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Amphi-Atlantic cold-seep Bathymodiolus species complexes across the equatorial belt

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Abstract:

Deep-sea bivalves of the subfamily Bathymodiolinae (family Mytilidae) are very widespread and form dense beds in reduced environments such as hydrothermal vents and cold seeps. Bathymodiolus mussels recently discovered on African cold seeps strangely resemble Gulf of Mexico and Barbados seep species. This raises intriguing questions regarding their taxonomic relationships and their dispersal capabilities across the Atlantic equatorial belt. The morphological study of the shell and soft parts of mussels from either sites of the Atlantic shows that they form two distinct groups: the Bathymodiolus boomerang group (also including Bathymodiolus heckerae and a species from Africa), and the Bathymodiolus childressi group (also including Bathymodiolus mauritanicus and one species from Barbados). Phylogenetic relationships inferred from the nucleotide sequences of the ribosomal DNA internal transcribed spacer (ITS-2) and mitochondrial cytochrome c oxidase subunit I (COI) confirmed morphological analyses and the existence of two amphi-Atlantic complexes of species. Both ITS2 and COI phylogenies indicate almost no difference between the two eastern Atlantic seep mussels (Bathymodiolus sp. A and B. mauritanicus) and their western Atlantic counterparts (B. boomerang and Bathymodiolus sp. B; Barbados Prism cold seeps). In the B. boomerang complex, B. heckerae seems to differ from the Barbados and the African species, whereas these latter two are not distinguishable. In the B. childressi complex, relationships are less clear and do not support the description of new species from the Barbados. Past and present-day connections across the Atlantic are discussed in the light of both larval dispersal capabilities of the mussels and the equatorial Atlantic circulation to appreciate whether these species could represent true amphi-Atlantic species.

Keywords: *Bathymodiolus*; Cold seeps; Amphi-Atlantic species; Mitochondrial cytochrome oxydase; rDNA ITS2

Introduction

Within the family Mytilidae, all vent and seep mussels belong to the subfamily Bathymodiolinae, genus Bathymodiolus Kenk and Wilson 1985; Gigantidas Cosel and Marshall 2003; and Tamu Gustafson et al.

1998, or to the subfamily Modiolinae, genera *Idas* Jeffrey 1876. The mussels dominate numerous hydrothermal-vent and cold-seep communities worldwide with a total of 35 species or morphospecific entities of Bathymodiolinae mussels (R. von Cosel, unpublished list). In the Atlantic, nine mytilid species so far have been described from deep chemosynthetic environments, including seven from cold seeps. In addition, two records from seeps remain to be identified, one on the Barbados accretionary prism and one from the Gulf of Guinea. Five mussels belonging to the genera *Bathymodiolus*, *Tamu* and *Idas* are known from cold seeps of the Gulf of Mexico (Gustafson et al., 1998), including one species, *Bathymodiolus heckerae* Gustafson, Turner, Lutz and Vrijenhoek 1998, found at the West Florida Escarpment (Hecker, 1985, Won et al., 2002) and later reported from the Blake Ridge seep sites off North Carolina (Van Dover et al., 2003). Two species, *Bathymodiolus boomerang* Cosel and Olu 1998 and the undescribed *Bathymodiolus* sp. B, co-occur at the Barbados prism (Jollivet et al., 1990; Olu et al., 1996). Two vent species, *Bathymodiolus azoricus* Cosel and Comtet 1999 and *Bathymodiolus puteoserpentis* Cosel, Métivier and Hashimoto 1994, have been described from the Mid-Atlantic Ridge (Desbruyères et al., 2000) where they locally hybridize (O'Mullan et al., 2001). Until recently, very few records were available from the European and African margins of the eastern Atlantic, apart from one *Idas* sp. collected from the Mediterranean ridge (Olu-Le Roy et al., 2004) and the report of "chemosynthetic mussels" in the Gulf of Cadiz (D. Masson et al. unpublished). However, recent ROV expeditions allowed us to discover dense populations of a large and very elongate bathymodioline mussel on a giant pockmark in the Gulf of Guinea named REGAB, which morphologically resembles the slender western Atlantic mussels *B. heckerae* and *B. boomerang*. Moreover, commercial trawling along the African margin off Banc d'Arguin, Mauritania, led to the sampling of *Bathymodiolus mauritanicus* Cosel 2002, presumably associated with methane cold seeps. This species displays morphological similarities with the Gulf of Mexico species *Bathymodiolus childressi* Gustafson, Turner, Lutz and Vrijenhoek 1998 and was included in the same morphological "group" by Cosel (2002). More recently, two species of mytilids were collected by box cores along the Nigerian margin, with one of them resembling *B. heckerae* and others being very close to *B. mauritanicus or B. childressi* (Cordes et al., 2007). This strong morphological resemblance despite the

great transatlantic distance between the Caribbean seep sites and their African counterparts raises questions about the genetic similarity and the dispersal abilities of the western and eastern Atlantic mussels. Moreover, it generates hypotheses about the greater or lesser diversification of cold-seep species as opposed to the vent species over great spatial scales (i.e. the Atlantic equatorial belt vs. the Mid-Atlantic Ridge) (Figure 1).

In this paper, the morphologies of *Bathymodiolus* sp. B. and *B. boomerang* from the Barbados prism are first compared to *B. mauritanicus* and *Bathymodiolus* sp. A from the West African seeps and to other closely-related species from the Gulf of Mexico and the Florida Escarpment. Detailed phylogenetic studies using sequences of both the rDNA internal transcribed spacer (ITS2) and the mitochondrial cytochrome c oxidase subunit I gene (COI) of these species and of additional Nigerian specimens were then performed to confirm the hypothesis of a genetic relatedness between eastern and western Atlantic seep mussels.

Materials and Methods

Sampling sites and animal collections

The specimens used for new morphological and molecular studies, as well as previously published sequences included in phylogenetic analyses are listed in Table 1.

Specimens of the slender West African *Bathymodiolus* sp. A were sampled during the ZAIROV, BIOZAIRE I and BIOZAIRE II cruises by the ROV Victor 6000 on the REGAB site, a pockmark 900 m in diameter located 8 kilometers north of the Zaire canyon, at 3170 m depth (Ondréas et al. 2005). This site is characterized by high methane concentrations in the seawater and by blocks of methane hydrate protruding from the sediment (Charlou et al., 2004; Olu-Le Roy et al., 2007). Mussel beds occur in the most active part of the area, where methane percolates (up to 33 µM close to the mussels) and carbonate concretions are abundant. *Bathymodiolus* sp. A forms dense beds (592-929 ind/m^2), which range from a few meters to 30 meters in diameter (Olu-Le Roy et al., 2007). Five mytilid aggregates were selected for sampling, but no morphological differences have been noticed across the different sampling areas. The

mussels were living on carbonate crusts or in depressions filled with soft sediment, or, rarely, they were isolated and partially buried in sediment. *Bathymodiolus* sp. A was found in close association with the siboglinid polychaete *Escarpia southwardae* Andersen, Hourdez, Marie, Jollivet, Lallier and Sibuet 2004. Two large species of vesicomyid bivalves also occur at the same REGAB site. The Barbados mussels, *B. boomerang* and the methanotrophic *Bathymodiolus* sp. B were collected together by the submersible Nautile from the Orénoque A diapiric ridge (both species), the Orénoque B Dome 13 (*B. boomerang*) and the El Pilar C Dome 2 (*Bathymodiolus* sp.B) during the DIAPISUB cruise. These sites and the seep ecology of *B. boomerang* are described in Olu et al. (1996) and Cosel and Olu (1998).

The five specimens of *B. childressi* used for ITS2 sequences were collected from two distinct localities of the Gulf of Mexico: “Brine Pool” along the Louisiana continental slope (Johnson Sea-Link dive A4038) and Alaminos Canyon together with two specimens of *B. brooksi* (Alvin dive A2209). Three specimens from *B. heckerae* were collected from the Florida Escarpment during the Alvin/AtlantisII cruise 2000. Specimens of *Bathymodiolus thermophilus* Kenk and Wilson 1985 were collected on the East Pacific Rise (EPR) with the submersible Nautile (HOT96 and NAUDUR cruise). Specimens of *B. azoricus* and *B. puteoserpentis* were collected along the Mid-Atlantic Ridge (MAR) with the Nautile (MARVEL and MICROSMOKE cruises). Specimens of *Bathymodiolus brevior* Cosel, Métivier and Hashimoto 1994 and *Bathymodiolus elongatus* Cosel, Métivier and Hashimoto 1994 were collected with the submersible Shinkai 6500 during the cruise Yokosuka 1991.

Previously published COI sequences were used in phylogenetic analyses, including two recently collected *Bathymodiolus* species from the Nigerian slope, one morphologically close to *B. heckerae* and hereafter referred to as *B.* sp Niger A, and one preliminarily assigned to *B.* cf *mauritanicus* (Cordes et al., 2007).

Specimen preservation

The specimens for dissection were fixed on board in 4% formaldehyde solution diluted in seawater and then transferred to 75% ethanol. The valves were kept gaping but care was taken that the adductors were

not cut. The specimens for molecular work were directly preserved in 99% ethanol, frozen, or preserved in BLB (1M Tris-HCl, 0.5M EDTA, 10% SDS pH 8.0).

Molecular analyses

Two molecular markers were used to establish the phylogenies of the species: the non-coding transcribed spacer (ITS2) separating the 5.8S and 28S ribosomal genes as it is assumed to be neutral and to evolve at a high rate in invertebrates (1-1.2% substitutions per Myr: Schlötterer et al. 1994) and the often-used mitochondrial cytochrome oxidase I (mtCOI) for which additional sequences were obtained from GenBank from earlier studies (Table1).

DNA extraction

Tissue samples (10-50 mg) of *Bathymodiolus* specimens were digested overnight with proteinase K at 55°C in 0.5 ml PK-SDS lysis buffer (Tris-HCl 50 mM pH 8.0, NaCl 100 mM, 10 mM EDTA, 1% SDS). Genomic DNA was then extracted using a standard phenol/chloroform protocol and precipitated with ethanol. The pellet was resuspended and stored at 4°C in TE (10 mM Tris, 1 mM EDTA pH 8.0) for amplifications.

rDNA ITS spacer sequencing

The non-coding transcribed spacer (ITS2) was first amplified using a set of "universally" applicable primers designed by P.W.H. Holland and associates from a wide range of metazoan sequences (see Jollivet et al. 1998):

PH19 5' -CATC GACA CTTT/C GAAC GCA- 3'

ITS2 5' -AATC CTGG TTAG TTTC TTTT CCTC CGCT- 3'

and then with nested primers specifically designed for the genus *Bathymodiolus*:

BathF 5’-GCTTAAATTCAGCGGGTACT-3’

BathR 5’-ACATTGCGGCTTTGGGTCAC-3’

Amplification reactions were performed using a Perkin-Elmer GenAmp 9700 Robot-Cycler in a 25-µl reaction volume consisting of 1X PCR buffer; 2 mM $MgCl_2$; 0.12 mM dNTPs; 0.2 µM forward and reverse primers; 0.5 U of Thermoprime Plus *Taq* polymerase (ABgene); and 25 ng genomic DNA. The optimal PCR cycling parameters were a first step of 3 min at 96°C followed by 35 cycles: 1.15 min/50°C, 1 min /72°C, 1 min/96°C and 1 cycle of 2 min/50°C and 10 min/72°C. PCR products were visualized on 2% agarose gels containing ethidium bromide under UV light. PCR products were purified using a QIAquick™ PCR purification kit, T/A-end ligated into a BlueScript™ T-vector plasmid at 16°C overnight, and subsequently cloned into DH5α-competent cells. Positive clones were screened by PCR using vector-specific primers flanking the insertion site. Plasmids were then used as a template for sequencing. Both DNA strands were sequenced for at least three distinct clones per individual (more if the three clones differed in sequence) using either T3 or T7 TexasRed primers. Fragments were subjected to electrophoresis on a 5% Hydrolink Long Ranger™ gel on a 725 VISTRA™ DNA sequencer.

