Molecular evidence for the existence of five cryptic species within the Japanese species of *Marphysa* (Annelida: Eunicidae) known as “Iwa-mushi”

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Abstract: Molecular phylogenetic analyses were conducted to clarify how genetically homogeneous the common Japanese species of *Marphysa* known as “Iwa-mushi” is. This is a well-known polychaete used as a fishing bait that was first described as *Marphysa iwamushi* Izuka, 1907 (type locality: Japan and Taiwan) and later synonymized to *Marphysa sanguinea* (Montagu, 1813) (type locality: England). The nucleotide sequences of a nuclear gene (18S rRNA) and two mitochondrial genes (16S rRNA and cytochrome *c* oxidase subunit I (COI)) were compared between specimens newly collected from 14 localities in Japan including commercially sold fishing baits and DDBJ/ENA/GenBank data for congeneric species. Our results show that the Japanese “Iwa-mushi” is not a single species but a species complex comprising five genetically well-separated clades that were tentatively designated as five undetermined species (*Marphysa* spp. A, B, C, D, and E). It is unclear whether any of these species corresponds to *M. iwamushi*. The COI nucleotide sequence of *Marphysa* sp. A was almost identical to that of *M. victori* Lavesque, Daffé, Bonifácio & Hutchings, 2017 (type locality: France) and *M. bulla* Liu, Hutchings & Kupriyanova, 2018 (type locality: China), suggesting that they are conspecific and supporting the hypothesis of Lavesque et al. (2017) that the population in France was introduced from Japan. The COI sequence of *Marphysa* sp. B corresponds to that of *M. maxidenticulata* Liu, Hutchings & Kupriyanova, 2018 (type locality: China). *Marphysa* sp. E was found only in Tokyo Bay and may be an alien species that was introduced by importing live fishing bait. Our results indicate that the European species *M. sanguinea* is not distributed in Japanese waters.

Key words: alien species, fishing bait, *Marphysa sanguinea* complex, polychaete, 16S rRNA, 18S rRNA, COI

Introduction

“Iwa-mushi” is the common Japanese name for a specific species of *Marphysa*, previously identified as *M. sanguinea* (Montagu, 1813) or *M. iwamushi* Izuka, 1907. The species commonly inhabits intertidal zones in semi-enclosed environments in and around Japan (Izuka 1912, Okuda & Yamada 1954, Imajima 1965, 2007). The Japanese name “Iwa-mushi” (Iwa=stone/rock, mushi=worm) is derived

from their characteristic habit of boring into mudstones on rocky shores; however, “Iwa-mushi” is also known to inhabit burrows in the muddy and sandy sediments of tidal flats and in sediments under stones on boulder shores (Okuda & Ishikawa 1936, Ishikawa 1938, Takahashi 1960, Miura 1977, Suzuki et al. 2013).

Marphysa iwamushi was first described by Izuka (1907) in Japanese with the common Japanese name “Iwa-mushi.” Although Izuka (1912) described apparently the same species as *Marphysa* “*iwamusi*” [sic] with “n. sp.,” this name is no doubt a junior objective synonym of *M. iwamushi* Izuka, 1907. *Marphysa iwamushi* was then synonymized with

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M. sanguinea by Fauvel (1933, 1936) with little explanation, but his view has been followed by most of the subsequent publications in Japan (e.g., Okuda & Ishikawa 1936, Izuka 1947, Utinomi 1956, Imajima & Hartman 1964, Imajima 1965, Miura 1977, Uchida 1992, Imajima 2007).

Marphysa sanguinea had been well-known as one of the most famous examples of a cosmopolitan polychaete species (Hutchings & Kupriyanova 2018). This is mainly because of the incomplete original description by Montagu (1813) and the subsequent synonymization of many other species with *M. sanguinea* without detailed explanation or revision of the type specimens (Molina-Acevedo & Carrera-Parra 2015, Hutchings & Kupriyanova 2018). The situation has changed considerably since Hutchings and Karageorgopoulos (2003) re-described and designated a neotype for *M. sanguinea* based on the topotypic material and suggested that the distribution of this species is restricted to northern Europe: taxonomic revisions of species that were previously referred to as *M. sanguinea* have been conducted worldwide (Lewis & Karageorgopoulos 2008, Molina-Acevedo & Carrera-Parra 2015, Zanol et al. 2016, Lavesque et al. 2017, Liu et al. 2017, 2018, Elgetany et al. 2018, Wang et al. 2018).

Using RAPD-PCR, Lewis & Karageorgopoulos (2008) showed that *M. sanguinea* is not a cosmopolitan species but a suite of genetically differentiated species among geographically separated populations, including that in Japan. Nishi & Tanaka (2009) also suggested that “Iwa-mushi” and *M. sanguinea* sensu stricto are morphologically different, e.g., in chaetal morphology, and the two are possibly different species. In response to these studies, the name *M. iwamushi* (or *M. cf. iwamushi*) has been used again as the specific name for “Iwa-mushi” in Japan (e.g., Nishi et al. 2009, Suzuki et al. 2013, Saito et al. 2014, Masumoto et al. 2017).

However, using *M. iwamushi* for “Iwa-mushi” is still problematic because (1) the identity of *M. iwamushi* remains unknown owing to the incomplete original descriptions of Izuka (1907, 1912) with regard to important morphological characteristics in the current taxonomy of *Marphysa* (Glasby & Hutchings 2010, Molina-Acevedo & Carrera-Parra 2015), and (2) there is a possibility that the present Japanese “Iwa-mushi” population may comprise several distinct species.

Another problem pointed out by Suzuki et al. (2013) is the possible presence of established populations of non-indigenous cryptic species of *Marphysa* in Japan brought by the import of live fishing baits. In Japan, at least two species of *Marphysa* were heavily exploited by local fishermen as fish bait (Izuka 1912, Ishikawa 1938, Takahashi 1960, Imajima 1971); however, the exploitation has steadily decreased and cultivation of the worms has been limited owing to several technical difficulties (Kitamori 1972, Imai 1982, Maeda & Tominaga 2008). Import of live *Marphysa* from Korea to Japan as fishing baits started in 1969; since the 1990s, Japan has shifted import of these worms

from China (Weekly Sunday Fishing 1997, Hayashi 2001, Saito et al. 2011, Saito 2016). Japanese native “Iwa-mushi” and *Marphysa* imported from Korea and China have been heretofore considered as the same species under the names of *M. sanguinea* or *M. cf. iwamushi* (Nishi & Kato 2004, Nishi 2005, Saito et al. 2011, 2014, Saito 2016).

