



A New Species of *Osedax* (Annelida: Siboglinidae) Associated with Whale Carcasses off Kyushu, Japan

Authors: Fujikura, Katsunori, Fujiwara, Yoshihiro, and Kawato, Masaru

Source: Zoological Science, 23(8) : 733-740

Published By: Zoological Society of Japan

URL: <https://doi.org/10.2108/zsj.23.733>

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

A New Species of *Osedax* (Annelida: Siboglinidae) Associated with Whale Carcasses off Kyushu, Japan

Katsunori Fujikura*, Yoshihiro Fujiwara and Masaru Kawato

Marine Biology and Ecology Research Program, Japan Agency for Marine-Earth Science and Technology (JAMSTEC), 2-15 Natsushima-cho, Yokosuka, Kanagawa 237-0061, Japan

A new whale-bone-eating polychaete species of the genus *Osedax* was found on sperm whale carcasses submerged off Cape Nomamisaki, Kyushu, Japan, at a depth of approximately 200 m. The new species, *Osedax japonicus*, is the fourth known species of the genus *Osedax* and the first species from the western Pacific. Female *O. japonicus* specimens (1) form dense clusters on whale carcasses; (2) have a body composed of crown, trunk, and root structure; (3) lack a digestive tract; and (4) have bacterium-like particles in the tissue of the root structure. *Osedax japonicus* shares all these characteristics with *O. rubiplumus* and *O. frankpressi*, and items (1) to (3) with *O. mucofloris*. *Osedax japonicus* is easily distinguished from the other three known species by oviduct morphology, body length, and palp coloration in females. No males of *O. japonicus* have yet been found.

Key words: *Osedax japonicus*, whale carcass, Siboglinidae, root structure, bacterium-like particles, Annelida, Polychaeta, ROV

INTRODUCTION

Decomposing whale carcasses on the deep-sea floor are home to unique and dense biological communities. These communities derive energy from initially large concentrations of fresh organic matter that result in substantial amounts of sulfide. Such communities, like deep-sea vent and seep communities that are supported by chemosynthetic production, are classified as chemosynthetic (Smith *et al.*, 1989; Smith, 1992; Naganuma *et al.*, 1996; Tunnicliffe *et al.*, 1998). At least 43 mega/macro-benthic species were reported to be associated with whale carcasses at a depth of 1,240 m in the Santa Catalina Basin off California (Bennett *et al.*, 1994).

Two polychaete species of the genus *Osedax*, *O. rubiplumus* Rouse, Goffredi, and Vrijenhoek, 2004 and *O. frankpressi* Rouse, Goffredi, and Vrijenhoek, 2004, were described from the bones of a gray whale carcass at a depth of 2891 m in Monterey Bay, California (Rouse *et al.*, 2004). A third species, *O. mucofloris* Glover, Källström, Smith, and Dahlgren, 2005 was subsequently described from an implanted Minke whale carcass at a depth of 125 m in Kosterfjord, Sweden, the North Sea (Glover *et al.*, 2005). *Osedax* is placed in the annelid family Siboglinidae (Rouse *et al.*, 2004), which also includes vestimentiferans and frenulates (pogonophores), all of which lack a digestive tract in adults. The *Osedax* worms have root structures running within the matrix of the bone substratum, and have dwarf males. *Osedax rubiplumus* and *O. frankpressi* have endo-

sympiotic bacteria in their root-structure tissues, and analyses of stable isotopes and fatty acids have revealed that the endosymbionts provide the nutrition of these species (Goffredi *et al.*, 2005).

On 1 February 2002, 12 sperm whale carcasses (11.8–16.0 m in body length) that had been stranded on the southwestern coast of Kyushu, southern Japan, were submerged at a depth of approximately 200 m off Cape Nomamisaki, Kagoshima, Kyushu. Two successive investigations using the remotely operated vehicle (ROV) *Hyper-Dolphin* were conducted at this site in 2004 to study the faunal composition and community succession associated with whale carcasses. Two whale bones were recovered by the ROV and subsequently maintained in a laboratory aquarium, and worms were observed in dense clusters on the whale-bone surfaces. This worm species appeared to have characteristics of the genus *Osedax* and is described here.

MATERIALS AND METHODS

Sample collection and *in-situ* observation

Dives of the ROV *Hyper-Dolphin* of the Japan Agency for Marine-Earth Science and Technology (JAMSTEC) were conducted from 24 July to 1 August 2004, at sites where four whale carcasses had been deposited at depths of 224–250 m off Cape Nomamisaki, approximately 20 km offshore from the East China Sea coast of Kagoshima Prefecture, southwestern tip of Kyushu Island, Japan (Fig. 1). These surveys were conducted 2.5 years after deposition of the whale carcasses on the sea floor. The bottom water temperature was 14–15°C. *In-situ* observations were made using a digital Hi-Vision TV camera mounted on the ROV. Two whale bones, a vertebra and an ulna, were collected with the ROV manipulator at 31°20.72' N, 129°59.29' E at a depth of 227 m, and at 31°18.84' N, 129°59.52' E at a depth of 245 m, respectively. These recovered bones were kept in artificial seawater (Rothemarine, Rei-Sea, Tokyo, Japan) at 13–14°C in the laboratory. Several worm speci-

* Corresponding author. Phone: +81-46-867-9560;
Fax : +81-46-867-9525;
E-mail: fujikura@jamstec.go.jp
doi:10.2108/zsj.23.733

