

NEW RECORD OF “*BATHYMODIOLUS*” *MAURITANICUS* COSEL 2002 FROM THE GULF OF CADIZ (NE ATLANTIC) MUD VOLCANOES

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ABSTRACT The “*Bathymodiolus*” *childressi* group is the most geographically diverse assemblage of deep-sea mussel species. In this paper we consider several possible hypotheses to explain the present biogeographic distribution of the “*B.*” *childressi* species complex. Mussels were collected for the first time from mud volcanoes in the Gulf of Cadiz (NE Atlantic Ocean) during the training through research (TTR) 16 research expedition in 2006. Preliminary observations of the shell features indicate that they belong to the “*B.*” *childressi* species complex, which has been recognized as morphologically and genetically distinct from other *Bathymodiolus* species. Molecular analyses of two mitochondrial genes (*COI-5* and *ND4*) were used to characterize the new mussel population from the Gulf of Cadiz (GOC) and to determine their phylogenetic relationships with other members of the “*B.*” *childressi* group. The results indicate that the GOC mussels are conspecific with “*Bathymodiolus*” *mauritanicus* Cosel (2002), described from West Africa margin, and support a previous hypothesis that “*B.*” *mauritanicus* is an ampho-Atlantic species

KEY WORDS: deep-sea, mytilids, ampho-Atlantic taxa, mitochondrial DNA

INTRODUCTION

Exploration of chemosynthetic environments during the past three decades has contributed substantially to the known biodiversity of deep-sea ecosystems, with more than 600 morphological species being described from hydrothermal vents and cold seeps worldwide (Van Dover et al. 2002). The mytilid genus *Bathymodiolus* Kenk & Wilson, 1985, and related genera *Gigantidas* and *Tamu* within the subfamily Bathymodiolinae (Mollusca: Bivalvia), are among the most widespread of the vent and seep taxa (Fig. 1), comprising 23 named species and six genetically distinct entities distributed in the Atlantic, Pacific and Indian Oceans (Kenk & Wilson 1985, Cosel et al. 1994, Hashimoto & Okutani 1994, Cosel et al. 1997, Cosel et al. 1999, Cosel & Olu 1998, Gustafson et al. 1998, Cosel 2002, Cosel & Marshall 2003, Hashimoto 2001, Hashimoto & Yamane 2005, McKiness & Cavanaugh 2005, McKiness et al. 2005, Sasaki et al. 2005). Long distance dispersal capabilities of bathymodiolins is inferred from observations of small oocytes (40–80 µm), large embryonic shells (100–120 µm) and larval shells (380–520 µm), and from their reproductive dynamics, which together suggest a planktotrophic larval phase that extends for five to six months (Turner et al. 1985, Comtet et al. 2000, Le Pennec & Beninger, 2000, Colaço et al. 2006, Dixon et al. 2006, Kádár et al. 2006, Tyler et al. 2007). Most bathymodiolins are known to host symbiotic methanotrophs, thiotrophs, or both types of bacteria simultaneously in their gill tissues, but they still retain a filter-feeding ability (Fisher et al. 1987, Page et al. 1991, Pile & Young 1999). Together, these reproductive and feeding strategies may explain the broad ecological success of bathymodiolin species in chemosynthetic environments.

Morphological and molecular phylogenetic studies have identified several natural groupings among species that have been assigned to *Bathymodiolus*, showing that the genus constitutes a paraphyletic taxon and needs systematic revision (Gustafson et al. 1998, Cosel, 2002, Iwasaki et al. 2006, Jones et al. 2006). Gustafson et al. (1998) first suggested that a new genus name could be used for “*Bathymodiolus*” *childressi* and recommended enclosing the genus name in quotation marks until its relationships with other bathymodiolins is better resolved. Researchers have since recognized that “*Bathymodiolus*” *childressi* is the first described member of a species complex that is morphologically and genetically distinct from the other *Bathymodiolus* species. Based on morphological criteria, Cosel (2002) includes three Atlantic species in the “*B.*” *childressi* species complex: “*B.*” *childressi*, “*B.*” sp. B (Barbados) and “*B.*” *mauritanicus*, as well as one NW Pacific species: “*B.*” *platifrons*. Molecular phylogenetic analyses (Jones et al. 2006, Jones & Vrijenhoek 2006) added more species to this complex: “*B.*” *japonicus*, “*B.*” *hirtus* and “*B.*” *securiformis* from the seeps and vents near Japan, “*B.*” *tangaroa* from the Kermadec Arc, SW Pacific and Edison Seamount, and “*B.*” n. sp. from Edison Seamount. Based on phylogenetic analyses, it remains unclear whether *Gigantidas gladius* from the Kermadec Arc should also be considered a member of the “*B.*” *childressi* complex, or its closest relative. Further phylogenetic analyses involving additional morphological and molecular characters are needed to resolve the generic status of this grouping, so for now we continue to refer to members of this complex as “*Bathymodiolus*.”

