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A new species of *Mesochaetopterus* (Annelida, Chaetopteridae) from Hong Kong, with comments on the phylogeny of the family

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Abstract

We described a new species, *Mesochaetopterus tingkokensis*, based on 14 specimens collected from an intertidal area in Hong Kong. This species is large (body length of complete specimens 17.9–24.1 cm), with 9, 2 and 36–41 chaetigers in region A, B and C, respectively. It belongs to a small group of *Mesochaetopterus* species with an expanded wing-shaped notopodia in chaetiger B2. The new species can be distinguished from other *Mesochaetopterus* species in this group by having a pair of palps with two longitudinal stripes formed by suture-like discontinuous orange bands, more teeth in the uncini of region B and C neuropodia, and presence of a bundle of simple chaetae in region C notopodia. Comparison with other chaetopterids based on partial cytochrome oxidase I (COI), 18S and 28S rRNA gene sequences confirmed the placement of *M. tingkokensis* **n. sp.**within *Mesochaetopterus* and its distinction from other members of this genus with the available DNA sequences. The phylogenetic tree base on COI showed that *Mesochaetopterus* and *Chaetopterus* are paraphyletic, but that based on concatenated data, 18S and 28S showed they are monophyletic with low supporting values.

Key words: taxonomy, polychaete, *Mesochaetopterus*, new species, Hong Kong

Introduction

Chaetopteridae is a small family of approximately 65 species of tube-building polychaete worms. Members of this family have been reported from the intertidal areas to the deep-sea hydrothermal vents, and from the tropics to the polar regions (Fauchald 1977; Rouse and Pleijel 2001; Morineaux *et al.* 2010). Chaetopteridae is divided into four genera based on whether their median notopodia are bilobed or fused, whether tentacular cirri are present, and whether palps are small. *Mesochaetopterus* can be distinguished from the other genera of chaetopterids by the presence of large palps and non-fused notopodia, and absence of tentacular cirri (Fauchald 1977), but this genus is considered paraphyletic based on analysis of a fragment of the cytochrome oxidase I (COI) gene (Martin *et al.* 2008; Morineaux *et al.* 2010). Previous analyses based on COI, 18S and 28S rRNA genes also showed that the other two genera *Phyllochaetopterus* and *Spiochaetopterus* are paraphyletic (Osborn *et al*. 2007; Martin *et al*. 2008; Morineaux *et al*. 2010).

Sixteen species of *Mesochaetopterus* are considered valid world-wide (Nishi 1999; Martin *et al.* 2008; Nishi *et al.* 2009; Nishi & Hsieh 2009). These species can be further divided into three groups based on the morphology of region B notopodia: 1, slightly modified and triangular; 2, conical; 3, extended and pointed (Nishi 1999), though the grouping scheme has not been subjected to a proper phylogenetic analysis. In the third group of *Mesochaetopterus* there are three large-sized species; two of them [*M. japonicus* Fujiwara, 1934, *M. selangolus* (Rullier, 1976)] are distributed in the western Pacific. *Mesochaetopterus selangolus* has been recorded from Malaysia only, whereas *M. japonicus* has been recorded widely from Japan (Nishi 1999), mainland China (Yang & Sun 1988) and Taiwan (Nishi & Hsieh 2004). Here we report *Mesochaetopterus tingkokensis* **n. sp.** from Hong Kong in southern China. It is the fourth species in the third group of *Mesochaetopterus*. We describe the morphology of this species, and present a phylogenetic analysis of chaetopterids based on partial sequences of the COI, 18S rRNA and 28S rRNA genes of *M. tingkokensis* and the corresponding sequences of these genes in other related species available in GenBank.

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TABLE 1. Sequences of chaetopterids with GenBank accession numbers used for phylogenetic analysis. The outgroup *Owenia* sp. is labelled as *Owenia fusiformis* in NCBI database.

Species	COI	18S	28S	Reference
Outgroup				
Owenia sp.	GU672205.1	AY176298.1	AY612634.1	Carr *et al*. 2011(COI); Direct submission (18S and 28S)
Ingroup				
Chaetopterus cf. *luteus*	DQ209253.1	DQ209220.1	DQ209234.1	Osborn *et al*. 2007
Chaetopterus pugaporcinus PB1	DQ209257.1	DQ209224.1	DQ209238.1	Osborn *et al.* 2007
Chaetopterus pugaporcinus PB2	DQ209256.1	DQ209223.1	DQ209237.1	Osborn *et al*. 2007
Chaetopterus sarsi	DQ209254.1	DQ209221.1	DQ209235.1	Osborn *et al*. 2007
Chaetopterus sarsi 1	N/A	DQ779642.1	N/A	Direct submission
Chaetopterus sp. 1	DQ209252.1	DQ209219.1	DQ209233.1	Osborn *et al*. 2007
Chaetopterus sp. 2	DQ209255.1	DQ209222.1	DQ209236.1	Osborn *et al*. 2007
Chaetopterus sp. KP-2005	DQ087501.1	N/A	N/A	Peterson & Butterfield 2005
Chaetopterus sp. NKP-2014	N/A	KM206141.1	N/A	Direct submission
Chaetopterus sp. SEG_CHARREA	N/A	AH001603.1	N/A	Field *et al*. 1988
Chaetopterus variopedatus CvB-Fr	AM503095.1	AJ966759.1	N/A	Martin *et al.* 2008
Chaetopterus variopedatus CvN-It	AM503094.1	AJ966758.1	N/A	Martin *et al.* 2008
Chaetopterus variopedatus CvNo-Uk	AM503096.1	N/A	N/A	Martin *et al.* 2008
Chaetopterus variopedatus CVU67324	N/A	U67324.1	N/A	Direct submission
Mesochaetopterus japonicus	N/A	DQ209218.1	N/A	Osborn *et al*. 2007
Mesochaetopterus rogeri	AM503098.1	AJ966762.1	N/A	Martin *et al.* 2008
Mesochaetopterus taylori	DQ209251.1	DQ209217.1	DQ209232.1	Osborn *et al*. 2007
Mesochaetopterus tingkokensis **n. sp.**	KP222296	KP222297	KP222298	This study
Mesochaetopterus xerecus	AM503097.1	AJ966763.1	N/A	Martin *et al.* 2008
Phyllochaetopterus polus	GQ891958.1	N/A	N/A	Morineaux *et al.* 2010
Phyllochaetopterus prolifica BAMPOL0306	HM473565.1	N/A	N/A	Carr *et al.* 2011
Phyllochaetopterus prolifica BAMPOL0308	HM473567.1	N/A	N/A	Carr *et al.* 2011
Phyllochaetopterus socialis	DQ209247.1	DQ209212.1	DQ209227.1	Osborn *et al.* 2007
Phyllochaetopterus socialis PsB-Fr	N/A	AJ966761.1	N/A	Martin *et al.*, 2008
Phyllochaetopterus gigas	DQ209248.1	DQ209213.1	DQ209228.1	Osborn *et al.* 2007
Phyllochaetopterus sp. 2	DQ209250.1	DQ209216.1	DQ209231.1	Osborn *et al.* 2007
Phyllochaetopterus sp. 1	DQ209249.1	DQ209215.1	DQ209230.1	Osborn *et al.* 2007
Phyllochaetopterus sp. SAM E3512	N/A	DQ779665.1	DQ779703.1	Rousset *et al.* 2007
Phyllochaetopterus sp. SAM E3513	N/A	DQ779666.1	DQ779704.1	Rousset *et al.* 2007
Spiochaetopterus bergensis	N/A	DQ209214.1	DQ209229.1	Osborn *et al.* 2007
Spiochaetopterus solitarius SsPV-Fr	N/A	AJ966760.1	N/A	Martin *et al.* 2008
Spiochaetopterus sp.	N/A	AF448165.1	N/A	Direct submission