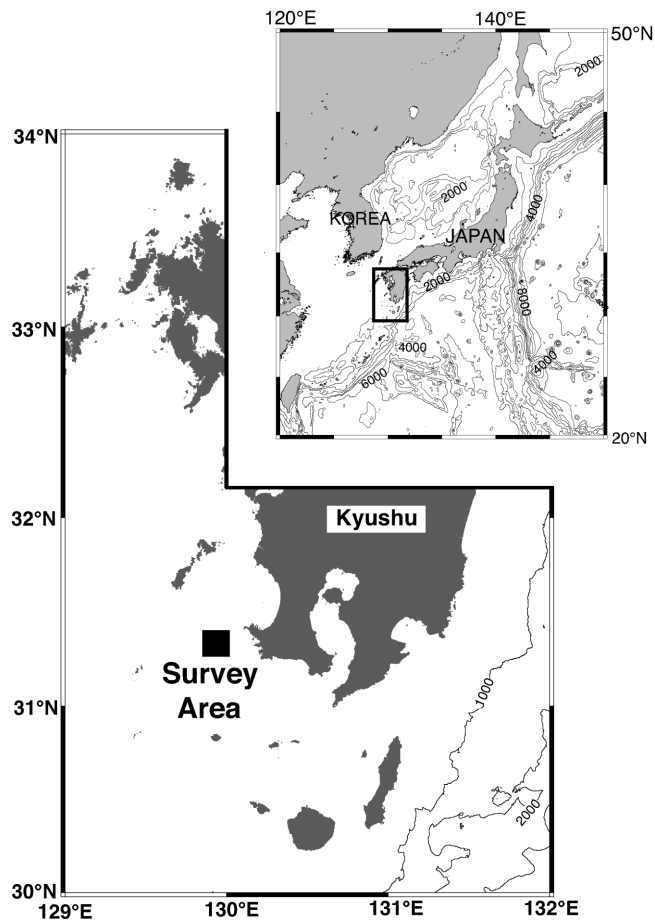


Fig. 1. Location map of the ROV *Hyper-Dolphin* dive off Cape Nomamisaki, the East China Sea coast of Kagoshima Prefecture, Kyushu, Japan.

mens were excavated from both bones and were preserved for morphological, molecular biological, and histological analyses. The type materials are deposited in the National Science Museum, Tokyo (NSMT) and the University Museum, University of Tokyo (UMUT).

Morphological analysis

After specimens were extracted from the whale bones, three specimens were fixed in 10% buffered seawater-formalin, five were fixed in 10% buffered seawater-formalin for a week and then transferred to 70% ethanol, and two were fixed in 99.5% ethanol for a week and then transferred to 70% ethanol. For detailed observations of internal organs, the tissues of three specimens were fixed in Bouin's solution for 24 h. The tissues were dehydrated with 100% ethanol, embedded in paraffin, sectioned in 4- μ m-thick strips, and stained with hematoxylin-eosin following standard procedures.

DNA preparation

DNA was extracted from the trunk and root structure from each of 4 worm specimens separately with a DNeasy Tissue kit (QIAGEN Japan, Tokyo, Japan). To eliminate surface contaminants, each tissue sample was washed thoroughly in autoclaved and filtered (pore size 0.22 μ m) seawater.

PCR amplification

The eukaryotic cytochrome c oxidase subunit I gene (COI) was amplified by PCR using an Ex Taq PCR Kit (TaKaRa, Kyoto,

Japan). Two oligonucleotide primers (0.2 μ M each) and <1 μ g of DNA template were added to the reaction mixtures. Thermal cycling conditions were denaturation at 96°C for 20 sec, annealing at 50°C for 45 sec, and extension at 72°C for 2 min for a total of 35 cycles. The oligonucleotide primers used for COI amplification were LCO1490 and HCO2198 (Folmer *et al.*, 1994). The molecular size of the PCR products was checked using 1.2% Agarose S (Nippon Gene, Toyama, Japan) gel electrophoresis.

Sequencing of amplified products

Sequencing reactions using the PCR products as template were performed with a BigDye Terminator Cycling Sequencing Ready Reaction Kit (PE Applied Biosystems, Foster City, CA, USA), using primers LCO1490 and HCO2198. Sequencing was accomplished with an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems Japan Ltd., Tokyo, Japan). The sequences reported here have been deposited in the DDBJ database under accession numbers AB259569.

Sequence and phylogenetic analyses

A partial sequence of COI was analyzed using the gapped-BLAST search algorithm (Altschul *et al.*, 1997; Benson *et al.*, 2000) to estimate the degree of similarity to other sequences deposited in the GenBank database. For the similarity analysis, 507 bp of homologous sites of the COI gene were used. Sequences were manually aligned, and the phylogenetic analysis was restricted to nucleotide positions that were unambiguously alignable in all sequences. We analyzed phylogenetic relationships among the new and the other siboglinid species using COI gene sequences deposited in the DDBJ database under accession numbers AB073491, AF34267, AY129128, AY12914, AY129140, AY326304, AY586490,

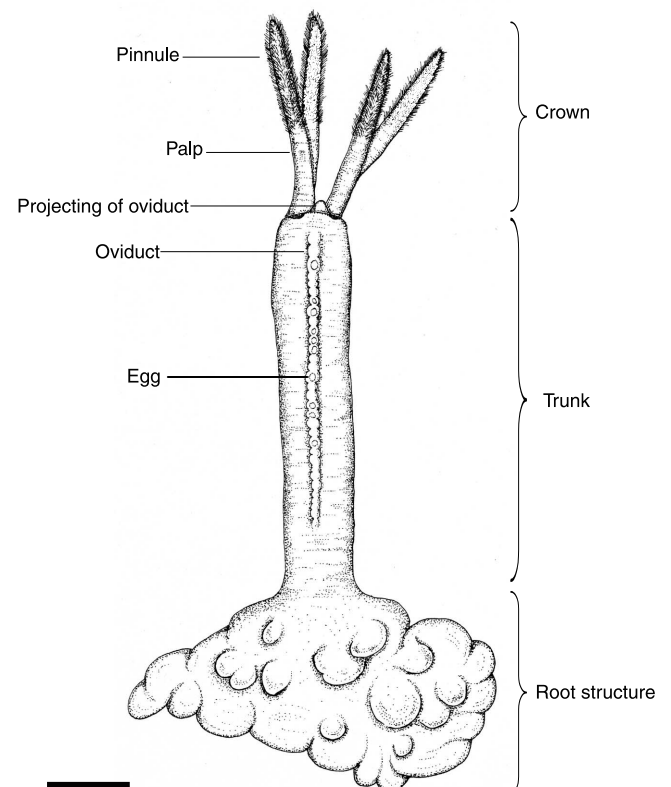


Fig. 2. *Osedax japonicus* n. sp. Holotype, female, drawing of preserved specimen in ventral view (gelatinous translucent cylindrical tube and transparent mucus removed). Scale bar=1 mm.

