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An overview of chemosynthetic symbioses in bivalves from the North Atlantic and Mediterranean Sea

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Abstract

Deep-sea bivalves found at hydrothermal vents, cold seeps and organic falls are sustained by chemosynthetic bacteria which ensure part or all of their carbon nutrition. These symbioses are of prime importance for the functioning of the ecosystems. Similar symbioses occur in other bivalve species living in shallow and coastal reduced habitats worldwide. In recent years, several deep-sea species have been investigated from continental margins around Europe, West Africa, East America, the Gulf of Mexico, and from hydrothermal vents on the Mid-Atlantic Ridge. In parallel, numerous more easily accessible shallow marine species were studied. We here provide a summary of the current knowledge available on chemosymbiotic bivalves in the area ranging west-to-east from the Gulf of Mexico to Marmara Sea, and north-to-south from the Arctic to the Gulf of Guinea. Characteristics of symbioses in 51 species from the area are summarized for each of the five bivalve families documented to harbor chemosynthetic symbionts (Mytilidae, Vesicomyidae, Solemyidae, Thyasiridae and Lucinidae), and compared among families with special emphasis on ecology, life cycle, and connectivity. Chemosynthetic symbioses are a major adaptation to ecosystems and habitats exposed to reducing conditions, yet relatively little is known regarding their diversity and functioning apart from a few "model species" on which effort has focused over the last 30 yr. In the context of increasing concern about biodiversity and ecosystems, and increasing anthropogenic pressure on Oceans, we advocate for a better assessment of bivalve symbioses diversity in order to evaluate the capacities of these remarkable ecological and evolutionary units to withstand environmental change

1 Introduction

Bivalve mollusks occur in a variety of marine and freshwater ecosystems, at all depths and latitudes. Among other adaptations, they have evolved various strategies for nutrition, including filter-feeding using their gills, particle uptake using elongated labial

palps, and carnivory through reduction of gills to muscular pumping elements. In some remarkable species (e.g. families Tridacnidae and Cardiidae), nutrition involves symbiotic interactions with micro-organisms such as photosynthetic dinoflagellates. Symbiotic nutrition through gill-associated bacteria is the most recently discovered nutrition mode. It was demonstrated in large clams and mussels found around deep-sea hydrothermal vents, which were discovered in 1977 (Lonsdale, 1977). Authors were puzzled when they discovered large fauna around chimneys emitting high-temperature, toxic fluids. In fact, bivalves as well as some giant tubeworms (Annelida: Siboglinidae) were shown to harbor chemoautotrophic bacteria in their tissues. These bacteria oxidize reduced sulfur from the fluids, and use the energy acquired to fix inorganic carbon, which is subsequently transferred to their animal host (Cavanaugh, 1983; Rau and Hedges, 1979). Similar and often related organisms and symbioses were then identified at cold seeps and large organic falls occurring in the deep sea (Pailleret et al., 2007; Paull et al., 1984; Smith and Baco, 2003) and subsequently, illustrating the occasionally strange paths which a developing scientific field can take, in much more readily accessible habitats such as seagrass beds, mangroves, fjords, deltas, coastal reduced sediments, and even sewage sludge (Dubilier et al., 2008; Fisher, 1990). As will become evident in the following text, the term "symbiosis" must be interpreted in the broadest sense here, because isolating and evaluating costs and benefits for each partner is far from an easy task (Douglas, 1994).

The occurrence of chemosynthetic symbioses is one of the characteristic features of cold seeps and hydrothermal vent faunas, because they are responsible for most in situ primary production and fuel the high-biomass communities. That said, symbioses have often been overlooked in other habitats, in which symbiotic species tend not to be dominant and somewhat smaller. Nevertheless, the ability to associate with chemosynthetic bacteria is a recurring feature in the evolution of bivalves, since it has appeared independently in at least five families, the Mytilidae, Vesicomyidae, Solemyidae, Lucinidae and Thyasiridae, and possibly more (e.g. Nucinellidae). Tight evolutionary relationships exist between metazoans and their associated symbionts in various

habitats, as evidenced by several studies supporting that deep-sea vent and seep bivalve species tend to derive from shallow-water ancestors (Craddock et al., 1995; Little and Vrijenhoek, 2003; Lorion et al., 2010; Williams et al., 2004). Examining the intra- and interspecific diversity displayed by chemosynthetic symbioses in as many species as possible, in parallel with the functional, ecological, biogeographical and evolutionary trends that exist in symbiotic systems will provide greater insight into this critical adaptation to reducing habitats.

The Atlantic Ocean opened relatively recently (~ 180 MY), after fragmentation of the Pangaea. Today, the northern hemisphere the Atlantic is connected with the Mediterranean Sea and the Gulf of Mexico. The Mediterranean only re-connected with the Atlantic following the Messinian Salinity Crisis (5.96–5.33 MYA) during which it essentially evaporated (Duggen et al., 2003). The Gulf of Mexico became exclusively connected to the Atlantic after the rise of the Isthmus of Panama about 5 MYA, restricting exchanges between the Pacific and Atlantic (Woods Hole Oceanographic Institution website). In this context, the North Atlantic makes for an interesting focus area for investigating colonization, evolution and biogeography of chemosynthetic symbioses. This is supported by the fact that shallow-water coastal areas of the northern Atlantic, Gulf of Mexico and Mediterranean have been investigated in detail since a long time, and information regarding the diversity of bivalves are some of the most accurate available, although certainly not exhaustive. On the continental shelf and in the deep-sea, most of the sampling effort has focused on a relatively small number of areas subject to hot and cold venting, which have been studied using manned submersibles and Remotely Operated Vehicles (Fig. 1). Many cold seep sites have been explored at varying depths in the Gulf of Mexico (see Cordes et al., 2010 for review) and several hydrothermal vents sites have been explored on the Mid-Atlantic Ridge (MAR) including Lucky Strike, Menez Gwen, Rainbow, Broken Spur, Lost City, TAG, Snake Pit, Logatchev, 5° S and 9° S (Desbruyères et al., 2000; van der Heijden et al., 2012). Other areas have been less intensively or more recently investigated. These include deep vents in the Caribbean Sea (Piccard vent field, Mid-Cayman Ridge), cold seeps on the Barbados prism, the

east American margin (Blake ridge), in the Marmara sea, the eastern Mediterranean (Olimpi mud field, Anaximander mounds, Nile deep-sea fan), the Gulf of Cadiz (various mud volcanoes), the Norwegian margin (Haakon Mosby Mud Volcano and Storegga slide), the Gulf of Guinea (Guiness, Regab, Kouilou), the Arctic Margin (Loki's castle), and Caribbean sea of Colombia and Laurentian deep-sea fan (Connelly et al., 2012; von Cosel and Olu, 2009; Van Dover et al., 2003; Gebruk et al., 2003; Gracia et al., 2012; Olu et al., 1996; Olu-LeRoy et al., 2007a; Sahling et al., 2008, Cunha et al., 2012).

A survey of the literature indicates that approximately 200 bivalve species occurring in the north Atlantic, Gulf of Mexico and Mediterranean Sea belong to families or subfamilies specifically reported to be associated with chemosynthetic bacteria. This number certainly underestimates the true diversity, as new species are often discovered upon exploration of new sites. Moreover, some described species could be hiding several cryptic species, which are hard to delimit because this requires detailed genetic study on numerous specimens, which are typically not available for the deep-sea. Cryptic species have for example been documented in deep-sea Mytilidae (Jollivet et al., 1990; Jones et al., 2006; Lorion et al., 2010). Aside from species detection and delimitation, the taxonomical background of chemosymbiotic bivalves itself is also under debate. Genera were indeed often defined morphologically at times when only very few species were available. Many taxonomic characters employed at that time have lost thir diagnostic value as the number of new available species increased, and new knowledge on features (such as allometric growth) became available. As a consequence, new molecular phylogenies often reveal polyphyly within defined genera (Decker et al., 2012; Jones et al., 2006; Krylova and Sahling, 2010; Taylor and Glover, 2006). Another problem is that many species have been described based on shell material only, for which there is no longer soft tissue for their validation through molecular techniques. Efforts are currently being made for connecting "old" names (such as *Idas modiolaeformis* Sturany 1896) with recent molecular work, but ultimately a reassessment of chemosymbiotic bivalves taxonomy combining morpho-anatomical and molecular

characters is still needed (Lorion and Samadi, 2010). This must be kept in mind when dealing with species and genera names (Table 1).

Herein we provide an overview of the current knowledge available on chemosymbiotic bivalve species in the region ranging west-to-east from the Gulf of Mexico (GoM) to Marmara Sea, and north-to-south from the Arctic to the Gulf of Guinea (GoG, Fig. 1). Characteristics of symbioses are summarized for each of the five bivalve families in which chemosynthetic symbionts are documented (with a note on the recently investigated Nucinellidae), and followed by a synthesis focusing on species from the area. Although some features are shared among families, the degree of dependency between host and symbionts or the mode of symbiont acquisition and transmission may vary among families. The last part of this paper is devoted to to compare these features in the different families to uncover general trends in bivalve chemosynthetic symbioses. Interestingly, several authors pointed to unexpected trans-Atlantic distribution of certain chemosymbiotic bivalve species or sister-species along the Atlantic Equatorial Belt (AEB), and a specific goal was to evaluate whether this hypothesis also holds true for their associated symbionts (Cordes et al., 2007; Génio et al., 2008; Olu et al., 2010; Olu-LeRoy et al., 2007b). A check list of species in which symbiosis has been documented is also provided (Table 1).

2 Chemosymbiotic bivalves and their symbioses

2.1 Mytilidae

One clade within the family Mytilidae has successfully colonized the deep-sea. At depths, species are restricted to ecosystems and habitats in which most organic matter is derived from chemosynthetic primary production or methanotrophy, such as cold seeps and hydrothermal vents, or from the slow degradation of refractory organic matter such as bone lipids of large cetacean carcasses or sunken wood. This deep-sea mussel clade is often referred to as the sub-family Bathymodiolinae typified by the

genus *Bathymodiolus* Kenk and Wilson, 1985, a large vent and seep mussel (Kenk and Wilson, 1985). In reality, several genera of smaller mussels such as *Idas*, *Adipicola* (formerly *Myrina)*, *Benthomodiolus*, or *Tamu*, some of which were described long before the discovery of *Bathymodiolus* and chemosynthetic ecosystems, are also part of the clade despite the fact that they are included in the subfamily Modiolinae, along with *Modiolus modiolus*, a species often used as an outgroup in deep-sea mussel phylogenies (Distel et al., 2000; Jones et al., 2006; Lorion et al., 2010; Samadi et al., 2007). Notably, deep-sea mytilids are almost absent from background abyssal fauna, except for few small species within genus *Dacrydium*, a group in which symbiosis has not been investigated (Duperron, 2010; Salas and Gofas, 1997). Contrary to most other chemosymbiotic bivalves discussed in this paper, mussels are epibenthic, and occur attached via a byssus to hard substrates including carbonates, basalts, tubes of large tubeworms (Annelida: Siboglinidae) or shell of other specimens. Exceptions include *Bathymodiolus boomerang* which lives partly buried in the sediment at the Barbados seeps (Von Cosel and Olu, 1998; Duperron, 2010). Mussels occur mostly in areas of diffuse fluid flow, and avoid the hottest or most anoxic niches. Genera themselves (e.g. *Bathymodiolus*, *Idas*, or *Adipicola*) remain poorly defined based on morphological characters, and are clearly non-monophyletic (Carney et al., 2006; Jones et al., 2006; Lorion et al., 2010). Cosel (2002) re-evaluated most of the species of *Bathymodiolus*, based on morphological characters of the shells and soft parts, suggesting division into four groups (*Bathymodiolus thermophilus* group, *B. brevior* group, *B. heckerae/boomerang* group and the *B. childressi* group) (Von Cosel, 2002). Keeping in mind these limitations, several common features are conserved within the group, such as the overall modioliform shape, the gross anatomy, the presence of often reduced digestive tract and labial palps, and of hypertrophied gills in most species (reviewed in Duperron, 2010). Nevertheless the most striking shared feature is the presence of bacterial symbionts associated with epithelial cells in the lateral zone of gill filaments in all species. A possible exception is *Idas argenteus* collected from woods in the North

Atlantic, which feed on larvae of wood boring bivalves as evidenced by the presence of remnants in their gut (Ockelmann and Dinesen, 2011).

