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Rearing and observation methods of vestimentiferan tubeworm and its early development at atmospheric pressure

Hiroshi MIYAKE[1], Junzo TSUKAHARA[2], Jun HASHIMOTO[3], Katsuyuki UEMATSU[4] and Tadashi MARUYAMA[5]
(1) *Enoshima Aquarium, 2-19-1 Katase-kaigan, Fujisawa, Kanagawa, Japan, 251-0035.*
Tel. +81-466-29-9967, Fax+81-466-29-9974, E-mail: miyake@enosui.com
(2) *Department of Chemistry and Bioscience, Faculty of Science, Kagoshima University, Kagoshima, Japan*
(3) *Marine Biology and Dynamics Division, Faculty of Fisheries, Nagasaki University, Nagasaki, Japan*
(4) *Marine Technology Center, Department of applied Ocean Engineering Research Facility Group, Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Kanagawa, Japan*
(5) *Marine Biology and Ecology Research Program, Extremobiosphere Research Center, Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Kanagawa, Japan*

Abstract: Vestimentiferan tubeworms are kept year-round at the Enoshima Aquarium and JAMSTEC. The tanks of vestimentiferan tubeworms have controlled low dissolved oxygen concentrations and added Na_2S and CO_2 to keep the animals in good condition. In addition, in order to observe vestimentiferan tubeworm behavior in its tube, vestimentiferan tubeworms have been placed into transparent vinyl tubes. The larval development of the vestimentiferan species *Lamellibrachia satsuma* was observed. Monospermy fertilization of oocytes by internal fertilization was observed. Fertilized eggs developed into trochophores and settled on the bottom of culture vessels. Scanning electron microscopy revealed a mouth in the trochophore stage after settlement. Bacteria were observed on the cilia of the mouth and in the guts of trochophores. Trochophores may eat microorganisms such as bacteria. Larvae which did not attach to suitable substrata swam for more than one month without any degeneration of the cilia ring. The larvae of *L. satsuma* were maintained in the laboratory for 45 days. *L. satsuma* have been found at Kagoshima Bay, Nankai Trough, and Izu-Ogasawara Ridge. The fast current speed of the Kuroshio (Black Current) and the length of the planktonic larval life of *L. satsuma* may have enabled this species to distribute in the Kuroshio Subgyre area.

Keywords: Hydrothermal Vents • Vestimentifera • Larval development • *Lamellibrachia satsuma* • Rearing methods • Planktonic life

Introduction

Vestimentiferan tubeworms are common organisms in chemosynthetic ecosystems around hydrothermal vents and cold seeps. They are mouthless and gutless organisms that live in association with endosymbiotic chemoautotrophic bacteria. Vestimentiferan tubeworms which live at hydrothermal vents or cold seeps need to constantly move and re-colonize other hydrothermal vents and cold seeps that are distributed in patches on the deep sea floor. There are two major unanswered questions regarding the life cycle of vestimentiferan tubeworms. The first question is how larvae reach new habitats and settle there and the other question is when and how the symbiosis between the worms and bacteria is established. Southward (1988) and Jones & Gardiner (1989) studied the early development of

a *Ridgeia* species using newly settled juveniles. On the other hand, Young et al. (1996) studied embryology in *Lamellibrachia* sp. and *Escarpia* sp. from the zygote to trochophore stage using eggs and sperm that were dissected from adults. Also Marsh et al. (2001) reported embryogenesis and larval dispersion of *Riftia pachyptila* Jones, 1981 from East Pacific Rise. However, the observation from the trochophore larvae stage to settlement of trochophore has not been reported. To answer the remaining questions regarding vestimentiferan life cycles, investigations of all development stages from egg to adult and the development of a culturing/rearing system of vestimentiferan tubeworms are needed.

Materials and methods

Sampling vestimentiferan tubeworm

Vestimentiferan tubeworms *Lamellibrachia satsuma* Miura et al., 1997 were collected at a depths of 82 ~ 105 m in Kagoshima Bay (31°39'N, 130°48'E) using manipulators of the ROVs *Dolphin-3K* and *Hyper-Dolphin* of JAMSTEC. The habitat of this species is hydrothermal vent and volcanic fumaroles (Hashimoto et al., 1993; Miura et al., 1997).

