Symbioses between deep-sea mussels (Mytilidae: Bathymodiolinae) and chemosynthetic bacteria: diversity, function and evolution

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Abstract

Mussels of the subfamily Bathymodiolinae thrive around chimneys emitting hot fluids at deep sea hydrothermal vents, as well as at cold seeps and on sunken organic debris (sunken wood, whale falls). Despite the absence of light-driven primary production in these deep-sea ecosystems, mussels succeed reaching high biomasses in these harsh conditions thanks to chemosynthetic, carbon-fixing bacterial symbionts located in their gill tissue. Since the discovery of mussel symbioses about three decades ago our knowledge has increased, yet new findings are published regularly regarding their diversity, role and evolution. This article attempts to summarize current knowledge about symbiosis in Bathymodiolinae, focusing on mussel species for which information is available regarding both hosts and symbionts. Moreover, new data obtained from small mussels inhabiting sunken woods around the Philippines are provided. Indeed, mussel species from organic falls remain poorly studied compared to their vent and seep relatives despite their importance for the understanding of the evolution of symbiosis in the subfamily Bathymodiolinae.

Résumé

Symbioses entre mytilidés des environnements marins profonds (Mytilidae : Bathymodiolinae) et bactéries chimiotrophes : diversité, fonction et évolution. Les mytilidés de la sous-famille Bathymodiolinae s’épanouissent autour des cheminées hydrothermales sous-marines émettant des fluides brûlants, ainsi qu’autour des sources de fluides froids et des débris organiques coulés tels le bois ou les carcasses de baleines. En dépit de l’absence de production primaire photosynthétique autochtone dans ces écosystèmes marins profonds, ces mytilidés peuvent atteindre des biomasses élevées grâce aux bactéries chimiotrophes fixatrices de carbone localisées dans leurs branchies. Notre connaissance de ces « systèmes symbiotiques » a progressée depuis leur découverte il y a une trentaine d’années, mais de nouvelles données sont régulièrement publiées concernant la diversité, le rôle et l’évolution de ces symbioses. Cet article présente une synthèse de ces connaissances, en se focalisant sur les espèces pour lesquelles des données sont disponibles concernant les hôtes et les bactéries symbiotiques. Par ailleurs, de nouvelles données sont présentées, tirées de...

Keywords: Bathymodiolus; Idas; Symbiosis; Hydrothermal vents; Cold seeps; Sunken woods

Mots-clés: Bathymodiolus; Idas; Symbiose; Cheminées hydrothermales; Sources de fluides froids; Bois coulés

1. Introduction

Most ecosystems on Earth depend on photosynthetic primary production for the transformation of inorganic carbon into organic compounds and its entry into food chains. Because light does not penetrate very deep in the seawater, very low biomasses occur at the seafloor where organisms depend upon the meteoritic input of sinking organic matter. However, high biomasses of invertebrates were discovered in the vicinity of deep-sea hydrothermal vents in the late 1970s, and later, on cold seeps, raising questions about how inorganic carbon was converted into organic matter at high rates in these ecosystems. The existence of symbioses between the dominant fauna and bacteria was proven to be the key adaptation explaining the high biomasses observed. Many invertebrates indeed live in association with bacteria which ensure a light-independent type of primary production named ‘chemoautotrophy’ [1,2]. Chemoautotrophic symbionts use reduced compounds, such as hydrogen sulphide present in the environment, as electron donors and oxygen as an electron acceptor to generate large amounts of energy required to fix inorganic carbon, providing a source of carbon to their metazoan host. The co-occurrence of energy-rich electron donors and oxygen is unusual because reduced compounds normally get oxidized in oxygenated seawater. However, the presence of hydrothermal vents and cold seeps is linked with processes generating huge amounts of reduced compounds. Briefly, hydrothermal vents result from geothermally-driven circulation of seawater in the crust linked to magmatic activity at oceanic ridges, and cold seeps occur where hydrocarbons and/or gas hydrates are present in the subsurface at continental margins. Both environments are characterized by emissions of fluids enriched in reduced compounds originating from the subsurface such as hydrogen sulphide, methane and dihydrogen which fuel diverse chemotrophic prokaryotes (for reviews see [3–5]).

