Branchial chamber tissues in two caridean shrimps: the epibenthic *Palaemon adspersus* and the deep-sea hydrothermal *Rimicaris exoculata*

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Abstract

The structure of the epithelia of the branchial chamber organs (gills, branchiostegites, epipodites) and the localization of the Na⁺,K⁺-ATPase were investigated in two caridean shrimps, the epibenthic *Palaemon adspersus* and the deep-sea hydrothermal *Rimicaris exoculata*. The general organization of the phyllobranchiate gills, branchiostegites and epipodites is similar in *P. adspersus* and in *R. exoculata*. The gill filaments are formed by a single axial epithelium made of H-shaped cells with thin lateral expansions and a basal lamina limiting hemolymph lacunae. In *P. adspersus*, numerous ionocytes are present in the epipodites and in the inner-side of the branchiostegites; immunofluorescence reveals their high content in Na⁺,K⁺-ATPase. In *R. exoculata*, typical ionocytes displaying a strong Na⁺,K⁺-ATPase specific fluorescence are observed in the epipodites only. While the epipodites and the branchiostegites appear as the main site of osmoregulation in *P. adspersus*, only the epipodites might be involved in ion exchanges in *R. exoculata*. In both species, the gill filaments are mainly devoted to respiration.

Keywords: Osmoregulation; Ionocyte; Gills; Na⁺,K⁺-ATPase; *Palaemon*; *Rimicaris*

1. Introduction

Osmoregulation is one of the most important adaptive physiological processes permitting the successful establishment of a species in a given habitat (Charmantier, 1998). In marine Crustaceans, this mechanism is based on active ion transport performed by highly differentiated osmoregulatory epithelia of the branchial chambers hosting specialized cells or ionocytes (Taylor and Taylor, 1992). Although a large amount of data exists on the pattern of osmo- and/or ionoregulation in different species of caridean shrimps (review in Mantel and Farmer, 1983), the structure of the ionoregulating tissues of the Caridea is poorly documented (see review in Martinez, 2001). The available data are limited to the gills in *Palaemonetes varians* (Allen, 1892), *Crangon vulgaris* (Debaiseux, 1970), *Macrobrachium olfersii* (Freire and McNamara, 1995; McNamara and Lima, 1997) and to the epipodites in *Crangon vulgaris* (Debaiseux, 1970). No information is available on the branchiostegites, which are known as osmoregulatory structures in decapod species such as the thalassinid shrimp *Calanus attenuatus* (Felder et al., 1986), the penaeid shrimps *Penaeus aztecus* (Talbot et al., 1972) and *P. japonicus* (Bourratch et al., 1994) and the lobster *Homarus gammarus* (Haoud et al., 1998; Lignot et al., 1999; Lignot and Charmantier, 2001). In addition there is no information on the structure of the branchial chamber tissues in the shrimps that live around deep hydrothermal vents. *R. exoculata* (Williams and Rona, 1986), family Alvinocarididae (Christoffersen, 1986) is the dominant species among the fauna of the Mid-Atlantic Ridge.
hydrothermal vent sites (Galkin and Moskalev, 1990; Gebruur et al., 1997; Tunicliffe et al., 1998; Van Dover, 2000). This species forms exceptionally dense clusters (up to 3000 individuals per m²) on sulfide mounds associated with active venting (Segonzac et al., 1993; Van Dover, 1995). The adult shrimps live permanently in or very close to variable and extreme environmental conditions such as high temperature, high sulfide and metal content, high level of carbon dioxide, low oxygen level, and low pH (Truchot and Lallier, 1998; Sarradin et al., 1998a; Sarradin et al., 1998b; Sarradin et al., 1998a,b; 1999). Temperatures appear to range between 10 and 30 °C with an upper limit at 70 °C (Segonzac et al., 1993; Lakin et al., 1997; Lallier and Truchot, 1997; Truchot and Lallier, 1998). From the scarce and controversial available information, salinity of the hydrothermal fluid seems variable from 2.8 to 68% depending to the sites (Chevaldonné, 1997; Truchot and Lallier, 1998; Van Dover, 2000).

