The role of the digestive tract of *Calyptogena laubieri* and *Calyptogena phaseoliformis*, vesicomyid bivalves of the subduction zones of Japan

Digestive tract
Nutrition
Vesicomyidae
Subduction zones
Tube digestif
Nutrition
Vesicomyidae
Zones de subduction

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ABSTRACT

During the 1985 Kaiko expedition on the subduction zones off Japan, four new species of bivalves were collected between 3000 and 6000 m depth. Two of them, *Calyptogena laubieri* and *Calyptogena phaseoliformis*, were studied in the laboratory. Results obtained reveal that several modifications of the digestive tract are related to the method of nutrition. The labial palps are reduced, the stomach seems to have no crystalline style and the intestine is narrow and straight. The main characteristic is the extreme reduction of the digestive gland. However, the presence of diatoms in the stomach of *C. laubieri* and various particles in the digestive gland could indicate that the digestive tract is functional. With the reductions and simplifications of the digestive tract observed in both species, the heterotrophic pathway of nutrition is probably minor compared to the autotrophic pathway by symbiotic relationships with bacteria.

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RÉSUMÉ

Rôle du tube digestif de *Calyptogena laubieri* et *Calyptogena phaseoliformis*, bivalves vesicomyidae des zones de subduction du Japon

Lors de l'expédition Kaïko, effectuée en 1985 sur les zones de subduction proches du Japon, quatre nouvelles espèces de bivalves ont été récoltées entre 3000 et 6000 m de profondeur. Deux d'entre elles, *Calyptogena laubieri* et *Calyptogena phaseoliformis*, ont été étudiées en laboratoire. Les résultats obtenus mettent en évidence des modifications du tractus digestif en relation avec le régime nutritionnel. Les palpes labiaux sont réduits, l'estomac semble dépourvu de stylet cristallin et l'intestin est un tube mince et rectiligne. La caractéristique essentielle est la forte réduction de la glande digestive. Cependant, la présence de diatomées dans l'estomac de *C. laubieri* et de diverses particules dans la glande digestive semble indiquer que ce tractus digestif est fonctionnel. Compte tenu des réductions et simplifications du tractus digestif observées chez les deux espèces, la voie hétérotrophique de nutrition est vraisemblablement mineure par rapport à la voie autotrophique par l'intermédiaire des bactéries symbiotiques.

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INTRODUCTION

During summer 1985, several heterospecific animal communities dominated by bivalves were discovered in the subduction zones around Japan. Four species were represented, from the genus *Calyptogena* which is widely distributed in the Pacific Ocean (Boss, Turner, 1980). They were *C. kaikoi*, *C. laubieri*, *C. nautilei* and *C. phaseoliformis* (Okutani, Métivier, 1986; Métivier *et al.*, 1986).

These bivalves are found in dispersed populations with densities which vary from few individuals to 2000/m^2. Their crude biomass is also variable and can be extremely high, ranging from 16 to 51 kg/m^2 (Laubier *et al.*, 1986). The high value of the biomass is one of the characteristics of this ecosystem compared with identical ocean depths.

These *Calyptogena* are strictly dependent on interstitial fluid emissions which diffuse through the oceanic base under action of pressures generated by the subduction phenomena of the plates. The presence of these animals, in such localities, poses several questions, but of particular interest is their nutritional pathway.

The data that we possess on the diet of the deep-sea Vesicomyidae mainly relate to *Calyptogena magnifica* which inhabits hydrothermal vents of the East Pacific rise. This species feeds by symbiosis with chemosynthetic endocellular bacteria located in the gill epithelium (Cavanaugh, 1983; Fiala-Médioni, 1984; Fiala-Médioni, Métivier, 1986). These sulpho-oxidizing bacteria may obtain their energy according to the metabolic scheme proposed by Felbeck *et al.* (1981; 1983). The use of this nutritional pathway is probably related to the modifications of the digestive tract which tend

