**Lamellibrachia sagami** sp. nov., a new vestimentiferan tubeworm (Annelida: Siboglinidae) from Sagami Bay and several sites in the northwestern Pacific Ocean

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**Abstract**

A new vestimentiferan tubeworm species of the genus *Lamellibrachia* Webb, 1969 is described. It was collected from cold seep areas off Hatsushima in Sagami Bay and at the Daini Tenryu Knoll in the Nankai Trough (606–1170 m depth). *Lamellibrachia sagami* sp. nov. differs from seven congeneric species in the following character states; showing a wider range of diameter of vestimental and trunk plaques than *L. barhami*, *L. luymesi*, *L. satsuma* and *L. anaximandri*; and having more numerous sheath lamellae (3–6 pairs) than *L. juni* (2–3 pairs) but fewer than *L. victori* (7 pairs) and *L. columna* (8–16 pairs).

**Key words:** *Lamellibrachia sagami*, Siboglinidae, Sagami Bay, vestimentiferan tubeworms

**Introduction**

Vestimentiferan tubeworms are considered to belong to the family Siboglinidae, Annelida (e.g. Rouse & Fauchald 1997; Halanych 2005; Struck et al. 2011; Weigert et al. 2014). The family contains the four lineages: frenulates, *Osedax*, *Sclerolinum* and vestimentiferan tubeworms (Hilário et al. 2011). Vestimentiferan tubeworms are often dominant in chemosynthetic communities. They depend on organic compounds supplied by endosymbiotic chemoautotrophic bacteria and lack a mouth and gut in the adult phase. These unusual features have driven many scientists to study their ecology (e.g. Laubier & Desbruyères 1985; Tunnicliffe et al. 1990), development (e.g. Jones & Gardiner 1989; Rimskaya-Korsakova & Malakhov 2010), larval dispersal (e.g. Young et al. 1996; Marsh et al. 2001), physiology (e.g. Miura et al. 2002; Carney et al. 2007), and phylogenetic relationships (e.g. Williams et al. 1993; Halanych 2005; Struck et al. 2011; Weigert et al. 2014).

The genus *Lamellibrachia* Webb, 1969 is characterized by sheath lamellae (=lamellar sheaths in Jones, 1985) and a divided posterior vestimental fold. The genus is more widely distributed horizontally and vertically than other vestimentiferan genera (McMullin et al. 2003).


Although the existence of several undescribed vestimentiferan species in the northwestern Pacific has been
indicated by molecular studies (Kojima et al. 2001; Kojima et al. 2002; McMullin et al. 2003; Kojima et al. 2003), their morphological characters remain to be examined. Two of these undescribed vestimentiferan species have been collected from Sagami Bay, Central Japan. One of them, temporarily named *Lamellibrachia* sp. L1, has similar morphological characters to known species of the genus *Lamellibrachia*. It is grouped with other *Lamellibrachia* species in the molecular phylogenetic tree and has been found at other sites close to Japan (Fig. 1), namely the Nankai Trough and the Okinawa Trough (Kojima et al. 2001).

Sagami Bay, located in the middle part of the Pacific coast of Japan, is one of the best studied deep bays in Japan. In 1984, a chemosynthetic ecosystem was found in Sagami Bay for the first time in Japanese waters (Okutani & Egawa 1985; Fujikura et al. 2008). Since 1985, several communities peculiar to the chemosynthetic ecosystem have been reported there (e.g. Hashimoto et al. 1989). The Nankai Trough is also a well studied area. A large number of sites which harboring cold seep communities were found there, between 270 and 4800 m depth (Fujikura et al. 2008). *Lamellibrachia* sp. L1 was found at some shallow sites in the Nankai Trough, e.g. the Kanesu-no-se Bank, the Ryuyo Canyon, the Omaezaki Spur and the Daini Tenryu Knoll (Kojima et al. 2001; Miura & Fujikura 2008). The Okinawa Trough is a representative back-arc basin with many hydrothermal vent fields in the western Pacific (Fujikura et al. 2008). *Lamellibrachia* sp. L1 is known from two vent fields in this basin, namely, the Iheya Ridge and the North Iheya Knoll. In this study, we examined the morphology of the siboglinid annelid which has been called *Lamellibrachia* sp. L1 and describe it as new to science.

