

# New distribution record of deep-sea mussel, *Bathymodiolus aduloides* (Mollusca: Bivalvia: Mytilidae) from a hydrothermal vent, Myojinsho

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*From the deep-sea hydrothermal vents and cold seeps ecosystems, more than 600 species containing many endemic species have been discovered. The deep-sea mussels of the genus Bathymodiolus are dominant species at deep-sea hydrothermal vents and cold seeps throughout the world. They are known to rely on the nutrients produced by the chemosynthetic symbiont in their gills. In 2009, we found a colony of bathymodiolin mussels at a hydrothermal vent of Myojinsho (Myojin Reef), which is a hardly investigated area. Myojinsho lies in the northern segment of the Izu-Ogasawara (Bonin) Arc and had been volcanically active until recently. We collected specimens from the colony using a remotely operated vehicle. We identified them as Bathymodiolus aduloides by morphological observation and phylogenetic analyses using mitochondrial DNA sequencing. We could not find any other species of mussels during the dive. This result is interesting because this species has not been discovered from hydrothermal vents in this region; the dominant species in this region, including the neighbouring Myojin Knoll Caldera, is B. septemdiarium.*

**Keywords:** *Bathymodiolus aduloides*, cold seep, hydrothermal vent, Myojinsho

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## INTRODUCTION

Deep-sea hydrothermal vents and cold seeps ecosystems, depending on chemoautotrophic primary production, occur in a variety of locations throughout the global ocean (Van Dover *et al.*, 2002). A variety of endemic organisms have been discovered from these environments.

The deep-sea mussels of the genus *Bathymodiolus* are one of the dominant groups of invertebrate at hydrothermal vent and cold seep ecosystems. Twenty-two species have been discovered thus far, from deep-sea hydrothermal vents on spreading ridges, back-arc basins and cold water seeps along subduction zones in the world (Miyazaki *et al.*, 2010): some of the species are vent-specific, some are seep-specific species and some are found in both environments. The mussels of this genus harbour chemosynthetic symbionts (thioautotrophic bacteria, methanotrophic bacteria, etc.) in their gills, and are essentially dependent on the organic substances produced by the symbionts (Felbeck, 1983).

It is considered that *Bathymodiolus* expanded their habitat from shallow to deep water using sunken woods and whale

carcasses, as an 'evolutionary stepping stone' (Distel *et al.*, 2000), and phylogenetic analyses have revealed the paraphyletic structure of the genus recently (Kyuno *et al.*, 2009; Lorion *et al.*, 2010). However, how bathymodiolin mussels have maintained and expanded their distribution area in the hydrothermal vent areas, which are patchily distributed and are temporally unstable, is still not fully understood.

Thus, it is important to accumulate the information about distribution of bathymodiolin mussels at various areas.

Japan is a point where the four tectonic plates, the Eurasian Plate, the Pacific Plate, the Philippine Sea Plate and North American Plate meet, and thus many hydrothermal vents and cold seeps have been discovered around there. These vents and seeps are a suitable field to study the ecology of chemosynthesis-dependent organisms. We are interested in the populations of deep-sea mussels around Japan. In 2009, we visited Myojinsho (Myojin reef), a submarine caldera located about 420 km south of Tokyo, approximately 9 km east-north-east of the Bayonnaise Rocks and 20 km south of Myojin Knoll caldera (Figure 1), and which lies in the northern segment of the Izu-Ogasawara (Bonin) Arc (Iizasa *et al.*, 1992). Myojinsho has erupted more than 20 times since 1869, including explosive eruptions accompanying dome growth and destruction that occurred during 1952–1953. Even after that eruptions were observed sporadically until the 1970s (Fiske *et al.*, 1998).

