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First Report of the Morphology and rDNA Sequences of Two *Pseudopolydora* Species (Annelida: Spionidae) from Japan

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The morphology of two *Pseudopolydora* species, *P*. cf. *reticulata* Radashevsky and Hsieh, 2000 and *P. achaeta* Radashevsky and Hsieh, 2000 are reported from Japan for the first time. *Pseudopolydora* cf. *reticulata* was collected from Japanese tidal flats, and individuals possess the characteristic netlike pigmentation on the dorsum of anterior chaetigers and the longitudinal black band along midline of caruncle. *Pseudopolydora achaeta* was collected from subtidal bottom mud of Onagawa Bay, and individuals have distinctive characteristics, such as intensive black pigmentation on dorsal and ventral sides of the anterior body and nearly straight vertical rows of major spines on the fifth chaetiger. The morphology of *P*. cf. *reticulata* is very similar to that of *P*. cf. *kempi*, with which it had been confused in Japan. We analyzed the 18S and 28S rRNA gene sequences of all five *Pseudopolydora* species recorded from Japan and found strong evidence that they are genetically distinct. Our analysis also suggests that boring polydorids have evolved among non-boring ones; the genus *Pseudopolydora*, which mostly shows the non-boring form, appears to have remained in a more ancestral condition.

Key words: Spionidae, polydorids, *Pseudopolydora*, molecular phylogeny, boring activity

INTRODUCTION

Spionids, one of the most abundant polychaete groups in terms of biomass and number of species, are found in a wide variety of habitats in the coastal benthic environment. The genus *Pseudopolydora* Czerniavsky, 1881 is a small group of spionid polychaetes, which comprises about known 20 species (Radashevsky and Migotto, 2009; Walker, 2011). *Pseudopolydora* species predominantly occur in the intertidal and shallow subtidal zone of coastal waters, and are mainly reported from the western Pacific (Radashevsky and Migotto, 2009). These worms play important roles in chemical and physical processes of material recycling due to their tube-building and feeding activities (Hentschel and Harper, 2006). *Pseudopolydora* species are also known as common dietary items for fish (Tomiyama et al., 2005, 2007; Tomiyama, 2012) and shorebirds (Wilson, 1994). Planktonic larvae belonging to the genus *Pseudopolydora* are an important component of coastal meroplanktonic communities (Abe et al., 2011, 2014; Omelyanenko and Kulikova, 2011).

Members of the genus *Pseudopolydora* possess falcate spines in the posterior row of notochaetae on the fifth segment, as do several other genera of spionids, collectively called the polydorids (Blake, 1996; Radashevsky, 2012). In most *Pseudopolydora* species, chaetiger 5 is only slightly modified, or is similar in appearance to neighboring chaetigers, making this the least modified group in the polydorids (Radashevsky and Migotto, 2009). Although many members of the polydorids are known for boring into various calcareous substrates, such as coralline algae, corals, mollusk shells, and barnacles (Blake and Evans, 1972; Blake, 1996), the *Pseudopolydora* species are typically non-borers. Some *Pseudopolydora* species also are reported from calcareous substrates, but the status of boring activity is unclear (Woodwick, 1964; Simon, 2009).

Taxonomy of *Pseudopolydora* species is often confused due to brief species descriptions and lack of adequate type specimens of some species, e.g. *P. kempi* (Southern, 1921). However, while accurate molecular identification may aid in elucidating taxonomic confusion regarding the *Pseudopolydora* species, there are few GenBank sequences available. In Japan, the morphology of three *Pseudopolydora* species, *P*. cf. *kempi*, *P*. *paucibranchiata* (Okuda, 1937), and *P*. *antennata* (Claparède, 1869) have been previously reported (Okuda, 1937; Imajima and Hartman, 1964; Sato-Okoshi, 2000) while ecological studies have simply recorded the occurrence of *Pseudopolydora reticulata* Radashevsky and Hsieh, 2000 from Japan (Abe et al., 2014; Kanaya et al., 2015a, b) without reporting on the morphology of this species in detail. In addition, *Pseudopolydora achaeta* Radashevsky and Hsieh, 2000 is newly recorded from Japan in the present study. The present study provides the first reports of the morphology of *P*. cf. *reticulata* and *P*. *achaeta* from Japan, their phylogenetic position within the family Spionidae, and implications for phylogenetic relationships among polydorid genera.

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MATERIALS AND METHODS

Morphological examination

Pseudopolydora cf. *reticulata, P. achaeta, and P.* cf. *kempi* were collected from Akkeshi Lake in Hokkaido; Gamo Lagoon, Sasuhama, and Onagawa Bay in Miyagi Prefecture; Matsukawa-ura Lagoon in Fukushima Prefecture; and Hachi-no-higata tidal flat in Hiroshima Prefecture from 2010 to 2014 (Fig. 1). Specimens were obtained by sampling bottom sediments using a scoop or Ekman–Birge grab sampler and sieving through 500-µm or 1-mm mesh sieves. Morphological characteristics were observed under a stereomicroscope (Olympus SZX 16) in both live specimens anesthetized in 7% magnesium chloride solution and preserved specimens (in 10% neutral formalin in seawater). Light micrographs were taken using a digital microscope camera (Olympus DP 73). In morphological analyses, 121, 71, and 61 specimens were used for *P.* cf. *reticulata*, *P.* cf. *kempi*, and *P. achaeta*, respectively. The number of specimens from each sampling site is given in parentheses following sampling date under the Materials examined sections below. A representative series of specimens were deposited in the National Museum of Nature and Science (NSMT), Tsukuba, Japan. Additional *Pseudopolydora* specimens collected from 1994 to 1996 from Manose Estuary, Omoi Estuary, Kotsuki Estuary, and Shigetomi tidal flats in Kagoshima Prefecture (Fig. 1) and previously deposited in Tohoku University (Sato-Okoshi, 2000) were also examined.

