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Two new deep-sea myzostomid species (Annelida: Myzostomida) from the Northwest Pacific off Japan

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Abstract: Two new deep-sea myzostomid species, *Pulvinomyzostomum unicorne* sp. nov. and *Eenymeenymyzostoma abyssale* sp. nov., are described from the Northwest Pacific off Japan. *Pulvinomyzostomum unicorne* sp. nov. was collected from the crinoid host *Pentametrocrinus varians* at a depth of 1506 m near Shima Spur, Japan, NW Pacific Ocean, characterized by its distinctive scalloped body margin with 20 equal-sized conical cirri, lack of dorsal ridges or color patterns, a smooth proboscis, and a single cirrus extending from the base of the parapodia. *Eenymeenymyzostoma abyssale* sp. nov., the deepest record for the genus, was found at 511 m depth on the black coral *Leiopathes* cf. *annosa* at the Nishi-Shichito Ridge, Japan, NW Pacific Ocean. This species exhibits an oval, thick body without marginal cirri, transparent and orange coloration internally when alive, and uniquely lacks parapodial cirri. Molecular phylogenetic analyses based on COI, 16S, and 18S sequences strongly supported the placement of these new species within their respective genera.

Key words: Myzostomida, Polychaeta, seamount, symbiosis, parasite

Introduction

Myzostomida Graff, 1877 are a unique group within Annelida, exhibiting highly modified morphologies distinct from typical annelids (Grygier 2000). They are primarily symbiotic with echinoderms, with approximately 200 valid species recorded from shallow to deep-sea environments worldwide (Summers et al. 2014; Read & Fauchald 2025). Most myzostomids exhibit host specificity, with only a few species known to associate with multiple host species (Summers et al. 2014). The majority of species are reported from feather stars (Isaychev et al. 2024), with a few exceptions found on sea stars (Jimi et al. 2017), brittle stars (Okada 1922), and stalked crinoids (Graff 1884a). Some rare cases of cnidarian-associated species have also been documented (Terrana & Eeckhaut 2017). Following more than a century of myzostomid taxonomic studies, Summers et al. (2014) proposed the use of *Turbo-taxonomy* for their classification. This approach mainly relies on photographs of live specimens, host, and COI barcode sequences. Given the morphological simplicity of myzostomids, this methodology facilitates rapid species identification and contributes significantly to biodiversity assessments.

The genus *Pulvinomyzostomum* Jägersten, 1940 is a small genus comprising only three species, with no reports from Japan (Jimi 2024). These species are known to associate with feather stars or stalked crinoids. For a

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Supplementary materials may be found in the online version of this article.