Rearing tank

A rearing tank was designed to maintain adult vestimentiferan tubeworms in good condition (Fig. 1). Temperature, dissolved oxygen, pH and hydrogen sulfide concentration are regulated at similar levels to those encountered in vestimentiferan tubeworm habitats. A canister filter (EHEIM) is used for water filtration. Dissolved oxygen is regulated at a low concentration (1.5ml.l^{-1}) by bubbling nitrogen gas using Dissolved Oxygen Concentration Control System (DOCCS; Miyake et al., 2005). When the DO concentration is too low (~ 0.0 ml.l^{-1}), oxygen is added by aeration. The pH is maintained at low levels (~ 6.8-7.0) by bubbling carbon dioxide using a pH controller. When the pH is high (> 7.0), an electromagnetic valve connected to the pH controller automatically opens and CO_2 input starts. When the pH is low (< 6.8), the valve closes and CO_2 input stops. CO_2 is also added for chemoautotrophic bacteria as a source of carbon. A solution of sodium sulfide (500 g Na_2S/20 L_{aq}) is added to the 40 L tank at a rate of 2.25 ml for every 20 minutes. The solution produces hydrogen sulfide (H_2S) in low pH rearing water. Mud collected from the original vestimentiferan tubeworm habitat was laid on the bottom of the tank at a thickness of ~ 5-10 cm.

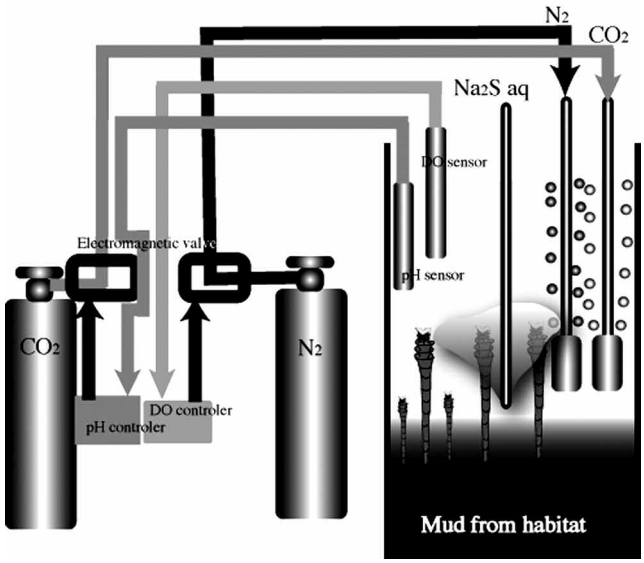

Figure 1. Rearing tank for vestimentiferan tubeworms.
Figure 1. Système d'élevage des Vestimentifères.

Methods of vestimentiferan tubeworms observation

The opaque chitinous tube that covers the worms makes *in situ* observations of the behavior of the worm impossible. We made observations possible by transplanting the *L. satsuma* into a transparent vinyl tube. At first, the original chitinous tube was gradually pared down from the tip until the worm could not retract into the tube. Subsequently, a transparent tube was connected to the cut end of the original tube. The tubeworm then moved on its own to the transparent tube (Fig. 2).

Larval development

Fertilized eggs of *L. satsuma* were collected by dissecting adult females. The culturing temperature was 16°C. Larvae and embryos were kept in twelve replicate petri dishes (6 cm in diameter and 4 cm high) filled with 0.22 µm filtered seawater that was changed daily. However, these culture dishes were not completely bacteria-free as the ambient water around larvae or fertilized eggs was contaminated at the starting point of cultivation. The observation of larvae was conducted by stereomicroscope, light microscope, scanning electric microscope, and transmission electron microscope.

Results and Discussion

Rearing

Before making our rearing tank, *L. satsuma* was kept in the tank with only regulated temperature and added Na_2S

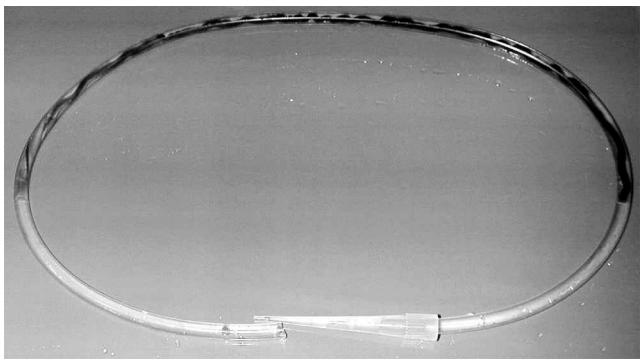

Figure 2. A vestimentiferan tubeworm *Lamellibrachia satsuma* introduced into transparent plastic tube.