Recently, Liu et al. (2017, 2018) and Wang et al. (2018) described five and one new species of *Marphysa*, respectively, from China. The following eight species of *Marphysa* are currently recognized from intertidal zones in China: *M. bulla* Liu, Hutchings & Kupriyanova, 2018, *M. digitibranchia* Hoagland, 1920, *M. hongkongensa* Wang, Zhang & Qiu, 2018, *M. maxidenticulata* Liu, Hutchings & Kupriyanova, 2018, *M. multipectinata* Liu, Hutchings & Sun, 2017, *M. orientalis* Treadwell, 1936, *M. tribranchiata* Liu, Hutchings & Sun, 2017, and *M. tripectinata* Liu, Hutchings & Sun, 2017. *Marphysa sinensis* Monro, 1934 was transferred to genus *Paucibranchia* by Molina-Acevedo (2018). Although *M. bulla*, *M. maxidenticulata*, and *M. tripectinata* were reported to be exploited as fishing baits in China (Liu et al. 2017, 2018), the number of species imported by Japan remains unknown. The intentional import of live fishing baits and their increasing use in recreational fishing contributes to introduction of non-indigenous species (Nishi & Kato 2004, Cole et al. 2018, Pombo et al. 2018) that may be difficult to detect because of the morphological similarity between the native and non-indigenous species (e.g. Nishizawa et al. 2014).

In this study, we conducted molecular analyses on “Iwa-mushi” worms, which were newly collected from various regions or purchased at fishing stores in Japan, to reveal whether this species of *Marphysa* is a complex consisting of several cryptic species.

Materials and Methods

Specimens of *Marphysa* referable to “Iwa-mushi” were collected from intertidal to subtidal zones with rocky, boulder, sandy, or muddy substrata in 14 Japanese localities (Table 1, Fig. 1). A part of specimens was obtained by purchasing the fishing baits that were collected by local fishermen and regionally sold under the local market names: “Maeba” in Sendai and “Benten-jamushi” in Hamamatsu (Table 1). All specimens were fixed and preserved in 99% ethanol, and stored at 4°C. Preliminary morphological observations of the preserved specimens were conducted under a stereomicroscope (Olympus SZX 9).

Genomic DNA was extracted from a small piece of tissue (branchiae, pygidial cirri, or body wall) from each specimen 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). Depending on the DNA concentration, undiluted or 10-fold diluted DNA extract was used as a template for polymerase chain reaction (PCR). Partial sequences of nuclear 18S rRNA (18S) and mitochondrial 16S rRNA

Table 1. List of specimens collected including the sampling localities, environment, habitat of the specimens, date of collection or purchase, and number (n) of specimens used for molecular analyses.

Species	Market name	Locality	Geolocation (lat., long.)	Environment	Habitat	Sampling/purchase date	n
<i>Marphysa</i> sp. A	-	Moune Bay	38°53'42"N, 141°37'34"E	Boulder shore	Under stone	May 31, 2018	1
	-	Mangoku-ura Inlet	38°25'06"N, 141°22'52"E	Artificial tidal flat	Under mesh bag ^a	Apr. 20, 2018	1
	-	Soukanzan, Matsushima Bay	38°21'08"N, 141°3'32"E	Tidal flat	Under stone	Jul. 1, 2018	1
	Maeba	Shichigahama, Sendai Bay	-	-	-	Jul. 1, 2018	1
	-	Ninzaki, Nanao Bay	37°12'15"N, 136°55'07"E	Subtidal <i>Zostera marina</i> bed	In muddy sand	Oct. 10, 2018	1
	-	Moroiso Bay	35°09'29"N, 139°36'44"E	Rocky shore	In mudstone	Mar. 13, 2013	1
	-	Ena Bay	35°08'40"N, 139°39'50"E	Tidal flat	In mudstone	Sep. 7, 2018	1
	Benten-jamushi	Lake Hamana	-	-	-	Jul. 8, 2013	1
<i>Marphysa</i> sp. B	-	Arao tidal flat, Ariake Sea	32°57'32"N, 130°25'41"E	Tidal flat	In muddy sand	Apr. 18–20, 2018	5
<i>Marphysa</i> sp. C	-	Samegawa River tidal flat	36°54'34"N, 140°49'00"E	Tidal flat	Under stone	Aug. 28, 2018	2
	-	Tsurugizaki, Sagami Bay	35°08'26"N, 139°40'35"E	Rocky shore	In mudstone	Sep. 6, 2018	2
<i>Marphysa</i> sp. D	-	Katsuoazaki, Toyama Bay	37°08'56"N, 137°03'10"E	Subtidal bottom	In sand	Oct. 27, 2017	1
<i>Marphysa</i> sp. E	-	Sanbanze Ocean Park, Tokyo Bay	35°40'02"N, 139°58'14"E	Tidal flat	In muddy sand	Jun. 2, 2018	3
	-	Yoro River tidal flat, Tokyo Bay	35°32'37"N, 140°03'56"E	Tidal flat	In muddy sand	Mar. 29, 2017	3

^a: mesh bags for spat collection of Manila clam *Ruditapes philippinarum* (see Toba et al. 2017)

(16S) and cytochrome *c* oxidase subunit I (COI) genes were amplified by PCR using the following primer pairs: 18S-1F1/18S-1R632, 18S-2F576/18S-2R1209, and 18S-3F1129/18S-R1772 for 18S, Marl6S-F/Marl6S-R for 16S, and MarCOI-F/polyHCO for COI (Table 2). Marl6S-F/Marl6S-R and MarCOI-F were designed for *Marphysa* in this study and are modifications of the 16Sar/16Sbr (Palumbi et al. 1991) and ACOIAF primers (Colgan et al. 2001), respectively. PCR was performed in a 15 μ L reaction mixture containing 1.0–2.0 μ L of template DNA, 1 $\times$ KOD One PCR Master Mix (TOYOBO, Osaka, Japan), and 0.3 μ M of each primer pair. The PCR cycling conditions were 28–36 cycles of 98°C for 10 s, 60°C for 5 s, and 68°C for 1 s for 18S; 32–36 cycles of 98°C for 10 s, 52–57°C for 5 s, and 68°C for 1 s for 16S; and 32–38 cycles of 98°C for 10 s, 40–43°C for 5 s, and 68°C for 1 s for COI. The PCR products were purified using ExoSAP-IT (Affymetrix, Cleveland, OH) and sequenced by Eurofins Genomics (Tokyo, Japan). The forward and reverse complementary sequences and contigs were assembled using GeneStudio ver. 2.2.0.0 (GeneStudio, Inc. Suwanee, GA). The gene sequence data obtained in this study have been deposited in the DDBJ/ENA/GenBank databases with accession num-

bers LC467718–LC467789 (Table 3).

To reconstruct the molecular phylogeny, sequences of the 18S, 16S, and COI genes were aligned with the sequences of other species of *Marphysa*, *Paucibranchia*, and outgroup taxa available in the DDBJ/ENA/GenBank databases (Table 3) using the MAFFT online service ver. 7 with the L-INS-i algorithm (Katoh et al. 2019). Ambiguously aligned regions were eliminated using the Gblocks server ver. 0.91b (Castresana 2000). The final lengths of the aligned sequences were 1762 bp for 18S, 519 bp for 16S, and 663 bp for COI. Four phylogenetic trees were constructed based on the concatenated sequences of 18S, 16S, and COI, and the sequences of each gene region separately using maximum likelihood (ML) methods. ML analyses were performed using RAxML ver. 8.0.26 (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 by rapid bootstrap analysis with 1,000 replicates.

