Zoological Studies **63**:41 (2024)
doi:10.6620/ZS.2024.63-41

Zoological Studies

Open Access

A New Species of *Natsushima* (Annelida: Chrysopetalidae) Living in the Mantle Cavity of a Deep-Sea Solemyid Clam

Fan Hui[1], Yi-Tao Lin[1], Maeva Perez[1], Jian-Wen Qiu[1], and Yanan Sun[2,*]

[1]*Department of Biology, Hong Kong Baptist University, Hong Kong SAR, China. E-mail: 23482648@life.hkbu.edu.hk (Hui); linyitao0@outlook.com (Lin); maeperez@hkbu.edu.hk (Perez); qiujw@hkbu.edu.hk (Qiu)*
[2]*Laboratory of Marine Organism Taxonomy and Phylogeny, Qingdao Key Laboratory of Marine Biodiversity and Conservation, Institute of Oceanology, Chinese Academy of Sciences, Qingdao, China. *Correspondence: E-mail address: sunyn@qdio.ac.cn (Sun)*

urn:lsid:zoobank.org:pub:79634D57-D17C-4797-A8A3-C5374440C19F
Received 3 May 2024 / Accepted 8 August 2024 / Published 5 December 2024
Communicated by Benny K.K. Chan

Natsushima is a genus of deep-sea Chrysopetalidae (Annelida) characterized by numerous bifurcate chaetae. It is poorly known, with three species living in the mantle cavity of bivalves in chemosynthetic habitats. Here we describe *Natsushima nanhaiensis* n. sp. based on an integrative morphological and molecular phylogenetic analysis of specimens collected from the Haima cold seep in the South China Sea. Morphologically, the new species can be distinguished from its congeneric species by the shape and number of the neuropodial hooks and bifurcate chaetae, the shape of the parapodia, and the long dorsal cirri. Sequence comparison and phylogenetic analysis based on the mitochondrial *COI* and *16S rRNA* gene sequences supported the placement of *Natsushima nanhaiensis* n. sp. in *Natsushima* and its status as a distinct species. We also present a key to species of *Natsushima* and discuss their biogeography.

Key words: Haima cold seep, Deep sea, *Natsushima*, Polychaeta, Symbiotic species

BACKGROUND

Chrysopetalidae Ehlers, 1864 is a family of marine Annelida with a broad morphological, ecological and habitat diversity. There are around 110 species in 31 genera and three subfamilies: Calamyzinae Hartmann-Schröder, 1971, Chrysopetalinae Ehlers, 1864 and Dysponetinae Aguado, Nygren & Rouse, 2013 (Rouse et al. 2022). Most chrysopetalids have historically been considered free-living forms inhabiting hard substrates from shallow-water coral reefs to deep-sea hydrothermal vents (Russell 1986 1997; Böggemann 2009; Watson et al. 2014). However, there are fifteen genera within the subfamily Calamyzinae (Watson et al. 2016) which are either endosymbiotic in molluscs or ectosymbiotic on polychaetes and octopuses (Jimi et al. 2019 2022).

Natsushima Miura and Laubier, 1990 is a genus in the subfamily Calamyzinae. Members of *Natsushima* are obligate symbionts living in the mantle cavity of deep-sea chemosynthetic bivalves. They have subbiramous parapodia with simple ventral hooks, single embedded acicula and many bifurcate simple setae (Miura and Laubier 1990). To date, only three species of *Natsushima* have been described: (1) *Natsushima bifurcata* Miura & Laubier, 1990 from a cold seep of Sagami Bay, western Pacific Ocean at 1170 m water depth, with *Acharax* sp. as the host (Miura and Laubier 1990); (2) *Natsushima graciliceps* Miura & Hashimoto, 1996 from Kagoshima Bay, western Pacific Ocean at 98 m water depth, associated with an undescribed solemyid clam (Miura and Hashimoto 1996); and (3) *Natsushima sashai* Aguado & Rouse, 2011 from a methane seep off Costa Rica, eastern Pacific Ocean at 1001 m water depth, hosted by *Acharax* sp. (Aguado and Rouse 2011).

Citation: Hui F, Lin YT, Perez M, Qiu JW, Sun Y. 2024. A new species of *Natsushima* (Annelida: Chrysopetalidae) living in the mantle cavity of a deep-sea solemyid clam. Zool Stud **63**:41. doi:10.6620/ZS.2024.63-41.

The South China Sea is a large marginal sea in the Western Pacific (Liang et al. 2017; Feng et al. 2018). The Haima cold seep, discovered in 2017 on the northwestern slope of the South China Sea, hosts a distinct chemosynthetic fauna (He et al. 2023). The dominant macrofauna at this site are epibenthic bivalves and annelids, which harbour endosymbiotic chemosynthetic bacteria, including bathymodioline mussels *Gigantidas haimaensis* (Xu et al. 2019), vesicomyid clams *Archivesica marissinica* (Chen et al. 2018), glass scallops *Catillopecten margaritatus* (Lin et al. 2023), and siboglinid tubeworms *Sclerolinum annulatum* (Xu et al. 2022) and *Paraescarpia echinospica* (Sun et al. 2021).

During a research cruise to the Haima cold seep in 2021, we collected a specimen of solemyid clam later identified as *Acharax haimaensis* (Yang et al. 2024). Dissection of the clam onboard the research vessel revealed the presence of five polychaete worms in its mantle cavity. A preliminary analysis of these worms suggested they belong to an undescribed species of *Natsushima*. Thus, the objectives of this study are to describe the species and determine its phylogenetic relationships with other congeneric species. This study expands our understanding of the diversity of *Natsushima* in the western Pacific Ocean. Given that solemyid clams are widespread across chemosynthetic habitats globally (Cavanaugh et al. 2006), many more species of *Natsushima* may likely be awaiting discovery.

MATERIALS AND METHODS

Sample collection

The worm specimens were obtained from the Haima cold seep in the South China Sea at a depth of 1385 m (16.73216°N, 110.46122°E) in 2021 using the *Challenger Deep* – a Human Occupied Vehicle (HOV) – on-board the research vessel (R/V) *Exploration 2* of the Institute of Deep-sea Science and Engineering, Chinese Academy of Sciences. One specimen of solemyid clam later described as *Acharax haimaensis* (Yang et al. 2024) was immediately dissected upon arrival on the main deck of the R/V, and all the chrysopetalids inside the host were preserved in 100% ethanol.