Invertebrates associated with chemotrophic symbionts include mostly bivalves (within families Lucinidae, Mytilidae, Solemyidae, Thyasiridae, and Vesicomyidae), gastropods (Provanidae), annelids (Alvinellidae, Siboglinidae) and arthropods (Alvinocaridae) (for reviews, see [6] and [7]). Among these groups, mussels of the subfamily Bathymodiolinae (Bivalvia: Mytilidae) are of particular interest for several reasons. They are dominant at many vents and seep sites with species reaching lengths up to 40 cm, and smaller species (up to 3 cm) are also found at large falls of decaying organic matter such as sunken wood and whale bones, where hydrogen sulphur is present as a by-product of the degradation of organic matter [8]. Mussels attach by their byssal threads to hard substrates just above the interface between bottom seawater and sediment or rocks. Most of the species harbour chemoautotrophic sulphur-oxidizing symbionts, as do many other invertebrates cited above, but some also display chemotrophic methane-oxidizing bacteria, and some display multiple symbioses within their gill tissue [9–11]. Bathymodiolines thus constitute good models to study the functioning and evolution of associations involving multiple partners. Symbiosis is important in the nutrition of mussels, as shown by the reduction of the gut observed in some species, but filter feeding can also play a role [12,13]. Information is scarce regarding the physiology and metabolism of bacterial symbionts due to the unability to cultivate them, but numerous studies have investigated various aspects of mussel symbioses. In this article we summarize the current knowledge about the phylogeny of mussels and their symbionts, and the diversity and role of bacteria. Trends in the evolution of mussel symbioses are discussed, focusing on mussel species for which information is available for both host and symbionts (Table 1). New TEM (Transmission Electronic Microscope) observations and sequence data obtained from small mussels associated with sunken woods collected around the Philippines are included.

2. Phylogenetic relationships within the subfamily Bathymodiolinae

Bathymodioline mussels form a monophyletic group within the mytilid family. Following early work by Craddock and co-workers, detailed accounts of the phy-
Species of Bathymodiolinae presented in this paper.

<table>
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<tr>
<th>Area</th>
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<th>HAB</th>
<th>Sites</th>
<th>Symbionts</th>
<th>Reference for symbionts</th>
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<th>Reference for hosts</th>
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<tr>
<td></td>
<td><em>B. heckeri</em></td>
<td>S</td>
<td>West Florida Escarpment <em>(Gulf of Mexico)</em>, Blake Ridge</td>
<td>M, S1, S2, My</td>
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<td>[17]</td>
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<td><em>B. brooksi</em></td>
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<td>Alaminos Canyon, Atwater Canyon <em>(Gulf of Mexico)</em></td>
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<td></td>
<td><em>&quot;B.&quot; childressi</em></td>
<td>S</td>
<td>Alaminos canyon, Louisana Slope <em>(Gulf of Mexico)</em></td>
<td>M</td>
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<td><em>B. thermophilus</em></td>
<td>H</td>
<td>East Pacific Rise, Galapagos Ridge</td>
<td>S</td>
<td>[64]</td>
<td>M99445</td>
<td>[76]</td>
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<td>H</td>
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<td></td>
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<td>W</td>
<td>Bohol sea (8.815°N, 123.627°E, 357–372 m depth)</td>
<td>S</td>
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<td>Bohol sea (9°25.6′N, 124°02.1′E, 1750–1763 m depth)</td>
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<td></td>
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<td>W</td>
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<td>S</td>
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<td>this study</td>
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Species for which molecular information is available regarding both hosts and bacterial symbionts are presented here. HABitat: hydrothermal vents (H), cold seep (S), sunken wood (W). Some sites where species occur are cited (not exhaustive). Symbionts: methane- (M) and sulphur-oxidizers (S, labelled S1 and S2 when two distinct are present), methylotrophs (My), gammaproteobacterium of unknown affinity (G) and Bacteroidetes (CFB). References and accession numbers of the sequences used to produce the trees are indicated. Specimens of *A. longissima*, MOTU 5 and MOTU 16 were collected during the Panglao cruise (May 2005, chief scientist: P. Bouchet). New 16S rRNA sequences from bacterial symbionts and new cytochrome c oxidase subunit 1 (COI) sequences from hosts were obtained following the protocols described in [26] and [21], respectively.
Fig. 1. Phylogenetic reconstruction of bathymodioline species based on cytochrome c oxidase subunit I (COI) nucleotidic sequences. The tree was built using PHYML (GTR substitution model, estimated proportion of invariable sites, four categories of substitution rates, 500 bootstrap replicates) [73], and rooted on *Modiolus modiolus*. New specimens from this study appear in bold. Scale bar represents 2% estimated base substitution. Bold nodes represent the ‘thermophilus’ and ‘childressi’ clades (see text). The most terminal nodes are well supported, but their relationships are much less clear, due to the poor resolutive power of the COI. The localization of the black spot relative to the white circle indicates whether symbionts are intra- (within) or extracellular (outside). HABitat: hydrothermal vents (H), cold seep (S), sunken wood (W). The presence of sulphur- (SOX) and methane-oxidizing (MOX) symbionts is indicated, a parenthesis indicating that the symbionts are most closely related to one another based on their 16S rRNA sequence (see text).