The COI sequences labeled as *Marphysa* sp. 1, 2, and 3 (DDBJ/ENA/GenBank accession numbers KY323709, KY328276, and KY328279) were referred to as *M. tripectinata*, *M. bulla*, and *M. maxidenticulata*, respectively, in

Fig. 1. Map showing sampling localities of *Marphysa* in Japan (A) and detailed maps for northeastern Japan (B), the Noto Peninsula (C), and the vicinity of Tokyo Bay (D). Symbols indicate the sampling localities of each species of *Marphysa* analyzed in this study.

Table 2. List of primers used for PCR amplification in this study.

Gene	Primer	Direction	Sequence (5'–3')	Reference
18S rRNA	18S-1F1	Forward	AACCTGGTTGATYCTGCCAG	Nishitani et al. (2012)
	18S-1R632	Reverse	ACTACGAGCTTTTAAACYGCARC	Nishitani et al. (2012)
	18S-2F576	Forward	GGTAATTCCAGCTCYAATRG	Nishitani et al. (2012)
	18S-2R1209	Reverse	AAGTTTYCCCGTGTTGARTC	Nishitani et al. (2012)
	18S-3F1129	Forward	GCTGAAACTTAAAGRAATTGACGG	Nishitani et al. (2012)
	18S-R1772	Reverse	TCACCTACGGAAACCTTGTTACG	Nishitani et al. (2012)
16S rRNA	Mar16S-F	Forward	CTGTTTAWCAAAAACATYGCC	This study
	Mar16S-R	Reverse	CGGTCTGAACCTCAGKTCA	This study
COI	MarCOI-F	Forward	TYWACAAAYCAYAAAGAYATTGG	This study
	polyHCO	Reverse	TAMACTTCWGGGTGACCAAARAATCA	Carr et al. (2011)

accordance with Wang et al. (2018). Although the deposited sequences were not included in the original description by Liu et al. (2017, 2018), correspondence between the sequences and species names were confirmed by the first author (Zhi Wang, personal communication). The DDBJ/ENA/GenBank sequence labeled as *M. sanguinea*

(KF733802) was referred to as *Marphysa* sp. since the sequence is divergent from the authoritative sequences of *M. sanguinea* (GQ478157 and GQ497547; Zanol et al. 2010); this species could have been misidentified (Lavesque et al. 2019). Some DDBJ/ENA/GenBank sequences labeled as *Marphysa* species were referred to as *Nicidion* or *Pauci-*

Table 3. List of terminal taxa used in the phylogenetic analyses with their sampling country, DDBJ/ENA/GenBank accession numbers, and references. The gene sequences obtained from this study are highlighted in boldface type.

Classification	Species	Country	DDBJ/ENA/GenBank accession number			Reference
			18S	16S	COI	
<i>Marphysa</i>	<i>Marphysa</i> sp. A	Japan	LC467718– LC467725	LC467742– LC467749	LC467766– LC467773	This study
	<i>Marphysa</i> sp. B	Japan	LC467726– LC467730	LC467750– LC467754	LC467774– LC467778	This study
	<i>Marphysa</i> sp. C	Japan	LC467731– LC467734	LC467755– LC467758	LC467779– LC467782	This study
	<i>Marphysa</i> sp. D	Japan	LC467735	LC467759	LC467783	This study
	<i>Marphysa</i> sp. E	Japan	LC467736– LC467741	LC467760– LC467765	LC467784– LC467789	This study
	<i>Marphysa aegypti</i>	Egypt	-	-	MF196968	Elgetany et al. (2018)
	<i>Marphysa bifurcata</i>	Australia	-	-	KX172177	Zanol et al. (2016)
	<i>Marphysa brevitentaculata</i>	Mexico	GQ497503	GQ478158	GQ497548	Zanol et al. (2010)
	<i>Marphysa bulla</i> (as <i>Marphysa</i> sp. 2)	China	-	-	KY328276	Liu & Hutchings (unpubl.)
	<i>Marphysa californica</i>	USA	GQ497507	GQ478162	GQ497552	Zanol et al. (2010)
	<i>Marphysa corallina</i>	South Africa	-	-	KT823257	Kara & Macdonald (unpubl.)
	<i>Marphysa fauchaldi</i>	Australia	-	-	KX172165	Zanol et al. (2016)
	<i>Marphysa graveleyi</i>	India	-	-	KP258205	Gomathy et al. (unpubl.)
	<i>Marphysa hongkongensa</i>	Hong Kong	-	MH598527	MH598525	Wang et al. (2018)
	<i>Marphysa kristiani</i>	Australia	-	-	KX172141	Zanol et al. (2016)
	<i>Marphysa maxidenticulata</i> (as <i>Marphysa</i> sp. 3)	China	-	-	KY328279	Liu & Hutchings (unpubl.)
	<i>Marphysa mossambica</i>	Philippines	-	JX559747	JX559751	Zanol et al. (2014)
	<i>Marphysa mullawa</i>	Australia	-	-	KX172166	Zanol et al. (2016)
	<i>Marphysa pseudosessilola</i>	Australia	-	-	KY605405	Zanol et al. (2017)
	<i>Marphysa regalis</i>	Brazil	GQ497510	GQ478165	GQ467562	Zanol et al. (2010)
	<i>Marphysa sanguinea</i>	France	GQ497502	GQ478157	GQ497547	Zanol et al. (2010)
	<i>Marphysa tamurai</i>	-	-	MG205526	MG205526	Chen et al. (unpubl.)
	<i>Marphysa tripectinata</i> (as <i>Marphysa</i> sp. 1)	China	-	-	KY323709	Liu & Hutchings (unpubl.)
	<i>Marphysa victori</i>	France	-	MG385000	MG384997	Lavesque et al. (2017)
	<i>Marphysa viridis</i>	Brazil	GQ497508	GQ478163	GQ497553	Zanol et al. (2010)
	<i>Marphysa</i> sp. (as <i>M. sanguinea</i>)	China	-	KF733802	KF733802	Li et al. (2016)
<i>Paucibranchia</i>	<i>Paucibranchia bellii</i> (as <i>M. bellii</i>)	France, Spain	AF412789	AY838835	KT307661	Struck et al. (2002, 2006), Aylagas et al. (2016)
	<i>Paucibranchia</i> cf. <i>bellii</i> (as <i>M.</i> cf. <i>bellii</i>)	Greece	GQ497511	-	-	Zanol et al. (2010)
	<i>Paucibranchia disjuncta</i> (as <i>M. disjuncta</i>)	USA	GQ497504	GQ478159	GQ497549	Zanol et al. (2010)
	<i>Paucibranchia fallax</i> (as <i>M. fallax</i>)	Italy	GQ497505	GQ478160	-	Zanol et al. (2010)
Outgroup	<i>Nicidion angeli</i> (as <i>M. angeli</i>)	Belize	GQ497506	GQ478161	GQ497550	Zanol et al. (2010)
	<i>Nicidion</i> cf. <i>hentscheli</i> (as <i>M.</i> cf. <i>hentscheli</i>)	Brazil	GQ497509	GQ478164	GQ497551	Zanol et al. (2010)

branchia species according to Molina-Acevedo & Carrera-Parra (2017) and Molina-Acevedo (2018).