The objective of the study was to compare the structure of the organs of the branchial chambers in two caridean shrimps living in demanding but different habitats: Palaemon adspersus, an epibenthic shrimp which lives in coastal lagoons where salinity is very variable, able to strongly hyperregulate (Martinez, 2001) and the deep-sea hydrothermal R. exoculata. The presence of typical cells involved in ion exchanges or ionocytes was investigated through light and electron microscopy; immunocytochemistry was used to localize Na⁺,K⁺-ATPase, one of the main enzyme involved in active ion transport.

2. Materials and methods

2.1. Animals

Adults of Palaemon adspersus were caught in the Mauguio lagoon (Hérault, France). The shrimps were maintained at the Montpellier laboratory for ca. two weeks in 3000 l tanks containing aerated and re-circulated natural seawater (37 ± 1% at 20 ± 1 °C) under a 12 h L/12 h D photoperiod. They were fed three times a week with defrosted cooked mussels. The shrimp mean cephalothoracic length was 10.14 ± 0.86 mm.

Adults of R. exoculata were collected by French and American submarines at a depth of about 3000 m, on the Rainbow and Logatchev sites of the Mid-Atlantic Ridge [36°11′N–33°57′W and 14°45′N–44°58′W (Tunicliffe et al., 1998; Van Dover, 2000), respectively, during the MARVEL (1997), and ATOS and DIVERSE Expedition (2001) missions. The shrimp cephalothorasic length was 10-25 mm. Only a small number of shrimps was available. The hydrothermal shrimps, which seem incapable of long-term survival outside the high-pressure environment of the deep-sea, were dissected and fixed as soon as they reached the boat. For both species, the observations were conducted in stage C specimens selected after microscopic examination of the tip of a pleopod according to the method of Drach and Tchernigovtseff (1967).

2.2. Light microscopy

The cephalothorax from freshly killed individuals (by section of the cerebroid ganglia) was longitudinally cut into two halves which were fixed for 24 h in Bouin’s fixative. This fixation was performed on board for R. exoculata. The specimens were then fully dehydrated in a graded ethanol series and embedded in paraplast. Sections (5 μm) were cut on a Leitz Wetzelar microtome, collected on albumine-glycerine slides and stained with Masson Trichrome (variant Goldner) (Martoja and Martoja-Pierson, 1967).

Immunostaining localization of Na⁺,K⁺-ATPase was performed with a mouse monoclonal antibody IgG5 raised against the α-subunit of the chicken Na⁺,K⁺-ATPase (Takeyasu et al., 1998). This antiserum, previously used on Porcellio scaber (Ziegler, 1997), Carcinus maenas (Lucu and Flik, 1999), Astacus leptodactylus (Barradas et al., 1999b) and Homarus gammarus (Lignot et al., 1999; Lignot and Charmantier, 2001) was kindly provided by D.M. Fambrough (Baltimore, Md., USA).

Transverse sections of the tissues (3 μm) were collected on poly-l-lysine-coated slides. The sections were pre-incubated for 10 min in 0.01 m Twenn 20, 150 mM NaCl in 10 mM phosphate buffer saline (PBS), pH 7.3, treated with 50 mM NH₄Cl in 20 mM phosphate buffer saline (PBS), pH 7.3 for 5 min, then incubated for 10 min with a blocking solution (BS) containing 1% bovine serum albumine (BSA) and 0.1% gelatin in 20 mM PBS, pH 7.3. Droplets of the primary antibody (dilution: 20 μg ml⁻¹) were placed on the sections that were incubated for 2 h in a wet chamber at room temperature. Control sections were incubated without primary antibody. After being washed in BS, the sections were incubated for 1 h in droplets of the secondary antibody, fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG (H&L; Jackson Immunoresearch, West Baltimore, Md.).
sections were mounted in 80% glycerine, 20% PBS plus 2% N-propyl-gallate to retard photobleaching. They were examined on a fluorescent microscope (Leitz Diaplan coupled to a Ploemopak 1-Lambda lamp) with the appropriate filter set (450–490 nm band-pass excitation filter).

