Structural adaptations in the gill of the Japanese subduction zone bivalves (Vesicomyidae) *Calyptogena phaseoliformis* and *Calyptogena laubieri*

Ultrastructure
Gill
Bacteria symbiosis
Bivalves
Japanese subduction zones

Ultrastructure
Branchie
Association bactérienne
Bivalves
Zones de subduction japonaises

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ABSTRACT

Histological and ultrastructural work on the gill of two new species of *Calyptogena* (*C. phaseoliformis* and *C. laubieri*), found only in association with fluid emissions in subduction zones off Japan, show modifications related to the predominant role of this organ in nutrition. In both species, the dominant cell-type is the bacteriocyte, which appears to be a general feature of the genus *Calyptogena* and probably of the entire family *Vesicomyidae.*

The endocellular bacteria of the gill resemble, in size, structure and dividing mode, the sulphur-oxidizing bacteria found in *C. magnifica*; the presence of abundant elemental sulphur crystals in the gill endorses the hypothesis of an active sulphur oxydizing metabolism associated with these bacteria.

The presence in the bacteriocytes of abundant lipidic inclusions as well as numerous lysosomes apparently reflects the active trophic relationships between the endocellular bacteria and their host.

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RÉSUMÉ

Adaptations structurales de la branchie de *Calyptogena phaseoliformis* et *C. laubieri*, mollusques bivalves des zones de subduction au large du Japon

L'étude histologique et ultrastructurale de la branchie de deux nouvelles espèces de *Calyptogena* (*C. phaseoliformis* et *C. laubieri*), exclusivement associées aux émissions de fluides froids des zones de subduction au large du Japon, montre de profondes modifications en relation avec le rôle prédominant de cet organe dans les processus de nutrition. Chez les deux espèces, le type cellulaire prédominant est le bactériocyte. L'importance trophique de l'association bivalves-bactéries endocellulaires apparaît donc comme une caractéristique générale du genre *Calyptogena*, et probablement de toute la famille des *Vesicomyidae.*

Les bactéries endocellulaires de la branchie semblent, par leur taille, leur structure et leur mode de division, proches des bactéries sulfo-oxydantes associées au *C. magnifica*; la présence d'abondants cristaux de soufre élémentaire étaye l'hypothèse d'un métabolisme actif lié à l'oxydation des sulfures associés à ces bactéries.

La présence dans les bactériocytes d'abondantes gouttelettes lipidiques ainsi que de nombreux lysosomes, atteste probablement de relations trophiques actives entre les bactéries endocellulaires et leur hôte.

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INTRODUCTION

Exploration of the subduction zones off Japan has revealed the presence in high numbers of giant clams of the genus *Calyptogena* (*Vesicomyidae* family), which indeed are the dominant organisms of benthic populations at abyssal depths from 3 800 to 5 960 m (Swinbanks, 1985 *a; b*; Laubier *et al.*, 1986). Submersible dives in the Japan and Kurile trenches have demonstrated the widespread existence of cold seepage of pore waters, rich in thermogenic methane and light hydrocarbons, associated with the clam colonies (Boulègue *et al.*, 1986 *b*). The high levels of biomass appear to reflect the ability of these organisms to utilize a chemosynthetic food source. This is consistent with ecological observations (Laubier *et al.*, 1986; Sibuet *et al.*, 1986) and with geochemical analyses (Boulegue *et al.*, 1986 *a; b*).

This paper presents a detailed study of the gill of two species of *Calyptogena*, which constitutes the deepest species observed in intense geological activity sites.

MATERIAL AND METHODS

Calyptogena laubieri, Okutani and Métivier (1986) belongs to the first population type extending from 3 800 to 4 020 m in the Tenryu Canyon. *Calyptogena phaseoliformis* Métivier *et al.* (1986) forms the second population type observed from 5 130 to 5 960 m in the landward slope of the Japan Trench.

Both species were sampled by the submersible "Nautile" during the Franco-Japanese *Kaiko* expedition in summer 1985. *C. laubieri* was alcohol fixed (70%) and *C. phaseoliformis* formalin and glutaraldehyde (2.5%) fixed on board by Prof. Boulègue. After dissection, specimens were post-fixed in osmium tetroxide (1%). Some were dehydrated in a graded acetone series and prepared for scanning electron microscopy (SEM) by critical point drying with carbon dioxide. Other specimens were dehydrated in a graded ethanol series, followed by propylene dioxide, embedded in Epon and sectioned for light and transmission electron microscopy (TEM). The SEM specimens were mounted on supports, coated with Argon-sputtered gold-palladium and observed in a Hitachi 5520 at 20 KV. The sectioned material was stained with toluidine blue for light microscopy. Uranyl acetate and lead citrate were used for TEM. Observations were made in a Hitachi 600 transmission electron microscope. Microanalyses of the crystals were carried out using energy dispersion spectroscopy with a tracor system.

