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The digestive system of *Euphausia krohni* (Crustacea, Euphausiacea): functional morphology and phylogenetic relevance

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Abstract: This report deals with the structure and ultrastructure of the labrum, foregut, midgut and midgut gland of *Euphausia krohni*. The labrum appears to play a major role in the first step of the digestive process. It is involved in both mechanical and chemical breakdown of the food. Structural, ultrastructural and cytochemical data on the labrum reveal similarities with the labrum of the primitive Mysidacea *Lophogaster typicus* (Lophogastrida). The foregut acts predominantly in triturating the food and in separating fine particles and fluids from coarse material by filters. Morphological features indicate that the foregut of euphausiids might have evolved from the same ancestral morphological type as the mysidaceans, i.e. close to *Gnathophausia gracilis* (Lophogastrida). The midgut exhibits typical ultrastructural features known in the Malacostraca, i.e. an epithelium composed of a single cell type which is implicated in absorption and production of peritrophic membranes. The midgut gland exhibits F-cells, considered to secrete enzymes in Decapoda, and not truly evidenced in the Mysidacea. Therefore, this study suggests that the digestive system of Euphausiacea has both primitive features evidenced in the lower Malacostraca (Lophogastrida), and also evolved ones found in the higher Malacostraca (Decapoda).

Résumé : *Le système digestif de* Euphausia krohni *(Crustacea, Euphausiacea) : morphologie fonctionnelle et intérêt phylétique*. La structure et l'ultrastructure du labre, de l'estomac, de l'intestin et de l'hépatopancréas ont été étudiées chez *Euphausia krohni*. Le labre joue un rôle majeur dans les premières phases du cycle digestif. Il prépare les aliments d'une manière mécanique et chimique. Les données structurales, ultrastructurales et cytochimiques montrent des similitudes avec le labre du mysidacé primitif *Lophogaster typicus* (Lophogastrida). Dans l'estomac, les aliments sont malaxés et les particules les plus fines et les fluides sont filtrés. La morphologie indique que l'estomac des euphausiacés a vraisemblablement évolué à partir d'un type morphologique ancestral proche de celui du mysidacé *Gnathophausia gracilis* (Lophogastrida). L'intestin moyen présente des caractéristiques ultrastructurales connues chez les malacostracés, à savoir un épithélium composé d'un seul type cellulaire, impliqué dans l'absorption et la production des membranes péritrophiques. L'hépatopancréas possède des cellules F, connues pour sécréter des enzymes chez les décapodes, et dont l'existence n'a pas été clairement démontrée chez les mysidacés. Ainsi, cette étude suggère que le système digestif des euphausiacés possède des caractères primitifs, qui existent chez les malacostracés inférieurs (Lophogastrida), mais aussi des caractères évolués que l'on retrouve chez les malacostracés supérieurs (Decapoda).

Keywords: ultrastructure, labrum, foregut, F-cells, Crustacea, Euphausiacea.

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Introduction

Many studies focusing on the digestive system in euphausiids, one of the most important groups in marine pelagic food webs, deal with the morphology of the peri-oral structures and of the foregut or with the feeding habits of these crustaceans (Mauchline & Fisher, 1969; Kils, 1983; Ikeda et al., 1984; Ishii, 1986; Hamner, 1988; Suh & Nemoto, 1988; Ritz et al., 1990; Ullrich et al., 1991; Suh & Toda, 1992; De Jong-Moreau et al., 2001; Meyer et al., 2002). However, the ultrastructural features of the labrum, midgut and midgut gland of euphausiids have never been investigated. In euphausiids and other Malacostraca, it has been reported that the foregut morphology is more related to the phylogenetic history than to the diet of species (Suh & Nemoto, 1988; Felgenhauer & Abele, 1989; De Jong-Moreau & Casanova, 2001). On the contrary, the euphausiid and mysid mandibles exhibit the same morphological adaptations according to the diet of the species (De Jong-Moreau et al., 2001).

Among Malacostraca, the phylogenetic relationships are still a matter of debate. In a previous study, it has been proposed that the Euphausiacea are more closely related to the Mysida than to the Decapoda (Jarman et al. 2000) as it is generally thought (Schram, 1981; Abele, 1982; Dahl, 1983; Hessler, 1983; Schram & Hof, 1998; Casanova et al., 2002). Since congruence between different characters is the best tool to determine evolutionary history, this work was aimed at investigating the main sites implicated in the digestive function of an euphausiid and at comparing them with other Malacostraca in view to a complementary approach in phylogeny.

For this purpose, the structure and ultrastructure of the labrum, foregut, midgut and midgut gland of an omnivorous species (Casanova, 1974) of euphausiids, *Euphausia krohni* Brandt, 1851, have been investigated.

Material and methods

Specimens of *Euphausia krohni* Brandt, 1851 were collected off Marseille (43°08'07N; 5°15'5E, May 2001, at a depth of 600 m). For scanning electron microscopy (SEM) observations, animals were preserved in 70% ethanol solution and dissected under a stereomicroscope. Labrum and foreguts were critical point-dried, sputter coated with gold and examined with an environmental SEM (Philips XL 30). Other specimens were fixed in 0.2 M sodium cacodylate buffer (pH 7.3) containing 2% glutaraldehyde, 1% paraformaldehyde, 30% filtered seawater, at 4°C (osmolarity 1200 mOsM). Labrum, midgut and midgut gland were then dissected, washed in 0.2 M cacodylate buffer, postfixed in buffered 1% osmium tetroxide (Arnaud et al., 1988) and embedded in Epon. Semi-thin sections were then stained with 50% Unna blue for light microscopy. For ultrastructural observations, thin sections were stained with uranyl acetate followed by lead citrate (Reynolds, 1963) before examination with a Zeiss EM 912 electron microscope at an accelerating voltage of 80 kV. Other thin sections were used for the PATAg cytochemical test (Thiéry & Rambourg, 1974) in view to detect polysaccharides in glandular secretions. For this purpose, sections were mounted on gold grids and treated as follows: oxidation in 1% periodic acid solution for 25 min, incubation in 0.2% TCH (thiocarbohydrazide, Serva) in a 20% acetic acid solution for 6 min to 72 h and final treatment with 1% silver proteinate in distilled water for 20-30 min. In control sections, the periodic acid was substituted by 10% H_2O_2.