Direct sequencing of mtCOI PCR products

Partial sequences (ranging from 595 to 630 bp, depending on species) of the mitochondrial cytochrome oxidase subunit I gene (mtCOI) were obtained using specifically designed primers:

BathCOI-F: 5'-TGTGGTCTGGAATAATTGGAAC-3'

BathCOI-R: 5'-ATAAAAAGATGTATTRAARTGACG- 3'

based on an alignment of a few bathymodiolin sequences previously obtained with the 'universally' applicable primers described by Folmer et al. (1994). DNA amplifications were performed under the following conditions: an initial denaturation step at 94°C for 2 min followed by 5 cycles: 35 s/94°C, 35 s/48°C and 70 s/72°C followed by 35 cycles: 35 s/94°C , 35 s/52°C, 70 s/72°C and a final elongation at 72°C for 10 min. PCR reactions were performed into a 25-µl reaction volume consisting of 1X PCR buffer; 2 mM $MgCl_2$; 0.12 mM dNTPs; 0.2 µM of forward and reverse primers; 0.5 U of Thermoprime Plus *Taq* polymerase (ABgene); and 25 ng genomic DNA. The PCR products were then purified and

directly sequenced on an ABI 3100 automated sequencer using BigDye© (Perkin Elmer) terminator chemistry.

Sequence analyses

Sequences used in phylogenies were selected according to three main criteria: (1) performing ITS2 and COI trees with the same species in order to compare them more accurately, (2) collecting a maximum of sequences for species suspected of belonging to the two complexes under study and, (3) getting a few morphologically distinct vent/seep species as a reference to assess species divergence. Forward and reverse sequences were proofread in Trace Viewer and corrected manually using Se-Al 2.0 Sequence Alignment Editor (Rambault, 1996) after being automatically aligned using ClustalX (Thompson et al. 1997). Both 472 bp rDNA ITS2 and 380 bp mtCOI sequence alignments were used to produce ML, MP and NJ phylogenetic trees with the PAUP 4.0b software (Swofford, 2002), using all informative sites and gaps when present and visualized using TreeView (Page, 1996). Substitution models used in phylogenies were selected according to Modeltest 3.7 (Posada and Crandall, 2001) with the Bayesian Information Criterion (BIC) at a level of significance α of 0.01. K2P distances were obtained using the DNAdist package of PHYLIP 3.573 (Felsenstein, 1981) to calculate frequency distributions of pairwise COI sequence differences in order to test for geographically-differentiated population admixture in both complexes of species. Sequence saturation was graphically tested from our dataset by plotting transitions versus transversions for the non-coding ITS2 region and by plotting the K2P (first two codon positions) versus the K2P (all three codon positions) for mtCOI (coding sequence).

Ribosomal ITS2

The neighbor-joining tree was obtained using the K2P distance (Kimura, 1980) and 10, 000 resampling bootstraps. The MP tree was obtained using the Branch-and-bound search approach with a furthest sequence addition procedure. Because gaps were numerous and found informative, they were treated as a fifth base. Each gap, however, was weighted as a single substitution. The optimal parsimony criterion was

that of Goloboff fit (K=2). An additional bootstrap 50% majority-rule consensus MP tree was obtained using a heuristic approach and 100 resampling bootstraps. For each dataset resampling, starting trees were obtained via stepwise addition as the closest and branches were furthermore swapped using the tree-bisection-reconnection (TBR) algorithm. Branches creating polytomies were collapsed if maximum branch length was equal to zero.

Mitochondrial COI gene

The neighbor-joining (NJ) tree was obtained using the K2P distance and 10 000 resampling bootstraps using the BIONJ algorithm for either the three codon positions or only the first two codon positions. The maximum likelihood tree was obtained from a heuristic approach using the HKY85+G model (Hasegawa et al. 1985) selected by Modeltest 3.7 (-LnL = 1662.4, K=96, BIC=3895.06) and a rate of substitution assumed to follow a gamma distribution (4 rate categories, α=0.1855). The search for the best tree was performed using a tree-bisection-reconnection (TBR) branch-swapping algorithm. Starting trees were obtained via stepwise addition with a random sequence addition (10 replicates) with starting branch lengths obtained using the Rogers-Swofford approximation method. The subsequent ML topology was then tested using a bootstrap method with a fast-heuristic search and 100 replicates following the same model parameters (i.e. HKY+G model).

Because Jones et al. (2006) found that methylotrophic symbiont-bearing mussels and thioautotrophic symbiont-bearing mussels represent 'true' monophyletic clades, all trees were rooted using the midpoint rooting method (*B. childressi* complex of species vs. other vent/seep mussels). This allowed us to exclude external mytilid outgroups (e.g. *Tamu fisheri* or *Modiolus modiolus*) that are far too divergent in order to avoid nucleotidic saturation and long-branch attraction.

Results

Morphological descriptions

Bathymodiolus sp. A from the REGAB site in the Gulf of Guinea has a thin and very elongate shell with a narrowly rounded anterior margin and a broadly rounded posterior margin (Figure 2). The ventral margin is straight in young individuals, becoming more and more concave in larger specimens. The anatomy of the soft parts is indistinguishable from that of *B. boomerang* and *B. heckerae*: the foot-byssus retractor muscle complex extends with the shell but the anterior byssus retractor is short. The posterior bundle of the posterior byssus retractor is very long and thin and passes at a very low angle (about 15°) from the longitudinal shell axis towards the attachment point in the postero-dorsal part of the valves next to the adductors. The heart is located in the posterior part of the body, the digestive tube is simple, the stomach is small, and the midgut runs straight and median from the stomach towards the posterior end to under the ventricle, entering it without any coiling or loop.

Shell shape and soft-part morphology place this mussel in the *B. heckerae* group, as defined by Cosel (2002). Because of the anteriority of the description of *B. boomerang*, however, we refer to this group as the *B. boomerang* complex hereafter. According to the size of the individuals, which have a straighter than concave antero-ventral margin, the morphologically closest species is *B. heckerae* from the Florida Escarpment (Figure 4) and the REGAB mussel was therefore treated as *Bathymodiolus* aff. *heckerae* in previous literature (Duperron et al., 2005, Ondréas et al., 2005). The only difference between the Gulf of Guinea specimens and *B. boomerang* (Figure 3) is the absence of very large individuals, which have a more pronounced “boomerang” shape, a more concave antero-ventral margin and a more broadly rounded anterior margin. However, an empty broken shell of about 300 mm and a height of 88 mm was sampled during the ZAIROV 2 cruise on another pockmark located south of the Zaire canyon at about 10 miles from the REGAB site and at 1900 m depth (K. Olu, R. von Cosel, A. Ondréas unpubl. data). The maximum known length of *B. heckerae* specimens from the Florida Escarpment is 230 mm as documented by Van Dover et al. (2003), whereas the largest *B. boomerang* was 360 mm in length (Cosel and Olu 1998). The largest skewed specimen of *B. heckerae* from the Blake Ridge diapir, however, had a length of 364 mm (Van Dover et al., 2003) and thus equals the size of *B. boomerang*. Apart from the size,

the shape variability is about the same across the four localities (Florida Escarpment, Barbados, Blake Ridge and Gulf of Guinea) with no morphological difference between them.

Bathymodiolus sp. B from the Barbados prism (Figures 5 and 6) has a short and stout shell that is variable in outline, is up to 127 mm long, and varies from rather thin but solid to thick-shelled. Juvenile specimens are broader still and less tumid than adults. Length-height ratio is 1.77. The beaks are slightly subterminal, at one-twelfth to one-fifteenth of the total shell length. The anterior margin is more or less narrowly rounded, and the ventral margin in adult specimens is markedly concave in the middle part, occasionally with a crease-like concave angle. The posterior margin is ventrally more or less broadly rounded, with the postero-dorsal margin slightly to markedly convex; the postero-dorsal corner is rather broadly rounded. The umbones are broad and somewhat flattened, the ligament plate slightly arched to nearly straight. The anterior adductor scar is small and placed just in front of the umbo. The large posterior adductor scar is united with the scar of the multibundle posterior pedal and byssus retractor muscle. The anterior byssus retractor scar is located in the posterior part of the umbonal cavity, behind the beaks. The larval shell is 200 microns long and high, circular, purplish red and sharply separated from the whitish postlarval shell. The foot-byssus retractor muscle complex has a rather long anterior retractor. The posterior byssus retractor is short and consists of two very strong muscle bundles, which are close together. The heart has large auricles that are fused posteriorly but for a short distance, and the ventricle is small and rather narrow. The stomach is very small and elongate, with extremely thin walls, and hardly recognizable chambers; style sac and midgut are conjoined. The midgut runs midline towards under the ventricle, changing direction there twice in a short S-shaped curve that is directed towards dorsal and slightly to the right side before entering the ventricle.

Slight differences in outline and inner mantle fold features were observed between *Bathymodiolus* sp. B specimens from Orenoque A sector and El Pilar sector, separated by a distance of about 150 km. The anterior end with umbo in the El Pilar specimens is somewhat narrower, and the shells are shorter. In most juveniles from the El Pilar domes there is a weak papilla on the valvular siphonal membrane, as in

B. childressi, whereas in all the specimens from the Orenoque sector, the margin is smooth. El Pilar specimens are generally smaller than specimens from Orenoque, with mean shell lengths of 54.9 and 92.2 mm, respectively. Comparison of allometric curves between 51 individuals from each locality showed slight differences in growth; individuals from Orenoque become stouter with increasing length than specimens from El Pilar. These differences are also well represented using the length-height (l/h) ratio versus shell lengths (Figure 7), but l/h ratios (sub-samples of 51 specimens of various sizes for each site) were significantly different between sites according to both the non-parametric Mann-Whitney U-test ($p=0.02$) and the Kolmogorov-Smirnov test ($p=0.037$). Length/tumidity ratios were, however, not significantly different. Additional measurements of the anterior part length (i.e. length from the anterior margin to the umbo) evidenced much shorter (about half as long) anterior parts for El Pilar specimens; differences were highly significant according to Student test ($p=2.7\ 10^{-7}$) and Mann -Whitney U test ($p=2.2\ 10^{-11}$).

