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Gill-symbiosis in mytilidae associated with wood fall environments

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Abstract Bivalves belonging to the genera *Idas* and *Adipicola* were collected from wood fall environments in the west Pacific (Vanuatu islands) between 300 and 890 m depths in 2004. Bacterial symbionts were checked by three complementary techniques: histological and DAPI staining, in situ hybridization (FISH), and TEM. No bacteria were detected inside the gills of the two species, rejecting the endosymbiosis hypothesis. However, results from our study demonstrated the existence of ectosymbionts colonizing microvilli differentiated at the apical surface of the cells constituting the lateral zone of gill filaments. These ectosymbionts are γ-Proteobacteria due to their strong hybridization with the specific probe GAM42; in contrast no hybridization was obtained from either gills or other host tissues by using the oligonucleotide probes specific to α- β- and δ-Proteobacteria. Based on TEM observations, these Gram-negative bacterial symbionts are not methanotrophic due to the lack of concentric stacking of intracellular membranes in their cytoplasm. Such ectosymbionts may represent thioautotrophic bacteria as already described in various Mytilidae from hydrothermal vents and cold seeps. Unfortunately, no phylogenetic analysis could be done in this study to compare their DNA sequence to that of other marine invertebrate symbionts described to date.

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Introduction

Interactions between prokaryotic symbionts and marine invertebrate hosts have been described since the early 1980s (Felbeck et al. 1981; Cavanaugh et al. 1981). They were then reported to occur in various environments from shallow water to hydrothermal vents and from various taxa including annelids, bivalves, etc. Gill-endosymbiosis in Mytilidae was first described in *Bathymodiolus thermophilus* [Kenk and Wilson, 1985] in hydrothermal vents (Fiala-Médioni et al. 1986). Then, small symbiotic mussels belonging to the genus *Idasolas* were reported to be associated with whale bones (Deming et al. 1997). Thus, symbiont-containing mytilids are commonly found at hydrothermal vents and cold seep environments throughout the world (for review, see Duperron et al. 2005). Distel et al. (2000) have suggested, based on phylogenetic analyses on various Mytilidae, that decomposing organic substrates such as wood and bone may serve as steps for the introduction of mytilids into vent and seep environments. Moreover, they suppose that Mytilidae found in such environments may harbor chemoautotrophic bacterial symbionts.

The aim of this study was to look for bacterial symbionts in mussel specimens collected from wood fall environments, to test the hypothesis of chemoautotrophy initially proposed by Distel et al. (2000). Histological analyses were performed to obtain a general view of the mytilid tissues, and to allow an overall in situ detection of bacteria in whole animal sections by DAPI staining. Complementary analyses focused on the gills, organs known to harbor symbionts

in bivalves (Reid 1990), by using fluorescent in situ hybridization and TEM analyses.

Materials and methods

Sampling of the animals

Bivalves were collected during the BOA0 cruise in November 2004, using a beam trawl between depths of 300–890 m from wood fall environments in the Vanuatu area. Samples were collected between 15°41.78–15°44.05S latitude and 167°03.83–167°01.83W longitude attached to the exterior of small pieces of submerged wood. Samples were processed on the ship within 1 h after collection. Based on shell characters only, the three individuals examined in this study belong to two genera, *Idas* and *Adipicola*. As these two species are undescribed species (Von Cosel, personal communication), we gave them the *nomen nudum* name of *Idas woodia* and *Adipicola sunkenia*. The single individual of *A. sunkenia* was fixed specifically for histology, whereas complementary analyses were performed on *I. woodia*.

Histological techniques

An overall view and histological information were obtained from paraffin sections. After breaking the shell, one single whole individual of *Idas* and *Adipicola* were fixed in Bouin's fluid (Gabe 1968) for 24 h at room temperature before being embedded in paraplast. Sections (7 μm thick) were stained by Goldner's trichrome for morphological information, according to Gabe (1968).

Fluorescent in situ hybridization experiments

One single whole individual of *I. woodia* was fixed for 1–3 h at 4°C in 4% paraformaldehyde in 1× PBS buffer. The specimen was then washed three times for 10 min each at 4°C in 1× PBS, then stored in 0.5× PBS/50% ethanol at 4°C until it was embedded in paraplast. Four micrometer-thick sections were placed on precoated slides from Sigma before hybridization. Five oligonucleotide probes were used: Eub338 (5′-GCTGCCTCCCGTAGGAGT-3′), targeting most members of the eubacteria (Amann et al. 1990a, b), and more specific probes such as ALF968 for α-proteobacteria (5′-GGTAAGGTTCTGCGCGTT-3′), GAM42 for γ-bacteria (5′-GCCTTCCCACATCGTTT-3′), BET42a for β-proteobacteria (5′-GCCTTCCCACTTCGTTT-3′), and NON338 (5′-ACTCCTACGGGAGGCAGC-3′) as a negative control. Hybridization experiments were similar to those previously described by Dubilier et al. (1995, 1999). To visualize total symbionts as a control, gill sections, after FISH hybridization, were counter-stained with DAPI (1 μg/ml).