A new population of bathymodiolin mussels was discovered during the TTR16 research expedition in 2006 to cold seeps and mud volcanoes in the Gulf of Cadiz (GOC), Northeast Atlantic Ocean. A preliminary examination of shell features of the GOC mussels suggested that they are members of “*Bathymodiolus*” *childressi* species complex. The geographically closest known members of this complex are “*B.*” *mauritanicus* (Cosel, 2002),

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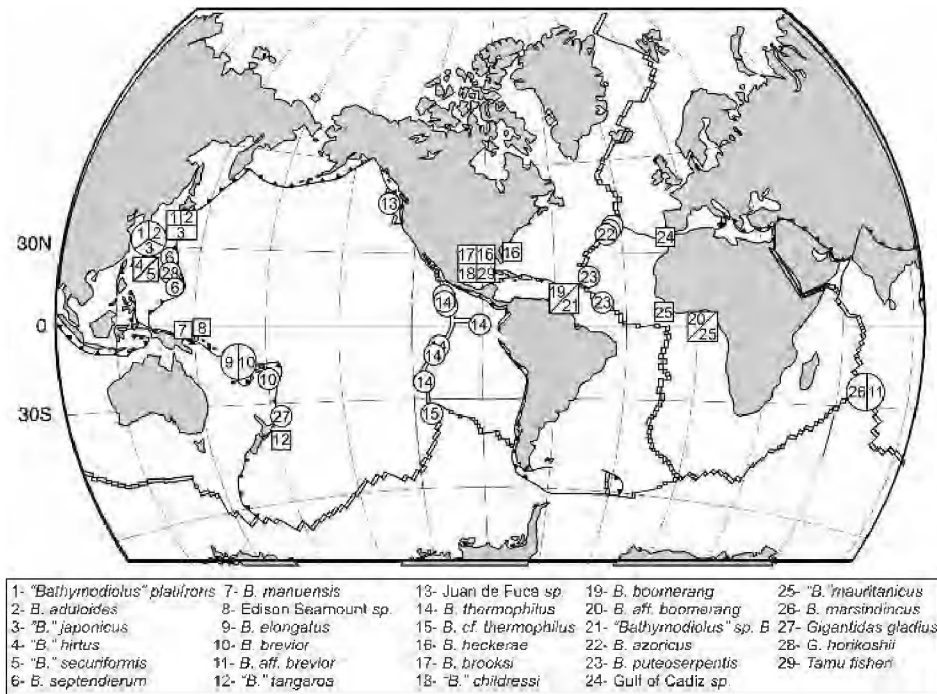


Figure 1. Distribution of described species of the bathymodiolin genus *Bathymodiolus* (Ken & Wilson 1985), *Gigantidas* (Cosel & Marshall 2003) and *Tamu* (Gustafson, Turner, Lutz & Vrijenhoek 1998) in cold seeps (square symbols) and vent sites (round symbols). Adapted and modified from Tyler & Young (1999).

from cold seeps along the Angola margin off W. Africa (WAF), and "*B. childressi*" (Gustafson et al. 1998), from Gulf of Mexico (GOM) hydrocarbon seeps. Genetically related "*Bathymodiolus*" populations also occur on the Barbados Accretionary Prism (BAP) and have been referred to as "*Bathymodiolus*" sp. B (Cordes et al. 2007, Olu-Le Roy et al. 2007). Molecular studies have revealed that the Barbados mussels are closely related to "*B. mauritanicus*" from the W. Africa margin and are probably conspecific, leading Olu-Le Roy et al. (2007) to hypothesize an amphi-Atlantic distribution for these populations. Here we characterize the GOC mussels genetically and morphologically and examine current hypotheses about the taxonomic status and biogeographic distribution of the Atlantic "*Bathymodiolus*" species at seep sites.

MATERIALS & METHODS

Study Area

The GOC is located westwards of the Strait of Gibraltar and is under the influence of the eastern end of the cross-Atlantic zonal jet and the Mediterranean inflow/outflow, which strongly influences the local circulation features, particularly because of the generation of the intermediate depth (750–1,250 m) Mediterranean outflow water mass and the Mediterranean water eddies (Peliz et al. 2006). The GOC (Fig. 2) is presently the most extensive cold seepage area known from the European margins, including a total of over 30 mud volcanoes at depths between 200 and 4,000 m (Pinheiro et al. 2003, Van Rensbergen et al. 2005). To date, biological samples have been collected from 18 of these mud volcanoes, but living mussels are only known from the Darwin mud volcano (1,115 m), which is covered by large

