The Phylogenetic Position of *Branchamphinome* (Annelida, Amphinomidae) with a Description of a New Species from the North Pacific Ocean

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Running head: A new species of Branchamphinome

Abstract

A new species of amphinomid polychaete, *Branchamphinome kohtsukai* sp. nov., is described from Japanese waters, 29–211 m in depth. The species is distinguishable from other congeners by the following features: *i*) branchiae with four–six filaments in midbody segments; *ii*) two pairs of eyes not coalescent; *iii*) the ventral side of the first four chaetigers broadly pigmented. This is the first record of *Branchamphinome* from the North Pacific Ocean. We provide a phylogenetic tree based on the sequences of four genes (COI, 16S, 18S, 28S) and discuss amphinomids' relationships.

Key words: Amphinomida, deep sea, Japan, Polychaeta, polychaetes, taxonomy

INTRODUCTION

Amphinomids are commonly known as fireworms to aquarists and divers because they have defensive dorsal calcareous chaetae and a complex mixture of toxins for defense against predators (Verdes et al., 2018). The family consists of approximately 180 nominal species in 22 genera (Borda et al., 2012; Barroso et al., 2017; Sun and Li, 2017), most of which are found under rocks or coral rubbles in shallow and tropical waters (Barroso et al., 2017).

The genus *Branchamphinome* Hartman, 1967 is a rare and enigmatic group of amphinomids. They have small bodies (about 1 cm long), orange body pigmentation, and branchiae from chaetiger 1. Currently, three *Branchamphinome* species are known from the shallow to bathyal area of the South Pacific, Atlantic, and Southern Oceans (*Branchamphinome antarctica* Hartman, 1967; *Branchamphinome islandica*, Detinova, 1986; *Branchamphinome tropicalis* Barroso, Ranauro, & Kudenov, 2017). Although several amphinomid species have been reported from the deep sea in Japan (e.g., Imajima, 2001, 2005, 2006, 2011; Jimi et al., 2018), there is no record of *Branchamphinome*.

Because of the presence of branchiae on all chaetigers and similar-shaped caruncles, *Branchamphinome* has been expected to be closely related to *Hermodice* Kinberg, 1857 or *Pherecardia* Horst, 1866 (Barroso et al., 2017, 2021). Molecular phylogenetic relationships among Amphinomida have been investigated in several previous studies (Wiklund et al., 2008; Borda et al., 2012, 2015), but a phylogenetic

position of Branchamphinome had not been clarified due to the lack of molecular data.

Eight specimens of *Branchamphinome* were collected from several sites during a survey of polychaetes in Japan. We describe the specimens as a new species and provide a phylogenetic tree based on four molecular sequences (COI, 16S, 18S, 28S). This is the first report of *Branchamphinome* from the North Pacific Ocean.

MATERIALS AND METHODS

Fresh specimens were collected by dredging from two areas: *i*) off Noto Peninsula (37°17.212'N to 37°16.989'N, 137°13.829'E to 137°13.737'E), 70–91 m in depth; *ii*) off Jyogashima (st. 1, 35°08.567'N to 35°08.651'N, 139°33.142'E to 139°32.783'E; st. 2, 35°08.329'N to 35°08.402'N, 139°32.857'E to 139°32.504'E), 105–211 m in depth; *iii*) off Okinoshima, Boso Peninsula (34°59.778'N to 34°59.441'N, 139°48.736'E to 139°48.650'E), 29 m in depth. ROV *Box Fish* collected another specimen from Omurodashi (34°32.814'N, 139°26.572'E), 196 m in depth. The live specimens were photographed with a Nikon D5200 digital camera. They were fixed in 70% ethanol. After preservation, these specimens were observed with a Nikon SMZ1500 dissecting microscope and an OLYMPUS BX51 compound microscope. All of the material has been deposited in the National Museum of Nature and Science, Tsukuba (NSMT). We followed the morphological terminology of Barroso et al. (2017) in the taxonomic description below.

