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ORIGINAL PAPER

Structural and ultrastructural analysis of the gills in the bacterial-bearing species *Thyasira falklandica* (Bivalvia, Mollusca)

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Abstract In this study, the cellular organization of the gill that harbors symbiotic bacteria is described in the thyasirid *Thyasira falklandica* collected from South Shetlands in Antarctic. Sections of the gills revealed that *T. falklandica* belongs to the gill type 3, as described by Dufour (Biol Bull, 208:200–212, 2005), with an elongated lateral zone along the frontal-abfrontal axis of the gill filaments. The ciliated and intermediary zones looked similar to those described in symbionts-bearing bivalves. The lateral zone is more complex in *T. falklandica* than in other Thyasiridae already described. Such a zone is composed of four different cell types. Bacteriocytes are abundant in the frontal and abfrontal positions, while the middle part of the lateral zone is occupied mostly by numerous granule cells devoid of bacteria. All along the lateral zone, TEM and SEM observations show some ciliated cells, which are regularly interspersed between bacteriocytes and/or granule cells. Such cells, according to the long double ciliary roots of their cilia, should have a sensory function. Intercalary cells, which have never been observed between bacteriocytes, are restricted to the middle part of the lateral zone where their expansions overlap the adjacent granule cells. Bacterial symbionts occur only extracellularly among long microvilli differentiated by the bacteriocytes. They are abundant, usually spherical in shape (around 0.7 μm length), and covered by the glycocalix from bacteriocyte microvilli. According to TEM views, the empty vesicles located in the periplasmic space should be sulfur storage, as known for other sulfur-oxidizing symbionts.

Keywords Bivalvia · Thyasiridae · Symbiosis · TEM · SEM

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Introduction

Thyasiridae comprises 11 genera of usually small bivalves, which have a wide range of distribution from shallow water to hadal depths (more than 7,000 m), from low sulphide to high-reduced sediments, and even in cold seeps (Southward 1986; Fujiwara et al. 2001; Fujiwara and Uematsu 2002; Imhoff et al. 2003). These bivalves have been described with reduced palps, rudimentary gut, and with an elongated burrowing foot (Allen 1958; Dufour and Felbeck 2003), and are known to establish symbiotic relationships with sulfur-oxidizing bacteria as described in several marine invertebrates since the last 25 years (Cavanaugh et al. 1981; Felbeck et al. 1981; Fischer 1990). The particularity of these symbiotic relationships in the thyasirids possessing symbionts, when compared to the other bivalve taxa known to harbor bacterial symbionts, is that in most of the observable cases bacteria occur extracellularly between a thin cuticle and the apical pole of the bacteriocytes (Southward 1986; Dufour 2005). In a few species, these bacteria are endosymbionts as in *T. sarsi* (Southward 1986) or *T. equalis* (Dufour 2005).

This bivalve family, based on morphological characters, belongs to the taxon Lucinoidea that contains the Ungulinidae, the Thyasiridae, and the Lucinidae (Allen 1958) and

three other families as the Fimbriidae, the Mactromyidae which is an entirely fossil group, and the Cyrenoididae (Williams et al. 2003). The position of these families against symbiosis is not equal. To date, no ungulinids have been reported with symbionts, but they are probably the less studied. All the lucinids described to date possess gill-endosymbiotic bacteria (for review see Taylor and Glover 2000; Gros et al. 2003; Glover et al. 2004), while the thyasirids present an intermediary position. Some genera of the later taxon, e.g. *Axinopsida, Genaxinus*, and *Mendicula*, never possess bacteria (Dufour 2005). On the other hand, species of *Conchocele*, *Thyasira*, and *Maorithyas* have been reported harboring bacterial symbionts (Fujiwara et al. 2001; Imhoff et al. 2003; Dufour 2005). Nevertheless, in the genus *Thyasira*, some species harbor symbionts while some other ones could be asymbiotic such as *T. eumyaria* [Sars, 1870], *T. ferruginea* [Winckworth, 1932], *T. granulose* [Monterosato, 1874] (Dando and Southward 1986; Southward 1986; Dufour 2005). This is why Fischer (1990) has suggested that sulfur-oxidizing symbiosis in thyasirids might represent a more primitive association between bacteria and bivalves when compared to the integrated system for gill-endosymbionts found in the Lucinidae.

Recently, the monophyly of these host bivalves believed to belong to the Lucinoidea was not supported by 18S- and 28S-rDNA gene sequence analyses (Williams et al. 2003). Thus, Ungulinidae and Thyasiridae are unrelated to the Lucinidae. Consequently, these authors conclude that Thyasiridae could represent an independent evolutionary pathway of bacterial chemosymbiosis along with the other bivalve groups. So, could these molecular data be confirmed by morphological and ultrastructural differences compared to Lucinidae strengthening a different and independent acquisition of bacterial symbiosis in this bivalve family?

Few data are available concerning the prokaryotes involved in such interactions. Dando et al. (1985) have suggested that thyasirid symbionts could be thioautotrophic due to the presence of elemental sulfur in their gills, while no sulfur could be detected for the foot. Such hypothesis was then confirmed by the study of the $^{13}C/^{12}C$ ratio (Spiro et al. 1986; Dando and Spiro 1993). Phylogenetic studies of such bacterial symbionts have been found in different clusters of thioautotrophic bacteria, but do not seem to form a common coherent group (Imhoff et al. 2003). However, they belong to the gamma subdivision of the Proteobacteria as all sulfur-oxidizing symbionts described to date in marine invertebrates (Distel et al. 1994).

According to Dell (1990), the geographical distribution of *Thyasira falklandica* can be summarized as follows: it extends from the Magellanic region through the islands of the Scotia Arc, and possibly to the Antarctic Peninsula. Narchi et al. (2002) gave detailed descriptions of their shells but without data concerning symbiosis or gill ultrastructure. The present work presents a well-detailed microscopic analysis (combining TEM and SEM views) of the gills of a thyasirid harbouring bacterial symbionts in order to check if an independent evolution of bacterial chemosymbiosis can be supported by structural differences in Thyasiridae compared to other bivalve species harbouring gill-symbionts.