carbonate slabs and crusts. The fissures among the slabs and depressions with scattered crust are filled by abundant shells of "*Bathymodiolus*" and *Neptunea contraria* and occasional small clumps of living "*Bathymodiolus*." Soft corals and other epifauna are occasionally present on the rocks and sediment surface. The extensive mussel graveyards found on the Darwin, Ginsburg (910 m), Student (955 m) and Yuma (975 m) mud volcanoes in the western Moroccan field suggest that this was an area of very active seepage, which has now waned. The geological characteristics of the study area are still being investigated (Gutscher et al. 2002, Medialdea et al. 2004, Duarte et al. 2005), but one of the most important structures is a thick (more than 5 km) Mio-Pliocene sedimentary sequence emplaced on the structurally complex convergent tectonic setting of the African and Eurasian plate boundary (Hensen et al. 2007). Mud volcanism is triggered mainly by the compressional stress along this boundary. Fluid geochemistry indicates a deep thermogenic source of the fluids caused by clay mineral dehydration followed by fluid mobilization along deeply rooted fault systems (Van Rensbergen et al. 2005, Hensen et al. 2007). The fluids are, on average, highly enriched in methane, but concentrations in the upper 30-cm layer of the sediment vary widely from site to site, and even locally (e.g., Nuzzo et al. 2005). However, the sulphide/methane gradient is usually located at more than 30-cm depth in the sediment and methane concentrations at the sediment/water interface are usually very low.

Sample Collection

Samples from the top of the Darwin mud volcano (Table 1) were collected with a TV-assisted grab during the TTR16 cruise aboard the RV *Prof. Logachev*, and with a suction sampler with

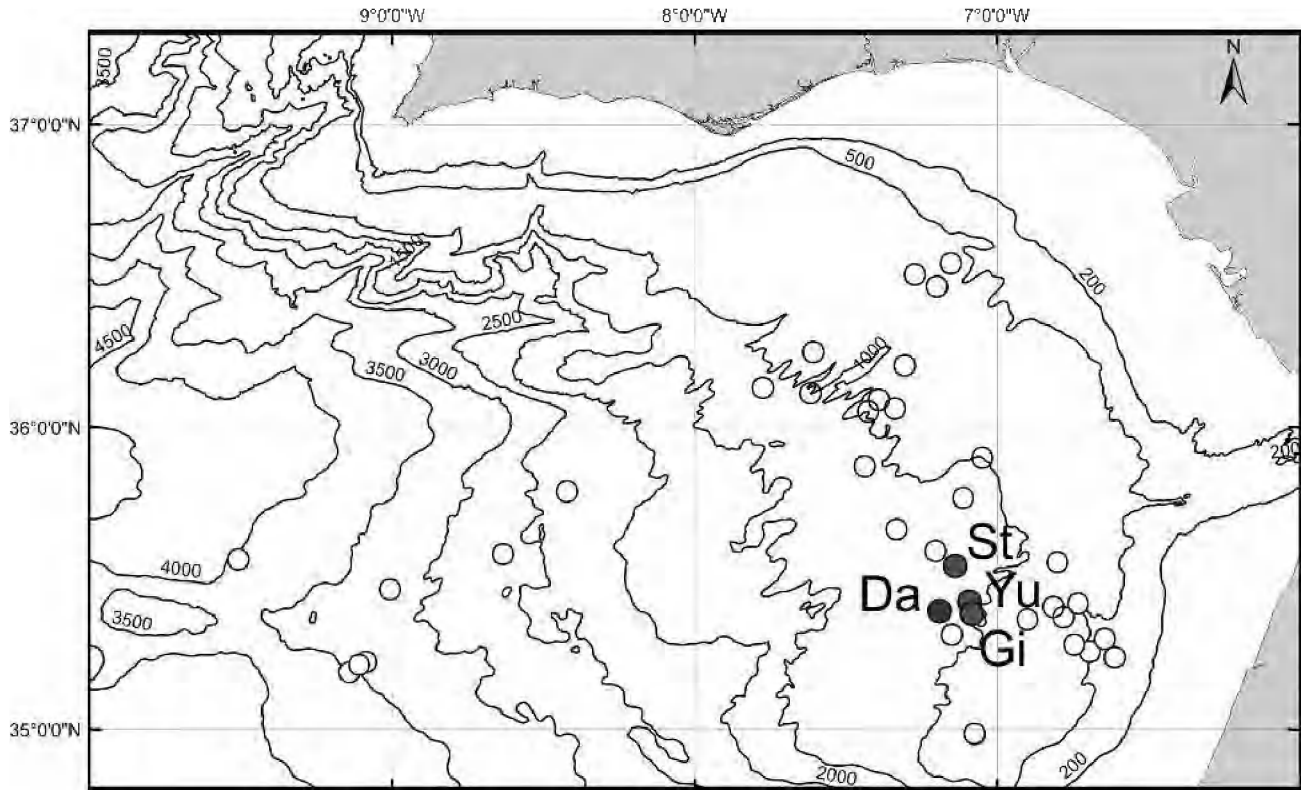


Figure 2. Location of Gulf of Cadiz mud volcanoes (white circles). Living specimens (black) of “*Bathymodiolus*” sp. were found in Darwin (Da) and empty shells (grey) were observed in Student (St), Yuma (Yu) and Ginsburg (Gi) mud volcanoes.

container mesh size of 2 mm, on Isis ROV Dive 41 during the JC10 cruise aboard the RSS *James Cook*. Shell morphological observations were made of 15 specimens and compared with previously described species.