Results

Labrum

The labrum is symmetrical (Fig. 1A). Enlargements of its external face show many pores (Fig. 1B, C). Labral glandular units related to those pores consist of numerous acini with three to four large cells (Fig. 1D, E). Two types of acini have been found, one with clear cells and the other one with dark cells (Fig. 1D, E). In both types, the glandular cells, enlarged in their basal part containing a round to oval nucleus, display abundant secretory granules occupying the whole mature cell cytoplasm (Fig. 2A, E). In clear immature cells, many Golgi complexes are found in the vicinity of the nucleus (Fig. 2A). These Golgi complexes are composed of three to four flattened saccules and exhibit a cis- and trans-Golgi network particularly dilated and filled with clear material. The last saccules of the trans-Golgi network produce clear vesicles at the origin of large clear heterogeneous granules (Fig. 2C). Clear mature granules fuse together (Fig. 2B) to form large clear areas that occupy almost all the cytoplasm of mature cells (Fig. 2A, D). Dark cells exhibit small numerous dark homogeneous granules that tend to fuse together to form larger ones (Fig. 2E, F). In dark mature cells, granules are so numerous that typical organelles such as rough endoplasmic reticulum (RER) and Golgi complexes are difficult to observe, and only small mitochondria are recognizable (Fig. 2G). In clear acini, the PATAg cytochemical test gives a strong positive reaction that already occurs after a 40 min incubation in TCH, which indicates an abundance of polysaccharides very sensitive to periodic acid (Fig. 2H, I). This reaction is located in the matrix of the clear granules and is more intense in the granules themselves than in large areas of fused granules. The content of the excretory ducts also exhibits a positive reaction (Fig. 2H, I). On the other hand, in dark acini, dark granules appear to be PATAg-negative (Fig. 2J, K).

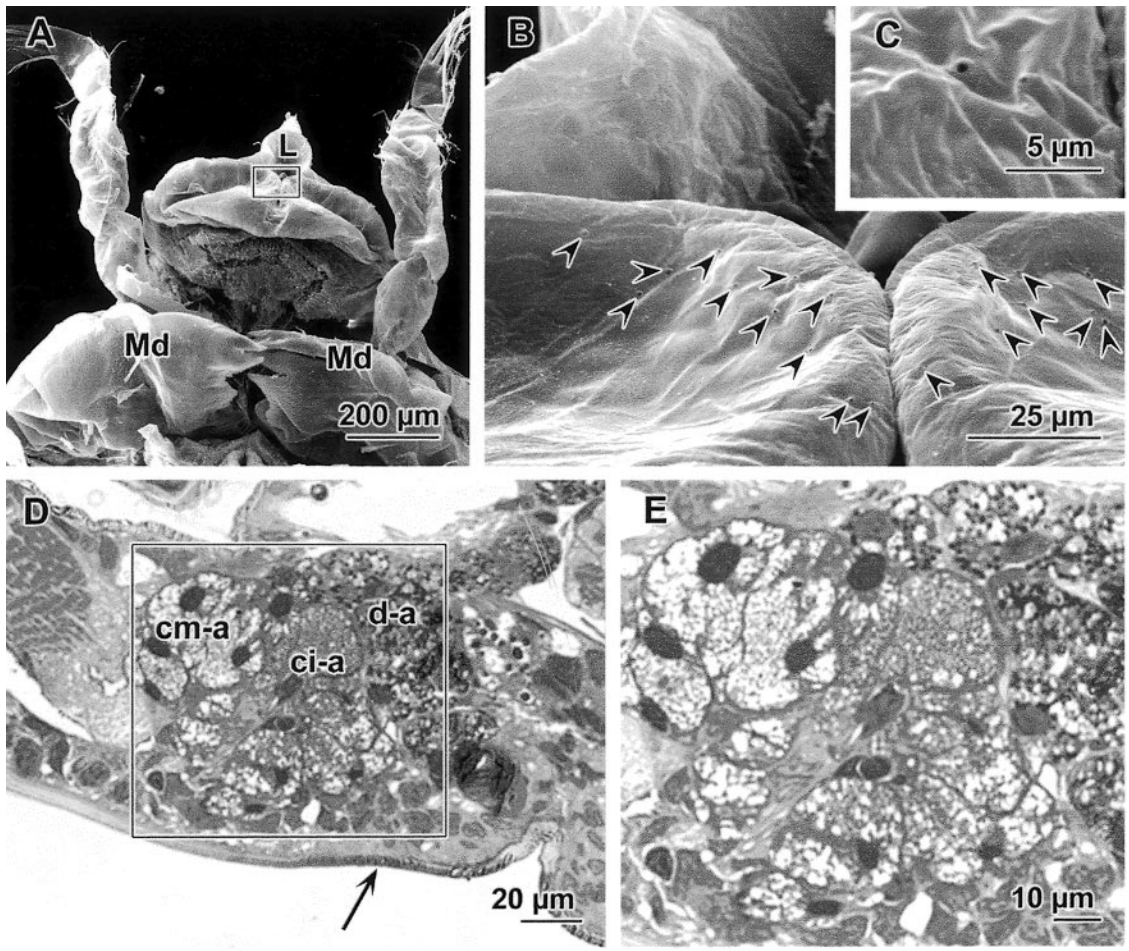


Figure 1. *Euphausia krohni*. **A-C**. Scanning electron micrographs of the labrum. **A**. External view. **B**. Detail of the labrum corresponding to the area boxed in figure 1A: note the presence of the numerous pores (*arrowheads*). **C**. Enlargement of a labral pore. **D, E**. Semi-thin sections of the labrum showing different labral glandular units. (*arrow*) edge of the labrum. **E**. Detail of the acini corresponding to the area boxed in figure 1D. (*ci-a*) clear immature acinus, (*cm-a*) clear mature acinus, (*d-a*) dark acinus, (*L*) labrum, (*Md*) mandible.