2.3. Transmission electron microscopy (TEM)

The technique of Pottu-Boumedil (1989) was used. Dissected pieces of gills, epipodites and branchiostegites were fixed on ice for 2 h with 2.5% glutaraldehyde in a mixture of seawater and freshwater, pH 7–4, adjusted to the hemolymph osmotic pressure to prevent osmotic shocks. The branchiostegites and epipodites were decalcified for five days in EDTA-Na₂ 0.2 M, pH 8. Samples were post-fixed for 2 h at 4°C in OsO₄, washed in distilled water and dehydrated in a graded ethanol series and propylene oxide, then embedded in Spur’s resin. Semithin and ultrathin sections were cut on a Reichert OMU3 ultramicrotome. The first sections were stained with toluidine blue. Ultrathin sections were contrasted with 2% uranyl acetate and embedded in an aqueous solution of Spur’s resin. Semithin sections were stained with 1% toluidine blue in 70% alcohol and lead citrate, and they were observed on a JEOL 1200 EX2 transmission electron microscope at 70 kV.

3. Results

3.1. Gills

The eight pairs of gills of P. adspersus are phyllobranchiate gills. The flattened lamellae are attached along the two outer faces of the triangular gill axis, forming two rows oriented at a 40° angle. The branchial lamellae are 20 μm thick (Fig. 1A and C). They are composed of H-shaped epithelial cells with a thick axial zone (6–10 μm) containing a voluminous round or oval central nucleus (3–6 μm). Lateral thin expansions (0.5 μm) extend under the thin cuticle (0.2–0.8 μm) and limit two rows of hemolymph lacunae, where few hemocytes are present. The cytoplasm of the epithelial cells is generally electron-dense, especially around the nuclei (Fig. 1C). The axial zone presents a dense system of membrane infoldings opened on the basal lamina and closely associated with numerous round or oval mitochondria (0.3–1.4 μm) (Fig. 1C). These infoldings are orientated parallel, rather than perpendicular, to the length of the lamellae (Fig. 1E). The lateral expansions of the epithelial cells contain few cytoplasmic organelles. Only sub-cuticular spaces, irregularly distributed, can be observed in the lamellae (Fig. 1C and G).

Regarding the immuno-localization of Na⁺,K⁺-ATPase, classical fixation and paraffin-embedding procedures yielded good antigenicity as observed with the fluorescent micrographs (Fig. 4A–D). Controls showed no specific binding within the epithelia of gills, branchiostegites (Fig. 4A) and epipodites (results not shown). Auto fluorescence was observed along the cuticle, mostly at the epicuticle level (Fig. 4D). Na⁺,K⁺-ATPase specific labelling was detected only in the axial zone of the epithelial cells of the gills; it was absent in their thin lateral expansions (Fig. 4B).

R. exoculata possess 10 pairs of phyllobranchiate gills. The branchial lamellae (15–40 μm thick) show a single layer of H-shaped epithelial cells which almost fully occupy the volume of the lamellae, bordered by a thin cuticle (0.3–0.5 μm) (Fig. 1B and D). The nuclei, round or oval (8–10 μm) are irregularly located in the central and dense perikaryon (13–20 μm thick) of the cells. Lateral cell expansions (2.5 μm thick) limit reduced lateral hemolymph lacunae lined by the basal lamina (Fig. 1B and D). The cytoplasm of the axial part of the epithelial cells contains numerous mitochondria (1 μm) with either well-shaped, regular and dense crests or short and disorganized crests; both are grouped and surrounded by a light system of membranes sometimes in relation with the basal lamina (Fig. 1F). Some mitochondria contain granules of 40 nm in diameter (Fig. 1F). Large vesicles or clear areas containing black particles are frequently located around the nucleus (Fig. 1D). The cytoplasm of the lateral expansions of the epithelial cells hosts few organelles except at the pilar junctions where bundles of long tonofilaments coming for the axial part are inserted on the cuticle (Fig. 1H). No Na⁺,K⁺-ATPase specific labelling was observed in the gills, neither in the axial zone nor in the lateral expansions of the epithelial cells (Fig. 4E).

