Leocratides (Annelida: Hesionidae) from the Pacific Coast of Middle Honshu, Japan, with a Description of *Leocratides kimuraorum* sp. nov.

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A new species of hesionid polychaetes, *Leocratides kimuraorum* sp. nov., is described based on material collected from the Shima Peninsula and Sagami Bay (middle Honshu, Japan), as well as museum specimens collected from Sagami Bay, Suruga Bay, and Shirahama. *Leocratides kimuraorum* sp. nov. is the third species in this genus and can be discriminated from the two congeners, *L. filamentosus* Ehlers, 1908 and *L. ehlersi* (Horst, 1921), by *i*) the length of the antennae, which are as long as palps, *ii*) the presence of pharyngeal terminal papillae, and *iii*) the absence of a papillose peristomial membrane. On the other hand, *L. filamentosus*, originally described from off Western Sumatra, Indonesia, was also collected from Sagami Bay, and represents a new record of this species for Japanese waters. Partial mitochondrial cytochrome *c* oxidase subunit I gene sequences from the holotype of *L. kimuraorum* sp. nov. and newly collected specimen of *L. filamentosus* are provided for reliable species identification in the future.

Key Words: Polychaeta, Phyllodocida, DNA barcode, COI, TRV *Seisui-maru*, JAMBIO, marine invertebrates, hexactinellid sponge, symbiosis, taxonomy.

Introduction

The hesionid polychaete genus *Leocratides* Ehlers, 1908 consists of two species, *L. filamentosus* Ehlers, 1908 and *L. ehlersi* (Horst, 1921), both of which were originally found among hexactinellid sponges from sublittoral substrates in Indonesian waters. The name-bearing types of the first species, *L. filamentosus*, were collected from *Aphrocallistes beatrix* Gray, 1858, off Nias Island at 677 m depth (Ehlers 1908). *Leocratides filamentosus* has also been reported from Japan (Imajima and Hartman 1964; Imajima 2003, 2005, 2006, 2007), New Caledonia (Rullier 1972), and Egypt (as *L. filamentosa* [sic]; Belal and Ghobashy 2012). The second species, originally described as *Leocrates ehlersi* and later combined with *Leocratides* by Fauvel (1932), was found among a species of *Aphrocallistes* Gray, 1858, collected at a depth of 274 m in Saleh Bay, north coast of Sumbawa, Indonesia (Horst 1921, 1924; Pettibone 1970). Besides the records for the type locality, *L. ehlersi* has also been reported from Sagami Bay of Japan (as *Leocratides ehlersi*; Hessle 1925), the Andaman Sea (Fauvel 1932, 1953), the Arabian Sea (Parulekar 1971), and the Red Sea (Fishelson and Rullier 1969; Amoureux *et al.* 1978).

The taxonomy within *Leocratides* is contentious. When Horst (1921) described *L. ehlersi*, he diagnosed the two *Leocratides* species as having one (in *L. filamentosus*) or two (in *L. ehlersi*) dorsal jaw plates. Based on examination of the two syntypes of *L. filamentosus*, however, Augener (1926) argued that *L. ehlersi* should be treated as a junior synonym of the former which has actually two dorsal jaw plates, instead of one. Later, Pettibone (1970) redescribed the syntypes of both species in detail and also concluded that they were synonymous. In contrast, also having examined the syntypes of the two, Pleijel (1998) restored *L. ehlersi* as a distinct species, concluding that the pharyngeal terminal papillae and the papillose peristomial membrane are both present in *L. filamentosus*, but are absent in *L. ehlersi*. In this study, we follow the taxonomy of Pleijel (1998), treating *L. filamentosus* and *L. ehlersi* as two distinct species.