Molecular examination and phylogenetic analysis

Genomic DNA was extracted from live or ethanol-preserved tissues and 18S and 28S rRNA gene analyses were performed, generally following the methods described by Sato-Okoshi and Abe (2012, 2013) and Teramoto et al. (2013), unless otherwise described. The 18S gene analysis was performed on five *Pseudopolydora* species collected from various locations in Japan (Table 1). Sequences of *Dipolydora bidentata* (Zachs, 1933), *Boccardiella hamata* (Webster, 1879), and *Boccardia proboscidea* Hartman, 1940 were also analyzed for inclusion in the molecular phylogenetic analysis of polydorid species. The number of specimens used for each analysis is included in parentheses after the "Accession Number" in Table 1.

The 28S rRNA gene analysis was conducted on three specimens of each of the five *Pseudopolydora* species (Table 1). The 28S rRNA gene was amplified with the forward (D1R: ACCCGCTGAATTTAAGCATA) and reverse (D2C: CCTTGGTCCGTGTTTCAAGA) primer pair (Scholin et al., 1994). PCR cycling conditions were 94°C for 2 min followed by 36 cycles of denaturation for 10 s at 98°C, annealing for 30 s at 52°C, and extension for 1 min at 68°C. The PCR reaction mixture and procedures for the 28S rRNA gene analysis were the same as that for the 18S rRNA.

Sequences of the 18S and 28S rRNA genes were aligned using MAFFT ver. 7.301b using L-INS-i algorithm (Katoh et al., 2002, 2005). Ambiguously aligned regions were eliminated by employing Gblocks ver. 0.91b (Castresana,

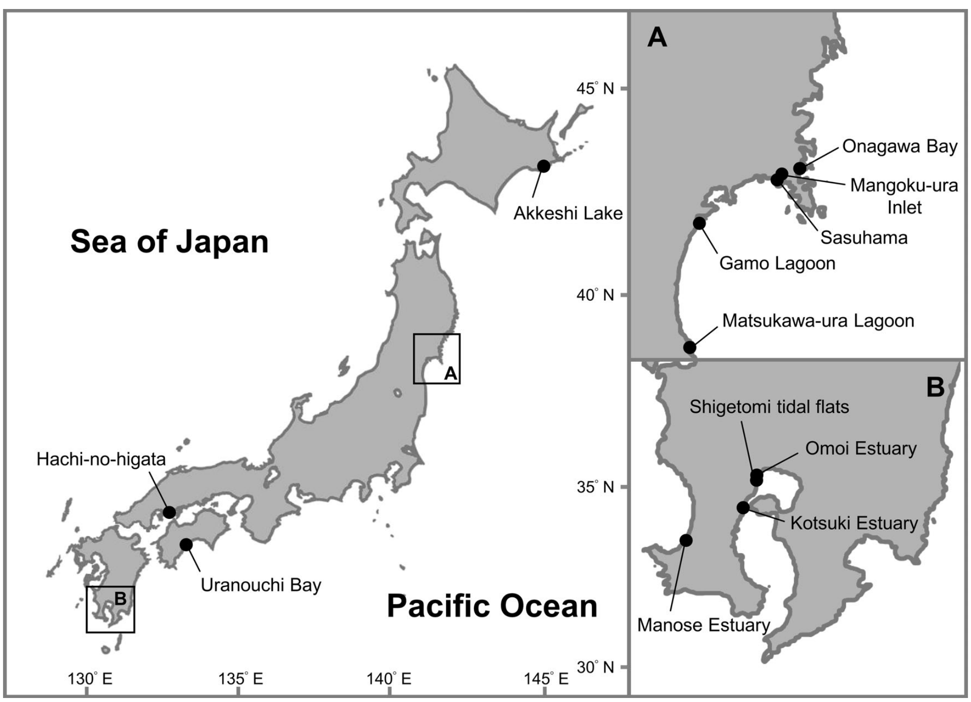

Fig. 1. Localities of sampling sites in Japan.

Table 1. Specimens belonging to *Pseudopolydora* and other genera used for molecular analysis and their GenBank accession and museum registration numbers. The number of individuals used for molecular analysis is indicated in parentheses after each accession number. Asterisks (*) indicate cases in which materials from different locations were pooled for identical sequences.

Species	Locality	Sampling date	Accession numbers		Museum registration numbers
			18S	28S	
Pseudopolydora cf. *reticulata*	Gamo Lagoon	Apr, 2013	LC019988 (11)*	LC019992 (3)*	NSMT-Pol 113039
	Sasuhama	Jul & Sep, 2011			
Pseudopolydora achaeta	Onagawa Bay	Dec, 2010; Sep & Dec, 2011	LC019989 (6)	LC019993 (3)	NSMT-Pol 113040
Pseudopolydora cf. *kempi*	Gamo Lagoon	Jan, May, & Dec, 2011; Apr, 2013	LC019990 (11)*	LC019994 (3)*	NSMT-Pol 113041
	Akkeshi Lake	Jun, 2013			
	Hachi-no-higata	Sep, 2014			
Pseudopolydora paucibranchiata	Onagawa Bay	Jan, Jul, and Aug, 2011	LC019991 (9)*	LC019995 (3)*	
	Mangoku-ura Inlet	Jul, 2014			
Pseudopolydora cf. *antennata*	Uranouchi Bay	Sep, 2011	AB973926 (3)	AB973934 (3)	
Dipolydora bidentata	Sasuhama	Jul, 2012	LC107609 (2)		
Boccardiella hamata	Sasuhama	May, 2011	LC107608 (2)		
Boccardia proboscidea	Sasuhama	May, 2011	LC107607 (2)		

2000; Talavera and Castresana, 2007). Maximum likelihood (ML) trees were reconstructed using MEGA ver. 6.06 (Tamura et al., 2013) under the Tamura–Nei model (Tamura and Nei, 1993) with gamma distribution and invariable sites (TN93+Γ+I) for the 18S rRNA and Kimura 2-parameter model (Kimura, 1980) with invariable sites (K2+I) for 28S rRNA gene, which were selected as the best-fit model by the Akaike information criterion in MEGA ver. 6. The reliability of the ML trees was evaluated by 1000 bootstrap replicates. Sequences of *Apistobranchus typicus* (Webster & Benedict, 1887) and *Magelona* sp. (18S rRNA), and *Tharyx* sp. (28S rRNA) were used for outgroup rooting. All the sequences newly generated in this study have been deposited in GenBank nucleotide sequence database. Accession numbers are listed in Table 1.