**Material and methods**

Vestimentiferan tubeworms were collected from cold seep sites off Hatsushima in Sagami Bay between 850–1170 m depths (Fig. 2), at the Daini Tenryu Knoll in the Nankai Trough at 606 m depth (Fig. 1). They are attached firmly to the fissures of rocks or angular boulders (Hashimoto et al. 1989) and about half-length of tubes were buried in sediment (Fujikura et al. 2008). Sometimes other vestimentiferan tubeworm an *Alaysia* species predominated over them (Fujikura et al. 1995). Specimens were collected during five cruises of the research vessel (RV) *Natsushima*, using the manned submersible *Shinkai 2000*, the remotely operated vehicle (ROV) *Dolphin 3k* and ROV Hyper-Dolphin of the Japan Agency for Marine-Earth Science and Technology (JAMSTEC) from 1986 to 2014. Details of samples are noted in the paragraph “Material examined”. To use for DNA analysis, a portion of one fresh specimen (JAMSTEC No. 1140043315) was cut off from the vestimentum and frozen at -80°C and preserved in a freezer. The remaining part of this specimen and all the other specimens were fixed in 4.7% neutralized formaldehyde solution and preserved in 70% ethyl alcohol. Although the duration of the formaldehyde-fixation was not recorded, generally it is a week but some may be several months. Cuticular plaques on the postero-central vestimentum surface near the trunk and on the anterior trunk surface near the vestimentum were observed and measured on thin pieces of epidermis cut out and placed on a glass slide with a few drops of lactic acid, which facilitated tissue observations. For each specimen, diameters of ten plaques on the posterior region of the vestimentum (vestimental plaques) and ten plaques on the anterior region of the trunk (trunk palques) were measured.

Total DNA was extracted from the vestimental tissue of specimen (JAMSTEC No. 1140043315) with the DNeasy Tissue Extraction Kit (QIAGEN, Hilden, Germany). A fragment containing a portion of the mitochondrial cytochrome oxidase I (COI) gene (626 bp) was amplified through the polymerase chain reaction (PCR) using the LCO1490/HCO2198 universal primers (Folmer et al. 1994). PCR reactions were performed using the following protocol; 94 °C for 40 sec.; then repeat 35 cycles consisting of 94 °C for 40 sec., 42 °C for 60 sec., and 72 °C for 90 sec. PCR products were purified by Exo-SAP-IT (United States Biochemical, Cleveland, OH). Nucleotide sequences were determined by ABI 3130 automated DNA sequencer (Applied Biosystems®, Life Technologies Corporation, Carlsbad, USA) with BigDye Terminator Cycle Sequencing Kit Version 3.1 (Applied Biosystems®). The nucleotide sequence will be available in DDBJ, EMBL and NCBI nucleotide sequence database (accession number: LC064365).

The holotype is deposited in National Museum of Nature and Science, Tokyo (NSMT). The paratypes are deposited in NSMT and JAMSTEC.
**Taxonomy**

**Family Siboglinidae Caullery, 1914**

**Genus Lamellibrachia Webb, 1969**

*Lamellibrachia sagami* sp. nov.
(Figs. 2–5)

*Lamellibrachia* sp. (Sagami Bay, 300 and 1200 m): Andersen et al. 2004, 980–999.

**Material examined.** Holotype: off Hatsushima, Sagami Bay, female, 39° 0.95′ N, 139° 13.32′ E, 853 m, Hyper-Dolphin Dive HPD#0928 during Natsushima NT08–25 cruise, 17 Dec 2008, NSMT-Pol H-593, JAMSTEC No. 079102. Paratypes: off Hatsushima, Sagami Bay during four dives, three males and two females, the same catch as the holotype, NSMT-Pol P-594, NSMT-Pol P-595, JAMSTEC No. 079069, 079073, respectively, and JAMSTEC No. 079098, 079100, 079109; two males and a female, 35° 0.00′ N, 139° 13.50′ E, 1170 m, Dolphin 3K Dive 3K#0190 during Natsushima NT94–04 cruise, 22 Sep 1994, NSMT-Pol P-596, JAMSTEC No. 79842; three males and a female, 35° 0.77′ N, 139° 13.65′ E, 937 m, Shinkai 2000 Dive 2K#1203 during Natsushima NT00–08 cruise, 10 Jul 2000, JAMSTEC No. 26480–26483; five females, 35° 0.76′ N, 139° 13.49′ E, 850 m, Shinkai 2000 Dive 2K#1203 during Natsushima NT00–08 cruise, 10 Jul 2000, NSMT-Pol P-597, JAMSTEC No. 026501–026507. Non-type specimens: a male, off Hatsushima, Sagami Bay, 35° 00.00′ N, 139° 13.50′ E, 1160 m, Natsushima NT86–02 cruise Shinkai 2000 Dive2K#222, 27 May 1986, JAMSTEC No. 80467; a female, the Daini Tenryu Knoll, the Nankai Trough, 34° 04.61′ N, 137° 47.27′ E, 606 m, Hyper-Dolphin Dive HPD#1655 during Natsushima NT14–07 cruise, 25 Apr 2014, JAMSTEC No. 114004313.