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bathymodioline species in the tree is Bathymodiolus but with a low bootstrap value (Fig. 1). The most basal branching in a relatively basal position in the tree, clustering in the multifurcation, and MOTU longissima from Juan de Fuca (JdF), but to positions, with MOTU 5 appearing as a sister taxon displayed in the tree presented here cluster in distinct close to the root of the tree[21]. Sunken wood species only on sunken wood. Two of these 5 species branched ing on organic substrates, out of which 5 were found recent study based on 4 genes including 7 species liv- ing on organic substrates, out of which 5 were found only on sunken wood. Two of these 5 species branched close to the root of the tree [21]. Sunken wood species displayed in the tree presented here cluster in distinct positions, with MOTU 5 appearing as a sister taxon to Idas sp. from the eastern Mediterranean, Adipicola longissima clustering in the multifurcation, and MOTU 16 branching in a relatively basal position in the tree, but with a low bootstrap value (Fig. 1). The most basal bathymodioline species in the tree is Bathymodiolus sp. from Juan de Fuca (JdF), but Benthiomodiolus lignicola, for which no information is available regarding symbi ons and which is thus not displayed, is more basal in published phylogenies [20]. Wood-associated mus sels displayed here are thus neither early-emerging, nor monophyletic.

Molecular estimations suggest a divergence time between 23.7 and 74.3 MY for the bathymodioline group [17]. This is congruent with the age of several fossils of sunken wood- and whale fall-associated mus sels belonging to the Idas genus recovered from the late Eocene (60 MY) to the Miocene (30 MY) and with the age of the oldest cold seep mussel, Bathymodiolus willapaensis, which was found in a geologic layer from the middle Eocene [22]. A recently-discovered fossil wood-fall community from the late Cretaceous did not yield mussels [23]. Unfortunately, the fossil record is still too patchy to really help answering whether mus sels associated with organic substrates appeared first, but habitats in which mussels were found strongly sug gest they already harboured bacterial symbi-ons. An origin around 60 MY could explain the absence of biogeographical patterns within lineages because at that time, the distribution of continental masses was different, allowing exchange between the future Pacific and Atlantic fauna.