RESULTS

Morphological descriptions of *Pseudopolydora* cf. *reticulata*, *P.* cf. *kempi* and *P. achaeta*

Family **SPIONIDAE** Grube, 1850
Genus ***Pseudopolydora*** Czerniavsky, 1881
Pseudopolydora* cf. *reticulata Radashevsky and Hsieh, 2000
[New Japanese name: amime-oni-supio]
(Fig. 2A–C)

?*Pseudopolydora reticulata* Radashevsky and Hsieh, 2000: 229–231, figs. 8, 11b; Zhou et al., 2010: 10.
Pseudopolydora cf. *kempi*: Sato-Okoshi, 2000: 448 in part.

Material examined. Manose Estuary, 31°26′N, 130°17′E, Kagoshima Pref., intertidal, sand flat, Apr. and Aug. 1996 (7); Omoi Estuary, 31°42′N, 130°37′E, Kagoshima Pref., intertidal, sand flat, May 1994 (4); Kotsuki Estuary, 31°34′N, 130°33′E, Kagoshima Pref., intertidal, sand flat, Apr. 1994 (55); Shigetomi tidal flats, 31°42′N, 130°37′E, Kagoshima Pref., intertidal, sand flat, Apr. and June 1994 (6), Mar. 1996 (4); Gamo Lagoon, 38°15′N, 141°00′E, Miyagi Pref., intertidal, sand flat, Jan. 2011, NSMT-Pol 113039 (34), Apr. 2013 (6); Sasuhama, 38°24′N, 141°22′E, Miyagi Pref., intertidal, sand, July (3) and Sep. 2011 (2).

Description. Maximum length 18.3 mm, 1.9 mm wide at chaetiger 5, in 51 chaetigers in formalin fixed specimens. Yellowish-white in color (live specimens), with black spots or transversal bands on antero-lateral edges of chaetigers 1 or 2 to 3–10 (both live and fixed specimens). Black, reticulated pigments present on dorsal side of chaetigers 1 to 8–17; thin, longitudinal black band present along midline of caruncle behind occipital antenna (Fig. 2A), usually remaining even in fixed specimens. Small, paired, ventral black spots absent or present along posterior edge of chaetigers 5–8 to 7–11. Dorsal, ventral, and lateral pigmentation often disappear in preserved material. Palps transparent, without white spots in living specimens (Fig. 2C).

Prostomium anteriorly bilobed; caruncle usually extended to middle of chaetiger 4, occasionally to end of chaetiger 3. Occipital antenna present on caruncle. Four black eyes arranged in trapezoidal pattern.

Chaetiger 1 with short notopodial lobe; notochaetae absent; winged capillary neurochaetae present on well-developed neuropodia. Notochaetae in anterior chaetigers varying in shape from short to long, curved to straight, and broadly to faintly limbate. Anterior row of notochaetae in chaetigers 3–6 heavily curved, pennoned, without subdistal constriction, modified when compared with notochaetae in chaetigers 2 and 7 onwards, with greatest modification on chaetiger 5. No special notochaetae in posterior chaetigers. Anterior neurochaetae broadly lanceolate, with very faint curvature replaced by bidentate hooded hooks from chaetiger 8. Hooded hooks not accompanied by capillaries, continuing to posterior end of body. Main fang of hooks located at right angle to shaft; apical tooth closely applied to main fang. Lower part of shaft curved at right angle; constriction present on upper part of shaft. Hooks number 25 to 36 per chaetiger. Branchiae from chaetiger 7 to 19–23, free from notopodial lamellae.

Chaetiger 5 bearing two types of major spines arranged in two, parallel, vertical, J-shaped rows on each side (Fig. 2B). Spines in anterior (outer) row pennoned with curved, pointed tips, without subdistal constriction; spines in posterior (inner) row simple and falcate. Companion chaetae absent. Noto- and neuropodial lamellae present on chaetiger 5 with well-developed noto- and neurochaetae, similar in shape and number to preceding and succeeding chaetigers.

Pygidium being large flaring disc with dorsal gap and erect process on each dorsolateral side, same color as body.

Glandular pouches from chaetiger 1, largest and paired on either side in chaetigers 6 and 7, single on either side in other chaetigers. No gizzard-like structure in digestive tract.

Remarks. Previously, Sato-Okoshi (2000) identified *P.* cf. *reticulata* as *P.* cf. *kempi*. All *Pseudopolydora* specimens collected from 1994 to 1996 from Manose Estuary, Omoi Estuary, Kotsuki Estuary, and Shigetomi tidal flats, southern Japan, were re-examined and determined as *P.* cf. *reticulata* in the present study.