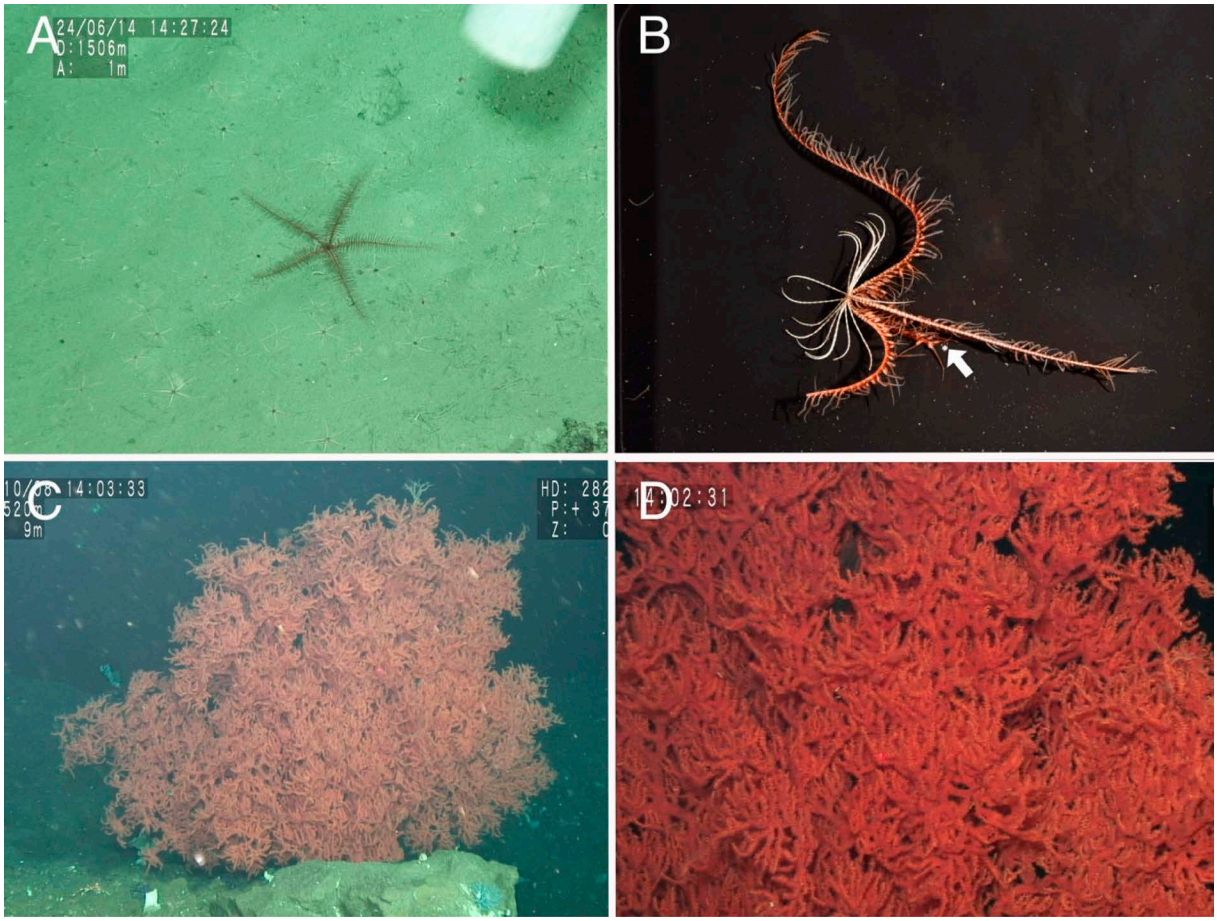

Fig. 1. Hosts of myzostomids in this study. A–B, *Pentametrocrinus varians*, at Shima spur. C–D, *Leiopathes* cf. *annosa* at Nishi-Shichito and of the Naka- and West Mariana ridges. Arrow indicates *Pulvinomyzostomum unicorne* sp. nov.

long time, the genus consisted solely of *P. pulvinar* (Graff, 1884) (Jägersten 1940; Grygier 2000; Summers & Rouse 2014). However, *P. inaki* Summers & Rouse in Summers et al., 2014, and *P. messingi* Summers & Rouse in Summers et al., 2014, were described by Summers et al. (2014), expanding the genus to three species. While the addition of these two species did not prompt a formal revision of the genus definition, their morphological and ecological characteristics differ in several aspects from the type species (e.g., presence of proboscis and cloaca on dorsal or ventral side). Currently, the genus is recognized as a phylogenetic clade rather than strictly by shared morphological traits (Summers et al. 2014; Summers & Rouse 2014).

The genus *Eenymeenymyzostoma* Summers & Rouse, 2015 was established based on a distinct clade revealed in molecular phylogenetic analyses (Summers & Rouse 2014, 2015; Summers et al. 2014). To date, only two species, *E. cirripedium* (Graff, 1885) and *E. nigrocorallium* Terrana & Eeckhaut, 2017, have been described. *Eenymeenymyzostoma cirripedium* is associated with stalked crinoids, while *E. nigrocorallium* is uniquely found on black corals (Antipatharia) (Terrana & Eeckhaut 2017). Host association with cnidarians is an extremely rare trait in myzostomids, making *E. nigrocorallium* the only confirmed species exhibiting this relationship. Morphological traits within *Eenymeenymyzostoma* are highly variable, and the genus is currently defined solely based on molecular phylogenetic placement. In the Pacific Ocean, the only recorded species is *E. cirripedium*, which has been found in Japan (Graff 1885; Jimi 2024).

In this study, we describe two new myzostomid species from the deep-sea region of Japan. *Pulvinomyzostomum unicorne* sp. nov. and *Eenymeenymyzostoma abyssale* sp. nov. add to the growing knowledge of deep-sea myzostomid diversity and highlight the importance of further exploration in symbiotic annelids.

