

## Research article

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**Morphology and phylogeny of a new polychaete,  
*Prionospio expansa* (Annelida: Spionidae)  
from the intertidal zone of the Yellow Sea, Korea**

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**Abstract.** A new spionid polychaete, *Prionospio expansa* sp. nov., collected from the intertidal habitat of the Yellow Sea in Korea, is described. The new species is closely related to *P. japonica* Okuda, 1935 from Northeast Asia both morphologically and genetically. They share four pairs of branchiae which are cirriform and apinnate, whereas the new species differs from *P. japonica* in the length of the branchiae, expansion of the anteriormost body, and size of the first notopodial postchaetal lamellae. Herein, a detailed description and illustrations of *P. expansa* sp. nov. are provided, with molecular data for three gene fragments: mitochondrial cytochrome *c* oxidase subunit I, 16S ribosomal DNA (rDNA), and nuclear 18S rDNA. A phylogenetic analysis was conducted based on mitochondrial and nuclear gene fragments.

**Keywords.** Molecular analysis, phylogenetic analysis, pigmentation, Polychaeta, Korean waters.

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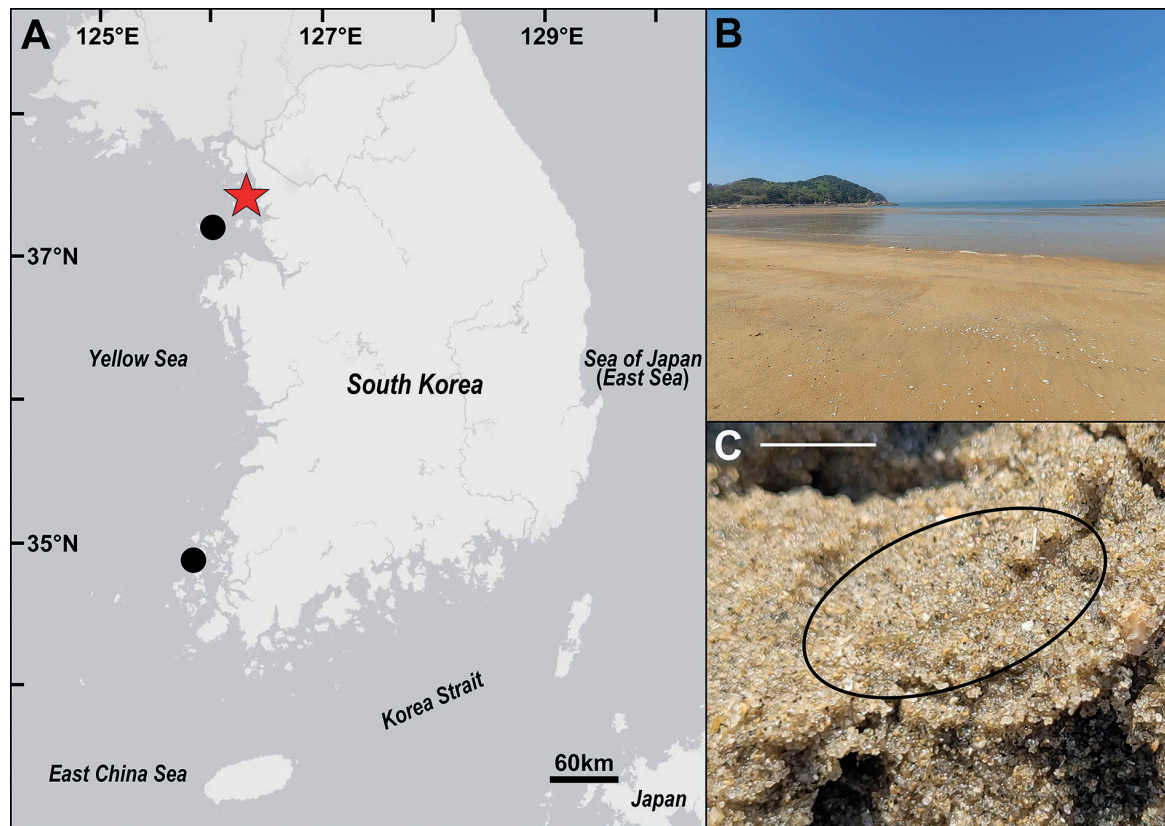
## Introduction

*Prionospio* Malmgren, 1867 is one of the most abundant and specious groups of spionids, commonly found in intertidal to deep-sea zones, comprising more than 100 described species (Blake *et al.* 2020). Members of this genus are characterized by a prostomium that is typically broadly rounded to truncate anteriorly, and branchiae that are either apinnate or pinnate (or combinations of these) on the anterior body (Peixoto & Paiva 2020).

An attempt to clarify the phylogenetic relationships within the *Prionospio*-complex based on cladistic methodology was performed by Sigvaldadóttir (1998). Since then, her proposal has been widely accepted by several authors (e.g., Radashevsky 2015; Paterson *et al.* 2016; Delgado-Blas *et al.* 2018; Peixoto & Paiva 2020), but not by other authors (e.g., Dagli & Çınar 2009, 2011; Zhou & Li 2009; Delgado-Blas *et al.* 2019). A systematic treatment of *Prionospio*-complex has not been explicitly done (Radashevsky 2015). Further morphological studies combined with molecular analyses are needed to confirm their phylogenetic relationships (Dagli & Çınar 2009).