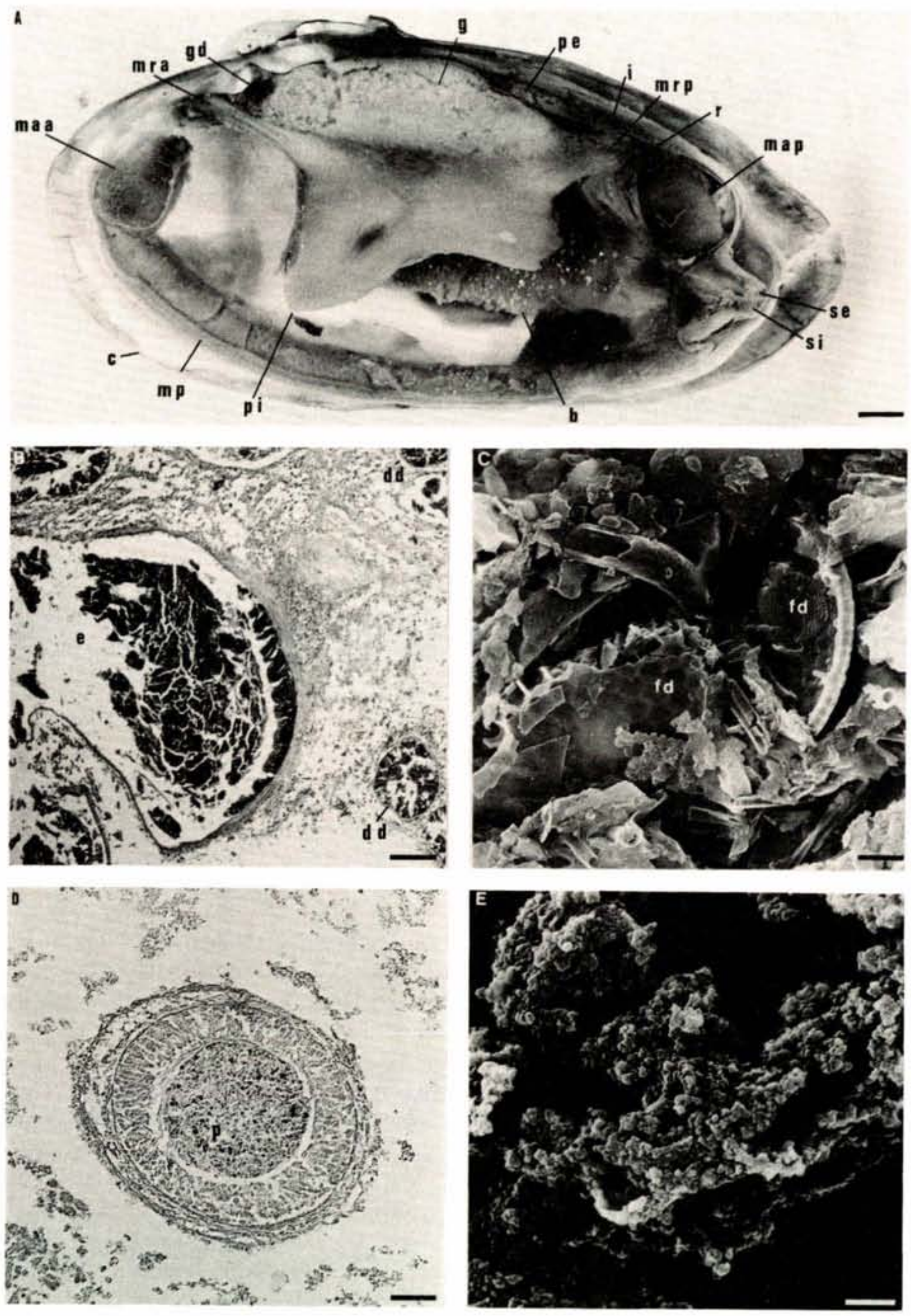

Figure 1

Calyptogena laubieri: *A: Lateral view of a whole animal dissected to show the gross anatomical features. The left gill has been removed; b: gill; c: shell; gd: digestive gland; g: gonad; i: intestine; maa: anterior adductor muscle; map: posterior adductor muscle; mp: pallial muscles; mra: anterior pedal retractor muscle; mrp: posterior pedal rectractor muscle; pe: pericardium; pi: foot; r: kidney; se: excurrent siphon; si: incurrent siphon. Scale bar = 0.5 cm; B: Transverse section through the stomach (e). Some digestive diverticula are present (dd). Scale bar = 1 mm; C: Stomach contents mainly composed of diatom remains (fd). SEM. Scale bar = 2,5 µm; D: Cross section of intestine in its median portion. The epithelial cells surround a lumen filled with particles (p). Scale bar = 80 µm; E: Intestine contents filled with bound particles. SEM. Scale bar = 2 µm.*