Material and methods

Specimens of *Mesochaetopterus tingkokensis* were collected from the intertidal zone of Ting Kok (22°28′09″N, 114°12′42″E), Hong Kong on 28 May 2014. Ting Kok was designated as a Site of Special Scientific Interest (SSSI) by Hong Kong Government in 1985 due to the presence of a mangrove stand and associated fauna on a 37-hectare sandy beach. Samples were collected from the beach (Figure 1A). The upper part of the *M. tingkokensis* tubes were observed near the low water mark at low tide (Figure 1A). The sediment at the site was silty sand. The tubes were straight, with only the top (~ 1–2 cm) protruding from the sediment surface (Figure 1B). The samples were collected by digging quickly down to 40–45 cm around the tubes. Specimens within their tubes were retrieved after gently opening the tubes using a scalpel. They were preserved in 75% ethanol after fixation in 6% formaldehyde for two days. Photographs of the whole body and tubes were taken using a Canon 550D camera. Photographs of morphological characters were taken using a Nikon Digital Sight DS-SM camera mounted on an Olympus SZX 16 stereomicroscope. Several parapodia from two paratypes were dissected for observation of chaetal morphology. The parapodia were dehydrated using graded concentrations of ethanol, critical point dried using a BAL-TEC CPD 030 Critical Point Dryer, and observed under a LEO 1530 FESEM scanning electron microscope. The types are deposited in Institute of Oceanology, Chinese Academy of Science (IOCAS), Qingdao, China, and Australian Museum (AM), Sydney, Australia (Table 2). Specimens of *M. japonicus* and *M. selangolus,* deposited in the Natural History Museum and Institute, Chiba, were used for morphological comparison (*M. japonicus*: CBM-ZW521–524, 620, 623, 624; *M. selangolus*: CBM-ZW601, 603, 605).

One specimen of *M. tingkokensis* **n. sp.** (catalog number IOCAS MBM240659) was preserved in 100% ethanol directly for phylogenetic analysis. Genomic DNA was extracted using the CTAB method and sequenced on an Illumina Hiseq 1500 to produce 5.7 Gb paired-end reads with a read length of 100 bp. Adaptors and low quality reads were removed using Trimmomatic V0.32 and the clean reads were assembled by CLC Genomics Workbench version 7.0.3. COI, 18S rRNA and 28S rRNA sequences used in phylogenetic analysis were found using local BLAST V2.2.24.

All available chaetopterid COI, 18S rRNA and 28S rRNA sequences in GenBank were used in phylogenetic analysis (Table 1). *Owenia* sp. was chosen as the outgroup. COI, 18S rRNA and 28S rRNA sequences were aligned by Mesquite using the MUSCLE algorithm, respectively. Poorly aligned positions and divergent regions were removed with the Gblocks Server. Sequence Matrix was used to concatenate data. The most suitable models of molecular evolution for each gene and the concatenated data were determined using jModeltest2 based on the Akaike Information Criterion (AIC) (Darriba *et al*. 2012). This resulted in the choice of GTR+I+G for COI, 28S and the concatenated data, and GTR+I for 18S. Maximum Likelihood (ML) analysis was conducted with RaXML GUI1.3 and Maximum Parsimony (MP) analysis was conducted with PAUP* V4.0 using settings previously reported by Osborn *et al*. (2007).

Results

Systematics

Genus *Mesochaetopterus* Potts, 1914

Mesochaetopterus tingkokensis n. sp..

(Figures 1–5, Table 2–3)

Materials examined. 14 specimens (Table 2). Holotype: IOCAS MBM240650, one complete specimen with tube. Paratypes: IOCAS MBM240651-240659, AM W.47811-47814. IOCAS MBM240650 and AM W.47811, complete specimens with tube; AM W.47812-47814, IOCAS MBM240652-240658, incomplete specimens with at least regions A and B. Several notopodia and neuropodia were removed from paratypes 02, 12 and 13 for observation of chaetal morphology. IOCAS MBM240659, incomplete region A, was cut into pieces and preserved in 100% ethanol for molecular analysis. All samples were collected on 28 May 2014.

Etymology. The specific epithet "*tingkokensis*" comes from Ting Kok, a beach in Tolo Harbor, Hong Kong, the type locality of this species.