Materials and Methods

Myzostomid hosts were collected using the HOV *Shinkai 6500* operated by R/V *Yokosuka* (Fig. 1). Specimens were obtained during two research cruises: from dive #1783 at depth of 1506 m on Shima Spur, Japan, NW Pacific Ocean, during cruise YK24-09S (14 June 2024), and from dive #1819 at a depth of 511 m on the Nishi-Shichito and of the Naka-Mariana ridges, NW Pacific Ocean, located within the offshore seabed nature conservation area of Japan, conducted during cruise YK24-15C on 8 October

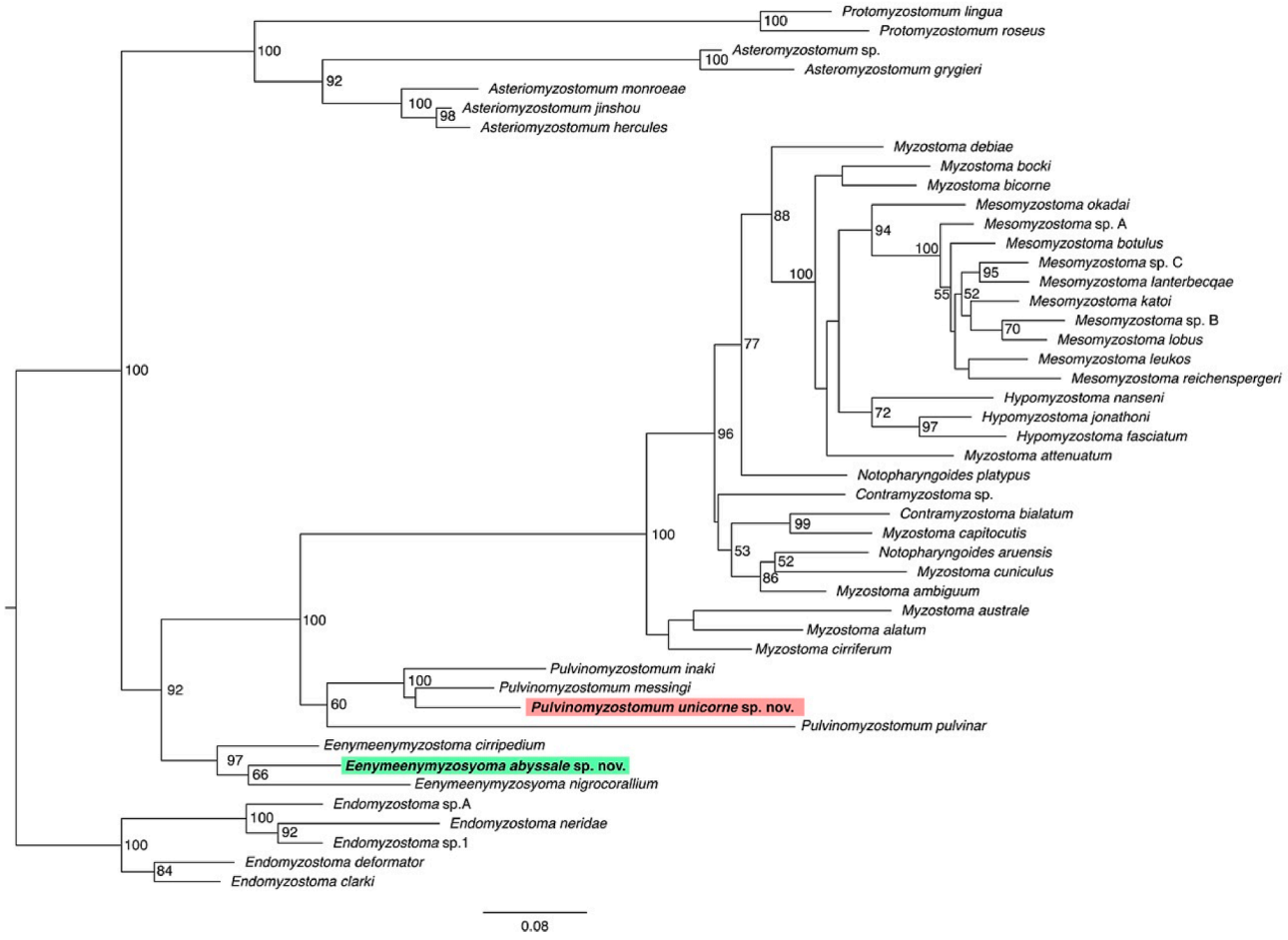

Fig. 2. Maximum-likelihood (ML) phylogenetic tree of Myzostomida based on COI, 16S, and 18S sequences. Nodal support values (bootstrap support value) higher than 50% are indicated on each branch. Red and green highlights indicate the new species described in this study.