For the purposes of this report, we apply the following placeholder names to the operational taxonomic units (OTUs) examined in this study (Table 2): “*B.*” sp. GOC, “*B.*” *childressi* GOM (“*B.*” *childressi* from the Louisiana Slope and Alaminos Canyon), “*B.*” *mauritanicus* WAF (West Africa margin), and “*B.*” sp. BAP (= “*B.*” sp. B from the Barbados Accretionary Prism).

Molecular Methods

Genomic DNA was isolated with the Qiagen DNeasy DNA extraction kit (Qiagen Inc., Valencia, CA) from adductor mussels from 18 ethanol-preserved specimens. *Bathymodiolus*-specific primers were designed by C. Braby (Univ. of Oregon) to amplify a ~600 bp region of the 5’ end of mitochondrial *cytochrome c oxidase subunit I (COI-5)*, the region commonly used for DNA barcoding of invertebrate species (Hebert et al. 2003):

COIG: 5’-GTATTGAATTAGCACGTCCTGGAA-3’
 COIH: 5’-ATACTATTCCAAACCCGGGTAAAAT-3’.

We also amplified a ~710 bp region of mitochondrial *NADH dehydrogenase subunit 4 gene (ND4)*:

Arg BL: 5’-CAAGACCCTTGATTTCGGCTCA-3’ (Bielawski & Gold 1996)
 NAP 2H: 5’-TGGAGCTTCTACGTGRGCTTT-3’ (Arevalo et al. 1994).

PCR was conducted in a 25 µL solution that included 30–100 ng of template DNA, 2.5 µL of × 1 of PCR buffer (supplied by manufacturer), 2.5 µL of 2.5 µM MgCl₂, 1 µL of each primer (10 µM final conc.), 2.5 units *Taq* polymerase (AmpliTaq Gold, Applied Biosystems Inc., Foster, CA), 2.5 µL of 2 mM stock solution of dNTPs, and sterile water to final volume and occurred with a Cetus 9600 DNA Thermal Cycler (Perkin-Elmer Corp. CT). We used an initial denaturation of 95°C/10 min, followed by 35 cycles of 94°C/1 min, 55°C/1 min, and 72°C/1 min, and a final extension at 72°C/7 min. PCR products were diluted in 40 µL sterile water and cleaned with Multiscreen HTS PCR 96 Filter plates on a vacuum manifold (Millipore

TABLE 1.
 Gulf of Cadiz station data.

Cruise	Dive	Station	Latitude	Longitude	Depth	Date
TTR16		AT608GR	35°23.531’N	07°11.475’W	1,115 m	05/31/2006
JC10	Isis 41	Stn 076-SUS2	35°23.523’N	07°11.479’W	1,109 m	06/01/2007

TABLE 2.
Operational taxonomic units (OTUs). Accession numbers for sequences that are new to this study are indicated in boldface type.

"Bathymodiolus" OTU	Accession no.		Location	Latitude, Longitude	Depth (m)	Reference
	COI-5	ND4				
sp. GOC	EU288159-172	EU288176-179	Gulf of Cadiz off Morocco	35°24'N, 07°11'W	1,115	This study
<i>mauritanicus</i> WAF	AY649801	AY649810	West Africa	0°55'N, 5°28'W	1,000–1,267	Jones et al. 2006
sp. NigerB*	EF051241		Nigerian seep	4°59'N, 4°08'W	1,000–1,700	Cordes et al. 2007
sp. BAP	DQ513425-440		Barbados Accretionary Prism:	10°20'N, 58°54'W	1,690	Olu et al. 2007
sp. B*			Orenoque A site	11°14'N, 59°22'W	1,200	
aff. <i>childressi</i> *			El Pilar site			
<i>childressi</i> GOM	EU288273-175	EU288180-184	Brine Pool	27°43'N, 91°16'W	649	
	DQ177878-884		Louisiana Slope:	27°46'N, 91°07'W	642	Carney et al. 2006
			GC 929 site	27°47'N, 91°31'W	540	
			Bush Hill			
			Alaminos Canyon			
	EF051245, 246			26°21'N, 94°29'W	540–2,222	Cordes et al. 2007
	AY649800	AY130248				Jones et al. 2006
<i>platifrons</i> SAG	AB101419, 420	AB175286, 287	Sagami Bay off Hatsushima	35°60'N, 139°14'E	1,180	Miyazaki et al. 2004
	AB101421		Iheya Ridge, MidOkinawa Trough	27°47'N, 126°54'E	1,028	
		AB175288	Sagami Bay off Hatsushima	35°60'N, 139°14'E	1,180	Iwasaki et al. 2006
<i>tangaroa</i> KER	AY608439	AY649811	Cape Turnagain, Kermadec Arc, New Zealand	40°26'S, 178°58'E	920–1,205	Jones et al. 2006

* Name attributed to "*Bathymodiolus*" specimens in the source reference.