Materials and methods

Specimens' collection

Living specimens of *Thyasira falklandica* (Smith 1885) (Thyasiridae, Bivalvia) with an average shell-length of 1.5 cm were obtained from bottom samples collected by a Van Veen grab during the January–April/2001 period from depths of 10–30 m in an area adjacent to the Brazilian Antarctic Station "Comandante Ferraz" (EACF) at the Martel Inlet, Admiralty Bay (58°21′W, 62°04′S). The samples were sieved through a 0.5 mm mesh, and the trapped specimens were kept in aquaria with natural sediment and circulating seawater (33‰) at 0 ± 1°C.

Histological preparations

Histological methods were performed according to Gabe (1968). Specimens were fixed in Bouin's fluid for 24 h, and then stocked in 70°C alcohol before examination. Back to the laboratory, samples were dehydrated through alcohol 100°C before embedding in paraplast. Sections (7 µm thick) were usually stained by a Goldner's trichrome staining. Alcian blue staining (pH 0.5 or 3) and periodic acid Schiff (PAS) reaction allowed identification of various mucosubstances.

Electron microscopic preparations

Scanning electron microscopy

Living specimens were dissected in the EACF by the removal of one valve with the aim of improving the infiltration of the anaesthetic and fixative solutions into the animals' tissues. Specimens were kept for 12 h in each of two anaesthetic solutions, the first with 16 mg and the second with 80 mg of 3-aminobenzoic acid ethyl ester diluted in 1 l of filtered (70 µm mesh) seawater. Individuals were prefixed in a solution of 2% paraformaldehyde, 2.5% glutaraldehyde, 2.5 mM calcium chloride, and 0.58 M sucrose, and buffered with 0.1 M sodium cacodylate, pH = 7.4 adjusted with a 0.2 N HCl solution. Immersed in excess of this

fixative solution, the samples were transported to Brazil where they were washed in three changes of cacodylate buffer solution and post-fixed in a buffered 1% osmium tetroxide solution for 45 min. After a short (15 min) bath in a solution of 1% tannic acid diluted in the same buffer, they were washed in distilled water, dehydrated in an acetone series, and critical point dried. Finally, the specimens were mounted with silver paint on aluminium stubs, gold coated, and viewed with a DSM 940 Zeiss Scanning Electron Microscope at the Instituto de Biociências, Universidade de São Paulo (IBUSP).

Transmission electron microscopy

Small pieces of gills were prefixed for a minimum of one hour at 4°C following the same procedure as for SEM, then fixed for 45 min at room temperature in 1% osmium tetroxide in the same buffer before being rinsed in distilled water, and post-fixed with 2% aqueous uranyl acetate for one more hour. After a rinse in distilled water, each sample was dehydrated through a graded ethanol series, and embedded in Epon-Araldite according to Mollenhauer (in Glauert 1975). Sections were cut using an UltracutE Leica ultramicrotome; thin sections (70 nm thick) were contrasted 30 min in 2% aqueous uranyl acetate and 10 min in 0.1% lead citrate before examination in a TEM Hitachi H-8000 at 100 kV in Guadeloupe (French West Indies).

Results

General morphology

The organs of the mantle cavity of *T. falklandica* are shown in Fig. 1; they are similar to those already described for other Lucinoidea. The anterior adductor muscle is characteristically elongated and the foot is long and slender. The reduced labial palps have only 4–5 inconspicuous folds. The deep-red, homorhabdic gills consist of outer and inner demibranchs, which cover lateral pouches of the visceral mass containing portions of the digestive diverticula and gonads (Figs. 1, 2). A faint groove is present along the ventral free margin of the inner demibranch (not shown). Both demibranchs are thick due to the length of the lateral zone of the filaments, and a transverse section through the whole animal shows that the space between the two lamellae composing of a demibranch contains a material strongly stained by alcian blue (Fig. 2). Moreover, semi-thin sections (Fig. 3) strongly suggest that each gill filament is composed of a frontal ciliated zone, and a large lateral zone characterized in its middle part by numerous large cells containing granules.