Mussel species investigated to date associate with one to six distinct types of bacterial symbionts. Each type displays a distinct 16S rRNA-encoding gene sequence, which is used as a barcode for identifying and characterizing phylogenetic relationships in bacteria (Fig. 2). Most frequently they correspond to sulfur-oxidizing (thiotrophic), or in some species methane-oxidizing (methanotrophic) Gammaproteobacteria (Cavanaugh, 1983; Cavanaugh et al., 1987; Felbeck et al., 1981; Rau and Hedges, 1979). Dual symbioses involving both types simultaneously have been reported (Distel et al., 1995; Fiala-Médioni et al., 2002; Fisher, 1993; Olu et al., 1996). More recently, other phylotypes were documented corresponding to methylotrophs, to a clade named Gammaproteobacteria G, and to Bacteroidetes (Duperron et al., 2007b, 2008a). Briefly, sulfur-oxidizing symbionts are small bacteria which occur either inside gill epithelial cells, within vacuoles, or outside the cells, within a layer of microvilli, depending on the host species (Duperron et al., 2008b, 2009; Gros and Gaill, 2007; Lorion et al., 2009; Miyazaki et al., 2010). They oxidize reduced sulfur compounds (sulfide, thiosulfate) and use the energy to perform carbon fixation via the Calvin cycle, using a Type I RubisCO (Fisher et al., 1988; Nelson et al., 1995; Pimenov et al., 2002). Recently, mussel-associated thiotrophs were shown to use hydrogen as an alternative energy source at the hydrogen-enriched ultramafic vents of Logatchev, but also at the hydrogen-poor southern MAR (5° S and 9° S). Pacific Antarctic Ridge mussel symbionts also displayed the gene, but the gene was not amplified from Gulf of Mexico seep mytilids (Petersen et al., 2011). Methane-oxidizers are related to free-living Type I methanotrophs; they are intracellular and larger than thiotrophs, and display Type I-typical stacked internal membranes within their cytoplasm (Cavanaugh et al., 1987). They possess methane monooxygenase, an enzyme found only in methanotrophs, and use methane as both a carbon and energy source (Cary et al., 1988; Childress et al., 1986; Duperron et al., 2007b; Pernthaler and Amann, 2004). Evidence on the occurrence of a RubisCO gene, and thus possibly an alternative autotrophic pathway of carbon assimilation, was

presented in a species from the Pacific Ocean (Elsaied et al., 2006). The metabolism of other symbionts (methylotrophs, Gammaproteobacteria G and Bacteroidetes) has not yet been investigated, and hypotheses are based on weak phylogenetic inferences (Duperron et al., 2007b, 2008a). Methylotrophs in *B. heckerae* were for example hypothesized to take up methanol from the methane-oxidizers close to which they occur.

Within the area considered in the present review, large *Bathymodiolus* are reported from cold seeps in the Gulf of Mexico (*B. childressi*, *B. brooksi*, *B. heckerae*), the Barbados accretionary prism (*B. boomerang*), the Blake Ridge diapir (*B. heckerae*), the Mid Atlantic Ridge hydrothermal vents (*B. azoricus*, *B. puteoserpentis*, and relatives of uncertain species status at sites 5° S and 9° S, displaying distinct COI haplotypes closely-related to *B. azoricus*), seeps off Mauritania and the Gulf of Cadiz (*B. mauritanicus*) and off Nigeria (*B*. aff. *mauritanicus* and *B*. aff. *boomerang*, with no detail regarding symbiosis), and deep seeps in the Gulf of Guinea (*B*. aff. *boomerang*, Fig. 1). They are not yet documented from the Mediterranean. Interestingly two groups of closely-related species, *B. boomerang*/*B*. *heckerae* and *B*. *mauritanicus*/*B*. *childressi*, display an amphi-Atlantic distribution which led authors to postulate a dispersal route following the Atlantic Equatorial Belt, the AEB hypothesis discussed in Cordes et al., 2007; Génio et al., 2008; Olu et al., 2010; Olu-LeRoy et al., 2007b. Speciation could also have occurred along this belt. All but *B. childressi*, which has only methanotrophs, harbor multiple symbionts, with simultaneous occurrence of one sulfur- and one methane-oxidizer, and in the case of *B. heckerae*, addition of a second distinct sulfur-oxidizer and a methylotroph (DeChaine et al., 2006; Distel et al., 1995; Duperron et al., 2005, 2007b; Fisher et al., 1993) (Fig. 2). In fact, all *Bathymodiolus* species documented to harbor two or more different symbionts with distinct metabolisms are documented from seeps and vents in the Atlantic and Gulf of Mexico. Multiple symbioses have not yet been documented in *Bathymodiolus* species from other oceans. Multiple symbiosis is a common feature of mussels within a clade that includes *B. azoricus*, the two mytilids from 5° S and 9° S MAR *B. puteoserpentis, B. heckerae, B. boomerang* and *B*. aff *boomerang*, a group that diverged around 6.2 MYA (Miyazaki et al., 2010).

This could be a phylogenetic trend, but multiple symbioses also occur in *B. brooksi* which is only distantly related to this group (Jones et al., 2006; Lorion et al., 2010), and in *B. mauritanicus* whereas the sister species *B. childressi* harbors only methanotrophs (Rodrigues et al., 2012). Symbioses involving two or more types of bacterial metabolisms were hypothesized to confer flexibility to their host, by allowing fine-tuning of the symbiont population for optimized resource use in habitats with strong spatial and temporal variability (Distel et al., 1995). This hypothesis was confirmed by several studies on *B. azoricus* and *B. puteoserpentis* from MAR vents. Symbiont densities based on TEM counts were shown to differ between sites in *B. azoricus* (Fiala-Médioni et al., 2002). More recently, using more quantitative approaches with statistical support, differences in absolute and relative abundance of sulfur- and methane-oxidizers were demonstrated (Halary et al., 2008). Relative abundances of symbionts qualitatively mirror the respective amount of energy available from the distinct substrates used by each symbiont in locale mussel habitats on MAR vents (Le Bris and Duperron, 2010). Experiments confirmed that a short sulfur or methane pulse stimulates growth of thiotrophic or metanotrophic symbionts, respectively (Halary et al., 2008; Riou et al., 2008), and also showed that symbiont densities vary over time. At cold seeps in the Gulf of Mexico, methane-oxidizers dominate the symbiont population, but self-evident differences in symbiont abundances were observed between *B. heckerae* and *B. brooksi*, and for the latest, between specimens from Alaminos Canyon which displayed lower symbiont densities compared to those from Atwater Canyon (Duperron et al., 2007b). Variability in relative abundances linked with the characteristics of local habitats was documented in more detail in *B*. aff. *boomerang* at the Regab and DIAPIR sites (Gulf of Guinea). Methanotrophs dominated in gills of all specimens, with higher relative abundances at a site with direct evidence of high flux (bubbling, high degassing activity), and highest methane concentration in bottom waters (Duperron et al., 2011). Stable isotope signatures correlated with these measurements, suggesting that specimens with more methane-oxidizers derived a substantially higher fraction of their carbon from methane, and a lower fraction of their sulfur from sulfide. This confirms that multiple symbioses

are highly adaptable to changing habitats, both in time and space, someway optimizing the use of resources. In *Bathymodiolus*, heterotrophy plays a limited role, but a certain fraction of carbon is derived from particulate and dissolved organic matter at shallower sites, as seen for *B. azoricus* at the 800 m deep Menez Gwen site (Riou et al., 2010), or during early life (Martins et al., 2008).

Symbioses in smaller mytilids from the Atlantic, Gulf of Mexico and Mediterranean have been far less documented. *Tamu fisheri*, which inhabits the base of *Lamellibrachia luymesi* (Annelida: Siboglinidae) aggregations and beds of *B. childressi*, and *Idas simpsoni* from the North Sea both harbor extracellular sulfur-oxidizing bacteria associated with their gill epithelial cells. This observation was based on electron microscopy data, but no molecular characterization is available yet (Southward, 2008). Conversely, the sulfur-oxidizing bacteria associated with *Idas macdonaldi*, occurring at intermediate depths (~ 650 m) on the Louisiana Slope were characterized by 16S rRNA gene sequencing only (Won et al., 2008). Interestingly, this species has a well-developed digestive system in comparison with others from the group, suggesting mixotrophy (Gustafson et al., 1998). Two *Idas* species were investigated in more detail, combining molecular and microscopic confirmative approaches data. These are an unnamed species recovered in the Marmara Sea tentatively ascribed to the genus *Idas*, and *Idas modiolaeformis*, a species identified in the eastern Mediterranean cold seeps with very close relatives recently sampled and investigated in the north east Atlantic (Duperron et al., 2008a; Lorion et al., 2012; Ritt et al., 2012; Rodrigues et al., 2012). Despite its apparent morphological resemblance with *Idas modiolaeformis*, similar habitat and depth range, *Idas* sp. Marmara at best distantly related to other *Idas* lineages and branches quite far from other Bathymodiolinae based on COI analysis (17 % divergence from any other mussel). *Idas* sp. Marmara hosts dense populations of sulfur-oxidizing bacteria in its gill epithelial cells. *Idas modiolaeformis* was initially described in the late 19th century, and was re-sampled only recently (Olu-LeRoy et al., 2004). It harbors 6 distinct bacterial 16S rRNA phylotypes in its gills, including two sulfur- and one methane-oxidizers, one methylotroph, one Bacteroidetes and one