Electron microscopy preparations

One single whole individual of *I. woodia* was prefixed on ship for 1 h at 4°C in 2.5% glutaraldehyde in 0.1 M pH 7.2 caccodylate buffer, adjusted to 900 mOsM with NaCl and $CaCl_2$ in order to improve membrane preservation. Following a brief rinse, it was stocked in the same buffer at 4°C until it was brought to the laboratory where gills were dissected. One was fixed for 45 min at room temperature in 1% osmium tetroxide in the same buffer, then rinsed in distilled water and post-fixed with 2% aqueous uranyl acetate for one more hour before embedding and observation as described previously (Gros et al. 2003). The other gill was dehydrated in an ascending series of acetone dilutions and critical point dried using CO_2 as transitional fluid for the SEM procedure. Samples were then sputter coated with gold before observation in a Hitachi S-2500 SEM at 20 kV.

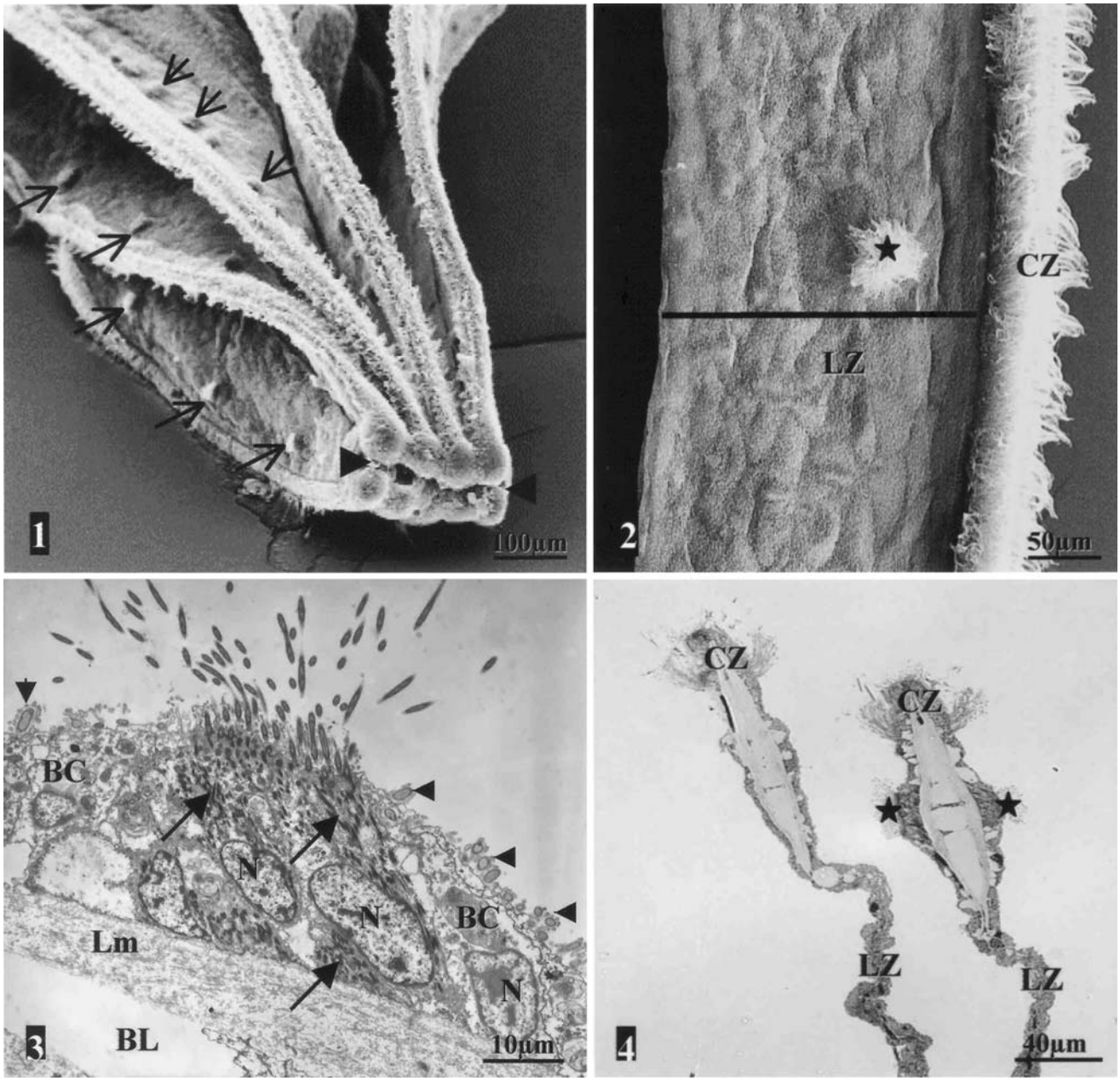
Results

Gill morphology

Both genera analyzed in this study possesed filibranch gills which are homorhabdic and non plicate. Based on histological sections, both individuals present gills characterized by two demi-branchs, each of which consists of an ascending and a descending lamellae. Upon dissection, the gill filaments of *I. woodia* individual were tangled and disjoined, indicating a low degree of inter-filamentar cohesion. SEM observations confirmed that first impression by revealing that each demibranch presents widely spaced filaments (Fig. 1). The gill filaments are weakly united by ciliary discs that are arranged at regular interval (Figs. 1, 2). Each ciliary disc consists of tufts of long cilia (Figs. 2, 3, 4). TEM views show that these cilia possess a ciliary axonemal structure with long ciliary roots (Fig. 3) reaching the basal pole of the cell. Moreover, cilia from one disc can reach across and link with those of the opposing disc as described by Morse and Zardus (1997). Each filament is composed of a ciliated zone (~40 μm) and a longer lateral zone containing the ciliary discs (Fig. 4). Gills from *Adipicola sunkenia* have a greater degree of cohesion between the filaments. Gill filaments were characterized by a long lateral zone while remaining thin (Figs. 4, 7) compared to the ciliated zone.