AY586505, AY645989, AY646000, AY827562, ESU84262, LCU74061, OAU74069, RPU87979, and U74066. Determination of the distance matrix and a neighbor-joining (NJ) tree was performed with CLUSTAL X (Thompson *et al.*, 1997).

Transmission electron microscopic observations of associated bacteria

Small pieces of tissue from the palps, trunks, and bulbous

structure from two specimens were sliced into 2-mm sections. The tissues were prefixed with 3% glutaraldehyde and 3% acrolein in filtered artificial seawater for 12 h at 4°C; post-fixed for 3 h in 2% OsO₄ in filtered artificial seawater; rinsed eight times for 15 min with distilled water; stained with 2% uranyl acetate for 90 min; rinsed again three times for 15 min with distilled water; dehydrated in a series of 30%, 50%, 70%, 80%, 90%, 95%, and 100% ethanol; and embedded in EPON 812 resin (TAAB, Berkshire, England). Ultra-

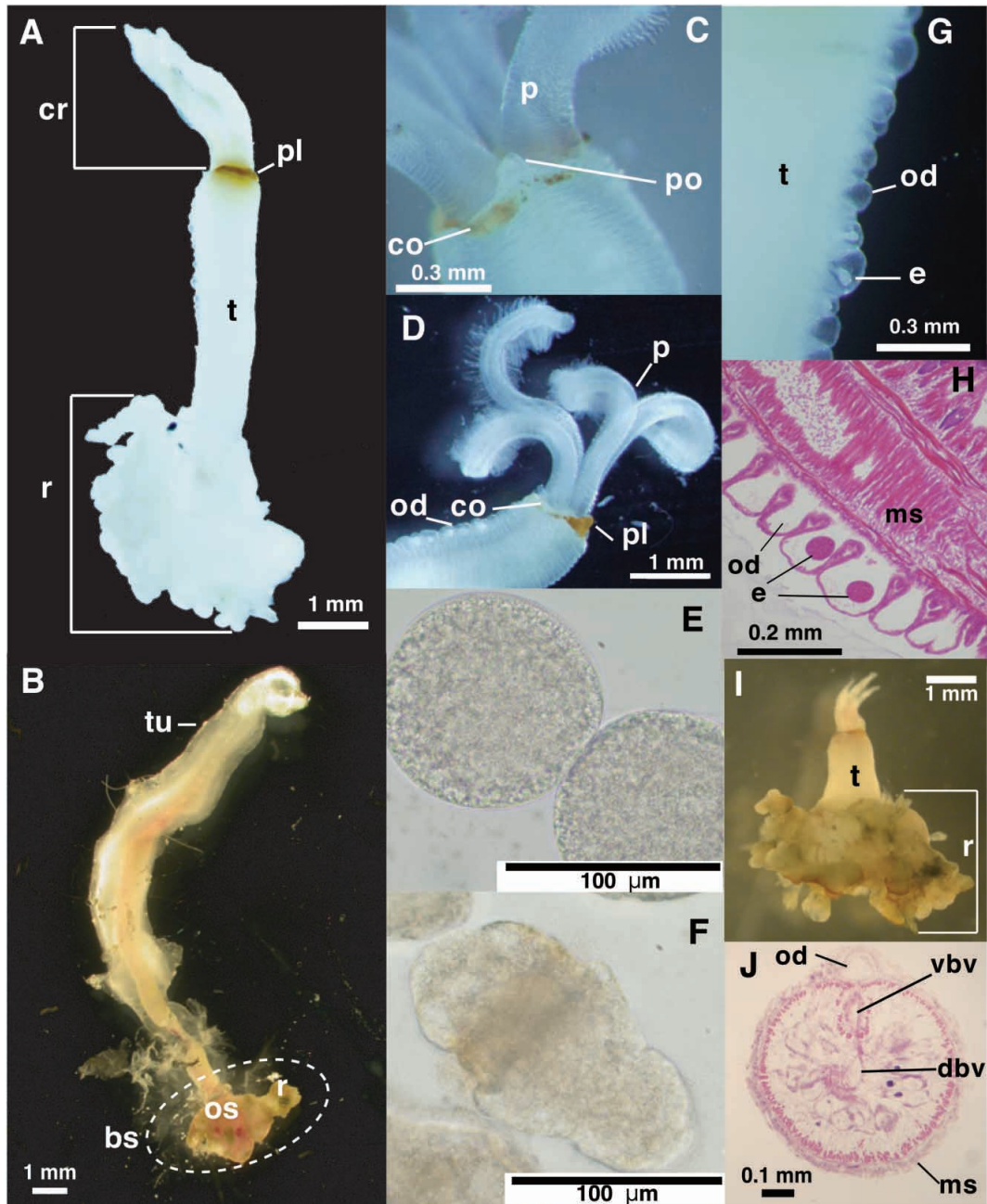


Fig. 3. *Osedax japonicus* n. sp. (A) Holotype, female, preserved individual; body from the dorsal side, gelatinous translucent cylindrical tube and transparent mucus removed. (B) Paratype 1, dissected from whale bone, fresh specimen, no preservation; contracted individual in gelatinous translucent cylindrical tube, with transparent mucus removed; root structure circled by a dashed line. (C) Holotype, close-up view of the boundary between trunk and crown. (D) Holotype, close-up view trunk and crown. (E) Eggs in mucus. (F) Trochophore-stage embryos in mucus. (G) Holotype, close-up view of the oviduct along trunk; eggs are present in the oviduct. (H) Vertical section of the ventral part of the trunk. (I) Paratype 2, fresh specimen, contracted small individual, gelatinous translucent cylindrical tube and transparent mucus removed. (J) Transverse section of the trunk. Abbreviations: bs, bulbous structure; co, collar; cr, crown; dbv, dorsal blood vessel; e, egg; ms, muscle; od, oviduct; os, ovisac; p, palp; pl, plaques; po, projecting of oviduct; r, root structure; t, trunk; tu, tube; vbv, ventral blood vessel.