Pseudopolydora reticulata was originally described from shallow water environments in Taiwan and is very close to *Pseudopolydora bassarginensis* (Zachs, 1933) from Peter the Great Bay in Russia (Radashevsky and Hsieh, 2000). Both species have a black band on the caruncle and reticulate netlike pigmentation on the dorsum of anterior chaetigers, but they differ in the length of the caruncle and the presence or absence of the black paired spots on the ventral side of the anterior chaetigers (Radashevsky and Hsieh, 2000). While the caruncle reaches the end of the chaetiger 5 in *P. reticulata*, it usually extends to the middle of the chaetiger 4, rarely to the end of chaetiger 4 in *P. bassarginensis*; the ventral black paired spots are present in *P. reticulata*, but absent in *P. bassarginensis*. The Japanese specimens share features of both species; namely, the caruncle usually extends to the middle of chaetiger 4 and some specimens have ventral black paired spots. The Taiwanese specimens have a longer caruncle than the Japanese ones, despite their body length being only half that of the Japanese specimens. Since the original description of *P. bassarginensis* is very brief and the status of the species remains uncertain (Radashevsky and Hsieh, 2000), we tentatively identify this species as *P.* cf. *reticulata*.

Distribution. This species has been reported only from the western North Pacific: Taiwan (Radashevsky and Hsieh, 2000), China (Zhou et al., 2010), and Japan (present study).

Pseudopolydora* cf. *kempi Southern, 1921
[Japanese name: doro-oni-supio]
(Fig. 2D–G)

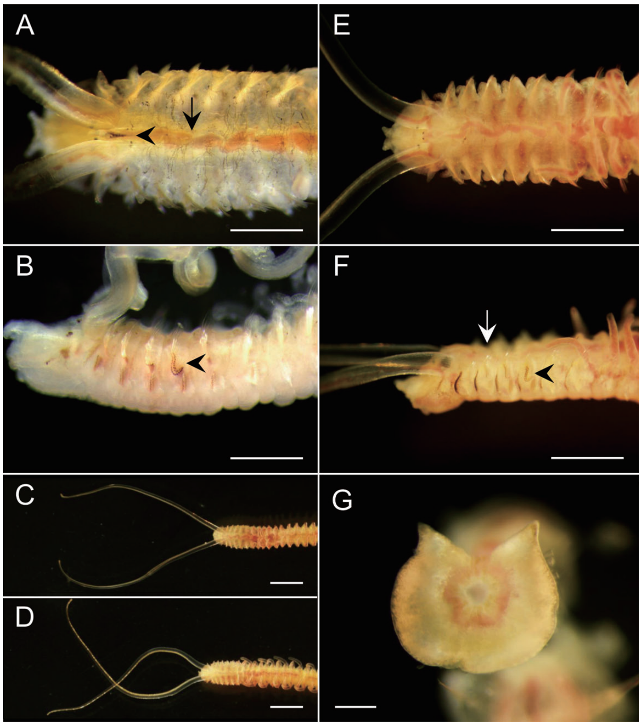

Fig. 2. Light micrographs showing morphology of *Pseudopolydora* cf. *reticulata* **(A–C)** and *Pseudopolydora* cf. *kempi* **(D–G)**. **(A)** Anterior, dorsal view of living *P*. cf. *reticulata*; arrowhead and arrow indicate the longitudinal black band on the caruncle and the end point of caruncle, respectively. **(B)** Anterior, lateral view of formalin-fixed *P*. cf. *reticulata*; arrowhead indicates the major spines in the fifth chaetiger. **(C)** Anterior and palps of living *P*. cf. *reticulata*; the palps exhibit no irregular small white spots. **(D)** Anterior and palps of living *P*. cf. *kempi*; the palps exhibit irregular small white spots. **(E)** Anterior, dorsal view of living *P*. cf. *kempi*. **(F)** Anterior, lateral view of living *P*. cf. *kempi*; arrowhead and arrow indicate the major spines in fifth chaetiger and the end point of caruncle, respectively. **(G)** Pygidium of living *P*. cf. *kempi*. Scale bars: **(A, B, E, F)** = 500 μm; **(C, D)** = 100 μm; **(G)** = 1 mm.

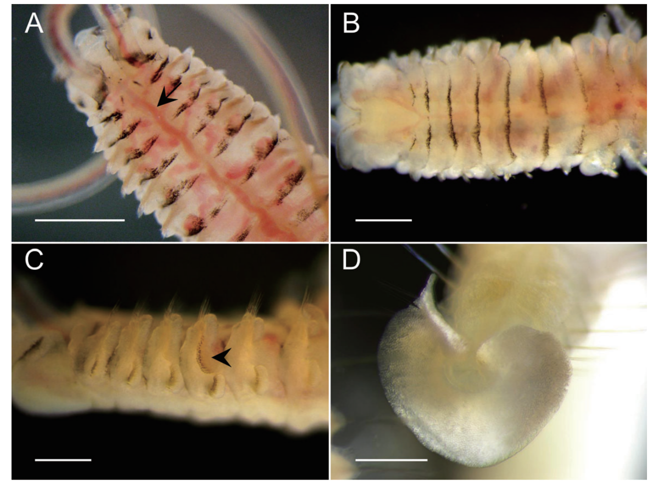

Fig. 3. Light micrographs showing morphology of *Pseudopolydora achaeta*. **(A)** Anterior, dorsal view of living specimen; arrow indicates the end point of caruncle. **(B)** Anterior, ventral view of living specimen. **(C)** Anterior, lateral view of living specimen; arrowhead indicates the major spines in the fifth chaetiger. **(D)** Pygidium of formalin-fixed specimen, erect process on right dorsolateral side is lacking. Scale bars: 500 μm.

?*Polydora* (*Carazzia*) *kempi* Southern, 1921: 636–628, fig. 20.
Pseudopolydora kempi: Blake and Woodwick, 1975: 118–124; Blake and Kudenov, 1978: 268–269: Light, 1978: 157–160, fig.158; Zhou et al., 2010: 8–9; Hiebert et al., 2015.
Pseudopolydora cf. *kempi japonica*: Radashevsky and Hsieh, 2000: 221–223, fig. 3.
Pseudopolydora cf. *kempi*: Sato-Okoshi, 2000: 448 in part.