Corp. Billerica, MA). Purified PCR products were sequenced bidirectionally with the same primers used in PCR on an ABI3100 capillary sequencer using BigDye terminator v3.1 chemistry (Applied Biosystems Inc., Foster, CA). DNA sequences were proofread using SEQUENCHER v4.7 (Gene Codes Corp. Inc., Ann Arbor, MI) and edited by eye using MACCLADE v4.08 (Maddison & Maddison 2004, Maddison & Maddison 2005). Statistical analyses were conducted using DNAsp v4.0 (Rozas 2003).

Phylogenetic Analysis

Phylogenetic analyses were conducted with the program MR. BAYES v3.1.3 (Huelsenbeck & Ronquist 2001). Appropriate substitution models for *COI-5* and *ND4* were determined with standard procedures in PAUP (Swofford 1998) using MRMODELTEST (www.ebc.uu.se/systzoo/staff/nylander). Bayesian analyses used six chains and were conducted separately for each gene and then in a combined analysis. The combined analyses were partitioned for each gene. After a burn-in period of 2,500 iterations, each analysis was run for 50 million generations and sampled at intervals of 1,000. Each analysis was repeated five times. Output data were visualized using TRACER v1.3 (Rambaut & Drummond 2003) to determine the appropriate burn-in interval and ensure that the data had reached convergence. Trees were visualized using FIGTREE v1.0 (www.tree.bio.ed.ac.uk). Prior phylogenetic analysis of the "*Bathymodiolus*" *childressi* complex (Jones and Vrijenhoek 2006) revealed that the most appropriate outgroups for our analyses are "*B.*" *platifrons* and "*B.*" *tangaroa*, for which *COI-5* and *ND4* sequences were already available (Table 2).

RESULTS

Morphological Observations of Shells

The Gulf of Cadiz mussels, "*Bathymodiolus*" sp. GOC, exhibit shell features (Fig. 3) that generally characterize the "*B.*" *childressi* group, as provisionally defined by Cosel (2002). They have terminal umbones, a rather narrow anterior margin and a broad posterior part that give the valves a wedge-shaped outline. They have a very small anterior adductor scar, a continuous posterior byssus retractor scar and an anterior retractor scar that is situated in the posterior part of the umbonal cavity behind the beaks. "*B.*" sp. GOC possesses a thick and solid shell, much like "*B.*" *mauritanicus* and "*B.*" sp. BAP, whereas "*B.*" *childressi* GOM and "*B.*" *platifrons* have thin and fragile shells. Like "*B.*" *mauritanicus* WAF and "*B.*" *platifrons*, "*B.*" sp. GOC possesses an anteriorly situated terminal umbone with the distance from anterior shell margin to anterior edge of umbo being <1 mm in small specimens and 1–2 mm in larger specimens. In contrast, "*B.*" *childressi* GOM and "*B.*" sp. BAP have slightly subterminal umbones. In addition to their anterior location, the umbones of "*B.*" sp. GOC are broad and somewhat flattened, a characteristic shared by "*B.*" *mauritanicus* WAF and "*B.*" sp. BAP.

Mitochondrial DNA Analyses

Our genetic analyses of 18 "*B.*" sp. GOC mussel specimens generated 14 novel *COI-5* sequences of ~531 bp length

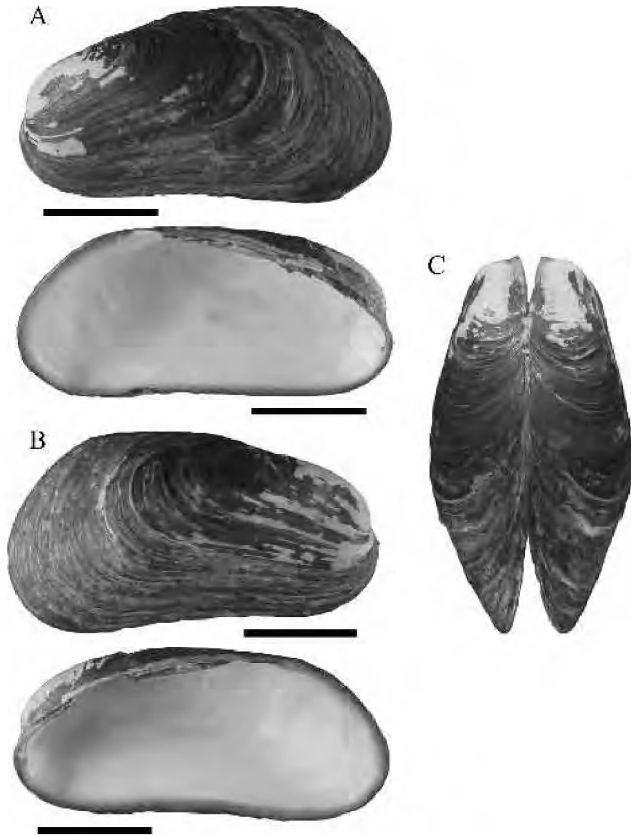


Figure 3. Gulf of Cadiz “*Bathymodiolus*” sp. (a) Exterior and interior view of left valve, (b) exterior and interior view of right valve, (c) dorsal view. Scale bar 1 cm.