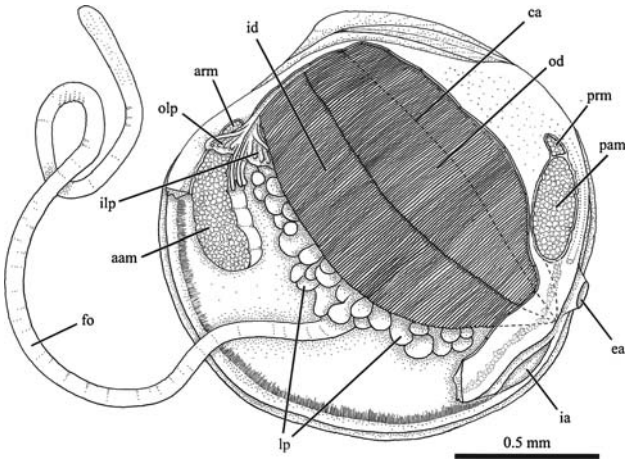
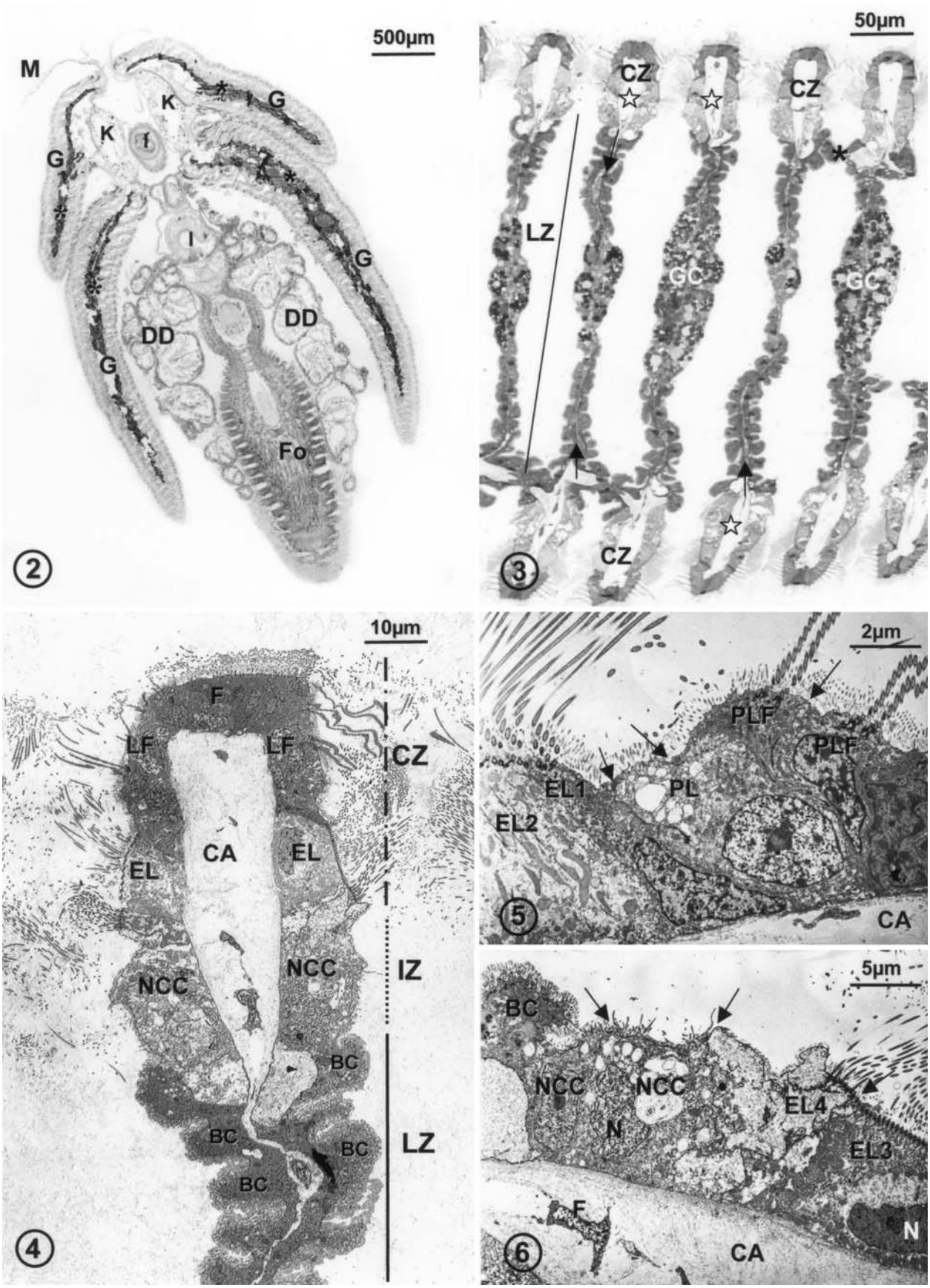


Fig. 1 *Thyasira falklandica*—Organs of the mantle cavity viewed from the left side after removal of the left shell valve and mantle lobe. The *dashed line* indicates the high of the ascending lamella of the inner demibranch. *aam* anterior adductor muscle; *arm* anterior pedal retractor muscle; *ca* ctenidial axis; *ea* exhalant aperture; *fo* foot; *id* inner demibranch; *ilp* inner labial palp; *lp* lateral pouches of the visceral mass containing portions of the digestive diverticula and gonads; *od* outer demibranch; *olp* outer labial palp; *omf* outer mantle fold lined by the periostracum; *pam* posterior adductor muscle; *prm* posterior pedal retractor muscle

Gill filament ultrastructure

The ciliated and intermediary zones looked similar to those described in symbiont-bearing bivalves (Figs. 3, 4). The ciliated zone is short with differentiated cell types as described following the terminology defined by Owen and McCrae (1976). Frontal cells bear short cilia without a precise orientation, while narrow prolaterofrontal cells bear two rows of long orientated cilia (Figs. 4, 5). Prolateral cells are devoid of cilia and have long regular microvilli (Fig. 5). The eulateral cells, which bear up to 30 rows of long independent cilia, constitute a functional group composed of three cell types (Figs. 4, 5, 6). The first one, located near the prolateral cells (Fig. 5), possesses cilia only on a small part of its apical area. The second one consists of two cells (EL2 and EL3) that exhibit cilia all along their apical surface, and the cytoplasm is occupied by many mitochondria (Figs. 4, 5, 6). The third cell type (EL4) is much like the first one but, being larger, is even more conspicuous, with clear cytoplasm, an apical or lateral nucleus, and several rows of cilia on a narrow apical pole compared to the second type (Figs. 4, 6). The intermediary zone appears quite long with non-ciliated cells differentiating long microvilli; such cells being located between the ciliated zone and the lateral zone characterized by the first cells bearing bacteria (Fig. 6).