Morphological analysis

The whole *Natsushima* individuals were photographed under a digital camera (Canon EOS 5D Mark IV, Japan). An NSZ-608T digital stereomicroscope (Jiangnan Novel Optics, China) coupled with a SEYE v2.0 digital camera (Shenzhen Young Win Technology, China) and a CX41 compound light microscope (Olympus, Tokyo, Japan) with a digital camera (Canon EOS 760D, Japan) were used to examine morphological features. Different body parts were dissected for scanning electron microscopy (SEM). The dissected samples were treated with a gradient of ethanol solutions (*i.e.*, 50, 75, and 100%, each for 10 min), followed by HMDS (hexamethyldisilazane) for 5–7 min to dry the samples inside a fume hood, mounted on a conductive carbon adhesive tape, sputter-coated with gold, and observed under a LEO 1530 Field Emission Scanning Electron Microscope (LEO Elektronenmikroskopie GmbH, Oberkochen, Germany).

All specimens used in this study are deposited in the Tropical Marine Biodiversity Collections of the South China Sea (TMBC), Chinese Academy of Sciences, Guangzhou, China.

DNA extraction, amplification and sequencing

The genomic DNA of five specimens of *N. nanhaiensis* n. sp. was extracted from the midbody tissues using the DNeasy Blood & Tissue Kits (Qiagen). DNA quality was examined with 1.0% agarose gel electrophoresis, and its concentration was measured using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, USA).

Two gene markers were amplified using TakaRa PCR Master Mix (Japan) following the manufacturer's protocol. Primers used to amplify the target gene fragments are listed in table 1 (Sjölin et al. 2005; Carr et al. 2011). The PCR temperature profiles were as follows: 95°C/5 min, 35 cycles of 94°C/30 s, 48°C/60 s and 72°C/60 s, followed by 72°C/7 min

Table 1. Primers used in PCR amplification and sequencing

Gene	Primer	5'-3'	Reference
COI	F-LCO1490	GGTCAACAAATCATAAAGATATTGG	Carr et al. 2011
	R-HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	
16S	AnnF	GCGGTATCCTGACCGTRCWAAGGTA	Sjölin et al. 2005
	AnnR	TCCTAAGCCAACATCGAGGTGCCAA	

for mitochondrial cytochrome oxidase I (*COI*) gene; 94°C/2 min, 35 cycles of 94°C/40 s, 60°C/40 s and 72°C/45 s, followed by 72°C/5 min for mitochondrial *16S rRNA* (*16S*) gene.

The PCR products were examined by electrophoresis using 1.0% agarose gel and purified using the ZymocleanTM Gel DNA Recovery Kit (Zymo Research, USA) following the manufacturer's protocol. The purified PCR products were bi-directionally sequenced on an ABI PRISM® 3730xl DNA Analyzer (Thermo Scientific, USA), using the same primers as for PCRs. The obtained sequences were manually examined and assembled using the DNASTAR Lasergene package (DNASTAR, USA). All sequences are deposited in GenBank with the accession numbers listed in table 2.

Phylogenetic analyses and genetic distance estimation

A total of 10 sequences of *COI* and *16S* genes from the new species, as well as 49 sequences of the two genes from 26 species of chrysopetialids downloaded from the GenBank (https://www.ncbi.nlm.nih.gov/) (Table 2), were used for phylogenetic analyses. Two species of Phyllodocidae, *Eulalia viridis* and *Notophyllum foliosum*, were included as outgroups (Ravara et al. 2019). Alignments were performed using MAFFT v7.505 (Katoh and Standley 2013) with the default parameters, then trimmed using Gblocks v0.91b (Gblocks parameters: minimum length of a block = 5; allowed gap positions = with half) (Talavera and Castresana 2007). A concatenation of

Table 2. Sequenced specimens and GenBank accession numbers

Subfamily/Species	*COI*	*16S*	Reference
Calamyzinae			
Boudemos ardabilia	EU555052	EU555051	Ravara et al. 2019
Boudemos flokati	EU555065	EU555034	Ravara et al. 2019
Calamyzas amphictenicola	JX078956	JX093563	Ravara et al. 2019
Calamyzinae sp.	JX078957	JX078951	Ravara et al. 2019
Calamyzas crambon	ON763130.1		Ravara et al. 2019
Craseoschema thyasiricola	MK988420	MK988419	Ravara et al. 2019
Iheyomytilidicola lauensis	JF304502	JX078952	Ravara et al. 2019
Laubierus alvini	JF304494	JX078950	Ravara et al. 2019
Micospina auribohnorum	JX093564	JX078949	Ravara et al. 2019
Natsushima bifurcata	JF304492	JX078953	Ravara et al. 2019
Natsushima sashai	JF304496	JX078954	Ravara et al. 2019
Natsushima nanhaiensis n. sp. (holotype)	**PP792856**	**PP790211**	This study
Natsushima nanhaiensis n. sp. (paratype 1)	**PP792857**	**PP790212**	This study
Natsushima nanhaiensis n. sp. (paratype 2)	**PP792958**	**PP790213**	This study
Natsushima nanhaiensis n. sp. (paratype 3)	**PP792859**	**PP790214**	This study
Natsushima nanhaiensis n. sp. (paratype 4)	**PP792860**	**PP790215**	This study
Shinkai fontefridae	JF304499	JX078948	Ravara et al. 2019
Shinkai longipedata	JF304500		Ravara et al. 2019
Spathochaeta octopodis	LC381959	LC381961	Ravara et al. 2019
Vigtorniella zaikai	KU057939	KU057933	Ravara et al. 2019
Chrysopetalinae			
Dysponetus populonectens	JQ623495	JX078955	Ravara et al. 2019
Dysponetus bulbosus	JQ623501	DQ442570	Ravara et al. 2019
Dysponetus caecus	AF221568	EU555047	Ravara et al. 2019
Dysponetus caecus 2		GQ426603	Ravara et al. 2019
Dysponetus sp.	EU555055	EU555048	Ravara et al. 2019
Dysponetinae			
Arichlidon reyssi	EU555054.1	EU555045.1	Ravara et al. 2019
Chrysopetalum debile	AF221567	EU555046	Ravara et al. 2019
Paleanotus sp.	EU555056	EU555050	Ravara et al. 2019
Bhawania heteroseta	EU555053	EU555044	Ravara et al. 2019
Outgroup			
Eulalia viridis	AY996122	AY996064	Ravara et al. 2019
Notophyllum foliosum	AY996117	DQ779627	Ravara et al. 2019

