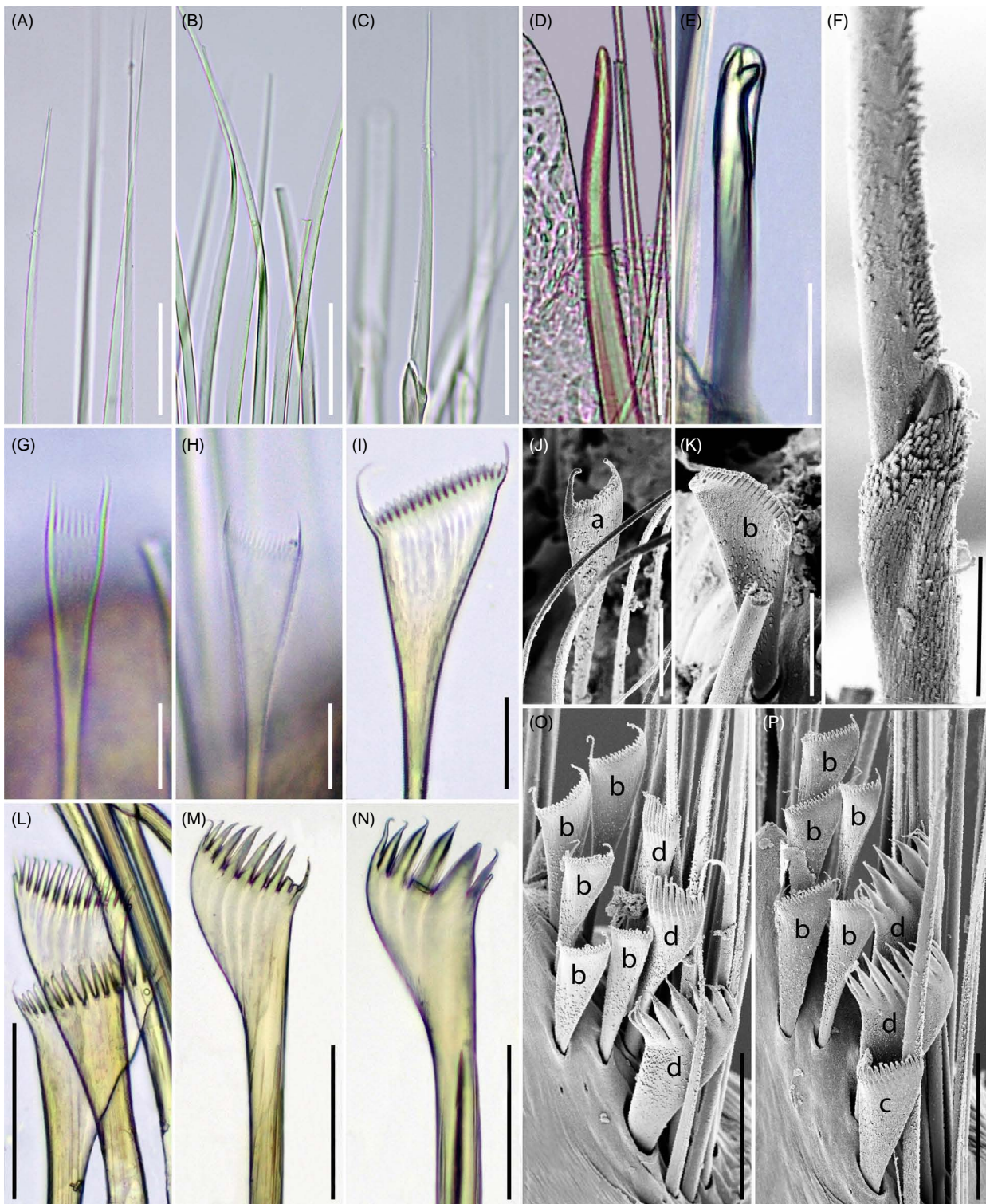


Fig. 3. *Marphysa hongkongensis* n. sp. chaetae. A-C, G-I, L-N: paratype (SWIMS-ANN-18-014); D-E: paratype (SWIMS-ANN-18-023); F, J-K, O-P: holotype (SWIMS-ANN-18-012). (A) chaetiger 30, capillaries; (B) posterior chaetiger, capillaries; (C) chaetiger 30, CS; (D) chaetiger 75, unidentate SH; (E) chaetiger 165, bidentate SH; (F) chaetiger 30, CS; (G) chaetiger 2, AFI; (H) chaetiger 70, FI; (I) chaetiger 160, PFI; (J) chaetiger 26, AFI; (K) chaetiger 103, PFI; (L) chaetiger 160, PMA; (M-N) chaetiger 165, PLA; (O-P) chaetiger 147-148, arrangement of pectinates, showing PFI, PMA and PLA. *Abbr.* AFI: anterior fine-toothed isodont (a); CS: compound spiniger; FI: fine-toothed isodont; PFI: posterior fine-toothed isodont (b); PLA: posterior large-toothed anodont (d); PMA: posterior median-toothed anodont (c); SH: subacicular hook. Scale bars: A-D, L-P = 50 μ m; E, G-K = 20 μ m; F = 10 μ m.

(71th vs. 26th-58th chaetiger), more teeth (30-40 vs. 15-23) in posterior fine-toothed isodonts, and fewer teeth (3-5 vs. 7-13) in posterior large-toothed anodonts. *M. maxidenticulata* Liu, Hutchings & Kupriyanova, 2018 has smaller maximal number of branchial filaments (3 vs. 5-10), and fewer teeth (3-6 vs. 7-13) in posterior large-toothed anodonts.

