Zoosymposia 19: 103–120 (2020)
https://www.mapress.com/j/zs
Copyright © 2020 · Magnolia Press

ISSN 1178-9905 (print edition)
ZOOSYMPOSIA
ISSN 1178-9913 (online edition)

https://doi.org/10.11646/zoosymposia.19.1.13
http://zoobank.org/urn:lsid:zoobank.org:pub:CA913C47-5576-45DD-912A-B1831A4AD343

Novel symbiotic relationship between a spionid polychaete and *Lingula* (Brachiopoda: Lingulata: Lingulidae), with description of *Polydora lingulicola* sp. nov. (Annelida: Spionidae)

HIROKAZU ABE[1]* & WAKA SATO-OKOSHI[2]

[1] *Department of Biology, Center for Liberal Arts & Sciences, Iwate Medical University, Iwate 028-3694, Japan*
habe@iwate-med.ac.jp, abehiro13@gmail.com, https://orcid.org/0000-0002-7753-9368
[2] *Laboratory of Biological Oceanography, Graduate School of Agricultural Science, Tohoku University, Sendai 980-8572, Japan*
waka.sato-okoshi.d3@tohoku.ac.jp, https://orcid.org/0000-0003-4351-3036
** Corresponding author: Hirokazu Abe*
Email: habe@iwate-med.ac.jp

Abstract

A new spionid species *Polydora lingulicola* **sp. nov.**, a novel symbiont of *Lingula anatina* Lamarck, 1801 is described here based on materials collected from the Yatsushiro Sea and Ariake Sea, southern Japan. *Polydora lingulicola* sp. nov., morphologically closest to *P. glycymerica* Radashevsky, 1993 and *P. vulgaris* Mohammad, 1972, is distinct from latter two shell-boring species by its smaller size, long maximum caruncle length, non-boring lifestyle, presence of subspherical yellow chromatophores on chaetiger 5 (visible in living specimens), and larval morphology. The new species was observed to construct mud tubes on the surfaces of *L. anatina* shells, with the tube apertures located near the lateral inhalant pseudosiphon of the hosts, and utilizes the water currents created by the filter-feeding host for feeding suspended food particles. *Polydora lingulicola* **sp. nov.** represents the first polychaetous annelid reported to exhibit an obligate symbiotic relationship with a lingulid brachiopod. Epibiotic polychaetes previously reported as *P. cornuta* Bosc, 1802 to occur on *L. anatina* shells from Japan in 1902 may belong to *P. lingulicola* **sp. nov.** The possibility that the wild populations of *P. lingulicola* **sp. nov.** may be vulnerable to extinction because they only associate with *L. anatina*, wild populations of which are near threatened by habitat loss, coastal pollution, and fishing pressure.

Keywords: commensalism, epibiont, *Lingula anatina*, symbiosis, threatened species, tidal flat, tube-dwelling

Introduction

Symbiotic relationships between animals are ubiquitous and diverse in marine environments and play an important role in shaping the spatial pattern and structure of marine biodiversity (Williams & McDermott 2004; Anker *et al*. 2005; Macdonald *et al*. 2006). Many of the polychaetous annelids that belong to the genus *Polydora* (family Spionidae) have symbiotic associations with other marine benthic invertebrates (Martin & Britayev 1998, 2018) and are capable of boring into hard calcareous substrates, such as mollusk shells (Blake & Evans 1973). These species are considered as harmful shell borers in mollusk aquaculture since heavy *Polydora* infestations can reduce the commercial value (*i.e*., growth rate and meat yield) of farmed species, as well together with high rates of mortality (*e.g*. Sato-Okoshi 1999; Simon & Sato-Okoshi 2015; Clements *et al*. 2018; Rice *et al*. 2018). Evidence of boring by *Polydora* and related genera (*i.e*., polydorids) is ubiquitous in fossil and recent substrates, and the host spectrum of these boring taxa is broad, including members of various phyla: Arthropoda, Brachiopoda, Bryozoa, Cnidaria, Echinodermata (fossil record only: Wisshak & Neumann 2006), Mollusca, and Porifera (Martin & Britayev 1998, 2018; Rodrigues *et al*. 2008). Boring worms can also bore into non-biogenic hard substrata, such as limestone, mudstone, and sandstone, and even though most borers are opportunists that are associated with a range of host species, some borers are specialists or commensals that are associated with a specific host species or with a group of similar host species (Martin & Britayev 1998, 2018; Williams & McDermott 2004; Radashevsky 2012). Records of trace fossils attributed to

Submitted: 30 Sept. 2019; Accepted by Sara Lindsay: 21 Apr. 2020; published: 28 Dec. 2020

the boring activities of spionid polychaetes (known as some ichnogenera including *Caulostrepsis*) reach back to the Paleozoic (Cameron 1969) and are much better documented for the Holocene and fossil mollusk shells (Taylor & Wilson 2003). Their traces have also been discovered in the shells of rhynchonelliform brachiopods from as far back as the Devonian (Taylor & Wilson 2003) and the association is also known in modern species (Rodrigues *et al*. 2008). However, the association between spionid polychaetes and Linguliform brachiopods has not been reported heretofore.

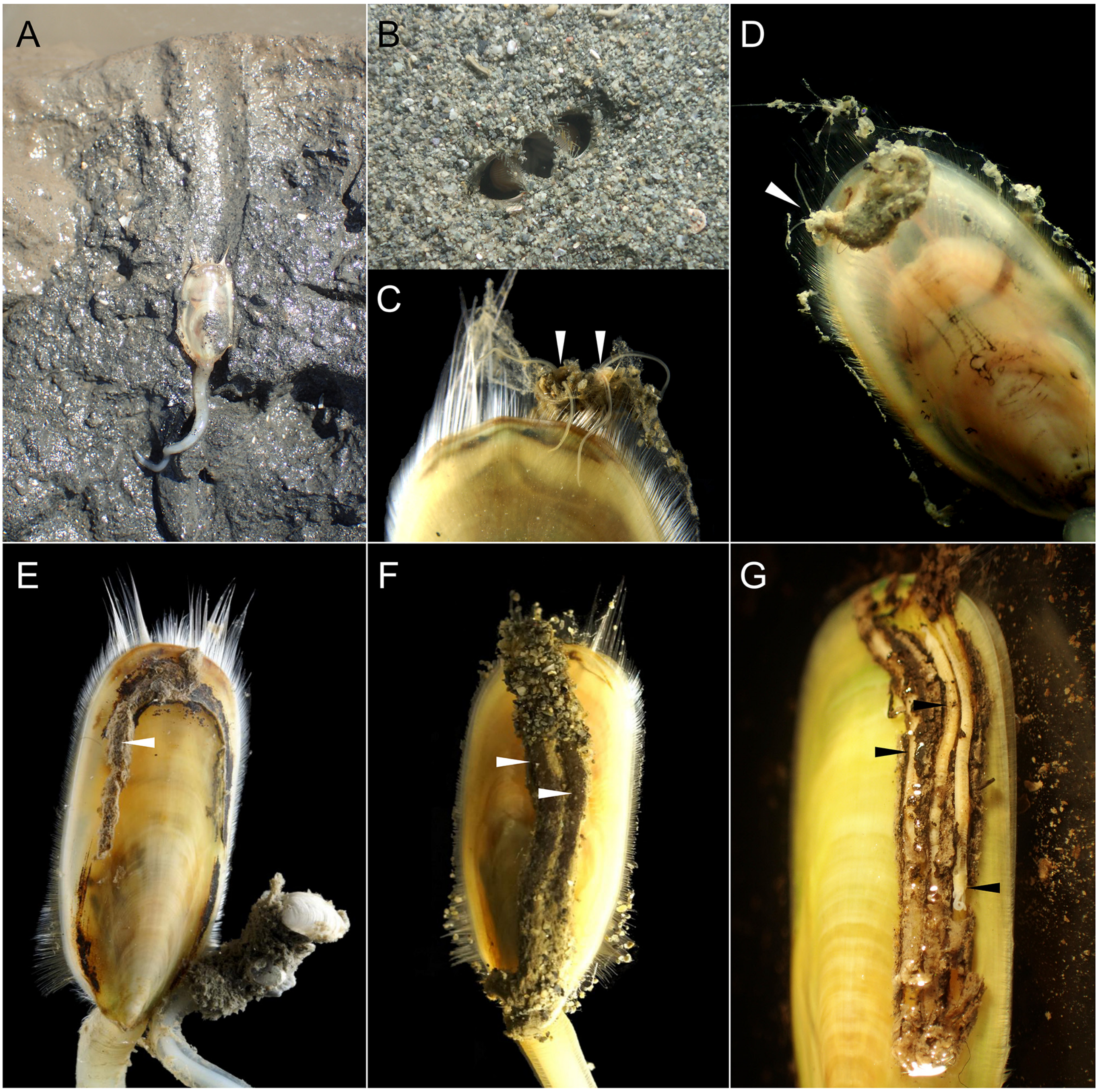

FIGURE 1. *Polydora lingulicola* **sp. nov.** and its host *Lingula anatina*. A, vertical burrow of *L. anatina* in the soft sediment of a tidal flat. B, three pseudosiphon holes of *L. anatina* on the sediment surface. C, two *P. lingulicola* **sp. nov.** individuals on a *L. anatina* shell, with palps extending from tubes. D, *P. lingulicola* **sp. nov.** individual attached to the upper-part of *L. anatina* shell. E, F, *P. lingulicola* **sp. nov.** mud tubes attached to lateral (E) and central (F) parts of shells its host *Lingula anatina*. G, *Polydora lingulicola* **sp. nov.** inside of their tubes (preserved specimen). Arrowheads indicate *P. lingulicola* **sp. nov.** individuals or tubes.