the gene fragments was conducted using PhyloSuite v1.2.3 (Zhang et al. 2020), with missing genes filled with "-". The Maximum likelihood (ML) analysis of the concatenated data set (*16S*+*COI*) was conducted using IQ-TREE v2.2.0 (Nguyen et al. 2015), with the GTR+R4+F model selected by ModelFinder (Kalyaanamoorthy et al. 2017) implemented in IQ-TREE, and ran for 5,000 ultrafast bootstraps (Minh et al. 2013). Bayesian Inference analysis was performed using MrBayes v3.2.7a (Ronquist et al. 2012) under GTR+I+F model (2 parallel runs, 2000000 generations, sampling every 1000 generations, in which the initial 25% of sampled data were discarded as burn-in). The phylogenetic trees and node labels were graphically edited with iTOL (Letunic and Bork 2007).

The Kimura's 2-parameter (K2P) model was then used for genetic distance calculation (Kimura 1980) in MEGA7 (Kumar et al. 2016) under the default settings.

RESULTS

SYSTEMATICS

Phylum Annelida Lamarck, 1802
Family Chrysopetalidae Ehlers, 1864
Genus *Natsushima* Miura & Laubier, 1990
Type species *Natsushima bifurcata* Miura & Laubier, 1990

Natsushima nanhaiensis n. sp.

(Figs. 1–2)
urn:lsid:zoobank.org:act:7AE5F9DA-92A3-4285-8A47-81012767803D

Type specimens: Holotype and paratypes are deposited in the Tropical Marine Biodiversity Collections of the South China Sea (TMBC), Chinese Academy of Sciences, Guangzhou under catalogue numbers TMBC031043 (holotype) and TMBC031044–TMBC031047 (paratypes 1–4).

Type locality: Haima cold seep, 1383 m water depth, off southern Hainan Island, on the northwestern slope of the South China Sea.

Etymology: The species is named after Naihan, the Chinese name for the South China Sea. The Chinese name corresponding to the Latin name of this species is "南海松島蟲".

Diagnosis: Body long, tapering posteriorly, flattened ventrally, arched dorsally. Prostomium short, with pair of antennae, no eyes. Parapodia subbiramous. Notopodia and neuropodia similar length, notoacicula absent. Neuropodia stout, with long neuroacicula, 2–6 hooks and numerous bifurcate chaetae with similar tooth length. Pygidium rounded, without anal appendages.

Description: Holotype 46 mm long, 2.7 mm wide including parapodia, 112 segments. Longest paratype 52 mm long, 2.0 mm wide with 175 segments (Fig. S1). Holotype and all paratypes complete except paratype 2.

Body vermiform, flattened ventrally and arched dorsally, tapering anteriorly and posteriorly (Fig. 1A–E). Specimens preserved in alcohol pale, body surface smooth. Living specimens pink-red.

Prostomium oval, short, with pair of small antennae, without eyes (Figs. 1D; 2A–B). Mouth located between prostomium and first chaetiger, without jaw (Figs. 1E; 2B). First chaetiger partially fused to prostomium, with dorsal and ventral cirri, and numerous neuropodial chaetae (Figs. 1C; 2A–B). Posterior segments similar in width as anterior segments (Fig. 1A). Pygidium simple, rounded, without anal cirri (Figs. 1D; 2F).

Parapodia subbiramous throughout, notopodia conical, dorsal cirri well-developed, basally swollen and distally pointed, without notoacicula (Fig. 1F). Neuropodia stout, with short ventral cirri (Fig. 2A, C–D). Each neuropodium supported by single very long, straight and thick embedded neuroacicula with pointed tip (Fig. 1F). Neuropodia similar in length with notopodia in midbody parapodia (Fig. 1B, F).

Chaetae simple, consisting of stout hooks and smaller bifurcate chaetae. Hooks few (2–6), with long handle, slightly curved distal end and swollen subdistal knob (Figs. 1G–H; 2H). Bifurcate chaetae numerous, located below hooks (Figs. 1F, H; 2A–E, G). Distal teeth of bifurcate chaetae curved, height of two teeth approximately equal, one tooth slightly thinner and sharper than the other (Figs. 1I–K; 2I). Middle segments with more hooks and bifurcate chaetae than anterior and posterior segments.

Distribution: Currently known only from the Haima cold seep.

Remarks: Three species (*N. bifurcata*, *N. graciliceps*, *N. sashai*) have been described in the genus *Natsushima*. *Natshushima nanhaiensis* n. sp. can be distinguished from its congeneric species by a combination of morphological characters of the chaetae and parapodia, such as the length, shape and number of neuropodial hooks. *Natshushima nanhaiensis* n. sp. has up to six neuropodial hooks per midbody neuropodium (Fig. 1H) while only two to four are present in the other *Natsushima* species: 3–4 in *N. sashai*, 2–4 in *N. bifurcata*, three in *N. graciliceps* (Miura and Laubier 1990; Miura and Hashimoto 1996; Aguado and Rouse 2011). The hooks of *N. nanhaiensis* n. sp. are significantly longer and thinner than those of *N. sashai*, similar to those in *N. bifurcata* (considering the length between the distal tip and subdistal knob):