**Description.** Tube length 277.0–661.5 mm (mean=545.7 mm, n=4); outer width of top funnel opening 9.5–11.2 mm (n=4); width of basal end 2.8–7.8 mm (mean=4.5 mm, n=4). All tubes incomplete, lacking considerable parts of basal regions. External characters of tube variable along its length (Fig. 3). Anterior part straight or slightly curved, but not coiled, with many short collars. Inter-collar distance generally small but varying among specimens. Posterior part sinuous, curled, smooth, without collars.

Obturaculum length 5.8–22.5 mm (mean=17.0 mm, n=18); width 4.4–10.8 mm (mean=8.1 mm, n=18), with bare anterior face, lacking any secreted structure (Figs. 4A, B). Lateral surface of obturaculum surrounded by branchial plumes. Three to six pairs of outer sheath lamellae (mean=4.4, n=17) present. Branchial lamellae with ciliated pinnules enclosed by sheath lamellae, number of pairs 19–26 (mean=23.2, n=17). Ratio of number of branchial lamellae pairs to obturaculum width (BL/OW) varying from 2.0–5.5 (mean=3.0, n=17).

Vestimentum length 32.0–84.5 mm (mean=58.0 mm, n=18); width 3.5–7.3 mm (mean=5.0 mm, n=18). Anterior edge of vestimentum forming centrally split collar-like fold extending outwardly (Fig. 4A). Dorsal paired genital ciliated grooves running along most length of vestimentum and flanked by conspicuous narrow epidermal folds in males; without epidermal folds in females. Ventral surface of vestimentum covered by numerous cuticular plaques, with narrow central ciliated field (Figs 4C, 5A). Posterior ends of vestimental folds separated centrally and rounded distally with tongue-like extensions.

All specimens lacking considerable posterior trunk parts. Trunk (Fig. 4D) filled with fragile tissue; surface covered entirely by cuticular plaques (Fig. 5B) except midventral and middorsal seam-like lines. Opisthosome not observed.

Vestimental plaques from 17 specimens ranging in diameter from 59–101μm (mean=77.4 μm; SD=8.3; n=170). Trunk plaques from 17 specimens ranging in diameter 67–130 μm (mean=94.0 μm; SD=11.7; n=170).

**Type-locality.** Off Hatsushima in Sagami Bay, 853 m deep.
FIGURE 1. Distribution of *Lamellibrachia sagami* **sp. nov.** around Japan. Stars, *Lamellibrachia sagami* **sp. nov.** examined in this study; circles, *L. sagami** sp. nov.; Open symbols stand for cold seep area; closed symbols stand for hydrothermal vent fields (Fujikura *et al*. 2008).
FIGURE 2. Field photographs of *Lamellibrachia sagami* sp. nov. with *Alaysia* sp. in the cold seep area off Hatsushima. A, Taken during the dive when the holotype was collected, NT08–25 cruise of RV *Natsushima* dive HPD#0928; B, Many branchial plumes of *L. sagami* sp. nov. were popping out of tubes. Taken during the NT06–23 cruise of RV *Natsushima* dive HPD#636. Anterior tube diameter of *L. sagami* sp. nov. are approximately 10 mm.
**Etymology.** The specific epithet *sagami*, as noun in apposition, refers to the province name of the Edo period for Kanagawa, the coastal area of Sagami Bay, the type locality.

