Research Article

Dispersal and Differentiation of Deep-Sea Mussels of the Genus *Bathymodiolus* (Mytilidae, Bathymodiolinae)

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Received 22 February 2009; Revised 27 May 2009; Accepted 30 July 2009

Recommended by Horst Felbeck

We sequenced the mitochondrial ND4 gene to elucidate the evolutionary processes of *Bathymodiolus* mussels and mytilid relatives. Mussels of the subfamily Bathymodiolinae from vents and seeps belonged to 3 groups and mytilid relatives from sunken wood and whale carcasses assumed the outgroup positions to bathymodioline mussels. Shallow water mytilid mussels were positioned more distantly relative to the vent/seep mussels, indicating an evolutionary transition from shallow to deep sea via sunken wood and whale carcasses. *Bathymodiolus platifrons* is distributed in the seeps and vents, which are approximately 1500 km away. There was no significant genetic differentiation between the populations. There existed high gene flow between *B. septemdierum* and *B. brevior* and low but not negligible gene flow between *B. marisindicus* and *B. septemdierum* or *B. brevior*, although their habitats are 5000–10 000 km away. These indicate a high adaptability to the abyssal environments and a high dispersal ability of *Bathymodiolus* mussels.

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1. Introduction

Deep-sea hydrothermal vents and their attendant dense biological communities were first discovered along the Galapagos Rift [1]. Since then, various deep-sea communities surviving under reductive environments rich in sulfide and methane have been discovered in hydrothermal vents on spreading ridges and back-arc basins and in coldwater seeps along subduction zones. These communities contain many endemic species whose primary production is based on bacterial chemosynthesis. Mussels of the genus *Bathymodiolus* are among the dominant macroorganisms in these communities. They rely primarily on chemoautotrophic endosymbionts for their nutrition similar to the other dominant groups of macroorganisms, such as vesicomyid clams and vestimentiferan tubeworms. The deep-sea mussels belong to one of the subfamilies, Bathymodiolinae, in the family Mytilidae of molluscan Bivalvia. Since the description

of *B. thermophilus* in 1985 [2], 19 species of the genus *Bathymodiolus* have thus far been described [3–12]. Three bathymodioline species belonging to genera, *Tamu* and *Gigantidas*, have been described [6, 10, 13].

Patchy and ephemeral deep-sea hydrothermal vents and coldwater seeps are separated from each other by various distances, for example, vent sectors are usually separated by a few hundred kilometers and within a vent sector, vent fields including sites which undergo the same temporal variations are separated by hundreds of meters to a few kilometers [14]. It is likely for the organisms of chemosynthesis-based communities to be genetically isolated in these discontinuous habitats; however, in Japanese waters, identical *Bathymodiolus* species are distributed in the Sagami Bay and the Okinawa Trough, which are approximately 1500 km away from each other [4]. On the other hand, there is no species shared between the Sagami Bay and the Izu-Ogasawara Island-arc, which are approximately 500 km away from each other.

Thus, speciation events do not necessarily depend on the geographical distances between habitats. Genetic differentiation and consequent speciation of deep-sea organisms in the community are caused by a combination of factors shared by diverse taxa (topography, geological histories, and oceanic currents) and those unique to their respective taxa (dispersal ability, physiology, and settlement cues) [15]. The dispersal ability of *Bathymodiolus* mussels is suggested to be high based on the larval shell morphology [16] and small egg size [17], which favors colonization in patchy and ephemeral habitats. Studies on genetic population structures can provide useful information on evolutionary processes such as dispersion, isolation, and speciation of deep-sea macroorganisms. It is tempting to examine the intraspecific relationships of *Bathymodiolus* mussels to search for factors that lead to speciation and populational differentiation.

Hydrothermal vents and cold-water seeps are driven by different geological processes. Hydrothermal vents are located at spreading centers and back-arc basins and emit water that is heated by the underlying magma chambers. Cold-water seeps are situated in passive margins along subduction zones and supply seawater, which is as cold as the ambient deep-sea water. Seeps are relatively stable, while vents persist for only a few decades [18]. Only 3 *Bathymodiolus* species in Japanese waters are capable of inhabiting both vents and seeps [4], although many species of chemosynthesis-based communities are restricted to either. This study examines whether the seep and vent populations of these *Bathymodiolus* species are genetically differentiated as a consequence of adaptation to highly different environments.

Dispersal ability and adaptability to the deep-sea environments have been found to be associated with speciation and thus the evolutionary process of deep-sea organisms [15, 19, 20]. Few studies of genetic population structures aimed at gaining an insight into dispersal ability have been done so far. Exceptions are for the northern and southern *Bathymodiolus* species of the East Pacific Rise [21] and the Mid-Atlantic Ridge [22]. There was no evidence of dispersal of northern species to the territory of southern species and vice versa [21, 22], with hybrid zones on the boundary of the territories in the case of the Atlantic mussels [22]. Genetic population structures of neoverrucid barnacles showed that they are unable to migrate between the Izu-Ogasawara Island-arc and the Okinawa Trough [23]. A study using East Pacific annelids showed that genetic population structures differed among species and suggested that those annelids had their own distinct abilities to disperse [24].