Species of the *B. childressi* group as defined by Cosel (2002) differ from each other by weak but consistent differences. The shell of *Bathymodiolus* sp. B from Barbados varies from modioliform, as in *B. childressi*, to more wedge-shaped, as in *B. mauritanicus* (Figure 8). The thickness of the shell in most observed specimens is also intermediate between the thin shell of *B. childressi* and the rather thick shell of *B. mauritanicus*. The position of the umbones is slightly subterminal to terminal, like those of *B. childressi*, whereas they occupy a terminal position in *B. mauritanicus*. *Bathymodiolus* sp. B from Barbados shares with other species of the *B. childressi* group an adult hinge line that is thickened below and anterior to the umbones, but it is shorter than in *B. childressi*, *B. mauritanicus*, and *Bathymodiolus platifrons* Hashimoto and Okutani 1994, another species of this group. The posterior end of the ligament is abrupt, as in *B. childressi*, whereas it is tapered in *B. mauritanicus*. The comparison of shells among species of the *B. childressi* group suggests that *Bathymodiolus* sp. B from Barbados and *B. childressi* differ less from each other than they do from *B. mauritanicus*. However, some characters are shared by *Bathymodiolus* sp. B and *B. mauritanicus* and not by *B. childressi*, e.g. small anterior adductor scar, a continuous posterior retractor scar and a very posteriorly-located anterior retractor scar. Moreover, the

fact that some individuals of both *B. mauritanicus* and *Bathymodiolus* sp. B have very thick and strong shells for their size is not common in bathymodiolin mussels. Finally, soft parts have been compared to those of *B. childressi* and *B. platifrons* only, as the animal of *B. mauritanicus* was not available. The papilla in the middle of the valvular siphonal membrane is almost absent in specimens from Barbados, as in *B. childressi*. It is, however, present in *B. platifrons*, as in the *B. thermophilus*, *B. heckerae* and *B. brevior* groups. The intestine lacks a short loop, as in *B. childressi*, but is short-S-shaped and not straight, as in *B. platifrons* or other *Bathymodiolus* spp. The form of the intestine is, however, highly variable in the Bathymodiolinae and is likely dependent on the balance between filter feeding and symbiosis in nutrition.

Molecular analyses

Mitochondrial COI gene

Accession numbers for new COI sequences are given in Table 1. The two trees yielded essentially the same topologies, with the exception of deep branches that were poorly supported (low boostrap values). Only one tree is presented here with bootstrap values for both Neighbor Joining and Maximum Likelihood (ML) methods(fig. 9). Both trees indicate the occurrence of two well-supported complexes of species within which the level of divergence did not exceed 0.05. Using only the first two codon positions in the analysis (not shown) produced a similar topology but with less resolution within the clades. The maximum likelihood tree was obtained (Likelihood tree: LnL = - 1481.38895, Figure 9) with a number of distinct data patterns of 100 under the HKY+G model over the 380 bases (sequence length) with no invariable sites and a gamma distribution shape parameter of 0.1855. Estimated base frequencies revealed a large excess of T (A=0.2225, C=0.1595, G=0.1902 and T=0.4277), with an estimated Ti/Tv ratio = 5.0391 (kappa = 11.05). The two major clades (average K2P distance between clades = 0.18) correspond to the *B. childressi* complex of species on the one hand (all the species that are strictly symbiotic with methanotrophic bacteria) and the *B. boomerang* complex of species on the other hand (comprising all the seep species with both sulfide-oxidizing and methanotrophic symbiotic bacteria). The African species *B.*

mauritanicus and specimens collected along the Nigerian coast all fall within the clade containing the methanotrophic *Bathymodiolus* sp. B (Barbados). This clade is closely related to *B. childressi* from the Gulf of Mexico but differs by 9 transitions and 1 transversion, (K2P distances ranged from 0.023 and 0.035.) COI sequence data indicate that the cluster *B. mauritanicus/Bathymodiolus* sp. B is genetically isolated from *B. childressi*. Within the group of *B. childressi*, sequences from the deepest site (Alaminos Canyon) differed by one transition from the shallower sites (Bush Hill, GC234, MS_Canyon). The K2P frequency histograms obtained for the *B. childressi* complex of species with and without *B. childressi* (Figure 10) showed that this complex of species represents an admixture of geographically isolated populations. Indeed, the histogram is clearly bimodal when *B. childressi* is included. In this figure, the peak centered at 0.03 (black circles) represents all pairwise differences between *B. childressi* and the other species. When *B. childressi* individuals are removed (white circles), a small peak remains but is not attributable to geographical differences between the African and Barbados mussels, suggesting that these latter mussels could represent a valid amphi-Atlantic species.

In species with sulfide-oxidizing symbionts, morphologically distinct species display K2P distances close to 0.15 with the exception of *B. heckerae, B. boomerang* and mussels collected from the Gulf of Guinea and Nigeria, which form a homogeneous clade (inter-specific K2P distances < 0.02). The species *B. heckerae*, however, derived from this group by 3 transitions and 1 transversion. Given the low number of sequences for this species we cannot state whether it represents a true homogenous group. The two fixed differences between B. *boomerang* and *Bathymodiolus* sp. A sequences suggest that these mussels are at least isolated from each other. The K2P frequency histograms obtained for the *B. boomerang* complex of species with and without *B. heckerae* (Figure 11) also confirmed that populations from the Florida Escarpment are genetically isolated from their southernmost amphi-Atlantic counterparts. In contrast to *B. childressi*, the histogram remains clearly bimodal after the removal of the two individuals of *B. heckerae* with a peak centered around 0.01. This peak encompasses all pairwise differences between African and Barbados mussels and thus indicates that there is a less pronounced, but still manifest, genetic break between mussels across the Atlantic when compared to the *B. childressi* complex of species.

Ribosomal ITS2

Because phylogenetic relationships between bathymodiolin mussels cannot be restricted to the mitochondrial genome as it is very often subjected to selective sweeps (Bazin et al., 2006) and thus prone to adaptive radiation, the validity of species complexes has also been assessed using a nuclear marker that presents an evolutionary rate nearly similar to the COI gene. The accession numbers for ITS2 sequences are given in Table 1. Both phylogenetic methods essentially provide the same tree topologies except for deep branches that are supported by low bootstrap values. The best Maximum Parsimony (MP) tree (Goloboff fit = -70.929) was obtained based on 456 characters (gaps included) from which 346 were invariant, 27 were variable but not found informative, and 81 were parsimony-informative (Figure 12). The tree is characterized by good consistency (CI) and retention (RI) indices of 0.709 and 0.895, respectively. The tree presented in figure 12 displays bootstrap values obtained from both the NJ and MP methods. As for the COI phylogeny, this tree supports the occurrence of the two species complexes corresponding to the *B. childressi* (0 < K2P distances < 0.028) and *B. boomerang* (0 < K2P distances < 0.015) clusters that were previously reported with the mtCOI gene. However, it indicates the occurrence of two distinct species within *B. brooksi* and also seems to support the occurrence of types a and b (shallow and deep, respectively) within the *B. childressi* sequences .The individual Bchildb2 is found in both clusters and could correspond to a hybrid. The inclusion of the gaps as a fifth character greatly improved the resolution of the phylogenetic signal across bathymodiolin species and led to a single MP tree with higher bootstrap values. Grouping strongly suggests that *Bathymodiolus* sp. B (Barbados) corresponds to *B. childressi*, with one individual from Orenoque area falling within *B. childressi* type a and the other from El Pilar in *B. childressi* type b. Within the *B. boomerang* clade, *B. boomerang* is well separated from *B. heckerae/B.* sp. A (from Africa) by a clear indel signature and 3 transversions, whereas *B. heckerae* seems to derive from *B.* sp. A by only one transition.

Interestingly, *B. puteoserpentis* does not form a sister group of *B. azoricus*, as was expected from the COI phylogeny. However, this sequence was found only in a single individual when the other individuals

yielded typical *B. azoricus* sequences. This could indicate either the presence of a cryptic species, the persistence of an ancient lineage in the *B. puteoserpentis* allele coalescent, or the occurrence of a selective sweep that favored the spread of the *B. azoricus* allele in the *B. puteoserpentis* population.

Discussion

Occurrence of two amphi-Atlantic Bathymodiolus species complexes

Morphological and phylogenetic analyses of *Bathymodiolus* species from several cold seeps across the Atlantic - the tropical West African and the Caribbean zoogeographic provinces - revealed the occurrence of two amphi-Atlantic species complexes: the *B. childressi* complex and the *B. boomerang* complex. This shows a clear connection between cold-seep communities from Barbados and the Gulf of Mexico on the one hand, and between these Western Atlantic areas and the tropical West African province on the other. These species complexes are representative of two major clades of the genus *Bathymodiolus*, one hosting a symbiosis strictly with methanotrophic bacteria (the *B. childressi* complex) and the second displaying dual symbioses with both methanotrophs and sulfide-oxidizers (the *B. boomerang* complex). Based on morphological characters of shell and soft parts of the mussels, these two main groups have already been described by Cosel (2002) and are referred to as the *B. childressi* and the *B. heckerae/B. boomerang* groups. Most speciation events appear to be fairly recent within both clades and suggest a concomitant history of colonization in the Atlantic Ocean that probably occurred during the last Miocene-Eocene tectonic reorganization of the Panama seaway (Coates and Obando, 1996, Roth et al., 2000).

In the present paper, the molecular examination of the recently-discovered long and slender seep mytilid from the Gulf of Guinea (*Bathymodiolus* sp. A) clearly assigns it to the *B. boomerang* complex. Apart from the size, there is no obvious morphological difference to discriminate among specimens from the three geographic areas. Shell morphology is almost the same in the three populations. This finding supports well the occurrence of a widespread complex of cold-seep species that clearly represents a sister group to the vent species *B. azoricus*, which possesses a dual symbiosis. Interestingly, based on a

bacterial 16S phylogeny, Duperron et al. (2005) also reported that the methanotrophic symbiont of *Bathymodiolus* sp. A (Africa) is most closely related to the symbiont of *B. heckerae* (Florida Escarpment). Larger series of adult specimens are necessary, however, before we can assign the African specimens to *B. boomerang*. Indeed, molecular analyses do not strictly support the hypothesis of an amphi-Atlantic species. Within the *B. boomerang* clade, phylogenetic trees based on mtCOI clearly separate *B. heckerae* from *Bathymodiolus sp.* A (Africa) and *B. boomerang* from Barbados. Based on the ITS2 marker, the phylogeny suggests a slightly different interpretation in which *B. heckerae* differs from *Bathymodiolus* sp. A by only one transition but clearly differs from *B. boomerang* by three transversions and one indel. K2P frequency histograms also indicate that the *B. boomerang* complex is not homogeneous and is probably composed of genetically-isolated groups of individuals that correspond to *B. heckerae* on the one hand, and to *B. boomerang*/*Bathymodiolus* sp. A, on the other. The occurrence of few fixed differences between Barbados and African mussels therefore suggests at least the existence of isolated populations of a single species or sibling species in which *B. heckerae* derives from *B. boomerang*.