While several authors have recorded *Leocratides* species from Japanese waters, the taxonomic identity of the Japanese taxon (or taxa) requires further scrutiny. Hessle (1925) reported six specimens of hesionids under the name “*Leocrates ehlersi*” from Sagami Bay (from 150 m to 600 m depths). Subsequently, Imajima and Hartman (1964) emended that record to “*Leocratides filamentosus*” without any explanation. Imajima (2003, 2005, 2006, 2007) also

identified a number of hesionid specimens as *L. filamentosus*; these were collected from the Pacific coast of middle Honshu (around Sagami Bay and Suruga Bay) and South West Japan (off the Tokara Islands and the Amami Islands), collected at the depth of 74 m to 297 m. Okanishi *et al.* (2016) reported “*Leocratides* sp.” near Shirahama (from 164–169 m depth). As to the Japanese *Leocratides* species, however, the diagnostic characters mentioned earlier were neither explicitly mentioned by Hessle (1925) and Okanishi *et al.* (2016) nor clearly illustrated by Imajima (2003, 2005, 2006, 2007), making their taxonomic identity blurred.

NJ obtained specimens of *Leocratides* during a research cruise onboard the training and research vessel (TRV) *Seisui-maru* (Mie University) off the Shima Peninsula. Another specimen of *Leocratides* was also collected by MT in Sagami Bay. A close examination in the laboratory revealed that these specimens are morphologically consistent with the description and illustration of “*L. filamentosus*” by Imajima (2003, 2007) and also the illustration of “*Leocrates ehlersi*” by Hessle (1925), whereas they do not correspond with the original descriptions of *L. filamentosus* and *L. ehlersi* (Ehlers 1908; Horst 1921). In this paper, we describe these specimens along with some of the voucher material examined in Imajima (2003, 2007) and Okanishi *et al.* (2016) as the third member of *Leocratides*, providing barcode sequence of the holotype for the new species to facilitate future reliable identification.

In addition to the new species, we also found a number of specimens of *L. filamentosus* in the sense of Ehlers (1908) but not Imajima (2003, 2007) from Sagami Bay. In this paper, we give a redescription of *L. filamentosus* based on these specimens as representing a truly new record for Japanese waters.

Materials and Methods

Fresh specimens were newly collected from Sagami Bay and off the Shima Peninsula, Japan (Table 1). Some of the live specimens were photographed onboard with a digital camera (Nikon D60), then fixed in 10% formalin-seawater or 70% ethanol. After preservation, these specimens were observed with a microscope (Nikon SMZ1500 and OLYMPUS BX51) and photographed with a digital camera (Nikon D5200). All the material has been deposited in the

National Museum of Nature and Science, Tsukuba (NSMT). Additionally, a part of *Leocratides* specimens investigated in Imajima (2003, 2007) and Okanishi *et al.* (2016), which have been kept in the NSMT, were also examined.

DNA extraction, and sequencing for partial sequences of mitochondrial cytochrome *c* oxidase subunit I (COI) were carried out following the method of Jimi and Fujiwara (2016). Newly obtained sequences have been deposited in the DNA Data Bank of Japan.

Systematics

Genus *Leocratides* Ehlers, 1908

[New Japanese name: hanakago-otohime-gokai-zoku]

Leocratides kimuraorum sp. nov.

[New Japanese name: Kimura-hanakago-otohime-gokai (previously called “oni-otohime-gokai”)]

(Figs 1, 2)

?*Leocrates ehlersi* (non Horst, 1921); Hessle 1925: 14–15, fig. 3. *Leocratides filamentosus* (non Ehlers, 1908); Imajima and Hartman 1964: 82–83; Imajima 2003: 136–138, fig. 80 (in part); Imajima 2007: 444, fig. 138 (in part).

Leocratides sp.; Okanishi *et al.* 2016: 14.

Material examined. Holotype: NMST-Pol H-622, 29 mm long, 5 mm wide, sex unknown, off Shima Peninsula, St. 1, 103–104 m depth, 12 October 2016, collected by NJ (right parapodium of chaetiger 5 was removed for observation; it is preserved in 70% ethanol in a 2.0 ml plastic tube, which is contained in the same glass vial together with the rest of the body). Paratypes (14 specimens): NSMT-Pol P-623–624, five specimens, 11–19 mm long, 2 mm wide, sex unknown, off Shima Peninsula, St. 1 (NSMT-Pol P-623, four specimens) and St. 2 (NSMT-Pol P-624, one specimen), 12 October 2016, collected by NJ; NSMT-Pol P-625, one specimen, 25 mm long, 4 mm wide, male (sperm present in each segment’s gonads), Sagami Bay St. 2, 104–111 m depth, 27 April 2016, collected by MT; NSMT-Pol P-626, three specimens examined by Okanishi *et al.* (2016), 6–16 mm long, 2 mm wide, sex unknown, off Shirahama, 164–169 m depth, 27 May 2015, collected by NJ; NSMT-Pol R 176, two specimens examined by Imajima (2003, 2007), 21–24 mm long, 3 mm wide, sex unknown, Sagami Bay, 110–120 m

Table 1. Collection data of *Leocratides* spp. in this study.