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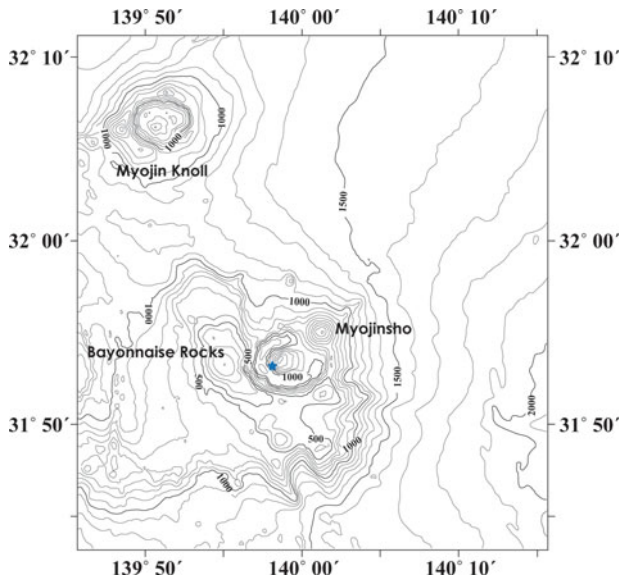


Fig. 1. Bathymetry around Myojinsho and Myojin Knoll. Star indicates the sampling point of the mussels.

In this paper, we report the discovery of a colony of a deep-sea mussel at a hydrothermal vent in Myojinsho. We examined the mussel morphologically and also using mitochondrial DNA sequence and identified it to be *B. aduloides*. This is an unexpected result because the dominant species of other hydrothermal vents in this area is *B. septemdiarium* and *B. aduloides* has not been previously recorded in hydrothermal vents in the Izu-Ogasawara Arc.

## MATERIALS AND METHODS

### Material examined

Six specimens were collected by the remotely operated vehicle (ROV) 'Hyper-Dolphin', which was operated by the research vessel (RV) 'Natsushima', at Myojinsho (31°53'N 139°58'E; Figure 1), at the depth of 900.5 m on 19 April 2009, during the research cruise NT09-05. The colony contained 44 mussels of countable size (Figure 2A). They are found to bury a part of their shells into the sediment.

### Morphological analysis

Morphological observations were performed using 2 specimens (shell length: 51.9 mm and 39.0 mm) according to Hashimoto & Okutani (1994). Shell feature was measured

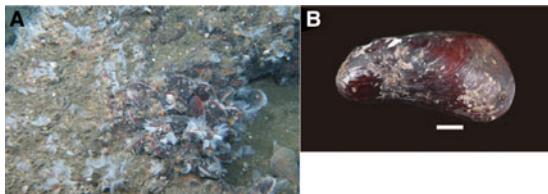


Fig. 2. (A) A picture of the mussel colony in Myojinsho. This image was taken by ROV 'Hyper-Dolphin' in April 2009. White cirri of *Neoverruca* sp. are observed around the mussels; (B) specimen of *Bathymodiolus aduloides*. White bar indicates 10 mm.

by caliper. Muscle scars and gut were observed under a stereoscopic microscope.

### DNA sequencing

Total DNA was extracted from the gill tissues with DNeasy Blood and tissue kit (Qiagen GmbH, Hilden, Germany). Mitochondrial cytochrome c oxidase I (COI) was amplified using the universal primers LCO1490: 5'-GGTCAACAAAT CATAAAGATATTGG-3' and HCO 2198: TAAACTTCA GGGTGACCAAAAAATCA-3' (Folmer *et al.*, 1994). The polymerase chain reaction (PCR) was performed using ExTaq (TaKaRa Bio, Otsu, Japan) under the following conditions: 1  $\mu$ l of 100  $\mu$ g genomic DNA was amplified in a 25  $\mu$ l reaction mixture containing 0.08  $\mu$ M dNTP, 0.02  $\mu$ M forward and reverse primers, 2.5  $\mu$ l 10  $\times$  ExTaq buffer and 0.625 U of ExTaq DNA polymerase (TaKaRa Bio, Otsu, Japan). Amplification was achieved through 35 cycles of denaturing at 95°C for 10 seconds, annealing at 50°C for 15 seconds and extension at 72°C for 1 minute. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen GmbH, Hilden, Germany). Sequences were determined with an automated sequencer (Applied Biosystems, model 31301  $\times$  1). The obtained sequence was compared using nucleotide-nucleotide BLAST (blastn) with default settings using the DDBJ/EMBL/GenBank database.