Only some *Bathymodiolus* species from restricted areas have been subjects in earlier molecular phylogenetic studies [25–28]. Then, given increasing sequence data, molecular phylogenetics searched for the phylogeny of about ten species [29, 30], and mytilid relatives from sunken whale carcasses and wood were included to trace the origin of *Bathymodiolus* mussels [31, 32]. In our previous studies [33], we showed, by sequencing of the mitochondrial COI (cytochrome *c* oxidase subunit I) gene of more than 15 nominal and cryptic bathymodioline species, that mussels in the subfamily Bathymodiolinae comprised 3 groups with one exception

of *Tamu fisheri*. The first group (Group 1) consisted of the West Pacific and Atlantic *Bathymodiolus* (Group 1-1) and *Gigantidas* mussels (Group 1-2). The second group (Group 2) consisted of *Bathymodiolus* mussels, which were subdivided into 3 subclusters (the Indo-West Pacific, Atlantic, and East Pacific species). The third group (Group 3) consisted of the West Pacific *Bathymodiolus* mussels. In the present study, we employed the faster-evolving mitochondrial ND4 (NADH dehydrogenase subunit 4) gene to investigate the genetic population structure and assess the dispersal ability and adaptability to deep-sea environments of *Bathymodiolus* mussels. We also investigated the phylogenetic relationships of deep-sea *Bathymodiolus* mussels and their mytilid relatives to understand the evolutionary processes of deep-sea animals.

2. Materials and Methods

2.1. Materials. The specimens used in this study are listed in Table 1, and the collection sites are mapped in Figure 1. All deep-sea mussels of the genus *Bathymodiolus* and *Gigantidas* (the subfamily Bathymodiolinae), except the East Pacific species and 3 Atlantic species, were collected during dives by submersibles from the Japan Agency for Marine-Earth Science and Technology (JAMSTEC). The East Pacific species *B. thermophilus* and the 2 Atlantic species *B. puteoserpentis* and *B. azoricus* were collected during the cruise of the scientific research vessel *Akademik Mstislav Keldysh* belonging to the Institute of Oceanology of the Russian Academy of Sciences. The Atlantic species *B. childressi* was collected in an oil-seep in the Gulf of Mexico during R/V Seward Johnson cruise (dive number 4568). The undescribed West Pacific species from off New Zealand (herein referred to as NZ *B. sp.*) was collected as described previously [27]. *Adipicola pacifica*, *A. crypta*, and *Benthomodiolus geikotsucola* (the subfamily Modiolinae) were collected from sunken whale carcasses during dives by submersibles from JAMSTEC. The mussels attached to sunken wood (modioline *A. iwaotakii* and *Idasola japonica*) were obtained by trawling. All the mussels collected for this study were frozen and preserved at -80°C or preserved in 100% ethanol, and deposited in JAMSTEC.

2.2. Sequencing of the Mitochondrial Gene. Total DNA was prepared from the foot muscle, gill, or mantle as described previously [26, 29, 33]. To amplify the 710 bp fragment including tRNAs and ND4, PCR was performed using a reaction mixture containing the template DNA and KOD dash (TOYOBO Co., Osaka) under the following conditions: 30 cycles of denaturation for 30 seconds at 94°C , annealing for 5 or 10 seconds at 45 or 56.5°C , and extension for 10 or 40 seconds at 74°C (depending on the samples). We used the ND4 primers described previously for amplification of fish ND4 [34, 35], that is, sense ArgBL (5'-caagacccttgatttcggtca-3') and antisense NAP2H (5'-tgagcttctacgtgrgcttt-3'). We also designed 2 sets of primers, that is, sense ND46S (5'-gctcatgccccgaatatgtct-3') and antisense ND47A (5'-caacctaaacaattatctctccc-3') and

TABLE 1: Sample list.