belonging to the "Gammaproteobacteria G" clade (Duperron et al., 2008a) (Fig. 2). Depending on sampling years or substrates, either sulfur- or methane-oxidizers dominate in the gills, but eventual relation with the variation in the local geochemistry was not investigated (Lorion et al., 2012). The species displays protandric hermaphrodism with possible gender transitions during adult life, and symbionts are absent from the gonad tissue which support environmental acquisition later during development or after larval settlement. The larval shell (Fig. 3f) suggests planktotrophic larvae with some dispersal capabilities (Gaudron et al., 2012). *Idas modiolaeformis* is the sister species of *Idas macdonaldi* known from the Glf of Mexico and possibly diverged around 1.84 MYA. This amphi Atlantic distribution led to the prediction that populations of mussels branching within the *I. modiolaeformis*/*I. macdonaldi* clade should occur at locations in between the eastern Mediterranean and the Gulf of Mexico. Recently, small-*Idas* like mussels were indeed recovered from the north east Atlantic on organic substrates at mud volcanoes in the Gulf of Cadiz (Darwin and Meknès MVs) and in the Gorringe Bank (Gettysburg Seamount) (Rodrigues et al., 2012a). The studied specimens display COI sequences identical (Gorringe) or almost identical (Meknès, Darwin MVs) to the specimens from the east Mediterranean, suggesting they belong ot the same species. Possible occurrence is also reported into wood block colonization experiments deployed in the Western Mediterranean Canyons at 500 m deep (pers. obs., Le Bris, N., personal communication, 2012). Interestingly, none of these seem to harbor methane-oxidizing symbionts. Furthermore, specimens from Meknès and Gorringe sites do not seem to have sulfur-oxidizers either. Identified bacteria belong to the "Gammaproteobacteria G" group, present in *I. modiolaeformis*, to a clade unrelated to any known symbiont (clone G-4), and to the Bacteroidetes. Overall, symbiosis in the *I. macdonaldi*/*I. modiolaeformis* clade appears unexpectedly variable. This could be linked with the wide range of habitats colonized, which include alfalfa and wood colonization devices, natural wood falls, carbonates close to reduced sediment, and siboglinid tubes. The plasticity of the *I. macdonaldi*/*I. modiolaeformis* clade makes it a very good model to study at which stage of the life cycle (spawned oocytes, veliger stages, post-larvae, aposymbiotic juveniles

or adult stages) and how symbionts establish, transmit and evolve over a relatively short evolutionary time scale.

Besides documented chemosynthetic gill-associated symbionts, deep-sea mytilids were shown to be affected by several potentially pathogenic micro-organisms, including viruses, prokaryotes, and ciliates (Ward et al., 2004). Yet the most intriguing potential pathogen is a lineage of intranuclear Gammaproteobacteria related to various sequences recovered from clone libraries from metazoans (Zielinski et al., 2009). *Candidatus* "Endonucleobacter bathymodioli" invades and disrupts nuclei of symbiont-free gill intercalary cells in several *Bathymodiolus* species. This group is thought to be widespread in deep-sea metazoans, but remains to be confirmed in other chemosymbiotic bivalves.

2.2 Vesicomyidae

The family Vesicomyidae consists of over 100 known species, distributed worldwide at depths from 100 to 9500 m (Baco et al., 1999; von Cosel and Olu, 2009; Krylova and Sahling, 2010). As in other families of chemosymbiotic bivalves, currently applied genera are under intense discussion (Krylova and Sahling, 2010). The subfamily Vesicomyinae, genus *Vesicomya*, consists of smaller species, up to 1 cm shell length which inhabit the abyssal plain, while representatives of the Pliocardiinae can reach much larger size (up to 30 cm) and colonize a variety of deep-sea reduced habitats including cold seeps, hydrothermal vents, and vertebrate carcasses (Krylova and Sahling, 2010). Most species are infaunal and possess siphons of varying length. They live with their anterior part buried into reducing sediment, although some vent species inhabit the cracks or small crevices on bare basalts. They thus live at the oxic-anoxic interface, bridging the gap between the two (Cavanaugh et al., 2005). This way they can access both oxygen and dissolved carbon, as well as reduced compounds including sulfide which seep from the subsurface (at vents) or are concentrated within the underlying sediment layers (at seeps) that they can reach by their vermiform foot (Childress and Mickel, 1982). Characteristic features of the Pliocardiinae are the medium-to-large

white shell, the reduced labial palps, simple gut (Fiala-Médioni and Le Pennec, 1987), sub-filamental tissue in the gills (Krylova and Sahling, 2010), large and thick gills, and the presence of very dense intracellular sulfur-oxidizing autotrophic Gammaproteobacteria located within gill epithelial bacteriocytes. Certain species of Vesicomyidae possess hemoglobin molecules capable of transporting hydrogen sulfide via the hemolymph, from the foot to the the gill symbionts (Childress et al., 1993). Oxygen is acquired directly in the gill and in some species transported by circulating hemoglobin (Terwilliger et al., 1983). In some species, the gills are also organized in tubes or channels (Le Pennec et al., 1988). Several species can co-occur at a given seep of vent site, and sometimes even within a single clam aggregate, as observed in the Gulf of Guinea with *Christineconcha regab* and *Laubiericoncha chuni* (Decker et al., 2012; Krylova and Sahling, 2010). Physiological differences between some species could account for a slight ecological niche differentiation and prevent competition in these situations (Goffredi and Barry, 2002; Decker et al., unpublished).

Symbionts associated with the Vesicomyidae are related to sulfur-oxidizing symbionts of the Mytilidae, and of sponges, and to various free-living bacteria (Fig. 2). They use reduced sulfur as an energy source and fix carbon through the Calvin cycle, but contrary to mussel symbionts, they employ a Type II instead of Type I RubisCO (Robinson and Cavanaugh, 1995). Vesicomyid symbionts form a tight clade that displays a high homogeneity among 16S rRNA sequences, most dissimilar sequences differ only by 8.1 % (Rodrigues et al., 2012b), despite the fact that the family is quite ancient (estimates varying from 100MYA based on the fossil record to 22-44MYA with molecular estimated (Little and Vrijenhoek, 2003). Symbionts are transmitted directly from mother to offspring as evidenced by PCR and in situ hybridization (ISH) tests using symbiont-specific primers and probes (Endow and Ohta, 1990; Krueger et al., 1996b). Host and symbiont phylogenies can be superimposed, suggesting rather strict co-speciation (Peek et al., 1998a, b). However, a limited amount of environmental transfer does exist and may permit gene exchanges between symbiont lineages (Stewart et al., 2008, 2009b). To date, Vesicomyidae are the only deep-sea chemosymbiotic

metazoans for which symbiont genomes are fully sequenced, in two species from the Pacific, *Candidatus* "Vesicomyosocius okutanii" and *Candidatus* "Ruthia magnifica", (Kuwahara et al., 2007; Newton et al., 2007). Genome sequencing indicates the loss of several genes with strong genome reduction compared to free-living relatives. This trait, shared with many insect symbionts, is characteristic for maternally-inherited bacteria with no free-living stage, and thus congruent with previous findings (Gil et al., 2004; Wernegreen et al., 2003). In *Cand.* "Vesicomyosocius okutanii", the loss of the *ftsZ* gene involved in bacterial division suggests for example strong host control over the symbiont cycle (Kuwahara et al., 2007). Significant variations also exist among metabolisms of closely related symbionts. *Cand.* Ruthia magnifica does not harbor the membrane-bound nitrate reductase present in *Cand.* Vesicomyiosocius okutanii, suggesting the former is not able to respire nitrate, while the latter can (Kleiner et al., 2012). Another consequence of genome alteration is that the existence of active free-living forms of vesicomyid symbionts is questionable. Although the issue is not settled, it must be noted that no environmental sequence from an active bacterium yet clusters inside the group. The only exception is the symbiont sequence from a thyasird, *Thyasira vulcolutre*, which was recently shown to cluster within this group, suggesting a host shift (Fig. 2) (Rodrigues and Duperron, 2011).

Despite the fact that approximately 28 Vesicomyidae species are documented in the Atlantic and Mediterranean Sea, symbiosis has been investigated in only 11 species (Table 1). Among these is *Abyssogena southwardae*, which occurs at seeps on the west coast of Florida and at the Barbados prism, with large populations on very deep mud volcanoes as well as at vent sites Logatchev, 5° S and 9° S, whose old shells were dredged on a seamount near Canary Islands. Although this species may not encountered contitions for a stable population at the Logatchev site, as the small field observed in 1997 had disappeared in 2007 (Gebruk et al., 2010). Interestingly, they could represent populations of an amphi-Atlantic species, and MAR vents and Florida species have very closely related symbionts (van der Heijden et al., 2012; Krylova et al., 2010). The genus *Laubiericoncha* could be amphi-atlantic, with *Laubiericoncha*

myriamae from Barbados (Jollivet et al., 1990; von Cosel and Olu, 2008) and *L. chuni* in the Gulf of Guinea, although recent molecular data question whether these species should be included in distinct genera (Decker et al., 2012). Although ultrastructural evidence exists for sulfur-oxidizing symbiosis in *L. myriamae*, no molecular data has been released regarding symbiont (Olu et al., 1996). In both *A. southwardae* and *L. myriamae*, genetic data regarding symbiosis is not available for specimens from the eastern Atlantic. Another species, *Pliocardia atalantae* (formerly *Isorropodon atalantae*), has been documented from the Gulf of Guinea to the Mid-Atlantic Ridge but no data is available regarding symbiosis.

In the Gulf of Mexico, vesicomyids are represented by *Pliocardia ponderosa* (formely *Calyptogena ponderosa)* and *Pliocardia chordate* (formely *Vesicomya chordata)* (Table 1). They both display closely related sulfur-oxidizing symbionts (Fig. 2) (Brooks et al., 1987; Distel et al., 1994; Stewart et al., 2009b) and abundant hemoglobin (Scott and Fisher, 1995). Species from seeps in the Gulf of Guinea have recently received attention. These include *Elenaconcha guiness*, *Isorropodon bigoti* and *Calyptogena valdiviae*. *E. guiness* was found to display a very typical vesicomyid symbiosis, as documented in many other species around the world (Duperron et al., 2012). With regards to connectivity, small clams of the genus *Isorropodon* yielded interesting findings. Specimens of three species, *I. perplexum*, *I. megadesmus* and *I. bigoti*, collected respectively in the eastern Mediterranean, Gulf of Cadiz and Gulf of Guinea, seemed to be closely related, forming a distinct clade in COI-based phylogeny (Von Cosel and Salas, 2001; Rodrigues et al., 2012). Their symbionts also appeared as close relatives (Fig. 2), and the association was very similar to that documented in larger clams. The genus *Isorropodon* consists of various species colonizing a wide range of depths (150–6800 m), and could represent a suitable case study into the barriers influencing the biogeography and evolution of vesicomyid symbioses, as demonstrated by the recent discovery of *I. perplexum* in the Marmara Sea (Ritt et al., 2012). Finding closely related symbionts in closely related species is somewhat expected, although instances of non-parental acquisition are reported (Stewart et al., 2008, 2009b). The homogeneity

of symbiont populations within a single species was also questioned recently by the finding of distinct bacterial 16S rRNA lineages in distinct specimens of *Calyptogena valdiviae* (Fig. 2). Overall, new data about symbioses in the Atlantic Vesicomyidae further support the existence of a certain level of symbiont heterogeneity within species, and of environmental acquisition of non-parental symbiont strains in co-occurring host species, which can potentially lead to symbiont co-occurrence displacement, or genetic recombination among symbionts. These phenomena might be of great significance for the evolution of vesicomyid symbioses.