The lateral zone constitutes the main part of the filament and accounts for the thickness and color of the gill (Figs. 3, 7). It is composed of four cell types. The most prevalent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◀ **Figs. 2–6** General organization of the gills of *T. falklandica*. **2, 3. Fig. 2.** Light micrograph. Cross section through the whole animal. Inner and outer demibranchs cover quite entirely the visceral mass on both sides. Compounds positively stained by alcian blue (*asterisks*) are present all along the space between the two lamellae of each demibranch. **Fig. 3.** Semi-thin transverse section of outer demibranch near its free ventral margin. Interlamellar junctions link ascending and descending portions of filaments. Each gill filament consists of a simple epithelium in which the lateral zone (*LZ*) is in contact with a blood lacuna (*arrows*) and is separated from the short ciliated zone (*CZ*) by a large intermediary zone (*stars*). Adjacent filaments are linked by tissular bridges (*asterisk*). Note zone devoid of bacteriocytes in the middle of the lateral zone rich in granule cells (*GC*). Seawater circulates through the inter-gill filament spaces. **Fig. 4.** TEM view of the ciliated zone (*CZ*), the intermediary zone (*IZ*), both devoid of bacteria, and the beginning of the lateral zone (*LZ*) which is characterized by numerous bacteriocytes (*BC*). Frontal (*F*), laterofrontal (*LF*), and eulateral (*EL*) ciliated cells are main cell types of ciliated zone (*CZ*) organized along a collagen axis (*CA*). Large non ciliated clear cells (*NCC*) make up the main part of the intermediary zone (*IZ*) and are in contact with bacteriocytes (*BC*) of lateral zone (*LZ*). **Fig. 5.** TEM. Prolatero-frontal cells (*PLF*) bear two rows of long orientated cilia while prolateral cells (*PL*) lack cilia. These prolateral cells are located between prolatero-frontal cells and the first ciliated eulateral cell (*EL1*). The second eulateral cell (*EL2*) bearing several rows of long independent cilia is characterized by numerous mitochondria (*m*) inside its cytoplasm. Eulateral cells constitute a functional group similar to those already described in bivalves (Owen and McCrae 1976). *Arrows* indicate junction complexes. **Fig. 6.** TEM of the intermediary zone. Large cell (*EL4*) possesses six rows of cilia and belongs to third functional group of eulateral ciliated cell. Few non-ciliated cells (*NCC*) are interspersed between this ciliated cell and first bacteriocyte (*BC*) from lateral zone. *Arrows* indicate junction complexes. *CA* collagen axis; *DD* lobes of the digestive gland; *F* fibroblast-like cell; *Fo* foot; *G* gills; *I* digestive tract; *K* kidney; *M* mantle; *N* nucleus

one is represented by cells which exhibit bacteria on their apical surface. Such proximity with bacteria leads us to define them as bacteriocytes similar to the cells harboring intracellular bacteria in other bivalve taxa (Reid 1990). Such bacteriocytes, which have a basal nucleus of a standard length of 5–10 µm (Fig. 7), are usually much wider than high. Their apical surface differentiates long microvilli linked by a fibrous glycocalyx in which bacteria are located (Figs. 7, 8, 9). The bacteriocytes organelles are represented by mitochondria, typical lysosome-like structures, and microbodies with whorls of membranes as large as the nucleus (Fig. 8). Occasionally, a few bacteria can be observed inside the cytoplasm (Fig. 9) suggesting a possible endocytosis phenomenon of these extracellular bacteria that could 4furthermore be destroyed by lysosomal enzymes.

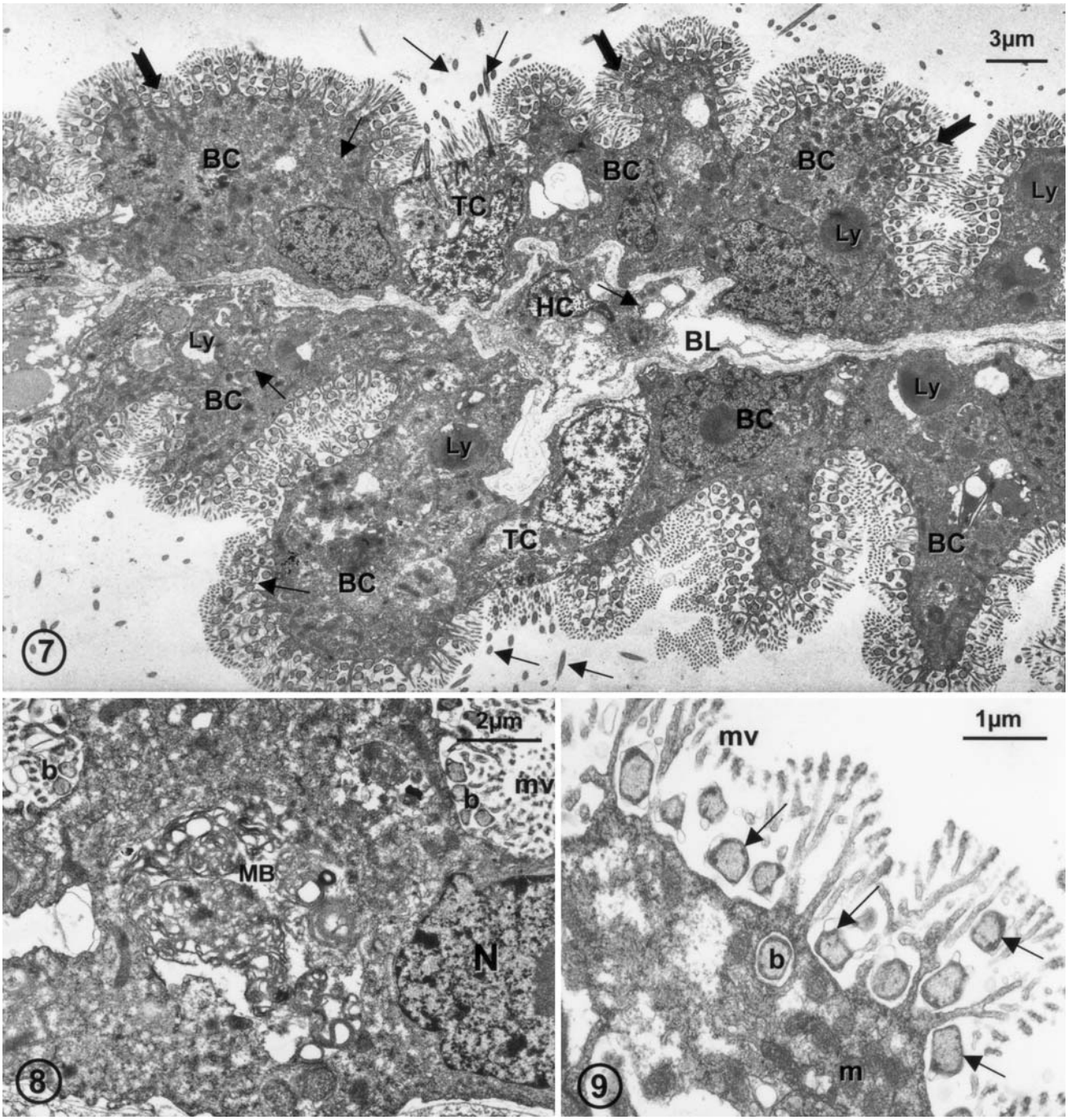
Bacterial symbionts are usually small (around 0.7 µm in length), with the typical double-membrane of Gram negative bacteria (Fig. 9). They are located between the long microvilli of the bacteriocytes in quite direct contact with environmental seawater (Figs. 7, 9). The bacterial cytoplasm contains some periplasmic membrane-bound vesicles (Fig. 9) that may storage sulfur globules as usually observed in other thioautotrophic bacterial symbionts.