Twelve species of *Prionospio* (*P. bocki* Söderström, 1920; *P. caspersi* Laubier, 1962; *P. depauperata* Imajima, 1990; *P. elongata* Imajima, 1990; *P. japonica* Okuda, 1935; *P. kirrae* Wilson, 1990; *P. krusadensis* Fauvel, 1929; *P. membranacea* Imajima, 1990; *P. multibranchiata* Berkeley, 1927; *P. paradisea* Imajima, 1990; *P. pulchra* Imajima, 1990; and *P. saccifera* Mackie & Hartley, 1990) have been reported in Korean waters (Paik 1989; Jung *et al.* 1998; Song *et al.* 2017; Lee *et al.* 2018, 2020; Lee & Min 2022). Herein, we report an undescribed species of *Prionospio*, *P. expansa* sp. nov., with detailed descriptions and illustrations. Sequences of three gene fragments, partial mitochondrial cytochrome *c* oxidase subunit I (COI), 16S ribosomal DNA (rDNA), and nuclear 18S rDNA, were also determined. Phylogenetic analysis was conducted based on the mitochondrial and nuclear gene fragments.

## Material and methods



**Fig. 1.** Map of the sampling sites and habitat of *Prionospio expansa* sp. nov. **A.** Map of sampling locations: type locality (red star) and other examined specimens collected (black circle). **B.** General view of type locality. **C.** Live worm inhabiting sandy sediment, scale bar: 5 mm.

### Sampling and morphological observations

Adult samples were collected from the intertidal muddy sand and fine sand habitats (Fig. 1B) of the Yellow Sea in Korea (Fig. 1A). Field samples of *Prionospio* and sediments (Fig. 1C) were collected using a scoop or spade and washed in the field on a 200 µm-mesh sieve. Morphological observations were performed on both live and fixed materials. Live specimens were relaxed in a 10% MgCl<sub>2</sub> seawater solution. Morphological characteristics were observed under a stereo microscope (Leica MZ125, Germany). After live observation, the specimens were fixed in 4% formaldehyde for additional morphological studies and subsequently transferred to 70% ethanol. The width (measured at chaetigers 4 and 9) and length of the specimens were based on the formalin-fixed specimens. The worms were photographed under a stereo microscope using a digital camera (Tucsen Dhyana 400DC, China) with a capture program (Tucsen Mosaic ver. 15, China). Formalin-fixed specimens were transferred to distilled water and stained with a methylene green (MG) distilled water solution. After 15 min in the MG solution, the specimens were transferred to 70% ethanol for at least 30 min for observation. Dissected appendages were mounted using Eukitt Quick-hardening mounting medium (Sigma-Aldrich, St. Louis, MO, USA) onto permanent slides and photographed under an optical microscope (Leica DM2500, Germany) using a digital camera (EOS 6D Mark II, Canon, Japan). Images of multiple focal layers were stacked using Helicon Focus software (Helicon Soft Ltd). Specimens used for scanning electron microscopy (SEM) were dehydrated using a t-BuOH freeze dryer (VFD-21S Vacuum Device Ibaraki, Japan), covered with platinum, and observed using a Hitachi SEM model S-4300SE (Hitachi, Japan). SEM observation of *P. japonica* for morphological comparison was performed based on the specimens from Lee *et al.* (2020) (Fig. 4J–K). To remove the hoods of hooks before SEM process, some specimens (e.g., in this study NIBRIV0000901890–1891) were rinsed in contact lens cleaning solution and sonicated in distilled water for 15–25 s at 45 kHz, according to the method of Peixoto & Paiva (2020). All the type and voucher specimens examined in this study were deposited at the National Institute of Biological Resources (NIBR), Incheon, Korea, and a distribution map was made with ArcGIS software (ESRI Inc.).

### Abbreviations for morphological terms

The following abbreviations are used (if the specimens were incomplete):

af = anterior fragment  
mf = middle fragment  
pf = posterior fragment

### Molecular analysis

Specimens used for molecular analysis were fixed in 95% ethanol and preserved at -20°C. Genomic DNA was extracted from the palps of four paratypes (NIBRIV0000900994–0997) using a LaboPass Tissue Mini (Cosmo GENETECH, Seoul, South Korea) according to the manufacturer's instructions. PCR amplification of the mitochondrial COI, 16S rDNA, and nuclear 18S rDNA gene fragments was performed using the following primer sets: polyLCO/polyshortCOIR (Carr *et al.* 2011), 16Sar/16Sbr for 16S rDNA (Kessing *et al.* 1989), and 18E/18B (outer) and 18F997/18L (inner) for 18S rDNA (Blank & Bastrop 2009; Mincks *et al.* 2009). Sequence editing and contig assembly of the obtained DNA sequence data were performed by Geneious ver. 8.1.9 (Biomatters, Auckland, New Zealand). Maximum likelihood (ML) trees were constructed based on the three gene regions using IQ-TREE with the GTR+F+I+G4, TIM2+F+I+G4, and TNE+R2 models with 1000 replicates (Kalyaanamoorthy *et al.* 2017; Hoang *et al.* 2018).