The modern lingulid brachiopod *Lingula anatina* Lamarck, 1801 is widely distributed in the western Pacific and has been reported from Japan, Korea, China, the Philippines, Australia, and India (Williams *et* *al*. 2000; Japanese Association of Benthology 2012). The taxonomy of *Lingula* species in Japan is confusing. Nishizawa *et al*. (2010) distinguished four populations of *L. anatina* as different species: *L. nipponica* Hayasaka, 1931 (Mutsu Bay), *Lingula* sp. 1 (Amami-Oshima Island), *Lingula* sp. 2 (Ariake Sea), and *Lingula* sp. 3 (Izu Shimoda). Afterward, Kuramochi *et al*. (2012) identified the *Lingula* population in the Ariake Sea as *L. reevei* Davidson, 1880, based on shell morphology. However, their results have not been followed by subsequent publications, such as those by the Japanese Association of Benthology (2012), Itoh (2017), and Fujii *et al*. (2019). Therefore, in the present study, the *Lingula* populations in the Ariake Sea and Yatsushiro Sea are referred to as *L. anatina*.

Lingula anatina inhabits mucus-lined vertical burrows in shallow-water soft sediments (Fig. 1A), and individual organisms use their elongated pedicles to anchor themselves at the bottoms of their burrows (Savazzi 1991; Emig 1997). The species is also a filter feeder, with two shell valves and three pseudosiphon holes at the anterior end of its mantle (Fig. 1B). A variety of ectosymbionts have been reported from *Lingula*; however, most of them are merely facultative, the result of chance selection of the hard substratum provided by the host shells as a settlement site (Hammond 1984). Exceptionally, obligate symbiotic bivalves that attach to the anterior end of *L. anatina* shells have been reported (Savazzi 2001; Lützen *et al*. 2009; Sato *et al*. 2011; Goto *et al*. 2014).

In the present study, a novel obligate ectosymbiotic association between a *Polydora* species and *L. anatina* is reported for the first time from southern Japan. A new species, *Polydora lingulicola* **sp. nov.**, is described here based on specimens collected from the Yatsushiro Sea and Ariake Sea, southern Japan.

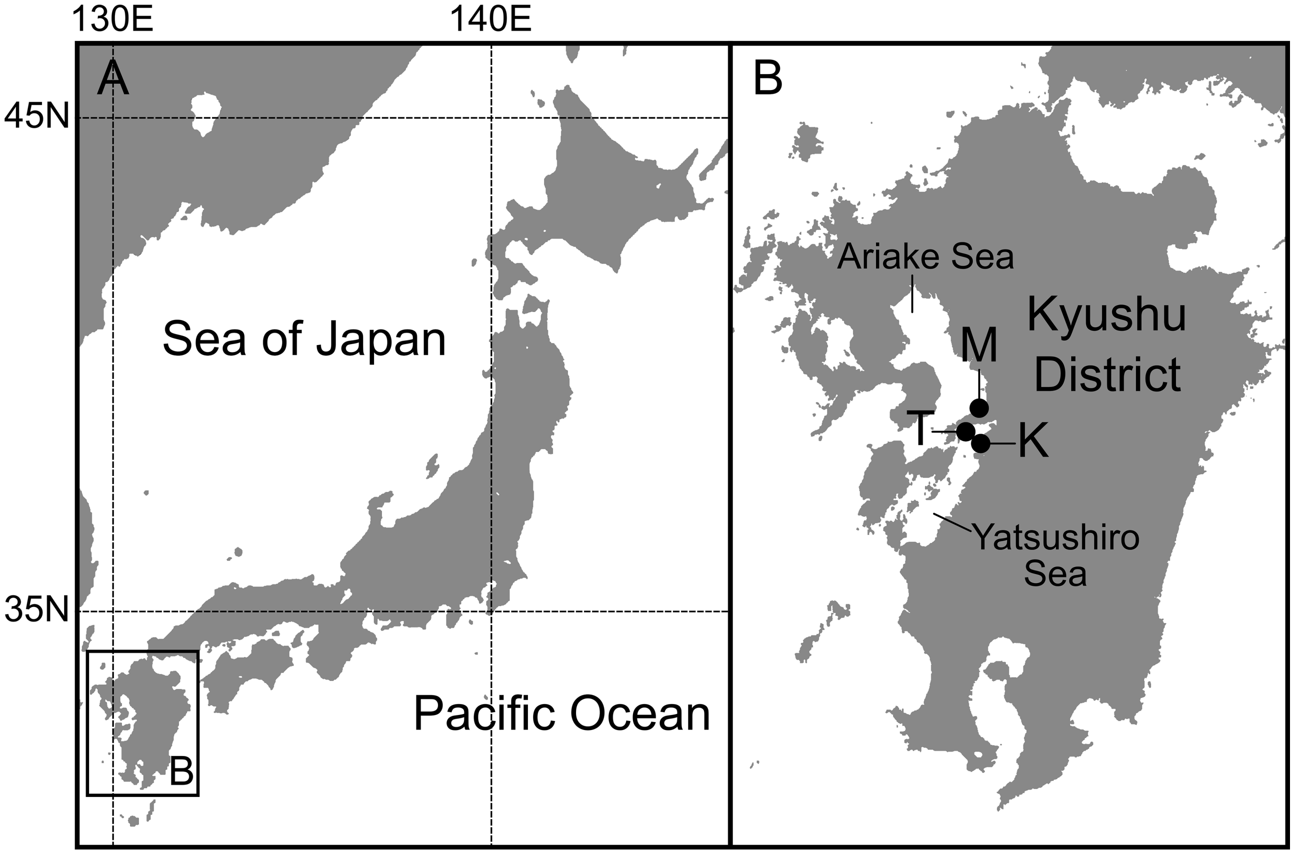

FIGURE 2. *Polydora lingulicola* **sp. nov.** sampling locations. A, map of Japan. B, map of Kyushu District. K: Kumagawa River Estuary (type locality); T: Tobase Island; M: Midorikawa River Estuary.

Materials and Methods

Specimen collection and morphological analysis

Lingula anatina were collected from the Kumagawa River Estuary (32°32′ N, 130°33′ E) and Tobase Island

(32°35′ N, 130°29′ E) in the Yatsushiro Sea, and the Midorikawa River Estuary (32°42′ N, 130°34′ E) in the Ariake Sea, Kumamoto Prefecture, Japan, on September 2016 (Fig. 2). *Lingula* samples were collected from bottom sediments using a hand scoop, and *Lingula* specimens with *Polydora* tubes attached to their shell surfaces (Fig. 1C–G) were fixed in 10% formalin (in seawater) before being stored in 70% ethanol for morphological analyses or were preserved in 99% ethanol for molecular analyses. To observe the behavior of the symbiotic *Polydora* species on the shell surfaces of the infaunal *Lingula*, living specimens were examined in the laboratory. During specimen collection, the number of *L. anatina* encountered with and without *Polydora* tubes were counted to calculate the prevalence of *Polydora* infestation. *Polydora* specimens were extracted from their mud tubes, and the morphologies of both live (anesthetized in 7% $MgCl_2$) and preserved specimens were observed using a stereomicroscope (SZ40 and SZX9; Olympus, Tokyo, Japan) and light micrographs were obtained using a digital camera (α6000; Sony, Tokyo, Japan) that was attached to the microscope using a c-mount camera adapter (SA20; Wraymer, Osaka, Japan). During the morphological examination, sections of the fifth chaetiger with major spines were mounted on microscope slides and observed using a phase-contrast light microscope (Eclipse 80i; Nikon, Tokyo, Japan). The specimens of *P. lingulicola* **sp. nov.** and host *L. anatina* have been deposited in the National Museum of Nature and Science (NSMT), Tsukuba, Japan, under the museum registration numbers NSMT-Pol H-790, P-791–P-805 (*P. lingulicola* **sp. nov.**) and NSMT-Te 1214–1226 (*L. anatina*), respectively. The number of specimens from each sampling site is given in parentheses following museum registration numbers under the "Type material" section below.

Molecular analysis

Genomic DNA was extracted from the ethanol-preserved tissue of seven *Polydora* specimens that were collected from the shells of *L. anatina* in the Ariake Sea by grinding and heating at 95°C for 20 min in 50 µL TE buffer (pH 8.0) with 10% Chelex 100 (Bio-Rad Laboratories, Hercules, CA; Richlen & Barber 2005), and analyses of nuclear (18S and 28S rRNA) and mitochondrial (16S rRNA) genes were performed according to the methods of previous studies (Sato-Okoshi & Abe 2012, 2013; Teramoto *et al*. 2013; Abe *et al*. 2016; Sato-Okoshi *et al*. 2017; Simon *et al*. 2019). The 28S and 16S rRNA gene sequences of *Polydora aura* Sato-Okoshi, 1998 and *Polydora cornuta* Bosc 1802 specimens that were collected from Hiroshima Bay, the Seto Inland Sea and Sasuhama, Ishinomaki Bay, Japan, respectively, were also analyzed for inclusion in the molecular phylogenetic analysis of *Polydora* species because these species closely resemble *P. lingulicola* **sp. nov.** All the newly generated sequences in the present study have been deposited in the DDBJ/ENA/GenBank databases under accession numbers LC500909–LC500931 and LC541483–LC541485 (Table 1).

To reconstruct the molecular phylogeny of *Polydora*, the 18S, 28S, and 16S rRNA gene sequences were aligned with sequences from other *Polydora* species and outgroup taxa (*Dipolydora*) that had been obtained from DDBJ/ENA/GenBank (Table 1) using MAFFT (online service ver. 7) with the L-INS-i algorithm (Katoh *et al*. 2017), and ambiguously aligned regions were eliminated using Gblocks (server ver. 0.91b) with the least stringent settings (Castresana 2000; Talavera & Castresana 2007). The final lengths of the aligned sequences were 1743, 768, and 471 bp for the 18S, 28S, and 16S rRNA genes, respectively. A maximum likelihood (ML) phylogenetic tree was constructed using concatenated 18S, 28S, and 16S rRNA gene sequences by RAxML ver. 8.1.2 (Stamatakis 2014), as implemented in raxmlGUI ver. 1.5 (Silvestro & Michalak 2012), under the GTR+GAMMA+I model. The robustness of the ML trees was evaluated using rapid bootstrap analysis with 1000 replicates. Ecological information on the lifestyle of each species based on available literature (Table 1) was mapped onto the phylogenetic tree.