Material examined. Akkeshi Lake, 43°3′N, 144°51′E, Hokkaido, intertidal, sand flat, June 2013 (5); Gamo Lagoon, 38°15′N, 141°00′E, Miyagi Pref. intertidal, sand flat, Jan. 2013, NSMT-Pol 113041 (39), May (1) and Dec. (1) 2011, Apr. 2013 (12); Matsukawa-ura Lagoon, 37°48′N, 140°58′E, Fukushima Pref., intertidal, sand flat, Apr. 2014 (1); Hachi-no-higata tidal flat, 34°19′N, 132°53′E, Hiroshima Pref., intertidal, mud flat, Sep. 2014 (12).

Description. Maximum length 16.2 mm, 1.7 mm wide at chaetiger 5, for 51 chaetigers in formalin fixed specimens. Light tan in color (live specimens), dorsal pigmentation of anterior chaetigers absent (Fig. 2E) or two rows of faint black spots present on chaetigers 3–5 to 9–12 (both live and fixed specimens). No pigment on caruncle, but some individuals have a small mid-dorsal black spot on caruncle behind occipital antenna. Black spots or transverse bands on antero-lateral edges in chaetigers 1 or 2 to 4–10 (Fig. 2F). Scant black pigmentation present on ventral side of peristomium and usually absent in other ventral part except in chaetigers 1 to 6–11 on some specimens. Dorsal, ventral, and lateral pigmentations often absent on fixed individuals. Palps of living specimens transparent, exhibit irregular small white spots under reflected light (Fig. 2D). These spots fade after fixation.

Prostomium anteriorly bilobed, caruncle usually extends to middle of chaetiger 3, occasionally to end of chaetiger 2. Occipital antenna on caruncle. Four black eyes arranged in trapezoidal pattern (Fig. 2E).

Chaetiger 1 with short notopodial lobe, no notochaetae; winged capillary neurochaetae present on well-developed neuropodia. Anterior row of notochaetae of chaetigers 3–6 modified in shape compared with notochaetae of chaetigers 2 and 7 onwards, greatest modification on chaetiger 5. No special notochaetae in posterior chaetigers. Bidentate hooded hooks in neuropodia beginning from chaetiger 8, replacing fascicle of neurochaetae, not accompanied by capillaries. Main fang of hooks at a right angle to shaft, with apical tooth closely applied to main fang. Lower part of shaft curved at right angle, constriction present on upper part of shaft. Hooks numbered from 10 to 33 per chaetiger. Branchiae from chaetiger 7 to 17–25, free from notopodial lamellae.

Chaetiger 5 not distinctly modified, with two types of major spines arranged in two, parallel, vertical, J-shaped rows on each side (Fig. 2F). Spines in anterior row pennoned with curved pointed tips, without subdistal constriction; spines in posterior row simple and falcate. Companion chaetae absent. Noto- and neuropodial lamellae present in chaetiger 5, well developed noto- and neurochaetae, same in shape and number to preceding and succeeding chaetigers.

Pygidium large, flaring, disc-like with dorsal incision and short erect process on each dorsolateral side (Fig. 2G), white colored or same color as body.

Glandular pouches present from chaetiger 1, largest and paired on either side in chaetigers 6 and 7, single on either side in other chaetigers. No gizzard-like structure in digestive tract.

Remarks. The original description of *Pseudopolydora kempi* (Southern, 1921) from brackish water in India was brief, based on incomplete specimens. Okuda (1937) described the same species from Japan. Later, Imajima and Hartman (1964) established a new subspecies, *P*. *kempi japonica*, for the Japanese population as these specimens were larger and had a longer caruncle than those of *P*. *kempi* from India, although it has been indicated that the description was probably based only on Okuda (1937) (Radashevsky and Hsieh, 2000). Sato-Okoshi (2000) regarded the Japanese population as *P*. cf. *kempi*, as the length of the caruncle can be variable and is often related to the length of the worm, although the author confused *P*. cf. *reticulata* and *P*. cf. *kempi* and cited them as *P*. cf. *kempi* (see above). Since neither Okuda (1937) nor Imajima and Hartman (1964) described the pigmentation pattern in the Japanese specimens, and the type specimens of *P*. *kempi japonica* were not deposited (Radashevsky and Hsieh, 2000), the status of *P*. *kempi japonica* remains unclear. However, the characteristics described by Okuda (1937) for his "*P*. *kempi*" and those by Imajima and Hartman (1964) for their "*P. kempi japonica*" are similar to those in *P*. cf. *reticulata* described in the present study, in that the caruncle extends back to the anterior margin of the fourth chaetiger. It is possible that Okuda's specimens may have lost their pigmentation following fixation. Irregular white spots on palps are present in our specimens, as in the Californian specimens described by Blake and Woodwick (1975).

Distribution. This species has been reported to have a wide geographical distribution. However, it is unclear if the specimens reported in previous papers are conspecific or not, and there might be several cryptic species. Currently confirmed distribution of this species is Taiwan (Radashevsky and Hsieh, 2000), China (Zhou et al., 2010), Japan (present study), and USA (Blake and Woodwick, 1975; Light, 1978; Hiebert et al., 2015). The Californian populations were allegedly introduced with oysters from Japan (Light, 1978). The status of the species reported from Mozambique (Day, 1955, 1967), India (Southern, 1921), Australia (Blake and Kudenov, 1978), Korea (Okuda, 1937), Russia (Radashevsky, 1993; Buzhinskaja, 2013) and Canada (Banse, 1972) need to be clarified.

Pseudopolydora achaeta Radashevsky and Hsieh, 2000
[New Japanese name: tora-oni-supio]
(Fig. 3)

Pseudopolydora achaeta Radashevsky and Hsieh, 2000: 223–226, fig. 4, 5, 11a.