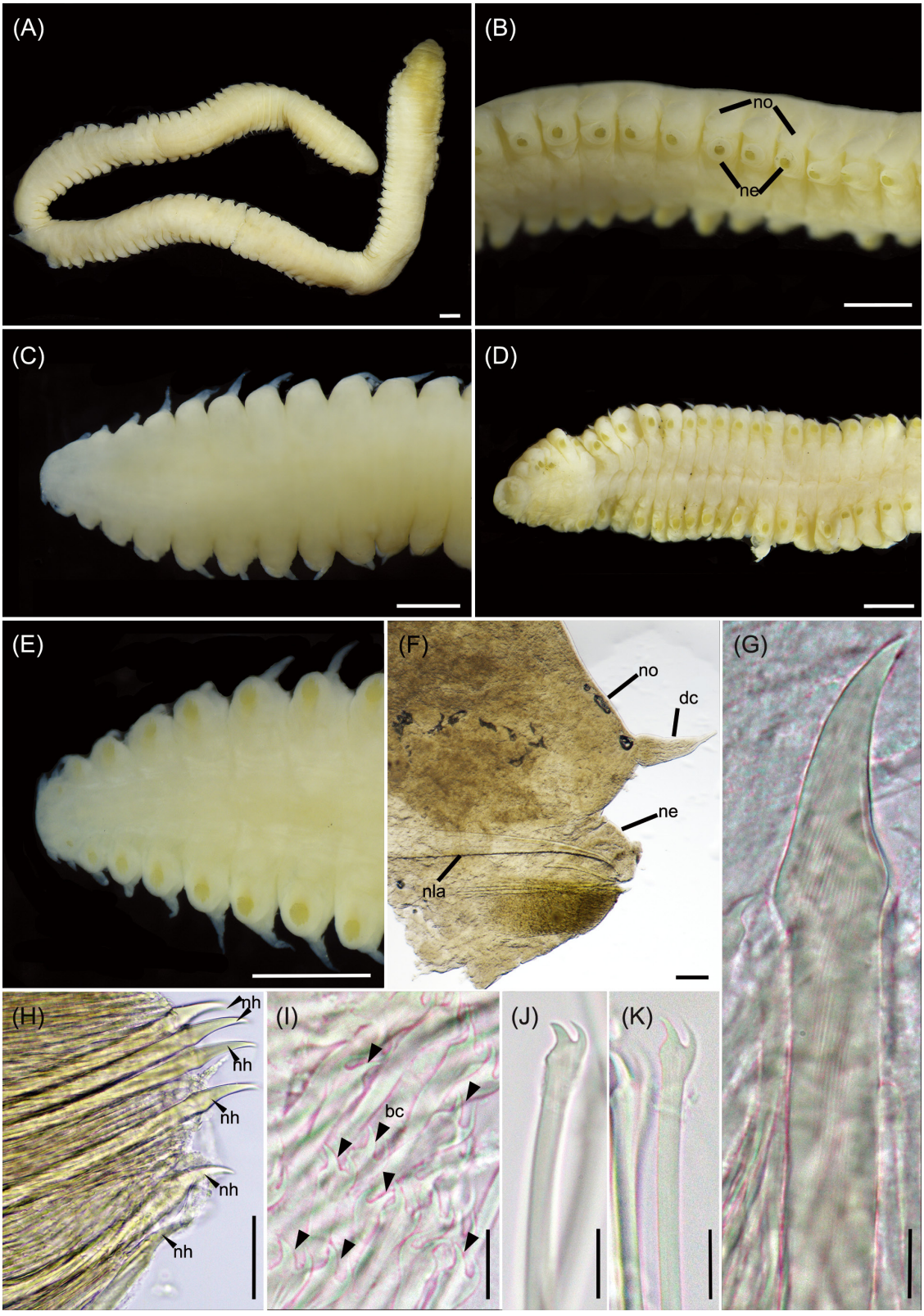

Fig. 1. *Natsushima nanhaiensis* n. sp. A–B, D, F–G, holotype (TMBC031043); C, E, paratype 3 (TMBC031046). A, whole specimen, dorsal view; B, midbody segments, ventral lateral view; C, anterior end, dorsal view; D, posterior end, ventral view; E, anterior end, ventral view; F, midbody parapodium, posterior view; G–H, midbody ventral hooks; I–K, ventral bifurcate chaetae. Abbreviations: no, notopodia; ne, neuropodia; nh, neuropodial hooks; bc, bifurcate chaetae; dc, dorsal cirri; nla, neuroacicula. Scale bars: A–E = 1000 μm; F = 100 μm; H = 50 μm; G, I–K = 10 μm.