Species	Sample abbreviation	Sampling site (locality number in Figure 1)	Depth (m)	Habitat type
Bathymodiolinae				
<i>Bathymodiolus aduloides</i>	AK1-5	Off Kikaijima Island (23)	1 451	seep
<i>B. azoricus</i>	AZL1,2	Lucky Strike, Mid-Atlantic Ridge (31)	unknown	vent
<i>B. brevior</i>	BN1-17,19-22	Mussele Valley, North Fiji Basin (6)	unknown	vent
	BN23-30	White Lady, North Fiji Basin (6)	unknown	vent
<i>B. childressi</i>	ChiG1,2	Gulf of Mexico (36)	1 859	seep
<i>B. hirtus</i>	HK1-5	Kuroshima Knoll, Off Yaeyama Islands (25)	637	seep
<i>B. japonicus</i>	JH1,2,4-13	Off Hatsushima, Sagami Bay (19)	1 170–1 180	seep
	JH14-17	Off Hatsushima, Sagami Bay (19)	908	seep
	JH18-21	Off Hatsushima, Sagami Bay (19)	unknown	seep
<i>B. marisindicus</i>	MK1-19	Kairei Field, Southern Central Indian Ridge (28)	2 443–2 454	vent
<i>B. platifrons</i>	PH1-10	Off Hatsushima, Sagami Bay (19)	1 170–1 180	seep
	PH11,12	Off Hatsushima, Sagami Bay (19)	unknown	seep
	PH13-20	Off Hatsushima, Sagami Bay (19)	1 029	seep
	PI1-4	North Iheya Ridge, Mid-Okinawa Trough (24)	1 028	vent
	PT1-10,12-15	Hatoma Knoll, Okinawa Trough (26)	1 523	vent
	PY1,2	Dai-yon Yonaguni Knoll, southern Okinawa Trough (27)	1 336	vent
<i>B. puteoserpentis</i>	PUS1,2	Snake Pit, Mid-Atlantic Ridge (32)	3 023–3 510	vent
<i>B. securiformis</i>	LK1-5	Kuroshima Knoll, Off Yaeyama Islands (25)	641	seep
<i>B. septemdirum</i>	SM1,2	Myojin Knoll, Izu-Ogasawara Island-arc (17)	1 288–1 290	vent
	SM3-10	Myojin Knoll, Izu-Ogasawara Island-arc (17)	1 346	vent
	SS1-11	Suiyo Seamount, Izu-Ogasawara Island-arc (14)	1 373–1 382	vent
<i>B. thermophilus</i>	ThE1	9N East Pacific Rise (39)	2 524	vent
Chamorro <i>B. sp.</i>	C1-3	South Chamorro Seamount, Mariana (9)	2 899	seep
Eifuku <i>B. sp.</i>	EF1-5	Northwest Eifuku Seamount (11)	1 625	vent
Kikaijima <i>B. sp.</i>	Kikaijima	Off Kikaijima Island (23)	1 430	seep
Lau <i>B. sp.</i>	Lau1,3,4,6,8	Hine Hina, Lau Basin (1)	1 818	vent
<i>B. manusensis</i>	BE1-5	PACKMANUS Field E, Manus Basin (7)	1 627–1 629	vent
NF <i>B. sp.</i>	NF1	White Lady, North Fiji Basin (6)	unknown	vent
NZ <i>B. sp.</i>	Ne1-5	Off New Zea land (unknown)	unknown	vent
Sissano <i>B. sp.</i> 1	Si2-1-4	Sissano, Papua New Guinea (8)	1 646	seep
	Si3-5	Sissano, Papua New Guinea (8)	1 881	seep
Sissano <i>B. sp.</i> 2	Si1-1, Si3-1,2,4,6	Sissano, Papua New Guinea (8)	1 881	seep
Sissano <i>B. sp.</i> 3	Si3-3	Sissano, Papua New Guinea (8)	1 881	seep
<i>Gigantidas horikoshii</i>	Kaikata	Kaikata Seamount (13)	486	vent
Aitape <i>G. sp.</i>	Aitape1,2	Aitape, Papua New Guinea (8)	470	seep
Ashizuri <i>G. sp.</i>	Ashizuri	Off Ashizuri Cape (21)	575	seep
Nikko <i>G. sp.</i>	NK1-5	Nikko Seamount (12)	485	vent
Sumisu <i>G. sp.</i>	Su1-5	Sumisu Caldera (16)	676–686	vent
Database				
<i>B. azoricus</i>	Baz1-3 (DB)	Menez Gwen (31)	866–2 330	vent
<i>B. brevior</i>	<i>B. brevior</i> MT (DB)	Mariana Trough (10)	3 589	vent
<i>B. brooksi</i>	<i>B. brooksi</i> AC (DB)	Alamiños Canyon (38)	2 222	seep
	<i>B. brooksi</i> WFE (DB)	West Florida Escarpment (35)	3 314	seep
<i>B. childressi</i>	<i>B.childressi</i> (DB)	Alamiños Canyon (38)	540–2 222	seep
<i>B. heckerae</i>	<i>B.heckerae</i> BR (DB)	Blake Ridge (34)	2 155	seep
	<i>B.heckerae</i> WFE (DB)	West Florida Escarpment (35)	3 314	seep

TABLE 1: Continued.

Species	Sample abbreviation	Sampling site (locality number in Figure 1)	Depth(m)	Habitat type
<i>B. marisindicus</i>	MK (DB)	Kairei Field, Southern Central Indian Ridge (28)	2 415–2 460	vent
<i>B. mauritanicus</i>	<i>B. mauritanicus</i> (DB)	West Africa (30)	1 000–1 267	seep
<i>B. puteoserpentis</i>	Bpu1-3 (DB)	Snake Pit, Mid-Atlantic Ridge (32)	3 023–3 510	vent
<i>B. tangaroa</i>	<i>B. tangaroa</i> (DB)	Off Turnagain Cape, New Zealand (4)	920–1 205	seep
<i>B. thermophilus</i>	<i>B. thermophilus</i> A (DB)	9N East Pacific Rise (39)	2 460–2 747	vent
	<i>B. thermophilus</i> B (DB)	7S East Pacific Rise (40)	2 460–2 747	vent
<i>B. sp. East Pacific</i>	<i>B. aff. thermophilus</i> (DB)	32S East Pacific Rise (41)	2 331	vent
<i>B. sp. NZ3</i>	<i>B. sp. NZ3</i> (DB)	Macauley Cone (3)	200	vent
<i>Gigantidas gladius</i>	<i>Gigantidas gladius</i> (DB)	Rumble III (5)	300–460	vent
<i>Tamu fisheri</i>	<i>Tamu fisheri</i> (DB)	Garden Banks (37)	546–650	seep
Modiolinae and Mytilinae				
<i>Adipicola crypta</i>	ACN1-3,328-1,2	Off Noma Cape, Kagoshima (22)	225–229	whale bone
<i>Adipicola iwaotakii</i>	AIH1-5	Off Nakaminato, Ibaraki (18)	490	wood
<i>Adipicola pacifica</i>	APN1-3,328-25,26	Off Noma Cape, Kagoshima (22)	225–229	whale bone
<i>Idasola japonica</i>	IJN1,2	Off Noma Cape, Kagoshima (22)	400–425	wood
<i>Modiolus nipponicus</i>	<i>Modiolus nipponicus</i>	Off Oura harbor, Shizuoka (20)	—	shallow
<i>Benthomodiulus geikotsucola</i>	Tori1-1-5	Torishima Seamount (15)	4 051	whale bone
Database				
<i>Benthomodiulus lignicola</i>	<i>Benthomodiulus lignicola</i> (DB)	Chatham Rise (2)	826–1 174	whale bone, wood
<i>Idas macdonaldi</i>	<i>Idas macdonaldi</i> (DB)	Garden Banks (37)	650	seep
<i>Idas washingtonia</i>	<i>Idas washingtonia</i> (DB)	Monterey Bay (42)	960–1 910	whale bone, wood, vent
<i>Mytilus edulis</i>	ME (DB)	Chester Basin, Nova Scotia, Canada (33)	—	shallow
<i>Mytilus galloprovincialis</i>	MG (DB)	Saronic Gulf, Greece (29)	—	shallow
<i>Mytilus trossulus</i>	MT (DB)	Chester Basin, Nova Scotia, Canada (33)	—	shallow

