ASED http://e-ASED.org
Animal Systematics, Evolution and Diversity

Anim. Syst. Evol. Divers. Vol. 39, No. 4: 309-313, October 2023
https://doi.org/10.5635/ASED.2023.39.4.018

Short communication

First Record of the Family Polygordiidae (Annelida: Polychaeta) in Korean Fauna

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ABSTRACT

Polygordiidae is recorded as a new record in the Korean fauna. Twenty-three specimens of *Polygordius pacificus* were collected from the low intertidal zone with coarse sandy bottom on the eastern coasts of Korea. Morphologically, there was no doubt that the specimens are close to the holotype of *P. pacificus*, particularly due to the presence of elongated and longitudinal pygidial glands, and the length to width ratio. The prostomium of the Korean specimens have a rounded shape that is similar to subsequent research using scanning electron microscope. The DNA sequence comparison of the mitochondrial cytochrome *c* oxidase subunit I (*COI*) between Korean and Japanese population supports the identification of present specimens.

Keywords: Archiannelida, knot worm, DNA barcode, meiofauna, interstitial

INTRODUCTION

The family Polygordiidae Czerniavsky, 1881 is an interstitial polychaete group distributed the Antarctica and the Atlantic, Indian, and Pacific Oceans (Ramey-Balci et al., 2018, 2020; Tustison et al., 2020). Polygordiid worms are called "Knot Worms" because they usually tie themselves in knots upon collection (Tustison et al., 2020). They are often misunderstood as Nematoda or Nemertea, due to the lack of chaetae, parapodia, and external signs of segmentation (Tustison et al., 2020). In addition, Perrier (1875) thought Polygordiidae should be considered a new type of worm for the following reasons: (1) it is not an annelid because of the absence of chaetae, (2) it is not a nemertean because of the absence of integumentary cilia and the presence of septation, and (3) it is not a nematode because no characters are similar. However, M'Intosh (1875) noted a remarkable similarity to annelids in the presence of septation. Additionally, he pointed out that Polygordiidae is morphologically similar to the annelid Ophellidae in the lacking external signs of segmentation and lacking the chaetae in some species (M'Intosh, 1875). In recent molecular phylogeny research, Polygordiidae are nested within Protodriliformia clade with Saccocirridae, Protodrilidae, and Protodriloidae (Worsaae et al., 2021).

To date, 17 species in a single genus, *Polygordius* Schneider, 1868, have been recorded from Pacific Ocean, Indian Ocean, Mediterranean Sea, Black Sea, Atlantic Ocean, and Southern Ocean (Ramey-Balci et al., 2020). Polygordiidae have been found in sedimentary environments from the intertidal to subtidal zone (Ramey-Balci et al., 2018). Among them, two species, *P. ijimai* Izuka, 1903 and *P. pacificus* Uchida, 1935, were found in the East Asian waters of Japan (Izuka, 1903; Uchida, 1935). In this study, we report the first occurrence of *P. pacificus* in Korean waters using morphological diagnosis and molecular data.

Specimens were collected from Gangneung-si, and Donghae-si on the eastern coast of Korea and Ulleungdo Island. The collection sites consisted of rock and sand. Sand was collected around rocks at a depth of 30 cm. In the laboratory, 10 spoons of sand were placed in an Erlenmeyer flask, and an anesthetic such as 40–50‰ magnesium chloride solution was added. According to the method described by Pfannkuche and Thiel (1988), an Erlenmeyer flask was inverted up and down to sufficiently anesthetize the organisms. The supernatants were passed through a 63 μm mesh and washed