3.2. Epipodites

Each branchial chamber of P. adspersus contains three epipodites associated with each maxilliped: the first is very small, the second (65 μm thick) has a common base with the podobranch, the third is a well-developed lamellar organ (100 μm thick). Both sides of the epipodites are lined by a thick regular epithelium made up of prismatic cells (20–25 μm high) with voluminous oval nuclei (5 μm in diameter) (Fig. 2A). The central axis of the organs is occupied by a lamellar septum made up of connective tissue and hemolymph lacunae; the basal laminae of the two facing epithelial layers are close in some places. No pillar structures have been observed between them. The cytoplasm of the epithelial cells contains numerous elongated mitochondria.

Fig. 2. Epipodites of Palaemon adspersus (A, C, E, G) and Rimicaris exoculata (B, D, F, H); semi-thin sections (A, B) and transmission electron micrographs (C–H). A, B: General organization of the two facing epithelia. C, D: Epithelial cells. E, F: Basal area with membrane infoldings and mitochondria. G, H: Apical protrusions of the lamellae. Scale bars: (A, B) 50 μm; (C–H) 1 μm. AM: Apical microvilli; B: bacteria; BI: basal infolding; BL: basal lamina; C: cuticle; E: epithelium; M: mitochondria; N: nucleus; S: septum; V: vesicle.
(2.5 μm in average length), closely associated with dense and deep infoldings of the basal cell membrane orientated perpendicularly to the surface of the epithelium (Fig. 2C and E). Under the thin cuticle (1 μm), the apical membrane of the cells forms numerous microvilli (1–1.5 μm high), sometimes associated with small mitochondria (Fig. 2G).

A strong Na⁺,K⁺-ATPase immunolabelling was detected in the two layers of the epithelium of the appendages, mostly in the basal and median parts of the cells. No immunoreactivity was detected in the axial septum (Fig. 4C).

The three pairs of appendages of *P. adspersus* are lamellar organs with an irregular thickness (80–160 μm). The first is a well-developed ovoid lamella; the second is rudimentary and elongated; the third is reduced and forms an outgrowth at the base of the third maxilliped. Their general organization is illustrated in Fig. 2B. They are limited by two single and dense epithelial layers made up of high prismatic cells (30–70 μm thick) with big ovoid nuclei (10–20 μm) at the apical side. A thin and dense conjunctive tissue with numerous nuclei and small hemolymph lacunae is present in the axial zone of the appendages. No pillar cell was observed. Both epithelia are limited by a thick cuticle (5 μm) covered by bacteria. The cytoplasm of the epithelial cells contains numerous mitochondria displaying various shapes (round, oval or elongated) and sizes (3 μm long, 0.3–1.5 μm in diameter) (Fig. 2D). They are closely associated with a dense network of deep basal infoldings. These well-developed infoldings have a variable length according to the cells or cellular areas (Fig. 2D and F). Abundant and irregular apical microvilli (3 μm high) are observed under the cuticle, sometimes associated with small vesicles (Fig. 2D and H). The epipodites showed a strong and regular Na⁺,K⁺-ATPase fluorescence in the cells of the two epithelial layers. The immunolabelling was absent in the axial septum (Fig. 4F).

### 3.3. Branchiostegites

The general organization of the branchiostegite of *P. adspersus* is illustrated in Fig. 3A. This structure, approximately 135 μm thick, comprises two irregular thick epithelia maintained by pillar cells which cross a voluminous central hemolymph lacuna. The outer epithelium is made up of high prismatic irregular cells (about 25–40 μm high) under a thick cuticle (40–50 μm) which forms part of the lateral carapace of the cephalothorax. The epithelium lining the inner side of the branchiostegite (Fig. 3C), irregular and thick (20–40 μm high), is covered by a thin cuticle (0.5 μm). In both epithelia, the nuclei are big (10 μm in diameter) and irregularly shaped. The outer epithelium contains mainly clear vesicles of various sizes and no specific differentiation except bundles of tonofilaments (not illustrated). The cells of the inner epithelium contain numerous round or oval mitochondria (0.5–3 μm) orientated perpendicularly to the surface of the epithelium. They are closely associated with dense and deep basal cellular infoldings which almost reach the apical side of the cells (Fig. 3E and G). No apical microvilli were observed (Fig. 3G). The inner epithelium of the branchiostegite showed a very strong Na⁺,K⁺-ATPase fluorescence particularly at the basal side of the cells. The enzyme specific labelling was absent from the central zone of the pillar structures. No fluorescence was detected in the outer epithelium of the branchiostegite (Fig. 4D).