RESULTS

In both species, the gill is thick and large, covering the entire visceral mass. It is composed of two ctenidia each with only one demibranch. Each demibranch of fused ascending and descending lamellae exhibit homorabdic filaments (Fig. 1, 3 *A*). These are connected by interfilamentary junctions fused with the longitudinal

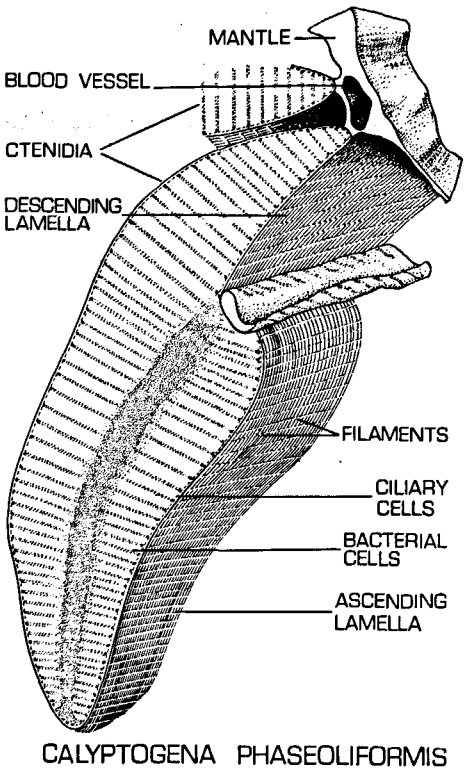

Figure 1
Calyptogena phaseoliformis. *Schematic representation of the gill in transverse section.*
Calyptogena phaseoliformis. Représentation transversale schématique de la branchie.

bars, which lie within the frontal edge of each filament and support the ciliated cells. The filaments of the ascending lamella are fused with the opposite filaments of the descending lamella by interlamellar septa in *C. phaseoliformis* (Fig. 2). On SEM pictures, the adjacent filaments show an abundant ciliation in their frontal region (Fig. 3 *B*). A very thin groove is present at the ventral margin of each demibranch (Fig. 3 *C*). The lateral faces of the filaments have a granular aspect

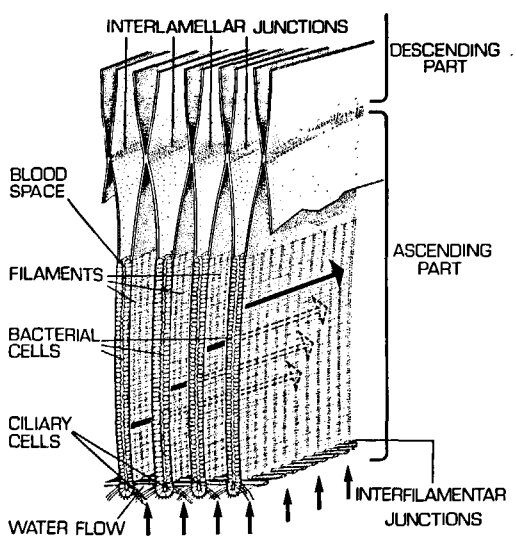

Figure 2
Calyptogena phaseoliformis. *Schematic representation of a cross-section through the filaments. The water current (arrows) crosses the ciliary cells and flows laterally over the bacteriocytes.*
Calyptogena phaseoliformis. Représentation schématique d'une coupe transversale des filaments. Le courant d'eau (flèches) traverse la trame ciliaire et circule latéralement en baignant les bactériocytes.