The genetic examination of the *B. childressi* species complex led to similar conclusions and supports the hypothesis of vicariant patterns of colonization between the two seep mussel complexes. Although slight morphological differences do exist, the mtCOI marker was not able to discriminate *B. mauritanicus* and the two *B.* aff. *mauritanicus* (Nigerian slope) from *Bathymodiolus* sp. B (from Barbados). However, *B. childressi* seems to be genetically isolated from its two related counterparts based on mitochondrial, but not nuclear, data. Such a discrepancy between the mitochondrial and nuclear genome evolution is not too paradoxical since the former has been shown to evolve faster. These latter populations therefore fall into the *B. childressi* complex, as defined by Cosel (2002), which also includes *B. platifrons* from vent sites in the western Pacific back-arc basins and represents a morphologically quite homogeneous group, which is also supported by genetic data (Miyasaki et al., 2004; Jones et al., 2006; Jones and Vrijenhoek, 2006). This group clearly differs from other *Bathymodiolus* species by several characters such as a small adductor scar, an almost continuous posterior byssus retractor scar, rather low and almost terminal

umbones, and an anterior retractor scar positioned more posteriorly within the umbonal cavity compared to *B. puteoserpentis*, *B. azoricus* or *B. thermophilus* (Cosel, 2002). Within this complex, *B. mauritanicus* and *Bathymodiolus* sp. B are very close and well separated from *B. childressi* from the Gulf of Mexico. In contrast, phylogenetic trees based on the ribosomal internal spacer (ITS2) indicate that *Bathymodiolus* sp. B belongs to the species *B. childressi* which, in turn, represents a dichotomic clade, supporting the occurrence of morphological types a and b as previously proposed by Gustafson et al. (1998). This result does not agree with another recent genetic study (Carney et al., 2006), which reported no genetic differentiation between *B. childressi* populations using microsatellites and RFLP markers in the Gulf of Mexico. Such a discrepancy may be explained by hybridization patterns. Indeed, at least one individual contained both an ITS2 type "a" allele and an ITS2 type "b" allele, suggesting hybridization between the two morphs. On the Barbados accretionary prism, the Orenoque and the El Pilar mussel populations exhibit slight differences in morphology and significant differences in the shell shape. Past exchanges across the Caribbean zone may thus have been interrupted over a wide area, leading to the emergence of two *B. childressi* ecotypes that hybridized later on.

Morphological differences are not very marked but are consistent within the *B. childressi* complex when compared to other *Bathymodiolus* spp. (Cosel 2002). These small differences in shell characteristics were used for the taxonomic description of *B. mauritanicus* (Cosel, 2002). The shell comparison between species of the *B. childressi* complex indicates that *Bathymodiolus* sp. B (from Barbados) and *B. childressi* share some shell characteristics that differ in *B. mauritanicus*. They both have a more modioloform shape, a broader anterior part, and a less terminal position of umbones than *B. mauritanicus*. These observations are consistent with the ITS2 phylogeny in which *Bathymodiolus* sp. B is more closely related to *B. childressi*. It is, however, noteworthy that African specimens collected for phylogenetic purposes (including *B. mauritanicus*) were not collected from the type locality of *B. mauritanicus*.

Morphological similarities between the two Caribbean species are balanced by some other characters shared by *Bathymodiolus* sp. B and *B. mauritanicus*, and not by *B. childressi*. Although specimens from

Barbados display a shorter hinge, the two latter species are characterized by a small anterior adductor scar, a continuous posterior retractor scar, and a very posteriorly-located anterior retractor scar. The two species also have a heavier and thicker shell than *B. childressi* from the Louisiana Slope. *B. childressi* seems to represent a distinct species when the mtCOI gene and some shell characteristics are considered. The nuclear marker ITS2, however, seems to group *B. childressi* and *B.* sp. B (Barbados). Therefore we cannot rule on the existence of distinct species in this complex without additional phylogenetic and morphological data.

Trans-Atlantic similarities: recent colonization routes or long-distance larval dispersal?

The lack of clear genetic differences between mussel populations in the Atlantic within the two species complexes indicates past connections between the western Atlantic and eastern Atlantic seep fauna and may support the hypothesis of recent exchanges between these geographically well-isolated populations, at least along the Atlantic equatorial belt. These colonization pathways appear clearly different from those that led to the colonization of the Mid-Atlantic Ridge and the emergence of *B. azoricus* and *B. puteoserpentis*, and they unambiguously show that hydrothermal vent fields from the MAR could not have acted as stepping stones across the Atlantic for the seep mussels. Three non-exclusive hypotheses may explain the occurrence of amphi-Atlantic species: (i) the occurrence of contemporary gene flow across the Atlantic equatorial belt via teleplanic larvae or larval exchanges along a continuum of seepages, sunken wood or whale carcasses, (ii) the very recent colonization of the Atlantic by the modern seep fauna via transform faults that offset the Mid-Atlantic Ridge and connect the western and eastern deep oceanic basins of the Southern Atlantic or (iii) the reflection of an evolutionary slow-down that prevented mussel lineages from diverging rapidly after they separated. The second and the third hypotheses may represent a valid explanation for species associated with cold seeps and have been previously invoked to reconcile paleontological and molecular data for vestimentiferan tubeworms (Little and Vrijenhoek, 2003). Chevaldonné et al. (2002) used Northern and Eastern Pacific sibling species to calculate the mtCOI substitution rate of vent annelids and found that it could be one order of magnitude

lower (0.2% per Myr) than that of vicariant coastal/intertidal Panamanian species (Knowlton and Weigt, 1998). Based on this kind of estimate, Jones et al. (2006) proposed that species diversification is recent in the genus *Bathymodiolus* and may have occurred during the progressive closing of the Panama Seaway, 10 to 3 M yrs ago (Ross and Scotese, 1988; Coates and Obando, 1996). This closing may have seriously affected both bottom and intermediate water circulation (Haddad and Droxler, 1996; Roth et al, 2000) and could have thus reinforced transverse circulation between eastern and western Atlantic basins along the equatorial belt, which in turn might have promoted migration across the Atlantic and the concomitant diversification of the genus *Bathymodiolus,* at least in the Atlantic. This view is supported by Gill et al. (2005), who reported the occurrence of bathymodioline mussels in the Eocene-Miocene carbonates of the Caribbean region. Interestingly, two siboglinid species, *Escarpia spicata* (Pacific coast of North America) and *E. laminata* (Gulf of Mexico) display very low levels of mtCOI divergence despite their isolation across the Panama Isthmus, suggesting that the substitution rate is extremely low in at least some seep species (McMullin et al., 2003).

The first hypothesis involves effective exchanges of propagules in the Atlantic Equatorial Belt. In highly fragmented and unstable environments, long-lasting larvae are a key element to ensure the efficient re-colonization of patchy habitats and thus a powerful tool to minimize the risk of a species becoming extinct. To date, dispersal capabilities of deep-sea organisms have been indirectly estimated for the vent fauna only, based on reproductive studies, larval transport models, and genetic structure (Mullineaux and France, 1995; Chevaldonné et al., 1997; Vrijenhoek, 1997; Comtet et al., 2000; Mullineaux et al., 2000, 2005; Marsh et al., 2001). The ability of vent species to recolonize new sites clearly shows their great potential for long-distance dispersal capabilities (Tunnicliffe et al., 1997; Shank et al., 1998). This assumption is in agreement with the lack of genetic structure that has been observed for numerous vent taxa over oceanic ridge scales (Jollivet, 1996). Effective larval transport across the Atlantic requires that larvae stay long periods of time in the water column. Generally speaking, more than 80% of marine invertebrates that possess a planktotrophic larva have an amphi-Atlantic distribution, and this is the case for, respectively, 10 and 30% of mollusks of the eastern Atlantic or from both coasts (Cosel, 1982;

Vermeij, 2005), with some being "pseudo amphi-Atlantic", using islands as "stepping stones" and "real" amphi-Atlantic species, with long-distance planktonic larvae. *Bathymodiolus* possesses a protoconch I of about 100-120 µm and a protoconch II of 380-520 µm long, supporting the idea of a long planktonic phase (Cosel et al., 1994; Cosel and Olu, 1998), with a duration of the free-swimming larval phase comparable to that of the blue mussel (Bayne 1965). Experiments on *B. childressi* larvae or indirectly on the reproductive dynamics of *B. azoricus* suggested that larvae can remain in the plankton for 5-6 months (Berger and Young, 2006; Tyler et al., 2005; Dixon et al., 2006).

In contrast to oceanic ridges, where negatively-buoyant larvae can easily be channeled by currents over great distances inside the axial valley (Mullineaux and France, 1995; Chevaldonné et al., 1997; Metaxas and Giffin, 2004), larval transport across deep oceanic basins seems more uncertain for the seep fauna. The Antarctic Bottom Water (AABW) enters the Guinea basin via the Romanche Fracture Zone and the Chain Fracture Zone (Mercier and Speer, 1998; Stephens and Marshall, 2000). The Brazil basin represents a vortex zone from which the AABW is subdivided into additional jets that move northward against the western flank of the Mid-Atlantic Ridge to reach the Florida Escarpment (Amos et al., 1971) and enters the Mid-Atlantic Ridge using the Vema Fracture Zone (Heezen et al., 1964; Stephens and Marshall, 2000). Longitudinal flow and movement of North Atlantic Deep Water (NADW) flowing between 1200 and 4000 m (Arhan et al., 1998) may represent a relay for long-lasting propagules. However, this deep current and stronger intermediate jets extending more broadly on both sides of the Atlantic (Arhan et al., 1998; Bourles et al., 2003; Schmid et al., 2005) have very low velocities (2-3 cm s^{-1} and 10 cm s^{-1}, respectively) that theoretically would not allow west-east particle transport in less than a few years (Richardson and Fratantoni, 1999; Andrié et al., 2002). The role of time-variable components, mixing, and eastward extra-equatorial jets is nevertheless unknown, and the transport times could be much lower than those predicted by models (Arhan, pers.com.). In contrast, shallower currents can be orders of magnitude faster, e.g. 0.8 to 1 m s^{-1} for the equatorial counter-current (Weiberg et al., 1987), representing crossing times of a few months, closer to the residence time of larvae in the water column. It is not known whether the larvae remain at the depth where the adults live. The larvae may very well get

close to the surface. Siboglinid larvae were indeed captured at depths as shallow as 5, 90 and 110 m (Marie et al., 2006). The temperature may be not a limiting factor for upward migration, high thermal tolerance (up to 20°C) having been observed in adult *B. childressi* and other seep gastropods (Van Gaest and Young, 2005; Berger and Young, 2006).