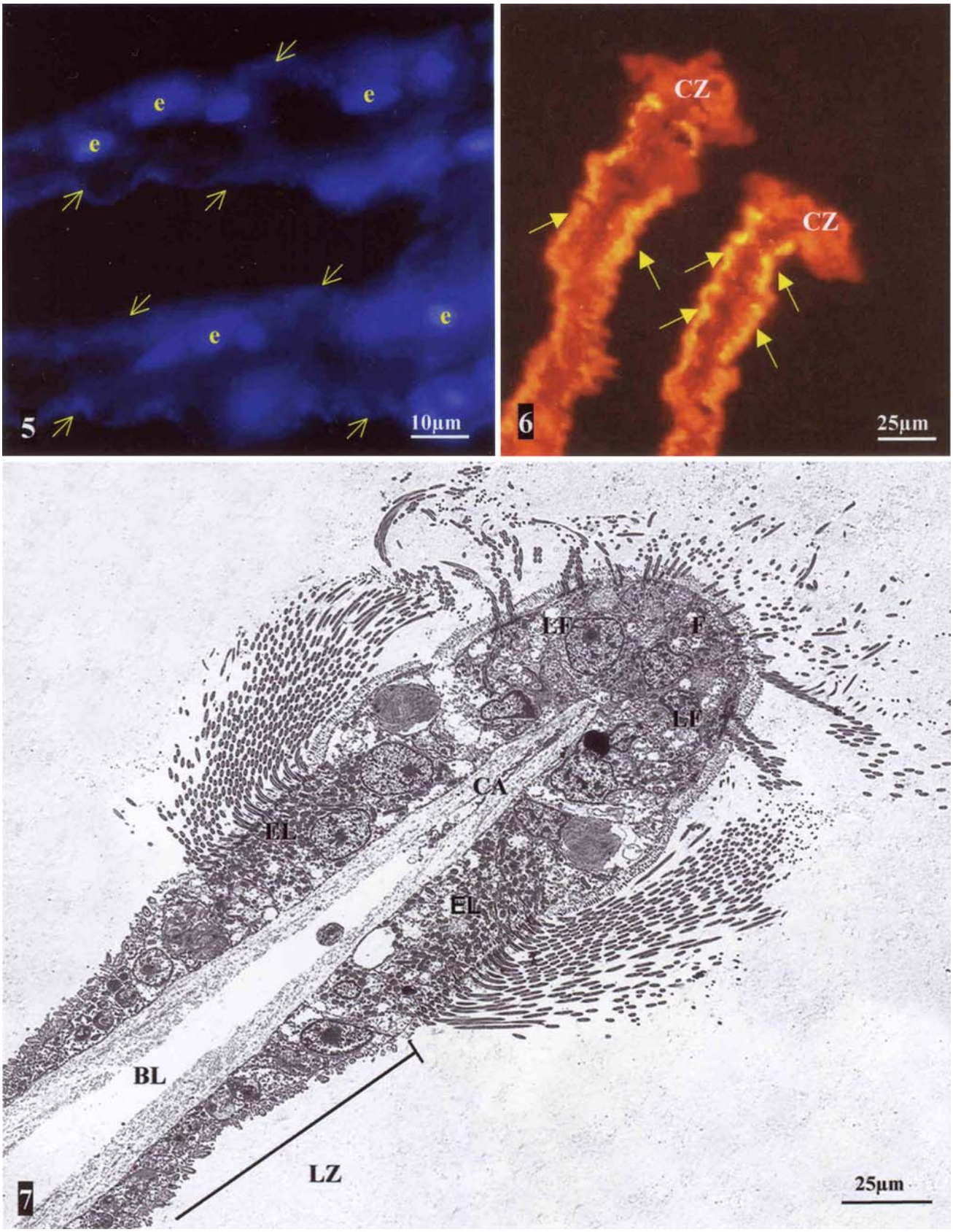
In situ hybridization

Following DAPI stainning, the lateral zone of *I. woodia* is characterized by numerous blue dots (~1.5 μm) smaller