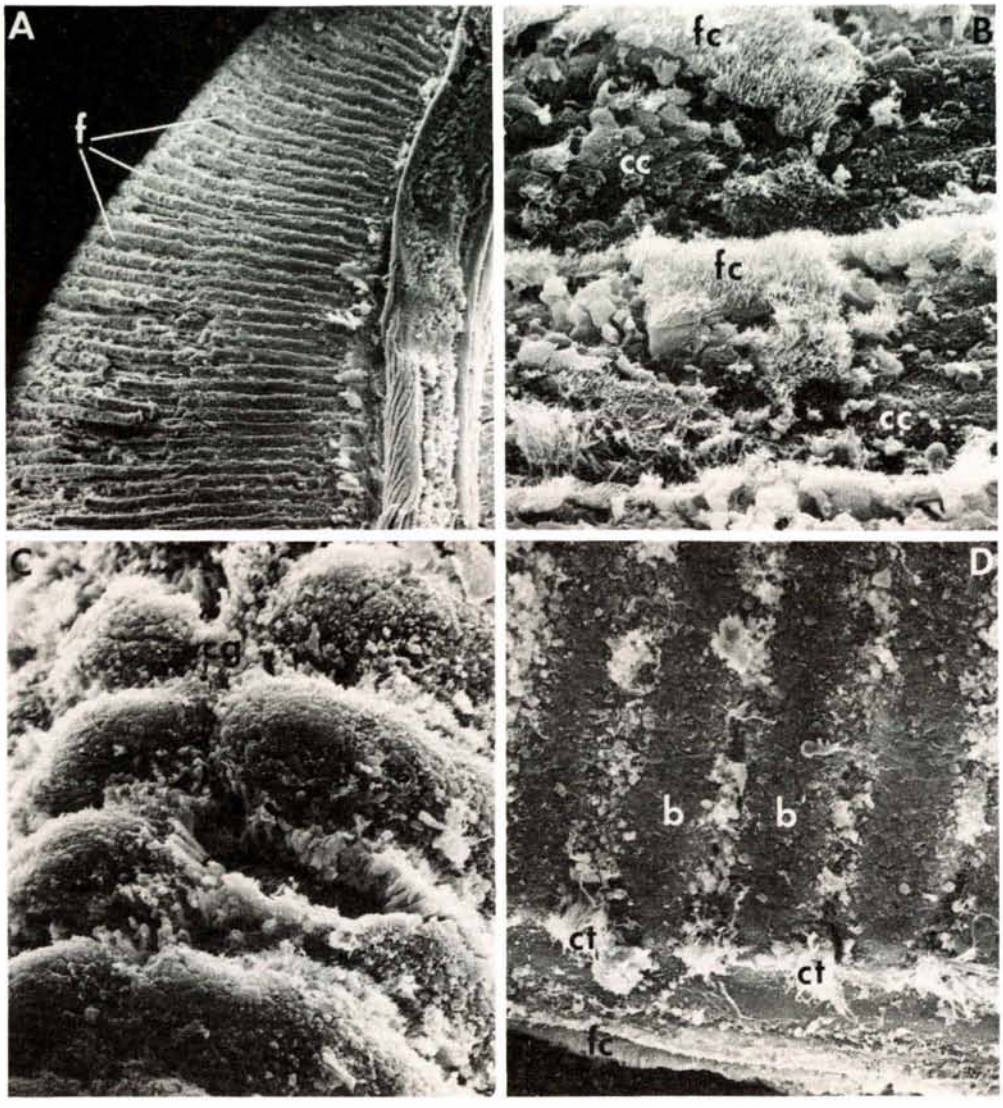

Figure 3

Calyptogena phaseoliformis. *Scanning electron micrographs of freeze-dried gill. A: lateral face of a lamella showing the adjacent ciliated filaments (f); B: high magnification of the filaments; fc: frontal cilia; cc: ciliated cells; C: residual groove (cg) at the ventral margin of a branch; D: lateral face of a filament with bacteriocytes (b) in files; ct: ciliary tufts; fc: frontal ciliation.*

Calyptogena phaseoliformis vues au microscope à balayage de la branchie passée au point critique. A : face latérale d'une lamelle montrant les filaments ciliés adjacents (f) ; B : agrandissement des filaments ; fc : cils frontaux ; cc : cellules ciliées ; C : gouttière résiduelle (cg) au bord ventral d'une branche ; D : face latérale d'un filament avec les bactériocytes (b) en files ; ct : touffes ciliaires ; fc : ciliature frontale.

related to bacteriocytes arranged in files in *C. phaseoliformis* (Fig. 3 *D*), or with a pavement-like aspect in *C. laubieri*. Light microscopy reveals abundant, translucent yellow crystals emerging from the lateral faces of the filaments in *C. laubieri*. On SEM pictures, these crystals, which measure from 50 to 150 µm (Fig. 4 *D*), present different geometries. Microanalysis shows them to be composed of pure elemental sulphur (Fig. 4 *C*). In *C. phaseoliformis*, branched accumulations of yellow crystals are also present inside the bacteriocytes.

Transverse thin sections through the filaments of both species confirm their general structure to be similar to those of *C. magnifica* (Fiala-Médioni, Métivier, 1986; Morton, 1986) with two different parts (Fig. 2, 4 *A*, 5 *A*, 5 *B*). The apical region is occupied by frontal, latero-frontal and lateral ciliated cells; only the long lateral cilia are well conserved (Fig. 5 *C*). The epidermis of the voluminous inner part of the filament is composed of a single layer of bacteriocytes separated by very thin bacteria free intercalary cells.

Ciliated cells and bacteriocytes are at their internal base limited by a basal lamina surrounding the blood lacuna with abundant hemocytes (Fig. 4 *B*, 5 *B*).

No mucus cells are observed, but this might be explained by insufficient conservation.