Other invertebrate species sampled at the REGAB site (Congo seeps) have close relatives at seep sites in the Caribbean province. The *Alvinocaris* shrimp is morphologically and genetically (COI) indistinguishable from *Alvinocaris muricola* Williams 1988 initially described from the Florida Escarpment (Komai and Segonzac, 2005; Hourdez, unpublished data). The vestimentiferan tubeworm *Escarpia southwardae*, also from REGAB, displays less than 0.2% divergence in COI with its escarpiid relatives from the Gulf of Mexico (Andersen et al., 2005). The commensal polynoid found in *Bathymodiolus* sp. A is genetically similar to *Branchipolynoe seepensis* Pettibone 1986 occurring at the Florida Escarpment (D. Jollivet, unpublished data). Finally, other deep-sea species such as the galatheid *Munidopsis geyeri* Pequegnat and Pequegnat, 1970 or the holothurian *Chiridota heheva* Pawson and Vance 2004, known from the Gulf of Mexico, the Columbia and Venezuela Basins, or the Florida Escarpment seeps, have also been sampled on seeps of the African margin (Pawson, 2004; MacPherson and Segonzac, 2005).

Other reduced environments may also act as stepping stones for larvae of amphi-Atlantic seep species. While sunken wood and whale falls are probably less common when moving away from the continental shelves, it remains plausible that low-temperature vents or seeps occur along the transform faults crossing the MAR or along the ridge itself (D. Desbruyères, pers. comm.). Hydrothermal activity may also occur in pronounced transform valleys (German et al., 1996) and may serve as stepping stones for dispersal (Van Dover, 2002). Dense beds of large Vesicomyidae have already been observed in the VEMA transform fault (M. Segonzac, pers. comm.), and other active fluid seeping areas have been observed off-axis along the MAR (Y. Fouquet pers. comm.). Some faunal exchange between MAR vents and seeps of

both the Gulf of Mexico and Barbados prism seems to occur as suggested by the sampling of the same species of ophiurids at these three locations (Stör and Segonzac, 2005). Nevertheless, the distribution of methane- and sulphide-rich sites in the Atlantic is poorly known, particularly in the South Atlantic. As an example, “chemosynthetic mussels” have recently been observed in the Gulf of Cadiz (D. Masson et al., unpublish.). As suggested by Vermeij (2005), amphi-Atlantic molluscs may have originated from brief episodes particularly favorable to transatlantic expansion, acting as potential stepping stones (e.g. relatively warm Arctic Ocean for shallow-water species). However, this author reports that molecular studies for several shallow-water molluscs all pointed to differences between eastern and western populations that imply substantial genetic isolation, in contrast with the deep- chemosynthetic mussels.

The two *Bathymodiolus* species complexes studied here seem to display the same patterns of dispersal (i.e. identical migration routes) but in different depth ranges. The *B. childressi* complex indeed seems to occur at two relatively “shallow” depths, ranging between 540 and 2200 m along the Louisiana slope, between 1000 and 1700 m on the Barbados prism, and between 1000 and 1260 m off Mauritania. Conversely, the *B. boomerang* complex has been recorded from deeper areas between 1700 and 2000 m on the Barbados prism and the Blake Ridge diapir and more than 3000 m off Congo and at the Florida Escarpment. Propagules are thus likely to be entrained through different water layers according to their depth range, and it would now be very interesting to study population differentiation within these two different complexes of species to quantify levels of gene flow between and along the American and African margins.

Conclusion

Based on morphological and molecular analyses, we showed the occurrence of two complexes of amphi-Atlantic bathymodiolin mussels (the *Bathymodiolus childressi* and the *Bathymodiolus boomerang* complexes) associated with cold seeps. Within the *B. boomerang* complex, the COI phylogeny suggests that *B. heckerae* is genetically different from the two others. However, two isolated populations of

Amazonian and African *B. boomerang* may exist. We thus propose to provisionally name the *Bathymodiolus* sp.A from the Gulf of Guinea cold-seep site REGAB *Bathymodiolus* aff. *boomerang* until we obtain larger specimens for morphological, especially allometric, comparisons. Similarly, *B. mauritanicus* from Mauritania to Nigeria and *Bathymodiolus* sp. B (Barbados) could represent a true amphi-Atlantic species.

These complexes of cold-seep species seem to share a similar colonization history across the Atlantic and thus are likely to display recent vicariant events. Although some teleplanic larvae may still be crossing the Atlantic using equatorial jets or shallower currents, the more likely explanation of the maintenance of amphi-Atlantic species is the very recent colonization of these zones together with a very low evolutionary rate of this peculiar fauna.

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Figure caption

Table 1: Specimen collection sites and GenBank accession numbers for Bathymodiolinae studied in this paper or included in phylogenetic analyses.

Figure 1: Areas of the Atlantic equatorial belt selected by the Census of Marine Life/ChEss program (from D. Desbruyères, available on the ChEss website, http://www.soc.soton.ac.uk/chess/equatorial_belt.html). The bathymodiolins included in this paper correspond to five encircled areas, which are, north to south and west to east, the northern Gulf of Mexico, the Florida Escarpment, the Barbados Accretionary Prism, the Banc d'Arguin off Mauritania, and the Gulf of Guinea.

Figure 2: **a.** *Bathymodiolus* sp. A from off Congo. Top: Exterior of left valve, BIOZAIRE 2 PL 147-10, REGAB, 5°47.78'S, 9°42.65'E, 3151-3159 m. Shell length 166.8 mm. Bottom: Exterior of right and left valve, interior of right valve, BIOZAIRE 2 PL 146-9, REGAB 5°47.82'S, 9°42.73'E, 3160-3158 m. Shell length 166.5 mm. **b.** Half-schematic drawing of the interior of right valve of *Bathymodiolus* sp. A from off Congo. Foot-byssus retractor muscle complex and its position in the shell. BIOZAIRE 2 PL 146-9, REGAB, 5°47.82'S, 9°42.73'E, 3160-3158 m. Shell length 166.5 mm, scale 10 mm.

Figure 3: Top**:** *Bathymodiolus boomerang* Cosel & Olu, 1998, Holotype MNHN, exterior and interior of left valve, Barbados Accretionary Prism, DIAPISUB PL 04, Orenoque A site, 10°19.65N-58°53.5 W, 1690m . Shell length 340.0 mm. Bottom: Juveniles *Bathymodiolus boomerang* Cosel & Olu, 1998, exterior of left and interior of right valves, Barbados Accretionary Prism, DIAPISUB, PL DS 10 (10°19.95'N-58°37.30'W, 1950m). Shell length 115.1 mm. **b.** Half-schematic drawing of the interior of the right valve of *Bathymodiolus boomerang* showing the foot-byssus retractor muscle complex and its position in the shell. Juvenile specimen. Barbados Accretionary Prism, DIAPISUB, PL DS 10, Orémoque B, 1950 m. Scale: 10 mm.

Figure 4: *Bathymodiolus heckerae* Gustafson, Turner, Lutz & Vrijenhoek, 1998, Top: Paratype 2542-13 MNHN, exterior and interior of right valve, ALVIN dive N° 2542,West Florida Escarpment, 26°01.8'N, 84°54.6'W, 3314 m. Shell length 164.1 mm Bottom: Paratype 2196-59 MNHN, interior and exterior of both valves, ALVIN dive N° 2186, West Florida Escarpment, 26°02.4'N, 84°54.4'W, 3314 m, Shell length 84.6 mm

Figure 5: **a.** *Bathymodiolus* sp. B (Barbados). Upper row and middle: exterior and interior of right valve, dorsal view, Barbados accretionary prism, DIAPISUB, PL DS 05 Orénoque A (10°19.65N-58°53.5 W, 1690m). Shell length 119.0 mm. Lower row: exterior of left valve, interior of right valve, Barbados Accretionary Prism, DIAPISUB, PL DS 05 Orénoque A. Shell length 80.9 mm. **b.** *Bathymodiolus* sp. B. Upper row and middle: exterior and interior of right valve, dorsal view, Barbados Accretionary Prism, DIAPISUB PL DS 15/16 El Pilar (11°14N-59°22W, 1060 m). Two specimens of shell length 99.5 mm and 124.9 mm. Note the broad shells, which are thick and heavy.

Figure 6: Half-schematic drawings of the interior of right valves of *Bathymodiolus* sp. B. **a.** Barbados Accretionary Prism, Orénoque A site. shell length 80.5 mm. **b.** Barbados Accretionary Prism, El Pilar site, foot-byssus retractor muscle complex and its position in the shell. Shell length 99.5 mm. Scale: 10 mm.

Figure 7: Length-height ratio (l/h) versus shell lengths of *Bathymodiolus* sp. B specimens from Orenoque and El Pilar sites on the Barbados Accretionary Prism (n=51).

Figure 8: **a.** *Bathymodiolus mauritanicus* Cosel, 2002, Holotype MNHN, interior and exterior of right valve, off Banc d'Arguin, Mauritania, 1200 m, Shell length 98.1 mm. **b.** *Bathymodiolus childressi* Gustafson, Turner, Lutz & Vrijenhoek, 1998, paratype 3129-52 MNHN, exterior and interior of right valve. Johnson Sea-Link 1, dive 3129, Bush Hill hydrocarbon seep, 27°46.9'N, 91°30.4'W, 566 m. Shell length 83.6 mm. **c**.: *Bathymodiolus childressi* Gustafson, Turner, Lutz & Vrijenhoek, 1998, paratype 2211-59 MNHN/ exterior of both valves, interior of right valve, Alaminos Canyon, 26°21.3'N, 94°29.7'W, 2222 m. Shell length 61.7 mm

Figure 9: NJ tree based on the COI mitochondrial sequences (380 bp). with bootstrap values obtained from 10 000 resamplings of the dataset. Figures in brackets correspond to bootstrap values obtained with the Maximum Likelihood (ML) method with 100 replicates. The trees have been oriented using the midpoint rooting method using clades previously proposed by Jones et al. (2006) but could also be viewed as unrooted trees. Bmaurit=*B. mauritanicus* from off Côte d'Ivoire; Bniger6 & 7: *B.* cf. *mauritanicus* from off Nigeria; BathDSxx and BathPIL: *B.* sp.B from Barbados prism (DS: Orenoque site; PIL=Pilar site); Bchild=*B. childressi* from the Gulf of Mexico (GoM); Bbrook=*B. brooksi* (GoM); Bputeo=*B. puteoserpentis* (MAR); Bazori=*B. azoricus* (MAR); BathZaire=B. sp. A from REGAB site (Congo seeps); Bniger1&5=B. sp. Niger A from off Nigeria; Bboo=*B. boomerang* from Barbados;

Bhecker=*B. heckerae* from Florida Escarpment; Bthermo=*B. thermophilus* (EPR); Bbrevior=*B. brevior* from Lau Basin/Mariana Trough.

Figure 10: K2P-frequency histogram between specimens collected within the *B. childressi* complex of species with (black circles) and without (white circles) the 6 *B. childressi* mtCOI sequences.