TABLE 1. Species, sequences, and ecological information of *Polydora* and outgroup (*Dipolydora*) taxa used for molecular phylogenetic analyses. Bold text indicates the gene sequences generated by the present study.

Genus	Species	GenBank accession number			Ecological information			Reference of ecological information
		18S	28S	16S	Life style	Host / Habitat	Nature of symbiosis	
Polydora	*P. lingulicola* sp. nov.	**LC500909 –LC500915**	**LC500916 –LC500922**	**LC500924 –LC500930**	Non-boring symbiotic (Tube-dwelling)	*Lingula anatina* (Brachiopoda)	Obligatory, Permanent	This Study
	P. aura	AB705409	**LC500923**	**LC500931**	Symbiotic boring	Mollusk shell, Coralline alga	Facultative, Permanent	Sato-Okoshi 1999; Sato-Okoshi & Abe 2012
	P. aura	KR052141	-	-	-	*Anadara uropigimelana*	-	Ye *et al*. 2019
	P. cornuta	**LC541483**	**LC541485**	**LC541484**	Free-living (Tube-dwelling) /Sessile	Soft sediment /hard substrates	-	Abe *et al*. 2019
	P. brevipalpa	AB705407	-	-	Symbiotic boring	Mollusk shell	Facultative, Permanent	Sato-Okoshi 1999; Sato-Okoshi & Abe 2012
	P. calcarea	AB705403	-	-	Symbiotic boring	Mollusk shell	Facultative, Permanent	Sato-Okoshi & Abe 2013
	P. hoplura	AB705408	LC101851	LC101867	Symbiotic boring	Mollusk shell	Facultative, Permanent	Sato-Okoshi 1999; Sato-Okoshi *et al*. 2017
	P. lingshuiensis	KF562240	KF562246	KF562233	Symbiotic boring /Free-living (Tube-dwelling)	Mollusk shell	Facultative, Permanent?	Ye *et al*. 2015
	P. neocaeca	AB705404	KF562248	KF562234	Symbiotic boring	Mollusk shell	Facultative, Permanent	Sato-Okoshi & Abe 2013; Ye *et al*. 2015; Malan *et al*. 2020
	P. cf. *nuchalis*	KY677902	-	-	Free-living (Tube-dwelling)	Soft sediment		Williams *et al*. 2017

...Continued on the next page

TABLE 1. (Continued)

Genus	Species	GenBank accession number			Ecological information			Reference of ecological information
		18S	28S	16S	Life style	Host / Habitat	Nature of symbiosis	
	P. onagawaensis	AB691768	-	-	Symbiotic boring	Mollusk shell	Facultative, Permanent	Teramoto *et al*. 2013
	P. triglanda SB	JN048718	JN048731	JN048705	Symbiotic boring	Mollusk shell	Facultative, Permanent	Radashevsky & Pankova 2013
	P. triglanda TD	JN048723	JN048736	JN048710	Free-living (Tube-dwelling)	Soft sediment	-	Radashevsky & Pankova 2013
	P. websteri	AB705402	-	-	Symbiotic boring	Mollusk shell	Facultative, Permanent	Sato-Okoshi 1999; Sato-Okoshi & Abe 2013
Dipolydora	*D. bidentata*	LC107609	JX228085	JX228103	Symbiotic boring	Mollusk shell, Hermit crab shell, Coralline alga, Serpulid tube	Facultative, Permanent	Radashevsky 1993; Radashevsky & Pankova 2013
	D. capensis	KY677896	-	-	Symbiotic boring	Mollusk shell	Facultative, Permanent	Williams *et al*. 2017
	D. cardalia	JX228073	JX228093	JX228113	Free-living (Tube-dwelling)	Soft sediment	-	Radashevsky & Pankova 2013
	D. carunculata	JN048711	JN048724	JN048698	Symbiotic boring /Free-living (Tube-dwelling)	Mollusk shell, Hermit crab shell, Barnacle shell, Sponge, Coralline alga, Soft sediment	Facultative, Permanent?	Radashevsky 1993; Radashevsky & Pankova 2013
	D. cf. *socialis*	KY677899	-	-	Primarily free-living (Tube-dwelling)	Soft sediment		Williams *et al*. 2017

Results

Descriptions

Family Spionidae Grube, 1850

Genus *Polydora* Bosc, 1802

Polydora lingulicola **sp. nov.**
(Figs. 3, 4)
urn:lsid:zoobank.org:act:4AC36CC3-C898-4595-9965-C229EF20E040

Type material. Holotype: NSMT-Pol H-790, Kumagawa River Estuary, Yatsushiro Sea, Kumamoto Pref., Japan, 32°32′N, 130°33′E, isolated from *Lingula anatina*, intertidal, sand flat, coll. H. Abe, M. Taru, M. Tanaka, & T. Unagami, Sep. 11, 2016. Paratypes: NSMT-Pol P-791–P-792 (2), Kumagawa River Estuary, Yatsushiro Sea, Kumamoto Pref., 32°32′N, 130°33′E, isolated from *Lingula anatina*, intertidal, sand flat, coll. H. Abe, M. Taru, M. Tanaka, & T. Unagami, Sep. 11, 2016; NSMT-Pol P-793 (2), Tobase Island, Yatsushiro Sea, Kumamoto Pref., 32°35′N, 130°29′E, isolated from *Lingula anatina*, intertidal, mudflat, coll. H. Abe, M. Taru, M. Tanaka, & T. Unagami, Sep. 14, 2016; NSMT-Pol P-794–P-804 (27), Midorikawa River Estuary, Ariake Sea, Kumamoto Pref., 32°42′N, 130°34′E, isolated from *Lingula anatina*, intertidal, mudflat, coll. H. Abe, M. Taru, M. Tanaka, & T. Unagami, Sep. 15, 2016, NSMT-Pol P-805 (6), coll. H. Nakata, Oct. 26, 2015.

Adult morphology. Up to 18 mm long, 0.8 mm wide for 115 chaetigers; holotype 12 mm long, 0.6 mm wide for 83 chaetigers. Body of living specimen light tan in life (Fig. 3A); body pigmentation absent in holotype, scattered brown pigmentation present in dorsal, lateral, and/or ventral side of middle and posterior chaetigers in some paratypes (Fig. 4B); palp pigmentation absent. Prostomium weakly incised on anterior margin (Figs. 3B, 4A). Eyes absent in holotype, usually absent but occasionally present in paratypes, especially in small individuals (Fig. 3G); four black eyes arranged as a trapezoid, with anterior pair more widely spaced than posterior pair. Caruncle extending posteriorly to end of chaetiger 2 in holotype, up to end of chaetiger 3 in paratypes, with fingerlike median antenna, present on caruncle at level of chaetiger 1 (Figs. 3C, 4A).

Chaetiger 1 with noto- and neuropodial postchaetal lamellae; notochaetae absent, short capillary neurochaetae present. Prechaetal lamellae absent in all parapodia. Parapodial lobes and postchaetal lamellae well developed on anterior chaetigers except chaetiger 5, gradually reducing on posterior chaetigers. Neuro- and notochaetae of chaetiger 2–4, 6 winged capillaries, arranged in two rows; capillaries in anterior row more curved and winged than that in posterior row. Number of capillaries per fascicle and wings in capillaries in notopodia of succeeding chaetigers gradually diminishing; rows of notochaetae becoming indistinct in posterior chaetigers. Posterior notochaetae with only capillaries, modified posterior notochaetae absent. Hooded hooks on neuropodia from chaetiger 7, not accompanied by capillaries; hooks bidentate, with main fang at right angle to shaft and acute angle with apical tooth; shaft slightly curved, having constriction in upper part; curve and constriction of shaft weak in hooks from anterior chaetigers, but well developed in hooks from posterior chaetigers (Fig. 4C); hooks numbering up to 8 in series.

Chaetiger 5 larger than either chaetiger 4 or 6, with well-developed dorsal musculature slightly overlapping chaetiger 6; with curved horizontal row of up to 8 major spines alternating with pennoned companion chaetae (Fig. 4D); dorsal superior capillaries absent; ventral tuft of several short, winged capillaries present. Major spines falcate with lateral flange, some spines simple falcate without lateral flange; lateral tooth invariably absent. One pair of subspherical yellow chromatophores present on anterior dorso-lateral sides of chaetiger 5 in living specimens (Fig. 3B). Pair of whitish spots, apparently groups of epidermal mucous cells (Radashevsky 1993), present on ventral side of chaetiger 7 (Fig. 3A). Subspherical yellow chromatophores and whitish spots unrecognizable in preserved specimens.

Branchiae from chaetiger 7 to end of body (Fig. 3D), absent from 6 to 10 posteriormost chaetigers; full-sized from chaetiger 9 or 10; free from notopodial postchaetal lamellae, with flattened surfaces oriented laterally. Nototrochs from chaetiger 7 onwards.

Pygidium white and cup-shaped, with dorsal gap (Figs. 3D, 4B). No gizzard-like structure in digestive tract. Grandular pouches from chaetiger 7.