Unfortunately, the alcohol fixation did not allow TEM observations on *C. laubieri*. TEM observations on *C. phaseoliformis* show the bacteriocytes completely filled with bacteria and numerous inclusions at their periphery (Fig. 6 *A*).

The bacteria measure about 0.6 to 1 µm in diameter (transverse sections) and present a gram-negative type of double cell wall, a dense cytoplasm at their periphery, and a well developed nucleoid (Fig. 6 *B*). Dividing stages can be observed (Fig. 6 *B* arrow). Groups of bacteria are surrounded by peribacterial membranes. Different types of inclusions are present in the bacteriocytes cytoplasm: those of clear appearance (1 to 3 µm in diameter) react positively to histological lipid tests and contain mineral crystals. Dark ones are phago-

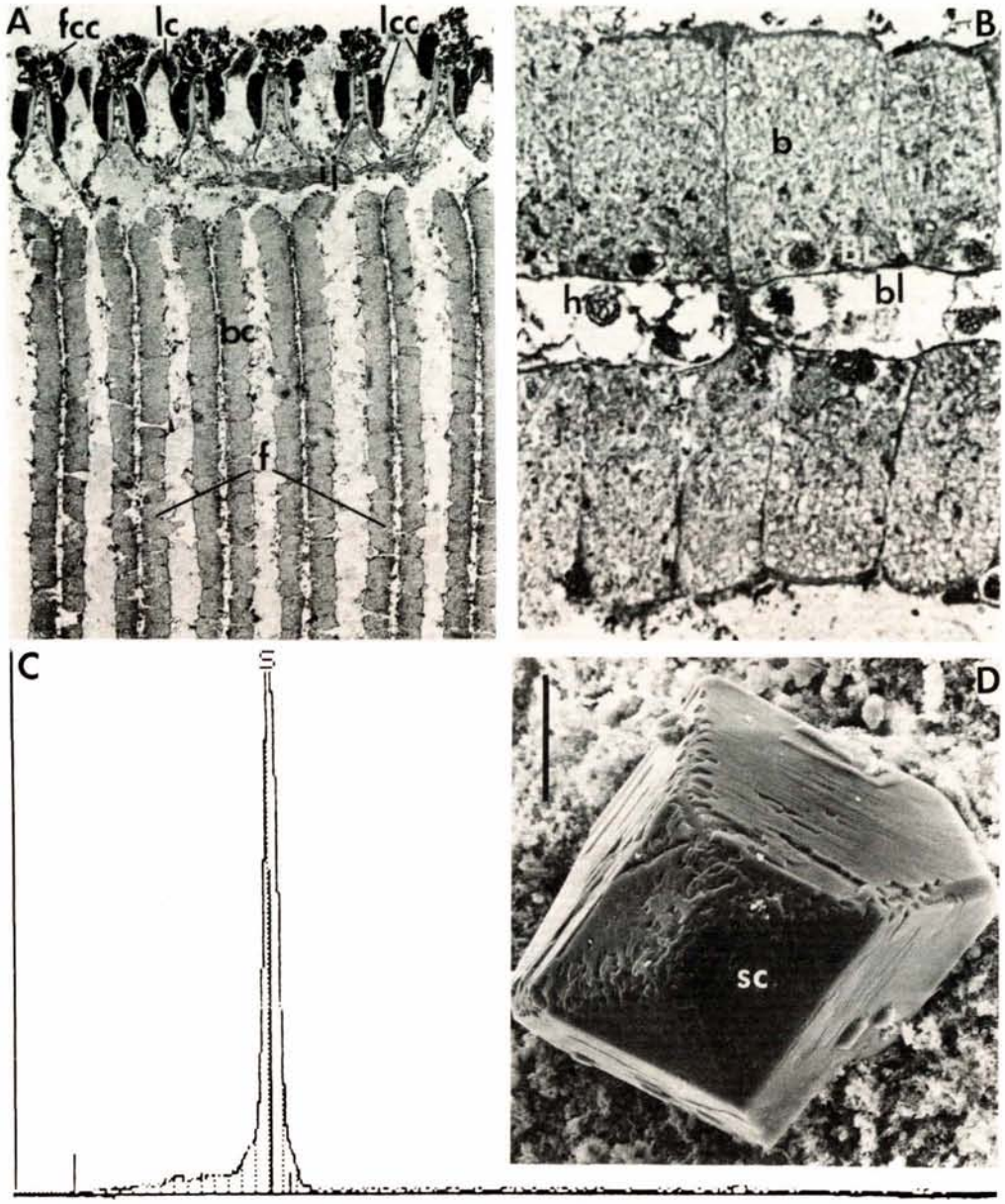

Figure 4

Calyptogena laubieri. *A: light micrograph of a cross-section through a lamella showing adjacent filaments (f) with ciliated frontal (fcc) and lateral (lcc) cells at the frontal part and bacteriocytes (bc) forming the highly developed inner part; ij: interfilamentary junctions; lc: lateral cilia; B: high magnification of the bacteriocytes; b: bacteria; BL: basal lamina; bl: blood lacuna; h: hemocyte; C: microanalysis of the crystal showing a peak of elemental sulphur (K = 2.31 KEV); D: scanning electron micrograph of a crystal (sc) emerging from the lateral face of a filament scale bar: 50 µm.*