2024. In situ images of the hosts were captured using the digital camera system aboard *Shinkai 6500*.

Specimens were observed under a Nikon SMZ1500 stereomicroscope and a Nikon Ni-U compound microscope and then deposited in the National Museum of Nature and Science, Tsukuba (NSMT). For scanning electron microscopy (SEM), specimens were dehydrated using a t-BuOH freeze dryer (Vacuum Device VFD-21S), coated with osmium using an osmium plasma coater (Nippon Laser & Electronics Lab OPC80), and examined with a JEOL JSM-7610F scanning electron microscope operating at 5.0 kV at the Division for Medical Research Engineering, Nagoya University Graduate School of Medicine. Brief host identification was provided by Reimer et al. (2024). The description of myzostomids followed the criteria outlined by Summers & Rouse (2014) in the Methods section of their study.

Genomic DNA was extracted from half body of the paratypes using the DNeasy Blood & Tissue Kit (Qiagen, USA) following the manufacturer's protocol. Partial sequences of mitochondrial cytochrome c oxidase subunit I (COI) and 16S ribosomal RNA (16S), and nuclear 18S ribosomal RNA (18S) and Histone H3 (H3) genes were amplified and sequenced according to the methods described in Jimi et al. (2021). Additionally, the COI region of the host crinoid was newly sequenced. Genomic DNA was extracted from genital pinnules of the host crinoids using the DNeasy Blood & Tissue Kit (Qiagen, USA) following the manufacturer's protocol. Two primer pairs were used for PCR amplification and sequencing: COIceF (5′-ACTGCC CACGCCCTAGTAATGATATTTTTATGGTNATGCC-3′) and COIceR (5′-TCGTGTGTCTACGTCCATTCCTAC TGTRAACATRTG-3′) for COI gene (Hoareau & Boissin 2010) and 16Sbr (5′-CGCCTGTTTACCAAAAACAT-3′) and m16Sar (5′-CCGGTCTGAACTCAGATCACGT-3′) for 16S rDNA region (Palumbi, 1996 modified by Okanishi et al. 2023). The PCR amplification, sequencing and assembly methods followed Ogawa et al. (2020).

The newly obtained sequences have been deposited in GenBank. Additional myzostomid sequences used for phylogenetic analyses were retrieved from GenBank (see Jimi et al. 2017; Table 1 in Terrana & Eeckhaut 2017). The phylogenetic analysis was conducted following the methods described in Jimi et al. (2021), using *Endomyzostoma* spp. as outgroups, based on the phylogenetic tree of Jimi et al. (2017). Sequences from 46 species across 11 genera, covering four gene regions, were included in the analyses (see