Locality	Latitude	Longitude	Depth (m)	Collection date	Collected species	Contained sponges
Sagami Bay, St. 1	35°07.132'N to 35°07.388'N	139°33.709'E to 139°33.365'E	213–255	29 June 2011	<i>Leocratides filamentosus</i>	Unknown
Sagami Bay, St. 2	35°07.5787'N to 35°07.5583'N	139°33.7096'E to 139°33.8756'E	104–111	27 April 2016	<i>Leocratides kimuraorum</i> sp. nov.	Hexactinellid sponge
Sagami Bay, St. 3	35°06.929'N to 35°06.631'N	139°33.814'E to 139°33.734'E	292–375	15 February 2017	<i>Leocratides filamentosus</i>	<i>Aphrocallistes</i> sp.
Off Shima Peninsula, St. 1	34°11.63'N to 34°11.66'N	136°42.56'E to 136°42.69'E	103–104	12 October 2016	<i>Leocratides kimuraorum</i> sp. nov.	Demosponge and hexactinellid sponge
Off Shima Peninsula, St. 2	34°11.81'N to 34°11.82'N	136°41.80'E to 136°41.95'E	107	12 October 2016	<i>Leocratides kimuraorum</i> sp. nov.	Demosponge and hexactinellid sponge

depth, 14 March 1954, collected by the late Emperor Showa; NSMT-Pol R 968, one specimen examined by Imajima (2003, 2007), 21 mm long, 3 mm, sex unknown, Sagami Bay, 85–90 m depth, 24 January 1965, collected by the late Emperor Showa; NSMT-Pol P-627: one specimen examined by Imajima (2007), 16 mm long, 3 mm wide, sex unknown, Suruga Bay, 92 m depth, 4 October 1995, collected by the late Dr. Minoru Imajima (MI); NSMT-Pol P-628: one specimen examined by Imajima (2007), 24 mm long, 3 mm wide, sex unknown, Suruga Bay, 86–91 m depth, 8 February 1978, col-

lected by MI.

Sequence. LC258082, COI gene, 630bp, determined from the holotype.

Description. Holotype 29 mm long, 5 mm wide in chaetiger 7 (not including parapodia), 21 segments, 16 chaetigers. Body cylindrical, tapered in posterior region (Fig. 1A, B), transparent in life (Fig. 1A) and whitish in ethanol; dorsal integument annulated, with 15–17 transverse wrinkles per segment. Dorsum with numerous, thin, transverse discontinuous brown lines, extended into lateral cushions,

Fig. 1. *Leocratides kimuraorum* sp. nov., holotype, NSMT-Pol H-622. A, Live specimen, dorsal view; B, same, ventral view; C, integument and lateral cushions in chaetigers 4–5, dorsal view; D, prostomium, dorsal view; E, pharynx and peristomium, ventral view, showing facial tubercle (white arrow), cushion-shaped appendages (black arrows), and dorsal jaw plates (arrowheads); F, pharynx with terminal papillae, ventral view. Scale of the ruler: A, B, 1 mm. Scale bars: C–F, 1 mm.