DNA extraction, sequencing, alignment, and removing ambiguous positions for specimens were carried out following the method detailed in Jimi et al. (2019). The length of amplicons was ~670 bp (*COI*), ~550 bp (*16S*), ~1800 bp (*18S*), ~1300 bp (*28S*). The newly obtained sequences *COI* (682 bp), *16S* (550 bp), *18S* (1704 bp), *28S* (888 bp) were deposited in GenBank (Table 1). A total of 67 sequences (18 species) were used for molecular analyses. The trimmed sequences of the four genes, *COI* (665 bp), *16S* (308 bp), *18S* (1736 bp), and *28S* (879 bp), were concatenated using the Kakusan program (Tanabe, 2007), which recommended a GTR+G evolutionary model for each of the genes. A phylogenetic tree was constructed using the maximum likelihood (ML) method in the RAxML-VI-HPC program (Stamatakis, 2006). The robustness of the ML tree was evaluated by 1000 bootstrap pseudo-replicates (F option). Bayesian Inference (BI) analysis was conducted using MrBayes 3.2.2 (Ronquist et al., 2012), with Markov chains of 10 million generations. The model choice for each partition was also based on the Kakusan results. Run convergence was analyzed using Tracer v1.6 (Rambaut et al., 2018); the first one million generations were discarded as burn-in. The OTUs used in this study belong to Amphinomidae, while *Euphrosine foliosa* and *Euphrosine* sp. belongs to Euphrosinidae, so *E. foliosa* and *Euphrosine* sp. were used as a reference group according to Borda et al. (2015).

RESULTS

Systematics

Family **Amphinomidae** Lamarck, 1818 [Japanese name: umikemushi-ka] Genus *Branchamphinome* Hartman, 1967 [New Japanese name: era-umikemushi-zoku] *Branchamphinome kohtsukai* Jimi, sp. nov. [New Japanese name: Kohtsuka-era-umikemushi] (Figs. 1, 2, 3)

Material examined. Holotype: NSMT-Pol H-838, 5 mm long, 1 mm wide (without chaetae, in widest chaetiger), 22 chaetigers, off Jyogashima (st. 1, 35°08.567'N to 35°08.651'N, 139°33.142'E to 139°32.783'E; st. 2, 35°08.329'N to 35°08.402'N, 139°32.857'E to 139°32.504'E), 105–211 m in depth, 30 July 2020, collected by NH. Paratypes: NSMT-Pol P-839, six specimens, 3–7 mm long, 0.5–1 mm wide (without chaetae, in widest chaetiger), 22-30 chaetigers, collection data is the same as that of the holotype. NSMT-Pol P-840, only anterior end (posterior part was used for DNA extraction), one specimen, 2 mm long, 1 mm wide (without chaetae, in widest chaetiger), off Noto Peninsula (37°17.212'N to 37°16.989'N, 137°13.829'E to 137°13.737'E), 70-91 m in depth, 19 November 2019, collected by NJ. NSMT-Pol P-841, one specimen, 7 mm long, 1 mm wide (without chaetae, in widest chaetiger), 30 chaetigers, Omurodashi (34°32.814'N, 139°26.572'E), 196 m in depth, 23 March 2021, collected by NJ. NSMT-Pol P-842, one specimen, 6 mm long, 1 mm wide (without chaetae, in widest chaetiger), 28 chaetigers, off Okinoshima, Boso Peninsula (34°59.778'N to 34°59.441'N, 139°48.736'E to 139°48.650'E), 29 m in depth, 26 April 2021, collected by NJ.

Description. Body depressed, elongated, whitish in life and after fixation,

longitudinal midventral groove absent. Orange pigmentation present (Figs. 1, 2): three irregular rectangular patches on dorsal side of each chaetiger (Figs. 1A, C, E; 2A); rectangular interparapodial patches on ventral side of notopodia (Fig. 2A, C); large rectangular patches on ventral side of neuropodia on chaetigers 1–4 (Fig. 2B); small rounded spots on ventral side of neuropodia on chaetiger 4 and following chaetigers (Fig. 2B).

Prostomium oval, slightly longer than wide (Figs. 2A, D; 3A). Eyes present, two pairs, reddish, anterior pair eyes twice larger than posterior ones (Figs. 2A, D; 3A). Pairs of lateral antennae and palps present, blunt conical, smooth (Fig. 3A); palps as long as lateral antennae. A median antenna present, digitate, twice longer than lateral antennae (Fig. 3A). Caruncle chevron-shaped (Fig. 3A), consists of median keel and four lateral lobes, median keel extends to chaetigers 2–3 (depending on fixation), unattached in posterior part. Mouth opening between chaetigers 2 and 3. Pharynx eversible.

Parapodia biramous, notopodia and neuropodia well separated (Fig. 2C). Dorsal and ventral cirri occur singly on notopodium and neuropodium, conical, pale, arising from body wall, present in all chaetigers. Branchiae dichotomously branching tufts from chaetiger 1, present in all chaetigers, filaments digitiform, four–six filaments per branchia in middle body chaetigers (Fig. 3B).

Notochaetae contain three types: *i*) long, thick harpoon chaetae, with serration limited to one side (Fig. 3C); *ii*) long, serrated capillaries (Fig. 3D); *iii*) notoaciculae, stout, abruptly tapered spines, numbering four–five per parapodium (Fig. 3E). Notopodial hooks absent in all specimens. Neurochaetae comprise three types: *i*) bifurcate chaetae, longer tine seven times longer than shorter one, serrated along its inner side (Fig. 3F); *ii*) long serrated capillaries (Fig. 3G); *iii*) neuroaciculae, numbering two–three per parapodia (Fig. 3H).