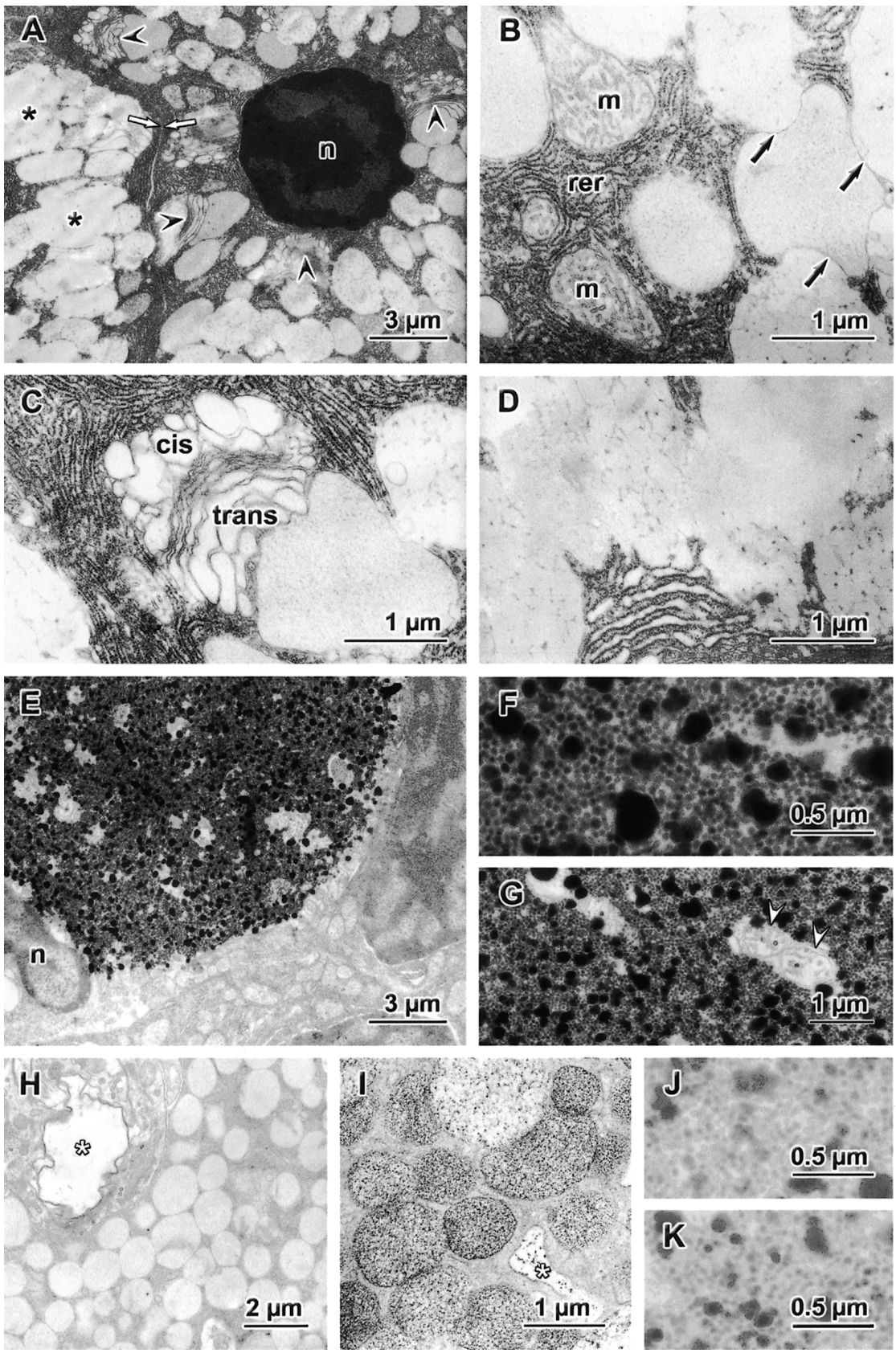
Figure 1. *Euphausia krohni*. **A-C**. Micrographies de microscopie électronique à balayage du labre. **A**. Vue externe. **B**. Agrandissement de la région du labre encadrée dans la figure 1A : noter la présence de nombreux pores (*pointes de flèches)*. **C**. Détail d'un pore labral. **D**, **E**. Coupes semi fines du labre montrant les différentes unités glandulaires. (*flèche*) bord du labre. **E**. Agrandissement des acini de la figure 1D. (*ci-a*) acinus clair immature, (*cm-a*) acinus clair mature, (*d-a*) acinus sombre, (*L*) labre, (*Md*) mandibule.

Foregut

The foregut exhibits an elongated shape. It is composed of a long cardiac chamber ending in a narrow area where the gastric mill (the dorso-lateral teeth and cluster spines) protrudes (Fig. 3A, B, C). The cardiac chamber filters the grinding food towards a very efficient primary filter provided with plumose setae (Fig. 3D, E). The gastric mill lacks a median dorsal tooth and the foregut is devoid of a secondary filter. Indeed, the pyloric chamber is reduced to its pyloric valves and a dorsal funnel region (Fig. 3A, F).

Midgut

All the midgut intestinal cells are of the same type with absorptive and secretory functions. They display a basal nucleus (not shown), normally developed microvilli (height ~ 500 nm), mitochondria with tubular cristae, both rough and smooth endoplasmic reticulum made up of short cisternae, and several Golgi complexes with 5 or 6 flattened saccules producing secretory granules (Fig. 4A-C). Apical secretory granules are present. They are of a single type and display light or moderately dense homogeneous contents; they are not very numerous and are present in the first half of the midgut. They are similar to the peritrophic membranes surrounding the faecal pellets (Fig. 4A). The intestinal epithelium lies on a thick basal lamina (~ 460-500 nm) composed of a median layer (~ 36-40 nm) with light homogeneous material completed on both sides with a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dense fibrogranular layer that is thicker on the outer side (~ 370-410 nm) than on the inner subepithelial one (~ 45-50 nm) (Fig. 4D).

Midgut gland

The midgut gland consists of two voluminous lobes which occur on either side of the midgut and extend along the cephalothorax. Each lobe, ramified into small numerous tubules, is connected separately to the junction of the foregut and midgut by a primary duct (data not shown).

The three main cell types (R, B and F) reported for other Malacostraca, and especially for Decapoda, are present in the midgut gland of *Euphausia krohni*.

R-cells (resorptive-cells) are the most numerous. They display long apical microvilli (2-3 µm), numerous mitochondria and a well-developed network of smooth endoplasmic reticulum at the base of the cell (not shown). Huge lipid droplets surrounded by RER occupy a large part of each cell (Fig. 5A).

B-cells (blister-like-cells) are dominated by one large vacuole encompassed with a thin layer of cytoplasm (Fig. 5B). The nucleus is restricted to the basal part of the cell (Fig. 5B). The fine structure of B-cells comprises short and irregular microvilli, and many apical tubules and vesicles implicated in endocytosis (Fig. 5C).