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eISSN 2234-8190

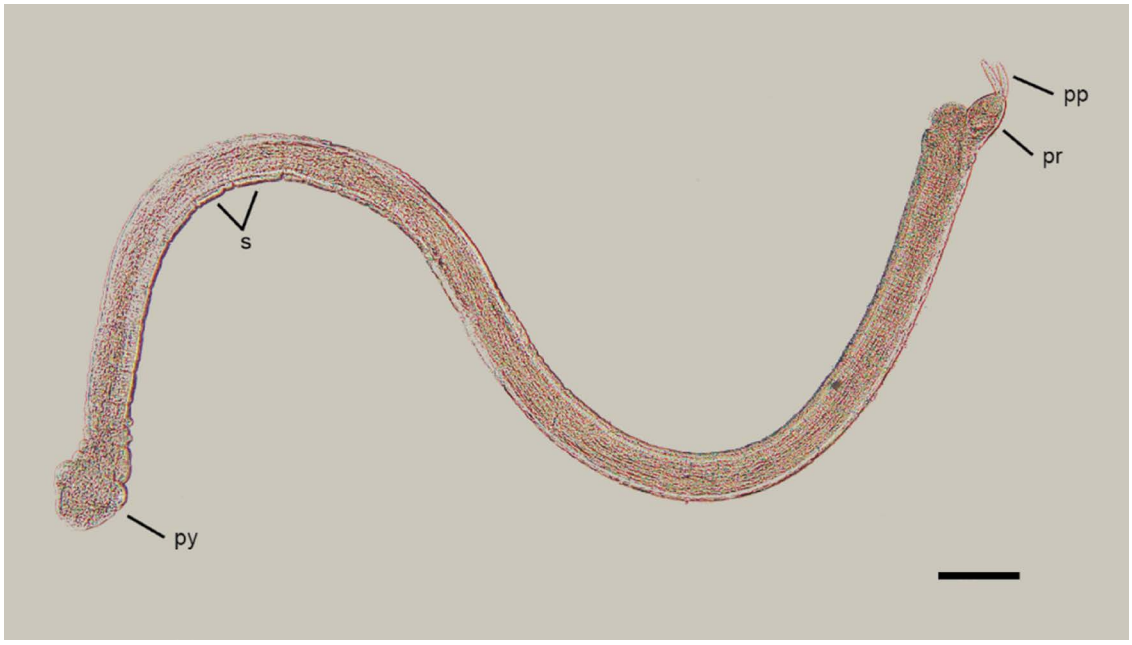

Fig. 1. Light microscopy images of *Polygordius pacificus*. pp, palp; pr, prostomium; py, pygidium; s, segments. Scale bar = 200 μm.

with seawater in a Petri dish. Specimens were extracted under a stereomicroscope and fixed using absolute ethanol for molecular analysis and 10% formaldehyde for morphological observation. Twenty-three individuals were mounted in anhydrous glycerine between two coverslips on a double-sided plate for morphological observation. The slide-mounted specimens were observed and photographed using a BX53 research microscope (Olympus, Tokyo, Japan) equipped with a differential interference contrast system. Measurements were performed using CellSens software (Olympus). Genomic DNA from three specimens were extracted using a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) for molecular analyses. Cytochrome *c* oxidase subunit I (*COI*) gene was amplified using PolyLCO and PolyHCO primers (Carr et al., 2011). Thermal cycling conditions were as follows: initial denature step at 94°C for 2 min, denaturation at 94°C for 1 min, annealing at 46–50°C for 1 min, extension at 72°C for 2 min, and final extension at 72°C for 7 min. Denaturation, annealing, and extension steps were performed 35 times. Sequences were aligned using Geneious Prime 2020.1 (Biomatters, Auckland, New Zealand). All species of Polygordiidae registered in GenBank were mined for comparison with the Korean *Polygordius* specimens. Pairwise genetic distances were calculated using the Kimura two-parameter (K2P) distance method (Kimura, 1980).

SYSTEMATIC ACCOUNTS

Phylum Annelida
Class Polychaeta Grube, 1850
[1]*Family Polygordiidae Czerniavsky, 1881

[2]*Genus *Polygordius* Schneider, 1868

Diagnosis. Body elongated and slender with invisible numerous segments resembling nematodes. Head with small rounded or conical prostomium. Prostomium with a pair of palps. Pigmented eyes absent. Head fold deep or shallow. Parapodia and chaetae absent. Pygidium inflated or not; glands present or absent; cirri present or absent.