## Results

### Taxonomy

Phylum Annelida Lamarck, 1809  
Family Spionidae Grube, 1850  
Genus *Prionospio* Malmgren, 1867

#### *Prionospio expansa* sp. nov.

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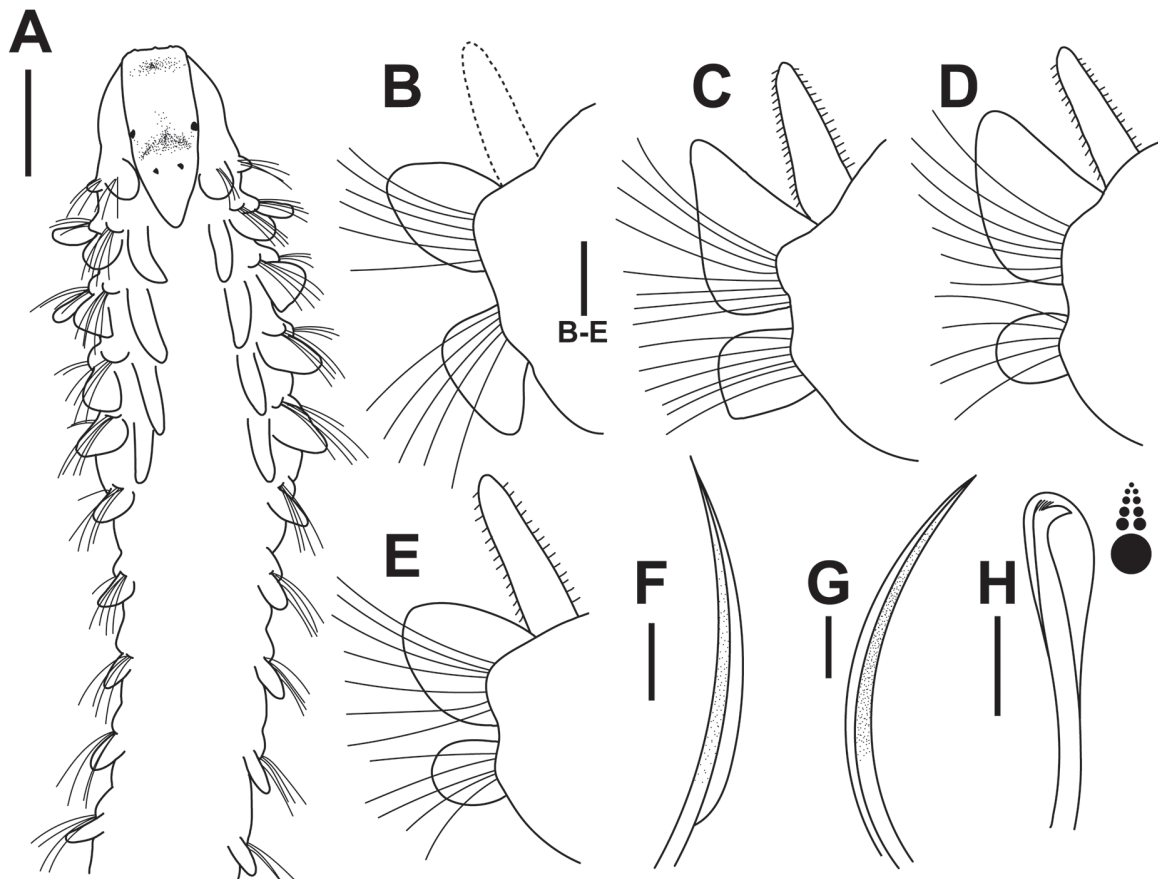
Figs 2–4

### Diagnostic features

Prostomium with orangish-brown pigmentations, anteriormost body conspicuously expanded (Fig. 3C–D), four pairs of short, apinnate, and cirriform branchiae, dorsal crests and ventral flaps absent.

### Etymology

The specific epithet '*expansa*' refers to the conspicuously expanded body in the anteriormost chaetigers of the new species.



**Fig. 2.** Drawing of *Prionospio expansa* sp. nov., paratype (NIBRIV0000900992). **A.** Anterior body without palps. **B–E.** Parapodium from chaetigers 2–5, front view. **F.** Unilimbate capillary from first notopodium. **G.** Ventral sabre chaeta from chaetiger 12. **H.** Neuropodial hooded hook from chaetiger 17. Scale bars: A = 0.2 mm; B–E = 0.1 mm; F–H = 20 μm.