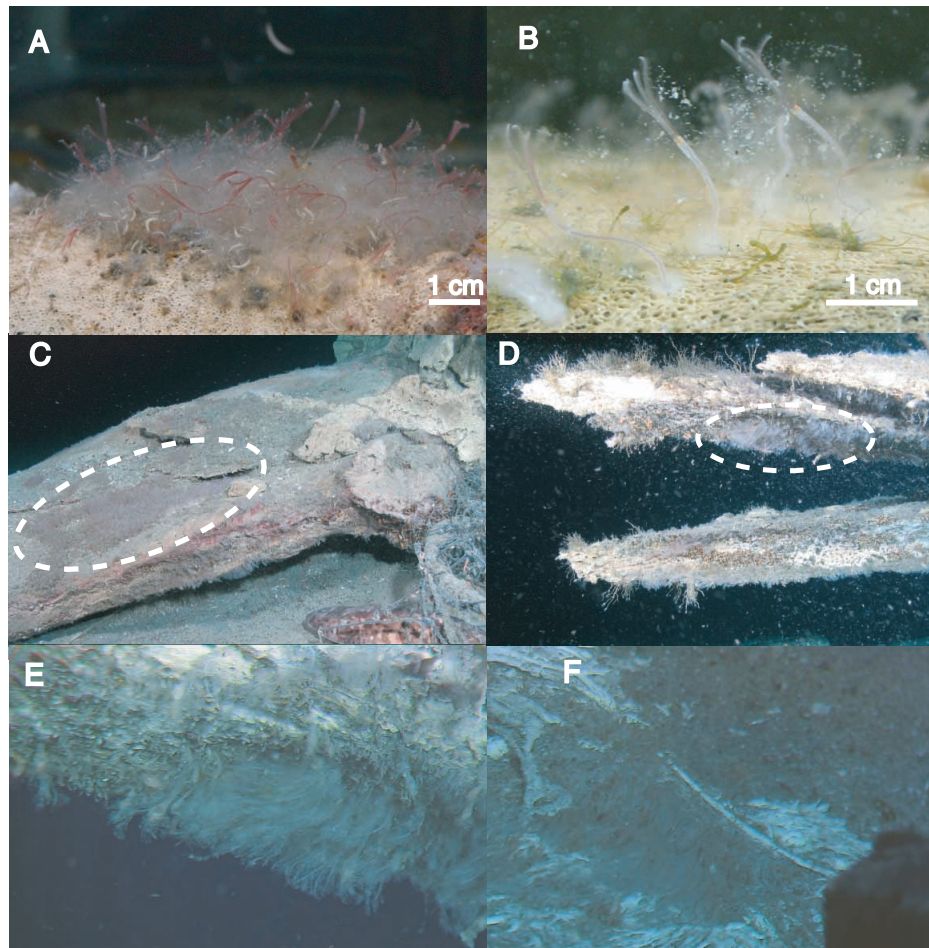
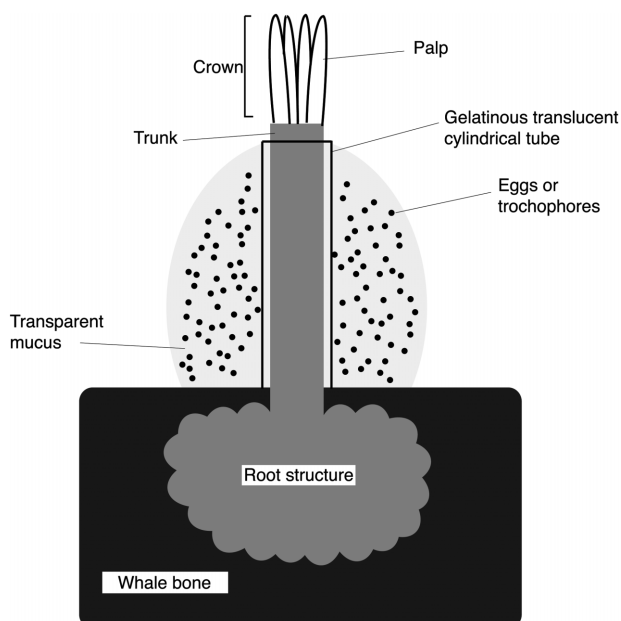


Fig. 4. Habitat of *Osedax japonicus* n. sp. (A) Worms emergent from a whale bone (ulna) in an aquarium. (B) Close-up view of emergent worms; the worms secreted the transparent mucus; numerous white particles (eggs or trochophore-stage embryos) were present in mucus. (C) Maxilla *in-situ*; a dense cluster of worms is circled by a dashed line. (D) Jawbones *in-situ*; emergent worms circled by a dashed line. (E) Close-up view of the dashed circle in D; numerous specimens formed a dense cluster. (F) Numerous living specimens on the surface of a tainted spermaceti.



thin sections of the specimens were stained with aqueous uranyl acetate and lead citrate, and observed under a transmission electron microscope (JEOL JEM-1210, Tokyo, Japan).