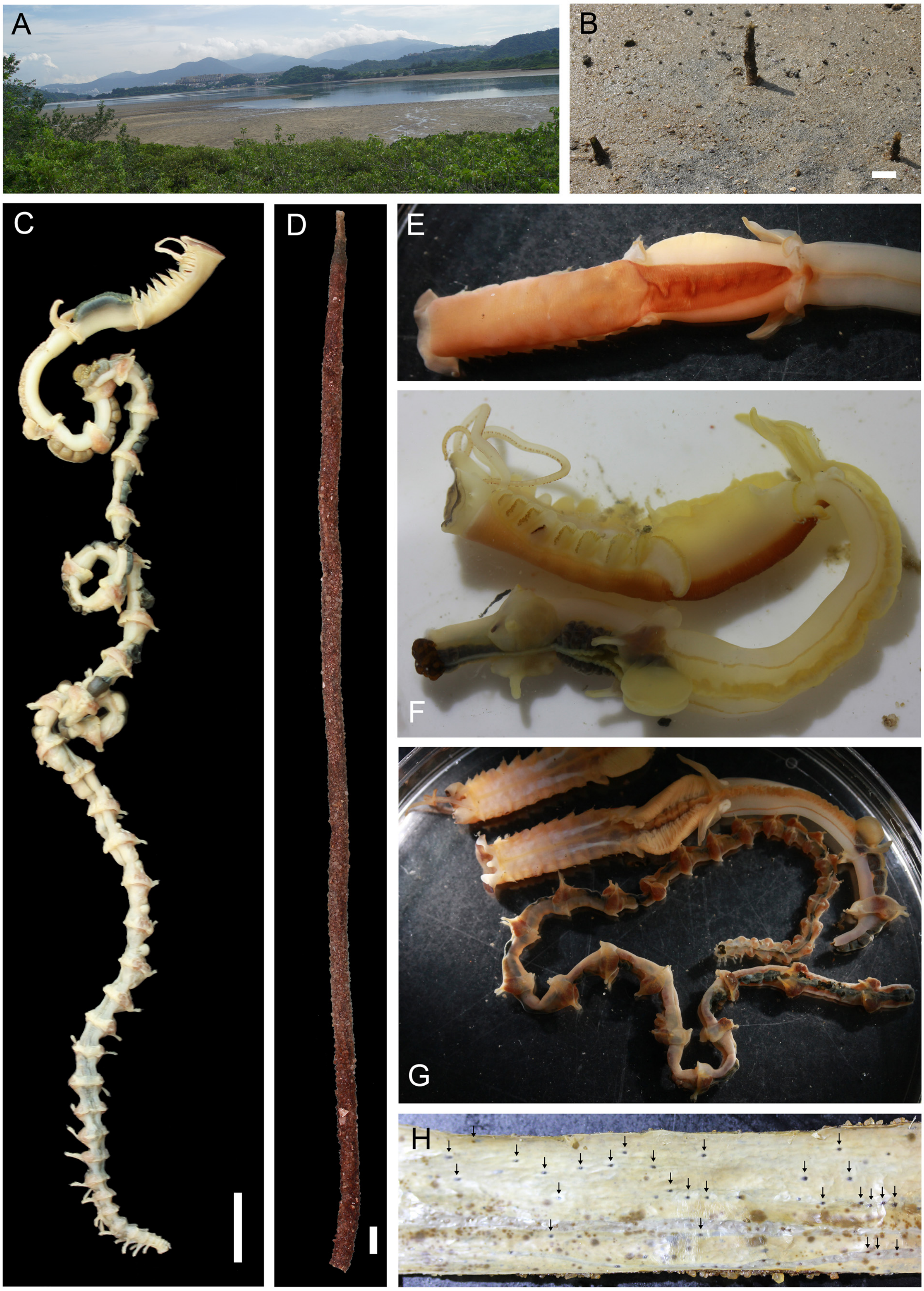

FIGURE 1. *Mesochaetopterus tingkokensis* **n. sp.** A, A photograph of Ting Kok showing the mangrove stand in the foreground and the mudflat where the type specimens were collected. B, Three tubes on the beach, with the top 1–2 cm above the sediment surface. C, A whole worm (holotype). D, A whole tube. E, Ventral view of the anterior body of a live specimen. F, Lateral view of the anterior body of a live specimen. G, Newly collected live worms in a 9-cm Petri dish. H, Inner surface of tube with arrows showing perforations. C and E, holotype IOCAS MBM240650; F and G, paratype AM W.47811. B, C and D: Scale bars = 1 cm.