### Phylogenetic analyses

Reference sequences of the mussels were obtained from the DDBJ/EMBL/GenBank database (Table 1).

The newly-obtained and reference sequences were aligned using the Clustal W program in MEGA software ver. 3.1 to detect insertion, deletion and base substitution.

The neighbour-joining and maximum parsimony trees were conducted with the MEGA software program ver. 3.1. Kimura's two-parameter model was used for the neighbour-joining tree. Bootstrap replication number was 1000 times for each method.

## RESULTS

### Morphological observations

The Myojinsho mussels exhibit shell and gut features of *B. aduloides* described in Hashimoto & Okutani (1994). They have thick, solid and elongate shells, modioliform, inflated, equivalve (Figure 2B). Shell height/length ratio: 0.50 (length 51.9 mm), 0.52 (length 39.0 mm), width/length ratio: 0.34 (length 51.9 mm), 0.33 (length 39.0 mm). The umbones subterminal situated at about one-tenth of the shell length. Ligament strong, extending about seven-tenths of dorsal margin length with slightly convex ligament plate. Anterior adductor muscle scar large and posterior adductor muscle scar rounded trapezoid. Gut with a single clockwise loop.

### Mitochondrial COI sequence and phylogenetic analyses

We obtained 667-base pairs (bp) of mitochondrial DNA sequence of COI region from Myojinsho mussel and the

**Table 1.** List of sequences used for phylogenetic analyses.

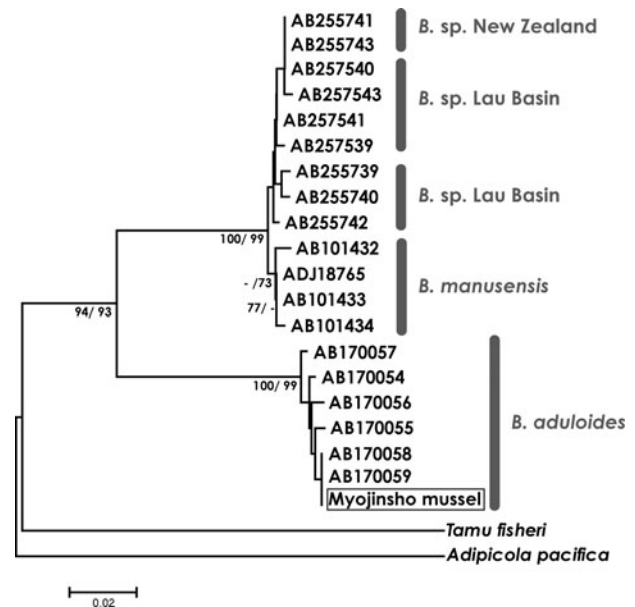
Species	Accession number	Sampling site	Habitat type
<i>Bathymodiolus aduloides</i>	AB170054	Iheya Ridge	Vent
	AB170055	Off Kikaijima Island	Seep
	AB170056	Off Kikaijima Island	Seep
	AB170057	Off Kikaijima Island	Seep
	AB170058	Off Kikaijima Island	Seep
	AB170059	Off Kikaijima Island	Seep
<i>B. sp. manusensis</i> -JM-2003	AB597557	Myojinsho (present study)	Vent
	AB101432	Manus Basin, PACKMANUS Field E	Vent
	AB101433	Manus Basin, PACKMANUS Field E	Vent
<i>B. sp.</i>	AB101434	Manus Basin, PACKMANUS Field E	Vent
	AB257539	Hine Hina, Lau Basin	Vent
	AB257540	Hine Hina, Lau Basin	Vent
	AB257541	Hine Hina, Lau Basin	Vent
	AB257543	Hine Hina, Lau Basin	Vent
	AB255739	Off New Zealand	Vent
	AB255740	Off New Zealand	Vent
	AB255741	Off New Zealand	Vent
	AB255742	Off New Zealand	Vent
	AB255743	Off New Zealand	Vent
<i>Tamu fisheri</i>	AY649803	Garden Banks	Seep
<i>Adipicola pacifica</i>	AB170040	Off Noma Cape	Whale bone