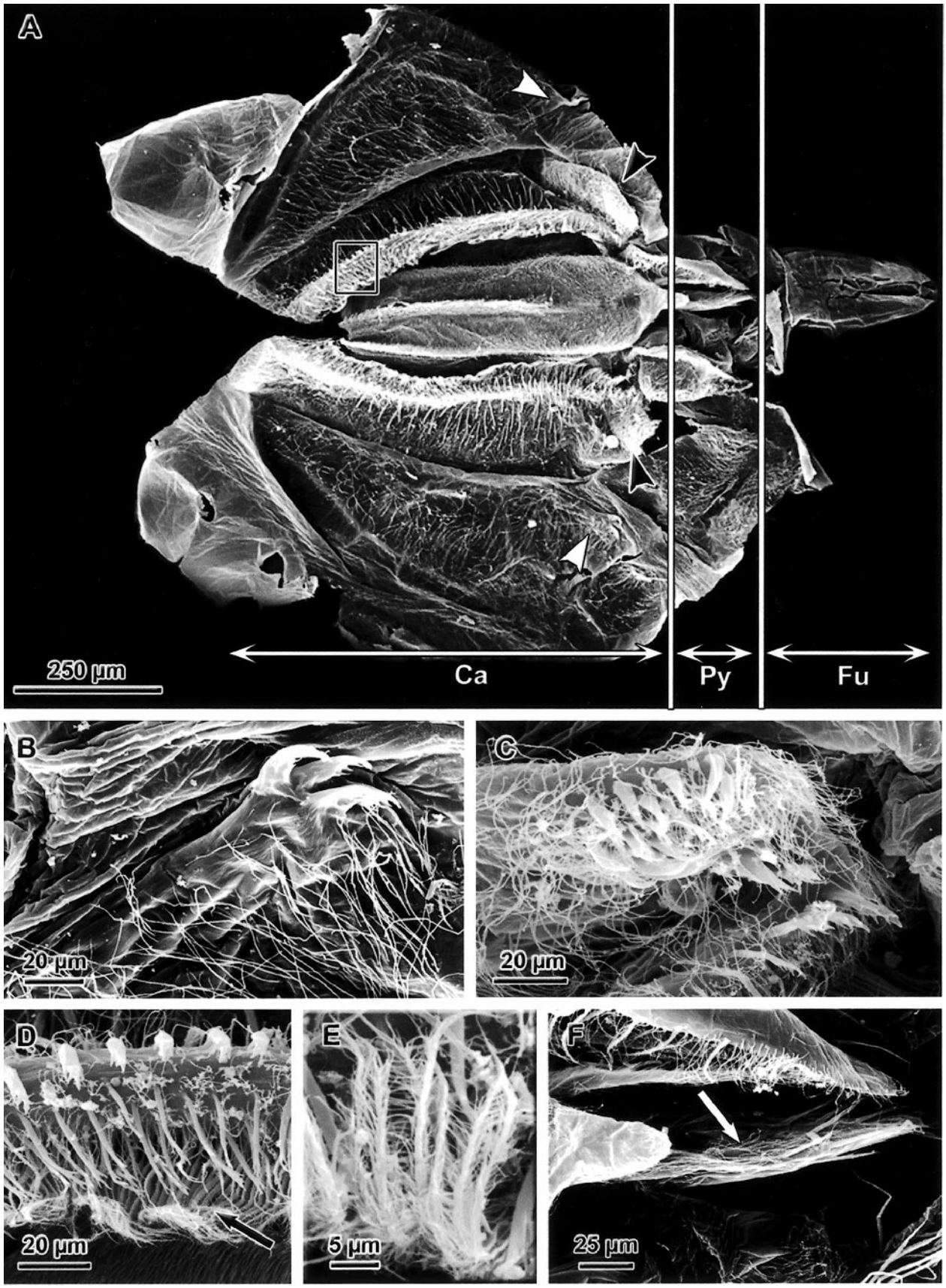
F-cells (fibrillar-cells) display a very extensive RER arranged in long parallel cisternae and numerous mitochondria (Fig. 5D). Golgi complexes are common (Fig. 5E) and produce many vacuoles at the origin of typical enzyme-like granules (Fig. 5F).

Discussion

In *Euphausia krohni*, the presence of numerous excretory pores on the external face of the labrum, related to numerous labral glandular units, is an interesting finding. Indeed, presence and distribution of pores have been described over the entire integument in pelagic crustaceans and euphausiids (Mauchline, 1977) and tegumental glands have been described in Decapoda (Felgenhauer, 1992). These dermal glands are generally scattered across the surface of the body. However, they could be concentrated in such areas as paragnaths, pereiopods and pleopods, oesophagus, foregut, hindgut, statocyst region and gills (Felgenhauer, 1992). To our knowledge, concentration of these units at the labral level has never been reported before in other Malacostraca, except recently for the Lophogastrida *Lophogaster typicus* M. Sars, 1857 (Mysidacea) (De Jong et al., 2002). More striking are the strong similarities in the structural and the ultrastructural organization of these glandular units between the two taxa: (1) the labral glandular units are organized in small acini of three to four large cells; (2) two kinds of glandular units are recognizable, i.e. clear and dark acini. These features of the labral glandular units differ from those of other tegumental glands found in various body regions of Decapoda. For these latter, isolated unicellular glands are more common than multicellular glands (Felgenhauer, 1992) and each glandular unit produces cytoplasmic electron-dense and electron-lucent secretory granules. These differences can be explained by a peculiar function according to the body region. Indeed, several functions have been attributed to tegumental glands: secretion of epicuticle, tanning of the

Figure 2. *Euphausia krohni*. Transmission electron micrographs of labral glandular units. **A-D**. Ultrastructure of clear cells. **A**. Immature (right) and mature (left) clear cells: note numerous large clear granules. *Opposing arrows* indicate plasma membranes of these two adjacent cells, (*arrowheads)* Golgi complexes, (*asterisks)* large areas of clear fused granules in mature cell. **B**. Immature clear cell. *Arrows* indicate the membranes of three granules ready to fuse together. **C**. Golgi complex. Note the dilated cis- and trans-Golgi network and the presence of a large clear vesicle against the last trans-Golgi saccule. **D**. Fused clear granules in a mature cell. **E-G**. Ultrastructure of dark cells. **E**. Portion of a dark cell. **F**. Fusion of small dark granules. **G**. Small mitochondria among dark granules (*arrowheads*). **H-K**. PATAg cytochemical test on labral glandular cells. Control (**H**) and PATAg test (**I**) in clear cells after 40 min incubation in TCH. Reactive clear granules and reactive content of the excretory duct (*asterisk*). Control (**J**) and PATAg test (**K**) in dark cells after 72 h incubation in TCH: no reactivity is evidenced. (*cis*) cis-Golgi network, (*m*) mitochondria, (*n*) nucleus, (*rer*) rough endoplasmic reticulum, (*trans*) trans-Golgi network.

