**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) also having examined the types of both species in detail and also concluded that they are synonymous. In contrast, having examined the types of both species in detail and also concluded that they were synonymous. In contrast, also having examined the types 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. Hesse (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 “*Leocrates 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 (2005, 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**

*Leocratides kimuraorum* sp. nov.

*Leocratides* filamentosus sp. nov.


**Material examined.** Holotype: NSMT-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 depth.

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

<table>
<thead>
<tr>
<th>Locality</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Depth (m)</th>
<th>Collection date</th>
<th>Collected species</th>
<th>Contained sponges</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sagami Bay, St. 1</td>
<td>35°07.132′N to</td>
<td>139°33.709′E to</td>
<td>213–255</td>
<td>29 June 2011</td>
<td><em>Leocratides filamentosus</em></td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>35°07.388′N</td>
<td>139°33.365′E</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Sagami Bay, St. 2</td>
<td>35°07.578′N to</td>
<td>139°33.709′E</td>
<td>104–111</td>
<td>27 April 2016</td>
<td><em>Leocratides kimuraorum</em> sp. nov.</td>
<td>Hexactinellid sponge</td>
</tr>
<tr>
<td></td>
<td>35°07.558′N</td>
<td>139°33.875′E</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sagami Bay, St. 3</td>
<td>35°06.929′N to</td>
<td>139°33.814′E</td>
<td>292–375</td>
<td>15 February 2017</td>
<td><em>Leocratides filamentosus</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>35°06.631′N</td>
<td>139°33.734′E</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Off Shima Peninsula,</td>
<td>34°11.63′N to</td>
<td>136°42.56′E to</td>
<td>103–104</td>
<td>12 October 2016</td>
<td><em>Leocratides kimuraorum</em> sp. nov.</td>
<td>Demosponge and hexacti-</td>
</tr>
<tr>
<td>St. 1</td>
<td>34°11.66′N</td>
<td>136°42.69′E</td>
<td></td>
<td></td>
<td></td>
<td>nellid sponge</td>
</tr>
<tr>
<td>Off Shima Peninsula,</td>
<td>34°11.81′N to</td>
<td>136°41.80′E to</td>
<td>107</td>
<td>12 October 2016</td>
<td><em>Leocratides kimuraorum</em> sp. nov.</td>
<td>Demosponge and hexacti-</td>
</tr>
<tr>
<td>St. 2</td>
<td>34°11.82′N</td>
<td>136°41.95′E</td>
<td></td>
<td></td>
<td></td>
<td>nellid sponge</td>
</tr>
</tbody>
</table>
Two species of *Leocratides* from Japan

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**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.

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**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, palpophore 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; anterior 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

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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.
Two species of Leocrates from Japan

Leocrates filamentosus Ehlers, 1908
[New Japanese name: hanakagoko-otohime-gokai]
(Figs 3, 4)


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); NMST-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 NMST-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,
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-
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 Aphrocallistidae, etc., (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 *Leocraterides*. Further efforts combining the records of *Leocraterides* species from various localities are required to reveal the nature of species distribution.

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**References**


Two species of *Leocrates* from Japan