**Comparison with specimens from non-type localities.** The COI sequence and the morphology of a specimen collected from the Daini Tenryu Knoll (JAMSTEC No. 1140043315) was examined. Its COI sequence (626bp) was identical to the sequence of 22 specimens (Haplotypes A and D) from the type locality of the new species (Kojima et al. 2001). Judging from the diameter of vestimental and trunk plaques and the number of branchial lamellae and sheath lamellae, it was also thought to be the new species (Table 1).

**Distribution.** Based on the COI sequence (Kojima et al. 2001; Miura & Fujikura 2008), the species is known from cold seep areas off Hatsushima and on the Okinoyama Bank, Sagami Bay, the Kanesu-no-se Bank, the Ryuyo Canyon, the Omaezaki Spur and the Tenryu Knoll, the Nankai Trough, and the Kuroshima Knoll, the Ryukyu Trench between 270–1300 m (Fig. 1). It is also known from hydrothermal vent fields on the Iheya Ridge and the North Iheya Knoll, the Okinawa Trough, and the Sumisu Caldera, the Izu-Bonin arc between 900–1500 m depth (Kojima et al. 2001; Miura & Fujikura 2008).

**Remarks.** All species of the genus *Lamellibrachia* are distinguishable by the sequence data for COI (Southward et al. 2011) with the exception of *L. victori* for which sequence data are not available. In this study, we compare morphologically the new species with other congeneric species.

*Lamellibrachia sagami* sp. nov. differs morphologically from other known congeneric species in the diameter of cuticular plaques, the number of branchial lamellae and sheath lamellae (Table 1). There was no significant correlation between mean of the vestimental plaques diameters and the body size (the length and width of obturatorum and vestimentum), and between the trunk plaques diameters and the body size in each specimen of *L. sagami* sp. nov. (Spearman rank correlation, 17 type specimens and two non-type specimens, P>0.05). Also the number of branchial lamellae and sheath lamellae were not correlated with the body size (Spearman rank correlation, 18 type specimens and two non-type specimens, P>0.05). These suggest that in adults, the diameter of plaques, and the number of branchial lamellae and sheath lamellae are independent of the growth and we, therefore used them for morphological comparison in the genus.
FIGURE 4. *Lamellibrachia sagami* sp. nov., holotype. A, anterior region, ventral view; B, anterior region, dorsal view; C, vestimental region, ventral view; D, whole specimen, dorsal view. Scale bars = 5 mm (A, B); 10 mm (C, D).
**TABLE 1.** Comparison of range for several diagnostic characters in plural specimens of all described species of the genus *Lamellibrachia.*

<table>
<thead>
<tr>
<th>Species</th>
<th>Specimen</th>
<th>OL (mm)</th>
<th>OW (mm)</th>
<th>BL (mm)</th>
<th>SL (mm)</th>
<th>VP plaque (μm)</th>
<th>Diameter plaque (μm)</th>
<th>TP plaque (μm)</th>
<th>Diameter plaque (μm)</th>
</tr>
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<td>1</td>
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<td>10.5</td>
<td>25</td>
<td>5</td>
<td>10</td>
<td>67–81</td>
<td>10</td>
<td>91–102</td>
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<td>5.8–22.5</td>
<td>4.4–10.8</td>
<td>19–26*</td>
<td>3–6*1</td>
<td>160</td>
<td>59–101*1</td>
<td>160</td>
<td>67–130*1</td>
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<td>10.2</td>
<td>6.8</td>
<td>23</td>
<td>5</td>
<td>5</td>
<td>58–67</td>
<td>5</td>
<td>70–104</td>
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<tr>
<td>The Daini Tenryu Knoll, the Nankai Trough</td>
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<td>4.5–12</td>
<td>–25</td>
<td>–5</td>
<td>60–150*3</td>
<td>115–160*3</td>
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<td><em>Lamellibrachia barhami</em></td>
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<td>4.5–12</td>
<td>–25</td>
<td>–5</td>
<td>60–150*3</td>
<td>115–160*3</td>
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<td><em>Lamellibrachia haymesi</em></td>
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<td>3.5–9</td>
<td>15–22</td>
<td>4–8</td>
<td>55–60*2</td>
<td>75–85*2</td>
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<td><em>Lamellibrachia victori</em></td>
<td>2</td>
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<td>70–120*6</td>
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<td>0–4*5</td>
<td>35–63*6</td>
<td>51–82*6</td>
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<td><em>Lamellibrachia juni</em></td>
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<td>5.2–8.3</td>
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<td>2–3</td>
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<td>80–98*7</td>
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<td>1.8–6</td>
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<td>3–9</td>
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<td>60–95*6</td>
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<tr>
<th>Species</th>
<th>BL/OW range</th>
<th>BL/OW mean</th>
<th>References</th>
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<tr>
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<td>3.4</td>
<td>This study</td>
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<td>van der Land &amp; Norrevang 1975; Gardiner &amp; Hourdez 2003; Southward et al. 2011</td>
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<td>2.7–4.6*8</td>
<td>3.7</td>
<td>Miura &amp; Kojima 2006</td>
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<tr>
<td>Lamellibrachia anaximandri</td>
<td></td>
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<td>Southward et al. 2011</td>
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</table>