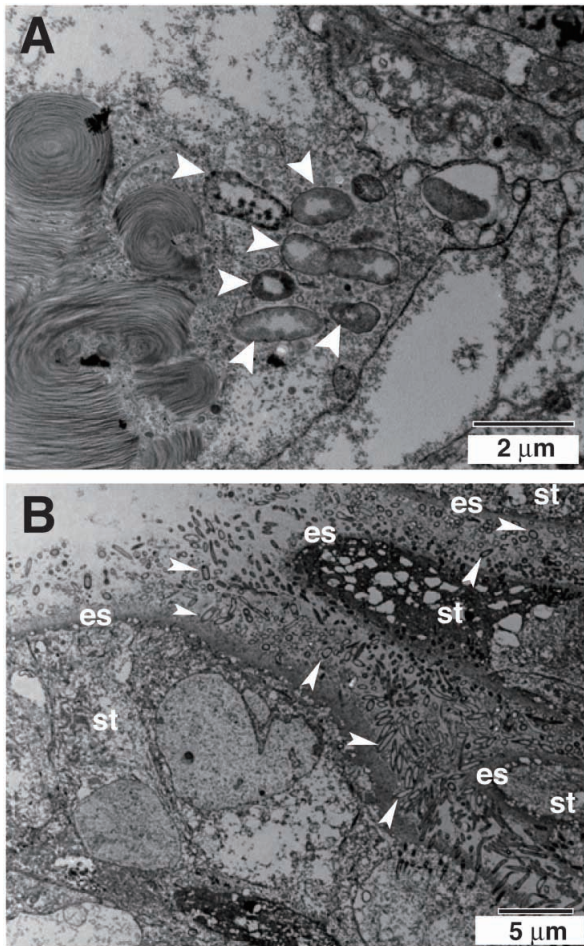
RESULTS

Taxonomic account

Phylum Annelida Lamarck, 1809
 Family Siboglinidae Caullery, 1914
 Genus *Osedax* Rouse, Goffredi and Vrijenhoek, 2004
Osedax japonicus n. sp.
 (Honekui-hanamushi in Japanese)

Type series. Holotype (NSMT-Pol H 478): complete mature female (body length, 8.8 mm; palp length, 2.5 mm; trunk length, 4.3 mm; trunk width, 1.0 mm; root structure

Fig. 5. Schematic diagram of the habitat of *Osedax japonicus* n. sp. The root structure penetrates the whale bone; the trunk lies within a gelatinous, translucent cylindrical tube emergent from the whale bone; the crown is exposed above the secreted the tube and transparent mucus; the mucus contains numerous eggs or trochophore-stage embryos.



length, 2.0 mm; root structure width, 3.2 mm), extracted from an ulna of a dead sperm whale off Cape Nomamisaki, Kagoshima Prefecture, Kyushu, Japan (31°18.84' N, 129°59.52' E, 245 m depth), 28 July 2004, collected by ROV *Hyper-Dolphin* of JAMSTEC, Dive No. 333. Paratype-1 (UMUT-RW29011): complete mature female (body length, 18.0 mm; palp length, 7.0 mm; trunk length, 8.7 mm; trunk width, 2.1 mm; root-structure length, 2.3 mm; root-structure width, 3.6 mm), extracted from a vertebra of a dead sperm whale off Cape Nomamisaki, Kagoshima Prefecture, Kyushu, Japan (31°20.72' N, 129°59.29' E, 227 m depth), 28 July 2004, collected by ROV *Hyper-Dolphin* of JAMSTEC, Dive No. 331. Paratype-2 (NSMT-Pol H 479): complete mature female (body length, 4.4 mm; palp length, 1.4 mm; trunk length, 1.1 mm; trunk width, 1.0 mm; root-structure length, 1.9 mm; root-structure width, 3.8 mm), extracted from a vertebra of a dead sperm whale off Cape Nomamisaki, Kagoshima Prefecture, Kyushu, Japan (31°20.72' N, 129°59.29' E, 227 m depth), 28 July 2004, collected by ROV *Hyper-Dolphin* of JAMSTEC, Dive No. 331.

Non-type material. Seven complete mature females deposited at JAMSTEC: (body length, 4.0–12.0 mm; palp length, 1.0–2.1 mm; trunk length, 1.2–8.8 mm; trunk width, 0.7–1.0 mm; root-structure length, 1.0–4.3 mm; root-structure width, 3.2–3.8 mm).

Fig. 6. Transmission electron micrographs of transverse sections of *Osedax japonicus* n. sp. Arrowheads indicate bacterium-like particles. (A) Intracellular bacterium-like particles in the sheath tissue of the root structure. (B) Numerous bacterium-like particles in deep grooves in the epidermis of the trunk; these particles are attached to the external surface of the body. Abbreviations: es, epidermis of sheath; st, sheath tissue.

Table 1. Characteristics comparison of *Osedax japonicus* n. sp. with the three known species, *O. rubiplumus*, *O. frankpressi* and *O. mucofloris*

	Species			
	<i>O. japonicus</i> n. sp.	<i>O. rubiplumus</i>	<i>O. frankpressi</i>	<i>O. mucofloris</i>
Size and Morphology				
Trunk length (mm)	1.1–8.8	38	4.5	6–8
Color of palps	White to pink	Red	Red with two longitudinal white-striped	White to pink
Pinnules	Outer margin of palps	Outer margin of palps	Inner margin of palps	unknown
Oviduct	No extension between palps, protuberance-like in shape. Eggs in oviduct	Long between palps, cylindrical in shape	Long between palps, convoluted upon contraction	Long between palps. Eggs in oviduct
Trunk-ovisac junction	Absent	Absent	Green bacteriocyte-filled sheath present	Absent
Root structure	Lobulate without branching	Four discrete root	Lobulate without branching	Lobulate, mycelial form
Tube and mucus	Gelatinous translucent cylindrical tube in transparent mucus emergent	Cylindrical tube	Gelatinous hemispherical tube	Gelatinous mucous tube rather than rigid cylindrical tube
Locality				
Area	Off Kyushu, Japan	Monterey Bay, USA	Monterey Bay, USA	Kosterfjord, Sweden
Depth (m)	224–250	2891	2891	125
Whale species of carcass	Sperm whale, <i>Physeter macrocephalus</i>	Gray whale, <i>Eschrichtius robustus</i>	Gray whale, <i>Eschrichtius robustus</i>	Minke whale, <i>Balaenoptera acutorostrata</i>
References	Present study	Rouse <i>et al.</i> (2004)	Rouse <i>et al.</i> (2004)	Glover <i>et al.</i> (2005)

ture width, 0.9–2.8 mm), extracted from a vertebra or an ulna of dead sperm whale off Cape Nomamisaki, Kagoshima Prefecture, Kyushu, Japan (31°20.72' N, 129°59.29' E, 227 m depth or 31°18.84' N, 129°59.52' E, 245 m depth), 28 July 2004, collected by ROV *Hyper-Dolphin* of JAMSTEC Dive No. 331 or 333.

Etymology: The species name “japonicus” refers to the occurrence of this species in Japan.