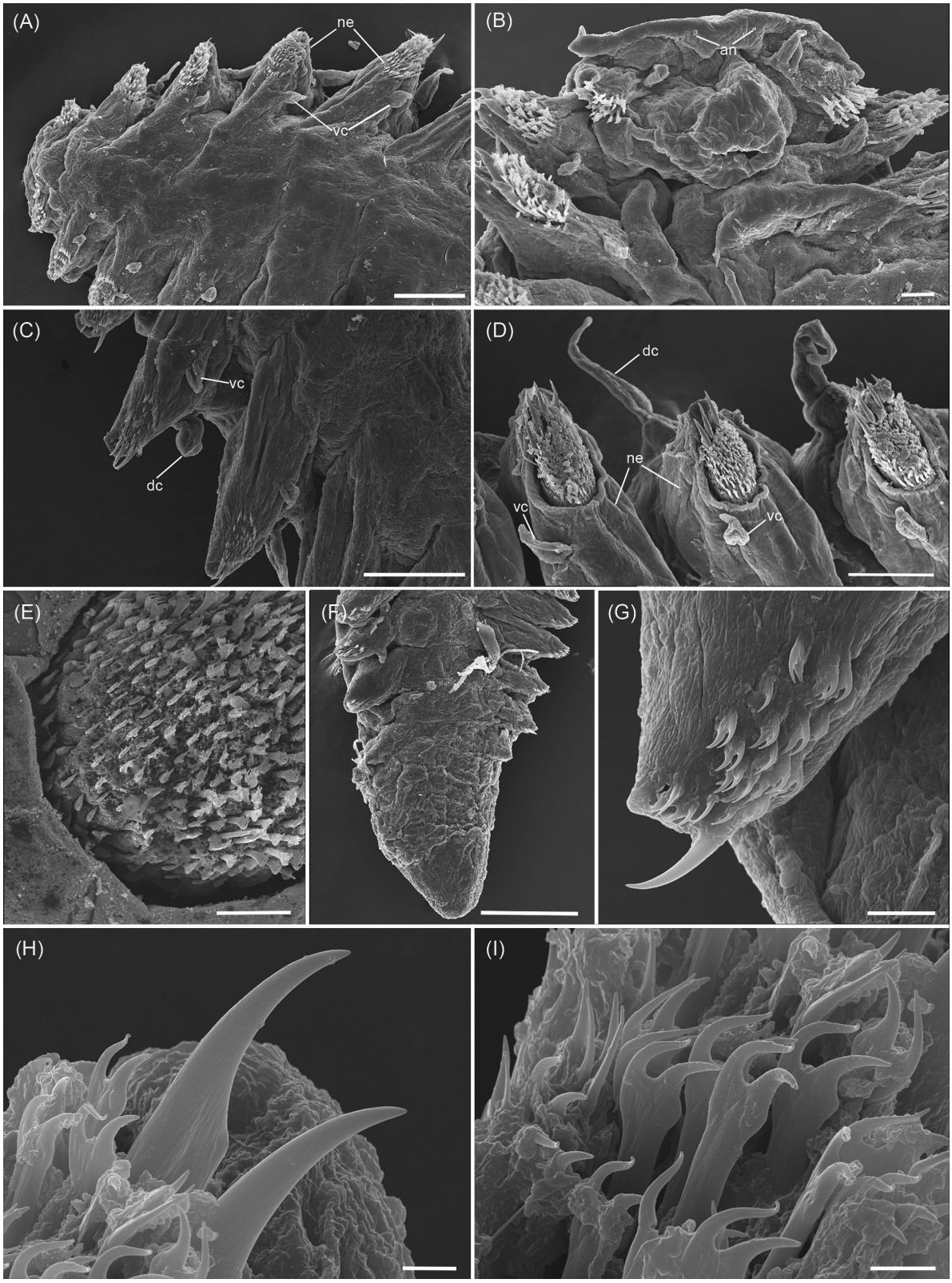

Fig. 2. *Natsushima nanhaiensis* n. sp. A, C, F–I, paratype 4 (TMBC031047); B, D, paratype 1 (TMBC031044); E, paratype 3(TMBC031046). A–B, anterior end, ventral view; C, posterior parapodia, ventral view; D, anterior segments parapodia, ventral view; E, midbody segments ventral chaetae; F, posterior end, ventral view; G, posterior parapodia, dorsal view; H, ventral hook; I, ventral chaetae. Abbreviations: ne, neuropodia; dc, dorsal cirri; vc, ventral cirri; an antenna. Scale bars: A, C–D, F = 100 μm; B, E = 30 μm; G = 10 μm; H–I = 3 μm.

those of *N. sashai* and *N. graciliceps* are ~18 and 13 μm, respectively, but those of *N. bifurcata* and *N. nanhaiensis* n. sp. are ~30 and > 35 μm, respectively (Miura and Laubier 1990; Miura and Hashimoto 1996; Aguado and Rouse 2011).

The two teeth of the bifurcate chaetae are similar in length in *N. nanhaiensis* n. sp., *N. bifurcata* and *N. graciliceps*. In both *N. nanhaiensis* n. sp. and *N. bifurcata*, one tooth is slightly thinner and pointed while the other is thicker and distally blunt. However, in *N. graciliceps*, both teeth are similar in size (Miura and Hashimoto 1996). In *N. sashai*, the two teeth are remarkably different, with one of them much thinner and shorter than the other, both with conspicuously pointed tips (Aguado and Rouse 2011).

In both *N. nanhaiensis* n. sp. and *N. sashai*, the notopodia and the neuropodia are similar in length in each parapodium. By contrast, in *N. bifurcata* the notopodia are shorter than the neuropodia, whereas in *N. graciliceps*, the notopodia are more than twice as long as the neuropodia. Among the four *Natsushima* species, *N. bifurcata*, *N. sashai*, and *N. nanhaiensis* originate from cold seeps, while the type of chemosynthetic environment (cold seep or hydrothermal vent) in Kagoshima Bay was not recorded for *N. graciliceps* (Miura and Laubier 1990; Miura and Hashimoto 1996; Aguado and Rouse 2011). *N. bifurcata* was collected from the mantle cavity of *Acharax johnsoni*, which was misidentified as a species of the genus *Solemya* in the original description (Miura and Laubier 1990; Miura and Hashimoto 1996). On the other hand, the other three species were found among the gill lamellae in the mantle cavity of their hosts (both *N. sashai* and *N. nanhaiensis* inhabit *Acharax* sp. specimens, while *N. graciliceps* was found in *Solemya*) (Miura and Hashimoto 1996; Yang 2007; Aguado and Rouse 2011).

Key to species of *Natsushima* Miura & Laubier, 1990

1a. Notopodia much shorter than neuropodia ..... *N. bifurcata* Miura & Laubier, 1990
1b. Notopodia much longer than neuropodia ..... *N. graciliceps* Miura & Hashimoto, 1996
1c. Notopodia and neuropodia of similar length 2
2a. Teeth of bifurcate chaetae of similar length ..... *N. nanhaiensis* n. sp.
2b. Teeth of bifurcate chaetae remarkably different, one tooth much larger and longer than the other tooth ..... *N. sashai* Aguado & Rouse, 2011

Phylogenetic relationships and genetic distances

Sequencing the target gene fragments from the five individuals of *N. nanhaiensis* n. sp. produced 651-bp *COI* and 314-bp *16S rRNA* sequences. Alignment and concatenation of the two gene fragments of the new species and other chrysopetalids generated a dataset of 965 bp for phylogenetic analyses. The ML and BI trees are largely consistent in their topology, with the 29 chrysopetalids divided into three major clades (Fig. 3), corresponding to the systematic scheme with three subfamilies (Ravara et al. 2019). *Natshushima nanhaiensis* n. sp. is consistently clustered among *Natsushima* species. The five specimens of *N. nanhaiensis* n. sp. formed a single clade, sister to *N. sashai* with strong support in both BI and ML analyses (BS = 95, pp = 0.92).

