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The First Record of *Leocratides kimuraorum* (Annelida, Hesionidae) from Korea, with DNA Barcode Data

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ABSTRACT

A hesionid species, *Leocratides kimuraorum* Jimi, Tanaka and Kajihara, 2017 is newly reported from the sublittoral zones (100 m depth) of the Korean coasts. This species is characterized by lateral antennae as long as the palps, peristomial membrane without papillose, peristomial dorsolateral tubercles with two round marginal lobes, and pharyngeal with terminal papillae. The intra-specific genetic distance among the cytochrome *c* oxidase subunit I (*COI*) sequences of *L. kimuraorum* specimens from Japan (type locality) and Korea (this study) was in the range of 0.002–0.005. The inter-specific genetic distance between *L. kimuraorum* and other hesionid species were 0.166–0.307. The present study is the first record of *Leocratides* species in Korean fauna. This paper also provides a morphological description and photographs of *L. kimuraorum*, with partial sequences of the mitochondrial *COI* based on Korean specimens.

Keywords: *COI*, Korean waters, *Leocratides kimuraorum*, Polychaeta, taxonomy

INTRODUCTION

The members of the genus *Leocratides* Ehlers, 1908, belonging to family Hesionidae Grube, 1850, generally found in hexactinellid sponges collected from sublittoral zone (Jimi et al., 2017b; Salazar-Vallejo, 2020). They are characterized by having 16 chaetigers, eight pairs of tentacular cirri, bi-articulated palps, a median antenna, facial tubercle, and sesquiramous parapodia (with dorsal cirri but without notochaetae) (Rizzo and Salazar-Vallejo, 2014; Salazar-Vallejo, 2020).

Leocratides is a small group containing four valid species, *L. ehlersi* (Horst, 1921), *L. filamentosus* Ehlers, 1908, *L. jimii* Salazar-Vallejo, 2020, and *L. kimuraorum* Jimi, Tanaka and Kajihara, 2017 (WoRMS, 2021). This genus was established by Ehlers (1908) with *L. filamentosus* collected from Indonesian waters. In taxonomy of *Leocratides*, the terminal papillae on pharynx and a papillose peristomial membrane had been recognized as invalid (Ehlers, 1908; Horst, 1921; Augener, 1926; Pettibone, 1970). However, Pleijel (1998) suggested that they are key characters distinguishable the species because he found significant difference of these characteristics in formerly synonymized species, and *L. kimuraorum* of the present study was recorded as a valid species of *Leocratides* based on the presence of pharyngeal terminal papillae and absence of papillose peristomial membrane from Japanese waters (Jimi et al., 2017b).

In Korean waters, four hesionid species, *Hesione reticulata* Marenzeller, 1879, *Hesiospina aurantiaca* (M. Sars, 1862), *Micropodarke dubia* (Hessle, 1925) and *Oxydromus pugettensis* (Johnson, 1901), have been reported (Paik, 1989), but no *Leocratides* species have been reported to data. In this study, we provide a detailed description and images of *L. kimuraorum*, new to Korean fauna, with DNA barcode data on this species.

MATERIALS AND METHODS

Sampling and morphological observations

Specimens were collected from the benthos of the sublittoral (100 m depth), fixed with 95% ethyl alcohol for both morphological and genetic analyses. Morphological observation

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Table 1. List of hesionid species used in molecular analysis and references

Species	Type locality	Collection locality	GenBank accession No.	Voucher No.	Reference
Hesione reticulata	East coast of Enoshima Island, Kanagawa, Japan	Zaimokuza, Japan	LC169753	NSMT Pol N-620	Jimi et al. (2017a)
Hesiospina aurantiaca	Norwey	Croatia	JF317197	SMNH-113855	Pleijel et al. (2012)
Leocrates chinensis	China Sea, Hong kong	Lifou, New Caledonia	DQ442565	SMNH 83510	Ruta et al. (2007)
Leocratides filamentosus	Indonesia	Sagami Bay, Japan	LC258083	NSMT-Pol 113220	Jimi et al. (2017b)
Leocratides kimuraorum	off Shima Peninsula, Japan	off Shima Peninsula, Japan	LC258082	NMST-Pol H-622	Jimi et al. (2017b)
Leocratides kimuraorum	off Shima Peninsula, Japan	Off Kii Peninsula	LC480519	–	Goto et al. (2019)
Leocratides kimuraorum	off Shima Peninsula, Japan	South Korea	MZ311581	MABIKNA00156552	Present study
Leocratides kimuraorum	off Shima Peninsula, Japan	South Korea	MZ311582	MABIKNA00156553	Present study
Leocratides kimuraorum	off Shima Peninsula, Japan	South Korea	MZ311583	MABIKNA00156554	Present study