Marphysa hongkongensa n. sp. can also be distinguished from the other 18 species of *Marphysa* of the Group B2 that were originally described from localities beyond Chinese waters. Nine species [*i.e.* *M. acicularum* Webster, 1884 (Molina-Acevedo & Carrera-Parra, 2015); *M. brasiliensis* (Hansen, 1882); *M. elityeni* Lewis & Karageorgopoulos, 2008; *M. fauchaldi* Glasby & Hutchings, 2010; *M. gravelyi* Southern, 1921; *M. kristiani* Zanol, da Silva & Hutchings, 2016; *M. mullawa* Hutchings & Karageorgopoulos, 2003; *M. schmardai* Gravier, 1907 and *M. viridis* Treadwell, 1917 (Molina-Acevedo & Carrera-Parra, 2015)] have bidentate subacicuar hook, but *M. hongkongensa* n. sp. has unidentate subacicuar hook. *M. victori* Lavesque, Daffe, Bonifácio & Hutchings, 2017 has no subacicuar hook. *M. januarii* (Grube, 1881) and *M. teretiuscula* (Schmarda, 1861) have a smaller maximal number of branchial filaments (4 vs. 5-10). *M. borradailei* Pillai, 1958 (Glasby and Hutchings, 2010) has a larger maximal number of branchial filaments (10-20 vs. 5-10). Three species (*i.e.* *M. macintoshi* Crossland, 1903; *M. mangeri* Augener, 1918 and *M. tamurai* Okuda, 1934) have an undivided prostomium but *M. hongkongensa* n. sp. has a bilobed prostomium. *M. sanguinea* (Montagu, 1813) and *M. furcellata* Crossland, 1903 have subacicuar limbate capillaries but this type of

chaetae are absent in *M. hongkongensa* n. sp. *M. simplex* (Langerhans, 1884) (Crossland 1903) has fewer teeth in Mx II than *M. hongkongensa* n. sp. (3 + 3 vs. 5 + 5-6).

Etymology: The specific epithet *hongkongensa* refers to the type locality of Hong Kong.

Habitat: Lower intertidal zone on sandy shores.