sense toriI-6S (5'-ttcgtctgctttacacgaagaagt-3') and antisense toriI-6A (5'-agtcaactaaacctatcacctct-3'). Direct sequencing was performed by using an ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems Inc., Calif, USA) and the primers for PCR on a Model 377 DNA sequencer (Applied Biosystems Inc., Calif, USA) according to the manufacturer's instructions. The ND4 sequence of the Indo-Pacific species *B. brevior* from DDBJ ([36]; AY046277-9, specimens from the Indian Ocean) has been cited herein as that of *B. marisindicus*.

2.3. Analysis. The DNA sequences were edited and aligned using DNASIS (Hitachi Software Engineering Co., Ltd., Tokyo) and MEGA 3.1 [37] and were corrected by visual inspection. We used 423-bp ND4 sequences and constructed dendrograms by the neighbor-joining (NJ) and maximum parsimony (MP) methods using PAUP*4.0 beta10 [38]. Genetic distances were computed according to Kimura's two-

parameter method [39]. The reliability of the trees was evaluated by producing 1,000 bootstrap replicates. The majority-rule consensus MP tree was constructed by conducting a heuristic search based on the 1,000 bootstrap replicates with an unweighted ts/tv ratio. The Bayesian tree was constructed using MrBayes version 3.1 [40] based on the model evaluated by the Mrmodel test 2.2 [41]. The Monte Carlo Markov chain (MCMC) length was 1×10^6 generations, and we sampled the chain after every 100 generations. MCMC convergence was assessed by calculating the potential scale reduction factor, and the first 2×10^3 generations were discarded. We used *Modiolus nipponicus* (the subfamily, Modiolinae) as an outgroup species.

We estimated the genetic divergences (F_{st}) and the bi-directional mean rates of gene flow (N_m ; the virtual average number of migrants exchanged per generation) between the populations using Arlequin 3.1 [42]. We evaluated the significance of F_{st} by calculating 1×10^6 values. We also calculated the mismatch distribution [43] and constructed

FIGURE 1: The sampling sites of the deep-sea *Bathymodiulus* mussels and their relatives used in this study. Refer to Table 1 for the details of the sampling sites (1 to 42). (a) worldwide map; (b) the magnified map showing the areas around Japanese waters. ○, hydrothermal vent; ●, cold-water seep; ■, wood/whale bone; ▲, shallow.

the minimum spanning tree [44] using Arlequin 3.1. We performed goodness-of-fit tests to evaluate discrepancy between the observed and model values of the mismatch distribution. The age of demographic expansion ($\tau = 2ut$) is proportional to the number of generations (t) since a population at equilibrium of size entered a demographic expansion phase, although the mutation rate (u) of mytilid mussels is unknown. For analysis of the genetic population structures, we determined the ND4 sequences of 20 specimens each of *B. platifrons* from the Sagami Bay and the Okinawa Trough and *B. japonicus* from the Sagami Bay. We also determined the ND4 sequences of 20 specimens of *B. marisindicus* from the Kairei field, 29 specimens of *B. brevior* from the North Fiji Basin, and 21 specimens of *B. septemdierum* from the Myojin Knoll and the Suiyo Seamount.

3. Results

3.1. Phylogenetic Relationships among the Bathymodiulus Mussels. The partial DNA fragments of the mitochondrial ND4 gene (423 bp) were sequenced from 1 to 5 specimens (if available, more than 5) of *Bathymodiulus* species and their mytilid relatives. The sequence data were deposited in the DDBJ, EMBL, and GenBank databases under accession numbers AB478422-AB478475. A part of the sequence data was previously reported (AB175280-AB175326, [29]). No deletions or insertions were found in the sequences after excluding the sequences of *Modiolus* and *Mytilus*. Sequences encoding 2 amino acids were deleted or inserted when *Modiolus* and *Mytilus* species were included.