2.3 Solemyidae

All documented Solemyidae live in obligate symbiosis with sulfur-oxidizing Gammaproteobacteria. They drill U- or Y-shaped burrows that allow access to both the bottom seawater, rich in oxygen, and sediment pore-waters which contain reduced sulfur (Fisher, 1990; Stewart and Cavanaugh, 2006). Due to their infaunal lifestyle, deep-sea Solemyidae are rarely sampled in great numbers, which limits the possibility to investigate them in great detail, and most data was obtained from coastal species. Although a weak suspension-feeding capability is retained, dependency upon symbionts-based nutrition is maximal as evidenced by the drastic reduction (*Solemya velum*) or even absence (*Solemya reidi*) of the digestive system and reduction in labial palps. Authors estimated that more than 97 % of the carbon is derived from symbionts (Conway et al., 1989; Krueger et al., 1992). Protobranch gills account for more than 35 % of total weight, with a greater surface area to volume ratio than those documented for other bivalves (Scott, 2005). Symbionts are located inside bacteriocytes of the gill epithelium, which alternate with symbiont-free intercalary cells. The symbiont cycle may be complex as bacterial shape is variable from coccoid- to rod-shaped, and dividing stages are rarely seen, suggesting strong host control. Carbon is fixed via the Calvin cycle (Cavanaugh, 1983; Cavanaugh et al., 1988), and using energy derived from the oxidation of reduced sulfur, most likely through the APS pathway for which they possess enzymes (Kleiner et al., 2012; Stewart and Cavanaugh, 2006). Ammonia is the nitrogen

source assimilated via a host-encoded glutamine synthetase (Lee et al., 1999). Vertical transmission of the symbionts is documented in the genus *Solemya* based on amplification of symbiont DNA from ovaria, eggs and larvae, although confirmation with in situ hybridizations was not obtained from oocytes and eggs (Cary, 1994; Krueger et al., 1996b). However, contrary to Vesicomyidae, host symbiont co-speciation is not observed.

Shallow water Atlantic species for which information is available regarding symbiosis include *Solemya velum*, the model species for the group, found in coastal sediments from Florida to Canada (Nova Scotia) with densities reaching 253 individuals per square meter in eelgrass beds on the coast of Massachusetts (Eisen et al., 1992; Krueger and Cavanaugh, 1997; Stewart and Cavanaugh, 2006). Molecular and ultrastructural information is also available from *S. occidentalis*, a small tropical Atlantic species, 1–3 mm long (Conway et al., 1992; Krueger et al., 1996a). Their symbionts belong to the Gammaproteobacteria (Fig. 2). In *S. velum*, four populations on the New England coast of the USA were show to harbor distinct symbiont genotypes despite host populations were connected, suggesting decoupling between host and symbiont history (Stewart et al., 2009a). This could either be due to the limited resolution of the host marker gene, or a consequence of the lateral acquisition of locally-adapted bacteria. Enzymatic and ultrastructural data also exists for *S. borealis*, another small species (Conway et al., 1992). Several species have been documented at deeper depths in the area. In the Mediterranean, a single specimen of an undetermined *Solemya* is for example the only living Solemyidae species reported in the deep Mediterranean (Rodrigues et al., 2011). It was recovered from a soft sediment core close to a cold seep area of the Nile deep sea fan, at a depth of 1697 m. Unfortunately, no data is available regarding symbiosis, despite attempts to amplify symbiont genes. *Acharax gadirae* and *Solemya (Petrasma) elarraichensis* have been documented from deep (Yuma, Ginsburg, Jesus Baraza, Captain Arutyunov, Carlos Ribeiro, Porta MVs, 960–3902 m depth) and shallower MVs (Mercator, Meknès, Gemini, Kidd, Yuma, Ginsburg, Darwin, 358–1105 m depth) respectively in the Gulf of Cadiz (Oliver et al., 2011). Another member

of the genus *Acharax*, not yet assigned to a species, was collected at the Guiness site (580 m depth) in the Gulf of Guinea. Sequences from symbionts of the two *Acharax* species (GoC and GoG) are almost identical to sequences of several *Acharax* from the Oregon, Pakistan and Indonesia margins at depths from 780 to 2940 m (Imhoff et al., 2003). *S. elarraichensis* symbiont sequences are, on the other hand, closely related to those of the shallow *Solemya velum* (Eisen et al., 1992; Rodrigues et al., 2010). *Acharax* shells, but no living specimens, are documented from various locations including the Norway Plateau or in the Barbados seeps (Ivanov et al., 2010; Olu et al., 1996). In addition several other species occur in the area, however no information is available regarding their symbioses (Table 1).

Overall, *Acharax*-associated symbionts form a very tight cluster despite the geographical distance between collection sites, meanwhile *Solemya* symbionts are spread over at least three distinct clades of Gammaproteobacteria, related to Lucinidae and Thyasiridae symbionts (Fig. 2). This distinct clustering could reveal significant differences in the symbioses between the different host genera, and even among species within genus *Solemya*. For example, carbon fixation in symbionts of *Solemya velum* is ensured by a Type IA RubisCO, meanwhile a Type II RubisCO is present in *Acharax* sp. Guiness (Duperron et al., 2012; Robinson and Cavanaugh, 1995; Schwedock et al., 2004). Other differences could exist besides the type of RubisCO, which definitely warrant further study.

Additional 16S rRNA sequences were recovered besides that of gammaproteobacterial symbionts in *Solemya elarraichensis* and *Acharax gadirae* branching within the Epsilonproteobacteria, Betaproteobacteria, Chlamydiae, Firmicutes, and Actinobacteria. Their status as symbionts or pathogens was not resolved, but at least for certain sequences, they were most likely contaminants. In the Atlantic area, a family related to Solemyidae, the Nucinellidae, was recently shown to display chemosynthetic sulfur-oxidizing bacteria in their gills based on detailed electron microscopy approaches, but without supporting molecular data (Oliver and Taylor, 2012).

2.4 Thyasiridae

Around one hundred Thyasiridae species are described worldwide (59 in the area), which occupy various habitats from intertidal to hadal waters, including the deepest reported chemosymbiotic species, *Maorithyas hadalis* (Fujiwara et al., 2001). They burrow into suboxic to anoxic sediment, in particular in habitats rich in hydrocarbons or fluids (cold seeps, hydrothermal vents) and in Oxygen Minimum Zones. They also occur in organic-enriched habitats such as sunflower seeds in a wrecked ship off Vigo (*Spinaxinus sentosus*, Oliver and Holmes, 2006), colonizing wood artificial substrates in the eastern Mediterranean close to seeps (*Thyasira* sp. Gaudron et al., 2010), and in Canyons under reducing and organic enriched conditions (Cunha et al., 2011). As in Vesicomyidae and Bathymodiolinae, genera are rather poorly defined, leading to frequent confusions (Taylor et al., 2007). Contrary to other bivalve groups discussed here, not all members of the Thyasiridae harbour bacterial symbionts. In fact, several degrees of association, ranging from absence of symbionts to occurrence of very dense bacterial populations in the lateral zone of gill filaments are documented (Dufour, 2005). This illustrates differing degrees of dependence upon symbiont-based nutrition (Dando and Spiro, 1993), and suggest that symbioses appeared several times during the evolution of the family. Species with symbionts harbor bacteria in large cells located in the gill epithelium, and bacteria are tightly packed into a large vacuole that is devoid of host cell cytoplasm (Fig. 3a) and separated from the outside by a net-like structure which could consist of modified microvilli (Dufour, 2005). These species burrow deeper than non-symbiotic species and use their super-extensile foot to mine for sulphide-rich pockets in the sediment, creating a network of tunnels (Dufour and Felbeck, 2003). Some species such as *Thyasira flexuosa* and *T. gouldi* lack siphons and maintain communication with the surface seawater by a long tube made of mucus rings created by the foot. Symbiont chemoautotrophy is supported by the occurrence of APS reductase-encoding genes in symbionts of some species (Rodrigues and Duperron, 2011), and by the carbon stable isotope signatures of animal tissue which are in the range of values

measured in chemosymbiotic metazoans harbouring sulfur-oxidizing symbionts. Variability in symbiont abundances and host nutrition strategy depend upon experimental conditions (presence of sulfide and particles), as evidenced in *Thyasira flexuosa*, *T. sarsi* and *Parathyasira equalis*, confirming their ability to withstand fluctuating environments (Dando and Spiro, 1993; Dufour and Felbeck, 2006).

Symbiosis has been investigated in 9 species from coastal and deep sediment, including cold seeps, but molecular data on symbionts remains very scarce (Table 1). In most investigated species, only ultrastructure was characterized. *Thyasira* (*Parathyasira*) *equalis* (Barents Sea) and *Axinulus croulinensis* (North Sea) harbour moderate amounts of extracellular symbionts, while *T. trisunuata* (Florida), *T. sarsi* (White Sea, North Sea), *T. flexuosa* (North Sea), *T. gouldi* (Arctic region) harbor dense bacterial populations in their gills (Dufour, 2005). Available 16S rRNA sequences all belong to the Gammaproteobacteria and are related to free-living bacteria and sulfur-oxidizing symbionts of several metazoan groups (Fig. 2). In the area considered herein, these include *T. flexuosa* from the Plymouth Sound, at 15 m depth, *Thyasira* aff. *flexuosa* from the Central Zone in the Nile Deep-sea fan cold seeps, *Thyasira vulcolutre* from the Carlos Ribeiro (2200 m depth) and Sagres (1562 m depth) MVs in the Gulf of Cadiz, *Thyasira* n. sp. from the Regab site, at 3167 m depth in the Gulf of Guinea, and *Thyasira* n. sp. Guiness from the Guiness site, at 580 m depth in the Gulf of Guinea (Brissac et al., 2011; Distel and Wood, 1992; Duperron et al., 2012; Rodrigues and Duperron, 2011). Each of these has a single dominant bacterial 16S rRNA phylotype (Fig. 2). A certain level of within-species strain heterogeneity could exist, as evidenced by the co-occurrence of two highly similar yet distinct phylotypes in *Thyasira* n. sp. Guiness (Duperron et al., 2012). Interestingly, symbionts cluster in at least three clearly distinct clades within the Gammaproteobacteria, four if we include Symbiont I of *Maorithyas hadalis* (Fig. 2). Sequences from *T. flexuosa* and *Thyasira* aff. *flexuosa* from eastern Mediterranean cluster with various sequences from Lucinidae and Siboglinidae symbionts, and with environmental sequences. The symbiont of *Thyasira* sp. Guiness clusters with that of *Thyasira* n. sp. Regab and of *Maorithyas hadalis* close to environmental

sequences from reducing habitats. Interestingly, the symbiont of *T. vulcolutre* clusters right within the clade of Vesicomyidae-associated symbionts, suggesting a recent host shift from Vesicomyidae to Thyasiridae (Rodrigues and Duperron, 2011). Although Vesicomyidae symbionts have a reduced genome and are predominantly maternally inherited, instances of environmental acquisition are reported (Stewart et al., 2008), while *T. vulcolutre* co-occurs with Vesicomyidae in the Gulf of Cadiz, which could provide a route for such an event. Symbiont phylogeny thus suggests multiple independent origins of symbiotic bacteria associated with Thyasiridae, possibly from a pool of environmental bacteria presenting features allowing them to establish interactions with metazoans (Imhoff et al., 2003; Rodrigues and Duperron, 2011). This observation agrees well with the hypothesis of multiple origin of symbiosis in Thyasiridae hosts. *Thyasira flexuosa* in particular seems to be a species with a wide geographical distribution, as occurring in the western Atlantic in Florida, off the coast of Norway, extending down to the English Channel and Galicia (Spain), and in the Mediterranean near the Iberian peninsula (Dufour, 2005; Southward, 1986). This could make *T. flexuosa* a good target species to investigate the relevance of the AEB hypothesis to Thyasiridae.