The K2P genetic distances between the examined chrysopetalids range from 11.43% to 52.95% for *COI* and 9.34% to 49.22% for *16S*. Between the examined species within the subfamily Calamyzinae, the K2P distance ranges from 11.43% to 25.67% for *COI* and 9.34% to 22.10% for *16S* (Tables S1–S2). Among the species examined, *N. nanhaiensis* n. sp. is most closely related to *N. sashai*, with a K2P genetic distance of 11.43% for *COI* and 9.34% for *16S*. The intraspecific K2P distance (*i.e.*, between *N. nanhaiensis* n. sp. specimens) ranges from 0.29% to 0.86% for *COI* and 0 to 0.58% for *16S*.

DISCUSSION

Our morphological analysis shows that the chrysopetallid specimens collected from the Haima cold seep belong to an undescribed species of *Natsushima*. This species, described as *N. nanhaiensis* n. sp. herein, can be distinguished from its congeneric species by a combination of features in parapodia and chaetae. These are the similar length of notopodia and neuropodia; the morphology of the bifurcate hooks with distal teeth of the same length but different width and shape (one of the teeth is slightly thinner and pointed while the other thicker and distally blunt), and the presence of up to six neuropodial hooks per midbody neuropodium.

Our molecular phylogenetic analysis revealed that *N. nanhaiensis* n. sp. is closely related to *N. sashai* and both species live in the mantle cavity of *Acharax* in cold seep habitats. Both the small intraspecific K2P distances of *N. nanhaiensis* n. sp. and the large interspecific distances with its most closely related *N. sashai* support the recognition of the new species.

Interestingly, the genetic distances across *Natsushima* species do not correlate with their geography. Indeed, *N. nanhaiensis* n. sp. is geographically closer to *N. bifurata* and *N. graciliceps* inhabiting cold seeps in the western Pacific but morphologically and phylogenetically closer to *N.*

sashai which inhabits cold seeps off the coast of Costa Rica in the eastern Pacific (Miura and Laubier 1990; Miura and Hashimoto 1996; Aguado and Rouse 2011). The phylogeographic distribution of *Natsushima* is however concordant with that of their *Acharax* hosts.

The *Acharax* genus is divided into two distinct genetic clusters: the JAC cluster with species located in the Java, Aleutian Trench, and Costa Rica continental margins; and the MOP cluster with species from the Makran, Oregon, and Peru continental margins (Neulinger et al. 2006; Sharma et al. 2013). The hosts of both *N. nanhaiensis* n. sp. and *N. sashai* belong to the JAC group, while the host of *N. bifurcata*, belong to the MOP cluster of *Acharax johnsoni*, which is now considered a species complex (Yang et al. 2024). Taken together, our results support a shared speciation history between *Acharax* and *Natsushima* and the host-symbiont co-evolution hypothesis put forward by Aguado and Rouse (2011).

Given that *Acharax* is widely distributed in deep-sea vent and seep ecosystems globally (Neulinger et al. 2006), and three of the four species of *Natsushima* are reported from the western Pacific Ocean, we expect that examination of the symbiotic annelids in *Acharax* in other regions may lead to the discovery of more *Natsushima* species. Such discoveries will enable an understanding of these annelids' biogeographical and divergence histories.

CONCLUSIONS

In this study, we reported a new species of symbiotic annelids, *Natsushima nanhaiensis* n. sp., hosted by the solemyid clam, *Acharax haimaensis* collected from the Haima cold seep in the South China Sea. We described the species based on morphological and molecular evidence. Our study increased the number of *Natsushima* species from three to four. Given that *Acharax* spp. have been widely reported from deep-sea hydrothermal vents and cold seeps worldwide, future studies of the symbionts of solemyid clams may reveal more species of *Natsushima* and understand how the hosts and symbionts co-evolve.