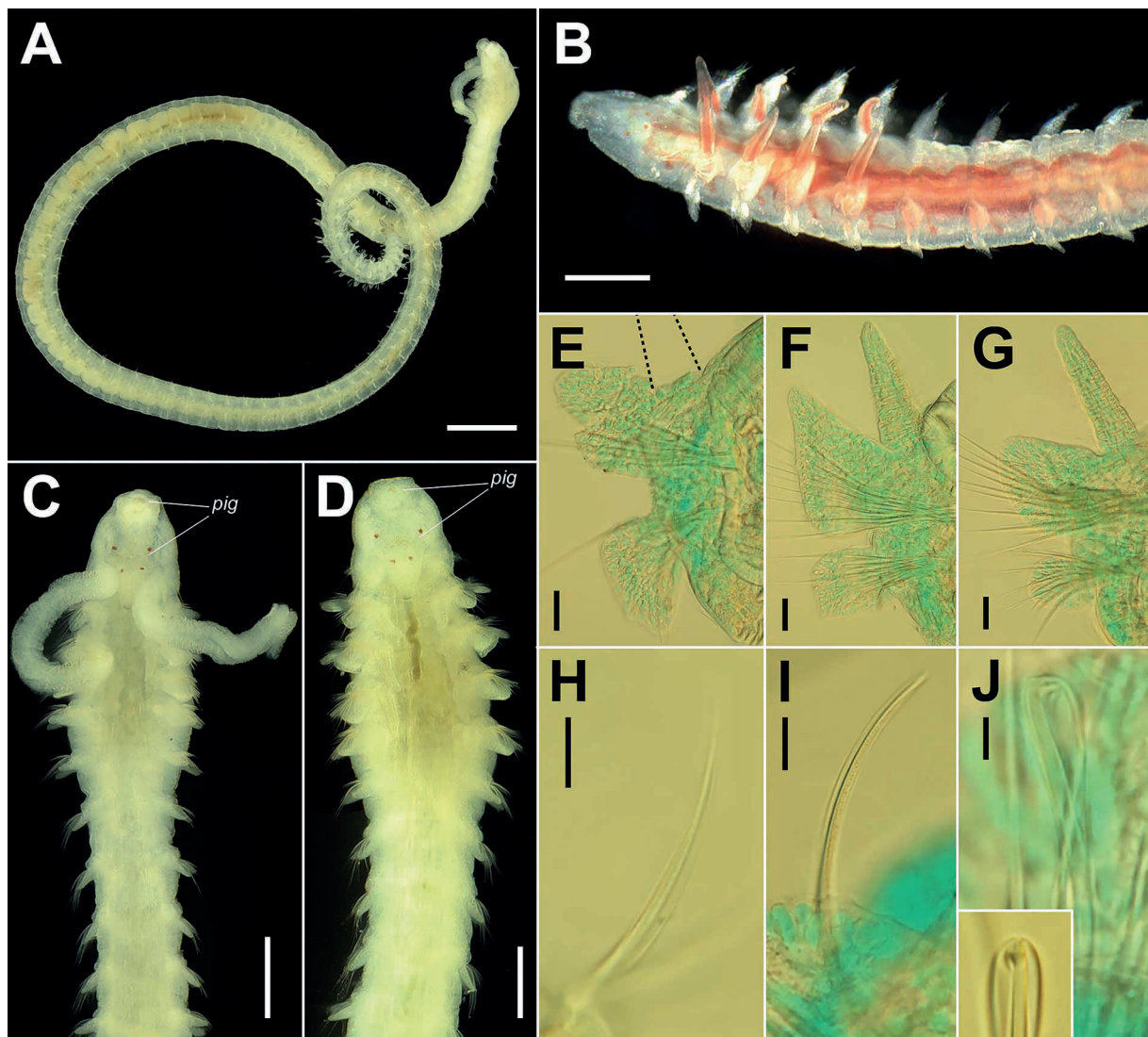
## Material examined

### Holotype

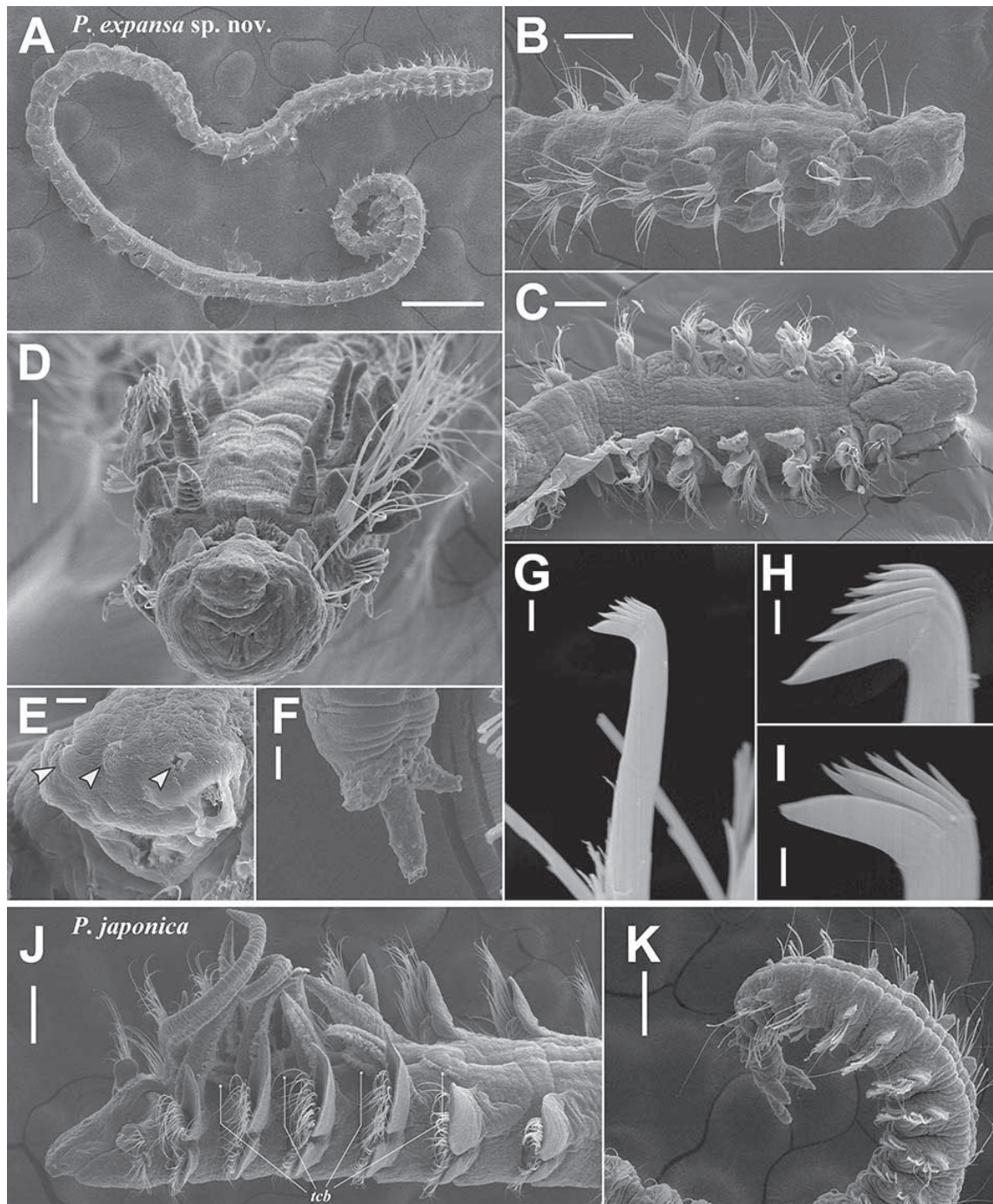
KOREA • 1 complete spec. with palps; Yellow Sea, Yeongjongdo Is., Eurwangni Beach; 37.4472° N, 126.3705° E; 1 Apr. 2022; Geon Hyeok Lee leg.; intertidal, silty sand; NIBRIV0000900991.