Occasionally, additional bacterial sequences are recovered, mostly Epsilonproteobacteria and Bacteroidetes, but fluorescence in situ hybridization has yet to support these as being significant symbionts (Brissac et al., 2011; Rodrigues and Duperron, 2011). Microscopy also indicates possible Spirochete-like morphotypes located extracellularly on the apex of some bacteriocytes, which could be epibionts. Interestingly, at least four studies report the presence of dense virus-like inclusions within bacteria of *Thyasira* from three different locations, namely *T. flexuosa* from the Plymouth Sound (15 m depth), off Long Beach, Florida (Dufour, 2005) and *Thyasira* sp. at cold seeps in the eastern Mediterranean (Central Zone, site 2A, 1693 m depth) (Brissac et al., 2011; Dando and Southward, 1986; Southward and Southward, 1991). In all cases, dodecahedral inclusions occur only in some bacteriocytes, but are abundant when present. Brissac and coworkers indicated that these “infected” bacteriocytes displayed large

lysosomal structures possibly involved in the destruction of infected symbionts. Viral infection of symbionts thus seems to be a recurrent feature of Thyasiridae symbioses.

2.5 Lucinidae

Lucinidae were formerly classified along with Thyasiridae within the Lucinoidea, mainly because of shared morphological features. However, recent molecular evidence have shown that the families, although displaying convergent features, were not closely related (Taylor and Glover, 2000; Williams et al., 2004). Lucinidae are burrowing bivalves occurring over a wide range of depths, from coastal to at least 2500 m, and latitudes (60° N to 55° S), and with more than 400 (330 living) species described, 84 in the area investigated here, are the most diverse of the chemosymbiotic bivalves (reviewed in Taylor and Glover, 2006; Taylor et al., 2011). All investigated Lucinidae harbor chemoautotrophic, sulfur-oxidizing Gammaproteobacteria located within vacuoles occurring in gill epithelial cells from the lateral zone of the gill filament, suggesting obligatory association (Frenkiel and Moueza, 1995). Despite that, lucinids retain the capability to filter feed (Duplessis et al., 2004). Gills represent about a third of an animal's weight, are thick, opaque, and organized in bacteriocyte channels (Distel and Felbeck, 1987). Moreover, bacteria can store sulfur granules. Symbiosis studies from littoral species have been initiated in the west coast of France (e.g. *Loripes lucinalis*) showing the translocation of fixed carbon from bacteria to the host tissues (Herry et al., 1989) but most of our knowledge regarding the functioning of lucinid symbioses is available from species from the Caribbean. In vivo experiments on *Codakia orbicularis* demonstrated symbiont absence in ovaries, testis, eggs, veliger larvae and metamorphosed juveniles reared in sterile sand (Gros et al., 1996, 1997). Symbiont-specific PCRs also failed to reveal bacterial DNA from the testis and ovaries and TEM did not reveal bacterial shapes in *Lucinoma aequizonata* (Gros et al., 1999). Overall this supports environmental acquisition of symbionts after larval metamorphosis. Free-living forms of symbionts were subsequently shown to be abundant in the *Thalassia testudinum* seagrass environment and water (Gros et al., 2003b). The functioning of symbiosis is also intriguing.

Bacteria replicate their genomic DNA but do not seem to divide actively within hosts cells (Caro et al., 2007), and are not released by adult bivalves (Brissac et al., 2009), suggesting a strong host control. What's more, symbiont reacquisition after starvation is not triggered by within-host division of bacterial cells, but rather by capture of new bacteria courtesy of a life-long continuous ability to acquire symbionts (Gros et al., 2012). In oxygen-depleted environments, symbionts of some species can avoid competition with their host for oxygen resources by growing on nitrate (Duplessis et al., 2004). Carbon transfer would then most likely involve the digestion of symbionts.

In the area considered here, symbioses were investigated in several coastal species, mainly from the Caribbean, near Guadeloupe and Martinique. These include *Lucina pectinata* which lives in black reducing sediment of mangrove swamps, and *Codakia orbiculata*, *C. pectinella*, *C. orbicularis*, *C. costata*, and *Linga pensylvanica* which live in *Thalassia testudinum* seagrass beds. All five seagrass bed species share a single 16S rRNA symbiont phylotype (Distel et al., 1994; Durand et al., 1996). *Anodontia alba* (Brissac et al., 2011) and *Divaricella quadrisulcata* (Gros et al., 2003a), which live in reduced sediment near seagrass beds, also have identical symbiont as those of *Codakia orbicularis*. The symbiont from *L. pectinata* not only diverges from those of other lucinids, but its tissue also displays high concentration of hemoglobin which suggests that the symbiosis might function in a different way (Frenkiel et al., 1996). Another species, *L. nassula*, occurs in seagrass beds near Florida and has a very similar symbiont sequence (Durand and Gros, 1996) Additionally, symbiosis was characterized based on ultrastructure of gill tissue or molecular evidence in a variety of other coastal reduced sediment or seagrass bed species including *Myrtea spinifera*, *L. floridana*, *L. borealis*, *Loripes lucinalis* (synonymized with *Loripes lacteus*, for which symbiont 16S sequences are in GENBANK but unpublished), with estimates that the latter could be responsible for up to 16 % of the primary production observed in seagrass bed habitats in a lagoon in upper Corsica (Dando et al., 1994, 1985, 1986; Distel et al., 1988; Johnson and Fernandez, 2001; Johnson et al., 2002).

Symbiosis has also been investigated in some deep-water species from the eastern Mediterranean Basin cold seeps. *Lucinoma* aff. *kazani* occurs at depths between 500 and 1709 m in the Anaximander Mountains between Rhodes and Cyprus, the Olimpi area south of Greece on the Mediterranean Ridge, and the Nile Deep-sea Fan (North Alex MV and pockmarks in the Central area); it was also recently reported from the Marmara Sea (Bayon et al., 2009; Olu-LeRoy et al., 2004; Ritt et al., 2010; Salas and Woodside, 2002). The second species, *Myrtea* sp., possibly *M. amorpha* described in Olu et al. (2004) and Ritt et al. (2010), is closely related to *M. spinifera* based on 18S and 28S gene sequences, and is labeled *Myrtea* aff. *spinifera* in the tree. It was sampled from the same areas as *Lucinoma kazani*, including the Marmara Sea. Both *Lucinoma* aff. *kazani* and *Myrtea* aff. *spinifera* harbor sulfur-oxidizing bacteria in their gills (Fig. 3b), and the ultrastructure of the association in terms of bacterial shape, presence of sulfur granules, and host tissue organization, resembles that of shallow water lucinids. Another deep-sea species with sulfur-oxidizing symbionts, *Lucinoma asapheus*, is documented from Mercator MV in the Gulf of Cadiz (Rodrigues et al., 2010). In *L*. aff.*kazani*, a dominant bacterial endosymbiont was identified, and the presence of APS rductase-and RubisCO-encoding genes confirmed the chemoautotrophic potential of symbionts. Spirochete sequences were reported for *L*. aff. *kazani* and *L. asapheus* but without further evidence they are unlikely to represent significant symbionts and were suggested as potential pathogens.

Seep lucinids emerged during the Jurassic period. Symbiosis has often been considered as a critical step in allowing their diversification, and could possibly even date back to the Silurian (Boss, 1970; Distel et al., 1994; Little and Vrijenhoek, 2003). From the point of view of bacteria, the situation is more complex. The host-symbiont relationship is not highly specific, as interspecific infection of aposymbiotic juveniles can occur (Gros et al., 2003a). Because symbionts do not divide inside host cells and cannot be released, symbiosis appears as an evolutionary dead-end for bacteria. Besides, multiple related symbiont strains most likely co-occur within a given host. Although 16S rRNA usually displays no intra-individual variation, two related but distinct RubisCO

form IA sequences were for example obtained from *L*. aff. *kazani* which could suggest multiple strains (Duperron et al., 2007a). Recombination between symbionts and environmental bacteria also likely occurs, as evidenced by the incongruence between the APS reductase and 16S rRNA phylogenies from the *L*. aff. *kazani* symbiont. Indeed, 16S rRNA indicates a Gammaproteobacterium, while APS has a Betaproteobacterium as a closest relative (Duperron et al., 2007a). To understand the evolution of lucinid symbionts will require extended knowledge of free-living forms, as symbiosis is probably just one of several niches available for the bacteria, and possibly not an optimal one with regards to fitness.

3 Comparing symbioses from North Atlantic, Gulf of Mexico and Mediterranean bivalves

3.1 Ecological trends in bivalve symbioses

Most chemosymbiotic bivalves associate with sulfur-oxidizing bacteria (Fig. 2). They usually live in habitats existing within the oxic-anoxic interface, where animals can acquire oxygen from seawater as well as reduced sulfur compounds available in the underlying substrate (e.g. reduced sediment, decaying organic matter, seeping fluid,...). In normal deep-sea sediment settings, this oxic-anoxic interface does not exceed a few centimeters, and even a few millimeters in undisturbed cold seep sediment with little-to-no mixing between the two layers (de Beer et al., 2006; Wenzhofer and Glud, 2002). Burrowing chemosymbiotic bivalves thus artificially extend it by their behavior and their morphological characteristics (long siphons, extensile foot), increasing their access to both oxygen and reduced compounds (Cavanaugh et al., 2005). Mytilidae are an exception not only because they can have additional symbionts, notably methanotrophs (Fig. 2), but also because they are epibenthic and usually do not burrow into the substrate. They thus need reduced compounds to be actively emitted at the sediment surface where they mix with oxygenated seawater in order to reach their gills. This might

explain their success at many vents and seeps characterized by active venting, including organic falls which emit reduced compounds, but also their absence at some other seeps and among background fauna, contrary to other families which have some representatives in reduced sediments away from active seeping sites (deep-sea or even coastal sediments).