Figure 11: K2P-frequency histogram between specimens collected within the *B. boomerang* complex of species with (black circles) and without (white circles) the 2 *B. heckerae* mtCOI sequences.

Figure 12: Maximum Parsimony (MP) tree based on the rDNA spacer ITS 2 (472bp) with bootstrap values (in brackets) obtained from 100 replicates. Figures without brackets correspond to bootstrap values obtained with the Neighbor-Joining (NJ) method with 10 000 resamplings of the dataset. The tree is oriented using the midpoint rooting method using clades previously proposed by Jones et al. (2006) but could also be viewed as unrooted trees. Bbar: *B.* sp.B from Barbados prism (DS: Orenoque site; PIL=Pilar site); Bchild=*B. childressi* from the Gulf of Mexico (GoM); Bboo=*B. boomerang* from Barbados; Bzaire=B. sp. A from REGAB site (Congo seeps); Bheckeaer=*B. heckerae* from GoM.

Table 1

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Species	Abbreviation in trees	Location	Lat/Long	Depth	Accession Number	Reference
Bathymodiolus sp.A	BathZaire (COI) Bzaire (ITS)	Congo-Angola margin, REGAB site	5°47.8'S, 9°42.7'E	3170 m	COI: DQ513442-43-45-47-50-51 ITS2 : DQ513493 to -497	This study
B. *boomerang*	Bboo (COI/ITS2)	Barbados prism Orenoque B site	10°19.9'N, 58°37.3'W	1950 m	COI: Q513444-46-48-49 ITS2 : Q513486	This study
B. heckerae	Bhecke	Florida Escarpment	26°01.8'N, 84°54.9'W 26°01.8'N, 84°54.9'W 26°01.8'N, 84°54.9'W	3290m 3290m 3290m	AY649794, AY649793 COI:DQ513441 ITS2 : DQ513490-492	Jones et al. 2006 This study This study
Bathymodiolus sp.B	BathDS BathPIL	Barbados prism Orenoque A site, Barbados prism El Pilar site	10°19.6'N, 58°53.5'W 11°14'N, 59°22'W	1690 m 1200 m	COI:DQ513425 to 440 ITS2: DQ513484-85	This study
B. *childressi* (ITS)	Bchilda Bchildb	Louisiana slope,BrinePool (2 ind) Louisiana slope,Alaminos canyon	27°43.4'N, 91°16.3'W 26°21.3'W, 94°29.8'N	650m 2200m	ITS2: DQ513475- 478 to -483	This study
B. *childressi* (COI)	BchildMS1 BchildGC1 BchildBH1 BchildAL1	Louisiana slope, Alaminos canyon Louisiana slope, GC 929 site Louisiana slope, Bush Hill Alaminos canyon	28°01'N, 89°43'W 27°46'N, 91.07'W 27°47'N, 91°30.5'W 26°21'N- 94°29'W	2222 m 642 m 540 m 2222m	AY649800 DQ177879-884 DQ177879-884 EF051241-EF051246	Jones et al. 2006 Carney et al. 2006 Carney et al. 2006 Cordes et al. 2007
B. mauritanicus	B. maurit	South of the Côte d'Ivoire	0° 53'N, 5°28'W	1000–1267m	AY649801	Jones et al. 2005
B. sp. NigerA	BNiger1&5	Nigerian slope	4°59'N, 4°08'E	1000-1700m	EF051242-243	Cordes et al. 2007
B. sp. NigerB = *B.* cf *mauritanicus*	BNiger6&7	Nigerian slope	4°59'N, 4°08'E	1000-1700m	EF051241	Cordes et al. 2007
*B. brooks*i	Bbrook	Alaminos Canyon	26°21.3'N, 94°29.7'W	2222 m	ITS:DQ513476 and –477 COI :AY649797-798	This study Jones et al. 2006
B. thermophilus	Bthermo	EPR 9°50'N Riftia field 18°S/EPR Animal farm	9°50.96'N, 104°17.7W 18°35'S, 13°25'W	2515 m 2673m	ITS2: DQ513452 and 454 COI:AF456282	This study Won et al. 2003
B. azoricus	Bazori	MAR, Menez Gwen site MAR, Lucky Strike site	37°50.6'N, 31°31 3'W 37°17.6'N,32°16 96'W	824m	ITS2: DQ513464 and 467 COI: AY649795	This study Jones et al. 2006
B. puteoserpentis	Bputeo	MAR, Logatchev site	14°45.2'N,44°58.76'W	3065 m	ITS2: DQ513458 COI: AY649796	This study Jones et al. 2006
B. brevior	Bbrevior	North Fiji Basin, White Lady Lau Back Arc basin Mariana Through	16°59.4'S, 173°54 9'E	1990 m	ITS2: DQ513470 COI : AY275544 COI: AY649799	This study Smith et al. 2005 Jones et al. 2006
B. elongatus	Belongatus	North Fiji Basin, Sunset	18°49'S, 173°29'E	2720 m	ITS2: DQ513471	This study

Figure1

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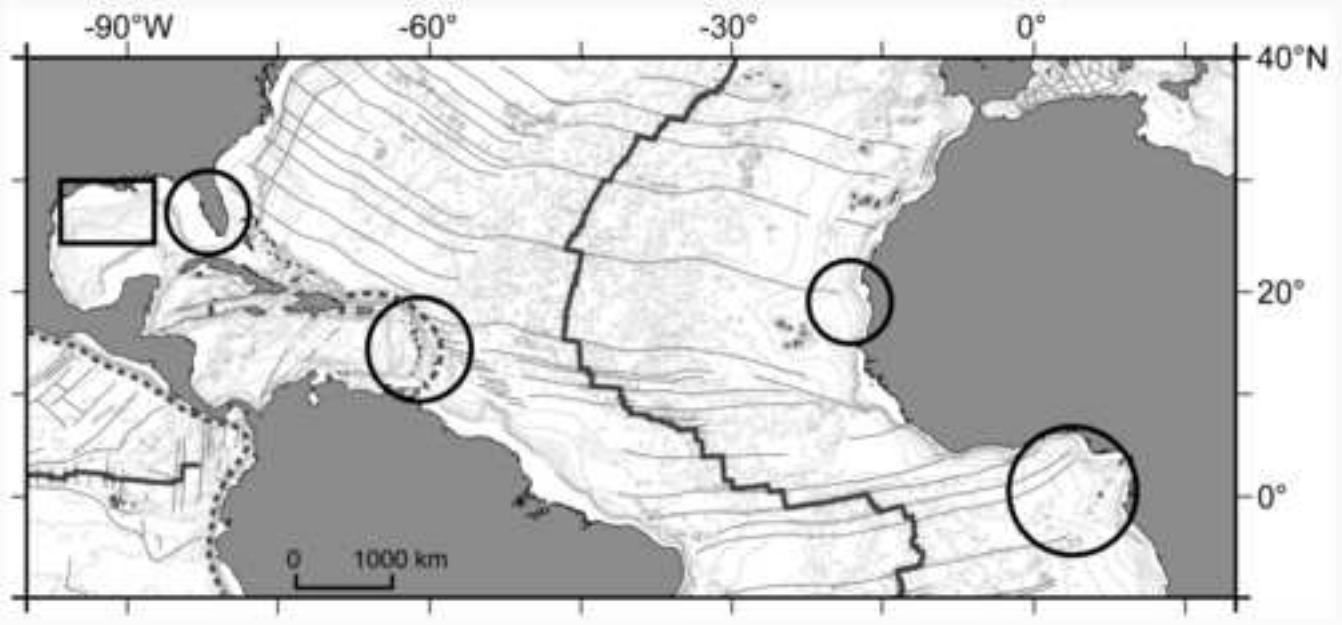

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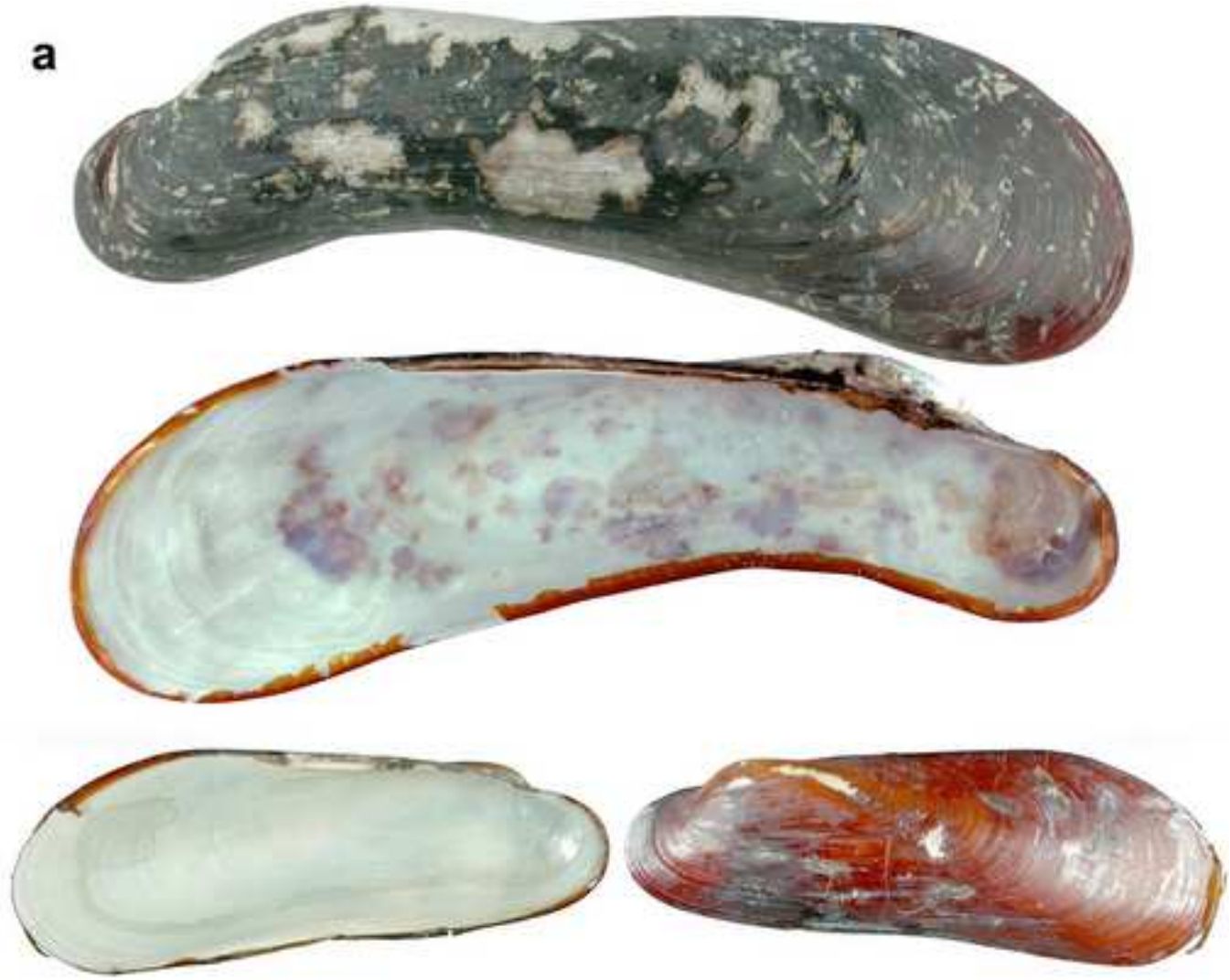