Figure 2. *Euphausia krohni*. Micrographies de microscopie électronique à transmission d'unités glandulaires labrales. **A-D**. Ultrastructure des cellules claires. **A**. Cellules claires immature (à droite) et mature (à gauche) : notez les nombreux granules clairs volumineux. Les *flèches opposées* indiquent les membranes plasmiques des deux cellules adjacentes, (*pointes de flèches*) dictyosomes, (*astérisques*) larges plages de granules clairs fusionnés dans la cellule mature. **B**. Cellule claire immature. Les *flèches* montrent les membranes de trois granules prêts à fusionner. **C**. Dictyosome. Notez les saccules cis- et trans-golgiens dilatés et la présence d'une grande vésicule claire coté trans. **D**. Granules clairs fusionnés dans une cellule mature. **E-G**. Ultrastructure de cellules sombres. **E**. Portion d'une cellule sombre. **F**. Fusion de petits granules sombres. **G**. Petites mitochondries (*pointes de flèches*) parmi les granules sombres. **H-K**. Test PATAg au niveau des cellules glandulaires labrales. Témoins (**H**) et test PATAg (**I**) après 40 min d'incubation des cellules claires dans du TCH. Marquage des granules clairs et du contenu d'un canal excréteur (*astérisque*). Témoins (**J**) et test PATAg (**K**) après 72 h d'incubation des cellules sombres dans du TCH : absence de marquage. (*cis*) réseau cis-Golgien, (*m*) mitochondrie, (*n*) noyau, (*rer)* réticulum endoplasmique granulaire, (*trans*) réseau trans-Golgien.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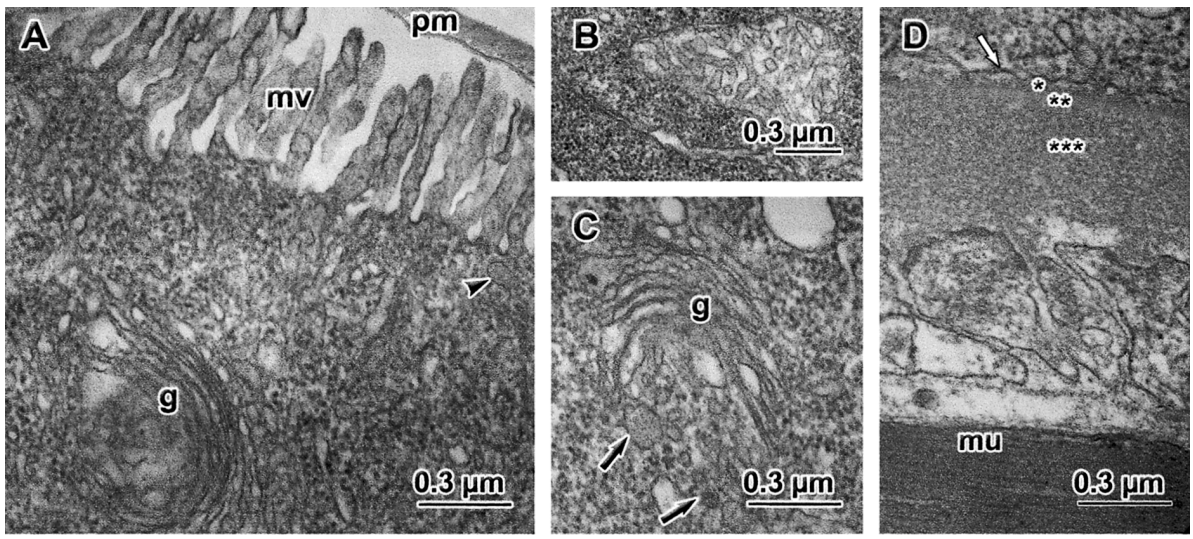

Figure 4. *Euphausia krohni*. Transmission electron micrographs of the midgut epithelium. **A**. Apical part of an intestinal cell. (*arrowhead*) secretory granule. **B**. Mitochondria. **C**. Golgi complex, (*arrows*) Golgi vesicles filled with homogeneous material. **D**. Detail of the large basal lamina, (*arrow*) plasma membrane, (*asterisk*) inner layer, (*double asterisk*) median layer, *(triple asterisk*) outer layer. (*g*) Golgi complex, (*mu*) muscles, (*mv*) microvilli, (*pm*) peritrophic membrane.

Figure 4. *Euphausia krohni*. Micrographies de microscopie électronique à transmission de l'épithélium intestinal. **A**. Partie apicale d'une cellule intestinale. (*pointe de flèche*) granule de sécrétion. **B**. Mitochondrie. **C**. Dictyosome, (*flèches*) vésicules golgiennes contenant du matériel homogène. **D**. Détail de l'épaisse lame basale, (*flèche*) membrane plasmique, (*astérisque*) couche sous épithéliale, (*double astérisque*) couche centrale, (*triple astérisque*) couche externe. (*g*) Dictyosome, (*mu*) muscles, (*mv*) microvillosités, *(pm*) membrane péritrophique.

integument and mucus production especially for the paragnaths (Felgenhauer, 1992). In Crustacea other than Malacostraca, labral glandular units are also observed e.g. in Copepoda (Arnaud et al., 1988; Nishida & Ohtsuka, 1996), Cladocera (Zaffagnini & Zeni, 1987), Branchiopoda (Zeni & Stagni, 2000) and Cephalocarida (Elofsson et al., 1992). However, they are composed of one or two cells only and are generally organized in syncitia. Among crustaceans, the organization of the labral glandular units into true acini seems to be an unusual feature only described so far in two Malacostraca, i.e. *Lophogaster typicus* (De Jong et al., 2002) and *E. krohni* (present work). Similarities are observed with the salivary glands of insects where the units are also organized in acini (Sauer et al., 2000). In *L. typicus* and *E. krohni*, the appearance of the clear granules that contain heterogeneous material, and the strong positive PATAg reaction indicate the presence of polysaccharides. These acini might be involved in the synthesis of mucopolysaccharides (De Jong et al., 2002). Furthermore, in dark cells, the slight positive PATAg reaction of the granules after a 72 h incubation in TCH supports the hypothesis of a glycoproteinic composition. As proposed for *L. typicus*, dark cells of *E. krohni* may be involved in high production of zymogen-like proteins. The mucopolysaccharide production could serve for agglutination of the soft food particles, while glycoproteinic digestive enzymes could be involved in the initial phase of digestion. Thus, the labrum seems to play an important role in the digestive function of the euphausiid crustacean *E. krohni*. It is involved both in the mechanical, with the help of mandibles,