varying in length, decreasing in size laterally (Fig. 1A, C); ventrally, black spots of different size and shape arranged along mid-ventral groove, in chaetal lobe regions, two larger spots better developed along chaetigers 4–15 (Fig. 1B). Prostomium rectangular, slightly wider than long, mid-dorsally with shallow depression, square in shape from dorsal view (Fig. 1D). Median antenna cirriform, smooth, tapered, inserted in central part of prostomium, extended beyond anterior prostomial margin, 1.1 times longer (and thinner) than lateral antenna, surpassing palpophores. Lateral antennae tapered, smooth, on anterior edge of prostomium. Palps bi-articulated, 0.9–1 times longer than lateral antennae, palpopore two times longer than palpostyle, bent laterally, pointed to body sides, external to antennae (Fig. 1D). Eyes brownish, two pairs, on mid-lateral part of prostomium; an-

terior pair slightly larger and more separated than posterior one (Fig. 1D). Facial tubercle present mid-ventrally on prostomium; cushion-shaped appendage present between palps and tentacular cirri on each side (Fig. 1E); papillose peristomial membrane absent.

Tentacular cirri eight pairs, long, thick; longest one reaching chaetiger 10. Lateral cushions low, barely projected dorsally, slightly projected laterally, undivided, with 17–18 longitudinal wrinkles per side (Fig. 1C).

Parapodia uniform throughout; with chaetal lobes tapered, truncate, as long as wide (Fig. 2A); dorsal cirri with cirrophores, latter being cylindrical, smooth, about twice longer than wide (Fig. 2B); cirrostyle very long, whip-like, smooth basally, annulated medially and distally, longer than body width (including parapodia). Ventral cirri basally

Fig. 2. *Leocratides kimuraorum* sp. nov., right parapodium of chaetiger 5, holotype, NSMT-Pol H-622. A, General appearance of parapodium, anterior view; B, cirrophore; C, ventral cirrus; D, acicular lobe, arrow indicating its tip, the base side of photo is the parapodial tip; E, bundle of neurochaetae; F, blade of neurochaeta. Scale bars: A, 2 mm; B–E, 300 µm; F, 50 µm.

smooth, rugose medially and distally, surpassing chaetal lobe, reaching up to half length of neurochaetal bundle (Fig. 2C).

Neuropodial acicula black, tapered; acicular lobe single, thick, digitate, tapered into a small mucro (Fig. 2D). Neurochaetae about 20 per bundle (Fig. 2E); handle greenish; blade pale brownish, bidentate, 5 times longer than wide; with subdistal tooth short, blunt, 0.4 times longer than apical tooth; guard tooth absent (Fig. 2F).

Cirri of prepygidial segment broken; pygidium smooth, depressed, with paired cirri; anus located dorso-terminally, with about 10 anal papillae.

Pharynx dissected in holotype, about 20 low cushion-like terminal papillae present (Fig. 1F); dorsal jaw two plates, ventral jaw one plate.

Variation. Dorsal transverse lines and ventral black spots faded in some paratype specimens.

Etymology. The specific name is a noun in the genitive plural, after a Japanese marine ecologist Dr. Taeko Kimura and a malacologist Mr. Shoichi Kimura, who organized the cruise of the TRV *Seisui-maru* of Mie University, during which a part of the type specimens, including the holotype, of the new species were collected.

Distribution. Pacific coast of middle Honshu, Japan: 85–169 m depth, collected with hexactinellid sponges.

Remarks. Our specimens undoubtedly belong to *Leocratides* because their body consists of 21 segments, the anterior cirri are in eight pairs, the neurochaetae are bidentate, the palps are bi-articulated, the parapodia are uniramous, and the jaws are present, all agreeing with the generic diagnosis provided by previous researchers (Pettibone 1970; Pleijel 1998; Rizzo and Salazar-Vallejo 2014; Salazar-Vallejo 2016).

Leocratides kimuraorum sp. nov. differs from *L. filamentosus* in the lateral antennae because they are as long as palps in *L. kimuraorum* sp. nov. but shorter than palps in *L. filamentosus*, and there is no papillose peristomial membrane in *L. kimuraorum* sp. nov. whereas it is present in *L. filamentosus*. It can also be distinguished from *L. ehlersi* by the pharyngeal terminal papillae (present in *L. kimuraorum* sp. nov. vs. absent in *L. ehlersi*) (Horst 1921; Pleijel 1998).

Because we were not able to examine Hessle's (1925) voucher material from Misaki, identified as "*Leocrates ehlersi*", its taxonomic identity remains uncertain, inasmuch as the morphological features illustrated in his figure (Hessle 1925: fig. 3) apply both to *L. ehlersi* and *L. kimuraorum* sp. nov.