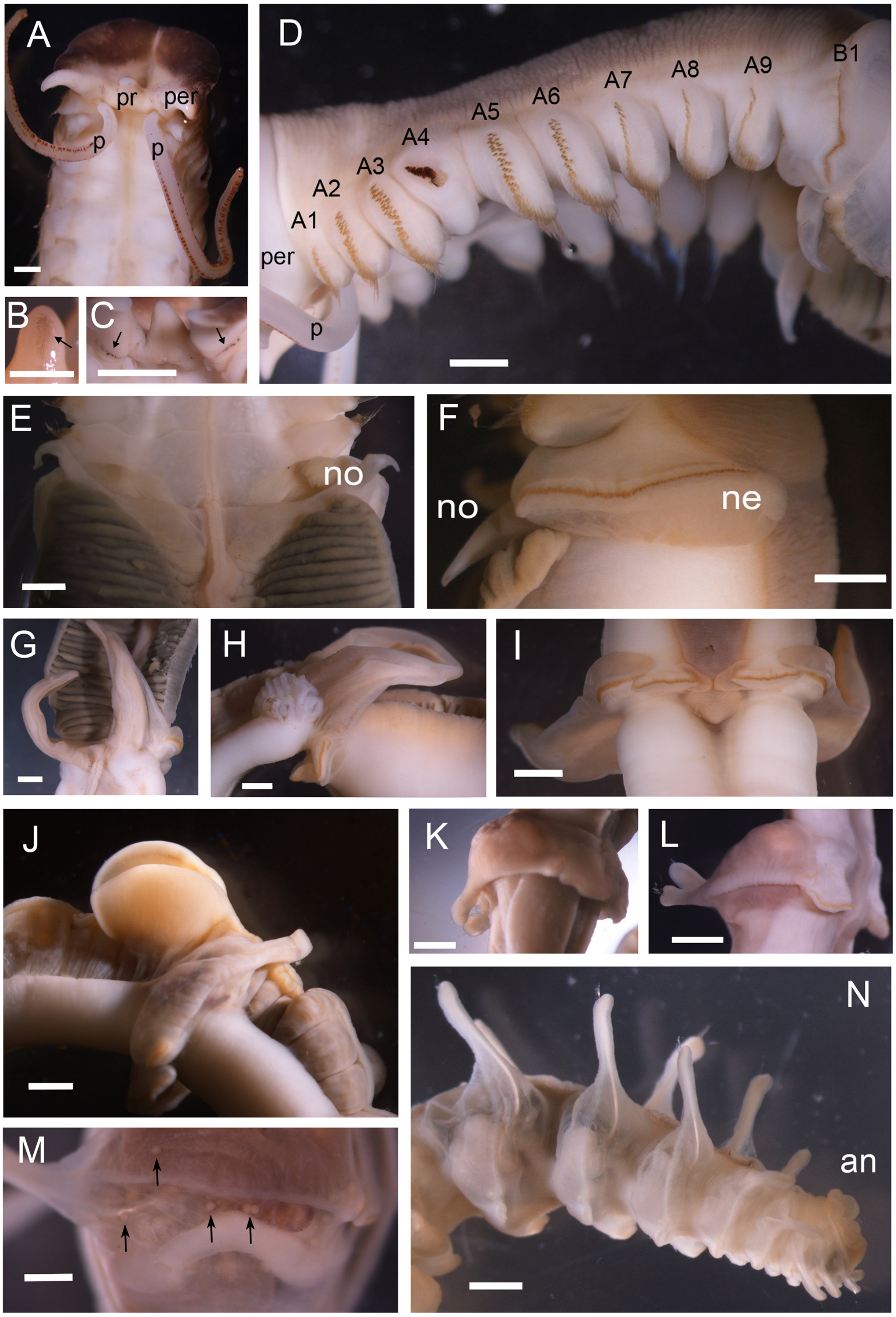

FIGURE 2. *Mesochaetopterus tingkokensis* **n. sp.** B, C, J, K, L and N: holotype; A, D, F and H: paratype AM W.47813; E, G and I: paratype AM W.47812; M: paratype IOCAS MBM240651. A, dorsal view of anterior with palps. B, pigment on ventral prostomium. C, dorsal view of the junction between prostomium and A1, with arrows showing the pigments. D, lateral view of the region A and B1. E, dorsal view of anterior B1. F, lateral view of anterior B1. G, dorsal view of anterior B2. H, lateral view of anterior B2. I, ventral view of anterior B2. J, lateral view of posterior B2 and anterior C1, showing the auricular accessory feeding organs (cupules). K, parapodia of C8 (anterior region C). L, parapodia of C20 (middle of region C). M, parapodia of C24 (near posterior end) with arrows showing eggs. N, posterior end and pygidium. Abbreviations: p, palp; per: peristomium; pr: prostomium; no: notopodia; an: anus. All scar bars represent 1 mm.

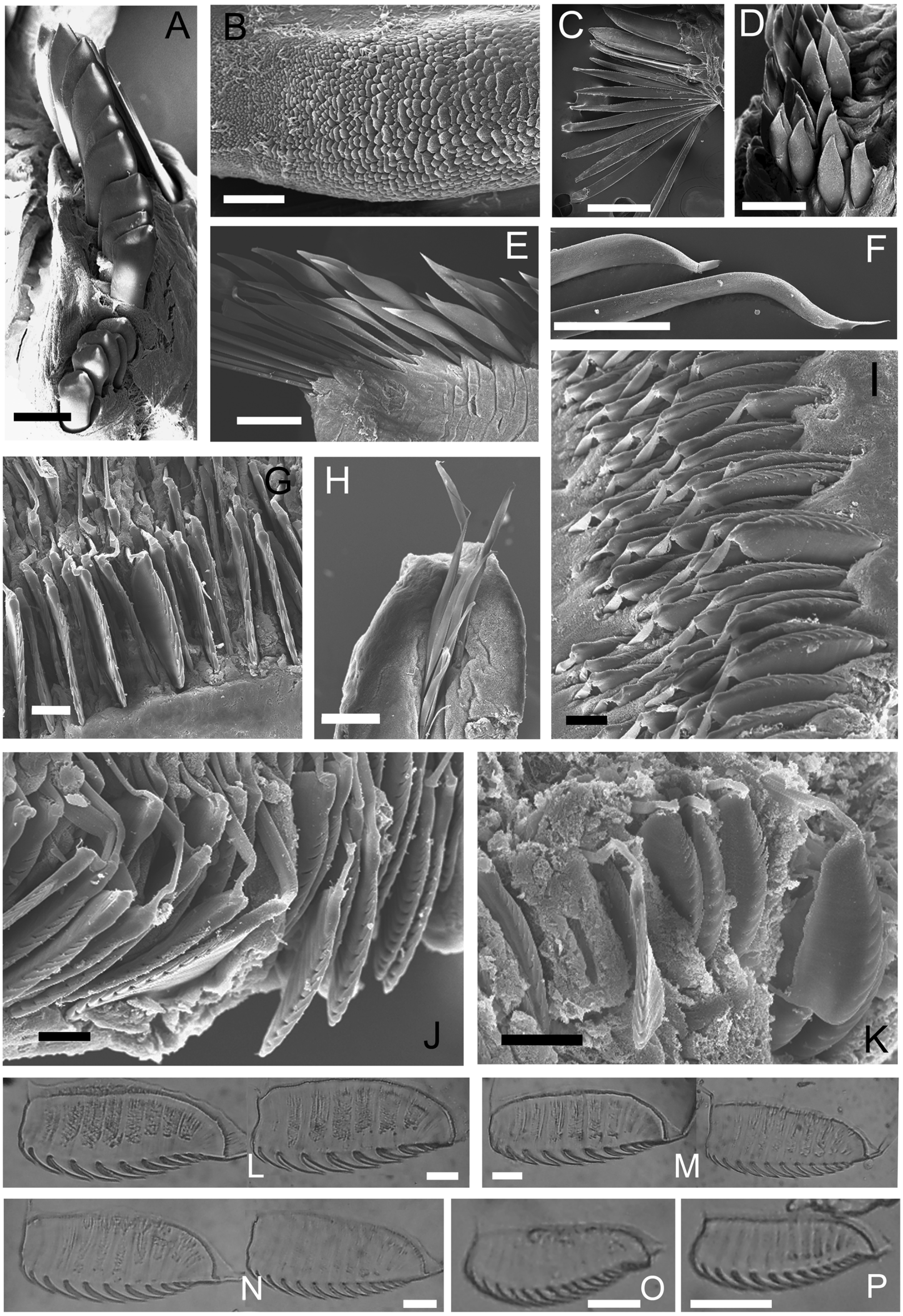

FIGURE 3. The chaetae of *Mesochaetopterus tingkokensis* **n. sp.** A–K, SEM micrographs; L–P, optical micrographs showing the shape of uncini. A and B, D–K: paratype IOCAS MBM240651; C: paratype IOCAS MBM240658; L–P: paratype AM W.47811. A, modified chaetae of A4. B, scale-like top surface of modified chaetae of A4. C, knife-like simple chaetae of A4. D, oar-like chaetae with pointed top on lateral side of A5. E, middle oar-like chaetae of A6. F, dorsal lateral lanceolate chaetae with rows of small projections from A3. G, uncini from ventral tori of B1 neuropodia. H, simple chaetae of C1 notopodia. I, uncini from ventral tori of C1 neuropodia. J, uncini in neuropodia from the middle of region C (chaetiger C20). K, uncini from neuropodia of the posterior of region C (chaetiger C34). L, Uncini from B1 neuropodia. M, Uncini from ventral (left) and lateral (right) tori of B2 neuropodia. N, Uncini from ventral (left) and lateral (right) tori of C1 neuropodia. O, Uncini from ventral tori of C20 (middle in region C). P, Uncini from ventral tori of C34 (posterior in region C). Scale bars: A, C–E: 200 µm; B, F, J–P: 20µm; G and I: 30µm; H: 100 µm.

