

# A New Record of *Prionospio depauperata* (Annelida: Polychaeta: Spionidae) with DNA Barcoding Data of Four *Prionospio* Species in South Korea

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## ABSTRACT

In this study, *Prionospio depauperata* Imajima, 1990 is newly reported in Korean fauna. *Prionospio depauperata* can be distinguished from other relatives by the four pairs of branchiae which are pinnate on chaetigers 2 and 5, and apinnate on chaetigers 3 and 4; caruncle extending to the end of chaetiger 2; and moderate dorsal crest present on chaetigers 7–13. The morphological diagnosis of *P. depauperata* are provided with the photographs of four *Prionospio* species. The mitochondrial cytochrome c oxidase subunit 1 (*COI*), 16S ribosomal DNA (16S rDNA), and the nuclear 18S ribosomal DNA (18S rDNA) sequences of four *Prionospio* species from Korean waters, *P. depauperata* Imajima, 1990, *P. japonica* Okuda, 1935, *P. krusadensis* Fauvel, 1929, and *P. membranacea* Imajima, 1990, were determined for the first time. The inter-specific genetic distances among the congeners of four *Prionospio* species were 22.3–29.6% in *COI*, 10.5–25.0% in 16S rDNA, and 0.3–3.6% in 18S rDNA.

**Keywords:** *Prionospio depauperata*, *COI*, 16S rDNA, 18S rDNA, Korea

## INTRODUCTION

The genus *Prionospio* Malmgren, 1867 is one of the largest groups of spionid polychaetes commonly found in the intertidal to deep sea (Dagli and Çinar, 2009). They are characterized by the prostomium broadly rounded to truncate anteriorly (except *P. cerastae* Radashevsky, 2015), and branchiae that are pinnate or apinnate, or combinations of these, present in the anterior region of the body (Radashevsky, 2015). More than 110 *Prionospio* species are known worldwide, and only 10 species have been recorded in Korean waters (Paik, 1975, 1982, 1984, 1989; Jung et al., 1998; Song et al., 2017; Lee et al., 2018).

*Prionospio depauperata* Imajima, 1990 was originally described from Japanese waters by Imajima (1990) and subsequently found from the Turkish Mediterranean coast by Dagli and Çinar (2009). This is the first record of *P. depauperata* in Korean waters and was collected from muddy sand of intertidal zone.

The sequences of mitochondrial cytochrome c oxidase subunit 1 (*COI*), 16S ribosomal DNA (16S rDNA), and the

nuclear 18S ribosomal DNA (18S rDNA) have been used as DNA barcoding markers for the molecular identification of spionid polychaetes (Meißner et al., 2011; Radashevsky and Pankova, 2013). Despite the abundance of *Prionospio* species, its DNA barcoding information is very poor.

In this paper, three DNA barcoding gene regions of four *Prionospio* species from Korean waters, *P. depauperata* Imajima, 1990, *P. japonica* Okuda, 1935, *P. krusadensis* Fauvel, 1929, and *P. membranacea* Imajima, 1990 were determined for the first time.

## MATERIALS AND METHODS

The specimens were collected from intertidal fine to muddy sand using 500 µm mesh sieves. All voucher specimens were deposited at the National Institute of Biological Resources. Morphological observation was carried out in live specimens under a stereo microscope (SZX12; Olympus, Japan) and optical microscope (DM2500; Leica, Germany). The specimens were fixed in 10% formaldehyde for morphological studies

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and in 96% ethanol for genetic studies.

PCR amplification was performed with primers for three gene regions: polyLCO and polyshortCOIR for *COI* (Carr et al., 2011), 16Sar and 16Sbr for 16S rDNA (Kessing et al., 1989), and Uni 18S F and HET 18S R for 18S rDNA (Dzikowski et al., 2004). The newly determined sequences of four *Prionospio* species were registered in the GenBank (Table 1). Pairwise genetic distances were calculated by MEGA X (Kumar et al., 2018) using Kimura two-parameter distance (K2P) (Kimura, 1980) for the sequences of three gene regions.