Lucinidae and Vesicomyidae are all associated with sulfur-oxidizing symbionts, whatever their habitat or depth, although direct evidence is still lacking for the genus *Vesicomya* sensu Krylova (Decker et al., 2012; Krylova and Sahling, 2010; Taylor and Glover, 2000). Symbiont phylogeny indicates the absence of depth- or habitat-related clades (Fig. 2). In contrast, in Vesicomyidae hosts, recent phylogenies suggest possible recurrent events of "stepwise speciation" from shallow to deep waters in different ocean basins, consistent with narrow vertical ranges for most of the vesicomyid genera (Decker et al., 2012; Krylova and Sahling, 2010). All Solemyidae also associate with sulfur-oxidizers, but the genus *Acharax* which occurs at deeper sites than *Solemya*, is associated with a different clade of sulfur-oxidizing bacteria than members of the shallower genus (Imhoff et al., 2003). It is hard to conclude whether this difference is linked with host genus or with distinct depth. In Thyasiridae, even if not all species have symbionts, symbiosis exists in both shallow and deep-sea species, and in the various types of habitats colonized by this family. There are also non-symbiotic species in all habitats and depths (Dufour, 2005). Southward for example demonstrated that individuals of *Thyasira ferruginea* found at 1500 m did not harbor symbionts (Southward, 1986), and Decker did not find evidence for a major input of symbionts in the nutrition of the host based on stable isotope analysis (Decker and Olu, 2012). In Mytilidae, multiple symbioses involving 2 to 6 bacterial types occur in the area considered in this study, and seem to be peculiar to this area, as species from other regions have either sulfur- or methane-oxiziders alone. Yet, it occurs both at seeps and vents, and methanotrophs mostly at methane-rich sites (Duperron et al., 2009). Most mussels associated with organic falls harbor thiotrophic symbionts, but some may have methanotrophs (identified on some *Idas modiolaeformis* specimens) or other symbiont types

such as Gammaproteobacteria G, and may not have thiotrophs (pers. obs.; Rodrigues et al., 2012a). Bacterial densities can be rather low, suggesting that additional nutritional sources are probably significant. Overall depth or habitat type does not seem to strongly influence the diversity of symbioses except in Mytilidae, in which the presence of methanotrophs is clearly linked with methane-enriched habitats, or by-products of wood degradation such as methanol.

Symbiont abundances on the other hand are clearly influenced by local characteristics of the habitat. This has been demonstrated in vent and seep Mytilidae, in which relative amounts of sulfur- versus methane-oxidizing bacteria reflect the availability of their respective substrates, and can also display age- or time-related variations (Le Bris and Duperron, 2010; Fiala-Médioni et al., 2002; Halary et al., 2008; Riou et al., 2008). Although they only have sulfur-oxidizing bacteria, Thyasiridae can also display inter-habitat variability in symbiont densities, with higher densities when sulfide is more abundant in the environment (Dufour and Felbeck, 2006). Dando and Spiro have shown using carbon stable isotopes ratios that the contribution chemoautotrophic bacteria could vary inter-annually in *Thyasira sarsi* and *T. equalis*, in relation to environmental change in the habitat (Dando and Spiro, 1993). In some specimens, carbon signatures of these symbiotic thyasirids were identical to those of non-symbiotic bivalves in which the diet was based upon phytoplankton-derived material. This was interpreted as a consequence of mixotrophy with animals that derive distinct fractions of their diet from symbionts and from heterotrophy.

3.2 Reproduction, development and dispersal strategies of hosts

Most documented symbiotic bivalves from the area under study are gonochoric, and a few of them are hermaphrodites alternating between male and female such as *Idas modiolaeformis*, a protandric hermaphrodite (Gaudron et al., 2012). Most of them seem to be broadcast spawners with no brooded-development detected so far. Oocytes can be large and rich in vitellus (Fig. 3c), indicative of lecithotrophic larval development and potentially enabling a significant larval dispersal under oligotrophic conditions in the

surrounding deep-sea (Beninger and Le Pennec, 1997). Alternatively, oocytes can be small and devoid of vitellus (Fig. 3d–e), indicative of planktotrophic larvae where the veliger larvae will actively feed upon dissolved organic matter, phytoplankton, or microorganisms (Arellano and Young, 2009). Planktotrophic larvae have historically been thought to spend an extended period in the plankton and disperse great distances, while lecithotrophic larvae live on limited reserves of vitellus which permit limited dispersal time only before metamorphosis and settlement (Thorson, 1950). This distinction might however be at least partially inaccurate in the cold deep-sea, as developmental and metabolic rates decrease with temperature, enabling longer dispersal time for lecithotrophic larvae prior to competency and settlement than previously thought (Le Pennec and Beninger, 2000; Tyler and Young, 1999). Much is believed to depend upon the seasonality of reproduction and whether larval dispersal takes place in cold deep waters or in warmer surface waters. Currently, however, such information is scarce for chemosymbiotic bivalves.

Vesicomyidae, Solemyidae and Lucinidae bivalves have large oocytes (from 100 µm up to 600 µm diameter) rich in yolk (Table 1, Fig. 3c). Most Vesicomyidae seem to be continuous spawners, but can also display periodicity such as for *Calyptogena pacifica* and *Calyptogena kilmeri* from bathyal depths in Monterey Bay (Lisin et al., 1997). The large oocyte size in several species indicates that the veliger larva is likely lecitotrophic but to date no data is available regarding their dispersal capacities (Lisin et al., 1997; Parra et al., 2009). The protobranch family Solemyidae has a pericalymna larva. In *Solemya velum* the embryo develops within a sticky and negatively buoyant gelatinous capsule on the seafloor and yields a juvenile stage resembling the adult and possessing gill symbionts (Gustafson and Lutz, 1992; Krueger et al., 1996b). This capsule, being adhesive, may disperse in the ocean if it has been initially attached to organic matter or sediment particles that may be resuspended and transported by water currents. The shallow-water *S. velum* is widespread in the North-West Atlantic, from Northern Florida to Nova Scotia, and larval dispersal conforms to that of a passive particle. Gelatinous egg capsules are also observed in the tropical Lucinidea from the Caribbean such as

Codakia orbicularis (Gros et al., 1997), and in the shallow-water Thyasiridae from the North Atlantic *Thyasira gouldi* (Blacknell and Ansell, 1974). Little data exists regarding dispersal capacities in both families. Mytilidae have planktotrophic larvae and the time spent in the water column has been inferred from the relative sizes of prodissoconch I and II (Fig. 3f). The granular prodissoconch I is produced from energy reserves of the oocytes, while the Prodissoconch II develops incrementally, using energy from food during the free-swimming larval form (Arellano and Young, 2009; Gaudron et al., 2012; Lutz et al., 1980; Ockelmann, 1965). Experimental evidence demonstrated that *Bathymodiolus childressi* from the Gulf of Mexico produced "teleplanic" larvae capable of spending one year in the water column before settlement, supporting long-distance larval dispersal capabilities (Arellano and Young, 2011). Besides, temperature does not limit the vertical migration of larvae up to 100 m or more above the cold seeps in the Gulf of Mexico.

Overall, investigating dispersal patterns of bivalve hosts is problematic. Early larvae and dispersal stages of chemosymbiotic bivalves are difficult to identify in the water column, despite various methodologies based on diagnostic PCR, or in situ hybridization of larvae using specific probes having been developed (Comtet et al., 2000; Le Goff-Vitry et al., 2007; Pradillon et al., 2007). Consequently, the distribution of larvae and their positioning in the water masses are not known. The factors triggering larval settlement to a new site are poorly understood, although several authors suggest that sulfide, or plume compounds may play a relevant role (Cowen and Sponaugle, 2009; Tyler and Young, 1999; Won et al., 2003). Theoretical approaches have attempted to evaluate dispersal potential, but with severe limitations (Martins et al., 2008). For example, a recent Lagrangian-based dispersal model implemented for the Gulf of Mexico mussel *Bathymodiolus childressi* indicated that despite long larval life, larvae released from relatively shallow seeps habitats of the species (100 and 500 m) tended to be retained in the Gulf of Mexico, limiting the potential for trans-Atlantic dispersal (Young et al., 2012). Scheltema (1971) in stark contrast demonstrated that several planktotrophic gastropods were capable of crossing and dispersing throughout the Atlantic

using diverse currents and that dispersion from Brazil to the Gulf of Guinea would take only 96 days (Scheltema, 1971). Dispersal potential also depends upon the depth at which larvae live and critically, whether or not they rise up the water column. Evaluating biological parameters needed to inform dispersal, including data concerning buoyancy, swimming behavior, duration of larval stage, age at competency, transport mode, or feeding strategy, is critical for building accurate predictions, thus emphasizing the need for more experimental work. The distribution of adults, although patchy and far from being fully documented, provides some clues but is not sufficient.

3.3 Acquisition of symbionts

Bacteria have been detected in the ovaries and follicle cells of Vesicomyidae, in ovaries, eggs, larvae and in juvenile stages of *Solemya reidi* (in the ciliated epithelial calymna surrounding the 3-day pericalymna larvae; inoculation by digestion of the calymna during metamorphosing of the larvae was proposed), whereby symbionts were subsequently transported to the developing gills (Gustafson and Reid, 1988; Stewart and Cavanaugh, 2006). As already discussed, maternal inheritance in Vesicomyidae has triggered genome reduction in symbionts, potentially limiting their ability to occur free-living. This has not yet been identified in *Solemya* symbionts (Kleiner et al., 2012). Environmental acquisition seems to imply the existence of free-living forms of the symbionts. Acquisition can start early in life, but the ability to acquire bacteria seems to be retained during adult life in certain bivalves. Indeed, symbiont-depleted Lucinidae were shown to reacquire symbionts exclusively via endocytosis throughout their life, and subsequently bacteria do not divide inside the gill (Brissac et al., 2009; Caro et al., 2007). In Mytilidae, many authors suggest that symbiont uptake occurs early in life (Salerno et al., 2005) but on the other hand, repeated observations of abundant endocytosis vacuoles engulfing bacteria in adult gill epithelial cells suggest that symbiont uptake may also occur in adults (Dubilier et al., 1998; Fiala-Médioni et al., 2002). Increase in symbiont numbers after a period of starvation also occurs through within-gill bacterial division (Kadar et al., 2005). Recent results indicate that maternal and environmental

acquisitions are not mutually exclusive. In Vesicomyidae, acquisition of non-parental symbiont strains, probably promoted by physical closeness, has been documented in several species (Stewart et al., 2008).