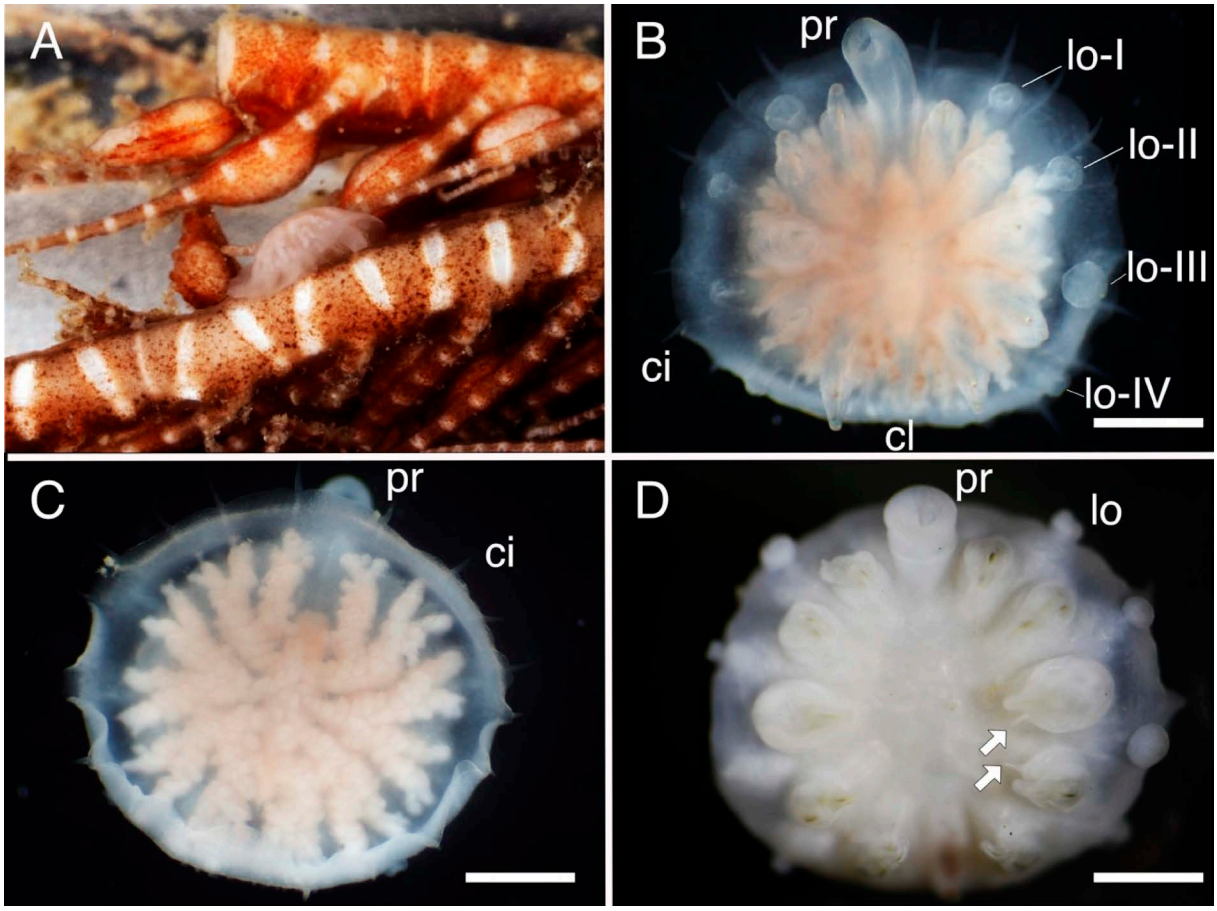

Fig. 3. *Pulvinomyzostomum unicorne* sp. nov., holotype. A, live specimen with crinoid host *Pentametrocrinus varians*, lateral view; B, live specimen, ventral view; C, live specimen, dorsal view; D, fixed specimen, ventral view. In B–D, upper side indicates anterior; lower side indicates posterior. Arrows indicate parapodial cirri. Scale bars: 0.5 mm. Abbreviation: ci, cirri; cl, cloaca; lo, lateral organ; pr, proboscis.

Supplementary File 1).

Results

Molecular analysis

In the resulting phylogenetic tree (Fig. 2), *Pulvinomyzostomum unicorne* sp. nov., *P. inaki*, and *P. messingi* formed a strongly supported clade with a bootstrap support value of 100%. Similarly, *Eenymeenymyzostoma abyssale* sp. nov., *E. cirripedium*, and *E. nigrocorallium* formed a well-supported clade with a bootstrap support value of 97%

Systematics

Family Pulvinomyzostomidae Jägersten, 1940
***Pulvinomyzostomum* Jägersten, 1940**
***Pulvinomyzostomum unicorne* sp. nov.**
[New Japanese name: yubi-tsuki-suikuchimushi]
(Figs. 1B, 3, 5A–B)

Material examined. Holotype (NSMT-Pol H-991): 1.5 mm in length, 2 mm in width, Shima spur, Japan (33°50.6964N, 136°51.5151E), 1506 m depth, 14 June 2024, collected from the crinoid host *Pentametrocrinus varians* (Carpenter, 1882) (NSMT E-15176) by Naoto Jimi (Figs. 1B, 3A). Paratypes: 2 specimens (NSMT-Pol P-992), 1.5 mm in length, 2 mm in width, collected from the same locality and host individual as the holotype; used for DNA analysis and SEM observation.

Diagnosis and Description (based on holotype). Holotype body oval, thin. Body margin scalloped and folded in dorsal side, with 20 equal-sized conical cirri (Fig. 3B, 3C). Body whitish in life (Fig. 3A–3C) and after fixation (Fig. 3D), smooth, without color bands, dorsal papillae, and ridges (Fig. 3C). Mouth and cloaca submarginal, not in dorsal, in line with parapodia (Fig. 3B, 3C). Extended proboscis smooth (Figs. 3B, 5A). Five pairs of parapodia (Figs. 3D, 5A–B), two-thirds of the way to body margin, with short conical parapodial cirri on ventral side (Figs. 3D, 5B). Lateral organ four pairs (Figs. 3B, D, 5A).