The thick (130 μm) branchiostegite of *R. exoculata* is lined by two irregular epithelial layers partly separated by clusters of a central hemolymph lacuna (Fig. 3B). The outer side of the epithelium (25–40 μm thick) is situated below a thick cuticle (30 μm) forming the lateral carapace of the cephalothorax. The inner epithelium (15–50 μm thick) is covered by a thin cuticle (0.5 μm). The nuclei are ovoid, variable in size (5–15 μm) and irregularly placed. Dense pillar structures join the two cuticles. The cytoplasm of the internal epithelial cells contains numerous small and round mitochondria (0.5–0.8 μm) (Fig. 3D). The basal side of the cells shows short (2.5–4 μm) and scarce basal infoldings (Fig. 3F). Toward the central hemolymph lacuna, some cells present voluminous basal bulges containing vesicles loaded with black particles of different densities (Fig. 3D). They are part of the cell and boarded by the basal membrane of the epithelium. Mitochondria are irregularly located beneath apical microvilli (1 μm high) (Fig. 3H). No Na⁺,K⁺-ATPase immunoreactivity was detected in the inner and outer epithelia of the branchiostegites (Fig. 4G).

### 4. Discussion

#### 4.1. Gills

*P. adspersus* and *R. exoculata* possess phyllobranchiate gills as the other palaemonid shrimps and the brachyuran crabs (Cuénot, 1893; Debaixieux, 1970; Taylor and Taylor, 1992).

The branchial lamellae of *P. adspersus* and *R. exoculata* show an axial epithelium with H-shaped cells. The perikarya of the cells are situated in the longitudinal medial septum while the lateral expansions form thin sheets along the inner surface of the cuticle. The basal lamina of the epithelial

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Fig. 3. Branchiostegites of *Palaemon adspersus* (A, C, E, G) and *Rimicaris exoculata* (B, D, F, H); semi-thin sections (A, B) and transmission electron micrographs (C–H). A: General organization; C: D: Epithelial cells; G: H: Apical part. Scale bars: (A, B) 50 μm; (C–D) 5 μm; (E–H) 1 μm. ASM: Apical microvilli; BL: basal lamina; BP: basal particles; H: hemocyte; HL: hemolymph lacuna; IC: inner cuticle; IE: inner epithelium; M: mitochondria; N: nucleus; OC: outer cuticle; OE: outer epithelium; PC: pillar cell.
Fig. 4. *P. adspersus* and *R. exoculata*. Immunolocalization of Na⁺,K⁺-ATPase in the gills, epipodites and branchiostegites. A, B, C, D: *P. adspersus*: A: control branchial chamber including gills and branchiostegite; B: gill lamellae; C: epipodite; D: branchiostegite. E, F, G: *R. exoculata*: E: gill lamellae; F: epipodite; G: branchiostegite. Scale bars: 50 μm. B: Bacteria; Br: branchiostegite; C: cuticle; E: epithelium; GL: gill lamellae; HL: hemolymph lacuna; IC: inner cuticle; IE: inner epithelium; LE: lateral expansion; MC: marginal channel; N: nucleus; OC: outer cuticle; OE: outer epithelium; PC: pillar cell; S: septum.
cells limits lateral hemolymph lacunae. The organization of the branchial lamellae of \textit{R. exoculata} is similar to that observed in \textit{P. adspersus} and in other palaeomonid and crangonid shrimps (Papathanassiou and King, 1983; Doughtie and Rao, 1984; Papathanassiou, 1985; Patil and Kalival, 1989; Freire and McNamara, 1995). This structure differs from the gill organization of brachyuran crabs in two ways: (i) the epithelium, which is axial in \textit{P. adspersus} and \textit{R. exoculata}, is laterally located in the brachyura (Fino and Croghan, 1983; Compère et al., 1989; Goodman and Cavey, 1990; Farelly and Greenaway, 1992); (ii) the axial conjunctive septum described in most brachyurans as located between the two epithelial layers, seems absent in \textit{P. adspersus} and \textit{R. exoculata}.