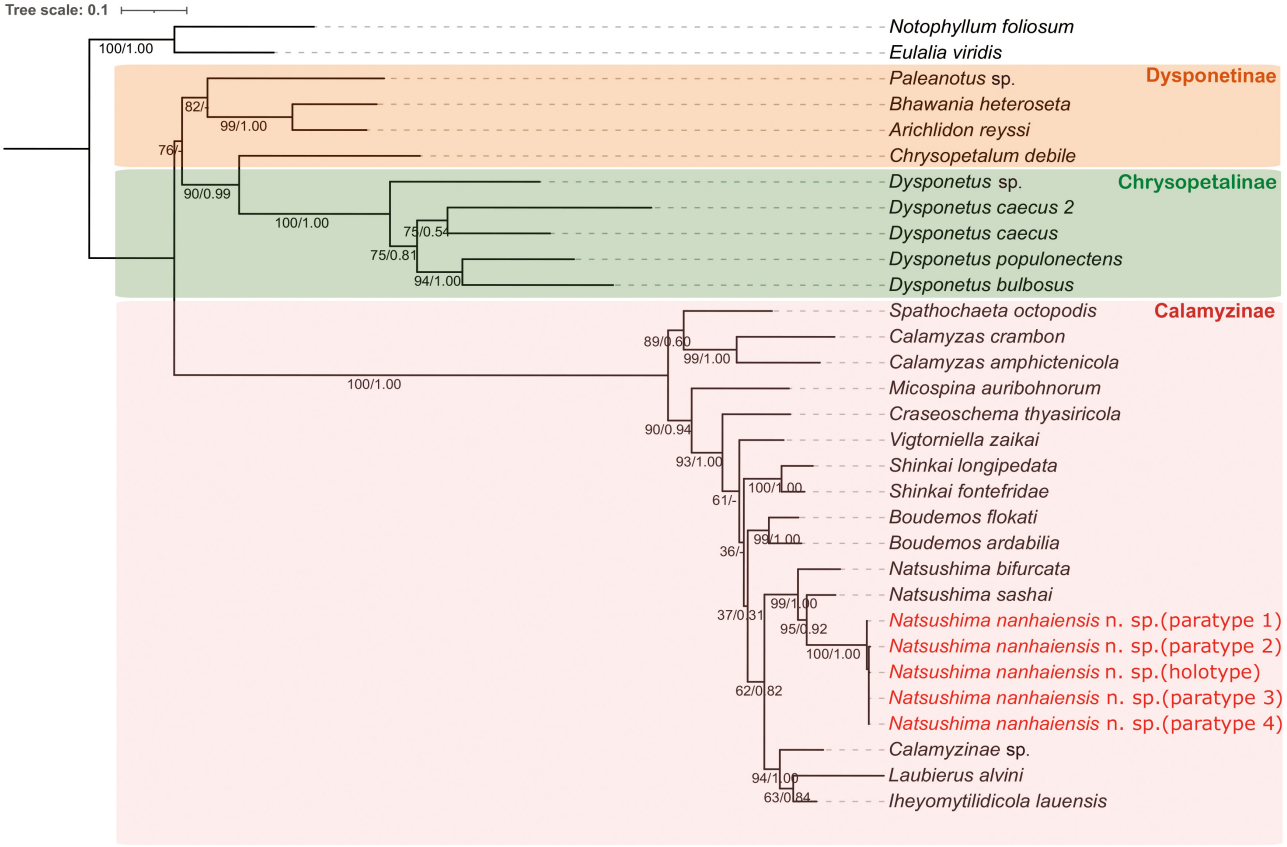

Fig. 3. Phylogenetic tree of Chrysopetalidae based on the concatenated sequences of the *COI* and *16S* gene fragments. The topology is based on ML analysis. Bootstraps values from the ML analysis and posterior probabilities values (0–1) from BI analysis are shown at the nodes. Dashes (-) indicate inconsistent branches. Individuals of the new species described in this paper, including holotype (H) and paratypes (P1–P4) are highlighted in red.

Acknowledgments: We thank the captain and crew of the (R/V) Exploration 2 and the operation team of the HOV Challenger Deep for their technical support during the cruise. We thank Zhaoming Gao from the Institute of Deep-sea Science and Engineering for collecting the samples, and Yu Sheung Law and Yee Man Lee from the Department of Biology at Hong Kong Baptist University for technical support. This study was supported by the Collaborative Research Fund (C2013-22GF) and the General Research Fund (12101021, 12102222) of the Hong Kong SAR.

Authors' contributions: YNS and JWQ initiated the study. FH drafted the manuscript. All authors revised the manuscript.

Competing interests: FH, YTL, MP, JWQ and YNS declare they have no conflict of interest.

Availability of data and materials: Type specimens are deposited in Tropical Marine Biodiversity Collections of the South China Sea (TMBC), Chinese Academy of Sciences, Guangzhou. The gene sequences are deposited on GenBank under the accessions PP792856-PP792860 (*COI*), PP790211-PP790215 (*16S RNA*).

Consent for publication: All authors agreed to publish the paper.

Ethics approval consent to participate: Not applicable.

REFERENCES

Aguado MT, Rouse GW. 2011. Nautiliniellidae (Annelida) from Costa Rican cold seeps and a western Pacific hydrothermal vent, with description of four new species. Syst Biodivers **9:**109–131. doi:10.1080/14772000.2011.569033.

Böggemann M. 2009. Polychaetes (Annelida) of the abyssal SE Atlantic. Org Divers Evol **9:**251–428. doi:10.1016/j.ode.2009.10.001.

Carr CM, Hardy SM, Brown TM, Macdonald TA, Hebert PD. 2011. A tri-oceanic perspective: DNA barcoding reveals geographic structure and cryptic diversity in Canadian polychaetes. PLoS ONE **6:**e22232. doi:10.1371/journal.pone.0022232.

Cavanaugh CM, McKiness ZP, Newton IL, Stewart FJ. 2006. Marine chemosynthetic symbioses. The Prokaryotes **1:**475–507. doi:10.1007/0-387-30741-9_18.

Chen C, Okutani T, Liang Q, Qiu J-W. 2018. A noteworthy new species of the family Vesicomyidae from the South China Sea (Bivalvia: Glossoidea). Venus **76:**29–37. doi:10.18941/venus.76.1-4_29.

Ehlers EH. 1864. Die Borstenwürmer (Annelida Chaetopoda) nach systematischen und anatomischen Untersuchungen dargestellt. W. Engelmann, Leipzig.

Feng D, Qiu J-W, Hu Y, Peckmann J, Guan H, Tong H, Chen C, Chen J, Gong S, Li N. 2018. Cold seep systems in the South China Sea: An overview. J Asian Earth Sci **168:**3–16. doi:10.1016/j.jseaes.2018.09.021.

He X, Xu T, Chen C, Liu X, Li Y-X, Zhong Z, Gu X, Lin Y-T, Lan Y, Yan G. 2023. Same (sea) bed different dreams: Biological community structure of the Haima seep reveals distinct biogeographic affinities. Innov Geosci **1:**100019. doi:10.59717/j.xinn-geo.2023.100019.

Jimi N, Moritaki T, Kajihara H. 2019. Polychaete meets octopus: symbiotic relationship between *Spathochaeta octopodis* gen. et sp. nov. (Annelida: Chrysopetalidae) and *Octopus* sp. (Mollusca: Octopodidae). Syst Biodivers **17:**1–6. doi:10.1080/14772000.2018.1520753.

Jimi N, Tsuchida S, Watanabe HK, Ohara Y, Yokooka H, Woo SP, Fujiwara Y. 2022. Worm on worm: Two rare genera of Calamyzinae (Annelida, Chrysopetalidae), with a description of new species. Parasitol Int **90:**102619. doi:10.1016/j.parint.2022.102619.

Kalyaanamoorthy S, Minh BQ, Wong TK, Von Haeseler A, Jermiin LS. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Methods **14:**587–589. doi:10.1038/nmeth.4285.

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol **30:**772–780. doi:10.1093/molbev/mst010.

Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol **16:**111–120. doi:10.1007/BF01731581.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol **33:**1870–1874. doi:10.1093/molbev/msw054.

Lamarck JB de. 1802 (privately published, reprinted 1906). Discours d'Ouverture, Prononcé le 27 floréal An 10, au Muséum d'Histoire naturelle. Recherches sur l'organisation des corps vivans. Bulletin Scientifique de la France et de la Belgique, 5th series, **40:**483–517.

Letunic I, Bork P. 2007. Interactive Tree of Life (iTOL): an online tool for phylogenetic tree display and annotation. Bioinformatics **23:**27–128. doi:10.1093/bioinformatics/btl529.