### Paratypes

KOREA • 1 complete spec.; Yellow Sea, Jaen Is.; 34.9200° N, 126.0572° E; 24 Sep. 2021; Geon Hyeok Lee leg.; low intertidal, muddy sand; NIBRIV0000900998 • 4 af; Yellow Sea, Deokjeok Is.; 37.2065° N, 126.1743° E; 24 Oct. 2021; Geon Hyeok Lee leg.; low intertidal, muddy sand NIBRIV0000900999



**Fig. 3.** Images of *Prionospio expansa* sp. nov. **A, C.** Holotype (NIBRIV0000900991). **B.** Paratype (NIBRIV0000901000). **D–J.** Paratype (NIBRIV0000900992). **A.** Entire body with palps. **B.** Live specimen, dorsolateral view. **C.** Anterior end with palps, dorsal view. **D.** Anterior end without palps, dorsal view. **E.** Chaetiger 2, front view, branchia missing. **F.** Chaetiger 3, front view. **G.** Chaetiger 5, front view. **H.** Unilimbate capillary of first monopodium. **I.** Sabre chaeta of chaetiger 12. **J.** Lateral view of hooded hook in chaetiger 17, inset indicating front view of hooded hook. Abbreviation: pig = pigmentation. Scale bars: A = 0.5 mm; B–D = 0.2 mm; E–G = 50 µm; H–I = 20 µm; J = 10 µm.



**Fig. 4.** SEM images of two *Prionospio* species from Korean waters. **A–I.** *Prionospio expansa* sp. nov. **A–B, D–F.** Paratype (NIBRIV0000901890). **C, G–I.** Paratype (NIBRIV0000901891). **J–K.** Korean *Prionospio japonica* Okuda, 1935, non-type. **A.** Entire body without palp, dorsal view. **B.** Anterior end with seven chaetigers, dorsolateral view. **C.** Anterior end with eight chaetigers, dorsal view. **D.** Anterior end, front view. **E.** Frontal margin of prostomium, arrows indicating frontal small peaks. **F.** Pygidium with middorsal cirrus and paired ventral lappets. **G.** Hook of middle chaetiger, hood removed, lateral view. **H.** Hook of posterior chaetiger, lateral view. **I.** Hook of posterior chaetiger, ventrolateral view. **J.** Anterior end with seven chaetigers, dorsolateral view. **K.** Pygidium. Abbreviation: tcb = transverse ciliated bands. Scale bars: A = 0.5 mm; B–D = 0.1 mm; E = 10 µm; F = 20 µm; G = 2 µm; H–I = 1 µm; J–K = 0.2 mm.



• 10 complete specs, 9 af, 10 mf, 7 pf; same collection data as for holotype; 1 Apr. 2022; Geon Hyeok Lee leg.; NIBRIV0000901890–1891 (2 complete specs), NIBRIV0000900992–0993 (2 complete specs), other for NIBRIV0000901000 • 1 complete spec.; Yellow Sea, Deokjeok Is.; 37.2165° N, 126.1120° E; 16 Apr. 2022; Geon Hyeok Lee leg.; low intertidal, muddy sand; NIBRIV0000901001 • 7 complete specs, 21 af, 5mf; same collection data as for holotype; 2 May. 2022; NIBRIV0000901002 • 1 af; same collection data as for holotype; 15 Jan. 2021; GenBank COI gene OQ672519, GenBank 16S gene OQ685963, GenBank 18S gene OQ685953; NIBRIV0000900994 • 3 complete specs; same collection data as for holotype; 1 Apr. 2022; GenBank COI gene OQ672520–2522, GenBank 16S gene OQ685964–5966, GenBank 18S gene OQ685954–5956; NIBRIV0000900995–0997.

## Description

Holotype complete with 90 chaetigers, about 0.41 mm wide at chaetiger 4 and about 14.2 mm long. Paratypes complete with 61–105 chaetigers, up to 0.51 mm wide at chaetiger 4 and about up to 15.5 mm long. Body conspicuously expanded dorsoventrally in chaetigers 2–6 (Fig. 3C–D), cylindrical afterwards, tapered towards pygidium (Fig. 4A).

Prostomium subtriangular, with three small peaks on anterior margin (Fig. 4E), extending posteriorly to posterior end of chaetiger 1 as a distinct caruncle; two pairs of reddish and rounded to oval eyes arranged in trapezoid, anterolateral pair larger and wider apart than posterior pair (Figs 2A, 3C–D). Peristomium reduced, fused to chaetiger 1, not forming lateral wings. Palps reaching up to about chaetiger 25 with longitudinal groove lined with fine cilia. Nuchal organs U-shaped, reaching posterior end of chaetiger 1, separated by caruncle (Fig. 4C). Transverse ciliated bands and intersegmental transverse ciliation indiscernible (Fig. 4B–C).

Chaetiger 1 moderately developed, with large, rounded notopodial postchaetal lamellae and small rounded neuropodial postchaetal lamellae; notopodial postchaetal lamellae similar in size to second notopodial postchaetal lamellae; only a few chaetae on both rami; prechaetal lamellae absent (Fig. 4B).