3. Diversity and role of bacterial symbioses in bathymodiolines

3.1. Sulphur oxidizing symbiosis

Most bathymodioline mussels harbour chemoa u-trophic, sulphur oxidizing, bacterial symbi-ons. Bac teria appear as ~0.5 µm diameter inclusions display ing double membranes typical of Gram-negative bac teria [24]. In most mussel species, symbionts occur in vacuoles produced by the host cells and localized within specialized epithelial cells of the lateral zone of the gill filament, hence named bacteriocysts (Figs. 2 and 3) [25]. Vacuoles contain between one to several bacteria (for example, in B. cf. boomerang (Fig. 3E) and B. azoricus respectively [26,27]), and dividing bac teria were observed in most species analyzed to date (see Fig. 3E). Interestingly, recent work has shown that thiotrophs occur extracellularly in small mussels associated with sunken woods collected in the Bohol Sea (Philippines), namely morphotypes BC 279 (Adipi cola longissima), BC 288, BC 294 (herein referred to as MOTU 5), BC 1007, and MOTU 16 (this study Fig. 3A, B, F, [28–30]). In these species, bacteria are located between long microvilli developed by bivalve cells throughout the lateral zone of each gill filament, and thickness of the bacterial layer depends upon the species and probably on local environmental condi tions (Fig. 3) [29]. Similar extracellular bacteria were also reported from I. simpsoni from a whale skull, and I. washingtoniana and Adipicola sp. from the Juan de Fuca hydrothermal vents, although the thiotrophic na ture of bacteria was only inferred from morphological evidence [31]. Two additional mussels with possible extracellular bacteria are Bathymodiolus sp. from the Juan de Fuca Ridge (vents), although results from TEM were ambiguous according to authors [32], and Tamu fisheri from the Gulf of Mexico (Hourdez, pers. comm.).

Evidence from enzymatic tests, immunology and gene sequencing indicate that symbi-ons harbour and express enzymes involved in the metabolism of sul phur such as adenosine phosphosulfate (APS) reducta-se and ATP sulfurylase, and in the Calvin Benson cycle such as ribulose 1,5 bisphosphate carboxylase oxygenase (RubisCO) [27,33–37]. Incubation exper iments indicating that sulphide or thiosulfate stimulate CO₂ uptake under aerobic conditions, for exam ple, in the thiosulfate specialist B. thermophilus, con firmed that symbi-ons use reduced sulphur compounds as electron donors to produce the energy necessary to fix dissolved inorganic carbon, although data from more species would be needed [38,39]. While sulphur oxidizing symbi-ons associated with many metazoans such as siboglinid tubeworms and lucinid clams deposit inorganic sulfur in granules, symbi-ons of mussels usu ally do not [25,33]. Debris of bacterial origin have been identified within phagolysosome-like structures located at the basis of bacteriocytes in mussel species with endosymbiotic thiotrophs, confirming the nutritional role of bacteria [40]. The nutritional role of thiotrophs is less clear in the case of mussels with extracellular bacteria, as degraded bacteria were not convincingly identified within phagolysosomes in these species [29]. Because sulphide poisons cytochrome oxidase in metazoans, an other possible role for thiotrophs would be to detoxify reduced sulphur compounds, an adaptation to variable
environments with temporary bursts of sulphide-rich fluids [26,41,42].

3.2. Methane-oxidizing symbiosis

Shortly after the discovery of thiotrophic symbionts, methane-oxidizing endosymbionts were shown to occur in the gill epithelium of several mussel species from the Gulf of Mexico, in vacuoles within bacteriocytes [10, 43,44]. To date, mussel-associated methanotrophs have only been identified in cold seep and hydrothermal vent mussel species (Table 1). Cavanaugh and co-workers demonstrated the presence of bacteria-like inclusions, 1.5 µm diameter, displaying stacked internal membranes typical of Type I methanotrophs in B. heckeri. Based on their morphology and highly negative stable carbon isotope signatures displayed by gill tissue, close to that...
Fig. 3.
of biogenic methane, symbionts were suggested to be methanotrophs (typical morphology of methanotrophs can be seen in Fig. 3C and E). Gills of “B. childressi” were shown to consume methane, and mussel growth was observed with methane as both a carbon and energy source [43,44]. Methane was confirmed to be the dominant source of carbon in several cold seep mussels [10,45]. Experiments using radiolabelled methane indicated rapid transfer of carbon from the symbionts to the host tissue, and lipids typical of methanotrophs were found in the foot of “B.” childressi, a tissue that contrary to the gill does not contain symbionts [46–48]. Methanol dehydrogenase activity was identified early on, but this enzyme is also found in non methane-consuming methylotrophs [10,46]. Presence of methane monooxygenase, the specific enzyme responsible for the first step of methane oxidation, was confirmed using immunology and sequence analysis [45,49]. Although methane can be used as both an electron donor and a carbon source by symbionts, recent evidence show that one methanotrophic symbiont also possesses RuBisCO, which could indicate the presence of a functional Calvin cycle [50]. Besides carbon, methanotrophs have been suggested to participate in nitrogen uptake in “B.” childressi [51,52].