Figs. 1–4 General view of the gill morphology. **Fig. 1.** SEM. General view of the gill of *Idas woodia*. Gill filaments are joined at the ventral edge of the homorhabdic gill, forming a faint marginal groove (delimited by *arrowheads*). Some tufts of cilia (*arrows*), regularly distributed through the gill, are seen on the lateral face of each gill filament. **Fig. 2.** SEM. Higher magnification of one gill filament of *Idas woodia*. Frontal cells of the ciliated zone (*CZ*) are characterized by long cilia used for filtering of particulate matter while the lateral zone (*LZ*) appears smooth excepted the tufts of cilia which represent ciliary disc (*star*). These structures, which are involved in the attachment process of gill filament in filibranchs are located in the frontal most third of the lateral zone, underneath the ciliated zone. **Fig. 3.** TEM view of a ciliary disc showing the ciliary axonemal structure characterized by long roots (*arrows*) reaching the basal pole of the cell. Bacteria (*arrow heads*) from the apical end of adjacent bacteriocytes (*BC*) closely surround the tuft of cilia, living no free apical cell surface from the lateral zone in direct contact with seawater. *Lm* basal lamina; *N* nucleus. **Fig. 4.** Light micrograph of a semi-thin transverse section of the outer demibranch. Gill filaments are linked by ciliary discs (*star*) located below the ciliated zone (*CZ*), which consists of a single layer of epithelial cells organized around a collagen axis. The lateral zone (*LZ*) appears heavily stained by toluidin blue compared to the ciliated zone

than host cell nuclei which measure approximately 5 µm (Fig. 5). These dots are present all along the lateral zone of each gill filament, and are located mostly at the periphery of the cells. The single specimen of *A. sunkenia* observed also presents such a pattern after DAPI staining (not shown). These results suggest that bacteria are associated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◀ **Figs. 5–7** **Fig. 5**. Gill filaments of *Idas woodia* and in situ hybridization of the bacterial symbionts DAPI staining of the lateral zone of a gill filament of *I. woodia*. The eukaryotic nuclei (*e*) are stained along with a thin fluorescent layer (*arrows*), located mostly at the periphery of the lateral zone. The *small dots* composing this layer were present all along the lateral zone of each gill filament. **Fig. 6**. Gill filaments of *Idas woodia* and in situ hybridization of the bacterial symbionts Gill filament of *I. woodia* after a positive hybridization with the universal probe EUB338. The ciliated zone (*CZ*) which is devoid of bacteria represents an internal negative control. Positive hybridization appears in *yellow* at the periphery of the lateral zone (*arrows*) while negative hybridization appears in *orange*. **Fig. 7**. Gill filaments of *Idas woodia* and in situ hybridization of the bacterial symbionts TEM view of the ciliated and lateral zones. Frontal (*F*), laterofrontal (*LF*) and eulateral (*EL*) ciliated cells are the main cell types of the ciliated zone which is devoid of bacteria. These cells are organized as an epithelium along a collagen axis (*CA*). The ciliated zone is directly in contact with cells from the lateral zone (*LZ*), which contain bacteria (*arrows*) on their apical surface. *BL* blood lacuna

with gill tissue in both species. To confirm this, in situ hybridizations were attempted with different probes. Positive hybridizations were obtained with the probes EUB338 and GAM42 in each sample analyzed (Fig. 6) indicating the presence of γ-Proteobacteria in gill cells all along the lateral zone of each gill filament. These bacteria could be located inside or outside the host cells as it is difficult to conclude only from FISH hybridization pictures (Fig. 6). No hybridization was obtained with either other host tissues or with the oligonucleotide probes against α- β- and δ-Proteobacteria. Similar results were obtained with the unique sample of *Adipicola* specimen analyzed which harbored also γ-Proteobacteria at the periphery of gill-cells all along the lateral zone of each gill filament (not shown).