Notopodial postchaetal lamellae foliaceous on chaetigers 2–5, becoming rounded in middle chaetigers, then subtriangular in posterior chaetigers; notopodial postchaetal lamellae largest in chaetigers 3 and 4, then abruptly decreasing in size posteriorly (Fig. 4B–C). Neuropodial postchaetal lamellae foliaceous on chaetiger 2, subrectangular on chaetiger 3, rounded from chaetiger 3; neuropodial postchaetal lamellae largest in chaetigers 2 and 3, then gradually decreasing in size posteriorly. Low and rounded prechaetal lamellae in both rami present in anterior chaetigers, but absent in middle and posterior chaetigers.

Anterior notochaetae all unilimbate capillaries, heavily granulated (Figs 2F, 3H), arranged in three rows; from about chaetiger 16, notochaetae arranged in two rows, then becoming arranged in a bundle posteriorly; anterior neurochaetae unilimbate capillaries rather thin, heavily granulated, arranged in two rows; sheaths of capillaries most broad at first 6–7 chaetigers; granulation disappeared in posterior chaetigers. Hooded hooks in notopodia appearing from chaetigers 28–38 (usually 35–38), numbering 1–2 at first, increasing up to four per fascicle; hooks in neuropodia usually appearing from chaetigers 15–17 (usually 17), numbering 1–2 at first, increasing up to six per fascicle (Fig. 5); hooks multidentate (Figs 2H, 3J, 4G), with three (Fig. 4I) or four (Fig. 4H) pairs arranged in two vertical rows and a smallest uppermost tooth above main fang; hooks in neuropodia accompanied by 1–4 thin, long non-limbate capillaries. Ventral sabre chaetae broadly unilimbate, heavily granulated with sheaths (Figs 2G, 3I), appearing from chaetiger 10.

Dorsal branchiae short, cirriform with rounded tip, four pairs on chaetigers 2–5 (Fig. 4D), first pair sometimes longer than last three pairs (Fig. 3B); first pair usually 1.5–2 × as long as notopodial postchaetal lamellae, up to about 3 × as long as, but not extending over two segments (Figs 2B, 3B);

second and third pairs similar in length or slightly longer than notopodial postchaetal lamellae, usually extending one segment, but slightly over than one segment in large specimens (Figs 2C–D, 3F); last pair distinctly usually  $2 \times$  as long as notopodial postchaetal lamellae; branchiae with heavy ciliation at inner and outer margins (Figs 2E, 3G); branchiae completely free from notopodial postchaetal lamellae.

Dorsal crest, lateral pouches, and ventral flaps absent.

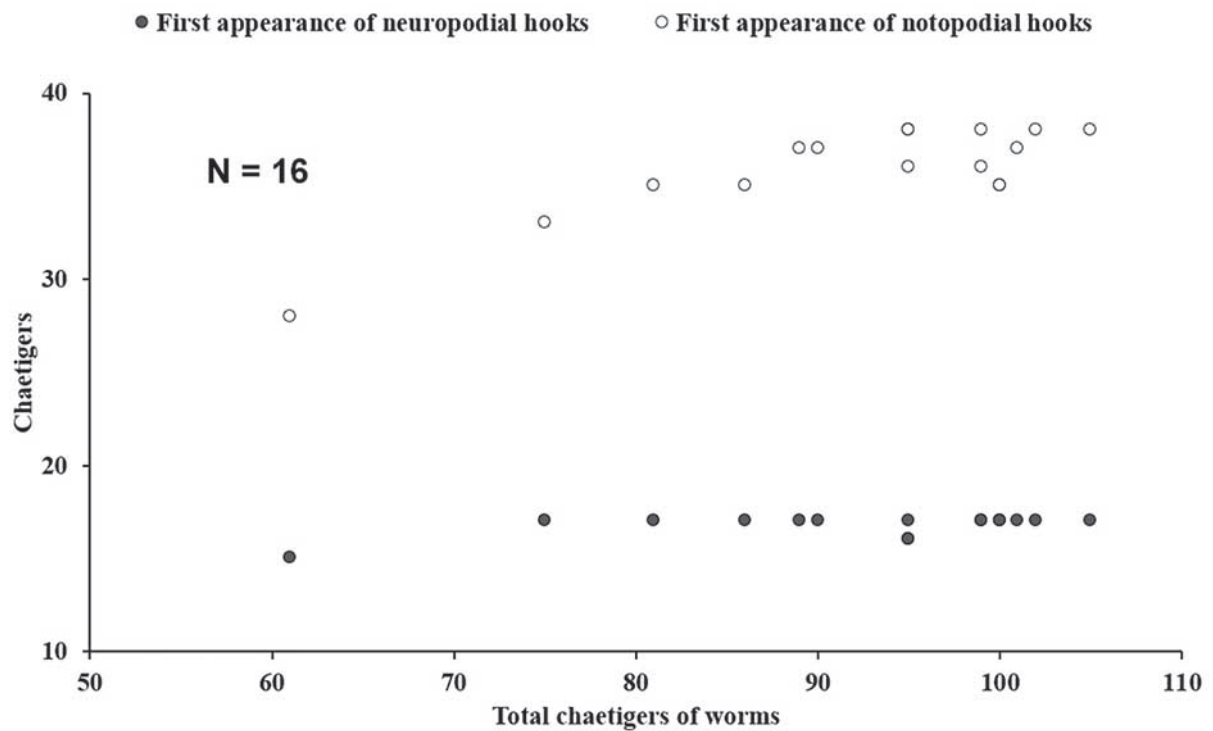
Oocytes unknown.