Distribution: Currently only known from Tolo Harbour, Hong Kong. Given its common occurrence on several beaches in Tolo Harbour (as found in this study) and the wide presence of "*Marphysa sanguinea*" recorded from local sea shores (Morton and Morton 1983), it is expected that this species is also distributed on other shores along the eastern coasts of Hong Kong.

Molecular analysis

Partial DNA sequences of *COI* (435bp) and 16S RNA (466bp) were used for phylogenetic analysis based on the Maximum Likelihood (ML) method (Fig. 4). Results based on the two single genes showed that *Marphysa* species form a monophyletic clade; however, support for the clade is weak for both single genes (bootstrap values < 65). The results of *COI* and 16S concatenated sequences are consistent with each single gene in that *Marphysa* species form a monophyletic clade, but the support value was higher (bootstrap values = 82). These results are in agreement with the results of Zanol et al. (2010 2014). Phylogenetic analysis placed *M. hongkongensa* n. sp. as sister to *M. tripectinata* Liu, Hutchings and Sun, 2017 based on the *COI* gene; and as sister to a clade

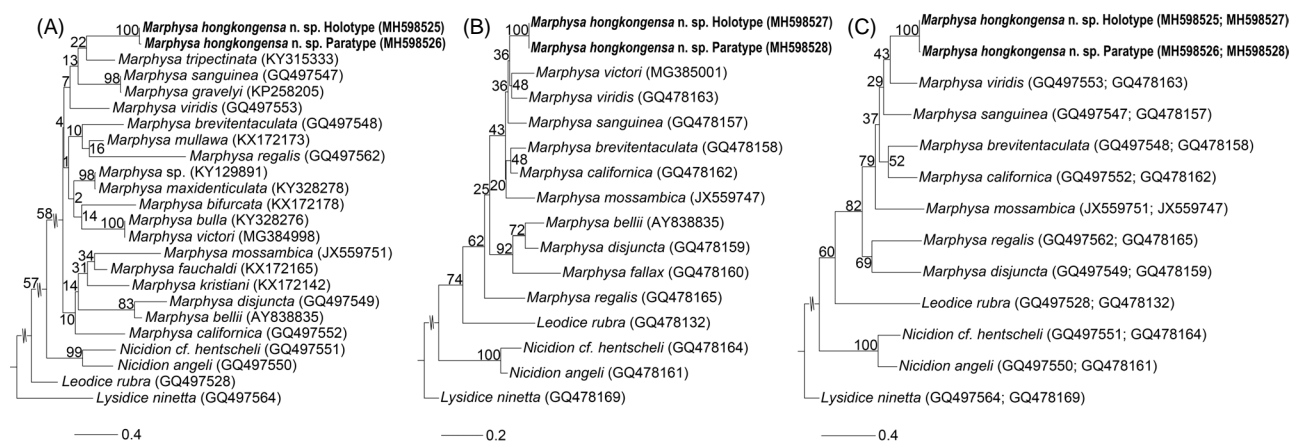


Fig. 4. Phylogenetic tree generated by maximum likelihood (ML) method based on *COI* (A), 16S (B) and their concatenated sequences (C). Numbers on the branches represent ML bootstrap values (maximum: 100) based on 1000 replicates. Genbank accession numbers of the *COI* and 16S genes used are shown in parentheses.

consisting of *M. victori* Lavesque, Daffe, Bonifácio & Hutchings, 2017 and *M. viridis* Treadwell, 1917 based on 16S gene with low bootstrap values (bootstrap values < 50). Nevertheless, there are much larger interspecific divergences in *COI* sequences (19.5%) and 16S sequences (12.1%) between *M. hongkongensa* n. sp. and the closest related *Marphysa* species than the intraspecific divergences (< 1%) of both two genes in *Marphysa* species. These analyses, therefore, support *M. hongkongensa* n. sp. as a valid species.