sequence data were deposited in the DDBJ/EMBL/GenBank databases under the accession number AB597557. A BLAST search revealed that the sequence of the Myojinsho mussel was identical with that of *B. aduloides* collected at the cold seep at Off Kikaijima (DDBJ/EMBL/GenBank accession number AB170058). We constructed phylogenetic trees using 430-bp from 21 specimens, including *B. aduloides*, *B. manusensis* and unidentified bathymodiolin mussels inhabiting Off New Zealand and Lau Basin, all of which are included in a single clade in the previous studies (Kyuno *et al.*, 2009; Lorion *et al.*, 2010; Miyazaki *et al.*, 2010). *Tamu fisheri* was added because it was suggested to be phylogenetically close to these species (Lorion *et al.*, 2010; Miyazaki *et al.*, 2010). *Adipicola pacifica* was used as outgroup. Neighbour-joining and maximum parsimony trees showed the same topology. *Tamu fisheri* branched first and others formed a single clade consisting of two subclades; Myojinsho mussel formed one subclade with *B. aduloides* of Off Kikaijima and Iheya ridge, and *B. manusensis* and *B. sp.* from Lau Basin and New Zealand formed another subclade (Figure 3).

## DISCUSSION

In addition to the morphological observations of the shell features, muscle scars, gut and mitochondrial DNA sequence from the COI region showed that the Myojinsho mussel is *Bathymodiolus aduloides* Hashimoto & Okutani 1994. It is interesting that the sequence was identical to that of *B. aduloides* of Off Kikaijima. This may indicate gene flow between the two populations. If this is the case, the Kuroshio Current may be involved. Gene flow in Myojinsho and Sagami Bay and/or Suruga Bay is also possible but we could not prove it because no sequence data are available for the mussels from these sites.

Phylogenetic analyses revealed that *B. aduloides* specimens including the Myojinsho mussel form a single subclade and *B. manusensis* and *B. sp.* from Lau Basin and New Zealand



**Fig. 3.** Phylogenetic relationships of Myojinsho mussel and their relatives based on the COI sequences. The neighbour-joining (NJ) tree was constructed based on the genetic distances calculated according to Kimura's two-parameter method using *Adipicola pacifica* as an outgroup species. The maximum parsimony (MP) tree presented essentially the same topology as the NJ tree. Only the NJ (left) and MP (right) bootstrap values >70% are specified. See Table 1 for accession numbers of specimens.

another subclade. These two subclades formed a single clade supported by high bootstrap values. These results support the previous studies (Kyuno *et al.*, 2009; Lorion *et al.*, 2010; Miyazaki *et al.*, 2010) that proposed the independence of the lineage of *B. aduloides* and *B. manusensis* among *Bathymodiolus* spp., and similarity of the unidentified mussels of Lau Basin and New Zealand to *B. manusensis*.

Distribution of *B. aduloides* at Myojinsho is interesting because this species has been recorded mainly from cold

seeps near Japan, i.e. at Off Kikajima, Okinoyama Bank in Sagami Bay and Kanasunose Bank in Suruga Bay. *Bathymodiolus aduloides* has been also recorded in hydrothermal vents but the location is the relatively distant Minami-Ensei Knoll and Iheya Ridge in the mid-Okinawa Trough, an active back-arc basin under extensional stress and in an incipient stage of back-arc spreading related to subduction of the Philippine Sea Plate along the Nansei Shoto Trench. Consequently, this is the first report on the new distribution of *B. aduloides* at the Izu-Ogasawara area, an island arc that extends longitudinally along the edge of the north-eastern Philippine Sea Plate (Figure 4).