Diagnosis. Large-sized *Mesochaetopterus*, exceeding 17.5 cm in body length for complete worms. A pair of palps with two longitudinal orange stripes. Region A with nine chaetigers; modified chaetae of A4 dark brown, 13 to 15 in number. Region B with two chaetigers; B1 with large wide neuropodia; B2 with large wing-shaped notopodia. Region C with 35–41 chaetigers (complete specimens), each chaetiger with club-shaped notopodia bearing 8–10 conspicuous capillary chaetae. Uncini with 8 to 10 teeth in B1, 10 to 12 teeth on ventral lobe and 12–14 teeth on lateral lobe in B2; uncini with 10 to 12 teeth on ventral lobe and 12–14 teeth on lateral lobe in C1.

Description. Holotype complete with tube (Figure 1C–D). Body width 6.0 to 8.0 mm, total length of complete specimens 17.9 to 24.1 cm, 47 to 52 chaetigers (9 in region A, 2 in region B and 36–41 in region C); all incomplete specimens lacking region C (Table 2).

TABLE 2. Major morphological characters in *Mesochaetopterus tingkokensis* **n. sp.**

	Catalogue no.	Body length (cm)	Regions contained	Length of region (cm)			Body width (mm)	Modified A4 chaetae (left/ right)	Length of palp (cm)	Sex
				A	B	C				
Holotype	IOCAS MBM240650	18.3	A1–C36	1.4	2.4	14.5	6.5	14/14	1.2	?
Paratypes	IOCAS MBM240651	17.9	A1–C37	1.4	2.5	14	8	14/14	2.2	♀
	AM W.47811	24.1	A1–C41	1.3	2.1	20.7	6.8	15/14	2	?
	AM W.47812	13.6	A1–C9	1.6	3	9.0+n.r.	7.5	14/14	1.4	?
	AM W.47813	2.4	A1–B1	1.4	1	n.r.	7	13/14	1.4	?
	AM W.47814	4.2	A1–C2	1.4	2.2	0.6+n.r.	6	13/13	n.r.	?
	IOCAS MBM240652	3	A1–B2	1.2	1.8	n.r.	7.2	14/14	2	?
	IOCAS MBM240653	3.2	A1–B2	1.1	2.1	n.r.	6.5	13/15	1.6	?
	IOCAS MBM240654	2.9	A1–B2	1.2	1.7	n.r.	6.8	13/13	1.5	?
	IOCAS MBM240655	4	A1–B2	1.5	2.5	n.r.	7.8	14/15	1.8	?
	IOCAS MBM240656	3.5	A1–B2	1.3	2.2	n.r.	7	14/14	1.9	?
	IOCAS MBM240657	4.6	A1–C2	1	2.5	1.1+n.r.	6.5	13/13	n.r.	?
	IOCAS MBM240658	1.8	A1–B1	1	0.8	n.r.	7	14/14	n.r.	?
	IOCAS MBM240659*									

n.r. character not recorded due to loss of the posterior part or the palps.
* Region A preserved in 100% ethanol for molecular analysis.

Region A long and narrow with 9 chaetigers, 1.0 to 1.6 cm long in preserved specimens (Table 2). Living specimens with a reddish orange ventral shield (Figure 1E–F); ventral shield color faded in preserved specimen (Figure 1C, 2D). Prostomium small, with a patch of light brown pigment on ventral side, without eyespots (Figure 2A). Peristomium extended, forming a wide collar surrounding prostomium, with two wing-shaped blackish patches facing peristomium (Figure 2A). Two palps arising dorsally from junction of prostomium and peristomium, slender (0.9 to 1.7 times of region A) with a mid-dorsal longitudinal groove and two lateral stripes of suture-like short orange bands. A line of brown pigment spots present dorsally on junction of peristomium and chaetiger 1(Figure 2C). A mid-dorsal ciliated groove on regions A to C (Figure 1E). Region A with 9 chaetigers; parapodia stout, cream-colored, with notopodia only (Figure 2D), with chaetae arranged in one row on A1 and A7–A9 and in two or three irregular rows on A2–A3 and A5–A6 (Figure 2D). Chaetae becoming gradually longer from lateral to dorsal side; on lateral side A1–A3 and A5–A9 notopodia, oar-like chaetae with short exposed part (Figure 3D); on dorsal side, lanceolate chaetae with long exposed part (Figure 3E); lanceolate chaetae long, asymmetrical, with rows of small knobs on one side, similar to modified chaetae on A4 (Figure 3F). Notopodia of A4 modified, shorter than those of A3 and A5, with chaetae on lower lateral side only; chaetae two types: 13 to 15 dark brown modified chaetae stout, knoblike, with a scaly surface on the top (Figure 3A–B), and 11 to 12 slender knife-like simple chaetae (Figure 3C), posteriorly to upper stout chaetae (Figure 3A).

Region B with two chaetigers, both longer than A and C ones. B2 longer (15.0–19.0 mm vs. 10.0–12.0 mm) and broader (11.0–14.0 mm vs. 5.0–6.5 mm) than B1. Dorsal luminous gland on B1, yellow in living worms (Figure 1G) grey in preserved specimens (Figure 2E and G), with transverse elevated ridges. Parapodia of region B biramous. B1 notopodia unilobed, small, pointed, digitiform, with 22 to 25 embedded chaetae but without exposed chaetae (Figure 2E–F). B1 neuropodia unilobed, with a row of uncini along ridge (Figure 2F). B2 notopodia elongated, large wing-shaped, with 80 to 90 embedded chaetae but without exposed chaetae (Figure 2G–I).A pair of white fluffy ball-shaped structures at base of, and immediately behind, B2 notopodia (Figure 2H). Associated feeding organ (cupule) on posterior side of B2 and anterior side of C1 (Figure 2J). B2 neuropodia bilobed, with ventral and lateral tori (Figure 2I) having one row of uncini each. Uncini of B1 neuropodia with 8 to 10 teeth (Figure 3F). Ventral uncini of B2 neuropodia with 10 to 12 teeth, and lateral uncini with 12 to 14 teeth (Figure 3F, L and M). Length of uncini 104 to 114 µm.