The deep-sea mussels of the subfamily Bathymodiolinae formed a poorly supported cluster consisting of 3 major groups (Figure 2). The ND4 trees constructed by the NJ, MP, and Bayesian methods yielded fundamentally the same topology. Previously published COI trees [33] also presented essentially the same topology (Figure 3). The first group (Group 1) was marginally supported (73, 57, and 0.97 for NJ, MP, and Bayes, resp.) and was subdivided into 2 clades. Group 1-1 was well supported (98, 91, 1.00) and contained the *Bathymodiulus* mussels exclusively, including the 7 nominal species, namely *B. hirtus*, *B. japonicus*, *B. platifrons*, and *B. securiformis* from Japanese waters, *B. tangaroa* from the West Pacific, and *B. mauritanicus* and *B. childressi* from the Atlantic along with 5 unidentified mussels from Sissano (*B. sp.1*, *B. sp.2*, and *B. sp.3*), the Chamorro Seamount (*B. sp.*), and off Kikaijima Island (*B. sp.*) in the West Pacific. Group 1-2 was well supported (96, 83, 0.97) and included the 2 nominal species, namely *Gigantidas horikoshii* and *G. gladius*, and 2 unidentified mussels from Aitape (*Aitape G. sp.*) and off Ashizuri Cape (*Ashizuri G. sp.*) in the West Pacific. We regarded the mussels from the Sumisu Caldera (*Sumisu G. sp.*) and the Nikko Seamount (*Nikko G. sp.*) as conspecific with *G. horikoshii*, because they were very closely related to each other. *Adipicola crypta* belonging to the subfamily Modiolinae formed a marginally supported cluster together with Group 1-2. On the other hand, *A. crypta* was a sister taxon to the cluster including Groups 1 and 2 in the COI tree (Figure 3).

Group 2 was well supported (94 and 88 for NJ and MP, respectively), but the topology of the Bayesian tree was different from those of the NJ and MP trees. This group consisted of the 8 nominal species, namely *B. septemdierum* and *B. brevior* from the West Pacific, *B. marisindicus* from the Indian Ocean, *B. azoricus*, *B. puteoserpentis*, *B. heckerae*, and *B. brooksi* from the Atlantic, and *B. thermophilus* and 1 undescribed (morphologically examined but not described yet) *Bathymodiulus* species from the East Pacific (East Pacific *B. sp.*). *Bathymodiulus septemdierum*, *B. brevior*, and *B. marisindicus* comprised the closely related species group (Cluster A). Mussels from the Eifuku Seamount were included in Cluster A. Group 2 was subdivided into 3 well-supported clades comprising the Indo-West Pacific, Atlantic, and East Pacific species. The only exception was the Atlantic species *B. brooksi*, which diverged basally to the Indo-West Pacific clade and the Atlantic clade including the other Atlantic species. In the COI tree (Figure 3), *B. brooksi* diverged basally to the Indo-West Pacific, Atlantic, and East Pacific clades. *Tamu fisheri* was found to be distantly related to the other bathymodioline species in our previous study based on COI sequences (Figure 3); however, the species was closely related to the *Bathymodiulus* species of Group 2 although the alliance was poorly supported. Group 3 was well supported (100, 99, 1.00) and consisted of the 2 nominal species *B. aduloideus* and *B. manusensis* from the West Pacific. Mussels from the Lau Basin (*Lau B. sp.*), North Fiji Basin (*NF B. sp.*), and off New Zealand (*NZ B. sp.*) should be conspecific with *B. manusensis*, because they were very closely related to each other. The distribution of Group 3 was restricted to the West Pacific.

The species of the subfamily Modiolinae obtained from sunken wood and whale carcasses and seeps, namely, *Benthomodiulus lignicola*, *Benthomodiulus geikotsucola*, *Idas macdonaldi*, *I. washingtonia*, *Adipicola iwaotakii*, *A. pacifica*, *Idasola japonica*, and 1 unidentified mussels from Macauley Cone (*B. sp. NZ3*), were outside the cluster including the bathymodioline species and modioline *A. crypta*. The relationships were also shown in the COI tree (Figure 3). *B. sp. NZ3* was reported to belong to the genus *Bathymodiulus* [30]. However, it was distantly related to *Bathymodiulus* as in the COI tree, and its phylogenetic position remains to be studied.

3.2. Genetic Population Structure. A minimum spanning tree was constructed based on the ND4 sequences of a total of 60 specimens of *B. platifrons* and *B. japonicus* (Figure 4(a)). The new sequence data were deposited in the DDBJ, EMBL, and GenBank databases under accession numbers AB480561-AB480578. As expected, no haplotype was shared by *B. platifrons* and *B. japonicus*. Significantly high F_{st} ($0.968 = (0.965 + 0.972)/2$) and small N_m ($0.016 = (0.018 + 0.014)/2$) were estimated between the 2 species (Table 2). On the other hand, the major haplotype was shared by 24 (60%) specimens of *B. platifrons* from the seeps of the Sagami Bay and the vents of the Okinawa Trough. Negative F_{st} (-0.007) and N_m of infinity were

FIGURE 2: The phylogenetic relationships of the deep-sea *Bathymodiolus* mussels and their relatives based on the 423-bp mitochondrial ND4 sequences. The NJ tree was constructed based on the genetic distances calculated according to Kimura's two-parameter method using *Modiolus nipponicus* as an outgroup species. The MP and Bayesian trees presented essentially the same topology as the NJ tree. Only the NJ (left) and MP (middle) bootstrap values >50% and Bayesian posterior probabilities (right) >0.50 are specified. The scale bar indicates 0.01 substitutions per site. See Table 1 for abbreviations of *Bathymodiolus* mussels and their relatives. ○, hydrothermal vent; ●, cold-water seep; ■, wood/whale bone; ▲, shallow.