Diagnosis. Live female with pink or red contractile and discrete crown composed of four palps; white to pink contractile trunk; and root structure (Figs. 2, 3A, 3B, 4A, 4B). Crown and trunk within gelatinous translucent cylindrical tube in transparent mucus emergent from whale-bone surface, palps typically extended from tube in live specimens (Figs. 4A, 4B, 5). Short and terminal oviduct at the end of trunk (Figs. 2, 3C).

Description. Palps 1.0–7.0 mm in length (contracted), of equal length in each specimen, emergent from upper part of trunk, numerous pinnules 0.1 mm long on approximately distal half outer margins of each palp (Figs. 2, 3D). Cylindrical and whitish translucent trunk 1.1–8.8 mm in length and 0.7–2.1 mm in width (contracted), composed mostly of muscles (Figs. 3H, 3J). Collar with brown plaques along edge of anterior trunk, except for ventral portion (Figs. 3A, 3C, 3D). Protuberances along the oviduct on trunk surface contain some eggs (Figs. 2, 3G, 3H). Oviduct terminates at distal end of trunk, level with base of palps (Figs. 2, 3C). In dissected specimens, ventral and dorsal blood vessels are obvious inside the trunk (Fig. 3J). Neither mouth nor obvious gut present. Root structure embedded within whale-bone matrix (Fig. 5). Bulbous or lobulate root structure (1.0–4.3 mm in length and 0.9–3.8 mm in width, contracted) cov-

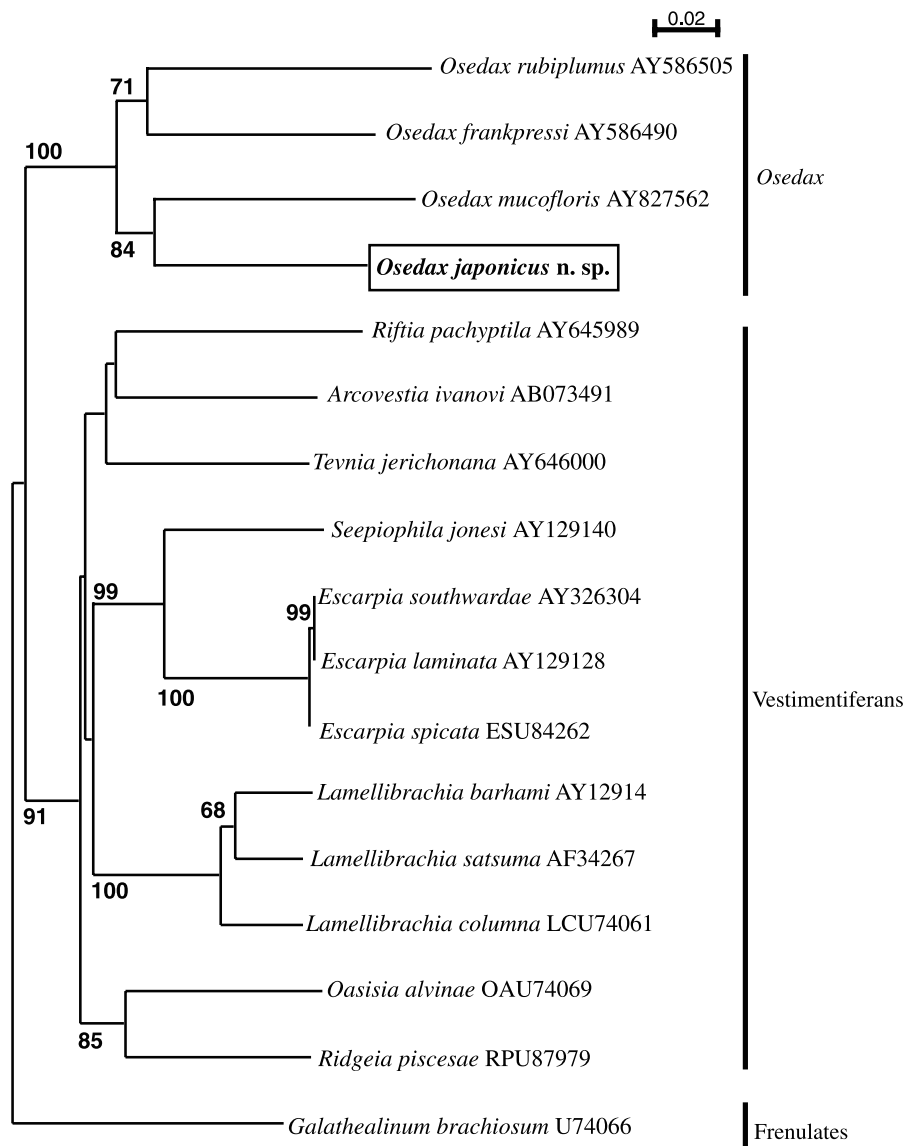


Fig. 7. Phylogenetic tree inferred by neighbor-joining analysis of 507 homologous positions of cytochrome c oxidase subunit I gene (COI) sequences of *Osedax japonicus* n. sp. and representative annelids including frenulate and vestimentiferan tubeworms. Scale bar represents 0.02 nucleotide substitutions per sequence position. Numbers near nodes indicate the percentage of 1000 bootstrap resamplings that include the nodes; only values >50% are shown. The new *Osedax* species examined in this study is indicated by a box.

ered by brownish-green sheath in life and continuous with root-like protuberances of varying length (Figs. 3A, 3B, 3I). No chaetae or segmentation apparent. Males unknown.

Habitat and distribution. Specimens were usually found living in dense clusters on sperm whale bones (Fig. 4) and were clearly visible to the naked eye in the aquarium (Figs. 4A, 4B). They penetrated into the bone matrix using the root structure (Fig. 5). On ROV video images, many living individuals were also observed on the surfaces of tainted spermaceti (Fig. 4F). All whale carcasses were located in a region of sand sediment. The only known occurrence of *Osedax japonicus* n. sp. is the present study area, off Cape Nomamisaki, southwestern tip of Kyushu Island, Japan, at a depth of 227–245 m.

Reproduction. In nine of 10 type and non-type specimens, transparent mucus surrounding living bodies contained several hundred eggs with a diameter of approximately 100 μm (Figs. 3E, 4B). In one specimen, the transparent mucus contained many trochophore-stage embryos with a body length of approximately 150 μm . Eggs and embryos were not contained in a gelatinous, translucent cylindrical tube (Fig. 3F). No males containing sperm were found.

