

New species of bone-eating worm *Osedax* from the abyssal South Atlantic Ocean (Annelida, Siboglinidae)

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Abstract

A new species of bone-eating annelid, *Osedax braziliensis* **sp. n.**, found in a sunken whale carcass at a depth of 4,204 m at the base of the São Paulo Ridge in the South Atlantic Ocean off the Brazilian coast is described. The organism was retrieved using the human-occupied vehicle *Shinkai 6500* during the QUELLE 2013 expedition. This is the 26th species of the genus and the first discovery from the South Atlantic Ocean, representing the deepest record of *Osedax* worldwide to date. This species morphologically resembles *Osedax frankpressi* but is distinguished by the presence of a yellow bump or patch behind the prostomium and its trunk length. Molecular phylogenetic analysis using three genetic markers (*COI*, *16S*, and *18S*) showed that *O. braziliensis* **sp. n.** is distinct from all other *Osedax* worms reported and is a sister species of *O. frankpressi*.

Keywords

Polychaeta, São Paulo Ridge, taxonomy, whale-fall ecosystem

Introduction

Whale falls provide an extensive food supply to the oligotrophic deep-sea environments and harbour a unique biological assemblage, which is considered a “whale-fall ecosystem” (Smith et al. 1989, 2015). This ecosystem is known to be chemosynthesis-based, similar to hydrothermal vent and hydrocarbon seep ecosystems, but dynamic succession has been reported (Smith and Baco 2003). Numerous scavengers such as deep-sea sharks, hagfish, and small crustaceans devour the soft whale tissues when the carcasses reach the deep-sea floor (mobile-scavenger stage) (Smith and Baco 2003). After consumption of most soft tissues, organically enriched sediments and exposed bones are colonised by dense assemblages of opportunistic polychaetes and crustaceans (enrichment opportunist stage) (Smith and Baco 2003). Reducing chemicals such as sulphide are produced through anaerobic bacterial decomposition of organic materials in bones and support chemoautotrophs and chemoautotrophic symbiont-harboring invertebrates as energy sources of primary production (sulphophilic stage) (Smith and Baco 2003). After the depletion of organic materials in the whale bones, the exhausted bones are thought to act as colonisation substrata primarily for suspension feeders (reef stage) but have never been observed in situ (Smith and Baco 2003).

One of the most unique organisms that has appeared in the whale-fall environment is an annelid polychaete of genus *Osedax* Rouse, Goffredi & Vrijenhoek, 2004 (Annelida, Siboglinidae), commonly known as bone-eating worms, discovered in whale carcasses in Monterey Bay (Rouse et al. 2004). Unlike other siboglinids, *Osedax* lack a discrete trophosome, the organ housing symbiotic bacteria in vestimentiferans and pogonophorans (Rouse et al. 2004). Instead, female *Osedax* possesses a vascularised “root” system penetrating the bone marrow (Rouse et al. 2004). *Osedax* worms are believed to acquire nutrition from the bones through their roots (Tresguerres et al. 2013, Miyamoto et al. 2017). Intracellular heterotrophic bacteria localise in the roots, but their role remains unclear (Goffredi et al. 2014). Surprisingly, all the visible worms are female, and the males are dwarfs, with the exception of one species (Rouse et al. 2015). Morphological characterisation of *Osedax* has been minimal thus far. The body size, palp colour, presence/absence of pinnules and their location on palps, presence/absence of oviduct and its length, and root form are examples of the limited characteristics used for species identification (Vrijenhoek et al. 2009).

To the best of our knowledge, 25 *Osedax* species have been described to date, and some additional undescribed species were reported from the West and East Pacific, North and South Atlantic, Mediterranean, Sub-Antarctic, and Antarctic (Rouse et al. 2004, Glover et al. 2005, Fujikura et al. 2006, Braby et al. 2007, Fujiwara et al. 2007, Goffredi et al. 2007, Rouse et al. 2008, Vrijenhoek et al. 2008, Vrijenhoek et al. 2009, Glover et al. 2013, Amon et al. 2014, Rouse et al. 2015, Taboada et al. 2015, Sumida et al. 2016, Rouse et al. 2018). Two species, i.e., *Osedax rubiplumus* Rouse, Goffredi & Vrijenhoek, 2004 and *Osedax frankpressi* Rouse, Goffredi & Vrijenhoek, 2004, were

first described from the bones of a grey whale carcass at a depth of 2,891 m in Monterey Bay, California, in 2004 (Rouse et al. 2004). *Osedax mucofloris* Glover, Källström, Smith & Dahlgren, 2005 and *Osedax japonicus* Fujikura, Fujiwara & Kawato, 2006 were reported from environments with relatively shallow water at depths of 125 m in Swedish waters and 220 m near Japan, respectively (Glover et al. 2005, Fujikura et al. 2006). *Osedax antarcticus* Glover, Wiklund, Taboada, Avila, Cristobo, Smith, Kemp, Jamieson & Dahlgren, 2013, *Osedax crouchi* Amon, Wiklund, Dahlgren, Copley, Smith, Jamieson & Glover, 2014, *Osedax deceptionensis* Taboada, Cristobo, Avila, Wiklund & Glover, 2013, *Osedax nordenskjoldi* Amon, Wiklund, Dahlgren, Copley, Smith, Jamieson & Glover, 2014, and *Osedax rogersi* Amon, Wiklund, Dahlgren, Copley, Smith, Jamieson & Glover, 2014 were described from the Antarctic Ocean at depths between 10 and 1,446 m (Glover et al. 2013, Amon et al. 2014, Taboada et al. 2015). *Osedax roseus* and *Osedax priapus* were reported from Monterey Bay, and the latter showed some unique morphological features, i.e., only two palps (four are typical) and no male dwarfism (Rouse et al. 2008, 2015). Recently, 14 species from Monterey Bay were simultaneously described (Rouse et al. 2018). Additionally, the genomes of several undescribed species were sequenced and deposited in the international nucleotide sequence databases.