Figure 3. *Euphausia krohni*. Scanning electron micrographs of the foregut. **A**. Dorsal view with dorsal wall removed. The gastric mill, i.e. cluster spines (*black arrowheads*) and dorso-lateral teeth (*white arrowheads*), is located at the posterior end of the cardiac chamber. **B**. Left dorso-lateral teeth. **C**. Right cluster spines. **D**. Detail of the right ventro-lateral ridge corresponding to the area boxed in the figure 3A. The plumose setae (*arrow*) constitute the efficient primary filter. **E**. Enlargement of the plumose setae. **F**. Pyloric valves showing the absence of plumose setae (*arrow*). (*Ca*) cardiac chamber, (*Fu*) funnel region, (*Py*) pyloric region.

Figure 3. *Euphausia krohni*. **A-C**. Micrographies de microscopie électronique à balayage de l'estomac. **A**. Vue dorsale après ouverture de la paroi dorsale. Le moulin gastrique, i.e. structures épineuses (*pointes de flèches noires*) et dents dorso-latérales (*pointes de flèches blanches*), est situé à l'extrémité postérieure de la chambre cardiaque. **B**. Dent dorso-latérale gauche. **C**. Structure épineuse droite. **D**. Agrandissement du repli ventro-latéral droit correspondant à l'encart de la figure 3A. Les soies plumeuses (*flèche*) forment le filtre primaire efficace. **E**. Agrandissement des soies plumeuses. **F**. Valves pyloriques montrant l'absence de soies plumeuses (*flèche*). (*Ca*) Chambre cardiaque, (*Fu*) région tunnel, (*Py*) région pylorique.

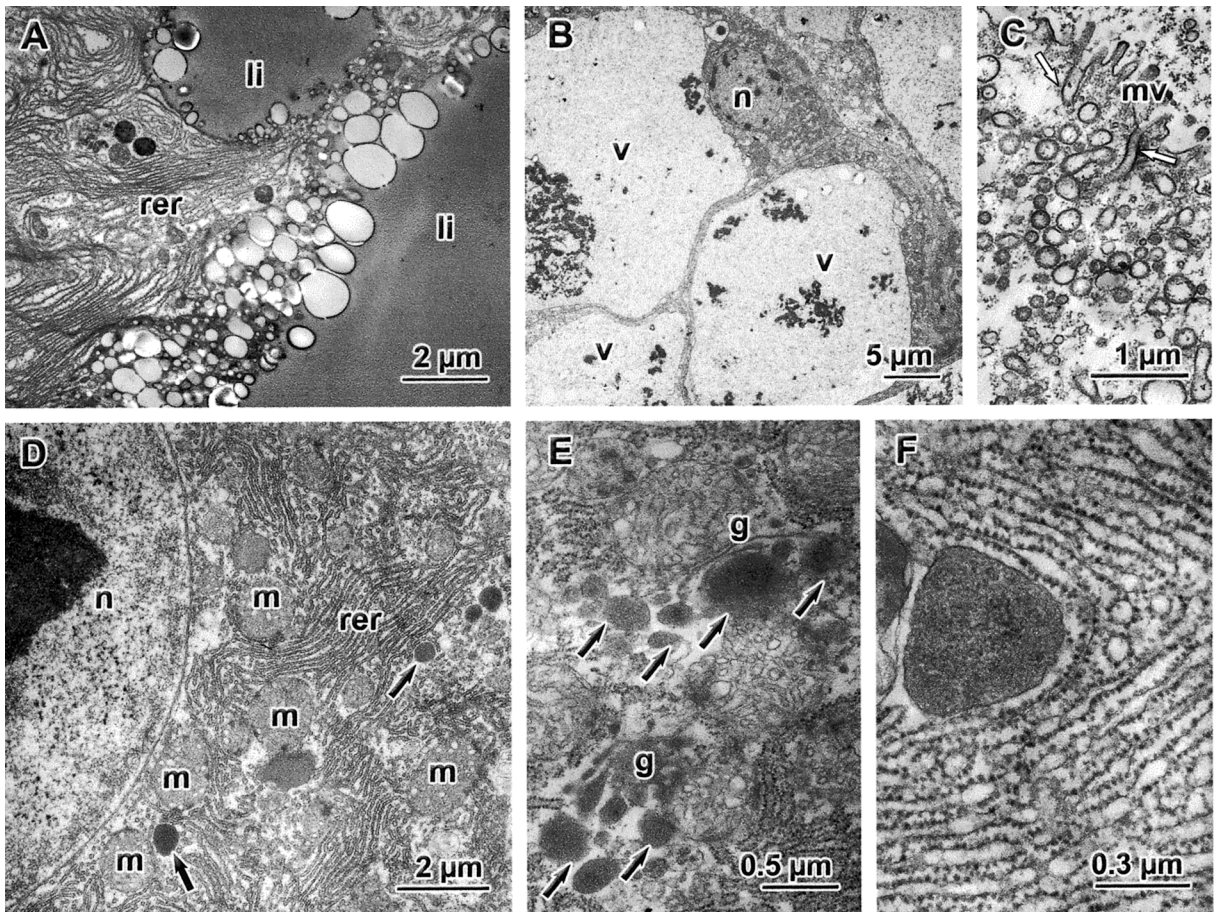

Figure 5. *Euphausia krohni*. Transmission electron micrographs of midgut gland cells. **A**. R-cell. **B**. B-cells with their large vacuole. **C**. Apical part of a B-cell showing the formation of numerous endocytotic vesicles (*arrows*). **D-F**. F-cells. **D**. Typical enzyme-like granules (*arrows*) at the vicinity of the nucleus. **E**. Golgi complexes with Golgi vesicles at the origin of the typical enzyme-like granules (*arrows*). **F**. Typical enzyme-like granule. (*g*) Golgi complex, (*li*) large lipid droplet, (*m*) mitochondria, (*mv*) microvilli, (*n*) nucleus, (*rer*) rough endoplasmic reticulum, (*v*) vacuole.