Etymology. The specific epithet "*unicorne*" (neuter adjective) derives from the Latin adjective "*unicornis*", meaning "having a single horn". It refers to the short, conical parapodial cirri characteristic of the new species. The ending "-e" matches the neuter gender of the genus name *Pul-*

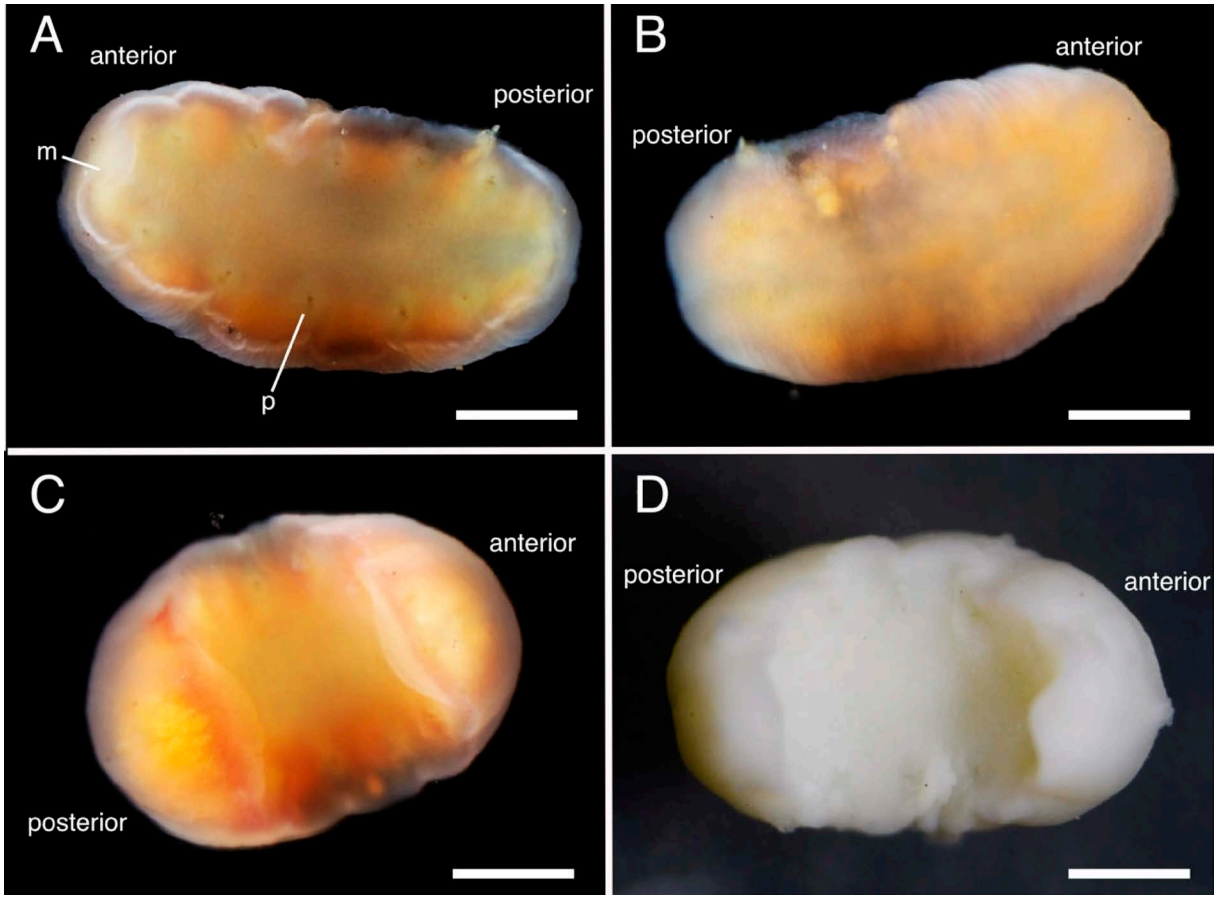

Fig. 4. *Eenymeenymyzostoma abyssale* sp. nov., holotype. A, live specimen, ventral view; B, live specimen, dorsal view; C, live specimen, ventral view, bended; D, fixed specimen, ventral view, bended. Scale bars: 0.5 mm. Abbreviation: m, mouth; p, parapodia.

vinomyzostomum.

Sequences. *Pulvinomyzostomum unicorne* sp. nov.: PV384156 (COI), PV385055 (18S); sequences for 16S and H3 could not be obtained. *Pentametrocrinus varians*: PV384158 (COI), PV385054 (16S). A BLAST search of the COI sequence of *P. varians* against the NCBI database revealed that the closest match was *Thaumatocrinus* sp., another member of Pentametrocrinidae, with 93% identity as the top hit. The gene sequences of *P. varians* are newly determined in this study and reported here for the first time.