Material examined. Onagawa Bay, 38°26′N, 141°27′E, Miyagi Pref., 20 m, mud, Jan., Feb., Apr., July, Aug. 2010, Feb., May, Sep., Oct. 2013, NSMT-Pol 113040 (61).

Description. Large bodied, maximum length 33.0 mm, 2.0 mm wide at chaetiger 5, for 93 chaetigers in formalin fixed specimens. Yellowish-white in color (live specimens), with black pigments on lateral edges of prostomium and laterally between anterior chaetigers (Fig. 3C). Black bands present dorsally on posterior edges of chaetigers from 1 to 9–16 (Fig. 3A). Small individuals with mid-dorsal small black spots on chaetigers 3–10, reducing with increasing size of specimen. Ventral black pigments present along posterior edges of chaetigers 1 to 9–12 (Fig. 3B). Dorsal, ventral, and lateral pigmentations may be lost in fixed individuals. Palps transparent in living specimens.

Prostomium weakly incised, caruncle usually extending to end of chaetiger 2. Peristomium ventrally forming folded lip, the edge of which extending from ventral midline to lateral surface of peristomium on each side (Fig. 3B). Occipital antenna present on caruncle. Four black eyes arranged in trapezoidal pattern.

Chaetiger 1 with short notopodial lobe lacking notochaetae; with 10–13 fine hair-like neurochaetae present on well-developed neuropodia. These fine hair-like neurochaetae difficult to observe under low magnification. Neurochaetae being winged capillaries in chaetigers 2–7. Anterior row of notochaetae in chaetiger 4 slightly modified, intermediate in shape between winged capillaries in chaetiger 3 and pennoned spine in chaetiger 5. No special notochaetae in posterior chaetigers. Bidentate hooded hooks in neuropodia from chaetiger 8 backward, replacing fascicles in neurochaetae, not accompanied by capillaries. Main fang of hooks at a right angle to shaft and apical tooth closely applied to main fang. Lower part of shaft curved at right angle and constriction present on upper part of shaft. Hooks numbering from 21 to 25 per ramus. Branchiae in chaetigers 7–16, free from notopodial lamellae.

Chaetiger 5 not distinctly modified, bearing two types of major spines arranged in two, parallel, vertical, slightly curved rows on each side (Fig. 3C). Spines in anterior row pennoned with curved pointed tips, without subdistal constriction; spines in posterior row simple and falcate. Companion chaetae absent. Noto- and neuropodial lamellae present on chaetiger 5, well developed noto- and neurochaetae identical in shape and number to preceding and succeeding chaetigers.

Pygidium large, flaring, disc-like with dorsal gap and erect process on each dorsolateral side (Fig. 3D), white-colored.

Glandular pouches present from chaetiger 1, largest and paired on either side in chaetigers 6 and 7, single on either side in other chaetigers. No gizzard-like structure in digestive tract.

Remarks. *Pseudopolydora achaeta* was originally described from shallow-water environments in Taiwan (Radashevsky and Hsieh, 2000). In the present study, it was collected only from muddy sediment at 20 m depth in the innermost part of Onagawa Bay. These populations differ in their body size; the length of the Japanese specimens was more than triple that of the Taiwanese specimens. This species has diffuse black pigmentation on the dorsal and ventral sides of the body and a characteristic, nearly straight, verti-

Table 2. Comparison of morphological characteristics among five *Pseudopolydora* species from Japan. Bold shows distinguishable key characters of the species.

Species	Prostomium	Length of caruncle	Pigmentation				Major spines arrangement	References
			Palps	Caruncle	Dorsal side	Anterior interchaetiger		
P. cf. *kempi*	Bilobed	End of chaetiger 2	**Irregular white small spots**	Absent or small black spot	Absent or paired black spots	Present	J-shaped	This study
P. cf. *reticulata*	Bilobed	Middle of chaetiger 4	Absent	**Longitudinal black band**	**Black reticulated**	Present	J-shaped	This study
P. achaeta	Weakly incised	End of chaetiger 2	Absent	Absent	**Intensively black pigmented**	Present	**Slightly curved vertically**	This study
P. paucibranchiata	**Rounded**	Middle of chaetiger 3	**White bars**	Absent	Absent	Absent	U-shaped	Sato-Okoshi, 2000
P. cf. *antennata*	Bilobed	**End of chaetiger 6**	Absent	Absent	Absent	Absent	U-shaped	Sato-Okoshi, 2000