was carried out with appendages dissected in a petri-dish using a pair of dissection forceps and surgical knives under a stereomicroscope (Carl Zeiss Axioskop II, Göttingen, Germany). The dissected materials were mounted onto temporary slides using glycerol or permanent slides using polyvinyl lactophenol solution. Photographs were captured by an image system, LAS V4.7 (Leica Microsystems, Heerbrugg, Switzerland). For scanning electron microscopy (SEM) the materials were dehydrated by t-butyl alcohol freeze dryer, VFD-21S (Vacuum Device, Ibaraki, Japan). They were mounted on stubs and coated with gold-palladium. Observation was carried out using a scanning electron microscope, SU3500 (Hitachi, Tokyo, Japan). The voucher specimens were deposited in the National Marine Biodiversity Institute of Korea under specimen numbers, MABIK NA00156552–00156555.

Molecular analysis

Genomic DNA (gDNA) of three specimens were extracted from the tissue of muscle in posterior segments using a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacture's protocol. Amplifications of partial sequences of the mitochondrial cytochrome *c* oxidase subunit I (*COI*) were carried out by polymerase chain reaction (PCR) method using a set of primers: LCO 1490 5′-GGTCAACAAATCATAAAGATATTGG-3′ and HCO 2198 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′ in *COI* amplification (Folmer et al., 1994). PCR amplification was conducted in a total volume of 20 μL: 10 μL of 2× DyeMIX-Tenuto (Enzynomics, Daejeon, Korea), 0.5 μL of each primer, 1 μL of gDNA, and 8 μL of sterile water. Hotstart-PCR was carried out according to the following cycling program: 94°C for 5 min, 94°C for 1 min, 40°C for 1 min and 72°C for 1 min, followed by 35 cycles, and final extension at 72°C for 7 min. The PCR products were purified with a QIAquick PCR Purification Kit (Qiagen, Chatsworth, CA, USA). Sequences for the PCR products were obtained by an Applied Biosystems 3730*xl* DNA sequencer and deposited in National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/) under GenBank accession numbers, MZ311581–MZ311583. The newly obtained *COI* sequences of *L. kimuraorum* were aligned with those of other hesionid species (Table 1) using Geneious Pro v.9.1.8 (Biomatters, Auckland, New Zealand). The genetic distances were calculated using the Kimura-2-parameter model in MEGA X software (Kumar et al., 2018).

SYSTEMATIC ACCOUNTS

Order Phyllodocida Dales, 1962
Family Hesionidae Grube, 1850
[1*]Genus *Leocratides* Ehlers, 1908

[2*]*Leocratides kimuraorum* Jimi, Tanaka and Kajihara, 2017 (Figs. 1, 2)

Korean name: [1*]긴수염갯지렁이속 (신칭), [2*]두돌기긴수염갯지렁이 (신칭)