Figure 5. *Euphausia krohni*. Micrographies de microscopie électronique à transmission des cellules hépatopancréatiques. **A**. Cellule R. **B**. Cellules B avec leur grande vacuole. **C**. Partie apicale d'une cellule B montrant la formation de nombreuses vésicules d'endocytose. **D-F**. Cellules **F**. **D**. Granules ayant l'aspect de granules enzymatiques près du noyau (*flèches*). **E**. Dictyosomes avec leurs vésicules à l'origine des granules enzymatiques (*flèches*). **F**. Granule enzymatique typique. (*g*) Dictyosome, (*li*) grande gouttelette lipidique, (*m*) mitochondrie, (*mv*) microvillosités, (*n*) noyau, (*rer*) réticulum endoplasmique granulaire, (*v*) vacuole.

and the chemical breakdown of the food. This chemical role of the labrum is, to our knowledge, unknown in other Malacostraca, except for the primitive Lophogastrida *L. typicus* (Crustacea, Peracarida) (De Jong et al., 2002).

The foregut of *E. krohni* is very similar to that of *E. superba* Dana, 1850 previously studied by Ullrich et al. (1991). Therefore, only the main morphological features are described here and comparisons are discussed with regard to a phylogenetic background. As previously demonstrated for the Mysidacea (Casanova et al., 1998; De Jong-Moreau & Casanova, 2001), the morphological features of the foregut are of a great interest as a helpful tool for studying phylogenetic relationships. Ullrich et al. (1991) have pointed out that the foregut of the Euphausiacea *Bentheuphausia amblyops* G. O. Sars, 1885, the only species in the Bentheuphausiidae family, possesses a secondary filter (also named pyloric filter), whereas this filter is absent in all the euphausiid species examined so far (Suh & Nemoto, 1988). The authors agree with Ullrich et al. (1991) stating that "the loss of a secondary filter may be related to the formation of a complex and effective primary filter, which substituted the pyloric apparatus". The plumose setae found in the primary filter of *E. krohni* are exactly of the same type as those found in the secondary filter of all the Mysidacea and Decapoda. These latter crustaceans that exhibit a secondary filter possess a less efficient primary filter devoid of such plumose

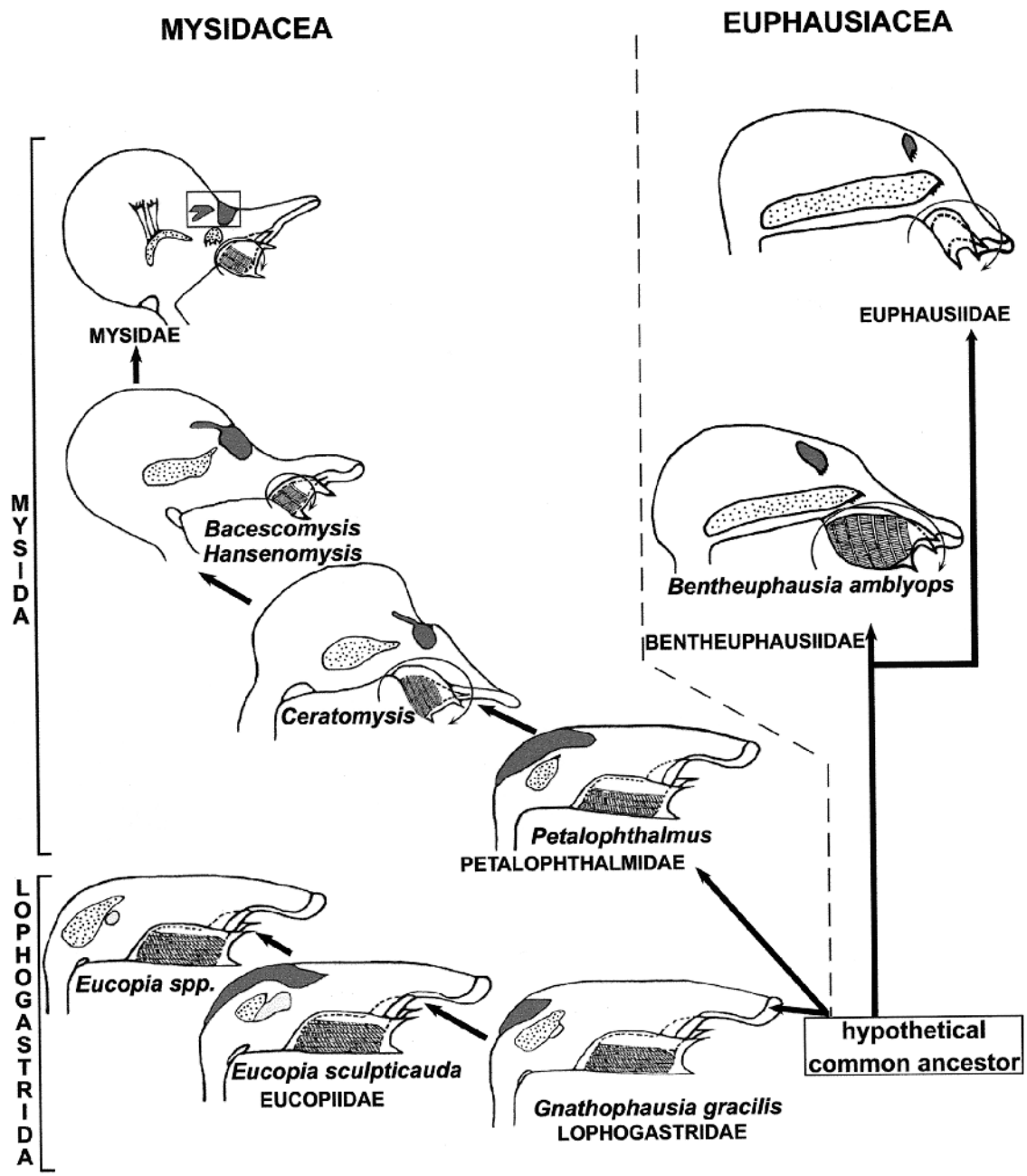

Figure 6. Synoptic diagram of the main evolutionary features of the foregut morphology within the Mysidacea and the Euphausiacea. Only the right structures have been represented. *Dotted area*: lateral teeth of the Mysidacea corresponding to the cluster spines of the Euphausiacea; *grey area*: median dorsal tooth of the Mysidacea corresponding to the paired dorso-lateral teeth of the Euphausiacea. In Mysidae, the dorso-lateral teeth (*left element in the square*) result from the splitting of the median dorsal tooth (*right element in the square*). The gastric mill migrates from the anterior to the posterior cardiac region of the foregut; *hachured area*: secondary pyloric filter. Note the bending of the pyloric region (*curved arrows*), the reduction of the secondary pyloric filter in the Mysidae and its missing in the Euphausiidae.