Region C with 36 to 41 chaetigers in complete specimens. Parapodia all biramous. Notopodia unilobed, digitiform, with 8 to 10 conspicuous simple chaetae (Figure 2J–N; Figure 3H). Neuropodia bilobed as in B2, but lateral tori much smaller (only 1/3 the length of lateral tori of B2). Uncini of C1 100–105 µm long, smaller than that of B2 but with more teeth: 10–12 for ventral tori (Figure 3I) and 12–14, (Figure 3N) for lateral tori. Uncini in chaetiger 20 (middle region C, paratype IOCAS MBM240651) around 65µm long, with 12–14 teeth in both ventral and lateral tori, much smaller than those of C1 (Figure 3I). Uncini in posterior region near anus around 45 µm long, with 13–15 teeth on both lateral and ventral tori (Figure 3J). Oocytes present in neuropodia of IOCAS MBM240651 (Figure 2M).

Tube. Straight (Figure 1D), perpendicular to sediment surface. Upper 1–2 cm grey to light brownish, extending out of sediment surface, tapering towards opening (3–4 mm in diameter). Most of the tube buried in sediment, similar throughout (6–8 mm in diameter), dark brown. Outer surface rough, coated with sand (Figure 1D). Inner surface smooth, membranous, with perforations (Figure 1H) throughout entire tube.

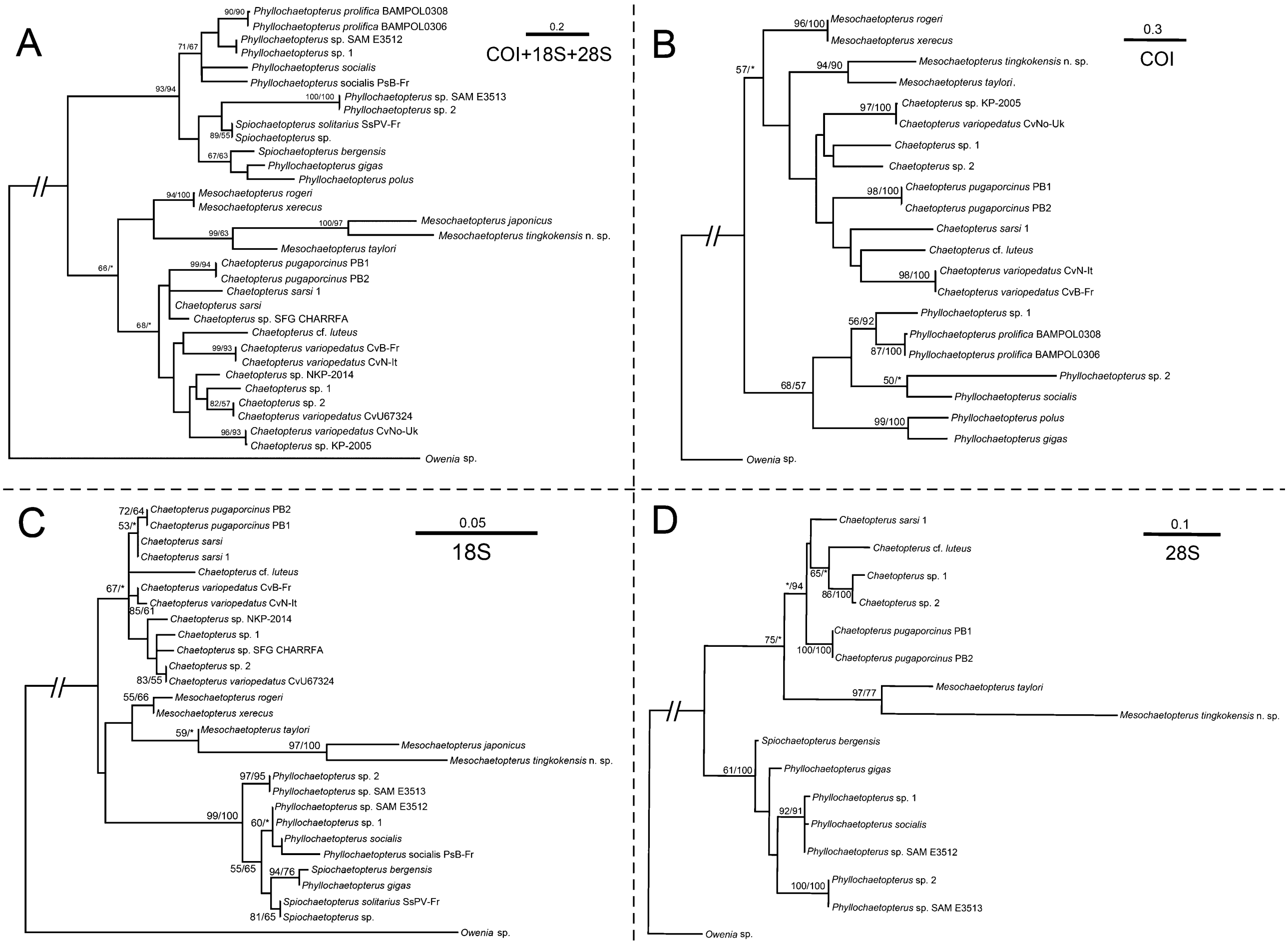

FIGURE 4. Phylogenetic tree generated by two methods of analysis (ML/MP). A, based on concatenated COI, 18S rRNA and 28S rRNA gene sequences; B–D, based on individual genes. Numbers above the branches represent ML/MP bootstrap values based on 1,000 replicates. Highest possible support is 100. Values below 70 are considered weak, and values below 50 are not shown. Clade with an asterisk indicates bootstrap value below 50.

Distribution. Currently only known from Tolo Harbor, Tai Tam Tuk, and Tsing Lung Garden in Hong Kong.

Molecular analysis. Partial sequences of COI (572 bp), 18S rRNA (646 bp) and 28S rRNA (779 bp) and their concatenated sequences (1997 bp) were used for phylogenetic tree construction based on the ML and MP analysis. The results from the concatenated sequences and 18S (Figure 4A, C) show that the various *Mesochaetopterus* spp. form a clade, which is consistent with the finding of Martin *et al.* (2008). However, the support of the clade is weak, as the bootstrap values are below 50. But the results from COI indicate that genus is paraphyletic (Figure 4B). Nevertheless, all results from concatenated and individual gene sequences (Figure 4) show that *M. tingkokensis* **n. sp.** is most closely related with at least one other species of *Mesochaetopterus*, supporting the placement of this new species into the genus.