Figure 2. Un individu de l'espèce vestimentifère *Lamellibrachia satsuma* introduit dans un tube plastique transparent.

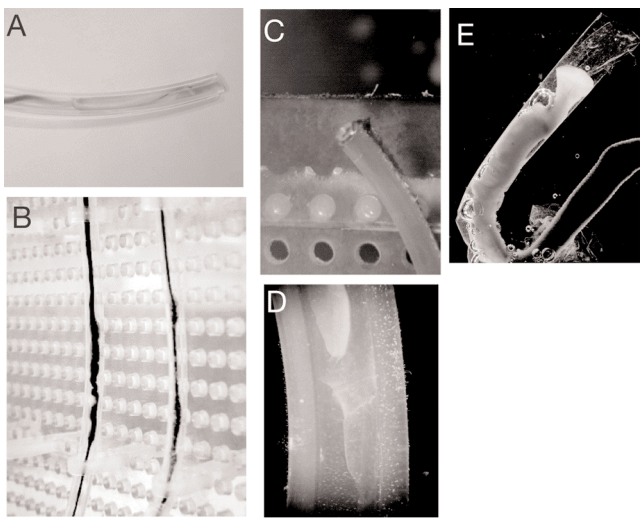

Figure 3. Observation of vestimentiferan tubeworm behavior in transparent tube. **A.** Vestimentum behavior. **B.** Trunk behavior. **C.** New tube production visible at the tip of the plastic housing. **D.** Tube production at the posterior end of vestimentiferan tubeworm. **E.** Newly produced tube with tube worm removed from plastic housing.

Figure 3. Observation du développement d'un vestimentifère dans un tube transparent. **A.** Aspect du vestiment. **B.** Aspect du tronc. **C.** Nouvelle production du tube visible à l'extrémité de l'enveloppe plastique. **D.** Production du tube dans la partie postérieure du vestimentifère. **E.** Tube nouvellement produit par le ver retiré de l'enveloppe plastique.

(Miura et al., 1997). In this tank, branchial filaments of *L. satsuma* were not always extended. *L. satsuma* grew by tube elongation at the anterior and posterior tips of tubes. The vestimentiferan tubeworms extended their branchial filaments in the tanks as observed in the field. Our rearing system was shown to provide an adequate environment not only for tubeworms, but also for hydrothermal vent crabs (*Austinograea yunohana* Takeda et al., 2000), hydrothermal vent shrimps (*Opaepele* sp.), hydrothermal vent squat lobsters (*Shinkaia crosnieri* Baba & Williams, 1998), and hydrothermal vent and cold seep mussels (*Bathymodiolus septemdierum* Hashimoto & Okutani, 1994, *Bathymodiolus hirtus* Okutani et al., 2004 and *Bathymodiolus securiformis* Okutani et al., 2004). They are displayed under atmospheric pressure year-round in the Deep-Sea Exhibition at the Enoshima Aquarium.

Observation method

The vestimentiferan tubeworm *L. satsuma* moved towards the opening of the vinyl tube in a slow peristaltic motion using the muscles of the vestimentum (Fig. 3A). The retraction towards the inner part of the tube however, was rapid due to fast contraction of the muscles of the trunk and contraction of the vestimentum. This very quick retraction response may be a part in tubeworm escape behavior. The trunk did not always appear as regular cylinder; sometimes the trunk had constricted parts and resembled a string of beads (Fig. 3B). Behavior such as peristaltic movements and occasionally wave-like movements in the trunk were observed. These actions may be helpful in the circulation of body fluids, and/or in making a suitable environment for endosymbiotic bacteria. Tube production was observed at the tip of the transparent tubes and inside of the tubes (Fig. 3C-D). Behavior of vestimentiferans in their tubes, the growth of individuals, and the process of tube production may be clarified by using the method outlined in this report, and in addition, is a very effective exhibition method for facilities such as public aquaria.