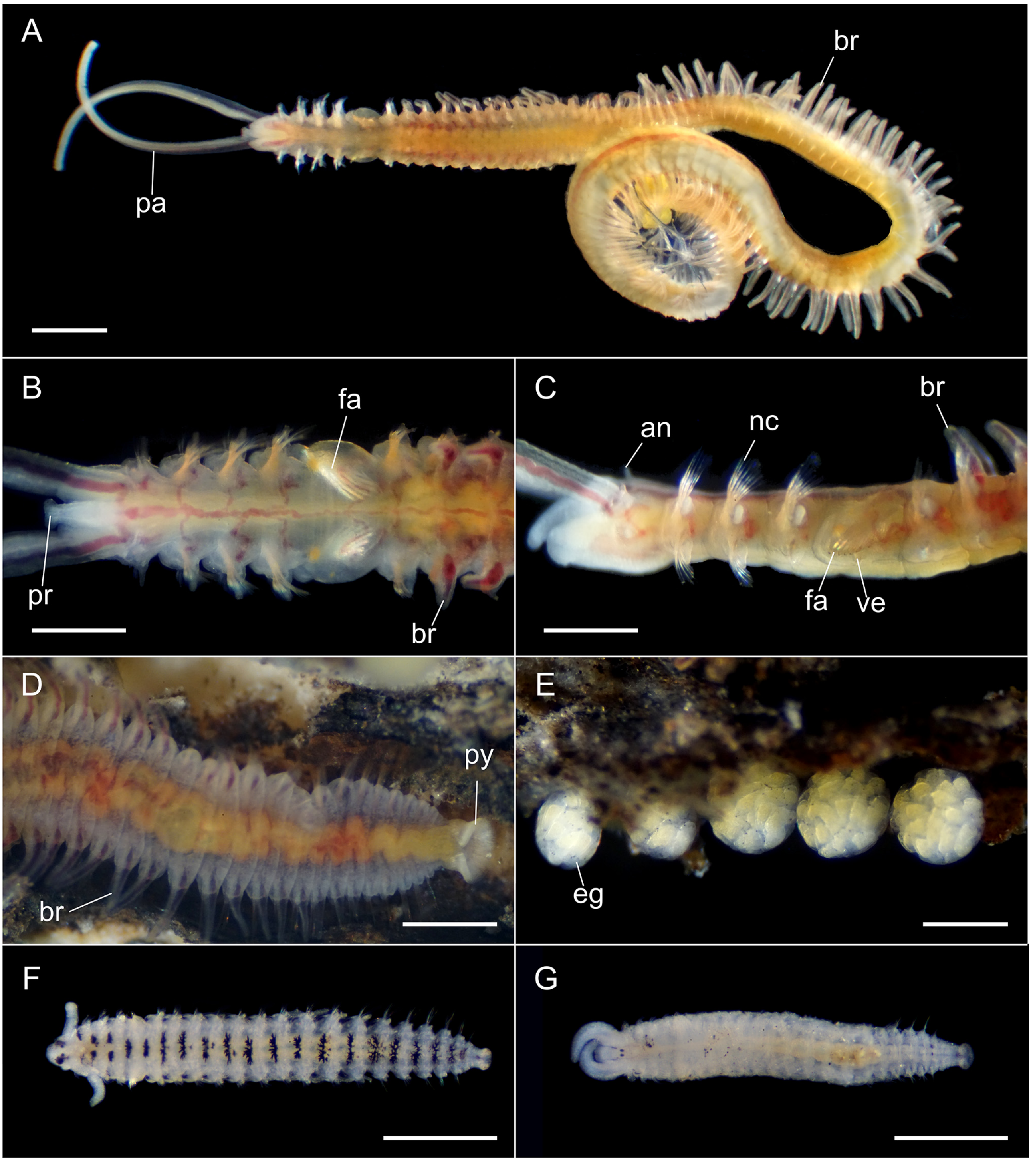

FIGURE 3. *Polydora lingulicola* **sp. nov.** Light micrographs of living (A–E) and preserved specimens (F, G). A–D: NSMT-Pol H-790 (holotype); E: NSMT-Pol P-802 (paratype); F, G: NSMT-Pol P-798 (paratype). A, entire body, anterior portion is in ventral view. B, anterior end, dorsal view. C, anterior end, lateral view. D, posterior end, dorsal view. E, egg capsules. F–G, pre-metamorphosed 21-chaetiger larvae (F) and metamorphosed 21-chaetiger juvenile (G) collected from the mud tubes attaching to the shell of *L. anatina*. br, branchia; eg, egg capsule; fa, major falcate spines; nc, notopodial capillaries; pa, palp; pr, prostomium; py, pygidium; ve, ventral capillaries. Scale bars: (A) = 1 mm; (B–G) = 500 µm.

Larval morphology. Pre-metamorphosed larvae at 21-chaetiger stage collected from inside of mud tubes have three pairs of eyes, most lateral pairs double-eyes (Fig. 3F); ramified melanophores between first and second innermost pair of eyes present. Two rows of melanophores on chaetigers 2 to 4 or 5, median row of star-shaped ramified melanophores from chaetiger 5 or 6 onward. Dorso-lateral black pigment spot from chaetiger 5 or 6 onward, ventral pigment absent; black pigment spot on the pygidium. Modified chaetae in chaetiger 5. Larval pigmentation lost immediately after metamorphosis (Fig. 3F).

Host association. Host *Lingula anatina* Lamarck, 1801 (Brachiopoda: Lingulidae). *Polydora lingulicola* **sp. nov.** constructed mud tubes on the surfaces of *L. anatina* shells, with their tube apertures located near anterior chaetae that project along the anterior end of the host shell (Fig. 1C–G). Tube positioning was variable, attached to the upper, lateral, or central parts of host shells (Fig. 1D–G). Tubes attached to upper shell parts were generally small and inhabited by juvenile worms (Fig. 1D). The number of the worms per host was usually one to three, but sometimes up to nine (2.5 ± 2.2 ind., N = 15). The prevalence of *Polydora* infestation of *L. anatina* varied according to sampling site: very low prevalence rates at Tobase Island (1.9%, N = 107) and the Kumagawa River Estuary (0.8%, N = 119) and 25.3% at the Midorikawa River Estuary (N = 83). The *Polydora* species typically attached to a single *Lingula* valve: 62.5% on the ventral valve, 33.3% on the dorsal valve, and 4.2% on both valves (N = 24). In the Midorikawa River Estuary, more than 100 individuals of sympatric infaunal bivalve species (*e.g.*, *Mactra quadrangularis* Reeve, 1854) were examined; however, *P. lingulicola* **sp. nov.** was only observed on *Lingula* shells.

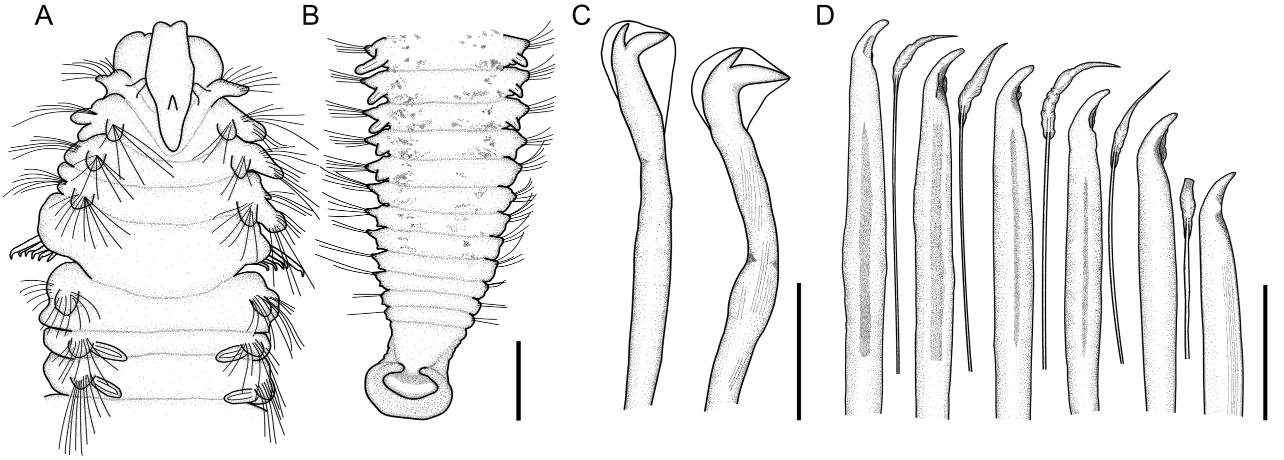

FIGURE 4. *Polydora lingulicola* **sp. nov.** A: NSMT-Pol H-790 (holotype); B: NSMT-Pol P-799 (paratype); C, D: NSMT-Pol P-794 (paratype). A, anterior end, dorsal view; B, posterior end, dorsal view; C, bidentate hooded hook from neuropodium of chaetiger 7 (left) and 72 (right); D, major spines and pennoned companion chaetae of chaetiger 5, ventral view. Scale bars: (A, B) = 300 µm; (C) = 20 µm; (D) = 100 µm.

Reproduction. Egg strings were found inside the *Polydora* mud tubes in September 2016 (Fig. 3E). The egg capsules were spherical and independent, without joining one another, and line up in series of egg capsules, sometimes touching, but not attached to neighboring capsules, and attached to the inner wall of the tube by thin filaments. The egg capsules were 500–580 µm (518.8 ± 48.8 µm, N = 8) in diameter in live condition and up to 32 capsules were contained in each string. The eggs were 110–120 µm (115.8 ± 6.7 µm, N = 12) in diameter in live conditions, without nurse eggs, and all embryos developed simultaneously (Fig. 3E). One- and two-chaetiger larvae within egg capsules were 180–230 µm (195.2 ± 11.9 µm, N = 25) in length in live conditions.

Molecular phylogeny. There was no intraspecific variation (100% sequence similarity) in the 18S and 28S rRNA gene sequences of seven *P. lingulicola* **sp. nov.** specimens, except for a hetero-peak site in the 18S rRNA gene sequence of a single specimen, and single nucleotide substitutions were identified at two sites in the 16S rRNA gene sequences. The sister clade relationship of *P. lingulicola* **sp. nov.** and *P. aura* from Hiroshima Bay, Japan, was well-supported (Fig. 5). The newly generated 18S rRNA genes sequences of *P. lingulicola* **sp. nov.** shared 99.8–99.9% identity with the DDBJ/ENA/GenBank sequence labeled as *P. aura* (DDBJ/ENA/GenBank ID: KR052141), which was collected from an *Anadara uropigimelana* (Bory de Saint-Vincent, 1827) shell in Weihai, China (Ye *et al.* 2019), although there was no mention of whether it was attached to or had bored into the shell.