Bacteriocytes are abundant in the frontal (Fig. 4) and abfrontal positions (Fig. 7), while the middle part of the lateral zone is occupied mostly by two other cell types devoid of bacteria (Figs. 3, 11). The first type corresponds to a secretory cell type characterized by a cytoplasmic volume filled with large membrane-bound inclusions (Figs. 3, 10, 11, 12, 13, 14) similar to the granule cells already described in various lucinid species. The second type consists of inconspicuous intercalary cells, which are interspersed between these granule cells (Figs. 11, 12). They are characterized by a trumpet shape with a narrow basis, an apical nucleus, and an enlarged apical area covered by microvilli (Fig. 12). Such cells encroach upon the apical area of adjacent granule cells restricting their contact with the pallial seawater (Fig. 12). The distribution of these intercalary cells is restricted to the middle part of the lateral zone in contact with granule cells (Fig. 11). No intercalary cells were observed interspersed between bacteriocytes (Fig. 7), as usually described in gill-endosymbiosis bivalves. Unusually in symbiont-bearing bivalve gills, no mucocytes were observed in the lateral zone of the gill filament either from histological sections or from TEM sections. The material present inside the space delimiting the two lamellae of each demibranch (Fig. 2) does not correspond to proteoglycan products secreted by mucuous cells but probably to secretory contents of granule cells.

All along the lateral zone, there are some ciliated cells that are regularly interspersed between bacteriocytes (Fig. 7) and/or granule cells (Fig. 11). According to TEM views, their cilia possess a ciliary axonemal structure with long double ciliary roots characterized by transversal striations (Fig. 14). Such roots sink up to the nucleus. SEM views confirm the fact that such cells differentiate on their apical part long cilia that give them a tuffty aspect (Figs. 10, 13).

Discussion

Thyasira falklandica possesses red gills as previously reported in other bivalves known to harbor gill-endosymbionts (Dando et al. 1985; Southward 1986; Frenkiel et al. 1996). In the Thyasiridae, *Thyasira ferruginea* and *T. gouldi* [Philippi, 1845] have been reported by Southward (1986) to possess gill's color from red to dark brown. Read (1962) believed that such color was due to a large supply of hemoglobin inside yellow-brownish dense inclusions. More recently, Frenkiel et al. (1996) have shown that in *Lucina pectinata* [Gmelin, 1791], the hemoglobin was located in dark areas of the bacteriocyte's cytoplasm. However, such patchy aspect of the cytoplasm was probably an artefact of the chemical fixation used because this patchy aspect is not



Figs. 7–9 TEM details of the lateral zone containing the bacteriocytes. **Fig. 7.** Lateral zone of *T. falklandica* gill filament. Bacteriocytes (*BC*) bordering blood lacuna (*BL*) contain lysosomes (*Ly*) at various degrees of maturity in their cytoplasm while the bacterial symbionts (*large arrows*) are located outside cell between microvilli. Few tufty cells (*TC*), devoid of bacterial ectosymbionts, can be observed with their long non-oriented cilia (*thin arrows*) between adjacent bacteriocytes. **Fig. 8.** Higher magnification of microbody (*MB*) filled with whorls of membranes probably corresponding to lysosome-like structure or to SOB described in some lucinids. **Fig. 9.** Host cells can enclose bacteria in phagocytic vacuoles (*b*) at their apical pole, while most of the bacterial symbionts (*arrows*) stay outside between the microvilli (*mv*) linked by a thin glycocalyx. *b* bacterial symbionts; *HC* Hemocyte. *m* mitochondria inside the bacteriocyte cytoplasm; *mv* microvilli; *N* nucleus

observed in cryofixed samples (Lechaire et al. 2006). Conversely to *L. pectinata* and/or *Calyptogena magnifica* (Boss and Turner 1980) (Fiala-Médioni and Métivier 1986), bacteriocytes of *T. falklandica* do not present a patchy aspect with dark areas. Anyway, it is difficult to conclude about the presence of cytoplasmic hemoglobin in this thyasirid only from TEM views. Further experiments are needed to perform cytochemical analyses using EDX on bacteriocyte

sections as done before in *L. pectinata* (Frenkiel et al. 1996) or by cryo-EFTEM as done recently by Lechaire et al. (2006).

The symbiotic bacteria described in *T. falklandica* are extracellular remaining in close contact with the apical pole of the bacteriocytes. The lateral zone of the gill filament is constituted by a thin simple epithelium suggesting that some exchanges occurred between the bacteriocytes and the environment. Moreover, bacteriocytes develop microvilli all along their apical pole probably to: (1) improve the area of cellular membrane representing an important site of uptake of dissolved organic compounds present in the water, and (2) offer the largest surface to harbor ectosymbiotic bacteria. Because of their particular location, such bacteria, which are probably sulfur-oxidizing bacteria, could protect host cells taking up the reduced sulfur compounds from the environment. These ectosymbionts are probably regularly endocytosed by bacteriocytes and digestion may occur inside lysosomal structures, giving the host cells an input of organic compounds.