Discussion

According to the grouping scheme proposed by Nishi *et al.* (2009), *M. tingkokensis* n. sp. belongs to group 3 with large wing-shaped notopodia. This group currently has four species: *M. japonicus* Fujiwara, 1934; *M. selangolus* Rullier, 1976; *M. ecuadorica* Nishi *et al.* 2009 and *M. tingkokensis* n. sp. They are all large worms, having nine chaetigers in region A (rarely ten in *M. selangolus* and *M. ecuadorica*), two parapodia in region B (with B1 wide and B2 slender) and a luminous organ with transverse ridges in B1. The chaetae of region A (including A4 modified ones), although variable in number inter- and intra-specifically, are morphologically similar.

Mesochaetopterus ecuadorica and *M. selangolus* have been reported only from Galapagos Islands and Malaysia, respectively; while *M. japonicus* occurs in Japan (Nishi 1999), mainland China (Yang & Sun 1988) and Taiwan (Nishi & Hsieh 2009). Taiwan is only roughly 600 km away from Hong Kong, with a similar climate. In Hong Kong, information about the taxonomy of Chaetopteridae is scarce (Sun & Qiu 2014). *Mesochaetopterus japonicus* was reported from protected beaches, with the symbiotic pinnotherid crab *Tritodynamia rathuni* living inside the tube (Morton & Morton 1983), but the exact sampling locations were not given. However, our recent sampling of nearly a dozen of Hong Kong protected beaches revealed *M. tingkokensis* n. sp. as the only large sized *Mesochaetopterus*. It is most abundant at Ting Kok, but is also found in small numbers at Tai Tam Tuk, and Tsing Lung Garden in Hong Kong waters. Among the samples collected, there were no crabs or other commensal animals inside the tube, which indicates that *M. tingkokensis* **n. sp.** may not host commensal organisms. Nevertheless, since the relationship between symbionts and their polychaete hosts are often species specific (Martin & Britayev 1998), the report of a pinnatherid crab inside the tube of a chaetopterid in Hong Kong by Morton & Morton (1983) may indicate that they had collected another large-sized chaetopterid species.

Mesochaetopterus tingkokensis **n. sp.** can be distinguished from the other three species in group 3 by several morphological characters (Table 3 and Figure 5).

1. Palps. In *M. tingkokensis* **n. sp.** palps have two longitudinal orange stripes, still visible in 6-month preserved specimens (Figure 5C). In *M. ecuadorica*, *M. selangolus* and *M. japonicus*, the palps do not bear longitudinal colour stripes. In fact, the palp colour pattern in *M. tingkokensis* **n. sp.** is singular among all currently recognized species of *Mesochaetopterus.*

2. Prostomium. In *M. tingkokensis* **n. sp.**, it is much larger (height:width about 1:1) than in *M. japonicus* and *M. selangolus* (height:width about 1:4) (Figure 5A–C), but it is similar to *M. ecuadorica* based on Nishi *et al.* (2009). The inner side of prostomium of *M. tingkokensis* **n. sp.** has brown pigments, but that of the other two species is unpigmented.

3. Number of A4 modified chaetae. It ranges from 13 to 15 in *M. tingkokensis* **n. sp.**, 8 to13 in *M. japonicus*, 8 to 15 in *M. selangolus*, and 8 to 10 in *M. ecuadorica*;

4. B1 notopodia. In *M. tingkokensis* **n. sp.**, they are short (B1 notopodia length:B1 trunk width = ~1:4–1:5), with a pointed end (Figure 2E); whereas in *M. japonicus* and *M. selangolus* they are longer (B1 notopodia length:B1 trunk width = ~1:2), with a blunt end (Figure 5D–F), as well as in *M. ecuadorica* according to Nishi *et al.* (2009).

5. Teeth of uncini in regions B and C. In B2, the neuropodial uncini of *M. tingkokensis* **n. sp.** have 10 and 12 teeth in the ventral lateral tori, respectively; while *M. japonicus* has 8 to 9 in both tori, *M. selangolus* has 7 to 8 in both tori (Nishi 1999), and *M. ecuadorica* has 7 teeth (although Nishi *et al*. 2009 did not report whether this number was from ventral or lateral tori). Similar results apply to the first few region C neuropodia.

6. Size of uncini. In region B, uncini of *M. tingkokensis* **n. sp.** are 104–114 µm long, while those of *M. selangolus* are 60–85 µm long and those of *M. japonicus* are 55–85 µm long. The same pattern occurs for the uncini

TABLE 3. Comparison among four similar species of *Mesochaetopterus*. Data on *M. japonicus*, *M. selangolus* and *M. ecuadorica* were from Martin *et al.* (2008) and Nishi (1999). Asterisks indicate distinctive characters for *M. tingkokensis* **n. sp.** Other characters overlap with other species.

Characters	*M. tingkokensis*	*M. japonicus*	*M. selangolus*	*M. ecuadorica*
Body length (cm)	17.9–25	25	25 (up to 60)	12–18
Body width (mm)	6–8	4–5	6–8	7–8
Trunk colour in living worm	brownish	cream-coloured	?	brownish
Prostomium height:width, colour	~ 1:1, with light and dark brown pigment	~1:4, unpigmented	~1:4, unpigmented	~1:1, unpigmented?
*Palp colour	two longitudinal orange bands	unpigmented	uniform pale brownish	uniform pale brownish
Palp:region A length	0.9–1.7:1	1:1	1.5:1	?
Segments in region A	9	9	9 (rarely 10)	9 (rarely 10)
A4 modified chaetae	13–15	8–13	8–15	8–10
*B1 notopodia	short and conical with a pointed end	long and digitiform with a blunt end	long and digitiform with a blunt end	short and digitiform with a blunt end
B1 notopodium length : B1 trunk width	~1:4–1:5	~1:1.5–1:2	~1:2	~1:4
Uncini teeth in region B	8–13	9–10	7–8	8–9
Uncini teeth in B2	ventral: 10; lateral: 12	8–9	7–8	8–9
Length of uncini in B1 (μm)	104–114	72–85	70–85	?
*Notopodia chaetae of region C	8–10 long capillary chaetae	7–9 embedded aciculae	6–8 embedded aciculae	6–8 embedded aciculae
Uncini teeth in C1 (ventral/lateral)	10–12/12–14	10–12/10–12	9–11/9–11	10–11/10–11
Anus	tapered	tapered	open	tapered
Tube	dark brown, parchment-like with perforations; coated with sand grains on outside; slightly tapered opening	long, straight or L-shaped and parchment-like; generally dark coated with sand grains; straight opening	J-shaped, parchment-like with embedded sand and periodic annulated rings and perforations at the lower end; slightly tapered opening	J-shaped, nearly straight orientation, delicate and bears some minute perforations; slightly tapered opening
Type locality	Hong Kong	Japan	Malaysia	Galapagos Islands

from C1, with 100–105 μm, 35–60 μm and 40–65 μm long in *M. tingkokensis* **n. sp.**, *M. selangolus* and *M. japonicus*, respectively.