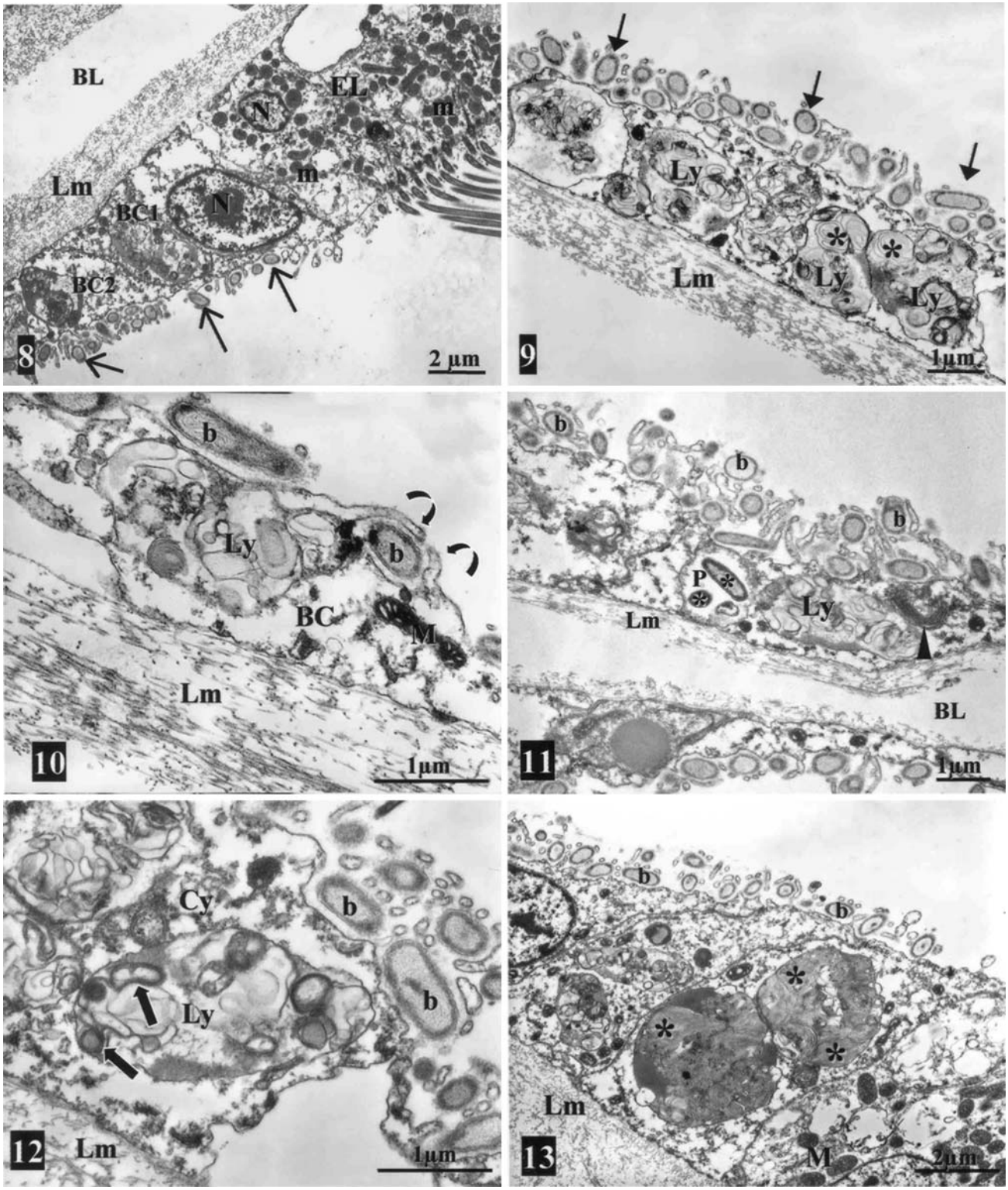
Gill filament ultrastructure

The ciliated zone looks similar to that of previously described symbiont-bearing bivalves (Fig. 7) and appears devoid of bacteria. The ciliated zone is short and contains the different cell types described by Owen and McCrae (1976). Frontal cells bear short cilia with no discernible orientation. Narrow prolaterofrontal cells bear two rows of long orientated cilia. Prolateral cells are devoid of cilia and have long, regular microvilli. The eulateral cells bear rows of long independent cilia (Fig. 7). There is no obvious intermediary zone as the first cell in contact with the last eulateral cell contains bacteria on its surface (Figs. 7, 8).

The lateral zone forms the main part of the filament and accounts for the thickness of the gill. This lateral zone appears quite simple because of it is composed of only three cell types (Figs. 8, 9). The most prevalent one consists of cells containing bacteria at their apical surface. These bacteria are interspersed between microvilli differentiated by the host cells (Figs. 8, 9). Such a proximity, associated with the bacterial density, lead us to define them as bacteriocytes, as are named cells harboring endocellular bacteria in other bivalve families (Fisher 1990). In the two specimens analyzed here, the bacteriocytes, which have a basally-located nucleus are much wider than high, and possess long microvilli, among which bacteria are located (Figs. 8, 9). The bacteriocytes organelles consist of few mitochondria and numerous lysosome-like structures characterized by whorls of membranes as large as the nucleus (Figs. 9, 12). These large secondary lysosomes contain putative remains of bacteria (Figs. 9, 12) suggesting a possible digestion of bacterial ectosymbionts by the invertebrate host. Host cells, using plasmic membrane expansions, can enclose bacteria in phagocytic vacuoles at their apical pole (Fig. 10). Thus, the aspect of the lysosome-like structures depends on their maturity from early endosomes just after the phagocytosis of extracellular bacteria (Fig. 11) to classical secondary lysosomes (Fig. 12) and even residual bodies (Fig. 13).

Bacterial symbionts are usually small and rod shaped (1 µm length, 0.3 µm high), with the typical double-membrane of Gram-negative bacteria (Figs. 14, 15, 16). The ovoid-shaped figures are probably due to transverse sections as there are no spherical bacteria observed with SEM (Fig. 17). Bacteria are always located between the long microvilli of the bacteriocytes in direct contact with seawater (Figs. 7, 8, 9). As evidenced by TEM observations, these symbionts are not methanotrophic bacteria due to the fact that their cytoplasm lacks the concentric stacks of intracellular membranes typical of methanotrophs (Figs. 14, 15, 16). The bacterial cytoplasm contains essentially DNA and ribosomes (Figs. 14, 15, 16) but electron-dense, non-membrane bound granules, located in the periplasmic space, can also be observed (Fig. 15).

There are no intercalary cells interspersed between bacteriocytes, as usually described in bivalves known to harbor bacteria (Figs. 7, 8, 9). Two secretory cell types were observed through the lateral zone of the gill filament of the *Idas* sample examined in this study. The first one has a cytoplasm filled with large, membrane bound, osmiophilic inclusions which are PAS positive on histological sections (not shown). Only a few of these granule cells were observed in the lateral zone of each gill filament even if they may be present throughout the length of the gills. The second secretory cell type consists of mucocytes, which are scarce in the lateral zone of the gill filaments. Most of the mucocytes observed are those which are located at the frontal crest of the filaments (not shown) inside the ciliated zone. Both cell types secrete acid mucopolysaccharides (stained in blue with alcian blue) and the mucous contents seem to be present along the surface of each gill filament, indicating that particle capture may occur in this species. Another possibility is that such mucous presence could help to protect gill cells against toxins in the environment.