The pairwise genetic distances were calculated between four *Prionospio* species and their congeners, *Aurospio dibranchiata* Maciolek, 1981, *A. foodbancsia* Mincks, Dyal, Paterson, Smith & Glover, 2009, *Prionospio cirrifera* Wirén, 1883, *P. dubia* Day, 1961, and *P. steenstrupi* Malmgren, 1867, mined from GenBank (Struck et al., 2008; Mincks et al., 2009; Carr et al., 2011). The sequences of *Spio filicornis* (Müller, 1776) are used for outgroup (Meißner et al., 2011).

## RESULTS AND DISCUSSION

Order Spionida Grube, 1850  
Family Spionidae Grube, 1850  
Genus *Prionospio* Malmgren, 1867

### <sup>1</sup>\**Prionospio depauperata* Imajima, 1990

*Prionospio depauperata* Imajima, 1990: 114, figs. 6–7;  
Dagli and Çinar, 2009: 3, fig. 2.

**Material examined.** Korea: 5 inds., incomplete, Gyeong-sangnam-do: Tongyeong-si, Sanyang-eup, Sinjeon-ri, 128°20' 18.8"E, 34°56'45.9"N, 20 Sep 2017, intertidal; 1 ind., incomplete, Jeollanam-do: Yeosu-si, Samsan-myeon, Dongdo-ri, 127°23'12.0"E, 34°03'32.0"N, 6 Jun 2017, intertidal, sand; 1 ind., complete specimen (NIBRIV0000876636), Gyeong-sangnam-do: Tongyeong-si, Yongnam-myeon, Samhwa-ri, 128°26'41.2"E, 34°53'19.9"N, 20 Jul 2020, intertidal, muddy sand.

**Diagnosis.** Color opaque white in alcohol. Prostomium truncate anteriorly, extending posteriorly to end of chaetiger 2 as prominent caruncle. Two pairs of black eyes arranged in trapezoid; one pair of large crescent-shaped and one pair of small rounded eyes. Peristomium fused to chaetiger 1 dorsally, forming lateral wings. Branchiae four pairs on chaetigers 2–5; pinnate on chaetigers 2 and 5 and apinnate on chaetigers 3 and 4. Parapodia of chaetiger 1 with lanceolate notopodial postchaetal lamellae and subrectangular neuropodial lamellae. Notopodial postchaetal lamellae triangular, largest on branchial region; neuropodial postchaetal lamellae small, subtriangular on anterior chaetigers; both rami becoming low and broadly rounded on posterior chaetigers. Moderate dorsal crests present on chaetigers 7–13; largest on chaetiger 7 (arrowed in Fig. 1A). Interparapodial pouches and ventral flaps absent. Capillary unilimbate, arranged in two rows. Hooded hooks multidentate, with 4–5 pairs of thin, long upper teeth arranged in a row above main fang; Hooks in neuropodia present from chaetiger 16 and hooks in notopodia from chaetigers 40–45. Ventral sabre chaetae in neuropodia from chaetiger 10. Pygidium with thin, long cirrus and one pair of short and thick ventral cirri.