Pygidium with one elongated, thick middorsal cirrus and one pair of short, thick ventral lappets, all bearing numerous non-motile sensory cirri up to 60  $\mu\text{m}$  long (Fig. 4F).

**COLORATION AND PIGMENTATION.** Whitish color in live specimens with orangish brown pigmentations presented on the anterior part of prostomium, between anterolateral eyes of prostomium (Fig. 3B), and lateral paired ventral lappets of pygidium. In formalin- or ethanol-fixed specimens yellowish white color, pigmentation on prostomium usually remained (Fig. 3C–D), but pigmentation on pygidium usually fades or is completely lost.

#### Methyl green staining pattern (MGSP)

Twelve complete specimens were examined for MGSP. Anterior margin of prostomium, dorsal and lateral sides of peristomium, caruncle, margins of postchaetal lamellae and branchiae, pygidium weakly stained; staining almost faded out in about 2–3 hours and completely disappeared in about 2–3 days. Lateral and ventral sides of chaetigers 8–22 intensely stained, and narrow transverse bands along anterior edges of chaetigers 10–18 most intensely stained; the patterns remained for at least one week and faded out after about a month.



**Fig. 5.** Relationships between the total chaetigers and the first appearances of neuro- and notopodial hooks in *Prionospio expansa* sp. nov.



### Habitat and distribution

Adults of this new species were found in muddy and silty sand in the intertidal zone of the Yellow Sea.

### Genetics

Sequences of three gene fragments (COI, 16S rDNA, and 18S rDNA) were determined from four adult specimens of *Prionospio expansa* sp. nov. The length of obtained DNA sequences were 605 bp for COI, 532 bp for 16S rDNA, and 1,762 bp for 18S rDNA. The newly determined sequences have been registered in GenBank with the accession numbers OQ672519–22 (COI), OQ685963–6 (16S rDNA), and OQ685953–6 (18S rDNA). The intraspecific genetic distances were 0–0.2% in both COI and 16S rDNA, and no variation was detected in 18S rDNA. Based on available gene data of *Prionospio* species from GenBank, the new species is genetically closest to *P. japonica* from Northeast Asia (squares in Fig. 6). The genetic differences between sequences of the new species and those of *P. japonica* from Japan and Korea were 20.7% (97/469 bp, MW054868) in COI, 10.4% (59/469 bp, LC595695) in 16S rDNA, and 0.1% (1/1,619 bp, LC545865) in 18S rDNA.

### Discussion

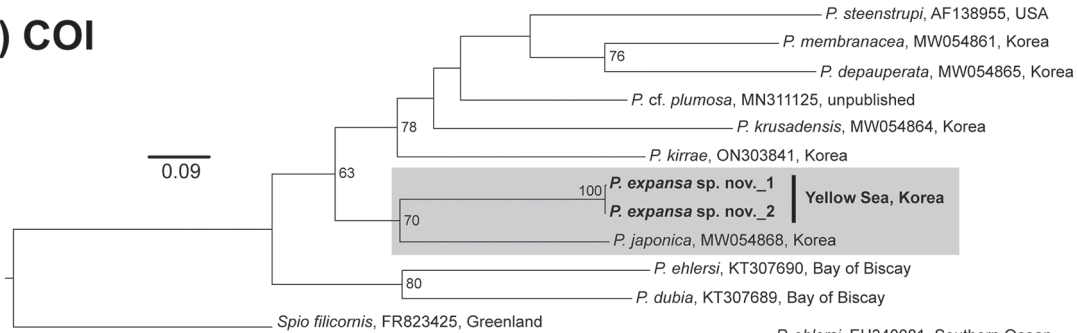
*Prionospio japonica* is one of the commonly found species from Northeast Asian waters (Okuda 1935; Imajima & Hartman 1964; Paik 1989; Imajima 1990a; Zhou & Li 2009; Lee *et al.* 2020). The new species is morphologically similar to *P. japonica* in having four pairs of cirriform and apinnate branchiae, an absence of dorsal crest or flap, and shape of pygidium. However, the new species clearly differs from *P. japonica* by the length of the branchiae on chaetiger 2 (similar or slightly longer than notopodial postchaetal lamellae in new species vs at least 3 × as long as in *P. japonica*) and size of the first notopodial postchaetal lamellae (almost similar in size to second lamellae vs distinctly smaller than second lamellae) (Okuda 1935; Imajima & Hartman 1964; Paik 1989; Imajima 1990a; Zhou & Li 2009; Lee *et al.* 2018). Additionally, the SEM observation showed that the two species differ by presence of transverse ciliated bands (indiscernible in new species vs present in *P. japonica*) (Fig. 4B–C, J).

The new species is also similar to *P. biancoi* Peixoto & Paiva, 2020 from Brazil in having four pairs of short and apinnate branchiae and absence of a dorsal crest or flap (Peixoto & Paiva 2020). However, the new species differs from *P. biancoi* by the shape of the prostomium (subtriangular in the new species vs rectangular in the Brazilian species), shape of branchiae of chaetigers 3–4 (cirriform vs robust and flattened), the first appearance of notopodial hooded hooks (usually 35–38 vs 67), and dentition above the main fang of hooks (7 or 9 vs 10) (Peixoto & Paiva 2020).