Discussion

Wood falls represent a massive input of food into a marine environment at depths where food is typically scarce due to the absence of sunlight. Wood debris can provide a huge amount of organic matter, drifting down from surface waters before being transported by submarine currents through various canyons at greater depths. Such organic matter could be used by microbial mats or by eukaryotic macrofauna directly (i.e. xylophagous organisms) or indirectly

◀ **Figs. 8–13** TEM. Bacteriocytes and intracellular digestion of the extracellular symbionts in *Idas woodia*. **Fig. 8.** Bacteriocytes with numerous bacterial ectosymbionts located between microvilli (*arrows*). The first bacteriocytes of the lateral zone (*BC1*, *BC2*) are in contact with the last eulateral cell (*EL*). The various symbiont shapes observed (rod-shaped or ovoid-shaped figures) could be due to the section orientation. *BL* blood lacuna; *Lm* basal lamina; *m* mitochondria; *N* nucleus. **Fig. 9.** Bacteriocyte cytoplasm is filled with secondary lysosomes (*Ly*), characterized by their heterogeneous aspect and whorls of membranes (*asterisks*). Extracellular symbionts (*arrows*) are located on the apical surface of the bacteriocytes in contact with microvilli. *Lm* basal lamina. **Fig. 10.** Some plasmic membrane expansions (*curved arrows*) enclosing bacterial ectosymbionts (*b*) in phagocytic vacuoles at the apical pole of the bacteriocyte (*BC*). *Lm* basal lamina; *Ly* lysosome-like structure; *M* mitochondria. **Fig. 11.** Bacteria envacuolated (*asterisks*) inside a phagosom (*P*) prior to addition of lysosomal enzymes brought by primary lysosomes produced by the Golgi apparatus (*arrow head*). *b* bacterial ectosymbionts; *BL* blood lacuna; *Ly* secondary lysosome. **Fig. 12.** Higher magnification of a complex lysosomal structure (*Ly*) showing partly destroyed-bacteria (*arrows*) inside the bacteriocyte cytoplasm (*CY*). Ectosymbionts (*b*) are located between the microvilli of the bacteriocyte. *Lm* basal lamina. **Fig. 13.** Residual bodies resulting from the intracellular digestion of ectosymbionts are characterized by whorls of membranes (*asterisks*). *b* bacterial ectosymbionts; *M* mitochondria

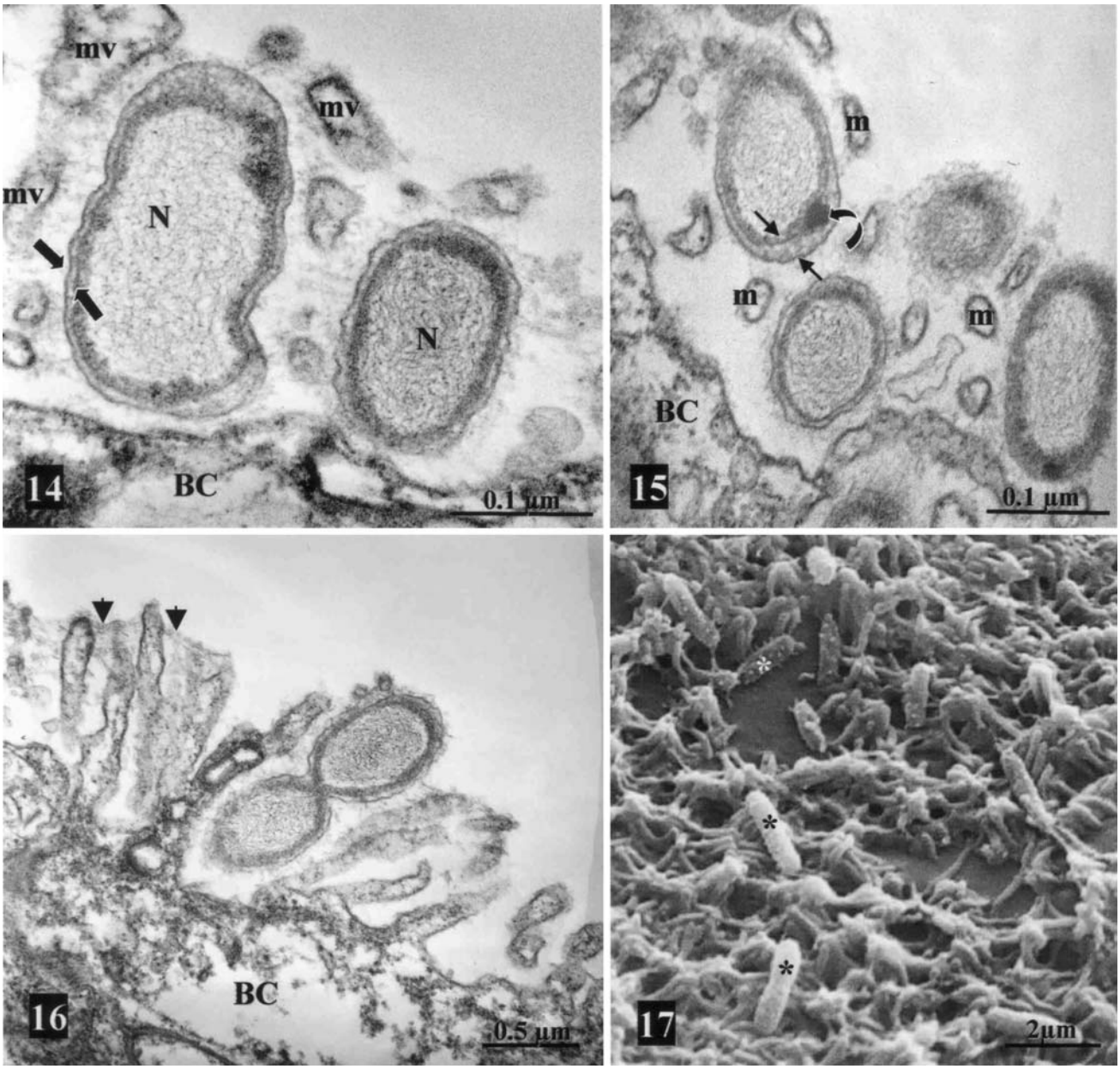
(heterotrophic organisms eating the microbial mats degrading the wood). Moreover, Leschine (1995) has shown that sulfide represents the principal product of the cellulose degradation in marine environment. Thus, such a continuous production of hydrogen sulfide could support chemosynthetic communities as previously described with bacterial decomposition of lipids from whale bones in the deep sea (Deming et al. 1997).