Calyptogena laubieri. A : vue en microscopie optique d'une section transversale d'une lamelle montrant les filaments adjacents (f) avec des cellules ciliées frontales (fcc) et latérales (lcc) sur la partie frontale et des bactériocytes composant la partie interne développée; ij : jonctions inter-filamentaires. lc : ciliature latérale; B : agrandissement de la partie bactérienne; b : bactéries; bl : lacune sanguine; BL : lame basale; h : hémocyte; C : pic de soufre élémentaire obtenu lors de la microanalyse d'un cristal (K = 2,31 KEV); D : cristal (sc) émergeant de la face latérale d'un filament vu au microscope à balayage; échelle : 50 µm.

lysosome-like organelles characterized by concentric pseudo-myelic fibres (Fig. 6 *C*). Small electron-dense grains (about 0.05 µm) are also present in the bacteriocytes; however they are more abundant in the blood lacuna near the basal lamina and in hemocytes including phagosomes (Fig. 6 *D*).

As the external surface of the filament epidermis does not appear well fixed, it is not possible to determine the presence or absence of microvillies as observed in *C. magnifica* (Fiala-Médioni, Métivier, 1986).

DISCUSSION AND CONCLUSIONS

The large size and the structure of the gill suggest that it is the main organ carrying out the nutritional processes. The enlargement of the gill related to the development of the bacterial part appears to be a general feature of the genus *Calyptogena*. It is observed in *C. magnifica* (Fiala-Médioni, Métivier, 1986) which exploits an unusual epifauna in the rocky habitat of the East Pacific hydrothermal vents (Boss, Turner, 1980), the sediment burrowing species *C. phaseoliformis*, *C. laubieri* from the Japanese subduction zones (Laubier *et al.*, 1986), *C. soyoe* from the sagami bay (Sakai *et al.*, 1986), *C. pacifica* (Cavanaugh, 1983) and *C. elongata* (Vetter, 1985).

This special adaptation of the gill probably characterizes the entire family *Vesicomyidae*, which is widely distributed in the Pacific Ocean (Boss, Turner, 1980), explaining its ability to densely colonize oligotrophic

deep-sea zones abundantly supplied with chemical energy.

Although all the usual components of the organs of digestion are present and show evidence of functional cells (Morton, 1986; Le Pennec, Fiala-Médioni, 1986 and this issue), the development of the gill, for this family, is generally correlated with a simplification or a reduction of the structures involved in particulate nutrition: labial palps are small, the feeding groove of the ventral margins of the gill are residual, the stomach non plicate, the digestive gland reduced and the narrow intestine straight (Boss, Turner, 1980; Fiala-Médioni, Métivier, 1986; Le Pennec, Fiala-Médioni, 1986 and this issue). The particulate transit is negligible in *C. magnifica* (Boss, Turner, 1980; Fiala-Médioni, Métivier, 1986) and very weak in *C. phaseoliformis* (Le Pennec, Fiala-Médioni, 1986 and this issue). According to stomach content observations, *C. laubieri* seems to have conserved the ability to transport particles (Le Pennec, Fiala-Médioni, 1986; this issue).

The histological and ultrastructural observations clearly demonstrate the quantitative importance of the endo-