This distribution pattern is similar to those of two other bathymodiolin mussels, *B. japonicus* and *B. platifrons*: these mussels inhabit both vents in Okinawa Trough and cold seep in Sagami Bay, of which the distance is approximately 1500 km (Kyuno *et al.*, 2009). However, as these two species harbour methanotrophic symbionts (Fujiwara *et al.*, 2000), their distribution is limited by availability of methane. As *B. aduloides* harbours thiotrophic symbionts, it seems that the distribution of *B. japonicus* and *B. platifrons* are limited by chemical condition rather than physical environment.

The distribution pattern of *B. aduloides* is similar to *Calyptogena* clams, which inhabit both Sagami Bay and Iheya Ridge in Okinawa Trough. It is also interesting that both harbour thiotrophic symbionts and have behaviour to bury the shells into sediment. However, they have different strategies for sulphide uptake: *Calyptogena* clams bury themselves deeper than *B. aduloides* inserting the foot deeply into the sulphide-rich sediment because they absorb sulphide from their foot and transport it to the symbionts in the gills.

For this purpose, they have evolved special haemoglobin (Childress *et al.*, 1993; Goffredi & Barry, 2002). Thus, they must inhabit the place where sulphide is in the sediment. Indeed, it is reported that *Calyptogena soyoae* inhabit a location where pore water containing hydrogen sulphide exists in the sedimentary layer (Hashimoto *et al.*, 1995; Tsunogai *et al.*, 1996). In contrast, *B. aduloides* do not have such carrier protein and they must absorb hydrogen sulphide in the water, not in the sediment, through the gill. In this context, *Calyptogena* clams are rather similar to vestimentiferan tubeworms, which absorb sulphide in the sediment (Julian *et al.*, 1999).

*Bathymodiolus septemdiernum* is not found at Myojinsho, whereas they inhabit at the depth of 1200–1300 m in Myojin Knoll, only 20 km distance from Myojinsho (Figure 1). It is interesting that *B. septemdiernum* and *B. aduloides* do not show sympatric distribution although they are congeners harbouring thiotrophic symbionts. Concerning sulphur isotopic composition of soft tissue of *B. septemdiernum* and *B. aduloides*, the former is similar to hydrogen sulphide in the hydrothermal fluids and the sulphide minerals in the chimney and sediments around their habitats, whereas the latter is similar to the hydrogen sulphide derived from sulphate in the seawater, dissimilarly reduced by sulphate-reducing bacteria (Yamanaka *et al.*, 2000). Thus, it is considered that the reason why *B. septemdiernum* and *B. aduloides* show allopatric distribution is that those mussels use a different source of sulphide. Indeed, at the eastern part of Myojin Knoll, there are many active chimneys and brecciated massive sulphide (Iizasa *et al.*, 1997), around which many large and dense colonies of *B. septemdiernum*