In 2013, the Iatá-Piúna Expedition, a collaborative scientific cruise between Japan and Brazil, was conducted within the framework of the around-the-world research cruise Quelle 2013 (Quest for the Limit of Life) of Japan Agency for Marine-Earth Science and Technology (JAMSTEC) using the HOV *Shinkai 6500* (Sumida et al. 2016). A sunken whale carcass was discovered at a depth of 4,204 m at the base of the São Paulo Ridge in the South Atlantic Ocean (Sumida et al. 2016). This was the first record of a natural whale fall in the deep Atlantic Ocean (Sumida et al. 2016). Forty-one benthic taxa including many new species were documented from the carcass in which galatheid crabs, *Rubyspira* gastropods, and polychaete annelids were dominant (Silva et al. 2015, Sumida et al. 2016, Shimabukuro et al. 2017a, 2017b). The skeleton belonged to an Atlantic minke whale (*Balaenoptera bonaerensis*) and was composed of nine caudal vertebrae, four of which were colonised by *Osedax* worms (Sumida et al. 2016, Alfaro-Lucas et al. 2017). Vertebrae not colonised by *Osedax* were well preserved and in a highly sulphophilic stage with chemosynthetic bacterial mats and numerous epifaunal organisms that fed on them. In contrast, vertebrae colonised by *Osedax* were heavily degraded and did not exhibit evidence of a sulphophilic stage, harbouring a distinct epifaunal assemblage (Alfaro-Lucas et al. 2017). A molecular phylogenetic analysis using mitochondrial *COI* sequences showed that the *Osedax* species from the São Paulo Ridge did not match any other sequences previously reported; therefore, the specimen was thought to be a new species.

Here we report a new species of *Osedax* collected from the South Atlantic at the deepest point recorded for this genus. Morphological and molecular phylogenetic characteristics are described.

Materials and methods

Specimen collection

Whale vertebrae harbouring *Osedax* worms were collected at a depth of 4,204 m at the base of the São Paulo Ridge (28°31.12'S, 41°39.41'W), southwest Atlantic Ocean during the HOV *Shinkai 6500* dives on April 24, 2013 (dive #1334), and April 26, 2013 (dive #1336), in the YK13-04 leg1 cruise using R/V *Yokosuka* (Figs 1–2). Upon recovery, the bones were immediately transferred to fresh chilled seawater (4 °C). *Osedax* worms were carefully removed from the bones under an on-board microscope just after the bone retrieval.

Treatment for electron microscopic observation

Whole bodies of *Osedax* worms ($n = 21$) were fixed with 2.5% glutaraldehyde in filtered seawater for 24 h and preserved in filtered seawater with 10 mM sodium azide at 4 °C. Samples were then washed in filtered seawater and fixed with 2% osmium tetroxide in filtered seawater for 2 h at 4 °C. For scanning electron microscopic observation, each sample was rinsed with distilled water and incubated with 1% aqueous tannic acid (pH 6.8) for 1 h for conductive staining. These samples were again washed with distilled water and treated with 1% aqueous osmium tetroxide for 1 h. The worms were dehydrated in a graded ethanol series and critical point-dried using a JCPD-5 critical point dryer (JEOL, Akishima, Japan). The samples were coated with osmium using a POC-3 osmium plasma coater (MEIWAFOSS Co., Osaka, Japan). The coated tissues were then observed using a JSM-6700F field-emission scanning electron microscope (JEOL) at an acceleration voltage of 5 kV.

DNA preparation

DNA was extracted from the root tissues of nine the *Osedax* worms. To reduce surface contaminants, each tissue sample was thoroughly washed in autoclaved and filtered (0.22 µm) seawater. DNA extraction from tissue samples was conducted separately using the DNeasy Tissue Kit (Qiagen Japan, Tokyo, Japan), following the instruction provided by the manufacturer.

Polymerase chain reaction (PCR) amplification, cloning, and sequencing

PCR amplifications were conducted using an Ex Taq PCR kit (TaKaRa, Kyoto, Japan) for three different molecular markers: cytochrome *c* oxidase subunit I (*COI*), 16S rRNA

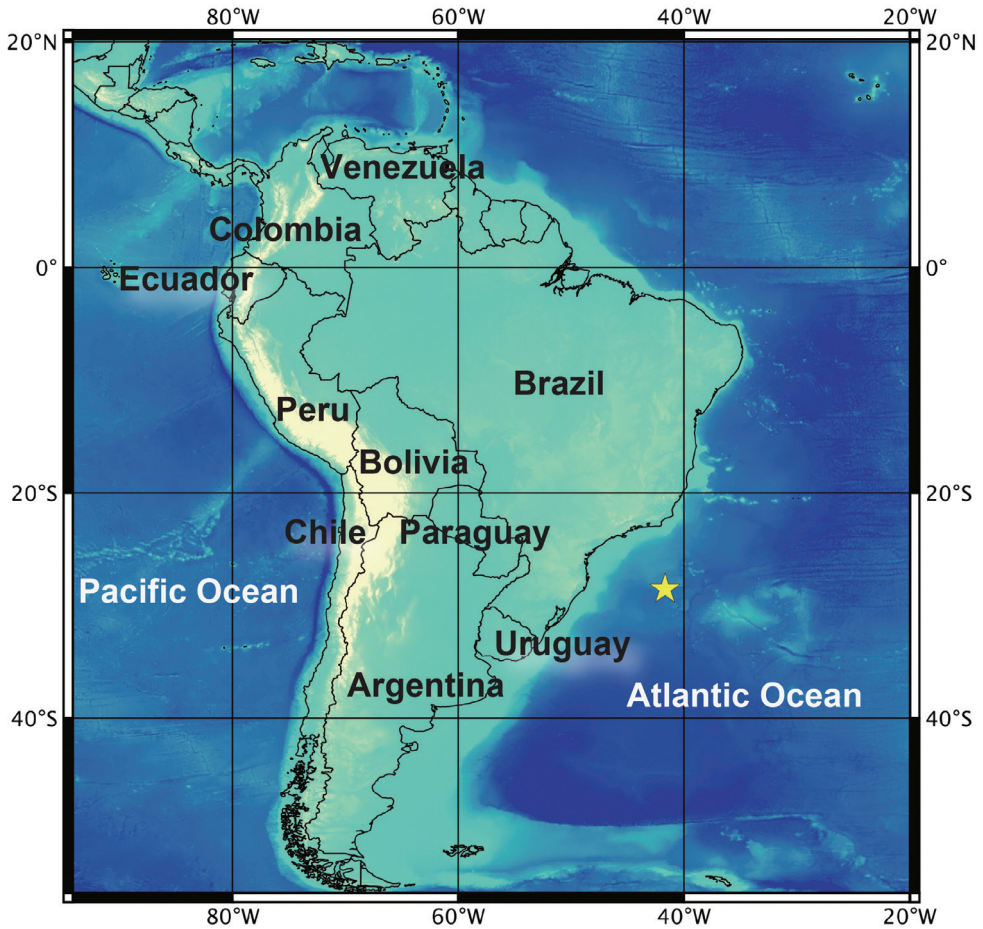


Figure 1. Sampling location of *Osedax braziliensis* sp. n. Solid star indicates the sampling location where a whale carcass was discovered at a depth of 4,204 m.