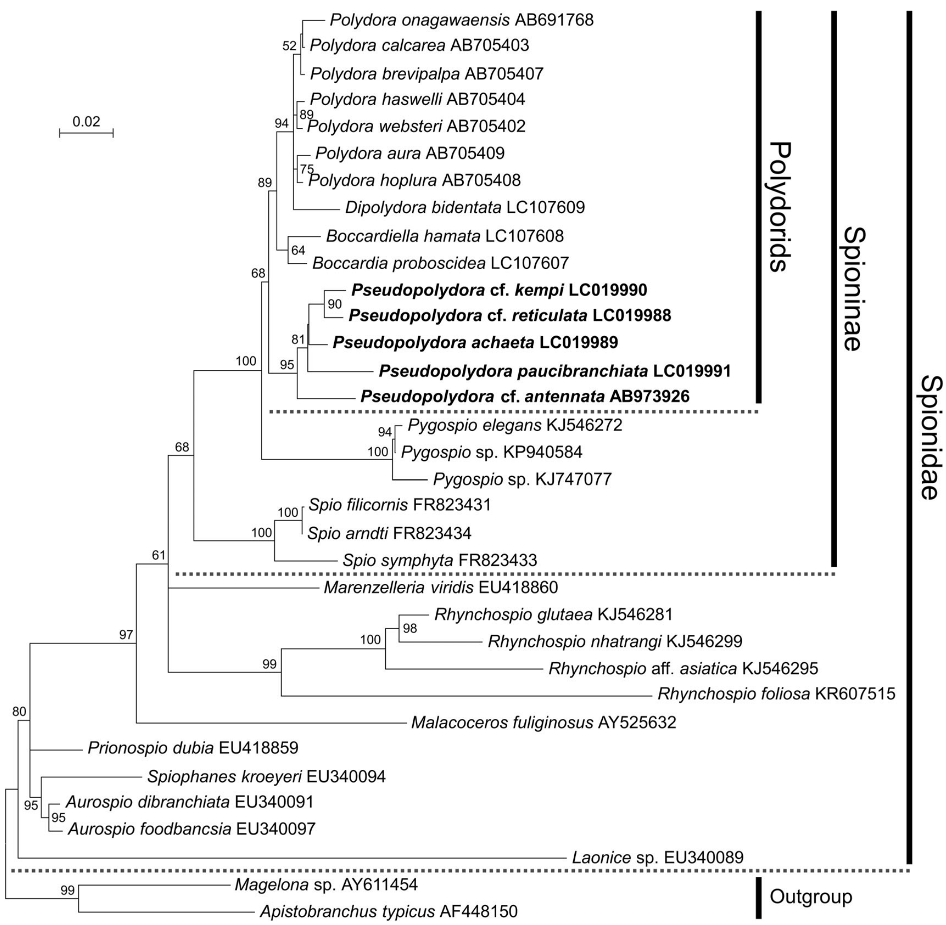

Fig. 4. A maximum likelihood tree inferred from the nuclear 18S rRNA gene sequences of spionid polychaetes. The gene sequences of the five *Pseudopolydora* species obtained in the present study are highlighted in boldface type. Bootstrap values of > 50% as percentage of 1,000 bootstrap replicates are given at the respective nodes. The scale bar represents the number of substitutions per site. Sequences of *A. typicus* and *Magelona* sp. are used for outgroup rooting.

cal rows of major spines (not J-shaped) on chaetiger 5. It is easy to distinguish the species from other *Pseudopolydora* species in Japan (Table 2).

Distribution. This species has been reported from the western North Pacific and the western South Atlantic: Taiwan (Radashevsky and Hsieh, 2000), Japan (present study), Russia (Buzhinskaja, 2013) and Brazil (Lana et al., 2006).

Phylogenetic analysis of the 18S and 28S rRNA gene sequences

The nuclear 18S (1772–1784 bp) and 28S (768–775 bp) rRNA gene sequences were successfully obtained from five *Pseudopolydora* species (Table 1). The 18S rRNA gene sequences of *P.* cf. *kempi* showed single nucleotide polymorphisms at three sites, and G/A, T/C, and C/A hetero-peaks were identified at nucleotides 176, 230, and 1,371 from the 5′ end, respectively, in both forward and reverse strands. These heterozygotes were observed in most of the *P.* cf. *kempi* individuals analyzed in this study. No heterozygous sites were observed in other species. There was no intraspecific variation in the 18S and 28S rRNA gene sequences for each of the five *Pseudopolydora* species except the above mentioned single nucleotide polymorphisms. The sequences were completely distinct between all the five *Pseudopolydora* species recorded from Japan. Two morphologically similar species, *P.* cf. *reticulata* and *P.* cf. *kempi*, were recovered as sister species in the phylogenetic tree (Figs. 4, 5). Species belonging to the genus *Pseudopolydora*, the polydorids, and the subfamily Spioninae formed a monophyletic group in the phylogenetic tree using the available 18S and 28S rRNA gene sequences of spionid polychaetes (Figs. 4, 5). The monophyly of *Pseudopolydora* spp. was well supported in the 18S tree (95% bootstrap support). However, it was less well supported in the 28S tree (55% bootstrap support). The supporting value of the monophyly of polydorids was sufficient (81%) in the 28S tree while that of the 18S tree was rather low (68%; Figs. 4, 5).

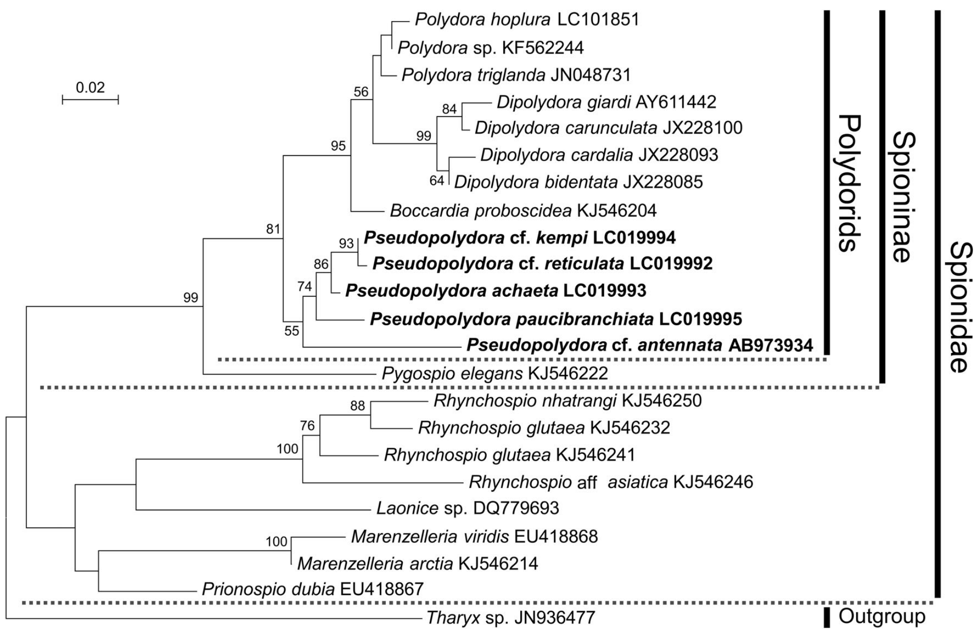

Fig. 5. A maximum likelihood tree inferred from the nuclear 28S rRNA gene sequences of spionid polychaetes. The gene sequences of the five *Pseudopolydora* species obtained in the present study are highlighted in boldface type. Bootstrap values as percentage of 1,000 bootstrap replicates are given at the respective nodes. The scale bar represents the number of substitutions per site. Sequence of *Tharyx* sp. is used for outgroup rooting.