TABLE 2. Morphology of known *Polydora* species that have a single median antenna on the caruncle and chaetiger 5 with ventral capillaries but lacking dorsal superior capillaries. *Polydora cornuta*, larger individuals of which lack ventral capillaries in chaetiger 5, is included as recently settled juveniles of the species occasionally possess one or two short ventral capillaries.

Species	Prostomium	Cruncle (maximal length)	Branchiae from chaetiger 7 to	Chaetiger 5		Modified posterior notochaetae	Pygidium	Pigmentation	Life style	Reference
				Major spine	Companion chaetae					
***Polydora lingulicola* sp. nov.**	**Incised**	**End of chaetiger 2–3**	**Near the end**	**Falcate with lateral flange**	**Present**	**Absent**	**Disc-like with middorsal gap**	**Absent or present (Scattered brown pigment on middle and posterior body)**	***Lingula* symbiotic**	**This study**
P. aura Sato-Okoshi, 1998	Incised	End of chaetiger 2–3	Near the end	Falcate with lateral flange	Present	Present	Wide flaring disc	Brown and orange pigment on body	Shell-borer	Sato-Okoshi 1998; Sato-Okoshi & Abe 2012
P. cornuta Bosc, 1802	Bifurcated and flaring laterally	End of chaetiger 3	Near the end	Falcate with lateral tooth and subdistal flange or keel	Present	Absent	Flaring cup to disc with middorsal gap to narrow incision	usually black spots on lateral sides of chaetigers from 7–10 to 10–19	Tube-dweller	Radashevsky 2005
P. fusca Radashevsky & Hsieh, 2000	Incised	End of chaetiger 2	About 3/4 of body	Falcate with small lateral tooth	Present	Present	Cup-shaped with dorsal gap	Scattered black pigment on head and body	Tube-dweller	Radashevsky & Hsieh 2000
P. glycymerica Radashevsky, 1993	Incised	Middle of chaetiger 2	Near the end	Falcate with lateral flange or sheath	Present	Absent	Disc-like with wide dorsal gap	Absent	Shell-borer	Radashevsky 1993

...Continued on the next page

TABLE 2. (Continued)

Species	Prostomium	Cruncle (maximal length)	Branchiae from chaetiger 7 to	Chaetiger 5		Modified posterior notochaetae	Pygidium	Pigmentation	Life style	Reference
				Major spine	Companion chaetae					
P. latispinosa Blake & Kudenov, 1978	Incised	End of chaetiger 2	Continuing to posterior end	Falcate with lateral sheath or flange	present	Present	Large flaring disc with wide dorsal gap	Brown pigment along edges of caruncle	Shell-borer	Blake & Kudenov 1978
P. nanomon Orensky & Williams, 2009	Incised	End of chaetiger 2	2/3 of body	Falcate with two lateral teeth	Present	Absent	Cup-shaped	Females sometimes exhibit irregular pigmentation patches on anterior and posterior dorsal side	Shell-borer	Orensky & Williams 2009
P. robi Williams, 2000	Rounded	End of chaetiger 2	End of body	Falcate with small lateral flange	Present	Present	With anal cirri	Absent	Shell-borer	Williams 2000
P. vulgaris Mohammad, 1972	Incised	Middle of chaetiger 2	Near the end	Falcate with lateral flange	Present	Absent	Saucer-shaped with a dorsal notch	-	Shell-borer	Mohammad 1972; Manchenko & Radashevsky 1994

Remarks. *Polydora lingulicola* **sp. nov.** resembles *P. aura*, *P. fusca* Radashevsky & Hsieh, 2000, *P. latispinosa* Blake & Kudenov, 1978, *P. nanomon* Orensky & Williams, 2009, and *P. robi* Williams, 2000, in that all possess median antenna on the caruncle and chaetiger 5 with ventral capillaries but without dorsal superior capillaries. However, *P. lingulicola* **sp. nov.** could be distinguished from these similar species by its maximum caruncle length, consecutive branchial chaetigers, and body pigmentation, as well as by the presence or absence of modified posterior notochaetae (Table 2). *Polydora lingulicola* **sp. nov.** also closely resembles *P. glycymerica* Radashevsky, 1993 and *P. vulgaris* Mohammad, 1972 (Table 2). Although *P. vulgaris* was originally described as possessing modified posterior notochaetae (Mohammad 1972), Manchenko & Radashevsky (1994) reexamined the holotype and revealed that this species lacks modified posterior notochaetae. The paper also noted that *P. glycymerica* and *P. vulgaris* are morphologically indistinguishable (Manchenko & Radashevsky 1994). *Polydora lingulicola* **sp. nov.** is distinct from *P. glycymerica* and *P. vulgaris* by their size and lifestyle: the former is a small-sized non-boring symbiont of lingulid brachiopod measuring up to 18 mm long with about 100 chaetigers, whereas the latter two are both large-sized shell borers (Table 1) measuring about 60 mm long with >200 chaetigers. Additionally, although the caruncle length is generally size-dependent in polydorid species (*e.g*. Radashevsky 1999; Williams & Radashevsky 1999; Sato-Okoshi & Takatsuka 2001), the smaller-sized former species have longer maximum caruncle length than larger-sized latter two species (Table 2). A pair of subspherical yellow chromatophores on chaetiger 5, which visible in living specimens of the new species (Fig. 3B), is not mentioned in the description of *P. glycymerica* and *P. vulgaris* even though the description of *P. glycymerica* was based on living material (Radashevsky 1993). The dorsal pigment pattern of the larvae of *P. lingulicola* **sp. nov.** resembles but slightly differs from those of *P. glycymerica* described in Radashevsky (1989): the two rows of melanophores were continued to chaetiger 4 or 5 in former (Fig. 3F) but are ramified star-shaped and continued more posteriorly in the later (until chaetigers 7 to 10).

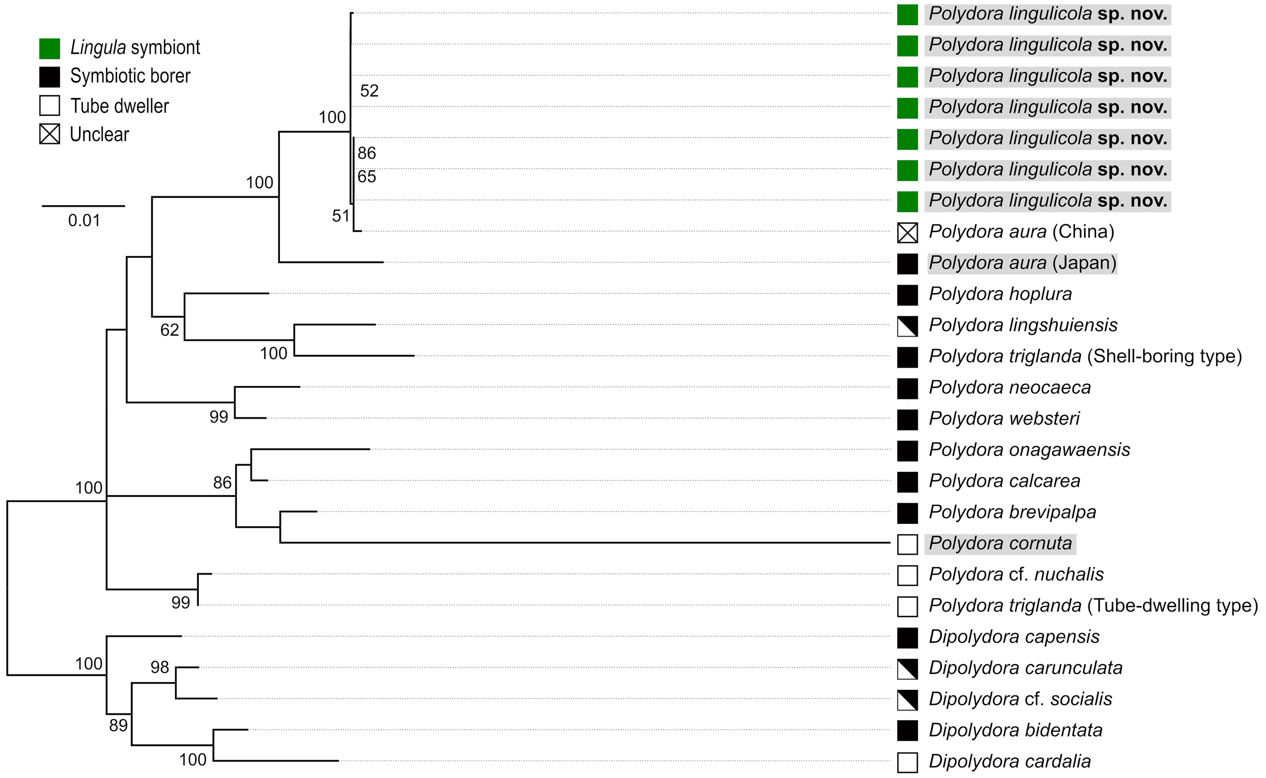

FIGURE 5. Maximum likelihood tree of *Polydora* rRNA gene sequences. Phylogenetic analysis was performed using the concatenated sequences of two nuclear (18S and 28S rRNA) and one mitochondrial (16S rRNA) genes with sequences from *Dipolydora* species for outgroup rooting. Shaded boxes indicate sequences that were generated by the present study, and symbols indicate species lifestyle, which was determined by consulting the literature (Table 1). The node values indicate maximum likelihood bootstrap support values (>50%) and the scale bar represents the number of substitutions per site.