(16S), and 18S rRNA (18S). Two oligonucleotide primers (0.2 μ M each) and <1 μ g of DNA template were added to the reaction mixtures. Thermal cycling was performed as follows: denaturing at 96 $^{\circ}$ C for 20 s, annealing at 55 $^{\circ}$ C for 45 s, and extension at 72 $^{\circ}$ C for 2 min for a total of 35 cycles. The oligonucleotide primer sequences used for the PCR amplification are shown in Table 1. The molecular sizes of the PCR products were confirmed with 1.2% Agarose S (Nippon Gene, Toyama, Japan) gel electrophoresis. The PCR products were purified using the Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA). The DNA sequencing reaction was performed using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Specific primers for each gene were used in sequencing reactions according to the manufacturer's recommended procedure (Table 1). Sequencing was performed using an ABI PRISM 3100 genetic analyser (Applied Biosystems).

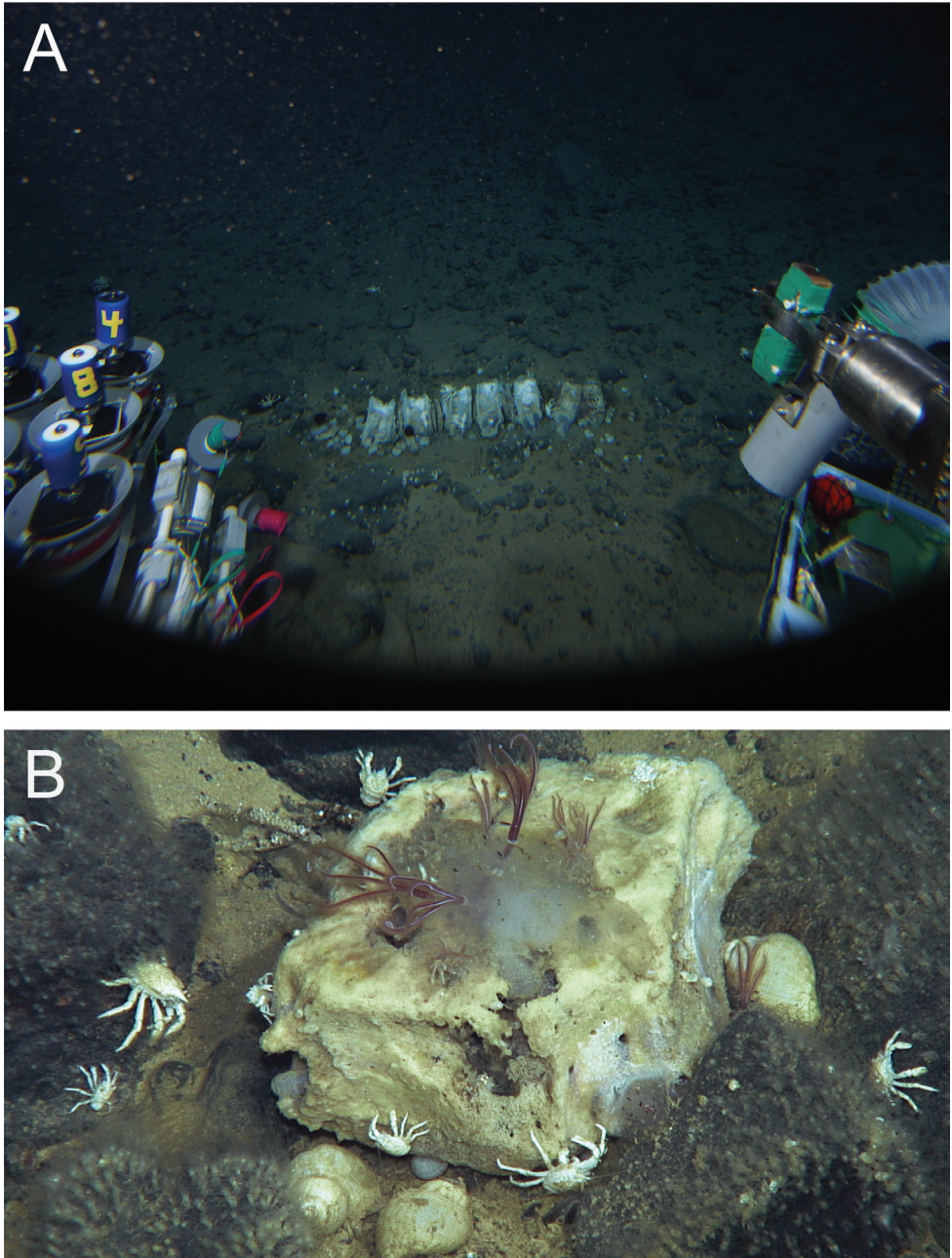


Figure 2. Whale skeleton discovered at a depth of 4,204 m in the South Atlantic Ocean. **A** Sunken whale skeleton of the Atlantic minke whale (*Balaenoptera bonaerensis*). Seven vertebrae are visible in this field of view. *Osedax braziliensis* sp. n. had colonised the first two bones on the right **B** Close-up view of a vertebra colonized by *O. braziliensis* sp. n. Galatheid crabs (*Munidopsis* spp.), amphipods (*Stephonyx* sp.), and gastropods (*Rubyspira* sp.) were also seen on and around the bones.