Calyptogena laubieri : A : Section médiane et longitudinale d'un animal vu dans sa valve droite ; b : branchie ; c : coquille ; gd : glande digestive ; g : gonade ; i : intestin ; maa : muscle adducteur antérieur ; map : muscle adducteur postérieur ; mp : muscles palléaux ; mra : muscle rétracteur antérieur du pied ; mrp : muscle rétracteur postérieur du pied ; pe : péricarde ; pi : pied ; r : rein ; se : siphon exhalant ; si : siphon inhalant. Échelle = 0,5 cm ; B : Coupe transversale de l'estomac (e). Quelques diverticules digestifs sont présents (dd). Échelle = 1 mm ; C : Contenu stomacal constitué principalement de frustules brisées de diatomées (fd). (MEB). Échelle = 2,5 µm ; D : Coupe transversale de l'intestin dans sa partie médiane. Les cellules épithéliales bordent une lumière remplie de particules (p). Échelle = 80 µm ; E : Contenu intestinal représenté par de petites particules non identifiables (MEB). Échelle = 2 µm.

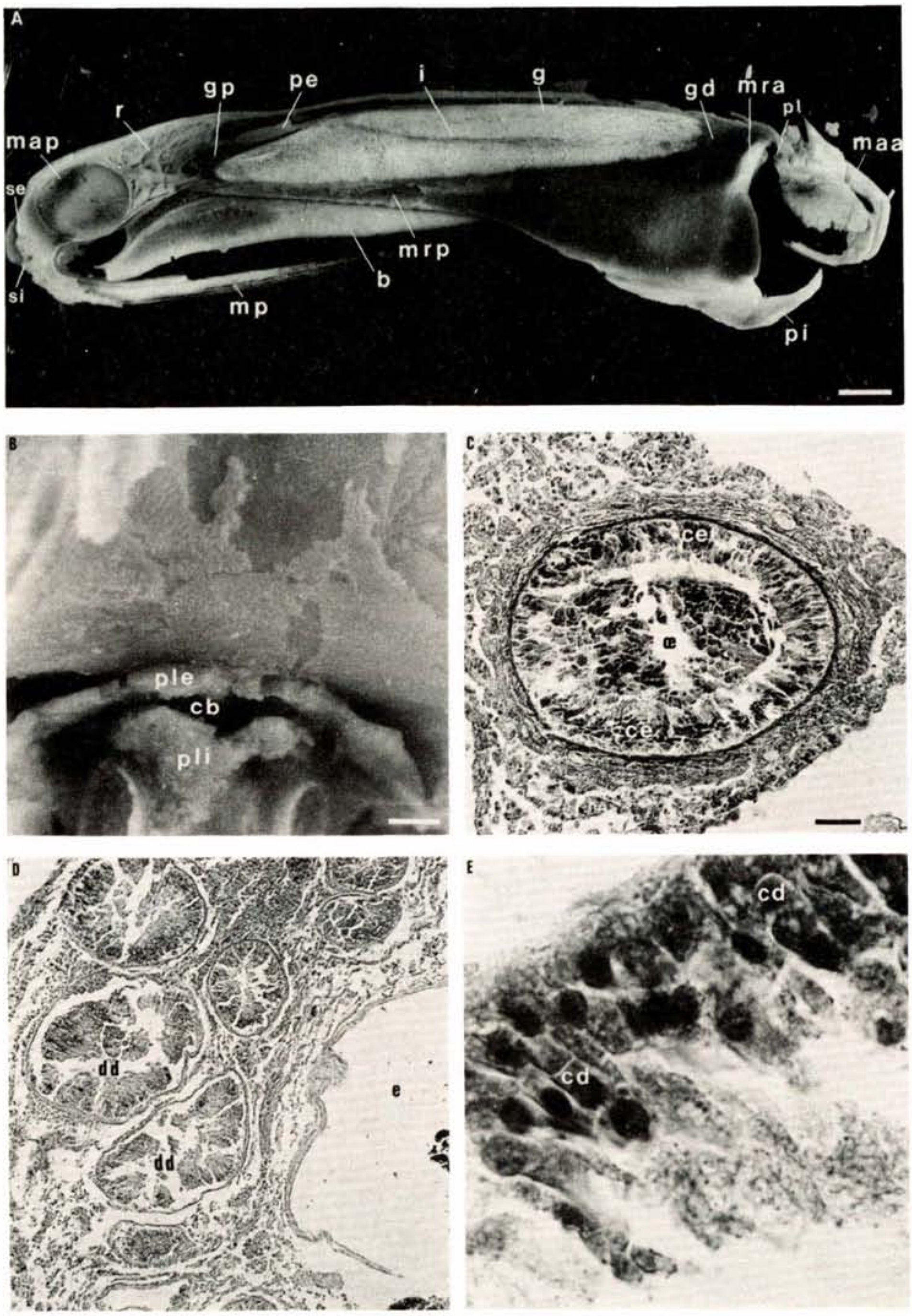

Figure 2

Calyptogena phaseoliformis: *A: Lateral view of a whole animal partly dissected; b: gill; gd: digestive gland; gp: pericardial gland; g: gonad; i: intestine; maa: anterior adductor muscle; map: posterior adductor muscle; mp: palleal muscles; mra: anterior foot retractor muscle; mrp: posterior foot retractor muscle; pe: pericardium; pi: foot; pl: labial palps; r: kidney; se: excurrent siphon; si: incurrent siphon. Scale bar = 0.6 cm; B: Mouth* (cb) *surrounded by the external labial palps* (ple) *and the internal labial palps* (pli)*; maa: anterior adductor muscle. Scale bar = 1 mm; C: Cross-section through the oesophagus* (oe)*. The epithelial cells* (ce) *are tall. Scale bar = 60 µm; D: Cross-section through the digestive diverticula* (dd) *and the stomach* (e)*. Scale bar = 100 µm; E: Detail of the wall of the digestive diverticula consisting only of digestive cells* (cd)*. Scale bar = 40 µm.*