Distribution and host. Only known from the type locality. The crinoid host *Pentametrocrinus varians* (Pentametrocrinidae) is known from deep-sea area of Indo-West Pacific region (OBIS 2025).

Remarks. This species can be distinguished from other myzostomid species by its white body color in life, the absence of any ridges or color patterns on the dorsal side, the folding of the dorsal margin, the presence of 20 small, equally sized marginal cirri, a single cirrus extending from the base of the parapodia, its host, and COI sequences.

Pulvinomyzostomum pulvinar was described from the Atlantic at 110–150 m depth but differs clearly in possessing a dorsal proboscis (Graff 1884b). *Pulvinomyzostomum inaki*, recorded from the Pacific at 1866 m depth, can be distinguished by the presence of dorsal ridges, its host (*Antedon* sp.), and COI sequences (Summers et al. 2014). *Pulvinomyzostomum messingi*, described from the Pacific at 1650 m depth, differs in having an elongated body, a furrowed dorsal surface, its host (the crinoid *Lamberticrinus messingi* (Roux & Lambert, 2011)), and COI sequences (Summers et al. 2014).

Some species in *Myzostoma* Leuckart, 1836 also share morphological similarities. *Myzostoma crenatum* Graff, 1883 resembles it in having 20 equally long cirri and a whitish, circular body. However, it differs by possessing thick marginal cirri and by being associated with a different crinoid host, *Comactinia meridionalis* (Graff 1883). *Myzostoma antarcticum* Stummer-Traunfels, 1908 also shares the characters of 20 equally long cirri and a whitish, circular body, but can be distinguished by its unfolded dorsal margin (Stummer-Traunfels 1908).

Family Eenymeenymyzostomatidae Summers & Rouse, 2015
***Eenymeenymyzostoma* Summers & Rouse, 2015**
***Eenymeenymyzostoma abyssale* sp. nov.**
[New Japanese name: goshinboku-suikuchimushi]
(Figs. 4, 5C–D)

Material examined. Holotype (NSMT-Pol H-993):