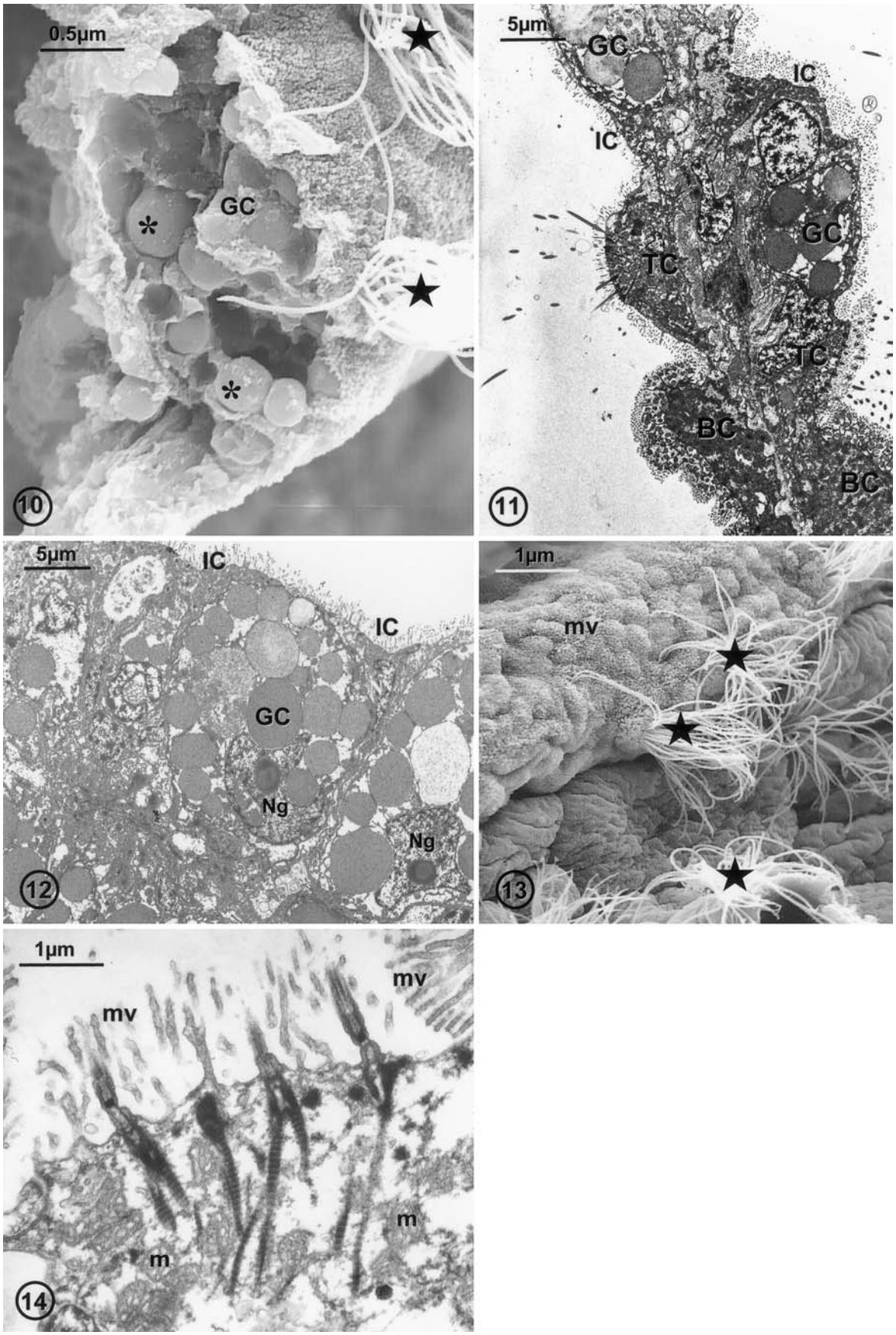
Ultrastructural studies have revealed that in *T. falklandica*, bacteriocytes contain lysosome-like inclusions as large as nucleus, which have been previously reported in symbiotic bivalves (for review see Frenkiel et al. 1996) and in *T. equalis* [Verril & Bush, 1898] (Dufour 2005) with various interpretations. They usually are considered as lysosomes involved in the digestion of a limited portion of bacterial endosymbionts (Giere 1985; Distel and Felbeck 1987; Le Pennec et al. 1988; Frenkiel et al. 1996). Their presence here should rule out this hypothesis in *T. falklandica* due to the fact that the bacteria are located outside the bacteriocytes. They could be involved in a recycling process of the cytoplasmic hemoglobin as described in *L. pectinata* (Frenkiel et al. 1996; Liberge et al. 2001).

The presence of numerous "granule cells" in the middle part of the lateral zone could be involved in some detoxification processes. This cell type has been observed in the thyasirid *Mendicula ferruginea* [Forbes, 1844] (Dufour 2005) and also in some lucinids (Giere 1985; Southward 1986; Frenkiel and Mouëza 1995). Frenkiel and Mouëza (1995) have strongly suggested that the abundant "granule cells" observed in *C. orbicularis* represent a cell type distinct from the other cells commonly found in the lateral zone such as bacteriocytes, mucocytes, and intercalary cells. Observations performed on aposymbiotic juveniles of *C. orbicularis* (from 250 µm to 2.5 mm long) obtained in laboratory culture (Gros et al. 1997) have definitively confirmed that "granule cells" represent a distinct cell type, probably involved in detoxification processes, appearing before the establishment of the bacterial symbiosis which occurs in all adults and/or juveniles in the wild (Gros et al. 1997, 1998). The "granule cells" observed in *T. falklandica* could also be involved in detoxification process.

According to Dufour (2005), there are three gills types recognized in Thyasiridae species, two of them harboring symbionts. The gill type 2 concerns filibranch gill-filaments with a relatively short lateral zone harboring ectosymbionts. The gill type 3 is characterized by elongated gill filaments with the whole cells below the ciliated zone harboring extracellular bacterial symbionts. *T. falklandica* undoubtedly belong to this gill type 3, confirming the observations of Dufour (2005). However, bacteriocytes are located only in the frontal and abfrontal parts of the lateral zone, the middle part lacking symbiont-bearing cells. Moreover, the presence of "sensory" ciliary cells has not been reported before in thyasirids. *T. falklandica* could represent a new subdivision inside the gill type 3 due to the particular organization of the lateral zone of its gill filaments. This ultrastructural study of the gill filaments of *Thyasira falklandica* allows the emphasis of a more diversified cellular structure of the lateral zone than described in other thyasirid species to date.