[3]****Polygordius pacificus*** **Uchida, 1935 (Tables 1, 2, Fig. 1)**

Polygordius pacificus Uchida, 1935: 119, fig. 1.

Material examined. Korea: 8 inds., Gangwon-do: Gangneung-si, Gangmun Beach, 37°47′53.2″N, 128°55′00.6″E, 16 Sep 2017, low intertidal coarse sand; 1 ind., Gangneung-si, Gangmun Beach, 37°47′53.2″N, 128°55′00.6″E, 2 May 2019, low intertidal coarse sand; 11 inds., Donghae-si, Gase Beach, 37°31′46″N, 129°07′15″E, 21 Jul 2018, low intertidal coarse sand; 1 ind. (NIBRIV0000903038), Gyeongsangbuk-do: Ulleung-gun, Hyunpo-ri, 37°31′37.6″N, 130°49′48.8″E, 13

Korean name: [1]*매듭갯지렁이과(신칭), [2]*매듭갯지렁이속(신칭), [3]*태평양매듭갯지렁이(신칭)

Table 1. Comparison of characters among species of the *Polygordius* from East Asia

	P. ijimai	*P. pacificus*	*P. pacificus* (redescription in Tustison et al., 2020)	*P. pacificus* from Korea
Collecting locality	Misaki Mar. Biol. Lab., Japan (Type locality)	Misaki Mar. Biol. Lab., Japan (Type locality)	Seto Mar. Biol. Lab., Japan	Gangneung; Donghae; Ulleungdo
Habitat	Intertidal	Intertidal	Intertidal	Intertidal
Sediment type	Sand, pebbles	Fine sand	Pebbles	Coarse sand
Body length (mm)	70–77	30–35	18.15–20.75	3.6–18.5
Body width (mm)	0.6–0.8	0.4–0.5	0.25–0.46	0.10–0.28
Segment	No data	No data	90–108	>61
Palp length (mm)	1	No data	0.18–0.23	0.08–0.25
Prostomium shape	No data	Conical	Rounded	Rounded
Prostomium length	No data	No data	0.09–0.11	0.09–0.14
Ratio (palp : prostomium)	No data	No data	~2 : 1	1 : 1
Head fold	No data	No data	Deep	Deep
Pygidium shape	Inflated	Inflated	Inflated	Inflated
Pygidium width	No data	No data	0.35–0.38	0.1
Pygidial glands	No data	No data	44–75	45
Pygidial gland shape/orientation	Elongate/no data	Elongate/longitudinal	Elongate/longitudinal	Elongate/longitudinal
Glandular belt (width) (μm)	No data	150	100	72
Gland width (μm)	No data	No data	10–15	13
Gland ratio (length : width)	No data	5 : 1	7–10 : 1	5.5 : 1

Jul 2021, low intertidal coarse sand; 2 inds., Ulleung-gun, Seo-myeon, 37°27′37″N, 130°51′23″E, 13 Jul 2021, low intertidal coarse sand. All specimens except one from Hyun-po-ri, Ulleung-gun, are stored in the Ecological Genetics Laboratory, Ewha Womans University.

Description. Body long and slender, 3.6–18.5 mm long, 0.1–0.28 mm wide with numerous invisible segments (Fig. 1). Head with rounded prostomium (0.09–0.14 mm long), oval nuchal organs, mouth, and paired antennae (0.08–0.25 mm long). Eyes absent. Ratio of antenna to prostomium 1 : 1. Head fold deep. Pygidium inflated, 0.1 mm wide. Pygidial glands present, elongate and longitudinal, 13 μm wide; glandular belt 72 μm wide; gland ratio of length to width 5.5 : 1. Pygidial cirri absent (Table 1).