3.4 Connectivity

Several metazoan species or sister species were shown to occur on both sides of the Atlantic, suggesting the existence of preferential dispersal pathways across the Atlantic Equatorial Belt (Cordes et al., 2007; Olu et al., 2010; Olu-LeRoy et al., 2007b). In chemosymbiotic bivalves for which symbiosis is documented, at least three Mytilidae groups, namely the species complexes *Bathymodiolus childressi*/*B. mauritanicus* (Gulf of Mexico, Barbados prism, Nigerian Margin and Gulf of Cadiz), *B. boomerang*/*B. heckerae* (Gulf of Mexico, Barbados prism, Blake Ridge and Gulf of Guinea), and *Idas macdonaldi*/*I. modiolaeformis* (Gulf of Mexico, Gulf of Cadiz and Mediterranean) (Cordes et al., 2007; Van Dover et al., 2003; Génio et al., 2008; Lorion et al., 2012; Olu-LeRoy et al., 2007b). These examples provide important insights on the speciation and an evolutionary context for the present biogeography of chemosynthetic mytilids in the area of interest. Given the planktotrophic larval development and the estimated duration of larval stages of 1 yr in *B. childressi* and at least 5 months in *Idas modiolaeformis*, there is potential for larvae to cross the Atlantic. Barriers to dispersal probably exist, but for example the Strait of Gibraltar does not prevent *Idas modiolaeformis* from occurring on both the Atlantic and Mediterranean side, suggesting occurrence of larvae in upper seawater layers (shallowest depth: 300 m). A non-exclusive alternative would be that intermediate populations occur at sites situated between the extremes of the reported distribution. This hypothesis was raised upon discovery of the relationship between *Idas modiolaeformis* and *I. macdonaldi*, and confirmed by the discovery of *I. modiolaeformis* specimens in the Gulf of Cadiz, and in the Lacaze-Duthiers Canyon in the western Mediterranean (Rodrigues et al., 2012; Le Bris, N. personal communication, 2012). *Abyssogena southwardae*, occurring from the west coast of Florida, the Barbados prism, along the MAR from 5° S to 14° N, to near the Canary islands, could

be example of a Vesicomyidae with amphi-Atlantic distribution, but molecular data is not available from all locations preventing rigorous testing of the hypothesis (van der Heijden et al., 2012; Krylova et al., 2010). *A. southwardae* probably has lecitotrophic larvae with a limited dispersal potential, but locations are separated by relatively short distances which may remain within the milits of larval dispersal capacity, particularly considering lecitotrophy the aforementioned advantage of lecitotrophy in oligotrophic deep-sea waters (Tyler and Young, 1999). Volcanic seamounts (Henry Seamount off Canary Islands), transform faults or other sulfide-enriched habitats such as organic remains may act as ecological and/or evolutionary stepping stones between the known seeps and vent localities. *Thyasira flexuosa* is found from Florida to the North Sea and in Mediterranean, suggesting it is not only an amphi-Atlantic species, but also capable of crossing the Strait of Gibraltar. Similarly, lecithotrophic larvae of the deep-sea lucinid *Lucinoma kazani* from the eastern Mediterranean seem to be able to cross the strait of Dardanelles, since the species has been recovered in cold seeps in the Marmara Sea, as has the small deep-sea vesicomyid *Isorropodon perplexum* (Brissac et al., 2011; Ritt et al., 2010; Rodrigues et al., 2012). Regarding their symbionts, a few clades show similar trans-Atlantic distributions (Fig. 2). Both sulfur- and methane-oxidizing symbionts of the amphi-Atlantic Mytilidae clade *Bathymodiolus heckerae* (GoM)/*B*. aff. *boomerang* (GoG) are closest relatives. The methanotroph associated with *B. mauritanicus* (GoC) is not closely-related to that of its neighbouring species *B. childressi* (GoM), but is related to bacteria associated with *Bathymodiolus* from southern Mid-Atlantic Ridge vent sites 5° S and 9° S. Methanotrophs of *B. brooksi* (GoM) and *Idas modiolaeformis* (Med) are also closest relatives. In Vesicomyidae symbionts, the only example is that of the *Elenaconcha guiness* (GoG) symbiont which is closely related to those of *Vesicomya cordata* and *Calyptogena ponderosa* (GoM), though with low support (Fig. 2). In Solemyidae, symbionts of *Solemya velum* (WA) are closely related to symbionts of *Solemya (Petrasma) elarraichensis* (GoC). No such relationships occur among reported Thyasiridae and Lucinidae symbionts. Apart from these examples, other 16S rRNA symbiont phylotypes are mixed with no self-evident trend.

Symbiont-containing clades include a mixture of phylotypes associated with hosts from various locations. This might be due to the limited amount of variation of the 16S rRNA gene, and maybe faster evolving genes could provide better estimates of symbiont biogeographical trends, as shown in Solemyidae from the coast of New England (Stewart et al., 2009a). However at this stage, and unlike the observed in some host bivalves, there is no apparent trend towards closer phylogenetic relationships between symbionts from the western and eastern Atlantic. Apart from the few examples listed above, the hypothesis of a preferential dispersal or diversification route along the Atlantic Equatorial Belt does not appear to be relevant to bacterial symbionts of bivalves.

Published symbiont phylogenies illustrate the close relatedness between the different clades of symbionts and various free-living bacteria (Fig. 2, Petersen et al., 2012). Symbiosis with bivalve hosts has appeared independently in several clades of Gammaproteobacteria, generally consisting of sulfur-oxidizing bacteria. In most cases, symbionts derive from free-living bacteria, but in a few instances symbionts seem to have passed from one host family to another, as seen for the symbiont of *Thyasira vulcolutre* which seems to have been derived from Vesicomyidae-associated bacteria (Rodrigues and Duperron, 2011). Host shifts might also have occurred in the group that includes symbionts from Solemyidae, Lucinidae and Thyasiridae, and possibly from Mytilidae to Vesicomyidae (Fig. 2). Based on the few genomes available, symbionts tend to share highly similar pathways for sulfur-oxidation, and a slightly altered Calvin-Benson-Bassham cycle for autotrophy using either Type I or Type II RubisCO (Kleiner et al., 2012). These pathways are less diverse than documented in available genomes from related free-living bacteria. Furthermore, phylogenies obtained for functional genes (for example encoding APS reductase, RubisCO, particulate methane mono-oxygenase) usually do not superimpose well with those obtained based on the classical marker 16S rRNA. This suggests that pathways convergence among symbionts result from lateral gene transfer of pathways rather than common ascendance. In fact recombination is documented among closely related symbionts (Stewart et al., 2009b). Loss of genes is a consequence of genome reduction, documented in maternally-inherited

Vesicomyidae symbionts, as well as in environmentally acquired mussel-associated sulfur-oxidizers (Kleiner et al., 2012). Free-living forms of thiotrophic symbionts are properly documented in Lucinidae in coastal seagrass beds, and strongly suspected in Mytilidae based on data from hydrothermal vent mats and mats attached to the back of annelids, although their abundance, metabolism, and exact ecological niche are not known (Crépeau et al., 2011; Gros et al., 2003b; Petersen et al., 2012). Overall, it seems that apart from a few cases (maternally-inherited Vesicomyidae symbionts), symbionts evolve quite freely from their hosts. Many symbionts have or probably had free-living forms, meanwhile hosts require their bacteria. This asymmetry between hosts and symbionts must be better acknowledged in evolutionary studies, and a better understanding of symbiont evolution will require addressing these free-living forms.

4 Conclusions

Chemosymbiotic bivalves are diverse. Four of the five well-documented families are associated with sulfur-oxidizing Gammaproteobacteria, meanwhile members of the family Mytilidae are characterized by a broader diversity of associated bacteria. Approximately 200 sepecies belonging to these families (considering only subfamily Bathymodiolinae for Mytilidae) are documented in the North Atlantic, Gulf of Mexico and Mediterranean, and data regarding symbiosis is available for 26.5 % of these species. Despite many similarities, important differences exist between bivalve families with regards to host behavior, reproductive biology and larval dispersal capabilities. Important differences also exist among their symbioses with regards to the mode of acquisition, the establishment and the functioning of their bacterial partners. Altogether, the level of host symbiont specificity and inter-dependency is variable among bivalve hosts. Different families thus illustrate different patterns of host symbiont association. It is therefore crucial for future to consider the particular question of asymmetry in the relationship between host and symbionts. Indeed, in many cases the symbiosis is obligatory for one partner but not for the other, as seen in Mytilidae or Lucinidae which apparently

need their symbionts, whereas bacteria can also live outside their host. This certainly has far-reaching consequences for the ecology, biogeography, and evolution of these symbiotic systems.

Most of today's knowledge has been obtained on a very limited number of "model species" presented as paradigms for the characteristics of their respective families. This approach had yielded detailed knowledge of these systems. However, recent results emphasize significant within-family variability in symbiotic interactions, which may have been overlooked in the past. In this synthesis, it appears that the more diverse families are those for which the lower proportion of species has been investigated: 19.0 % and 15.2 % of Lucinidae and Thyasiridae (84 and 59 species, respectively) against 39.3, 54.5 and 61.1 % of Vesicomyidae, Solemyidae and Mytilidae (clade Bathymodiolinae). Even focusing only on the northern Atlantic, Gulf of Mexico and Mediterranean, much more can be learnt from within- and among-families comparison in terms of common trends and peculiarities. Aside from the necessary in-depth studies carried out on selected representative species, family-scale comparative studies appear equally important to develop an integrated view of bivalve symbioses encompassing the true diversity of species and associations within each family. This broader perspective is intrinsic to concerns about marine biodiversity and conservation, which have triggered considerable effort through programs such as the Census of Marine Life (German et al., 2011). At a time when many human activities including pollution, overfishing, and offshore gas, petroleum, and minerals exploitation threaten the world's oceans, a better understanding of bivalve symbioses is needed to evaluate the capacities of these remarkable ecological and evolutionary units to withstand environmental change.

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Table 1. Summary of chemosymbiotic bivalve species for which data is available regarding symbiosis in the Atlantic, Gulf of Mexico, and Mediterranean Sea. Numbers in parenthesis indicate the number of species for which symbiosis is documented using electron microscopy (species names are in italic) and molecular data, possible including in situ hybridization of symbionts (grey overlay). Synonymized taxa, according to the World Register of Marine Species (WORMS, www.marinespecies.org), are included as many former names were in use when symbioses were initially documented, and thus appear in the tree (Fig. 2). Symbiont (Sym.) types: (S)ulfide-oxidizer, (M)ethane-oxidizer, (My) methylotrophs, (B)acteroidetes, and (G) for Gammaproteobacteria unrelated to other documented symbiont groups. Location (Loc.) corresponds to (N)orth, (S)outh, (E)ast, (W)est (A)tlantic, (Med)iterranean. Type (Ty.) of chemosynthesis-based ecosystem is mentioned for deep-sea species, (CS) cold seeps, (HV) hydrothermal vents, and (OF) organic falls. A non-exhaustive list of sampling sites is provided for vent and seep species (vent and seep sites illustrated in Fig. 1). Reproduction (Rep.), when documented is either (G)onochoric or (H)ermaphrodite (-p then means protandric), and can be seasonal (-s). For references, see text.