Table 1. List of primers used for PCR and sequencing.

| Primer | Target gene | Sequence (5'–3') | Application | Reference |
|-----------|-------------|-----------------------------|---|---------------------------------------|
| P_CO1f | <i>COI</i> | TCMACTAATCAYAARGAYATTGGNAC | PCR, Sequencing | Nelson and Fisher (2000) |
| P_CO1r | <i>COI</i> | CCDCCTAGWCCCTARRAARTGTTGNGG | PCR, Sequencing | Nelson and Fisher (2000) |
| Euk_42F | 18S rRNA | CTCAARGAYTAAGCCATGCA | PCR, Sequencing | Takishita et al. (2007) |
| Euk_1520R | 18S rRNA | CYGCAGGTTACACCTAC | PCR, Sequencing | Takishita et al. (2007) |
| Euk_555F | 18S rRNA | AGTCTGGTGCCAGCAGCCGC | Sequencing (internal) | Takishita et al. (2007) |
| Euk_555R | 18S rRNA | GCGGCTGCTGGCACCAGACT | Sequencing (internal) (Complement of Euk_555F) | Modified from Takishita et al. (2007) |
| Euk_1269R | 18S rRNA | AAGAACGGCCATGCACCAC | Sequencing (internal) | Takishita et al. (2007) |
| 16SarL | 16S rRNA | CGCCTGTTTAACAAAAACAT | PCR, Sequencing | Palumbi et al. (2000) |
| 16SbrH | 16S rRNA | CCGGTCTGAACTCAGATCACGT | PCR, Sequencing | Palumbi et al. (2000) |

Phylogenetic analysis

Partial sequences of the *COI*, *16S*, and *18S* genes were analysed using the gapped-BLAST search algorithm (Altschul et al. 1997, Benson et al. 2000) to estimate the degree of similarity to other related sequences. Additional sequences of siboglinids for phylogenetic analyses were obtained from the non-redundant nucleotide sequence database of the DNA Data Bank of Japan (Kodama et al. 2018) (Table 2). Sequences were aligned using CLUSTAL X (Larkin et al. 2007), followed by automatic editing of the resulting alignments using the GBLOCKS program for all the genetic markers under the options allowing smaller final blocks, gap positions within the final blocks, and less strict flanking positions (Castresana 2000, Talavera and Castresana 2007). The alignments (34 OTUs / 3,112 bp in total) are available upon request from the corresponding author. The maximum likelihood (ML) analysis was performed using the RAxML-VI-HPC program (Stamatakis 2006). Evolutionary models for each marker (GTR + γ) were separately estimated using KAKUSAN4 software (Tanabe 2007). The ML bootstrap analyses (1,000 replicates, -f option) were constructed as in the model and using the settings described earlier in this section.

COI genetic distance

Minimum genetic distances based on Kimura 2 parameters (K2P) model were calculated between *Osedax* species using MEGA7 software (Kumar et al. 2016). These distances were calculated using the *COI* alignment used in the phylogenetic analyses without gaps.

Table 2. List of operational taxonomic units included in the phylogenetic analysis, together with accession numbers in DDBJ.

| Taxon | COI | 16S rRNA | 18S rRNA |
|-----------------------------------|----------|----------|----------|
| <i>Lamellibrachia columna</i> | DQ996645 | FJ347646 | FJ347679 |
| <i>Riftia pachyptila</i> | KJ789166 | KP119573 | KP119591 |
| <i>Sclerolinum brattstromi</i> | KJ789167 | FJ347645 | FJ347680 |
| <i>Osedax antarcticus</i> | KF444422 | KF444418 | KF444420 |
| <i>Osedax braziliensis</i> sp. n. | LC381421 | LC381422 | LC381424 |
| <i>Osedax bryani</i> | JX280610 | KP119580 | KP119593 |
| <i>Osedax crouchi</i> | KJ598038 | KJ598032 | KJ598035 |
| <i>Osedax deceptionensis</i> | KT860545 | KF444419 | KF444421 |
| <i>Osedax doericketts</i> | FJ347626 | FJ347650 | FJ347688 |
| <i>Osedax frankpressi</i> | FJ347607 | FJ347658 | FJ347682 |
| <i>Osedax jabba</i> | FJ347638 | FJ347647 | FJ347693 |
| <i>Osedax japonicus</i> | FM998111 | LC381423 | FM995535 |
| <i>Osedax knutei</i> | FJ347635 | FJ347648 | FJ347692 |
| <i>Osedax lehmani</i> | EU223323 | FJ347660 | FJ347689 |
| <i>Osedax lonnyi</i> | FJ347643 | FJ347651 | FJ347695 |
| <i>Osedax mucofloris</i> | KJ806976 | N.A. | AY941263 |
| <i>Osedax nordenskjoldi</i> | KJ598033 | KJ598033 | KJ598036 |
| <i>Osedax packardorum</i> | FJ347629 | FJ347661 | FJ347690 |
| <i>Osedax priapus</i> | KP119564 | KP119575 | KP119594 |
| <i>Osedax randyi</i> | FJ347615 | FJ347659 | FJ347684 |
| <i>Osedax rogersi</i> | KJ598040 | KJ598034 | KJ598037 |
| <i>Osedax roseus</i> | FJ347609 | FJ347657 | FJ347683 |
| <i>Osedax rubiplumus</i> | EU852488 | FJ347656 | FJ347681 |
| <i>Osedax ryderi</i> | KP119563 | KP119574 | KP119597 |
| <i>Osedax sigridae</i> | FJ347642 | FJ347655 | FJ347694 |
| <i>Osedax talkovici</i> | FJ347621 | FJ347654 | FJ347685 |
| <i>Osedax tiburon</i> | FJ347624 | FJ347653 | FJ347687 |
| <i>Osedax ventana</i> | EU236218 | FJ347652 | FJ347686 |
| <i>Osedax westernflyer</i> | FM998110 | FJ347649 | FJ347691 |
| <i>Osedax</i> sp. 'MB16' | JX280612 | KP119581 | KP119592 |
| <i>Osedax</i> sp. 'mediterranea' | KT860548 | KT860551 | KT860550 |
| <i>Osedax</i> sp. 'sagami3' | FM998081 | N.A. | FM995537 |
| <i>Osedax</i> sp. 'sagami4' | FM998082 | N.A. | FM995541 |
| <i>Osedax</i> sp. 'sagami5' | FM998083 | N.A. | FM995539 |

Systematics

Family Siboglinidae Caullery, 1914

Genus *Osedax* Rouse, Goffredi & Vrijenhoek, 2004

Osedax braziliensis sp. n.