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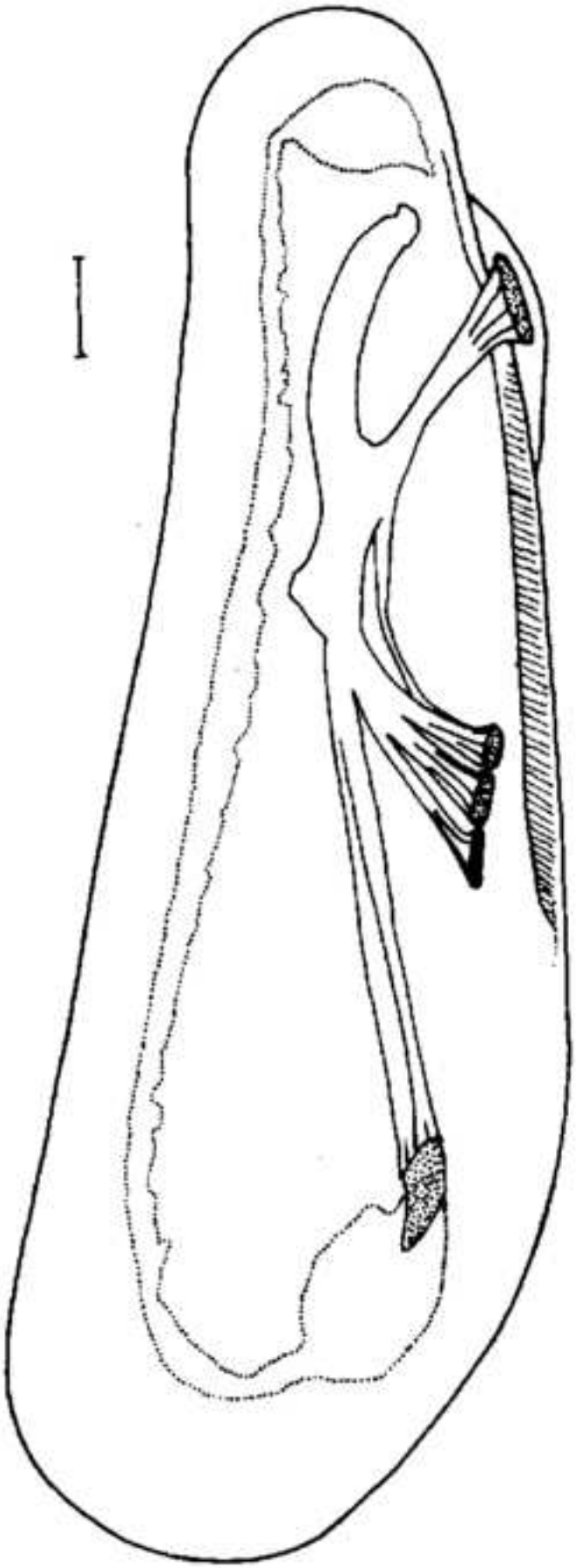

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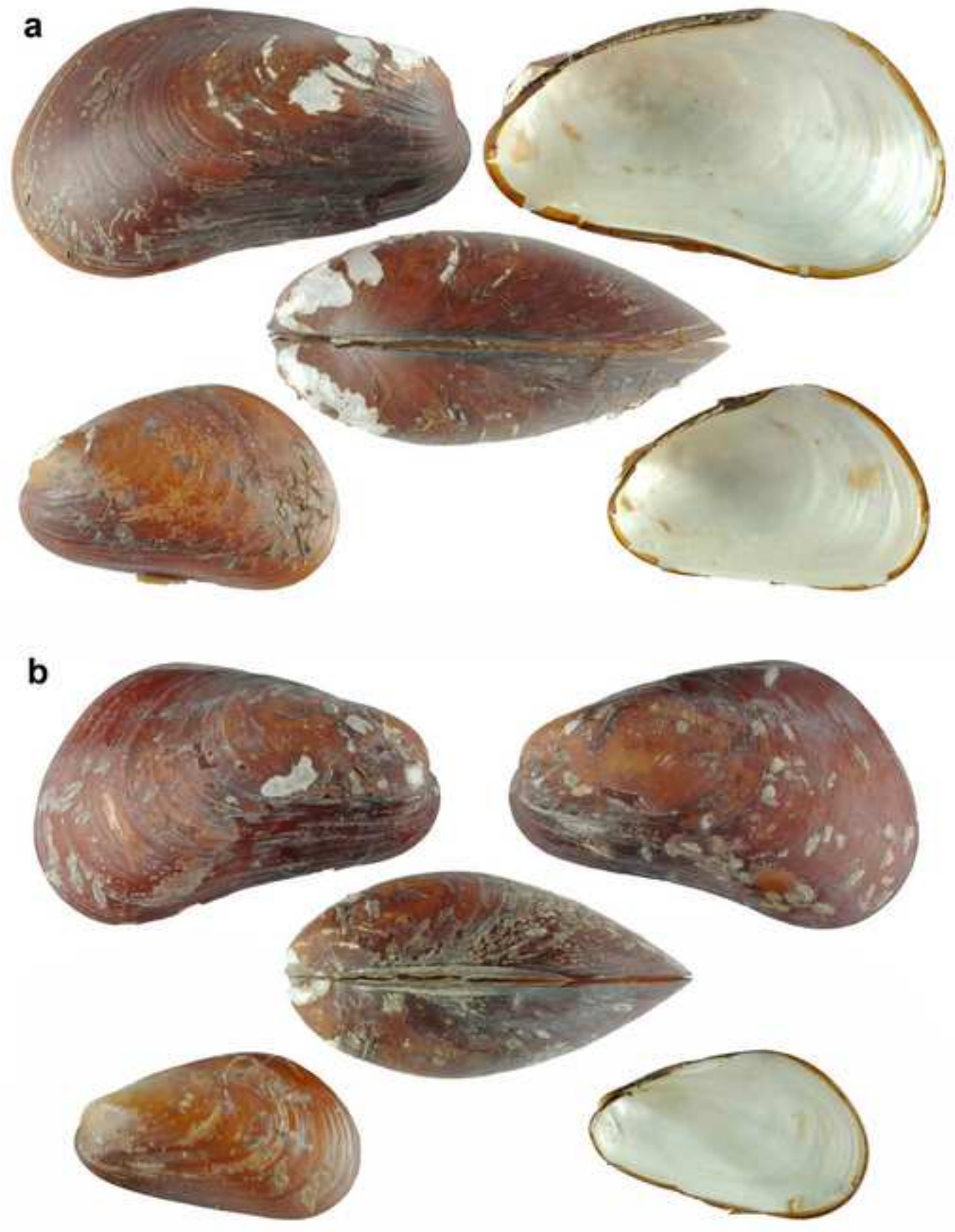

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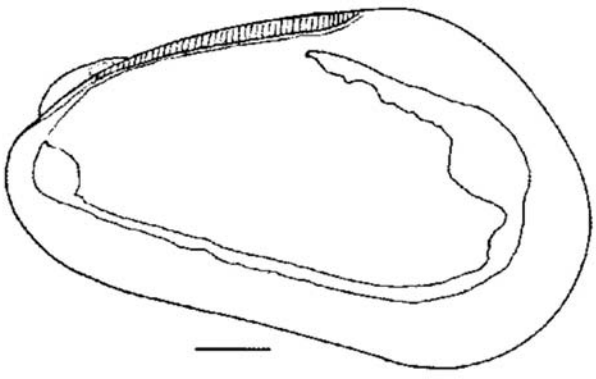

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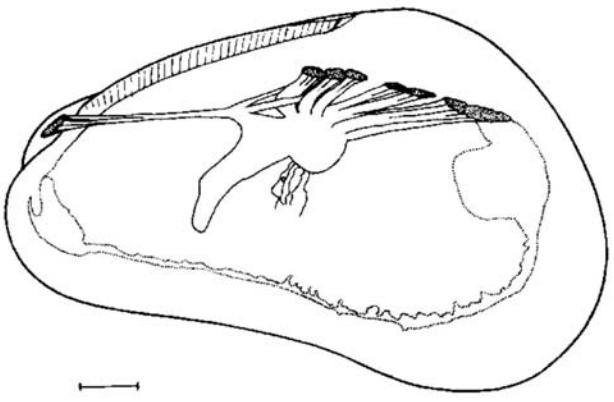

Figure7

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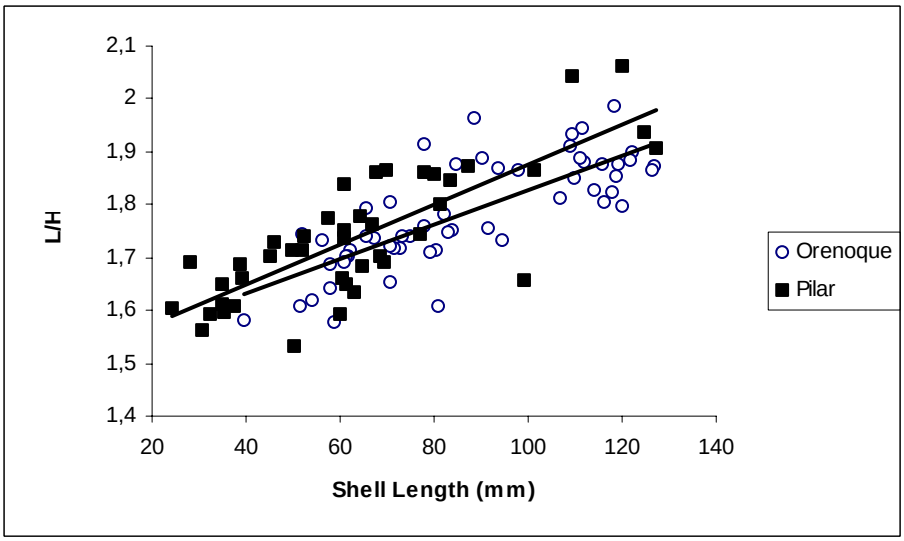