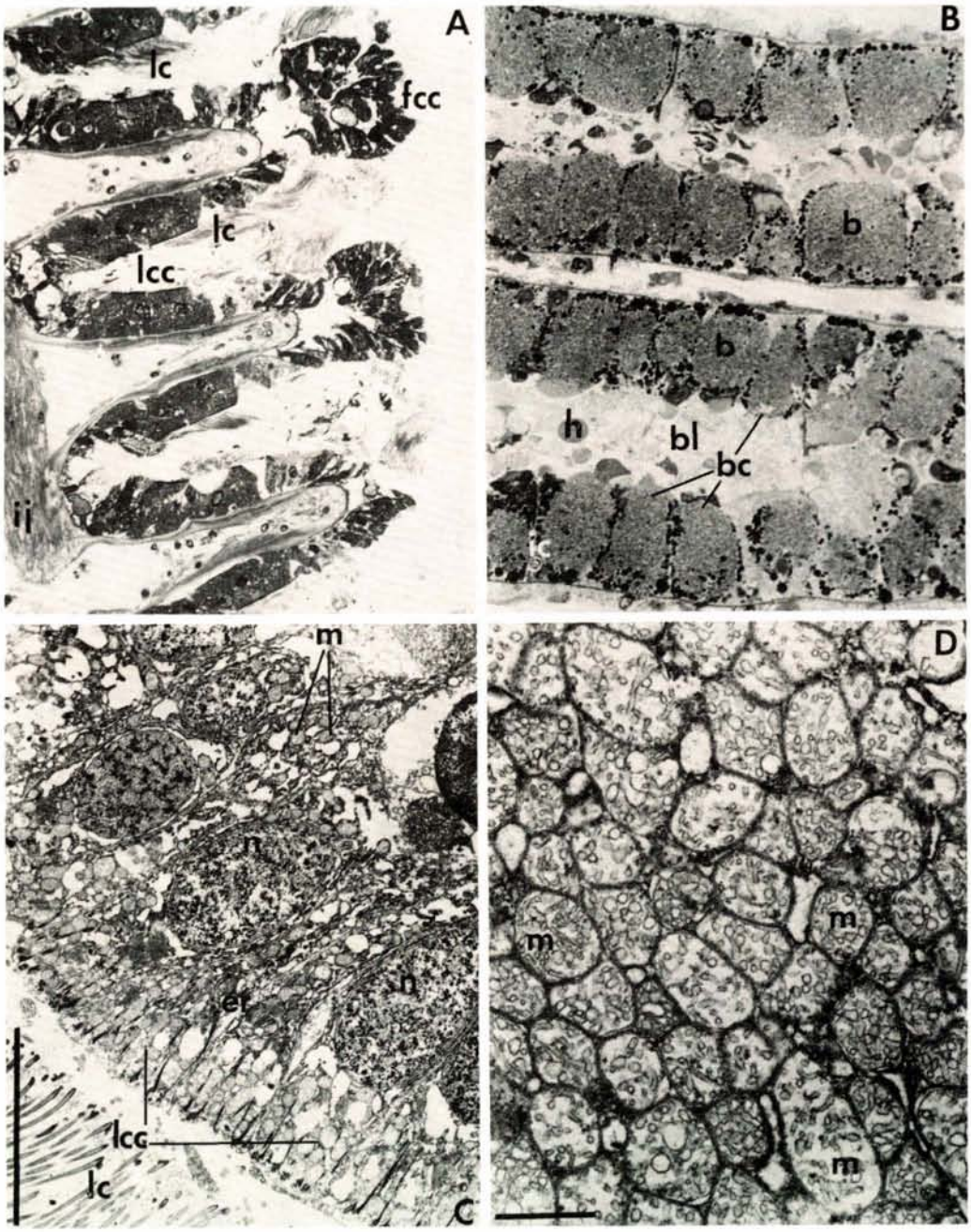

Figure 5

Calyptogena phaseoliformis. *A, B: light micrographs of cross-sections through a lamella; A: frontal (fcc) and lateral (lcc) cells in the frontal part; lc: lateral cilia; ij: interfilamentary junctions; B: bacteriocytes (bc) forming the inner part of the filaments; b: bacteria; bl: blood lacuna; h: hemocyte; ic: bacteria-free thin intercalary cell; C: electron micrographs of cross-section through the lateral ciliated cells (lcc); lc: lateral cilia; n: nucleus; m: mitochondria; re: endoplasmic reticulum; scale bar:* 10 μm*; D: high magnification of dense mitochondria (m) in the ciliated cells; scale bar:* 1 μm.

Calyptogena phaseoliformis. A, B : vues en microscopie optique de sections transversales à travers une lamelle ; A : cellules ciliées frontales (fcc) et latérale (lcc) sur la partie frontale ; lc : ciliature latérale ; ij : jonctions interfilamentaires ; B : bactériocytes (bc) constituant la partie interne des filaments ; b : bactéries ; bl : lacune sanguine ; h : hémocyte ; ic : cellules intermédiaires sans bactérie ; C : vue en microscopie électronique à transmission d'une coupe transversale de cellules ciliaires latérales (lcc) ; lc : ciliature latérale ; n : noyau ; m : mitochondrie ; re : reticulum endoplasmique ; échelle : 10 μm ; D : agrandissement de mitochondries (m) dans les cellules ciliaires ; échelle : 1 μm.