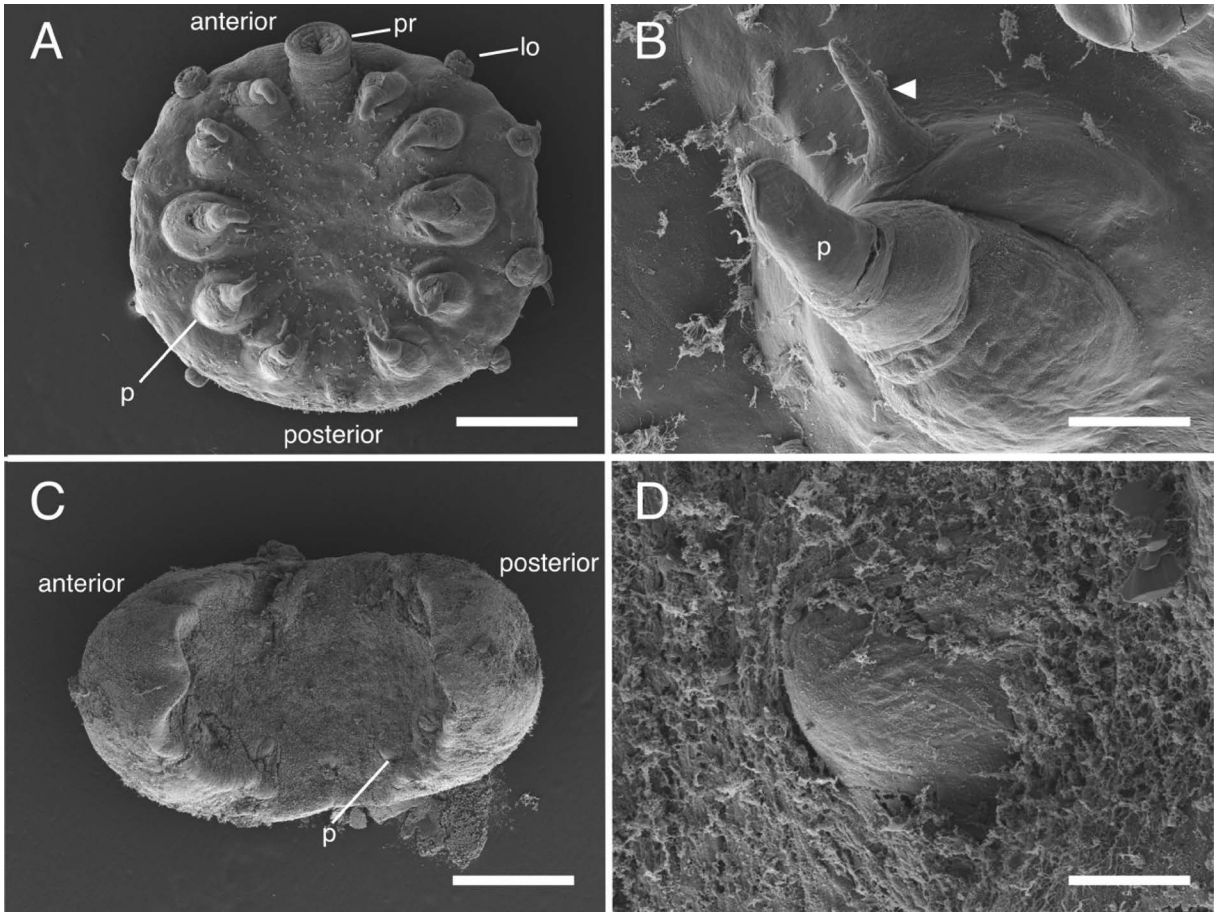

Fig. 5. SEM observation of the two new species. A–B, *Pulvinomyzostomum unicorne* sp. nov., paratype. C–D, *Eenymeenymyzostoma abyssale* sp. nov., paratype. A, whole specimen, ventral view; B, parapodium, ventral view; C, whole specimen, ventral view, bended; D, parapodium, ventral view. Arrowhead indicates short conical parapodial cirri. Scale bars: A, C, 0.5 mm; B, D, 0.05 mm. Abbreviation: lo, lateral organ; p, parapodia; pr, proboscis.

2 mm in length, 1 mm in width, Nishi-Shichito and of the Naka- and West Mariana ridges, Japan (to ensure the conservation of the host, precise latitude and longitude are withheld), 511 m depth, 8 October 2024, collected from the black coral *Leiopathes* cf. *annosa* Wagner & Opresko, 2015 (Fig. 1C–D) by Naoto Jimi. Paratypes: 2 specimens (NSMT-Pol P-994), 2 mm in length, 1 mm in width, collected from the same locality and host individual as the holotype; used for DNA analysis and SEM observation.

Diagnosis and Description (based on holotype). Holotype body oval, thick. Body margin scalloped, without cirri (Fig. 4). Body transparent and orange inside in life and whitish after fixation, smooth, with whitish line in margin of body in life (Fig. 4A–C), without dorsal papillae, and ridges (Fig. 4B). Mouth and cloaca marginal, not in dorsal, not in line with parapodia (Fig. 4A–C). Extended proboscis not seen. Five pairs of small parapodia submarginal (Fig. 4A), without parapodial cirri. Lateral organ not seen.

Etymology. The specific epithet "*abyssale*" (neuter adjective) is derived from the Latin adjective "*abyssalis*", meaning "pertaining to the abyss or deep sea," reflecting the species' habitat. The ending "-e" matches the neuter gender of the genus name *Eenymeenymyzostoma*.

Sequences. *Eenymeenymyzostoma abyssale* sp. nov.: PV384157 (COI), PV385053 (16S), PV385056 (18S); sequences for 16S and H3 could not be obtained. *Leiopathes* cf. *annosa*: PP806640 (ND2), PP806639 (ND5 and ND1) (registered by Reimer et al. 2024).

Distribution and habitat. Currently known only from the type locality. The black coral host, *Leiopathes* cf. *annosa* (Leiopathidae), has been observed growing on the upper part of the West Mariana Ridge (Fig. 1C–D).

Remarks. This species represents the third known member of the genus *Eenymeenymyzostoma*. *Eenymeenymyzostoma cirripedium*, recorded from 128 m depth of Sagami Bay, Japan, can be distinguished from the new species by its host (stalked crinoids) and the presence of marginal cirri (Graff 1885). *Eenymeenymyzostoma nigrocorallium*, described from 15–35 m depth in Madagascar, shares the same host type (black corals) but differs in having an orange, flattened body, being found in shallower waters, its host (Antipathidae), and COI sequences (Terrana & Eeuckhaut 2017). The new species represents the deepest record for the genus.

Electronic supplementary materials

The online version of this article (doi: 10.3800/pbr.20.241) contains supplementary material: Supplementary File 1. Data table of sequences used in the phylogenetic analyses.

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ZooBank registration

The ZooBank LSID for this publication is: urn:lsid:zoobank.org:pub:05A0CB57-3DBE-4135-B41F-23CBB73F741C

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