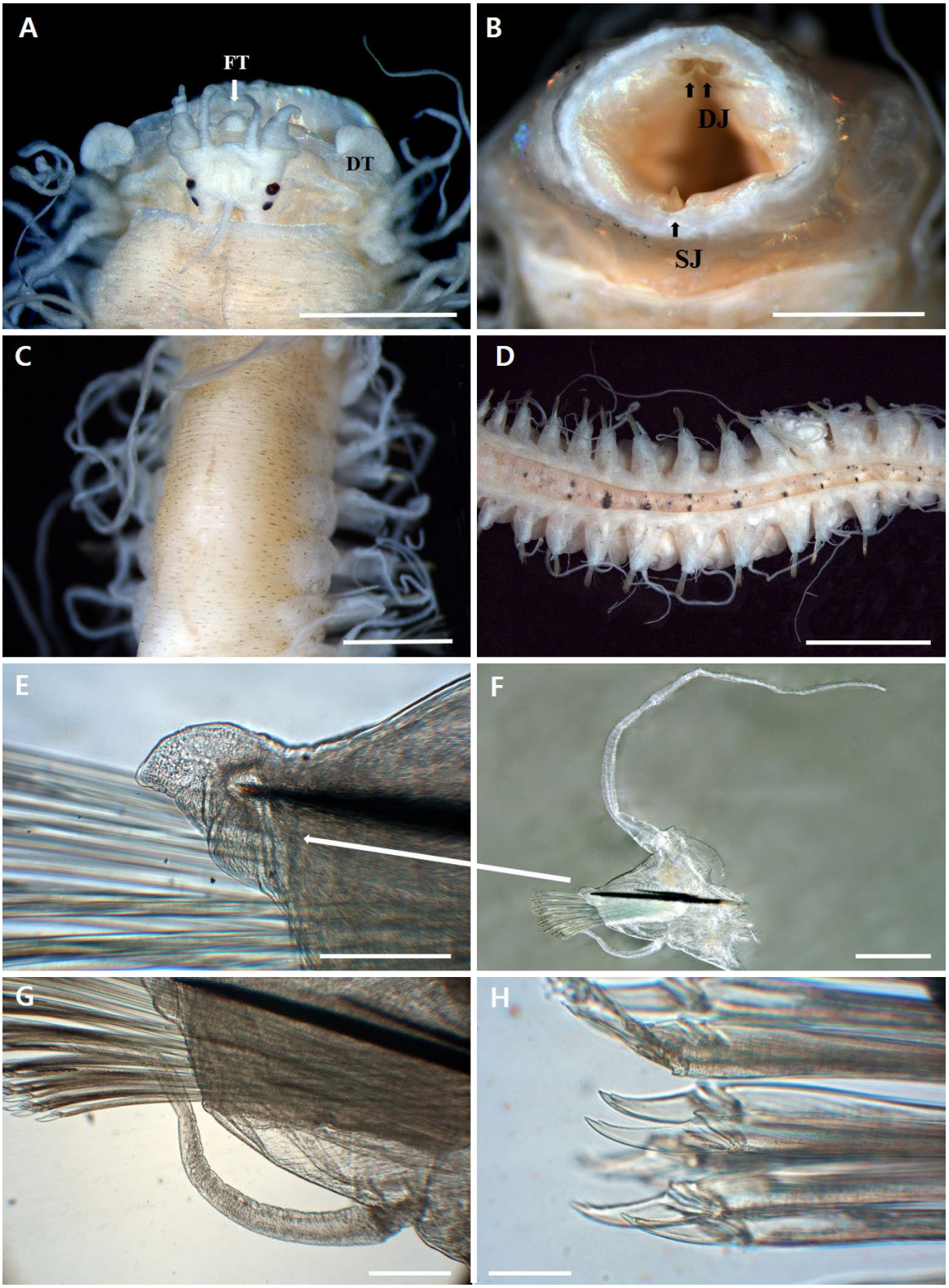

Fig. 1. Light microscopy images of *Leocratides kimuraorum* Jimi, Tanaka and Kajihara, 2017: A, Anterior end, dorsal view; B, Anterior end, ventral view; C, Integument and lateral cushions, Dorsal view; D, Ventral view; E, Acicular lobe (left parapodia of chaetiger 5); F, Parapodium (left parapodia of chaetiger 5); G, Ventral cirrus (left parapodia of chaetiger 5); H, Blade of neurochaeta (left parapodia of chaetiger 5). A, E–G, MABIKNA00156552; C, H, MABIKNA00156553; B, D, MABIKNA00156554. DJ, double jaw; DT, dorsolateral tubercles; FT, facial tubercle; SJ, single jaw. Scale bars: A–D = 2.0 mm, E, G = 300 μm, F = 1.0 mm, H = 50 μm.

Leocratides kimuraorum Jimi, Tanaka and Kajihara, 2017: 134–137, figs. 1–2.

Material examined. South Korea: four individuals (MABI KNA00156552–00156555), 33°35′26.43″N, 127°24′53.45″E, 6 May 2019; all specimens were collected using the benthic trawl mounted on RV Tamgu 22 of National Institute of Fisheries Science (NIFS) from Korea.

Description. Body complete, cylindrical, tapered posteriorly, with 16 chaetigers. Dorsum with numerous, thin, transverse discontinuous brown lines, extended into lateral cushions, decreasing in size laterally (Fig. 1A, C); ventrally, black spots of different size and shape arranged along mid-ventral groove, two larger spots better developed along chaetigers 4–16 (Fig. 1D).