FIGURE 3: The phylogenetic relationships of the deep-sea *Bathymodiolus* mussels and their relatives based on the 401-bp mitochondrial COI sequences. The tree was modified from our published data [33] for reference. ○, hydrothermal vent; ●, cold-water seep; ■, wood/whale bone; ▲, shallow. (c) Malacological Society of Japan.

FIGURE 4: The minimum spanning trees revealing the genetic population structure based on the 423-bp mitochondrial ND4 sequences. The tree (a) was constructed using total 60 specimens of *B. platifrons* from the seeps of the Sagami Bay (PH1~20), *B. platifrons* from the vents of the Okinawa Trough (PT1~10, 12~15, PI1~4, PY1, 2), and *B. japonicus* from the seeps of the Sagami Bay (JH1, 2, 4~21). Black, haplotypes possessed by *B. platifrons* specimens from the vents; gray, haplotypes shared by *B. platifrons* specimens from the seeps and vents; white, haplotypes possessed by *B. platifrons* or *B. japonicus* specimens from the seeps. The tree (b) was constructed using a total of 70 specimens of *B. septemdierum* from the Myojin Knoll (SM1~10) and the Suiyo Seamount (SS1~11), *B. brevior* from the North Fiji Basin (BN1~17, 19~30), and *B. marisindicus* from the Kairei field (MK1~19, MK from a database). Black, haplotypes possessed by *B. marisindicus* specimens; gray, haplotypes shared by the three species; white with the bold outline, haplotypes possessed by *B. brevior*; white with the thin outline, haplotypes possessed by *B. septemdierum* or those shared by *B. brevior* and *B. septemdierum*.

FIGURE 5: Mismatch distribution based on the 423-bp mitochondrial ND4 sequences. (a) *B. japonicus* from the Sagami Bay; *B. platifrons* from the Sagami Bay; *B. platifrons* from the Okinawa Trough; (d) *B. marisindicus* from the Kairei field; (e) *B. septemdiernum* from the Myojin Knoll and the Suiyo Seamount; (f) *B. brevior* from the North Fuji Basin.

TABLE 2: F_{st} (bold values) and N_m (light values). JH, *B. japonicus* from the Sagami Bay; PH, *B. platifrons* from the Sagami Bay; PIPTPY, *B. platifrons* from the Okinawa Trough; MK, *B. marisindicus* from the Kairei Field; SMSS, *B. septemdierum* from the Myojin Knoll and the Suiyo Seamount; BN, *B. brevior* from the North Fiji Basin.

(a)			
	JH	PH	PIPTPY
JH		0.01823	0.01433
PH	0.96483*		inf
PIPTPY	0.97215*	-0.00668	
(b)			
	MK	SMSS	BN
MK		1.48622	1.44545
SMSS	0.25173*		29.61904
BN	0.25701*	0.0166	

estimated between the 2 populations. These results showed high gene flow between the seep and vent populations. Neither the seep nor the vent population was monophyletic, and the members of both populations were intermingled (Figure 2), indicating that the type of environment is not the primary factor responsible for habitat segregation.

A minimum spanning tree was also constructed based on the ND4 sequences of a total of 70 specimens of *B. septemdierum*, *B. brevior*, and *B. marisindicus* (Figure 4(b)). The new sequence data were deposited in the DDBJ, EMBL, and GenBank databases under accession numbers AB485606-AB485629. The haplotype of the greatest majority was shared by 21 (30%) specimens of *B. septemdierum* from the Myojin Knoll and the Suiyo Seamount of the Izu-Ogasawara Island-arc and *B. brevior* from the North Fiji Basin of the Southwest Pacific. One of the 2 major haplotypes was possessed exclusively by 6 specimens (8.6%) of *B. marisindicus* from the Kairei field of the Southern Central Indian Ridge, and the other was shared by 7 specimens (10%) of the 3 species, although they are distinct species. Low F_{st} (0.017) and large N_m (29.619) were estimated between *B. septemdierum* and *B. brevior*, while significantly high F_{st} ($0.254 = (0.252 + 0.257)/2$) and small N_m ($1.466 = (1.486 + 1.445)/2$) were estimated between *B. marisindicus* and the 2 West Pacific species (Table 2).

Mismatch distribution showed that the τ values were 2.197 for *B. japonicus* and 2.111 and 1.967 for *B. platifrons* from the Sagami Bay and the Okinawa Trough, respectively (Figures 5(a)–5(c)), and the τ values of 3.752, 2.383, and 1.394 were assigned to *B. marisindicus*, *B. septemdierum*, and *B. brevior*, respectively (Figures 5(d)–5(f)). Goodness-of-fit tests showed no significant differences between the observed and model values ($P = .42$ for *B. japonicus*; 0.91 for the Sagami Bay population of *B. platifrons*; 0.20 for *B. marisindicus*; 0.79 for *B. septemdierum*; 0.86 for *B. brevior*) except for the Okinawa Trough population of *B. platifrons* (0.00).