Figure8
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Figure 9

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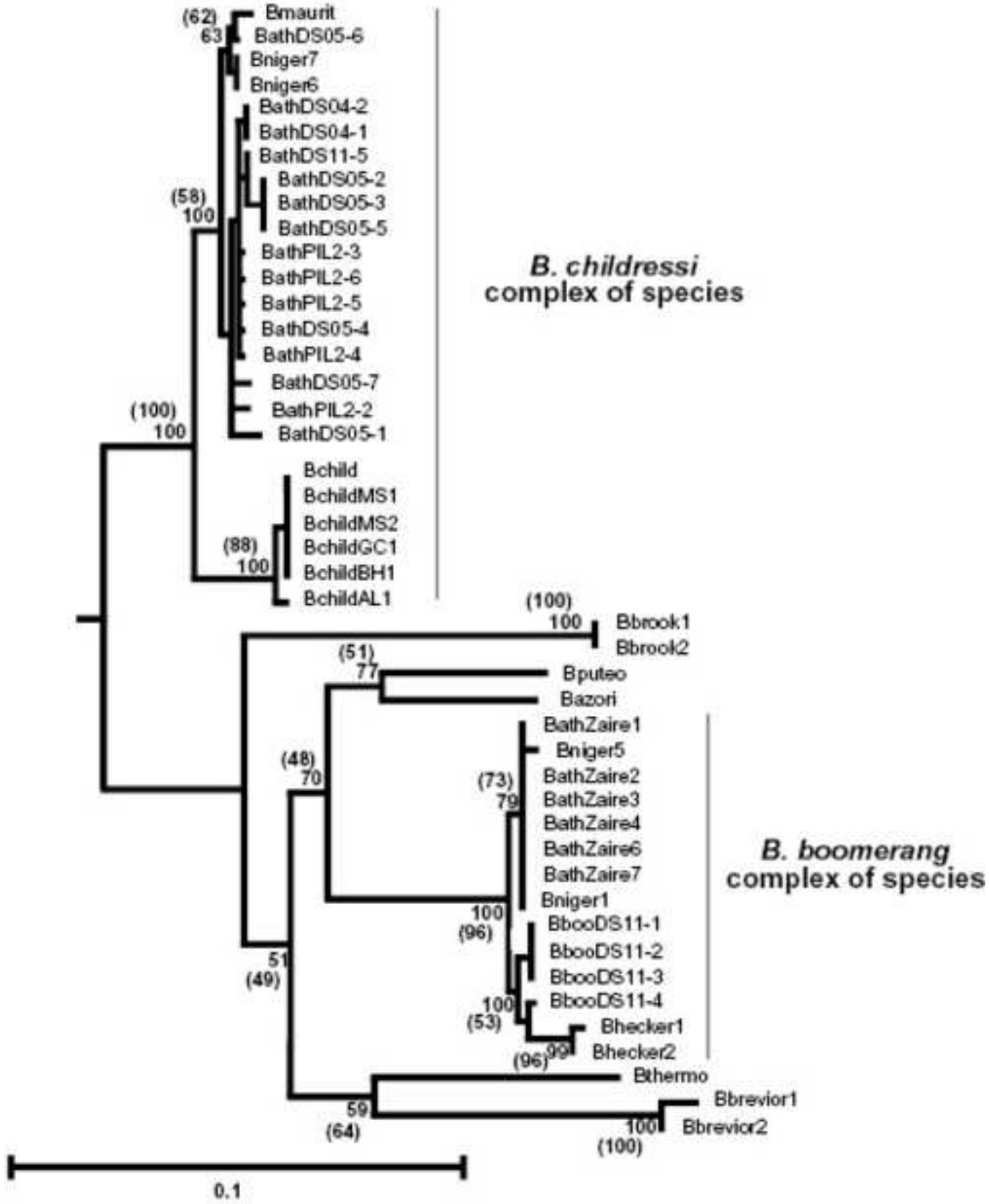

Figure 10

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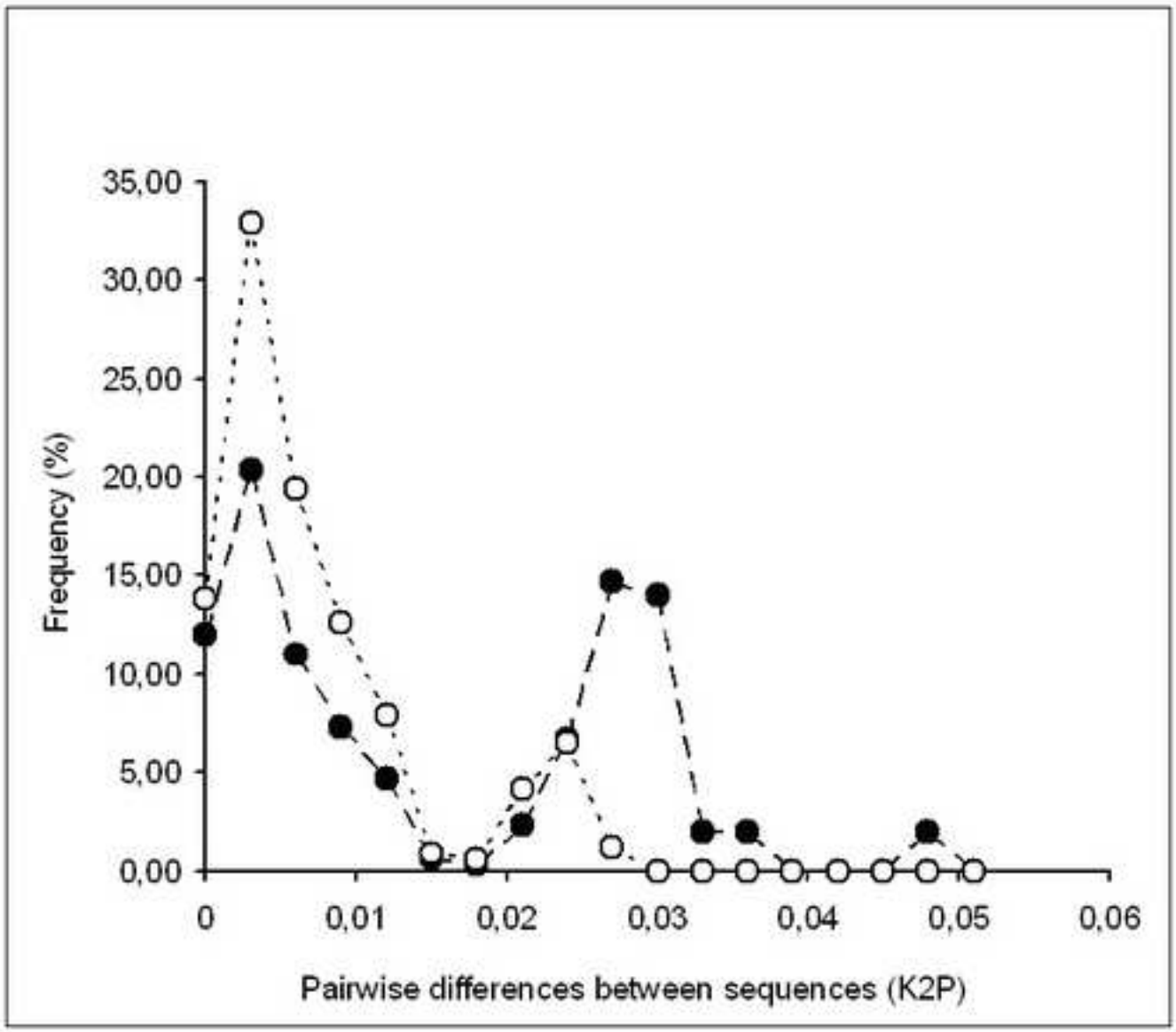

Figure 11

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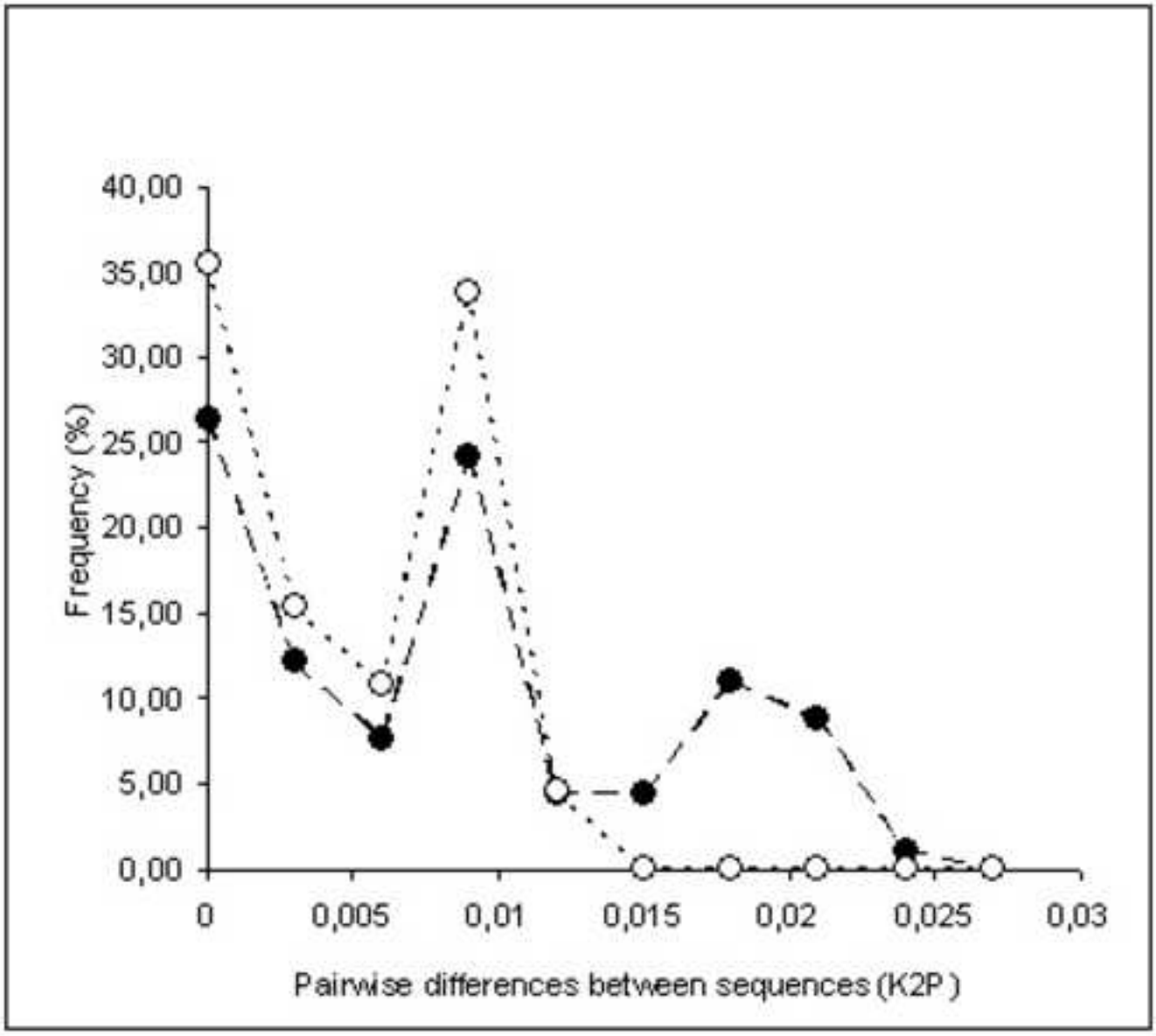

Figure 12

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