In Bivalvia, most of the taxa known to possess sulfur-oxidizing bacteria belong to Filibranchia and Eulamellibranchia. In the Protobranchia, only the Vesicomyidae is known to possess such bacterial gill-symbionts. The most common location for these thiotrophic symbionts in the bivalve species belonging to these three orders is intracellular. Thus, most of the Mytilacea (filibranch) harbor gill endosymbionts, which are either sulfur-oxidizing or methanotrophic bacteria, while in some cases a dual symbiosis has been reported with a mixed population composed of both bacteria inhabiting the same tissue (for review see Duperron et al. 2005). However, McKiness et al. (2005) have described extracellular bacterial symbionts in one bathymiodoline mussel collected at 2,200 m depth at the Juan de Fuca hydrothermal vents.

In Eulamellibranchia, gill-symbionts are intracellular excepted for some members of the Thyasiridae in which most of the symbionts are extracellular (Dufour 2005). Because endosymbiosis represents a more integrated state between two partners than ectosymbiosis, thioautotrophic symbiosis relationships occurring in Thyasyridae are probably more recent than those described in the other Eulamellibranchia species. This could indicate an independent evolutionary acquisition of the symbionts compared to other taxa known to harbor sulfur-oxidizing gill-symbionts. Moreover, in the Thyasiridae, there are structural modifications that improve the accommodation of ectosymbionts such as elongation of gills and presence of elongated microvilli of epithelial cells to retain bacteria. As reported by Dufour (2005), some non-symbiotic thyasirids present also such morphological adaptations suggesting that these species are not incompatible with thioautotrophic symbiosis. So, in the Thyasiridae, such morphological adaptation could be a precondition to symbiosis. Consequently,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◀ **Figs. 10–14** Other cell types composing the lateral zone of *T. falklandica*. **Fig. 10.** SEM view of a fractured granule cells (*GC*). Note that granules (*asterisks*) inside the cytoplasm are not endocellular bacteria. *stars*: cilia from tufty ciliated cells. **Fig. 11.** TEM. Middle part of the lateral zone. Granule cells (*GC*) and tufty ciliated cells (*TC*) lack bacterial symbionts conversely to bacteriocytes (BC). Intercalary cells (*IC*) can be observed between these two kinds of cells while never observed between bacteriocytes. **Fig. 12.** TEM. Granule cells (*GC*) characterized by their numerous large membrane-bound inclusions at various degrees of maturity according to their density. Intercalary cells (*IC*) are usually characterized by a trumpet shape with a narrow basis, an apical nucleus (*N*), and an enlarged apical area covered by microvilli without ectosymbionts. **Fig. 13.** SEM view of tufty ciliated cells (*stars*) throughout the lateral zone. **Fig. 14.** TEM view of the double ciliary roots characterized by transversal striations of cilia from tufty ciliated cells. *m* mitochondria; *mv* microvilli; *Ng* nucleus of granule cells

symbiosis does not occur randomly in bivalve taxa but require specific gill modifications before establishment of such relationships that could vary depending on the groups. In conclusion, morphological and ultrastructural differences reported in Thyasiridae strengthen the fact that this taxon represents an independent acquisition of chemosymbiosis as proposed by Williams et al. (2003) according to molecular data.

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References

Allen JA (1958) On the basic form and adaptations to habitat in the Lucinacea (Eulamellibranchia). Philos Trans R Soc Lond B 684:421–482

Cavanaugh CM, Gardiner SL, Jones ML, Jannasch HW, Waterburry JB (1981) Prokaryotic cells in the hydrothermal vent tube worm *Riftia pachyptila* Jones: possible chemoautotrophic symbionts. Science 213:340–342

Dando PR, Southward AJ (1986) Chemoautotrophy in bivalve Molluscs of the genus *Thyasira*. J Mar Biol Ass UK 66:915–929

Dando PR, Spiro B (1993) Varying nutritional dependence of the thyasirid bivalves *Thyasira sarsi* and *T. equalis* on chemoautotrophic symbiotic bacteria, demonstrated by isotope ratios of tissue carbon and shell carbonate. Mar Ecol Progr Ser 92:151–158

Dando PR, Southward AJ, Southward EC, Terwilliger NB, Terwilliger RC (1985) Sulphur-oxidizing bacteria and haemoglobin in gills of the bivalve mollusk *Myrtea spinifera*. Mar Ecol Progr Ser 23:85–98

Dell RK (1990) Antarctic Mollusca. With special reference to the fauna of the Ross Sea. R Soc N Z Bull 27:1–311

Distel DL, Felbeck H (1987) Endosymbiosis in the lucinid clams *Lucinoma aequizonata, Lucinoma annulata*, and *Lucina floridana*: a reexamination of the functional morphology of the gills as bacteria-bearing organs. Mar Biol 96:79–86

Distel DL, Felbeck H, Cavanaugh CM (1994) Evidence for phylogenetic congruence among sulfur-oxidizing chemoautotrophic bacterial endosymbionts and their bivalve hosts. J Mol Evol 38:533–542

Dufour SC (2005) Gill anatomy and relationship to chemoautotrophic symbiont presence in the bivalve family thyasiridae. Biol Bull 208:200–212

Dufour SC, Felbeck H (2003) Sulphide mining by the superextensile foot of symbiotic thyasirid bivalves. Nature 426:65–67

Duperron S, Nadalig T, Caprais JC, Sibuet M, Fiala-Médioni A, Amann R, Dubilier N (2005) Dual symbiosis in a Bathymodiolus mussel from a methane seep on the Gabon continental margin (South East Atlantic): 16S rRNA phylogeny and distribution of the symbionts in the gills. Appl Environ Microbiol 71:1694–1700