Results

Molecular phylogenetic analysis of the concatenated sequences revealed that the “Iwa-mushi” samples were divided into five genetically distinct clades (CL1–CL5, Fig. 2A) corresponding to five species namely *Marphysa* spp. A–E as described below. Interspecific phylogenetic relationships were ambiguous due to low bootstrap values (below 50%) at most of the higher internal nodes (Fig. 2A–D).

(1) *Marphysa* sp. A (Fig. 3A, B)

Eight specimens of *Marphysa* sp. A were collected from various localities along the coasts of the Pacific Ocean and Sea of Japan (Fig. 1). These specimens can be found in muddy and sandy sediments under stones on tidal flats and boulder shores, inside mudstones, and occasionally from burrows in the muddy sediments in eelgrass beds of *Zostera marina* Linnaeus, 1753 (Table 1).

Body lengths of the preserved specimens ranged between 5.5–11 cm with 178–307 chaetigers in complete specimens and 3–8 cm with 106–211 chaetigers in incomplete anterior or posterior fragments. Bodies of the worms were beige to reddish brown with dark brown mottled pig-

Marphysa sp. A was categorized in CLI together with *M. bulla* and *M. victori* Lavesque, Daffe, Bonifácio & Hutchings, 2017 (Fig. 2A). The COI sequences of *Marphysa* sp. A shared 99.5–100% identity with those of *M.*

(2) *Marphysa* sp. B (Fig. 3C, D)

The five specimens of *Marphysa* sp. B were collected

Fig. 3. Light photographs showing morphology of living specimens of *Marphysa* sp. A (A, B: same individual), *Marphysa* sp. B (C, D: same individual), *Marphysa* sp. C (E, F: same individual), posterior fragment of ethanol fixed specimen of *Marphysa* sp. D (G), posterior fragment of a living specimen of *Marphysa* sp. E (H), and a fecal mound excreted by *Marphysa* sp. E on the sediment in the tidal flat (I). Scale bars are not shown but the lengths of the living specimens in this figure were ca. 15 cm for *Marphysa* sp. A (A), ca. 10 cm for *Marphysa* sp. B (C), ca. 5 cm for *Marphysa* sp. C (E), and c.a. 2 cm and >30 cm for the incomplete posterior fragment of *Marphysa* sp. D (G) and *Marphysa* sp. E (H), respectively. The diameter of the burrow at the center of the fecal mound was ca. 5 mm (I).

from burrows in the muddy sand sediment in tidal flats off Arao in the Ariake Sea, Kyushu, southern Japan (Table 1, Fig. 1).

Body lengths of the preserved specimens were 11–12 cm with 265–316 chaetigers in complete specimens and 7–10 cm with 69–113 chaetigers in incomplete anterior fragments. The worms looked beige to reddish pink with increasing iridescence towards the anterior in both live and preserved specimens (Fig. 3C, D). The specimens had limbate capillaries and compound spinigers in neurochaetae without compound falcigers.

CL2 comprised *Marphysa* sp. B, *M. maxidenticulata*, and *Marphysa* sp. (Fig. 2A). The COI sequences of *Marphysa* sp. B shared 99.8–100% identity with those of *M. maxidenticulata* and *Marphysa* sp. (KF733802) from China (Table 3, Fig. 2D).

(3) *Marphysa* sp. C (Fig. 3E, F)

CL3 consisted of four samples of *Marphysa* sp. C found under stones in the sandy tidal flats in the estuarine region of the Samegawa River, southern Tohoku District and inside mudstones on the rocky shore of Tsurugizaki, Miura Peninsula, Sagami Bay (Table 1, Fig. 1).

Body lengths of the preserved specimens ranged between 5–16 cm with 221–422 chaetigers in complete specimens and 3–4 cm with 115–124 chaetigers in incomplete anterior fragments. Body color of the worms was pinkish

orange to beige with dark brown mottled pigmentation and increasing iridescence towards the anterior in both live and preserved specimens. Anterior mottled pigmentation was faint in a few small individuals (Fig. 3E, F). The specimens had limbate capillaries and compound spinigers in neurochaetae without compound falcigers.

CL3 consisted exclusively of *Marphysa* sp. C and formed a sister group with *M. viridis* Treadwell, 1917 (Fig. 2A). There were no 16S and COI sequences available in DDBJ/ENA/GenBank that were identical to those of the samples (Fig. 2C, D). Although their 18S sequences were completely identical (Fig. 2B), *Marphysa* sp. B, *Marphysa* sp. C, and *M. viridis* from Brazil (Table 3) were clearly distinguishable by their 16S and COI sequence analyses (Fig. 2C, D).

(4) *Marphysa* sp. D (Fig. 3G)

A single incomplete posterior fragment of *Marphysa* sp. D was collected from the sandy subtidal zone in Katsuo-zaki, Toyama Bay, Sea of Japan (Table 1, Fig. 1).

The length and width of the preserved posterior fragment was ca. 2 cm and 4 mm, respectively, with 71 chaetigers and the color was yellowish white (Fig. 3G). The specimen had limbate capillaries and compound spinigers in neurochaetae without compound falcigers.

CL4 was only represented by *Marphysa* sp. D and there were no sequences available in DDBJ/ENA/GenBank data-

bases that matched this species (Fig. 2).

(5) *Marphysa* sp. E (Fig. 3H)

Six specimens, all incomplete posterior fragments of *Marphysa* sp. E, were collected from burrows in the muddy sand tidal flat in Tokyo Bay (Sanbanze Ocean Park and mouth of the Yoro River) (Table 1, Fig. 1).

The anterior-most part of this worm could not be collected owing to its long body (>30 cm) and deep burrowing habit. The length of the posterior fragments analyzed were between 3–8 cm with 72–230 chaetigers. The live and preserved worms were reddish pink and yellowish white or beige, respectively (Fig. 3H). The specimens have limbate capillaries in neurochaetae without compound chaetae. Conical mounds of fecal pellets excreted by this worm were frequently observed at the collection sites in Tokyo Bay (Fig. 3I).

Marphysa sp. E was equivalent to CL5 that was the sister group of *M. mossambica* (Peters, 1854) (Fig. 2A). There were not any sequences that were identical to those of CL5 in the DDBJ/ENA/GenBank databases (Fig. 2).

Discussion

Molecular analyses in this study clearly distinguished five clades within the Japanese species of *Marphysa* known as “Iwa-mushi.” These five clades were regarded as different species since the genetic differentiation among the clades were comparable to that among several nominal species previously known in *Marphysa* (Fig. 2). Although the COI sequences of CL1 and CL2 shared similarity with identified sequences from DDBJ/ENA/GenBank, we refer to the five clades as undetermined *Marphysa* spp. A–E. To accurately identify these species, detailed morphological analyses and comparisons of these five species are required along with establishing the identity of *M. iwamushi* based on the re-description of its name-bearing types.