<http://zoobank.org/7AD6CE45-4585-42C6-BB5E-22587BB2307F>

Figures 3, 4

New Japanese name: Burajiru-honekuihanamushi

Osedax sp. nov.: Sumida et al. 2016: 1–6, figs 3–4, Table 1. *Osedax*: Alfaro-Lucas et al. 2017: 1–9, fig. 2B.

Type material. Holotype: NMST-Pol H-685, trunk 14 mm long, 2 mm wide, female, 4,203 m depth, 26 April 2013, collected by YF, DDBJ No. LC381421,

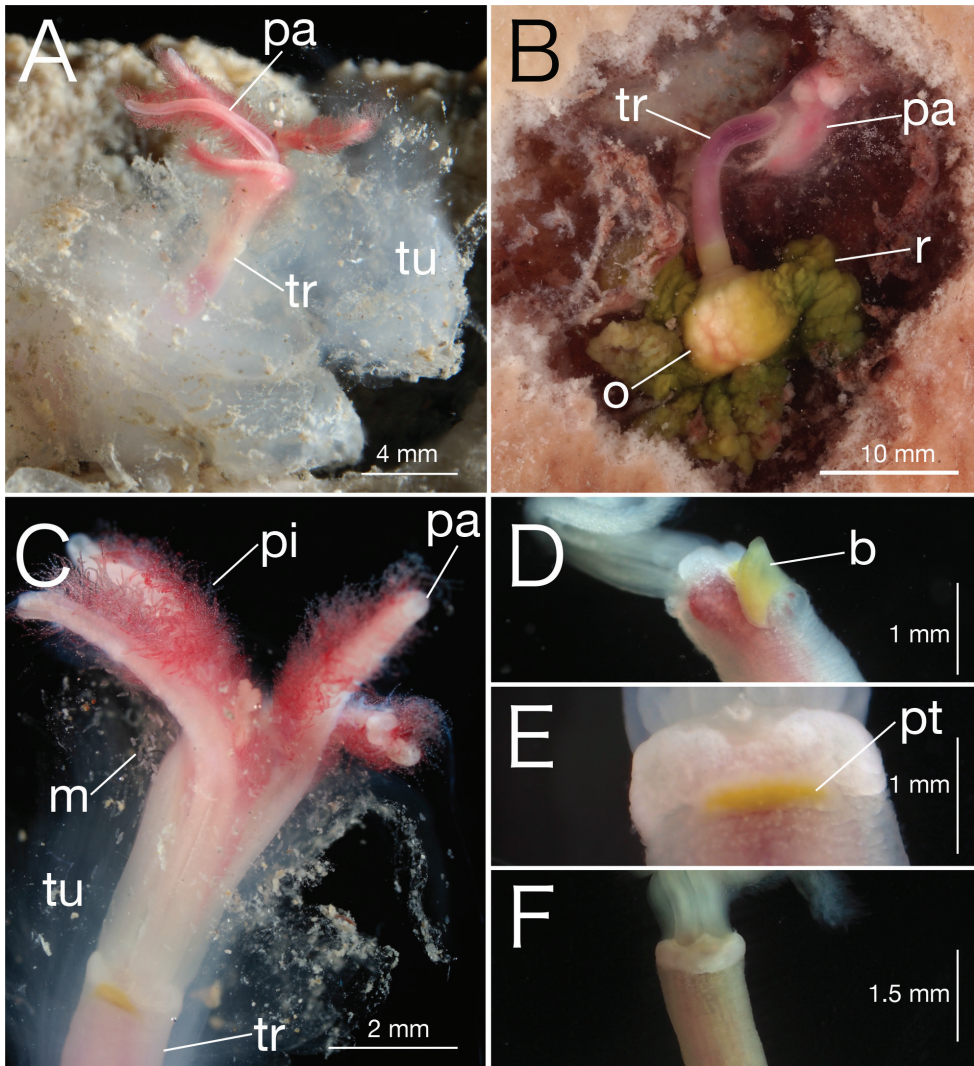


Figure 3. Photographs of unfixed *Osedax braziliensis* sp. n. **A** Palps (*pa*), trunk (*tr*) and a gelatinous hemispherical tube (*tu*) **B** lateral view of palps (*pa*), trunk (*tr*), ovary (*o*), and root (*r*) **C** ventral view of palps (*pa*) with pinnules (*pi*) and trunk (*tr*). Dwarf males (*m*) inhabit a gelatinous tube (*tu*), and **D** ventro-lateral view of an individual possessing a yellow bump (*b*) present behind prostomium **E** Ventral view of holotype (NMST-Pol H-685) possessing a yellow patch (*pt*) **F** Ventral view of an individual without yellow bump.

LC381422, LC381424. Paratypes (14 specimens): NSMT-Pol P-686–690 and JAMSTEC-1130038806, 1130039105, 1130039113, 1130039116, 1130039146, 1130039163, 11 specimens, female, 24 and 26 April 2013, collected by YF, DDBJ No. LC381777, LC381766, LC381767, LC381768, LC381769, LC381771; JAMSTEC-1130057454, 1130057457, 1130057458, 3 specimens, male, 26 April 2013, collected by YF.