We examined some of Imajima's (2003, 2007) voucher material from Sagami and Suruga Bays and noticed that his descriptions contain several errors. For instance, Imajima (2003, 2007) noted that there were two to five transverse wrinkles on the dorsal integument in each segment, whereas there are actually 15–17 wrinkles per segment. When it comes to the pharyngeal terminal papillae, Imajima (2003, 2007) stated that these were absent, although the papillae are in fact present in his voucher specimens. Therefore, we can confidently regard his material as belonging to *L. kimuraorum* sp. nov.

Leocratides filamentosus Ehlers, 1908

[New Japanese name: hanakago-otohime-gokai]

(Figs 3, 4)

Leocratides filamentosus Ehlers, 1908: 63, pl. 6, figs 8–12;

Pettibone 1970: 230–231, figs 27–28; Pleijel 1998: 112.

?*Leocratides filamentosus*; Rullier 1972: 58; Imajima 2005: 82; 2006: 351; Belal and Ghobashy 2012: 178 (as *L. filamentosa* [sic]).

Material examined. Nine specimens: NMST-Pol 113219, one specimen, sex unknown, Sagami Bay, St.1, 213–255 m depth, 29 June 2011, collected by MT (right parapodium of chaetiger 10 removed for observation; preserved in 70% ethanol in a 2.0 ml plastic tube, kept in the same vial together with the rest of the body); NSMT-Pol 113220, eight specimens, one female and others of unknown sex, Sagami Bay, St. 3, 292–375 m depth, 15 February 2017, collected by NJ, MT, HK.

Sequence. LC258083, COI gene, 592 bp, determined from a specimen of NSMT-Pol 113220.

Description. 14–30 mm long, 2–4 mm wide in chaetiger 7 (not including parapodia), 21 segments, 16 chaetigers; right parapodium of chaetiger 10 removed for observation. Body cylindrical, tapered in posterior region, transparent in live (Fig. 3A, B), whitish in ethanol; dorsal integument annulated, with 12–17 transverse wrinkles per segment. Brown lines on dorsal surface absent (Fig. 3A, C); ventrally with black spots of varying size and shape arranged along median groove, in some places roughly on two rows, in chaetigers 5–16; some spots being particularly larger than others, arranged laterally in pair along line between parapodia in each segment, especially prominent in chaetigers 7–11 (Fig. 3B). Prostomium rectangular, longer than wide, mid-dorsally with shallow depression, square in shape from dorsal view (Fig. 3D). Median antenna conical, smooth, in central part of prostomium, not reaching anterior prostomial margin, 1.3 times longer than lateral antennae. Lateral antennae conical, smooth, on anterior edge of prostomium. Palps bi-articulated, 1.2 times longer than lateral antennae, palpophore four times longer than palpostyle, ventral to antennae (Fig. 3D). Eyes brownish, two pairs, on mid-lateral part of prostomium, anterior pair slightly larger and more separated than posterior one (Fig. 3D). Facial tubercle present mid-ventrally on prostomium; papillose peristomial membrane present (Fig. 3E).

Tentacular cirri eight pairs, long, thick; longest one reaching chaetiger 6. Lateral cushions low, barely projected dorsally, slightly projected laterally, undivided, with 7–8 longitudinal wrinkles per one lateral cushion (Fig. 3C).

Parapodia uniform throughout; chaetal lobes cylindrical, truncate, as long as wide (Fig. 4A); dorsal cirri with cirrophores cylindrical, smooth, 1.3 times longer than wide (Fig. 4B); cirrostyle whip-like, smooth, annulated, longer than body width (without parapodia). Ventral cirri basally rugose, surpassing chaetal lobe, reaching up to half length of neurochaetal bundle (Fig. 4C).