Anus opening dorsally on terminal chaetiger; pygidium with a median grooved unpaired papilla.

Etymology. The species is named after Mr. Hisanori Kohtsuka. He has been engaged in marine biology for 30 years in Japan and revealed brilliant biodiversity of Japanese coastal waters. The specific name is a noun in the genitive case.

Confirmed distribution. Shallow to deep-sea waters of Japan, 29–211 m in depth.

Phylogenetic analysis

The topologies (Fig. 4) recovered by ML and BI analyses were identical. *Branchamphinome kohtsukai* sp. nov. and *Pareurythoe borealis* formed a clade with 97% bootstrap support in ML and 1.00 posterior probability in BI. *Pherecardia* cf. *striata* is sister to the *Branchamphinome–Pareurythoe* clade, with a high support value (95% BS, 1.00 PP).

DISCUSSION

The new species has a small body, branchiae in all chaetigers, and a chevron-shaped caruncle. These morphological characters are identified with the diagnosis of *Branchamphinome* in Barroso et al. (2017). The new species can be discriminated from other congeners by having four–five branchial filaments in the midbody segment, two separated pairs of eyes, and pigmentation of the first four chaetigers (see Key to species). The members of *Branchamphinome* have been reported from the shallow to deep-sea waters of the Southern, Atlantic, and South Pacific Oceans. This is the first record of *Branchamphinome* from the Northwestern Pacific Ocean.

The phylogenetic position of *Branchamphinome* has been discussed in Borda et al. (2015) and Barroso et al. (2017). Borda et al. (2015) provisionally classified *Branchamphinome* into Amphinominae. Barroso et al. (2017) inferred that *Branchamphinome* is closely related to *Pherecardia* based on the caruncles' shape. The caruncles are similar to each other in arrangements, although *Branchamphinome* lacks the intricate array of lateral lobes (Barroso et al., 2017). Our phylogenetic tree indicated that *Branchamphinome* belongs to Amphinominae, and *Pareurythoe* is closer than *Pherecardia* to *Branchamphinome* (Fig. 4). This result indicates two patterns of caruncle evolution: *i*) common ancestor species of the *Pherecardia–Branchamphinome–Pareurythoe* clade has a caruncle with lateral lobes, like *Pherecardia*, and lateral developed lobes were secondarily reduced in the *Branchamphinome–Pareurythoe* clade; *ii*) common ancestor species of the *P-B-P* clade has a caruncle with poorly developed lateral lobes, and lateral developed lobes were secondarily reduced in the *Branchamphinome–Pareurythoe* clade; *iii*) common ancestor species of the *P-B-P* clade has a caruncle with poorly developed lateral lobes, and lateral developed lobes were secondarily gained in the *Pherecardia* clade. For understanding the evolution of caruncle ornamentation, most parsimonious reconstructions of morphological characters based on a more robust

phylogenetic tree are needed.

Notopodial hooks are absent in *B. kohtsukai* sp. nov., although they are present in the two species *B. antarctica* and *B. tropicalis*. Barroso et al. (2017) mentioned that the presence of this character is size-dependent. Our phylogenetic tree indicated that this character is not synapomorphy of *Branchamphinome* and *Paramphinome* since the new species formed a clade with *Pareurythoe* (Fig. 4). We agree with the speculation of Barroso et al. (2017) that the presence of notopodial hooks in chaetiger 1 might be a sizerelated feature, which is ultimately lost during the later ontogenetic stage.

The reduction of body size might be a synapomorphy of the *Branchamphinome–Pareurythoe* clade in Amphinominae. Members of the clade have a small body (approximately 1 cm in length) (e.g., Silva, 1965; Hartman, 1967), while others have a large one (approximately 5–10 cm in length) (e.g., Çinar, 2008; Yáñez-Rivera and Salazar-Vallejo, 2011). We suppose that this miniaturization is likely to be related with adaptation to their habitat preference. Most species of Amphinominae are found under rocks or on the seafloor, while the members of the *Branchamphinome–Pareurythoe* clade inhabit sediments (based on NJ's observations in sampling and speculation). Evolutionary history of the lifestyle and body size reduction will be revealed based on broader OTUs sampling with habitat information in future studies.