In \textit{P. adspersus}, the axial zone of the epithelial cells of the gills possesses a network of basal internal membranes closely associated with numerous mitochondria. Na$^+$.K$^-$-ATPase is also slightly present. These cells appear as atypical ionocytes. They differ from typical ionocytes by the low number of membrane infoldings, their irregular orientation and their scarce openings on the basal lamina (reviews in Taylor and Taylor, 1992; Péqueux, 1995), but the presence of the principal enzyme involved in active ion transport is indisputable. These cells are thus probably involved in osmoregulatory active ion pumping. Anatomical evidence has lead to the same hypothesis in other caridean shrimps (Doughtie and Rao, 1978, 1983; Papathanassiou and King, 1983; Rao and Doughtie, 1983; Papathanassiou, 1985; Freire and McNamara, 1995; McNamara and Lima, 1997; McNamara and Torres, 1999).

The general structure of the gill lamellae in \textit{R. exoculata} is similar to the corresponding organization in \textit{P. adspersus}. However, in \textit{R. exoculata}, the axial zone of the epithelial cells contains fewer mitochondria and does not reveal the presence of Na$^+$.K$^-$-ATPase. Thus the axial epithelium of the gills of \textit{R. exoculata} does not seem involved in active ion exchanges.

The lateral expansions of the epithelial cells of the gills of \textit{P. adspersus} and \textit{R. exoculata} are similar, thin, poorly differentiated and covered by a thin cuticle. They lack Na$^+$.K$^-$-ATPase in both species. They present the features of respiratory epithelia as described in other species, e.g. (i) in the anterior and posterior gills of osmocomping crabs as \textit{Can- cer pagurus} (Péqueux et al., 1988); (ii) in the anterior gills of osmoregulating crabs as \textit{Callinectes sapidus} (Copeland and Fitzjarrell, 1968), \textit{Eriocheir sinensis} (Barra et al., 1983; Péqueux et al., 1988), \textit{Carcinus maenas} (Compère et al., 1989; Goodman and Cavey, 1990), and \textit{Gecarcinus lateralis} (Copeland, 1968); (iii) in the gills of the lobster \textit{Homarus gammarus} (Haond et al., 1998) and in some filamentous gills of the crayfish \textit{Astacus leptodactylus}, \textit{Austropotamo- bius pallipes} (Dunel-Erb et al., 1982, 1997) and \textit{Procambarus clarkii} (Dickson et al., 1991).

In summary, the gills of \textit{P. adspersus} seem to have a double function, respiration and osmoregulation, respectively effected by the lateral expansions and the axial part of the cells. This situation is different from, but reminiscent of the functional separation between the respiratory anterior gills and the osmoregulatory posterior gills in brachyuran crabs (Copeland and Fitzjarrell, 1968; Barra et al., 1983; Péqueux et al., 1988; Compère et al., 1989) and between different filaments of the same gill in crayfish (Barradas et al., 1999a).

The gills of \textit{R. exoculata} appear involved in respiration only. Compared to the situation in epibenthic species, their single function may be interpreted as an adaptation to the relative hypoxia of the deep hydrothermal environment. This hypothesis is supported by the higher number of gills in \textit{R. exoculata} compared to \textit{P. adspersus} (10 pairs versus 8 pairs) and by the comparatively high oxygen affinity of the hemocyanin in \textit{R. exoculata} (Lallier and Truchot, 1997; Lallier et al., 1998). The gills of \textit{R. exoculata} present other peculiarities such as the presence of bacteria on the cuticle, the thickness of their epithelium, the presence of black particles in perinuclear areas, the mitochondrial granules and the reduced and disorganized mitochondrial crests. They might represent other adaptations to their particular environment such as sulfide detoxication for some of them. This detoxication function has already been hypothesized in the gills of \textit{R. exoculata} through sulfide-oxidising bodies (SOBs) (Compère et al., 2002). Mitochondria containing small dense granules also slightly present in the gills of the “Pompeii worms” \textit{Alvinella pompejana} and \textit{Paralvinella grasslei} (Jouin and Guill, 1990) and in the meiobenthic thiotrophic turbellarian \textit{Solenofilosomorpha funilis}, a species able to detoxify sulfides (Duffy and Tyler, 1984).