Gill structure and bacterial location

The bacteria described in the specimens of the two genera presented here (*Adipicola* and *Idas*) are located extracellularly while remaining in close contact with the apical pole of gill cells along the entire lateral zone of the gill filaments. By comparison with previous descriptions of gill endosymbiosis in bivalves or of ectosymbiosis as observed in individuals belonging to the families Thyasiridae (Dando and Southward 1986; Dufour 2005; Dias Passos et al. 2007) and Bathymiodiolinae (McKiness et al. 2005), such cells establishing preferential interactions with extracellular bacteria will be called bacteriocytes. The lateral zone consists of a thin, simple epithelium, suggesting that exchanges can occur between the bacteriocytes and the environment. Moreover, bacteriocytes develop microvilli all along their apical pole probably to (1) increase the surface of the cellular membrane, which is an important site of uptake of dissolved organic compounds present in the water and (2) offer the largest surface to harbor ectosymbiotic bacteria. Thus, bacteria live in close contact with host cells and with the flow of water circulating through the lateral zone of gill filaments. Because of their particular location, such bacteria, could protect host cells by taking up reduced sulfur compounds from the environment. Moreover, these ectosymbionts are probably regularly endocytosed by bacteriocytes and digested inside lysosomal structures providing the host cells with an input of organic compounds as previously described in various marine symbiosis models (Fiala-Médioni et al. 1994; Liberge et al. 2001). TEM analysis has revealed that in the *Idas* woodia individual, bacteriocytes contain numerous lysosome-like structures as large as nuclei. These structures appear to be filled with membrane whorls. Such structures have been previously reported in symbiotic bivalves (for review see Frenkiel et al. 1996; Dufour 2005) with various interpretations. They usually are considered to be lysosomes involved in the digestion of a limited portion of bacterial endosymbionts (Giere 1985; Distel and Felbeck 1987; Frenkiel et al. 1996). In our case, they may be involved in the digestion of ectosymbionts after their phagocytosis by host cells. This phenomenon may also be involved in a larger process: the control of the proliferation of the bacterial symbiont community on the cell surface. However, such structure with membrane whorls have also been described as sulfide-oxidizing bodies (SOBs) in marine invertebrates from sulfide-rich environments (Powel and Somero, 1985; Liberge et al. 2001). Thus, further cytochemical analyses of gill tissues will be necessary to conclude as well as stable isotope analyses and/or enzyme assays to detect of sulfur-based chemoautotrophic enzymes and to the ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco) to clarify the mode of nutrition of the bacteria and bivalves.

Are the bacterial ectosymbionts chemoautotrophic?

This is the first study to document gill-symbiosis in marine invertebrates associated with sunken wood environments. Mytilidae from cold seeps or hydrothermal vents usually harbor gill endosymbionts which are either sulfur-oxidizing symbionts (Fiala-Médioni et al. 1986; Distel and Cavanaugh 1994; Fujiwara et al. 2000) or methanotrophic bacteria (Childress et al. 1986; Cavanaugh et al. 1987; Fujiwara et al. 2000), while in some cases a dual symbiosis has been reported, with a mixed population composed of methanotrophic and thiotrophic bacteria inhabiting the same tissue (Fisher et al. 1993; Duperron et al. 2005). Recently, McKiness et al. (2005) have described extracellular bacterial symbionts in a Bathymiodolinae collected at 2,200 m depth at the Juan de Fuca hydrothermal vents. The 16S rDNA sequence obtained from the gills clustered with other mytilid chemoautotrophic symbionts previously described. Thus, species belonging to the family Mytilidae can harbor