Figure 6. Schéma synoptique retraçant les principales caractéristiques évolutives de la morphologie de l'estomac chez les mysidacés et les euphausiacés. Seules les structures latérales droites ont été représentées. *Zone pointillée* : dents latérales des mysidacés correspondant aux structures épineuses des euphausiacés ; *zone grisée* : dent dorsale médiane des mysidacés correspondant à la paire de dents dorso-latérales des euphausiacés. Chez les Mysidae, les dents dorso-latérales (*élément de gauche dans l'encart)* sont issues de la dent médiane dorsale (*élément de droite dans l'encart*). Le moulin gastrique migre de la partie antérieure vers la partie postérieure de la région cardiaque de l'estomac ; *zone hachurée* : filtre pylorique secondaire. Notez l'inclinaison de la région pylorique (*flèches courbes*), la réduction du filtre pylorique secondaire chez les Mysidae et son absence chez les Euphausiidae.

setae. In euphausiids, the lack of a secondary filter and the efficiency of the primary one are apomorphic characters that are derived from an ancestral foregut closer to that of *Bentheuphausia amblyops*. In Euphausiacea, the pyloric region is bent compared with the cardiac one. Such a bending of the pyloric chamber appears progressively in the phylogenetic history of the Mysidacea (Fig. 6). Moreover, the cardiac chamber is elongated in the Euphausiacea as also observed in the primitive Lophogastrida (see De Jong-Moreau & Casanova, 2001 for review). The location of the gastric mill, at the end of the cardiac chamber, corresponds to that observed in the Mysidae, the most evolved family of Mysidacea, and also in the Decapoda. The progressive migration of the gastric mill from the anterior to the posterior part of the cardiac chamber is related to a better efficiency of the grinding function (De Jong-Moreau & Casanova 1999, 2001). Therefore, it is possible that the foreguts of the Mysidacea, Euphausiacea and Decapoda arose from a common ancestral form and evolved independently in the three taxa. According to previous studies, it can be hypothesized that the species *Gnathophausia gracilis* W.-Suhm, 1875 is at the base of the Lophogastrida phylogenetic tree (Casanova et al. 1998, De Jong-Moreau et al., 2001). The lack of a median tooth in the Euphausiacea has also been noticed in the Eucopiidae (Lophogastrida), while it is always present in the Decapoda. Indeed, in the monogeneric family, Eucopiidae, all the species lack this median tooth, except *Eucopia sculpticauda* Faxon, 1893 considered at the root of the family (Casanova et al., 1998), the presence of this tooth being considered as a plesiomorphic character. Moreover, in the Mysida, the median tooth is at the origin of the formation of dorso-lateral teeth that are only present in Mysidae. The location of these teeth corresponds to that of the dorso-lateral teeth observed in Euphausiacea. In the same way, the cluster spines of Euphausiacea correspond to the lateral teeth of Mysidacea. The main features of the foregut morphology of both Mysidacea and Euphausiacea are summarized in an evolutionary diagram (Fig. 6).

Since 1904, Calman used the morphology of the midgut gland as a character to separate the Peracarida ("hepatic caeca few and simple") and the Eucarida ("hepatic caeca much ramified"). The present study shows that the gross morphology of the midgut gland of *Euphausia krohni* is similar to that of the Decapoda (Icely and Nott, 1992), which is in agreement with the statement of Calman (1904).

The specific role of the different cell types found in the midgut gland has been investigated in the Decapoda on the basis of morphology, histology, fine structure and feeding experiments (see Icely & Nott, 1992 for review). In brief, digestive enzymes are produced in F-cells and are released periodically after ingestion of food to act externally in the tubule lumen and in the foregut. B-cells absorb luminal molecules by intensive endocytosis, resulting in the development of an enormous vacuolar apparatus corresponding to a giant phagosome. R-cells absorb large amounts of fat precursors, which are stored in large or small droplets. The presence of characteristic F-cells in the *Euphausia krohni* midgut gland is the most interesting ultrastructural finding. Indeed, the presence of this cell type has never been truly demonstrated in the Mysidacea (Friesen et al., 1986; De Jong-Moreau et al., 2000).

The phylogenetic relationships within the Malacostraca crustaceans are still a matter of debate. Recently, a new phylogeny based on 28S rDNA sequences analysis has been stated (Jarman et al., 2000; Jarman, 2001). The Euphausiacea might be more closely related to the Mysida, than to the Decapoda as it is generally thought. If molecular biology is an indisputable relevant tool for studies on phylogenetic relationships, it must be reminded that many rare species cannot be used for such studies. This is actually the major problem of this modern method, especially when studying taxa including rare species such as euphausiids and mysids. Indeed, most of them are deep living organisms, present in small numbers in collections and preserved in fixative solutions not always suitable today for molecular studies. Until now, the primitive rare species, as those of the Petalophthalmidae (Mysidacea, Mysida) or *Bentheuphausia amblyops* (Euphausiacea), were included in phylogenetic studies based only on morphological data (Suh & Nemoto, 1988; De Jong-Moreau & Casanova, 2001) and not with the help of molecular tools.

The results of the present study on the digestive system of *Euphausia krohni* show the complexity of the phylogenetic relationships among the Malacostraca. Indeed, they suggest that the digestive system of this Euphausiacea has both primitive and derived features. The chemical role of the labrum is an interesting finding, only described to date in the primitive *Lophogaster typicus* among the Malacostraca Crustaceans. The morphology of the foregut suggests a common ancestral form for the Mysidacea, Euphausiacea and Decapoda. The structure and ultrastructure of the midgut gland are similar to that observed in the higher Malacostraca, i.e. the Decapoda.

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