Yatsu (1902a) reported that some *L. anatina* shells from Hyakkan-zeki (located at the mouth of the Tsuboi River, 32°46′ N, 130°37′ E), Kumamoto Prefecture, Kyusyu District, Japan, were covered by the tubes of polychaetous annelids, but the identity of the polychaete was not provided. Hammond (1984) later identified similar worms from the same locality as *P. ligni* (currently synonymized with *P. cornuta*). *Polydora cornuta* is a non-boring species and possesses a single median antenna on the caruncle and chaetiger 5 without dorsal superior capillaries like *P. lingulicola* **sp. nov.** Although 18–22-chaetiger recently settled juveniles of *P. cornuta* occasionally have one or two short ventral capillaries (Radashevsky 2005), these two species are distinguishable as ventral capillaries in chaetiger 5 are always absent in larger individuals of *P. cornuta* whereas the ventral capillaries in chaetiger 5 are always present in *P. lingulicola* **sp. nov.** The dorsal pigment pattern of the larvae of *P. cornuta* and *P. lingulicola* **sp. nov.** are also different as the former have paired melanophores from chaetiger 3 onwards (Radashevsky 2005), whereas the latter have a median single row of melanophores from chaetiger 5 or 6 onwards. The results of the present study suggest that the previous records are attributable to *P. lingulicola* **sp. nov.**, which is described here, owing to the proximity of the sampling localities and the fact that no *P. cornuta* specimens were collected from *Lingula* shells in the present study.

Etymology. The specific name *lingulicola* is a combination of two words, the host genus name *Lingula* plus Latin word -cola (= dweller), which refers to the symbiotic relationship of the species with a brachiopod species belonging to the genus *Lingula*.

Habitat. Middle to lower intertidal zones of mud and sand flats.

Distribution. Ariake Sea and Yatsushiro Sea, southern Japan.

Discussion

Symbiotic ecology

Polydora lingulicola **sp. nov.** is unique among its genus in that it exhibits a symbiotic relationship with the lingulid brachiopod *L. anatina*. Polydorid species, which include the genus *Polydora*, have two types of lifestyles: some dwell in semi-permanent tubes (*i.e.*, tube-dwelling type), whereas the others bore into shells and other hard substrata (*i.e.*, shell-boring type); and very few species have both shell-boring and tube-dwelling modes of life (Radashevsky & Pankova 2013). *Polydora lingulicola* **sp. nov.** is considered as a tube-dwelling species that specialize in adhesion to *Lingula* shells and has only been observed on *Lingula* shells, despite co-occurring with a variety of other bivalves at the sampling sites. This suggests that the species has an obligate symbiotic association with *L. anatina*. Similarly, *Pseudopolydora diopatra* Hsieh, 1992 is reported as a tube-dwelling species that adhesion to tube-caps of the onuphid *Diopatra bilobata* (Hsieh 1992, 1994). However, this species also utilizes the shell surface of the oyster *Crassostrea gigas* (Radashevsky & Hsieh 2000) and considered as facultative symbionts.

Our molecular analysis suggests that a *Polydora* species that was collected from an *A. uropigimelana* shell in China (Ye *et al*. 2019) and labeled as *P. aura* (KR052141) is closely related to *P. lingulicola* **sp. nov.** by the similarity of the 18S rRNA gene sequences (99.8–99.9% identity; Fig. 5). As the sequence from China was divergent from the Japanese *P. aura* 18S rRNA gene sequence (AB705409; Sato-Okoshi & Abe 2012), the specimen labeled as *P. aura* from China was possibly misidentified (Fig. 5). Because the 18S rRNA gene is often considered too conservative to resolve species-level genetic divergence and Ye *et al*. (2019) did not provide a morphological description of the species, it remains unclear whether the *P. lingulicola* **sp. nov.** specimens in the present study and *P. aura* sensu Ye *et al*. (2019) are the same species. If *P. aura* sensu Ye *et al*. (2019) represents the new species, it has important implications on host specificity, suggesting that the species has a broader range of hosts.

Few obligate ectosymbionts of *Lingula* have been reported, but some galeommatoidean bivalves are known to participate in obligate symbiotic associations with *L. anatina*. These bivalves attach to the anterior end of *Lingula* shells and collect food particles from the mantle of the host using their adhesive feet (*Koreamya arcuata*; Savazzi 2001) or using the water currents created by the host for filter-feeding (*K. setouchiensis*; Goto *et al*. 2014). Similar to *K. setouchiensis*, *P. lingulicola* **sp. nov.** individuals utilize the water currents created by their filter-feeding host and collect suspended food particles using their palps. To execute this feeding strategy, the *P. lingulicola* **sp. nov.** individuals form their tube apertures near the anterolateral chaetae of *Lingula* (Fig. 1C–G). *Lingula anatina* uses three pseudosiphon holes on the anterior end of the mantle for filter-feeding (Fig. 1B): the mantle chamber is subdivided into one median exhalant and two lateral inhalant chambers, and form the water currents by ciliary action (Chuang 1956; Emig 1997). In contrast to *K. setouchiensis* (Goto *et al*. 2014), *P. lingulicola* **sp. nov.** individuals typically form tubes with openings near the anterolateral chaetae of the host (Fig. 1D–G), which suggests that *P. lingulicola* **sp. nov.** prefers to use the inhalant current created by the host. Considering that *P. lingulicola* **sp. nov.** collects suspended food particles and takes advantage of particulate fluxes in the inhalant feeding currents of its host, the association between *P. lingulicola* **sp. nov.** and its host should be considered commensalism. It is unclear if *Polydora lingulicola* **sp. nov.** also feed on mucus secreted by its host like *K. arcuata*.

In addition to the advantage of food particulate fluxes, the symbiotic *P. lingulicola* **sp. nov.** is presumed to receive important benefits from its host. For example, the host (*L. anatina*) provides a depth refuge from predation for their symbionts by the rapid predator-avoidance withdrawal reflex into the burrow (Yatsu 1902a; Hammond 1983; Emig 1997). The host also provides a stable habitat, on which the ectosymbionts can complete their lifecycle because *L. anatina* individuals can move their shells up and down within burrows and can burrow upwards after being smothered by quick sedimentation (Yatsu 1902a; Emig 1981; Hammond 1983), which should save their symbionts from burial. In addition, *L. anatina* possess surprisingly long life-spans (Emig 1997), which have been estimated as >4 years in Kasari Bay, Amami-Oshima Island (Fujii *et al*. 2019), ≥7 years in Ariake Sea, southern Japan (Ito 2017), ≥5 years in Misaki, central Japan (Yatsu 1902b), 13–20 years in Asamushi, northern Japan (Akuta 1995), and ≤12 years in Queensland, northeastern Australia (Kenchington & Hammond 1978). Because the life span of *Polydora* species is generally 1–2.5 years or less, with some exceptions (Sato-Okoshi *et al*. 1990; Dualan & Williams 2011; Teramoto *et al*. 2013), the *Lingula* host provides a relatively stable substrate and a sufficient period of time for symbiotic *Polydora* species to complete their lifecycles.

It is likely that *P. lingulicola* **sp. nov.** exhibits long-term planktonic larval development as they form egg capsules without nurse eggs, and all embryos developed simultaneously (Fig. 3E). *Polydora* species form egg capsules within parental tubes and, subsequently, incubate the egg capsule during their movements within the tubes; the fertilized eggs develop into larvae that hatch from the capsules and become planktotrophic after utilizing their intrinsic yolk reserves (*i.e*., long-term planktonic larval development) in the species that form egg capsules without nurse eggs (Blake & Arnofsky 1999). The position of the *P. lingulicola* **sp. nov.** tube on the surfaces of the *L. anatina* shells was variable (Fig. 1 D–G), but the tubes attached to the upper part of the *L. anatina* shells were small and inhabited by juvenile worms (Fig. 1D). Therefore, after larval settlement and metamorphosis on *L. anatina*, it is likely that the juvenile *P. lingulicola* **sp. nov.** first make their tubes on the upper part of *Lingula* shells and then elongate their tubes as they grow, with variation in the direction of tube growth. This tube-forming behavior would be beneficial for their feeding ecology as *P. lingulicola* **sp. nov.** collects suspended food particles from the inhalant feeding currents of its host.

Population sustainability

Wild populations of *P. lingulicola* **sp. nov.** may be vulnerable to extinction because the species is associated only with *L. anatina*, which is considered “near threatened” by the *Threatened Animals of Japanese Tidal Flats: Red Data Book of Seashore Benthos*, which was edited by the Japanese Association of Benthology (2012). In Japan, *L. anatina* is widely distributed, from northern (Mutsu Bay) to southern (Amami Island) Japan. However, wild populations of the species are threatened by habitat loss and coastal pollution, and the species is rare except in the Ariake Sea and Yatsushiro Sea (Kyushu district) and Kasari Bay (Amami Island; Japanese Association of Benthology 2012; Fujii *et al*. 2019). In addition to this, *L. anatina* populations in the Ariake Sea and Yatsushiro Sea are threatened by fishing pressure as they are a traditional food source. Currently, *P. lingulicola* **sp. nov.** is only known to occur in the Ariake Sea and Yatsushiro Sea and has not been observed from the *Lingula* population of the Tekebu tidal flat in Kasari Bay (Fujii, per. comm.). The same or similar *Polydora* species may also be distributed in Korea because similar mud tubes attached to *Lingula* shells were observed in the Saemangeum tidal flat (Hong & Sato, per. comm.). However, that habitat is also seriously threatened by a huge reclamation project around the Saemangeum area (Hong *et al*. 2007). Another previous study reported the finding of an unidentified tubicolous polychaete on the shell of *Lingula reevii* in Hawaii (Hammond 1984).