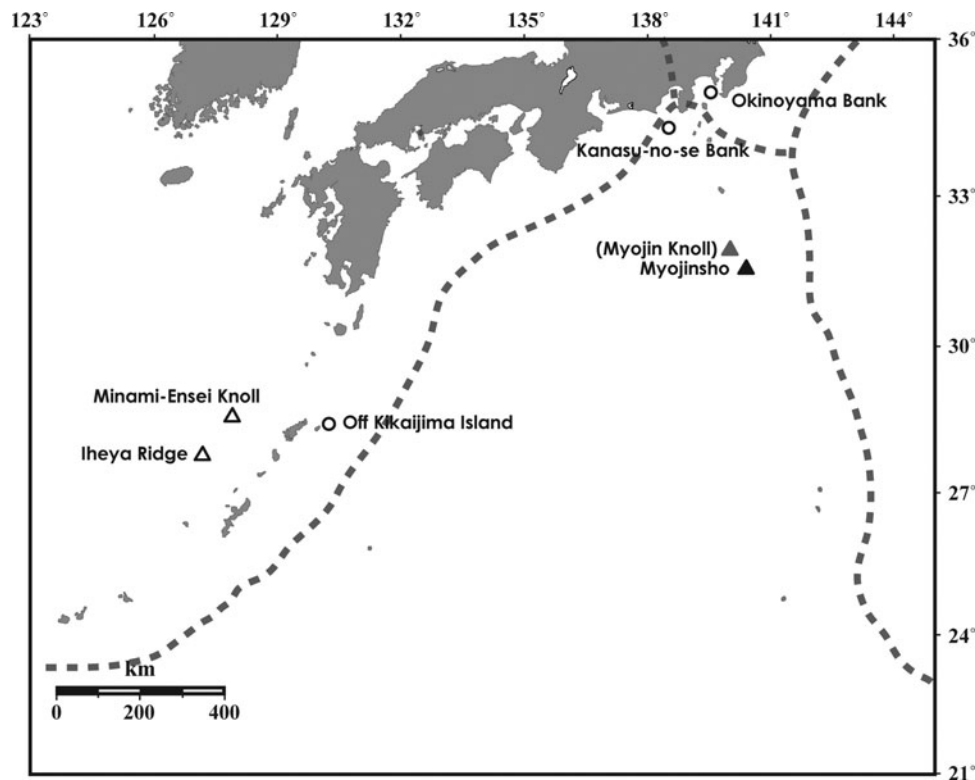


Fig. 4. Habitat location of *Bathymodiolus aduloides*: closed triangle indicates the location of the present study, Myojinsho. Previously reported habitats are indicated by open triangles (hydrothermal vent) and open circle (cold seep). Myojin Knoll (grey triangle) was also shown as the reference of the distance although *B. aduloides* has not been found there. Dashed lines indicate the plate boundaries.

are found, forming rich fauna with the barnacle *Neoverruca* sp. and a number of the hydrothermal vent-specific crab *Gandalfus yunohana* (Kojima, 2002). In contrast, Myojinsho seafloor is mainly occupied by boulder to pebble-sized rocks covered thinly with sediment (Iizasa *et al.*, 1992), and we did not find hydrothermal vent fluid from chimneys. Therefore, sulphide supply may not be sufficient for *B. septem-dierum*. The population of *Neoverruca* sp. and *G. yunohana* which is also likely to be dependent on hydrothermal fluid (Kojima, 2002) was also poor at Myojinsho. Instead, numerous bivalves of the family Pectinidae were observed at the sandy bottom of Myojinsho; it has not been recorded at Myojin Knoll. Thus, species composition and geological character are different between the two calderas despite the close distance, and the difference is likely to be due to the difference of the chemical environments.

It is also possible that periodic eruptions destroyed the previous biota of Myojinsho and *B. aduloides*, which has been carried from Okinawa Trough and some other locations, and has settled into the open niche. In this case, *B. septem-dierum* may appear in Myojinsho in the future. Thus, Myojinsho offers a good model to investigate the succession of a hydrothermal vent-specific community, and continuous observation should be performed.