Felbeck H, Childress JJ, Somero GN (1981) Calvin benson cycle and sulphide oxidation enzymes in animals from sulphid-rich habitat. Nature 293:291–293

Fiala-Médioni A, Metivier C (1986) Ultrastructure of the gill of the hydrothermal vent bivalve *Calyptogena magnifica*, with a discussion of its nutrition. Mar Biol 90:215–222

Fischer CH (1990) Chemoautotrophic and methanotrophic symbioses in marine invertebrates. Rev Aquat Sci 2:399–436

Frenkiel L, Mouëza M (1995) Gill ultrastructure and symbiotic bacteria in *Codakia orbicularis* (Bivalvia, Lucinidae). Zoomorphology 115:51–61

Frenkiel L, Gros O, Mouëza M (1996) Gill ultrastructure in *Lucina pectinata* (Bivalvia: Lucinidae) with reference to hemoglobin in bivalves with symbiotic sulphur-oxidizing bacteria. Mar Biol 125:511–524

Fujiwara Y, Uematsu K (2002) Microdistribution of two endosymbionts in gill tissue from a hadal thyasirid clam *Maorithyas hadalis*. Cah Biol Mar 43:341–343

Fujiwara Y, Kato C, Masui N, Jujikura K, Kojima S (2001) Dual symbiosis in the cold seep thyasirid clam *Maorithyas hadalis* from the hadal zone in the japan trench, western pacific. Mar Ecol Progr Ser 214:151–159

Gabe M (1968) Techniques histologiques. Masson, Paris

Giere O (1985) Structure and position of bacterial endosymbionts in the gill filaments of Lucinidae from Bermuda (Mollusca, Bivalvia). Zoomorphology 105:296–301

Glauert AM (1975) Practical methods in electron microscopy. 3 (1): fixation, dehydration and embedding of biological specimens. Elsevier, Amsterdam

Glover EA, Taylor JD, Rowden AA (2004) *Bathyaustrellia thionipta*, a new lucinid bivalve from a hydrothermal vent on the Kermadec ridge, New Zealand and its relationship to shallow-water taxa (Bivalvia: Lucinidae). J Molluscan Stud 70:283–295

Gros O, Frenkiel L, Mouëza M (1997) Embryonic, larval, and post-larval development in the symbiotic clam, *Codakia orbicularis* (Bivalvia : Lucinidae). Invertebr Biol 116:86–101

Gros O, Frenkiel L, Mouëza M (1998) Gill filament differentiation and experimental colonization by symbiotic bacteria in aposymbiotic juveniles of *Codakia orbicularis* (Bivalvia: Lucinidae). Invertebr Reprod Dev 34:219–231

Gros O, Liberge M, Felbeck H (2003) Interspecific infection of aposymbiotic juveniles of *Codakia orbicularis* by various tropical lucinid gill-endosymbionts. Mar Biol 142:57–66

Imhoff JF, Sahling H, Süling J, Kath T (2003) 16S rDNA-based phylogeny of sulfur-oxidising bacterial endosymbionts in marine bivalves from cold-seep habitats. Mar Ecol Progr Ser 249:39–51

Lechaire J-P, Frébourg G, Gaill F, Gros O (2006) In situ localization and characterization of sulfur in the thioautotrophic symbiotic model *Lucina pectinata* (Mollusca: Bivalvia) by cryofixation and EFTEM analysis. Biol Cell 98:163–170

Le Pennec M, Diouris M, Herry A (1988) Endocytosis and lysis of bacteria in gill epithelium of *Bathymodiolus thermophilus, Thyasira*

flexuosa, and *Lucinella divaricata* (Bivalve, Molluscs). J Shellfish Res 7:483–489

Liberge M, Gros O, Frenkiel L (2001) Lysosomes and sulfide-oxidizng bodies in the bacteriocytes of *Lucina pectinata*, a cytochemical and microanalysis approach. Mar Biol 139:401–409

McKiness ZP, Mc Mullin ER, Fisher CR, Cavanaugh CM (2005) A new bathymiodoline mussel symbiosis at the Juan de Fuca hydrothermal vents. Mar Biol 148:109–116

Narchi W, Domaneschi O, Passos FD (2002) Bivalves Antárticos e Subantárticos coletados durante as Expedições Científicas Brasileiras à Antártica I a IX (1982–1991). Rev Brasiliana Zool 19:645–675

Owen G, McCrae JM (1976) Further studies on the latero-frontal tracts of bivalves. Proc R Soc Lond B 194:527–544

Read KRH (1962) The hemoglobin of the bivalved Mollusc, *Phacoides pectinatus* Gmelin. Biol Bull 123:605–661

Reid RGB (1990) Evolutionnary implications of sulphide-oxidizing symbioses in bivalves. In: Morton B (ed) The Bivalvia—Proceedings of a memorial symposium in Honour of Sir CM Yonge, Edinburgh, 1986. Hong Kong University Press, Hong Kong, pp 127–140

Smith EA (1885) Report on the Lamellibranchiata collected by H.M.S. Challenger during the years 1873–1876. Report on the Scientific Results of the Voyage of H.M.S. Challenger. Zoology 13:1–341

Southward EC (1986) Gill symbionts in thyasirids and other bivalve mollusks. J Mar Biol Ass UK 66:889–914

Spiro B, Greenwood PB, Southward AJ, Dando PR (1986) $^{13}C/^{12}C$ ratio in marine invertebrates from reducing sediments: confirmation of nutritional importance of chemoautotrophic endosymbiotic bacteria. Mar Ecol Progr Ser 28:233–240

Taylor JD, Glover EA (2000) Functional anatomy, chemosymbiosis and evolution of the Lucinidae. In: Harper EM, Taylor JD, Crame A (eds) The evolutionary biology of the bivalvia. Geol Soc Lond Spec Publ 177:207–225

Williams ST, Taylor JD, Glover EA (2003) Molecular phylogeny of the Lucinoidea (Bivalvia): non-monophyly and separate acquisition of bacterial symbiosis. J Molluscan Stud 70:187–202