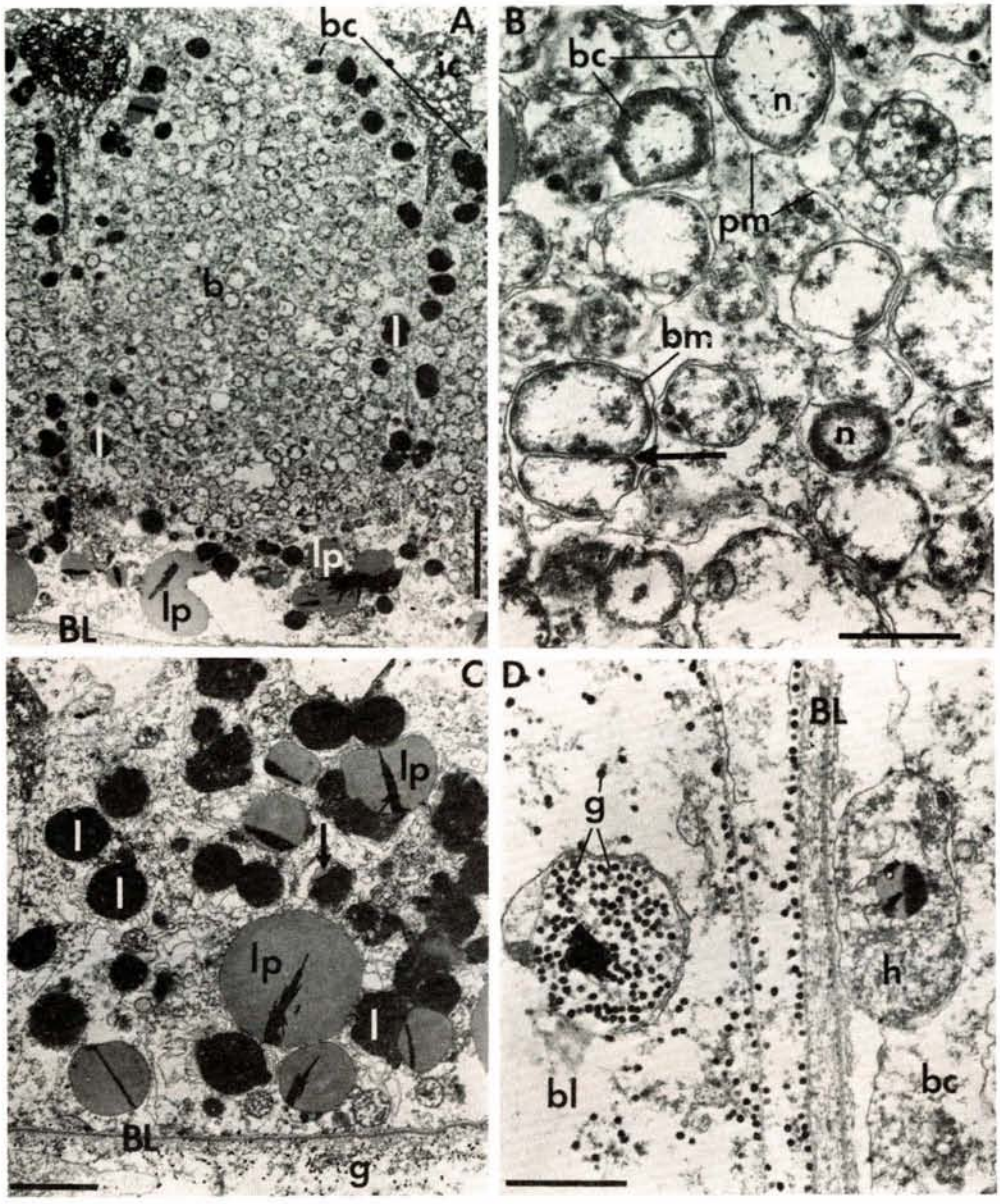

Figure 6

Calyptogena phaseoliformis. *Electron micrographs of cross-section through a filament. A: bacteriocytes (bc) are separated by very thin intercalary bacteria-free cells (ic); b: bacteria; lp: lipids; l: lysosomes; BL: basal lamina; scale bar:* 5 µm*; B: high magnification of bacteria; n: nucleoid; bc: bacterial cytoplasm; bm: bacterial membrane; pm: peribacterial membrane; arrow showing a dividing stage; scale bar:* 1 µm*; C: high magnification of the different inclusions; lp: lipids; l: lysosome; BL: basal lamina; g: granules arrow showing myelinic fibers of a lysosome; scale bar:* 2 µm*; D: abundant electron dense grains are present in the blood lacuna (bl) and hemocyte (h); BL: basal lamina; bc: bacteriocyte; scale bar:* 1 µm.