4. Discussion

4.1. Phylogenetic Relationships of the Bathymodiolus Mussels and their Relatives. The present study based on the ND4 sequences presented fundamentally the same phylogenetic relationships of the *Bathymodiolus* mussels and their relatives as those reported by our previous study based on the COI sequences [33]. Although the discrepancy was found in the positions of *Tamu fisheri* and *Adipicola crypta*, it did not affect the major conclusions presented in our previous study. The bathymodioline *Tamu fisheri* was closely related to the *Bathymodiolus* mussels of Group 2 in the ND4 study, while it was shown to be distantly related to the other bathymodioline species in the COI study. Although the modioline *Adipicola crypta* was closely related to Group 1-2 in the ND4 study, it was more closely related to the cluster consisting of Groups 1-1, 1-2, and 3 in the COI study. Both studies suggested that the subfamily Bathymodiolinae and the genus *Bathymodiolus* were not monophyletic because the monophyly of the former and that of the latter were refuted by the existence of *A. crypta* and two *Gigantidas* species, respectively. More extensive morphological investigations are needed to reevaluate the classification. The branching orders in Groups 1 to 3 and in the 3 subclusters of Group 2 differed between the ND4 and COI studies. However, their divergences appeared trichotomous because of the short branch lengths between the nodes leading to the groups and clusters.

4.2. Adaptation of the Mussels to the Abyssal Environment. The present study supports the “Evolutionary stepping stone hypothesis” [25, 45]. According to this hypothesis, the ancestors of *Bathymodiolus* mussels exploited sunken wood and whale carcasses in their progressive adaptation to the deep-sea environment with regard to nutrition and tolerance to high pressure, cold seawater, and toxicity of hydrogen sulfide. Both ND4 and COI [33] trees showed that species from sunken wood and whale carcasses assumed the outgroup position to the *Bathymodiolus* and *Gigantidas* mussels from the vents and cold seeps, with only the exception of *A. crypta* from the whale carcasses. Shallow water mytilid mussels such as *Modiolus nipponicus*, *Mytilus edulis*, *M. galloprovincialis*, and *M. trossulus* were positioned more distantly to the vent/seep mussels. The findings indicate an evolutionary transition from the shallow water to vent/seep sites via the wood/whale carcass sites, and a reversion to the whale carcass sites from the vents or seeps in the case of *A. crypta*. The studies also suggest independent invasion into the seeps in case of *Idas macdonaldi* and into the vents in case of *I. washingtonia* and *B. sp. NZ3*.

Most species of the deep-sea chemosynthesis-based communities are restricted either to seeps or vents. Only three known *Bathymodiolus* species endemic to Japanese waters can inhabit both seeps and vents. *Bathymodiolus japonicus* and *B. platifrons* live in the seeps in the Sagami Bay and the vents in the Okinawa Trough. We analyzed the genetic population structure to examine whether the seep and vent populations of *B. platifrons* were genetically differentiated owing to their adaptation to the highly different habitats.

The present results showed no significant genetic differentiation between the seep and vent populations, indicating a high adaptability of the species to the abyssal environment. Genetic similarity between populations from the Sagami Bay and the Okinawa Trough was also shown in deep-sea bresiliid shrimp *Alvinocaris longirostris* [46].

Habitat segregation is not caused by habit types (seep versus vent), but depth in some species of the chemosynthesis-based community. The vestimentiferan tubeworm *Escarpia* sp. inhabits the seeps in Japanese waters and the vents in the Manus Basin, and no genetic differentiation was detected between their seep and vent populations, although their habitats are approximately 4400 km away from each other [47]. The populations from the seeps in the Nankai Trough and the vents in the Lau Basin were not distinguished genetically in the vestimentiferan *Lamellibrachia columna*, although their habitats are 7500 km away from each other [48]. Habitation of *Calyptogena* clams in Japanese waters is constrained by depth, but not restricted by the type of environment, and their colonization is not limited by geographical distances between habitats [49]. Habitat segregation by depth in the *Calyptogena* clams is probably ascribed to the differences in their physiological tolerance to pressure [49, 50].

It is unlikely that habitation is constrained by depth [26] or colonization is limited by geographical distances (as described below) in some species of the genus *Bathymodiolus*. Instead habitat segregation and colonization of deep-sea mussels can be ascribed to their preference to one (or some) specific ambient condition(s). *Bathymodiolus* species that harbor only methanotrophic endosymbionts occur in cold-water seeps and hydrothermal vents with higher methane concentrations, and the deep-sea mussels that depend on thioautotrophic endosymbionts for their nutrition occur in vents with lower methane concentrations [51]. This suggests that the chemical environment in their habitats is one of the factors restricting the distribution of the *Bathymodiolus* species.

4.3. Dispersal of the Deep-Sea Mussels. High gene flow ($N_m = \text{infinity}$) was detected between the populations from the Sagami Bay and the Okinawa Trough in *B. platifrons*, although the sites are more than 1500 km away from each other. An N_m value of more than 1 is indicated to be sufficient to maintain genetic continuity among populations [42].