Prostomium, slightly wider than long, square in shape from dorsal view (Figs. 1A, 2A). Lateral antennae tapered, smooth, on anterior edge of prostomium, as long as palps. Median antenna cirriform, smooth, tapered, inserted in central part of prostomium, extended beyond anterior prostomial margin, slightly longer than lateral antenna. Palps bi-articulated, palpophore slightly longer and markedly wider than palpostyle, bent laterally, external to antennae (Figs. 1A, 2A).

Eyes brownish, two pairs; anterior pair slightly larger and more separated than posterior one (Fig. 1A).

Facial tubercle present mid-ventrally on prostomium; Peristomial dorsolateral tubercles present between palps and tentacular cirri on each side (Figs. 1A, 2A), with two round marginal lobes, wider than prostomium; papillose peristomial membrane absent.

Pharynx exposed, about 20 low round terminal papillae present; Upper jaw double, lower jaw single (Figs. 1B, 2B).

Tentacular cirri eight pairs, long, thick; longest one reaching chaetiger 10.

Parapodia uniform; with chaetal lobes tapered, truncate, as long as wide (Fig. 1F); dorsal cirri with cirrophores, latter being cylindrical, smooth, slightly longer than wide (Fig. 1F); cirrostyle very long, whip-like, smooth basally, annulated medially and distally, longer than body width including parapodia. Ventral cirri basally smooth, rugose medially and distally, surpassing chaetal lobe, reaching up to half length of neurochaetal bundle (Fig. 1F, G).

Neuropodial acicula black, tapered; acicular lobe single, thick, digitate, tapered into a small mucro (Fig. 1E). Neurochaetae about 20 per bundle; bidentate, blades about 5 times longer than wide (Figs. 1H, 2C).

Pygidium smooth, with paired cirri; anus located dorso-terminally.

Remarks. *Leocratides kimuraorum*, originally described from the Pacific coast of middle Honshu in Japan (Jimi et al., 2017b), is characterized by a combination of following features: (1) the length of the lateral antennae is as long as the palps; (2) the papillose peristomial membrane is absent;