Calyptogena phaseoliformis : A : Animal hors de sa coquille. Coupe médiane et longitudinale ; b : branchie ; gd : glande digestive ; gp : glande péricardique ; g : gonade ; i : intestin ; maa : muscle adducteur antérieur ; map : muscle adducteur postérieur ; mp : muscles palléaux ; mra : muscle rétracteur antérieur du pied ; mrp : muscle rétracteur postérieur du pied ; pe : péricarde ; pi : pied ; pl : palpes labiaux ; r : rein ; se : siphon exhalant ; si : siphon inhalant. Échelle = 0,6 cm ; B : Cavité buccale (cb) entourée des palpes labiaux externes (ple) et des palpes labiaux internes (pli) ; maa : muscle adducteur antérieur. Échelle = 1 mm ; C : Coupe transversale de l'œsophage (oe). Les cellules épithéliales (ce) sont hautes. Échelle = 60 µm ; D : Coupe transversale au niveau de diverticules digestifs (dd) et de l'estomac (e). Échelle = 100 µm ; E : Détail de la paroi de diverticule digestif uniquement constituée de cellules digestives (cd). Échelle = 40 µm.

towards a reduction or loss of function of the various anatomic components (Boss, Turner, 1980).

In *C. laubieri* and *C. phaseoliformis*, the presence of endocellular chemosynthetic bacteria in the gill tissues has been demonstrated (Fiala-Médioni, Le Pennec, 1986) and preliminary observations conducted on the digestive tract have shown that this system is also modified to some extent (Le Pennec, Fiala-Médioni, 1986).

The anatomy of the digestive tract ot these two bivalves was studied using structural and ultrastructural analysis, permitting a fuller understanding of the role played by this system in their nutrition.

MATERIAL AND METHODS

Samples of *Calyptogena* were collected by the submersible Nautile during the French-Japanese Kaiko cruise in June, July and August 1985.

C. laubieri was taken between 3 800 and 4 020 m in Tenryu Canyon and at the top of basement swell of Zenisu Ridge, both of which are located in the eastern Nankai subduction zone. *C. phaseoliformis* was collected in the landward wall of the Japan trench between 5 130 and 5 960 m.

Five specimens of *C. laubieri*, 7 cm length, were fixed in 70% ethanol. Three specimens of *C. phaseoliformis*, between 13 and 15 cm length, were fixed in 10% formol saline and only 1 specimen, 14 cm length, in 6% glutaraldehyde.