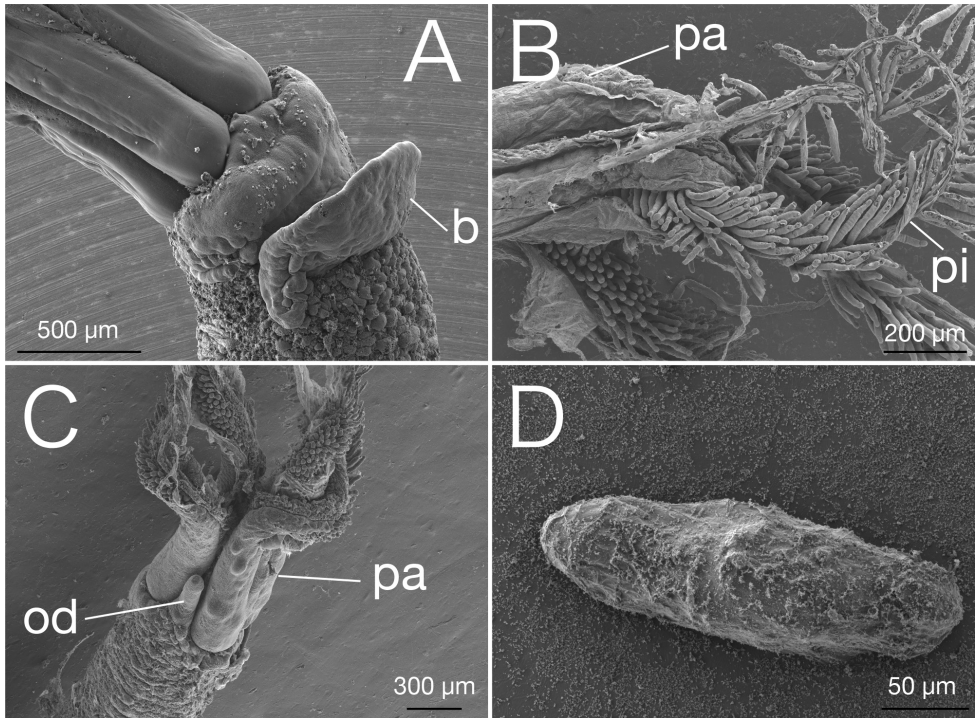


Figure 4. Scanning electron micrographs of *Osedax braziliensis* sp. n. **A** Ventro-lateral view of bump (*b*) **B** lateral view of palps (*pa*) with pinnules (*pi*), and **C** dorsal view of palps (*pa*) and oviduct (*od*) **D** Dwarf male taken from the tube of a female.

Type locality. São Paulo Ridge, Brazil, 4,204 m depth.

Diagnosis. Trunk length long. Gelatinous hemispherical tube encases trunk and base of palps. Yellow bump or patch present (absent in some specimens). Pinnules on inner margin of palps. Root lobulated without branching.

Description. Genetic data (*COI*, *16S*, and *18S*) deposited in DDBJ (LC106303, LC381421, LC381422, LC381424, and LC381765–LC381787). Trunk length up to 22 mm, width at collar 0.5 mm, reddish purple while alive and whitish after fixation (Fig. 3A–C); gelatinous hemispherical tube encases trunk and base of palps, 1–2 mm thick, contains eggs and dwarf males (Fig. 3A, C). Prostomium whitish while alive, present at top of trunk. Yellow bump or patch present behind prostomium: this yellow bump or patch size varies among individuals, biggest bump reaches top of trunk, and is absent in some specimens (Fig. 3D–F). Crown consists of four palps; palps about 1.5 mm length, red colour while alive with two whitish stripes on the inner side, fused for about 30% of length; pinnules on inner margin of palps, about 50–250 µm, 7–8 pinnules in transverse rows (Figs 3A, C, 4B, C). Oviduct free to base, adjoined to the trunk at opposite side of prostomium region, reaching up to 20–30% of palp length (Fig. 4C). Ovisac whitish; trunk–ovisac junction about 15% of trunk length, light green while alive (Fig. 3B). Root lobulated without branching, yellow greenish while alive and whitish after fixation; intracellular symbiotic bacteria in root tissue (Fig. 3B). Eggs about 150 µm diameter ($n = 20$), whitish while alive (Fig. 3B).