Larval development (Fig. 4)

The fertilization of oocytes in the ovisac and oviduct by internal fertilization occurs at high rates in *L. satsuma* (Tsukahara et al., 2004). Brooding of larvae was not observed. These results were the same as reported by Hilario et al. (2005). The fertilized egg size had a diameter of 100 µm (Fig. 4A). The first cleavage occurred four hours after dissection (Fig. 4B). Two hours after the first cleavage, the embryo was in the four-cell stage (Fig. 4C). After 24 hours from dissection, the embryo was in the blastula stage and began to swim using cilia (Fig. 4D). Five-day old embryos developed into trochophores (160 µm in length, 130 µm in diameter, (Fig. 4E).) and then after two more days (seven days total), trochophores elongated their body to 200 µm in length and become pear-shaped (Fig. 4F). Trochophore larvae had prototrochal rings, telotrochal rings and ventral lines of cilia (Fig. 4G). 10 day old larvae settled on the bottom of a petri dish, bacterial films on the bottom or water surface of a petri dish or on dust particles originating from the air. In 12 day old larvae

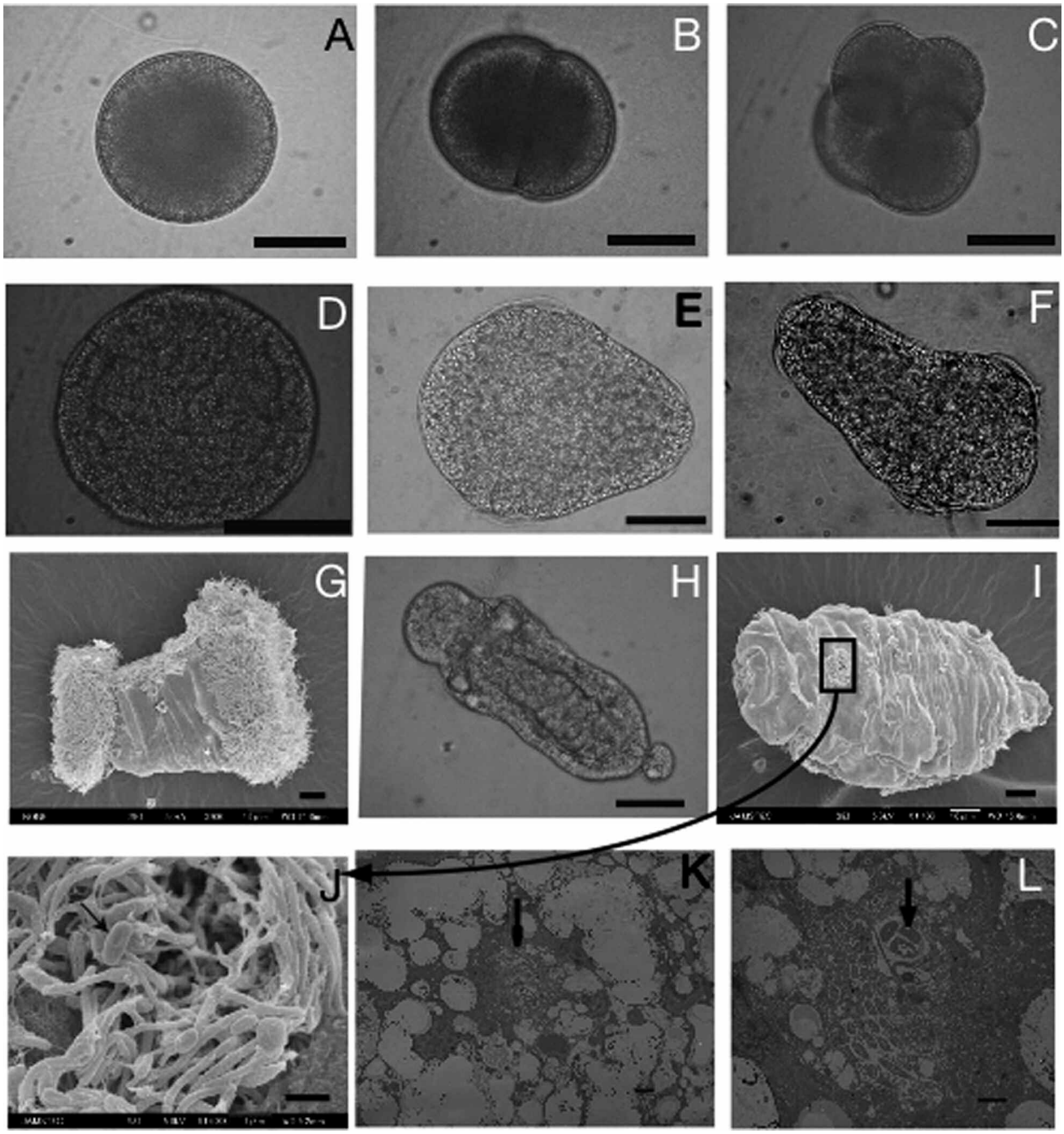

Figure 4. Development of larval vestimentiferan tubeworms, *Lamellibrachia satsuma*, cultured at atmospheric pressure. **A.** Fertilized egg just after dissection. **B.** The two-cell stage (four hours old). **C.** The four-cell stage (six hours old). **D.** The blastula stage (one day old). **E.** Early trochophore stage (five days old). **F.** Trochophore stage (seven days old). **G.** SEM image of a trochophore (seven days old). **H.** Trochophore stage just after settlement (12 days old). **I.