According to Glasby & Hutchings (2010), the species belonging to *Marphysa* are classified into five informal groups based on the following type of compound chaetae present in the subacicular neurochaetae, i.e. the Mossambica-group (=Group A sensu Fauchald 1970) includes species lacking any compound chaetae; the Sanguinea-group (=Group B) includes species with compound spinigers only; the Aeana-group (=Group C) includes species with compound falcigers only; the Belli-group (=Group D) includes species with both compound falcigers and spinigers; and the Teretiuscula-group includes species with compound spinigers restricted to the anterior chaetigers only. Based on this categorization, *Marphysa* spp. A–D and *Marphysa* sp. E were classified into the Sanguinea-group and the Mossambica-group, respectively, even though the *Marphysa* sp. D–E samples were incomplete posterior fragments. Besides *M. sanguinea* and *M. iwamushi*, the following seven species of *Marphysa* are currently recognized in Japan: *M. bifurcata* Kott, 1951, *M. depres-*

sa (Schmarda, 1861), *M. macintoshi* Crossland, 1903, *M. mortenseni* Monro, 1928, *M. tamurai* Okuda, 1934, and *Marphysa* sp. B and C sensu Imajima, 2007 (Okuda 1934, Iwase et al. 1990, Uchida 1992, Imajima 2006, 2007). *Marphysa bellii* (Audouin & Milne Edwards, 1833), *M. conferta* Moore, 1911, *M. disjuncta* Hartman, 1961, *M. kinbergi* McIntosh, 1910, *M. stragulum* (Grube, 1878), and *Marphysa* sp. A sensu Imajima, 2007 previously recorded from Japan (Imajima 1970, 2006, 2007, 2011, Miura 1977, 1979) were recently transferred to genus *Paucibranchia* (Molina-Acevedo 2018). Among above seven *Marphysa* species in Japan, *M. macintoshi* and *M. tamurai* belong to the Sanguinea-group. Since they possess a prostomium with a very faint middle notch (Okuda 1934, Iwase et al. 1990), they are at least different from *Marphysa* spp. A–C with the distinct middle notch-containing prostomium (Fig. 3A–F). Other Japanese species *M. bifurcata* and *M. mortenseni* belong to the Aeana-group; *M. depressa* and *Marphysa* sp. C sensu Imajima, 2007 to the Belli-group; and *Marphysa* sp. B sensu Imajima, 2007 to the Mossambica-group. Although they both belong to the Mossambica group, it is unclear whether *Marphysa* sp. E (from our study) and *Marphysa* sp. B sensu Imajima, 2007 are the same species.

The COI sequences of *Marphysa* sp. A were almost identical to those of *M. bulla* and *M. victori* obtained from the DDBJ/ENA/GenBank databases (Fig. 2), suggesting that they may be the same species. This presumption was also supported by our 16S sequence data that showed complete identity between *Marphysa* sp. A and *M. victori*. *Marphysa bulla* and *M. victori* were described based on specimens collected from the Yellow Sea, China by Liu et al. (2018) and Arcachon Bay, France by Lavesque et al. (2017), respectively. These species have only been found in their respective type localities inhabiting rock crevices (*M. bulla*) and around oyster reefs, abandoned oyster farms, or galleries in old pieces of driftwood (*M. victori*). Both species were morphologically recognized to belong to the Sanguinea-group similar to *Marphysa* sp. A; however, in contrast to the molecular analyses, the descriptions of *M. bulla* and *M. victori* indicate some morphological differences between these two species, e.g., in maxillary formula, presence of subacicular hooks, and the number of types of pectinate chaetae (Lavesque et al. 2017, Liu et al. 2018).

In the original description of *M. victori*, it was hypothesized that this species may not be indigenous to France and could have been transferred by “hitchhiking” on exotic Pacific oyster *Crassostrea gigas* (Thunberg, 1793) that were transported to Arcachon Bay (type locality of *M. victori*) from Japan or Canada (Lavesque et al. 2017) as is the case with several non-indigenous species (Gouilleux et al. 2016). After the mass mortality of the Portuguese oyster *Crassostrea angulata* (Lamarck, 1819) in France, restoring oyster culturing involved the mass export of oyster spat (*C. gigas*) from Japan, especially from Sendai Bay, to France between 1969 and 1979 (Koganezawa 1984, Koike 2015). This oyster spat was also introduced into Arcachon Bay

between 1971 and 1975 (Grizel & Héral 1991). Interestingly, *Marphysa* sp. A was collected from Sendai Bay and its branches, i.e. Mangoku-ura Inlet and Matsushima Bay (Table 1). Our results support the hypothesis by Lavesque et al. (2017) that the *M. victori* population in France was introduced from Japan.

Marphysa sp. E excretes fecal pellets on the surface of tidal flats and forms distinctive fecal mounds (Fig. 3I). These fecal mounds are frequently found in the Sanbanze Ocean Park and mouth of the Yoro River in Tokyo Bay. The fecal pellets have been used to study the chemistry of organic environmental toxicants (identified as *M. sanguinea*; Onozato et al. 2010, 2012, Nishigaki et al. 2013). Owing to their unusual distribution, we cannot eliminate the possibility that *Marphysa* sp. E is a non-indigenous alien species introduced via imported live bait: this species was only found in Tokyo Bay (Table 1) that is one of the biggest “melting pots” of alien species in Asia (Asakura 1992, Iwasaki 2006, Abe et al. 2019). To ascertain the indigeneity of this species, it is important to compare its morphology and gene sequences with those of the species of *Marphysa* imported from China as live fishing bait. The results of this study indicate that the confusion in taxonomy of *Marphysa* probably arises from the intentional and unintentional introduction of exotic species by transporting oyster and import of live fishing bait.

Marphysa sp. B and *M. maxidenticulata* are likely to be the same species since their COI sequences were almost identical. *Marphysa maxidenticulata* was originally categorized in the Belli-group (Liu et al. 2018). However, Wang et al. (2018) re-categorized this species to the Sanguinea-group because the short-bladed compound falcigers (sensu Liu et al. 2018) in *M. maxidenticulata* (as well as in *M. orientalis* shown by Liu et al. 2018) were re-interpreted as compound spinigers: spinigers and falcigers in *Marphysa* are recognized by unidentate sharp tips and bidentate hooded tips, respectively (Zhi Wang, personal communication). Their interpretation is consistent with our results; *Marphysa* sp. B is a part of the Sanguinea-group. The current distribution of *M. maxidenticulata* is restricted to Cao-feidian (Bohai Sea) and the Qingdao coast (Yellow Sea), north China (Liu et al. 2018). Our study may expand the known distribution range of *M. maxidenticulata* to include the Ariake Sea, south Japan. The distribution of *Marphysa* sp. B suggests that it may be a potentially relict species that was isolated from the original Asian continental coast population by geohistorical events and has existed only in the Ariake Sea, a semi-closed shallow sea with a large tidal flat and macro-tidal environment similar to the Bohai and Yellow Sea (Sato & Takita 2000, Sato 2010, 2017).