DISCUSSION

We report the morphology of the two *Pseudopolydora* species, *P*. cf. *reticulata* and *P*. *achaeta*, for the first time from Japan. Although *P*. cf. *reticulata* is morphologically very similar to *P*. cf. *kempi*, the 18S and 28S rRNA gene sequences of the two species are clearly different (Figs. 4, 5). Furthermore, the two species can be clearly distinguished by the larval developmental mode: larvae in *P*. cf. *reticulata* hatch out at 3-chaetiger stage and have a long planktonic larval period, while those in *P*. cf. *kempi* hatch out at 12–14-chaetiger stage and have a short planktonic larval period (Kondoh et al., unpublished data). The only diagnostic morphological character to distinguish these two species collected in Japan is the pigmentation pattern on the caruncle and on the dorsal surface of the anterior chaetigers (Table 2, Fig. 2). Irregular white spots on the palps are only observed in *P*. cf. *kempi*; however, identification of fixed specimens should be made with caution, because the white spots gradually fade out after fixation. Fortunately, the longitudinal black band along the midline of the caruncle in *P*. cf. *reticulata* is generally remained after fixation and is therefore useful for identification of the species. Some individuals of *P*. cf. *kempi* also possess a small mid-dorsal black spot on the caruncle.

Sato-Okoshi (2000) identified *P*. cf. *reticulata* as *P*. cf. *kempi*. *Pseudopolydora* cf. *kempi* has been recorded from numerous ecological studies (e.g., Kanaya et al., 2011; Tanaka et al., 2013; Kanaya, 2014), and has, until the present report, been considered to be one of the most common species inhabiting estuaries and tidal flats in Japan. *Pseudopolydora* cf. *reticulata* and *P*. cf. *kempi* seemed to occur sympatrically as observed in Gamo Lagoon (present study). We thus cannot rule out the possibility that this confusion has also occurred in other studies. It is necessary to elucidate dominant species in various habitats in Japanese waters, and careful attention to these two *Pseudopolydora* species is especially required for future coastal studies in Japan and other countries.

The taxonomy and ecology of *P. kempi* is confused due to the brief morphological description (Southern, 1921), lack of type materials, and uncertain status of the species. Myohara (1979) suggested that *P. kempi japonica* from Japan and *P. kempi* from California described by Blake and Woodwick (1975) can be distinguished by characters from both their embryology and morphology. Myohara (1979) pointed out that *P. kempi* from California has irregular white small spots on the palps, while *P. kempi japonica* from Japan lacks white spots. In the present study, it was confirmed that the Japanese *P*. cf. *kempi* has irregular white small spots on their palps (Table 2, Fig. 2). It is possible that the *P*. *kempi japonica* discussed by Myohara (1979) was *P*. cf. *reticulata* as described in this study. Various types of larval development have been reported in *P*. *kempi* (Blake and Woodwick, 1975; Srikrishnadhas and Ramamoorthi, 1977; Myohara, 1979; Radashevsky, 1985), and it appears that these reports suggest the existence of sibling species (Blake and Arnofsky, 1999). To clarify the status of *P*. *kempi*, further studies on morphology and larval development are required, particularly those of materials collected from the type locality, and molecular genetic analysis would be an effective and important tool for resolution of the problem.

Polydorids all have characteristic modified chaetae in the modified fifth chaetiger and many members have an ability to bore into various calcareous substrates. However, species belonging to the genus *Pseudopolydora* typically have the least modified fifth chaetiger in the polydorids and almost all species belonging to the genus are non-boring. From the morphological and ecological features of polydorids, the evolutionary direction is an interesting subject (Sato-Okoshi and Takatsuka, 2001). However, the phylogenetic relationships among polydorid genera have not yet been accurately estimated, although the generic relationships of spionids have been estimated from parsimony analyses of morphological (Sigvaldadóttir et al., 1997) and morphological and reproductive characters (Blake and Arnofsky, 1999). The polymorphism of spines on chaetiger 5 has also been an obstacle to recognizing the monophyletic origin of polydorids (Radashevsky and Fauchald, 2000).

This study reports for the first time the molecular phylogeny of *Pseudopolydora* species. The monophyly of the *Pseudopolydora* clade and the polydorid clade (*Polydora*, *Dipolydora*, *Boccardia*, *Boccardiella*, and *Pseudopolydora*) is either moderately or well supported by the bootstrap values in the 18S and 28S trees (Figs. 4, 5). The phylogenetic trees indicate that polydorids have a derived position within the family Spionidae, the members of which are mostly nonborers, with the exception of some species belonging to several polydorid genera. That the non-boring five species of *Pseudopolydora* appeared to be sister to the clade comprised of other four polydorid genera (Figs. 4, 5) suggests that *Pseudopolydora* retains a more ancestral condition among polydorids; i.e. the non-boring form is plesiomorphic within the polydorids. A greater understanding of phylogenetic relationships among all the nine currently recognized polydorid genera (*Polydora*, *Dipolydora*, *Pseudopolydora*, *Boccardia*, *Polydorella*, *Tripolydora*, *Boccardiella*, *Carazziella*, and *Amphipolydora*) is required to finally reveal the origin and evolution of the boring activity in polydorids.

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