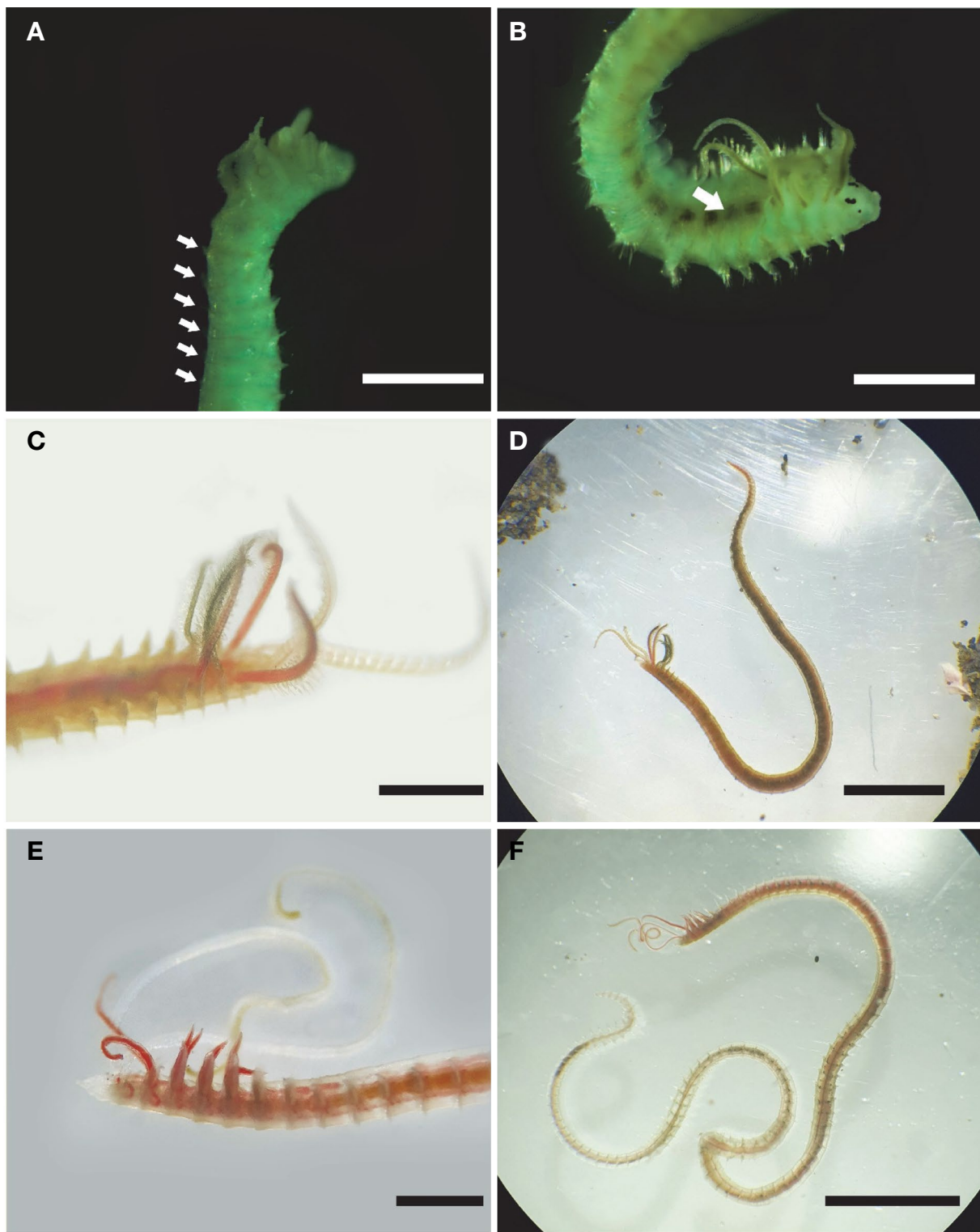
**Remarks.** The Korean specimens of *P. depauperata* are well agreed with the original description based on the following characteristics combined: (1) the four pairs of branchiae which are pinnate on chaetigers 2 and 5, and apinnate on chaetigers 3 and 4; (2) two pairs of distinct eyes; (3) caruncle extending to end of chaetiger 2; and (4) moderate dorsal crest present on chaetigers 7–13 (Fig. 1A). In Korean waters, *P. depauperata* is morphologically similar to *P. membranacea* in having the same arrangement of branchiae, but can be distinguished by the moderate dorsal crest present on chaetigers 7–13 (vs. high dorsal crest present on chaetiger 7 in *P. membranacea*) and caruncle extending to the end of chaetiger 2 (vs. end of chaetiger 1 in *P. membranacea*) (Fig. 1A, B). Genetic distances between two species were 22.3% in *COI*, 10.5% in 16S rDNA, and 0.3% in 18S rDNA (Tables 2–4).

The DNA barcoding sequences of *COI*, 16S rDNA, and 18S rDNA from *P. depauperata* (1 ind., NIBRIV0000876636),

**Table 1.** Sequence data of four *Prionospio* species and their GenBank accession numbers

	<i>P. depauperata</i> (NIBRIV0000876636)	<i>P. japonica</i> (NIBRIV0000876629–31)	<i>P. krusadensis</i> (NIBRIV0000876633–35)	<i>P. membranacea</i> (NIBRIV0000876632)
<i>COI</i>	MW054865	MW054866–68	MW054862–64	MW054861
16S rDNA	MW077202	MW077203–05	MW077199–201	MW077198
18S rDNA	MW077210	MW077211–13	MW077207–09	MW077206

Korean name: <sup>1</sup>\*빈약예쁜얼굴갯지렁이



**Fig. 1.** Korean *Prionospio* species analyzed by this study. A, *Prionospio depauperata* (NIBRIV0000876636) stained with methyl green, dorsal crests (arrowed) from chaetiger 7; B, *Prionospio membranacea* (NIBRIV0000876632) stained with methyl green, dorsal crest (arrowed) on chaetiger 7; C, D, *Prionospio krusadensis* (NIBRIV0000876633), anterior end (C), specimen in sea water (D); E, F, *Prionospio japonica*, anterior end (E), specimen in sea water (F). Scale bars: A, B=0.5 mm, C, E=1.0 mm, D=4.0 mm, F=5.0 mm.