Although we do not know whether any of the five undetermined species correspond to *M. iwamushi*, we can infer from their habitats and sampling localities that *Marphysa* sp. A is most likely to be *M. iwamushi*. In the original description, Izuka (1907) mentioned that *M. iwamushi* is found in various regions of Japan and the Pescadores

(=Penghu) Islands, Taiwan and lives inside intertidal muddy sand and soft rocks. Izuka (1912) also stated that *M. iwamushi* is “one of the most common littoral annelids in Japan” (p. 131) and “found usually burrowing in tertiary rocks, but sometimes in sandy flats” (p. 133). In our study, we found *Marphysa* sp. A often burrowed into mudstones as well as muddy or muddy sand sediment especially under stones half-buried in tidal flats and inhabited various regions in Japan. Although *Marphysa* sp. C also burrows into mudstones, as compared to *Marphysa* sp. A this species was less frequently collected in our study (Table 1) and seemed to prefer habitats facing the open ocean: most of the benthic species living around the collection site of *Marphysa* sp. C in tidal flats in the Samegawa River estuary were those typically found in rocky shores in outer bay environments (Kanaya et al. 2019), and Tsurugizaki at the southeastern end of the Miura Peninsula is also a rocky shore environment facing the open ocean (Fig. 1). To determine whether *Marphysa* sp. A and *M. iwamushi* are the same species, it is imperative to re-describe the type specimens of *M. iwamushi* deposited in the University Museum, the University of Tokyo (Nishi & Tanaka 2011) and compare their morphologies. However, this is beyond the scope of this study and will be addressed in the future.

Note that molecular analyses showed that none of the sequences of *Marphysa* obtained from our study matched with the authoritative sequences of *M. sanguinea* from France (Zanol et al. 2010). Thus, there is currently no evidence to support the presence of *M. sanguinea* in Japan, further strengthening the validity of *M. iwamushi* as a different species.

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References

- 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. *Mar Biodiv Rec* 12: 7.
- Asakura A (1992) Recent introductions of marine benthos into Tokyo Bay (review): process of invasion into an urban ecosystem with discussion on the factors inducing their successful introduction. *J Nat Hist Mus Inst Chiba* 2: 1–14. (in Japanese with English abstract)
- Aylagas E, Borja Á, Irigoien X, Rodríguez-Ezpeleta N (2016) Benchmarking DNA metabarcoding for biodiversity-based monitoring and assessment. *Front Mar Sci* 3: 96.
- Carr CM, Hardy SM, Brown TM, Macdonald TA, Hebert PDN (2011) A tri-oceanic perspective: DNA barcoding reveals geographic structure and cryptic diversity in Canadian polychaetes. *PLoS ONE* 6: e22232.
- Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol Biol Evol* 17: 540–552.
- Cole VJ, Chick RC, Hutchings PA (2018) A review of global fisheries for polychaete worms as a resource for recreational fishers: diversity, sustainability and research needs. *Rev Fish Biol Fisher* 28: 543–565.
- Colgan DJ, Hutchings PA, Brown S (2001) Phylogenetic relationships within the Terebellomorpha. *J Mar Biol Assoc UK* 81: 765–773.
- Elgetany AH, El-Ghobashy AE, Ghoneim AM, Struck TH (2018) Description of a new species of the genus *Marphysa* (Eunicidae), *Marphysa aegypti* sp. n., based on molecular and morphological evidence. *Invertebr Zool* 15: 71–84.
- Fauchald K (1970) Polychaetous annelids of the families Eunicidae, Lumbrineridae, Iphitimidae, Arabellidae, Lysaretidae and Dorvilleidae from western Mexico. *Allan Hancock Monogr Mar Biol* 5: 1–335.
- Fauvel P (1933) Annelides polychètes Golfe du Pei Tcheu Ly de la collection du Musée Hoang ho Pai ho. *Publ Mus Hoang ho Pai ho Tien Tsin* 15: 1–67.
- Fauvel P (1936) Annelides polychètes du Japon. *Mem Coll Sci Kyoto Imp Univ* 12: 41–92.
- Glasby CJ, Hutchings PA (2010) A new species of *Marphysa* Quatrefages, 1865 (Polychaeta: Eunicida: Eunicidae) from northern Australia and a review of similar taxa from the Indo-West Pacific, including the genus *Nauphanta* Kinberg, 1865. *Zootaxa* 2352: 29–45.
- Gouillieux B, Lavesque L, Blanchet H, Bachelet G (2016) First record of the non-indigenous *Melita nitida* Smith, 1873 (Crustacea: Amphipoda: Melitidae) in the Bay of Biscay (NE Atlantic). *BioInvasions Rec* 5: 85–92.
- Grizel H, Héral M (1991) Introduction into France of the Japanese oyster (*Crassostrea gigas*). *J Cons Int Explor Mer* 47: 399–403.
- Hayashi I (2001) Recent progress in polychaete ecology 32. Useful and harmful functions to human activities (2). *Aquabiology* 23: 617–621. (in Japanese with English abstract)
- Hutchings PA, Karageorgopoulos P (2003) Designation of a neotype of *Marphysa sanguinea* (Montagu, 1813) and a description of a new species of *Marphysa* from eastern Australia. *Hydrobiologia* 496: 87–94.
- Hutchings PA, Kupriyanova E (2018) Cosmopolitan polychaetes—fact or fiction? Personal and historical perspectives. *Invert Syst* 32: 1–9.
- Imai T (1982) The early development and breeding of *Marphysa sanguinea* (Montagu). *Benthos Res* 23: 36–41. (in Japanese)
- Imajima M (1965) 'Iwa-mushi' *Marphysa sanguinea* (Montagu). In: *New Illustrated Encyclopedia of the Fauna of Japan* [I] (eds Okada Y, Uchida S, Uchida T). Hokuryukan, Tokyo, p. 512. (in Japanese)
- Imajima M (1970) Errant polychaetous annelids collected from the areas around the Tsushima Islands. *Mem Natl Sci Mus Tokyo* 3: 113–122.
- Imajima M (1971) *Tsuri Esa Mushi no Iroiro* [A variety of fishing bait worms]. *Natural Science and Museums* 38: 171–183. (in Japanese)
- Imajima M (2006) Polychaetous annelids from Sagami Bay and Sagami Sea, central Japan. *Mem Natl Sci Mus Tokyo* 40: 317–408.
- Imajima M (2007) *Kankei-doubutsu Tamou-rui III* [Annelida, Polychaeta III]. Biological Research Corporation, Tokyo, 560 pp. (in Japanese)
- Imajima M (2011) Polychaetous annelids collected from Sagami Bay toward the Ogasawara Islands, Japan. *Mem Natl Mus Nat Sci Tokyo* 47: 145–218.
- Imajima M, Hartman O (1964) The polychaetous annelids from Japan. Part 2. *Allan Hancock Found Publ Occ Pap* 26: 239–452.
- Ishikawa K (1938) *Jikken Ouyou Tsuriesha-mushi Riyou no Kenkyu* [Studies on the Worms used for Fish Bait]. *Tsurishiryoku Kenkyu-kai* [Association for Fish Bait Studies], Fukuoka, 253 pp.
- Iwasaki K (2006) Human-mediated introduction of marine organisms in Japan: a review. In: *Assessment and Control of Biological Invasion Risks* (eds Koike F, Clout MN, Kawamichi M, De Poorter M, Iwatsuki K). Shoukadoh Book Sellers, Kyoto and IUCN, Gland, pp. 104–112.
- Iwase F, Nomura K, Fukuda T, Misaki H, Uchida H (1990) *Okinawa Kaichu Seibutsu Zukan Vol. 11: Hitode, Namako, Gokai, Isoginchaku, Sonota* [Illustrated Marine Organisms in Okinawa Islands, Vol. 11. Lesser Animals]. Southern Press, Naha, 272 pp. (in Japanese)
- Izuka A (1907) On two new species of the eunicid annelid. *Zool Mag* 19: 139–143. (in Japanese)
- Izuka A (1912) The errantiate Polychaeta of Japan. *J Coll Sci Imp Univ Tokyo* 30: 1–262.
- Izuka A (1947) 'Iwa-mushi' *Marphysa sanguinea* (Montagu). In: *Revised Edition Illustrated Encyclopedia of the Fauna of Japan* (Exclusive of Insects) (eds Uchida S et al.). Hokuryukan, Tokyo, p. 1328. (in Japanese)
- Kanaya G, Taru M, Yuhara T, Unagami T, Miura O, Nakai S, Itoh H, Suzuki T (2019) Macrozoobenthos of the Samegawa River (Iwaki, Fukushima Prefecture) and the Momiya River (Hitachi, Ibaraki Prefecture) tidalflats, Japan: diversity shifts following the 2011 earthquake and effects of ongoing restoration works. *Jpn J Benthol* 73: 84–101. (in Japanese with English abstract)

- Katoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Brief Bioinform* 20: 1160–1166.
- Kitamori R (1972) Some problems on studies of life history and ecology of polychaetous annelids. *Benthos Res* 5/6: 20–22. (in Japanese)
- Koganezawa A (1984) *Matsushima Gaki no Konjyaku* [History of oyster culture in Matsushima]. *News of Tohoku Nat Fish Res Inst* 26: 2–3. (in Japanese)
- Koike Y (2015) An example of friendship and cooperation between France and Japan: oyster farming in Sanriku area (Tohoku region, northern Japan) before and after tsunami – restoration and technical adaptation of culture systems. In: *Marine Productivity: Perturbations and Resilience of Socio-ecosystems* (eds Ceccaldi HJ, Hénocque Y, Koike Y, Komatsu T, Stora G, Tusseau-Vuillemin MH). Springer, Cham, pp. 3–14.
- Lavesque N, Daffe G, Bonifácio P, Hutchings P (2017) A new species of the *Marphysa sanguinea* complex from French waters (Bay of Biscay, NE Atlantic) (Annelida, Eunicidae). *ZooKeys* 716: 1–17.
- Lavesque N, Daffe G, Grall J, Zanol J, Gouillieux B, Hutchings P (2019) Guess who? On the importance of using appropriate name: case study of *Marphysa sanguinea* (Montagu, 1813). *ZooKeys* 859: 1–15.
- Lewis C, Karageorgopoulos P (2008) A new species of *Marphysa* (Eunicidae) from the western Cape of South Africa. *J Mar Biol Assoc UK* 88: 277–287.
- Li S, Chen Y, Zhang M, Bao X, Li Y, Teng W, Liu Z, Fu C, Wang Q, Liu W (2016) Complete mitochondrial genome of the marine polychaete, *Marphysa sanguinea* (Polychaeta, Eunicida). *Mitochondrial DNA Part A* 27: 42–43.
- Liu Y, Hutchings PA, Kupriyanova E (2018) Two new species of *Marphysa* Quatrefages, 1865 (Polychaeta: Eunicida: Eunicidae) from northern coast of China and redescription for *Marphysa orientalis* Treadwell, 1936. *Zootaxa* 4377: 191–215.
- Liu Y, Hutchings PA, Sun S (2017) Three new species of *Marphysa* Quatrefages, 1865 (Polychaeta: Eunicida: Eunicidae) from the south coast of China and redescription of *Marphysa sinensis* Monro, 1934. *Zootaxa* 4263: 228–250.
- Maeda Y, Tominaga O (2008) Seed production and aquaculture of *Marphysa sanguinea*, annelide Polychaeta. *J Fukui Pref Univ* 30: 203–213. (in Japanese)
- Masumoto T, Yuhara T, Taru M, Furota T, Nishi E, Komai T, Kurozumi T, Sasaki M, Nakagawa M (2017) Differences in species found using different examination methods in a large-scale benthic zone in the Banzu tidal flat of Tokyo Bay (adjacent to the mouth of Obitsu River) and comparative community analysis. *Bull Chiba Pref Univ Health Sci* 8: 41–51. (in Japanese with English abstract)
- Miura T (1977) Eunicid polychaetous annelids from Japan. II. *La Mer* 15: 61–81.
- Miura T (1979) Eunicid polychaetous annelids from Japan. III. *La Mer* 17: 33–42.
- Molina-Acevedo IC (2018) Morphological revision of the Subgroup 1 Fauchald, 1970 of *Marphysa* de Quatrefages, 1865 (Eunicidae: Polychaeta). *Zootaxa* 4480: 1–125.
- Molina-Acevedo IC, Carrera-Parra LF (2015) Reinstatement of three species of the *Marphysa sanguinea* complex (Polychaeta: Eunicidae) from the Grand Caribbean Region. *Zootaxa* 3925: 37–55.
- Molina-Acevedo IC, Carrera-Parra LF (2017) Revision of *Marphysa* de Quatrefages, 1865 and some of *Nicidion* Kinberg, 1865 with the erection of a new genus (Polychaeta: Eunicidae) from the Grand Caribbean. *Zootaxa* 4241: 1–62.
- Montagu G (1813) Descriptions of several new or rare animals, principally marine, discovered on the south coast of Devonshire. *Trans Linn Soc Lond* 11: 18–21.
- Nishi E (2005) Invasive marine and brackishwater invertebrates in Kanagawa Prefecture. *Actinia (Bull Manazuru Mar Lab Sci Educ Fac Educ Human Sci Yokohama Nat Univ)* 16: 25–29. (in Japanese with English abstract)
- Nishi E, Kato T (2004) Introduced and globally invaded polychaetous annelids. *Jpn J Benthol* 59: 83–95. (in Japanese with English abstract)
- Nishi E, Tanaka K (2009) Chapter 6: *Gairaisyu toshite no Tamourui* [Polychaete as alien species]. In: *Marine Aliens introduced by Human Activities and Their Impacts on Ecosystems and Industries* (eds The Plankton Society of Japan, The Japanese Association of Benthology). Tokai University Press, Hadano, pp. 91–98. (in Japanese)
- Nishi E, Tanaka K (2011) Catalogue of invertebrate collection deposited in the Department of Zoology, the University Museum, the University of Tokyo. Phylum Annelida (Classes Polychaeta and Myzostomida): a catalogue of Akira Izuka's type and non-type polychaete collection in the University Museum, the University of Tokyo. *Mat Rep Univ Mus Univ Tokyo* 90: 1–57.
- Nishi E, Masumoto T, Taru M, Nakayama S, Kazama-Wakabayashi M, Monju M, Suyama N, Aida M, Sato M, Tanaka K, Kupriyanova EK (2009) Polychaete fauna and diversity around Haneda, Tokyo Bay, a result of environmental assessment of Haneda Airport re-expansion project. *Proc Ocean Engineer Symp OES21-191*: 1–8. (in Japanese with English abstract)
- Nishigaki A, Muramatsu A, Onozato M, Ohshima S (2013) Determination of polycyclic aromatic hydrocarbons in the fecal materials of *Mactra veneriformis* and excrement of *Marphysa sanguinea*. *Polycycl Aromat Comp* 33: 151–160.
- Nishitani G, Nagai S, Hayakawa S, Kosaka Y, Sakurada K, Kamiyama T, Gojobori T (2012) Multiple plastids collected by the dinoflagellate *Dinophysis mitra* through kleptoplastidy. *Appl Environ Microbiol* 78: 813–821.
- Nishizawa R, Sato M, Furota T, Tosuji H (2014) Cryptic invasion of northeast Pacific estuaries by the Asian polychaete, *Hediste diadroma* (Nereididae). *Mar Biol* 161: 187–194.
- Okuda S. 1934. A new species of errantiate polychaete, *Marphysa tamurai* n. sp. *Proc Imp Acad* 10: 521–523.
- Okuda S, Ishikawa K (1936) Polychaete worms used as baits in Japan (II). *Bot Zool* 4: 1861–1868. (in Japanese)
- Okuda S, Yamada M (1954) Polychaetous annelids from Matsushima Bay. *J Fac Sci Hokkaido Univ Ser VI, Zool* 12: 175–199.
- Onozato M, Nishigaki A, Ohshima S (2010) The fate and behavior of polycyclic aromatic hydrocarbons (PAHs) through feeding and excretion of annelids. *Polycycl Aromat Comp* 30: 334–345.
- Onozato M, Sugawara T, Nishigaki A, Ohshima S (2012) Study on the degradation of polycyclic aromatic hydrocarbons (PAHs) in the excrement of *Marphysa sanguinea*. *Polycycl*