Key to species of *Branchamphinome* Hartman, 1967 (emended from Barroso et al. [2017])

Branchiae with 15 to 18–20 filaments in midbody segment

 B. antarctica Hartman, 1967
 Branchiae with four–eight filaments in midbody segment
 2

 Two pairs of eyes nearly coalescent, forming a figure '8'; body not characteristically pigmented.
 B. islandica Detinova, 1968
 Two pairs of eyes, not coalescent and not forming a figure '8'; body characteristically pigmented.
 3
 Ventral side of first four chaetigers broadly pigmented, unlike following chaetigers
 B. kohtsukai sp. nov.
 Ventral patches are equally pigmented in all chaetigers
 B. tropicalis Barroso et al., 2017

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COMPETING INTERESTS

The authors have no competing interests to declare.

AUTHOR CONTRIBUTIONS

NJ, NH, KT, and RY collected the samples, NJ carried out morphological observation, NJ conducted molecular analyses, SI helped with the observation and analyses, NJ wrote the text, and all authors approved the manuscript.

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FIGURE LEGENDS AND TABLE CAPTION

Fig. 1. *Branchamphinome kohtsukai* sp. nov. Holotype (NSMT-Pol H-838). **(A)** whole body, dorsal view, **(B)** whole body, ventral view. Paratypes (NSMT-Pol P-839): **(C)** whole body, dorsal view, **(D)** whole body, ventral view, **(E)** whole body, dorsal view, **(F)** whole body, ventral view. Scale bars: 2 mm.

Fig. 2. Branchamphinome kohtsukai sp. nov. Holotype (NSMT-Pol H-838). (A) anterior end, dorsal view, (B) anterior end, ventral view. Paratype (NSMT-Pol P-839):
(C) anterior end, lateral view, (D) anterior end, frontal view. Scale bars: 1 mm.

Fig. 3. *Branchamphinome kohtsukai* sp. nov. Holotype (NSMT-Pol H-838). (**A**) anterior end, dorsal view, (**B**) branchia, chaetiger 10, (**C**) tip of long thick harpoon notochaeta, chaetiger 10, (**D**) tip of serrated capillary notochaeta, chaetiger 10, (**E**) tip of notoacicula, chaetiger 10, (**F**) tip of bifurcate neurochaeta, chaetiger 10, (**G**) tip of serrated capillary neurochaeta, chaetiger 10, (**H**) tip of neuroacicula, chaetiger 10. Scale bar: (**A**) 250 μm, (**B**) 100 μm, (**C**–**H**) 50 μm.

Fig. 4. Maximum-likelihood (ML) phylogenetic tree of Amphinomidae based on COI, 16S, 18S, and 28S sequences. *Euphrosine foliosa* and *Euphrosine* sp. were used as 'outgroups' for the rest of the amphinomids. Nodal support values (bootstrap support [BS] value) higher than 50% are indicated on each branch. Posterior probability of each branch is also shown behind the bootstrap value.

Table 1. List of amphinomids and outgroup species included in the phylogeneticanalysis, together with accession numbers in GenBank.

18S 16S Species 28S COI References Archinome storchi JN086533 JN086523 JN086543 JN086552 Borda et al. (2012) Archinome rosacea EF076777 EF076778 JX028111 JX028046 Wiklund et al. (2008); Borda et al. (2015) Archinome jasoni LC466627 JX028112 KM979540 Borda et al. (2015) JX028118 Archinome tethyana KM055049 JX028140 JX028113 JX028053 Borda et al. (2015) Archinome levinae KM055048 JX028134 KY684724 Borda et al. (2015) JX028037 Amphinome rostrata JN086534 JN086524 JN086544 JN086560 Borda et al. (2012) Branchamphinome MZ568461 MZ568459 MZ568411 MZ568464 This study kohtsukai sp. nov. Chloeia flava JN086536 JN086526 JN086554 Borda et al. (2012) Chloeia viridis JN086537 JN086527 JN086546 JN086555 Borda et al. (2012) Cryptonome barbada NC 037947 NC 037947 Barroso et al. (2018) Cryptonome conclava JN086535 JN086525 JN086545 JN086553 Borda et al. (2012) Eurythoe complanata JN086539 JN086529 JN086548 JN086557 Borda et al. (2012) Hermodice carunculata JN086540 JN086530 JN086549 JN086558 Borda et al. (2012) Hipponoa gaudichaudi Borda et al. (2012) JN086542 JN086532 JN086551 JN086561 Paramphinome jeffreysii AY838856 AY838865 AY838875 AY838840 Struck et al. (2006) Pareurythoe borealis JN086541 JN086531 JN086550 JN086559 Borda et al. (2012) Pherecardia cf. striata MZ568462 MZ568460 MZ568412 MZ568463 This study Outgroup Euphrosine foliosa JN086538 JN086528 JN086547 JN086556 Borda et al. (2012) DQ779687 DQ779649 Euphrosine sp. DQ779613 Rousset et al. (2007)

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