### 4.2. Epipodites

In \textit{P. adspersus} and \textit{R. exoculata}, the epipodites present two thick lateral epithelial layers without pillar structures, separated by an axial septum presenting small hemolymph lacunae. This structure is similar to those described in \textit{Crangon crangon} (Debaiseaux, 1970) and in the pened shrimp \textit{Peneus japonicus} (Bouaricha et al., 1994). The epipodites of the lobster \textit{Homarus gammarus} (Haond et al., 1998) and the lamina (equivalent structure) of the crayfish \textit{Astacus leptodactylus} and \textit{Austropotamo- bius pallipes} (Dunel-Erb et al., 1982, 1997) present the same organization of two facing epithelia, but with the addition of frequent pillar cells and of a voluminous central lacuna.

The epithelial cells of the epipodites of \textit{P. adspersus} and \textit{R. exoculata} are typical ionocytes displaying apical microvilli and numerous mitochondria closely associated with deep basal infoldings which reveal a strong presence of Na$^+$.K$^-$-ATPase. Ionocytes have also been described in the epipodites of \textit{Peneus japonicus} (Bouaricha et al., 1994), \textit{Austropotamo- bius pallipes} and \textit{Astacus leptodactylus} (Dunel-Erb et al., 1982, 1997) and \textit{Homarus gammarus} (Haond et al., 1998). The presence of Na$^+$.K$^-$-ATPase was also detected in the last species (Lignon et al., 1999). The epipodites of \textit{P. adspersus} and \textit{R. exoculata} may both have an osmoregulatory function. Compared to \textit{P. adspersus}, the epipodites of \textit{R. exoculata} seem to present particular features potentially associated with their function in the deep-sea hydrothermal
vents. Well-developed apical microvilli are present in both species: in *R. exoculata* they are associated with numerous vesicles, which are scarce or even absent in *P. adspersus* (this study) and in *Palaemon serratus* (Papathanassiou and King, 1983). These vesicles have been interpreted as pinocytic vesicles (Copeeland and Finjarrell, 1968; Bubel and Jones, 1974; Bubel, 1976; Papathanassiou and King, 1983). Their membranes in *R. exoculata* are close to numerous mitochondria and thus may constitute “mitochondrial pumps” as it has been hypothesized by Maina (1990) in the crab *Palaemon nitidus*. The epithelium of the epipodites of *R. exoculata* and *P. adspersus* may thus play a role in osmoregulation and also in other ionic exchanges, e.g. in acid-base regulation (Truchot, 1983; Henry and Wheatly, 1992).

4.3. Branchiostegites

The branchiostegites of *P. adspersus* and *R. exoculata* present cellular structures similar to those described in the same organs in the thalassemid *Callianassa jamaicense* (Felder et al., 1986), *Peneus ezeicus* (Talbot et al., 1972), *P. japonicus* (Bouaricha et al., 1994) and *Homarus gammarus* (Haond et al., 1998): two thick and irregular lateral layers of epithelium separated by pillar cells which cross a voluminous central hemolymph lacuna. To our knowledge, our study is the only one concerning this site in caridean shrimps.

The internal epithelium of the branchiostegites of *P. adspersus* reveals the ultrastructural features of typical ionocytes associated to a strong immunolabelling for Na⁺,K⁺-ATPase. The presence of this enzyme has been already reported in the branchiostegites of juvenile *Homarus gammarus* (Lignot et al., 1999; Lignot and Charmantier, 2001). It points to an involvement of these structures in osmoregulation in the lobster and the shrimp.