- Aromat Comp 32: 238–247.
- Palumbi S, Martin A, Romano S, McMillan WO, Stice L, Grabowski G (1991) The Simple Fool's Guide to PCR. Version 2.0. Department of Zoology and Kewalo Marine Laboratory, University of Hawaii, Honolulu, 45 pp.
- Pombo A, Baptista T, Granada L, Ferreira SMF, Gonçalves SC, Anjos C, Sá E, Chainho P, Fonseca LCda, Costa PFe, Costa JL (2018) Insight into aquaculture's potential of marine annelid worms and ecological concerns: a review. *Rev Aquacul* doi: 10.1111/raq.12307
- Richlen ML, Barber PH (2005) A technique for the rapid extraction of microalgal DNA from single live and preserved cells. *Mol Ecol* 5: 688–691.
- Saito H (2016) Current state of annelids sold as recreational fishing bait in Japan. *Kaiyo Monthly/Special* 57: 83–92. (in Japanese)
- Saito H, Kawai K, Umino T, Imabayashi H (2014) Fishing bait worm supplies in Japan in relation to their physiological traits. *Mem Mus Vic* 71: 279–287.
- Saito H, Niwa N, Kawai K, Imabayashi H (2011) Current state of aquatic animals sold as sport fishing bait in Western Japan. *Bull Hiroshima Univ Mus* 3: 45–57. (in Japanese with English abstract)
- Sato M (2010) Anthropogenic decline of the peculiar fauna of estuarine mudflats in Japan. *Plankton Benthos Res* 5 (Suppl): 202–213.
- Sato M (2017) The values of very soft mudflats in the Isahaya Bay in the Ariake Sea, Kyushu, Japan. *Environ Fish Ariake Sea* 4: 7–12.
- Sato M, Takita T (2000) Fauna and general environments in Ariake Sea. In: *Life in Ariake Sea: Biodiversity in Tidal Flats and Estuaries* (ed Sato M). Kaiyu-sha, Tokyo, Japan, pp. 10–35. (in Japanese)
- Silvestro D, Michalak I (2012) RaxmlGUI: a graphical front-end for RAxML. *Org Divers Evol* 12: 335–337.
- Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313.
- Struck T, Westheide W, Purschke G (2002) Progenesis in Eunicida (“Polychaeta,” Annelida)—separate evolutionary events? Evidence from molecular data. *Mol Phylogenet Evol* 25: 190–199.
- Struck TH, Purschke G, Halanych KM (2006) Phylogeny of Eunicida (Annelida) and exploring data congruence using a partition addition bootstrap alteration (PABA) approach. *Syst Biol* 55: 1–20.
- Suzuki T, Kimura S, Kimura T, Mori K, Taru M (2013) Benthos of the Tidal Flat. *Wetland International Japan*, Tokyo, Japan, 257 pp. (in Japanese)
- Takahashi K (1960) ‘Iwa-mushi’ *Marphysa sanguinea* Montagu[sic]. In: *Encyclopaedia Zoologica Illustrata in Colours* (eds Okada K, Uchida T et al.). Hokuryukan, Tokyo, p. 154, pl. 77. (in Japanese)
- Toba M, Kobayashi Y, Higano J, Ishii R, Hayashi T, Okamoto R (2017) Characteristics of mesh bag and artificial turf as a spat collector of natural asari clam *Ruditapes philippinarum* in Banzu tidal sand flat in Tokyo Bay. *J Fish Tech* 9: 101–112. (in Japanese with English abstract)
- Utinomi H (1956) Coloured Illustrations of Sea Shore Animals of Japan. Hoikusha, Osaka, xvii+167 pp. (in Japanese)
- Uchida H (1992) Polychaeta. In: *Guide to Seashore Animals of Japan with Color Pictures and Keys*, Vol. 1 (ed Nishimura S). Hoikusha, Osaka, pp. 310–373, pls. 63–72. (in Japanese)
- Wang Z, Zhang Y, Qiu J-W (2018) A new species in the *Marphysa sanguinea* complex (Annelida, Eunicidae) from Hong Kong. *Zool Stud* 57: 48.
- Weekly Sunday Fishing (1997) *Mamushi Busoku ha mou Okorainai—Chuugoku-san Yunyuu Zou* [Shortage of the ‘Mamushi’ (worms of *Marphysa*) will not happen again—importation of the worms from China is increased]. *Weekly Sunday Fishing* 12: 38–39.
- Zanol J, Halanych KM, Struck TH, Fauchald K (2010) Phylogeny of the bristle worm family Eunicidae (Eunicida, Annelida) and the phylogenetic utility of noncongruent 16S, COI and 18S in combined analyses. *Mol Phylogenet Evol* 55: 660–676.
- Zanol J, Halanych KM, Fauchald K (2014) Reconciling taxonomy and phylogeny in the bristleworm family Eunicidae (polychaete, Annelida). *Zool Scr* 43: 79–100.
- Zanol J, Silva T, Hutchings P (2016) *Marphysa* (Eunicidae, Polychaeta, Annelida) species of the *sanguinea*-group from Australia, with comments on pseudo-cryptic species. *Invert Biol* 135: 328–344.
- Zanol J, Silva T, Hutchings P (2017) One new species and two redescriptions of *Marphysa* (Eunicidae, Polychaeta, Annelida) species of the Aenea-group from Australia. *Zootaxa* 4268: 411–426.