3.3. Multiple symbioses

The first multiple symbiosis discovered in a chemosynthesis-based ecosystem was ‘dual symbiosis’ in which both sulphur- and methane-oxidizing symbionts co-occurred within the same host cells. Dual symbiosis is reported in at least 5 to 6 mussel species: B. azoricus and B. puteoserpentis (Mid Atlantic Ridge (MAR) vents), B. heckerae, B. brooksi and B. boomerang (Gulf of Mexico (GoM) seeps), and B. cf. boomerang (Gulf of Guinea (GoG) seeps) (see Fig. 1 and Table 1) [10,26,27,45,53,54]. Because sulphur- and methane-oxidizing symbionts have distinct requirements with regards to electron donors and carbon sources, their co-occurrence is thought to increase the flexibility of the association, which could improve the fitness of their host in varying environments such as seeps with possible bursts of sulphide or vents with variable fluid regimes. Both sulphur- and methane-oxidizers contribute significantly to the carbon nutrition of their host, as shown in a study where respective contributions of 20% and 15–25%, respectively, were estimated based on the analysis of lipid δ13C signatures in B. azoricus [55].

In recent years, the screening of large 16S rRNA clone libraries and the use of fluorescence in situ hybridization techniques led to the discovery of 4 and 6 co-occurring symbionts in the gills of B. heckerae and Idas sp., respectively [11,45]. Both species harbour 3 symbionts which belong to the two groups discussed above: a methanotroph and two related, but distinct sulphur-oxidizers (labelled S1 and S2 in Table 1). Both also harbour a bacterium related to free-living methylotrophs of the genus Methylphaga, i.e. bacteria able to oxidize many C1 compound except methane. Despite the lack of direct evidence, it has been suggested that these symbionts could consume methanol, a compound present in fluids, but also a by-product of methanotrophs activity. Idas sp. shelters two additional symbionts, a gammaproteobacterium with no cultivated close relatives and a member of the Bacteroidetes (CFB) related to pathogens and cellulose-oxidizing bacteria. While their role is unknown, a stimulating hypothesis was that the later would be a symbiont able to degrade sunken wooden substrates because close relatives of Idas sp. occur in such environments. However, no CFBs were identified in 16S rRNA clone libraries obtained from wood-associated mussels [29].

3.4. Dynamics of symbiont populations

Bathymodiolines from hydrothermal vents experience local environments where hydrogen sulphur concentrations are variable and sometimes high, reaching for example 325 µM at the Rose Garden vent field, and more typically comprised between 1 and 40 µM [56]. Seep species such as B. cf. boomerang live in environments displaying high methane concentrations, reaching, for example, up to 33.7 µM around mussels at the Régab site in the Gulf of Guinea [26]. Although accurate and relevant measurements are hard to perform in situ at the deep-sea, it is clear that reduced compounds are abundant around mussels, although spatial and temporal variability is high [57,58]. Some data confirm that these compounds are necessary for the survival of mussels and their symbionts. B. thermophilus specimens from the waning vent site Animal Farm (South East Pacific Rise) displayed thinner bacteriocytes with fewer bacteria and lower condition indexes than specimens from an active site, indicating mussels in bad condition; in the same study, thiotrophic symbionts disappeared quickly after transfer of specimens from an active site to an inactive zone of barren basalt [59]. Starvation experiments on B. azoricus specimens kept in aquarium confirm that the loss of symbionts is rapid, but also that it is reversible [60]. Symbionts are thus quickly lost when reduced compounds are not available anymore, and mussels having lost their symbionts are not healthy. These results indicate that symbiosis is
obligatory for the mussels. This is congruent with the observation that early post-larval stages of *B. azoricus* already display their typical two symbionts [61].