** SEM image of settled larva (15 days old). The rectangular area is the mouth. **J.** Bacteria (arrow) on the cilia of mouth. **K.** The intestinal (arrow) tract of 13 day larva. **L.** Bacteria (arrow) in the interstinal tract of 13 day larva. Scale bar = 50 µm (A, B, C, D, E, F, H), 10 µm (G, I) and 2 µm (K) and 1 µm (J, L).

Figure 4. Développement larvaire du vestimentifère *Lamellibrachia satsuma* élevé à pression atmosphérique. **A.** Oeufs fécondés juste après dissection. **B.** Stade deux cellules (4 h). **C.** Stade quatre cellules (6 h). **D.** Stade blastula (1 jour). **E.** Jeune stade trochophore (5 jours). **F.** Stade trochophore (7 jours). **G.** Photographie au MEB d'une trochophore (7 jours). **H.** Stade trochophore après la sédentarisation (12 jours). **I.** Photographie au MEB d'une larve sédentarisée (15 jours). Le rectangle indique la bouche. **J.** Bactérie (flèche) sur un cil de la bouche. **K.** Le tractus intestinal (flèche) d'une larve de 13 jours. **L.** Bactérie (flèche) dans le tractus intestinal d'une larve de 13 jours. Echelles = 50 µm (A, B, C, D, E, F, H), 10 µm (G, I) and 2 µm (K) and 1 µm (J, L).

(200 µm in length), constriction around telotrochal ring and structure similar to an intestinal tract were observed (Fig. 4H). These cilia degenerated after settlement except for in the region around the mouth (Fig. 4I). Scanning electron microscopy revealed a mouth in the trochophore stage (Fig. 4I). Bacteria were observed by SEM on the cilia of the mouth (Fig. 4I, J) and by TEM in the intestinal tract (Fig. 4K, L) in later stage larvae. Larvae that did not attach to suitable substrata swam for more than one month without any degeneration of the cilia ring.