**Table 2.** Pairwise genetic distances based on 549 bp size of *COI* sequences of six spionid polychaetes

No.	Species (GenBank accession No.)	1	2	3	4	5	Data source
1	<i>Prionospio depauperata</i> (MW054865)						Present study
2	<i>P. membranacea</i> (MW054861)	0.223					Present study
3	<i>P. krusadensis</i> (MW054862)	0.239	0.275				Present study
4	<i>P. steenstrupi</i> (HQ024205)	0.274	0.248	0.268			Carr et al. (2011)
5	<i>P. japonica</i> (MW054866)	0.296	0.259	0.282	0.313		Present study
6	<i>Spio filicornis</i> (FR823431)	0.348	0.295	0.324	0.316	0.292	Meißner et al. (2011)

**Table 3.** Pairwise genetic distances based on 510 bp size of 16S rDNA sequences of seven spionid polychaetes

No.	Species (GenBank accession No.)	1	2	3	4	5	6	Data source
1	<i>Prionospio depauperata</i> (MW077202)							Present study
2	<i>P. membranacea</i> (MW077198)	0.105						Present study
3	<i>P. krusadensis</i> (MW077199)	0.182	0.182					Present study
4	<i>Aurospio foodbancsia</i> (EU340076)	0.232	0.242	0.177				Mincks et al. (2009)
5	<i>A. dibranchiata</i> (EU340084)	0.236	0.255	0.202	0.191			Mincks et al. (2009)
6	<i>P. japonica</i> (MW077203)	0.224	0.208	0.228	0.227	0.262		Present study
7	<i>P. cirrifera</i> (EU340079)	0.250	0.258	0.236	0.227	0.190	0.225	Mincks et al. (2009)

**Table 4.** Pairwise genetic distances based on 1,694 bp size of 18S rDNA sequences of seven spionid polychaetes

No.	Species (GenBank accession No.)	1	2	3	4	5	6	Data source
1	<i>Prionospio depauperata</i> (MW077210)							Present study
2	<i>P. membranacea</i> (MW077206)	0.003						Present study
3	<i>P. japonica</i> (MW077211)	0.011	0.012					Present study
4	<i>P. cirrifera</i> (EU340093)	0.014	0.015	0.018				Mincks et al. (2009)
5	<i>P. dubia</i> (EU418859)	0.035	0.036	0.038	0.039			Struck et al. (2008)
6	<i>P. krusadensis</i> (MW077207)	0.036	0.035	0.037	0.042	0.053		Present study
7	<i>Spio filicornis</i> (FR823431)	0.075	0.077	0.078	0.076	0.080	0.093	Meißner et al. (2011)

*P. japonica* (3 inds., NIBRIV0000876629–31), *P. krusadensis* (3 inds., NIBRIV0000876633–35), and *P. membranacea* (1 ind., NIBRIV0000876632), were determined. The intra-specific genetic distances of *P. krusadensis* were 0.3–0.6% in *COI*, 0.0–0.6% in 16S rDNA, and no variation in 18S rDNA. No intra-specific variation was detected in three gene regions among the *P. japonica* specimens. On the contrary, the inter-specific genetic distances among the congeners of *Prionospio* species were 22.3–29.6% in *COI*, 10.5–25.0% in 16S, and 0.3–3.6% in 18S (Tables 2–4).

**Distribution.** Japan (type locality), Korea, Mediterranean Sea.

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## CONFLICTS OF INTEREST

Seong Myeong Yoon and Gi-Sik Min, contributing editors of the *Animal Systematics, Evolution and Diversity*, was not involved in the editorial evaluation or decision to publish this article. The remaining author has declared no conflicts of interest.

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