(GenBank accession numbers EU288159 to EU288172). For comparative purposes (Table 2), we trimmed our sequences to 372 bp to match the length of published sequences for other “*Bathymodiolus*.” This length of *COI-5* exhibited 18 polymorphic sites, four as singletons and 14 as parsimony-informative

sites. The same 18 specimens generated eight novel *ND4* sequences that we trimmed to 456 bp length for comparative purposes (GenBank accession numbers EU288176 to EU288179). The *ND4* sequences exhibited 43 polymorphic sites, 25 as singletons and 18 as parsimony-informative sites. The best-fit substitution model, according to the AIC criterion (Akaike 1974), was the HKY + SS for both *COI-5* and *ND4*, when treated separately. But for the combined analyses, GTR + I + G provided the best fit.

Pairwise comparisons of sequence divergence between different OTUs (d_b ; Table 3) revealed that “*B.*” sp. GOC was very similar to both “*B.*” *mauritanicus* WAF ($d_b = 0.04\%$ for *COI-5* and $d_b = 0.65\%$ for *ND4*) and “*B.*” sp. BAP ($d_b = 0.42\%$ for *COI-5*). These divergence values were comparable with the values seen within OTUs for which we had adequate sample sizes: “*B.*” *childressi* GOM, $d_w = 1.06\%$ for *COI-5* and $d_w = 0.39\%$ for *ND4*; “*B.*” sp. BAP, $d_w = 0.70\%$ for *COI-5*. Sequence divergence among other OTUs was higher among the named OTUs, “*B.*” *tangaroa*, “*B.*” *platifrons*, “*B.*” *childressi* GOM, and “*B.*” *mauritanicus* WAF, which differed minimally by 1.41% for *COI-5* and 3.83% for *ND4*. The two Pacific species “*B.*” *tangaroa* and “*B.*” *platifrons* were the most divergent taxa with respect to *ND4*.

The three phylogenetic trees yielded essentially the same topologies, therefore we show the combined tree of *COI-5* and *ND4* genes (Fig. 4). Phylogenetic analyses identified a well-supported grouping that included all the Atlantic OTUs: “*B.*” *childressi* GOM, “*B.*” *mauritanicus* WAF, “*B.*” sp. BAP and “*B.*” sp. GOC. Within this grouping, “*B.*” *childressi* GOM is clearly differed from the W. Atlantic species, which all clustered together. “*B.*” sp. GOC could not be discriminated from “*B.*” *mauritanicus* WAF or “*B.*” sp. BAP.

DISCUSSION

In addition to the morphological observations of the shell features, mitochondrial DNA sequences from two genes (*COI-5* and *ND4*) showed that the GOC mussels are essentially identical with “*Bathymodiolus*” *mauritanicus* Cosel (2002), from

TABLE 3.

Mean sequence divergence within (on diagonal) and between “*Bathymodiolus*” Operation Taxonomic Units (OTUs). Names are followed by 3-letter abbreviations for sample locality: GOC, Gulf of Cadiz; WAF, West Africa margin; BAP, Barbados Accretionary Prism; GOM, Gulf of Mexico; SAG, Sagami Bay; KER, Kermadec Arc. Sample sizes are given in parentheses after each gene.

“ <i>Bathymodiolus</i> ” species	sp. GOC	<i>mauritanicus</i> WAF	sp. BAP	<i>childressi</i> GOM	<i>platifrons</i> SAG	<i>tangaroa</i> KER
<i>COI-5</i>	(15)	(2)	(16)	(10)	(4)	(1)
sp. GOC	0.0008					
<i>mauritanicus</i> WAF	0.0004	0.0000				
sp. BAP	0.0042	0.0038	0.0070			
<i>childressi</i> GOM	0.0498	0.0494	0.0533	0.0106		
<i>platifrons</i> SAG	0.0145	0.0141	0.0180	0.0605	0.0040	
<i>tangaroa</i> KER	0.0697	0.0693	0.0701	0.1045	0.0586	0.0000
<i>ND4</i>	(15)	(2)	(0)	(10)	(4)	(1)
sp. GOC	0.0019					
<i>mauritanicus</i> WAF	0.0065	0.0000	—			
<i>childressi</i> GOM	0.0383	0.0402	—	0.0039		
<i>platifrons</i> SAG	0.0669	0.0704	—	0.0863	0.0000	
<i>tangaroa</i> KER	0.2555	0.2554	—	0.2513	0.2602	0.0000

the W. Africa margin. These DNA sequences also confirmed that the mussels from the Barbados Accretionary Prism ("*B.*" sp. B of Olu-Le Roy et al. 2007) are very close to "*B.*" *mauritanicus*. All pairwise distances among the three OTUs were comparable with sequence divergence within adequately sampled OTUs. Consequently, we consider the Gulf of Cadiz and Barbados populations of these mussels as conspecific with "*B.*" *mauritanicus*, a result that clearly supports the hypothesis

of Olu-Le Roy et al. (2007) that "*B.*" *mauritanicus* is an "amphi-Atlantic species".