Molecular information. Maximum intra-specific variation in terms of genetic divergence was 1.6%, which was observed between Gangneung (OQ719755) and Ulleungdo (OQ719756) specimens. When comparing the Japanese specimens, there is a maximum genetic variation of 1.5% and a minimum of 1.0%. Minimum inter-specific genetic divergence was 19.2% between *P. pacificus* and *P. neapolitanus* (Table 2).

Remarks. In this study, Polygordiidae is reported for the first time in Korean waters. In East Asia, only two species have been recorded to date, and both are known to be distributed in Japan: *P. pacificus* Uchida, 1935 and *P. ijimai* Izuka, 1903. According to each original descriptions, both species were found in the same collection site: Misaki Marine Biological Station of the University of Tokyo, Miura, Kanagawa, Japan. The most significant morphological difference between two species in the presence/absence of pygidial cirri. All material examined in this study lacked pygidial cirri as *P. pacificus*. The morphological features of Korean specimens are found to be identical to original description (Uchida, 1935): (1) ratio of antenna to prostomium (1 : 1), (2) presence of pygidial glands, (3) absence of pygidial cirri, and (4) gland ratio of length to width (5 : 1). Although the original description of this species indicated that the shape of the prostomium was conical, a redescription by Tustison et al. (2020) based on scanning electron microscope information indicated that the prostomium had a rounded shape. Similarly, the prostomium of the Korean specimens have a rounded shape that was longer in width than in length. In DNA barcoding analysis (Table 2), a comparison with the *COI* molecular data obtained by Tustison et al. (2020) for *P. pacificus* in Japan revealed high similarity with *P. pacificus* from Korea. Among the sequences reported to date, it shows a genetic difference of 19.2–19.4% from *P. neapolitanus* (Italy), 19.4–19.8% from *P. eschaturus* (Brazil), and more than 20% from other *Polygordius* species.

Habitat. Low intertidal zone, coarse sand.

Distribution. Japan and Korea (Gangneung; Donghae; Ulleungdo).

Table 2. Genetic distances (*p*-distance) and accession number of GenBank

No.	Species	Genbank accession No.	1	2	3	4	5	6	7	8	9	10	11	12	Data source
1	*P. pacificus*	OQ719755													Present study
2	*P. pacificus*	OQ719756	0.016												Present study
3	*P. pacificus*	OQ719757	0.011	0.008											Present study
4	*P. pacificus*	MT263741	0.011	0.015	0.010										Tustison et al. (2020)
5	*P. jouinae*	MG603405	0.232	0.228	0.228	0.233									Ramey-Balci et al. (2018)
6	*P. kurthcarolae*	MT263735	0.227	0.223	0.223	0.220	0.241								Tustison et al. (2020)
7	*P. erikae*	MT263740	0.213	0.210	0.213	0.209	0.228	0.222							Tustison et al. (2020)
8	*P. jenniferae*	MT263733	0.220	0.216	0.220	0.218	0.213	0.203	0.195						Tustison et al. (2020)
9	*P. eschaturus*	MG603404	0.197	0.199	0.199	0.194	0.218	0.255	0.207	0.230					Ramey-Balci et al. (2018)
10	*P. appendiculatus*	KF808170	0.202	0.200	0.199	0.195	0.210	0.232	0.218	0.198	0.205				Aylagas et al. (2014)
11	*P. lacteus*	MG603411	0.218	0.212	0.213	0.212	0.228	0.238	0.236	0.206	0.223	0.177			Ramey-Balci et al. (2018)
12	*P. neapolitanus*	MG603434	0.194	0.192	0.194	0.192	0.250	0.241	0.233	0.225	0.215	0.199	0.146		Ramey-Balci et al. (2018)
13	*Pharyngocirrus uchidai*	LC440427	0.246	0.244	0.242	0.244	0.265	0.256	0.277	0.278	0.273	0.265	0.268	0.254	Park et al. (2019)

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CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGMENTS

This work was supported by grants from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR 202231206, NIBR202203106).

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Received July 27, 2023
Revised September 25, 2023
Accepted October 27, 2023