7. Notopodial chaetae of region C. In *M. tingkokensis* **sp. n.**, they are simple, conspicuous even to the naked eye. In *M. ecuadorica* and *M. selangolus*, region C notopodia bear embedded aciculae only, with no chaetae extending outside the epidermis (Nishi 1999). In *M. japonicus,* region C notopodia were reported to bear minute chaetae (Nishi 1999). All seven specimens of *M. japonicas*, and 2 out of 3 of *M. selangolus*, we examined had region C. However, all their corresponding notopodia do not bear externally visible chaetae (Figure 5G and H).

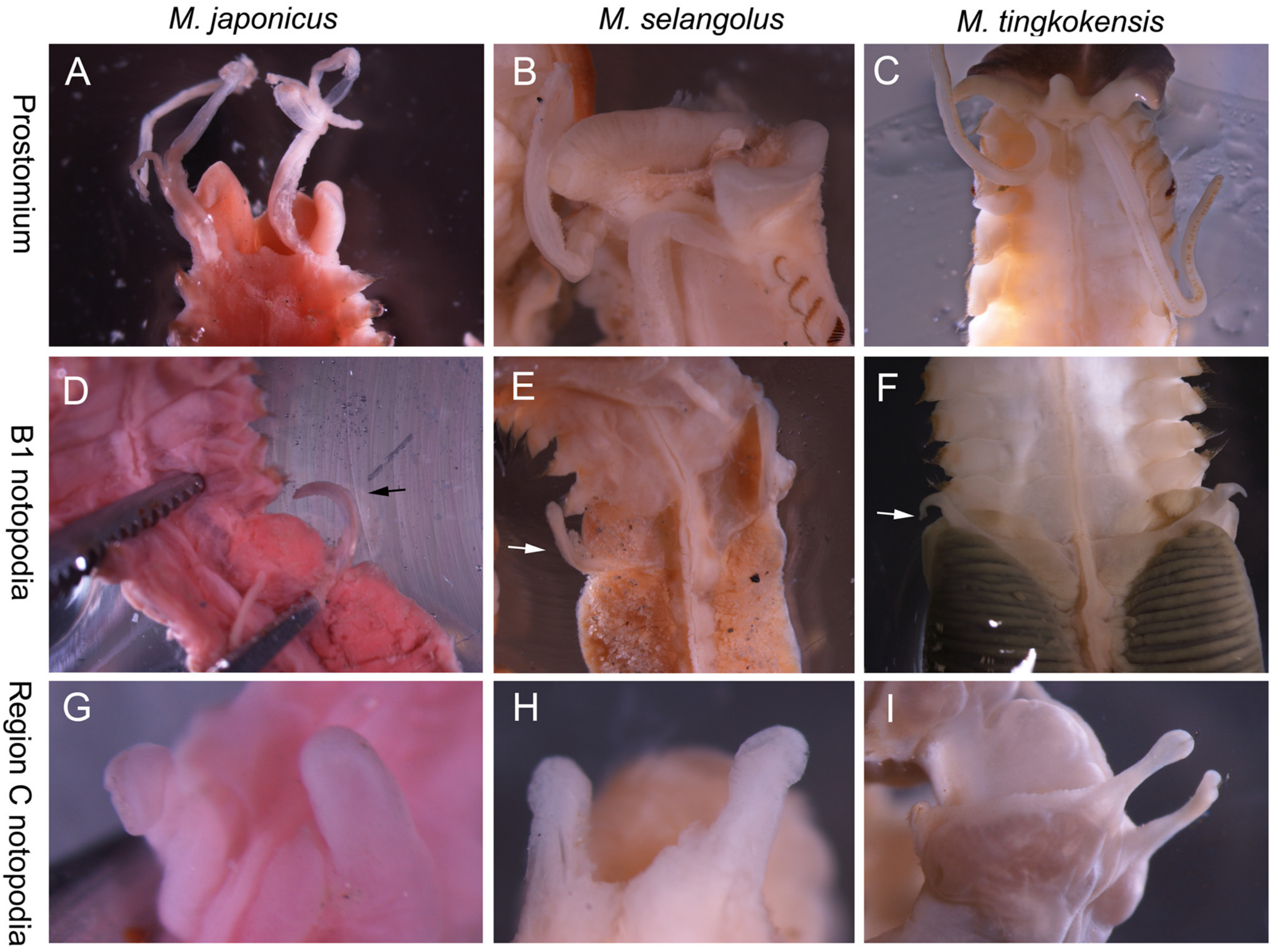

FIGURE 5. Comparison of *Mesochaetopterus japonicus*, *M. selangolus* and *M. tingkokensis* **n. sp.** A and D: CBM-ZW522; G: CBM-ZW523; B, E and H: CBM-ZW601; C and F: paratype AM W.47813; I: paratype IOCAS MBM240651.

Our molecular analysis used two slowly evolving genes (18S and 28S rRNA) and a faster evolving gene (COI). These genes have been used in the phylogeny of chaetopterid worms (Osborn *et al*. 2007; Martin *et al.* 2008; Morineaux *et al*. 2010). In this study, these three gene fragments were concatenated to generate a phylogenetic tree by both ML and MP analyses. The two analyses placed all *Mesochaetopterus* species, including *M. tingkokensis* **n. sp.**, in the same clade, with the new species being most closely related to *M. japonicus*. The other *Mesochaetopterus* species with wing-shaped B2 notopodia were not included in the analyses due to the lack of molecular data. Based on Nishi's grouping scheme (2009), *M. rogeri* with small triangular notopodia in region B belongs to group 1; *M. xerecus* and *M. taylori* are both described as large worms with conical parapodia in region B and belong to group 2. However, *M. xerecus* is most closely related to *M. rogeri* instead of *M. taylori* (Figure 4). Furthermore, *Mesochaetopterus* and *Chaetopterus* are sister groups, whereas *Phyllochaetopterus* and *Spiochaetopterus* are paraphyletic (Figure 4).

It should be noted that the *Mesochaetopterus* clade has only a low bootstrap support. In the phylogenetic analyses with COI, this clade is not supported (Figure 4B), which is consistent with previous reports (Osborn *et al.* 2007; Morineaux *et al.* 2010). Further taxon sampling and more gene sequences will thus be needed to resolve the phylogenetic relationship among the different genera of Chaetopteridae, whether *Mesochaetopterus* is monophyletic, and whether the 3-group scheme of *Mesochaetopterus* based on the shape of region B notopodia agrees with the natural phylogenetic history of this genus.

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