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## REFERENCES

- Childress J.J., Fisher C.R., Favuzzi J.A., Arp A.J. and Oros D.R. (1993) The role of a zinc-based, serum-borne sulphide-binding component in the uptake and transport of dissolved sulphide by the chemoautotrophic symbiont-containing clam *Calyptogena elongata*. *Journal of Experimental Biology* 179, 131–158.
- Distel D.L., Baco A.R., Chuang E., Morrill W., Cavanaugh C. and Smith C.R. (2000) Do mussels take wooden steps to deep-sea vents? *Nature* 403, 725–726.
- Felbeck H. (1983) Sulphide oxidation and carbon fixation by the gutless clam *Solemya reidi*: an animal–bacteria symbiosis. *Journal of Comparative Physiology* 152, 3–11.
- Fiske R.S., Cashman K.V., Shibata A. and Watanabe K. (1998) Tephra dispersal from Myojinsho, Japan, during its shallow submarine eruption of 1952–1953. *Bulletin of Volcanology* 59, 262–275.
- Folmer O., Black M., Hoeh W., Lutz R. and Vrijenhoek R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3, 294–299.
- Fujiwara Y., Takai K., Uematsu K., Tsuchida S., Hunt J.C. and Hashimoto J. (2000) Phylogenetic characterization of endosymbionts in three hydrothermal vent mussels: influence on host distributions. *Marine Ecology Progress Series* 208, 147–155.
- Goffredi S.K. and Barry J.P. (2002) Species-specific variation in sulfide physiology between closely related vesicomid clams. *Marine Ecology Progress Series* 225, 227–238.
- Hashimoto J. and Okutani T. (1994) Four new mytilid mussels associated with deep-sea chemosynthetic communities around Japan. *Venus* 53, 61–83.
- Hashimoto J., Ohta S., Fujikura K., Fujiwara Y. and Sukizaki S. (1995) Life habitat of vesicomid clam, *Calyptogena soyoeae*, and hydrogen sulfide concentration in interstitial waters in Sagami Bay, Japan. *Journal of Oceanography* 51, 341–350.
- Iizasa K., Yuasa M. and Yokota S. (1992) Mineralogy and geochemistry of volcanogenic sulfides from the Myojinsho submarine caldera, the Shichito-Iwojima Ridge, Izu-Ogasawara Arc, north-western Pacific. *Marine Geology* 108, 39–58.
- Iizasa K., Naka J., Yuasa M. and Fiske R.S. (1997) Sulphide chimneys and massive sulfide at Myojin knoll caldera, Izu-Ogasawara arc. *JAMSTEC Journal of Deep Sea Research* 13, 443–456. [In Japanese with English abstract.]
- Julian D., Gaill F., Wood E., Arp A.J. and Fisher C.R. (1999) Roots as a site of hydrogen sulfide uptake in the hydrocarbon seep vestimentiferan *Lamellibrachia* sp. *Journal of Experimental Biology* 202, 2245–2257.
- Kojima S. (2002) Deep-sea chemoautosynthesis-based communities in the north-western Pacific. *Journal of Oceanography* 58, 343–363.
- Kyuno A., Shintaku M., Fujita Y., Matsumoto H., Utsumi M., Watanabe H., Fujiwara Y. and Miyazaki J.-I. (2009) Dispersal, differentiation of deep-sea mussels of the genus *Bathymodiolus* (Mytilidae, Bathymodiolinae). *Journal of Marine Biology* 72. Doi:10.1155/2009/625672.
- Lorion J., Buge B., Cruaud C. and Samadi S. (2010) New insights into diversity and evolution of deep-sea Mytilidae (Mollusca: Bivalvia). *Molecular Phylogenetics and Evolution* 57, 71–83.
- Miyazaki J.-I., Martins LdO., Fujita Y., Matsumoto H. and Fujiwara Y. (2010) Evolutionary process of deep-sea *Bathymodiolus* mussels. *Plos One* 5, 1–11
- Tsunogai U., Ishibashi J., Wakita H., Gamo T., Masuzawa T., Nakatsuka T., Nojiri Y. and Nakamura T. (1996) Fresh water seepage and pore water recycling on the seafloor: Sagami Trough subduction zone, Japan. *Earth and Planetary Science Letters* 138, 157–168.
- Van Dover C.L., German C.R., Speer K.G., Parson L.M. and Vrijenhoek R.C. (2002) Evolution and biogeography of deep-sea vent and seep invertebrates. *Science* 295, 1253–1257.
- and
- Yamanaka T., Mizota C., Maki Y., Fujikura K. and Chiba H. (2000) Sulfur isotope composition of soft tissues of deep-sea mussels, *Bathymodiolus* spp., in Japanese waters. *Benthos Research* 55, 63–68.

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