In *R. exoculata*, the inner epithelium of the branchiostegite, covered by a thin cuticle, comprises high prismatic cells presenting apical microvilli and numerous mitochondria but with only short and scarce basal infoldings and no evidence of Na⁺,K⁺-ATPase. The branchiostegites of *R. exoculata* are therefore probably not involved in active sodium transport.

The branchiostegites of *P. adspersus* and *R. exoculata* have therefore different functions. They most probably are osmoregulatory organs in *P. adspersus*. Their role in *R. exoculata*, still hypothetical, may be linked to the presence of the large basal protuberances filled with black particles. They could participate to sulfide detoxication in addition to the gills.

4.4. Localization of the osmoregulatory structures

Since the early studies on crustacean osmoregulation, the gills have been considered as the primary site for ionic and osmotic regulation (review in Robertson, 1960; Lockwood, 1962; Gilles, 1975; Croghan, 1976; Mantel and Farmer, 1983; Péqueux et al., 1984; Towlé, 1984; Péqueux, 1995). In some species, osmoregulation is anatomically separated from the respiratory function. In strongly osmoregulating brachyurans such as *Pachygrapsus marmoratus*, the anterior gills are specialized in respiration and the posterior gills have an osmoregulatory function (Péqueux, 1995). In these species, the epipodites and branchiostegites do not display any osmoregulatory structure. In crayfish, on each gill, the filaments are specialized either in ionic regulation or in gas exchanges (Taylor and Taylor, 1992; Barradas et al., 1999a,b). A few studies have revealed that differentiated osmoregulatory tissues can be present in the branchial cavity of some decapods, located at two sites different from the gills. These sites include the branchiostegites (Talbot et al., 1972; Felder et al., 1986; Bouaricha et al., 1994; Haond et al., 1998; Lignot et al., 1999) and/or the epipodites (Duneè-Erb et al., 1982, 1997; Kikuchi and Matsumasa, 1993; Bouaricha et al., 1994; Haond et al., 1998; Barradas et al., 1999b; Lignot et al., 1999). They may be temporary at certain stages of development. The observations reported here regarding *P. adspersus* and *R. exoculata* bring additional structural and functional evidence of the existence of extrabranchial ion-transporting tissues in adult decapod crustaceans. *P. adspersus* is a strong hyper-hypo-osmoregulatory species (Martínez, 2001) like most epibenthic carides (Mantel and Farmer, 1983). Its capacity to osmoregulate originates from a combined activity of the gills, the epipodites and the branchiostegites. The two latter sites might play a predominant role if one considers their high number of typical ionocytes including a high content of Na⁺,K⁺-ATPase. They probably are the major site for active ionic exchanges in this shrimp. In the gills, the axial zone of the epithelial cells, similar to atypical ionocytes and with Na⁺,K⁺-ATPase, may also participate to active ionic exchanges, whereas the lateral expansions would be involved in respiration. In *R. exoculata*, ionocytes and Na⁺,K⁺-ATPase have been found only in the epipodites, and not along the gills and the branchiostegites. We hypothesize that the ability to osmoregulate in *R. exoculata* is comparatively low, considering the small surface of the six epipodites compared to those of the gills and/or the branchiostegites in strong osmoregulating decapods including epibenthic shrimps.

This species may have favoured the respiratory mechanism at the expense of the osmotic regulation in order to deal with the hypoxia of the deep hydrothermal environment. Their frequent movements probably maintain the shrimps in an environment where temperature conditions (recently determined to be below 25 °C by Ravaux et al., 2003) and salinity are both within tolerable range. These constant movements are also favorable for the search of food. This set of hypothesis seems in agreement with in situ observations which describe the shrimps *R. exoculata* as very and constantly active animals, all the more active as they get closer to the warmest emission sources (Segonzac et al., 1993; Van Dover et al., 1998, 1999; Sarradin et al., 1999). The structural evidence for limited osmoregulation in *R. exoculata* reported here should be complemented by direct measurements of hemolymph osmolality.
on freshly captured shrimps, exposed on board to different salinities in pressurized aquaria (Ravaux et al., 2003). It will be a nice step to understand the extent of the osmoregulatory adaptations of the caridean vent shrimps.

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