"*Bathymodiolus*" *childressi* from the Gulf of Mexico (Louisiana Slope and Alamiños Canyon off Texas) does differ from the three "*B.*" *mauritanicus* OTUs. However, divergence levels were relatively low (*COI-5*: 4.94–5.3%; and *ND4*: 3.83–4.02%) compared with divergence between pairs of other named bathymodiolin species, which typically are greater than 10%

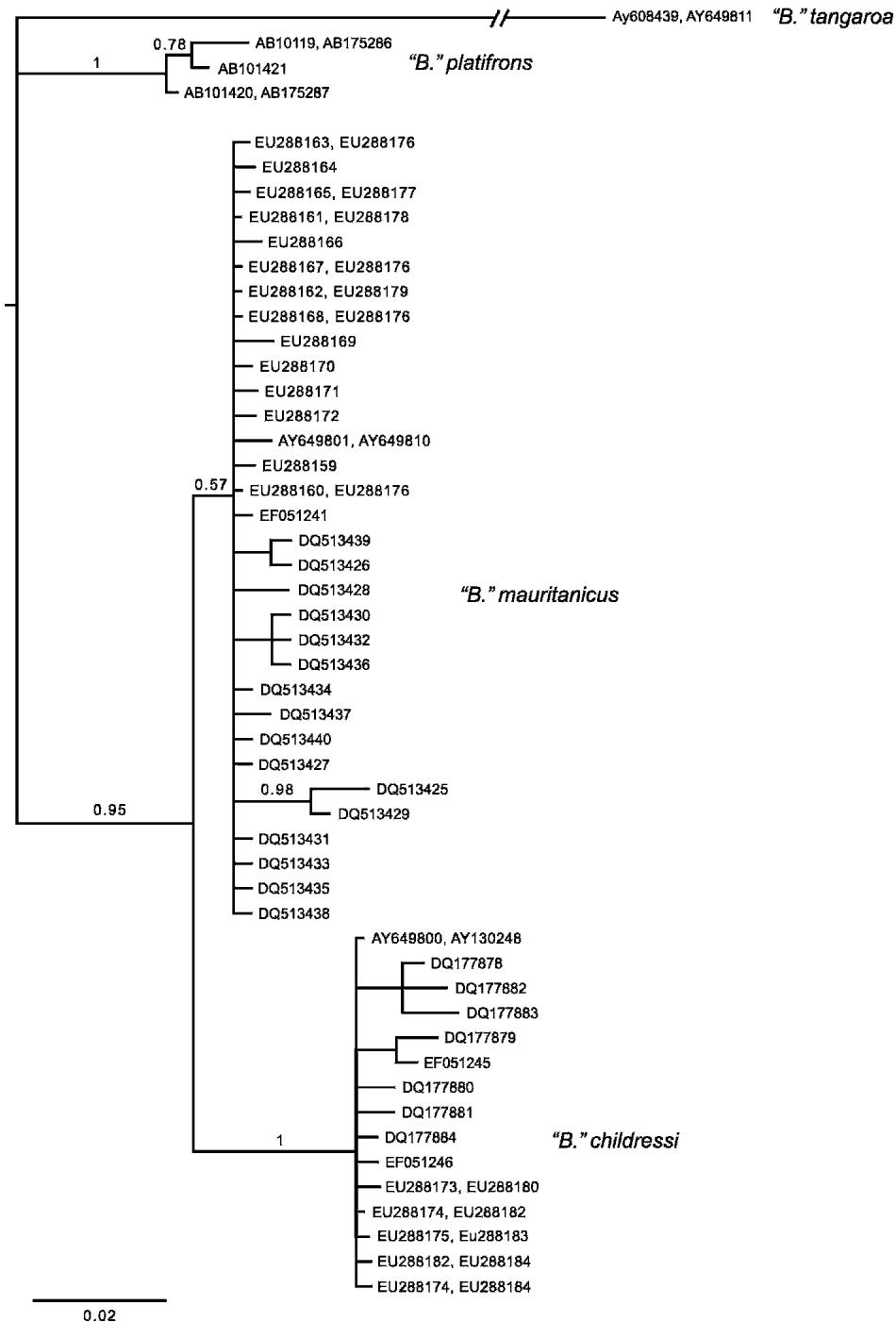


Figure 4. Bayesian tree of *COI-5* and *ND4* combined dataset. Scale bar indicates percent sequence divergence. Bayesian posterior probabilities (BPP) are shown.

(Jones & Vrijenhoek 2006). Yet, the *COI-5* divergence level between “*B.*” *mauritanicus* and “*B.*” *childressi* was very similar to that found between the sister-species *Bathymodiolus thermophilus* and *B. aff. thermophilus* (~4.4%) from the East Pacific Rise vent sites (Won et al. 2003). Although Olu-Le Roy et al. (2007) reported the same pattern of mitochondrial divergence between their “*B.*” sp. B (Barbados) and “*B.*” *childressi*, they puzzled over the identity of these taxa because they shared identical nuclear ribosomal *ITS2* sequences. However, ongoing comparisons of sister-species pairs of mussels for *ITS2* (*B. azoricus* versus *B. puteoserpentis* and *B. thermophilus* versus *B. aff. thermophilus*) have also failed to find divergence (Y. J. Won, personal communication). This untranscribed spacer region of the nuclear ribosomal operon is conserved in many taxa, and appears not to be a reliable marker of species divergence in mussels.