Acknowledgments

We would like to express our sincere gratitude to Masanori Taru, Kenji Okoshi (Toho Univ.), Masaatsu Tanaka (Kagoshima Univ.), and Tomoo Unagami (Center for Environmental Studies) for their help with fieldwork and specimen collection; Misuzu Aoki (Wetlands International Japan), Yasuhisa Henmi, Haruhiko Nakata (Kumamoto Univ.), Masanori Sato (Kagoshima Univ.), and Taichi Wada (Nankou Wetland Conservation Group) for providing the *Polydora* specimens; Jae-Sang Hong (Inha Univ.), Shin'ichi Sato (Shizuoka Univ.), and Ryoko Fujii (Kagoshima Univ.) for information about mud tubes on *Lingula* shell in Korea and Amami Island; Goh Nishitani (Tohoku Univ.) for support for gene sequencing analysis; Takao Suzuki (Michinoku Research Institute for Benthos) for providing literature; James A. Blake and an anonymous reviewer for helpful comments and suggestions. This study was partly supported by JSPS KAKENHI (Grant Number: JP18K05777, JP15K07540, JP19K15899) and Environment Research and Technology Development Fund (Grant Number: JPMEERF20204R01) of the Environmental Restoration and Conservation Agency of Japan.

References

Abe, H., Kondoh, T. & Sato-Okoshi, W. (2016) First report of the morphology and rDNA sequences of two *Pseudopolydora* species (Annelida: Spionidae) from Japan. *Zoological Science*, 33, 650–658.
https://doi.org/10.2108/zs160082

Abe, H., Takeuchi, T., Taru, M., Sato-Okoshi, W. & Okoshi, K. (2019) Habitat availability determines distribution patterns of spionid polychaetes (Annelida: Spionidae) around Tokyo Bay. *Marine Biodiversity Records*, 12, 7.
https://doi.org/10.1186/s41200-019-0167-4

Akuta, K. (1995) *Ecological distribution and life history of* Lingula anatina *(Brachiopoda, Inarticulata) in Asamushi, northern Japan*. Unpublished M. Sci. Thesis, Tohoku University, Sendai, 42 pp. [in Japanese]

Anker, A., Murina, G.V., Lira, C., Vera Caripe, J.A., Palmer, A.R. & Jeng, M.S. (2005) Macrofauna associated with echiuran burrows: a review with new observations of the innkeeper worm, *Ochetostoma erythrogrammon* Leuckart and Rüppel, in Venezuela. *Zoological Studies*, 44, 157–190.

Blake, J.A. & Arnofsky, P.L. (1999) Reproduction and larval development of the spioniform Polychaeta with application to systematics and phylogeny. *Hydrobiologia*, 402, 57–106.
https://doi.org/10.1023/A:1003784324125

Blake, J.A. & Evans, J.W. (1973) *Polydora* and related genera as borers in mollusk shells and other calcareous substrates. *The Veliger*, 15, 235–249.

Cameron, B. (1969) Paleozoic shell-boring annelids and their trace fossils. *American Zoologist*, 9, 689–703.
https://doi.org/10.1093/icb/9.3.689

Castresana, J. (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution*, 17, 540–552.
https://doi.org/10.1093/oxfordjournals.molbev.a026334

Chuang, S.H. (1956) The ciliary feeding mechanisms of *Lingula unguis* (L.) (Brachiopoda). *Proceedings of the Zoological Society of London*, 127, 167–189.
https://doi.org/10.1111/j.1096-3642.1956.tb00468.x

Clements, J.C., Bourque, D. McLaughlin, J., Stephenson, M. & Comeau, L.A. (2018) Wanted dead or alive: *Polydora websteri* recruit to both live oysters and empty shells of the eastern oyster, *Crassostrea virginica*. *Journal of Fish Diseases*, 41, 855–858.
https://doi.org/10.1111/jfd.12794

Dualan, I.V. & Williams, J.D. (2011) Palp growth, regeneration, and longevity of the obligate hermit crab symbiont *Dipolydora commensalis* (Annelida: Spionidae). *Invertebrate Biology*, 130, 264–276.
https://doi.org/10.1111/j.1744-7410.2011.00234.x

Emig, C.C. (1981) Observations sur l'écologie de *Lingula reevei* Davidson (Brachiopoda: Inarticulata). *Journal of Experimental Marine Biology and Ecology*, 52, 47–61.
https://doi.org/10.1016/0022-0981(81)90170-2

Emig, C.C. (1997) Ecology of inarticulated brachiopods. *In*: Kaesler, R.L. (Ed.), *Treatise on Invertebrate Paleontology, Part H. Brachiopoda, Revised. Vol. 1*. Boulder, Colorado and Lawrence, Kansas: Geological Society of America and University of

Kansas, KS, pp. 473–495.
https://doi.org/10.17161/dt.v0i0.5558

Fujii, R., Ueno, R. & Yamamoto, T. (2019) Breeding season and life history of *Lingula anatina* after settlement in Amami-Oshima Island, Kagoshima, Japan. *Plankton & Benthos Research*, 14, 45–51.
https://doi.org/10.3800/pbr.14.45

Goto, R., Ishikawa, H., Hamamura, Y., Sato, S. & Kato, M. (2014) Evolution of symbiosis with *Lingula* (Brachiopoda) in the bivalve superfamily Galeommatoidea (Heterodonta), with description of a new species of *Koreamya*. *Journal of Molluscan Studies*, 80, 148–160.
https://doi.org/10.1093/mollus/eyu009

Hammond, L.S. (1983) Experimental studies of salinity tolerance, burrowing behavior and pedicle regeneration in *Lingula anatina* (Brachiopoda, Inarticulata). *Journal of Paleontology*, 57, 1311–1316.

Hammond, L.S. (1984) Epibiota from the valves of recent *Lingula* (Brachiopoda). *Journal of Paleontology*, 58, 1528–1531.

Hong, J.-S., Yamashita, H. & Sato, S. (2007) The Saemangeum Reclamation Project in South Korea threatens to extinguish an unique mollusk, ectosymbiotic bivalve species attached to the shell of *Lingula anatina*. *Plankton & Benthos Research*, 2, 70–75.
https://doi.org/10.3800/pbr.2.70

Hsieh, H.L. (1992) *Pseudopolydora diopatra*, a new species (Polychaeta, Spionidae) from Taiwan. *Proceedings of the Biological Society of Washington*, 105, 630–635.

Hsieh, H.L. (1994) Larval development and substrate preference at settlement in *Pseudopolydora diopatra* (Polychaeta: Spionidae). *Invertebrate Reproduction & Development*, 25, 205–214.
https://doi.org/10.1080/07924259.1994.9672387

Ito, S. (2017) Growth and maturation of *Lingula anatina* in the inner part of Ariake Sound. *Bulletin of Saga Prefectural Ariake Fisheries Research and Development Center*, 28, 105–110. [in Japanese with English abstract]

Japanese Association of Benthology (2012) *Threatened Animals of Japanese Tidal Flats: Red Data Book of Seashore Benthos*. Tokai University Press, Tokyo, 285 pp. [in Japanese]

Katoh, K., Rozewicki, J. & Yamada, K.D. (2017) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics*, bbx108.
https://doi.org/10.1093/bib/bbx108

Kenchington, R.A. & Hammond, L.S. (1978) Population structure, growth and distribution of *Lingula anatina* (Brachipoda) in Queensland, Australia. *The Transactions of the Zoological Society of London*, 184, 63–81.
https://doi.org/10.1111/j.1469-7998.1978.tb03266.x

Kuramochi, T., Koi, A., Kashihara, K. & Naganuma, T. (2012) Taxonomic revision of Japanese *Lingula anatina* with *L. reevei* (Brachiopoda: Lingulata). *Biosphere Science: Journal of the Graduate School of Biosphere Science, Hiroshima University*, 51, 27–35. [in Japanese with English abstract]

Lützen, J., Hong, J.-S. & Yamashita, H. (2009) *Koreamya arcuata* (A. Adams, 1856) gen. nov. (Galeommatoidea; Montacutidae), a commensal bivalve associated with the inarticulate brachiopod *Lingula anatina*. *Journal of Conchology*, 39, 669–699.

Macdonald, K.S., Ríos, R. & Duffy, J.M. (2006) Biodiversity, host specificity, and dominance by eusocial species among sponge-dwelling shrimp on the Belize Barrier Reef. *Diversity and Distributions*, 12, 165–178.
https://doi.org/10.1111/j.1366-9516.2005.00213.x

Manchenko, G.P. & Radashevsky, V.I. (1994) Genetic differences between two allopatric sibling species of the genus *Polydora* (Polychaeta: Spionidae) from the West Pacific. *Biochemical Systematics and Ecology*, 22, 767–773.
https://doi.org/10.1016/0305-1978(94)90079-5

Malan, A., Williams, J.D., Abe, H., Sato-Okoshi, W., Matthee, C.A. & Simon, C.A. (2020) Clarifying the cryptogenic species *Polydora neocaeca* Williams & Radashevsky, 1999 (Annelida: Spionidae): a shell-boring invasive pest of molluscs from locations worldwide. *Marine Biodiversity*, 50, 51.
https://doi.org/10.1007/s12526-020-01066-8

Martin, D. & Britayev, T.A. (1998) Symbiotic polychaetes: review of known species. *Oceanography and Marine Biology: An Annual Review*, 36, 217–340.

Martin, D. & Britayev, T.A. (2018) Symbiotic polychaetes revisited: an update of the known species and relationships (1998–2017). *Oceanography and Marine Biology: An Annual Review*, 56, 371–448.
https://doi.org/10.1201/9780429454455-6

Mohammad, M.-B.M. (1972) Infestation of the pearl oyster *Pinctada margaritifera* (Linne) by a new species of *Polydora* in Kuwait, Arabian Gulf. *Hydrobiologia*, 39, 463–477.
https://doi.org/10.1007/BF00046740

Nishizawa, A., Sarashina, I., Tsujimoto, Y., Iijima, M. & Endo, K. (2010) Artificial fertilization, early development and chromosome numbers in the brachiopod *Lingula anatina*. *Special Papers in Palaeontology*, 84, 309–316.