Accepted species name	Synonymised taxa	Sym.	Loc.	Ty.	Sampling sites (vent/seep species)	Rep.
SOLEMYIDAE (6/11)						
Acharax sp.		S	EA	CS	Gulf of Guinea (Guiness site)	
Acharax gadirae		S	WA	CS	Gulf of Cadiz	
Solemya elarraichensis		S	NEA	CS	Gulf of Cadiz	
Solemya velum		S	NWA			G-s
Solemya borealis		S	WA			
Solemya occidentalis		S	WA			
LUCINIDAE (16/84)						
Anodontia (Loripinus) fragilis		S	EA, Med			
Anodontia alba		S	WA			
Codakia orbicularis		S	NWA			G-s
Ctena orbiculata	*Codakia orbiculata*	S	WA			G
Divalinga quadrisulcata	*Divaricella quadrisulcata*	S	WA			
Loripes lucinalis	*Loripes lacteus*	S	A			G-s
Lucina pensylvanica	*Linga pensylvanica*	S	WA			G
Lucinisca nassula	*Lucina nassula*	S	WA			
Lucinoma asapheus		S	NEA	CS	Gulf of Cadiz	
Lucinoma borealis		S	NEA			
Lucinoma kazani		S	Med	CS	Mediterranean, Marmara Sea	
Myrtea spinifera		S	NEA, Med	CS	Mediterranean, Marmara Sea	
Parvilucina costata	*Codakia costata*	S	WA			
Parvilucina pectinella	*Codakia pectinella*	S	WA			
Phacoides pectinata	*Lucina pectinata*	S	WA			H-p-s
Stewartia floridana	*Lucina floridana*	S	WA			

Table 1. Continued.

Accepted species name	Synonymised taxa	Sym.	Loc.	Ty.	Sampling sites (vent/seep species)	Rep.
THYASIRIDAE (9/59)						
Axinulus croulinensis		S	EA; WA			G
Thyasira equalis	*Parathyasira equalis*	S	EA; WA			G
Thyasira flexuosa		S	EA; WA, Med			
Thyasira gouldi		S	EA;WA			
Thyasira sarsi		S	EA; WA			
Thyasira trisinuata		S	WA			G
Thyasira vulcolutre		S	NEA	CS	Gulf of Cadiz	
Thyasira n. sp. Regab		S	EA	CS	Gulf of Guinea (Regab site)	
Thyasira sp. Guiness		S	EA	CS	Gulf of Guinea (Guiness site)	G
MYTILIDAE, clade Bathymodiolinae (11/18)						
Bathymodiolus azoricus		M, S	A	HV	Mid Atlantic Ridge	G-s
Bathymodiolus boomerang		M, S	A	CS	Barbados, Gulf of Guinea	
Bathymodiolus brooksi		M, S	WA	CS	Gulf of Mexico	
Bathymodiolus childressi		M	WA	CS	Gulf of Mexico	G-s
Bathymodiolus heckerae		M, 2S, My	WA	CS	Gulf of Mexico	
Bathymodiolus mauritanicus		M, S	NEA	CS	Gulf of Cadiz. Mauritania	G-s
Bathymodiolus puteoserpentis		M, S	A	HV	Mid Atlantic Ridge	G-s
Idas macdonaldi		S	WA	CS, OF	Gulf of Mexico	
Idas modiolaeformis		M, 2S, My, G, B	NEA	CS, OF	Mediterranean, Gulf of Cadiz	H-p
Idas simpsoni		S	NEA	OF		
Idas sp. Marmara		S	Mar	CS	Marmara Sea	
VESICOMYIDAE (11/28)						
Abyssogena southwardae		S	A	HV, CS	Florida, Mid-Atlantic Ridge, Canary Islands	
Calyptogena valdiviae	*Vesicomya valdiviae; V. longa*	S	EA	CS	Gulf of Guinea	G
Christineconcha regab	*Calyptogena regab*	S	EA	CS	Gulf of Guinea	
Elenaconcha guiness		S	EA	CS	Gulf of Guinea	
Isorropodon bigoti		S	EA	CS	Gulf of Guinea	G
Isorropodon megadesmus		S	NEA	CS	Gulf of Cadiz	
Isorropodon perplexum		S	Med	CS	Mediterranean, Marmara Sea	
Laubiericoncha chuni	*Callogonia chuni*	S	EA	CS	Gulf of Guinea	
Laubiericoncha myriamae		S	NA	CS	Barbados	
Pliocardia cordata	*Vesicomya cordata*	S	WA	CS	Gulf of Mexico	
Pliocardia ponderosa	*Calyptogena ponderosa*	S	WA	CS	Gulf of Mexico; Colombia	

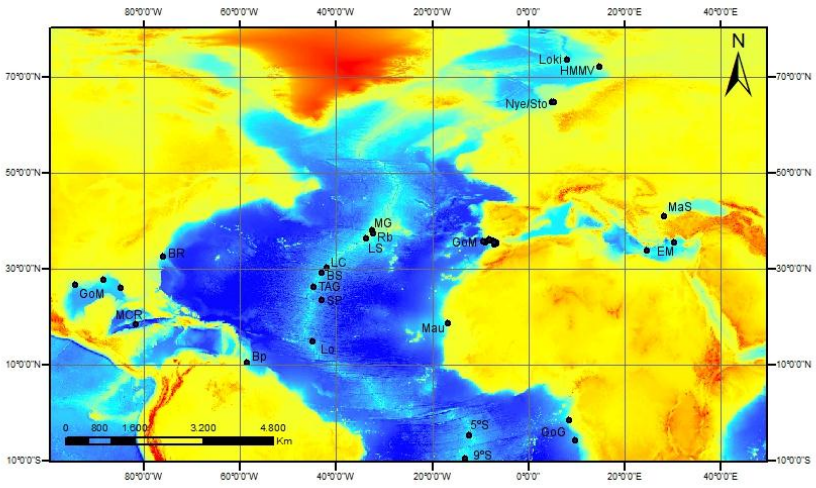


Fig. 1. Map displaying the deep-sea hydrothermal vent and cold seep sites from which chemosymbiotic bivalves were sampled in the north Atlantic and Mediterranean. West to east, labels correspond to Gulf of Mexico (GoM), Mid-Cayman Ridge (MCR), Blake Ridge (BR), Barbados Prism (Bp), Trans-Atlantic Geotraverse (TAG), Logatchev (Lo), Broken Spur (BS), Lost City (LC), Snake Pit (SP), Lucky Strike (LS), Rainbow(Rb), Menez Gwen (MG)Mauritania (Mau), 9° S and 5° S, Gulf of Cadiz (GoC), Nyegga/Storegga (Nye/Sto), Loki's Castle (Loki), Gulf of Guinea (GoG), Haakon Mosby (HMMV), East Mediterranean (EM) and Marmara Sea (MaS).

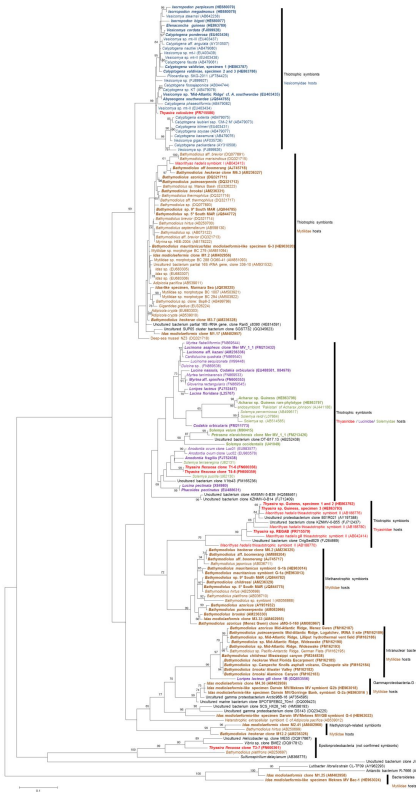


Fig. 2. (Caption on next page.)

Fig. 2. Phylogeny of the Gammaproteobacteria associated with bivalve hosts based on analysis of 16S rRNA-encoding genes. Epsilonproteobacteria presented include two sequences obtained from animal tissues, but not confirmed as actual symbionts, contrary to the Mytilidae-associated Bacteroidetes. Colors relate to the family to which the host belongs, sequences in bold are from species present in the area of focus for the review. For clarity, only host species name is mentioned without mention of "symbiont". Properly identified bivalve-associated 16S rRNA sequences above 1200 bp were used, short sequences available for *Bathymodiolus tangaroa*, *Acharax gadirae* and *Idas macdonaldi* were not included. Tree was reconstructed using a Maximum Likelihood algorithm from 1129 nucleotide positions. Based on Bayesian Information Criterion, a Tamura 3-parameters with Gamma-distribution of rates (T92+G) was employed. Node support values (> 60 % shown) are based on 500 bootstrap replicates using the same methodology. Analyses were run using MEGA 5.0 (Tamura et al., 2011). Although some names have recently taken on new synonyms (see Table 1), we used names included in the original GENBANK records.

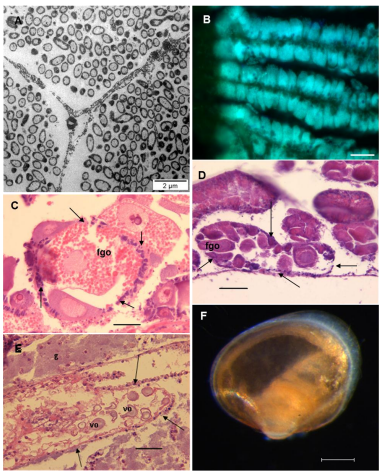


Fig. 3. (a) Detail of sulfur-oxidizing symbionts associated with gill filaments of *Thyasira* sp. Regab (Thyasiridae) under the TEM. **(b)** Fluorescence in situ hybridization using a symbiont-specific probe on a cross-section through 3 gill filaments of *Myrtea spinifera* (Lucinidae) showing the dense populations of bacteria in the lateral zone. Scale bar = 50 µm. **(c–e)** Histological sections of female gonads stained with hematoxylin-eosin. Scales bars are 50 µm. **(c)** Detail of an acinus (arrows delimiting) encompassing a large lecithotrophic oocyte (fgo: fully grown oocytes of ~ 150 µm diameter) in *Isorropodon bigoti* (Vesicomyidae). **(d)** Detail of an acinus (arrows delimiting) encompassing smaller fully grown oocytes (fgo; ~ 60 µm diameter) in *Idas modiolaeformis* (Mytilidae) that has a planktotrophic larvae. **(e)** Detail of a gonad (arrows delimiting) encompassing small vitellogenic oocytes (vo) ~ 20 to 30 µm diameter in *Thyasira* sp. nov. from the Guiness site (Thyasiridae) that probably has a planktotrophic larvae (g for gills). **(f)** Early juvenile of *Idas modiolaeformis*, with prodissoconch II visible in orange and the surrounding young dissoconch in white. Notice the low number of gill filaments visible by transparency. Scale bar = 100 µm.