Several genetic studies have investigated gene flow among deep-sea mussel populations at chemosynthetic provinces (Craddock et al. 1995, Vrijenhoek 1997, Comtet & Desbruyères 1998, O’Mullan et al. 2001, Won et al. 2003, Carney et al. 2006), but the mechanisms that lead to the observed diversity of bathymodioliins are not yet fully understood. Won et al. (2003) suggested that the formation of the Easter Microplate could have created a geographical barrier between mussel populations along the East Pacific Rise (EPR), leading to the evolution of the sister species *B. thermophilus* and *B. aff. thermophilus* north (7°C–17°S) and south (31°C–32°S) of the microplate, respectively. However, Won et al. (2003) found no evidence for isolation-by-distance between Galapagos Rift and EPR (13°N to 11°S) populations, which appear to be genetically homogeneous. A similar study of the mussel populations distributed along the Mid-Atlantic Ridge vent sites revealed a hybrid zone between the two mussel species, *B. azoricus* and *B. puteoserpentis* (O’Mullan et al. 2001). The recent study by Carney et al. (2006) of “*B.*” *childressi* populations occurring at hydrocarbon and brine seeps in the Gulf of Mexico revealed that these populations are not differentiated genetically, despite the broad range of depths at which they occur (540–2,200 m). Moreover, trans-Atlantic larval dispersal across the equatorial belt region was suggested to explain the occurrence of genetically related “*Bathymodiolus*” populations at West Africa and Barbados seeps (Cordes et al. 2007, Olu-Le Roy et al. 2007). A limited analysis of the GOC population shows that mussels were undergoing reproduction and that all specimens examined were in the early stage of gametogenesis (P. Tyler, unpublished data). These observations suggest a seasonal pattern correlated with surface primary production, as seen in “*B.*” *childressi* (Tyler et al. 2007) and *B. azoricus* (Colaço et al. 2006, Dixon et al. 2006). Such reproductive patterns give further support to the high dispersal capabilities of the planktotrophic larvae of bathymodioliin species. Together, these studies show that there is not a single pattern for species differentiation among chemosynthetic mussels and that habitat specific biotic and physical characteristics may play important roles, leading to population divergence at vents and seeps and subsequent speciation events.

The discovery of “*B.*” *mauritanicus* at the Gulf of Cadiz mud volcanoes not only constitutes the northernmost record for this species in the NE Atlantic, but also gives new insights into the biogeographic distribution of “*B.*” *childressi* group of mussels. The current phylogeny of “*B.*” *childressi* group (Jones &

Vrijenhoek 2006) shows that the basal members live in the West Pacific Ocean, whereas the more derived members live in the Atlantic, suggesting the group has diversified from the Pacific to the Atlantic. Here we consider three possible routes for this diversification. One is *via* the Tethys seaway, which previously linked the Indo-Pacific and Atlantic Oceans and closed during the early Cenozoic by the movement of Africa and India towards Eurasia. There were plenty of seep habitats in the western Tethys area during the Cenozoic, as evidenced by the many Miocene communities in the Apennine region of Italy (Taviani 2001, 2003). Before the Messinian Salinity Crisis at the end of the Miocene, these communities strongly resembled modern seep communities in the West Africa and Gulf of Mexico, containing large mussels, possibly related to *Bathymodiolus*. However, after the salinity event and the disappearance of deep corridors between the Mediterranean and the Atlantic basins, Mediterranean seep communities developed a distinctive character, lacking several representatives of the typical oceanic cold seep communities, such as the large mussels, large vesicomid clams, and provannid gastropods (Taviani 2003, Olu-Le Roy et al. 2004, Salas et al. 2004).

A second possible Pacific-to-Atlantic diversification route for the “*B.*” *childressi* group was through the Central America region before the closure of the Isthmus of Panama, around five million years ago. This may be supported by the presence of the oldest known representatives of bathymodioliins from Eocene to Oligocene seeps in Washington State, USA (Kiel 2006, Kiel & Little 2006) and bathymodioliin fossils from Paleogene and Neogene seeps in the Caribbean region (Gill et al. 2005, Kiel & Peckmann 2007). Finally, a more recent colonization of the Atlantic seep sites by the “*B.*” *childressi* group may have occurred around the African margins, as has been recently suggested for species of *Calyptogena* (Krylova & Sahling 2006).

As well as being the most geographically diverse assemblage of the deep-sea mussel species, the “*B.*” *childressi* complex contains species living at both cold seeps and hydrothermal vents, such as “*B.*” *platifrons* and “*B.*” *japonicus*. Further biogeographic analyses and population genetic studies of all the Pacific and Atlantic populations of the “*B.*” *childressi* species complex will help to understand better their evolutionary history and to resolve current systematic problems within this taxon.

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