In the case of dual symbiotic mussels, several studies have pointed the link between environmental variables and abundances and contributions of symbionts. *B. azoricus* and *B. puteoserpentis* from the Mid Atlantic Ridge were shown to harbour a smaller proportion of methanotrophs versus thiotrophs at sites where end-member fluids contained less methane [27, 36, 62]. Total abundances of symbionts were shown to be variable between specimens of the dual symbiotic species *B. brooksi* from Atwater Canyon and Alaminos Canyon, two cold seep sites in the Gulf of Mexico, although methanotrophs were dominant over thiotrophs at both sites [45]. Recently, short pulses of sulphur were shown to increase the density and relative abundance of sulphur-oxidizers in bacteriocytes of *B. azoricus* using quantification techniques based on fluorescence *in situ* hybridization (FISH) [63]. Such quantifications of symbiont abundances will have to be coupled with detailed investigations of the chemical characteristics in the immediate environment of mussels to really quantify the effect of *in situ* environmental parameters, but it is becoming clear that the dynamics of symbiont populations are influenced by the environment.

4. Phylogenetic relationships of bacterial symbionts based on 16S rRNA sequences and evolution of symbiosis in bathymodioline

All sulphur-oxidizing symbionts of mussels belong to a single large, well-defined clade within the Gammaproteobacteria. This clade also includes several sequences from free-living bacteria and several from other bivalve symbionts, namely thysanoid-associated euctosymbionts and vesicomyid-associated endosymbionts, as shown in trees from the following references [7, 26, 64, 65]. Reconstructed symbiont phylogenies are usually poorly resolved and display very few supported nodes [45]. The poor resolution is probably a consequence of the low variability of 16S rRNA in this group (only up to 4.9% divergence between sequences within the group), and the lack of a good (i.e. close) outgroup clearly hampers the quality and reliability of the reconstruction. Altogether, this indicates that 16S rRNA is not an accurate marker to investigate symbiont phylogeny, and that markers containing more phylogenetic information, such as 23S rRNA or Internal Transcribed Spacers, would be more appropriate as shown by two studies [66, 67].

Because the two paraphyletic, deep-branching, mussels *Bathymodiolus* sp. (Juan de Fuca) and MOTU 16, as well as all bathymodioline but three (“B.” *japonicus*, “B.” *platifrons*, and “B.” *childressi*, representing a single clade) display sulphur-oxidizing symbionts (see Table 1 and Fig. 1), it can be suggested that thiotrophic symbiosis was present in the latest common ancestor of bathymodiolines. It could have represented one of the key adaptations favouring the diversification of the group, also explaining why today’s bathymodiolines are restricted to reduced environments.

The occurrence of extracellular bacteria in the gills of sunken wood mussels was considered significant because ectosymbiosis probably represents the earliest type of association on the evolutionary route to endosymbiosis, supporting the hypothesis of a progressive process of symbiont internalization leading to true endosymbiosis found in vent and seep mussel species. Host phylogeny presented in Fig. 1, however, shows that there is no evident shift from ecto- to endosymbiosis. This is either not the case in published symbiont phylogenies [29]. Extracellular bacteria are related but not paraphyletic to endosymbionts, indicating that localization is not a driving force of the evolution of symbionts. The ambiguous localization of symbionts in *B.* sp. JdF is problematic, and detailed studies about symbiont localization in deep-branching mussel species such as *Benthomodiolus ligincola* would be needed to figure out whether ‘ectosymbiosis’ could be ancestral in bathymodiolines. At present, it appears that certain species, mostly small mussels which do not form a coherent group, display extracellular sulphur-oxidizing bacteria. Whether bacterial localization depends upon host species or a habitat could be tested if mussel species colonizing several types of habitats (vents, seeps, organic falls) were discovered.