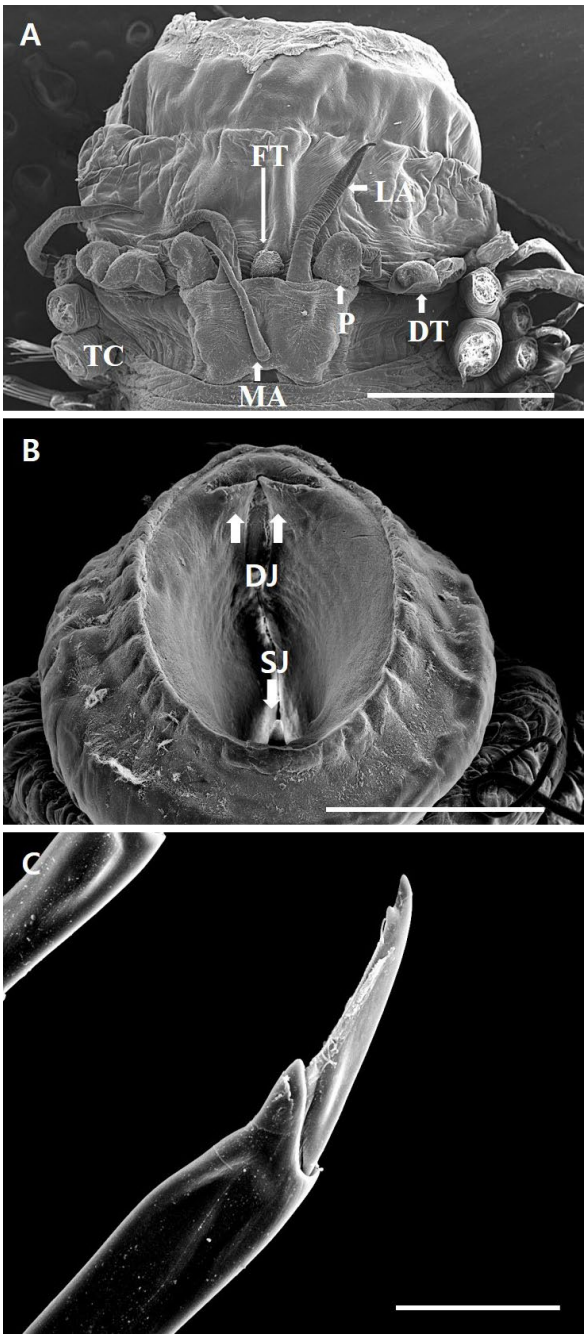

Fig. 2. Scanning electron microscopy images of *Leocratides kimuraorum*: A, Anterior end, dorsal view; B, Anterior end, ventral view; C, Blade of neurochaeta (left parapodia of chaetiger 4). A–C, MABIKNA00156555. DJ, double jaw; DT, dorsolateral tubercles; FT, facial tubercle; LA, lateral antennae; MA, medium antenna; SJ, single jaw; TC, tentacular cirri; P, palps. Scale bars: A, B = 1.0 mm, C = 50 μm.

Table 2. Pairwise genetic distance (K2P) based on mitochondrial cytochrome *c* oxidase subunit I (*COI*) sequences among five hesionid species

	Species	Accession No. (Voucher No.)	1	2	3	4	5	6	7	8	9
1	*Leocratides kimuraorum*	MZ311581 (MABIKNA00156552)									
2		MZ311582 (MABIKNA00156553)	0.005								
3		MZ311583 (MABIKNA00156554)	0.002	0.003							
4		LC480519 (−)	0.003	0.002	0.002						
5		LC258082 (NMST-Pol H-622)	0.002	0.002	0.002	0.002					
6	*Leocratides filamentosus*	LC258083 (NSMT-Pol 113220)	0.169	0.171	0.169	0.171	0.166				
7	*Leocrates chinensis*	DQ442565 (SMNH 83510)	0.222	0.222	0.222	0.222	0.222	0.206			
8	*Hesione reticulata*	LC169753 (NSMT Pol N-620)	0.253	0.253	0.253	0.253	0.253	0.263	0.231		
9	*Hesiospina aurantiaca*	JF317197 (SMNH-113855)	0.287	0.292	0.290	0.290	0.286	0.295	0.307	0.294	

(3) peristomial dorsolateral tubercles have two round marginal lobes; and (4) pharynx has terminal papillae (Jimi et al., 2017b). These diagnostic features agree well with Korean specimens. However, Korean specimens are slightly different to the type materials from Japan. In the Korean specimens, ventral black two larger spots were developed along chaetigers 4−16, but were developed along chaetigers 4−15 in Japanese specimens (Jimi et al., 2017b).

Leocratides kimuraorum is similar to *L. ehlersi* in having peristomial dorsolateral tubercles with a few or no lobes and some ventral pigmentation (Jimi et al., 2017b; Salazar-Vallejo, 2020). However, *L. kimuraorum* is distinguished from *L. ehlersi* in the shape of neuro-acicular lobes and ventral pigmentation patterns. *Leocratides kimuraorum* has mucronate neuro-acicular lobes as long as wide and ventral pigmentation with the spots composed of various sizes, whereas *L. ehlersi* has non-mucronate neuro-acicular lobes twice longer than wide, and ventral pigmentation of a long striped pattern (Jimi et al., 2017b; Salazar-Vallejo, 2020).

The DNA barcode sequence of the mitochondrial *COI* gene from three Korean specimens of the species were determined (GenBank accession numbers MZ311581−MZ311583). The lengths of all sequences obtained were 658 bp in total. The *COI* sequences of *L. kimuraorum* obtained in this study were analyzed with *COI* sequences from Japanese specimens of *L. kimuraorum* and those of other hesionid species available in GenBank (Table 1). The intra-specific genetic distance among the *L. kimuraorum* specimens from Japan (type locality) and Korea (this study) ranged from 0.002 to 0.005 (Table 2). The inter-specific distances between *L. kimuraorum* and other hesionid species ranged from 0.166 to 0.307 (Table 2). Combination of morphological data and *COI* DNA barcode data will be useful in increasing the taxonomic accuracy of hesionid polychaetes.

Distribution. Korea (100 m depth, Korea Strait), Japan (103−104 m depth, Off Shima Peninsula; 85−169 m depth, Pacific coast of middle Honshu).

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CONFLICTS OF INTEREST

Gi-Sik Min, contributing editors of the Animal Systematics, Evolution and Diversity, were 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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