Orensky, L.D. & Williams, J.D. (2009) Morphology and ecology of a new sexually dimorphic species of *Polydora* (Polychaeta: Spionidae) associated with hermit crabs from Jamaica, West Indies. *Zoosymposia*, 2, 229–240.
https://doi.org/10.11646/zoosymposia.2.1.17

Radashevsky, V.I. (1989) Ecology, sex determination, reproduction and larval development of commensal polychaetes *Polydora commensalis* and *Polydora glycymerica* in the Japanese Sea. *In*: Sveshnicov, V.A. (Ed.), *Symbiosis in marine animals*.

Russian Academy of Sciences, Moscow, pp. 137–164. [in Russian with English abstract]

Radashevsky, V.I. (1993) Revision of the genus *Polydora* and related genera from the North West Pacific (Polychaeta: Spionidae). *Publications of the Seto Marine Biological Laboratory*, 36, 1–60. https://doi.org/10.5134/176224

Radashevsky, V.I. (1999) Description of the proposed lectotype for *Polydora websteri* Hartman in Loosanoff & Engle, 1943 (Polychaeta: Spionidae). *Ophelia*, 51, 107–113. https://doi.org/10.1080/00785326.1999.10409402

Radashevsky, V.I. (2005) On adult and larval morphology of *Polydora cornuta* Bosc, 1802 (Annelida: Spionidae). *Zootaxa*, 1064, 1–24. https://doi.org/10.11646/zootaxa.1064.1.1

Radashevsky, V.I. (2012) Spionidae (Annelida) from shallow waters around the British Islands: an identification guide for the NMBAQC Scheme with an overview of spionid morphology and biology. *Zootaxa*, 3152, 1–35. https://doi.org/10.11646/zootaxa.3152.1.1

Radashevsky, V.I. & Hsieh, H.-L. (2000) *Polydora* (Polychaeta: Spionidae) species from Taiwan. *Zoological Studies*, 39, 203–217.

Radashevsky, V.I. & Pankova, V.V. (2013) Shell-boring versus tube-dwelling: is the mode of life fixed or flexible? Two cases in spionid polychaetes (Annelida, Spionidae). *Marine Biology*, 160, 1619–1624. https://doi.org/10.1007/s00227-013-2214-8

Rice, L.N., Lindsay, S. & Rawson, P. (2018) Genetic homogeneity among geographically distant populations of the blister worm *Polydora websteri*. *Aquaculture Environment Interactions*, 10, 437–446. https://doi.org/10.3354/aei00281

Richlen, M.L. & Barber, P.H. (2005) A technique for the rapid extraction of microalgal DNA from single live and preserved cells. *Molecular Ecology Notes*, 5, 688−691. https://doi.org/10.1111/j.1471-8286.2005.01032.x

Rodrigues, S.C., Simões, M.G., Kowalewski, M., Petti, M.A.V., Nonato, E.F., Martinez, S. & Del Rio, C.J. (2008) Biotic interaction between spionid polychaetes and bouchardiid brachiopods: paleoecological, taphonomic and evolutionary implications. *Acta Palaeontologica Polonica*, 53, 657–668. https://doi.org/10.4202/app.2008.0410

Sato, S., Owada, M., Haga, T., Hong, J.-S., Lützen, J. & Yamashita, H. (2011) Genus-specific commensalism of the galeommatoid bivalve *Koreamya arcuata* (A. Adams, 1856) associated with lingulid brachiopods. *Molluscan Research*, 31, 95–105.

Sato-Okoshi, W. (1998) Three new species of polydorids (Polychaeta, Spionidae) from Japan. *Species Diversity*, 3, 277–288. https://doi.org/10.12782/specdiv.3.277

Sato-Okoshi, W. (1999) Polydorid species (Polychaeta: Spionidae) in Japan, with descriptions of morphology, ecology and burrow structure. 1. Boring species. *Journal of the Marine Biological Association of the United Kingdom*, 79, 831–848. https://doi.org/10.1017/S0025315498001003

Sato-Okoshi, W. & Abe, H. (2012) Morphological and molecular sequence analysis of the harmful shell boring species of *Polydora* (Polychaeta: Spionidae) from Japan and Australia. *Aquaculture*, 368–369, 40–47. https://doi.org/10.1016/j.aquaculture.2012.08.046

Sato-Okoshi, W. & Abe, H. (2013) Morphology and molecular analysis of the 18S rRNA gene of oyster shell borers, *Polydora* species (Polychaeta: Spionidae), from Japan and Australia. *Journal of the Marine Biological Association of the United Kingdom*, 93, 1279–1286. https://doi.org/10.1017/S002531541200152X

Sato-Okoshi, W., Abe, H., Nishitani, G. & Simon, C.A. (2017) And then there was one: *Polydora uncinata* and *Polydora hoplura* (Annelida: Spionidae), the problematic polydorid pest species represent a single species. *Journal of the Marine Biological Association of the United Kingdom*, 97, 1675–1684. https://doi.org/10.1017/S002531541600093X

Sato-Okoshi, W., Sugawara, Y. & Nomura, T. (1990) Reproduction of the boring polychaete *Polydora variegata* inhabiting scallops in Abashiri Bay, North Japan. *Marine Biology*, 104, 61–66. https://doi.org/10.1007/BF01313158

Sato-Okoshi, W. & Takatsuka, M. (2001) *Polydora* and related genera (Polychaeta, Spionidae) around Puerto Montt and Chiloé Island (Chile), with description of a new species of *Dipolydora*. *Bulletin of Marine Science*, 68, 485–503.

Savazzi, E. (1991) Burrowing in the inarticulate brachiopod *Lingula anatina*. *Palaeography, Palaeoclimatology, Palaeoecology*, 85, 101–106. https://doi.org/10.1016/0031-0182(91)90027-O

Savazzi, E. (2001) A review of symbiosis in the Bivalvia, with special attention to macrosymbiosis. *Paleontological Research*, 5, 55–73.

Silvestro, D. & Michalak, I. (2012) RaxmlGUI: a graphical front-end for RAxML. *Organisms Diversity & Evolution*, 12, 335–337. https://doi.org/10.1007/s13127-011-0056-0

Simon, C.A. & Sato-Okoshi, W. (2015) Polydorid polychaetes on farmed molluscs: distribution, spread and factors contributing to their success. *Aquaculture Environment Interactions*, 7, 147–166.

https://doi.org/10.3354/aei00138

Simon, C.A., Sato-Okoshi, W. & Abe, H. (2019) Hidden diversity within the cosmopolitan species *Pseudopolydora antennata* (Claparède, 1869) (Spionidae: Annelida). *Marine Biodiversity*, 49, 25–42. https://doi.org/10.1007/s12526-017-0751-y

Stamatakis, A. (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, 30, 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Talavera, G. & Castresana, J. (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology*, 56, 564–577. https://doi.org/10.1080/10635150701472164

Taylor, P.D. & Wilson, M.A. (2003) Palaeoecology and evolution of marine hard substrate communities. *Earth−Science Reviews*, 62, 1–103. https://doi.org/10.1016/S0012-8252(02)00131-9

Teramoto, W., Sato-Okoshi, W., Abe, H., Nishitani, G. & Endo, Y. (2013) Morphology, 18S rRNA gene sequence and life history of a new *Polydora* species (Polychaeta: Spionidae) from northeastern Japan. *Aquatic Biology*, 18, 31–45. https://doi.org/10.3354/ab00485

Williams, A., Cohen, B.L., Cusack, M. & Long, S. (2000) Provenance of Atlantic lingulid brachiopods. *Palaeontology*, 43, 999–1018. https://doi.org/10.1111/1475-4983.00159

Williams, J.D. (2000) A new species of *Polydora* (Polychaeta: Spionidae) from the Indo-West Pacific and first record of host hermit crab egg predation by a commensal polydorid worm. *Zoological Journal of the Linnean Society*, 129, 537–548. https://doi.org/10.1111/j.1096-3642.2000.tb00616.x

Williams, J.D. & McDermott, J.J. (2004) Hermit crab biocoenoses: worldwide review of the diversity and natural history of hermit crab associates. *Journal of Experimental Marine Biology and Ecology*, 305, 1–128. https://doi.org/10.1016/j.jembe.2004.02.020

Williams, J.D. & Radashevsky, V.I. (1999) Morphology, ecology, and reproduction of a new *Polydora* species from the east coast of North America (Polychaeta: Spionidae). *Ophelia*, 51, 115–127. https://doi.org/10.1080/00785326.1999.10409403

Williams, L.G., Karl, S.A., Rice, S. & Simon, C. (2017) Molecular identification of polydorid polychaetes (Annelida: Spionidae): is there a quick way to identify pest and alien species? *African Zoology*, 52, 105–118. https://doi.org/10.1080/15627020.2017.1313131

Wisshak, M. & Neumann, C. (2006) A symbiotic association of a boring polychaete and an echinoid from the Late Cretaceous of Germany. *Acta Palaeontologica Polonica*, 51, 589–597.

Yatsu, N. (1902a) On the habits of the Japanese *Lingula*. *Annotationes Zoologicae Japonenses*, 4, 61–67.

Yatsu, N. (1902b) On the development of *Lingula anatina*. *The Journal of the College of Science, Imperial University of Tokyo, Japan*, 17, 1–112.

Ye, L.T., Tang, B., Wu, K., Su, Y., Wang, R., Yu, Z. & Wang, J. (2015) Mudworm *Polydora lingshuiensis* sp. n is a new species that inhabits both shell burrows and mudtubes. *Zootaxa*, 3986 (1), 88–100. https://doi.org/10.11646/zootaxa.3986.1.4

Ye, L.T., Yao, T., Wu, L., Lu, J. & Wang, J. (2019) Morphological and molecular diagnoses of *Polydora brevipalpa* Zachs, 1933 (Annelida: Spionidae) from the shellfish along the coast of China. *Journal of Oceanology and Limnology*, 37, 713–723. https://doi.org/10.1007/s00343-019-7381-0