The topology of the thiotrophic symbiont tree is significantly distinct from that of the host tree (AU test using CONSEL, *p* < 0.001 [68]). There is no co-speciation between hosts and symbionts. This is congruent with results from a recent study and is not surprising as strict co-speciation is expected only when symbionts are strictly maternally-inherited, such as in vesicomyids, while thiotrophs are environmentally acquired in mussels [19, 66, 69]. Lack of co-speciation is also consistent with the co-occurrence of two distinct thiotrophic phylotypes in the gills of *B. heckerae* and *Idas* sp., and with the extracellular localization of bacteria in some mussels [11, 29, 45]. At least three common nodes are however strongly supported in both host and symbiont trees (Fig. 1). The two MAR species *B. azoricus* and *B. puteoserpentis* are closest relatives, and
display the same thiotrophic symbiont 16S rRNA phyla-
type. B. cf. boomerang and B. hekerae are also closest
relatives, and so are the dominant thiotroph of the for-
mer and the thiotroph of B. cf. boomerang, despite the
great geographical distance between the Gulf of Mex-
ico and the Gulf of Guinea. Finally symbionts of the
closely related vent mussels from the western Pacific,
B. brevior and B. septemdierum, are also closest rela-
tives. These results indicate that co-speciation could be
significant for very closely related species, or at short
time scales.

Mussel-associated methanotrophs belong to a gam-
maproteobacterial clade which, to date, only includes
mussel symbionts, suggesting a single common origin
of methanotrophic symbionts which would imply
that the ability to establish symbiosis appeared once
in this group of bacteria [70]. Their closest relatives
are free-living Type I methanotrophs, as could be ex-
pected from symbiont morphology displaying typical
internal stacked membranes (see Fig. 3E) [71]. All
symbiont 16S rRNA sequences are closely related,
displaying a maximum divergence of 3.2%. As for
thiotrophs, B. azoricus and B. puteoserpentis share a
single 16S rRNA methanotroph phylotype, and methan-
otrophs associated with B. cf. boomerang and B. heck-
erae are closest relatives [36,45]. Apart from these two
clades, topologies of host and methanotroph trees do
do not display common well-supported nodes (AU test:
p < 0.001). For example, the methanotroph from MAR
mussels is the most closely related to methanotrophs
from B. brooksi and Idas sp.

Few elements are available that would explain this
lack of co-speciation, because the question of how
methanotrophs are acquired is not resolved. For ex-
ample, a puzzling observation is that B. brooksi and
“B.” childressi co-occur within the same mussel beds
at Alaminos Canyon (GoM) but display clearly distinct
methanotroph phylotypes [45]. Overall, symbioses in-
volving methanotrophs are present in less than half of
the mussel species, and are spread over the host tree.
From the point of view of the host, these symbioses
could reflect opportunistic associations rather than real
evolutionary trends. Indeed, methanotrophic symbioses
are found in mussel species exposed to moderate-to-
high methane concentrations, in particular at cold seeps.
In this regard, the absence of methanotrophic symbioses
in environments such as organic falls could be due to the
absence of significant methane sources in these areas.
The possibility to establish opportunistic associations
could also explain the presence of additional symbionts
besides sulphur- and methane-oxidizers [11,45]. It may
well be that a diversity of bacteria which are able to
establish associations with metazoans occur in the en-
vironment.

5. Conclusion and future directions

More information is becoming available regarding
the diversity, role and functioning of mussel symbio-
sis. Symbiosis seems to be obligatory for the host, but
thiotrophic symbionts, at least, are environmentally ac-
quired and both sulphur- and methane-oxidizers do not
coopulate with their hosts. Our understanding of the
evolution of mussels and their symbionts is however
still fragmentary. It is limited by the need for expensive
cruises, and biased towards focused research areas such
as the mid Atlantic, the eastern and western Pacific, and
the Gulf of Mexico. Mussel as well as symbiont phy-
logenies are not well resolved and more marker genes
and species are needed. Similarly, the true diversity of
bacterial symbionts is probably underestimated.

How important and integrated is the interaction for
the host and its associated bacteria, and how it is in-
fluenced by the environment remain to be determined.
Although suspected due to indications of environmen-
tal acquisition of symbionts, the existence of free-living
forms of symbionts is not yet proven, and how hosts
and symbionts recognize each other and establish the
interaction is not elucidated. Finally, how symbiosis
shapes the evolution of mussel hosts and their associ-
bated bacteria is not known. For example, symbiosis was
recently shown to have led to genome reduction in the
maternally inherited thiotrophic symbiont of Calypto-
gea magna [72]. Genomics are thus the next big
step forward needed to better understand mussel sym-
bioses.

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