Neuropodial acicula black, tapered; acicular lobe single,

Fig. 3. *Leocratides filamentosus* Ehlers, 1908. A, B, F; NSMT-Pol 113220, C–E; NSMT-Pol 113219. A, Live specimen inside a fragment of an *Aphrocallistes* sp. hexactinellid sponge, dorsal view; B, same specimen, ventral view; C, integument and lateral cushions in chaetigers 2–4, dorsal view; D, prostomium, dorsal view; E, pharynx and peristomium, ventral view, showing facial tubercle (white arrow) and papillose peristomial membrane (black arrows); F, pharynx with terminal papillae, ventral view. Scale bars: A, B, 10 mm; C–F, 1 mm.

wider basally, tip digitate, long (Fig. 4D). Neurochaetae about 20 per bundle (Fig. 4E); handle greenish; blade yellowish, bidentate, six times longer than wide; with subdistal tooth 0.5 times longer than apical tooth; guard absent (Fig. 4F).

Cirri of prepygidial segment broken; pygidium smooth, depressed, with paired cirri; anus dorso-terminal, with about eight anal papillae.

Pharynx dissected, about 20 terminal low cushion-like terminal papillae present (Fig. 3F); dorsal jaw two plates, ventral jaw one plates.

Confirmed distribution. From Sagami Bay, Japan to Indonesia, 213–677 m depth, in hexactinellid sponges.

Remarks. Imajima (2003, 2007) reported “*Leocratides filamentosus*” from Japanese waters. However, as indicated above Imajima’s (2003, 2007) specimens from Sagami and Suruga Bays actually belong to *L. kimuraorum* sp. nov. Imajima (2005, 2006) also reported “*L. filamentosus*” from off Izu-Oshima Island, Sagami Bay, and several other localities off the Nansei Islands, southwestern Japan, but neither morphological accounts nor the basis of his identifications were provided. We searched unregistered polychaete collec-

Fig. 4. *Leocratides filamentosus* Ehlers, 1908. Right parapodium of chaetiger 10, NSMT-Pol 113219. A, General appearance of parapodium, posterior view; B, cirrophore; C, ventral cirrus; D, acicular lobe; E, bundle of neurochaetae; F, blade of neurochaeta. Scale bars: A, 1 mm; B–E, 300 μ m; F, 100 μ m.

tion in NSMT for the voucher specimens that substantiate Imajima's (2005, 2006) records of his "*L. filamentosus*" from Izu-Oshima Island, Sagami Bay, and the Nansei Islands, but were not able to locate them. This makes the validity of these records of "*L. filamentosus*" rather obscure. Thus, we conclude that there has been no reliable distributional records of *L. filamentosus* from Japanese waters and this study represents the first record of the species for Japan.

Out of the type locality and Japanese waters, Rullier (1972) and Belal and Ghobashy (2012) also reported "*Leocratides filamentosus*" from extremely shallow depths (up to 8 m depth) in New Caledonia and Lake Timsah of the Suez Canal, Egypt, respectively. However, these studies provided only a brief account or an inadequate illustration and we consider these records are doubtful.

Discussion

An ecological association of *Leocratides* with hexactinellid sponges has been indicated because the two previously known species, *L. filamentosus* and *L. ehlersi*, were both found among *Aphrocallistes* hexactinellids (e.g., Pettibone 1970; this study). Our specimens of *L. kimuraorum* sp. nov. were also found among sediments which contained fragments of hexactinellid sponges, indicating that *Leocratides* species are generally associated with hexactinellids. Because dredging inevitably disturbs natural habitat, future studies aiming to observe specimens *in-situ* using a remotely operated vehicle, could reveal the ecology of *Leocratides* and the interaction between the worms and the host hexactinellid sponges.

In this study, we found that the bathymetric distributions of *L. kimuraorum* sp. nov. and *L. filamentosus* seem to be clearly separated. In Sagami Bay, the two species were obtained almost sympatrically, but *L. kimuraorum* sp. nov. occurred in shallow waters, around 100–150 m depth, while *L. filamentosus* tended to occur in deeper waters, more than 200 m depth (Table 1). Recent studies focused on cryptic diversity of polychaetes indicate that depth may play an important role in speciation (Nygren *et al.* 2005, 2010; Schüller 2011; Nygren 2014; Oug *et al.* 2017); this scenario may be true for *Leocratides*. Further efforts combining the records of *Leocrates* species from various localities are required to reveal the nature of species distribution.

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