OL: obturacular length, OW: obturacular width, BL: number of branchial lamellae, SL: number of sheath lamellae, VP/TP: vestimental/trunk plaques. Blanks: no data. *1: specimen n=16; *2: specimen n=1; *3: specimen n=5; *4: specimen n=53; *5: specimen n=57; *6: specimen n not mentioned in the paper; *7: specimen n=4, referred to the plaque width in the text; *8: calculated in this study.
FIGURE 5. A, plaques on vestimentum; B, plaques on trunk of holotype in grey scale. Scale bar = 100 µm (A, B).

At the continental margins in the Pacific, *L. sagami* sp. nov., *L. barhami* and *L. satsuma* differ one another in the diameter of vestimental plaques (Table 1); 59–101 µm in *L. sagami* sp. nov., 60–150 µm in *L. barhami* (Southward et al. 2011) and 35–63 µm in *L. satsuma* (Miura et al. 1997), and in that of the trunk ones; 67–130 µm, 115–160 µm and 51–82 µm, respectively.

In the South Pacific, *L. sagami* sp. nov., *L. columna* and *L. juni* are distinguishable by the number of sheath lamellae (Table 1); 8–16 pairs in *L. columna* and two or three pairs in *L. juni* (Southward 1991) while three to six pairs in *L. sagami* sp. nov.. Although some specimens of *L. juni* and *L. sagami* sp. nov. have three pairs of sheath lamellae (Miura & Kojima 2006), the new species has a smaller number of branchial lamellae.

*Lamellibrachia sagami* sp. nov. differs from *L. luymesi* known from the Atlantic and *L. anaximandri* found in the Mediterranean in the diameter of plaques (Table 1). The diameter of vestimental plaques vary 59–101 µm in *L. sagami* sp. nov., whereas 55–60 µm in *L. luymesi* and 55–70 µm in *L. anaximandri*; that of trunk ones 67–130 µm in *L. sagami* sp. nov. whereas 75–85 µm in *L. luymesi* (Southward et al. 2011). *Lamellibrachia anaximandri* has fewer branchial lamellae than *L. sagami* sp. nov.. *Lamellibrachia victori* has seven pairs of sheath lamellae (Mañé-Garzón & Montero 1985) and differs from *L. sagami* sp. nov., in having three to six pairs (Table 1).

Additionally, the BL/OW ratio significantly differs from each other in ranging 2.0–5.5 in *L. sagami* sp. nov., 4.7–7.8 in *L. satsuma*, 2.7–4.6 in *L. juni* (Steel-Dwass test, n=17, 6, 8, respectively, P<0.05).

*Lamellibrachia sagami* sp. nov., has been thus demonstrated to be new to science.

Acknowledgments

Figure 2A and 2B were taken during the NT08–25 and NT06–23 cruise of RV *Natsushima*, the Japan Agency for Marine-Earth Science and Technology. We express heartfelt thanks to Dr. Shinji Tsuchida for providing us specimens for study of morphology. A special note of thanks goes to Dr. Katsunori Fujikura and Dr. Hiromi Watanabe (JAMSTEC) for their support to our study. We also thank Asako Hatakeyama, Yuko Uchida, Takashi Hosono and Marika Ichiyanagi and other staffs of JAMSTEC for their sincere assistance in procedure for using specimens which were kept in JAMSTEC. We are grateful to Dr. Eve Southward, Dr. Anja Schulze and an anonymous reviewer for giving us invaluable comments on earlier draft.

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