Associated bacterium-like particles. The root-structure tissues contained small assemblages of intracellular rod-shaped bacterium-like particles, 2.7 μm long along the long axis (Fig. 6A). The numbers of particles here was very low. The epidermis of the trunk had numerous microvilli and was deeply invaginated. Numerous bacterium-like particles were present in these deep grooves on the trunk surface (Fig. 6B). The particles also were present between the pinules of the palps, but fewer than on the trunk surface.

Remarks: *Osedax japonicus* n. sp. resembles *O. mucofloris*, recorded from shallow water (125 m deep) of the Kosterfjord in the North Sea, except in oviduct form. *Osedax mucofloris*, *O. rubiplumus*, and *O. frankpressi* have a long oviduct projecting between the palps, whereas *Osedax japonicus* n. sp. has the oviduct terminating at the end of the trunk (Table 1; Figs. 2, 3C). *Osedax japonicus* n. sp. and *O. rubiplumus* have a similar tube form, and both lack a trunk-ovisac junction, but they differ in size, color of palps, form of oviduct, and root structure (Table 1). *Osedax japonicus* n. sp. and *O. frankpressi* have the same form of root structure, but differ in color of palps, forms of oviduct and gelatinous tube, and presence or absence of trunk-ovisac junction in *O. frankpressi* (Table 1). *Osedax japonicus* n. sp. and *O. rubiplumus* both have numerous pinules on outer margins of each palp, but *O. frankpressi* has pinules on inner margins of each palp (Table 1). These marked morphological differences seem to be significant for delineating species of *Osedax*. Moreover, the validity of our determination has been confirmed by a molecular phylogeny using partial COI sequences of *O. rubiplumus*, *O. frankpressi*, and *O. mucofloris* (see below).

Phylogenetic position inferred from COI gene sequences

Partial sequences (~560 bp) of the cytochrome c oxidase subunit I gene from four specimens of *O. japonicus* n. sp. were identical at homologous positions. A phylogenetic analysis performed by the neighbor joining (NJ) method placed the COI sequence from *O. japonicus* n. sp. within the Family Siboglinidae containing frenulate and vestimentiran

tubeworms that lack digestive organs (Fig. 7). The COI sequence of *O. japonicus* n. sp. differed from those of other siboglinid polychaetes. *Osedax japonicus* formed a clade with the three other *Osedax* species, supported by a bootstrap value of 100%.

DISCUSSION

Osedax japonicus n. sp. is the fourth known species in the genus *Osedax* and the first of this genus from the western Pacific. *Osedax rubiplumus* and *O. frankpressi* from Monterey Bay, California, were shown to have the following characteristics by Rouse *et al.* (2004): 1) associated with whale carcasses in the deep sea, 2) have root structures running within whale-bone marrow, 3) lack a digestive tract, 4) have endosymbiotic bacteria in root-structure tissues and derive nutrition from the degradation of bone-marrow hydrocarbons by the endosymbionts, and 5) have dwarf males. *Osedax japonicus* n. sp. has been shown here to share characteristics 1, 2, and 3.

Small assemblages of intracellular rod-shaped bacterium-like particle were observed in the root structure tissues (Fig. 6). These particles are almost same shape and size as symbiotic bacteria of other Siboglinidae, such as *O. rubiplumus* (Rouse *et al.*, 2004) and *Sclerolium brattstromi* (Southward, 1982). These similarities suggest *O. japonicus* n. sp. also has endosymbiotic bacteria in its root-structure tissues. Other animals harboring symbiotic bacteria, such as *Calypotogena* clams and vestimentiferan tubeworms in vent and seep environments, depend nutritionally on diverse symbiotic bacteria to compensate for minimal or lacking digestive systems. Analyses of stable isotopes and fatty acids have revealed that the endosymbionts are responsible for the nutrition of *O. rubiplumus* and *O. frankpressi* (Rouse *et al.*, 2004; Goffredi *et al.*, 2005). However, very few endosymbiotic bacteria-like particles were distributed in tissues of *O. japonicus* n. sp. If these particles are truly endosymbiotic bacteria, it seems that so few bacteria could not provide sufficient nutrients to the worm. On the other hand, TEM observations showed numerous bacterium-like particles on the epidermis of the trunk (Fig. 6). Future investigations are needed to determine the role of these epidermal bacteria, and whether or not the bacterium-like particles in the root tissues are symbionts.

Females of *O. rubiplumus* and *O. frankpressi* were found to contain microscopic males in their tubes (Rouse *et al.*, 2004). Rouse *et al.* (2004) hypothesized that sex may be environmentally determined in *Osedax*, with the larvae that settle on exposed bones maturing as females and those that land on females becoming males. However, despite detailed examination of the tubes of 10 mature female specimens, we could not find dwarf males in *O. japonicus* n. sp. Glover *et al.* (2005) likewise did not detect in males their examination of at least 50 mature specimens of *O. mucofloris*. We observed numerous eggs in the transparent mucus surrounding gelatinous translucent cylindrical tube of *O. japonicus* n. sp. (Figs. 3E, 4B, 5). Additionally, we found many trochophore-stage embryos in the mucus of one of 10 specimens (Fig. 3F). Rouse *et al.* (2004) showed that the dwarf males of *O. rubiplumus* and *O. frankpressi* resemble typical siboglinid trochophores. However, we judged that all the small individuals contained in the mucus mass of a

female of *O. japonicus* n. sp. (Fig. 3F) were not dwarf males, but rather were true trochophore-stage embryos, because no spermatozoa were observed in the trochophore body. Further investigation will be needed to discover the males.

Polychaetes exhibit diverse reproductive strategies, and gelatinous encapsulation of eggs and/or larvae is common (Rouse and Pleijel, 2001). However, since *O. japonicus* n. sp. is restricted in its habitat to whale carcasses, where it lives in dense clusters (Fig. 4), long distance dispersal may be necessary for embryos of *Osedax* to reach other whale carcasses. It is unknown when the embryos hatch out from the gelatinous mass, or how long the larvae swim freely in water column.