In the laboratory, various parts of the digestive tract were dissected and embedded in paraffin after dehydration in ethanol, 5 µm sections were cut with a Minot type microtome and colored with Masson trichome for microscope observations. Some histological sections were critical-point dried, mounted on copper tape, coated under vacuum with approximately 300 Å of gold and examined under the Jeol 35 SEM. Other samples were postfixed in 1% osmic acid and embedded in Spurr. Semi-thin sections were stained with toluidine blue for light microscopy and ultra thin sections were contrasted with uranyl acetate and lead citrate for TEM observations with the Jeol 100 C.

RESULTS

In both species, the digestive tract is poorly representated in the visceral mass. However, the four parts classically defined in bivalves are present: labial palps, mouth and oesophagus; stomach; digestive gland; intestine and anus (Fig. 1 A; Fig. 2 A).

— The labial palps are extremely reduced and flattened against the internal base of the anterior adductor muscle. The two pairs, external and internal, are of unequal size, the internal palps being less developed (Fig. 2 B).

— The opening of the mouth is lenticular.

— The oesophagus is short and the epithelial cells of its wall are columnar (Fig. 2 C). Their nucleus is median.

— The stomach is utricular but no crystalline style was detected. The wall is plicated, mainly in the posterior region and the epithelial cells are of unequal sizes but always tall and narrow on the lateral wall (Fig. 1 B).

— The digestive diverticula are reduced. They are mainly observed ventrally and laterally to the stomach (Fig. 1 A, B; Fig. 2 A, D). Their walls are composed of tall cells (Fig. 2 E).

Ultrastructural observations of the cellular types of the digestive diverticula were only conducted in *C. phaseoliformis*. They reveal the presence of only one cellular type: the digestive cells. Their cytoplasm possesses numerous vacuoles, some of which contain lipids. Mitochondria are abundant. The apical pole of the cells has few if any microvilli. The basal membrane is thin and regular. Numerous electron-dense granulations are observed under this membrane.

— The intestine, in both species, is a long, thin tube without convolutions (Fig. 1 D; Fig. 2 A). The cells of the wall become longer the farther they are from the stomach. The intestine goes through the pericardium. The rectum passes round the posterior adductor muscle (Fig. 3 B) and ends in the anus.

Scanning observations reveal, in *C. laubieri*, the presence of a great quantity of diatom frustules which almost fill the entire stomach cavity (Fig. 1 C). Numerous particles are also observed in the intestine, but they are very degraded and unidentifiable (Fig. 1 E). No bacteria were detected in the digestive tract. In *C. phaseoliformis* the entire digestive tract contains particles from the oesophagus (Fig. 3 C) to the rectum (Fig. 3 F). In the stomach cavity there are numerous small particles which are impossible to identify with certainty (Fig. 3 D), but it would appear that remains of diatom frustules are present.

Occasionally, aggregates of these degraded particles can be seen in the intestine (Fig. 3 E). In both species mineral particles are frequently observed in the digestive tract.

DISCUSSION AND CONCLUSIONS

In bathyal and abyssal depths the quantity of nutritive particles normally present is small, and the organic matter originating from the photic zones of the ocean is not sufficient to allow the establishment of a high invertebrate biomass. According to Somero *et al.* (1983), the values of the biomass for all the fauna normally found at 2 500 m are from 0.1 to 10 g/m^2.

The bivalves are well represented in this scattered fauna. It is by means of various modifications of several systems, but especially the digestive tract, that the bivalves have adapted to great depths. According to Allen and Sanders (1973), these adaptive modifications occur mainly in the labial palps which grow in size; in the stomach, where several chambers are formed; and in the intestine where the surface of contact with nutritive particles is greatly increased by the convolutions. For example, in species of the genus *Abra*, there is a linear relation between the size of the intestine and depth (Allen, Sanders, 1966). On the other hand, the gill of most of deep-sea bivalves is generally abundantly ciliated, this characteristic being related to the transport of nutritive particles, especially diatoms (Allen, Sanders, 1966).

Recent studies of the two dominant species of bivalves of the hydrothermal vents ecosystem of the East Pacific rise, *Bathymodiolus thermophilus* and *Calyptogena magnifica*, have revealed the existence of separate nutritional strategies different from those of other deep-sea bivalves.