Figs. 14–17 **Fig. 14.** Bacterial symbiont ultrastructure in *Idas woodia*. Symbionts, extracellularly located between the microvilli (mv) of the bacteriocyte and possess a typical double membrane (*arrows*) of Gram-negative bacteria. The DNA (*N*) occupies most of the volume of the bacterial cytoplasm, which also contains numerous non-membrane-bound granules (ribosomes or glycogenic storage) in contact with the internal membrane. The periplasmic space does not seem to contain empty vesicles (such as sulfur granules) usually observed in thioautotrophic bacteria encountered in symbiotic bivalves. *BC* bacteriocyte. **Fig. 15.** Bacterial symbiont ultrastructure in *Idas woodia*. TEM. Some ectosymbionts harbor osmiophilic dense granules (*curved arrow*) between the two membranes (*arrows*) delimiting the periplasmic space of Gram-negative bacteria. *BC* bacteriocyte cytoplasm; *m* microvilli. **Fig. 16.** Bacterial symbiont ultrastructure in *Idas woodia*. TEM of dividing ectosymbionts located between microvilli which are linked by a thin glycocalyx (*arrow head*). *BC* cytoplasm of the bacteriocyte. **Fig. 17.** Bacterial symbiont ultrastructure in *Idas woodia*. SEM view of the apical surface of the lateral zone of a gill filament of *I. woodia*. Bacterial ectosymbionts (*stars*) are mostly a rod shaped. The bacteria are located between the host cell microvilli

extracellular chemoautotrophic bacteria. Chemoautotrophic bacteria also occur as ectosymbionts in most of the members of the family Thyasiridae (Dufour, 2005). The distribution of the bacteria throughout the lateral zone of each gill filament and their specific location between microvilli of the host cells below the glycocalyx strongly suggest that these bacteria are not ordinary fouling microorganisms that could be found on any inert surface associated with sunken wood environment. They have established particular relationships with the bivalve, which are probably symbiotic, as described in marine invertebrates colonizing sulfide-rich habitats.

The two types of symbionts (thioautotrophic and methanotrophic) belong to the γ-proteobacteria (Cavanaugh 1994) and it is difficult to determine if the ectosymbionts found in here are chemoautotrophic based only on their ultrastructure. Ectosymbionts described in the bivalve family Thyasiridae are sulfur-oxidizing bacteria (Dufour 2005) while in shrimps the bacterial community described in the gill cavity of *Rimicaris exoculata* [Williams & Rona, 1986] belongs to the ε-proteobacteria (Madrid et al. 2001; Zbinden et al. 2004). In our case, we can rule out the methanotrophic metabolism due to the lack of concentric stacks of intracellular membranes in the bacterial ectosymbionts; however, further investigations will be necessary (phylogenetic or metabolic studies) to determine their metabolic pathways.

Symbiont transmission mode

The fact that these symbionts are located outside the bacteriocytes should indicate that the transmission mode of such ectosymbionts is environmental. Thus, symbionts come from an environmental stock of free-living, symbiosis-competent bacteria which colonize aposymbiotic juveniles of the new host generation as described in the family Lucinidae (Gros et al. 1996, 1998). This is supported by the recent suggestion, based on genetic data, that thioautotrophic gill endosymbionts are environmentally acquired in some Mytilidae (Won et al. 2003). New investigations are in progress in our lab to check for the presence of symbionts in members of the family Mytilidae associated with sunken wood collected at deeper sites, between 500 and 2,000 m depth. The localization of the gill symbionts in such specimens may be important in understanding the history of colonization of vent and seep environments. In fact, all Mytilidae described to date harbor endocellular bacteria which may be environmentally transmitted to the new host generation, based on genetic and cytological observations obtained from deep-sea mussels of the genus *Bathymodiolus* (Won et al. 2003). However, endosymbiosis represents a more integrated state between two partners than ectosymbiosis; the best integration is represented by intracellular symbionts vertically transmitted to the new host generations. In this case, the prokaryotic symbionts usually have lost a part of their genome and are dependent on the eukaryotic host cells, as previously described for eukaryotic organelles such as mitochondria (for review see Douglas 1994). If the hypothesis that decomposing wood may serve as a step for the introduction of mytilids to vents and seeps, species found at intermediate depths (500–800 m) could progressively internalize their symbionts in deeper environments as an adaptation to increasing environmental constraints. Once internalized, gill-endosymbionts would be initially environmentally transmitted before being vertically transmitted through the host gametes as in a more integrated symbiosis, such as the one described for bivalves belonging to the family Vesicomyidae (Cary and Giovannoni 1993; Hurtado et al. 2003).

In conclusion, this study represents the first report of gill-symbiosis in metazoan species associated with sunken wood environments, strengthening the hypothesis that these sites could serve as steps for the colonization of chemosymbiotic species into either vents or shallow water environments. However, new investigations are needed to improve our knowledge of the symbiotic relationships occurring in bivalve species inhabiting this specific environment, especially regarding symbiont phylogeny. We look forward to getting new specimens in order to pursue this investigation.

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