Key to species in the *Marphysa sanguinea* complex from Chinese waters
(modified after Liu et al. 2017)

1. Subacicular hooks present after chaetiger 70 2
1. Subacicular hooks present before chaetiger 60 3
2. Subacicular hooks present from posterior chaetigers, Mx II = 3 + 3, branchiae from chaetigers 35-45, up to 3 filaments; pectinates from chaetiger 193, posterior pectinate 3 types: asymmetrical isodont approximately 30 teeth, median teeth anodont 16 teeth, large-toothed anodont 4-5 teeth *M. orientalis*
2. Subacicular hooks present from 170th chaetiger, Mx II = 5 + 5, branchiae from chaetiger 15-24, up to 6-8 filaments; Pectinates from first few chaetigers, four types, anterior isodonts approximately 10 teeth, posterior pectinates 3 types: fine-toothed isodont > 30 teeth, median teeth anodont 14-18 teeth, large-toothed anodont 5-7 teeth *M. tripectinata*
2. Subacicular hooks present from approximately 71th chaetiger, Mx II = 4 + 4, branchiae from chaetiger 29-36, up to 5 filaments; pectinates from first few chaetigers, four types, anterior isodonts 13-17 teeth, posterior pectinates 3 types: fine-toothed isodont approximately 30-40 teeth, median-toothed anodont approximately 14 teeth, large-toothed anodonts 3-5 teeth, ventral cirri with a swollen base and a globular tip *M. bulla*
3. Subacicular hooks present after 20th chaetiger, pectinates present from first few chaetigers, 4 types 4
3. Subacicular hooks present from 20th chaetiger, pectinates present after 20th chaetiger, 3 types 5
4. Subacicular hooks present from chaetiger 25, Mx II = 4 + 6, branchiae from chaetiger 28, up to 3 filaments; anterior isodonts 10-12 teeth; posterior pectinates 3 types: fine-toothed isodont approximately 25 teeth, median-toothed anodont 14 teeth, large-toothed anodont 3-6 teeth *M. maxidenticulata*
4. Subacicular hooks present from 26 to 58 chaetigers, Mx II = 5 + 5-6, branchiae from chaetiger 14-35, up to 5-10 filaments, 1-3 anterior isodonts, 8-15 teeth, posterior pectinate 3 types: approximately 5 fine-toothed isodonts arranged in 2 rows, approximately 23 teeth; 1-2 median-toothed anodonts, approximately 15 teeth; 2-3 large-toothed anodonts, 7-13 teeth *M. hongkongensa* n. sp.
5. Mx II = 4 + 4, branchiae present from chaetigers 16-26, up to 2-3 filaments; pectinate from approximately 20th chaetiger, anterior fine-toothed isodonts approximately 12 teeth, posterior pectinate 2 types: fine-toothed isodont approximately 17 teeth, median-toothed anodont 14 teeth, large-toothed anodont absent *M. tribranchiata*
5. Mx II = 3 + 3, branchiae present from chaetigers 29-32, up to 3-5 filaments; pectinate from approximately chaetiger 70, anterior fine-toothed isodonts absent; posterior pectinate 3 types: fine-toothed isodont approximately 12-16 teeth, median-toothed anodont 14 teeth, large-toothed anodont approximately 4 teeth *M. multipectinata*

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Authors' contributions: JWQ initiated the study. ZW, YZ and JWQ designed the study and wrote the manuscript.

Competing interests: ZW, YZ and JWQ declare they have no conflict of interest.

Availability of data and materials: The manuscript has been incorporated in ZooBank (urn:lsid:zoobank.org:pub:13CF278B-A8A8-4EE5-A9BF-1BA0D3F7BF78). Type specimens are deposited in the Swire Institute of Marine Science, the University of Hong Kong (SWIMS) and the Australian Museum (AM).

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Ethics approval consent to participate: Not applicable.

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Supplementary Material

Fig. S1. Sampling sites (red dots) of *Marphysa hongkongensa* n. sp. in the intertidal zone of Tolo Harbour, Hong Kong. (download)