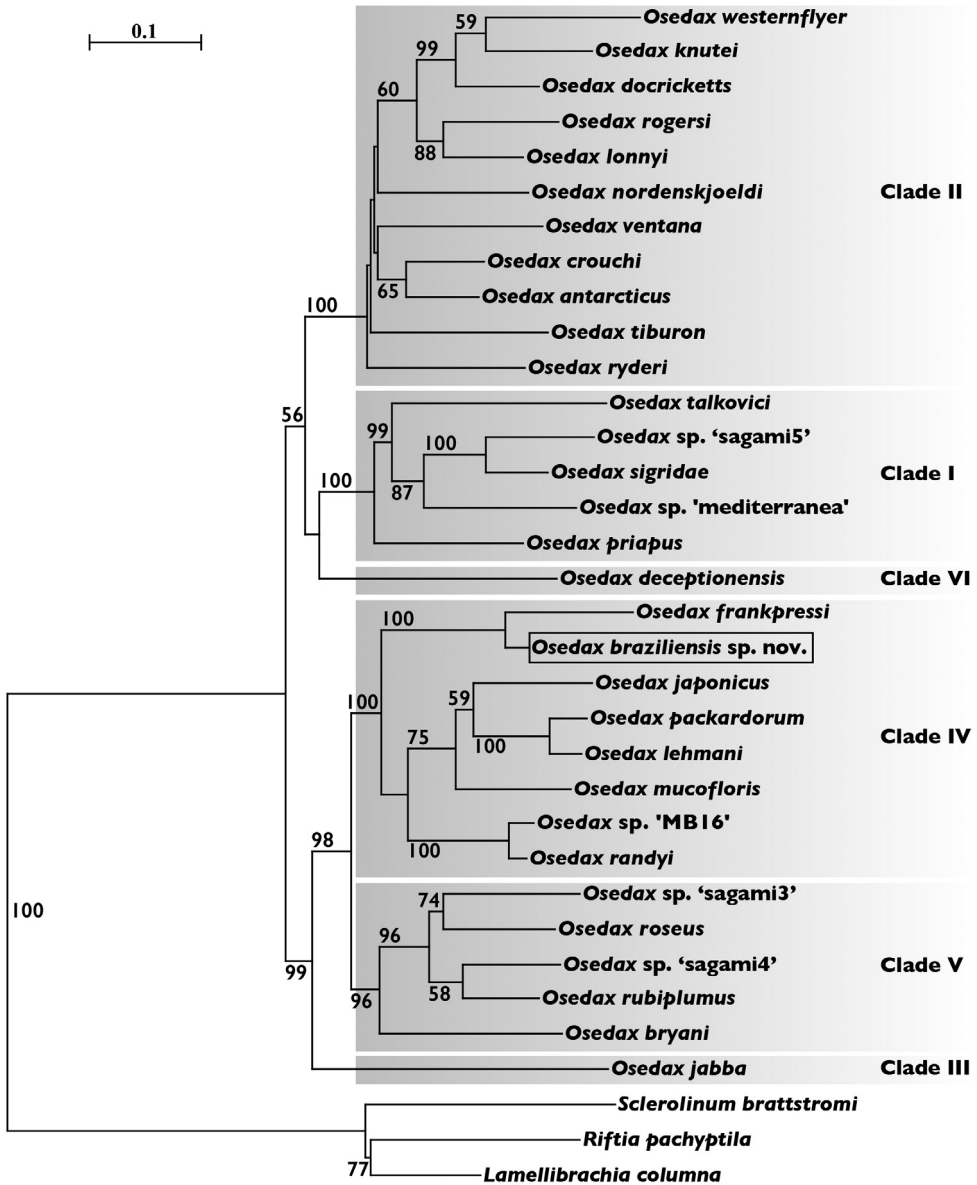


Figure 5. Phylogenetic placement of *Osedax braziliensis* sp. n. based on nucleotide sequences on the concatenated *COI*, *16S*, and *18S* markers, using maximum likelihood. Scale bar represents 0.1 nucleotide substitutions per sequence position. Only bootstrap values greater than 50 are shown for each branch. *Osedax braziliensis* sp. n. is boxed.

Dwarf male about 250 μm in length ($n = 20$), fusiform, whitish while alive, prostomium and pygidium rounded, no appendage organs; posterior hooks present, two pairs (5–7 hooks per bundle) arranged in three rows (Figs 3C, 4D).

Etymology. This species is named after the type locality, Brazil. This name is an adjective used as a substantive in the genitive case.

Table 3. COI divergence values (Kimura 2 parameters) between *Osedax* species and OTUs.