The mytilid, *B. thermophilus*, has a mixed diet based both on a symbiotic relationship with chemosynthetic endocellular bacteria (Cavanaugh, 1983; Fiala-Médioni, 1984; Le Pennec, Hily, 1984; Le Pennec *et* *al.*, 1985; Fiala-Médioni *et al.*, 1986) and the digestion of various nutritive particles entering the digestive tract (Le Pennec *et al.*, 1983; Le Pennec, Prieur, 1984; Hily *et al.*, 1986; 1987). The gill assumes a double trophic role, firstly in the capture and transportation of nutritives particles toward the buccal opening, which is carried out by the ciliated cells of the distal parts of filaments. Secondly, the lateral cells which constitute the main part of filaments form bacteriocytes where symbiosis occurs. The digestive tract of this mytilid is almost identical to those of deep-sea bivalves independent of hydrothermal vents and subduction zones. The main difference occurs in the intestine which is straight in *B. thermophilus* and generally has convolutions in deep-sea bivalves. Moreover, it seems that the presence of extracellular enzymes in the intestinal lumen contributes to a complementary digestion of nutritive particles and reinforces the role of the intestine (Hily *et al.*, 1987). The impact of this trophic pathway is not known, as the nutritional quality of particulate material sinking at that depth has not been established.

In *C. magnifica* the digestive tract is highly modified.