Calyptogena phaseoliformis. Vues en microscopie électronique à transmission d'une coupe transversale à travers un filament. A : les bactériocytes (bc) sont séparés par d'étroites cellules intermédiaires sans bactérie (ic) ; b : bactéries ; lp : lipides ; l : lysosomes ; bm : membrane basale ; échelle : 5 µm ; B : agrandissement des bactéries ; n : nucléoide ; bc : cytoplasme bactérien ; bm : membrane basale ; pm : membrane péribactérienne ; la flèche indique un stade de division bactérienne ; échelle : 1 µm ; C : agrandissement de différentes inclusions ; la flèche montre les fibres myéliniques d'un lysosome ; lp : lipides ; l : lysosomes ; BL : lame basale ; g : granules ; échelle : 2 µm ; D : d'abondants grains denses aux électrons sont présents dans la lacune sanguine (bl) et les hématocytes (h) ; BL : lame basale ; bc : bactériocyte ; échelle : 1 µm.

cellular bacterial association. Except for the frontal area occupied by ciliary cells, the gill is predominantly composed of bacteriocytes with abundant, normally reproducing bacteria. These observations, in agreement with the hypothesis of a vital importance for the clam of symbiotic relationships with its endocellular bacteria, corroborate the ecological observations of the populations strictly dependent upon the hydrothermal fluid emissions (Hessler, Smithey, 1983; Laubier, Desbruyères, 1984; Hessler *et al.*, 1985; Sibuet *et al.*, 1986) and the isotope analyses demonstrating very low 13_C, about −30 to −40 (Rau, 1981; 1985; Boulègue *et al.*, 1986 *a*) in relation with a predominantly chemoautotrophic source of carbon.

The general sulphide-based metabolic scheme of the relationships between bacteria and number of molluscs and other invertebrates in sulphide-rich habitats (Cavanaugh *et al.*, 1981; Felbeck *et al.*, 1981; 1983; Dando, Southward, 1986; Dando *et al.*, 1986; Southward, 1986) has been also proposed for *C. magnifica* (Felbeck *et al.*, 1983; Powell, Somero, 1985). In *C. phaseoliformis* and *C. laubieri*, the primary energy source for the bacteria remains to be elucidated. These species live half buried in sulphide-rich sediments (Boulègue *et al.*, 1986 *a*) but methane is also abundant in the water emissions (Dron *et al.*, 1986). From a geochemical analysis, Boulègue *et al.* (1986 *b*) postulate oxidative processes of methane; the energy liberated in

this manner might allow incorporation of CO_2. Methane can also be used as a direct carbon source and has recently been found to support mussels off Florida (Childress *et al.*, 1986). Ultrastructural pictures show the bacteria of *C. phaseoliformis* to be similar in size, structure and mode of division to those of *C. magnifica*; stacks of intracytoplasmic membranes characterizing type I methanotrophs of the Florida mussel (Childress *et al.*, 1986) are not observed. In addition, the presence of abundant elemental sulphur (Fiala-Médioni, Le Pennec, 1986) endorses the hypothesis of an active sulphur metabolism associated with the gill, and this, even if the possibility of the crystals being reprecipitated forms related to the fixation procedure cannot be excluded. Elemental sulphur found in abundance in the gill of *C. magnifica* (Felbeck *et al.*, 1983) as well as in a number of other bivalves living in sulphur-rich sediments and harbouring endocellular bacteria (Childress, Mickel, 1982; Berg, Alatalo, 1984; Powell, Somero, 1985; Vetter, 1985; Reid, Brand, 1986; Dando, Southward, 1986; Dando *et al.*, 1985; 1986) is supposed to constitute either endogenous reserves or an end product of the sulphide bacterial metabolism.

The sulphides present in the mud (Dron *et al.*, 1986) might be absorbed by the foot of the half buried clams and carried to the bacteria via the blood as suggested by Arp *et al.* (1984).

The signification of the electron-dense grains, abundant in the blood lacuna, hemocytes and in lesser concentration in bacteriocytes of all *Calyptogena* species studied (Fiala-Médioni, 1984; Fiala-Médioni, Métivier, 1986; Fiala-Médioni, Le Pennec, 1986) has also to be clarified.

As they have been observed also in *Bathymodiolus thermophilus* from the 13°N site in the East Pacific Rise and in both species of *Calyptogena* from the Japanese subduction zones, accumulation of lipid inclusions and lysosomes seems to play a general role related to symbiotic relationships between the bacteria and their host. Lipids might represent reserve inclusions for the clams; *in situ* experiments on *B. thermophilus* have demonstrated an active incorporation of H_3CO_2 14_C in lipids (Fiala-Médioni *et al.*, 1986 *b*).

The lysosomes, clearly involved in a lysosomic resorption of the bacteria (Fiala-Médioni, 1984; Fiala-Médioni *et al.*, 1986 *a*; Fiala-Médioni, in press), might represent, as well as the direct organic molecular transfer postulated by Felbeck *et al.* (1983), a trophic path for the clam. This is one of the many questions that remain to be answered on the biochemical patterns and processes characterizing the relationships between the bacteria and their hosts.

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