| | OTU | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
|----|-----------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 1 | <i>Osedax frankipressi</i> | | | | | | | | | | | | | | | | | |
| 2 | <i>Osedax</i> sp. 'sagamis8' | 0.230 | | | | | | | | | | | | | | | | |
| 3 | <i>Osedax tiburon</i> | 0.206 | 0.188 | | | | | | | | | | | | | | | |
| 4 | <i>Osedax</i> sp. 'sagamis7' | 0.207 | 0.221 | 0.241 | | | | | | | | | | | | | | |
| 5 | <i>Osedax kinari</i> | 0.238 | 0.167 | 0.231 | 0.230 | | | | | | | | | | | | | |
| 6 | <i>Osedax</i> sp. 'sagamis4' | 0.193 | 0.214 | 0.214 | 0.162 | 0.212 | | | | | | | | | | | | |
| 7 | <i>Osedax mucrofloris</i> | 0.198 | 0.224 | 0.209 | 0.204 | 0.190 | 0.214 | | | | | | | | | | | |
| 8 | <i>Osedax antarcticus</i> | 0.209 | 0.209 | 0.218 | 0.235 | 0.211 | 0.201 | 0.255 | | | | | | | | | | |
| 9 | <i>Osedax talkovici</i> | 0.225 | 0.219 | 0.234 | 0.244 | 0.214 | 0.220 | 0.220 | 0.267 | | | | | | | | | |
| 10 | <i>Osedax randsji</i> | 0.207 | 0.219 | 0.244 | 0.006 | 0.233 | 0.165 | 0.209 | 0.231 | 0.238 | | | | | | | | |
| 11 | <i>Osedax crouchi</i> | 0.209 | 0.213 | 0.180 | 0.178 | 0.196 | 0.177 | 0.203 | 0.205 | 0.221 | 0.180 | | | | | | | |
| 12 | <i>Osedax</i> sp. 'MB16' | 0.207 | 0.230 | 0.253 | 0.057 | 0.219 | 0.178 | 0.212 | 0.240 | 0.236 | 0.059 | 0.175 | | | | | | |
| 13 | <i>Osedax signata</i> | 0.192 | 0.198 | 0.190 | 0.214 | 0.207 | 0.207 | 0.230 | 0.212 | 0.215 | 0.208 | 0.188 | 0.217 | | | | | |
| 14 | <i>Osedax lbhmani</i> | 0.193 | 0.229 | 0.211 | 0.178 | 0.223 | 0.175 | 0.178 | 0.264 | 0.202 | 0.175 | 0.200 | 0.175 | 0.195 | | | | |
| 15 | <i>Osedax ventana</i> | 0.193 | 0.187 | 0.165 | 0.217 | 0.196 | 0.208 | 0.225 | 0.221 | 0.220 | 0.222 | 0.155 | 0.222 | 0.207 | 0.228 | | | |
| 16 | <i>Osedax roseus</i> | 0.172 | 0.209 | 0.201 | 0.209 | 0.230 | 0.168 | 0.206 | 0.196 | 0.226 | 0.201 | 0.222 | 0.211 | 0.201 | 0.191 | 0.203 | | |
| 17 | <i>Osedax</i> sp. 'MB16' | 0.230 | 0.187 | 0.236 | 0.206 | 0.204 | 0.185 | 0.227 | 0.168 | 0.233 | 0.208 | 0.188 | 0.200 | 0.209 | 0.222 | 0.201 | 0.195 | |
| 18 | <i>Osedax brazilensis</i> sp. n. | 0.117 | 0.203 | 0.203 | 0.184 | 0.211 | 0.217 | 0.196 | 0.193 | 0.236 | 0.183 | 0.206 | 0.190 | 0.187 | 0.217 | 0.198 | 0.170 | 0.214 |
| 19 | <i>Osedax rubiplanius</i> | 0.190 | 0.211 | 0.222 | 0.195 | 0.230 | 0.161 | 0.230 | 0.206 | 0.224 | 0.203 | 0.183 | 0.203 | 0.203 | 0.190 | 0.228 | 0.185 | 0.193 |
| 20 | <i>Osedax</i> sp. 'incertiraneta' | 0.230 | 0.187 | 0.236 | 0.206 | 0.204 | 0.185 | 0.227 | 0.168 | 0.233 | 0.208 | 0.188 | 0.200 | 0.209 | 0.222 | 0.201 | 0.195 | 0.000 |
| 21 | <i>Osedax</i> sp. 'incertiraneta' | 0.214 | 0.241 | 0.212 | 0.206 | 0.264 | 0.217 | 0.241 | 0.259 | 0.203 | 0.201 | 0.227 | 0.190 | 0.155 | 0.206 | 0.230 | 0.192 | 0.238 |
| 22 | <i>Osedax packardorum</i> | 0.201 | 0.216 | 0.208 | 0.185 | 0.237 | 0.170 | 0.181 | 0.258 | 0.191 | 0.177 | 0.219 | 0.199 | 0.201 | 0.082 | 0.219 | 0.196 | 0.227 |
| 23 | <i>Osedax byroni</i> | 0.209 | 0.213 | 0.180 | 0.178 | 0.196 | 0.177 | 0.203 | 0.205 | 0.221 | 0.180 | 0.000 | 0.175 | 0.188 | 0.200 | 0.155 | 0.222 | 0.188 |
| 24 | <i>Osedax nondensipoloidi</i> | 0.203 | 0.190 | 0.002 | 0.244 | 0.230 | 0.177 | 0.209 | 0.218 | 0.237 | 0.246 | 0.177 | 0.255 | 0.188 | 0.200 | 0.163 | 0.204 | 0.238 |
| 25 | <i>Osedax westernrhyffer</i> | 0.233 | 0.004 | 0.188 | 0.221 | 0.170 | 0.211 | 0.224 | 0.212 | 0.222 | 0.219 | 0.216 | 0.230 | 0.200 | 0.229 | 0.193 | 0.206 | 0.193 |
| 26 | <i>Osedax ryderi</i> | 0.209 | 0.213 | 0.180 | 0.178 | 0.196 | 0.177 | 0.203 | 0.205 | 0.221 | 0.180 | 0.000 | 0.175 | 0.188 | 0.200 | 0.155 | 0.222 | 0.188 |
| 27 | <i>Osedax</i> sp. 'sagamis6' | 0.193 | 0.175 | 0.185 | 0.238 | 0.152 | 0.219 | 0.203 | 0.203 | 0.225 | 0.233 | 0.175 | 0.224 | 0.221 | 0.236 | 0.153 | 0.222 | 0.178 |
| 28 | <i>Osedax jubba</i> | 0.228 | 0.172 | 0.222 | 0.203 | 0.206 | 0.251 | 0.195 | 0.238 | 0.228 | 0.209 | 0.216 | 0.228 | 0.222 | 0.241 | 0.188 | 0.220 | 0.213 |
| 29 | <i>Osedax abrickletts</i> | 0.198 | 0.172 | 0.183 | 0.236 | 0.157 | 0.219 | 0.206 | 0.206 | 0.225 | 0.230 | 0.170 | 0.227 | 0.218 | 0.233 | 0.148 | 0.222 | 0.176 |
| 30 | <i>Osedax priapus</i> | 0.217 | 0.191 | 0.186 | 0.206 | 0.210 | 0.180 | 0.230 | 0.211 | 0.231 | 0.209 | 0.160 | 0.195 | 0.155 | 0.195 | 0.183 | 0.198 | 0.178 |
| 31 | <i>Osedax</i> sp. 'sagamis5' | 0.193 | 0.214 | 0.207 | 0.222 | 0.204 | 0.206 | 0.212 | 0.228 | 0.226 | 0.222 | 0.198 | 0.224 | 0.122 | 0.203 | 0.209 | 0.217 | 0.219 |
| 32 | <i>Osedax</i> sp. 'sagamis3' | 0.196 | 0.206 | 0.242 | 0.209 | 0.219 | 0.181 | 0.217 | 0.220 | 0.186 | 0.201 | 0.206 | 0.209 | 0.211 | 0.194 | 0.234 | 0.187 | 0.224 |
| 33 | <i>Osedax japonicus</i> | 0.201 | 0.238 | 0.220 | 0.175 | 0.236 | 0.172 | 0.173 | 0.216 | 0.204 | 0.172 | 0.188 | 0.165 | 0.206 | 0.149 | 0.206 | 0.185 | 0.211 |
| 34 | <i>Osedax deceptionensis</i> | 0.214 | 0.230 | 0.236 | 0.273 | 0.235 | 0.271 | 0.246 | 0.254 | 0.221 | 0.273 | 0.242 | 0.270 | 0.209 | 0.233 | 0.236 | 0.241 | 0.236 |

Distribution. Only known from a whale carcass of the type locality. São Paulo Ridge, off Brazilian coast, 4,204 m depth.

Phylogenetic analysis. The final lengths of the aligned sequences were 1,004 bp (*COI*), 486 bp (*16S*), and 1,604 bp (*18S*). The phylogenetic position of *O. braziliensis* sp. n. determined from our ML analysis recovered, with total support, a distinct species from that of all other *Osedax* species reported (Fig. 5). The six *Osedax* clades proposed by Rouse et al. (2018) were recovered. The phylogenetic analysis showed that *O. braziliensis* sp. n. falls into Clade IV, and is a sister species of *O. frankpressi* known from Monterey Bay at depths between 1,820 m and 2,898 m (Fig. 5).

Remarks. This species resembles *Osedax frankpressi* in the pinnules distributed only at the inner margin of palps, lobulated root structure without branching, gelatinous hemispherical tube, and dwarf males (Rouse et al. 2004). However, it can be discriminated from *O. frankpressi* by the presence of the yellow bump or patch behind the prostomium, trunk length, and genetic data. In *O. braziliensis* sp. n., the yellow bump or patch was present in some specimens including holotype, and the trunk length is long (6–22 mm), whereas in *O. frankpressi*, the bump or patch is absent in all specimens, and the trunk length is shorter (4.5 mm). *COI* genetic distances between *O. braziliensis* sp. n. and *O. frankpressi* are 0.111–0.117, which are greater than intraspecific values in *O. braziliensis* sp. n. (0.001–0.006). Genetic distances between *O. braziliensis* and the rest of the *Osedax* taxa for the *COI* ranged from 0.117 to 0.236 (Table 3).

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