L. satsuma has been found at Kagoshima Bay (between 82-105 m, 31°39'N, 130°48'E), the Kanesu-no-Se Bank in Nankai Trough (between 34°17'-18'N, 138°15'E, 290-330 m depth) (Kojima, 2002), the Nikko Seamount (23°05'N, 142°20'E, 430 m) (Kojima, 2002) and the Daikoku Seamount (21°19'N, 144°11'E, 410 m) (Tsuchida et al., 2006) at Izu-Ogasawara Ridge and north Mariana Trough, respectively. The distribution of *L. satsuma* was in the Kuroshio Subgyre, which consists of the Kuroshio Current and the Kuroshio Counter Current. The distribution of scyllarid phyllosoma larvae and larval recruitment has been explained by the hydrodynamics of the Kuroshio Subgyre (Inoue & Sekiguchi, 2005). Marsh et al. (2001) estimated dispersal distance rarely exceeded 100 km based on the metabolic life span. The larvae of *L. satsuma* were maintained in the laboratory for 45 days. The current speed of Kuroshio's upper 1000 m depth layer is over 0.5 $m.s^{-1}$ (Chaen & Ichikawa, 2001). The buoyancy of eggs were neutral at atmospheric pressure, and we estimate the dispersal distance in 45 days to be approximately 2000 km. Adult *L. satsuma* live in environments of around 12-16°C and can endure high temperatures to 25°C (personal observation). This temperature tolerance matches the temperature environment of the Kuroshio. The length of life of planktonic larvae of *L. satsuma* observed in these experiments may explain their distribution in the Kuroshio Subgyre area. Planktonic larvae cultured in filtered seawater contaminated with obvious microorganisms such as bacterial films and protozoa lived longer than larvae in filtered sea water with less contamination. This suggests that vestimentiferan larvae may eat microorganisms such as bacteria. Trochophores attached to any substrate upon which they settled. However, larvae did not develop into tubeworms. Metamorphosis into the juvenile stage may require the acquisition of symbionts.

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References

Chaen M. & Ichikawa H. 2001. *Kuroshio*. Shun-en-do: Kagoshima, Japan. 228 pp. (japanese)

Hashimoto J., Miura T., Fujikura K. & Ossaka J. 1993. Discovery of vestimentiferan tube-worms in the euphotic zone. *Zoological Science*, **10**: 1063-1067

Hilario A., Young S.M. & Tyler P.A. 2005. Sperm storage, internal fertilization, and embryonic dispersal in vent and seep tubeworms (Polychaeta: Siboglinidae: Vestimentifera). *Biological Bulletin,* **208**: 20-28.

Inoue N. & Sekiguchi H. 2005. Distribution of scyllarid phyllosoma larvae (Crustacea: Decapoda: Scullaridae) in the Kuroshio Subgyre. *Journal of Oceanography*, **61**: 389-398.

Jones M. & Gardiner L. 1989. On the early development of the vestimentiferan tube worm *Ridgeia* sp. and observation on the nervous system and trophosome of *Ridgeia* sp. and *Riftia pachyptila*. *Biological Bulletin*, **177**: 254-276.

Kojima S. 2002. Deep-sea chemoautosynthesis-based communities in the northwestern Pacific. *Journal of Oceanography*, **58**: 343-363.

Marsh A.G., Mullineaux L.S. Young C.M. & Manahan D.T. 2001. Larval dispersal potential of the tubeworm *Riftia pachyptila* at deep-sea hydrothermal vents. *Nature*, **411**: 77-80.

Miura T., Tsukahara J. & Hashimoto J. 1997. *Lamellibrachia satsuma*, a new species of vestimentiferan worms (Annelida: Pogonophora) from a shallow hydrothermal vent in Kagoshima Bay, Japan. *Proceedings of the Biological Society of Washington*, **110**: 447-456.

Miyake H., Yamamoto H., Kitada M., Ueda I., Okoshi K., Kitamura M., Matsuyama K. & Tsuchida S. 2005. Attempts to rear the deep-sea white clams *Calyptogena soyoae* and *Calyptogena solidissima*. *Oceanography in Japan*, **14**: 645-651. (Japanese with English abstract and figure captions)

Southward E. 1988. Development of the gut and segmentation of newly settled stage of *Ridgeia* (Vestimentifera): implications for relationship between Vestimentifera and Pogonophora. *Journal of the Marine Biological Association of United Kingdom*, **68**: 465-487.

Tsuchida S., Yamaguchi H., Nakamura K., Inagaki F., Fujikura K., Dower J.F. & Embley R.W. 2006. Geographical distribution of hydrothermal vent communities in northern Mariana Arc. *Abstracts of the 22th Shinkai Symposium*: 227.

Tsukahara J., Miyake H., Hashimoto J., Miura T., Hatano N. & Hara M. 2004. Internal fertilization of vestimentiferan tubeworm, *Lamellibrachia satsuma*. *Abstracts of the 20th Shinkai Symposium*: 127-128.

Young C., Vázquez E., Metaxas A. & Tyler P. 1996. Embryology of vestimentiferan tube worms from deep-sea methane/sulphide seeps. *Nature*, **381**: 514-516.