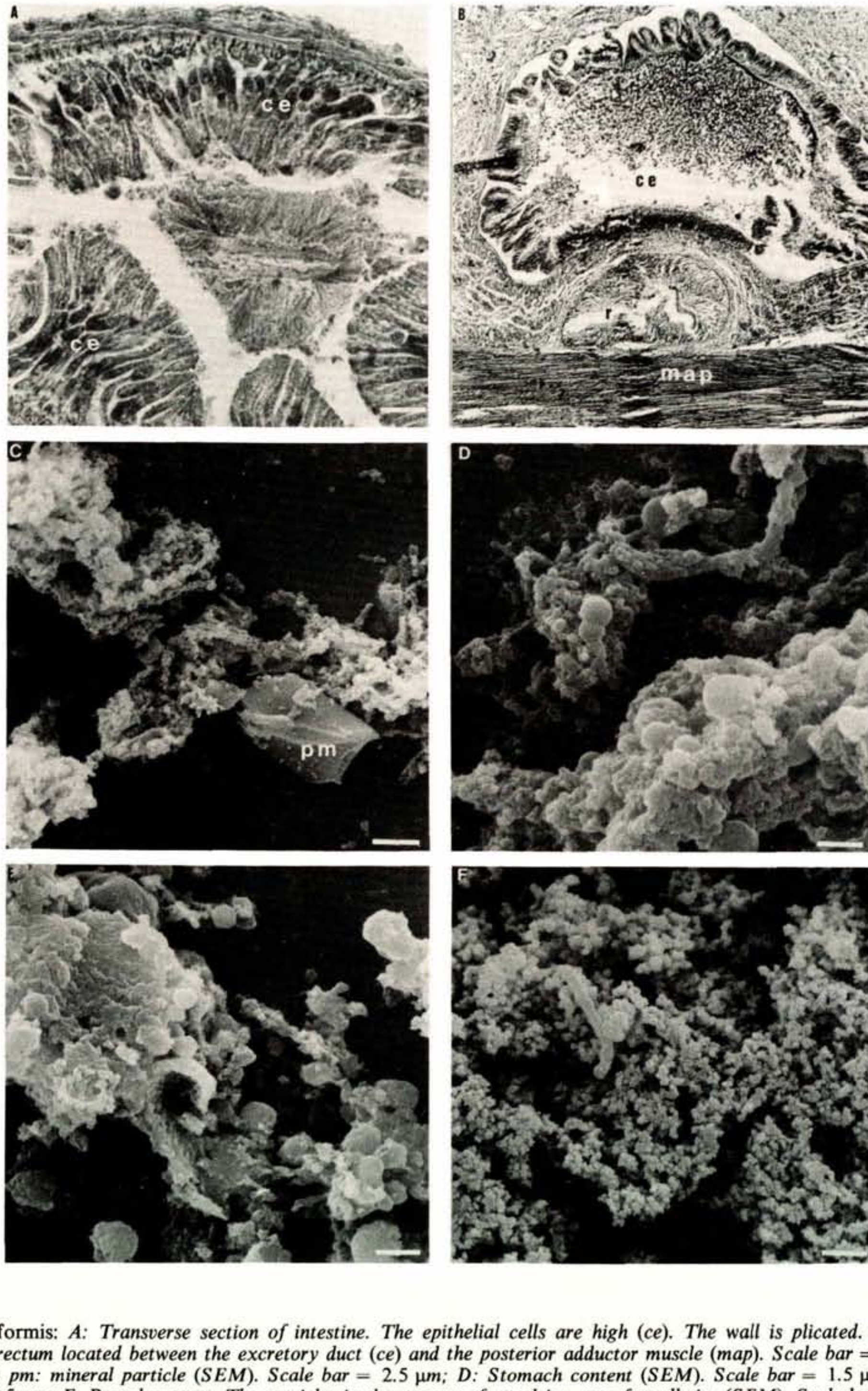

Figure 3

Calyptogena phaseoliformis: *A: Transverse section of intestine. The epithelial cells are high (ce). The wall is plicated. Scale bar = 25 µm; B: Transverse section of rectum located between the excretory duct (ce) and the posterior adductor muscle (map). Scale bar = 70 µm; C: Particles in the oesophagus lumen; pm: mineral particle (SEM). Scale bar = 2.5 µm; D: Stomach content (SEM). Scale bar = 1.5 µm; E: Intestinal content (SEM). Scale bar = 2.5 µm; F: Rectal content. The particles in the process of expulsion are of small size (SEM). Scale bar = 2.5 µm.*

Calyptogena phaseoliformis : A : Coupe transversale de l'intestin. Les cellules épithéliales sont hautes (ce). La paroi est plissée. Échelle = 25 µm; B : Coupe transversale du rectum (r), compris entre un canal excréteur (ce) et le muscle adducteur postérieur (map). Échelle = 70 µm; C : Particules en transit dans l'œsophage; pm : particule minérale (MEB). Échelle = 2,5 µm; D : Contenu stomacal (MEB). Échelle = 1,5 µm; E : Contenu intestinal (MEB). Échelle = 2,5 µm; F : Contenu rectal. Les particules en voie d'expulsion sont de très petites tailles (MEB). Échelle = 2,5 µm.

The labial palps are extremely reduced, the stomach is simple, utricular and without a crystalline style and the intestine has no convolutions (Boss, Turner, 1980). Scanning observations of the stomach contents show that it is always empty, except for a clear whitish mucous-like material. The rectum is also empty and flattened throughout its length (Boss, Turner, 1980). The digestive tract of this species is completely or virtually non-functional and the gill appears to be the only site which provides the nutritional requirements of the bivalve (Cavanaugh, 1983; Fiala-Médioni, 1984; Fiala-Médioni, Métivier, 1986).

In *C. laubieri* and *C. phaseoliformis* the digestive tract is also highly modified. The labial palps are very small, the stomach is a simple chamber probably without a crystalline style and the intestine is a narrow and straight tube. Above all, it is the weak development of the digestive gland which is the main characteristic of this atrophic digestive system. However, it seems that the nutritional role of this system differs from one species to another.

The great quantity of diatom frustules observed in the stomach of *C. laubieri* and the presence of degraded particles in the intestinal tract seem to indicate that the digestive system plays a role in the feeding of this bivalve. There is, however, no doubt that the main trophic function is assumed by the gill, a greatly developed organ in *Calyptogena* and which facilitates the symbiosis with chemosynthetic bacteria.

The digestive apparatus of *C. phaseoliformis* is perhaps functional, as we observed the presence of particulate material in the stomach, in the lumen of digestive diverticula and in the gut. But the digestive gland is reduced to a few acini situated near the stomach and its role is limited. On the other hand, the presence of only one cellular type in the digestive diverticula is uncommon and further observations are needed before any conclusion can be reached. In this species the main trophic role is played by the voluminous gill where chemoautotrophic bacteria are present.

To date, *C. magnifica*, which lives around 2 500 m depth, constitutes the only deep sea Vesicomyidae studied in which the trophic role is entirely assumed by the gill. In *C. laubieri* and, to a lesser extent, in *C. phaseoliformis* which lives deeper, it appears that the digestive tract is able to carry out a limited amount of assimilation and digestion of nutritive particles.

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