The species in Cluster A were very closely related to one another, although they are distributed over vast distances, including *B. marisindicus* in the Kairei field, *B. brevior* in the North Fiji Basin, and *B. septemdierum* in the Myojin Knoll and the Suiyo Seamount. Our previous study [29] showed that their interspecific genetic distances were considerably smaller than those of species except the Cluster A species and approximated to intraspecific genetic distances of the latter. High gene flow ($N_m = \text{ca. } 30$) existed between *B. septemdierum* from the Myojin Knoll and the Suiyo Seamount and *B. brevior* from the North Fiji Basin. The localities of the two species are approximately 5,000 km

away. Furthermore, the gene flow between *B. septemdierum* and *B. marisindicus* from the Kairei field ($N_m = \text{ca. } 1.5$) and that between *B. brevior* and *B. marisindicus* ($N_m = \text{ca. } 1.4$) were not negligible despite significantly high genetic divergences ($F_{st} = \text{ca. } 0.25$ and 0.26 , resp.). The locality of *B. marisindicus* is approximately 10,000 km away from those of *B. septemdierum* and *B. brevior*. Therefore, the present results showed that (1) gene flow was present between *B. septemdierum* and *B. brevior* and (2) although *B. marisindicus* was not isolated from *B. septemdierum* and *B. brevior*, gene flow was relatively limited. These results indicate the high dispersal ability of deep-sea mussels, although various factors such as oceanic circulation patterns, water temperature, and sea-floor topography may change the actual dispersal distances.

Bathymodiolus mussels are suggested to have the high dispersal ability, based on their larval shell morphology [16] and small egg size [17] that are indicative of planktotrophic (actively feeding planktonic larval) development. Developmental arrest at cold temperatures also appears to play an important role in extension of the planktonic stage and increasing the dispersal distance [52].

Some deep-sea animals are known to have an ability to disperse their larvae over very long distances. The East Pacific deep-sea mussel *B. thermophilus* and clam *Calyptogena magnifica* are estimated to have dispersal capabilities of at least 2,370 km and 3,340 km, respectively [53]. The vestimentiferans *Escarpia* sp. and *Lamellibrachia* sp. have larvae with long-distance dispersal capability [47, 48]. Kojima et al. [54] showed the existence of active gene flow between the populations in the Manus Basin and the North Fiji Basin (3500 km away from each other) in the vent-endemic gastropod *Alviniconcha* sp. *Riftia pachyptila* had a larval stage of approximately 38 d under conditions similar to the *in situ* environment, suggesting that larvae can disperse over 100 km albeit influenced by deep-water circulation regimes [55]. The larvae of the verruciform barnacle *Neoverruca* sp. had a planktonic period of over 70 d at 4°C under 1 atm, suggesting its high dispersal ability [56]. Since *Riftia pachyptila* and *Neoverruca* sp. have non-planktotrophic larvae, it is conceivable that *Bathymodiolus* mussels can disperse their planktotrophic larvae over longer distances.

Mismatch distribution showed that the τ values decreased in the order of Sagami Bay *B. japonicus*, Sagami Bay *B. platifrons*, and Okinawa Trough *B. platifrons* (Figures 5(a)–5(c)). The results suggest the immigration of ancestral *B. platifrons* into the Okinawa Trough from the Sagami Bay, which is consistent with the history of the Japanese archipelago. The Okinawa Trough has been habitable for animals in the chemosynthesis-based community since ca. 200 MYA, while the Sagami Bay since more than 500 MYA [57]. However, this immigration event appears unlikely because the difference in the τ values was small (2.111 versus 1.967), and the intense stream, due to the existence of the Kuroshio Current, runs from the Okinawa Trough to the Sagami Bay down to a depth of 1,000 m. Therefore, we suppose that immigration might have occurred to the Okinawa Trough and the Sagami Bay from an unknown

home location somewhere in the West Pacific. The τ values decreased in the order of *B. marisindicus*, *B. septemdierum*, and *B. brevior* (Figures 5(d)–5(f)), proposing that the ancestor of the 3 species might have migrated from the Southern Central Indian Ridge to the Izu-Ogasawara Island-arc via the Southwest Pacific. However, the present definite gene flow between *B. septemdierum* and *B. brevior* revealed that they were conspecific or sibling species that had recently differentiated, thus suggesting a greater probability that the Southern Central Indian Ridge might be the more ancient residence rather than the West Pacific. The possible routes for dispersal appear to be along the seeps and vents localized to north and south of Australia or through sunken wood and whale carcasses. The subduction zones reel along the South Asian Islands, and the Southeast Indian Ridge runs from the east to the west.

We suggest collecting novel or more specimens to answer the questions regarding the dispersal routes and barriers. Such future work will provide more information on the sea floor topography, oceanic circulation, distribution of unknown seeps and vents, and larval development.

Acknowledgments

The authors would like to express our thanks to Drs. Charles R. Fisher (Pennsylvania State University; the mussel was collected with support to C. Fisher from the Mineral Management Service and the NOAA Ocean Exploration Program), Tadashi Maruyama (JAMSTEC), and Peter Smith (National Institute of Water and Atmospheric Research Ltd.) for providing *Bathymodiolus* mussels. We wish to express our gratitude to Drs. Yurika Ujiie and Juichiro Ashi (University of Tokyo), Drs. Takashi Okutani, Katsuyuki Uematsu, Masaru Kawato, Shinji Tsuchida, and Katsunori Fujikura (JAMSTEC), Dr. Jun Hashimoto (Nagasaki University), Dr. Toshiyuki Yamaguchi (Chiba University), Dr. Yohey Suzuki (AIST) for their useful advice and support throughout this work. We also extend our thanks to the operation teams of the submersibles *Shinkai 2000*, *Shinkai 6500*, *Dolphin 3K*, *Hyper Dolphin*, and *Kaiko* and the officers and crew of the support vessels *Natsushima*, *Yokosuka*, and *Kairei* for their help in collecting the samples.

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