Liang Q, Hu Y, Feng D, Peckmann J, Chen L, Yang S, Liang J, Tao J, Chen D. 2017. Authigenic carbonates from newly discovered active cold seeps on the northwestern slope of the South China Sea: Constraints on fluid sources, formation environments, and seepage dynamics. Deep-Sea Res Part I **124:**31–41. doi:10.1016/j.dsr.2017.04.015.

Lin Y-T, Li Y-X, Sun Y, Tao J, Qiu J-W. 2023. A new species of the genus *Catillopecten* (Bivalvia: Pectinoidea: Propeamussiidae): morphology, mitochondrial genome, and phylogenetic relationship. Front Mar Sci **10:**1168991. doi:10.3389/fmars.2023.1168991.

Minh BQ, Nguyen MAT, Von Haeseler A. 2013. Ultrafast approximation for phylogenetic bootstrap. Mol Biol Evol **30:**1188–1195. doi:10.1093/molbev/mst024.

Miura T, Hashimoto J. 1996. Nautiliniellid polychaetes living in the mantle cavity of bivalve mollusks from cold seeps and hydrothermal vents around Japan. Publications of the Seto Marine Biological Laboratory **37:**257–274.

Miura T, Laubier L. 1990. Nautiliniellid polychates collected from the Hatsushima cold-seep site in Sagami Bay, with descriptions of new genera and species. Zool Sci **7:**319–325.

Neulinger SC, Sahling H, Süling J, Imhoff JF. 2006. Presence of two phylogenetically distinct groups in the deep-sea mussel *Acharax* (Mollusca: Bivalvia: Solemyidae). Mar Ecol Prog Ser **312:**161–168. doi:10.3354/meps312161.

Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating

maximum-likelihood phylogenies. Mol Biol Evol **32:**268–274. doi:10.1093/molbev/msu300.

Ravara A, Aguado MT, Rodrigues CF, Génio L, Cunha MR. 2019. Description of a new genus and species of Chrysopetalidae (Annelida: Polychaeta) from the NE Atlantic, with some further records of related species. Eur J Taxon **539:**1–21. doi:10.5852/ejt.2019.539.

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol **61:**539–542. doi:10.1093/sysbio/sys029.

Rouse GW, Pleijel F, Tilic E. 2022. Annelida, Oxford University Press, New York, pp. 418. doi:10.1093/oso/9780199692309.003.0001.

Russell CW. 1997. Patterns of growth and setal development in the deep-sea worm, *Strepternos didymopyton* (Polychaeta: Chrysopetalidae). Bull Mar Sci **60:**405–426.

Russell CW. 1986. *Paleaequor*, a new genus of polychaete worm (Chrysopetalidae). Rec Aust Mus **38:**153–174.

Sharma PP, Zardus JD, Boyle EE, Gonzalez VL, Jennings RM, McIntyre E, Wheeler WC, Etter RJ, Giribet G. 2013. Into the deep: a phylogenetic approach to the bivalve subclass Protobranchia. Mol Phylogenet Evol **69:**188–204. doi:10.1016/j.ympev.2013.05.018.

Sjölin E, Erséus C, Källersjö M. 2005. Phylogeny of Tubificidae (Annelida, Clitellata) based on mitochondrial and nuclear sequence data. Mol Phylogenet Evol **35:**431–441. doi:10.1016/j.ympev.2004.12.018.

Sun Y, Sun J, Yang Y, Lan Y, Ip JC-H, Wong WC, Kwan YH, Zhang Y, Han Z, Qiu J-W. 2021. Genomic signatures supporting the symbiosis and formation of chitinous tube in the deep-sea tubeworm *Paraescarpia echinospica*. Mol Biol Evol **38:**4116–4134. doi:10.1093/molbev/msab203.

Talavera G, Castresana J. 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Syst Biol **56:**564–577. doi:10.1080/10635150701472164.

Watson C, Chivers AJ, Narayanaswamy BE, Lamont P, Turnewitsch R. 2014. Chrysopetalidae (Annelida: Phyllodocida) from the Senghor Seamount, north-east Atlantic: taxa with deep-sea affinities and morphological adaptations. Mem Mus Vic **71:**311–325. doi:10.24199/j.mmv.2014.71.24.

Watson C, Ignacio Carvajal J, Sergeeva NG, Pleijel F, Rouse GW. 2016. Free-living calamyzin chrysopetalids (Annelida) from methane seeps, anoxic basins, and whale falls. Zool J Linn Soc **177:**700–719. doi:10.1111/zoj.12390.

Xu T, Feng D, Tao J, Qiu J-W. 2019. A new species of deep-sea mussel (Bivalvia: Mytilidae: *Gigantidas*) from the South China Sea: Morphology, phylogenetic position, and gill-associated microbes. Deep Sea Res Part I **146:**79–90. doi:10.1016/j.dsr.2019.03.001.

Xu T, Sun Y, Wang Z, Sen A, Qian P-Y, Qiu J-W. 2022. The morphology, mitogenome, phylogenetic position, and symbiotic bacteria of a new species of *Sclerolinum* (Annelida: Siboglinidae) in the South China Sea. Front Mar Sci **8:**793645. doi:10.3389/fmars.2021.793645.

Yang M, Li B, Gan Z, Dong D, Li X. 2024. New chemosymbiotic bivalve species of the genus *Acharax* Dall, 1908 (Bivalvia, Solemyida, Solemyidae) from the Haima cold seep of the South China Sea. Zookeys **1198:**185–192. doi:10.3897/zookeys.1198.112618.

Yang Z. 2007. PAML 4: phylogenetic analysis by maximum likelihood. Mol Biol Evol **24:**1586–1591. doi:10.1093/molbev/msm088.

Zhang D, Gao F, Jakovlić I, Zou H, Zhang J, Li WX, Wang GT. 2020. PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. Mol Ecol Resour **20:**348–355. doi:10.1111/1755-0998.13096.

Supplementary materials

Fig. S1. *Natsushima nanhaiensis* n. sp., (A) Whole holotype specimen (TMBC031043) dorsal view; (B) Whole paratype specimen (TMBC031046), dorsal view. (download)

Table S1. The K2P pairwise distance of chrysopetalids based on *COI*. (download)

Table S2. The K2P pairwise distance of chrysopetalids based on *16S RNA*. (download)