According to the molecular phylogenetic analysis, *O. japonicus* n. sp. forms a clade with the other three known *Osedax* species (Fig. 7). This lends support to placement of these species in a single genus, *Osedax*, which had been established by morphological analyses.

Studies are underway on the roles that whale-fall communities play in cycling organic materials in the deep sea, whale-fall community succession, and the dispersal of vent and seep community species (Smith *et al.*, 1989; Bennett *et al.*, 1994; Baco and Smith, 2003; Smith and Baco, 2003; Goffredi *et al.*, 2004). Our study site (Off Cape Nomamisaki) is only approximately 150 km, 400 km, and 420 km distant from the hydrothermal vent communities in Kagoshima Bay (Hashimoto *et al.*, 1993), the cold seep communities off Kikaijima (Kojima, 2002), and hydrothermal vent communities on Minami-Ensei Knoll in the Okinawa Trough (Hashimoto *et al.*, 1995), respectively. However, *O. japonicus* n. sp., two species of mussel, *Adipicola pacifica* and *A. crypta* (Okutani *et al.*, 2003), and a lancelet, *Asymmetron inferum* (Nishikawa, 2004), have been recorded only from the sperm whale carcasses off Cape Nomamisaki, and have not been found in the neighboring vent and seep communities. The whale-fall community off Cape Nomamisaki may therefore be a unique community with no relationship to neighboring vent and seep communities.

ACKNOWLEDGMENTS

We thank Mr. K. Uematsu of Marine Work Japan for TEM analysis; the captain and crew of the R/V *Natsushima*; the operation team of the ROV *Hyper-Dolphin*; Mr. Ayumu Kimura of NHK and the inhabitants of Oura Town, Kagoshima Prefecture; and Ms. Karen Jacobson for their cooperation and enthusiasm throughout the surveys. Our special thanks are also due to Dr. T. Maruyama, Dr. T. Okutani, Dr. J. Reimer, Dr. S. Tsuchida, and Ms. Y. Sasaki of JAM-STEAC for their enthusiastic contributions to the study. The manuscript was improved by careful review and corrections by Dr. G. Rouse of the South Australian Museum, Dr. R. Vrijenhoek of the Monterey Bay Aquarium Research Institute, an editor, and anonymous reviewers.

REFERENCES

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25: 3389–3402

Baco AR, Smith CR (2003) High species richness in deep-sea chemoautotrophic whale skeleton communities. *Mar Ecol Prog Ser* 260: 109–114

Bennett BA, Smith CR, Glaser B, Maybaum HL (1994) Faunal community structure of a chemoautotrophic assemblage on whale bones in the deep northeast Pacific Ocean. *Mar Ecol Prog Ser* 108: 205–223

Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Rapp BA, Wheeler DL (2000) GenBank. *Nucleic Acids Res* 28: 15–18

Folmer O, Black M, Hoeh W, Luts RA, Vrijenhoek RC (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol Biotechnol* 3: 294–299

Glover GA, Källström B, Smith CR, Dahlgren TG (2005) World-wide whale worms? A new species of *Osedax* from the shallow Biol Sci north Atlantic. *Proc Biol Sci* 272: 2587–2592

Goffredi SK, Paull CK, Fulton-Bennett K, Hurtado LA, Vrijenhoek RC (2004) Unusual benthic fauna associated with a whale fall in Monterey Canyon, California. *Deep Sea Res Part I Oceanogr Res Pap* 51: 1295–1306

Goffredi SK, Orphan VJ, Rouse GW, Jahnke L, Embaye T, Turk K, Lee R, Vrijenhoek RC (2005) Evolutionary innovation: a bone-eating marine symbiosis. *Environ Microbiol* 7: 1369–1378

Hashimoto J, Miura T, Fujikura K, Osaka J (1993) Discovery of vestimentiferan tube-worms in the euphotic zone. *Zool Sci* 10: 1063–1067

Hashimoto J, Ohta S, Fujikura K, Miura T (1995) Microdistribution pattern and biogeography of the hydrothermal vent communities of the Minami-Ensei Knoll in the Mid-Okinawa Trough, western Pacific. *Deep Sea Res Part I Oceanogr Res Pap* 42: 577–598

Kojima S (2002) Deep-sea chemoautotrophy-based communities in the Northwestern Pacific. *J Oceanogr* 58: 343–363

Naganuma T, Wada H, Fujioka K (1996) Biological community and sediment fatty acids associated with the deep-sea whale skeleton at the Torishima Seamount. *J Oceanogr* 52: 1–15

Nishikawa T (2004) A new deep-water lancelet (Cephalochordata) from off Cape Nomamisaki, SW Japan, with a proposal of the revised system recovering the genus *Asymmetron*. *Zool Sci* 21: 1131–1136

Okutani T, Fujiwara Y, Fujikura K, Miyake H, Kawato M (2003) A mass aggregation of the mussel *Adipicola pacifica* (Bivalvia: Mytilidae) on submerged whale bones. *Venus* 63: 61–64

Rouse GW, Pleijel F (2001) *Polychaeta*. Oxford University Press, New York

Rouse GW, Goffredi SK, Vrijenhoek RC (2004) *Osedax*: Bone-eating marine worms with dwarf males. *Science* 305: 668–671

Smith CR (1992) Whale falls. *Chemosynthesis on the deep sea floor*. *Oceanogr* 35: 74–78

Smith CR, Baco AR (2003) The ecology of whale falls at the deep-sea floor. *Oceanogr Mar Biol Annu Rev* 41: 311–354

Smith CR, Kukert H, Wheatcroft RA, Jumars PA, Deming JW (1989) Vent fauna on whale remains. *Nature* 341: 27–28

Southward E (1982) Bacterial symbionts in pogonophora. *J Mar Biol Ass UK* 62: 889–906

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 25: 4876–4882

Tunnicliffe V, McArthur AG, McHugh D (1998) A biogeographical perspective of the deep-sea hydrothermal vent fauna. *Adv Mar Biol* 34: 353–442

(Received February 21, 2006 / Accepted May 9, 2006)