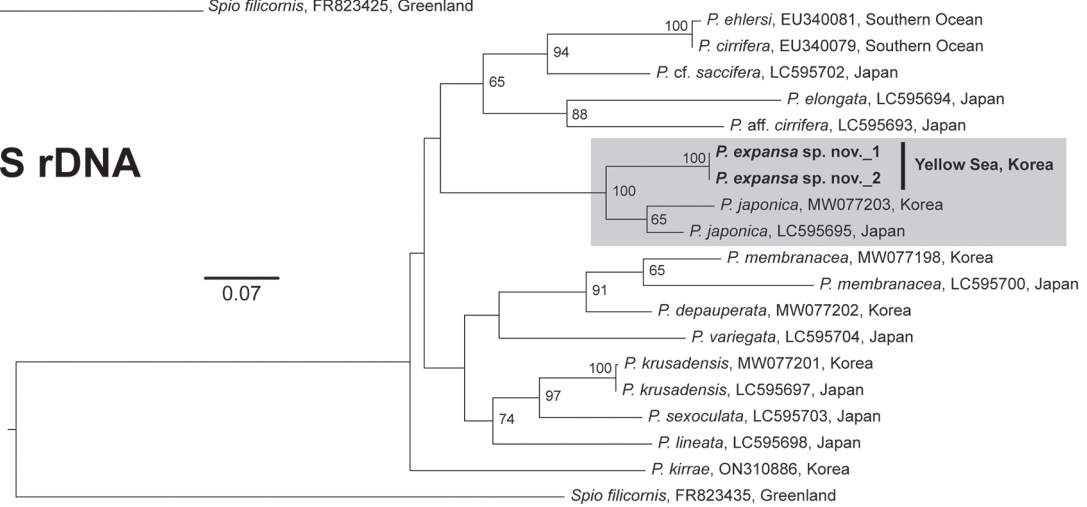
In Northeast Asia, *P. oshimensis* Imajima, 1990 is known to have pigmentation on the prostomium (Imajima 1990b), and the new species shares this feature. The new species is clearly distinguished from this species in the arrangement of branchiae (all apinnate in the new species vs pinnae on the first and fourth branchiae in the Japanese species).

Recently, some molecular data of the genus *Prionospio* from Japan and Korea were provided (e.g., Lee *et al.* 2020; Abe & Sato-Okoshi 2021; Lee & Min 2022). Based on currently available information, three ML trees in this study clearly showed that the new species is closely related to *P. japonica* from Northeast Asia (Fig. 6). Morphologically, they share the same branchiae shape (all apinnate) and arrangement (four pairs). This result could be a first step in understanding the phylogenetic relationships between species of *Prionospio*. However, in the 18S rDNA gene tree (Fig. 6C), the new species and *P. japonica* formed a monophyletic group with *P. kirrae* which has both apinnate and pinnate branchiae. So, it is still difficult to elucidate whether the *Prionospio*-complex can be considered a monophyletic group based on their branchial form and arrangement. Moreover, compared to the high species diversity of *Prionospio*

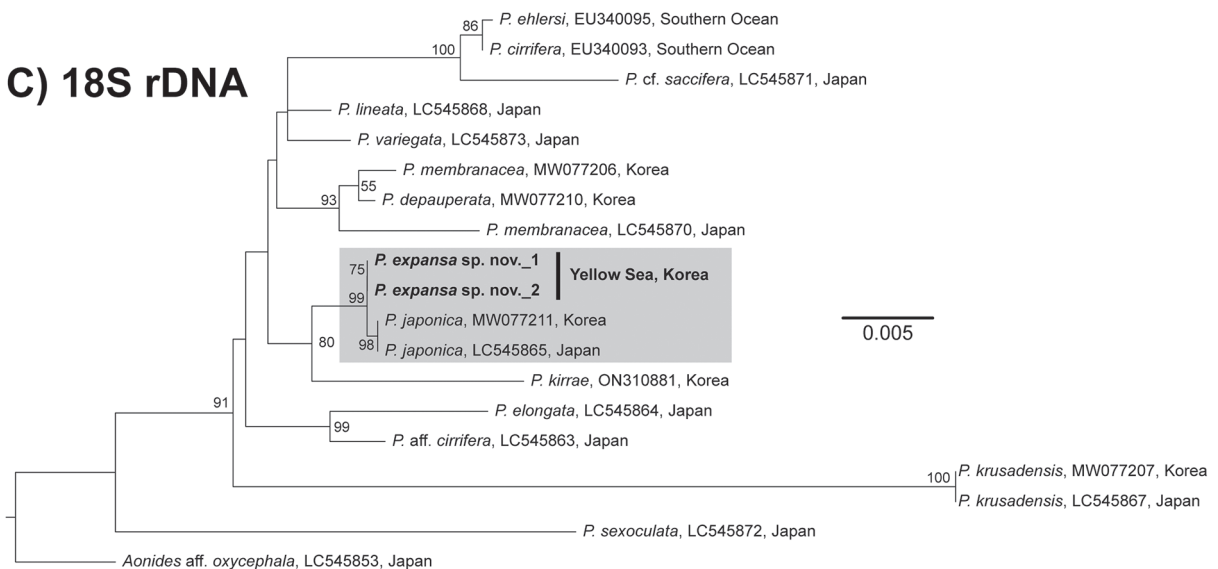
## A) COI



## B) 16S rDNA



## C) 18S rDNA



**Fig. 6.** Maximum likelihood (ML) trees inferred from gene fragments of COI (469 bp), 16S rDNA (470 bp), and 18S rDNA (1,619 bp). The sequences of *Prionospio expansa* sp. nov. obtained in the present study are highlighted in bold. Numbers beside branches indicate ML bootstrap values of  $\geq 50\%$  as a percentage of 1000 bootstrap replications. The sequences of *Spio filicornis* (Müller, 1776) and *Aonides aff. oxycephala* (Sars, 1862) were used as outgroup taxa.

(more than hundred species), the genetic data analyzed in this study were extremely poor. To clarify their phylogenetic relationships, additional molecular and morphological study of the genus is still needed.

## Acknowledgments

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